AAPS Open

RESEARCH

Open Access

Technical transfer and commercialisation of lyophilised biopharmaceuticals — application of lyophiliser characterisation and comparability

Sean Cullen[1*†], Emma Walsh[2], Valeria Gervasi[1†], Dikshitkumar Khamar[2] and Timothy R. McCoy[1†]

Abstract

A holistic approach was taken to characterise lyophilisers at both laboratory and commercial scale to design a focused validation strategy for commercialising parenteral drug products. Vial heat transfer coefficients (K_v) and equipment mass transfer boundaries were generated for a Lyostar II and three commercial scale IMA Lyomax lyophilisers. K_v studies were performed using gravimetric methodologies. K_v calculated for the Lyostar II was equivalent to the commercial equipment at 133 µBar however trended higher below 133 µBar and lower above 133 µBar potentially impacting primary drying product temperature during scale-up depending on the chamber pressure recipe set point. K_v profiles were consistent within and across the commercial equipment. Edge effect was most prominent at commercial scale with minimal shielding of the edge vials in contrast to the presence of a metal ring around the vial pack in the Lyostar II. Equipment capability studies for mass transfer showed commercial scale equipment could achieve lower chamber pressure and greater sublimation rates when compared to the Lyostar II. Furthermore, differences were also measured between large-scale lyophilisers based on condenser orientation (horizontal vs vertical). The results demonstrate greater equipment capability of the two-storey vertical configuration with respect to choked flow regime. Worst-case locations within a commercial lyophiliser were identified providing rationale for reduced sampling for product shelf-mapping locations. This work provides guidance on execution of commercial scale characterisation studies and application of the data to enhance scale-up, technical transfer and focused process validation strategies.

Keywords: Freeze-drying, Lyophilisation, Heat and mass transfer, Quality by design, Technical transfer

Introduction

Lyophilisation is a key manufacturing step for the manufacturing of stable biologic parenteral drug products. Lyophilisation, also referred to as freeze-drying, involves the freezing and drying of a liquid formulation converting it to a solid form (Patel 2011). It is employed in the biopharmaceutical industry to induce long-term stability of biologics such as proteins (Carpenter et al. 2002). Products are lyophilised in a number of different container systems including vials, syringes and ampoules (Patel 2011). The process involves exposing a liquid formulation to a range of pressures and temperatures during three stages,freezing, primary drying and secondary drying (Tang 2004). A review by Gervasi et al. showed that of 400 parenteral protein products approved by the EMA (within the European Union) between 1995 and 2018, 34% (90 in total) were presented in a lyophilised format (Gervasi et al. 2018).

Technical transfer of lyophilised drug products comes at great expense. Associated engineering and validation

†Sean Cullen, Valeria Gervasi and Timothy R. McCoy were employees of Sanofi, IDA Industrial Estate at the time work was performed

*Correspondence: sean.cullen5@mail.dcu.ie

[1] Sanofi, IDA Industrial Estate, Waterford, Ireland
Full list of author information is available at the end of the article

activities can take significant line time and consume large quantities of representative drug product formulation material; however, the extent of these activities may be simplified by a robust product and process characterisation data package. A single commercial scale filling slot including production materials for 30 K vials for a lyophilised drug product cost approximately $500 K. An assessment of product value has reported the average cost for recombinant protein manufacture to be US $307 per gram (with a range from US $50 to > US $1000) (BioPlan Associates I 2017). Considering a scenario where a 100 mg/vial product is being transferred, a lyophilisation engineering run executed at maximum capacity could cost $1.5 million ($500 K for manufacturing and US $1 million for product material). For further context, a site-to-site transfer of a biopharmaceutical including personnel resources and over-head spend would cost US $5 + million. Extensive understanding of the lyophilisation equipment can enable reduced engineering, validation and sampling requirements (Jennings 2002). Formulation and process development using the quality-by-design (QbD) approach is crucial to provide the key characteristics of laboratory and commercial equipment to support an efficient technical transfer.

To characterise a lyophiliser, it is important to have a good understanding of the equipment design and functionality. The basic components of a lyophiliser include a product chamber, condenser, refrigeration system and vacuum system. Shelf temperature (T_s) is controlled to transfer heat to and from the product vials. Chamber pressure (P_c) is controlled by a vacuum pump located downstream of the condenser and a modulated nitrogen feed valve in the product chamber. At the end of processing, the chamber is backfilled with an inert gas such as nitrogen to a partial vacuum, and the shelves facilitate automatic stoppering by compressing (Patel 2011; Tang 2004). All lyophilisers have generally the same basic components,however, differences in equipment and configurations can result in differences in heat and mass transfer characteristics. Examples of such differences include shelf design, condenser location, condenser chute dimensions, refrigeration capacity and product chamber dimensions (shelf surface area and number of shelves). Various methods for generating heat and equipment mass transfer characteristics as part of equipment characterisation have been outlined in the literature (Wegiel et al. 2018; Rambhatla et al. 2006; Searles JAJF-dlop, products b 2016; Patel et al. 2010). These include vial heat transfer coefficient (K_v), product dry layer resistance (R_p) and lyophiliser mass transfer limitation (Jameel et al. 2001).

Heat transfer coefficient (K_v) — background

Figure 1 illustrates the role of heat transfer for a vial during lyophilisation. K_v is defined as the ratio of the area normalised heat flow to the temperature difference between heat source and heat sink (Patel et al. 2010). The value of K_v is the sum of three components (Pikal

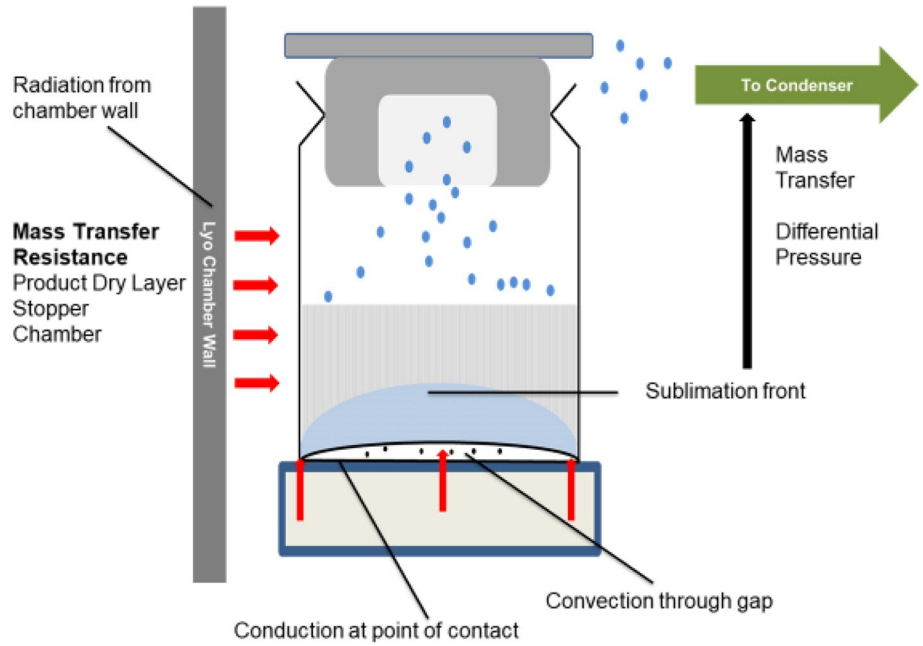


Fig. 1 Heat and mass transfer in a vial during freeze-drying

et al. 1984): (1) conduction from contact between the shelf and the vial base, (2) convection via gas molecules in the chamber and small gap between the shelf and the concave section of the vial base and (3) radiation from the chamber walls and shelves. It can be defined by Eq. 1 (Wegiel et al. 2018).

$$Kv = \frac{\Delta Hsdm/dt}{A_v(T_s - T_p)} \quad (1)$$

where dm/dt is the mass flow rate of water vapour and ΔHs is the specific heat of sublimation of ice, A_v is the area of the vial, T_s is the shelf temperature and T_p is the product temperature within the vial.

The relationship between K_v and P_c has been reported as non-linear (Kawasaki et al. 2019; Hibler et al. 2012). Determination of K_v is required for accurate mathematical modelling of primary drying (Pikal 1985). K_v is vial and lyophiliser specific, i.e. K_v of a specific vial for a laboratory equipment may be different at commercial scale. Differences in K_v result in different T_p profiles for the same process parameters used in different equipment,thus, the determination of K_v in different lyophilisers is critical for successful product technical transfers.

R_p is another factor used to characterise a product and process. R_p is a product characteristic and is defined by its impact to sublimation by resisting the flow of water vapour flow from the freeze-drying front through the pores of the dry layer forming above the diminishing frozen plug (Zhou et al. 2019).

Examples of K_v and R_p measurement and application have been provided previously using various methods. Tchessalov et al. presented a protocol for measuring K_v using the gravimetric approach, as well as providing industrial case studies demonstrating how the data was applied (Tchessalov et al. 2021). At Biogen, R_p calculated at laboratory scale coupled with K_v measured at commercial scale were successfully applied during a technical engineering batch to confirm a primary drying prediction model. Janssen performed K_v measurements at pilot and commercial scale and found statistically comparable results providing rationale for scalability. K_v and R_p coefficients were measured using manometric temperature measurements (MTM) and verified experimentally at BMS to create a design space (Tchessalov et al. 2021). Pisano et al. showed the application of mathematical modelling to scale-up from a laboratory scale Telstar LyoBeta 25 to a pilot scale GEA Lyovac FCM 40-D and an industrial scale GEA Lyovac FCM 500-D. They concluded that a change in recipe was required due to a higher edge effect at laboratory scale vs pilot scale where the centre vials once again showed comparable K_v (Pisano et al. 2013).

Mass transfer characterisation — background

Commercial operations generally require lyophilisation cycle duration to be minimised to enable optimal equipment capacity. Depending on equipment differences, during technical transfer, the receiving unit equipment may not be capable of maintaining the required sublimation rates for optimal primary drying which is typically the longest phase of the lyophilisation process (Tang 2004). Exceeding maximum sublimation rates results in a loss of P_c control due to choked flow at the exit condenser chute. Choke occurs when the water vapour reaches Mach I or the speed of sound at the condenser chute exits (Patel et al. 2010). Choke flow has further been described where the P_c control is lost due to exceeding the limitation of the lyophiliser equipment to transfer the required mass of water vapour through the condenser duct (Wegiel et al. 2018). Patel et al. discussed the origins of choke flow demonstrating the limitation itself is a function of the gas flow as opposed to mass flow (Patel et al. 2010). The resulting increase in P_c will drive an increase in T_p risking product collapse (Gervasi et al. 2019) or can potentially trigger an equipment pressure alarm placing the product into a safe mode which often means the shelves automatically revert to the freezing temperature set point (Tang 2004).

The maximum sublimation rate (choke point) is determined across a range of P_c where the relationship is linear and represents the equipment mass transfer design space boundary. Design and performance attributes that impact the equipment mass transfer limitation include the geometry of the condenser chute, condenser and refrigeration system capacities and the heating capacity for the shelf fluid (Searles JAJF-dlop, products b 2016). Traditionally, equipment mass transfer limitation is measured using ice slabs (Patel et al. 2010).

Examples of equipment mass transfer limitation studies have been provided previously using various methods. At Pfizer, mass transfer characteristics measured for a 42 m^2 industrial scale lyophiliser were calculated assuming highest sublimation rate during the beginning of primary drying (Tchessalov et al. 2021). Kuu et al. compared a Lyostar with an industrial scale 20 m^2 BOC Edwards. The data shows that the Lyostar provides a "worst-case" scenario having a sublimation rate 91% that of the industrial lyophiliser. They also concluded that heat transfer rates for both lyophilisers were approximately equal at centre location vials (Kuu et al. 2005). Kshirsagar et al. used computational fluid dynamics modelling of equipment mass transfer characteristics and compared the data generated with experimental data for a vertical configuration

23 m^2 lyophiliser. Using experimental data collected on vertical configuration — 2 configurations were simulated by CFD (vertical and horizontal). They concluded that the vertical condenser configuration showed greater equipment limitation (Kshirsagar et al. 2019). Rambhatla et al. reported operational qualification (OQ) sublimation tests that were performed on two laboratory lyophilisers, Durastop and Lyostar I, a pilot scale Edwards Lyofast S20 and an industrial Lyomax (BOC Edwards Inc). They concluded that the minimum controllable pressure varies from one lyophiliser to another,in this case, the minimum chamber pressure rises very steeply in the case of the pilot and the laboratory lyophiliser when compared with the industrial equipment (Rambhatla et al. 2006).

Combining lyophiliser equipment limitation data, K_v and product dry layer resistance R_p provide the three key inputs to generate a process design space that can be utilised to predict the primary drying behaviour for a specific product at laboratory or commercial scale manufacturing and during technical transfer. In addition to previous literature, this paper provides a practical approach to executing commercial scale studies and how to apply the data during technical transfer. The aim of this study was to measure K_v and equipment limitation data for one laboratory (Lyostar II) and three commercial scale (IMA Lyomax 28/29) lyophilisers at the same manufacturing facility using traditional gravimetric approaches. This is the first time that dedicated studies have been performed for both K_v and mass transfer limitation on a suite of equipment at a commercial facility. This data has been compiled to create a comprehensive package to simplify technical transfer and support manufacturing operational activities while establishing practical advice for industry.

Materials and methods

Materials

Specification for laboratory scale lyophiliser with stainless steel door (*Lyostar II, SP Scientific, Stone Ridge, NY*) and commercial scale (*Lyomax 28 or 29, IMA Life North America,* Tonawanda, NY) lyophilisers used in this study are outlined in Table 1. For K_v studies, 20 mL tubular vials (*Schott AG Pharmaceutical Systems, Muellheim-Hugelheim, Germany*) and 20 mm LyoTec Envision Stoppers B2 coating (*West Pharmaceuticals, Jersey Shore, PA, 17,740, USA*) were used. For vial filling, a peristaltic pump (520di, *Watson Marlow Fluid Technology Group, Ireland*) was used to deliver ultrapure water (*type 1, Milli-Q*) under laminar air flow. Temperature measurements during lyophilisation were monitored using resistance temperature detectors (RTDs) (*Ellab Tracksense Data loggers, Hillerød, Denmark)* secured in bespoke shuttles placed in the vial pack.

For the mass transfer limitation studies, commercial grade purified water was pumped into trays lined with 100-µm-thick food grade plastic sheeting and secured in place using plastic clips. Bespoke bottomless polyethylene frames were fabricated for the commercial studies, and stainless-steel vial pack rings were used for the Lyostar II. The total internal area within the bespoke trays designed specifically for the Lyomax 29 shelf stack was 26 m^2. The total internal area of the rings for the Lyostar II was 0.47 m^2.

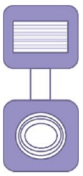
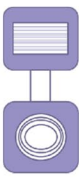
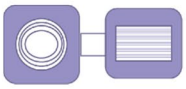
For commercial scale experiments, a weighing pallet truck (*Schneider, model robusto VL1000*) and a 1000 L intermediate bulk container were used to determine the water mass added to the tray prior to each experiment. On the contrary, for laboratory experiments, the mass of water was measured using an analytical balance (*Top pan balance, XP802S, Mettler-Toledo Ltd., Leicester, UK*). Kapton and metallic tapes were used to secure the RTD probes in position to monitor the shelf and ice temperatures.

Measurement of the vial heat transfer coefficient (K_v)

A gravimetric method was used to establish the K_v for 20 mL vials in both laboratory and commercial scale equipment. The methodology is based on guidance provided by Tchessalov et al. (2021) outlined as follows:

1) The 20 mL vials were filled with 10 mL of purified water using a peristaltic pump under a Laminar Air Flow unit and partially stoppered.
2) Each vial was then labelled using a marker to identify its lyophiliser, vial location and cycle number for traceability.
3) The labelled vials were preweighed. For the Lyostar II, one of the three shelves (middle shelf) was fully loaded with preweighed filled vials (Fig. 2A). Vial packs were arranged in a metal ring placed on the shelves. For commercial scale equipment, three shelves were fully loaded with filled vials — the top shelf (shelf 1), a middle shelf (shelf 4: horizontal configuration, shelf 7: vertical configuration) and the bottom shelf (shelf 13). Preweighed vials were then placed in the centre and edge locations as per the vial pack layout outlined in Fig. 2B.
4) Ellab RTDs were placed in contact with the bottom of the vial in order to measure ice temperature during the lyophilisation cycle. Ice temperature data from probed vials at the centre location, highlighted in red in Fig. 2, were used for K_v calculations. Ice temperature data from vials highlighted in orange in Fig. 2 at the edge locations was not used to calculate K_v but gathered for additional information.

Table 1 Laboratory and commercial scale — equipment specifications

Lyophilizer	Model	Number of Shelves	Total Shelf Surface Area (m^2)	Shelf Inter Distance (mm)	Shelf Capacity for 20 mL Vials	Condenser		Condenser Configuration
Commercial Scale Lyophilizer 01	IMA Life Lyomax 28	13	28	100	2185	Capacity Kg	767	Two storey vertical 
						Duct Length, m	2.4	
						Duct Diameter, m	0.7	
Commercial Scale Lyophilizer 02	IMA Life Lyomax 29	13	29	110	2332	Capacity, Kg	767	Two storey vertical 
						Duct Length, m	2.4	
						Duct Diameter, m	0.7	
Commercial Scale Lyophilizer 03	IMA Life Lyomax 29	13	29	110	2322	Capacity Kg	767	One storey horizontal 
						Duct Length m	1.7	
						Duct Diameter m	0.7	
Laboratory Scale Lyophilizer	SP Scientific Lyostar II	3	0.47	100	161	Capacity Kg	30	Vertical 
						Duct Length m	0.35	
						Duct Diameter m	0.10	

5) Data collected from the shelf surface inlet was used for the calculation of K_v. Shelf temperature data was gathered using three different methods. Two Ellab RTDs were attached to each shelf surface at the inlet and outlet. Probes were secured to the shelf inlet and outlet using Kapton and metallic tape. Three layers of Kapton tape were applied over the probes followed by three layers of metallic tape. This was performed to secure the probe to the shelf surface while also providing insulation and shielding from radiative heat input.
   For information and comparative purposes, T_s was also recorded from the shelf oil inlet as per the standard lyophiliser cycle data collection. T_s was also gathered from within the vial pack via RTDs inserted into brass pucks located in the under carriage of the Ellab shuttles resting in contact with the shelf surface.
6) K_v was determined at three different pressure set points (SP) with an altered sublimation duration to target a sublimation weight loss of 20–30%. This weight loss was targeted to avoid loss of contact between the ice and glass which may impact calculations due to the pressure dependent gaps between glass and ice (Tchessalov 2016). The K_v lyophilisation cycle recipe details are outlined in Table 2. The specific chamber pressure set point and sublimation duration for each freeze-drying cycle are outlined in

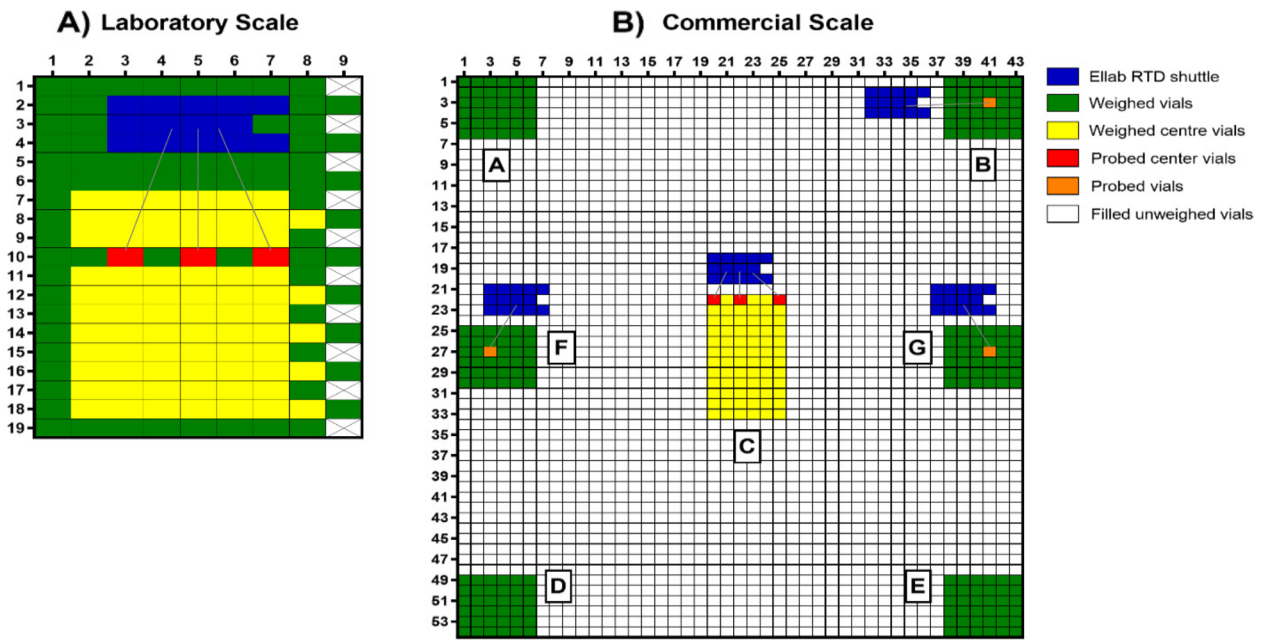


Fig. 2 Vial pack layout. **A** Laboratory scale lyophiliser and **B** commercial scale lyophiliser

Table 2 Freeze-drying recipe used for Kv determination at different pressure set points (SP)

Step	Shelf temp (°C)	Chamber pressure (µbar)	Step time (HH:MM)
1. Loading	5	N/A	N/A
2. Freezing ramp	−40	N/A	2:00
3. Freezing hold	−40	N/A	4:00
4. Evacuation	−40	SP1-SP3 (Table 3)	N/A
5. Sublimation ramp	0	SP1-SP3 (Table 3)	1:10
6. Sublimation hold	0	SP1-SP3 (Table 3)	SP1-SP3 (Table 3)

Table 3 Chamber pressure set points and sublimation duration for Kv cycles conducted in each freeze-dryer

Set point	Lyophiliser	Pressure, µBar	Sublimation time, h
SP1[a]	01, 02, Lyostar II	67 (50 mTorr)	4.5
SP2	01, 02, 03, Lyostar II	133 (100 mTorr)	4.0
SP3	01, 02, Lyostar II	200 (150 mTorr)	3.5

[a] SP1 for Lyo 02 was performed at a pressure of 46 µBar (35 mTorr)

Table 3. For lyophiliser 03, K_v was measured at SP2 only due to limited equipment availability.

7) Upon completion of the K_v lyophilisation cycle for each set point, the chamber was backfilled with nitrogen gas and automatically stoppered under a partial vacuum of 0.8 Bar. Following unloading, vials were opened to release the partial vacuum, and post-weighing was completed. For commercial equipment, an average weight loss was calculated for centre vials (yellow area in Fig. 2B). Distribution of K_v in the different locations was assessed by applying a normalisation factor based on weight loss of vials divided by the mean weight loss for centre vials. For the Lyostar II, an average weight loss was taken from vials located rank 2 or more from the edge and from the Ellab shuttle. This accounted for an average weight loss from 82 vials in total. Normalised weight loss was then applied to all vials to demonstrate the distribution of K_v across the Lyostar II shelf.

K_v was calculated as per Eq. 1 (Tchessalov et al. 2021).

$$Kv = \frac{2\Delta H_s (m_{ice})_{vial}}{S_{out} \sum_{i=1}^{n} (\Delta T_i + \Delta T_{i-1})(t_i - t_{i-1})} \quad (2)$$

where K_v is the vial heat transfer coefficient, (cal/s.cm^2.°C), ΔHs is the specific heat of sublimation (676 cal/g) for water, $(m_{ice})_{vial}$ is the total amount of ice in a individual vial (g), S_{out} is the external surface of vial, (cm^2), T is temperature (Kelvin) and t is time (seconds). K_v values were then converted to W/$m^2 \times$ °C.

8) For laboratory scale: one K_v was calculated using the T_p for each probed vial in the centre location using the shelf inlet measurement provided by the lyophiliser data. In total, three K_v values were obtained for the centre location of the middle shelf at each pressure set point.
   At commercial scale: Six K_v values were obtained for the centre location of each shelf at each pressure set-point: two K_v values were calculated using the T_p for each of the three probed vials in the centre location using data from the two T_s surface inlet Ellab RTDs. An overall K_v value for the lyophiliser was generated by averaging the results from each shelf, i.e. $n = 18$ where three shelves were assessed.
9) Non-linear regression analysis was performed using Sigma Plot®. K_v values generated experimentally at various P_c set points were plotted and non-linear regression curves were established. Non-linear regression provided a value of a, b, c coefficients that represent the best fit, refer to Eq. 3. An R^2 value of > 0.95 was selected as the acceptance criterion

$$K_v = a + \frac{b \times Pc}{(1 + c \times Pc)} \tag{3}$$

Method — equipment limitation studies

The equipment limitation study was conducted using the ice slab approach which consists of two parts (Searles JAJF-dlop, products b 2016). Part 1 involved determination of the minimum controllable pressure (MCP) for a set range of T_s. Data generated in part 1 of the study was used to assess the equipment choke flow regime and generate the equipment limitation boundary of maximum sublimation rate vs P_c. The method and parameters used were based on previous choke flow studies performed by Patel et al. (2010). The approach uses a stepwise elevation of T_s where P_c is set to the lowest value available on the equipment.

Part 2 of the study was performed to verify the sublimation rate data generated from the MCP assessment in part 1 by performing direct gravimetric measurements at specific T_s and P_c. The methodology is outlined as follows.

Part 1: Minimal controllable pressure

The methodology is outlined as follows:

1) Bottomless frames were lined with thin plastic secured in place around the outer frame using plastic clips (Fig. 3D). Ellab RTDs were used to monitor T_s, and the probe was placed 20 cm in from the front centre of the shelf and secured using thermal Kapton tape and metallic tape, to minimise the radiation effect (Fig. 3B).
2) The frame lined with plastic and was then positioned on the lyophiliser shelf. A second Ellab RTD was placed above the plastic, positioned 0.5 cm above the plastic to collect ice temperature data, as presented in Fig. 3 B and C. RTD positioning was conducted in duplicate for all shelves.
3) Purified water was pumped into the trays to a height of 2 cm using a peristaltic pump (weight of water approximately 40 kg at commercial scale and approximately 2 kg at laboratory scale). All shelves were loaded with trays filled with water (13 shelves for commercial scale and 3 shelves for laboratory scale) (Fig. 3 A and E).
4) The study parameters for part 1 were based on the work of Patel et al. are outlined in Table 4 (Patel et al. 2010).
5) Data generated in part 1 was used to calculate the sublimation rate to identify equipment boundaries. The sublimation rate is calculated using Eq. 4 (Rambhatla et al. 2006).

$$\frac{dm}{dt} = K_{bag} B (Ts - Tp) \tag{4}$$

where $\frac{dm}{dt}$ is the sublimation rate in Kg/hr/m^2, B is the latent heat of sublimation (53.25), T_s is the measured shelf surface temperature, T_p is the measured temperature of ice during sublimation. K_{bag} is the heat transfer coefficient of the plastic liner which was calculated using Eq. 5 provided by Rambhatla et al. (2006).

$$K_{bag} = 0.7 + 33.2Pc/1 + 2.88P_c \tag{5}$$

Part 2: Gravimetric verification cycles

Two direct measurements of sublimation rate were executed at T_s of − 20 °C and 20 °C utilising the correlating MCP measured in part 1. The methodology is outlined as follows:

1) Preparation of lined frames and addition of water were conducted as per part 1 ("Part 1: Minimal controllable pressure", steps 1–3). Additionally, for part 2, the mass of water added to each tray was recorded as

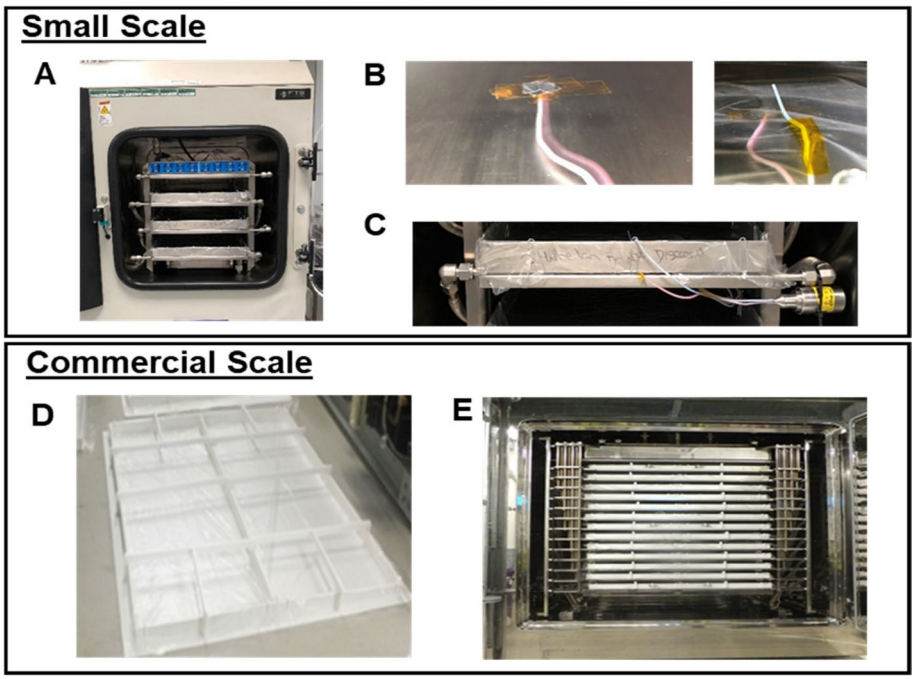


Fig. 3 **A**, **B**, and **C** Laboratory scale tray set-up. **A** Three shelves loaded with lined trays containing water. **B** Temperature probing on shelf surface and in the tray prior to water addition. **C** Tray loaded with water and temperature probes. **D** and **E** Commercial scale tray set-up. **D** Frame lined with plastic. **E** Thirteen shelves loaded with lined trays containing water

Table 4 Freeze-drying cycles used for MCP experiments

Phase	Shelf temp (°C)	Vacuum pressure set point (μBar)	Step hold time (HH:MM)
Freezing	−50	N/A	04:00
Drying	−50	01	01:00–02:00
Drying	−40	01	01:00–02:00
Drying	−20	01	01:00–02:00
Drying	0	01	01:00–02:00
Drying	10	01	01:00–02:00
Drying	20	01	01:00–02:00
Drying	30	01	01:00–02:00
Drying	40	01	01:00–02:00

pre-weight, using an analytical balance for laboratory scale study and a weighing pallet truck for commercial scale study.

2) The sublimation duration was altered to target a sublimation weight loss of 20% to ensure that the surface area was maintained. As sublimation progresses the edges of the ice slab sublime faster causing shrinkage and loss of total area. The study parameters used are outlined in Table 5.
3) Upon completion of the recipe, the shelves were set to − 20 °C for unloading, the remaining ice was removed from the trays and the post-weight mass was recorded. The total loss of water was then used to calculate the sublimation rate in Kg/hr/m^2 as per Eq. 4.

Design space generation

The Design Space was created as per guidance provided by Mockus et al. (2011) and Jameel et al. (2001). Calculations were completed using SP scientific Lyo calculator (Scientific 2016) with the following criteria:

- Vial diameter of 3 cm
- 10 mL fill volume
- 7.5% solid content
- R_p "General Material of Low Resistance, Resistance increases non-linearly with depth dried" provided by the SP Scientific material data base ($R=1$, $A_1=4$ and

Table 5 Freeze-drying recipe for part 2, gravimetric verification runs for lyophilisers (Lyo) 02 and 03

Phase	Shelf Temp (°C)	Vacuum pressure set point (μBar)		Step hold time (HH:MM)
Freezing	−50	N/A		2:00
Drying — verification cycle 1	−20	Lyostar II	63	10:00
		Lyo 02	28	
		Lyo 03	31	
Drying — verification cycle 2	20	Lyostar II	182	06:00
		Lyo 02	78	
		Lyo 03	104	

$A_2 = 1$) (Scientific 2016). Product critical temperature (collapse) $T_c = -25$ °C.

Deviations

There were two deviations to the original plan to note during execution of the commercial scale studies:

1) SP1 for lyophiliser 02 was conducted at a P_c of 49 μBar due to an inadvertent cut-off of the nitrogen gas supply inhibiting the lyophiliser control system to counter the overshoot of the 67 μBar set point upon initial evacuation. There is no impact to the study as the K_v is expressed as a curve.
2) Due to an issue during loading of SP1 on lyophiliser 01, data from shelves 7 and 13 could not be acquired. Thus, the K_v data in this case accounts for shelf 1 only. There is minimal impact to the overall study conclusion as two other cycles (SP2 and SP3) were conducted successfully for all three shelves to provide appropriate characterisation of the lyophiliser chamber. Calculated K_v data also meets the expected curve fitting rationale in line with data points for SP2 and SP3.

Results and discussion

The following sections presents the acquired K_v and equipment limitation data across the three commercial lyophilisers and the Lyostar II.

Figure 4A provides the overall mean K_v results for the centre location population of vials commercial scale data. It is the average of the three shelves assessed. K_v increases non-linearly with increased P_c. The non-linear relationship has been described previously, and the behaviour observed in the study is consistent with previous published data T (Kawasaki et al. 2019; Hibler et al. 2012). K_v was measured at SP2 for lyophiliser 03 only. The data

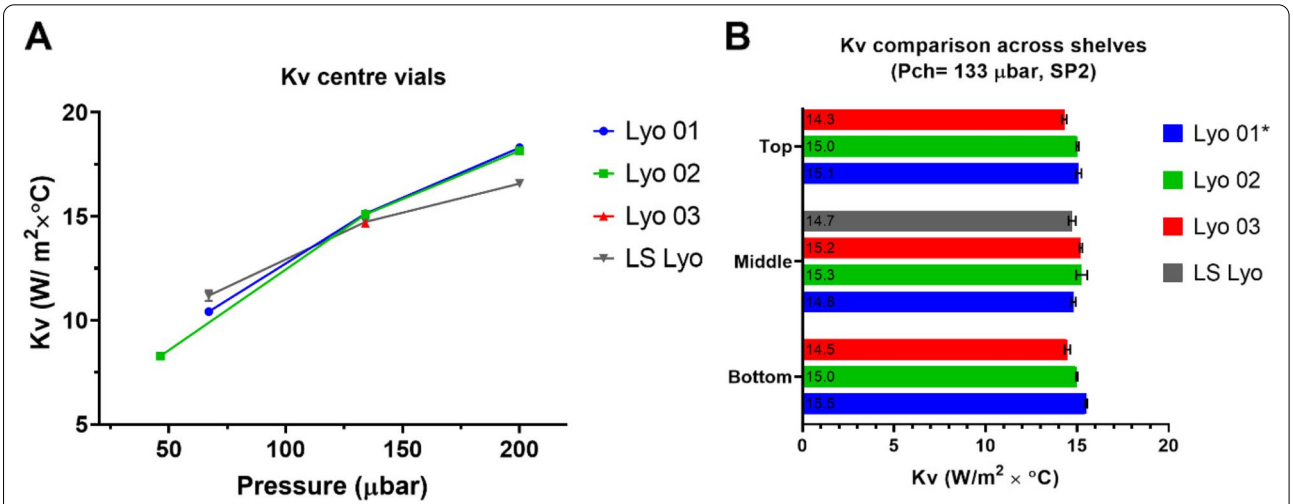


Fig. 4 K_v comparability data for commercial lyophilisers (Lyo) 01, 02, and 03 and laboratory scale (LS) lyophiliser. $N = 18$ data points for commercial scale lyophiliser (with the exception of SP1 Lyo 01), $N = 6$ data points for laboratory scale lyophiliser. **A** Centre vials K_v. **B** Comparison of centre vials K_v across shelves for SP2 (133 μBar)

shows an equivalent K_v profile for lyophilisers 01 and 02. The Lyostar II data demonstrates equivalency at 133 µBar (SP2), however exhibits a higher K_v below 133 µBar and lower K_v above 133 µBar when compared with the commercial units. Therefore, at the specific P_c set point of 133 µBar (100 mT), K_v measured was deemed to be equivalent across all our lyophilisers evaluated. Differences in profile curves are likely a function of lyophiliser design and the relevant equipment specific dependency on conductive and radiative heat input as convection changes with change in P_c. A similar trend has been shared previously by Tchessalov who compared K_v profiles as a function of P_c across multiple lyophilisers (Tchessalov 2016).

Figure 4B shows the K_v data by shelf for SP2. The graph illustrates not only the equivalence between the four lyophilisers but also the consistency within each individual lyophiliser for K_v from the top, middle and bottom shelves.

As outlined earlier in the manuscript and captured by Eq. 1, K_v is directly proportional to T_p. Once K_v is established, coupled with other inputs such as R_p and lyophiliser recipe set points, the T_p profile can be predicted. Based on the K_v values at 133 µBar, with an equivalent R_p, it would be expected that the resulting T_p would be equivalent for a given formulation across all four lyophilisers for main centre vial area. Where the primary drying P_c set point is set above or below 133 µBar, then T_s or P_c may need to be adjusted at commercial scale to generate an equivalent T_p profile in a given lyophiliser depending on K_v.

Table 6 provides the a, b and c coefficients as per the non-linear regression fitting formula provided by Eq. 3 for lyophilisers 01, 02 and the Lyostar II. Results are not displayed for lyophiliser 03 as only SP2 was conducted on this equipment. These coefficients can be utilised in primary drying prediction tools such as the one provided by SP scientific (Scientific 2016) or any other customised heat-mass transfer model.

Table 6 Coefficients for non-linear regression for 20 mL Schott vial lyophilisers 01 and 02 and Lyostar II

	Lyo 01	Lyo 02	Lyostar II
R^2	1	1	1
a	2.8066	2.1427	1.8591
b	0.1484	0.1668	0.2531
c	0.0046	0.0054	0.0122

Shelf temperature input for K_v calculation

Supplementary T_s data from the shelf fluid inlet, outlet and the shelf surface at the outlet and data collected from RTDs embedded in brass pucks within the Ellab shuttle located in the vial pack sitting in contact with the shelf surface is shown on Fig. 5. Figure 5A captures T_s data from lyophiliser 03, SP2. As described in the "Materials and methods" section, T_s surface at the inlet measured by RTD was used to calculate K_v. T_s surface at the inlet was chosen as it accounts for not only the shelf fluid but also the shelf stainless steel construction.

For the K_v recipes provided in Table 5, the T_s set point was 0 °C. The T_s surface measured at the outlet was typically around 1 °C lower than at the inlet. This may be explained as due to heat energy flowing from the thermal fluid to the product to facilitate sublimation resulting in a colder outlet temperature. This was not so evident in the shelf fluid inlet and outlet data in this case as these readings were recorded from the main thermal fluid path manifold, and only 3 of the 13 shelves contained product.

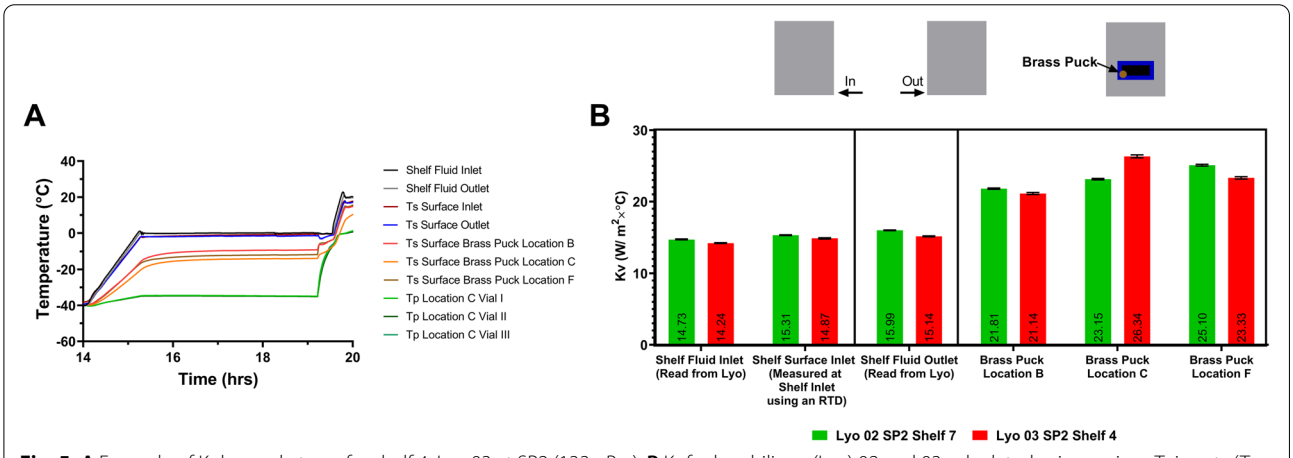


Fig. 5 **A** Example of K_v lyo cycle trace for shelf 4, Lyo 03 at SP2 (133 µBar). **B** K_v for lyophilisers (Lyo) 02 and 03 calculated using various T_s inputs (T_s inlet, T_s inlet surface, T_s outlet and T_s surface measured using the brass puck)

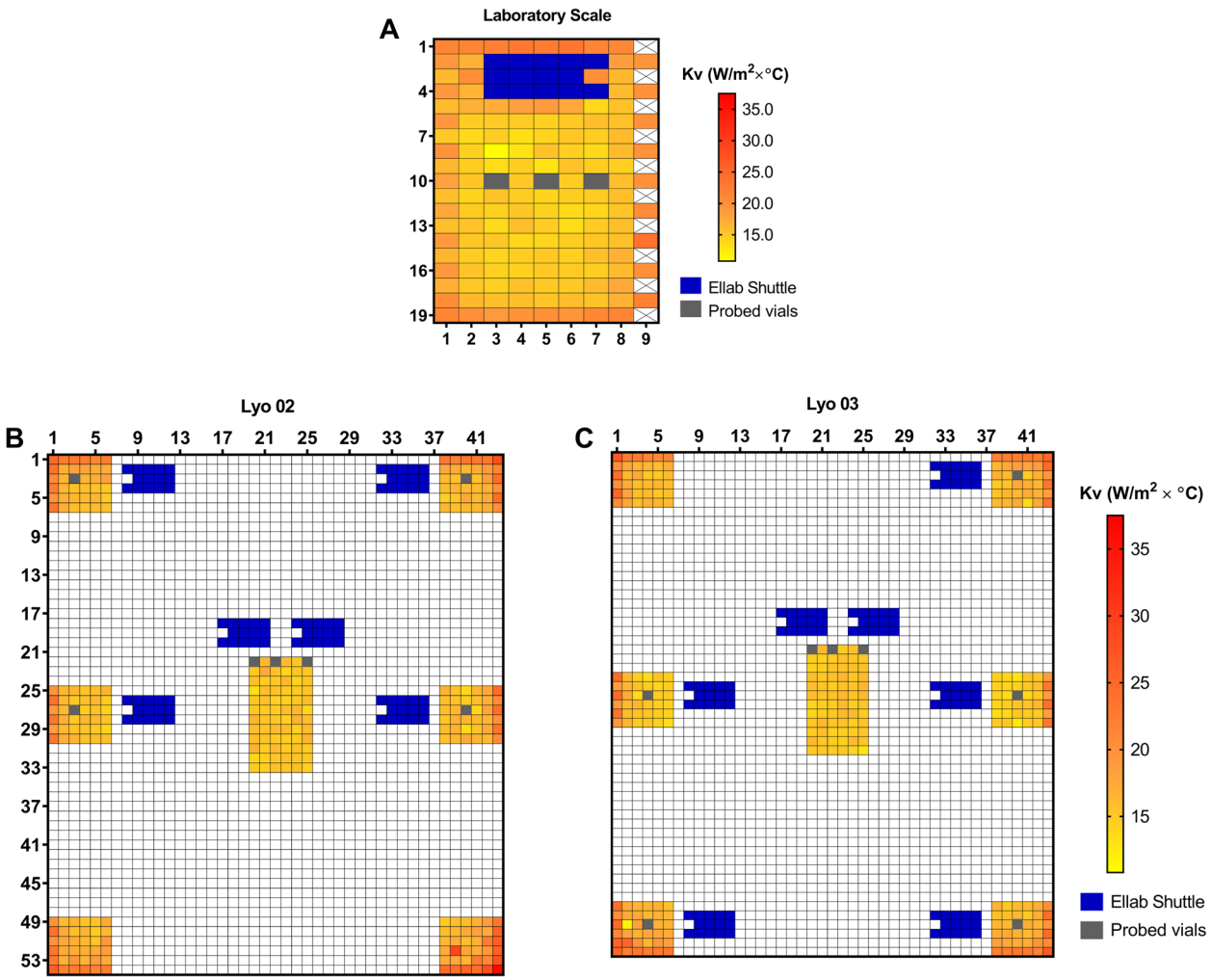


Fig. 6 Example of the K_v distribution outlined as heat map for commercial scale lyophiliser 02, shelf 07, lyophiliser 03 shelf 04 and the laboratory scale lyophiliser, middle shelf at SP2 (133 µBar)

T_s surface data collected from the brass pucks within the vial pack was 10 to 15 °C lower than the T_s set point. This may be attributed to the sublimation cooling impact of the vial pack impacting the shelf surface. Also, with this apparatus, the puck temperature is likely an average of the shelf temperature the bottom surface is resting on and the vapour temperature impacting the upper face that is not isolated. For this reason, we recommend using the T_s surface inlet for K_v calculations until further suitability of data collection via the brass puck method is established.

Figure 5B shows K_v data calculated using various T_s measurement inputs for lyophilisers 02 and 03. The data shows that whether using the T_s thermal fluid data or T_s surface data collected by the RTDs, the resulting K_v calculations are comparable. However, T_s surface recorded from the brass puck, which is arguably located more appropriately in proximity with the vials, results in double the K_v values following the calculation. Previously published K_v data has been calculated using the T_s inlet and is consistent with our data shown in Fig. 5 calculated using the T_s surface inlet (Tchessalov et al. 2021).

K_v edge vs centre

A distribution of K_v across the shelves was generated by normalising the weight loss recorded for vials at the edge locations against the mean weight loss from the central location. It has been shown in the literature (Pikal et al. 2016; Rambhatla 2003) the major factor for a higher K_v at the edge relative to the centre is the radiative heat due to proximity with product chamber walls. Materials of construction are also a consideration,for all laboratory and

commercial scale equipment used in this study, stainless steel walls and doors were used.

Figure 6 shows K_v heat maps generated for the Lyostar II (A), lyophiliser 02 (B) and lyophiliser 03 (C). The edge effect is more prominent in the commercial units in comparison with the Lyostar II. K_v at the edge for lyophilisers 02 and 03 is up to 2 times that of the centre but only up to 1.5 times in the Lyostar II. For the commercial units at 3 vials deep from the edge, K_v is more consistent with the centre vial (location C). Therefore, K_v data calculated for location C is representative of approximately 87% of the 2322 vials on each shelf. The Lyostar II data shows K_v is equivalent to that of the centre location at rank 2 vials deep. These differences in K_v raise questions regarding the representative nature of edge vials at laboratory scale and how they represent the edge effect at commercial scale. It should be noted that the vial pack was surrounded by a metal ring that sits in contact with the shelf surface in the Lyostar II. Pisano et al. discussed how metal bands provide an additional contribution to heat transfer via conduction due to contact with the edge vial while also shielding radiative heat input from the chamber walls, thus reducing the heat input to edge vials (Pikal et al. 2016). There is still a radiative contribution by the metal band,however, it is limited as the surface temperature is low (Pisano et al. 2013). In comparison, during lyophilisation in the Lyomax system, the vials are not shielded from radiation which may explain why the edge effect is more prominent in the commercial scale units. For lyophiliser 01, the distance from the side edges of the shelf to the side walls is approximately 400 mm,the distance from the front and back edges to the adjacent wall/door is approximately 85 mm. For lyophilisers 02 and 03, the distance from the side edges of the shelf to the side walls is also approximately 400 mm; however, the distance from the front and back edges to the adjacent wall/door is approximately 170 mm.

Figure 7 provides a summary of the average edge vs centre K_v across SP1–SP3 (A) and a breakdown of the normalised edge vs centre factor per shelf (B). The figure was generated using values from the outer row of vials. The data provides further evidence showing consistent higher edge effect impact at commercial scale when compared with the Lyostar II, particularly regarding lyophiliser 01. Figure 7B provides some further insight; shelf 1 of lyophiliser 01 shows the highest edge effect normalisation factor, where shelf 7 and 13 are comparable with that of lyophilisers 02 and 03. The closure proximity of the front and back shelf edges of lyophiliser 01 with the adjacent surfaces does not appear to impact the weight loss in these local areas when compared with lyophilisers 02 and 03. Further assessment of the geometry specifically the ceiling area of lyophiliser 01 is required to further understand this identified hotspot, and the elevated radiative contribution needs to be accounted for in process robustness. In each case, for all 3 commercial units', shelf 1 (top) presented the highest degree of edge effect, this is likely due to increased radiation as a result to proximity to not only the chamber walls but also the ceiling.

Other areas of consideration include the presence of sight glasses in the equipment walls and the contribution of radiative heat input from the Ellab shuttles. The Lyostar II has an integrated sight glass door. The commercial lyophilisers have an integrated sight glass adjacent to shelf 13 in the back engineering side door as well as both side walls. There was no evidence of additional contribution of radiative heat at these locations; in these cases, the design of the site glasses includes a peak extending over the top of the glass minimising direct light entry to the chamber. Furthermore, as discussed previously for the Lyostar II, vials in this vicinity are

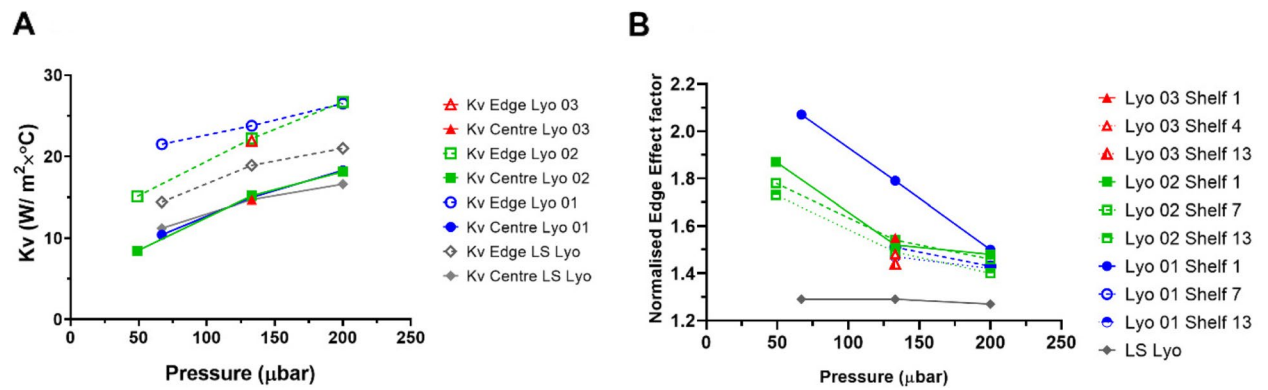


Fig. 7 **A** K_v difference between centre and edge vials and **B** normalised edge effect factor for K_v for lyophilisers (Lyo) 01, 02 and 03 and the Lyostar II (LS) lyophiliser. Normalised weight loss data was used to calculate representative K_v at the edge locations

shielded by a metal band. There were limited preweighed vials placed adjacent to site glasses in the commercial units; thus, there is opportunity to investigate further. Vials in direct contact with Ellab shuttles showed consistent weight loss with that of edge vials as detected during Lyostar II studies specifically with weight loss data associated with Fig. 5A. Taking this into account, weight loss from vials in contact with Ellab shuttles was not included for over K_v calculations for the Lyostar II. During commercial scale studies, Ellab shuttles were placed strategically away from the seeded vials measured for weight loss to mitigate any impact to the resulting data.

It has been reported that radiative heat transfer is independent of pressure (Kuu et al. 2005). During these experiments however, a gradient was observed for the commercial scale equipment where the edge effect was more pronounced at lower P_c. This may be explained because at lower pressures, the contribution of radiation to the K_v is more prominent at the edge where overall K_v across the vial pack becomes less dependent on convection. This observation is consistent for Lyomax lyophiliser data shared by Tchessalov (2016) who showed a more prominent edge effect for a Lyomax 6 at P_c of 30 mTorr vs 500 mTorr. In contrast however, the Lyostar II is in more agreement with literature and exhibits a consistent edge effect factor as a function of pressure. This may be due to the inclusion of the metal band around the vial pack resulting in an edge effect less dependent on pressure due to the reduction in radiative heat input. Overall, the data shows the requirements for consideration in scale-up, if a reduction in the P_c set point is used to achieve a lower T_p, but this might increase variability from edge to centre at commercial scale. Alternatively, lowering the T_s set point may be more beneficial to minimise the risk of exceeding a critical product temperature.

The impact of radiation at edge locations has been well explained for primary drying (Pikal et al. 2016; Rambhatla 2003). In our experience at locations that exhibit a hotspot such as shelf 1 of lyophiliser 01, T_p profiles measured during scale-up and technical transfer are impacted. Data collected from a scale-up technical batch for a sucrose-based formulation in the 20 mL SCHOTT vial used for these characterisation studies provided T_p data at the edge of shelf 1 location A and the centre location C for a Lyomax 29. In this technical batch, T_p measured data provided evidence of a hotspot at a similar location to that observed during the K_v assessment of lyophiliser 01. T_p profiles measured using Ellab RTDs on shelf 1 for location A ($n=2$) and for location C ($n=3$) demonstrated worst-case edge vs centre T_p profile from a Lyomax 29. During freezing, T_p at location A trended approximately 4 °C higher than T_p at location C. During primary drying, T_p trended approximately 2 °C higher at location A when compared with location C, and the duration of sublimation was approximately 15% shorter based on T_p equilibrating with T_s. During secondary drying, T_p trends about 3 °C lower at location A than location C (data available in the associated supplementary material).

The edge T_p characteristics observed during freezing and secondary drying during this technical batch may be attributed also to the chamber wall which in this case is not temperature controlled. Figure 8 shows data from lyophiliser 02 SP3 (200 µbar) where RTDs were fixed to the chamber wall adjacent to shelf 10 (empty shelf) and shelf 13 (bottom shelf containing vials of water). During freezing, with T_s at −40 °C, the wall surface temperature decreases gradually from ambient to between 0 and −5 °C. During primary drying with T_s at 0 °C and $T_p < -30$ °C, the wall surface temperature gradually increases to approximately 5 °C during 5 h of sublimation. This information provides rationale for the edge T_p behaviours described above, as the chamber wall surface temperature is higher than the T_s during freezing and lower than T_s transitioning from primary drying into secondary drying. This may directly impact T_p at the edge.

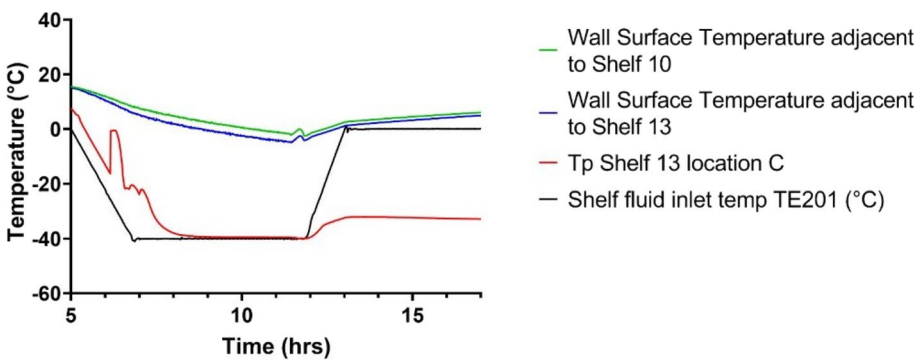


Fig. 8 K_v lyocycle trace for lyophiliser 02 SP3 showing chamber wall surface temperature adjacent to shelf 10 and shelf 13

As outlined previously, lyophiliser 03 is a single-storey configuration with the condenser chute opening adjacent to shelf 4. This is an area of interest as edge vials adjacent to the chute opening are not proximal to the chamber wall surface. This location may also have a localised vacuum pressure lower than anywhere else in the product chamber during primary drying due to proximity with the condenser (Kshirsagar et al. 2019). It was found that vials in this location exhibited comparable edge effect characteristics to other edge locations assessed (results not shown).

In summary, the data demonstrates a more significant edge effect at commercial scale where edge vials are not shielded from radiation in comparison with the Lyostar II where radiation is shielded by the steel ring surrounding the vials. K_v and T_p profiles of edge locations versus centre demonstrate the necessity for a robust formulation development and consideration during scale-up.

Impact of shelf inter-distance on K_v

Lyophiliser 01 has a shelf inter-distance of 100 mm. Lyophilisers 02 and 03 have a shelf inter-distance of 110 mm. Data in this study suggests shelf inter-distance does not impact K_v. This is in agreement with work performed by Ganguly et al. who proposed using CFD modelling that at an inter-shelf distance of 90 mm, there is a nearly uniform distribution in pressure (Ganguly et al. 2017).

Impact of load on K_v

As outlined in "Materials and methods", for commercial scale equipment, 3 of the 13 shelves were utilised for this study. The study design was considered to facilitate an assessment of the top, middle and bottom of the shelf stack under a limited commercial equipment capacity and primary packaging component availability. There are in this case some limitations to consider with respect to the data's representation of fully loaded lyophiliser production cycles.

It has been demonstrated that a full capacity load produces a lower K_v and thus lower T_p during sublimation with a longer primary drying duration (Patel et al. 2010). The calculated K_v in this study could be higher than what is expected under full load conditions as a function of the thermal impact of a larger number of vials subject to sublimation cooling in the product chamber. On the contrary, the calculated K_v in this study could also be lower than what is expected under full load conditions as a function of the gas composition in the product chamber. Under maximum load conditions during sublimation, there is a larger fraction of water vapour making up the gas composition in the product chamber. This reduces the nitrogen supply required to maintain vacuum pressure set-point control. During these experiments however, there was likely a larger fraction of nitrogen in the overall gas composition due to the lower load in the product chamber. Water vapour has a molecular conductivity about 60% higher than that of nitrogen (Nail et al. 2017). Considering an identical experimental approach was taken for each commercial scale lyophiliser, there is minimal impact to the study's comparability element. A confirmation K_v study under maximum load conditions would further verify the data (Barresi and Marchisio 2018).

Sublimation studies

Figure 9 shows the MCP trend from lyophiliser 02 as an example; trend data from lyophiliser 03 is not shown. As described in the method "Part 1: Minimal controllable pressure", this data was generated as per part 1 of the study where the outputs were used to assess the equipment choke flow regime and generate the equipment limitation boundary of maximum sublimation rate vs P_c.

The graph illustrates how the incremental increases in T_s influence not only the vacuum pressure but also the ice temperature, condenser coil temperature and T_s surface. The delta between the T_s shelf fluid inlet and the T_s surface measured by RTD increases as the degree of sublimation and the endothermic cooling increases showing the impact of ice under sublimation on the T_s surface.

Figure 10 shows an overlay of MCP vs sublimation rate for lyophilisers 02 (horizontal configuration) 03 (vertical configuration) and the Lyostar II.

The data shows the equipment capability boundaries of lyophilisers 02 and 03 and the LyosSar II, inside which the operational space should be defined. Outside this boundary, P_c control would be lost resulting in choked flow (Patel et al. 2010). An inflection point in the Lyostar II data represents the point at which the condenser is overloaded causing the unit to go into a "safe mode". At this point, the condenser was no longer able to trap vapour which risks moisture entering the vacuum pump. During the commercial scale studies, this was avoided by monitoring the condenser coil temperature to ensure there was no risk to damaging commercial equipment.

Lyophiliser 02 offers the highest water vapour mass transfer capacity, whereas the Lyostar II unit is the most limited piece of equipment. Literature articles often describe scale-up scenarios where commercial equipment is not capable of facilitating lyophilisation recipes developed at laboratory scale due to choke limitations (Pisano et al. 2013). In this example however, the most aggressive recipe developed on the Lyostar II will not pose a risk of choke at commercial scale. Also included in Fig. 10 for comparative purposes is normalised Lyostar II data generated using TDLAS by Mockus et al. which

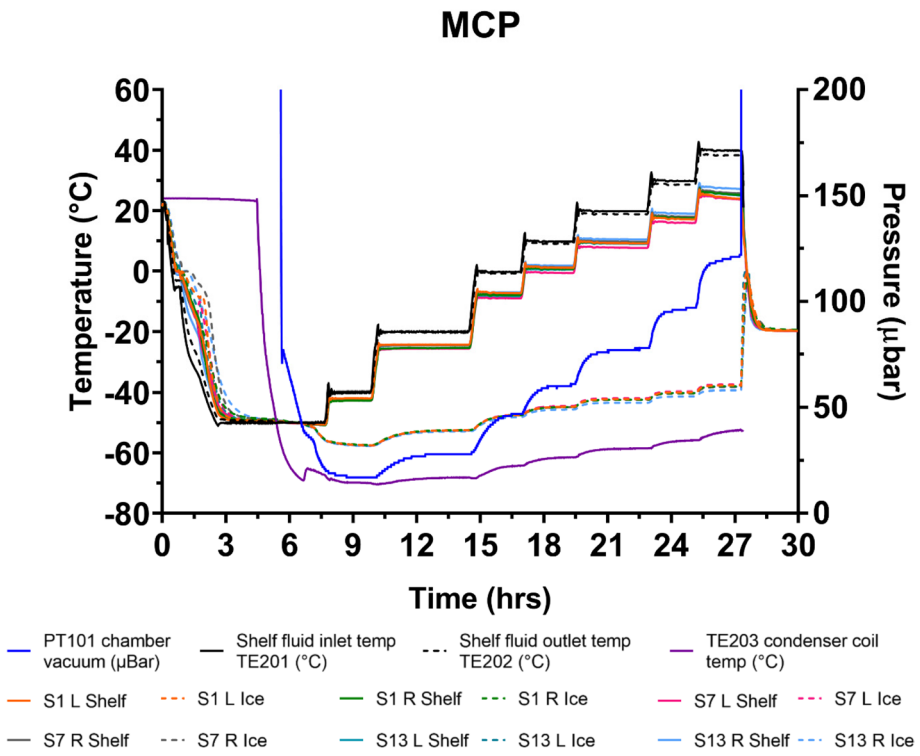


Fig. 9 MCP Lyophilisation trace for sublimation study. Part 1 on lyophiliser 02

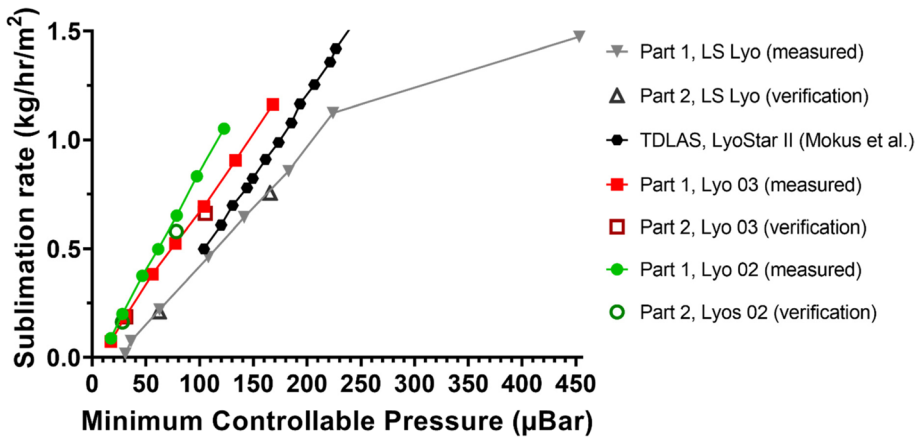


Fig. 10 Equipment limitation data of the LyoStar II (LS) and commercial scale lyophilisers (Lyo) 02 and 03 in comparison with literature TDLAS data for Lyostar II by Mockus et al. Part 1 — refers to minimum controllable pressure cycles. Part 2 — refers to the gravimetric verification cycles executed considering part 1 results

provides further evidence of the higher capability of the Lyomax units when compared with a Lyostar model (Mockus et al. 2011).

The data also poses the question on condenser location impact to performance. The K_v assessment provided evidence of comparability between lyophiliser 02 (Lyomax 29 vertical configuration) and 03 (Lyomax 29 horizontal configuration). However, the equipment limitation assessment identified differences in performance. In contrast to Kshirsagar et al., data generated in this study showed a broader equipment boundary with respect to the vertical condenser configuration. The CFD model presented by Kshirsagar assumes the absence of the mushroom valve at the condenser chute, whereas

the valve was intact during the studies described in this experiment. Further consideration for lyophilisers 02 and 03 with respect to the controlling capacitance manometer vacuum gauge is required. The controlling capacitance manometer is located in the ceiling of the product chamber; however, the distribution of P_c across the product chamber is likely to exhibit a gradient with proximity to the condenser which is more prominent in a vertical two-story configuration (Kshirsagar et al. 2019).

Another key variable to consider is the length and diameter of the condenser chute. Patel et al. demonstrated that gas velocity reaches the Mach I limit at the condenser chute exit under the choked flow conditions (Patel et al. 2010). The correlation between gas flow conductance and chamber pressure depends on the geometry of the chute where the dimensions are characterised by the ratio of chute length/diameter (L/D). For lyophiliser 02, the chute length is approximately 2.4 m with a diameter of about 0.7 m resulting in a L/D of 3.4. Lyophiliser 03 has chute length of approximately 1.7 m with a diameter of about 0.7 m resulting in a L/D of 2.4. Mach 1 and subsequent choked flow is observed in lyophiliser 03 at a higher P_c at a given T_s.

Applications and practical advice

The data provided in this paper has practical applications including building a design-space, scale-up, technical transfer and commercial performance. The value is understated where business requirements for multi-product facilities require optimised cycle duration and efficient technical transfer that minimises impact on commercial demands.

Scale-up and technical transfer

Technical transfer of a lyophilisation process can include both scale-up from a sending unit, i.e. process development to a receiving unit, i.e. commercial manufacturing or even between commercial entities. K_v and equipment performance information from both the sending and receiving unit equipment provides scientists and engineers a means of predicting whether (1) the receiving unit will exhibit a comparable T_p profile during primary drying, (2) the primary drying time is optimal, (3) the receiving unit has the mass transfer capability to facilitate the incoming recipe, and (4) the variability of the receiving unit equipment will impact product T_p and CQAs. This information along with other key product characteristics such as dry layer resistance, R_p, provides opportunity to mitigate the need for preprocess qualification engineering activities. In our experience, a process design space should be developed and verified at laboratory scale where differences in performance for commercial equipment should be considered to define scalability. It should be noted that this argument is only relevant when the vial, formulation and fill volume do not change. It also does not account the impact of environmental factors that lead to variability in R_p such as the particle-free class 100 commercial manufacturing areas that impacts the product nucleation temperature during freezing (Jameel et al. 2001).

Figure 11 presents a process design space developed as an outcome from using the characterisation (heat and mass transfer) data inputted into a primary drying prediction model. Included is the equipment limitation boundary of lyophilisers 02 and 03 along with T_s and T_p isotherms derived using the 20 mL SCHOTT K_v data from lyophiliser 02. Further inputs into the model are a hypothetical process set point, and operating range established for lyophiliser 02 where an equivalent K_v profile for lyophilisers 02 and 03 was assumed. The purpose of including the equipment boundary for both lyophilisers is to demonstrate if the process set point that is within in the equipment capability limit for lyophiliser 02 is at the edge of failure for lyophiliser 03. Thus, operating this process on both pieces of equipment may induce loss of pressure control due to choke flow in lyophiliser 03. To introduce a robust process in lyophiliser 03 may require a change in primary drying process parameters.

As described in Fig. 4, process transfer from the Lyostar II to either of the Lyomax 29 units using a P_c set-point above or below 133 μBar may require modification of either T_s or P_c to achieve an equivalent primary drying T_p profile. It is preferable to not change process parameters during technical transfer. If product specifications cannot be met with existing parameters, modifications may be necessary. However, if a product formulation is robust enough where known differences in equipment characteristics and performance do not impact product quality, retaining the original sending unit parameters is favourable to reduce complexity during regulatory submissions. This highlights the need for robust formulation and process development to ensure the delivery of commercialisation and technical transfer-friendly products.

Engineering batches

Engineering batches are performed to build confidence prior to process validation (PV). Engineering batches however often consume commercial production capacity. Lyophiliser characterisation data can reduce the workload required to demonstrate process performance prior to PV with the potential to even remove the requirement for engineering batches (assuming the appropriate process understanding of not only lyophilisation but all other unit operations such as thawing, compounding, filtration and filling).

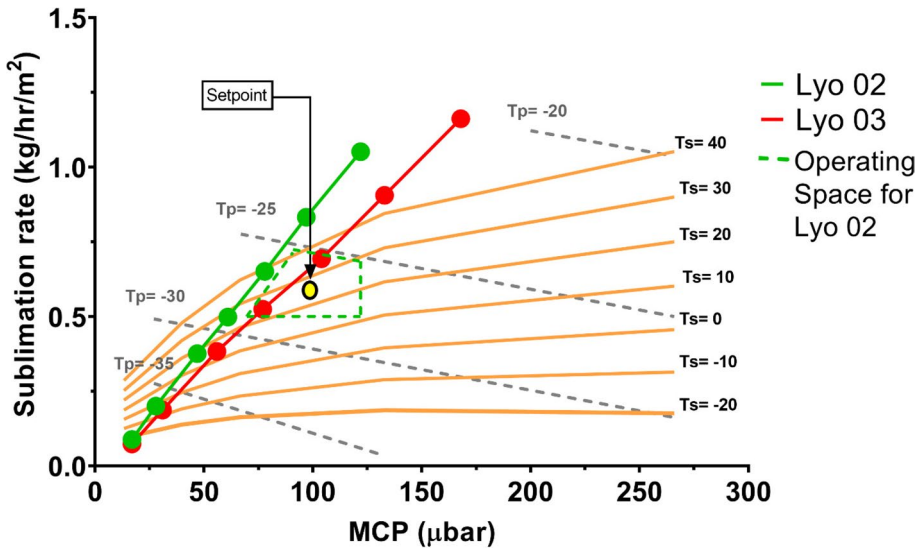


Fig. 11 Example of primary drying design space including choke flow limits for lyophilisers (Lyo) 02 and 03

When an engineering batch is required and there are product material constraints, a co-load approach is often employed. The co-load approach involves the filling of vials with a surrogate buffer that exhibits equivalent physical attributes such as solid content, density, viscosity and T_p profile (demonstrated at laboratory scale). This can be applied to maximise lyophiliser capacity while also providing the opportunity to seed product-filled vials into strategic locations for lyophilisation and retrieving them after for analysis such as residual moisture.

Another consideration for engineering batches is when a lyophiliser has been already characterised for mass transfer capability or historically has manufactured a product with known higher mass transfer rate demand. In this scenario, completing a reduced load engineering batch may be more beneficial to verify worst-case T_p conditions during sublimation. As discussed previously, a maximum capacity batch will sublime at a slower rate with a lower T_p and challenge the equipment, i.e. the condenser capacity and choke limit (Patel 2011). Thus, a minimum batch size represents worst case for the product, i.e. performing a minimum load assessment may provide an opportunity to assess worst-case high T_p during sublimation under reduced load conditions.

Supplementary scale-down process robustness assessments at the design space boundaries should also be conducted to provide further supporting data.

Process validation

As stated in the European Medicine Agency guidelines (Agency 1995) "data should be provided in the dossier on a number of consecutive batches at production scale prior to approval. The number of batches should be based on the variability of the process, the complexity of the process / product, process knowledge gained during development, supportive data at commercial scale during technology transfer and the overall experience of the manufacturer. Data on a minimum of 3 production scale batches should be submitted unless otherwise justified. Data on 1 or 2 production scale batches may suffice where these are supported by pilot scale batches and a justification as highlighted above". For initial commercialisation process performance qualification (PPQ) approaches, it is common to provide a data set from at least three batches executed on the same lyophiliser. However, as described in the extract above, agencies provide opportunity to simplify PPQ strategies based on providing further supporting data along with demonstrated technical competency. A good opportunity to apply this approach is when performing PPQ for more than one lyophiliser, product strength or batch size. A key element to this is a demonstration of equipment comparability which provides the basis for bracketed PV strategies. For instance, a position could be made to include two lyophilisers as part of the PV strategy utilising three PV batches (covering minimum and maximum loads) or a single PV batch for one lyophiliser. A comparability package for the qualification of two lyophilisers is proposed to provide a strong justification for such strategies:

- Equipment design and specification
- Initial equipment installation qualification and operational qualification data
- Annual equipment re-qualification

- Vial heat-transfer coefficient data
- Equipment mass transfer capability — differences may be acceptable as long the process will operate within the safe zone of the receiving unit's equipment design space.
- Benchmarking of engineering batch or other existing commercial product performance and T_p data if available
- Primary drying model — to simulate conditions as part of scale-up and qualification in the case of a new product, simulation of commercial process in another lyophiliser in the case of a commercial technical transfer.

Shelf mapping

During PV, it is often requested by agencies to provide shelf-mapping information, a validation of homogeneity demonstrating product quality is equivalent at various locations across the chamber. A typical approach is the "star" method where upon completion of a lyophilisation PPQ batch, vials area sampled from the four corners and the centre of each shelf and analysed (Jameel et al. 2001; Huang 2016). Lyophilisers 01, 02 and 03 contain 13 shelves each,thus, full shelf mapping would generate 65 locations to be analysed per batch. This is frankly not a practical scenario for a commercial production and analytical testing facility. K_v studies described in this paper provide a means of positioning reduced sampling requirements based on a data set identifying the best and worst locations within the product chamber. Consistently, throughout the studies, the highest K_v was observed at the edge locations of top shelf representing the worst-case location. The centre locations were equivalent at the top, middle and bottom shelves. This knowledge provides the basis for a simplified approach. For instance, one could propose the star method for the top shelf (5 locations; centre and the 4 corners) and supplement a number of other locations throughout the shelf stack to provide a sample set large enough to complete hypothesis testing, i.e. 20 locations in total. Generating power curves for a 2-sample *t*-test assessment for an identified product attribute with known vial-to-vial variability will provide a statistically significant sample number recommendation to detect a shift in a mean value between two pieces of equipment.

A justification to reduce the number of shelf locations required from 65 to 20 for a commercialisation PV programme results in product material and resource savings without compromising the integrity of the technical transfer. If we consider four analytical methods to be in scope during shelf mapping, we can reduce the laboratory test number from 260 to 80 per maximum load PV batch. Where three PV batches are being conducted, this would be a reduction from 780 to 240 analytical tests for the full campaign. Where validated laboratory quality analytical methods are required, daily throughput can be slow and analyst resources and laboratory equipment capacity limited; this approach provides a more efficient technical transfer while lowering opportunity for laboratory errors.

Further considerations

Manufacturing excursions

K_v coupled with R_p information are key inputs to primary drying predictive tools (Tchessalov et al. 2021; Leys et al. 2020).Outputs include T_p and primary drying duration. Manufacturing excursions impacting T_s and P_c directly impact T_p. T_p is the most critical parameter during lyophilisation yet not generally monitored during commercial operations. Up-front product and process characterisation provide a means to simulate the impact to T_p upon loss of P_c or T_s control during investigations.

Execution of equipment characterisation

Based on our experience, to conduct these studies on commercial scale equipment, the best opportunity is to do so during commissioning/installation. Retrospective characterisation requires further consideration such as impacting commercial manufacture capacity and quality requirements for placing new materials into GMP equipment. Associated clean rooms for filling and lyophiliser loading may also need to be disabled requiring further cleaning and sterilisation activities. Maintenance shutdowns may provide opportunity to generate supporting data from existing commercial data while minimising impact to commercial operations. Integration of T_p and other parameters obtained from engineering batches in existing heat-mass transfer models and comparison with small-scale models further enhances equipment characterisation understanding.

Use of gravimetric methodology vs process analytical tools (PAT)/quality by design (QbD) tools

While new PAT/QbD tools such as tunable diode laser absorption spectroscopy (TDLAS) are being established to provide real-time output of gas flow and derivative T_p, it provides an estimated batch average K_v (Jameel et al. 2001). The accuracy of the instrument for process output is dependent on more precise directly measured K_v information such as that generated from studies such as the gravimetric approach. Furthermore, the average methods such as TDLAS still do not provide a means of assessing the worst-case locations within a lyophiliser chamber. In summary, accurately measured K_v coupled with TDLAS provides a very strong data gathering system for

real-time performance. Where cost may be a limiting factor to acquire equipment limitation information using TDLAS, it is still a viable option to generate this data using traditional gravimetric methods.

Temperature monitoring equipment

Nail et al. recommended the use of fine gauge thermocouple for measurement of T_p which offers better opportunity to measure at the centre of the vial touching the base in comparison with other technologies such as RTDs (Nail et al. 2017). In our experience, we agree with this conclusion in the case where a T_p profile of pharmaceutical product is being measured. As discussed previously, the T_p profiles illustrated in Fig. 9 were recorded using RTD probes of the inflection point at the end of sublimation is not well defined due to point of measurement in the vial which presents a gradual T_p equilibration with T_s trend. For the purpose of K_v studies where T_p data for partial sublimation of purified water with no R_p is being collected using RTDs, our experience was positive. As illustrated in Fig. 4, T_p profiles acquired for K_v studies were consistent and reproducible. Another study comparing thermocouples, RTDs and the Tempris system showed variability in K_v calculation between the systems is within the variability of centre to edge K_v values (Gervasi 2020). Regardless, it makes practical sense to use the same systems where possible when comparing different pieces of equipment.

Conclusion

Lyophiliser characterisation is a valuable exercise that requires upfront investment of time and resources. The benefits demonstrate a premium level of process knowledge that drives enhanced technical support for technical transfer, commercial manufacturing and life cycle management. In this study, we aimed to assess and compare the heat and mass transfer attributes of one laboratory and three commercial scale lyophilisers using a 20 mL vial and apply the data to support technical transfer and operational activities.

Our characterisation data showed the K_v profile measured for the Lyostar II exhibited differences when compared with that of the commercial equipment with equivalency at an intersection at 133 µBar. The edge effect was shown to be most prominent at commercial scale with minimal shielding of the edge vials in contrast to the presence of a metal ring around the vial pack in the Lyostar II. Equipment limitation boundary was shown to be better for commercial scale equipment when compared to the Lyostar II. Furthermore, differences were also detected between the same model of commercial equipment with alternative condenser orientation (horizontal vs vertical).

Examples of equipment characterisation and predictive modelling are limited and not typically provided in an end-to-end package but rather in separate case studies. Collectively, our data was compiled in this study to provide a full package of heat and mass transfer data across our laboratory and commercial scale equipment. The application of this comprehensive database resulted in stronger justifications for the strategies used in commercial scale activities including the reduction of engineering and validation batches while reducing the risk of failure during scale-up and technical transfer. Existing guidance for the application of best practises for process characterisation and validation is in some cases not practical to apply due to the complexity, required time and general resources. The experience gained in these studies demonstrated realistic approaches and subsequent shared advice for building a strong technical package.

It is recommended to perform such experiments if possible, during commissioning/qualification. Hybrid studies can be executed in the event manufacturing equipment time is limited. It is also recommended where possible to include additional vial sizes and load sizes to create a more comprehensive data package especially for multi-product facilities. The generation of K_v at laboratory and commercial scale for a given vial along with performance characteristic data will help with better scale-up and PPQ strategies, thereby reducing commercial equipment capacity needs, resource requirements and overall cost. This will also be valuable in supporting manufacturing and better understand true impact in the event of manufacturing deviations during the lyophilisation cycle for a given clinical late stage or commercial batch.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41120-022-00059-0.

Additional file 1. Supplementary Materials: - Technical Transfer and Commercialisation of Lyophilised Biopharmaceuticals – Application of Lyophiliser Characterisation and Comparability.

Acknowledgements

This work represents an interdependent cross functional effort by multiple departments at Sanofi Waterford including MSAT, Maintenance, Process Engineering, Quality Control and Quality Assurance. Further support and encouragement was most welcome from the Global Lyo CoE within Sanofi BioDPD at Framingham MA and extended team at Paris, France. Guidance, advice and feedback was also provided by Arnab Ganguly of IMA Life, Inc., Tonawanda, New York, USA, as well as through Industry Lyophilisation forums by Steven Nail, Serguei Tchessalov, Kevin Ward and Michael Pikal.

Authors' contributions

Sean Cullen – Design of Work, acquisition, analysis and interpretation of data, accountable for all aspects of the work, final approval. Emma Walsh - Analysis and interpretation of data, drafting the work, final approval. Valeria

Gervasi - Analysis and interpretation of data, drafting the work, final approval. Dikshitkumar Khamar - Analysis and interpretation of data, drafting the work, final approval. Timothy R McCoy - Design of Work, analysis and interpretation of data, final approval.

Funding
All funding was provided by Sanofi, IDA Industrial Estate, Waterford, Ireland.

Availability of data and materials
Not applicable.

Declarations

Competing interests
All authors were employed by Sanofi while the studies were conducted. Sean Cullen. Valeria Gervasi and Timothy McCoy have since left the company.

Author details
[1]Sanofi, IDA Industrial Estate, Waterford, Ireland. [2]Drug Product MSAT, Sanofi, IDA Industrial Estate, Waterford, Ireland.

Received: 10 March 2022 Accepted: 16 June 2022
Published online: 01 September 2022

References

Agency EM. Process validation for finished products – information and data to be provided in regulatory submissions 1995 - 2020 [updated 2020. Available from: https://www.ema.europa.eu/en/process-validation-finished-products-information-data-be-provided-regulatory-submissions.
Barresi AA, Marchisio DL (2018) Computational fluid dynamics data for improving freeze-dryers design. Data Brief 19:1181–213
BioPlan Associates I (2017) A study of biotherapeutic developers and contract manufacturing organizations. report and survey of biopharmaceutical manufacturing capacity and production
Carpenter JF, Chang BS, Garzon-Rodriguez W, Randolph TW (2002) Rational design of stable lyophilised protein formulations: theory and practice. Springer, Rational design of stable protein formulations, pp 109–133
Ganguly A, Varma N, Sane P, Bogner R, Pikal M, Alexeenko A (2017) Spatial variation of pressure in the lyophilization product chamber part 1: computational modeling. AAPS PharmSciTech 18(3):577–585
Gervasi V, Agnol RD, Cullen S, McCoy T, Vucen S, Crean A et al (2018) Parenteral protein formulations: an overview of approved products within the European Union. Eur J Pharm Biopharm 131:8–24
Gervasi V (2020) Lyophilisation of high concentration protein formulations. University College Cork, Ireland
Gervasi V, Cullen S, McCoy T, Crean A, Vucen S (2019) Application of a mixture DOE for the prediction of formulation critical temperatures during lyophilisation process optimisation. Int J Pharm 572:118807
Hibler S, Wagner C, Gieseler H (2012) Vial freeze-drying, part 1: new insights into heat transfer characteristics of tubing and molded vials. J Pharm Sci 101(3):1189–201
Huang E. Lyophilization validation: a regulatory perspective 2016 [updated 2016. Available from: https://docplayer.net/49577706-Lyophilization-validation-a-regulatory-perspective-ellen-huang-cber-ocbq-dmpq-casss-cmc-strategy-forum-july-19-2016.html.
Jameel F, Alexeenko A, Bhambhani A, Sacha G, Zhu T, Tchessalov S, et al (2021) Recommended best practices for lyophilization validation—2021 part i: process design and modelling. AAPS PharmSciTech 22(7):1–18
Jennings TJAPR (2002) Transferring the lyophilization process from one freeze-dryer to another. ResearchGate 5(1):34–42
Kawasaki H, Shimanouchi T, Kimura YJJoC (2019) Recent development of optimization of lyophilization process. J Chem 2019;2019:9502856
Kuu WY, Hardwick Lm Fau - Akers MJ, Akers MJ (2005) Correlation of laboratory and production freeze drying cycles. Int J Pharm (0378–5173 (Print))
Kshirsagar V, Tchessalov S, Kanka F, Hiebert D, Alexeenko A (2019) Determining maximum sublimation rate for a production lyophiliser: computational modeling and comparison with ice slab tests. J Pharm Sci 108(1):382–90
Leys L, Vanbillemont B, Van Bockstal PJ, Lammens J, Nuytten G, Corver J, Vervaet C, De Beer T (2020) A primary drying model-based comparison of conventional batch freeze-drying to continuous spin-freeze-drying for unit doses. Eur J Pharm Biopharm 157:97–107
Mockus LN, Paul TW, Pease NA, Harper NJ, Basu PK, Oslos EA, et al (2011) Quality by design in formulation and process development for a freeze-dried, small molecule parenteral product: a case study. Pharm Dev Technol 16(6):549–76
Nail S, Tchessalov S, Shalaev E, Ganguly A, Renzi E, Dimarco F et al (2017) Recommended best practices for process monitoring instrumentation in pharmaceutical freeze drying—2017. AAPS PharmSciTech 18(7):2379–93
Patel SM, Chaudhuri S, Pikal MJ (2010) Choked flow and importance of Mach I in freeze-drying process design. J Pharm Sci 65(21):5716–27
Patel SM, Jameel F, Pikal MJ (2010) The effect of dryer load on freeze drying process design. J Pharm Sci 99(10):4363–79
Patel SM, Pikal MJJAP (2011) Emerging freeze-drying process development and scale-up issues. AAPS PharmSciTech 12(1):372–8
Pikal MJ (1985) Technology. Use of laboratory data in freeze drying process design: heat and mass transfer coefficients and the computer simulation of freeze drying. J Parenter Sci Technol 39(3):115–39
Pikal MJ, Bogner R, Mudhivarthi V, Sharma P, Sane P (2016) Freeze-drying process development and scale-up: scale-up of edge vial versus center vial heat transfer coefficients, Kv. J Pharm Sci. 105(11):3333–43
Pikal MJ, Roy M, Shah S (1984) Mass and heat transfer in vial freeze-drying of pharmaceuticals: role of the vial. J Pharm Sci 73(9):1224–37
Pisano R, Fissore D, Barresi AA, Rastelli M (2013) Quality by design: scale-up of freeze-drying cycles in pharmaceutical industry. AAPS PharmSciTech 14(3):1137–49
Rambhatla S, Pikal MJJAP (2003) Heat and mass transfer scale-up issues during freeze-drying, I: atypical radiation and the edge vial effect. AAPS PharmSciTech 4(2):22–31
Rambhatla S, Tchessalov S, Pikal MJ (2006) Heat and mass transfer scale-up issues during freeze-drying, III: control and characterization of dryer differences via operational qualification tests. AAPS PharmSciTech 7(2):E61–E70
Rey L, (Ed.) (2010) Freeze-Drying/Lyophilization of Pharmaceutical and Biological Products (3rd ed.). CRC Press.Searles JAJF, Chapter 18 Optimizing the throughput of freeze-dryers within a constrained design space. pp. 425–440
Scientific S. Lyo calculator 2016 [updated 2016. Available from: https://www.spscientific.com/LyoCalc/Lyocalculator.html.
Tang XC, Pikal MJJPr (2004) Design of freeze-drying processes for pharmaceuticals: practical advice. Pharm Res 21(2):191–200
Tchessalov S, Latshaw D, Nulu S, Bentley M, Tharp T, Ewan S et al (2021) Application of first principles primary drying model to lyophilization process design and transfer: case studies from the industry. J Pharm Sci 110(2):968–981
Tchessalov S (2016) Methodology of lyophilisers characterization to enable modeling of process parameters during cycle scale up (webinar). SP Scientific Webinar Archive https://www.spscientific.com/Methodology_Modeling/.
Wegiel LA, Ferris SJ, Nail SL (2018) Experimental aspects of measuring the vial heat transfer coefficient in pharmaceutical freeze-drying. AAPS PharmSciTech 19(4):1810–7
Zhou D, Shang S, Tharp T, Jameel F, Sinha K, Nere NK (2019) Leveraging lyophilization modeling for reliable development, scale-up and technology transfer. AAPS PharmSciTech 20(7):263

Publisher's Note