Effect of calcium phosphate synthesis conditions on its physico-chemical properties and evaluation of its antibacterial activity

Guerfi Souad1,2 , Souad Baghdadi3
1 Research Center in Industrial Technologies CRTI P.O.Box 64, Cheraga 16014 Algiers, Algeria
2 Advanced Materials Laboratory, Badji Mokhtar University, P . B. 12, Annaba 23000, Algeria
3 Semi-Conductors Laboratory, Badji Mokhtar University, P . B. 12, Annaba 23000, Algeria
E-mail: guerfisouad@yahoo.fr

Abstract
The antibacterial activity of non-stoichiometric calcium phosphate particles prepared by precipitation under controlled experimental conditions at pH ~ 9 and sintered at high temperature was studied against Staphylococcus aureus bacteria. The effects of operating parameters developed according to an experimental design of Plackett-Burman type on the physicochemical characteristics and the capacity to inhibit bacterial growth were identified using a thermal analysis (TGA-DTA-DSC), x-ray Diffraction (XR), Raman Spectroscopy, Scanning Electron Microscope (SEM) and the Kirby Bauer Method. The XRD spectrum shows that the synthetic crystalline nanoparticles powders consist of multiphasic calcium phosphate $\beta$-TCP/ $\beta$-CPP/OCP/HA and that the average particle size is between 56 and 123 nm calculated by the Debay–Shearer equation. The Raman spectrum of sintered powder shows the main absorption bands that are assigned to the asymmetric / symmetric P-O stretching vibrations in $PO_4^{3-}$ and the symmetric O-H stretching mode of the hydroxyl group in addition of Ca-PO$_4$ and Ca-OH modes. The samples were found to possess different morphologies consisting of nano-rods of different lengths, semi / spherical structures and fine granules, in addition to irregular clusters. The antibacterial tests results showed that the high concentration calcium phosphate powder exhibited better antibacterial activity against Staphylococcus aureus bacteria with inhibition zones ranging from 0.2–0.7 cm.

Introduction
Calcium phosphate (Ca-P) ceramics are the most widely used substances in the field of biomaterials, including hydroxyapatite (HA), tricalcium phosphate ($\alpha$, $\beta$-TCP), octacalcium phosphate (OCP) and dehydrated dicalcium phosphate (DCPD) [1–6]. Calcium phosphate is the major component of the human body bones and teeth [1, 7]. Due to its ability to form a direct biological link with living tissue by forming a bone-like apatite on its surface [1], it is used as a powder or coating [6, 8–11] in joint prostheses (hip, elbow), bone replacement for tumour or trauma, dental and bone filling, and maxillofacial reconstruction [2, 12, 13]. Calcium phosphate is currently used for medical purposes either to fill gaps or as a carrier accepted by the body to encompass certain drugs. Ca-P can be of natural [14–19] or synthetic origin [4, 6, 7, 11, 20, 21]. It can be prepared using several methods such as: aqueous precipitation [7, 17, 22], sol-gel method [10, 22–24], solid reaction [25], and hydrothermal [26, 27]. The synthetic Ca-P is an alternative to the natural material because of its chemical, biological and mechanical properties [4, 15, 28]. Calcium phosphate is a preferred class of biomaterials because of its good properties such as biocompatibility, osteoconductive, bioactivity and non-toxic properties [26]. However, bacterial infection is the main factor leading to implant failure, resulting in serious physiological damage with the possibility of additional surgery [29, 30]. Excessive colonization of bacteria causing postoperative inflammation has serious consequences for the biocompatibility of calcium phosphate [29]. Several studies have been reported on the antibacterial activity of the biomaterials, including hydroxyapatite against certain types of bacteria, S. pyogenes, S. aureus, S. epidermidis, B. subtilis, K. pneumonia, E. coli,
P. mirabilis, P. aeruginosa and B. cereus \[23, 29, 31–35\]. Staphylococcus aureus spherical is one of the most common and most dangerous bacterial species. He is present at the wound and at the skin. These bacteria often cause inflammation after the implantation of medical devices such as pacemakers, heart valves, artificial joints and bone implants \[26\]. It is also believed that the antibacterial properties of Ca-P are related to its morphological properties, crystal structure and its concentration, its pH and the sintering temperature \([18, 23, 26, 32, 36]\). More recently, the improvement of the physico-chemical properties of biomedical materials, both natural and synthetic, has preoccupied researchers and industrialists \([4, 15, 37–39]\). The purpose of this study is the synthesis of calcium phosphate particles by the aqueous precipitation method by following a set of experimental condition that permit to control the resulting calcium phosphate particle characteristics. Some of these conditions are reaction time, reaction temperature, stirring rate and phosphate solution addition rate. These factors were found to have determinant effects on the morphological characteristics, crystallite size, phase composition and the nature of the functional groups of the prepared powders. In addition, the evaluation of the antibacterial activity of non-stoichiometric calcium phosphate nanoparticles against staphylococcus aureus is also studied.

**Materials and methods**

**Syntheses of non-stoichiometric calcium phosphate**

Calcium phosphate powders were synthesized via the aqueous precipitation process, using the following reactants: dihydrate Calcium chloride \((\text{CaCl}_2·2\text{H}_2\text{O}), \text{Sigma-Aldrich,100%}, \text{CAS Nu:10035-04-8,USA}\) and dihydrate Sodium phosphate dibasic \((\text{H}_2\text{Na}_2\text{PO}_4·2\text{H}_2\text{O}, \text{Sigma-Aldrich,>99%, USA})\). This method consists of adding drop by drop a solution of calcium salt in a phosphoric solution in a controlled manner at pH = 9 and \(\text{Ca}/\text{P} = 2\) after dissolving the reagents in distilled water. The obtained precipitate is filtered using a Buhner funnel and a vacuum pump, washed with distilled water and finally dried. The powders were prepared according to Plackett-Burman experimental design using the following parameters: agitation speeds 100 and 300 rpm, reaction temperatures 80 and 100 °C, reaction durations of 30 and 60 min, and the phosphate solution addition flow rates of 5 and 10 ml s\(^{-1}\) (table 1). Finally, the prepared powder samples were treated at high temperature. The main stages of calcium phosphate powder synthesis are expressed in the following schematic flow diagram (figure 1):

**Characterizations of calcium phosphate powder**

The thermal analysis DTA-TGA-DSC were performed simultaneously using the TA instrument SDT- Q600, (New Castle, USA) apparatus with a speed of 10 °C/min up to 1300 °C. The obtained powders were characterized by x-ray Diffraction (XRD) using a Rigaku (Tokyo, Japan) type apparatus with a Cu radiation source \(\lambda_{\text{Cu}} = 0.15406\ \text{nm}\). The powders were analysed in the 2\(\theta\) range 10°– 90° at a scan speed of 0.03°/s and a step size of 0.01°. Identification of the calcium phosphate powder component phases was performed using the Panalytical X’Pert HighScore Plus software. The particle size was calculated using the Debye-Scherrer equation:

\[
D = \frac{K\lambda}{\beta \cos \theta}
\]

Where \(D\) is the average crystallite size (nm), \(K\) is the Scherrer constant estimated at 0.9, \(\lambda\) is the used x-ray wavelength, \(\beta\) is the full width at half maximum (FWHM) of the consideder peak and \(\theta\) is the value of Bragg’s angle (deg). The Raman spectra of the sintered powders were recorded in frequency domains 1200-0 cm\(^{-1}\), using a Horiba Raman spectrometer (Model INF-300, Japan) at 473 nm laser wavelength, at room temperature. The micro structural analysis is performed using a Quanta scanning electron microscope (Quanta FEG-250,FEI Inc, USA) which possesses a voltage acceleration in the range 0.2V-30 KV. The Kirby-Bauer disc diffusion method was used to determine the antibacterial activity of Ca-P powder against the bacterium Gram-positive

| Ca/P | Time (min) | Run-order | Flow rates (ml/s) | Agitation speeds (rpm) | Temperature total mass loss (% | Temperature total mass loss (%) |
|------|-----------|-----------|-------------------|------------------------|-------------------------------|-------------------------------|
| 2    | 30        | Ca-P1     | 5                 | 100                    | 100 | 4.90 |
|      |           | Ca-P2     | 10                | 100                    | 80  | 4.15 |
|      |           | Ca-P3     | 5                 | 300                    | 100 | 3.70 |
| 60   |           | Ca-P4     | 10                | 300                    | 80  | 5.50 |
|      |           | Ca-P5     | 5                 | 100                    | 80  | 4.90 |
|      |           | Ca-P6     | 10                | 300                    | 100 | 5.20 |

Table 1. Plackett-Burman plan and mass losses (%) of the powders at 10 °C/min up to 1300 °C.
Staphylococcus aureus ATCC 6538. The pure bacterial strains were first cultured in Mueller-Hinton nutrient broth medium (MHB) at 37 °C for 18 h, and diluted 100-fold in sterile water. Then, the bacterium of 10⁵ CFU (colony forming unit) was inoculated onto solidified Mueller-Hinton agar on a sterilized Petri dish. In this study all the samples with the concentrations 0.3 M, 0.7 M, 1.1 M and 1.5 M have been prepared as a suspension in sterile water solution with 50 vol.% dimethyl sulfoxide (100 μl). These suspensions were inoculated onto Muller-Hinton agar plates using sterile filter paper and incubated at 37 °C for 24 h. The antibacterial activity of the Ca-P samples was evaluated by measuring the diameter of the inhibition region for each sample.

**Results and discussion**

Figure 2 shows the thermogravimetric analysis curves (TGA) of the apatite powders having a molar ratio Ca/P = 2 and different reaction time t. (a) t = 30 min and (b) t = 60 min.

Staphylococcus aureus ATCC 6538. The pure bacterial strains were first cultured in Mueller-Hinton nutrient broth medium (MHB) at 37 °C for 18 h, and diluted 100-fold in sterile water. Then, the bacterium of 10⁵ CFU (colony forming unit) was inoculated onto solidified Mueller-Hinton agar on a sterilized Petri dish. In this study all the samples with the concentrations 0.3 M, 0.7 M, 1.1 M and 1.5 M have been prepared as a suspension in sterile water solution with 50 vol.% dimethyl sulfoxide (100 μl). These suspensions were inoculated onto Muller-Hinton agar plates using sterile filter paper and incubated at 37 °C for 24 h. The antibacterial activity of the Ca-P samples was evaluated by measuring the diameter of the inhibition region for each sample.

**Results and discussion**

Figure 2 shows the thermogravimetric analysis curves (TGA) of powder synthesized according to the Plackett-Burman experimental plan at Ph~9 and heat-treated at high temperature under an inert atmosphere. They show that the general appearance of these curves is the same for all the powders obtained with different synthesis parameters. The total mass losses associated with each sample are reported in table 1 and are found to vary from 3.70% to 5.50%. The curves of the calorimetric analysis (DSC) of the obtained powders (figure 3(a)) show an
endothermic peak at 69.69 °C, which can be explained by the evaporation of the water molecules and two exothermic peaks at 632.96 °C and 949.99 °C. The first peak corresponds to the crystallization of the powder and the second peak corresponds to the formation of calcium phosphate. The results of the differential thermal analysis (DTA) of the synthesized powders (figure 3(b)) show an endothermic peak and exothermic peaks due to the variation of the mass of calcium phosphate powder (Ca-P).

The XRD spectra of the synthesized powders that were sintered at 1300 °C for 3 h are presented in figure 4. These spectra indicate that the powder is well crystallized and multiphasic. The XRD spectra indicate that the powders are composed of octacalcium phosphate Ca₈H₂(PO₄)₆(H₂O)₅ (OCP), hydroxyapatite Ca₁₀(PO₄)₆(H₂O)₆(OH) of hexagonal structure, of tricalcium phosphate β-Ca₃(PO₄)₂ (β-TCP) and also of calcium pyrophosphate β-Ca₂P₂O₇ (β-CPP). According to the results of the literature [40], octacalcium phosphate decomposes thermally forming hydroxyapatite and dicalcium phosphate (equation (2)) at a temperature greater than 220 °C, while at 700–900 °C, the decomposition continues according to the following reaction (equation (3)):

\[
5\text{Ca}_8\text{H}_2(\text{PO}_4)_{6.5}\text{H}_2\text{O} = 4\text{Ca}_{10}(\text{PO}_4)_{6}(\text{OH})_2 + 6\text{H}_3\text{PO}_4 + 17\text{H}_2\text{O}
\]  
\[
\text{Ca}_8\text{H}_2(\text{PO}_4)_{6.5}\text{H}_2\text{O} = 2\text{Ca}_3(\text{PO}_4)_2 + \text{Ca}_3\text{P}_2\text{O}_7 + 6\text{H}_2\text{O}
\]

The histograms of the variation of the particle size of the synthesized powders are presented in figure 5 and show that the particles have a fine particle sizes ranging from 23.54 to 52.88 nanometers, while the largest particle sizes are between 233.52 and 352.93 nm. On the other hand, it is found that the average particle size of Ca-P3 sample was lower than that of other powders and was 56.39 nm. It is clear that the parameters used for synthesizing the powders have resulted in a change in the particle size from nanometers to a few micrometers. This suggests that the particle size distribution is multimode.
Figure 6 shows the result of Raman spectral analyses of non-stoichiometric calcium phosphate sintered at 1300 °C (All samples have similar properties). These analysis reveal the presence of the characteristic absorption bands of the phosphate groups at 420 cm⁻¹ (ν₂PO₄), 595 cm⁻¹ (ν₄PO₄), 974 cm⁻¹ (ν₁PO₄), 1017–1080 cm⁻¹ (ν₃PO₄), and hydroxide at 610 cm⁻¹ (ν₁OH). In addition, we note the presence of two bands at 114 cm⁻¹ and 234 cm⁻¹, corresponding to Ca-PO₄ mode and the 270–295 cm⁻¹ band were attributed to the stretching of ν₁Ca(II)-OH. An additional band was observed at 798 cm⁻¹ and can be attributed to the liberation of water molecules. Table 2 illustrates the positions and assignment of Raman vibration bands of granulated synthetic powder sintered at 1300 °C. The presence of these bands is in agreement with the literature and confirms the formation of calcium phosphate with hexagonal structure, β-TCP, β-CPP and OCP [41–44]. This is in agreement with the results of XRD analysis.

The SEM images of the synthetic powders that were obtained according to the processing parameters and sintered at 1300 °C are shown in figure 7. It can be observed that most of the particles of sample Ca-P1 are irregular groups, with the appearance of nanometric porous network and the formation of semi/spherical shaped particles with the size of around 0.5 μm for sample Ca-P6. In addition, we note the presence of tubes...
having at least 3.5 μm in length and 1.5 μm in diameter for sample Ca-P2, resulting from a conglomeration of semi-globular granules. We also note that the size of these granules increased significantly to 3 μm and 4 μm for Ca-P3 and Ca-P4 samples, respectively. For sample Ca-P5, the SEM images show that it is mainly constituted of spherical particles of around 1.5 μm in diameter.

The powders antibacterial test results obtained against gram-positive bacteria, Staphylococcus aureus are presented in figure 8 and show that for the low concentration phosphate calcium samples of 0.3 M, 0.7 M, 1.1 M, no inhibitory zone was observed. Whereas for the high concentration of 1.5 M, an inhibition zone was estimated at 0.3 cm, 0.4 cm, 0.5 cm, 0.7 cm and 0.2 cm for the Ca-P2, Ca-P3, Ca-P4, Ca-P5 and Ca-P6 respectively.

Figure 9 shows the histograms of the variation of the inhabitation zone of S. aureus for the 1.5 M concentration samples. It can be seen that the CaP5 sample shows an excellent inhibitory effect on the growth of S.aureus bacteria compared to other samples. Indeed the sample CaP5 is characterized by a spherical morphology, an average particle size of 60 nm and the most crystallized structure with the presence of octacalcium phosphate (OCP), tricalcium phosphate (β-TCP) and calcium pyrophosphate (β-CPP) with a triclinic crystalline structure, Rhombohedral and Tetragonal structure, respectively. It should be noted that the other samples have grains of irregular shapes or agglomerated as a rod or semi-spherical. The antibacterial resistance of the Ca-P5 sample can be explained by two main factors: the first concerns the concentration of calcium in the sample and the second is attributed to the physicochemical properties, the shape and the size of the sample particles, crystalline structure, phases formed, degree of crystallization and functional groups. Some researcher [23] have suggested that the antibacterial activity can be attributed as calcium phosphate powders that are composed of metallic elements such as Na or Cl, which are derived from the primary solutions that were used in the synthesis of the powder. Moreover, the variation of the antibacterial activity from one sample to another is due to the difference of the crystalline structure and the secondary phases [23]. While, Other researchers [31, 45] reported that the antibacterial activity was due to calcium ions and hydroxide which degrade the bacterial cell membrane and hence eliminate the Staphylococcus aureus.

| Raman bands (cm⁻¹) | Modes | Band assignment |
|-------------------|-------|----------------|
| 114, 234          | ν₃    | Ca-PO₄ stretching mode |
| 270–295           | ν₂    | Ca(II)-OH stretching mode |
| 420               | ν₃    | Symmetric O-P-O variable angle vibration. |
| 595               | ν₄    | Antisymmetric O-P-O variable angle vibration |
| 610               | ν₁    | Stretching mode of hydroxyl vibration |
| 798               |       | Bending mode of H-O-H in H₂O |
| 974               | ν₁    | Symmetric stretching mode of P-O in PO₄³⁻ |
| 1017–1080         | ν₃    | Asymmetric stretching mode of P-O in PO₄³⁻ |

Table 2. Positions and assignments of Raman vibration bands of granulated synthetic powder sintered at 1300 °C.
Optical microscopic observation of bacterial strains revealed the presence of bacteria in the form of small cocci having the appearance of clusters of 0.5 to 1.5 μm in diameter as shown in Figure 10. In addition, optical microscopic analysis of the 1.5 M sample showed that the concentration of inhibitory bacteria varies from sample to sample, which corresponds to the diameters of the inhibition zones of the previous results.

Figure 7. Microstructures of nonstoichiometric calcium phosphate powders synthesized and sintered at 1300 °C.
Conclusion

The antibacterial activity of the non-stoichiometric calcium phosphate bioceramic synthesized by the precipitation method against staphylococcus aureus bacteria was investigated using the disc diffusion technique. The samples with a high concentration of 1.5 M and sintered at 1300 °C showed that they were able to inhibit Staphylococcus aureus bacteria in contrast to other samples which have lower concentrations of 0.3 M, 0.7 M and 1.1 M. The Ca-P5 sample synthesized at 60 min, molar ratio Ca/P = 2, agitation 100 rpm, temperature 80 °C, the rate of addition of phosphate solution 5 ml s⁻¹ and sintered under air shows the strongest activity to inhibit bacteria than that of other sample having higher concentrations (1.5 M). The antibacterial resistance of
the Ca-P5 sample can be explained by two main factors: the first concerns the concentration of calcium in the sample and the second is attributed to the physicochemical properties, the shape and the size of the sample particles, crystalline structure, phases formed, degree of crystallization and functional groups. The synthesis factors and particularly the concentration of calcium phosphate powder can be considered to reduce inflammation resulting from bacterial colonization after implantation and can improve the antibacterial properties of a calcium phosphate biomaterial.

Acknowledgments

The authors are thankful to Pr D. Hamana and M A C I Chetibi, Mentouri University constantine, Mrs H Zeddouri, Head of the Science and Materials Engineering Departmental Laboratories of the National School of Mining and Metallurgy, Annaba, as well as the laboratory engineer Mr M Metiri and Mr H Brahmia, PhD student A Kout from chemistry department , Badji Mokhtar university in Annaba and PhD student K Guediri from chemistry department, Ferhat Abbas Sétif University 1 for their contribution to raman analyses, SEM observations and antibacterial activity experiments.

ORCID iDs

Guerfi Souad  https://orcid.org/0000-0002-8600-1658

References

[1] Biernat M, Jaegermann Z, Tymowicz-Grzyb P and Konopka G 2019 Influence of low-temperature reaction time on morphology and phase composition of short calcium phosphate whiskers *Processing and Application of Ceramics* 13 57–64
[2] Sheikh Z, Hamdan N, Abdallah M-N, Glogauer M and Grynpas M 2019 Natural and synthetic bone replacement graft materials for dental and maxillofacial applications *Advanced Dental Biomaterials* (Amsterdam: Elsevier) pp 347–76
[3] Kucko N W, Herber R-P, Leeuwenburgh S C and Jansen J A 2019 Calcium Phosphate Bioceramics and Cements *Principles of Regenerative Medicine* (Amsterdam: Elsevier) pp 591–611
[4] Ebrahimi M, Botelho M, Lu W and Monnaturapoj N 2019 Synthesis and characterization of biomimetic bioceramic nanoparticles with optimized physicochemical properties for bone tissue engineering *Journal of Biomedical Materials Research Part A* 107A 1654–66
[5] Boanini E, Gazzano M, Nervi C, Chierotti M R, Rubini K, Gobetto R and Bigi A 2019 Strontium and zinc substitution in β-tricalcium phosphate: an x-ray diffraction, solid state NMR and ATR-FTIR study *Journal of functional biomaterials* 10 20
[6] Chamard M, Marsan O, Charvillat C, Grossin D, Fort P, Rey C, Gitzhofer F and Bertrand G 2019 Effect of the deposition route on the microstructure of plasma-sprayed hydroxyapatite coatings *Surf. Coat. Technol.* 371 68–77
[7] Natale L C, Rodrigues M C, Alania Y, Chiari M D, Vilela H S, Vieira D N, Arana-Chavez V, Meier M M, Vichi F M and Braga R R 2019 Development of calcium phosphate/ethylene glycol dimethacrylate particles for dental applications *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 107 708–15
[8] Rodrigues L Jr, Tronco M, Escobar C, Rocha A and Santos I 2019 Painting method for hydroxyapatite coating on titanium substrate *Ceram. Int.* 45 14806–15
[9] Harun W, Asri R, Alias J, Zulkifli F, Kadirgama K, Ghani S and Shariffuddin J 2018 A comprehensive review of hydroxyapatite-based coatings adhesion on metallic biomaterials Ceram. Int. 44 1250–68
[10] Rahmani F, Es-Haghi A, Hosseini M-R M and Mollahosseini A 2019 Preparation and characterization of a novel nanocomposite coating based on sol-gel titanate hydroxyapatite for solid-phase microextraction Microchem. J. 145 942–50
[11] Stango S A X and Vijayalakshmi U 2019 Synthesis and characterization of hydroxyapatite / carboxylic acid functionalized MWCNTs composites and its triple layer coatings for biomedical applications Ceram. Int. 45 69–81
[12] Li J and Zreiqat H 2019 Tissue Response to Biomaterials Encyclopedia of Biomedical Engineering 270–77
[13] Aveix S, Dostalov R, Vogel M, Weber M, Buttlar P, Tonmai G P and Fischer H 2018 Microporous biomaterial scaffolds with defined interconnecting channel structure provide a mimetic 3D niche for bone marrow metastasized tumor cell growth Acta Biomater. 88 527–39
[14] Ferro A C and Guedes M 2019 Mechanocochal synthesis of hydroxyapatite using cuttlefish bone and chicken eggshell as calcium precursors Materials Science and Engineering: C 101 24–30
[15] Matinifar M, Megar A S and Mohammadi Z 2019 Evaluation of physicochemical, mechanical and biological properties of chitosan/ carboxymethyl cellulose reinforced with multiphasic calcium phosphate whisker-like fibers for bone tissue engineering Materials Science and Engineering: C 100 341–53
[16] Resmim C M, Dalpasquale M, Vielmo N I, Mariani F Q, Villalba J C, Anaisi F J, Caetano M M and Tusi M M 2019 Study of physico-chemical characteristics and in vitro antimicrobial activity of hydroxyapatites obtained from bone calcination Progress in biomaterials 8 1–9
[17] Horta M, Aguilar M, Moura F, Campos J, Ramos V and Quízida A 2019 Synthesis and characterization of green nanohydroxyapatite from hen eggshell by precipitation method Materials Today: Proceedings 14 716–21
[18] Wu S C, Hsu H C, Hsu S K, Tseng C P and Ho W F 2019 Effects of calcination on synthesis of hydroxyapatite derived from oyster shell powders J. Agric. Chem. Soc. Taiwan 55 1051–58
[19] Esmaeilkhani A, Sharifianjazi F, Abouchenari A, Rouhani A, Parvin N and Irani M 2019 Synthesis and characterization of natural nanohydroxyapatite derived from turkey femur–bone waste Appl. Biochem. Biotechnol. 189 919–32
[20] SIMSEK Y and AVCI S 2019 Synthesis and characterization of hydroxyapatite produced by microwave assisted precipitation technique Acta Physica Polonica, A 135 974–81
[21] Mohammed E, Bouazza T and Khilil E H 2018 A novel method for the elaboration of hydroxyapatite with high purity by sol-gel using the albumin and comparison with the classical methods Alp. Conf. Proc. 003013
[22] Tilkin R G, Mahy J G, Rêgueb N, Grandfile C and Lambert S D 2019 Optimization of synthesis parameters for the production of biphasic calcium phosphate ceramics via wet precipitation and sol-gel process ChemistrySelect 6 46334–41
[23] Phatai P, Futalan C, Kamonwanansit S and Khemthong P 2019 Structural characterization and antibacterial activity of hydroxyapatite synthesized via sol-gel method using glutinous rice as a template J. Sol-Gel Sci. Technol. 89 764–75
[24] Türk S, Altunsoy I, Efe G C, Ipek M, Ozacar M and Bindi C 2019 Effect of Solution and Calcination Time on Sol-gel Synthesis of Hydroxyapatite J. Bionic Eng. 16 311–8
[25] Kurniawati R, Hidayat N and Kurniawan R 2019 Variable-sintering synthesis of biphasic calcium phosphate/alumina ceramic composites and their mechanical behaviors JOP Conf. Ser.: Materials Science and Engineering 012095
[26] Balakrishnan S, Rajendran A, Kulandavelu R and Nellaiappan S N T 2019 Saponin-mediated synthesis of hydroxyapatite by hydrothermal method: characteristics, bioactivity, and antimicrobial behavior J. Agric. Chem. Soc. 55 953–67
[27] Karunakaran G, Kumar G S, Cho E B, Sunwoo Y, Kolesnikov E and Kuznetsov D 2019 Microwave-assisted hydrothermal synthesis of mesoporous carbonated hydroxyapatite with tunable nanoscale characteristics for biomedical applications Ceram. Int. 45 9707–70
[28] Saïd L 2013 Étude comparative des caractéristiques physicochimiques et mécaniques des biomatériaux à base de phosphate de calcium, d’alumine et de zircon: Caractérisation et modélisation
[29] Wang J, Gong X, Hai L and LTJ 2018 Synthesis of silver-hydroxyapatite composite with improved antibacterial properties Vaccum 152 132–7
[30] Salwiczek M, Qu Y, Gardiner J, Strugnell R A, Lithgow T, McLean K M and Thissen H 2014 Emerging rules for effective antimicrobial coatings Trends Biotechnol. 32 82–90
[31] Riaz M, Zia R, Iqaz A, Hussain T, Mohsin M and Malik A 2018 Synthesis of monoatomic Ag doped hydroxyapatite and evaluation of antibacterial activity Materials Science and Engineering: C 90 308–13
[32] Riaz M, Zia R, Saleemi F, Ikram H and Bashir F 2015 In vitro antimicrobial activity of ZnO based glass–ceramics against pathogenic bacteria J. Mater. Sci., Mater. Med. 26 268
[33] Coelho C C, Araújo R, Quadros P A, Sousa S R and Monteiro F J 2019 Antibacterial bone substitute of hydroxyapatite and magnesium oxide to prevent dental and orthopaedic infections Materials Science and Engineering: C 97 529–38
[34] Lamkho S, Phaya M, Jansakun C, Chandet N, Thongkorn K, Rujijanagul G, Bangrak P and Randorn C 2019 Synthesis of hydroxyapatite with antibacterial properties using a microwave-assisted combustion method Sci. Rep. 9 4015
[35] Mondal S, Hoang G, Manivasan P, Moorby M S, Kim H H, Phan T T V and Oh J 2019 Comparative characterization of biogenic and chemical synthesized hydroxyapatite biomaterials for potential biomedical application Mater. Chem. Phys. 228 344–56
[36] Pupo Y M, Nadal I M, Maluf D F, de Lara E L, Saito R E, Michel M D, Antunes S R, da Graça Toledo M, Gomes J C and Farago P V 2019 Effect of coating morphology and quaternary ammonium polymer co-incubation on bone strength, cytotoxicity, and cell morphology of self-etching adhesive Int. J. Adhes. Adhes. 92 7–15
[37] Shi D, Liu F, Yu Z, Chang B, Goff H D and Zhong F 2019 Effect of aging treatment on the physicochemical properties of collagen films Food Hydrocoll. 87 436–47
[38] Diaz S M, Mokhtarpour M, Shekaari H and Sharif S 2019 Hydroxyapatite-gelatin nanocomposite films; production and evaluation of the physicochemical properties Journal of advanced chemical and pharmaceutical materials (JACPM) 2 111–5
[39] Yelen-Yilmaz A and Yilmaz S 2018 Wet chemical precipitation synthesis of hydroxyapatite (HA) powders Ceram. Int. 44 9703–10
[40] Corbridge D E 2016 Phosphorus: chemistry, biochemistry and technology (CRC press)
[41] Crane N J, Popescu V, Morris D M, Steenhuis P and Igelüzi M A Jr 2006 Raman spectroscopic evidence for octacalcium phosphate and other transient mineral species deposited during intramembranous mineralization Bone 39 434–42
[42] Markovic M, Fowler B O and Tung M S 2004 Preparation and comprehensive characterization of a calcium hydroxyapatite reference material J. Res. Nat. Inst. Stand. Technol. 109 553
[43] Yilmaz B and Evis Z 2014 Raman spectroscopy investigation of nano hydroxyapatite doped with yttrium and fluoride ions Spectrosc. Lett. 47 24–9
[44] Gras P 2014 Étude physico-chimique et structurale de pyrophosphates de calcium hydratés: application aux micro-calciﬁcations associées à l’arthrose. Thesis University of Toulouse, National Polytechnic Institute of Toulouse (INP Toulouse) (http://ethesis.imp-toulouse.fr/archive/00002824/01/Gras)
[45] Xie Y and Yang L 2016 Calcium and magnesium ions are membrane-active against stationary-phase staphylococcus aureus with high speciﬁcity Sci. Rep. 6 20628