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Investigation of hyperfine interactions in hafnium compounds with F^{1-}, OH^{1-}, and EDTA ligands by perturbed γ-γ angular correlation

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ABSTRACT

In this study the hyperfine parameters, including the dynamical nature, Perturbed Angular Correlation (PAC) spectroscopy was used to measure the hyperfine parameters in molecules of ligand compounds in solutions. The measurements were carried out at 295 K and 77 K using $^{181}Hf \rightarrow ^{181}Ta$, as probe nuclei. Samples were prepared by adding a small volume of radioactive solution containing the probe nuclei in aqueous solution, buffer solution and ethylenediaminetetraacetic acid (EDTA) used as a ligand with pH between 4 and 5 which correspond to the pH of the saturated EDTA solution and in buffer solution with pH between 9 and 10. The results made possible to understand the impact of each method for PAC measurements. Finally a comparative analysis for the several methods of inserting of the probe nuclei in the sample was made, considering chemical and nuclear aspects. The lack of measurements in this kind of samples justifies the importance of the obtained results.

1. INTRODUCTION

The knowledge of the dynamic behavior of biomolecules is fundamentally important to better understand the different processes related to the metabolic dynamics of biological systems such as immunological processes and therapeutic processes. Another very important issue and not well described yet in metal-biomolecule complexes is how the functions of the biomolecules are affected by the structure and dynamics of the metal ion binding site once such ions are very reactive with the different molecules present in a cell, for instance [1]. Therefore, fundamental questions in the biochemistry of proteins containing metals is to precisely describe the site in the molecule wherein the metal is bound, the structure around it and the dynamic behavior of this protein [1].

Consequently, in order to better understand the behavior of a biological system it is necessary to describe what happens in a nanoscopic view by means of an investigation of the local interactions, i.e., at a molecular level.

One accurate and efficient way to study local interactions in metal-biomolecule complexes is using nuclear techniques to measure hyperfine interactions at the metallic ion. Among the techniques capable to measure hyperfine interactions, the perturbed gamma-gamma angular correlation (PAC) spectroscopy is particularly useful to be used in the investigation of metal-biomolecule complexes since it uses radioactive nuclei of metal as probe nuclei and, as a consequence, the metal which is bound to the biomolecule can be the probe itself. Details of PAC spectroscopy can be found else where in literature. PAC spectroscopy has been used

with success to investigate local interactions in metal ions chemically bound to biomolecules [1,2].
In the present work ^{181}Ta from the decay of radioactive ^{181}Hf in different solutions obtained from the dissolution of radioactive Hf in fluoridric acid (HF) and from the dissolution of hafnium oxychloride ($HfOCl_2.8H_2O$) in water were measured with PAC spectroscopy. The chief intention is to develop a methodology to prepare samples of ^{181}Hf-biomolecules complexes to be measured by PAC spectroscopy in order to study hyperfine interactions at Hf site in such complexes. Ethylenediaminetetraacetic acid (EDTA) was used as a ligand molecule, but the method can be extend to any other biomolecule such as amino acids, polypeptide and proteins.

2. EXPERIMENTAL

Samples of Sodium ethylenediaminetetraacetic ($C_{10}H_{14}N_2O_8Na_2.2H_2O$) with pH between 4 and 5 (pH of the EDTA saturated solution) and buffer solution with pH between 9 and 10). The EDTA solution with pH 10 was prepared by dissolving 10 g of EDTA (s) in 100 mL of buffer solution at pH 10.

The probe nuclei used in the PAC measurements were ^{181}Ta which is formed in the β^- decay of ^{181}Hf, with 42 days of half-life. However, in order to analyze the site location of the probe atoms it was considered the chemical properties of the parent isotope, ^{181}Hf instead of the daughter's, basically for two reasons. First because the ligand is bound to the parent ion, and second because, as soon as the daughter isotope is formed it emits the gamma cascade, which used in the PAC measurements, within a very short time, which may be shorter than the time required for the chemical environment of the metal to change to be suitable to the chemical properties of the daughter isotope ion. In conclusion, the daughter isotope emits the gamma cascade which carries information on the chemical environment and site structure around the parent isotope.

The ^{181}Hf (^{181}Ta) probe nuclei were obtained in two different chemical environment which are decribed as follow:

1. **Solution with ^{181}Hf(^{181}Ta) formed from the dissolution of radioactive metal Hf in HF.**

   In this case, natural metal Hf was irradiated with thermal neutrons (neutron flux ~5. 10^{13} neutrons.cm^{-2}.s^{-1}) in the IEA-R1 research reactor of IPEN during 60 hours. After irradiation the metal Hf was dissolved in concentrated fluoridic acid (HF) resulting in the stock solution # 1 (see Table 1).

   A aqueous solution (sample 1) was prepared by adding 5 μL of the stock solution to 45 μL of distilled water. EDTA solution (sample 2) was prepared by adding EDTA and 5 μL of the stock solution to 45 μL of distilled water. The resulting pH of this solution was between 4 and 5. After measured at 295 K and 77 K this samples was heated under infrared light in order to eliminate the water and crystallize Hf-EDTA solution (sample 3).

Table 1. Preparation of samples with ^{181}Hf from the dissolution of radioactive metal Hf in HF.

Sample	Stock solution #1	Distilled water	EDTA solution 100g/L	buffer EDTA solution 100g/L
1	5μL	45μL	-	-
2	5μL	-	45μL	-

2. **Solution with ^{181}Hf(^{181}Ta) formed from the dissolution of radioactive hafnium oxychloride in water.**

   Radioactive ^{181}Hf solution was obtained by other way. Hafnium oxychloride ($HfOCl_2.8H_2O$) powder was irradiated with thermal neutrons (neutron flux ~5. 10^{13} neutrons.cm^{-2}.s^{-1}) in the IEA-R1 research reactor of IPEN during 60 hours. After irradiation the powder was dissolved in deionized water forming a solution containing $^{181}Hf^{4+}_{(aq)}$ resulting in the stock solution # 2 (see Table 2).

Table 2. Preparation of samples with ^{181}Hf from the dissolution of radioactive $HfOCl_2.8H_2O$ in water

Sample	Stock solution # 2	Distilled water	Buffer solution	buffer EDTA solution 100g/L
4	5μL	50μL	-	-
5	5μL	-	50μL -	-
6	5μL	-	-	50μL -

A aqueous solution (sample 4) was prepared by adding 5 μL of the stock solution to 50 μL of distilled water. A buffer solution with pH 10 (sample 5) was prepared by adding 5 μL of the stock solution to 50 μL of the buffer solution. EDTA solution (sample 6) was prepared by adding EDTA and 5 μL of the stock solution to 50 μL of buffer solution.

Measurements of samples were carried out at 295 K and 77 K in the 4 BaF2 detectors PAC spectrometer which yields twelve coincidence spectra $W(\theta,t)$. These spectra were analyzed by the software [3] which generate the $A_{22}G_{22}(t)$ spectra through the following combination of $W_i(\theta,t)$ spectra:

$$A_{22}G_{22}(t) = 2\left[\frac{C(180^o,t) - C(90^o,t)]}{C(180^o,t) + 2C(90^o,t)}\right]$$

were,

$$C(180°,t) = \sqrt[8]{\prod_{i=1}^{8} W_i(180^o,t)} \quad \text{e} \quad C(90°,t) = \sqrt[4]{\prod_{i=1}^{4} W_i(90^o,t)}$$

$W_i(\theta,t)$ are spectra measured at angles θ = 90°, 180° were the accidental coincidence have been taken into account by $W_A(t)$: $W_i(\theta, t) = W_i(\theta,t) - W_A(t)$.

From the $A_{22}G_{22}(t)$ curve the transition frequencies ω_i are calculated, which correspond to the split of the intermediate level of the gamma cascade due to the interactions with the electric field gradient produced by charges around the probe nuclei [4,5].

Some PAC spectra were taken at 295 K in solutions, conditions under which the rotational diffusion of molecules are present. This phenomenon is measured by the rotational correlation time $\tau_{CR,}$ which describes the mobility of the molecule in solution. τ_{CR} depends on the viscosity of solution ξ, the temperature T and the molecule volume V, being therefore defined by $\tau_{CR} = V.\ \xi/(k_BT)$, where k_B is the Boltzmann constant [6]. The dynamic hyperfine intraction is stronger when $\omega_0\tau_{CR} \approx 1$, with ω_0 being the lower transition frequency. The effect on the PAC spectrum in this case is a fast damping in the anisotropy with time. There are two other possible situations: (1) when the quadrupole interaction fluctuation is fast ($\omega_0\tau_{CR}<<1$), in this situation the fluctuation time is short compared to the time scale of the quadrupole interaction characterized by ω_0, and the probe nuclei lose the phases coherence and the PAC spectrum is represented by a exponential decay, (2) the slow quadrupole interaction fluctuation ($\omega_0\tau_{CR}>>1$), when the fluctuation time is long when compared to the time scale of the quadrupole interaction. The effect on the PAC spectrum is a slow damping of the anisotropy and in the limit when $\tau_{CR} \rightarrow \infty$, the interaction is purely static. Only in this condition is possible to simultaneously determine the quadrupole frequency ν_Q and the asymmetry parameter η which describe the local structure of the probe nuclei sites in the biomolecule.

3. RESULTS AND DISCUSSION

The Fig. 1 shows PAC spectra for solutions prepared with ^{181}Hf from radioactive metal Hf dissolved in HF, whereas Fig. 2 shows PAC spectra measured in solutions prepared with ^{181}Hf obtained from irradiated $HfOCl_2$ dissolved in water.

Results of the fitting of experimental PAC spectra at 295 K and at 77 K (shown in Fig. 1) to the theoretical functions (see details in Shipinkova [7]) showed that for ^{181}Ta in aqueous solution as well as ^{181}Ta in EDTA solution with pH between 4 and 5 no rotational diffusion was present. The results for these two samples also show that ^{181}Hf effectively has strongly bound to the fluoride ion when dissolved in Fluoridic acid. One possibility is the formation of the complex íon $[HfF_6]^{2-}$ [8] and, as reported by ALDOUS [9], the ion $[HfF_7]^{3-}$ can also exist in aqueous solution. $[HfF_6]^{2-}$ íon can dimerize to form the $[Hf_2F_{12}]^{4-}$dimer, whereas the ion $[HfF_7]^{3-}$ can form $[Hf_2F_{13}]^{5-}$ and $[Hf_2F_{14}]^{6-}$ $2H_2O$ dimmers [10]. The two different quadrupole frequencies of 957 MHz and 545 MHz observed in the PAC spectra for ^{181}Ta in aqueous solution (see Table 3) can therefore be associated to the $[HfF_6]^{2-}$ e $[HfF_7]^{3-}$ dimmers.

Data for samples with ^{181}Ta in EDTA solution with pH between 4 and 5, also shown in Table 3, reveal the existence of two quadrupole frequencies as well. However, there is no evidence that the probe metal ions have bound to EDTA molecules because the major fractions at 295 K and 77 K have close frequencies, which agree with previous PAC measurements reported by CHAIN e PASQUEVICH [11]. As the frequencies for EDTA are different from those measured for aqueous solution one can conclude that the EDTA molecule has bound to the dimers $[Hf_4F_{12}]^{4-}$ e $[Hf_2F_{14}]^{2-}$ through hydrogen bridges [9], with a consequent change in the whole charge resulting in a different charge configuration around the metal.

The EDTA solution with pH between 4 and 5 was crystallized by water evaporation which probably made the sodium ions from EDTA to bind to $[Hf_2F_{13}]^{5-}$ and $[Hf_2F_{14}]^{6-}$ dimmers forming Na_3Hf_7 and $Na_5Hf_2F_{13}$ compounds [11]. As can be seen in Table 3 the frequencies measured for this sample at 77 K and 295 K are essential the same however different from those obtained for EDTA in solution.
By a visual inspection of the spectra in figure 2A and 2B, which shows PAC results for ^{181}Ta in aqueous solution formed from $HfOCl_2$ (sample 4), one can see the presence of dynamic interaction at 295 K (λ=168 MHz), which is a strong evidence that ^{181}Hf is bound to a molecule. However, as the solution does not contain any ligand, only water, it is reasonable to infer the occurrence of hydrolysis with the formation of Hf aqueous complexes [12].

The increase of pH as well as the presence of new ligands (CO_3^{2-} e HCO_3^-) from the buffer solution has driven to the formation of new compounds as can be seen in figure 2C and by the measured frequencies of 621 MHz and 739 MHz.

With the addition of EDTA to the buffer solution there is the formation of the Hf–EDTA complex, which is evidenced by the observation of dynamic interaction at 295 K, as shown in Fig. 2D. When this sample is frozen at 77 K, two fractions were observed. As the minor fraction (47%) has a frequency of 723 MHz close to that observed for the buffer solution, one can conclude that part of the ^{181}Hf has bound to the one ligand present in it. Consequently, we have assigned the major fraction (53%) with frequency of 832 MHz to ^{181}Hf bound to EDTA.

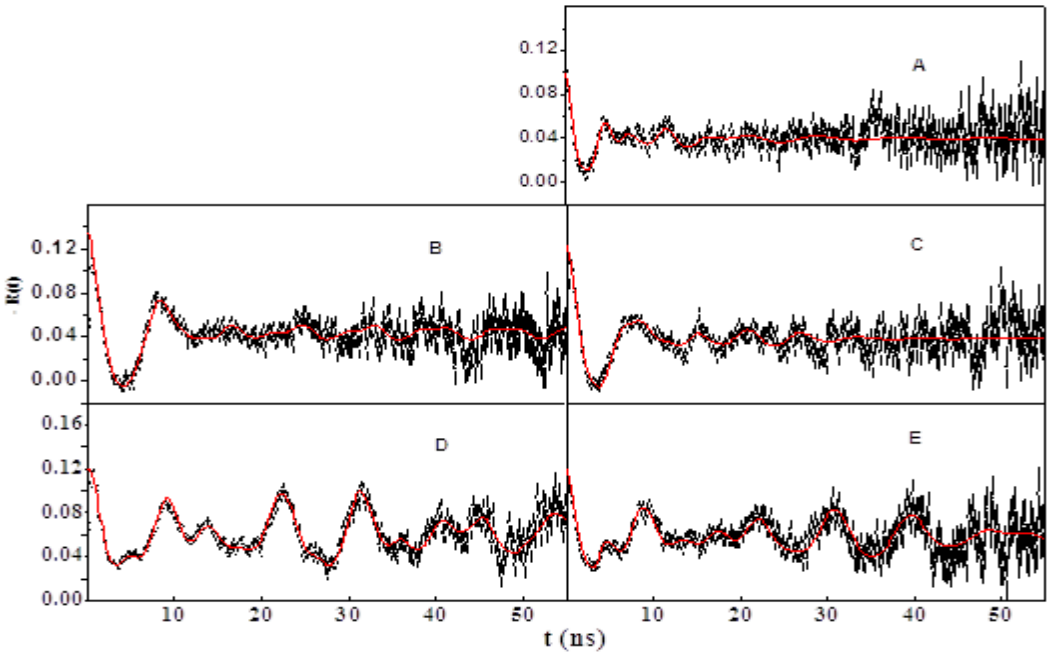

Figure 1 PAC spectra measured for samples 1 (A), 2(B and C), and 3(D and E) measured with ^{181}Ta at 295 K (left) and 77 K (right) from ^{181}Hf obtained from solution of radioactive metal Hf dissolved in HF

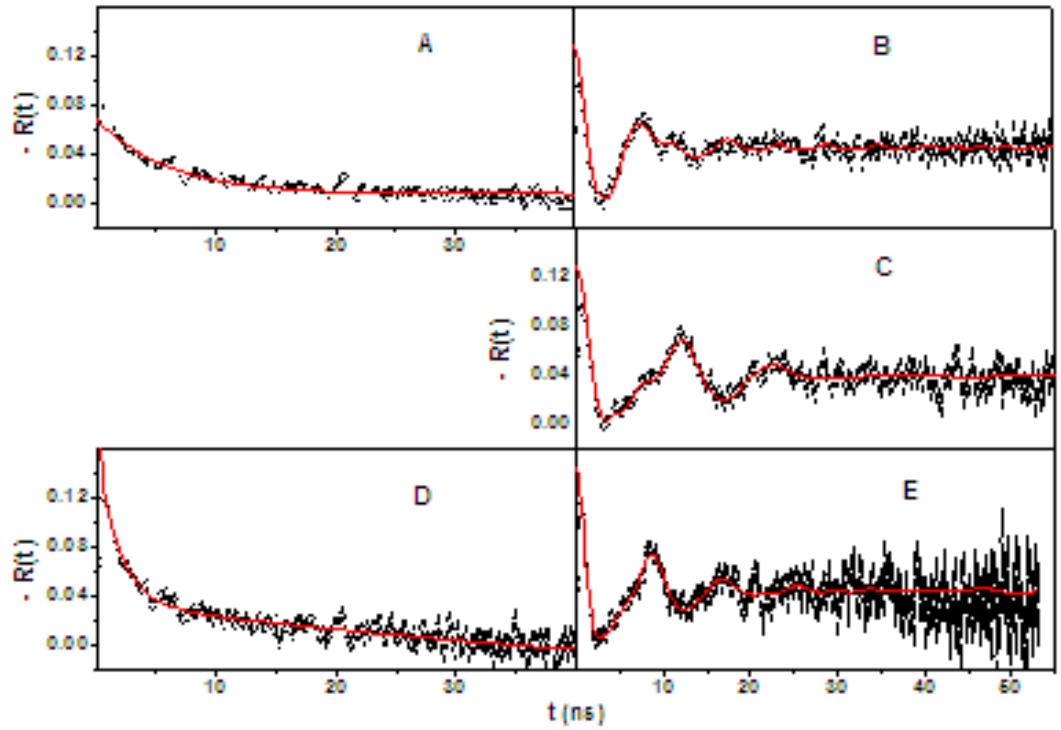

Figure 2: PAC spectra measured for samples 4 (A and B), 5(C), and 6(D and E) measured with ^{181}Ta at 295 K (left) and 77 K (right) from ^{181}Hf obtained from dissolution of radioactive $HfOCl_2$ dissolved in water.

Table 3. Hyperfine parameters, quadrupole frequency (ν_Q), asymmetry parameter (η), relaxation constant (λ) and frequency distribution (δ), measured with ^{181}Ta in different solutions.

Sample number	Sample description	Site	Temperature. (K)	Site fraction (%)	ν_Q (MHz)	η	λ (MHz)	δ
1	^{181}Ta in aqueous solution	1	77	80	957 ±45	0,6± 0,1	-	0,1
		2		20	545±10	0,2 ± 0,1	-	0,1
2	^{181}Ta in EDTA solution with pH between 4 and 5	1	77	87	517,5±20	0,6± 0,1	-	0,2
		2		13	678±15	0,6± 0,1	-	0,0
		1	295	89	457±30	0,7± 0,1	-	0,2
		2		11	802±55	0,2± 0,1	-	0,0
3	^{181}Ta in crystallized EDTA solution with pH between 4 and 5	1	77	78	458 ±20	0,6± 0,1	-	0,0
		2		22	818±30	0,8± 0,1	-	0,0
		1	295	85	458±20	0.6± 0,1	-	0,0
		2		15	722±50	0,8± 0,1	-	0,0
4	^{181}Ta ($HfOCl_2$) in aqueous solution	1	77	89	1035±51	0,5± 0,1	-	0,3
		2		11	656±62	0,7± 0,1	-	0,1
		1	295	100			168	
5	^{181}Ta ($HfOCl_2$) in aqueous solution + buffer solution with pH 10	1	77	20	621±30	0,3± 0,1	-	0,1
		2		80	739±11	0,3± 0,1	-	0,1
6	^{181}Ta ($HfOCl_2$) in EDTA buffer solution with pH 10	1	77	47	723±34	0,3± 0,1	-	0,1
		2		53	832±11	0,6± 0,1	-	0,1
		1	295	100	-	-	71,5	0.0

Obs. The uncertainties of δ parameter are so smaller when compared to δ values that they were omitted.

4. CONCLUSIONS

According to our results it is quite reasonable to conclude that first, the PAC spectroscopy showed to be an efficient technique to investigate hyperfine interactions, including dynamical parameters, in biologic molecules bond to metallic ion in order to obtain information on the site location of the ion as well as on the dynamic behavior of the molecules. Second, solution containing ^{181}Hf obtained from the dissolution of radioactive metallic hafnium in HF is not suitable to be used in the production of Hf-biomolecule samples to be measured with PAC

spectroscopy once the bond between Hf^{4+} and F^{1-} is very strong and, consequently it prevents Hf^{4+} ions to bind to any other ligand. And, third, irradiation of $HfOCl_2$ with thermal neutrons and subsequent dissolution in water in order to yield a solution containing ^{181}Hf has shown the best way to provide radioactive Hf^{4+} probe ions to be bound to EDTA molecules in order to be studied with PAC spectroscopy.

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