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1 **How vial geometry variability influences heat transfer and product**
2 **temperature during freeze-drying**

3

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10

11 **ABSTRACT:** Vial design features can play a significant role in heat transfer between the
12 shelf and the product and, consequently, in the final quality of the freeze-dried product. Our
13 objective was to investigate the impact of the variability of some geometrical dimensions of a
14 set of tubing vials commonly used for vaccine production on the distribution of the vial heat
15 transfer coefficients (K_v) and its potential consequence on product temperature. Sublimation
16 tests were carried out using pure water and eight combinations of chamber pressure (4 to 50
17 Pa) and shelf temperature (-40 °C and 0 °C) in two freeze-dryers. K_v values were individually
18 determined for 120 vials located in the center of the shelf. Vial bottom curvature depth and
19 contact area between the vial and the shelf were carefully measured for 120 vials and these
20 data were used to calculate K_v distribution due to variability in vial geometry. At low
21 pressures commonly used for sensitive products (below 10 Pa), the vial-shelf contact area
22 appeared crucial for explaining K_v heterogeneity and was found to generate, in our study, a
23 product temperature distribution of approximately 2 °C during sublimation. Our approach
24 provides quantitative guidelines for defining vial geometry tolerance specifications and
25 product temperature safety margins.

26

27 **Keywords:** Freeze drying/lyophilization; amorphous; drying; vaccines; distribution; vial heat
28 transfer coefficient; sublimation rate; vial design; inter-vial heterogeneity

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30

31

32 INTRODUCTION

33 Nowadays, freeze-drying is an essential and valuable preservation method to ensure the long-
34 term stability of the growing list of biopharmaceuticals such as antibodies, hormones,
35 vaccines, therapeutics peptides and proteins. This method makes it possible to remove the
36 majority of water at temperatures far below 0 °C (usually between -40 °C and -20 °C) by
37 sublimation, the phase transition from ice to water vapor.¹

38 Due to the really low temperature and pressure typically used, freeze-drying remains a time
39 consuming process often difficult to control and scale-up. The US Food and Drug
40 Administration has recently proposed a new regulatory philosophy to manage product
41 quality: the Quality by Design (QbD) initiative. Quality will be no more tested into the
42 product but designed into the process. The QbD approach is based on pre-defined quality
43 targets and on a deep understanding of how formulations and process interact to influence
44 critical quality attributes of pharmaceutical products.² In contrast to tablets, products intended
45 to be freeze-dried are conditioned in their final packaging system (vial or syringe) before the
46 process.³ The vial thus directly influences the freeze-drying process and impacts final product
47 quality.^{4,5} Furthermore, since the capacity of a manufacturing freeze-dryer can easily reach
48 100 000 vials, ensuring uniform product quality attributes (potency of the active ingredient,
49 residual moisture content, visual aspect of the freeze-dried cake) within the entire batch
50 represents a real challenge. Any variation in the design of the packaging system or other
51 parameters could result in product quality variation.

52 Product temperature is a key process parameter governing an important critical product
53 quality attribute, the visual aspect of the freeze-dried cake, which in turn could influence the
54 residual moisture, the stability of the active ingredient and the reconstitution time.⁶ During
55 the process the product temperature should be maintained below a critical value
56 corresponding to the glass transition temperature in amorphous product.^{3,7} However, the

57 product temperature profile cannot be directly controlled and depends on the process
58 operating parameters (i.e., shelf temperature, chamber pressure) and on the heat transfer
59 through the container (e.g., vial).^{4,7,8} Knowledge of the heat transfer characteristics of the vial
60 and the uniformity or non uniformity of this property within vial arrangement inside a freeze-
61 dryer is thus essential to be able to predict final quality of the product batch. Several authors
62 have reported that the heat transfer rate between the shelf and the product is dependent on the
63 vial position on the shelf.^{4,5,9-12} Pikal et al.⁵ showed that the vials located at the periphery of
64 the shelf exhibited sublimation rates 15% higher than vials located in the center. This
65 phenomenon, referred to as "edge effect", has been ascribed to additional heat transfer by
66 radiation from walls and doors.^{4,5,8,9,11} The higher heat flow rate of these periphery vials
67 could lead to product collapse due to increased product temperatures during the primary
68 drying phase. Furthermore, Pisano et al.¹⁰ recently observed a normal distribution in the vial
69 heat transfer coefficient evaluated for vials located in the center of the shelf.

70 The design of the vial also strongly influences the heat transfer efficiency between the shelf
71 and the product.^{4,5,7,12-15} Considering that the vials are placed directly on the shelf, the heat
72 flow transferred to the product can be described by three parallel mechanisms: conduction
73 from the shelf surface to the vial *via* points of direct contact between the vial bottom and the
74 shelf, conduction through the vapor entrapped in the vial bottom concavity and radiation.^{5,8,12}
75 Heat transfers *via* contact conduction and conduction through the gas are influenced,
76 respectively, by the dimension of the contact area between the shelf and the vial and the
77 depth of bottom curvature in which the gas is entrapped.^{5,8,12} Several studies^{13,16,17} have
78 demonstrated that the vial bottom curvature limits the heat transfer and, thus, the sublimation
79 flow rate that determines the duration of the primary drying. The concavity of the vial bottom
80 limits the direct surface contact between the vial and the shelf, accounting for most of the
81 resistance to conductive heat transfer.^{14,16} In pharmaceutical freeze-drying conditions, contact

82 conduction is more efficient than gas conduction, and an increase in the contact area leads to
83 a significant increase in the total heat transfer.^{5,14}

84 Our objective was to quantitatively investigate the role of vial geometry distribution on heat
85 transfer heterogeneity and subsequently on product quality by predicting product temperature
86 distribution during the primary drying step induced by variability in vial dimensions.
87 Proposing an approach to understand how vial design and operating conditions interact to
88 influence product quality is completely in the scope of the QbD approach.

89 In the present study, only vials located in the center of the shelf and surrounded by other vials
90 in the same conditions were considered so as to avoid any heterogeneity due to the additional
91 border heat transfer. The analysis of the heterogeneity was conducted in terms of vial heat
92 transfer coefficient (K_v) distributions. Based on theoretical analysis,^{5,8,12} attention was
93 focused on the role of two vial dimensions, the bottom curvature depth and the contact area
94 between the bottom vial and the shelf. Two shelf temperatures (-40 °C and 0 °C) and six
95 chamber pressures (4, 6, 9, 15, 40 and 50 Pa) were tested in two freeze-dryer pilot plants of
96 similar shelf emissivity to assess the impact of these operative parameters on the heat transfer
97 heterogeneity among central vials. Finally, as example of practical application of our work
98 for assessing the pharmaceutical product quality, the impact of the central vial K_v variability
99 on product temperature was evaluated. Product temperature distributions for a 5 % sucrose
100 solution were calculated from the simulated K_v distributions based on the vial geometry for
101 several operating conditions.

102

103 **MATERIALS AND METHODS**

104 **Materials**

105 Siliconized glass tubing vials (3 mL) were provided by Müller + Müller (Holzminden,
106 Germany). These vials are routinely used in commercial manufacturing. Distilled water was
107 used throughout the experiments.

108 Two pilot scale freeze-dryers differing mainly by their size, the type of valve connecting the
109 drying chamber to the condenser and their age were used for this study:

110 - a LyoVac GT6 (Finn-Aqua Santasalo-Sohlberg SPRL, Brussels, Belgium), referred to as
111 LYO A. It included 5 shelves with an area of 0.14 m² each, a distance between shelves of 56
112 mm, a drying chamber volume of 0.061 m³ and a butterfly valve between the chamber and
113 the condenser.

114 - an Epsilon 2-25D (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz,
115 Germany), referred to as LYO B. It included 7 shelves with an area of 0.27 m² each, a
116 distance between shelves of 55 mm, a drying chamber volume of 0.38 m³ and a mushroom
117 valve between the chamber and the condenser.

118 The pressure in the freeze-dryer chamber was monitored by a capacitive manometer. Since it
119 was not technically possible to install thermocouples in the drying chamber of the two freeze-
120 dryers, Tempris wireless sensors (IQ Mobil Solution GmbH, Holzkirchen, Germany) were
121 positioned in the bottom center of selected vials (Figure 1) to record ice temperature during
122 the experiments. The obtained signal was used to define the sublimation starting point.

123

124 **Ice sublimation experiments**

125 All experiments were performed using a 1.8 mL fill volume of distilled water (filling height:
126 11 mm). No stopper was inserted into the vial neck. The middle shelf was fully covered by
127 filled vials for all runs, corresponding to a total of 540 vials in LYO A and 950 vials in LYO
128 B. Bottomless trays were used.

129 The vials were quickly loaded on the pre-cooled shelf at -50°C. The presence of a dry laminar
130 flow in front of the freeze-dryer door made it possible to control the air relative humidity and
131 thus to limit condensation on the shelves. After a freezing step of 2 hours, the pressure was
132 decreased and the shelf temperature was increased by 1 °C/min. Experiments were carried out
133 at 4, 6, 9, 15, 40 and 50 Pa with a shelf fluid inlet temperature of 0 °C, and at 4 and 6 Pa with
134 a shelf fluid inlet temperature of -40 °C. The run performed at 0 °C and 6 Pa was repeated
135 three times. The cycles were run long enough to dry up to 20-25 % of the initial fill volume.
136 Subliming a larger quantity of ice could lead to loss of contact between the vial and the ice,
137 introducing uncertainty in the analysis.

138 The sublimation rate \dot{m} was measured gravimetrically for each vial and calculated as the
139 mass loss divided by the period of sublimation. A total of 100 vials, placed in the centre of
140 the shelf and surrounded by other vials in the same conditions, were individually weighed
141 before and after the experiment on a precision scale (± 0.001 g; Mettler Toledo, Zaventem,
142 Belgium). Sublimation time was measured from the moment when shelf temperature
143 exceeded product temperature, meaning that there was a net heat flux from the shelf towards
144 the vials. The arrangement of the weighed vials within the shelves is shown in Figure 1 for
145 the two freeze-dryers.

146

147 **Measurement of emissivity of the vial and of the shelf**

148 Emissivity measurements were performed by Themacs Ingénierie (Champs sur Marne,
149 France). The glass vial emissivity was determined using a Fourier transform infrared
150 spectrophotometer (Frontier, Perkin Elmer, Roissy, France) equipped with a Pike[®] integrant
151 sphere (Pike Technologies, Fitchburg, WI, USA). The measured emissivity varied from 0.78
152 to 0.80 within the range of temperatures tested (from -48 °C to 27 °C). Thus, a constant value

153 corresponding to the average observed product temperatures (between -48 to -24 °C) was
154 used in the data analysis, as reported in Table 1.

155 The shelf emissivity was measured using the emissometer EM-2, making it possible an in situ
156 measurement.¹⁸ The emissivity value of the shelves of LYO B was 0.18 ± 0.06 (Table 1).
157 Measurements were carried out on several pilot and production freeze-dryers and shelf
158 emissivity values in the range of 0.18-0.3 were obtained. Considering the relative standard
159 deviation of the method (0.06), the measured values are in agreement with values reported in
160 literature.⁵

161

162 **Dimensional analysis of a batch of vials**

163 The dimensions of 120 vials were precisely measured by the specialized company
164 Precis&Mans (Le Mans, France) using the micrometer Mitutoyo 3D (Mitutoyo Europe
165 GmbH, Neuss, Germany). The following geometrical parameters were determined with a
166 precision of 0.003 mm: the inner and outer bottom radius and the maximum bottom curvature
167 depth. These values were used to calculate additional vial dimensions: outer and inner vial
168 bottom area, vial shelf contact area (A_c) (named radius-based contact area in Table 1) and
169 mean bottom curvature depth (l) (Appendix).

170 Furthermore, the vial-shelf contact area was also estimated using the imprint method
171 proposed by Kuu et al.¹⁷ and Hibler et al.⁴ The vials were gently placed on an inkpad and then
172 on a sheet of white paper. ImageJ v.1.49 software (National Institutes of Health, Bethesda,
173 MD, USA) was used for the determination of the vial-shelf bottom contact area in pixels from
174 the imprint images. The scale factor of pixels in mm^2 was determined by evaluating the
175 number of pixels of a black shape of known area and was equal to $0.0153 \text{ mm}^2 \text{ pixel}^{-1}$. The
176 mean value and the relative standard deviation of these geometrical dimensions are reported
177 in Table 1. The two methods used for evaluating the vial-shelf contact area gave similar mean

178 values: 16.7 mm² for the imprint method and 17.8 mm² for the dimensional analysis.
179 However, the coefficient of variation of these methods appeared different and significantly
180 higher for the imprint method (23.9 % versus 12.0 % for the dimensional analysis). The
181 values of contact area determined using this latter method were selected for the analysis
182 considering that this method better accounts for intimate contact between vial and shelf.

183

184 **THEORY AND DATA ANALYSIS**

185 **Evaluation of the vial heat transfer coefficient K_v based on experimental data**

186 As widely reported in literature,^{4,5,8,12} the vial heat transfer coefficient K_v was calculated
187 using the following equation:

188

$$189 \quad K_v = \frac{\dot{Q}}{A_b(T_s - T_b)} = \frac{\Delta H \dot{m}}{A_b(T_s - T_b)} \quad \text{Equation 1}$$

190

191 where \dot{Q} is the heat flow received by the vial, A_b is the outer vial bottom area, T_s is the
192 average temperature between the inlet and outlet shelf fluid temperatures, T_b is the bottom
193 product temperature, ΔH is the latent heat of sublimation and \dot{m} is the sublimation rate.

194 Since it was not possible to implement thermocouples in the freeze-dryer pilot plant to have a
195 precise measurement of the product temperature, T_b was theoretically determined as:

196

$$197 \quad \dot{Q} = \frac{\lambda_{ice}}{L_{ice}} A_{in} (T_b - T_i) \quad \text{Equation 2}$$

198

199 where λ_{ice} is the ice thermal conductivity, A_{in} is the inner bottom area of the vial, L_{ice} is the
200 ice thickness and T_i is the ice-vapor interface temperature. The ice thickness was estimated as

201 the mean between the initial and final ice thickness values (calculated using the amounts of
202 initial and sublimed ice).

203 No stopper and pure water were used in this study in order to assume that the partial pressure
204 of vapor at the sublimation interface was equal to the chamber pressure. The temperature at
205 the ice-vapor interface T_i was thus calculated as a function of the interface pressure P_i using
206 the Clausius-Clapeyron relation:¹⁹

207

$$208 \quad T_i = \frac{6139.6}{28.8912 - \ln(P_i)} \quad \text{Equation 3}$$

209

210 The T_b value calculated was compared to the product temperature value given by the Tempris
211 probe and an excellent agreement between experimental and theoretical data was observed.

212

213 **Theoretical description of the vial heat transfer coefficient K_v**

214 The main objective of this work was to quantify the impact of vial dimensions distribution on
215 heat transfer variability and its resulting consequence on product temperature. To this end,
216 the vial heat transfer coefficient need to be theoretically expressed in function of specific vial
217 dimensions.

218 The vial heat transfer coefficient K_v can be described as the sum of three contributions:^{5,8,12}

219

$$220 \quad K_v = K_c + K_g + K_r \quad \text{Equation 4}$$

221

222 where K_c represents the thermal contact conduction between the shelf and the vial *via* the
223 direct contact area, K_g the thermal conduction through the gas entrapped in the vial bottom
224 curvature and K_r the thermal radiation between the vial and the top and bottom shelves.

225

226

227 *Heat transfer by thermal contact conduction K_c*

228 The expression of K_c has been discussed in the literature by only a few authors.^{16,17} Kuu et
229 al.¹⁷ proposed an evaluation of this parameter and showed that the larger the contact area is,
230 the larger the value of the contact conduction coefficient will be. Thus, K_c can be assumed to
231 be proportional to the contact area (A_c , evaluated by the imprint test method) through an
232 empirical constant (C_1):

233

$$234 \quad K_c = C_1 A_c \quad \text{Equation 5}$$

235

236 *Heat transfer by conduction through the gas K_g*

237 The coefficient K_g , representing the contribution of the conduction through the gas in K_v , can
238 be expressed as:^{5,12}

239

$$240 \quad K_g = \frac{C_2 P_C}{1 + \frac{C_2 l}{\lambda_{amb}} P_C} \quad \text{Equation 6}$$

241

242 where P_C is the chamber pressure, λ_{amb} is the molecular conductivity of the water vapor at
243 ambient pressure, l is the mean vial bottom curvature depth calculated as reported in the
244 Appendix, and the coefficient C_2 is equal to:

245

$$246 \quad C_2 = \Lambda_o \frac{\alpha_c}{2 - \alpha_c} \left(\frac{273.15}{T_{gas}} \right)^{0.5} \quad \text{Equation 7}$$

247

248 where Λ_o is the free molecular heat conductivity of the gas at 0 °C, T_{gas} is temperature of the
249 gas participating to heat conduction, calculated as average between the product temperature

250 at the sublimation interface and the shelf temperature values,¹² and α_c is the accommodation
251 coefficient.

252 *Heat transfer by thermal radiation K_r*

253 The heat transfer by radiation between the shelf and the vial \dot{Q}_r^{shelf} can be described by the
254 Stephen-Boltzmann equation:^{5,8,20}

255

$$256 \quad \dot{Q}_r^{shelf} = A_r \mathcal{F} \sigma (T_s^4 - T_b^4) \quad \text{Equation 8}$$

257

258 where A_r is the area exposed to the radiation from the shelves to be considered equal to the
259 vial bottom area A_b , \mathcal{F} is the visualization factor and σ the Stephan-Boltzman constant. After
260 mathematical rearrangement, Equation 8 can be expressed as:

261

$$262 \quad \dot{Q}_r^{shelf} = A_r \mathcal{F} \sigma (T_s^4 - T_b^4) = A_b \mathcal{F} \sigma (T_s + T_b)(T_s^2 + T_b^2)(T_s - T_b) \quad \text{Equation 9}$$

263

264 Thus, the heat transfer coefficient K_r for thermal radiation can be defined as:

265

$$266 \quad K_r = \mathcal{F} \sigma (T_s + T_b)(T_s^2 + T_b^2) \quad \text{Equation 10}$$

267

268 During the process, central vials are affected by two different radiative heat transfer
269 contributions: between (i) the shelf below the vial and the vial bottom, and (ii) the shelf
270 above the vial and the top of the vial.^{5,8,12} Hence, the visualization factor will be the sum of
271 two terms:

272

$$273 \quad \mathcal{F} = \mathcal{F}_b + \mathcal{F}_{top} \quad \text{Equation 11}$$

274

275 The visualization factor at the bottom of the vial \mathcal{F}_b can be evaluated considering the
 276 definition proposed by Bird et al.²⁰ and Pikal⁸ for the heat transfer by radiation between
 277 parallel surfaces:

278

$$279 \quad \mathcal{F}_b = \frac{1}{1 + \left(\frac{1}{e_v} - 1\right) + \left(\frac{1}{e_s} - 1\right)} \quad \text{Equation 12}$$

280

281 where e_v and e_s are the emissivities of the vial and shelf, respectively.

282 Considering vials located in the centre of the shelf and surrounded by other vials in the same
 283 conditions, it is possible to assume that (i) the vial area exposed to the top shelf is much
 284 smaller than the area of the shelf and (ii) the vial top does not receive radiations from the side
 285 walls of the chamber. Thus, the visualization factor between the top of the vials and the shelf
 286 \mathcal{F}_{top} can be estimated equal to the emissivity of the vial:^{5,8}

287

$$288 \quad \mathcal{F}_{top} = e_v \quad \text{Equation 13}$$

289

290 In agreement with the literature, the visualization factor at the vial top (equal to 0.78; Table
 291 1) is higher than the one at the vial bottom (equal to 0.16; Table 1).⁵

292

293 *Dependence of the vial heat transfer coefficient on vial geometry*

294 Equations 4, 5 and 6 were combined to highlight the dependence of K_v on the contact area
 295 (A_c), and bottom curvature depth (l):

296

$$297 \quad K_v = C_1 A_c + K_r + \frac{C_2 P_c}{1 + \frac{l}{\lambda_{amb}} C_2 P_c} \quad \text{Equation 14}$$

298

299 The term K_r was calculated from Equations 10-13 for each experimental condition.
300 Coefficients C_1 and C_2 were determined by fitting Equation 14 in a least-squares sense to
301 experimental K_v values determined by the gravimetric method. Calculations were performed
302 with Matlab R2014b software equipped with the Statistics Toolbox (The Mathworks Inc.,
303 Natick, MA, USA). The bottom curvature depth l and contact area A_c were evaluated from
304 dimensional analysis of the vial and imprint test, respectively.

305

306 **Calculation of K_v distributions based on vial geometry**

307 Two vial dimensions influence heat transfer: the contact area (A_c) and the mean bottom
308 curvature depth (l). The absence of correlation between the two geometrical dimensions l and
309 A_c was verified by calculating the correlation factor together with its statistical significance
310 (p-value > 0.5). It was thus possible to independently evaluate the impact of those parameters
311 on K_v . Using Equation 14, three K_v distributions based on vial dimension variations were
312 simulated: (i) curvature-based (l in Equation 14); (ii) contact area-based (A_c in Equation 14);
313 and (iii) their combination. The curvature-based K_v distribution was obtained by evaluating
314 Equation 14 with the 120 measured values of the mean bottom curvature depth (l), while the
315 contact area was maintained constant at its mean value. The contact area-based K_v
316 distribution was obtained by evaluating Equation 14 with the 120 measured values of the
317 imprint-based contact area (A_c), whereas the bottom curvature depth was maintained constant
318 at its mean value. Plugging both measured l and A_c values into Equation 14 gave the
319 combined contact area and curvature-based K_v distribution. The calculation was repeated for
320 all the studied chamber pressures and shelf temperatures. Chi-square goodness-of-fit tests were
321 performed on the simulated K_v distributions, establishing that the sample data were consistent
322 with a normal distribution at a 0.05 significance level.

323

324 **Simulation of the product temperature distributions using the K_v distribution**

325 Product temperature distributions were obtained from the calculated contact area and
326 curvature-based K_v distribution. The product temperature was calculated for a 5 % w/w
327 sucrose solution, considering a shelf temperature of -25°C and four chamber pressures (4, 6,
328 9, 15 Pa). Pressure at 40 and 50 Pa were not considered because the product temperature
329 exceeds the glass transition temperature of the product at such high pressure (i.e., -32 °C for a
330 5 % w/w sucrose solution).²¹ In the case of a real product, the mass flow rate can be
331 expressed as reported by Pikal et al.⁵:

332

333
$$\dot{m} = \frac{(P_t - P_c) A_{in}}{R_p} \quad \text{Equation 15}$$

334

335 where R_p represents the area-normalized product resistance. The value of R_p used for this
336 simulation is reported in Table 1 and was considered for a dried layer thickness of 0.5 cm.²¹
337 To simulate the product temperature distribution, the non-linear system composed of
338 Equations 1-3 and 14-15 was solved for each pair of measured l and A_c values. Chi-square
339 goodness-of-fit tests were performed on the product temperature distributions, demonstrating
340 that the simulated data were compatible with a normal distribution at a 0.05 significance
341 level.

342

343 **RESULTS AND DISCUSSION**

344 **Impact of equipment on K_v**

345 Vial heat transfer coefficient K_v of 100 vials located in the centre of the shelf was
346 experimentally determined for different chamber pressures (4 to 50 Pa), shelf temperatures (-
347 40 °C and 0 °C) and freeze-dryers (LYO A and LYO B). Figure 2 illustrates the evolution of
348 the average value of K_v with pressure. Equation 14 was fitted with the experimental data and

349 the resulting coefficients C_1 and C_2 are presented in Table 2 for the data obtained in LYO A,
350 LYO B and their combination. The accommodation coefficient α_c was also calculated from
351 Equation 7, considering an average value of the gas temperature obtained under the different
352 operating conditions tested ($T_{gas} = -35$ °C). The obtained values of the accommodation
353 coefficient appear to be in agreement with data in literature.⁸

354 In Table 2, LYO A and LYO B exhibited a similar value of the C_1 coefficient that is related
355 to the contact area coefficient K_c (Equation 5), but distinct values of the C_2 coefficient that is
356 related to K_g (Equation 6). LYO B exhibited a slightly higher accommodation coefficient
357 than LYO A, probably due to a different finish of the freeze-dryer shelf material.

358 When considering pressure values lower than 10 Pa (Figure 2), the K_v values obtained in the
359 two freeze-dryer appeared similar. This result was confirmed also by Pisano,¹² who reported
360 no significant difference in K_v value of central vials processed in a pilot and manufacturing
361 freeze-dryer at a pressure of 10 Pa. For pressure value higher than 10 Pa, the influence of
362 freeze-dryer configuration became significant with slightly higher values obtained in LYO B
363 (Figure 2). At 50 Pa, the K_v value was approximately 8% higher in LYO B than in LYO A.
364 Considering the different values of C_2 coefficient, the K_v difference between freeze-dryers at
365 high pressure can thus be ascribed to the increased rate of heat transfer through the gas over
366 the total heat flow. However, the physical origin of the differences in the pressure-dependent
367 component of K_v remains unclear. Possible hypotheses include: (i) differences in the shelf
368 surface finish that could induce differences in the gas-shelf heat transfer through the
369 accommodation coefficient⁸ (Equation 6-7) as well as (ii) differences in the gas convection
370 conditions, a mechanism responsible for a small part of the pressure-dependent heat
371 transfer.²² These results suggest that small differences between devices might become more
372 apparent at high pressures. When considering only vials not exposed to edge effect a cycle
373 designed with low operating pressure (below 10 Pa) could therefore be more suitable for safe

374 scale-up. However the behavior of the edge vials located at the periphery of the shelf or in
375 contact with metallic band need also to be considered and some elements were recently
376 proposed by Pikal et al.¹¹ to investigate the impact of the freeze-dryer configuration.

377

378 **Impact of chamber pressure and shelf temperature on K_v**

379 As reported in the literature and shown in Figure 2, chamber pressure had a strong impact on
380 K_v .^{4,5,8,12,13,15} The vial heat transfer coefficient increased approximately four times between 4
381 and 50 Pa. At the vial bottom, the presence of the curvature limits the intimate contact
382 between the shelf and the vial and create an empty space between the shelf and the vial that
383 acts as an insulator.^{13,16,17} At very low pressures typically used in the process, the heat
384 transfer contribution by gas convection is usually neglected,¹¹ whereas the contribution of gas
385 conduction has to be considered. This heat transfer mechanism, represented by the coefficient
386 K_g , is dependent on the chamber pressure and increases when increasing pressure, as shown
387 in Equation 6.^{5,8,12,15} Figure 3 shows the relative contributions of K_c , K_r and K_g on the total
388 K_v , calculated using the set C of the fitting coefficients reported in Table 2. The K_c and K_r
389 contributions go from about 30 % at 4 Pa to 10 % at 50 Pa, whereas the K_g contribution goes
390 from about 25 % at 4 Pa to 80 % at 50 Pa.

391 A moderate effect of shelf temperature on K_v was expected theoretically due to K_r (Equation
392 10) and to K_g through the gas temperature (Equations 6-7). Figure 4a displays the influence
393 of shelf temperature on K_v . Differences in K_v values due to temperature remained within the
394 standard deviation when considering pressure values lower than 10 Pa and when the
395 contribution of K_g in the total K_v is moderate (around 25 %, Figure 3).

396 In order to clarify the impact of the shelf temperature, the contributions of the single
397 coefficients K_c , K_r and K_g on K_v were calculated for two shelf temperatures (25 °C and -25
398 °C) and three chamber pressures (4, 6 and 50 Pa), as show in Figure 4b. The contact

399 conduction coefficient K_c does not depend on the shelf temperature (Equation 5) and thus has
400 a constant contribution in K_v for all the temperatures tested. The radiative coefficient K_r
401 depends on the third power of the shelf temperature and increases by about $1 \text{ W m}^{-2} \text{ K}^{-1}$
402 between $-25 \text{ }^\circ\text{C}$ and $25 \text{ }^\circ\text{C}$ for all pressures considered. The gas conduction coefficient K_g
403 depends on the gas temperature and decreases by $0.1\text{-}0.2 \text{ W m}^{-2} \text{ K}^{-1}$ at low pressures (4-6
404 Pa) and by $1.1 \text{ W m}^{-2} \text{ K}^{-1}$ at 50 Pa, between the two considered values of shelf temperature.
405 When increasing shelf temperature, the increase of K_r is partly compensated by the decrease
406 of K_g . These results confirm that the dependence of K_v on the shelf temperature is negligible,
407 especially if compared with the role played by the chamber pressure. Pisano et al.¹² and
408 Hottot et al.²³ reported similar K_v values for different shelf temperature conditions.

409

410 **Inter-vial heat transfer heterogeneity and the role of vial dimensions**

411 Figure 5 presents the experimentally observed distributions of the vial heat transfer
412 coefficient data of central vials at six chamber pressures. Since temperature- and equipment-
413 induced variations were minor, data obtained in LYO A and B and for the two shelf
414 temperatures were merged. A significant variability in the K_v values evaluated for central
415 vials was observed, the standard deviation increasing with pressure from 0.84 to
416 $2.46 \text{ W m}^{-2} \text{ K}^{-1}$. The values of standard deviation corresponded to coefficient of variation
417 comprised between 4 and 8 % depending on the operating conditions. The measurement error
418 associated to the determination of K_v was evaluated as the sum of the individual
419 measurement accuracy of each parameter entering in the calculation of K_v (Equation 1). The
420 measurement uncertainty was estimated to be $\sim 1 \%$. This value is in agreement with the
421 value reported by Pikal et al.⁵, who reported an uncertainty value of $\sim 1.2 \%$. The
422 measurement uncertainty alone did not allow to completely explain the variability of the K_v

423 data. An external factor responsible for inter-vial heat transfer heterogeneity had thus to be
424 considered.

425 Attention was focused on the container: the geometrical difference among the vials was
426 considered as a possible source of the heat transfer heterogeneity. This variability in the vial
427 dimensions can be due to production limits and could change as a function of the container
428 model and provider. For the tested vial set, the coefficient of variation was approximately
429 27.7 % for the mean bottom curvature depth (l) and 23.9 % for the imprint-based contact area
430 (A_c). Hence, the effect of the variability of these geometrical dimensions on K_v was evaluated
431 as proposed in the Theory and data analysis section using the set of coefficients C reported in
432 Table 2.

433 Figure 6 displays the simulated distributions of K_v based on the vial bottom dimensions.
434 These distributions showed a trend and range of K_v values similar to the experimental ones.
435 At low pressure, K_v variability is almost completely due to the contact area variability. The
436 importance of the contact area on the K_v value was also confirmed by Pikal et al.⁵ and
437 Cannon et al.¹⁶ Regarding the vial bottom curvature, the importance of its variability
438 increased when the pressure rose. This is due to the coefficient K_g that plays a major role in
439 the total value of K_v at 40 and 50 Pa, as shown in Figure 3. The role of the bottom curvature
440 dimension was previously investigated by Brülls and Radsmuson¹⁵ and Cannon et al.¹⁶ Brülls
441 and Radsmuson¹⁵ have shown that the bottom curvature has an impact on the heat transfer
442 only at chamber pressures higher than 30 Pa. This conclusion was confirmed by Cannon et
443 al.¹⁶, who found that bottom curvature had little impact when considering low pressure (< 27
444 Pa). Our results agree with these conclusions,^{15,16} confirming that the variability of the
445 bottom curvature depth has to be taken into consideration only if cycles at high chamber
446 pressure are performed (> 30 Pa).

447 Figure 7 displays the coefficient of variation of the experimental and calculated
448 K_v distributions at different pressures. For the experimental distributions, the coefficient was
449 calculated as an average between the LYO A and B datasets. The trend of the observed
450 coefficient of variations for both experimental and simulated K_v distributions decreased from
451 approximately 9 % to 4 % when increasing the chamber pressure. The variability of the
452 experimental K_v distributions is completely explained by the geometrical variability at low
453 pressures (i.e., 4, 6 Pa), whereas at higher pressures, the experimental coefficient of variation
454 appears to be slightly higher than the one calculated based on vial geometry. It is thus
455 possible that other sources of variability should be taken into consideration, for example
456 convection in the drying chamber could play a role if higher pressures are considered.²²
457 These considerations can guide the selection of the container as a function of the variability
458 of the vial dimensions. The results obtained show that at low chamber pressure (i.e., 4, 6, 9,
459 15 Pa), it is important to assess the variability of the contact area between the vial and the
460 shelf, whereas for cycles performed at high pressure (i.e., 40, 50 Pa), the variability of the
461 bottom curvature depth becomes a relevant parameter. Consequently, for pharmaceutical
462 processes that are usually carried out at pressures lower than 10 Pa, the contact area needs to
463 be taken into account more than the bottom curvature depth.

464

465 **Impact of K_v heterogeneity on the product temperature distribution within a batch of**
466 **vials located in the centre of the shelf and not exposed to edge effect**

467 In the case of freeze-drying, product temperature is one of the most important critical quality
468 parameters. During the process, product temperature must be maintained close to a limit
469 value (i.e., glass transition temperature for amorphous products, T_g) in order to optimize the
470 process time but not to exceed it so as to guarantee the visual aspect of the cake and the
471 product quality.

472 The vial-to-vial heat and mass transfer heterogeneity during the sublimation step causes
473 variability in the product temperature. Considering a constant and fixed value of mass
474 transfer resistance, it would be interesting to estimate the product temperature distribution
475 during the primary drying step resulting only from the variability in vial geometry. Product
476 temperature distributions were thus evaluated considering the contact area and curvature-
477 based K_v distributions. For this analysis, a 5 % w/w sucrose solution was considered,
478 processed at -25 °C and four different pressures (4, 6, 9 and 15 Pa). Relevant data concerning
479 product resistance and glass transition temperature (-32 °C) were found in the literature.²¹ As
480 expected, product temperature increased from 4 Pa to 15 Pa because of the higher value of
481 the vial heat transfer coefficient and higher ice sublimation temperature.

482 The variability of the product temperature was estimated to be approximately 0.9 °C at 15 Pa
483 and as large as 2.2 °C at 4 Pa, considering ± 3 times the standard deviation that includes 99 %
484 of the vials (Figure 8). A practical implication of these results is that, at the low pressures
485 commonly encountered in vaccine freeze-drying, a temperature safety margin of
486 approximately 2 °C has to be considered with respect to cycles designed on the basis of an
487 average K_v value and to vials not exposed to edge effect.

488 Vials located at the periphery of the shelf (i.e., edge vials) receive additional heating due to
489 the radiation from the chamber walls and the contact with the metallic guardrail. Thus, edge
490 vials present a higher sublimation rate and a higher product temperature respect central vials.⁵
491 Tang et al.²⁴ reports that the temperature difference between edge vials and central vial can be
492 up to 2 °C at a shelf temperature of 20 °C and up to 4 °C at -30 °C for a chamber pressure of
493 about 10 Pa. Depending on the operating conditions of the process, the safety product
494 temperature margin resulting from variability in vial dimensions could be in the same order
495 of magnitude than the safety margin imposed by the "edge effect".

496

497 **CONCLUSIONS**

498 Implementation of the Quality by Design initiative require a precise definition of the
499 acceptable range for all product and process variables ensuring the fulfillment of the critical
500 quality attributes of the final product. The impact of any variation of these variables on the
501 final quality need to be quantified in advance. In this work, the effect of the variability of
502 geometrical dimensions observed within a batch of vials (i.e., contact area between the shelf
503 and the vial and the mean bottom curvature depth) on product quality was explored. The
504 product quality was evaluating by predicting the product temperature knowing the vial heat
505 transfer coefficient K_v . An original approach was proposed to calculate K_v distribution based
506 on geometrical dimensions when considering a batch of vials located in the center of the shelf
507 not exposed to any edge effect. The impact of freeze-dryer configuration and operating
508 conditions was also considered. When considering low pressure (< 10 Pa), commonly used
509 for freeze-drying biopharmaceuticals, the influence of freeze-dryer configuration and shelf
510 temperature on heat transfer characteristics can be neglected and K_v distribution is
511 completely explained by the contact area distribution. Furthermore the variability of vial
512 dimension results in the definition of a product temperature safety margin of 2 °C. However,
513 additional sources of variability need to be included in QbD approach. In particular, a study
514 focused on the variability between edge and central vials and its role in cycle scale-up is
515 presently ongoing.

516

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519 reviewing this manuscript and Vincent Ronsse (technician) and Alain Philippart (operator)
520 (GSK Vaccines) for their help in the data acquisition.

521

522 **CONFLICT OF INTEREST**

523 Erwan Bourlés is an employee of the GSK group of companies. Bernadette Scutellà
524 participated in a postgraduate PhD program at GSK Vaccines. Stephanie Passot, Fernanda
525 Fonseca and Ioan Cristian Trelea report no financial conflicts of interest.

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531

532 **AUTHORS' CONTRIBUTIONS**

533 Bernadette Scutellà, Stephanie Passot, Erwan Bourlés, Fernanda Fonseca and Ioan Cristian
534 Trelea were involved in the conception and design of the study. Bernadette Scutellà and
535 Erwan Bourlés acquired the data. Bernadette Scutellà, Stephanie Passot, Erwan Bourlés,
536 Fernanda Fonseca and Ioan Cristian Trelea analyzed and interpreted the results. All authors
537 were involved in drafting the manuscript or revising it critically for important intellectual
538 content. All authors had full access to the data and approved the manuscript before it was
539 submitted by the corresponding author.

540

541

APPENDIX

542 The evaluation of the mean bottom curvature depth l was performed from geometrical
543 considerations on the semi-spherical calotte at the vial bottom, as represented in Figure A.

544 The bottom curvature depth depends on the radius of the vial bottom as follows:

545

$$546 \quad l(r) = R_c - a(r) \quad \text{Equation A1}$$

547

548 where R_c is the radius of the calotte and $a(r)$ is the distance between the shelf and the vial
549 bottom, measured normal to the vial bottom:

550

$$551 \quad a(r) = \sqrt{(R_c - l_{max})^2 + r^2} \quad \text{Equation A2}$$

552

553 R_c can be calculated as a function of the maximum bottom curvature depth l_{max} and the inner
554 vial bottom radius R_i :

555

$$556 \quad R_c^2 = R_i^2 + (R_c - l_{max})^2 \quad \text{Equation A3}$$

557

558 The area-mean bottom curvature depth can be defined as the integral of $l(r)$ on the calotte,
559 divided by the area:

560

$$561 \quad l = \frac{1}{A} \int l(r) dA \quad \text{Equation A4}$$

562

563 The relevant area for heat transfer by gas conduction is:

564

$$565 \quad A = \pi R_i^2 \quad \text{Equation A5}$$

566

567 and the area element:

568

569

$$dA = 2\pi r dr$$

Equation A6

570

571 Combining Equations A1-A6, l was calculated as:

572

573

$$l = \frac{2}{R_i^2} \int_0^{R_i} (R_c - \sqrt{(R_c - l_{max})^2 + r^2}) r dr$$

Equation A7

574

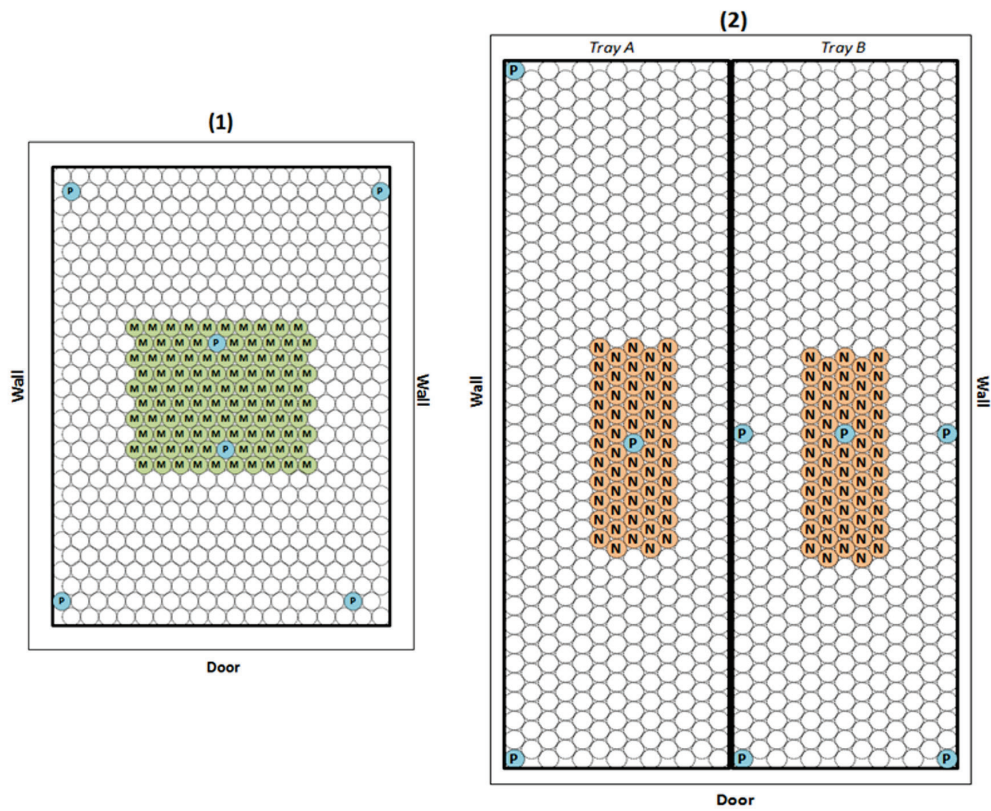
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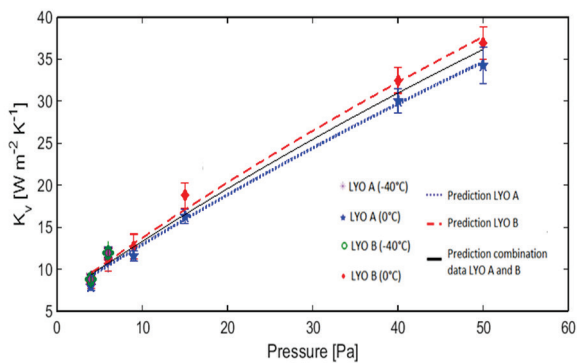
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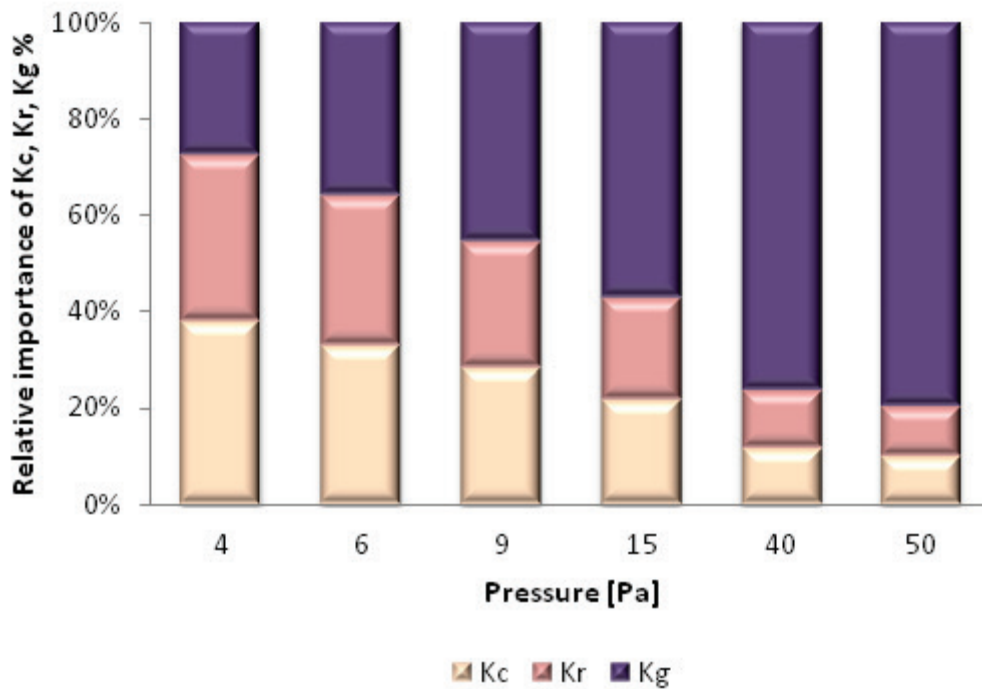
640 **Figure 1.** Vial arrangements in (1) LYO A and (2) LYO B. Gravimetrically-analyzed vials
 641 are marked with the letters M and N for LYO A and B, respectively. Vials in which wireless
 642 temperature probes were located are marked with the letter P. All vials were filled with 1.8
 643 mL of pure water.

644

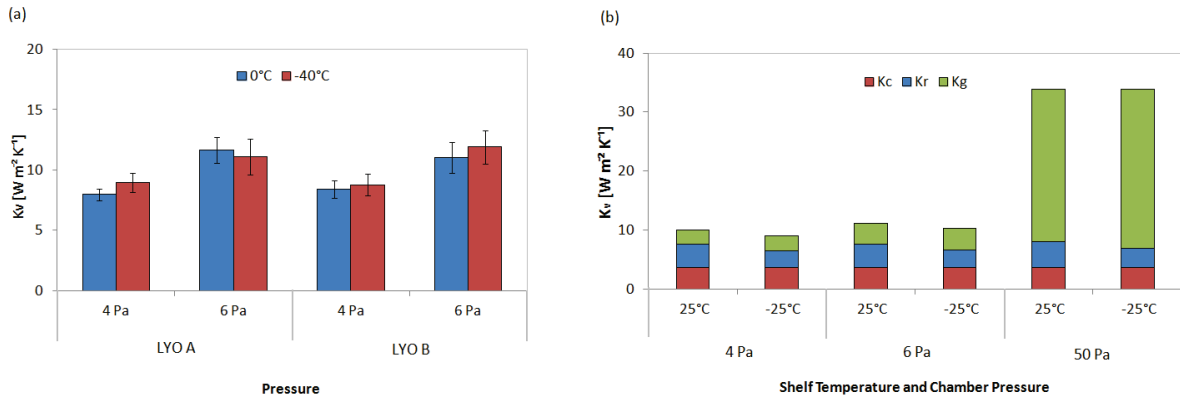


645

646 **Figure 2.** Vial heat transfer coefficient (K_v) values vs. chamber pressure (P_c). The markers
 647 refer to the K_v average values measured in LYO A and B at -40°C and 0°C . The lines
 648 correspond to the values calculated with Equation 14 with the data obtained from LYO A, B
 649 and their combination. Error bars represent standard deviations.
 650



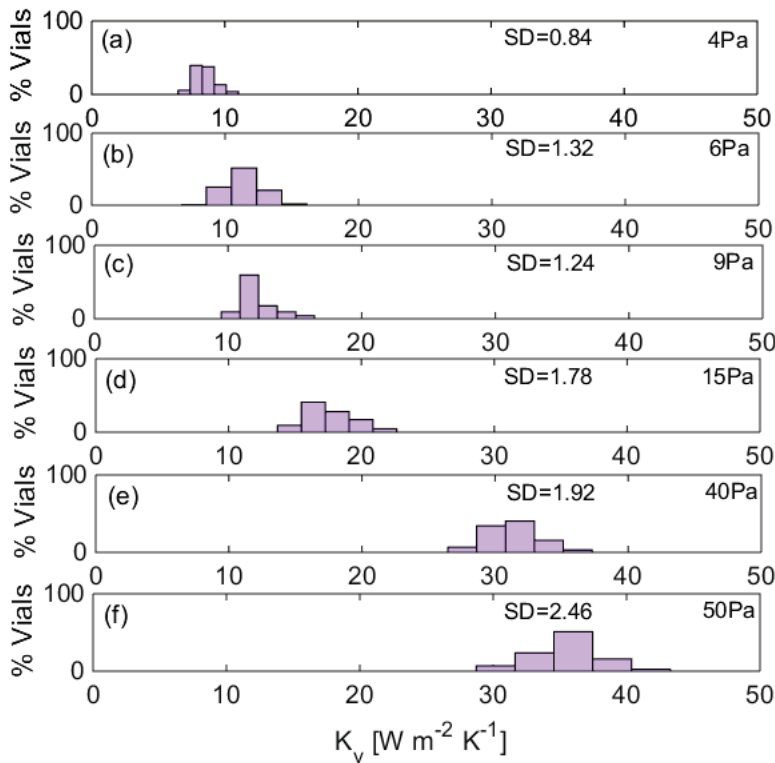
651
 652 **Figure 3.** Relative importance of the heat transfer coefficients by contact conduction (K_c),
 653 radiation (K_r) and conduction through the gas (K_g) as percentages of the total heat transfer
 654 coefficients (K_v). Average values of the contact area (A_c) and mean bottom curvature depth
 655 (l) were considered in this calculation. The set of coefficients C (Table 2) was used to
 656 evaluate K_c and K_g .
 657



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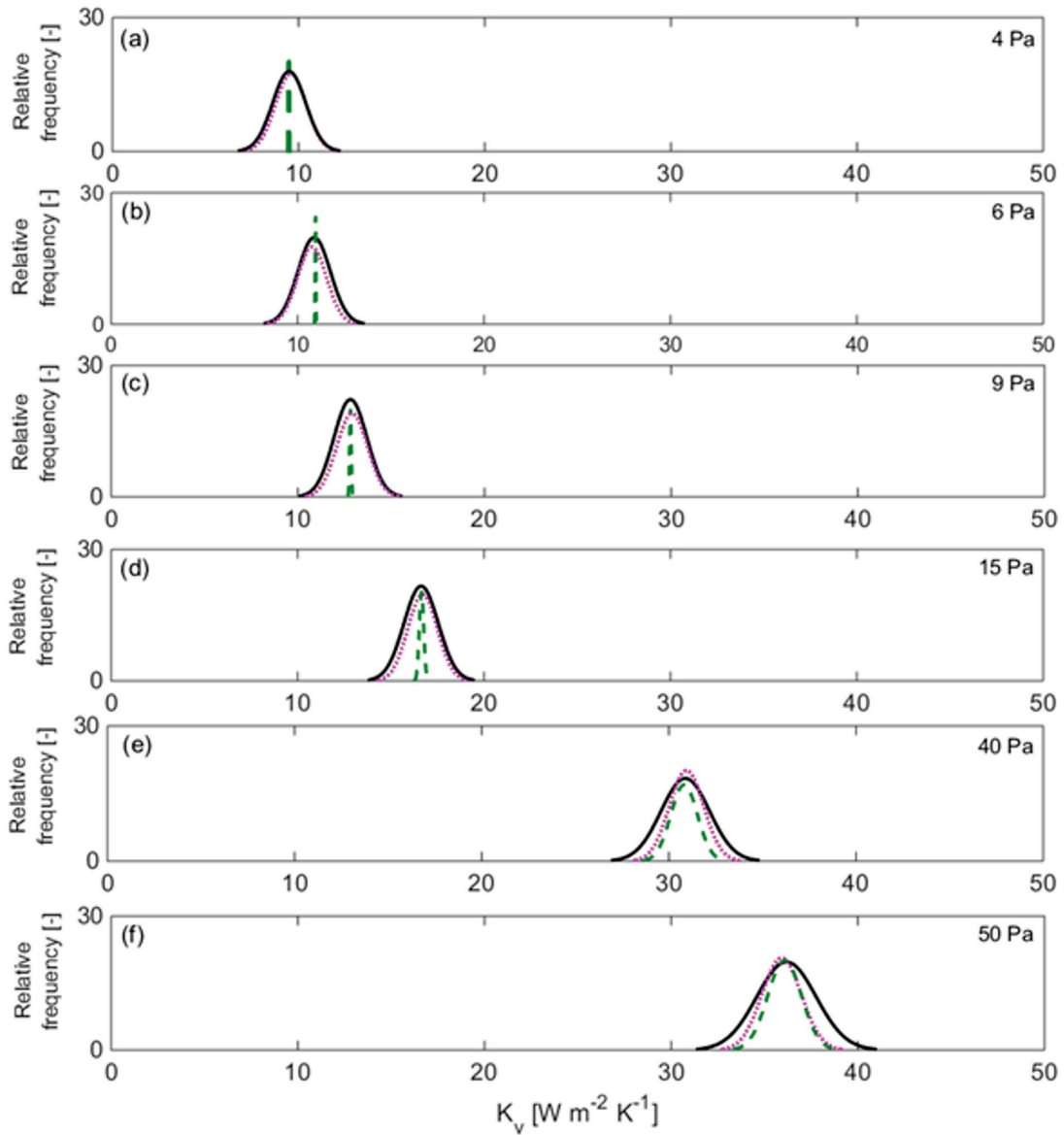
659 **Figure 4.** Influence of the shelf temperature on the vial heat transfer coefficients K_v . K_v
 660 values were evaluated at different shelf temperatures and chamber pressures (a) from
 661 experimental data obtained in LYO A and B and (b) from the coefficients K_c , K_r and K_g ,
 662 calculated using Equations 5, 6 and 10.

663



664

665 **Figure 5.** Experimentally-measured distribution of the vial heat transfer coefficients at 4 Pa
 666 (a), 6 Pa (b), 9 Pa (c), 15 Pa (d), 40 Pa (e) and 50 Pa (f). Data of LYO A and LYO B at
 667 different shelf temperatures (0°C and -40°C) were combined.



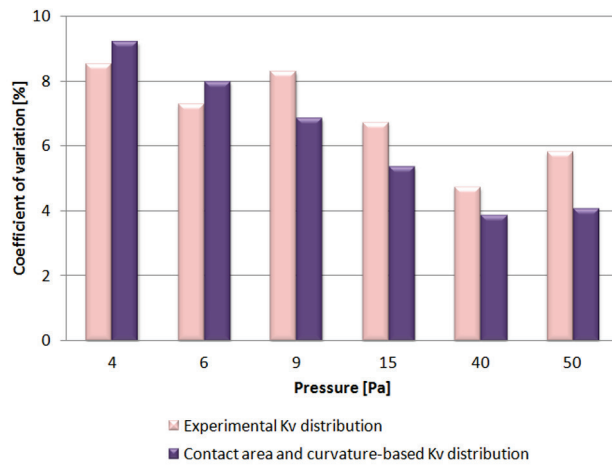
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670 **Figure 6.** Calculated K_v normal distributions: curvature-based (dashed green line - -),

671 contact area-based (dotted red line - - -) and combined curvature and contact area-based

672 (solid black line —) at 4 Pa (a), 6 Pa (b), (c) 9 Pa (c), 15 Pa (d), 40 Pa (e) and 50 Pa (f).

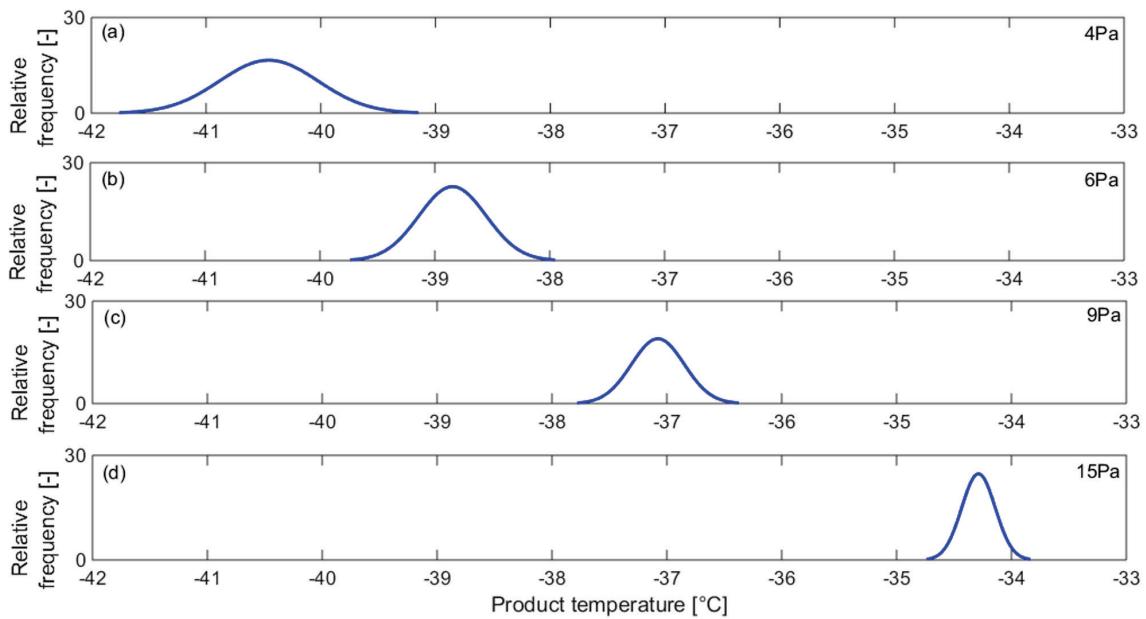
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674

675 **Figure 7.** Coefficients of variation of experimentally-measured K_v distribution (LYO A and
 676 B; light bars) and calculated (combined contact area and curvature-based) K_v distribution
 677 (dark bars) and at different pressures.

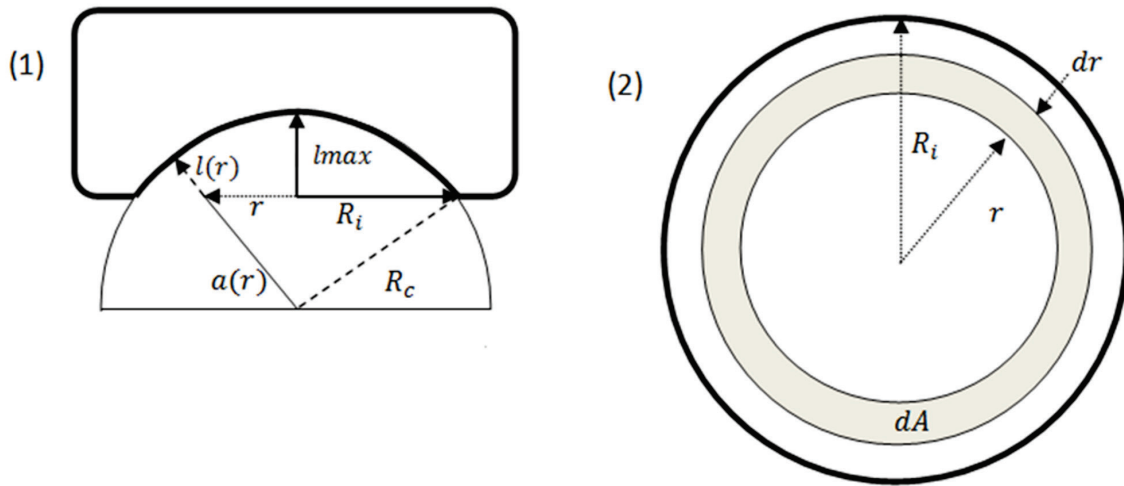
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680 **Figure 8.** Product temperature distributions obtained from the contact area and curvature-
 681 based distribution for a 5 % w/w sucrose solution processed at a shelf temperature of -25 °C
 682 and four chamber pressures: (a) 4 Pa, (b) 6 Pa, (c) 9 Pa and (d) 15 Pa.

683



684

685 **Figure A.** Side (1) and top (2) view of the vial bottom represented as a semi-spherical calotte.

686

687

688