STUDY OF DYNAMIC BEHAVIOR OF EDTA MOLECULE IN SOLUTION USING PERTURBED GAMMA-GAMMA ANGULAR CORRELATION SPECTROSCOPY

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ABSTRACT

In this work, PAC spectroscopy has been used to obtain the hyperfine parameters in EDTA molecules in solutions with pH 4.3 and pH 10.5 both measured at 77 K and 295 K using ^{181}Hf(^{181}Ta) as probe nuclei. Both dynamic and static interactions were measured in aqueous solution, crystallized and re-hydrated samples in order to examine the motion and structure of EDTA-molecules. The hyperfine parameters, quadrupole interaction frequency (ν_Q), asymmetry (η), and the dynamic interaction frequency (λ) were obtained. The outcomes show that the rotational correlation time (τ_{CR}) is larger than the half-life of the intermediate state of probe nuclei. For samples with pH 4.3 and pH 10.5, it was observed an increase in ν_Q when the temperature decreases, as expected, and also a variation of η, which is an evidence of a change in the EDTA molecule structure. ^{181}Hf is bound only to a single molecule site when the pH was 4.3, differently from the results for pH 10.5 sample, which showed two fractions with different ν_Q indicating the possibility of ^{181}Hf being bonded to two different sites of the molecule. Measurements of the dehydrated sample presented different results leading us to conclude that the preparation procedure can causes alterations in the chemical bounds. Concluding, these results showed a systematic behavior of the ^{181}Hf-EDTA, with the variation of pH from 4 to approximately 11, and they are important to the knowledge of the dynamic behavior of this molecule.

1. INTRODUCTION

The knowledge of ligand molecules behavior is fundamental to understand phenomena related to metabolic dynamics, immunological processes, therapeutic processes, etc. Many studies of such phenomena are interpreted from a macroscopic point of view (cellular level) whereas, in order to better understand them, it is necessary to investigate the microscopic interactions (molecular level).

The measurement of hyperfine interactions is one of the best ways to perform local investigations once such interactions yield information on the atomic neighborhood of a probe nucleus in a nanometric scale by means of interactions between the nuclear moments and the surrounding charges and spins. The Perturbed Angular Correlation (PAC) spectroscopy can measure hyperfine parameters regarding many phenomena that can occur in biologic system or single molecules [1,2].

The EDTA (*Ethylenediamine tetraacetic acid),* and its disodium salt are regularly used as chelate in order to form complexes with metal ions with stable bonds. EDTA can be successful used in different applications as in pharmaceuticals in order to assure the absorption of certain ions in medicines; to increase the bioavailability of certain minerals

(near 800 daltons). A chelated fertilizer is eight times more efficient for Mn and 5 times more efficient for Zn than soluble salts. EDTA reduces the chemistry reactions that affect the solubility of nutrients and it is compatible with the majority of insecticides and fungicides and can also be applied along them to reduce cost of application. It is of easy application causing no damages on the equipment or on the plants. In nuclear medicine EDTA form complexes with radioactive metals in order to allow their utilization in radio diagnostics or chemotherapy.

In this work, PAC spectroscopy has been used to obtain the hyperfine parameters in EDTA molecules in solutions with pH 4.3, pH 10.5 measured at 77 K and 295 K. The ^{181}Ta, formed from the β-decay of ^{181}Hf (obtained by neutron irradiation of natural hafnium) was used as probe nucleus. Samples were examined in solution, crystallized and hydrated, seeking to analyze the dynamic interaction as well as the molecule structure in each case.

2. EXPERIMENTAL

Samples of EDTA - Ethylenediamine tetraacetic acid ($C_{10}H_{14}N_2O_8Na_2.2H_2O$) solution with two different pH´s have been measured with PAC spectroscopy using ^{181}Ta as probe nuclei. The sample with 4.3 pH was prepared with the dissolution of 100 mg of $EDTA_{(s)}$ in 10 ml of distilled water at 25 ^{0}C, whereas in order to prepare the 10.5 pH sample 1.0 g of $EDTA_{(s)}$ was dissolved in 20 ml of distilled water followed by the addition of 22 ml from a 0.33 mol/l solution of sodium hydroxide.

In order to be measured in the PAC spectrometer, a volume of 48 μl has been separated from each solution and around 10 μl of a solution of hafnium fluoride has been added. The hafnium fluoride solution was prepared by dissolving 1 mg of natural metallic hafnium after being irradiated in with neutrons in the IEA-R1 research reactor of IPEN. The probe nucleus used in the PAC measurements is the ^{181}Ta, which is formed from the β$^-$-decay of ^{181}Hf ($T_{½}$ = 42.4 d). ^{181}Hf is yielded by thermal neutron capture reaction in ^{180}Hf..

Samples of each pH were measured in solution and dehydrated. Dehydrated samples were obtained from heating the solution under infrared radiation. A re-hydrated sample of 4.3 pH was also measured. This sample was prepared by adding distilled water to the dehydrated sample.

 PAC measurements were carried out using a BaF_2 four-detector spectrometer, which yields twelve coincidence spectra W(θ,t) that were analyzed by means of TDPAC [3] software. TDPAC output is the $A_{22}G_{22}(t)$ function given by the combination of W(θ,t) spectra:

$$A_{22}G_{22}(t) = 2[C(180^o,t) - C(90^o,t)]/[C(180^o,t) + 2C(90^o,t)] \tag{1}$$

where,

$$C(180°,t) = \sqrt[8]{\prod_{i=1}^{8} W_i(180°,t)} \quad \text{e} \quad C(90°,t) = \sqrt[4]{\prod_{i=1}^{4} W_i(90°,t)} \tag{2}$$

The coincidence spectra $W_i(\theta,t)$ are produced from signals of combinations of two detectors with angles $\theta = 90°$ or $180°$ between them, after subtracting the effects of unwanted accidental coincidences: $W_i(\theta, t) = W_i(\theta,t) - W_A(t)$. From $A_{22}G_{22}(t)$ function was possible to obtain ω_i transition frequencies corresponding to the splitting of intermediate energy level from probe nucleus gamma cascade due to the presence of an Electric Field Gradient originated from the electronic neighborhood [5].

PAC measurements were carried out with EDTA molecules in solution, in which the rotational diffusion effect is present. This effect is represented by the rotational correlation time τ_{CR}, which describes the mobility of a molecule in the solution. τ_{CR} depends on the viscosity (ξ), the temperature (T) and the volume of the molecule (V): $\tau_{CR} = V. \xi/(k_BT)$, where k_B is Boltzmann's constant. The influence of the dynamic interaction is stronger when $\omega_0\tau_{CR}\approx 1$, and as a consequence the effect on the PAC spectrum is a fast damping of the anisotropy. There are two possible situations: (1) when the quadrupole interaction fluctuation is fast, $\omega_0\tau_{CR}\ll 1$, since the fluctuation time is small when compared with time scale of the quadrupole interaction characterized by ω_0, hence, the nucleus loses the phase coherence and the perturbation function becomes an exponential decay; (2) and the quadrupole interaction fluctuation is slow, $\omega_0\tau_{CR}\gg 1$, the fluctuation time is long when compared with time scale of the quadrupole interaction, and the effect is a slow damping of the anisotropy. In the limit when $\tau_{CR}\rightarrow\infty$, the interaction is pure static. Only in this case it is possible to determine simultaneously the quadrupole frequency ω_0 and the asymmetry parameter η, the hyperfine parameters related to the local structure around the probe nucleus into the EDTA molecule.

3.   RESULTS AND DISCUSSION

Figures 1, 2 and 3 show, respectively PAC spectra for EDTA samples with pH 4.3 measured in solution, dehydrated and re-hydrated, at 295 K and 77 K each. PAC results also at 295 K and 77 K for samples with pH 10.5 in solution and dehydrated are respectively shown in figures 4 and 5.

Results for both pH 4.3 and 10.5 samples in solution show no evidence of rotational diffusion at 295 K. This can be ascribed to the fact that the half-life of the ^{181}Ta-cascade intermediate state would be much smaller than the rotational correlation time of the EDTA molecule in such solutions[6]. Therefore, for all results, with exception of that measured at 295 K for 4.3 pH re-hydrated sample, there is no damping from dynamic interactions and the quadrupole frequency ν_Q can be obtained.

Table 1 shows results of hyperfine parameters measured with PAC for 4.3-pH samples. A single fraction was observed for measurements of sample in solution, whereas for dehydrated samples two fractions have been observed for measurements at both 295 K and 77 K. The results show that ν_Q increases sharply when the temperature of EDTA sample in aqueous solution decreases from 295 K to 77 K, and the solution was frozen. However, ν_Q obtained for major fraction of dehydrated sample little decreases and the asymmetry parameter η little increases. This observation indicates that water has a big influence in the structure of EDTA, and the second fraction appearing in the dehydrated sample is evidence that the structure of some molecules has been much affected by the dehydration process. The re-hydration of the sample lead to complete different results when compared to the results early obtained for the

sample in solution, what shows that the damage in the dehydrated molecules are permanent and a change in the pH of the sample has occurred.
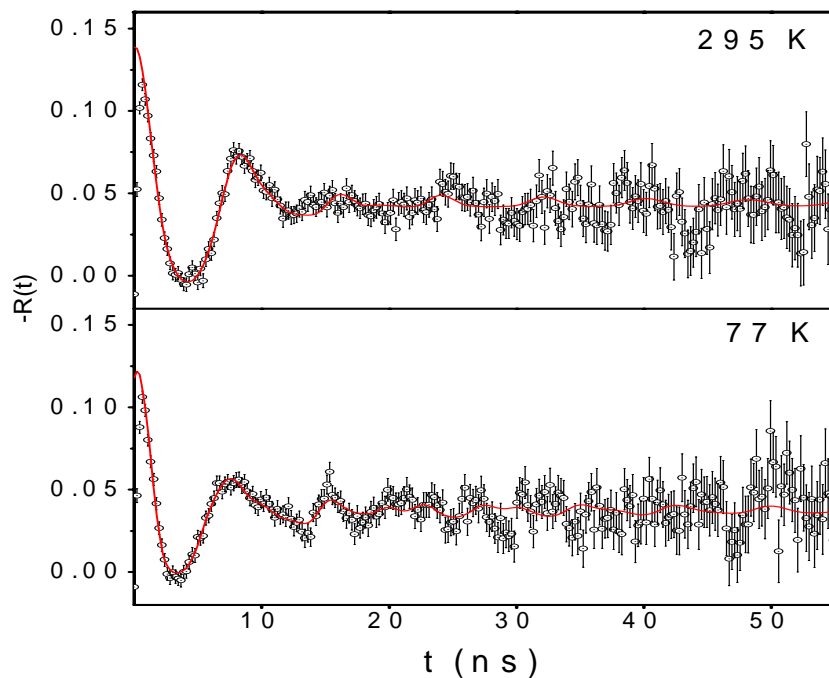

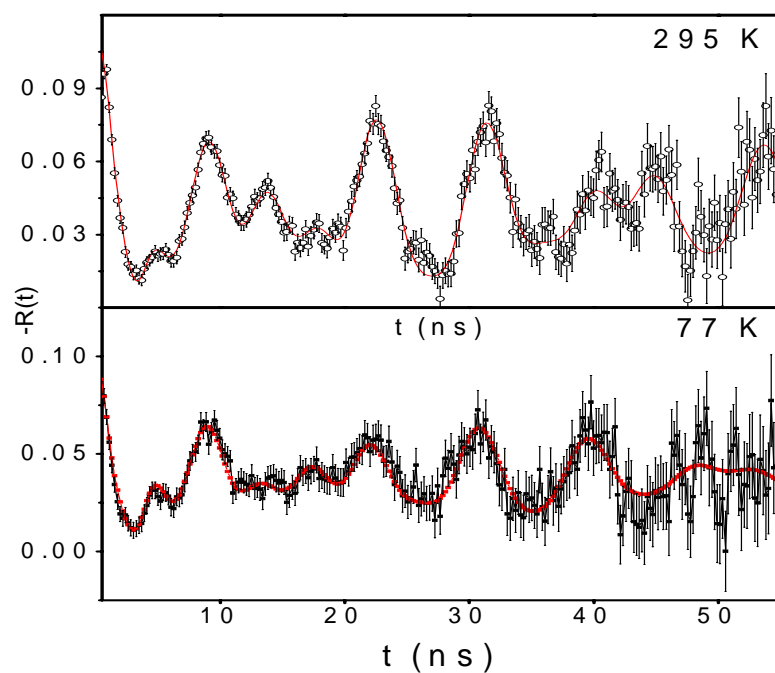**Figure 1. PAC spectra for EDTA sample measured with [181]Hf in pH 4.3 solution.**

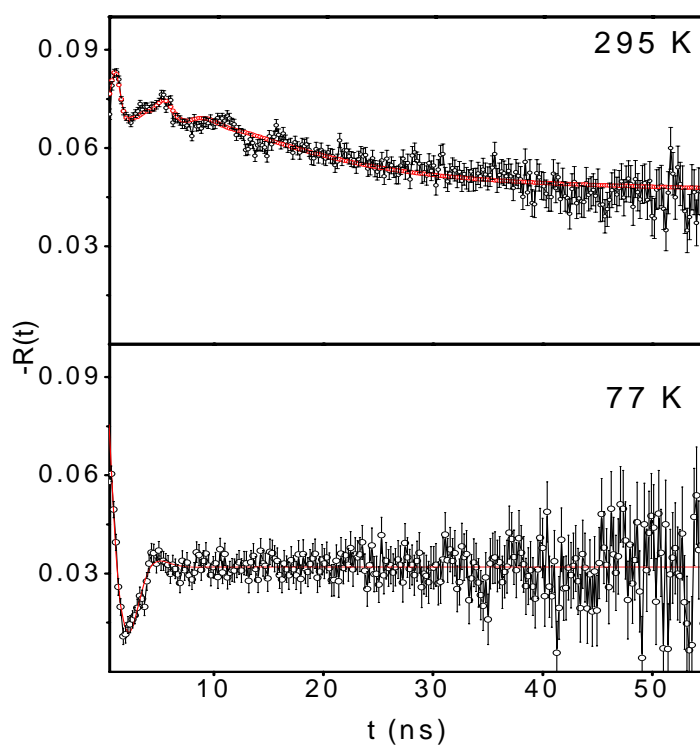**Figure 2. PAC spectra for dehydrated pH 4.3 EDTA sample measured with [181]Hf.**

Figure 3. PAC spectra for re-hydrated pH 4.3 EDTA sample measured with [181]Hf.

Table 1. Hyperfine interaction parameters for EDTA prepared with pH 4.3.

	Site 1			
Sample	Tempera-ture (K)	f%	ν_Q(MHz)	η
Solution	295	100	435(24)	0.89(16)
	77	100	610(11)	0.55(2)
Dehydrated	295	71	463(1)	0.62(1)
	77	60	461(1)	0.70(6)
	Site 2			
Dehydrated	295	29	879(24)	0.64(4)
	77	40	815(16)	0.77(6)

Results for 10.5-pH samples are shown in table 2 and two fraction were observed for all samples in both 295 K and 77 K temperatures which indicates that [181]Hf has bonded to two

different sites in the EDTA molecules. Differently from the 4.3-pH sample, values of ν_Q show a great variation for both fractions of sample in solution with temperature, and the same
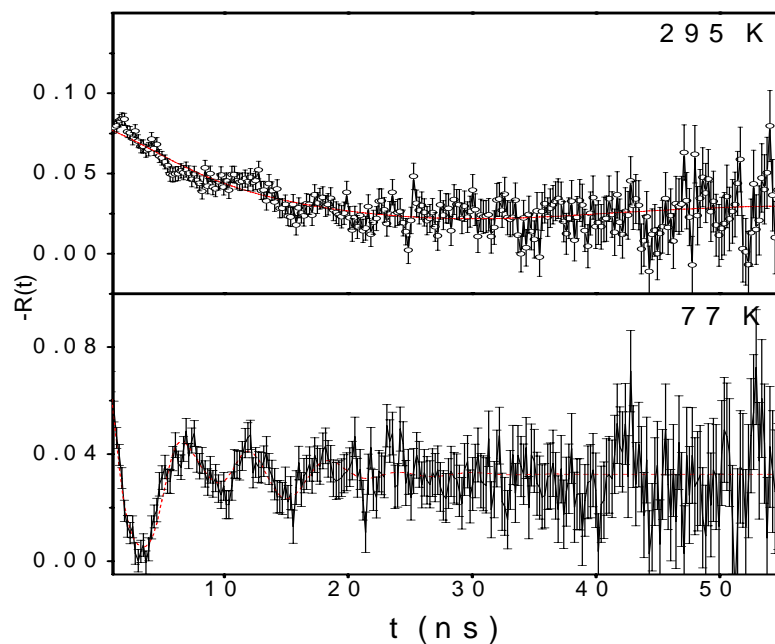

Figure 4. PAC spectra for EDTA samples measured with ^{181}Hf in pH 10.5 solution.
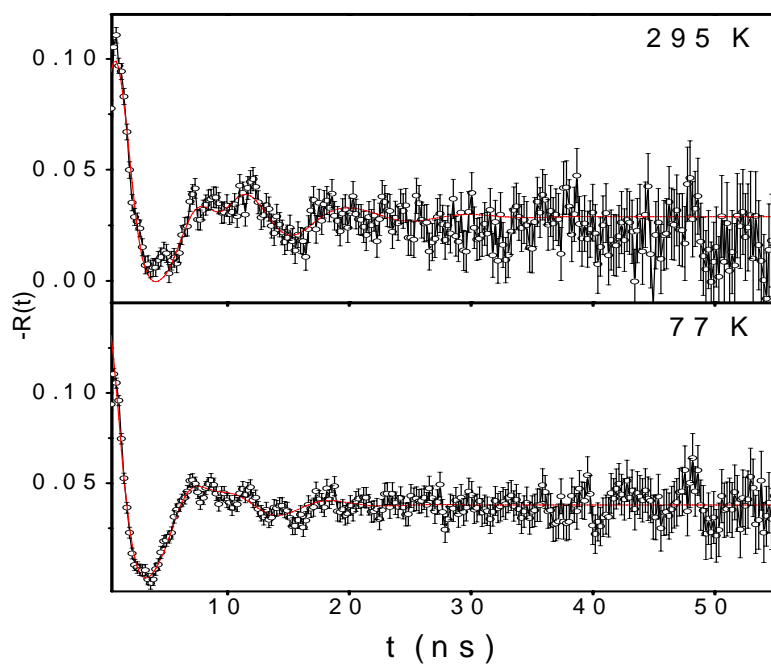

Figure 5. PAC spectra for dehydrated pH 10.5 EDTA sample measured with ^{181}Hf.

Table 2. Hyperfine interaction parameters for EDTA prepared with pH 10.5.

Site 1				
Sample	Temperature (K)	f%	ν_Q(MHz)	η
Solution	295	51	68(3)	0.48(12)
	77	42	622(7)	1
Dehydrated	295	58	481	1
	77	47	480	1
Site 2				
Solution	295	49	250(39)	1
	77	58	485(36)	0.66(8)
Dehydrated	295	42	587(13)	0.39(4)
	77	54	550(7)	0.55(2)

has been observed for η values, indicating a sharp change in the structure around the ^{181}Ta probe nuclei. However, the dehydrated samples have not presented such change in the hyperfine interaction parameters. The difference in the values obtained for different pH´s show that the solution pH has a great influence on the site where probe nuclei are bonded to the EDTA molecule.

4. CONCLUSIONS

In the present work, PAC spectroscopy has been used to investigate hyperfine interactions in EDTA molecules in solutions with different pH´s. The measurements were taken for samples in solution, dehydrated and re-hydrated.

Results have shown that ^{181}Hf can be chemically bonded to two different active sites in EDTA molecules, depending on the pH, the environment of the molecule(solution or dehydrated) as well as the temperature. The quite different results obtained for dehydrated and re-hydrated samples lead us to conclude that such procedures have provoked a great change in the molecules.

REFERENCES

1. L. Hemmingsen, K. N. Sas, E. Danielsen, Biological Applications of Perturbed Angular Correlations of γ-Ray Spectroscopy. *Chem. Rev.* **104**, pp 4027-4061(2004).

2. T. K, Leipert, J. D. Baldeschwieler, D. A. Shirley, Applications of gamma ray angular correlations to the study of biological macromolecules in solutions. *Nature*. **200**, pp.907-909(1968).
3. J. Mestnik filho, *Tratamento de Dados de Correlação Angular Preturbada*, **private communication**.
4. E. Karlsson, E. Matthias, S. Siegbahn, *Perturbed Angular Correlation*, North-Holland, Amsterdam (1965).
5. T. BUTZ, Analytic perturbation function for static interaction in perturbed angular correlations of γ-rays, *Hyperfine Interactions* **52**, pp.189-228(1989).
6. A. Abramgam, R. V. Pound, Influence of eletric and magnetic fields on angular correlations. *Phys Rev*. **92**, pp.943-962(1953).