2013 International Nuclear Atlantic Conference - INAC 2013
Recife, PE, Brazil, November 24-29, 2013
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: **978-85-99141-05-2**

Investigation of hyperfine interactions in DNA nitrogenous bases using perturbed angular correlation spectroscopy

Andréia dos Santos Silva[1], Artur Wilson Carbonari[1], André Luis Lapolli[1], Rajendra Narain Saxena[1] and Henrique Saitovitch[2].

[1]Instituto de Pesquisas Energéticas e Nucleares, Av. Prof. Lineu Prestes, 2242, 05508-000, São Paulo, SP, Brazil.

[2]Centro Brasileiro de Pesquisas Físicas, R. Dr. Xavier Sigaud, 150, 22290-180, Rio de Janeiro, RJ, Brazil.
asilva@usp.br

ABSTRACT

Perturbed γγ angular correlations (PAC) spectroscopy has been used to study the DNA nitrogenous bases (adenine, cytosine, guanine, thymine), using $^{111}In \rightarrow ^{111}Cd$ and $^{111m}Cd \rightarrow ^{111}Cd$ probe nuclei. One of the advantages of applying PAC technique to biological molecules is that the experiments can be carried out on molecules in aqueous solution [1], approaching the function of molecules under conditions that are close to in vivo conditions. The measurements were carried out for DNA nitrogenous bases molecules at 295 K and 77 K in order to investigate dynamic and static hyperfine interactions, respectively. The interpretation of the results was based on the measurements of dynamic interaction characterized by the decay constant from which valuable information on the macroscopic behavior of the molecules was obtained [2; 3]. On the other hand, PAC measurements at low temperature showed interaction frequency (ν_Q), asymmetry parameter (η) and the distribution of the quadrupole frequency (δ). These parameters provide a local microscopic description of the chemical environment in the neighborhood of the probe nuclei. Results showed differences in the hyperfine interactions of probe nuclei bound to the studied biomolecules. Such differences were observed by variations in the hyperfine parameters, which depended on the type of biomolecule and the results also showed that the probe nuclei bounded at the molecules in some cases and at others did not.

1. INTRODUCTION

The DNA is a type of nucleic acid that is composed of nucleotides which are linked thorough phosphodiester bonds [4]. Each nucleotide consists of a cyclic sugar (β-D-2' deoxyribose in DNA), which is phosphorylated in the 5' position of the sugar and carries a heterocyclic ring at the C1' position (β-glycosyl C1'-N bond). The nitrogenous bases are the purines bases guanine (G) and adenine (A), and the pyrimidine bases cytosine (C) as well as thymine (T). DNA usually occurs as an antiparallel double helix with base pairing through H bound formation between the complementary bases (C and G) and (A and T) [5]. See in figure 1 the simple structure of a DNA molecule and the possible sites of binding to metals on the nitrogenous bases. The nitrogenous bases are the purine bases guanine (G) and adenine (A) and the pyrimidine bases cytosine (C) and thymine (T) [5]. The nucleic acid monomers have different metal ion affinities [6]. Metal species can interact with nitrogenous bases by two following ways: First, directly via coordination to atoms of the nitrogenous bases (N, C, O). Second, indirectly via coordination to other ligands that includes H bond formation [5]. The

order of establish of 3d transition metal ion-base complex are: G > A, C > T. At physiological pH the preferred binding sites on the nitrogenous bases are: guanine N7, adenine N1 and /or N7, cytosine N3, thymine O4 [6].

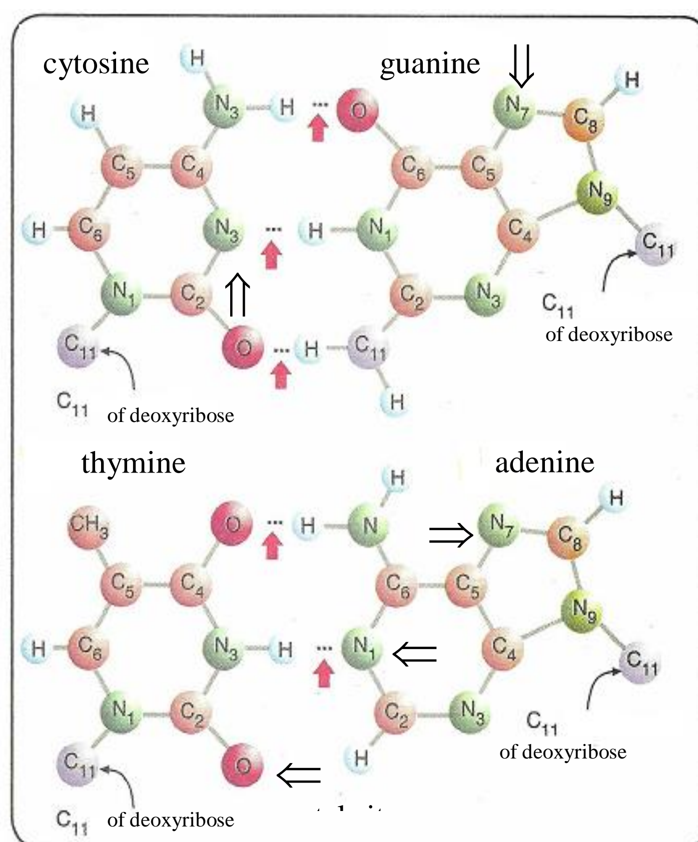

Figure 1: Simple structure of a DNA molecule and the possible sites of binding to metals on nitrogenous bases.

When the nitrogenous bases are incorporated into a duplex DNA matrix, the affinities towards metal ions are modified [7]. One of the advantages of applying PAC spectroscopy to biological molecules is that experiments can be carried out on molecules in aqueous solution, approaching to the biologic sample under conditions close to in vivo. A detailed description of the application of PAC spectroscopy to investigate biomolecules is given in a review article [3; 8]. Metal ion can interact with nitrogenous bases in two distinct modes of binding: diffuse binding and site binding, both of which are important for the structure and function of the nitrogenous bases. In the diffuse binding mode the metal and the nitrogenous bases retain their hydration layer and the interaction is through water molecules. In the site binding mode the metal is coordinated to specific ligand on the nitrogeneus bases [6].

The Perturbed Gama-Gama Angular Correlation (PAC) spectroscopy can be used in order to investigate biomolecules because it provides information about the physical and chemical environment around a probe nucleus [9; 2; 3]. PAC spectroscopy is an important toll to investigate the microscopic behavior of the biomolecules through hyperfine interactions that

occur between the electric nuclear quadrupole moment of the probe nucleus with the electric field gradient (EFG) from the surrounding charge distribution [1; 10].
The main objective of the present work is to investigate the neighborhood of the sites to which the ^{111}In-^{111}Cd or ^{111m}Cd→^{111}Cd probes are bond in the DNA nitrogenous bases by measuring nuclear quadrupole interactions in order to compare them and establish which site the probes are bonded to.

2. EXPERIMENTAL

The nitrogenous bases used in the present work were obtained commercially from Sigma-Aldrich. In order to be used in PAC measurements, a small amount of the powder of each nitrogenous bases was dissolved in deionized water, with a concentration of 5×10^{-5} Mol/ml, and stored at room temperature until its use in the experiment. An aliquot of 50μl of these solutions was mixed with a solution containing ~10μCi of the radioactive probe nuclei ^{111}In or ^{111m}Cd as $^{111}InCl_3$ or $^{111m}Cd(NO_3)_2$, respectively. The carrier free $^{111}InCl_3$ solution was purchased from MS Nordian, Canada. The $^{111m}Cd(NO_3)_2$ was obtained by neutron irradiation of 5 mg of Cd metal in the IEA-R1 research reactor at IPEN for 2 hours in a flux of $\sim 5 \times 10^{13}$ n/cm^2.s . After irradiation, the Cd metal was dissolved in 0.3 ml of 1M nitric acid, which was completely evaporated, the resulting salt was then dissolved in 0.5 ml of deionized water. PAC measurements were carried out using a BaF_2 four-detector spectrometer (see figure 2). The twelve measured coincidence spectra W(θ,t) are analyzed by TDPAC software [11] to create the spin rotation R(t) curve given by the combination of W(θ,t) spectra:

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

where,

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

being $W_i(\theta,t)$ the coincidence spectra for combinations of detectors in angles θ = 90° or 180°, after subtracting the effects of unwanted accidental coincidences (t). A22 is the angular correlation coefficient and G22(t) is the perturbation function that contains information about the hyperfine interactions and, in the case of static electric quadrupole hyperfine interaction, can be written as:

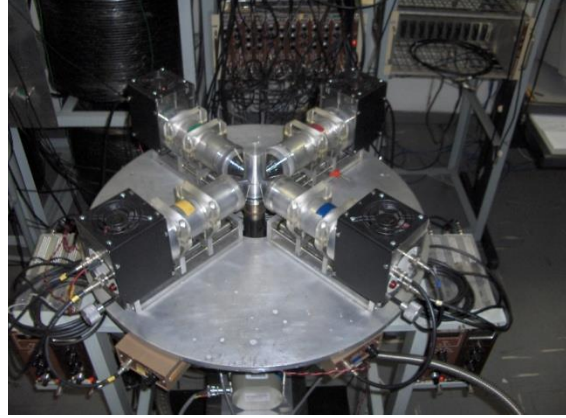

Figure 2: BaF_2 four-detector spectrometer

$$G_{22}(t) = S_{20} + \sum_{n=1}^{3} S_{2n} \cos(\omega_n t), \tag{3}$$

where S_{2n} are coefficients and ω_n are the transition frequencies corresponding to the splitting of the intermediate energy level of the probe nucleus gamma cascade due to the presence of the electric field gradient (efg) produced by the electronic neighborhood [10; 12]. The transition frequencies are related to the quadrupole frequency $\omega_Q = eQV_{zz}/4I(2I - 1)$ η. PAC measurements of the nitrogenous base samples were carried out in solution at 295 K, when samples are liquid and the presence of the rotational diffusion effect is expected, and at 77 K for solid samples at which static quadrupole interactions are present. The rotational diffusion is characterized by the rotational correlation time τ_{CR}, which describes the mobility of a molecule in a solution and depends on the viscosity (ξ), the temperature (T) and the volume of the molecule (V) as $\tau_C = V\xi/(k_B T)$, where k_B is Boltzmanns constant. The influence of the dynamic interaction is stronger when $\omega_0\tau_C \approx 1$, 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, $\omega_0\tau_C << 1$, 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, $\omega_0\tau_C >> 1$, 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 $\tau_C \to \infty$, 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 [2], the hyperfine parameters related to the local structure around the probe nucleus into the biomolecule.

3. RESULTS AND DISCUSSION

PAC spectra for nitrogenous bases measured with ^{111}In (^{111}Cd) at 295 K and 77 K are shown in figure 3. As expected, samples measured at room temperature showed dynamic interaction with fast relaxation of the quadrupolar interaction, resulting in an exponential decay of the

PAC spectra, which indicates the existence of mobility of the molecules in solution. However, samples measured at the liquid nitrogen temperature showed quite slow relaxation ($\lambda \sim 0$) of the quadrupolar interaction or only static interactions as expected at low temperatures.

It is clearly seen that all spectra at 295 K present a non-zero baseline at times higher than ~250 ns, which correspond to an "unperturbed fraction'' due to unbound probe atoms or some light molecules containing the probe atoms. Therefore, experimental data measured with at 295 K could be appropriately modeled by the following function [13]:

$$R(t) = a_0 + a_1 e^{-\lambda_{2(1)}} + a_2 e^{-\lambda_{2(2)}}, \tag{4}$$

where $\lambda_{2(i)}$ are the typical relaxation constant which is proportional to the square of the spin-independent quadrupole frequency $\nu_Q = eQV_{zz}/h$ and τ_C. For the spin of the intermediate level of the g-cascade I = 5/2, which is the case of both probe nuclei used in this work, the relaxation constant is [3]:

$$\lambda_2 = 2.49\nu_Q^2(1 + \eta^2 / 3)\tau_C, \tag{5}$$

The fit of equation (5) to experimental data at 295 K produced two fractions with quite different relaxation constant values as can be seen in table 1, which displays results for hyperfine parameters measured with PAC using $^{111}In \rightarrow ^{111}Cd$ in nitrogenous bases. One minor fraction with λ_2 values in the range ~7 MHz to 10 MHz and another major fraction with higher λ_2 values (table 2). This later fraction might represent the complex ^{111}In-nitrogeneous bases in transient state after ^{111}In decay into ^{111}Cd when the electric quadrupole interaction rapidly changes due to the chemical rearrangement of the complex. All spectra show an initial fast drop from 0 ns to around 15 ns and at later times a slow decay. In these cases we have fitted the spectra considering that $^{111}In \rightarrow ^{111}Cd$ probes are present in two types of complexes. One fraction of probes is in a complex which is rapidly rotating with a characteristic λ_2 and the other fraction is in a complex which must be rotating very slowly. At 77 k all spectra measured with $^{111}In \rightarrow ^{111}Cd$ probes were fitted with a model for static interactions considering only one site for which the quadrupole interaction frequencies ν_Q are highly distributed with δ values of ~ 45%. Results displayed in table 1 show quite similar ν_Q values for all nitrogenous bases, however very different from the ν_Q value observed for aqueous solution of the $^{111}In \rightarrow ^{111}Cd$ probes.

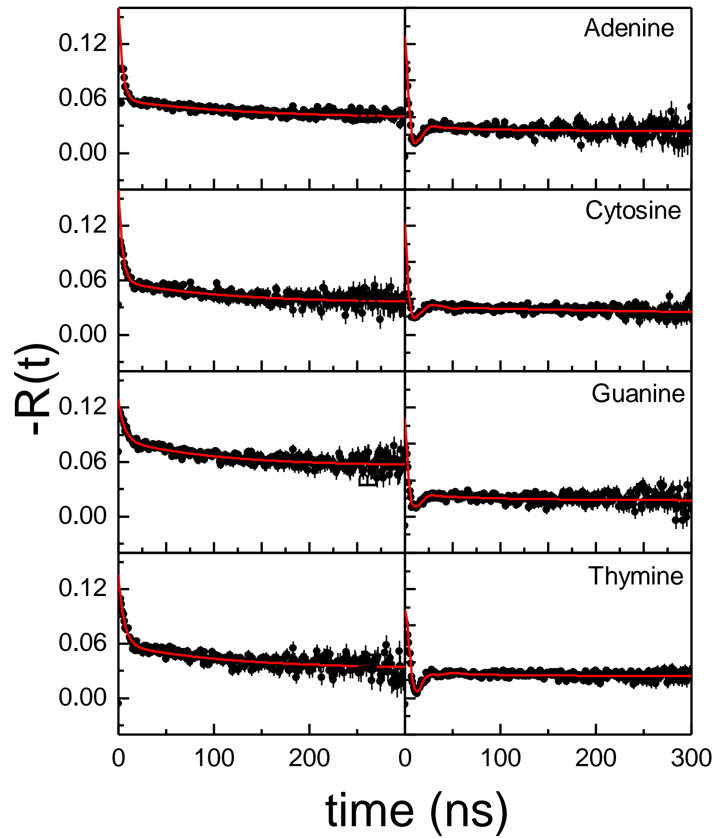

Figure 3: PAC spectra of indicated nitrogenous bases measured with ^{111}In (^{111}Cd) probes at 295K (left) and 77K (rigth).

Table 1. Hyperfine parameters for nitrogenous bases measured with $^{111}In \rightarrow ^{111}Cd$.

Bases	ν_Q (MHz)	V_{zz1} (x10^{21} V/m^2)	η	δ(%)	ν_Q (MHz)	V_{zz2} (x10^{21} V/m^2)	η	δ(%)
Adenine		-	-	-	142± 6	7.08± 0.30	0.57± 0.07	46
Cytosine		-	-	-	139± 8	6.91± 0.42	0.68± 0.10	47
Guanine		-	-	-	155± 8	7.71± 0.43	0.40± 0.09	46
Thymine		-	-	-	145± 8	7.23± 0.39	0.65± 0.08	40
Aqueous solution	120,3 ± 15,2	4.29± 0.16	0.6± 0.06	20.2				

Table 2. Hyperfine parameters extracted from the fits to experimental data measured with $^{111}In \rightarrow ^{111}Cd$. The calculated rotational correlation time (τ_C) for slow dynamic interaction is displayed as well.

	295K					77K	
	$\lambda_{2(1)}$ (MHz)	f_1 (%)	τ_C (10^{-11}s)	$\lambda_{2(2)}$ (MHz)	f_2 (%)	ν_Q (MHz)	η
Adenine	7.4±1.3	16	13.3±2.5	248±16	84	142±6	0.57±0.07
Cytosine	9.1±1.8	18	16.4±2.7	253±29	82	139± 8	0.68± 0.10
Guanine	10.0±1.6	39	15.9±2.5	163±21	61	155± 8	0.40± 0.09
Thymine	8.7±2.0	26	14.6±2.6	172±15	74	145± 8	0.65± 0.08
Aqueous solution	0.15±0.01	100	0.73±0.2	–	–	86±3	0.6±0.06

After-effects, which are caused by the electron capture process when ^{111}In decay to ^{111}Cd, is likely the main responsible for the broad frequency distribution observed at 77 K [13]. The after-effect, therefore, results in a dynamic process which can contribute to decrease the amplitude in the PAC spectra when time increases for the static interaction at 77 K. As a consequence, the broad frequency distribution makes the uncertainty in the quadrupole values to be high, which affects the accuracy in the determination of rotational correlation time.

Measurements of the quadrupole frequency using $^{111m}Cd \rightarrow ^{111}Cd$ probes eliminate the influence of the after-effect. As ^{111m}Cd is formed at one of the excited states of ^{111}Cd, this probe does not produce after-effects during PAC measurements and the uncertainty in the measured quadrupole frequency is small. We therefore measured the nitrogenous base samples using $^{111m}Cd \rightarrow ^{111}Cd$ probes at 77 K. The measured spectra are shown in figure 4.

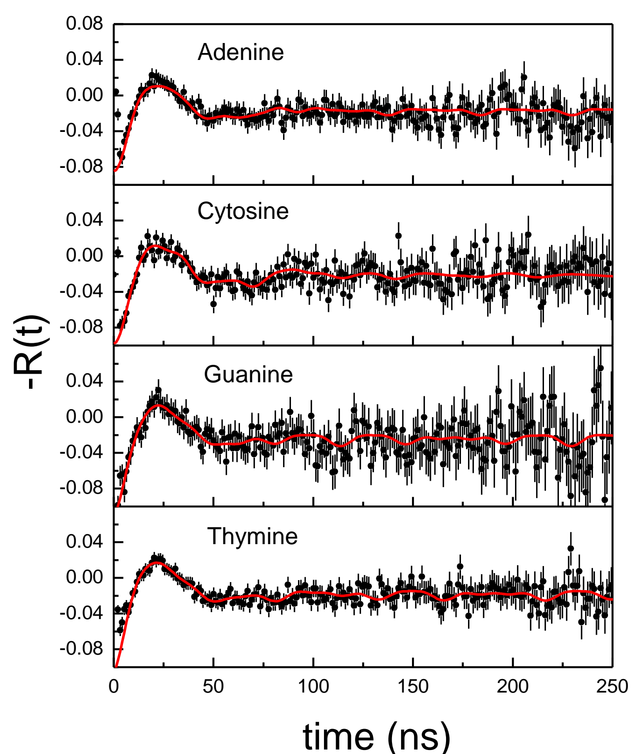

Figure 4 – PAC spectra of indicated nitrogenous bases measured with ^{111m}Cd probe at 77K.

Results were analyzed considering a model of pure static quadrupole interaction with two fraction sites. The major fraction for all nitrogenous bases present low frequencies similar to that for $^{111m}Cd \rightarrow ^{111}Cd$ diluted in a neutral aqueous solution, ν_Q = 85(3) MHz. We therefore assigned these major fractions to ^{111m}Cd probes bound to water molecules. The minor fraction, with higher frequency values, displayed in table 3, was assigned to complexes of ^{111m}Cd with the nitrogenous base molecules. These frequency values are well defined with a very small frequency distribution. We therefore use such values to re-calculate the rotational correlation time, and the values are also shown in table 4. The results show that guanine and thymine present higher τ_C values and the quadrupole frequencies for both are smaller than those for adenine and cytosine. The nitrogenous bases have Nitrogen and Oxygen in their composition and these atoms are available to form chemical binding with metals such as In and Cd [6]. We therefore propose that Cd probes are bound to the same ion in Adenine and Cytosine, however, different from the same ion to which guanine and thymine are bound to.

Table 3. Hyperfine parameters for nitrogenous bases measured with $^{111m}Cd \rightarrow ^{111}Cd$.

Bases	ν_Q (MHz)	V_{zz1} (10^{21} V/m^2)	η	δ(%)	ν_Q (MHz)	V_{zz2} (10^{21} V/m^2)	η	δ(%)
Adenine		4.34± 0.12	0.49± 0.04	21	145± 2	7.22± 0.07	0.10± 0.08	0
Cytosine		4.05± 0.17	0.55± 0.05	20	149± 3	7.40± 0.16	0.51± 0.04	3
Guanine		5.20± 0.15	0.49± 0.09	0	124± 3	6.17± 0.13	0	34
Thymine		3.89± 0.21	0.53± 0.06	29	126± 2	6.28± 0.06	0.49± 0.02	0
Aqueous solution	98,05 ± 2,8	4.29± 0.15	0.54± 0.04	18.3				

Table 4. Hyperfine parameters extracted from the fits to experimental data measured with $^{111m}Cd \rightarrow ^{111}Cd$. The calculated rotational correlation time (τ_C) for slow dynamic interaction is displayed as well.

	295 K ($^{111}In \rightarrow ^{111}Cd$)		77K ($^{111m}Cd \rightarrow ^{111}Cd$)	
	$\lambda_{2(1)}$ (MHz)	τ_C (10^{-11}s)	ν_Q (MHz)	η
Adenine	7.4±1.3	14.1±1.5	145± 2	0.10± 0.08
Cytosine	9.1±1.8	15.2±1.7	149± 3	0.51± 0.04
Guanine	10.0±1.6	26.1±1.6	124± 3	0
Thymine	8.7±2.0	20.4±1.3	126± 2	0.49± 0.02

4. CONCLUSIONS

In conclusion, results showed the existence of the interactions between biomolecules and the probe nucleus which the probe nucleus was in fact bound to the DNA nitrogenous bases in a specific site according each one . These results are part of an extensive work that has studied hyperfine interactions in DNA and antibodies which intended to contribute to better explain the different patterns of immunological response presented by the different mice lineages when infected by Y strain of *T. cruzi.*

ACKNOLEDGMENTS

Financial support for this research was partially provided by the Fundação de Amparo para Pesquisa do Estado de São Paulo (FAPESP). AWC and RNS thankfully acknowledge the support provided by CNPq in the form of research fellowship.

REFERENCES

1. LEIPERT, T. K., BALDESCHWIELER, J.D., SHIRLEY, D.A., Applications of gamma ray angular correlations to the study of biological macromolecules in solutions, **Nature** 200, 907-909, 1968**.**
2. ABRAGAM, A., POUND, R. V., Influence of electric and magnetic fields on angular correlations. **Phys. Rev.** 92,943-962, 1953.
3. HEMMINGSEN, L., SAS, K.N., DANIELSEN, E., Biological Applications of Perturbed Angular Correlations of γ-Ray Spectroscopy. **Chem. Rev**. 104, 4027-4061, 2004.
4. ALBERTS, B. **Molecular biology of cell**. 3. ed. New York: Gerland, 1994.
5. LIPPERT, B. **Multiplicity of metal ion binding patterns to nucleobases**. Coordination Chemisrty Reviews.200-202 (2000) 487-516.
6. HADJILIADIS, N. and SLETTEN, E. **Metal complex-DNA interactions**. Blackwell Publishing Ltda. 2009.
7. VINJE, J., PARKINSON, J. A., SADLER, P. J., BROWN, T., SLETTEN, E. Sequence-selective metalation of duble-helical olygodexyribonucleotides with Pt^{II}, Mn^{II} and Zn^{II} ions; **Chem. Eur. J**., 2003, 9, 1620-1630.
8. BAUER, R., Perturbed angular correlation spectroscopy and its application to metal sites in proteins: possibilities and limitations, **Quarterly Review Biophysics,** 18, 1-64, 1985.
9. HAAS, H., SHIRLEY, D. A. Nuclear quadrupole interactions studies by perturbed angular correlations. J. Chem. Phys. 58, 3339-3355, 1973.
10. BUTZ, T. Analytic perturbation function for static interaction in perturbed angular correlations of γ-rays, **Hyperfine Interactions** 52, 189-228, 1989.
11. MESTNIK, J. F. **Data treatment of perturbed angular correlation**. Private communication, 2002.
12. KARLSSON, E., MATTHIAS, E. SIEGBAHN, S. **Perturbed Angular Correlation,** North-Holland, Amsterdam, 1965.
13. SHPINKOVA, L. G, CARBONARI, A. W, NIKITIN, S. M. and MESTNIK-FILHO, J. Influence of electron capture after-effects on the stability of $^{111}In(^{111}Cd)$-complexes with organic ligands. **Chemical Physics** 279: 255-267, 2002**.**