THE EFFECT OF PROCESS PARAMETERS AND TREHALOSE ON GAMMA GLOBULIN STABILITY DURING LYOPHILIZATION

Heljo VP[1], Yohannes G[2], Harju H[1], Hartonen K[2], Riekkola M-L[2], Juppo AM[1]

[1] Industrial Pharmacy, Faculty of Pharmacy, University of Helsinki, Finland
[2] Laboratory of Analytical Chemistry, Department of Chemistry, University of Helsinki, Finland

BACKGROUND

Despite the fact that proteins are easily degraded by extreme temperatures and dehydration, pharmaceutical proteins are often lyophilized in an attempt to improve their stability. To aid in this, amorphous excipients such as trehalose (Fig. 1) are commonly used to protect proteins from dehydration induced degradation. The temperature of the frozen solution is normally held below the glass transition temperature of the maximally freeze concentrated amorphous phase (Tg') during the primary drying stage to keep its viscosity high.

According to the vitrification hypothesis, this slows down the rate of conformational changes in proteins. However, several studies have been published where no clear difference in protein structure has been observed between samples that have been lyophilized below or above their Tg'[(1-3)]. Therefore additional studies are needed to determine how the lyophilization parameters affect the stability of different pharmaceutical protein formulations.

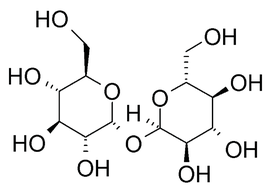

Figure 1. Structural formula of D-trehalose.

OBJECTIVES

1) To study the effect of process parameters on gamma globulin (**IgG**) *in vitro* activity and aggregation.

2) To assess the suitability of different analytical techniques in IgG analysis after lyophilization.

MATERIALS AND METHODS

Aqueous formulations prior to lyophilization:
1) 1 mg/ml IgG + 0.001 M Na- phosphate buffer + 0.015 NaCl in 1 ml H_2O
2) 1 mg/ml IgG + 5 mg/ml trehalose + 0.001 M Na-phosphate buffer + 0.015 NaCl in 1 ml H_2O

Solutions were lyophilized using Lyostar II (SP Industries Inc., USA), cycles shown in Figure 2.

Analysis:
1) IgG *in vitro* activity studied with "sandwich" enzyme-linked immunosorbent assay (**ELISA**) on 96-well plates using TECAN Genesis RSP 8/150 robotic workstation (Tecan Group Ltd., Swizerland) for pipetting

2) IgG monomers and oligomers studied with asymmetric field flow fractionation (**AsFFF**) using AF2000 (Postnova Analytics, Germany). The separation channel was connected to ultraviolet (**UV**) and multi angle light scattering (**MALS**) detectors.

3) Larger IgG aggregates studied with dynamic light scattering (**DLS**) using Zetasizer 3000HS (Malvern Instruments Ltd, UK)

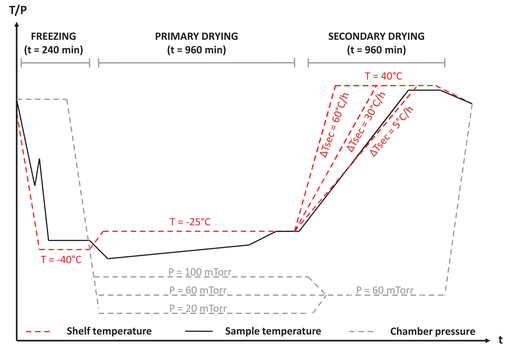

Figure 2. Primary drying chamber pressures (**Pprim**) and secondary drying shelf ramping rates (**Tsec**) used in the lyophilization experiments.

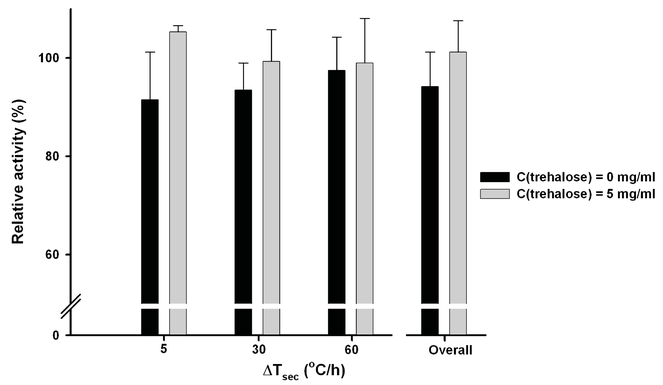

Figure 3. The effect of Tsec and trehalose on IgG activity (Pprim = 60 mTorr), measured with ELISA (n = 3).

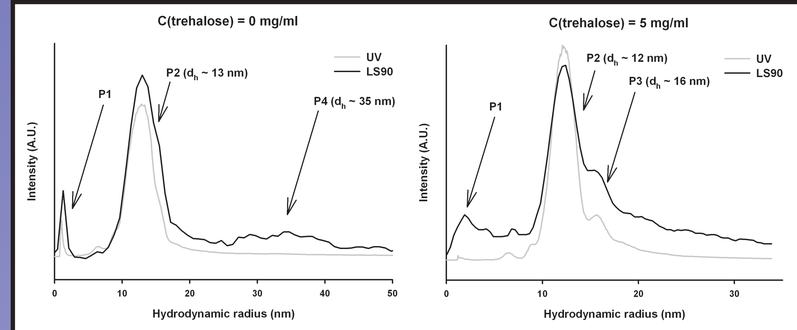

Figure 4. AsFFF graphs (UV and 90° light scattering) of samples that were dried using Tprim = 20 mTorr and Tsec = 60°C/h (n = 1).

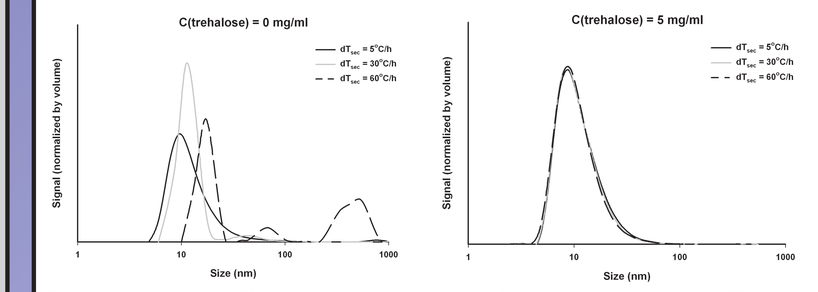

Figure 5. Average DLS graphs of samples that were dried using Tprim = 60 mTorr and Tsec = 5 - 60°C/h (n = 2).

REFERENCES
1. Sarciaux J-M, Mansour S, Hageman MJ, Nail SL. Effects of Buffer Composition and Processing Conditions on Aggregation of Bovine IgG during Freeze-Drying. J Pharm Sci, 88(12):1354-1361 (1999).
2. Chatterjee K, Shalaev EY, Suryanarayanan R. Partially crystalline systems in lyophilization: II. Withstanding collapse at high primary drying temperatures and impact on protein activity recovery. J Pharm Sci, 94(4):809-820 (2005).
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RESULTS AND DISCUSSION

ELISA analysis showed no significant differences in relative IgG activity due to lyophilization parameters (Fig. 3, only the effect of Tsec shown), but formulations containing trehalose had higher overall activities (t-test, $p < 0.05$) than those without it. However, large deviation in the activity values show that the method was not very accurate in estimating IgG degradation during lyophilization.

AsFFF analysis showed differences between IgG samples that were lyophilized with or without trehalose (Fig. 4). When C(trehalose) = 0 mg/ml, the first peak (P1) is caused by the largest aggregates eluting before the IgG monomers (P2) due to steric mode elution. As their distance from the accumulation wall is restricted by their large size, the elution time of the aggregates is short. The aggregate peak (P1) is smaller, and instead of an oligomer peak (P4), a dimer peak (P3) is visible in C(trehalose) = 5 mg/ml formulations. The results imply that trehalose decreases lyophilization-induced oligomerization and aggregation in IgG.

DLS measurements indicated that when not protected by trehalose, higher heating rate during secondary drying induced aggregation in IgG (Fig. 5). This was most likely caused by the higher residence time at T = 40°C. Trehalose protected IgG from such stress, as no differences were observed in samples that were lyophilized with different Tsec.

CONCLUSIONS

Trehalose was found to be effective in inhibiting *in vitro* activity loss and aggregation in IgG during lyophilization. Increasing Tsec was found to cause protein aggregation in those formulations that did not contain trehalose. *In vitro* activity measurements seemed less accurate than particle size analyses in assessing IgG degradation during lyophilization.

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