Comparison of characteristics of hydroxyapatite powders synthesized from cuttlefish bone via precipitation and ball milling techniques

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Abstract. The aim of this work was to compare characteristics of hydroxyapatite synthesized by precipitation and ball milling techniques. The cuttlefish bone powder was a precursor in calcium source and the di ammonium hydrogen orthophosphate powders was a precursor in phosphate source. The hydroxyapatite was synthesized by the both techniques such as precipitation and ball milling techniques. The phase formation, chemical structure and morphology of the both hydroxyapatite powders have been examined by X-ray diffractometer (XRD), Fourier transform infrared spectroscopie (FTIR) and field emission scanning electron microscope (FESEM), respectively. The results show that the hydroxyapatite synthesized by precipitation technique formed hydroxyapatite phase slower than the hydroxyapatite synthesized by ball milling technique. The FTIR results show the chemical structures of sample in both techniques are similar. The morphology of the hydroxyapatite from the both techniques were sphere like shapes and particle size was about in nano scale. The average particle size of the hydroxyapatite by ball milling technique was less than those synthesized by precipitation technique. This experiment indicated that the ball milling technique take time less than the precipitation technique in hydroxyapatite synthesis.

1. Introduction

Hydroxyapatite is the emerging most bio-ceramic that is the popularly used in various bio-medical application, mainly in orthopedics and medicine application [1]. Nevertheless, hydroxyapatite can used in agriculture and industrial applications, which used for the removal of heavy metal from contaminate soils and wastewater [2]. Chemical formula of hydroxyapatite is Ca₁₀(PO₄)₆(OH)₂. Hydroxyapatite can be synthesized from calcium and phosphate sources. Calcium source can found in natural and waste from community, such as rock, coral, eggshell and sea shell. Cuttlefish bone is a common waste material produced by the food industry, which cause environmental problems such as, odours generated during the treatment and localized element concentration in the landfills. Cuttlefish bones have chemical and crystallographic properties similar to coral. Cuttlefish bone has aragonite structure of CaCO₃ more than 90% [3]. In the hydroxyapatite synthesis, there are many techniques, for instance; hydrothermal, solid state and so-gel. Especially, the precipitation and ball milling technique is the popular, simple and widely researched technique for hydroxyapatite synthesizing [4]. Moreover, the both techniques can prepare...
hydroxyapatite at room temperature. This work is to compare the characteristics of hydroxyapatite from cuttlefish bone synthesized by precipitation and ball milling techniques. The characteristics of hydroxyapatite are analyzed by X-ray diffraction, Fourier transform infrared spectroscopy and scanning electron microscopy.

2. Methodology

Cuttlefish bones were washed with water several times and transferred to the oven at 90 °C to dry. The dried cuttlefish bones were ground into powder by agate mortar. Then, the powders were calcium carbonate (CaCO$_3$) phase and were transformed to calcium oxide (CaO) phase after the powders were calcined at 1300 °C for 4 h with a rate of 10 °C/min. The CaO and di ammonium hydrogen orthophosphate ((NH$_4$)$_2$HPO$_4$) powders were used for the preparation of hydroxyapatite by the precipitation and ball milling techniques. In the precipitation technique, the CaO powder was completely dissolved by nitric acid and diluted with distil water which this solution is Ca(NO$_3$) solution. The ((NH$_4$)$_2$HPO$_4$) powder was dissolved with distill water. The both solutions were adjusted pH between 10 and 12 by ammonium hydroxide (NH$_4$OH) solution. The ((NH$_4$)$_2$HPO$_4$) solution was slowly added into the Ca(NO$_3$)$_2$ solution using syringe. The mixed solution was stirred by a magnetic stirrer for 24 h. The obtained precipitate was washed using distill water for several times and was filtered by vacuum pump. For the ball milling technique, the CaO powder from heated cuttlefish bones and Di ammonium hydrogen orthophosphate ((NH$_4$)$_2$HPO$_4$) powder were mixed at molar ratio 5:3 with distill water. Milling performed in stainless steel containers and balls for 2 h. Then, the samples from the precipitation and ball milling techniques were dried at 90 °C and ground into powder. The phase formation was characterized by powder X-ray diffractometer (XRD; Philips X’ Pert PW3020) with CuKα ($\lambda$ = 1.5418 Å) radiation source generated at 30 mA and 30 kV in the 2-theta range 20 to 65 degrees at scan speed of 0.02 sec. The chemical structure of the all samples was characterized by a Fourier transform infrared spectrooscope (FTIR; Perkin Elmer spectrum two). The infrared spectra were recorded in between 500 and 4000 cm$^{-1}$ with resolution of 2 cm$^{-1}$. The morphology was carried out in a field-emission scanning electron microscope (FESEM; FEI Nova NanoSEM 450).

3. Results and discussion

The X-ray diffraction technique is to identify the crystal structure of sample and to characterize the phase formation of a particular constituent. Figure 1 shows the recorded XRD patterns of the samples from the both synthesis techniques has hydroxyapatite phases. The crystal structure present in the both samples was identified as hexagonal structure according to JCPDS file number 09-0432. The crystalline size of the both samples was calculated using XRD data were given in table 1 by Scherer’s equation [5].

$$D = \frac{k\lambda}{\mathrm{FWHM} \cos \theta}$$  \hspace{1cm} (1)

Where $k$ is 0.94, $\lambda$ is wavelength of X-ray, $\theta$ is Bragg angle of peak from diffraction and FWHM is the full width half maximum.

The crystalline size of sample synthesized by ball milling technique was more than those synthesized by precipitation technique because, the mechanical activation leads to the formation and growth of hydroxyapatite crystalline size [6].

| Sample from technique | Crystalline size (Å) |
|-----------------------|----------------------|
| Precipitation         | 100.62               |
| Ball milling          | 130.70               |
Figure 1. X-ray diffraction pattern of the hydroxyapatite synthesized by (a.) precipitation and (b.) ball milling techniques.

The infrared spectra of the synthesized powder samples show in the figure 2. The interaction bands of internal phosphate mode were appeared in three spectra; the bands at 565 and 603 cm\(^{-1}\) were vibration of \(v_4\) bending mode, the 964 cm\(^{-1}\) band in the spectra may be correlated with the vibration of \(v_1\) symmetric stretching and the doublet in the band between 1100 and 1000 cm\(^{-1}\) correspond to characteristic \(v_3\) antisymmetric stretching mode. The both samples had a hydroxyl (OH\(^{-}\)) group, vibration at 636 and 1734 cm\(^{-1}\) was assigned to stretching and \(v_2\) bending, respectively [7]. The peak at 1434 and 1366 cm\(^{-1}\) of sample synthesized by precipitation techniques was weak vibration than the sample synthesized by ball milling technique. The ball milling technique activated high energy which lead to reverse reaction in the CaO become to CaCO\(_3\). Moreover, this peak was appoint to \(v_3\) (carbonate group; CO\(_3^{2-}\)) bending which the CO\(_3^{2-}\) group in the spectra demonstrated the attendance of CO\(_2\) in the synthesized hydroxyapatite [8].

Figure 2. infrared spectra of the hydroxyapatite synthesized by (a.) precipitation and (b.) ball milling techniques.
Figure 3 shows the FESEM micrographs of the all samples. The both hydroxyapatite synthesized by precipitation and ball milling techniques was a spherical like shape [4]. The average of particle sizes of the hydroxyapatite synthesized by precipitation were about 60 to 120 nm but, the average particle sized of those sample were in the range of 60 to 70 nm. Furthermore, nanoparticle in sample from precipitation was dispersed more than a nanoparticle in hydroxyapatite from ball milling. However, nanoparticles of the both samples had interconnected and porosity.

Figure 3. FESEM micrograph of the hydroxyapatite synthesized by (a.) precipitation and (b.) ball milling techniques.

4. Conclusions
Hydroxyapatite powder was successfully synthesized by precipitation and ball milling techniques. The phase formation, chemical structure and morphology of hydroxyapatite from the both techniques were similar but, the average nanoparticle size of the sample from ball milling technique was less than other samples from precipitation. Furthermore, the ball milling is technique for rapid and simple in hydroxyapatite synthesis.

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