Contribution ID: **58** Type: **poster**

The development of a method for determining ortho-Positronium mean lifetime in extracellular vesicles using Positron Annihilation Lifetime Spectroscopy

Sunday, 10 October 2021 16:10 (20 minutes)

Positron annihilation lifetime spectroscopy (PALS) has been used less extensively in studies with biological material, although its use in interrogating free volume, voids and defects in polymers is well established. There exist a number of results, e. g. by E. Kubicz and the J-PET group [1], showing the correlation between cell structure and the PALS parameters, such as mean ortho-Positronium (o-Ps) lifetime and intensity. This technique has also been demonstrated to have utility as an in situ molecular probe in self-assembled biomimetic systems for which it is highly sensitive to conformational, structural and microenvironmental transformations [2].

Extracellular vesicles (EVs) are defined as bilayer cell membrane fragments released into the extracellular space by various types of cells. EVs play dual role throughout the body, due to their involvement in both physiological and pathological conditions [3]. Growing interest in these spherical structures emerges from their involvement in cell-to-cell communication, tumour progression and their possible application as biomarkers or drug delivery systems [4].

Applying PALS to study EVs required set-up modifications (chamber design) and calibrations, in order to adjust the system for studies of liquid samples in temperature controlled conditions. For that purpose, the system was equipped with the Lauda LOOP L100 thermostat. Temperature of investigated samples was estimated from calibration data obtained through extensive thermal testing.
Two EV samples derived from normal pancreatic beta-cell cultures suspended in PBS solution were examined: (1) from culture under normoglycemic and (2) hyperglycemic conditions. EV concentrations in the samples were determined using qNano technique and its values were respectively: (1) 9×10^{10} and (2) $6,9 \times 10^{10}$ particles/mL.

Preliminary results demonstrate strong correlation between mean o-Ps lifetime and EV concentration in the sample. Studied concentrations of EVs were too low, therefore it was mainly the PBS solution that was contributing to the resulting o-Ps lifetime value, and not the EVs itself.
Obtained result opens perspective for further research, when applying higher EVs to PBS ratio. Such experiments were performed e. g. by P. Sane et al. [5] and demonstrated that observing changes in o-Ps lifetime, corresponding to phase transitions of membrane lipids in vesicles (multilamellar DPPC), is feasible with PALS technique.

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Session Classification: Poster Session