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Accurate characterization of pure silicon-substituted hydroxyapatite powders synthesized by a new precipitation route

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26 **Abstract**

27 This paper presents a new aqueous precipitation method to prepare silicon-substituted
28 hydroxyapatites $\text{Ca}_{10}(\text{PO}_4)_{6-y}(\text{SiO}_4)_y(\text{OH})_{2-y}(\text{V}_{\text{OH}})_y$ (SiHAs) and details the characterization of
29 powders with varying Si content up to $y=1.25 \text{ mol mol}_{\text{SiHA}}^{-1}$. X-ray diffraction (XRD),
30 transmission electron microscopy (TEM), solid-state nuclear magnetic resonance (NMR) and
31 Fourier transform infrared (FTIR) spectroscopy were used to accurately characterize samples
32 calcined at 400°C for 2 h and 1000°C for 15 h. This method allows for synthesizing
33 monophasic SiHAs with controlled stoichiometry. The theoretical maximum limit of
34 incorporation of Si into the hexagonal apatitic structure is $y<1.5$. This limit depends on the
35 OH content in the channel, which is a function of the Si content, temperature and atmosphere
36 of calcination. These results, particularly those from infrared spectroscopy, express serious
37 reservations about the phase purity of SiHA powders, pellets or scaffolds prepared and
38 biologically evaluated in the literature.

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46 **Keywords:** biomaterials; silicon-substituted hydroxyapatite; precipitation method, infrared
47 spectroscopy, NMR spectroscopy.

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49 1. Introduction

50 According to the literature, silicon-substituted hydroxyapatite (SiHA) is a highly promising
51 material in the field of bioactive bone substitutes and bone tissue engineering. It is now well-
52 established that silicon plays an important role in the early stage of cartilage and bone growth
53 [1-4]. Soluble silicon species have been shown to stimulate spontaneous calcium phosphate
54 precipitation (*i.e.* the mineral bone phase) [5] and to increase bone mineral density [6].
55 Moreover, silicon has been reported to have a positive influence on the synthesis of type I
56 collagen by human osteoblast cells (MG-63 cell line) *in vitro* [7]. Thereby, it is expected that
57 silicon could enhance the hydroxyapatite (HA) bioactivity [8, 9], and silicon-substituted
58 hydroxyapatites (SiHAs) have become a subject of great interest in bone repair. The SiHA
59 structure corresponds to the substitution of phosphate ions (PO_4^{3-}) by silicate ions (SiO_4^{4-})
60 into the HA crystal structure. Different mechanisms for charge compensation have been
61 suggested [8, 10, 11]. The most cited one was proposed by Gibson et al. with the creation of
62 anionic vacancies at OH sites [8, 12]. This mechanism leads to silicon-substituted
63 hydroxyapatites with the general formula $\text{Ca}_{10}(\text{PO}_4)_{6-y}(\text{SiO}_4)_y(\text{OH})_{2-y}(\text{V}_{\text{OH}})_y$, where y
64 represents the molar number of silicate groups introduced into the apatitic structure ($0 \leq y \leq$
65 2) and V_{OH} stands for vacancies maintaining the charge balance. The incorporation of Si into
66 the HA structure in solid solution, *i.e.* without the formation of other phases, seems to be
67 limited. However, the value and the origin of this limitation are still not known, with for
68 instance the following values: 5 wt% ($\approx 1.7 \text{ mol}_{\text{Si}} \text{ mol}_{\text{SiHA}}^{-1}$) [13-15], 4 wt% ($\approx 1.4 \text{ mol}_{\text{Si}}$
69 $\text{ mol}_{\text{SiHA}}^{-1}$) [16, 17], 3.1 wt% ($\approx 1.1 \text{ mol}_{\text{Si}} \text{ mol}_{\text{SiHA}}^{-1}$) [18], 2 wt% ($\approx 0.7 \text{ mol}_{\text{Si}} \text{ mol}_{\text{SiHA}}^{-1}$) [11,
70 19], 1.0 wt% ($0.36 \text{ mol}_{\text{Si}} \text{ mol}_{\text{SiHA}}^{-1}$) [20] or 0.28 wt% ($0.1 \text{ mol}_{\text{Si}} \text{ mol}_{\text{SiHA}}^{-1}$) [21]. Additionally,
71 it has been suggested that the concentration of 0.8 wt% of Si ($\approx 0.28 \text{ mol}_{\text{Si}} \text{ mol}_{\text{SiHA}}^{-1}$) is
72 optimal to induce the development of important bioactivity [22-24]. A value of 2.2 wt% of Si
73 was also reported by Thian et al. [25]. Unfortunately, in spite of extensive studies in recent

74 years, these results remain heterogeneous, confusing and sometimes misleading. For instance,
75 Hing et al. revealed faster bone apposition and improved adhesion and proliferation of
76 osteoblast-like cells for SiHA compared to stoichiometric HA [23, 26], whereas Palard et al.
77 found no significant difference in the behavior of MG-63 osteoblast-like cells between pure
78 HA and SiHA pellets (three compositions: $y=0.2, 0.4$ and $0.6 \text{ mol}_{\text{Si}} \text{ mol}_{\text{SiHA}}^{-1}$) [27]. Recent
79 critical analyses of the published results regarding SiHAs have highlighted the lack of
80 experimental evidence which could explain the real effects of Si substitution on biological
81 activity in a biological environment [28, 29]. In particular, it has been criticized that the
82 physico-chemical characterizations of SiHA bioceramics are not detailed (purity, solubility,
83 rate of incorporation of Si inside the crystal lattice, etc.). Therefore, the available data do not
84 provide sufficient information to establish the origin of the improved biological performance
85 of SiHA: (i) a direct effect of SiHA by Si release, (ii) an indirect effect of SiHA by changes in
86 the physico-chemical properties of HA due to Si substitution (microstructure, superficial
87 chemistry, topography, etc.) or (iii) an effect of second phases (crystalline and/or amorphous).
88 According to Boanini et al., the term “ion-substituted” is quite often used without any
89 experimental proof regarding the incorporation of ions inside the crystal lattice of calcium
90 orthophosphates [28]. The unclear bioactivity of SiHA ceramics could be explained by
91 variations in the phase composition. The first evidence for this was provided by the few
92 accurate analyses available in the literature which show that SiHA powders can contain
93 crystallized [16, 30-35] and amorphous [16, 34, 36-38] impurities. The study by Kanaya et al.
94 is representative of the characterization problems of SiHA samples [38]. Indeed, while the X-
95 ray diffraction patterns show only the characteristic lines of the HA phase (PDF: 09-432), the
96 ^{29}Si MAS NMR spectrum revealed that only 10% of Si was incorporated into the HA lattice;
97 the rest was on the particle surface in the form of polymeric silicate species [38]. An
98 equivalent observation was made by Gasquères et al. [16]. Most studies do not evidently show

99 the purity of their SiHA samples (powders, pellets or scaffolds) [8, 9, 19, 21-24, 26, 27, 39-
100 58]. They generally provide an imprecise physico-chemical characterization with assumptions
101 based on X-ray diffraction patterns or incomplete infrared band assignment [8, 9, 19, 22, 32,
102 36, 39, 59-61]. Moreover, infrared vibrations at 692, 840, 890 and 945 cm^{-1} , detected on an
103 SiHA sample containing 1.6 wt% Si (1200°C for 2 h), were attributed without evidence by
104 Gibson et al. to the substitution of SiO_4 for PO_4 into the HA lattice [8, 12]. Unfortunately, this
105 article set the standard for SiHA analysis by infrared spectroscopy and has been widely cited
106 to prove the purity of SiHA powders synthesized using Gibson's method [9, 23, 39, 40, 43,
107 44, 46, 47, 53, 57] or displaying the same new infrared bands [14, 32, 36, 48, 50, 54, 62].

108 Several methods are used to prepare Si-substituted hydroxyapatites (SiHAs), such as
109 the sol-gel route [48], resuspension processes [20, 33, 34, 63-66], solid state reactions [10,
110 67], hydrothermal techniques [17, 68, 69], mechanochemical methods [70], magnetron
111 sputtering [14], pulsed laser deposition [51, 71], electrophoretic deposition [72] and
112 precipitation from aqueous solutions. Aqueous precipitation methods are the most often
113 described and set up in the literature. Two different procedures are used to produce Si-HA
114 from aqueous solutions: (i) the acid-base neutralization [8, 9, 16, 19, 23, 30, 35, 37, 39, 46,
115 47, 49, 50, 53, 56, 57, 73-75], and (ii) the use of phosphorus and calcium salts [13, 21, 32, 36,
116 37, 60, 74, 76, 77]. Generally, far less attention has been devoted to the silicon reagent. Two
117 organic compounds are mainly used as a source of silicate ions: tetraethylorthosilicate (TEOS,
118 $\text{Si}(\text{OC}_2\text{H}_5)_4$) and tetraacetoxysilane (TAS, $\text{Si}(\text{COOCH}_3)_4$) which are not miscible in water.
119 Powders synthesized through these methods are poorly crystallized and often contaminated by
120 second phases (e.g. α and β -Tricalcium phosphate, amorphous phase, silicocarnotite [16, 19,
121 30, 31, 36-38, 49, 59, 60, 63, 64, 67, 74, 76]) or undesired ions like Mg [56], S [77] or Na [78,
122 79].

123 In order to correctly describe the physical, chemical and biological properties of
124 SiHAs and to compare them to routinely implanted HA and β -TCP, well-characterized pure
125 SiHAs powders first need to be prepared. Therefore, this work was devoted to the
126 development of a new route to synthesize monophasic SiHA powders with controlled
127 stoichiometry. To this purpose, a solution of soluble silicate was first prepared from TEOS *via*
128 a sol-gel route, and then accurate powder analysis was carried out by means of ICP/AES, X-
129 ray powder diffraction, Rietveld refinement, high resolution electron transmission microscopy
130 (HR-TEM) with energy dispersive spectroscopy (EDS) as well as infrared (FT-IR/ATR) and
131 solid-state NMR spectroscopy. Two pH levels of precipitation were studied, as well as six
132 Si/P molar ratios.

133 **2. Materials and methods**

134 **2.1 Powder synthesis**

135 HA and SiHA powders were prepared through an aqueous precipitation method using a fully
136 automated apparatus. A diammonium hydrogen phosphate aqueous solution ($(\text{NH}_4)_2\text{HPO}_4$,
137 99%, Merck, Germany), and, if applicable, an alkaline silicate solution were added to a
138 calcium nitrate solution ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 99%, Merck, Germany) using peristaltic pumps.
139 The reaction was performed under an argon flow (4.8, AirLiquide) to prevent any excessive
140 carbonation of precipitates. The pH of the suspension was adjusted by the addition of a 28%
141 ammonia solution (Merck, Germany) by means of a pH stat (Hanna Instruments), and the
142 temperature was controlled and regulated automatically with an external T-probe. The
143 suspension was continuously stirred and refluxed. After complete introduction of the
144 solutions, the suspension was matured for 24 h, and then filtered under vacuum. Finally, the
145 precipitates were dried at 70°C overnight.

146 The solution of soluble silicates was prepared from tetraethylorthosilicate (TEOS
147 $\geq 99\%$, Aldrich, Germany) *via* a sol-gel route. The original step consisted of the preparation of

148 a silica gel through the hydrolysis and condensation of this precursor. First, the alkoxide
149 groups (Si-OEt) of TEOS were hydrolyzed to silanol groups (Si-OH). The reaction was
150 catalyzed by a nitric acid aqueous solution ($7.5 \cdot 10^{-3} \text{M}$). This generates a considerable amount
151 of monomers (Si-OH), which aggregate and form a colloidal suspension called the “sol”. This
152 reaction, based on Klein et al. [80], was carried out by maintaining the molar ratio of
153 TEOS/ethanol/acidified water at 1.0/8.5/4.0 and the solution at 25°C in an ambient
154 atmosphere. Second, the condensation of the silanol or alkoxide groups was initiated by
155 increasing the solution pH to a basic level, about pH 8, with a few drops of pure 28%
156 ammonia solution (Merck, Germany). This reaction creates siloxane bridges (Si-O-Si),
157 leading to the formation of a silica gel. The gelation reaction was continued for about 12 h at
158 25°C. Finally, a solution of soluble silicates was obtained by depolymerization of the silica
159 gel. This was achieved through the nucleophilic attack of siloxane linkages (Si-O) by
160 hydroxide ions in pure 28% ammonia solution (pH > 11). After filtration through a Millipore
161 filtration unit (Magma nylon, 0.8 μm), the soluble silicate ion concentration of these solutions
162 was measured by means of an ion-exchange chromatography system (DIONEX DX-500)
163 equipped with a SiO_4^{4-} ion exchange column (AS4A) with a UV/Vis detector (410 nm). The
164 soluble silicate ion concentration in these solutions was, on average, $1076 \pm 34 \text{ ppm}$ (n=14
165 solutions). This value is a function of the preparation process (e.g. hydrolysis, condensation
166 and depolymerization reactions). Thereby, the volume of the solutions of soluble silicates was
167 adjusted for each synthesis.

168 A pure HA powder $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ was first prepared to be used as a reference
169 material for further SiHA syntheses and analyses. This was obtained at a pH and temperature
170 of precipitation of 9.5 and 50°C, respectively, with a maturation time of 24 h, and a reagent
171 (Ca/P) molar ratio equal to 10/6.

172 The reagent ratios used to prepare the SiHA powders were calculated by assuming,
173 first, the general formula $\text{Ca}_{10}(\text{PO}_4)_{6-y}(\text{SiO}_4)_y(\text{OH})_{2-y}(\text{V}_{\text{OH}})_y$ and, second, a constant molar
174 ratio $\text{Ca}/(\text{P}+\text{Si})$ equal to 10/6. The volume of the calcium and phosphate aqueous solutions
175 was fixed at 500 mL and 250 mL, respectively. Table 1 summarizes the amount (mol) of Ca,
176 P and Si used for each (Si)HA powder prepared with y equal to 0, 0.25, 0.50, 0.75, 1.00, 1.25;
177 designated hereafter as HA, $\text{Si}_{0.25}\text{HA}$, $\text{Si}_{0.50}\text{HA}$, $\text{Si}_{0.75}\text{HA}$, $\text{Si}_{1.00}\text{HA}$ and $\text{Si}_{1.25}\text{HA}$, respectively.
178 Precipitations were achieved at 50°C for 24 h of maturation time, according to the preliminary
179 tests performed on pure HA. Two parameters of precipitation were examined, the pH of
180 precipitation and the stoichiometric number y , *i.e.*, the amount of silicate theoretically
181 incorporated into the apatite structure. Precipitations were conducted either at pH 9.5,
182 previously established for HA synthesis, or at $\text{pH } 10.80 \pm 0.05$. The former was chosen
183 according to phosphate and silicate speciation curves, in order to have HPO_4^{2-} [81] and
184 H_3SiO_4^- ions [82] in solution as the main phosphate and silicate species, respectively. The
185 studied parameters are listed in Table 1.

186 The as-synthesized powders were heated under air using an alumina crucible. Aliquots
187 of each sample were calcined both at 400°C for 2 h (designated hereafter as “raw powders”),
188 in order to remove synthesis residues, and at 1000°C for 15 h under air according to ISO norm
189 13779-3 in order to characterize powders with a well-defined structure. The heating and
190 cooling rate was fixed at 4°C min^{-1} .

191 **2.2 Powder characterization**

192 **2.2.1 X-ray powder diffraction and Rietveld refinement**

193 Crystalline phases were identified by means of a Siemens D5000 $\theta/2\theta$ X-ray diffractometer
194 (XRD) using $\text{CuK}\alpha$ radiation and operating at 40 kV and 20 mA. XRD patterns were first
195 collected over the 2θ range of 10-60° at a step size of 0.03° and counting time of 4 s per step
196 in order to determine the phase composition. Crystalline phases detected in the patterns were

197 identified by comparison to standard patterns from the ICDD-PDF (International Center for
198 Diffraction Data-Powder Diffraction Files). In addition, high resolution XRD scans were
199 obtained on calcined powders from 10° to 120° in 0.02° steps with a counting time of 10.5 s
200 per step. These patterns were used to calculate the lattice parameters and the crystalline size
201 by full pattern matching. In this mode, the positions of the diffraction peaks and the
202 systematic extinctions are calculated from the cell parameters and the space group. The
203 intensities of the lines are adjusted by the refinement program and not calculated from the
204 atomic positions. The refinements were performed using the space group of the HA structure
205 P63/m (PDF 09-432) by means of the Topas software (Bruker, Germany). The initial cell
206 parameters were taken as $a = 9.42\text{\AA}$ and $c = 6.88\text{\AA}$.

207 The evolution of the crystallinity of the samples after calcination at 1000°C for 15 h
208 was evaluated by means of the full width at half maximum (FWHM) of the (211) peak at
209 $2\theta=31.8^\circ$, as it had the highest intensity and minimal overlap with neighboring peaks.

210 **2.2.2 Infrared spectroscopy**

211 Fourier transform infrared (FT-IR) measurements were carried out with the use of a MIR TF
212 VERTEX 70 Spectrometer by means of the ATR system. The spectra were recorded over the
213 range of $450\text{-}4000\text{ cm}^{-1}$ with a resolution of 2 cm^{-1} . Spectra were obtained by signal averaging
214 32 successive scans. Every measurement was at least duplicated on two independent powder
215 samples crushed by hand, with an agate pestle and mortar, and deposited on the ATR system.
216 Spectra were normalized with respect to the ν_4 band of the phosphate group at about 602 cm^{-1} ,
217 according to a classical procedure [83, 84].

218 **2.2.3 NMR spectroscopy**

219 NMR spectra were recorded on a Bruker AVANCE 300 spectrometer: $B_0 = 7.05\text{ T}$,
220 $\nu_0(^1\text{H}) = 300.13\text{ MHz}$, $\nu_0(^{31}\text{P}) = 121.49\text{ MHz}$, $\nu_0(^{29}\text{Si}) = 59.62\text{ MHz}$, using a CP-MAS Bruker
221 probe with 4 mm rotors spinning at 14 kHz for ^1H and ^{31}P and with 7 mm rotors spinning at 5

222 kHz for ^{29}Si . Chemical shifts are referenced to TMS for ^1H and ^{29}Si and 85% aqueous H_3PO_4
223 for ^{31}P . Single pulse and CP (cross-polarization) MAS experiments have been used to study
224 these materials. CP MAS relies on the heteronuclear dipolar interaction between an abundant
225 spin X (^1H here) and a low abundant nuclei Y (^{31}P and ^{29}Si here). It allows the study of local
226 molecular motion. The NMR parameters are summarized in Table 2.

227 **2.2.4 Electron microscopy (HR-TEM, SAED and EDX)**

228 High resolution transmission electron microscopy (HR-TEM) was conducted on calcined
229 powders ($1000^\circ\text{C}/15\text{ h}$) using a JEOL 2010 F Microscope at a voltage of 200 kV. Samples
230 were prepared by dispersing the powders in ethanol. After sonication at 40 W for 5 min, a
231 small drop of the suspension was placed onto copper mesh grids coated with a holey carbon
232 film. Finally, a thin coating of gold (10 nm in thickness) was sputtered on half of the grid.
233 Gold was then distributed as crystallized nano-domains which were used as a reference in the
234 selected area electron diffraction (SAED) patterns to calculate as precisely as possible the
235 lattice parameters. The SAED patterns obtained from regions with or without gold on the HA
236 part were the same.

237 **2.2.5 Elemental analysis**

238 The silicon, phosphorous and calcium content in powders was determined by inductively
239 coupled plasma atomic emission spectrometry (ICP/AES) (HORIBA Spectrometer, Jobin-
240 Yvon, Activa model). Powder samples were dissolved in a nitric acid solution (0.5 M).
241 Solutions were prepared (e.g. powder mass, dilution) in order to limit the measurement
242 uncertainty as well as to determine the concentrations of Ca, P and Si within the highest
243 sensitivity range of the ICP/AES device (around 30 ppm). Lastly, the carbon content in the
244 powders was determined using an elemental analyzer with an infrared detector (LECO CS-
245 444 carbon and sulfur analyzer).

246 The stoichiometric number y was determined by means of Eq. 4, assuming that
 247 powders containing $0 \leq \text{wt\%Si} \leq 2.81$ (or $0 \leq y \leq 1.00$) are pure monophasic SiHAs
 248 $\text{Ca}_{10}(\text{PO}_4)_{6-y}(\text{SiO}_4)_y(\text{OH})_{2-y}(\text{VOH})_y$, without any second crystalline or amorphous phase.

$$249 \quad \% \text{ wt Si} = (m_{\text{Si}} 100 / m_{\text{Si}y\text{HA}}) = (\text{C}_{\text{mSi}} V_{\text{sol}} 100) / (m_{\text{Si}y\text{HA}}) \quad (1)$$

$$250 \quad n_{\text{Si}} = (\% \text{ wtSi } m_{\text{Si}y\text{HA}}) / (M_{\text{Si}} 100) \quad (2)$$

$$251 \quad M_{\text{Si}y\text{HA}} = M_{\text{HA}} - (19.895 y_{\text{Si}}) \quad (3)$$

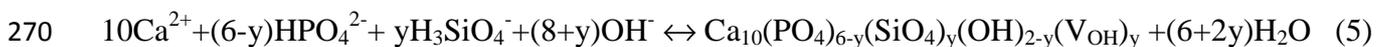
$$252 \quad y_{\text{Si}} = (n_{\text{Si}} M_{\text{Si}y\text{HA}}) / (m_{\text{Si}y\text{HA}}) = (\% \text{ wtSi } M_{\text{HA}}) / (100 M_{\text{Si}} + 19.985 \% \text{ wt Si}) \quad (4)$$

253 where % wt Si is the mass percentage of Si in the powder, C_{mSi} is the mass concentration of Si
 254 in the analyzed solution by ICP/AES (ppm or mg L^{-1}), V_{sol} is the volume of the analyzed
 255 solution (L), $m_{\text{Si}y\text{HA}}$ is the mass of the powder dissolved in the former solution (mg), and
 256 $M_{\text{Si}y\text{HA}}$, M_{HA} and M_{Si} are the molar masses of $\text{Si}y\text{HA}$, HA and Si, respectively (g mol^{-1}).

257 3. Results and discussion

258 3.1 Influence of pH on the SiHA phase composition

259 $\text{Si}_{0.50}\text{HA}$ powders were synthesized at 50°C with pH values equal to 9.50 ($\text{Si}_{0.50}\text{HA-9.5}$) and
 260 10.80 ($\text{Si}_{0.50}\text{HA-10.8}$). Fig. 1 shows the XRD diffractograms of powders calcined at 1000°C
 261 for 15 h. The pattern of the $\text{Si}_{0.50}\text{HA-9.5}$ sample presents two different crystalline phases
 262 matching the ICDD standard for hydroxyapatite (HA, PDF 9-432) and α -tricalcium phosphate
 263 (α -TCP, PDF 9-348). The $\text{Si}_{0.50}\text{HA-10.8}$ diffractogram displays only the characteristic
 264 diffraction lines of HA. This result indicates that the final composition of the powders is a
 265 function of silicate ion speciation. H_3SiO_4^- or more basic forms of silicate ions have to be
 266 maintained during the synthesis to obtain a thermally stable SiHA phase, *i.e.* a monophasic
 267 powder after heat treatment at $1000^\circ\text{C}/15$ h. Therefore, herein, the following results are
 268 presented for powders prepared at pH 10.8. Under these synthesis conditions, the general
 269 reaction of precipitation can be written as follows:



271 To our knowledge, this is the first time that a true precipitation reaction has been given for
272 SiHA.

273 **3.2 Influence of the Si content (y_{Si})**

274 **3.2.1 X-ray diffraction analysis**

275 Fig. 2 shows the diffraction patterns of HA and Si_yHA ($y_{\text{Si}} = 0.25, 0.50, 0.75, 1.00$ and 1.25
276 mol) raw powders. Each pattern matches well with the standard pattern of HA (PDF 9-432).
277 No other phase was detected. The samples exhibit broad diffraction lines which indicate very
278 small crystallites and/or low crystallinity. More, the FWHM increased with silicon content.
279 The diffractograms of heat treated powders ($1000^\circ\text{C}/15$ h) are displayed in Fig. 3. They
280 present no secondary phase besides hydroxyapatite for a silicon content up to $y_{\text{Si}} = 1.00$. On
281 the other hand, HA (PDF 9-432) and α -TCP (PDF 9-348) were detected in the pattern of
282 $\text{Si}_{1.25}\text{HA}$ calcined at 1000°C for 15 h. Moreover, as observed for the raw powders, the
283 crystallinity of Si_yHA calcined powders with $1 \geq y \geq 0$ decreased with an increase in the
284 silicate content. Indeed, the higher the Si content, the higher the FWHM of the diffraction
285 line. For instance, the FWHM of the diffraction line at 31.8° (2θ) increased as follow: 0.070
286 (HA) < 0.096 ($\text{Si}_{0.25}\text{HA}$) < 0.160 ($\text{Si}_{0.50}\text{HA}$) < 0.164 ($\text{Si}_{0.75}\text{HA}$) < 0.170 ($\text{Si}_{1.00}\text{HA}$). This is due
287 to both direct (decrease in the crystallinity) and indirect (decrease in the crystallite size)
288 effects of Si, but the individual contributions of these effects cannot be evaluated by X-ray
289 diffraction.

290 The XRD patterns indicate that whatever the amount of silicon ranging between $0 \leq$
291 $\text{wt\%Si} \leq 3.51$ (or $0 \leq y \leq 1.25$), the crystalline phase of the precipitates is a hydroxyapatite
292 (PDF 09-432). Thereby, silicon can be incorporated in the apatitic structure, or in an
293 amorphous phase or in both phases. However, only precipitates containing up to 2.81 wt%Si
294 (or $y = 1.00$) are thermally stable at 1000°C for 15 h.

295 **3.2.2 Lattice parameter refinement**

296 Refinement was performed on monophased heat-treated powders ($0 \leq y \leq 1.00$). The results,
297 displayed in Table 3, reveal changes in the apatitic structure parameters. The Si substitution
298 causes c -axis and unit cell volume expansion as well as a reduction in the mean crystallite
299 size. The same observation was reported in other studies [10, 17, 32, 70, 85]. On the other
300 hand, and in contrast to the linear increase of the lattice parameter c with an increasing
301 amount of Si, the a lattice parameter fluctuates at random between 9.416 Å and 9.428 Å.
302 These results highlight that all or part of the silicon is incorporated into the apatitic structure.

303 3.2.3 Elemental analysis

304 The experimental Ca/(P+Si) molar ratios of the final calcined powders are presented in Table
305 1. Values are in accordance with the expected ones (1.67) for all compositions, considering
306 experimental error. Moreover, the calcined powders were not carbonated since the carbon
307 content by weight was equal to 0.03, 0.07, 0.05, 0.05 and 0.05 wt% (± 0.02 wt%) for HA,
308 Si_{0.25}HA, Si_{0.50}HA, Si_{0.75}HA and Si_{1.00}HA, respectively.

309 The results, presented in Table 1, show that the chemical formulas of SiHA calculated
310 first by the measured silicon contents, then by the the theoretical formula $\text{Ca}_{10}(\text{PO}_4)_6$
311 $_y(\text{SiO}_4)_y(\text{OH})_{2-y}(\text{V}_{\text{OH}})_y$ and finally using Eq. 4 are in a good agreement with the nominal ones.

312 3.2.4 Electron microscopy

313 A low magnification bright-field image of heat-treated Si_{1.00}HA particles is shown in Fig. 4a.
314 SiHA powders are composed of round particles, smaller than 100 nm in diameter for
315 Si_{1.00}HA, whereas the HA powder shows bigger acicular crystals [86]. At higher
316 magnification, grains do not present any dislocation or disorder within the central region.
317 Similarly, high-resolution lattice images of the grain surface do not show any amorphous or
318 disordered layer (see Fig. 4b). A selected area electron diffraction (SAED) pattern obtained on
319 the same grain is displayed in Fig. 5a. SAED patterns show a diffraction pattern with well-
320 defined spots regardless of the selected area. This indicates the high crystallinity of the heat-

321 treated SiHA powders and the homogeneity of the crystals. Second, a very good agreement
322 appears between the SAED patterns and the PDF card 09-432 of HA as identified by X-ray
323 diffraction. Moreover, no halo ring was observed, confirming the absence of the amorphous
324 phase. Fig. 5b shows a SAED pattern for the [010] zone axis. Two other SAED patterns were
325 obtained for the [1-10] zone axis. Indexation of these patterns allows for calculating the c/a
326 ratio by means of the interval between two spots. Thus, for instance, according to the
327 hexagonal crystallographic structure identified by X-ray diffraction and the distance D_1 and
328 D_2 between two spots along the [h00] and [001] directions ([010] zone axis, Fig. 5b),
329 respectively, the c/a ratio was calculated as follows:

$$330 \quad (c/a)^2 = \frac{3}{4} (D_1/D_2)^2 \quad (6)$$

331 The results are the average of about ten intervals per pattern. A c/a ratio of $0.730 \pm$
332 0.005 was determined. Moreover, other experimental patterns for different zone axes (not
333 shown here) were obtained and compared to theoretical electron diffraction patterns
334 calculated by means of the Java Electron Microscopy Simulation (JEMS) software [87]. The
335 results indicate that the experimental and simulated patterns are perfectly superimposed for
336 $0.734 \geq c/a \geq 0.729$. Additionally, the c/a ratio value from PDF 09-432 (0.7309) is included in
337 this range.

338 **3.2.5. Solid-state NMR analysis**

339 **3.2.5.1. ^{31}P MAS NMR**

340 The ^{31}P MAS NMR spectra of HA, $\text{Si}_{0.50}\text{HA}$ and $\text{Si}_{1.00}\text{HA}$ calcined at 1000°C for 15 h are
341 shown in Fig. 6a. The spectra present a main narrow peak which corresponds to the single P
342 site of hydroxyapatite [88], and whose position shifts to higher frequencies with increasing
343 silicon content: 2.8 ppm for HA, 3.0 ppm for $\text{Si}_{0.50}\text{HA}$, and 3.1 ppm for $\text{Si}_{1.00}\text{HA}$. Likewise,
344 the FWHM broadens: $166 \text{ Hz} < 188 \text{ Hz} < 233 \text{ Hz}$ for raw HA, $\text{Si}_{0.50}\text{HA}$ and $\text{Si}_{1.00}\text{HA}$,
345 respectively (figure not included), and $55 \text{ Hz} < 132 \text{ Hz} < 233 \text{ Hz}$ for calcined HA, $\text{Si}_{0.50}\text{HA}$

346 and Si_{1.00}HA, respectively (Fig. 6a). The chemical shift and peak broadening indicate that the
347 SiHA structure is different from a perfect hydroxyapatite short-range structure. The increasing
348 line width suggests an increase in local disorder around those phosphate groups where Si is
349 incorporated in the HA structure. Besides the 2.8 ppm peak, two additional weak broad peaks
350 at 4.6 and 5.8 ppm were detected in the ³¹P spectra. The peak at 4.6 ppm was only revealed in
351 the ¹H → ³¹P CP MAS spectrum (Fig. 7b). HA powder did not present the 4.6 ppm signal (Fig.
352 7a). According to Hartmann et al. [89], the ³¹P MAS peak at 5.8 ppm corresponds to PO₄
353 tetrahedra in a proton-free region of a hydroxyapatite channel structure containing OH
354 vacancies (*i.e.* along the crystallographic *c*-axis). These proton-free regions, observed in
355 oxyhydroxyapatite Ca₁₀(PO₄)₆(OH)_{2-2x}(O)_x(V_{OH})_x (OHA), must exist in silicon-substituted
356 hydroxyapatite Ca₁₀(PO₄)_{6-y}(SiO₄)_y(OH)_{2-y}(V_{OH})_y (SiHA), or silicon-substituted
357 oxyhydroxyapatite Ca₁₀(PO₄)_{6-y}(SiO₄)_y(OH)_{2-y-2x}O_x(V_{OH})_{x+y} (SiOHA). The assignment of the
358 peak at 4.6 ppm, that seems related to the incorporation of Si in the apatite lattice, will be
359 discussed later on (section 3.2.5.3).

360 3.2.5.2. ¹H MAS NMR

361 The ¹H MAS NMR spectra of pure HA, Si_{0.50}HA and Si_{1.00}HA powders calcined at 1000°C
362 for 15 h are shown in Fig. 6b. Two signals were observed, one sharp at 0 ppm and one
363 broader at about 5.2 ppm. The former (0 ppm) is characteristic of OH groups in crystalline
364 HA [89]. Moreover, and similarly to the ³¹P signal at 2.8 ppm, the greater the Si content in the
365 apatitic structure of the calcined powders, the broader the FWHM of the signal at 0 ppm
366 (FWHM = 108 Hz for HA, 191 Hz for Si_{0.5}HA and 228 Hz for Si_{1.00}HA). This suggests a
367 change in the environment around the hydroxyl groups, highlighting the modification of O-H
368 bond distances due to Si. As postulated by Gomes et al., this concomitant broadening of the
369 ³¹P and ¹H resonances with Si content confirms the incorporation of Si atoms into the apatite
370 lattice [30]. The signal at 5.2 ppm was attributed by Hartmann et al. to OH⁻ positions missing

371 only one neighboring ion in OHA channels [89]. This type of OH⁻ group along the channels of
372 the apatite structure leads to the formation of “proton pairs” stabilized by a hydrogen bond.
373 The intensity of this proton position is higher in SiHA samples than in HA, but does not seem
374 to be an exclusive function of the Si content (see Fig. 6b). Therefore, ¹H MAS NMR indicates
375 the presence of two types of OH⁻ groups along the channels of the apatite structure: an
376 unchanged proton line position with continuous OH⁻ groups (0 ppm) and OH⁻ pairs
377 surrounded by vacancies (5.2 ppm).

378 3.2.5.3. ¹H-³¹P HETCOR CP MAS

379 To confirm that the entire proton position is located in the apatite channel structure, two-
380 dimensional ¹H-³¹P HETCOR CP MAS experiments were performed on raw and calcined
381 powders. Fig. 8 presents ¹H-³¹P HETCOR CP MAS spectra recorded on raw Si_{1.00}HA (Fig.
382 8a) and calcined Si_{0.50}HA (Fig. 8b) powders. The spectra show two main 2D correlation peaks
383 on both raw and calcined powders. The same peaks were observed for Si_{0.25}HA, Si_{0.50}HA and
384 Si_{1.00}HA (HETCOR CP MAS results for calcined Si_{0.25}HA and Si_{1.00}HA not included). The
385 stronger one (peak A, Fig. 8b), characteristic of an ideal HA structure, is due to the dipolar
386 interaction between the P sites ($\delta(^{31}\text{P}) = 2.8$ ppm) and the OH sites ($\delta(^1\text{H}) = 0$ ppm). It
387 corresponds to an undisturbed hydroxyapatite short-range structure [89]. The second one
388 (peak B, Fig. 8b) appears between the ³¹P signal at 4.6 ppm and the ¹H signal at 5.2 ppm. Its
389 intensity increases with heat treatment (Fig. 8) and the Si content (data not included). This
390 correlation is not observed for the HA sample (data not included). Moreover, a low extent of
391 peak B is observed with the main ³¹P peak at 2.8 ppm (peak C, Fig. 8b). The intensity of this
392 2D correlation peak becomes stronger with Si content as well. More generally, the higher the
393 amount of Si incorporated into the SiHA structure, the stronger the dispersion of the main
394 correlation between A and B along the ³¹P axis. Finally, as expected, the HETCOR spectra
395 show no correlation peak of the ³¹P line at 5.8 ppm, confirming that it corresponds to a

396 proton-free phosphate position. To conclude, first the broadening of peak A indicates an
397 increase in the distribution of P-H bond distances when silicate is incorporated into the HA
398 structure, corresponding to the loss of local ordering in the phosphate environment. Second,
399 peaks B and C correspond to two distinct P sites in the vicinity of isolated pairs of protons
400 (OH⁻ pairs) present in the channel structure of silicon-substituted oxyhydroxyapatite
401 $\text{Ca}_{10}(\text{PO}_4)_{6-y}(\text{SiO}_4)_y(\text{OH})_{2-y-2x}\text{O}_x(\text{V}_{\text{OH}})_{y+x}$ (SiOHA): (i) peak B represents a very strongly
402 distorted hydroxyapatite short-range structure and (ii) peak C represents a less distorted one.
403 Peak B is clearly related to SiO_4 substitution and proves once again that charge compensation
404 implies OH vacancies along the channels, leading to the formation of OH⁻ pairs in the vicinity
405 of the substituted PO_4 tetrahedra. Peak C can be due to SiO_4 substitution as well as
406 dehydration of SiHA, both leading to the formation of OH vacancies in SiOHA.

407 **3.2.5.4. ²⁹Si MAS NMR**

408 The ²⁹Si MAS NMR spectra of $\text{Si}_{1.00}\text{HA}$ powders, raw and calcined, are shown in Fig. 9. The
409 raw powders present two resonances: one sharp at about -72 ppm that is clearly attributed to
410 SiO_4^{4-} (Q^0) in the apatitic structure [90], and a broad peak at about -100/-110 ppm which
411 corresponds to Q^3/Q^4 silicon species [16]. The results confirm that the main part of the silicate
412 is incorporated into the HA lattice during precipitation, according to the main reaction Eq. 1.
413 The rest of the silicates are adsorbed at the crystallite surface, as observed for phosphates
414 during the precipitation of apatitic calcium phosphates [91, 92]. After calcination, the broad
415 peak at -100/-110 ppm disappears without the detection of new signals, similar to those
416 reported in the literature (Si-TCP or $\alpha\text{-Ca}_3\text{Si}_3\text{O}_9$) [16, 30, 38]. The same results were obtained
417 for the $\text{Si}_{0.25}\text{HA}$ and $\text{Si}_{0.50}\text{HA}$ samples (data not included).

418 **3.2.6. FT-IR analysis**

419 **3.2.6.1 As-synthesized samples**

420 The IR spectra of the raw HA and SiHA powders with a silicon content up to $y_{\text{Si}} = 1.25$ mol
421 (figure not included) present strong similarities and exhibit intense bands characteristic of
422 hydroxyapatite. They correspond to the four vibrational modes of phosphate groups (ν_1 , ν_2 , ν_3 ,
423 and ν_4), and the stretching (ν_S) and librational (ν_L) modes of the hydroxide groups [81, 92-94].
424 The intensity of the latter, at 630 cm^{-1} (ν_L) and 3570 cm^{-1} (ν_S), clearly decreased with the
425 amount of silicon. These results confirm that the as-synthesized precipitates exhibit the
426 hydroxyapatite phase, regardless of the amount of silicon ranging between $0 \leq \text{wt\%Si} \leq 3.51$
427 (or $0 \leq y \leq 1.25$), as observed on the diffractograms (Fig. 2). A more accurate description of
428 the infrared spectra of the as-synthesized SiHA powders is available in a complementary
429 article [95].

430 **3.2.6.2 Calcined samples – general observations**

431 Fig. 10 compares the infrared spectra of heat-treated HA and SiHA powders ($1000^\circ\text{C}/15 \text{ h}$).
432 The HA and Si_yHA with $0 \leq y \leq 1.0$ powders mainly present bands characteristic of
433 hydroxyapatite with the ν_1 (962 cm^{-1}), ν_2 (473 cm^{-1}), ν_3 (1021 and 1085 cm^{-1}) and ν_4 (562 and
434 600 cm^{-1}) modes of PO_4^{3-} , as well as the stretching (ν_S : 3572 cm^{-1}) and librational (ν_L : 629 cm^{-1})
435 modes of hydroxide groups [81, 92, 94]. A shoulder at 947 cm^{-1} was also observed for HA
436 and SiHA powders. The relative intensity of this shoulder was slightly higher for SiHA than
437 for HA, but did not seem to be a function of the amount of Si. Besides the low hydroxyapatite
438 vibrations, the spectrum of the $\text{Si}_{1.25}\text{HA}$ powder presents mainly the characteristic bands of α -
439 TCP and traces of β -tricalcium phosphates (β -TCP): the weak band at 495 cm^{-1} ascribed to
440 the O-P-O ν_2 vibrational mode of β -TCP, the bands due to the splitting of the ν_4 mode at 567
441 cm^{-1} in α -TCP (551 , 560 , 580 , 595 and 611 cm^{-1}), the bands at 945 and 955 cm^{-1} assigned to
442 the degenerated symmetric P-O stretching vibration of the phosphate ions (ν_1) in β - and α -
443 TCP, respectively, and the bands corresponding to the strong asymmetric P-O stretching
444 mode (ν_3) for β -TCP (988 , 1025 cm^{-1}) and α -TCP (988 , 1013 , 1025 , 1031 and 1055 cm^{-1}) [52,

445 96]. This result is complementary to the X-ray diffraction pattern (Fig. 3) and indicates that
446 the $\text{Si}_{1.25}\text{HA}$ apatitic precipitate decomposes during heat treatment with the formation of TCP
447 and amorphous silica species.

448 **3.2.6.3. Calcined samples – accurate IR band assignment of the pure SiHA phase**

449 As opposed to HA, the FT-IR spectra of the calcined Si_yHA powders, with $0 \leq y \leq 0.75$,
450 exhibit nine new bands or shoulders (sh) at 491 (sh), 504, 528, 750, 840, 893, 930 (sh), 985
451 (sh) and 1002 (sh) cm^{-1} . The intensity of these new vibrations increased with the amount of
452 Si, except for the weak band at 840 cm^{-1} and the shoulder at 930 cm^{-1} which remained
453 relatively constant. Conversely, the intensity of the absorption bands attributed to the four
454 vibrational modes of PO_4^{3-} and both modes of OH^- in hydroxyapatite decreased as the amount
455 of Si increased in the range $0 \leq y \leq 1.00$ (e.g. $\nu_S \text{OH}^- : 0.49 (\text{HA}) > 0.39 (\text{Si}_{0.25}\text{HA}) > 0.35$
456 $(\text{Si}_{0.50}\text{HA}) > 0.12 (\text{Si}_{0.75}\text{HA}) > 0.07 (\text{Si}_{1.00}\text{HA})$). More precisely, the ν_L mode decreased to the
457 detection limit for $y = 1$, while the stretching the OH^- band decreased and broadened. In fact,
458 two new vibrations at 3565 and 3552 cm^{-1} , close to the main one at 3572 cm^{-1} , were detected
459 (Fig. 10). This confirms the mechanism of charge compensation with the creation of OH^-
460 vacancies, and highlights the new environment and distribution of OH^- ions along the
461 channels of the SiHA structure [93, 97] and, more importantly, the formation of low hydrogen
462 bonding between closed O^{2-} and OH^- ions (H-bonds cause a shift of 20 cm^{-1} to the low
463 wavenumber of the main band at 3572 cm^{-1} [98]). The FT-IR spectrum of $\text{Si}_{1.0}\text{HA}$ displays
464 the same eight new bands as those detected on $\text{Si}_{0.25}\text{HA}$, $\text{Si}_{0.50}\text{HA}$ and $\text{Si}_{0.75}\text{HA}$. However,
465 their relative intensity decreased significantly, reaching the detection limit for the band at 750
466 cm^{-1} and with a complete disappearance of the bands at 491, 504, and 528 cm^{-1} . Additionally,
467 seven new vibrations appeared at 500, 515, 535, 683, 798 and 873 cm^{-1} . They were
468 accompanied by an intensification of the shoulder at 947 cm^{-1} . The bands at 683, 798 and 870
469 cm^{-1} were also detected in spectrum of $\text{Si}_{1.25}\text{HA}$ calcined powder. According to these results

470 and an accurate review of the bibliography, summarized in Table 4, it is clear that the nine
471 vibrations at 493, 504, 528, 750, 840, 893, 930, 985 and 1002 cm⁻¹ are related to the
472 incorporation of Si into the hydroxyapatite structure. The changes detected in the sample
473 Si_{1.0}HA were due to the start of decomposition of the SiHA phase, emphasized by a decrease
474 in the nine characteristic bands and the formation of an amorphous silica phase which
475 presents vibrations at 500, 515, 535, 683, 798 and 873 cm⁻¹. Silica was not detected by NMR
476 due to the low amount of ²⁹Si in the samples (high detection limit). The shoulder at 947 cm⁻¹
477 could be attributed to β-TCP, which exhibits a symmetric P-O stretching mode ν₁ at 945 cm⁻¹
478 [96]. However, this assumption is not convincing since other bands assigned to β-TCP are
479 absent. In fact, this shoulder is a contribution of the Si-O in plane stretching vibrations in Si-
480 OH and Si-O⁻ on the surface of the grains, and the symmetric stretching (ν₁) vibration of
481 orthophosphate groups in the neighborhood of OH vacancies along the channels. The former,
482 reported at 950 cm⁻¹ (see Table 4), shifted toward lower frequencies due to hydrogen bonding
483 between surface groups, thus creating the component at 930 cm⁻¹. The band at 950 cm⁻¹ is
484 commonly observed for oxy-hydroxyapatite Ca₁₀(PO₄)₆(OH)_{2-2x}O_x(V_{OH})_x, [93, 99-102].
485 Therefore, in the context of this study, OH⁻ vacancies (V_{OH}) are due first to the incorporation
486 of SiO₄ into the HA structure, and second to the partial dehydration of SiHA to SiOHA as
487 follows:



489 3.2.6.4. Calcined samples – OH vacancies and the limit of incorporation of Si in HA

490 The amount of hydroxide (%OH) in the silicon-substituted hydroxyapatite structure is
491 reported versus the amount of silicon (mol mol_{SiHA}⁻¹) in Fig. 11. The theoretical values of OH
492 were plotted by considering the formula Ca₁₀(PO₄)_{6-y}(SiO₄)_y(OH)_{2-y}(V_{OH})_y (*i.e.* %OH = 100 -
493 50 y). The experimental values were determined at room temperature on the calcined samples
494 (1000°C/15 h) by means of the integrated area of the ν_L and ν_S OH bands. The average of the

495 integrated areas obtained for HA ($y=0$) was assumed to be representative of the full
496 occupancy of the OH position within the channels (100% OH). The amount of OH vacancies
497 (V_{OH}) determined experimentally was always higher than the theoretical one with respect to
498 the chemical formula $Ca_{10}(PO_4)_{6-y}(SiO_4)_y(OH)_{2-y}(V_{OH})_y$. In other words, the dehydration of
499 the calcined powders was greater than expected, and a higher Si doping level led to a greater
500 difference. Thus, the incorporation of Si into the hydroxyapatite structure generates OH
501 vacancies first to maintain the charge balance ($Ca_{10}(PO_4)_{6-y}(SiO_4)_y(OH)_{2-y}(V_{OH})_y$) and second
502 from another phenomena, which is an increasing function of the amount of Si. A possible
503 explanation is an increase of the mobility of OH ions along the channel axis (local disorder)
504 and their subsequent rate of diffusion and debonding from the HA lattice [103]. HA
505 decomposition is a dynamic process, mainly controlled by the degree of dehydration [104-
506 106]. In fact, the thermal stability of hydroxyapatite was found to depend on the fraction of
507 V_{OH} and O^{2-} ions in the channels. Several “critical values” of V_{OH} in OHA $Ca_{10}(PO_4)_6(OH)_{2-}$
508 $2xO_x(V_{OH})_x$ have been reported beyond which the apatite channel structure is destroyed: $x \leq$
509 0.80 [103], $x \leq 0.75$ [107], $x \leq 0.50$ [89]. However, the most probable limit comes from work
510 of Heughebaert and Montel on the crystallization of calcium phosphates during precipitation
511 [108, 109]. They determined that the apatitic structure appears only when at least 25% of the
512 OH^- positions along the channels are really occupied. In other words, the apatitic structure
513 appears when $V_{OH} < 1.5$. Therefore, the theoretical maximum limit of incorporation of Si into
514 a hexagonal apatitic structure is $y < 1.5$ as regards the formula $Ca_{10}(PO_4)_{6-y}(SiO_4)_y(OH)_{2-}$
515 $y(V_{OH})_y$. However, due to the dehydration reaction (Eq. 7), this limit is a function of the
516 temperature and the atmosphere of heat treatment. This assumption can explain why the
517 $Si_{1.0}HA$ and $Si_{1.25}HA$ powders, which showed an apatitic structure after calcination at $400^\circ C$
518 for 2 h, were not thermally stable at $1000^\circ C$ for 15 h in contrast to $SiHA$ with a silicon
519 content ranging between $0 \leq y \leq 0.75$. Control of the degree of hydroxylation, *i.e.* the partial

520 steam pressure $p_{\text{H}_2\text{O}}$, is necessary to control the thermal stability of the SiHA phase. This
521 assumption is confirmed by the infrared spectra obtained from the $\text{Si}_{1.0}\text{HA}$ sample heat treated
522 at 1000°C for 15 h under different partial pressures of steam, $p_{\text{H}_2\text{O}}$, in mbar (Fig. 12); a higher
523 $p_{\text{H}_2\text{O}}$ led to a more stable silicon-substituted apatite phase. Thus, the $\text{Si}_{1.0}\text{HA}$ powder, after
524 calcination under high steam pressure ($p_{\text{H}_2\text{O}} = 200$ mbar), presented only the characteristic
525 bands of SiHA with traces of amorphous silica at the IR spectroscopy detection limit
526 ($\approx 0.1\text{wt}\%$), whereas the same sample heat-treated under dry argon gas ($p_{\text{H}_2\text{O}} = 0$ mbar)
527 contained amorphous silica species as well as TCP (α and β). Moreover, the vibrations related
528 to Si-O in SiHA (750 and 890 cm^{-1}) as well as the vibrational modes ν_3 and ν_4 of the phosphate
529 groups in HA decreased with a decrease in $p_{\text{H}_2\text{O}}$. Conversely, the vibrations due to amorphous
530 silica species, at 683 , 798 and 873 cm^{-1} , and TCP (e.g. 731 , 942 , 997 and 1137 cm^{-1}) increases
531 with a decrease in $p_{\text{H}_2\text{O}}$.

532 These results confirm that the formation of amorphous silica and the decomposition of
533 the SiHA phase are correlated. More precisely, the detection of amorphous silica indicates the
534 start of decomposition, which is followed by the formation of TCP (α and β).

535 Finally, Trombe and Montel reported the variation of a from 9.421 \AA to 9.402 \AA ,
536 without a significant variation in the c lattice parameter, when HA transforms into OHA,
537 $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{0.5}\text{O}_{0.75}(\text{V}_{\text{OH}})_{0.75}$ [102]. Thus, SiHA dehydration can explain the random
538 fluctuations in the a lattice parameter observed in this work and in the literature [30].

539 **4. Conclusion**

540 This paper presents a new route to synthesize monophasic silicon-containing hydroxyapatites
541 $\text{Ca}_{10}(\text{PO}_4)_{6-y}(\text{SiO}_4)_y(\text{OH})_{2-y}(\text{V}_{\text{OH}})_y$ (SiHAs) with controlled stoichiometry. The combination of
542 solid state nuclear magnetic resonance (NMR) and IR spectroscopy (IR) demonstrated that
543 silicate substitution for phosphate creates OH vacancies along the channels. Moreover, Si
544 substitution increases the local disorder and the mobility of OH ions along these channels and

545 their subsequent rate of diffusion and debonding from the HA lattice. The control of the
546 degree of hydroxylation of the SiHA phases, *i.e.* their thermal stability, can be carried out by
547 means of the partial steam pressure, p_{H_2O} . Additionally, an exhaustive description of the
548 infrared bands related to the incorporation of silicate groups into the HA structure was
549 established. It allowed us to determine that the literature has incorrectly attributed some
550 infrared bands to silicate groups in the apatite structure. These bands, e.g. 683, 798 and 873
551 cm^{-1} , due in fact to amorphous silica phases, raise questions regarding the phase purity of the
552 great majority of biologically evaluated SiHA bioceramics.

553

554

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558

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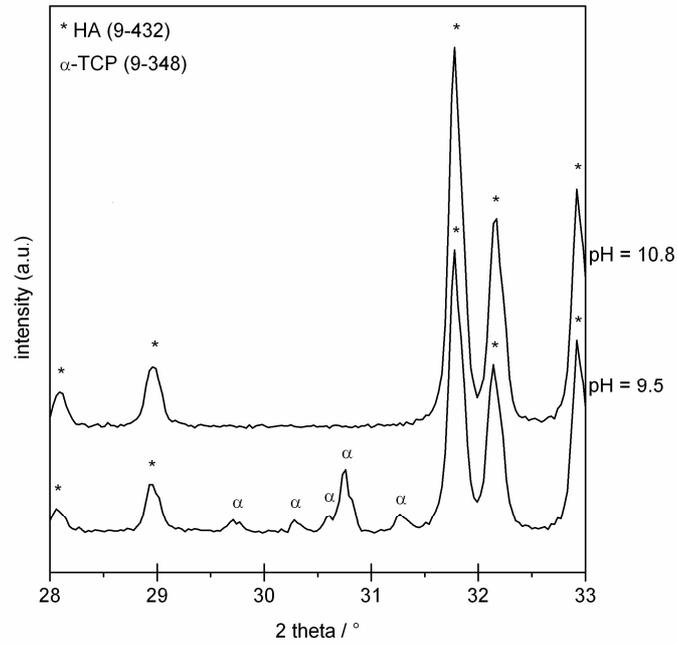


Fig. 1. XRD patterns of heat treated $\text{Si}_{0.50}\text{HA}$ powders prepared at 50°C and maintained at pH 9.5 and 10.8.

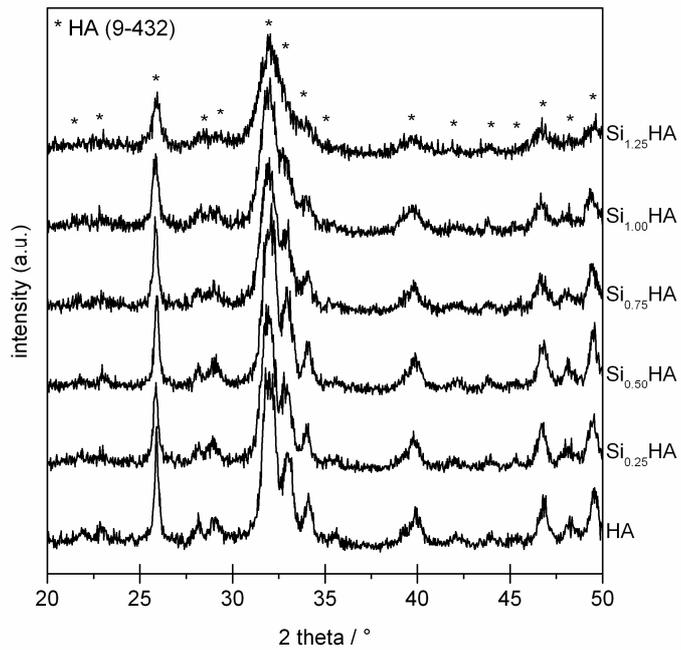


Fig. 2. X-ray diffraction patterns of raw SiHA powders synthesized at $\text{pH} = 10.8$ with different silicon concentration.

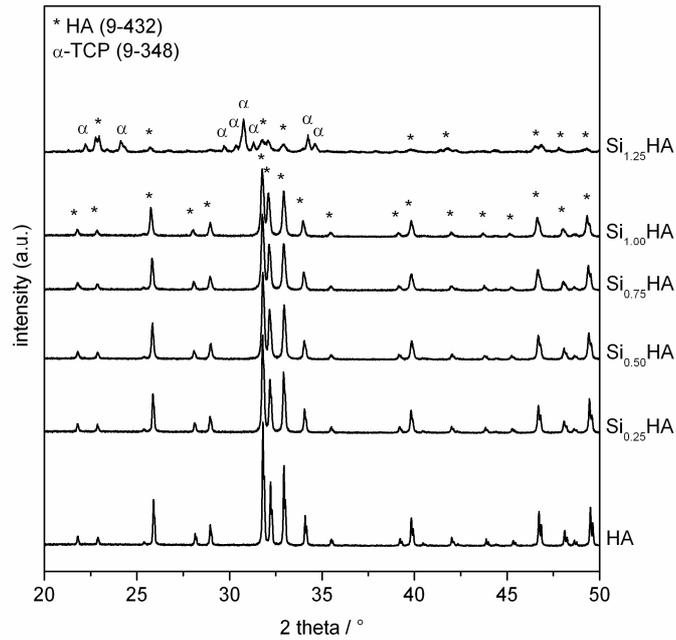


Fig. 3. X-ray diffraction patterns of heat treated (1000°C/15h) SiHA powders synthesized at pH = 10.8 with different silicon concentration.

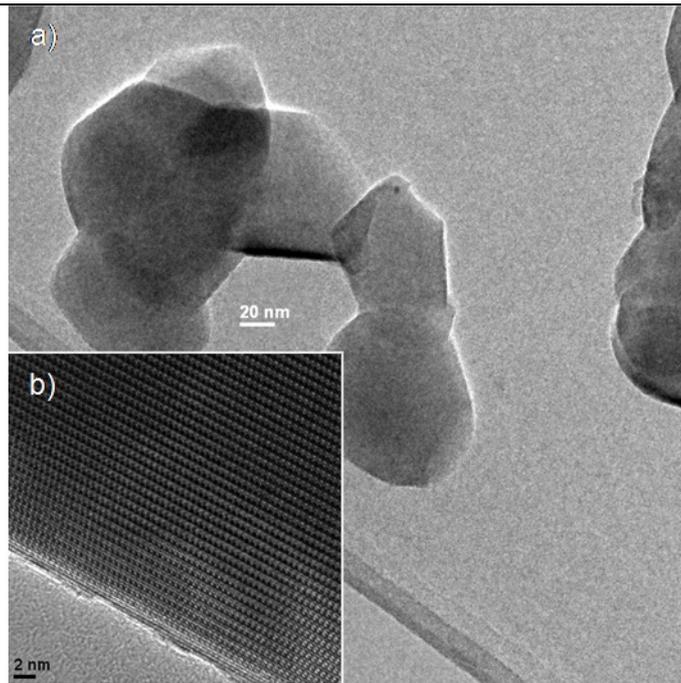


Fig. 4: (a) TEM micrograph of heat treated $\text{Si}_{1.0}\text{HA}$ crystallites (1000°C/15h), (b) High-resolution lattice image of a heat treated $\text{Si}_{1.0}\text{HA}$ particle (1000°C/15h) along the zone axis [01-1]; plane spacing of 8.2Å.

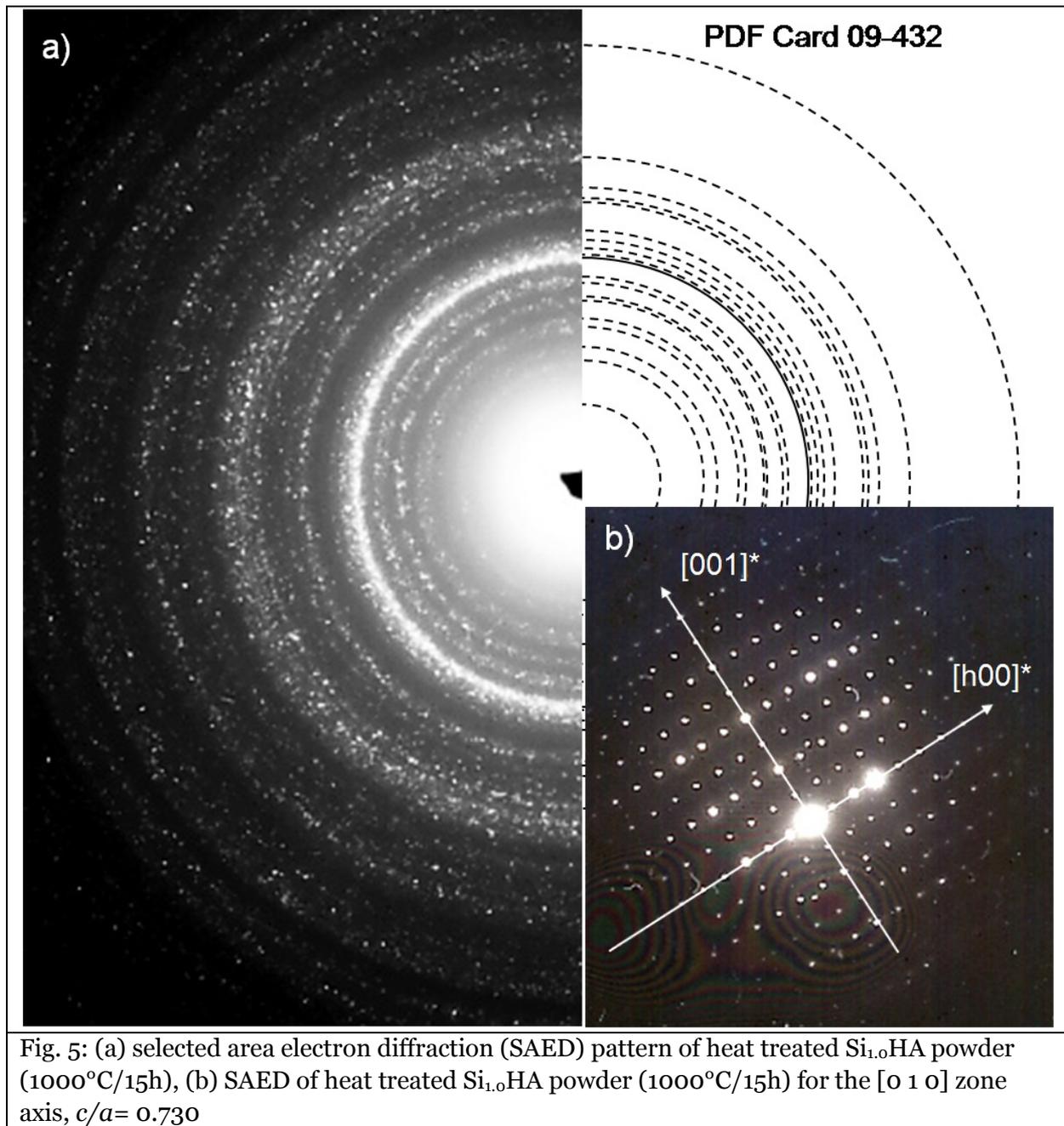


Fig. 5: (a) selected area electron diffraction (SAED) pattern of heat treated $\text{Si}_{1.0}\text{HA}$ powder (1000°C/15h), (b) SAED of heat treated $\text{Si}_{1.0}\text{HA}$ powder (1000°C/15h) for the $[0\ 1\ 0]$ zone axis, $c/a= 0.730$

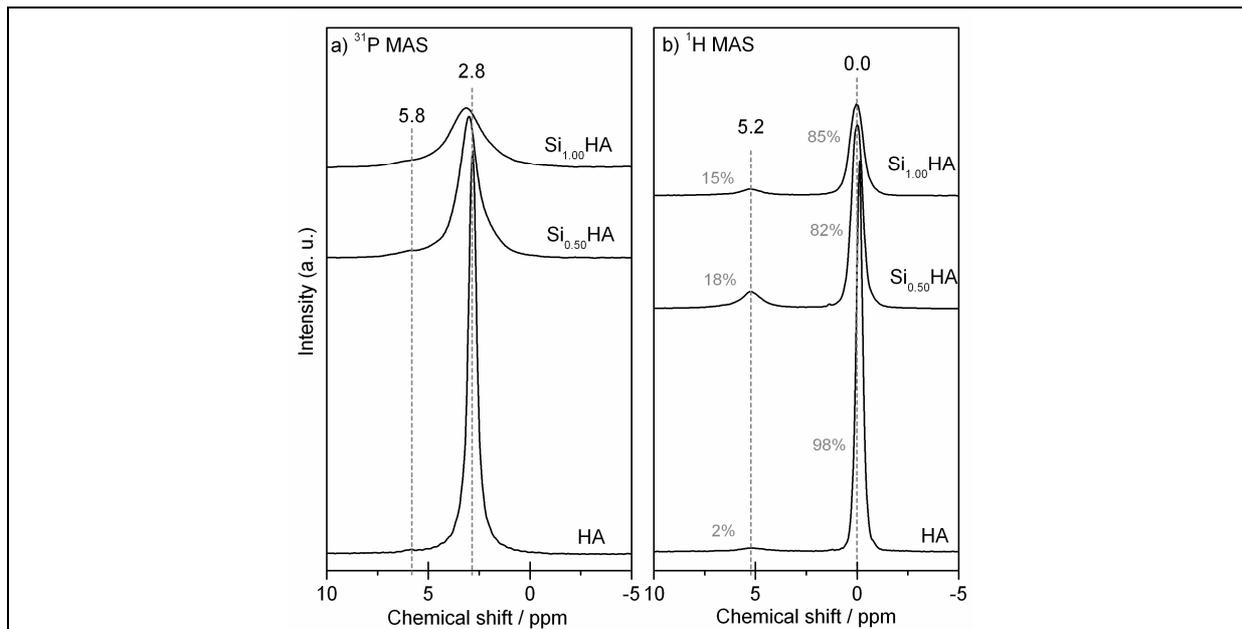


Fig. 6. (a) ^{31}P and (b) ^1H MAS-NMR spectra of the HA, $\text{Si}_{0.50}\text{HA}$ and $\text{Si}_{1.00}\text{HA}$ powders calcined at 1000°C for 15h.

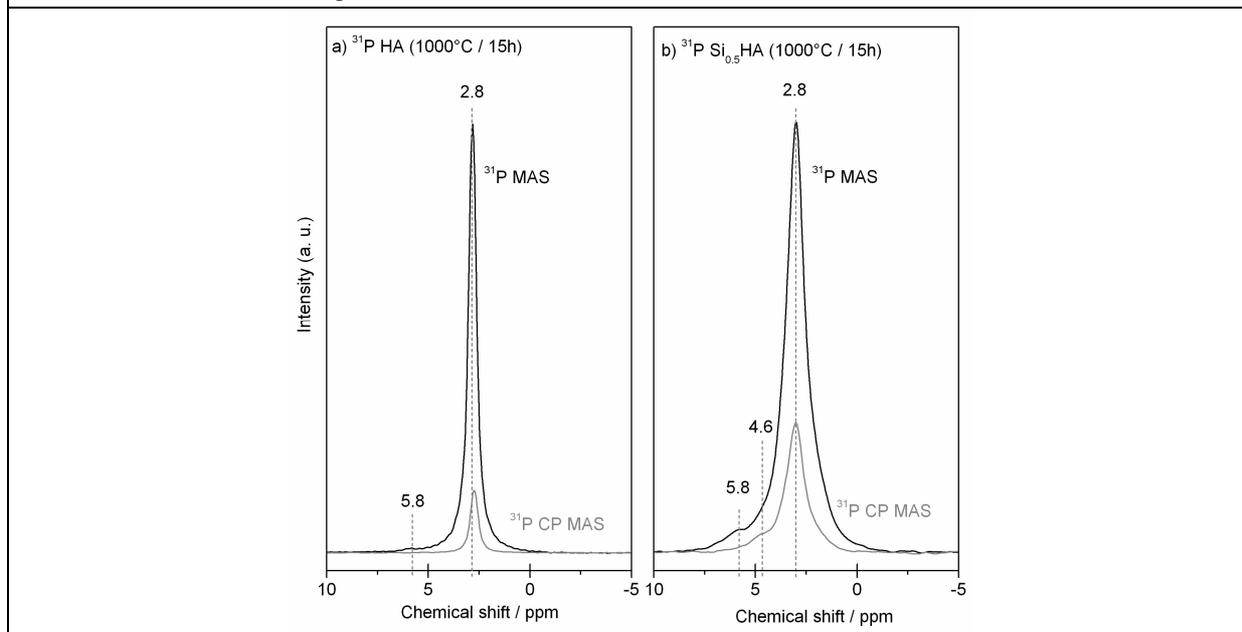


Fig. 7. Comparison between ^{31}P MAS-NMR and CP MAS NMR spectra of (a) HA and (b) $\text{Si}_{0.50}\text{HA}$ powders calcined at 1000°C for 15h.

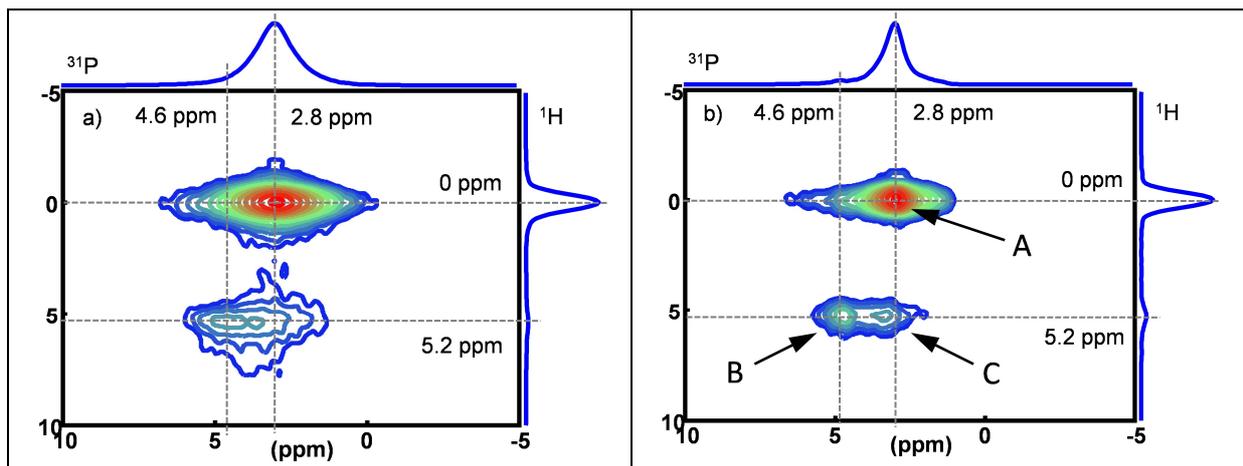


Fig. 8. ^1H - ^{31}P HETCOR CP MAS experiment recorded on (a) $\text{Si}_{1.00}\text{HA}$ powder calcined at 400°C for 2h, and (b) $\text{Si}_{0.50}\text{HA}$ powder calcined at 1000°C for 15h.

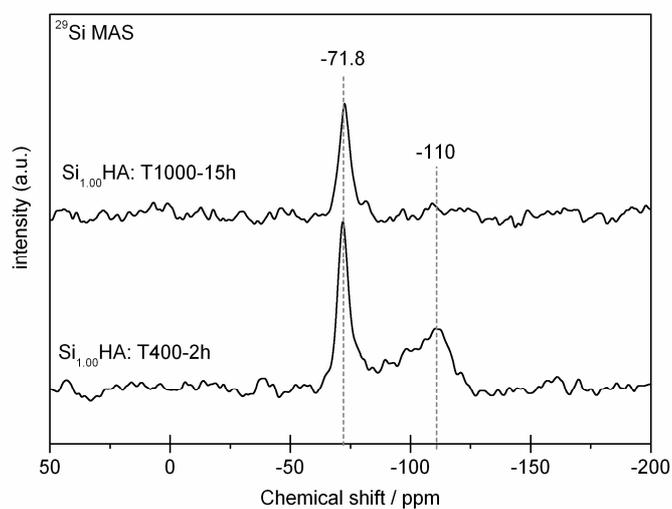


Fig. 9. ^{29}Si MAS-NMR spectra of the $\text{Si}_{1.00}\text{HA}$ powder calcined at 400°C for 2h and 1000°C for 15h

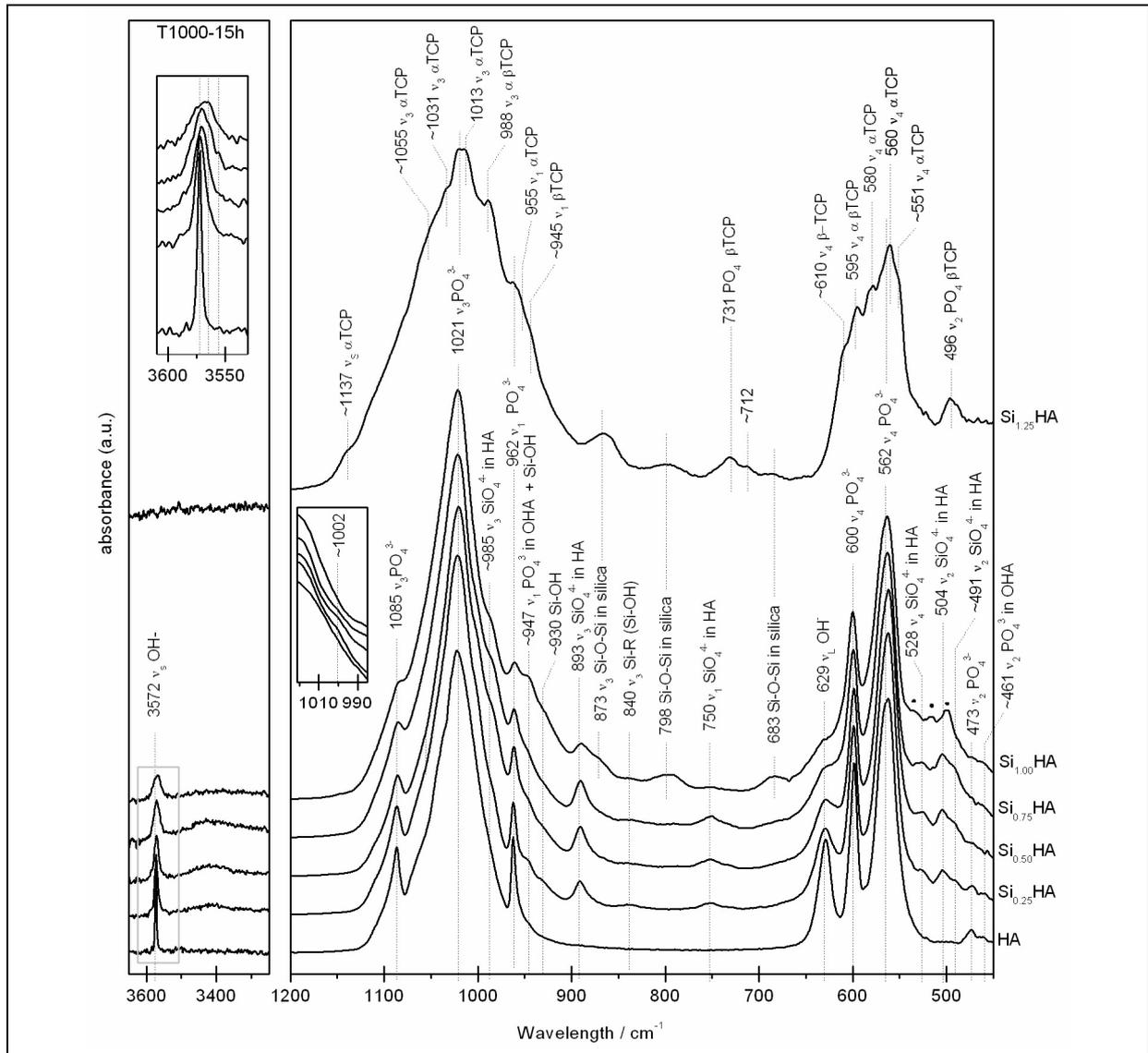


Fig. 10. FTIR spectra of calcined HA and SiHA powders (1000°C / 15h) collected at room temperature, symbol ● shows additional vibrations for Si_{1.0}HA at 500, 515, 535 cm⁻¹.

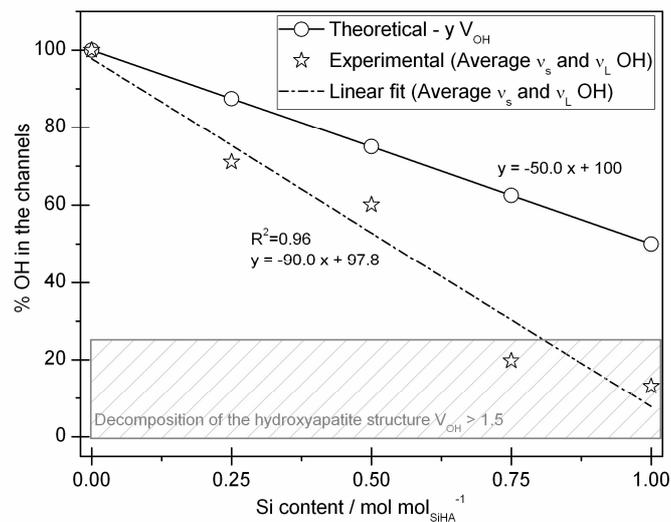


Fig. 11. OH content into the apatitic channels versus the Si content, for theoretical SiHA structure ($y V_{OH}$) and samples calcined at 1000°C for 15h (determined from integrated areas

of ν_L and ν_S bands).

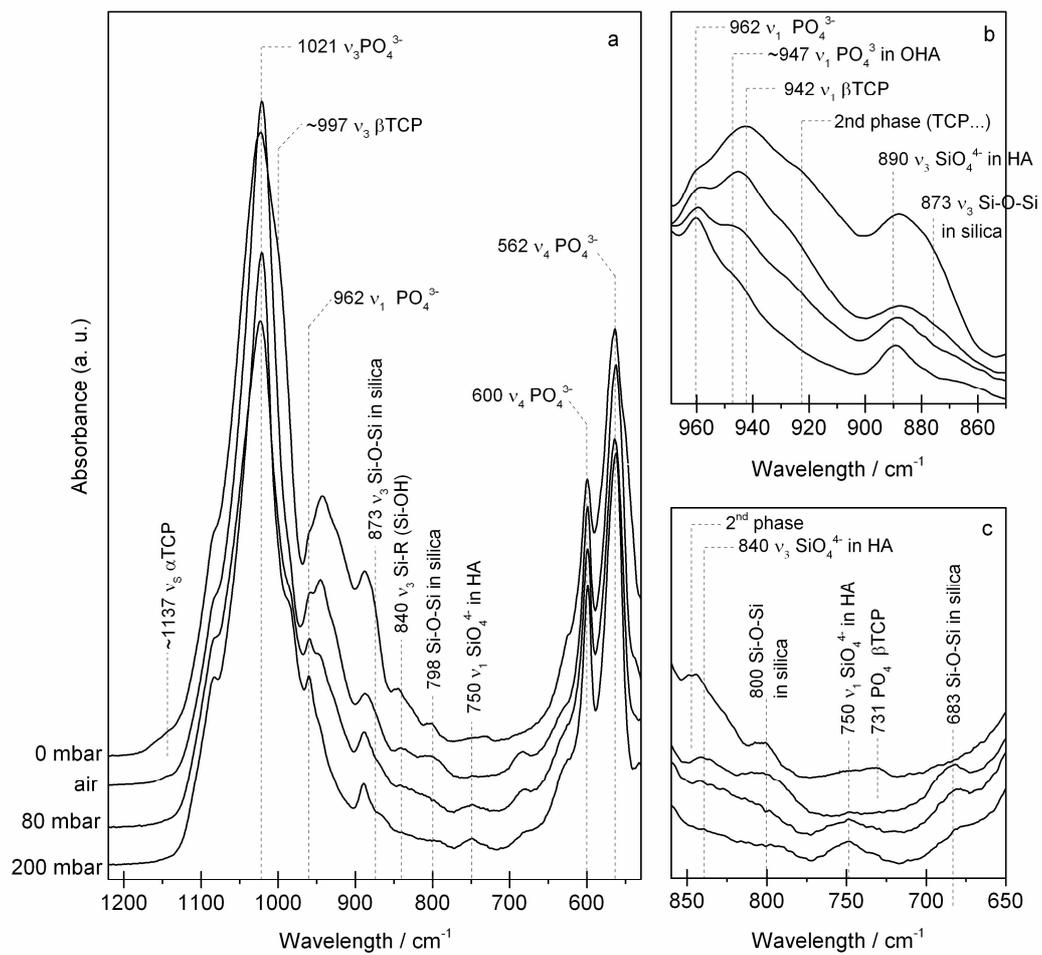


Fig. 12. FTIR spectra of calcined $\text{Si}_{1.0}\text{HA}$ powder (1000°C / 15h) under different partial pressure of steam ($p_{\text{H}_2\text{O}} = 0, 80, 200$ mbar and air).

Table 1

Chemical conditions of preparation of HA and Si_yHA samples and final composition of the calcined powders (1000°C/15h).

Sample	Synthesis parameters (T = 50°C, t _m = 24h)					Composition of the final calcined powders (1000°C/15h)		
	n _{Ca}	n _P	n _{Si}	pH	Y _{Si}	Y _{Si}	Chemical formula of Si _y HA	Ca/(P+Si)
					Expected	Determined - According to Equation Eq. 2		Determined by ICP/AES
	/ mol	/ mol	/ mol	-	/ mol mol ⁻¹ _{SiHA}	-		
HA	0.200	0.120	0	9.5	0	0	Ca ₁₀ (PO ₄) ₆ (OH) ₂	1.69 ± 0.05
Si _{0.25} HA	0.200	0.115	0.005	11.0	0.25	0.28±0.02	Ca ₁₀ (PO ₄) _{5.72} (SiO ₄) _{0.28} (OH) _{1.72} (V _{OH}) _{0.28}	1.69 ± 0.02
Si _{0.50} HA	0.200	0.110	0.010	9.5	0.50	N/A	HA + α TCP	N/A
Si _{0.50} HA	0.200	0.110	0.010	11.0	0.50	0.52±0.02	Ca ₁₀ (PO ₄) _{5.48} (SiO ₄) _{0.52} (OH) _{1.48} (V _{OH}) _{0.52}	1.68 ± 0.02
Si _{0.75} HA	0.200	0.105	0.015	11.0	0.75	0.72±0.02	Ca ₁₀ (PO ₄) _{5.28} (SiO ₄) _{0.72} (OH) _{1.28} (V _{OH}) _{0.72}	1.67 ± 0.03
Si _{1.00} HA	0.200	0.100	0.020	11.0	1.00	0.97±0.02	Ca ₁₀ (PO ₄) _{5.03} (SiO ₄) _{0.97} (OH) _{1.03} (V _{OH}) _{0.97}	1.67 ± 0.01
Si _{1.25} HA	0.200	0.095	0.025	11.0	1.25	N/A	HA+TCP	N/A

Table 2

NMR parameters.

NMR Experiments	t_{90°	number of scans	relaxation delay	^1H decoupling	contact time	t_1 increments
	/ μs	-	/ s	-	/ ms	
^1H MAS	4.6	4	300	-	-	-
^{31}P MAS	1.3	16	60	SPINAL-64 (54kHz)	-	-
^{31}P CPMAS	1.5	16	15		3	-
^1H - ^{31}P HETCOR CPMAS		32			1	128
^{29}Si MAS	1.5	~ 4000	60	SPINAL-64 (45kHz)	-	-

Table 3.

Lattice parameters, unit cell volume and mean size of the crystallites of heat-treated powders at 1000°C for 15h evaluated by Rietveld refinement.

Sample	Lattice parameters / Å		Volume of the unit cell / Å ³	Crystallites mean size / nm
	a-axis	c-axis		
PDF 9-432	9.418	6.884	1058	-
HA	$9.421 \pm 3 \cdot 10^{-5}$	$6.884 \pm 3 \cdot 10^{-5}$	1058 ± 1	243 ± 1
Si _{0,25} HA	$9.423 \pm 4 \cdot 10^{-5}$	$6.892 \pm 4 \cdot 10^{-5}$	1060 ± 1	157 ± 1
Si _{0,50} HA	$9.428 \pm 2 \cdot 10^{-4}$	$6.899 \pm 2 \cdot 10^{-4}$	1062 ± 1	116 ± 1
Si _{0,75} HA	$9.423 \pm 2 \cdot 10^{-4}$	$6.908 \pm 2 \cdot 10^{-4}$	1062 ± 1	103 ± 1
Si _{1,00} HA	$9.416 \pm 7 \cdot 10^{-5}$	$6.920 \pm 5 \cdot 10^{-5}$	1063 ± 1	107 ± 1

Table 4

IR bands and shoulders assignment proposed by our work

λ / cm^{-1}	Mode assignments proposed by this work	Literature support
1002*	Degenerated PO_4^{3-} asymmetric stretching (ν_3)	[94, 110]
985	Si-OH at SiHA surface / and/or / Si in SiHA	[110-112]
947	Oxyapatite	[93, 99, 100, 102, 113]
	Si-OH at SiHA surface	[114-116]
930**	Si-OH at SiHA surface / and/or / Si in SiHA	[114, 117-121]
893	Si in SiHA	[8, 113, 118]
873	Si in silica	[112, 113, 122, 123]
840	Si-R	[118, 124]
798	Si in silica	[34, 110, 112-115, 117, 120, 122, 125-127]
750	Si in SiHA	[32, 54, 74]
683	Si in silica	[113, 114, 116, 126, 127]
535	Si in silica	[128]
528	Si in SiHA	[111, 118, 121, 128]
515	Si in silica	[127-130]
504	Si in SiHA	[32, 56, 118]

* degeneration of the $\nu_3 \text{PO}_4^{3-}$ domain at 1020 cm^{-1} due to the SiO_4^{4-} in the environment of the phosphate ions

** degeneration of the band at 947 cm^{-1}