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Online Publication Date: 01 October 2009

To cite this Article Stojanović, Zoran, Veselinović, Ljiljana, Marković, Smilja, Ignjatović, Nenad and Uskoković, Dragan(2009)'Hydrothermal Synthesis of Nanosized Pure and Cobalt-Exchanged Hydroxyapatite',Materials and Manufacturing Processes,24:10,1096 — 1103

To link to this Article: DOI: 10.1080/10426910903032113
URL: http://dx.doi.org/10.1080/10426910903032113

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Hydrothermal Synthesis of Nanosized Pure and Cobalt-Exchanged Hydroxyapatite

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Pure and cobalt-exchanged hydroxyapatite (HAp and CoHAp) powders were synthesized by hydrothermal method. X-ray diffraction (XRD), Raman spectroscopy, particle size analysis, inductively coupled plasma (ICP) emission spectroscopy, and scanning electron microscopy (SEM) were used to study the microstructural and unit cell parameters, average particle size, particle size distribution, chemical composition, and morphology of the synthesized powders. XRD and Raman spectroscopy confirmed that the samples were free from impurities and other phases of calcium phosphates. It has been found that the increase in the cobalt amount in the crystal structure of HAp reduces unit cell parameters, as well as average crystallite size (from XRD measurements). All of the powders were nano-sized with narrow particle distribution (from particle size analyses). SEM investigations indicated that nano-sized particles were organized in soft micro-sized agglomerates, whose sizes increased with the increase in the content of Co in HAp crystal structure.

Keywords Hydrothermal method; Hydroxyapatite; Particle size distribution; Raman spectroscopy; XRD.

1. INTRODUCTION

Nowadays, hydrothermal processing of advanced materials is one of the most interesting techniques in the field of nanostructured materials and is also very attractive for large scale production due to its adaptivity, simplicity, low energy consumption, and good environmental aspects. In the past two centuries, hydrothermal technique was mainly applied to processes of single crystal growth and ore leaching, whereas nowadays hydrothermal technology has become one of the most important tools for advanced materials processing. This technique offers many advantages for the processing of nanostructured materials for a wide variety of applications in industry, such as ceramics, biomedical, catalytic, optoelectronics, etc. [1–5].

During the past several decades, there has been an ever increasing interest in materials with apatite structure. General chemical formula of stoichiometric mineral apatites is $M_{10}(XO_{4})_6Y_2$, where M is a divalent cation, X could be P, V, or Si, while Y is some halogen element or group like OH−. Phosphates with molecular formula $M_{10}(PO_4)_6OH_2$ are used for various medical applications as bone implants, in industry as absorbers for heavy or radioactive elements ions, ion exchangers in chromatographic columns, catalysts, or electrolytes for ceramic fuel cells [6, 7].

Natural calcium hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) is the main inorganic component of bones and teeth of vertebrates. For medical purposes, synthetic HAp material, with excellent biocompatibility, was produced for the use in bone and teeth reconstruction. Adequate biodegradation rate and nontoxicity make this material suitable for drug delivery systems providing controlled release [8, 9]. The structure of HAp allows the substitution of $Ca^{2+}$, $PO_4^{3−}$, and/or $OH^{−}$ with other ions. Calcium ions in an apatite structure can be substituted with various metal ions like K, Na, Mg, Sr, Ba, Pb, Mn, Co, Fe, etc. [10, 11]. Generally accepted affinity of divalent cations for substitution is $Cu^{2+} > Pb^{2+} > Zn^{2+} > Cd^{2+} > Co^{2+}$ [6]. It enables us to tailor HAp with modified properties, such as lattice parameters, crystallinity, morphology, and additional desired properties for a specific application. Regarding the material’s biocompatibility, there is also a possibility of preparing magnetic nanoparticles of HAp by substituting $Ca^{2+}$ with some magnetic ions such as Fe, Co, etc., for the application in magnetic resonance imaging, hyperthermia treatment, cell separation, and drug delivery [12]. Cobalt-substituted HAp shows catalytic activity for oxidative dehydrogenation of alcohols as well as for the process of styrene epoxidation [13]. Catalytic activity of CoHAp depends on the amount of cobalt substituted in the HAp lattice; moreover, its catalytic activity could be improved by loading with Ru [6, 14].

In this article, a series of pure calcium hydroxyapatite (HAp) and cobalt-substituted calcium hydroxyapatite (CoHAp; $Ca_{10−x}Co_x(PO_4)_6(OH)_2$, $x = 0.5, 1,$ and 2) powders were prepared by simple hydrothermal treatment of precipitates. X-ray diffraction (XRD), Raman spectroscopy, particle size analysis, inductively coupled plasma (ICP) emission spectroscopy, and scanning electron microscopy (SEM) were used to study the qualitative content, unit cell parameters, degree of crystallinity, average particle size, particle size distribution, chemical composition, and morphology of the synthesized powders.
2. Experimental

Pure HAp and cobalt-substituted hydroxyapatite (CoHAp) powders were prepared by hydrothermal treatment of precipitates. In the first set of experiments, pure HAp powders were prepared under different temperatures in order to investigate crystallization kinetics. HAp precipitate was prepared by adding filtered supersaturated alkaline solution of Ca(NO₃)₂ drop-wise in a mixture of H₃PO₄ and ammonia water at 50°C, under constant stirring of 700 RPM. About 11 of the as-obtained suspension was hydrothermally treated in 2 l Parr stainless steel stirred reactor under nonequilibrium conditions up to 250°C at a constant heating rate of 2°C/min, under constant stirring of 400 RPM. The samples were taken from the autoclave at 100, 150, 200, and 250°C. Raman spectroscopy analysis of the powders has shown that the increase in temperature over 200°C causes a partial conversion of HAp to β-tricalcium phosphate (β-TCP). The presence of β-TCP was not observed due to XRD analysis, probably because its amount was smaller than 2%. Therefore, hydrothermal treatment at a temperature of 200°C was chosen for the further synthesis of CoHAp powders. In the set of experiments the amount of reagents was adjusted to obtain powders in which cobalt would substitute calcium in 5, 10, and 20 at%, respectively. Supersaturated alkaline solution of Ca(NO₃)₂ and aqueous solution of Co(NO₃)₂ were simultaneously added drop-wise in solution mixture of H₃PO₄ and ammonia water under the same conditions as in the previous case. The ratio of (Ca + Co)/P was fixed to 1.67 in the starting solutions. Each prepared suspension (about 11 in volume) was then treated in the autoclave on 200°C for 8h under the pressure of 2 MPa, under constant stirring of 400 RPM. After the treatment, the autoclave was quenched down to room temperature. The precipitate was washed with distilled water to remove NH₄ ions and potentially adsorbed Co²⁺ ions, and then dried on 90°C in air for 24h.

The qualitative analysis of the synthesized powders was carried out by XRD using a Philips PW 1050 automated diffractometer with Cu tube (λCaKα = 1.54178 Å). The X-ray generator operated at 40 kV and 20 mA. The patterns were collected in the 2θ range 10–70° with a scanning step size of 0.05°, at room temperature. The mean crystallite size (D) of the powders were calculated from the half height width (β_m) of the XRD reflection of (002) plane (at 2θ = 25.8°), using the Scherrer’s Eq. (1),

\[ D = \frac{K\lambda}{\beta_m \cos \theta} \]  

(1)

where λ is the wavelength of X-ray radiation; K is the shape coefficient and is approximately equal to one; θ is the diffraction angle (°) [15, 16]. The unite cell parameters (a and c) were determined from XRD data using the least-squares method by LSUCRI computing program [17].

The Raman spectra were taken in the backscattering geometry using a μ-Raman system with a Jobin Yvon T64000 triple monochromator, equipped with a liquid nitrogen cooled charge-coupled-device (CCD) detector. The 514.5 nm line of an Ar-ion laser was used as an excitation source. The measurements were performed at laser power of 80 mW. The Raman spectra were recorded in the frequency interval 100–1500 cm⁻¹, with a resolution of 4 cm⁻¹. Each spectrum was a result of additional 10 scans. The amount of calcium and cobalt in the HAp and CoHAp powders were determined by inductively coupled plasma (ICP) emission spectroscopy analysis (ICAP Thermo Scientific 6300 spectrometer). The average particle size, particle size distribution, and the nature of agglomerates were determined by particle size analyzer (PSA). The used instrument was Mastersizer 2000 (Malvern Instruments Ltd., UK) particle size analyzer based on laser diffraction, which covers the particle size range of 0.02–200 μm. For the PSA measurements, the powders were dispersed in distilled water, in ultrasonic bath (low-intensity ultrasound, at a frequency of 40 kHz and power of 50 W), for 3 min. The morphology of the prepared HAp and CoHAp powders, previously gold-coated, was studied by scanning electron microscopy (JEOL SEM, JSM 6390), operating at 20 kV.

3. Results and discussion

3.1. XRD Analysis

XRD patterns of HAp powders synthesized by hydrothermal method at several different temperatures are presented in Fig. 1. XRD measurements confirmed pure apatite phase in all samples (according to JCPSD 09-0432) [18]. The wide peaks with weak intensity indicate low crystallinity and small crystallite size [19, 20]. With the increase in the temperature of hydrothermal synthesis from 100 up to 250°C diffraction maximums of the investigated powders become narrower, with higher intensity, which indicates better crystallinity and increase in crystallite size. XRD patterns of the investigated CoHAp powders, with different cobalt content, synthesized by hydrothermal method at 200°C are presented in Fig. 2. XRD measurements show a pure apatite phase in all samples. Diffraction peaks become wider and less intense as a consequence of the decrease in crystallite size resulting from the increase of the Co content in the obtained samples. Additionally, with the increase of the cobalt content in HAp samples, diffraction maximums are shifted to larger angles, i.e., unit cell parameters decrease [21]. These phenomena are related to the incorporation of Co²⁺ with smaller ionic radius (0.070 nm) than Ca²⁺ (0.099 nm) into the cationic crystallographic position in HAp structure. Lattice parameters (a and c), the volume of the unit cells, and values of mean crystallite size are listed in Table 1.

The number of Ca and Co atoms in the unit cell of CoHAp powders was calculated according to ICP emission spectroscopy analysis. Calculated stoichiometric formulas are listed in Table 1.

3.2. Raman Spectroscopy

One more technique is used to confirm the phase identification of CoHAp powders. The Raman spectroscopy is an excellent complementary (to XRD) characterization tool to identify the phase evolution as a function of cation substitution in HAp. This method could provide information on the short- and intermediate-range ordering in the solids [22]. Raman spectroscopy is an established analytical technique for biomedical application because: (a) the need
Figure 1.—XRD patterns of HAp powders synthesized by hydrothermal method at: (a) 100; (b) 150; (c) 200; and (d) 250°C.

Figure 2.—XRD patterns of powders synthesized by hydrothermal method at 200°C: (a) HAp; (b) Co5HAp; (c) Co10HAp; and (d) Co20HAp.

Figure 3 presents Raman spectra of pure HAp synthesized by hydrothermal method at different temperatures. Only the region with the phosphate (PO$_4^{3-}$) vibrational modes is studied, because of their significance for structural investigations. The spectra are almost identical for all investigated HAp powders. In all the cases, the following

Table 1.—Characteristics of CoHAp powders synthesized by hydrothermal method at 200°C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Stoichiometry*</th>
<th>Lattice parameters [Å]</th>
<th>Cell volume [Å$^3$]</th>
<th>Mean crystallite size [nm]</th>
<th>$d_{10}$</th>
<th>$d_{50}$</th>
<th>$d_{90}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAp</td>
<td>Ca$_{10}$(PO$_4$)$_6$(OH)$_2$</td>
<td>$a = 9.4296(3)$, $c = 6.8837(3)$</td>
<td>529.34(5)</td>
<td>47.71(7)</td>
<td>62</td>
<td>94</td>
<td>163</td>
</tr>
<tr>
<td>Co5HAp</td>
<td>Ca$<em>{9.57}$Co$</em>{0.43}$(PO$_4$)$_6$(OH)$_2$</td>
<td>$a = 9.4248(3)$, $c = 6.8787(2)$</td>
<td>527.36(9)</td>
<td>24.36(5)</td>
<td>34</td>
<td>63</td>
<td>117</td>
</tr>
<tr>
<td>Co10HAp</td>
<td>Ca$<em>{9.09}$Co$</em>{0.91}$(PO$_4$)$_6$(OH)$_2$</td>
<td>$a = 9.4238(5)$, $c = 6.8689(3)$</td>
<td>527.54(2)</td>
<td>15.26(14)</td>
<td>35</td>
<td>64</td>
<td>124</td>
</tr>
<tr>
<td>Co20HAp</td>
<td>Ca$<em>{8.85}$Co$</em>{1.15}$(PO$_4$)$_6$(OH)$_2$</td>
<td>$a = 9.4194(6)$, $c = 6.8514(3)$</td>
<td>526.67(2)</td>
<td>14.23(16)</td>
<td>31</td>
<td>71</td>
<td>136</td>
</tr>
</tbody>
</table>

*According to ICP results.
bands are assigned: 430, 448 (O-P-O doubly degenerated bending mode, \( \nu_2 \)); 582, 594, 609 (O-P-O triply degenerated bending mode, \( \nu_4 \)); 960 (P-O nondegenerated symmetric stretching mode, \( \nu_1 \); and 1031, 1048, and 1080 cm\(^{-1}\) (P-O triply degenerated antisymmetric stretching mode, \( \nu_3 \) [24, 25]), the only differences being those in the width and relative intensity of the bands. In particular, it is noticed that with the increase in the temperature of the hydrothermal synthesis (from 100 up to 250°C), bands become narrower and the intensity ratios \( \nu_1/\nu_2, \nu_3, \nu_4 \) increase, indicating structural ordering. Moreover, in the Raman spectra of HAp synthesized at 250°C the most intensive band at 960 cm\(^{-1}\) is broadened with the enhancement of the low-frequency side, resulting in a double-band structure [small shoulder at 950 cm\(^{-1}\) is marked by arrow in Fig. 3(d)]. The existence of a double-band structure can be correlated to the presence of another calcium phosphate phase mixed with hydroxyapatite. According to the literature data, the double-band structure corresponds to the superposition of pure HAp and \( \beta \)-tricalcium phosphate (\( \beta \)-TCP) phases [26, 27]. Different crystallographic structures of \( \beta \)-TCP and HAp [26] resulted in a distortion of the PO\(_4\) tetrahedra in the case of low symmetry \( \beta \)-TCP structure. Thus, the broadening of the \( \nu_1 \) vibrational modes in the Raman spectra of HAp indicates a partial conversion of HAp to \( \beta \)-TCP during the hydrothermal treatment at 250°C. That was the reason why we chose the temperature of 200°C as an optimal condition for further synthesis of pure HAp and CoHAp, without second phase(s).

Figure 4 presents Raman spectra of CoHAp synthesized by hydrothermal method at 200°C. As in the case of pure HAp, there are four vibrational modes for PO\(_4^{3-}\) tetrahedra. Comparing the spectra of CoHAp-s with that of pure HAp it can be seen that the Raman bands of CoHAp-s coincide with those of HAp. The overall spectrum does not change in the sense of the band number and the position. A considerable line broadening and decrease of intensity ratio \( \nu_1/\nu_2, \nu_3, \nu_4 \), from HAp to Co20HAp, are related to the incorporation of “foreign” Co\(^{2+}\) into Ca\(^{2+}\) crystallographic positions in HAp structure. These facts, the line broadening and the decrease of intensity ratio, confirm the disordering of the crystal structure of hydroxypatite (i.e., disordering on long-range scale).
3.3. Particle Size and Morphology

While XRD was used for the estimation of crystallite size, the average particle size and particle size distribution were studied by a laser particle size analyzer. Besides, the particle size analyzer was used for the investigation of the nature and extent of agglomerates in hydrothermally synthesized powders. The success of this technique depends on the dispersion of the powder [28, 29]. Since the synthesized powders are agglomerates of primary nanoparticles, it is difficult to disperse them as individual particles, and hence, in spite of true particle size, the results indicate size (and distribution) of agglomerates. In order to investigate the extent and nature of agglomeration, the particle size distribution of the powders was measured before and after ultrasonic treatment. Before ultrasonic treatment, all the powders consisted of agglomerates with sizes between 1 and 4 μm. After 3 min of ultrasound de-agglomeration, the sizes of agglomerates were reduced from micro to nano level. Thus, it can be inferred that these agglomerates are basically soft in nature; attractive forces between particles are weak van der Waals forces that can be easily broken down by low-intensity ultrasound.

The following results were obtained. The particle size distribution of pure HAp powder, prepared by hydrothermal method at 200°C, was very narrow (span = 1.075), with average particle size of 94 nm, while more than 90% of particles were smaller than 163 nm. The incorporation of 4.3 at% of Co²⁺ into Ca²⁺ crystal position provoked slight reduction of average particle size from 94 to 63 nm, while particle size distribution was broadened to (span =) 1.338. Further increase in the cobalt content, up to 9.5 at% in the crystal position of Ca²⁺ changed neither average particle size nor particle size distribution. The average particle size was 64 nm, whereas the span was 1.387. Furthermore, the incorporation of 12 at% of Co²⁺ into the crystal structure of HAp yielded powder with particles of \( d_{50} = 71 \text{ nm} \) and narrow particle size distribution with span 1.356. According to the particle size analysis and particle size distribution of CoHAp powders (distribution based on number), the increase in Co²⁺ in the HAp crystal structure does not influence average particle size, nor the width of particle size distribution. The observed average particle dimensions (\( d_{50} \)) are somewhat larger than the corresponding average crystallite sizes estimated from the XRD analysis. The discrepancy is due to the presence of aggregates in the particle-size analyzed particles, consisting of several crystallites. The particle size values at 10%, 50%, and 90% of cumulative weight are shown in Table 1. Particle size distribution of hydrothermally synthesized CoHAp powders after ultrasonication is presented in Fig. 5.

Furthermore, SEM analyses of CoHAp powders show structures consisted of nonuniform agglomerates with various average particle sizes (Fig. 6). Evidently, the introduction of cobalt cations into the structure of hydroxyapatite changed powder morphology. Pure hydroxyapatite powder consisted of particles of 94 nm in average size (due to PSA), scatterly organized in soft agglomerates with sizes up to 2 μm [Fig. 6(a)]. Powder Co5HAp contained particles of 63 nm in average size (due to PSA); those particles were arranged in soft loose
Figure 6.—The morphology of powders synthesized by hydrothermal method at 200°C: (a)–(a′) HA; (b)–(b′) Co5HA; (c)–(c′) Co10HA; and (d)–(d′) Co20HA. Left-hand side: (a)–(d) 5000 × magnification and right-hand side: (a′)–(d′) 20000 × magnification.
agglomerates with sizes from several hundreds nm up to 4 μm [Fig. 6(b)]. Further increase of the cobalt content in the crystal structure of HAp increased the agglomeration. Co10HAp powder consisted of particles of 64 nm in average size, arranged in agglomerates with sizes up to 6 μm [Fig. 6(c)]. Finally, Co20HAp contained particles with sizes between 40 and 140 nm, consolidated in large condensed agglomerates with sizes rising up to 14 μm [Fig. 6(d)].

Generally, according to XRD measurements, PSA, and SEM images, it can be noticed that the increase in Co2+ amount in the crystal structure of HAp induces a reduction of crystallite sizes. Furthermore, the increasing amount of Co2+ in HAp increases the tendency of crystallites to stick together, leading to the formation of large particles (aggregates). Finally, those large particles were consolidated in compact agglomerates. The size of agglomerates increased with the increase in the Co content, because cobalt promotes the agglomeration of nanoparticles in solution during synthesis [30]. However, even though the morphology of the agglomerates was changed due to the increase in the cobalt content in the crystal structure of HAp, their nature remained soft, as we previously showed.

4. Conclusion

Hydrothermal treatment is an effective method for the preparation of nanocrystalline cobalt-substituted hydroxyapatite (CoHAp). Data obtained by XRD and Raman spectroscopy show a change in the structure of HAp, which confirms that Ca2+ ions are substituted with Co2+ ions, without change in crystal symmetry. It was found that hydrothermally prepared powders were consisted of agglomerates that were broken down to the aggregate by low-intensity ultrasound. The aggregates (94–63 nm in average, from PSA) consisted of several crystallites with calculated sizes of 41–14 nm (from XRD). Crystallite size depends on the cobalt content in CoHAp. Precisely, the increase in Co2+ amount in the HAp structure provokes a slight reduction of both crystallite size and unit cell parameters. Furthermore, the incorporation of Co2+ into HAp structure reduces average particle size (i.e., aggregate size). SEM investigations revealed changes in morphology. Contrary to the average crystallite and particle size, on the large scale an increased amount of cobalt promotes the agglomeration of nanoparticles.

Acknowledgments

The Ministry of Science and Technological Development of the Republic of Serbia provided financial support under Grant No. 142006. The authors would like to thank M.Sc. B. Hadžić for Raman spectroscopy measurements, Prof. M. Kuzmanović for ICP measurements, and Prof. V. Pavlović for SEM images.

References

18. JCPDS File No. 09-0432 (HAp), International Center for Diffraction Data.


