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1 Article

2 Silver doping mechanism in bioceramics – from 3 Ag⁺:doped HAp to Ag⁰/BCP nanocomposite

4 Aurélie Jacobs¹, Morgane Gaulier¹, Alexis Duval¹ and Guillaume Renaudin^{1*}

5 ¹ Université Clermont Auvergne, CNRS, SIGMA Clermont, ICCF, F-63000 Clermont-Ferrand, France.

6 * Correspondence: guillaume.renaudin@sigma-clermont.fr; Tel.: 00 33 4 73 40 73 36, Fax.: 00 33 4 73 40 70 95

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8 **Abstract:** The results presented in this paper, based on the powder X-ray diffraction technique
9 followed by Rietveld analyses, are devoted to the mechanism of silver incorporation in Biphasic
10 Calcium Phosphates. Results were confirmed by SEM observation. Samples were synthesized via
11 the sol-gel route, followed by heat treatments. Two incorporation sites were highlighted: Ca²⁺
12 replacement by Ag⁺ into the calcium phosphates (HAp: hydroxyapatite and β-TCP: TriCalcium
13 Phosphate), and the other as metallic silver Ag⁰ nanoparticles (formed by autogenous reduction).
14 The samples obtained were thus nanocomposites, written Ag⁰/BCP, composed of closely-mixed Ag⁰
15 particles of about 100 nm at 400 °C (which became micrometric upon heating) and calcium
16 phosphates, themselves substituted by Ag⁺ cations. Between 400 °C and 700 °C the cationic silver
17 part was mainly located in the HAp phase of the composition Ca_{10-x}Ag_x(PO₄)₆(OH)_{2-x} (written
18 Ag⁺:HAp). From 600 °C silver cations migrated to β-TCP to form the definite compound
19 Ca₁₀Ag(PO₄)₇ (written Ag⁺:TCP). Due to the melting point of Ag⁰, the doping element completely
20 left our sample at temperatures above 1000 °C. In order to correctly understand the biological
21 behaviour of such material, which is potentially interesting for biomaterial applications, its complex
22 doping mechanism should be taken into consideration for subsequent cytotoxic and bacteriologic
23 studies.

24 **Keywords:** Biomaterial; Silver-doping; Silver nano-composite; Hydroxyapatite; Powder X-ray
25 Diffraction.

26

27 1-. Introduction

28 The utilization of synthetic materials for bone reconstructive surgery is generally necessary,
29 because autograft and allograft practice is limited by the quantity of available material, and in the
30 first case entails a second surgical procedure [1]. Among the numerous synthetic materials
31 investigated for bone replacement and/or prosthesis coating, hydroxyapatite (HAp, Ca₁₀(PO₄)₆(OH)₂)
32 is the most often-used material due to its chemical and structural similarities with the bone mineral
33 constituent [2-5]. Biological apatite refers to the main constituent of bone and hard tissue in mammals:
34 a poorly crystallized non-stoichiometric carbonate-containing HAp that composes about 65 weight
35 percent (wt %) of bone and about 90 wt % of dental enamel [6]. Apatite is a complex and diverse class
36 of materials, with a flexible structure that accepts many substitutions, either cationic or anionic [7].
37 Hydroxyapatite is therefore an interesting biocompatible and osteoconductive material, but it has
38 limited antibacterial properties [8], even though bacterial infections are the main cause of
39 postoperative problems [9]. Bacterial overgrowth on the surface of orthopaedic implants – that is,
40 biofilm formation – potentially leads to serious complications during reconstructive surgery, with
41 severe physiological damage, significant patient discomfort and additional costly surgical
42 procedures [10-12]. About 1% of total joint hip arthroplasties, and about 3% in the case of knees,

43 require a second (or multiple) surgical intervention(s) because of bone healing process complications,
44 which makes it a real societal problem on a global scale [13-17]. Nowadays, an antibacterial effect at
45 the surgical site is ensured by systematic antibiotic administration via the blood, which potentially
46 generates toxicity, poor penetration into the surgical site, and also the problem of bacterial resistance.
47 The delivery, or the presence, of a bactericidal agent directly at the surgical site would ensure a really
48 promising alternative [18,19]. Among the various possibilities, the use of silver – well known in
49 medicine since ancient times in the treatment of bacterial infection – seems very promising by
50 combining broad spectrum and long term antimicrobial activity with the absence of microorganisms
51 developing resistance [20-25]. A particularity of the antiseptic properties of silver is the possibility of
52 using it in metallic or ionic form, without a loss of efficiency [26-28,41]. For these reasons, research
53 on silver incorporation into hydroxyapatite has developed in recent years, and has shown the high
54 potential of the synthesized materials [17,22,29-35]. Although the results agree regarding the
55 beneficial biological effects, the literature on the topic does not address the fine description of the
56 synthesized materials and the mechanisms of silver incorporation. Many synthesis methods have
57 been described (co-precipitation [29,35,43] potentially followed by a thermal [8,22,33,36,38] or
58 reducing treatment [34,41], sol-gel [17,30,40,44], electrochemical [31,37]; hydrothermal [32],
59 microwave [26,39], sonochemical [42], plasma-spraying [43,45] and also impregnation [46,47]
60 methods), with the formation of two different kinds of material: either an Ag⁺:doped HAp compound
61 or an Ag⁰/HAp nanocomposite material. The difference is – of course – fundamental, since in the first
62 case the single-phase material contains Ag⁺ cations that substitute Ca²⁺ from the HAp crystal
63 structure, while in the second case metallic silver particles are intimately mixed in a multi-phase
64 composite. Although these two alternatives are listed and described in the literature [42] there is a
65 lack of understanding the mechanisms that lead to one or the other.

66 The purpose of this paper is to fully characterize the silver-HAp doping mechanism in the case
67 of sol-gel synthesis followed by gradual thermal treatment leading to silver auto-reduction. The
68 study is a continuation of our previous work on the BCP (biphasic calcium phosphate) doping
69 mechanism with cations from the first-row transition metals [48]. The case of zinc doping, with an
70 interstitial mode of incorporation into the HAp crystal structure, was first fully characterized [49-51],
71 then investigated for iron [52]. In the case of copper, it transpired that control of the doping
72 mechanism allowed an insight into behaviour in a biological environment [53]. For a complete
73 understanding of the present paper it is very important to note the difference between the notations
74 used: Ag⁺:HAp and Ag⁰/HAp. Ag⁺:HAp corresponds to the incorporation of Ag⁺ cations within the
75 crystal structure of hydroxyapatite (which is expected in the case of conventional cationic doping),
76 whereas Ag⁰/HAp means the formation of a composite containing two distinct phases, the
77 hydroxyapatite trapping nanoparticles of Ag⁰ metallic silver.

78 2-. Materials and methods

79 2.1. Sol-gel elaboration of silver containing BCP samples

80 The sol-gel route previously proposed by the authors was used to synthesize both undoped and
81 Ag-doped series of BCP samples [51]. Briefly, to produce 2 g of undoped BCP powder, 4.7 g of
82 Ca(NO₃)₂·4H₂O (Sigma-Aldrich) and 0.84 g of P₂O₅ (Sigma-Aldrich) were dissolved in ethanol under
83 stirring and refluxed at 85°C for 24 hours. The solution was then maintained at 55°C for 24 hours to
84 obtain a white consistent gel, and further heated at 80°C for 10 hours to obtain a white powder.
85 Finally, the powder was heat-treated for 15 hours. This thermal treatment was performed between
86 100°C and 1200°C. Samples from the undoped series were named the 0Ag-*T* series with *T* indicating
87 the temperature (from 400°C to 1200°C) of the following thermal treatment. Required amounts of
88 AgNO₃ (Sigma-Aldrich) were added to the solution simultaneously with Ca(NO₃)₂·4H₂O (Sigma-
89 Aldrich) in order to synthesize the Ag-doped series. Nominal compositions were calculated,
90 assuming Ag⁺ calcium substitution (i.e. assuming a (Ca+Ag)/P = 1.67 constant ratio) with two doping
91 levels: a 2.5% calcium substitution (Ag/(Ca+Ag) = 0.025 corresponding to the nominal composition
92 Ca_{9.75}Ag_{0.25}(PO₄)₆(OH)_{1.75}; named the 25Ag-*T* series) and a 10% calcium substitution (Ag/(Ca+Ag) = 0.1

93 corresponding to the nominal composition $\text{Ca}_9\text{Ag}_1(\text{PO}_4)_6(\text{OH})$; named the 100Ag-*T* series). As-
94 synthesized Ag-doped powders were pale yellow; heat treatments turn the colour to light grey, and
95 then to white above 1000°C.

96 2.2. Powder X-Ray Diffraction (PXRD) and Rietveld analyses

97 PXRD patterns were recorded on a Philips X'Pert Pro PANalytical diffractometer (Almelo,
98 Netherlands), with θ - θ geometry, reflection mode, equipped with a solid-state X-Celerator detector
99 and using Cu K α radiation ($\lambda = 1.54184 \text{ \AA}$). PXRD patterns were recorded at room temperature in the
100 interval $3^\circ < 2\theta < 120^\circ$, with a step size of $\Delta 2\theta = 0.0167^\circ$ and a counting time of 200s for each data
101 value.

102 Rietveld refinements were systematically performed for each measurement (13 temperatures for
103 the 3 series) using the FullProf.2k program [54]. The Rietveld strategy was detailed in previous
104 related works [48-53]. A new additional phosphate compound was considered here: $\text{AgCa}_{10}(\text{PO}_4)_7$,
105 which crystallizes in the trigonal $R3c$ space group with $a = 10.4372 \text{ \AA}$ and $c = 37.3379 \text{ \AA}$ [55]. It is
106 isotopic with members of the $M\text{Ca}_{10}(\text{PO}_4)_7$ ($M = \text{Li}, \text{Na}, \text{K}$ and Cs) series, and is closely related to the
107 structure of β -TCP (β - $\text{Ca}_3(\text{PO}_4)_2$, $R3c$ symmetry with $a = 10.4352 \text{ \AA}$ and $c = 37.4029 \text{ \AA}$ [56]). The half-
108 occupied Ca4 site of β -TCP [56], becomes fully occupied by silver cations in $\text{AgCa}_{10}(\text{PO}_4)_7$ [55];
109 explaining the charge compensation between Ca^{2+} and Ag^+ . The metal Ag° phase was also considered
110 in several sample; it is a compact cubic structure $Fm\bar{3}m$ with $a = 4.085 \text{ \AA}$ [57]. An example of a
111 Rietveld plot is shown in Figure SEI1.

112 2.3. Scanning Electron Microscopy (SEM)

113 Electronic microscopy observations used a ZEISS SUPRA 55VP with GEMINI Field Emission –
114 Scanning Electron Microscope and were carried out on pressed pellets after gold metallization. No
115 polishing was carried out to avoid the leaching of the silver cations and to avoid the deformation of
116 the ductile metallic silver particles. The main object of this analysis is not the quantification, but the
117 localization, of the silver element. Quantitative analyses were performed using an EDS (OXFORD
118 XMAX 80 N+ 80 mm² Si-detector combined with OXFORD AZtec Advanced V3.3 software) on Ca, P
119 and Ag elements. Measurements were made using an acceleration voltage of 20 kV and 30 seconds
120 of acquisition on three wide areas (magnification x100) before averaging. Some specific isolated
121 measurements were performed using x5000 magnification. A 4QBSD detector was used to acquire
122 chemical contrast images to highlight silver particles.

123 The following nine samples were characterized by SEM: 0Ag-400, 0Ag-700, 0Ag-1200, 25Ag-400,
124 25Ag-700, 25Ag-1200, 100Ag-400, 100Ag-700 and 100Ag-1200.

125 3-. Results: Materials characterization

126 3.1. Quantitative phase analysis

127 To correctly interpret the behaviour of our samples, their phase compositions were extracted
128 from Rietveld analyses (PXRD patterns from the 25Ag-*T* series are shown in Figure 1). Figure 1
129 evidences the gradual increase in crystallinity of HAP with respect to temperature by the decrease in
130 the diffraction peak width. Phase compositions of the undoped BCP series and of the two Ag-doped
131 BCP series ($x = 25$ and 100) are presented in Table 1, and Figure 2a represents the thermal composition
132 variation for the two main phases: HAP and β -TCP. Supplementary minor phases were observed. α -
133 CDP (diCalcium DiPhosphate with composition $\text{Ca}_2\text{P}_2\text{O}_7$) was observed up to 700°C, with a
134 maximum amount of 4 wt % at 500-600°C. CaO and CaCO_3 (calcite) were present in the samples.
135 They are indicated in CaO equivalent content in Table 1, as calcite decarbonation leads to CaO
136 formation at about 800°C. The amount of CaO equivalent becomes negligible at high temperatures,
137 where the BCP were mainly composed of HAP (corresponding to the nominal composition). All these
138 observations – on main and minor phases – were similar to those of our previous studies on first-row
139 transition metal doping [48-53]. The β -TCP phase is stabilized for intermediate temperatures, with a

140 maximum amount close to 20 wt % around 900 °C for the undoped series and around 700 °C for the
 141 two silver-doped series. The main difference compared to our previous works concerns the formation
 142 of metallic silver, Ag⁰, already at 400°C in the Ag-doped series (contrary to metal oxide formation for
 143 the first-row transition metals, including ZnO and CuO). The amount of metallic silver reached 1 wt
 144 % for the 25Ag-*T* series and 6 wt % for the 100Ag-*T* series (Table 1 and Figure 2b). These values are
 145 to be compared to the quantities introduced during the syntheses: 2.65 wt % for the nominal
 146 Ca_{9.75}Ag_{0.25}(PO₄)₆(OH)_{1.75} composition and 10.22 wt % for the nominal Ca₉Ag₁(PO₄)₆(OH) composition
 147 (respectively the 25Ag-*T* and the 100Ag-*T* series). Figure 2b highlights the fact that the entirety of the
 148 dopant introduced was not found exclusively in metallic form, but rather of the order of half of this
 149 quantity. The reduction of the incorporated Ag⁺ silver cation to Ag⁰ silver metal was autogenous, and
 150 monitored by the thermal treatment only, without any reducing agent.

151 The formation of Ag⁰/HAp nanocomposite by heating Ag⁺-incorporated hydroxyapatite has
 152 already been mentioned in the literature, either using a reducing agent (NaBH₄ [34,58], hydrazine
 153 [41]) or not [17,22,26,38,42]. However, the nanocomposite formation mechanism, the main subject of
 154 this paper, has never been studied and detailed. It may first be pointed out that a thermal study on
 155 silver nitrate (AgNO₃; not shown here) showed decomposition accompanied by reduction at 300 °C,
 156 which leads to the production of metallic silver (Ag⁰). To complete this information, the colour
 157 changes of our samples are also interesting. The undoped series remained constantly white, while
 158 the doped series were yellow at 400 °C (pale yellow for the 25Ag-*T* series and yellow-brown for the
 159 100Ag-*T* series), then turned grey up to 1000 °C before bleaching above 1100 °C. The grey colour can
 160 be attributed to the silver nanoparticles present in the Ag⁰/HAp composite. It is therefore normal to
 161 lose the grey colour above 1000 °C, since silver has a melting point of 962 °C. Thus it is difficult to
 162 design Ag⁰/HAp composites which could be obtained following heat treatments at more than 1000
 163 °C [8,36,39,59]. Our white 25Ag-1100, 25Ag-1200 and 100Ag-1200 samples were then exempt of silver
 164 particles, and certainly just composed of undoped HAp counterbalanced by a few wt % of β-TCP due
 165 to the introduced Ca/P ratio (1.625 for the 25Ag-*T* series and 1.5 for the 100Ag-*T* series instead of the
 166 1.67 value for HAp). By wetting calcium phosphate particles, metallic silver was still present in the
 167 25Ag-1000 sample (in very little amount) and in 100Ag-1100 sample (although the Ag⁰ amount
 168 decrease is very clear at above 900 °C).

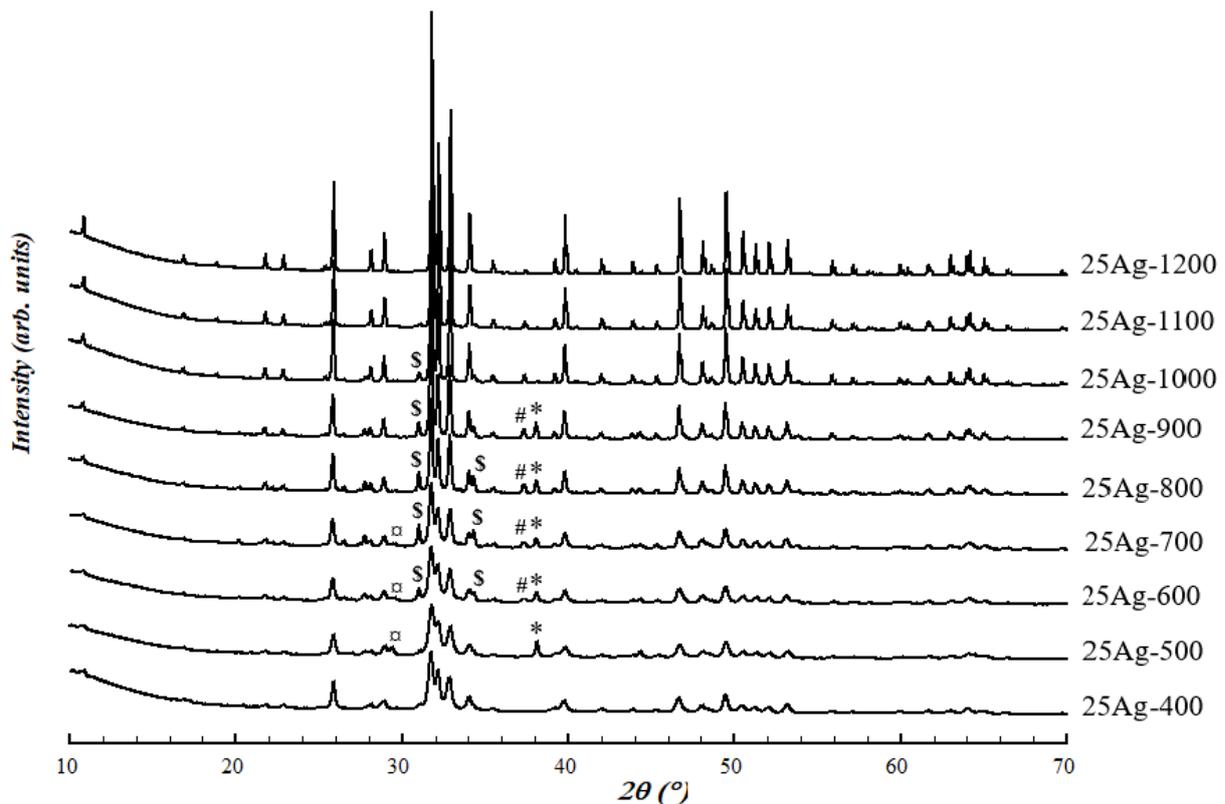
169 **Table 1.** Quantitative phase analysis (wt %) of the 27 samples; standard deviations are indicated in
 170 brackets (CDP: Ca₂P₂O₇ and 'Eq. CaO' considers both the CaO and CaCO₃ amounts).

Sample	Phase composition (wt %)				
	HAp	TCP	CDP	Eq. CaO	Ag ⁰
0Ag-400	97.1 (2.0)	2.9 (1.0)	/	/	/
0Ag-500	94.8 (2.0)	1.5 (0.5)	2.1 (0.5)	1.6 (0.5)	/
0Ag-600	91.8 (2.0)	3.3 (1.0)	3.8 (1.0)	1.2 (0.5)	/
0Ag-700	85.8 (2.0)	9.3 (1.0)	3.1 (1.0)	1.9 (0.5)	/
0Ag-800	81.9 (2.0)	16.0 (1.0)	/	2.1 (0.5)	/
0Ag-900	81.4 (2.0)	16.7 (1.0)	/	1.9 (0.5)	/
0Ag-1000	85.8 (2.0)	13.6 (1.0)	/	0.6 (0.5)	/
0Ag-1100	92.5 (2.0)	7.1 (1.0)	/	0.4 (0.2)	/
0Ag-1200	96.0 (2.0)	3.8 (1.0)	/	0.2 (0.2)	/
25Ag-400	92.7 (2.0)	2.5 (0.5)	3.1 (1.0)	1.2 (0.5)	0.3 (0.2)
25Ag-500	88.5 (2.0)	3.6 (1.0)	4.1 (1.0)	2.6 (0.5)	1.1 (0.5)
25Ag-600	81.9 (2.0)	11.3 (1.0)	3.5 (1.0)	2.4 (0.5)	0.8 (0.5)

25Ag-700	80.4 (2.0)	15.8 (1.0)	1.1 (0.5)	2.2 (0.5)	0.7 (0.5)
25Ag-800	82.9 (2.0)	14.0 (1.0)	/	2.2 (0.5)	0.9 (0.5)
25Ag-900	87.1 (2.0)	19.9 (1.0)	/	2.0 (0.5)	1.0 (0.5)
25Ag-1000	93.1 (2.0)	5.3 (1.0)	/	1.5 (0.5)	0.1 (0.2)
25Ag-1100	96.8 (2.0)	2.0 (0.5)	/	1.2 (0.5)	/
25Ag-1200	99.0 (2.0)	0.5 (0.2)	/	0.5 (0.2)	/
100Ag-400	92.3 (2.0)	/	/	2.4 (0.5)	5.3 (1.0)
100Ag-500	81.4 (2.0)	7.4 (1.0)	2.3 (0.5)	3.4 (1.0)	5.5 (1.0)
100Ag-600	76.8 (2.0)	12.5 (1.0)	1.8 (0.5)	3.0 (1.0)	5.9 (1.0)
100Ag-700	73.8 (2.0)	16.1 (1.0)	1.0 (0.5)	3.2 (1.0)	6.0 (1.0)
100Ag-800	77.3 (2.0)	13.9 (1.0)	/	2.9 (0.5)	5.9 (1.0)
100Ag-900	79.4 (2.0)	13.5 (1.0)	/	1.4 (0.5)	5.6 (1.0)
100Ag-1000	85.5 (2.0)	8.7 (1.0)	/	1.1 (0.5)	4.8 (1.0)
100Ag-1100	92.2 (2.0)	4.7 (1.0)	/	0.4 (0.2)	2.8 (0.5)
100Ag-1200	96.8 (2.0)	3.0 (1.0)	/	0.1 (0.2)	/

171

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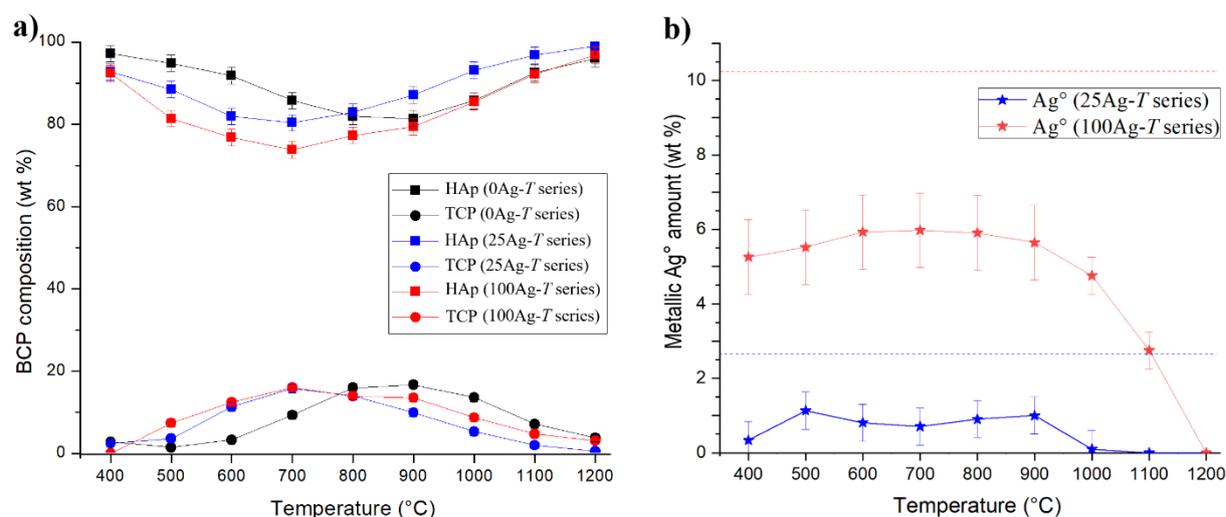
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Figure 1. PXRD patterns from the 25Ag-T series. Scattering signals are mainly those from the HAp phase, and other phases are identified thanks to their intense diffraction peaks (*: Ag°, #: CaO, x: CaCO₃ and \$: β-TCP).

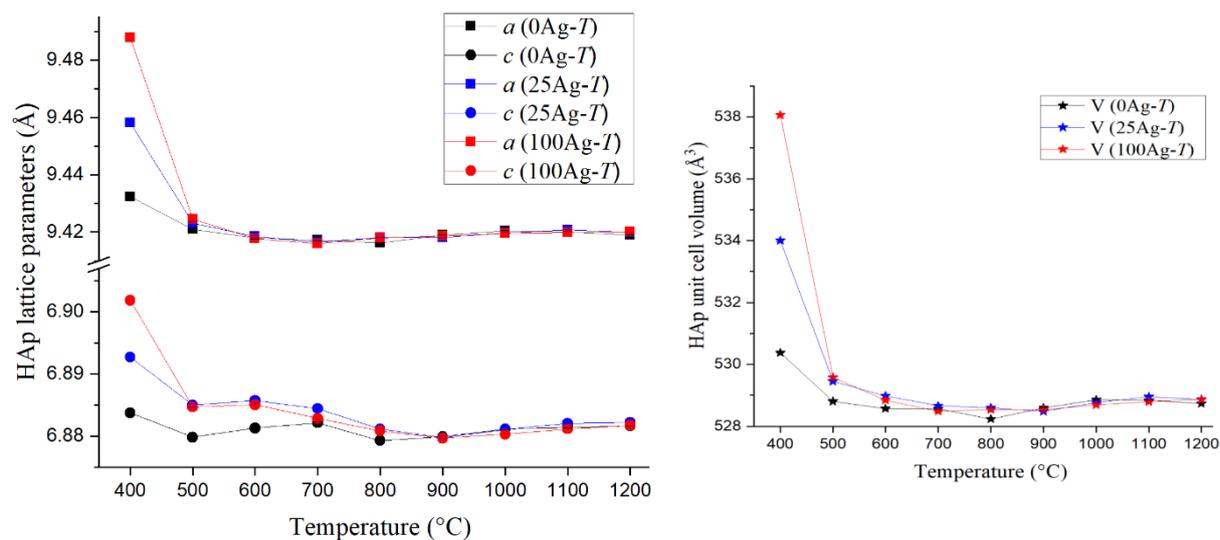


177

178 **Figure 2.** Thermal variation in a) the two main phase contents (squares: HAp; circles: TCP) from the
 179 BCP samples and b) the metallic silver amount (stars) for the undoped 0Ag-*T* series (black symbols),
 180 the 25Ag-*T* series (blue symbols) and 100Ag-*T* series (red symbols). Dotted lines indicate the nominal
 181 silver amounts, and lines are only guides for the eyes.

182 3.2. Thermal variation in the HAp structural parameters

183 Thermal variations in the HAp lattice parameters are shown in Figure 3 and collated in Table
 184 SEI1. Figure 3 clearly shows that the parameters of the HAp network evolved for the lowest
 185 temperatures and then showed no more variations. The undoped series presents a relatively weak
 186 contraction between 400 °C and 500 °C for both *a* and *c* lattice parameters (and consequently for the
 187 unit cell volume). This contraction is also observed between 400 °C and 500 °C on the basal *a* lattice
 188 parameter for the two silver-doped series, but much more markedly. Concerning the hexagonal *c*
 189 lattice parameter, the difference between the undoped and the two doped series extends up to 700
 190 °C, which of course also affects the unit cell volume difference up to 700 °C. For the lowest
 191 temperatures, an enlargement of the HAp network is in favour of a calcium-to-silver substitution,
 192 explained by the larger cationic radius of the Ag⁺ cation: 1.28 Å for Ag⁺ against 1.12 Å for Ca²⁺ in
 193 eightfold coordination [60]. This indication of a silver substitution mechanism to form Ag⁺:HAp with
 194 larger lattice parameters is consistent with previous literature results [32,33,38], but more marked in
 195 our study. Indeed, the large values found here (*a* = 9.488 Å and *c* = 6.901 Å for the 100Ag-400 sample)
 196 have never been evinced before. Despite the electronic contrast between Ag⁺ and Ca²⁺ cations, the last
 197 Rietveld refinement cycles failed to clearly locate the silver atoms in the hydroxyapatite structure.
 198 Nevertheless, the indications obtained support the notion of a substitution mechanism at the Ca1 and
 199 Ca2 sites: the results indicate substitution rates which are too weak, but which are present (silver
 200 occupancies about 3 % and 5 % – instead of the expected 10 % – for the Ca1 and Ca2 sites respectively),
 201 and show the absence of chemical elements at the interstitial site within the hexagonal channel (i.e.
 202 the 2*b* Wyckoff site). This leads us to privilege the Ca_{10-x}Ag_x(PO₄)₆(OH)_{2-x} stoichiometry for the doped
 203 Ag⁺:HAp phase, and not that proposed by Geng et al., Ca_{10-x}Ag_{2x}(PO₄)₆(OH)₂ [32], which implies a
 204 double mechanism by substitution and insertion. Our work on the structural parameters of
 205 hydroxyapatite enables us to conclude that the HAp silver-doped phase is present in our samples
 206 from 400 °C to 600 °C. This is correlated with the refined isotropic thermal parameters (see B_{iso} in
 207 Table SEI1) of hydroxyl anions, which are exaggerated in these samples: 25Ag-400, 25Ag-500, 25Ag-
 208 600, 100Ag-400, 100Ag-500 and 100Ag-600. We will consider below for these temperatures that the
 209 Ag⁺:HAp phase presents the nominal stoichiometry, that is to say Ca_{9.75}Ag_{0.25}(PO₄)₆(OH)_{1.75} for the
 210 25Ag-*T* series and Ca₉Ag₁(PO₄)₆(OH) for the 100Ag-*T* series, although there is no definitive
 211 experimental proof for these compositions.

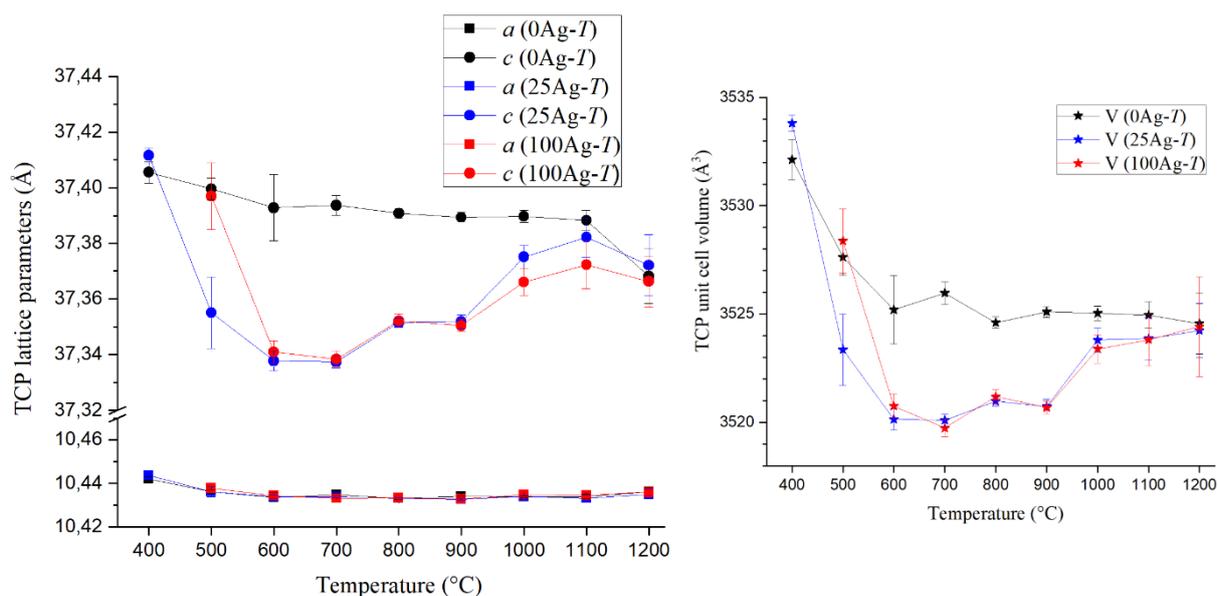


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213 **Figure 3.** Thermal variation in the HAp lattice parameters for the three series of samples. Left: *a*
 214 (squares) and *c* (circles) hexagonal lattice parameters, right: unit cell volume (stars). Lines are guides
 215 for the eyes only.

216 3.3. Thermal variation in the β -TCP structural parameters

217 Thermal variations in the TCP lattice parameters, shown in Figure 4 and collated in Table SEI2,
 218 are very different from those presented above for HAp. The basal *a* lattice parameter remained
 219 constant whatever the synthesis series and heat treatment temperature. On the other hand, variations
 220 in the axial *c* lattice parameter provide interesting information. The *c* lattice parameter – and
 221 consequently the unit cell volume – for the two silver-doped series is below the value refined for the
 222 undoped series, except for the temperature of TCP appearance (400 °C for the 25Ag-*T* series and 500
 223 °C for the 100Ag-*T* series) and for the higher temperatures (1100 °C and 1200 °C). It corresponds to
 224 the formation of the ordered calcium-to-silver substitution in β -TCP that leads to the $\text{AgCa}_{10}(\text{PO}_4)_7$
 225 compound. This compound is isotypic to β -TCP (trigonal $R3c$ space group, 18 independent atomic
 226 positions) with its half-occupied Ca4 site (at the special $6a$ Wyckoff site [56]) fully filled by Ag^+ cations
 227 [55]. The content of the unit cell is then $\text{Ag}_6\text{Ca}_{60}(\text{PO}_4)_{42}$; i.e. $\text{AgCa}_{10}(\text{PO}_4)_7$ corresponding to $\text{Ca}_{3-x}\text{Ag}_x(\text{PO}_4)_2$
 228 with $x = 0.143$. This ordered substitution mechanism, leading to a definite compound, is
 229 accompanied by a decrease in the axial *c* lattice parameter despite the larger size of Ag^+ : 37.403 Å for
 230 β -TCP [56] against 37.338 Å for $\text{AgCa}_{10}(\text{PO}_4)_7$ [55]. Thus, the value of the *c* axial lattice parameter
 231 allows us to determine whether the present TCP phase corresponds to the undoped β -TCP phase or
 232 the silver-containing $\text{AgCa}_{10}(\text{PO}_4)_7$ phase. Table SEI2 indicates the TCP phase attribution for each
 233 sample; either β -TCP (implied undoped $\text{Ca}_3(\text{PO}_4)_2$) or $\text{AgCa}_{10}(\text{PO}_4)_7$ (called Ag^+ :TCP here).



234
 235 **Figure 4.** Thermal variation in TCP lattice parameters for the three series of samples. Left: *a* (squares)
 236 and *c* (circles) trigonal lattice parameters; right: unit cell volume (stars). Lines are guides for the eyes
 237 only.

238 3.4. Ag location studied by SEM analyses

239 SEM analyses were used to evaluate the silver amount and location in selected samples from the
 240 doped series. Results from elemental quantifications made with EDS spectroscopy are summarized
 241 in Table 2. The calculated Ca/P ratio indicates that results should be considered as semi-quantitative
 242 only because of large deviations from the expected values as well as large variations within a single
 243 series. This must be connected to the unpolished surface state of the pellets and the use of internal
 244 calibration lines in the software. However, interesting additional information is provided by these
 245 analyses.

246 **Table 2.** Quantitative elemental analyses performed by EDS/SEM.

Sample	Analysis	Atomic composition (%)			Calculated ratio		Expected ratio	
		Ca	Ag	P	Ag/Ca	Ca/P	Ag/Ca	Ca/P
0Ag-400	global	65.63	0.00	34.37	0.00	1.91	0.00	1.67
0Ag-700	global	59.80	0.00	40.20	0.00	1.49		
0Ag-1200	global	68.02	0.00	31.98	0.00	2.13		
25Ag-400	global	66.47	2.17	31.37	0.03	2.12	0.03	1.62
25Ag-700	global	60.14	1.43	38.43	0.02	1.56		
25Ag-1200	global	65.48	0.00	34.52	0.00	1.90		
100Ag-400	global	62.39	3.94	33.68	0.06	1.85	0.11	1.50
100Ag-700	global	62.35	3.03	34.61	0.05	1.80		

100Ag-1200	global	60.59	0.00	39.41	0.00	1.54		
25Ag-400 (*)	local_1	65.21	1.24	33.55	0.019	1.94	0.03	1.62
	local_2	40.26	24.64	35.11	n.c. (**)	n.c.		
	local_3	7.40	89.95	2.65	n.c.	n.c.		
	local_4	64.25	1.39	34.36	0.022	1.87		
25Ag-700 (*)	local_5	63.88	0.00	36.12	0.00	1.77		

247 (*) corresponding images are shown in Figure 6.

248 (**) non-calculated ratio because the analysed area did not correspond to a calcium phosphate.

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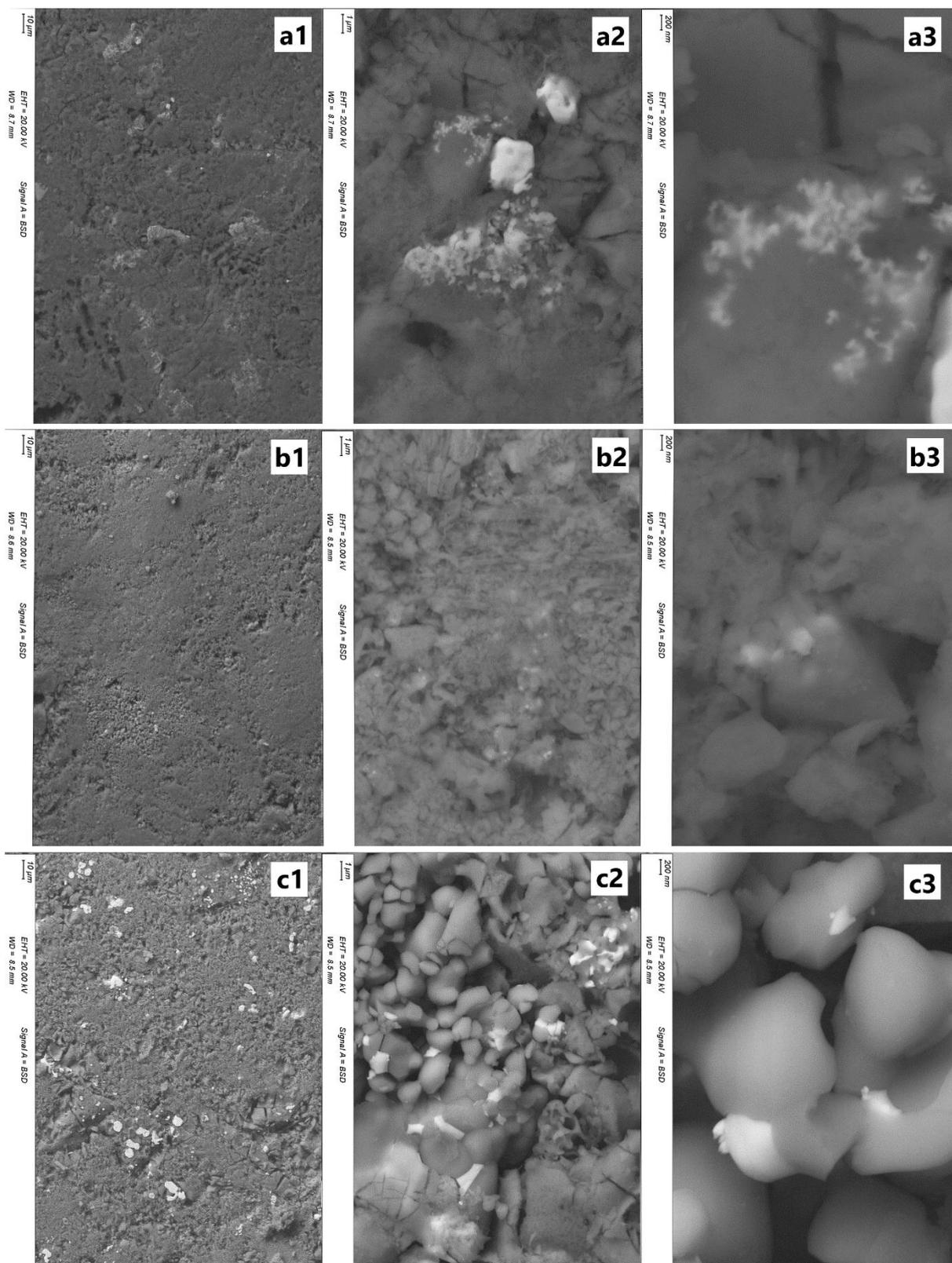
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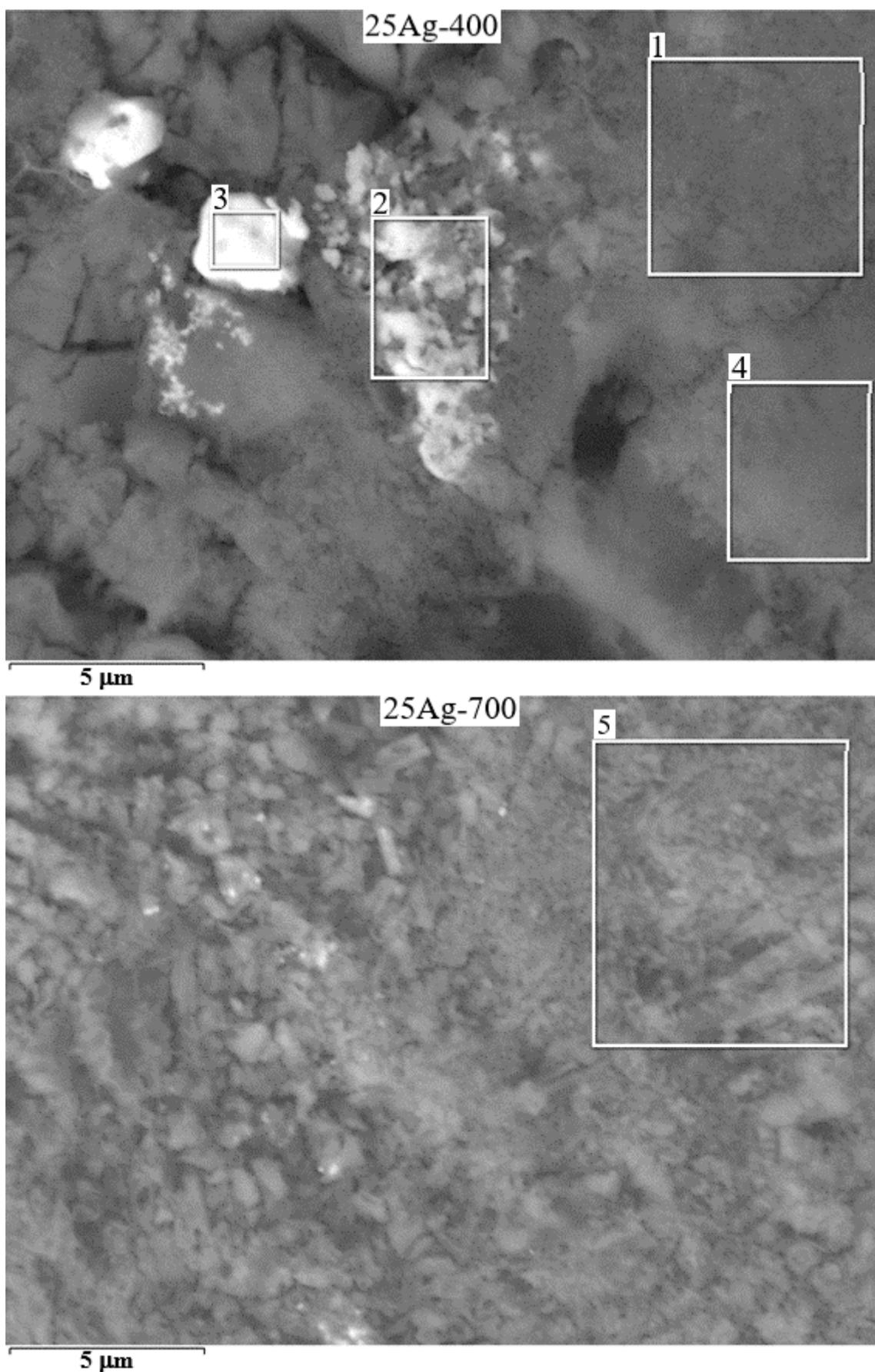
First, results from Table 2 confirm the complete disappearance of silver in doped series after heat treatment at 1200°C. We can conclude that above the melting point of silver, not only did the metallic Ag⁰ particles leave the sample, but the calcium phosphate phases were also completely free of Ag⁺ cations. The chemical contrast imaging performed with the backscattered electron detector makes it possible to highlight the notion of Ag⁰ particles (Figure 5). The use of low magnifications shows the presence of large, micrometric, particles (already present in the 25Ag-400 sample, and in large quantities in the 100Ag-700 sample). Then, thanks to higher magnifications, it is possible to visualize nanometric particles of small size. Figure 5a3 evidences the presence of silver particles of less than 100 nm diameter in 25Ag-400. These particles merged on heating to reach diameters of 200-400 nm in the 25Ag-700 sample (Figure 5b3) and 500-1000 nm in the 100Ag-700 sample (Figure 5c3). Images from Figure 5 clarify the composite feature of the samples.

Finally, local elemental analyses were performed to conclude the SEM characterization (see selected zones in Figure 6). Results from selected areas (EDS shown in Figure 7) are presented in Table 2 for the 25Ag-*T* series. These results confirm that nanoparticles were exclusively composed of silver: metallic Ag⁰ as shown in area 3 (and also area 2) in the 25Ag-400 sample. Indeed, the results from the local zone '3' are in favor of particle containing only the silver element (the minor Ca and P contents come from the not really punctual electron beam), and therefore of a metallic nature (i.e. Ag⁰ particles). These results also confirm the presence of silver in the phosphate calcium phases for the lowest heat treatment temperatures. Areas 1 and 4 indicate the presence of small amounts of silver, in combination with large amounts of calcium and phosphor at 400°C, corresponding to an Ag⁺:doped HAp phase. This is no longer true at 700 °C, where the calcium and phosphorus elements are completely separated from the silver element: area 5 in sample 25Ag-700 is only composed of Ca and P corresponding to undoped calcium phosphate compound (Figure 7 and Table 2).



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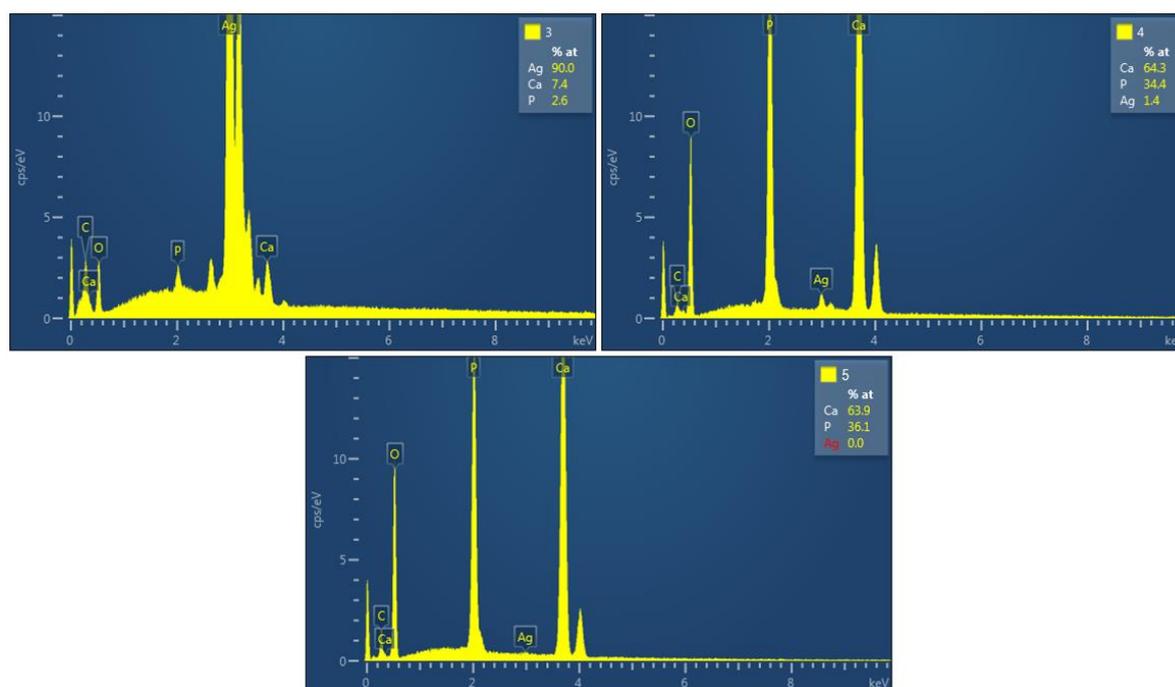
Figure 5. Electron Backscattered SEM images showing the metallic silver particles in the samples. a) 25Ag-400; b) 25Ag-700 and c) 100Ag-700 samples using 1: x500, 2: x5k and 3: x20k magnifications.



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Figure 6. Electron Backscattered SEM photographs used for local elemental analyses (presented in Table 2) of the 25Ag-400 and 25Ag-700 samples.

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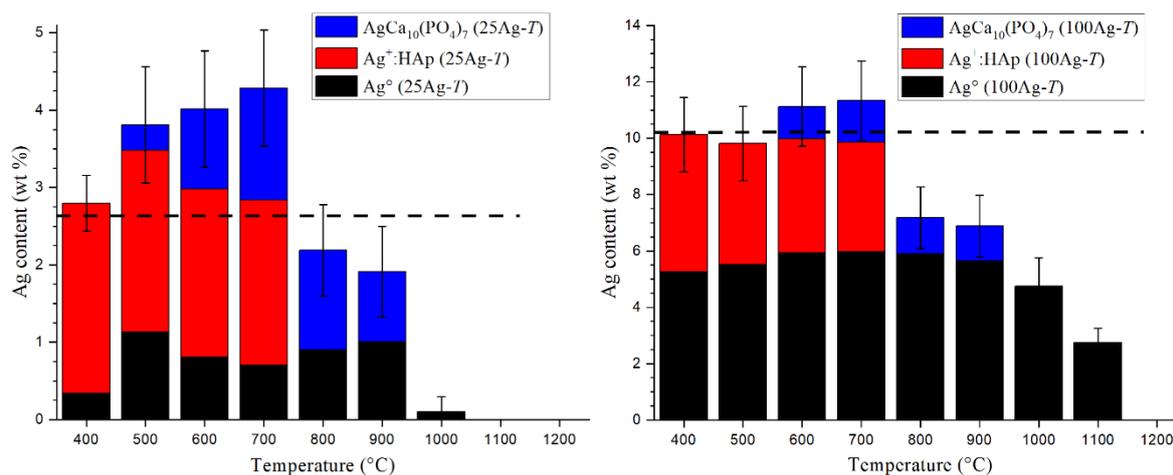
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282 **Figure 7.** Selected local EDS analyses (presented in Table 2) from zones '3' and '4' of the 25Ag-400
 283 sample and from zone '5' of the 25Ag-700 sample (shown in Figure 6).

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285 4-. Discussion

286 The results presented above evidence three distinct locations for silver atoms in the synthesized
 287 BCP samples, including two distinct chemical states (metallic and ionic). A part of the introduced
 288 silver cations is found in the metallic state, in the form of nanoparticles, already present at 400 °C.
 289 The remainder is in the cationic form in substitution for calcium cations, either in the HAp phase (at
 290 low temperatures: from 400 °C to 600 °C) or in the TCP phase (at the following temperatures and up
 291 to 1000 °C). From 1000 °C silver leaves the samples, especially because of the melting point of Ag⁰
 292 (962 °C). The cumulative amounts of located silver atoms are shown in Figure 8 for both the 25Ag-*T*
 293 and 100Ag-*T* doped series. It appears that calculations for the lowest (400 °C) temperature are in good
 294 agreement with the nominal introduced amounts (i.e. 2.67 wt % for the 25Ag-*T* series and 10.22 wt %
 295 for the 100Ag-*T* series). We then observe an overestimation of the silver content up to 600°C, before
 296 seeing it start to plummet at 700 °C. These fluctuations around the expected values indicate that the
 297 amount of silver in the HAp phase is overestimated above 500 °C, and conversely the amount of
 298 silver in substitution in β-TCP is certainly underestimated from 800 °C upwards.



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Figure 8. Cumulative silver amount taken from metallic Ag° (black), substituted HAp (red) and

$\text{AgCa}_{10}(\text{PO}_4)_7$ (or substituted TCP, blue) for the 25Ag-T series (left) and the 100Ag-T series (right).

Dotted lines indicate the nominal silver amounts, and error bars correspond to cumulative standard

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deviation considering the three phases.

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Nanoparticles of metallic silver therefore formed very quickly in our samples by the autogenous

reduction of silver nitrate. The set of samples can thus be considered as a $\text{Ag}^\circ/\text{BCP}$ composite.

However, at low temperatures, we also observe the formation of a silver-doped $\text{Ag}^+:\text{HAp}$ phase

whose composition is close to the nominal composition (i.e. $\text{Ca}_{9.75}\text{Ag}_{0.25}(\text{PO}_4)_6(\text{OH})_{1.75}$ for the 25Ag-T

series and $\text{Ca}_9\text{Ag}_1(\text{PO}_4)_6(\text{OH})$ for the 100Ag-T series). Beyond 400 °C, this doped $\text{Ag}^+:\text{HAp}$ phase

persists while gradually lowering its doping element content. The drop in the doping rate was

notably predictable by observing the variations in the HAp lattice parameters between 400 °C and

700 °C (Figure 3). Both a and c lattice parameters of the $\text{Ag}^+:\text{HAp}$ phase are vastly superior to those

from the undoped series at 400 °C. This difference fades at 500 °C and disappears at 700 °C. SEM

analyses confirmed the presence of dopant in the calcium phosphate phase at 400 °C, followed by its

exclusion at 700 °C. Concomitantly, cationic Ag^+ substitutes calcium cations into the β -TCP phase,

which stabilizes around 700 °C. First, the $\text{AgCa}_{10}(\text{PO}_4)_7$ definite compound is formed; it is

distinguishable from the undoped β -TCP phase thanks to the smaller c lattice parameter (Figure 4).

The underestimation of silver content from 800 °C upwards suggests that the Ag^+ amount in the TCP

phase continues to increase; however, no experimental proof has been brought to support this.

The characterization of the doped $\text{Ag}^+:\text{HAp}$ phase with increased lattice parameters, by

comparison with an undoped HAp lattice, has already been reported in the literature. This is notably

the case in the study of Geng et al. [32], where the $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ doped composition was

considered. This nominal composition suggests two kinds of doping mechanism: only half of the

silver cations (x value) can substitute calcium cations into the Ca1 or Ca2 crystallographic sites of the

HAp crystal structure. The second half of silver cations (to reach the $2x$ doping level) must be located

at interstitial sites. Our crystallographic study, based on Rietveld refinement, did not enable us to

highlight the presence of silver cations at interstitial crystallographic sites, not to mention that this

situation is not preponderant for large cations like Ag^+ . For these reasons we preferred to note the

chemical composition of the doped $\text{Ag}^+:\text{HAp}$ phase as follows: $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_{2-x}$, in which only

the substitution mechanism was considered in agreement with the paper by Badroun et al. [61].

However, we must admit that our Rietveld refinements did not make it possible to clearly quantify

the silver substitution rates at both the Ca1 and Ca2 crystallographic sites in the HAp structure.

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5-. Conclusion

During the past decade, several studies have been devoted to silver incorporation in HAp (or

BCP) samples due to their high potential for biomaterial applications, namely because of the well-

known bactericide properties of silver. Unanimously, the results have shown a very interesting

bactericidal effect following the doping of calcium phosphates with silver. However, the material

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337 aspect of the samples studied in the literature showed some disparities, in particular due to a lack of
338 understanding of the doping mechanism. Some works mention metallic silver nanoparticles; others
339 focus on cationic substitution in phosphate phases. The results presented in the present paper are
340 especially devoted to the description of the mechanism by which silver is incorporated into BCP
341 samples. This work follows the experience previously acquired on the BCP doping mechanism of the
342 first-row transition elements. It appears that both electronic states are simultaneously present in the
343 silver-doped samples: metallic Ag^0 and cationic Ag^+ . Synthesized samples are composites comprising
344 closely-mixed Ag^0 nanoparticles and Ag^+ -doped calcium phosphate phases. At the lowest
345 temperature (400 °C), it is the HAp phase which presents a silver-to-calcium substitution leading to
346 the doped $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_{2-x}$ compounds. Then, by increasing the temperature (above 600 °C) the
347 Ag^+ cations migrate to the β -TCP phase to form the definite $\text{Ca}_{10}\text{Ag}(\text{PO}_4)_7$ compound corresponding
348 to $\text{Ca}_{3-x}\text{Ag}_{2x}(\text{PO}_4)_2$ with $x = 0.143$ (i.e. $\text{Ca}_{2.857}\text{Ag}_{0.286}(\text{PO}_4)_2$). Finally, the composite aspect of the prepared
349 samples disappears above 1000 °C because the relatively low melting point of silver (962 °C). In the
350 light of these results, it appears important for the study of biological properties to distinguish the
351 behaviour in biological conditions of metallic silver nanoparticles from that of substituted cationic
352 Ag^+ , particularly in terms of bactericidal power and release rate/kinetics in the human body.

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