RESEARCH ARTICLE

Demonstrating Functional Equivalence of Pilot and Production Scale Freeze-Drying of BCG

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Abstract

Process analytical technology (PAT)-tools were used to monitor freeze-drying of Bacille Calmette-Guérin (BCG) at pilot and production scale. Among the evaluated PAT-tools, there is the novel use of the vacuum valve open/close frequency for determining the end-point of primary drying at production scale. The duration of primary drying, the BCG survival rate, and the residual moisture content (RMC) were evaluated using two different freeze-drying protocols and were found to be independent of the freeze-dryer scale evidencing functional equivalence. The absence of an effect of the freeze-dryer scale on the process underlines the feasibility of the pilot scale freeze-dryer for further BCG freeze-drying process optimization which may be carried out using a medium without BCG.

Introduction

Lyophilization or freeze-drying is often used for stabilization of biopharmaceuticals such as vaccines [1,2]. Besides improvement of the stability of vaccines, lyophilization is used to facilitate the production of new dosage forms of vaccines [3,4], such as bioneedles for intramuscular delivery [5–8] or powders for pulmonary delivery [9,10]. If properly formulated, lyophilized vaccines are less prone to chemical and physical degradation pathways owing to the removal of water and vaccine antigen vitrification in the formulation [11].

Bacille Calmette-Guérin (BCG) vaccine contains a non-infectious strain of Mycobacterium bovis and is used prophylactically against tuberculosis or for immune therapy against bladder cancer [1,12–14].

The process of freeze-drying includes besides a freezing step also two drying steps: primary drying, and secondary drying. During primary drying ice is removed by sublimation and during secondary drying water is removed by desorption [2,11,15].

The strategy for the design of a freeze-drying process is generally based on the physical properties of the formulation in order to aim at a freeze-dried product with an intact cake structure. The occurrence of cake collapse, although not necessarily detrimental for the
product, is often unwanted and may pose a reason for rejection of that vial. Collapse may occur during lyophilization by raising the product temperature (T_p) above a critical threshold, the onset collapse temperature (T_oc). The T_oc depends on the composition of the formulation and may be determined by freeze-drying microscopy (FDM) [15]. For amorphous solids, collapse may occur upon raising the product temperature to or slightly beyond the glass transition temperature of the maximally freeze-concentrated fraction (T_g0). In the case of crystalline excipients, collapse may occur upon increasing the T_p to the eutectic temperature (T_e). Both amorphous and crystalline components may be present in a single formulation [2].

Collapse can be prevented by choosing an appropriate shelf temperature and chamber pressure to ensure the T_p to remain below the T_oc during primary drying [2, 15]. Optimal process conditions for primary drying may be characterized by a minimum duration of primary drying and a maintained cake structure. This is relevant since freeze-drying is a lengthy process [2] especially in the case of non-optimized lyophilization cycles. In general, optimization of the duration of primary drying gives the biggest efficiency gain in lyophilization, resulting in less process time, and increased production capacity.

The aim of this study is to demonstrate product (RMC and BCG survival rate) and process (read: primary drying time) equivalence of pilot scale (total shelf area 2.7 m²) and production scale (total shelf area 43.2 m²) freeze-drying of BCG to support further BCG freeze-drying process optimization at pilot scale. This will be done by using both well-known PAT-tools, the pressure rise test (PRT) [16], T_p [17], a balance [18], and the condenser temperature [19], and a, to our knowledge, novel PAT-tool, which is the open/close frequency of the vacuum valve used for chamber pressure control. This frequency is indirectly determined from the saw-tooth pattern in the recorded graphs of the registered chamber pressure during freeze-drying. Process equivalence may also be demonstrated by showing that T_p-profiles (T_p vs time) are independent of the freeze-dryer scale [20–23], however, GMP-restrictions, did not allow measurement of the T_p at production scale. For that reason, alternative in-process parameters were considered as (scale-dependent) PAT-tools to monitor primary drying and determine the endpoint thereof.

The comparison of freeze-drying processes as a function of dryer scale has been documented before [24, 25]. This study contributes to the existing knowledge by providing a substantial experimental data set on two freeze-drying cycles, which gives insight in process variation and equipment related factors (if present).

Materials and Methods
Description of formulation, products and materials
After cultivation, BCG was pelleted by centrifugation and formulated in freeze-drying medium [14, 26]. One liter of freeze-drying medium (HGT-medium) contains 108 g glucose, 25 g poly-gelone (Thera Select unless stated otherwise.) and 0.05 g Tween80.

Prior to freeze-drying 23 mL vials are filled with either MiliQ (purified water), HGT-medium, or formulated BCG to reach a total volume of 10 mL/vial (fill height = 2.0 cm) which are equipped with stoppers (Helvoet Pharma, The Netherlands).

Freeze-drying was performed at pilot scale (Klee, shelf surface area: 2.7 m²) and/or production scale (Klee, shelf surface area: 43.2 m²). The pilot freeze-dryer contains 5 shelves, including a sampling shelf and balance shelf. The sampling shelf is suitable to be completely transported to the interior of a transparent Perspex chamber mounted on the freeze-dryer door from where vials can be closed and/or removed during the freeze-drying process using a sample thief.
Freeze-drying protocols

Freeze-drying was performed according to two cycles, I and II (see Table 1).

In these cycles, a PRT was performed as indicated. If the PRT-criterion (pressure rise < 0.03 mbar/min) of cycle II was not met, an extra drying time of 2 hours was added after which another PRT was performed ultimately till the end of primary drying was reached (the PRT-criterion was met) after which secondary drying was started.

The difference in chamber pressure during secondary drying between cycle I and II was presumed not to impact product quality since the water desorption rate in the cycles used is independent of the chamber pressure below 0.27 mbar [2].

Demonstrating equivalent drying behavior of HGT-medium and BCG in HGT-medium

Drying of HGT-medium and BCG in HGT-medium was investigated using a fully loaded pilot freeze-dryer. The freeze-dryer predominantly contained BCG in HGT-medium. On the sampling shelf, HGT-medium and BCG in HGT-medium were placed next to each other (in sets of three each). A sample thief was used to stopper and remove vials during freeze-drying according to cycle II.

Effect of chamber pressure on the sublimation rate

A series of independent experiments was performed in the pilot freeze-dryer to study the effect of the chamber pressure (from 0.01 to 0.5 mbar) on the sublimation rate. Vials were filled with either 10 mL MiliQ or HGT-medium, stoppered, and loaded onto the balance shelf (~ 79 vials) and the sampling shelf (~ 79 vials). In order to reduce the impact of external heat radiation, the Perspex chamber was covered with aluminum foil. Freezing was performed as described before (see Table 1). During primary drying at the studied chamber pressure, the shelves were held 1 hour at -35°C, then in 0.5 hour to -30°C and thereafter, up to 40 hours at -30°C. The average sublimation rate to remove 100 g of ice was calculated from the decrease in mass over time.

Table 1. Freeze-drying protocols as used in this study (PD = primary drying, and SD = secondary drying). The pressure rise test (PRT) in cycle I was used to confirm the end of primary drying. In cycle II the PRT was used during primary drying and after not meeting the criterion (maximum rise in pressure <0.03 mbar/min) the primary drying stage was prolonged by an additional 2 hours.

| Step   | Cycle I | Cycle II |
|-------|---------|----------|
|       | t (hours) | T (°C) | p (mbar) | t (hours) | T (°C) | p (mbar) |
| Loading | +4 | -1000 |  | +4       | -1000 |  |
| Equilibration | 3 | to -15 | -1000 | 3       | to -15 | -1000 |
| Freezing | 1 | -15 | -1000 | 1       | -15   | -1000 |
| PD | 1 | -35 | 0.045 | 0.5 | to -30 | 0.045 |
|     | 227, PRT | -30 | 0.045 | 90 | -30   | 0.09 |
|     |  | 2, PRT | -30 | 0.09 |
| SD | 30 | +30 | 0.045 | 30 | +30  | 0.09 |
|     | 30 | +30 | 0.007 | 30 | +30  | 0.046 |

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Analytical testing

BCG survival. For determination of the BCG survival, a dilution series was prepared in Sauton solution (freeze-dried material was resuspended in physiological salt solution) and the number of viable BCG bacteria, before and after freeze-drying, were determined by counting the colonies grown (at a temperature of 36 ± 0.5°C for at least 28 days) on Loewenstein agar plates.

Results are reported as the average BCG survival of three randomly selected vials (this represents an analysis result).

Residual moisture content. The residual moisture content (RMC) was determined using a Karl Fischer coulometric titrimeter (Model CA-06 moisture meter, Mitsubishi). The samples were weighed, subsequently reconstituted in 10 mL Coulomat A (Fluka, Switzerland) and a volume of 0.1 mL was injected into the titration vessel. The mass of the dried material was calculated by also determining the mass of the empty vial (+stopper). The RMC was calculated based on the measured water content, the mass of the dried material, the reconstitution volume, the injection volume and the water content of the blank.

To obtain an analysis result, the average RMC of three vials (randomly selected from the batch) was calculated.

Statistical analysis

The statistical analysis were performed using Student’s t-test with P < 0.05 as the minimal level of significance.

The number of experiments (n) refers to the number of analysis results, or primary drying times from independent experiments.

Average values are presented ± standard deviation.

Results

Scale-dependent PAT-tools for monitoring primary drying

Several in-process parameters, PAT-tools, are available for monitoring BCG primary drying at pilot and production scale (see Table 2) of which the PRT is available in both freeze-dryers.

At production scale, the PRT is only routinely used to confirm the endpoint after the fixed primary drying time of 228.5 hours. By using the PRT for process monitoring (see Fig 1) it can be seen that this fixed period of time is longer than strictly required, i.e. the required time for primary drying is 150–160 hours as by then the pressure rise is very low and almost constant.

Table 2. Overview of in-process parameters at pilot and production scale that are useful for monitoring primary drying and primary drying endpoint determination.

| Parameter                         | Feasibility for primary drying endpoint determination |
|----------------------------------|-------------------------------------------------------|
|                                  | Production scale | Pilot scale |
| Pressure rise test               | +              | +           |
| Product temperature (Tp)         | -              | +           |
| Mass (balance shelf)             | -              | +           |
| Outlet condenser temperature     | +              | -           |
| Vacuum valve*                    | +              | -           |

* Refers to changes in the chamber pressure caused by opening and closing of the vacuum valve.

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The observed trend in values from the PRT corresponds to time profiles of the outlet condenser temperature (see Fig 1A) and the vacuum valve open/close frequency (see Fig 1B) evidencing the use of these methods for monitoring the primary drying process.

In the pilot freeze-dryer, use of the balance shelf and external thermocouples were evaluated for determination of the endpoint of primary drying (data not shown). It appeared that passing the PRT criterion also coincided with: A) no further decrease in mass of vials placed on the balance shelf and B) a constant apparent product temperature that is generally above the shelf temperature.

Functional equivalence of BCG freeze-drying

Freeze-drying according to cycle I. Production scale freeze-drying of BCG is done according to a non-optimized freeze-drying cycle (cycle I) which consistently yields product (see Table 3) with a BCG survival rate of 7.4 ± 2.5% (n = 14), and an RMC of 0.78 ± 0.08% (n = 14).

Estimating the duration of primary drying using the vacuum-valve-method and the outlet-condenser-temperature-method indicated that a duration of ~162 hours (instead of 228.5 hours) appeared already sufficient to remove all ice. Pilot scale freeze-drying resulted in an average primary drying time of ~153 hours, which was not significantly different from the production scale value (p > 0.05).

Table 3. Overview of primary time (hours), RMC (%), and BCG survival rate (%) as a function of the freeze-drying cycle and freeze-dryer scale. Several methods were used for primary drying endpoint determination: the vacuum valve open/close frequency, the outlet condenser temperature, and PRT refers to the pressure rise test. The maximum load of the freeze-dryers is as follows: 12500 vials at production scale and 750 vials at pilot scale. See S1 Data for raw data.

| Description       | Vials | PD time (hours) | RMC (%)          | Survival (%)   |
|-------------------|-------|-----------------|------------------|----------------|
| **Cycle I**       |       |                 |                  |                |
| **Production scale** |      |                 |                  |                |
| Fixed time        | ~ 1050| 228.5           | 0.78 ± 0.08 (n = 14) | 7.4 ± 2.5 (n = 14) |
| Vacuum valve      |       | 164.2 ± 3.1 (n = 6) |                |                |
| Outlet cond. temp.|       | 158.3 ± 5.7 (n = 6) |                |                |
| Overall           |       | 161.3 ± 6.5     |                  |                |
| **Pilot scale**   |       |                 |                  |                |
| • PRT             | ~ 750 | 153.5 (n = 2)   | 0.82 (n = 2)     | -              |
| • PRT             | ~ 160 | 150.7 (n = 1)   | -                | -              |
| Overall           |       | 152.5 ± 5.5     |                  |                |
| **Cycle II**      |       |                 |                  |                |
| **Production scale** |      |                 |                  |                |
| • PRT             | ~ 1050| 129.0 ± 2 (n = 3) | 0.83 ± 0.06 (n = 3) | 8.4 ± 2.5 (n = 3) |
| **Pilot scale**   |       |                 |                  |                |
| • PRT             | ~ 750 | 120.6 ± 6 (n = 4) | 0.80 ± 0.07 (n = 3) | 7.7 ± 3.0 (n = 3) |
| • PRT             | ~ 160 | 131.3 (n = 1)   | -                | -              |
| Overall           |       | 122.9 ± 7.0     |                  |                |

* The supplier of the polygeline component was Piramal.
Selecting a more favorable chamber pressure. In order to study the effect of a process change as function of freeze-dryer scale, a more favorable chamber pressure was selected by confirming the well-known effect of the chamber pressure on the sublimation rate [25, 27]. Fig 2 shows that a higher sublimation rate may be obtained by applying a chamber pressure of 0.09 mbar (in cycle II) instead of 0.045 mbar (as used in cycle I) to freeze-dry both purified water and HGT-medium. In all cases, the HGT cake structure was maintained during the course of the experiment.

Freeze-drying according to cycle II. At production scale, a primary drying time of ~129 hours was obtained, a gain in process time of ~30 hours compared to cycle I (see Table 3). The BCG survival rate was 8.4 ± 2.5% and the RMC 0.83 ± 0.06%. The primary drying time, BCG survival rate, and RMC did not differ significantly (p > 0.05) from those observed at pilot scale. The results from cycle I and II were used to demonstrate functional equivalence of pilot and production scale freeze-drying of BCG.

The feasibility of the pilot scale freeze-dryer as a down-scale model for further freeze-drying cycle optimization was confirmed further by freeze-drying an experimental HGT-medium (without BCG).
The available and relevant in-process parameters showed an evident change after ~116 hours of primary drying (see the vertical line in Fig 3A–3C). This change likely illustrated a decrease in the overall sublimation rate, as concluded from the slower loss in mass of vials on the balance shelf in the pilot scale freeze-dryer beyond ~116 hours of primary drying (see Fig 3C). This time course may be explained by a decreasing number of vials still containing ice, i.e. the drying rate of edge vials is known to be higher than that of centre vials [24, 28–30]. Therefore, the vertical line in Fig 3A–3C apparently marks the moment at which vials at (and/or close to) the edge no longer contain ice. This suggests that primary drying of edge vials was independent of the dryer scale.

At production scale, the inlet condenser temperature (see Fig 3A) showed a constant minimum of ~-80°C. Apparently, there is a substantial amount of heat transferred to the condenser during solidification of water vapour onto the condenser, which is reflected by a higher initial outlet condenser temperature of ~-60°C. A lower overall sublimation rate (beyond ~116 hours) results in a lower outlet condenser temperature because less heat is transferred to the condenser. This means that the observed pattern in condenser outlet temperature is related to the cooling capacity of the equipment.

The observed pattern in the vacuum valve open/close frequency of the production scale freeze-dryer (see Fig 3B) may be explained by the decreasing need to adjust the chamber pressure near the end of primary drying.

The increase in pressure from the PRT was included as a control (see Fig 3A and 3B) and was found to parallel the outlet condenser temperature and the vacuum valve open/close frequency. This confirmed the suitability of both methods for primary drying endpoint determination as described earlier.

In the pilot scale freeze-dryer, the apparent product temperature of a centre vial on the sampling shelf is clearly below the shelf temperature of -30°C during sublimation (see Fig 3C). The product temperature increases as cooling of the vial decreases, beyond a primary drying time of ~116 hours. At the end of primary drying, the product reaches a temperature above the shelf temperature due to outside heat radiation.

In an independent experiment, it was tested whether freeze-drying of HGT-medium would be representative for BCG freeze-drying. Fig 4 shows that the removal of moisture, reflected by a decrease in vial content and RMC, is comparable. This shows that further cycle improvement may be performed with HGT-medium (or mostly HGT-medium and some vials containing BCG).

**Discussion**

**PAT-tools for monitoring primary drying and demonstrating process equivalence**

This study introduces a novel PAT tool, the vacuum valve open/close frequency for monitoring primary drying and determination of the primary drying endpoint. Opening and closing of the
A vacuum valve resulted in a typical saw-tooth pattern in the chamber pressure (not shown) which was registered during BCG freeze-drying. The fact that the production scale process is apparently not operated at a constant chamber pressure is not ideal and for that reason, most production scale freeze-dryers are currently equipped with a calibrated leak. In such a case, it is possible to use the bleed rate of (sterile) N\textsubscript{2} into the freeze-dryer as a PAT-tool for process monitoring [19].

Besides these two tools, there are many more available in the literature [16] of which use of the PRT [16], T\textsubscript{p} [17], balance [18], and condenser temperature [19] as used in this study are well-known examples.

Demonstrating process equivalence is preferred in case of process transfer to another freeze-dryer. In such a case, the product temperature vs time profiles should ideally be the same [20–23]. With respect to the duration of primary drying, the T\textsubscript{p} of centered vials is relevant as these are generally colder than edge vials and will need more time to dry. Edge vials

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**Fig 4. Freeze-drying of 10 mL HGT-medium and BCG in HGT-medium in the pilot freeze-dryer.** Depicted are: the vial content (g) of BCG in HGT-medium (black circle) and HGT-medium (clear circle) and the RMC (%) of BCG in HGT-medium (black square) and HGT-medium (clear square). Each dot represents an average value ± standard deviation (n = 3). The solid horizontal line shows represent the total process time. The stars mark the beginning of primary, and secondary drying.

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may be relevant for studying the visual appearance of the cake. In this study, the visual appearance (mostly intact with only small dents at the bottom) of the cake was independent of the dryer scale and position of the vial indicating that in all cases the $T_p$ was below the critical threshold and therefore visible cake collapse did not occur.

This study shows that besides measurement of $T_p$, also other in-process parameters appear useful to demonstrate process equivalence upon operating different freeze-dryers as also explained elsewhere [19].

**Effect of freeze-dryer scale and other factors on primary drying**

There are several examples described in the literature on the comparison of processes operated in different freeze-dryers [24, 25, 31]. Running the same cycle in different freeze-dryers is no guarantee for process equivalence as several (scale dependent) factors may affect the process [19–21].

A GMP production scale freeze-drying environment may be cleaner (less particles) than a research setting which in turn may result in a higher degree of supercooling during freezing at production scale. Compared to pilot scale freezing, this may result in the formation of smaller ice crystals, a higher average $T_p$, and a longer primary drying time approximating 1% of the total PD time/°C of extra supercooling [17, 18]. In this study, no difference in primary drying time was noted. Probably, the equilibration step, in which the vials are kept at -15°C for 1 hour before freezing, and/or the presence of sufficient (bacterial) nucleation sites [32] minimize(s) freezing differences.

Also the dryer load may affect the primary drying time [22]. In this study, this possibility was addressed by lowering the number of vials in the pilot scale freeze-dryer. It was found that a lower load of 20% (a fully loaded middle shelf, i.e. loaded with two cassettes) did not result in a reduced primary drying time (see Table 3) likely because the percentage of warm edge vials remained unchanged.

Interestingly, the observed variation in the determined primary drying time (see Table 3) is relatively small (RSD < 6%). This experimental base is in support of a theoretical approach, which allows calculation of for example the effect of shelf temperature on the primary drying time. Typically, such calculations appear satisfactory [23], suggesting that inherent freeze-drying process variation is small in general.

**Outlook towards process time reduction of BCG freeze-drying**

It was demonstrated here that pilot scale freeze-drying resembled the production scale process suggesting suitability of the pilot scale freeze-dryer for future process optimization. For optimization experiments, use of HGT-medium (for example supplemented with some BCG vials) is possible as drying was not affected by the presence of BCG (see Fig 4).

It is conceivable that process time reduction may be obtained by increasing the shelf temperature. A short study indicated that the use of a shelf temperature gradient during primary drying could significantly reduce the primary drying time without affecting the cake appearance (data not shown), but this cycle was not implemented at production scale.

Besides shortening the duration of primary drying it may also be possible to reduce the secondary drying time by for example increasing the shelf temperature ramp rate of the first secondary drying step (see Table 1) as explained elsewhere [2].

**Risks related to process transfer from a pilot to a production scale freeze-dryer**

The freeze-drying cycles studied here use a constant shelf temperature for primary drying. It was anticipated that this would mitigate risks involved in process transfer, i.e. avoid process
differences associated to uneven heating of shelves, and/or temperature lag of which both are conceivable in case of a (steep) shelf temperature gradient. The introduction of extra primary drying time (10 h) after the estimated endpoint of primary drying would mitigate the risk for the occurrence of melt back in vials located in the center of an array (not required in this study).

The risk for the occurrence of evident cake collapse in edge vials was not anticipated (at least in the case of BCG freeze-drying) because the impact of heat radiation is generally less in a production scale freeze-dryer.

Supporting Information

S1 Data. Raw data corresponding to Table 3. (XLSX)

Author Contributions

Conceived and designed the experiments: RtH KR PvH J-PA GK. Performed the experiments: RtH KR. Analyzed the data: RtH KR. Contributed reagents/materials/analysis tools: RtH KR. Wrote the paper: RtH KR PvH J-PA GK.

References

1. Gheorghiu M, Lagranderie M, Balazuc AM. Stabilisation of BCG vaccines. Dev Biol Stand. 1996; 87:251–61. PMID: 8854025.
2. Tang X, Pikal MJ. Design of freeze-drying processes for pharmaceuticals: practical advice. Pharmaceutical research. 2004; 21(2):191–200. PMID: 15032301.
3. Amorij J, Kersten G, Saluja V, Tonnis WF, Hinrichs WLJ, Slüter B, et al. Towards tailored vaccine delivery: Needs, challenges and perspectives. Journal of Controlled Release. 2012; 161:363–76. doi: 10.1016/j.jconrel.2011.12.039 PMID: 22245687
4. Kraan H, Vreling H, Czerkinsky C, Jiskoot W, Kersten G, Amorij J. Buccal and sublingual vaccine delivery. Journal of Controlled Release. 2014; 190:580–92. doi: 10.1016/j.jconrel.2014.05.060 PMID: 24911355
5. Hirschberg HJHB, van de Wijden GGP, Kelder AB, van den Dobbelsteen GPJM, Kersten G. Bioneedles as vaccine carriers. Vaccine. 2008; 26:2389–97. doi: 10.1016/j.vaccine.2008.02.067 PMID: 18402021
6. Hirschberg HJHB, van de Wijden GGP, Kraan H, Amorij J, Kersten G. Bioneedles as alternative delivery system for hepatitis B vaccine. Journal of Controlled Release. 2010; 147(2):211–7. doi: 10.1016/j.jconrel.2010.06.028
7. Christensen D, Lindenstrom T, van de Wijden GGP, Andersen P, Agger EM. Syringe Free Vaccination with CAF01 Adjuvanted Ag85B-ESAT-6 in Bioneedles Provides Strong and Prolonged Protection Against Tuberculosis. Plos One. 2010; 5(11):1–7. doi: 10.1371/journal.pone.0015043
8. Soema PC, Willems G, van Twillert K, van de Wijden GGP, Boog CJ, Kersten G, et al. Solid Bioneedle-Delivered Influenza Vaccines Are Highly Thermostable and Induce Both Humoral and Cellular Immune Responses. Plos One. 2014; 9(3):1–8.
9. Amorij J, Huckriede A, Willchut J, Frijlink HW, Hinrichs WLJ. Development of Stable Influenza Vaccine Powder Formulations: Challenges and Possibilities. Pharmaceutical research. 2008; 25(6):1256–72. doi: 10.1007/s11095-008-9599-6 PMID: 18938241
10. Saluja V, Amorij J, Kapteyn JC, de Boer AH, Frijlink HW, Hinrichs WLJ. A comparison between spray drying and spray freeze drying to produce an influenza subunit vaccine powder for inhalation. Journal of Controlled Release. 2010; 144:127–33. doi: 10.1016/j.jconrel.2010.02.026 PMID: 20219606
11. Franks F. Freeze-drying of bioproducsts: putting principles into practice. Eur J Pharm Biopharm. 1998; 45(3):221–9. PMID: 9653626.
12. Rahmat JN, Esuvannathan K, Mahendran R. Bacillus Calmette-Guerin induces cellular reactive oxygen species and lipid peroxidation in cancer cells. Urology. 2012; 79(6):1411 e15-20. doi: 10.1016/j.urology.2012.01.017 PMID: 22446348.
13. Vazquez-Lavista LG, Flores-Balcazar CH, Llorente L. [The bacillus Calmette-Guerin as immunomodu-
lator in bladder cancer]. Rev Invest Clin. 2007; 59(2):146–52. PMID: 17633803.
14. Debruyne FMJ, van der Meijden APM, Schreinemachers ADH, Geboers ADH, Franssen MPH, van
Leeuwen MJW, et al. BCG-RIVM intravesical immunophylaxis for superficial bladder cancer. Prog
Clin Biol Res. 1988; 269:511–24. PMID: 3134662
15. Liu J. Physical characterization of pharmaceutical formulations in frozen and freeze-dried solid states:
techniques and applications in freeze-drying development. Pharmaceutical development and technol-
ogy. 2006; 11(1):3–28. doi: 10.1080/10837450500463729 PMID: 16544906.
16. Patel SM, Doen T, Pikal MJ. Determination of end point of primary drying in freeze-drying process con-
trol. AAPS PharmSciTech. 2010; 11(1):73–84. doi: 10.1208/s12249-009-9362-7 PMID: 20058107;
PubMed Central PMCID: PMC2850457.
17. Roy ML, Pikal MJ. Process control in freeze drying: determination of the end point of sublimation drying
by an electronic moisture sensor. J Parenter Sci Technol. 1988; 43:60–6.
18. Barresi AA, Pisano R, Fissore D, Rasetto V, Velardi SA, Vallan A, et al. Monitoring of the primary drying
of a lyophilization process in vials. Chemical Engineering and Processing. 2009; 48:408–23.
19. Sane SU, Hsu CC. Strategies for successful lyophilization process scale-up. Am Pharm Rev. 2007;
41:132–6.
20. Barresi AA. Overcoming common lyophilization scale-up issues. Pharm Technol 2011; 23(7):29–32.
21. Jennings TA. Transferring the lyophilization process from one freeze-dryer to another. American Phar-
maceutical Review. 2002; 5:34–42.
22. Patel SM, Jameel F, Pikal MJ. The effect of dryer load on freeze drying process design. Journal of phar-
maceutical sciences. 2010; 99(10):4363–79. doi: 10.1002/jps.21232 PMID: 20737639.
23. Patel SM, Pikal MJ. Emerging freeze-drying process development and scale-up issues. AAPS
PharmSciTech. 2011; 12(1):372–8. doi: 10.1208/s12249-011-9599-9 PMID: 21347620; PubMed Cen-
tral PMCID: PMC3066344.
24. Kramer T, Kremer DM, Pikal MJ, Petre WJ, Shalaev EY, Gatlin LA. A procedure to optimize scale-up
for the primary drying phase of lyophilization. Journal of pharmaceutical sciences. 2009; 98(1):307–18.
doi: 10.1002/jps.21430 PMID: 18506820.
25. Kuu WY, Hardwick LM, Akers MJ. Correlation of laboratory and production freeze drying cycles. Inter-
national journal of pharmaceutics. 2005; 302(1–2):56–67. doi: 10.1016/j.ijpharm.2005.06.022 PMID:
16099610.
26. van Hemert P. Vaccine production as a unit process. Prog Ind Microbiol. 1974; 13:151–271. PMID:
4373785.
27. Fissore D, Velardi SA, Barresi AA. In-line control of a freeze-drying process in vials. Drying Technology.
2008; 26:685–94.
28. Rambhatla S, Pikal MJ. Heat and mass transfer scale-up issues during freeze-drying, I: atypical radia-
tion and the edge vial effect. AAPS PharmSciTech. 2003; 4(2):E14. doi: 10.1208/pi040214 PMID:
12916896; PubMed Central PMCID: PMC2750592.
29. Tang XC, Nail SL, Pikal MJ. Freeze-drying process design by manometric temperature measurement:
design of a smart freeze-dryer. Pharmaceutical research. 2005; 22(4):685–700. PMID: 15889467.
30. Tsinontides SC, Rajniak P, Pham D, Hunke WA, Placek J, Reynolds SD. Freeze drying—principles
and practice for successful scale-up to manufacturing. International journal of pharmaceutics. 2004;
280(1–2):1–16. doi: 10.1016/j.ijpharm.2004.04.018 PMID: 15265542.
31. Pisano R, Fissore D, Barresi AA, Rastelli M. Quality by design: scale-up of freeze-drying cycles in phar-
maceutical industry. AAPS PharmSciTech. 2013; 14(3):1137–49. doi: 10.1208/s12249-013-0003-9
PMID: 23884856; PubMed Central PMCID: PMC3755168.
32. Searles JA, Carpenter JF, Randolph TW. The ice nucleation temperature determines the primary drying
rate of lyophilization for samples frozen on a temperature-controlled shelf. Journal of pharmaceutical
sciences. 2001; 90(7):660–71. PMID: 11458335.