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LYOPHILIZATION CYCLE COMPARISON AND SCALE-UP OF ESOMEPRAZOLE SODIUM FOR INJECTION BETWEEN LAB SCALE AND SCALE-UP BATCHES VS FDM STUDY

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Keywords:

Esomeprazole Sodium, Lyophilisation, Collapse temperature, Freeze drying microscopy scale up.

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ABSTRACT: Lyophilization also known as freeze drying is a process in which water is removed from a product after it was frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase. The process consists of three separate, unique, and interdependent processes; freezing, primary drying (sublimation), and secondary drying (desorption). Esomeprazole sodium for Injection 20 mg and 40 mg formulation was developed by optimized Lyophilization cycle. The objective of the research work was to compare the lyophilsation process cycle between Lab scale and scale up batches. The collapse temperature (Tc) for the product was identified using Freeze-drying microscope (FDM). During manufacturing the samples were exposed to above said collapse temperature to study the impact on product quality. Lab scale manufacturing was executed in Tofflon Lyophilizer 0.5 and scale-up batches were executed in Tofflon Lyophilizer 13. The Lyophilisation cycle and analytical results for both the batches were compared.

INTRODUCTION: Freeze drying technique was adopted to develop Esomeprazole sodium for Injection. Esomeprazole is highly unstable drug in Liquid form & it is not stable in high temperature. This method also offers better accuracy of dose because the drug is filled in final containers in the solution form. Although the lyophilization possesses numerous advantages over other drying processes, it also has certain challenges. The process involves inherent destabilization forces that may unstabilize the drug eg: cold shock, ice-water interfaces, pH changes during freezing, dehydration stress, etc. Also, if Lyophilization cycle is not optimized, the process can be highly energy demanding and time consuming.

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Development of optimized lyophilization cycle to overcome these challenges of lyophilization process.

Of all pharmaceutical unit operations, the highest manufacturing costs arise from drying processes. Lyophilization is the most expensive of all drying operations, both in capital investment and in operation expenses. In this context the main focus in Optimization of Lyophilization (process development) is to minimize consistently drying times, while maintaining constant product quality. Obviously the primary drying step should be carried out at the highest temperature possible, which is limited by the so called "maximum allowable temperature". This temperature indicates the eutectic temperature for a solute that crystallizes during freezing or the "collapse temperature" for a system that remains amorphous. Therefore process control means control of the product temperature vs. time profile during Lyophilization. To reach this goal the balance

between heat and mass transfer which determines the product temperature must be more or less equal Heat and mass transfer are also key issues during scale-up of lyophilization processes. Differences in the degree of super cooling between laboratory, pilot and manufacturing plants, heat transfer owing to differences in dryer design, and the efficiency in the condenser or refrigerator system can result in substantial heterogeneity in sublimation rates and/or desorption rates and hence variation in drying time .

The present work was designed to address the following objectives: -

- Formulation of the injectable dosage form.
- Performing Lyophilization and study its parameters
- Evaluation of the collapse temperature for the formulation and its importance
- Successful scale-up of the formulation

MATERIAL AND METHODS:

Materials:

Esomeprazole sodium was procured from PICAS, Disodium EDTA and Sodium hydroxide procured from Merck, Vials from schott & stoppers procured from Aptar Stelmi.

Method:

Formulation of Esomeprazole Sodium for Injection 40mg was done by Lyophilization process. In lab scale development the batch size was 250 vials. The qualitative and quantitative composition for the injection was as similar to reference listed drug (NEXIUM IV of ASTRAZENECA PHARMS) The optimized composition for the injection is as below **Table 1**.

TABLE 1: FORMULA COMPOSITION FOR ESOMEPRAZOLE SODIUM FOR INJECTION 20 mg

Sl. No.	Ingredients	Function	Qty/Vial
1	Esomeprazole Sodium	Active	40.0mg
2	Disodium EDTA	Chelating Agent	1.5mg
3	Sodium Hydroxide	To adjust pH	Q.S
4	Water For Injection	Solvent	Q.S

Method of Preparation for Lab scale:

1. Take 337.5ml of WFI (water for Injection) in a beaker maintained a temperature 2^0-8^0C.
2. Add Disodium EDTA to WFI under stirring at 1800rpm for 10min. A clear solution with pH 4.78 found.
3. Adjust the pH to 10.30 using 1.375ml of 1mol/L sodium hydroxide solution. Added Esomeprazole sodium to Sodium hydroxide under stirring at 1800rpm and maintain the temperature 2^0-8^0C throughout the manufacturing process.
4. Stir for another 10min to get a clear solution.
5. Adjust the pH to 11.92 with 1.9ml 1mol/L NaOH solution. Adjust the final volume up to 375ml with WFI.
6. Stir for another 5 min to get homogeneous solution. Filtration of the solution was done by using 0.2µ PES filter (Parker).
7. Clear solution sent for in-process check.
8. Filling & Stoppering: samples were filled by calibrated micropipette into 8ml glass vial with fill volume 1.5ml. Each vials Filled volume was crosschecked by weighing (w/w) Limit: 1.48ml – 1.52ml. 13mm bromobutyl rubber stopper was used and it was half stoppered.

Lyophilization Cycle for lab scale:

The half stoppered vials were loaded in the Lyophilizer after pre-cooling at -15^0C for 20min. The parameters for Lyophilization cycle is as below in **Table 2.**

TABLE 2: PARAMETERS FOR LYOPHILIZATION CYCLE

Lyophilization stages	Temperature ©	Ramp Duration (min)	Soak Duration (min)	Pressure (Pa)
Pre-cooling	-45^0	60	60	-
Primary Drying	-30^0	30	30	13
	-10^0	60	480	13
	5^0	60	500	13
Secondary Drying	35^0	60	420	13

The samples were evaluated for Physical appearance, assay and pH analysis.

Collapse temperature (Tc) and FDM Study:

In order to design an optimum nanoparticles freeze drying process, process development scientists need to know the critical properties of the optimized formulation and how to apply this information to process design.The critical formulation properties include the glass transition temperature of the frozen sample (Tg′), the collapse temperature of the formulation (Tc), and the properties of the excipients used. The collapse temperature is the maximum allowable product temperature during primary drying [28]. Freeze-dried product loses macroscopic structure and collapses during freeze drying when it is heated to above the temperature of collapse (Tc). Tc is usually about 2 °C higher than Tg′, or equals the eutectic temperature (Teu). The temperature of product should be lower than the collapse temperature in the stage of primary drying depicted in **Fig. 1.**

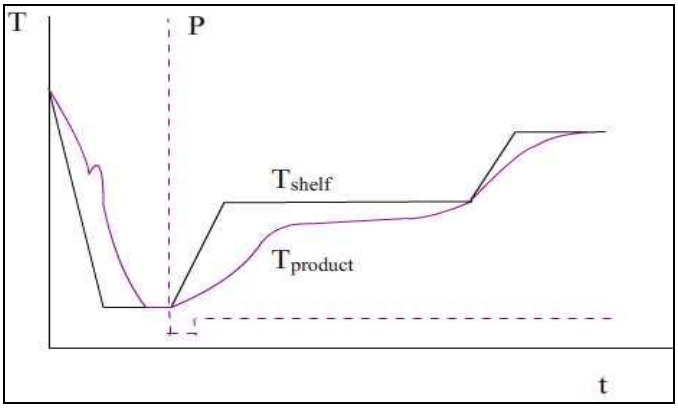


FIG. 1: RELATIONSHIP BETWEEN COLLAPSE TEMPERATURE AND PRODUCT TEMPERATURE DURING PRIMARY DRYING

When the formulation collapses, it produces a cake with unacceptable aspect. Furthermore, high residual water and prolonged reconstitution times are common consequences of collapse in a product. The reconstitution of collapsed cake was very difficult with the absence of porous structure.

The microscopic structure of the frozen matrix can be observed by different high resolution techniques such as transmission electron microscopy (TEM), atomic force microscopy (AFM), scanning electron microscopy (SEM) etc. This technique is very suitable for quantification of sublimation rate of very thin biological tissue specimen at various conditions by measurement of thickness of dried layer formed during the process. Moreover, freeze-drying microscopy (FDM) enables identification of temperatures at which visible changes occur and measurement of relative drying rates.

For Esomeprazole Sodium for injection freeze-drying microscopy (FDM) technique was used to identify the collapse temperature for the formulation.

The formulation was analyzed using the Lyostat2 Freeze-Drying Microscope, equipped with Linksys32 image and data capture software. The vacuum gauge was from adward. The chamber of FDM worked just like a freeze-drier. And the process of sublimation was set to heat up with the rate of 2^0C/min. The monitoring software Linksys32 kept recording the sample image every 5second. The sample information and parameters for the study is recorded in **Table 3** and **4**.

TABLE 3: SAMPLE INFORMATION FOR FREEZE-DRYING MICROSCOPE STUDY

Name	Solution of Esomeprazole sodium	
Specification	20mg	40mg
Formulation description	Esomeprazole sodium, EDTA-2Na	
Amount of Sample	20ml	20ml

TABLE 3: PARAMETERS SET FOR ESOMEPRAZOLE SODIUM IN COLLAPSE TEMPERATURE DETERMINATION

Sample	Temp Set (^{0}C)	Cooling Rate (^{0}C/min)	Holding Time (min)	Heating Rate (^{0}C/min)
20mg	-45	20	2	2
40mg	-45	20	2	2

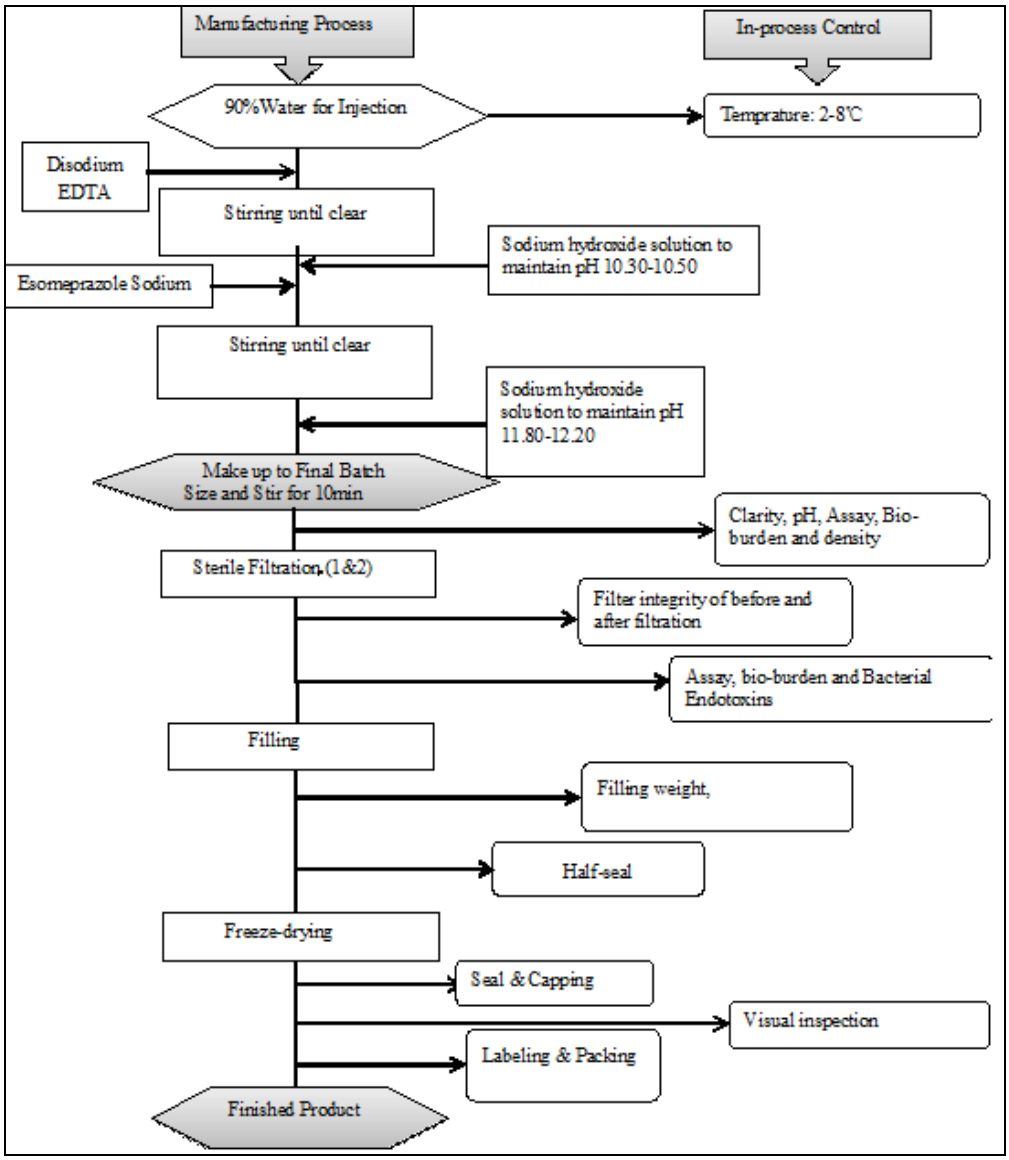
Lyophilization Cycle of Esomeprazole Sodium For Injection 40 mg - Scale-up batch:

Involvement of numerous process parameters at various stages of freeze-drying makes it a process where scale-up from laboratory scale to commercial or industrial scale through pilot scale is difficult to implement. An essential criterion during scale-up is to obtain the same product temperature history throughout the drying process independent of the dryer scale. Esomeprazole sodium for Injection 40mg Scale-up batch was manufactured with the batch size: 20,000 Vials by keeping all the manufacturing formulation & procedure similar as R&D trial batch. Only difference is this batch was filled by automated machine. The set parameters for freeze drying cycle is as recorded in **Table 4**.

TABLE 4: FREEZE DRYING CYCLE SET PARAMETERS ARE AS BELOW

Lyophilization stages	Temperature ©	Ramp Duration (min)	Soak Duration (min)	Pressure(mbar)
Pre-cooling	-45^0	60	60	999
Primary Drying	-30^0	30	30	0.13
	-10^0	60	480	0.13
	5^0	60	500	0.13
Secondary Drying	35^0	60	420	0.13

Process Flow Chart for Lyophiliation Cycle of Esomeprazole Sodium for Injection 40 mg:



RESULTS AND DISCUSSION:

Lab scale data:

The lab scale samples were analyzed for physical appearance, Assay and pH (pH after reconstitution with 0.9% NaCL. The analytical data or the samples are as reported in **Table 5**. The lyophilisation cycle for lab scale manufacturing is depicted in **Fig.2**

TABLE 5: ANALYTICAL DATA FOR LAB SCALE SAMPLES

Test	Acceptance Criteria	Results
Physical Appearance	Elegant appearance and uniform particle size distribution	Conforms
Clarity	Colorless to pale yellow, clear solution free from visible particles	Conforms
Assay	96.0% - 104.0%	101.9%
Density	For Information Only	1.0061

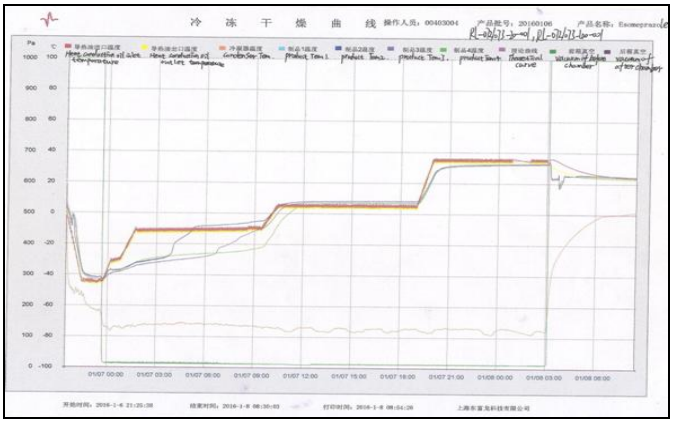


FIG. 2: GRAPHICAL PRESENTATION OF LYOPHILIZATION CYCLE DURING LAB SCALE MANUFACTURING

FDM study:

Collapse temperature data as recorded by FDM for the analysed Esomeprazole Sdoim for injection 20 mg and 40 mg is given below in **Fig. 3 – 5.**

FIG. 3: TYPICAL PICTURE OF COLLAPSE TOKEN BY FDM

FIG. 4: COLLAPSE TEMPERATURE OF 20MG ESOMEPRAZOLE SODIUM

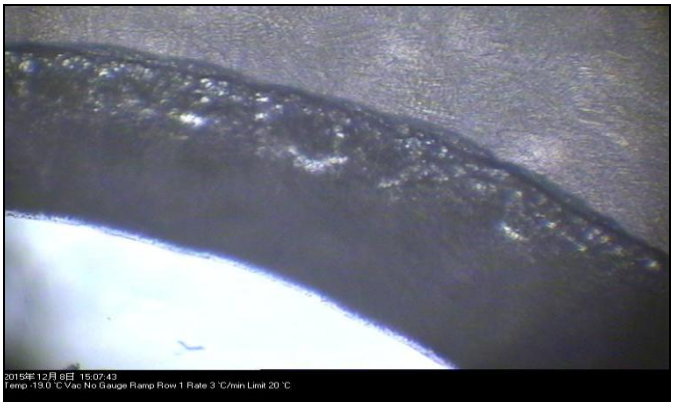


FIG. 5: COLLAPSE TEMPERATURE OF 40 MG ESOMEPRAZOLE SODIUM

The results of FDM analysis for Esomeprazole Sodium for Injection 20 mg and 40 mg is as below in **Table 6**.

TABLE 6: FDM ANALYSIS DATA FOR ESOMEPRAZOLE SODIUM FOR INJECTION

Sample	TC (^{O} C)
20mg	-19.2
40mg	-19.0

Based on the analysis of FDM detection images, the TC of Esomeprazole sodium solution is about -19^{0}C. If it is applied to the practical freeze-drying process, it can set first freeze-dried process curve according to the results of parameter detection and adopt a freezing way of quick frozen. And a sublimed temperature setting shall ensure that the sample temperature is lower than -19^{0}C, so that it can guarantee that the samples will be dried below TC.

Scale up batch data: The sample solutions before Lyophilization process were evaluated for their conformity to In-process specification. The data is below in **Table 7** and **8**.

TABLE 7: IN-PROCESS ANALYTICAL DATA BEFORE FIRST FILTRATION FOR ESOMEPRAZOLE FOR INJECTION 40 MG

Batch No		20160102
Testing items	**Acceptance criteria**	**Testing result**
pH value	11.00~12.00	11.87
Assay	96.0% - 104.0%	103.5%
Density	For information only	1.0085g/ml

TABLE 8: IN-PROCESS ANALYTICAL DATA BEFORE SECOND FILTRATION FOR ESOMEPRAZOLE FOR INJECTION 40 MG

Batch No		20160102
Testing items	**Acceptance criteria**	**Testing result**
Assay	96.0%-104.0%	103.3%

The Lyophilization cycle for scale up batch manufacturing is depicted in **Fig. 6.**

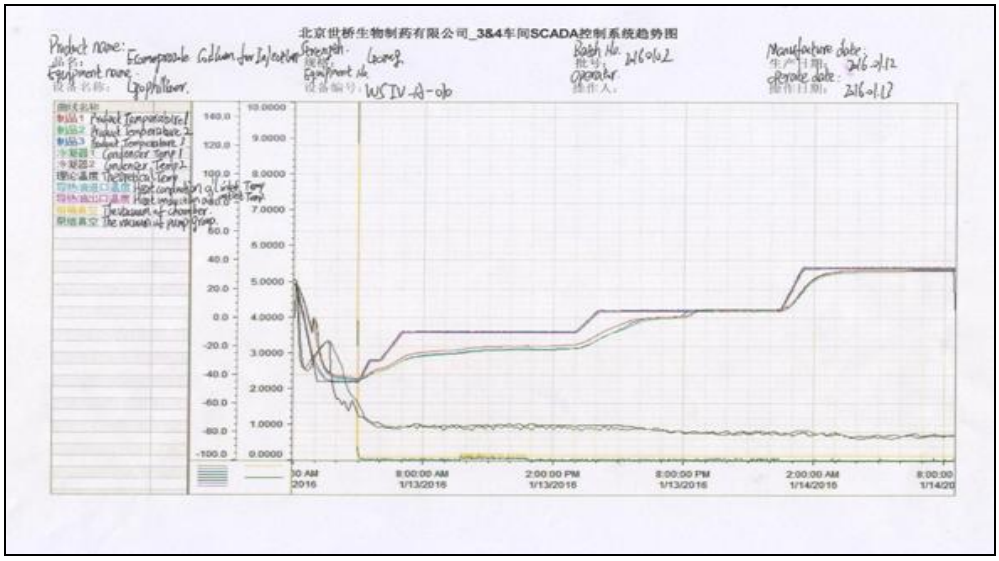


FIG. 6: GRAPHICAL PRESENTATION OF LYOPHILIZATION CYCLE DURING SCALE UP BATCH MANUFACTURING

The Blue lines in the graph depicted the product temperature during the initiation of Lyophilisation Cycle, Which is quite lower than the Collapsible temperature which was found during FDM Study. The lyophilied samples are diluted with required quantity of diluent solution to make up the concentration at 5 mg/ml of Esomeprazole Sodium. The samples are analysed for Physical appearance, Assay and pH after reconstitution. The data is as recorded in **Table 8**.

TABLE 8: ANALYTICAL DATA FOR SCALE UP BATCH SAMPLES

Batch No		20160102
Testing items	**Acceptance criteria**	**Testing result**
Physical Appearance	Elegant appearance and uniform particle size distribution	Conforms
Clarity	Colourless to pale yellow, clear solution free from visible particles	Conforms
pH value	11.00~12.00	0.9% NaCL : 10.92 5% Glucose : 10.45 Riger'sSolution : 10.87
Assay	96.0%-104.0%	102.6%

CONCLUSION: The present research work was designed to develop a lyophilized injectable dosage form of an Anti-Ulcer drug Esomeprazole Sodium. The drug is unstable if dispensed as liquid dosage form. Hence the present project was envisaged to overcome the drawbacks associated with Esomeprazole sodium and to formulate a stable and therapeutically effective formulation by lyophilization technique which provides extended shelf life. Based on the physicochemical properties of the drug, disodium Edetate (chelating agent) and Sodium Hydroxide (Solubilizing agent), Lyophilization technique was adopted to improve the cake characteristics of the lyophilized form of Esomeprazole sodium.

The optimized Lyophilization cycle developed in lab scale provided samples with consistent physical and chemical attributes as per the pre- determined specification limits. From the present study it can

conclude that LAB SCALE & Scale-up batches, Freeze dried cycle is similar with the set parameters in Lab Scale (Tofflon Lyophilizer 0.5) & Commercial Scale equipment (Tofflon Lyophilizer 13). The FDM study data indicated the collapse temperature of the formulation at -19.2 (^{O} C). The FDM study data had a profound influence in the development of Esomeprazole Sodium for Injection.

Hence it can be concluded that our objective of Lyophilization Cycle Comparison and scale-up Of Esomeprazole Sodium For Injection Between Lab Scale and Scale-Up Batches Vs FDM Study is achieved

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