BIOMATERIAUX
Chapitre 3 – Apatite

D. Bazin
Laboratoire de Physique des Solides UMR 8502,
Université Paris Sud, Bât 510 91405 Orsay Cedex, France.
Chapitre 3 Apatite

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<td>Chapitre 3D.3a :</td>
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<td>Chapitre 3D.4a : activation of bone repair materials</td>
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<td>p118</td>
<td>Chapitre 3D.4a : activation of bone repair materials</td>
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<td>Chapitre 3D.5 Localisation</td>
</tr>
<tr>
<td>p124 Cd$^{2+}$</td>
<td>Chapitre 3D.6 Localisation</td>
</tr>
<tr>
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<td>Chapitre 3D.6 Localisation</td>
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Chapitre 3D.1a Release of Zn$^{2+}$

Recently, the effects of Zn$^{2+}$ on osteogenesis stimulation have become major topics in the research fields of bone formation and organism essential elements. A WHO expert committee on trace elements in human nutrition reported that Zn stimulates bone formation in humans and many animals.

In this investigation, we studied the effects of ZnTCP added to apatite cement (AC) with respect to its setting reaction and proliferation of human osteoblastic cells as an initial evaluation for the feasibility of AC containing ZnTCP.

Des mélanges complexes : In brief, an equimolar mixture of tetracalcium phosphate (TTCP; Ca$_4$(PO$_4$)$_2$O), prepared from CaHPO$_4$ and CaCO$_3$, with a median particle size of 10.1 mm and ground dicalcium phosphate anhydrous (DCPA; CaHPO$_4$, J.T. Baker Chemical Co., NJ) with a medium particle size of 1.2 mm were mixed using a speed mill (SK-M2, Kyoritsuriko, Tokyo, Japan) as described previously.

Fig. 1. Powder X-ray diffraction patterns of
(a) unreacted powder phase of AC;
(b) ZnTCP with composition of $\text{Zn}_{0.3}\text{Ca}_{2.7}(\text{PO}_4)_2$;
(c) a mixture of unreacted powder phase of AC and 10% ZnTCP;
(d) set c- AC (0%) kept in an incubator at 37°C and 100% relative humidity for 24 h;
(e) set c-AC (6%) kept in an incubator at 37°C and 100% relative humidity for 24 h;
(f) set c-AC (10%) kept in an incubator at 37°C and 100% relative humidity for 24 h;
(g) a mixture of set c-AC (0%) kept in an incubator at 37°C and 100% relative humidity for 24 h and ZnTCP corresponding to c-AC (10%);
(h) poorly crystalline AP.

We concluded therefore, that addition of ZnTCP to AC is useful to enhance the osteoconductivity of AC when release of $\text{Zn}^{2+}$ can be carefully regulated.
Chapitre 3D.1b : release de protéine

Généralités : Numerous studies have shown that Nanoparticles (NPs) can not only
- improve the resistance of therapeutic agents against enzymatic degradation
- but also provide the possibility of transporting biomolecules to specific tissues, cells, and cell compartments in a controlled manner with a minimally invasive procedure.

Bovine serum albumin (BSA) protein incorporated with hydroxyapatite (HA) nanoparticles (NPs) were synthesized. 2 mol% Zn$^{2+}$ and Mg$^{2+}$ were used as dopants to synthesize Zn$^{2+}$/Mg$^{2+}$-doped HA-BSA NPs. In our study we used BSA as a model protein. The amount of BSA uptake by doped and undoped HA NPs and subsequent release of BSA from NPs were investigated.

Caractérisation des matériaux : XRD, FTIR, TEM

**Figure 1.** X-ray diffraction pattern of doped and undoped HA-BSA nanopowder.

**Table 1.** Peak Width Measurements of the (002) and (310) Reflections of BSA-Loaded Doped and Undoped HA Nanopowder

<table>
<thead>
<tr>
<th>sample</th>
<th>peak width (2θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA-BSA</td>
<td>(002) 0.456</td>
</tr>
<tr>
<td>MgHA-BSA</td>
<td>(002) 0.476</td>
</tr>
<tr>
<td>ZnHA-BSA</td>
<td>(002) 0.512</td>
</tr>
</tbody>
</table>

**Figure 4.** Particle size distribution of synthesized HA-BSA nanopowder.
Our study showed that the protein release rate from HA NPs can be controlled by the addition of suitable dopants, and doped HA-based NP systems can be used in bone growth factor and drug release study.
Ca$_6$Ca$_4$(PO$_4$)$_6$OH$_2$. Understanding of how trace elements are accommodated in the HAP structure is essential for evaluating and predicting its long-term stability as well as the bioavailability of incorporated trace elements$^{9,10,11,12}$. Despite the importance of Zn interaction and storage in biological tissues and minerals, the mode of Zn incorporation in HAP, specifically the binding site and its local structure, is still not clearly understood. One reason for this uncertainty is the presence of two structurally distinct cation sites, Ca1 and Ca2, in HAP that appear to be suitable for Zn substitution.

Additional uncertainty arises due to the different coordination environments that Zn can adopt, both tetrahedral and octahedral.

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Méthodes de Caractérisation

A faible concentration pour le dopant, la diffraction des rayons X est inopérante.

**Conclusion:** X-ray absorption near edge structure (XANES) spectroscopy results suggest one dominant coordination environment for the incorporated Zn, and no evidence was observed for other Zn-containing phases. Extended X-ray absorption fine structure (EXAFS) fitting of the synthetic samples confirms that Zn occurs in tetrahedral coordination, with two P shells at 2.85–3.07 Å, and two higher Ca shells at 3.71–4.02 Å.

These fit results are consistent with the most favored DFT model for Zn substitution in the Ca2 site.
### Table 3
First coordination sphere around Zn atoms determined through Xas in the case of synthetic apatites. Selected details regarding the preparation are given.

<table>
<thead>
<tr>
<th>Synthetic HAP</th>
<th>Uptake (%)</th>
<th>pH</th>
<th>$N_{ZnO}$</th>
<th>$R_{ZnO}$ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precursor : ZnCl₂ [46]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Zn]tot µM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>58.9</td>
<td>5</td>
<td>4.4</td>
<td>1.96</td>
</tr>
<tr>
<td>3000</td>
<td>68.5</td>
<td>5</td>
<td>5.1</td>
<td>1.98</td>
</tr>
<tr>
<td>5000</td>
<td>69.6</td>
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<td>5.3</td>
<td>1.98</td>
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<td>50</td>
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<td>250</td>
<td>97.6</td>
<td>7.3</td>
<td>4.3</td>
<td>1.97</td>
</tr>
<tr>
<td>5000</td>
<td>31.4</td>
<td>7.3</td>
<td>5.1</td>
<td>2.00</td>
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<td>100</td>
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</tr>
<tr>
<td>1000</td>
<td>99.4</td>
<td>9.0</td>
<td>5.2</td>
<td>1.99</td>
</tr>
<tr>
<td>Precursor : Zn(NO₃)₂ [47]</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Precursor : ZnCl₂ [48]</td>
<td>Fluoride</td>
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<td></td>
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<tr>
<td>Zn %</td>
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<td>4</td>
<td>1.99</td>
<td></td>
</tr>
<tr>
<td>1.70</td>
<td>/</td>
<td>4</td>
<td>1.98</td>
<td></td>
</tr>
<tr>
<td>1.69</td>
<td>138</td>
<td>4</td>
<td>1.98</td>
<td></td>
</tr>
<tr>
<td>1.64</td>
<td>1260</td>
<td>4</td>
<td>1.98</td>
<td></td>
</tr>
<tr>
<td>1.70</td>
<td>8540</td>
<td>4</td>
<td>1.99</td>
<td></td>
</tr>
<tr>
<td>1.68</td>
<td>22,100</td>
<td>4</td>
<td>1.99</td>
<td></td>
</tr>
<tr>
<td>[50]</td>
<td></td>
<td>2</td>
<td>1.79</td>
<td></td>
</tr>
<tr>
<td>Precursor ZnCl₂ [51]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Zn]tot mM</td>
<td>T°C</td>
<td>pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>75-80</td>
<td>10</td>
<td>3.4</td>
<td>1.95</td>
</tr>
<tr>
<td>0.5</td>
<td>75-80</td>
<td>10</td>
<td>4.4</td>
<td>1.96</td>
</tr>
<tr>
<td>1.</td>
<td>75-80</td>
<td>10</td>
<td>3.5</td>
<td>1.95</td>
</tr>
<tr>
<td>Precursor : Zn(NO₃)₂ 6 H₂O [57]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0</td>
<td>1.98</td>
<td></td>
</tr>
</tbody>
</table>
As the mechanical strength of both cortical and trabecular bone is related to the

- crystal size,
- crystallinity,
- composition of bone crystal\textsuperscript{15},

changes in the chemical composition of bone crystal could account for changes in the mechanical strength of both cortical and trabecular bone.

**Cristal size**: The size of individual Sr\textsuperscript{2+} HA crystallites were calculated from XRD data using the Scherrer equation and verified by measuring 200 particles by TEM. The peak at 25.9° 2\(\theta\) (002) was fit to define its full width at half maximum intensity (B\(_{1/2}\)(rad-2\(\theta\))) :

\[
d = \frac{k \lambda}{(B_{1/2}(\text{rad}-2\theta)) \cos \theta}
\]

where \(d\) is the crystal size, as calculated for the (hkl) reflection, \(\lambda\) is the wavelength of Cu K\(\alpha\) radiation (\(\lambda = 1.5418\text{Å}\)), and \(k\) is the broadening constant varying with crystal habit and chosen as 0.9 for the elongated apatite crystallites.

\textsuperscript{14} Z.Y. Li et al. Chemical composition, crystal size and lattice structural changes after incorporation of strontium into biomimetic apatite Biomat. 28 (2007)1452-1460.
\textsuperscript{15} Adele B, Bone mineral crystal size. Osteoporos Int 2003;14:16–21.
As the mechanical strength of both cortical and trabecular bone is related to the
- crystal size,
- **crystallinity**,
- composition of bone crystal\(^{16}\),
changes in the chemical composition of bone crystal could account for changes in the mechanical strength of both cortical and trabecular bone.

The crystallinity noted by \(X_c\), corresponds to the fraction of crystalline apatite phase in the investigated volume of powdered sample. An empirical relation between \(X_c\) and the \(B_{1/2}\) was deduced, according to the equation as below:

\[
B_{1/2} \times \frac{3}{\sqrt{X_c}} = K_A
\]

where \(X_c\) is the crystallinity degree, \(B_{1/2}\) is the full width of the peak at half intensity of (002) reflection in (degree-\(2\theta\)), \(K_A\) is a constant set at 0.24.

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Fig. 2. XRD patterns of HA (a) and Sr-HA (b-d) with different Sr incorporation. 1.5% and less Sr incorporation did not change the patterns of Sr-HA, while the peaks of 15% Sr-HA were broadened.

Table 2
Crystal size and crystallinity of Sr-HA reflected by XRD pattern

<table>
<thead>
<tr>
<th>Sample</th>
<th>Line width (002) FWHM, (rad)</th>
<th>Average crystal size d (nm) by Scherrer’s equation</th>
<th>Line width (002) FWHM, (deg)</th>
<th>Crystallinity (Xc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>0.0061</td>
<td>23.31</td>
<td>0.35</td>
<td>0.3224</td>
</tr>
<tr>
<td>Sr-HA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3%</td>
<td>0.0061</td>
<td>23.31</td>
<td>0.35</td>
<td>0.3224</td>
</tr>
<tr>
<td>1.5%</td>
<td>0.0061</td>
<td>23.31</td>
<td>0.35</td>
<td>0.3224</td>
</tr>
<tr>
<td>15%</td>
<td>0.0113</td>
<td>12.54</td>
<td>0.65</td>
<td>0.0503</td>
</tr>
</tbody>
</table>

FWHM: full-width at half-maximum intensity of peak (002).
Fig. 4. (a) TEM (Left) and HRTEM (Right) of HA;
(b) TEM (Left) and HRTEM (Right) of 0.3% Sr-HA;
(c) TEM (Left) and HRTEM (Right) of 1.5% Sr-HA;
(d) TEM (Left) and HRTEM (Right) of 15% Sr-HA; 1.5% and less Sr incorporation did not change the crystal shape and lattice spacing of Sr-HA (a–c). However, the crystal size and observed lattice spacing dramatically decreased with 15% Sr incorporation (d).
Conclusion : TEM images showed that the crystal length and width of 0.3% and 1.5% Sr-HA increased slightly. Meanwhile, the length and width distribution were broadened and the aspect ratio decreased from $10.68 \pm 4.00 \text{Å}$ to $7.28 \pm 2.80 \text{Å}$. The crystal size and crystallinity of 15% Sr-HA dropped rapidly, which may suggest that the fundamental crystal structure is changed.

The findings from this work indicate that current clinical dosage which usually results in Sr incorporation of below 1.5% may not change chemical composition and lattice structure of bone, while it will broaden the bone crystal size distribution and strengthen the bone.
Sr$^{2+}$ Chapitre 3D.1b Taille des cristaux 2/2 \(^{17}\)

Sr is a bone seeking element and 98% of the total body Sr content can be found in the skeleton\(^ {18}\).

Conclusion: in both mineralized cultures and synthetic HA, XRD and FTIR analysis showed a reduced crystallinity and altered crystal lattice at similar concentrations.

**Sr²⁺ Chapitre 3D.1c Solubilité de l’apatite**

Strontium may substitute at Ca sites, forming a continuous solid-solution (Srₓ-HAp) up to full substitution (Sr-HAp) due to chemical similarity.

However, the mechanism and behaviour of strontium in such systems are controversial.

- Grynpas²⁰ thought that the incorporation of strontium induced hypomineralization by weakening the apatite lattice and so increasing bone mineral solubility, while it was recently reported that no stimulation of new bone formation was found in ovariectomized rats after SrR intake for 3 months²¹.

- On the other hand, Christoffersen et al.²² and Dedhiya et al.²³ found that strontium significantly inhibited the dissolution of HAp, and a “surface complex”, Ca₃Sr₂(PO₄)₃OH (40% Sr-substituted HAp, Sr40-HAp), has been postulated to account for such dissolution retardation²⁴.

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Fig. 1. XRD pattern of the solids prepared by the hydrothermal method; standard XRD patterns of HAp (JCPDS 72-1243) and Sr-HAp (JCPDS 70-1511) for comparison.

Fig. 2. Typical particle morphologies for the strontium-substituted hydroxyapatites.

Influence de la teneur en Sr sur la morphologie des cristaux
**Conclusion:** Solubility increased with increasing strontium content. No phase other than strontium-substituted HAp, corresponding to the original titrant, was detected in the solid present at equilibrium; in particular, dicalcium hydrogen phosphate was not detected at low pH. The increase in solubility with strontium content is interpreted as a destabilization of the crystal structure by the larger strontium ion. Carbonated HAp was formed in simulated body fluid containing carbonate on seeding with Sr10-HAp, but the precipitate was strontium-substituted on seeding with Sr-HAp. Strontium-substituted HAp might be usable as a template for the growth of new bone, since nucleation appears to be facilitated.
Les carbonates : Among the hetero-ions substituting in biological HA, CO$_3^{2-}$ is the most abundant (2–8 wt.%$^{26}$), and partially substitutes both in the PO$_4^{3-}$ site (B-type HA) and the OH- site (A-type HA) of the HA structure, with a preference for the former.

The high reactivity of young bone could be related to the greater presence of B-CO$_3$ compared with in old bone.

B-type carbonated hydroxyapatites showed
- improved solubility,
- collagen deposition in vitro,
- and reabsorption in vivo,
compared with stoichiometric HA and/or type A CHA$^{27,28,29}$. 

Préparation : Sr and CO$_3$ co-substituted hydroxyapatite (SrCHA) nanopowder was synthesized by neutralization. The powder was characterized. The improved solubility in Hanks’ balanced solution of SrCHA granules (400–600 µm of dimensional range), potentially usable as bone filler, was assessed and compared with that of an analogous carbonate free granulate.

The X-ray diffraction (XRD) analysis of the synthetic SrCHA powder detected a broad spectrum, typical of nanocrystalline apatite (Fig. 1). The cell parameter values were evaluated as \(a = b = 9.429 \text{ Å}\) and \(c = 6.933 \text{ Å}\), and a \(c/a\) ratio value of 0.7350 was obtained (increased compared with the stoichiometric HA one of 0.7309, since \(a = b = 9.418 \text{ Å}\) and \(c = 6.884 \text{ Å}\)). The crystallite dimensions were \(D_{002} = 230 \text{ Å}\) and \(D_{300} = 150 \text{ Å}\), giving an aspect ratio of 1.53.
Conclusion: The possibility of widely modulating, by acting on the chemical–physical–geometrical features of the material, the prolonged in situ release of therapeutic Sr, together with the fundamental (Ca, PO₄) and main substituting (CO₃) ions that constitute the bone mineral phase, makes the use of SrCHA as resorbable bone filler or bone substitute scaffolds promising, especially when pathologies related with Sr deficiency are present. In vitro and in vivo tests are in progress.
Sr$^{2+}$ Chapitre 3D.2e Localisation 1$^{30}$

The unit cell of stoichiometric crystalline hydroxyapatite hosts 10 cations arranged in two nonequivalent positions: four at the M(1) site aligned in the column, each surrounded by nine oxygen atoms, and six at the M(2) site arranged at the apexes of “staggered” equilateral triangles, each surrounded by seven oxygen atoms$^{31,32,33}$.

Protocole de preparation: (Ca–Sr) hydroxyapatites (Ca–Sr–HA) with Sr/(Ca + Sr) molar ratios in the range from 0 to 1 were synthesized in N$_2$ atmosphere using 50 ml solutions with different Sr/ (Ca + Sr) ratios prepared by dissolving the appropriate amounts of Ca(NO$_3$)$_2$ · 4H$_2$O and Sr(NO$_3$)$_2$ in CO$_2$-free deionized water and adjusting the pH to 10 with NH$_4$OH.

2.2.1. Scherrer analysis

The line broadening of the 002 and 310 reflections was used to evaluate the length of the coherent domains ($\tau_{h k l}$) along the c-axis and along a direction perpendicular to it. $\tau_{h k l}$ values were calculated from the widths at half maximum intensity ($\beta_{1/2}$) using the Scherrer equation [20]:

$$\tau_{h k l} = \frac{K \lambda}{\beta_{1/2} \cos \theta}$$

where $\lambda$ is the wavelength, $\theta$ the diffraction angle and $K$ a constant depending on crystal habit (chosen as 0.9). The silicon standard peak 1 1 1 was used to evaluate the instrumental broadening.

Fig. 1. Powder X-ray diffraction patterns of (a) Sr0; (b) Sr10; (c) Sr30; (d) Sr50; (e) Sr70; and (f) Sr100. The strontium content in the solid phase is reported in Table 1.

La localisation du Sr s’effectue à partir d’une analyse fine des diagrammes de diffraction du type Rietveld.
Conclusion: The results of the structure refinements carried out using the Rietveld method indicate that whilst in most of the range of concentration strontium displays a slight preference for the M(2) cation site coherently with its ionic radius, at very low concentrations its occupancy of the smaller M(1) site is slightly higher.
Protocole de préparation :

Through this method, an aqueous solution containing Ca(NO$_3$)$_2$ and Sr(NO$_3$)$_2$ was added dropwise to a (NH$_4$)$_2$HPO$_4$ solution at a flow rate of 5 ml min$^{-1}$ and temperature of 90°C. The pH of 10 was maintained by NH$_3$OH addition.

Caractérisation :

- X-Ray diffraction (XRD) experiments were performed on the SrHA-X samples at the ID15B beamline of the European Synchrotron Radiation Facility (ESRF). The incident X-ray energy was 89.5 keV. Debye rings were collected with a two-dimensional MAR345 image plate with a diameter of 345 mm (2300 x 2300 pixels).

- EXAFS spectra were acquired at the strontium K-edge (16.105 keV) using the facilities of the LNLS storage ring (Campinas, Brazil), operated at the energy of 1.37 GeV with a maximum beam current of 120 mA.

Fig. 2 X-Ray diffraction data. (a) Raw intensities for 1, 5, 10 and 15 at% SrHAp. (b) Fitted fit and residuals for 5 at% Sr.

Fig. 4 EXAFS in vicinity of Sr K-edge for SrHAp. (a) $\chi^2/(\nu)$ K-space oscillations (black) and fit (red), (b) K-space Fourier transform magnitude (black) and fit (red).
Conclusion: Density functional theory periodic band-structure calculations indicate that the Ca^{2+} to Sr^{2+} substitution induces strong local distortion on the hydroxyapatite lattice: the nearest neighbor Sr–O bond structures in both cationic sites are comparable to pure SrHA, while Sr induces more distortion at site 2 than site 1.

Infrared vibrational spectroscopy (FTIR) and extended X-ray absorption fine structure (EXAFS) analysis suggest increasing lattice disorder and loss of OH with increasing Sr content.

Rietveld refinement of synchrotron X-ray diffraction patterns shows a preference for the Ca1 site at Sr concentrations below 1 at.%. The ideal statistical occupancy ratio Sr2/Sr1 = 1.5 is achieved for 5 at.%; for higher Sr concentrations occupation of the Ca2 site is progressively preferred.
Protocole de préparation

- Calcium nitrate 4-hydrate (Ca(NO$_3$)$_2$4H$_2$O) and/or strontium nitrate (Sr(NO$_3$)$_2$) were dissolved in 200 ml of distilled water.

- Calcium hydroxide (Ca(OH)$_2$) and strontium hydroxide (Sr(OH)$_2$) were dissolved in 500 ml of distilled water.

Fig. 1. (a) XRD traces and Rietveld refinement of the series (Sr$_x$Ca$_{1-x}$)$_5$(PO$_4$)$_3$OH, where $x = 0.00, 0.25, 0.50, 0.75$ and $1.00$: data points (XRD) and solid lines (Rietveld);
(b) Rietveld refinement for $x = 0.50$, with residual and positions of Bragg reflections.

Crystal sizes, $d$, were calculated from the full width at half maximum values (FWHMs) of the most intense Bragg reflection at around $2\theta = 25.9^\circ$, corresponding to the 002 $hkl$ reflection and using the formula equation shown below in Eq. (1) [1.6]

$$d = \frac{0.9 \lambda}{w \cos \theta_x}$$

Table 3
Parameters from Rietveld refinement of the series $(Sr_xCa_{1-x})_3[PO_4]_2OH$, where $x = 0.00, 0.25, 0.50, 0.75$ and 1.00, with standard deviations

<table>
<thead>
<tr>
<th>Sr</th>
<th>Ca</th>
<th>$a$ / Å</th>
<th>$2\sigma_a$ / Å</th>
<th>$c$ / Å</th>
<th>$2\sigma_c$ / Å</th>
<th>$V$ / Å$^3$</th>
<th>$2\sigma_V$ / Å$^3$</th>
<th>$\rho$/g.cm$^{-3}$</th>
<th>$d$ / nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>1.00</td>
<td>9.411</td>
<td>0.004</td>
<td>6.877</td>
<td>0.003</td>
<td>527.5</td>
<td>0.378</td>
<td>3.163</td>
<td>17.55</td>
</tr>
<tr>
<td>0.25</td>
<td>0.75</td>
<td>9.505</td>
<td>0.007</td>
<td>6.950</td>
<td>0.006</td>
<td>543.8</td>
<td>0.648</td>
<td>3.384</td>
<td>11.37</td>
</tr>
<tr>
<td>0.50</td>
<td>0.50</td>
<td>9.596</td>
<td>0.004</td>
<td>7.054</td>
<td>0.004</td>
<td>562.6</td>
<td>0.452</td>
<td>3.692</td>
<td>9.28</td>
</tr>
<tr>
<td>0.75</td>
<td>0.25</td>
<td>9.659</td>
<td>0.005</td>
<td>7.182</td>
<td>0.004</td>
<td>590.3</td>
<td>0.740</td>
<td>5.897</td>
<td>12.94</td>
</tr>
<tr>
<td>1.00</td>
<td>0.00</td>
<td>9.777</td>
<td>0.003</td>
<td>7.288</td>
<td>0.003</td>
<td>603.3</td>
<td>0.472</td>
<td>4.068</td>
<td>20.50</td>
</tr>
</tbody>
</table>

Sr in Ca (II)
**Sr$^{2+}$ Chapitre 3D.2h Localisation 4**

Fig. 2. XRD patterns of HA (a) and Sr-HA (b–d) with different Sr incorporation. 1.5% and less Sr incorporation did not change the patterns of Sr-HA, while the peaks of 15% Sr-HA were broadened.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Line width (002)</th>
<th>Average crystal size d (nm) by Scherrer’s equation</th>
<th>Line width (002) FWHM$_h$ (deg)</th>
<th>Crystallinity ($\lambda$$_c$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>0.0061</td>
<td>23.31</td>
<td>0.35</td>
<td>0.3224</td>
</tr>
<tr>
<td>Sr-HA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3%</td>
<td>0.0061</td>
<td>23.31</td>
<td>0.35</td>
<td>0.3224</td>
</tr>
<tr>
<td>1.5%</td>
<td>0.0061</td>
<td>23.31</td>
<td>0.35</td>
<td>0.3224</td>
</tr>
<tr>
<td>15%</td>
<td>0.0113</td>
<td>12.54</td>
<td>0.65</td>
<td>0.0503</td>
</tr>
</tbody>
</table>

FWHM: full-width at half-maximum intensity of peak (002).

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The crystallinity noted by $X_c$ corresponds to the fraction of crystalline apatite phase in the investigated volume of powdered sample. An empirical relation between $X_c$ and the $B_{1/2}$ was deduced, according to the equation as below:

$$B_{1/2} \times \frac{3}{\sqrt{X_c}} = K_A \quad (2)$$

where $X_c$ is the crystallinity degree, $B_{1/2}$ is the full width of the peak at half intensity of (0 0 2) reflection in (degree-2θ), $K_A$ is a constant set at 0.24.
Summary: Gallium, a group IIIa metal, is known to interact with hydroxyapatite as well as the cellular components of bone. In recent studies we have found gallium to be a potent inhibitor of bone resorption that is clinically effective in controlling cancer-related hypercalcemia as well as the accelerated bone resorption associated with bone metastases. To begin to elucidate gallium's mechanism of action we have examined its effects on bone mineral properties. After short-term (14 days) administration to rats, gallium nitrate produced measurable changes in bone mineral properties. Using atomic absorption spectroscopy, low levels of gallium were noted to preferentially accumulate in regions of active bone formation, \(0.54 \pm 0.07 \mu g/mg\) bone in the metaphyses versus \(0.21 \pm 0.03 \mu g/mg\) bone in the diaphyses, \(P<0.001\). The bones of treated animals had increased calcium content measured spectrophotometrically. Rats injected with radiolabeled calcium during gallium treatment had greater 45-calcium content compared to control animals. By wide-angle X-ray analyses, larger and/or more perfect hydroxyapatite was observed. The combined effects of gallium on bone cell function and bone mineral may explain its clinical efficacy in blocking accelerated bone resorption.

37. R. S. Bockman et al., Gallium increases bone calcium and crystallite perfection of hydroxyapatite, Calcified Tissue International 39, 1986,376-381.
Sufficient evidence has accumulated that lends credit to the possibility of direct and relevant participation of gallium in skeleton metabolism. Because of its strong affinity to bone tissue this trace element at present is widely used in the form of its radioactive isotope Ga-67 for diagnostic purposes. Gallium ions also are clinically effective against bone resorption and for the treatment of osteoporosis and cancer-related hypercalcemia.

Influence du précurseur

![X-ray diffractograms of gallium-doped hydroxyapatite. (I) Sample obtained using gallium nitrate; (II) sample obtained using sodium gallate; (III) commercial hydroxyapatite.](image)

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According to scanning electron microscopy images, gallium insertion does not cause any morphological alterations in hydroxyapatite structure.
**Conclusion**, Par analyse des diffractogrammes, Gallium does not replace calcium as a result of heterovalent substitution and consequently produces no distortions in the framework of hydroxyapatite matrix. It remains strongly fixed in the form of solid solution of intercalation. According to scanning electron microscopy images gallium insertion does not cause any morphological alterations in hydroxyapatite structure and the product developed meets physico-chemical criteria for biomaterial to be employed in orthopedic practice and local handling of traumatic injuries. Its future usage opens the opportunity to enhance osteosynthesis and calcium retention in local...
The surface activation of calcium phosphate-based biomaterials for bone repair is an emerging route for improving bone regeneration processes. One way for such activation is through the exchange of surface calcium ions with biologically-active cations such as Mg$^{2+}$ or Sr$^{2+}$.

In this work, the interactions of noncarbonated and carbonated nanocrystalline apatites with Mg$^{2+}$ and Sr$^{2+}$ were investigated by means of ion exchange experiments in solution. Langmuir-type isotherms were determined. For both Sr and Mg, a greater uptake was observed on the carbonated sample, and on both types of apatites the maximum strontium uptake was greater than that of magnesium.

Inverse exchanges showed that the proportion of reversibly fixed ions after surface exchange was close to 85% for Mg and 75–80% for Sr. The results are related to the presence of a surface hydrated layer on the nanocrystals and possible exchange mechanisms are discussed.

Autres travaux:

The presence of magnesium in apatite, for instance, causes a decrease in crystallinity with increasing Mg content$^{42,43}$.

The incorporation of foreign ions, such as Mg$^{2+}$, exhibiting a biological activity for bone regeneration is presently considered as a promising route for increasing the bioactivity of bone-engineering scaffolds.

The goal of this contribution is to investigate in further details the surface state of biomimetic apatite nanocrystals, exploring the particle morphology and following the interaction between water molecules and the surface of apatite nanocrystals. This study was carried out in the absence and in the presence of magnesium, incorporated by surface exchange with calcium ions, in order to eventually unveil modifications due to the presence of Mg$^{2+}$ ions.

44. L. Bertinetti et al., Surface Characteristics of Nanocrystalline Apatites: Effect of Mg Surface Enrichment on Morphology, Surface Hydration Species, and Cationic Environments Langmuir, 2009
It is worth noting that XRD analysis of such Mg-enriched apatite is still characteristic of nanocrystalline apatite and the presence of secondary crystalline Mg-containing phases was not observed.

The IR spectra related to Mg-free gels were found to exhibit a fine structuration (adjacent thin bands), especially in the range 900-1200 cm\(^{-1}\), characteristic of the \(\nu_1\nu_3\)\((PO_4)\) region, which was previously linked to the existence of a structured hydrated layer with characteristics close, although not identical, to those of octacalcium phosphate (OCP, as shown in Figure 2). It therefore reveals a strong alteration of phosphate chemical environments in the presence of magnesium.
Figure 4. TEM micrographs of hap-1d 0.9% Mg. (A) Mainframe: low-magnification image of the material (original magnification: 80K); black arrows indicate particles similar to those observed for the parent hap-1d material; **white arrows indicate new phase particles**; Inset: magnified view of the enframed region of an apatitic particle. (B-E) Magnified view of new phase particles constituted by 2, 3, and 4 planes (B-D, in that order), and exhibiting structural defects (E).

**Conclusion:** The IR study of the surface hydration indicated that the Ca$^{2+}$/Mg$^{2+}$ exchange resulted in an increase of the amount of water molecules absorbed on the surface, while the process also affected the “sub-surface layers” to some extent.
Calcium hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$, designated as Ca–HA, is a primary constituent of vertebral animal’s hard tissues. The synthetic Ca–HA has attracted our attention for its utility in the fields of bioceramics, catalysts, adsorbents, and so on\textsuperscript{46}. In the apatite structure, the Ca ions occupy two types of nonequivalent sites: the M (1) sites are at the fourfold symmetry 4(f) position and the M (2) sites are at the sixfold symmetry 6(h) position\textsuperscript{47}. The apatite structure has a high flexibility, so many metal ions can be incorporated into the structure. It is interesting to investigate the site of metal ions that are incorporated into the apatite structure.

\textbf{Table 1}

<table>
<thead>
<tr>
<th>Sample</th>
<th>$P$ (ppm)</th>
<th>$\text{Pb}$ (ppm)</th>
<th>$\text{Ca}$ (ppm)</th>
<th>(\text{Pb}/(\text{Ca+Pb})) molar ratio</th>
<th>(Ca+Pb)/P molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hf0</td>
<td>15.91</td>
<td>0.00</td>
<td>11.61</td>
<td>0.00</td>
<td>1.73</td>
</tr>
<tr>
<td>Hf10</td>
<td>14.11</td>
<td>6.77</td>
<td>9.19</td>
<td>0.12</td>
<td>1.77</td>
</tr>
<tr>
<td>Hf20</td>
<td>7.20</td>
<td>5.92</td>
<td>3.02</td>
<td>0.23</td>
<td>1.67</td>
</tr>
<tr>
<td>Hf30</td>
<td>10.88</td>
<td>12.77</td>
<td>5.27</td>
<td>0.32</td>
<td>1.69</td>
</tr>
<tr>
<td>Hf40</td>
<td>12.67</td>
<td>19.79</td>
<td>5.37</td>
<td>0.42</td>
<td>1.72</td>
</tr>
<tr>
<td>Hf50</td>
<td>11.35</td>
<td>20.76</td>
<td>3.92</td>
<td>0.51</td>
<td>1.65</td>
</tr>
<tr>
<td>Hf60</td>
<td>14.78</td>
<td>31.38</td>
<td>4.17</td>
<td>0.50</td>
<td>1.64</td>
</tr>
<tr>
<td>Hf70</td>
<td>12.17</td>
<td>33.82</td>
<td>2.32</td>
<td>0.74</td>
<td>1.73</td>
</tr>
<tr>
<td>Hf80</td>
<td>9.54</td>
<td>29.35</td>
<td>1.08</td>
<td>0.84</td>
<td>1.68</td>
</tr>
<tr>
<td>Hf90</td>
<td>7.29</td>
<td>25.88</td>
<td>0.34</td>
<td>0.94</td>
<td>1.74</td>
</tr>
<tr>
<td>Hf100</td>
<td>9.13</td>
<td>35.43</td>
<td>0.00</td>
<td>1.00</td>
<td>1.78</td>
</tr>
</tbody>
</table>

\textsuperscript{45} Zhu et al., Crystallographic study of lead-substituted hydroxyapatite synthesized by high-temperature mixing method under hydrothermal conditions, Inorganica Chimica Acta 363 (2010) 1785–1790


\textsuperscript{47} A. Hadrich, A. Lauti, T. Mhiri, J. Raman Spectrosc. 32 (2001) 33.
Fig. 3. TEM (a) 0 (b) 0.2 (c) 0.4 (d) 0.6 and SEM (e) 0.8 (f) 1 photographs of the samples with various Pb/(Pb+Ca) molar ratio prepared by HTMM.

The larger elongated crystals coexisted with the larger bulky crystals in the samples with vPb more than 0.6.

Conclusion:
It was found that Pb ions in the solid solutions preferentially occupied the M (2) site in the apatite structure.
Cadmium is dangerous for human health and ecosystem. A chronic intoxication by cadmium can have serious consequences on the kidneys, the lungs and the bones (osteoporosis). The main sources of cadmium in natural waters are industrial wastes and phosphate fertilizers.

The starting sample

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Fig. 4 shows the SEM micrographs of the starting HA and sample 78 hT30 obtained after 78 h in cadmium aqueous solution (6.97×10⁻³ M) at 30 °C. The starting HA powder is formed of rod-shaped agglomerated crystallites (Fig. 4a). After soaking in aqueous solution under stirring conditions, the crystallites are slightly more rounded than the starting HA, as shown in the example of sample 78 hT30 (Fig. 4b).
Conclusion

This study confirms that hydroxyapatites are efficient to immobilize cadmium in polluted aqueous solutions.

Cd$^{2+}$ cations substitute for the easiest accessible calcium at the HA surface. Then, these cadmium cations are incorporated into a solid solution Ca$_{10-x}$Cd$_x$(PO$_4$)$_6$(OH)$_2$ with 4.00$\geq x \geq$2.65.

This results in the formation of an apatite solid solution, which is very important because in this way decontamination and storage can be performed with the same material.
The use of contrast agents has expanded the role of imaging in medical diagnostic studies, and hence the size of the population exposed to chelated Gd.

In fact, since 2005 more than 20 million Gd-based contrast enhancement procedures are performed each year. Gd (z = 64) is a paramagnetic rare earth element (REE) commonly used as a contrast enhancement agent for clinical and diagnostic medical imaging. Gd was the first contrast agent introduced for diagnostic MRI studies because of its paramagnetic nature with an efficient (long) T1 relaxing mechanism.

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49. Darrah et al., Incorporation of excess gadolinium into human bone from medical contrast agents, Metallomics, 2009, 1, 479–488.
**Toxicology:** Free gadolinium (Gd$^{3+}$) is
- extremely toxic$^{55,56}$, 
- disrupts cellular processes$^{57,58}$, 
- inhibits stretch-activated ion channels$^{59}$, 
- is one of the most efficient known calcium antagonists$^{60}$.

Chelation of Gd in the MRI contrast media is intended to prevent toxic cationic Gd (Gd$^{3+}$) from directly interacting with cells and tissues, and to allow efficient and rapid removal of the contrast agent from the body following imaging studies$^{61}$.

Severe adverse health effects may occur with the use of Gd-based contrast agents if Gd dissociates from chelate structures in the bloodstream$^{55}$. Release from the chelating agent is known to occur via transmetallation reactions in which metals in blood and extracellular fluids (e.g. Cu, Zn, Fe, Ca) replace Gd within the chelate structure$^{62}$. Anions such as PO$_4^{3-}$, CO$_3^{2-}$, OH$^{-}$ compete with the chelates for the Gd cation.

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Fig. 3 Statistical distributions of normalized Gd anomaly (Gd/Gd*N) for cortical bone samples of control and Gd exposure patient groups (p = 0.001), and for both osteoarthritis and osteoporotic fracture patients exposed to Gd, which are also significantly different.

**Conclusion**: We find anomalously high gadolinium (Gd) concentrations in the femoral head bones of patients exposed to chelated Gd, commonly used as a contrast agent for medical imaging.