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Fabbricazione Industriale dei Medicinali - 8 CFU

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PROCESSO DI LIOFILIZZAZIONE

FREEZE-DRYING PROCESS
Principle and Practice

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Pharmaceutical Technologies
& Development

INTRODUCTION

Lyophilization, or freeze drying, is a process in which the solvent (usually water) is:

- first frozen and then
- removed by sublimation

in a vacuum environmental.

INTRODUCTION

Freeze drying is a widely used method for the stabilization of otherwise easily degraded substances:

- microorganisms

- foods

- biological products and

- pharmaceutical products.

INTRODUCTION

While the common application of pharmaceutical freeze drying is in the production of:

- injectable dosage forms.

The process is also used in the production:

- of diagnostics and
- for oral solid dosage forms,
  where a very fast dissolution rate is desired.

INTRODUCTION

Characteristics of the freeze dry process are:

1. minimization of chemical decomposition
   (drying takes place at low temperatures)

2. complete dissolution of dried product
   (resulting product has a very high surface area)

3. More compatibility with sterile operations
   (solution is sterile-filtered before filling vials)

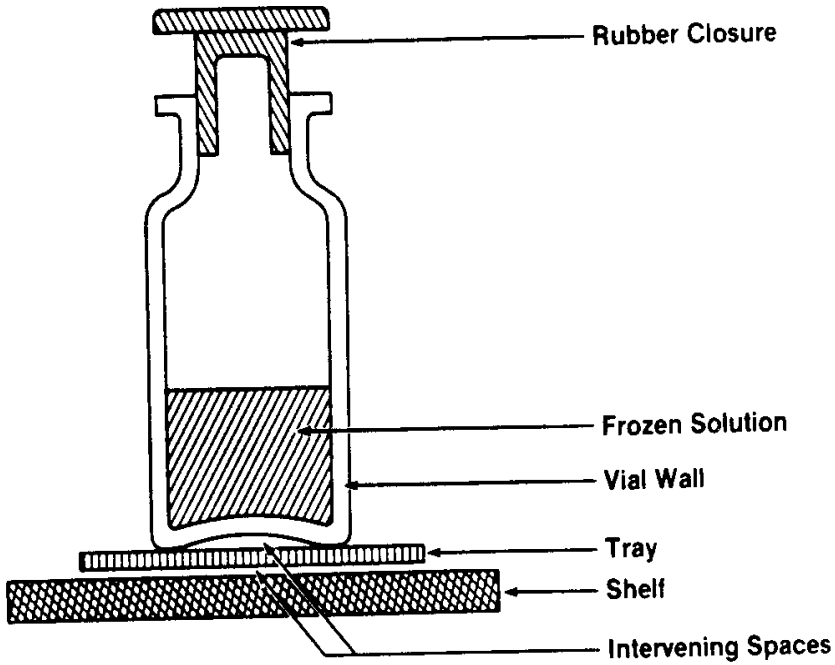
4. precise filling weight
   (fill weight control is more precise for liquid)

5. absence of powder
   (particulate contamination is minimized).

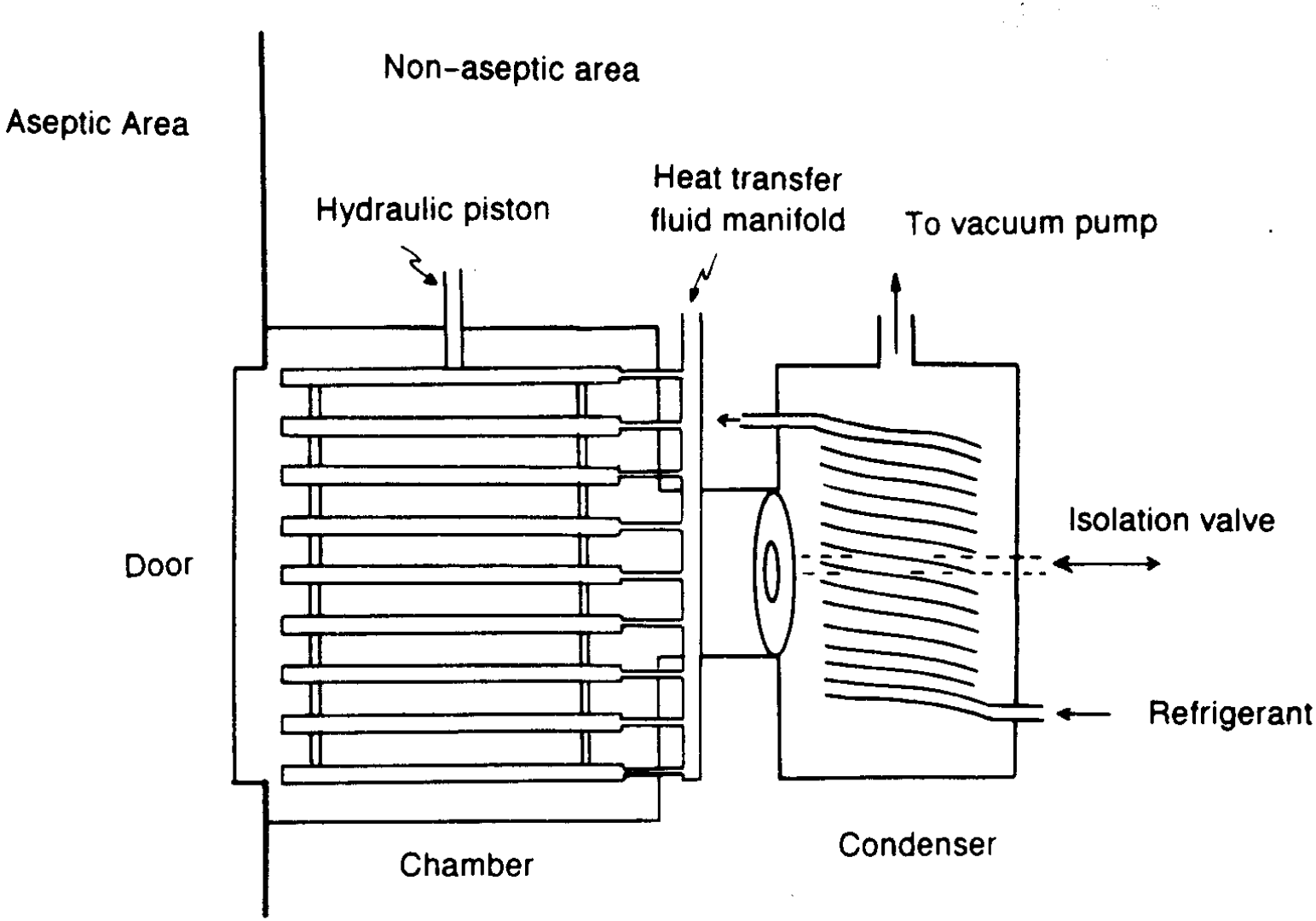
PROCESS OVERVIEW

Vials are aseptically filled with the solution (or suspension) to be freeze dried and partially stoppered with a special rubber closure that allows to escape the water vapor.

Then, vial are transferred under aseptic conditions to the freeze drier.



Schematic diagram of freeze drier



PROCESS OVERVIEW

Trays of product are placed on shelves containing internal channels allowing circulation of silicone oil or another heat transfer fluid.

Shelves may be pre-chilled or not.

The tray may have a removable bottom for direct contact on the shelf eliminating one resistance to heat transfer.

A temperature-measuring device may be placed in some vials for process monitoring/sequencing.

PROCESS OVERVIEW

The product is first frozen to a low enough temperature to allow complete solidification of the content 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.

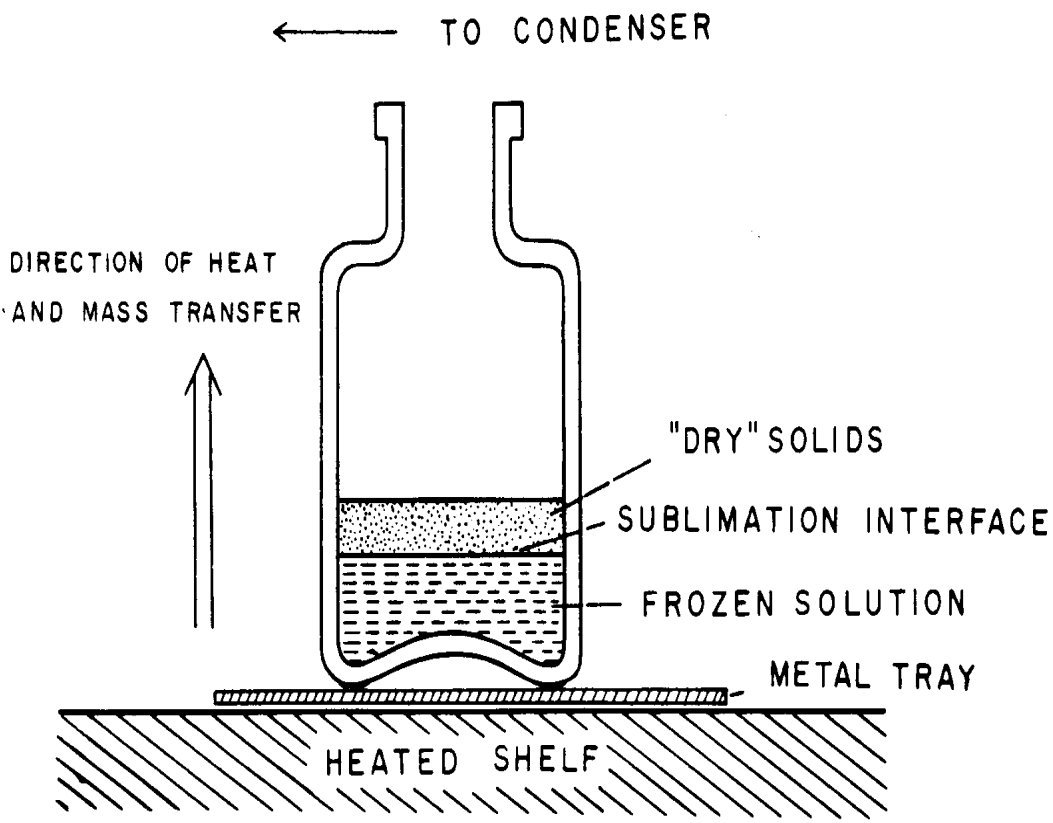
Table 2 Vapor Pressure of Water and Ice Below 0°C[a]

Temp. (°C)	Water	Ice
0	4.579	4.579
–1	4.258	4.217
–2	3.956	3.880
–3	3.673	3.568
–4	3.410	3.280
–5	3.163	3.013
–10	2.149	1.950
–15	1.436	1.241
–20	—	0.776
–25	—	0.476
–30	—	0.286
–40	—	0.097
–50	—	0.030
–60	—	0.008
–70	—	0.002
–80	—	0.0004
–90	—	0.00007

[a]Pressure of aqueous vapor over water and ice in mm Hg.

PROCESS OVERVIEW

DIAGRAM OF VIAL DURING PRIMARY DRYING



After this pressure is reached, heat is applied to the shelves to provide the energy required for sublimation of ice.

As drying proceeds, a receding boundary can be observed in the vial as the frozen layer decreases in thickness and the thickness of partially dried solids increases.

This phase is called primary drying

PROCESS OVERVIEW

When the ice is gone, additional drying time is request to remove water

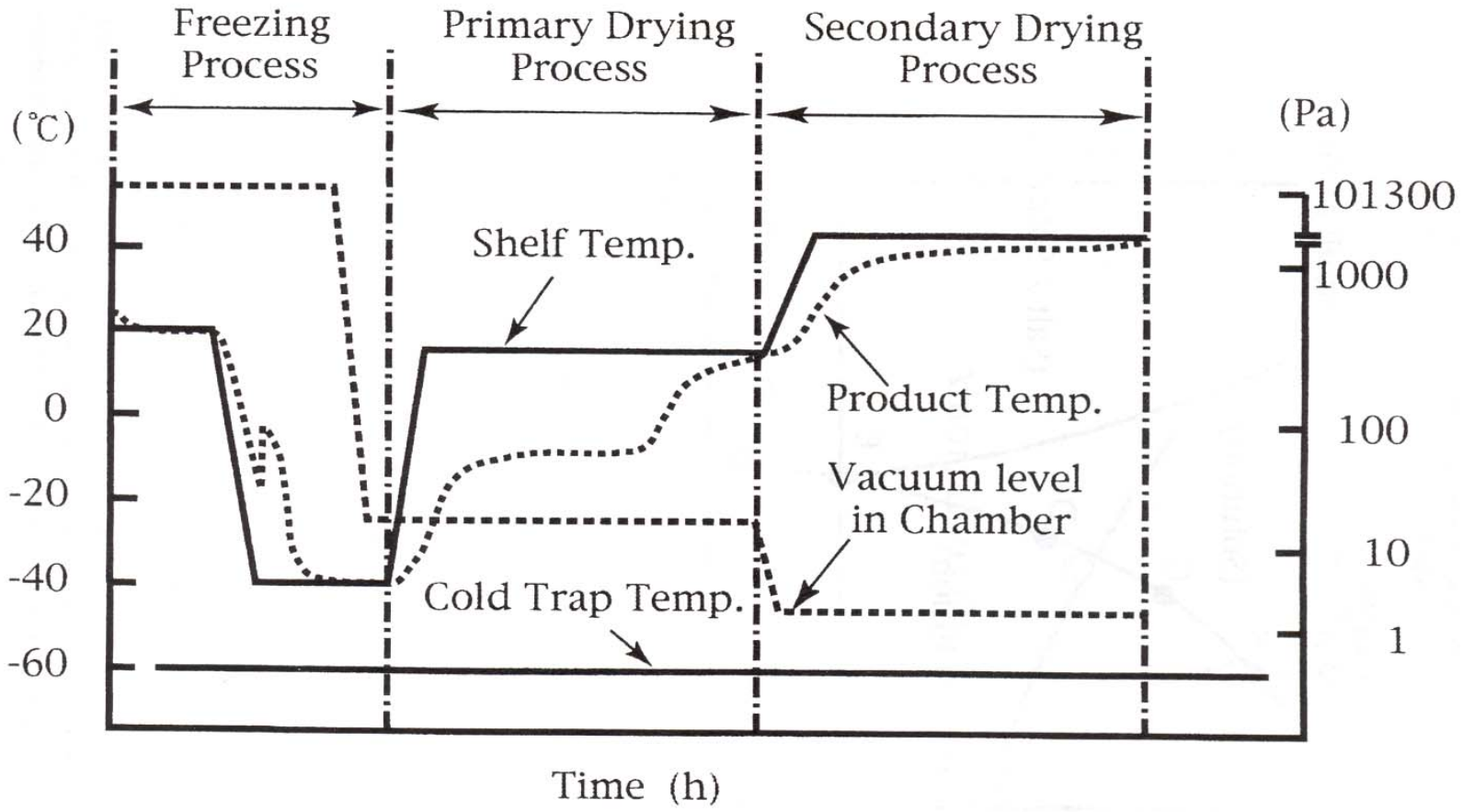
- adsorbed to, or

- trapped by,

the solid matrix.

This phase is called secondary drying.

PLOT OF PROCESS VARIABLES DURING FREEZE DRY CYCLE



PROCESS OVERVIEW

When the product is dry, the vials are stoppered in place within the drier by hydraulic compression of the shelf stack, pushing the stoppers to the fully inserted position.

This occurs either under a full vacuum or by back-filling the chamber with inert gas

PROCESS OVERVIEW

The most important objective in the developing a freeze dried product is to assure the quality requirements as:

- the original chemical or biological potency after reconstitution
- rapid and complete dissolution
- appropriate residual moisture level, and
- acceptable cake appearance.

This requirements have to be met not only initially but throughout the shelf life of the product.

PROCESS OVERVIEW

In addition, however, process conditions should be chosen to maximize process efficiency.

Of all drying operations, freeze drying is the most expensive both in:

- the capital investment and
- operating expense.

PROCESS OVERVIEW

Success in this challenge requires an understanding of:

- the physical chemistry of “frozen solution”
- heat and mass transfer under conditions encountered in freeze drying
- temperature and pressure measurement
- process monitoring
- general freeze drying system design considerations.

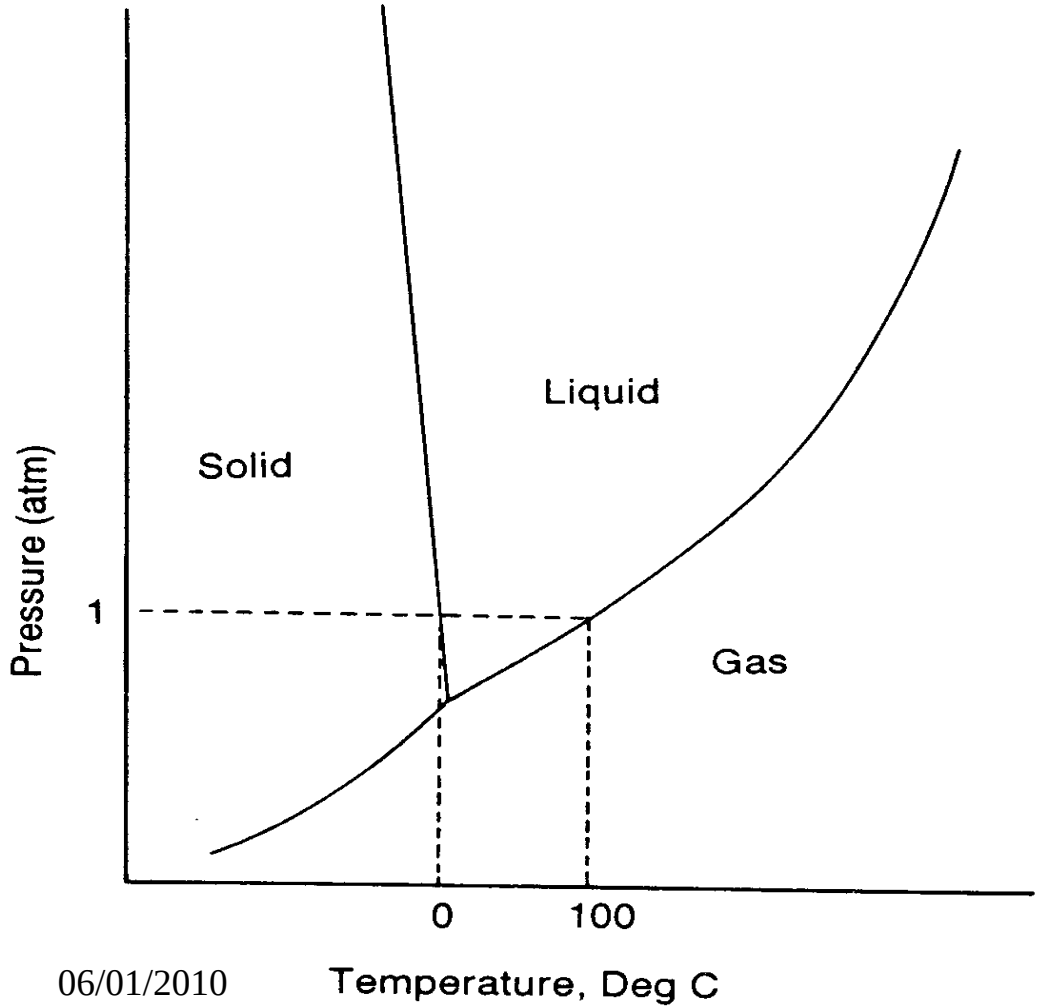
THE FREEZING PROCESS

Freezing is a critical step in the freeze drying process, since the microstructure (of both ice and solute) formed during freezing determines both:

- the quality of the final product, and
- its processing characteristics such as the rates of primary and secondary drying.

It is essential to know the physical events associated with freezing process-supercooling, ice crystallization (primary), concentration of the solutes during ice crystal growth and crystallization of solute (secondary crystallization).

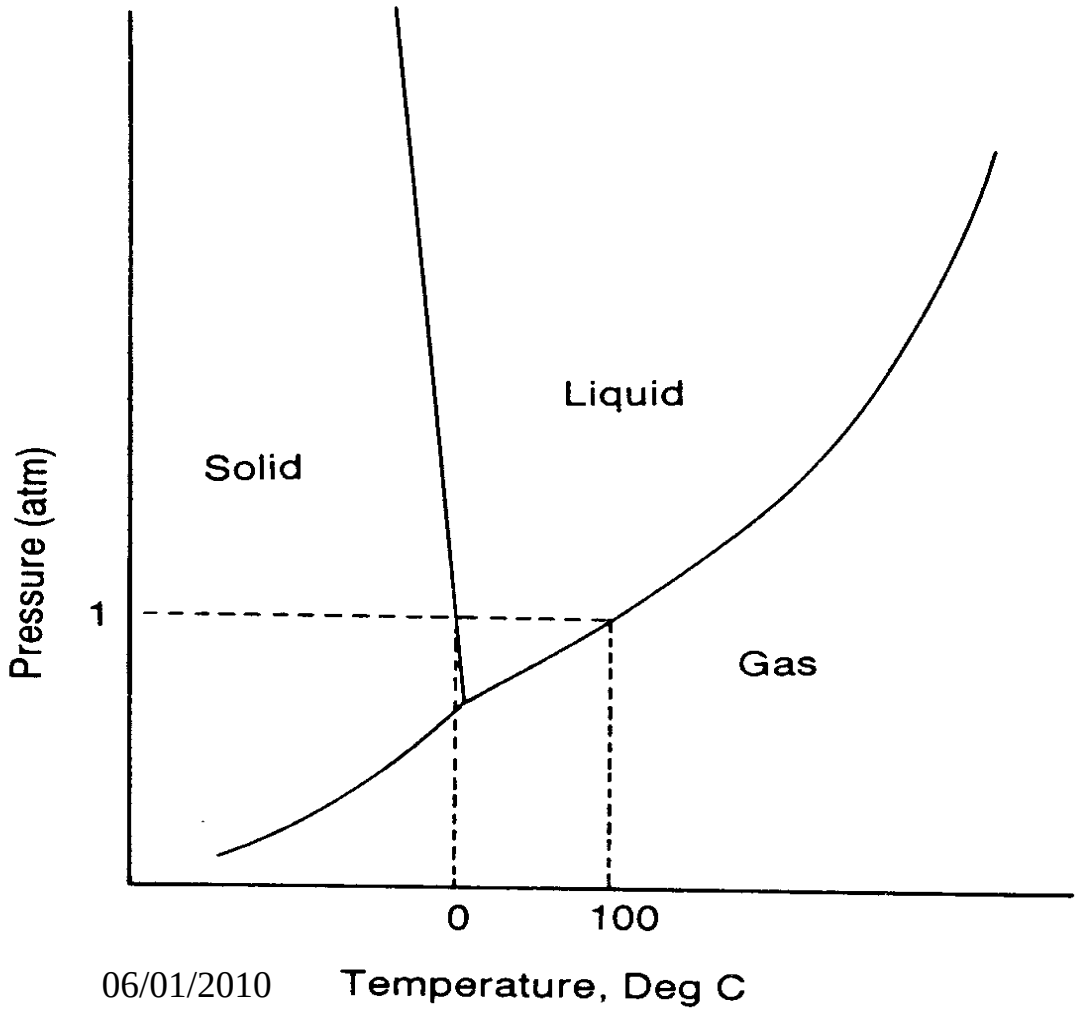
PHASE DIAGRAM OF WATER



At the triple point (*0.0098°C and 4.58 mm Hg*), ice , water and water vapor coexist in equilibrium.

Freeze drying takes place below the triple point, where water passes from solid phase directly to the vapor phase.

PHASE DIAGRAM OF WATER



The 4.58 mm Hg refers to the water vapor pressure, not the total system pressure.

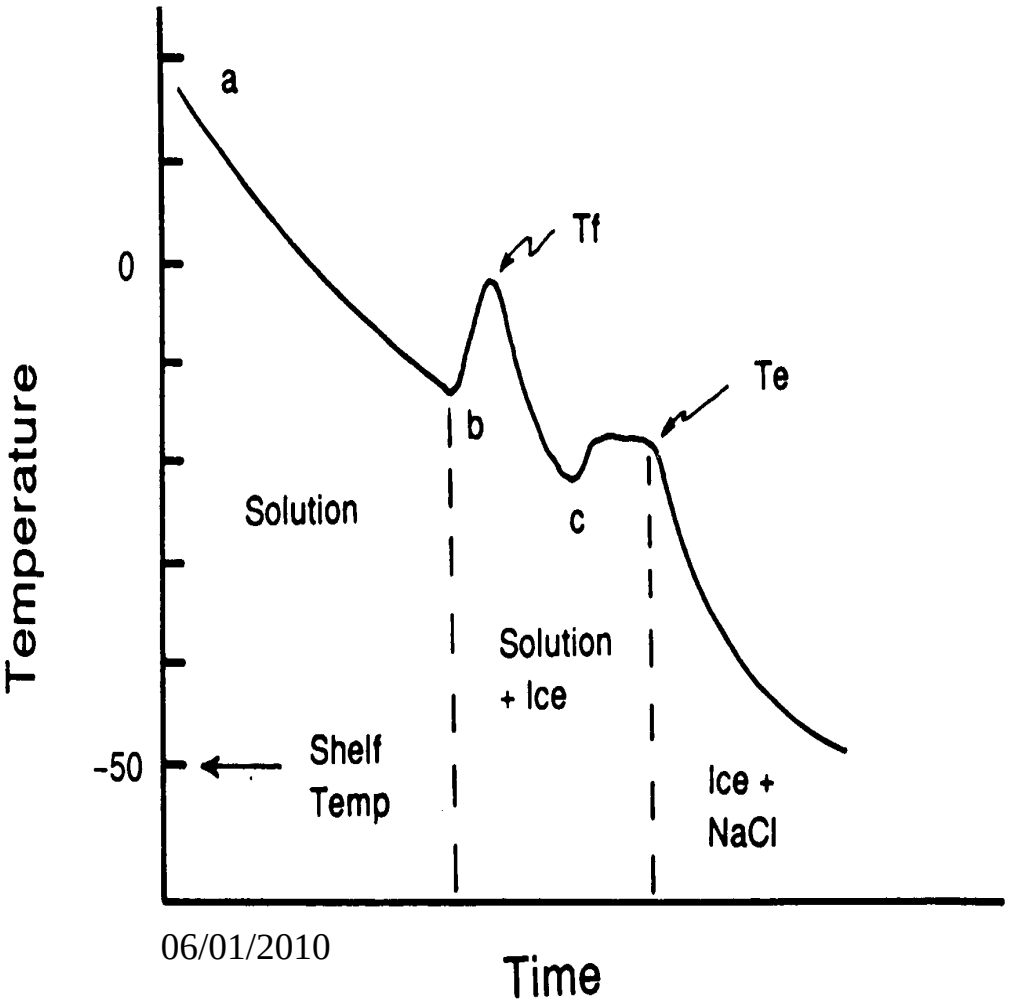
Sublimation can occur at atmospheric pressure as long as the water vapor pressure is below 4.58 mm Hg.

This "atmospheric freeze drying" is the phenomenon that causes "freezer burn" in home freezer.

It has been used by the Eskimos as an effective method of meat preservation.

FREEZING OF AQUEOUS SOLUTIONS

Temperature Vs. Time for Freezing of NaCl/water

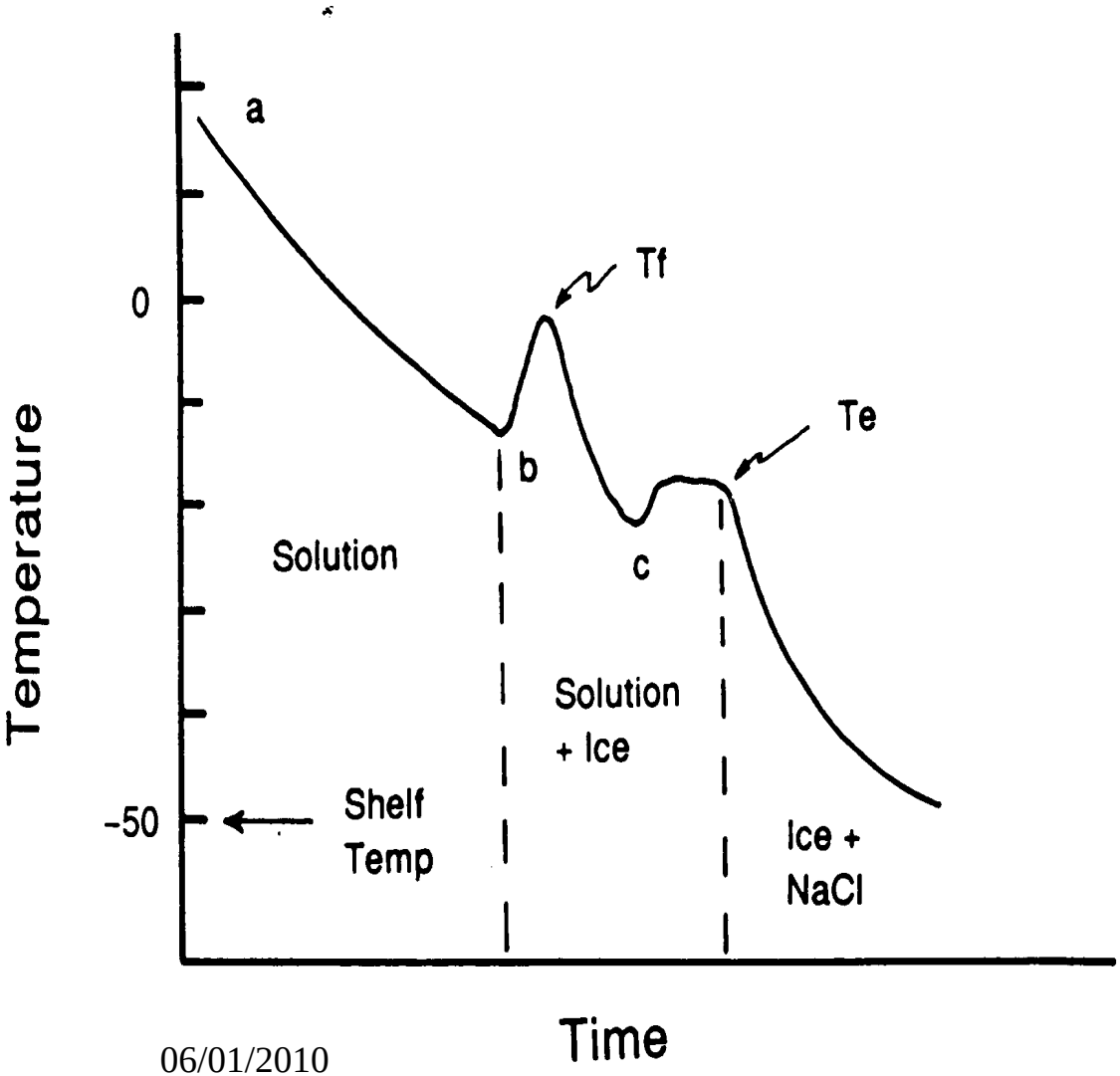


In the segment ab, the product temperature decreases to below the equilibrium freezing temperature ($\mathbf{T_f}$) of the product.

At the point b, nucleation of ice crystals occurs.

As nucleation and crystal growth of ice begins at b, energy is released (*the latent heat of fusion*) and the temperature increases to $\mathbf{T_f}$.

TEMPERATURE VS. TIME FOR FREEZING OF NACL / WATER

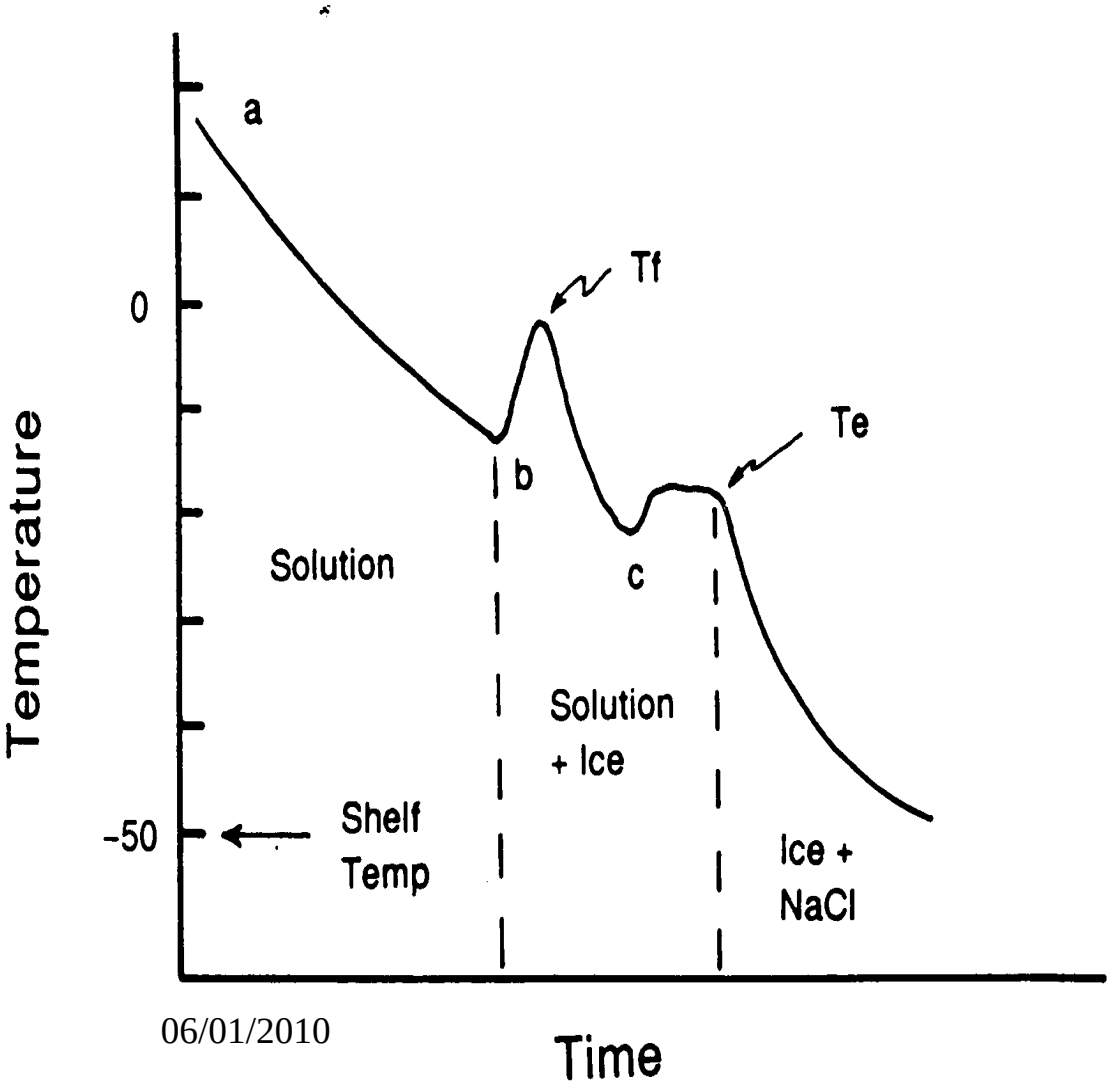


Cooling continues with ice crystal growing and the interstitial fluid becoming more concentrated.

At the point c, crystallization of concentrated interstitial fluid is initiated: an eutectic mixture of crystalline NaCl/ice.

A eutectic is an intimate physical mixture of two or more crystalline solids that melts as single pure compound

TEMPERATURE VS. TIME FOR FREEZING OF NACL / WATER

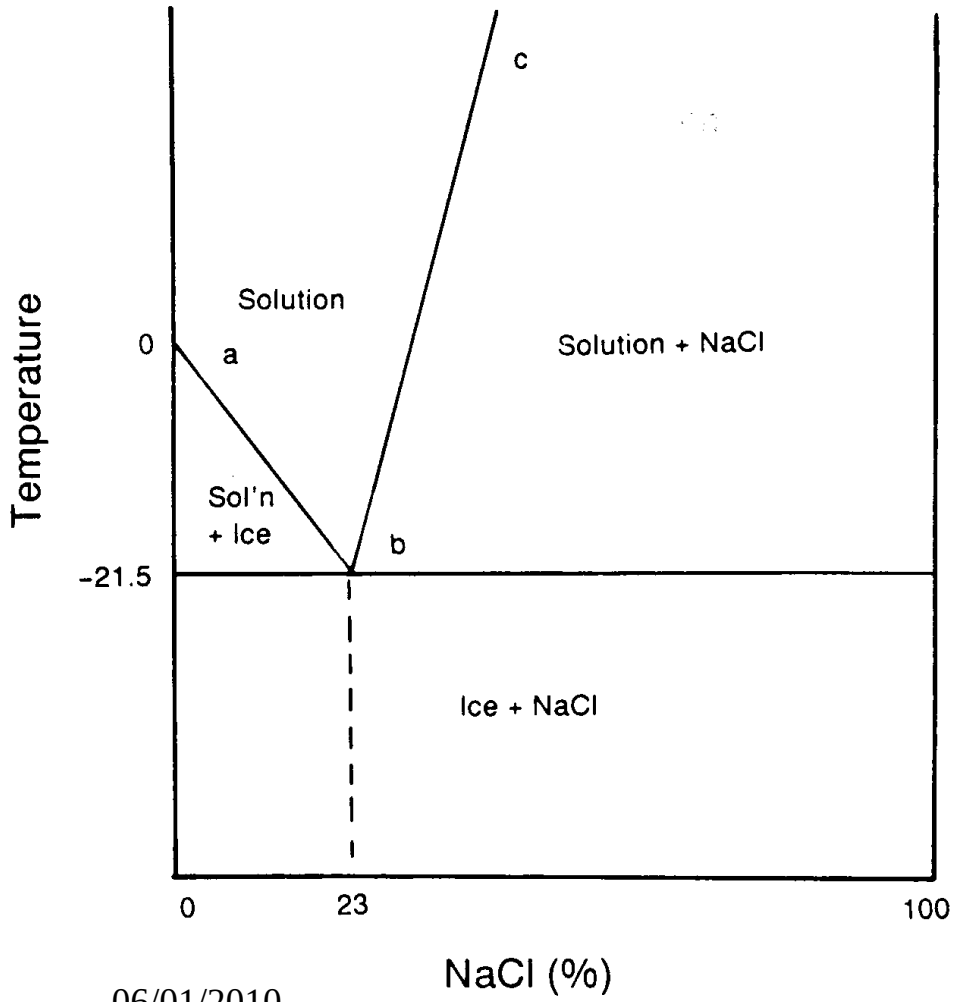


When eutectic crystallization is initiated, the temperature of the product increases to the eutectic temperature (**Te**).

After eutectic crystallization is completed at the point **Te**, no more liquid is present and no changes in microstructure of frozen system take place.

Then, the product temperature decrease more rapidly toward the shelf temperature.

Phase Diagram of NaCl/water



The line ab represents the equilibrium freezing temperature of water (**Tf**) as a function of NaCl concentration.

The line bc represents the solubility of NaCl in water.

The intersections of lines at point b is the eutectic point.

EUTECTIC TEMPERATURES FOR AQUEOUS SOLUTIONS OF VARIOUS COMPOUNDS

Citric acid	- 12.2°C
Glycine	- 3.5°C
Mannitol	- 1°C
Sodium acetate	- 18°C
Sodium carbonate	- 18°C
Sodiun chloride	- 21.5°C
Sodium phosphate, dibasic	- 0.5°C

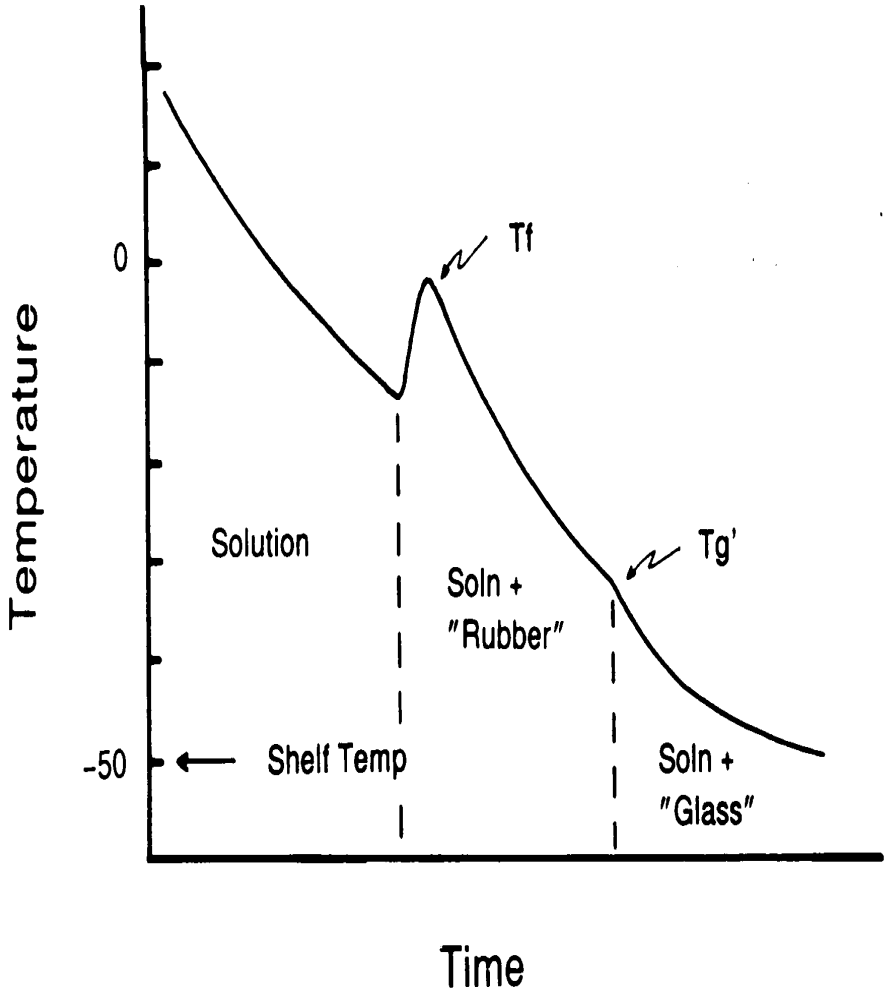
EUTECTIC TEMPERATURE

The eutectic temperature (*eutectic is an intimate physical mixture of two or more crystalline solids that melts as a single pure compound*) is important in freeze drying because represents the maximum allowable product temperature during primary drying.

If the product exceeds the Te, drying takes place from liquid instead of the solid.

However, eutectic behavior is only observed when the solute crystallizes.

TEMPERATURE VS. TIME FOR FREEZING OF AMORPHOUS SOLUTE



In the most cases, the solute does not readily crystallize during freezing.

The first part of curve is the same, then a secondary (eutectic) crystallization does not occur, but a slight change in slope of the temperature vs. time curve is observed at **Tg** (glass transition temperature).

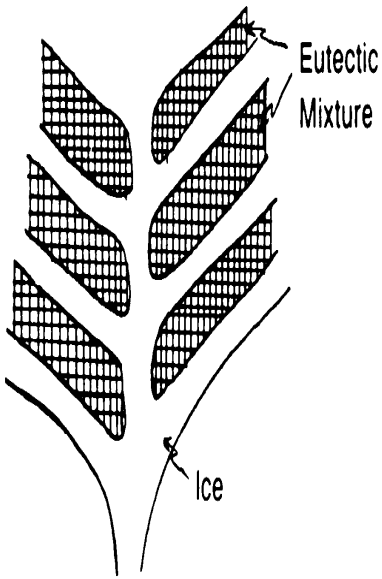
GLASS TRANSITION (OR COLLASPE) TEMPERATURE

For amorphous system, glass transition temperature (**Tg**) corresponds to a change in the viscosity of solution from a viscous liquid to a glass or an essentially solid solution of solute in water.

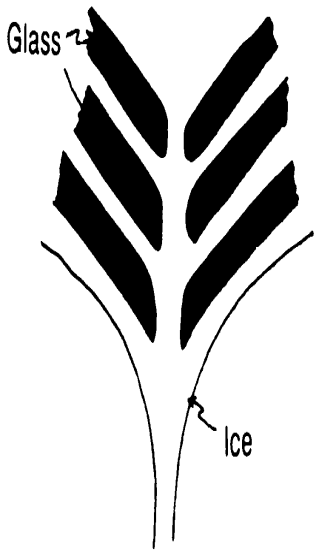
Tg is important for amorphous solute as **Te** for crystalline solute. It represents the maximum allowable product temperature during the primary drying.

If product temperature exceeds the glass transition temperature, the product will undergo collapse.

DRAWING OF MICROSTRUCTURE FOR CRYSTALLINE AND AMORPHOUS SOLUTES UPON FREEZING



a) Crystalline Solute



b) Amorphous Solute

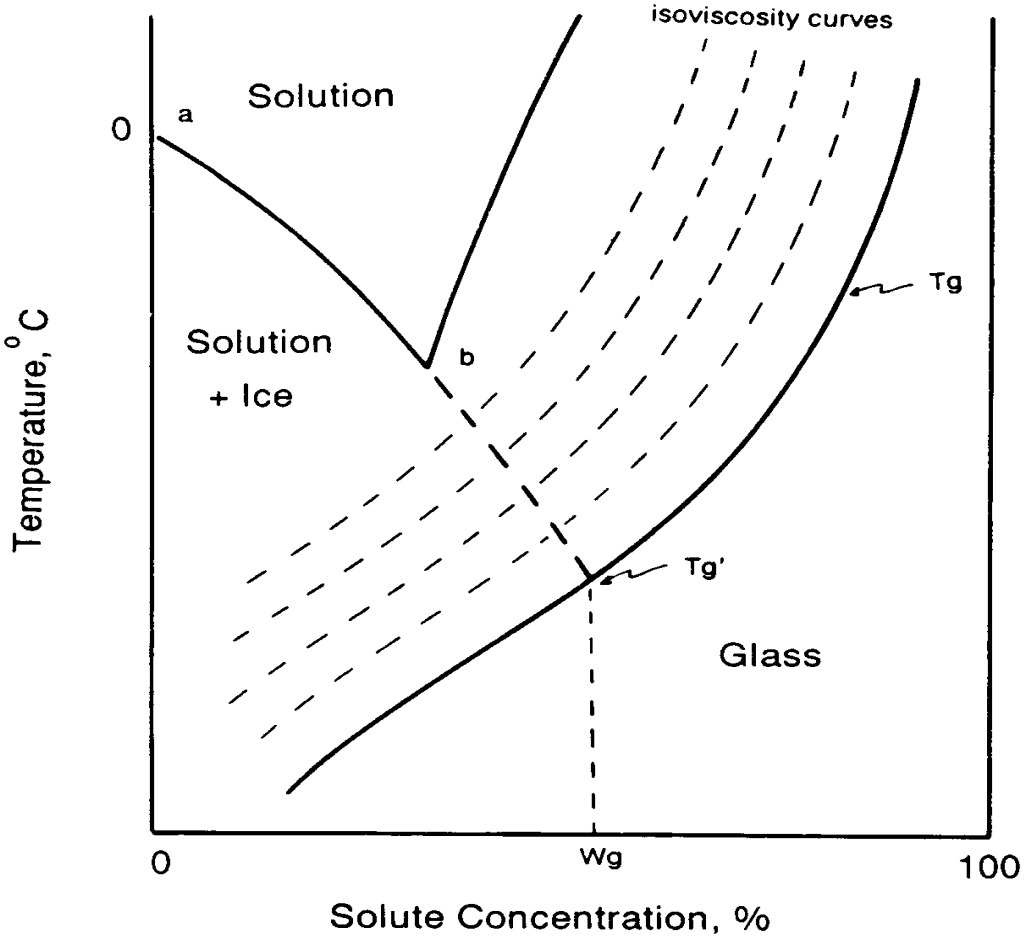
For the *crystalline* (a), the interstitial material consists of a mixture of eutectic ice and crystalline solute.

When the ice is removed by sublimation, a crystalline solid with 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 to keep the microstructure established during freezing.

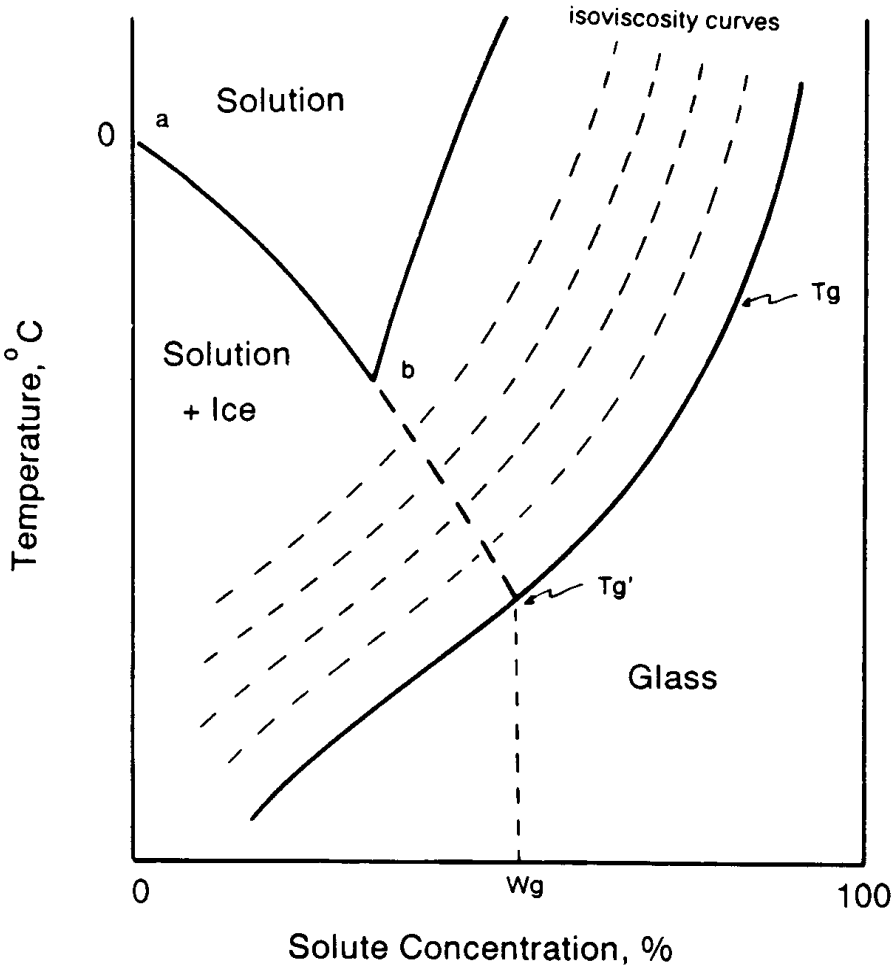
PHASE DIAGRAM FOR AN AMORPHOUS SOLUTE



The line ab represents the freezing temperature of water as a function of solute concentration.

Instead of the solute crystallizing at point b, the interstitial material remains as liquid, or freeze concentrate, and continues along line b-Tg.

PHASE DIAGRAM FOR AN AMORPHOUS SOLUTE

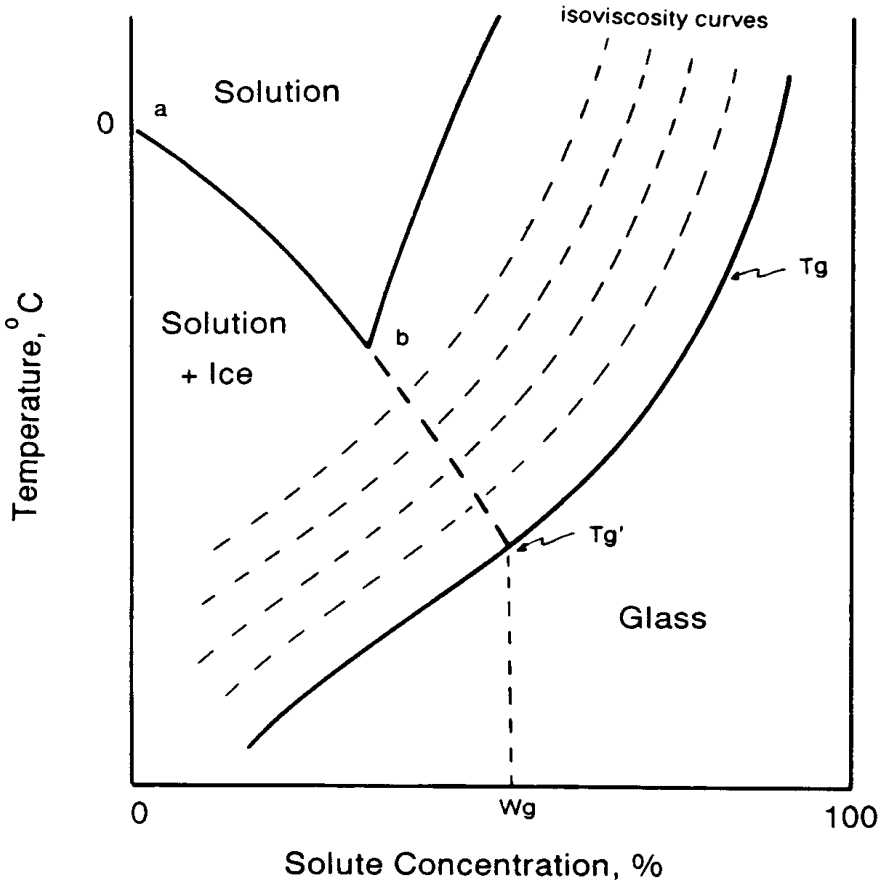


Ice crystals continue to grow, and the freeze concentrate become more concentrated and more viscous.

The family of curves shown by the dashed lines are iso-viscosity curves, i.e., combinations of solute concentration and temperature that result in the same fluid viscosity.

The solid line is the glass transition point of amorphous solid as a function of water content and is itself an iso-viscosity curve representing a viscosity of about 10^{14}poise.

PHASE DIAGRAM FOR AN AMORPHOUS SOLUTE



As freezing proceeds, the freeze concentrate becomes more viscous until the system reaches point Tg and the growth of ice crystal stops.

At the point Tg (the glass transition temperature of the freeze concentrate) the interstitial fluid changes from a viscous liquid or rubber to an elastic solid.

The concentration of unfrozen water in glass is represented by Wg.

GLASS TRANSITION TEMPERATURE OF VARIOUS COMPOUNDS

Dextran	- 9°C
Ficoll	- 19.5°C
Fructose	- 48°C
Glucose	-40 to 43°C
Sucrose	- 32 to 34°C
Maltose	-32°C
Trehalose	- 29.5°C
Sorbitol	-45 to 51°C
Lactose	- 32°C
Ovalbumin	- 10°C
Gelatin	- 8 to 10°C
Polyvinylpyrrolidone	-23 to 24°C
Methylcellulose	-9°C

THE PROCESS

The process is conventionally divided into three stages:

- Freezing
  Cooling the material until completely frozen

- Primary drying
  Sublimation of ice from product reducing pressure in the chamber and providing heat to the product

- Secondary drying
  Desorption of residual moisture from the product

PROCESS

The process of freezing involves:

(1) dissolving the drug and excipients in a suitable solvent, generally water

(2) sterilizing the bulk solution by passing it through bacteria-retentive filter (0.2 microns)

(3) filling into individual sterile containers

(4) freezing the solution by placing the open container on cooled shelves in a freeze drying chamber or pre-freezing into another chamber

(5) applying a vacuum to the chamber and heating the shelves in order to sublime the water from the frozen state.

CHARACTERISTICS

The desired characteristics of a freeze-dried pharmaceutical dosage form include:

(1) an intact cake occupying the same shape and size as the original frozen mass

(2) sufficient strength to prevent cracking, powdering, or collapse

(3) uniform color and consistency

(4) sufficient dryness to maintain stability (<2%)

(5) sufficient porosity and surface area to permit a rapid reconstitution.

CHARACTERISTICS

And, of course, freedom from contamination such as:

- micro-organisms (*sterile*),

- pyrogens *(5 Endotoxins Units/Kg),* and

- particulates (*less than 50 particles of 10 μm per container and less than 5 particles of 25 μm per container result)*

is an essential attribute.

The desired characteristics can be achieved by proper formulation of the product and by employing optimum freeze-drying cycles.

DEVELOPMENT

The development of a suitable formulation and a freeze-dry cycle requires knowledge of some basic properties, such as:

- eutectic temperature
- temperature effect on solubility
- thermal properties of the frozen solution
- degree of super-cooling
- heat transfer properties of the freeze-dryer shelves, the metal trays, the containers and the frozen product
- equipment design and equipment capability.

DEVELOPMENT A SUITABLE FORMULATION FORMULATION

In developing a formulation of freeze drying, the optimal formula will permit the overall cycle to be carried out in the least amount of time, while providing a stable and efficacious product which:

- contains a low moisture content,

- undergoes rapid reconstitution, and

- possesses the desired appearance.

SOLVENTS

The solvent is generally water.

Organic solvents can be added up to 20%:

- to promote wetting and/or solubilization of the drug
- to reduce the degradation rate of the drug in water during processing
- to induce crystallization of the drug in the frozen state.

ORGANIC SOLVENTS

Organic solvents may be present as a residual impurity (less than 2% in pharmaceutical powder).

Most commonly organic solvents include:

- ethanol,
- n-propanol,
- n- and tert-butanol,
- iso-propanol,
- ethyl acetate and
- dimethyl carbonate.

ORGANIC SOLVENTS

The presence of organic solvents affects the efficiency of the freeze-drying process:

- with frozen liquid residue (*e.g., tert-butanol*) the products dry mainly by direct sublimation and the drying process is usually rapid

- with unfrozen liquid residue (e.g., *ethanol, iso-propanol*) the products dry more slowly and there is a risk of meltback or collapse.

Solvent melting points:

- *tert-butanol, 25.5°C*
- *ethanol, - 110.5°C*
- *iso-propanol, - 85.8°C.*

ORGANIC SOLVENTS

Moreover, when lyophilizing organic-aqueous systems, the freeze-dryer needs to be modified to handle such solvents:

- the condenser have to be linked to a refrigerated solvent trap, otherwise an extra condenser is needed, more effective as refrigerating capacity

- the unit system must be equipped with selected solvent-resistant gaskets.

Although non-aqueous solvents have certain useful properties, their use is limited because they are not as easy to handle as water and give serious toxicity problems for the products administered parenterally.

ADDED SUBSTANCES

Bulking agents

For very low dose products, the minimum practical fill volume results in a solute concentration so low that the dried product layer formed does not have sufficient mechanical strength to withstand the force of flowing water vapor during primary drying: the dried product is “blown” all over the drier.

Too low an initial solids concentration may also result in dry cake of fluffy consistency, that fails to cohere and which is extremely hygroscopic.

The need for a suitable of bulking agent is often indicated to provide the necessary bulk and desired characteristics.

Generally, bulking agent should be above 2% and not exceed 30%, with 5 to 15% content being optimum.

ADDED SUBSTANCES

Bulking agents

Most commonly used bulking agents include:

- mannitol,
- lactose,
- dextran,
- sorbitol,
- sucrose,
- dulcitol,
- gelatin,
- bovine serum albumin,
- glycine,
- polyvinylpyrrolidone,
- sodium chloride,
- ficoll 70 (a branched polymer synthesized from sucrose and epichlorohydrin).

ADDED SUBSTANCES

Rigidizers or collapse temperature modifiers

If a substance is vulnerable to collapse, a rigidizer such as *glycine or mannitol* may need to added.

It is important to point out that dilution with a bulking agent is also a way to avoid meltback or collapse.

One may also try to raise the collapse temperature of a formulation.

Potential collapse temperature modifiers with the respective collapse temperature are:

- dextran (-10°C),
- ficoll (-20°C),
- human serum albumin (-9.5°C),
- gelatin (-8°C),

Sugar may contribute towards collapse because they do not crystallize.

ADDED SUBSTANCES

Cryoprotectant agents

If the damage during freezing is a problem, a cryo-protective agent such as sugars (*sucrose, trehalose*) or bovine serum albumin may be added.

Buffer agents

If degradation is a risk during freezing due pH change, buffers

(*acetate, citrate, phosphate, glutamate*) may be added to maintain the pH in a region desirable for the stability of a drug.

Tonicity adjusters

Tonicity adjusters to avoid hemolysis or crenation of red blood cells in isotonic solution, e.g., NaCl 0.9% and dextrose 5%, the cell maintain their “tone”

(*hypotonic solution < 280 mOsm/kg; Hypertonic solution >360 mOsm/kg*).

ADDED SUBSTANCES

Antioxidants

They prevent oxidation of the drug (*sodium bisufite and metabisulfite, tocopherols, butylhydroxyanisole, ascorbic acid,)*

Preservatives

They avoid risk of contamination in containers intended for multiple injections (*benzalkonium and benzethonium chloride, benzyl alcohol, methyl and propyl parabens, chlorobutanol*)

Surfactants

They help solubilization (poloxyethylene sorbitan monooleate, sorbitan monooleate).

ADDED SUBSTANCES

Especially for potent drugs, *adhesion* of the active substance to the glass surface is experienced.

In such cases, the vials may be internally coated with silicone.

The *depth of fill* in a container is critical.

While this depends on the volume of the container, a rule of thumb has been *1 to 2 cm* in depth, but never exceed *one-half* the capacity of the container.

FREEZING OF AN AQUEOUS SOLUTION

Freezing is the *reduction* of the temperature of the product to *induce* crystallization of the bulk of the contained water before primary drying.

The freezing process can have a very important *effect* on the appearance and the properties of the final product.

The *crystal size* formed during freezing can significantly affect the dissolution rate of the dried material.

FREEZING OF AN AQUEOUS SOLUTION

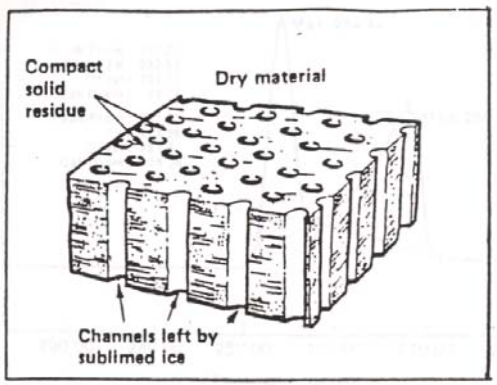
Generally the *slower rate* of freezing, the *larger* the ice crystal that form.

Slow freezing can subject the drug to concentrated solutions for longer periods of time, permitting maximum chance for crystal growth.

Usually, the *small crystals* formed during rapid freezing result in a product which has a *fast solution rate*.

A fast ice growth also help to prevent the denaturation of proteins (if present) which may result from prolonged exposure to strong concentrations of salts because of slow ice growth.

FREEZING OF AN AQUEOUS SOLUTION



Pores left by sublimation of pure ice in the solid residue after freeze-drying.

On the other hand, the *main pores* in the solid residue after freeze-drying are those left by the *sublimation of pure ice* and they form the principal channels for the escape of vapour.

During sublimation, very small ice crystals form smaller pores and pathways, which are more restrictive to vapour flow than those formed after slower freezing.

FREEZING OF AN AQUEOUS SOLUTION

Freezing of the solution to be freeze-drying is most conveniently accomplished in the chamber to be employed for drying (internal freezing), by placing the containers of solution (tray, vials ampoules) on a shelf that is cooled by a circulating refrigerant, such as Freon, Cellosolve or thrichloroethynene)

It is sometimes economical to carry out the freezing in a separate installation the frozen material is then transferred to the shelves of the freeze-dryer.

FREEZING OF AN AQUEOUS SOLUTION

Freezing in a separate installation is usually done for one of the following reasons:

- to store *unstable material* until a complete freeze-dryer load is ready
- to achieve *maximum utilization* of the freeze-dryer for drying
- successive freezing of *layers of different fluids* is more easily carried out
- filling time into the containers can be prolonged if filling *machine capacity* is limited.

FREEZING OF AN AQUEOUS SOLUTION

However, freezing products outside the chamber has certain disadvantages:

- increased chance of *contaminating* the product because of the need for extra handling
- possibility that the frozen product may *partially melt* during transfer to the freeze-dryer
- moisture from the atmosphere may *condense* and *form frost* on the containers of the frozen product or on the necessarily pre-cooled shelves of the freeze-dryer while it is being loaded.

  This frost:

  -makes loading difficult,

  -increases the quantity of ice to be sublimed in the early stages of primary drying,

  -may cause the containers to make poor thermal contact with the shelves in the freeze-dryer
- increased risk of *breakage* of the containers and loss of product
- increased *labour* is required, increased *floor area* is needed and more *electrical power* is consumed.

FREEZING OF AN AQUEOUS SOLUTION

Thus, in general, these are good reasons for preferring an installation in which freezing is carried out in the freeze-dryer.

In any case, where practicable, the depth or thickness of the material should be minimized for short freezing and freeze-drying cycles.

A typical thickness is 10-15 mm.

STAGES OF FREEZING

The initial freezing process is of critical importance since it will influence the pattern of the sublimation phase.

The latter phase must occur from the solid state throughout the cycle.

Thus, appropriate cooling cycles must be determined in order to obtain an appropriate structure of the frozen mass, which is a function of the rate of freezing and the final freezing temperature.

STAGES OF FREEZING

In general, the freezing of an aqueous binary solution consisting of a solute in water may be considered to occur as follow:

- assuming the *solubility of the solute* is high enough so that it is not deposited on cooling, *ice crystals* first form at a temperature usually *below 0°C* as a consequence of supercooling effect

- as the ice crystals form and grow, the remaining solution ("*interstitial fluid*") becomes more and more concentrated in the solute

FREEZING OF AN AQUEOUS SOLUTION

- if the solute forms a true eutectic with water, an eutectic phase - *consisting of finely divided crystals of the solute and ice* - crystallizes out.

  The *highest* temperature at which the whole system becomes solidified is termed the maximum *temperature of complete solidification*, $T_{cs.}$

  This is the temperature at which *no liquid states* exist in the product and it is a state that must be achieved if a solution is to be considered a freeze-dried.

FREEZING OF AN AQUEOUS SOLUTION

- If the solute does not crystallize (i.e., does not form a true eutectic), it is transformed into a *rigid glass* when the system is brought below the *glass transition temperature* of the amorphous phase (Tg).

  This parameter describes the temperature at which there is a fundamental change in the physical properties of the product, which does not reflect a change in state, but rather a change in the macromolecular mobility: below the Tg product mobility is severely restricted.

  The amorphous phase consists of *uncrystallized solute and uncrystallized water.*

THERMAL TREATMENT

Compounds that do not form true eutectic are *difficult to dry* successfully because during the drying process, as the product temperature rises, the glassy structures soften and the matrix collapses making it necessary to reject the batch.

It is *often possible* to crystallize a solute system that tends to remain amorphous or to promote the growth of the ice crystals by following a freezing procedure referred to as *thermal treatment* (or tempering or annealing).

THERMAL TREATMENT

Thermal treatment consists of:

- first freezing the product at low enough temperature

- warming it gradually to a predetermined temperature well above the glass transition temperature

- holding there for a sufficient period of time to allow any metastable state to crystalize out,

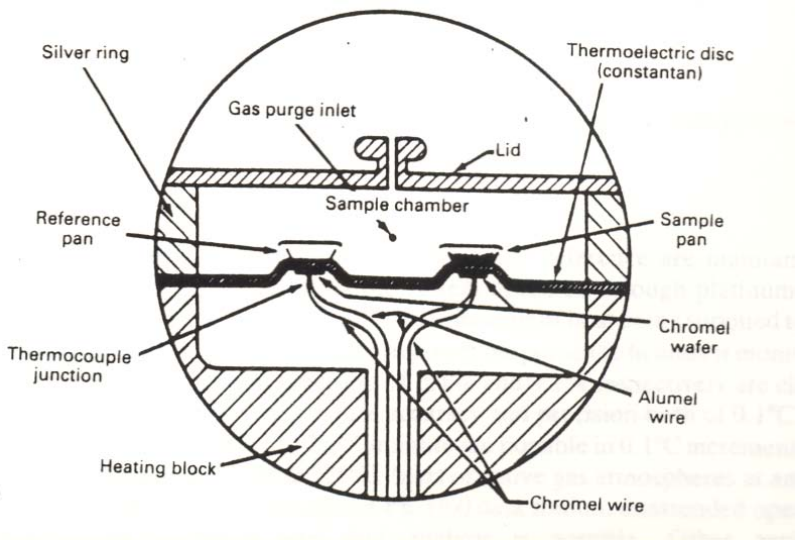
- and then cooling it again to suitable temperature before initiating primary drying .

TRANSITIONS OF PHASE

The phase transitions in the frozen state occur and influence the properties of the dried product.

A better understanding of the transitions which occur during the warming of frozen systems would permit *better control and optimization* of freeze-drying cycles in order to provide a finished product of higher quality.

TRANSITIONS OF PHASE



Differential Scanning Calorimetry (DSC) can be usefully employed to determine the phase transitions in the frozen systems.

In DSC, the temperatures of the sample and a suitable reference (*which does not undergo any transition in the temperature range of interest*) are compared as both are heated or cooled simultaneously and at the same rate.

The aim is to keep the temperatures of the sample and reference equal.

TRANSITIONS OF PHASE

When the sample undergoes *a transition*, its temperature differs from that of the reference and heat either flows to or away from the sample.

If heat flow occurs toward the sample, then it must have undergone an endothermic change and viceversa. The heat flow is displayed as a peak in the thermogram.

Second order transitions (e.g., *glass transition*), involving only a change in the heat capacity of the sample, are displayed as a change in the slope of the baseline.

The output signal is proportional to the difference in energy needed to keep the sample and reference temperatures equal. Thus, the DSC monitors thermal events quantitatively as well as qualitatively.

TRANSITIONS OF PHASE

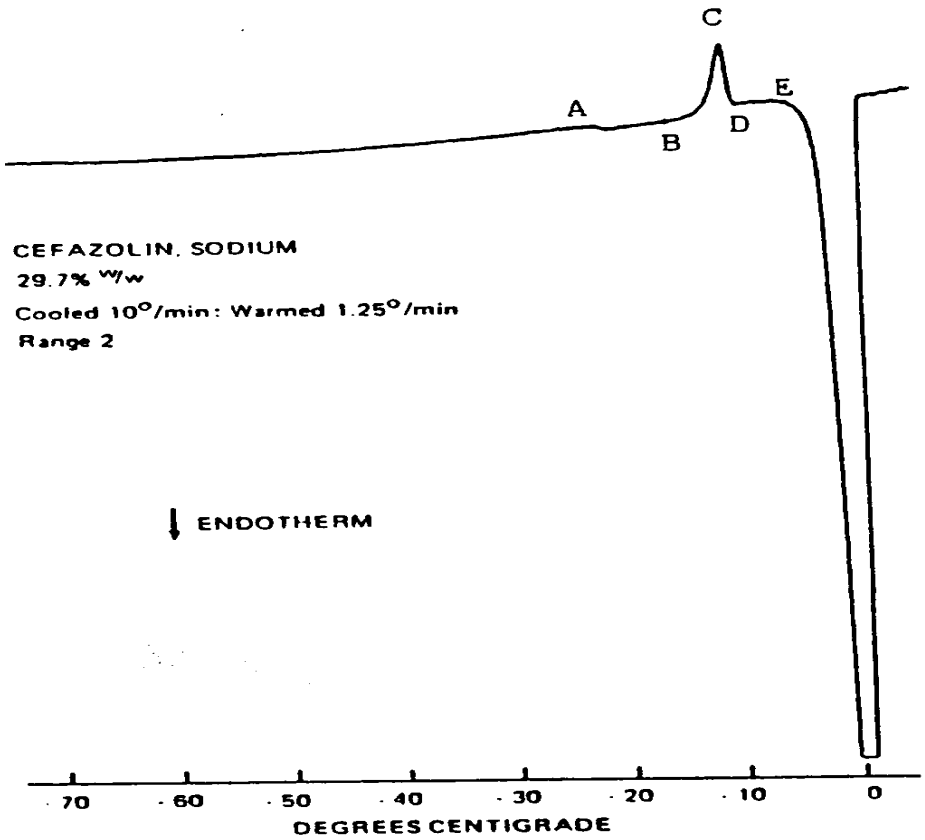
Transitions such as *melting* involve an equilibrium and therefore are *reversible.*

A reversible thermal event is one which following warming and recooling, the thermal event will reoccur when warmed again.

Transitions such as *crystallization* and *polymorphic changes* may not be in equilibrium and thus are *irreversible.*

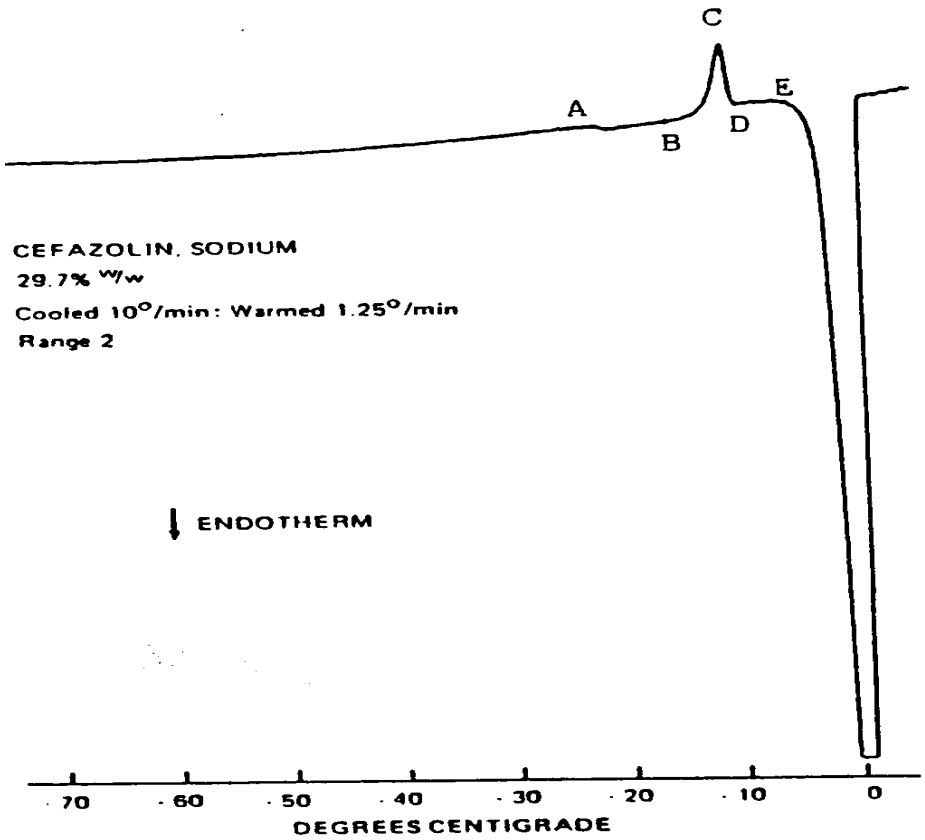
An irreversible thermal event is one which following warming and recooling, the thermal event will not reoccur when warmed again.

THERMAL TREATMENT



Gatlin and De Luca observed some features of the low temperature DSC thermograms of some antibiotic solutions, which provide a base for thermal treatment them to obtain crystals of the drug.

THERMAL TREATMENT



The thermogram shows:

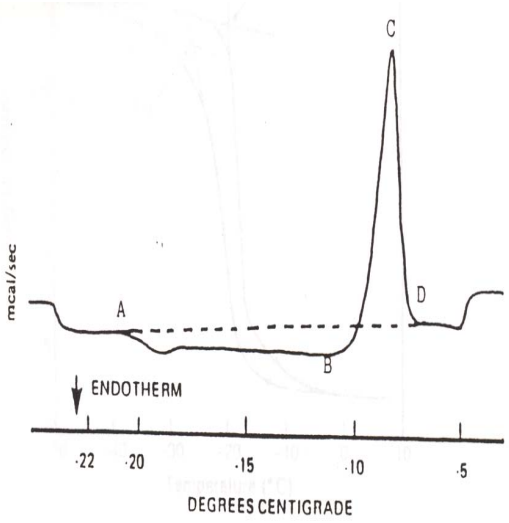
- a first endothermic shift occurring at -20°C (*Point A*)

- an irreversible exotherm beginning at -11°C (*Point B*)

(which can be interpreted as representing the crystallization of the solute during warming)

- melting of ice (endothermic shift) beginning at -4°C (*Point E*)

THERMAL TREATMENT



Endothermic and exothermic areas of the thermogram of cefazolin sodium. Solid curve corresponds to warming following freezing to -30° C; dashed line corresponds to the warming curve of the previous solution which was recooled after warming to -6° C.

Considering the portion of the curve beginning just below the initial endotherm and to just above the irreversible exotherm, if the frozen solution is warmed to just beyond the exotherm but below -4°C (say -6°C) and then the system is recooled to -25°C, upon rewarming, the thermogram in dashed line is obtained.

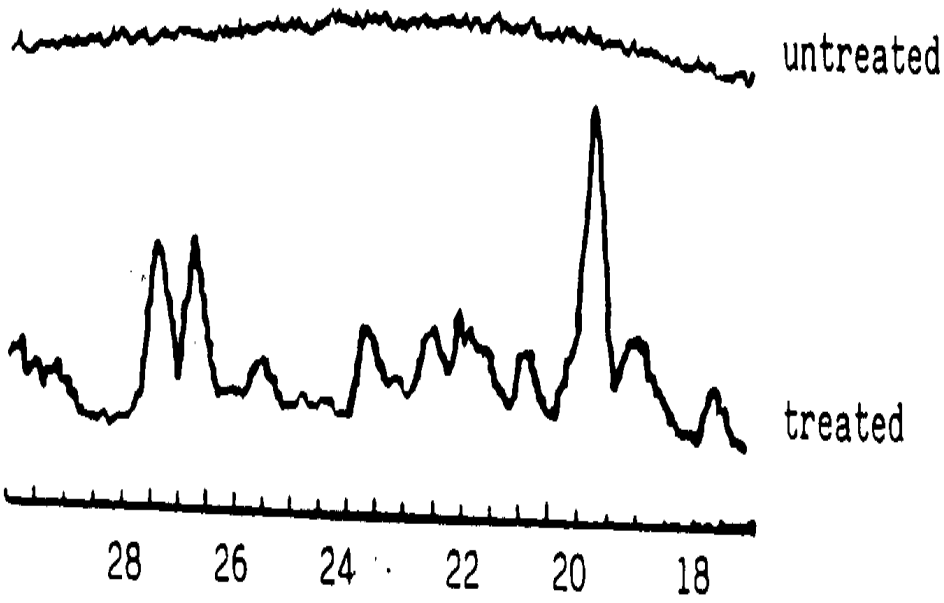
THERMAL TREATMENT

Material submitted to thermal treatment exhibits:

- birefringence under optical microscopy
- defined shape by SEM
- X-ray diffraction pattern consisting of peaks of various intensity.

All of these are indications of a crystalline structure.

X-RAY DIFFRACTION SPECTRA OF FREEZE DRIED CEFAZOLIN



The upper tracing is of material dried without thermal treatment.

The lower is of material which was thermally treated before drying.

INDUCTION TO THE CRYSTALLIZATION

Induction to the crystallization of pharmaceutical compound can be also accomplished by:

- humidity treatment
- excipient addition
- solvent addition.

CRYSTALLIZATION OF ACTIVE COMPOUND

Crystallization of active compound is desirable for the following reasons:

- firstly, the *stability* of a drug is usually *greater* if it is present in the crystalline form than the amorphous form
- secondly, the crystalline drug can be *dried* at a *higher temperature* than the amorphous
- thirdly, a crystalline solid can be *dried faster* than the corresponding amorphous form because of the higher melting temperature

Increase of the sublimation rate of the ice, thereby decrease the drying time.

On the contrary, a advantage is a possible decrease in solubility or an increase in reconstitution time.

CRITICAL TEMPERATURES

Before designing an optimum freeze drying cycle for a solution, two critical temperatures need to be determined:

- **Tcs** or *temperature of complete solidification*

It is the highest temperature at which any liquid state ceases to be present during cooling

- **Tim** or *temperature of incipient melting*

It is lowest temperature at which liquid state begin to appear during warming.

CRITICAL TEMPERATURES

These two values are used in the freeze drying cycle as follow:

- **Tcs** is the minimum temperature at which the solution must be cooled to have a cake completely frozen

- **Tim** is the maximum temperature at which the product must be kept during sublimation to avoid melting or other damages

METHODS FOR STUDYING FREEZING CHATACTERISTICS

The methods currently used to study the freezing drying characteristics of solutions are :

-Thermal Analysis
(DSC cooling and warming)

- Electrical Resistance
(mobility of the ions)

- Freezing/Freezing Drying microscope.

METHODS FOR STUDYING FREEZING CHATACTERISTICS

Thermal Analysis

It may be usefully employed:

- DSC cooling thermograms may be used to determine the temperature of complete solidification (T_{cs})

- DSC warming thermograms may give a direct measurement of Tg and the eutectic temperature

METHODS FOR STUDYING FREEZING CHATACTERISTICS

Electrical Resistance

This method involves the simultaneous monitoring of resistance and temperature of a frozen sample.

Resistance measurements offer an advantage over thermal analysis in the estimation of T_{cs} and T_{im} because conductance (*and, therefore, resistance*) in solution depends on the mobility of the conducting species.

When a solution is frozen, irrespective of whether it is in a glassy or crystalline form, its resistance increases because of the reduced mobility of the ions.

Electrical Resistance

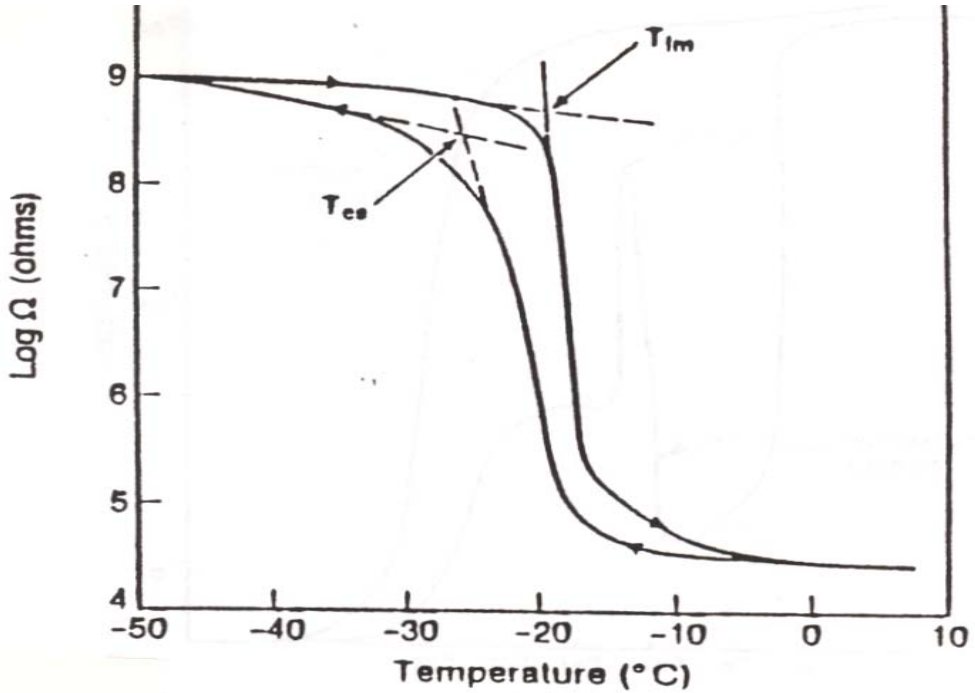


Diagram showing an "ideal" resistance-temperature profile for a solution (scales are arbitrary). Arrows on curves indicate cooling and warming cycles. T_{cs} : temperature of complete solidification; T_{im} : temperature of incipient melting.

METHODS FOR STUDYING FREEZING CHATACTERISTICS

Freezing/Freezing Drying microscope

The freezing microscope, unlike all the above methods, allows direct observation of the sample being frozen or warmed.

Typically, the solution is trapped between two glass slides or cover slips, and cooled (or warmed) while being observed.

A vacuum pump and a temperature control unit may be connected to the slide and the whole device becomes a freeze-drying microscope which permits observation of the entire lyophilization process.

This device has found very useful application in lyophilization, especially in the study of collapse in freeze-dried preparations.

SUPERCOOLING AND DEGREE OF CRYSTALLIZATION

When an aqueous solution is cooled, the water in the solution almost undergoes some degree of supercooling before crystallizing out as ice.

This means that no ice forms at the *thermodynamic or equilibrium freezing point*.

The ice usually nucleates and crystallizes after supercooling below the equilibrium freezing point.

The degree of supercooling depends on:

- the nature of the solutes
- the freezing procedure
- the container, and
- the presence of particulate matter.

SUPERCOOLING AND DEGREE OF CRYSTALLIZATION

The degree of supercooling is important in determining the size of ice crystals formed: *a higher degree of supercooling produces smaller ice crystals*.

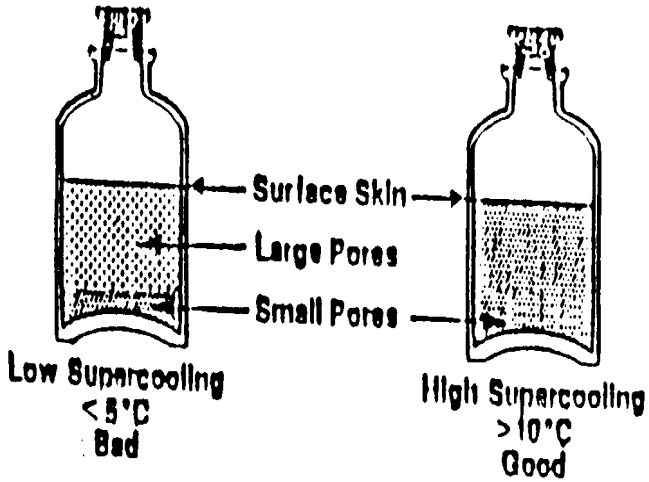
The size of the ice crystals determines the size of the pores (or channels) created during ice sublimation and determines the surface area of the porous solid produced by the sublimation process.

Thus, the degree of supercooling affects:

- the rate of sublimation (*large ice crystals create large pores, leading to rapid sublimation*), and

- the rate of secondary drying (*large ice crystals create a small surface area, leading to slow secondary drying*).

DEGREE OF SUPERCOOLING



According to Jennings:

- a *high* degree of supercooling leads to homogeneous network of fine pores
- a *low* degree of supercooling yields a heterogeneous plug structure with a *thick* skin on the surface and a *very fine pores* on the bottom.

The presence of a glaze on the surface of the product (which may result from slow ice growth from the bottom of the vial) may retard the sublimation of ice from product.

The presence of a fine structure/coarse structure boundary may be responsible for collapse at the boundary.

DEGREE OF SUPERCOOLING

Thus, in general, a moderate/high degree of supercooling (10-15°C) is desirable.

Most important, the degree of supercooling should be uniform, both within a given vial and within the entire batch of vials.

In practice, this is not so easy to obtain, because of the variations in the cooling process and in the product.

The following is a tentative methodology to obtain a moderate degree of uniform supercooling:

- Minimize solution depth
- Moderate shelf/solution temperature difference (~20C).

DEGREE OF SUPERCOOLING

If necessary, a tempering process may be followed to assure uniformity in degree of supercooling.

This procedure involves first cooling all product to a temperature lower than 0°C but higher than the temperature that will cause nucleation and crystallization (typically, -5° to -10°C).

The shelf temperature is then decreased (typically -20° to -30°C) to induce crystallization of ice in all containers.

When sufficient time has elapsed to result in ice crystallization in all containers, the shelf temperature is lowered below the temperature of complete solidification.

The final product temperature during freezing is typically about -40°C.

Once the system is completely solidified, primary drying may begin.

PRIMARY DRYING

Primary drying is a problem in coupled heat and mass transfer, and heat and mass transfer issues must be recognized to achieve process optimization.

Mass transfer may be discussed in terms of resistance to the flow water vapor through the various mass transfer barriers (*which are partially dried product, stopper openings, chamber-to-condenser pathway*).

The heat is supplied from shelf (*by means of circulating fluid*) and is transferred mainly by conduction through the frozen matrix to the sublimating front.

RESISTANCE TO THE FLOW

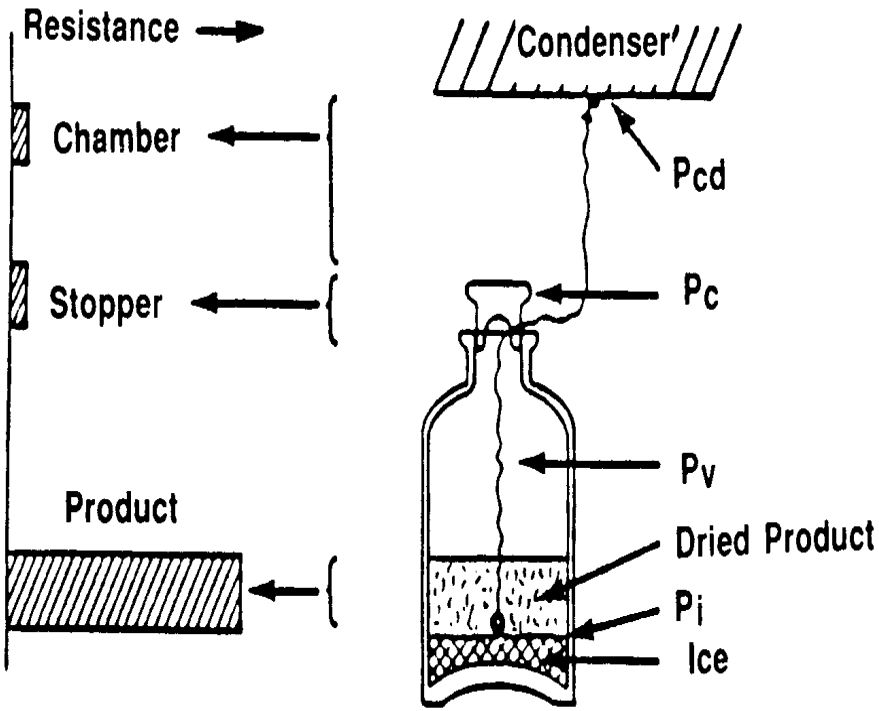
The resistance to the flow may be defined as the ratio

- of *driving force for sublimation* expressed by the pressure difference across the barrier

- to the *flow through the barrier* represented by the sublimation rate.

$$\text{Resistance to flow} = \frac{\text{Pressure difference across the barrier}}{\text{Sublimation rate}}$$

Typical pressures in primary drying and various resistances



P_i and P_{cd} are determined by the vapor pressure of ice at the front of sublimation and the condenser surface, respectively.

The resistance of dried product typically accounts for over 90 % of the total resistance to mass transfer

RESISTANCE OF THE DRIED PRODUCT

The resistance of the dried product accounts for over 90% of the total resistance and depends:

- the nature of the product
- the cross-sectional area of the product
- the thickness of dried product.

Dried product resistance decreases as:

- vials diameter increases
- product thickness decreases
- solute concentration decreases.

It also generally decreases:

- as the temperature of frozen product approaches the eutectic temperature or the glass transition temperature

- if larger ice crystals are produced by a tempering process during freezing.

SUBLIMATION RATE

The *sublimation rate* per vial (dm/dt) may be expressed in terms of *driving force* for transport of water vapor from ice-vapor interface to the chamber (p_o - p_c):

$$dm/dt = (p_o - p_c) / (r_p - r_s)$$

where:

p_o = equilibrium vapor pressure of ice at the temperature of the frozen product

p_c = pressure in the drying chamber

r_p = dried-product resistance

r_s = stopper resistance.

SUBLIMATION RATE

Because p_o - which is *equilibrium vapor pressure of ice* at the temperature of the frozen product - *increases exponentially* with the temperature, the sublimation rate *increases* dramatically as the product temperature increases

About 13% for each 1°C increase in temperature.

The key to successful drying is to remove the water vapor from the frozen cake *without allowing liquid water* to form.

In other words, operate conditions at or just *below* the eutectic or collapse point.

EUTECTIC MELTING AND COLLAPSE

Eutectic melting involves the melting of the eutectic phase and therefore occur throughout the frozen matrix. It results in drying by evaporation of water from liquid phase.

Collapse is essentially the *amorphous* system analog of a eutectic melt.

If the product temperature *rises above* the collapse temperature, the amorphous solute-water system gains *sufficient fluidity* to undergo viscous flow once the ice in that region has sublimed.

Thus, the dried region adjacent to the ice will “flow” and lose the structure.

EUTECTIC MELTING AND COLLAPSE

The collapse temperature and the glass temperature are closed related (for practical purposes are identical)

Methods of preventing collapse include:

- addition of a solute which crystallizes, or one with a high collapse temperature
- thermal treatment to cause the metastable water to crystallize out
- careful control of freeze-drying conditions.

HEAT TRANSFER

The transfer of heat to the product is generally done by means of circulating a fluid through the shelf on which vials (ampoules or trays) are placed.

Thus the heat is supplied from below and transferred mainly by conduction through the frozen matrix to sublimating front.

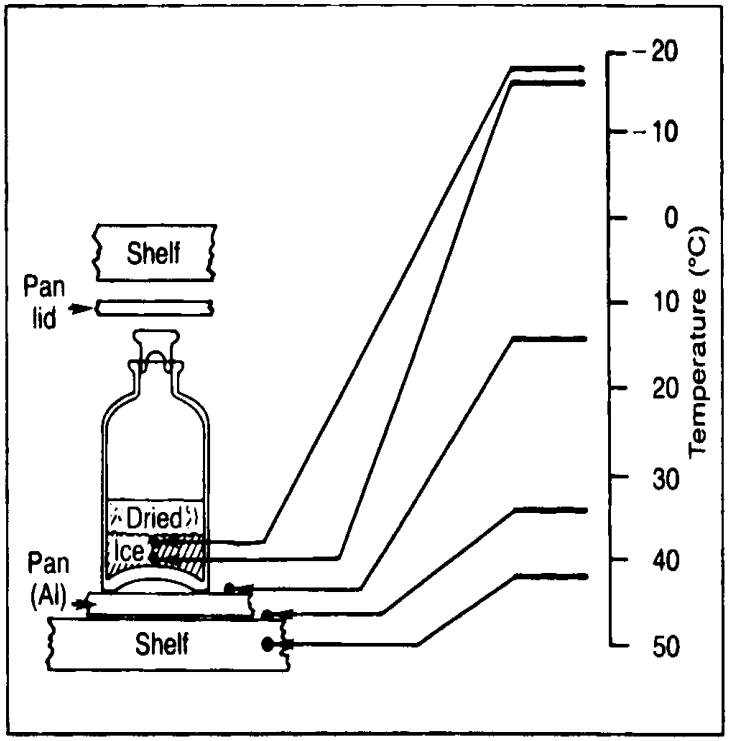
It has to pass through four barriers:

- the shelf
- the tray (if present)
- the glass vial
- the frozen solution.

The temperature of the frozen interface determines the vapor pressure of the ice and the driving force for sublimation.

This temperature is different from that of the supporting shelf, or the product container, as a temperature gradient is needed to ensure the flow of heat to the product.

Temperature profile in primary drying



- The temperature difference between the shelf surface and interior, 8°C, is a thermal barrier that represents imperfect heat transfer within the shelf itself.
- The temperature difference between the shelf surface and the top surface of the tray bottom is 20 °C,
- that between the product in the bottom of the vial and the pan surface is 30°C
  and
- that between the ice at the vial bottom and the ice at the sublimation interface is about 2 °C.

HEAT FLOW

For vials resting directly on the freeze-drier shelf, the vials heat coefficient, k_v, is defined by:

$$dq/dt = a_v\, k_v(t_s - t_b)$$

where:

dq/dt = heat flow (cal/s) from shelves to the product in a given vial,

a_v = *cross-sectional area of the vials calculated from the vial outer diameter,*

t_s = *temperature of the shelf surface, and*

t_b = *temperature of the product at the bottom center vial.*

Therefore, the *heat-transfer coefficient* (Kv) is defined as the ratio of the area-normalized heat flow to the temperature difference between heat source (the shelf) and heat sink (the frozen product).

HEAT-TRANSFER COEFFICIENT

The vial heat-transfer coefficient is the sum of three contributions representing three parallel heat-transfer mechanisms:

$$k_v = k_c + k_r + k_g$$

where:

k_c= contribution from direct conduction between shelf and glass at the points of actual contact,

k_r= contribution from irradiative heat transfer, and

k_g= contribution from conduction through the gas between the shelf and the vial bottom.

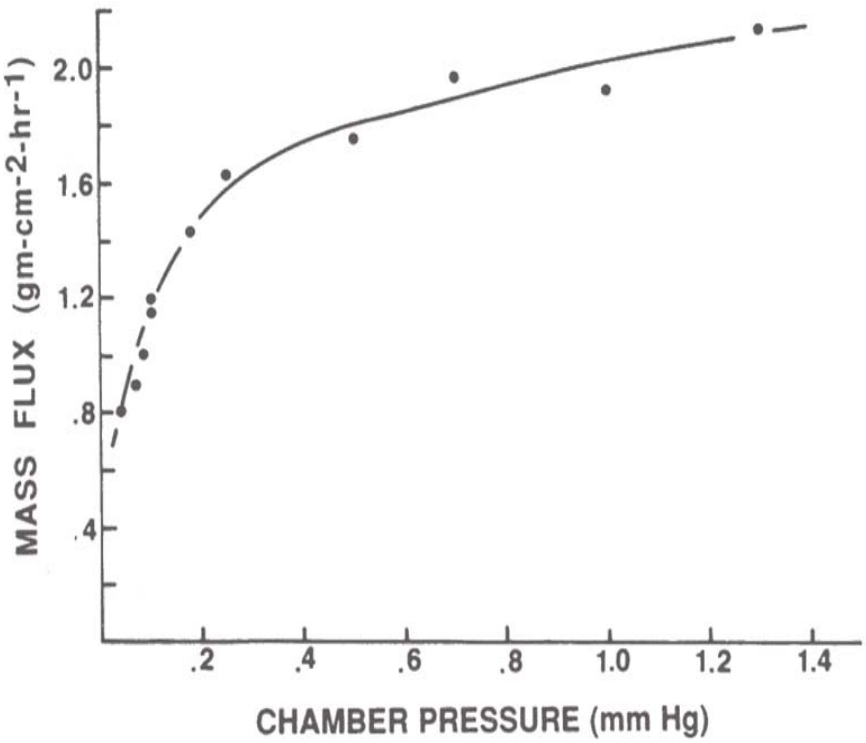
k_g increases with increasing pressure, due to the increased number of gas molecules to conduct heat through collisions between gas molecules and the two surfaces (vial bottom and shelf surfaces)

EFFECT OF CHAMBER PRESSURE ON PRIMARY DRYING

As the chamber pressure increases, the vial heat-transfer coefficient increases, thereby transporting more heat to the product at a fixed shelf temperature (*more gas molecules conducting heat*) and increasing the product temperature (*increasing p_o, therefore the driving force*).

For a given formulation, fill volume, container, and freezing process, the chamber pressure and shelf temperature sequence with time determine the product temperature (*essentially constant at a safe level below the eutectic or collapse temperature; safe margin is 2-5°C*).

EFFECT OF CHAMBER PRESSURE ON PRIMARY DRYING



Primary drying rate is more dependent upon pressure at low pressure than at higher pressure.

The primary drying rate at 0.5 mm Hg is approximately twice as fast as at 0.05 mm Hg at a constant shelf temperature.

EFFECT OF CHAMBER PRESSURE ON PRIMARY DRYING

The general rule is that chamber pressure should be significant lower than the vapor pressure of ice at the target product temperature (*in the range of 10-30% of the vapor pressure of ice*).

If the target temperature is -33°C (*that is, collapse -30°C*) the vapor pressure of ice is 0.21 mm Hg, and the chamber pressure should be about 0.06 mm Hg.

END POINT OF THE PRIMARY DRYING

Primary drying ends when all ice in all product containers has been removed.

Indications of the end of primary drying are:

- product temperature:

The product begins to warm up and its temperature rises to roughly reach that of the shelf

- pressure rise test:

The valve separating the driving chamber and the condenser chamber is periodically closed, and the rate pressure increase is monitored. If the rate significantly exceed the leak rate, ice is must still be present, and the valve is opened to continue with primary drying.

SECONDARY DRYING

Secondary drying involves the removal of absorbed water (*water which did not separate out as ice during freezing, even 20%)* from the product, to reduce the residual moisture to an optimum value for stability (*usually 0.5-2.0*).

Typical additional times are 0.35 to 0.5 times the primary drying times.

The product temperature is usually raised (*by increasing shelf temperature*) and the chamber pressure further reduced.

END POINT DETERMINATION OF SECONDARY DRYING

During the secondary drying the product temperature generally rises gradually and equals the shelf temperature.

Pressure rise test is an effective method to indicate the end point of secondary drying.

The chamber isolated from the condenser and pumping system and the rise in the chamber measured.

The rise in chamber pressure is directly proportional to the residual moisture of the product.

OPTIMIZATION OF THE LYOPHILIZATION PROCESS

Development of an optimized lyophilization cycle, designed on a scientific basis through the knowledge of the most important physico-chemical parameters which may affect product quality and stability.

The objective of freeze-drying process development is to minimize the process time while maintaining high product quality.

CYCLE TIME AND WORKING DAYS

In a typical industrial situation the working will allow for 220 to 240 days.

An ideal cycle is 22 hours, which gives 4 cycles per week.

34 hours or 46 hours cycles give respectively 3 or 2 cycles week.

USE OF MICROPROCESSOR

The use of microprocessor now makes it possible to run freeze-drying cycles automatically from beginning to end while controlling the heating rate and the chamber pressure.

While this is an important advancement, it does not remove the need for a thorough understanding of freezing characteristics of each drug solution, and the factors which influence the rate drying.

Understanding the freeze-drying process thoroughly can significantly reduce processing time and therefore reduce costs, ensuring the quality of the final product.

CONCLUSIONS

Freeze-drying has often been carried out in an empirical "trial and error" manner.

The preceding discussion attempts to show however that every stage of lyophilization is governed by certain principles which need to be understood if the process is to be optimized.

The study of thermal events and related phase transitions as well as the use of microprocessors are the foundations to approach rationally the freeze-drying process.

CONCLUSIONS

A strict cooperation a pharmaceutical technologist, thermal analysis scientist and the designer of freeze-dryer is essential to achieve the goal:

a freeze-dried product with the best attributes and stability.

FREEZE DRY EQUIPMENT

A freeze dry system for production of pharmaceutical dosage forms consists of:

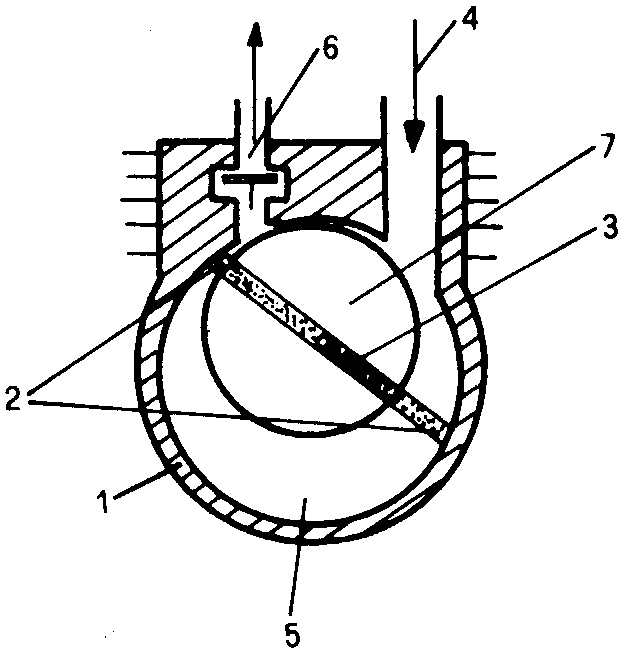
- a *chamber* containing shelves through which a heat transfer fluid can be circulated
- a system for *pumping, heating, and cooling* the fluid
- a *vacuum pumping* system
- a *condenser* for trapping water vapor, and
- a *refrigeration system* for cooling the condenser.

FREEZE DRY EQUIPMENT

In addition these essential components, pharmaceutical freeze dryers may incorporate systems for:

- sterilization of the chamber/condenser
- stoppering vials within the chamber
- automatic cleaning in place (CIP), and
- automatic loading and unloading of vials
- computerized monitoring and control.

VACUUM SYSTEM



The most common type of vacuum pump in freeze drying is the rotary oil pump.

It consists of a steel cylinder rotating eccentrically within a round casing.

The gas being pumped is admitted into the casing via an inlet valve, compressed, and forced out a discharge valve.

Oil serves both as a lubricant and a sealant to prevent back diffusion of gas past the rotating cylinder.

Rotary oil pump are able to achieve vacuum as low as about 1 μm Hg.

SPEED OF A VACUUM PUMP

The speed of a vacuum pump is given by pumping speed (S) in the equation:

$$dP/dt = S / C (P-Ps)$$

where:

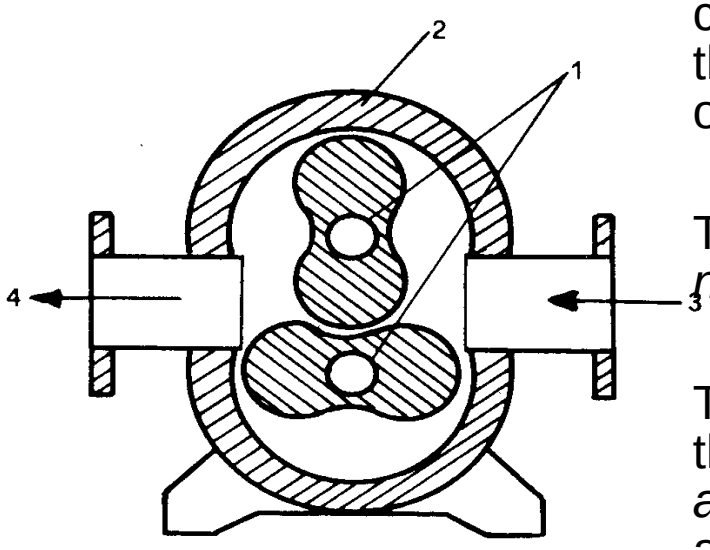
dP/dt= rate of change of pressure with time

S=pumping speed (volume/time)

C=volume of system to be evacuated

Ps=lowest attainable pressure.

VACUUM PUMP



Roots pumps are frequently used and comprise two figure eight-shaped rotors that counter-rotate without touching each other or the chamber walls.

There are *no inlet or discharge* valves, and *no oil* or other fluid for lubrication.

The function of roots pumps is to increase the speed of the pumping system by *about a factor 10*, and to also increase the lowest attainable vacuum.

They are always used in combination with another pump such as a *rotary oil pump.*

REFRIGERATION

It is required both for *cooling the shelves* during freezing of product and for *cooling the condenser* during drying.

The condenser is generally *cooled by direct expansion of the refrigerant*, usually a *fluoro-hydrocarbon*, in the condenser coils.

Generally, refrigeration can be switched from a condenser to the heat transfer fluid during freezing of product, and back to the condenser during drying.

REFRIGERATION

The refrigerant evaporates in the condenser coils, withdrawing the latent heat of sublimation from the condenser.

Vapor is drawn from the freeze drying condenser by a compressor and pumped to a condenser at a higher pressure.

In the condenser, cooling water causes the compressed vapor to liquefy, and the condensed refrigerant is collected in a receiver.

The liquid refrigerant is returned to the cooling coils via an expansion valve and the cycle is repeated.

HEAT TRANSFER FLUID

The most common types of heat transfer fluid are silicone oil, trichloroethylene (TCE) and Lexol, an oil similar to kerosene.

Silicon oil is by far the most common; TCE has been phased out due to safety concerns.

STERILIZATION OF FREEZE DRYERS

The most common method of sterilization of freeze dryers is steam under a pressure of about 15 psi, which corresponds to a temperature of about 121°C.

Some units are sterilized by ethylene oxide (under regulatory scrutiny).

VACUUM INTEGRITY TEST

The vacuum integrity of the freeze dryer chamber/condenser should be monitored, since a leak of non-sterile air into the system will compromise asepsis.

This is easily done by evacuating the system to a known pressure (100 μm), closing the valve between the vacuum pump and the freeze dryer, and monitoring the increase in pressure for at least 15 min.

SUMMARY

Freeze drying provides a valuable tool to the pharmaceutical scientist by permitted dehydration of heat-sensitive drug or biologicals at low temperature.

The final product is quickly and easily reconstituted, and the process is compatible with aseptic operations.

The trend in parenteral manufacturing is toward developing technology that automatically monitors the critical variables (temperature and pressure) and controls throughout the process removing the operators from direct interaction with products .