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BIOCOMPATIBILITY STUDIES OF SILVER DOPED HAP/ALUMINA BY SOL-GEL METHOD

Deep Shikha*, Rakesh Tirky* & S. K. Sinha**
* Department of Chemistry, Birla Institute of Technology, Mesra, Ranchi, Jharkhand
** Department of Physics, Birla Institute of Technology, Mesra, Ranchi, Jharkhand

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Abstract:

Silver doped hydroxyapatite (Ag- HAP) coated of alumina having antibacterial properties is of great interest for the development of new biomedical applications. In the current study pure hydroxyapatite (HAP), 1.5wt% (0.9gm)Ag doped in hydroxyapatite (Ag-HAP) coated on alumina disk were processed and characterized. Pure HAP and Ag-HAP were synthesized using the sol-gel method. Calcium nitrate tetrahydrate $Ca(NO_3)_2.4H_2O$ as a source of Ca precursor, phosphorous pentaoxide (P_2O_5) as a source of P precursor and Silver Nitrate ($AgNO_3$) as a source of Ag precursor were used. Samples were characterized using SEM, FTIR, ECA (in Ringer's solution) and OCA. SEM study shows the granular like Ag-HAP. The formation HAP and Ag-HAP was confirmed using FTIR and XRD. Wettability study shows that the surface of Ag-HAP is more hydrophilic as compared to pure HAP Samples. Corrosion study reveals that Ag-HAP has lesser corrosion rate as compared to HAP. There is no toxic effect of Ag-HAP as found by bacterial cell culture test.

Key Words: HAP, Corrosion & Biocompatibility

Introduction:

For the past decade, ceramic materials are increasingly being used for the repair and reconstruction of skeletal disorders and diseases [1]. The most common for bone tissue regeneration is hydroxyapatite (HAP) which is a crystalline calcium phosphate ($Ca_{10}(PO_4)_6(OH)_2$) present in bones. Depending on its source, it can be natural or synthetized, for example, it can be produced from calcium carbonate and ammonium phosphate at ambient pressure from natural sources [2, 3]. It exhibits very similar properties to bone with osteoconductive characteristics allowing connective tissue surrounding and start the regeneration process. It has been extensively used as implant materials because of its similarity with composition of human bone and therefore its ability to form a strong bond with the human hard tissue. However amino acids, proteins and few other organic substances are easily adsorbed on HAP, which in turn favors the adsorption and replication of the bacteria in HAP. Silver-loaded HAP gel has shown antibacterial effects both in nutrient-rich and in poor environments. Even though the exact mechanism of the antibacterial action of Ag particles is not completely understood, there are reports seen in the literature that electrostatic attraction between positively charged nanoparticles and negatively charged bacterial cells is crucial for the activity of nanoparticles as bactericidal materials.

Several different techniques for the synthesis of HAP have been developed in recent years. These techniques include mechanochemical synthesis and, combustion preparation. Although, various type of wet chemistry techniques such as electrochemical deposition, sol–gel procedures direct precipitation from aqueous solutions, hydrothermal synthesis, and emulsion or micro-emulsion routes are also widely used [4]. These processes require high temperature operation and produces multi-phase powder. A comparatively simplified sol–gel process using ethanol as solvent has also been reported to obtain stoichiometric, nanocrystalline single phase HAP. Synthesis of nano-HAP powders (having stoichiometric ratio) by sol–gel method is relatively easy. It gives greater homogeneous composition, higher product purity, and comparatively low synthesis temperatures than other methods.

Due to the good osteoconductivity and biocompatibility, HAP is used in medical implants for repairing and reconstructing diseased or damaged hard tissue. One of the most common problems of using implants is the risk of developing post operatory infections or having the implant refused by the body. To make sure that the implant is not rejected by the human body, nowadays implants are covered with highly biological compatible substances with extraordinary biological properties. Recent studies have reported that substitution of Ca^{2+} with other metal ions such as Ag^{+}, Cu^{2+}, Ce^{3+}, and Zn^{2+} is one of the most effective ways to improve the properties of HAP. Li et al [5] indicated that the HAP is ideal biomaterial to embed silver ions because the Ca^{2+} ions can be easily substituted by Ag^{+} ions within the matrices, creating a material with high biocompatibility and antibacterial properties.

In the present work, we synthesis Silver Doped Hydroxyapatite (Ag- HAP) coated on alumina by sol gel method and characterize its structural and biocomapatibility properties. The characterization processes

involved are Scanning Electyron Microscope (SEM), Fourier Transform Infra-red spectroscopy (FTIR), Electrochemical Analyzer (ECA) (in Ringer's solution) and Optical Contract Angle (OCA). Toxic effect of the sample has also been studied on the basis of Bacteria cell culture test.

Experimental:

Ag-HAP was prepared using Sol Gel method using Calcium Nitrate ($Ca(NO_3)_2.4H_2O$) as a Calcium(Ca) precursor, Phosphorous Pentoxide (P_2O_5) as Phosphorous(P) precursor and Silver Nitrate($AgNO_3$) as Silver(Ag) precursor; using ethanol as a solvent. HAP was doped using 1.5 weight% silver(Ag). For 1.5wt% Ag-HAP (0.173M $AgNO_3$ solution), 0.935g of Ag, 4M $Ca(NO_3)_2.4H_2O$ solution was prepared by dissolving 94.46g in 100 mL ethanol gives yellowish colour which were stirred vigorously for 24hrs. 2M P-containing solution was also prepared by adding 28.39g of P_2O_5 and 0.9gm $AgNO_3$ in 40 mL ethanol 60 ml distilled water colour changes to brown which were stirred vigorously for 24 hrs. After the stirring process,{ P+Ag}-containing solution was added dropwise into the Ca solution and stirred for 30 mins. At this viscous solution of Ag-HAP sol is formed which is aged for 24 hrs in incubator.

For thin film coating, alumina samples were cleaned in ultrasonic bath with acetone for 30 minutes. After that samples were again washed with running distilled water and kept for drying at 100° C for 15 min in hot air oven. The dried alumina samples were further used for Ag-HAP thin film coating. The alumina substrate were dipped into the solution with the withdrawing rate of 40 mm/min using Dip Coater unit (Single Dip Coating Unit Model No.:SDC2007C, Apex Instruments Co., Kolkatta, India) and were dried at 100-200° C for 15mins. At last dried gel coated samples is placed directly into an electric furnace at 500 C in air and is removed from the furnace after 15 minutes [2].

FTIR was taken using IR-prestige 21 (Shimadzu corporation, Japan) in Attenuated total reflectance (ATR) mode, in the range of 650-4000 cm^{-1}. SEM was taken using JSM-6390LV (Jeol, Japan). ECA was done using CH Instruments, Model 680B (USA). Wettability studies were carried out to check whether the surface has become hydrophobic or hydrophilic after coating. The contact angle was measured by dropping 5µm distilled water on the surface of the sample. The instrument employed for the measurement of contact angle was from Data Physics Germany, model OCAH-230. Corrosion resistance of the samples were studied using Electrochemical Analyzer (ECA), model 660 Series, CHI Instruments, made in USA, available at BIT, Mesra, Ranchi. XRD were carried out by Rigaku, Japan, SmartLab 9kW instruments using Cu K_α of wavelength 1.54 Å. Bacteria cell culture test has been performed for toxicity study of the samples to check the surface features and biological performance of the materials.

Results and Discussions:

Compound formation is studied by FTIR and XRD. Fig. 1 shows the FTIR of Ag doped HAP thin film coated on alumina. Presence of functional groups of OH- in the range of 3124.68, 3410.14; CO_3^{2-} in the range of 1365.60, 1427.75 and PO_4^{3-} in the range of 921.97- 1226.72 cm^{-1} confirm the formation of Ag-HAP. Small Peaks at 3124 and 3410 cm^{-1} correspond to O-H stretching in pure HAP. Broad peaks observed in the range of 3100-3400cm^{-1} correspond to stretching of O-H bond in case of Ag-HAP. Small peaks observed in the range 1300-1600 cm^{-1} correspond to the small amount of CO_3^{2-} (carbonate) group which may be formed by the entrapment of atmospheric carbon dioxide during either reflux or calcinations process, while broad peaks observed in the range of 900 to 1250cm^{-1} correspond to the symmetric as well as asymmetric stretching modes of P-O bond in PO_4^{3-} (phosphate) group.

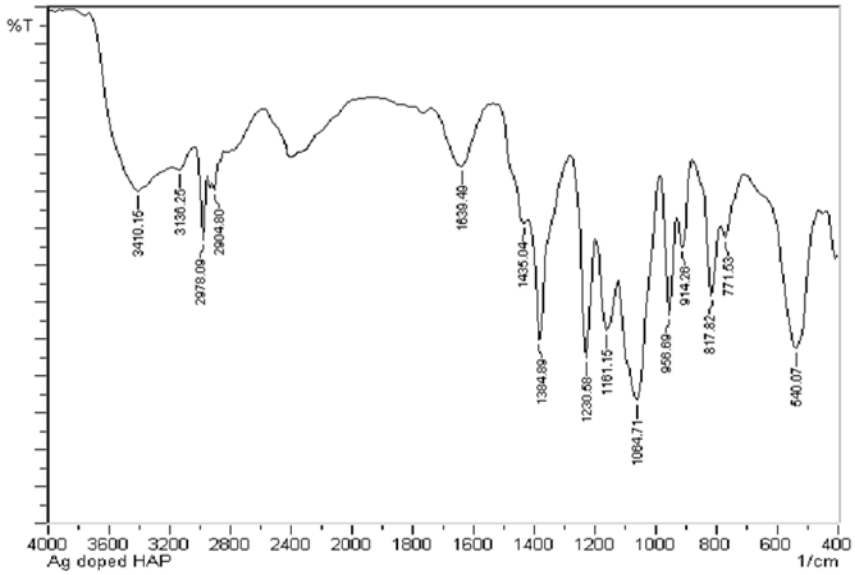

Figure 1: FTIR of pure Ag-HAP deposited on alumina

According to JCPDS (International center for Diffraction Data) for PCPDF, the XRD pattern of silver doped HAP on alumina is shown in Fig. 2. Peaks of HAP were observed at diffraction angles 37.66(032), 56.45(143), 59.59(513). This confirms the compound formation of HAP alumina. All the other unmarked peaks corresponds to different phases of alumina.

Figure 3(a) and Figure 3(b) show the SEM images of HAP coated alumina and Ag-doped HAP on alumina. Ag-HAP/alumina seems to be more homogeneous than only HAP/alumina. Grains size also becomes smaller after Ag doping.

Figure 4 shows the Energy-Dispersive X-Ray Spectroscopy (EDX) data. Presence of small amount 1.5% of Ag is also observed confirming the silver doping.

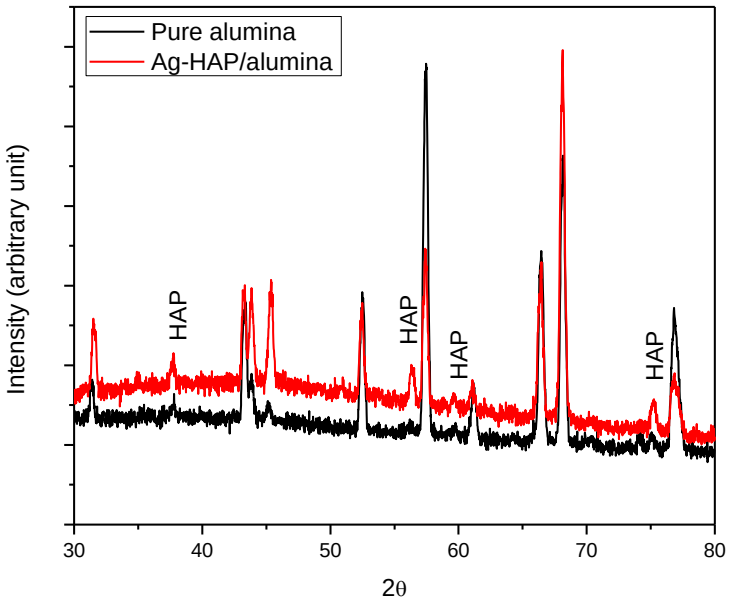

Figure 2: XRD of Ag-HAP coated on alumina

Corrosion analysis were carried put using ECA. Sample was dipped in Ringer's solution (composition – 9g NaCl, 0.425 KCl, 0.119g $CaCl_2$, 0.1g $NaHCO_3$ in 1L distilled water), kept at a constant temperature of 37^oC and their corrosion behaviour was studied. The corrosion potential (E_{corr}) and corrosion current (i_{corr}) were determined from the Tafel plot. This is shown in Fig. 5. Using the i_{corr} value, corrosion rate of all the three samples were calculated. The results were compared with respect to HAP coated on alumina [2]. Table I shows the comparison between the two samples. A marked improvement in corrosion rate after silver doped HAP/alumina is observed.

The wetting characteristic was studied by measuring the optical contact angle. Fig 6(a), (b) and (c) show the wettability behavior of alumina, HAP/alumina and Ag-HAP/alumina respectively. The oprical contact angle is presented in the table II. It is clear from the OCA figures that the surface of Ag-HAP is more hydrophilic as compared to pure HAP, i.e. wettability increased after Ag-doping.

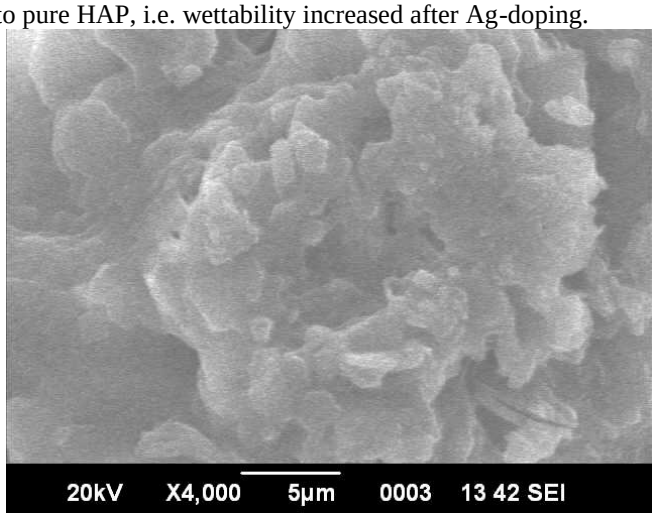

Figure 3(a): SEM of HAP/alumina

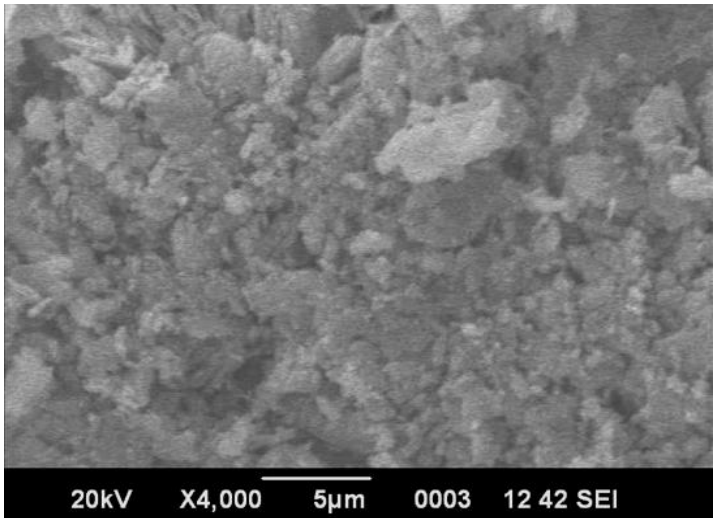

Figure 3(b); SEM of Ag-HAP/alumina

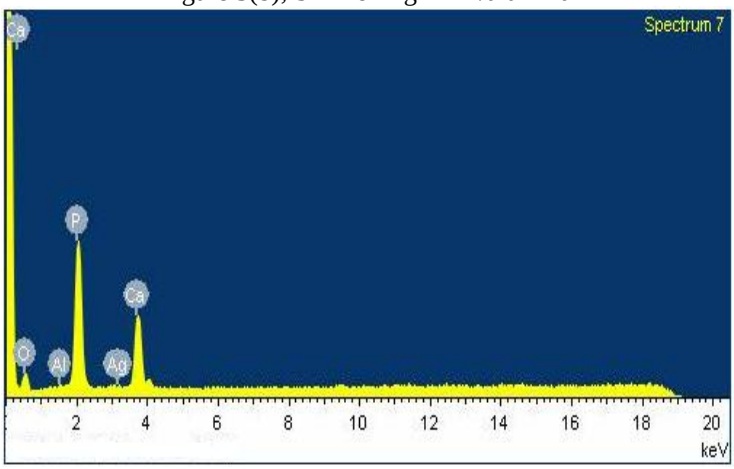

Figure 4: EDX of Ag-HAP/alumina showing the presence of Ag.

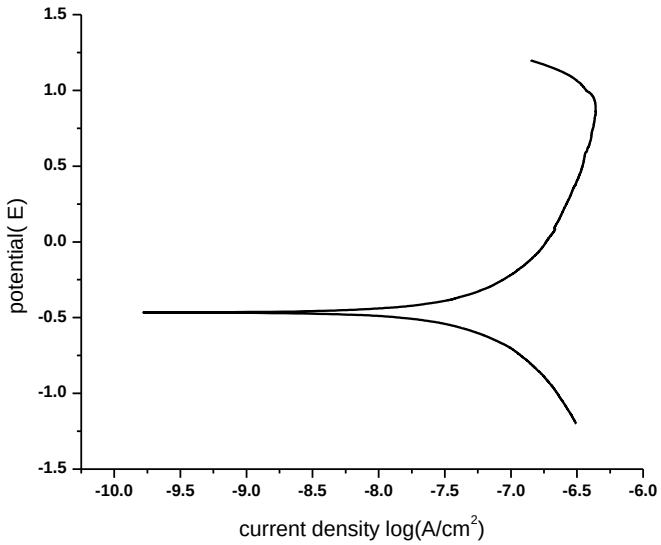

Figure 5: Tafel plot of corrosion behaviour of 1.5wt% Ag-HAP

Table 1: Corrosion rates of pure and doped HAP

Sample	**$E_{corrosion}$ (mV)**	**$i_{corrosion}$ (uA/cm^2)**	**Corrosion rate (mmpy)**
Pure HAP	-641	172.5	2.82×10^{-3}
1.5% Ag-HAP	-10.15	0.0398	1.73×10^{-6}

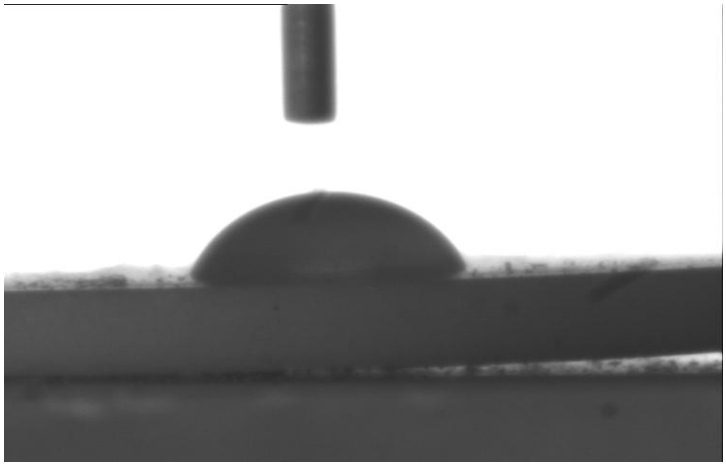

Figure 6.(a): OCA of HAP/alumina

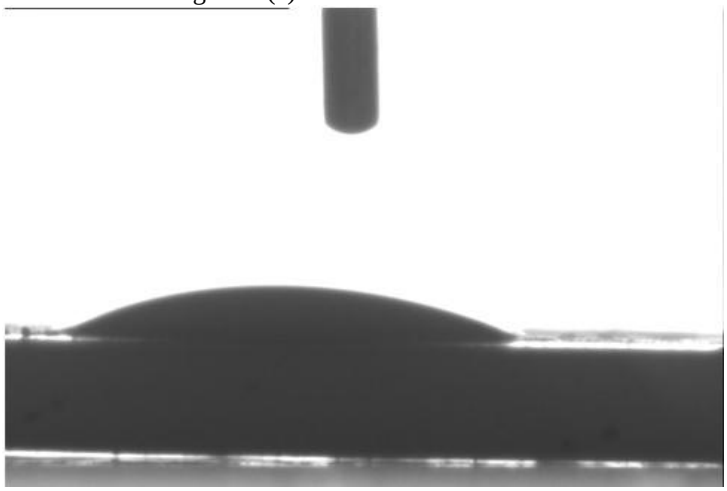

Figure 6.(b): OCA of pure HAP/alumina

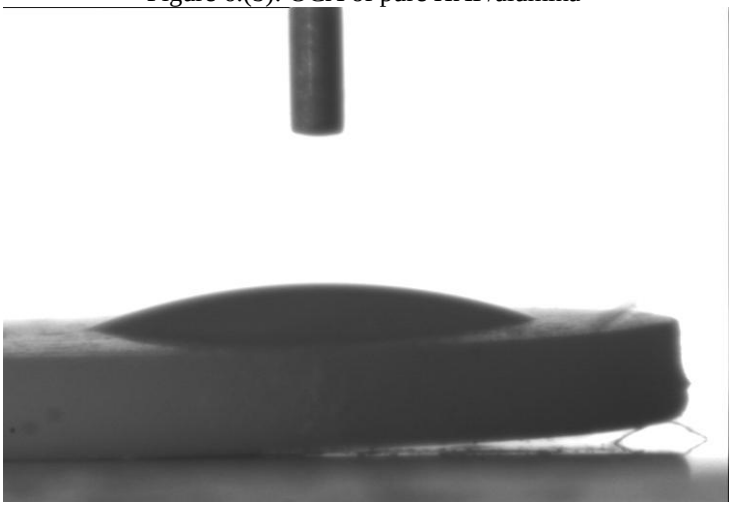

Figure 6(c): OCA of 1.5wt% AgHAP/alumina

Table 2: OCA of pure and Agdoped HAP

Sample	Optical Contact Angle
Alumina	73.33±1.35
Pure HAP	25.30±1.24
1.5% AgHAP(reflux)	28.12±1.05

For toxicity investigation, *Pseudomonas aeruginosa* a Gram (-ve bacteria is used to check the toxicity of materials. *Pseudomonas aeruginosa* were cultured in the Nutrient Agar media. Nutrient Agar is used for the cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids. The powdered sample, scratched from the surface of the Ag-HAP/alumina sample was put and growth was observed. The P^H of the nutrient Agar media was maintained at 7.4 ±0.2 and autoclaved, and cooled to about 60˚C.

Figure 7: Tioxicity investigation

After the medium was allowed to stand for 10 min at 60˚C to reduce foaming and then 20ml of medium was poured into each glass Petri dish. *Pseudomonas aeruginosa* strains were collected from Dept. of Bioengineering, BIT Mesra, Ranchi. The growth condition was aerobic and the surface features of Ag- HAP/alumina sample were identified on spread plates after incubation for 48 hours at 37˚C. The growth of microorganism implies that our sample was anti-toxic.

Conclusions:

Ag-HAP/alumina is prepared by sol gel method. Compound formation, surface structure, corrosion resistance and wettability behavior have been studied. Grain size of Ag-HAP/alumina is observed to be smaller than HAP/alumina as seen using SEM. Corrosion resistance is also higher with respect to HAP/alumina sample. Wettability also becomes higher in Ag-HAP/alumina coated sample, i.e., surface is becoming more hydrophilic in behaviour. Samples were observed to be non-toxic in nature.

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