Phase stability of silver particles embedded calcium phosphate bioceramics

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Abstract. In this paper, we report the compositional variation-dependent phase stability of hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) on doping with silver. The transformation of hydroxyapatite to (β/α) tricalcium phosphate phases during sintering has been explored using Raman spectroscopy and X-ray diffraction techniques. The optical absorption spectroscopy analysis reveals the presence of Ag^+ ions at low doping levels. As the doping increases, abundance of Ag particles is enhanced.

Keywords. Biomaterial; hydroxyapatite; Raman spectroscopy.

1. Introduction

Hydroxyapatite (HA or $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) has been studied extensively due to its identical composition with calcified hard tissue of vertebrates.^{1,2} Biocompatibility, low solubility and bone integration properties make it a suitable bio-implant material along with other calcium phosphate bioceramics.^{3–5} Apart from HA, tricalcium phosphate (TCP) has also been used for prosthetic implants and dicalcium phosphates have applications in bone cement and restorative materials.⁶ During the synthesis of HA, some of these calcium phosphates form as secondary phases.^{3,7} In order to obtain desired microstructure, the combination of powder synthesis route and sintering conditions play an important role.⁸ We have used a wet chemical method to synthesize parent and silver-doped HA. The advantages of this process include homogeneous molecular mixing, comparatively low synthesis temperature, ability to generate shape and size controlled nanosized particles.^{9,10}

It is known that HA crystallizes in hexagonal symmetry, which is constructed by the columns along hexagonal axis made-up of calcium ions and oxygen atoms.¹¹ The exchangeability of cations has been regarded as a typical characteristic of HA due to the presence of Ca^{2+} ions in the columns. To improve the antimicrobial activity of HA, attempts have been made to substitute Ca^{2+} ion with several ions. Cu^{2+} (antimicrobial), Zn^{2+} (antimicrobial), Ni^{2+} and Co^{2+} , whose ionic radii and coordination numbers differ from those of Ca^{2+} , are reported to substitute partially and this is

accompanied by the reduction in crystallinity and enhancement in cytotoxicity.^{10,12} On the other hand, the size of the Ag^+ ion is comparable to Ca^{2+} ion and forms four to eight coordination complexes. Silver-based antimicrobial materials received much attention because of the low toxicity of active Ag^+ ions and metallic silver to the human cells.¹³ HA composites of Ag metal, Ag ions incorporated in HA and Ag supported HA thin films have been explored extensively for its mechanical, electrical and biocompatibility properties.^{14–19} However, the aspects of stability of HA in Ag-doped apatites as well as compositional-dependent antibacterial property have not been investigated so far.

In this study, we report the results of the structural transformations of silver-substituted $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ ($0.0 \leq x \leq 1.5$) compositions using X-ray diffraction and room temperature Raman spectroscopic studies. A critical analysis of the obtained results enable us to document the following aspects: (i) the systematic transformation of HA into TCP with the increase in Ag content in 800/1200°C sintered samples and (ii) synthesis of nano/micron-sized silver particles embedded HA/TCP and its antimicrobial effects on the growth of Gram-negative bacteria *Escherichia coli* (ATCC#25922).

2. Experimental

Parent (undoped) HA and Ag-doped HA were synthesized using a wet chemical method.¹⁸ X-ray diffraction patterns were recorded using the CuK_α radiation ($\lambda = 1.5405 \text{ \AA}$ ($\text{K}_{\alpha 1}$), $1.5443 \text{ \AA}$ ($\text{K}_{\alpha 2}$)) to evaluate the phase assemblage. Raman spectra were recorded using an argon ion laser source (514.5 nm). The scattered light was analysed using a

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spectrograph (Model: Acton AM505F). The laser power was kept low (7 mW) to avoid sample heating and subsequent damage. The spectra were recorded in the wavenumber region of 200–3700 cm^{-1} . The scattered radiation was collected at 180° (backscattered geometry) to the incoming beam and detected using a CCD cooled to approximately -120°C . The spectral resolution of the Raman experimental setup was better than 1 cm^{-1} and the total integration time was ~ 1 min for each spectrum. Optical absorption spectra of samples were recorded at room temperature using a UV–visible double beam spectrophotometer (Hitachi Model U-3310). Transmission electron microscopic images of selected powder samples placed on copper grids were recorded using a Phillips 200 kV Transmission Electron Spectrometer. Scanning electron microscopic images were recorded using a Field Emission scanning electron microscope (FESEM, Model—SUPRA 40VP). The bacterial strain of *E. coli* (ATCC#25922) was used to study the *in vitro* antimicrobial properties of some selected silver-doped bioceramics samples.

3. Results and discussions

Figure 1a shows the X-ray diffraction patterns of the parent HA $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ ($x = 0.0$) and silver-doped composition ($x = 1.5$). At low sintering temperatures (800°C), sintered parent HA sample shows the pure phase of HA. All the peaks are indexed for hexagonal symmetry of the HA.¹¹ We have found that HA phase is stable upto 1450°C sintering temperature. Nevertheless, weak peaks of β -TCP and CaO were observed.²⁰ The silver-doped composition ($x = 1.5$) shows the major phase of β -TCPs and metallic silver along with HA phase after 800°C sintering. On increasing the sintering temperature to 1200°C for $x = 1.5$ composition, all the β -TCPs and HA phase converted into α -TCPs.²¹ The X-ray diffraction peaks are indexed for α -TCPs for 1200°C sintered silver-doped composition ($x = 1.5$) of HA.²¹

Figure 1b shows the X-ray diffraction patterns of the $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ ($0.3 \leq x \leq 1.0$) bioceramic samples, sintered at 800 and 1200°C for 2 h. For $x = 0.3$ composition, no secondary phases of β -TCPs and α -TCP were observed. With the increase in silver content, the β -TCP forms as a second phase in the 800°C sintered samples, as shown in the left column of figure 1b. In the 800°C sintered samples of $x = 0.8$ and $x = 1.0$ compositions, we also found traces of the silver peak, shown by the # symbol in the diffraction patterns. In the right column of figure 1b, we document the X-ray diffraction patterns of 1200°C sintered samples of silver-doped HA. The presence of α -TCP phase was found in $x = 0.5$, $x = 0.8$ and $x = 1.0$ compositions. The β -TCP converts into α -TCP on sintering the silver-containing samples at 1200°C for 2 h. The $x = 1.0$ composition shows the presence of α -TCP as a major phase along with HA as secondary phase. For the composition of $x \geq 0.5$, the presence of silver increases the β -TCP on

Figure 1. X-ray diffraction (XRD) patterns of 800 and 1200°C sintered $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ ($0.0 \leq x \leq 1.5$) bioceramics. Phases other than pure HA are marked as follows: *— α -tricalcium phosphate, \$— β -tricalcium phosphate, •—CaO and #—silver.

sintering at 800°C , which converts into the α -TCP upon sintering the samples at 1200°C . For composition of $x = 1.5$, the HA phase entirely converted into α -TCP phase upon sintering at 1200°C for 2 h. Silver-substituted compositions

($x \geq 1.0$ and 1.5) also exhibit the presence of TCPs. Further, to explore the structural transformation at a microscopic level, we have done Raman spectroscopy of parent and silver-doped samples.

Figure 2 shows the room temperature Raman spectrum of parent and silver-doped HA $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ ($0.0 \leq x \leq 1.5$), sintered at 1200°C for 2 h. The symmetric mode (ν_1) corresponding to the HA phase persists between $0 < x < 0.8$ composition samples. The $x = 1.0$ composition shows the presence of two peaks at frequencies of 960 and 971 cm^{-1} , which correspond to HA (major) phase and TCP phase (minor), respectively. The $x = 1.5$ composition shows intense peaks at 970 and 962 cm^{-1} , suggesting concomitant increase in the TCP phase. On increasing the Ag content in the HA lattice, the intensity of one of the doubly degenerate bending peak centred at $\sim 444\text{ cm}^{-1}$ increased. Apart from the increase in one of the peak intensity from doubly degenerate mode, one more new peak originated at $\sim 410\text{ cm}^{-1}$ in the $x = 1.0$ and $x = 1.5$ compositions. The signature of new peak at $\sim 410\text{ cm}^{-1}$ and increase in intensity of 444 cm^{-1} frequency mode in $x = 1.5$ composition suggest the presence of Ag–O bond in the Ag-doped samples.²² The new peak at $\sim 410\text{ cm}^{-1}$ and shifting of the mode towards higher frequency is the signature of Ag–O stretching mode in oxygen deficiency.²² Further, in the triply degenerate mode (ν_4) of the $x = 1.5$ composition of HA, a new intense peak appears near to $\sim 625\text{ cm}^{-1}$. It has been reported that when oxygen interacts with the Ag metal at higher temperatures the two

layers of the oxygen remain in contact with Ag metal and giving rise to the characteristic band.²³ In the triply degenerate asymmetric mode (ν_4) of Ag-doped HA, we have found new peaks, which correspond to the TCP phase. The Raman peaks of $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ ($0.0 \leq x \leq 1.5$) composition validate the observance of X-ray diffraction patterns. For composition $x = 1.5$, we have found intense peaks corresponding to α -TCP. The intense peak of Ag–O bond in the composition $x = 1.5$ shows the route for the formation of TCP from HA. Silver adsorbs oxygen from the hydroxyl group of the HA lattice and converts HA into TCP, which was not decomposed even after sintering of parent HA at 1450°C . The inset of figure 2 shows the absence of OH hydroxyl group in $x = 1.5$ composition.

During the synthesis of the silver-doped HA, we observed the colour change of the silver-doped samples only after sintering at 800°C irrespective of dopant content. It has been already reported that colloidal silver gives yellow colour, while pink colour corresponds to silver nanoparticles.²⁴ To find out the origin of pink colour in our 800°C sintered $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ ($0.3 \leq x \leq 1.5$) HA compositions,

Figure 2. Raman spectra of $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ ($0.0 \leq x \leq 1.5$) hydroxyapatite samples sintered at 1200°C .

Figure 3. Optical absorption spectra of $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ ($0.3 \leq x \leq 1.5$) 800°C sintered bioceramics. Inset shows TEM image of 800°C sintered ($x = 0.5$) powder sample.

we have recorded optical absorption measurements for 800°C sintered samples of $x = 0.3, 0.5, 0.8, 1.0$ and 1.5 compositions in the wavelength range 200–900 nm (figure 3). All the compositions show a broad absorption band starting from ~ 350 to 700 nm. The $x = 0.3$ composition shows absorption maximum at ~ 511 nm. With the increase in silver content, the absorption band shifts towards lower wavelength, which corresponds to an increase in particle size with the increase in silver content.^{25–27} Apart from the higher wavelength-centred absorption band, sharp bands at ~ 222 nm and another at ~ 250 nm were observed in Ag-doped compositions. These peaks are similar to those observed

in aqueous solution of Ag^+ ions.²⁶ Such absorption was ascribed to parity-forbidden transitions involving electron promotion from the $4d^{10}$ ground state to some levels in the $4d^9 5s^1$ configurations.²⁸ These observations also suggest uniform distribution of Ag^+ ions and silver nanoparticles in 800°C sintered samples.

Furthermore, we have carried out antimicrobial tests on the 800°C sintered pelletized samples of silver-doped HA. The effect of nanoparticles of silver and Ag^+ ions on the antimicrobial activity of the silver-doped samples was observed by monitoring the growth of *E. coli* bacteria using FESEM observations of the pelletized samples of $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ ($x = 0.0, 0.8, 1.0$ and 1.5) compositions. Figure 4 shows the FESEM images of the samples of $x = 0.0, 1.0$ and 1.5 compositions, which were kept for incubation for 4 h in the Gram-negative bacterial strain of *E. coli*. We observed a number of bacteria adhered on the surface of parent HA, while the ceramic compositions of $x = 0.8$ and $x = 1.0$ exhibit adherence of very few bacteria on the surface. On increase in silver content, $x = 1.5$ composition does not show the presence of any bacteria. These results confirm that silver nanoparticles and Ag^+ ions indeed significantly improve the antimicrobial property of HA.

4. Conclusions

In summary, the following conclusions can be drawn. (a) The wet chemical synthesis of silver-doped HAs $[\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2]$ exhibit interesting trend in terms of the compositional-dependent phase stability at the selected sintering temperatures. Independent of sintering temperature, HA phase is found as the major phase upto $x = 0.8$ silver-doped composition and α -TCP as major phase in $x = 1.5$ composition. (b) Raman spectroscopic data reveal the presence of symmetric mode of oxygen adsorbed on silver surface in 1200°C sintered samples, which provides the origin of TCP phase in silver-doped compositions. The presence of silver nanoparticles in 800°C sintered samples is confirmed using optical absorption measurements. (c) Importantly, $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ ceramics with $x \geq 0.8$ composition exhibit extensive antimicrobial property, as *E. coli* bacteria does not adhere even after 4 h of incubation in bacterial growth medium.

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Figure 4. SEM images of the upper surface of the $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ **a**—($x = 0.0$), **b**—($x = 1.0$), **c**—($x = 1.5$) pellets (sintered at 800°C) incubated in *Escherichia coli* (ATCC#25922) bacterial suspension. Note the size of silver particles ~ 1 micron as shown in inset in **b**.

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