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REVIEW ARTICLE

Lyophilization: An Emerging Trend in Formulation of Parenterals

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ABSTRACT

Most often drugs in solution forms are susceptible to degradation and thus have reduced stability and shelf life. Gelsiation also known as Cryodessication or lyophilization is a well-established process that helps in improving the stability of labile pharmaceuticals. It is one of the recent emerging techniques for formulation of powder for injection. Many of the antibiotics, e.g., semi-synthetic penicillins, cephalosporins, doxycycline and chloramphenicol are manufactured by lyophilization process. Other drugs such as hydrocortisone sodium succinate, methylprednisolone sodium succinate and anti-cancer drugs are also formulated as lyophilized products. It involves sublimation of ice from the frozen product at low temperature and pressure resulting in a low water content which in turn helps in decelerating the physical, chemical and biological degradation reactions. Apart from enhancing the stability of the product, lyophilized formulations have several advantages, such as fast reconstitution, easy to handle during storage and shipping etc. However, the lyophilization of parenterals is not problem free. Rejection may be attributed to delay in reconstitution time, melt back or non-uniformity in color of the dried product. Since the process is cost intensive, optimization is important not only for saving the cost and time, but also to prevent rejection of the product. This article discusses design of lyophilization along with understanding the critical parameters that help in optimizing the process.

KEYWORDS

Lyophilization, Stability, Optimization, Reconstitution Time

INTRODUCTION

Lyophilization is a term coined by Rey. He attributed the name to the porous nature and the 'lyophil' character of the final product that enables easy reconstitution and restoration to solution form.[1] Lyophilization can be defined as a stabilizing process in which, the substance is first frozen and then the solvent is removed in two stages, first being sublimation (primary drying) and later desorption (secondary drying).

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The levels of solvents are reduced to such an extent where physical, chemical or biological reactions are not supported.[1]

The advantages of lyophilization include[2]

- Enhances stability of the product in dry state.
- Removal of solvent without excessive heating the product.
- Rapid and easy dissolution of the reconstituted product.
- Ease of handling and storage.

The disadvantages of this process are[2]

- Technical expertise and skill
- Cost and complexity of equipment.
- Increased handling and processing time.
- Need for sterile diluent upon reconstitution.

Applications[3]

The applications of lyophilization are not limited to the pharmaceutical industry. Lyophilization can be used for processing of food, ceramic industry and restoration of water damaged documents. In the pharmaceutical industry; lyophilization is used for the production of,

- Injectable dosage forms
- Solid oral dosage forms (Ex: Ondansetron-rapid orally dissolving tablets).
- Diagnostics.

Characteristics of a Lyophilized Product[4]

The nature of a lyophilized product is dependent on each step of the process. An ideal product is the one which has the following characters

- The original chemical or biological potency after reconstitution.
- Intact shape and size as that of the frozen product.
- Uniform colour and consistency.
- Freedom from contamination.
- Sufficient dryness to maintain stability.
- Sufficient porosity to permit rapid reconstitution.
- Reconstitution time (RCT) meeting specification limits.

Delayed reconstitution time, reduction in potency and formation of turbid solutions is an indication of improper lyophilization process or malfunction of the equipment.[1]

Methods of Lyophilization[5]

Depending on the type of product required and its final configuration, different methods of drying may be used, they include;

- Manifold drying
- Batch drying and
- Bulk drying

Manifold drying: in this technique, prefrozen vials are placed in flasks or attached individually to the ports of the drying chamber. This is generally used for products with small volumes and high collapse or eutectic temperatures. It is advantageous since each vial or flask has a direct connection to the collector. Thus, the drying is relatively faster compared to the other methods, increasing the drying efficiency. Since each flask or vial is attached individually, it can be removed when required without interrupting the other flasks.

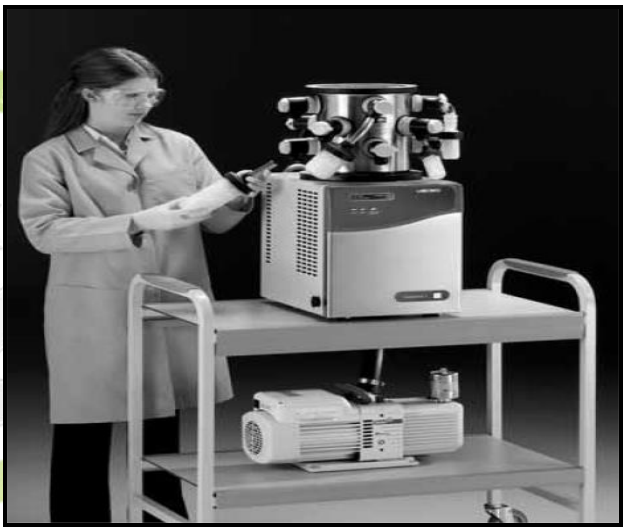

Figure 1: Manifold drying

Batch drying: This method is used for a large number of vials of similar size and containing the same product. It allows for efficient control of pressure and temperature. Since all the vials are exposed to similar environment, the variability between vials is minimized. Closure of the vials within the equipment after releasing vacuum and backfilling ensures sterility. This method is widely used in the industry.

Bulk drying: The product is poured as a single unit in a tray and placed in the tray dryer in this method of bulk drying. Uniform drying of the product does not occur. Also, the product does not lend itself to the aseptic sealing. The product is usually packed in air tight containers after

removal from the equipment. This process is used for those products that are not sensitive to oxygen or moisture.

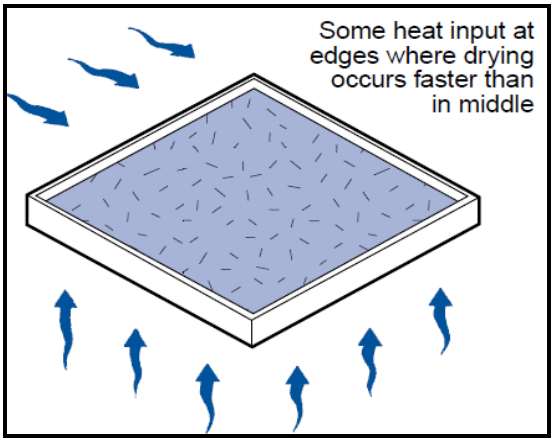

Figure 2: Bulk drying

Figure 3: Batch drying

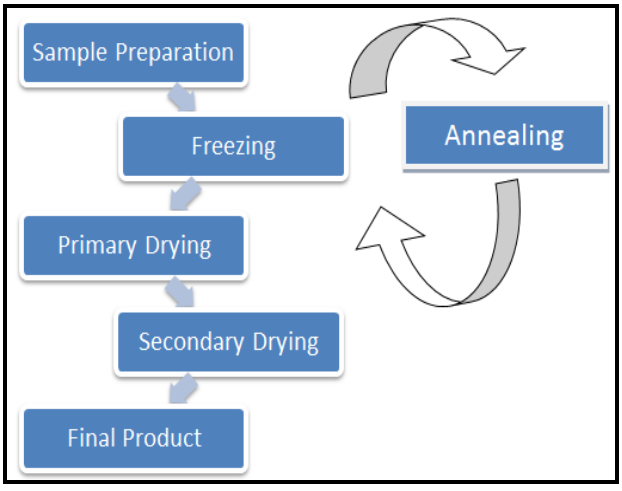

Figure 4: The Freeze Drying Process

Freezing

The freezing stage in lyophilization is simple by concept, but is the most complex and least understood step. The optimization and careful control of this stage determines the efficiency with which drying occurs and the type of product formed.

Freezing can be defined as an efficient desiccation step, where the solvent, usually water is separated from the solute so as to form ice.[7] The solute remains in the interstitial space between the ice crystals.[1] This ice is subsequently removed during the drying steps. The process of freezing is a critical step since the microstructure of the ice crystals formed will affect the quality of the final product and the processing characters of primary and secondary drying process.[3]

There are three physical events associated with freezing;

(i) Super cooling: The main aim of freezing process is to ensure that all the content of vial is converted into a stable solid form. Freezing point depression and lack of nucleation centers in the solution play an important role in failure of this step.[8] The presence of concentrated pockets of the solution will lead to depression of freezing point. These may remain in liquid state itself. Also since there is no prior ice upon which the crystallization can take place (i. e., no nucleation site), cooling the solution only up to the freezing point is not sufficient. Thus super cooling is required, i.e., the temperature should be lower than the equilibrium ice formation temperature for complete freezing to occur.

(ii) Primary crystallization: The nucleation and formation of crystals of the solvent is termed as primary or ice crystallization.[3]

(iii) Secondary crystallization: Concentration of the solutes during ice crystal growth and crystallization of solute is termed as secondary crystallization.[3]

At the end of these events, the contents of the vial are said to be completely solid.

The temperature required to obtain complete freezing depends on the nature of the solvent and the solute characteristics. The two factors that one has to keep in mind during freezing are the cooling rate and temperature &time of freezing.

A. Cooling Rate

The cooling rate determines the type of ice crystals formed. Rapid cooling results in the formation of small ice crystals which are helpful in preserving structures for microscopic observation, such as cells. But they are difficult to dry subsequently. For pharmaceutical injections, rapid drying is important. Slower cooling rate leads to the formation of larger ice crystals which are not only easy to dry but also lead to formation of less restrictive channels in the matrix during the drying process.[5]

B. *Temperature & Time of Freezing*

One of the important factors to be kept in mind is the eutectic temperature (T_{eu}) for crystalline substances or the glass transition temperature (T_g') in case of amorphous substances. During freezing one has to keep in mind that the product temperature should be way below the T_{eu} or T_g' to ensure that complete freezing occurs.

Because of the limited thermal conductivity between the shelf and the glass vial, freezing requires significant time. The optimum time is determined by trial and error. The fill depth also plays an important role. As the depth increases, the time required for freezing also increases proportionately[7].

Annealing

Lyophilization is essentially for high value pharmaceuticals. Thus, product consistency is essential. Variation in vial condition and the position of the vials in the lyophilizer will result in differences in the ice nucleation further leading to heterogeneities in the drying rate and product quality[9].

Annealing can be defined as the process involving controlled heating and cooling of the substance to encourage crystallization. It is a step within freezing. Usually annealing is done so as to crystallize the bulking agent or to assist in the formation of large ice crystals.[7] Annealing eliminates variation in initial ice crystal size distributions induced by variable nucleation temperatures and the resulting heterogeneity in drying rates[9].

During annealing, the increase in temperature will result in the melting of crystals and subsequent cooling will lead to crystallization. Smaller ice crystals will melt easily compared to larger ones and thus, will recrystallize on the larger ones. Thus larger ice crystals are formed[6].

Proper selection of the annealing time and temperature leads to an effective process that substantially reduces the time for primary drying and improves the appearance as well as the dissolution rate of the resulting lyophilized cake[7, 9].

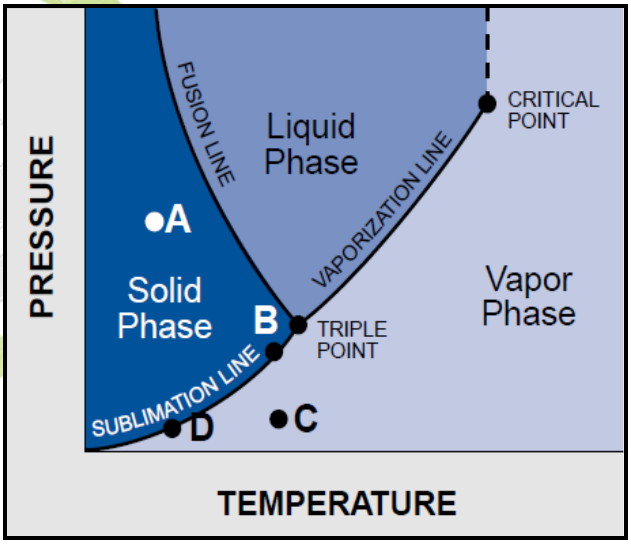

Figure 5: Typical phase diagram

Primary Drying [4, 5, 7]

The next step in the process of lyophilization is primary drying. In this stage the ice formed during the freezing step is made to sublime i.e., the ice converts to vapor state without passing through the liquid state. This vapor is made to condense on the condenser plates. The three basic requirements of primary drying are shelf temperature, target product temperature and chamber pressure. The primary drying conditions must be established in such a way in which sublimation from the frozen product

occurs, resulting in a dry and structurally intact product.

During primary drying, sublimation of ice starts from the surface of the frozen product and continues towards the bottom of the vial. Thus, the evolving vapor must pass through the pathway created by the sublimed ice on the upper layers.

Chamber pressure: Molecules migrate from the region of high pressure to low pressure. This fact is taken into consideration in the process of primary drying. For this reason, the chamber is evacuated such that the pressure is less than that of the vapor pressure of ice at any given temperature of the product. The variable here is the chamber pressure, which affects the heat and mass transfer processes. This in turn impacts the sublimation rate. The relation between the sublimation rate and chamber pressure can be given by using the following equation,[7]

$$dm\backslash dt = \frac{P_{ice} - P_c}{R_p + R_s}$$

Where, dm\ dt is sublimation rate in g\hr per vial,

P_{ice} is equilibrium vapor pressure of ice at sublimation interface,

P_c is the chamber pressure,

R_p is the resistance of the dry layer to the vapor and

R_s is the resistance offered by the stopper to vapor.

The sublimation rate is proportional to the pressure differential between the vapor pressure of ice and the partial pressure of water in the chamber (P_i). During primary drying, P_i is same as that of the chamber pressure. Thus, at a given product temperature or ice vapor pressure, the smallest chamber pressure will eventually result in the highest sublimation rate. However, very low chamber pressure may lead to contamination of the product with volatile components of the stopper or the pump oil. Also heterogeneity in heat transfer between different batches of a vial is also associated with very low chamber pressure. The ideal chamber pressure for the process should be in the range of 50-200 mTorr. The following equation gives the optimum chamber pressure to be used for a given target product temperature. [7]

$$P_c = 0.29.10^{(0.019.T_p)}$$

Where, T_p is the target product temperature and

P_c is the chamber pressure.

Target product temperature: For a structurally elegant and desirable product, it is necessary that the components of the vial do not collapse during the process. In view of this criterion, the formulator has to set the target product temperature. To prevent collapse, the product temperature should be several degrees below the collapse temperature (T_c). The temperature difference between the target product temperature and the collapse temperature is called the temperature safety margin.

For faster drying rate, the temperature of the product must be high. Therefore, an optimized cycle uses a target product temperature which is high as possible, but which is below the collapse temperature. The temperature safety margin must be set keeping in mind the duration of cycle. A small safety margin of 2˚ C is recommended for a cycle that is long (<2 days) and a high safety margin of about 5˚ C for a short cycle (>10 hours) and an intermediate of 3˚C for a cycle between 10 hours and 2 days.

Shelf temperature: The energy for the process of sublimation is derived from the heat provided by the shelf. Hence, shelf temperature is an important process parameter. It also determines the product temperature. Determination of an optimum shelf temperature: time profile that helps to achieve the desired target product temperature is one of the essential steps in freeze drying process. The shelf temperature during primary drying is always higher than the temperature of the vial, except when the process is coming to an end. The temperature difference may range from 5-40˚C. The shelf temperature to achieve the desired target product temperature can be determined by the equation given below[7].

$$T_s = T_p + \frac{1}{A_V} \cdot \frac{dQ}{dt} \cdot \left(\frac{1}{K_V} + \frac{l_{ice}}{k_I}\right)$$

Where, T_s is the temperature of the shelf to be set,

T_p is the target product temperature,

l_{ice} is the ice thickness,

k_I is the thermal conductivity of ice,

K_V is the heat transfer coefficient of the vials,

A_V is the outer area of the vial bottom and

dQ\dt is the heat transfer rate.

It is said that the product temperature changes by 1-2˚ C for every 5˚ C rise in the shelf temperature. Since the collapse of the product depends on the temperature, it is recommended that the shelf temperature not to be changed until more than two-thirds of the primary drying is done, as a rough rule.

Determination of the End Point of Primary Drying[10]

Freeze drying is a time and energy intensive process, most of which is due to the time taken for sublimation of ice or primary drying. Trying to reduce the primary drying time by increasing the shelf temperature to secondary drying temperature, before all of the ice is sublimed may result in collapse of the cake or eutectic melt. It is thus vital to determine the end point of primary drying so as to shorten the overall cycle time so as to reduce the cost and time consumed. Typically, one uses a thermocouple or temperature probe in one of the outer vials to determine the end point of primary drying. A sharp increase in temperature is seen, as the process approaches completion. On completion, the vial temperature is same as that of the shelf since no heat is required for sublimation. But this is not indicative of all the vials. Since the probe is placed in the outer vial for sterility assurance, it might finish primary drying faster due to radiative heat from the chamber wall. This is not seen for vials which are placed in the interior of the chamber. When these are being used for indicating the end point, it is always safe to include an additional soak time of at least 10-20% of the primary drying time to ensure that all ice has sublimed.

Advent of technology has led to the development of various techniques that does not require an additional soak time and also be representative of the whole batch without compromising on sterility. Few of them are discussed herewith:

Techniques based on gas composition in the product chamber include-

- Pirani gauge
- Dew point monitor
- Measuring water concentration using tunable diode laser absorption spectroscopy(TDLAS)
- Lyotrack

Others include:

- Product thermocouple response
- Condenser pressure
- Manometric temperature measurement

A. Pirani Gauge

Pirani vacuum gauge is an instrument that works on the principle of measurement of thermal conductivity of the gas in the drying chamber. Primary drying involves sublimation of ice into vapor. Thus, the chamber contains mostly water vapor during this phase. This vapor condenses on the condenser subsequently. When the ice is completely sublimed, the percentage of nitrogen in the chamber starts to increase and vapor falls. The difference in the thermal conductivity helps in determination of the end point. The thermal conductivity of water vapor is about 1.6 times that of nitrogen. The point where the pirani pressure starts to decrease sharply indicates that the gas composition is changing from vapor to that of nitrogen.

B. Dew Point Measurement

Frost point or dew point is the temperature at which the equilibrium vapor pressure of ice is same as that of the partial pressure of water. Electronic monitor sensors are used to monitor

this temperature. The sensor uses a thin film of aluminum oxide whose capacitance is measured. Measurement of the change in capacitance of the film due to the adsorption of water at a given partial pressure and conversion of this capacitance to voltage forms the basis of this sensor. A sharp fall in reading indicates that the water vapor concentration in the chamber is falling and that primary drying is essentially complete.

C. TDLAS

TDLAS stands for tunable diode laser absorption spectroscopy. It is helpful in determining the water vapor concentration as well as the rate of sublimation. TDLAS consists basically of two laser beams, one of which is directed towards the vapor flow and the other against the vapor flow in the duct connecting the chamber and condenser. The wavelength of the beam is adjusted according to the absorption lines of the gas in the duct i.e., water vapor. This helps in determination of the concentration of vapor. The Doppler shifted vapor absorption spectrum is used so as to measure the gas flow velocity. It basically is used to determine the shift in the peak absorption wavelength between the two beams or the shift in absorption relative to the reference zero velocity of the gas sample. The sublimation rate can thus be determined by using the above parameters i.e., concentration and velocity. The point where the concentration of the vapor decreases sharply indicates that the sublimation is essentially over or primary drying is complete.

D. *Lyotrack*

Lyotrack is also known as gas plasma spectroscopy. It works on the principle of optical emission spectroscopy and helps in determination of water vapor concentration. It consists of a plasma generator which generates radio frequency whose energy is transmitted into the plasma tube filled with gas. Absorption of this energy leads to conversion of the gas into plasma. This plasma mainly consists of molecules, atoms either in exited or ground state and also ions, electrons and photons. Since the atoms or molecules are not in the exited state, they emit energy and return to its initial energy level. The wavelength of the emitted energy in the form of light is characteristic of the atom or molecule and hence is useful in the detection of the concentration. The point when the concentration of the water vapor starts to fall indicates the ending of the process of sublimation.

Problems Associated With Lyophilization[2]

The cake formed during lyophilization shows two common problems, they are cake collapse or meltback. Cake collapse occurs when the vial is subjected to a temperature above the collapse temperature before all water sublimes.

Meltback can be termed as a form of collapse which is caused due to change in state from solid to liquid.

Secondary Drying[2,7]

The removal of water from the product by the process of desorption is termed as secondary drying. This process is generally used so as to remove the unbound water or the water which has been adsorbed on to the internal surface of the product. This ensures that the water content of the product is low enough such that any biological growth and chemical reactions are not supported. There is no clear demarcation between primary and secondary drying process but the water content at the end of primary drying is high that the desired stability is not achieved. Thus, secondary drying is done with an objective of removing the residual water and reducing the water level to values that ensure stability of the final product. This process depends upon the materials adsorption isotherm.

Secondary drying is slower compared to primary drying although the quantity of water removed is less. Thus, it takes a larger portion of drying time compared to primary drying. This is because in secondary drying, the water is desorbed and moves by the process of diffusion from the region of higher concentration to a region of lower concentration. The low acceptable water content levels and the slow transport mechanism combined result in requirement of a longer drying time.

Research demonstrated that water levels were low on the cake surface and also on the walls of the vial compared to the rest of the cake. This is mainly due to shrinkage of the cake from the walls. This provides a path for the escape of water vapor. However, storage under normal conditions will result in the uniform distribution of moisture throughout the cake. If shrinkage of the cake does not occur, then the lowest moisture levels occur at the top of the cake.

Parameters that Govern Secondary Drying

The parameters that govern secondary drying are similar to that of primary drying, namely, product temperature, shelf temperature and chamber pressure, also time required for secondary drying and ramp rate also play an important role.

A. Target Product Temperature

The target product temperature or T_p is the final temperature of the product after secondary drying. Determination of target product temperature is done based on two factors, namely, the stability of the drug at the final temperature and time of secondary drying. The T_p must be set in such a way that the drug on being exposed to that temperature for a prolonged period of time does not degrade. Also, the temperature should be such that the desired levels of moisture should be attained.

Since we already know that the process of freeze drying is a time intensive process, one has to make sure that the time involved is short. So it is feasible to use the highest possible temperature for the shortest possible time. However, reducing the time such that the desired water content is not attained will compromise the stability of the product. The crystallinity of the product also plays an important role in the determination of the secondary drying time. Amorphous products have a higher level of water content and require longer secondary drying time than that of crystalline products.

B. Shelf Temperature

The shelf temperature is the source of energy for the removal of water. Since the process of desorption requires lesser energy than that of primary drying, the shelf temperature need not be increased much. However, the temperature should be high enough to ensure removal of the unbound water in a short time without damaging the product.

C. Chamber Pressure

The process of secondary drying involves diffusion. If the chamber pressure is not maintained low as it is done in primary drying, then the process would have been dependent solely on the process of diffusion. Since the pressure is maintained low, the vapor makes its way out of the vial through pressure differential system. Thechamber pressure has to be maintained constant so as to ensure that the water vapor evolving is removed from the vial. The chamber pressure is maintained constant by the means of slow release of nitrogen gas to compensate for the reduction in the water vapor.

D. Heat Transfer Rate

Heat transfer rate determines the time for which the drug or product is exposed to a particular temperature. Rate of heat transfer is determined on the basis of the amount of residual moisture in the cake after primary drying. If the water content is high at the end of primary drying, a low rate of transfer (R_s) will result in the exposure of the drug to heat for a long time resulting in degradation. In such cases the rate should be increased so as to ensure thermal stability of the product. The rate of heat transfer plays a major role in the secondary drying of amorphous products. Amorphous products have a high degree of residual moisture as well as a low transition temperature, thus having high rate of heat transfer might result in the collapse of the cake. Thus, for amorphous drugs or products the heat transfer rate must be kept as low as possible. Once the ambient temperature is reached for amorphous products, then the ramp rate need not be slow. This problem is not seen with crystalline drugs and thus high rates of heat transfer are allowed.

The process of secondary drying can be done using the sensors used to determine the end

point of primary drying, i.e., pirani gauge, residual gas analysis and humidity sensor.

Evaluation of Lyophilized Products[2]

The following tests are conducted on the finished lyophilized product:

A. Appearance

The cake should not be collapsed on removal from the lyophilizer. It should occupy the same volume as that of the liquid filled before the lyophilization process.

B. Reconstitution Time and Clarity of Reconstituted Solution

One of the important advantages of lyophilization is that the surface area of the cake increases and thus the solubility increases. Reconstitution of the cake should take the least time as possible and also upon complete reconstitution, the solution formed should be clear, without any visible particles.

C. Water Content

The objective of lyophilization is to ensure that the product has the lowest water content as possible. The water content is determined by Karl Fischer titration method.

D. Assay

Assay shall be within specification limits.

E. Stability

The main concern of lyophilized product is the amount of moisture present in the product and its effect on the stability of the product. Stability testing has to be done by placing the vials under long term and at accelerated conditions as per ICH requirements. The vials have to be tested for all the above mentioned parameters.

F. Sterility

Since the lyophilized product is reconstituted for parenteral use, Water for Injection is used or any other sterile solvent can be used for reconstitution. One has to ensure that the product is sterile by performing sterility testing. The results which show contamination of the product should be identified and reviewed.

CONCLUSION

Despite oral route being the most convenient choice for administration of drugs, the risk of failure of therapy has led to the advent of formulation of parenterals. Lyophilization method addresses the need for production of stable formulation for drugs that are water sensitive and thermo labile. The stability and ease of handling of the lyophilized product is increased and thus it is one of the desirable techniques for the production of parenterals. However, the desire to reduce the development time for the formulation of pharmaceuticals requires thorough understanding of the product and process characteristics. Development of proper technology and process monitoring will eventually result in the formation of a good and consistent lyophilized product.

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