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NANOSIZED BIOCERAMIC HYDROXYAPATITE POWDERS VIA SOL-GEL METHOD

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ABSTRACT

High purity hydroxyapatite powders have been synthesized through sol-gel technique using calcium nitrate tetrahydrate and di-ammonium hydrogen phosphate as the precursors for calcium and phosphorus, respectively. The monomers were heated in an 11 v/v% ammonium solution at 100°C until white gels of hydroxyapatite mixture were obtained. The obtained gel was then dried at ca. 350°C and subsequently subjected to calcination. XRD measurement shows that the powders contain hydroxyapatite crystals with β-TCP and calcium oxide as secondary phases. Hydroxyapatite powder of higher purity, i. e., the correct Ca/P ratio has been obtained, by adding an appropriate amount of diammonium hydrogen phosphate and heating with stirring. Morphological evaluation by SEM measurement shows that the particles of the HA are tightly agglomerated, globular in shape with an average size of 1-2 μm. The primary particulates have average diameters of 50-200 nm, as detected by SEM and nanoparticle sizer. Purity (almost 100%) of the obtained hydroxyapatite was confirmed by XRD analysis.

***Keywords*:** Hydroxyapatite Powder, Sol-Gel, Bioceramics, Synthesis, Characterization

INTRODUCTION

The submicroscopic crystal of calcium phosphate in bones and teeth resembles the crystal structure of synthetic hydroxyapatite (HA). Owing to excellent biological compatibility with these tissues, hydroxyapatites have become a particularly attractive material for hard tissue substitutes, leading to its intensive development worldwide for last 30 years (Hence, 1991; Suchanek & Yoshimura, 1998; Sopyan *et al.,* 2007; Sopyan, 2008a; Willman, 1996; Sopyan, 2008b; Toibah *et al.,* 2008; Ramesh *et al.,* 2008). Since the use of hydroxyapatite for the first time in 1981 for periodontal lesion filling, its use in the medical field has extended to solid blocks, solid components, and films for dental implants. Many studies have shown that HA ceramics show no toxicity, inflammatory response, pyrogenetic response, or fibrous tissue formation between implant and bone. Also, these materials have the ability to bond directly to the host bone (Ramesh *et al.,* 2008). 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 should be bioinert. Hydroxyapatite, on the other hand, has high bioactivity, with many medical applications in the form of porous, dense, granules, and, as coatings (Ramesh *et al.,* 2008; Sopyan & Rahim, 2008). 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 (Correia *et al,* 1996; Tas *et al,* 1997; Fulmer *et al,* 1992; Li *et al,* 1992; Slosaeczyk *et al,* 996; Bako & Kotsis, 1992), hydrothermal technique (Hattori et al, 1989; Hattori &Iwadate, 1990; Fujishiro et al, 1995), and hydrolysis (Monma & Kamiya, 987) 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 (Masuda *et al,* 1990; Russels *et al*, 996; Liu *et al,* 2001; Weng & Baptisa, 1998; Jillavekatesa & Condrate, 998; Layrolle *et al,* 1998), spray pyrolysis (Hwang & Lim, 1999; Inoue & Ono, 1987; Luo & Nieh, 995; Aizawa *et al,* 1995a; Aizawa *et al,* 1995b) , mechano-chemical method (Toriyama *et al,* 1995; Toriyama *et al,* 1996), 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, Sumio Sakka and co-workers (Masuda *et al,* 1990). 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 study, we have developed sol-gel procedures for 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 homogenity of the final product. We report herein a novel sol-gel method for preparing extraordinarily fine hydroxyapatite powders, utilizing easily obtainable raw materials of relatively low cost. 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 has been also carried out.

EXPERIMENTAL

Materials

For preparation of hydroxyapatite powders, calcium nitrate tetrahydrate and di-ammonium hydrogen phosphate (reagent grade) were used as calcium and phosphorus precursors, respectively. Both reagents were purchased from Merck KGaA, Germany. Urea (R&M Chemicals, UK) was used as gelling and ammonium donor agent. EDTA (Merck KGaA) was used as chelating agent to prevent immediate precipitate formation calcium ions in the course of gel formation. The reaction was conducted in basic solution using ammonium solution (R&M Chemicals, UK) as solvent.

Preparation of the stoichiometric hydroxyapatite powder

Principally, the experimental procedure employed in this study is described in the diagram as shown in Figure 1. An ammoniun solution was heated and 90.5 g EDTA was added while stirring until it dissolved, and then the heating was stopped. Into this, 200 mL aqueous solution of 64.5 g calcium nitrate tetrahydrate was poured, and then 19.9 g of di-ammonium hydrogen phosphate and 22.6 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 350°C under ambient static air and subsequently subjected to an 900°C calcination under flowing air. The powder was examined by X-ray diffraction techniques to determine the phases formed.

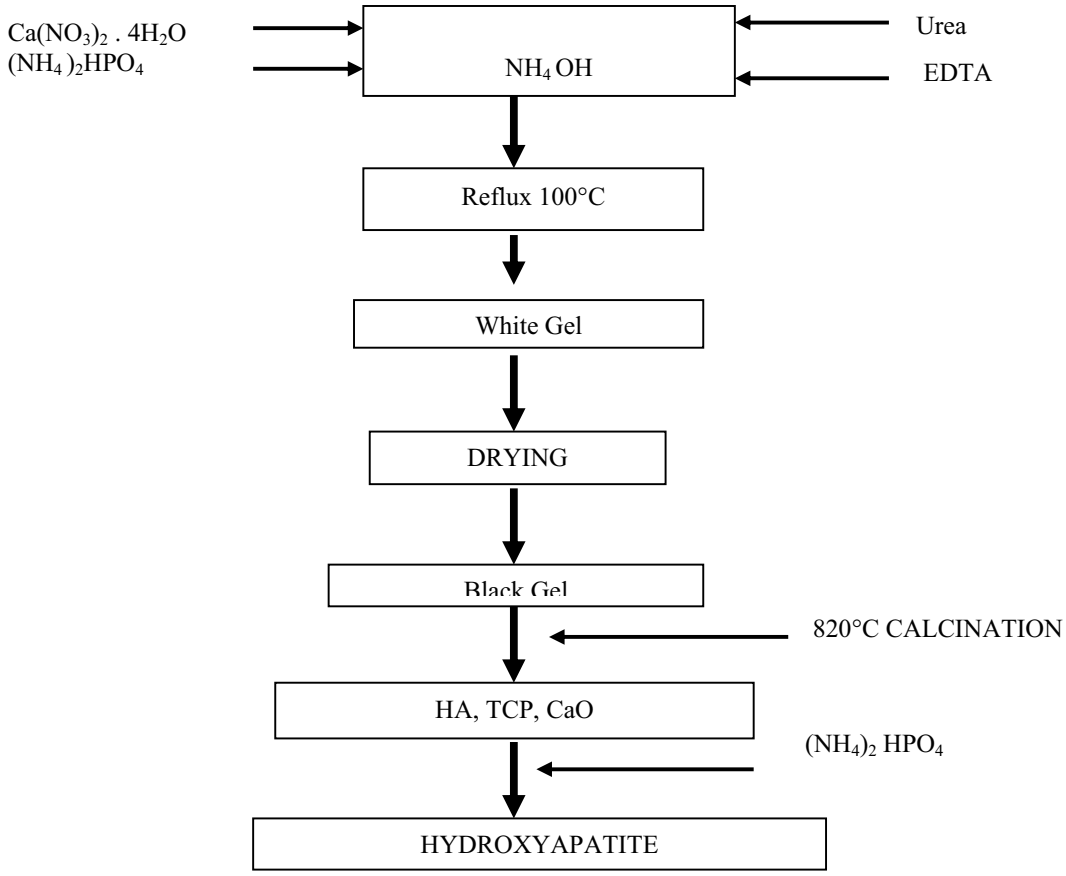

Figure 1: Scheme of Hydroxyapatite preparation via Sol-Gel Method.

It was observed 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 diammonium hydrogen phosphate, followed by suspending in water and heating at 90°C with rigorous stirring for ca. 4 h. This procedure has restored the Ca/P ratio of hydroxyapatite powder to 1.67. After drying for 15 h, pure hydroxyapatite powder was obtained.

Characterization

Scanning Electron Microscopic measurement for morphology evaluation of the powder was performed on a JEOL FESEM, JSM 6700F model. Specific surface area of the as-prepared HA powder was measured by BET nitrogen adsorption method (Quantachrom, Model 8201 PC). The measurement of particle size distribution was performed using back scattering method on a Malvern Instruments Nanosizer, Zen 1600 model. Differential and thermogravimetric analysis was performed on the dried gel in ambient air using a Perkin Elmer TG/DTA apparatus, Pyris Diamond model with a 10°C/min heating rate. 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^{-1}. XRD patterns obtained were utilized for quantitative phase analysis according to the manner already published elsewhere (Tampieri *et al,* 2000).

RESULTS AND DISCUSSION

Reaction of hydroxyapatite formation can be expressed as follows:

$$5Ca(NO_3)_2 + 3(NH_4)_2HPO_4 + 4NH_4OH \longrightarrow Ca_5(PO_4)_3OH + 10NH_4NO_3 + 3H_2O \quad (1)$$

The black dried gel obtained was then subjected to TG-DTA for thermal characterization. DTA-TG curves of the gel dried at 350°C (Figure 2) shows the first weight drop of 10% at 100°C due to water evaporation. A subsequent decrease in weight of *ca.* 50% occurs until 700°C which is attributed to decomposition and elimination of ammonia, nitrate, urea, organic compounds, and carbon dioxide.

There are two exothermic peaks in the curve; the first ranging from 350 – 420°C, is attributed to decomposition of ammonia and organic compounds. The second one, from 420 – 500 °C is a large exothermic peak which may be due to decomposition of urea and carbon dioxide. Subsequently, calcination of dried gel at 820°C for 2h under flowing air converted it into hydroxyapatite powder. The yields of the HA powders were 90 –95%. As listed in Table 1, the yields of the HA powders ranged between 25 to 26 grams (90~93%). Except for the result of Experiment I, all the results have showed good purity; 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 (Bertoluzza *et al,* 1995).

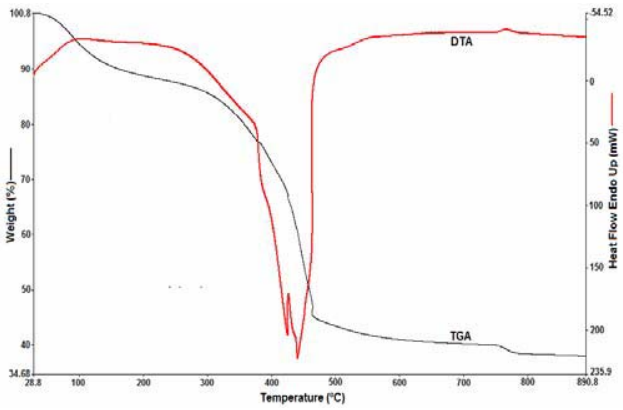

Figure 2: DTA-TG curve of the gel dried at 340°C.

The evaluation of crystalline phase of the powder was conducted by XRD. Figure 3 shows X-ray diffaction pattern of hydroxyapatite powders after the heat treatment at 820 °C. It is shown in the pattern that hydroxyapatite is the main component in the powder (ca. 75%).

Table 1. Result of Preparation of hydroxyapatite powder.

Run	Yield[a)](g)	Ca/P ratio[b)]	Purity of HA[b)]
I	53.1	1.655	93%[c)]
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 (Bertoluzza *et al,* 1995). Thus, HA powder from Exp. I was not used for further processing.

Calcium oxide and β-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, β-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. Into the suspension of the mixture HA, di-ammonium 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 possiblility of the presence of calcium hydroxide in the powder. A phenolphtalein test shown that no the hydroxide is present in our powder.

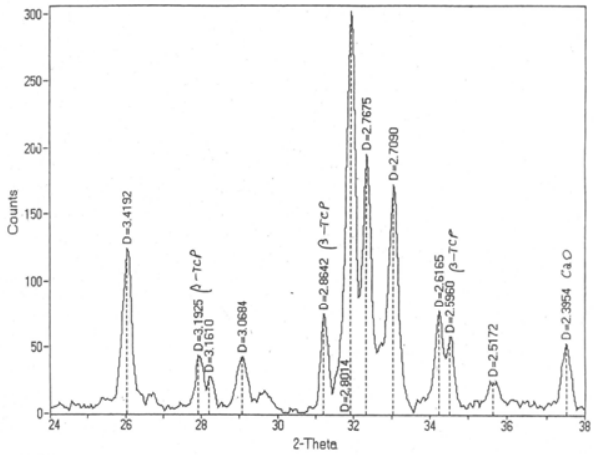

Figure 3: XRD pattern of hydroxyapatite powder mixed with TCP and CaO obtained before the purification process.

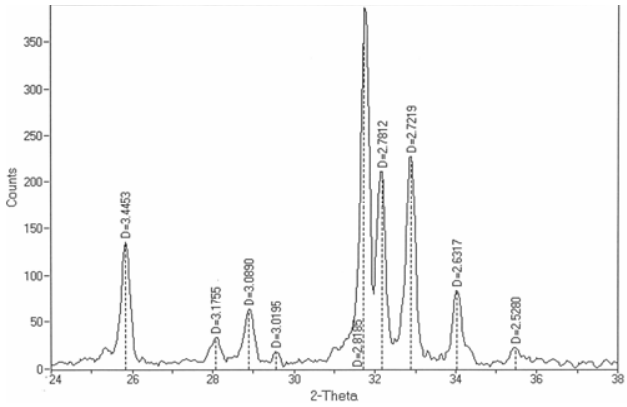

Figure 4: XRD pattern of pure hydroxyapatite powder obtained after the purification process.

Figure 5 shows SEM picture of the as-prepared HA powder. Individual hydroxyapatite particles formed in globular shape with an average size of about 50-200 nm in diameter. The nanometric primary particles agglomerated tightly into micrometric aggregates of various shape and size. On the other hand, the particle size distribution of the hydroxyapatite powder as measured by nanoparticle sizer (shown in Figure 6) shows two separate distributions: the lower distribution ranging from ca. 50 until 500 nm might attributed by individual particles and the higher distribution from 2000 to 7000 nm might attributed by tightly bonded particle agglomerates. It is likely difficult to disperse all agglomerates even after rigorous stirring for hours. On the other hand, the specific surface area measured by BET method gave a low value of 7 m^2/g. This value is unusual for particles as fine as hundreds nanomaters level; hence it is considered that the surface area measured by BET is 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 (Masuda *et al,* 990).

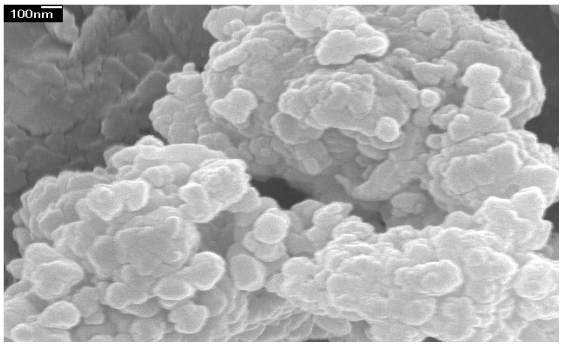

Figure 5: SEM photograph of hydroxyapatite powder.

Figure 7 shows the IR spectra of the dried gel and hydroxyapatite powder after calcinations at different temperatures. The FTIR spectrum of the dried gel shows broad peaks of amorphous products including carboxylic and carbonate (1400-1600 cm^{-1}), phosphate (500, 900-1100 cm^{-1}), amino (1400, 1600 and 3200 cm^{-1}) and acetate (2800, 2300 cm^{-1}) groups. When it was subjected to heat treatment, peaks of 3200 cm^{-1} amino group disappeared gradually and those of phosphate (900-1100 cm^{-1}) become sharper. FTIR spectrum of the powder after 800°C treatment shows the characteristic peaks corresponding to OH^- (630 and 3560 cm^{-1}) and $PO43^-$ (960, 1050, 1090 cm^{-1}) vibrations, together with weak bands of CO_3^{2-} group (870, 1415, 1450 and very weak 1540 cm^{-1}), which indicates that it is slightly B-carbonated hydroxyapatite (partial substitution of OH by

CO_3^{2-} group), as a consequence of the use of organic reagents in this procedure. At this stage, we have succedded in preparing hydroxyapatite (HA) powders with particles having nanosized structures suitable for medical applications. With extraordinarily fine individual particles, the sol-gel derived HA powders showed promising potentiality for use as raw materials for making any bioactive hard tissue prostheses either in porous or dense forms.

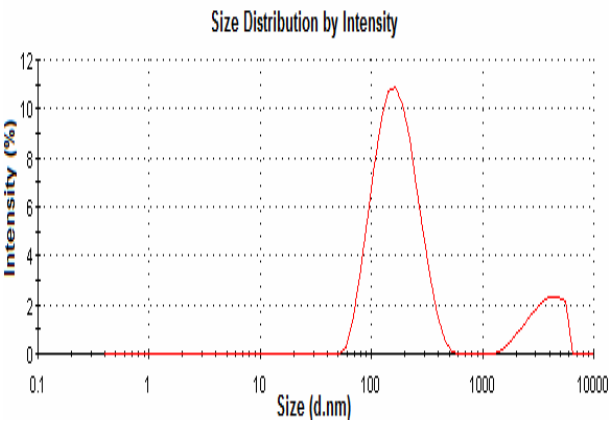

Figure 6: Distribution particles of hydroxyapatite powder as measured using nanoparticle sizer.

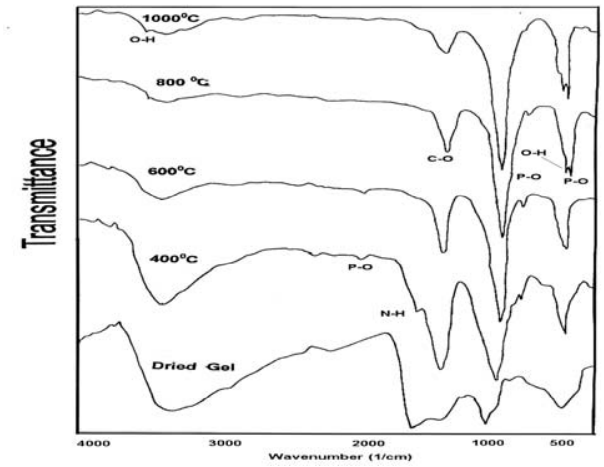

Figure 7: FTIR spectra of dried black gel and the sol-gel hydroxyapatite powder obtained after calcinations at temperatures of 400, 600, 800, and 1000°C.

CONCLUSIONS

Hydroxyapatite (HA) powders with particles having nano size were succesfully 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 (nearly 100%), thus fulfilling the medically required specification. The primary particulates have globular shape with a diameter of 50-200 nm in average, as detected by SEM and nanoparticle sizer. Hydroxyapatite powders we prepared using the sol-gel method showed promising potentiality for use as raw materials for making any bioactive hard tissue prostheses either in porous or dense forms.

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