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INTRODUCTION

This technical note provides an overview of freeze drying from small container systems, with a focus on the Virtis Freeze Drying System (FDS). Lyophilization from small container systems, e.g. 96 well PCR plates, is particularly attractive for highly potent API's such as peptides or cytostatic agents. Other interesting applications include high throughput screening and long time storage of minimal amounts of samples sensitive to heat and moisture. This procedure eliminates the need for deep-frozen storage in liquid state which is expensive and space-intensive. Protection from moisture requires rapid adequate sealing of the sample after completion of the freeze drying process, e.g. sealing in aluminum bags or stoppering of the container.

The FDS is a novel system for lyophilization of sample volumes between 100 and 500 µL in an array of 96 small glass vials that are enclosed in a custom-made aluminum block designed for optimal heat transfer. Each glass vial in the array can be stoppered at the end of the freeze drying process using the LyoCap™ 96 Well Capmat Lyophilization Stopper, either under vacuum or after backfilling with nitrogen or air. The FDS including the stoppers is shown in Figure 1.

Figure 1: Virtis Freeze Drying System (FDS)

MATERIALS AND METHODS

The vial heat transfer coefficient is a measure for the amount of heat transferred from the shelf through the vial to the product. Optimized heat transfer, e.g. good contact and small separation distance between vial and shelf, yields homogeneous and controlled supply of heat to the product. Heat transfer in freeze drying mainly involves three mechanisms: direct conduction at points of contact, heat conduction through the gas, and radiative heat transfer [1]. Depending on the heat transfer characteristics, shelf temperature and chamber pressure need to be optimized to obtain a suitable product temperature and process time.

A recent publication [2] describes a series of experiments aimed at determining the heat transfer for the Virtis FDS via a sequence of sublimation tests. This is a well established methodology based on filling and weighing the container system in question with pure water which is subsequently frozen. After a brief sublimation period under vacuum and heating, the product is thawed and re-weighed. For improved accuracy it is advisable to subtract the mass removal during the ramping phase which needs to be determined in separate experiments from the total mass flow. In combination with temperature measurements of ice and shelf surface, the heat transfer coefficient can be calculated using equation 1:

$$K_V = \frac{(dm/dt) \cdot \Delta H_S}{A_V \cdot (T_S - T_b)}$$

where K_v is the vial heat transfer coefficient, dm/dt is the mass removal over time (e.g. g/vial/h), ΔH_s is the heat of sublimation of ice, A_v is the vial area, and T_s-T_b is the temperature difference between shelf (i.e. heat source) and product (i.e. heat sink).

K_v values are typically measured individually over a range of chamber pressures. Fitting of these results allows delineation of the contribution of individual factors (i.e. pressure dependent vs. pressure independent) and interpolation of K_v values. This way the heat transfer to the product can be calculated over a wide pressure range, and serve as a basis for the optimization of drying conditions in development and transfer and scale-up of lyophilization processes.

RESULTS AND DISCUSSION

The container geometry is a critical factor for the heat transfer to the product. For instance, conventional 96 well plates exhibit come significant disadvantages as a container for freeze drying. Their point-shaped bottom in contact with the shelf leads to a large fraction of heat transfer via radiation and heterogeneous drying profiles. Additionally the material may be distorted due to temperature changes from -40 to 40°C. This makes it difficult to ensure uniform product temperatures throughout primary drying in all wells and necessitates the use of relatively inefficient drying conditions.

Von Graberg studied the heat transfer characteristics of several small container systems, including the Virtis FDS [2]. She found that the FDS tubes exhibit a very flat bottom, as can be seen in ink print tests (i.e. placing the tube first on an ink pad and then on a sheet of paper). The majority of the tubes showed a contact area between 60 and 72%, which is very high compared to the results for standard vials [3]. Additionally a part of the outer tube wall was found to be in contact with the aluminum block, yielding an average contact area of 22% between tube bottom and walls and the aluminum block for tubes in both edge and center positions. This fraction allows direct conduction of heat from the aluminum block to the tube. In the remaining part of the tube the heat is either transferred through the gas phase or via radiation. The heat transfer is expected to be uniform to all glass vials in the array, relatively independent of their position.

Sublimation tests with the Freeze Drying System were also performed by von Graberg [2] over a range of chamber pressures between 30 and 500 mTorr. The mass flow of numerous individual tubes was measured, and shelf temperature and product temperature were determined using thermocouples. The K_v values are shown in Figure 2. The contribution to heat transfer from pressure-independent pathways (direct conduction and radiation) was approximately 1.4 (10^4 cal s^{-1} cm^{-2} K^{-1}), as indicated by the curve intercept at 0 mTorr. At increasing pressure the heat transfer increases rapidly through the contribution of gas conduction. These results are comparable to data measured for 20 mL serum tubing vials [4], and allow calculation of the energy introduced.

At chamber pressures above 50 mTorr, the majority of heat is transferred through gas conduction. This observation is in good agreement with the small separation distance of only 0.02 cm calculated for these measurements. The separation distance is an indication of the average distance a gas molecule travels between the heat source (i.e. aluminum block) and the glass tube.

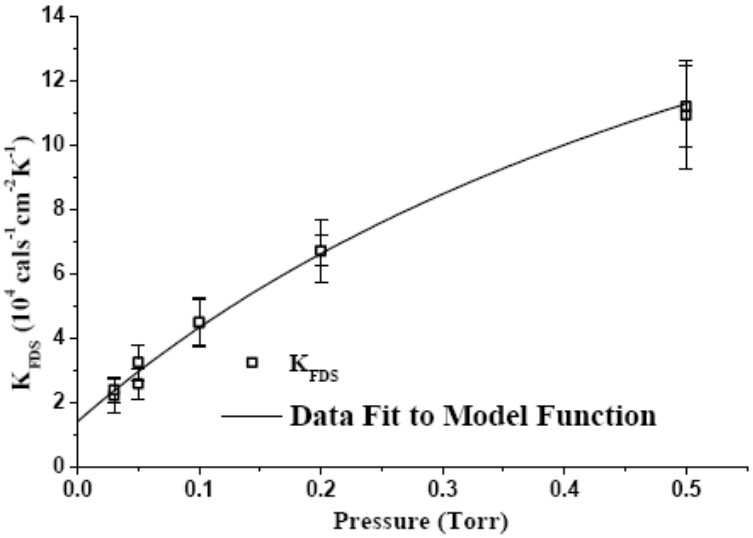

Figure 2: K_v values determined for the Virtis FDS (from 2)

As a reference, Pikal [3] reported separation distances of 0.04 – 0.05 cm for several serum tubing vials, and results up to 0.22 cm for molded vials. As the FDS tubes fit very closely into the aluminum block, gas molecules can transfer heat very efficiently from the aluminum block to the glass wall and the product inside.

SUMMARY AND CONCLUSION

Freeze drying from small container systems is an important new field (e.g., for highly potent drugs and small amounts of new rare substances). While well plates are still the predominantly used container system in these applications, the Virtis FDS offers several advantages, such as improved heat transfer to the tubes through the precise-cut aluminum block, and the possibility to stopper the glass tubes under vacuum. This facilitates the design of efficient and well-controlled processes.

REFERENCES:

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