Influence of pH values on preparation of hydroxyapatite/gelatin composites

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Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) biomaterials have been studied as the substitute materials for the hard tissue because of its biocompatibility, bioactivity, and osteoconductivity [1]. Unfortunately, HA biomaterials cannot be used for heavy load-bearing applications due to their low mechanical properties [2]. Therefore the mechanical properties of HA biomaterials have been frequently improved by including a series of organics, such as collagen, gelatin, and Polylactide (PLA) [3– –6]. It is well known that the hard tissue is composed of HA crystals and the organic matrix, of which the main constituent is type I collagen [7]. But the biggest practical problem with collagen is its cost and commercial availability. Gelatin is the metaprot of collagen and is commercially available. Same as collagen it also shows good biocompatibility and osteoconductvity [8, 9]. Therefore, gelatin has always been used instead of collagen to prepare composites with HA in many investigations. Chang *et al.* had developed a chemical co-precipitation process to synthesize HA/gelatin nanocomposites [10]. However, a detailed study of the effect of pH of reactive solution on the synthesis of HA/gelatin composites had not yet been studied till date. The pH values would affect both the HA formation and the gelatin incorporation. The aim of the present study was to investigate the effect of pH values on the structure, phase constitutes, and gelatin content of HA/gelatin composites.

The staring materials were $Ca(NO_3)_2$ (analytic grade), $(NH_4)_2HPO_4$ (analytic grade) and gelatin (MW250,000), and they were all dissolved in distilled water. $NH_3{\cdot}H_2O$ (analytic grade,) was added to above solutions to adjust the pH. Then the gelatin and $(NH_4)_2HPO_4$ solutions were gradually added to the stirred $Ca(NO_3)_2$ solution, respectively. White precipitates of HA/gelatin was formed when PO_4^{3-} solution was dropped into the Ca^{2+} solution according to the following equation.

$$\begin{aligned}&5Ca(NO_3)_2 + 3(NH_4)_2HPO_4 + 4NH_4OH\\ &\quad = Ca_5(OH)(PO_4)_3\downarrow + 10\ NH_4NO_3 + 3H_2O\end{aligned}$$

The temperature of reaction mixture was kept at 25 °C. The weight ratio of HA/gelatin was set at 80/20. HA/gelatin composites were prepared under various pH values of 7, 8, 9, and 10, respectively. Correspondingly, the samples were named as HA/gelatin-7, HA/gelatin-8, HA/gelatin-9, and HA/gelatin-10. The HA/gelatin slurries was washed with distilled water until pH reached 7.0. After this it was aged at 25 °C for 24 hr. The obtained HA/gelatin cakes were naturally dried in the air at room temperature. Thermal Analysis (TG-DTA) (Ptc-10A, Rigalu, Japan), X-Ray Diffraction (XRD) (X'Pert Pro MPD, Philips, Netherlands) and Raman Spectra (RM 3000 Micro-Raman System, Renishaw, British) were employed to analyze the components and crystal structure of HA/gelatin composites.

Fig. 1 shows the total weight loss of various HA/gelatin samples when heated from room temperature to 800 °C (at the heating rate of 10 °C/min). In the present experiment, the weight loss was divided into two parts. One resulted from the loss of adsorbed water and another was from the decomposition of the organic phase, gelatin. As could be seen from the diagram, the total weight loss of HA/gelatin-8 was the highest among the four samples, which reached up to 28%. It indicated that the gelatin content in the HA/gelatin-8 was the highest. The total weight loss ratio of as-prepared HA/gelatin composites decreased with the increase in pH values when the pH was over 8. Especially when the pH was 10, the weight loss was the lowest, which indicated the content of gelatin of the HA/gelatin-10 was the lowest. It was reported that gelatin could dissolve partially in the alkali solution [11]. The higher pH of reactive solution would result in the lower content of gelatin in HA/gelatin composites in the present experiment. Apart from the water loss, the total gelatin weight losses of all the as-prepared composites were less than 20% designed in the present trail. The DTA curves of various HA/gelatin composites showed that an endothermic peak followed by a lager exothermic peak (data not shown). The endothermic peak was associated with the water loss. The lager exothermic peak

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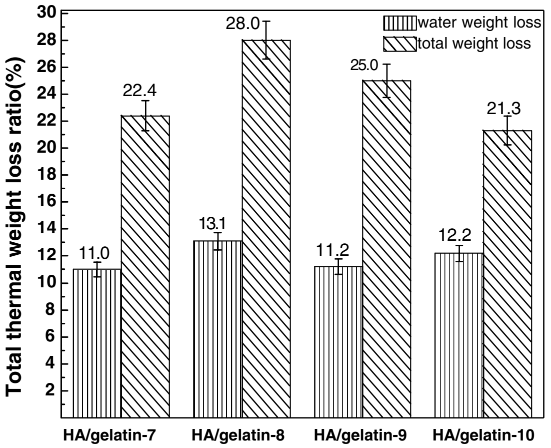

Figure 1 Diagram for total weight loss and water weight loss of HA/gelatin-7, HA/gelatin-8, HA/gelatin-9, and HA/gelatin-10.

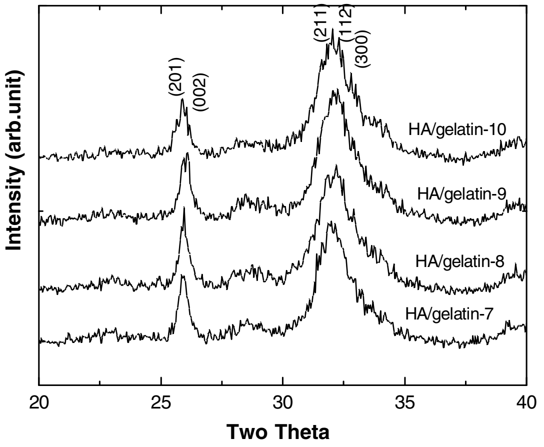

Figure 2 XRD patterns for HA/gelatin-7, HA/gelatin -8, HA/gelatin-9, and HA/gelatin-10.

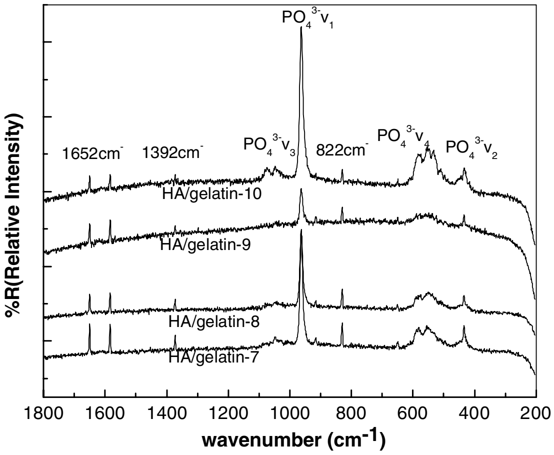

Figure 3 Raman spectra for HA/gelatin-7, HA/gelatin-8, HA/gelatin-9, and HA/gelatin-10. The peaks assigned to HA are indicated.

was assigned to the thermal decomposition of HA/gelatin composites, which began at approximately 245 °C.

The XRD results confirmed that there were two broader diffraction peaks near $2\theta = 26^\circ$ and 31.8°, which were assigned to the main diffraction peaks of HA (Fig. 2). It also demonstrated that there were no other Ca–P phases in as-prepared HA/gelatin as shown in XRD. Moreover, these XRD patterns possessed the diffusion background comparable to those of HA ceramics sintered at high temperature (data not shown). The broader diffraction peaks was due to the smaller grain size based on the Debye–Scherrer formula. The diffusion background of XRD patterns indicated that they possessed the poorer crystallinity.

Raman spectra of different HA/gelatin composites are shown in Fig. 3. The peaks between 400 and 1200 cm^{-1} were assigned to the υ_1, υ_2, υ_3, and υ_4 of PO_4^{3-} of HA as indicated in Fig. 3 [12]. Additionally, the peaks at 1652, 1392 and 822 cm^{-1} were assigned to the function group of gelatin in the Raman Spectrum, which indicated the existence of gelatin in HA/gelatin composites [13]. The position of the υ_1 P–O symmetry stretching vibration at about 960 cm^{-1}, for instance, showed small but recognizable differences among the as-prepared HA/gelatin composites. The peaks of the υ_1 P–O of HA/gelatin-9 and HA/gelatin-10 were shifted to 962 cm^{-1}, which were similar to that of the synthetic pure HA standard. Although the peak of the υ_1 P–O of HA/gelatin-8 and HA/gelatin-7 were at 960 cm^{-1}, it was characteristic of all the biological apatite, i.e., bone, dentin, and enamel [12]. Additionally, as-prepared HA/gelatin composites also differed in the absolute positions and relative intensities of some other phosphate vibrational modes. The peaks of υ_3 and υ_4 of PO_4 were much weaker in HA/gelatin-8 and HA/gelatin-9 compared to those of HA/gelatin-7 and HA/gelatin-10. These characteristics of Raman spectrum could be attributed to the distortion caused in the PO_4^{3-} tetrahedral, which indicated by the incorporation of gelatin into neighboring tetrahedral.

On the basis of the results of TA, there were much more gelatin incorporated into HA/gelatin-8 and HA/gelatin-9, which would result in more distortion of PO_4^{3-} tetrahedral compared to those of other as-prepared composites. As could be seen from Fig. 3, the relative peak intensity of PO_4^{3-} of HA/gelatin-9 was the lowest among four composites, whereas the peak intensity of PO_4^{3-} of HA/gelatin-10 was the largest. The relative intensity of function groups of gelatin decreased with the increase in pH. The higher pH would facilitate formation of HA. On the other hand, the alkali solution would dissolve much gelatin, which would give rise to the lower precipitation of gelatin [11].

In the bone formation process, osteoblast synthesize the collagenous matrix that subsequently become mineralized by the formation of HA crystals within and between the collagen fibrils [14]. The pH played an important role on this biological mineralization. Accordingly, pH also served as the key factors to prepare HA/gelatin composites. Lower pH was not beneficial to produce HA crystals. In contrast, higher pH led to partial dissolution of gelatin and resulted in the lower gelatin incorporation. The present results elicited that HA/gelatin-8 was the optimum as-prepared when composites takes account of both the HA and gelatin precipitation.

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