Effects of 42khz Ultrasonic Treatment to the Crystallite Size and Morphology of Chemically-Prepared Carbonated-Hydroxyapatite

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ABSTRACT

Background: Carbonate-substituted hydroxyapatites (carbonated-hydroxyapatite) are the primary mineral constituents of human and animal bone tissues. These materials are increasingly used in medical fields due to their biocompatibility and high bioactivity to osseous tissue.

Objectives: To investigate the effect of ultrasonic treatment to the composition, crystallite size and morphology of the hydroxyapatite powders obtained using Ca(OH)₂ and (NH₄)₂HPO₄ using wet chemical precipitation technique with and without post-synthesis ultrasonic (42KHz) treatment, characterized using Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM).

Results: From FTIR spectra, carbonated hydroxyapatites were depicted with A and B-type dominated carbonate substitution, and crystal sizes equal to 0.537, 0.481, 0.453 and 0.432 for 0, 20, 30, 40 minutes ultrasonic treatment, respectively. The rate of change of crystallite size with ultrasonic exposure time is 0.00267 mm/minute. From SEM images, agglomerated nano-sized particles were observed with increasing time ultrasonic treatment. Conclusions: Ultrasonically-treated for 20 to 40 minutes and untreated hydroxyapatite powders were both A and B-type carbonate-substituted hydroxyapatite with B-type dominance. These hydroxyapatite powders exhibited crystallite size that decreases linearly (R²=0.994) from 0.537 to 0.432 nm with agglomeration of particles with increasing ultrasonic treatment.

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INTRODUCTION

Significant attention has been given to carbonate-substituted hydroxyapatite, also known as carbonated hydroxyapatite, in the field of medical due to its excellent biocompatibility as it makes up most of the inorganic component of the human bones and teeth (Ivanova et al., 2001). Moreover, carbonated hydroxyapatites appear to be an excellent material for bone substitutes because of their high bioactivity to osseous (Poinern et al., 2011).

The presence of carbonates in the hydroxyapatite structure can lead to the distortion of its crystal lattice, thus, changing its essential properties like morphology, crystallinity, and etc. which are the determining factors of its possible application (Poralan et al., 2013, Porter et al., 2005). The incorporation of carbonate could take place during chemical reaction between primary precursors used. A number of literature have already been reported in synthesizing carbonated hydroxyapatite which includes wet chemical process, hydrothermal technique, and sonochemical (ultrasonic treatment) method. It has been reported elsewhere (Poralan et al., 2013), that carbonated hydroxyapatites were successfully fabricated under wet chemical precipitation using Ca(OH)₂ and (NH₄)₂HPO₄ mixture with subsequent exposure to ultrasonic treatment for 20 minutes. However, investigation on the effects of ultrasonic treatment on the properties of hydroxyapatites for longer exposure has not yet been made and reported.

It is believed that sonochemical effects enhance the incorporation of carbonates on the as-grown hydroxyapatite powders upon introduction of ultrasonic treatment. The negative pressure caused by the ultrasonic wave produced acoustic cavitations or voids in which carbonates could be trapped inside (Santos et
al., 2009). With these, carbonates may substitute on either “a” or “b” site of the hydroxyapatite structure as the cavitation bubbles penetrate into the hydroxyapatite matrix and collapse (Lafon et al., 2008).

In addition, the formation, growth, and implosion of the cavitation bubbles induces intense localized heating (~5,000K), high pressures (~1,000atm), heating and cooling rates (~107 K/sec), and liquid jet streams (~400km/h) which associated for the chemical and physical effects of ultrasonic treatment (Hielscher, 2005). This intense localized phenomenon could probably excite hydroxyapatite particles that could lead to coalescence of particles as smaller particles disperse from the surface and aggregate on the surface of larger particles to lower the overall surface energy. Thus, sonochemical effects resulting from the collapsed cavitation bubbles directly influence the particle size and morphology during growth phase.

In this present work, carbonated hydroxyapatite is fabricated using calcium hydroxide (Ca(OH)2) and diammonium hydrogenphosphate ((NH4)2HPO4) with and without post-synthesis exposure to ultrasonic (42 KHz) from 20 to 40 minutes. The chemical purity of the samples using Perkin Elmer Spectrum 100 FTIR Spectrometer under KBr pellet technique. The FTIR transmittance spectra are derived from 4,000 - 2,000 cm^-1 infrared spectral region. The spectra obtained from FTIR spectroscopy are then compared to the reference materials, and standard KBr. For more precise assignments of the observed vibrational peaks, FTIR spectra are compared to correlation charts.

I. Methodology:
A. Materials:
Hydroxyapatite powders are prepared using calcium hydroxide (Ca(OH)2) and di-ammonium hydrogenphosphate ((NH4)2HPO4) as our chemical precursors. A Cole-Palmer (08891-06) (100W, 42 KHz +/- 6%) is used as ultrasonic transducers in the post synthesis ultrasonic treatment.

B. Methods:
In preparing hydroxyapatite bioceramic, calcium hydroxide Ca(OH)2 and di-ammonium hydrogenphosphate (NH4)2HPO4 are weighed first using a digital analytical balance in amounts corresponding to the Ca/P ratio of 1.67. The weighed powders of calcium hydroxide and di-ammonium hydrogenphosphate are then diluted with distilled water in two separate 250 ml beakers to make a 100 ml solution. The container of calcium hydroxide solution is bath with distilled water and then stirred using a hot plate (Corning) with magnetic stirrer apparatus at 360 rpm and heated up to 70 °C. Subsequently, di-ammonium hydrogenphosphate solution is added dropwise into the calcium hydroxide solution in air environment. Then, the chemical bath is maintained to 70 °C for 3.5 hours in order to let the precursors to react. Thus, mixture of calcium hydroxide and di-ammonium hydrogenphosphate would then form hydroxyapatite, ammonium hydroxide and water that is 10Ca(OH)2 + 6(NH4)2HPO4 → Ca10(PO4)6(OH)2 + 12NH4OH + 6H2O. Afterwards, the synthesized as grown powders are introduced to the ultrasonic transducers at various time from 20-40 minutes with ten minutes interval using the operating ultrasonic frequency 42kHz (Cole-palmer). After exposure, the mixed solution is filtered and dried in a convection oven (Memmert) to eliminate the water content of the sample.

C. Characterization:
After drying the sample, Fourier Transform Infrared (FTIR) analysis is carried out to investigate the chemical purity of the samples using Perkin Elmer Spectrum 100 FTIR Spectrometer under KBr pellet technique. The FTIR transmittance spectra are derived from 4,000- 400 cm^-1 infrared spectral region. The spectra obtained from FTIR spectroscopy are then compared to the reference materials, and standard KBr. For more precise assignments of the observed vibrational peaks, FTIR spectra are compared to correlation charts. For the morphological features of the synthesized hydroxyapatite, Scanning Electron Microscopy (SEM) is used. The hydroxyapatite powder were observed using JEOL JSM-6510LA by coating platinum on the surface of the sample. The resolution of the SEM images is taken at 30,000 magnifications.

D. Determination of the crystallite size:
According to some researchers (Poralan et al., 2013), the percent B (%B) which is calculated by taking the area of symmetrical and asymmetrical stretching of phosphate vibration at 900-1200 cm^-1 spectral region from the Gaussian-fitted FTIR spectra was found to correlate with the crystallite size derived from XRD results. The best fitted result comes up with correlation coefficient (R²) always greater than 0.99. The peak associated to the area of phosphate-substitution site is located around 1060 cm^-1 spectral region. In the present, the FTIR spectra of as-grown hydroxyapatite at 900-1200 cm^-1 exposed to ultrasound at various times (0, 20, 30 and 40 mins) is graph and fitted to obtain the %B of the samples. Using Gaussian fitting of the QtiPlot application (http://www.qtiplot.ro/), the calculated %B is equal to 35.96, 42.35, 45.59 and 48.01, for 0, 20, 30 and 40 mins of exposure, respectively. It has been reported that the any changes with the value of the calculated %B (equation 1) would imply change with the crystal size of the hydroxyapatite as well, that is a decrease of %B indicates increase in the crystal size of hydroxyapatite sample. Equation (1) is given as

\[ \%B = \left( \frac{A_p}{A_t} \right) \times 100\% \]  

(1)
where \( A_P \) is the area of phosphate substitution site found in the subbands at around 900-1200 cm\(^{-1}\) and \( A_T \) is the total area of the subbands. It was found in the previous study on the same type of material using XRD and FTIR for the crystallite size and the \( \%B \) determination, respectively, that the \( \%B \) behaves linearly with crystallite size \( C(s) \) expressed as (Poralan et al., 2013):

\[
Y(C) = -114.4C + 97.4 \quad (R^2 = 0.993) \quad \text{for} \quad 0.67 \leq C \leq 0.75
\]

where \( Y(C) \) is the \( \%B \) and \( C \) is the crystallite size \( C(s) \). Using equations (1) and (2), the crystallite size of the present samples in this study were determined.

2. Results:

Figure 1 shows the FTIR spectra of the samples exposed at various ultrasonic times. The obtained spectra of the samples depict all the characteristic absorption bands for hydroxyapatite compound. The bands observed at 500-620 cm\(^{-1}\) and 900-1,200 cm\(^{-1}\) are associated to the bending, and the symmetric and anti-symmetric stretching of \( \text{PO}_4 \) vibrations, respectively. Moreover, the first order rotational motion of OH ions and the stretching of OH ions in the hydroxyapatite lattice were attributed to the peaks around 631 and 3,567-3,569 cm\(^{-1}\) spectral region, respectively. Thus, it implies that all fabricated samples with and without ultrasonic exposure are all hydroxyapatite-based.

![FTIR spectra of the obtained samples treated at various time of ultrasonic exposure](image1.png)

**Fig. 1:** FTIR spectra of the obtained samples treated at various time of ultrasonic with evident peaks at [a] 500 – 620, [b] 620-650, [c] 875, [d] 900-1,200, [e] 1,400-1,472, [f] 1,620-1,660, [g] 3,300-3,557 and [h] 3,567-3,569 cm\(^{-1}\).

Furthermore, the peaks at 1,620-1,660 cm\(^{-1}\) and the broad peaks around 3,300-3,557 were related to the stretching of the OH of water. However, additional peaks were observed at 875 and 1,400-1,472 cm\(^{-1}\) spectral region which are attributed to the bending and asymmetrical stretching of carbonates confirming the presence of carbonates on all samples. Hence, we can say that all samples are carbonated-hydroxyapatites.

It is very apparent in the FTIR spectra that there is an increased of the absorbance of the bands corresponding to the carbonate functional group as we exposed our sample to ultrasound for longer period. The peaks associated to carbonates correspond to what type of substitution that takes place in the hydroxyapatite lattice. The doublet peaks of carbonates (Figure 2) at 1400-1472 are classified as “B” and “A” type carbonate substitution while the single peak at 875 corresponds to “B” type substitution (Lafon et al., 2008).

![FTIR spectra of the doublet peak of carbonates’ asymmetrical stretching](image2.png)

**Fig. 2:** FTIR spectra of the doublet peak of carbonates’ asymmetrical stretching (peak 1 and 2 at 1421 and 1454 cm\(^{-1}\), respectively).
As the exposure time increases, the “B” type carbonate asymmetrical stretching also increases relative to unexposed sample, except for 20 minutes exposed sample (Figure 3). The increase of the concentration of “B” type carbonates in the hydroxyapatite material is about 9.4 and 15.5 % after ultrasonic treatment for 30 and 40 minutes, respectively, and for “A” type carbonate asymmetrical stretching is about 10.1 and 12.8 % increase after 30 and 40 minutes ultrasonic treatment also.

![Absorbed carbonates on ultrasonically-treated hydroxyapatite.](image)

**Fig. 3:** Absorbed carbonates on ultrasonically-treated hydroxyapatite.

In addition, the concentration of “B” type carbonate at 875 cm\(^{-1}\) (Figure 4) in the hydroxyapatite material after 30 and 40 minutes ultrasonic treatment increases around 23 and 13 % (Figure 5), respectively. Thus, it can be concluded from the figure that “B” type carbonates dominated in the hydroxyapatite lattice compared to “A” type carbonate.

![FTIR spectra of “b” type carbonate bending vibration at 875 cm\(^{-1}\).](image)

**Fig. 4:** FTIR spectra of “b” type carbonate bending vibration at 875 cm\(^{-1}\).

![Absorbed type b-carbonates at 875 cm\(^{-1}\).](image)

**Fig. 5:** Absorbed type b-carbonates at 875 cm\(^{-1}\).
Meanwhile, using the obtained equations 1 and 2 for the calculation of crystallite size of the fabricated samples with various ultrasonic exposures for 0, 20, 30, and 40 minutes and plotted as shown in Figure 6. The crystallite size of the samples decreases from 0.537 to 0.432 nm with increasing time of ultrasonic treatment. It is noticeable that the crystallite size is linearly correlated to time of ultrasonic treatment with correlation coefficient close to unity ($R^2 = 0.994$) according to the equation

$$C(s) = -0.00267s + 0.536 \text{ for } 0 \leq s \leq 40 \text{ minutes}$$  \hspace{1cm} (3)

where $C(s)$ is the crystallite size and $s$ is the ultrasonic treatment time. The rate of change of crystallite size with time of ultrasonic treatment of the samples is very slow that is 0.00267 nm/minute. So far, no report of the same material prepared using the same chemical precursors and processed ultrasonically having the same behavior can be found.

![Graph showing crystallite size vs. ultrasonic exposure time](image)

**Fig. 6:** Crystal size of apatite samples with and without ultrasonic exposure.

On the other hand, the SEM micrographs of the samples are presented in Figure 7. The surface morphology of the untreated and ultrasonic-treated samples as seen in Figure 7 show agglomerated nano-sized particles. These agglomerated nano-sized particles are evidently exhibited by the untreated and the 20 and 40 minutes ultrasonic treated samples. However, for 30 minutes ultrasonically-treated hydroxyapatite powders, more agglomerated nano-sized particles are observed.

![SEM images of ultrasonically-treated apatite at various exposure time, 0, 20, 30 and 40 minutes at 30000 magnification](image)

**Fig. 7:** SEM images of ultrasonically-treated apatite at various exposure time, 0, 20, 30 and 40 minutes at 30000 magnification.

3. **Discussions:**

The absorbed water in the FTIR spectra is most probably due to the absorption of moisture during characterization. Meanwhile, the presence of carbonates in the sample is probably due to the reaction of carbon dioxide ($\text{CO}_2$) in the surrounding with hydroxyapatite during synthesis that can substitute either in the hydroxyl or in the phosphate site in the hydroxyapatite lattice. There are two types of carbonates that can occur, namely,
A and B-type carbonate substitution. “A” type carbonates can substitute on OH site of apatite structure leading to expansion of its a and b lattice parameters with shortening of its c-axis parameters while the “B” type can substitute into one the phosphate site leading to shortening of either a or b parameters with lengthening of the c-axis (Ivanova et al., 2001).

Accordingly, the observed increase of carbonate content in hydroxyapatite after ultrasonic exposure for 30 and 40 minutes ultrasonically-exposed sample relative to unexposed one is possibly due to the sonochemical reaction that have occurred in the solution. The dissolved CO$_2$ in the mixture of the chemical precursors that are trapped in the hydroxyapatite matrix might have undergone chemical reaction to form carbonic acid (H$_2$CO$_3$) and the ultrasonic treatment might have hastened the formation and decomposition of H$_2$CO$_3$. The localized heating that occurs during ultrasonic treatment could be the reason for the decomposition of more carbonic acid (H$_2$CO$_3$) that leads to the formation of carbonates. These carbonate ions then possibly react to the hydroxyapatite matrix resulting to the increase of concentration of carbonates in hydroxyapatite material. For the 20 minutes exposed sample, the “B” type carbonates decreases relative to unexposed sample which is possibly because of the onset of the diffusion of carbonates. These results indicate that we can control the CO$_3$ substitution using ultrasonic treatment to the as-prepared hydroxyapatite powder particles.

Moreover, the observed increase of %B indicates that incorporation of carbonates in the phosphate site of hydroxyapatite matrix increases affecting its crystallite size. These observation follows on the behavior of crystal size of the samples, in which crystallite size decreases linearly (linear correlation coefficient, $R^2=0.994$) with increasing time of ultrasonic treatment. Furthermore, the slow rate of change of crystal size with increasing ultrasonic treatment is may be due to low constant ultrasonic frequency (42 kHz) used in the study that can atomize surfaces of exposed hydroxyapatite particles. Hence, these observed decreased of crystal size is an indication that crystalline nature of as-grown hydroxyapatite can be controlled by exposing to ultrasonic treatment.

On the other hand, the minute increase of particle sizes along with increasing ultrasonic treatment might be due to the coalescence of particles. These agglomeration on the surface of the material is probably due to the intense localized heating induced by the implosion of the cavitation bubbles during ultrasonic treatment. Moreover, this increase can also be attributed to the competing incorporation of carbonates into the hydroxyapatite structure as observed in their FTIR result.

4. Conclusions:
Carbonate-substituted hydroxyapatites powders from the reaction of calcium hydroxide Ca(OH)$_2$ and di-ammonium hydrogenphosphate (NH$_4$)$_2$HPO$_4$ through wet chemical precipitation technique were successfully synthesized. The ultrasonic treatment of the as-prepared hydroxyapatite particles from 20 to 40 minutes decreased the crystallite size of the agglomerated hydroxyapatite nano-particles with increasing CO$_3$ substitution as a general trend. All hydroxyapatite powders were both “B” and “A” type carbonate-substituted with “B” type dominance. Furthermore, the crystal size decreases linearly from 0.537 to 0.432 nm with coalescence of particles with increasing ultrasonic treatment time.

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