Contents lists available at ScienceDirect







journal homepage: www.elsevier.com/locate/apmt

Defect-related luminescence properties of hydroxyapatite nanobelts

Verónica J. Huerta^a, Paloma Fernández^b, Virginia Gómez^c, Olivia A. Graeve^d, Manuel Herrera^{e,*}

^a Centro de Investigación Científica y de Educación Superior de Ensenada, Carretera Ensenada-Tijuana No. 3918, C.P. 22860, Ensenada, Baja California, México

^b Departamento de Física de Materiales, Universidad Complutense de Madrid, Facultad de Física, C.P. 28040, Madrid, Spain

^c Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria, C.P. 04510, Ciudad de México, México

^d Department of Mechanical and Aerospace Engineering, University of California San Diego, 9500 Gilman Drive - MC 0411, La Jolla, CA 92093-0411, United States

^e Centro de Nanociencias y Nanotecnología, Universidad Nacional Autónoma de México, Km. 107 Carretera Tijuana-Ensenada, C.P. 22800, Ensenada, Baja California, México

ARTICLE INFO

Article history: Received 20 June 2020 Revised 3 September 2020 Accepted 5 September 2020

Keywords: Hydroxyapatite Point defect Luminescence Nanobelt Electron paramagnetic resonance

ABSTRACT

In this work, we study the generation of visible and near-infrared (NIR) luminescence associated with the formation of point defects in hydroxyapatite (HAp) nanobelts. Three different HAp samples were synthesized by a modified hydrothermal method at growth times of 24, 30, and 36 h. Transmission electron microscopy measurements revealed the presence of stacking faults in the nanobelts grown during shorter times. X-ray diffraction and Raman spectroscopy measurements confirmed the high crystalline quality of the samples, showing a hexagonal calcium deficient (Ca-D) HAp phase, supported by the Ca/P ratio of 1.2 and 1.3 revealed by X-ray photoelectron spectroscopy and energy dispersive spectroscopy measurements, respectively. Electron paramagnetic resonance, photoluminescence, and cathodoluminescence measurements demonstrated the presence of hydroxyl ion vacancies (V_{OH}^{-}), calcium vacancies (V_{Ca}), and oxygen vacancies (V_{O}) in HAp nanobelts generates a strong luminescence in the visible and NIR ranges.

© 2020 Elsevier Ltd. All rights reserved.

1. Introduction

Hydroxyapatite (HAp), with chemical formula $Ca_{10}(PO_4)_6(OH)_2$, is a biomaterial with a structure similar to the mineral component found in bones and teeth [1]. Thus, this biocompatible, bioactive, and osteoconductive material has been used for a variety of biomedical applications, including bone replacement [2], drug and gene delivery agent [3,4], bioactive coating on metallic implants [5,6], and dental materials [7]. HAp crystallizes in a monoclinic structure (P2₁/b) when chemically synthesized at low temperatures (\leq 100°C). However, the presence of structural defects, either because of the synthesis process or the incorporation of carbonate ions from the atmosphere, leads to the formation of vacancies within the lattice, with calcium and oxygen being the most common. Because of its high structural flexibility, HAp allows these types of deformations [8]. As a result, it is able to crystallize in a hexagonal structure (P6₃/m) at temperatures as low as 100°C [9-11], which would regularly only occur at high temperatures (200-400°C) [12].

electronic configuration of HAp [13] and, as a consequence, luminescent properties can arise. Pure or stoichiometric HAp shows no luminescence. Therefore, it is doped with rare-earth ions to become a contrast agent for magnetic resonance [14,15], X-ray [16,17], and near-infrared reflection imaging [14,16]. However, the ions used as luminescent centers are expensive to produce and not environmentally friendly [11,18], limiting the application of these materials in the biomedical area.

The formation of vacancy defects produces alterations in the

Since it has been reported that defective, or non-stoichiometric HAp, exhibits strong intrinsic photoluminescence (PL) in the visible spectral range [11–13,19,20], the use of this HAp as a contrast agent, without dopants, represents a nontoxic, eco-friendly alternative. Furthermore, the near-infrared luminescence of this material remains unexplored. In this work, we report the defect-related cathodoluminescence in the visible and near-infrared range of HAp nanobelts, as well as the photoluminescence emission spectra in the visible range for comparison with the cathodoluminescence spectra. Moreover, we confirm the presence of vacancy defects by electron paramagnetic resonance measurements.

* Corresponding author.

E-mail address: zaldivar@cnyn.unam.mx (M. Herrera).

Table 1

Reaction conditions and atomic compositions of HAp samples obtained by hydrothermal synthesis.

Sample	Temperature	Time	Elemental composition* (at. %)			Ca/P atomic ratio
			Ca	Р	0	
S1	100°C	24 h	16.63	12.43	70.93	1.34
S2		30 h	17.62	13.26	69.12	1.33
S3		36 h	15.24	11.61	73.14	1.31

*Measured by energy dispersive spectroscopy (EDS).

2. Experimental

HAp powders were synthesized using a modified hydrothermal method. Calcium nitrate tetrahydrate [Ca(NO₃)₂•4H₂O 99 %, Alfa Aesar, A16645] and di-ammonium phosphate [(NH₄)₂HPO₄ 98 %, Alfa Aesar, 11597] were used as precursors. Deionized water solutions (0.1 M) of Ca(NO₃)₂•4H₂O (Ca solution) and (NH₄)₂HPO₄ (P solution) were prepared by maintaining a Ca/P volume ratio of 1.67. First, the Ca solution was heated under magnetic stirring at a temperature of 100°C. Then, the P solution was added drop by drop into the heated Ca solution. The resultant solution was maintained at 100°C under magnetic stirring and reaction time was varied between 24, 30, and 36 h, to obtain three different samples labeled samples S1, S2, and S3, respectively (Table 1). After the reaction was finished, the solution was cooled to room temperature. Subsequently, the sediment was filtered and washed with deionized water until the aqueous waste reached a pH of 7. Finally, the product was dried at 50°C for one hour under an atmospheric pressure to obtain the final HAp powder.

The crystalline structure of the HAp powders was identified by X-ray diffraction (XRD, Philips, X'pert MPD) with monochromatic CuK α radiation ($\lambda = 1.5405$ Å) and a step size of 0.02° in a 2θ range between 20° and 70° . The vibrational modes of HAp were analyzed by Raman spectroscopy (Horiba Jobin-Yvon, LabRam HR800 micro-Raman system), exciting the samples with a 633 nm He-Ne laser. The elemental composition and Ca/P ratio were determined by energy dispersive spectroscopy (EDS, Oxford Instruments, X-Max spectrometer) with a detector size of 20 mm², attached to a JEOL JIB-4500 SEM. The elemental quantification of samples was calculated by Inca Software (Oxford Instruments) using a standard-based sequence. All measurements were obtained by operating the SEM at 15 keV, electron beam current of 3.1×10^{-8} A, and scanning a sample area of about $1.1 \times 10^4 \ \mu m^2$. To support EDS results, X-ray photoelectron spectroscopy spectra (XPS, SPECS system) were obtained using a PHOIBOS WAL analyzer and an aluminum anode, and subsequently analyzed with the CasaXPS software. The morphology of the samples was observed by field emission scanning electron microscopy (FE-SEM, JEOL, JSM-7800F), and also by transmission electron microscopy (TEM, JEOL, JEM-2010) operated at 15 kV and 200 kV, respectively. The micro-Raman system mentioned above was used to obtain the photoluminescence (PL) spectra of the samples in the range between 300 and 1100 nm using a 325 nm He-Cd laser. Cathodoluminescence (CL) spectra in the visible range (200-900 nm) were obtained using a Gatan MonoCL4 system in the JEOL JIB-4500 SEM. The CL spectra in the near-infrared range (800-2500 nm) were obtained using a nitrogen-cooled Oriel 74100 IR monochromator and a Photocool Series PC176 system equipped with a R5509-73 photomultiplier, both adapted to a Hitachi S2500 SEM. The paramagnetic species present in the HAp samples were identified by electronic paramagnetic resonance (EPR) using a JEOL, JES-TE300 system, acquiring spectra with an absorption frequency of 9.4 GHz at ambient (298 K) and liquid nitrogen (77 K) temperatures, varying the microwave power from 1 to 20 mW. The EPR spectra obtained for the HAp



Fig. 1. XRD patterns of the different synthesized HAp samples.

powders were simulated and fitted using the EasySpin MATLAB toolbox (supplementary information) [21].

3. Results and discussion

Table 1 shows the reaction conditions and atomic compositions of the synthesized HAp samples. The XRD patterns (Fig. 1) exhibit high crystallinity and peaks that correspond to the HAp $P6_3/m$ hexagonal phase, according to PDF card #09-0432. Since the Ca/P ratios displayed in Table 1 are below 1.67, it is inferred that calcium-deficient HAp (Ca-D HAp) phase is also present in the samples. However, it is difficult to identify unequivocally due to the similarities in the diffraction patterns of both phases. Also, small peaks corresponding to the monenite phase (DCP, CaHPO₄) appear in sample S2 (30 h), attributed to the participation of this phase in the formation process of HAp and other apatites [1,3].

Fig. 2 illustrates the Raman spectra obtained for the HAp powders, composed only of signals corresponding to vibrational modes of the hydroxyapatite structure. The PO_4^{3-} vibration frequency at 962 cm⁻¹ (ν_1) corresponds to the symmetric stretching of P–O bonds [22]. The bands at 430 cm⁻¹ (ν_2), and 590 cm⁻¹ (ν_4) are attributed to the O–P–O symmetric and asymmetric bending modes, respectively, while the band found at 1043 cm⁻¹ (ν_3) corresponds to the P–O asymmetric stretching mode [9,23,24]. Furthermore, a small peak at 322 cm⁻¹ is present, attributed to Ca²⁺ ion translational modes [25]. Fig. 2 also shows that the signals are consistent, both in intensity and wavenumber, throughout all the Raman spectra of the S1-S3 samples, indicating that the morphology of the HAp nanobelts in the different samples is homogeneous.

The chemical state of the elements in the samples was determined by XPS. Fig. 3 shows the survey spectrum for sample S1 (24



Fig. 2. Raman spectra of the different studied samples revealing four vibrational modes associated with the HAp structure.



Fig. 3. Survey XPS spectrum for sample S1 (24 h).

h), representing the typical behavior of all samples. The O 1s, Ca 2p, and P 2p signals were identified by their corresponding binding energies of 531, 347, and 133 eV, respectively. Also, the appearance of a carbon signal (C 1s) was attributed to C-C and C-H bonds of carbon impurities. The peak for C 1s at 284.6 eV [26] was used to correct the shift on the rest of the peaks due to the charging of the sample [27]. Likewise, the Auger signals for carbon (C KLL), calcium (Ca LMM), and oxygen (O KLL) were detected at 1223, 1197, and 978 eV, respectively. Figure 4 shows the high-resolution XPS deconvoluted spectra for O 1s, Ca 2p, P 2p, and C 1s signals. Figure 4a shows three components for the O 1s signal corresponding with binding energies of O-H₂ bonds (534.7 eV), O-P and O-H bonds associated with phosphates and hydroxyl groups (532.7 eV), and O-C bonds associated with oxide groups (531.7 eV) [28]. Figure 4b shows three components for the Ca 2p signal, the Ca $2p_{1/2}$ (351.3 eV) and Ca $2p_{3/2}$ (347.8 eV) signals, as well as a component at 347.1 eV attributed to Ca-CO₃ bonds [29,30]. The P 2p signal, shown in Figure 4c, exhibits the two components associated with the spinorbit splitting $2p_{1/2}$ and $2p_{3/2}$. For the C 1s signal, the deconvoluted XPS peak reveals three components (Fig. 4d), C-O₃ bonds

Table 2
XPS quantitative analysis of the different HAp samples.

Sample	Time	Elemen	tal compo	Ca/P atomic ratio	
		0 1s	Ca 2p	P 2p	
S1	24 h	57.82	23.48	18.70	1.25
S2	30 h	57.86	23.69	18.44	1.28
S3	36 h	57.26	24.12	18.62	1.29

associated with carbonates groups (288.6 eV), C-OH bonds (286.1 eV), and C-C bonds associated with aromatic groups (284.6 eV) [28].

The spectra shown in Fig. 4 were used to quantitatively analyze the Ca/P atomic ratio of the surface and compare it with the EDS quantitative analysis. Table 2 shows that all the HAp powders present a lower Ca/P atomic ratio on the surface compared to the values observed by EDS (Table 1), suggesting that the formation of Ca vacancies is higher on the surface than on the bulk of the samples. This effect is probably due to the HAp nanobelts were grown in an aqueous solution. Previously, Astala and Stott reported that Ca loss in exchange for two H on HAp (001) and Ca-rich (010) surfaces is energetically favorable [31], supporting qualitatively Ca desorption as the driving mechanism for dissolution, as proposed by other authors [32]. These authors also suggest that Ca release by the Ca-H exchange should be most influential in HAp samples with large surface-to-volume ratio, such as fine powders.

Fig. 5 illustrates typical SEM images obtained for the HAp S1 (24 h) and S3 (36 h) samples. As exhibited in Fig. 5a, the nanostructures are inhomogeneous in size and shape at a reaction time of 24 h. However, uniform nanobelts with a width of 10-50 nm and a length of 0.5-3 μ m are obtained at 36 h.

TEM images obtained from the HAp S1 (24 h) sample revealed that the synthesized nanobelts are well defined (Fig. 6a-c), narrow in thickness, and porous as observed in the zones delimited by yellow dotted lines in Fig. 6b-c. Furthermore, the HAp nanobelts exposed to relatively long times at the high energy electron beam (200 keV) of the TEM instrument revealed their deformation, resulting in the generation of holes and creases that appear after 60 s of exposure to the beam, as observed in Fig. 6d-e. Fig. 7a shows a typical high-resolution TEM image of sample S2 (30 h), were the crystal lattice and the growth direction of the nanobelts are identified, revealing a distance of 3.1 Å between the atomic rows stacked along the [001] growth direction (Fig. 7b), which is near the theoretical value of 3.4 Å for the stoichiometric HAp. This last value was calculated by simulating the crystal structure using the VESTA software, with a projection on the (100) crystalline plane (Fig. 7c). However, a detailed TEM study on HAp nanobelts revealed the presence of several stacking faults between the hexagonal basal planes (001), as shown in Fig. 7d for the S2 (30 h) sample, which we attribute to the absence of Ca atoms during the HAp crystal growth, associating this phenomenon with the low stoichiometry observed on the HAp nanobelts (Tables 1 and 2).

The EPR technique was employed to identify the presence of different paramagnetic radicals associated with point-defects in HAp nanobelts. Fig. 8 shows the spectra of sample S3 (36 h) obtained at room temperature and varying microwave power, which led us to identify three different paramagnetic species. The species g tensor values were used to simulate the spectrum taken with a microwave power of 10 mW, presented in Fig. 9. The first signal (the central one) is attributed to CO_2^- with an orthorhombic symmetry with $g_x = 2.0029$, $g_y = 2.0020$, and $g_z = 1.9972$ [33–35]. These radicals are a very stable type of defect that appears in carbonate-containing apatites due to CO_2 adsorption from the air [26,34], which is supported by the presence of carbonate, as detected by XPS (Fig. 4), and the high concentration of calcium vacancies (V_{Ca}) on the surface of the nanobelts. The second signal is



Fig. 4. High-resolution XPS spectral deconvolution for a) O 1s, b) Ca 2p c) P 2p, and d) C 1s of sample S1 (24 h).



Fig. 5. SEM images of HAp samples prepared with a reaction time of a) 24 h (sample S1) and b) 36 h (sample S3).

associated with O⁻ radical vacancies (V_O-) that show an isotropic symmetry with g = 1.9989 [36,37]. Finally, the third signal has a g = 2.0039 and is associated with organic radicals [35]. Furthermore, as observed in Fig. 8, the intensity of this signal increases as

the applied power decreases (contrary to the CO_2^- signal), a behavior previously reported in the literature [38]. Table 3 summarizes the paramagnetic species observed by EPR (at 300 K and 77 K) for HAp and compares between the *g* values calculated and the ones reported in the literature.

On the other hand, Fig. 10 presents the low-temperature spectrum (77 K) obtained with a microwave power of 3 mW for sample S3 and its corresponding simulated results using tensor g values for five different paramagnetic signals. Two of these are V₀and CO2⁻ radicals, previously observed in the EPR spectrum obtained at 300 K (Fig. 9). A third broad signal, observed between 310 and 315 mT, corresponds to $^{13}\mathrm{CO}_2{}^-$ radicals since the $^{13}\mathrm{C}$ nucleus has a magnetic moment different from zero and nuclear spin of 1/2 [39,40]. The remaining two signals are only seen at temperatures below 90 K, the first one being attributed to an O_2^- anion adjacent to a V_{Ca} , originated by vacancies of the hydroxyl anions (V_{OH}^{-}) as precursors [41]. This radical has an orthorhombic symmetry with g_{x} = 2.0380, g_{y} = 2.0129, and g_{z} = 2.0040, labeled as $g_{x,y,z}^{O_{2}^{-}}$ in Fig. 10. The second one also presents an orthorhombic symmetry with $g_x =$ 2.0680, $g_y = 2.0579$, and $g_z = 2.0009$, labeled as $g_{x,y,z}^{0^-}$ in Fig. 10, and has been assigned to the formation of an O⁻ ion located on a hydroxyl site [42]. Moreover, Moens et al. have reported that the precursor of this O⁻ radical is a carbonate group, which introduces a hydroxyl vacancy (V_{OH}) in the process [43].

Photoluminescence (PL) and cathodoluminescence (CL) techniques were used to determine the luminescence properties of the HAp samples and corroborate the presence of point defects identified by EPR. To date, most of the PL spectra reported for hydroxyapatite nanostructures synthesized via the hydrothermal method, consist of a single broad emission band that has its maximum at 2.95 eV, which varies slightly depending on the pH at which the synthesis is carried out [19]. This band centered at 2.95 eV has



Fig. 6. a) Panoramic and b-c) single nanobelt TEM images of sample S1 (24 h), which show the porous nature of the HAp nanobelts. TEM images of the sample S2 (30 h) acquired after exposure to the electron beam for d) 10 s and e) 60 s, showing the formation of holes and creases in the latter.

Table 3

Comparison between the g values calculated and the ones reported in the literature for the paramagnetic species found in HAp by EPR.

Paramagnetic specie CO2 ⁻	EPR spectra temperature 77 K and 300 K	g values (calculated) $g_x = 2.0029$	g values (literature) $g_x = 2.0030$	Reference [34,35]
2		$g_y = 2.0020$	$g_y = 2.0019$	
		$g_z = 1.9972$	$g_z = 1.9974$	
Vo ⁻	77 K and 300 K	g = 1.9989	g = 1.999	[36,37]
Organic radical	300 K	g = 2.0039	g = 2.0040	[35]
$O_2^ V_{Ga}$	77 K	$g_x = 2.0380$	$g_x = 2.0380$	[41]
		$g_y = 2.0129$	$g_y = 2.0130$	
		$g_z = 2.0040$	$g_z = 2.0040$	
0-	77 K	$g_x = 2.0680$	$g_x = 2.0680$	[42,43]
		$g_y = 2.0579$	$g_y = 2.0579$	
		$g_z = 2.0009$	$g_z = 2.0009$	



Fig. 7. a) High-resolution TEM image of sample S2 (30 h), b) inset of the upper left corner of the TEM image, c) illustration of the HAp structure modeled with Vesta software and projected on the [010] - [001] crystalline plane, and d) HRTEM image revealing stacking faults of the (001) planes for nanobelts of the S2 (30 h) sample.



Fig. 8. EPR spectra of S3 (36 h) sample obtained at various microwave power values.



Fig. 9. a) EPR experimental spectrum taken at 300 K (10 mW) and b) corresponding simulated spectrum for the S3 (36 h) sample.

been attributed to hydroxyl ion vacancies (V_{OH^-}) [44], given that the intensity of the signal increases as the concentration of V_{OH^-} increases when applying a heat treatment to HAp. However, as seen in Fig. 11, the PL spectra obtained for our samples revealed that the luminescence of the HAp nanobelts is composed of more



Fig. 10. a) EPR experimental spectrum from the S3 (36 h) sample acquired at 77 K and 3 mW, and b) corresponding EPR simulation showing the anisotropic g values associated with O_2^- and O^- ions.

than one emission band, showing that both the number of components and their relative intensities remain constant regardless of the reaction time used to synthesize the different samples. The components were calculated by the deconvolution of the PL spectra using Gaussian curves centered at 2.10, 2.40, 2.57, and 2.95



Fig. 11. PL spectra in the visible range of a) S1 (24 h), b) S2 (30 h), and c) S3 (36 h) samples.



Fig. 12. CL spectra in the visible range of a) S1 (24 h), b) S2 (30 h), and c) S3 (36 h) samples.

eV, to match with the energies of the defect-related emissions reported by other authors. The Full Width at Half Maximum (FWHM) of these components was adjusted to obtain a fitting with a value for the coefficient of determination R² nearest to unity. The values obtained for this parameter were 0.9954, 0.9952, and 0.993 after fitting the PL spectra of S1, S2, and S3 samples, respectively, by assigning an FWHM of 0.4 eV to each Gaussian component. It is essential to consider that the FWHM usually represents the homogeneous and inhomogeneous broadening of the linewidth of an electronic transition simultaneously. The homogeneous or natural broadening (represented with a Lorentzian curve) is generated by electron-phonon coupling in perfect crystals. In contrast, the inhomogeneous broadening (commonly represented with a Gaussian curve) is generated by crystallinity disorder associated with impurities, anti-site defects, or vacancies [45,46]. In this work, we have assigned the same FWHM value of 0.4 eV for all defect-related emissions in HAp, attributing that each of them corresponds to individual electronic transitions (see Fig. 14) with a comparable inhomogeneous broadening. Moreover, we propose that local field effects do not contribute significatively on the broadening of the electronic transitions between the different defect-related energy levels, because following the theory reported by Klingshirn [46], such effects only are significative on solids with a high density of oscillators, such as optical phonons, plasmons, or excitons. Considering that electron-hole pairs also are represented as oscillators to evaluate the optical response of solids [47], and since the HAp is a dielectric material [48], we expect the generation of a low density of them in the HAp samples. Recently, Soares da Silva *et al.* reported practically the same FWHM value (~ 0.5 eV) for three defect-related components centered at 2.15, 2.44, and 2.75 eV, calculated by the deconvolution of PL measurements from HAp nanorods [49]. Moreover, the FWHM value of 0.4 eV assigned in this work is in range with the FWHM values (between 0.15 and 0.43 eV) reported by Machado *et al.* for five defect-related components calculated by the deconvolution of PL spectra from HAp nanoparticles [13]. Besides, these authors reported the HAp components centered at 2.40 and 2.57 eV shown in Fig. 11, associating their origin with defects located in the bulk of the material such as calcium vacancies (V_{Ca}), interstitial defects, and distortions in the phosphate groups.

Fig. 12 shows the CL spectra obtained for the HAp samples. The deconvolution of the CL spectra was calculated using Gaussian curves centered at 1.83, 2.15, 2.57, 2.95, 3.32, 3.67, and 4.05 eV, with a single FWHM value of 0.4 eV. The coefficient of determination R^2 values obtained for these calculations ranged between 0.9953 and 0.9967. It was observed that the bands centered at 2.15, 2.57, and 2.95 eV in the CL spectra correspond to those found by PL. However, the relative intensity of these CL bands shows variations regardless of the growth times, as shown in Fig. 12a-c. This effect reveals that the point defect densities, associated with the bands shown in the CL spectra, are different across all samples due to the absence of control in their generation. Fig. 12 also shows that the component of 2.95 eV exhibits the higher intensity in the CL spectra, revealing that V_{OH^-} are found in greater concentration



Fig. 13. CL spectra in the NIR range of the (a) S1 (24 h), (b) S2 (30 h), and S3 (36 h) samples revealing an emission of 0.81 eV partially resolved due to the limited sensibility of the detector.

within the bulk of the samples, where the CL signal is generated. Since the O_2^- and O^- ions detected by EPR (Fig. 10) are related to the generation of V_{OH}^{-} , there could be a significant amount of these vacancies in the nanobelts, explaining the high intensity of the 2.95 eV component assigned to V_{OH}⁻. Moreover, the component centered at 3.67 eV has been reported by Bystrov et al.[44] and also assigned to V_{OH^-} defects, which suggests that the components identified in both PL and CL emission curves can be associated with more than one type of defect [13]. On the other hand, since the excitation source for PL is a UV laser and for CL is an electron beam, it can be established that PL is a superficial technique in comparison with CL. According to this argument, it is proposed that the type of defect responsible for the emission of 2.40 eV (which only appears in the PL emission curve) is found in a much higher concentration on the surface than in the bulk of the HAp samples. Taking into account the detection of V_{Ca} by EPR (Fig. 10), as well as the EDS and XPS results, we suggest that this component could be related to surface V_{Ca}, since the Ca/P ratio was lower on the surface (Ca/P \approx 1.2) than in the bulk of the samples $(Ca/P \approx 1.3).$

Fig. 13 exhibits the CL spectra of the HAp samples obtained in the near-infrared (NIR) range, revealing an emission centered at 0.81 eV partially resolved due to the limited sensibility of the detector. Fig. 13 also shows that the sample S2 (30 h) recorded the lower intensity for this emission, which is possibly associated with its higher calcium concentration (Table 1), and consequently with a lower concentration of V_{Ca} in S2 (30 h) sample than expected for S1 (24 h) and S3 (36 h) samples. On the other hand, Machado et al. recently proposed that shallow levels near the HAp conduction band are created mainly by V_{Ca} defects [13]. Besides, Sun *et al.* have reported that V_{Ca}^{2+} is the most stable defect in the HAp lattice [50]. We expect the formation of V_{Ca} defects in our HAp samples with a concentration that depends on the intensity of the 0.81 eV emission, and we propose that this defect generates energy levels at 0.81 eV under the conduction band (CB). As a result of the PL and CL recorded emissions, and considering a HAp band gap energy (E_g) of 5.5 eV [13,44,51–53], with several reports about the energy levels associated with vacancy defects, we propose the electronic structure scheme shown in Fig. 14. The donor-like level proposed for V_{OH} corresponds with the energy reported for the charge transition (+/0) of V_{OH} at 3.5 eV above the valence band (VB) [54], with the level around 1 eV under the CB for V_{OH}^{-} [51], and with the level about 2 eV under the CB for V_{OH} [55]. The level proposed for the complex defect composed of and OH vacancy next to and H interstitial $(V_{OH} + H_i)$ is the most stable defect between the different oxygen vacancy configurations in HAp when it is double-positive charged, exhibiting an energy level at about 0.95 eV under the CB, as reported by Bystrov et al. [53]. The level for V₀ in phosphate ions has been calculated theoretically with an energy of 1.70 eV above the VB [53], which we proposed exhibits an energy around 2.10 eV above the BV (Fig. 14). Finally, the level for V_0 in hydroxyl ions corresponds with the energy value of 0.71 eV above the VB, as reported by Bystrov et al. [53]. Both V_O levels proposed are mainly supported by the presence of



Valence band

Fig. 14. Schematic representation of the proposed electronic structure for HAp nanobelts.

this type of defect in the HAp nanobelts, as determined by EPR (Fig. 9 and Fig. 10). Table S3 (supplementary information) summarizes the relationship between the point-defects detected as paramagnetic radical by EPR and as emissions detected by PL and CL techniques.

4. Conclusions

Hydroxyapatite (HAp) nanobelts were synthesized by a modified hydrothermal method varying the growth time to evaluate the formation of several crystal defects. Three samples were synthesized at growth times of 24, 30, and 36 h (samples S1-S3). XRD measurements revealed the formation of the hexagonal Ca-D HAp phase in samples, with a residual monenite phase observed only in sample S2. Raman spectroscopy revealed four vibrational modes associated with the HAp structure in all samples, corresponding to the symmetric stretching of P-O bonds (962 cm⁻¹), symmetric and asymmetric bending of O-P-O bonds (430 and 590 cm⁻¹, respectively), asymmetric stretching of P-O bonds, and Ca²⁺ ion translation (322 cm⁻¹). Quantification of the HAp nanobelt composition revealed calcium deficiency. EDS and XPS measurements showed Ca/P ratios of around 1.3 and 1.2, respectively. SEM images revealed that homogeneity on the aspect ratio of the HAp nanobelts was achieved for a growth time of 36 h, while TEM images showed the presence of stacking faults between the (001) planes of the HAp nanobelts synthesized at a growth time of 30 h (S2). The optical properties of the samples studied by PL and CL techniques revealed the presence of several emissions in the visible and NIR range, attributed to the presence of hydroxyl ion vacancies (V_{OH}⁻), calcium vacancies (V_{Ca}), and oxygen vacancies (V₀) in phosphate and hydroxyl ions. Finally, EPR measurements confirmed the presence of oxygen and calcium vacancies in the HAp nanobelts, revealing signals generated by spin $\frac{1}{2}$ systems that correspond with CO_2^- and $V_{\rm O}{}^-$ radicals, $O_2{}^-$ anions adjacent to $V_{Ca},$ and O^- anions.

Credit Author Statement

1. Verónica J. Huerta, Data analysis and interpretation. Drafting the article

- 2. Paloma Fernández, Critical revision of the article
- 3. Virginia Gómez, Data collection
- 4. Olivia A. Graeve, Critical revision of the article

5. Manuel Herrera¹, Data analysis and interpretation. Critical revision of the article. Final approval of the version to be published

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by grants from Conacyt (No. 284667), PAPIIT-UNAM (No. IN101917), and the National Science Foundation (No. 1911372). VH thanks Conacyt for a research grant (No. 692540). The technical support of E. Aparicio, F. Ruiz, and D. Domínguez is greatly appreciated. M. I. Pérez Montfort and A. Cortes corrected the English version of the manuscript. The authors are also grateful to the Spanish Ministry of Science, Innovation and Universities for support via the project MINECO/FEDER-MAT2015-65274-R and University Complutense via project UCM-Santander 2019 (PR87/19-22613).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.apmt.2020.100822.

References

- [1] M. Sadat-Shojai, M.T. Khorasani, E. Dinpanah-Khoshdargi, A. Jamshidi, Synthesis methods for nanosized hydroxyapatite with diverse structures, Acta Biomater 9 (2013) 7591-7621, doi:10.1016/j.actbio.2013.04.012.
- M. Vallet-Regí, J.M. González-Calbet, Calcium phosphates as substitution of bone tissues, Prog. Solid State Chem. 32 (2004) 1–31, doi:10.1016/j. progsolidstchem.2004.07.001.
- [3] V. Uskoković, D.P. Uskoković, Nanosized hydroxyapatite and other calcium phosphates: Chemistry of formation and application as drug and gene delivery agents, J, Biomed. Mater. Res. - Part B Appl. Biomater. 96 B (2011) 152-191, doi:10.1002/ibm.b.31746.
- [4] L.A. Zavala-Sanchez, G.A. Hirata, E. Novitskaya, K. Karandikar, M. Herrera, O.A. Graeve, Distribution of Eu2+ and Eu3+ lons in Hydroxyapatite: A Cathodoluminescence and Raman Study, ACS Biomater. Sci. Eng. 1 (2015) 1306-1313. doi:10.1021/acsbiomaterials.5b00378.
- M.H. Fathi, F. Azam, Novel hydroxyapatite/tantalum surface coating for metallic [5] dental implant, Mater, Lett. 61 (2007) 1238–1241, doi:10.1016/j.matlet.2006.07
- [6] C. Auclair-Daigle, M.N. Bureau, J.G. Legoux, L. Yahia, Bioactive hydroxyapatite coatings on polymer composites for orthopedic implants, J, Biomed. Mater. Res. - Part A. 73 (2005) 398–408, doi:10.1002/jbm.a.30284.
- S. Mistry, D. Kundu, S. Datta, D. Basu, Comparison of bioactive glass coated and hydroxyapatite coated titanium dental implants in the human jaw bone, Aust. Dent. J. 56 (2011) 68-75, doi:10.1111/j.1834-7819.2010.01305.x
- V. Uskoković, The role of hydroxyl channel in defining selected physicochemical peculiarities exhibited by hydroxyapatite, RSC Adv. 5 (2015) 36614-36633, doi:10.1039/C4RA17180B
- I.S. Neira, Y.V. Kolen'ko, O.I. Lebedev, G. Van Tendeloo, H.S. Gupta, F. Guitián, [9] M. Yoshimura, An effective morphology control of hydroxyapatite crystals via hydrothermal synthesis, Cryst. Growth Des. 9 (2009) 466-474, doi:10.1021/ cg800738a
- [10] L. An, W. Li, Y. Xu, D. Zeng, Y. Cheng, G. Wang, Controlled additivefree hydrothermal synthesis and characterization of uniform hydroxyapatite nanobelts, Ceram. Int. 42 (2016) 3104-3112, doi:10.1016/j.ceramint.2015.10.099.
- [11] T.R. Machado, J.C. Sczancoski, H. Beltrán-Mir, I.C. Nogueira, M.S. Li, J. Andrés, E. Cordoncillo, E. Longo, A novel approach to obtain highly intense self-activated photoluminescence emissions in hydroxyapatite nanoparticles, J, Solid State Chem 249 (2017) 64-69, doi:10.1016/j.jssc.2016.12.018.
- [12] G. Gonzalez, C. Costa-Vera, L.J. Borrero, D. Soto, L. Lozada, J.I. Chango, J.C. Diaz, L. Lascano, Effect of carbonates on hydroxyapatite self-activated photoluminescence response, J, Lumin 195 (2018) 385-395, doi:10.1016/j.jlumin.2017.11.058.
- [13] T.R. Machado, J.C. Sczancoski, H. Beltrán-Mir, M.S. Li, J. Andrés, E. Cordoncillo, E. Leite, E. Longo, Structural properties and self-activated photoluminescence emissions in hydroxyapatite with distinct particle shapes, Ceram. Int. 44 (2018) 236-245, doi:10.1016/j.ceramint.2017.09.164
- [14] F. Chen, P. Huang, Y.J. Zhu, J. Wu, D.X. Cui, Multifunctional Eu 3+/Gd 3+ dualdoped calcium phosphate vesicle-like nanospheres for sustained drug release and imaging, Biomaterials 33 (2012) 6447-6455, doi:10.1016/j.biomaterials. 2012.05.059
- [15] P. Warncke, A. Tesch, F.A. Müller, D. Fischer, J.R. Reichenbach, K.-H. Herrmann, C. Wenisch, Luminomagnetic Eu3+- and Dy3+-doped hydroxyapatite for multimodal imaging, Mater. Sci. Eng. C. 81 (2017) 422-431, doi:10.1016/j.msec. 2017.08.032

- [16] A. Ashokan, D. Menon, S. Nair, M. Koyakutty, A molecular receptor targeted, hydroxyapatite nanocrystal based multi-modal contrast agent, Biomaterials 31 (2010) 2606–2616, doi:10.1016/j.biomaterials.2009.11.113.
- [17] M. K, S. K., T S, M.Sanjeed T, Silver and Gadolinium Ions Co-substituted Hydroxyapatite Nanoparticles as Bimodal Contrast Agent for Medical Imaging, Bioceram. Dev. Appl. (2014) 04, doi:10.4172/2090-5025.1000079.
- [18] C. Zhang, J. Lin, Defect-related luminescent materials: Synthesis, emission properties and applications, Chem. Soc. Rev. 41 (2012) 7938–7961, doi:10.1039/ c2cs35215j.
- [19] C. Zhang, J. Yang, Z. Quan, P. Yang, C. Li, Z. Hou, J. Lin, Hydroxyapatite nanoand microcrystals with multiform morphologies: Controllable synthesis and luminescence properties, Cryst. Growth Des. 9 (2009) 2725–2733, doi:10.1021/ cg801353n.
- [20] G. Kumar, E. Girija, A. Thamizhavel, Synthesis and Photoluminescence Study of Flower-like Hydroxyapatite Nanostructure for Bioprobe Applications, AIP Conf. Proc. 1512 (2013) 250–251, doi:10.1063/1.4914792.
- [21] S. Stoll, A. Schweiger, EasySpin, a comprehensive software package for spectral simulation and analysis in EPR, J, Magn. Reson. 178 (2006) 42–55, doi:10.1016/ j.jmr.2005.08.013.
- [22] J.A. Darr, Z.X. Guo, V. Raman, M. Bououdina, I.U. Rehman, Metal organic chemical vapour deposition (MOCVD) of bone mineral like carbonated hydroxyapatite coatings, Chem. Commun. 10 (2004) 696–697, doi:10.1039/b312855p.
- [23] P.N. De Aza, F. Guitián, C. Santos, S. De Aza, R. Cuscó, L. Artús, Vibrational Properties of Calcium Phosphate Compounds. 2. Comparison between Hydroxyapatite and β-Tricalcium Phosphate, Chem. Mater. 9 (1997) 916–922, doi:10.1021/cm9604266.
- [24] D. Yamini, G. Devanand Venkatasubbu, J. Kumar, V. Ramakrishnan, Raman scattering studies on PEG functionalized hydroxyapatite nanoparticles, Spectrochim. Acta - Part A Mol. Biomol. Spectrosc. 117 (2014) 299–303, doi:10.1016/ j.saa.2013.07.064.
- [25] R. Cuscó, F. Guitián, A. De Aza, L. Artús, Differentiation between hydroxyapatite and β -tricalcium phosphate by means of μ -Raman spectroscopy, J, Eur. Ceram. Soc. 18 (1998) 1301–1305, doi:10.1016/s0955-2219(98)00057-0.
- [26] H.B. Lu, C.T. Campbell, D.J. Graham, B.D. Ratner, Surface characterization of hydroxyapatite and related calcium phosphates by XPS and TOF-SIMS, Anal. Chem. 72 (2000) 2886–2894, doi:10.1021/ac990812h.
- [27] G.N. Raikar, J.L. Ong, L.C. Lucas, Hydroxyapatite Characterized by XPS, Surf. Sci. Spectra. 4 (1996) 9–13, doi:10.1116/1.1247808.
- [28] S. Kačiulis, G. Mattogno, L. Pandolfi, M. Cavalli, G. Gnappi, A. Montenero, XPS study of apatite-based coatings prepared by sol-gel technique, Appl. Surf. Sci. 151 (1999) 1–5, doi:10.1016/S0169-4332(99)00267-6.
- [29] R.J. Chung, M.F. Hsieh, R.N. Panda, T.S. Chin, Hydroxyapatite layers deposited from aqueous solutions on hydrophilic silicon substrate, Surf. Coatings Technol. 165 (2003) 194–200, doi:10.1016/S0257-8972(02)00731-4.
- [30] H.Y. Shin, J.Y. Jung, S.W. Kim, W.K. Lee, XPS analysis on chemical properties of calcium phosphate thin films and osteoblastic HOS cell responses, J, Ind. Eng. Chem. 12 (2006) 476–483.
- [31] R. Astala, M.J. Stott, First-principles study of hydroxyapatite surfaces and water adsorption, Phys. Rev. B - Condens. Matter Mater. Phys. 78 (2008) 1–11, doi:10. 1103/PhysRevB.78.075427.
- [32] I.S. Harding, N. Rashid, K.A. Hing, Surface charge and the effect of excess calcium ions on the hydroxyapatite surface, Biomaterials 26 (2005) 6818–6826, doi:10.1016/j.biomaterials.2005.04.060.
- [33] G. Bacquet, V.Q. Truong, M. Vignoles, J.C. Trombe, G. Bonel, ESR of CO2- in Xirradiated tooth enamel and A-type carbonated apatite, Calcif. Tissue Int. 33 (1981) 105–109, doi:10.1007/BF02409421.
- [34] G.C. Vanhaelewyn, R. a Morent, F.J. Callens, P.F. Mathys, Xand Q-band electron paramagnetic resonance of CO2- in hydroxyapatite single crystals, Radiat. Res. 154 (2000) 467–472 doi:10.1667/0033-7587(2000)154{[]0467:XAQBEP]2.0.CO;2.
- [35] Z. Kabacińska, L. Yate, M. Wencka, R. Krzyminiewski, K. Tadyszak, E. Coy, Nanoscale Effects of Radiation (UV, X-ray, and γ) on Calcite Surfaces: Implications for its Mechanical and Physico-Chemical Properties, J, Phys. Chem. C. 121 (2017) 13357–13369, doi:10.1021/acs.jpcc.7b03581.

- [36] M.A. Fanovich, M.S. Castro, J.M. Porto López, Structural analysis of modified hydroxyapatite powders, Mater. Res. Bull. 36 (2001) 487–496, doi:10.1016/ S0025-5408(01)00526-8.
- [37] I.P. Vorona, V.V. Nosenko, N.P. Baran, S.S. Ishchenko, S.V. Lemishko, I.V. Zatovsky, N.Y. Strutynska, EPR study of radiation-induced defects in carbonatecontaining hydroxyapatite annealed at high temperature, Radiat. Meas. 87 (2016) 49–55, doi:10.1016/j.radmeas.2016.02.020.
- [38] H.K. Kundu, H. Sato, A. Ganas, M. Ikeya, ESR studies on calcite encrustation on Fili neotectonic fault, Greece, Appl. Magn. Reson. 29 (2005) 185–194, doi:10. 1007/BF03167006.
- [39] S.S. Ishchenko, I.P. Vorona, S.M. Okulov, N.P. Baran, 13C hyperfine interactions of CO2- in irradiated tooth enamel as studied by EPR, Appl. Radiat. Isot. 56 (2002) 815–819, doi:10.1016/S0969-8043(02)00049-0.
- [40] V.V Rudko, N.P. Baran, I.P. Vorona, S.S. Ishchenko, S.M. Okulov, L.S. Chumakova, Structure and properties of CO 2 – centers in biological and synthetic hydroxyapatite, IOP Conf. Ser. Mater. Sci. Eng. 15 (2010) 012032, doi:10.1088/ 1757-899x/15/1/012032.
- [41] P. Sharrock, G. Bonel, Colour centres in plasma-sprayed hydroxyapatite, Biomaterials 13 (1992) 755-758, doi:10.1016/0142-9612(92)90013-E.
- [42] M. Mengeot, R.H. Bartram, O.R. Gilliam, Paramagnetic holelike defect in irradiated calcium hydroxyapatite single crystals, Phys. Rev. B - Condens. Matter Mater. Phys. 11 (1975) 4110–4124.
- [43] P. Moens, F. Callens, S. Van Doorslaer, P. Matthys, ENDOR study of an ion observed in x-ray-irradiated carbonated hydroxyapatite powders, Phys. Rev. B -Condens. Matter Mater. Phys. 53 (1996) 5190–5197, doi:10.1103/PhysRevB.53. 5190.
- [44] V.S. Bystrov, C. Piccirillo, D.M. Tobaldi, P.M.L. Castro, J. Coutinho, S. Kopyl, R.C. Pullar, Oxygen vacancies, the optical band gap (Eg) and photocatalysis of hydroxyapatite: Comparing modelling with measured data, Appl. Catal. B Environ. 196 (2016) 100–107, doi:10.1016/j.apcatb.2016.05.014.
- [45] B. Henderson, Spectroscopic effects of disorder in laser materials, Contemp. Phys. 43 (2002) 273–300, doi:10.1080/00107510110120812.
- [46] C.F. Klingshirn, Semiconductor optics, Fourth, Springer-Verlag Berlin Heidelberg, Germany, 2012, doi:10.1007/978-3-642-28362-8.
- [47] V. Chernyak, S. Mukamel, Size-consistent quasiparticle representation of nonlinear optical susceptibilities in many-electron systems, J, Chem. Phys. 104 (1996) 444–459, doi:10.1063/1.470843.
- [48] S.A.M. Tofail, D. Haverty, K.T. Stanton, J.B. McMonagle, Structural order and dielectric behaviour of hydroxyapatite, Ferroelectrics 319 (2005) 117–123, doi:10. 1080/00150190590965523.
- [49] J. Soares Da Silva, T.R. MacHado, T.A. Martins, M. Assis, C.C. Foggi, N.G. MacEdo, H. Beltrán-Mir, E. Cordoncillo, J. Andrés, E. Longo, α-AgVO 3 Decorated by Hydroxyapatite (Ca 10 (PO 4) 6 (OH) 2): Tuning Its Photoluminescence Emissions and Bactericidal Activity, Inorg. Chem. 58 (2019) 5900–5913, doi:10.1021/acs. inorgchem.9b00249.
- [50] J.P. Sun, Y. Song, G.W. Wen, Y. Wang, R. Yang, Softening of hydroxyapatite by vacancies: A first principles investigation, Mater. Sci. Eng. C. 33 (2013) 1109– 1115, doi:10.1016/j.msec.2012.12.001.
- [51] K. Matsunaga, A. Kuwabara, First-principles study of vacancy formation in hydroxyapatite, Phys. Rev. B - Condens. Matter Mater. Phys. 75 (2007), doi:10. 1103/PhysRevB.75.014102.
- [52] J.B.R. Trigos, Y. Jiménez-Flores, V. Suárez, M. Suárez-Quezada, U. Nogal, Sol-Gel Synthesis of Calcium-Deficient Hydroxyapatite: Influence of the pH Behavior during Synthesis on the Structural, Chemical Composition and Physical Properties, Powder Technol (2018), doi:10.5772/intechopen.76531.
- [53] V.S. Bystrov, L.A. Avakyan, E.V. Paramonova, J. Coutinho, Sub-Band Gap Absorption Mechanisms Involving Oxygen Vacancies in Hydroxyapatite, J, Phys. Chem. C. 123 (2019) 4856–4865, doi:10.1021/acs.jpcc.8b11350.
- [54] L.A. Avakyan, E.V Paramonova, J. Coutinho, S. Öberg, V.S. Bystrov, L.A. Bugaev, Optoelectronics and defect levels in hydroxyapatite by first-principles, J, Chem. Phys. 148 (2018) 154706, doi:10.1063/1.5025329.
- [55] V.S. Bystrov, J. Coutinho, A.V. Bystrova, Y.D. Dekhtyar, R.C. Pullar, A. Poronin, E. Palcevskis, A. Dindune, B. Alkan, C. Durucan, E.V. Paramonova, Computational study of hydroxyapatite structures, properties and defects, J, Phys. D. Appl. Phys. 48 (2015), doi:10.1088/0022-3727/48/19/195302.