CHAPTER II

LITERATURE REVIEW

Freeze - Drying

Freeze – drying is a process for drying the material by freezing the solvent first and then removed the solvent by sublimation in vacuum environment. Freeze drying technique is developed into a widely used method for stabilization of the easily degraded substance. There are several characteristics of the freeze dry process that make it desirable over other drying methods. First, since drying take place at low temperature, chemical decomposition is minimized. Second, the resulting product has a very high specifics surface area, which promotes fast and complete dissolution of the dried product. This is the critical quality attribute for freeze dried drugs used in emergency situation. Third, freeze-drying is more compatible with sterile operations than dry powder filling, since a solution can be sterile filtered immediately before filling vials. Liquid filling is more precise than powder filling and the absence of powder at the filling step minimizes problems with particulate contamination in clean, aseptic environment (9). However freeze-drying is very effective means of drying product, it also used high capital cost of equipment, high energy cost and lengthy process time. So it should be optimized between the product requirements and the cost effective of this process. In summary, the principle advantage of freeze-drying are also, minimum damage to and lost of activity in delicate heat labile materials, creation of a porous and friable structure, possibility of accurate and clean dosing into final product container, and , speed and completeness of rehydration. On the other hand, the principle disadvantages are high capital cost of equipment, high energy costs, and, lengthy process time.

Process overview of freeze-drying

Before the freeze-drying process take place, the vial is filled with the solution to be freeze dried and was usually partially stoppered with a special slotted rubter closure that allow escape of water vapor when the stopper is inserted halfway into the neck of the vial. Vials are transferred in metal trays to the freeze dryer. Trays of product are placed on shelves containing internal channels allowing circulation of silicone oil or another suitable heat transfer fluid. A temperature-measuring device may be placed in several vials spaced throughout the chamber

prior to freezing and connected to an external device for process monitoring and sequencing of the freeze dry cycle.

The product is first frozen to a low enough temperature to allow complete solidification of the contents of each vial. Then the chamber is evacuated until the pressure is less than the vapor pressure of ice at the temperature of the product. After this pressure is reached, heat is applied to the shelves to provide the energy required for sublimation of ice. As drying proceeds, a recording boundary can be observed in the vial (Figure 2) as the frozen layer decreases in thickness and the thickness of the partially dried solids increases. This phase is called primary drying. When the ice is gone, additional drying time is required to remove water adsorbed to, or trapped by, the solid matrix. This is called secondary drying. A representative plot of product temperature along with shelf temperature and chamber pressure during freezing, primary drying and secondary drying is shown in figure 3. When the product is sufficiently dry, the vials are usually stoppered in place within the dryer by hydraulic compression of the shelf stack, which pushes the stoppers to the fully inserted position, either under a full vacuum or by back-filling the chamber with an inert gas (9).

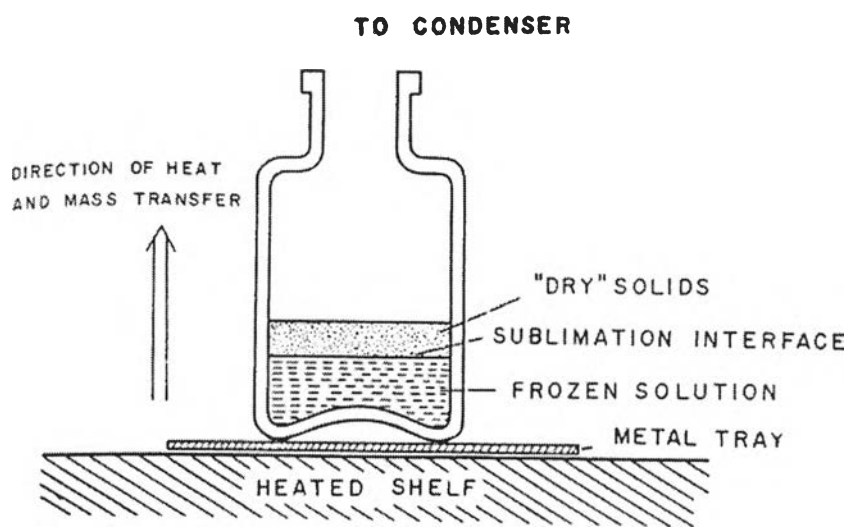

Figure 2 Diagram of vial during primary drying.(9)

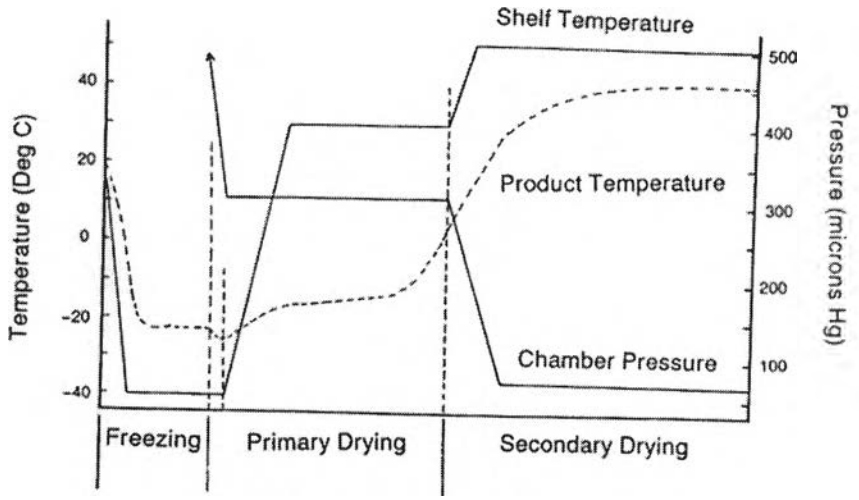

Figure 3 Plot of process variable during freeze drying cycle.(9)

The most important objective in developing a freeze dried product is to assure that quality requirements are met not only initially but throughout the shelf life of the product. In addition, however, process conditions should be chosen to maximize process efficiency. Success in this challenge requires an understanding of the physical chemistry of frozen solutions, heat and mass transfer under conditions encountered in freeze drying, temperature and pressure measurement, process monitoring, and general freeze dry system design considerations.

The Freezing Process

Freezing is a critical step in the freeze drying process, since the microstructure of ice and solute formed during freeze determine both the quality of the final product and its processing characteristics, such as the rate of primary and secondary drying. The discussing below presents physical events associated with the freezing process-supercooling, ice crystallization, concentration of solute with respect to freeze drying behavior and final product quality.

The phase diagram of water is illustrated in figure 4. At the triple point (0.0098^{o}C and 4.58 mmHg), ice, water, and water vapor coexist in equilibrium freeze drying takes place below the triple point, where water passes from the solid phase directly to the vapor phase without an intermediate liquid phase appearing.

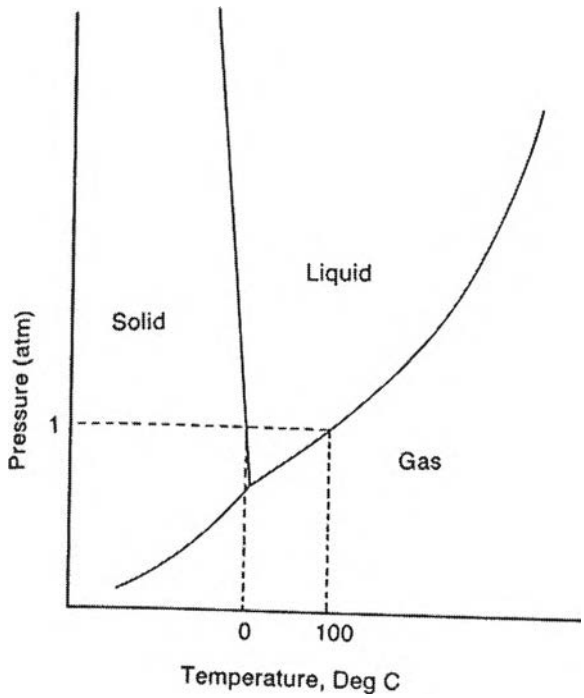

Figure 4 Phase diagram of water (9)

Freezing of aqueous solution

In simple two-compartment systems water and solute may both crystallize. In this case, freezing proceed as follows. Cooling from the initial temperature to below $0^{\circ}C$ eventually results in the nucleation of ice. The release of heat of crystallization raises the temperature toward $0^{\circ}C$. Crystallization then proceeds at a progressively falling temperature related to the equilibrium melting point of the concentration solution as shown in figure 6 which shows the equilibrium phase diagram for NaCl and water.

As the temperature falls, the solution becomes closer to saturation so that crystals of solute are precipitated. Eventually, a eutectic point is reached where the one cryatallizable solute, a similar situation exists but the eutectic point is lower than that of any two components combined.

In the majority of practical cases, the solute does not actually crystallize but form an amorphous glass. There is no eutectic temperature but a maximum product temperature for freeze-drying with retention of microstructure. This is called the collapse temperature. If product temperature exceeds the glass transition temperature, the product will undergo collapse (23).

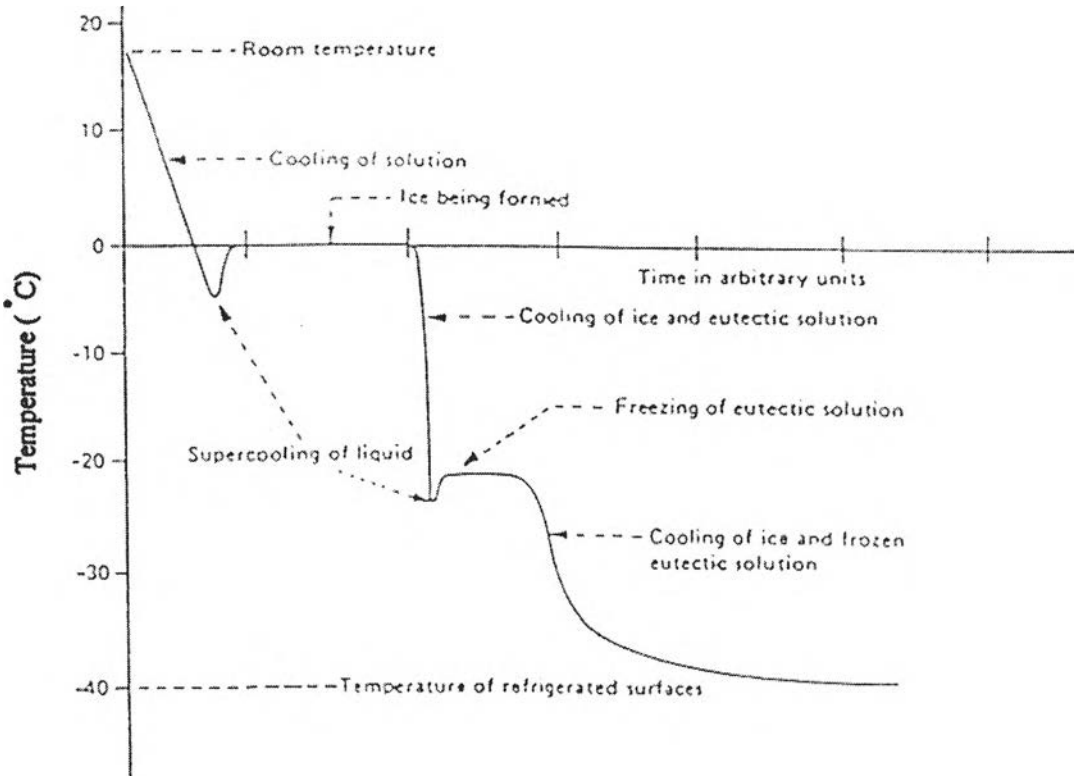

Figure 5 Equilibrium phase diagram for NaCl and water (24)

The concept of the glass transition applies to amorphous systems and, for our purposes, corresponds to a change in the viscosity of the solution from a viscous liquid to a glass, or an essentially solid solution of solute in water. The glass transition is important for an amorphous solute in the same way that the eutectic temperature is important for a crystalline solute- it represents the maximum allowable product temperature during primary drying.

In order to obtain the desirable properties of a freeze dried product, the microstructure formed during freezing must be retained after removal of the ice.

The eutectic temperature is important in freeze drying because it represents the maximum allowable product temperature during primary drying. If product temperature exceeds the eutectic temperature while ice is still present, drying takes place from the liquid state instead of the solid state, and the desirable properties of a freeze dried product are lost. However, eutectic behavior is only observed when the solute crystallizes. In most cases, the solute does not readily crystallize during freezing, and a different type of behavior is observed.

Figure 6 shows the diagram of the microstructure of a frozen solution containing a crystalline and amorphous solute. The interstitial material in part (a) consists of a mixture of eutectic ice and crystalline solute. When ice (both eutectic ice and pre-eutectic ice) is removed by sublimation, a crystalline solid containing very little water is left. For the amorphous system (b), the interstitial glassy material must be rigid enough to support its own weight after the ice is removed in order for the micro structure which was established during freezing to be retained after drying. Below the glass transition temperature, the viscosity is high enough that, on a practical time scale, no flow of the material is observed. Above the glass transition temperature, the solute flows after the supporting ice structure is removed, this can result in collapse of the product (9).

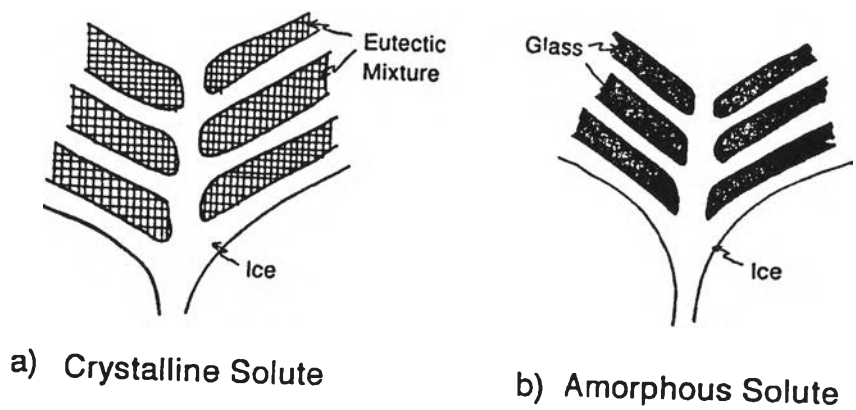

Figure 6 Microstructure for solute which (a) crystallizes upon freezing and (b) remains amorphous with freezing (9)

The freezing behavior of a solution containing a solute that will not crystallize upon freezing cannot be represented by an equilibrium phase diagram, since it is not equilibrium.

Thermal treatment

A solute crystallizes readily from solution during freezing. Many other solutes do not crystallize as easily. Because of super-cooling and the resulting rapid rate of freezing once ice crystals are nucleated, a metastable amorphous solute phase may be formed initially. If the system is then warmed to some temperature above the glass transition temperature but below the melting temperature, the solute may crystallize. This process is called thermal treatment, or annealing.

Other product characteristics can also be affected by crystallinity of the solute, such as reconstitution time and cake appearance.

From the study of characterization of glycine frozen solution by Chongprasert et al, annealing at controlled temperature in the melting region prior to recooling the system was useful not only in interpreting the complex DSC thermogram, but also in controlling the glycine polymorph resulting from freeze drying(12). A neutral glycine exists in 3 polymorphs forms. The melting temperature of the ice/glycine eutectic mixture has been report as -3.4 to -3.6°C. From the annealing method of this study, glycine produced the α, β and γ polymorphs showed by x-ray powder diffraction.

The Primary Drying Process (9, 21, 25-26)

Primary drying is characterized by a receding boundary layer of ice in the vial. The driving force for sublimation of ice during primary drying is the difference between the vapor pressure of ice at the sublimation front and the partial pressure of water vapor in the freeze dry chamber. Since the driving force is determined only by difference in vapor pressure of water within the system, and not by the total system pressure, it is misconceptions that freeze drying can take place only under high vacuum. However, freeze drying at atmospheric pressure must take place by molecular diffusion of water against a pressure gradient (air), which is slow process. Freeze drying did not become a commercial practical process until advance in vacuum pump technology allowed the freeze dry chamber to be rapidly pumped down to a total pressure of less than the vapor pressure of ice in the product. The vapor pressure increase by roughly 70% for every 5°C increased in temperature. Thus, it is important for maximum process efficiency to keep the product temperature as high as possible without exceeding the maximum allowable product temperature during drying. As discussed above, the maximum allowable product temperature is determined either by the eutectic melting temperature or by the collapse temperature of the product. In either case, exceeding the maximum allowable product temperature is resulted in an unacceptable product.

After freezing, the partial pressure of water vapor must be reduced below the triple point pressure of water for sublimation. If there is a free escape path for water molecules, the speed of sublimation at the beginning of primary drying is largely limited by the rate at which heat can be transfer to the freeze-drying front.

If the temperature of the lowest eutectic exhibited by the material is exceeded during drying, then melting can occur. This rises to gross material faults such as melting, shrinking and puffing. These are sometimes confused with another phenomena known as collapse, which occur when one or more of the solutes does not crystallize but forms an amorphous glass associated with unfrozen water. The glass is supported by the pure water ice crystals so the product appears to be rigid, but as the pure water ice crystals sublimate away, the support is removed and apparently dry product collapse to form an impermeable mass.

Secondary drying or desorption (9, 27-28)

When ice crystals have been removed from the product by direct sublimation, the product temperature increases sharply, since the heat of sublimation is no longer required and heat continues to be applied to the product. The abrupt increase in product temperature signals the beginning of secondary drying.

Secondary drying is the removal of unfrozen water. It follows that the amount of the residual water to be removed during secondary drying depends mostly on whether the solute, or solutes, crystallize before primary drying. For a crystalline solute, essentially all of the water is present either as eutectic ice or pre-eutectic ice. When all ice has sublimed, the only water remaining is water adsorbed to the surface of the solute crystals, unless water of hydration is present within matrix. In this case, the product is essentially dry when primary drying is complete, and secondary drying is brief.

For an amorphous solute, however, the solute is present in a glassy matrix containing perhaps as much as 40 % water. Since there are few, if any, open channels for mass transfer from the interior of the glass phase on the surface, this process must take place by molecular diffusion. Because of the large amount of water to be removed and the slow mechanism of transfer, secondary drying can be the most time-consuming phase of freeze drying for an amorphous solute.

The general practice in freeze drying is to increase the shelf temperature somewhat secondary drying and to decrease drying and to decrease chamber pressure in the lowest attainable level. This practice is based on the idea that since ice is no longer present and there is no concern about "melt back" the product can withstand higher heat input. Also, the water remaining during secondary drying is more strongly bound, thus requiring more energy for its

removal. Decreasing the chamber pressure to the minimum attainable vacuum has traditionally been thought to favor desorption of water.

The approach to establishing optimum process conditions during secondary drying is the same as described in the discussion of primary drying above, i.e., identify the limiting resistance and use conditions that minimize this resistance. Possible resistances include heat transfer in the evaporation zone, diffusion of water within the solid to the surface, evaporation at the surface, transport of water vapor within the porous plug of solids, or transport of water vapor from the head space of the vial to the condenser.

Strong dependency of secondary drying rate on specific surface area of solids suggests that the rate of freezing should have a significant impact on the rate of secondary drying. Slow freezing is expected to produce relatively large ice crystal and interstitial solid material of low specific surface area; hence a lower rate of secondary drying. Conversely, fast freezing produces small ice crystals and a relatively high specific surface area of solids; thus a faster secondary drying rate. The effect of freezing on drying rate would be expected to be different the primary and secondary drying.

Characterization of Freezing Behavior (9)

An important step in the development of freeze dried dosage forms is characterization of the behavior of the formulation when frozen. This analysis can provide information which will help determine whether the active component crystallizes upon freezing and the effect of excipients on freezing behavior. Analysis of the formulation can also provide a good estimate of the maximum allowable product temperature during primary drying-information that is needed to optimize cycle conditions during drying.

The common methods of analysis of formulations to be freezed dried are thermal analysis, thermoelectric (or electrokinetic) analysis, and freeze dry microscopy. These methods are viewed briefly below.

Thermal Analysis (9)

Physical or chemical changes that occur in a material with changes in temperature are generally accompanied by the absorption or release of energy in the form of heat. In differential thermal analysis (DTA), the temperature of the sample and a thermally inert reference material (such as glass beads) are measured as a function of temperature. Any transition that sample undergoes will result in liberation or absorption of energy by the sample, causing a difference in

temperature (T) between the sample and the reference material. Plotting this differential temperature against the programmed temperature of the system gives information on the temperature at which a transition occurs and whether the transition is exothermic (release of heat) or endothermic (absorption of heat). A technique that is closely related to DTA is differential scanning calorimetry, or DSC. In DSC, the system is also subjected to a carefully controlled programmed temperature, but whenever a transition occurs that results in a temperature difference between the sample and the reference material, heat is added to or taken from the sample so as to maintain both sample and reference at the same temperature. The energy added to or subtracted from the easily measured, and is equal to the energy absorbed or evolved in the thermal transition; thus, recording this energy results in a direct calorimetric measurement of the transition energy.

Types of thermal transitions which can be detected for frozen solutions are shown in figure 8. The output is a plot of ΔT vs T for DTA and heat applied to the sample (mW) vs temperature for DSC. Endotherms and exotherms are distinguished by the direction of a peak on the thermogram. In this discussion, an upward peak is indicative of an exotherm, and an endotherm is a downward peak. Some instruments use the opposite convention. The heat capacity of the sample is proportional to the displacement from the blank baseline. A shift in the baseline toward a higher heat capacity is indicative of a glass transition, and denotes a decrease in order within the system. Increasing of rotational energy at the molecular level at temperature above this transition may give flexibility and elastomeric properties to polymeric materials. Endotherms generally indicate physical changes, such as melting, rather than chemical changes. Sharp endotherms are characteristic of melting of pure compounds. Exothermic behavior is associated with increased molecular order of a system, such as crystallization of a metastable system.

Thermal transitions can be further characterized as reversible or irreversible. For a reversible event, the transition recurs as the sample is cooled and rewarmed, e.g., the melting of ice at 0°C. Crystallization, on the other hand, is an example of an irreversible event, i.e., a crystallization exotherm will not be observed again if the sample is cooled and rewarmed through the same temperature range.

For the thermal analysis experiment, a small amount of sample is placed in an aluminium pan. The sample is then placed in the thermal cell along with a thermally inert reference material. An empty pan is usually a suitable reference material. The sample is then cooled to a predetermined temperature, such as -50°C, and slowly warmed (1-5°C/min) to a temperature

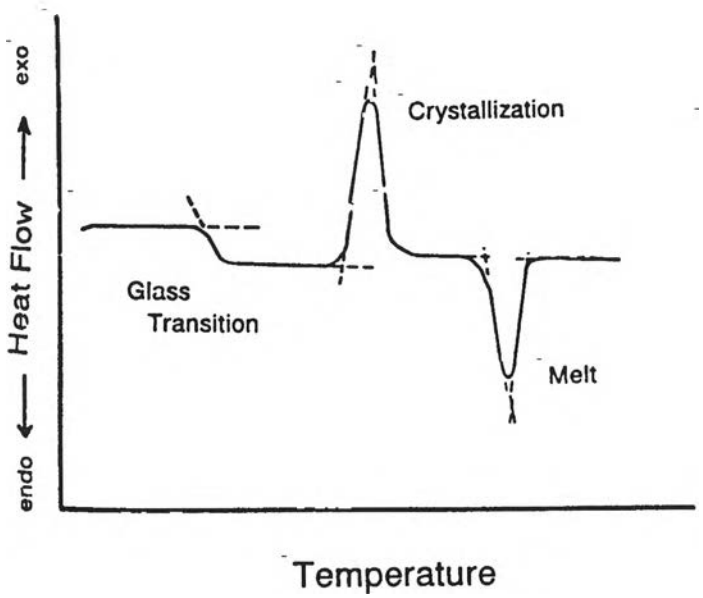

Figure 7 Types of thermal transitions relevant to frozen solutions (9)

above the freezing point of the sample above the freezing point of the sample. It is important that the analysis be carried out during warming of the sample, since transitions recorded during cooling would not be accurate due to supercooling. The sample cell should also be flushed continuously with a small stream of dry nitrogen to prevent formation of ice from atmospheric moisture condensation between the sample and the temperature detector (9).

For example, the thermal analysis of frozen glycine solution was studied by Chongprasert et al, DSC thermogram represented the low temperature thermal characteristic of glycine after quench cooled and heated at 0.1°C/min. The DSC thermogram of the low temperature region, Figure 8, shows a complex glass transition region that two separates exotherms and 5 endotherms was observed (12).

Freeze Dry Microscopy (9)

The most unambiguous method for characterizing the freezing and freeze drying behavior of a formulation is by direct observation under a microscope. The system comprise a microscope, a freeze drying stage, a cooling system, and probe for sample measurement, and a vacuum system.

The freeze drying stage is the most critical part of the system. Designs vary widely, but most stages consist of a metal with windows on each side to allow light transmission through the sample. A microscope slide that supports the sample is usually placed on a metal block containing

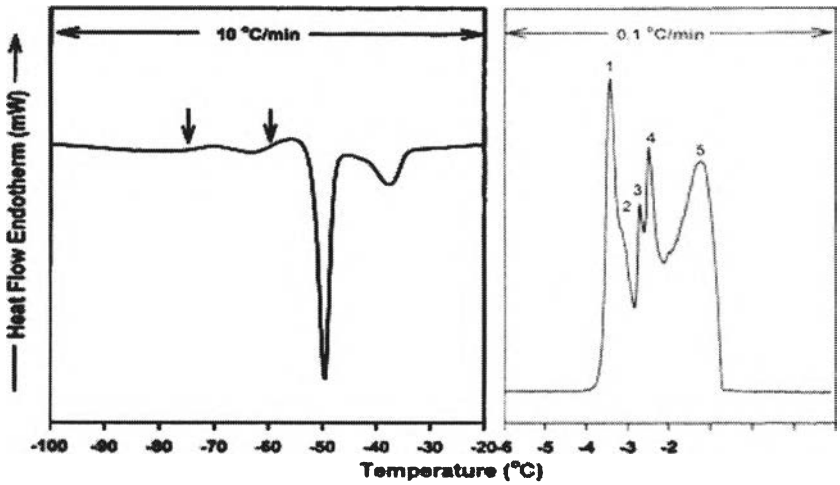

Figure 8 A complex glass transition region of glycine solution (12)

channels for circulation of a cooling or heating fluid. The fluid may be either a gas, such as dry nitrogen, which passes through a cooling coil, or a liquid such as alcohol. Heating of the sample is accomplished either by increasing the temperature of the incoming fluid stream, using a resistance heater between the cooled block and the sample, or by placing a conductive coating on a glass slide as a compensatory heater.

Most freeze drying microscope stages are designed such that the temperature is nearly constant across the sample. This minimizes uncertainty in sample temperature arising from placement of the thermocouple.

A polarized light microscope is useful for observing phase transitions during freezing and during freeze drying. Because of the birefringence of ice crystals, different colors are observed depending on the orientation of the ice relative to the incident light. Similarly, crystallinity of the freeze dried solid can be observed directly.

Experimentally, a few micro liters of sample is placed on a microscope slide, a cover slip is placed over the sample, and the slide is placed on a microscope slide, a cover slip is placed over the sample, and the slide is place on the controlled temperature block. The stage is then covered with a metal lid containing a viewing window. The vacuum seal between the lid and the body of the stage is generally accomplished by an O ring. A stream of dry nitrogen or air is usually blown across the windows to prevent condensation of atmospheric moisture on the windows. After the sample is frozen to a predetermined temperature, the stage is evacuated to less than about 0.5 torr, and the sample is heated. A distinct drying front can be seen moving across the sample as drying

proceeds, and changes in sample morphology can be observed as a function of temperature and time.

The main advantage of microscopy as an analytical tool is the easy observability of collapse as compared with both thermal analysis and thermoelectric analysis. In thermal analysis, the glass transition involves very low energy; thus it can be difficult to detect. For thermoelectric analysis, the applicability to detection of glass transitions has not been established.

Freeze-dried product requirements (29)

The desirable freeze-dried products include:

1. Retain acceptable activity
2. Remain clean and sterile (injectable dosage form)
3. Rapid solution when the solvent is added during constitution
4. Uniformity
5. A porous cake occupying the same volume as the original liquid volume
6. Pharmaceutical elegance
7. Sufficient mechanical strength in the cake to prevent collapse
8. Isotonicity and pH adjustment (injectable dosage form)
9. Justify the high cost of freeze drying process

The desired characteristics can be achieved by proper formulation of the product and by employing optimum freeze drying cycles. The development of a suitable formulation and a freeze drying cycle requires knowledge of some basic properties, such as

1. Eutectic temperature
2. Temperature effect on solubility
3. Thermal properties of the frozen solution
4. Degree of supercooling
5. Heat transfer properties of the freeze-dryer shelves, metal trays, glass vials and the frozen product (30)
6. Equipment design and equipment capability

Physicochemical Characteristics of freeze-dried products

Freeze-dried product has its own unique set of physicochemical characteristics and stability problems (30) that can be categorized into several groups.

1. Effect of residual moisture (32-35)

Since freeze-drying process is chosen for drying poor solution stability drugs, the stability profile of freeze-dried product will depend on residual moisture. At low moisture levels, degradation can be expected to approach zero order conditions since the reaction will depend on the essentially constant amount of moisture available to dissolve the drug. There is always an excess of drug in the solid phase, this adsorbed moisture layer will remain saturated with drug throughout and zero order kinetics will result.

2. Effect of excipients

As with simple solution, excipients used in a freeze-dried product can markedly affect the stability of labile components in the dried product.

3. Effect from bulk solution

Manufacturing of freeze-dried product generally requires production of a bulk solution prior to aseptic filling and freeze drying. Besides the minimum time necessary for such processing operations, it is a frequent practice to assay this solution before filling in vials in order to allow any necessary per vial potency adjustment to be made in fill volume. The drug may be held in the relatively unstable solution state from a few hours up to several days before filling into vials and freezing, for particularly labile drugs, even this relatively short period may result in substantial decomposition, shortening shelf life of the products. One method to minimize this type of in process instability is to include the volatile organic cosolvent in the bulk solution to depress the solvolysis rate.

4. Effect of freeze drying cycle condition (36-39)

An interesting aspect of lyophilized product is the potential for processing conditions, such as cooling and heating rates in the freeze drying cycle, to effect the chemical stability, crystalline content and appearance of the freeze dried product. The well known accelerated decomposition of some penicillin in frozen solution illustrates the potential for prolonged or poorly controlled freezing cycles to yield variable and possibly un acceptable decomposition. More effect of freeze drying cycle condition on product stability is the solid drug in a variety of physical states, i.e., amorphous or in different crystalline polymorph.

Summary (9)

Freeze drying provides a valuable tool to the pharmaceutical scientist by permitting dehydration of heat-sensitive drugs and biological at low temperature. The final product is quickly and easily reconstituted, and the process is compatible with aseptic operations.

Freezing is a critical step, since the microstructure established by the freezing process usually represents the microstructure of the dried product. The product must be frozen to a low enough temperature to be completely solidified. If the solute crystallizes during freezing, this temperature is the eutectic temperature. If the solute remains substantially amorphous with freezing, the relevant temperature is the collapse temperature. Understanding the physical form of the solute-crystalline or amorphous-after freezing can be important from the standpoint of drying characteristics, appearance of the final product, and even product stability during storage. Supercooling is a significant factor in freezing of formulations intended for freeze drying-prior to both primary and secondary (eutectic) crystallization.

The driving force for freeze drying is the difference in vapor pressure of ice between the sublimation zone and the condenser. Because the vapor pressure of ice increase sharply with increased product temperature, it is important from the standpoint of process efficiency to maintain product temperature as high as possible during primary drying without damaging the product. The upper limit of product temperature during primary drying again depends on the physical form of the solute. Exceeding either the eutectic temperature (crystalline solute) or the collapse temperature (amorphous solute), it results in loss of the desirable properties of a freeze dried product.