DOUBLE STEP STIRRING: A NOVEL METHOD FOR PRECIPITATION OF NANO-SIZED HYDROXYAPATITE POWDER

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The effect of three different stirring techniques (magnetic, ultrasonic and double-step stirring) on the properties of hydroxyapatite (HA) prepared from calcium hydroxide $[Ca(OH)_2]$ and phosphoric acid $[H_3PO_4]$ was investigated. The employed precipitation processes were found to be suitable for the production of nano-sized pure HA powders. Sample characterization was achieved by powder X-ray diffraction (XRD), Fourier transformed infrared (FT-IR) transmission spectroscopy, scanning electron microscopy (SEM), density and surface area measurements. The double-step stirring technique played an important role to progress the homogenous reaction and the preparation of pure and fine HA powder.

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1. Introduction

Hydroxyapatite [HA: $Ca_{10}(PO_4)_6(OH)_2$] is well known as the mineral component of bones and teeth. Thus, it has a considerable interest in dental and medical research [1]. In the past few decades, HA was the subject of many scientific researches due to its high chemical and thermal stability, unique sorption properties and biocompatibility [2.3]. To produce high quality HA bioceramics for artificial bone substitution, ultrafine HA powder was usually employed. But it is difficult to make the high purity HA because of calcium phosphates have different derivates and the synthesis of calcium phosphates strongly dependent on the reaction conditions [4]. Up to the present, various techniques have been developed to synthesize hydroxyapatite powders such as acid-base reaction, precipitation, sol-gel method, hydrothermal method, mechanochemical synthesis, combustion method and various techniques of wet chemistry [5-12]. In these methods, wet chemical process was generally used to prepare HA powder because it is a simple and versatile economic route. Separately, during the reactions, the reaction media involves no foreign elements except water, the only by product. For these reasons, it is of great importance to develop inexpensive HA synthesis methods focused on the precise control of particle size, morphology and chemical composition [13-15]. However, it needs highly qualified and controlled parameters such as pH and temperature of the solutions, nature and composition of the starting materials, reagent's concentration and addition rate, stirring technique and stirring speed, maturation and presence of impurities to obtain HA monophase [16-17].

Chemical precipitation of nano-sized powders from salt solutions is among the simplest techniques for rapid synthesis of large amounts of material in a controlled manner [18].

The choice of the effective stirring technique is the main problem of chemical precipitation method [19, 20]. Hence, the different stirring methods were developed to prevent the particle agglomeration/aggregation and to precipitate the ultrafine particles which has highly HA

phase. Since the function of a stirrer is to agitate solutions for speeding up reactions or improving mixtures, these stirring methods were resulted in different particle sizes and homogenous solutions.

Generally, for HA synthesis, three stirring techniques are used: mechanical, magnetic and ultrasonic. In many previous studies mechanical [1, 2, 9, 10, 17] and magnetic [8, 9, 11, 15, 21] stirring techniques were used to precipitate hydroxyapatite powder. In the recent years, nano-sized particles gained importance in many fields. Thus, nanoparticles can be produced bottom-up in liquids by means of precipitation. In this case, a supersaturated mixture starts forming the solid particles out of the highly concentrated material, particles grows and finally precipitates. In order to achieve the convenient particle/crystal size and morphology, controlling the precipitation factors is essential.

In recent researches, various ultrasonic stirring techniques were used to obtain stoichiometric and highly pure HA powder [22, 23]. These techniques involve dissolution and precipitation of solids through particle size reduction and surface activation by intensive stirring. However, a double-step stirring technique (DSS: magnetic+ultrasonic) including magnetic stirring and ultrasonic irradiation techniques to synthesize hydroxyapatite has not been reported in the literature yet. In the present work, HA powders were synthesized by magnetic, ultrasonic and double-step stirring techniques, using $Ca(OH)_2$ and H_3PO_4 as raw materials and the effects of stirring types on the properties of HA powder were investigated.

2. Materials and methods

HA powder samples were prepared by wet chemical precipitation using different stirring techniques. Analytical grade Ca(OH)₂ (Merck, 96%) and H₃PO₄ (Merck, 99%) were weighted at molar ratio of Ca/P=1.67 and dissolved in distilled water to prepare homogenous solution. H₃PO₄ solution was added slowly to Ca(OH)₂ solution and stirred using the magnetic, ultrasonic and double-step techniques (Table 1).

Sample	Stirring technique	Stirring conditions	Density	SSA	Mean Particle
			(g/cm^3)	(m^2/g)	Size (µm)
M-HA	magnetic	300 rpm, 1hour	3.51	6.69	6.29
U-HA	ultrasonic	320W, 35KHz, 1hour	3.38	14.31	2.52
DSS-HA	Double-step stirring	(300 rpm, 1hour)	3.81	5.32	8.70
	(Magnetic+Ultrasonic)	+			
		(320W, 35KHz, 1hour)			

Table 1. Stirring techniques, stirring conditions and properties of hydroxyapatite powders.

Magnetic stirrer (Heidolph MR 3001, Germany) and ultrasonic apparatus (Bandelin Sonorex, Germany) have a stirring rate range of 0-1000 rpm and 35 KHz frequency and 160-320 W power, respectively. The activation temperature changed in the range of 25°C-60°C during the stirring processes and all stirring times were set to 60 minutes. The wet cakes were washed with distilled water three times and final pH values were measured by pH-meter (Inolab). Then the powder was dried with lyophilizator (Telstar Cryodos) (Fig. 1).



Fig. 1. Flow chart of the precipitation process.

The phase composition of prepared HA powders were analyzed by X-ray diffraction (XRD, Philips X-Ray Diffractometer). The specific surface areas of the precipitated powder samples were measured by a BET technique (Autosorb-1, Quantachrome Co., USA). Size distributions of the powder samples were analyzed by a particle size analyzer (Malvern Mastersizer 2000, UK). The chemical nature and molecular bond structure of the synthesized HA powder were determined by using FT-IR (Perkin-Elmer Spectrum One B V 5,01). The measuring resolution was 4 cm⁻¹ and interactions were performed for 256 times in the range of 450–4000 cm⁻¹. The sample compartment of the FT-IR spectrophotometer was purged with dry nitrogen. The morphology study performed on the "as prepared" HA particles using a scanning electron microscope (SEM: JEOL, JSM 5910 LV).

3. Results and discussion

Stirring techniques, stirring conditions and properties of hydroxyapatite powders are presented in Table 1 and XRD diffraction patterns of the samples are shown in Fig. 2. The XRD pattern of the precipitated powder using magnetic stirring technique (M-HA) at 300 rpm for 1 h is shown in Fig. 2a. This pattern clearly indicates that the precipitated particles have a crystal structure consisting of Ca(OH)₂, CaHPO₄ and HA phases. The powder precipitated using the ultrasonic stirring technique (U-HA) has $Ca(OH)_2$, CaHPO₄ and phases HA (Fig. 2b). When the magnetic and ultrasonic stirring techniques were used together (double-step stirring: DSS), the XRD spectrum of the powder (DSS-HA) included only HA phase (Fig. 2c). The sample precipitated by DSS technique did not contain any Ca(OH)₂ peaks as shown in Fig. 2c. The presence of Ca(OH)₂ peaks in the powder structure were attributed to especially weak stirring effect.



Fig. 2. XRD patterns of HA powders: (a) M-HA; (b) U-HA; (c) DSS-HA.

The possible chemical reactions for wet chemical process may be expressed as follows [22];

$$3Ca(OH)_2 + 3H_3PO_4 \rightarrow 3CaHPO_4 + 6H_2O \tag{1}$$

$$Ca(OH)_2 + 3H_3PO_4 \rightarrow \frac{1}{2}Ca_{10}(PO_4)_6(OH)_2 + 9H_2O$$
 (2)

 $2Ca(OH)_2 + 3CaHPO_4 + 6H_2O \rightarrow \frac{1}{2}Ca_{10}(PO_4)_6(OH)_2 + 9H_2O$ (3)

When the three stirring techniques were compared with each other, it seems that the reactions given in Eqs (1), (2) and (3) are dominant in magnetic and ultrasonic activation techniques while the reactions given in Eq. (2) and Eq. (3) are dominant in DSS activation technique. Fig. 2c shows lower HA crystallinity and a large amount of amorphous phase for DSS-HA sample. Some researchers reported that this is a good result because amorphous components would present improved biodegradation behaviour [15, 21]. XRD patterns of the M-HA and U-HA samples exhibited crystalline structures (Fig. 2a and 2b), because the crystalline structure has narrower peaks in XRD spectrums [24].

In Fig. 2a and Fig. 2b two major peaks observed at 2 Θ angles of 26.3 and 30.2 degrees as a result of the magnetic activation corresponding to the (002) and (120) peaks of CaHPO₄ phase, respectively while the major peaks at 28.9 degrees correspond to HA phase. In Fig. 2c the most prominent peak is situated at ~31.8 degrees, corresponding to HA peak (211) and no other phases observed. This suggests that pure HA phase have been formed as a result of the DSS activation.

BET analysis of the powders is given in Table 1. Specific surface area (SSA) value of M-HA powder was 6.69 m²/g. DSS-HA sample showed the lowest surface area as 5.32 m²/g. However, U-HA powder had the highest surface area value of $14.31m^2$ /g. Although DSS-HA powder had the highest HA content, it showed the lowest surface area according to the results of the BET analysis.

Fig. 3 and Table 2 show particle diameters of samples which were determined with particle size analyzer. DSS-HA and U-HA showed bimodal particle size distributions (Fig. 3a and 3b), while sample M-HA had a unimodal particle size distribution (Fig. 3c).



Fig. 3. Particle size distribution of HA powders: (a) M-HA; (b) U-HA; (c) DSS-HA.

In general, the formation of agglomerated HA particles can be explained by nucleation–aggregation–agglomeration-growth mechanism [1]. The powder densities detected by ultrapicnometer were between 3.3 and 3.8 g/cm³. The densest sample was DSS-HA (3.8 g/cm^3). This sample had also the highest particle size. These results can be attributed to the strong agglomeration in the DSS-HA sample.

	First peak			Second peak		
Sample	Range (µm)	Peak (µm)	Volume (%)	Range (µm)	Peak (µm)	Volume (%)
M-HA	1.0-60	15	100	_	_	_
U-HA	0.8-10	2	82	10.0-90	20	18
DSS-HA	1.0-10	5.0	49	20-400	80	51

Table 2. Mean parameters of particle size for hydroxyapatite powders.

BET (Table 1) and laser particle size (Table 2) analyzes exhibited micro-sized HA powders although nano-sized particles were seen in SEM images for the same powders (Fig. 4 and Fig. 5). The spherical particle shapes of these powders were also evaluated on these SEM micrographs. The SEM micrographs of M-HA and U-HA powders display the particles with size above 100 nm as shown in Fig. 4A and Fig. 4B. DSS-HA powder exhibits a particle size at about 50 nm (Fig. 4C and Fig. 5). M-HA, U-HA and DSS-HA powders consist of agglomerates. Micro-sized particles occurred as a result of strong agglomeration of nano-sized particles. This strong agglomeration was developed because of the water that could not be removed with freeze drying process. Freeze drying process has two stages; at the first stage physical water is removed and at the second stage chemical water is removed from the sample.



Fig. 4. SEM micrographs of HA powders: (A) M-HA, (B) U-HA and (C) DSS-HA (a: x5000; b: x50000).



Fig. 5. SEM micrographs of DSS-HA powder (x100000).

FT-IR plot is also showed water existence in all three samples that caused agglomeration of nano-sized particles (Fig. 6). This existence of water can be attributed to insufficient freeze drying time. Freeze drying method was preferred for this study because it provides controlling the particle size, crystallinity and microporosity. At low temperatures, no crystallization or further reaction occurs. Thus, the lyophilized powder retains the reactive sites and amorphous crystalline state of the precipitate [25].



Fig. 6. FT-IR spectrum of the powders precipitated using different stirring techniques: (a) M-HA; (b) U-HA; (c) DSS-HA.

Fig. 6 illustrates the FT-IR spectrum of HA powders synthesized by different stirring techniques and Table 3 shows the wave numbers in the FT-IR spectra of chemical groups belonging to precipitated HA samples obtained with various stirring techniques [2, 5, 8, 9, 11, 16, 23]. Diffusive reflectance of the IR was measured for powders diluted with KBr powder of spectroscopic grade by one-tenth background noise was corrected with pure KBr data. OH liberation band (628 cm⁻¹) and a broad hydrated OH peak (3436, 3437, 3461 and 3425 cm⁻¹) that thought to be sourced by water absorbed from media can be seen from the figures of three stirring techniques clearly (Fig. 6). The peaks obtained for HPO₄²⁻ groups were at around 718-720 cm⁻¹ and 1164-1143 cm⁻¹ wave numbers for M-HA and U-HA samples while no such peaks were seen for DSS-HA. There are also several characteristic sharp peaks at 1085-1092; 1033-1035; 1000; 933-962; 500-611 cm⁻¹ come from PO₄³⁻. Here, PO₄³⁻ and HPO₄²⁻ indicate hydroxyapatite existence in the powders. As a result of FT-IR spectrums of the samples, it can be concluded that the consistence of hydroxyapatite in DSS-HA powder is much more pronounced than that in M-HA and U-HA powders.

Chemical bonding for HA	Wave number (cm ⁻¹)
Hydrated O-H	3436-3461; 2924-2926
О-Н	1642; 628.31
HPO ₄ ²⁻	1140–1164.7; 718.5–720
PO ₄ ³⁻	1085–1092; 1033–1035; 1000; 933–962; 560–602.5

Table 3. The wave numbers in the FT-IR spectra of chemical groups belonging to precipitated HA samplesobtained at various stirring techniques from Ref.s 2, 5, 8,9, 11, 16 and 26.

4. Conclusion

The HA powders can be synthesized from aqueous suspensions containing H_3PO_4 and $Ca(OH)_2$ with an aid of magnetic, ultrasonic and double-step stirring (magnetic + ultrasonic stirrings) techniques under atmospheric pressure and room temperature. The present study compared the powder properties of hydroxyapatite prepared by wet chemical method using these stirring techniques.

XRD analysis and FT-IR spectrums of the samples showed that hydroxyapatite consistence of DSS-HA powder is much more than M-HA and U-HA powders. HA particles

exhibited a spherical shape with a particle size below 100 nm. Hydroxyapatite powder prepared with double-step stirring technique (DSS-HA) had the highest HA content and was nano-sized.

HA powder prepared using double-step stirring technique exhibited better chemical composition; therefore it can be applied to HA synthesis.

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