

Preparation and Morphology Control of Hydroxyapatite by Aqueous Precipitation

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Abstract

Hydroxyapatite, with excellent biocompatibility, biological activity, and biological affinity, is the main inorganic component of human bones and teeth. It can promote bone tissue regeneration and has important applications in bone tissue engineering fields such as bone defect repair and bone replacement. In this study, calcium acetate solution and diammonium dihydrogen phosphate solution had been mixed to prepare calcium phosphate through aqueous precipitation reaction. The testing results of scanning electron microscopy and X-ray diffraction show that the morphology of the product can be controlled by changing the pH, the temperature, and the method of adding reactant solution dropwise. When the temperature of the reaction system is 20 °C, plate-like calcium hydrogen phosphate is mainly obtained; block-like hydroxyapatite is obtained when the temperature rised to 40 °C; and needle shaped hydroxyapatite is obtained at 60 °C. The length and diameter of two-dimensional hydroxyapatite crystals become smaller when the temperature and the pH value of the reaction system increase.

Keywords

Hydroxyapatite; Precipitation Method; Crystal Morphology.

1. Introduction

Hydroxyapatite (HAP, $(\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2)$) is an important inorganic biomaterial, which composition is similar to the mineral composition of human teeth and bones, therefore it has good biological activity and biocompatibility. HAP is widely used in the fields of bone repair and bone regeneration. HAP can be used as a bone filling material and reconstruction implant for the treatment of fractures, bone defects, etc. Combining HAP with cells or bioactive substances can construct artificial bone scaffolds or support bodies, providing support for the growth and differentiation of bone cells. In the field of dentistry, HAP can be used to produce artificial tooth roots and toothpaste. In addition, HAP is also used for drug sustained-release and biosensing. Therefore, investigating the growth principle and influencing factors of HAP crystals is of great significance for studying the biomineralization process of HAP and improving its application value[1,2].

Aqueous precipitation method is advantageous due to the low cost and simplicity [3-9], while the influence of adding reagents method has seldom been studied. HAP was synthesized by an aqueous precipitation method in this work. The effects of synthesis temperature, pH value and the adding reagents method on the crystal structure and micrograph of HAP powders were characterized by SEM and XRD.

2. Experiment

2.1. HAP Preparation

All chemicals ($(\text{NH}_4)_2\text{HPO}_4$, $\text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$, $\text{H}_2(\text{CH}_3\text{COO})_2$, NH_4OH) solution were of analytical grade. Double distilled water was used in preparation.

First, 0.03 mol $(\text{NH}_4)_2\text{HPO}_4$ and 0.05 mol $\text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ were dissolved in 100 mL distilled water respectively. The molar ratio $\text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ to $(\text{NH}_4)_2\text{HPO}_4$ ($n(\text{Ca}^{2+})/n(\text{PO}_4^{3-})$) was 1.667 which is the stoichiometric $n(\text{Ca}^{2+})/n(\text{PO}_4^{3-})$ ratio of HAP. The employed pH values of 6, 7, and 7.5 were adjusted by addition of NH_4OH solution. Synthesis temperature was maintained at 20 °C, 40 °C, 60 °C and 80 °C, respectively. After mixing, the solution was stirred for about 3h, then aged for 48h at room temperature. Finally, the precipitate was centrifuged and washed by double distilled water for three times consecutively. The wet powders were dried at 80 °C for 6 h [3,4].

2.2. Characterization

The morphological features of the precipitated powders were investigated with a Phenom G3 Pro scanning electron microscope, the phases of the samples were investigated by D/MAX2500PC X-ray diffraction diffractometer with $\text{CuK}\alpha$ radiation.

3. Results and Discussion

3.1. The Influence of Synthesis Temperature on HAP Morphology

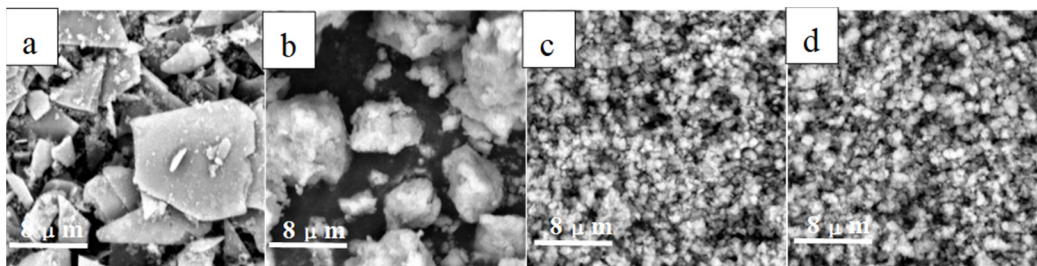


Fig 1. SEM images of calcium phosphate powder prepared prepared at 350r/min, pH=7.5 with different reaction temperature: (a) 20 °C; (b) 40 °C; (c) 60 °C; (d) 80 °C

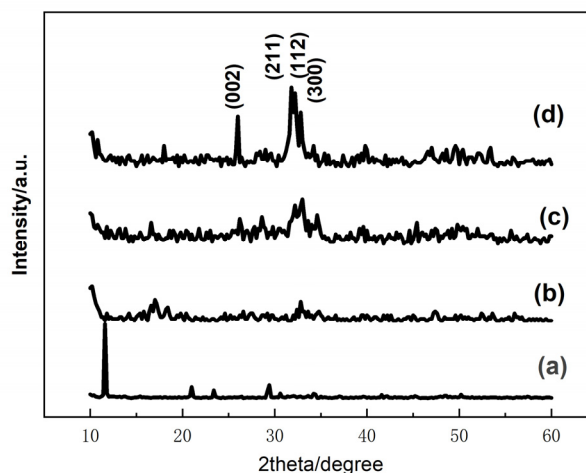


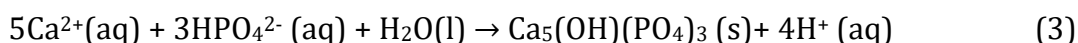
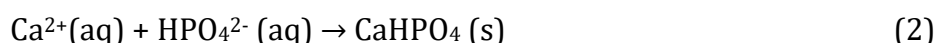
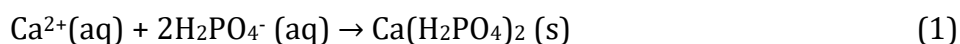
Fig 2. XRD patterns of calcium phosphate powder prepared at 350r/min, pH=7.5 with different reaction temperature: (a) 20 °C; (b) 40 °C; (c) 60 °C; (d) 80 °C;

Temperature has a significant impact on the reaction process. Fig. 1 shows the morphology of the calcium phosphate varied greatly with the synthesis temperature. The samples were flake-like shape (Fig. 1(a)), block like shape (Fig. 1(b)), and needle like shape (Fig. 1(c) and 1(d)). Further XRD testing and analysis had be conducted to investigate its root cause[5]..

Fig. 2 shows the XRD patterns of samples prepared at different synthesis temperature. The XRD diffraction peaks in Fig. 2(a) are different from those of the other three samples (Fig. 2(b), 2(c) and 2(d)).The main phase(Fig. 2(a)) was calcium hydrogen phosphate. The strongest peaks of powders (Fig.2(b), 2(c), and 2(d)) were between 31.50 and 32.0, their main phase was hydroxyapatite (JCPDS 09-0432)[6-8].

Calcium hydrogen phosphate was generated at 20°C, hydroxyapatite samples was prepared when the temperature rised to 40°C. The XRD diffraction peaks with the synthesis temperature at 40°C were weaker and less sharp, indicating poor crystallinity of the hydroxyapatite; When the synthesis temperature rised to 60°C, all diffraction peaks (002), (211), (112), (300) appeared, and the two main diffraction peaks (211) and (112) have separated, indicating that the crystallinity of HAP particles is high and there is a trend of preferential growth along the c-axis.

Phosphoric acid is a ternary acid, three acid ions exist in ammonium hydrogen phosphate aqueous solution, H₂PO₄⁻, HPO₄²⁻, PO₄³⁻. There are also a variety of precipitation reactions in the mixed solution of calcium acetate and ammonium hydrogen phosphate:



Although HAP is more stable than CaHPO₄ in aqueous solution(the solubility product of HAP is 2.34×10⁻⁵⁹ at 37°C)[4], the concentration of HPO₄²⁻ ions is higher in (NH₄)₂HPO₄ solution, CaHPO₄is easy produced through reaction equation(2); the HAP generated when the temperature raised higher because that the degree of water dissociation increases and the concentration of OH⁻ions increases, on the other hand the driving force ΔG_{HAP}, for the formation of HAP increases with increasing temperature. The driving force ΔG_{HAP} is given by the following equation[4],

$$\Delta G_{HAP} = -\frac{RT}{n} \ln\left(\frac{Q}{K}\right) = -\frac{RT}{n} \ln\left(\frac{[\alpha]e}{[\alpha]}\right) = -\frac{RT}{n} \ln\left(\frac{C_e}{C}\right) \tag{4}$$

$$\lg K_{sp} = \frac{8219}{T} - 1.6657 - 0.098215T \tag{5}$$

Where R is the universal gas constant; T is the absolute temperature; Q is the ionic product of the precipitating salt, n is the number of ions, and Ksp is the solubility product, [α] is ion activity, C is ion concentration, [α] ≈C when the solution is very thin. Therefore, the driving force and the growth rate increased with increasing temperature (Eq.4).According to the theory the growth rate of HAP were fast as the temperature increased, which were in accordance with the SEM and XRD results.

3.2. The Influence of pH Value on HAP Morphology

Table 1. pH of reaction systems before and after reaction (80 °C)

pH before reaction	7.5	6	5
pH after reaction and aging for 48h	5.67	4.57	4.22

The pH value of the solution affects the dissociation of HPO_4^{2-} and H_2O in the reaction system, as well as the reaction process and product morphology. Table 1 shows that the pH value of the system decreases after the reaction, which was due to the consumption of OH^- ions during the formation of HAP.

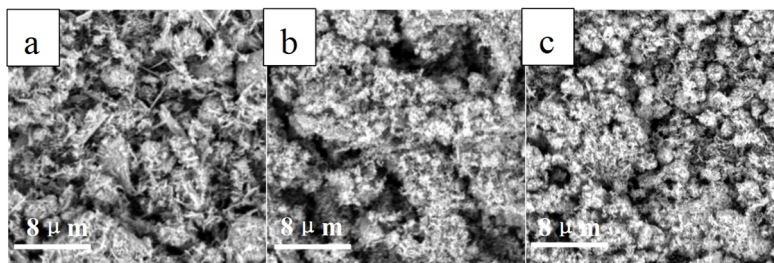


Fig 3. SEM images of powders prepared at 100r/min, 80 °C with different pH values: (a) pH=5; (b) pH=6; (c) pH=7.5.

Fig. 3 shows the morphology of the HAP varied with the pH value of the solution. All samples are needle shaped crystals. Fig. 3(a) shows that the size of the sample obtained at pH=5 is approximately 3-5 μm in length, the aspect ratio is about 8-10; and the interface edge is clear. Fig. 3(b) and 3(c) indicate that the crystal length shortens to 1-3 μm as the pH value of the solution increased, these products become finer and has a distinct filamentous morphology.

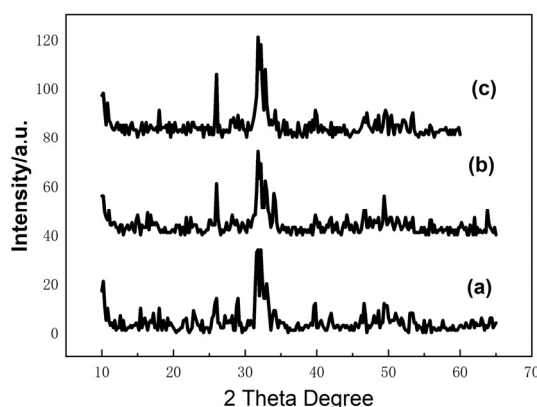


Fig 4. XRD patterns of powder after two days of reaction at 100r/min, 80°C with different pH values: (a) pH=5; (b) pH=6; (c) pH=7.5;

Fig. 4 shows the XRD patterns corresponding to the three experimental products in Fig. 3. Three products are all HAP. Diffraction peaks at the (002) crystal plane indicating a trend of preferential growth of HAP along the c-axis during the formation process. Diffraction peaks at the (002) crystal plane in Fig. 4(b) and 4(c) were stronger than 4(a), Therefore, the obtained HAP powder in exhibits a needle like or filamentous morphology.

3.3. Influence of the Method of Adding Reagents

Three ways of adding reagents to prepare HAP were used. Fig. 5 shows that the HAP products prepared by three drip addition methods are all filamentous products. The length of product in Fig. 5(a) and Fig. 5(b) was about 1-2 μm . The sizes were smaller and with certain degree of agglomeration. On the contrary, the product generated under the conditions of simultaneously adding calcium and phosphorus sources (Fig. 5(c)) has a length of approximately 5-6 μm , the products are arranged in a network, and the agglomeration phenomenon is relatively light, with clear interface edges. In the way of simultaneous addition of two reactant, the initial number of reactant ions (Ca^{2+} , PO_4^{3-} , OH^-) is relatively fewer, resulting in fewer crystal nuclei. Continuing to add the reagents mainly cause crystal growth rather than forming more crystal nuclei, then the product grains grow more fully, resulting in a larger size of the product.

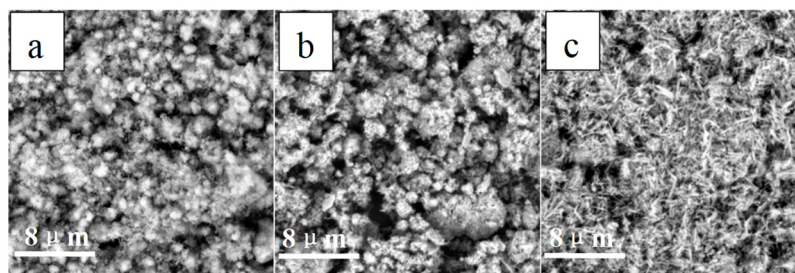


Fig 5. SEM images of powders prepared at 350r/min, 70°C, pH=7.5 by different drip addition methods:

(a) $(\text{NH}_4)_2\text{HPO}_4 \rightarrow \text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$; (b) $\text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O} \rightarrow (\text{NH}_4)_2\text{HPO}_4$; (c) Add $\text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ and $(\text{NH}_4)_2\text{HPO}_4$ dropwise simultaneously.

4. Conclusion

When calcium phosphate is prepared by precipitation method using calcium acetate as the calcium source and diammonium hydrogen phosphate as the phosphorus source, dicalcium phosphate are formed at lower temperature and HAP are formed at higher temperature. The HAP also formed in the acidic reaction system. When the pH value of reaction system was lower, the HAP became needle-like shape; however, when the pH value increased, the HAP became filamentous shape. The way of adding reagents also has an effect on the morphology of the HAP product, adding calcium sources and phosphorus sources simultaneously give larger needle-like products with coarser particle sizes.

Acknowledgments

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