Structural, Optical and Antimicrobial Activity of Copper and Zinc Doped Hydroxyapatite Nanopowders using Sol-Gel Method

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ABSTRACT. Antimicrobial materials based on hydroxyapatite nanopowders are functionally attractive in a wide variety of medical applications. The Cu^{2+} and Zn^{2+} incorporated HAp nanopowders were synthesized by simple Sol-Gel method. The structural study, optical study and biological activity of the prepared nanopowders were characterized by X-ray diffraction, UV-Vis absorption spectroscopy and disc diffusion method respectively. The XRD results are demonstrated that the presence of copper and zinc doped hydroxyapatite nanopowders and size of the crystalline was found for pure HAp, 10%, 25% and 50% of Zn^{2+}/ Cu^{2+} metal ions incorporated HAp respectively. The optical absorption analysis was used to estimate band gap value of pure HAp 3.86eV and Zn^{2+}/ Cu^{2+} doped HAp 3.77eV (10%), 3.69eV (25%) and 3.30eV (50%) respectively. Antibacterial activity of synthesized nanocomposite against human pathogenic bacteria was tested by disc diffusion method on Muller- Hinton agar medium. The pure HAp powder has excellent antibacterial activity and the antibacterial rate gradually rise with the increase in Copper and Zinc concentrations in the HAp nanopowders.

Introduction. Inorganic antimicrobial materials are made of dense metal ions having biocidal action such as silver, zinc, copper and calcium phosphate [1-5]. Calcium phosphate, synthetic hydroxyapatite (HAp, $Ca_{10} (PO_4)_6 (OH)_2$), is one of the most promising material because of its biocompatibility, good cation exchange rate with metals and high affinity for the pathogenic microorganisms [6-8]. In recent years, incorporation of metallic antibacterial agents (such as Cu^{2+}, Zn^{2+}, Ag^{+} and Ce^{4+}) in bioceramics is mainly implemented because of their antibacterial property, which aids in inhibiting microbial growth at the implant site and their lack of cytotoxicity at low concentrations. HAp is incorporated with metal ions can be synthesized by various methods, among them Sol-Gel technique is a versatile method for the synthesis of ion substituted HAp nanopowders. Hence, the present work is designed to synthesis Zn^{2+}/Cu^{2+} metal ions incorporated into HAp nanopowders and to improve the antibacterial efficiency.

Experimental details

Preparation of HAp nanopowders. Calcium acetate ($Ca (C_2H_3O_2)_2$, Orthophosphoric acid (H_3PO_4), Copper oxide (CuO), Zinc oxide (ZnO) were used as starting materials. Ethanol and double distilled water were used as solvents and acetic acid was used as a stabilizing agent. 0.55M solution of calcium acetate was prepared by dissolving the appropriate amount of calcium acetate $Ca (C_2H_3O_2)_2$ salt and it was mixed with 125 ml of solvent (100 ml double distilled water and 25 ml of ethanol). The solution was stirred for 2 hrs under vigorous conditions at room temperature while obtained $Ca (OH)_2$ Sol-Gel solution. A required amount of Zinc oxide and Copper oxide was dissolved in 0.33M of H_3PO_4 and then add drop by drop in above prepared Sol-Gel solution. Finally, the transparent solution was formed after stirring for 4 hrs. During the preparation of HAp solution the pH of the solution was maintained at 10.5 by adding aqueous ammonia. Then various proportions of the powders were annealed at 500˚C in a muffle furnace at a constant heating rate under an air atmosphere for about 1 hr and then grained in a mortar and pestle. Finally, pure form of powder of HAp, 10% Zn/Cu-HAp, 25% Zn/Cu-HAp and 50%Zn/Cu-HAp obtained. Antibacterial activity of synthesized nanocomposite against human pathogenic bacteria was tested by disc diffusion method on Muller- Hinton agar medium (MHA) ([g/L]: Beef extract – 3, Casein acid hydrolysate – 17.5, Starch – 1.5 and Agar – 17) (Nagarajan et al., 2014). The human pathogenic bacterium Escherichia coli MTCC 443 and Salmonella typhi MTCC 733 were obtained from Microbial Type Culture Collection (MTCC) Chandigarh, India. The above said two human pathogens were inoculated in 25mL of sterile Nutrient broth and it was incubated at 37 °C for 10 hrs. A 50μL of human pathogens were inoculated on MHA medium, cultures were swabbed with the help of sterile cotton buds. A disc with 120 mg/mL concentration of nanocomposite was used in this study with tetracycline (2 mg/ml) as reference control. Inoculated plates were incubated for 24 hrs at 37 °C. After 24 hrs of incubation, different levels of zone of inhibition (ZOI) were measured using a meter ruler. The experiments were performed in triplicates.

Result and Discussion

Structural study. Fig.1 (A) shows the XRD patterns of the synthesized pure form of HAp and Fig.1 (B) shows Zn/Cu metal ions doped HAp with different amount, such as 10% Zn/Cu-HAp, 25%Zn/Cu-HAp and 50% Zn/Cu-HAp.

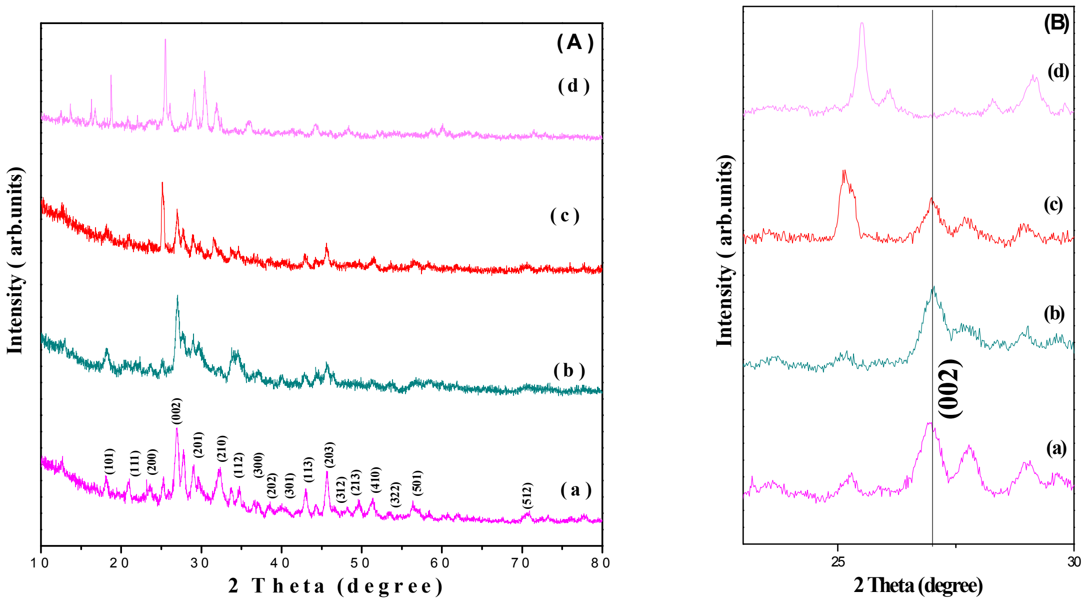

Fig. 1. (A) XRD patterns of hydroxyapatite powders prepared under different doping concentrations. a) pure form of HAp, b) 10% Zn/Cu-HAp, c) 25% Zn/Cu-HAp and d)50% Zn/Cu-HAp; (B) Enlarged XRD patterns of (0 0 2) plane.

It was found that the XRD results confirmed the hexagonal HAp (Space group $P6_3/m$, JCPDS file no.09-432) to be the main apatite phase and sharp peaks confirm that they are well crystallized. In the case of doped powders, the sharp and shallow peaks denoted that increased crystallinity as well as crystalline size, owing to the incorporation of Zn and Cu in Zn/Cu-HAp powders. The diffraction peak (0 0 2) plane was selected for local comparison because the plane was separated from other peaks. In particular, for Zn/Cu-HAp (Fig.1 (B)), the diffraction peak position shifted towards smaller angles from the standard XRD pattern for HAp. From the graph, the value of FWHM are decreased for the plane (0 0 2) due to increase the Zn and Cu concentrations with respect to increase the crystallinity and peaks gets sharpened. The 50% Zn/Cu-HAp has more new peaks which denoted that peaks correspond to Zn and Cu in HAp sample.

Table 1. Crystalline sizes for Hydroxyapatite powders prepared underdifferent doping concentrations.

Sample name	Crystalline size in nm
HAp	15.88
10% Zn/Cu-HAp	16.05
25% Zn/Cu-HAp	21.21
50% Zn/Cu-HAp	21.52

Optical study. Fig. 2 (A) shows that the optical absorption analysis of synthesized pure form of HAp and Fig. 3 (A) shows that the optical absorption analysis of synthesized various ratios of (Zn/Cu) doped HAp powder samples. According to Lambert–Beer law, absorption coefficient (α) is proportional to absorbance. Thus, the energy intercept of the curve in $(\alpha h\upsilon)^2$ vs hυ plot gives the value of Eg when the tangent line is extrapolated to the zero ordinate. Fig. 2 (B) pure form of HAp composite sample gives the band gap value is 3.86eV [as per literature band gap energy value of HAp is 5.3eV] [8]. Fig. 3 (B) shows the band gap values of the corresponding doped HAp composites were found to be 3.77eV, 3.69eV and 3.30eV for 10%Zn/Cu-HAp, 25%Zn/Cu-HAp and 50%Zn/Cu-HAp respectively, which were smaller than pure HAp (3.86eV). From the UV-Vis absorption analysis, the observed band gap values decrease with increase in doping concentrations. From the absorption spectrum of doped samples, absorption edge is slightly shifted towards the higher wavelength (red shift), as compared to the pure form of HAp. This could be attributed to the uniform doping of Cu^{2+} and Zn^{2+} ions in the HAp lattice. The decrease in the band gap with increase in the concentrations of Cu^{2+} and Zn^{2+} ions can be explained by the *p-d* spin-exchange interactions between the band electrons and the localized *d* electrons of the transition metal ion substituting the host metal ion. There is a strong p-d mixing of O, Cu and Zn ions in HAp lattice. This could also be a possible reason for the narrowing of the band gap.

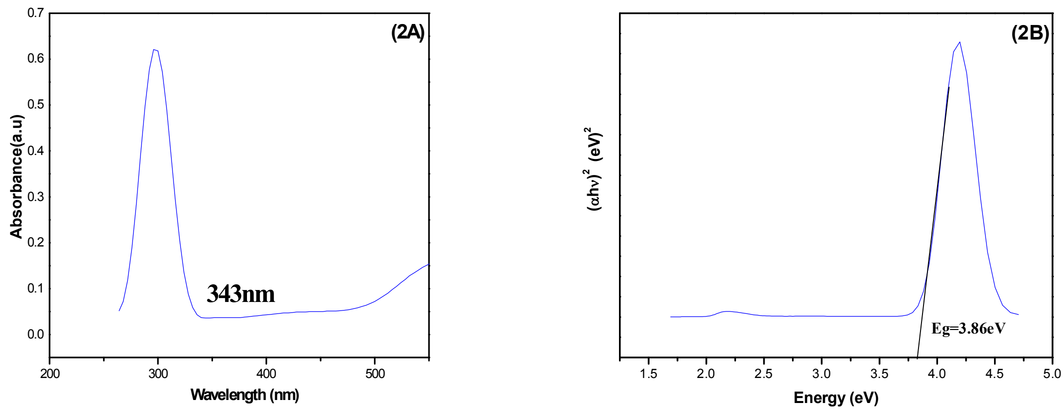

Fig. 2. (A) UV-Vis absorption spectrum of pure form of HAp and (B) Tauc extrapolation plots for the pure form of HAp sample.

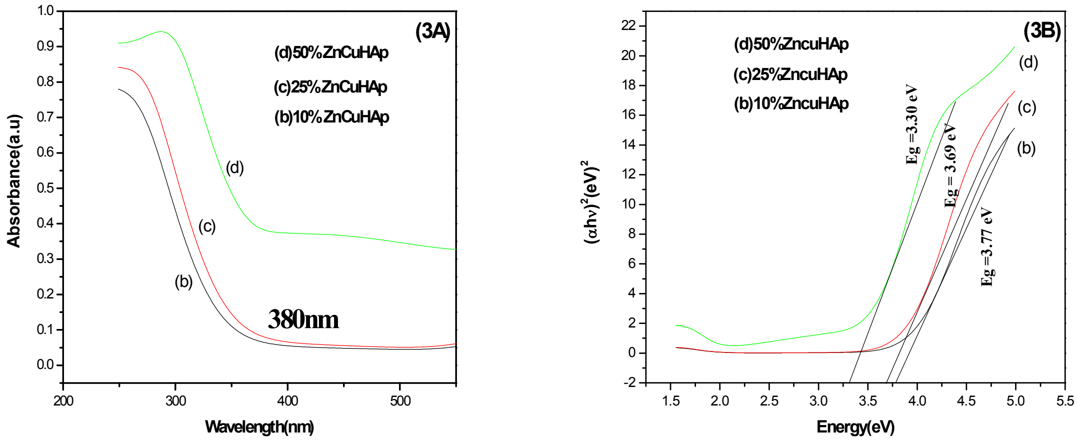

Fig. 3. (a) UV-Vis absorption spectrum of (b)10%Zn/Cu-HAp, (c)25%Zn/Cu-HAp and (d)50%Zn/Cu-HAp and (B) Tauc extrapolation plots for the (b)10%Zn/Cu-HAp, (c)25%Zn/Cu-HAp and (d)50%Zn/Cu-HAp samples.

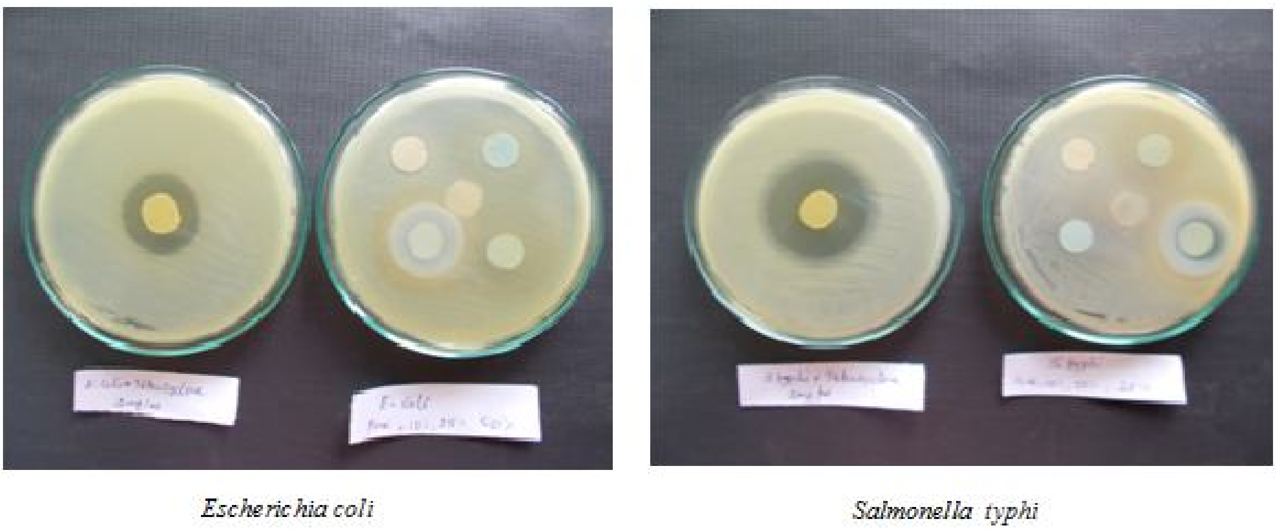

Fig. 4. Antibacterial activity of human pathogens against nanopowders (zone of inhibition in mm).

Antibacterial activity. Fig.4 shows the bactericidal activity of the Zn^{2+}/Cu^{2+}-HAp composites were evaluated by the inhibition of bacterial growth of *S.typhi* and *E.Coli*. Both pathogens are Gram-negative bacteria. It is conclude that HAp doped nanocomposite was checked for antibacterial activity against human pathogens. Among the four compounds, two compounds shows there is no zone of inhibition compared with Tetracycline as a reference control, because doping concentration is low compare to other compounds. The remaining two compounds showed antibacterial activity against *E. coli* at 50% Zn/Cu-HAp (24 mm) and pure HAp (16 mm) compared with the reference control (Table-2). Also the compound 50% Zn/Cu-HAp showed antibacterial activity (26 mm) against *S. typhi* compared with Tetracycline as a reference control. Results showed good inhibition on two types of bacteria *E.Coli* and *S.typhi*.

Table 2. Antibacterial activity of human pathogens against nanopowders (zone of inhibition in mm).

S. No.	Sample name	*Escherichia coli* MTCC443	*Salmonellatyphi* MTCC733
1	50% Zn/Cu-HAp	24	26
2	Pure HAp	16	-
3	Tetracycline	27	36

Summary. The XRD analysis confirmed that sharp and shallow peaks denoted that increased crystallinity as well as crystalline size, owing to the incorporation of Zn and Cu in HAp powders. The optical analysis denoted that the bandgap values of the corresponding doped HAp composites were found to be 3.77eV, 3.69eV and 3.30eV for 10%Zn/Cu-HAp, 25%Zn/Cu-HAp and 50%Zn/Cu-HAp respectively which were smaller than pure HAp (3.86eV). The above results confirmed that uniform doping of Cu^{2+} and Zn^{2+} ions in the HAp lattice. The Antibacterial activity of human pathogens reveals that good inhibition on two types of bacteria E.coli and S.typhi.

References

[1] Vojislav Stanic, Suzana Dimitrijevic, Jelena Antic-Stankovic, Miodrag Mitric, Bojan Jokic, Ilija B.Plecas, Slavica Raicevic, Synthesis and characterization and antimicrobial activity of copper and zinc-doped hydroxyapatite nanopowders, Applied Surface Science 256, 2010, 6083-6089.10.1016/j.apsusc.2010.03.124.

[2] J. Husheng, H. Wensheng, W. Liqiao, X. Bingshe, L. Xuguang, The structures and antibacterial properties of nano-SiO2 supported silver/zinc–silver materials, Dent. Mater. 24, 2008, 244–249. 10.1016/j.dental.2007.04.015.

[3] G. Zhou, Y. Li, W. Xiao, L. Zhang, Y. Zuo, J. Xue, J.A. Jansen, Synthesis characterization and antibacterial activities of a novel nanohydroxyapatite/zinc oxide complex, J. Biomed. Mater. Res. A 85, 2008, 929–937. 10.1002/jbm.a.31527.

[4] Y. Zhou, M. Xia, Y. Ye, C. Hu, Antimicrobial ability of Cu2+-montmorillonite, Appl. Clay Sci. 27, 2004, 215–218. 10.1016/j.clay.2004.06.002.

[5] K.C. Carson, J.G. Bartlett, T.J. Tan, T.V. Riley, In vitro susceptibility of methicillinresistant Staphylococcus aureus and methicillin-susceptible Staphylococcus aureus to a New antimicrobial, copper silicate, Antimicrob. Agents Chemother. 51, 2007, 4505–4507. 10.1128/AAC.00771-07.

[6] R.Z. LeGeros, Calcium phosphate-based osteoinductive materials, Chem. Rev.108, 2008, 4742–4753. 10.1021/cr800427g.

[7] I. Smiˇciklas, A. Onjia, J. Markovi´ c, S. Raiˇcevi´ c, Comparison of hydroxyapatite sorption properties towards cadmium, lead, zinc and strontium ions, Mater.Sci. Forum 494, 2005, 405–410.10.4028/www.scientific.net/MSF.494.405.

[8] S. Shanmugam and B. Gopal, Copper substituted hydroxyapatite and fluorapatite: synthesis, characterization and antimicrobial properties, Ceram. Int., 2014, 40, 15655– 15662. http://dx.doi.org/10.1016/j.ceramint.2014.07.086.

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