Hyperfine Interactions Measurements in Hydroxiapatite: Considerations and Perspectives

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1) Introduction - The use of nanoparticles, here enclosed their delivery by specific carriers, in current medicine are under systematic investigation(1). The possible advantages proposed by these systems are very impressive and the results may be quite schemer(2). One of such systems is the Hydroxiapatite (HA) ceramics [Ca_{10}(PO_4)_6(OH)_2], pure and doped with rare-earths, used as carriers for bone treatments in healthcare(3). The synthesized HA is a pure phase that is well established for bone replacement material in orthopedics and dentistry(4). More recently, it's highly active surface started to being used for doping it with several elements, for instance Holmium atoms (Ho), which are introduced in HA during its sintesization; with the expectation that, because of the HA similarity to bones structures, it could be successfully used as a Ho delivery system in bone's diseases(5). But, these substitutions may provoke, until present unknown, changes in the HA's surface structure and charge, and in such a way influencing it's ability to play it's role on natural bone remodeling processes. In this scenario, it is certainly interesting to enlarge the knowledge of the actions the atoms of the nanosystem's crystalline structures, as well as their changes, dopped or non-dopped, are playing to allow the consequent medical applications. And such changes, which occur in an atomic level can be, even considering the eventual difficulties of such approaches in the case of biological compounds, studied with the so called Hyperfine Interactions (HI)(6).

In this study HA nanoparticles, with and without doping Ho atoms, were prepared with diffused isotope-probe ^{111}In in order to use the Time-Differential Perturbed Angular Correlation (TDPAC) nuclear spectroscopy for measuring HI(7).

2) Materials & Methods

2.1 - Samples: the compounds annalized in these experiments were: a) nano-Hydroxyapatites (HA) sintesized at 310K (HA-310K) and 370K (HA-370K); b) nano-HA doped with Ho (HA-Ho) during it's
sintesization [all the previous mentioned samples were measured as non-thermalized and thermalized (heated at $T= 1,273K$ for 12h) structures]; c) Tricalcium Phosphate (TCP) non-thermalized.

2.2 – Structural Characterizations: generically the HA's calcium and phosphorous concentrations (Ca/P= 1.66) may be determined by X-Ray Fluorescence Spectroscopy (XFS), as well as their OH – groups can be identified by Fourier Transform Infrared Spectrophotometry (Fig.1-FTIS) in transmission mode from 400 cm$^{-1}$ to 4000 cm$^{-1}$, with their crystallites mean size ($\tau$) along HA, (002) and (300) directions, determined by Debye-Sherrer formula, $\tau= K.\lambda/\beta_{1/2}.\cos\theta$, where $\beta_{1/2}$ is the peak line width (values in radians) of the reflection and $K= 0.9$. More specifically, concerned the present case, all the samples here used were characterized by X-Ray Diffractometry (XRD) with CuK-radiation 40 kV-40 mA.

2.3 - Time Differential Perturbed Angular Correlation (TDPAC): when the electromagnetic field gradients (EFG) $V_{zz}$ present in the interior of a crystalline net interact with the nuclear electric quadrupole (Q) and/or magnetic ($\mu$) moments of the intermediate nuclear state of a $\gamma_1$-$\gamma_2$ decaying nuclear cascade of a suitable isotope-probe, it occurs what is being called HI which appear like wiggles in the TDPAC spectrum, here represented by the decay-line of the intermediate level of those $\gamma_1$-$\gamma_2$ cascade. The electromagnetic fields that contribute to the HI originate from charges and "spins" of the inner atomic levels of the isotope-probe influenced by the atoms of the next neighborhood to this isotope-probe, leading to the characterization of these neighborhoods. The HI may be described by a perturbation factor $G_{22}(t)$ which, in the case of $V_{zz}/Q$ static interactions in policrystalline samples, can be expressed as an overlapping of oscillatory components:

$$G_{22}(t)= [\sigma_{20}+\Sigma\sigma_{2n}.F_1(\delta,\omega_n,t).F_2(\tau_d,\omega_n).\cos(\omega_n t)].\exp(-\lambda_2 t) \quad (eq.1)$$

$\omega_n(\nu_0,\eta)$: transition frequencies between the magnetic sub-levels related to the "spin" of the intermediate nuclear state of the probe-isotope; $F_1$: function associated to the nuclear interaction frequency distribution (usually lorentzian or gaussian); $F_2$: function associated to the resolution time $\tau_d$ of the measuring setup; $\sigma_{2n}$: expansion coefficients; $\lambda_2$: relaxation parameter
The TDPAC experiments were carried out with a four-detector BaF$_2$ setup with an electronic coincidences measuring system based on the “slow-fast” electronic coincidences principle (Fig.2/photo) and the measurements were performed on the nuclear $\gamma_1$-$\gamma_2$ cascade of the probe-isotope $^{111}$In→$^{111}$Cd (170 keV-250 keV) -intermediate nuclear state level half life-time $\tau_{1/2}$= 84ns- as part of a InCl$_3$ solution which was dropped on the samples ($\approx$ 10$^{-4}$at.%), then samples sealed in quartz tubes; diffusion done at 1,073 K during 8hs. All the experiments were performed at “room temperature (RT)”.

3) Measurements & Results: in order to get comparable results concerning all the present measured systems, all the experiments were performed in the same measuring conditions. The non-thermalized samples: HA-310K, HA-370K, HA-Ho (Figs.3,5,7) and the thermalized sample HA-370K (Fig.6) display TDPAC adjusted spectra shapes, as already well known, related to distribution of nuclear electric quadrupole interaction frequencies which may indicate, even non committing the general crystalline structure of the compound, the presence of small structural distortions around the isotope-probes sites; the rest of thermalized samples: HA-310K, HA-Ho (Figs.4,8) display TDPAC adjusted spectra shapes related to well defined crystalline structures around the isotope-probe sites. A spectrum of similar nature is displayed by a pure TCP [Ca$_3$(PO$_4$)$_2$] non-thermalized sample (Fig.9), a reasonable evidence of the TCP* presence in the two thermalized samples: HA-310K, HA-Ho (Figs.4,8). Concerning XRD, the spectra peaks related to non-thermalized samples, as shown in Figs. (3,5,7) -with exception of non-thermalized TCP (Fig.9)- even in the same graph positions of the thermalized samples [Figs.(4,6,8)], seem to be less defined, with larger half-widths when compared to XRD of the thermalized samples.

The TDPAC and XRD measured spectra are displayed below.
HA-370K – non-thermalized/thermalized

Fig.5: HA370K non-thermalized - TDPAC

Fig.5A: HA370K non-thermalized - XRD

Fig.6: HA370K thermalized - TDPAC

Fig.6A: HA370K thermalized - XRD

HAHo – non-thermalized/thermalized

Fig.7: HAHo non-thermalized - TDPAC

Fig.7A: HAHo non-thermalized - XRD

Fig.8: HAHo thermalized - TDPAC

Fig.8A: HAHo thermalized - XRD

TCP non-thermalized

Fig.9: TCP non-thermalized - TDPAC

Fig.9A: TCP non-thermalized - XRD
4) Discussion: the present results indicate that the TCP found in some HA thermalized samples, as announced by the TDPAC measurements, even with the crystalline site of the isotope-probe $^{111}$In -possibly substituting Ca in some of it's sites- still unknown, is very low to be displayed in the XRD spectra. Such result emphasize, in this case, the characterization role played in some situations by TDPAC when compared to XRD. By other side, such eventual TCP presence seems not to alterate the HA therapeutic performance. Nevertheless, the eventual effects of the TCP, even in a microscopic scale as presently announced, in human body is still unknown and therefore should be better studied.

The HA's sintesized at 370 K unchanged structure by the thermalization process, as displayed by XRD and TDPAC measurements, may indicate it's a more favourable sample to use for medical treatment. For the study of this structure other means of introducing the probe isotope should be tried: for instance, directly during the synthesis of HA could be a good way.

According to present results, where in some cases HA occurs simultaneously with TCP -and such simultaneous occurrence was already announced$^{(8)}$- new ways of thermalization processes could be tried in order to better separate both compounds.

5) Further Remarks: The present growing of the use of carriers and delivery systems, as happens with nano-HA and other nano-radiopharmaceuticals in general, are improovingly changing the present context of nuclear medicine, for instance in bone cancer imaging and treatment. In this context nuclear spectroscopies, with the informations they are able to apport concerning atomic local sites, as well as identifying small amounts of compounds below XRD resolution as was the case in the present contribution, may play a valuable role. Further developments of the present attempt are under way.

Table I – HA Hyperfine Interactions Parameters

<table>
<thead>
<tr>
<th>sample</th>
<th>$\nu_0$ (NQI) (MHz)</th>
<th>$\eta$</th>
<th>$\delta$ (MHz)</th>
<th>$\lambda_2$ (MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA 310K</td>
<td>210 (4)</td>
<td>0</td>
<td>0.42 (0.008)</td>
<td>7.1 (0.3)</td>
</tr>
<tr>
<td>HA-310K therm.</td>
<td>106 (0.2)</td>
<td>0.24</td>
<td>0.048 (0.0016)</td>
<td>7.3 (0.2)</td>
</tr>
<tr>
<td>HA 370K</td>
<td>210 (4)</td>
<td>0</td>
<td>0.41</td>
<td>8.0 (0.5)</td>
</tr>
<tr>
<td>HA-370K therm.</td>
<td>168 (3)</td>
<td>0</td>
<td>0.55 (0.05)</td>
<td>5.27 (1.5)</td>
</tr>
<tr>
<td>HAHo</td>
<td>249 (8)</td>
<td>0</td>
<td>0.58 (0.02)</td>
<td>5.0</td>
</tr>
<tr>
<td>HAHo-therm.</td>
<td>112 (0.2)</td>
<td>0.28 (0.004)</td>
<td>0.045</td>
<td>13.2</td>
</tr>
<tr>
<td>TCP</td>
<td>107.04 (0.4)</td>
<td>0.23</td>
<td>0</td>
<td>10.1 (0.8)</td>
</tr>
</tbody>
</table>
REFERENCES


7- “The Study of Hyperfine Interactions on Hydroxyapatite Compounds by Gamma Spectroscopy Perturbed Angular Correlation – PAC” - Osmar F.S. LeiteNeto, Fabio H.M. Cavalcante, Jose T. P.D. Cavalcante, Henrique Saitovitch, Ralph Santos-Oliveira; abstract accepted by 5th Joint Int. Conf. on HI/NQI; Australia, October/2014.


(*) The possible existence of TCP in present HA samples, even eventually and in small amounts, was suggested by Dr. Alexandre M. Rossi (Lab. Materiais Biocerâmicos/APL/CBPF)

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