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An overview of lyophilization: troubleshooting the challenges and pharmaceutical applications

Hetal A. Pandya [1], Shital Butani [1, *]

[1]Department of Pharmaceutics, Institute of Pharmacy, Nirma University, SG Highway, Chharodi, Ahmedabad: 382481, Gujarat, India

*corresponding author e-mail address: ***shital.butani@nirmauni.ac.in*** | Scopus ID 6507187686

ABSTRACT

Although, lyophilization is well known technique for drying, recently its applications in pharmaceutical field is tremendously increased. The quality product with low moisture content, higher solubility and easy reconstitution lead to wide spread applications of the process. The formulation is frozen, followed by sublimation and desorption to yield dry powder ready for reconstitution. This technique is critical, therefore optimization of many parameters is mandatory to get quality product. The improper insight of parameters like freezing temperature, drying temperature, vacuum, fill volume, etc. may result into collapse, melt back, blow out, shrinkage of the cake which directly affects product reconstitution time, moisture content, assay, and ultimately the stability of the final product. In this review, the challenges experienced in development of freeze drying cycle and its appropriate remedies are discussed at a length. Additionally pharmaceutical applications with the regulatory concerns are also reviewed.

Keywords: *lyophilization; freeze drying; Challenges; applications.*

1. INTRODUCTION

The term lyophil was used for the first time by Rey for the product which is porous, reabsorbs the liquid and return to its original state. The "Lyophilization" is a process in which the substance is frozen in first step (freezing) and then solvent is been sublimated by applying vacuum and increasing temperature in second stage (primary drying) followed by desorption i.e. third stage (secondary drying) to the significant that will no longer sustain biological growth or chemical reaction [1]. Ready-to-use liquid dosage forms of many biopharmaceuticals are not available in market due to their instability in liquid state. Such unstable liquid formulation or unstable drugs can be converted into free flowing powder which further can easily be liquefied at the time of use. Thus, freeze drying technique yields dry product with sufficient stability [2]. Many biopharmaceutical products that can be freeze dried like antibodies, enzyme, hormones, antibiotics, vitamin and typically vaccines. This is also a key process in conservation of blood plasma. Apart from pharmaceutical applications, freeze drying is primarily used in stabilizing food products like coffee, herbs and fruits [3].

In last few years, nano carriers are highly explored in pharmaceutical industry for the drug delivery. However, the major drawback of nano formulation is its long term instability, which can be increased significantly through lyophilization process. Converting colloidal suspension into freeze dried product results into considerable advantage to avert particle aggregation, degradation of polymer and leakage of drug which is enveloped in nanoparticle [4]. Nowadays, vesicular system is also gaining superiority in pharmaceutical industry as a drug carrier. Marketed formulation such as AmbiSome® (Amphotericin B) and DOXIL® (Doxorubicin) are well known examples. Its chemical and physical instability in aqueous medium (e.g., hydrolysis and oxidation of phospholipids, encapsulated solute leakage and vesicle aggregation) for long period of time is still a challenge, where freeze drying technique plays a crucial role for stabilizing the vesicular product [5]. Chonlada Charoenviriyakula also proved that freeze drying is an effective method to improve the stability of exosomes [6]. Further, freeze drying is also used to overcome the obstacles encountered during stabilizing live virus vaccines to fight against infectious diseases like Bacille Calmette Guerin (BCG), Smallpox, Chicken pox, Influenza, Polio, Tetanus and so on. There are many parameters like pH, temperature, suspension medium, contact to light, freezing, thawing, anti-microbial, and inactivating agent interrupt with the stability of vaccines in liquid state. However, it can be overcome by converting vaccines into a stable state through lyophilization [7]. Many pharmaceutical companies try to stabilize the live virus vaccines through altering formulation [8]. Stability and long term storage of proteins and DNA/RNA are also considerably improved through this technique [9]. Sometimes, protein undergo degradation during lyophilization process itself due to freezing, where physical environment of protein changes and leads to instability. Hence optimization of all process parameters in freeze drying is very crucial [10].

Freeze-drying holds various advantages over other different drying processes like drying at low temperature, shelf life of the product is high due to very low moisture content, the product properties which make it easy to reconstitute and convert into liquid dosage form, sterile product manufacturing throughout the process etc [7].

In this review, the three major steps of the process are briefly described. It seems simple, however careful optimization of each parameter is necessary for getting a product with required quality. There are many challenges observed during process optimization. Such challenges, the probable causes and the ways to overcome the same are discussed at a length. Further, lyophilization of various products, regulatory aspects and marketed status are also included.

2. LYOPHILIZATION PROCESS

In 1906, the frozen product was first dried with the help of vacuum. However, lyophilization was established by shackell in 1920, as a stabilizing process for heat-liable materials. This technique was used for the preservation of plasma during the second world war too [9].

Lyophilization is a critical method, in which the process is mainly divided into three stages: Freezing step, Primary drying step and Secondary drying step (Fig 1). In this technique, the substance is freezed, sublimated and at the end desorption takes place. The substance i.e. active ingredient with solvent is first frozen (ice formation) and then solvent is removed by sublimation (primary drying) followed by desorption (secondary drying) of the remaining solvent. Steps of freeze drying are discussed below:

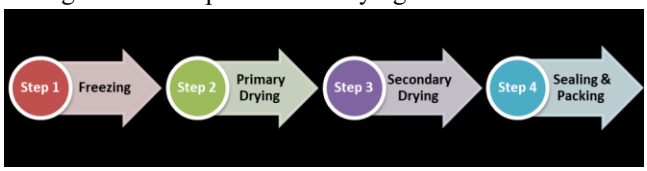

Figure 1. Steps in lyophilization.

2.1. Freezing.

Freezing is the first step where solvent, usually water, is been frozen. It is defined as the process of ice crystallization from super-cooled water. The freezing step initially involves the cooling of the solution until ice nucleation occurs. Ice crystals begin to grow at a certain rate, which results in formation of freeze concentration of the solution [11].

Freezing step is generally carried out at -40°C if Tg or Teu is higher than -38˚C otherwise 2˚C less than Tg or Teu is used. The freezing time depends on fill volume; larger fill volume takes more time to freeze. Usually freezing occurs in 1 h if fill volume is less than or equal to 1 cm [9].

The occurrence of this event takes place in the following steps:

2.1.1. Super-Cooling. The withholding of the liquid state below the equilibrium freezing point of the solution is entitled "Super-Cooling". It is important to know that there are two types of super-cooling: Global super-cooling (complete solution is homogeneously super-cooled) and local super-cooling (only a portion of the liquid is super-cooled). Slow cooling rate generally leads to global super-cooling, while local super-cooling is observed above a critical cooling rate [12]. The degree of super-cooling control the rate of nucleation and so determines the shape and number of ice crystals formed, which directly affects the porosity of the freeze-dried cake [13]. When high degree of super-cooling is performed, irregular needle shaped many small ice crystals or dendrites are observed when water molecule arranges itself randomly around ice nuclei, whereas in low degree of super-cooling, regular hexagonal few ice crystals called dendrites are formed when water molecule arranges itself systematically around ice nuclei [14]. The number of ice crystals increases, the pore size decreases and thus, primary drying time increases. Therefore, the effect of degree of super-cooling is both remarkable and variable on drying step [13].

2.1.2. Ice Nucleation. In the super cooled liquid water, density variation from Brownian motion was observed, due to which molecules of water form clusters with long living hydrogen bond with similar molecular arrangement as in ice crystal [3]. The appearance of initial nuclei is called primary nucleation and when growth of nuclei continue with definite velocity is called as secondary nucleation [12]. The onset of ice nucleation is a random event and is dependent on factors like the degree of super-cooling, the solution properties and process conditions [15]. Ice nucleation can be controlled in order to form larger pores which apparently minimize the primary drying time up to 55% [16]. Whereas uncontrolled or random nucleation results into longer cycle and higher cost [15]. R. Geidobler proved that controlled nucleation plays a crucial role in freezing step and can be induced through different techniques like vacuum induced nucleation, ice fog technique, ultrasound technique, agitation induce nucleation, gap freezing and so on [12].

In vacuum induce technique, the pressure within the chamber is reduced for a short time, during freezing. This reduction in pressure produces the partial evaporation of water, which causes a reduction in product temperature and promotes the nucleation of ice [17]. Irene Oddon studied that this technique precisely control the nucleation temperature and promotes the reduction of total drying time [16].

In the ice fog method, the vials are initially cooled to the temperature below the equilibrium freezing point and the pressure is reduced to around 50 Torr. Cold nitrogen gas is then introduced through a liquid nitrogen heat exchanger into the chamber. The cold gas in the chamber forms an ice fog, which is enforced into the vials to seed ice crystallization in the super-cooled solution. In short, ice fog originates "seed" crystals that are in the vials creating the "nucleus" around which ice crystals form during nucleation [18].

Nakagawa et al have shown that ultrasound technique is possible in glass vials by connecting an ultrasound generator and transducer to a temperature controllable aluminum plate on which the vials were placed. The vials are given ultrasonic wave for only 1 or 2 seconds at a fixed temperature using silicon oil as good connector between vials and plate. Anyway, up-scaling of this ice nucleation technique is quite onerous [19].

Agitation induce nucleation can be performed by connecting the shelf to the hydraulic unit of the freeze dryer which is mounted on swing-metal connections on top of the product chamber. A vibrator agitates the whole shelf via the hydraulic piston and the vibrator can be adjusted in frequency. Until now, there are no reports on this theory and the equipment, including vibrator and swing metal connector as this modification is quite expensive [12].

Gap freezing is the technique where gap is created by keeping spacer between tray and lower shelf. The purpose of the gap is to control the heat conduction from the shelf surface to vials by separating the shelf and the tray a certain distance. The objective of this arrangement is to freezes the sample from the bottom to top which avoids formation of a highly concentrated amorphous layer on top of the vial [20].

2.1.3. Ice Crystal Growth. Once the mass of ice nuclei is formed, crystallization of ice occurs in the system which leads to development of stable ice crystals. As soon as, the stable ice crystals are formed, their growth continues to increase by the

addition of molecule to the interface. In crystallization process, the product temperature accelerates quickly to near the equilibrium freezing point. Additional heat is removed from the solution after the primary ice crystals are formed by further cooling and the residual solvent freezes as the ice crystals grows [11].

Annealing is the process which can help to modify the ice crystal growth. However, it is not necessarily applied in all types of formulations. The frozen formulation is kept above the freezing temperature for a defined time is known as "Annealing". The temperature of annealing should be between melting temperature and glass transition temperature, but hold time to the particular temperature can vary depending upon the formulation and heating rate. The studies showed that annealing directly affects the particle size distribution of the ice crystals and thus primary drying time [21].

2.2. Primary Drying.

In primary drying, sublimation of ice crystals takes place with the help of various pressure and temperature conditions. According to phase diagram of water, sublimation of water takes place only if the temperature and vapor pressure are below the triple point of the water, that is, below 611.73 Pa and 0.01˚C respectively (Fig 2)[22]. However, presence of excipient modifies the pressure and temperature required for sublimation. The sublimation will starts from surface of the sample and continues towards the bottom. Thus, thickness of sample also affects the time require for completion of drying process [23]. Mostly, 95% of the water is sublimed during primary drying process.

The major factor which plays a vital role during this step is "collapse temperature/glass transition temperature and eutectic melting temperature". The temperature of the product above the critical formulation temperature is called as Eutectic Melting Temperature Teu, for crystalline material and Tc or Tg for amorphous material. Drying temperature above Tc/Tg/Teu leads to loss of cake structure. Thus, for this reason, optimization of pressure and temperature conditions of the primary drying step is important.

In addition to optimization of drying conditions, the determination of end point for the primary drying process is also essential. Sandip Khairnar has discussed in detail about end point determination by various methods like Comparative pressure measurement, Dew point monitor, Process water concentration from tunable diode laser absorption spectroscopy, Lyotrack, Product thermocouple response, Condenser pressure, Pressure rise test etc.

2.3. Secondary Drying.

In secondary drying step, water desorption occurs at 20°C to 40°C temperature. Moderate ramp rate i.e. from 0.1°C/min to 0.3°C/min should be kept in secondary drying step to avoid surpassing the Tg of the freeze dried cake and pertaining cake collapse. Secondary drying, is the step where fall in moisture content is obtained within the freeze dried cake to less than 1% [24]. After the product is been freeze dried, packing is done in the vials/containers with rubber stopper to constrain re-absorption of water molecule or oxygen from the atmosphere. Thus drugs that are unstable/less stable/degradable/decomposable in the presence of oxygen or moisture are been converted into dried stable powder by freeze drying [10].

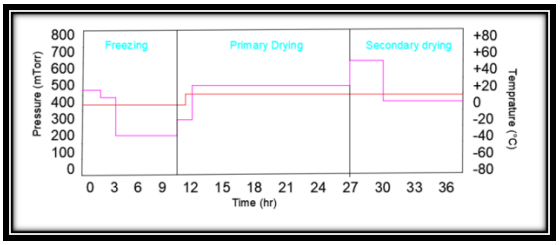

Figure 2. The processing conditions during various stages of freeze drying (temperature, vacuum).

3. IN-PROCESS CHALLENGES

The main objective to develop lyophilized cycle for any pharmaceutical formulation is to achieve compatible, stable and acceptable product. Final product with low moisture content and minimum reconstitution time is considered to be the ideal features for any lyophilized product. Development of cycle is totally based on trial-and-error of main parameters that are affecting the final product characteristics. During trial-and-error method, many challenges are faced which may directly or indirectly affects the moisture content, reconstitution time, cake appearance and stability (Fig 3). These may be due to improper freezing time/temperature, dying time/temperature or ramp rate. Collapse, shrinkage, melting, powder ejection etc. might also observed due to improper processing. Some of the challenges regarding process parameters are discussed below:

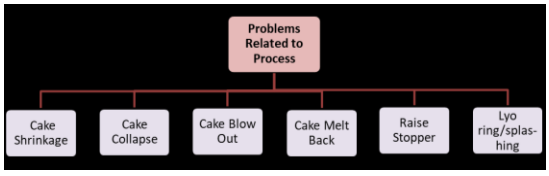

Figure 3. Challenges in process parameters.

3.1. Shrinkage and Cracking.

The cake is detached from the walls and bottom of the vial when the unfrozen water in the frozen matrix dries and creates stress which results in shrinkage or cracking of the cake (Fig 4 A). The cake gets separated from the bottom when inverted due to release of stress [25]. However, there is an opposite relation between shrinkage and cracking. The drying stress which was built up by removal of water, get relax, is known as shrinkage which is correlated with cracking. If shrinkage is prevented, then more cracking is seen, to relax the drying tension. The addition of higher disaccharide concentration, may reduce shrinkage and increase cracking [26]. Shrinkage and cracking may also be affected by fill volume. Higher cracking was seen in 10R vials than 2R vials with constant fill volume [25]. Srivalli Telikepalli studied that shipping stress can play a major role in breakage of the cake [27]. To prevent this defect product temperature can be kept lower in drying step and cycle optimization should be done with trial-and-error method. However, this defect does not significantly alter product characteristics [25].

3.2. Cake Collapse.

Collapse of the powder is observed when viscosity of amorphous phase decreases, due to temperature exceeding above Tg in primary drying step. Micro-collapse takes place at small scale, which leads to an increase in pore size of powder, ultimately, rise in sublimation rate and drops the primary drying time. Macro-collapse comparatively, affects primary drying

negatively, as it constrains sublimation rate [28]. The specific surface area (SSA) reduces due to loss of porous structure i.e. cake collapse and these lead to slow rate of desorption during secondary drying [29]. However, a few scientists reported that stability of protein is not affected by collapse of cake.[30,31,32]. While some reports prove that reconstitution time and moisture content are also not majorly affected by this phenomenon. Even though there are not many publication on negative effect of collapse cake on stability, this type of products are not preferred to be marketed [29]. Freeze dry microscopy, thermal analysis and electric resistance measurement are techniques to determine collapse temperature [33]. Cryoprotectant is widely used to prevent cake collapse by increasing freezing temperature [34]. In conclusion, the product performance is insignificantly affected by cake collapse but it increases secondary drying time and the appearance of product is unacceptable as shown in Fig 4 B.

3.3. Melt Back.

Melt back is the condition where melting of frozen ice occurs during freeze drying. Collapse and melt back are interlinked terms. Melt back is observed when residual ice melts upon rising temperature to 25°C.[35] It is mostly seen at the end of primary drying step or at starting of secondary drying step. Melt back defect is observed due to the improper formulation and processing parameters. This defect is not accepted because it directly affects the quality and appearance of the final freeze dried product (Fig 4 C) [25]. It can be eliminated by formulation and freeze drying cycle optimization using suitable design of experiment.

3.4. Lyo Ring or Splashing.

Lyo ring or splashing in the formation of ring slightly above the cake or on the neck/shoulder of the vial as shown in Fig 4 D. Splashing is not considered to be a serious defect because it can easily be eliminated by proper handling of vials before freeze drying. After manufacturing of the formulation, during filling process some liquid may adhere surface of the vials. The lyo ring is observed in freeze dried products due to surface adhered liquid. Thus, it totally depends on the filling setup and design which affects the residual product around the neck of vial. Another reason, may be the agitation during transportation of the vials to the lyophilizing chamber which results in formation of lyo ring after freeze drying [36]. Therefore, filling and transportation of vials should be done appropriately to avoid splashing or lyo ring.

3.5. Blow Out.

Blow out is the defect where the formulation during primary drying step ejects out of the vial (Fig 4 E). Blown powder might be totally dried or partially dried which adhere to the neck or shoulder area of the vial. The freeze-dried powder from the co-solvent system is much less cohesive than the powder obtained from freeze-drying from water. Particles start escaping from the surface, which results in powder ejection from vials [37]. There might be many reasons for blow out such as sudden vacuum application, smaller vial size, higher fill volume and may be due to instrumental error. As fill volume is more and vials are small, chances of powder ejections are high. Deep jyoti Das proved that drying of the powder too quickly with high vacuum lead to blowing out [38]. Formulation containing organic solvents are more prone to powder ejection from the vial.[39] Fly off from vial is considered to be the major defect, which may directly affect the drug content, reconstitution time, moisture content, stability and eventually the quality of the product. Hence, blown out should be completely impeded by optimization of freeze drying cycle.

3.6. Raised Stopper.

Raised stopper may directly affect the level of moisture content in the final product which leads to alteration of product quality. Hence, vials with raised stopper after final stoppering is mostly rejected. When the design of vials and stopper is not proper then it may pop out of the vial after final stoppering. It also may go off the vial when stoppering lead moves in the upward direction. During cycle optimization, this challenge should be understood well and must be overcome.

(A) (B) (C) (D) (E)

Figure 4. Image A is shrinkage, image B is collapse, image C is melting back, image D is formation of lyo ring, and image E is blow out.

4. FREEZE DRYING OF VARIOUS FORMULATIONS

Over the last few decades, freeze drying technique is having tremendous usage in stabilizing various formulations like Nanoparticles, Liposomes, Viruses, Vaccines, Proteins, etc. Lyophilization technique increases stability of many drugs like Acyclovir, Mycamin, Azithromycin, Vancomycin Hydrochloride, Pantoprazole, and so on. This method is used to manufacture stable and acceptable product with long term stability. In addition to process related challenges as discussed, the optimization of formulation is also very crucial. Therefore, the challenges encountered in lyophilization of different formulation with the remedies to overcome them are discussed at a length.

4.1. Small Molecules.

Small molecules are those that are having low molecular weight and small size. Some of the formulations such as Nanoparticles, Nanocapsules, Nanosphere, Microsphere, Liposomes, Niosomes, Exosomes, Aquasomes for different drug require lyophilization.

Particles of size in nano scale are prepared for various reasons to like to improve bioavailability, for targeting, to improve stability of different drug molecules [40]. Particle size reduction increases surface free energy which result in aggregation and destabilization of nanoparticals in suspension. Aggregation/fusion, sedimentation or creaming, crystal growth, change in crystal state are the major reason behind instability of nanoparticle in aqueous solvent [41]. Hence, to improve the long term stability of nanoparticles, colloidal suspension is converted into dry powder form using lyophilization. During freeze drying, crystallization of ice produces stress which may destabilize the formulation. Thus,

many data are published where cryoprotectants are added in suspension itself to preserve the matrix [4]. Research also proves that cryo/lyo-protectant directly affects aggregation, moisture content, particle size, reconstitution time and appearance [42, 43]. Commonly used cryoprotectants are fructose, sucrose, dextran, trehalose, glucose, PEG-2000, PEG-10,000, lactose, mannitol, sorbitol, from which trehalose and sucrose are most widely used [40]. However, Abdulaziz Almalik shows that PEG-2000, PEG-10,000 and mannitol take longer reconstitution time and increase particle size when coated on nanoparticles. Excess amount of cryoprotectant may shows agglomeration and therefore, type and quantity of cryoprotectant should be considered and optimized well [44]

Vesicular drug delivery systems are minute spherical vesicles made up of phospholipids bound with water molecule and encapsulated with drug. However, many challenges like storage of vesicles in cool temperature, leakage of drug at room temperature, aggregation/fusion of vesicles, and other physical and chemical problems, they are lyophilized. During freeze drying, cycle is optimized such a way that lipid bilayer does not damage and remains intact during freezing step. Adding optimum cryoprotectant is mandatory to maintain its matrix throughout the process. Cryoprotectant likes sugars, will replace the water molecule by breaking the bond between water and phospholipids. This theory is known as water replacement theory. As per another vitrification model, glassy solid of saccharide trap the liposomes and ultimately increase the stability [5,45]. Moreover, hydroxypropyl-β-cyclodextrin (HPβCD), a cyclic oligosaccharide is also been used as cryoprotectant having higher Tg to stabilize vesicles during freeze drying. HPβCD has shown tremendous stabilization of PEGylated liposomes in both spray drying as well as freeze drying [46]. However, van den Hoven JM reported that liposomes produced by freeze drying and spray drying process were stable [47].

Generally, co-solvent is used to increase solubility and stability of certain active pharmaceutical ingredient. Some of the solvents such as ethanol, acetone, dimethyl sulfoxide (DMSO) modify the shape and size of the crystal formed in freezing and ultimately boost the primary drying time. But, level of co-solvents in the system is the major concern due to its toxicological effect in body. And hence, United States Pharmacopeia (USP) and International Conference on Harmonization (ICH) have specified their maximum concentration of residual solvent to be used for any formulation. Primary drying condition and annealing are effective methods to reduce level of residual solvent. But, secondary drying step is not suggested to decrease the solvent level because, at a particular point, moisture content is so low that increasing temperature does not affect the solvent since the solvent is trapped in micro region of freeze dried solids rather than adsorbed on the surface [48,49,50].

4.2. Large Molecule/Biopharmaceuticals.

Paradigm shift is observed in the treatment of many diseases using biopharmaceuticals and biosimilars instead of small molecules. Many proteins, peptides, hormones, vaccines etc are administered through parenteral route. Majority of these molecules are unstable in liquid state and hence, are converted to dry powder using freeze drying process. However, selection of suitable cryoprotectant and careful optimization of freeze drying cycle can only produce a stable product.

4.2.1. Proteins and Peptides. Proteins and peptides are large biomolecules made up of amino acids having very low physical and chemical stability due to which they are only administered through injection. They are temperature sensitive and are only stable at a definite temperature. At high temperature, the denature/unfold of protein occurs and at lower temperature cold denaturation takes place.[51] Stress during freezing and drying, results in denaturation of protein and hence, stabilizer/ cryoprotectant is preferred to preserve it from stress. Thus, selection of appropriate cryo/lyo-protectant is required [52]. Sumit Luthra investigated that addition of sucrose completely stabilize the Lactate Dehydrogenase protein using mini freeze dryer [53]. Fonte et.al., compared the protein-loaded PLGA nanoparticles with and without cryoprotectant and concluded that adding lyoprotectant increases the stability of the formulation. They also studied the importance of annealing to decrease primary drying time and reported that it reduced cycle time by 26% [54].

4.2.2. Vaccines. Vaccines are mostly stored at low temperature to augment its shelf life. There may be several factors like pH, exposure to light, temperature, aqueous medium directly affects the long term stability of vaccines. Therefore, converting liquid vaccines into freeze dried products enhances thermal stability during storage and transportation and ultimately the shelf life of the formulation. Laurent Hansen studied the effect of temperature using accelerated stability testing of freeze dried vaccines. They concluded that such vaccines are mostly unstable at higher temperatures and moisture content which influence stability and undoubtedly the quality of the product [55]. Shouvik Roy studied the effect of pH on stability of botulinum neurotoxin, serotype A vaccines and proved that trehalose as cryoprotectant with pH 8 is optimum for stability [56]. However, Sorbitol as cryoprotectant is suggested during lyophilization of several viruses like varicella-zoster virus, herpes simplex virus, yellow fever vaccines and respiratory syncytial virus [57]. Kumar Jha et.al., experimented and concluded that lyophilized product of adenovirus infectivity can be retained over 6 months at room temperature as well as at 4˚C. However, viral load increased at 4˚C after 6 months storage.[58]

4.2.3. Blood products. The freeze dried plasma has been used since world war II however freeze drying of red blood cells, platelets, fibrinogen etc are also investigated. Lyophilization method obliterates the use of cold storage of red blood cell and provide the extended shelf life. In civilian setting the blood was stored in citrate phosphate dextran anticoagulant to prevent aggregation of RBC. However, nowadays, freeze drying using cryoprotectant, majorly trehalose, is in use which can eliminate need of low temperature storage.

Platelets are extremely sensitive to low temperature storage. They change their shape, increase intracellular calcium and secrete alpha granules when stored below 20˚C. Preservatives such as Me_2SO, hydroxyethyl starch, glycerol-glucose are been used, from which Me_2SO is superior in preservation but has poor in-vivo recovery and high clearance. Trehalose is also used as cryoprotectant for lyophilization of platelets as it replaces water and makes product stable.

Freeze dried fibrinogen is been evaluated predominantly in cardiac surgery but unfortunately, the use of lyophilized fibrinogen does not result in noteworthy difference when compared to conventional treatment of cardiopulmonary bypass [59,60].

5. MARKETED FORMULATIONS

The freeze drying process is extensively explored to stabilize variety of products. Currently, more and more applications of freeze drying are seen in biopharmaceuticals. In 1998, 11.9% lyophilized products were marketed but presently, half of the marketed products are been lyophilized. Pharmaceuticals, dairy industry, food industry, neutraceuticals and many other fields have employed freeze drying techniques. Fig 5 shows the general applications of freeze drying technique in such fields and Table 1 includes various marketed products.

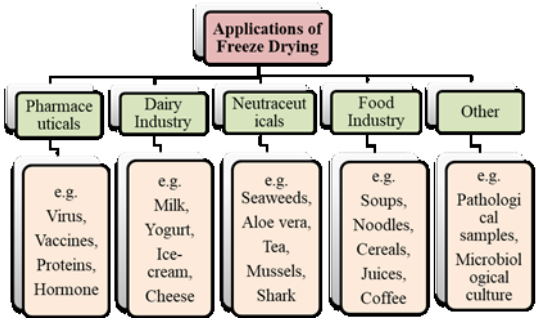

Figure 5. Applications of freeze drying in various fields

Table 1. Lyophilized marketed formulations.

Sr no.	Drug	Brand name	Manufacturer	Use
1	Paclitexel	Taxol	Pfizer	Anti-cancer
2	Pantoprazole	Protonix	Takeda	Proton pump inhibitor
3	Vancomycine Hydrochloride	Vancocin	Mylan	Antibiotic
4	Chlorthiazide	Diuril	Merck	Diuretic
5	Thiopental sodium	Pentothal sodium	Hospira	Anesthetic agent
6	Cisplastin	Platinol	Bristol Myers Oncology	Anti-neoplastin
7	Acyclovir	Zovirax	Bedford Pharmaceuticals	Anti-viral
8	Pemetrexed	Alimta	Eli Lilly & Co. Ltd	Anti-cancer
9	Tigecycline	Tygacil	Amneal Biosciences Llc	Antibiotic
10	Ceftazidime	Fortaz	Pfizer	Antibiotic

6. REGULATORY ASPECTS

The freeze drying is explored more and more in the various fields such as food industries, pharmaceutical industries, neutraceuticals, dairy industries and so on. Yet majority of the regulatory bodies have limited experience with the review and approval of freeze dried products specially biopharmaceuticals. The in depth understanding of the formulation and processing parameters can decrease the concerns raised by the regulatory bodies. Regulatory approvals are primarily based on quality of the product, effectiveness and safety. With the growing concern of lyophilization of different formulation, U.S. Food and Drug Administration (USFDA) published a guiding document in which they have discussed problems during manufacturing and controlling it during the freeze drying process. To maintain sterility is one of the major difficulty experienced during lyophilization. In certain old equipment, stoppering may be done manually rather than using machine. Due to which risk of contamination in the final product is much greater. Additionally, filling operation and transportation of filled vials should carefully be done to avoid content variation as well as contamination. Hence, validation of this operation is suggested through media filling. Further, different techniques are used for sterilization of dryer like gas sterilization, moist heat under pressure sterilization or through chemical treatment. As per USFDA document, the moist heat under pressure is considered as superior method over other two. Further, USFDA released various guidelines like Process Analytical Technology (PAT) and Quality by Design (QbD) to build quality in product. Hence, for meeting up the level of quality, moisture content, assay, reconstitution time, appearance, good pharmaceutical practice is mandatory [61].

7. ADVANCES IN FREEZE DRYING TECHNIQUE.

Recently, Spray freeze drying (SFD) technique is explore where a combination of both techniques spray drying and freeze drying is implicated. In SFD, a formulation or solution is atomized and converted into droplet, get solidified by cold fluid and sublimated at low temperature and pressure. S. Padma Ishwarya studied that there are different technique for atomization such as one fluid (hydraulic), two fluid (pneumatic), or ultrasonic nozzles which directly affects the size of particle which is not possible in freeze drying. A number of various methods have been established for spray freezing which is spray freezing into vapour, spray freezing into vapour over liquid, spray freezing into liquid etc. SFD has list of advantages like decreases drying time, percentage oxidation and has good oxidative stability with good rehydration property. In contrast, encapsulation efficiency and cost is the key snag of SFD. Protein powder, coffee powder, egg albumin, bovine DNAse drugs like, Carbamazepin, Danazole, Trypsinogen, Albuterol sulphate, Ciprofloxacin etc. were manufactured by SFD [62,63,64].

As a part of PAT, the freeze drying process was modeled using various approaches to minimize the processing time without deteriorating product quality. El-Maghlany et al., compared 1D (thickness), 2D (thickness and width) and 3D (thickness, width, and depth) models for radiation-based heating methods at different aspect ratio and compared with microwave heating method. The drying time is reduced at a lower aspect ratio in case of radiation assisted heating model. They concluded that complex 3D models

are mandatory at lower aspect ratio, however, simple 1D and 2D models are efficient at a higher aspect ratio (>10) for radiation heating method while it is immaterial for microwave heating method [65].

8. CONCLUSION

The lyophilization technique is widely explored to develop stable injectable dosage form with optimal moisture content, assay and rapid reconstitution for thermolabile drugs. However, the process is very critical and requires careful optimization to get desired quality product. Several difficulties like shrinkage, collapse, melt back, lyo ring, and blow out is been encountered during the process. This article briefs the troubleshooting of these challenges. The cryoprotectant used for drying of small molecules as well as biopharmaceuticals plays an important role in stabilization. Therefore, the correct understanding and knowledge about freeze drying is primary requirement to produce stable pharmaceutical lyophilized products.

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10. ACKNOWLEDGEMENTS

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