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INFLUENCE OF A SMALL AMOUNT OF GLYCEROL ON THE TREHALOSE BIOPROTECTIVE ACTION ANALYZED *IN-SITU* DURING FREEZE-DRYING OF LYZOZYME FORMULATIONS BY MICRO-RAMAN SPECTROSCOPY

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ABSTRACT

Micro-Raman spectroscopy gives the original opportunity to monitor simultaneously the operating process and the protein structure from *in-situ* investigations along the three stages of the freeze-drying (FD) process. This opportunity was used for determining how a small amount of glycerol enhances the bioprotective efficiency of trehalose during freeze-drying of lysozyme formulations. Three lysozyme formulations were analyzed: lysozyme dissolved in D_2O (wt% 1:9), in Trehalose-D_2O mixture (wt% 1:1:8) and in the Trehalose-Glycerol-D_2O mixture (wt% 1:1:0.17:7.93). Raman mapping performed during each stage of the FD process have provided important information about the preferential interaction between water, trehalose and lysozyme in relation with the protein stability. It was found that the addition of a small amount of glycerol had a plasticizing effect on the glassy trehalose-water matrix during the primary drying stage and then reduced the bioprotective effect of trehalose. By contrast, during the secondary drying stage, glycerol significantly enhanced the stabilizing effect of trehalose in the same sample, by replacing water-trehalose H-bonds with stronger glycerol-trehalose H-bonds and stiffening the amorphous trehalose matrix. The action of glycerol is also related with its capability to prevent aggregation of trehalose making the structure of the frozen product more homogeneous, by changing the hydrogen-bond networks in the liquid formulation before the freezing stage.

Keywords: bioprotection, protein stability, freeze-drying, Raman imaging, excipients, *in-situ* monitoring by Raman spectroscopy