Efficient Drug Delivery

Development of Hydroxyapatite Microspheres for Biomedical Applications

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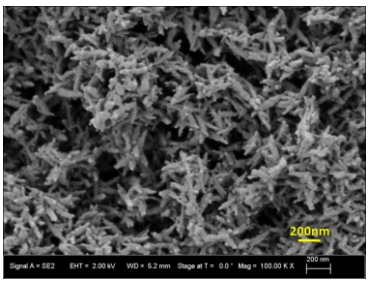

SEM micrograph of as-synthesized hydroxyapatite nano-particles

ABSTRACT

Porous biocompatible microspheres of hydroxyapatite (HAp), are potential candidates for various bio-medical applications including drug delivery and bone tissue engineering. We have synthesized porous HAp microspheres with diameter ranging 3-5μm consist of needle/rod shape HAp nanoparticles with an average length of 200nm using spray drying technique. We have shown that solely by controlling the spray drying temperature the outer shape of the microspheres can be tuned from spherical to doughnut. Their phase structure and outer morphology has been studied using X-ray diffraction and scanning electron microscopy. The absorption of bovine serum albumin protein by the synthesized granules has been tested as a potential application.

KEYWORDS: *Hydroxyapatite, Biomaterials, Microspheres, Spray drying, XRD, SEM, Nanoparticles*

Introduction

Hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$), major mineral component of human bones and teeth plays an important role in bone tissue engineering such as implant coatings and bone substitutes and variety of bio-medical industrial applications including matrices for drug release control etc[1,2]. The close chemical similarity between HAp and mineralized bone of human tissue has led to extensive research efforts in synthetic HAp and based ceramic. These materials have excellent biocompatibility, osteoconductivity, and osteotransductivity. To meet the needs of different applications, HAp based bone graft substitutes are being used in the form of powders, microspheres, sintered porous blocks, cements, putties and sponge/foams and as a coating over metallic implants. Among various morphologies, especially HAp granules has been proven to be ideal vehicles for drug delivery or scaffold for cell delivery[3] due to their relatively low cost, broad availability, good biological properties.

The pore size distribution and porosity of spherical granules is of major importance for drug delivery and for orthopedic and dental applications. The main focus is on the space between the particles. Most granular CaP bone graft substitutes have a diameter in the range of 0.5 to 5 mm because blood vessels and cells should be able to invade the space in between the particles to promote ceramic resorption and bone formation throughout the defect. Drug delivery is usually controlled by dissolution, diffusion and surface interaction, hence precipitated HAp crystals become promising due to its very large specific surface areas (typically above $50m^2/g$)[2].

There have been several methods reported in literature for preparation of HAp microspheres such as microwave assisted hydrothermal route[4], ion-assisted mineralization method[5], biopolymer assisted assembly from HAp nano rods[6] and glass conversion method[7]. However, these methods require numerous processing steps and are time consuming and complicated in nature. On the other hand, spray drying method[8] offers the inherent flexibility in operation and allows modification of process parameters which makes it possible to engineer the powder properties to predefined specifications, such as microsphere size, morphology and density. There have been few reports of HAp microspheres prepared by spray drying[8,9] in literature.

The main objective of this study is to synthesize HAp microspheres by modifying the spray drying process parameter i.e. temperature and evaluate the effect of drying temperature on the outer morphology of dried microspheres. The protein adsorption by HAp microspheres is investigated using model protein Bovine Serum Albumin (BSA), which was chosen as a test protein because of its better stability and availability at high purity. Details of this work can be obtained from reference [10].

Method

HAp nanoparticles were prepared by precipitation method according to the reaction described as follows:

$$10Ca(NO_3)_2 + 6(NH_4)_2HPO_4 + 8NH_4OH \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 20NH_4NO_3 + 6H_2O \quad (1)$$

Under continuous stirring aqueous solutions of 0.2M calcium nitrate ($Ca(NO_3)_2$) was gradually added into 0.2M ammonium hydrophosphate ($(NH_4)_2HPO_4$) solution. Their stoichiometric ratio Ca/P was kept at ~ 1.67. Temperature of the water bath was adjusted at 55°C and pH of solution was maintained at 10 till complete precipitation. The experimental steps were shown in the flow sheet given in Fig.1. The HAp nanoparticles were subsequently characterized for phase purity, size and shape.

HAp microspheres were obtained by drying the HAp colloidal dispersion solution using LU 228 (M/s. LABULTIMA, India) spray dryer. The stability of feed mixture was determined

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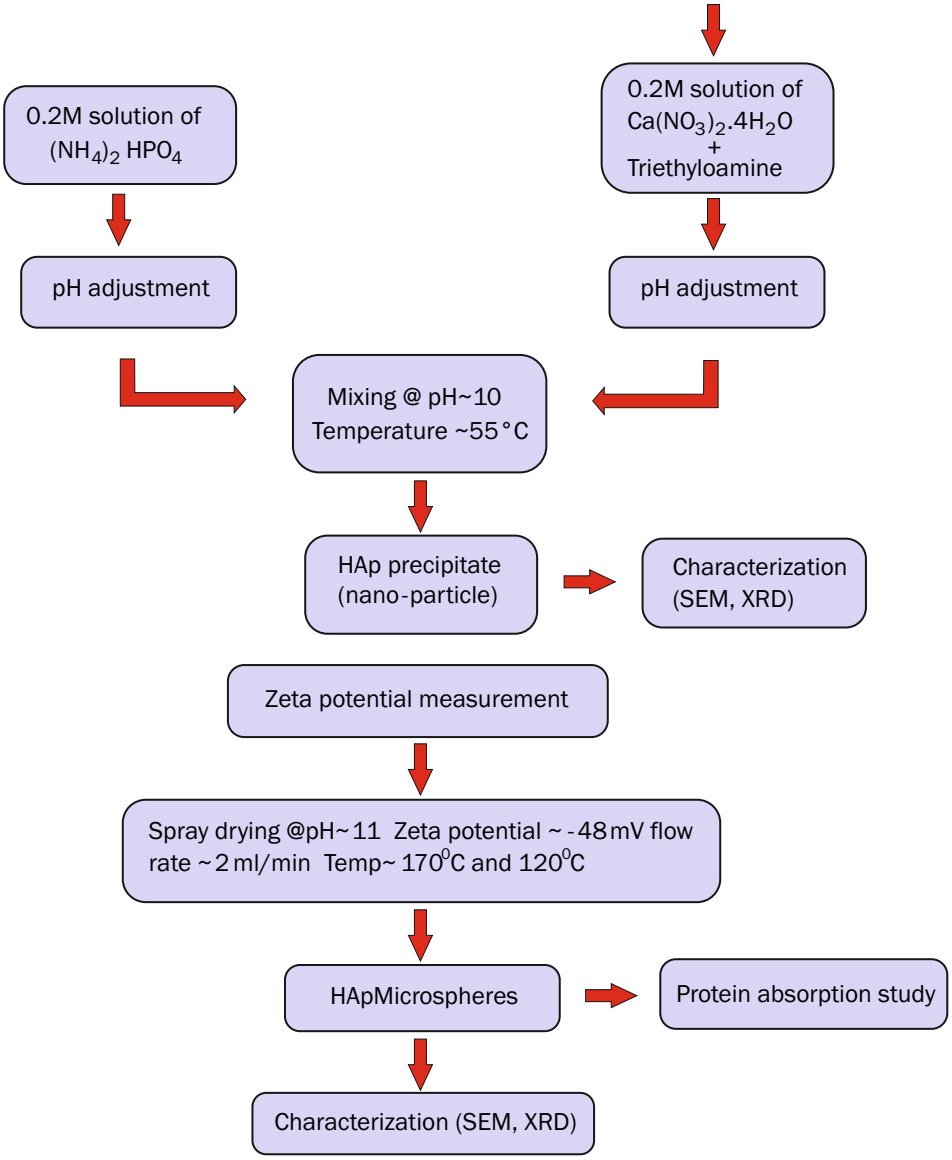

Fig.1: Flow sheet of experiments.

prior to spray drying by measuring zeta potential (ζ) and maintained at ζ~49mV at pH~11. Spray drying was carried out with compressed air at selected air pressure of 2 kg/cm^2 during atomization and flow rate was kept 2 ml/min. The aspiration rate was kept fixed at 50 m^3/h. The final values of these parameters were obtained after series of experimental trials. In the initial trial of spray drying it has been observed that large volume of power was not converted into microsphere and it remained in the form of agglomerated powder chunks. In the further trials by changing initial slurry concentration and flow rate of feed, microspheres could be formed but there was huge agglomeration of microspheres. After various trials spray drying parameters were optimized for disperse microspheres in the size range of 3-5μm. Later on, in spray drying experiment inlet temperature was varied at 120°C and 170°C, respectively to understand the effect of drying temperature on the morphology of dried granules.

The phase analysis of HAp nanoparticles was carried out using x-ray diffractometer (D8 discover, Bruker) using Cu K_α radiation (λ= 0.15406 nm). Rietveld analysis of XRD pattern was done using "Fullprof" software for crystal structural analysis. The morphology of HAp nanoparticles and microstructure of external surface of spray dried HAp microspheres was examined using Carl Zeiss Auriga FE-SEM.

Crystallized and lyophilized BSA (Grade A9418, Sigma Chem-ical Co.,) protein with molecular weight of 69 kDa and iso-electric point ~4.74 was used for the protein adsorption study. Initially, 300 mg Hap granule (S1 sample) was added in 10 ml BSA with concentration of 25 mg/ml and incubated at 37°C for different time schedule from 3h to 96h. The supernatant solutions were collected for each conjugate after centrifugation at 800 rpm and kept at temperature 4°C. UV–vis spectroscopy (Perkin Elmer Lambada 35) at 562 nm was used to characterize the absorbance peaks and BSA concentration was quantified through the use of a pre-determined standard concentration–intensity calibration curve. The amount of adsorbed protein was calculated from solution depletion.

Results and Discussion

Phase analysis

XRD patterns of as-synthesized Hap nano-particles (Fig.2a) shows broad and overlapped diffraction peaks which indicate low crystallinity and small crystallite size of nano-particles. However, broadening and overlapping of Bragg peaks is significantly reduced after calcination at 800°C.

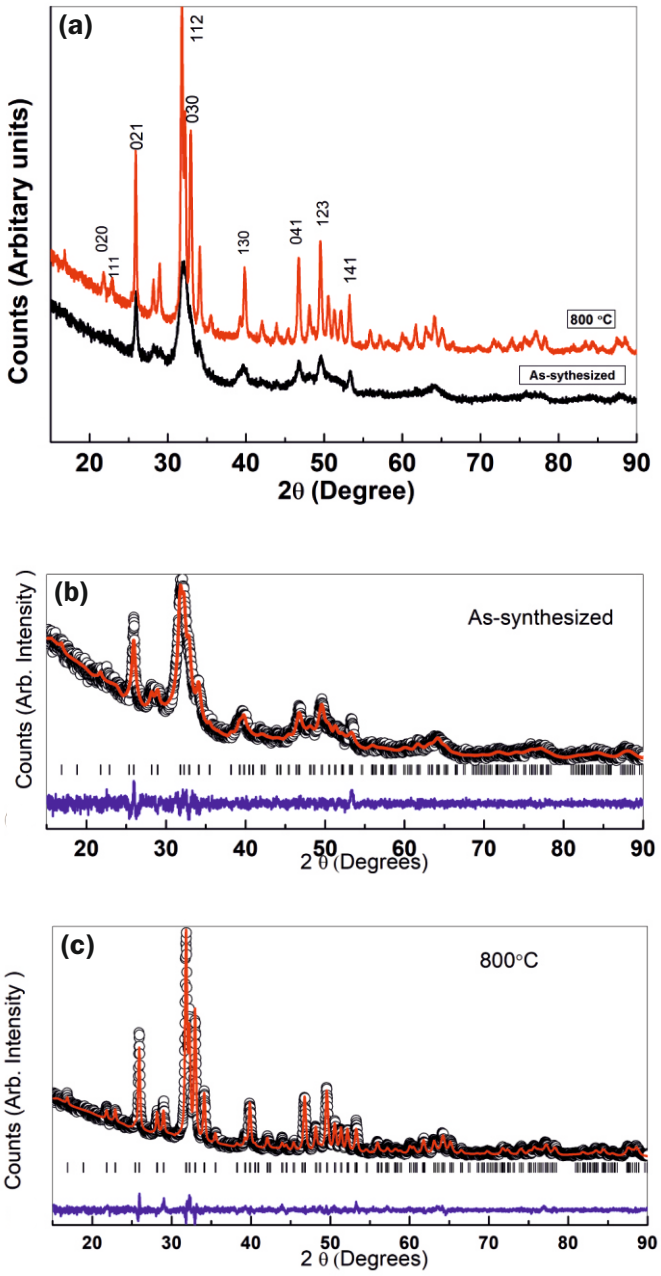

Fig.2: (a) XRD pattern of as-precipitated and after calcination at 800°C. Rietveld refinement plots of x-ray diffraction data of HAp nano-particles (b) as-synthesized dried (c) after calcination at 800°C. Experimental data, simulated data and difference plots are represented as open circles, continuous line (red) and continuous line (blue) respectively. Vertical bar symbol represents Bragg peak positions.

Calcined XRD pattern is determined to be pure HAp phase by comparing it with standard JCPDS card (09-432). The average crystallographic data was determined by Rietveld refinement of XRD data. Starting unit cell and structural parameters came from the refinement performed with neutron data by Kay *et al*.,[11] for HAp phase. First, scale factor, background, peak profile (pseudo-voigt function) and lattice parameters were simultaneously refined, later atomic positions and isotropic displacement parameters were refined one by one for each atom. Fig.2 shows the Rietveld plot of HAp powder obtained by refinement with space group P63/m. The refined lattice parameters and volume for as-synthesized powder are: a=b=9.4279(31), c=6.8683(25), V=528.70(31), and for calcined HAp powder a=b=9.4128(2), c=6.8755(2) and V=527.56(2) respectively. Error associated with refined parameters is much higher for as-synthesized powder due to its low counting rate and presence of nano scale crystallite size.

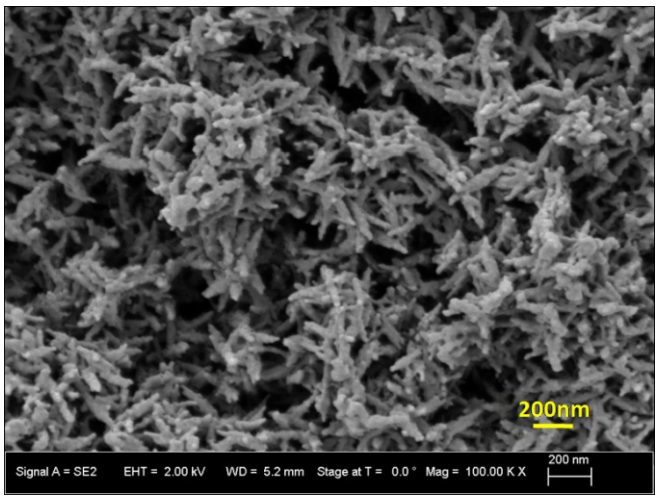

Fig.3: SEM micrograph of as-synthesized hydroxyapatite nano-particles.

Microstructural characterization

SEM micrograph of as-synthesized HAp nano-particles (Fig.3) suggests that nano-particles have reasonably small poly-disparity as most of the nano-particles have similar size and shapes. They exhibit ellipsoidal morphology with ~200nm length and aspect ratio of ~0.3. The nanometer size of HAp as-synthesized particles easily explain the presence of broad and overlapping peaks in XRD pattern shown in Fig.2. It is observed from the figure that these nano-particles are soft agglomerates which can be easily dispersable and friable. Fig.4 shows the SEM micrographs of HAp granules prepared by spray drying at 120°C (a-b) and 170°C (c) respectively with diameter 3-5µm. HAp granules have spherical morphology when spray drying is performed at temperature 120°C and doughnut-like morphology for spray drying temperature of 170°C (Fig.4c).

At lower drying temperature, HAp nano-particles with in a droplet have sufficient time to redistribute themselves by diffusion throughout evaporating droplet and yields in a final arrangement inside an. assembled granule corresponding to a packing due to random jamming of the constitute particles. Fig.5 shows schematically the possible mechanisms for the dense homogeneous sphere. On the other hand, higher drying temperature accelerates the droplet evaporation and water (solvent) on the surface runs away quickly. HAp nano-particles have insufficient time to diffuse from surface to the center of the droplet and instead accumulate near the drying front of the droplet and dimpled or doughnut shaped morphology forms (schematic Fig.5).

BSA protein adsorption on HAp microsphere

Fig.6 exhibits the BSA protein adsorption on HAp granules at different time interval. It is clearly visible that with increasing time the concentration of adsorped BSA is increasing. Their mutual interaction and adsorption behavior is dependent upon hydrophobicity of individual HAp and BSA molecule[12].

Conclusions

HAp microspheres of diameter ranging 3-5 µm were synthesized using precipitation method followed by spray

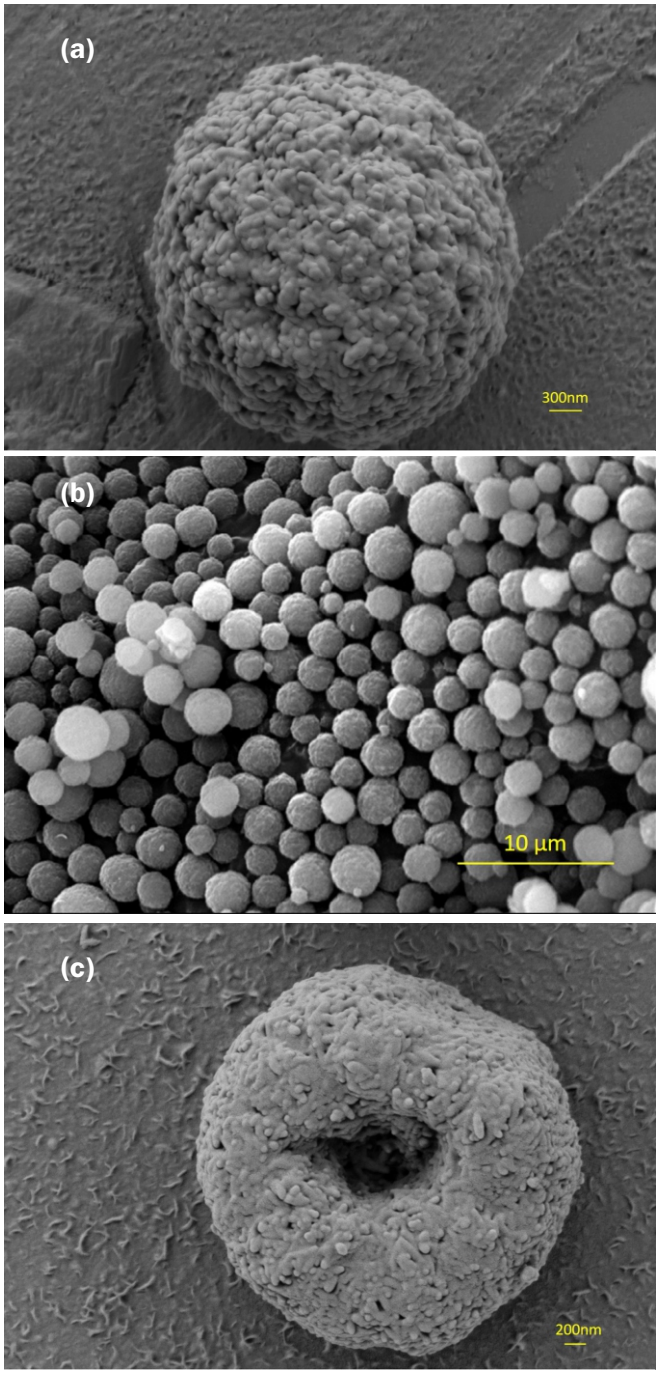

Fig.4: SEM micrographs of spray dried HApmicrospheres dried at drying temperature (a)-(b) 120°C and (c) 170°C.

drying of aqueous solution of precipitate. These microspheres were consisted of needle/rod shape HAp nano particle of 200nm length. XRD results reveal that as-precipitated HAp powder was amorphous and calcination at 800°C transformed it to phase pure HAp with crystalline nature.

It has been shown that morphology of spray dried HAp microspheres can be simply tuned from spherical to doughnut-like by solely changing the drying temperature. Adsorption of BSA protein on spray dried HAp granules is also demonstrated as a potent application.

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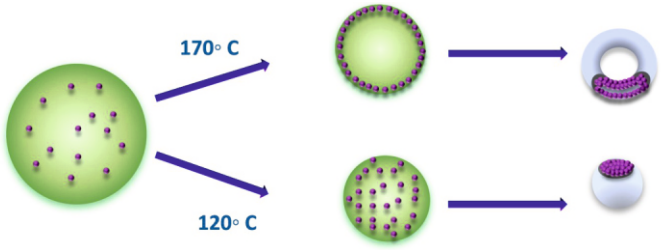

Fig.5: The schematic diagram of the granule morphology in two extreme cases (at 120°C and 170°C).

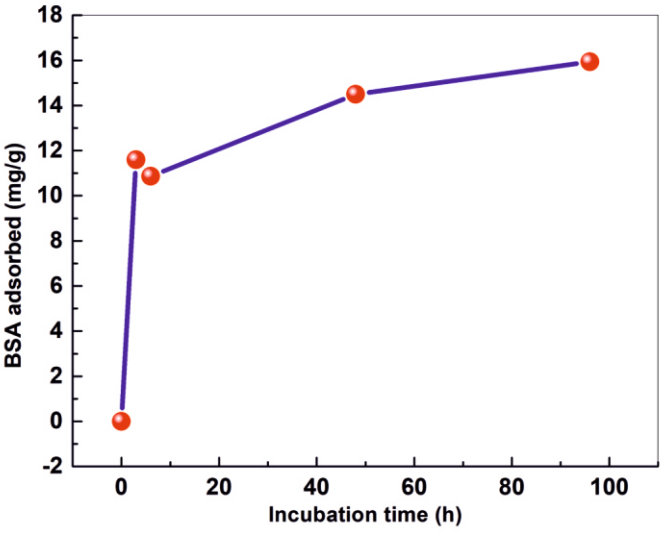

Fig.6: BSA adsorption by HAp granule at different incubation time.

References

[1] L.L. Hench, Bioceramics: from concept to clinic, Journal of the American Ceramic Society, 1991, 74, 1487-1510.

[2] M. Bohner, S. Tadier, N. van Garderen, A. de Gasparo, N. Do¨belin, G. Baroud, Synthesis of spherical calcium phosphate particles for dental and orthopedic applications, Biomatter, 2013, 3(2), e25103.

[3] H.-H. Lee, S.-J. Hong, C.-H. Kim, E.-C. Kim, J.-H. Jang, H.-I. Shin, H.-W. Kim, Preparation of hydroxyapatite spheres with an internal cavity as a scaffold for hard tissue regeneration, Journal of Materials Science: Materials in Medicine, 2008, 19, 3029-3034.

[4] K.W. Wang, Y.J. Zhu, X.Y. Chen, W.Y. Zhai, Q. Wang, F. Chen, J. Chang, Y.R. Duan, Flower-Like Hierarchically Nanostructured Hydroxyapatite Hollow Spheres: Facile Preparation and Application in Anticancer Drug Cellular Delivery, Chemistry–An Asian Journal, 2010, 5, 2477-2482.

[5] W. Xia, K. Grandfield, A. Schwenke, H. Engqvist, Synthesis and release of trace elements from hollow and porous hydroxyapatite spheres, Nanotechnology, 2011, 22, 305610.

[6] S.D. Jiang, Q.-Z. Yao, G.-T. Zhou, S.-Q. Fu, Fabrication of hydroxyapatite hierarchical hollow microspheres and potential application in water treatment, The Journal of Physical Chemistry C, 2012, 116, 4484-4492.

[7] W. Xiao, H. Fu, M.N. Rahaman, Y. Liu, B.S. Bal, Hollow hydroxyapatite microspheres: A novel bioactive and osteoconductive

carrier for controlled release of bone morphogenetic protein-2 in bone regeneration, Acta biomaterialia, 2013, 9, 8374-8383.

[8] R. Sun, Y. Lu, K. Chen, Preparation and characterization of hollow hydroxyapatite microspheres by spray drying method, Materials Science and Engineering: C, 2009, 29, 1088-1092.

[9] P. Luo, T. Nieh, Synthesis of ultrafine hydroxyapatite particles by a spray dry method, Materials Science and Engineering:C-Biomimetic Materials Sensors and Systems, 1995, 3, 75-78.

[10] R. Garg, D. Sen, A. Sahu, K. Madangopal,Morphological Tuning of Nanostructured Hydroxyapatite (HAp) Porous Microgranules by Evaporation-Induced Assembly, Journal of Nanoscience and Nanotechnology, 2020, 20, 1631-1642.

[11] M.I. Kay, R. Young, A. Posner, Crystal structure of hydroxyapatite, Nature, 1964, 204, 1050.

[12] K. Kandori, M. Mukai, A. Fujiwara, A. Yasukawa, T. Ishikawa, Adsorption of bovine serum albumin and lysozyme on hydrophobic calcium hydroxyapatites, Journal of Colloid and Interface Science, 1999, 212, 600-603.