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Spontaneous fabrication of octacalcium phosphate: synthesis conditions and basic characterizations

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Abstract. Octacalcium phosphate (OCP), an encrusted calcium phosphate complex, has appealed consideration in the field of biomaterial and apothecary, owing to its exceptional biocompatibility. However, not much is known about the effect of Na ion for the formation of OCP, irrespective of pH. Consequently, in this study, we considered the part of the Na ion in OCP growth from dicalcium phosphate dihydrate (DCPD) via hydrolysis by using 0.1 M CH_3COONa solutions with adjustment of various pH. Novelty of the study is, expending this way we can synthesize OCP irrespective of pH and most importantly at 37°C, which is an important parameter for acceptable biomaterials. Throughout the study, no foreign phase was observed except OCP. Morphological images are also evident for the attractive OCP flowers.

Keywords. DCPD; OCP; acetate buffer; pH.

1. Introduction

Octacalcium phosphate (OCP), $Ca_8H_2(PO_4)_6{\cdot}5H_2O$, has organizational comparisons with hydroxyapatite, HAP, $Ca_{10}(PO_4)_6(OH)_2$, though conflicting in their Ca/P molar ratios (1.33 and 1.67 for pure OCP and pure HAP, respectively). Owing to the physical comparisons amid OCP and HAP and despite the calcium-deficient nature of biological apatites, OCP has been offered to be a biological originator for the formation of apatites in enamel, dentine and bone [1,2]. OCP is not only a biomineral, but also a biomaterial having ability of bone regeneration. In a past research, OCP was implanted to calvaria and thigh muscle of mice and found that implanted OCP was transformed into low crystalline hydroxyapatite [3,4]. The advanced osteoconductive behaviour of OCP, accompanying the structural change to apatite phase, has been demonstrated to promote osteoblastic cell differentiation *in vitro* and facilitate bone regeneration *in vivo* [5]. OCP enhances bone regeneration and is resorbed *in vivo* more than HA or βTCP [6]. However, OCP has limited usability because it is produced in granular form owing to its chemical structure.

It has been shown that, in the course of OCP conversion to hydroxyapatite (HAP), the crystal lattice of OCP experiences topological changes as a result of Ca^{2+} and PO_4^{3-} diffusion [7,8]. Being a metastable phase, OCP is difficult to synthesize for kinetic reasons such as: (i) suitable calcium and phosphate ion concentrations, pH values and temperature, which together determine the necessary level of supersaturation, and (ii) appropriate start for dropping the energy in the case of non-homogeneous nucleation. OCP loses water starting at a temperature near 90°C, heating to 180°C leads to interlayer bond breaking in the structure of OCP, and heating to 300°C causes transformations accompanied by pyrophosphate formation from OCP [9]. Thus, it is impossible to fabricate ceramics from such materials by conventional methods that involve sintering.

In this article, we have discussed about the synthesis of OCP by the hydrolysis of dicalcium phosphate dihydrate (DCPD), permitting the hydrolysis period to be reduced, in buffer solutions and observe the influence of pH on the composition and morphology of the final product. The main objective of the study is to determine that by varying synthesis parameters one can obtain OCP precursors at various pH conditions.

2. Materials and methods

All reagents were procured from Wako Pure Inc., Japan. $CaCO_3$ was used as obtained. Ultrapure water (prepared using Direct-Q, Nihon Millipore K.K., Tokyo, Japan) was employed for experimental purposes. To an aqueous 1 M $NH_4H_2PO_4$ solution was added $CaCO_3$ powder in the ratio 1:100 (g ml^{-1}). The pH and temperature of the mixture were maintained at 4.6 and 25°C, respectively. The reaction product was kept for drying at room temperature for 24 h.

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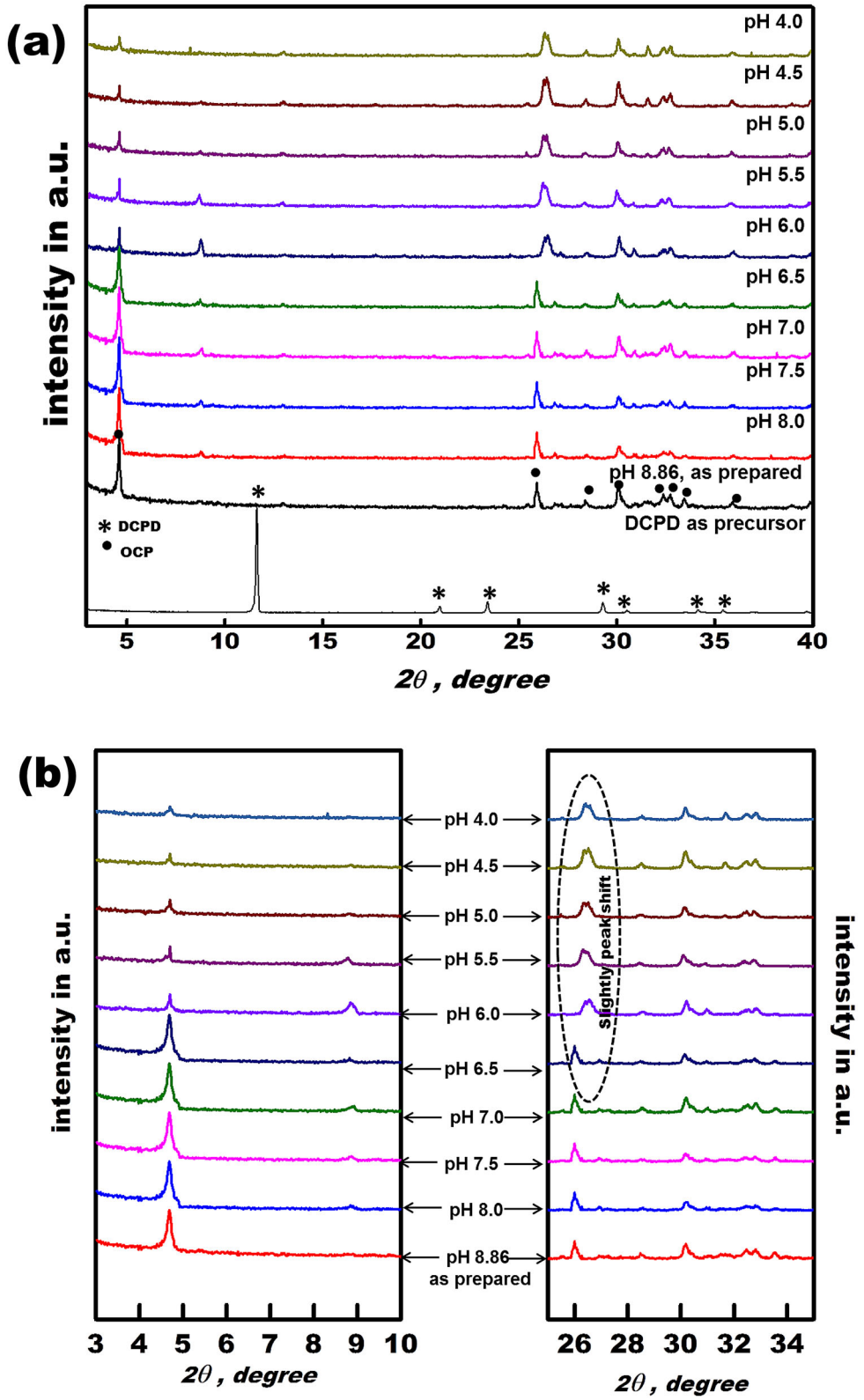

Figure 1. (**a**) Powder XRD patterns at different pH solutions of CH_3COONa: transformation of DCPD to octacalcium phosphate (OCP). (**b**) Closer view to evidence the slight peak shifts at certain pH solution.

Table 1. Percent yield for each set of pH condition.

pH	% Yield
8.89	90.5
8.0	90.0
7.5	85.2
7.0	85.2
6.5	85.0
6.0	82.5
5.5	82.2
5.0	80.2
4.5	80.0
4.0	80.0

X-ray diffraction examination showed that the powder consisted of phase pure DCPD.

$$CaCO_3 + NH_4H_2PO_4 + H_2O \rightarrow CaHPO_4 \cdot 2H_2O + NH_3 \uparrow + CO_2 \uparrow$$

DCPD was further hydrolysed with 0.1 M aqueous sodium acetate solution ($CH_3COONa{\cdot}3H_2O$) with varying pH from 8.8 to 4.0 at temperatures of 37°C, according to scheme (1)

$$CaHPO_4 \cdot 2H_2O \xrightarrow{CH_3COONa+H_2O} Ca_8(HPO_4)_2(PO_4)_4{\cdot}5H_2O$$

The surface microstructure of the samples were characterized using a scanning electron microscope (SEM; Model S-3500N; Hitachi Co., Tokyo, Japan), an energy dispersive X-ray analyser (EDX; Model EX-400; Horiba Co., Kyoto, Japan), X-ray diffractometer (XRD; MXP3V; Mac Science Ltd., Yokohama, Japan) and Fourier-transform infrared spectrometer (FT-IR, FT/IR-6100, JASCO Co., Tokyo, Japan). For FT-IR, the obtained OCP were first ground and mixed with KBr powder at a mass ratio of 1:100 and a thin film was prepared by uniaxially pressing the mixed powder to measurement.

3. Results and discussion

The powder XRD patterns of the DCPD and OCP obtained at different pH solution are shown in figure 1. For DCPD, all the peaks belonged to the pure DCPD phase (peak positions matching perfectly well with those given in JCPDS no. 11-293). DCPD was obtained by using $CaCO_3$ and $NaH_2PO_4{\cdot}2H_2O$ solutions (data for $CaCO_3$ not shown). OCP peaks (JCPDS no. 26-1056) appeared after immersion in CH_3COONa solution. As shown in figure 1a, beyond the CH_3COONa solution of pH 6.5 to pH 4.0 there was slight shift in the peak, which can be more closely evident in figure 1b. It shows that the peak shift is from 25.9 to 26.2 ± 0.1. There is no such evidence for this gradually shifted peak, but there is possibility of some ionic incorporation

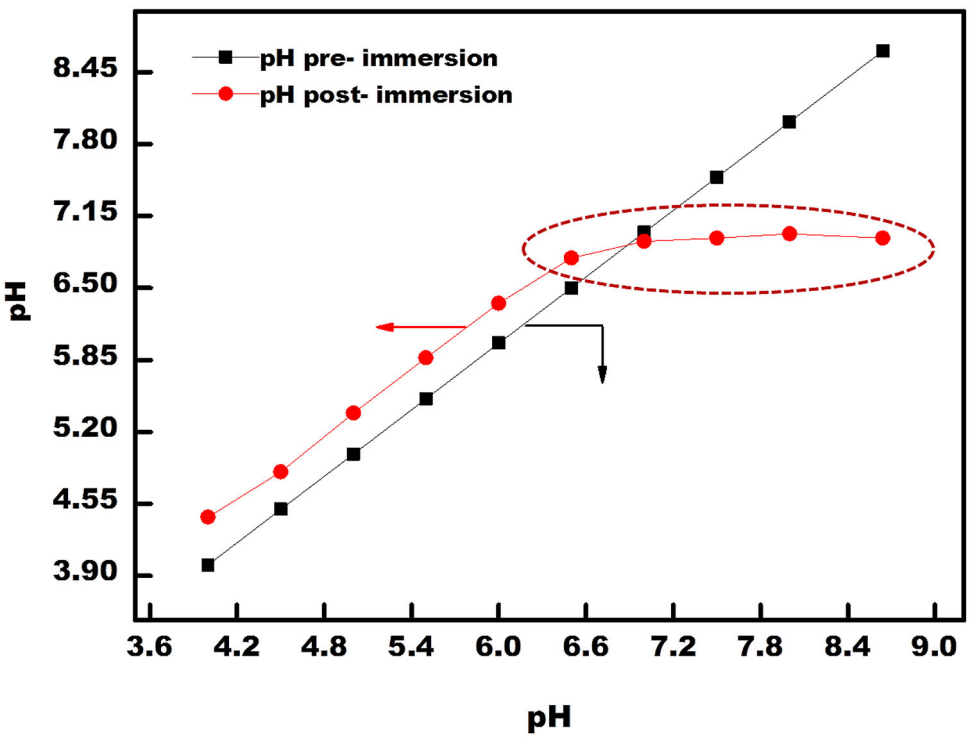

Figure 2. Graphical illustration of the pH of prepared solution: pre- and post-immersion of DCPD to obtain OCP (area in red: OCP formation range).

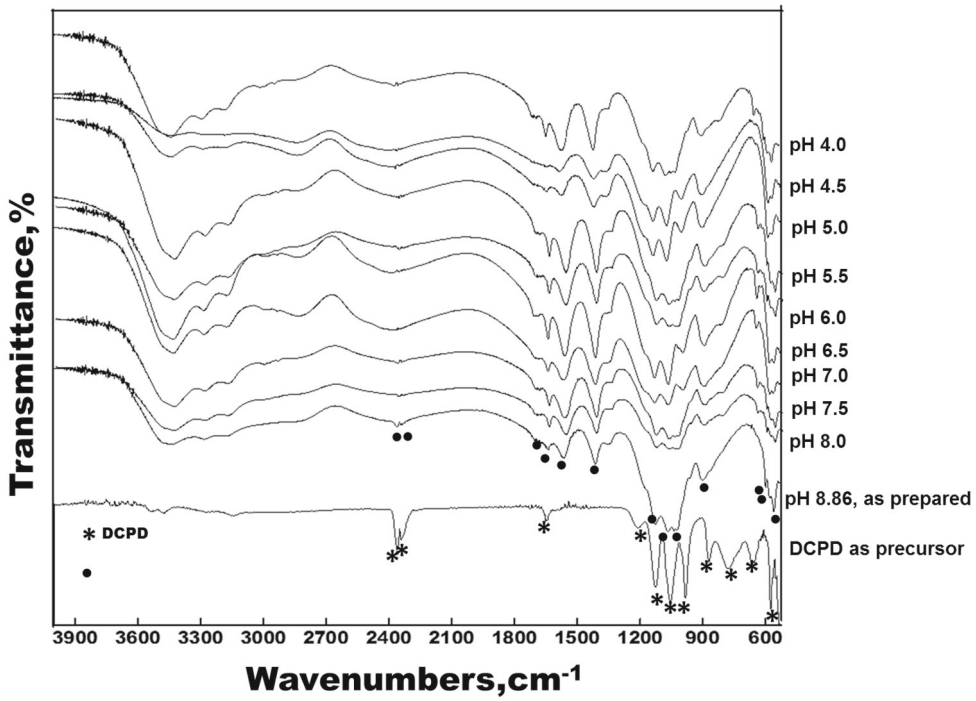

Figure 3. FT-IR patterns of DCPD precursor and obtained octacalcium phosphate (OCP) at different pH.

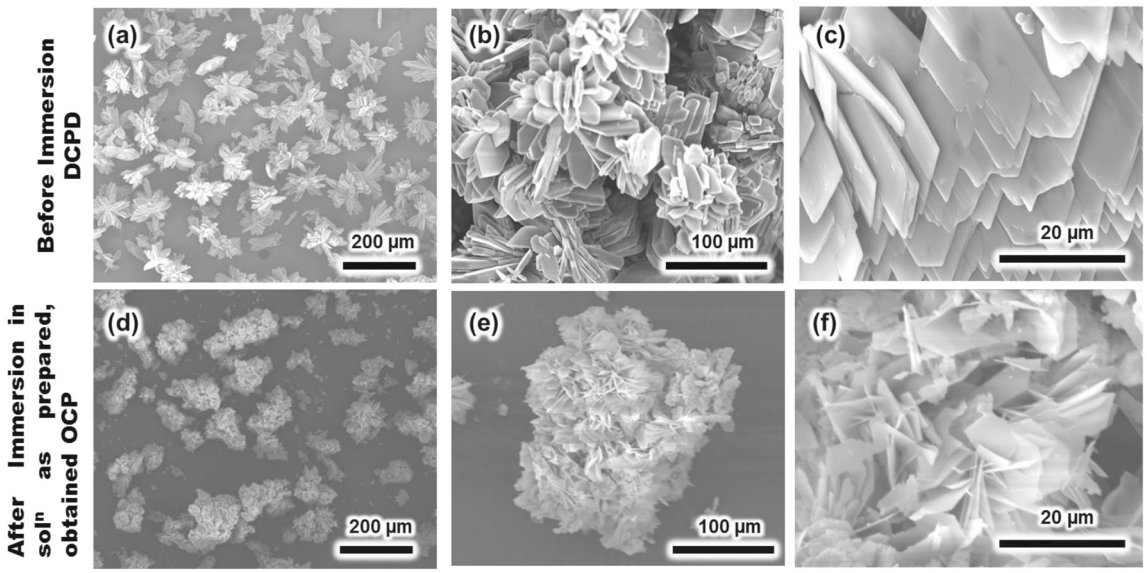

Figure 4. SEM images of DCPD precursor and obtained OCP.

with DCPD while converting to OCP. The % yield for the obtained OCP for each set of pH condition has been summarized in table 1. The yield ranged between 90 and 80% for various pH conditions.

Figure 2 summarizes the change in pH of prepared solution, pre- and post-immersion of DCPD to obtain OCP. The highlighted area in red is the most stable pH range for the formation of OCP in general. Remarkably, in this article we are reporting and providing evidence for the formation of OCP beyond this pH range and even more acidic condition. OCP is favourably formed under weak acidic environments [10,11]. It is well known that the pH changes after phase transformation and also marked in the current case. Apart from the highlighted area, other solutions also obtain OCP but with the minor peak shift and in a few cases a minute residue of DCPA. It is well known that the stability

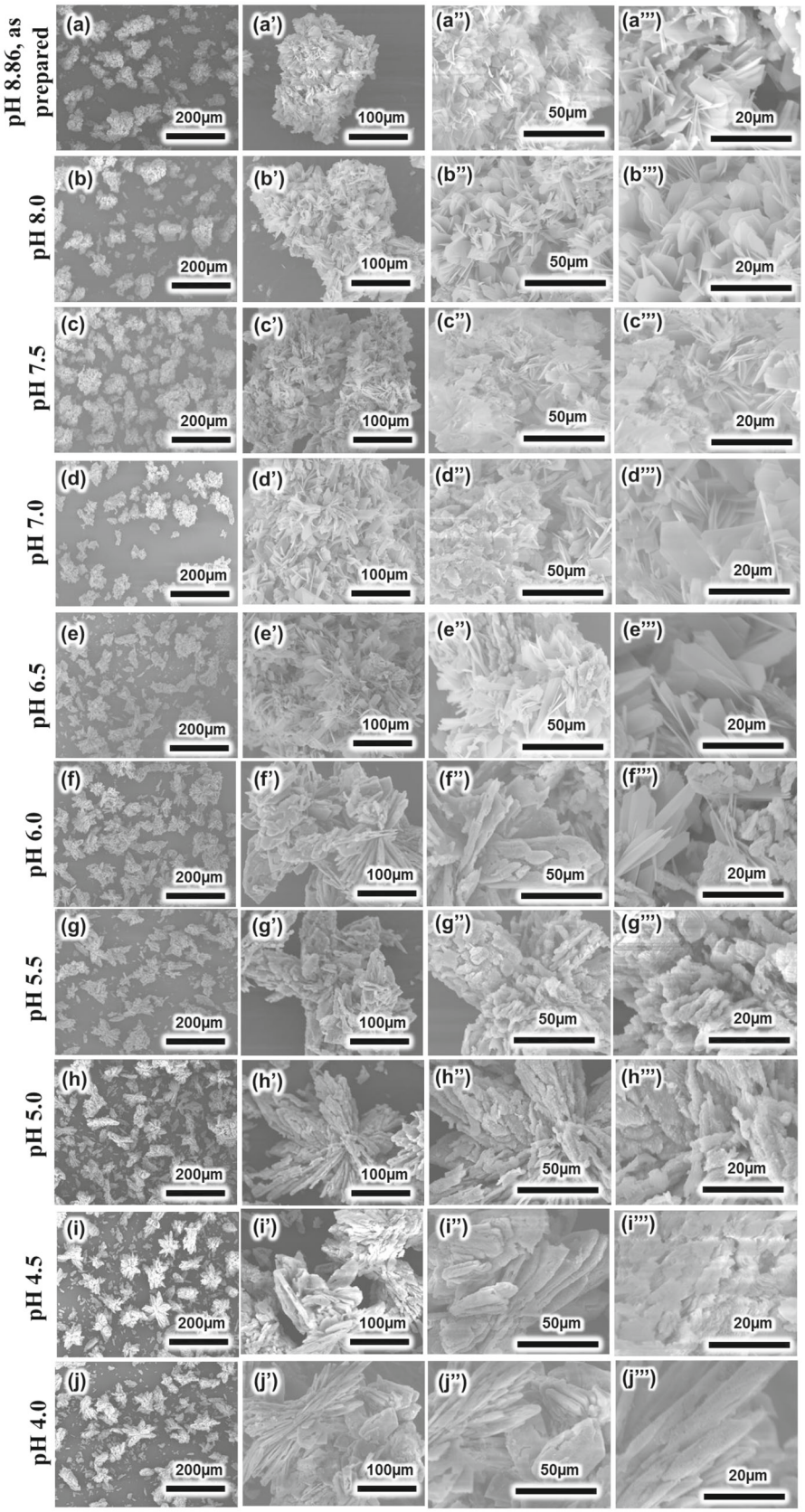

Figure 5. SEM images of obtained OCP at different pH solutions of CH_3COONa.

of Ca/P phase is mainly based on pH, so the final pH of the solutions after conversion of DCPD to OCP could be used to estimate the Ca/P products.

The FTIR spectra were taken in the range between 400 and 4000 cm^{-1} for DCPD and OCP crystals at room temperature. Figure 3 displays the FTIR scan of DCPD and developed OCP crystals under several solution conditions. They showed the wide envelope between 2000 and 3900 cm^{-1}, which may perhaps be allocated to OH stretching of HPO_4^{2-} and H_2O [12]. The bands that occurred at 986, 990, 1060, 1063, 1067, 1129, 1134 and 1136 cm^{-1} correspond to HPO_4^{2-} vibration. The PO_4^- bending mode peaks appeared at 666, 668, 791, 794, 872 and 874 cm^{-1}. The indexing of the absorption peaks of OCP was postulated to the previous report [13,14]. In pure OCP, 1193, ~1120, ~1034, 601 and 560 cm^{-1} absorption peaks were allocated to the –OH in plane-bending mode of HPO_4 in the hydrated layer, HPO_4 stretching mode, PO_4 stretching mode, and PO_4 bending mode, respectively [15]. Akin to XRD results, here also in FTIR we did not sign any foreign phase throughout.

Figure 4 shows SEM images of the precursor DCPD and obtained OCP (in as-prepared CH_3COONa solution) at lower and higher magnifications. Fine-looking flowers of DCPD depict the formation of pure crystalline DCPD both at lower and higher magnification images. Similarly, aggregated petals of OCP illustrate the pure OCP formation during phase transformation.

As mentioned earlier, we have adjusted the pH of CH_3COONa solution ranging from 8.89 to 4.0, to make variables. We immersed the DCPD into all set of solution and further analysed if there is formation of OCP or any other phase. The morphological results of obtained products are summarized in figure 5. Results illustrated that we obtained large OCP petals till pH 6.0, later it was difficult to observe the clear OCP. There is possibility of very small-sized OCP petals, which have been deposited on the base structure of DCPD. Contradictly, in XRD and FTIR, clear peaks of OCP were present. This might be due to the difference of analysis techniques and examination level.

This study has confirmed the part of Na ions in the development of OCP in limited conditions by means of unique 0.1 M CH_3COONa solution system at 37°C. It needs to be remarked here that this study is very simple and easy way to obtain the OCP without any impediment at wide range of pH ranging from as-prepared solution to 4.0. Ten different pH solutions of CH_3COONa have been prepared to check whether it can transform DCPD to OCP, and surprisingly yes, it can work well.

Upcoming studies would consider the part of further ions in OCP formation. It is yet indistinct in what way the Na or other counter ions will affect the OCP crystal lattice and integrates their behaviour. The procedure defined here will suggestively subsidize to the expansion of usages for OCP in numerous fields.

4. Conclusion

In this article, we have confirmed that for the period of OCP formation from hydrolysis reaction of DCPD, sodium (Na) affects OCP development and its crystallographic construction irrespective of pH. This method is very simple and quite effective for OCP formation in laboratory or industry. We have studied the chemical conversion of calcium carbonate into DCPD, followed by DCPD hydrolysis to OCP. The process enables the preparation of phase pure OCP. DCPD hydrolysis in an aqueous sodium acetate solution of 0.1 M involves OCP formation. Working temperature is very important parameter for biomaterials, and in this study all experiments were performed at room temperature (solution preparation) and 37°C.

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