

Amino acid-assisted synthesis of strontium hydroxyapatite bone cement by a soft solution freezing method

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Abstract. Among many cations that can substitute for calcium in the structure of hydroxyapatite, strontium provokes an increasing interest because of its beneficial effect on bone formation and prevention of bone resorption. Strontium-incorporated calcium phosphates show potential in biomedical application, particularly the doped strontium may help in new bone formation. We have synthesized strontium hydroxyapatite powders at 2 °C by a soft solution freezing method using glycine as the template. The structural and morphological characterizations were carried out on the as obtained powders using Fourier transform infrared spectroscopy, X-ray diffraction analysis and scanning electron microscopy techniques. Strontium was quantitatively incorporated into hydroxyapatite where its substitution for calcium provoked a linear shift of the infrared absorption bands of the hydroxyl and phosphate groups. The strontium substituted bone cement has potential for use in orthopaedic surgeries. The present study shows that the addition of glycine plays an important role in reducing the particle size of strontium hydroxyapatite which could be used for biomedical applications.

Keywords. Strontium hydroxyapatite; freezing synthesis; template; biomaterials; bone cement; bioceramics.

1. Introduction

Hydroxyapatite (HAp), $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$, has been widely studied as an important biocompatible material because of its chemical similarity to the natural calcium phosphate mineral present in a biological hard tissue (Gomez-Vega *et al* 2000; Bezzi *et al* 2003; Wang *et al* 2008). Calcium phosphate cements are broadly used as bone substitutes in orthopaedic applications for their high biocompatibility as well as osteoconductivity (Guo *et al* 2005). Among the calcium phosphate cements, hydroxyapatite is reported to show high bone bonding ability. Moreover, HAp is the most thermodynamically stable phase in physiological conditions (Sanosh *et al* 2010). But pure HAp cannot be used in load bearing applications due to its low mechanical strength (Mardziah *et al* 2009). It has been demonstrated that the properties of HAp can be tailored over a wide range by modifying the composition through ionic substituents (Suchanek *et al* 2004; Kannan *et al* 2008). Several studies have been reported on the synthesis of metal ion substituted HAp and have been shown to improve its structural stability and biocompatible properties (Webster *et al* 2004; Kalita and Bhatt 2007; Xue *et al* 2006; Capuccini *et al* 2008). A great number of substitutions, both cationic and anionic are possible since Ca^{2+} ion sites can be replaced by various divalent cations including Sr^{2+} , Ba^{2+} , Pb^{2+} , Mg^{2+} , Zn^{2+} etc. Among these, strontium

is believed to play an important role in the treatment of osteoporosis and enhancement of bone remineralization. Moreover, strontium HAp $[\text{Sr}_{10}(\text{PO}_4)_6(\text{OH})_2]$ has received enormous attention because of its crystallochemical similarity to calcium hydroxyapatite (Balamurugan *et al* 2009). Strontium is also believed to play an important role in the enhancement of bone remineralization as it is associated with a reduction of bone resorption, an increase in the formation of new bone fracture (Pan *et al* 2009). Recently, strontium hydroxyapatite has shown to hold a great potential for coating titanium implants and as filler for bone cements and toothpastes (Lam *et al* 2010). Strontium hydroxyapatite, a bioactive bone cement, is used in spinal and bone fracture surgery and it is also used in bone replacement, bone fillings, bone adhesives and for treatment of osteoporosis (Ni *et al* 2006). The synthesis of strontium hydroxyapatite can be accomplished by using various methods of synthesis like sol-gel (Balamurugan *et al* 2009), solid titrations (Pan *et al* 2009), wet chemical (Bigi *et al* 2007) and sol-gel- supercritical fluid drying (SCFD) methods (Yingguang *et al* 2008). However, drawbacks of these methods include partial reproduction, inhomogeneity of the architecture and high cost. One approach to overcome these drawbacks is the template direct method (Li *et al* 2008), which promises ultra fine high quality powders and provides no phase transformations due to decomposition.

To the best of our knowledge there are no reports about synthesis of strontium hydroxyapatite bone cement by freezing method. Sr-HAp bioactive bone cement promotes

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osteoblast attachment and mineralization *in vitro* as well as bone growth and osteointegration *in vivo*. Local delivery of strontium with HAp in cement has been shown to induce new bone formation and to be effective in reducing fracture risk in osteoporosis. The oral strontium salts have recently been used as a drug associated with treating osteoporosis. Sr-HAp was synthesized with the purpose of being used locally as a filling material for bone cement (Wong *et al* 2009). We have now synthesized strontium hydroxyapatite that possesses architecture similar to that of natural bones, which is more bioactive when compared to non-substituted hydroxyapatite. The strontium substituted calcium phosphate cement has potential for use in orthopaedic surgeries. The present paper reports about the successful synthesis of strontium hydroxyapatite bone cement that could be widely used in orthopaedic applications to anchor implants to existing bone, reconstruct bone and deliver bioactive agents to the body by using glycine as template and freezing at 2 °C. The main advantage of this soft solution freezing method is its simplicity and low cost.

2. Experimental

Strontium nitrate, $\text{Sr}(\text{NO}_3)_2$ and diammonium hydrogen phosphate ($(\text{NH}_4)_2\cdot\text{HPO}_4$) were used as the precursors for Sr and P, respectively. Glycine was used as a template for overall synthesis. All the chemicals were of analytical grade and aqueous solutions were made by dissolving them in deionized water. Firstly, 0.05 M strontium nitrate, $\text{Sr}(\text{NO}_3)_2$, was mixed with varied concentrations of glycine, 0.01 to 1 wt%. The mixtures were continuously stirred under magnetic stirring for 1 h. The precursor mixture, 0.03 M of diammonium hydrogen phosphate was added drop wise to the above mixture until a homogenous white suspension was obtained and this was kept under stirring for about 12 h. pH of the suspension was adjusted to 9 using NaOH. A milky white precipitate was obtained, which was placed in a freezer for 48 h at 2 °C and the precipitate was then dried at 70 °C for 24 h. The obtained precipitate was calcined for 6 h to remove the template and finally sintered at 600 °C for 2 h. The flow chart of the experimental procedure is depicted in figure 1.

To characterize the synthesized strontium substituted hydroxyapatite powders, Fourier transform infrared spectra (FT-IR) were recorded using Nicolet 380 FT-IR spectrophotometer over a range from 4000 to 400 cm^{-1} with number of scans being 32 and resolution, 4 cm^{-1} . For this, small amounts of Sr-HAp powders were blended with KBr and then pressed into discs for measurement. X-ray diffractions (XRD) for the synthesized powders were obtained by Bruker D-8 Advanced-Germany spectrometer. The morphology of the synthesized powders was obtained through scanning electron microscopic technique (SEM) performed by JSM 840A scanning microscope, JEOL, Japan.

The phase composition and crystallinity of the calcined and sintered Sr-HAp powders were determined by X-ray diffractions (XRD) (Bruker D-8 Advanced-Germany spec-

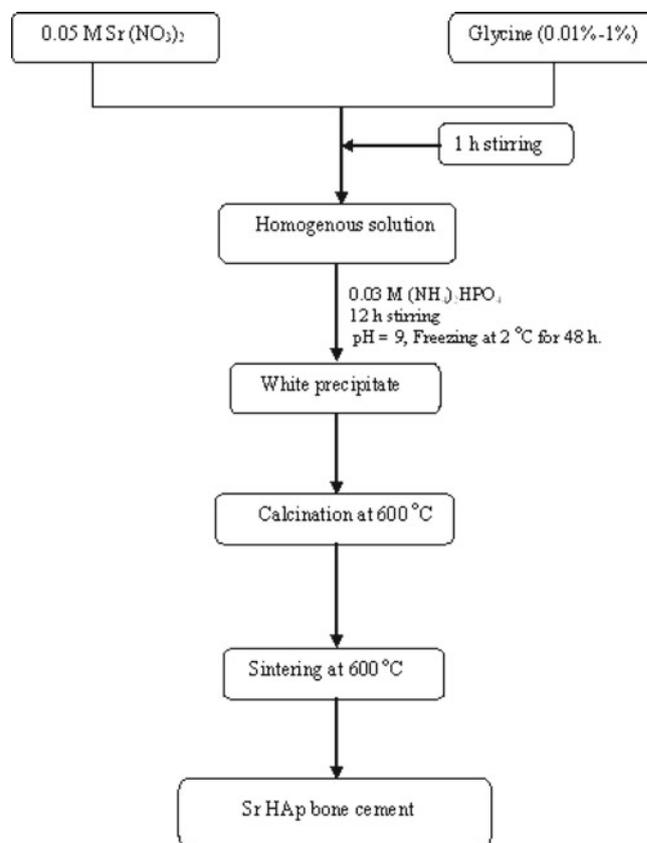


Figure 1. Schematic representation of synthesis of Sr-HAp bone cement by soft solution freezing method.

trometer), with Cu $K\alpha$ radiation, $\lambda = 1.5406 \text{ \AA}$, generated at 35 kV and 25 mA. Data were collected over the 2θ range 20–60° with a step size of 0.010° and a count time of 0.2 s. The crystallite size of the powder was calculated from the X-ray diffractions data using Scherrer's equation (Pang and Bao 2003).

$$X_S = 0.9\lambda / \text{FWHM} \cos \theta, \quad (1)$$

where X_S is the crystallite size (nm), λ the wavelength of monochromatic X-ray beam (nm) ($\lambda = 1.5406 \text{ \AA}$ for Cu $K\alpha$ radiation), FWHM is the full width at half maximum for the diffraction peak under consideration and θ the diffraction angle (°).

The fraction of crystallinity, X_c , of the strontium hydroxyapatite powders were determined (Degirmenbasi *et al* 2006) from the equation:

$$X_c = (0.24/\beta)^3, \quad (2)$$

where β is the FWHM.

3. Results and discussion

FT-IR spectra of the as-synthesized powders obtained with different glycine concentrations (0.01 %, 0.1 %, 0.5 % and

1 wt. %) are shown in figure 2. The characteristic peaks found in the spectra are attributed to strontium hydroxyapatite and no other peaks corresponding to glycine or any trace of impurities could be detected as shown in figures 2 (a–d). To account for them, the peaks appeared at around 1640 cm^{-1} and 3400 cm^{-1} due to the bending mode of adsorbed water, while the OH^- stretching band for Sr–HAp is shifted to higher wave numbers around 3586 cm^{-1} and the OH^- libration band shifts from around 600 to 564 cm^{-1} (Fowler 1974). The IR absorption band due to phosphate groups shift to lower wave numbers for Sr–HAp (Bigi *et al* 2007). Apart from that the characteristic phosphate bands observed at ν_1 from 962 cm^{-1} (Ca–HA) to around 940 cm^{-1} (Sr–HA) and ν_4 from 603 cm^{-1} (Ca–HA) to around 586 cm^{-1} (Sr–HA) in the spectra were attributed to the phosphate (PO_4^{3-}) characteristic absorption (Bigi *et al* 2007; Gopi *et al* 2008, 2009). This change is attributed to the complete substitution of Sr^{2+} for Ca^{2+} into the lattice of apatite phase. Upon increasing the concentration of glycine, the hydroxyl stretching ($-\text{OH}$) and bending modes are never lost as shown in figures 2(a–d). This is a strong indication that the formation of strontium hydroxyapatite is favoured under these concentrations. FT-IR results indicated that the samples exhibit excellent purity of the as-synthesized Sr–HAp.

Figure 3 represents XRD pattern of strontium substituted hydroxyapatite obtained with different glycine concentrations. All the XRD patterns obtained for the samples are in

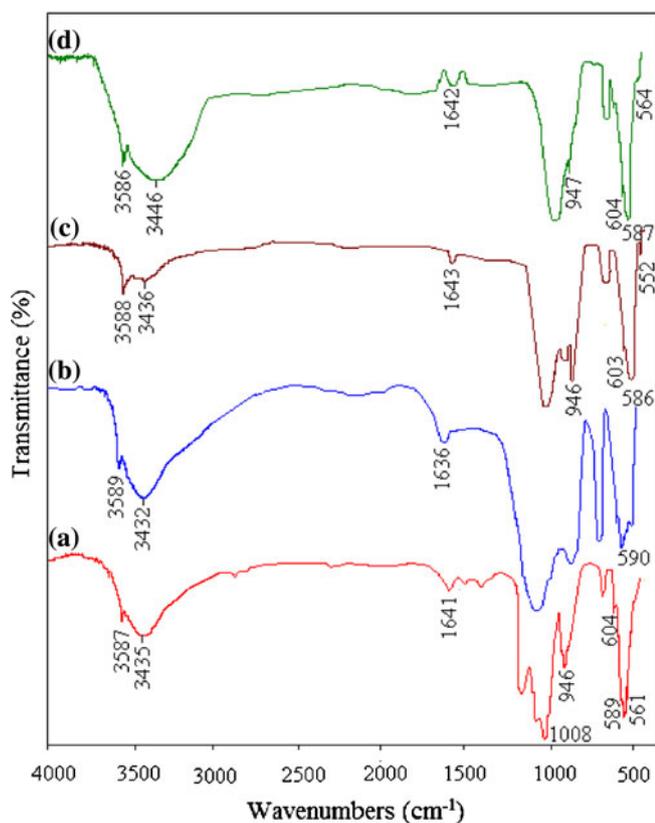


Figure 2. FT-IR spectra of Sr–HAp bone cement using various concentrations of glycine in wt.% (a) 0.01, (b) 0.1, (c) 0.5 and (d) 1.

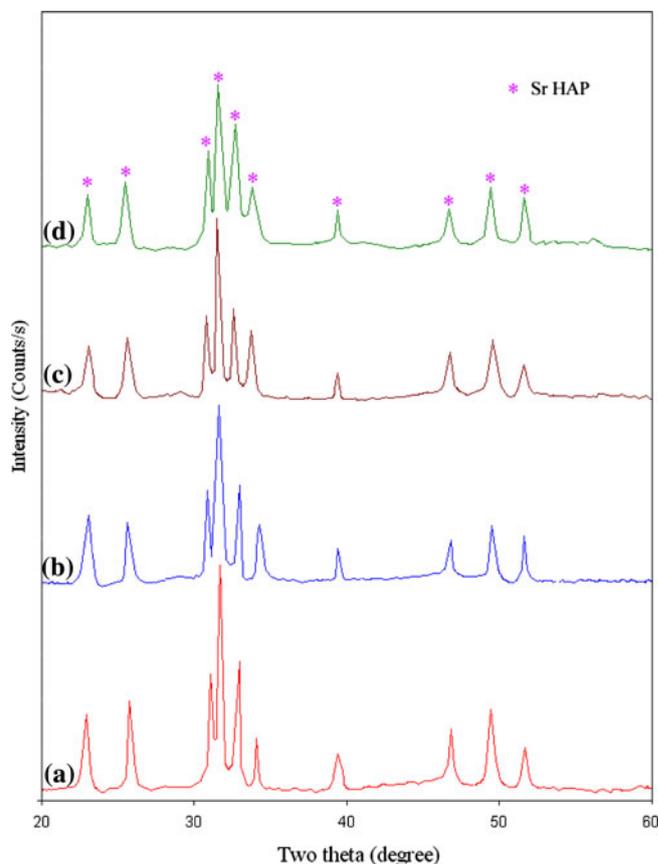


Figure 3. XRD patterns of Sr–HAp bone cement using various concentrations of glycine in wt.% (a) 0.01, (b) 0.1, (c) 0.5 and (d) 1.

Table 1. Structural properties of Sr–HAp obtained from Scherrer's equation at different concentrations of glycine in wt.% (a) 0.01, (b) 0.1, (c) 0.5 and (d) 1.

Concentration (%)	Plane	FWHM	Crystallite size, X_s (nm)
0.01	002	0.309	24.99
0.1	002	0.440	17.33
0.5	002	0.501	17.17
1	002	0.414	16.87

good agreement with the stoichiometric strontium hydroxyapatite (JCPDS; 33–1348). All the XRD patterns obtained at different concentrations of glycine exhibit peaks belonging only to strontium hydroxyapatite, but of different intensity and sharpness. The intensity and sharpness of XRD peaks are more pronounced for the lower glycine content (0.01 wt. %) and tend to gradually decrease with increasing concentrations of the template. As stated above, peaks broadening accompanied by a decrease in peak height are clear symptoms of a decreasing trend in crystallinity as predicted by the Scherrer's equation (Pang and Bao 2003). This trend is confirmed by the data reported in table 1 showing that increasing added amounts of glycine resulted in smaller crystallites in the as synthesized Sr–HAp powders.

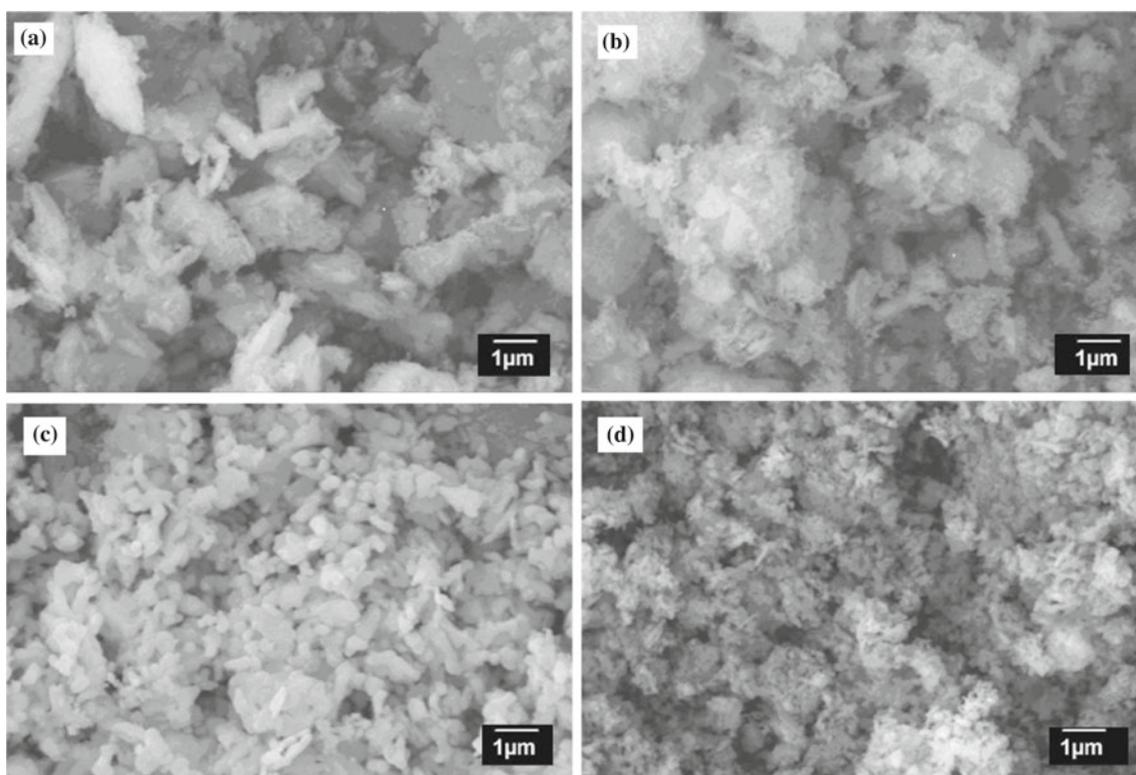


Figure 4. SEM images of Sr-HAp bone cement using various concentrations of glycine in wt% (a) 0.01, (b) 0.1, (c) 0.5 and (d) 1 at 600 °C.

SEM micrographs presented in figure 4 show the effects of addition of glycine on the morphology and microstructure of the as calcined and sintered strontium hydroxyapatite powders. The morphology of strontium hydroxyapatite powder synthesized in the presence of 0.01 wt% of glycine reveals a highly agglomerated strontium apatite powder (figure 4a). When the concentration of glycine was slightly increased (0.1 wt. %), the extent of agglomeration decreased in the Sr-HAp powders (figure 4b). On further increasing the glycine concentration to 0.5 wt.%, Sr-HAp powder with well defined grain structure resulted accomplished by the significant reduction in the particle size (figure 4c). This trend was even exacerbated by further doubling the concentration of glycine to 1 wt. %. Figure 4d shows that the resulting particles are smaller and appear less agglomerated. These morphological observations are consistent with the evolution of the structural parameters as a function of glycine concentration presented in table 1. From these results, it seems reasonable to hypothesize that the reactivity of the system towards precipitating the Sr-HAp powder has been decreased with increasing added amounts of glycine. Its pK values are 2.35 and 9.78 (<http://en.wikipedia.org/wiki/Glycine>), so within the pH range of the solutions/precipitating suspensions. The glycine species exist as anionic amine ($\text{NH}_2\text{-CH}_2\text{-CO}$) (Recommendations 1983, 1984) having the ability to act as a chelating agent towards the Sr^{2+} cations and/or to adsorb at the surface of the precipitated Sr-HAp particles. The

chelating role is directly responsible for the reduced reactivity towards precipitating the Sr-HAp, while the adsorption at the surface of the precipitated Sr-HAp particles will enhance their colloidal stability through the repulsive inter-particle forces generated. Considering that adsorption of glycine species is expected to occur from the early stage of nucleation and that the extent of adsorption should increase with increasing added amounts of glycine, this means that the growth/agglomeration rate of particles precipitated under these conditions is expected to decrease. XRD data (figure 3) and SEM observations (figure 4) concur very well with this interpretation. Hence, increasing the concentration within the 0.01–1.0 wt % range studied in the present work leads to smaller crystallites and particle sizes/agglomerates of the precipitated Sr-HAp powders. This means that both kinetics of the reaction to form Sr-HAp and the kinetics of particles growth/agglomeration are expected to decrease with increasing amounts of chelating agent.

4. Conclusions

Strontium hydroxyapatite bone cement with high purity could be easily prepared at 2 °C by the soft solution freezing method. The formation of Sr-HAp was confirmed by FT-IR, XRD and SEM results. The addition of glycine was found to be beneficial, as it resulted in fine sized Sr-HAp

powders. The FT-IR and XRD results have proved the formation of pure Sr-HAp powders, while the resulting crystallites and particles/agglomerates formed became smaller with increasing added amounts of glycine as clearly evident by XRD results and SEM analysis. These effects were attributed to a chelating role of glycine soluble species towards the Sr²⁺ cations that slows down the reactivity towards precipitating Sr-HAp, while their adsorption at the surface of the precipitated Sr-HAp particles from the early stage of nucleation enhance their colloidal stability. The relevance of these effects increased with the increasing added amounts of glycine, explaining why the size of both the crystallites and particles/agglomerates formed became gradually smaller with increasing amounts of chelating agent. This means that the amino acid-assisted synthesis has potential application in tailoring the characteristics of Sr-HAp bone cement.

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