Crystal growth of hydroxyapatite microplates synthesised by Sol–Gel method

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Hydroxyapatite microplates were synthesised by the Sol–Gel method using a mixture of calcium nitrate and potassium phosphate. The reaction took place at room temperature for 48 h, 300, 400, 500 and 600°C calcination temperatures were made in order to study the effect of this temperature on the growth and morphology of the nanostructures. Crystalline composition, functional groups and surface morphology were studied by X-ray diffraction (XRD), Fourier transform infrared spectroscopy and scanning electron microscopy. Hydroxyapatite microplates with average dimensions of 40 μm × 13 μm was obtained, the morphology of the surface is well defined in the form of a plate composed of a large number of small nanofibers. According to the studies by XRD, preferential orientations in the directions (200) and (002) were observed which is attributed to the conditions of synthesis.

1. Introduction: Hydroxyapatite (HAp) is a class of calcium phosphate widely investigated in compounds to be used as substitutes for bone tissue [1–3]. The synthesis of HAp nanostructures is of great interest due to its similarity with natural bone, it is a material with high biocompatibility [4]. In many biomedical applications, it is required that the HAp compounds have certain properties such as mechanical strength, adsorption capacity, etc. These properties depend on the particle size, morphology and surface area of the nanostructures [5]. In recent years research has been developed to find methods to control the morphology of HAp powders. HAp powders with various morphologies had been synthesised by means of solid-state reaction [6, 7], template-directed method [8, 9], hydrothermal method [10, 11] and emulsion method [12, 13] or emulsion technique [14]. However, the method of chemical precipitation is the most convenient way to synthesise HAp powders since reactions take place between calcium and phosphorus ions under a controlled environment of pH and temperature in a solution.

Sol–gel method has been popular because of well-known inherent advantages such as homogeneous molecular mixing, low processing temperature and ability to generate nanocrystalline powders, bulk amorphous monolithic solids and thin films [15]. This method has several advantages such as reaction speed, morphology control and quantity of final material compared to other synthesis processes. The HAp sol–gel synthesis is a convenient technique due to the small amount of equipment used, high performance, homogeneous composition and low synthesis temperature [16, 17]. In this work, we present a simple synthesis by sol–gel method to obtain HAp nanostructures. The powders obtained were subjected to high temperatures in order to study the influence of temperature on the size and morphology of the nanostructures. The structure and crystallinity were studied by X-ray diffraction (XRD), while the functional groups and morphology were studied by Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM), respectively.

The goal of this study is to find the reproducibility in the synthesis process that allows obtaining a material with possible biomedical applications in the regeneration of bone tissue. The novelty of this study is the obtaining of microplates with controlled morphology using a simple synthesis method with greater performance. The preferential growth of the nanostructures is of great importance for possible biomedical applications.

2. Experimental section

2.1. Synthesis of HAp nanostructures: Calcium nitrate Ca(NO3)2 (Quimica Meyer) and potassium phosphate K2HPO4 (Quimica Meyer) were used as precursors in the synthesis process. A solution 1 M of calcium nitrate and 0.67 M of potassium phosphate was made to dissolve the crystals in deionised water. The calcium nitrate solution is added slowly to the potassium phosphate solution while stirring. The solution was dried for 24 h with a pH = 7. A white solution was obtained, then filtered and washed with distilled water after 24 h. The material obtained was dried at 60°C for 24 h. Finally, the obtained powders were subjected to a calcination process between 300 and 600°C. The calcination temperatures were 300, 400, 500 and 600°C for HAp-1, HAp-2, HAp-3 and HAp-4, respectively.

2.2. Characterisation of HAp nanostructures

3.1. Phase composition: XRD: X-ray powder diffraction was used to identify the crystalline phases contained in all samples. Wide-angle X-ray experiments were carried out in a Rigaku Dmax 2100 diffractometer using the CuKα radiation (λ = 1.5406 Å), an accelerating voltage of 30 kV and a current of 20 mA. Diffractograms were recorded from 5 through 80° on a 2θ scale with a rate of 10° per minute. Spectrum analysis software, MDI Jade V 5.0.37, was used.

3.2. Functional groups: FTIR: In order to identify the presence of functional groups corresponding to HAp a FTIR spectrometer Spectrum GX of Perkin Elmer was used. The infrared spectra were recorded in medium infrared between 650 and 4000 cm⁻¹ with a resolution of 1 cm⁻¹.

3.3. Morphology and microstructure: SEM: Morphological, topological and microstructural analyses of all samples were carried

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out in a JEOL JXA – 8530F Scanning Electron Microscope. The analysis was performed using 20 kV electron acceleration voltage and the images were formed by secondary electrons. All the samples were placed on a stainless-steel plate with a separation of 5 mm and fixed with silver paint and covered with a gold thin film done by sputtering to avoid the electrostatic charge accumulation.

3.4. Elemental composition: XRF: In order to obtain the elemental composition and the Ca/P ratio of nanostructures, Bruker S2 Picofox was used. The operation conditions were: a voltage of 50 kV and a potassium molybdenum radiation, the samples were dispersed in 1 ml of isopropyl alcohol and iron was added as an internal standard.

4. Results and discussion

4.1. Structural characterisation

4.1.1. Crystalline phases: The study of the crystal structure was done by XRD. In Fig. 1 the diffraction patterns for each sample are observed. In all samples the peaks corresponding to the hexagonal stoichiometric HAp were indexed according to PDF # 84-1998. The Bragg reflections present in the samples HAp-1 and HAp-2 correspond to planes (200), (111), (002), (102), (301), (400), (401) and (231), the intensities varied from one sample to another. In both samples it is observed that the most intense peak corresponds to the diffraction of the plane (200) which follows a greater presence of these planes, indicating a preferential crystallographic orientation in ‘a’ axis. The FWHM of the peaks in these samples is not observed very narrow suggesting a low crystallinity according to the Debye-Scherrer equation where, if the FWHM decreases the crystallinity increases [18]. For HAp-3 and HAp-4, the presence of fewer reflections is observed in relation to the previous samples (upper part of Fig. 1), the observed reflections correspond only to planes (200), (111), (002) and (301), a clear difference can be observed since the peak corresponding to the plane (200) decreased considerably, in both samples it is observed that now the most intense peak corresponds to the planes (111) and (002). In addition, the FWHM for these samples is broader, suggesting that the increase in temperature modifies the crystal structure of the HAp. According to previously reported works [11], the results obtained in all the diffractograms suggest a preferential crystalline growth in the directions [100] and [001] of the structure of the HAp.

The crystallinity of the samples is directly related to the crystallite size, to identify the level of crystallinity, the crystallite size was measured using the Williamson Hall method [19]. The crystallite sizes for HAp-1, HAp-2, HAp-3 and HAp-4 are shown in Fig. 2. It is observed that the crystallite size decreases as the calcination temperature increases, the values calculated were 7.78, 7.42, 6.02, 5.90 and 5.62 nm for calcination temperatures of 300, 400, 500, 600 and 700°C, respectively. The calculation at 700°C was included in order to observe more clearly the decreasing behaviour of the crystallite size. These results are consistent with the XRD studies.

In Fig. 2 it can be seen that the crystallite size is inversely proportional to the FWHM, so in HAp-1 and HAp-2 the peaks are narrower compared to HAp-3 and HAp-4, so the size crystallite is larger. The HAp obtained by other synthesis methods report a smaller crystallite size, as compared to the sol–gel method. The crystallite size in those papers is of the order of tens of nanometers [20, 21]. In previous reports [22], nanostructures were synthesised under similar conditions of synthesis with low crystallinity and without preferential orientation of growth, in this work were synthesised nanostructures with high crystallinity and a preferential orientation, in this case the calcination temperature plays an important role in the direction of growth of nanostructures (Fig. 3).

4.1.2. Functional groups: To identify the functional groups, present in the samples, FTIR studies were done as a complementary technique to XRD. The absorption band located at 561 cm⁻¹ is attributed to triply degenerated bending mode, νbc, of the O–P–O bonds of the phosphate group [19, 21], the absorption band in 962 cm⁻¹ corresponds to nondegenerated symmetric stretching mode, νs, of the P–O bonds of the phosphate group [22, 23], the bands located at 1032, 1046 and 1087 cm⁻¹ are attributed to triply degenerated asymmetric stretching mode, νas₁, νas₂, νas₃, respectively of the P–O bond of the phosphate group [24–26]. In all the samples, the absorption bands characteristic of the molecular structures of the HAp were observed, the spectra show bands with different widths and intensities.

The absorption bands have a well-defined shape indicating a high degree of crystallinity due to the calcination process [27], which is in agreement with the XRD studies. In the spectra, no absorption bands corresponding to the OH⁻ groups were observed due to the

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**Fig. 1** XRD patterns for HAp-1, HAp-2, HAp-3 and HAp-4 samples

**Fig. 2** Calcination temperature versus crystallite size. A decrease in the crystallite size is observed as the calcination temperature increases

**Fig. 3** FTIR for HAp-1, HAp-2, HAp-3 and HAp-4 samples
synthesis process and the calcination temperatures that eliminate all the presence of water inside the samples.

4.2. Morphological characterisation

4.2.1. Surface microstructure: SEM is an excellent technique to study the surface microstructure of samples. Fig. 4 shows the micrographs of all the samples in a 250× amplification. In all cases microstructures in the form of plates are observed, in some cases these plates are interlaced to form larger microstructures in the shape of a star. Table 1 shows the average dimensions of the nanoplates observed; with this analysis it can be observed that the size of the nanoplates was decreasing as the temperature of the synthesis increased. This suggests that the increase in temperature caused the rupture of the nanoplates into smaller plates. In the micrographs, a higher density of nanoplates was observed as the temperature of the synthesis increases.

In Fig. 5 micrographs of the samples with amplification of 500× are observed. The images can be observed with more precision the morphology of the nanoplates. The nanoplates are formed of many nanofibers, the growth of these structures is according to what was observed in the X-ray analysis. The petal-like morphology is observed due to the agglomeration of several nanoplates. In other literary works, the growth of nanoplates has been made under the influence of glutamic acid as an agent to guide the growth of the nanostructures [20], in this work the synthesis process is free of glutamic acid obtaining similar nanostructures. Other methods have been used to obtain HAp crystals such as microwaves or chemical vapour deposition [20, 28], however the sol–gel method has the advantage of using a lower synthesis temperature and a high performance, as mentioned previously.

In order to complete the information obtained by XRD and SEM, XRF studies were done to determine the elemental composition of each sample. In Fig. 6 the XRF spectra are shown and the Ca/P ratio was calculated for each sample obtaining the following values: 1.70, 1.74, 1.66 and 1.69 for HAp-1, HAp-2, HAp-3 and HAp-4, respectively. The Ca/P ratio is of great importance for biocompatibility applications, the values obtained here are in the range for stoichiometric HAp.

5. Conclusion: In this work, HAp nanoplates were obtained under the sol–gel method, the synthesis conditions allowed the reproducibility of the microstructures. XRD studies showed a high crystallinity, whereas in the images obtained by SEM a well-defined morphology was observed. The calcination temperature was the factor that determined the size of the nanostructures, since at higher temperatures the dimensions of the nanoplates were smaller. The nanoplates obtained at low calcination temperatures (300 and 400°C) present a preferential growth in the direction (200) which suggests an improvement in their mechanical properties in addition to obtaining this preferential growth without the presence of a reagent to guide the growth. The elemental composition suggests the use of this material in the manufacture of composite materials with possible biomedical applications and bone tissue regeneration applications. The nanoplates can be used in the development of a bioceramic composite by adding the organic phase, with the aim to study their mechanical and biocompatibility properties.

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7 References

[1] Hench L.L.: ‘Bioceramics: from concept to clinic’, J. Am. Ceram. Soc., 1991, 74, (7), pp. 1487–1510
[2] Jarcho M.: ‘Calcium phosphate ceramics as hard tissue prosthetics’, Clin. Orthop., 1981, 157, pp. 259–278
[3] Legeros R.Z.: ‘Calcium phosphate materials in restorative dentistry: a review’, Adv. Dent. Res., 1988, 2, pp. 164–168
[4] Roeder R.K., Converse G.L., Leng H., et al.: ‘Kinetic effects on hydroxyapatite whiskers synthesized by the chelate decomposition method’, Am. Ceram. Soc., 2006, 89, (7), pp. 2096–2104
[5] Bose S., Saha S.K.: ‘Synthesis and characterization of hydroxyapatite nanopowders by emulsion technique’, Chem. Mater., 2003, 15, pp. 4464–4469
[6] Ramachandra Rao R., Roopa H.N., Kannan T.S.: ‘Solid state synthesis and thermal stability of HAp and HAp–b-TCP composite ceramic powders’, J. Mater. Sci., Mater. Med., 1997, 8, (8), pp. 511–518
[7] Pramanik S., Agarwal A.K., Rai K.N., et al.: ‘Development of high strength hydroxyapatite by solid-state-sintering process’, Ceram. Int., 2007, 33, pp. 419–426
[8] Wang Y.J., Zhang S.H., Wei K., et al.: ‘Hydrothermal synthesis of hydroxyapatite nanopowders using cationic surfactant as a template’, Mater. Lett., 2006, 60, pp. 1484–1487
[9] Prélot B., Zemb T.: ‘Calcium phosphate precipitation in catanionic templates’, Mater. Sci. Eng. C, 2005, 25, pp. 553–559
[10] Ioku K., Yamauchi S., Fujimori H., et al.: ‘Hydrothermal preparation of fibrous apatite and apatite sheet’, Solid StateIon., 2002, 151, pp. 147–150
[11] Lozano N.M., Velázquez-Castro R., Rivera Muñoz E.M., et al.: ‘Crystal growth and structural analysis of hydroxyapatite nanofibers synthesized by the hydrothermal microwave-assisted method’, Ceramics Int., 2017, 43, pp. 451–457
[12] Lim G.K., Wang J., Ng S.C., et al.: ‘Processing of hydroxyapatite via microemulsion and emulsion routes’, Biomaterials, 1997, 18, pp. 1433–1439
[13] Sun Y.X., Gao G.S., Tao D.L., et al.: ‘Reverse microemulsion-directed synthesis of hydroxyapatite nanoparticles under hydrothermal conditions’, J. Phys. Chem. Solids, 2007, 68, pp. 373–377
[14] Lim G.K., Wang J., Ng S.C., et al.: ‘Formation of nanocrystalline hydroxyapatite in nonionic surfactant emulsions’, Langmuir, 1999, 15, pp. 7472–7477
[15] Kim S., Kunta P.N.: ‘Sol–gel synthesis and characterization of nanostructured hydroxyapatite powder’, Mater. Sci. Eng. B, 2004, 111, pp. 232–236
[16] Simon V., Cavalia S., Simon S., et al.: ‘Surface functionalisation of sol–gel derived aluminosilicates in simulated body fluids’, Solid State Ion., 2009, 180, pp. 764–769
[17] Chen J., Wang Y., Chen X., et al.: ‘A simple sol–gel technique for synthesis of nanostructured hydroxyapatite, tricalcium phosphate and biphasic powders’, Mater. Lett., 2011, 65, pp. 1923–1926
[18] Rukusoljant A., Parangat K., Rujjanagul G., et al.: ‘Synthesis and characterization of nanocrystalline hydroxyapatite from natural bovine bone’, Curr. Appl. Phys., 2008, 8, pp. 270–272
[19] Mote V.D., Purushotham Y.: ‘Williamson–hall analysis in estimation of lattice strain in nanometer-sized ZnO particles’, J. Theor. Appl. Phys., 2012, 6, p. 6
[20] Cabrera J.L., Velázquez-Castro R., Rivera Muñoz E.M.: ‘Synthesis of hydroxyapatite nanostructures using microwave heating’, J. NanoSci. Nanotechnol., 2011, 11, pp. 1–7
[21] Oh S.-H., Finnöes R.R., Dario C., et al.: ‘Growth of nano-scale hydroxyapatite using chemically treated titanium oxide nanotubes’, Biomaterials, 2005, 26, pp. 4938–4943
[22] Liu D.-M., Troczynski T., Tseng W.J.: ‘Water-based sol–gel synthesis of hydroxyapatite: process development’, Biomaterials, 2001, 22, pp. 1721–1730
[23] Klew WE., Engel G.: ‘Infrared spectra of the phosphate ions in various apatites’, J. Inorg. Nucl. Chem., 1970, 32, pp. 1837–1843
[24] Fowler B.O.: ‘Infrared studies of apatites I. Vibrational assignments for calcium, strontium, and barium hydroxyapatites utilizing isotopic substitution’, Inorg. Chem., 1974, 13, pp. 194–207
[25] Joris S.J., Amberg C.H.: ‘Nature of deficiency in nonstoichiometric hydroxyapatites. II. Spectroscopic studies of calcium and strontium hydroxyapatites’, J. Phys. Chem., 1971, 75, pp. 3172–3178
[26] Bhalia N., Di Lorenzo M., Pula G., et al.: ‘Protein phosphorylation detection using dual-mode field-effect devices and nanoplasmic sensors’, Sens. Actuators, B, 2015, 5, p. 5867
[27] Giraldo-Betancur A.L., Espinosa-Arbeleza D.G., del Real-López A., et al.: ‘Comparison of physicochemical properties of bio and commercial hydroxyapatite’, Car. Appl. Phys., 2013, 13, pp. 1383–1390
[28] Krumdieck S.P., Royngoud B.P., Barnett A.D., et al.: ‘Deposition of bio-integration ceramic hydroxyapatite by pulsed-pressure MOCDV using a single liquid precursor solution’, Chem. Vap. Deposition, 2010, 16, pp. 55–63