INFLUENCE OF DMSO ON CRYSTALLIZATION OF WATER AT LOW TEMPERATURES AND ITS IMPACT ON CELL SURVIVAL

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INTRODUCTION

In the past, our group published study of solidification and thawing processes of water-DMSO mixtures (up to 10% vol. of DMSO), which represent a cryopreservation medium for cells in cell therapy.[1] Application of positron annihilation lifetime spectroscopy (PALS) allowed monitoring temperature dependence of orthopositronium lifetime, which is sensitive to phase changes or changes in structure. It has been shown that with increasing concentration of DMSO in water, there is an increased induction of the amorphous fraction in the freezing mixture.[2] However, this fraction recrystallizes during slow heating, which is manifested by hysteresis between the temperature-dependent lifetime of the ortho-positronium probe during heating and cooling. This recrystallization can be avoided by rapid heating.

AIMS OF THIS WORK

In this work we study phase behaviour of system consisting of lipid bilayer (dimyristoyl phosphocholine) and water-DMSO mixture. This is model system of cells in cryopreservation medium. We study crystallization behaviour of water confined in liposomes what are closed structures of sizes ranging from 200 nm to few μm. We study temperature and time stability of amorphous fraction induced by DMSO as well.

CONCLUSIONS

Crystallization of water confined in liposomes is triggered by homogenous nucleation at -38°C (235 K) for medium containing only water. Addition of DMSO induced depression of this temperature which is proportional to concentration of DMSO in medium.

The presence of recrystallization induced by slow heating in the temperature range -110 °C to -63 °C (163 K – 210 K) was confirmed in system containing lipid bilayer as well (Fig.1). This temperature range lies within limits of glass transition of water-DMSO mixture and melting of eutectic water-DMSO complexes. Specifically, we link this phase behaviour to recrystallization and melting of DMSO trihydrate. We were able to positively correlate stability of amorphous freeze-concentrated phase with increased cell post-thaw viability.[3]

The ability of DMSO to induce amorphization of water is an advantageous property as this form of frozen water does not pose a risk to membrane systems and can form a protective layer around the cells against ice crystals.

References:
[1] J. Lakota and P. Fuchsberger, *Bone Marrow Transplantation* **18(1)**, 262-3 (1996)
[2] K. Čechová, I. Maťko, J. Rusnák et al., *RSC Advances* **9**, 34299-34310 (2019)
[3] W. F. Rall, D. S. Reid and C. Polge, *Cryobiology* **21(1)**, 106–121 (1984)

Positron Annihilation Lifetime Spectroscopy

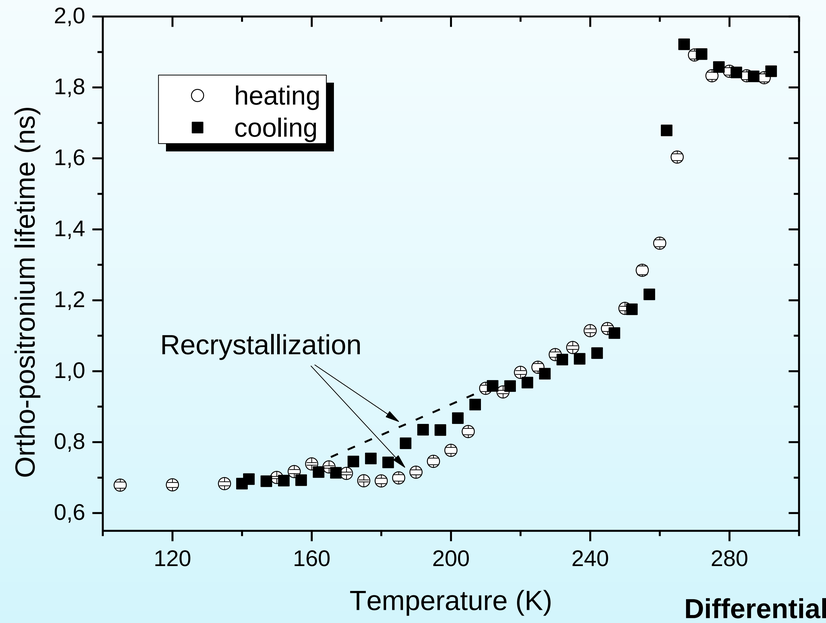

Fig. On the left: Temperature dependence of orthopositronium lifetime in 10 % DMSO with lipid bilayer. Dashed line represents hypotetical state of mixture of ice and fully amorphous freeze-concentrated phase. Picture shows recrystallization of amorphous freeze-concentrated phase at temperatures higher than 165 K.

Differential Scanning Calorimetry

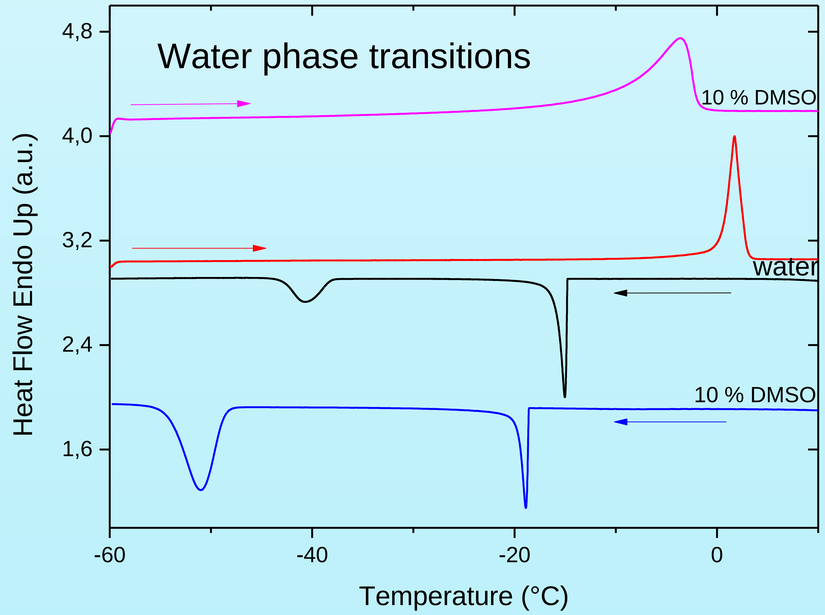

Fig. On the right: DSC thermograms of lipid bilayer with water and 10 % DMSO. Picture show depression of ice melting and crystallization temperature induced by DMSO

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