DISSERTATION ZUR ERLANGUNG DES DOKTORGRADES

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Nests and Chamber Wall Temperature Control as Tools in Pharmaceutical Freeze-Drying – Evaluation of Heat Transfer, Edge-Vial-Effect and Drying Kinetics

SARAH EHLERS (geb. DALLER)

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ERKLÄRUNG

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..SARAH EHLERS.....

Sarah Ehlers (geb. Daller)

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1. Gutachterin / 1. Gutachter: Prof. Dr. Wolfgang Frieß

2. Gutachterin / 2. Gutachter: Prof. Dr. Gerhard Winter

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ENJOY THE MOMENT.

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TABLE OF CONTENT

List of Abbreviations

FDA	U.S. Food and Drug Administration
CIP	cleaning in place
DSC	Differential Scanning Calorimetry
EPD	End of primary drying
Kc	Heat transfer via direct contact
Kg	Heat transfer via convection
Kr	Heat transfer via radiation
Kv^{app}	Apparent vial heat transfer coefficient
Kv	Vial heat transfer coefficient
P_C	Chamber pressure
PEEK	Polyether ether ketone
POM	Polyoxymethylene
SIP	Sterilization in place
Tc	Collapse temperature
T_{cage}	Radiation cage temperature
Tg'	Glass transition temperature
T_P	Product temperature
T_{shelf}	Shelf temperature

CHAPTER 1 GENERAL INTRODUCTION

1.1 The freeze-drying process

Freeze-drying is a well-established technique to reduce the water content in biopharma industry and to stabilize biological products as well as to prolong their shelf-life compared to the liquid form. [1, 2] The number of FDA approved freeze-dried products on the market nowadays rises. [3] The reduction of water minimizes chemical degradation processes such as oxidation, deamidation, hydrolysis, ß-elimination and isomerization and enhances the colloidal and conformational stability of a protein. [4-6] The freeze-drying process mainly consists of the three steps freezing, primary drying and secondary drying (Figure 1-1).

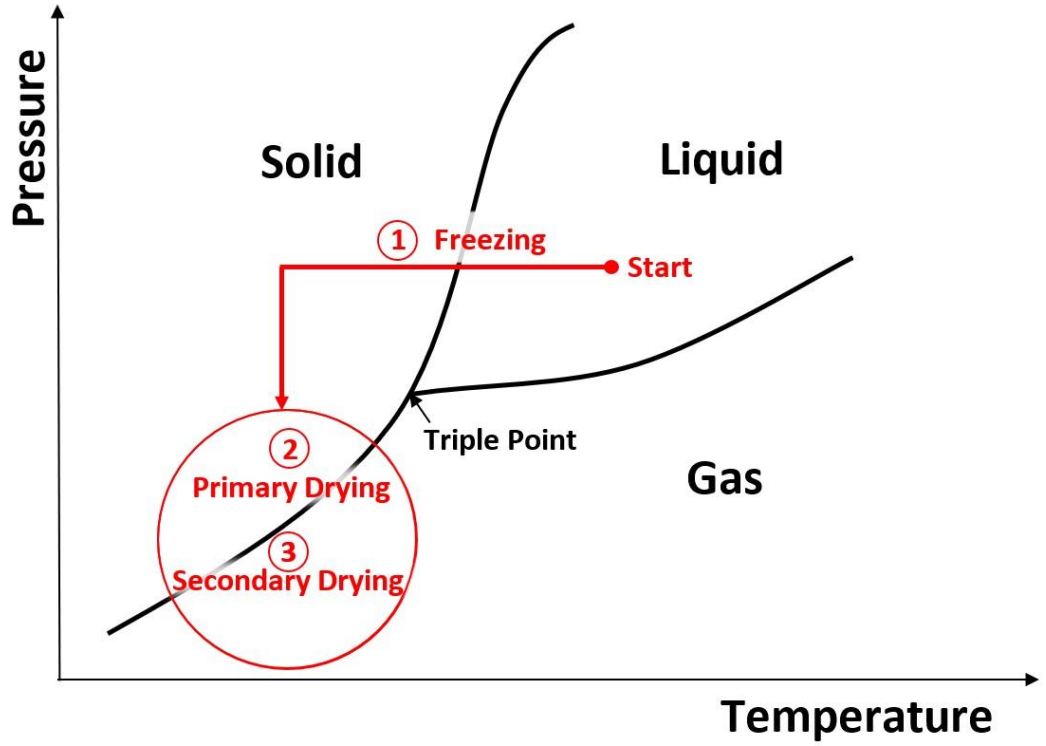

Figure 1-1. Physical state of water during main steps of freeze-drying: freezing, primary drying and secondary drying.

In the freezing step, the product is frozen below its eutectic point, in case of crystalline excipients, or glass transition temperature of the maximally freeze concentrated solution in case of amorphous excipients. [7] During freezing, ice crystals form resulting in a matrix, which later reflects the pore structure of the freeze-dried cake. Since the ice crystal formation is an uncontrolled spontaneous process, various techniques for controlled ice nucleation were

developed. [8-10] During primary drying 90-95 % of the available water molecules are sublimed by pressure decrease and a slight temperature increase of the product. [11] Remaining bound water is sublimed in secondary drying by further temperature increase. Freeze-dried cakes with less than 1 % and an upper limit of 3 % residual water content are pursued. [12-15]

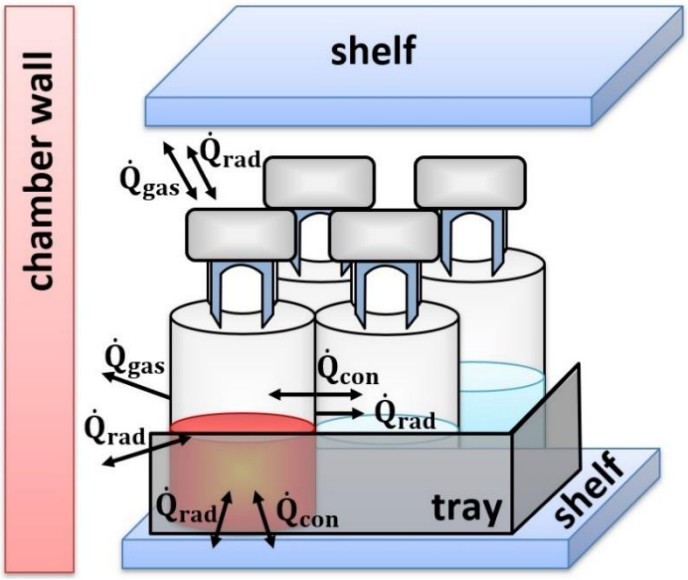

Figure 1-2. Heat transfer modes in vials during freeze drying

The sublimation process during primary drying is of endothermic nature and thus heat has to be transferred from the shelves to the product. There are three modes of heat transfer: conduction via direct contact (K_c), radiation (K_r) and gas convection (K_g) (figure 1-2). [16] Conduction via direct contact occurs between vial and shelf as well as in between neighboring vials. Due to the curvature of the vial bottom and the resulting cavity between vial and shelf, heat is also transferred through radiation and gas conduction from shelf to vial. [17] However, the main source of radiation heat are warmer construction elements of the freeze dryer e.g. the chamber wall. Gas conduction is the strongest heat transfer mode at higher pressures. [18-20] Understanding total K_V and the contribution of the individual modes are important for full understanding of a freeze-drying process.

1.2 New upcoming vial holding systems

Two current trends in biopharma industry are personalized medicine and therapeutic niche applications. [21] Individually targeted antibodies, antibody drug conjugates and dual variable domain antibodies are potentially inferior in stability causing challenges. [4, 22] In addition with patient individual and therapeutic niches products the batch sizes for clinical and market product decrease. To ensure flexible, time and cost-effective as well as high quality aseptic fill/finish manufacturing, innovative machinery with a higher level of control and automation is required. Compared to standard equipment, these new flexible units come with different vial handling options using robotics, single-use product contact parts, ready-to-use primary packaging materials and racks. In this context lyophilization gets special attention since it allows to cope with a potentially lower stability of pharmaceuticals even in later phases of development. Moreover, there is a higher uncertainty of product stability due to a less in-depth development program. Especially for automated filling lines, nested containers are provided. [23] Freeze-drying of vials in nests and racks has rarely been analyzed. A vial nest may have the potential for up to 30 % reduction of lyophilization time compared to non-nested processing. [24] Rack type of holder systems have been studied in the context of freeze-drying in dual chamber cartridges, but the results cannot be easily transferred to nested vials. [25-28] Vials nested in a rack do not have direct contact to each other, different from the commonly used setting of vials in bulk and understanding of the importance of the different modes of heat transfer is necessary to ensure high quality.

1.3 Critical product temperature and role of the edge-vial-effect

During primary drying a critical product temperature exists, which marks the threshold above which the cake starts to collapse leading to inappropriate visual appearance, retarded drying and potentially protein instability (Figure 1-3). [29-31] Previously, the glass transition temperature Tg' of the maximally freeze-concentrated solution was considered to be this critical product temperature. [32] At Tg' the rigid amorphous glass becomes a viscous rubber. [33] Tg' usually is measured by differential scanning calorimetry (DSC). [34] Recently, more focus is on the collapse temperature Tc, which marks the beginning of collapse as studied by freeze-drying microscopy. [35-37] Freeze-drying above Tg' but below Tc can result in elegant cakes with well-preserved protein stability. [33, 38]

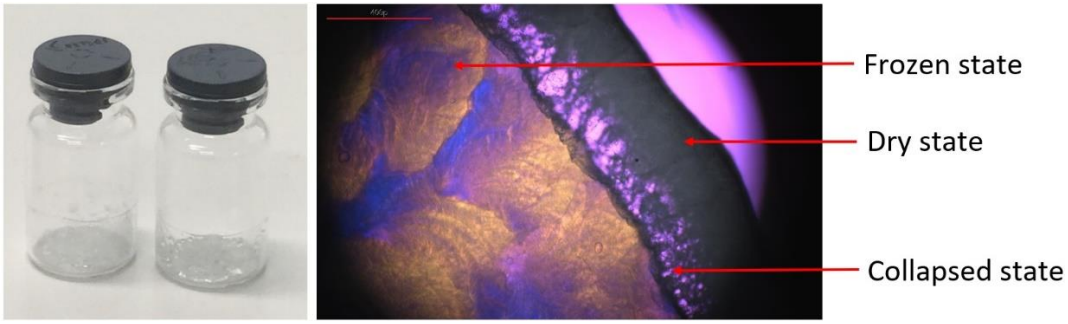

Figure 1-3. Left: fully collapsed freeze-dried cakes; right: collapse in a freeze-drying microscope

Ideally the product temperature should be the same for all vials during the whole freeze-drying process. But vials at the corners and edges of a shelf load tend to run warmer and to dry faster than center vials. [39] This edge-vial-effect arises from additional heat received by corner and edge vials which is assumed to come mainly from the chamber wall through radiation. [40] Center vials are shielded against this effect through surrounding neighbor vials. [41] The researcher has to balance the risk between potential collapse of warmer edge and corner vials and more conservative primary drying conditions keeping the edge and corner vials further below the critical product temperature but leading to even colder center vials which take even longer to dry. [42]

1.4 The chamber wall as source for radiation

Radiation from the freeze dryer chamber walls has been described as the main driving force for the edge-vial-effect. [16, 41, 43] The extent of radiation is dependent on the emissivity of a surface and may vary due to its polish. [44] The radiation impact is specifically noticeable in freeze dryers with an acrylic glass door coming with 0.9 emissivity compared to units with a stainless steel door with an emissivity of stainless steel about 0.63. [44] To avoid radiation impact, aluminum foil is often wrapped around the vial setting in laboratory experiments or the plastic doors are covered with aluminum foil. [45] Obeidat et al. reported on the usage of temperature-controlled chamber walls in a mini freeze dryer. [46] Control of the chamber wall temperature on product temperature resulted in similar product temperature and Kv values of corner and center vials. Nevertheless, a difference between corner and center vials of 2-

3 °C in product temperature and 6-9 % in Kv value respectively remained. Other studies with prevention of radiation showed still a difference between corner and center vials in sublimation rates, K_V values or product temperature curves. [47] Similarly a radiation cage constructed of four additional stainless steel shelf like walls parallel to the chamber wall could be used. [48] If the corner vials would get less energy by radiation, they run colder at center vial level resulting in a more homogeneous batch temperature profile. This would enable to run the freeze drying process at slightly more aggressive primary drying conditions for the entire batch and thus faster processes.

1.5 Transfer of freeze-drying processes in biopharmaceutical industry

Scale-up and transfer of freeze-drying processes is a crucial challenge in biopharma industry. A freeze-drying cycle developed on a smaller laboratory freeze dryer, often cannot be transferred 1:1 to a larger unit as differences in construction, loading and performance between the freeze dryers lead to different heat and mass transfer conditions. [49, 50] For instance, differences in location and construction of the condenser can lead to different water vapor flow. [51] Heat transfer by radiation may differ due to different emissivity values of components and other distances between chamber wall and shelves in the freeze dryers. [52] Differences in product resistance can originate from differences in freezing processes caused by variation of the nucleation events due to differences in vibrations or clean air conditions. [53] A direct transfer of the freeze-drying cycle would require two parameters to be similar: product temperature history and product resistance. Especially the drying kinetics of corner and edge vials are more difficult to transfer since they are impacted by parts of the freeze dryer through radiation. [54] In case of vials positioned in racks the holding systems and its effect on the heat transfer adds another level of complexity. To minimize many trial and error experiments for transfer and scale-up, a good understanding of the equipment as well as the freeze-drying cycle is crucial.

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CHAPTER 2 Objectives of the thesis

The scope of this study is to I) investigate the heat transfer and the drying behavior of product vials placed in rack systems as compared to the standard bulk setup and II) analyze the impact of the freeze dryer chamber wall temperature on drying kinetics. In the common bulk setting, vials are subject to the edge-vial effect and the heat transfer is well understood. In a rack, vials lack direct contact to each other and are potentially shielded from direct radiation coming from other sources e.g. the chamber wall by the rack. Thus, heat transfer could be faster and more controlled compared to vials set in bulk setting. Therefore, product temperature of placebo vials was measured in rack compared to tray at shelf temperatures varying from -10 °C to -25 °C and chamber pressures varying from 0.066 mbar to 0.267 mbar. The shielding effect of the rack was investigated in comparing product temperature of vials in a rack vs. separated vials without rack. Furthermore, Kv mapping was performed with at different chamber pressure values in rack and tray. Upscaling from one rack per shelf to four racks per shelf was performed and drying behavior was evaluated. Finally, a comparability study from rack to tray and between different freeze dryers should demonstrate necessary changes in chamber pressure and shelf temperature.

The radiation cage is supposed to minimize radiation from the chamber wall. The optimum temperature for the radiation cage during freeze-drying has to be defined in order to gain a homogeneous batch. Thus, drying behavior was determined during radiation cage temperatures from -25 °C to -45 °C compared to a passive radiation cage. The impact of the radiation cage temperature on vials shielded by a rack was investigated. A Kv mapping should demonstrate the impact of reduced radiation especially on corner and edge vials compared to center vials. For comparison of a higher radiation cage temperature during secondary drying to a passive, colder radiation cage, a residual moisture mapping was performed. Moreover, the impact of a reduced radiation cage temperature on drying behavior was compared to freeze-drying cycles without radiation cage to define directions for a successful process transfer.

As elimination of the chamber wall radiation does not completely reduce the edge-vial-effect, we hypothesized that neighboring vial affect each other substantially which contributes to the edge-vial effect. Since corner and edge vials have less neighbors than center vials, the cold neighbor vial configuration may significantly impact the product temperature. The drying

behavior of a vial surrounded by nothing, empty vials or heat-conductive paste containing vials was studied. Additionally, Kv values of vials standing at varying distance from 1 to 100 mm to each other were determined. To further evaluate the cooling effect of a vial, the number of direct neighbors was varied from 0 to 6.

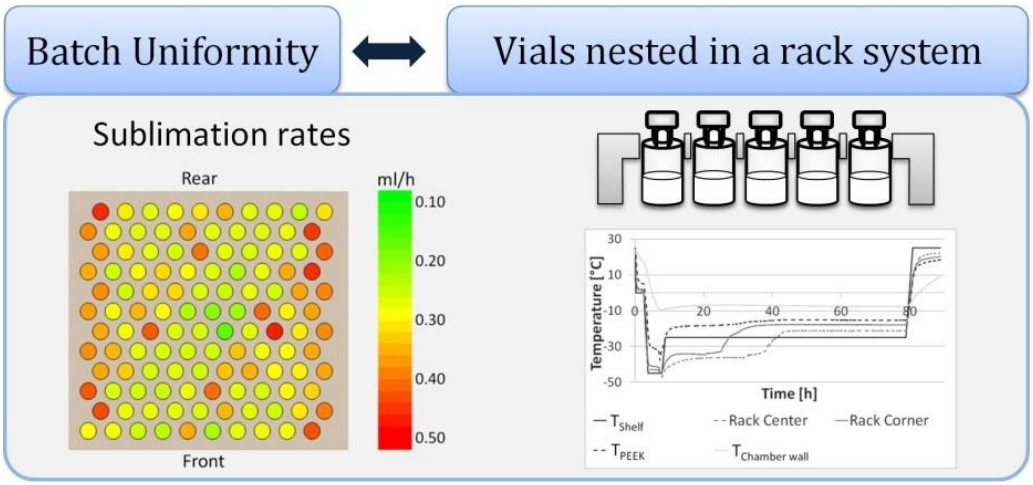
CHAPTER 3 Energy Transfer in Vials Nested in a Rack System During Lyophilization

The following chapter has been published in the Journal Pharmaceutics and appears in this thesis with the journal`s permission:

Sarah Daller, Wolfgang Friess and Rudolf Schröder

DOI: 10.3390/pharmaceutics12010061

3.1Graphical abstract



Keywords: lyophilization, freeze drying, rack system, heat transfer, sublimation rates, edge-vial-effect, T_P, pressure, radiation, direct contact

3.2 Abstract

Small batch sizes are a consequence of more personalized medicine and reflect a trend in the biopharmaceutical industry. Freeze drying of vials nested in a rack system is a tool used in new flexible pilot scale processing lines. Understanding of heat transfer mechanisms in the rack loaded with vials not in direct contact with each other is necessary to ensure high quality. Lyophilization in the rack vial system enables a homogeneous drying with a reduced edge-vial-effect and shielding against radiation from surrounding components, e.g., the chamber wall. Due to the separation effect of the rack, direct shelf contact contributes approx. 40% to the overall energy transfer to the product during primary drying. Hence overall the rack is a flexible, robust tool for small batch production, which ensures a controlled heat transfer resulting in a uniform product.

3.3 Introduction

Patient centered manufacturing instead of a bulk approach is trending in the biopharmaceutical industry [1]. Notably in the fields of oncology, immunology and neurology, biologics are in high demand and their contribution is still growing [2,3]. To ensure flexibility, time, and cost-effective aseptic fill/finish manufacturing at high quality, new machinery with high automation and control is being developed [4,5]. Lyophilization is often required to achieve adequate stability of the biopharmaceutical. Consequently, it is necessary to include lyophilization as part of the fill/finish process in these new flexible units which come with different vial handling compared to standard equipment using robots, disposables, ready-to-use materials, and racks.

Lyophilization is a time consuming and critical step [6]. One challenge in lyophilization is the inhomogeneous heat transfer across a shelf and related edge vial effect. During lyophilization energy can be transferred through direct contact, specifically between vial and shelf, radiation, and gas conduction. Rambhatla noted radiation from the freeze dryer walls as the main driving force for the edge vial effect [7]. It leads to higher product temperature (T_P) during lyophilization and therefore higher potential for collapse. Consequently, freeze-drying processes may be run more conservative than is necessary for the vast majority of product vials. This must be considered most especially for freeze dryers with a cleaning-in-place system.

Nests with vials filled with the liquid formulation are one approach utilized in flexible automated production for transfer into the freeze dryer. This represents both challenges and opportunities which must be thoroughly understood. It is necessary to evaluate heat and mass transfer mechanisms to ensure high quality manufacturing. Especially for transfer and scale-up, understanding of energy transfer is essential to achieve adequate quality of biopharmaceutical products. The basis for determination of sublimation rates is the supposition of a steady state in heat and mass transfer as stated by Pikal et al. [8]. The contributions of gas conduction, direct contact, and radiation in a standard setting have been thoroughly summarized by Brülls and co-workers [9].

The scope of this study is to evaluate the effect of a 120-hole polyether ether ketone (PEEK) vial rack system during lyophilization on energy transfer and T_P during primary drying. The impact of the rack as well as of the lack of direct contact between the vials on modes of energy transfer were analyzed. We determined sublimation rates with water. Additionally, the drying

homogeneity and the edge vial effect in the rack system were investigated. Finally, a second, smaller rack system of different material and dimensions was tested for comparison.

3.4 Materials and Methods

3.4.1 Equipment and Materials

A pilot scale freeze dryer (Hof, Lohra, Germany) equipped with four shelves with 1.0 m2 total surface area was used. In addition to the installed Ni/CrNi thermocouples (type K), a wireless temperature sensor system (iQ-mobile solutions, Holzkirchen, Germany) was utilized.

3.4.2 Vial holding Systems

A commercial polyether ether ketone (PEEK) rack for 6R vials of 30 × 30 cm with 12 × 10 bottomless holes of 2.3 cm diameter, was used (Hof, Lohra, Germany) (Figure 3-1). For all experiments one fully loaded rack holding 120 6R vials was used. Additionally, a flexible 6R vial holding system of 23 × 19 cm with 8 × 6 bottomless holes, was evaluated (Schott, Mainz, Germany) (Figure 3-1) [10]. For temperature mapping, thermocouples were attached to the top of the rack (*n* = 8), chamber wall (*n* = 5, one at the center and four at the corners), and shelves (*n* = 18, 6 on each shelf, two at the center and four at the corners) using Cryo-Babies (sticky labels), and covered with aluminum foil (Figure 3-2).

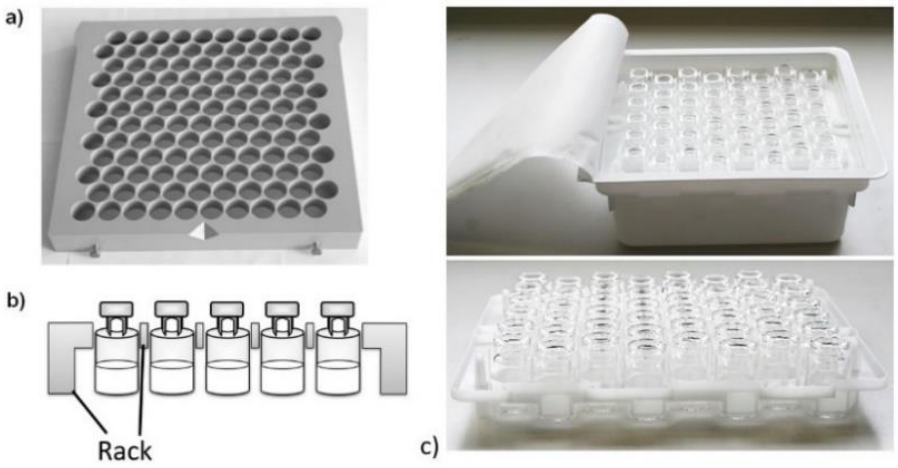


Figure 3-1. Polyether ether ketone (PEEK) rack, (a) top side, (b) cross section, (c) tub with nested vials from Schott, AdaptiQ.

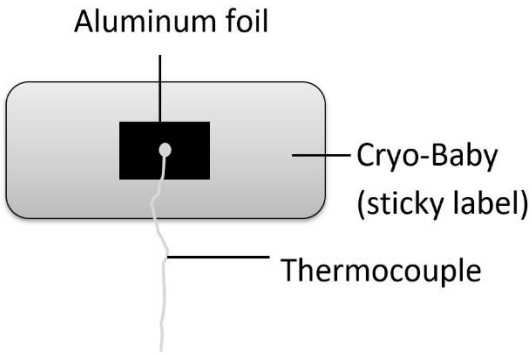


Figure 3-2. Placement of thermocouples with Cryo-Babies.

3.4.3 Excipients

Either water for injection or a placebo composed of 4.6% sucrose/0.23% histidine (both from Merck, Darmstadt, Germany), pH 6.0 formulation containing 0.01% Polysorbate 80 (J.T. Baker, Pa, USA) were used. The 6R vials (Fiolax Clear, Schott AG, Mainz, Germany) were filled with 2.5 mL. Stoppering was automatically performed in the freeze dryer at 0.5 bar nitrogen pressure with 20 mm stoppers (Dätwyler, Schattdorf, Switzerland).

3.4.4 Determination of Glass Transition Temperature Tg' and Collapse Temperature Tc

The glass transition temperature, Tg', of the placebo was measured using Differential Scanning Calorimetry (Netzsch, Selb, Germany) in aluminum crucibles during heating from −75 °C to 20 °C at 10 K/min (n = 3). The collapse temperature, Tc, was measured by freeze drying microscopy (Biopharma Systems, Winchester, UK), cooling the sample to −40 °C at 20 K/min, applying 0.001 mbar vacuum, and heating to 20 °C at 0.25 K/min (n = 5).

3.4.5 Freeze Drying Procedure

Samples were frozen to −45 °C and primary drying was performed at −25 °C, followed by secondary drying at 25 °C both at 0.066 mbar (Table 3-1). All temperature ramps were performed at 1 K/min. Corner vials were defined as vials with fewer neighbors than center vials, which were arranged in a hexagonal neighbor packaging. T_P was measured with thermocouples or wireless sensors placed at the bottom center of the vials according to literature [11].

Table 3-1. Freeze drying cycle for temperature measurement experiments.

Step No.	Time [hh:mm]	Temperature [°C]		Vacuum [mbar]
		Shelves	Condensor	
1 Loading	00:01	20.0	n/a	1000.0
2 Freezing	00:20	0.0	n/a	1000.0
3 Freezing	02:10	0.0	n/a	1000.0
4 Freezing	01:20	-45.0	n/a	1000.0
5 Freezing	3:00	-45.0	n/a	1000.0
41 Evacuation	01:00	-45.0	-85.0	0.066
42 Primary Drying	70:00	-25.0	-85.0	0.066
43 Primary Drying	02:00	-25.0	-85.0	0.066
92 Secondary Drying	00:15	25.0	-85.0	0.036
93 Secondary Drying	08:00	25.0	-85.0	0.036
94 Secondary Drying	00:20	5.0	-85.0	0.036
95 Storage	00:01	5.0	-85.0	0.036

3.4.6 Determination of Sublimation Rates

Sublimation rates (n = 1 both for rack and separated vials) were determined with water by weighing all 120 vials before and after freeze drying. Samples were frozen to −40 °C. Sublimation was performed at 5 °C shelf temperature (T_{shelf}) for 7 h at 0.066, 0.133, 0.200, and 0.267 mbar. Vials were also weighed after freezing and evacuation only. For sublimation rate (dm/dt) determination, the mass loss per vial (m_t) was corrected for the mass loss per vial after freezing and evacuation only (m_e) with t = 7 h according to Equation (1).

$$\frac{dm}{dt} = \frac{(m_t - m_E)}{t} \quad (1)$$

For temperature measurements one wireless sensor for center temperature and one for corner temperature were placed at a corner and a center positioned vial.

3.4.7 Modes of Energy Transfer and Impact of the Rack

To investigate the impact of the rack on heat transfer, T_P was measured in separated vials. For this purpose, vials were placed in the rack and afterwards the rack was removed while the vials remained in the same arrangement. To analyze the contribution of direct contact between shelf and vial, a 0.5 cm Styrofoam (extruded polystyrene) plate was paced under the vials standing in a rack and sublimation rates were determined. The heat transfer coefficient, K_v, was determined from sublimation rate, heat of sublimation of ice (ΔH_s), the vial outer cross-sectional area (A_v), the shelf surface temperature (T_s), and the temperature at the center bottom of the vial (T_b) [8,12] according to Equation (3-2).

$$K_v = \frac{\frac{dt}{dm} \cdot \Delta H_s}{A_v \cdot (T_s - T_b)} \tag{3-2}$$

3.5 Results and Discussion

3.5.1 Characterization of the Solution

The glass transition temperature of the placebo was −30.5 °C with an onset at −32.4 °C. The collapse temperature was similar to −33.0 °C. To stay well below the critical product temperature, the freeze drying cycle mentioned in the method section was employed. Edge vials, which are known as the hot spots in a batch [12] showed a T_P of −35 °C and no collapse during primary drying.

3.5.2 Behavior of the Rack during Rreeze Drying

Temperature mapping showed higher temperature at the corners of the top side of the rack and lower temperatures at the bottom side of the grid differing in temperature by approx. 10 °C (Figure 3-3). Temperatures of the rack, product, and chamber wall are summarized in Table 3-2.

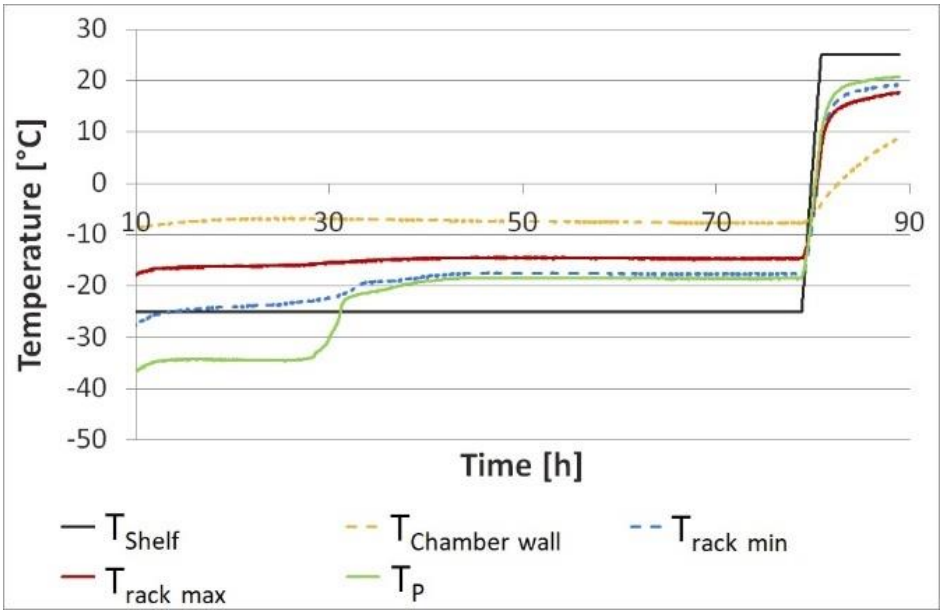


Figure 3-3. Temperature mapping: cold and hot spots of the rack in comparison to T_P and $T_{chamber\ wall}$.

Table 3-2. Temperatures of rack, product, and chamber wall during primary drying.

Position	Temperature [°C]
Top side of the rack	−15
Bottom side of the rack	−23
Shelf	−25
Chamber wall	−8
Product	−35

During primary drying the top of the rack was 10 °C warmer than the shelves and the bottom side of the grid was −23 °C. The rack, especially the outside of the rack is impacted by the radiation coming from the warmer chamber wall. Due to the low heat transfer coefficient of PEEK of 0.25 $\frac{\mathrm{W}}{(\mathrm{m}\cdot\mathrm{K})}$ [13], the rack adapts to T_{shelf} slowly. During primary drying, energy transfer from the rack to the vial via radiation and gas conduction is to be expected. In the primary drying phase, the chamber wall was approx. 10 °C warmer than the rack which itself is warmer than the product by 10–20 °C. At the same time the massive rack reduced radiation from the wall directly onto the product, potentially reducing the edge vial effect.

3.5.3 Modes of Energy Transfer in Separated Vials

Separated vials, meaning vials positioned on a shelf with the aid of the rack but with the rack removed after positioning, showed an earlier beginning of the endpoint of sublimation in primary drying (Figure 3-4). Temperatures of the rack, the chamber wall and product in both the rack and separated vials are summarized in Table 3-3.

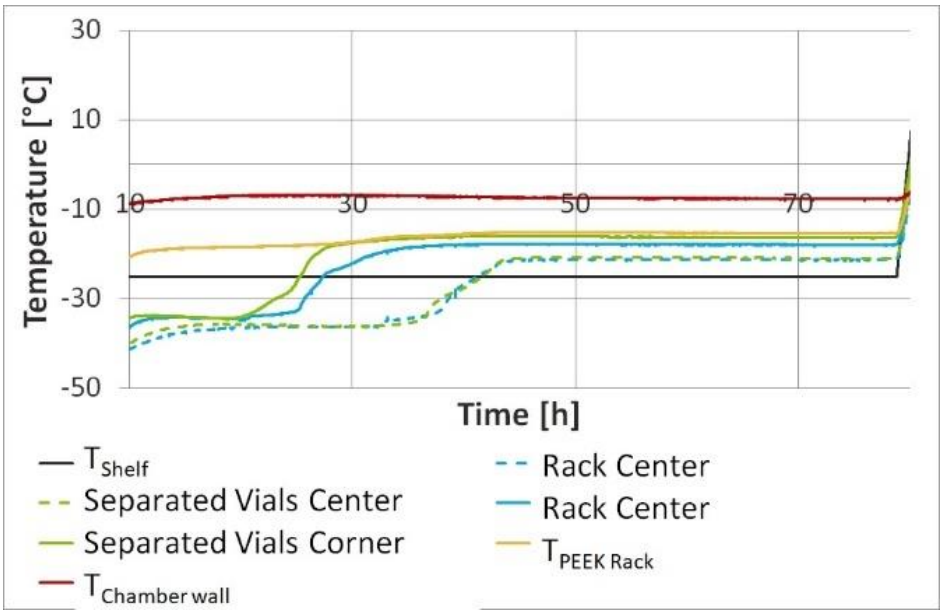


Figure 3-4. T_P of separated vials compared to T_P of vials in the rack. Definition of separated vials: vials positioned on a shelf with the aid of the rack but with the rack removed after positioning.

Table 3-3. Measured T_P in the rack compared to separated vials.

Position	Temperature (°C)		End of Steady State Phase (h)	End of Primary Drying (h)	Difference between Corner and Center at the End of Primary Drying (%)
	During Steady State Phase	At the End of Primary Drying			
Rack	−20	−15	n/a	n/a	n/a
Shelf	−25	−25	n/a	n/a	n/a
Chamber wall	−8	−8	n/a	n/a	n/a
Separated vials corner	−33	−15	20	27	40
Separated vials center	−35	−21	33	45	
Rack corner	−33	−18	25	33	27
Rack center	−35	−21	33	45	

In both settings the center vials had a similar behavior during freeze drying and reached an endpoint after 45 h. For corner vials in the rack the steady state of primary drying ended after 25 h as compared to 20 h for separated vials. In separated vials the energy transfer in primary drying mainly involves direct contact with the shelf, radiation coming from the chamber wall, and gas convection (Figure 3-5).

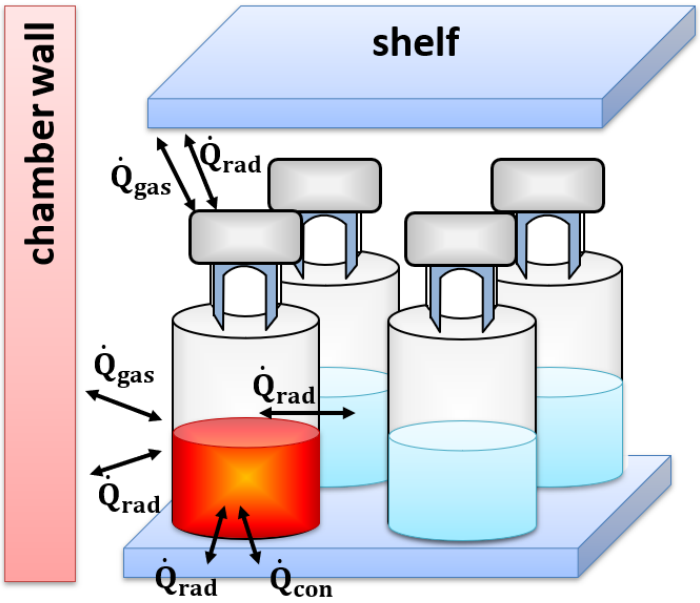


Figure 3-5. Energy transfer of a separated corner vial.

Lacking a radiation shield, separated corner vials dried at a 3 °C higher temperature during primary drying as compared to vials standing in a rack. The difference in drying time between corner and center vials in the rack was 27% as compared to 40% in the case of separated vials without a rack. Thus, the rack enables a homogeneous T_P and primary drying of corner and center vials.

3.5.4 Energy and Mass Transfer in a Rack System

Sublimation rate mapping of vials filled with water in the rack at 0.066 mbar chamber pressure and T_{shelf} of −25 °C showed that the mass transfer in corner vials is 24% higher than in center vials (Figure 3-6).

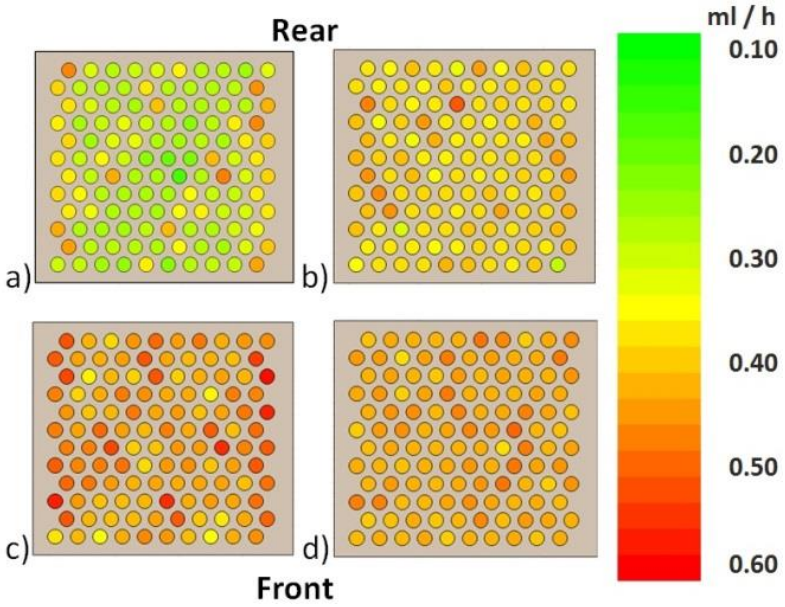


Figure 3-6. Mapping of sublimation rates in the rack at (a) 0.066 mbar, (b) 0.133 mbar, (c) 0.200 mbar, and (d) 0.267 mbar.

Corner vials left and right showed higher sublimation rates and 3–4 °C higher T_P compared to corner vials at the front and rear sides. The mean sublimation rates at 0.066, 0.133, 0.200, and 0.267 mbar (n = 1) are shown in Figure 3-7.

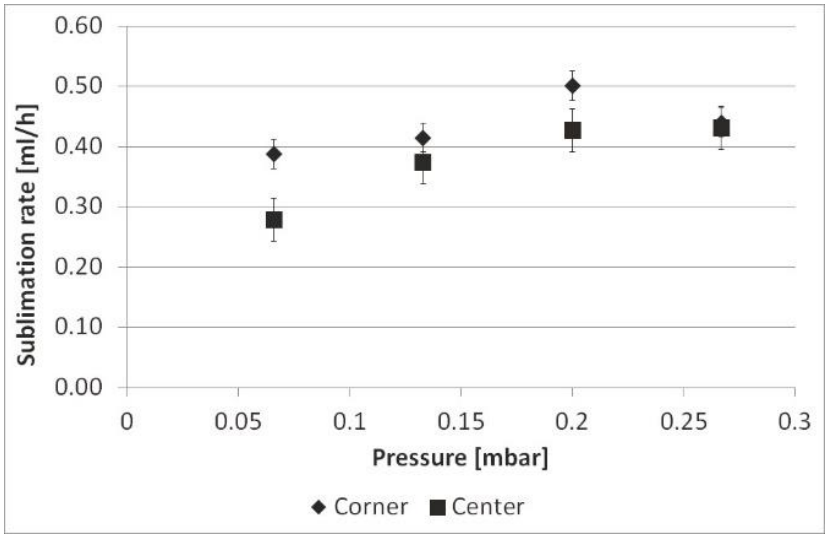


Figure 3-7. Mean sublimation rates in the rack at 0.066 mbar, 0.133 mbar, 0.200 mbar and 0.267 mbar.

As illustrated by Pisano et al., sublimation rates increase with increasing pressure [14]. At 0.066 mbar the sublimation rates between corner and center differed by 28 %. These differences diminished with a higher pressure of 0.267 mbar to 2.3%. Due to a lack of direct contact between the vials, gas molecules are able to reach edge and center vials in the same

manner. With increasing pressure, gas conduction becomes a stronger contributor to heat transfer [9], resulting in reduced edge effect. Separated vials in the same arrangement without a rack showed higher sublimation rates as they were not shielded from radiation (Figure 3-8).

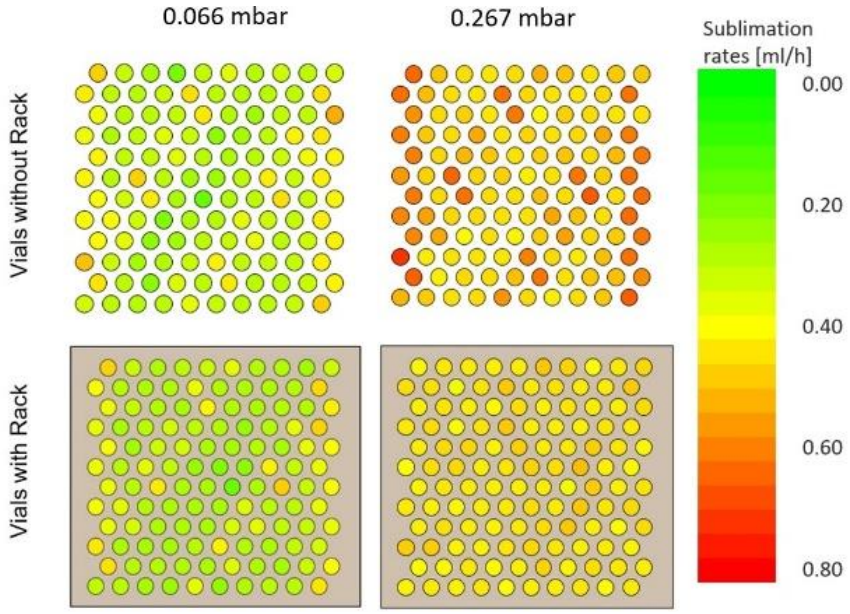


Figure 3-8. Sublimation rate mapping for vials with and without a rack at 0.066 mbar and 0.267 mbar.

The difference between corner and center vials was 31% at 0.066 mbar, which decreased to 22% at 0.267 mbar.

Ganguly et al. found a 17% contribution from direct contact between vial and shelf to the total heat transfer at low pressures which decreased to 10% at high pressures [15]. An additional experiment with a Styrofoam plate placed under the vials was performed. Due to the low thermal conductivity of 0.029 $\frac{\mathrm{W}}{\mathrm{m}^2 \cdot \mathrm{K}}$ [16], the Styrofoam plate was assumed to minimize energy transfer via direct contact to a minimum. The sublimation rate of 0.066 mbar decreased by 28% for corner vials and 54% for center vials (Figure 3-9).

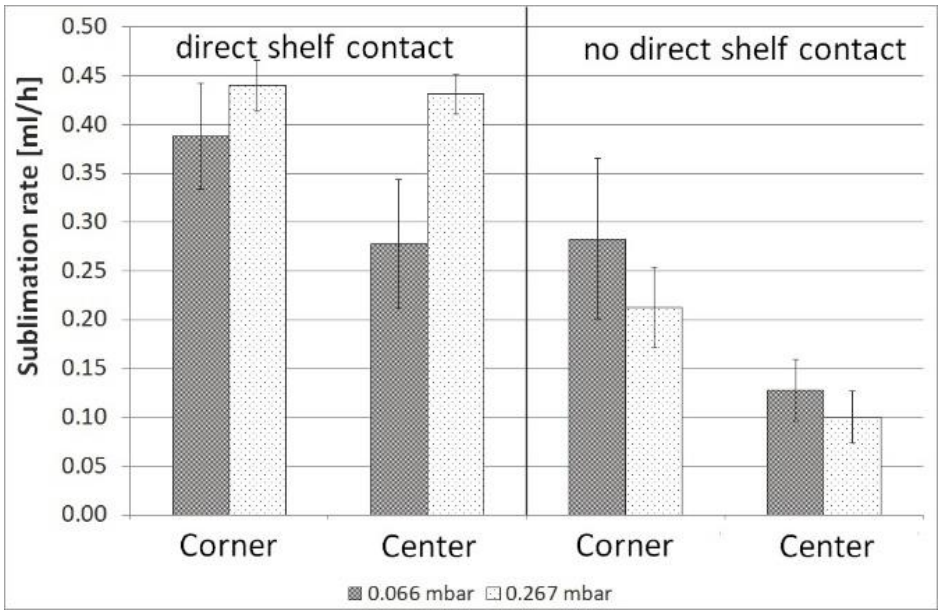


Figure 3-9. Mean sublimation rates in center and edge vials, either with direct contact to the shelf or without, at 0.066 mbar, error bars represent standard deviation (*n* = 10).

Therefore, the contribution of direct contact to the total heat transfer in the rack can be assumed to be 42% on average. At 0.267 mbar, sublimation rates decreased by 52% in corner vials and by 77 % in center vials when direct contact between vial and shelf was prevented by a Styrofoam plate. Contribution of direct contact to total heat transfer is higher at higher pressures. In the rack, the heat transfer via direct contact is reduced to a vial-to-shelf contact. For center vials, the impact of radiation from the chamber wall becomes negligible due to surrounding vials.

3.5.5 Comparison of the Rack System to Another Nested Vial System

The utilization of flexible small-scale manufacturing lines makes vial holding systems necessary. We consequently evaluated another flexible holding system of different geometry and made of polyoxymethylene (POM) instead of PEEK. The smaller POM nest system had no contact with the shelf and no band surrounding the vials. Including 24 corner and 24 center vials, the ratio of corner to center is higher compared to the rack, which includes 40 corner and 80 center vials.

Compared to corner and center vials standing in the PEEK rack system, for which primary drying ended after 24 h and 36 h respectively, the steady state of primary drying ended 10 % earlier, after 21 h for corner and 31 h for center vials, in the flexible POM nest (Figure 3-10).

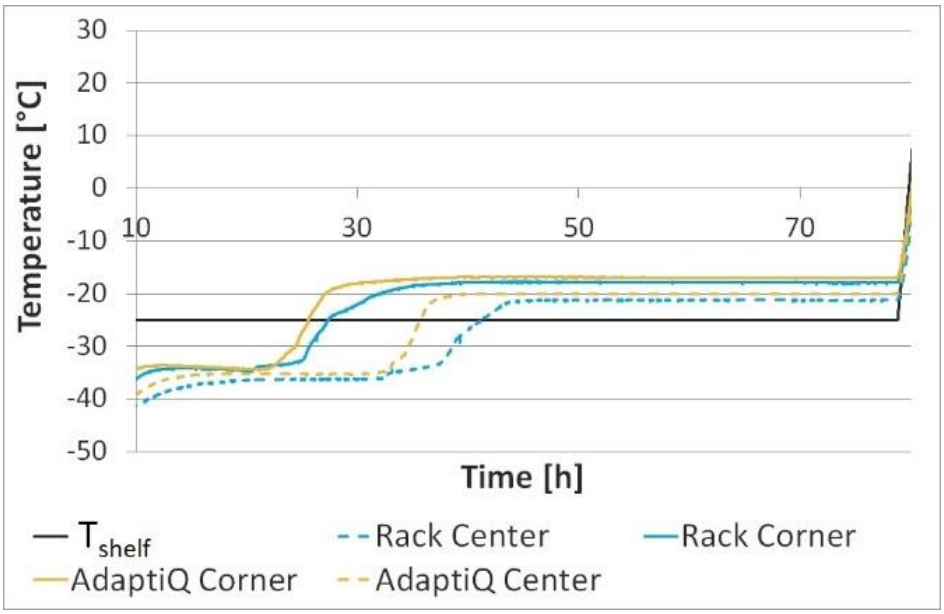


Figure 3-10. T_P of vials in a rack and AdaptiQ nest.

There is less shielding from radiation provided by the POM nest compared to the rack. In AdaptiQ, radiation coming from the chamber wall is able to impact vials at the corners. Therefore, AdaptiQ corner vials behave similarly to separated corner vials without a rack.

3.6 Conclusions

Heat transfer for sublimation in vials nested in a rack system is dominated by direct contact between vial and shelf and radiation coming from the rack itself. Heat transfer through direct contact is limited to contact between vial and shelf. Contribution of direct contact is higher and radiation effect from the chamber wall is less than in the standard configuration. This allows a reduction in the difference of T_P between corner and center vials from 39 % to 27 %. Separated corner vials without a rack showed a 6 h shorter primary drying time as they lacked the radiation shielding provided by the rack. With increasing pressure, the difference in sublimation rates between corner and center vials in the rack decreased due to a higher contribution of gas conduction, leading to a reduced edge-vial-effect. Compared to another smaller, more flexible, nested vial system without shelf contact, the primary drying time is reduced by 10 %. Finally freeze drying of vials nested in the rack system is an important tool in flexible manufacturing units which require a good understanding of heat transfer. They can provide a controlled heat transfer with reduced edge vial effect. Future research will investigate a 1:1 comparison of rack with bulk setting and focus on transfer of freeze-drying cycles within and between different vial arrangements.

3.7 References

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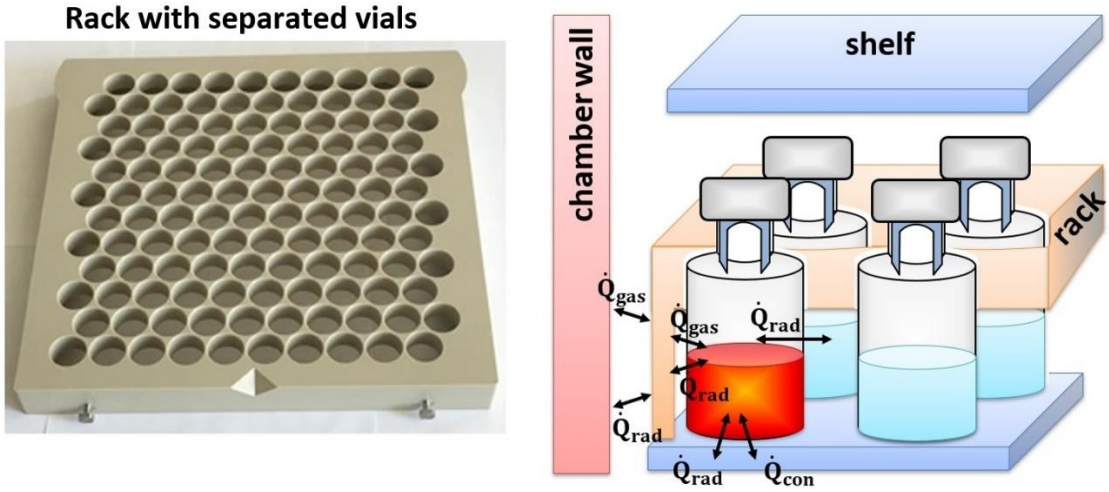
CHAPTER 4 Chapter 4: Process Optimization and Transfer of Freeze-drying in Nested Vial Systems

The following chapter has been published in the European Journal of Pharmaceutics and Biopharmaceutics and appears in this thesis with the journal`s permission:

Sarah Ehlers (née Daller), Rudolf Schröder and Wolfgang Friess

DOI: 10.1016/j.ejpb.2021.01.002

4.1 Graphical abstract



Keywords: Lyophilization, freeze-drying, rack system, nested vial, heat transfer, sublimation rates, edge-vial-effect, product temperature, radiation, direct contact

4.2 Abstract

Scale-up and transfer of freeze-drying processes is a crucial challenge in biopharma industry. With the success of small batch processing lines utilizing rack vial holding systems, further detailed knowledge about freeze-drying cycles and their scale-up for vials in a rack is required. Therefore, product temperature (T_P) profiles as well as Kv values of vials nested in a Polyetheretherketon (PEEK) rack were compared to those of vials placed in a commonly used stainless steel tray. Additionally, both setups were challenged with varying fill volume and partially versus fully loaded rack. Additionally, a process developed for rack was compared to a tray freeze-drying cycle.

Freeze-drying in vials placed in the rack is markedly faster for center vials and more homogeneous compared to vials in bulk tray setting, as indicated by T_P and Kv values. Due to the more homogeneous drying the rack is more flexible regarding variation of the fill volume. The key point for the transfer of a freeze-drying cycle from rack to tray is to consider the higher sublimation rates in the rack by adapting chamber pressure or shelf temperature for the tray. Furthermore, transfer from one rack per shelf in a laboratory freeze-dryer to pilot scale with four racks per shelf was successful. Thus, understanding of the process in rack and tray setup was enhanced to ensure efficient scale-up and transfer of freeze-drying processes.

4.3 Introduction

Freeze drying of biopharmaceuticals is a common technique to achieve high quality for these sensitive products. [1] Typically, a freeze-drying cycle has to be scaled-up from laboratory development to clinical and commercial manufacturing. Scale-up and transfer of freeze-drying processes is challenging as various parameters affect the process and product e.g. freeze-dryer size, design and load, fill volume of the vial, vial arrangement as well as formulation characteristics like glass transition temperature and solid content. [2-4] Different modeling techniques were developed to avoid trial and error experiments in process development and scale-up. [5-7] A new situation arises with the utilization of fully automated fill/finish lines with rack-type vial holding systems which separate the vials on the shelf. [8] Development and transfer of freeze-drying processes with rack systems, e.g. made out of PEEK, is rarely analyzed and has to be further investigated with focus on variations of process parameters, transfer between different holding systems and scale-up. [9]

Since freeze-drying is an endothermic process, energy has to be transferred from shelf to vial and from vial to product. There are three heat transfer mechanisms: gas conduction K_g, direct contact K_c and radiation K_r. [4, 10-12] K_c typically includes direct contact between neighboring vials as well as between vial and shelf. In the rack system direct contact between vials is not given. Especially the rate limiting center vials are supposed to dry faster compared to a tray setup, where a center vial has a direct energy exchange with six neighbors. Due to the separation effect of the rack, there is more space for gas molecules, which can easily reach every vial in the rack setting. Thus, K_g could have a stronger contribution to the vial heat transfer at higher pressures. Furthermore, the rack acts as a radiation shield against radiation mainly coming from the chamber wall. Therefore, radiation from the chamber wall is supposed to have a reduced effect in a rack setting. [13]

Additionally, the drying rate depends on the product resistance which itself is impacted by the formulation and the fill height. [14] In a rack setup the shielding and vial to rack contact changes over the product height. Therefore, the impact of the fill volume has to be taken into consideration. If material is limited, process optimization might be performed in a partial load setting, where product containing vials are either surrounded by vials filled with placebo or the rest of the rack is left empty. The neighboring vials heavily impact each other. [15] Additionally, an edge vial of a full vs. partial load standing closer to the chamber wall could receive more radiation. Using a small rack this effect could be even more pronounced

depending on the positioning of the rack on the shelf. But the rack is at the same time assumed to be a radiation shield. Moreover, it has been shown that the pressure may be higher in the center of the shelf compared to the outer regions. [5] Therefore, it is crucial to compare the product temperature (T_P) of a partial load with a full load with a focus on the edge vials. [16] The purpose of this study was to characterize freeze-drying of vials separated in a rack system. Therefore, the rack setup was confronted with the following challenges:

a) varying fill volume,
b) partial load vs. full load without surrounding vials or with surrounding placebo vials,
c) varying chamber pressure (p_C), and
d) varying shelf temperature (T_{shelf}).

We focused on rack temperature, primary drying time and T_P. Furthermore, K_V was evaluated at different pressures and the progression of primary drying was further characterized by measuring T_P at vial top, middle, and bottom, assuming that the end of primary drying is characterized by convergence of T_P and T_{shelf}. T_{shelf} and p_C were optimized for a rack freeze-drying process and compared to a tray setup. Scale-up from one rack per shelf to four racks on a shelf was performed.

4.4 Materials and Methods

4.4.1 Materials

If not stated otherwise 2.5 ml of an aqueous solution composed of 4.6 % sucrose (low endotoxin sucrose, Merck, Darmstadt, Germany), 0.23 % L-histidine (Merck) and 0.01 % Polysorbate 80 (J. T. Baker, PA, USA), pH 6.0 (pH adjusted with 0.1 % HCl) was filled into 6R vials (Schott AG, Mainz, Germany) (Tg': -30.5 °C, onset: -32.4 °C). 20 mm diameter lyo stoppers (brombutyl rubber stopper, siliconized, Datwyler, Schattdorf, Switzerland) were used.

4.4.2 Freeze drying unit

Lyophilization experiments were performed in a pilot scale freeze dryer (HOF Sonderanlagenbau GmbH, Lohra, Germany) equipped with four shelves and with a total surface area of 1.0 m^2. For scale-up a freeze dryer (HOF Sonderanlagenbau GmbH, Lohra,

Germany) with 4 shelves and 1.5 m^2 total surface area linked with a semi-automatic filling line was used. Both freeze dryers were equipped with capacitance manometers for pressure control and Pirani gauge. T_P measurements were performed with wireless sensors (Tempris®, iQ-mobile solutions GmbH, Holzkirchen, Germany) and Thermocouples at the center bottom of the vials. The mean value of minimum 4 temperature measurements was documented.

4.4.3 Vial setup in bulk tray and rack system

The 300 x 300 mm bottomless PEEK rack (HOF Sonderanlagenbau GmbH, Lohra, Germany) can hold 120 6R vials, equipped for 40 edge and corner vials and 80 center vials as shown in Figure 4-1.

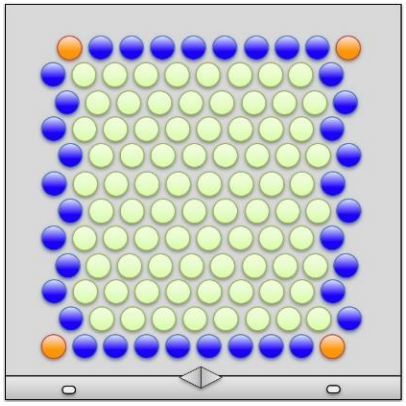


Figure 4-1: Schema of a PEEK rack.

For bulk tray setting a stainless-steel frame of 470 x 245 mm and 15 mm height surrounded 252 6R vials, 65 edge and 187 center vials. Center vials are defined as vials surrounded by six other vials, whereas edge vials lack this hexagonal surrounding. Corner vials form a subset of the edge vials and have only two to three neighbor vials.
In the current work, partial load is defined as 14 6R vials filled with placebo placed in the rack and the bulk system respectively (Figure 4-2).

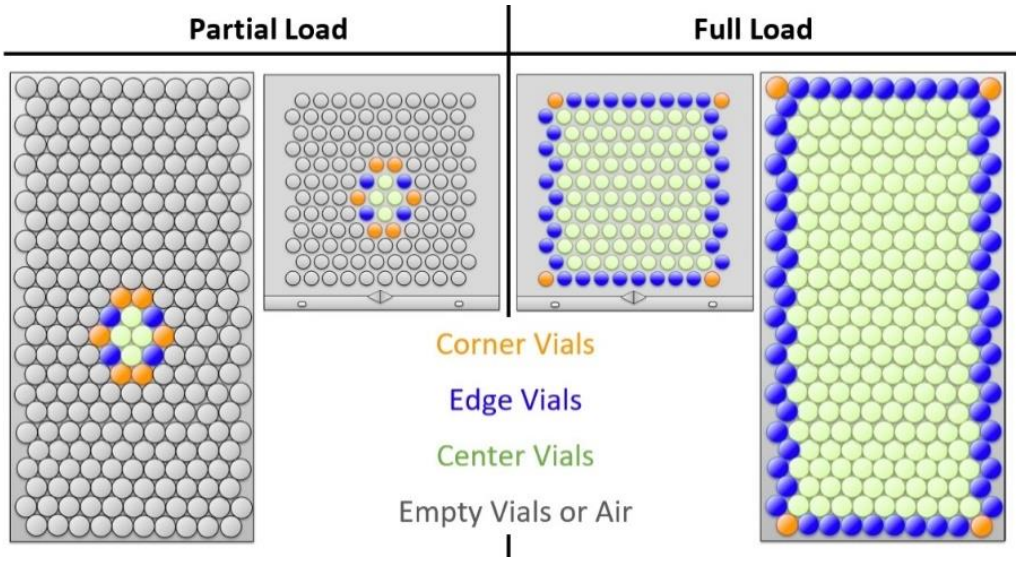


Figure 4-2. Position and definition of corner, edge and center vials.

In this experiment a corner vial is defined as a vial standing at the corner of the 14-vial cluster. Full load is defined as rack loaded with 120 placebo vials and tray filled with 252 placebo vials respectively.

4.4.4 Freeze-drying process

Vials were frozen to -45 °C. After 2 h hold, primary drying was performed at T_{shelf} of -25 °C and p_C of 0.066 mbar. Secondary drying was performed at T_{shelf} of 25 °C and p_C of 0.066 mbar. To evaluate the impact of rack position on the shelf, primary drying was performed with T_{shelf} at -20 °C and p_C at 0.133 mbar. All temperature ramps were performed at 1 K/min. Vials were automatically stoppered at 0.5 bar nitrogen partial pressure.

Convergence of T_P and T_{shelf} marks the end of primary drying (EPD). [17] In this study EPD was determined by the inflection point of the product temperature curve. Furthermore, the plateau at the beginning of primary drying was determined and called steady state in this study. EPD could also be identified by comparative pressure measurement as, Pirani gauge and capacitance manometer converge [18, 19] but this batch method cannot distinguish between center and corner positions.

To further evaluate the rate of sublimation thermocouples were placed at bottom, half and top fill height in selected vials.

In order to study the impact of p_C and T_{shelf} on T_P during primary drying, p_C was set to 0.066 mbar, 0.133 mbar, 0.200 mbar and 0.267 mbar respectively at a constant T_{shelf} of -25 °C (Table 4-1).

Table 4-1: Experimental setting to determine the impact of p_C and T_{shelf} on T_P.

	p_C [mbar]	T_{shelf} [°C]
Study 1	0.066	-25
	0.133	
	0.200	
	0.267	
Study 2	0.066	-25
		-20
		-15
		-10

In a second study, T_{shelf} was set to -20 °C, -15 °C and -10 °C at a constant p_C of 0.066 mbar.

4.4.5 Kv and residual moisture determination

For Kv determination vials filled with 5.0 ml deionized water were weighed before and after drying for 4 h at T_{shelf} of 5 °C and p_C of 0.066, 0.133, 0.200 and 0.267 mbar [19], [20] in rack and bulk tray.

Residual moisture was measured with Karl Fischer (874 oven sample processor, Metrohm, Herisau, Germany) with a heating temperature of 100 °C in the oven method according to literature. [21, 22] Differences between tray and rack setting were further characterized by stopping selected freeze-drying process already after primary drying.

For both Kv and residual moisture values a one-way analysis of variance (one-way ANOVA) was performed with a significance level of $\alpha < 0.05$.

4.5 Results and Discussion

4.5.1 Drying behavior of vials nested in a rack system compared to bulk setting

At first, heat transfer and drying behavior of vials in a 300 x 300 mm full rack positioned in the center of the shelf as compared to a full tray with vials occupying the whole shelf were determined. At the beginning of primary drying, the chamber wall was much warmer than the shelfs leading to a substantially higher rack temperature than T_P (17 °C) (Figure 4-3).

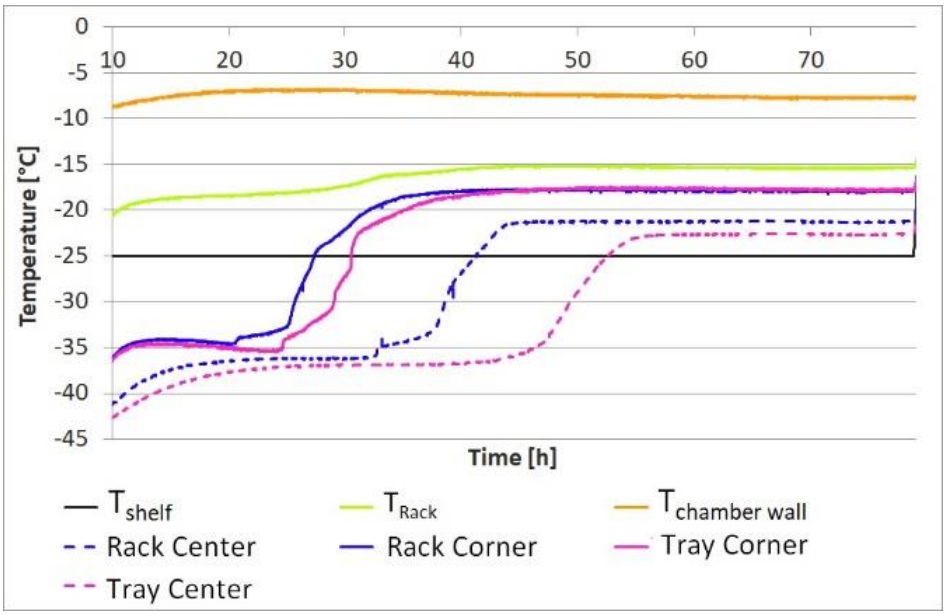


Figure 4-3. Product temperatures of vials in rack and bulk tray setup during primary drying.

Due to radiation effects T_P of corner vials both in rack and tray at the end of primary drying was 7 °C above T_{shelf}. For corner vials the T_p in the steady state of primary drying and EPD of rack and tray were similar. In contrast, the center vials in the rack always ran about 1-2 °C warmer than in the tray and EPD was reached after 39 h in the rack and after 50 h in the tray. Tray center vials have direct contact to six neighboring vials, which cool each other. In comparison the spacing between the vials in the rack reduces this cooling effect which increases the primary drying rate substantially. [15] The corner vials in the tray have only 2-3 neighbors which substantially reduces the cold neighbor influence in combination with enhanced radiation from the chamber wall. Additionally, vial separation of the rack and the rack itself acting as a radiation shielding enables rack vials to dry faster than tray vials. This brings the drying rate close to the situation in the rack with limited neighborhood. However, with the smaller rack positioned in the center of the shelf, rack corner vials have a larger

distance to the chamber wall compared to tray corner vials and therefore be less affected by radiation coming from the warm chamber wall. We therefore additionally tested freeze-drying in the rack positioned at the edge of the shelf. T_P and EPD were not significantly affected by the position of the rack (Figure 4-4).

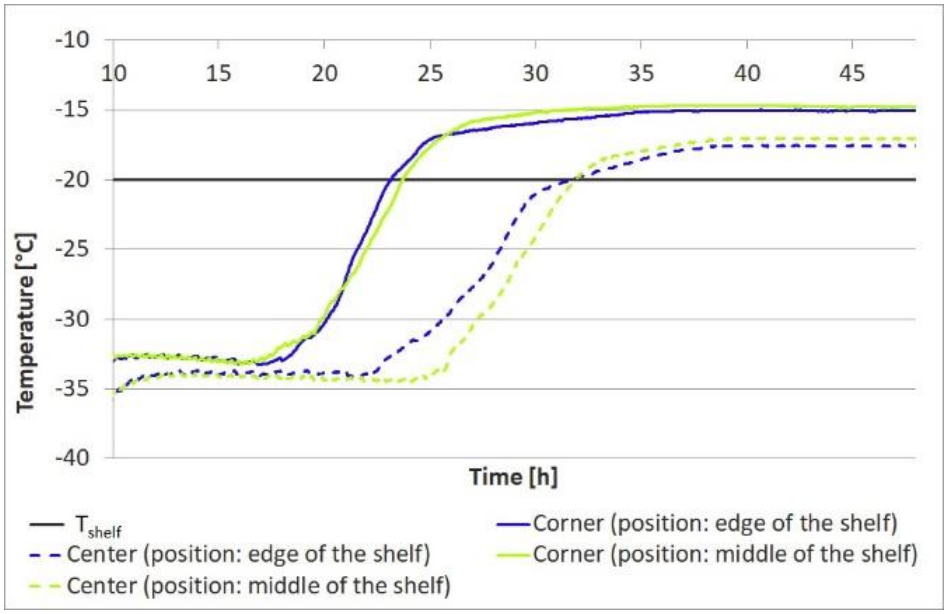


Figure 4-4. T_P during primary drying: rack positioned at the edge or at the middle of the shelf.

We additionally determined Kv values with water in the tray setting at different pressure settings operating at a higher T_{shelf} to reach the steady state faster. The Kv values were compared to them in the rack system. [13] Kv values were higher for corner vials compared to center vials both in rack and tray and overall, the Kv values for vials in the rack were 10 to 15 % higher than for the vials in the tray (Figure 4-5).

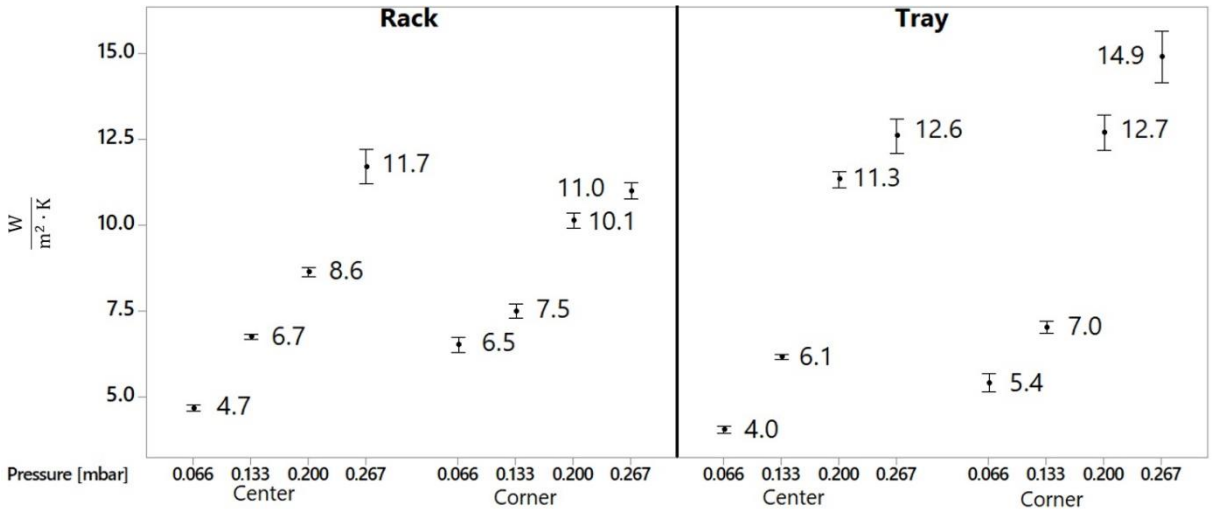


Figure 4-5. Kv values determined with water of corner and center vials in the tray compared to the rack.

With increasing pressure gas conduction improves and Kv both in rack and tray increases. The vials ran 1 – 2 °C warmer in the rack leading to a substantially higher sublimation rate closing the gap between center and corner vials.

We further characterized the drying behavior by placing thermocouples at top, middle and bottom of vials with 5.0 ml fill volume. Temperature curves of rack and tray vials were overall similar for center vials and drying was longer in the tray setting (Figure 4-6).

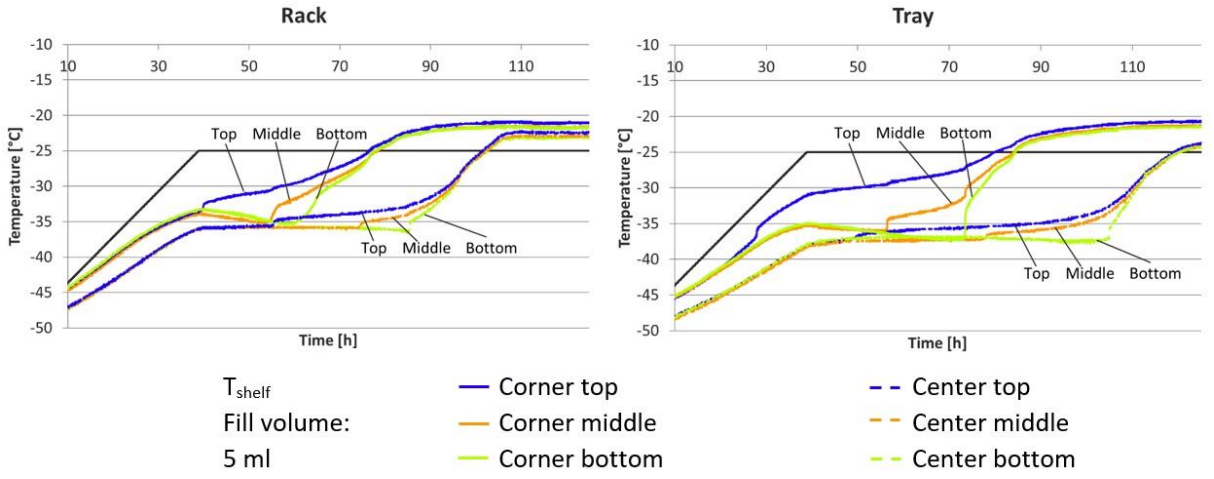


Figure 4-6. Temperature profile during primary drying at top, center and bottom of corner and center vials in rack and tray setting.

In corner vials, middle and bottom ran rather similar. However, we noticed a difference in the top region. At 5.0 ml fill volume in 6R vials the upper level is at the height of the warmer rack

plate and therefore this top region ran at significantly higher temperature and dried much faster.

Since the temperature at the end of primary drying was rather different between the rack and the tray setup we additionally analyzed the residual moisture of the cakes. The residual moisture level was only slightly higher and only a little bit less homogeneous in the colder tray vials with 4.4 ± 0.43 % compared to 4.2 ± 0.34 % (Figure 4-7).

In summary, the differences between corner and center vials in the rack are less than in a tray. Since center vials dry markedly faster in the rack than in the tray, the total freeze-drying time is shorter. In addition, T_P is more homogeneous. But this comes at the expense of a reduced number of vials per shelf area.

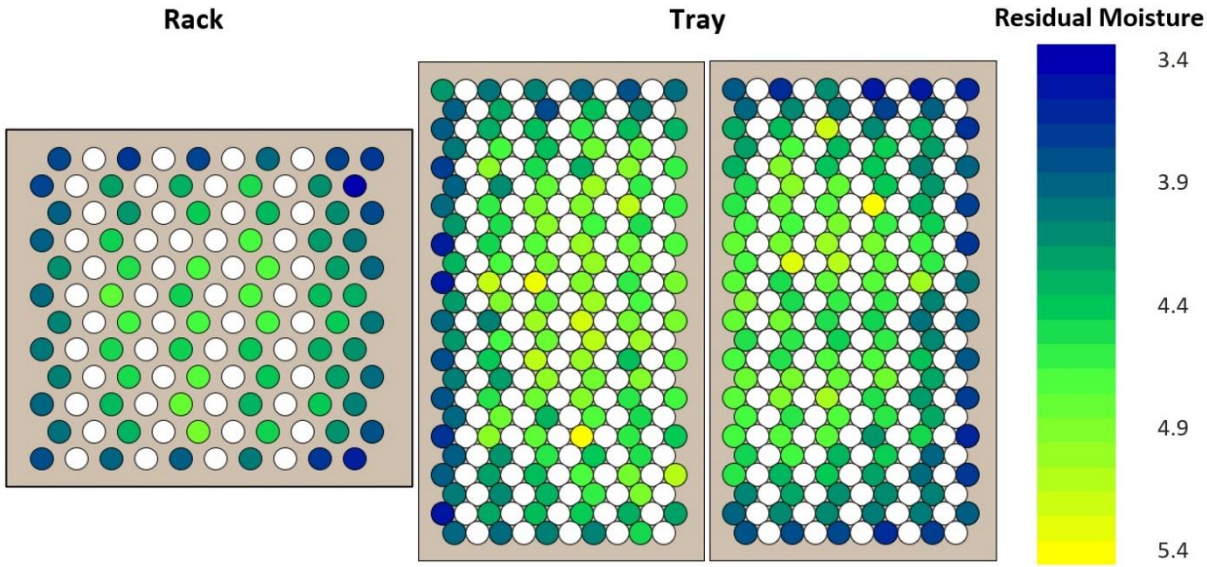


Figure 4-7. Residual moisture mapping after primary drying.

4.5.2 Evaluation of rack process parameters for different fill volume and partial load

Due to the higher temperature in the top region of a 6R vial with extremely high fill volume of 5.0 ml reaching up to the rack plate, we further challenged and compared rack and tray by drying with 1.0 ml / 3 mm, 2.5 ml / 7 mm and 5.0 ml / 13 mm fill volume /height. Overall, the T_P profiles in rack and try ran rather similar (Figure 4-8).

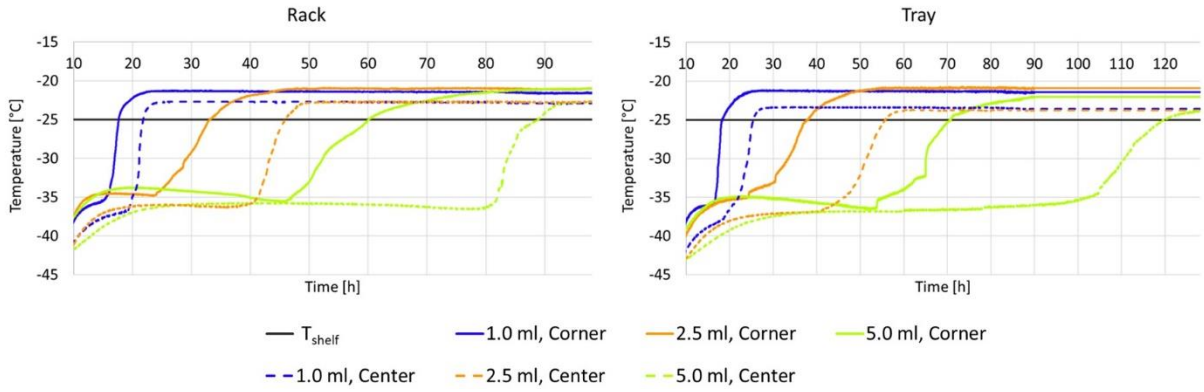


Figure 4-8. T_P of vials with different fill volume (1.0 ml, 2.5 ml, 5.0 ml).

Rack vials ran consistently at slightly higher T_P. Corner vials went into steady state within a few hours. It took much longer for center vials, specifically for the vials in the tray. Primary drying was finished earlier at low fill volumes and the differences between center and corner as well as between rack and tray became more pronounced with higher fill volume (Table 4-2).
EPD difference between center and corner vials for 5.0 ml in rack setup with 84 h vs. 54 h was less than in tray setup with 110 h vs. 65 h. Thus, the overall drying behavior in the rack is not critically affected by the fill volume despite that the product reaches the level of the rack plate and transfer between tray and rack should be similar for different fill volumes.

Table 4-2: PD for rack and tray corner and center vials with varying fill volume.

Position		Fill volume [ml]	EPD [h]
Rack	Corner	1.0	17
		2.5	31
		5.0	54
	Center	1.0	21
		2.5	44
		5.0	84
Tray	Corner	1.0	18
		2.5	35
		5.0	65
	Center	1.0	24
		2.5	51
		5.0	110

In many cases material is limited in process development and freeze dryers are only partially loaded with product vials. The remaining space directly surrounding the product vials could be left empty or the vial holding system is filled up with buffer containing vials. The usage of buffer containing vials implies substantial additional time and resource consumption. But it is clearly necessary for a representative drying process because of shielding and temperature effect of the neighbor vials. [15] In the rack, the vials are not in direct contact with each other and potentially empty positions do not have to be filled with placebo vials but instead could be filled with empty vials or left completely empty. Therefore, a process was run in partial load with remaining space either left empty, or filled with empty vials, and compared to a fully loaded rack. Difference in T_P between corner and center vials during steady state disappeared in both partial load settings without additional vials or with empty vials both in rack and tray. Center vials dried much faster than in a full load setting (Figure 4-9).

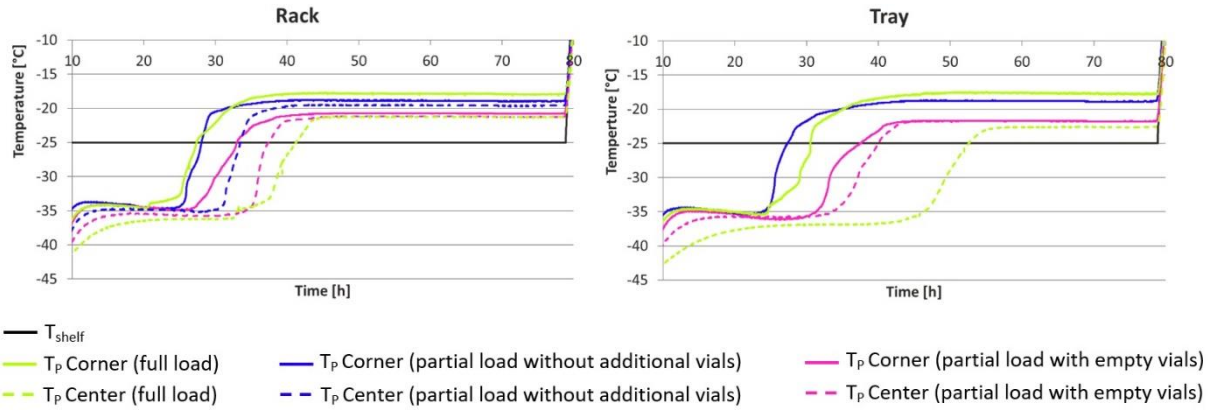


Figure 4-9. T_P during primary drying of vials in a full load or partial load setting, rack compared to tray.

Corner vials in partial load setting with no additional vials behaved like corner vials in a full load setting in both rack and tray. Thus, despite spacing between the vials, a rack which is only partially equipped with product vials should always be filled up with placebo vials to have not only representative corner vial but also center vial behavior.

4.5.3 Optimization, transfer and scale-up of freeze-drying in a rack

To further understand the impact of chamber wall radiation and the effect of the rack temperature we extended the freezing hold at -45 °C from 3 h48 h in order to reduce the chamber wall and rack temperature during primary drying. Although the wall temperature

was approx. 10°C lower at the beginning of primary drying in the modified cycle, T_{rack} at the beginning of primary drying did not differ (Figure 4-10). It took about 20 h longer for T_{rack} to come into the plateau phase but this slight difference did not result in a change in EPD. Although the rack temperature affects the product temperature upon very high filling, it did not impact the product at a regular fill.

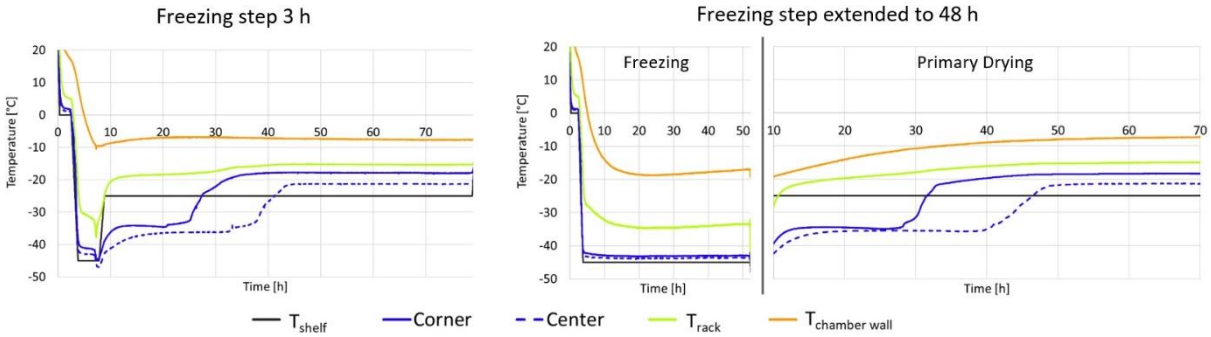


Figure 4-10. Temperature curves of corner and center vials in a rack; 3 h freezing step (left) compared to freeze-drying cycle with 48 h extended freezing step (right).

In order to further characterize and optimize the freeze-drying process in the rack system, p_C at a T_{shelf} of -25 °C and T_{shelf} at a p_C of 0.066 mbar were systematically varied in primary drying. T_P of corner vials ran at -32 °C in steady state during primary drying at higher T_{shelf} = -15 °C or higher p_C = 0.200 mbar both in rack and tray and showed shrinkage and minor collapse as T_P exceeded the onset of Tg' at -32.4 °C (Figure 4-11). Both in rack and tray an increase of p_C or T_{shelf} resulted in significantly faster drying which is known by literature. Additionally, there was a slightly reduced difference in EPD between corner and center vials (Table 4-3).

Table 4-3: EPD of rack and tray corner and center vials at different T_{shelf} and p_C.

T_{shelf} [°C]	p_C [mbar]	EPD Rack [h]		EPD Tray [h]	
		Corner	Center	Corner	Center
-25	0.066	27	39	30	50
	0.133	25	33	30	38
-20	0.066	26	35	29	42
	0.133	22	29	22	34
	0.200	20	23	21	31
	0.267	19	24	22	31
-15	0.133	20	25	21	29
-10	0.133	19	26	19	24

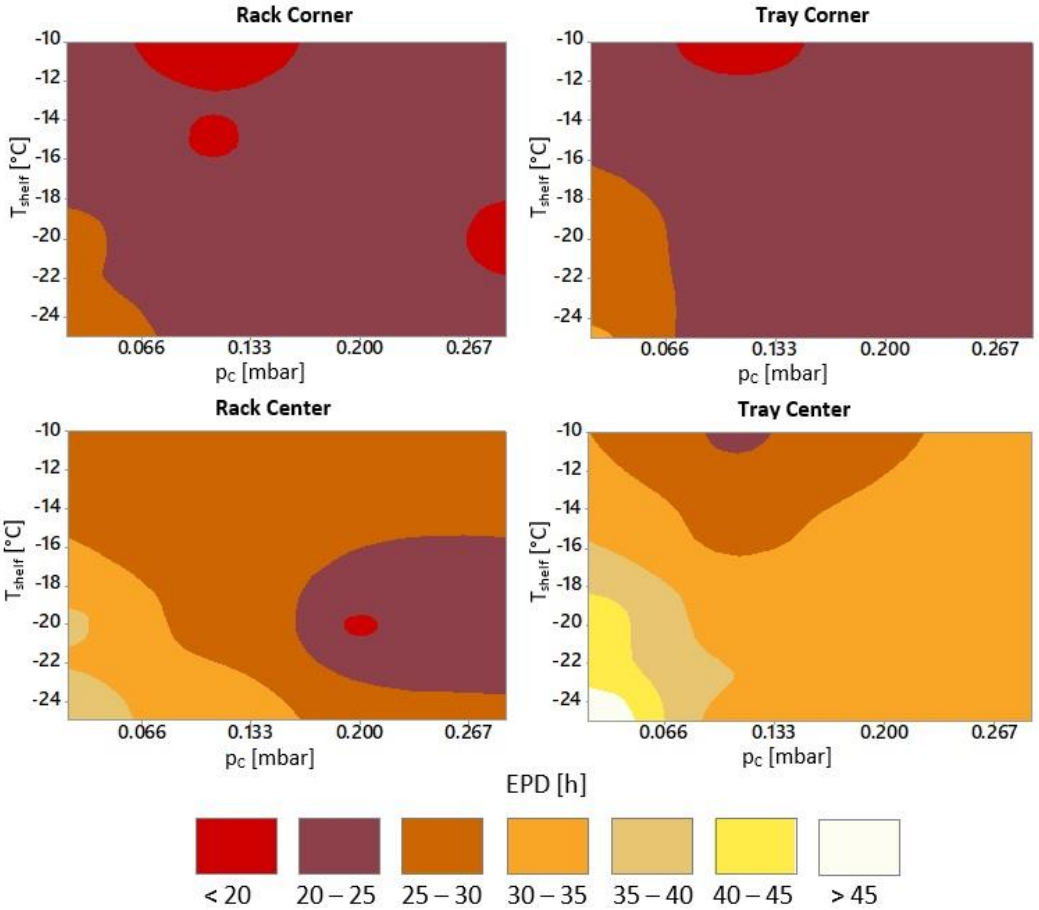


Figure 4-11. Impact of p_C and T_{shelf} on EPD; rack compared to tray.

Rack center vials showed a higher impact of pressure than T_{shelf} variation on EPD compared. The shortest EPD of 29 h for the limiting center vials and a T_P below Tg' of the critical corner

vials at -33 °C was possible with a p_c of 0.133 mbar and a T_{shelf} of -20 °C. With this setting total primary drying time was reduced by 10 h compared to the previous standard cycle with a p_c of 0.066 mbar and a T_{shelf} of -25 °C. This setup established with one rack per shelf was additionally tested in a 1.5 m^2 pilot freeze dryer with 4 racks placed on a shelf. Center vials showed similar T_P profiles in the laboratory and in the pilot scale setup (Figure 4-12). Corner vials in the pilot scale experiment reached EPD 3 h earlier than in laboratory experiment. The upscaled vials showed elegant cakes without shrinkage or cracking.

For a transfer of a rack cycle to bulk setting two key points have to be taken in account: stronger edge-vial-effects due to lacking radiation shielding and longer primary drying time of center vials due to closer vial packing in the tray. This may make adjustment of T_{shelf} and p_C necessary.

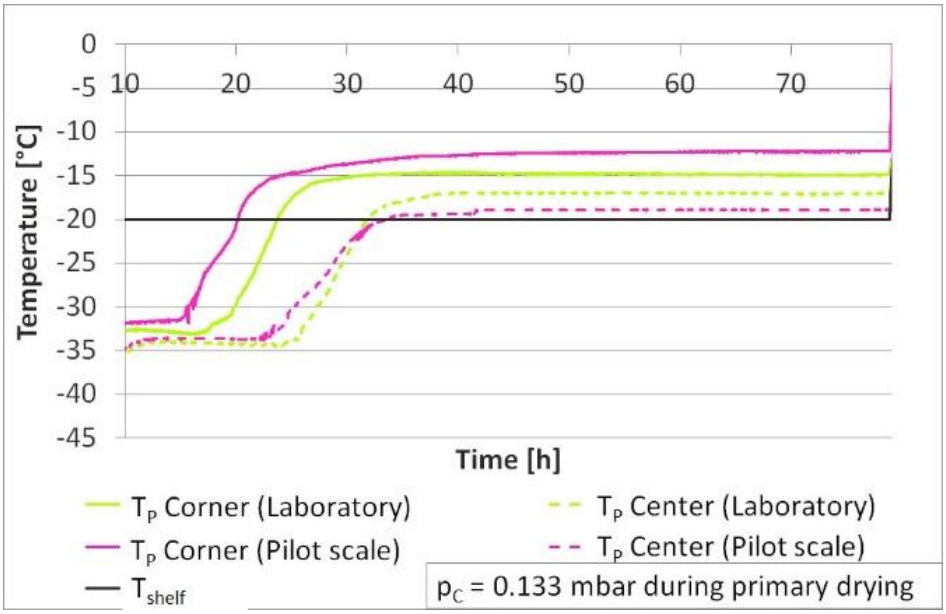


Figure 4-12. Temperature profiles during primary drying in a rack: Laboratory with one rack per shelf compared to pilot scale with four racks per shelf.

If a shorter freeze-drying cycle optimized for rack lyophilization would be used for lyophilization in the tray, center vials in the tray may collapse and be not completely dried. The aim of a comparability experiment was to reach similar EPD without collapse in both vial setups. An increase of pressure during primary drying from 0.066 mbar to 0.133 mbar was necessary to achieve T_P profiles in the tray with a maximum of -33 °C in corner vials similar to those in the rack (Figure 4-13). Center vials were dried after approx. 40 h with both setups.

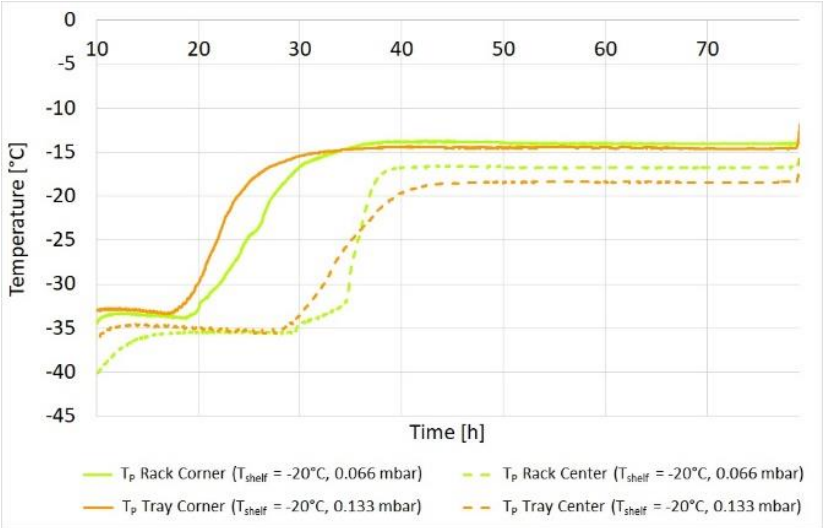


Figure 4-13. T_P during primary drying at T_{shelf} = -20 °C and p_C at 0.066 mbar (rack setup) or 0.133 mbar (tray setup).

4.6 Conclusion

The aim of this study is a better understanding of lyophilization in rack vial holding systems. Compared to a standard tray setup, where corner vials have 2-3 and center vials 6 neighbors with direct contact, vials in the rack are separated by 2-3 mm. In addition, the rack generates a radiation shield which itself runs at a temperature above T_p. Thus, freeze-drying in the rack is faster and more homogeneous with markedly decreased differences of EPD and T_P between corner and center vials compared to the tray. Whereas corner vials of rack and tray behave similar, rack center vials dry much faster than tray center vials, resulting in shorter primary drying. Therefore, transfer of a freeze-drying cycle from rack to tray may require prolongation of cycle time or increase of p_C to yield similar EPD and T_P. There were no pronounced differences between rack and tray when varying fill volume. Despite the homogenization effect of the rack, a partial product load should always be fill up with placebo to represent a full load; leaving the positions empty or placing empty vials there is not adequate. In summary, the rack is a robust tool for freeze-drying coming along with a shorter homogeneous primary drying, flexibility regarding fill volume and robustness towards scale-up compared to commonly used trays.

4.7 References

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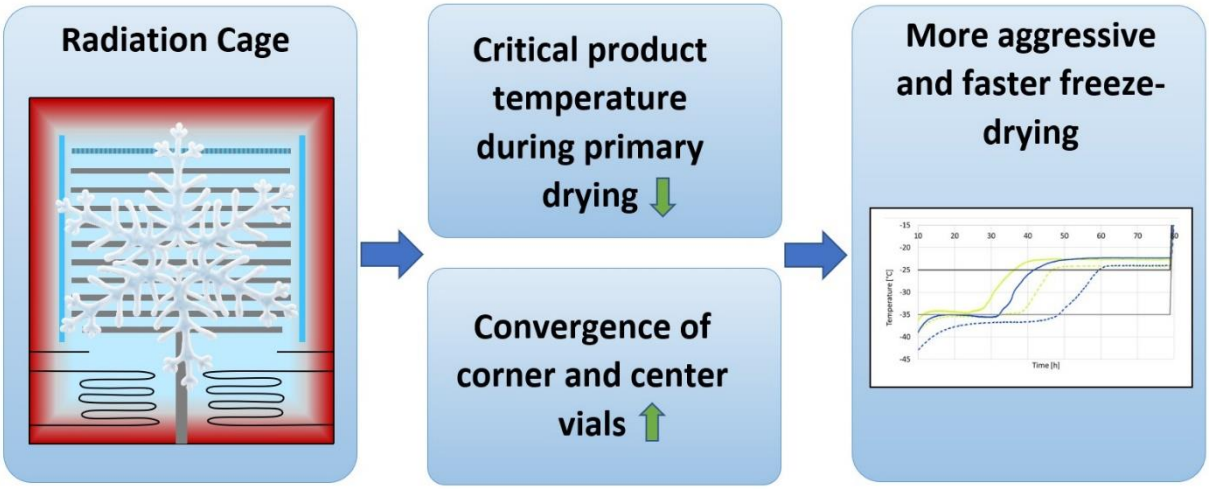
CHAPTER 5 Impact of Chamber Wall Temperature on Energy Transfer During Freeze-Drying

The following chapter has been published in the International Journal of Pharmaceutics and appears in this thesis with the journal`s permission:

Sarah Ehlers (née Daller), Wolfgang Friess and Rudolf Schröder

DOI: 10.1016/j.ijpharm.2020.120025

5.1 Graphical abstract



Keywords: Lyophilization, freeze-drying, radiation cage, heat transfer, edge-vial-effect, radiation, chamber wall

5.2 Abstract

Minimization of radiation coming from the chamber wall during lyophilization has the potential to reduce the edge-vial-effect. The edge-vial-effect is a phenomenon in which vials positioned at the shelf edges and corners tend to run warmer compared to center vials. A higher product temperature may result in product collapse in these vials. Consequently, more conservative and time-consuming freeze-drying cycles with lower shelf temperatures and pressures are chosen to ensure a product temperature below the collapse temperature in all vials. The edge-vial-effect is of even higher impact in small batches, where the ratio of corner and edge to center vials is higher compared to large scale manufacturing. The chamber wall is often discussed as the primary source of radiation impacting corner and edge vials. A radiation cage was set at different low temperatures to determine the impact of chamber wall temperatures below 0 °C on product temperature. At the end of primary drying, product temperature of corner vials could be reduced by 6 °C through the radiation cage but primary drying was elongated. Compared to vials in a tray, the chamber wall temperature had less impact on vials nested in a rack system due to a shielding effect of the rack itself. Corner and center vials ran more homogeneous with radiation cage since the edge and corner vials were slowed down. The difference in primary drying time between corner and center vials in the tray could be significantly reduced by 18 % by means of 7 h when the radiation cage was controlled at product temperature and combined with a higher shelf temperature. In summary, the radiation cage is a useful tool for a more homogeneous batch with the potential to reduce primary drying time. Nevertheless, the drying difference between corner and center vials could only be reduced and was not completely eliminated.

5.3 Introduction

Radiation coming from the chamber wall during freezing and primary drying is a main factor for the “edge-vial-effect”, where corner vials receive more energy by radiation coming from warmer parts of the freeze dryer, specifically the chamber wall, compared to center vials. [1-5] Radiation can be even more relevant in cases of a too short waiting time after a cleaning in place and sterilization in place (CIP/SIP) process. During primary drying, corner and edge vials may run warmer and exceed critical collapse temperature. Consequently, more conservative freeze-drying cycles, with lower shelf temperature (T_{shelf}) and lower chamber pressure (p_C) rendering a lower product temperature (T_P) for all vials, are utilized. This, however, results in longer cycle times. In small scale experiments, the higher ratio of corner to center vials makes the effect even more critical compared to large scale lyophilization. [6] To control radiation coming from the chamber wall, a radiation cage was invented by Sennhenn et al. It is composed of stainless-steel shelf-like walls which are parallel to the chamber walls and door. The temperature of the radiation cage can be controlled (Figure 5-1). [7]

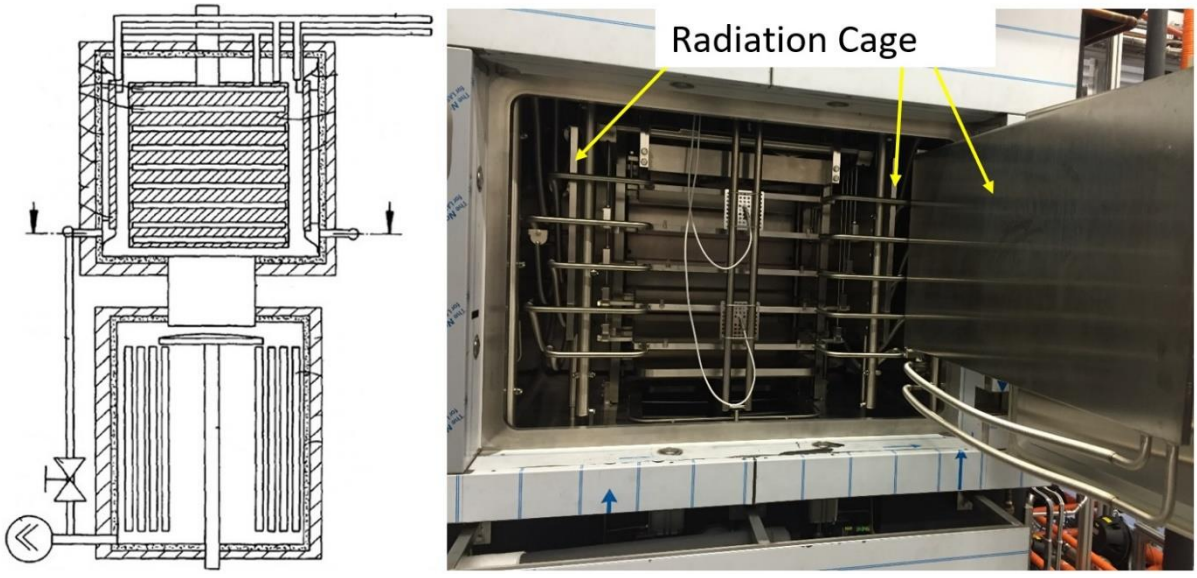


Figure 5-1. Prevention of radiation coming from the chamber wall by the radiation cage in the freeze dryer [7].

Thus, the cage may be useful to limit unwanted radiation from the wall during primary drying. In addition, during freezing, radiation coming from the chamber wall could impact nucleation, ice crystal formation and pore size and thus ultimately drying behavior. [2, 8] If the radiation

cage temperature (T_{cage}) is controlled at T_{shelf} during freezing, a more homogeneous batch may be expected.

The purpose of this study was to evaluate the impact of T_{cage} on T_P of a placebo in a pilot scale freeze dryer especially during primary drying. T_{cage} was set to passive (not controlled), -25 °C (T_{shelf} of the standard process used), -35 °C (typical T_P for the standard process used) and -45 °C (theoretical temperature of ice at the sublimation interface for the standard process p_C of 0.066 mbar [9]) during primary drying. In addition, distribution of residual moisture and heat transfer coefficient Kv were analyzed. Heat transfer for corner vials would still involve radiation coming from the cage if T_{cage} is controlled at T_{shelf}. Therefore, there should still be an edge-vial-effect. If T_{cage} is controlled at T_P, there should be no additional heat transfer by radiation coming from the cage since temperature difference between cage and product is eliminated. The temperature of the subliming ice front is lower, e. g. -45 °C at p_C of 0.066 mbar [9] and T_{cage} controlled at this temperature should lead to lower T_P in corner and edge vials and may even reverse the edge-vial effect. Additionally, due to upcoming new vial holding systems the standard tray setup was compared to a vial separating Polyetheretherketon (PEEK) rack. The rack shields its vials from radiation coming from the chamber wall. [10] It is not the vial, but the rack itself, which is impacted by the chamber wall temperature. Thus, T_{cage} is supposed to have less impact on T_P in the rack setup. Finally, scale-up and transfer within different vial holding systems (rack and tray) and between freeze dryers with high and with low chamber wall temperatures were evaluated. We used a flexible pilot filling line which includes a freeze dryer equipped with the radiation cage and a robotic handling system that makes racks obligatory. Consequently, we analyzed the transfer of a tray cycle established in a laboratory freeze dryer without a radiation cage to the pilot freeze dryer with radiation cage using four racks on a shelf.

5.4 Materials and Methods

5.4.1 Instrumentation

A laboratory freeze dryer equipped with four shelves (1.0 m2 total shelf area) was used (HOF Sonderanlagenbau GmbH, Lohra, Germany). Scale-up experiments were performed in a pilot scale freeze dryer (HOF Sonderanlagenbau GmbH, Lohra, Germany) embedded in a flexible

fill-finish unit with four shelves (1.5 m2 total shelf area) and a capacity of 1920 6R vials in rack systems. Both freeze dryers come with a capacitance manometer for pressure control and an additional Pirani gauge. The temperature of the radiation cage of the pilot scale freeze dryer is controlled by a thermal fluid circuit which winds throughout the cage and is separated from the shelf fluid circuit. [7] Stated radiation cage temperature is the mean of the temperature measured at the inlet and outlet. The temperature of the thermal fluid was measured by PT-100 temperature sensors at the inlet and outlet. In case of a passive radiation cage, thermal fluid does not circulate.

T_P was measured by wireless temperature measurement system (Tempris®, iQ-mobile solutions GmbH, Holzkirchen, Germany) and thermocouples at the center bottom of the vials. Temperature of the rack itself and the surface of the radiation cage was measured by thermocouples sticked to the surface of rack and radiation cage and covered with aluminum foil.

5.4.2 Materials

If not stated otherwise, experiments were performed with 2.5 ml of a 4.6 % sucrose (low endotoxin sucrose, Merck), 0.23 % histidine (L-histidine, Merck) and 0.01 % Polysorbate 80 (J. T. Baker) solution in 6R glass vials (Schott AG, Mainz, Germany) (Tg': -30.5 °C, onset: -32.4 °C).

5.4.3 Freeze-Drying

Samples were frozen at T_{shelf} = -45 °C, followed by primary drying at T_{shelf} = -25 °C and p_C of 0.066 mbar and secondary drying at 25 °C and 0.066 mbar. All temperature ramps were performed at 1 K/min. Stoppering was performed automatically by the freeze dryer at 0.5 bar (abs.) nitrogen pressure with 20 mm diameter stoppers (Datwyler Sealing Solutions International AG, Schattdorf, Switzerland).

For tray setting a stainless-steel tray (HOF Sonderanlagenbau GmbH, Lohra, Germany) with a metal band of 2 mm height holding up to 65 corner and 187 center 6R vials was used. Furthermore, impact of chamber wall temperature on vials set in a rack was studied. The rack is a bottomless 300 x 300 mm PEEK 6R vial holding system (HOF Sonderanlagenbau GmbH,

Lohra, Germany) including 120 vials and has previously been described in detail. [10] In this study, center vials are defined as vials surrounded by six other vials, whereas corner vials are defined as vials standing at the corner and edges lacking a hexagonal surrounding of neighbor vials. Vials standing at the corners of the rack are also called corner vials, thus resulting in 40 corner and 80 center vials. Nevertheless, keep in mind, that vials in the rack have no contact to each other and therefore a rack corner vial is not the same as a tray corner vial and the same applies for center vials.

Usually, the end of primary drying is marked by convergence of T_P and T_{shelf}. [9] Due to varying shapes of the temperature curves, "end of primary drying" (EPD) was defined as the time span between the beginning of primary drying by pulling vacuum and the inflection point of the final convergence of T_P and T_{shelf}. At the beginning of primary drying the energy input and output is balanced, resulting in a T_P plateau, which is termed the 'steady state phase' throughout the present study. We used this method to distinguish between corner and center vials. Another method to define the end of primary drying is the comparative pressure measurement, where convergence of Pirani gauge and capacitance manometer marks the end of primary drying. [11, 12] Since this method is a batch method and cannot distinguish between corner and center positions, the convergence of T_P and T_{shelf} was preferred.

5.4.4 Kv determination

Vials filled with 5.0 ml deionized water were weighed before and after drying for 4 h for Kv measurements according to literature. [13, 14] Freezing to T_{shelf} = -40 °C followed by a sublimation step at T_{shelf} = 5 °C and 0.066, 0.133, 0.200, or 0.267 mbar with the radiation cage set to passive or at -35°C was performed. A further experiment included Kv mappings at a chamber pressure of 0.267 mbar and a comparison of different radiation cage temperatures of -25 °C, -35 °C and radiation cage set to passive.

5.4.5 Determination of residual moisture

The residual moisture of every second vial on a tray was analyzed. Samples were heated to 115 °C and evaporating moisture was quantified by Karl Fischer titration in methanol (874 oven sample processor and titrator, Metrohm, Herisau, Germany). Samples were analyzed for

T_{cage} passive after primary drying and for T_{cage} set to -35 °C / 25 °C during primary / secondary drying after primary drying and after secondary drying.

5.4.6 Transfer and scale-up of freeze-drying cycles with radiation cage and various vial holding systems

At first, the impact of an increase of p_C and T_{shelf} on T_P and visual appearance of the lyo cake was determined. The p_C was set to 0.066 mbar and 0.133 mbar in primary drying at a constant T_{shelf} of -25 °C. In a second study, T_{shelf} was increased from -25 °C to -20 °C at a constant p_C of 0.066 mbar. T_{cage} was set to -35°C and tray setting was compared to rack setting. For the transfer experiment, a freeze-drying cycle optimized for tray was transferred from a laboratory freeze dryer without a radiation cage to a pilot freeze dryer with a radiation cage.

5.5 Results and discussion

5.5.1 Impact of T_{cage} on energy transfer in a tray setting

Radiation coming from the chamber wall can critically impact corner vials resulting in higher T_P and increasing the risk of product collapse. [15] Heat transfer in tray systems have been thoroughly described, differentiating between gas conduction, direct heat transfer and radiation. [16-18] Studies with a cooled chamber wall indicate that control of the chamber wall temperature on product temperature results in similar product temperature and Kv values of corner and center vials. [1, 19, 20] Nevertheless, a difference between corner and center vials of 2-3 °C in product temperature and 6-9 % in Kv value respectively remained. Temperature mapping of a passive radiation cage showed a radiation cage surface temperature of -6 °C at the end of primary drying. A passive radiation cage means that the heat transferring medium neither is moved through the cage nor the medium is actively heated or cooled. The setting with a passive radiation cage is assumed to be comparable to a freeze-dryer without cage since the cage is made from stainless steel used for freeze-dryer building . Overall, CFD modeling of freeze dryers with radiation cage set to shelf temperature suggests that the pressure at the center of a shelf is higher than at the edges, which could impact drying rates. [21] EPD with passive radiation cage was 31 h in corner vials and 50 h in center vials. T_P of corner vials was at -35 °C in steady state phase and at -18 °C at the end of primary drying compared to -37 °C and -23 °C respectively of center vials.

Controlling T_{cage} at lower temperature during primary drying prolonged primary drying in both corner and center vials (Figure 5-2).

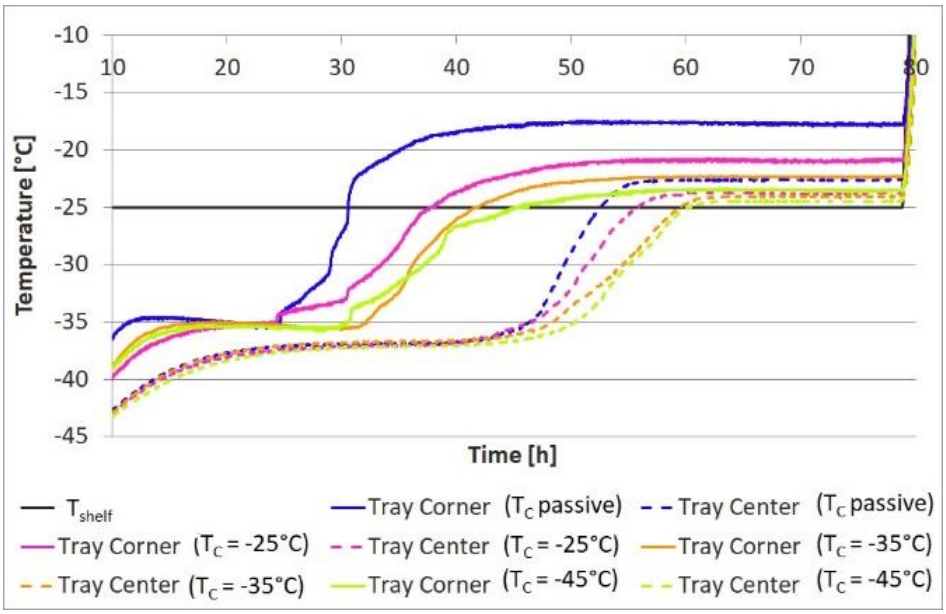


Figure 5-2. Product temperature during primary drying of vials in a tray at T_{cage} = passive, T_{cage} = -25°C, T_{cage} = -35°C and T_{cage} = -45°C.

Table 5-1 summarizes T_P and EPD values for the different radiation cage settings.

Table 5-1. T_P and EPD of tray corner and center vials with T_{cage} = passive, T_{cage} = -25 °C T_{cage} = -35 °C and T_{cage} = -45 °C.

T_{cage}	Parameter	Tray Corner	Tray Center
passive	T_P during steady state	-35 °C	-37 °C
	T_P at the end of primary drying	-17 °C	-23 °C
	EPD	31 h	50 h
-25 °C	T_P during steady state	-36 °C	-37 °C
	T_P at the end of primary drying	-21 °C	-24 °C
	EPD	35 h	51 h
-35 °C	T_P during steady state	-36 °C	-37 °C
	T_P at the end of primary drying	-22 °C	-24 °C
	EPD	37 h	54 h
-45 °C	T_P during steady state	-36 °C	-37 °C
	T_P at the end of primary drying	-23 °C	-24 °C
	EPD	38 h	54 h

With a radiation cage controlled at -25 °C, -35 °C and -45 °C EPD for center vials was reached after 51 h, 54 h, 54 h, respectively. T_P during steady state phase was -37 °C and -24 °C at the end of primary drying for all cage settings. This indicates that the radiation cage has only a little impact on T_P of center vials. Because center vials are surrounded by other vials shielding them against radiation, minimization of radiation has little effect on them. For corner vials EPD was reached after 35 h, 37 h, 38 h, respectively. Prolongation of EPD is most pronounced with changing T_{cage} from passive to -25 °C indicating that a substantial amount of energy is transferred via radiation if the radiation cage is passive. T_P of corner vials during steady state phase was -36 °C for all cage settings. Thus, the radiation cage does not affect the steady state temperature of both center and corner vials. The steady state duration increased from 12 h at T_{cage} passive to 15 h at T_{cage} = -35 °C or -45 °C. At the end of primary drying T_P was higher with -17 °C at T_{cage} passive compared to -23 °C at T_{cage} = -45 °C, again demonstrating that radiation is minimized when the radiation cage is used. With radiation minimized for corner vials by controlled T_{cage}, T_P of corner and center vials correspond at the end of primary drying. Thus, a combination of the radiation cage with an increased T_{shelf} during primary drying could enable to shorten primary drying with T_P remaining below Tg'.

T_P during secondary drying is shown in figure 5-3, where T_{cage} is set to either passive or 25 °C.

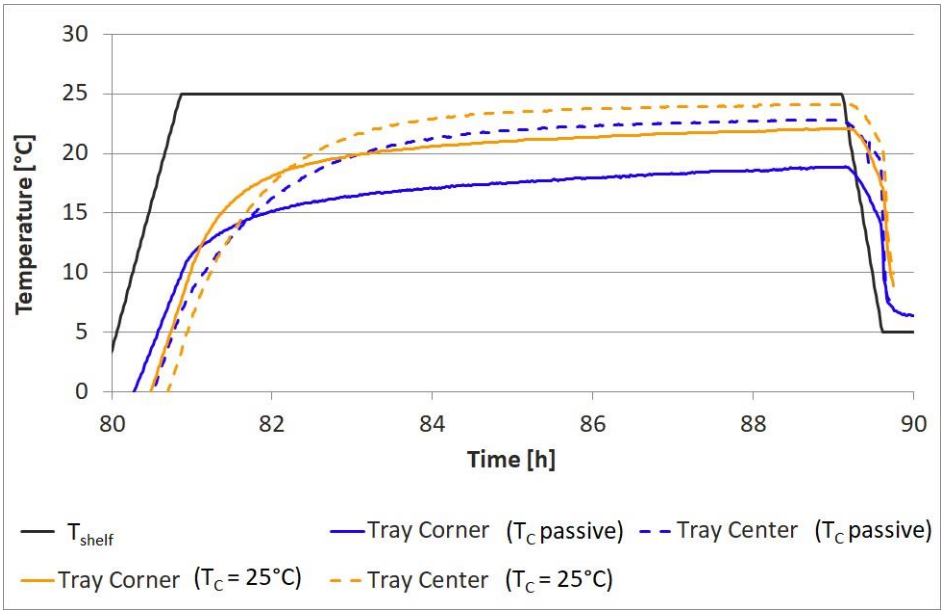


Figure 5-3. Product temperature during secondary drying of vials in a tray at T_{cage} = 25 °C and T_{cage} passive.

If T_{cage} is set passive, the radiation cage has a temperature of maximum 9 °C during secondary drying. T_P reaches T_{shelf} faster when the radiation cage is set to T_{shelf} of 25 °C during secondary drying. T_P of corner vials is 22 °C at T_{cage} = 25 °C compared to 19 °C when the radiation cage is passive. Range in T_P of corner and center vials at the end of secondary drying decreased by 2 °C when T_{cage} is set to 25 °C. Therefore, secondary drying and residual moisture could be more homogeneous when the radiation cage is set at T_{shelf}. Overall, the radiation cage has the potential to shorten secondary drying.

With a radiation cage set to 25 °C during secondary drying, T_P of corner vials is 2 °C higher compared to radiation cage set passive. This observation is reflected by higher Kv values at the corners at 0.066 mbar (Figure 5-4).

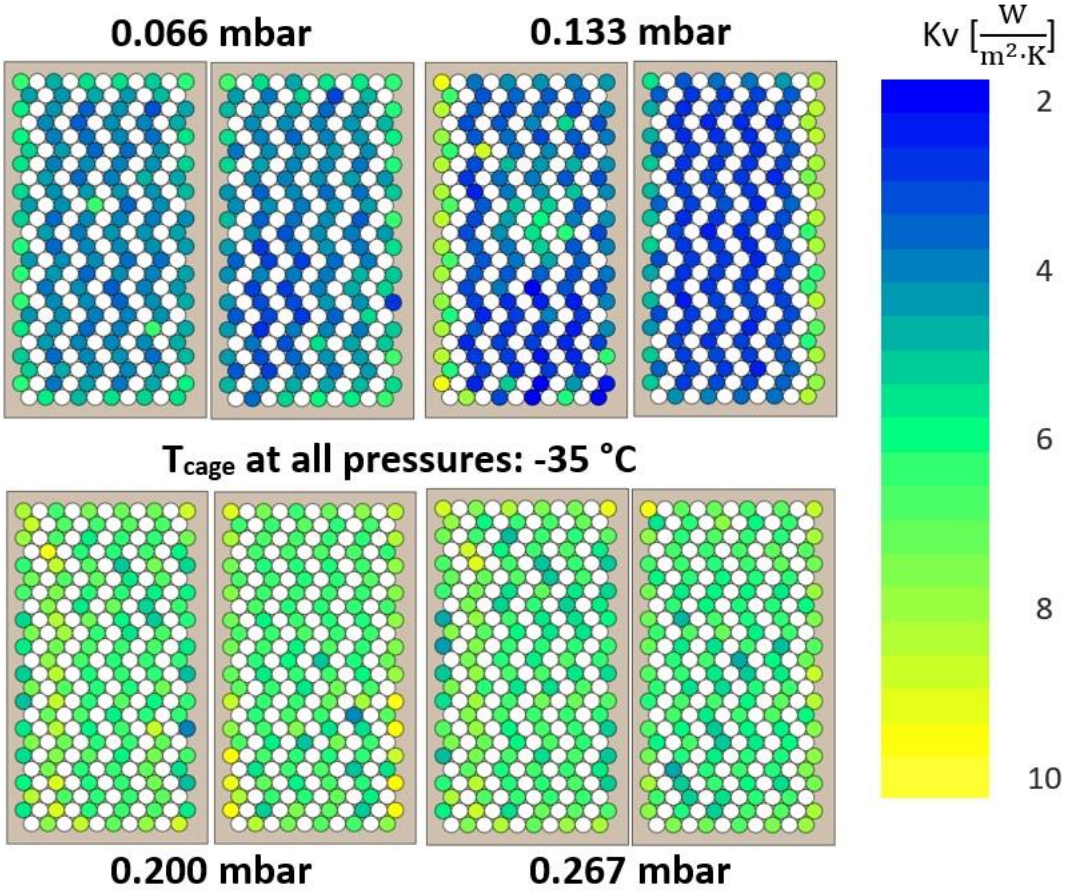


Figure 5-4. Kv mapping in the tray at 0.066 mbar, 0.133 mbar, 0.200 mbar and 0.267 mbar.

Radiation has negligible impact on the center vials due to a radiation shielding by neighbor vials. Especially at 0.133 mbar, there is a remarkable difference between corner vials at the left and the right side of a single tray. Always two trays are placed on a shelf and therefore corner vials on the right side of the left tray behave similar to center vials due to the short

distance to the next tray. For corner vials the effect of the chamber wall temperature on Kv was pressure dependent. At low p_C of 0.066 mbar Kv was on average 5.4 $\frac{W}{m^2 \cdot K}$, which is similar to Kv without radiation cage reported in another study. [22] At 0.267 mbar Kv was reduced from 14.9 to 9.6 $\frac{W}{m^2 \cdot K}$ with the use of a lower $T_{cage.}$ In addition, Kv difference between corner and center vials was decreased from 6.2 $\frac{W}{m^2 \cdot K}$ to only 0.8 $\frac{W}{m^2 \cdot K}$. Thus, Kv is more homogeneous when the radiation cage is used at higher p_C.

Nevertheless, it is known by literature that Kv becomes more homogeneous at higher pressures. Therefore, Kv distribution at 0.267 mbar and different cage temperatures was determined (Figure 5-5).

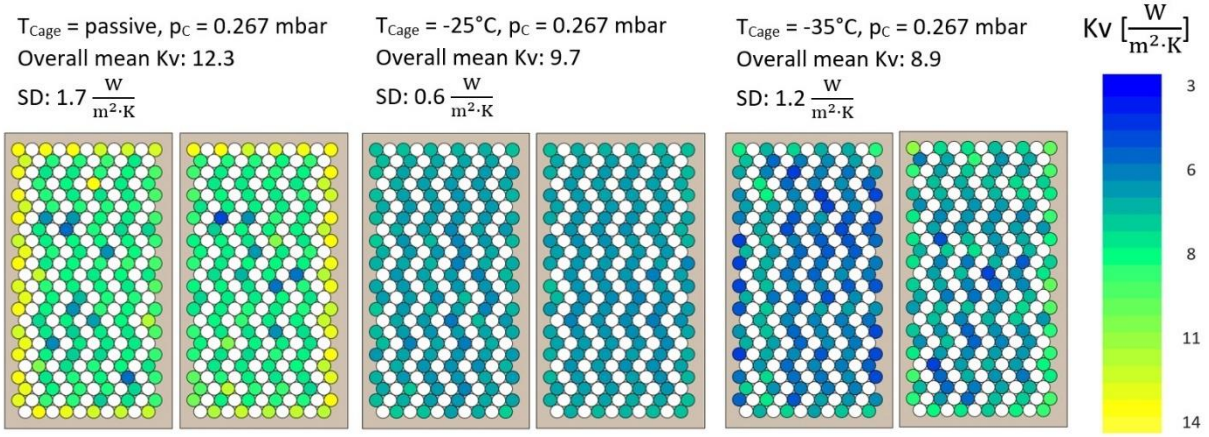


Figure 5-5: Kv mapping: comparison of different radiation cage settings at a chamber pressure of 0.267 mbar; T_{cage} = passive, T_{cage} = -25 °C and T_{cage} = -35 °C from left to right; SD = Standard deviation.

At a passive radiation cage corner vials showed higher Kv values compared to center vials which perfectly confirms the faster drying of corner vials seen in the temperature measurements before. With T_{cage} set to -25 °C or -35 °C, these differences diminish.

5.5.2 Impact of T_{cage} on residual moisture

The residual moisture was mapped to learn about the effect of the chamber wall temperature on the equilibrium moisture levels at the end of primary and secondary drying (Figure 5-6).

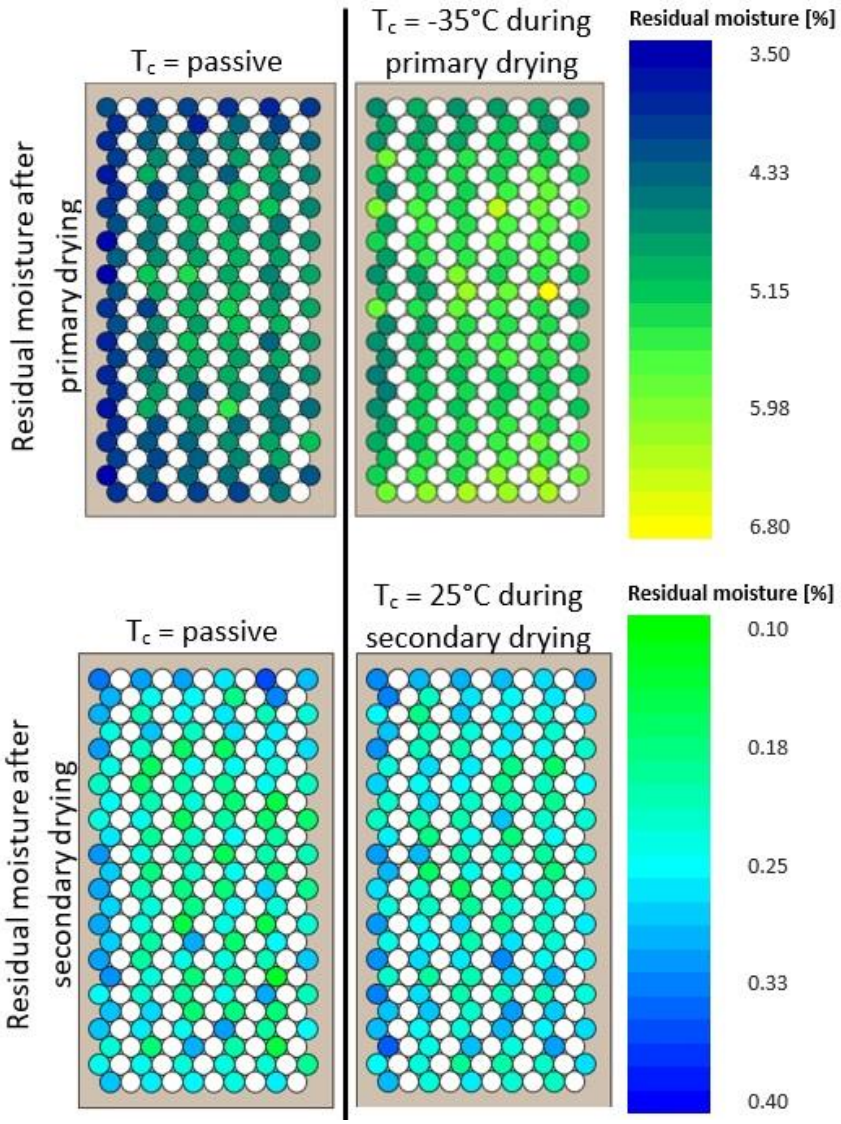


Figure 5-6. Residual moisture mapping in tray, T_{cage} passive compared to T_{cage} = -35 °C, after primary drying compared to after secondary drying.

Residual moisture after primary drying was 1 % higher in corner vials if T_{cage} was decreased resulting in a 5 °C lower T_P. Residual moisture differences between corner and center vials were halved when T_{cage} was set to -35 °C. After secondary drying residual moisture was similar independent of T_{cage} with 0.1 % for corner and 0.4 % for center vials, although T_P of corner vials was 3 °C higher at T_{cage} = 25 °C.

5.5.3 *Impact of T_{cage} on energy transfer for vials in a rack setting*

Racks may be used in flexible fill/finish lines. They differ from traditionally used trays in two main points: every vial is isolated and the whole vial cluster is surrounded by the rack. Therefore, energy transfer is assumed to be more controlled due to a non-existing direct contact between vial neighbors. Furthermore, the rack acts as a radiation shielding for the vial array. It is not the vial but the rack itself, which is impacted by radiation coming from the chamber wall. Thus, the impact of the chamber wall temperature on energy transfer of vials in a rack may be limited. Temperature profiles of the lyophilization process run with vials in a rack are shown in figure 5-7. T_P during steady state, at the end of primary drying as well as EPD are summarized in Table 5-2.

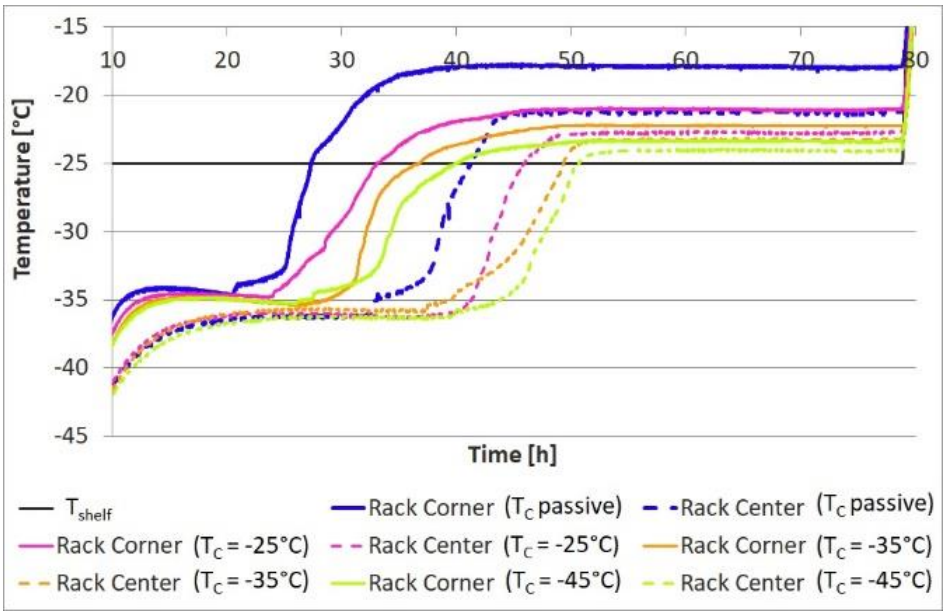


Figure 5-7. Product temperature during primary drying of vials separated in a rack at T_{cage} = passive, T_{cage} = -25°C, T_{cage} = -35°C and T_{cage} = -45°C.

Table 5-2. T_P and EPD of rack corner and center vials with T_{cage} = passive, T_{cage} = -25 °C T_{cage} = -35 °C and T_{cage} = -45 °C.

T_{cage}	Parameter	Rack Corner	Rack Center
passive	T_P during steady state	-35 °C	-36 °C
	T_P at the end of primary drying	-18 °C	-21 °C
	EPD	27 h	39 h
-25 °C	T_P during steady state	-35 °C	-36 °C
	T_P at the end of primary drying	-21 °C	-23 °C
	EPD	31 h	44 h
-35 °C	T_P during steady state	-35 °C	-36 °C
	T_P at the end of primary drying	-22 °C	-23 °C
	EPD	33 h	47 h
-45 °C	T_P during steady state	-35 °C	-37 °C
	T_P at the end of primary drying	-23 °C	-24 °C
	EPD	35 h	48 h

When T_{cage} was passive, at -25 °C, -35 °C or -45 °C EPD for center vials was 39 h, 44 h, 47 h, and 48 h, respectively. T_P during steady state phase was -36 °C in all cases. At the end of primary drying T_P decreased from -21 °C for a passive cage to -24 °C for the lowest T_{cage} setting. T_P of rack center vials during steady state and at the end of primary drying was similar to tray center vials. Due to a radiation shield composed of the rack itself as well as other center vials, minimization of radiation through the radiation cage has little effect on T_P of center vials.

For corner vials, EPD was 27 h, 31 h, 33 h, and 35 h respectively when T_{cage} was set passive or at -25 °C, -35 °C or -45 °C. Steady state T_P was at -35 °C for all settings. At the end of primary drying T_P was -18 °C for T_{cage} passive and -23 °C at the lowest cage temperature. We observed an approx. 13 h shorter EPD for corner compared to center vials in the rack at all cage settings. As seen in tray vials, T_P difference during steady state between corner and center vials is not improved and thus points to the fact that the effect of the radiation cage on corner vials is limited.

Heat transfer in the rack system involves radiation coming from the rack and direct contact between vial and shelf. [10] Since the vials are not in direct contact, radiation from the rack

impacts both corner and center vials. This impact is stronger on corner vials. Correspondingly T_P of corner vials at the end of primary drying becomes lower with a decrease of T_{cage}. Furthermore, the rack temperature is affected by the chamber wall temperature and was 17 °C above T_P with a passive cage as compared to 13 °C and 11 °C respectively when T_{cage} was set to -25 °C or -35 °C (Figure 5-8).

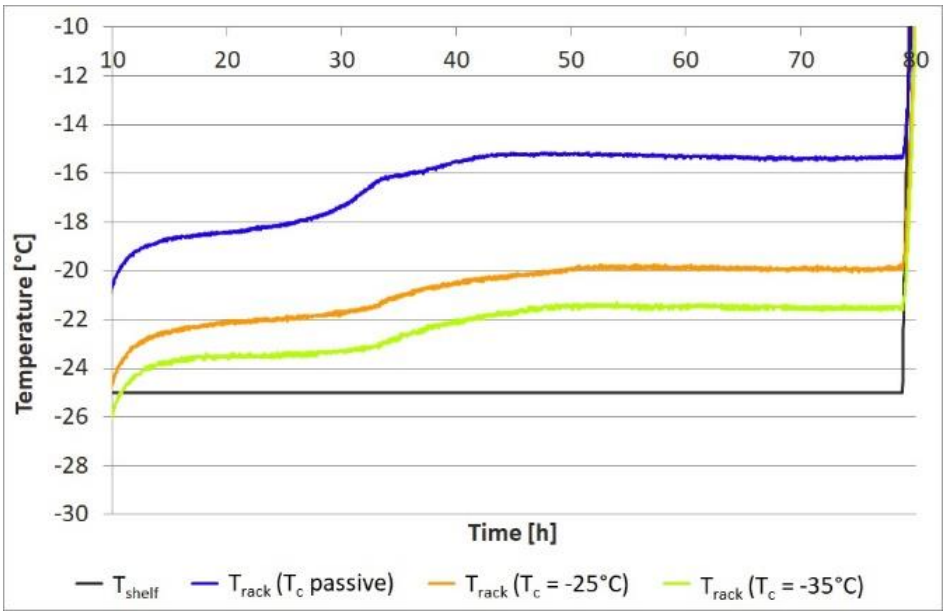


Figure 5-8. Temperature profile of the rack during primary drying at T_{cage} = passive, T_{cage} = -25°C and T_{cage} = -35°C.

Overall, center vials in the rack are more affected by the wall setting compared to center vials in the tray setting.

The Kv of rack vials with T_{cage} set at -35 °C increased with increasing p_C (Figure 5-9) from 5.6 $\frac{W}{m^2 \cdot K}$ at 0.066 mbar to 9.9 $\frac{W}{m^2 \cdot K}$ at 0.267 mbar.

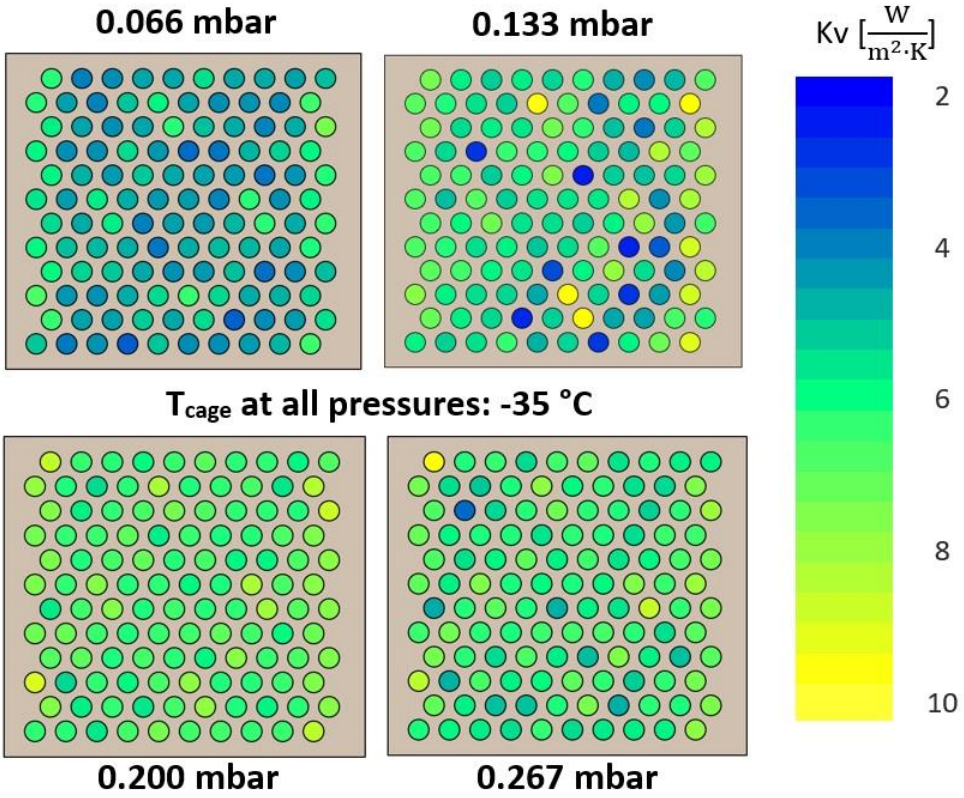


Figure 5-9. Kv mapping in rack system at 0.066 mbar, 0.133 mbar, 0.200 mbar and 0.267 mbar.

As seen for T_P and EPD, the difference between corner and center vials in Kv was less compared to the tray setting. Thus, the shielding and separation effect of the rack makes drying more homogeneous and the rack opens the door for potentially more aggressive freeze-drying parameter setting. This could be further supported by a chamber wall controlled at lower temperature.

5.5.4 Transfer of radiation cage settings with different vial holding systems

In order to optimize the above described freeze-drying cycle, the impact of p_C and T_{shelf} on T_P in rack and tray with T_{cage} = -35 °C was investigated. Therefore, T_{shelf} was increased from -25 °C to -20 °C during primary drying and p_C was increased from 0.066 mbar to 0.133 mbar (Figure 5-10 and 5-11).

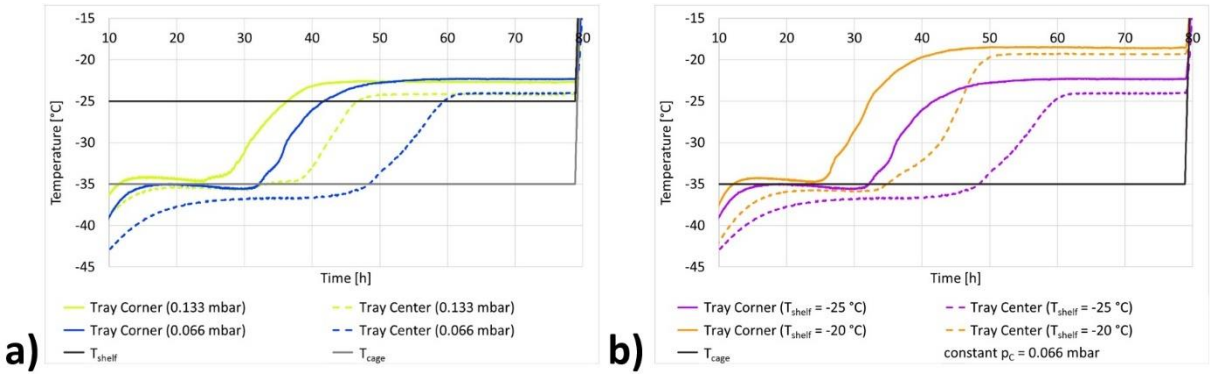


Figure 5-10. Product temperature during primary drying in the tray with varying a) p_C and b) T_{shelf} during primary drying.

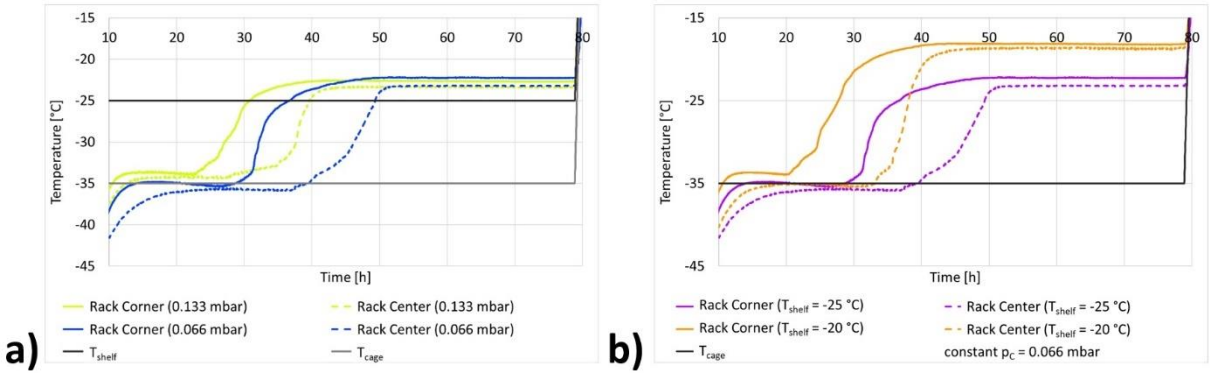


Figure 5-11. Product temperature during primary drying in the rack with varying a) p_C and b) T_{shelf} during primary drying.

In tray setting, the higher pressure resulted in a decrease of EPD from 37 h for corner vials and 54 h for center vials to 32 h and 42 h, respectively. T_P during steady state remained similar both in corner and center vials. A comparable change in T_P and EPD was observed with an increase of T_{shelf}. Whereas T_P at the end of primary drying was not affected by pressure, it was higher with increased T_{shelf}. A similar effect resulted in the rack setup. EPD in center vials decreased from 47 h to 38 h and in corner vials from 33 h to 28 h both by an increase of T_{shelf} or p_C. In summary, p_C or T_{shelf} variations had a stronger effect on tray center vials than on rack center vials leading to an 11 h reduction in EPD. Thus, primary drying can be strongly shortened. Additionally, the difference between corner and center vials can be reduced with moving T_P closer to Tg' in corner vials when increasing p_C or T_{shelf} while controlling the chamber wall temperature.

Transfer between freeze dryers with and without radiation cage and between tray and rack setting may be necessary. Therefore, EPD and T_P in perspective to the critical product temperature have to be kept in mind. With the radiation cage set to T_{shelf} or T_P primary drying will take longer with less energy transfer via radiation. Without a radiation cage T_P in corner and edge vials may exceed the critical product temperature due to higher radiation. A vial separating rack will have a radiation shielding effect and therefore the impact of a radiation cage will be reduced. We consequently performed a transfer aiming at similar T_P during steady state and at the end of primary drying as well as at a comparable EPD for two freeze dryers with and without radiation cage and the different vial settings. A process was to be transferred from a laboratory freeze dryer with 500 x 500 mm shelf area without radiation cage to a pilot freeze dryer with 620 x 620 mm shelf area with radiation cage. In the laboratory freeze dryer two trays per shelf were used and in the pilot freeze dryer four racks per shelf. With the radiation cage at -35 °C primary drying takes 7 h (13 %) and 4 h (11 %) longer respectively for corner and center vials compared to rack at the same setting. An increase of chamber pressure has a stronger effect on center vials, increasing T_P and speeding up primary drying, but it does not change T_P at the end of primary drying. Therefore, two settings were selected: a) radiation cage controlled at -35 °C, T_{shelf} at -20 °C and p_C of 0.133 mbar during primary drying, rack; b) without radiation cage, T_{shelf} at -20 °C and p_C of 0.066 mbar during primary drying, tray (Figure 5-12).

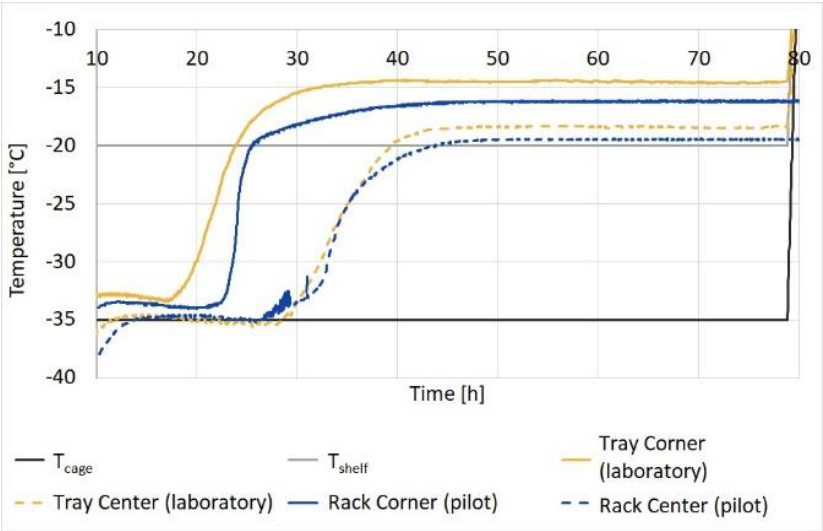


Figure 5-12. T_P during primary drying: laboratory = tray setting without radiation cage, T_{shelf} = -20°C, p_C = 0.133 mbar; pilot = rack setting with radiation cage, T_{shelf} = -20°C, p_C = 0.066 mbar.

T_P during steady state differed by only 1 °C between the two runs and was well below Tg'. At the end of primary drying T_P of center vials differed by 1 °C whereas corner vials showed a difference of 3 °C between the two settings. EPD was similar with 21 h for corner and 34 h for center vials. Thus, the decrease of chamber pressure from 0.133 mbar to 0.066 mbar in combination with the cage, which reduces radiation coming from the chamber wall, enabled similar T_P profiles in laboratory and pilot setting including a transfer from tray to rack.

5.6 Conclusion

In freeze-drying radiation from the chamber wall can lead to a higher T_P of corner vials, resulting in faster drying at a higher risk for collapse compared to center vials. A reduction of chamber wall radiation has more impact on tray corner vials compared to center vials which are surrounded by neighbors. Utilizing a radiation cage at low temperatures led to a decreased Kv and T_P but also longer primary drying especially in corner vials. The T_P reduction of the critical corner vials during primary drying allows for a more aggressive freeze-drying cycle while T_P remains below Tg'. Thus, higher T_{shelf} and higher chamber pressure could be applied and faster drying than in a non-cage setting is possible. Overall, the difference in primary drying time between corner and center vials can be substantially reduced with the radiation cage in combination with an increase of T_{shelf} or chamber pressure. With radiation cage the corner vials are slowed down and therefore corner and center vials ran more homogeneous. Thus, the overall drying time could be kept. The improved homogeneity of the batch allows to adapt the process to run corner and center vials faster at a reduced risk of the product temperature in the corner vials exceeding a critical product temperature. Nevertheless, the longer primary drying time triggered by the radiation cage has to be balanced with a higher T_{shelf} or p_C. It has to be evaluated if the resulting faster drying can compensate the additional running costs of the radiation cage. In the vial separating rack the radiation cage had a similar but less pronounced effect due to the rack surrounding and homogenizing the vials.

Transfer between freeze dryers with and without radiation cage and between different vial holding systems targeting the same EPD and T_P can be achieved. The reduced radiation energy with a cold radiation cage can be compensated by a chamber pressure increase enabling a transfer from tray to rack at the same time.

Finally, the radiation cage can enhance our understanding of the edge-vial mechanism. The radiation cage is a useful tool for the simulation of different chamber wall temperatures and to gain new insights into the nature of radiation impacts even at varying T_{shelf} and p_C. Further studies with different vial size, formulations and process conditions are interesting to demonstrate the benefit of the cage and future case studies are planned. Furthermore, additional experiments to investigate the robustness and the general applicability have to be conducted.

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CHAPTER 6 Trouble with the Neighbor During Freeze-Drying: Rivalry about Energy

The following chapter has been published in the Journal of Pharmaceutical Sciences and appears in this thesis with the journal`s permission:

Sarah Ehlers (née Daller), Rudolf Schröder and Wolfgang

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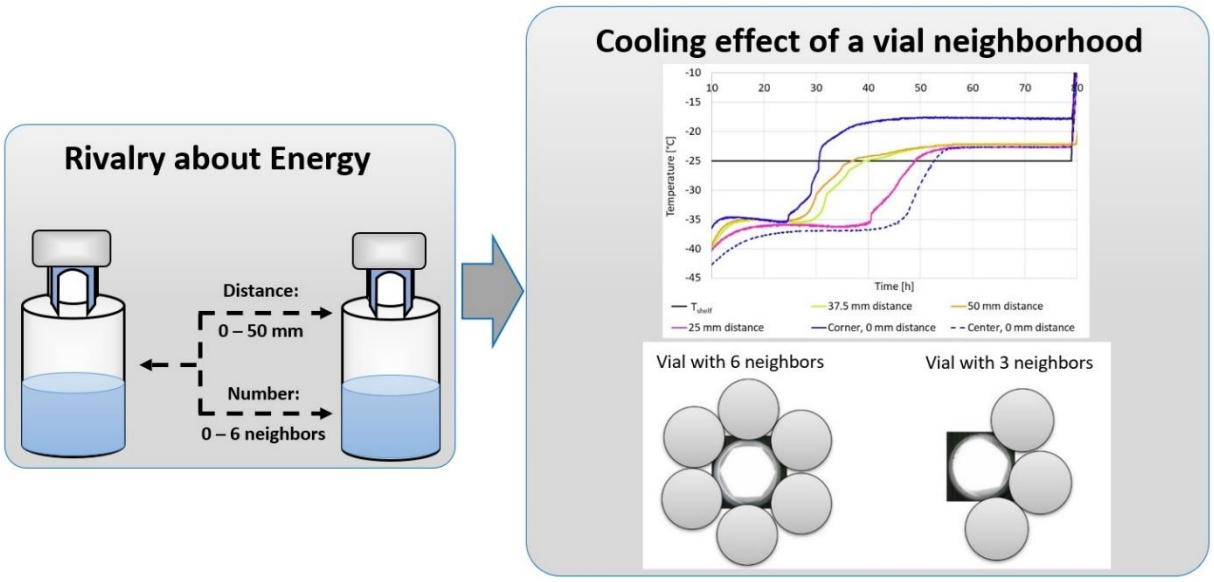
6.1 Graphical abstract



Keywords: Lyophilization, freeze-drying, heat transfer, neighbor vial, edge-vial-effect, product temperature, radiation, direct contact

6.2 Abstract

Batch homogeneity during lyophilization is crucial to ensure products with high quality. Known as edge-vial-effect, vials at the corners and edges tend to run warmer than center vials during primary drying. This is associated with risk of collapse or increased costs due to use of more conservative, longer drying conditions resulting in lower product temperature. The edge-vial-effect has been attributed to radiation coming from the chamber wall. We could show that the neighbor vial has a dominant impact on product temperature during lyophilization. Depending on the number of neighbors as well as the distance to a neighbor vial, the neighbor vial exerts a remarkable cooling effect. Energy transfer by gas conduction enables the cooling effect of a neighboring vial over a distance up to 10 mm. This not only leads to prolonged primary drying but also impacts cake appearance. Thus, to avoid trouble during lyophilization you have to watch out for the neighborhood.

6.3 Introduction

Freeze-drying is a gentle method to enhance storability for sensitive biological products. [1] During lyophilization water is removed minimizing degradation processes. [2, 3] Homogeneity in terms of product temperature during freeze-drying within lyophilization batches is critical to ensure product quality. Due to the edge-vial-effect corner and edge vials on a shelf tend to be warmer than center vials during primary drying. [4] The main reason for the edge-vial-effect is radiation coming from the chamber wall. Whereas center vials are shielded by a hexagonal array of other vials with almost no view from the chamber walls, corner vials lack this shielding and heat is transferred to them. [5-8] This higher energy input for corner and edge vials comes with a higher risk of exceeding a critical product temperature e.g. the collapse temperature. [9] To avoid this risk more conservative primary drying conditions, resulting in a lower product temperature of the whole batch, are applied. This leads to longer cycle time and ultimately higher costs. Corner vials differ from center vials not only in the impact of radiation. Corner vials as well as many edge vials have only 2 to 3 direct neighbors whereas center vials are surrounded by 6 other product vials.

On the one hand, vials nested in a rack system without direct contact to each other show an edge-vial-effect due to radiation effects which points towards less importance of the surrounding by other vials. [6, 10-13] On the other hand, using a radiation cage placed in front of the chamber walls at product temperature during primary drying, eliminating radiation, could reduce but not fully suppress the edge-vial-effect. [14]

The purpose of this study is to determine the role of the neighbor vials in freeze-drying, specifically to obtain new insights into factors driving the edge-vial-effect. Therefore, product temperature measurements were performed in separated vials without contact to each other combined with using a radiation cage. Additionally, the neighborhood of a vial was varied comparing neighbor vials filled with product to vials filled with heat-conductive paste and unfilled. Furthermore, the number of empty rows i.e. no vials surrounding a vial was varied and the number of direct neighbor vials filled with product was modified from zero to six. Measurement on the apparent vial heat transfer coefficient Kv^{app} were performed with vials having a distance of 0 to 100 mm to further characterize the cooling effect of a single neighbor vial. Moreover, the impact of two or six neighbors on shrinkage was investigated.

6.4 Materials and Methods

6.4.1 Excipients

2.5 ml Placebo composed of 4.6 % sucrose (low endotoxin sucrose, Merck, Darmstadt, Germany), 0.23 % histidine (L-histidine, Merck) and 0.01 % Polysorbate 80 (J. T. Baker, PA, USA) in water was filled into 6R vials (Schott AG, Mainz, Germany).

6.4.2 Freeze drying

Lyophilization experiments were performed in a pilot scale freeze dryer (HOF Sonderanlagenbau GmbH, Lohra, Germany) equipped with four shelves, total surface area of 1.0 m2, Pirani and capacitance manometer for pressure control.

Product temperature was measured by thermocouples (HOF Sonderanlagenbau GmbH, Lohra, Germany) and wireless temperature measurement system (Tempris®, iQ-mobile solutions GmbH, Holzkirchen, Germany) with sensors placed at the bottom center of the vials. Freezing temperature was -45 °C. During primary drying sublimation occurred at a shelf temperature (T_{shelf}) of -25 °C and chamber pressure of 0.066 mbar. Secondary drying was performed at 25 °C and 0.066 mbar. All temperature ramps were performed at 1 K/min. The freeze dryer performed stoppering automatically at 0.5 bar nitrogen partial pressure with 20 mm diameter stoppers (brombutyl rubber stopper, siliconized, Datwyler, Schattdorf, Switzerland).

Convergence of product temperature (T_P) and T_{shelf} marks the end of primary drying for the whole freeze-dryer load. [10] The endpoint of primary drying for the different vials in the same freeze-drying cycle was determined via the product temperature profile. Due to varying shapes of the temperature curves end of primary drying was determined by the inflection point of the final convergence of the T_P. The time from the end of freezing to the inflection point will be called ‘end of primary drying’ (EPD) throughout the present study. At the beginning of primary drying energy input and energy output is in balance, leading to a steady state and showing a plateau in temperature curves. This phase is called ‘steady state phase’ throughout the present study.

A stainless-steel tray with 20 mm band holding 252 6R vials was used as vial holding system Center vials are defined as vials surrounded by six other vials, whereas corner vials are defined

as vials standing at the edges lacking a hexagonal surrounding by neighbor vials. To separate vials, 6R vials were positioned in a 3 mm thick polypropylene grid holding 120 vials (Figure 6-1). The distance between the holes is 2-3 mm so that direct contact between the vials is prevented.

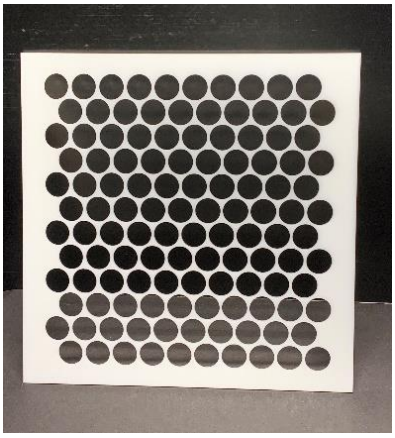


Figure 6-1. Polypropylene grid for holding 120 6R vials.

To characterize the cooling and freezing impact of a direct neighbor, the position of corner and center vials was changed after freezing. Thus, vials with thermal history of a corner vial during freezing and of a center vial during primary drying, named 'Freezing in corner / PD in center position', and the other way around, named 'Freezing in center / PD in corner position', were generated.

6.4.3 Kv^{app} determination

The apparent heat transfer coefficient Kv^{app} was determined in a simplified way and therefore differs from Kv of other studies. [15] For Kv^{app} measurements, vials filled with 5.0 ml deionized water were weighed before and 4 h after the stopped freeze-drying cycle. [16, 17] The vials were frozen to -40 °C followed by a sublimation step at 5 °C and 0.267 mbar. The time span to reach a temperature equilibrium during sublimation was neglected. To determine the impact of the vial distance two vials placed at 0, 1, 3, 5, 10, 25, 50 and 100 mm distance (n = 4) were compared to a single vial on a shelf.

6.4.4 Effect of vial spacing on product temperature

Product temperature in vials surrounded by either empty vials or without neighboring vials was measured (Figure 6-2). Furthermore, T_P in a vial surrounded by 1 (≙ 25 mm distance), 2 (≙ 50 mm distance) and 3 (≙ 75 mm distance) empty rows were measured. The applied freeze-drying cycle was described above under section freeze-drying.

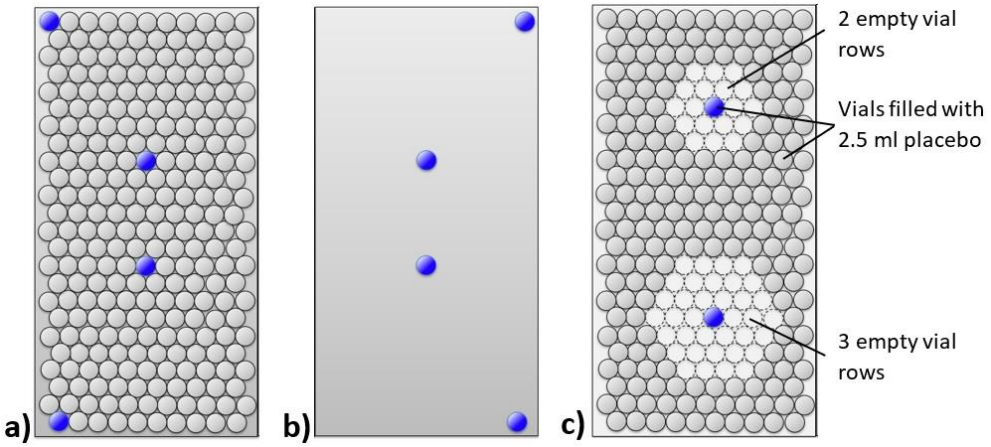


Figure 6-2. Experimental setting for corner and center vials set in a neighborhood either with empty vials (a) no vials (b) and a vial surrounded by 2 or 3 empty rows (c).

Additionally, a placebo vial was surrounded either by nothing or by vials containing 2.5 ml heat-conductive paste in a hexagonal setting, further surrounded by 3 rows of vials filled with placebo (Figure 6-3). The product temperatures of the centered vial as well as of one of the heat-conductive paste filled vials were measured.

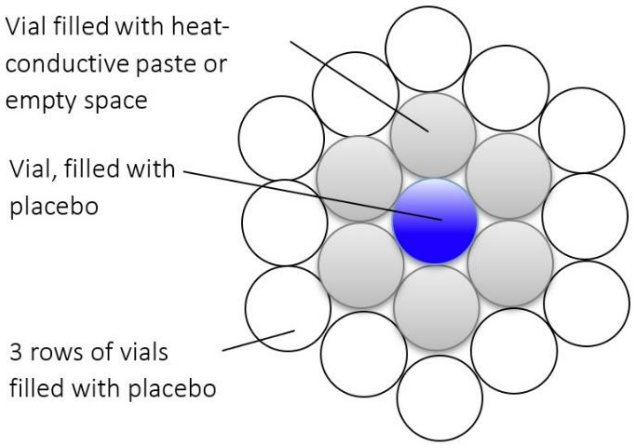


Figure 6-3. Experimental setting for a vial either surrounded by air or heat-conductive paste.

6.5 Results and Discussion

6.5.1 Impact of radiation on the edge-vial-effect

Radiation, which mainly affects corner vials, is the primary cause for the edge-vial-effect according to literature. In the regular tray setting T_P during steady state of corner vials was -35 °C and -38 °C for center vials and EPD differed by 20 h respectively. [13] To minimize radiation coming from the chamber wall we employed the radiation cage controlled at T_{shelf} of -25 °C during primary drying. T_P difference of corner and center vials during steady state remained the same and EPD difference decreased by 3 h, when the radiation cage was used. [14] Additionally, with the aid of a grid, direct contact between vials was eliminated generating a 2-3 mm spacing. By using a grid without radiation cage, T_P of corner and center vials increased by 1-2 °C compared to regular setting and EPD difference between corner and center vials decreased by 5 h compared to regular setting (Figure 6-4). Despite vial separation and minimized wall radiation we found a marked edge-vial-effect with the corner vials running at 2-3 °C higher T_P in the steady state phase and at the end of primary drying as well as a 15 h earlier completion of primary drying.

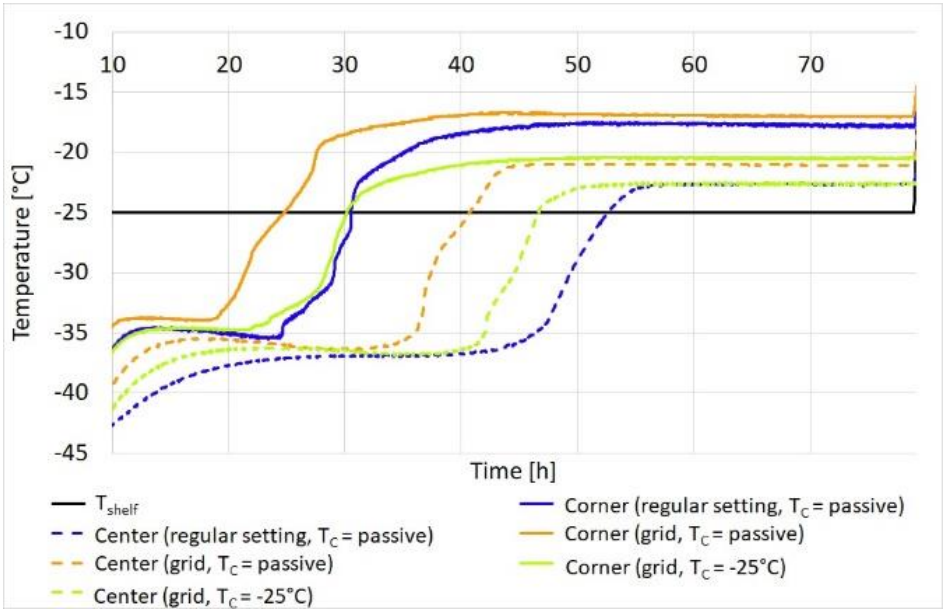


Figure 6-4. T_P during primary drying of corner and center vials separated by a grid with a cage setup to either passive or -25°C.

In an additional setting, vials filled with placebo were placed at the corner and the center of a tray and the tray was filled with empty vials or the tray area left completely empty. The

radiation cage was set to -25°C. There was no difference between the setting with empty vials and air (Figure 6-5).

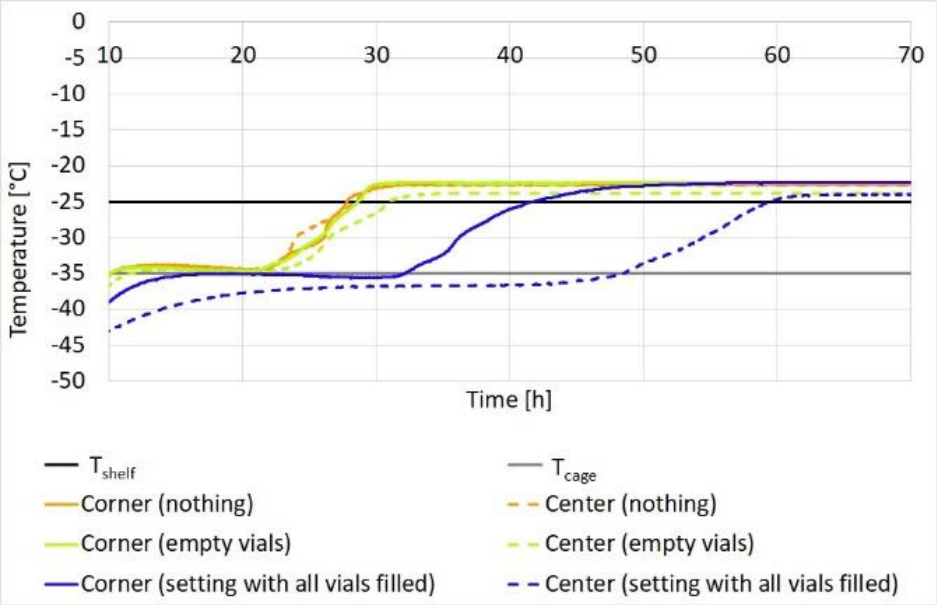


Figure 6-5. T_P during primary drying; in corner and center vial surrounded either by regular filled vials (blue), empty vials (green) or nothing (red).

Steady state T_P was at -35 °C, T_P was at approx. -23 °C at the end of primary drying and EPD was reached after 26 h for all vials, both center and corner. Thus, in contrast in a tray full of placebo vials, the edge-vial-effect was gone in absence of a cooling direct neighbor.

In the previous experiment radiation was minimized through the radiation cage setting. We consequently performed a test without the radiation cage, surrounding the vial of interest by passive vials filled with a heat-conductive paste which do not show the self-cooling effect of sublimation but convey mass and radiation shielding in close vicinity (Figure 6-6).

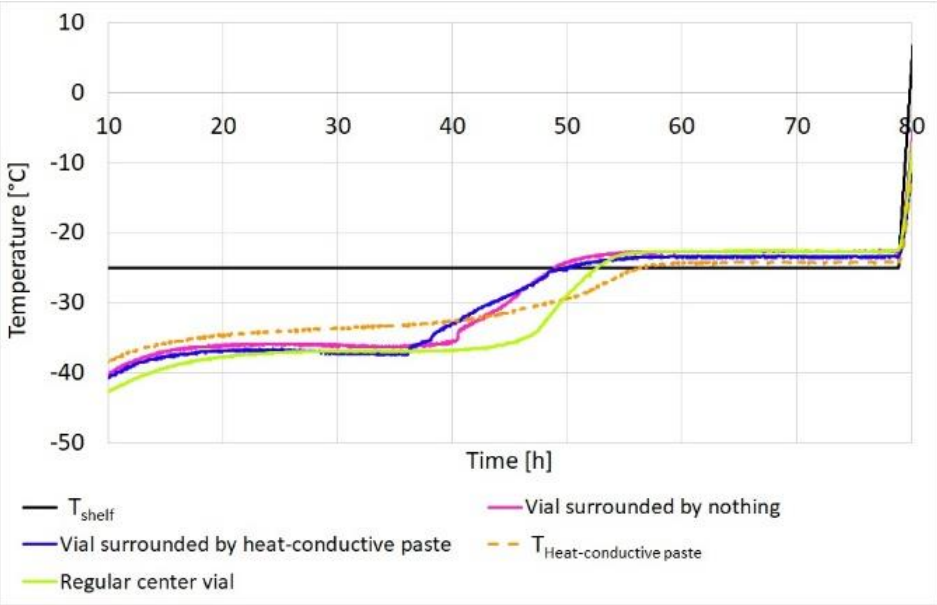


Figure 6-6. T_P during primary drying in a vial either surrounded by air or vials filled with heat-conductive paste (passive) in comparison to a regular center vial.

Both vials surrounded by heat-conductive paste and surrounded by air showed the same T_P during steady state and at the end of primary drying as well as same EPD. The heat-conductive paste itself showed a similar thermal history as the placebo vials. Their temperature is an outcome of T_{shelf} and T_P of the direct neighbors without self-cooling due to sublimation. While sublimation is ongoing the temperature of the passive vials is governed by the cold neighbor vials. Upon completion of sublimation at the end of primary drying, this cooling effect goes away and the vials approach T_{shelf}. The passive vial itself did not impact the centered placebo vial as we did not observe a difference in the behavior of the center placebo vials surrounded by air of the passive vials.

6.5.2 Impact of distance between neighbors

To understand whether the cold neighbor vials impact the vial of interest only via direct contact or over a longer distance, an experiment with 25, 37.5 and 50 mm distance (corresponding to 1 – 3 empty rows) between a centered vial and surrounding vials was performed and compared to standard setting without distance between the vials (Figure 6-7).

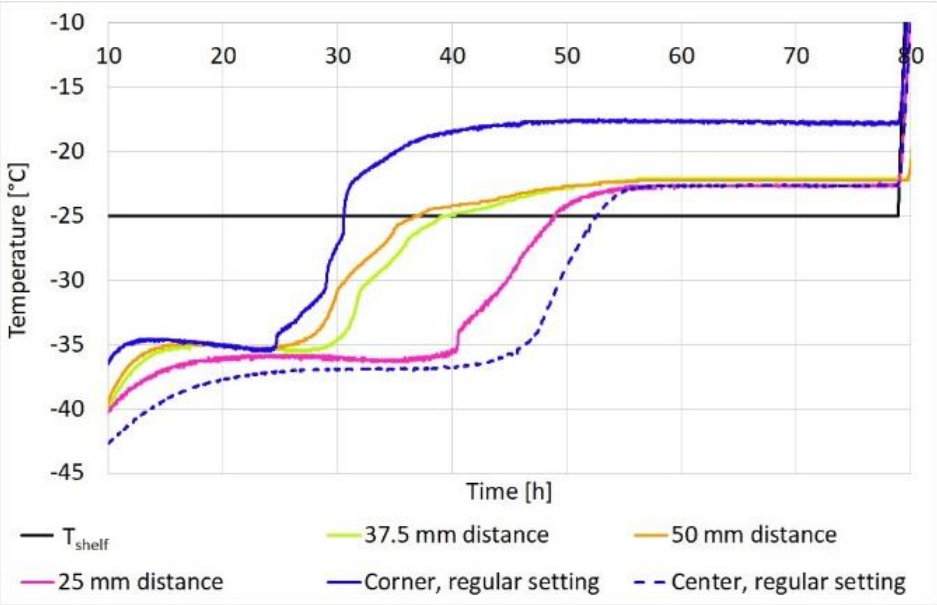


Figure 6-7. T_P during primary drying: vial distance of 25, 50 and 75 mm compared to standard tray setting.

T_P reached steady state 4 h earlier when vials were set in a distance of 25 mm and 9 h earlier with a distance of 37.5 mm. At a distance of 37.5 mm T_P during steady state was comparable to that of a corner vial in a regular setting. At the end of primary drying T_P of a regular corner vial was 5 °C higher compared to vials with any distance or a regular center vial. With increasing distance EPD decreased from 50 h for a regular center vial surrounded by filled neighbors to 45 h and finally approx. 33 h with 37.5 and 50 mm distance, which is similar to a corner vial in a regular tray setting (Table 6-1). Thus, the cooling effect of the neighboring vials was still noticeable over 25 mm distance but disappeared at 37.5 mm.

Table 6-1: EPD of regular setting compared to vials with 25, 37.5 and 50 mm distance.

Distance to surrounding vials	EPD [h]
25 mm distance	45
37.5 mm distance	34
50 mm distance	32
Regular center vial	50
Regular corner vial	30

To evaluate to what extent one single vial impacts its neighbor, we determined the Kv^{app} of two vials with varying distance of 0 - 10 mm (Figure 6-8).

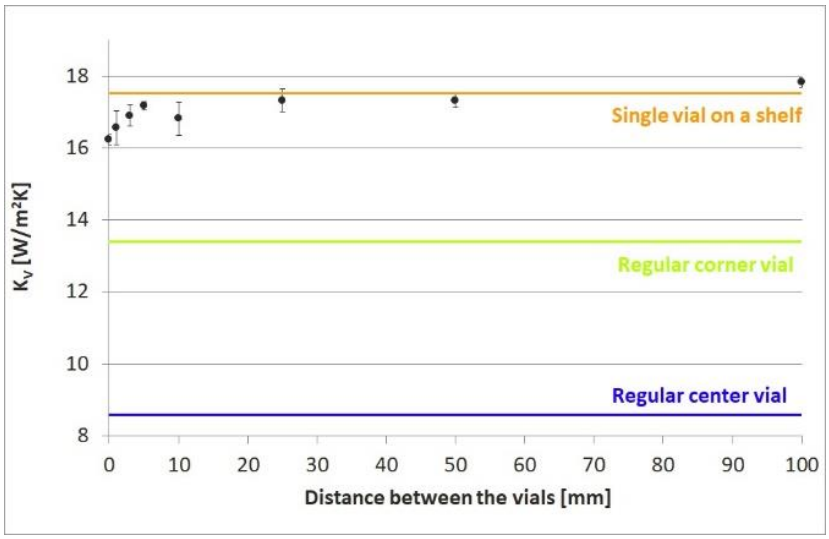


Figure 6-8. K_V^{app} of vials in 0 – 100 mm distance, compared to a single vial on a shelf, a regular corner vial and a regular center vial.

A single vial on a shelf showed a Kv^{app} of 17.7 $\frac{W}{m^2 \cdot K}$. There was a remarkable difference of 4.3 $\frac{W}{m^2 \cdot K}$ between a single vial on a shelf and a standard corner vial, underlying the huge impact of only two direct cooling neighbors. The gap between a single vial and center vial in Kv^{app} is enormous with 8.6 $\frac{W}{m^2 \cdot K}$. The difference of 1.5 $\frac{W}{m^2 \cdot K}$ between a single vial and a setting with two directed neighbors (Kv^{app} of 16.2 $\frac{W}{m^2 \cdot K}$) indicates the substantial impact of just one neighbor vial. Assuming 2-3 neighbor vials for corner the cold neighbors perfectly explain the 4.3 $\frac{W}{m^2 \cdot K}$ difference between corner vials and a single vial. The 6 neighbors lead to the 8.6 $\frac{W}{m^2 \cdot K}$ difference between center vials and a single vial and ultimately the delta in Kv^{app} between corner and center vials of 4.8 $\frac{W}{m^2 \cdot K}$.

Separating the two vials leads to an increase in Kv^{app} from 16.2 to 17.2 $\frac{W}{m^2 \cdot K}$ and the neighbor vial impacts over almost 25 mm which corresponds to the finding above. This experiment shows clearly that the impact of a neighbor vial has to be taken into account during freeze-drying.

6.5.3 Impact of number of direct neighbors

A direct cooling neighborhood has a strong impact on Kv^{app} which increases with closer distance and number of neighbors. It also affects T_P and primary drying rate. A vial without neighbors dried at a T_P during primary drying of -36 °C and primary drying was completed after 35 h (Figure 6-9).

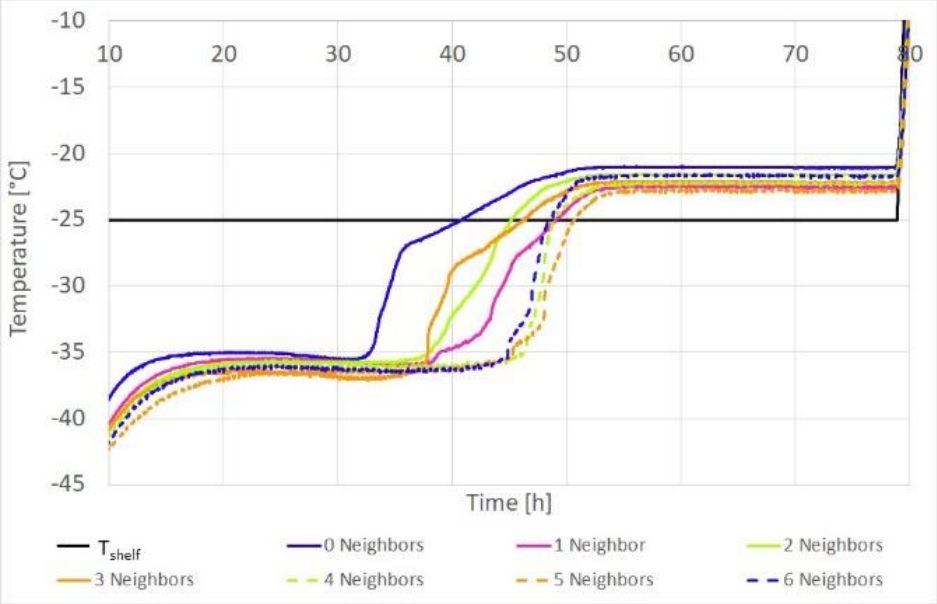


Figure 6-9. Primary drying: T_P of a center vial with 0-6 neighbors surrounding it.

Vials with 1, 2 or 3 neighbors reached EPD after 43 h and vials with 4, 5 or 6 neighbors after 48 h. The time for reaching steady state depends on the number of neighbors and confirms the cooling effect of direct neighbors discussed above. Steady state phase in primary drying elongated with increasing number of neighbors (Table 6-2).

Table 6-2. End of steady state phase during primary drying depending on the number of direct neighbors.

Number of direct neighbors	Steady state phase [h]
0	32
1	40
2	37
3	38
4	47
5	47
6	46

Both T_P and length of steady state showed that there is no difference between 1, 2 or 3 neighbors and 4, 5 or 6 neighbors, respectively.

The cold neighboring vials additionally impact cake appearance. Shrinkage is allocated in regions of direct contact to the neighbors resulting in a hexagonal cake appearance for center vials (Figure 6-10). Shrinkage can develop during primary and secondary drying or during ramping into primary drying and may imply a micro collapse. [18, 19] Due to temperature differences between product and glass vial, the cake separates from glass wall and can shrink. A differentiation between shrinkage and collapse is not standardized. Nevertheless, shrinkage could be a first hint to collapse. It is obvious that the direct neighbors are associated with shrinkage and further studies including different vial locations and vial types are necessary. Shrinkage is also known to depend on the formulation, the solid content and the product resistance. [18, 20, 21] Moreover, studies on the time point at which shrinkage occurs during primary drying or in the course of the transition into secondary drying would be beneficial to understand the nature of the phenomenon.

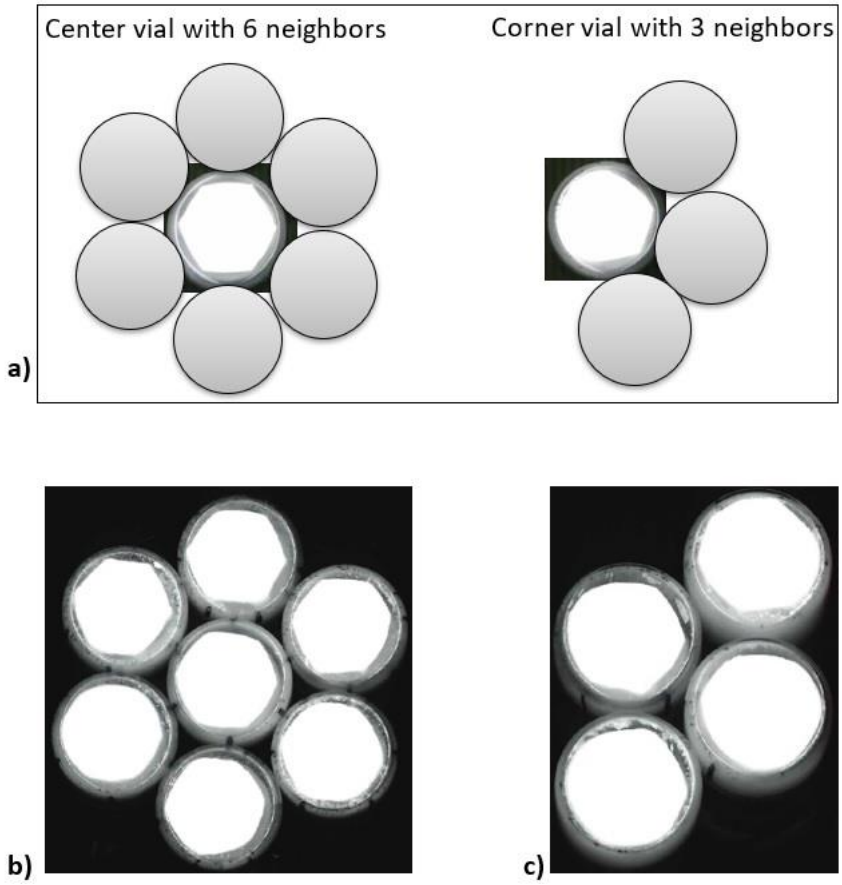


Figure 6-10. Visual cake appearance with shrinkage of corner vial compared to a center vial.

Although the freezing process itself may be influenced by the neighboring vials, we did see an impact of freezing scenario. When the corner and center position were changed after freezing, vials frozen in corner or center position behaved the same upon primary drying in corner position with T_P of -41 °C during freezing and EPD of 31 h or in center position with T_P of -43 °C and EPD of 50 h (Figure 6-11).

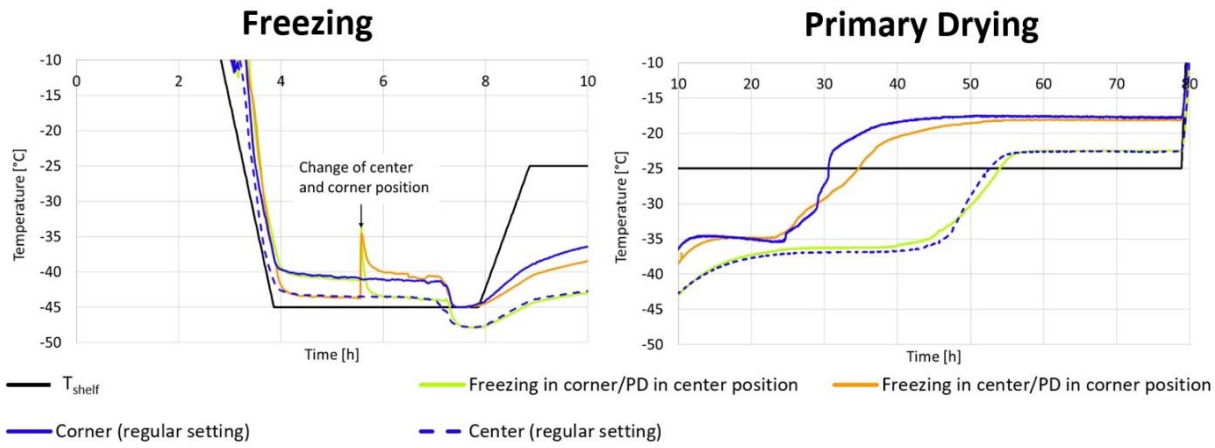


Figure 6-11. T_P of corner and center vials, position changed during freezing compared to corner and center vials in a regular setting.

6.6 Conclusion

The edge-vial-effect is the reason for more conservative freeze-drying cycles in order to prevent collapse of corner vials, which run warmer than center vials. We could show that radiation is not the main cause for corner vials drying faster and at a higher temperature compared to center vials. Instead, a cooling effect by the neighboring vials is the main driving of the different behavior. Filled vials show a lower product temperature than T_{shelf} due to the sublimation process. The effect of the cold neighbor ranges over 25 mm distance between two separated vials. A vial with only 2-3 direct neighbors dries faster than a vial with 4-6 neighbors. Even a single direct neighbor has a cooling effect slowing down primary drying. This direct impact of the neighboring vial is also the reason for the frequently observed shrinkage at distinct regions, specifically resulting in a hexagonal form, as the neighbor vials may impact the temperature differences between product and glass of the vial upon going into secondary drying. In summary, differences in drying time between corner and center vials are mainly caused by the cooling effect of the direct neighbor vial and not by radiation coming from the chamber walls. Nevertheless, contribution of radiation to the edge-vial-effect is still not negligible. It is good practice in freeze-drying development to surround vials with minimum of one row of product or placebo filled vials. This simulates the center vial behavior in a tray. In order to simulate edge and corner vials we recommend to place only 2 to 3 neighbors next to the vial of interest as this may be more relevant especially for later scale up to a different freeze-dryer with different geometry and chamber wall temperature.

6.7 References

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CHAPTER 7 Final Summary

To cope with the challenge of new formats of biologics, personalized medicine and batch sizes becoming smaller, the pharmaceutical industry operates more with flexible fill/finish units. They often come with special robotic and handling systems like nested vials or customized racks. One time-consuming part in fill/finish processes is freeze-drying. Freeze-drying may be necessary to achieve adequate stability of the products. The utilization of racks during freeze-drying is rather new. In contrast to the standard stainless steel trays, positioning of vials in racks and nests results in spacing of the vials. This may substantially impact the drying process and a thorough understanding is required with respect to drying kinetics. Additionally, homogeneity within a shelf has to be assured. Differences in drying behavior between the vials on a shelf can results from the edge-vial effect. Receiving more energy by radiation from warmer parts in the freeze dryer like the chamber wall, corner and edge vials run warmer than center vials during primary drying potentially resulting in collapse or application of more conservative drying cycles to keep the temperature of these vials well below a critical temperature. Radiation from the chamber wall could be controlled and reduced by implementation of an additional radiation cage in the drying chamber. The radiation cage is temperature controlled, similar to a shelf, but is operated with a separate fluid cycle which allows separate temperature control and enables to simulate different chamber wall temperatures. Operating with the cage allows to study and understand the impact of the chamber wall on edge-vials. It may furthermore enable to perform freeze-drying cycles with higher quality in terms of identical thermal history of the product vials and less collapse. Moreover, the impact of the combination of radiation cage and rack system has to be addressed before operating with new flexible lines equipped with a cage. The question about the importance of radiation effects and vials spacing ultimately calls for investigations of the role of the neighbor vial surrounding an individual vial. Differences in product temperature and drying time between corner and center vials may not only originate from radiation affecting corner vials but also from corner vials having less neighbors than center vials. Filled product vials may on the one hand side self-cool each other and shield from radiation on the other hand side.

A Polyetheretherketon (PEEK) rack holding 120 6R vials (chapter 3) was tested. Compared to a bulk tray setting, the rack separates the vials and forms a radiation shield. Energy transfer in

the rack is dominated by direct contact between vial and shelf and higher compared to a tray setup. The second heat source is the radiation emanating from the rack itself whereas radiation from the chamber wall is reduced compared to vials set in bulk trays. Primary drying time is significantly extended in vials in the rack as compared to the same arrangement but without the rack underlining the shielding effect of the rack. The difference in sublimation rates between corner and center vials in the rack decreased at higher pressures due to a higher contribution of gas conduction.

To enable process optimization and transfer between different vial holding systems freeze-drying behavior in the rack was compared to the commonly used stainless steel tray (chapter 4). Vials dry significantly faster in the rack with less difference in primary drying time between corner and center vials. The rack temperature is higher than the product temperature in the steady state phase of primary drying which impacts both corner and center vials through radiation. In tray setting only the corner and edge vials receive substantial radiation energy from the warm chamber wall. Center vials from rack and tray differ in the distance to the neighbors as well as the radiation shielding: whereas tray center vials have six direct neighbors representing a radiation shielding, rack center vials lack direct contact with spacing of approx. 3 mm and radiation shielding is provided by the rack. Interestingly, larger fill volumes resulted in more pronounced extension of the duration of primary drying in the tray as compared to the rack. A partial load of a vial holding system may be favorable in case of limited material availability to simulate the thermal behavior of corner and center vials. If material is limited, trays and racks can be filled up with placebo vials. However, this comes with significant effort. Although the rack showed a homogenization effect, it is still necessary to complement partial product load with placebo vials to represent a full load setting. Since edge vials run at slightly higher temperature and dry faster in a tray setting whereas the edge-vial-effect is less pronounced in the rack setup, transfer of a freeze-drying cycle from rack to tray required a slight extension of the primary drying time by decreasing the chamber pressure.

As discussed above, radiation impacting edge and corner vials mainly comes from the chamber wall, which is usually not temperature controlled. The radiation cage described in chapter 5 has the potential to minimize radiation and thus reduce the product temperature and the risk of collapse of corner vials during primary drying. As expected, the radiation cage leads to a stronger decrease of Kv compared to a passive radiation cage, a lower product temperature and slower drying for corner vials when set to -25 °C or -45 °C compared to a passive setting

with a surface temperature of -6 °C at the end of primary drying. Only little effect was seen for center vials. The product temperature of corner vials at the end of primary drying decreased with decreasing radiation cage temperature demonstrating the effect of the cage. A more aggressive cycle with increased shelf temperature and chamber pressure could be performed resulting in faster primary drying as compared to a setting without cage without increasing the risk of collapse and product temperature for edge vials. The extension of primary drying time through lowering the temperature of the radiation cage has to be balanced by an increase of chamber pressure or shelf temperature.

Despite minimized radiation from the chamber wall when using the cage, edge and corner vials dry faster than center vials, both in tray, rack and separated vials without rack setting. Therefore, in chapter 6 the impact of direct neighbors on product temperature and drying rate was investigated. Freeze-drying in a vial with the direct neighbors missing but further surrounded by rows of filled vials as well as freeze-drying in only two vials on a whole shelf showed that filled vials cool each other during primary drying. The cooling effect lowered the ice sublimation rate substantially if the distance is less than 25 mm. Experiments with vials filled with heat-conductive paste, not showing the self-cooling effect by ice sublimation, underlined this strong cooling impact. A single vial on a shelf showed a significantly higher Kv^{app} value than a vial with 2-3 direct neighbors reflecting an edge or corner vial and a vial with 6 direct neighbors representing a center vial, which has the lowest Kv^{app}. Already one direct neighbor decreases Kv^{app} compared to a single vial. The direct neighbors additionally appear to impact the temperature differences between product and glass resulting in local cake shrinkage. This explains the frequently observed phenomenon of a hexagonal cake form. Thus, in order to simulate center vials in a tray on small scale with limited material, it is recommended to surround the vial of interest with at least one row of placebo filled vials whereas for corner vial simulation only 2 to 3 neighbors should be placed in direct contact with the vial of interest.

In summary, this work presented new insights into the heat and mass transfer of vials in a rack vial holding system as part of a flexible fill/finish line as well as impact of lower chamber wall temperatures represented by the radiation cage on drying behavior. Both rack and tray can contribute to a more homogeneous freeze-drying. Another key finding is that a vial's direct neighborhood highly impacts its drying behavior and that the neighbors are much more important for the edge-vial effect than radiation from the chamber wall.

Appendix

7.1 Research Articles

Daller, S., W. Friess, and R. Schroeder, Energy Transfer in Vials Nested in a Rack System During Lyophilization. Pharmaceutics, 2020. 12(1).

Ehlers, S. (née Daller), W. Friess, and R. Schroeder, Process optimization and transfer of freeze-drying in nested vial systems. European Journal of Pharmaceutics and Biopharmaceutics, 2021.

Ehlers, S. (née Daller), W. Friess, and R. Schroeder, Impact of Chamber Wall Temperature on Energy Transfer During Freeze-Drying. International Journal of Pharmaceutics, 2020. 592.

Ehlers, S. (née Daller), R. Schroeder, and W. Friess, Trouble with the Neighbor: Rivalry about Energy. of Pharmaceutical Sciences, 2020.

7.2 Poster Presentations

Daller S., Frieß W., Schroeder R.; New tools for flexible and more controlled lyophilization, 11th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Granada, Spain, March 19-22, 2018.

Daller S., Frieß W., Schroeder R.; Separated-vial lyophilization in semi-automated production lines, Conference on Freeze Drying of Pharmaceuticals & Biologicals, Garmisch-Partenkirchen, Germany, September 18–21, 2018.

Daller S., Schroeder R., Frieß W.; Heat transfer in vials separated in a rack system during lyophilization, 9th International Symposium on Lyophilization of Pharmaceutials, Ghent, Belgium, September 2–6, 2019.

7.3 Oral Presentations

Daller S.; Improving lyophilization in a small scale production line, International PhD Students/Postdoc Meeting of the German Pharmaceutical Society (DPhG), Bad Dürkheim, Germany, March 14-16, 2018.