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Application of Quality by Design in a Commercialized Lyophilized Vaccine

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Abstract

The pharmaceutical industry has being implementing regulatory practices to assure that consumers obtain products with quality, safety, and efficacy. The use of Quality by Design (QbD) for products on development has increased though the years to avoid issues related to quality parameters and has been suggested by Regulatory Agencies to standardize globally the documentation for the registration of new products. Although the concepts of QbD gain importance, it is still not a widespread practice to existing systems and products already on the market. This work aims to propose a case study with a vaccine using QbD concepts on the lyophilization unit operation production step to provide robustness and increase efficiency, leading to a lyophilization cycle time reduction. To this end, a reverse way of the use of QbD principles were applied based on historical batches database, down scale experiments, and finally in industrial scale to establish new boundaries in the lyophilization cycle. Experimental batches samples were analyzed through accelerated and real time stability study. At the end, this case became a possibility to establish new ranges to lyophilization process predicting risks and assure robustness to this production step with the maintenance of quality and safety of vaccine.

Keywords: Quality by Design; Freeze-Drying; Vaccine; Lyophilization.

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1. Introduction

Biological drug production is a complex operation involving many agents, materials, equipment, and technologies, transforming the pharmaceutical industry into a highly regulated entity through quality policies and regulation authorities. Although the first Quality by Design (QbD) approach was outlined almost thirty years ago [1], the increase in the pharmaceutical industry began after the creation of the guidelines of International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the Regulatory Agencies from Europe (EMA) and EUA (FDA) starting to emphasize the QbD component as part of regulatory filing for new developed drugs. FDA started to shift from the traditional approach, which includes a rigorous testing of the final product (Quality by Test), to a risk-based Quality by Design (QbD) approach [2 – 3]. QbD is focused on process design; the relationship with a risk-based approach has been discussed in ICH Q8 and Q11 [4-5]. The step by step to implement QbD for development of pharmaceutical products has been established [5-10] in Figure 1.

Figure 1: QbD General Application Roadmap – Adapted [5-10]

The most important components of the QbD used in the pharmaceutical industry were [4, 6, 11]: Quality target product profile (QTPP): A prospective summary of the quality characteristics of a drug product that will, ideally, if achieved ensure the desired quality, taking into account safety and efficacy of the drug product [4], Critical quality attributes (CQAs): A physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality [4], Critical process parameters (CPP): A process parameter for which the variability has an impact on a CQA and therefore should be monitored or controlled to ensure the process produces the desired quality [4], Critical material attributes (CMAs): A physical, chemical, biological or microbiological property or characteristic of an input material that should be within an appropriate limit, range, or distribution to ensure the desired quality of output material [11]. However, guidelines from ICH do not mention the use of QbD for products already on the market. In this paper, the QbD principles and its components will be applied for a vaccine lyophilization production step that has been produced in Brazil since 1937, first by the Oswaldo Cruz Institute and later in the 1980s by the Institute of Immunobiological Technology - Bio-Manguinhos [12]. After more than thirty years in the market, the process for this vaccine respond to epidemic outbreaks but was not revised by the QbD concepts. The lyophilization process for this vaccine was established before the QbD principles. At that time, the variation of CPP influencing the CQA was based on the lyophilization cycle setpoints (temperature, pressure, and time) and the capability of the freeze dryer in automatic mode to control those setpoints. This capability and sources of variability that could have a negative influence in product quality was addressed by risk analysis by a multidisciplinary team of specialists. Freeze-drying is a complex and multistage process that needs to be adjusted for each product, making its primary characteristic – drying in a frozen state – a desirable feature. Lyophilization process represents a viable alternative formulation strategy to improve biologic products stability and long-term storage as well as their ease of shipping and handling. A traditional lyophilization cycle consists

of the freezing step, the primary step and secondary drying step [13]. Although lyophilization leads to the stabilization of biological products, the high investment costs associated with the acquisition of large-scale freeze-drying equipment, high-energy demand, and high process operation times, make freeze-drying a challenge for the pharmaceutical industry [14 - 17]. For commercial purpose, 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 [18]. The primary focus of this study was to use Qbd principles on the lyophilization unit operation to allow the vaccine production with wide ranges of lyophilization CPP to achieve the same CQA results in order to maintain the quality of the final product. To measure and guide the methodology regarding the case study for the vaccine, a SWOT analysis for the lyophilization unit operation using QbD principles were developed.

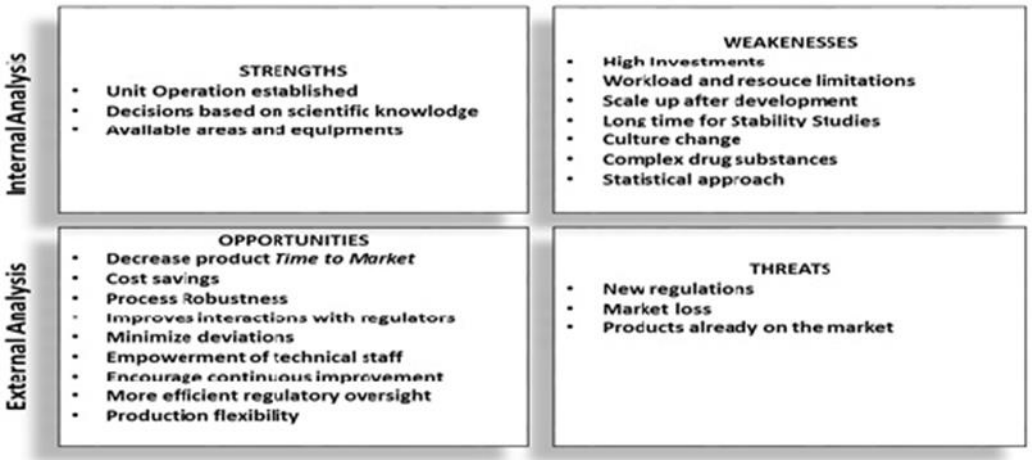

Figure 2: QbD SWOT analysis for the lyophilization unit operation. Strengths, weaknesses, opportunities, and threats available on the vaccine producer.

With the SWOT analysis applied the QbD principles for the lyophilization unit operation, a trade-off of weakness and opportunities had the major bullets. Clearly, there are a bunch of opportunities that QbD can bring to the unit operation, however, a lot of work, investment, and time consuming are expected as weaknesses for all process. It could be an opportunity to apply QbD principles focusing on lyophilization unit operation for products in the market updating documents and understanding better the freeze dryer cycle CPP and the impacts in CQAs of the product. Once, this knowledge is absorbed, increase productivity can be verified with new lyophilization cycle times. The authors in [19] developed and optimized a lyophilized formulation of simvastatin thought the successful application of QbD approach relationshiping the influence of two developed formulation and process parameters on the CQAs of lyophilization of simvastatin determined using DoE. The influence of several risk factors (three formulation factors and two process parameters over the critical quality attributes of lyophilized long circulating liposomes with simvastatin) was investigated within the current study using the design of experiments tool of QbD. Moreover, the design space was determined, in which the established quality requirements of the product are met, provided that the risk factors vary within the established limits. Other authors in [20], described a rational procedure, based on mathematical modeling, for properly choosing the freezing conditions according to the QbD approach to build the design space to describe the impact of freezing conditions on product ice crystal size and drying performances demonstrating thought the results the power of

QbD. A deep understanding of the freezing phenomena was required, and, according to the QbD approach, this knowledge was used to build the design space allowing control of the freezing process and fast selection of appropriate operating conditions. In this paper the goal is to review the process to establish new ranges for the lyophilization cycle CPP using QbD concepts proceeding with a review of commercial batches database, propose hypotheses, and with pilot and industrial scale freeze dryer proceed with experiments, based on previous knowledge of critical temperature and other product specifications reached by Differential Scanning Calorimetry, Freeze dryer Microscope and electric resistance. According to Brazilian National Regulatory Agency (ANVISA) regulations, changes in the formulation of the product are classified as level 2 or 3 for product registration and can lead to clinical trials to confirm that the modifications will not affect the quality, efficiency, and safety of the vaccine [21]. To avoid investment in clinical trials, in this work the formulation and fill-and-finish production process steps were not modified. The study focused on the freeze-drying step of a vaccine and a review of batches database from 2008 up to 2014 to apply QbD principles and suggest cycle experiments on the lyophilization unit operation to establish new ranges of CPP. After formulation and proper conditioning in the lyophilizer, a programmed cycle initiates the 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 [22 - 26]. Although Brazil is the biggest producer of the studied lyophilized product worldwide, there is still a risk supply discontinuity to meet the demand in the country for the National Immunization Program (PNI) and Africa if the epidemiological scenario becomes worse. In terms of process management, lyophilization process step is a bottleneck to increase the vaccine production during outbreaks. To reach robustness in the freeze-drying cycle in industrial scale, the following aspects were conducted: (a) The application of specific QbD principles applied on the vaccine already on the market; (b) Based on commercial batches database the suitability of the present freeze-drying cycle for the vaccine and changes in CPP to determine the new ranges necessary to make production more flexible and minor risks of deviations; (c) The proposal of new lyophilization cycle and conduction of experimental batches on a pilot and industrial scale, based on the same formulation and fill and finish procedures; (d) The sampling of the experimental batches drug product on an industrial scale to analyze residual moisture content, aspect, pH, potency and long-term thermostability; and (e) The similarities between the results against commercial batches, thus confirming the benefits of QbD approach.

2. Material and methods

2.1. Unit operation observations, deviations and proposed CPP values

To provide wide ranges on CPP in the lyophilization unit operation the first step of the methodology was a review of lyophilization commercial batches cycles and deviations in the database of quality system of the product regarding the lyophilization step. The standard procedures for commercial batches are loading the freeze dryer manually allowing the product measure with resistance temperature detectors (RTDs) inside the product vials monitoring the product temperature along the lyophilization process and recording in the freeze dryer database. The profile of the product in commercial batches in freezing, primary and secondary drying step are presented in Figure 3.

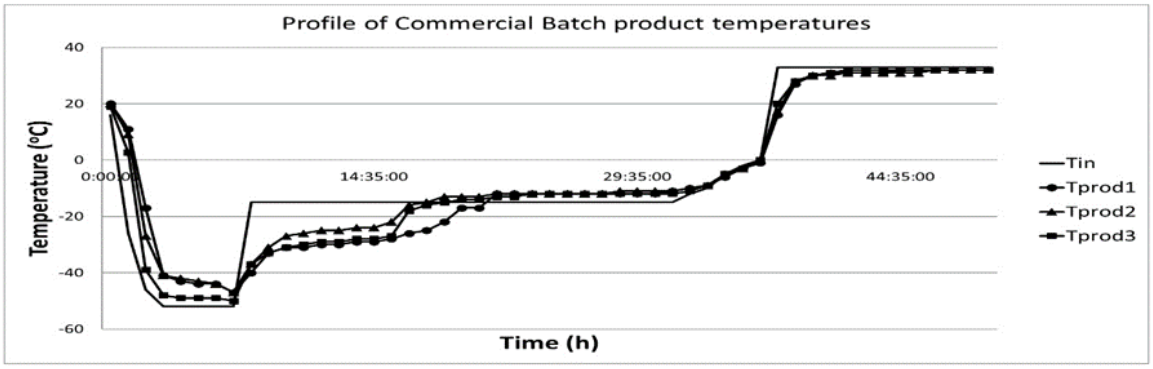

Figure 3: Registration of three product temperatures monitored along a commercial production.

The data from Figure 3 presents no variability between product temperatures (Tprod1, Tprod2, Tprod3). The industrial freeze dryer shelves temperature (Tin) are controlled automatically in temperature ranges of ±1.5°C from the shelf setpoint. Temperatures out of this range represents a deviation on quality system of the company. The product freeze dryer cycle loading temperature is positive and product temperatures during freezing step are below -40ºC achieved after 2:30h. The posterior time during freezing step is important to have temperature homogeneity of the vials, although no product temperatures significantly changes are observed. During primary step, the product is kept for 8h with no product temperatures significant temperature changes observed and in secondary step when the product reaches a constant temperature over time. With those observations the regions of the lyophilization cycle that could enhanced were mapped and will be challenged with experiments. The magnitude of the deviation regarding the CPP (temperature, time, and pressure) in each cycle step were correlated to the CQA results from Quality Control in each experiment.

2.2. Proposal modifications in freeze dryer cycle CPP

Based on the correlation results and the product temperature database from commercial lyophilization cycles available, a new lyophilization cycles experiments were proposed to predict the impact on CQAs with the modification on CPPs setpoints of the lyophilization unit operation of the vaccine. The decision was supported by an impact analysis (Table I) approach conduct as a second step. The strategy was first conducted using downscaled batches in pilot freeze dryer and after adjusted the parameters to the industrial equipment. The suitability of the present freeze-drying cycle to changes in CPPs was aligned for new ranges necessary for flexibility of production without deviations.

Table 1: Resume of impact analysis for proposed CPP modifications on lyophilization Cycle

Lyophilization Cycle Step	CPP Modification	Impacted CQA	Product Impact
Loading	Temperature	Aspect, Potency, Residual Moisture, pH	High*
Freezing	Temperature and Time		High*
Primary Drying	Time		High*
Secondary Drying	Temperature		High*

*Product Impacts: High – Modification has high probability to results out of range of CQA

2.3. Experimental batches

Six experimental batches were designed as the third step. Three experiments were produced using an automatic pilot freeze dryer IMA Lyoflex with capacity of 2,000 vials full loaded and three experimental batches using automatic industrial freeze dryers IMA Lyomax with capacity of 36,000 vials full loaded. Both freeze dryers are equipped with a capacitance manometer (Barocell; mks, USA) and has a setpoint temperature deviation of ±1.5°C and pressure setpoint deviation of ±0,50μHg. The product temperature was measured by RTD probes and endpoint of primary drying by the manometric temperature measurement. All the experiments were performed following the same vaccine primary raw materials (20mL amber Schott vials and 20mm West rubber stoppers), formulation ratio, and quantities of API, sucrose, glutamate, sorbitol, hydrolyzed bovine gelatin, erythromycin, kanamycin, and water for injection, fill-and-finish steps in Bosch filling line Model, lyophilization in IMA freeze dryers, production procedures, documentation traceability and validations established for commercial batches. Lyophilization cycle evaluation proposed new CPP ranges for the shelf temperature and time cycle step. In this study, pressure during lyophilization was not modified from the original freeze dryer cycle.

Experiment 1 – Downscaled Batches

During the production of three commercial batches, 720 vials of each commercial batch were sent to IMA pilot freeze-dryer and the batches were named as A1, A2 and A3. The Experimental 1 batches were produced with different CPP set points (Table 2), reducing or increasing shelves temperature and time compared to the current cycle for industrial batches.

Table 2: Proposed changes in commercial freeze dryer cycle CPP for experiment 1

Batch	Loading Temperature	Freezing Time Reduction	Freezing Temperature Reduction	Primary Drying Time	Secondary Drying Temperature Increasing	Cycle Total Time Reduction
A1	15°C reduction	1h	4°C	14h	3°C	17h
A2	Sub-zero	1h	4°C	14h	3°C	17h
A3	Sub-zero	2h	4°C	16h	3°C	20h

The objective of those experiments was to preview if the product could be loaded at subzero temperatures, if the observation from lyophilization cycles database could be applied to lower the shelf temperature and time of the freezing step, if the dead time observed in the three phases of the lyophilization cycle could be reduced and, at least, if the product could handle high temperatures in the secondary step.

Experiment 2 – Industrial scale freeze dryer cycle

To perform experiments in industrial freeze dryers, the lyophilization cycle in experimental 1 was scaled-up and three identical experiments (B1, B2, B3) were produced (Table 3).

Table 3: Proposed changes in freeze dryer cycle CPP for experiment 2

Batch	Loading Temperature	Freezing Time	Freezing Temperature	Primary Drying Time	Secondary Drying Temperature	Cycle Total Time Reduction
B1 B2 B3	Sub-zero	2h reduction	4°C reduction	12h reduction	3°C increased	15h

2.4. Analytical determinations

To confirm the results, each batch was analyzed regarding CQA aspect, residual moisture, potency, and pH (Table 4).

Table 4: CQA analyzed for each experiment

CQA	Analysis Method	Specification	Reference
Aspect	Automatic and visual inspection / Reconstitution by adding sterile water	Compact cake / opalescent suspension slightly pinkish-yellow	Producer
Residual moisture	Karl-Fischer coulometric titration	Maximum 3%	WHO [27]
Potency	Methodology for 50% Cell Culture Infective Dose	Equal or higher than 3.73 log 10 PFU/HD	WHO [27]
pH	Methrom 780 pHmeter	from 6.5 to 7.5 at 25°C.	Producer

Following to experiment 1, six months accelerated stability studies and twenty-four months real time stability study were conducted to confirm the suitability of the lyophilization cycle before experiment 2 industrial scale. For the experiment 2, six months accelerated stability studies and 36 months real time stability study were conducted in 2-8°C and -20°C storage conditions according to ANVISA and WHO regulations [21, 27].

3. Results and discussion

The proposed industrial freeze dryer experiments loading the product on sub-zero shelf temperature and with reduced freezing step temperature in 4°C with less than two hours leads to changes in the product temperature profile during the freezing step (Figure 4).

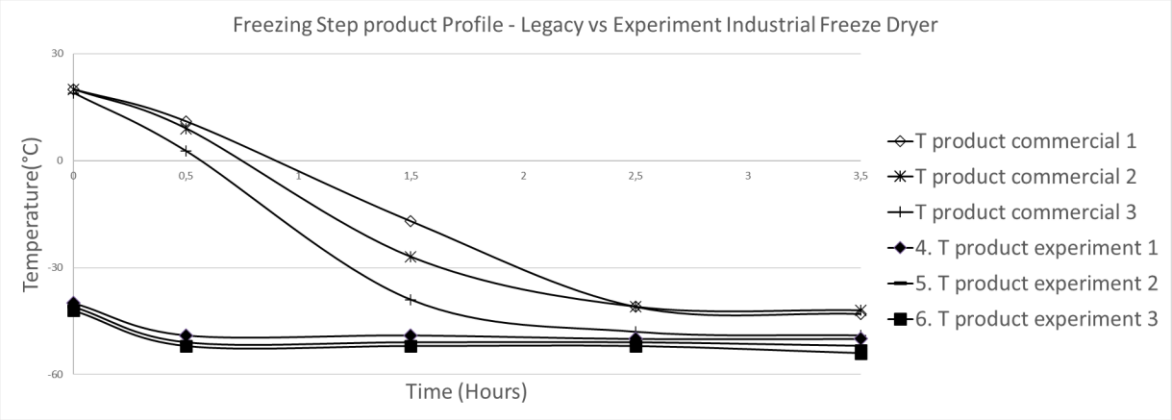

Figure 4: Comparison freezing step product profile commercial (legacy) vs experiment.

Lines 1, 2, and 3 represent the product profile monitored during commercial production (legacy) in freezing lyophilization cycle step. Lines 4, 5 and, 6 represent the new product profile in freezing step during Experiment 2 in the industrial freeze dryer. As expected, from figure 4, the product from experiment 2 achieves negative temperatures faster and lower than commercial batches avoiding the time related to decrease product temperature necessary on commercial batches. Although, it could lead to different ice morphology and impacts the degree of super-cooling and, for last, the primary step time [28]. From the primary drying step perspective, the shelf temperature and chamber pressure setpoints were the same used in commercial batches. It was observed that the product profile in experiments in industrial freeze dryer changes increasing product temperatures along this lyophilization step (Figure 5).

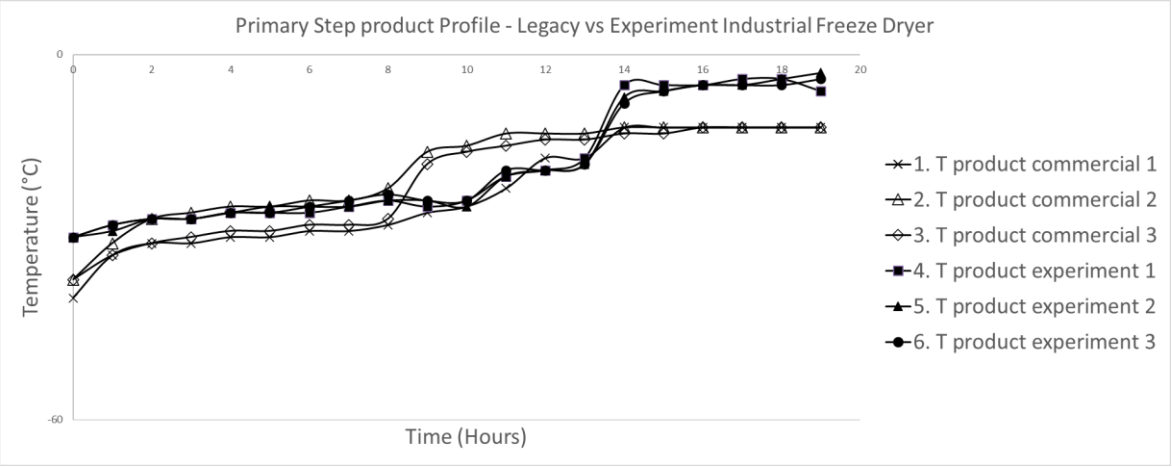

Figure 5: Comparison primary drying product profile commercial (legacy) vs experiment.

Lines 1, 2, and 3 represent the product profile during commercial production (legacy) in primary drying lyophilization cycle step. Lines 4, 5, and 6 represent the new product profile monitored in primary drying step during Experiment 2 in the industrial freeze dryer. From figure 5, the Experiment 2 product achieves superior temperatures than the commercial batches, but with a shorter primary drying time step of 12h. This could be also be related to the degree of super-cooling and resistance of mass transfer influencing primary drying times

[28]. No different profile was observed in the secondary drying step with the proposal of increase the shelf temperature in the experiments maintaining the same chamber pressure setpoint used in commercial batches (Figure 6).

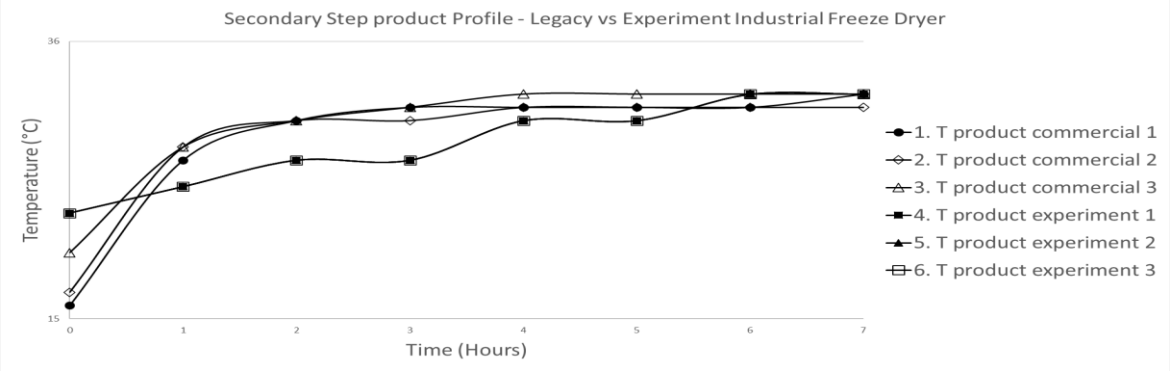

Figure 6: Comparison secondary drying product profile commercial (legacy) vs experiment.

Lines 1, 2, and 3 represent the product profile during commercial production (legacy) in secondary drying lyophilization cycle step. Lines 4, 5, and 6 represent the new product profile monitored in secondary drying step during Experiment 2 in the industrial freeze dryer. From figure 6, the increase in shelf temperature setpoint in 3°C with the same pressure setpoint and step time used in commercial batches during secondary lyophilization step do not represent a new product profile for the experimental batches. From legacy, previous stability studies with the vaccine produced in commercial scale were evaluated in three different storage conditions during long term and accelerated stability studies for thirty-six months (Table 5).

Since the CQA can change along the shelf time, stability studies in different storage temperatures were conducted in Experiment 2 batches to compare with the vaccine commercial batches studies to evaluate the freeze dryer cycle modifications proposed with the same accelerated stability conditions during six months at 25ºC, normal storage conditions at 2-8ºC for 36 months and low temperature at -20ºC for 36 months (Table 6).

Table 5: CQA results of accelerated and long-term stability studies of commercial batches

Assay	Specification	Storage Temperature	#Commercial Batch	Time (months)								Average
				0	3	6	9	12	18	24	36	
Average Residual moisture	≤ 3%	25ºC	Com-1	0.75	1.24	1.25						1.08±0.08
			Com-2	0.76	1.21	2.05						1.34±0.10
			Com-3	0.68	1.18	1.16						1.01±0.08
		2-8ºC	Com-1	0.75	0.70	1.15		1.51	1.36	1.98	1.08	1.22±0.08
			Com-2	0.76	0.80	0.89		1.10	0.98	1.16	0.67	0.91±0.09
			Com-3	0.68	0.73	0.94		1.42	1.09	1.02	1.43	1.04±0.08
		-20ºC	Com-1	0.75	0.90	0.73	0.91	1.02	0.94	0.95	0.69	0.86±0.07
			Com-2	0.76	0.88	0.76	0.84	1.06	0.67	0.77	0.73	0.81±0.09
			Com-3	0.68	0.63	0.68	0.68	0.69	0.62	0.62	0.69	0.66±0.12
Average Potency	≥ 3.0 Log 10 LD50/dose.	25ºC	Com-1	4.94	4.65	4.79						4.79±0.03
			Com-2	5.04	4.59	4.77						4.80±0.05
			Com-3	4.83	4.84	4.78						4.82±0.08
		2-8ºC	Com-1	4.94	5.04	4.84		4.78	5.14	4.68	4.48	4.84±0.08
			Com-2	5.04	4.97	4.80		4.80	5.05	4.64	4.75	4.86±0.06
			Com-3	4.83	4.76	5.0		5.25	5.12	4.82	4.97	4.96±0.06
		-20ºC	Com-1	4.71	4.58	4.71	4.60	7.48	4.57	4.75	4.36	4.60±0.09
			Com-2	5.04	4.76	4.95	4.96	4.77	5.14	5.02	4.61	4.91±0.04
			Com-3	4.83	5.01	5.01	4.96	4.82	5.21	4.97	4.67	4.94±0.08
pH	6.5 to 7.5 at 25°C	25ºC	Com-1	6.8	6.8	6.75						6.78±0.06
			Com-2	6.80	6.80	6.76						6.79±0.04
			Com-3	6.80	6.80	6.74						6.78±0.06
		2-8ºC	Com-1	6.80	6.80	6.80		6.74	6.75	6.77	6.79	6.78±0.03
			Com-2	6.80	6.76	6.85		6.80	6.75	6.78	6.79	6.79±0.06
			Com-3	6.80	6.70	6.80		6.83	6.70	6.80	6.73	6.77±0.05
		-20ºC	Com-1	6.80	6.80	6.80	6.78	6.82	6.70	6.80	6.77	6.78±0.04
			Com-2	6.80	6.70	6.80	6.76	6.79	6.80	6.80	6.80	6.78±0.02
			Com-3	6.80	6.80	6.79	6.79	6.79	6.74	6.80	6.78	6.79±0.05

Table 6: CQA results of accelerated and long-term stability studies of Experimental 2

Assay	Specification	Storage Temperature	#Experiment 2	Time (months)								Average
				0	3	6	9	12	18	24	36	
Average residual moisture	≤ 3%	25ºC	B1	0.51	1.36	1.08						0.98±0.09
			B2	0.61	1.66	1.11						1.13±0.10
			B3	0.64	1.57	1.64						1.28±0.09
		2-8ºC	B1	0.51	0.62	0.91	1.09	0.94	1.30	1.41	1.39	0.99±0.11
			B2	0.61	0.81	1.10	1.35	1.18	1.91	1.92	1.54	1.30±0.10
			B3	0.64	0.77	1.10	1.17	1.18	1.17	1.42	1.60	1.13±0.10
		-20ºC	B1	0.51	0.79		0.86	0.82	1.01	1.10	1.21	0.90±0.09
			B2	0.61	0.98		0.96	1.32	1.09	1.26	1.18	1.60±0.11
			B3	0.64	1.01		0.98	1.35	1.20	1.33	0.82	1.05±0.11
Average potency	≥ 3.0 Log 10 LD50/dose.	25ºC	B1	4.90	4.34	4.73						4.66±0.06
			B2	4.91	4.38	4.73						4.67±0.08
			B3	4.82	4.47	4.89						4.73±0.06
		2-8ºC	B1	4.90	4.54	4.64	4.85	4.74	4.63	4.41	4.48	4.65±0.04
			B2	4.91	4.81	4.70	4.61	4.70	4.62	4.49	4.50	4.67±0.06
			B3	4.82	5.09	4.76	4.78	4.91	4.81	4.67	4.64	4.81±0.04
		-20ºC	B1	4.90	4.85		4.55	4.57	4.68	4.85	4.37	4.68±0.06
			B2	4.91	4.86		4.53	4.55	4.74	4.59	4.61	4.68±0.06
			B3	4.82	4.89		4.64	4.71	4.96	4.79	4.86	4.81±0.08
Average pH	6.5 to 7.5 at 25°C	25ºC	B1	6.85	6.66	6.78						6.76±0.04
			B2	6.85	6.53	6.74						6.71±0.02
			B3	6.85	6.75	6.77						6.79±0.05
		2-8ºC	B1	6.85	6.72	6.80	6.80	6.89	6.90	7.06	6.88	6.86±0.04
			B2	6.85	6.78	6.70	6.90	6.89	6.90	7.04	6.84	6.86±0.06
			B3	6.85	6.78	6.80	6.90	6.88	6.90	7.05	6.84	6.88±0.04
		-20ºC	B1	6.85	6.77		6.90	6.80	6.90	6.50	6.95	6.78±0.05
			B2	6.85	6.90		6.90	6.80	6.90	6.94	6.91	6.89±0.06
			B3	6.85	6.96		6.90	6.80	6.90	6.88	6.92	6.89±0.03

From Table 4, average CQA residual moisture, potency and pH for all Experiment 2 batches were according with the specifications and presents similar results compare with Commercial Batches confirming that CPP changes in the lyophilization cycle does not change the CQA of the product. In all the Experiment 2 batches, the aspect of the cake appeared to be compact and without collapsed cakes (Figure 7) after evaluation by visual inspection and reconstitution analysis performed.

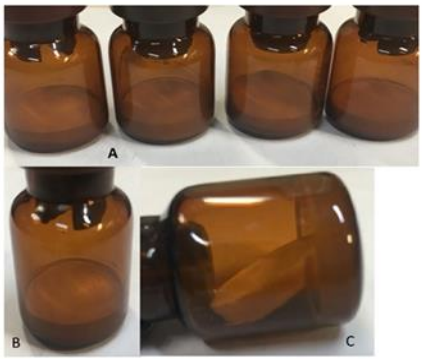

Figure 7: Product aspect of commercial and experimental 2. A) Commercial product cake B) and C) Experimental 2 product cake.

No excipients were introduced into the system or the formulation step to improve the appearance of the cake or to enhance any other property of the product in both experiments. After 100% visual inspection, a yield of 94% of the vials were approved of each experimental 2 batches and no impact on CQA aspect of the cake or after reconstitution was observed during the stability studies with different storage conditions. An improvement on the freeze-drying process for a tuberculosis vaccine by obviating the need for maintenance of the product at low temperatures was reached [26]. 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. In a review about the freezing stage of lyophilization [18], the consequences of freezing step were studied on the overall performance of the freeze-drying process, and the quality of biopharmaceutical products and 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. The authors in [29] 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. A procedure of emulsification with lyophilization, where 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 [23]. Aggregation was observed 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 those 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 [30]. Many authors, to achieve better results in the lyophilized product stability proceed with changes in the formulation of the products. On other hand, in this study no formulation changes were applied and the average CQA results for residual moisture, potency, and pH during accelerated and long-term stability studies in different storage conditions for Experimental 2 batches showed quite satisfactory and similar to commercial batches in industrial scale. Figures 8 show the profile stability study in different storage conditions for CQA residual moisture and Figure 9 for potency from Experimental 2 batches and from commercial batches of vaccine.

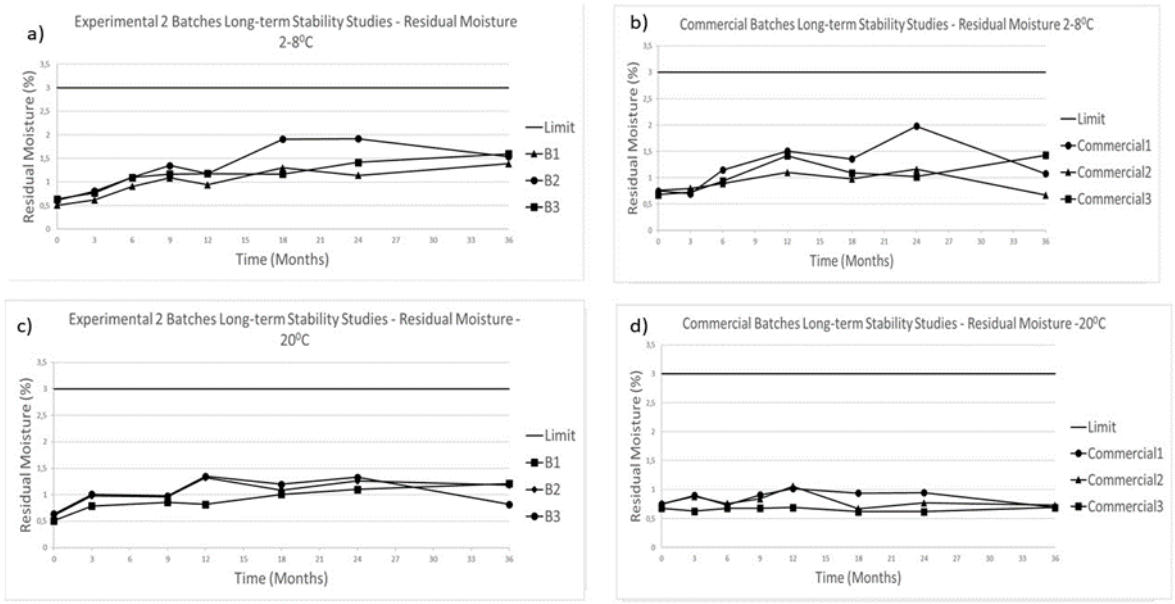

Figure 8: Profile of long-term residual moisture stability study in different storage conditions. a) b) long-term stability for residual moisture at 2-8ºC for Experimental 2 and commercial batches c) d) long-term stability for residual moisture at -20ºC for Experimental 2 and commercial batches

The profile of long-term stability result from Experimental 2 batches and from commercial batches of vaccine are very similar with crescent growing of residual moisture below the maximum limit of 3% and below 2% along the thirty-six months of stability study under 2-8°C storage. From the CQA potency perspective, the profile of long-term stability result from experimental 2 batches and from commercial batches of the vaccine are very similar with variations of potency above the minimum limit of 3.0 Log 10 LD50/dose and with values above 4,14 Log 10 LD50/dose demonstrating comparability and no significant changes in the thermostability profile of the vaccine with proposed CPP changes for the lyophilization unit operation cycle. In relation to the loss associated with accelerated thermostability of the experimental batches as a function of the freeze-drying time, all the results were satisfactory (all losses were less than or equal to 1 log PFU/HD). Regarding pH, all the experimental batches results are within the requirements and maximum value is 6.9 when vaccine is storage at -20ºC. Comparing with commercial batches there were no significant changes.

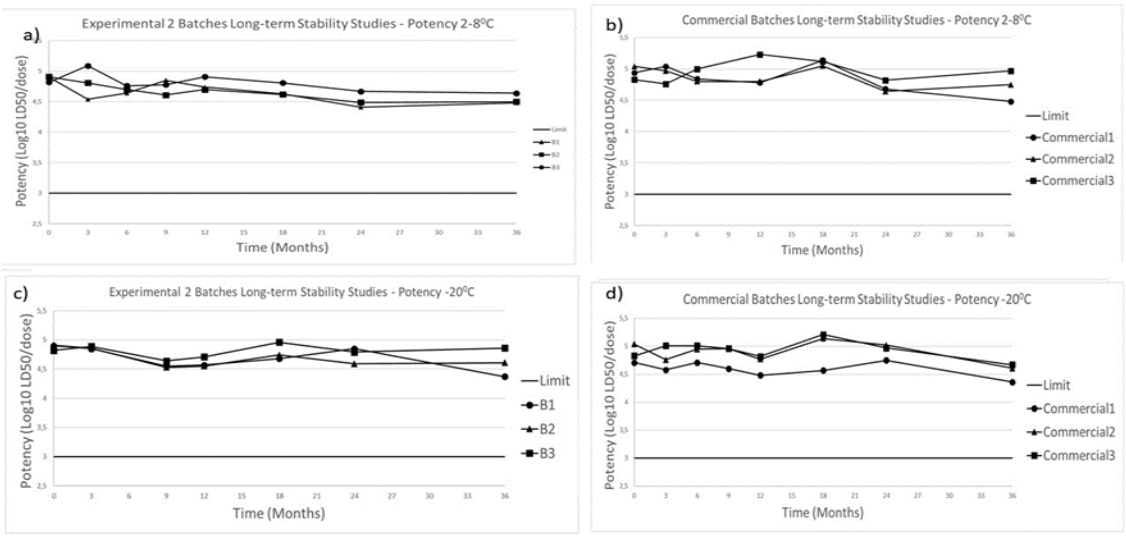

Figure 9: Profile of long potency stability study in different storage conditions. a) b) long-term stability for potency at 2-8ºC for Experimental 2 and commercial batches c) d) long-term stability for potency at -20ºC for Experimental 2 and commercial batches.

This suggests that the freezing and postfreezing CPP changes in the freeze-drying cycle with 15h less were effective for the viral vaccine test in this work and will be beneficial for this product and the CQA potency were not impacted. Performing the reduction of cycle time in the sequence of vaccine manufacturing showed benefits in cost investment of new equipments to increase production, vaccine availability, lead-time to market and others once the lyophilization is usually a bottleneck in production activities. Vaccine producers face challenges associated with maintaining consistent supply due to complexity and high fixed costs of vaccine manufacturing, regulations, and commercial requirements to supply these vaccines at affordable prices [31]. If installed, capacity of vaccine manufacturing is too large, the fixed costs increase per-unit dose cost and, on the other hand, capacity that is lower than the market can lead to lack of flexibility of supply as market conditions change. 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 [18, 30 - 34]. With the actual commercial freeze dryer and the Experiment 2 results a range for the CPP temperature and time for the lyophilization steps could be stablished.

Table 7: CPP Ranges for vaccine Freezing Dryer Cycle

Freeze Dryer CPP	CPP New Range
Temperature (Loading Step)	Positive to Sub-zero
Time (Freezing Step)	5h to 2h
Temperature (Freezing Step)	From Commercial setpoint cycle to less 4°C
Time (Primary Drying Step)	From 24h to 12h
Temperature (Secondary Drying Step)	From Commercial setpoint cycle to more 3°C

From the results, Table 7 describe ranges of CPP possible to be used for the vaccine lyophilization cycle in this study. Loading step temperature could be positive or negative, but the freezing step time will depend on that for a homogenous freeze state of the vials in the freeze dryer. From the standpoint of temperature in the freezing step, variations below 4°C from the setpoint can be implemented. If the freezing step is well succeeded, the primary drying step can vary from 12h to 24h. Lastly, variations above 3°C in secondary dry step can be implemented. Since these boundaries represents values which the set point can vary it these variations will not represent risks to product quality.

4. Conclusions

This work describes how QbD principles and contents can be applied on a product that has been commercialized for a long time and propose, based on scientific knowledge, improvements on the lyophilization production step for the vaccine. The use of QbD principles were supported by commercial batches results database and the suitability of lyophilization cycle change. With no modifications in the formulation of the product, a criticality analysis was performed in the freeze dryer cycle followed to an impact analysis to establish experiments in small scale providing scientific evidences for proposed changes on CPP ranges temperature and time on the industrial lyophilization unit operation. The experiments results in a small scale freeze dryer were according with the product CQA specification and provided more data to propose a lyophilization cycle scale up for the commercial scale industrial freeze-dryers. The industrial scale batches with the suggested CPP boundaries analyzed for thirty-six months real time stability at 2-8ºC and at -20ºC demonstrated profile and results in accordance with WHO minimum requirements and similar results with current freeze dryer cycle. Wider ranges for cycle CPPs were established for the lyophilization cycle so that the producer can guarantee, under the new limits, no interference on product quality if variations occurs during the lyophilization process. Thus, by decreasing 15h the lyophilization cycle time the number of produced batches per year can be increased with the same number of industrial freeze dryers. Those improvements are aligned with the strategies of the Brazilian National Immunization Program and WHO to contribute to the global stockpile of vaccines for emergency outbreaks. The concept of QbD and the methodology suggested in this paper can be applied to others biological lyophilized products on the market with scientific data acquisition, less product deviations and productivity increase.

5. Recommendations

Even though initial investments may be required for analysis of product specifications and process critical parameters to apply QbD concepts in small scale, the understanding of process multivariate parameters, the possibility of continuous improvement, risk mitigation of batches failures and alignment with Regulatory Authorities, who charge manufacturers for knowledge and control of his own process based on scientific matters, are some of the advantages that goes beyond of initial financial return. The use of batches database, risk analysis and Corrective Action Preventive Action could be the first step to planning future applications of the QbD principles aiming the redesign of existing systems of pharmaceuticals products already on the market and could avoid, initially, Design of Experiments due to the wide range of information already available in the manufacturer site. For future works, advances in others lyophilization CPP could be related to CQA of the

product.

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References

[1]. J.M. Juran. Juran on Quality by Design. New York, NY: Free Press, 1992, pp. 407–425.

[2]. Food and Drug Administration (2004, Sep.). "Guidance for Industry PAT – a framework for innovative pharmaceutical development, manufacturing and quality assurance". U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine, Office of Regulatory Affairs. [On-line]. 1 (1), pp. 1-19. Available: https://www.fda.gov/media/71012/download. [Mar. 20].

[3]. Food and Drug Administration (2004, Sep.). "Pharmaceutical cGMPs for the 21st century – a risk based approach". U.S. Department of Health and Human Services, Food and Drug Administration. [On-line]. 1 (1), pp. 1-32. Available: https://www.fda.gov/media/77391/download. [Mar. 20].

[4]. International Conference on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH (2009, Aug.). Q8(R2) Pharmaceutical Development. [On-line]. 1 (1), pp. 1-28. Available: https://database.ich.org/sites/default/files/Q8_R2_Guideline.pdf. [Jun. 20].

[5]. International Conference on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH (2012, May.). Q11 Pharmaceutical Development. [On-line]. 1 (1), pp. 1-30. Available: https://database.ich.org/sites/default/files/Q11%20Guideline.pdf. [Jun. 20].

[6]. S. Mao, L. Zhang. "Application of quality by design in the current drug development". Asian J. Pharm. Sci, vol. 12, pp. 1-8, Aug. 2017.

[7]. L.X. Yu. "Pharmaceutical quality by design: product and process development, understanding, and control". Pharmaceutical Research, vol. 25, pp. 781-791, Apr. 2008.

[8]. S. Rathore. "Roadmap for implementation of quality by design (QbD) for biotechnology products". Trends Biotechnology, vol. 27, pp. 546-553, Sep. 2009.

[9]. E. Tomba, P. Facco, F. Bezzo, M. Barolo. "Latent Variable modeling to assist the implementation of Quality-by-Design paradigms in pharmaceutical development and manufacturing: a review". International Journal of Pharmaceuticals, vol. 457, pp. 283-297, Sep. 2013.

[10]. M. Maniruzzaman, A. Ross, T. Dey, A. Nair, M.J. Snowden, D.A. Douroumis. "Quality by design (QbD) twin - screw extrusion wet granulation approach for processing water insoluble drugs". International Journal of Pharmaceuticals, vol 526, pp. 496-505, Jun. 2017.

[11]. J. Maguire, D. Peng. "How to identify critical quality attributes and critical process parameters", presented FDA/PQRI 2nd Conference North Bethesda, Maryland 2015.

[12]. R.M. Martins, A.L.B. Pavão, P.M.N. Oliveira, P.R.G. Santos, S.M.D. Carvalho, R. Mohrdieck, A.R.

Fernandes, H.K. Sato, P.M. Figueiredo, V.R. Doellinger, M.L.F. Leal, A. Homma, M.L.S Maia. "Adverse events following yellow fever immunization: Report and analysis of 67 neurological cases in Brazil". Vaccine, vol. 32, pp. 6676- 6682, Jan. 2014.

[13]. X. Tang, J. Pikal. "Design of freeze-drying process for pharmaceuticals: practical advice". Pharmaceutical Research, vol. 21, pp. 191–200, Feb. 2004.

[14]. K.Ryu, S. Kim, H. Nam. "Current status and perspectives of biopharmaceutical drugs". Biotechnology and Bioprocess Engineering, vol. 17, pp. 900-911, Oct. 2012.

[15]. D.H. Nam, D.D.Y. Ryu. "Biomolecular engineering and drug development". Biotechnology and Bioprocess Engineering, vol. 4, pp. 83-92, Jun. 1999.

[16]. H. Kang, V. Saraswat, J. Lee, H. Park. "Production of lyophilized culture of Lactobacillus acidophilus with preserving cell viability". Biotechnology and Bioprocess Engineering, vol. 4, pp. 36-40, Apr. 1999.

[17]. G. Kim, S. Yang. "Current trends in edible vaccine development using transgenic plants". Biotechnology and Bioprocess Engineering, vol. 15, pp. 61-65, Mar. 2010.

[18]. C. Kasper, W. Friess. "The freezing step in lyophilization: Physico-chemical fundamentals, freezing methods and consequences on process performance and quality attributes of biopharmaceuticals". European Journal of Pharmaceutics and Biopharmaceutics, vol. 78, pp. 248-263, Mar. 2011.

[19]. A. Porfile, M. Muntean, L. Rus, B. Sylvester, I. Tomut. "A quality by design approach for the development of lyophilized liposomes with simvastatin". Saudi Pharmaceutical Journal, vol. 25, pp. 981–992, Nov. 2017.

[20]. A. Arsiccio, R. Pisano. "Application of the Quality by Design Approach to the Freezing Step of Freeze-Drying: Building the Design Space". Journal of Pharmaceutical Sciences, vol. 107, pp. 1586-1596, Jun. 2018.

[21]. Brasil Ministério da Saúde. Agência Nacional de Vigilância Sanitária (2011, Sep. 20). "Resolução RDC nº 50, de 20 de setembro de 2011. Dispõe sobre os procedimentos e condições de realização de estudos de estabilidade para registro ou alterações pós-registro de produtos biológicos e dá outras providências". Diário Oficial [da República Federativa do Brasil]. [On-line]. 1 (183), pp. 694. Available: http://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2013/rdc0050_20_09_2011_rep.html [Mar 20, 2020].

[22]. C. Mariner, A. House, E. Sollod, C. Stem, M. VAN der ende, A. Mebus. "Comparison of the effect of various chemical stabilizers and lyophilization cycles on the thermostability of a vero cell-adapted rinderpest vaccine". Veterinary Microbiology, vol. 21, pp. 195-209, Jan. 1990.

[23]. W. Wang. "Lyophilization and development of solid protein pharmaceuticals". International Journal of Pharmaceuticals, vol. 203: pp. 1-60, Aug. 2000.

[24]. C. Chen, D. Han, C. Cai, X. Tang. "An overview of liposome lyophilization and its future potential". Journal of Controlled Release, vol. 142, pp. 299-31, Mar. 2010.

[25]. P. Freixeiro, E. Diéguez-Casal, L. Costoua, B. Seijo, M. Ferreirós, T. Criado, S. Sánchez. "Study of the stability of proteoliposomes as vehicles for vaccines against Neisseria meningitidis based on recombinant porin complexes". International Journal of Pharmaceutics, vol 443, pp. 1-8, Feb. 2013.

[26]. T. Orr, M. Kramer, V. Barnes, M. Dowling, L. Desbien, A. Beebe, D. Laurance, B. Fox, G. Reed, N.

Coler, S. Vedvick. “Elimination of the cold-chain dependence of a nanoemulsion adjuvanted vaccine against tuberculosis by lyophilization”. Journal of Controlled Release, vol. 177, pp. 20-26, Mar. 2014.

[27]. W.H.O. (2012, Aug. 1). Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines. (1st edition). [On-line]. Vol. 964 (59). Available: https://www.who.int/biologicals/WHO_TRS_964_web.pdf [Mar 21, 2020].

[28]. S. Rambhatla, R. Ramot, C. Bhugra, M. J. Pikal. “Heat and Mass Transfer Scale-up Issues during Freeze Drying: II. Control and Characterization of the Degree of Supercooling”. American Association of Pharmaceutical Scientists, vol. 5, pp. 58, Aug. 2004.

[29]. A. Hawe, W. Friess. “Impact of freezing procedure and annealing on the physico-chemical properties and the formation of mannitol hydrate in mannitol–sucrose–NaCl formulations”. European Journal of Pharmaceutics and Biopharmaceutics, vol. 64, pp. 316-325, Dec. 2006.

[30]. J. Hassett, C. Cousins, A. Rabia, M. Chadwick, M. O’Hara, P. Nandi, N. Brey, J. Mantis, F. Carpenter, W. Randolph. “Stabilization of a recombinant ricin toxin A subunit vaccine through lyophilization”. European Journal of Pharmaceutics and Biopharmaceutics, vol. 85, pp. 279-286, Apr. 2013.

[31]. S. Plotkin, J.M. Robinson, G. Cunningham, R. Iqbal, S. Larsen. “The complexity and cost of vaccine manufacturing – An overview”.Vaccine, vol. 35, pp. 4064-4071, Jun. 2017.

[32]. A. Clausi, P. Chouvenc. “Formulation approach for the development of a stable, lyophilized formaldehyde-containing vaccine”. European Journal of Pharmaceutics and Biopharmaceutics, vol. 85, pp. 272-278, Oct. 2013.

[33]. T. Kodama, M. Takeuchi, N. Wakiyama, K. Tearada. “Optimization of secondary drying condition for desired residual water content in a lyophilized product using a novel simulation program for pharmaceutical Lyophilization”. International Journal of Pharmaceuticals, vol. 1, pp. 59-66, Jul. 2014.

[34]. W.F. Tonnis, J.P. Amorij, M.A. Vreeman, H.W. Frijlink, G.F. Kersten, W.L.J. Hinrichs. “Improved storage stability and immunogenicity of hepatitis B vaccine after spray-freeze drying in presence of sugars”. European Journal of Pharmaceutical Sciences, vol. 55, pp. 36-45, May 2014.