

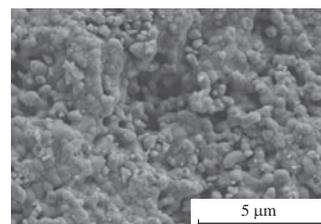
Everything old is new again: a reinspection of solid-state method for the fabrication of high quality calcium hydroxyapatite bioceramics

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DOI: 10.1016/j.mencom.2019.05.010

The simple, fast and cost-efficient solid-state synthesis was developed for the preparation of calcium hydroxyapatite bioceramics. The obtained materials were characterized by powder X-ray diffraction analysis, IR spectroscopy, and scanning electron microscopy. The samples prepared in the temperature range of 800–1000 °C were almost single-phase (*i.e.*, only minor impurities were observed) high-crystalline calcium hydroxyapatites.



Synthesized hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$

The basic bone mineral is calcium hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ (CHAp) possessing the Ca:P ratio of 1.67. Hence, synthetic CHAp has received great attention in medical applications.^{1–5} Bioactivity of CHAp depends on its morphology, particle size, and phase purity, which consequently depend on the synthesis route.^{5–9} Variations in synthetic methods and conditions affect the formation of impurities and additional products. For example, common impurity phases in synthetic apatites prepared *via* a precipitation from supersaturated aqueous solutions are calcium phosphate compounds, such as amorphous calcium phosphates of variable compositions $\text{Ca}_3(\text{PO}_4)_{2-2x}(\text{HPO}_4)_{3x} \cdot n\text{H}_2\text{O}$, octacalcium phosphate $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$, and calcium hydrogen phosphate dihydrate $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$.

CHAp powders can be obtained *via* a solid state reaction, wet synthesis, precipitation, sol-gel, and microwave assisted and hydrothermal methods.^{10–12} The solid state reaction is a relatively simple and chemically hazard free process, which can yield large

amounts of material with desired structure and properties.¹³ This procedure relies on diffusion of ions among powder raw materials and thus requires a high temperature processing (1200–1300 °C) to initiate the reaction.¹⁴ However, the solid state process for the CHAp powders exhibits a low reproducibility, therefore, some sophisticated improvements were also implemented. The solid state protocols, which comprise ball milling and pulse electric discharge, or microwave-assisted solid state reaction, or computer modelling, were developed.^{15–17} The solid state reaction often provides biphasic bioceramics in addition to the risk of contamination during the milling or mechanochemical processing.^{18–20}

This work was mainly aimed at the demonstration of the simplicity and cost-effectiveness of classical solid-state synthesis method for the production of calcium hydroxyapatite bioceramics by the selection of appropriate starting materials and sintering temperature.

Figure 1 shows the XRD patterns of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ synthesized by solid-state method at different temperatures.[†] The recorded patterns are in a good agreement with the reference data for $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ (PDF [72-1243]). As one can see, the XRD pattern of sample prepared at 800 °C revealed the formation of almost monophasic CHAp. Only one very weak reflection at $2\theta \approx 37^\circ$ attributable to a CaO phase (PDF [37-1497]) could be detected. The increase in annealing temperature up to 900 °C led

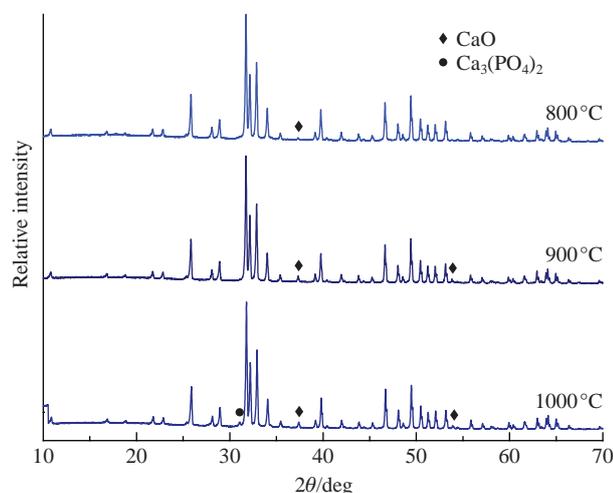


Figure 1 XRD patterns of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ synthesized at different temperatures. The impurity phases are marked therein.

[†] $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ samples were synthesized by the conventional solid-state method. The starting materials, $\text{Ca}(\text{AcO})_2 \cdot \text{H}_2\text{O}$ (5.285 g) and $\text{NH}_4\text{H}_2\text{PO}_4$ (2.066 g), were carefully mixed in agate mortar with acetone (30 ml) for ~20 min until the evaporation of solvent. The Ca:P molar ratio of 1.67 was always maintained in the synthesis. The powders were preheated at 600 °C for 5 h with a heating rate of 1 K min⁻¹. The intermediate powders were annealed at three different temperatures (800, 900 and 1000 °C) for 8 h with a heating rate of 2 K min⁻¹. The obtained products were characterized by powder X-ray diffraction (XRD) analysis using a Rigaku MiniFlex II diffractometer working in Bragg–Brentano ($\theta/2\theta$) geometry. SEM images were obtained on a Hitachi SU-70 microscope. IR spectra were recorded using a PerkinElmer Frontier FTIR spectrometer.

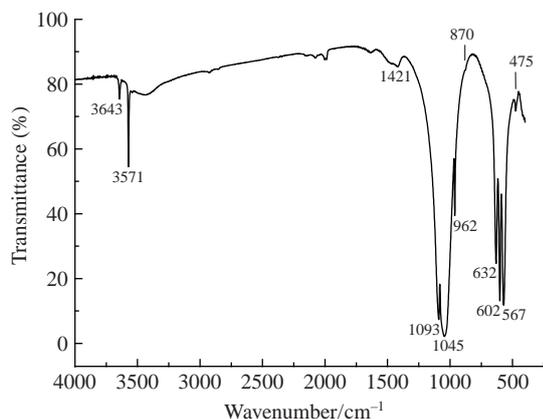


Figure 2 FTIR spectrum of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ prepared at 900 °C.

to a slightly increased amount of CaO phase. At 1000 °C, a minor amount of calcium phosphate $\text{Ca}_3(\text{PO}_4)_2$ (PDF [29-359]) phase (reflection at $2\theta \approx 30.7^\circ$) along with calcium oxide has been formed. This could be caused by changes in equilibria between the major calcium phosphate compounds, such as calcium hydroxyapatite, monocalcium phosphate, dicalcium phosphate, amorphous tricalcium phosphate, octacalcium phosphate, α - and β -tricalcium phosphates, and tetracalcium phosphate.²¹ Thus, the XRD results revealed that the calcination products of mechanical mixture of $\text{Ca}(\text{AcO})_2 \cdot \text{H}_2\text{O}$ and $\text{NH}_4\text{H}_2\text{PO}_4$ obtained at different temperatures within the range 800–1000 °C were very similar, *e.g.*, the temperature increase from 900 to 1000 °C resulted in just one more compound appeared in the composition. The CHAp sample synthesized at 800 °C exhibited the highest phase purity. The lattice parameters of the synthesized $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ were obtained from the diffraction pattern by fitting the peaks of identified reflections. The hexagonal lattice parameters and cell volume determined for the sample obtained at 800 °C were found as $a = 9.423(3)$ Å, $c = 6.880(2)$ Å, and $V = 529.05(3)$ Å³, which correspond to the literature data for a stoichiometric calcium hydroxyapatite. The XRD results have clearly indicated that small amounts of calcium oxide and calcium phosphate were formed additionally to the main CHAp phase, however they are non-toxic and compatible to be used as a bone substitute material with sufficient cell proliferation and alkaline phosphatase activity.^{22,23}

FTIR spectroscopy is highly sensitive to the impurities and substitutions in the structure of apatite.⁸ However, all the three FTIR spectra of samples obtained in the temperature range of 800–1000 °C were almost identical. Figure 2 shows the representative FTIR spectrum of CHAp sample prepared at 900 °C. In this spectrum, a band of the asymmetric stretching vibration of the phosphate group at 1000–1100 cm^{-1} dominates. In the region of symmetric stretching vibration of phosphate group at 940–970 cm^{-1} , the peak at 962 cm^{-1} characteristic of β - $\text{Ca}_3(\text{PO}_4)_2$ could be observed. The peaks assigned for carbonate substitution in the apatite structure at 1400–1550 cm^{-1} are weakly expressed that is characteristic of apatites calcined at ≥ 1000 °C.

Table 1 IR signals and their assignments for the calcium hydroxyapatite obtained at 900 °C.^a

Signal position, ν/cm^{-1}	Assignment	Phase
3643	OH ⁻	Ca(OH) ₂
3571	ν_{S} OH ⁻	CHAp
632	ν_{L} OH ⁻	CHAp
1476, 1421	ν_3 CO ₃ ²⁻	CCAp
870	ν_2 CO ₃ ²⁻	CCAp
1093, 1045, 1011	ν_3 PO ₄ ³⁻	β -TCP, CHAp
1063	ν_3 PO ₄ ³⁻	β -TCP
962	ν_1 PO ₄ ³⁻	β -TCP, CHAp
956	ν_1 PO ₄ ³⁻	β -TCP
622, 566	ν PO ₄ ³⁻	β -TCP
602, 567	ν_4 PO ₄ ³⁻	CHAp
505	ν_2 PO ₄ ³⁻	β -TCP
727, 944, 1188, 1213	ν_1 – ν_4 PO ₄ ³⁻	β -TCP

^aTCP is tricalcium phosphate.

The peak wavenumbers and their assignments for the calcium hydroxyapatite samples obtained at 900 °C are summarized in Table 1. The FTIR spectra support the XRD results, since the absorbing bands indicate the formation of a typical CHAp structure containing sharp O–H and P–O peaks.^{24,25}

The morphology of all the synthesized CHAp samples was examined using scanning electron microscopy (SEM). Interestingly, their surface morphologies are very similar regardless of the synthesis temperature (Figure 3). One can see that CHAp powders consist of volumetric spherical particles varying in size from approximately 200 to 300 nm (at 800 °C) and from 200 to 400 nm (at 900–1000 °C). Thus, the submicronic nature of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ powders with the narrow size distribution of crystallites was observed even in the case of solid-state fabrication of this compound. The good connectivity between the grains indicates the formation of micrograin networks and shows a high level of agglomeration of particles. These morphological features of synthesized CHAp promote the formation of different bioactive and biocompatible composites and glasses.^{26–30}

In conclusion, we have developed the simple solid-state synthetic route to obtain calcium hydroxyapatite bioceramics from calcium acetate monohydrate [$\text{Ca}(\text{AcO})_2 \cdot \text{H}_2\text{O}$] and ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$). The polycrystalline single-phase CHAp has been prepared by annealing the homogenized and preheated mixture of starting materials at 800 °C for 8 h. The hexagonal lattice parameters and cell volume were determined. The phase purity of prepared ceramics was found being slightly dependent on the synthesis temperature in the range of 800–1000 °C. The CHAp materials obtained at higher temperatures contained the minor amount of CaO and/or $\text{Ca}_3(\text{PO}_4)_2$ as secondary phases. The FTIR spectra fully supported the XRD results. The SEM measurements demonstrated that CHAp powders consist of volumetric spherical particles with the narrow size distribution. Therefore, the reported results can be of practical interest due to

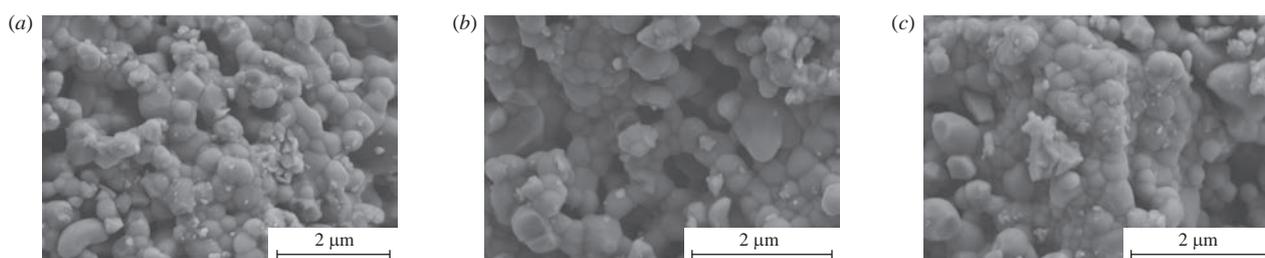


Figure 3 SEM micrographs of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ synthesized at various temperatures: (a) 800, (b) 900 and (c) 1000 °C.

the synthesis simplicity, cost-efficiency and importance of CHAP materials for real medicinal applications.

This work was supported by the National Research Programme ‘Healthy ageing’ (SEMAT, grant no. SEN-02/2016) from the Research Council of Lithuania. The authors are grateful to Dr. Irma Bogdanoviciene for helpful discussions and technical assistance.

References

- 1 S. R. Paital and N. B. Dahotre, *Mater. Sci. Eng., R*, 2009, **66**, 1.
- 2 L. L. Hench, *J. Am. Ceram. Soc.*, 1991, **74**, 1487.
- 3 E. Mohseni, E. Zalnezhad and A. R. Bushroa, *Int. J. Adhes. Adhes.*, 2014, **48**, 238.
- 4 S. M. Best, A. E. Porter, E. S. Thian and J. Huang, *J. Eur. Ceram. Soc.*, 2008, **28**, 1319.
- 5 J. Cizek and K. A. Khor, *Surf. Coat. Technol.*, 2012, **206**, 2181.
- 6 E. Garskaite, L. Alinauskas, M. Drienovsky, J. Krajcovic, R. Cicka, M. Palcut, L. Jonusauskas, M. Malinauskas, Z. Stankeviciute and A. Kareiva, *RSC Adv.*, 2016, **6**, 72733.
- 7 R. A. Surmenev, M. A. Ryabtseva, E. V. Shesterikov, V. F. Pichugin, T. Peitsch and M. Epple, *J. Mater. Sci.: Mater. Med.*, 2010, **21**, 1233.
- 8 E. Garskaite, K.-A. Gross, S.-W. Yang, T. C.-K. Yang, J.-C. Yang and A. Kareiva, *CrystEngComm*, 2014, **16**, 3950.
- 9 P. Usinskas, Z. Stankeviciute, G. Niaura, J. Maminskas, G. Juodzbalys and A. Kareiva, *J. Sol-Gel Sci. Technol.*, 2017, **83**, 268.
- 10 I. Mobasherpour, M. S. Heshajin, A. Kazemzadeh and M. Zakeri, *J. Alloys Compd.*, 2007, **430**, 330.
- 11 I. Grigoraviciute-Puroniene, K. Tsuru, E. Garskaite, Z. Stankeviciute, A. Beganskiene, K. Ishikawa and A. Kareiva, *Adv. Powder Technol.*, 2017, **28**, 2325.
- 12 S. Shanmugam and B. Gopal, *Ceram. Int.*, 2014, **40**, 15655.
- 13 S. Pramanik, A. K. Agarwal, K. N. Rai and A. Garg, *Ceram. Int.*, 2007, **33**, 419.
- 14 R. Borsa, M. Freche, J.-L. Lacout, G. Cosmeleata and S. Ciucu, *Ann. Chim.-Sci. Mat.*, 2008, **33**, 469.
- 15 H. Kimura, K. Kitahara and M. Naka, *J. Jpn. Inst. Met.*, 2006, **70**, 1.
- 16 A. Safavi and M. Sorouri, *Mater. Lett.*, 2013, **91**, 287.
- 17 M. Mackevičius, F. Ivanauskas, A. Kareiva and I. Bogdanovičienė, *J. Math. Chem.*, 2013, **51**, 1249.
- 18 S. H. Rhee, *Biomaterials*, 2002, **23**, 1147.
- 19 A. Serret, M. V. Cabanas and M. Vallet-Regi, *Chem. Mater.*, 2000, **12**, 3836.
- 20 N. V. Bulina, M. V. Chaikina, I. Yu. Prosanov, D. V. Dudina and L. A. Solovyov, *J. Solid State Chem.*, 2017, **252**, 93.
- 21 C. Rey, C. Combes, C. Drouet and D. Grossin, in *Comprehensive Biomaterials*, eds. P. Ducheyne, K. E. Healy, D. W. Huttmacher, D. W. Grainger and C. J. Kirkpatrick, Elsevier, Amsterdam, 2011, vol. 1, pp. 187–221.
- 22 M. Wu, T. Wang, Q. Wang and W. Huang, *Mater. Lett.*, 2019, **236**, 120.
- 23 M. Shahrezaee, M. Raz, S. Shishehbor, F. Moztarzadeh, F. Baghbani, A. Sadeghi, K. Bajelani and F. Tondnevis, *Silicon*, 2018, **10**, 1171.
- 24 H. Arce, M. L. Montero, A. Sáenz and V. M. Castaño, *Polyhedron*, 2004, **23**, 1897.
- 25 M. Malakauskaite-Petruleviciene, Z. Stankeviciute, G. Niaura, E. Garskaite, A. Beganskiene and A. Kareiva, *Vib. Spectrosc.*, 2016, **85**, 16.
- 26 S. V. Rempel, A. A. Valeeva, E. A. Bogdanova, H. Schroettner, N. A. Sabirzyanov and A. A. Rempel, *Mendelev Comm.*, 2016, **26**, 543.
- 27 R. Golubevas, A. Zarkov, L. Alinauskas, Z. Stankeviciute, G. Balciunas, E. Garskaite and A. Kareiva, *RSC Adv.*, 2017, **7**, 33558.
- 28 A. P. Solonenko and V. V. Boksgorn, *Russ. Chem. Bull., Int. Ed.*, 2017, **66**, 439 (*Izv. Akad. Nauk, Ser. Khim.*, 2017, 439).
- 29 Z. Chen, J. Zhai, D. Wang and C. Chen, *Ceram. Int.*, 2018, **44**, 10204.
- 30 L. Xie, Y. Yang, Z. Fu, Y. Li, J. Shi, D. Ma, S. Liu and D. Luo, *RSC Adv.*, 2019, **9**, 781.

Received: 31st October 2018; Com. 18/5735