Calcium deficiency in hydroxyapatite and its drug delivery applications

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In this work, calcium deficient hydroxyapatites (HAPs) $[Ca_{10-x}(PO_4)_6(OH)_2]$ [where $x = 0 - 0.3$] were synthesised by precipitation method and calcined samples were characterised by Fourier transformation infrared spectroscopy, powder X-ray diffraction, scanning electron microscopy and energy-dispersive X-ray spectroscopy techniques to check phase purity, calcium deficiency, particle size and shape. The results indicate that the structure of the HAP can tolerate a calcium deficiency up to 0.2. The calcium deficient HAP ($x = 0.1$) powder was found to be highly porous with a particle size below 200 nm. This powder was used as a drug delivery carrier for the drug, ciprofloxacin and sustained release of the drug in the phosphate buffer solution was investigated.

1. Introduction: Calcium phosphate bioceramics have served to human beings for many years as excellent biomaterials since they mimic the natural bone constituent hydroxyapatite (HAP) $\{Ca_{10}(PO_4)_6(OH)_2$ [1.67]$\}$. Drug delivery systems using calcium phosphate bioceramics have been developed in the recent years, particularly for the treatment and regeneration of bone infections and bone defects. Based on calcium/phosphorous ratio, these calcium phosphates exist in different phases such as, α-tricalcium phosphate $Ca_3(PO_4)_2$ [1.5], β-tricalcium phosphate $Ca_3(PO_4)_2$ [1.5], octa calcium phosphate $Ca_8(HPO_4)_2(PO_4)_4.5H_2O$ [1.33], amorphous calcium phosphate $Ca_x(PO_4)_y{\cdot}nH_2O$ [1.2–2.2], dicalcium phosphate dihydrate (brushite) $CaHPO_4{\cdot}2H_2O$ [1.0], dicalcium phosphate anhydrous (monetite) $CaHPO_4$ [1.0], monocalcium phosphate monohydrate $Ca(H_2PO_4)_2{\cdot}H_2O$ [0.5] and anhydrous monocalcium phosphate $Ca(H_2PO_4)_2$ [0.5]. These calcium phosphates usually appear as impure phases during the synthesis of HAP [1].

Besides stoichiometric HAP, bone minerals are essentially composed of calcium deficient HAP (CDHAP). The CDHAP is (Ca/P ratio of about 1.5) structurally similar to stoichiometric HAP, whereas chemically and compositionally similar to tricalcium phosphate. It is worth to mention that the CDHAP has a higher specific surface area compared to tricalcium phosphate and HAP. Further, CDHAP is more soluble in water/body fluids when compared to HAP and it exhibits reproducible seeding efficacy [2, 3]. From the above considerations, it is evident that the C/P ratio plays a major role in regulating calcium phosphate characteristics used in biomedical applications, hence more emphasis is given on the CDHAP preparation and property studies. Sampath Kumar and co-workers [3] reported the microwave assisted synthesis method for CDHAP nanoparticles useful for biomedical applications, while Kim and Kim [4] suggested CDHAP synthesis in the presence of amphiphilic triblock copolymer. In addition, Kasten *et al.* [5] reported more favourable properties of CDHAP in terms of seeding efficacy in comparison with human bone marrow stromal cells seeded on β-tricalcium phosphate and demineralised bone matrix.

In the drug delivery applications, CDHAP has been studied as a platform for the specific delivery of germinal bisphosphonates, a class of drugs to the bone which finds application in bone and tooth repair [6]. Previously, Kamitakahara *et al.* [7] reported the CDHAP granules as drug carriers with controlled surface microstructure. In this Letter, we prepared the CDHAPs and studied its drug delivery applications in addition to investigating the ability of the HAP structure in tolerating calcium deficiency.

2. Experimental procedure

2.1. Materials: $Ca(NO_3)_2{\cdot}4H_2O$ (SDFCL), $(NH_4)_2HPO_4$ (SDFCL), aqueous ammonia (25%) (SDFCL), acetone (SDFCL), PVA (SDFCL), KBr (SDFCL), K_2HPO_4 (SDFCL), NaOH (SDFCL) and ciprofloxacin hydrochloride were commercially purchased. All chemicals are of analytical grade; 98–100% pure and used as such without further purification.

2.2. Synthesis of CDHAP: CDHAPs $[Ca_{10-x}(PO_4)_6(OH)_2]$ $[x = 0 - 0.3]$ were prepared by (Fig. 1) precipitation method at room temperature using stoichiometric amounts of aqueous solutions of $Ca(NO_3)_2{\cdot}4H_2O$ and $(NH_4)_2HPO_4$. Both the solutions were made alkaline (pH between 10 and 11) using aqueous ammonia (25%). The $(NH_4)_2HPO_4$ solution was added drop wise to the $Ca(NO_3)_2{\cdot}4H_2O$ solution and stirred for 24 h for precipitation. The precipitate was filtered, washed with water/acetone, and dried in hot air oven at 100°C for 24 h. The dried powder was ground and calcined at 900°C for 2 h to obtain CDHAP [8].

2.3. Characterisation: Powder-XRD (X-ray diffraction) patterns were recorded using (Bruker, D8 advance) X-ray diffractometer with Cu Kα, Ni-filtered radiation and Fourier transformation infrared (FTIR) spectrum were recorded in Shimadzu FTIR spectrometer using KBr. Sample images after gold sputtering were recorded using Carl Zeiss, EVO MA 15 model SEM (scanning electron microscopy). Elemental composition was obtained using (Inca Penta FET x3 models, Oxford instruments) EDS (energy-dispersive X-ray spectroscopy) instrument.

2.4. Drug delivery studies: About 90 mg of the CDHAP compound ($x = 0.1$), 1 ml of drug solution (1 mg/1 ml conc. ciprofloxacin

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hydrochloride) and 0.1 ml of 10% PVA were mixed all together and dried at room temperature for one day. Then the mixture was ground and made as a pellet using 8 mm die by applying 10 kg/cm^2 pressure. Potassium phosphate buffer solution was prepared by dissolving 4.0820 g of the monobasic salt (K_2HPO_4) and 18.114 g of the dibasic salt (KH_2PO_4) in 2 l of double distilled water. The pH of this solution was adjusted to 7.4 by adding small amounts of NaOH (1 M).

The prepared pellet was soaked in 50 ml of phosphate buffer solution in a conical flask at 37°C. Every 1 h, 5 ml of the buffer solution was taken from the flask and refilled with fresh buffer. Up to 36 h, the samples were collected and UV readings were recorded at 278 nm. The release of the ciprofloxacin drug from CDHAP was calculated from the UV readings.

3. Results and discussion: Since the calcium deficiency in HAP finds promising applications in the field of biomedical sciences it is worth to find how much calcium deficiency the structure of HAP can tolerate. For this purpose, we prepared CDHAPs [$Ca_{10-x}(PO_4)_6(OH)_2$] [$x = 0 - 0.3$] using a simple precipitation method with alkaline (pH = 10–11) condition and Fig. 1 shows the synthetic route for the CDHAP preparation. The XRD diffraction patterns for the obtained samples were shown in Fig. 2. The patterns were indexed to JCPDS Card No. 74-0565 and matched perfectly, which confirms the formation of pure HAP for samples with $x = 0 - 0.2$. The HAP crystallises in a hexagonal structure with lattice parameters $a = 9.424$ Å and $c = 6.879$ Å and the diffraction peaks were broadened identifying the nanocrystalline nature. The shift in the major intensity peak [2θ value 32.0/(211) plane] suggests that the calcium deficiency has occurred in HAP structure. For $x = 0.3$, one extra peak at a 2θ value of 31.30 was found, indicating the formation of β-tricalcium phosphate as an impurity [9, 10]. This clearly indicates that the structure is not stable at $x = 0.3$. Fig. 3 shows the FTIR spectrum of CDHAPs calcined at 900°C. As shown in Fig. 3, a broad band at 3420 cm^{-1} is due to moisture, and a sharp peak at 3571 cm^{-1} is due to the stretching vibration of the −OH group. A shoulder peak at 631 cm^{-1} is due to the bending vibration of the −OH group and the intensive bands at 1092 and 1032 cm^{-1} are due to m3 vibrational modes of a phosphate group. Further, the band at 961 cm^{-1} corresponding to the m1 vibrational mode of a phosphate group, can be observed in all spectra of CDHAP and the weak band at 473 cm^{-1} is attributed to the m2 vibrational mode of the phosphate group. Especially, phosphate m4 bands were observed at 633, 602 and 565 cm^{-1} [9, 10]. We did not observe much difference in the FTIR spectra of all CDHAPs, but a small shift in the stretching vibration of the −OH group may be due to the calcium deficiency in the HAP structure. In order to find the morphological features and elemental composition of the sample CDHAP ($x = 0.1$), the gold coated samples were tested by SEM coupled with EDX instrument. The SEM images are shown in Figs. 4*a* and *b* at different magnifications. It shows the highly crystalline nature of the particles and distribution of a large number of small particles concentrated along the pores. The dimensions of the particles were in the nanoregime. The average size of the particles was about 185 nm. Fig. 4*c* shows the elemental composition and the presence of Ca, P, O elements without any impurity.

In general, the drugs can be incorporated in whole calcium phosphate material, which facilitates the release of drugs in the sustained mode for longer durations. Previously, Castro *et al.* reported the antibiotic drug release mechanism from the implants contain ciprofloxacin, HAP, poly (DL-lactide), tricalcium phosphate, and observed the erosion, swelling, and disintegration of the implants. Queiroz *et al.* studied the drug absorption and release of sodium ampicillin in *in-vitro* conditions from HAP and glass-reinforced HAP composites. They reported sodium ampicillin was adsorbed more on HAP then the glass reinforced – HAP composites [11, 12]. It was found that the composition of the matrix, particle size and nature of the drug influences the drug release profile of the

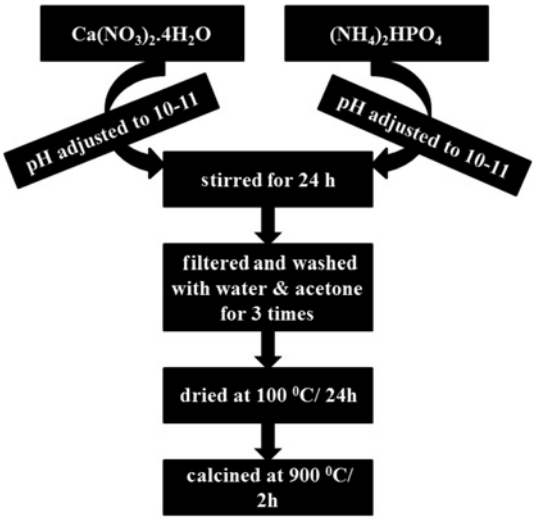

Fig. 1 *Synthetic route of HAP*

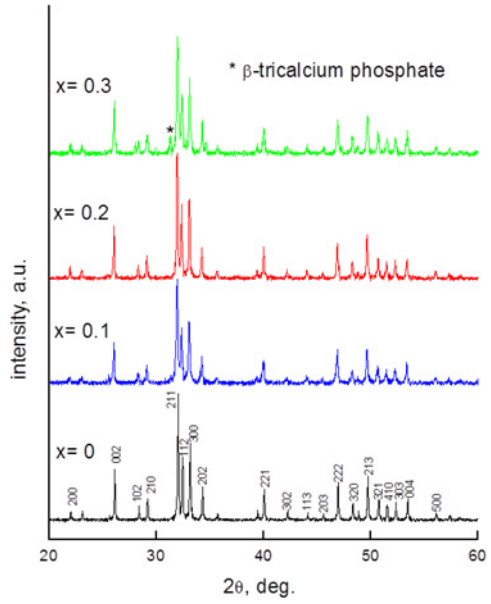

Fig. 2 *XRD patterns of synthesised CDHAPs (x = 0 – 0.3)*

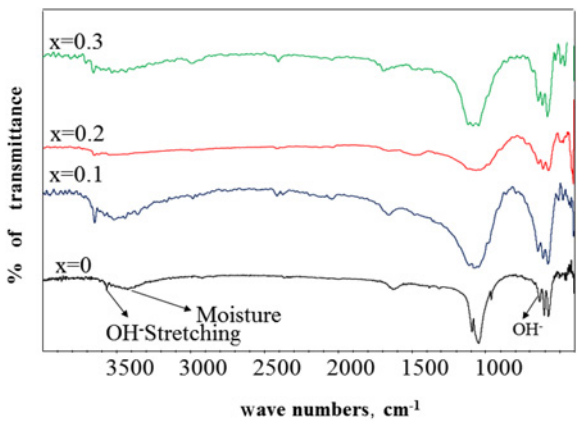

Fig. 3 *FTIR patterns of synthesised CDHAPs (x = 0 – 0.3)*

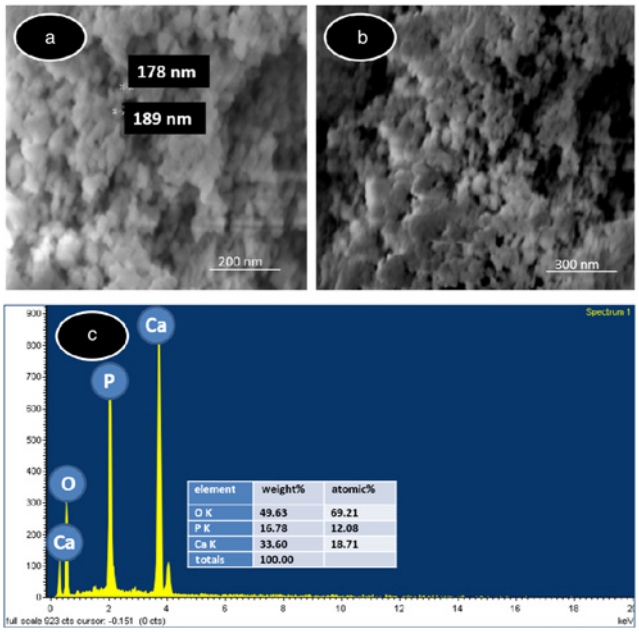

Fig. 4 *SEM images of CDHAP ($x = 0.1$)*
a At *x* 50.00 K magnification
b At *x* 30.00 K magnification
c EDX spectrum

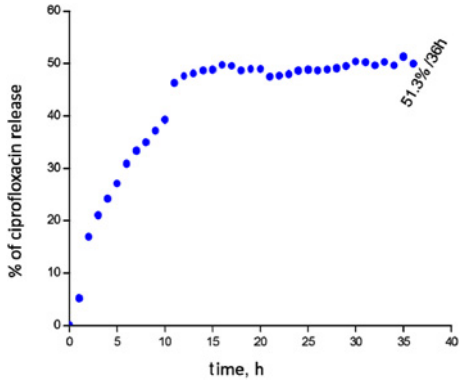

Fig. 5 *Ciprofloxacin release profile*

HAP carrier. However, so far CDHAP was not investigated as a drug carrier and here we attempted to study the release profile of ciprofloxacin. To evaluate the drug delivery kinetics, CDHAP ($x = 0.1$) was used as ciprofloxacin hydrochloride antibiotic drug carrier in phosphate buffer solution of pH 7.4. As described in Section 2, the UV absorbance readings were recorded for all sample solutions at 278 nm and from the data, the amount of the drug released was calculated as given in Fig. 5. In the early stage, the extent of the drug release was about 47% in 12 h, however, sustained release of ciprofloxacin drug was observed in *in-vitro* conditions and 51.3% of the drug was released in 36 h in phosphate buffer solution. Even after one and half years, the used pellet was observed to be stable at room temperature. This also suggests the additional advantage of calcium deficiency for drug delivery applications.

4. Conclusion: Synthesised CDHAPs [$Ca_{10-x}(PO_4)_6(OH)_2$] [where $x = 0 - 0.3$] were of single phasic up to $x = 0.2$ and structure of HAP can tolerate a deficiency up to $x = 0.2$. CDHAP ($x = 0.1$) was in nanoregime with porous and agglomerated nature. *In-vitro* drug release profile shows the sustained release of 51% ciprofloxacin hydrochloride drug in 35 h from CDHAP ($x = 0.1$). All these findings suggest the CDHAPs as potential biomaterials for drug delivery applications.

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