

# Synthesis and characterization of nanocrystalline hydroxyapatite obtained by the wet chemical technique

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Hydroxyapatite was synthesized by the wet chemical technique. Diammonium hydrogen phosphate and calcium nitrate 4-hydrate were used as starting materials and sodium hydroxide solution was used as the agent for pH adjustment. The powder sample was characterized by the commonly used bulk techniques of scanning electron microscopy (SEM), transmission electron microscopy, Fourier transform infra-red spectroscopy, differential thermal analysis, thermal gravimetric analysis, X-ray diffraction, atomic absorption spectroscopy and EDTA titration analyses. With respect to the results achieved from the above analyses, it was found that nanocrystalline hydroxyapatite can successfully be produced through the wet precipitation method. The bulk Ca/P molar ratio of synthesized hydroxyapatite was determined as 1.71, which was higher than the stoichiometric ratio (1.667) that is expected for a pure HA phase. SEM investigations revealed that, there is a distribution of small particles and large agglomerates consisting of fine particles cold welded together. Lastly, the TEM technique demonstrated that the crystallites of the prepared powder were nanosized and had a needle-like morphology. The aspect ratio of the crystallites was about 8.

Keywords: *hydroxyapatite; precipitation; nanocrystalline material; synthesis*

## 1. Introduction

In recent years, significant effort has been devoted to developing inorganic nanocrystals because of their potential applications in biology, electronics, optics, transport and information technology. Although there have been some investigations into ways of fabricating such nanocrystals, ways now need to be found of controlling their size, shape and crystallinity. This requires the identification of the various parameters influencing these features [1].

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Synthetic ceramic materials based on calcium phosphates (CaP), particularly those in the composition of tricalcium phosphate (TCP,  $\text{Ca}_3(\text{PO}_4)_2$ ) and hydroxyapatite (HA,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ), have been extensively studied and clinically used. These ceramics have been the focus of over three decades of biomaterials research in orthopedics and dentistry [2–7]. They are considered to be suitable biomaterials due to their biological affinity and activity to surrounding host tissues, when implanted [5]. Furthermore, according to the literature, calcium phosphates are widely used in medicine and oral biology, due to the apatite-like structure of enamel, dentin and bones, usually called “hard tissues”. To date, in spite of the availability of several sophisticated characterization techniques for the investigation of tooth and bone tissues, their exact composition, phase distribution and structure remain unresolved [3]. The aim of the preparation of synthetic CaP is to understand the properties and physicochemical behaviour of biological mineral phases found in human hard tissues because they share many similarities [4]. In addition, these materials are also important in the study of biomineralization, since they are precursors and are the major components of bone and teeth [8]. In order to gain insights into the complex structure found in biological mineral phases, it would be necessary to establish a well-defined system for the characterization of the synthetic CaP, in which the composition, crystallinity and nanostructure would have to be properly addressed. These properties play a major role in the bioactivity of CaP based materials in terms of enhanced contact areas and degradation [5]. Detailed characterization indicates that an apatite layer is usually formed on the ceramic surface when implanted. This layer consists of a carbonate-ion-containing apatite, named “bone-like” apatite, forming a bond with the human bone [2]. These ceramic materials can also be used as coating on implants to improve the biocompatibility [1, 8] and can be injected in bone with non-invasive surgical techniques [9]. Bioactivity of CaP materials is dependent on many factors during the synthesis procedure, such as precursor reagents, impurities, crystal size and morphology, concentration and mixture order of reagents, pH and temperature. Also, the bioactivity response of CaP materials will depend on the type of thermal treatment selected for drying and sintering. These conditions are controlled by parameters of synthesis and consequently for each application a specific route is selected [6, 10].

In this investigation we report on the synthesis of nanocrystalline hydroxyapatite by the wet precipitation technique.

## 2. Experimental

*Materials and methods.* The flowchart for the synthesis of the hydroxyapatite is shown in Fig. 1. 0.09 M diammonium hydrogen phosphate ( $(\text{NH}_4)_2\text{HPO}_4$ , 99%, Merck; No. 1207) and 0.15 M calcium nitrate 4-hydrate ( $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 98%, Merck PROLABO, No. 22 384 298) were prepared and the pH of the both solutions was brought to about 11, by adding 1 M sodium hydroxide (NaOH, 99%, Merck, No. 5 226 926). The phosphate solution was added drop-wise into the calcium ni-

trate solution, resulting in the precipitation of HA according to reactions (1) and /or (2) [11].

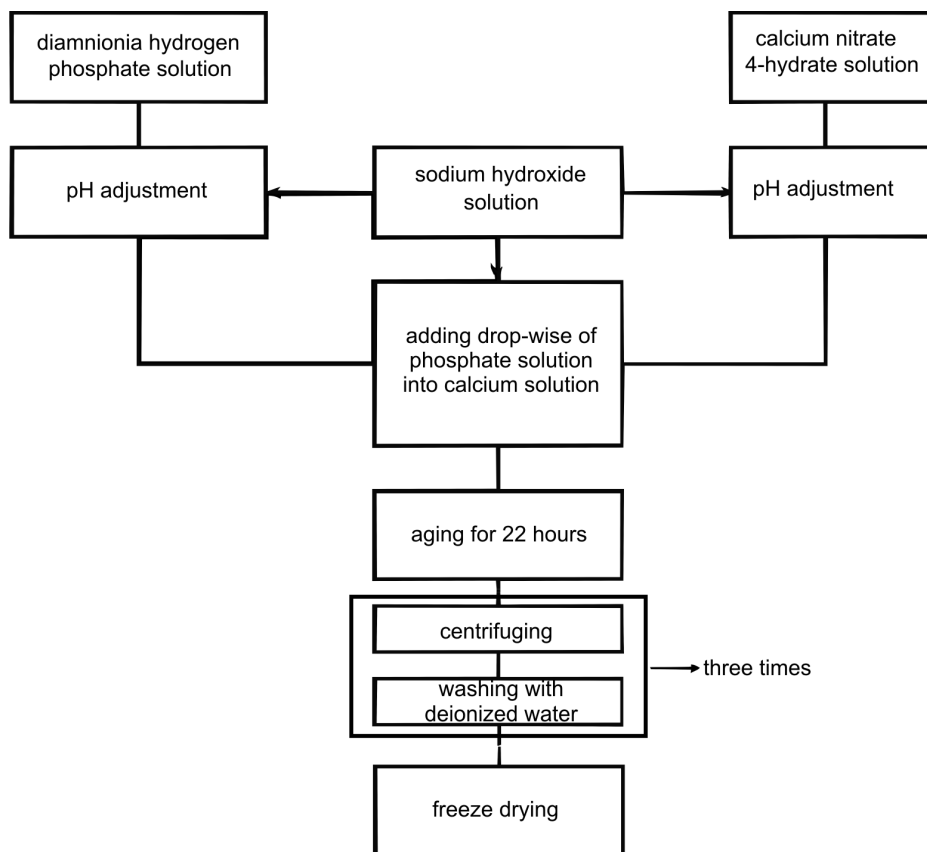
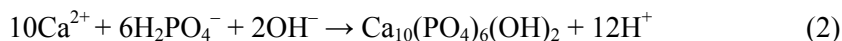
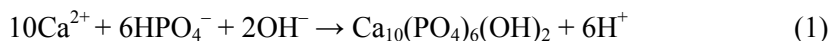


Fig. 1. Flowchart for the synthesis of the hydroxyapatite powder

The precipitate was aged for 22 h at room temperature. In the next step, the precipitated HA was centrifuged and then washed with de-ionized water. The processes of centrifuging and washing were carried out three times. The resulting powder was dried in a freeze-drier system (Alpha 1-2 LD, Germany) for 10 h. Lastly, dried powder was calcined in an electrical box furnace at 900 °C for 1 h at the rate of 5 °C/min in air.

*Powder characterization.* The powder sample was subjected to Fourier Transform infra-red analysis with a Bomem MB 100 spectrometer. For IR analysis, at first 1 mg

of the powder sample was carefully mixed with 300 mg of KBr (infrared grade) and palletized under vacuum. The pellets were analyzed in the range of 500 to 4000  $\text{cm}^{-1}$  at the scan speed of 23 scan/min at the resolution of 4  $\text{cm}^{-1}$ .

The resulting powder was analyzed by the X-ray diffraction (XRD) with a Siemens-Brucker D5000 diffractometer. This instrument works with voltage and current settings of 40 kV and 40 mA, respectively, and uses  $\text{CuK}_\alpha$  radiation (1.540600 Å). For qualitative analysis, XRD diagrams were recorded in the interval  $7^\circ \leq 2\theta \leq 60^\circ$  at the scan speed of 2  $^\circ\text{C}/\text{min}$ , the step size being 0.02 $^\circ$  and the time step being 1 s.

The thermal behaviour of hydroxyapatite was studied by simultaneous thermal analysis (STA). A thermoanalyzer (Polymer Laboratories PL-STA 1640) was started at room temperature and measurements were subsequently taken up to 1200  $^\circ\text{C}$ ; the heating rate of 10  $^\circ\text{C}/\text{min}$  was used to record the conventional DTA and TGA curves.

In order to calculate the Ca/P molar ratio of the precipitated powder, the contents of Ca and P were chemically analyzed by the quantitative chemical analysis via the EDTA titration technique and atomic absorption spectroscopy (AAS) with a Shimadzu UV-31005 instrument, respectively.

The powder sample was coated with a thin layer of Gold (Au) by sputtering (EMITECH K450X, England) and then the microstructure of the powder sample was observed under a scanning electron microscope (SEM, Tescan Vega 2XMU) that operated under the acceleration voltage of 15 kV.

Transmission electron microscopy (TEM, CM200-FEG-Philips) was used for characterizing the particles. For this purpose, particles were deposited onto Cu grids, which support a carbon film. The particles were deposited onto the support grids by deposition from a dilute suspension in acetone or ethanol. The particle shapes and sizes were characterized by diffraction (amplitude) contrast and, for the crystalline materials, by high resolution (phase contrast) imaging.

### 3. Results and discussion

#### 3.1. FTIR analysis

Figure 2 shows the FT-IR spectrum of HA powders. Characteristic bands (listed in Table 1) exhibited in the sample spectrum are as follows: Two bands were observed at 3555  $\text{cm}^{-1}$  and 622  $\text{cm}^{-1}$  and were due to the stretching mode of hydrogen-bonded  $\text{OH}^-$  ions and librational mode of hydrogen-bonded  $\text{OH}^-$  ions, respectively. The band at 1040  $\text{cm}^{-1}$  has been attributed to  $\nu_3 \text{PO}_4$ , the bands at 603  $\text{cm}^{-1}$  and 561  $\text{cm}^{-1}$  to  $\nu_4 \text{PO}_4$ . The FTIR analysis showed all typical absorption bands of hydroxyapatite. In addition, some carbonate content also was seen ( $\text{CO}_3^{2-}$  peak around 1600  $\text{cm}^{-1}$ ), which is an indication of the presence of carbonate apatite. This might originate from absorption of carbon dioxide from the atmosphere [12, 14]. Therefore according to this reasoning, it is obvious that the synthesized powder is certainly hydroxyapatite.

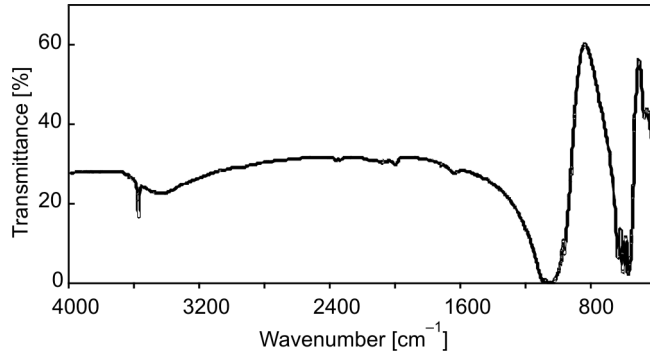


Fig. 2. FT-IR spectrum of the synthesized hydroxyapatite

Table 1. Infrared frequencies for the synthesized hydroxyapatite powder

Frequency [cm <sup>-1</sup> ]	Assignment
561	PO <sub>4</sub> bending $\nu_4$
622	OH structural
1040	PO <sub>4</sub> bending $\nu_3$
3555	OH structural

### 3.2. XRD analysis

The XRD analysis was performed using the X-ray diffractometer. The straight base line and sharp peaks of the diffractogram in Fig. 3 confirmed that the product was well crystallized. The XRD pattern indicated that hydroxyapatite was formed in this sample and traces of other calcium phosphate impurities were not detected by this technique. This can also be seen in this figure, secondary CaO phase was observed.

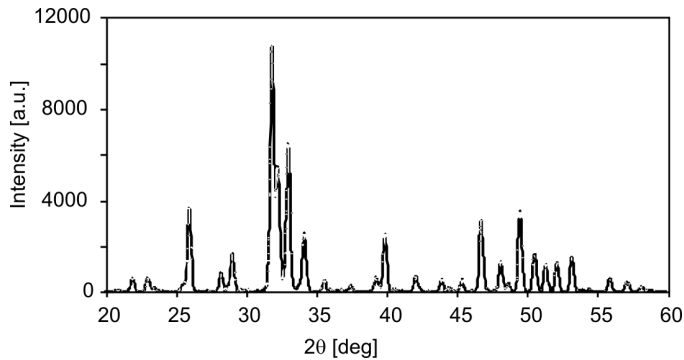


Fig. 3. XRD pattern of the synthesized hydroxyapatite

The intensity of the (200) CaO peaks on the XRD pattern of HA powder was used as a direct indicator of its purity as in the research of Afshar et al. [10]. The ratio of

the peak intensities in the XRD pattern of CaO/HA ( $I_{(200)} \text{ CaO}/I_{(002)} \text{ HA}$ ) was calculated. The amounts of ( $I_{(200)} \text{ CaO}/I_{(002)} \text{ HA}$ ) in the sample was about 0.08. The CaO presence, however, does not necessarily mean that HA has lower biocompatibility [15]. The control of parameters of the synthesis is the key to developing HA purity as well as to indentifying other phases in bioceramics [15].

### 3.3. Thermal analysis

Exemplary DTA and TGA curves for the hydroxyapatite powder are shown in Fig. 4. The first endothermic region ranges from 90 to 295 °C with a peak at about 250 °C, which corresponds to the dehydration of the precipitating complex and loss of physically adsorbed water molecules of the hydroxyapatite powder. The weight loss in this region is 16%. With increase in temperature from 295 to 1200 °C, no peak was observed, except that a weight loss of 6% is observed in the TGA curve in the temperature range which is assumed to be the result of gradual dehydroxylation of hydroxyapatite powder. This can be explained by the following reaction [13]:

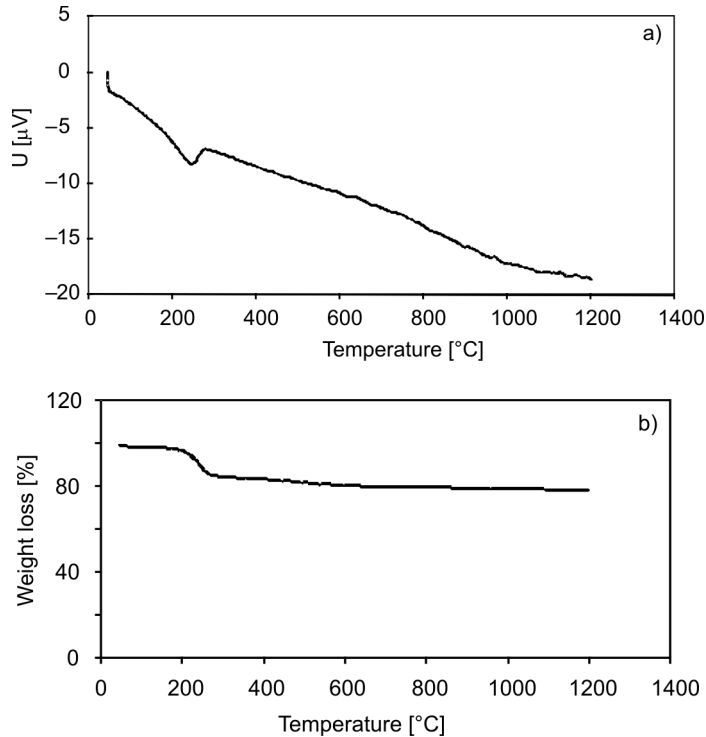


Fig. 4. DTA (a) and TGA (b) traces of the hydroxyapatite powder

### 3.4. Elemental analysis

The results of measurement for the elemental composition (Ca and P content) and Ca/P molar ratio are given in Table 2.

Table 2. Ca and P content in the synthesized hydroxyapatite powder and Ca/P molar ratio

Element	Measured content [wt%]	Ca/P molar ratio
Ca	38.63	1.71

The bulk Ca/P molar ratio was determined as 1.71. The measured Ca/P ratio for this synthesized powder was higher than the stoichiometric ratio (1.667) expected for a pure HA phase that can arise from the local presence of carbonate apatite in which the Ca/P molar ratio can be as high as 3.33 [14] or from the presence of impurities such as CaO. According to the XRD pattern that showed the existence of small amounts of CaO phase, the second case is much more probable.

### 3.5. SEM observations

The microstructure of the powder prepared by the present process was analyzed using SEM. SEM micrographs of synthesized powder under two magnifications are shown in Fig. 5.

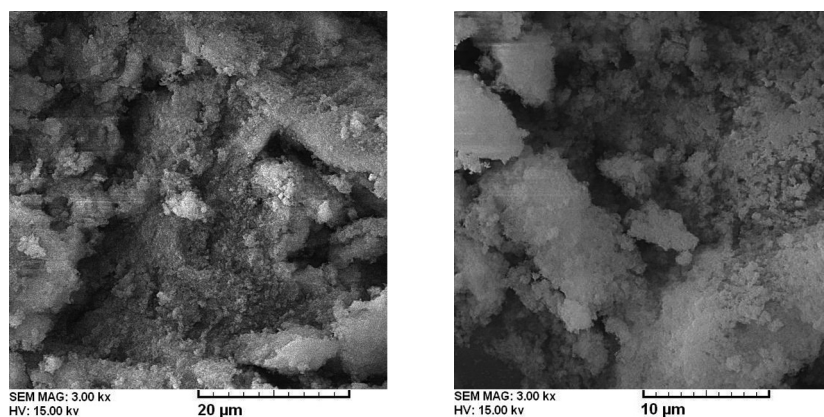


Fig. 5. SEM of the synthesized hydroxyapatite powder: magnification 3000 $\times$  (left) and 5000 $\times$  (right)

As can be seen from the morphologies of particles, there is a distribution of small particles and large agglomerates. These agglomerates consist of fine particles that are cold welded together.

### 3.6. TEM observations

TEM was used to examine the hydroxyapatite crystallites. TEM micrographs of the hydroxyapatite powder under two magnifications are shown in Fig. 6. The microstructure of the hydroxyapatite crystallites was observed to be almost like a needle, with the mean crystallite size of 60 nm. The aspect ratio of needle-like hydroxyapatite was measured to be about 8.

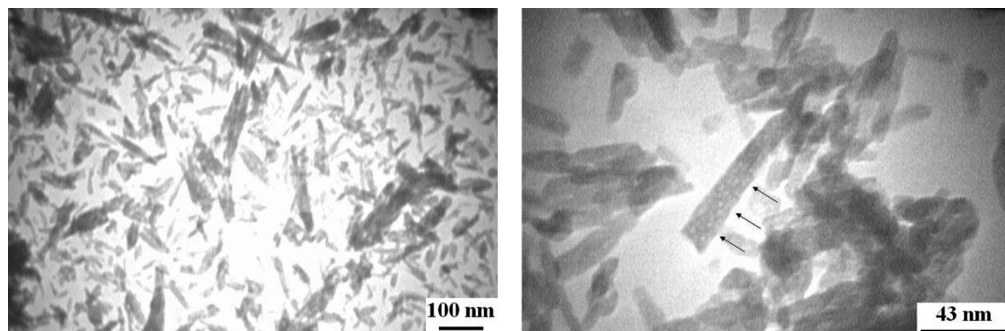


Fig. 6. TEM micrographs of the synthesized hydroxyapatite powder magnification 3500 $\times$  (left) and 100 000 $\times$  (right)

## 4. Conclusions

Hydroxyapatite was synthesized by the wet precipitation method. The hydroxyapatite powder was characterized at a macroscopic level by XRD, FTIR, DTA, TGA and chemical analysis (AAS and EDTA titration technique), while SEM and TEM provided detailed information at the microscopic (individual grain) level. The XRD analysis showed that the prepared hydroxyapatite sample was nearly a pure hydroxyapatite. Only low levels of specific impurities (such as CaO) were detected. FTIR investigations also showed all typical absorption characteristics of hydroxyapatite. The thermal behaviour of hydroxyapatite was studied by simultaneous thermal analysis (STA) which confirmed that the obtained powder was hydroxyapatite. Chemical analyses (for example AAS and EDTA titration) were used for the determination of Ca/P molar ratio. The bulk Ca/P molar ratio was determined as 1.71, which showed that the measured Ca/P ratio for the synthesized powder was higher than the stoichiometric ratio (1.667) expected for a pure HA phase. SEM revealed that there is a distribution of small particles and large agglomerates, and these agglomerates consist of fine particles that are cold welded together. Finally, TEM analysis ascertained that the crystallites of prepared powder were nano-sized and needle-like, with an aspect ratio of about 8.



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