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Research Article

MICROWAVE SYNTHESIS OF Au/Ag Co - DOPED HYDROXYAPATITE FROM DEAD MUSSEL SHELL AND BIO COMPATIBILITY TEST BY SIMULATED BODY FLUID

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ABSTRACT

Nano hydroxyapatite $(Ca_{10}(PO_4)_6(OH)_2$ was produced from the bio-waste material such as 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 hydrochloric acid and di-ammonium hydrogen phosphate was slowly added to the mixture while maintaining the pH at 10 using ammonium hydroxide(NH_4OH), followed by microwave irradiation for 30 min. The HAp powder calcined at 900° C for 2h and soaked in simulated body fluid (SBF) for several period of time probably 7, 14 and 30 days correspondingly. After the exact period of soaking; the residue was collected, washed and dried at hot air oven and characterized by XRD, FTIR, FE-SEM, FE-TEM and XPS analysis.

INTRODUCTION

In the current years, the damaged bones and tooth can be simply exchanged by various bio-ceramics materials containing hydroxyapatite. In this study, hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ is one of the main part of indispensable bio-material which has been generally used for replacing the wounded part of the human body, mainly for bones and tooth etc. (Dasgupta Adak *et al*., 2011; Sobczak-Kupiec *et al*., 2014). Specifically in recent times, it has been evidenced that the usages of calcium phosphates in biomedical field is an wonderful perform which is importance plenteous uses on biomaterial sector as dentistry, bone interchange, bone repair materials and drug relief methods (Prabaharan *et al*., 2005).

Really, the main piece of apatite calcium phosphate of bone mineral is made up of calcium carbonate. Owing to this concerning, the synthesized Au/Ag co-doped nano HAp initiates to have superior bio-activity and bio-compatibility to the suitable as expectable calcium phosphate (Remant Bahadur *et al*., 2008). For suitable applications in the field of medicine, the well densification of HAp is significant (Bose *et al*. 2010; Park *et al*., 2008; Jalota *et al*., 2004). The method associated with microwave-irradiation is one of the finest techniques to synthesize nano size HAp, which covers shorter reaction time and lesser-energy utilization and etc. In the earlier period, there have been establishing many information which deal the production of nano sized HAp through microwave-irradiation. Though, this process requires being novel, acceptable to improve the biological devastate and control over the contamination (Gopi *et al*., 2013).

In addition, agreeing to Kokubo and Takadama's investigational outcomes, a bioactivity of HAp could be evidenced by immersing in simulated body fluid for permitting to access apatite development. Aimed by the above work, we tried and tested the biocompatibility of as synthesized HAp to be recognized to bone connecting species and to form *in-vivo* apatite activities. The formation can be reproduced in a simulated body fluid with ion concentrations nearly equal to that human blood plasma (Kokubo *et al*., 2006).

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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

Synthesis of Simulated Body Fluid (SBF)

SBF is known as a metastable buffer solution containing calcium and phosphate ions already supersaturated with respect to the apatite. Therefore SBF is prepared as follows, and even a small, undesired variance 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.

Put 750 ml of deionized water in to a 1000 ml of beaker (polyethylene beaker). Stir the water and keep its temperature at 37^{o}C with magnetic stirrer with heater. Add each chemicals given in the table 2 in to water until # 8, one by one in the order given in the table 2, after each reagent was completely dissolved. Add #9 should be 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^{o}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 stored the solution at 5-10^{o}C in the refrigerator (Kokubo *et al.*,1990).

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

Order	Chemicals	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 (About 90% of HCl to be added)	40 Ml
7	$CaCl_22H_2O$	0.278
8	Na_2SO_4	0.071
9	$(CH_2OH)_3CNH_2$	6.057

MATERIALS AND METHOD

Synthesis of HAp

The skeletons of mussel shells were collected and washed with tap water, followed by distilled water to get rid of excess deposits and mucks. They were dried in direct sun light (Kalita *et al.*, 2010; Nedunchezhian *et al.*, 2015; Singh *et al.*, 2012) for 24h and crushed by pestle and mortar, 200 size mesh used to sieve then obtain fine powder (Nedunchezhian *et al.*, 2016). The mussel-shell powder was dissolved in dilute hydrochloric acid and the mixture was added to a solution of di-ammonium hydrogen phosphate. $AuCl_3$ and $AgNO_3$ each 0.1gm were added to the solution prepared before and the pH was maintaining at 10 using ammonium hydroxide. The mixed solution was stirred for 1hr and quickly transferred to a domestic microwave oven (LG, India) and irradiated at 800W energy of frequency employing 2.45 GHZ for 30 min continuously. After the irradiation, the residue was washed with de-ionized water three times and then dried in a vacuum air oven at 60° C for 12h (Murugan *et al.*, 2003).

Characterization

The phase analysis of nano sized hydroxyapatite was analyzed by X-ray powder diffraction technique using Cu-kα radiation and the powder morphology was observed by FE- SEM (JEOL JSM 6701-F USA) fitted 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 analysis carried out (Multilab 2000, Thermoscientific, UK) for elemental and molecular information of the samples.

RESULTS AND DISCUSSION

Phase Analysis

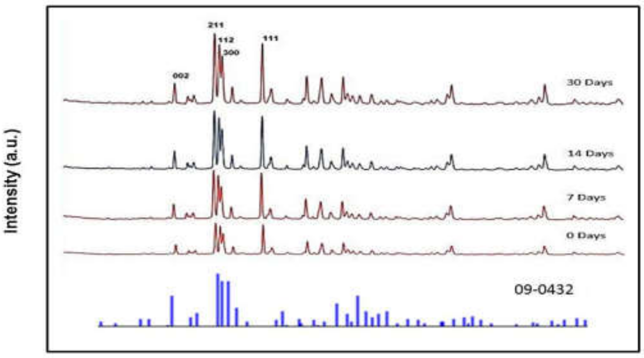


Fig 1 XRD- Analysis Au/Ag doped HAp powders
Before immersion (0 days), after soaked in SBF 7 days, 14 days and 30 days

The XRD diffraction patterns of HAp powder calcined at 900° C and immersed in SBF in various periods were shown in Fig.1. The result shows that all the observed major peaks designate the presence of nanosized HAp in all the samples (Murugan *et al.*, 2005. The observed peaks at 25.7°, 31.7°, 32.1°, 32.9° and 38.1° occurs connected to the planes (002), (211), (112), (300), and (111) which represents the high crystallinity of the (211) plane it confirms the formation of HAp. Clearly, these outcomes were good in agreement with standard JCPDS Card No.09-0432.

Table 1 Calculation of crystallite size of Au/Ag doped Nano sized HAp

S.No.	Sample	Crystallite Size (nm)
1	Au /Ag doped HAp Before Immersed in SBF	16.87
2	Au /Ag doped HAp Immersed after 7 days	16.98
3	Au /Ag doped HAp Immersed after 14 days	25.08
4	Au /Ag doped HAp Immersed after 30 days	25.91

$D = k \lambda / \beta \cos \theta.$

Where $\beta = FWHM \times \pi/180$, $K=0.94$, $\lambda=1.5406$ Å

The crystallite size was calculated using Debye- Scherer formula for HAp powders, which discloses that the typical crystallite size of HAp before immersed in SBF was 16.87 nm, 16.98 nm for HAp immersed after 7 days, 25.08 nm and 25.91 nm for 14 and 30 days of immersion in SBF. Hence the better crystallite sizes of the HAps are depicted in the table 1 (Dasgupta Adak *et al*., 2011).

Chemical composition

The FTIR spectrum of prepared Au and Ag doped calcined HAp before and after immersion of SBF solution as shown in Fig.2. The characteristic peaks matching to stretching vibration of PO_4^{3-} ions at around 1088.54cm^{-1} -1058.22 cm^{-1} and the peaks at 569.10 cm^{-1} - 604.59 cm^{-1} are assigned to the deformation of PO_4^{3-} ions (Syed Sibte Asghar Abidi *et al*.,2014). The presents of OH- stretching band around 3571.90 cm^{-1} suggest the adsorption of H_2O molecules. The observed doublet peak around 604.59 cm^{-1} – 569.10 cm^{-1} are recognized the formation of apatite. These peaks are due to bending mode of P-O bonds in phosphate group, The peaks at 960.47 cm^{-1} are the stretching mode of CO_3^{2-} ions. This may be weak bonding between carbon and oxygen in the present study (Gross *et al*.,2013; Sushma Jadalannagari *et al*.,2014; Igbal *et al*., 2014;Dezhi Qin *et al*.,2015).

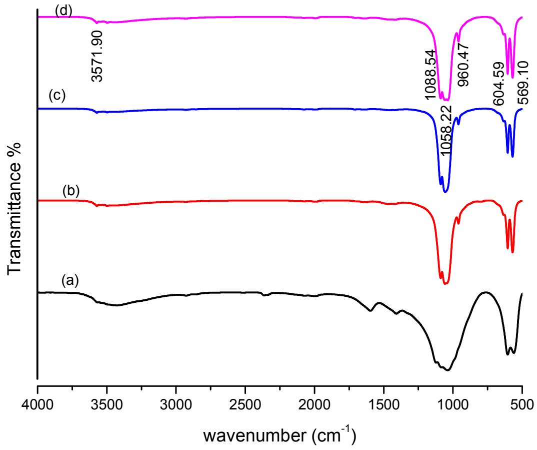


Fig 2 FTIR Spectrum of Au/Ag doped HAp powders before and after soaked in SBF. (a) 0 day (b) 7 days (c).14 days (d).30 days

Morphological studies

Fig.3 shows the morphology of HAp before and after soaked in SBF. It reveals that the Au/Ag doped HAp has solid apatite, excellent crystallite like structure morphology with less crystallite of highly agglomerated powders (Nedunchezhian *et al*.,2015). The changes in the morphology of the Au/Ag doped calcined nano sized HAps are due to the increase in the crystallinity of the powder included with the calcined temperatures. The HAp powder with soaked in SBF achieve the round morphology with homogenous microstructure. This is due to the decreasing the P ion and gradually increased Ca ion of the concentration of SBF solution (Himeno *et al*., 2003). After soaking in SBF for (7,14 and 30) days Ca/P ratio increased to1.73, indicating that the HAp surface gathered Ca, P, Au and Ag ions in the fluid up to this soaking period. The presents of Ca, P, O, Au and Ag to confirm the formation of Au/Ag doped apatite. The presented elements and their atomic, weight percentages are inset in the Fig. 4 (Deepa *et al*., 2013).

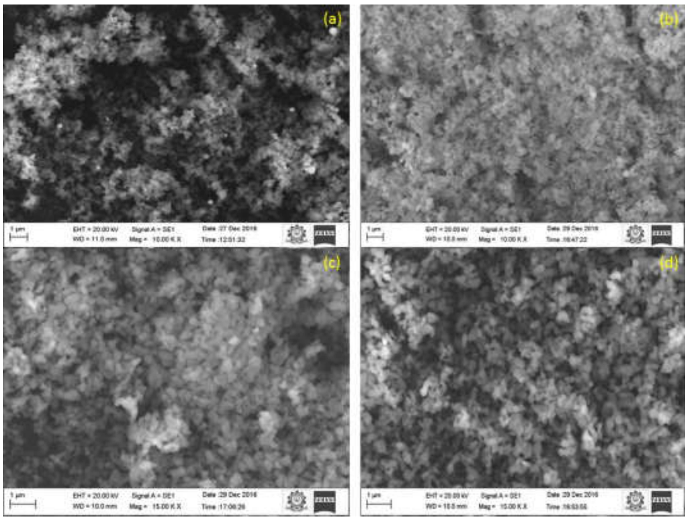
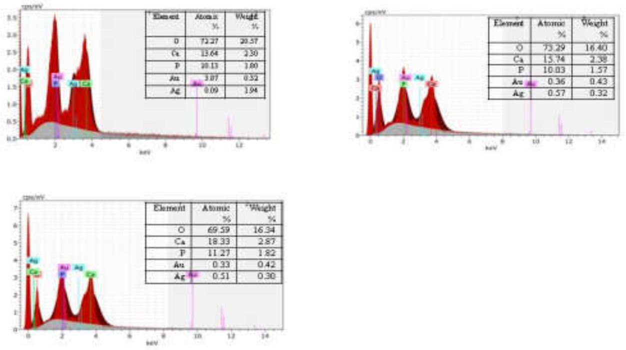


Fig 3 FE-SEM images of Au/Ag doped HAp powders before and after soaked in SBF. (a) 0 day (b) 7 days (c).14 days (d).30 days



Element	Atomic %	Weight %
O	72.27	20.57
Ca	13.64	2.30
P	10.13	1.80
Au	3.87	0.32
Ag	0.09	1.94

Element	Atomic %	Weight %
O	73.29	16.40
Ca	13.74	2.38
P	10.03	1.57
Au	0.36	0.43
Ag	0.57	0.32

Element	Atomic %	Weight %
O	69.59	16.34
Ca	18.33	2.87
P	11.27	1.82
Au	0.33	0.42
Ag	0.51	0.30

Fig 4 EDAX Analysis of Au /Ag doped HAp

TEM Analysis

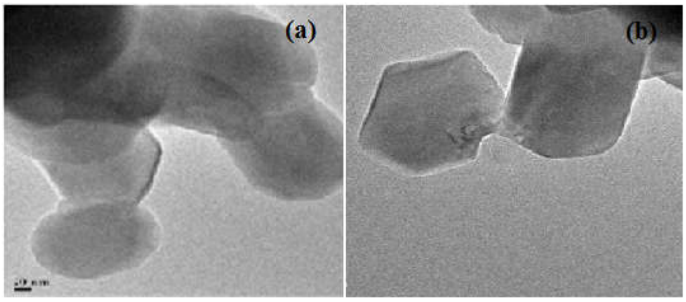


Fig 5 (a-b) FE- TEM images of HAp powder.

Fig. 5 (a-b) Shows the TEM analysis of shape and size of the Au/Ag doped HAp powder. The as synthesized particles seem to be more of roundish morphology containing rod-like hexagonal shape of crystals size about 25 nm. This result was more hopeful with the grain size calculated by Debye- Scherer formula (Kateryna Artyushkova *et al*., 2001).

X-ray Photoelectron Spectroscopy (XPS) analysis

The XPS method deals the chance of distinguishing amongst different kinds of chemical bonds of outer atoms on the basis of the chemical shifts and change in shape of the XPS peaks. The investigation spectra 0 to 1400 eV and the high resolution spectra of the, O1s, Ca 2p, P 2p, Ag 3d, Au 4f were obtained in the above fig(6). The surface analysis of Au and Ag doped HAp performed by XPS are illustrated in the Fig (7).

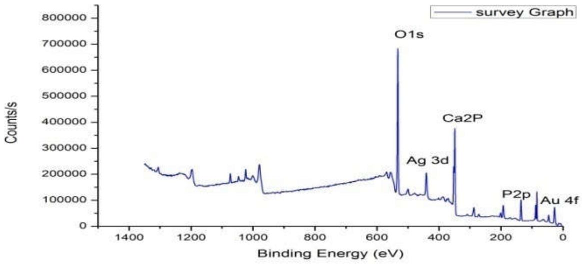


Fig 6 XPS image of Au and Ag doped Hydroxyapatite

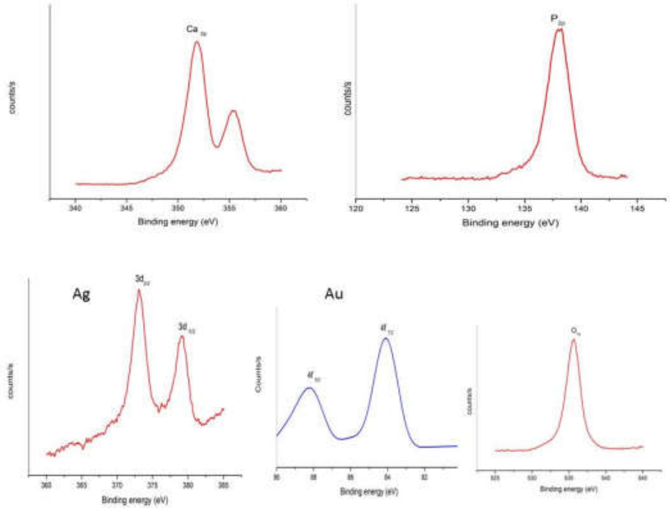


Fig 7 XPS surface Analysis of HAP

The Deconvaluted spectra corresponding to different elements (Ca, P, O, Au and Ag) are discussed. The relative concentration of most important elements and XPS transitions (O1s, Ca 2p, P 2p, Ag 3d, and Au 4f) for Au and Ag doped HAp. The Ca 2p spectrum reveals a doublet with Ca $2p_{3/2}$ binding energy of 351.5 eV and Ca $2p_{1/2}$ with binding energy 356eV. The P2p spectrum reveals that a single P $2p_{3/2}$ peak, the binding energy position at 137.9 eV. The Ag 3d spectrum reveals that a doublet with Ag $3d_{3/2}$ and $3d_{5/2}$ with binding energy of 372.6 eV and 378.5 eV. The Au 4f spectrum reveals a doublet with Au 4f $_{7/2}$ binding energy of 84.0 eV and Au $4f_{5/2}$ with binding energy 88.0 eV. The transition O 1s, Ca 2p, P 2p is confirming the formation of apatite. In addition to present Ag 3d and Au 4f is confirm the formation of gold and silver doped nano hydroxyapatite (Chader *et al*., 1985; Myung Chul Chang *et al*., 2002).

CONCLUSIONS

In this study we conclude Au/Ag doped hydroxyapatite hexagonal shape of nano platelet were successfully synthesized assisted by microwave irradiation using dead mussel shell powder as calcium source with di-ammonium hydrogen phosphate as phosphate source. The synthesized HAp powder was characterized by different systematic techniques to prove the formation of apatite. The fabricated powders immersed in SBF and check their bio activity these hydroxyapatite powder signifying that the novel materials are suitable for bone and tooth replacement materials.

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. Prabaharan K, Balamurugan A, Rajeshwari S, (2005) Development of calcium phosphate based apatite from hens egg shell, *Bull.mater.Sci.* 28(2): 115-119.
4. Remant Bahadur K. C, Kim C.K, Khil M .S, Kim H. Y and Kim I .S, (2008) synthesis of hydroxyapatite crystal using titanium dioxide electrospun nano fiber, *J. Mater. Sci. Eng C.* 28: 70-74.
5. Bose S, Dasgupta S, Tarafder S, Bandyopadhyay A, (2010) Microwave processed nano crystalline hydroxyapatite simultaneous enhancement of mechanical and biological properties, *Acta. Biomater.* 6: 3782-3790.
6. Park Y, Ryu Y, Yoon S, Stevens R, Park H, (2008) Preparation of whisker shaped hydroxyapatite,β-Tri calcium phosphate composite, *Mater. Chem. Phys*. 109: 440-447.
7. Jalota A , Tas C, Bhaduri S .B, (2004) In-vitro testing of calcium phosphate HA TCP and HA TCP whiskers, *J. Mater. Res.* 19: 1876-1881.
8. Gopi D, Indhira J , Nithiya S , Kavitha S. L , Kamachi Mudali U , Kanimozhi K, (2013) Influence of surfactant concentration on nano hydroxyapatite growth, *J.Bull. Mater. Sci.* 36: 799-805.
9. Kokubo T, Takadama H, (2006) How useful is SBF in predicting in-vivo bone bioactivity, *J.biomaterials* 27: 2907-2915.
10. Kokubo T, Kushitani H, Sakka S, Kitsugi T, Yamamuro T, (1990) *J.Biomed.Mater.Res*, 24 : 721-734
11. Kalita S. J, Varma V, (2010) nano crystalline hydroxyapatite bio –ceramic using microwave radiation, *Mater.Sci Eng.C*; 30: 295-303.
12. 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,* 1(4): 345-354.
13. 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.
14. 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.
15. Murugan R, Panduranga Rao V, Sampathkumar T. S, (2003) Microwave synthesis of bio resorbable carbonated hydroxyapatite using goniopora, *Key Engineering Materials*, 242: 51-54.
16. Murugan R, Ramakrishnan S, (2005) Acquiesces mediated synthesis of bio resorbable nano crystalline hydroxyapatite, *J. Cryst. Growth,* 274: 209-213.

17. 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.
18. 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.
19. Gross K .A, Komarovska L and Viksna A, (2013) Efficient zinc incorporation hydroxyapatite through crystallization of an amorphous phases could extended, *Journal of Astralian Ceramic Society* 49(2): 129-135.
20. 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.
21. Dezhi Qin, L. I. Zhang, Xian Du, Guangrui Yang, and Qiuxia Zhang: (2015) fabrication of worm like Ag 2s nano crystalline under mediation of protine, *Bull.Mater.Sci* 38: 1665-1671.
22. Igbal N, Abdul Kadir M. R , Mahmood N. H, Salim N , Froemming G. R. A, Balaji H. R., Kamarul T, (2014) Antibacterial in-vitro compatibility of zinc -silver doped hydroxyapatite nano particles prepared through microwave irradiation ,*Ceramic International*, 40: 4507-4513.
23. 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.
24. 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.
25. Deepa C, Nishara Begum A, Aravindan S, (2013) Preparation and antimicrobial observationsof zinc doped nano hydroxyapatite, *Nanosystems:Physics,chemistry, Mathematics*, 4(3): 370-377.
26. Kateryna Artyushkova, Julia E. Fulghum, (2001) *Journal of Electron Spectroscopy and Related Phenomena* 121: 33-35.
27. 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.
28. Chader S, Fuerstenau D.W (1985) An XPS study of the fluoride uptake by HAP, J.*Colloids and Surfaces* 13: 137- 144.

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