

Paramagnetic Iron-Doped Hydroxyapatite Nanoparticles with Improved Metal Sorption Properties. A Bioorganic Substrates-Mediated Synthesis

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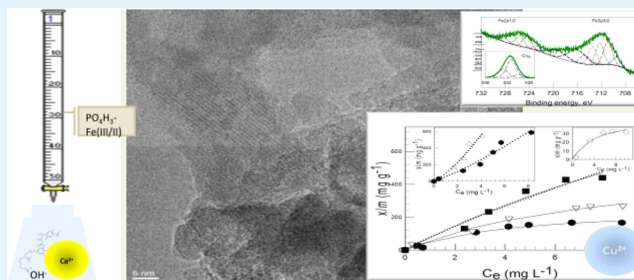
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Supporting Information

ABSTRACT: This paper describes the synthesis of paramagnetic iron-containing hydroxyapatite nanoparticles and their increased Cu^{2+} sorbent capacity when using Ca^{2+} complexes of soluble bioorganic substrates from urban wastes as synthesis precursors. A thorough characterization of the particles by TEM, XRD, FTIR spectroscopy, specific surface area, TGA, XPS, and DLS indicates that loss of crystallinity, a higher specific area, an increased surface oxygen content, and formation of surface iron phases strongly enhance Cu^{2+} adsorption capacity of hydroxyapatite-based materials. However, the major effect of the surface and morphological modifications is the size diminution of the aggregates formed in aqueous solutions leading to an increased effective surface available for Cu^{2+} adsorption. Maximum sorption values of 550–850 mg Cu^{2+} per gram of particles suspended in an aqueous solution at pH 7 were determined, almost 10 times the maximum values observed for hydroxyapatite nanoparticles suspensions under the same conditions.

KEYWORDS: magnetic nanomaterials, Cu^{2+} sorption, effective surface, bioorganic substrates, surface chemistry



INTRODUCTION

Hydroxyapatite (Ap), $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, the main component of the bone tissue of vertebrates, is one of the most promising sorbent materials for removing actinides and heavy metals from wastewaters and soils. Apatites of the most different origins, such as cow bone charcoal,¹ nano-Ap (nAp),² waste oyster shells,³ and bacteria,⁴ were used for the removal of heavy metals. Ap is an ideal material for long-term sequestration in aqueous reservoirs because of its chemical stability across a wide range of geological conditions,⁵ high biocompatibility, strong ability to fix actinides and heavy metals,⁶ and maximum metal ion sorption capacity at pH 6–7.²

Humates are also widely applied to clean up toxic waste sites. Formation of humates is based on the ability of carboxyl and hydroxyl groups of humic and/or fulvic acids to bind metal cations. In particular, Ca^{2+} are effective precipitators of humates and provide a route for the transfer of trace metals from the solution to the solid phase.⁷ Moreover, formation of stable, water-soluble phosphate- Ca^{2+} -humic complexes⁸ were reported. Urban biowastes (UBW) have been reported to contain soluble humic-like substances known as bioorganic substances (SBO)

which show promise as chemical auxiliaries for a number of technological applications in the chemical industry and in environmental remediation.⁹ UBW-SBO are mixtures of substances with molecular weights in the range $(1-3) \times 10^5$ Da, formed by long aliphatic C chains substituted by aromatic rings and COOH, NCO, C=O, PhOH, O-alkyl, O-aryl, OCO, OMe, and alkyl amines functional groups. Therefore, the use of UBW-SBO as templates in the synthesis of Ap materials may be expected to affect Ap morphology and surface chemistry, as observed for organic matrix-mediated fabrication of nanostructured hydroxyapatite biocomposites.¹⁰

Nanomaterials showing high specific surface area and tunable surface chemistry were suggested to offer significant improvement as metal adsorbents.¹¹ In particular, magnetite-incorporated hydroxyapatite composites attract much attention as a magnetic functional material for developing adsorbents and catalysts since they could be easily removed by application of a

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magnetic field.¹² However, to date, the metal adsorption capacity of these materials was not assessed.

In this context, in the present communication we describe the synthesis of Ap-based magnetic nanoparticles and their increased metal sorption capacity when synthesized using Ca^{2+} complexes of UBW-SBO as synthesis precursors.

■ EXPERIMENTAL SECTION

Reactants. Calcium hydroxide 95 wt %, phosphoric acid 85%, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ > 99 wt %, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 97 wt %, were supplied by Sigma Aldrich and used without further purification. Deionized water was Milli-Q purified (18.2 M Ω cm and 6 ppb TOC). When required, pH was stabilized to 7.0 with 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid, sodium salt (HEPES-Na), and nitric acid.

The bioorganic substances herein used were available from a pilot plant from Studio Chiono & Associati in Rivarolo Canavese, Italy. The fraction of soluble biorganic substances used, namely CVT230, was extracted from home gardening and park trimming residues aerated for 230 days for aerobic microbiological treatment; the resulting biomass was digested with an alkali solution at 65 °C for several hours, and the remaining supernatant was filtered by an UF membrane operating with tangential flow to yield a retentate with 5–10% dry matter content. The concentrated retentate (CVT230) was finally dried at 60 °C. CVT230 was 72.1% solid residue and composed of 38.25 ± 0.09 % w/w C and 4.01 ± 0.03 % w/w N. Among the important mineral residues are the following: 256 ppm Zn, 202 ppm Cu, 92 ppm Ni, 85 ppm Pb, 19 ppm Cr, and minor amounts of Si, Fe, and Ca. CVT230 carbon fractions are as follows: 37% aliphatic, 20% aromatic, 14% alkoxy, 12% carboxylic, and 7% are in an α position to amines.¹³ SBO were reported to be nontoxic to *Vibrio fischeri*, activated sludge bacteria, *Daphnia magna*, and *Pseudokirchneriella subcapitata*. Their reported biological to chemical oxygen demand ratio, BOD:COD < 0.1, indicates an extremely low biodegradability.

Addition of excess Ca^{2+} to SBO aqueous solutions of pH 11 leads to the formation of insoluble SBO- Ca^{2+} products which became solubilized on decreasing the pH to 5. The observed behavior resembles that of humic and fulvic acids complexation with Ca^{2+} ions.¹⁴

Synthesis of Hydroxyapatite Magnetic Nanoparticles. A modification of literature methods was used for the synthesis of hydroxyapatite nanoparticles, nAp.¹⁵ Briefly, a suspension of $\text{Ca}(\text{OH})_2$ (5 g in 40 mL of H_2O) was stirred and heated to 80 °C. A phosphoric acid solution (4.44 g in 60 mL of H_2O) was added dropwise into the calcium hydroxide suspension over a period of 2 h under constant heating and stirring, to obtain a suspension of nAp. The precipitate was separated from the mother liquor by centrifugation, washed several times with deionized water, and dried at 80 °C.

The previous process was adapted for the synthesis of Fe-containing hydroxyapatite nanoparticles, denoted as Fe-nAp. A $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (1.27 g), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (1.79 g) phosphoric acid (4.44 g in 60 mL of H_2O) solution was added dropwise into a $\text{Ca}(\text{OH})_2$ suspension (5 g in 40 mL of H_2O) over a period of 2 h under constant stirring and heating at 80 °C. The total amounts of iron to calcium ions were adjusted to obtain an analytical molar ratio of Fe/Ca = 0.2. The reaction products were kept in suspension with constant stirring for 24 h at room temperature. The precipitate was separated from the mother liquor either by centrifugation or by means of a laboratory magnet bar of ca. 2000 G, then washed several times with deionized water, and dried at 80 °C.

For the synthesis of SBO-templated iron-containing hydroxyapatite nanoparticles, either 20, 200, or 1000 mg of SBO were dissolved in a $\text{Ca}(\text{OH})_2$ suspension (5 g in 40 mL of H_2O), and the resulting suspension was stirred for 2 h at 80 °C. A $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (1.27 g), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (1.79 g) phosphoric acid (4.44 g in 60 mL of H_2O) solution was then added dropwise into the $\text{Ca}(\text{OH})_2$ -SBO suspension over a period of 2 h under constant stirring and heating at 80 °C. The reaction mixture was kept in suspension with constant stirring for 24 h at room temperature. The precipitate was separated from the mother liquor by centrifugation and by means of a laboratory magnet bar and

then washed several times with deionized water until no SBO was observed in the washing liquors by, both, UV-vis spectroscopy and total organic carbon assays. All the particles were dried at 80 °C. The particles were named 0.05SBO-Fe-nAp, 0.5SBO-Fe-nAp, and 2.5SBO-Fe-nAp, according to the SBO/Ca w/w ratio used in the synthesis. When required, 2.5SBO-Fe-nAp powders were further calcined at 600 °C for 3 h. Calcined particles are therefore named 2.5SBO-Fe-nAp-cal.

Particle Characterization. The crystalline phase content of samples was assessed by X-ray Diffraction (XRD) by using a PW3040/60 X'Pert PRO MPD X-ray diffractometer from PANalytical, in Bragg-Brentano geometry, equipped with the high power ceramic tube PW3373/10 LFF source with Cu anode. The qualitative phase analysis was performed with the EVA software package and compared to the ICDD-PDF-2- database. The effect of Fe and SBO treatment on nAp crystallites domain size, D_v , were evaluated. A lower limit value of D_v was calculated employing the Scherrer equation relating the size of submicrometre particles in a solid to the broadening of diffraction pattern main peaks¹⁷ and considering $k = 0.9$ as the shape factor and $\lambda = 0.154056$ nm as the wave length of the X-rays for Cu $K\alpha_1$ radiation.

High resolution transmission electron microscopy (HRTEM) studies were carried out on a JEOL JEM 3010UHR (300 kV) TEM fitted with a single crystal LaB_6 filament. Samples were dry deposited on Cu "holey" carbon grids (200 mesh).

The thermal behavior of the powders was studied by thermal gravimetric analysis (TGA) using a Q600 TA Instruments. The program used a nitrogen environment and involved a one minute initial temperature of 50 °C, followed by a 20 °C/min ramp up to 800 °C, and maintained at this temperature for 10 minutes. For some samples, an oxygen environment was also used.

The functional groups present in the prepared nanoparticles were identified by their IR spectrum obtained with a Bruker IFS28 spectrophotometer equipped with a Global source and a DTGS detector working at 128 scans with 4 cm^{-1} resolution in the overall range 4000–400 cm^{-1} . Samples were prepared by pressing a grounded mixture of 1% of the particles powder with 99% dry KBr at a load of 5 tons.

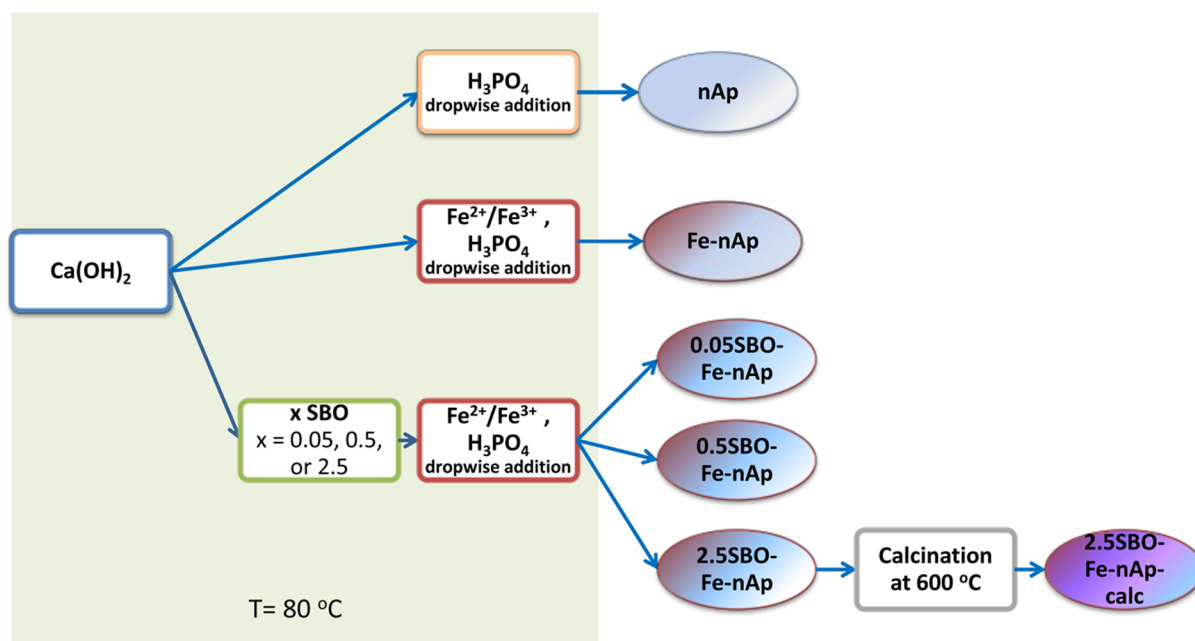
Nitrogen gas-volumetric adsorption experiments for specific surface area determinations (BET model) were performed at 77 K by means of a ASAP2020 by Micromeritics model. Before each measurement, samples were outgassed overnight at 80 °C at a residual pressure of about 10^{-2} mbar to guarantee a good cleaning of the sample surface.

The X-ray photoelectron spectroscopy (XPS) spectra were obtained under UHV with a XR50 Specs GmbH spectrometer using Mg $K(\alpha)$ as the excitation source and a PHOIBOS 100 half sphere electron energy analyzer. A two-point calibration of the energy scale was performed using sputtered cleaned gold (Au 4f7/2, binding energy (BE) 84.00 eV) and copper (Cu 2p3/2, BE: 932.67 eV) samples. Internal calibration to correct for surface charging was performed with the C 1s peak at BE = 284.6 eV due to adventitious carbon. High resolution XPS spectra were taken to get a better insight into the chemical environment of the different atoms. A Shirley-type background from each spectrum was used to remove the effect of the extrinsic structure loss and the spectrum resolved by Gaussian-Lorentzian fitting, keeping χ^2 values to their minimum values.

The electrophoretic mobility was measured by Laser Doppler Velocimetry-Photon Correlation Spectroscopy using a DELSA 440 spectrometer (Beckman Coulter Electronics, Hialeah, FL) equipped with a 5 mW HeNe laser (632.8 nm).

The magnetic characteristics of the particles were assessed using a Lakeshore 7403 vibrating sample magnetometer. Magnetization of dried samples was performed at room temperature up to a maximum field of 1.9T.

Metal determinations were performed with a Perkin Elmer Optima 7000 DV Model inductively coupled plasma atomic emission spectrometer (ICP-AES). The instrument is equipped with a Echelle monochromator, a cyclonic spray chamber, and a PTFE Mira Mist nebulizer. The instrumental conditions were as follows: plasma power 1.3 kW; sample aspiration rate 1.2 mL/min; argon cooling flow 1.5 L/min; argon sampling flow 0.6 L/min. The overall P:Ca:Fe ratio of the

Scheme 1. Flow Chart of the Synthesis Pathways Used for Obtaining the Different Particles^a

^a“x” denotes the SBO/Ca w/w analytical ratio after mixing.

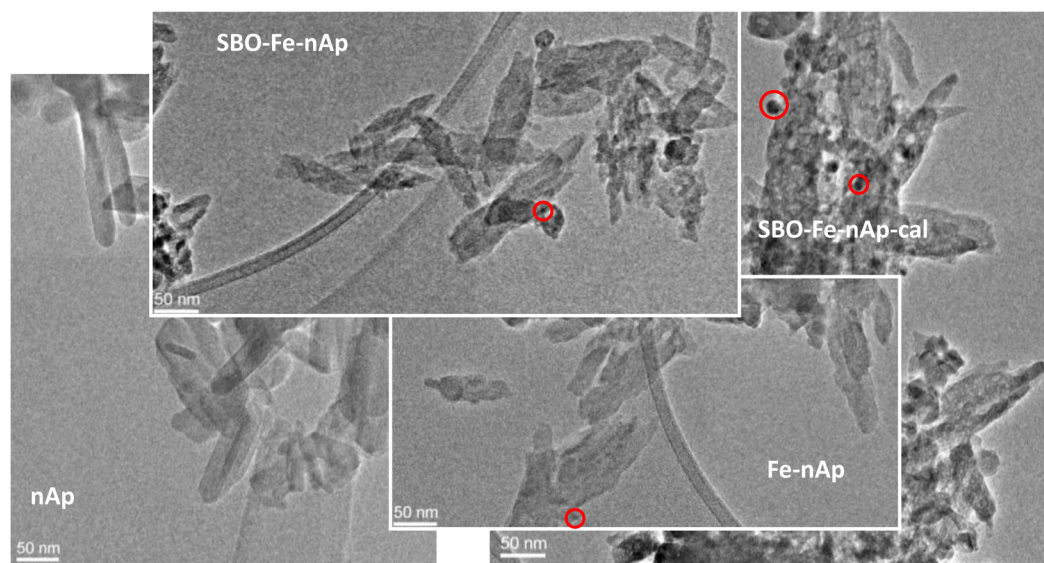


Figure 1. HRTEM micrographs. Circles show iron-containing domains.

synthesized particles was also measured by ICP after acid digestion of the particles.

Metal Adsorption Experiments. Adsorption studies were conducted in batch experiments. Variable mL-aliqouts of a $\text{Cu}(\text{NO}_3)_2$ stock solution of 1000.0 mg L^{-1} were mixed with 5 mL of a 100 ppm solution of either nAp, Fe-nAp, 0.05SBO-Fe-nAp, 0.5SBO-Fe-nAp, 2.5SBO-Fe-nAp, or 2.5SBO-Fe-nAp-cal and further diluted to a final volume of 25 mL with a solution of 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid sodium salt (HEPES-Na) of pH 7 and 0.01 M ionic strength. The highest $[\text{Cu}^{2+}]$ used was limited by Cu^{2+} solubility.¹⁸

The suspensions were shaken at room temperature ($25 \pm 2^\circ\text{C}$) for several hours until equilibration was observed. Preliminary experiments showed that equilibration takes place already after one hour. The supernatant and solid residues were separated by centrifugation at 4,000 rpm for 10 min. The Cu concentration in the supernatant solutions was determined by ICP-AES. The amount of adsorbed Cu

was calculated according to $x/m = v \times (C_0 - C_e)/m$, where x/m (mg g^{-1}) is the weight in mg of Cu^{2+} ions adsorbed per g of particles, C_0 and C_e ($\text{mg}\cdot\text{L}^{-1}$) stand for the initial and equilibrium Cu^{2+} concentrations in solution, respectively, v (L) is the solution volume used in the experiment, and m (g) is the mass of particles used. Data were expressed as the mean of three replicates.

RESULTS AND DISCUSSION

A flow chart of the pathways followed for the synthesis of the different particles is described in Scheme 1.

Particle Characterization. The effect of SBO and Fe on the physical and chemical properties of nAp was studied and compared with untreated nAp. Figure 1 shows comparative HRTEM micrographs of the prepared powders. Due to substantial agglomeration it is not possible to obtain a statistic analysis of the particles size. However, the micrographs show

cylindrical, 100–150 nm length, crystalline nAp particles, as indicated by the presence of interference fringe patterns observable at high resolution. Fe-nAp particles show somewhat shorter (50–100 nm) acicular shapes of irregular contour. The latter observation is in line with the reported effect on the morphology of rodlike nanometer size crystalline nAp which became elongated and the crystallinity slightly lowered upon addition of Fe(III) ions,¹⁹ *vide infra*. Inhibition of hydroxyapatite *c*-axis and promotion of the *a*-axis by added Fe(II) during a coprecipitation synthesis was also reported.²⁰

The use of 2.5 w(SBO)/w(Ca) as template in the synthesis of the particles leads to an even more heterogeneous sample of irregular acicular particles. Calcination of 2.5SBO-Fe-nAp leads to an increased size heterogeneity with a predominant population of enlarged particles. In all the micrographs relative to Fe-containing samples, it is possible to observe the presence of globular lumps characterized by higher optical density assignable to iron-containing domains of nanometric size (<10 nm), as also reported for Fe-containing particles obtained by similar synthetic procedures.^{16,17} The lumps are distributed quite homogeneously in the materials, indicating that the synthesis in the presence of Fe²⁺/Fe³⁺ salts brought about a good dispersion of iron-containing phases on Ap-based materials. This characteristic seems to be enhanced in the SBO patterned particles.

The XRD diffractograms of nAp-based materials are shown in Figure 2. Hydroxyapatite XRD patterns are observed in all

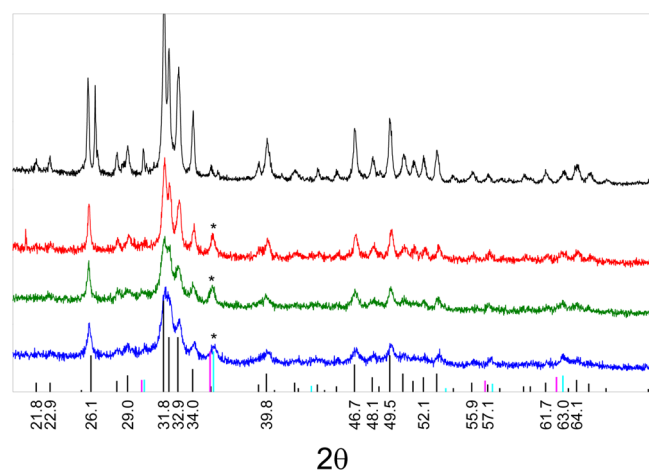


Figure 2. X-ray diffractograms of (from top to down) nAp, Fe-nAp, 2.5SBO-Fe-nAp, and 2.5SBO-Fe-nAp-cal. Bars stand for hydroxyapatite (black), maghemite (cyan), and magnetite (pink) principal reference patterns. Asterisks indicate the $2\theta = 35.5$ diffraction peak.

the prepared powders with no significant shifting of peak positions, thus suggesting that Fe²⁺/Fe³⁺ incorporation did not greatly modify the hydroxyapatite structure. A marked broadening increase of the XRD peaks of Fe-nAp, and particularly of 2.5SBO-Fe-nAp and 2.5SBO-Fe-nAp-cal powders, strongly indicates an important reduction in the crystalline structure of the particles. In addition, these samples exhibit more intense diffraction peaks at $2\theta = 35.5$ as well as peaks of lower intensity at $2\theta = 30.2$ and 62.5 , characteristic of magnetite and maghemite structures (depicted in Figure 2). Despite the presence of magnetite and maghemite crystalline phases is being supported, they could not be unambiguously differentiated, since their XRD diffraction spectrum mainly differ in the relative intensity of the peaks. In fact, the obtained

XRD data closely resembles those reported for Fe-containing hydroxyapatites from different synthetic methods.^{15,16,21}

The FTIR spectra of the particles are shown in Figure 3. nAp spectrum shows the fundamental vibration modes of PO₄

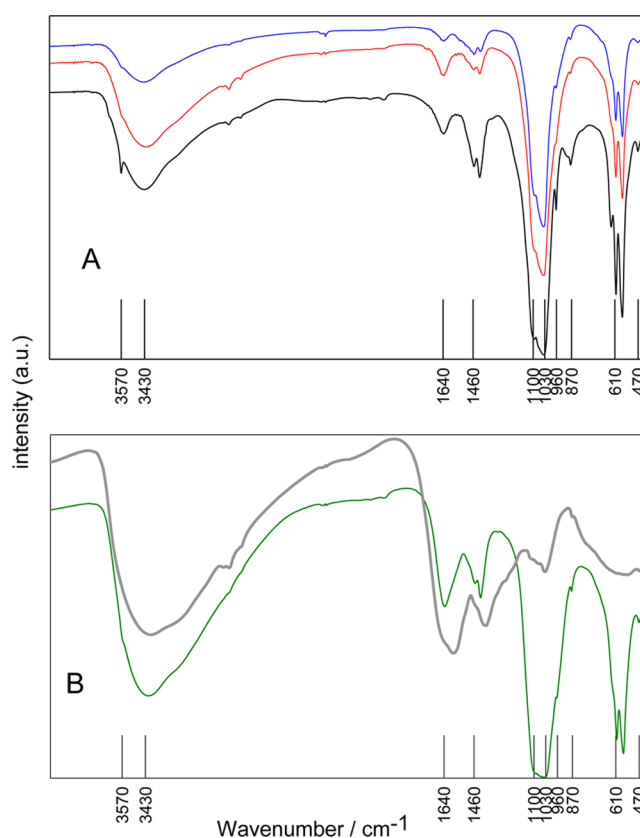


Figure 3. FTIR spectra (from down to top): (A) nAp, Fe-nAp, and 2.5SBO-Fe-nAp-cal. (B) SBO and 2.5SBO-Fe-nAp.

groups of the apatitic structure at about 470, 560, 610, 960, and 1030–1100 cm⁻¹.²² Moreover, the bands at 870 and 1460 cm⁻¹ attributed to residual carbonate from the synthesis process are also observed. The broad bands in the regions 1640 and 3430 cm⁻¹ correspond to δ H–O–H bands and ν OH signals due to HOH bands of lattice and physisorbed water molecules. The IR spectrum of Fe-nAp shows mainly peaks attributed to nAp. The characteristic peaks due to iron oxides in the 600–400 cm⁻¹ range²³ may be occluded by those of nAp due to the low amount of iron present in the samples. The 2.5SBO-Fe-nAp spectrum shown in Figure 3B evidences nAp signals superimposed to those of SBO (also shown in the figure). The intense bands at 3430 and 1649 cm⁻¹ indicate the presence of HO groups of phenols, alcohols, and carboxyl groups and of C=O bonds of carboxyl and carbonyl moieties, respectively. On the other hand, the FTIR absorption peaks of 2.5SBO-Fe-nAp-cal may be assigned mainly to those of nAp, thus indicating that 3 h calcination at 600 °C of 2.5SBO-Fe-nAp powders is effective in eliminating SBO residues. nAp spectrum also shows the sharp hydroxyl bands at 632 and 3572 cm⁻¹, typical of nAp with a high degree of crystallinity. The absence of these peaks in the spectra of all the other particles suggests that the powders show poor crystallinity, in agreement with XRD data also supporting a decreased hydroxyapatite crystallinity upon Fe addition and use of SBO as organic template.

The TGA curves (see Figure 4) obtained in a N₂ environment in the temperature range from 80 to 800 °C for

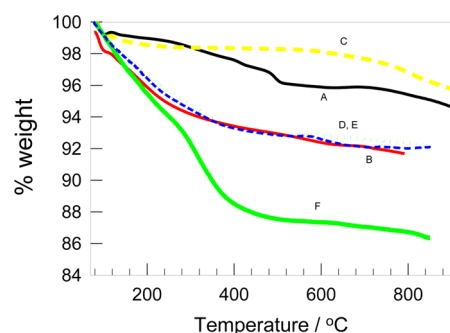


Figure 4. TGA performed in a N₂ atmosphere, curves (A) nAp, (B) Fe-nAp, and (C) 2.5SBO-Fe-nAp-cal. TGA performed in an O₂ atmosphere, curves (D) 0.0SBO-Fe-nAp, (E) 0.5SBO-Fe-nAp, and (F) 2.5SBO-Fe-nAp. Corresponding derivative curves are shown in S1.

Ap-based materials yield information on the adsorbed substrates, as decomposition of the Ap structure is expected at temperatures over 900 °C.²² The 5.9% total mass loss of nAp is attributed to adsorbed water ($T \leq 100$ °C), dehydration of hydroxides (380–500 °C), and elimination of surface carbonate-like groups (500–800 °C), in good agreement with literature reports for nano-sized Ap powders.²² Fe-nAp shows an 8% total mass loss between 80 and 800 °C attributed to adsorbed water, dehydration of Fe hydroxides (380–500 °C), and elimination of surface carbonate-like groups. On the other hand, 2.5SBO-Fe-nAp powder shows a 14% total mass loss suggesting an important contribution of SBO pyrolysis (curve not shown), in agreement with the fact that only a 4% weight loss is observed for the same particles calcined for three hours at 600 °C. The latter observations are in agreement with the FTIR spectra indicating the presence of SBO in 2.5SBO-Fe-nAp and a complete elimination of the organic template in the calcined powders. An estimation of the particles carbon content may be obtained from the mass loss in the temperature range from 200 to 380 °C observed in TGA curves performed in an O₂ environment. Approximately 5% mass content due to SBO is estimated for 2.5SBO-Fe-nAp. TGA curves of 0.5SBO-Fe-nAp and 0.0SBO-Fe-nAp performed in the presence of O₂ agree, within the experimental error, with that of Fe-nAp, indicating that no significant amounts (<1%) of organic matter are present in the materials.

Table 1 depicts the specific surface area of the synthesized powders as determined by the BET model and the relative crystallites domain size as estimated by the Scherrer equation

using the XRD data. Crystalline nAp shows the lowest surface area, while almost a twofold increase is observed for Fe-nAp, and a further 15% increase is observed for 2.5SBO-Fe-nAp. Calcination of the latter reduces the surface area by 15%. The BET isotherms show hysteresis mainly at high P/P^0 values related to N₂ condensation inside interparticle macropores of ca. 100 nm (see S2). Crystallite domain sizes decrease in the trend nAp < Fe-nAp < 2.5SBO-Fe-nAp, while a further increase is observed for 2.5SBO-Fe-nAp-cal. All together, the results suggest a reduced crystallite domain size with increasing specific surface area and particle size diminution. The increase of specific surface area besides crystallinity diminution is also reported for nanoparticulated apatite.²⁴

The average hydrodynamic particle size distribution determined by DLS measurements, shown in Figure 5,

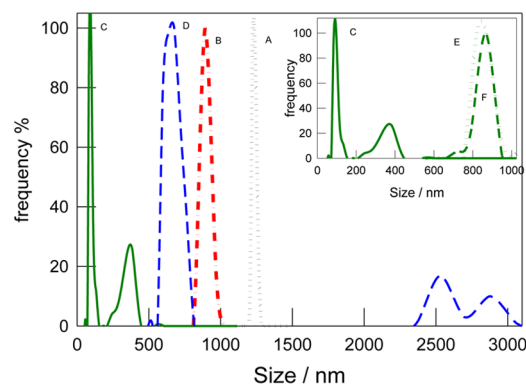


Figure 5. Hydrodynamic particle size distribution as obtained by DLS for nAp (A), Fe-nAp (B), 2.5SBO-Fe-nAp (C), 2.5SBO-Fe-nAp-cal (D), 0.5SBO-Fe-nAp (inset, E), and 0.0SBO-Fe-nAp (inset, F).

indicates that the particles hydrodynamic sizes are several fold-times higher than the sizes observed in TEM micrographs. Thus, important aggregation in aqueous solutions at pH 7 and 0.01 M ionic strength takes place. The amount of SBO used as template in the particles synthesis strongly influences aggregation, as smaller hydrodynamic sizes are observed for increasing SBO concentrations. Calcination of 2.5SBO-Fe-nAp particles increases the aggregates size, in line with the population of larger particles depicted in the corresponding HRTEM micrographs (vide supra). Aggregate sizes in the range of 100 nm are observed for 2.5SBO-Fe-nAp particles, and interparticle macroporosity of almost the same size is evidenced by N₂ gas-volumetric adsorption, thus indicating that macropores formed in the solid state do not have real significance in aqueous suspensions of the particles.

Table 1. Specific Surface Area As Obtained from BET Isotherms, Lower Limit Size As Estimated by the Scherrer Equation, Surface Stoichiometric Ratio Obtained by XPS Analysis, % C from TGA Experiments, Electrophoretic Mobility (μ) in the pH Range from 6.5 to 7, Bulk Ca and Fe Percentages, and Fe to Ca Molar Ratio in the Particle Bulk

particle type	specific surface area/m ² g ⁻¹	crystallite domain size/ nm	surface composition	% w/w SBO	μ (mS/cm)	bulk		
						% Ca w/w	% Fe w/w	Fe/ Ca
nAp	34	30–50	Ca ₁ P _{0.7} O _{3.1} Fe ₀	0	+0.95	38.4	<0.02	0
Fe-nAp	64	20–40	Ca ₁ P _{0.9} O _{4.6} Fe _{0.14}	0	–1.8	28.4	7.8	0.2
0.0SBO-Fe-nAp	68	----	----	< 1	–1.5	31.2	7.4	0.17
0.5SBO-Fe-nAp	70	----	Ca ₁ P _{0.7} O _{3.7} Fe _{0.09}	< 1	–1.6	29.0	6.9	0.17
2.5SBO-Fe-nAp	71	10–30	Ca ₁ P _{0.75} O _{3.7} Fe _{0.09}	~5	–1.8	28.3	6.6	0.17
2.5SBO-Fe-nAp-cal	64	20–60	Ca ₁ P _{0.8} O _{3.3} Fe _{0.06}	0	-----	30.9	7.2	0.17

The electrophoretic mobility of the particles obtained in the pH range from 6.5 to 7 is depicted in Table 1. nAp and Fe-nAp values of +0.95 and -1.8 mS/cm, respectively, indicate that the incorporation of 20% Fe on the hydroxyapatite particles brings about an increased surface charge density and a predominance of negative surface charges. Moreover, SBO-patterned particles show electrophoretic mobilities similar to those observed for Fe-nAp. The contrast between the similar mobilities of these particles with their different aggregates size strongly suggests an increased surface hydrophilicity in SBO-patterned particles due to the presence of uncharged surface groups which strongly stabilize the aqueous suspensions of the particles.

The XPS survey spectra of the particles (see S4) depict the main lines for Ca, O, P, and Fe (except for nAp). Beside these peaks, a C(1s) "adventitious carbon" peak was observed which was used for binding energy correction due to charging effects by setting it to 284.6 eV. Carbonate-type carbon, incorporated during synthesis, was also observed. All powders show the Ca2p_{1/2} (350.8 eV) and Ca2p_{3/2} (347.4 eV) lines, and the P2s line at 191 eV due to calcium phosphate in apatite (see S5). On the other hand, analysis of Fe2p_{3/2} peaks is more controversial. The use of broad peak shapes has proved useful for the identification of pure compounds but of lesser use for the identification of mixtures due to the spectral overlap of most Fe(III) compounds which show similar binding energies but varying peak shapes and satellite intensities.²⁵ In fact, such analysis leads to a peak at about 710.5 eV with a satellite displaced 4 eV and a second broad peak at about 712.5 eV with no satellite contribution unless overlapped to the Fe2p_{1/2} portion of the spectrum. These peaks did not match the reported data of neither pure Fe oxides²⁶ nor of phosphated iron.²⁷ Therefore, Fe2p_{3/2} peaks for all the iron-containing samples herein synthesized were modelled using a combination of ferrous and ferric multiplet patterns for the lower and higher energy portion of the spectrum, respectively,^{25,28} as shown in Figure 6 for Fe-nAp. Fe2p_{3/2} signal adjustment was performed

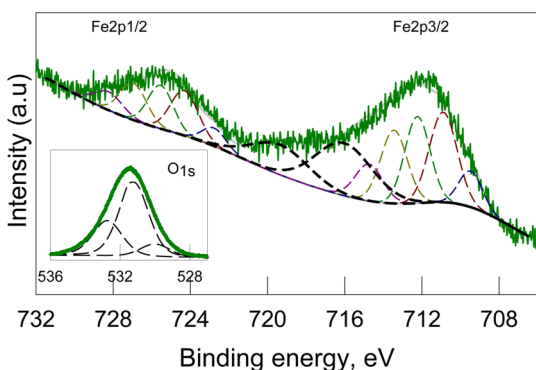


Figure 6. XPS Fe2p_{3/2} and Fe2p_{1/2} signals (full line) for 2.5SBO-Fe-nAp and contributing peaks (dashed lines). *Inset:* O1s signals (full line) and contributing species (dashed lines).

assuming a Shirley type baseline and using the lowest number of peaks allowed with about 1.7 eV FWHM, except for the peak attributed to the Fe II satellite which was left free. All samples show contributing peaks at 709.5, 710.9, 712.2, 713.4, 714.9 eV, and a Fe²⁺ satellite peak shifted by 4.8–5 eV, in line with the patterns reported for Fe(II)-PO₄ surface oxidized to Fe(III)-OH.²⁸ Moreover, no metallic component can be observed in the spectrum indicating its absence in the surface layer. The observed satellite peak at ca. 719 eV, may be assigned to the

contribution of iron oxides.^{26,29} Observed Fe2p_{3/2} and Fe2p_{1/2} signals separations, ΔE (BE 2p_{3/2} –BE 2p_{1/2}), of about 13.0–13.3 eV are in agreement with reported data for Fe oxides and salts.²⁶ Therefore, the XPS data points to a complex mixture of Fe²⁺/Fe³⁺ phosphates and oxides on the particles surface.

Deconvolution of the O1s peak of n-Ap shows the main contribution of a band at 531.8 eV (96%) and a minor contribution at 532.8 eV. According to literature reports, these peaks may be assigned to oxygen atoms in phosphates and hydroxyls and to adsorbed water, respectively, in agreement with reported data for hydroxyapatite.²⁷ On the other hand, O1s peaks of Fe-containing particles are best modelled when the contribution of a third peak at ca. 530.1 eV characteristic of O atoms in iron oxide environments is considered,²⁶ as depicted in Figure 6 *inset* and S5. Peak contributions of 22% and 13% were observed for Fe-nAp and 2.5SBO-Fe-nAp-cal, respectively, while contributions of ca. 5% were observed for 0.5SBO-Fe-nAp and 2.5SBO-Fe-nAp. The latter observations are in line with the larger peak area of Fe2p_{3/2} multiplets of BE > 711 eV observed for Fe-nAp and 2.5SBO-Fe-nAp-cal, normally identified with Fe³⁺ ions.

Considering the experimental sensitivity factors relative to the different elements, a surface Ca:P:O stoichiometric ratio was determined for each particle type as depicted in Table 1. The P/Ca ratio for nAp is somewhat higher than the expected theoretical Ca(PO₄)_{0.6}O_{0.2} stoichiometric ratio but close to the values reported in the literature for several hydroxyapatite materials and attributed to the presence of surface acid phosphates.²⁹ On the other hand, the O/P ratio is, within the experimental error, in line with the expected value for hydroxyapatite. Fe-nAp shows higher P/Ca and O/Ca ratios than nAp. All together, the data seems to indicate that surface iron increases phosphate content due to the formation of Fe(II)-phosphates, while surface Fe³⁺ seems to lead to the formation of iron oxides. In fact, Fe(III)-substituted hydroxyapatite nanoparticles were reported to show an increased surface hydrophilicity as a consequence of the formation of surface Fe–OH groups, though the number of original Ca(II) atoms was reduced.¹⁹

On the other hand, lower Fe/Ca surface ratios were observed when SBO was used as a template in the particle synthesis. 0.5SBO-Fe-nAp and 2.5SBO-Fe-nAp show P/Ca ratios similar to those of nAp, though the respective O/Ca ratios are significantly higher, thus suggesting the presence of surface OH groups. Upon calcination, 2.5SBO-Fe-nAp show a decreased content in surface oxygen and iron.

Hysteretic M–H curves of Fe-nAp and 2.5SBO-Fe-nAp at 300 K (see S3) exhibit a superparamagnetic behavior with zero coercivity and zero remanence on the magnetization curves. For both particles, the saturation magnetization was of 4 emu/g of particles, in line with reported values for Fe-nAp nanopowders.^{15,16} Considering a 7% w/w iron content in the particles (see Table 1), the saturation magnetization per gram of iron is 57 emu/g Fe, half the value expected for magnetite nanoparticles.³⁰ Therefore, formation of significant amounts of iron compounds not contributing to the overall paramagnetism is supported.

The described results clearly indicate that introduction of Fe³⁺/Fe²⁺ ions and the use of SBO-Ca²⁺ complexes as templates during hydroxyapatite synthesis leads to a diminution in the apatite crystalline structure, a negatively charged surface, and an increased surface hydrophilicity. Formation of magnetite

and/or maghemite is observed by XRD. However, iron oxides formation is not the only fate of iron. The loss of crystallinity of the apatite structure might be a consequence of Ca^{2+} exchange by $\text{Fe}^{2+}/\text{Fe}^{3+}$ ions. In fact, XPS analysis clearly indicates the presence of surface Fe^{2+} phosphates salts and oxidized Fe^{3+} . Formation of surface FeO and OH groups is suggested from the O2s peak analysis. A comparison between Fe:Ca contents in the particles volume and at the surface, see Table 1, indicates that Fe ions are preferentially incorporated in the particles core.

Metal Adsorption Capacity. Figure 7 shows the measured Cu^{2+} adsorption isotherms. The one-adsorption site Langmuir

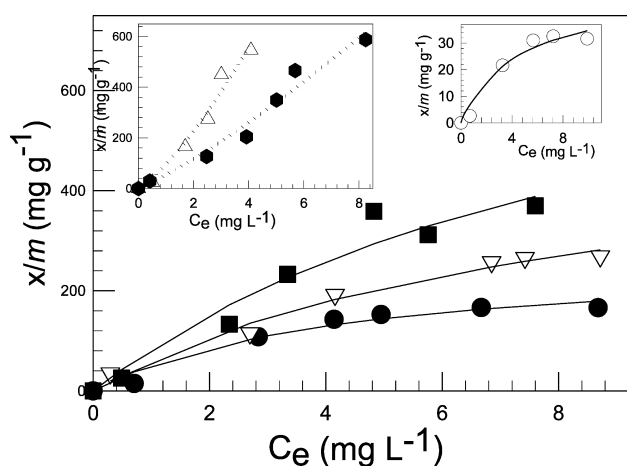


Figure 7. Cu^{2+} adsorption isotherms at 25 °C. Main figure: Fe-nAp (●), 0.05SSBO-Fe-nAp (▽), and 0.5SSBO-Fe-nAp (■). Inset left: 2.5SSBO-Fe-nAp (△) and 2.5SSBO-Fe-nAp-cal (●). Inset right: nAp (○). The solid lines and dashed lines stand to the fitting of the data to eqs 1 and 2, respectively.

model, eq 1, fits the isotherms of Cu^{2+} adsorption on nAp, Fe-nAp, 0.05SSBO-Fe-nAp, and 0.5SSBO-Fe-nAp with regression coefficients $r^2 > 0.95$. The obtained fitting parameters are depicted in Table 2. The affinity coefficient of Cu^{2+} adsorption, k_L , shows the trend nAp \approx Fe-nAp $>$ 0.05SSBO-Fe-nAp \approx 0.5SSBO-Fe-nAp, while the maximum number of sites b increases in the order nAp $<$ Fe-nAp $<$ 0.05SSBO-Fe-nAp $<$ 0.5SSBO-Fe-nAp. On the other hand, adsorption on 2.5SSBO-Fe-nAp and 2.5SSBO-Fe-nAp-cal particles were better fitted by the Freundlich model, eq 2. This experimental isotherm has been interpreted to arise from a heterogeneous surface showing a distribution of different adsorption sites, where $1/n \leq 1$ is an experimentally determined exponent and k_F is the Freundlich constant. However, within the experimental error, obtained values of n are close to 1, thus suggesting the applicability of the Langmuir isotherm under the condition $1 \gg k_L \times C_e$ and $k_F = k_L \times b$. In fact, the maximum Cu^{2+} adsorption could not be

reached for these particles due to the limited Cu^{2+} solubility.¹⁸ Taking $k_L \times b = 90 \text{ mg g}^{-1}$ for 2.5SSBO-Fe-nAp and considering $k_L \approx 0.11$ for SBO-templated particles (vide supra), results in $b \approx 850 \pm 400 \text{ mg g}^{-1}$.

$$x/m = \frac{k_L \times b \times C_e}{1 + k_L \times C_e} \quad (1)$$

$$x/m = k_F \times C_e^{1/n} \quad (2)$$

Isolation of the Cu^{2+} -adsorbed nAp, Fe-nAp, and 0.5SSBO-Fe-nAp and further resuspension in an aqueous solution of pH 7 show 0.2, 0.1, and 0.02% Cu^{2+} leaching after two hours, respectively. Thus indicating that Cu^{2+} is strongly chemisorbed at pH 7.

The obtained value of b for nAp is in line with that reported for Cu^{2+} adsorption on commercial nanohydroxyapatite at pH 7.2 (64.8 mg g^{-1}), though the reported² $k_L = 0.80$ is almost 4 times higher than that found here. An even number of regularly spaced, positively charged, calcium ions and negatively charged oxygen groups on the surface of crystalline Ap support metal cation retention due to ion exchange and/or coordination with P-sites and surface HO. Moreover, Cu^{2+} precipitation with free PO_4^{3-} ions as a consequence of the high Ap solubility at pH 5 is also reported.³¹ In our experiments, the amounts of calcium and iron ion concentrations measured in solution during the Cu^{2+} adsorption experiments are ca. 17 and $0.5 \mu\text{g/g}$ NP, respectively, for all the particles, independently of the adsorbed amount of Cu^{2+} ions. Therefore, Cu^{2+} exchange for Ca^{2+} and Fe^{2+} in the hydroxyapatite structure seems not to significantly contribute to Cu^{2+} adsorption under our experimental conditions.

Moreover, the observed increase in the specific surface area of Fe-nAp and SBO-Fe-nAp containing particles seems not to be the cause of the increased Cu^{2+} adsorption of these materials (vide infra), as normalization of the adsorption isotherms by the specific surface area of the materials does not modify the observed trend.

Reported Cu^{2+} maximum adsorption capacities for different magnetite composites are of the order of 1.9 for aqueous suspensions of pH 5.5,³² 56.71 for magnetite-immobilized chitin,³³ and 140–150 mg/g for magnetite-immobilized *Pseudomonas putida* 5X cells at pH 7.³² These values are below those observed for the materials herein synthesized. On the other hand, Cu^{2+} ions have been reported to readily form nonlabile complexes with fulvic acids and dissolved natural organic matter, DOM,³⁴ as amino groups, phenolic hydroxyls, and adjacent aromatic carboxylates provide lone pairs of electrons for chelating Cu^{2+} ions. The reported total number of binding sites range from 1 to $100 \mu\text{M mg C}^{-1}$ depending on pH, ionic strength, and the DOM origin and concentration. A rough estimation of the adsorption sites provided by SBO may be

Table 2. Fitting Parameter Values of the Langmuir and Freundlich Models^a

particle	eq	$b/\text{mg g}^{-1}$	k_L	$1/n$	$k_F/\text{mg g}^{-1}$	r
nAp	1	50(25)	0.22(0.1)			0.967
Fe-nAp	1	265(60)	0.24(0.02)			0.969
0.05SSBO-Fe-nAp	1	550(200)	0.12(0.04)			0.978
0.5SSBO-Fe-nAp	1	850(400)	0.10(0.07)			0.953
2.5SSBO-Fe-nAp	2			1.3(0.2)	90(22)	0.974
2.5SSBO-Fe-nAp-cal	2			1.2(0.2)	50(15)	0.970

^aError bars are denoted in parentheses.

obtained if it is considered that 5% mass of 2.SSBO-Fe-nAp is due to SBO organic residues, from which 38.5% in weight is due to C atoms. Therefore, taking the maximum amount of 100 μM sites mg C^{-1} , $\sim 122 \text{ mg g}^{-1}$ Cu^{2+} ions would be adsorbed by the organic matter, well below the obtained values (see Table 2). Consequently, the increased Cu^{2+} adsorption capacity observed for SBO-patterned particles cannot be assigned solely to surface-trapped SBO, neither to the small magnetite domains observed on the particles surface. These observations are further supported by the significant Cu^{2+} adsorption capacity still observed for 2.SSBO-Fe-nAp-cal despite the elimination of all C-containing residues due to calcination.

As discussed above, the main differences observed between nAp and Fe-containing apatites involve a reduction in the crystallinity of hydroxyapatite domains, a reduced Ca^{2+} content, and the formation of surface phosphated iron and magnetite/maghemite phases. An increased O-content is also observed at the surface of all SBO-Fe-nAp materials. Such surface and structural modifications might support an increased adsorption efficiency of the iron-containing hydroxyapatite materials due to Cu^{2+} complexation with surface PO_4 and OH groups. It results striking though, that despite both 0.SSBO-Fe-nAp and 2.SSBO-Fe-nAp showing similar surface composition, except for the small C content of the latter, Cu^{2+} adsorption ability of 2.SSBO-Fe-nAp is significantly higher. We suggest, therefore, that the smaller aggregates formed by 2.SSBO-Fe-nAp in aqueous suspensions makes the particles surface more available for Cu^{2+} adsorption. In fact, strong electrostatic interactions might prevent Cu^{2+} ions to approach the binding sites of the particles surface within the aggregates. Surface area measurements of dry materials do not necessarily represent the effective surface area of suspended particles in aqueous solution because of the particles agglomeration in suspension.^{35,36}

A rough estimation of the effective surface area may be obtained if it is assumed that Cu^{2+} is mainly adsorbed at the agglomerates surface and taking the agglomerates hydrodynamic radius distribution measured by DLS and the density value of hydroxyapatite (3.156 g/cm^3)³⁷ for all the particles. Normalization of the isotherms shown in Figure 6 by the estimated effective agglomerate area (1.55, 2.1, 2.2, 2.3, 6.5, and $2.8 \text{ m}^2 \text{ g}^{-1}$ for nAp, Fe-nAp, 0.05SSBO-Fe-nAp, 0.5SSBO-Fe-nAp, 2.SSBO-Fe-nAp, and 2.SSBO-Fe-nAp-cal, respectively) yields adsorption isotherms (see Figure 8) which may be classified in two main groups: nAp and Fe-containing apatites. Iron-

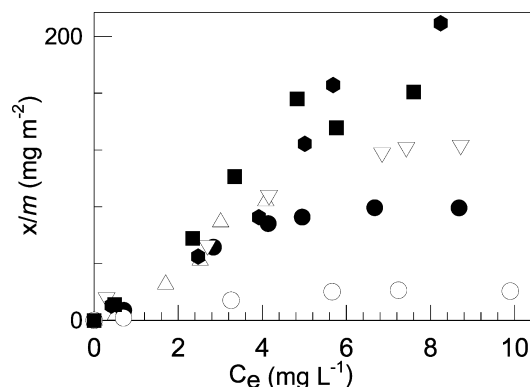


Figure 8. Cu^{2+} adsorption isotherms at 25 °C normalized by the effective area (see text). nAp (O), Fe-nAp (●), 0.05SSBO-Fe-nAp (V), 0.5SSBO-Fe-nAp (■), 2.SSBO-Fe-nAp (Δ), and 2.SSBO-Fe-nAp-cal (\circ).

containing particles, all depicting similar k_L values, show improved adsorption capacity with respect to pure nAp, in line with their described surface characteristics, vide supra. Differences among the adsorption isotherms of the iron-containing particles are mainly observed at high Cu^{2+} loadings, probably due to Cu^{2+} adsorption on the particles surface facing the interior of the agglomerates at higher concentrations, a situation not considered in the simplified estimation of the effective surface area.

CONCLUSIONS

The present results strongly indicate that hydroxyapatite nanoparticles doped with iron and synthesized using SBO as template show significant changes in the particles morphology and surface chemistry. Among the observed effects are the following: a reduction in the crystallinity of hydroxyapatite domains, elongated and smaller particles with higher specific surface area, reduced Ca^{2+} content, formation of surface phosphated iron and iron oxides, increased oxygen content at the surface, and an overall negative surface charge at pH 7.0. Moreover, formation of magnetite oxide seems responsible for the particle paramagnetism. Such surface and structural modifications support the formation of stable homogeneous aqueous suspensions of the particles, which show a reduction in the size of the agglomerates when increasing the SBO content in the synthesis procedure.

A significant improvement in the Cu^{2+} adsorption capacity was observed upon iron-doping of the hydroxyapatite particles and, particularly, for the SBO-templated particles. Such improvement is correlated with a negatively-charged surface and the presence of phosphate and Fe–OH surface groups. However, a second important effect appears to be the agglomerates size, as agglomeration seems to severely reduce the effective surface available for adsorption in aqueous suspensions.

The materials herein synthesized are a potential, self-sustaining, environmentally friendly, and efficient material for Cu^{2+} remediation in aqueous effluents. Future work involves metal sorption selectivity studies and metal desorption conditions for particle reuse.

ASSOCIATED CONTENT

Supporting Information

TGA derivatives, N_2 adsorption isotherms and BJH mesopores analyses, magnetization curves, XPS survey spectra, and elements high resolution peaks. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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