Study of DNA and Protein Structures Through Perturbed Angular Correlations

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Abstract. The Perturbed Angular Correlation (PAC) technique is largely used in Condensed Matter Physics experiments. Recently it has also been used in applications on biological problems, as the determination of structural characteristics of DNA and Protein molecules. We are implementing a PAC Spectrometer at the University of São Paulo Pelletron Laboratory. The main motivation is the study of DNA and Protein structures. We have already 6 BaF_2 detectors for gamma rays, which are adequate due to the good time resolution. The spectrometer mechanical structure is ready and tests have been performed in order to choose the best configuration for the data acquisition electronics, composed by a multiparameter CAMAC system. At the same time, we have performed, at IPEN-CNEN/SP, measurements to study the structure of organic systems like lipids and surfactants. If some characteristics of these molecules are known, then some mechanisms of interaction between them and protein molecules can be understood.

1 Introduction

The application of the PAC technique to Biological and Biochemical problems has brought very interesting results in the study of DNA and protein molecules structures [1, 2]. One of the advantages of this technique is the possibility of performing measurements with samples in solution and observing dynamical characteristics [3].

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We are implementing the PAC technique at the University of São Paulo Pelletron Laboratory with the objective of studying problems involving DNA and protein structure and functions. Allied to the charged particle external beam facility constructed by our research group [4], this technique can provide a versatile system. It is possible to produce structural modifications in protein or DNA molecules and by using the PAC technique, investigate them through the monitoring of the samples before and after irradiation.

2 Theory of Perturbed Angular Correlation of γ-Rays

The theory and the method of perturbed angular correlations of γ-rays are described in detail in [5]. We will show here only relevant points.

A γ-ray emitted from a radioactive nucleus carries angular momentum away from the nucleus in the nuclear decay. The total angular momentum is conserved in the nuclear decay, and this is the origin of the angular correlation of γ-rays. Experimentally, the technique consists in inserting a radioactive nucleus, like ^{111}In, in a specific medium and then observing the temporal variation or perturbation of the distribution of the γ-rays emitted in the nuclear decays, due to the electric field gradient (EFG) generated by the structure of the external medium. Figure 1 shows an example of the decay of ^{111}In and ^{111m}Cd.

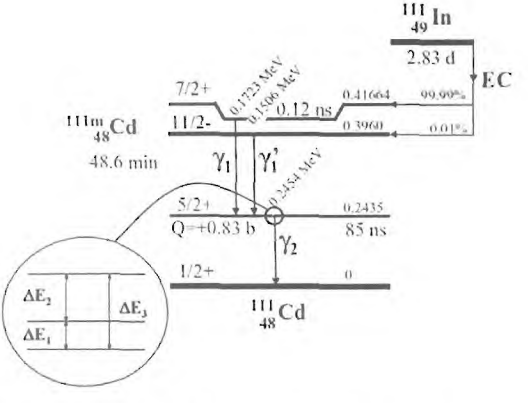

Figure 1 ^{111}In and ^{111m}Cd decays. The inset shows the three possible energy levels for the 5/2 spin intermediate state due to the influence of a electric field gradient (EFG). ΔE_1, ΔE_2 and ΔE_3 are the energy differences among the states.

The distribution of the second γ-ray with respect to the first is given by:

$$W(\theta, t) = \frac{e^{-t/\tau_N}}{4\pi\tau_N}(1 + A_2 G_2(t) P_2(\cos\theta)) \qquad (1)$$

where τ_N is the intermediate state mean life, A_2 describes the anisotropy of the angular correlation between the two γ-rays. P_2 is given by: $P_2(\cos\theta) = \frac{1}{2}(3\cos^2\theta - 1)$. $G_2(t)$ describes the temporal evolution of the probability to detect the second γ-ray in an interval t. For the case when the EFG is static we have:

$$G_2(t) = a_0 + \sum_{n=1}^{3} a_n \cos(\omega_n t) \tag{2}$$

The angular frequencies ω_1, ω_2 and ω_3 above correspond to the energy differences ΔE_1, ΔE_2 and ΔE_3 (Figure 1). It is possible to obtain them directly through a $G_2(t)$ Fourier transform. These frequencies are, in the static case, directly proportional to the quadrupolar frequency, ω_Q.

The EFG can be described by two parameters [6]. In the Quantum Theory, the EFG is given by a 3 × 3 tensor, represented here by V. If an adequate choice of coordinate system is made, in which $|V_{zz}| \geqslant |V_{yy}| \geqslant |V_{xx}|$, the EFG is generally described by the component $|V_{zz}|$ (z component of V), which is directly proportional to the quadrupolar frequency ω_Q, and by the asymmetry parameter η, given by:

$$\eta = \frac{V_{xx} - V_{yy}}{V_{zz}} \tag{3}$$

If there is a temporal dependence of the EFG, i.e. the orientation of the EFG changes in the time between the emissions of the two γ-rays, Equation (2) must be modified. This can be the case for molecules which are submitted to rotational diffusion due to Brownian motion in the sample, for molecules that are not rigid but change conformation, or undergo other dynamic processes in the time period between the emission of the two γ-rays. The term $G_2(t)$ passes to have an exponential dependence with time, by the factor e^{-t/τ_c}, for slow reorientation, and $e^{-2.8\omega_0^2\tau_c(1+\eta^2/3)t}$, in the fast limit. τ_c is denominated rotational correlation time, which for spherically symmetric molecules depends on the viscosity of the medium (ξ), the temperature (T) and the molecule volume (V), of the following mode:

$$\tau_c = \frac{V.\xi}{T.k_B}, \tag{4}$$

where k_B is the Boltzmann constant.

3 Experimental Facility

It is possible to measure the distribution $W(\theta, t)$ through coincidences between pairs of detectors. With the 6 detector PAC spectrometer we are constructing (see Figure 2), we have 6 combinations for $\theta = 180°$, and 24, for $\theta = 90°$

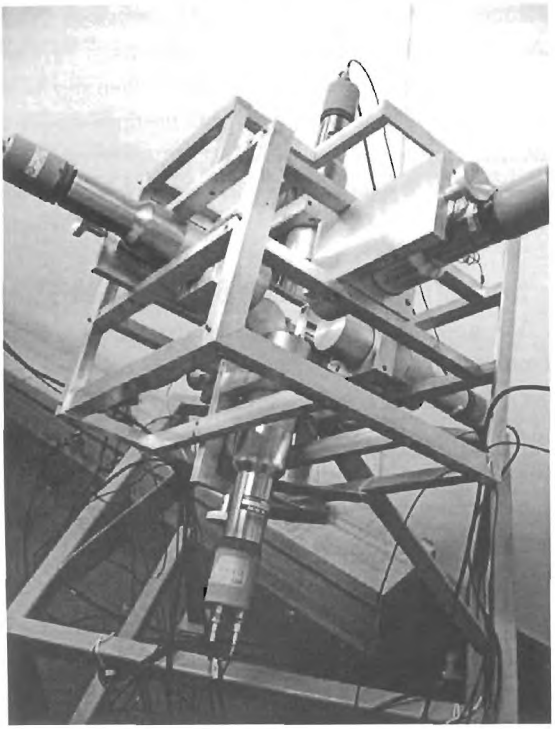

Figure 2 new PAC spectrometer structure with BaF_2 detectors at the Pelletron Laboratory.

The experimental perturbation function is given by:

$$A_2G_2(t) = 2\frac{W(180°,t) - W(90°,t)}{W(180°,t) + 2W(90°,t)} \tag{5}$$

W(180°,t) and W(90°,t) represent the geometric averages of the coincidences measurements for 180° and 90°, respectively.

Generally, PAC spectrometers work with data acquisition electronics with a single channel analyzer to each detector and a router module [7] to separate, electronically, the coincidences of the two γ-rays and the energy spectra are not acquired.

Recently a new PAC spectrometer was constructed in which all the process are computational [8]. In this case the energy spectra are acquired.

Finally, the PAC spectrometer we are constructing uses a different electronics concept, in which the energy spectra are also acquired and the coincidences are also selected computationally, but through a multiparameter acquisition CAMAC

system. The electronics schematic for two detectors is shown in Figure 3. The generalization of this example to 6 detectors is direct.

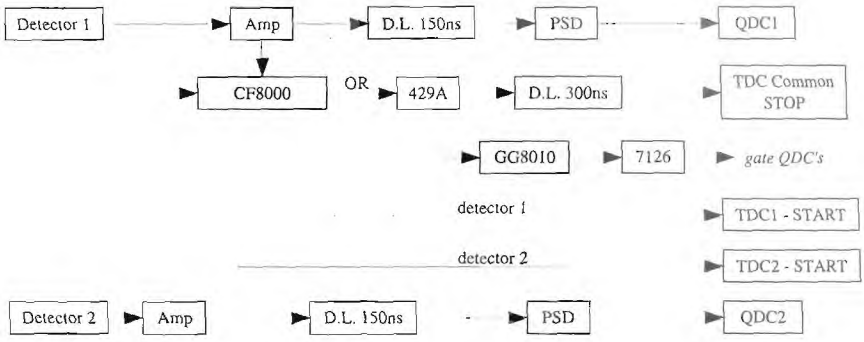

Figure 3 scheme of the data acquisition electronics system to the new PAC spectrometer at the Pelletron Laboratory, for two detectors. Amp is a 612AM LeCroy amplifier; D.L. is a cable delay line; PSD is a pulse shape discrimation module, used to attenuate the energy signal; QDC is a charge to digital converter Phillips 7166H. CF8000 is an ORTEC constant fraction discriminator; 429A is a LeCroy logic fan in fan out; GG8010 is a ORTEC gate & delay generator; 7126 is a Phillips level translator. The TDC module used is a time to digital converter Phillips 7186.

4 Measurements at IPEN/SP

We performed experiments at the *Instituto de Pesquisas Energéticas e Nucleares* (IPEN-CNEN/SP), where a 4 BaF_2 detectors PAC spectrometer [7] is available. Cardiolipin:phosphatidylcoline 1:1 vesicles [9] and *sodium dodecyl sulfate* (SDS) [10] samples were used.

For the experiments with vesicles the objective was to verify if the PAC technique could sense differences related to variation of their dimensions, provoked by adding a surfactant (SDS) to a vesicles sample, tranforming them into micelles. We have done measurements with vesicles at 295K and 77K. Also, with vesicles plus SDS at 295K and 77K.

After that, other measurements were performed with SDS samples in different enviroments (water and water plus methanol) in which the SDS concentrations of the solutions were varied to verify if the PAC parameters were sensitive to the molecular agregation or even to critical micellar concentration (CMC).

4.1 Materials and Methods

For the solution of vesicles, first it was diluited 5mg of cardiolipin and 5mg of phosphatidylcoline-98% in 0.5mL of chloroform. After that, the solution was dried with argon gas and then diluted in 2mL of mQ water. This solution was passed through a 100nm filter to form vesicles with this dimension. The sample, to be used in the spectrometer, was prepared by depositing 4.0μL of acqueous solution traced with $InCl_3$ in 40.0μL of vesicles solution. Two measurments were performed, the first one, at 295K and the second, at 77K.

The vesicles plus SDS sample was prepared adding 0.57mg of SDS to 40,0μL of vesicle solution (similar to the previous one), forming a micelles solution. In this manner, we would have smaller (approximately 10nm) molecules than in the first kind of sample. As in the previous sample, 4.0μL of acqueous solution traced with $InCl_3$ was added. The objective was to verify if the PAC thechnique would be capable to sense differences in the dimensions of the molecules.

In order to verify the sensitivity of PAC technique to the critical micellar concentration, we have performed several measurments with SDS samples, all at 295K. In these cases, we varied the SDS concentration. The $InCl_3$ solution to be added was diluted either in methanol or in water.

For all SDS series of measurments first it was prepared a 50.0mM acqueous stock solution, by adding 0.361g of SDS in 25.0mL of water. Each specific concentration of SDS samples was obtaind through the dilution of one small volume of the stock solution in one separated recipient with a specific water volume, as the final SDS concentration was smaller than 50.0mM.

In the case of $InCl_3$ diluted in water, measurements were performed with 0.05mM, 0.2mM, 1.0mM, 2.67mM, 4.0mM, 8.0mM and 10.0mM concentrations of SDS.

When $InCl_3$ was diluted in methanol, the volumes of SDS solutions and the volume of methanol were different for each sample, according to the ^{111}In activity and concentration os SDS.

4.2 Results and Discussion

4.2.1 *Vesicles Samples*

The graphs in figures 4 and 5 refer to vesicles samples, the first one at 295K and the second, at 77k.

Following, Figures 6 and 7 are about vesicles plus 50mM SDS samples, at 295K and 77K. Visually, these graphs are similar to the previous ones.

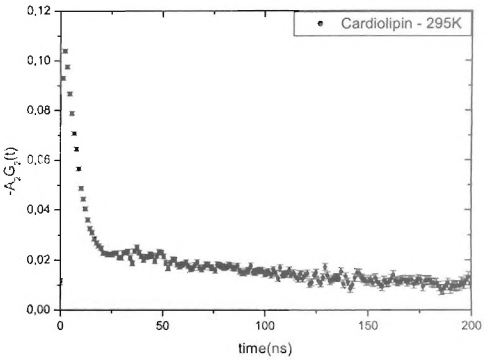

Figure 4 Perturbatoin function for vesicles sample at 295K.

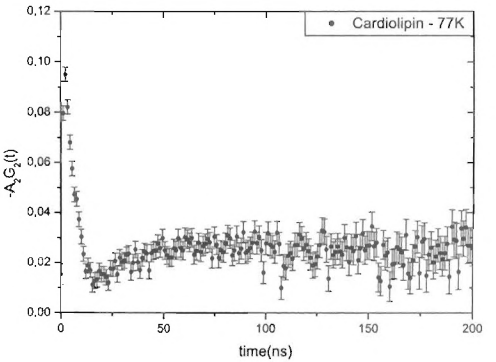

Figure 5 Perturbation funciton for vesicles sample at 77K.

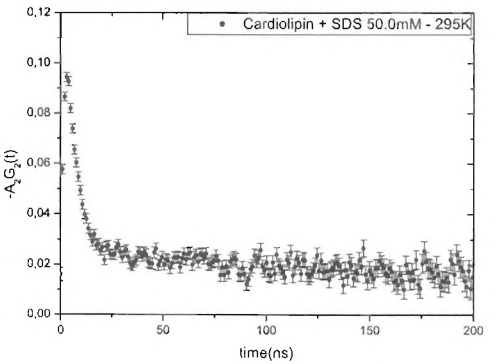

Figure 6 Perturbation function for vesicles plus SDS 50mM at 295K.

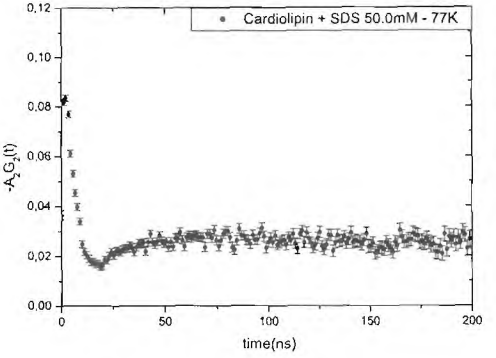

Figure 7 Perturbation function for vesicles plus SDS 50mM at 77K.

The most relevant parameters obtained by fitting the perturbation functions of the four graphs above are presented in Table 1:

Table 1 Results for vesicles at 295K (sample 1) and 77K (sample 2) and vesicles plus SDS at 295K (sample 3) and 77K (sample 4). The asymmetry parameter (η) is zero for the four samples. ν_Q is the quadrupolar frequency in MHz, δ is the width of ν_Q distribution and λ is the inverse of τ_c in Equation (4).

Sample	ν_Q (MHz)	δ	λ (ns^{-1})
1	81.4(5)	0.770(10)	15.6(7)
2	127.6(11)	0.570(10)	0
3	82.1(10)	0.620(20)	22.9(19)
4	127.7(10)	0.620(10)	0

At 295K there are dynamical interactions in the two samples, indicated by the values of λ (inverse of τ_c in Equation (4)), different from zero, in Table 1. This effect can also be noticed in the shape of the curves, which present exponential dampings.

The quadrupolar frequency is better determined at 77K, when the EFG doesn't change, and consequently, the value of λ is compatible with zero. This means we are dealing with a static situation.

If we compare the results for the two samples, we can notice that the significant difference between them is the value of λ. The quadrupolar frequencies don't change after adding SDS to the vesicles solution, indicating that the neighbourhood of ^{111}In didn't change conformation when the molecules passed from vesicle form to micellar form. In this manner, it is possible to believe that the EFG changes at

295K, due to rotational diffusion caused by the Brownian movement in the samples and the value of λ is bigger for the second sample because its molecules are smaller.

4.2.2 *SDS Samples*

For the experiments with SDS samples it is not possible to notice through the PAC technique the critical micellar concentration, which for acqueous solution at 295K, occurs around 7mM [11]. As previously mentioned, the concentration was varied from 0.05mM to 10.0mM. In the fits of the respective perturbation curves no significant changes were observed. Nevertheless, it was possible to observe that the medium, water or water plus methanol, modifies the behavior of the parameters.

In Figures 8 and 9 are presented two examples, 1.0mM that correspond to a sample with SDS concentration under CMC, and 10.0mM, that is above CMC. The shape of the perturbation curves don't change significantly, neither for the other samples in water solution. This indicates that there are no big changes in behavior of the molecules.

It was possible to adjust two binding sites for the ^{111}In (due to the charge of ^{111}In), and then the values of the quadrupolar frequencies fluctuated around 60MHz for the most populous site. The samples don't present dynamic interactions. Also, the quadrupolar frequencies were not well defined. It was not possible to adjust a free value of the parameter δ, that represents the width of the distribution of frequencies.

In Figure 10 it is shown a graph of a sample in which it was added 10μL of methanol to 1.0mM (10μL) acqueous solution sample. Then, we could obtain a SDS concentration, in such manner, intermediate between the two previous, for which we did not expect big changes if the solution was acqueous.

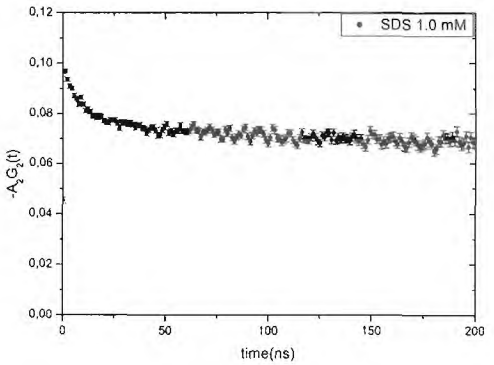

Figure 8 Perturbation function for SDS 1.0mM in water at 295K.

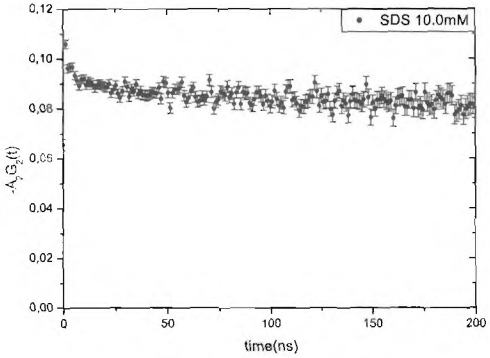

Figure 9 Perturbation function for SDS 10.0 mM in water at 295 K.

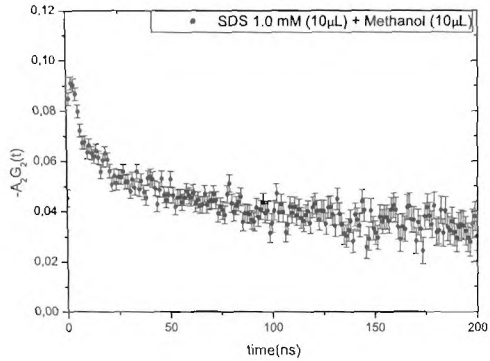

Figure 10 Perturbation function for SDS plus metanol at 295 K.

As we can see, the shape of the curve in Figure 10 is clearly different from the perturbation function in Figures 8 and 9, what indicates that the medium, water or methanol, influences the SDS molecules bahavior. This can be explained by the changing of viscosity of the medium.

In the case of the samples that contained methanol, it was possible to fit one single site for ^{111}In. As in the acqueous samples, the frequencies in general were not well defined. This didn't allow us to find some systematics in the behaviors with the SDS concentration.

5 Conclusion

In this work we have presented a new PAC spectrometer being constructed at the University of São Paulo Pelletron Laboratory with an acquisition system based

on multiparameter CAMAC system. We have also presented some results with cardiolipin:phosphatidylcoline vesicles and SDS samples from measurments at IPEN-CNEN/SP. For the vesicle samples, we can observe that the PAC technique is sensitive to differences between two samples for which the dimension of the molecule changes due to the presence of SDS surfactant, which tranforms one vesicle into a micelle, with out changes in the probe radioactive nucleus neighbourhood. On the other hand, the technique is not capable of sensing the changes caused by agregation of SDS molecules while the concentration of the solution is varied until forming micelles, and is not sensitive to the critical micellar concentration. Finally, we could observe that the behavior of SDS molecules changes due to the exchange of the medium, water or methanol. One of the possibilities to explain this bahavior is the changing of solvent viscosity.

Acknowledgements

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