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# Effect of Different Calcium Precursors on Biomimetic Hydroxyapatite Powder Properties

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Hydroxyapatite is the main component of human hard tissues such as bones and teeth. Because it has a great biocompatibility with human organism, it is used as biomaterial with the purpose of form and repair hard tissues. Hydroxyapatite is formulated as $Ca_{10}(PO_4)_6(OH)_2$ and shows high stability under physiological conditions. In this study, biomimetic hydroxyapatite powder has been synthesized using by synthetic body fluids which has the same composition as human blood plasma. Moreover, the effect of different precursors on properties of synthesized powders has been investigated. For that purpose calcium nitrate tetrahydrate [$Ca(NO_3)_2{\cdot}4H_2O$], calcium chloride [$CaCl_2$], calcium hydroxide [$Ca(OH)_2$] and diammonium hydrogen phosphate [$(NH_4)_2HPO_4$] were used as precursors. Mainly literature focused on the synthesis of hydroxyapatite powders was carried out using chemical method. However, in this study, the synthesis of hydroxyapatite powder is carried out using biomimetic method. Chemical structures of synthesized powders have been investigated by the Fourier transform infrared and X-ray diffraction methods. Results showed that synthesized powders have a pure hydroxyapatite structure. Surface area of the synthesized powders was measured by the Brunauer–Emmett–Teller method. Morphological structures have been characterized by using scanning electron microscopy. Furthermore, particle size of powders was calculated using the Brunauer–Emmett–Teller method. It was clearly seen that morphological properties of biomimetic hydroxyapatite have affected the use of different calcium precursors.

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## 1. Introduction

Hydroxyapatite (HA) is the main inorganic phase of human bones and teeth [1–3] which consist of nearly 72 wt% [4] and 96 wt% [5] apatitic materials, respectively. Calcium to phosphate ratio of HA is 1.67 [1]. Due to its biocompatibility and bioactivity [5], HA does not exhibit any cytotoxic effects. Moreover, HA can be directly bound to the living tissues. According to these characteristic properties HA has had wide acceptance in medicine and dentistry recently [2, 6, 7]. Unfortunately, due to its low mechanical properties, especially in wet environments, HA cannot be applicable to use for heavy load bearing applications, like artificial teeth or bones [5].

HA powders can be synthesized using a number of techniques including sol–gel processing, emulsion techniques, hydrothermal process, mechanochemical and co-precipitation techniques [8]. The last one is the most useful and easy technique to synthesize hydroxyapatite [9]. This technique is based on precipitation of hydroxyapatite in aqueous solution. It is known that calcium hydroxyapatite is the least soluble and the most stable calcium phosphate in the aqueous solution at pH values higher than 4.2. HA has been synthesized either in neutral or highly alkaline media to ensure the thermal stability of the formed phase after high temperature sintering [9, 10]. Since simulated body fluids (SBF) have almost the same ionic concentration as extracellular human blood plasma, it has been used instead of aqueous solution to precipitate hydroxyapatite powders in recent years. In the literature this technique is called biomimetic method. Biomimetic method offers more bioactive, biocompatible and stable products by comparison with the conventional precipitation method. It is also possible to improve the properties of HA ceramic by controlling important parameters of powder precursors such as particle size and shape, particle distribution and agglomeration [11].

## 2. Experimental

Before preparing SBF solution Chitsiku et al. [12] offered cleaning procedure to obtain more stabilized SBF solutions. For that purpose each material (such as beaker, flask etc.) were cleaned according to his recipient. SBF solutions were prepared according to the Tas SBF solution [13]. The reagents used to prepare one liter of SBF are NaCl (6.547 g/L, 99.5%), $NaHCO_3$ (2.268 g/L, 99.5%), KCl (0.373 g/L, 99.0%), $Na_2HPO_4{\cdot}2H_2O$ (0.178 g/L, 99.5%), $MgCl_2{\cdot}6H_2O$ (0.305 g/L, 99.0%), $CaCl_2{\cdot}H_2O$ (0.355 g/L, 99.0%), $Na_2SO_4$ (0.355 g/L, 99%), $(CH_2OH)_3CNH_2$ (6.057 g/L, 99.5%), and HCl (37 vol.%). All reagents were purchased from Merck. Reagents were added one by one, after each reagent was completely dissolved in water with given order. Also after $MgCl_2{\cdot}6H_2O$ and $(CH_2OH)_3CNH_2$ were dissolved, desired amount of 1 M HCl (37 wt% Merck) solution was added to adjust pH value 7.4 [13].

Calcium nitrate tetrahydrate [$Ca(NO_3)_2 \cdot 4H_2O$], (CNT) calcium chloride [$CaCl_2$] (CC), calcium hydroxide [$Ca(OH)_2$] (CH), and diammonium hydrogen phosphate [$(NH_4)_2HPO_4$] (DHP) were supplied from Sigma-Aldrich and used as calcium and phosphorus precursors. Firstly, 0.167 M calcium nitrate tetra (CAT) and 0.1561 M DHP solutions were prepared in SBF. Then DHP solutions were added to CAT solutions drop by drop. The final suspension was called S1. The same experiments were carried out using CC and CH-calcium precursors and final suspensions were called S2 and S3, respectively. Ca/P ratio of HA in the all suspensions was calculated as 1.67. Finally, these suspensions were aged for a day at 37°C. Following the aging process precipitates were filtered and washed with ultrapure water. The resulting precipitates were dried at 80°C for 8 h and then ground using agate mortar. Dried powders were sintered in air atmosphere at 900°C for 2 h with 10°C/min heat rate in a kiln oven (Protherm furnaces). Heat treated powders were called S1S, S2S, and S3S, respectively.

Chemical structures of synthesized powders have been examined by the Fourier transform infrared (FTIR) technique (Perkin-Elmer Spectrum-100) and X-ray diffraction (XRD) (Shimadzu, XRD-2000, TUBITAK, MAM). Surface area measurements and morphological structures have been characterized by using the Brunauer–Emmett–Teller (BET) (Quardrosorb SI, Malvern) method and scanning electron microscopy (SEM) (JEOL-JSM-6335F, TUBITAK, MAM) analysis, respectively. Also ultrapicnometer (Quardrosorb Ultrapyc 1200e, Malvern) was used to measure the density of dried and sintered powders. According to BET measurement particle sizes of the powders were calculated. As a result, using different precursors has affected the particle size and morphological properties of BHA.

## 3. Result and discussion

The reactions involved in the formation of HA during the precipitated from SBF solutions can be expressed as follows:

$$10Ca(NO_3)_2 \cdot 4H_2O + 6(NH_4)_2HPO_4 + 20NH_4OH \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 12NH_4NO_3 + 8HNO_3, \quad (1)$$

$$10CaCl_2 + 6(NH_4)_2HPO_4 + 8NH_4OH \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 20NH_4Cl + 6H_2O, \quad (2)$$

$$10Ca(OH)_2 + 6(NH_4)_2HPO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O + 12NH_3. \quad (3)$$

The FTIR spectra of dried and sintered powders are plotted in Fig. 1. $OH^-$ at 3580 cm$^{-1}$ and $PO_4^{3-}$ at 630 cm$^{-1}$ are characteristic peaks of HA structure. However, band position of $OH^-$ was not clearly seen in Fig. 1 because of its low concentration in the structure. Also 1020 cm$^{-1}$, 960 cm$^{-1}$ and 1093 cm$^{-1}$ are referred to $PO_4^{3-}$ groups in the HA. A very small hump at the wave number 1454 cm$^{-1}$ in this figure arose from vibrations of $CO_3^{2-}$ ions. However, carbonate peaks become insignificant with sintered powders. The 1454 cm$^{1-}$ peak is the characteristic bond of $CO_3^{2-}$ groups. If $CO_3^{2-}$ group substitution with $OH^-$ groups of HA resulting powders called as A-type hydroxyapatite and substation with $PO_4^{3-}$ sites in the apatite structure is formed B-type hydroxyapatite. At 900°C, the intensity of the $CO_3^{2-}$ peaks at 1454 and 1414 cm$^{-1}$ was decreased. This could be interpreted as a release of carbonate from $PO_4^{3-}$ positions in the apatite structure.

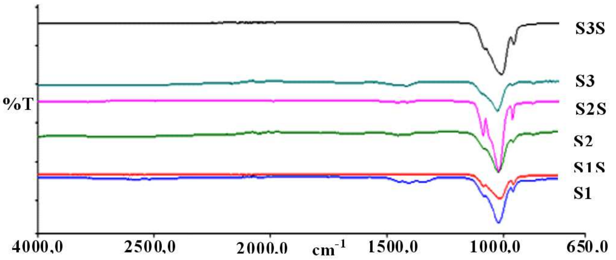

Fig. 1. The FTIR spectrum of dried samples at 80°C (S1, S2, S3) and sintered samples at 900°C (S1S, S2S, S3S).

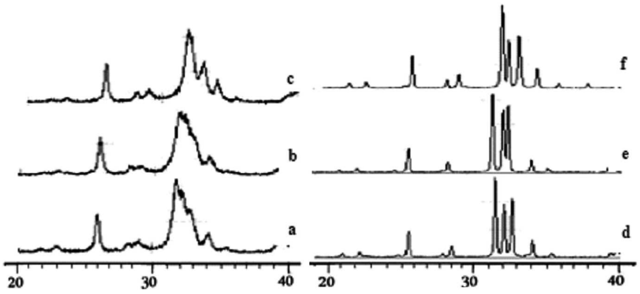

Fig. 2. XRD spectrum of HA samples (a) S1 at 80°C--dried, (b) S2 at 80°C-dried, (c) S3 sample 80°C-dried, (d) S1S at 900°C-sintered, (e) S2S at 900°C-sintered, (f) S3S at 900°C-sintered.

Figure 2 shows the XRD patterns of HA powders. HA peaks occurred at 2$\theta$ of 28° and 32°, which are consistent with the standard XRD peaks for HA. The XRD patterns of all the samples indicate that the main phase is HA, even if literature said that HA could be synthesized with some impurities at initial pH values higher than 10. According to some experiments, the phase of crystal prepared at initial pH 8.0 was most of $\beta$-three calcium phosphate (TCP), with weak diffraction peaks of HA [14]. To compare with this result, our investigation proved that pure HA could be synthesized at pH 8.0. Moreover, XRD patterns of synthesized amorphous HA structure could be turned to crystalline phase when it had been treated at higher temperature without any or negligible transition. Also it can be clearly seen that S1, S1S and S3, S3S had the same patterns and some impurities associated with $\beta$-TCP. However S2 and S2S

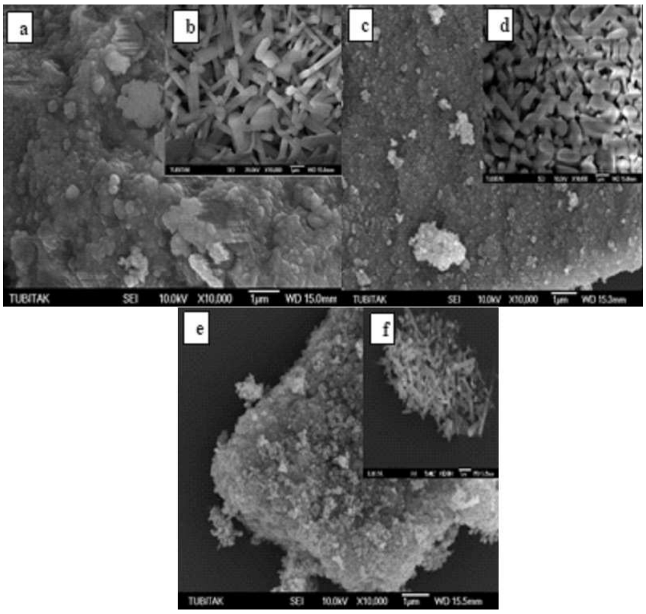

Fig. 3. SEM micrographs of synthesized powders: (a) S1 at 80°C-dried, (b) S1S at 900°C-sintered, (c) S2 at 80°C-dried, (d) S2S at 900°C-sintered, (e) S3 at 80°C-dried, (f) S3S at 900°C-sintered.

had a pure structure which means that the precursors could affect the final powders purity of synthesized HA.

The morphology of the HA powders was investigated by SEM. Typical SEM micrographs of 80°C-dried and 900°C-calcined samples was given in Fig. 3. These powders showed a significant level of agglomeration and amorphous properties without heat treatment at 900°C. However, S1 powders have a very fine particle size when it is compared with the other powders. On the other hand, S1S and S3S samples had more regular and rod-like structure unlike the irregular structure of S2S powders.

Table shows the specific surface area of the powders. According to these measurements mean size of the HA particles was calculated by the following equation [15]:

$$\text{particle size} = 6/(\rho S_{\text{BET}}), \tag{4}$$

where $\rho$ is the density of the pure HA sample (3.16 g/dm$^3$) and $S$ refers to specific surface area of powders obtained from BET method. The result showed that all the grain samples particle size was the nanometer scale and powders could be densified with heat treatment at high temperature. As it could be seen in Table, the S2S sample has smallest particle size and reached exactly the same density as HA when sintered at 900°C.

## 4. Conclusion

Nanosized and chemically homogeneous hydroxyapatite powders have been precipitated by using calcium nitrate tetrahydrate, calcium chloride and calcium hydroxide with diammonium hydrogen phosphate in simulated body fluid solutions, at the physiological and biomimetic conditions of pH 8.0 and 37°C. By comparison with calcium precursors effect, by using calcium chloride we would be able to produce pure HA structure at both low and high temperature. Moreover, synthesized biomimetic HA powder has the smallest particle size and the same density as the commercial HA. It was concluded that different precursors affect the HA structure and using different combinations could be a useful method to obtained such samples with appropriate properties. Also this study proved that higher pH values are not necessary to obtain pure HA. As we know there are some studies focused on synthesis of HA at low pH values. This study has proved that HA powders could be easily synthesized at low pH values. It is a promising technique to obtain more biocompatible HA structure for biomedical applications.

TABLE

Surface area, density and particle size values of green and sintered samples.

| Sample | Surface area [m$^2$/g] | Density [g/cm$^3$] | Particle size [nm] |
|---|---|---|---|
| S1 | 86.688 | 2.78 | 21.85 |
| S2 | 121.461 | 2.73 | 15.63 |
| S3 | 86.084 | 2.75 | 22.06 |
| S1S | 2.551 | 3.18 | 744.31 |
| S2S | 2.072 | 3.16 | 916.38 |
| S3S | 4.807 | 3.21 | 394.99 |

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## References

[1] H. Baumann, K. Bethge, G. Bilger, D. Jones, I. Symietz, *Nucl. Instrum. Methods Phys. Res. B* **196**, 286 (2002).

[2] F.N. Oktar, *Ceram. Int.* **33**, 1309 (2007).

[3] J. Song, Y. Liu, Y. Zhang, L. Jiao, *Mater. Sci. Eng. A* **528**, 5421 (2011).

[4] C. Yang, Y. Guo, M. Zhang, *Trans. Nonferrous Met. Soc. China* **20**, 254 (2010).

[5] I. Mobasherpour, M.S. Heshajin, A. Kazemzadeh, M. Zakeri, *J. Alloys Comp.* **430**, 330 (2007).

[6] P. Tschoppe, D.L. Zandim, P. Martus, A.M. Kielbassa, *J. Dentistry* **39**, 430 (2011).

[7] E. Menendez-Proupin, S. Cervantes-Rodriguez, R. Osorio-Pulgar, M. Franco-Cisterna, H. Camacho-Montes, M.E. Fuentes, *J. Mech. Behav. Biomed. Mater.* **4**, 1011 (2011).

[8] S.K. Padmanabhan, A. Balakrishnan, M.C. Chu, Y.J. Lee, T.N. Kim, S.J. Cho, *Particuology* **7**, 466 (2009).

[9] D. Bayraktar, A.C. Tas, *J. Europ. Ceram. Soc.* **19**, 2573 (1999).

[10] A.C. Tas, *J. Europ. Ceram. Soc.* **20**, 2389 (2000).

[11] S. Best, W. Bonfield, *J. Mater. Sci. Mater. Med.* **5**, 516 (1994).

[12] C. Ohtsuki, T. Miyazaki, M. Kamitakahara, M. Tanihara, *J. Eur. Ceramic Society* **27**, 1527 (2007).

[13] A.C. Tas, Turkish Patent Institute No. 99/0037, 11 January 1997.

[14] M. Goubin, L.Y. Xiang, *Crystal Growth Design* **9**, 2991 (2009).

[15] S.J. Roll, M.Sc. Thesis, National Inst. of Tech., Rourkela 2010.