

## PREPARATION OF HYDROXYAPATITE POWDERS FOR MEDICAL APPLICATIONS VIA SOL-GEL TECHNIQUE

Iis Sopyan

Ceramics Technology Group, Materials Technology Center  
Agency for the Assessment and Application of Technology (BPPT)  
Gedung BPPT II Lantai 22, Jalan M.H. Thamrin 8, Jakarta 10340

### ABSTRACT

**PREPARATION OF HYDROXYAPATITE POWDERS FOR MEDICAL APPLICATIONS VIA SOL-GEL TECHNIQUE.** Hydroxyapatite (HA) powders have been prepared via sol-gel procedure using calcium nitrate tetrahydrate and di-ammonium hydrogen phosphate as the precursors for calcium and phosphorus, respectively. An ammoniacal solution of the monomers was heated at 100°C for about 3–4 h until a white gel of hydroxyapatite mixture were obtained. The obtained gel was then dried at 340°C for 7 h and subsequently subjected to an 820°C calcination for 2 h. The yield of the powder mixture ranged between 50 to 53 g. XRD measurement shown that the powder contained hydroxyapatite crystal with b-TCP (15-20%) and calcium oxide (4-6%) as secondary phases. Further treatment was conducted to gain hydroxyapatite powder of higher purity, thus the correct Ca/P ratio, by adding an appropriate amount of di-ammonium hydrogen phosphate and heated while stirring at 90°C for 4 h. Good purity (almost 100%) of the obtained hydroxyapatite was confirmed by XRD analysis. This result proved that our HA powder meets the medical prerequisite: a HA purity of above 95%. Morphological evaluation by SEM measurement shows that the particles of the HA agglomerate are globular in shape with an average size of 1-2 mm in diameter. Meanwhile, the primary particles have a diameter of 70-150 nm in average. Surface area of the powder is 7 m<sup>2</sup>/g and the mean particle size as measured using X ray sedimentation method is ca. 1 mm. The measurements are not in good agreement with the morphological investigation. We consider, accordingly, that the particles presumably have high surface potentials, thus forming very tightly bonded agglomerates

**Key words :** Hydroxyapatite Powder, Sol-Gel, Medical Application, Preparation, Characterization

### ABSTRAK

**PREPARASI SERBUK HIDROKSIAPATIT UNTUK APLIKASI MEDIS DENGAN TEKNIK SOL-GEL.** Serbuk hidroksiapatit (HA) telah dipreparasi melalui prosedur sol-gel menggunakan kalsium nitrat tetrahidrat dan di-amonium hidrogen fosfat, berturut-turut sebagai prekursor untuk kalsium dan fosfor. Larutan amonia dari monomer-monomer tersebut dipanaskan pada 100°C selama 3-4 jam sampai diperoleh gel putih campuran hidroksiapatit. Kemudian gel ini dipanaskan pada 340°C selama 7 jam dan terus dikalsinasi pada 820°C selama 2 jam. Rendemen campuran serbuk yang diperoleh berkisar antara 50 ~ 53 g. Pengukuran difraksi sinar-x (XRD) memperlihatkan bahwa serbuk tersebut mengandung kristal hidroksiapatit yang bercampur dengan b-TCP (15-20%) dan kalsium oksida (4-6%) sebagai fasa sekunder. Hasil ini memberikan campuran kalsium fosfat dengan rasio Ca/P sekitar 1,8. Perlakuan lebih jauh dilakukan untuk mendapatkan serbuk hidroksiapatit dengan tingkat kemurnian yang lebih tinggi, dengan demikian rasio Ca/P yang tepat (1,67), dengan menambahkan sejumlah kristal di-amonium hidrogen fosfat dan kemudian memanaskannya pada 90°C selama 4 jam sambil diaduk. Analisis XRD membuktikan bahwa hidroksiapatit yang diperoleh mempunyai kemurnian yang baik, hampir 100%. Evaluasi morfologis melalui pengukuran SEM membuktikan bahwa partikel-partikel dari aglomerat HA berbentuk bulat dengan diameter rata-rata 1-2 mm. Sementara itu, partikelnya sendiri memiliki diameter rata-rata 70-150 nm. Luas permukaan dari serbuk tersebut adalah 7 m<sup>2</sup>/g dan diameter partikel rata-rata yang diukur menggunakan metode sedimentasi sinar-X sekitar 1 mm. Hasil-hasil pengukuran ini tidak konsisten dengan hasil investigasi morfologis menggunakan SEM. Oleh karena itu, diperkirakan bahwa partikel-partikel HA mungkin mempunyai potensial permukaan yang tinggi, yang dengan demikian memudahkan terbentuknya aglomerat-aglomerat yang terikat sangat kuat.

**Kata kunci :** Serbuk Hidroksiapatit, Sol-Gel, Aplikasi Medis, Preparasi, Karakterisasi

### INTRODUCTION

Hydroxyapatite (HA) is a particularly attractive material for bone and tooth implants since it closely resembles human tooth and bone mineral and has proven

to be biologically compatible with these tissues [1-9]. Their Ca/P ratio of 1.5-2.0 makes them an excellent choice for most dental and orthopaedic application. Moreover,

hydroxyapatite is thermodynamically the most stable calcium phosphate at the pH, temperature, and composition of physiological fluids. Since hydroxyapatite was applied for the first time in 1981 for periodontal lesion filling, its use in the medical field has expanded to solid blocks, solid components, and films for dental implants. Many studies have shown that HA ceramics show no toxicity, no inflammatory response, no pyrogenetic response, no fibrous tissue formation between implant and bone, and the ability to bond directly to the host bone [9].

The main limitation of HA ceramics as well as all other bioactive materials is that they have poor mechanical properties. Basically, all bioceramics which have good mechanical properties and suitable for load bearing applications are bioinert. Hydroxyapatite, on the other hand, has high bioactivity, with many proved medical applications in the forms of porous and dense bodies, granules, and, the most frequently applied, in coating forms [9-11].

Several research groups have reported on development of preparative procedures for hydroxyapatite. Traditionally, two main methods were employed for preparation of HA powders: wet (chemical) method (including precipitation method [12-20], hydrothermal technique [21-25], and hydrolysis [26]) and dry (solid state reaction) method (its references are cited in the Literature 3). Difference in the preparative routes resulted in a variety in morphology, stoichiometry, and level of crystallinity. Other methods, such as sol-gel [27-32], spray pyrolysis [34-37], mechano-chemical method [38-39], and so on were also developed newly as well documented in the review.

Sol-gel procedure was firstly employed for the preparation of HA by a Japanese research group, Prof. Sumio Sakka and co-workers [27]. They used calcium diethoxide and phosphorus triethoxide as starting materials. Hydrolysis – polycondensation of the monomers in neutral and acidic solutions gave rise to HA powders of high purity. Extraordinarily fine amorphous particulates with less than 10 nm in diameter were obtained from precipitation of the solutions, and enlarged to only ca. 100 nm after 900°C calcination. Since that, sol-gel derived-hydroxyapatite powders were also developed by other groups.

In this present research work, we also developed sol-gel procedures in preparing HA powder. It is well known that sol-gel techniques have several advantages for producing ceramic particulates of high purity, high crystallinity, nano sizes, and high reactivity. Sol-gel process, however, has some drawbacks such as expensive raw materials and low homogeneity of the final product in the case the highly distinct rate of monomers hydrolysis. This report presents a preliminary study on development of a novel sol-gel method for preparing extraordinarily fine hydroxyapatite powders, utilizing the raw materials of relatively low cost and easily

obtainable. Simplicity of experimental execution, in respect of methods employing wet chemical reaction, is one of the most important advantages offered by this method. Physico-chemical characterization of the hydroxyapatite powders obtained from the sol-gel procedure was conducted.

## METHOD

### Materials

Calcium nitrate tetrahydrate, reagent grade, was obtained from Aldrich. Di-ammonium hydrogen phosphate and urea (analytical grade) were purchased from Merck. EDTA and 30% v/v ammonium solution were obtained from Carlo Erba Reagenti. All the chemicals were used without further treatment.

### Preparation of the Stoichiometric Hydroxyapatite Powder

Principally, the experimental procedure employed in this study is described in the diagram as shown in Figure 1.

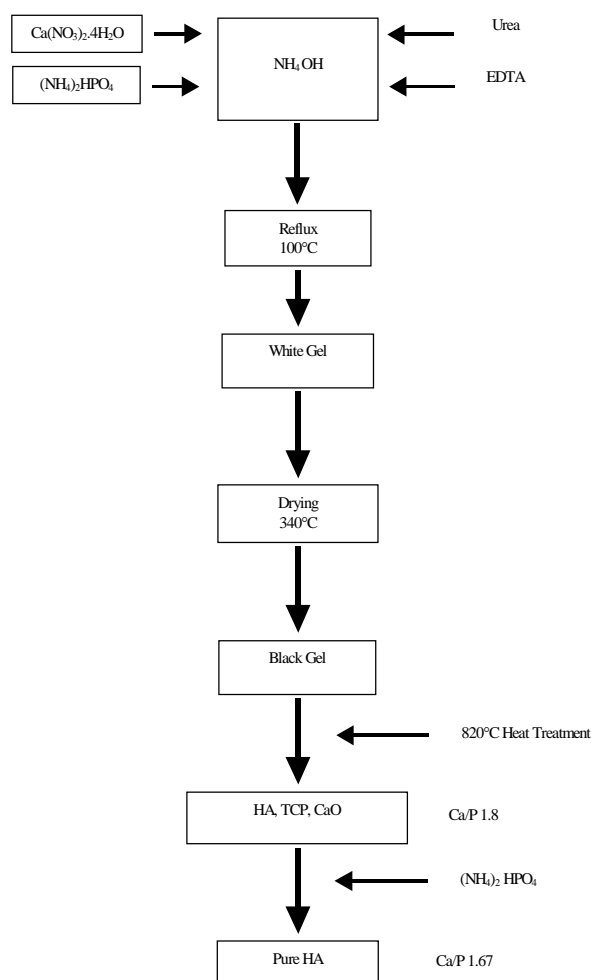


Figure 1. Scheme of Hydroxyapatite preparation via Sol-Gel Method.

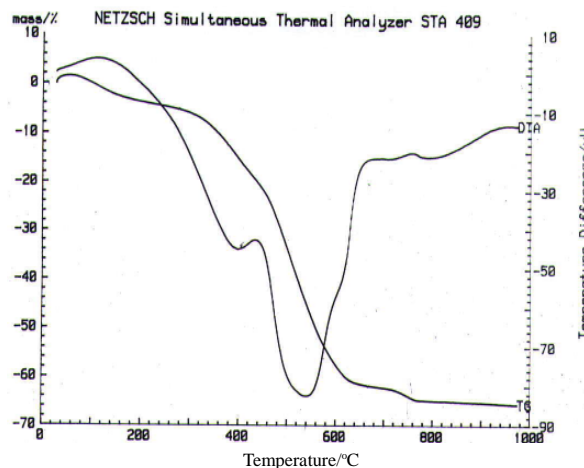
An ammonium solution was heated at 60°C, and 181 g EDTA was added while stirring until it dissolved, and then the heating was stopped. Into this, 200 mL aqueous solution of 129 g calcium nitrate tetrahydrate was poured, and then 39.83 g of di-ammonium hydrogen phosphate and 45.20 g of urea were subsequently added. The mixture was then heated at 100°C for 3-4 h. The obtained gel was then dried at 340°C under ambient static air and subsequently subjected to an 820°C calcination under flowing air. The powder was examined by X-ray diffraction techniques to determine the phases formed. It was shown that Ca/P molar ratio is ca. 1.8. Accordingly, to compensate the upward deviation from the stoichiometric ratio (1.667), the powder was mixed with an appropriate amount of di-ammonium hydrogen phosphate, and followed by suspending in water and heating at 90°C with rigorous stirring for ca. 4 h. This procedure restored the Ca/P ratio of hydroxyapatite powder to be 1.67. After drying for 15 h, the pure hydroxyapatite powder was obtained.

### Characterizations

Scanning Electron Microscopic measurement for morphology evaluation of the powder was performed on a Leica Cambridge of Stereoscopy 360 model. Specific surface area of the as-prepared HA powder was measured by the BET method (Sorpty 1750, Carlo Erba). The measurement of particle size distribution and mean particle size was performed using X ray sedimentation method on a Micromeritics SediGraph 5100 model after ultrasonic exciting for 80 min. The crystalline phases composition of the powders and of the dense samples were evaluated in a Rigaku diffractometer with copper  $K\alpha$  radiation and a scan rate of 2° in 2θ min<sup>-1</sup>. XRD patterns obtained were utilized for quantitative phase analysis according to the manner already published elsewhere [40]. Differential and thermogravimetric analysis was performed on the as-prepared of HA powders and dried gel in ambient air using Netzsch STA 409 apparatus with a 10°C/min heating rate.

### RESULT AND DISCUSSIONS

After refluxing the reaction mixture at 100°C for ca. 4 h, concentrated sol formed, subsequently it converted to white gel through *in situ* solvent evaporation. The gel was dried at 340°C using a very slow heating rate of 50°C/h under ambient static air. The black dried gel obtained was then subjected to TG-DTA to follow its thermal characterization. Figure 2 shows the DTA-TG curve of the gel dried at 340°C. A decrease in weight of ca. 65% was attributed to decomposition and elimination of water, ammonia, and carbon dioxide. There are two exothermic peaks in the curve. The first at 390°C, is attributed to decomposition of organic compounds. The second at 540 °C is a large exothermic peak which can be due to decomposition of hydroxide.



Gambar 2. DTA-TG curve of the white gel dried at 340°C.

Subsequently, calcination of dried gel at 820°C for 2h under flowing air converted it into white hydroxyapatite powder. As listed in Table 1, the yields of the HA powders ranged between 50 to 53 grams. Except for the result of Experiment I, all the results have showed good specification; where the Ca/P ratios were 1.667 and the purity was above 95%. These samples fulfilled the Standard Specifications of the ASTM where a 95% minimum amount of HA is prerequisite [8, 41].

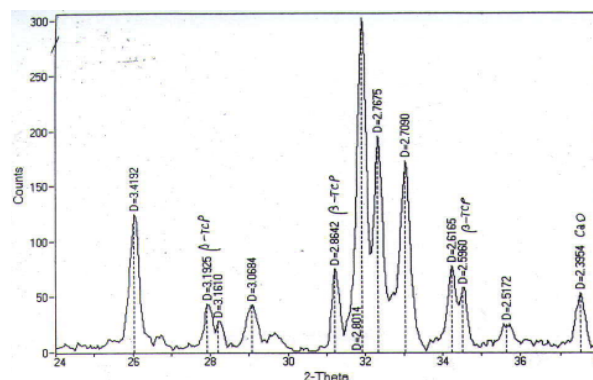


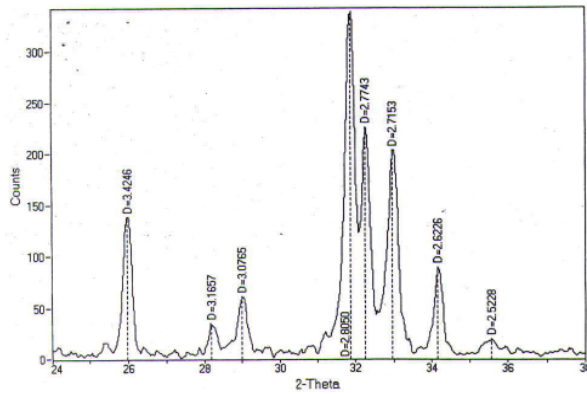
Figure 3. XRD Pattern of hydroxyapatite mixed with TCP and CaO (Before treatment HA).

The evaluation of crystalline phase of the powder was conducted by XRD. Figure 3 shows X-ray diffraction pattern of the powder. It is shown that hydroxyapatite is the main component in the powder (ca. 75%). Calcium oxide and  $\beta$ -tricalcium phosphate were present as secondary phases with an amount of 5 and 20%, respectively. Generally, the powder mixture obtained at this stage contained 75-85%, 15-20%, and 4-6% of HA,  $\beta$ -TCP, and CaO, respectively. At such composition, the Ca/P molar ratio is ca.1.8. On the other hand, it is well known that the optimum Ca/P molar ratio must be 1.667. Thus, to compensate the upward deviation of Ca/P, the right amount of di-ammonium hydrogen phosphate was added with a multiplying factor of 0.07304 in respect of the yield of the HA mixture as shown in Table 1.

**Table 1.** Result of Preparation of hydroxyapatite powder.

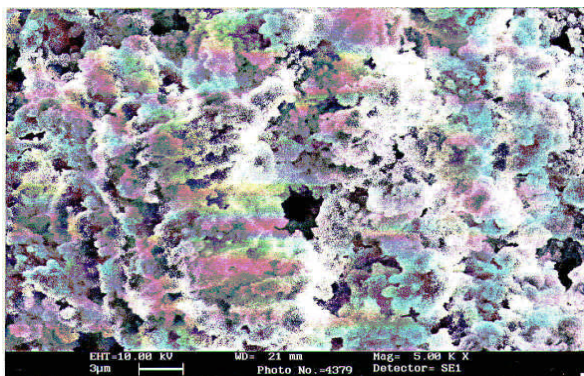
Experiment	Yield <sup>a)</sup> (g)	Ca/P ratio <sup>b)</sup>	Purity of HA <sup>b)</sup>
I	53.1	1.655	93% <sup>c)</sup>
II	51.2	1.667	100%
III	52.0	1.667	98%
IV	50.4	1.667	98%

- a) Yields for mixture HA after calcination at 820°C (Before correction of the Ca/P ratio).
- b) Values after the correction of the Ca/P ratio as calculated from the XRD analysis.
- c) This sample does not fulfill the Standard Specifications of the ASTM where a 95% minimum amount of HA is prerequisite [8,41]. Thus, HA powder from Exp. I was not used for further processing.



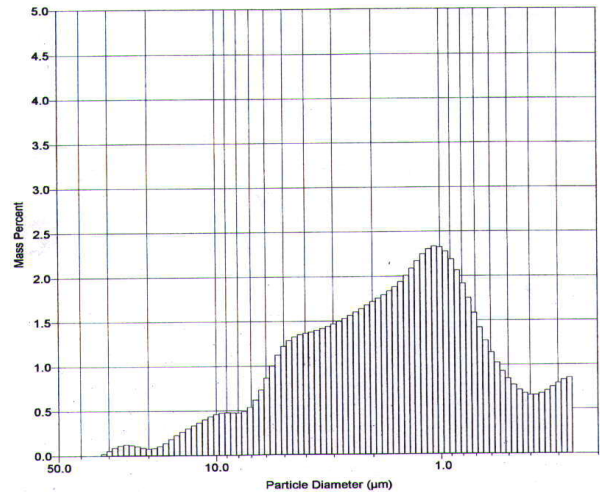
**Figure 4.** XRD Pattern of Pure Hydroxyapatite

Into the suspension of the mixture HA, diammonium hydrogen phosphate was added and heated at 90°C while rigorous stirring for ca. 4 h until the solvent was removed. Subsequently, the powder was dried in a furnace also at 90°C for 15 h, followed by 120°C drying for 2h. Figure 4 presents XRD pattern of the powder obtained after this treatment. It was shown that peaks of β-TCP and CaO disappeared, proving a 100% purity of the hydroxyapatite powder. We also checked the possibility of the presence of calcium hydroxide in the powder. A phenolphthalein test shown that no the hydroxide is present in our powder.



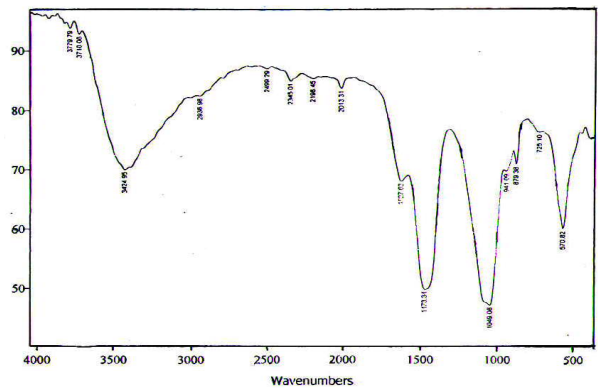
**Figure 5.** SEM picture of the as-prepared HA powder.

Figure 5 shows SEM picture of the as-prepared HA powder. Particles formed in globular shape with an average size of about 70-150 nm in diameter. On the other hand, Figure 6 presents the particle size distribution as measured by sedimentography. From this measurement, the main particle size are 1.0 µm in diameter, which are much larger than that observed by SEM.



**Figure 6.** Particle distribution of HA powder

Accordingly, it is considered that the result of sedimentography is presumably the result for “agglomerates measurement” rather than “particle measurement”. On the other hand, the specific surface area measured by BET method gave a low value of 7 m<sup>2</sup>/g. This value is unusual for particles as fine as hundreds nanometers level. The appropriate interpretation concerning this result is not clear yet, however, it is considered that individual particles in the powders have very high surface potential, resulting in tightly bonded agglomerates. Thus, surface area measured by BET is also the value for agglomerates instead of particles. We could state the particle size of the HA powder obtained in this study is considerably fine, as confirmed by SEM measurement, in respect of the HA powder prepared by sol-gel technique. Many reports stated that sol-gel derived HA powders have particle size of about 100 nm in diameter [27,30].



**Figure 7.** FTIR Spectrum of HA powder.

Figure 7 shows an IR spectrum of the sol-gel derived hydroxyapatite powders. The spectrum shows the characteristics peaks corresponding to OH<sup>-</sup> (630 and 3560 cm<sup>-1</sup>) and PO<sub>4</sub><sup>3-</sup> (960, 1050, 1090 cm<sup>-1</sup>) vibrations. At this stage, we have succeeded in preparing hydroxyapatite (HA) powders with particles having fine size for medical applications. Further studies are required for obtaining HA powders with no agglomeration disturbing characterization of the material. Presumably, a kind of additive is necessary to add to neutralize surface charge on the HA particulates so the powder could be well dispersed. This will lead to a good agreement between the measurements of surface area and mean particle size as well as morphological evaluation.

## CONCLUSION

Hydroxyapatite (HA) powders with particles having fine size were successfully prepared via a novel, relatively simple sol-gel procedure using calcium nitrate tetrahydrate and di-ammonium hydrogen phosphate as the precursors. All the powders obtained have good purity (almost 100% HA), thus fulfilling the specification required medically. The primary particulates have globular shape with a diameter of 70-150 nm in average, as detected by SEM. The results of the measurements of surface area (7 m<sup>2</sup>/g) and mean particle size (1 μm) are not in good agreement with the morphological investigation by SEM. We consider, accordingly, that the particles presumably have high surface potentials, thus forming very tightly bonded agglomerates.

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