Optimization of a freeze-drying cycle of a viral vaccine based on changes in temperature, time and geometry of the vials

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ABSTRACT

The freeze-drying process is used in pharmaceutical industry, however in terms of process management, the process must be avoided at all cost because it has several disadvantages such as high equipment investment, high energy demand, a process that requires long times and products easily to moisturize and fragile and needs to be carefully packed and stored. This work aims at reducing the freeze-drying cycle time of a viral product and consider loading this product at subzero temperatures to increase productivity of this product. Experiments were carried out with freeze-drying cycles with less 15 and 20h in comparison to the original cycle. The experiments were performed with the same formulation of the commercial batch and using a pilot freeze dryer. Modifications were made in the physical properties of the current freeze-drying cycle (temperature, pressure and time) and the loading temperature of the product, without changing the formulation of the vaccine or primary loading. Samples of the lyophilized product trial were analyzed for their appearance, performance, residual moisture, strength and accelerated thermostability in the amount currently used by the company quality control. All the results were within the specifications of the company were close to or better when compared to commercial batches.

INTRODUCTION

Lyophilization, or freeze-drying, is a widely used process to dehydrate numerous materials, such as feedstock, pharmaceutical products, biotechnological products and vaccines. Lyophilization is a unit operation, where water is frozen and then subjected to a high vacuum. It is commonly used for products where stability of the active product is important, a particular situation where viral vaccines apply (Berk, 2013). If they are protected from oxygen, humidity and light, lyophilized products can be stored for long periods of time once they are dried. Due to their highly porous structure, lyophilized products can, reabsorb into their original solvents at any moment and still keep their primary physicochemical characteristics. Although lyophilization leads to the stabilization of biological products, the high investment costs associated with the acquisition of freeze-drying

chambers, high energy demand and high process operation times, make freeze-drying challenging for the pharmaceutical industry (Ryu et al. 2012, Nam and Ryu, 1999, Kang *et al.,* 1999, Kim and Yang, 2010). Freeze-drying is a complex, multistage process that needs to be adjusted for each product, making its primary characteristic – drying in a frozen state – a desirable feature. Drying in a frozen state is particularly important for stabilization of hydrated and labile organic components of biological origin (Rey and May, 1990). A freeze-drying cycle can be divided into three distinct stages: freezing, where the liquid product is frozen; primary drying, where the frozen solvent is removed by sublimation; and secondary drying, where the solvent is removed by desorption. For commercial use, process costs are as important as product quality, which leads to a desire to optimize the process, particularly the heat and mass transfer and the formulation of the product to be lyophilized (Kasper and Friess, 2011). In essence, the freeze-drying cycle is a sequence of three distinct processes: (1) freezing the product below its freezing temperature to maximize ice formation; (2) sublimation of ice at a temperature below the

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freezing point (usually performed under reduced pressure); and (3) removing residual water that did not solidify in solution. An optimized freeze-drying procedure can be found by controlling parameters, such as the pressure of the drying chamber and the shelf temperature, thus minimizing decreases in quality of the lyophilized product. The primary focus of our study was to optimize the freeze-drying process by reducing the drying time but minimizing the effects on the quality of the final product.

Mody et al. (2014) improved the manufacturing process for several vaccines by adding silicone mesoporous nanoparticles (functionalized with amino groups) to the process. This addition stabilized the products in the range of 23 to 27°C.

The introduction of elements and adjuvan substances is the most common approach to freeze-drying optimization, which aims to increase the production of the lyophilized product without compromising its original characteristics or activity. However, very few studies focus on changing the current process by altering process variables, such as temperature and cycle time, to increase productivity.

Improving the freeze-drying process consists of changing the cycle time, temperature and pressure. Another optimization approach is to change the morphology of the vial where lyophilization occurs.

Decreasing the freeze-drying cycle time may lead to higher availability of the lyophilizer, consequently increasing the number of batches that can be produced within a specific period of time (Kasper and Friess, 2011, Clausi and Chouvenc, 2013, Hassett *et al.,* 2013, Kodama *et al.,* 2014, Tonnis *et al.,* 2014).

Recently, a viral lyophilized product with double dosage was launched in Brazil, in a packing vial with a larger diameter and double the volume capacity. To accelerate the time to market, the same freeze-drying cycle was used for the original and doubled-dosage batches, and the results were satisfactory. However, investigation of the freeze-drying cycle time for the same product at double dosage showed that the cycle times used were longer than the necessary.

Regarding the freeze-drying of vaccines, formulation is the first step in the final processing of immunobiological products. In formulation, the active pharmaceutical ingredient, the solvent, stabilizers, cryoprotectors, buffers and the remaining substances are added to a previously sterilized tank such that each individual formulation is unique, thus producing a batch of the desired product.

After formulation and proper conditioning in the lyophilizer, a programmed cycled initiates freeze-drying of the vaccine, which ultimately increases its shelf life. The cycle time depends on the specific product being lyophilized and usually requires more than three days to obtain a product that meets quality control specifications (Mariner *et al.,* 1990, Wang, 2000, Chen *et al.,* 2010, Freixeiro *et al.,* 2013, Orr *et al.,* 2014). Based on these observations, this work aimed to study decreases in the freeze-drying cycle time of a viral vaccine by

changing specific physical parameters for increased flexibility and vaccine production. Based on these assumptions, it is proposed that changing the freeze-drying of the viral vaccine by changing the geometry of the vial, temperature of freeze-drying and time needed to obtain the lyophile is important.

To reach improve the freeze-drying cycle, the following aspects were investigated: (a) the suitability of the present freeze-drying cycle for a product with double the original dosage, in a larger vial that is used for commercial lots, and changes in temperature, time and pressure to determine the necessary specifications to the commercialize the product; (b) the effects of milder drying temperatures and sub-zero temperatures on the vaccine, compared with the results with for commercial lots with the standard freeze-drying cycle; (c) the lyophilizing of experimental lots on a reduced scale, based on pre-established amounts for commercial lots; (d) the sampling of the experimental lot to analyze residual humidity content, potency, accelerated thermostability and quality of the final product; and (e) the similarities between the results for the experimental and commercial lots, thus confirming the benefits of changes to the freeze-drying technique.

MATERIALS AND METHODS

Decrease in the freeze-drying time of a viral vaccine

The main purpose of this work was to test a viral lyophilized product launched into the market, containing double the original dosage, using the same freeze-drying cycle but a vial with a larger diameter. Figure 1 shows the geometry of both vials that were used for these experiments.

A comparison between the vials indicates that the vial used for the double-dosage product was 7 mm higher and with a base bulge of 0.3 mm. The height of the liquid vaccine inside each of the vials was equal, although the total volume of the product was larger in one vial than in the other.

Because the thickness of the bottom of both vials was the same, the only significant differences between the vials were the hollow of the vials and the base ratio: the 6.5 mL vial presents 0.30 mm hollow and a base ratio two times higher. Consequently, this vial presented a plane bottom and a higher contact area in the shelf of the lyophilizer. This, theoretically, contributes for the thermal conduction between the vial and the shelf of the equipment.

The type of vial and the amount of product to be lyophilized directly affect freezing, ice crystal formation and sublimation. These changes in the vial for lyophilization can help decrease the freeze-drying cycle.

Changes in the present freeze-drying cycle

After bottling the vials, the product is introduced in the lyophilizer at a positive shelf temperature, a procedure that can eventually decrease the potency of the viral vaccine if the lyophilizer requires long periods to be filled.
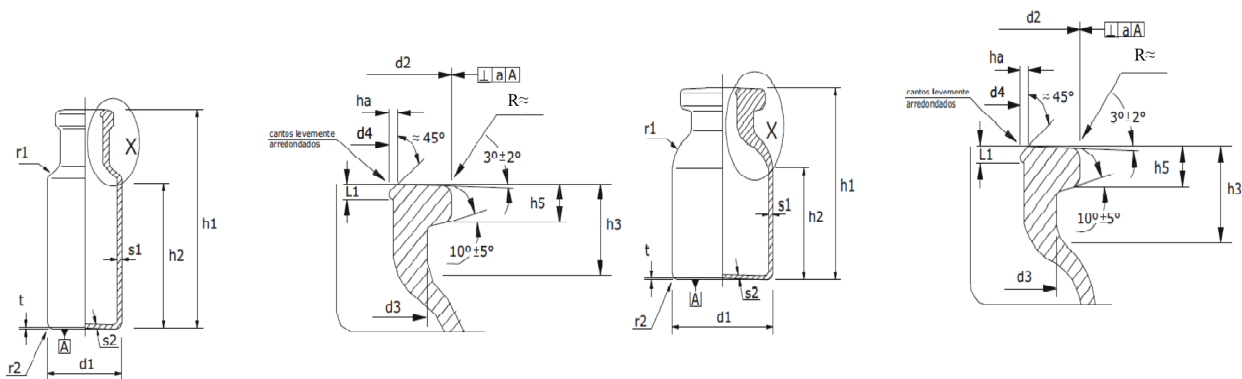
Type I vial hydrolitic class, colorless, 4.0 mL volume.   Type I vial hydrolitic class, amber, 6.5 mL volume.

Dimensional (mm)
d1 = Body diameter ....16.50±0.20
d2 = Mouth external diameter...........................12.95+0.20
d3 = Bottle neck diameter (max).......................11.00
d4 = Mouth internal diameter...........................7.0±0.20
h1 = Total height..41.50±0.50
h2 = Body height (minimum)............................28.50
h3 = Mouth height...10.00±0.50
h5 = Flange height...3.60±0.20
s1 = Body thickness..1.00±0.04
s2 = Bottom thickness.......................................0.70
r1 = Shoulder ratio..3.00
r2 = Bottom ratio..1.50
t = Bottom hollow..0.50±0.20
L1 = Inner ring width..1.50±0.50
ha = Inner ring height.......................................0.25

Dimensional (mm)
d1 = Body diameter ....23.50±0.20
d2 = Mouth external diameter...........................19.85+0.20
d3 = Bottle neck diameter (max).......................16.30
d4 = Mouth internal diameter...........................12.60±0.20
h1 = Total height..34.00±0.50
h2 = Body height (minimum)............................20.00
h3 = Mouth height...8.60
h5 = Flange height...3.60±0.20
s1 = Body thickness..1.00±0.04
s2 = Bottom thickness.......................................0.70
r1 = Shoulder ratio..3.50
r2 = Bottom ratio..3.00±0.50
t = Bottom hollow..0.30 (max)
L1 = Inner ring width..1.50±0.50
ha = Inner ring height.......................................0.25

Fig. 1: Vials Used for the Production of the Vaccine Before (left) and After (right) Freeze-drying Changes.

Therefore, developing a freeze-drying cycles that operates that at milder or negative temperatures is crucial. The freeze-drying cycle starts by ramping down to the desired freezing temperature, followed by an incubation period once homogeneous distribution of the temperature is detected in the vials. In the experimental vials, the final freezing temperature will be lowered to reduce the length of this phase by exploiting the thermal properties of the industrial lyophilizer and the original vial. Following the pressure decrease in the freeze-drying chamber, primary drying begins with an increase in temperature. There is a specific point in the cycle when the product no longer absorbs enough energy to sublimate the ice in the vials. For the experimental cycle, the time required to reach that point will also decrease. After primary drying, the temperature is increased to accelerate desorption (secondary drying). The temperature for secondary drying will be changed for the experimental cycle to compensate for the decrease in the proposed cycle and to obtain a product with potency and residual humidity that meets the quality control requirements. At the end of the cycle, the vials are closed under inert atmosphere conditions and are ready to be collected from the lyophilizer. The vials are then inspected and sampled for analytical tests. Commercial vaccine batches, where

vials were removed for experimental tests, were named VAC-1 (subdivided into batches VAC-A and VAC-B) and VAC-2. The main experimental changes proposed are briefly described below.

Experiment 1

The first experimental run was performed on a tray of product from VAC-1. In that case, 360 vials were lyophilized in a reduced cycle. This batch was then named VACEXP-1.

The shelf temperature was adjusted to be a positive temperature, 75 % smaller than the shelf temperature in the present freeze-drying cycle. This change was made to investigate whether the vaccine could be lyophilized at a lower temperature without compromising aspect and quality requirements, specifically the residual humidity. Smaller crystals are less likely to form at lower temperatures, which could jeopardize sublimation in the primary phase or lead to a non-satisfactory residual humidity in the final product. This temperature change could also lead to a collapse of the product during secondary drying, thus increasing product loss or heterogeneous qualifications of the product in each vial, across different batches. These inconsistencies in product quality are unacceptable.

One advantage of filling the vials at lower temperatures is a decreased risk of reducing the vaccine's potency, particularly for cases when extended times are required to fill the vial. Temperature plays a key role in lyophilization of a viral vaccine because it can accelerate the degradation of the virus in the final product, which must be confirmed in the potency test.

To achieve this goal, the temperature was changed to a negative temperature, and this new temperature was used to test lyophilization, as described in Experiment 2. It was clear that the sublimation time was higher than necessary based on use of the present, commercial freeze-drying cycle for product with double the standard amount of doses. This outcome was indicated by the results of previous batches where there were no more energy changes between the product and the shelf of the equipment, thus proving that the process was consuming energy rather than resulting in sublimation.

The time needed for primary drying under the new experimental conditions, in comparison to the commercial cycle, was reduced by 4 hours and 30 minutes without changing the temperature of the lyophilizer shelf in this stage.

To prove that greater contact between the vial and the shelf can contribute to a decreased freeze-drying cycle length, the pressure in the primary and secondary phases were the same as those used for commercial cycles. These pressures were kept constant during the entire drying phase. With the modifications suggested, the first cycle proposed decreased the freeze-drying process by 17 hours.

Experiment 2

The second experimental run was performed on a tray of product from VAC-1. 360 vials of product were lyophilized in a reduced cycle. This batch was then named VACEXP-2.

The tray was placed in the lyophilizer in the negative temperature from Experiment 1. This experiment deemed the immediate start of the freeze-drying cycle at a negative temperature. When placing the tray in the lyophilizer at a negative temperature and immediately starting the freeze-drying cycle, it could be determined whether the time programmed would be enough to homogeneously distribute the temperature for all the vials during the freezing stage as, for commercial batches, the freeze-drying starts immediately after the lyophilizer is full.

Because the experimental lyophilizer has four shelves, one shelf was used to perform Experiment 1 and a different shelf was used to perform Experiment 2. The main objective of this step was to determine whether the vaccine could withstand negative temperature without compromising the aspect and final quality of the product. Due to the rapid freezing performed, risks associated with quality parameters are likely. However, when the product is frozen at negative temperatures, the length of the freeze-drying cycle is greatly decreased, This large decrease is the result of the initial temperature being closer to the final freezing temperature needed, thus avoiding a freezing ramp time of several hours. In this way, Experiments 1 and 2 (from the same lyophilizer) were

processed under the same experimental freeze-drying cycle, and therefore, both tests could be easily compared. The freeze-drying cycle was then started in the equipment from the negative temperature of the shelf, with the time needed for primary drying, in relation to the commercial cycle, reduced without changes in the temperature of the shelf in this phase, and without changes in the pressure in primary and secondary phases. With the modifications suggested in this experiment, the proposed cycle would have 17 hours less than the present one.

Experiment 3

The third experiment was performed on a commercial viral vaccine from the VAC-2 batch. From this batch, two trays (360 vials each) were lyophilized under a reduced cycle in a pilot lyophilizer. This lot was named VACEXP-3. Based on the previous results of residual humidity and aspect from the two former experiments, both trays for Experiment 3 were placed in the lyophilizer when the temperature of the shelf was at the same negative temperature used in Experiments 1 and 2. The cycle started when the programmed freezing phase temperature could be reached as fast as possible. In relation to the previous experiments, the shelf temperature for the freezing state was further decreased to freeze the product faster. After an evaluation of the thermal behavior of the product in experiments 1 and 2 and their results of residual humidity and aspect, the following additional changes were proposed to decrease the commercial cycle by 20 hours: (a) an additional decrease in the time needed for primary drying; (b) a gradual increase in the temperature ramping from the primary to the secondary drying phase, to accelerate the time gap between the drying phases of lyophilization; (c) an increase in shelf temperature, during the secondary drying (due to a decrease in the cycle length); (d) maintenance of the same shelf temperatures and the same pressure as used in experimental cycles 1 and 2. It was expected that these changes would decrease the freeze-drying cycle of a viral vaccine by 20 hours and free up the lyophilizer for additional operations, thus increasing the production capacity for vaccines. Based on the changes in temperature and freezing and heating rates and time, the freeze-drying cycle was markedly reduced. To check the final quality of the product associated with crystal formation during freezing, product thickening in the vial, characteristics of the vials and the speed of sublimation, the obtained products were tested after freeze-drying according to the internationally accepted standard parameters.

Analytical determinations

Aspect

The aspect of the final product was evaluated according to guidelines provided internally by the producer of the vaccine. Vials containing abnormal tablets were discarded and counted as losses due to lyophilization. The remaining losses, such as vials with altered volumes, broken glasses, and vials without covers after freeze-drying, were not counted due to the changes promoted in the freeze-drying cycle.

Residual humidity

　　　Tests for residual humidity on the experimental lyophilized vaccine were based on the Karl-Fischer coulometric titration, according to protocols adopted for commercial vaccines (Karl Fischer Coulometric KF titrator", Mettler Toledo, model C30).

　　　The test consisted of analysis of a blank, using 1.0 mL of Karl-Fischer reagent, and including the amount of water found. The masses of five vials of vaccine were measured and the average value was calculated. The experimental vaccines were then dissolved in 2.0 mL of Karl-Fischer reagent and 1.0 mL of this sample was injected into the titration cell of the equipment. According to the WHO's minimum requirements for residual humidity of a vaccine, the control national authority must specify both the analytical method and the upper limit for humidity. In this case, humidity levels below 2 % are considered satisfactory, and the maximum accepted residual humidity is 3 % (WHO, 2015).

Potency and Thermostability

　　　Potency tests of the viral component of the experimental and commercial vaccines were determined based on the methodology for 50 % Cell Culture Infective Dose - $CCID_{50}$, the same used for the quality control of the commercial batches.

　　　This test determines the concentration of viral particles in a human immunization dose when kept at 2ºC to 8ºC or - 70ºC and when removed at the formulation stage.

　　　For the test, each vaccine sample was diluted by a factor of at least 10 (1:10 to 1:100000) and inoculated in rats (under brain anesthesia) from the National Institute of Health lineage. 0.03 mL of the vaccine sample was inoculated in at least 6 rats for each dilution, and their death rates were observed for 21 days.

　　　The thermostability test determined the number of viral particles sufficient for human immunization that were present in each lyophilized vaccine sample. The thermostability tests were conducted at 37ºC for 14 days.

　　　For the thermostability tests, the lyophilized vaccine was subjected to a temperature of 37ºC for 14 days. This step was followed by preparation of a cell suspension of Vero cells, dilution of the lyophilized vaccine, and inoculation of the diluted vaccine in plates with cell suspensions and finally incubation of the plates in a controlled atmosphere. After viral adsorption, the overlay was added, and with the help of a negatoscope, the colonies developed in each wheel of the plates were counted.

　　　The losses of thermostability (the difference between the viral dose of the formulated vaccine and the lyophilized vaccine after incubation at 37ºC for 14 days) were also monitored and evaluated.

　　　Potency, thermostability and viral dose are very important for the final approval of the batches produced. Potency is related to the amount of virus needed for human immunization, thermostability is related to the conservation of the vaccine during its shelf life and viral dosages of the samples before and after freeze-drying can predict the loss of viral particles during lyophilization.

　　　Potency and thermostability tests were conducted in 4 vials each of vaccines from experiments 1, 2 and 3, and 4 vials of the commercial viral vaccine. Due to the fractioning of lot VAC-1, determinations were performed for the same number of vials for both VAC-A and VAC-B.

　　　According to the WHO's minimum requirements, values are considered satisfactory for potency and thermostability when the average dosages of the vials of the lyophilized vaccine, stored at 2ºC to 8ºC and kept at 37ºC for 14 days, are equal or higher than 3.73 log 10 PFU/HD, which is equivalent to 3 log 10 LD_{50} per dose used in rats.

Performance of the cycle

　　　The performance of the experimental freeze-drying cycle was evaluated using the historical operation of the commercial lots obtained up to the present. In other words, 95 % of the vials were approved/batch after proper inspection of the aspect and physico-chemical and biological analysis conducted in the sampled vials. All the protocols here used comply with the particular recommendation and proper approval were obtained.

RESULTS AND DISCUSSION

　　　From the results, the freeze-drying cycles of the commercial lots containing doubled doses of the vaccine and using the vials with a different geometry, contained some regions where the product's temperature was higher than the shelf temperature of the lyophilizer. The product temperature was kept constant and no changes were implemented in the shelf of the lyophilizer. This led to the conclusion that the amount of energy required to sublimate the ice was no longer satisfactory in those regions.

　　　To prove that the cycle length could be reduced, small amounts of commercial vaccine (minimum of 360 vials per experiment) were removed from the commercial line of production and the freeze-drying tests were performed in a pilot lyophilizer. In this lyophilizer, freeze-drying cycles could be programmed for changes in temperature, pressure and time, in comparison to the standard conditions used for commercial lots.

Aspect of the tablets of lyophilized vaccine

　　　In both experiments, the lyophile appeared to be compact, without fractures and easily removed from the vials. These results were observed for more than 98 % of the vials, without changes in the formulation.

　　　Jamil et al. (2014) also tried to implement modifications pre-freeze-drying in viral vaccines using two distinct stabilizers: one based on dehydrated trehalose and another one containing sucrose, human albumin and sorbitol. In these cases, Jamil et al. (2014) tested the obtained product for accelerated stability at various temperatures and concluded that under extreme conditions, the stability was confirmed only when trehalose was introduced.

　　　In the case of the present work, no products were introduced into the system to improve the appearance of the lyophile or to enhance any other property of the product. The

results of the residual humidity, potency and thermostability tests of the commercial and experimental lots are presented in Table 1. Those results are exclusively obtained based on the changes described in experiments 1 to 3. In short, these were changes in freeze-drying time and geometry of the vials.

Table 1 Results of Residual Humidity, Potency and Thermostability of Commercial and Experimental Lots of a Viral Vaccine.

Average residual humidity (%)		
Commercial batches		Experimental batch
Experiment 1		
VAC-A	VAC-B	VACEXP-1
0.87 ± 0.15	0.78 ± 0.09	0.51 ± 0.13
Experiment 2		
0.87 ± 0.15	0.78 ± 0.09	0.74 ± 0.11
Experiment 3		
0.77 ± 0.08		0.77 ± 0.15
Average potency (log 10 PFU/DH)		
Commercial batches		Experimental batch
Experiment 1		
VAC-A	VAC-B	VACEXP-1
4.97±0.05	5.13±0.07	5.05±0.02
Experiment 2		
4.97±0.05	5.13±0.07	5.11±0.05
Experiment 3		
5.04±0.06		4.77±0.08
Accelerated thermostability (log 10 PFU/DH)		
Commercial batches		Experimental batch
Experiment 1		
VAC-A	VAC-B	VACEXP-1
4.70±0.07 (0.27)*	4.79±0.03 (0.34)	4.67±0.08 (0.38)
Experiment 2		
4.70±0.07 (0.27)	4.79±0.03 (0.34)	4.64±0.04 (0.47)
Experiment 3		
4.68 ± 0.08 (0.36)		4.26 ± 0.08 (0.51)

* Numbers in parenthesis correspond to the average loss of activity as a function of thermostability. Experiment 1: Positive temperature of the shelf of the lyophilizer during loading of the vaccine and 17 hours of reduction in the time of lyophilization; Experiment 2: Negative temperature of the shelf of the lyophilizer and 17 hours of reduction in the time of lyophilization; Experiment 3: Negative temperature of the shelf of the lyophilizer and 20 hours of reduction in the time of lyophilization.

Residual humidity

Table 1 presents the results of residual humidity of the commercial batch (subdivided into VAC-A and VAC-B) and experimental lots 1, 2 and 3. As observed from data for experiment 1, the commercial VAC-A and VAC-B lots presented a distinct residual humidity, ranging from 0.72 to 1.0 3% and 0.70 to 0.91 %, respectively, across the 5 replicate vials examined. The experimental lot, on the other hand, presented a residual humidity ranging from 0.40 to 0.73 %.

As previously reported, the limit for residual humidity post-freeze-drying, established by the Brazilian regulation, is equal to or less than 3 %. All batches tested were below 3 % residual humidity, and the experimental batch presented an average value less than the commercial ones. Kodama *et al.* (2014) tried to optimize a freeze-drying cycle with the help of a simulation program based on basic heat transfer formulas, several empirical formulas and Fick's second law. The program proved to be useful. In regard to the residual water content, the simulation produced values fairly similar to the observed experimental results.

In a review about the freezing stage of lyophilization, Kasper and Friess (2011) studied the consequences of this step on the overall performance of the freeze-drying process, and the quality of biopharmaceutical products. The authors emphasized that a deep understanding of the freezing stage and the ability to control freezing more efficiently, are key factors in improving the quality and stability of pharmaceutical products.

Potency of the final bulk product

The potency test for a vaccine consists of determining the concentration of viral particles contained in a human dose, when stored at 2° to 8°C and lyophilized or when stored at - 70°C and then removed after formulation to prepare for human immunization. The test is performed on an aliquot of the formulated product (bulk product), and its potency must be greater than or equal to 5 log PFU/mL. The potency of commercial lot VAC-1 was 6.18 log PFU/mL and therefore passed on to the next step of the process, filling.

As shown in Table 1, the average potency for the experimental lot was between the two average values of the VAC-A and VAC-B commercial lots. Both the commercial and the experimental lots meet the required product specifications. Furthermore, after the thermostability test the experimental lot had a lower average potency than the commercial batch. Consequently, the experimental lot presented an average loss in potency higher than that of the commercial batch, although both lots met the regulations.

The specific regulation for potency of the final bulk product, that is, the formulated viral vaccine, must be greater than or equal to 5 log 10 PFU/mL. This was true for both the VAC-1 and VAC-2 lots; therefore, they were approved for filling.

Thermostability of the final lyophilized product

Using the potency value of the product just after lyophilization, and the potency value after the accelerated thermostability test of the lyophilized product (at 37°C for 14 days) it is possible to calculate the loss of potency of the vaccine. This loss of potency can be used to determine whether the vaccine can still immunize a person, 2 years after potency validation.

The specific regulation requires that the lyophilized product have a potency greater than or equal to 3.73 log 10 PFU/HD and that after thermostability testing the potency loss is less than or equal to 1 log 10 PFU/HD.

As shown in Table 1, the average potency of the experimental batch was always less than the potency of the commercial batch; however, both batches are in accordance with the required specifications. In addition, the experimental lot showed a lower average potency after the thermostability test than the commercial batch. Consequently, the experimental lot showed a greater average loss higher than the commercial lots. However, both lots meet the average loss specifications.

Orr *et al.* (2014) improved the freeze-drying process for a tuberculosis vaccine by obviating the need for maintenance of the product at low temperatures. This change was for a freeze-drying

process that used an abrupt change of storage conditions of the product at low temperatures. The authors were able to maintain the activity and stability of the vaccine before and after the introduction of the changes in the freeze-drying cycle.

Hawe and Friess (2006) studied the thermal stability of a mannitol formulation by introducing sodium chloride to the lyophile. The authors concluded that the presence of sodium chloride contributed to higher stability of the formulated product, thus counteracting problems associated with change in the aspect of the lyophile, particularly crystallization.

Wang *et al.* (2014) used a procedure of emulsification with lyophilization. Adjuvants were prepared as albumin carriers and produced a dry product whose stability was confirmed by storage at room temperature. The product formed was able to induce systemic immune responses, efficiently acting as potent vaccines without the need for storage at cold temperatures.

Hasset et al. (2013) implemented the used of solid and dry formulations of recombinant vaccines using colloidal aluminum hydroxide as an adjuvant. Similar to tests in the present work, authors used lyophilized formulations prepared after rapid cooling during lyophilization. Regarding the aspect of the produced lyophile, Hasset et al. (2013) observed aggregation due to the presence of colloidal aluminum hydroxide in formulations processed after rapid cooling. Rats immunized with the reconstituted vaccine produced specific antibodies and toxin neutralizers, irrespective of the duration of the high temperature storage or the level of aggregation of the adjuvant during lyophilization.

In their rat studies, lyophilized formulations of the vaccine protected against lethal doses of ricin, even when formulations were stored at 40°C for 4 weeks. On the other hand, the liquid formulation of the same vaccine, stored under similar conditions, was not effective against ricin.

The thermostability regulations must include losses less than or equal to 1 log 10 PFU/HD. The average results for residual humidity, potency and thermostability for both the commercial and experimental lots showed that the results are quite satisfactory for residual humidity ($\leq$ 3 %).

For experiments 1 and 2, where both batches of samples were lyophilized at the same time in the same lyophilizer, and the only difference was the shelf temperature in the equipment, residual humidity of the vaccine at negative temperature (0.74) was higher than the humidity of the vaccine at positive temperature (0.51). These results emphasize the importance of the freezing rate of the product as rapid freezing can lead to the formation of smaller crystals, thus making primary drying difficult. However, it must be emphasized that the average results of residual humidity for experimental lots 1 and 2 were better than the results for the commercial lots, which suggests that the post-freezing changes in the freeze-drying cycle were effective for the viral vaccine test in this work.

In experiment 3, the average residual humidity of the commercial and experimental lots were similar, showing that decreasing lyophilization by 20 hours and using a negative

temperature at the start of the process was beneficial for this product. When the freeze-drying cycle was reduced by 17 hours the potency of the experimental lots was practically unaltered or showed improved results compared with the commercial lots. Reducing the cycle by 20 hours resulted in a product with lower potency, among all others tested. One possible explanation for this result may be associated with the change in the secondary drying temperature used in experiment 3 and/or the changes implemented in heating rate from the primary to secondary drying phase.

However, the results from experiment 3 show that for an adequate evaluation of potency, the suggested cycle (20 h) and the negative temperature at the start of the process, is well projected for this viral vaccine.

In relation to the loss associated with accelerated thermostability of the commercial and experimental batches as a function of the freeze-drying time, all the results were satisfactory. In other words, all losses were less than or equal to 1 log PFU/HD. The shorter the duration of the freeze-drying cycle, the higher the loss due to accelerated thermostability.

For experiment 1, which was loaded in the freeze dryer at a positive temperature, there was a small increase in the potency loss due to thermostability of the product. This loss was similar to the results for the commercial batches. A comparison between experiments 1 and 2 indicates that the loss due to thermostability tests was much higher for experiment 2. Accordingly, experiment 3 produced the highest loss after thermostability testing, in all cases. The sequence of operations in each of the new cycles, consisting of a reduction in cycle time and a new geometry of the vials was beneficial for the production of the viral vaccine. Assuming that there are four consecutive working weeks in a month and four lyophilizers in the production area (each one processing 400,000 doses of vaccine per lyophilization cycle) 32 batches of the product can be produced each month. If the cycle with a duration reduced by 20 hours is adopted, a marked increase in productivity could occur (Table 2).

Table 2: Increased Productivity of a Viral Vaccine, due to Changes in the Freeze-drying Cycle.

Status	Batches/month	Vaccine doses/month
Actual cycle	32	12,800,000
Improved cycle	40	16,000,000
Increased productivity (%)		25

The 25% increase in productivity corresponds to the production of 3,200,000 doses of the vaccine per month. By decreasing the cycle time, the whole production process improves by increasing the number of batches produced per month and a greater product supply for the market. Usually, lyophilizers are not dedicated solely to one product; therefore, the equipment must be made available to produce other vaccines.

CONCLUSIONS

By changing two aspects of a freeze-drying process (the size of the vial and the volume of the viral vaccine in the vial)

based on changes in temperature and pressure, the freeze-drying cycle of a viral vaccine was markedly decreased.

The results obtained after the changes were made were in accordance to the international regulations, namely residual humidity, aspect, accelerated thermostability and potency. Accordingly, the international regulations were also met after decreasing the freeze-drying cycle by 17 or 20 h.

The new freeze-drying cycle increased the production capacity of the vaccine by 25%, corresponding to an increase of 3,200,000 doses produced per month.

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