Chapter -4

Ca/P Ratio Controlled Phase Morphology of Calcium Phosphate Nanoparticles
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Ca/P RATIO CONTROLLED PHASE MORPHOLOGY OF CALCIUM PHOSPHATE NANOPARTICLES

ABSTRACT

Calcium phosphate nanostructures with different morphology and crystal structure have been successfully synthesized via the emulsion process by varying the Ca/P ratio and thus varying the pH of the reaction. The as synthesized products have been extensively characterized by fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), field emission transmission electron microscopy (FETEM). The phase and morphological changes have marked dependence on the Ca/P ratio. The final morphology is the result of a complex, time dependent self-assembly and growth process. Most of the studies report the synthesis of pure hydroxyapatite at a Ca/P ratio of 1.67 but in the present study phase pure hydroxyapatite is formed at the lowest Ca/P ratio.
4.1 INTRODUCTION

Calcium phosphates are bioactive materials which have the potential to be used as materials for artificial bones, scaffolds for tissue engineering, chromatographic packing, because of its high bioactivity and particular adsorbability for various ions and organic molecules, in drug delivery etc [129, 214-216]. The bioactivity, biocompatibility, stability and mechanical properties of calcium phosphates are determined by their morphology, crystallite size, composition and structure which influences in turn influences their performance in various applications. Fibrous and needle-like morphologies with a high specific surface area are advantageous for adsorption and ion exchange, while high mechanical strength due to a rigid structure is required for other applications [217, 218]. Dicalcium phosphate and monetite has been found to be suitable precursors for nanoscopically controlled HAP crystals [84, 219]. Therefore, the development of simple, fast and low cost synthesis methods for control over the morphology and phase of calcium phosphate is of great importance for broadening and improving its biomedical applications.

There are many studies reporting wet chemical routes which have been applied for the morphological and phase control of calcium phosphate crystals [63, 220, 221]. Monetite whiskers and hydroxyapatite needles can be obtained by hydrothermal treatment [220]. Direct reaction of calcium chloride and sodium phosphate solution resulted in plate-like brushite nanocrystals [63]. The flower like morphology of hydroxyapatite can be obtained using Na₂ – EDTA as the template directing reagent under hydrothermal treatment [221]. To the best of our knowledge,
there is no study reporting the effect of Ca/P ratio on the phase and morphology of calcium phosphate nanoparticles synthesized via emulsion route.

In the present study we demonstrate a detailed investigation of nanoscale morphologies and different phases of calcium phosphate nanocrystals obtained by emulsion process (section 2.2.2). The final nanoscale morphologies are found to get influenced by the concentration of calcium and phosphate ions. The Ca/P molar ratio has been varied to study its effect on the nanomorphology and phase of calcium phosphate. New findings presented in this study on the formation of calcium phosphate nanostructures will be informative for the science and technology of wet-chemical materials processing.

4.2 RESULTS AND DISCUSSION

4.2.1 Spectral Analysis

FTIR. FTIR spectra of all the samples viz. E₁, E₂, E₃, E₄ & E₅ are shown in Figure 4.1. Theoretically, there are four vibration modes (ν₁-ν₄) present for the PO₄³⁻ ion. The ν₄ (asymmetric bending) bands at 520-600 cm⁻¹, as well as the ν₁ (symmetric stretching) and ν₃ (asymmetric stretching) spectral bands positioned in the 900-1200 cm⁻¹ range, are useful in identifying the structure of calcium phosphates [222]. The ν₂ band of PO₄³⁻ is positioned around 469-473 cm⁻¹.

Figure 4.1(a) represents the spectrum of nanoparticles obtained from the system E₁. The spectra possess absorption peak around 560 cm⁻¹ ascribed to the ν₄ bending vibration mode of PO₄³⁻ group [222]. A small absorption peak at 525 cm⁻¹ does appear in this spectrum. This peak may be assigned to the bending mode of the HPO₄²⁻ group, which is a characteristic of crystalline acid phosphate, thus indicating the presence of
Figure 4.1: The FTIR spectra of calcium phosphate nanoparticles obtained from the system (a) E₁, (b) E₂, (c) E₃, (d) E₄, & (e) E₅.
brushite or monetite [223]. The band at 890 cm\(^{-1}\) is assigned to HPO\(_4^{2-}\) [222, 223]. The two small and broad peaks around 1068 and 1130 cm\(^{-1}\) are ascribed to the \(v_3\) stretching vibrations of PO\(_4^{3-}\) group and P-O stretching in brushite, respectively [222].

On further investigation small bands around 1645 cm\(^{-1}\) and 2925 cm\(^{-1}\) are observed which are ascribed to bending mode (\(\delta\)) and stretching mode of OH\(^-\) group [222, 223]. The broad bands observed between 3000 - 3500 cm\(^{-1}\) (characteristic peak of brushite) are ascribed to OH\(^-\) stretches, **Figure 4.1(a)** [199, 200].

FTIR spectrum also shows absorption peak at 1384 cm\(^{-1}\), which can be attributed to the presence of adsorbed (surface) CO\(_3^{2-}\) [195]. These bands are ascribed to B-type of carbonated hydroxyapatite (CO\(_3^{2-}\) group gets incorporated into hydroxyapatite crystal structure and replaces PO\(_4^{3-}\) group). The small peak around 2350 cm\(^{-1}\) is indicative of atmospheric CO\(_3^{2-}\) [224].

Thus, the FTIR spectrum of nanoparticles obtained via system E\(_1\) reveals the formation of brushite or monetite with little carbonate impurity. However, FTIR alone cannot reveal the exact phase of calcium phosphate.

**Figure 4.1(b)** shows the spectrum of the sample prepared via the system E\(_2\). The FTIR spectrum reveals the peak attributed to PO\(_4^{3-}\) ions (\(v_4 - 563\) cm\(^{-1}\), \(v_3 - 1064\) cm\(^{-1}\) and 1128 cm\(^{-1}\)) [222, 223]. The absorption band around 525 cm\(^{-1}\) assigned to bending mode of HPO\(_4^-\) has been observed [223]. The absorption bands ascribed to the adsorbed (surface) CO\(_3^{2-}\) (B-type substitution - 1384 cm\(^{-1}\)) is also observed [195]. Four sharp peaks between 3000 cm\(^{-1}\) and 3600 cm\(^{-1}\) (due to physisorbed water and structural OH- group) and one around 1650 cm\(^{-1}\) (due to bending mode of OH-group) are absent indicating the formation of anhydrous form of dicalcium phosphate,
monotite (dicalcium phosphate anhydrous, DCPA) [199, 200]. Thus the nanoparticles obtained from the system E₂ are of monotite.

The FTIR spectrum of nanoparticles obtained from systems E₃, E₄ and E₅ are represented in Figure 4.1(c), (d) & (e). In all the samples (E₃, E₄ & E₅), the small absorption ν₂ band of PO₄⁢³⁻ positioned around 470 cm⁻¹ has been observed [223]. The bands at 563 cm⁻¹ and 603 cm⁻¹ are attributed to the ν₄ asymmetric bending mode of PO₄³⁻ [222, 223]. The appearance of absorption band around 962 cm⁻¹ and 1032 cm⁻¹ attributed to ν₁ symmetric stretching and ν₃ asymmetric stretching of PO₄³⁻ points toward the formation of stoichiometric hydroxyapatite in these samples [222]. In all these samples the band around 525 cm⁻¹, attributed to HPO₄⁻, disappears exempting the possibility of brushite/monotite formation. The weak bands around 898 cm⁻¹ and 1400 cm⁻¹ corresponds to A-type (CO₃²⁻ replaces OH⁻ anions) and B-type (CO₃²⁻ replaces PO₄³⁻ ions) carbonate substitution, respectively [127, 221, 225]. The absorption band at 1650 cm⁻¹ corresponds to the ν₂ bending mode of the H₂O molecule [226-228]. In the FTIR spectra of samples obtained from the system E₃, E₄ & E₅ instead of four bands between 3000 – 3600 cm⁻¹ the presence of two characteristic bands around 3250 and 3425 cm⁻¹, ascribed to the ν₃ and ν₁ stretching modes of the hydrogen bonded H₂O molecule. The characteristic peak around 3570 cm⁻¹, assigned to structural OH⁻ ions, confirms the formation of hydroxyapatite [229]. An additional small sharp band around 2360 cm⁻¹ has been observed which corresponds to the atmospheric CO₃²⁻ [224].

Thus FTIR analysis reveals that AB-type carbonate substituted hydroxyapatite has been formed from the system E₃, E₄ & E₅.
4.2.2 Crystallographic Studies

XRD. The XRD technique has been employed to assess the phase and crystallographic changes of the calcium phosphate biomaterials derived from varied Ca/P ratios and pH, as described in experimental procedure. Figure 4.2 represents the XRD patterns of calcium phosphate prepared in this study. Figure 4.2(a) depicts the XRD patterns of sample E1 prepared with Ca/P ratio of 1.67. It has been mentioned in the literature that at Ca/P ratio of 1.67 hydroxyapatite is formed [229]. The resulting diffractogram demonstrates that the as synthesized product is dual phase, i.e. there are diffraction maxima that correspond to the brushite (JCPDS – 71-1759) and monetite (JCPDS – 72-1240) structure [83]. The reflections around 13.10, 25.70, 26.49, 28.65, 30.19, 32.57, 35.65, 39.10, 39.89 and 39.92° corresponding to (100), (111), (200), (112), (120), (201), (022), (301), (003) and (221) planes, respectively, indicates that monetite constitutes the main phase of the product [83]. The additional reflections due to (020), (121), (040) and (141) planes at 11.56, 20.82, 23.44 and 29.0° reveals the presence of brushite [83]. An intense and sharp (020) peak (interplanar distance = 7.6 Å) in the X-ray diffraction patterns reveals that the nanostructures grow anisotropically along the b-axis of the monoclinic structure of brushite.

As the Ca/P ratio is lowered (Ca/P = 1, pH = 7-8), with the increase in phosphate ion concentration the pH of the aqueous phase increases slightly. With the increase in phosphate ion concentration and the corresponding pH of the reaction system the relative intensities of the diffraction peaks attributed to brushite phase starts to disappear.
Figure 4.2: The XRD pattern of calcium phosphate nanoparticles obtained from the system (a) E₁, (b) E₂, (c) E₃, (d) E₄, & (e) E₅.
Figure 4.2(b) represents the XRD patterns of the sample E₂. At the Ca/P = 1 (pH = 7-8) the diffraction pattern indicates that the E₂ sample consists of a single phase of crystalline monetite with a triclinic structure (JCPDS-71-1759) [83]. The chemical reaction for the formation of monetite can be simplified as follows:

$$\text{Ca}^{2+} + \text{HPO}_4^{2-} \leftrightarrow \text{CaHPO}_4$$

Figure 4.2(b) shows that relative intensity of the (200) diffraction peak of monetite is maximum with an interplanar distance of 3.37 Å revealing that the nanocrystals grow along the (100) plane, i.e. growth along the $a$-axis. Also the (200) diffraction peak in the XRD pattern of the sample E₂ is slightly broadened as compared to E₁ indicating towards the small crystal size [195]. Figure 4.2(b) also reveals the presence of two splitted diffraction peaks at 32.1° and 32.6° corresponding to the higher degree of crystallinity. The increase in phosphate ion concentration, i.e., low Ca/P ratio makes the diffracted peaks more sharp near 32°, which is a good sign of the improved crystallinity of calcium phosphate nanoparticles.

Figure 4.2(c) reveals that hydroxyapatite is the main constituent at Ca/P ratio of 0.67, pH = 7-8 (sample E₃). The diffraction peaks around 26°, 28.5°, 32° 34°, 36°, 39.8° are observed corresponding to the hydroxyapatite phase (JCPDS No. 09-0432) [67]. The diffractogram shows a broad peak over the characteristic region 31°-33° which is expected to be formed by the diffusion of the three individual peaks each at ~ 31.8°, 32.1° and 32.9° ($^\circ$) corresponding to (211), (112) and (300) planes, respectively. The intensities of the peaks around 26° corresponding to (002) plane and around 32° corresponding to (300) plane are found to be 100% and 99% respectively. Thus, these peaks are expected to control the morphology of the particles. The diffraction maxima at
26° indicate that it has a preferred growth orientation along the (002). The high intensity of these planes indicates the growth along the c-axis which may be related to their one dimensional morphology. **Figure 4.2(c)** also shows two small peaks around 13° and 30.1° indicating the presence of small amount of monetite [83].

**Figure 4.2(d)** shows the XRD patterns of the product obtained at Ca/P ratio of 0.50, pH = 8 (sample E₄). The resulting diffractogram demonstrates that all the diffraction peaks corresponds to hydroxyapatite except for a little peak at 30.33° corresponding to β-TCP [67, 195]. The broad peak in the region 31°-33° has the maximum intensity (again formed by the diffusion of the three individual peaks each at ~ 31.8°, 32.1° and 32.9° corresponding to (211), (112) and (300) planes respectively). The crystallinity of the nanostructures is lower than that of other products as revealed by the broadened diffraction peaks.

**Figure 4.2(e)** depicts the representative XRD pattern of the product E₅ obtained at Ca/P ratio of 0.40, pH = 8-9 (E₅). All the Bragg diffraction peaks can be indexed to pure hexagonal phase with space group P6₃m coinciding well with the standard data for hydroxyapatite (JCPDS No. – 09-0432) [67, 195]. The crystallinity of the nanostructures is lower than that of other products as revealed by the relatively broadened diffraction peaks. The appearance of the three individual peaks each at ~ 31.8°, 32.1° and 32.9° (2θ) as one diffused broad peak near 32° region again reveals the low crystallinity of the hydroxyapatite nanostructures. However, the crystallographic structure of hydroxyapatite obtained via E₅ system is quite similar to biological apatite [67, 230]. From XRD result, it has been found that the intensity
ratio between (211) plane and the other peaks is high, suggesting that the nanocrystals are characterized by dominant (211) plane.

Thus, XRD analysis divulge that with the decreasing Ca/P ratio the metastable phases like brushite, monetite and β-TCP starts to disappear and leads to the formation of impurity free and most thermodynamically stable hydroxyapatite phase.

4.2.3 Morphological Analysis

Field emission transmission electron microscopy analyses provide further insight into the nanometer scale details of the calcium phosphate particles. Hollow nanospheres have been obtained from the system E₁, **Figure 3.4(b)** (section 3.3.2). **Figure 4.3 to 4.6** represents the field emission transmission electron microscopy analysis of the products obtained by system E₂, E₃, E₄ & E₅. **Figure 4.3(a), (b) & (c)** indicates that the monetite obtained from sample E₂ consists of short rod-like particles which has a diameter of about 5 nm and a length of about 15 nm. As disclosed by the corresponding FETEM, the interplanar distance between the adjacent lattice fringes is determined as 0.335 nm, **Figure 4.3(d)**. This plane can be well indexed as d-spacing value of the (200) plane of the monetite nanoparticles. **Figure 4.4(a), (b) & (c)** illustrates the representative FETEM image of the sample obtained via system E₃. The image shows the presence of well-defined nanorods with a diameter of 10 nm and length of 56 nm. The nanorods obtained from system are larger than those obtained from system E₂. The corresponding FETEM image recorded indicates lattice fringes with interplanar spacings of 0.336 nm for (002) and 0.275 nm for (300) planes of hydroxyapatite nanorods, **Figure 4.4(d)**. The length of nanorods further increases to 82 nm and diameter decreases to 6 nm as the phosphate ion concentration increases in
Figure 4.3 (a, b): FETEM micrographs of the sample obtained from the system $E_2$. 
Figure 4.3: Magnified FETEM micrograph (c) and lattice fringes (d) of the sample obtained from the system E₂.
Figure 4.4 (a, b): FETEM micrographs of the sample obtained from the system $E_3$. 
Figure 4.4: Magnified FETEM micrograph (c) and lattice fringes (d) of the sample obtained from the system $E_3$. 
system $E_4$, Figure 4.5(a) & (b). A typical FETEM image shows interplanar spacing of about 0.282 nm which corresponds to (300) planes of hydroxyapatite, Figure 4.5(c). The hydroxyapatite nanorods obtained from system $E_5$ are the longest of all the system with its minor axis of 6 nm and major axis of about 100 nm, Figure 4.6(a) & (b). The interplanar distance measured in segments of the FETEM micrograph is 0.284 nm corresponding to the interplanar spacing of the (211) planes of the hexagonal hydroxyapatite, Figure 4.6(c).

These results corroborate the XRD results.

43 CONCLUSIONS

In conclusion, via emulsion process a general synthesis route has been demonstrated for the synthesis of calcium phosphate nanoparticles. The investigation revealed the formation of rod like morphologies with varied dimensions except for the system with lowest phosphate concentration. The FETEM images clearly show good crystallinity and defect free crystals. Detailed experimental studies suggest that the phase and morphology of calcium phosphate can be controlled judiciously by varying the Ca/P molar ratio and hence the pH of the reaction.
Figure 4.5 (a, b): FETEM micrographs of the sample obtained from the system $E_4$. 
Figure 4.5 (c): Lattice fringes of the sample obtained from the system $E_4$. 

Figure 4.6 (a, b): FETEM micrographs of the sample obtained from the system $E_5$. 
Figure 4.6 (c): Lattice fringes of the sample obtained from the system E₅.