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Processing and Characterization of Natural Hydroxyapatite Powder from Bovine Bone a

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ABSTRACT

In this paper, hydroxyapatite powder is produced via annealing of natural bovine bone. The as received bovine bone was annealed at three different temperatures of 400 °C, 700 °C, and 1000 °C after a primary preparation stage. The powders obtained from annealed bovine bone were analyzed and characterized by Scanning Electron Microscope (SEM), X-Ray Diffraction (XRD), and Energy-Dispersive X-ray (EDX) spectroscopy. The XRD analysis showed that the annealed bone at 1000°C is pure hydroxyapatite, at 700 °C, it is mostly hydroxyapatite, and at 400°C, there are not enough hydroxyapatite crystals. The morphology of the samples was analyzed by SEM. It was observed that the bone annealed at 1000 °C exhibits human bone-like matrix particle shapes while at 400 °C, and 700 °C, the irregular shape exists. EDX analysis showed that Ca, P, C, and O were detected in the samples, while Ca and P were the major components. Also, the Ca/P ratios were more than 1.67, which is the ratio of stoichiometric hydroxyapatite.

Keywords: *Hydroxyapatite, Bovine bone, Annealing, Characterization*

1. Introduction

So many factors can cause damage to the structure of bones in the human body; Such as age, physical activity, and injuries. Bone tissue can repair itself; However, when the injuries are serious, this ability is not always sufficient. Therefore, other techniques and treatments are needed for repair and regeneration of bones [1–3]. Studies have shown that hydroxyapatite promotes osseointegration between bone and orthopedic implants. Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is an essential material in the dental implant industry and orthopedic prostheses, as it has a similar structure and bioactivity of natural bone [1–5]. For example, metallic dental implants can be fixed to the bone permanently or temporarily by using mechanical screws, or acrylic bone cement. However, these methods can cause problems such as implant-bone loosening. Therefore, hydroxyapatite coating can be a solution for this, as it is a bioactive surface [6–9]. Various forms of HAp, such as powders, porous blocks, and hybrid composites, are used as an implant material, due to their exceptional biocompatibility, good bioactivity, and osteoconductivity. They can be integrated into bone without stimulating an immune reaction [10].

Hydroxyapatite can be produced by different methods such as thermal decomposition, alkaline hydrolysis, and combination method. Abundant sources are available to extract natural hydroxyapatite as well; Fishbone, limestone, camel bone, and even some plants are some of these sources [11–13]. For example, Gergely et al. [14] produced HAp by using recycled eggshells.

Heat treatment of bovine bone is a method to obtain HAp. Controlling heat treatment's

temperature and duration are the most important parameters [15]. Zhou et al. [16] reported that synthetic HAp would be stable for the Ca/P mixing ratios near to 1.67 for temperatures below 1200°C. The prior relevant study on the properties of HAp was done by Ooi et al. [13]. They produced porous hydroxyapatite by heat treatment of bovine bone at temperatures between 400°C and 1200°C.

In this research, hydroxyapatite is produced from bovine bone. The powder was exposed to three different temperatures of 1000 °C, 700 °C, and 400 °C. The resulting HAp powders were then analyzed and characterized by XRD, SEM, and EDX analysis.

2. Experimental procedures

2.1. Sample preparation

A bovine bone is the as-received raw materials of this study. First, the bones were meticulously cleaned and scrubbed to remove impurities, such as meat, completely. During the next step, the bones were left in acetone bath for a day and they were then rewashed with water. Afterwards, the bones were boiled in water for one day. As a result, they were easily crushed and then milled into smaller particles. Finally, the pulverized bone samples were divided in three groups and each were annealed in a furnace at three specific temperatures of 400 °C, 700 °C, and 1000 °C for two hours. The schematic of the processing stages can be observed in Figure 1.

2.2. Characterization

The crystallinity of the annealed powder was analyzed via X-ray diffractometer and HighScore Plus software. The composition of phases was analyzed by the X-Ray diffraction (XRD) method with the use of the Rigaku ULTIMA IV diffractometer, which had a graphite monochromator with Cu Kα radiation (λ = 0.154 nm) and Ni filter. The XRD spectra were taken at 40 kVp and 40 mA over a range of 2θ angles from 20 to 50 degrees. The annealed bone were analysed at room temperature at a scan rate of 4 degrees per minute with a step scan of 0.02. Using the XRD data, crystallite size was related to peak broadening of the XRD graphs, using Scherrer's formula which indicates that the peak width is inversely proportional to crystalline size. The composition of crystalline phase was determined based on standard JCPDS cards available in the system software. The ratio of calcium (Ca) to phosphorus (P) was estimated from compounds containing both elements.

Different annealing temperatures affected the morphology of the powder. The annealing temperature effect on the evolution of microstructure was investigated by a scanning electron microscope (SEM: S-4160 Hitachi). To be able to take SEM micrographs, the samples were coated with a layer of gold (Au). Also, energy dispersive analysis of X-rays was done by energy-dispersive X-ray spectroscopy (EDX).

3. Results and discussions

The first obvious change within the annealed powder was their color change. The primary prepared bovine bone was yellow. However, when it was annealed at 400 °C, its color changed to black. At 700 °C, the color of the sample became white, and at 1000 °C, the color was almost the same as 700 °C. This suggests that at temperatures 700 °C and above, the organic substances, such as protein, are completely removed. This is in agreement with the results of the study by Manalu et al. [17],

Fig. 1- Schematic of the processing stages starting from as-received bone to hydroxyapatite powder.

stating that the lowest optimal temperature to produce HAp with close to no organic substances has been 700 °C. They found a gradual change in the degree of organic substances with the increase of the annealing temperature from 200 °C to 700 °C. Asaduzzaman et al. [18], also observed similar finding both for bovine bone and human bone with the reported temperature of 600 °C as the temperature at which annealing would remove the organic substances completely. More tests can be done using the annealing temperatures between 400 °C and 700 °C to find a more precise threshold at which the HAp has close to no organic substances.

The derived HAp crystals phase and purity were confirmed using XRD analysis. XRD results of powders annealed at the three different temperatures are shown in Figure 2. The width of peaks at 400 °C, presented in red, demonstrates the deficiency of hydroxyapatite crystals in the sample. In other words, the fluorapatite phase was mostly observed at this temperature. This XRD pattern is similar to the XRD patterns previously reported by researchers showing the poorly crystallized or amorphous fraction, having nearly 2° shift of the broad maximum to a lower Bragg angles compared to the peak produced by the HAp crystalline [19]. This shift is observable when comparing the XRD of the annealed powder at 400°C (red) with the XRD patterns of the samples annealed at 700 °C (blue) and 1000 °C (green). The XRD patterns of the annealed powder show that as the temperature increases, the peaks' width decreases, and their height increases. By implementing heat treatment to the bones, organic components will be burnt out, and organic components will be re-crystallized [20]. Narrower, sharper, and more intense diffraction peaks show an increase in the rate of crystallinity, and it could be concluded that the crystallite size qualitatively increased [13–16,19–21]. The 2θ angles were similar to that of standard HAp with minor shifting that might be related to the removal of OH radicals due to the dehydroxylation of the HAp phase [17]. It can be seen that the XRD pattern related to the samples annealed at 1000 °C (green) that the no other peak apart from the HAp is obtained. This shows that the chemical structure of the HAp has not been changed and the bone matrix was not disordered with annealing up to 1000 °C.

As mentioned, 70 % hydroxyapatite phase and 30% fluorapatite phase were observed at 700 °C, and at 1000 °C, hydroxyapatite was the only phase (100%, as shown in the figure). At 700 °C and 1000 °C, the XRD patterns were almost the same. This comparison shows that at 1000 °C, the sample was pure hydroxyapatite.

In order to further compare the degrees of crystallinity in the annealed samples equation (1) was used [22,23]:

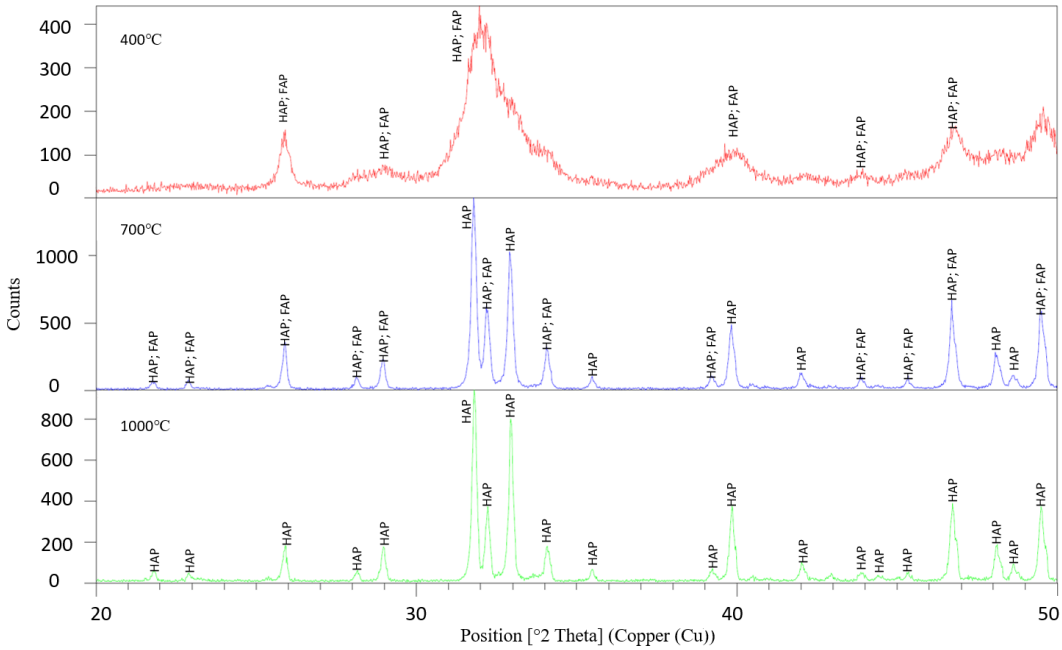

Fig. 2- XRD patterns of the samples annealed at 400 °C, 700 °C, and at 1000 °C (FAP=fluorapatite and HAP=hydroxyapatite).

$$Xc\ (\%) \approx (1- (V_{112/300} / I_{300})) \times 100 \quad (1)$$

Where $V_{112/300}$ is the valley intensity between peaks 112 and 300 planes, I_{300} is the peak intensity 300 plane, and Xc is a fraction of crystalline phases existing in the sample.

The crystallinities were found to be 90.6 % and 93.1 % for samples annealed at 700 °C and 1000 °C respectively showing the high crystallinity samples annealed at both temperatures. The peaks were not separated between 112 and 300 planes for the case of 400 °C, indicating the sample annealed at this temperature being amorphous.

The increasing temperatures affected the morphology of the samples. SEM images of the samples annealed at 400 °C, 700 °C, and 1000 °C are shown in Figure 3 and 4. As shown in the figure, the size of the grains increased with increase in annealing temperature. In addition, at 400 °C, the shape of the particles was irregular; This

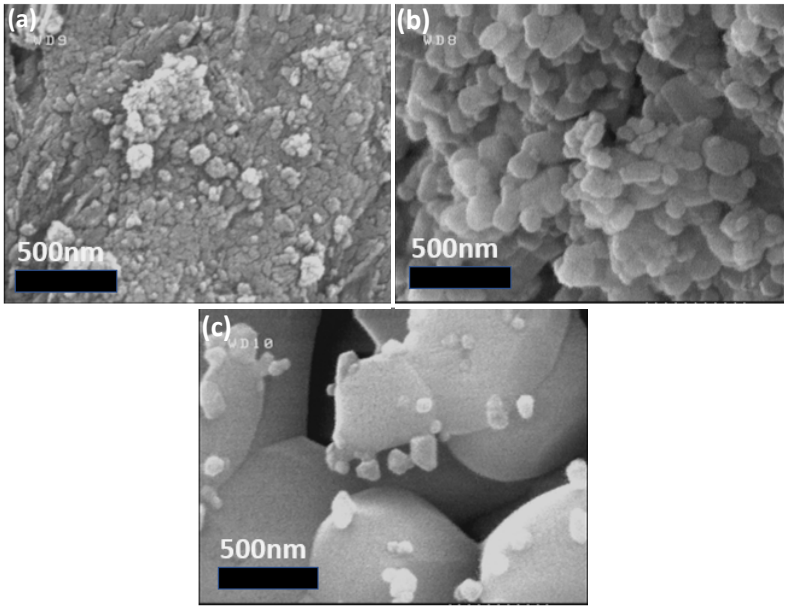

Fig. 3- SEM image of bone annealed at (a) 400 °C (b) 700 °C (c) 1000 °C with 60.0K magnification.

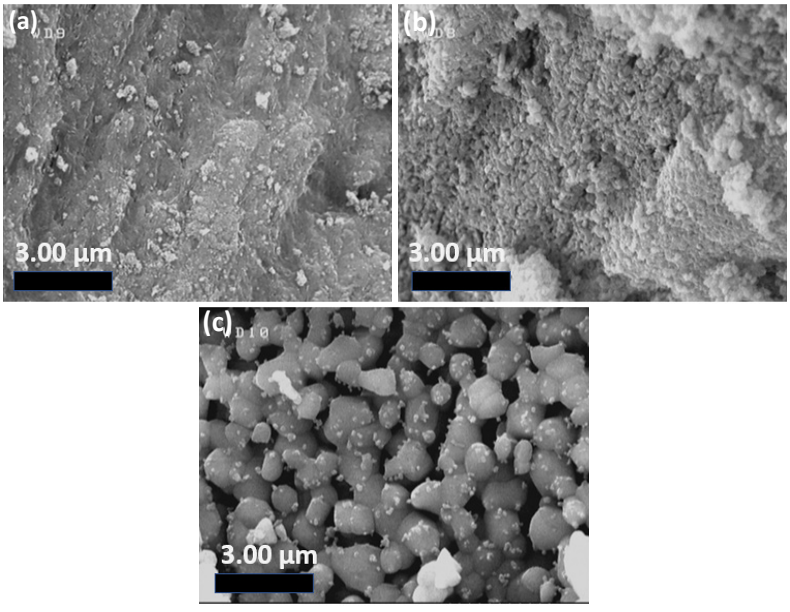

Fig. 4- SEM image of bone annealed at (a) 400 °C (b) 700 °C (c) 1000 °C with 10.0K magnification.

demonstrates that the organic substances were not completely removed at this temperature. At 700 °C, the structure was less irregular, while at 1000 °C, a typical bone-like matrix as shown in Figure, was observed [13], Showing complete removal of organic substances [24]. The average formed microparticles of samples annealed at 700 °C, and 1000 °C, were found to be roughly 310 nm and 941 nm respectively. The microstructures observed in the samples annealed at the higher temperatures is due the tendency of the particles to cluster at high temperatures [17]. The SEM micrographs results are in agreement with the results from XRD analysis.

The EDX analyses of the samples are shown in Figure 5. The figure demonstrates the different elements in the calcined bones, which Ca and P are the major components amongst them; Other elements such as C and O were also seen as minor components.

The Ca/P weight and molar ratios of all three samples were calculated; The weight ratios are 2.24, 2.72, 2.37 for bones annealed at 400 °C, 700 °C, and 1000 °C, respectively. The Ca/ P molar ratios are 1.73, 2.10, 1.83 for HAP-400 °C, HAP-700 °C, and HAP-1000 °C, respectively. Figueiredo et al. [25] articulated that natural HAP is non-stoichiometric, having a Ca/P ratio greater than 1.67. The high Ca/P molar ratio values may have resulted because

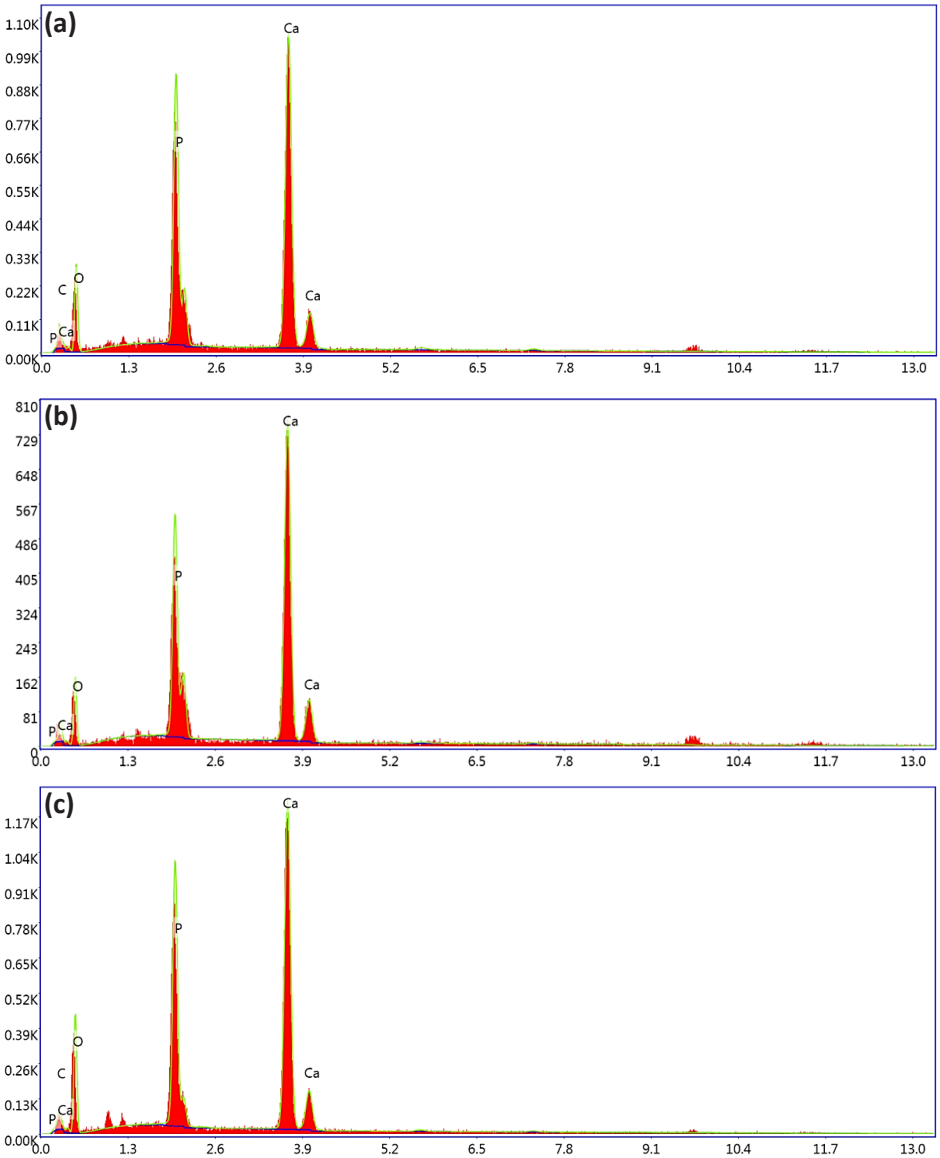

Fig. 5- The EDX analysis of a bone sample at (a) 400 °C (b) 700 °C (c) 1000 °C.

other Ca and P compounds occurred instead of hydroxyapatite [26]. Moreover, the approach of extracting hydroxyapatite from bovine bone by calcination, the temperature, and the conditions may have an impact on the Ca/P ratio [11].

4. Conclusion

In this study, hydroxyapatite was extracted from the bovine bone in three different temperatures; 400 °C, 700 °C, and 1000 °C, and the following results could be concluded.

- Pure hydroxyapatite powder was successfully processed via annealing of bovine bone at 1000 °C.
- XRD and SEM analyses show that at 1000 °C, the organic substances were completely removed, and pure hydroxyapatite was produced. In other words, the structure of the sample at this temperature was similar to natural bone.
- The fluorapatite phase was also seen in the powder annealed at 400 °C, and 700 °C.
- Ca and P were detected as the major components in all processed powders
- The Ca/P ratio of all powders was more than the ratio of stoichiometric hydroxyapatite (1.67).

References

1. Yılmaz P, öztürk Er E, Bakırdere S, ülgen K, özbek B. Application of supercritical gel drying method on fabrication of mechanically improved and biologically safe three-component scaffold composed of graphene oxide/chitosan/hydroxyapatite and characterization studies. Journal of Materials Research and Technology. 2019;8(6):5201-16.
2. Reshadi F, Faraji G, Baniassadi M, Tajeddini M. Surface modification of severe plastically deformed ultrafine grained pure titanium by plasma electrolytic oxidation. Surface and Coatings Technology. 2017;316:113-21.
3. Zhang J, Jia J, Kim JP, Shen H, Yang F, Zhang Q, et al. Ionic Colloidal Molding as a Biomimetic Scaffolding Strategy for Uniform Bone Tissue Regeneration. Advanced Materials. 2017;29(17):1605546.
4. Reshadi F, Faraji G, Moghtaderi H, Faghihi S. Surface and Bulk Modification of Titanium Grade 2 Substrates for Enhanced Biological Activity. JOM. 2020;72(2):721-9.
5. Teh SJ, Lai CW. Carbon nanotubes for dental implants. Applications of Nanocomposite Materials in Dentistry: Elsevier; 2018. p. 93-105.
6. Goodacre CJ, Bernal G, Rungcharassaeng K, Kan JYK. Clinical complications with implants and implant prostheses. The Journal of Prosthetic Dentistry. 2003;90(2):121-32.
7. Herberts P, Malchau H. Long-term registration has improved the quality of hip replacement: A review of the Swedish THR Register comparing 160,000 cases. Acta Orthopaedica Scandinavica. 2000;71(2):111-21.
8. Hemmerl J, n ag A, Ert rk S. Ultrastructural features of the bone response to a plasma-sprayed hydroxyapatite coating in sheep. Journal of Biomedical Materials Research. 1997;36(3):418-25.
9. Sun L, Berndt CC, Gross KA, Kucuk A. Material fundamentals and clinical performance of plasma-sprayed hydroxyapatite coatings: A review. Journal of Biomedical Materials Research. 2001;58(5):570-92.
10. Alagarsamy K, Vishwakarma V, Kaliaraj GS, Viswanathan K, Chavali M. Implant application of bioactive nano-hydroxyapatite powders—a comparative study. Materials Research Express. 2018;5(1):015405.
11. Mohd Pu'ad NAS, Koshy P, Abdullah HZ, Idris MI, Lee TC. Syntheses of hydroxyapatite from natural sources. Heliyon. 2019;5(5):e01588-e.
12. Pramanik S, Hanif ASM, Pingguan-Murphy B, Abu Osman NA. Morphological Change of Heat Treated Bovine Bone: A Comparative Study. Materials (Basel). 2013;6(1):65-75.
13. Ooi CY, Hamdi M, Ramesh S. Properties of hydroxyapatite produced by annealing of bovine bone. Ceramics International. 2007;33(7):1171-7.
14. Gergely G, Wéber F, Lukács I, Tóth AL, Horváth ZE, Mihály J, et al. Preparation and characterization of hydroxyapatite from eggshell. Ceramics International. 2010;36(2):803-6.
15. Joschek S, Nies B, Krotz R, Göpferich A. Chemical and physicochemical characterization of porous hydroxyapatite ceramics made of natural bone. Biomaterials. 2000;21(16):1645-58.
16. Zhou J, Zhang X, Chen J, Zeng S, De Groot K. High temperature characteristics of synthetic hydroxyapatite. Journal of Materials Science: Materials in Medicine. 1993;4(1):83-5.
17. Lamsihar Manalu J, Soegijono B, Indrani DJ. Characterization of Hydroxyapatite Derived from Bovine Bone. Asian J Appl Sci 2015;03:2321-0893.
18. Asaduzzaman SM. Extraction of Hydroxyapatite from Bovine and Human Cortical Bone by Thermal Decomposition and Effect of Gamma Radiation: A Comparative Study. International Journal of Complementary & Alternative Medicine. 2017;8(3).
19. Prevéy PS. X-Ray Diffraction Characterization of Crystallinity and Phase Composition in Plasma-Sprayed Hydroxyapatite Coatings. Journal of Thermal Spray Technology. 2000;9(3):369-76.
20. Sobczak-Kupiec A, Malina D, Kijkowska R, Wzorek Z. Comparative study of hydroxyapatite prepared by the authors with selected commercially available ceramics. Digest Journal of Nanomaterials and Biostructures. 2012 Jan 1;7(1):385-91.
21. Khoo W, Nor FM, Ardhyananta H, Kurniawan D. Preparation of Natural Hydroxyapatite from Bovine Femur Bones Using Calcination at Various Temperatures. Procedia Manufacturing. 2015;2:196-201.
22. Landi E, Tampieri A, Celotti G, Sprio S. Densification behaviour and mechanisms of synthetic hydroxyapatites. Journal of the European Ceramic Society. 2000;20(14-15):2377-87.
23. Pang YX, Bao X. Influence of temperature, ripening time and calcination on the morphology and crystallinity of hydroxyapatite nanoparticles. Journal of the European Ceramic Society. 2003;23(10):1697-704.
24. Murugan R, Rao KP, Kumar TSS. Heat-deproteinated xenogeneic bone from slaughterhouse waste: Physico-chemical properties. Bulletin of Materials Science. 2003;26(5):523-8.
25. Bano N, Adzila S, Jikan SS, Basri H, Kanasan N. Extraction of Biological Apatite from Cow Bone at Different Calcination Temperatures: A Comparative Study. Key Engineering Materials. 2019;796:46-52.
26. Doostmohammadi A, Monshi A, Fathi MH, Braissant O. A comparative physico-chemical study of bioactive glass and bone-derived hydroxyapatite. Ceramics International. 2011;37(5):1601-7.