Critical Factors for Freeze-Drying Cycle Design When Using the Gore™ Lyoguard® Vial Isolator System

INTRODUCTION

Gore™ Lyoguard® Vial Isolators (GVI) are single-use protective caps which comprise a proprietary semi-permeable membrane and are placed on the top of a vial to ensure that product contamination, cross-contamination and blow-out is reduced to a minimum [1]. It is well known that routinely used stoppers for freeze-drying impose some resistance to water vapor flow from the product to the condenser [2]. Since the stopper resistance is very small if the stopper is placed correctly, this resistance to water vapor flow is typically neglected in freeze-drying cycle design considerations. Keeping this in mind, the goal of the present study is to investigate if a use of GVI’s impact product temperature profiles during sublimation. Residual moisture content and product appearance and/or morphology was examined right after the freeze-drying cycle. Two model formulations were processed with and without GVI. Also, a sublimation test was conducted to calculate the additional resistance to water vapor flow during primary drying to ensure the puree moisture content was not only performed at the end of secondary, but also at the end of primary drying as the maximum mass flow occurs during the sublimation phase. Here, a potential difference in residual moisture content might be more easily observable and might reveal if cycle modifications are truly inevitable.

MATERIALS & METHODS

Materials
Sucrose and Dextran (70 kD) of analytical grade were purchased from Sigma (Sigma Chemical Company, Germany). Water for injection was purchased from Braun (Braun-Melsungen, Germany). A 1% serum tubing vials (15R) were obtained from Lutz GmbH (Wertheim, Germany) and 20 mm West flute® lyophilization stoppers from West Pharmaceutical Services, Inc. (Lonvile, PA, USA). Gore™ Lyogurad® Vial Isolators were provided by Gore & Associates, Inc. (USA).

Differential Scanning Calorimetry, DSC
Determination of T_g in the model formulations was performed by using a DSC 282e, Mettler Toledo. Data acquisition for the liquid formulations was performed from -80°C to 5°C. The cooling rate was 20°C/min and the heating rate was 5°C/min.

Freeze-Drying Experimental Design
2 mL of each formulation was filled into a glass vial and then stopped with a standard stopper. Moreover, 50% of the effective vials were equipped with a GVI. Subsequently, freeze-drying experiments were performed on a laboratory scale freeze-dryer (Virtis Advantage Plus, SP Scientific, USA). Freeze-drying was performed at -40°C (shelf inlet temperature) for 60 min including equilibrating steps at +5°C and -5°C for 15 min. The temperature profile during primary drying was controlled at 100 mTorr and shelf temperature was adapted to freeze-dry close to the critical formulation temperature of the corresponding model formulations. Product temperature was determined using thermocouples placed center-bottom in a vial. For those vials containing a GVI cap a pinhole was drilled into the upper vial wall to insert the thermocouple wire. The pinhole was then sealed with a silicon rubber to assure a tight closure.

Scanning Electron Microscopy, SEM
Pieces of the lyophilized samples were fixed on Al stubs and subsequently gold-sputtered at 20 mA / 5 kV (Hummer JR Techniques). Cake morphology was then examined using Amray 1810T Scanning Electron Microscope at 20 kV with magnifications between 6x and 500x.

Karl Fischer Titration,KF
Residual moisture content were determined using a Metromeh Karl Fischer Coulometer 831 KF with titration solvent Hydranal Coulomat. Samples were weighed into a glass vial and then inserted into an oven purging the sample vessel with dry nitrogen. The samples were first heated to 120°C for a defined time period and the water vapor was accumulated in the titration solvent.

RESULTS & DISCUSSION

Sublimation Test With Pure Water
To get a first estimate of how water vapor flow is potentially impeded when using a GVI a sublimation test (primary drying: 0°C shelf inlet temperature) with pure water was performed. A distinct increase of the product temperature of about 5°C was found when using a GVI, an observation which is in line with equation 1 [2]. It was found out that applying a GVI resulted in an increase of R_p of about 0.31 cm² Torr hr g⁻¹, which, in turn, is more than twice the resistance of the individual West flute® stopper (R_s = 0.13 cm² Torr hr g⁻¹).

Evaluation of the Practical Implication of the Additional Resistance
A 50 mg/g sucrose solution in a subsequent experiment, a mixture of 25 mg/g sucrose and 25 mg/g dextran were freeze-dried close to their critical formulation temperature. DSC analysis revealed for the pure sucrose formulation a T_g of -32°C and a T_g of -25°C for the 1:1 mixture of sucrose and dextran. Here, dextran acts as a collapse temperature modifier and increases significantly the critical formulation temperature of a pure sucrose solution [4]. As a result product temperature can be elevated to generate a higher water vapor flow during primary drying without the risk of structural loss. In addition, these excipients remain amorphous and thus exhibit remarkable residual moisture contents after primary drying (cf. Table 1). Here, a possible difference when using a vial isolator should be more easily discernable.

Impact on Cake Appearance, Morphology and Residual Moisture Content
The cake appearance with a GVI was in every case less elegant compared to the freeze-dried product without a GVI. Especially the puree sucrose formulations showed a higher and more uneven degree of shrinkage. Note that sucrose is well-known for its susceptibility to shrinkage [6] (cf. Figure 3). The cake morphology could be confirmed by SEM analysis of the cake structure. The cake structure of the model was determined in every single case which was again more pronounced for the sucrose formulations (cf. Figure 4).

CONCLUSIONS

The present study clearly depicts that GVI’s significantly impede water vapor flow during the primary drying step, even at low product temperatures. It was observed that shrinkage and microporosity was more pronounced for the GVI products, specifically when the process is designed very close to the GVI inducing temperature. Furthermore, the GVI products exhibited always a higher residual moisture content. As a result, process adaption might be necessary when using GVI’s, a decision which is also based on formulation properties and primary packaging materials.

REFERENCES