



LYOPHILIZATION: PROCESS, METHODS AND APPLICATION

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ABSTRACT

Lyophilization is the common, but cost-intensive process. Freezing is an equally important step in Lyophilization, as it affects both process efficiency and quality of the product. However simple concept, freezing is probably the most complex step in lyophilisation. Therefore, to get a more comprehensive understanding, the lyophilization methods and application are first summarized. The different techniques or method that can be used in lyophilisation are also reviewed.

The purpose of this review is to make the reader aware not only of the significance but also of the complexities of the freeze stage in lyophilisation. In this review article there are many method introduced with different method of lyophilisation and used of lyophilization production in different area.

KEYWORDS:-Theory of Lyophilization, Principle, The process steps, Traditional Lyophilization technology, Different Methods of Lyophilization, Applications.

1. INTRODUCTION

Lyophilization is the one of the greatest innovations in pharmaceutical industry for enhancing the long-term safety of drug products and simplifying the shipping and handling of drugs. It is a 3-stage process involving freezing, primary drying (ice sublimation), and secondary drying (unfrozen desorption of the water). It is a time-and energy-intensive process that can take days to complete, where a large part of the cycle time is spent on primary drying. During primary drying, the product temperature must be kept below the critical product temperature of the formulation, such as collapse temperature (T_c) for crystalline structures with amorphous or eutectic temperature (T_e), So as to guarantee that the application is applied.^[1,7] Freeze-drying is commonly used to gain long term stability in the processing of biopharmaceutical products, and is a time and energy-consuming process.^[8] Is a time and energy-consuming process which can allow day or week finish when most cycle time is spent during primary drying. The optimization first stage therefore has become an important focal point for process development scientists to reduce operating costs and increase production throughput.^[9-15] The objective during stage 1 to minimize drying time (PDT) while keeping the product temperature (TP) below the critical product temperature of the formulation, such as collapse temperature (T_c) for amorphous system or eutectic temperature (T_e) for crystalline systems. The formulations describe the critical product temperature to be the optimum primary drying TP, which is used as the

Upper limit to identify process parameters that typically produce a primary drying temperature below that critical product temperature below 1-2 degree Celsius.^[15,17] The longest primary drying process is period of the Lyophilization cycle, during which ice sublimation takes place under conditions of vacuum and low temperature. During primary drying the product temperature is controlled by changing Temperature on shelf and the pressure of the room. An increased in product temperature above a critical temperature will adversely affect product quality, likely resulting in collapsed, so that measurement of the material of the temperature during stage 1 important. Using thermocouples, which has several significant limitations, is the standard approach to product temperature calculation on a commercial dryer.^[18] In the pharmaceutical industry, Lyophilization is used to minimize formulations of liquid products in a non-destructive way to their solid constituents.^[19-20] This method aims to increase the stability and therefore the shelf-life of chemically or biologically unstable drugs in a solution.^[21,24] While some time Lyophilization has been use, the recent increase in the production of biopharmaceutical drugs, which are often not stable in a liquid formulation, have resulted in a significant increase in the number of drugs developed in a lyophilized manner.^[24,25] This is an undesirable relationship because these leachable substance impurities in the drug product and therefore have the potential to impact its health, efficacy and identification. Regulatory bodies 8, 9 are for these purposes.^[26] The lyophilized formulations are more

susceptible to leaching substances from stoppers used to seal their primary container if other factors are equivalent. Nevertheless, it is not clear what the patient's exposure to these substances, and hence the patient's risk, might be. For example, in its primary packaging method, usually, the lyophilized drug is reconstituted with an aqueous medium, transferred and further diluted to a secondary aqueous medium bottle, and administered via a secondary container and tertiary method. There are at least 2 pathways for the elimination of the leachable from the drug solution when reconstituted and administered in this manner.^[27]

2. THEORY OF LYOPHILIZATION

2.1 Freeze Drying

Freezing Is In the pharmaceutical industry, freezing is often used to ensure long-term safety of the parenteral products which are sensitive to moisture. There are three main processes of lyophilization: During the freezing process, freezing, primary drying, and secondary drying phase transition from liquid to solid state takes place. This is accompanied by primary drying, sublimating the frozen water using low temperatures and heat. Accompany secondary drying by primary drying, in which thermal desorption extracts the majority of the remaining water through exposing the mostly dry substance to warmer temperatures while still in vacuum condition. The optimal method of Lyophilization produces a stable product with high power yield, High porosity, low moisture content and a smooth pharmaceutical feel. A major concern in the pharmaceutical industry is the uniformity of samples, in addition to the manufactured product with the desired characteristics mentioned above. The root causes of variability in the lyophilization cycle have been extensively studied and are commonly due to freezing variations and environmental variations around each vial during freezing processes. The freezing process in major causes of in homogeneity within a batch as well as between lots. Freezing can occur at or below the product's freezing temperature; super cooling is the difference between the nominal freezing point and the actual freezing temperature of the product. The degree of super cooling is linked to freezing method, nucleation process molecular dynamics, cooling rate, and the product's particulate content. The extent of super cooling in any individual vial can differ from the extent of super cooling in adjacent vials. The degree to which super cooling occurs changes the ice structure of the frozen substance greatly, which usually results in smaller ice crystals.^[28,29] The primary drying process consists of the ice sublimation from the frozen product. Where the structure produced during the freezing process, the solution components permitted above the glass transition are destroyed or partially destroyed during primary or secondary drying due to a viscous migration. Collapse can lead to incomplete drying, resulting in moisture content higher than desired and an undesirable product appearance. The collapsed product can differ in appearance, but a slightly discolored, shrunken cake

frequently characterizes it.^[30]

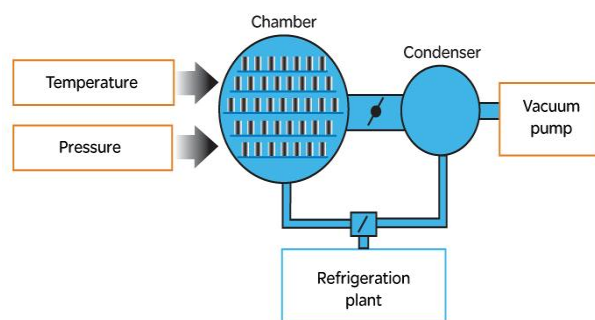


Fig.no.1 Freeze drying process.

3. PRINCIPLE

It also called sublimation in important principle involved in freezing drying, where water transfers directly from the solid state (ice) to vapor state without going through the liquid layer. Water sublimation can occur at pressures and temperatures below the triple point i.e. 4,579 mm Hg and 0.0099 degrees Celsius.^[31] The drying material freeze then Heated under a high vacuum, to leave only solid, dried components of the original liquid. The water vapor concentration gradient between the drying front and the condenser is the driving force for water removal during Lyophilization.^[32]

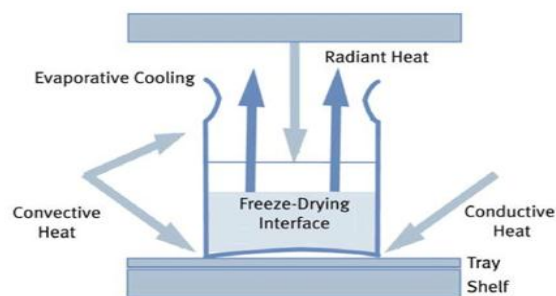
4. THE PROCESS STEP

4.1. Freezing: Frozen goods, this provides the necessary condition for drying at low temperatures.

4.2. Vacuum: The substance is then put under vacuum after freezing. It helps the freezing substance to vaporize without going through the liquid phase, which is a process called sublimation.

4.3. Heat: Heat is added to frozen product in order to speed up sublimation.

4.4. Condensation: The vaporized liquid is removed by low-temperature condenser plates from the vacuum chamber by transforming it back into a solid. It completes process of separation.^[33]



Heat Transfer in a Shelf Freeze Dryer

Fig. 2: The process steps.

5. EXCIPIENT IN LYOPHILIZATION

5.1. Bulking agent: Bulking agents make up bulk lyophilized substance and give the cake a proper structure. These are commonly used for low-dose (high-potency) drugs that do not have the bulk needed to support their own structure. These are especially important when the overall solid content is less than 2 per cent (5). In such cases, a bulking agent is applied to the matrix for the formulation.^[42] The mostly used bulking agent are mannitol and glycine, followed by glucose, saccharose, lactose, trehalose and dextran.^[43] Nevertheless, Crystallization of the bulking agent can adversely affect the product's physical stability some cases, which would prefer an amorphous bulking agent.^[44]

5.2. Buffering Agent: PH regulation is important to prevent product degradation during reconstitution, manufactured and storage requiring the Added buffering agent to lyophilized formulations.

The choice of buffer depend on active ingredient's pH stability profile, since the drug must be reconstituted and processed before it can be given to the patient for some time. The pH of the drug's optimal stability should be established and maintained to this end. For sensitive molecules it is important to select an appropriate buffer and its concentration. The buffering agent should have a high temperature of failure, be non-volatile and a high glass transition temperature (TG).^[45]

5.3. Stabilizers: - lyophilization system in addition being a disaccharide, bulking agent form amorphous glass of sugar and has proved to be the most efficient in stabilizing materials such as proteins and liposome during lyophilization. Trehalose and sucrose are inert and used to stabilize formulations of liposomes, proteins and viruses. Glucose, lactose and maltose eliminate sugars, and proteins can be reduced by the means for a mallard reaction.

5.4. Tonicity adjusters- [Lyophilization system] in a number of cases, an isotonic formulation may be required. The need for such a formulation can be calculated either by the needs of quality of the bulk solution or by those for the route of administration. Excipient such as glycine, mannitol, sucrose, glycerol and sodium chloride, are effective tonicity adjusters. Glycine will lower the temperature of the glass transition if held in amorphous phase Tonicity modifiers may also be included as diluents instead of formulation.

6. TRADITIONAL LYOPHILIZATION TECHNOLOGY

This is very complex process requiring careful product, equipment and processing techniques to be lyophilization was used for nearly about 30 years to stabilize many forms of chemical components. Chemical reagents and biochemical are unstable in liquid form, chemically or biological active, sensitive to temperature, and chemically reactive to each other. Because of these

features, Chemicals may have very short shelf-life, may need freezing, or may degrade unless they are stabilized. The Lyophilization method, when properly done, solves these problems by placing reagents in a state of suspended operation.^[34] Lyophilization provides long shelf-life for unstable chemical solutions when handled at room temperature. The process gives excellent product solubility characteristics; allowing fast reconstitution Compounds which are sensitive to heat and moisture maintain their viability. During the process, most proteins do not denature, and the bacterial growth and enzyme activity usually occurring in aqueous preparations may be removed. Lyophilization thus ensures complete preservation of biological and chemical pureness.^[35]

6.1. Lyophilization process architecture has historically been broken down into three parts

6.1.1. Freezing phase- the liquid composition is cooled until the ice starts to nucleate and ice growth follows. It results in the removal of most of the water from a mixture of glassy and/or crystalline solutes into ice crystals.^[36,37]

6.1.2. Primary drying- Sublimation removes the crystalline ice that was formed during freezing. Hence the pressure of chamber is reduced below the vapor pressure of the shelf temperature and ice is increased to provide the heat generated by the ice sublimation.^[38]

When primary drying completed After the product of primary drying still contain approximately 15-20% of the unfrozen stage, usually at high temperatures and low pressures, so that the desired low humidity content is finally achieved.^[39] Lyophilization is typically an extremely time and energy intensive drying cycle.^[40]

6.1.3. Secondary drying- Compared to primary drying (every day), it is relatively short (every hour) For this reason, the development of the Lyophilization process has usually focused on optimizing the primary drying stage, i.e. shortening the primary drying time by adjusting the shelf temperature and/or chamber pressure without affecting product quality.^[37,38] Though freezing is one of the most critical stages during lyophilization, in the past the significance of the freezing process has been rather overlooked.^[41]

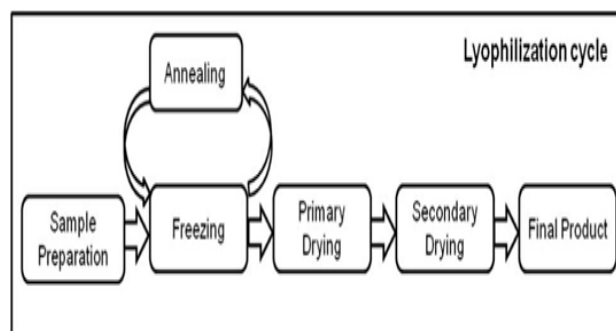


Fig no.3 Lyophilization process.

7. DIFFERENT METHODS OF LYOPHILIZATION

1. Lyophilization^[65]

Experiments were conducted using lab- (LyoStar II; SP Industries) and pilot-scale (IMA Life North America

Inc.) lyophilizers with approximately 0.45 m² and 2 m² of shelf surface, respectively.

Table1. Selected Laboratory Specifications-(LyoStar II) and Pilot Lyophilizers and TDLAS Sensor Assembly.

| Content | Lyostart II | Pilot-scale dryer |
|---|------------------------------|------------------------------|
| Chamber volume L | 107.5 | 950.3 |
| Duct diameter cm | 9.72 | 33.70 |
| Duct area cm ² | 74.36 | 892.00 |
| Total shelf surface area m ^[2] | 0.46 | 2.25 |
| Path length cm | 13.15 | 50.10 |
| Measurement angle | 45.0 degree and 135.0 degree | 47.4 degree and 132.6 degree |

2. Sample preparation and lyophilization

Briefly, HP storage solutions at 500, 1000, 3000 and 5000 ppm were prepared with Milli-Q water from 3 per cent solution. These freshly prepared HP stock solutions were sharpened to protein formulation and placebo at a final concentration of 0, 0.5, 1.0, 3.0 or 5.0 ppm. The spiked samples were mixed gently, dispensed at 10 mL / vial into a 20cc glass vial, and stopped entirely. After storage in dark at room temperature for 25 hours, the protein and placebo vials were loaded together onto a lyophilize shelf that was pre-cooled to 5 precautions. Thermal couples were placed in both protein and placebo vials to monitor product temperature during Lyophilization. Before the temperature was ramped to 0C, the vials were frozen to about 040C and kept for 13 hours. The temperature was then ramped for 18 h to 15 premises before the secondary drying at 30 premises for 4 h was started. Over the entire cycle a steady pressure of 100 mTorr was used. All vials were filled with nitrogen at the end of the lyophilization process, completely stopped, and crimped with seals of aluminium. For up to 6 months, the crimped vials from each of the groups were incubated to test their stability after lyophilization either at 2 auxiliary C-8C or 25 auxiliary C.^[15]

3. Lyophilization process parameters^[66]

For all freeze-drying tests, aseptically filled 3.5 mL of 0.22-mm filtered Protein X formulation into 10-mL vials and partially stopped with 20-mm stoppers. The freeze-drying cycles were conducted at 20 per cent of the total lyophilizer shelf load on a Lyostar 3 freeze-dryer

(SP Scientific, Stone Ridge, NY). Prior to the start of the freeze-drying process, a 15-minute equilibration at 5trecc was used. Despite the use of various freezing methods (Table 2), the primary drying steps (almost 30C, 100 mTorr) and secondary drying (40 C, 100 mTorr) were kept identical for all cycle tested. The pressure was regulated by means of a manometer capacitance gage. In all freeze-drying cycles, The secondary drying phase time was set at 6 h, whereas the duration / end of the primary drying phase was determined by a comparative pressure calculation in which the Pirani pressure measurement converges, within 10 mT, to that of the chamber power manometer. During freeze-drying the product temperature was monitored using thermocouples (TCs) and also calculated via Manometric Temperature Measurement (MTM). A total of 4 TCs were put in the center of selected vials at the edge of each shelf (2 vials) and middle (2 vials). The TCs were spread over the shelf to track any difference in product temperature as a function of shelf position. The freeze-drying cycles were designed to vary in the freezing step in order to assess the effect of the freezing step on overall process output and product quality. One of the freezing cycles included an annealing stage with a temperature setting point of approximately 015BC (referred to as Anneal), whereas the other cycle used ControlLyoTM Technology10 for a regulated ice nucleation step (referred to as CN) at a temperature setting point of approximately 08BC instead of annealing. Finally, a third cycle was tested without either annealing or controlled ice nucleation. The freeze-drying method parameters are summarized in Table 2.

Table no.2 Lyophilization the Anneal, No Anneal and Controlled Ice Nucleation (CN) cycle process parameters.

| Process parameter/cycle | Anneal cycle | No anneal cycle | CN cycle |
|--|------------------|------------------------|------------------------|
| Ramp to freezing | 1 degree c/min | 1°C/min | 1°C/min |
| Freezing temperature /hold time | -40 degree c/4 h | -40°C/4 h | -40°C/4 h |
| Annealing temperature /hold time | -15 degree c/2 h | NA | NA |
| Controlled ice nucleation temperature /hold time | - | NA | -8°C/4 hr |
| Ramp to primary drying | - | 0.1°C/min | 0.1°C/min |
| Primary drying temperature/pressure/hold time | - | -30°C/100 mTorr/Pirani | -30°C/100 mTorr/Pirani |
| Ramp to secondary drying | - | 0.3°C/min | 0.3°C/min |
| Secondary drying temperature/pressure /hold time | - | 40°C/100 mTorr/6 h | 40°C/100 mTorr/6 h |

4. Preparation of tablet with lyophilized nanosuspensions^[67]

Nanosuspensions were prepared with slight modification by nanoprecipitation process. In short, product (250 mg) has been dissolved in amount of pure ethanol (1.5 ml). The process of anti-solvent was prepared by dissolving the mannitol and PVA in water and cooled in a bath of ice. Nanosuspensions were prepared with slight modification by sono-precipitation process. In short, product (250 mg) has been dissolved in pure ethanol (1.5 ml). The process of antisolvent was prepared by dissolving PVA and mannitol in water and cooled in ice bath. The resulting nano-suspensions were easily poured into the pockets of a blister pack of PVC to obtain 50 mg dose of drug per tablet. The tablet blister packs were then moved to a -80 ° C freezer, and held for 6 hours. The freeze tablet were then dried to freeze for 24 h. Use Lyophilizer Hetotrap CT 60e lyophilize. The produced the lyophilization nanosuspension tablet were stored at room temperature in densely closed amber-yellow containers until further use.

5. Monoclonal antibody (MAB) formulation and lyophilization^[68]

There are two concentration of IgG1 formulation, 1 mg/ml and 20 mg/93ml were prepared by adding adequate amounts of stock mAb 94 in acetate buffer (pH 5.5), sucrose (170 mM), acetate buffer (25 mM 95 sodium acetate buffer, pH 5.5) and polysorbate 20 (0.01 per cent w / v). Both formulations were 0.22 mm filtered and a bulk solution of 5 ml fill in the vials under the laminar air flow. Vials were stopped in part by and put on the lyophilizer shelf. Lyophilization of the formulations was carried out using a Laboratory Scale Freeze-100 Dryer, LyoStar 3 (FTS Systems, Stone Ridge, NY) equipped with 101 SMARTTM technology and ControlLyo on Demand technology (Danbury, CT). The software SMARTTM Technology allowed manometric temperature measurements (MTM) to be performed while the on-demand ControlLyo technology permitted controlled nucleation by pressurizing and depressurizing the lyophilization chamber using argon gas. Filled vials were cooled at ramp rate of 1 °C / min from room temperature to 5 °C for lyophilization cycles with uncontrolled nucleation and held at that point for 30 min. The vials were 110 then cooled to -5 degrees C (ramp rate of 1 degree C / min) and kept for 30 min to 111 to reach approximate temperature equilibrium in all samples and 112 then ramped to -42 degrees C at 1 degree C / min and keeping for 120 min until 113 ramping to primary drying. The 115 vials were cooled from room temperature to 5 degrees C at a ramp rate of 116 1 degree C / min for lyophilization cycles using controlled ice nucleation, and held for 30 minutes.

6. Tangential flow filtration^[69]

The vaccination was administered using a tangential flow filtration method MinimateTM with a cut-off pore size of 5 KD. The TFF cassette had been sanitized with 0.5 M NaOH at 45 u-CS for 45 min before use as recommended

by the manufacturer. The tangential flow filtration was subsequently rinsed with highly purified water until a neutral pH of a SevenEasy1 pH-meter was measured. The vaccine concentration was carried out at a temperature of 2–8 degrees C after saturation of the TFF-cassette membrane with 200 ml of circulating vaccine solution over a 60 min time period. The injection of the H1N1 influenza vaccine was performed at a flow rate of 100 ml / min, as mentioned by the manufacturer. The membrane chamber was pressurized by a peristaltic pump while the vaccine solution was in contact with the membrane. The initial volume of the vaccine has been reduced to 4%, resulting in a concentration of hemagglutinin 10-13 times higher than the original dose (final concentration 150 mg / ml). The concentrated vaccine was subsequently dialyzed against a 10 mM phosphate buffer (0.22 mm filtered) to reduce salts to prevent a phase separation during freeze-drying.

7. Fractional factorial screening design^[70]

A 24-1 fractional factorial design (Table 2) was used to determine the key effects of NaCl, Polysorbate 20, pH and lactic acid on the concentration of mAb (A280 nm), glass transition temperature (Tg), unfolding temperature (Tm) and the presence of aggregation (A410 nm) in lyophilized formulations. Preliminary tests were performed to determine the variables and their levels for these studies, as well as sufficient buffer systems and pH range resulting in limited precipitation of the mAb. Arginine buffer (50 mM, pH 8), Tris (100 mM, pH 8), phosphate buffer (100 mM, pH 7), histidine buffer (50 mM, pH 6), citrate (100 mM, pH 6), and acetate buffer (100 mM, pH 5) were checked for this reason. Studies have also been performed to identify the correct lyoprotectants from sucrose, trehalose, mannitol, and glycine. In those cases, depending on preliminary studies, the concentration and pH range of the formulation buffer system selected was either 50 mM of histidine (pH 6) or arginine (pH 8) with 100 mM of NaCl. The concentration of sucrose was calculated for each formulation, based on the difference between the NaCl related other variables of the formulation and the remaining amount needed of isotonicity. Based on the experimental design, the monoclonal antibody stock solutions were prepared by dialyzing the mAb into the correct buffer system based on the optimal pH level to produce a final concentration of 2 mg / mL.

The excipient stock solutions were also prepared by properly dissolving weighted excipients in deionized water and adjusting them to the desired volume. Active formulations were prepared by applying a 1:1 excipient ratio and solutions with mAb stock to achieve a 1 mg / mL mAb formula. Based on the survey of marketed lyophilized mAb products (concentration range: 1–200 mg / mL) and the availability of purified mAb in the laboratory, the target concentration was determined. In order to minimize possible contamination of ingredients, all tests and preparations were conducted in the Biological Safety Cabinet (BSC) and all tubes, vials and

stoppers used for experiments were autoclaved for 30 minutes at 121 degrees C and allowed to cool before use.

Table no.3 Optimisation method parameters.

| # | Pattern | Ph | NaCL9mM) | Polysorbate 20(mM) |
|----|---------|----|----------|---------------------|
| 1 | 000 | 7 | 30 | 0.04 |
| 2 | 0-- | 7 | 10 | 0.008 |
| 3 | +0+ | 8 | 30 | 0.08 |
| 4 | -0+ | 6 | 30 | 0.08 |
| 5 | 0++ | 7 | 50 | 0.08 |
| 6 | 0+- | 7 | 50 | 0.008 |
| 7 | ++0 | 8 | 50 | 0.04 |
| 8 | --0 | 6 | 10 | 0.04 |
| 9 | -+10 | 6 | 50 | 0.04 |
| 10 | +0- | 8 | 30 | 0.008 |
| 11 | 000 | 7 | 30 | 0.04 |
| 12 | 000 | 7 | 30 | 0.04 |
| 13 | -0- | 6 | 30 | 0.008 |
| 14 | 0+ | 7 | 10 | 0.08 |
| 15 | +0 | 8 | 10 | 0.04 |

Table no.4 Lyophilized Microparticles containing lysozyme.

| formula | Lysozyme (w/w, %) | HP- β -CD (W/W, %) | Biocompatible polymer (w/w, %) | TPGS 1000 (W/W, %) | Sugar (w/w, %) |
|---------|-------------------|--------------------------|--------------------------------|--------------------|----------------|
| F1 | 1 | 1 | WCS 0.2% | - | - |
| F2 | 1 | 1 | WCS 0.2% | - | SUCROSE 2% |
| F3 | 1 | 1 | WCS 0.2% | - | MALTOSE 2% |
| F4 | 1 | 1 | WCS 0.2% | - | Tetrahalose 2% |
| F4-1 | 1 | 1 | HPMC 0.2% | - | Tetrahalose 2% |
| F4-2 | 1 | 1 | HPMC0.2% | 0.5 | Tetrahalose 2% |

9. Spray freeze drying (SFD)^[72]

Essentially, the SFD procedure was performed in accordance with the method described above, but with some modifications. A single disperser droplet generator with a nozzle plate diameter of 100 μ m used for the formation of droplets. Nanoparticles dispersions were co-sprayed in a cold air column (-120 ° C), surrounded by a cooling jacket of liquid nitrogen, at a fixed concentration of 5 percent w / v of various cryoprotectants. Throughout their flight, the droplets are frozen in the cooled air and the frozen particles were collected in a cooled container after sedimentation, placed directly at the bottom of the spray column to allow the frozen particles to be easily transported to the freeze dryer for subsequent drying. Freeze drying FD had prepared similar control samples. The freezing step was performed at -30 ° C in a shelf-refrigerator. In an Alpha 1-4 LSC Plus freeze dryer (Martin Christ, Germany) operating at a vacuum level of 0.1 mbar and the collector at a temperature of -52 ° C, the frozen dispersions and SFD particles were lyophilized for 48 h. Reconstitution of the spray dried and freeze dried nanoparticle the reconstitution potential of FD and SFD lyophilizates was assessed by re-dispersing 50 mg of each sample into 1 ml of distilled water with gentle shaking and corresponding particle size calculation after re-dispersion. The difference in the size of the nanoparticles before (Si) and after (Sf) re-

8. Preparation of lyophilized microparticles containing lysozyme^[71]

The freeze drying process prepared the biocompatible microparticles. In short, 1% (w / w) of the HP-bis-CD solution was prepared in distilled de-ionized water (DDW) containing lysozyme (about 14.4 kDa), WCS, HPMC, TPGS 1000 and sugars (Table 1). The solution was then stirred for 24 h and passed through a filter of 0.2 mm membranes. To obtain microparticles, the filtrate was lyophilized. The lyophilization was carried out as follows; the vials containing the filtrate were frozen for 2 hours at -20/2C and stored for 24 h at -80/C. The samples were then lyophilized into a freeze dryer (OPERON FDU-8612, Korea) at 40 mbar vacuum for 48 h. The lyophilized samples were then dried for 48 h to remove the residual water content. Lyophilized microparticulated containing lysozyme composition.

dispersion was used to characterize the lyophilized reconstitution potential, where a Sf / Si value of 1 indicates full reconstitution, whereas a Sf/Si value of > 1 indicates weak reconstitution.

10. Precipitation-lyophilization-homogenization (Plh) method^[73]

As previously reported, clarithromycin nanocrystals were prepared using the precipitation– lyophilization – homogenization (PLH) method. In short, SLS (0.1 percent w / v) and poloxamer 407 (2 and 5 percent w / v, respectively, for Formulations A and B, were dissolved into the sterile water. Meanwhile, 1 percent w / v of clarithromycin have dissolved completely into excess acetone. The solution was mixed with Clarithromycin in the surfactant. The mixture was stirred continuously at about 8–10 ° C until acetone was evaporated completely. The suspension was eventually pre-frozen at 12 a.m.

It was then primarily dried at -36 degrees C and 0.200 mbar for 24 hours and then secondarily dried by freeze dryer at -50 degrees C and 0.040 mbar for 8 h. In the sterile water the lyophilized material was redispersed and mixed homogeneously. Using a high pressure homogenizer the nanosuspension was then obtained by applying the homogenization step in order of 5 cycles at 50 bar, 5 cycles at 100 bar, 10 cycles at 500 bar, 10

cycles at 1000 bar and 30 cycles at 1500 bar. For the coarse suspension of clarithromycin, one-step preparation was done by homogeneously dispersing clarithromycin powder (1 percent w / v) in the Formulation A surfactant solution Afterwards, under the aforementioned condition, the obtained nanosuspension and coarse suspension were eventually converted to the dried powder form by lyophilization.

11. Preparation of odt (orally disintegrate tablet) by matrix former^[74]

Nimesulide ODTs were prepared using gelatin as a former matrix, a sugar alcohol (sorbitol or mannitol) and a defense against collapse (glycine). Gelatin was used at three different concentrations (1%, 2% and 3% w / v), whereas the two sugar alcohols and glycine were used at 0.886% w / v. During the formulation process, the percentage of sugar alcohol and glycine used has been optimized to produce a strong and elegant tablet that can be treated with ease. To achieve the necessary concentration, gelatin was dissolved in distilled at 40°C. At predetermined concentration, sorbitol (or mannitol) and glycine was then applied to the gelatin solution. An

appropriately measured volume of NM powder was spread through a magnetic stirrer in the prepared aqueous solution, resulting in a dosage of 50 mg NM per 1 ml. Then, One milliliter of the suspension was poured into each pocket of a PVC blister pack of 13 mm in diameter with a depth of 3 mm resulting in 50 mg per pill. At -22 degree C, The tablet blister packs were then transferred to a freezer and kept for 24 hours in the fridge. The frozen tablets were put in a lyophilizer for 24 hours using a Novalyph-NL 500 Freeze Dryer with a condenser temperature of -45 degrees C and a pressure of 7 likely 10-2 mbar. The better of these formulations was taken to the next level where a water-soluble surface active agent or polymer was added to improve the time and friability of the disintegration. The accelerators of such disintegration were sodium lauryl sulfate (SLS); three grades of PEG, i.e. PEG 400, PEG 4000, and PEG 6000; 3 grades PVP, i.e. PVP K25, PVP K30 and PVP K90; and Two classes of Tweens, i.e. Between the 20th and the 80th Four. All of these were introduced at a concentration of 1 percent w / v except for SLS, added at 0.05 percent w / v. the exact composition of the ODTs.

Table no.5 Preparation of ODT.

| Formulation | Gelatine % w/v | Sorbitol % w/v | Mannitol % w/v | Glycine % w/v | Disintegration accelerators | NM % w/v |
|-------------|----------------|----------------|----------------|---------------|-----------------------------|----------|
| G1 | 1 | 0.886 | - | 0.886 | - | 5 |
| G2 | 1 | - | 0.886 | 0.886 | - | 5 |
| G3 | 2 | 0.886 | - | 0.886 | - | 5 |
| G4 | 2 | - | 0.886 | 0.886 | - | 5 |
| G5 | 3 | 0.886 | - | 0.886 | - | 5 |
| G6 | 3 | - | 0.886 | 0.886 | - | 5 |
| G7 | 2 | - | 0.886 | 0.886 | - | 5 |
| G8 | 2 | - | 0.886 | 0.886 | 0.005% SLS | 5 |
| G9 | 2 | - | 0.886 | 0.886 | 1% PEG 400 | 5 |
| G10 | 2 | - | 0.886 | 0.886 | 1% PEG 4000 | 5 |
| G11 | 2 | - | 0.886 | 0.886 | 1% PEG 6000 | 5 |
| G12 | 2 | - | 0.886 | 0.886 | 1% PVP K25 | 5 |
| G13 | 2 | - | 0.886 | 0.886 | 1% PVP K90 | 5 |
| G14 | 2 | - | 0.886 | 0.886 | 1% Tween 20 | 5 |
| G15 | 2 | - | 0.886 | 0.886 | 1% tween 80 | 5 |

12. Micro-encapsulation process^[75]

The L strain freeze-dried. In MRS broth, in casei BNCC 134415 the freeze-dried powder was completely mixed in a culture medium of 0.4 mL and then grown on MRS agar plates under anaerobic conditions for 48 hours. The activated bacteria it was further grown in a 100 mL MRS broth and incubated under the same conditions for obtaining the probiotic at the stationary state for 48 h. The culture has been centrifuged at 6000 g at 4 ° C for 15 min, the supernatant was discarded, and the precipitated cells were washed with PBS twice (pH 7.4), followed by centrifugation. In PBS (pH 7.4), the cells have been reconstituted to provide 5.0tre108 CFU / mL for cell count. The probiotic suspension was used for the process of microencapsulation.

13. Lyophilization of solid dispersion^[76]

The selected solid dispersions of cyclohexanol were dissolved in a minimum amount. This solution was easily solidified by moving small portions with a Pasteur pipette to a cold Labconco interior surface. Flask spinning in a -50°C methanol bath. The Labconco flask was attached to the lyophilizer vacuum adapter after a certain layer thickness was obtained. At a pressure of 8-10 mmHg the solvent was sublimated and condensed to a condenser of -75 ° C. Upon complete removal of the solvent, the powder residue appeared as porous; the mass is light and fluffy. At room temperature, the lyophilized preparations were placed in a desiccator.

8. APPLICATION

8.1 Application in biotechnology and food processing.

Freeze-drying (lyophilization) was developed to preserve bioactive molecules (DNA, enzymes, and proteins), pharmaceutical products (antibiotics), and other sensitive impregnated solvent materials.^[46] Freeze-drying will become an increasingly common method for the long term preservation of various biological materials. Because lyophilization currently produces a variety of foods, pharmaceuticals, etc., a successful application for the long-term survival of living systems, such as cells, remains one of the greatest challenges in this field for scientists.^[47] Freeze-drying therefore tends to be a promising technique for the dehydration of heat-sensitive materials, such as fruit.^[48] The manufacturing conditions influence the consistency standards used to test freeze-dried fruits and the freezing time. In addition, two criteria (rehydration and texture) are closely related for the best use of the freeze product, and cannot be viewed as absolute criteria.^[49] The physical properties of freeze-dried materials during freeze-drying have been shown to depend on the temperature Collapse happens above the glass transition temperature for all materials and the effect becomes more extreme as the temperature rises, regulating the bulk density and porosity of the dried materials.^[50] The freezing step explains the product's instant rehydration efficiency, and the loss of texture due to damage to the cell wall. Nevertheless, the freezing rate does not affect the quality of freeze-dried strawberry, nor the freezing time.^[49] Freeze-drying, including pre-crystallization and rapid freezing, enables preservation of the functional properties of egg yolk without additives. Since full contact rapid freezing enables freeze-dried egg yolk to be produced without liquid nitrogen, even at marketable costs.^[51] High rehydration potential limits freeze-dried application of strawberries in liquid carriers as the texture of such dried strawberries can collapse. Therefore, an effective coating needs to be implemented to slow down the rehydration rate of freeze-dried strawberries in order to preserve their flavor for a long time to come. Because of its physicochemical properties, a whey protein coating solution has been shown to reduce the rehydration rate of freeze-dried strawberry pieces.^[52] The rehydration and sorption properties were related to structural changes in frozen strawberries during osmotic dehydration and freeze-drying.^[53]



Fig. no. 4 Lyophilized food.

2. Application of novel pharmaceutical lyophilization.

Lyophilization was a thing of the past primarily limited to drying out labile pharmaceutical products such as antibiotics and, since the 1980s, preferably reconstituted proteins before administration. Nowadays, however, the scope of pharmaceutical applications dependent on lyophilisation has expanded, allowing for useful pharmaceutical advancements, but also resulting in more and more complicated lyophilisation. Fig.5

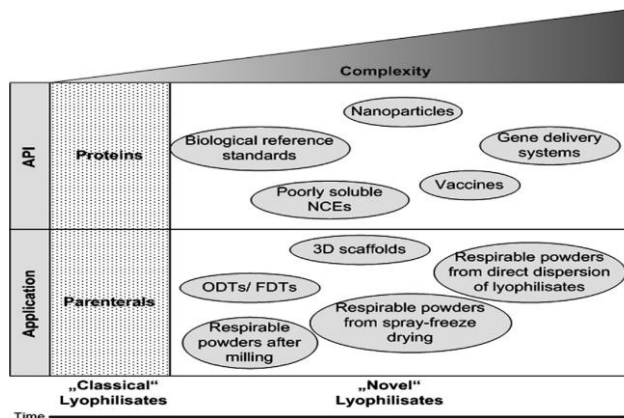


Fig.no. 5 Overview on the broadened spectrum of pharmaceutical application manufactured by lyophilisation resulting in increased complexity.

3. Lyophilisation beyond the drying of pharmaceutical proteins.

The majority of literature on the drying of pharmaceutical proteins in the pharmaceutical lyophilisation field is generally available. Work has also begun to explore the use of lyophilization for several other pharmaceutical applications. Next, lyophilization is commonly used to achieve long-term safe formulations of the vaccines.^[54] In addition, cell lyophilization, e.g. red blood cells, is a beneficial target.^[55] Many micro- and nanoparticulate structures have been developed to deliver small molecules, proteins, or peptides effectively with drugs.^[56] Lyophilization has been developed in that sense as a common approach to overcoming their physical and/or chemical instabilities.^[56,58] In this case, the production of formulations can be quite difficult, as stabilization mechanisms and excipients developed for proteins do not necessarily apply to these very stress-sensitive systems.^[59] In the area of biological medicines, biological reference criteria have also been introduced recently. Often these biological standards are derived from a single big batch of well-characterized biological material. Here, lyophilization, often carried out in bulbs rather than vials, helps to ensure long-term stability and ease of distribute.



Fig. no. 6 drying of pharmaceutical protein.

4. Pharmaceutical application of lyophilization in the solid state

Lyophilization's most common use is the manufacture of parenterals which are administered after a simple step of reconstitution. However, there have also been a few other applications which take advantage of the unique properties of lyophilizate in solid state. First, the popularity of orally disintegrating tablets (ODTs) or quick dissolving tablets (FDTs) has grown. Market-based preparation technologies such as Zydis™ (R.P. Scherer, UK) technology, Lyoc™ (Farmalyoc, France) technology, or Quicksolv™ (Janssen Pharmaceutica, USA) technology are available. FDTs prepared by lyophilization have a specific porosity that ensures rapid dissolution in the saliva without water, but can display reduced mechanical strength and stability of storage. Nevertheless, the gentle lyophilization preparation method now makes FDTs a promising solution, for example to the development of tablet vaccines.^[61] Recently, the porosity of the scaffolds has been shown to be controllable by modifying the freezing conditions during the freezing lyophilization step.^[62] In general, the lyophilization of biopharmaceuticals can also be used as a preliminary stage in the development of coarse powders, which must subsequently be milled to produce dry inhalation particles.^[63] Similar approaches have also been used to formulate a lyophilized dry powder vaccine for nasal delivery.^[64]

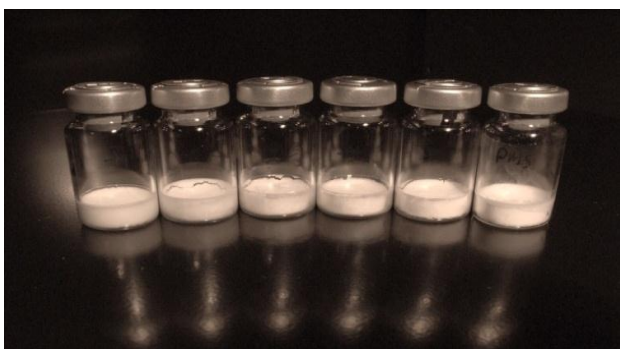


Fig. no.7 Lyophilized solid state powder.

9. CONCLUSION

In This article demonstrate that the studied or performed the different Method with the help of primary drying, secondary drying. In this all methods the different lyophilization products were performed. The

lyophilization process is also demonstrated, and how the traditional method was used as compared the latest lyophilization method. The lyophilization product was used in different area, There are different application was also be demonstrate.

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