Preparation and Characterization of Magnetic Gd$^{3+}$ Doped Hydroxyapatite Nanoparticles

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Abstract: Hydroxyapatite (HAP), the major inorganic composition of mammal bones, has good biocompatibility, biological activity and osteoconductivity. Gadolinium (Gd) with seven unpaired electron on 4f orbit can be used for MRI contrast agent due to its large electron spin magnetic moment and relaxivity. In this study, the magnetic Gd$^{3+}$ doped HAP nanoparticles (Gd-nHAP) were synthesized via coprecipitation method. Results showed that the magnetization of Gd-nHAP was gradually enhanced along with the increase of doping content of Gd$^{3+}$, which indicated magnetic property depended on doping content. Moreover, with a fixed doping content of Gd$^{3+}$, the increase of reaction temperature (60°C-280°C) did not generate significant effect on magnetization of synthesized Gd-nHAP. The result showed that magnetization of Gd$^{3+}$ has no strong correlation with crystallinity and crystalline size of HAP.

1. Introduction

Hydroxyapatite (HAP), as the main inorganic component of vertebrate teeth and bones [1], has good biocompatibility/bioactivity and bone conductivity, which is non-toxic and does not cause inflammation and rejection [2]. In the field of biomedicine, HAP can be used as bone tissue repair materials, drug delivery carriers [3], bioactive coatings, dental materials and middle ear implants.

Recent studies have shown that the introduction of magnetic materials in the process of bone repair can promote the proliferation and differentiation of osteoblasts, accelerate bone integration and new bone formation [4-7], increase bone density and calcium content, and accelerate the healing of fractures [8-11]. For example, in calcium phosphate porous ceramics and hydroxyapatite/collagen scaffolds, composite superparamagnetic iron tetroxide can give bone repair materials magnetic properties [12], thereby enhancing the effect of magnetic therapy. The magnetic rare earth element gadolinium, which has seven unpaired electrons in the 4f orbital, can provide high electron magnetic moments. It has been used as a magnetic resonance imaging (MRI) contrast agent (e.g. gadolinium diethylenetriamine pentaacacetate, gadolinium tetraazacyclododecane tetraacetate) in clinic [13]. By incorporating gadolinium ions into HAP crystal lattice, HAP can be endowed with magnetic properties, which is expected to be used as a magnetic bone repair material or an MRI contrast agent.

In this paper, we prepared the magnetic gadolinium ion (Gd$^{3+}$) doped HAP nanoparticles (Gd-nHAP) by coprecipitation method and the properties were characterized. Gd-nHAP with different doping content and crystallinity was prepared by adjusting the concentration of gadolinium ion and synthesis temperature. The effect of gadolinium doping content and crystallinity on the magnetic properties of Gd-nHAP was discussed.
2. Experiment

2.1. Preparation

Gd-nHAP was synthesized by coprecipitation method using (NH₄)₂HPO₄, CaCl₂ and Gd(NO₃)₃ (purity > 99.9%) as raw material. Firstly, controlling the molar ratio of (Ca+Gd)/P as 1.67, 30 ml (NH₄)₂HPO₄ (PO₄³⁻concentration is 0.06mol/L) solution was added to the mixture solution of 30 ml CaCl₂ and Gd(NO₃)₃ (total concentration of Ca²⁺ and Gd³⁺ is 0.1 mol/L). Then, after adjusting its pH to about 10 by ammonia water, the solution was stirred for one hour at a certain temperature. When the reaction was completed, the precipitated products were washed with deionized water for three times to remove impurity ions. Finally, Gd-nHAP powder was obtained after freeze-drying. The molar ratios of Gd/(Gd+Ca) were set from 0% to 100%, and the reaction temperatures were set from 60°C to 280°C. The reactions below 100°C were carried out in water bath pots, and those from 100°C to 280°C were carried out in high temperature autoclaves.

2.2. Characterization

X-ray diffraction (XRD, D8 Advance) was used to analyze the phase composition and structure of crystal materials. Crystallinity can be calculated by equation

\[ X_c = 1 - \frac{V_{112}/300}{I_{300}} \]

where the \( V_{112}/V_{300} \) is the intensity of the bottom of the peak between the (112) and (300) diffraction peaks and \( I_{300} \) is the intensity of the (300) diffraction peaks [14].

The grain size of Gd-nHAP can be calculated by Scherrer equation

\[ D = \frac{k \lambda}{\beta \cos \theta} \]

where \( \lambda \) is the wavelength of X-ray, \( \beta \) is half-peak width of diffraction peak, \( \theta \) is the angle of Bragg diffraction, \( k = 0.9 \) is Scherrer constant [15].

Fourier transform infrared spectroscopy (FTIR, Nicolet 6700) was used to detect the characteristic functional groups of the samples. The particle size, agglomeration and morphology were observed by field emission scanning electron microscopy (FE-SEM). The particle size was analyzed by laser particle size analyzer. The elements were analyzed by Energy dispersive spectrometer (EDS). X-ray photoelectron spectroscopy were used to investigate the surface or interface composition of the sample. Physical property measurement system (PPMS-9T) was used to analyze the magnetic property of samples at room temperature with the change of external magnetic field.

3. Results and analysis

3.1. Phase composition and structure analysis

Figure 1 (a) showed the XRD spectra of Gd-nHAP synthesized at 80°C with different Gd³⁺ doping content. The results showed that the characteristic peaks of HAP crystals (standard card PDF 074-0565#) appeared in the samples with different Gd³⁺ doping content (up to 20%). In addition, no obvious impurity peaks were found even when the doping content of gadolinium reached 20%. The doping of Gd³⁺ did not change the crystal phase composition of the products. However, with the increase of Gd³⁺ doping ratio (from 0% to 20%), the three main peaks ((211), (112) and (300) crystal planes) became insignificant and tended to merge together, which indicated that the crystallinity of samples decreased with the increase of Gd³⁺ doping ratio. Moreover, with Gd³⁺ doping content increasing continuously (from 20% to 100%), the main peaks of HAP gradually disappeared and other impurity phases began to manifest. Accordingly, it indicated that Gd³⁺ (0%-20%) doped HAP nanoparticles with single crystal phase could be prepared by coprecipitation method.
The XRD spectra of Gd(4%)-nHAP synthesized at different temperatures are shown in Figure 1 (b). The results showed that all the samples synthesized at different temperatures were HAP crystalline phases. With the increase of temperature, the peak shape became sharper and the three main peaks became more prominent, indicating that the increase of reaction temperature promoted the crystallization. With the reaction temperature increasing from low temperature (60°C ~ 80°C) to high temperature (240°C ~ 280°C), the crystallinity increased rapidly from about 0.2 to about 0.8. As shown in Table 1, the grain size of Gd-nHAP along the long axis (002) and diameter direction (300) increased with the increase of reaction temperature.

![Figure 1](image1.jpg)

**Figure 1.** (a) XRD patterns of samples synthesized at 80°C with different Gd³⁺ doping content (b) XRD patterns of Gd(4%)-nHAP synthesized at different temperature.

| Temperature (°C) | D(002) | D(300) |
|------------------|--------|--------|
| 120              | 26.7   | 19.6   |
| 160              | 28.9   | 20.8   |
| 200              | 29.0   | 21.9   |
| 240              | 31.7   | 27.0   |
| 280              | 37.4   | 29.0   |

**Table 1.** Crystallite size synthesized at different temperature.

![Figure 2](image2.jpg)

**Figure 2.** (a) Infrared spectra of samples synthesized at 80°C with different Gd³⁺ doping content (b) Infrared spectra of Gd(4%)-nHAP synthesized at different temperature.
3.2. Infrared analysis

Figure 2 (a) showed the FTIR spectra of HAP nanoparticles with different doping content at 80°C. Figure 2 (b) showed FTIR spectra of HAP nanoparticles doped with 4% Gd³⁺ at different temperatures. The broad absorption peak at 3430 cm⁻¹ was the stretching vibration peak of the intermolecular hydrogen bond (O-H) of water adsorbed by Gd-nHAP product, and the bending vibration peak at 1641 cm⁻¹ was the in-plane bending vibration peak of hydroxyl (-OH) of adsorbed water. The absorption peaks at 3573 cm⁻¹ and 630 cm⁻¹ were the stretching vibration peaks of hydroxyl groups in HAP lattices, which belong to the characteristic peaks of HAP. The absorption peaks at 1093 cm⁻¹ and 1032 cm⁻¹ were the phosphate ν₁ stretching vibration mode, the 602 cm⁻¹ and 564 cm⁻¹ bands were phosphate ν₄ bending vibration mode, the 962 cm⁻¹ band arised from ν₁ PO₄ and the 472 cm⁻¹ band arised from ν₂ PO₄. Combined with the PO₄³⁻ group’s strong characteristic absorption peaks at 550-650 cm⁻¹ and 950-1100 cm⁻¹, it can be concluded that the product was HAP. In addition, the characteristic peaks of 1486 cm⁻¹, 1420 cm⁻¹ and 871 cm⁻¹ of CO₃²⁻ were observed in FTIR spectra of all samples. During the process of the reaction, small amount of CO₂ entered the solution and formed CO₃²⁻ which partly substituted PO₄³⁻ in HAP lattice [16].

![Figure 3](image3.png)

**Figure 3.** SEM micrographs of Gd-nHAP synthesized at 80°C with different Gd³⁺ doping content: (a) 0%, (b) 4%, (c) 10%, (d) 20%, (e) 40%, (f) 60%, (g) 80%, (h) 100%.

![Figure 4](image4.png)

**Figure 4.** SEM micrographs of Gd(4%)-nHAP synthesized at different temperature: (a) 60°C; (b) 120°C; (c) 160°C; (d) 200°C; (e) 240°C; (f) 280°C.
3.3. **SEM observation**

Figure 3 showed field emission scanning electron microscopy (FESEM) image of HAP nanoparticles with different Gd\(^{3+}\) doping content synthesized at 80\(^\circ\)C. The synthesized Gd-nHAP nanoparticles were nanosize. Compared with pure HAP, the size of Gd(\(~10\%)\)-nHAP nanoparticles did not change much, and they were needle-like, and the nanoparticles tended to agglomerate together because of high surface energy. However, with the increase of Gd\(^{3+}\) doping content from 20\% to 100\%, the needle-like shape was substituted by dot-like shape. The amount of Gd-nHAP nanoparticles decreased and impurity phases began to emerge.

Figure 4 exhibited FESEM image of HAP powder samples doped with Gd\(^{3+}\) (molar content set as 4\%)(Gd(4\%)-nHAP) synthesized at different temperatures. It can be observed that with the increase of reaction temperature, the synthesized Gd-nHAP particles changed from needle-like to short column-like, and the length and diameter of the particles increased gradually.

3.4. **DLS analysis**

The results in Figure 5 showed that Gd-nHAP with different Gd\(^{3+}\) doping content dispersed by heparin sodium were well dispersed in water. As is shown in Figure 5, the average particle size of Gd-nHAP was around 100nm.

![Figure 5. Size distribution of Gd-nHAP synthesized at 80\(^\circ\)C with different Gd\(^{3+}\) doping content in water: (a) 0\%; (b) 4\%; (c) 8\%.](image)

![Figure 6. EDS spectra of Gd(4\%)-nHAP.](image)
3.5. Element Content and Composition Analysis

The element analysis of Gd(4%)-nHAP powder samples synthesized at 80°C was carried out by EDS. Results from Figure 6 showed that the Ca/P mole ratio and (Ca+Gd)/P mole ratio were 0.043 and 1.64 which were close to the setting values of 0.04 and 1.67.

Figure 7. XPS spectra of Gd(4%)-nHAP: (a) Survey scan spectrum, (b), (c), (d), (e) are high resolution scan spectra of Gd 3d, Ca 2p, P 2p and O 1s, respectively.
Figure 7 showed the XPS survey spectrum of Gd (4%) - nHAP. The element composition on the surface of the sample and the binding energies and valence states of Gd, Ca, P and O elements were analyzed. The binding energies of 347.05 eV, 133.02 eV and 530.91 eV were the characteristic peaks of Ca 2p, P 2p and O 1s. In the XPS high resolution scanning spectra of Gd elements, there were obvious characteristic peaks at 1197.05 eV. Combining the results of XRD and EDS, we can confirm that Gd$^{3+}$ has been successfully doped into HAP lattice.

Figure 8. (a) Magnetization curves of Gd-nHAP synthesized at 80°C with different Gd$^{3+}$ doping content (b) Figure of magnetization changed along with doping content of Gd$^{3+}$ at 3T.

Figure 9. (a) Magnetization curves of Gd(4%)-nHAP synthesized at different temperature (b) Figure of magnetization changed along with temperature at 3T.

3.6. Analysis of magnetic properties

Figure 8 (a) was the M-H magnetization curves of HAP nanoparticles with different gadolinium doping content synthesized at 80°C. Figure 8 (b) was the variation figure of magnetization changed with Gd$^{3+}$ doping content at 3T. Figure 9 (a) was the M-H magnetization curve of Gd(4%)-nHAP synthesized at different temperatures. Figure 9 (b) was the temperature dependence figure of magnetization at 3T. From the M-H magnetization curve, it can be seen that the magnetization of Gd-nHAP synthesized at fixed temperature increased with the increase of Gd$^{3+}$ doping content. The
relationship between magnetization and concentration can be regarded as linear correlation at low doping content (from 0% to 20%). Magnetization still increased with the increase of doping content (from 20% to 100%), but the relation was not linear correlation and the growth rate dropped continuously. The magnetization reached maximum when the doping content was 80%. Combined with the aforementioned results of XRD and FTIR, it can be concluded that HAP can enhance the effect of magnetization. Setting the doping content of Gd$^{3+}$ as 4%, the magnetization of Gd-nHAP synthesized at $60^\circ$C ~ $240^\circ$C increased slightly with the increase of reaction temperature, which indicates that the crystallinity of matrix material HAP has a certain effect on the magnetic properties. This may be due to the diffusion of Gd$^{3+}$ to HAP crystal lattice and occupying the calcium site in HAP crystal lattice, but the field effect of HAP crystal did not seem to affect the magnetic properties. As the reaction temperature continued to rise to $280^\circ$C, the magnetization of the synthesized Gd-nHAP decreased, which was inconsistent with the effect of crystallinity, suggesting that the magnetic properties may also be related to the grain size of HAP, showing a negative correlation. With the increase of grain size, the magnetic properties of Gd-nHAP caused by size factors became significantly weakened.

4. Conclusions
Gd-nHAP was prepared by coprecipitation method from calcium nitrate, gadolinium nitrate and diammonium hydrogen phosphate. Keeping the synthesis temperature unchanged, increasing the doping amount of gadolinium ion to 20%, the crystalline phase was single phase, the particle size did not change much, and the magnetization increased linearly with the doping content of gadolinium ion. The growth rate of magnetization decreased with the increase of doping content (from 20% to 100%). Magnetization reached maximum when the doping content reached 80%. Increasing the synthesis temperature, the crystallinity and grain size gradually increased with the doping content of gadolinium ion fixed at 4%. With the increase of synthesis temperature to $240^\circ$C, the magnetization increased slightly. When the temperature further increased to $280^\circ$C, the magnetization decreased slightly. However, the effect of reaction temperature did not have a great influence on the magnetization compared to the effect of doping content. The results showed that the magnetic properties of Gd-nHAP were significantly correlated with the content of gadolinium ions, but the influence of synthesis temperature on the magnetic properties was not significant.

References
[1] Currey J 2001 *Nature* **414** 699
[2] Zhou H and Lee J 2011 *Acta Biomater* **72** 769
[3] Kumar S and Randhawa J K 2013 *Mater Sci Eng C* **33** 1842
[4] Bassett C A, Schink-Ascani M and Lewis S M 1989 *Clin Orthop Relat Res.* **246** 172
[5] Santini M T, Rainaldi G, Ferrante A, *et al.* 2003 *Bioelectromagnetics* **24** 327
[6] Mcleod K J and Collazo L 2000 *Radiat Res.* **153** 706
[7] Jansen J H, vanderJagt O P, Punt B J, *et al.* 2010 *BMC Musculoskel Disord.* **11** 188
[8] Fini M, Cadossi R, Cane V, *et al.* 2002 *J. Orthop Res.* **20** 756
[9] Zhang X Y, Xue Y and Zhang Y 2006 *Bioelectromagnetics* **27** 1
[10] Chang K and Chang W H 2003 *Bioelectromagnetics* **24** 189
[11] Taylor K F, Inoue N, Raffee B, Tis J E, McHale K A and Chao E Y 2006 *J Orthop Res.* **24** 2
[12] Bock N, Riminucci A, Dionigi C, *et al.* 2010 *Acta Biomater* **6** 786
[13] Li Y, Beiha M, Laurent S, *et al.* 2012 *Macromolecules* **45** 4196
[14] Landi E, Tampieri A, Celotti G and Sprio S 2000 *J Eur Ceram Soc.* **20** 2377
[15] Rusu V M, Ng C-H, Wilke M, Tiersch B, Fratzl P and Peter M G 2005 *Biomaterials* **26** 5414
[16] Markovic M, Fowler B O and Tung M S 2004 *Res Natl Inst Stand Technol.* **109** 553