



STRUCTURAL AND LUMINESCENT PROPERTIES OF EUROPIUM-DOPED AND UNDOPED HYDROXYAPATITE POWDERS SINTERED BY SPARK PLASMA

G. GARCÍA DOMÍNGUEZ*, [#]A. GARRIDO HERNÁNDEZ**, G. CERÓN MONTES**, A.J. MORALES RAMÍREZ*, S. DÍAZ DE LA TORRE*

*Instituto Politécnico Nacional, CIITEC IPN, Cerrada de Cecati S/N, CP 02250, Col. Santa Catarina Azcapotzalco Ciudad de México, México **Universidad Tecnológica de Tecámac, UTTEC, Carretera Federal México, Pachuca Km 37.5, CP 55740, Col. Sierra Hermosa, Tecámac, Estado de México, México

[#]E-mail: agarridoh@uttecamac.edu.mx

Submitted August 30, 2018; accepted December 10, 2018

Keywords: Ceramics, Luminescence, SPS

This paper studies the structural and photoluminescent properties of hydroxyapatite (HA) and europium-doped hydroxyapatite (HA:Eu) synthesised by the hydrothermal method and sintered by the spark plasma sintering (SPS) technique. HA and HA:Eu powders, synthesised at pH of 10, 11 and 12, were characterised by means of infrared spectroscopy, X-ray diffraction, scanning electron microscopy, Raman spectroscopy and luminescence spectroscopy. The HA and HA:Eu samples were sintered using the SPS technique at 900 and 1200 °C. It was determined that the HA and HA:Eu powders crystallised in the hexagonal phase, which is stable until 900 °C. The presence of Eu³⁺ ions tended to stabilise the hexagonal phase of hydroxyapatite at 1200 °C. Undoped hydroxyapatite sintered at 1200 °C revealed a significant amount of tricalcium phosphate (β -TCP) as a result of its decomposition. By increasing the heat treatment temperature, different emission lines (${}^{5}D_{0} \rightarrow {}^{7}F_{1}$ and ${}^{5}D_{0} \rightarrow {}^{7}F_{2}$ transitions) were obtained due to calcium site substitution by the europium ions in the HA structure.

INTRODUCTION

Among bio-ceramics for use in biomedical applications, hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂) is the most investigated, since it is the main inorganic component of human tissues such as bones and teeth [1]. Hydroxyapatite (HA) is the most stable calcium phosphate compound in body fluids under physiological conditions [2]. Therefore, HA is widely used as a biomaterial in orthopaedic and dental applications; moreover, nanosized hydroxyapatite with excellent biocompatibility and thermal stability also has its possible applications in bioimaging and nanomedicine [3]. Recently, HA has been proposed to be useful in applications such as gene transfection [4, 5], hyperthermia therapy [6], drugs delivery [7, 8] and molecular imaging [9]. For these purposes, it is necessary to functionalise the hydroxyapatite nanoparticles by incorporating organic dyes, quantum dots, and rare earth elements [10, 11].

Various studies have demonstrated that Eu³⁺ and Gd³⁺ dopants can affect bone metabolism and might be useful for treating bone density disorders such as osteoporosis [12]. Eu ions are used as doping activators in other calcium-based materials because of their low level of toxicity, photostability, high thermal and chemical stabilities as well as their high quantum luminescence yield [13, 14].

Over the past decades, gel systems [15], liquidsolid-solution synthesis [16], molten salt synthesis [17], electrochemical deposition [18], hydrolysis [19] and hydrothermal routes [20] have been used for preparing HA with a controlled structure and morphology. Among these methods, it is worth mentioning wet chemical methods, which have the advantages of controlling the morphology and particle size. These methods have demonstrated the precise control over the microstructure and morphology of some ceramics [21]. A typical wet process, normally carried out at room temperature to temperatures higher than the boiling point of water, involve the dropwise addition of one reagent to another under stirring [22]. Of the wet processes, the hydrothermal method is the most promising and convenient method since different parameters (solvent, pH, concentration, temperature reaction time, surfactant and others) can be tuned in order for it to result in the desired phase and morphology of the HA. Additionally, the hydrothermal approach is also known as an efficient method to synthesise defect-free single crystals of high crystallinity with narrow particle size distribution and tunable morphology [23].

Due to the importance of hydroxyapatite as a biomaterial, a large number of research studies have been carried out on manufacturing HA products by sintering methods, which can be classified as non-pressure and pressurised, the conventional hot-pressing (HP) and the spark plasma sintering (SPS) techniques, respectively [24]. Although SPS has been widely used to consolidate samples to high densities for metal and engineering ceramics [25, 26], there are few reports on the application of this technique to produce dense ceramics for biomedical engineering and luminescent applications. During the SPS process, a strong electromagnetic field is produced in the gaps left by the particles, which will eventually trigger the chemical potential at the surface of the oxide, promoting their consolidation. Thereby, the chemical reactions take place during this process. Munir et al. [27] stated the use of lower temperatures and shorter holding times allows sintering nanometric powders to near theoretical density values with little grain growth. The effect of the applied pressure leads a particle re-arrangement which contributes to the improved sintering. Recently, Cuccu et al. [28] reported a fully dense material of hydroxyapatite without secondary phases using SPS at 900 °C; the sintered product, consisting of sub-micrometre hydroxyapatite grains, displayed optical transparency and good mechanical properties.

It is well known that one of the main disadvantages of the thermal processing of HA is related to its thermochemical instability. In fact, HA decomposition takes place when high temperature conditions are encountered during the condensation of the powders. Consequently, negative effects on the mechanical and biological characteristics of HA appear [29, 30]. In this context, SPS sintering is a suitable method to obtain ceramic products with a high density under lower sintering conditions and a shorter processing time compared to other methods such as hot pressing.

In the present study, both the structural and photoluminescent properties of HA and HA:Eu powders synthesised by the hydrothermal method are presented. A detailed comparison of their HA and HA:Eu counterparts sintered by SPS is also presented.

EXPERIMENTAL

In the HA and HA:Eu syntheses, commercial-grade calcium nitrate tetrahydrate $CaNO_3 \cdot 4H_2O$ (Mallinck-rodt 99 %) and diammonium hydrogen phosphate $(NH_4)_2HPO_4$, (Baker Analyzed 98.7 %) were used in hydrothermal method as starting materials. Europium oxide, Eu_2O_3 , was used as the starting material for doping.

First, an $(NH_4)_2HPO_4$ solution at 0.5 M was added dropwise to a CaNO₃·4H₂O solution at 0.5 M; during the addition, the Ca solution was kept under stirring at 50 °C. (For the doped samples, a europium ions at 10 mol. % (via addition of Eu₂O₃) was incorporated into the Ca solution). Then, the combined solutions were adjusted to pH = 10, 11 and 12 using a 1.5 M NaOH solution. After setting the pH, the solutions were aged in the mother liquor at room temperature for 15 hours. Subsequently, the final solution was poured into a Teflon[®]-lined autoclave at 200 °C for 15 hours. Once the hydrothermal reaction finished, the autoclave was cooled to room temperature. The powders obtained were washed by means of centrifugation at 4000 rpm, 15 min, and distilled water was used for each wash, three washing cycles were employed. Finally, these powders were dried in an oven at 120 °C for 15 hours.

A total of 1.5 g of undoped hydroxyapatite (HA) and europium-doped hydroxyapatite at 10 mol. % (HA:Eu) powders were used for sintering in a SPS1050 model DR. SINTER[®] machine. The powder was placed into a graphite disk of a 10 mm internal diameter in order to obtain the final sintered material. The powders were sintered at either 900 °C or 1200 °C for further investigation. All of the SPS experiments were carried out at 20 MPa mechanical pressure (P), 6 min of holding time (t_D) and a 75 °C·min⁻¹ heating rate. The SPS parameters such as mechanical load, vertical displacement, temperature, current and voltage between the machine electrodes were recorded in real time.

The X-ray diffraction patterns were recorded at room temperature on a Bruker d8 advance eco diffractometer, using Cu K α radiation ($\lambda = 1.54184$ Å). To determine the crystalline phase of the samples, a 2θ scan ranging from 4°-60° was used. The infrared spectra were obtained at room temperature with a Perkin Elmer spectra two spectrophotometer. The spectra were recorded at 32 scans in the 4000 - 450 cm⁻¹ range. The SEM micrographs were recorded using a Jeol JSM-IT100 scanning electron microscope operating under a low accelerating voltage (5 kV) and a high vacuum. A secondary electron detector (High-Sensitivity Low-Vacuum SE detector) and backscattered electron detector were used. Raman spectra were recorded using a T64000 Jobin-Yvon confocal micro-Raman Spectrometer with a 514 nm wavelength line green laser excitation source (Coherent model 70C5 Ar⁺) operating at 800 mW. The emission and excitation spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer at room temperature.

RESULTS AND DISCUSSION

Structural characterisation of the HA obtained by the hydrothermal method

It has been stated that HA can be obtained at different pH values; however, the basic media ($pH \ge 9$) was preferred because it has been reported that well-crystallised HA can be obtained at pH values between 10 and 12 [31]. Therefore, to evaluate the synthesis conditions of HA as a function of pH, three different pH values were tested (10, 11 and 12).

Figure 1 shows the diffraction patterns of the HA synthesised at different pH values. The diffraction profile of the samples corresponds to a hexagonal structure which matches very well with the JCPDS (Joint Committee on Powder Diffraction Standards) File No: 09-432 (space group P6₃/m). All of the XRD patterns do not present additional peaks beyond those of the hexagonal phase. Therefore, no secondary phase was detected. According to the XRD pattern, a better crystalline HA was obtained at pH = 11, since the splitting of the peak located at $2\theta = 32.84^{\circ}$ corresponding to the (300) plane was observed.

In order to identify the functional groups, FT-IR analyses were conducted. The infrared spectra were interpreted according to the data from the literature [32]. All of the infrared spectra of the powders in Figure 2 revealed hydroxyapatite formation, as all of the typical absorption bands appeared. In particular, the absorption band located at 1089 cm⁻¹ and 1025 cm⁻¹ can be assigned



Figure 1. XRD patterns of the HA powders synthesised at pH 10, 11 and 12.

to the asymmetric stretching vibrations of the P-O band. The absorption band located around 962 cm⁻¹ can be attributed to the symmetric stretching of P-O.

Sadat-Shojai et al. [22] demonstrated that the anionic sites within the HA crystals are very susceptible to carbon dioxide originating from the atmosphere; in fact, these carbonate ions (CO_3^{2-}) can substitute for hydroxy or phosphate groups. Besides this, they also found



a) pH = 10



b) pH = 11



Figure 2. The FT-IR spectra of the HA powders synthesised at pH 10, 11 and 12.



c) pH = 12

Figure 3. SEM micrographs of HA as-synthesised at pH = 10, 11 and 12.

small amounts of CO_3^{2-} groups when the samples were synthesised at pH = 9, due to the solubility of CO_2 in alkaline solutions. Despite this reported behaviour, in this research using basic conditions at pH = 10, 11 and 12, no absorption bands belonging to the groups were detected.

According to the literature, there is a similarity between the X-ray patterns of OCP (octacalcium phosphate, $Ca_8(HPO_4)_2(PO_4)_45H_2O$), and HA at high angle regions; in order to avoid the misidentification between these phases, researchers have suggested the use of IR spectra as an alternative for detecting the presence of OCP [33]. In particular, peaks appearing at 1193 and 868 cm⁻¹ have been assigned to the OH inplane bending and P–(OH) stretching modes of groups in the OCP structure, respectively [34]. However, those absorption bands were not identified. Thus, likely, at most, only a very small amount of OCP could be present in the HA samples as an impurity. Finally, the absorption bands centred at 630 cm⁻¹ are attributed to the vibrational mode of the hydroxyl group.

As seen in the SEM micrographs of the as-synthesised HA powders (Figure 3), the HA powders obtained at pH 10 are constituted of agglomerated rodshaped particles. These particles are about 100 - 200 nm in diameter and 300 - 600 nm in length. It is clear that these powders are not homogeneous particles in size. Changing the pH, it was possible to modify the particle agglomerations, morphology and the particle size homogeneity, since the pH affects the superficial charge of particles. Thus, the HA particles present different agglomeration, particles size homogeneity and morphology. The rod-shaped particles similar to pH 10, around 100 - 150 nm in diameter and 300 - 500 nm in length are seen for the HA powders synthesised at pH = 11. At pH 12, the rod-shaped morphology of HA is transformed into spherical particles with a diameter around 70 - 120 nm, it is clear that the agglomeration of the HA particles synthesised at pH 12 is higher when compared to the HA powder obtained at pH 10 and 11.



Figure 4. Temperature and sample shrinkage time profiles recorded during the consolidation of the powders by SPS.

Structural and optical characterisation of HA and HA:Eu synthesised by the hydrothermal method and sintered pellets by SPS

Figure 4 depicts the common outputs of the sample shrinkage (δ) and temperature profiles recorded during the SPS sintering process (at 900 °C, 75 °C min⁻¹,



Figure 5. XRD patterns of the as-synthesised, heat-treated and sintered HA:Eu (10 mol. % Eu) powders.

 $t_D = 6 \text{ min and } P = 20 \text{ Mpa}$) of the HA powders. Normally, the sample densification improves as the sintering temperature increases, but it is clear that europium affects the HA behaviour during the sintering process, since different shrinkage values were reached.

The undoped HA powders sintered at 900 °C (HA 900S) reached a constant shrinkage at 7.2 mm in 16 min, as did the powders sintered at 1200 °C (HA1200S). Both samples of undoped hydroxyapatite had the same shrinkage value at each sintering temperature; this effect could be related to the phase transformation of HA into β -TCP and the arrangement of the powders before the sintering process. Meanwhile, the europium-doped HA powders (HA:Eu900S) developed shrinkage stability around 8 mm at 14 min and the HA:Eu1200S reached a constant shrinkage (about 8.8 mm) at 16 min. The HA:Eu samples did not present a phase transition like the HA samples did. Thus, higher shrinkage values were reached.

Figure 5 shows the diffraction patterns of the undoped and europium-doped hydroxyapatite. All of the as-synthesised samples mainly crystallised in the hexagonal phase; this crystalline phase matched with the JCPDS card no. 09-432. No second phase related to the Eu³⁺ ion appeared, therefore, the Eu³⁺ ion was successfully incorporated into the HA structure. This result can be understood in terms of the very small difference in ionic radii between Ca and Eu, 1.80 Å and 1.85 Å, respectively; therefore, hydroxyapatite is a suitable matrix for incorporating europium ions [35]. In order to compare the structural stability of HA and HA:Eu, the powders were annealed at 700 °C for 3 hours, since this temperature promotes crystallisation. The heat-treated powders at 700 °C presented a small presence of β -TCP which can be confirmed by the (300), (217) and (220) planes located around $2\theta =$ = 29.62° , 31.09° and 34.34° , respectively. On the other hand, a better crystalline hexagonal phase was reached when the europium-doped HA was annealed at 700 °C for 3 hours (HA:Eu700).

Because of their similarity in the XRD patterns at the high angle region, there is a misidentification possibility between the OCP and HA structures. In particular, the OCP structures have a characteristic peak located at $2\theta = 4.7^{\circ}$ in addition to the absorption bands discussed in the FT-IR results. However, the characteristic peak located around 4.7° was not found, as shown in Figures 5a and b. Thus, it confirms the proper identification of the HA structure. The OCP stability decreases as the temperature and pH increase [33], as a result, the OCP phase in the heat-treated powders at 700 °C will decrease since recent thermodynamic analyses have shown that OCP is a metastable phase of HA [36]. Cuccu et al. [28] also reported β-TCP formation by thermal decomposition (> 800 °C) of the calciumdeficient HA (CDHA). Besides the chemical preparation route, β -TCP can be obtained by calcining bones; for



Figure 6. Infrared spectra of: a) the doped b) the undoped HA powders.



Figure 7. Raman spectra of the heat-treated and sintered HA powders.

this reason, β -TCP is also called "bone ash" [37]. At higher temperatures, β -TCP is transformed into α -TCP, which is stable at temperatures between 1180 - 1400 °C.

Therefore, the sintering temperature in the SPS technique causes decomposition of the HA powders at elevated temperatures of 900 °C and 1200 °C. After the sintering process at 900 °C, the undoped HA powders presented extra diffraction peaks when compared with the hexagonal structure of HA. These peaks match

with the β -TCP phase (JCDPS data file no. 09-348). At 1200 °C, the sintered HA powders (HA1200S) had more diffraction peaks than the sintered powders at 900 °C, suggesting a decomposition of HA and the transformation of β -TCP into α -TCP. On the other hand, the sintered europium-doped HA at 900 and 1200 °C, (HA:Eu900S and HA:Eu1200S, respectively) presented very similar diffraction patterns to each other, which are associated with the hexagonal phase of HA (Figure 5b).



Figure 8. Micrograph of the as-synthesised HA and HA:Eu (10 mol. % Eu) powders.

Thus, it is evidenced that europium dopant stabilises HA, since the presence of α -TCP and β -TCP could not be detected.

The infrared spectra in Figure 6 presents the ATR-FTIR spectra of the HA and HA:Eu samples. Figures 6a and b depict the broader absorption bands located around 1030 cm⁻¹ as a function of the sintering temperature. As noted above, the temperature promotes the decomposition of HA. Therefore, the broader absorption band is related to the presence of β -TCP and the increasing crystallite size due to the temperature effect. The absorption bands are assigned to the vibrations of the phosphate group and are located around 1090 and 1022 cm⁻¹ (v₃), 961 cm⁻¹ (v1), 602 and 560 cm⁻¹ (v₄). The absorption bands centred at 630 cm⁻¹ are attributed to the vibrational mode of the hydroxyl group.

Raman spectroscopy provides complementary information to the FT-IR analysis, since it describes variations in the mineral crystallinity [38]. Thus, the heat-treated and sintered HA powders were analysed to describe their structural characteristics. The positions of all the peaks in Figure 7 are in good agreement with the hexagonal HA phase [39]. The peak centred at 961 cm⁻¹ corresponds to the v_1 mode, the peaks located in the position of 390 - 470 cm⁻¹ are assigned to the v_2 mode, the bands between 560 and 625 cm⁻¹ to the v_4 mode, and the bands between 1010 and 1100 cm⁻¹ to the v_3 mode, and all of these vibration modes (v_1 , v_2 , v_3 and v_4) correspond to the group. Finally, the OH⁻ symmetric stretching mode is centred at 3572 cm⁻¹.

In order to evaluate the morphology and composition of the samples, the ceramic powders were analysed by SEM-EDS analysis. Figure 8 shows the EDS analysis of the undoped and europium-doped HA. All the atoms associated with the Ca, P, O and Eu atoms of hydroxyapatite are presented in the EDS mapping, these zones confirm the homogeneous composition, indicating that the dopant ions were well incorporated into the HA matrix. The HA and HA:Eu morphology are constituted by rod-like particles that appeared to be affected by europium, as a reduction in the particle size was detected (around 70 - 120 nm in diameter and 200 - 500 nm in length).

The grain size affects the flow stress for the grain boundary sliding, and hence, the porosity of the sintered changes, as shown in Figure 9. As a result, the density of the sintered samples changed. The apparent density of the sintered samples was calculated using Archimedes' principle. The HA:Eu sintered powders likely had a higher density than the HA sintered powders because





c) 3.128 g·cm⁻³/900 °C

d) 3.135 g·cm⁻³/900 °C

of their structural stability, since a lower response of the secondary phase was found in the XRD analysis. As expected, the density of the samples increased as the temperature of the sintering process increased. The density values were very close to that of the theoretical density of HA (3.16 g·cm⁻³), reaching relative densities of 99.81 %, 99.19 %, 99.19 % and 99.5 % for HA900S, HA1200S, HA:Eu900S and HA:Eu1200S, respectively.

The sintered HA powders present a white colour, while the sintered HA:Eu powders changed their colour due to the europium doping. For both of the samples, HA and HA:Eu opaque sintered materials were obtained, this finding agreed with the sintered HA material containing an additional phase obtained for Cuccu et al [28]. Thus, the opaque sintered HA could be associated with the HA decomposition. On the other hand, they also obtained



Figure 10. Photoluminescence spectra of the heat-treated (700 °C) and sintered HA:Eu (10 mol. %) powders a) excitation b) emission.

a transparent sintered material corresponding to HA without a secondary phase. In this work, although the europium dopant stabilised the hexagonal structure of HA, the transparent sintered material was not obtained. This result suggests that the chemical composition of the samples affect the lack of transparency in the sintered material; furthermore, the use of different SPS machines, particle size and morphology affect the final features of the sintered material.

Figure 10 shows the excitation and emission spectra of the europium-doped heat-treated and sintered HA powders excited at 394 nm. The heat-treated HA:Eu presents two broad emission peaks centred at 592 nm and 616 nm. These peaks correspond to the typical ${}^{5}D_{0} \rightarrow {}^{7}F_{1}$ and ${}^{5}D_{0} \rightarrow {}^{7}F_{2}$ transitions of the Eu $^{3+}$ ion (Figure 10b). These characteristic emission bands correspond to the europium ions occupying the Ca $^{2+}$ (I) sites [40,41].

The emission peak position and the emission intensity of the Eu^{3+} transitions lines depend on the local symmetry in the nanocrystal [42]. According to the findings [43], europium-doped hydroxyapatite synthesised at low temperature presents emission lines attributed for Ca²⁺ (I) substitution similar to the emission spectrum of the heat-treated HA:Eu shown in Figure 11.

The temperature used in the sintering process modified the europium emission spectra, i.e., at 900 and 1200 °C, a new intense peak at 574 nm corresponding to the ${}^{5}D_{0} \rightarrow {}^{7}F_{0}$ transitions was observed. This peak indicates the presence of Eu³⁺ in other calcium sites of symmetry [44] as result of Eu³⁺ migration from Ca²⁺ (I) to Ca²⁺ (II), which was activated by the diffusion process. Similar observations were reported by Silva et al. [45], who studied the migration of Eu³⁺ from Ca²⁺ to Ca²⁺ (II) by calcining of europium-doped samples and



Figure 11. Characteristic emissions from the Eu^{3+} (I) and Eu^{3+} (II) centres.

found a change in the emission spectra when powders were annealed at 800 °C for 3 hours, while at 600 °C no change was reported. These findings are in complete agreement with the results of this research since the europium doped HA at 700 °C for 3 hours (HA:Eu700) presents the emission peaks centred at 592 nm and 616 nm, which are characteristic with the europium ion occupying the $Ca^{2+}(I)$ site. On the other hand, a complete migration of the Eu³⁺ ions could be achieved at 900 °C, since the emission spectrum of the europium-doped hydroxyapatite at 1200 °C presented similar emission peaks. This means that most of the europium ions were located at the Ca²⁺ (II) site. Normally, similar emission lines of Eu³⁺ ions are obtained for HA:Eu prepared by combustion synthesis [41], and the heat-treated samples (at temperatures more than 800 °C) [44, 46]. The emission lines and the calcium sites are described in Figure 11. The emissions at 590 nm and 574 nm are the characteristic lines of the Eu³⁺(I) and Eu³⁺ (II) centres, respectively.

CONCLUSION

Undoped and europium-doped hydroxyapatite powders were obtained by a hydrothermal method. The HA and HA:Eu powders were sintered by SPS. Detailed structural analyses demonstrated the hexagonal phase of the HA powders changed into β -TCP as a function of the sintering temperature. Europium stabilised the hexagonal phase of hydroxyapatite. The europium ions also led to the reduction of grain size changing the density of the sintered samples. The temperature in the sintering process promoted the migration of Eu³⁺ from Ca²⁺(I) to Ca²⁺(II) changing the emission lines of the europium ions.

Acknowledgments

The authors gratefully acknowledge CIITEC–IPN and CCAI–UTTEC for the use of the facilities and the characterisation analyses. We also thank María Jiménez Santillán for her assistance during the writing process.

REFERENCES

- Dorozhkin S.V. (2010): Bioceramics based of calcium orthophosphates. Biomaterials, 64, 442-447. doi:10.1007/s10717-007-0109-1
- Kalita S.J., Bhardwaj A., Bhatt H A. (2007): Nanocrystalline calcium phosphate ceramics in biomedical engineering. Materials Science and Engineering: C,, 27, 441-449. doi:10.1016/ j.msec.2006.05.018
- Liong M., Lu J., Kovochich M., Xia T., Ruehm S.G., Nel A.E., Tamanoi F., Zink J.I. (2008): Multifunctional inorganic nanoparticles for imaging targeting and drug delivery. ACS Nano, 2, 889-896. doi:10.1021/nn800072t

- Tenkumo T., Vanegas Sáenz J., Takada Y., Takahashi M., Rotan O., Sokolova V., Epple M., Sasaki K. (2016): Gene transfection of human mesenchymal stem cells with a nanohydroxyapatite-collagen scaffold containing DNA-functionalized calcium phosphate nanoparticles. Molecular Biology Society of Japan, 21, 682-695. doi:10.1111/gtc.12374
- Wu G.J., Zhou L.Z., Wang K.W., Chen F., Sun Y., Duan Y.R., Zhu Y.J., Gu H.C. (2010): Hydroxylapatite nanorods: an efficient and promising carrier for gene transfection. Journal of Colloid and Interface Science, 345, 427-432. doi:10.1016/j. jcis.2010.01.048
- Hou C.H, Hou S.M, Hsueh Y.S, Lin J., Wu H.C., Lin F.H. (2009): The in vivo performance of biomagnetic hydroxy-apatite nanoparticles in cancer hyperthermia therapy. Bio-materials, 30, 3956-3960. doi:10.1016/j.biomaterials.2009.04.020
- Zhang C., Li C., Huang S., Hou Z., Cheng Z., Yang P., Peng P., Lin J. (2010): Self-activated luminescent and mesoporous strontium hydroxyapatite nanorods for drug delivery. Biomaterials, 31, 3374-3383. doi:10.1016/j.biomaterials. 2010.01.044
- Ye F., Guo H., Zhang H., He X. (2010): Polymeric micelletemplated synthesis of hydroxyapatite hollow nanoparticles for a drug delivery system. Acta Biomaterialia, 6, 2212-2218. doi:10.1016/j.actbio.2009.12.014
- Ashokan A., Menon D., Nair S., Koyakutty M. (2010): A molecular receptor targeted, hydroxyapatite nanocrystal based multi-modal contrast agent. Biomaterials, 31, 2606-2616. doi:10.1016/j.biomaterials.2009.11.113
- Yao C., Tong Y. (2012): Lanthanide ion-based luminescent nanomaterials for bioimaging. TrAC Trends in Analytical Chemistry, 39, 60-71. doi:10.1016/j.trac.2012.07.007
- Sun Y., Yang H., Tao D. (2011): Microemulsion process synthesis of lanthanide-doped hydroxyapatite nanoparticles under hydrothermal treatment. Ceramics International, 37, 2917-2920. doi:10.1016/j.ceramint.2011.03.030
- Cannillo V., Esposito L., Rambaldi E., Sola A., Tucci A. (2009): Microstructural and mechanical changes by chemical ageing of glazed ceramic surfaces. Journal of the European Ceramic Society, 29, 1561-1569. doi:10.1016/j.jeurceramsoc. 2008.10.018
- Arellano-Jiménez M.J., García-García R., Reyes-Gasga J. (2009): Synthesis and hydrolysis of octacalcium phosphate and its characterization by electron microscopy and X-ray diffraction. Journal of Physics and Chemistry of Solids, 70, 390-395. doi:10.1016/j.jpcs.2008.11.001
- Wiglusz R.J., Bednarkiewicz A., Strek W. (2011): Synthesis and optical properties of Eu³⁺ ion doped nanocrystalline hydroxyapatites embedded in PMMA matrix. Journal of Rare Earths, 29, 1111-1116. doi:10.1016/S1002-0721(10) 60608-4
- 15. Tanahashi M., Kamiya K., Suzuki T., Nasu H. (1992): Fibrous hydroxyapatite grown in the gel system: effects of pH of the solution on the growth rate and morphology. Journal of Materials Science: Materials in Medicine, 3, 48-53. doi:10.1007/BF00702944
- Wang X., Zhuang J., Peng Q., Li Y. (2006): Liquid-solidsolution synthesis of biomedical hydroxyapatite nanorods. Advanced Materials, 18, 2031-2034. doi:10.1002/adma. 200600033
- 17. Tas A.C. (2001): Molten salt synthesis of calcium hydroxyapatite whiskers. Journal of the American Ceramic Society, 84, 295-300. doi:10.1111/j.1151-2916.2001.tb00653.x
- Wei M., Wang X.X. (2007): Ribbon-like and rod-like hydroxyapatite crystals deposited on titanium surface with electrochemical method. Materials Letters, 64, 4062-4065. doi:10.1016/j.matlet.2007.01.040

- 19. Yoon S.Y., Park Y.M., Par S.S., Stevens R., Park H.C. (2005): Synthesis of hydroxyapatite whiskers by hydrolysis of α -tricalcium phosphate using microwave heating. Materials Chemistry and Physics, 95, 48-53. doi:10.1016/j. matchemphys.2004.10.049
- Neira I.S., Kolenko Y.V., Lebedev O.I., Van-Tendeloo G., Gupta H.S., Guitia F., Yoshimura M. (2009): An effective morphology control of hydroxyapatite crystals via hydrothermal synthesis. Crystal Growth and Design, 9, 466-474. doi:10.1021/cg800738a
- Norton J., Malik K.R., Darr J.A, Rehman I. (2006): Recent developments in processing and surface modification of hydroxyapatite. Advances in Applied Ceramics, 105, 113-139. doi:10.1179/174367606X102278
- 22. Sada-Shojai M., Atai M., Nodehi A. (2011): Design of experiments (DOE) for the optimization of hydrothermal synthesis of hydroxyapatite nanoparticles. Journal of the Brazilian Chemical Society, 22, 571–582. doi:10.1590/S0103-50532011000300023
- Byrappa K., Yoshimura M. (2007): Progress in Crystal Growth and Characterization of Materials. A Technology for Crystal Growth and Materials Processing, 53, 117-166. doi:10.1016/j.pcrysgrow.2007.04.001
- 24. Escudero A., Calvo M.E., Rivera-Fernández A., de la Fuente J.M., Ocaña M. (2013): Microwave-assisted synthesis of biocompatible europium doped calcium-hydroxyapatite and fluoroapatite luminescence nanospindles functionalized with poly (acrylic acid). Langmuir, 29, 1985-1994. doi:10.1021/ la304534f
- Omori M. (2000): Sintering, consolidation, reaction and crystal growth by the spark plasma sintering (SPS). Materials Science and Engineering: A, 287, 183-188. doi:10.1016/ S0921-5093(00)00773-5
- 26. Nygren M., Shen Z.J. (2003): On the preparation of bio-, nanoand structural ceramics and composites by spark plasma sintering. Solid State Sciences, 5, 125-131. doi:10.1016/ S1293-2558(02)00086-9
- 27. Munir Z.A., Anselmi-Tamburini U., Spinolo O. (2006): The effect of electric field and pressure on the synthesis and consolidation of materials: A review of the spark plasma sintering method. Journal of Materials Science, 41, 763-777. doi:10.1007/s10853-006-6555-2
- Cuccu A., Montinaro S., Orrù R., Cao G., Bellucci D., Sola A., Cannillo V. (2015): Consolidation of different hydroxyapatite powders by SPS: Optimization of the sintering conditions and characterization of the obtained bulk products. Ceramics International, 41, 725-736. doi:10.1016/j.ceramint.2014.08.131
- Champion E. (2013): Sintering of calcium phosphate bioceramics. Acta Biomaterialia, 9, 5855-5875. doi:10.1016/j. actbio.2012.11.029
- 30. Cihlar J., Buchal A., Trunec M. (1999): Kinetics of thermal descomposition of hydroxyapatite bioceramics. Journal of Materials Science, 34, 6121–6131. doi:10.1023/ A:1004769820545
- Thomann J.M., Voegel J.C., Gramain Ph. (1990): Kinetics of dissolution of calcium hydroxyapatite powder. III: pH and sample conditioning effects. Calcified Tissue International, 46, 121-129. doi:10.1007/BF02556096
- 32. Slosarczyk A., Paszkiewicz Z., Paluszkiewicz C. (2005): FTIR and XRD evaluation of carbonated hydroxyapatite powders synthesized by wet methods. Journal of Molecular Structure, 744-747, 657-661. doi:10.1016/j.molstruc.2004.11.078

- 33. Sadat-Shojai M., Khorasani M.T., Jamshidi A. (2012): Hydrothermal processing of hydroxyapatite nanoparticles – A Taguchi experimental design approach. Journal of Crystal Growth, 361, 73-84. doi:10.1016/j.jcrysgro.2012.09.010
- 34. Koutsopoulos S. (2002): Synthesis and characterization of hydroxyapatite crystals: a review study on the analytical methods. Journal of Biomedical Materials Research, 62, 600-612. doi:10.1002/jbm.10280
- 35. Kim C.W., Kang H., Heo N., Seff K. (2014): Encapsulating Photoluminescent Materials in Zeolites. Crystal Structure of Fully Dehydrated Zeolite Y (Si/Al = 1.69) Containing Eu³⁺. The Journal of Physical Chemistry C, 118, 11014-11025. doi:10.1021/jp501826x
- 36. Wang L., Nancollas G.H. (2008): Calcium orthophosphates: crystallization and dissolution. Chemical Reviews, 108, 4628-4669. doi:10.1021/cr0782574
- Dorozhkin S.V. (2011): Calcium orthophosphates occurrence, properties, biomineralization, pathological calcification and biomimetic applications. Biomatter, 1, 121-164. doi:10.4161/ biom.18790
- 38. Stewart S., Shea D.A., Tarnowski C.P., Morris M.D., Wang D., Francheschi R., Lin D.L., Keller E. (2002): Trends in early mineralization of murine calvarial osteoblastic cultures: a Raman microscopic study. Journal of Raman Spectroscopy, 33, 536-543. doi:10.1002/jrs.892
- Neira I.S., Guitián F., Taniguchi T., Watanabe T., Yoshimura M. (2008) Hydrothermal synthesis of hydroxyapatite whiskers with sharp faceted hexagonal morphology. Journal of Materials Science, 43, 2171–2178. doi:10.1007/s10853-007-2032-9
- 40. Gaft M., Reisfeld R., Panczer G., Shovala S., Champagnon B., Boulon G. (2005). Modern luminescence spectroscopy of minerals and materials. 2nd ed. Springer.
- Graeve O.A., Kanakala R., Madadi A., Williams B.C., Glass K.C. (2010): Luminescence variations in hydroxyapatites doped with Eu²⁺ and Eu³⁺ ions. Biomaterials, 31, 4259-4267. doi:10.1016/j.biomaterials.2010.02.009
- 42. Wei Z., Sun L., Liao C., Yin J., Jiang X., Yan C. (2002): Sizedependent chromaticity in YBO₃:Eu nanocrystals: correlation with microstructure and site symmetry. The Journal of Physical Chemistry B, 106, 10610-10617. doi: 10.1021/ jp025967z
- 43. Harshani-Perera T.S., Han Y., Lu X., Wang X., Dai H., Li S. (2015): Rare earth doped apatite nanomaterials for biological application. Journal of Nanomaterials, 2015, 1-6. doi:10.1155/2015/705390
- 44. Xuan T.C., Trung N.N., Pham V.H. (2015): Comparative characterization of microstructure and luminescence of europium doped hydroxyapatite nanoparticles via coprecipitation and hydrothermal method. Optik-International Journal for Light and Electron Optics, 126, 5019-5021. Doi:10.1016/j.ijleo.2015.09.136
- 45. Silva F., de Lima N.B., Bressiani A., Courrol L., Gomes L. (2015): Synthesis, characterization and luminescence properties of Eu³⁺-doped hydroxyapatite nanocrystal and the thermal treatment effects. Optical Materials, 47, 135-142. doi:10.1016/j.optmat.2015.07.006
- 46. Long M., Hong F., Li W., Li F., Zhao H., Lv Y., Li H., Hu F., Sun L., Yan C., Wei Z. (2008): Size-dependent microstructure and europium site preference influence fluorescent properties of Eu³⁺-doped Ca₁₀(PO₄)₆(OH)₂ nanocrystal. Journal of Luminescence, 128, 428-436. doi:10.1016/j.jlumin.2007. 09.012