Murine biological tissues characterized by PALS Measurements

Roberto Sebastián Tomás [1], Cecilia Yamil Chain [2] and Laura Cristina Damonte[3]

1Laboratorio de Terapias Fotoasistidas, Centro de Investigaciones sobre Porfirinas y Porfirias (CIPYP) CONICET-Htal de Clínicas José de San Martín-Universidad de Buenos Aires (UBA) Córdoba 2351; Ciudad Autónoma de Buenos Aires, 1120AAF, Argentina, 2-Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA, CONICET La Plata), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 1900 La Plata, Argentina, 3-Departamento de Física and Instituto de Física La Plata (IFLP, CONICET La Plata), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CC 67, 1900 La Plata, Argentina.

Positron Lifetime Spectroscopy (PALS) allows to obtain information about the electronic densities and defects in a sample. In materials where positronium can appear (as it is the case of biomaterials and biological tissues), it is also possible to gain information about the size of the cavities and the chemical and physical properties of the sample. PALS technique has been applied to biological systems since the sixties, but the reported data is dispersed and the analysis lacks of rigurosity. In the last years, the improvement of the experimental techniques and the computational methods have contributed to increase the research in different materials, in particular in those related with life sciences. The aim of this work is to establish the importance of the PALS technique in the characterization of biological tissues. From this perspective, here we have been used PALS to find the annihilation parameters that better characterize the biological tissues of rodents from Muridae family. For this, different pieces of the same organ, different organs from the same animal and the same organs from different animals were analyzed. Finally, from the results obtained (lifetimes and relative intensities and percentage of loss water) a statistical study was carried out to analyze the relationship between the main components of a data matrix. This analysis made it possible to describe the variability and correlations between the parameters measured in the samples under study.

Experimental Design

- The specimens were anesthetized and sacrificed for dissection and organ extraction. Each anatomical structure was preserved at 4°C embedded in a 70% ethanol solution, in order to fix its basal structure.
- The tissues were dried at atmospheric pressure in a desiccator. The water mass loss percentage was measured at defined time intervals.
- PALS measurements were done using a ^{22}NaCl source onto a kapton film (1.42 g/ml) in a typical sandwhich arrangement.

Figure 1: Wistar rodents

Figure 2: PALS experimental device. FWHM of 202 ps.

- The obtained spectra were analyzed with the POSITRONFIT programme yielding three exponential decays that characterize three positron annihilation sites.
- From the resulting annihilation parameters a statistical procedure based on principal component analysis (PCA) was carried out.

PALS Results

Table 1: Positron annihilation parameters for different murine tissues

Sample	τ_1	τ_2	τ_3	τ_{prom}	I_1	I_2	I_3
Liver	0.15_6	0.377_7	1.88_1	0.63_2	9_2	73_2	18.4_2
Lung	0.16_1	0.46_1	1.84_1	0.56_3	38_2	47_2	15.5_2
Heart	0.13_8	0.362_1	1.82_3	0.56_4	9_3	75_3	15.3_3
Brain	0.20_3	0.37_1	1.88_3	0.46_5	20_8	68_8	11.1_3
Liver (Mouse 2)	0.11_5	0.357_9	1.78_4	0.52_3	20_8	68_8	11.1_3
Heart (Mouse 2)	0.11_7	0.33_1	1.71_6	0.52_3	11_4	80_4	9.30_5

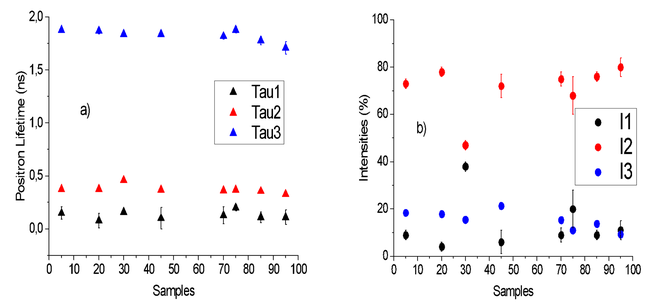

Figure 3: a) Positron lifetime components, b) Positron lifetime intensities. The horizontal axis represents the different tissues: 5 y 20 for liver samples with and without source; 30 y 45 for lung samples with and without source, ; 70 heart; 75 brain, 85 y 95 liver and heart samples of a different specimen.

- First and second positron lifetime component takes into account positron annihilation in sites with different electronic densities.
- The first lifetime component includes also annihilation of p-Ps (125 ps).
- The long live component has a constant value around of 1.8 ns, which represents voids of 0,27 nm (Tau-Eldrup model). It is assigned to annihilation of o-Ps inside water cavities while the variability of their intensities indicate the humidity degree of each sample.
- At first glance the annihilation lifetimes do not allow us to extract a definitive conclusion about a specific characterization of the tissues.

Statistical Analysis

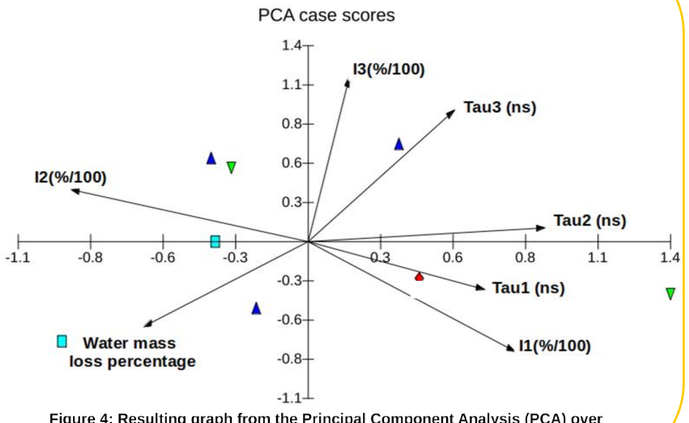

Figure 4: Resulting graph from the Principal Component Analysis (PCA) over the initial data matrix from the MVSP program. ▲ Liver ▼ Lung ■ Heart ◆ Brain

- A high correlation between τ_2 and the first main component of the system (that cumulates, by definition, the main variability of the model) is observed. Thus, τ_2 is the variable that best characterizes the samples under study.
- An inverse relation between τ_2 and its intensity is verified.
- The analysis of the second main component yields an inverse relation between I_3 and the percentage of drying of the samples. In other words, as drier is the sample, lower the number of positrons that annihilates in water.
- The MVSP analysis allows to find correlations between the variables and to identify the ones that more contribute to characterize the samples under study.

This finding is in contrast to those provided by reported publications which focus their attention only on τ_3 and I_3 for the characterization and structural differentiation of normal and tumor cells. Therefore, in order to characterize biological tissues, all the annihilation parameters must be considered together, not only the component corresponding to the formation of o-Ps.

Conclusions

★ PALS measurements on biological tissues are characterized by three positron lifetimes, being the long lived one assigned to positron annihilation in water.

★ The long-lived component is not a positron annihilation parameter adequate to sense the structural particularities of biological tissues; since it is not sensitive to the internal defects these materials have.

★ The use of a statistical analysis is needed for the comprehension of the whole positron parameters behavior.

★ From statistics based on PCA it was concluded the second lifetime component with its corresponding intensity is the best parameter to characterize the biological tissues. It is observed an inverse relationship between the component τ_2 and its respective intensity.

★ The high complexity and physicochemical diversity of biological systems makes difficult their absolute characterization through a PALS study. The complementarity between the PALS technique and statistical analysis was demonstrated.