Synthesis and Characterization of Nanoporous Biphasic Calcium Phosphate

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Abstract. For the past few years, many researchers are focusing on biomaterials fabrication in porous form. The research on porous calcium phosphate has been investigated due to its excellent biocompatibility and better osseointegration. This research paper presented nanoporous biphasic calcium phosphate (BCP) synthesised using chemical precipitation method. Triblock co-polymer F127 was used as pore directing agent. The chemical compositions of pure BCP samples were examined using X-ray diffraction (XRD) analysis which shows common peak of BCP. The pore size distribution (PSD) on the other hand shows that the pore size of the samples mainly distributed at 52.8 nm, 49.6 nm and 32 nm. BCP pellets were soaked in phosphates buffered saline (PBS) and distilled water (DW) for 15 days. The pH of the soaking medium decreases throughout the soaking period due to degradation of BCP pellets, which release hydrogen ions into the PBS and distilled water. BCP degrades faster in distilled water than in PBS. After soaking for 15 days, materials were examined with a scanning electron microscope (SEM) to assess the morphological structure before and after in vitro degradation. Apatite formation was discovered on the surface of the BCP pellet that had been immersed in the PBS solution.

1. Introduction

Biphasic calcium phosphate (BCP) consists of two individual calcium phosphate phases which are hydroxyapatite (HA, Ca10(PO4)6(OH)2) and β-tricalcium phosphate (β-TCP, β-Ca3(PO4)2) in different proportions [1, 2]. HA is a stable phase with low degradation rate, while β-TCP is the soluble phase with higher degradation rate [2, 3]. This combination made BCP possessed more advantages compared to the single phase of HA and β-TCP [4-8] by allowing a better control of degradation rate, improved bioactivity and promoting osseointegration. For many years, HA has been widely applied in bone tissue
engineering due to the similarity in its chemical composition with the inorganic mineral of biological bone [9].

Numerous synthesis techniques have been established to produce BCP bioceramics with varied HA/β-TCP ratios that mimic the physical and biological features of biological bones. There are two major approaches to produce BCP, the first one is sintering of natural or synthetic non-stoichiometric calcium phosphate powder such as calcium deficient HA (CDHA) at temperature above ≈ 700 °C [10-13]. Another preparation approach is involved mechanical mixing of HA and β-TCP powders at elevated temperature [14, 15]. This approach is based on solid-state reaction between two solid compounds. The materials reactivity is influenced by the degree of calcium deficiency (Ca/P molar ratio > 1.67), where the lower the ratio, the stronger the reactivity [16].

The preparation of pure nanoporous BCP with Ca/P ratio of 1.5 and degradation properties has not been reported in the literature and hence the aim of this study is to develop a simple pathway for preparation of this material. In this study, nanoporous BCP was synthesized using wet precipitation method by using surfactant as pore template.

2. Methodology

2.1. Materials
Calcium nitrate tetrahydrate (Ca(NO$_3$)$_2$·4H$_2$O) and diammonium hydrogen phosphate ((NH$_4$)$_2$HPO$_4$) were the chemicals used as calcium and phosphate precursors, respectively. Ammonium hydrogen carbonate (NH$_4$HCO$_3$) was used as a carbonate precursor. Pluronic® F127, a non-ionic triblock copolymer donated by BASF, USA, was used as the pore template's structure directing agent. In addition, sodium hydroxide (NaOH) was used to keep the pH of the mixtures at 11.

2.2. Synthesis of BCP powders
Triblock copolymer, Pluronic® F127 was diluted in 100 ml of distilled water and stirred until a clear surfactant solution was obtained. The amount of calcium and phosphate precursor used was determined based on Ca/P molar ratio is 1.5. The surfactant-calcium mixed solution was then obtained by adding 9.446 g of Ca(NO$_3$)$_2$·4H$_2$O to the surfactant solution and stirring for 30 minutes. Concurrently, 3.526 g of (NH$_4$)$_2$HPO$_4$ was dissolved in 60 ml of distilled water to form a phosphate solution. The phosphate solution was then slowly dripped into the surfactant-calcium solution while continuously stirring until a milky suspension was obtained. 1M NaOH was used to set the pH to 11 throughout the mixing process. The milky solution was then aged for 24 hours at room temperature. The solution was then centrifuged at 3000 rpm for 20 minutes and washed 5 times with 100 ml of distilled water. Following that, the white precipitates were dried in an oven at 100°C for 24 hours before being ground into fine powders with a mortar and pestle. Finally, the white powders were calcined in a furnace at 550°C for 6 hours.

2.3. Characterizations of nanoporous BCP powders
X-ray diffraction (XRD) spectrometer was used to determine phase and crystallographic structures of the calcined powders. Surface area and pore characteristics of the samples were evaluated using Micromeritics TriStar nitrogen adsorption-desorption analysis machine. Brunauer-Emmett-Teller (BET) equation was used to calculate the specific surface area of the samples. The pore size distribution (PSD) of the samples was calculated from the desorption data of the isotherms using the Barrett-Joyner-Halenda (BJH) model.

2.4. In vitro degradation study of nanoporous BCP
Calcined powders were pressed into 1.6 mm thick and 10 mm diameter pellets using a uniaxial press at 150 MPa for 30 seconds. The pellets were immersed in 10 ml of 0.1 M phosphates buffered saline (PBS) solution, pH 7.4 and in distilled water (DW), pH 7 as the control samples. The pellets were incubated at 37°C for 3, 6, 9, 12, and 15 days without replacing the PBS solution. At the end of each soaking day,
pH of the PBS and DW was measured. BCP pellets were removed, rinsed with distilled water and dried in desiccator to determine water absorption and weight loss.

The weight of the wet pellet and the weight of the dried pellet were used to calculate the water uptake, as shown in equation 1 [17]. \( W_w \) is the weight of the final sample (wet) and \( W_i \) is the weight of the initial sample (dry).

\[
\text{Water uptake (\%) } = \frac{W_w - W_i}{W_i} \times 100\%
\]  

The pellets were then dried in a desiccator for 24 hours before the weight changes were calculated using equation 2. [17]. \( W_f \) is the weight of the final sample (after 24 hours drying) and \( W_i \) is the weight of the initial sample (dry).

\[
\text{Weight loss (\%) } = \frac{W_i - W_f}{W_i} \times 100\%
\]

The morphological surface of the BCP pellets before and after the soaking period in PBS and distilled water was examined by using scanning electron microscopy (SEM).

3. Results and Discussion

3.1. Characterization of nanoporous BCP powders
The crystallographic phases present in the BCP samples was determined using XRD. Figure 1 shows the XRD pattern of three batches of BCP powders. The XRD result shows that the HA and \( \beta \)-TCP peaks were appeared in calcined BCP samples. The peaks at \( 2\theta = 23.2^\circ, 25.9^\circ, 29.7^\circ, 31.8^\circ, 32.9^\circ, 34.0^\circ, 39.8^\circ, 41.9^\circ, 46.7^\circ, 49.5^\circ \) and \( 53.2^\circ \) matched closely with the diffraction peaks of stoichiometric HA (ICDD 090432). The peaks of \( \beta \)-TCP appear at \( 2\theta = 25.9^\circ, 29.7^\circ, 34.0^\circ, 39.8^\circ, 46.7^\circ \) and \( 53.2^\circ, 57.1^\circ \) (ICDD 090169). Two main peaks which represent plane (2 1 1) of HA and (0 2 1) of \( \beta \)-TCP were present at \( 31.8^\circ \). No other peaks of other phases were found in the spectrum. Hence, the samples consist of HA and \( \beta \)-TCP phases at percentage of 40% and 60% respectively. The broad peak in between \( 30^\circ \) and \( 35^\circ \) indicate that the BCP was less crystalline. This was postulated by the addition of F127 surfactant during synthesising process which altered the long-range order of the crystal structure hence resulting in lower crystallinity.

![XRD pattern for BCP.](image)

Figure 1. XRD pattern for BCP.
Figure 2 shows nitrogen adsorption desorption isotherms of BCP samples. Similarly, the patterns shown are exhibit to the Type IV isotherms pattern with H1 hysteresis loop. The H1 hysteresis is often associated with porous materials which consist of agglomerates or compacts form of nearly uniform spheres with fair regular array. This on the other hand disclosed that the BCP powders have narrow pore size distributions [30]. Though, the quantity adsorbed and desorbed are slightly distinct between these three BCP samples. These differences could be seen clearly at the relative pressures (P/Po) 0.8 to 0.99. These slight differences are due to the differences in the surface area and average pore size of the samples as shown in Table 1. Figure 3 shows the pore size distribution (PSD) of the samples, which demonstrates that the pore size is mainly distributed at 52.76 nm, 49.55 nm, and 31.99 nm which within the range of nanopores (1-100 nm).

Table 1. Pore characteristics of synthesized BCP powders.

| Sample | Surface Area, \( S_{BET} \) (m²/g) | Average pore Size, BJH (nm) | Pore Size (Mode), BJH (nm) | Pore Volume, BJH (cm³/g) |
|--------|----------------------------------|-----------------------------|-----------------------------|--------------------------|
| BCP 1  | 24                               | 37.64                       | 52.76                       | 0.110                    |
| BCP 2  | 32                               | 34.99                       | 49.55                       | 0.122                    |
| BCP 3  | 42                               | 26.51                       | 31.99                       | 0.100                    |

Figure 2. Nitrogen adsorption-desorption isotherm of BCP.

Figure 3. Pore size distribution (PSD) of the BCP powders.
3.2. Degradation of BCP pellets

In this discussion, graph named as Experimental sample represent pellets immersed in phosphate buffer saline (PBS) solution while graph named as Controlled sample represent pellet immersed in distilled water (DW).

3.2.1 pH changes

Figure 4 shows the changes in degradation behaviour in respect to pH value over degradation period. The pH value of PBS drops significant initially from 7.4 to approximately 6.9 and stabili
ses at pH around 6.7 after soaking for 3 days. Distilled water (DW) demonstrated similar trend as shown in Figure 4. The solution become acidic due to hydronium ions were released from the BCP pellet into the PBS [32]. The chemical reaction can be explained as shown in equation 3. PBS solution is a buffer that is resistant to severe pH fluctuations when an acid or a base is added. As a result, relatively minor pH alterations were found in PBS as compared to DW.

\[
H_3O^+_{(ag)} + HPO_4^{2-}_{(ag)} \rightarrow H_2PO_4^-_{(ag)} + H_2O\tag{3}
\]

![Figure 4. pH changes in Experimental sample (PBS) and Controlled sample (DW).](image)

![Figure 5. Water uptake in Experimental sample (PBS) and Controlled sample (DW).](image)

3.2.2 Water uptake

Water uptake is crucially for bioactive materials for diffusion and exchange of nutrients and waste. Mechanical performance of the biomaterial will also be affected by the amount of water absorbed [33]. Hydrophilicity of a material surface is greatly influenced the degradation of the material as the absorption of water molecule contributes a favourable environment for hydrolytic actions. Figure 5 shows the water uptake of BCP pellets that immersed in PBS and DW at 37 °C for 15 days. The graph shows that the water uptake in PBS medium is increase steadily from 28.5% on day 3 to 38% on day 12. However, the percentage of water suddenly drop to 31.5% on the last day of soaking (day 15). The samples immersed in PBS and DW demonstrated similar trend of water uptake, but it can be seen that BCP pellets are more permeable to PBS than DW. This result most probably due to the concentrated ions in PBS solution that increased the difference in osmotic pressure between the pellets network and PBS thus resulting in a swollen pellet.

3.2.3 Weight loss

Figure 6 shows the weight loss of pellets after immersed in PBS and DW. The degradation rates are highly depending on the pH since calcium phosphate species are more soluble in acidic environments compared to neutral environment. By referring to Figure 4, the pH of the DW become more acidic in comparison to PBS solution due to absence of buffer effect in DW. Hence, the degradation rate of BCP
is highly take place in distilled water compared to PBS medium. As the degradation rate is higher, the weight loss also higher.

Graphs in Figure 6 shows that after 3 days of immersion, the weight loss for pellets immersed in PBS is 4.5% while in DW is 7.5%. Throughout the soaking period, pellets that immersed in PBS solution demonstrated much lower weight lost compared to the pellets that immersed in DW. Interestingly, after 6 days of the immersion, both graphs gradually decrease due to the higher dissolution of Ca\(^{+}\) in the solution. This consequently favourable for some degree of re-precipitation, which can also contribute to the decrease in the degradation rate. At this stage, it can be assumed that the degradation rate is occurs slowly compared to the apatite forming rate. However, after 15 days of immersion, the weight losses for both samples were increase again as the degradation rate was faster than the apatite formation rate.

The degradation behaviour of BCP also ruled by other factors include the in vitro solution environment, such as type and concentration of the buffered or non-buffered solutions, the degree of saturation, ionic strength, porosity, surface morphology of material such as cracks, loading conditions, duration of immersion and the presence of proteins and cells. Besides that, the physicochemical such as crystallography (crystallinity, crystal particle size), density and chemical composition may also affect the degradation performance of the BCP [34].

![Graph](image_url)

**Figure 6.** Weight loss in experimental sample (PBS) and controlled sample (DW).

### 3.2.4 Scanning Electron Microscopy (SEM)

Figure 7 and Figure 9 shows SEM images of BCP pellet before immersed in DW and PBS. According to Figure 8, no apatite formed on the pellet surface after immersed in DW for 15 days. Meanwhile, SEM images in Figure 10 revealed that concentrated spherulites [35] deposited on the BCP surface representing the growth of apatite crystals after 15 days of PBS immersion. Furthermore, a spongy-like structure also formed on the pellets surface due to the increasing number of deposited particles. The release of Ca\(^{+}\) from the BCP pellets during first 3 days of soaking period (from day 0 until day 3) promote the apatite formation. As mention earlier, weight loss reduction from day 6 until day 12 of immersion was most probably due to the BCP degradation rate is lower than apatite formation rate. The apatite layer was formed on the BCP pellet surface after 6 days of immersion and continue to growth until day 15 of immersion. The growth of apatite layers on the surface of the BCP pellets mimic the process of mineralization in the bone. According to Mohammad [18], mineralization of osteoblast only took place after 16 to 20 days of cell culture.
4. Conclusion
Nanoporous BCP powders were successfully synthesised by using chemical precipitation method with non-ionic surfactant F127 as pore template. The degradation study revealed that pH of solution and amount of water absorb/uptake effect degradation rate of the BCP. The degradation rate of BCP is highly take place in acidic medium compared to alkaline medium. Thus, in this study, BCP degrades faster in distilled water than in PBS. As degradation rate is higher, the weight loss increases. Apatite formation was discovered on the surface of the BCP pellet that had been immersed in the PBS solution.

Acknowledgement
This research work was supported by Research Materials Fund (RESMATE, 9001-00626) from Universiti Malaysia Perlis.

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