The Effect of pH Variation on Fluor-Hydroxyapatite Nano Crystal Synthesis with Microwave Irradiation Method

Hafiz¹, Nurlely¹, Y W Sari²

¹Departement of Physics, University of Indonesia, 16424, Depok, Indonesia
²Departement of Physics, Bogor Agricultural University, 16680, Bogor, Indonesia
* Email: nurlely@sci.ui.ac.id

Abstract. Fluoride-substituted hydroxyapatite (FHA) can be used for biomaterial applications because it has biocompatible properties. FHA is formed by replacing fluor ion (F⁻) with hydroxyl ion (OH⁻) from hydroxyapatite. This work aims to see the influence of changes in the form of acidity (pH) at the FHA synthesis process using microwave irradiation method. FHA nano-powder was synthesized using a titration of calcium hydroxide solution with diammonium hydrogen phosphate and ammonium fluoride, which will vary the level of fluoride at FHA by 0.9 and 1.3. Subsequently, HCl 1 M or NaOH 1 M to give a variation of pH value. X-ray diffraction (XRD), Fourier transform infrared (FTIR), scanning electron microscopy (SEM), and energy-dispersive X-ray (EDX) spectroscopy analysis techniques were utilized to evaluate the characteristic of synthesized FHA nano-powder. The XRD and FTIR results showed that the powder with pH under control conditions had several additional formed phases such as chlorapatite and carbonate. The value of crystallinity had the distinction of each powder in each pH, but only pH control had crystallinity values, such as tooth enamel. Using the Scherrer formula, the average crystallite size was found around 21-80 nm. SEM and EDX characterization results showed the Ca/P ratio formed was 1.34. The results showed that the addition of acid resulted in the formation of a new phase which dominated and revamped some crystal parameters. Therefore, the synthesis in these conditions is not recommended.

Keywords: Fluor, Fluor-hydroxyapatite, biomaterial, pH

1. Introduction

Problems with cavities are a scourge for public health. Data from the Indonesian Ministry of health in 2012 showed that in the 12-year age group, having cavities and not treated, were 43.4%, and those who had cavities were 67.2%. While the general picture of the Indonesian entire population on average has 5 cavities in each person [1]. The presence of bacterial activity and food waste results in the dissolution of mineral structures in the enamel layer causing cavities [2], and this can be corrected (remineralization) by the presence of fluoride or fluorine (F). In preventing the occurrence of cavities, Fluor can work systemically (in the body) and topically (outside the body). Systemically fluoride is obtained through drinking water, food, and fluorine supplements. Meanwhile, topically it is obtained from toothpaste, mouthwash, and also the application of fluorine which is done by dentists [3].
Calcium apatite is one of the main constituent elements of bones (65%) and teeth (95%), which exists along with minor constituents of metal ions to balance biological and mechanical activities [4]. Hydroxyapatite (HA) made synthetically \[\text{Ca}_{10} (\text{PO}_4)_6 (\text{OH})_2\], closely resembles the mineral phase of human bones and teeth but requires an increase in mechanical strength to be used as an implant. The addition of fluorine in HA to fluor-hydroxyapatite (FHA – \text{Ca}_{10} (\text{PO}_4)_6 (\text{OH})_2, \text{F}_x\) also increases thermal stability and mechanical strength of the material. FHA synthesis can be made through precipitation method, and in the process, the synthesis of acidity degrees can be varied to see the characteristics of FHA nanocrystals formed, this can be useful to find a good pH condition value for a particular FHA crystal characteristic.

In this study, FHA synthesis will be carried out by precipitation and microwave irradiation methods, which use microwaves to accelerate the process, of forming FHA nanocrystals. In the synthesis process, the pH value of the mixture will be varied at different levels of fluoridation. This is intended to compare the characteristics of crystals formed through characterization with XRD, FTIR, SEM, and EDX.

2. Materials and methods.

2.1 Materials.

This research used several chemical reagents for FHA nanocrystal synthesis. These materials included calcium hydroxide (Ca(OH)\(_2\)), ammonium fluoride (NH\(_4\)F), diammonium hydrogen phosphate ((NH\(_4\))\(_2\)HPO\(_4\)), and aquades as solvents. Next, to reduce or increase the pH value, the mixture was added with 1 M hydrochloric acid (HCl) or 1 M sodium hydroxide (NaOH).

2.2 Synthesis.

The synthesis process of FHA used precipitation and irradiation methods. The synthesis began with the titration of NH\(_4\)F and (NH\(_4\))\(_2\)HPO\(_4\) solutions into a solution of Ca(OH)\(_2\) simultaneously at a rate of 5 mL/minute. During the titration process, Ca(OH)\(_2\) was stirred with a magnetic stirrer and the pH value changes were monitored using a pH meter and a universal indicator. The ratio of Ca/P used was 1.67 and x or the level of fluoridation, which is a reference for the concentration of NH\(_4\)F used for titration. The X was 0.7 and 1.3 where these values indicate the number of indexes of the hydroxyl (OH) group replaced with fluor ion (F\(^-\)). During the titration process, HCl and NaOH solutions would be added to change the pH value to 5, 7, 9, and 11. The process of adding an acid/base was stopped when the pH did not change, and the titration was complete. In addition, it wasalso made 1 mixture which was not added with HCl or NaOH, this solution was named a control sample.

The result of the titration mixture was then irradiated with microwaves using 720 watts of power for 15 minutes. After irradiation, the mixture was poured into a petri dish and dried using an oven to remove the water content. The drying process is considered complete if there is no more mass reduction from the sample. The samples were dried, then weighed using a digital balance, then mashed and filtered using mesh filter # 100 to equalize the particle size. The results of the sample in the form of powder would then be used for characterization purposes.

2.3 Characterization.

The synthesized powder was characterized by several tests such as XRD, FTIR, SEM, and EDX. XRD analysis was carried out by an X-ray diffractometer (XRD, Panalytical X'Pert Pro MPD) using 0.02 mm Nickel (Ni) filtered with monochromatic Cu K\(\alpha\) where the value of \(\lambda\text{CuK}\alpha\) was around 0.15406 nm, and the radiation beam setting was at 40 kV and 30 mA. The particles were examined in a range of 2\(\theta\) from 20° to 60° with a step rate of 0.4°/s. The results of the XRD were then processed using HighScore Plus software to search for several parameters from FHA crystals and then compared with the standards compiled by The International Center Diffraction Data (ICDD), which was added Powder Diffraction File (PDF) No.09-0432 to HA and No.15-0876 for FHA.

To find the functional groups contained in the synthesized powder, testing was performed using Fourier transform infrared spectroscopy (FTIR) applied to find functional groups in the wavenumber range.
ranging from 500 - 4000 cm\(^{-1}\). FTIR testing was carried out at the pharmaceutical and medical technology laboratory, PTFM BPPT Serpong. Data obtained from FTIR testing were in the form of graphs and between wavenumbers (cm\(^{-1}\)) with transmittance for each pH tested. These results will be processed using Origin Pro software to find the position of the detected functional groups using wavenumbers, as shown in the FTIR result pattern.

The morphology or outer form of the synthesized crystals was investigated using an electron microscope (SEM, FEI Inspect 10) at the Metallurgical and Materials Engineering Laboratory, University of Indonesia. SEM was used by setting the electron beam at a voltage of 20 kV, WD around 6 mm, the spot around 5.0, and magnification of 200 kx or a scale of 500 nm. The percentage of the elements possessed by the synthesized powder was investigated by dispersive energy X-ray spectroscopy (EDX) in the test range from 0.1 to 5 keV, this result would be used to find the Ca/P value of each tested sample.

3. Results.

3.1 XRD Analysis

The pattern of the XRD test results is shown in Figure 1. The pattern given at both pH below and pH control at x = 0.7 or 1.3 shows the emergence of shifts and impurity peaks, indicating a phase other than FHA or HA. Using the HighScore Plus software, other which that appear at pH 5, 7, 9 were chlorapatite, and chlorinated hydroxyapatite. Whereas pH control and pH 11 had a FHA formation phase greater than pH under control.

![Figure 1](image)

**Figure 1.** XRD pattern of FHA powders was synthesized at different pH and x values (a) pH = 5, 7, 9 at x = 0.7, (b) pH = control, 11 at x = 0.7, (c) pH = 5, 7, 9 at x = 1.3.

The results of x and c lattice parameter values were unstable up or down, both at x = 0.7 and 1.3. At x = 0.7, the value of a fell from pH = 5 to 7 but returned to pH = 9 and returned to pH = control and 11. Meanwhile for x = 1.3, the value of a decreases from pH = 5 to pH = 7 then rose again at pH = 9 and dropped back at pH control. The decrease in the length of a side occurred due to strong electronegativity of fluorine s which could increase the building among the crystalline forming elements of FHA. The size of F and OH ions were also different, causing changes in the distance among atoms [2]. However, in the results of this study, the value of a only decreased from pH = 5 to 7, then from pH = 9 to 11.
For the length of the c axis in both values of x with pH = 5, there were differences where at x = 0.7 the value of the length c axis tended to rise while at x = 1.3 it tended to decrease. Similar to the value of a, the change in the value of c also occurred at pH = 7 to 9, where the value changed from the condition that previously rose to decrease, vice versa. An increase in the length of the c axis could occur because the position of F ion (z position) was higher than that of OH ion [2]. Thus, the replacement of the position of OH ion with F will cause the elongation (extension) of the c axis, this occurred at x = 0.7. Crystal size at pH = 5, both at x = 0.7 and 1.3 showed a very large number of 80.43 nm and 80.30 nm respectively, which far exceeded the size of the crystal at another pH. Then, the crystal size decreased to 26.80 nm at x = 0.7 and 37.1 nm for x = 1.3. The size of the crystal returned to the control pH and fell back to pH = 11.

The largest crystalline/crystallinity index value was also possessed at pH = 5 in both x values which were equal to 13.66 and again decreased with increasing pH. Dental enamel had a crystallinity index of 0.80 to 1.21 [6] so that from all the results of the crystallinity data, only pH control entered the crystallinity range of dental enamel.

3.2 FTIR Analysis

FTIR testing was carried out at x = 0.7 with all variations in pH values. In the results of testing using FTIR, a band was obtained indicating the presence of other groups which should not present in the FHA compound. This shows that the resulting sample was not pure, because another group was formed.

![FTIR spectra](image)

**Figure 2.** (a) The IR spectrum of FHA crystals for pH = 5, 7, and 9 at x = 0.7. (b) IR spectrum of FHA crystals for pH = control and 11 at x = 0.7

At all pH values in the range of wavenumber 873 cm$^{-1}$ – 1417 cm$^{-1}$, a narrow band formed indicated the presence of the CO$_3^{2-}$ group [7]. The carbonate group was formed as a result of the reaction between water (solvent) and carbon dioxide (CO$_2$) gas around it [8]. The presence of carbonate in the apatite structure itself is considered less profitable because it can affect its thermal stability. The carbonate group could be reduced by synthesizing with inert atmospheric conditions. The apatite structure of all samples showed the presence of bands indicating several variations of the vibrational phosphate mode (PO$_4^{3-}$), PO$_4^{3-}$ v$_4$ vibration mode-asymmetric and symmetric deformation mode (bending mode) appeared in the range of wavenumbers 554 cm$^{-1}$ and 600 cm$^{-1}$. Asymmetric stretching mode PO$_4^{3-}$ v$_3$ vibration mode was showed at wavenumbers around 1006 to 1019 cm$^{-1}$, while the
symmetric stretch mode or \( \nu_1 \) PO\(_4\) was found in the range of 950 cm\(^{-1}\). For the indentation or \( \nu_2 \) variation mode, PO\(_4\) appeared in the range of the wavenumber 470 cm\(^{-1}\), but among the 4 modes of vibration, \( \nu_3 \) and \( \nu_4 \) were the greatest in terms of intensity. At pH = 7, 9, control, and 11, there was the absence of bands in the range 630-740 cm\(^{-1}\), this indicates the presence of fluorine substitution replacing the OH\(^-\) group.

3.3 SEM analysis

The FHA morphology of the test results using SEM (Scanning Electron Microscope) at pH = 7 and 9 for \( x = 0.7 \) is shown in figures 3 (a) and (b).

![SEM images](image)

Figure 3. (a) SEM of FHA crystals for pH = 7 at \( x = 0.7 \). (b) SEM of FHA crystals for pH = 9 at \( x = 0.7 \).

The particle size was calculated using Fiji ImageJ software and the measurements were 22.37 nm and 136 nm. This size was very far for the pH range tending to be close, but according to the described morphology when crystals form at pH = 7, they tend to be round and agglomerate (agglomerate), while at pH = 9, particles were rod-shaped and more separate.

4. Discussion

The results showed the influence of pH changes can provide a variation in the characteristic value of the resulting FHA crystals. This research is still limited to 1 base pH value above normal FHA pH when the synthesis is 10.21 so it has not answered how the characteristics of crystals are formed at a higher pH than in acidic or control conditions.

This research can be developed by replacing the used acid solution to see if the other dominant phase is formed as the addition of HCl and its effect on F ion substitution [3], or the addition of pH value variation above 11. In this study, several other phases, such as chlorapatit can be formed due to changes in pH carried out before the process of microwave irradiation has been done, and the provision of microwaves itself is to accelerate the process of crystal formation [3]. This arises a new question, whether the influence of pH occurs only when the process of adding acid or alkaline solution is done during the synthesis process only. How to change the pH process performed after the irradiation process of microwaves or after aging is also needed to be reviewed. Then in addition to the comparison of pH values, because the purpose of the use of FHA is in the field of dental health, it is necessary to check quantitatively the quality comparators with other biomaterials such as apatite carbonate which has crystallinity value, such as bones so that it can be reabsorbed by the body in the process [5].
5. Conclusion

The synthesis process of FHA has been carried out in various variations of pH and different x values, but due to the addition of acidic and basic solutions, all these variations form several new phases, such as chlorapatite, chlorine substituted hydroxyapatite, and carbonate-hydroxyapatite, and only samples with control pH which results in the formation of the largest FHA. From the XRD results, it is obtained that the size of the produced crystals has a range of values 21-80 nm with the largest size owned by pH = 5. While the greatest crystallinity is also owned by pH = 5, and the value tends to decrease and rise again at pH = 9, the crystallinity is close to tooth enamel only at pH control both at the value of x = 0.7 and 1.3 with values of 1.076 and 0.81 respectively. From the results of FTIR, it is found a function group of carbonates which should not be present in FHA, phosphate, and water. The Ca/P ratio at pH = 7 and 9 has a value of 1.34, and the results still show a value which is not close to apatite stoichiometry of 1.67 so that it shows that FHA is not suitable to be synthesized in acidic conditions which will greatly change its composition and produce another crystal phase.

Acknowledgment

This work is supported by the grant of PITTA 2019 by DRPM of University of Indonesia with contract No. NKB-0653/UN2.R3.1/HKP.05.00/2019

References.

[1]. Apsari, W. (2015). Fluoride, mineral penting untuk kesehatan gigi. Difa Oral Health Center Education Series No : 04/DifaOH/C/XII/20

[2]. Gunawam, Harun.A. (20016). Pengaruh tingkat pH larutan teri terhadap perubahan dimensi dan kelarutan Kristal apatit. Jurnal Anatom Indonesia, Vol. 01, No. 01, page 25-29.

[3]. Nabiyouni, M., Zhou, H., Luchini, T.J.F., Bhaduri, S.B. (2014). Formation of nanostructured fluorapatite via microwave assisted solution combustion synthesis. Mater. Sci. and Eng. C. 37, 363–368.

[4]. Sanyal, V. Raja, C.R. (2016). Synthesis, Characterization and in-vitro studies of strontium - zinc - Co - substituted fluorohydroxyapatite for biomedical application. Journal of Non-Crystalline Solids. 445–446, 81–87

[5]. Nurlatifah, MZ., Cahyanto A. (2016). An introduction to carbonateapatite as a biocompatible material in density.

[6]. Reyes-Gasga, J., Martinez-Pineiro, E.L., Rodriguez-Alvarez, G., Tiznado-Orozco, G.E., Garcia-Garcia, R., Bres, E. (2013). XRD and FTIR crystallinity indices in sound human tooth enamel and synthetic hydroxyapatite. Mater. Sci. and Eng. C. 33. 4568–4574.

[7]. Sasani, N., Khadivi, Ayask. H., Zebrarjad, S. M., Vahdati, Khaki. J. (2013). Characterization of rod-like high-purity fluoroapatite nanopowders obtained by sol-gel method. Journal of Ultimate Grained and Nanostructured Material, Vol. 46, No. 1, 2013, page 31-37.

[8]. Suryadi. (2011). Sintesis dan karakterisasi biomaterial hidroksiapatit dengan proses pengendapan kimia basah. Skripsi. Teknik Metalurgi dan Material, Universitas Indonesia
To Whom It May Concern

This is to acknowledge that the manuscript written by Hafiz, Nurlely, Y. W. Sari:

The Effect of pH Variation on Synthesis of Fluor-Hydroxyapatite Nano Crystal Synthesis wWith Microwave Irradiation Method.

was proofread by the staff member of UPT Pusat Bahasa ITB.

Bandung, 2 January 2020

[Signature]

[Name]
Editor of UPT Pusat Bahasa ITB

[Title]