УНИВЕРЗИТЕТ
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ШТИП

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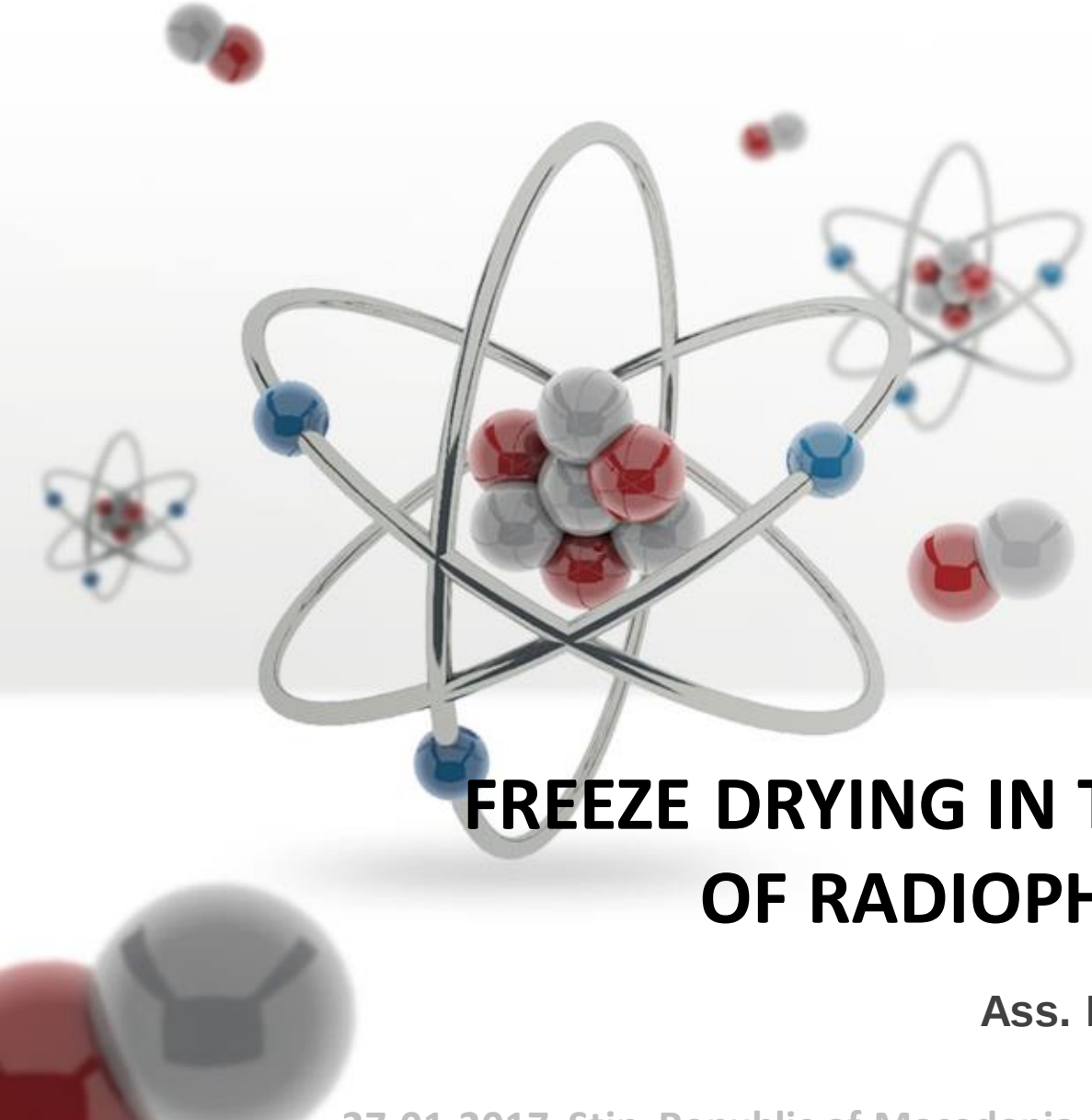

FREEZE DRYING IN THE PRODUCTION OF RADIOPHARMACEUTICALS

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MAIN COMPONENTS OF GMP

- Adequate premises, space, equipment and materials, qualified and trained personnel
- Clear definition of manufacturing processes and validation of critical steps in the process and any significant changes to the process
- Approved instructions and procedures for production, quality control (QC), product release, etc..
- Quality assurance (QA) and QC activities, independent of production
- Records of manufacture and complete batch history
- Controlled product release
- Suitable storage and transport of finished products
- Means to recall any batch from sale or supply, examination of complaints and investigation of quality defects

Kit production

- ✓ clearly defined procedures as detailed in the SOPs and the batch processing record
- ✓ production must be performed and supervised by competent, well trained people.
- ✓ QC at defined points

Validation steps in kit production

- Sterile filtering of the solution containing the active ingredient and excipients
- Dispensing of the sterile filtered solution
- Freeze-drying

GENERAL PROCEDURES FOR PRODUCTION OF KITS

- Washing and sterilization of glassware and stoppers
- Preparation of bulk solution
- Sterile filtration
- Dispensing
- Freeze-drying
- Crimping
- QC

CLEAN AIR CLASSIFICATIONS WITH REFERENCE TO ISO-14644 STANDARDS

ISO class	Grade (class)	Maximum permitted number of particles/m^3 of air			
		At rest		In operation	
		Particles ≤0.5 µm	Particles ≤5 µm	Particles ≤0.5 µm	Particles ≤5 µm
5	A (100)	3 500	<1	3 500	<1
6	B (1 000)	3 500	<1	350 000	<1
7	C (10 000)	350 000	2 000	3 500 000	20 000
8	D (100 000)	3 500 000	20 000	Not defined	Not defined

Washing and sterilization of glassware and stoppers

- **Grade D** environment is required for washing of glassware and stoppers.
- After final sterilization, the glassware should be placed in a **grade C** environment
- Sterilized glass vials and stoppers should be placed in a **grade A** environment with a **grade B** background shortly before filling.

VIals:

1. Washing with hot purified water.
2. Washing with WFI
3. Drying
4. Heating in an oven at 250^{o}C for 3 h for depyrogenation.

Washing and sterilization of glassware and stoppers

Rubber stoppers:

1.2.3. same as vials

4. Steam sterilization in an autoclave at 121^{o}C for 30 min.

- ✓ materials should be processed (with a small excess amount) for a single batch at a time
- ✓ After sterilization, the materials should be used for production on the same day and not be stored for later use
- ✓ Sterilization records should be filed for each sterilization run and should be approved as a part of the batch release procedure.

Preparation of bulk solution

*Weighing, dissolution, mixing of starting materials (approved by QC previously) in a **grade C** environment*

Stock solutions for the manufacture of kits should be prepared in a grade C environment **in a laminar flow bench**

- Weighing and dissolution of excipients (stannous chloride, buffer components, etc..) in appropriate dilluent (water, dilute hydrochloric acid, etc..)
- Weighing and dissolution of the ligand (active substance)
- Mixing, heating and stirring of the dissolved ingredients
- Adjustment of the pH to the required value

Additional preparation – sterile nitrogen bubbling in RP to be labeled with Tc-99m

Sterile filtration

- Sterilization by filtration through **0.22 µm filters** is performed using filter holders.
- Preferably, *filter integrity* will be checked before and after filtration to make sure that the filter has not been damaged during the process.
- The product should be filtered into a previously sterilized container located close to the filling point.
- The *same filter should not be used* for more than one batch filtration (within one working shift).

Dispensing

Sterilized stock solution should be dispensed into the sterile glass vials in a *grade A environment with a grade B background*.

Usually the dispensed volume is 1 mL of stock solution.

Directly after dispensing the solution into the vials, sterile stoppers should be placed on the vials (with the necks open for freeze-drying) and the vials should be placed on the precooled shelves of the freeze-dryer.

If the freeze-dryer is not equipped with the desired precooling option, the kits should first be frozen and then placed in the freeze-dryer

The parameters of freeze-drying are individually adjusted depending on the product. Freeze-dryer parameters such as pressure and temperature should be monitored during the process

Freeze drying

- The freeze-drying cycle is started and carried out in the time required to complete the process.
- The parameters of freeze-drying are individually adjusted depending on the product.
- Freeze-dryer parameters such as pressure and temperature should be monitored during the process.
- When the process is finished, the freeze-dryer chamber is filled with sterile nitrogen (or another inert gas).
- Closing of the stoppers is done automatically by shifting the shelves and pressing the stoppers in.

Steps in the freeze-drying process

- Introducing the vials containing the dispensed solution into the freeze dryer
- Freezing the dispensed solution (alternatively, this can be done outside, either in a deep-freezer or by immersing the vials in liquid nitrogen and introducing the frozen samples into the freeze-dryer)
- Cooling the condenser to below –40°C
- Evacuating the system to less than 13 Pa
- Providing controlled heat input to the product during the freeze-drying cycle
- Sealing the vials (under vacuum or under nitrogen gas) in the freeze-dryer after completion of the drying cycle

Crimping

- The crimping device should be located in a **grade B** environment.
- The sealed vials containing the product are transferred from the freeze-dryer to the crimping device for capping.
- Sterilized aluminum caps are placed on the rubber stoppers and crimped.
- The quality of crimping should be checked/ visual inspection of the vials containing the product.

Validation of freeze-drying

❖ **Heat distribution study**

- **empty chamber** of the freeze-dryer.

How it is done?

The temperature is measured on the trays at a minimum of **five different points** at **three different temperatures** (–40, –10 and +40°C).

Acceptance criteria

the average difference in the measured temperature between two measuring points must be less than ±2°C and the deviation must be less than ±4°C.

❖ **Vacuum leak testing**

- should be done at –40°C

After switching off the vacuum, the **pressure in the chamber** is measured for **up to 30 min**.

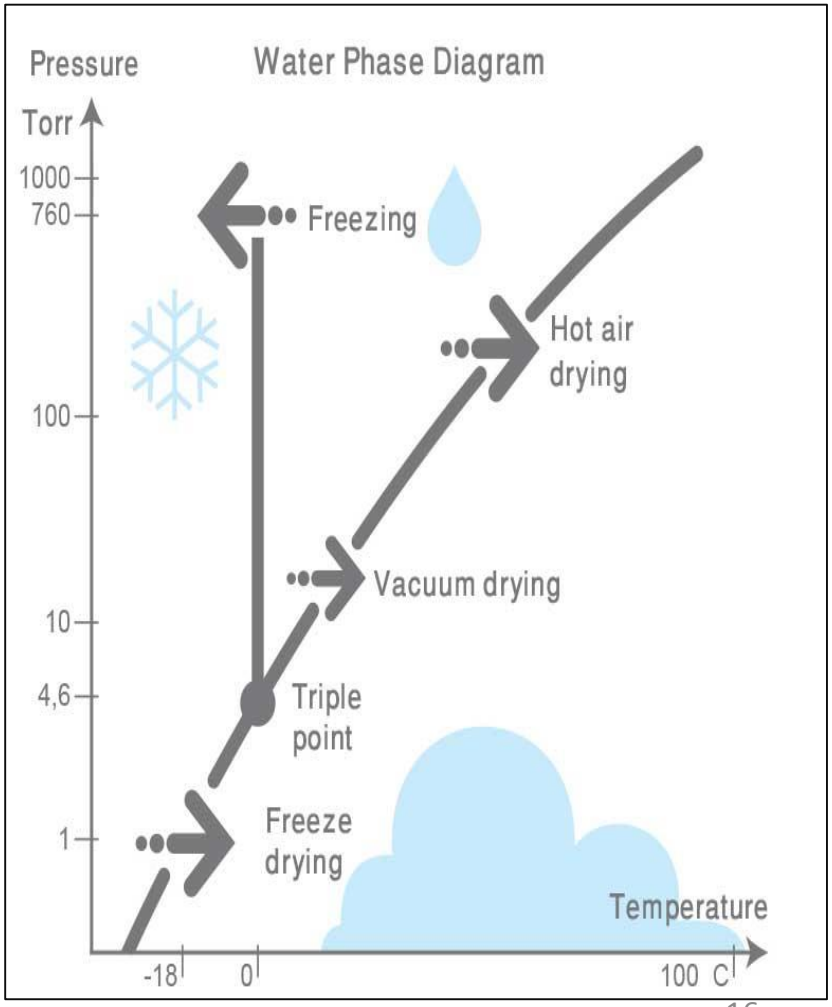
Acceptance criterion

the rise of the pressure in the chamber must be not more than 500 µbar during a 30 min period.

Freeze drying - overview

Freeze drying involves the removal of water or other solvent(s) from a frozen product by a process called *sublimation.*

Sublimation occurs when a frozen liquid goes directly to the *gaseous* state without passing through the liquid phase.



Freeze drying - overview

FIRST STEP- freezing (cooling below eutectic crystallization and glass transition temperatures)

! The rate of cooling may affect the degree of super cooling and the size/type of ice crystals

- Crystalline bulking agent
- Annealing step: elevating and holding the temperature above glass transition temperature of the bulking agent to promote crystallization

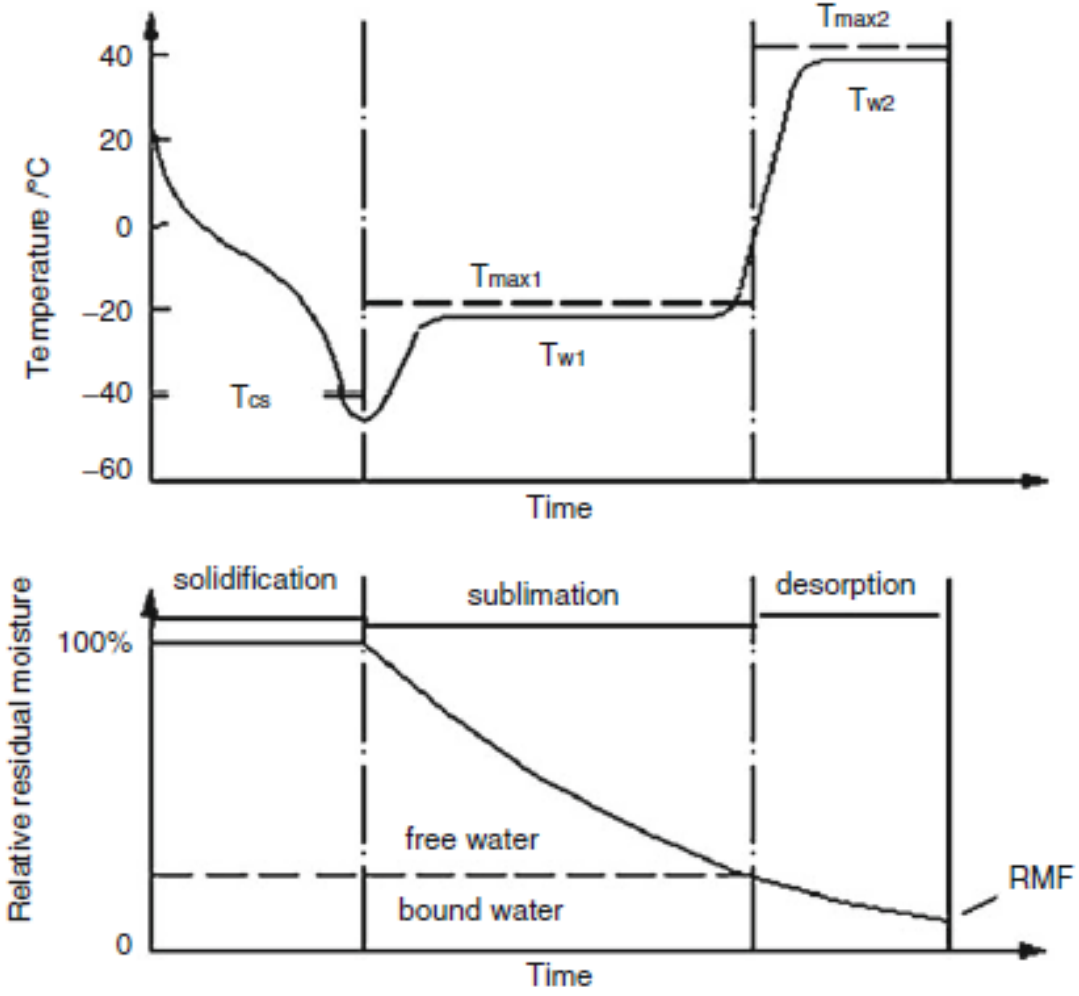
Freeze drying - overview

SECOND STEP- primary drying,

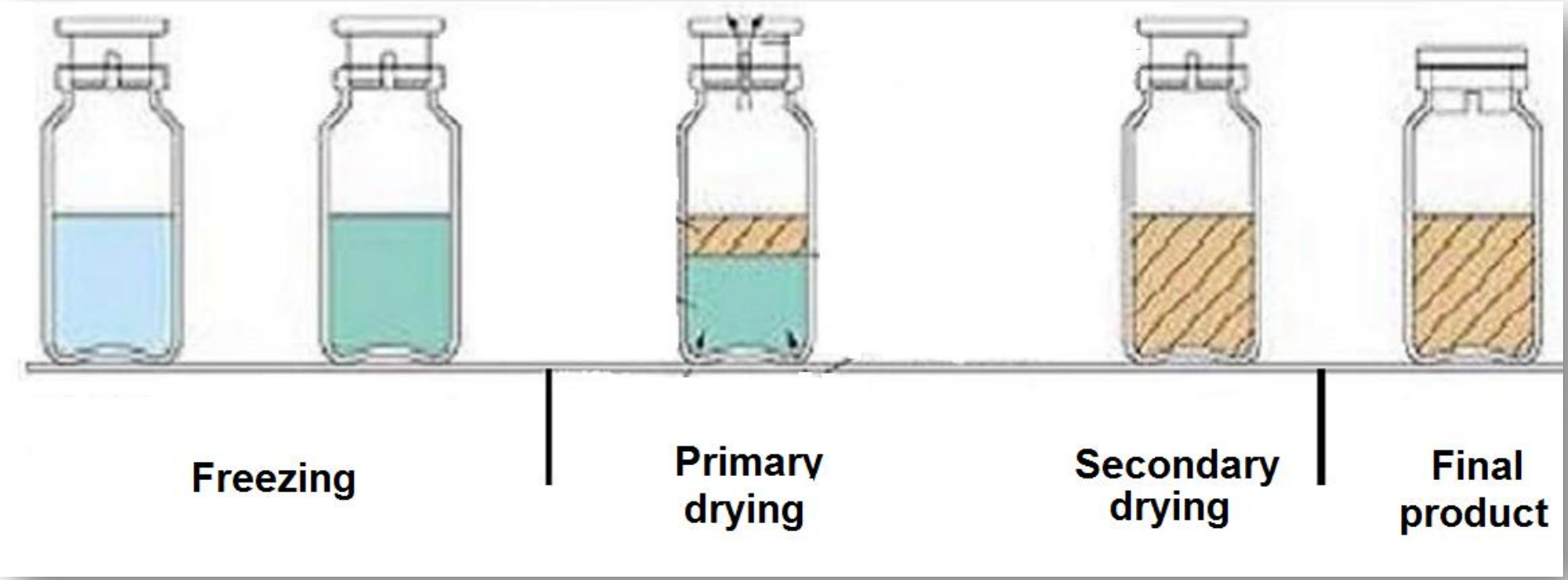
sublimation of frozen and unbound water, below its collapse temperature (keeping intact the microscopic structure)

THIRD STEP- secondary drying: removal of bound or adsorbed water

Variations in sample temperature and moisture content during freeze-drying processes



Freeze-drying: step by step



Advantages of freeze drying

- ✓ increased stability
- ✓ easy to handle product
- ✓ easy and fast solubility
- ✓ increased shelf-life
- ✓ suitable for substances prone to thermal degradation, oxidation

Changes that may occur during freeze drying

- *Low-temperature stress*
- *Concentration effect*
- *Formation of ice-water interface*
- *pH changes during freezing*
- *Phase separation during freezing*
- *Dehydration stresses*
- *Aggregation*
- *Chemical reactions (e.g. oxydation, deamidation in proteins)*
-
-

Disadvantages of freeze drying

- expensive and specific equipment and conditions
- duration of the process
- scale up and feasibility

Design of the freezing step

Freezing – cooling below the temperature of glass transition of the sample and its transformation to solid state

- Crystals of water (ice) are formed

Cooling – how?

- ✓ liqiud nitrogen
- ✓ deep freezer
- ✓ freeze-dryer

Rapid cooling results in small ice crystals, useful in preserving structures to be examined microscopically, but resulting in a product that is more difficult to freeze dry. **Slower cooling** results in larger ice crystals and less restrictive channels in the matrix during the drying process.

To consider:

- ➢ Minimal freezing temperature needed
- ➢ Rate of cooling
- ➢ Duration of the step

Types of freezing

Eutectic samples

Mixture of substances that freeze at lower temperatures than the surrounding water

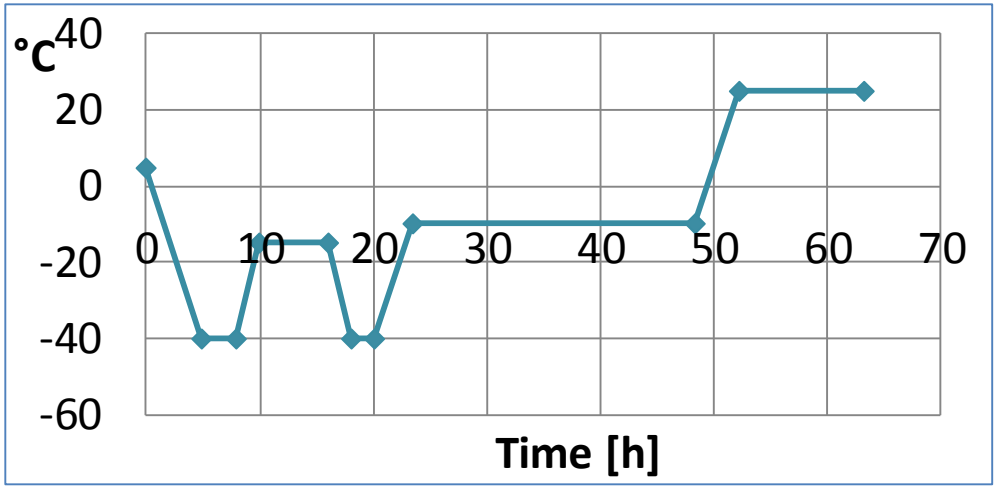
- Creation of more concentrated areas of solute which have lower freezing temperature than the water
- The product is NOT completely frozen
- Eutectic temperature – the temperature when ALL of the eutectic mixture is frozen

Glass formation samples

Suspension that undergoes glass formation during the freezing process.

- the entire suspension becomes increasingly viscous as the temperature is lowered
- Finally the product freezes at the *glass transition point forming a vitreous solid.*
- *EXTREMLY DIFFICULT to freeze- dry*

Annealing step



- Crystalization of excipients (bulking agents)
- Increases the speed of the primary drying
- Produces larger crystals that are easier for sublimation
- Improves homogenicity
- Improves the appearance of the final product
- Prevents vial brake

Design of the primary drying phase

Prymary drying – removal of the largest amount of the water

- Includes vacuum – high consumption of energy
- The most time-consuming phase

Parameters important for the process

- Shelf temperature
- Chamber pressure

In order to achieve max. speed of sublimation, a combination of ***highest possible temperature*** and ***lowest possible pressure*** is necessary .

‼ Very important – determining the final point of the phase.

Design of the phase of secondary drying

- The final step of the freeze-drying process

Includes removal of the remaining (bonded) water

Usually **not all** water is removed (residual moisture content may be as high as 7-8%)

- Diffusion (desorption) process
- Raising the temperature with certain rate
- The chamber pressure has a significantly smaller impact to the process

Lyoprotectors may be added in order to prevent over drying in protein radiopharmaceutical kits.

Design of the phase of secondary drying

Continued drying is necessary at the warmer temperature to reduce the residual moisture content to optimum values.

The bound water is ***desorbed*** from the product = ***isothermal desorption.***

- The temperature of secondary drying must be compatible with the sensitivity of the product.
- The other conditions (pressure and collector temperature) need not to be changed.

the vacuum - as low as possible

the collector - as cold as can be attained

Secondary drying consumes approximately 1/3 to 1/2 the time required for primary drying

Commonly used excipients in radiopharmaceutical kits

Stannous chloride dihydrate (reducing agent)

Ascorbic acid (for stabilizing the reducing agent)

Sodium chloride (isotonicity)

Hydrochloric acid (pH adjusting)

Sodium hydroxide (pH adjusting)

Buffers: Phosphate buffer (monobasic and dibasic sodium phosphate), Acetate buffer (sodium acetate and acetic acid).

Glycine, arginine

Bulking agents, cryo- and lyoprotectants

Cryoprotectants and lyoprotectants

Purpose – to protect a protein from freezing (cryoprotection) and/or dehydration (lyoprotection) damage

1. Sugars and polyols

➢ the hydroxyl groups in the stabilizers can form hydrogen bonds to the surface of (protein) molecules as water does, and "substitute" for the hydrogen bonding interaction with water that is lost during drying

2. Polymers

Polymers are used as protective agents because they can increase the glass transition temperature of the solution

Cryoprotectants and lyoprotectants - sugars and polyols-

Type	Name	Formula	MW (g/mol)	T_g (°C)	T_{col} (°C)
Mono-saccharide	Glucose	$C_6H_{12}O_6$	180.16	–43	–41
	Galactose			–41	
	Mannose			–41	
	Fructose			–42	
	Ribose	$C_5H_{10}O_5$	150.13	–47	
	Xylose			–48	
Oligo-saccharide	Sucrose	$C_{12}H_{22}O_{11}$	342.30	–32	–31
	Lactose				30.5
	Maltose monohydrate	$C_{12}H_{22}O_{11}\cdot H_2O$	360.32	–30	
	Trehalose dehydrate	$C_{12}H_{22}O_{11}\cdot 2H_2O$	378.34		–29
	Raffinose pentahydrate	$C_{18}H_{32}O_{16}\cdot 5H_2O$	594.53		–26
[redacted]	Mannitol	$C_6H_{14}O_6$	182.17	–27	
	Glycerol	$C_3H_8O_3$	92.09	–65	
	Sorbitol	$C_6H_{14}O_6$	182.17	–44	–54
	Xylitol	$C_5H_{12}O_5$	152.15	–47	
	Inositol	$C_6H_{12}O_6$	180.16		

Cryoprotectants and lyoprotectants -polymers-

Name	Formula	MW (g/mol)	T_g (°C)	T_{col} (°C)
Polyethylene glycol (PEG)	$H[OCH_2CH_2]xOH$	$2–400 \times 10^2$		–13
Dextran	$[C_6H_{10}O_5]x$	$1–200 \times 10^4$		–10
Hydroxyethyl starch (HES)			–12	> –5
Ficoll		$7–40 \times 10^4$		–20
Gum arabic (acacia)		25×10^5		
Gelatin				–8
Polyvinylpyrrolidone (PVP)	$[CHN(CH_2)_4CO]x$	$3–36 \times 10^4$		–24 to –27
Cellulose				
β-Cyclodextrin	$C_{48}H_{80}O_{40}$	1,135.00		
Methocel		$4–18 \times 10^4$		–9
Maltodextrin 860				
Sephadex G200				–10
Bovine serum albumin (BSA)		67,000	–11	

Cryoprotectants and lyoprotectants

3. *Surfactants :* Surfactants are wetting agents composed of hydrophilic and oleophilic groups that can reduce the surface tension of a liquid and reduce the interfacial tension between two liquids.
In the freeze-drying of proteins, surfactants can reduce denaturation during freezing and dehydration. The surfactants also act as humidifying agents during the rehydration process.

4. Amino acids: having both amine and carboxyl functional groups, can inhibit pH changes of the solution during low -temperature storage and freeze- drying of proteins

5. *Antioxidants,* such as vitamin D, vitamin E, protein hydrolysate, and sodium hyposulfite, are used to prevent oxidation during freeze-drying and storage

6. *Buffer and chelating agents*, such as phosphoric acid, amino acids, and EDTA, regulate the pH value of the material and sequester ions.

Cryoprotectants and lyoprotectants - surfactants-

Name
Tween 80
Triton X-100
Sucrose fatty acid monoester
3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS)
Hydroxypropyl-β-cyclodextrin (HP-β-CD)
Sodium dodecanesulfonate (SDS)
Brij35 Brij30
Lubrol-px
Pluronic F127

Cryoprotectants and lyoprotectants -amino acids-

Name	Formula	MW (g/mol)	T_g (°C)
Proline	$(CH_2)_3NHCHCOOH$	115.13	
Glycine	CH_2NH_2COOH	75.07	–37
Glutamic acid	$(CH_2)_2NH_2CH(COOH)_2$	147.13	–17
Histidine	$NHCHNCHCCH_2CH(NH_2)COOH$	155.16	–32
Arginine	$HNC(NH_2)NH(CH_2)_3CH(NH_2)COOH$	174.20	
4-Hydroxyproline	$NHCH_2CH(OH)CH_2CHCOOH$	131.13	
L-Serine	$HOCH_2CH(NH_2)COOH$	105.09	
β-Alanine	$CH_2(NH_2)CH_2COOH$	89.09	–65
Lysine hydrochloride	$H_2N(CH_2)_4CH(NH_2)COOH{\cdot}HCl$	182.65	
Lysine	$H_2N(CH_2)_4CH(NH_2)COOH$	146.19	
Sarcosine	CH_3NHCH_2COOH	89.09	
γ-Aminobutyric acid	$H_2NCH_2CH_2CH_2COOH$	103.12	

Cryoprotectants and lyoprotectants -antioxidants-

Name	Formula	MW (g/mol)
Antisterility factor (vitamin E)	$C_{29}H_{50}O_2$	430.72
Ascorbic acid (vitamin C)	$C_6H_8O_6$	176.13
Lecithin	$C_{40}H_{82}NO_9P$	752.08
D(–)-Isoascorbic acid	$C_6H_8O_6$	176.13
L-Ascorbic sodium	$C_6H_7\ NaO_6$	198.11
Sodium thiosulfate anhydrous	$Na_2S_2O_3$	158.11
3-Tert-butyl-4-hydroxyanisole	$C_{11}H_{16}O_2$	180.25
Butylated hydroxy toluene	$CH_3C_6H_2(OH)[C(CH_3)_3]_2$	220.36
Propyl gallate	$(HO)_3C_6H_2COOCH_2CH_3$	212.20
Ethylene diamine tetraacetic acid disodium salt dihydrate (Na_2EDTA)	$C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$	372.24

Cryoprotectants and lyoprotectants

5. *Bulking agents* are substances that can prevent the effective components of the formulation from escaping along with the water vapor, and promote fixation of the effective components in the material.
Typical bulking compounds are mannitol, lactose, and gelatin.

Additional functions:
✓Provide adequate mechanical support for the final freeze-dried products
✓improve the appearance of the freeze-dried product
✓increase the solubility of solutes
✓prevent the freeze-dried products from collapse or overflow

Cryoprotectants and lyoprotectants -bulking agents-

Name	Formula	MW (g/mol)	T_g (°C)	T_{col} (°C)
Citric acid monohydrate	$C_6H_8O_7 \cdot H_2O$	210.14	–54	
Phosphoric acid	H_3PO_4	98.00		
Ethylenediamine tetraacetic acid (EDTA)	$(CH_2)_2N_2(CH_2COOH)_4$	292.24		
Tartaric acid	$C_4H_6O_6$	150.09		
4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)	$C_8H_{18}N_2O_4S$	238.31	–63	
Histidine	$NHCHNCHCCH_2$ $CH(NH_2)COOH$	155.16	–33	
Potassium acetate	CH_3COOK	98.14	–76	
Potassium citrate	$HOC(COOK)$ $(CH_2COOK)_2$	306.42	–62	
Potassium phosphate monobasic	KH_2PO_4	136.09		–55
Sodium acetate	CH_3COONa	82.03	–64	
Sodium bicarbonate	Na_2CO_3	105.99	–52	
Sodium citrate	$HOC(COONa)$ $(CH_2COONa)_2$	258.10	–41	
Sodium phosphate	NaH_2PO_4	119.98	–45	
Tris base			–55	
Tris HCl			–65	

Collapse phenomenon

Aqueous sucrose solutions can undergo structural changes during the drying process - phenomenon known as *collapse.*

- Although the product is frozen below its eutectic temperature, warming during the freeze drying process can affect the structure of the frozen matrix at the boundary of the drying front → collapse of the structural matrix.
- To prevent collapse of products containing sucrose, *the product temperature must remain below a critical collapse temperature during primary drying*.

Equipment

Some frequently employed protocols

In production of radiopharmaceutical kits

Acknowledgement and further reading

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