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Design and Development of Lyophilization Cycle for Polymyxin B

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Abstract

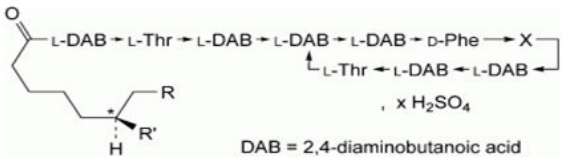
The Lyophilisation is also called as Freeze drying where water can be removed from the product with the application of temperature and vacuum. The process of Lyophilisation consists of three steps freezing, primary drying and secondary drying. In the primary drying unbound water is removed from the product after it is froze when placed under vacuum and in secondary drying the bound water which is associated with the drug molecule can be removed by desorption. Determination of the Critical events is very necessary to design and optimize the lyophilisation cycle.

Different studies are involved into the determination of critical events for the design and development of lyophilisation cycle, like FDM, DSC and DTA and impedance analysis. The aim of this study is optimization of the Lyophilisation cycles for Polymixin B using different formulations.

Keywords: Polymyxin B; N gonorrhoeae; chemicals; Lyophilisation

Introduction

Polymyxin B is the active substance and is official in USP; Polymyxin B is manufactured by M/S Xellia. Polymyxin B for injection is Polymyxin B for Injection, USP is one of a group of basic polypeptide antibiotics derived from B polymyxa (B aerosporous). Polymyxin B sulfate is the sulfate salt of Polymyxins B and B , which are produced by the growth of Bacillus polymyxa (Prazmowski) Migula (Fam. Bacillacea). It has a potency of not less than 6000 polymyxin B units per mg, calculated on the anhydrous basis. The structural formulae are C56H100N16O17S



Polymyxin B sulfate has a bactericidal action against almost all gram-negative bacilli except the Proteus group. Polymyxins increase the permeability of bacterial cell wall membranes. All gram-positive bacteria, fungi, and the gram-negative cocci, N gonorrhoeae and N meningitidis, are resistant.

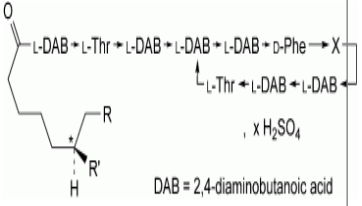
Susceptibility plate testing: If the Kirby-Bauer method of disc susceptibility testing is used, a 300-unit polymyxin B disc should give a zone of over 11 mm when tested against a polymyxin B susceptible bacterialstrain.

Polymyxin B sulfate is not absorbed from the normal alimentary tract. Since the drug loses 50 percent of its activity in the presence of serum, active blood levels are low. Repeated injections may give a cumulative effect. Levels tend to be higher in infants and children. The drug is excreted slowly by the kidneys. Tissue diffusion is poor and the drug does not pass the blood brain barrier in to the cerebrospinal fluid. In therapeutic dosag e, polym yxin B sulfate causes some nephro toxicity with tubule damage to a slight degree [1,2].

Physico-chemical characteristics:

The Physico-chemical characteristics of Polymyxin B for injection drug substance, as reported in the literature are tabulated below [3,4].

Table1: Physico-chemical characteristics of the Active Pharmaceutical Ingredient.

CAS No.	**1405-20-5**
Chemical Name	(N-(4-amino-1-((1-((4-amino-1-oxo-1-((6,9,18-tris(2-aminoethyl)-15-benzyl-3-(1-hydroxyethyl)-12-isobutyl-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19- heptaazacyclotricosan- 21-yl)amino)butan- 2- yl)amino)- 3-hydroxy- 1- oxobutan- 2- yl)amino)- 1-oxobutan- 2- yl)- 6- methyloctanamide sulfate
Structural Formula	
Molecular Formula	C56H98N16O13 • H2SO4
Molecular Weight	1301.56.
Solubility	Polymyxin B sulfate in water at 50 mg/mL is clear to very slightly hazy,

	colorless to yellow in appearance.2 Polymyxin B sulfate has only minimal solubility in any organic solvent, for example, 0.115 mg/mL in ethanol
Loss on drying	Dry it at 60° for 3 hours
Specific rotation	-78.0° to -90.0°

Materials and Methods

Materials

Polymyxin B was generously supplied as a gift sample by outsourced from M/S Xellia pharmaceuticals aps, India, Mannitol supplied by Merck, (Hyderabad, India. All other chemicals were of analytical reagent grade and were used as received [6,7].

Methods

Lyostat Analysis

The formulation was analysed by Lyostat freeze drying microscope for the determination of the critical events and corresponding temperatures that are specific to that material. This is the eutectic temperature for a crystalline material or a collapse temperature for an amorphous material. A 2µl sample of the formulation was pipetted between a quartz and glass cover slip to create a 70µm thick 'sandwich' of sample – this ensures that the sublimation front can be observed so that the critical temperature can be established. The principal information obtained from FDM analysis is the collapse (Tc) or Eutectic (Teu) point of the formulation.. Images of the FDM analysis were taken at appropriate intervals and are provided in the figure 1.

DTA and impedance analysis

The formulation was analysed using the Lyotherm 3 combined thermal analyser, in accordance with standard operating procedure.Two 3ml samples of the formulation were loaded on the Lyotherm 3 apparatus alongside a 3ml AnalaR grade water reference, before the system was immersed in liquid nitrogen and cooled to below -100.0°C. The temperatures of the samples and the impedance of the sample together with the temperature of the reference were measured at 3-second intervals during cooling and heating and the raw data was exported directly to Microsoft Excel for analysis. The heating profile of the formulation was compared with that of the respective reference sample to determine the critical temperatures of the significant events, which could be attributed to thermal changes occurring in the solution [8-10].

Table 2: Composition of Polymyxin B for injection 50mg/vial

S.No.	Raw materials	Pharmacoepial reference/ standard	mg/Vial
1.	Polymyxin B	USP/Ph.Eur	50.00ng
2.	Water for Injection	IP	q.s to 2.0 mL

Brief Manufacturing procedure

- Required quantities of ingredients were dispensed as per the above composition table.
- About 90% (of total batch size) of Water for Injection was collected in a beaker and purged with filtered nitrogen and remaining Water for Injection was kept aside for the rinsings and the final volume make up.
- The solution was cooled to 20°C-25°C and maintained the same for throughout the batch
- Required qty of Polymyxin B was added to step 3 under continuous stirring and stirred the solution for about 10 minutes.
- Final solution was made up to 100% with water for Injection from step 2 and stirred for 10 minutes.
- Filtered the solution through 0.22µ PVDF hydrophilic membrane filter.
- The filtered solution was filled into 10 mL/20 mm clear Tubular glass vials with the fill volume of 02 mL and partially stoppered with 20 mm Bromobutyl doubled slotted rubber stoppers and subject to Lyophilization as per below recipe.

Lyophilization cycle development:

This section summarizes the activity aimed at development and optimization of Lyophilization cycle for manufacture of clarithromycin for injection 500 mg/vial and evaluating the effect of Lyophilization cycle on the product characteristics.

The brief explanation of Lyophilization cycle development for Polymixin B for Injection 50mg/vial is below [11].

Typical freeze-drying process consists of three stages: that is,

- Freezing
- Primary drying(sublimation)
- Secondary drying (desorption)
- Freezing is an efficient dessication step where most of the solvent, typically water, is separated from the solutes to form ice. As freezing progresses, the solute phase becomes highly concentrated and is termed the "freeze concentrate" by the end of freezing.
- Primary drying, or ice sublimation, begins whenever the chamber pressure is reduced and the shelf temperature is raised to supply the heat removed by ice sublimation. During primary drying the chamber pressure (vacuum) is well below the vapor pressure of ice and ice is transferred from the product to the condenser by sublimation and crystallization on to the cold coils/plates (<-50°C) in the condenser.
- Secondary drying is the stage where the water is desorbed from the freeze concentrate, usually at elevated temperature and low pressure. Some secondary drying occurs at the very beginning of primary drying as ice is removed from a region, but the bulk of secondary drying occurs after primary drying is over and the product temperature has increased.

Below is the Lyophilization parameters are considered in the process development:

Stage of process	Freezing	Primary drying	Secondary drying
Critical parameters	Freezing set temperature	Primary drying set temperature Vacuum during primary drying	Secondary drying set temperature Vacuum during secondary drying
Critical parameters during process	Shelf temperature Product temperature	Shelf temperature Product temperature Vacuum vs temperature	Shelf temperature Product temperature Vacuum Vs temperature

Process Step: Freezing

The freezing step was initiated by decreasing the shelf temperature (from 5 °C) over 30 minutes and held at 0°C for 120 minutes to ensure that all the vials get homogenous temperature throughout the bulk solution/in-between vials, once all the vials solution obtained 0°C, decrease the shelf temperature to -40°C with slow freezing (cooling) to ensure the complete crystallization of the formulation components and to avoid thermal expansion as the temperature is increased during the temperature ramp to primary drying [12-14].

This also maintains the homogeniety between the vials on shelves in lyophilizer. This usually produces uniform ice structure in vials.

Process Step: Primary drying

At the completion of freezing, primary drying is initiated with the lowering of the chamber pressure and an increase in the shelf temperature. The shelf temperature was increased to -20°C over 200 minutes (0.1°C/ min) and held for 300 minutes.

The selected temperature is well below the freezing point and equilibrium freezing temperature to avoid melting of the frozen cake, which would otherwise result in collapse of the cake and improper drying of the same.

The slow heating rate during early steps ensures a gradual heating of the product vial such that melt back does not happens during the process.

The vacuum/chamber pressure was selected as 200 milli Torr, which is well below the vapor pressure of ice at this temperature. This vacuum is derived from ice vapor pressure data at optimized temperature.

During ramp up the shelf temperature from -20°C to +55°C, the chamber pressure was increased from 200 milli Torr to 50 milli Torr. This is done gradually by increasing the chamber pressure in a programmed manner. This increased chamber pressure is necessary to ensure that the product does not melt back during the process.

Process Step: Secondary drying:

At the completion of primary drying, secondary drying is initiated with the lowering of the chamber pressure and an increase in the shelf temperature. The shelf temperature was increased to +55°C over 40 minutes (0.5°C/ min) and held for 300 minutes.

The chamber pressure was maintained at 50 mT. During secondary drying there will be a rapid loss of free moisture from the product, hence a low chamber pressure (i.e. higher vacuum) would ensure the moisture is removed efficiently [15].

After completion of Lyophilization cycle, stoppering was done by backfilling the chamber with 0.22µm filtered nitrogen.

Lyo Cycle development : Optimization batches

Table 3: Lyo Cycles Optimization batches.

Lyo recipe	POL-01			POL-02				POL-03				
	Temp (°C)	RAMP (mins)	HOLD (mins)	Vacuum (mT)	Temp (°C)	RAMP (mins)	HOLD (mins)	Vacuum (mT)	Temp (°C)	RAMP (mins)	HOLD (mins)	Vacuum (mT)
Loading	5	--	--	--	5	--	--	--	5	--	--	--
Freezing	-45	60	300	--	-45	60	240	--	-45	60	300	--
	-15	30	240	--	-15	30	180	--	-15	30	240	--
	-45	60	300	--	-45	60	240	--	-45	60	300	--
Primary Drying	-30	300	500	150	-30	300	400	200	-30	120	240	50
	-15	200	840	150	-20	300	420	150	-15	60	300	100
	-5	300	300	150	-5	420	660	150	-5	60	300	100
	10	20	300	150	20	20	300	150	10	300	420	100
	--	--	--	--	--	--	--	--	20	600	500	100
	--	--	--	--	--	--	--	--	--	--	--	--
Secondary Drying	25	40	360	50	25	40	300	50	25	40	300	50

Results and Discussion

Lyo Stat Analysis

Freeze-drying microscopy analysis using the Lyostat freeze drying microscopy apparatus revealed the formulation to display collapse zone behaviour, where the structure of the drying sample is gradually lost over a temperature range, rather than at a distinct point.

The upper and lower temperature limits could be established, as given in Table 4 below. Selected images from the Lyostat analyses can be found in figure 1.

Table 4: Summary of observations made in Lyostat analysis.

Formulation	Temperature at which the drying structure began to collapse	Temperature at which total collapse of the drying structure was observed
Tenecteplase	-25.3°C	-22.3°C
Tenecteplase (annealed)	-25.6°C	-22.4°C

Figure 1: Images of Lyostat analysis.

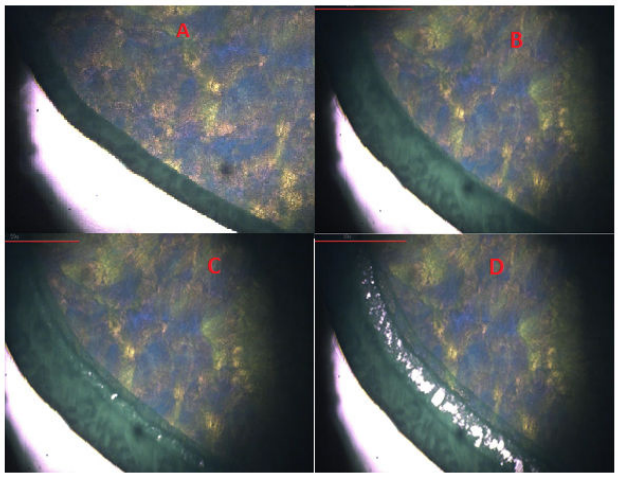


Image A: A 2 µl sample of Tenecteplase dried with good structure at -40.0°C

Image B: First signs of collapse onset observed as the temperature of the sample reached - 25.3°C

Image C: The loss of drying structure became more apparent as the temperature reached -25.9°C.

Image D: As the temperature reached -24.3°C, total collapse of the drying structure was observed

DTA and impedance analysis

Figure 2: Impedance and DTA analysis results for Polymixin B solution.

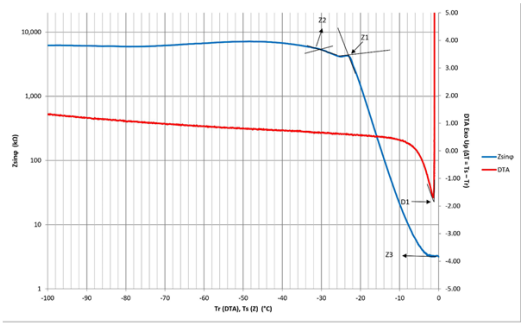


Table 5: Observations of Impedance (Zsinϕ) analysis.

Analysis	Event	Temperature	Description of events/general comments
Impedance (ZSinφ)	Z1	-70.0°C	Levelling of downward gradient of the impedance curve indicative of a stabilisation within the frozen material
	Z2	-73.0	Increase in downward gradient of the impedance curve indicative of a softening within the frozen material
DTA	D1	-1.0°C	Ice melt endotherm

Table 6: Water content values of Polymixin B for Injction.

Test	Specifications	Water Content Results		
		Polymixin B for Injection B. No: POL-01	Polymixin B for Injection B. No: POL-02	Polymixin B for Injection B. No: POL-03
Water Content	Not More than 3.0 %	0.48 0.34 0.37	0.31 0.34 0.33	0.74 0.57 0.60
	Average	0.39	0.32	0.63

Table 7: Assay results of Polymixin B in Polymixin B for Injection.

Sr. No	Batch Number	Assay %	Marketed Sample
1	POL-01	101.3	100.1%
2	POL-02	102.0	
3	POL-03	99.8	

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