

Solid state synthesis and characterization of calcium phosphate for biomedical application

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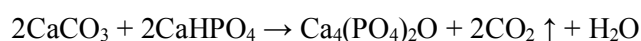
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Abstract: In recent years calcium phosphates (CaPs) consisting of tricalcium phosphate (TCP) and hydroxyapatite, have attracted significant interest in different applications like scaffolds (orthopaedic, oral and maxillofacial surgery), dental cement (adhesives, void fillers), bone cement (coating of implants, bone grafting, void fillers) and delivery systems (BMPs, proteins, drugs). Objective of this work is to synthesis a calcium phosphate by solid state reaction at high temperature using the mixture of calcium carbonate (CaCO₃) and di-calcium phosphate anhydrous (CaHPO₄), and evaluate the biphasic calcium phosphate containing tricalcium phosphate (Ca₃(PO₄)₂) and hydroxyapatite (HA) for cementing application. The resulting mixtures were characterized and determined by using X-ray diffraction analyses confirmed the phase purity, particle size, phase difference, and crystallinity. Cross-links and functional groups between mixture of solids were determined using Fourier transform infrared spectroscopy (FTIR). It can be concluded that the synthesised hydroxyapatite had a significant clinical advantage, and might have potential to be applied in orthopaedic, reconstructive and maxillofacial surgery, dental void fillers especially for minimally invasive techniques.

Keywords: Calcium phosphate, Calcium carbonate, di-calcium phosphate anhydrous, Hydroxyapatite.

1. Introduction

The need for bone repair in dental, craniofacial and orthopaedic applications has increased as the world population ages¹. Tissue engineering approaches are being developed with promising results for regenerative medicine applications³⁻⁸. However, is still a major orthopedic challenge for tissue engineering strategies due to inadequate vascularization in the reconstruction of large skeletal defects⁹. Injectability and biocompatibility are the two promising parameters in calcium phosphate cements for bone repair^{2, 5, 10-12}. A calcium phosphate cement comprising a mixture of tetra calcium phosphate [TTCP: Ca₄(PO₄)₂O] and di-calcium phosphate anhydrous lead to be known as CPC^{10, 13}. CPC was approved in 1996 by the Food and Drug Administration due to its excellent osteoconductivity and bone replacement capability, for repairing craniofacial defects in humans, thus becoming the first CPC available for clinical use¹⁰. When the starting CaCO₃-CaHPO₄ mixture has an overall Ca/P of 2.0 and homogeneity is achieved, pure TTCP forms at 1500°C for 6 h followed by rapid quenching at room temperature. Quenching is necessary to avoid the transformation of TTCP to phases that are more stable at lower temperature¹⁴, according to the following reaction:



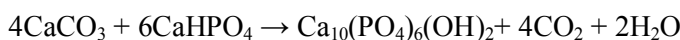
In this paper, we present a modified method for the preparation of calcium phosphate powder using solid state reaction at high temperature. The calcium phosphate powder synthesised by solid state reaction where evaluated by phase composition, and chemical bonds in the powder.

2. Materials and methods

The conditions for the formation of hydroxyapatite (HA) using solid state reaction were initially optimized. Analytical grade calcium carbonate (CaCO_3) (HIMEDIA, India) and di-calcium phosphate anhydrous (CaHPO_4) (HIMEDIA, India) were used as the starting materials.

2.1. Synthesis of HA at 1300 °C

Equiratio of CaCO_3 and CaHPO_4 mixed using mortar and pestle for 2 hours for the formation of homogeneity of the powder. This powder was fired in a high temperature furnace (Indfurr, India) for 7 h at 1300 °C in alumina crucibles, followed by rapid cooling at room temperature. In these conditions, when the starting CaCO_3 and CaHPO_4 mixture has an overall Ca/P of 2.0 and homogeneity is achieved, the formation of HA is achieved according to the following reaction:



The 1300 °C sample named as 13CP powder were examined with a high resolution X-ray powder diffractometer in Guinier geometry using monochromatic Cu K α radiation ($\lambda = 1.54059 \text{ \AA}$, XRD 3003TT, GE Inspection Technology, Germany). The X-ray diffraction patterns were recorded in steps of 0.02° interval with 1 s counting time at each step. Fourier transform infrared (FT-IR) spectra were obtained over the region $400\text{-}4000 \text{ cm}^{-1}$ in the pellet form for the powdered samples mixed with spectroscopic grade KBr (SHIMADZU Model: IR-Affinity 1, with resolution of 4).

3. Results and discussions

3.1. XRD analysis

The XRD patterns of the powdered samples for the solid state reaction of the sample 13CP at 1300°C furnace cooled to room temperature, and HA are shown in Figure 1. All the patterns show uniformly broad peaks around the characteristic peak regions indicating that the samples are microcrystalline in nature. The crystallinity of the sample may increases by heating the sample at high temperature. The diffractograms of 13CP samples correspond to HA (JCPDS 09-0432), where 13CP patterns have no additional peaks compared to those of HA. The broad patterns around at (002) and (211) indicate that crystallites are very tiny in nature with much atomic oscillations in 13CP XRD patterns. In Figure1 In compare between 13CP and HA, 13CP pattern do not show any extra peak of β - and α -TCP. The XRD patterns around the main peak ($2\theta = 24^\circ - 36^\circ$) of 13CP sample were expanded and are shown in Figure. 2. Using Debby Scherrer formula the particle size is calculated for (002) peaks where the FWHM (Full-width HalfMaximum) is 0.13278 for 13CP gives the crystallite size of 60.74 nm in size. There are no separate patterns for CaCO_3 in XRD spectra of the 13CP sample.

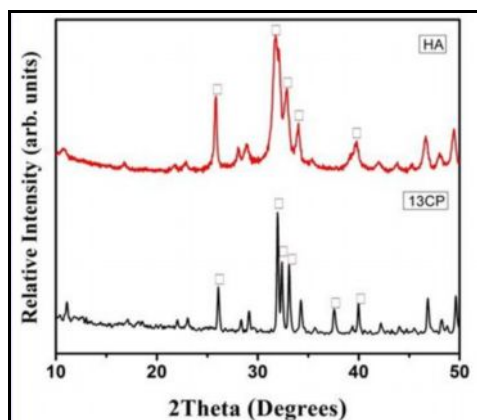


Figure. 1. XRD patterns of (13CP) synthesized at 1300 °C and (HA) pure HA. (□) indicates the main peaks of HA.

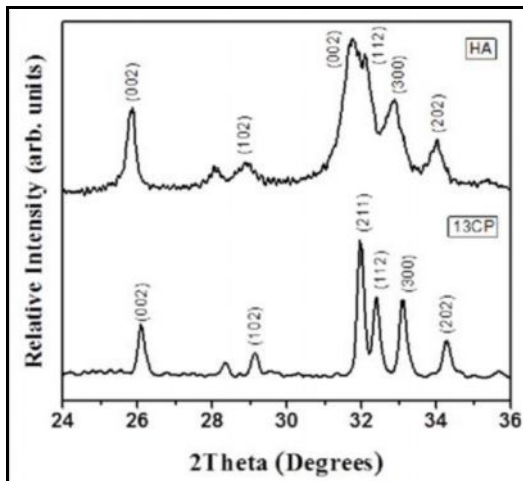


Figure 2: XRD patterns around the main peak($2\theta = 24^\circ - 36^\circ$) of 13CP and HA marked with Miller indices.

3.2 FTIR analysis

The FT-IR spectroscopy of the two samples 13CP shows the HA phase formation. The FT-IR spectra of pure HA and 13CP samples are shown in Figure 3. All the samples show vibrational modes corresponding to the phosphate, hydroxyl and carbonate groups. The hydroxyl bond stretch is observed at 3421 cm^{-1} in all the samples. There is a broad envelope between 3421 and 3489 cm^{-1} in both the sample of 13CP due to the O-H stretch of water and HA. The O-H groups are hydrogen bonded. The sharp peak at 3641 cm^{-1} in 13CP sample is referring to unhydrogen bonded free O-H stretch which may be presented at the surface of the crystallites. There is no absorption band at 1382 cm^{-1} , which shows there no presence of carbonate and/or hydrogen phosphate in both the samples.

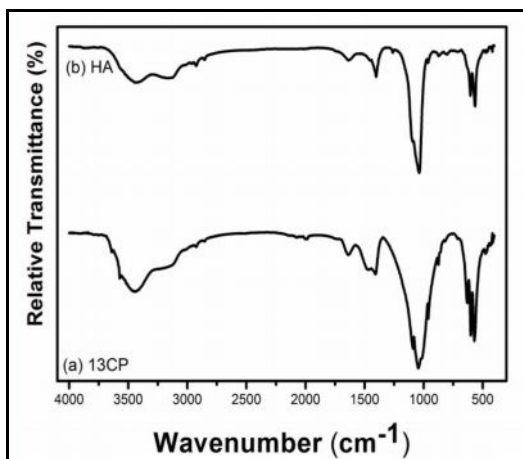


Figure. 3. FTIR spectra of (a) 13CP, (b) HA

The intense broad peak between 900 and 1100 cm^{-1} is referring to PO_4^{3-} . The stretching and the bending modes of PO_4^{3-} appeared at 601 and 570 cm^{-1} as intense sharp peak at 13CP sample. For 13CP samples, in figure 3, the peaks due to CO_3^{2-} (875 and 1402 cm^{-1} for 13CP), are very evident and it might be due to the absorption of atmospheric CO_2 on the surface of HA. The peak at 630 and 3570 cm^{-1} are the characteristic peaks for stoichiometric HA¹⁵. These are present in the samples in all samples, confirming the XRD results for pure stoichiometric HA.

4. Conclusion

In this study presents an alternative method to form pure, stoichiometric, stable, and crystalline HA phase at high temperature. This method provides the synthesis of pure stoichiometric HA at 1300°C in solid state reaction using calcium carbonate (CaCO_3) and di-calcium phosphate anhydrous (CaHPO_4). The effect of solid state reaction temperature on the phase and morphology of 13CP were investigated by XRD and FTIR

shows the formation of HA. This conclude that only by heating two different mixtures (CaCO_3 and CaHPO_4) at 1500°C for 7-12 h, followed by rapid quenching at room temperature will give pure TTCP. The powders consisted of equiaxed crystals, which formed larger aggregates resulting in median particle size in the range of 69.77 nm. The 13CP samples show vibrational modes corresponding to the phosphate, hydroxyl and carbonate groups using FTIR analysis.

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References

1. Aoki H. Science and medical applications of hydroxyapatite: Ishiyaku Euroamerica; 1991.
2. Hench LL. Bioceramics: from concept to clinic. *Journal of the American Ceramic Society*. 1991;74:1487-510.
3. Suchanek W, Yoshimura M. Processing and properties of hydroxyapatite-based biomaterials for use as hard tissue replacement implants. *Journal of Materials Research*. 1998;13:94-117.
4. Tenhuisen KS, Martin RI, Klimkiewicz M, Brown PW. Formation and properties of a synthetic bone composite: hydroxyapatite–collagen. *Journal of biomedical materials research*. 1995;29:803-10.
5. Shibutani T, Moriwaki Y, Kajimoto T, Iwayama Y. Sintered carbonate apatites as bioresorbable bone substitutes. *Journal of biomedical materials research*. 1998;39:603-10.
6. LeGeros R. *Calcium Phosphates in Oral Biology and Medicine* (1991). San Francisco, California.
7. Iwanaga H, Shibutani T, Moriwaki Y, Iwayama Y. Osteoclastic responses to various calcium phosphates in cell cultures. *Journal of biomedical materials research*. 1999;47:424-33.
8. Redey SA, Nardin M, Bernache–Assolant D, Rey C, Delannoy P, Sedel L, et al. Behavior of human osteoblastic cells on stoichiometric hydroxyapatite and type A carbonate apatite: role of surface energy. *Journal of biomedical materials research*. 2000;50:353-64.
9. Barralet J, Akao M, Aoki H. Dissolution of dense carbonate apatite subcutaneously implanted in Wistar rats. *Journal of biomedical materials research*. 2000;49:176-82.
10. LeGeros RZ, Bonel G, Legros R. Types of “ H_2O ” in human enamel and in precipitated apatites. *Calcified tissue research*. 1978;26:111-8.
11. de Groot K. *Bioceramics of calcium phosphate*: CRC; 1983.
12. Williams D. *Biocompatibility of Tissue Analogs*, vol. 11. CRC Press, Boca Raton; 1985.
13. Brown WE, Chow LC. *Dental restorative cement pastes*. Google Patents; 1985.
14. Costantino PD, Friedman CD, Jones K, Chow LC, Pelzer HJ, Sisson GA. Hydroxyapatite cement: I. Basic chemistry and histologic properties. *Archives of Otolaryngology–Head & Neck Surgery*. 1991;117:379-84.
15. Friedman CD, Costantino PD, Jones K, Chow LC, Pelzer HJ, Sisson Sr GA. Hydroxyapatite cement: II. Obliteration and reconstruction of the cat frontal sinus. *Archives of Otolaryngology—Head & Neck Surgery*. 1991;117:385.
