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LYOPHILIZATION TECHNOLOGY THE THEORY AND PRACTICE OF FREEZE-DRYING OF PHARMACEUTICALS

Pawar Amol R.[1]*, Dipti G. Phadtare[2], R.B.Saudagar[2]

1.Department of Quality Assurance Technique, R.G.Sapkal College of Pharmacy Anjaneri, Nasik, 422003

2.Department of Pharmaceutical Chemistry, R.G.Sapkal College of Pharmacy Anjaneri, Nasik, 422003

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For Correspondence:

Pawar Amol R.

Department of Quality Assurance Technique, R.G.Sapkal College of Pharmacy Anjaneri, Nasik, 422003

E-mail:

amolpharma9@gmail.com

ABSTRACT

This review deals with the excipients used in various lyophilized formulations of the molecules. The role of excipients such as bulking agents, buffering agents, tonicity modifiers, antimicrobial agents, surfactants and co-solvents has been discussed. On 21st century, in pharmaceutical field lyophilization has become important subject to ongoing development and its expansion. This technology is developed for individual pharmaceutical products for preservation and to improve the stability of product during its storage condition. This review also focused on the recent advances and its targets in near future. At first, the principle, steps involved, formulation aspects and importance of lyophilization was explained.

INTRODUCTION:-[3,4]

Lyophilization or freeze drying is a process in which water is frozen, followed by its removal from the sample, initially by sublimation (primary drying) and then by desorption (secondary drying). Freezedrying is a process of drying in which water is sublimed from the product after it is frozen [3]. It is a drying process applicable to manufacture of certain pharmaceuticals and biologicals that are thermolabile or otherwise unstable in aqueous solutions for prolonged storage periods, but that are stable in the dry state. The term "lyophilization" describes a process to produce a product that "loves the dry state" [4].

Freeze-drying process:-

Freeze drying is mainly used to remove the water from sensitive products, mostly of biological origin, without damaging them, so they can be preserved easily, in a permanently storable state and be reconstituted simply by adding water [8]. Examples of freeze dried products are: antibiotics, bacteria, sera, vaccines, diagnostic medications, proteincontaining and biotechnological products, cells and

tissues, and chemicals. The product to be dried is frozen under atmospheric pressure. Then, in an initial drying phase referred to as primary drying, the water (in form of ice) is removed by sublimation; in the second phase, called secondary drying, it is removed by desorption. Freeze drying is carried out under vacuum[9].

For freeze drying? [2]

1. Low temperature drying process.
2. Compatible with aseptic/sterile processing.
3. To get amorphous form of drug desirable for solubility in freeze drying.
4. To stabilize highly degradable protein drugs.
5. To stabilize heat sensitive and chemically unstable solutions.
6. Low particulate contamination.

Desirable freeze drying characterstics [1]

1. Uniform color of products.
2. Sufficient drying of products.
3. Sufficient porosity of finally dried products.
4. Chemical stability of products.
5. Intact cake of products.
6. Sufficient strength in terms of assay pH.

Advantages [1]

- Enhanced product stability in a dry state.
- Ease of processing a liquid, simplifies aseptic handling.
- More compatible with sterile operations than dry powder filling.
- Heat labile costly drug product can be stabilized in dry form.
- Increases shelf life of product during storage condition Chemical decomposition is minimized.
- Removal of water without excessive heating.

Disadvantages of lyophilization [1]

- Increased handling and processing time.
- Volatile compounds may be removed by vacuum.
- Very costly technology increases the cost of drug product.
- Require monitoring of each and every step of lyophilization and time consuming process.
- Need for sterile diluents upon reconstitution.

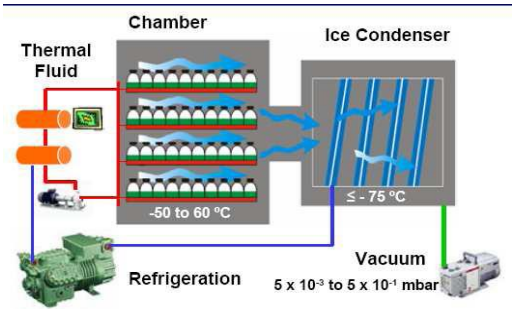
Reasons for Freeze Drying?

- Material chemically unstable in solution
- Low temperature drying process
- Compatible with protein pharmaceuticals
- The amorphous form of the drug is desirable (i.e., solubility)
- Low particulate contamination
- Compatible with aseptic/sterile processing

Lyophilizer

Fig: A benchtop freeze dryer, front view[14].

Freeze Dryer Design:-



Essential Components:-

Chamber:-

This is the vacuum tight box, sometimes called the lyophilization chamber or cabinet. The chamber contains shelf or shelves for processing product. The chamber can also fit with a stoppering system. It is typically made of stainless steel and usually highly polished on the inside and insulated and clad on the outside [37]. The door locking arrangement by a hydraulic or electric motor.

Shelves:-

A small research freeze dryer may have only one shelf but all others will have several. The shelf design is made more complicated because of the several functions it has to perform. The shelf act as a heat exchanger, removing energy from the product during freezing, and supplying energy to the product during the primary and secondary drying segments of the freeze drying cycle. The shelves will be connected to the silicone oil system through either fixed or flexible hoses. Shelves can be manufactured in sizes up to 4 m2 in area [38].

Process Condenser:-

The process condenser is sometimes referred as just the condenser or the cold trap. It is designed to trap the solvent, which is usually water, during the drying process. The process condenser will consist of coils or sometimes plates which are refrigerated to allow temperature. These refrigerated coils or plates may be in a vessel separate to the chamber, or they could be located within the same chamber as the shelves. Hence there is designation "external condenser" and "internal condenser". Physically, the external condenser is traditionally placed behind the chamber, but it may be at the side, below or above [39]. The position of the condenser does not affect trapping performance. For an internal condenser the refrigerated coils or plates are placed beneath the shelves on smaller machines, and behind the shelves on larger machines, but again there is no performance constraint, only the geometry of the chamber.

Shelf fluid system:-

The freeze-drying process requires that the product is first frozen and then energy in the form of heat is applied throughout the drying phases of the cycle. This energy exchange is traditionally done bycirculating a fluid through the shelves at a desired temperature [41]. The temperature is set in an external heat exchange system consisting of cooling heat exchangers and an electrical heater. The fluid circulated is normally silicone oil. This will be pumped around the circuit at a low pressure in a sealed circuit by means of a pump.

Refrigeration system:-

The product to be freeze dried is either frozen before into the dryer or frozen whilst on the shelves. A considerable amount of energy is neededto this duty. Compressors or sometimes-liquid nitrogen supplies the cooling energy. Most often multiply compressors are needed and the compressor may perform two duties, one to cool the shelves and the second to cool the process condenser.

Vacuum system:-

To remove solvent in a reasonable time, vacuum must be applied during the drying process. The vacuum level required will be typically in the range of 50 to 100μ bar. To achieve such a low vacuum, a two stage rotary vacuum pump is used. For large chambers, multiple pumps may be used.

Control system:-

Control may be entirely or usually fully automatic for production machines. The control elements required are as mentioned above, shelf temperature, pressure and time. A control program will set up these values as required by the product or the process. The time may vary from a few hours to several days. Other data such as a product temperatures and process condenser temperatures can also be recorded and logged [42].

The fundamental process steps [6,7]

1. Freezing : The product is frozen. This provides a necessary condition for low temperature drying.

2. Vacuum: After freezing, the product is placed under vacuum. This enables the frozen solvent in the product to vaporize without passing through the liquid phase, a process known as sublimation.

3. Heat: Heat is applied to frozen product to accelerate sublimation.

4.Condensation: Low temperature condenser plates remove the vaporized solvent from the vacuum chamber by converting it back to a solid. This completes the separation process [6]. Resulting product has a very large surface area thus promoting rapid dissolution of dried product [7].

Steps involved in Lyophilization cycle :-

A. Freezing:- Freezing of water into ice to produce a rigid frozen solute structure Solutes concentrate between ice crystals

B. Primary Drying:- Removal of ice via sublimation Product temperature less than Collapse temperature

C. Secondary Drying:-Remove adsorbed water Achieve moisture content needed for stability

Applications[33,34,35,36,40]

Pharmaceutical and biotechnology:- Pharmaceutical companies often use freeze-drying to increase the shelf life of products, such as vaccines and other injectables. By removing the water from the material and sealing the material in a vial, the material can be easily stored, shipped, and later reconstituted to its original form for injection.

Food Industry:-

Freeze-drying is used to preserve food and make it very lightweight. The process has been popularized in the forms of freeze-dried ice cream, an example of astronaut food.

Technological Industry:-

In chemical synthesis, products are often freezedried to make them more stable, or easier to dissolve in water for subsequent use. In bioseparations, freeze-drying can be used also as a late-stage purification procedure, because it can effectively remove solvents. Furthermore, it is capable of concentrating substances with low molecular weights that are too small to be removed by a filtration membrane.

Other Uses:-

Organizations such as the Document Conservation Laboratory at the United States
National Archives and Records Administration (NARA) have done studies on freeze-drying as a recovery method of water-damaged books and documents. In bacteriology freeze-drying is used to conserve special strains.

Traditional Lyophilization Technology:-

Traditional lyophilization is a complex process that requires a careful balancing of product, equipment, and processing techniques. For nearly 30 years, lyophilization has been used to stabilize many types of chemical components. In their liquid form, many such biochemicals and chemical reagents are unstable, biologically and chemically active, temperature sensitive, and chemically reactive with one another. Because of these characteristics, the chemicals may have a very short shelf life, may need to be refrigerated, or may degrade unless stabilized. When performed properly, the process of lyophilization solves these problems by putting reagents into a state of suspended activity. Lyophilization gives unstable chemical solutions a long shelf life when they are stored at room temperature. The process gives product excellent solubility characteristics, allowing for rapid reconstitution. Heat- and moisture-sensitive compounds retain their viability. Proteins do not denature during the process, and bacterial growth and enzyme action, which normally occur in aqueous preparations, can be eliminated. Thus, lyophilization ensures maximum retention of biological and chemical purity.

A. Freezing:-

Freezing is the first step of a freeze drying process, and the performance of the overall freeze drying process depends significantly on this stage. At the end of the freezing step, about 65%-90% of the initial moisture is in the frozen state and the rest remains at the adsorbed state in many cases [10].The freezing temperature, freezing rate and supercooling degree are all important factors influencing the overall drying time and product quality. Based on the physical and chemical properties of material, the freezing protocol can be optimized to produce the most favorable freeze drying results in terms of both high product quality and short drying time [11]. The characteristics of the frozen matrix strongly affect drying rates at primary and secondary stages [12]. It is generally accepted that the liquid material being frozen displays one of two different types of freezing behaviors as shown in Fig. 3: the liquid phase suddenly solidifies (eutectic formation) at a tempera temperature depending on the nature of solids in solutions, or the liquid phase does not solidify (glass formation), but rather it becomes more and more viscous until it finally takes the form of a very stiff substance, and becomes a highly viscous liquid [10].

B.Primary Drying:-

After the freezing stage, drying chamber is evacuated and the chamber pressure is reduced to a value that allows the ice sublimation to take place. This signifies the start of primary drying stage. Selection of the chamber pressure depends on the ultimate frozen temperature of the material. The pressure must be lower than the vapor pressure of ice in order for sublimation to take place. It was recommended that a reasonable chamber pressure during primary drying should be no more than about one-half and not less than about one-fourth of the vapor pressure of ice at the desired product temperature [13, 20]. As ice crystals sublimate, the sublimation front (sublimation interface), which starts on the outside surface, gradually recedes to the material, and a porous layer of the material remains after front advancing. The heat used for sublimation can be conducted through this dried layer to the sublimation front. The sublimated vapor travels by diffusion and convective flow through the porous layer and enters the drying chamber of freeze dryer. Since water sublimation requires a significant amount of heat, the temperature of material is usually further reduced. The primary goal of research on freeze drying is to improve process economics by reducing processing time. An important objective is the determination of the drying rate-limiting factors. Heat transfer and mass transfer are the two most likely rate-controlling factors. They depend on operating parameters such as temperature and pressure.

C. Secondary Drying:-

The secondary drying stage involves the removal of bound water by desorption. The amount of bound water remained is about 10%-35% of the total moisture content [10]. The effect of the bound water on the drying rate and overall drying time is significant. The time required to remove the bound water could be as long as or longer than the time spent for removal of the free water. The governing relation of moisture in porous media during the secondary drying is the adsorption-desorption equilibrium. It depends not only on temperature, but also on moisture content [22,23]. Fakes and co-workers described the moisture sorption behavior of mannitol, anhydrolactase, sucrose, D-(+)-trehalose, dextran 40 and providone (PVP K24) before and after freeze drying in a 10% solution [24]. The moisture content of dried product at the end of the secondary drying stage depends on the user requirement or the storage life of products with an acceptable product quality.The bound water is considered to be either bound at the surface of the crystals in a crystalline product or embedded in the highly viscous amorphous matrix [25]. The secondary drying stage for crystalline products may be shortened because higher drying temperature can be applied without the risk of damaging the product [20, 21]. In contrast, glassy products are limited in their drying rate at this stage by the slow molecular diffusion within the dried cake [15]. The amount of residual water may comprise 20% [26] or even up to 40% [26]. Therefore, it is very important to hold the product temperature well below its collapse temperature to prevent cake from deformation [14]. The shelf temperature for amorphous products should be increased slowly (0.1-0.15 °C·min−1), especially in the early stage when the moisture content is high. However, Pikal and Shah demonstrated that in the case of maintaining the shelf temperature at the same level as in primary drying for the first few hours, the glass transition temperature rises much faster than the product temperature [15]. This reveals that the shelf temperature during secondary drying should be as high as possible, which is normally in the range of 25 to 50 °C . It is not necessary to change the chamber pressure for secondary drying because the chamber pressure was found to have no measurable influence on drying rate [15]. A strong dependency of the secondary drying rate on the specific surface area is also described in literature similar to the primary drying section [26, 14]. Slow freezing produces large ice crystals with interstitial solid material of low specific surface. Hence, there is a fast drying rate during the primary stage, but conversely a decrease in drying rate during the secondary stage. This is consistent with the observation that the rate-limiting mass transfer process for drying an amorphous solid is either sublimation at the solid-vapor interface or diffusion of vapor within the solid

matrix. Thus the mass transfer will be affected by the specific surface area [14, 15]. A desorption process usually requires a raised ambient temperature or a much high vacuum pressure to promote because the amount of the bound water is strongly dependent on the ambient temperature, and the saturation vapor pressure of the bound water is closely related to the residual moisture content.

Freeze Drying Methods[16,17]

Three methods of freeze drying are commonly used

1) Manifold method:-

In the manifold method, flasks ampoules or vials are individually attached to the ports of a drying chamber. The product either frozen in a freezer, by direct submersion in a low temperature bath, or by shell freezing, depending on the nature of the product and the volume to be freeze dried. The pre frozen product is quickly attached to the drying chamber or manifold to prevent warming. The vacuum must be created in the product container quickly, and the operator relies on evaporative cooling to maintain the low temperature of the product. This procedure can only be used for relatively small volumes and product with high eutectic and collapse temperatures. Manifold drying has several advantages over batch tray drying. Since the vessels are attached to the manifold individually, each vial or flask has a direct path to the collector. This removes some of the competition for molecular space created in a batch system, and is most ideally realized in a cylindrical drying chamber where the distance from the collector to each product vessel is the same. Heat input can be affected by simply exposing the vessels to ambient temperature or via a circulating bath. For some products, where precise temperature control is required, manifold drying may not be suitable.

2) Batch method:-

In a batch drying, large numbers of similar sized vessels containing like product are placed together in a tray dryer. The product is usually pre frozen on the shelf of the tray dryer. Precise control of the product temperature and the amount of heat applied to the product during drying can be maintained. Generally all vials in the batch are treated during drying process, although some variation in the system can occur. Slight difference in heat input from the shelf can be expressed in different areas. Vials located in the front portion of the shelf may radiantly through the clear door. These slight variations can result in small difference in residual moisture. Batch drying allows closure of all vials in a lot at the same time, under the same atmospheric condition. The vials can be stoppered in a vacuum, or after back filing with inert gas [18]. Stoppering of all vials at the same time ensures a uniform environment in each

vial and uniform product stability during storage. Batch drying is used to prepare large numbers of ampoules or vials of one product and is commonly used in the pharmaceutical industry.

3) Bulk method:-

Bulk drying is generally carried out in a tray dryer like batch drying. However, the product is poured into a bulk pan and dried as a single unit. Although the product is spread through out the entire surface area of the shelf and may be the same thickness as product in vials, the lack of empty spaces within the product mass changes the rate of heat input. The heat input is limited primarily to that provided by contact with the shelf.

Issues Related to Improper Lyophilization Cycle:-

A. Melting of product

B. Shrunken freeze dried plug

C. Collapse product

D. Fly off

E. Cracking and breakage of vials

Components Needed for Lyophilization Development and Excipients In Lyophilized Formulation:-

For successful formulation and delivery of peptides and proteins, it is crucial that the formulation scientist has a thorough knowledge of several factors: how to optimize the physical and chemical stability of the active ingredient; how, rationally, to include specific excipients in the formulation; how to obtain the optimum conditions for stability; how to prevent stability issues during up-scaling; and, finally, how to design a formulation that is suitable for the route of administration, that is, one that allows the absorption barriers to be overcome. The choice of excipients is often based on previous experience, and on which excipients have been approved by the authorities. Excipients are chosen: to ensure that a certain function can be obtained, for example, controlled release; to ensure a successful end point for a preparation process, for example, allowing a dry powder to be obtained; to ensure that a liquid formulation remains at a constant pH value; or to stabilize the protein against a certain production-induced effect, for example, adsorption. However what may be useful for one protein can have detrimental effects on another. So, there is room and a need for guides to lead to the right choice of excipients. Excipients are added to formulations for several reasons, and some of them may have more than one effect or purpose for being part of the formulation (Wei Wang (2000))

Buffer:-[28,29,30]

Buffers are required in pharmaceutical formulations to stabilize pH. In the development of lyophilized formulations, the choice of buffer can be critical. Phosphate buffers, especially sodium phosphate, undergo drastic pH changes during freezing. A good approach is to use low concentrations of a buffer that undergoes minimal pH change during freezing such as citrate and histidine buffers. Lyophilized protein formulations often contain a buffering agent(s). Different proteins may need different buffering agents for maximum stabilization in solid state. The buffer concentration also influences storage stability of lyophilized proteins. (Wei Wang (2000). Buffer Selection.

- Low buffer concentration should be preferred
- A good target is 5 mM or less
- High concentrations can lead to pH shifts (crystallization) or low Tg's when they remain amorphous

Common buffers:-

- Phosphate
- Citrate
- Histidine .

Some buffers exhibit pH shifts during freezing; pH changes may occur in final reconstituted product also. Ex- Phosphate buffer, Glycine buffer, Tris buffer.

Bulking Agent:- [31,32]

The purpose of the bulking agent is to provide bulk to the formulation. This is important in cases in which very low concentrations of the active ingredient are used. Crystalline bulking agents produce an elegant cake structure with good mechanical properties. However, these materials oftren are ineffective in stabilizing products such as emulsions, proteins and liposomes but may be suitable for small chemical drugs and some peptides. If a crystalline phase is suitable,mannitol can be used. Sucrose or one of the other disaccharides can be used in a protein or liposome product. A crystallizing bulking agent(s) is usually needed in a solid protein formulation to have one or more of the following functions:

- To provide mechanical support of the final cake
- To improve product elegance
- To improve formulation dissolution
- To prevent product collapse and blowout

Stabilizer:-

In addition to being bulking agents, disaccharides form an amorphous sugar glass and have proven to be most effective in stabilizing products such as liposomes and proteins during

lyophilization. Sucrose and trehalose are inert and have been used in stabilizing liposome, protein, and virus formulations. Glucose, lactose, and maltose are reducing sugars and can be reduce proteins by means of the mallard reaction. The level of stabilization afforded by sugars or polyols generally depends on their concentrations. A concentration of 0.3 M has been suggested to be the minimum to achieve significant stabilization.

Choice of Stabilizers:-

Sugars or polyols have commonly been used to stabilize lyophilized proteins for long-term storage, such as

- Sucrose
- Trehalose
- Mannitol
- Sorbitol

i. Disaccharides:-

- Trehalose
- Sucrose
- Good for cryoprotection and dessicoprotection
- Typical levels of disaccharide: Proteins are at least 1:1 and often higher
- Avoid reducing sugars (i.e. lactose)

ii. Polymers:-

Stabilization of proteins by polymers can generally be attributed to one or more of these polymer properties: preferential exclusion, surface activity, steric hindrance of protein–protein interactions, and: or increased solution viscosity limiting protein structural movement. In recent years, additional properties of polymers have been implicated in stabilizing proteins during freeze-thawing and freeze-drying. Polymers such as dextran have been reported to stabilize proteins by raising the glass transition temperature of a protein formulation significantly and by inhibiting crystallization of small stabilizing excipients such as sucrose. PEG3350 or dextran T500 at 4% (w: w) has been found to inhibit a pH drop during freezing of a phosphate-buffered solution by inhibiting crystallization of disodium phosphate.

- Dextran
- PVP
- Good for cryoprotection–preferentially excluded
- Not typically sufficient for stabilization of proteins during drying –believed to be due to bulkiness and difficulty in H-bonding to proteins

iii. Non-Ionic Surfactants:-

The formation of ice-water interfaces during freezing may cause surface denaturation of proteins. Surfactants may drop the surface tension of protein solutions and reduce the driving

force of protein adsorption and: or aggregation at these interfaces. Low concentrations of nonionic surfactants are often sufficient to serve this purpose due to their relatively low critical micelle concentrations (CMC). Polysorbate 80 and 20

- Poloxamer 188
- Prevent denaturation of proteins at interfaces (i.e. ice-solute interface during freezing)
- May contain low levels of peroxides –best to keep concentration relatively low (0.1% (w/v) or less)
- Others Salts and amines have been used as cryoprotectants. Metal ions can protect certain proteins during lyophilization. Istonicity modifiers

Tonicity adusters:-

In several cases, an isotonic formulation might be required. The need for such a formulation may bedictated by either the stability requirements of the bulk solution or those for the route of administration. Excipients such as mannitol, sucrose, glycine, glycerol, and sodium chloride are good tonicity adjusters. Glycine can lower the glass transition temperature if it is maintained in the amorphous phase. Tonicity modifiers also can be included diluent rather than the formulation.

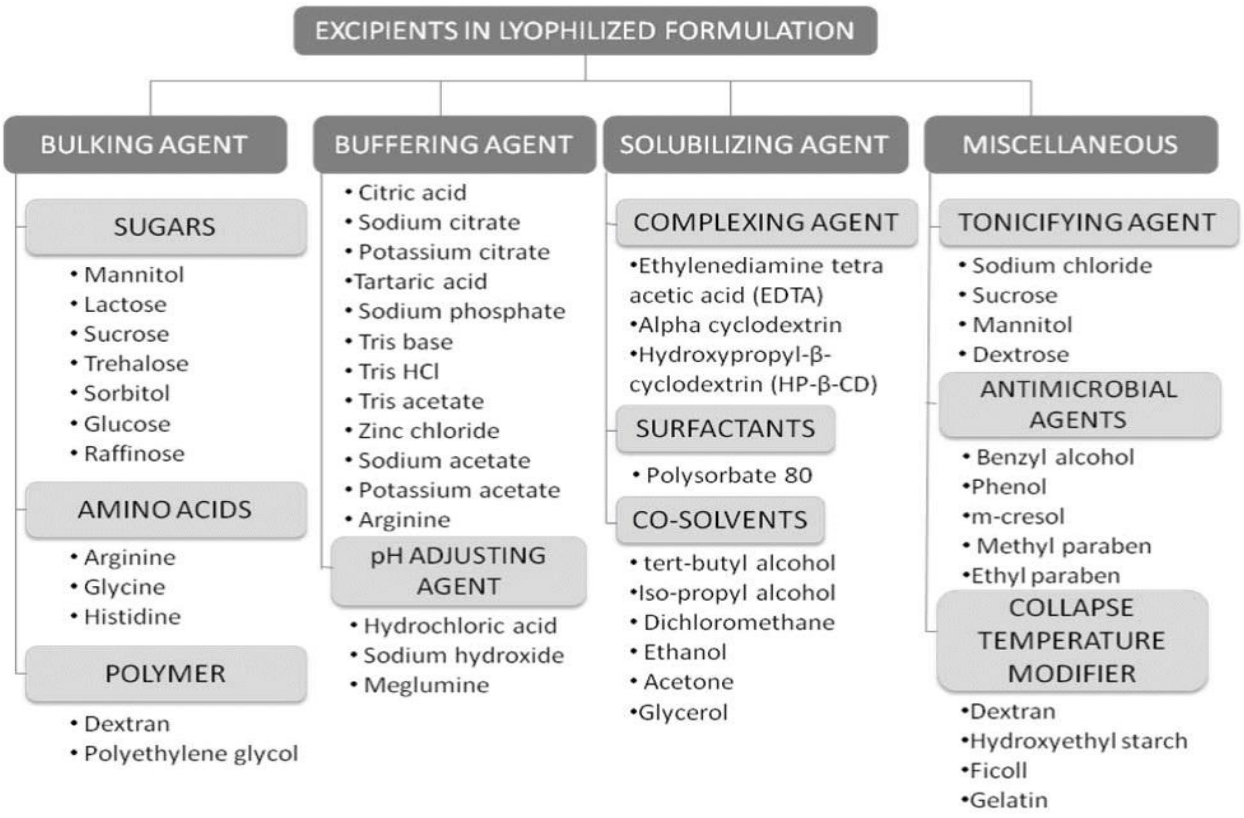


Figure 5 classification of commonly used excipients used in lyophilization of small molecules. A need based approach is utilized to select the appropriate excipient(s) for lyophilization.

Container closure system used in Lyophilization Technology:-

- **Vials** - for small lyophilized powder for injection generally tubular vials are used as it has greater heat transfer by contact conductivity, greater Kc value and these vials are clear transparent vial of lyo grade. For larger volume lyophilized powder for injection molded vials are used as these vials can tolerate higher vaccume pressure and heat transfer from the shelf. Type I glass is used. [27]
- **Rubber Stopper** - coated rubber stopper are generally used in lyophilization as they are non volatile in nature and prevent the drug product from contamination as in high vaccume pressure or by high heat volatile oil from rubber stopper may contaminate the product. This coating may be of Teflon, sulfur or other material compatible with the product. [27]

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