Effect of pH and ammonia concentration on the shape and size of fluorapatite nanoparticles obtained by chemical reaction

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ABSTRACT

Nanoparticles of fluorapatite (FAp Nps) were prepared by one step chemical reaction in aqueous solutions. The pH of the system was kept constant at 6 and 8. The FAp Nps were characterized with Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (SEM-EDAX), X-ray Diffraction (XRD), Transmission Electron Microscopy (TEM) and Fourier transform infrared spectroscopy (FTIR). The Ca/P calculated from EDAX analysis was 1.69. The results of XRD analysis and FTIR showed the presence of FAp phases. The DRX results confirm the formation of FAp with a hexagonal structure. TEM images show semispherical and elongated nanoparticles with mean diameter of 3.78 nm, 8.63 nm and 10.46 nm, when the pH and ammonia concentrations is varied. Ammonia concentration and pH influence the morphology and size of the FAp Nps.

1. Introduction

Bioceramics are an important subset of biomaterials which became an accepted group of materials for medical applications, mainly for implants in orthopedics, maxillo-facial surgery and dental implants [1]. Among them, hydroxyapatite, HAp, \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \) seems to be the most appropriate ceramic material for tooth applications owing to its excellent biocompatibility and bioactivity [2]. However, the incorporation of fluorine into HAp induces better biological response. It is possible to obtain nanometric fluorapatite \( \text{Ca}_5(\text{PO}_4)_3\text{F} \) with different morphologies and sizes using different process [1,3–6].

The size of nanoparticles has a direct connection with their application. In biomedicine, the most effective parameter is size, and, sometimes the shape or stabilizing agent. In this article we present the effect of pH on the size and shape of fluorapatite nanoparticles (FAp Nps) prepared by a modified method that involves a displacement reaction between hydroxyapatite and NaF [6].

2. Material and methods

2.1. Chemicals

Sodium fluoride, NaF (≥98%), hydrochloric acid, HCl (98%) and ammonium hydroxide, \( \text{NH}_4\text{OH} \) (90%) were purchased from Sigma-Aldrich Peru (Lima, Peru). All chemicals were of analytical purity and used as received without further purification. Milli-Q water (18 MΩ cm), obtained from a purification system (Millipore, Darmstadt, Germany), was used.

2.2. Preparation of fluorapatite nanoparticles (FAp Nps)

FAp Nps were synthesized by modified displacement chemical reaction method proposed by Taheri et al. [7,8]. HAp Nps previously prepared were suspended in Milli-Q water. The pH of the solution was adjusted to 2, adding a few mL of HCl (1.0 M). \( \text{NH}_4\text{OH} \) solution (0.5 M to 6.0 M) was added to increase the pH to the appropriate value (6 and 8). Next, a NaF solution (0.4 M) was dropped into the solution (rate of 3 ml/min; 60 °C). The process was carried out for 2 h with stirring. After the reaction, the colloids containing the nanoparticles were centrifuged at 10,000 rpm for 5 min using a micro centrifuge (Eppendorf 5804). To remove excess of different ions, the colloids were washed at least three times with Milli-Q water. A dried nanopowder of fluorapatite was obtained by freeze-drying. To characterize the nanoparticles, a certain amount of FAp Nps was suspended in deionized water using an ultrasonic cleaning container (Fisher Bioblock Scientific).

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2.3. Characterization techniques

2.3.1. Elemental composition analysis

Chemical compositions of the samples were analyzed by SEM using a FEI Quanta 650 in the secondary electron mode at an accelerating voltage of 3–10 kV (FEI Europe BV; Eindhoven, The Netherlands). EDAX analysis was performed with an Ametek EDAX TEAM system coupled to the SEM microscope.

2.3.2. Structural characterization

- XRD analysis

XRD analysis have been carried out using a BRUKER D8 ADVANCE (Karlsruhe, Germany) X-ray diffractometer equipped with a copper anticathode (λ • Cu Kα = 1.54056 Å). Data were obtained over the range 20 = 5°–70° using a step size of 0.03° and counting time of 10 s per step. Reference intensity ratios methodology from XRD was used in order to assign the phases observed in the X-ray pattern. The crystallite size measurements were also carried out using the Scherrer equation, D = kλ/βcosθ, where D is the crystallite size, k is a constant (=0.9 assuming that the particles are spherical), λ is the wavelength of the X-ray radiation, β is the line width at half maximum intensity of the peak and θ is the angle of diffraction.

- FTIR analysis

The functional groups of the FAp Nps were identified using a FTIR spectrometer (Perkin Elmer Frontier, Miami, USA). Approximately 1.0 % sample is well mixed into 200 to 250 mg of KBr powder and then finely pulverized and put into a pellet-forming die. The FTIR spectrum was scanned from 4000 to 400 cm⁻¹, with spectral resolution of 0.4 cm⁻¹.

2.3.3. Morphology analysis

The size and morphology of FAp Nps were performed by TEM using a Philips CM20-Ultra Twin microscope operating at 200 kV (Philips; Eindhoven, The Netherlands). Histograms of size distribution were calculated from the TEM images by measuring the diameters of at least 50 particles using the Imagej software. Using ultrasound equipment, a little portion of nanopowder was dispersed in ethanol (98%) during 1 min. Then a few drops of solution containing FAp Nps were placed on carbon-coated TEM gold grids.

3. Results and discussion

3.1. Effect of the pH

Fig. 1 shows the FTIR spectra of FAp-06 and FAp-08. Both samples exhibit the characteristic bands originating from PO₄³⁻, which had four distinct asymmetrical stretching vibration modes, appeared at 1097 cm⁻¹, 1036 cm⁻¹, 965 cm⁻¹, 605 cm⁻¹, 567 cm⁻¹ and 472 cm⁻¹. The week band at 1639 cm⁻¹ and the broad band around 3432 cm⁻¹ were
assigned to $\text{H}_2\text{O}$. A sharp and broad peak between 900 cm$^{-1}$ and 1100 cm$^{-1}$ is related to the PO$_4^{3-}$ group [1,9,10].

The EDAX spectra of FAp-06 (pH = 6) and FAp-08 (pH = 8) confirm the presence of elemental calcium; phosphorus, flour and oxygen signals (Fig. 2A and Fig. 2B). Furthermore, based on the elemental analysis of samples FAp-06 (Ca = 19.48, P = 11.47%) and FAp-08 (Ca = 23.60%, P = 14.22%), the Ca/P ratio can be calculated to be 1.69 and 1.65 respectively, which are close to theoretical value of 1.67 for FAp [1].

Fig. 2D and Fig. 2E shows the XRD patterns of the FAp-06 and FAp-08 samples that correspond to hexagonal phase of Ca$_5$(PO$_4$)$_3$F with a hexagonal structure (JCPDS card no. 015–0876). No other diffraction peaks arising from HAp or Fluor-hydroxyapatite (FHAp) appears in the XRD patterns.

TEM micrographs of all samples synthesized is presented in Fig. 3. The nanoparticles show narrow sizes between 2 and 22 nm. Sample FAp-06 (Fig. 3A) and FAp-08 (Fig. 3B) show semispherical and elongated forms respectively [1]. The histograms of each picture show nanoparticles ranging from 4 nm to 22 nm and 7 nm to 14 nm with the estimated mean diameter of 8.63 nm $\pm$ 2.66 nm and 10.46 nm $\pm$ 1.97 nm respectively. The crystallite size calculated using the approximate Scherrer’s formula was 30.64 nm $\pm$ 0.44 nm (FAp-06) and 11.62 nm $\pm$ 0.32 nm (FAp-08).

3.2. Effect of the ammonia concentration

Two samples at pH 8 with different concentration of NH$_4$OH, 6.0 M (FAp-08) and 0.5 M (FAp-08b) were obtained. The EDAX spectrum of the sample confirms the presence of elemental calcium; phosphorus, flour and oxygen signals (Fig. 2C). The Ca/P ratio calculated from EDAX analysis was 1.60, which is close to theoretical value of 1.67 for FAp [1].

The corresponding electron diffraction patterns (Fig. 2H) indicate that the nanoparticles have a typical FAp crystalline structure [1]. This result is consistent with the XRD patterns of the FAp-08b (Fig. 2F). TEM image of FAp-08b sample shows agglomerated nanoparticles (Fig. 3C), some of them are semi-spherical and very few are almost elongated. The nanoparticles showed sizes between 2 and 6 nm with a mean diameter of 3.78 nm $\pm$ 1.03 nm. In addition, the crystallite size was found to be 8.87 nm $\pm$ 0.29 nm. Then when a dilute ammonia solution is used, the size of the FAp crystal decreases. In this case, the nucleation of FAp Nps process is favored until the critical radius is reached, therefore the mean size of the nanoparticles decreases.
4. Conclusions

FAp Nps with narrow size distribution from 2 to 22 nm and hexagonal structure have been successfully synthesized in ammonia media. Depending on the pH adjustment, semispherical or elongated nanoparticles similar to nanowires were obtained. The influence of the ammonia concentration on the morphology and size of the nanoparticles was evidenced at pH = 8, where nanoparticles with 3.78 nm and 10.46 nm were synthesized.

Table 1
Mean diameter and crystallite size of each sample obtained.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean diameter by TEM (nm)</th>
<th>Distribution (nm)</th>
<th>Crystallite size by Scherrer (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA-06</td>
<td>8.63 ± 2.66</td>
<td>4-22</td>
<td>30.64 ± 0.44</td>
</tr>
<tr>
<td>FA-08</td>
<td>10.46 ± 1.97</td>
<td>7-14</td>
<td>11.62 ± 0.32</td>
</tr>
<tr>
<td>FA-08b</td>
<td>3.78 ± 1.03</td>
<td>2-6</td>
<td>8.87 ± 0.29</td>
</tr>
</tbody>
</table>

Fig. 3. TEM images and particle size distribution of FAp Nps prepared at different NH₄OH concentration a) 6.0 M (pH = 6); b) 6.0 M (pH = 8) and c) 0.5 M (pH = 8).

CRediT authorship contribution statement

Maribel Guzman: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing. Jean Dille: Supervision, Resources, Methodology, Writing - review & editing. Stephane Godet: Resources, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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