PDA Europe Virtual Training Course: Optimize Your Freeze-Drying Process

Andrew G. Bright, PhD, MChem
Senior Scientist, Biopharma Group

15th October 2020

PDA Parenteral Drug Association

CONNECTING PEOPLE SCIENCE AND REGULATION®

Speaker Profile

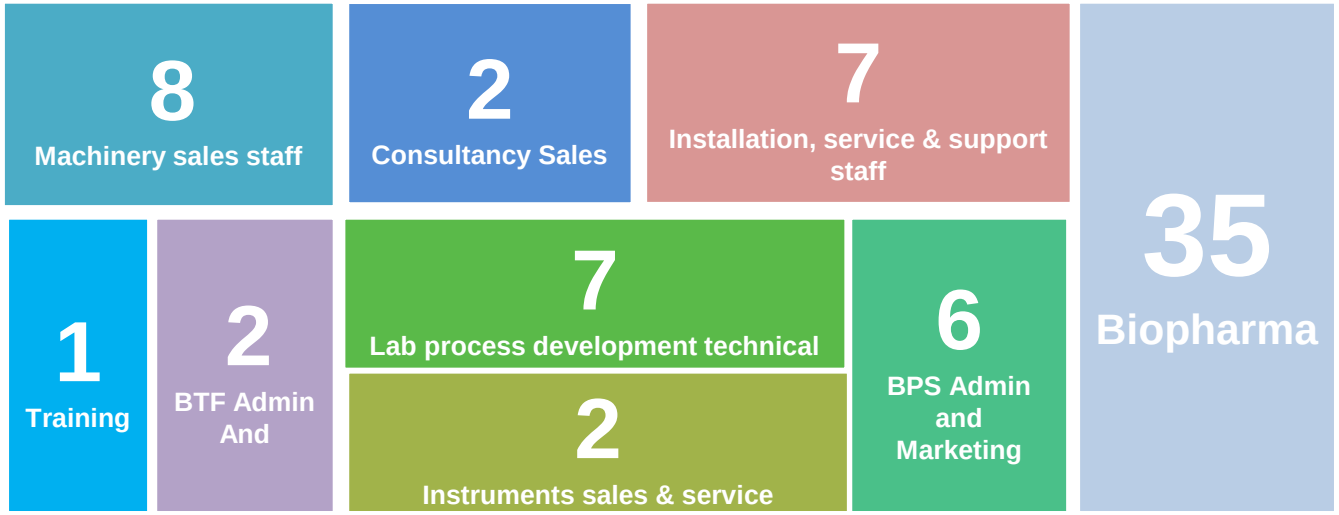
DR ANDREW BRIGHT

- Joined BTL in January 2018 as Senior Scientist.
- Developed the formulation and manufacturing process on a range of freeze dried products including; vaccines, small molecule drug products, large molecule drug products, diagnostic tests, cosmetics and standards.
- Run and support training courses on the development of freeze dried products.
- Ph.D. from the University of Bradford investigating freeze dried vaccine formulations with the thesis title “Mechanistic Insights into the Stabilisation of Biopharmaceutical Using Glycine Derivatives” and also holds a MChem in Chemistry with Pharmaceutical and Forensic Science.
- Andrew previously worked for 2 years as a Senior Scientist at Pfizer within liquid formulations specialising in freeze dried formulation design, process development, and scale up.

Biopharma Group – founded 1989 – SP agents since 1992

Biopharma Process System Limited

- Head Office in **Winchester**
- Location of **worldwide** process development laboratory
- Worldwide **Admin Centre**



Timetable

Thursday, 15 October 202014:00 – 17:00 CEST	
Time	Topic
14:00-14:05	Welcome, Opening Remarks and Introductions
14:05-14:40	Overview of the Freeze-Drying Process
14:40-15:00	Freeze Dried Formulations and Typical Use of Common Excipients
15:00-15:30	Frozen State Characterization
15:30-15:40	Comfort break
15:40-16:00	Overview of Typical Process Analytical Technology used in Monitoring Primary Drying
16:00-16:45	Cycle Development and Scale up: Use of the Iterative Approach and Use of SMART Software
16:45-17:00	Round-up Discussion and Questions

Objective

- During this presentation we will review the basics of freeze drying to aid in understanding what factors effect the process, and how these can be controlled and monitored to optimise the freeze drying process of a product.

Overview of the Freeze-Drying Process
(35 Minutes)

Terminology

- **Freeze-drying** implies a process where a product is first frozen and then dried.
- **Lyophilization** is the process of creating a lyophile (‘solvent loving’ material), which is one of the principal features of the process; terminology first used by Professor Louis Rey.
- While the definitions of Freeze Drying and Lyophilization are slightly different, both terms are invariably used to describe the same process.

Advantages of Freeze Drying

- The primary aim is to preserve by the removal of water
- It is a method of drying whilst maintaining the original structure and activity
- Provides for a long shelf life
- The product should easily reconstitute to return to its former state
- Liquid fill enables accurate dosing
- Allows manufacture under sterile conditions

Disadvantages of Freeze Drying

Cost

- Capital cost of procuring the equipment
- Costs of running equipment
  - Energy costs
  - Manufacturing under sterile conditions

Time

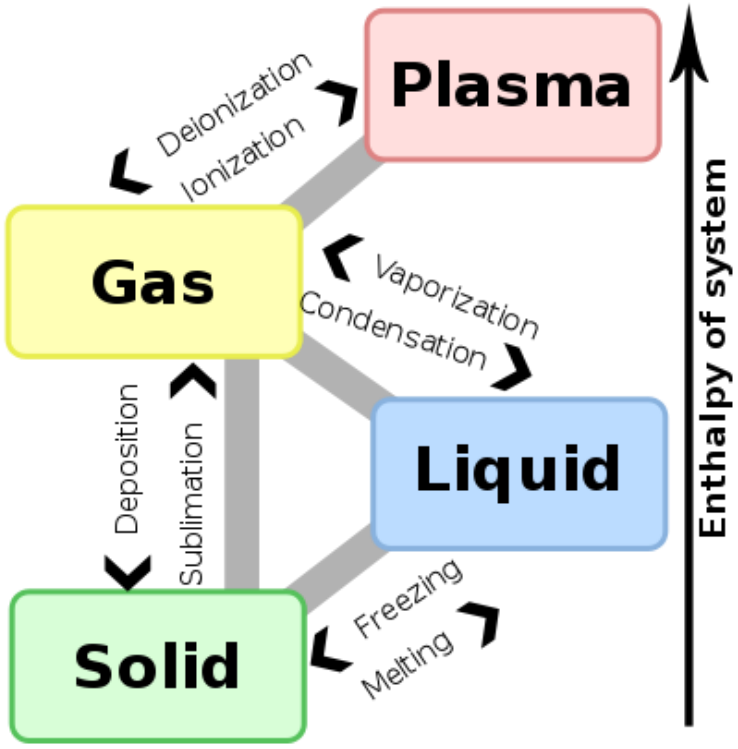
- Many processes can take days to complete
- Increased risk the longer a cycle

Phases of Water

The terminology used to describe the changes from one phase to another

The energy flow (heat input) to change from the solid phase to the plasma phase

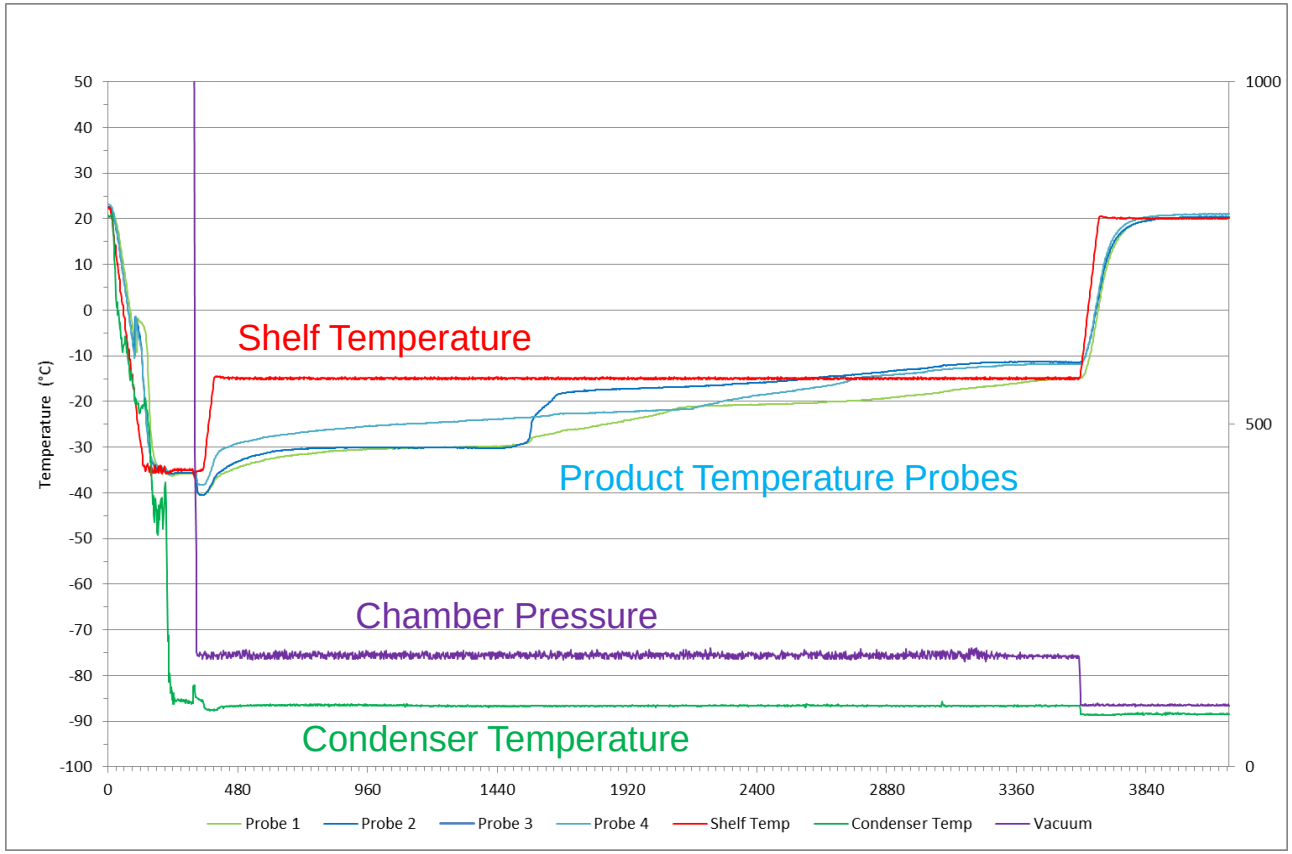
The process of going direct from the solid to the gaseous phase is a process known as SUBLIMATION



Major Phases of Freeze Drying

- Freezing
  - Freezes both the solvent and the solute
  - Immobilises the material
  - Defines the structure ready for drying
- Drying
  - Primary Drying
    - The removal of the **freezable** water by a process of sublimation – the evaporation of ice to vapour without passing through the liquid phase
  - Secondary Drying
    - The process of further drying of **unfrozen** water, by a process of desorption

Example of Cycle Trace



Lab, Pilot & Production Equipment

- The equipment used in production covers myriad sizes, functions and features.
- Not all freeze dryers have all features, although they all have some essential components in common.
- Some of these features are apparent in the following slides…

Benchtop Units

Trapping Capacity **6 litre/24 hr**

Shelf Area **0.3 m²**

R&D and pilot and small production units

Trapping capacity up to 50 Litres. Shelf Area up to 2.1 m^2

Clean Room Configurations

Small Production Machine

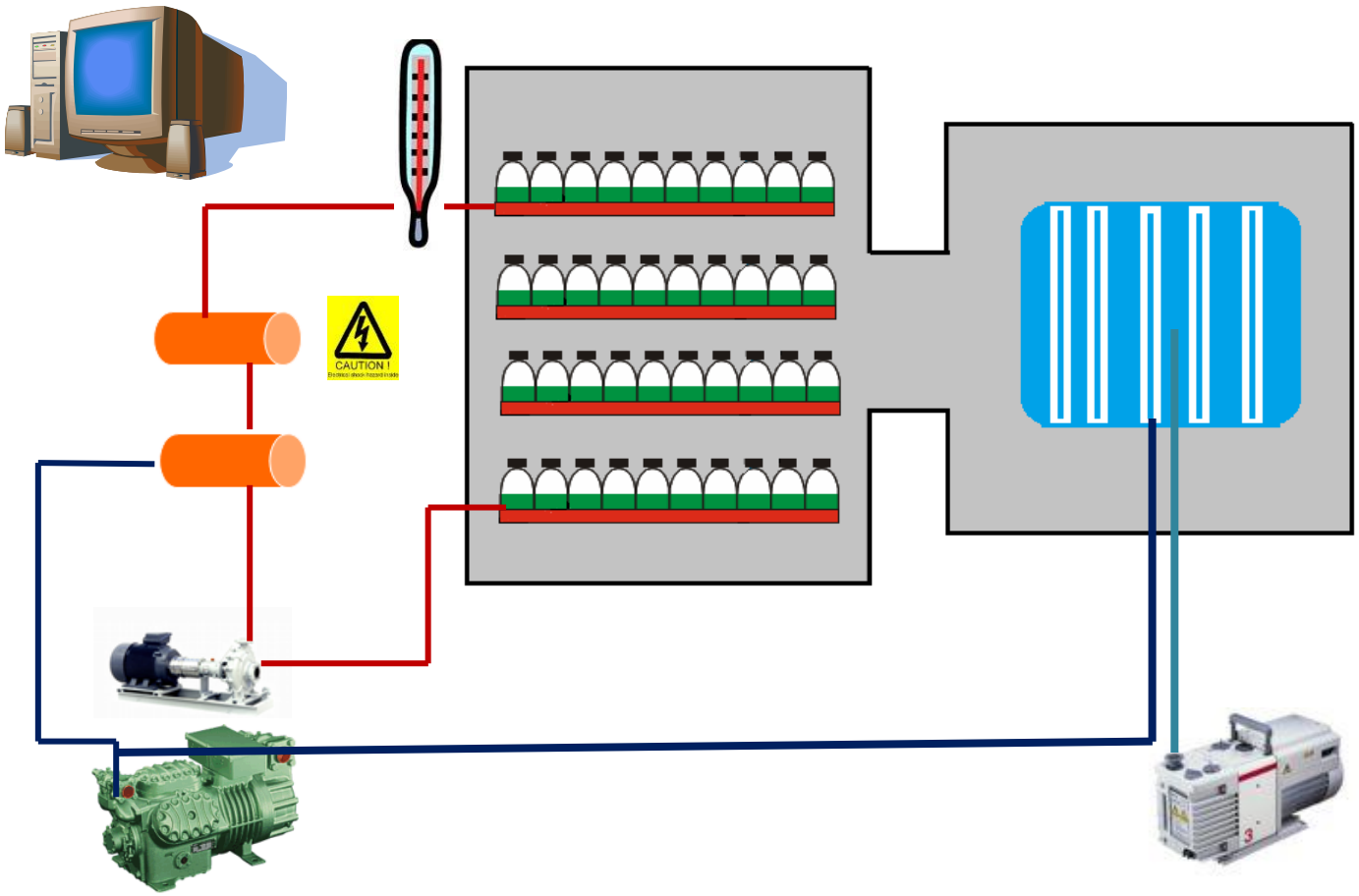
Large Production Machine

All the shelves are currently in the “parked” position at the bottom of the chamber.

This aids cleaning and is useful for loading at constant height.

Often, this design is suitable for automatic loading systems with a pizza type door

Machine Control–Shelf Temperature



Product Freezing – the basics

We need to consider:

SOLVENT BEHAVIOUR – helps define the structure and porosity of the material, but we will want to remove it later

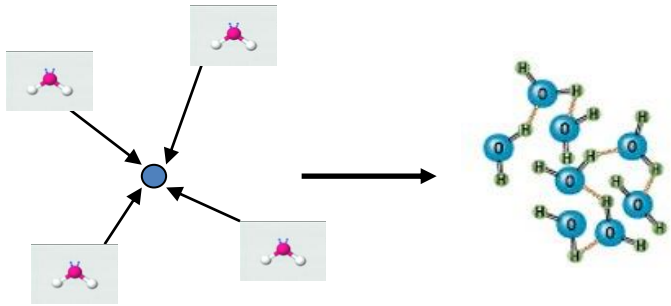
SOLUTE BEHAVIOUR – this is the part that will eventually become our product, so we need to make sure it freezes in the correct manner before we remove the ice

The effect of each of the above on the other

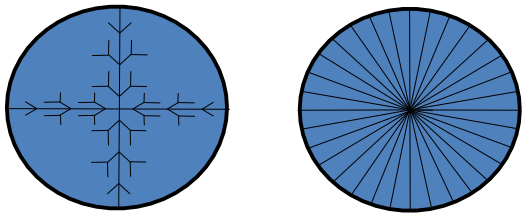
Ice Formation: a two-stage process

– Nucleation

(molecules arranging from random positions or 'gathering' around impurities)



– Crystal Growth



Dendritic Spherulitic

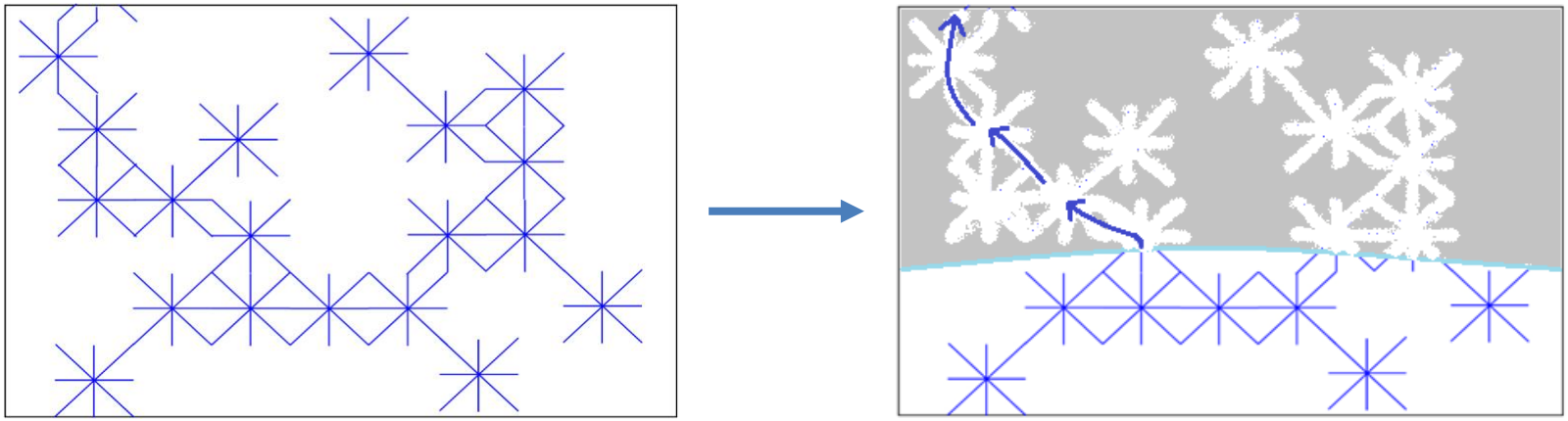
*However, in freeze-drying, we are more concerned with **crystal size** and networks than with basic **crystal shape***

Cooling Rate and Ice Structure

Generally, a slower* cooling rate leads to:

- ☑ Larger ice crystals (better for drying)
- ☑ Better ice “networking” (better for drying)
- Possible heterogeneity in solute distribution
- Chance of skin formation (impedes drying)

*A typical default cooling rate is 0.5°C/min
Anything slower than 0.25°C/min could be considered SLOW
Anything faster than 1.0°C/min could be considered FAST



Cooling Rate and Ice Structure

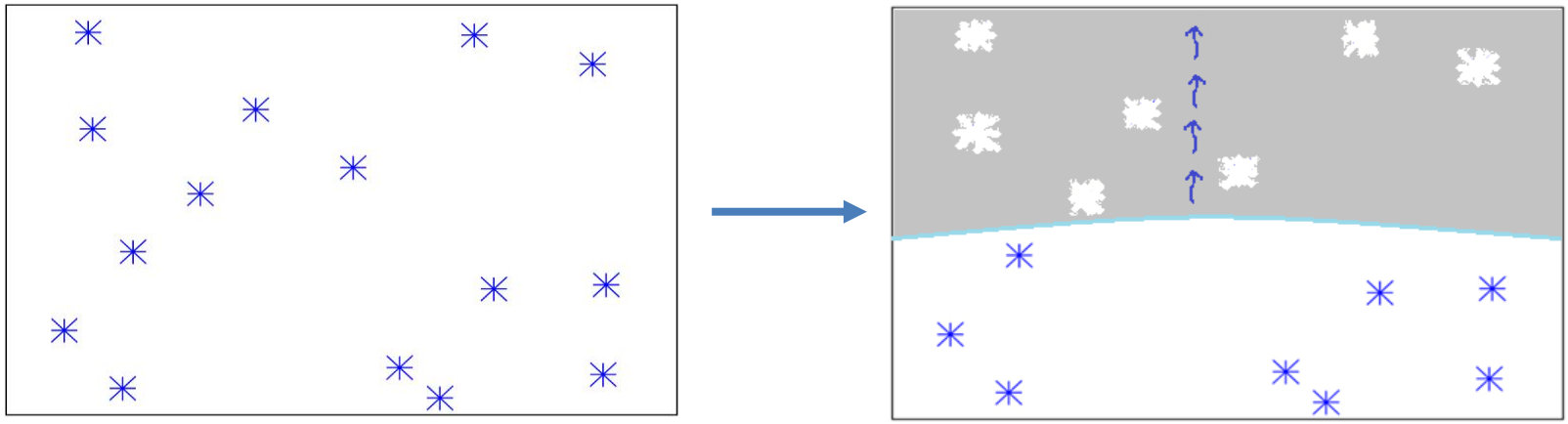
…while more rapid* cooling can give:

- ☑ A lesser potential for skin formation
- ☑ A more even distribution of solutes
- ➧ Limited ice “networking” (but instead, smaller, more isolated ice crystals, which are harder to dry)

*A typical default cooling rate is 0.5°C/min

Anything slower than 0.25°C/min could be considered SLOW

Anything faster than 1.0°C/min could be considered FAST



Solute Crystallisation – what happens and how to detect it

Liquid Formulation
(water + solutes)

COOL →

Freezable water converted to Pure Ice
Solute remains in liquid state with unfrozen water ('eutectic liquid')

COOL →

Solute crystallises
Now there is a mixture of pure ice + eutectic solid

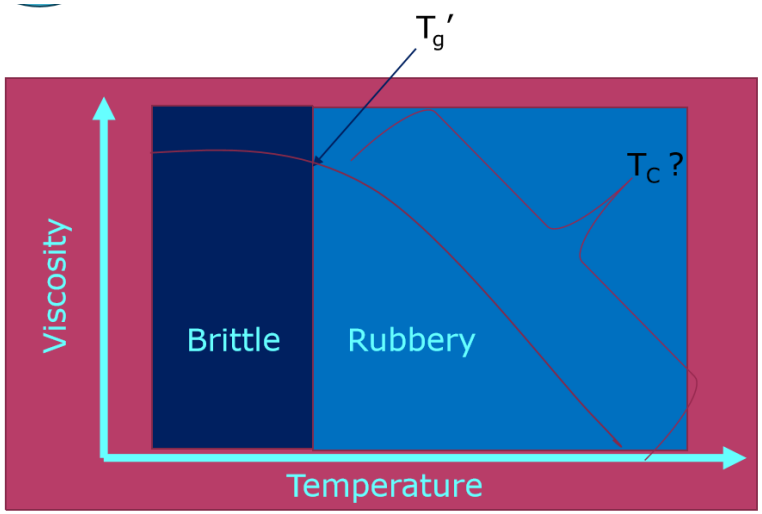
- Exothermic crystallisation process can be **detected** during cooling (e.g. using DSC or DTA – see later)
- However, thermal events are typically measured on warming, to avoid any artefacts related to cooling speed (so we would measure the eutectic **melt** in this case rather than the solidification itself)
- Freezing, crystallisation and melting can be **observed** by freeze-drying microscopy or optical DSC

Glass transition vs. Collapse Temperature

The glass transition temperature of a frozen material (Tg') is where the solute phase becomes flexible (a ***precursor*** to collapse)

The collapse temperature (Tc) is where the flexible solute phase becomes sufficiently mobile to ***visibly lose structure*** when the ice is removed ***during sublimation*** (defined as 'viscous flow')

Therefore, Tc ≥ Tg'



We consider Tg' to be the temperature at which *an amorphous frozen system changes from "brittle" to "rubbery" material. This is associated with a sudden change in viscosity and heat capacity.*

T_c can occur at the same or higher temperature as Tg', the collapse is where the viscosity of the material decreases to the point where it can no longer support it own weight and therefore loses structure.

Glass transition vs. Collapse Temperature

Measurement of glass transitions in frozen solutions:

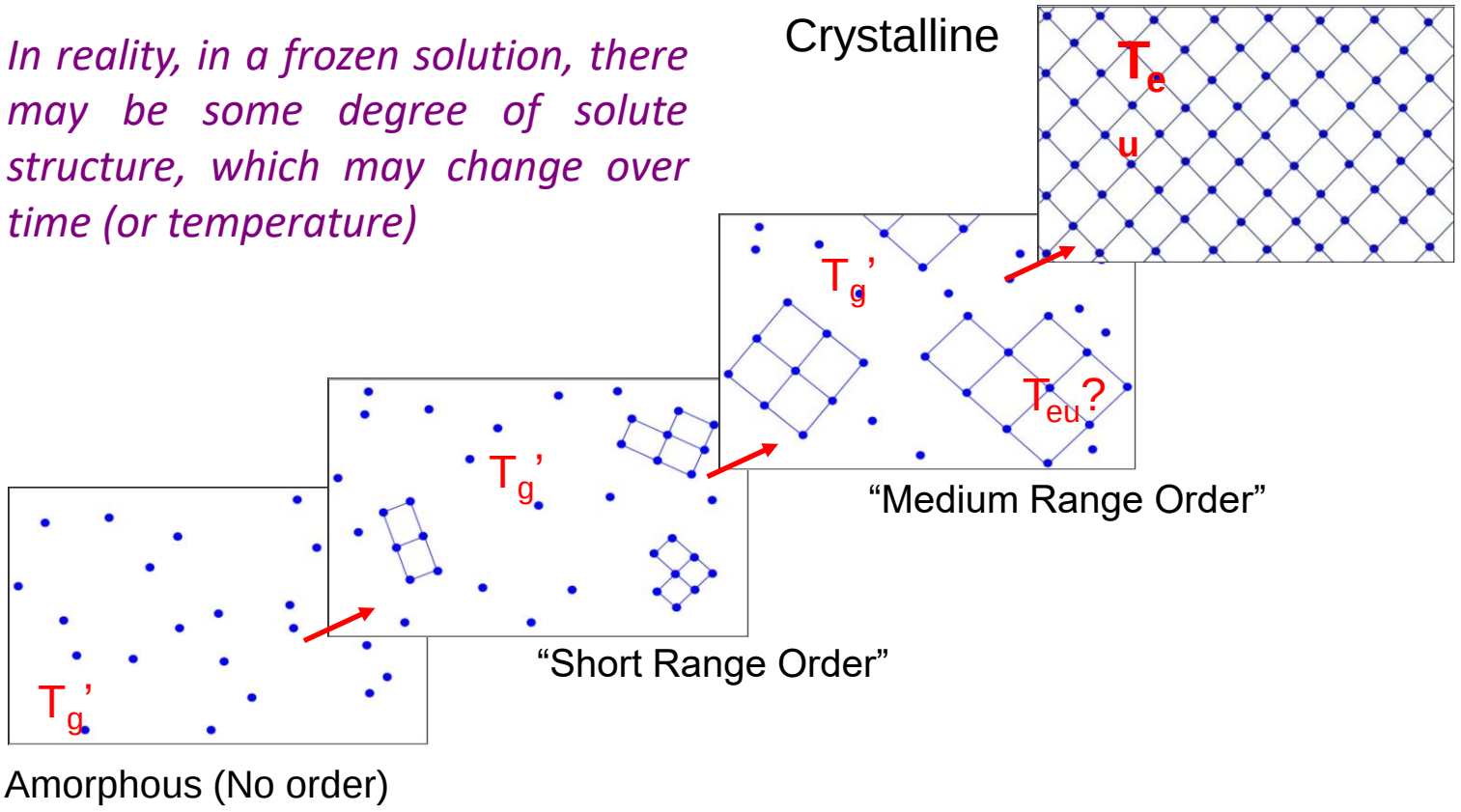
- differential scanning calorimetry (DSC)
- differential thermal analysis (DTA)
- Dynamic / Thermal Mechanical Analysis (DMA/TMA)
- Microcalorimetry
- Electrical impedance analysis (Zsinφ)

Determination of collapse and other visible events:

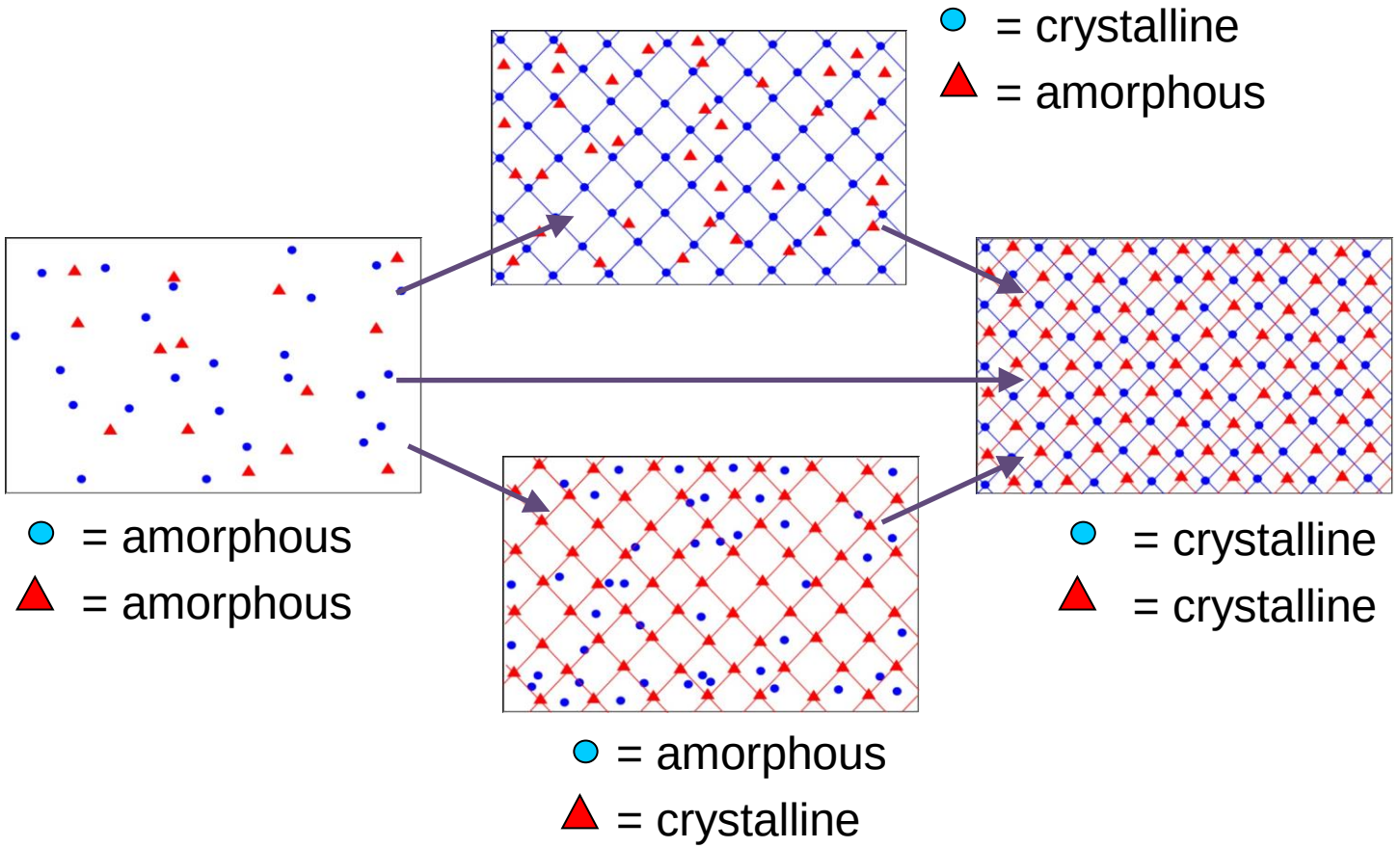
- Freeze-drying microscopy

Amorphous → Crystalline?

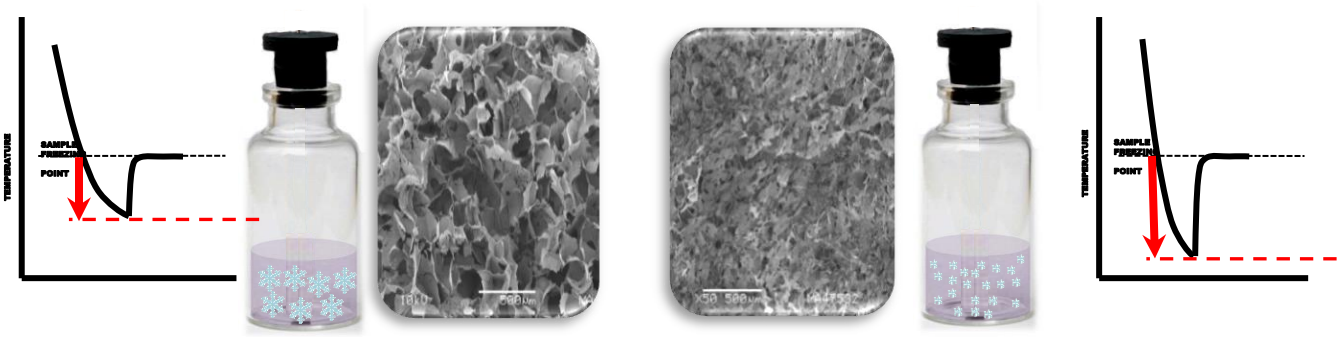
In reality, in a frozen solution, there may be some degree of solute structure, which may change over time (or temperature)



Multi-component solutions: *the outcomes can multiply!*



Supercooling & Nucleation



- Low degree of supercooling:
  - → Larger ice crystals + channel formation
  - → larger pores in the dried matrix
  - → **lower Rp** (product resistance)
- High degree of supercooling:
  - → Smaller ice crystals, limited channels
  - → smaller pores in the dried matrix
  - → **higher Rp**

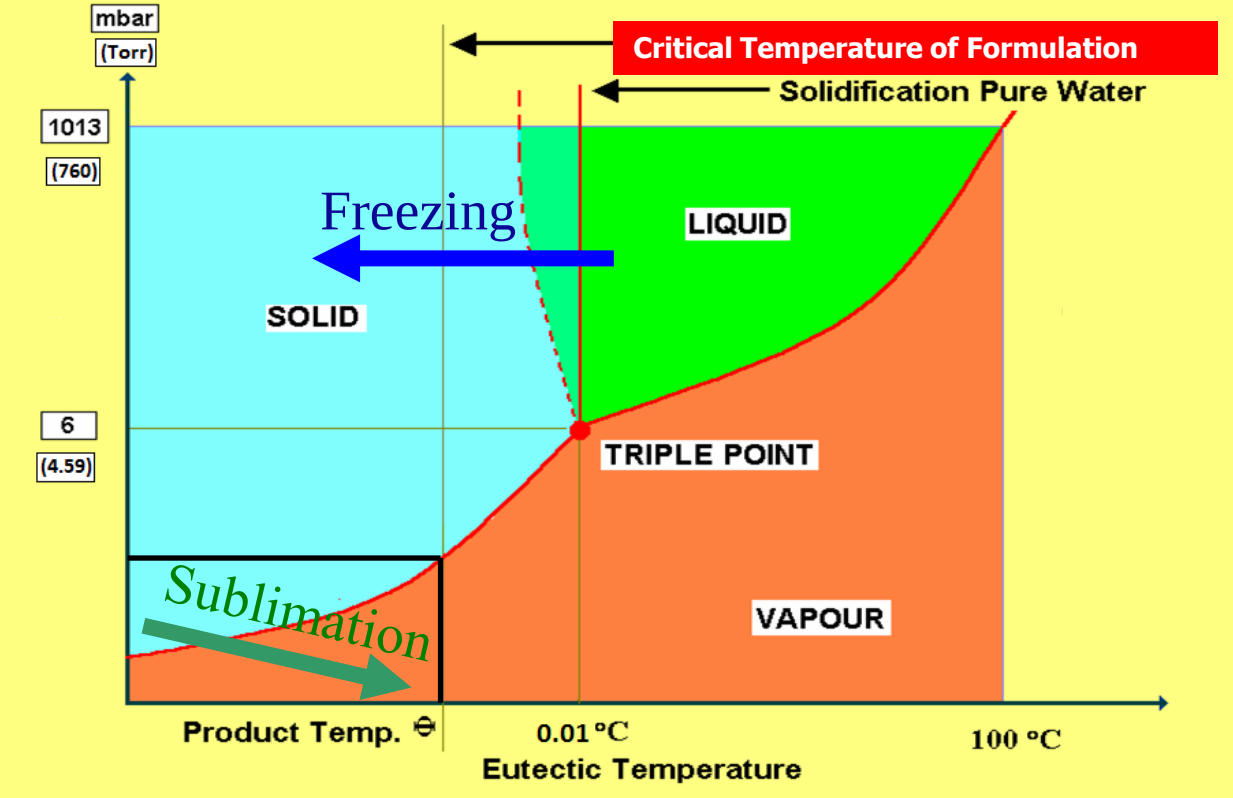
For every 1 degree increase in nucleation temperature, drying times are reduced by 3%. [1]

[1] Searles JA, Carpenter T, Randolph, TW., 2001. The Ice Nucleation Temperature Determines the Primary Drying Rate of Lyophilization for Samples Frozen on a Temperature Controlled Shelf. *J Pharm Sci* **90**: 860-871.

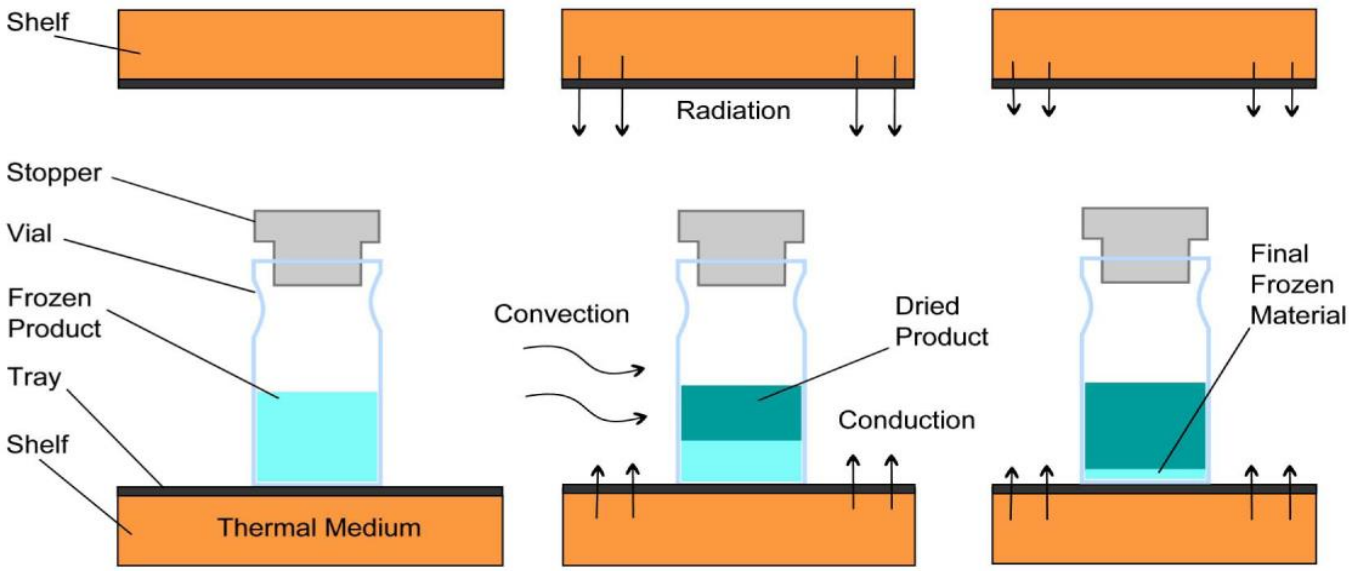
Primary and Secondary drying

- **Primary drying** is the removal of **bulk** solvent (usually ice) by **sublimation**
  - Sublimation is the change in state directly from solid to gas
  - Phase change requires energy (approx. 2800 J to convert 1 gram ice to vapour)
  - In freeze drying, this energy is provided by heating the shelves & maintaining pressure
- **Secondary drying** is the removal of **unfrozen** (adsorbed, associated, bound…) solvent (usually water) by **desorption** (and some **evaporation**)

Amended Phase Diagram for Pure Water



Mechanisms of heat transfer into the product



The **HEAT INPUT** is offset by the **HEAT OUTPUT (LOSS)** from the sublimation process (2,800 J/g)

Principles of Primary Drying

Primary drying is about achieving a balance:

- Too little energy input = Slow Drying Rate *(inefficient but probably safe)*
- More energy input than the product can physically lose (by sublimation) = increase in $T_{product}$ *(maybe more efficient but greater risk to product)*

We can calculate the (relative) driving force for the process by looking at the *Vapour Pressure Differential*

Vapour pressure differential

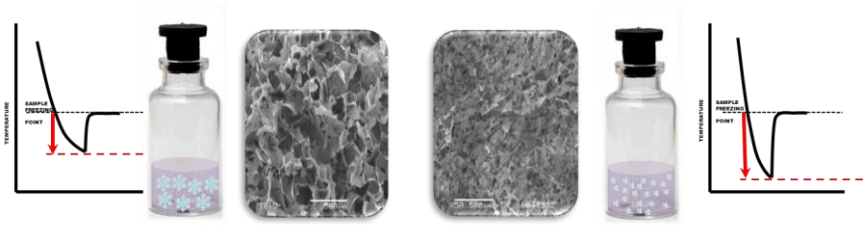
- All materials will exert a *vapour pressure* on their surroundings
- It describes the tendency of particles to escape from the liquid (or a solid).
- VP is related to temperature (see table of literature values given in Appendix)
- In freeze-drying, we may define the **driving force** for sublimation as *the difference in **VPs** between the ice leaving the product and the ice at the vapour trap*

Relationship between VP & Temperature for ice/water

Temperature °C	Pressure mbar	Pressure Torr	Temperature °C	Pressure mbar	Pressure Torr
100	1013	760	-30	0.38	0.28
20	23.6	17.7	-40	0.13	0.10
10	12.4	9.3	-50	0.04	0.03
0	6.1	4.6	-60	0.011	0.008
-10	2.6	1.9	-70	0.0026	0.0020
-20	1.03	0.77	-80	0.0006	0.0004

Impedance to vapour flow within the product

In reality, product resistance (Rp) accounts for up to 60-70% of the resistances to drying



- Low degree of supercooling:
  - → Larger ice crystals + channel formation
  - → larger pores in the dried matrix
  - → **lower Rp (product resistance)**

- High degree of supercooling:
  - → Smaller ice crystals, limited channels
  - → smaller pores in the dried matrix
  - → **higher Rp (product resistance)**

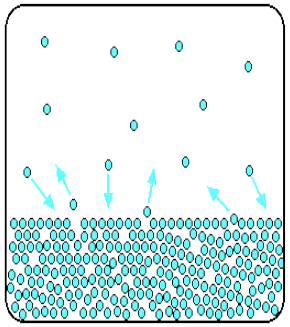
Rp will vary with:

- Starting concentration of formulation
- Depth of dry layer (which increases during process)
- Ice structure and networking / channels
- Presence / absence of surface skin / crust

Vapour Pressure

- The tendency for particle within a solid or liquid to be freed and join the gas phase
- At equilibrium, particles leaving the surface are balanced by those becoming trapped
- Vapour pressure of ice tables describe ***equilibrium*** conditions

- *So, how can we figure out what pressure to use in the dryer?...*

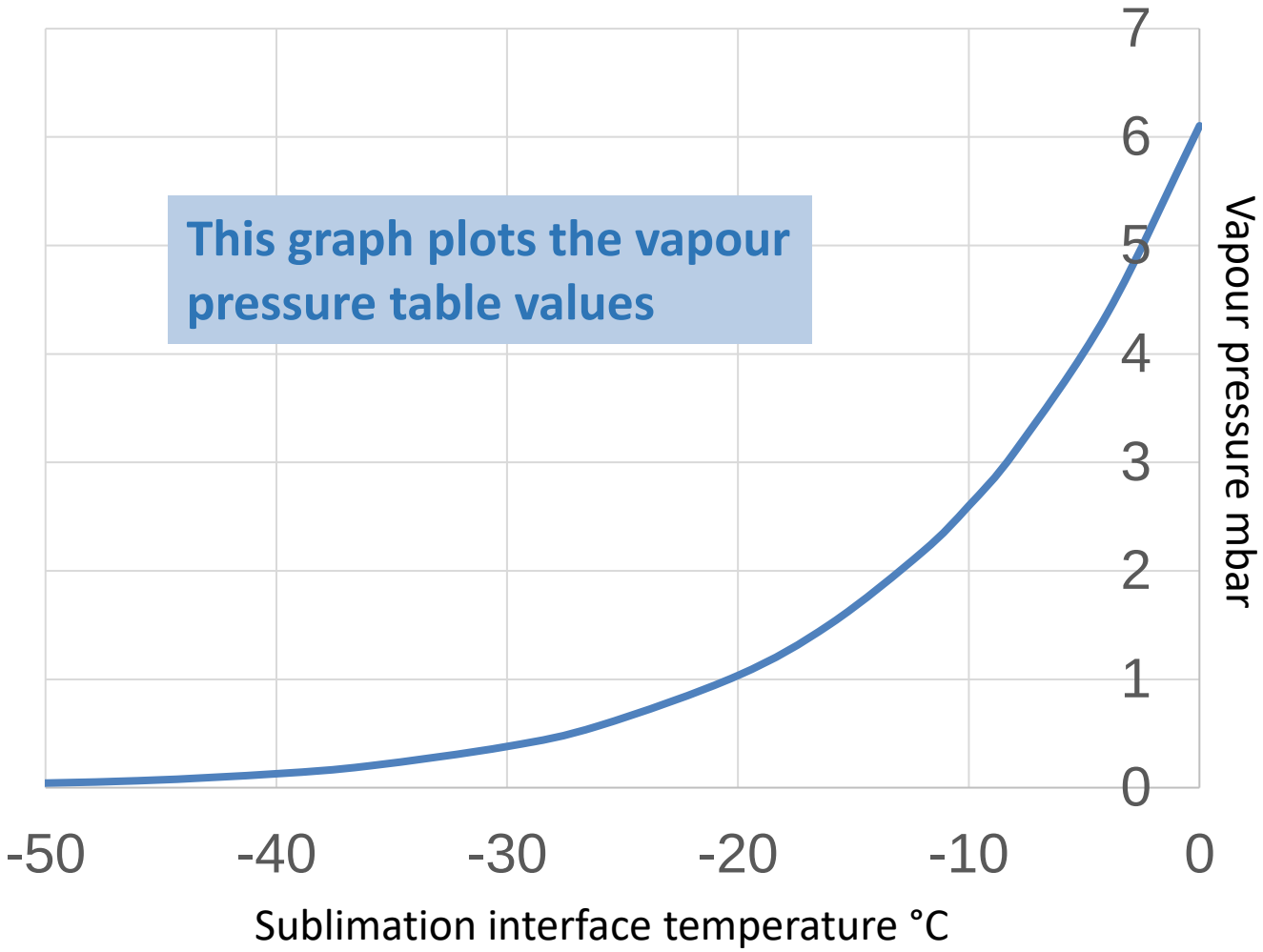


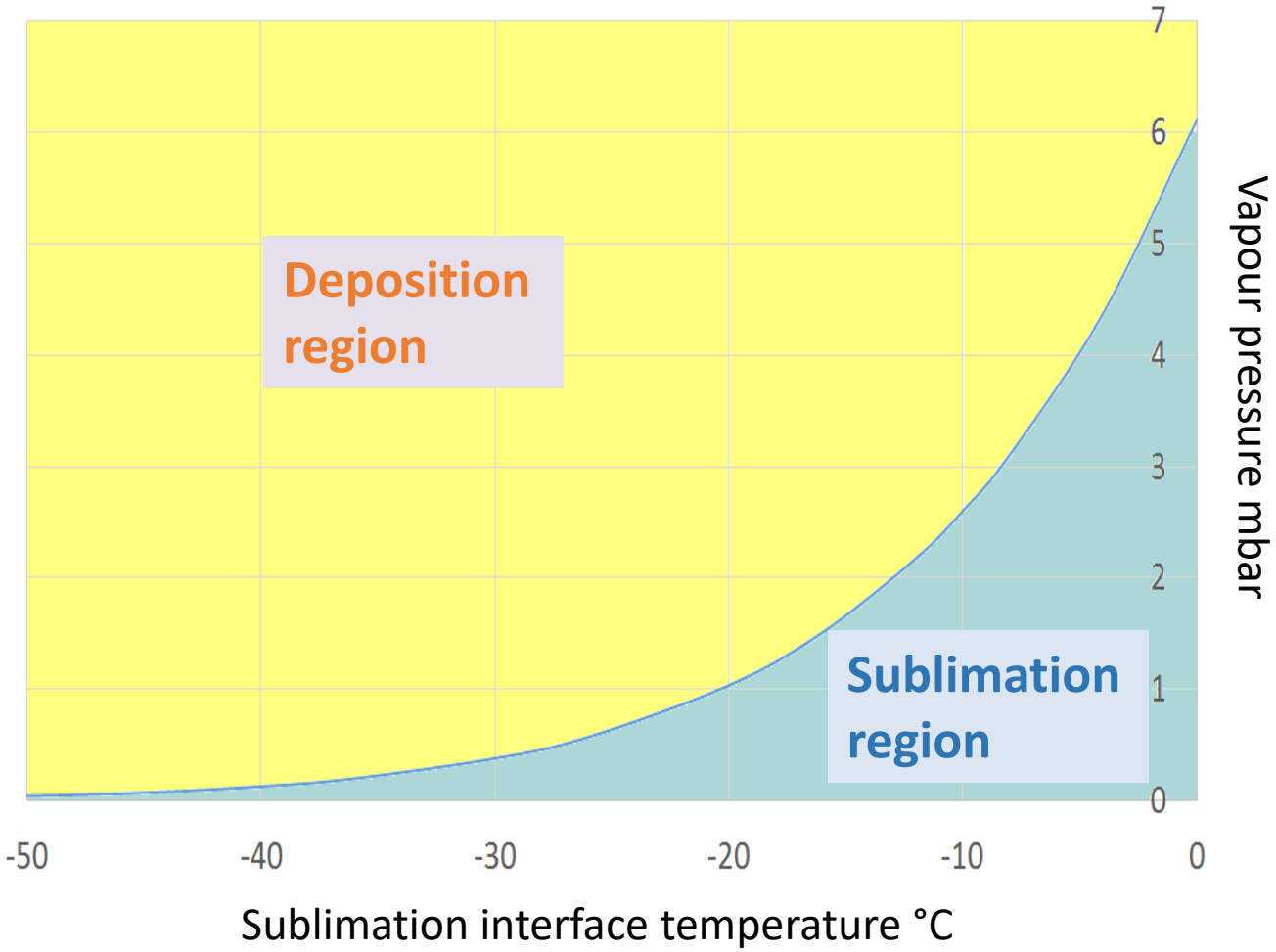
Vapor Pressure Over Ice Chart

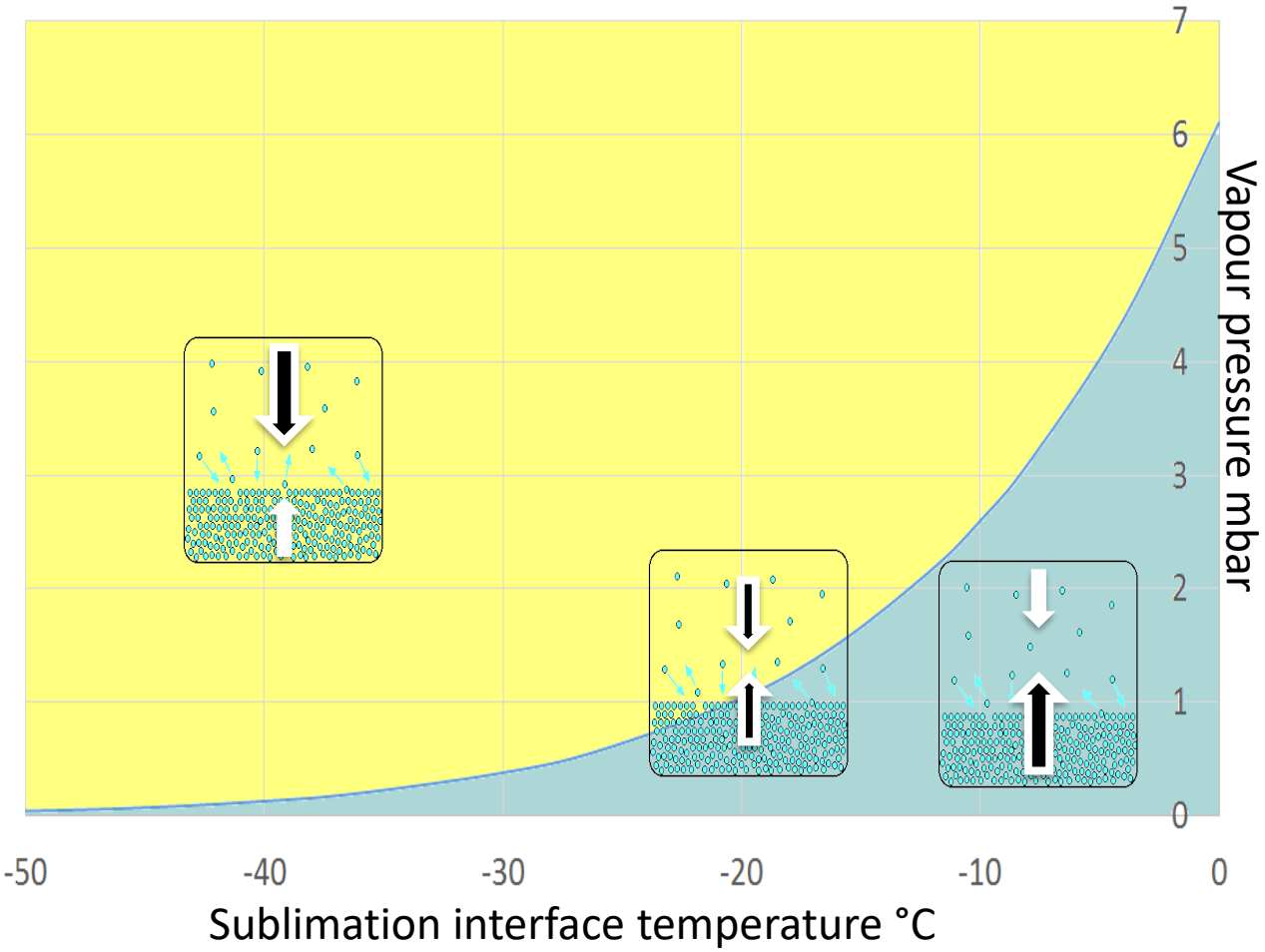
Temp	Vapor Pressure		Temp	Vapor Pressure	
Deg C	mTorr	mBar	Deg C	mTorr	mBar
0	4,584.00	6.111480	-50	29.500	0.039330
-2	3,883.00	5.176893	-52	23.000	0.030664
-4	3,281.00	4.374295	-54	17.900	0.023865
-6	2,765.00	3.686353	-56	13.800	0.018398
-8	2,325.00	3.099737	-58	10.600	0.014132
-10	1,949.00	2.598446	-60	8.100	0.010799
-12	1,630.00	2.173149	-62	6.160	0.008213
-14	1,359.00	1.811846	-64	4.660	0.006213
-16	1,130.00	1.506539	-66	3.510	0.004680
-18	936.80	1.248960	-68	2.630	0.003506
-20	774.40	1.032446	-70	1.960	0.002613
-22	638.20	0.850861	-72	1.450	0.001933
-24	524.30	0.699007	-74	1.060	0.001413
-26	429.40	0.572485	-76	0.780	0.001040
-28	350.50	0.467294	-78	0.570	0.000760
-30	285.10	0.380101	-80	0.410	0.000547
-32	231.20	0.308240	-82	0.290	0.000387
-34	186.80	0.249045	-84	0.210	0.000280
-36	150.30	0.200383	-86	0.150	0.000200
-38	120.60	0.160786	-88	0.100	0.000133
-40	96.30	0.128389	-90	0.072	0.000096
-42	76.70	0.102258	-92	0.049	0.000065
-44	60.80	0.081060	-94	0.034	0.000045
-46	48.00	0.063995	-96	0.023	0.000031
-48	37.70	0.050262	-98	0.015	0.000020

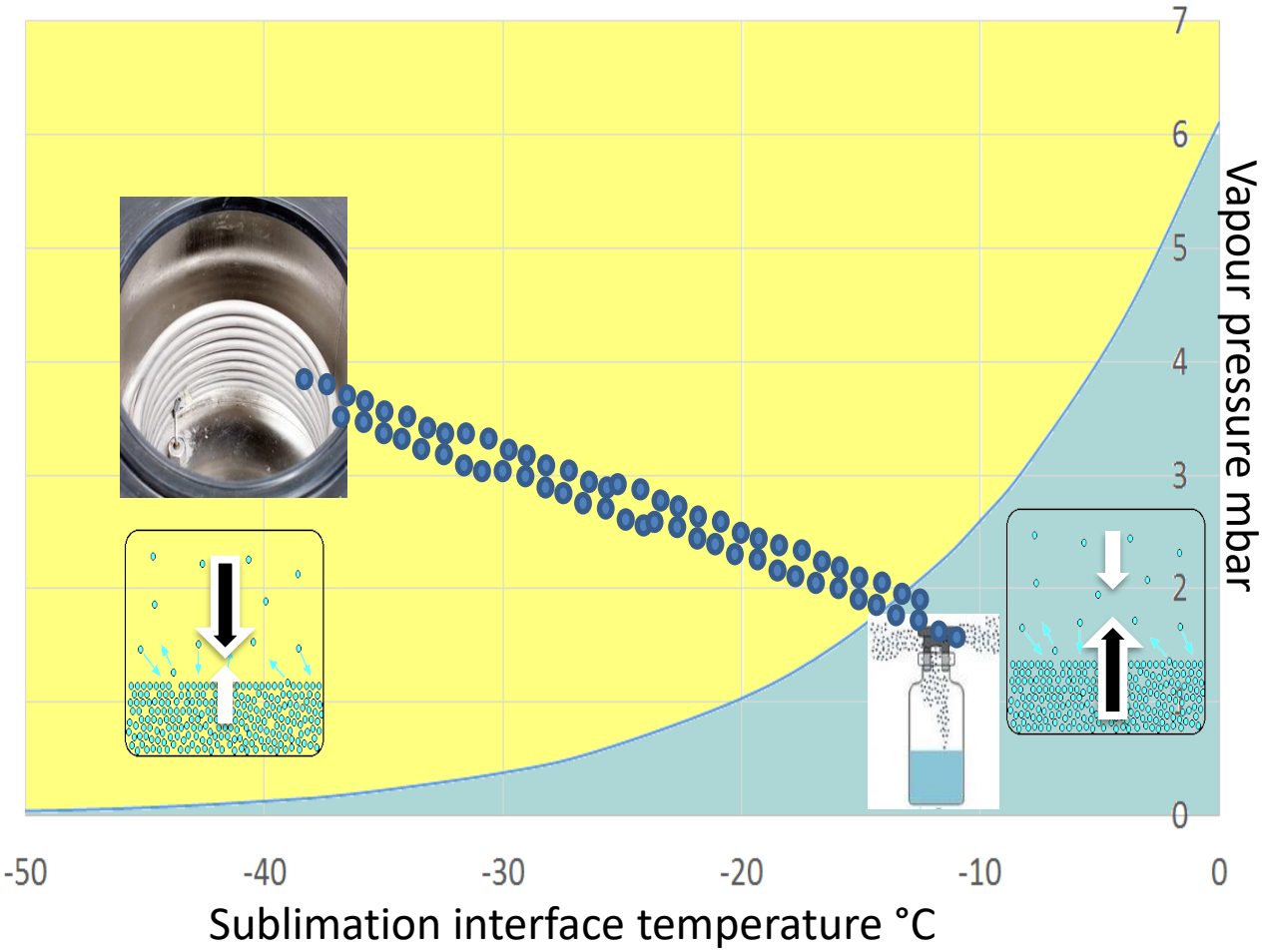
SP SCIENTIFIC
Making Time for Science

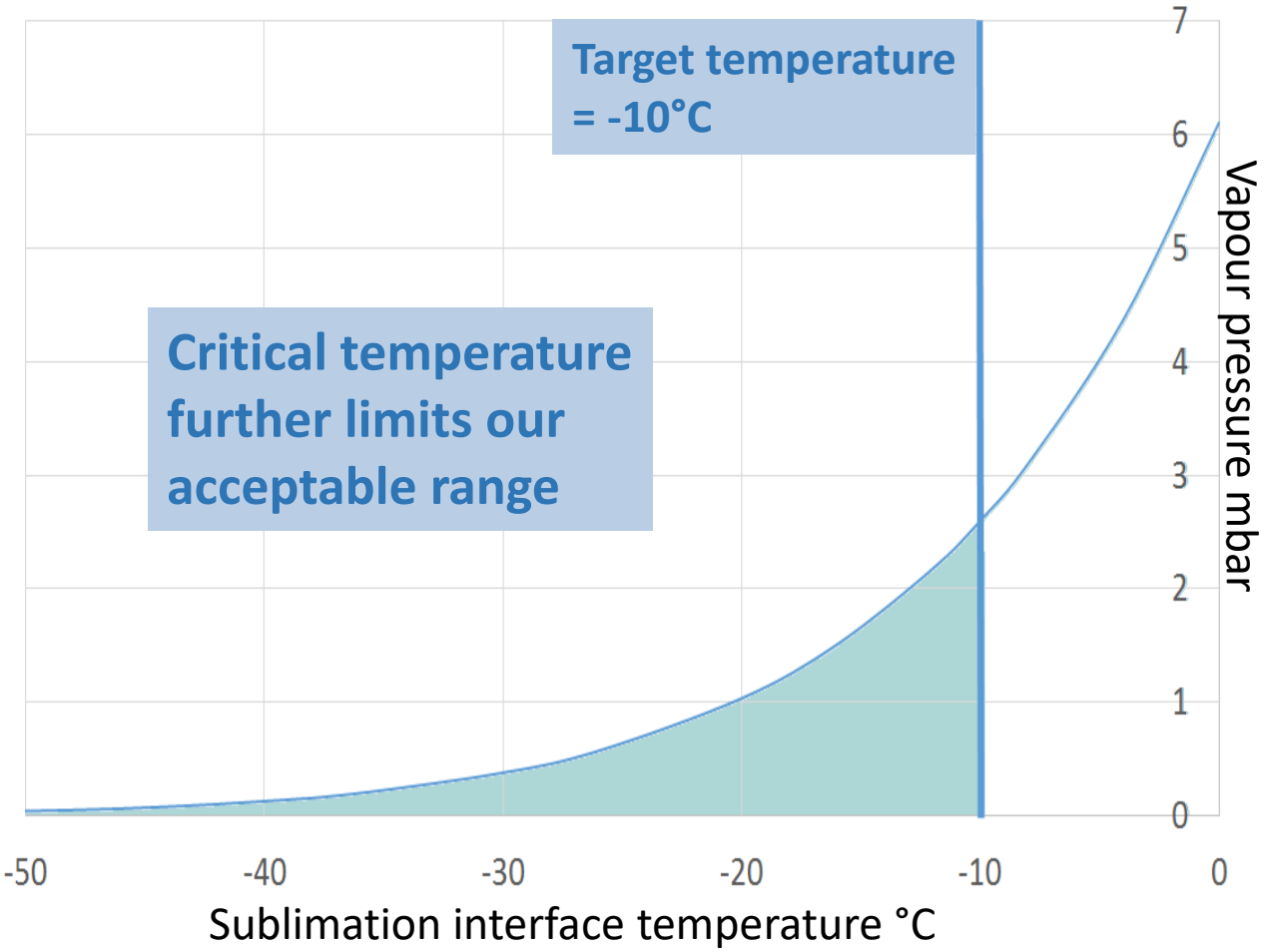
FTS
Hull
VirTis
Genevac
National-Hotpack

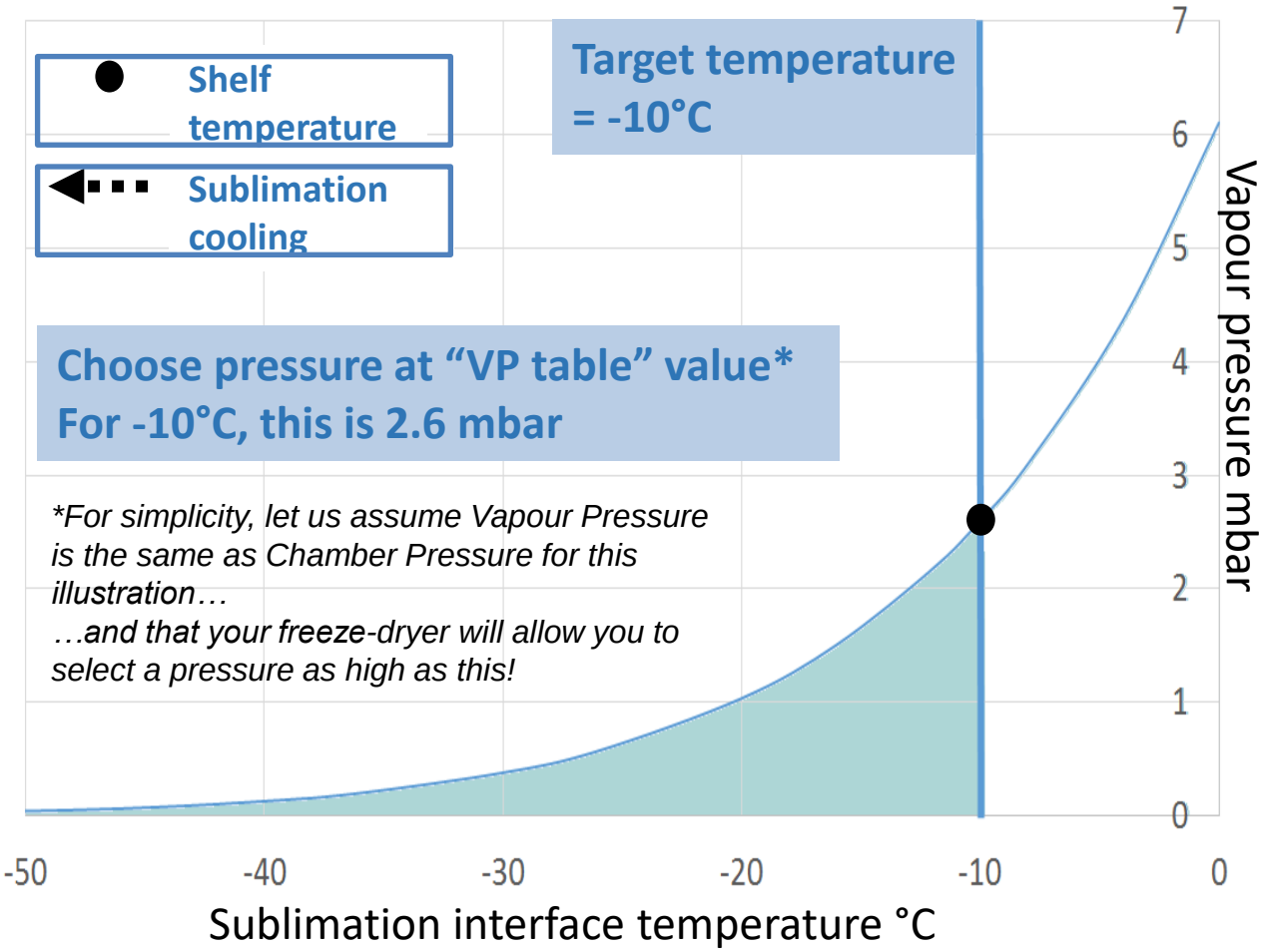

This graph plots the vapour pressure table values
7
6
5
4
3
2
1
0
Vapour pressure mbar
-50
-40
-30
-20
-10
0
Sublimation interface temperature °C

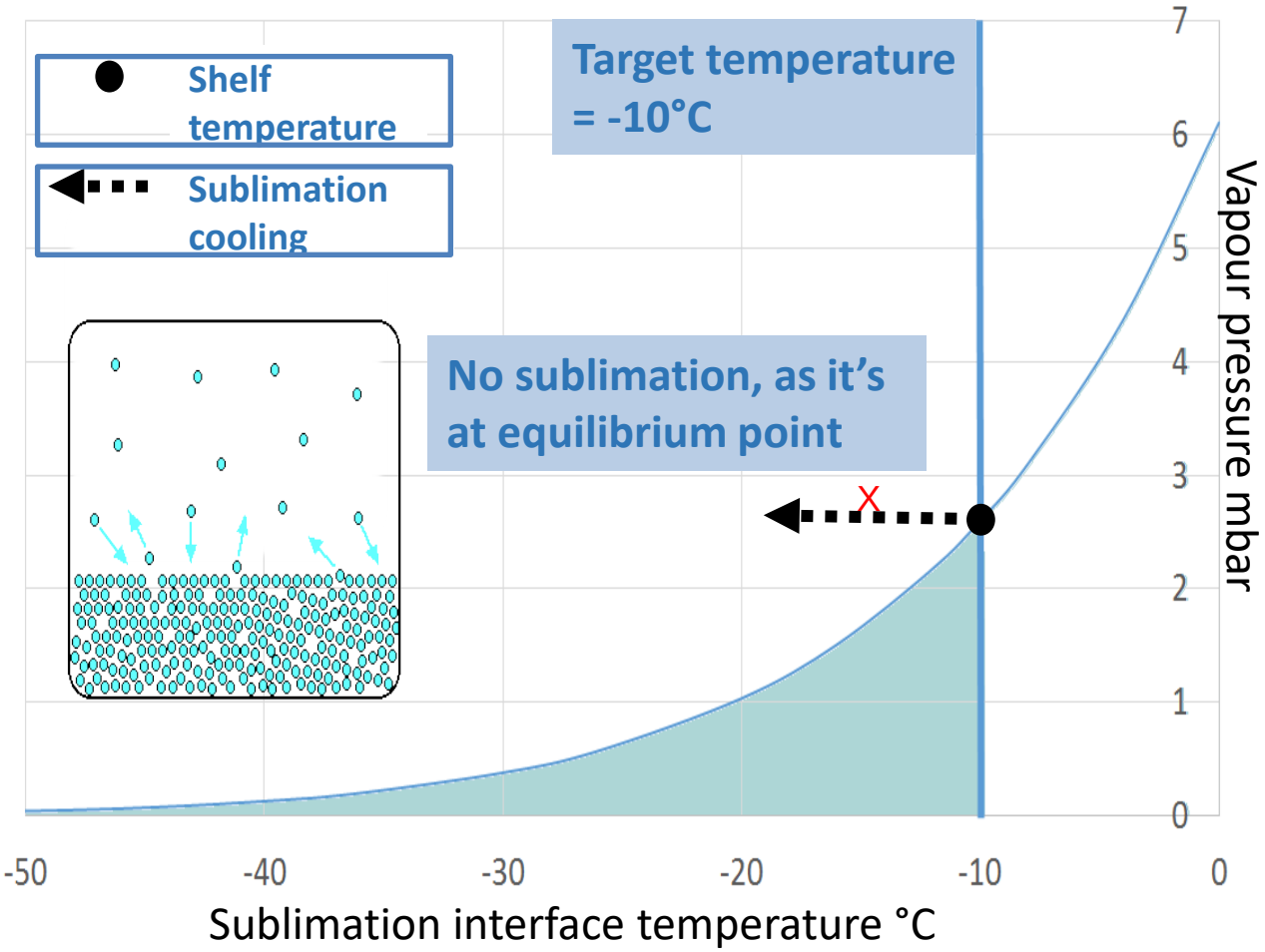

Deposition region
Sublimation region
7
6
5
4
3
2
1
0
Vapour pressure mbar
-50
-40
-30
-20
-10
0
Sublimation interface temperature °C

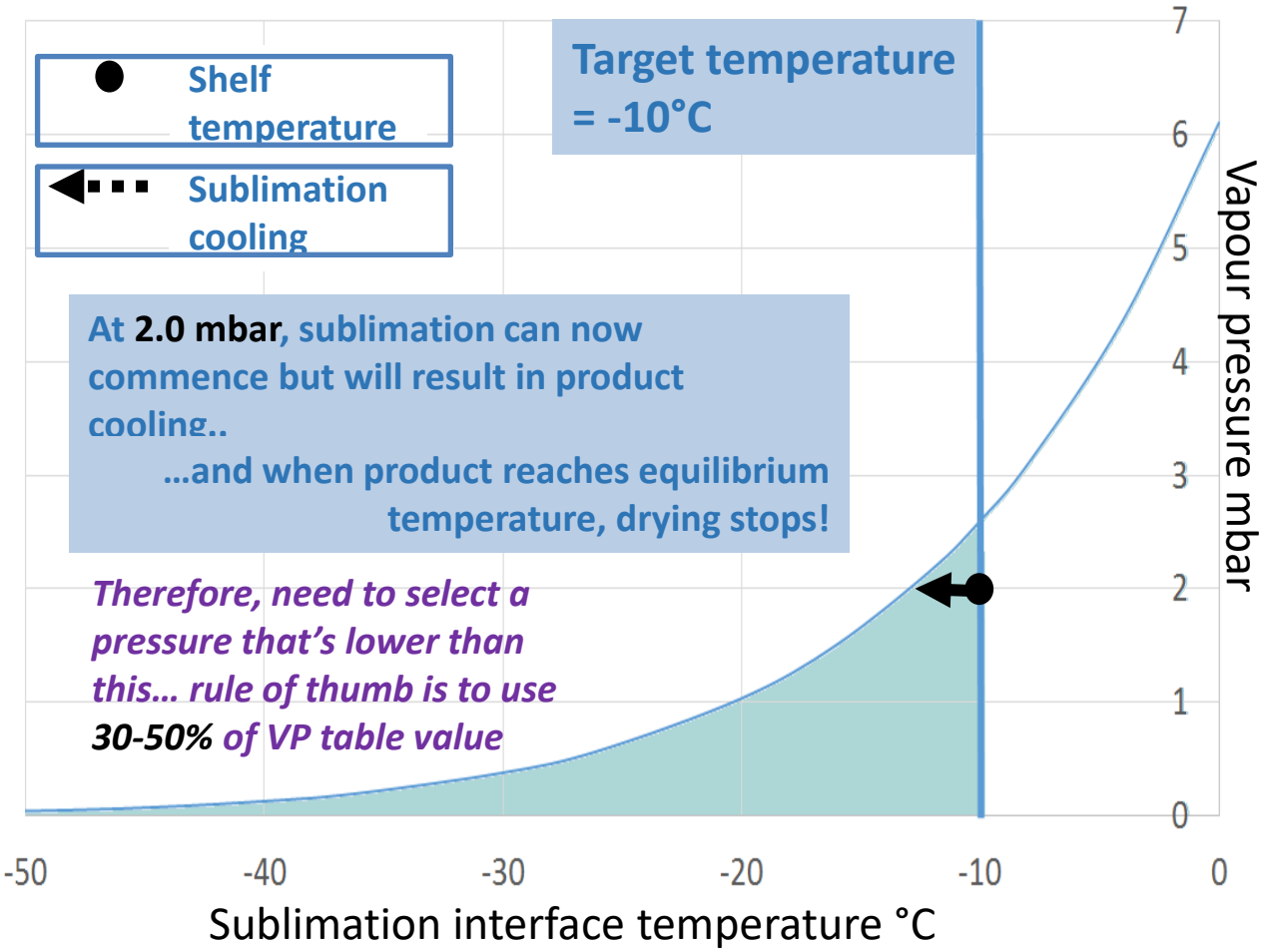

7
6
5
4
3
2
1
0
Vapour pressure mbar
-50
-40
-30
-20
-10
0
Sublimation interface temperature °C

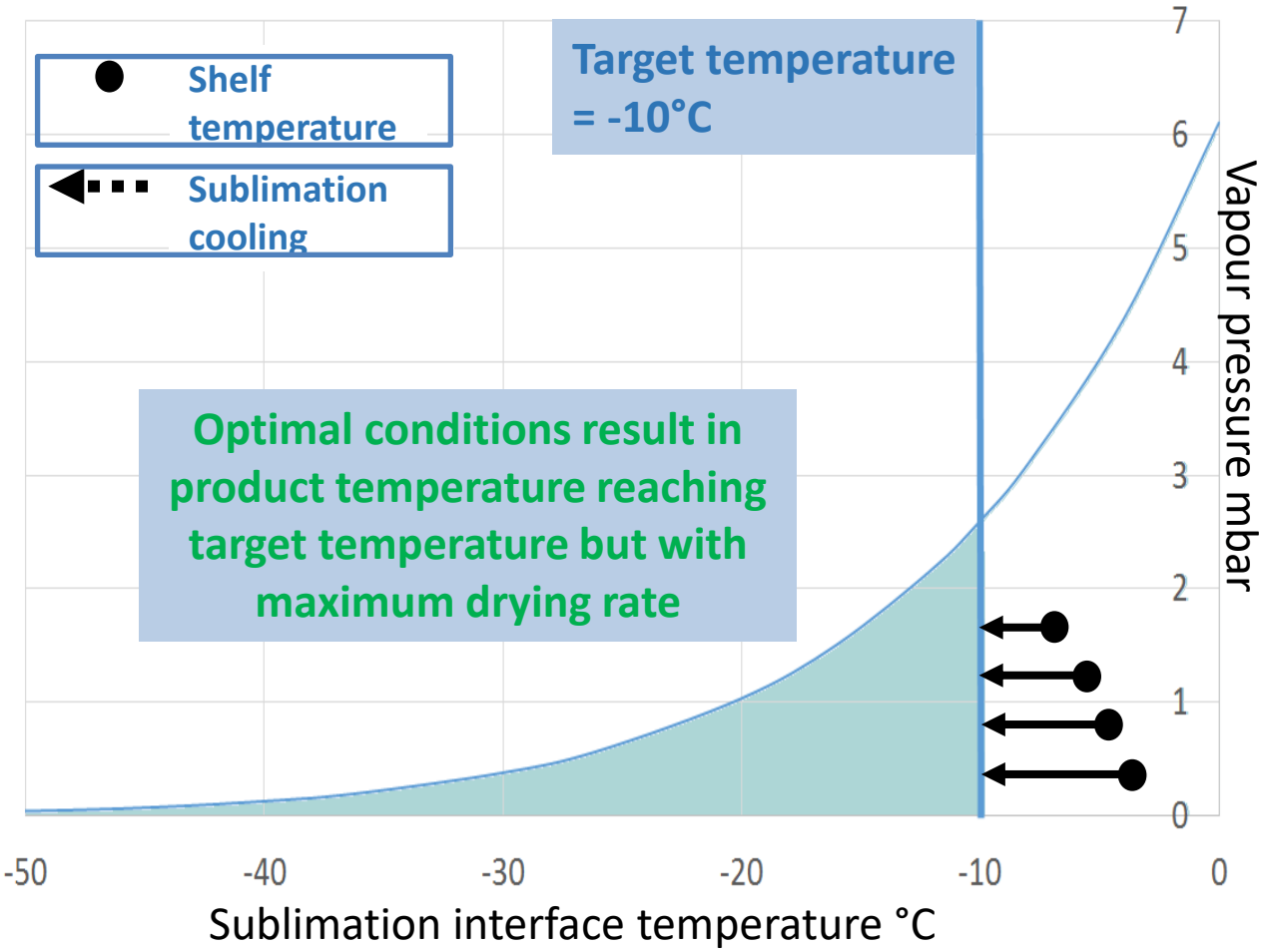

7
6
5
4
3
2
0
Vapour pressure mbar
-50
-40
-30
-20
-10
0
Sublimation interface temperature °C


Target temperature = -10°C
Critical temperature further limits our acceptable range
Vapour pressure mbar
0
1
2
3
4
5
6
7
-50
-40
-30
-20
-10
0
Sublimation interface temperature °C


Shelf temperature
Sublimation cooling
Target temperature = -10°C
Choose pressure at "VP table" value*
For -10°C, this is 2.6 mbar
*For simplicity, let us assume Vapour Pressure is the same as Chamber Pressure for this illustration…
…and that your freeze-dryer will allow you to select a pressure as high as this!
Vapour pressure mbar
0
1
2
3
4
5
6
7
-50
-40
-30
-20
-10
0
Sublimation interface temperature °C


Shelf temperature
Sublimation cooling
Target temperature = -10°C
No sublimation, as it's at equilibrium point
X
Vapour pressure mbar
0
1
2
3
4
5
6
7
-50
-40
-30
-20
-10
0
Sublimation interface temperature °C


Shelf temperature
Sublimation cooling
Target temperature = -10°C
At 2.0 mbar, sublimation can now commence but will result in product cooling..
...and when product reaches equilibrium temperature, drying stops!
Therefore, need to select a pressure that's lower than this... rule of thumb is to use 30-50% of VP table value
Vapour pressure mbar
0
1
2
3
4
5
6
7
-50
-40
-30
-20
-10
0
Sublimation interface temperature °C


Shelf temperature
Sublimation cooling
Target temperature = -10°C
Optimal conditions result in product temperature reaching target temperature but with maximum drying rate
Vapour pressure mbar
0
1
2
3
4
5
6
7
-50
-40
-30
-20
-10
0
Sublimation interface temperature °C

Other impedances to vapour migration

- Molecular interactions (collisions) between gas/vapour molecules
- Interactions between vapour molecules and the container (*e.g.* vial walls, stopper)
- Mechanical impedances by baffles, valves, bends in pipework in the freeze-dryer
- Approximately 20-30% of total resistances

- Formation of ice on the condenser will have an insulating effect
  - This will reduce the VPD, which may reduce the deposition rate of further ice
  - This effect is believed to be no more than 10% of the total resistances within the system, mainly due to the smaller VP differences at lower temperatures

Freeze Dried Formulations and Typical Use of Common Excipients

(20 Minutes)

Product properties that are strongly influenced by the Formulation

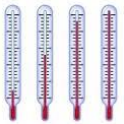
- Acceptable appearance
- Uniform from unit to unit, batch to batch
- Dried to a consistent moisture level
- Active… for its entire shelf life!
- Clean (sterile for an injectable product)
- Ethically acceptable
- Readily soluble and easy to reconstitute
- Compatibility with economical processing cycle

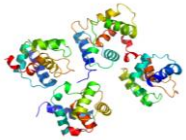
First Step: Understand the Active Ingredient…

- Is it crystalline (Teu) or amorphous (Tg' / Tc)?
- What are its bulk characteristics when freeze-dried alone?
- Solubility (are we near the limit already?)
- Can it survive FD without the use of stabilisers?
- pH-stability plot (and does the active ingredient have an intrinsic buffering capacity?)
- Hydrophilicity / hydrophobicity?

For more complex materials (e.g. proteins, liposomes, cells), there may be additional parameters to consider

Formulating specifically for Freeze-Drying





The choice of ingredients (excipients) is based on the requirements of the final product and any qualities or sensitivities of the active ingredient(s) that need to be taken into account:

- Small molecules often have low transition temperatures, which may require **thermal stabilisers** to increase the Tg' or Teu into the normal working temperature range of a lyophiliser
- Biomolecules (e.g. proteins – enzymes – antibodies) often need **lyoprotectants** to stabilise them during freezing (which involves concentration and possible pH change) and during drying, where water molecules important to the 3D structure of the active molecule may be removed
- **Bulking agents** may be added to enhance the physical properties of the lyophilised material (e.g. cohesiveness or powder flow properties) and may offset the effect of any moisture uptake

Typical examples:

Polymers or large saccharides such as dextran or PEG [poly-(ethylene glycol)]

Disaccharides such as sucrose, trehalose, lactose, maltose; using non-reducing sugars can help prevent Maillard reactions in dry state

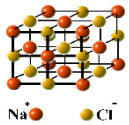
Mannitol, sorbitol, dextran, PEG, some amino acids

Some common ingredients and their roles in lyophilisation

Buffer systems	Thermal stabilisers	Lyoprotectants	Bulking agents
PBS	Dextran	Sucrose	Dextran
Citrate	Proteins	Trehalose	Mannitol
Tris	PEG (1kD+)	Lactose	Lactose
Glycine	Mannitol	Glucose	Sucrose
Histidine		Amino acids	
		Proteins	

Notes: (1) Some excipients fall into more than one category (e.g. dextran, mannitol, lactose, sucrose)
(2) The active ingredient can also fulfil additional roles itself (e.g. buffering effect)
(3) Lyoprotectants are only usually required for proteins and more complex biologicals
(4) Bulking agents may not be needed once other excipients have been added to the formulation

Solute Behaviour Patterns in Freeze-Drying

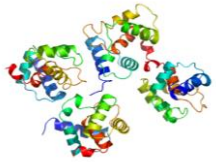


Readily crystallises, irrespective of cooling rate. Examples:

- Many crystalline drug substances (NB: some may have several crystal forms – 'polymorphs')
- Sodium chloride when used in sufficient quantities

Reluctantly crystallises, requiring slow cooling or annealing. Examples:

- Mannitol (3 anhydrous polymorphs + crystalline hemihydrate + amorphous form)
- Sodium chloride when in presence of larger amounts of other solutes
- Some small molecule drugs and – *occasionally* – buffer salts (see later)



Remains amorphous – does not / cannot crystallise at all during freeze-drying. Examples:

- Proteins (including enzymes and antibodies)
- Polymers
- Saccharides
- Some small molecule drugs

Mannitol

Mannitol is interesting because it can be the formulator's *friend* or *enemy*, depending on the circumstances… There are:

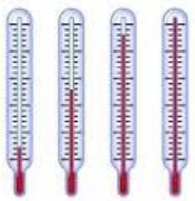
- 3 anhydrous crystal forms (polymorphs): α, β, δ
- Hemihydrate (2 mannitol molecules share one water molecule)
- Amorphous mannitol (which tends to be unstable)

We saw in the previous session that mannitol can provide a good cake, ***but*** that it can mask things that happen in the background… (*e.g.* microcollapse)

Using amorphous excipients with amorphous active ingredients can help avoid microcollapse

Thermal stabilisers

- Excipients may be added in order to improve thermal stability during processing
- The aim is to achieve a high **critical temperature** of the formulation
- The higher the temperature a product can survive without undergoing processing defects, the greater its drying rate



Rule of thumb: a Tp increase of 8°C doubles the sublimation rate

Proteins vs. Small Molecules

Proteins can be sensitive even to subtle changes in their micro-environment

- In the liquid state
- In the frozen state (or while becoming frozen)
- During the drying process
- In the dried state

The complexity of proteins is perhaps illustrated by the number of characterisation methods available and parameters of interest…

Protein Features and Characterisation Methods

Size

Electrophoresis

Size Exclusion Chromatography

Photon Correlation Spectroscopy

Concentration

Protein Assay

Quantitative amino acid analysis

UV absorption

ELISA

Surface Charge

Iso-Electric Focusing (IEF)

Ion Exchange Chromatography

Purity

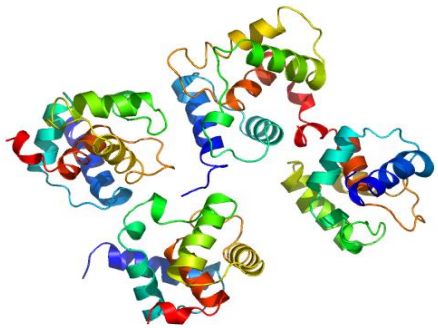
Reverse Phase HPLC

GPC

SDS-PAGE

ELISA

Immunoblotting



Structure / Sequence

N- and C- termini

Amino acid / peptide analysis

Mass Spectrometry

Disulfide linkage

Mapping carbohydrate analysis

CD, FT-IR

Shape

Circular Dichroism

Analytical Ultracentrifugation

Epitope detection

Specific binding

Identity

N-terminal sequence

Peptide mapping

Specific bioassay

Iso-electric focusing (IEF)

Activity

In-vitro bioassay

In-vivo bioassay

Specific binding / affinity assay

Titration microcalorimetry

Other Parameters

Fluorescence

Calorimetry

Clarity

Particle size / content

Osmolality, pH

How can freeze-drying help?

Low temperature processing such as lyophilisation can help minimise or ‘quench’ processes such as:

- Deamidation
- Isomerisation / Racemisation
- Hydrolytic-based degradation reactions
- Disulfide formation / exchange
- β-elimination
- Oxidation

However, freezing, drying and rehydration have also been shown to CAUSE problems…

Protein Destabilisation in Freeze-Drying…..

….and what can we do to solve it?

Cold Denaturation

Use slow cooling even while liquid

Freeze-Concentration effects:

- Increase in ionic strength
- Increase in protein concentration
- Preferential binding of salts
- pH shift

Rapid cooling may help, otherwise may need to reformulate…

Interfacial effects:

- Adsorption to ice-water interface
- Damage usually proportional to total ice crystal surface area

Slower cooling may help, otherwise may need to reformulate…

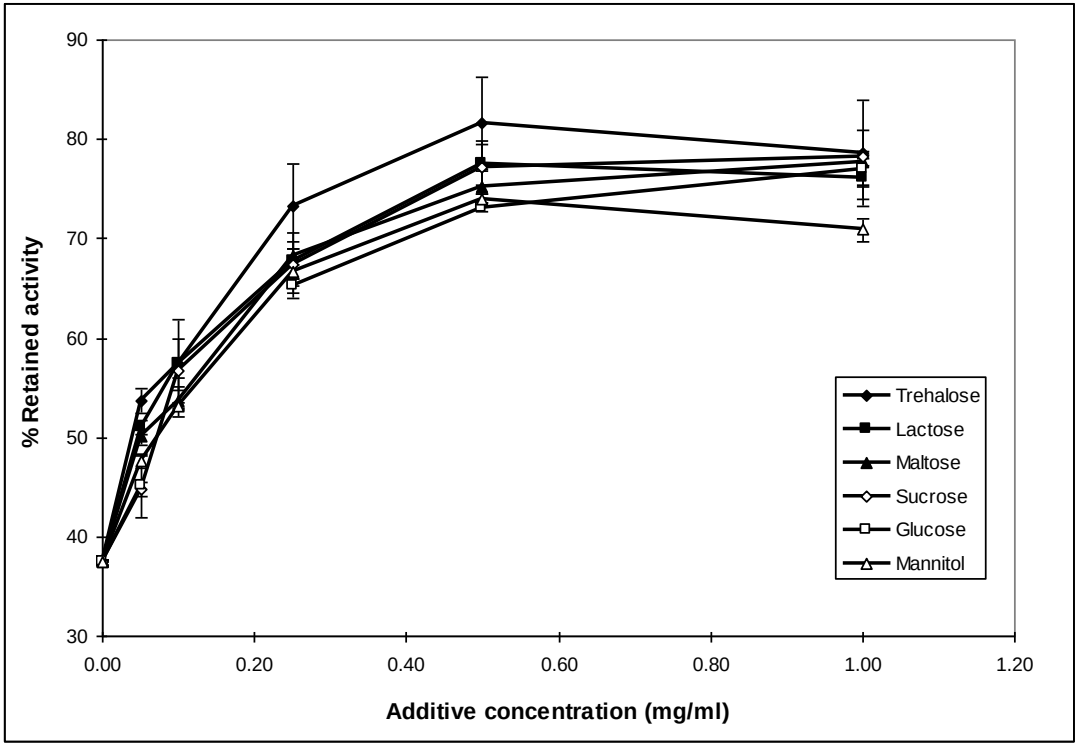
Dehydration Stresses

Use of Lyoprotectants, but cycle modification may also solve problem

Observations on Lyoprotectants

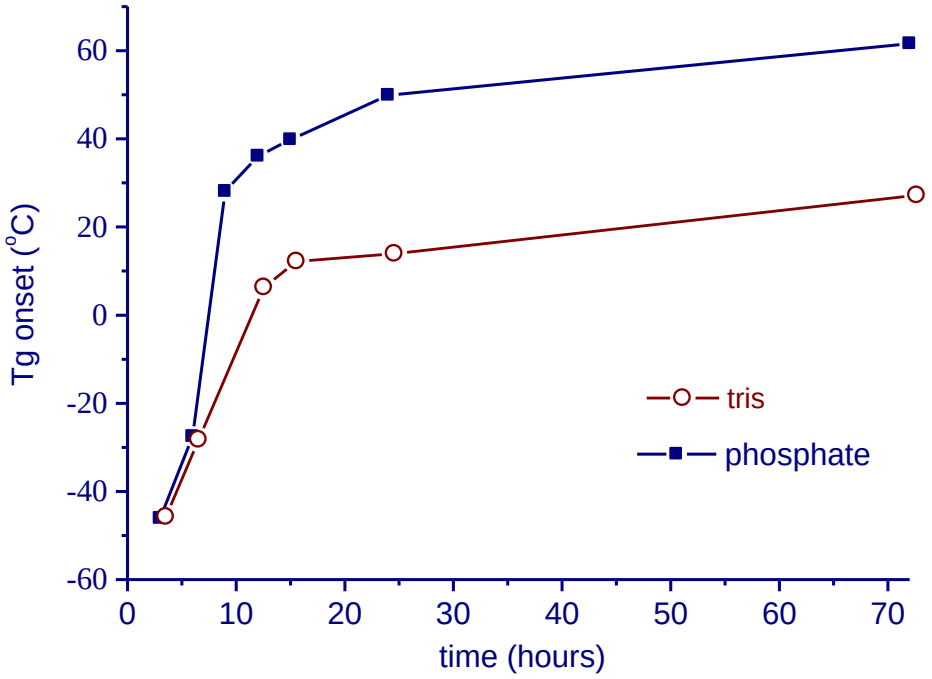
- Lyoprotectants are typically saccharides, polymers, amino acids or other proteins
- Some protectants work better than others with different proteins but this may also depend on the freeze-drying conditions used and what else is in the formulation
- Reducing sugars often best avoided due to the possibility of Maillard reactions, which can occur in the dried state and damage proteins
- Sucrose is often the disaccharide of choice due to availability and cost, but there are two scenarios where trehalose may offer greater long-term stability:
  - If drying to low moisture level still results in poor dry state thermal properties (Tg)
  - If the product needs to be maintained at a pH lower than 4.5 (sucrose may hydrolyse)

L-Asparaginase stabilised by a range of saccharides



Thermal Stability of the Dried Product

5ug/ml LDH + 5% sucrose + 10mM Buffer



Had phosphate buffer not caused problems with LDH activity, it would most likely have given a more thermally stable dried product (Tg ~60C)

Tris helped maintain activity but the dried product may not be stable at ambient temperature

“Overdrying” Proteins

Concept of *Overdrying*: Is this a legitimate concern?

- Hsu et al (1991) proposed that ideally, a monomolecular layer of water should remain on the surface of the protein molecule after lyophilisation. However, this is impossible from a practical standpoint!
- Pikal has demonstrated that overdrying is not always an issue, but the route to achieving a low moisture content can cause problems (e.g. high secondary drying temperature)
- Therefore, each protein should be assessed on a case-by-case basis and final dryness balanced with other parameters

For some proteins, the possibility of aggregation exists even in the secondary drying phase of FD

Common excipients: pros & cons

Excipient	Bulk	Thermal	Protection
Mannitol (when crystallised)	Good	Good	Poor
Disaccharides: Sucrose	Good	OK	Good*
Lactose	Good	OK	Good*
Trehalose	Good	OK	Good*
Maltose	Good	OK	Good*
Glucose	Poor	Poor	Good*
Dextran	Good	Good	?*
PVP	Good	Good	?*
PEG	Good	Good	?**
BSA / HSA	Good	Good	?*
Amino acids / dipeptides	Variable	Variable	Some good*

*fulfil the basic requirement of remaining amorphous but protective ability depends on API
**PEG often provides *cryo*protection but not necessarily *lyo*protection as it can crystallise

“Lyo-friendly” buffers (from Carpenter et al, 2004)

Citrate

Tris

Glycine / Histidine

Phosphate often best avoided due to acidic pH shift on freezing, resulting from di-sodium salt crystallising out

In all cases, there will be a freeze-concentration effect, so *even dilute buffers can cause problems*

Volatile Buffers

Particular care must be taken with components such as:

- Hydrochloric acid
- Acetic acid
- Trifluoroacetic acid
- Carbonic acid

Such components are volatile and may be removed by freeze-drying. The resulting product will then be at a **different (higher) pH** than the starting material

Stability post-Freeze Drying

Kinetics of change (crystallisation, polymorphic changes, degradation reactions) related to:

- Compatibility / reactivity of components
- Dried state thermal properties
- Storage temperature
- Moisture content (and whether this changes over time)

These should be considered during the formulation design process

Other issues affecting excipient selection

- Previous acceptance by regulatory bodies (FDA, MHRA etc.) for each mode of use / administration

  (e.g. *in-vitro*, oral, subcutaneous, IM, IV, intranasal…)

- Cost
- Chemical interactions
- Grade of quality / purity available
- Supply chain reliability / flexibility

Allowing for active ingredient batch variation

Batches of API may exhibit some variation in the levels of some components – especially those produced *via* biological processes (e.g. fermentation)

Varying levels of such components may lead to:

- Better or poorer lyoprotection of the active molecule
- Fluctuation (raising or lowering) of the collapse temperature
- Other changes in formulation behaviour (e.g. mannitol crystallisation)

If the upper and lower concentration limits of these components are known, it should be possible to make the formulation sufficiently robust to minimise the impact of any such effects

Critical temperatures of mixtures (1)

For **fully amorphous systems**, the Tc / Tg' of a mixture can be relatively predictable (it approximates to the weighted mean values for the individual components)

Also, as a general rule:

- Higher MW components (*e.g.* polymers, proteins, large saccharides) tend to have higher critical temperatures
- Lower MW components (*e.g.* salts, small saccharides) tend to have lower critical temperatures

Critical temperatures of mixtures (2)

For **fully crystalline systems**, the critical temperature is harder to predict. There may not be a true eutectic mixture, but as a rule, the Teu of the mixture will tend to be *lower than the lowest Teu of any component*

For a **crystalline / amorphous mix**, it is impossible to predict the critical temperature, as:

- It depends on the extent to which the amorphous components inhibit the crystallisation of the other components
- This may also depend on cooling rate and temperature

Amorphous + Crystalline components when mixed =

More predictable →

- Predictable phase separation
  (ice + glassy phase + crystalline phase)
- Be aware of possible microcollapse / micromelting
- Predictable inhibition of crystallisation
- Resulting metastable components that could change over time *(during freeze-drying, or in the dry state)*
- Completely unpredictable behaviour with elusive or less well defined “critical temperature”

Less predictable →

Summary

Only add excipients that fulfil a useful function, such as:

- Bulking agents (cohesive cake)
- Thermal stabilisers (high $T_{critical}$)
- Sensitivities of the molecule / entity of interest
- Protective Agents (retention of activity)
- Effects of mixing crystalline and amorphous components on $T_{critical}$

Summary

There are many other factors that influence choice of excipients (*i.e.* not strictly related to freezing or drying, but rather, commercial / strategic / regulatory factors)

Complex buffers best avoided, especially if other formulation components are sensitive to pH changes during freezing

Features such as high collapse temperature may often be designed into a formulation, but Teu is more difficult to predict

Care should be taken to give good (upstream) stability and product shelf stability, not just success during the freeze-drying process itself

Frozen State Characterization (30 minutes)

"How do we know what the Critical Temperature is for our product?"

The "Critical Temperature" will be:

- The **eutectic temperature (T_{eu})** for **crystalline materials**
- The **collapse temperature (T_c)** for **amorphous materials** (somewhere at or above the glass transition temperature)
- The **lower** of the above temperatures for **mixed systems** (depending on whether micro-collapse is acceptable)

We can analyse the critical temperature of a formulation before freeze-drying it, using, for example:

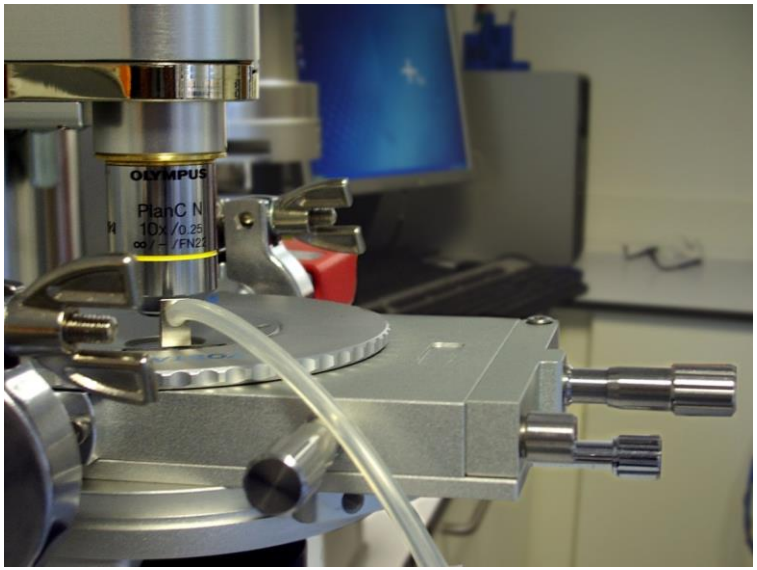
- Freeze-Drying Microscopy (FDM)
- Impedance ($Z\sin\varphi$) and Thermal Analysis

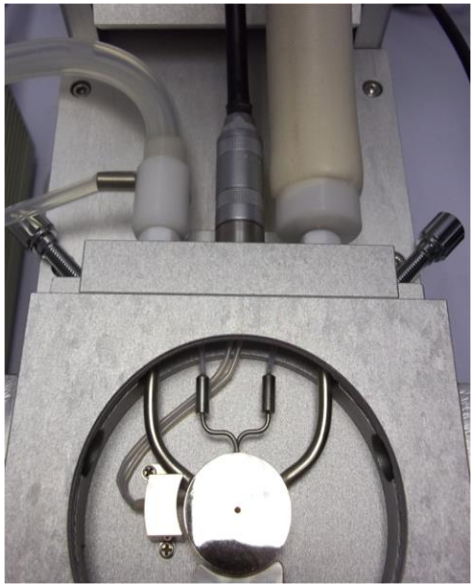
Freeze-drying microscopy (FDM)

FDM is the study of freeze-drying at the microscopic level

FDM allows determination of collapse, melting and other phenomena such as skin (crust) formation

What is a Freeze-Drying Microscope?





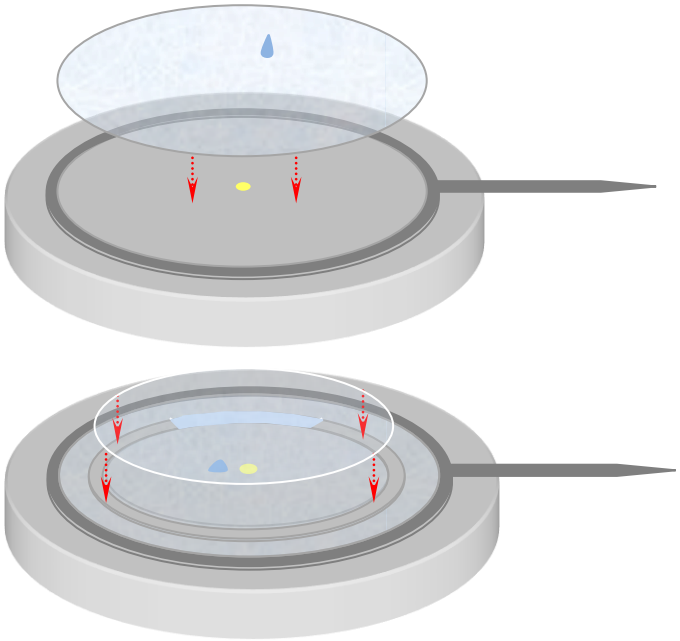
Effectively a ‘micro freeze-dryer’ where the freeze-drying of a small sample may be observed

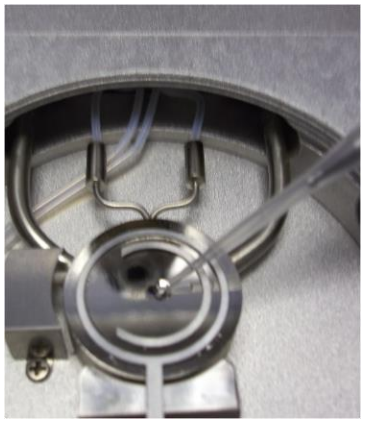
First designs back in the 1960s (Alan Mackenzie, Louis Rey)

FDM systems have been commercially available since the 1990s

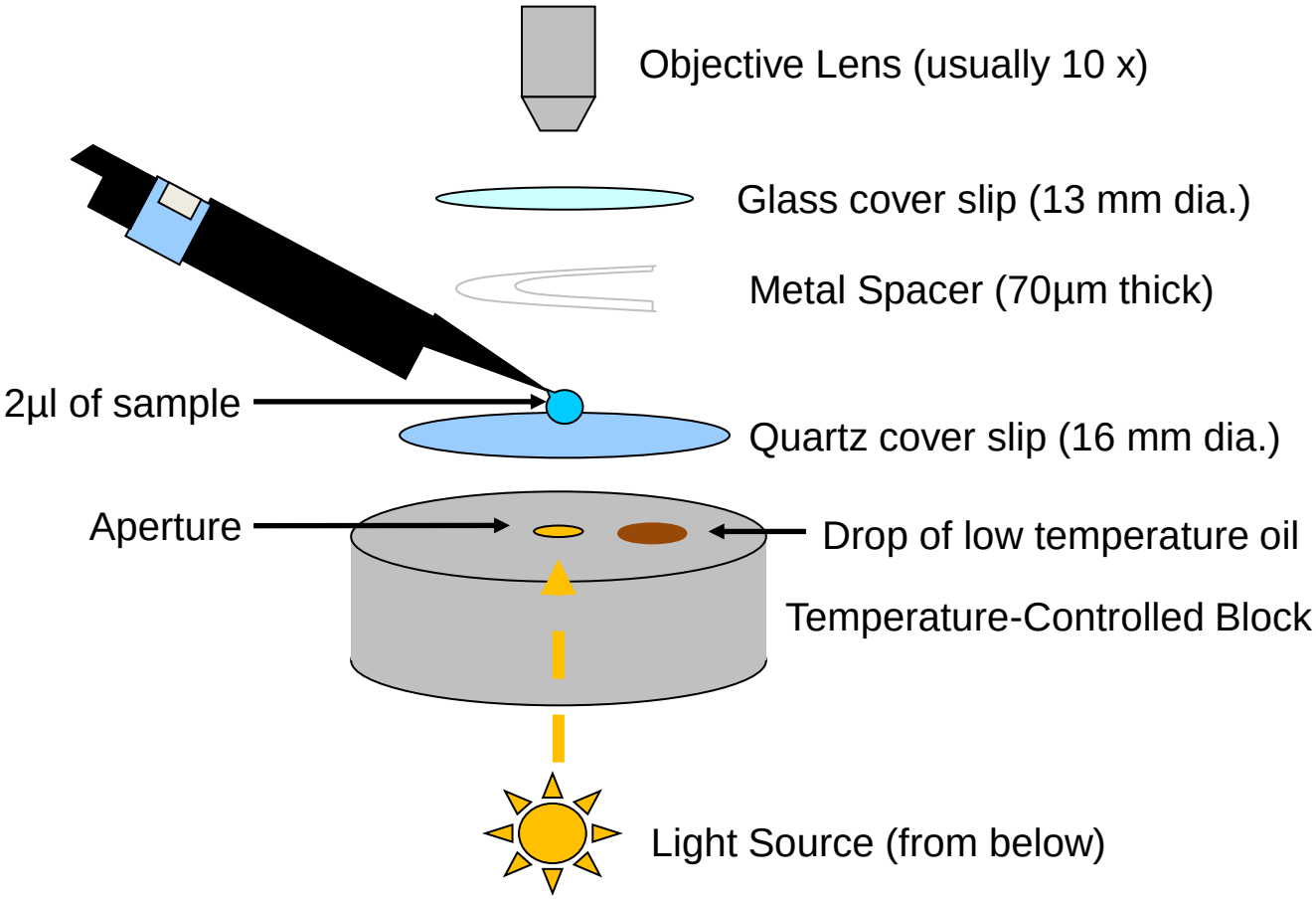
Sample preparation

A 1 – 2ul sample of the formulation loaded onto a quartz slide mounted on a silver block. A glass slide is placed on top of a 70um spacer to ‘sandwich’ the sample, which is then viewed from above.

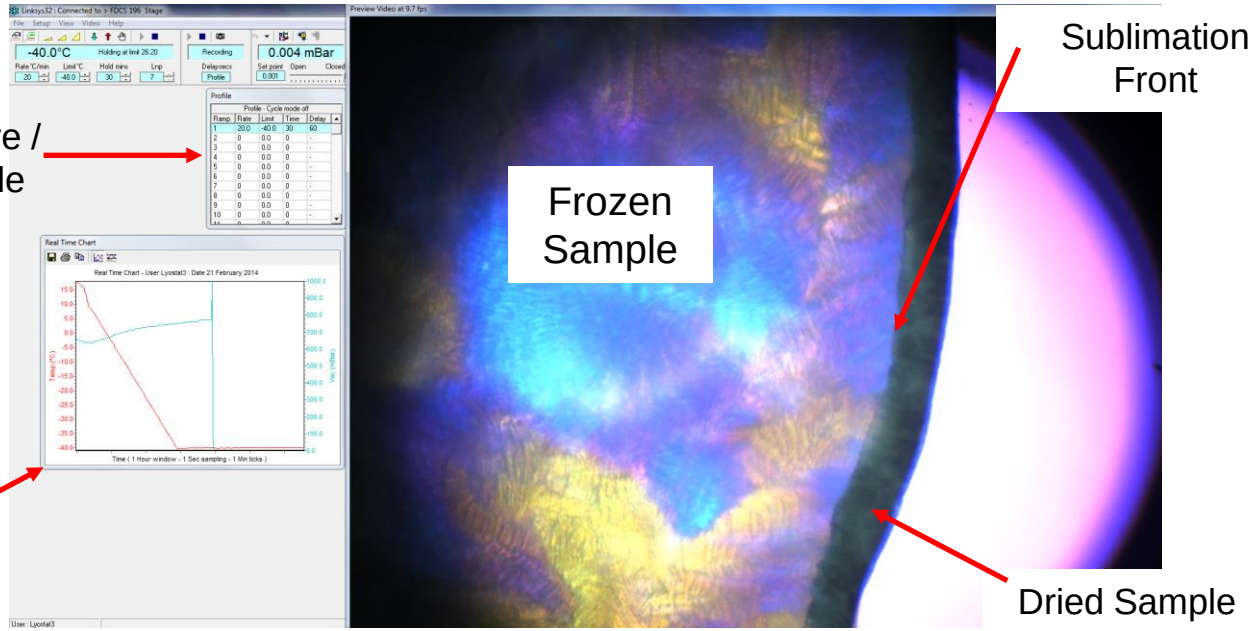




Sample Format in BTL's Lyostat FDM

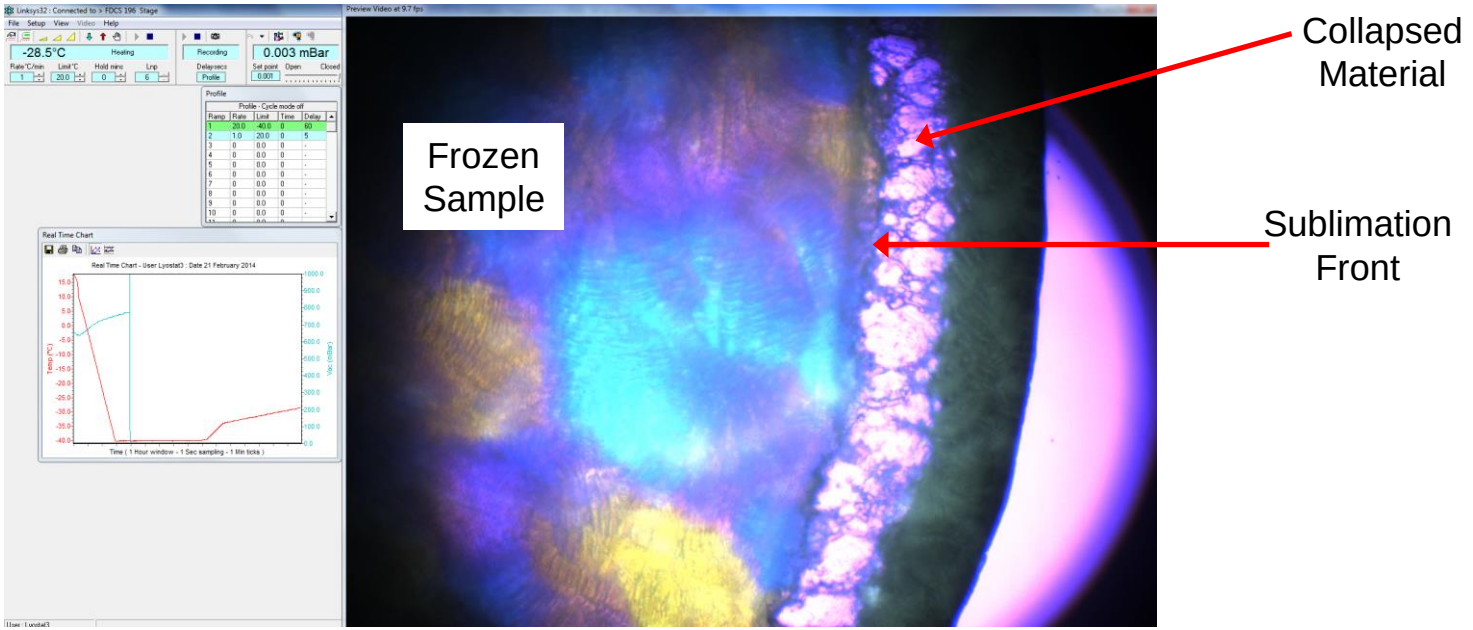


Initial FDM Image



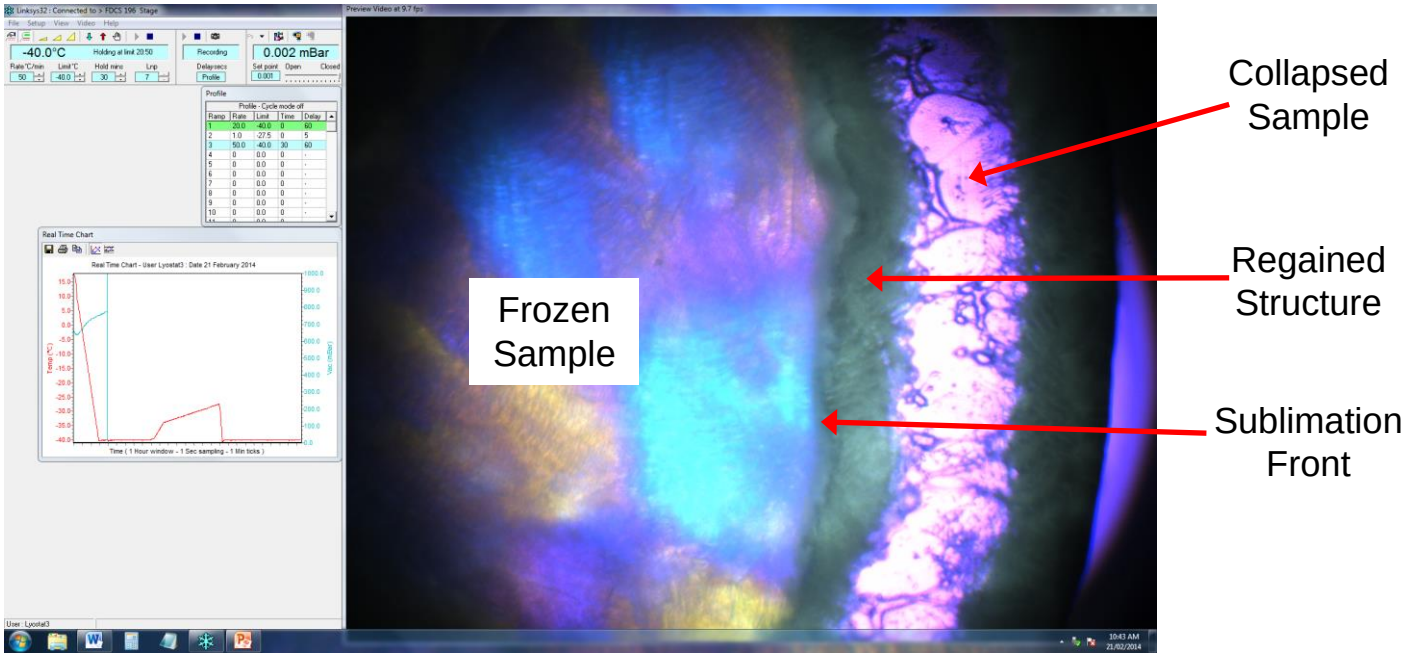
- Sample viewed from above (*"plan view"*)
- When sample reaches the holding temperature and has been observed to freeze, vacuum pump is switched on and drying begins.
- Sublimation interface can be seen moving through the frozen sample.

Identification of the Collapse event



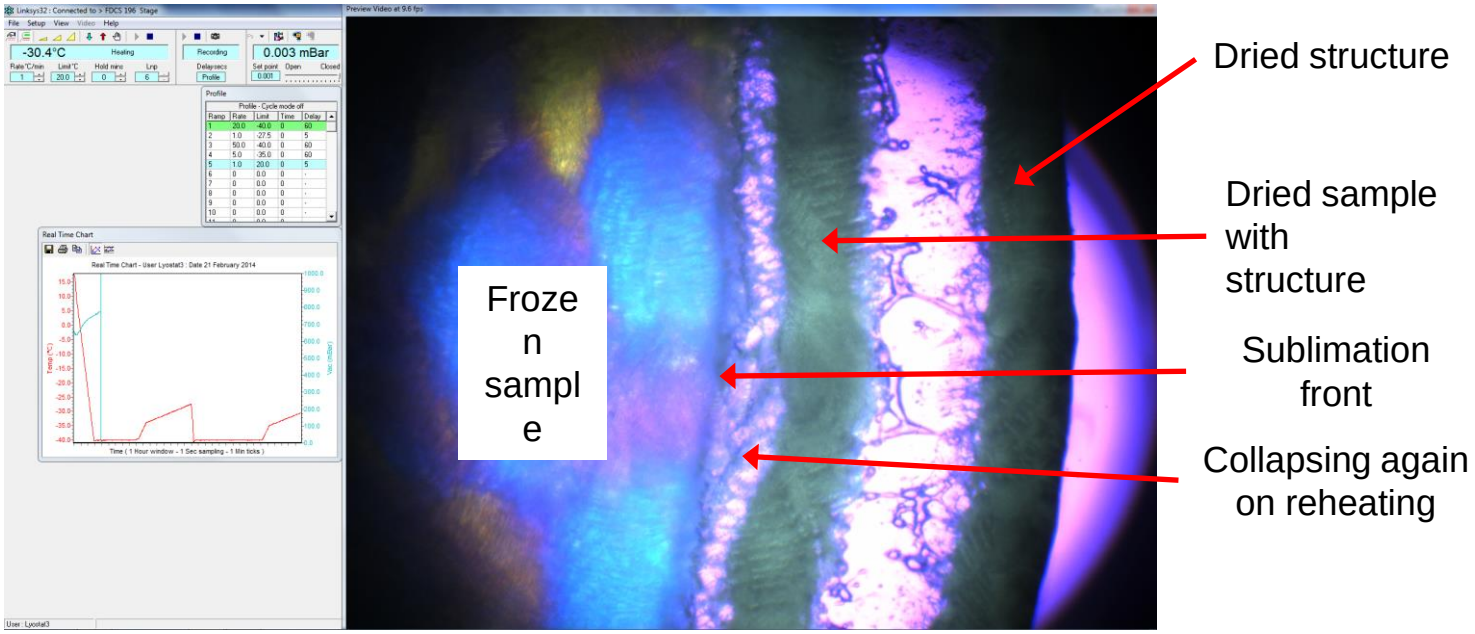
- Increasing or decreasing the temperature of the sample allows you to view the critical freeze-drying characteristics.
- By examining the freeze-dried structure behind the interface, the collapse temperature of the material can easily be determined.
- The temperature may be cycled in order to evaluate Tc more closely

Identification of the Collapse event



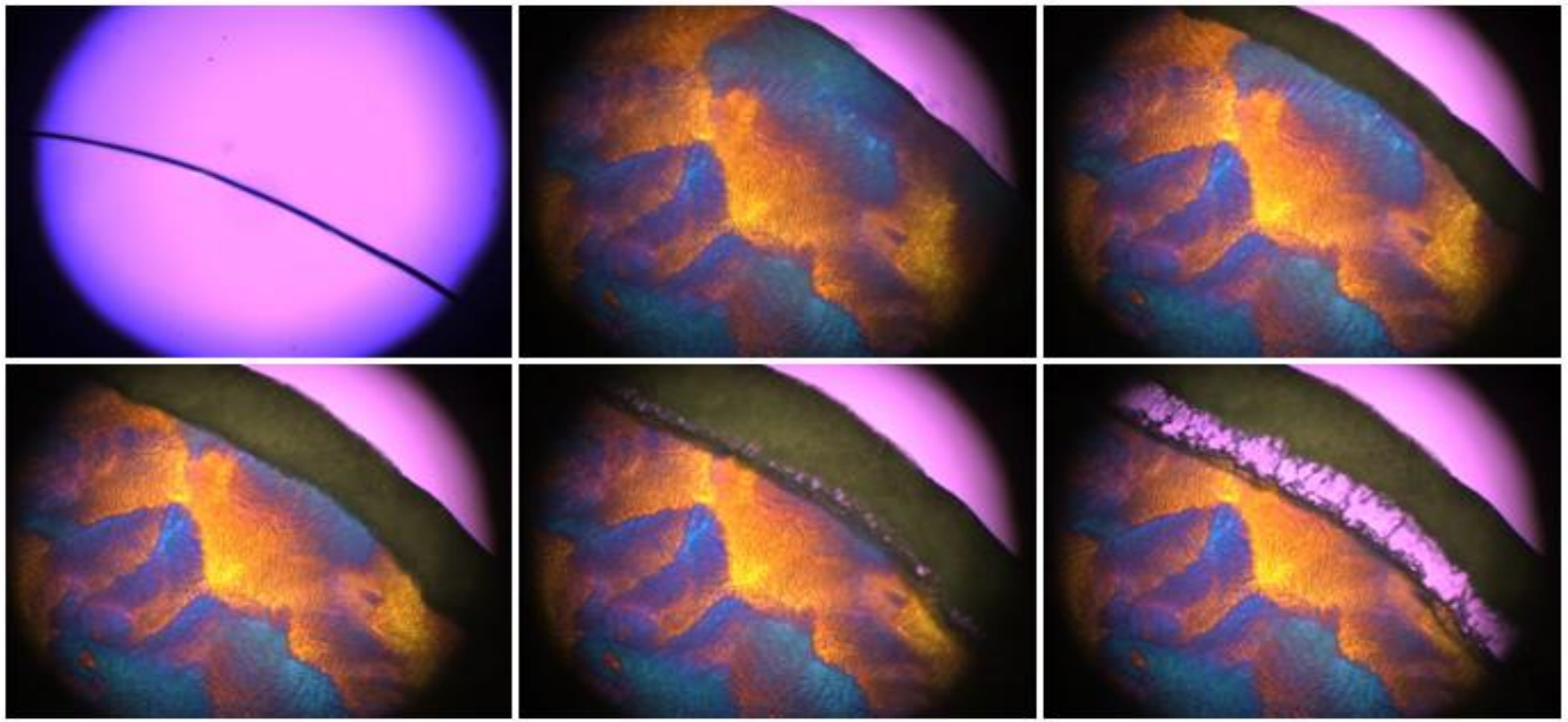
- Sample structure lost when the collapse temperature was exceeded.
- Structure regained as sample was re-cooled to below its collapse temperature.

Identification of the Collapse event



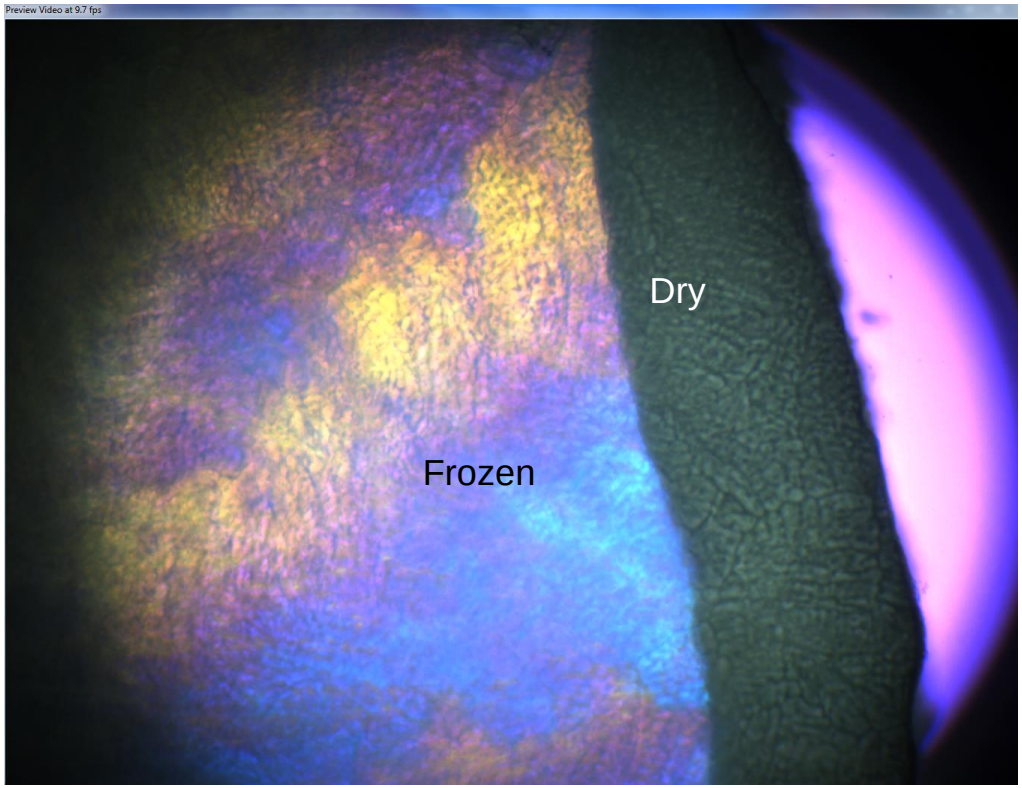
- 100% structure has been regained by lowering the sample temperature.
- Sample temperature was again increased to above its collapse temperature, causing the sample to collapse.

4% Sucrose solution drying on the Lyostat3

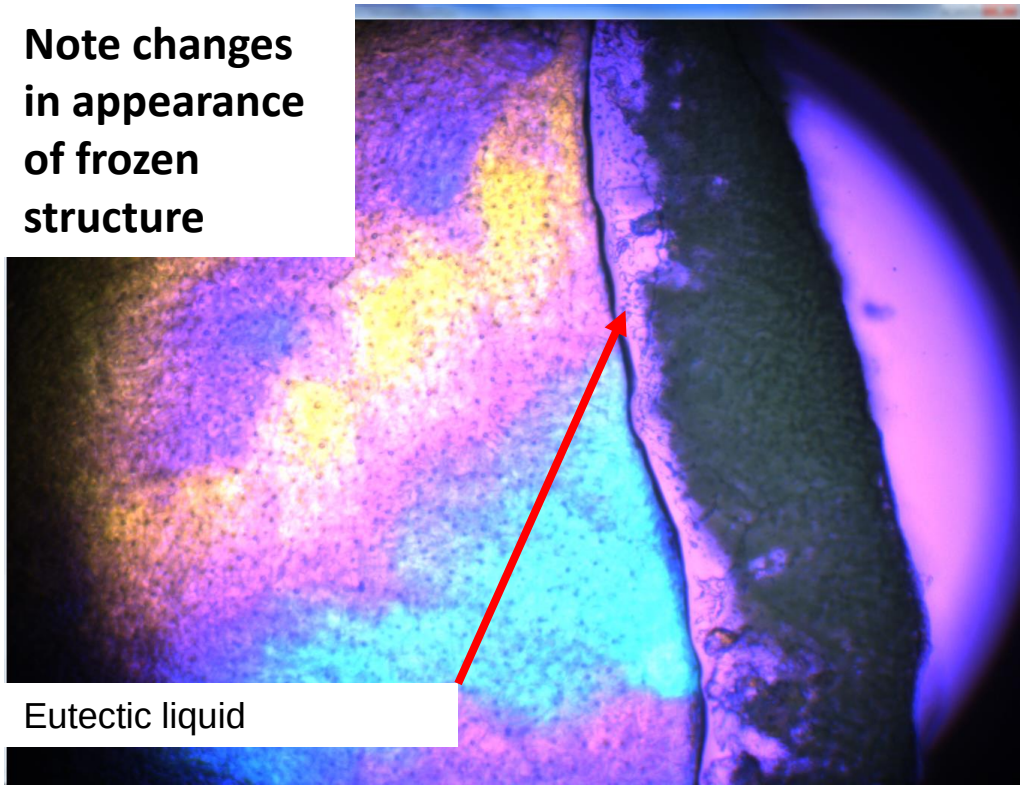


So, what else can FDM tell us?

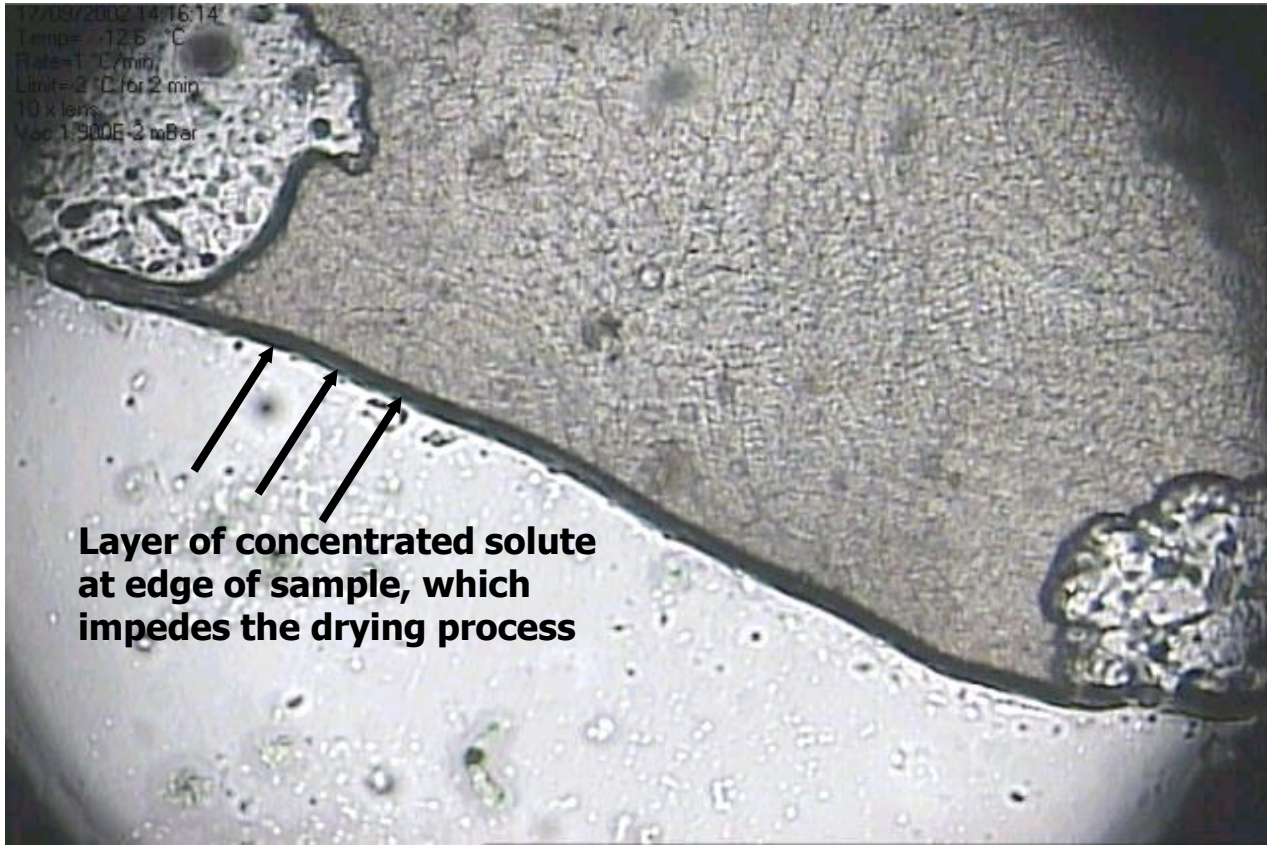
NaCl Below Eutectic Temperature



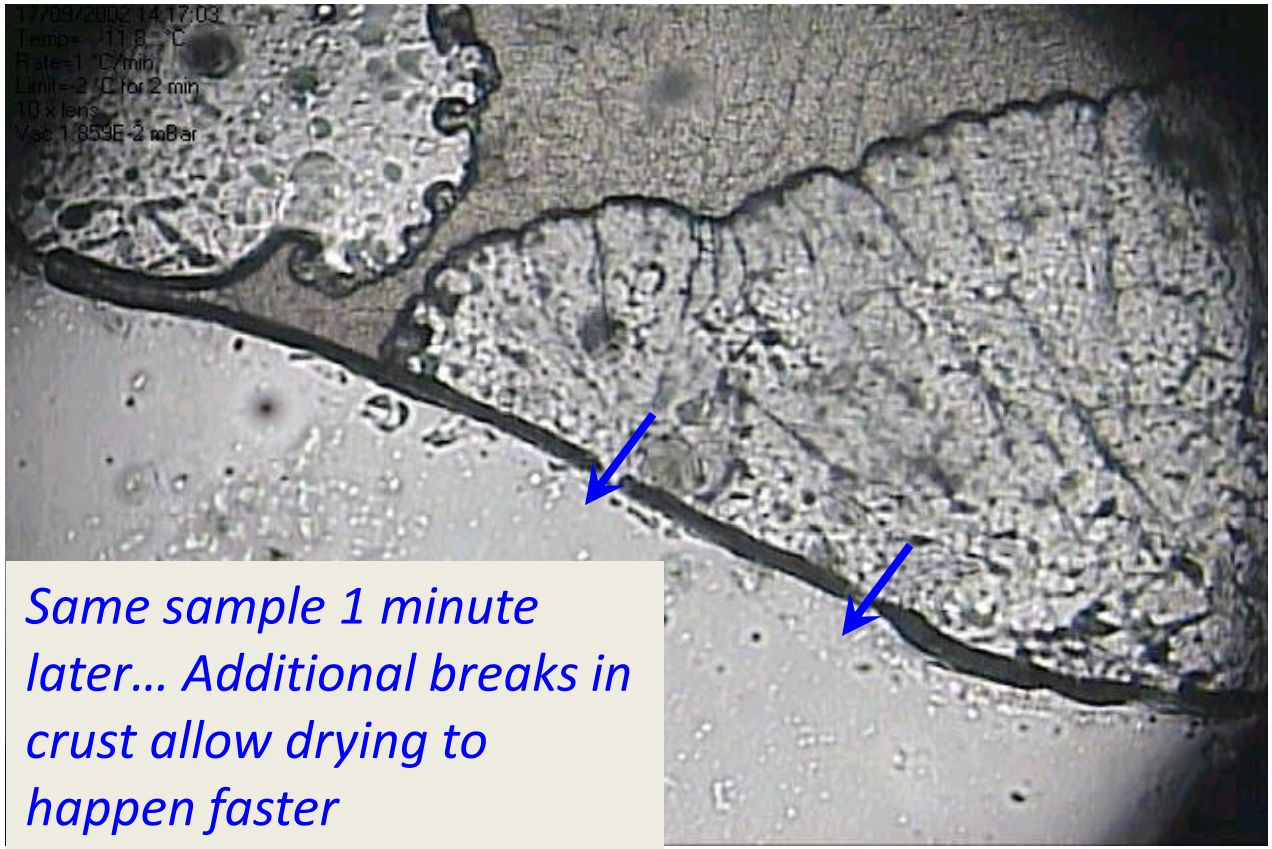
NaCl Above Eutectic Temperature



Skin (crust) formation (1)

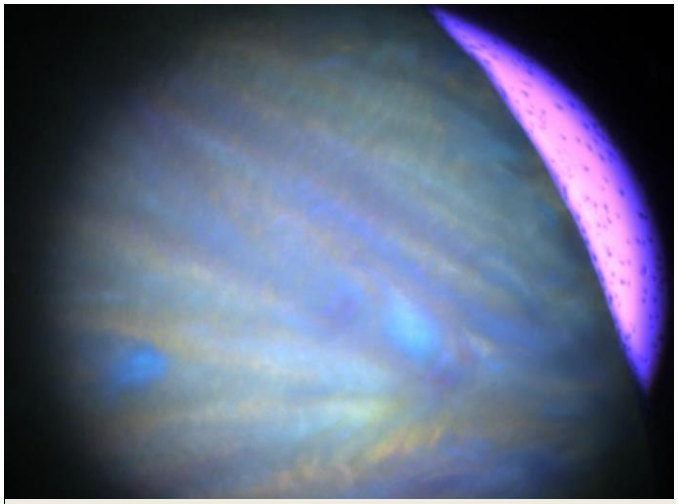


Skin (crust) formation (2)

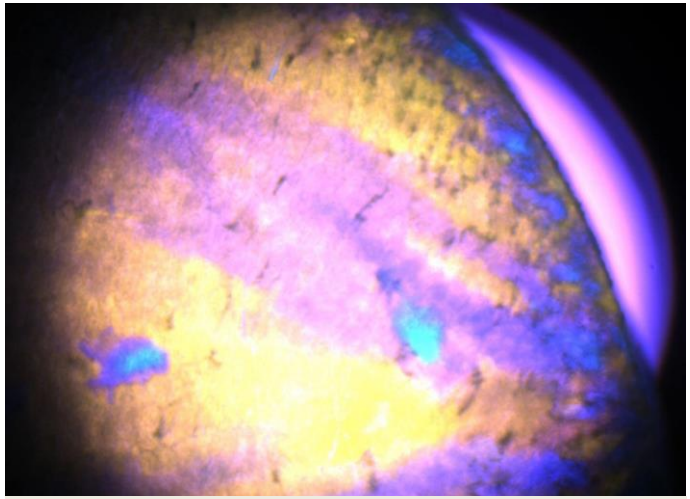


Same sample 1 minute later… Additional breaks in crust allow drying to happen faster

Effect of annealing on ice crystal size



Sample cooled to -40°C, then warmed to -10°C



Same sample after a further 10 minutes at -10°C

Experiments can be carried out at different temperatures, in order to establish what annealing temperature might be most efficient to use in the freeze-dryer.

Further applications of FDM

It is possible to examine differences in relative drying rates:

- For different formulations
- For a specific formulation at different temperatures

Ref: Zhai, S., Taylor, R., Sanches, R. and N.K.H. Slater (2003). Measurement of Lyophilisation primary drying rates by freeze-drying microscopy. *Chem. Eng. Sci.* **58**, 2313-2323

FDM in Summary

FDM can provide a **visual** indication of:

- Collapse temperature (Tc)
- Eutectic temperature (Teu)
- Skin formation potential
- Annealing effects: on ice structure, solute crystallisation, critical temperature
- Relative rates of drying for different formulations, or for the same formulation at different temperatures

All the above information can be useful for formulation & cycle development, but is this 100% of the story?...

Thermal Analysis - Overview

The basis of thermal analysis and its relevance to lyophilisation

More commonly used methods:

- Differential Scanning Calorimetry (DSC)
- Modulated DSC (MDSC)
- Differential Thermal Analysis (DTA)
- Electrical Impedance (Zsinφ) Analysis

94

The use of thermal analysis

Most changes occurring in a material (liquids, solids, gases) will be accompanied by a heat flow

Changes requiring heat are *ENDO*thermic. *E.g.*

- Melting (of ice or eutectic solids)
- Softening when warming through a glass transition

Changes emitting heat are *EXO*thermic. *E.g.*

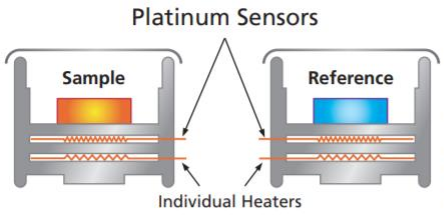
- Crystallisation events
- Some polymorphic changes (*e.g.* from a metastable form to a more stable one)

95

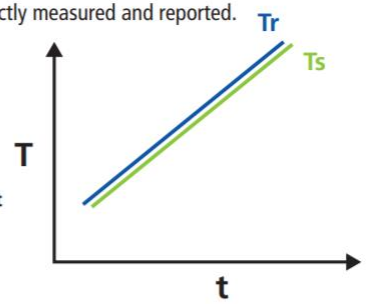
Differential Scanning

Double-furnace DSC

Two independent, small furnaces where energy change of the sample is controlled, directly measured and reported.

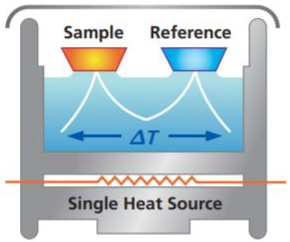


- Two independent small furnaces
- Measures heat flow directly
- True isothermal measurement
- Fastest heating and cooling
- Fastest response times

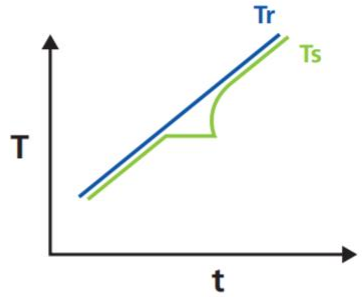


Single-furnace DSC

One large furnace containing both a sample and reference pan where temperature difference between the sample side and reference side are measured and calculations used to determine energy change in the sample.



- One large, single-furnace
- Heat flow derived from ΔT signal



What is DSC actually measuring?

$$P = \frac{dQ}{dt} = C_P \frac{dT}{dt} + f(t,T)$$

dQ/dt = Total heat flow (W/g = J/g*s)

C_p = Specific heat capacity (J/g*C)

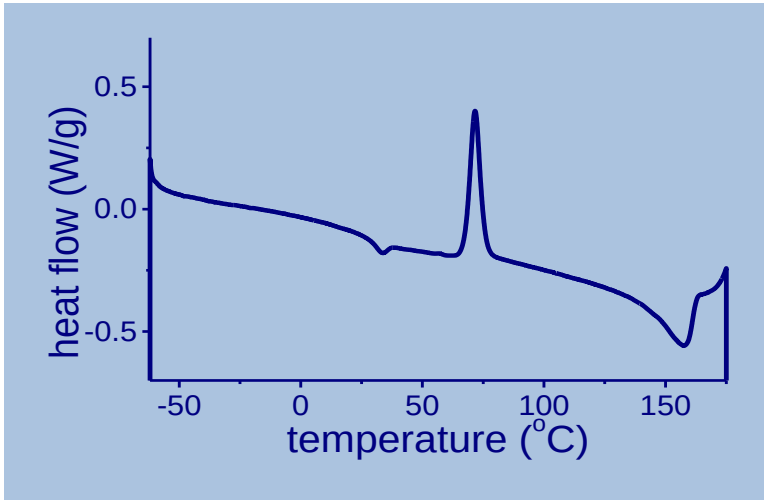
dT/dt = Heating rate (C/s)

f(t,T) = Time-dependent (kinetic) response (**f**unction of **t**ime and **T**emperature)

e.g. melting, crystallization, curing etc.

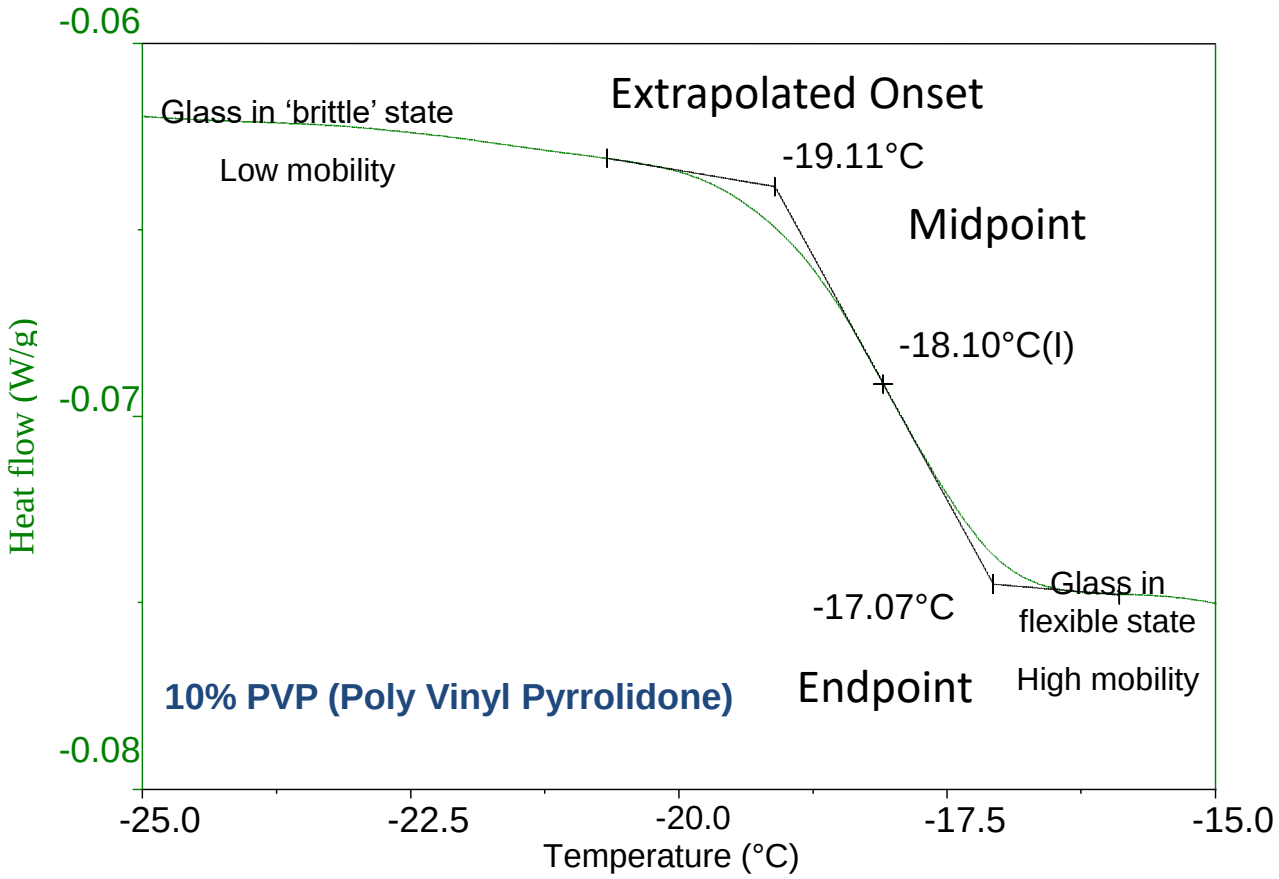
Specific heat (*C_p*) represents the quantity of energy needed to raise the temperature of a unit of mass of sample by 1°C

Conventional DSC plots Total heat flow against temperature...



...it is unable to distinguish between different thermal events that occur simultaneously or overlap. Some events can have an 'additive' effect, while others can 'cancel each other out' (in part or totally).

Glass transition terminology



Differential Thermal Analysis (DTA)

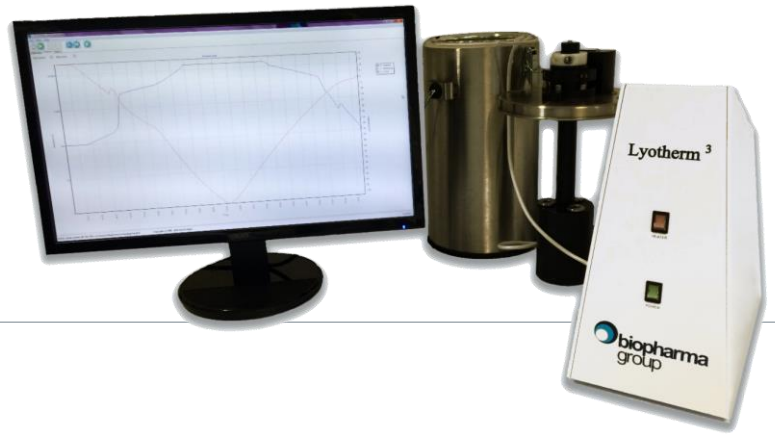
- Same principle as DSC but measures temperature differential, not heat flow
- Effective yet simple and inexpensive method of analysing frozen solutions
- Gives exothermic and endothermic events just like ***Total heat flow*** in DSC
- In our lab, we use this in combination with electrical impedance (Zsinφ) analysis to give a more complete picture of frozen state transitions
- This is a more sophisticated version of electrical resistance (R) analysis (used since the 1950s for looking at frozen solutions)
- Impedance (Z) is a combination of Resistance + Inductance + Capacitance
- Looking at Z (or more specifically Zsinϕ) can give more detailed information about frozen solute behaviour (Rey, 1999)

[1]L. Rey (1999) Glimpses into the Realm of Freeze-Drying: Classical Issues and New Ventures, In: Freeze-Drying/ Lyophilization of Pharmaceutical and Biological Products, L. Rey & J. May (eds), Marcel Dekker Inc., pp. 1-30

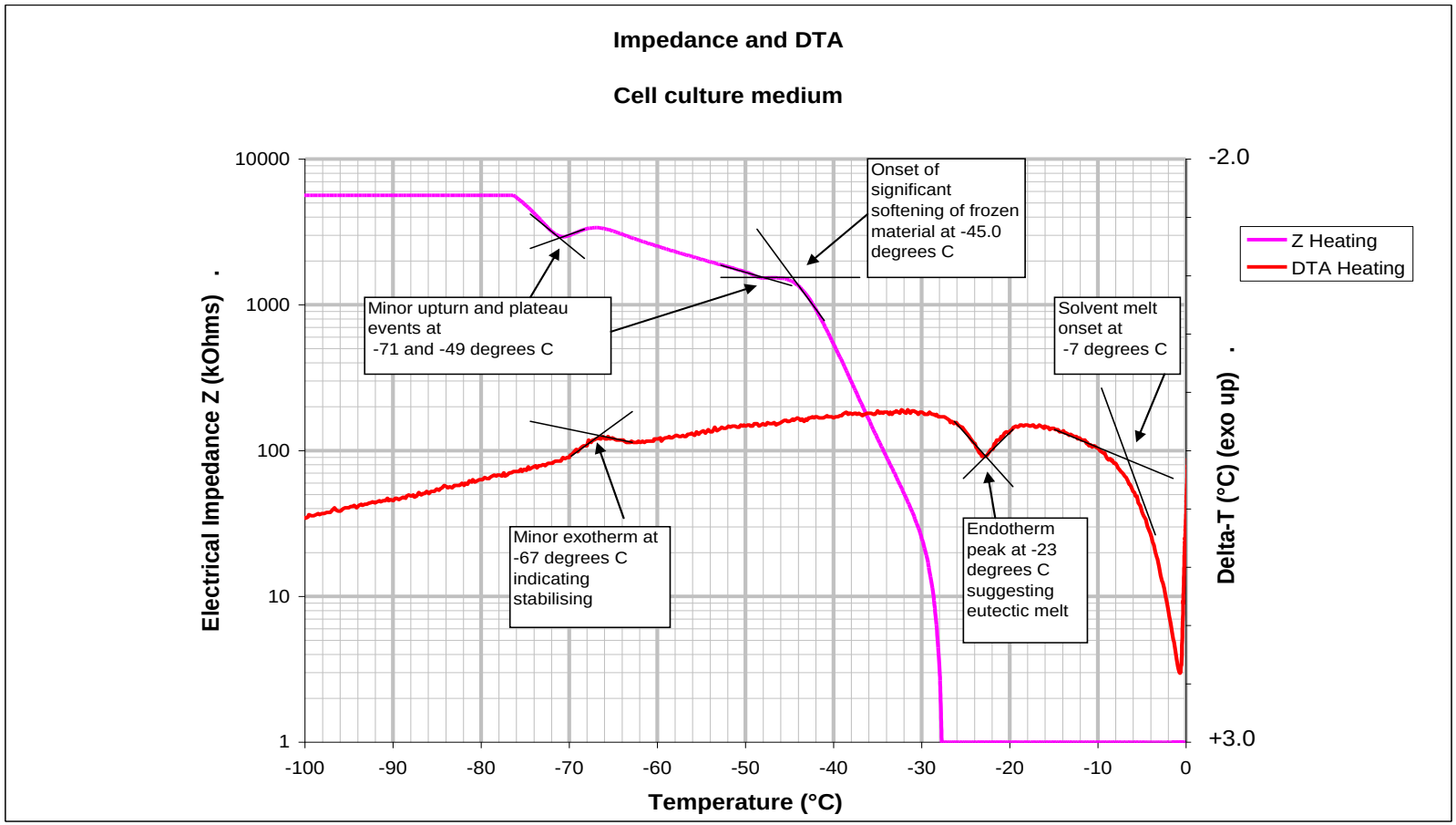
Investigating Zsinϕ

In collaboration with the late Prof. Louis Rey, we developed a device capable of analysing frozen state Impedance (Zsinϕ) at a frequency of 1000Hz

It also incorporates DTA to indicate any measurable thermal changes in the formulation



Example of Zsinϕ + DTA graph



Examples where onset of mobility increase (‘T_{zonset}’) observed significantly **below T_c**

Formulation (% values in w/v)	**Onset Tc (ºC)** [Lyostat FDM]	**T_{Zonset} (ºC)** [Zsinϕ analysis]
NaCl (0.9%) + HSA (0.5%)	-18.4	-46
NaCl (0.9%) + HSA (1.0%)	-20.4	-64
NaCl (0.9%) + HSA (5.0%)	-23.0	-60
Egg allantoic fluid (undiluted)	-50	-60
Potassium phosphate buffered HSA (0.2%) + trehalose (0.1%)	-50	-58
HSA (0.1%) + casein (0.3%) in PBS	-50	-59

What can be understood from these data?

Often, a value of $\mathbf{T_{Zonset}}$ can be obtained, even when conventional thermal analysis does not yield a clear glass transition

Identification of $\mathbf{T_{Zonset}}$ may assist with the prediction of **microcollapse**

What is clear is that collapse is not 100% of the story for all formulations, and that Zsinϕ analysis may fill in some of the gaps that thermal analysis does not

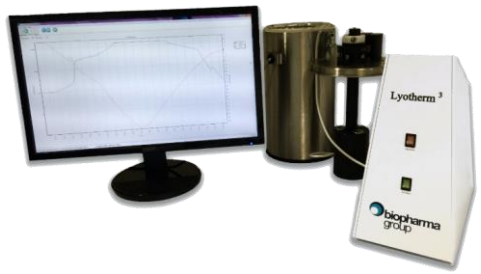
IN SUMMARY:

Thermal analysis useful for identifying events in sub-ambient region, which may not translate to visible defects in the product but may still be important

Helpful in choice of freezing/annealing temperatures

But remember*… we still need to know what is in the formulation (and how ingredients may be interacting) before we can fully interpret the data…*

Characterisation Summary



FDM can provide a visual indication of:

- Collapse temperature (T_c)
- Eutectic temperature (T_{eu})
- Skin formation potential
- Annealing effects: on ice structure, solute crystallisation, critical temperature...

Thermal methods can indicate:

- Endothermic changes such as melting or glass transitions
- Exothermic changes such as crystallisation
- Annealing effects: on solute crystallisation, critical temperature...
- Molecular mobility of components

Comfort break (10 minutes)

Overview of Typical Process Analytical Technology used in Monitoring Primary Drying

(20 Minutes)

Temperature Measurement

- Resistance Thermometers
- Thermocouples
- Issues with measuring Product Temperature

Process Analytical Technologies available

- Individual sample measurement methods
- Batch measurement methods
- Comparison of methods

Product Temperature Measurement

Problems with product temperature measurement

- Invariably it is a destructive measurement
- This makes it atypical of the remainder of the batch

Problems with product temperature data

- Are product probes “controlling” segment changes?
  - An average value of all probes is used
- Are product probes providing information only?
  - Product temperature traces on batch reports

There are other problems to be considered when measuring product temperature

Data Interpretation

With all PAT methods, it's important to recognise what the data really mean

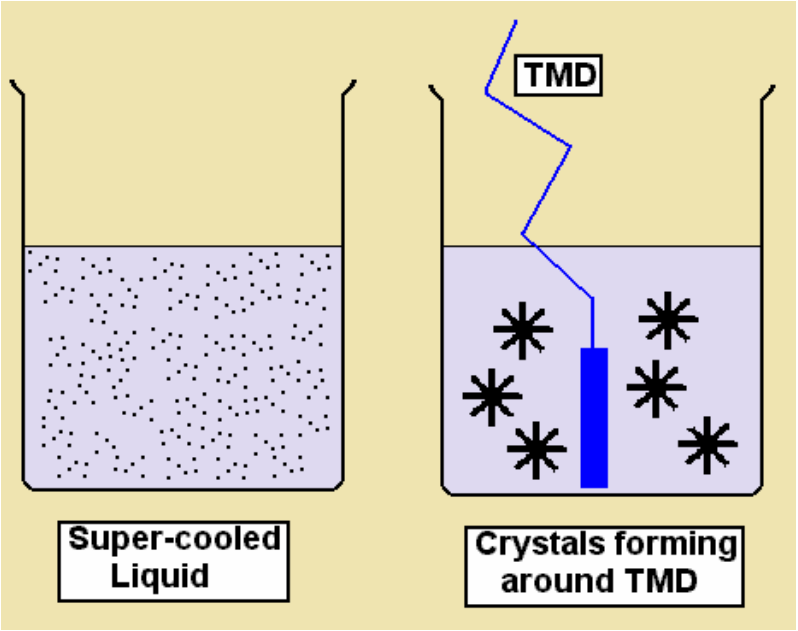
Methods that look at INDIVIDUAL samples can help give an idea of 'spread' in the dryer… so long as we need to pick the right locations!

Methods that look at the BATCH as a whole can help take into account the slowest to dry

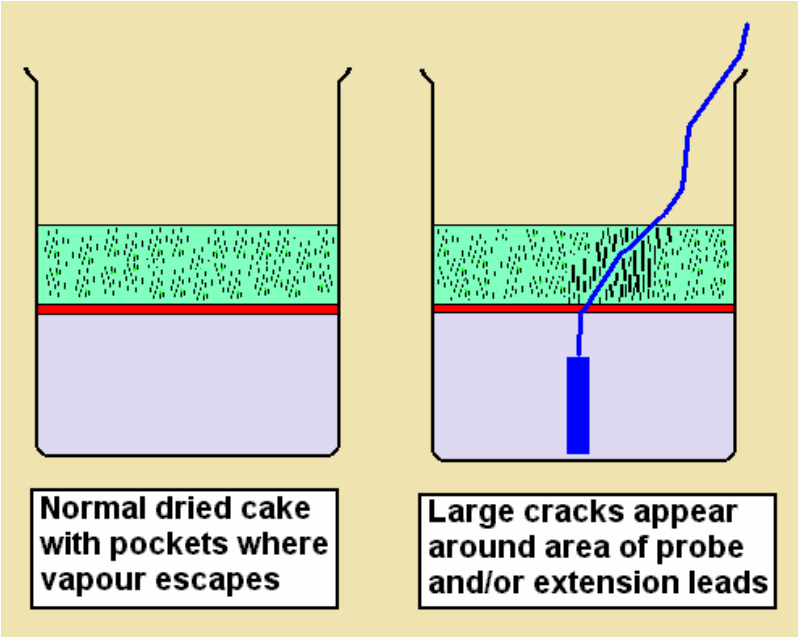
Even with the measurement of our Process Control Parameters (T_{shelf} and $P_{chamber}$), there are limitations / caveats to the data generated….

In Sublimation, these factors may promote faster sublimation in the measured containers (sublimation interface moves FASTER down the vial)

Nucleation

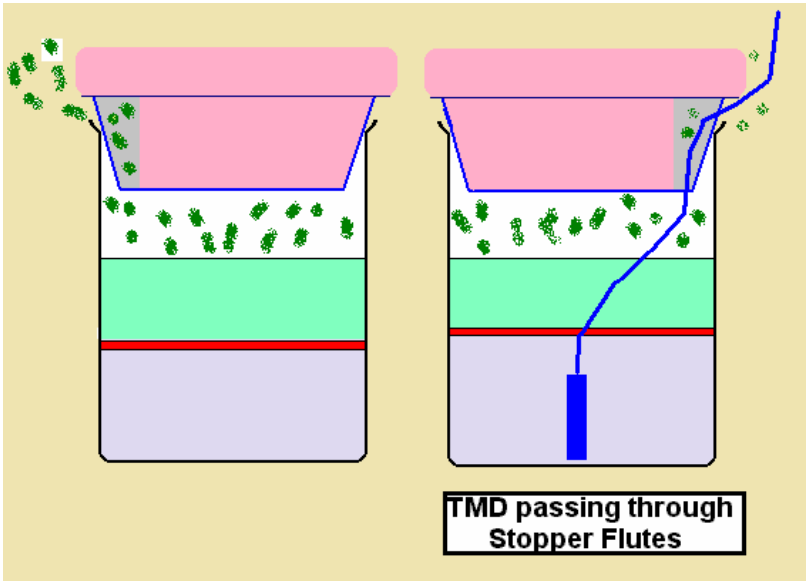


Cracking

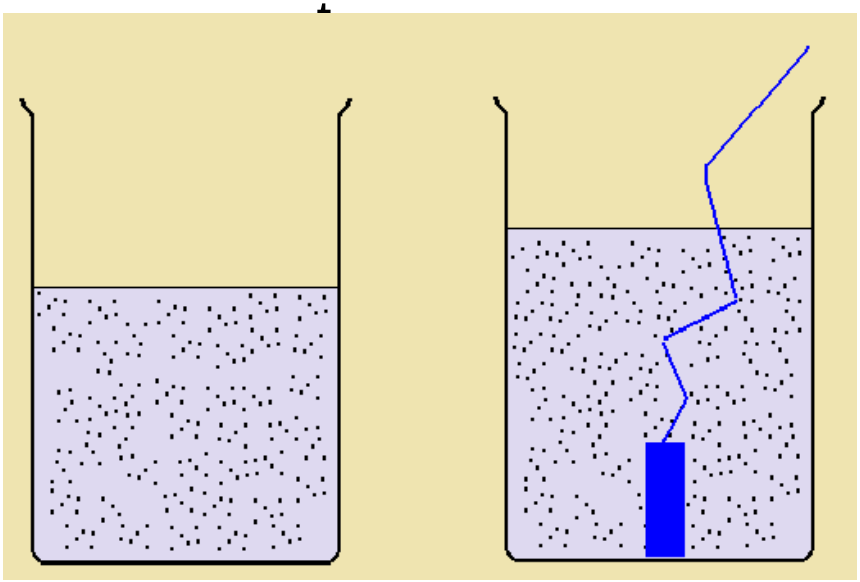


In Sublimation, these factors may promote slower sublimation in the measured containers (Sublimation interface moves SLOWER down the vial – or the distance has increased)

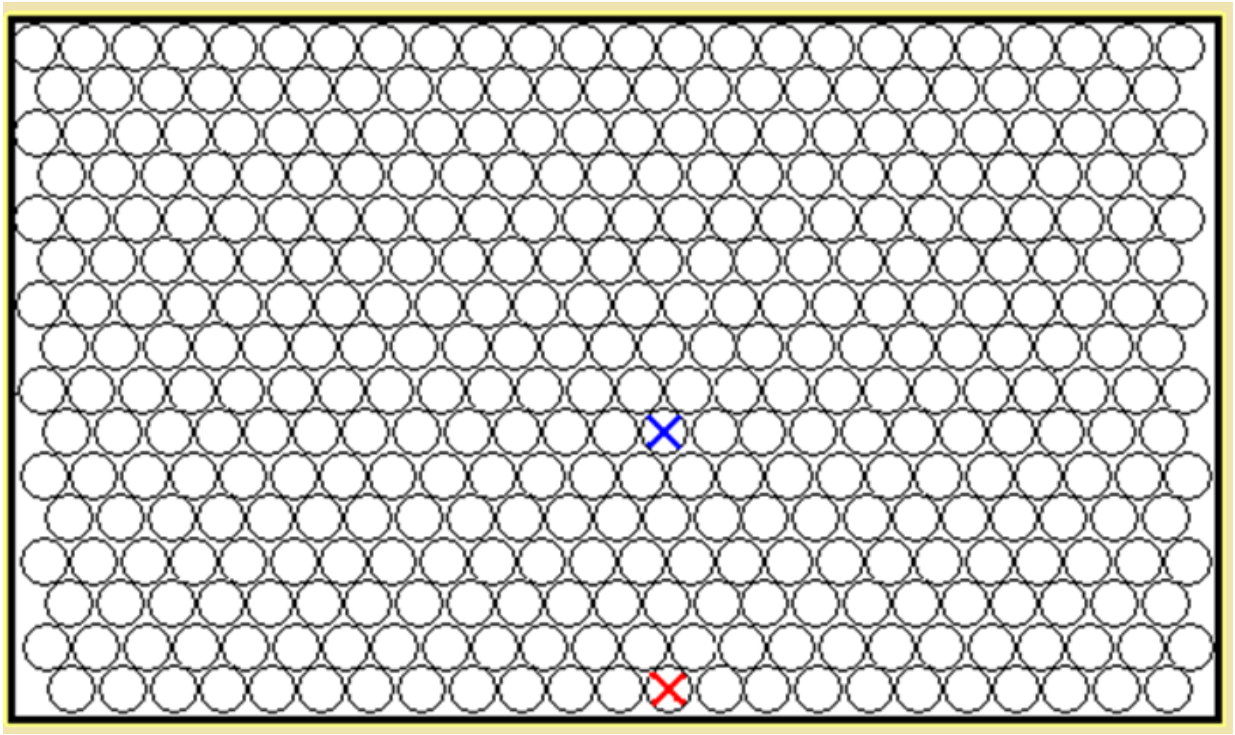
Impedance



Displacemen
t

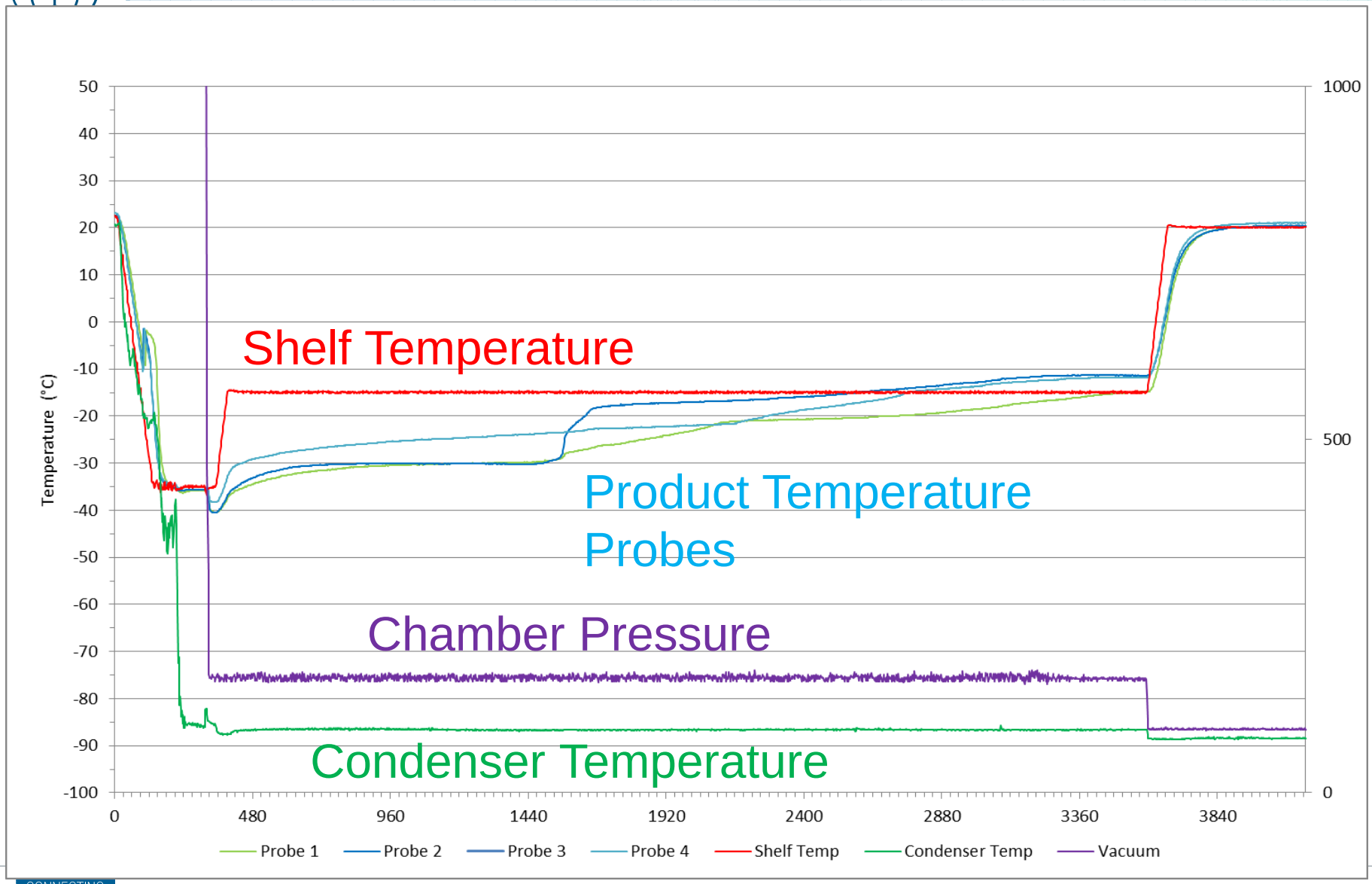


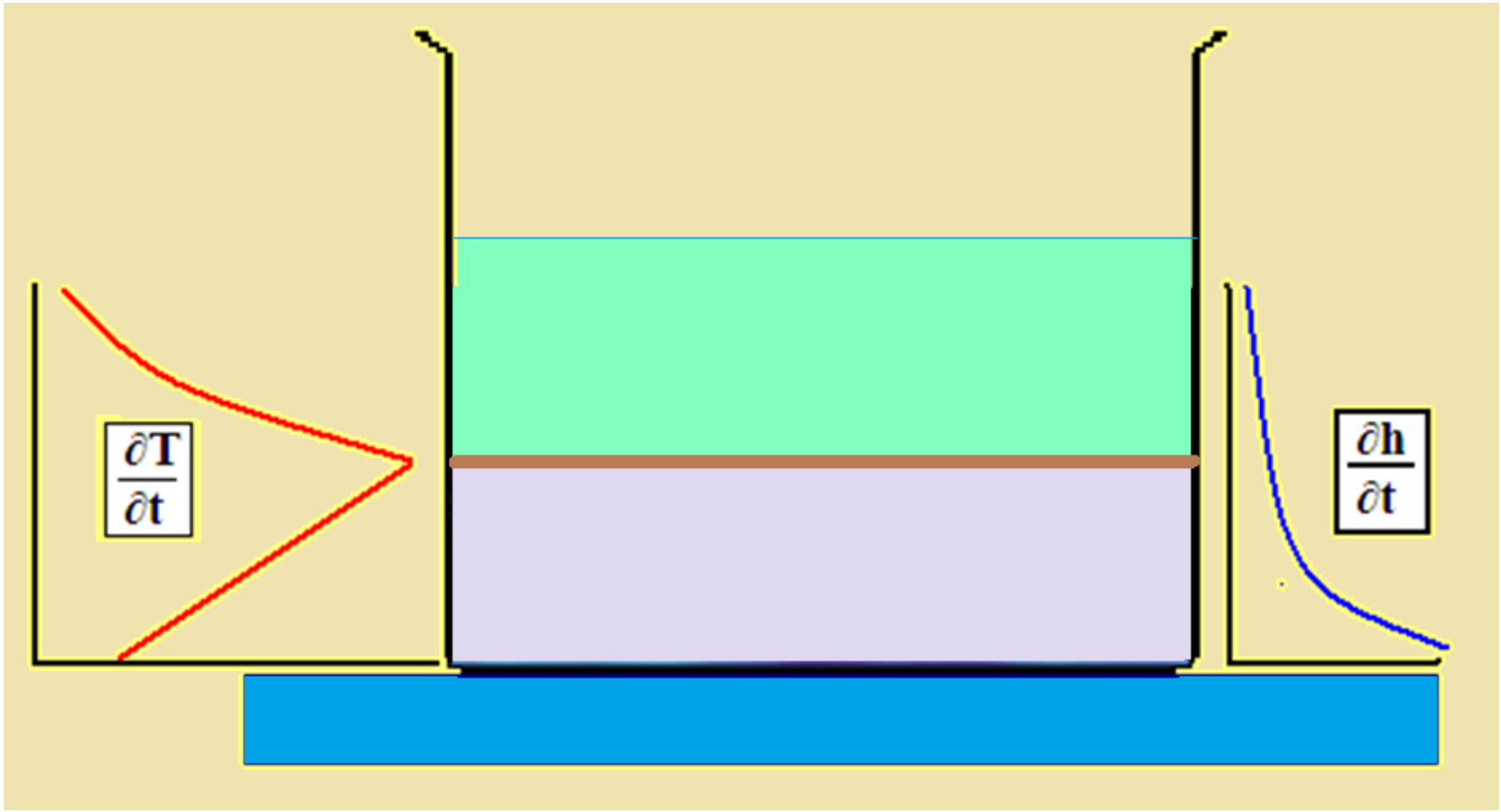
Position of vials can affect heat energy transfer in both Freezing and Sublimation



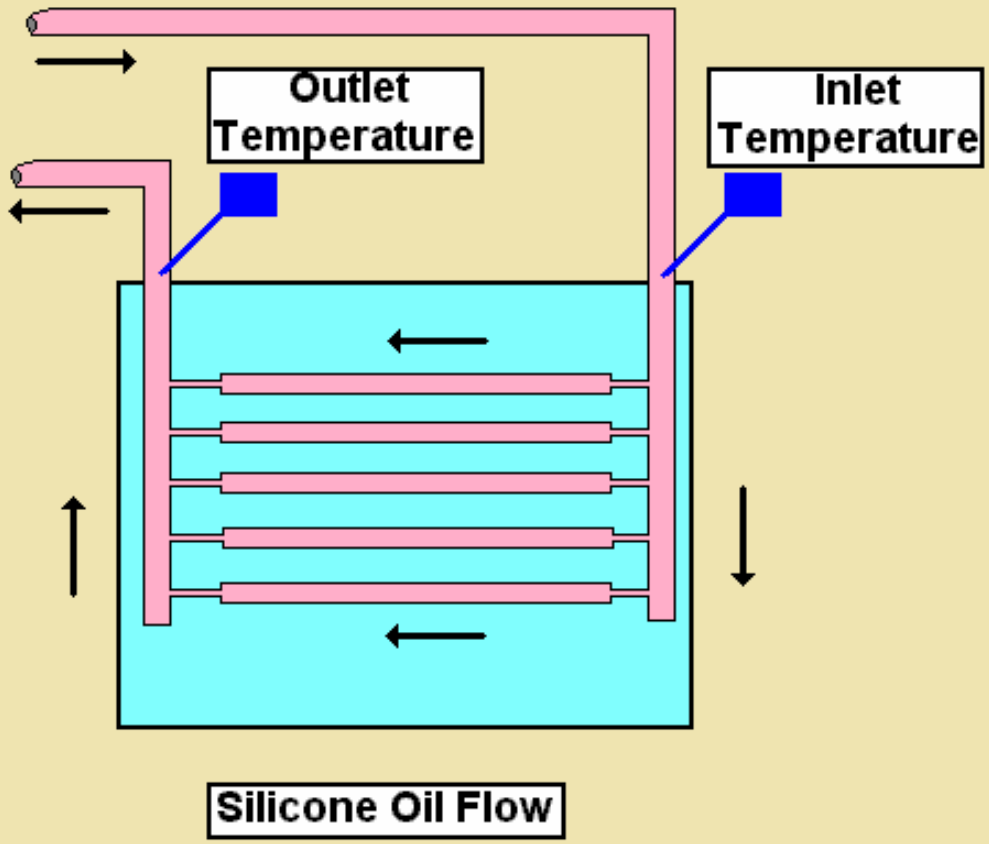
Insulation from Surrounding Vials (Blue x) can lead to a significantly different profile to that in edge vials (Red x)

Example of Cycle Trace





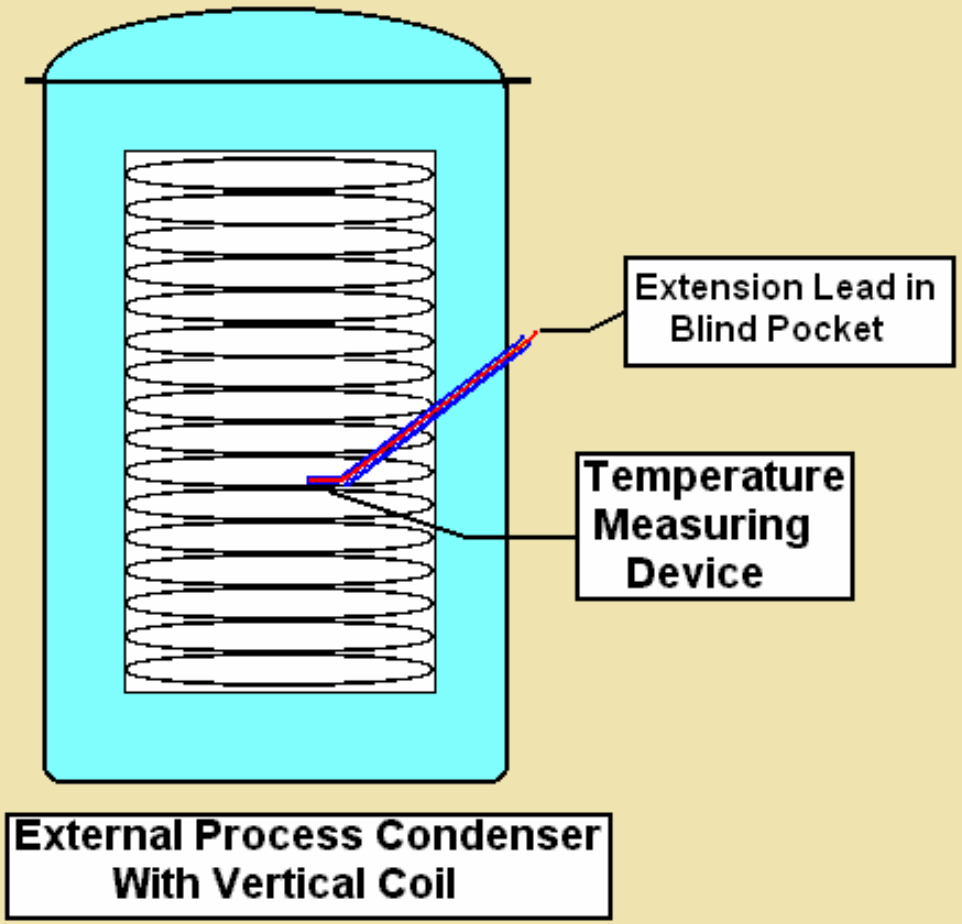
This forms the basis of Manometric / Barometric Temperature Measurement (MTM / BTM)



Here, the actual shelf temperature surface is not being measured. It is the temperature of the fluid in the Inlet manifold.

We can also measure the outlet temperature.

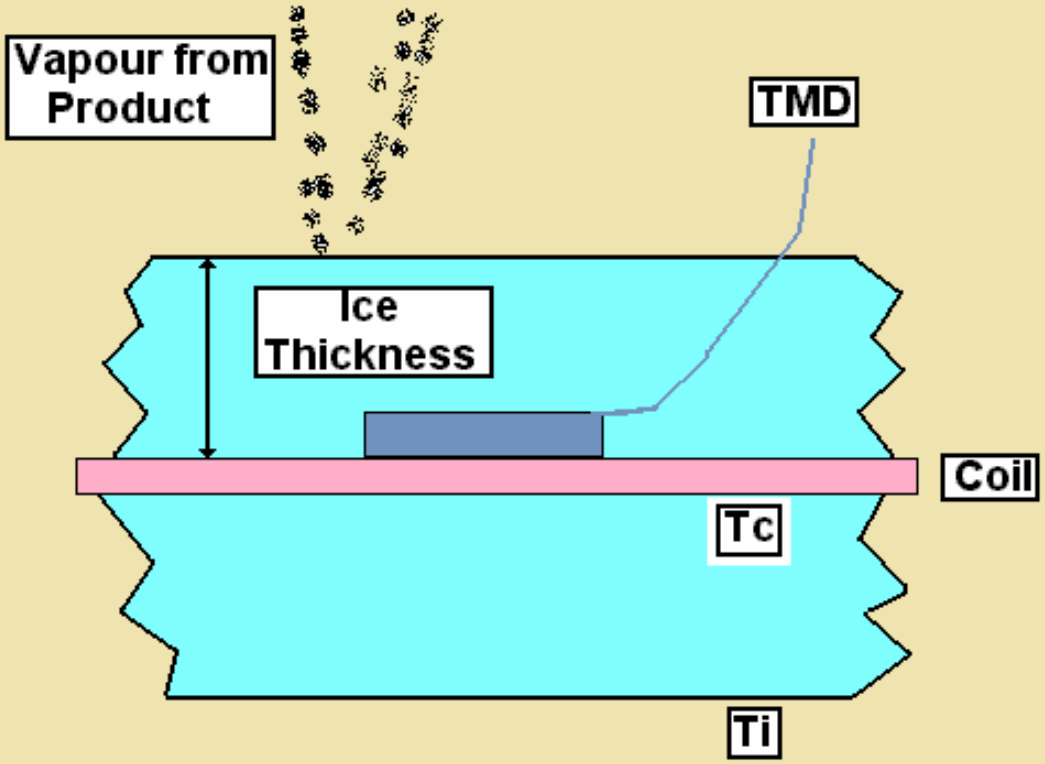
Machines always control on the inlet temperature



Temperature measured is the actual coil (or plate) temperature at the point where the probe is connected.

There will be a temperature gradient up and down the coil.

It is the (coldest available) ice surface that drives the process



Process Analytical Technology (PAT) in Freeze-Drying

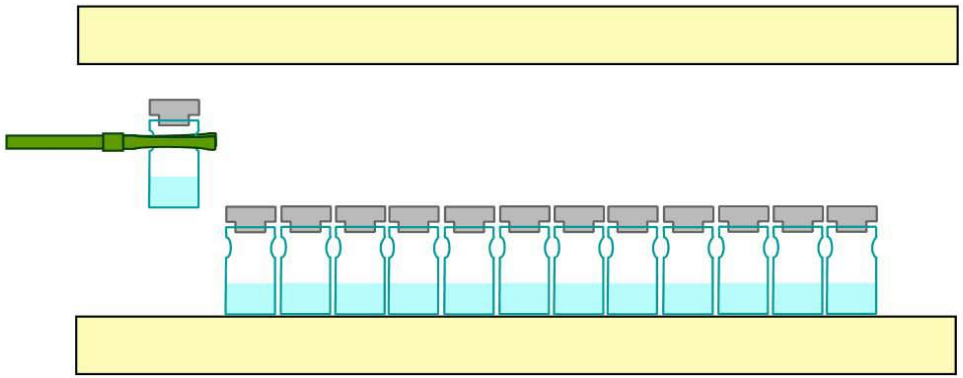
Due to the uncertainty and lack of scalability in some of the traditional data, a number of PAT methodologies have been developed

Single sample measurement methods include:

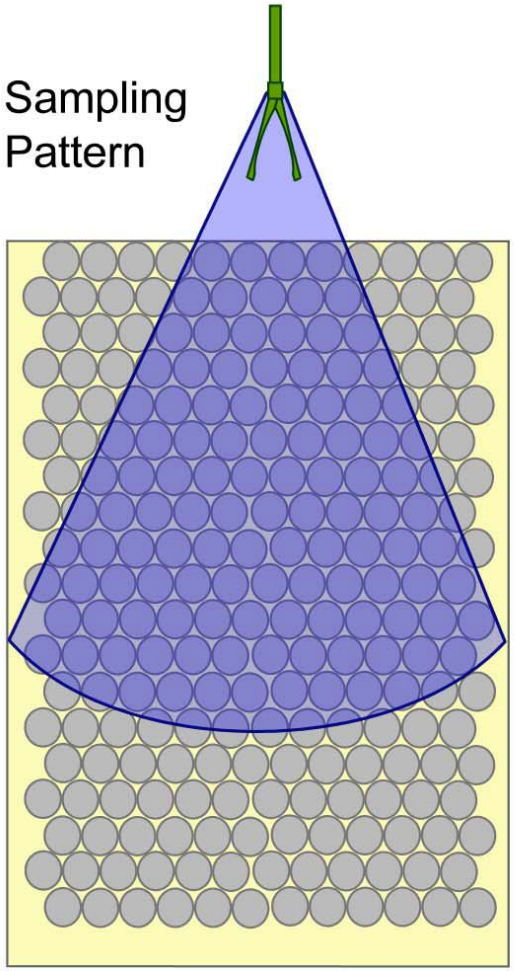
- Temperature probes (traditional or wireless)
- Sample weight loss monitoring during a cycle
- NIR / Raman probes aimed at specific samples
- Other offline methods via use of a sample thief

Use of Sample Thief

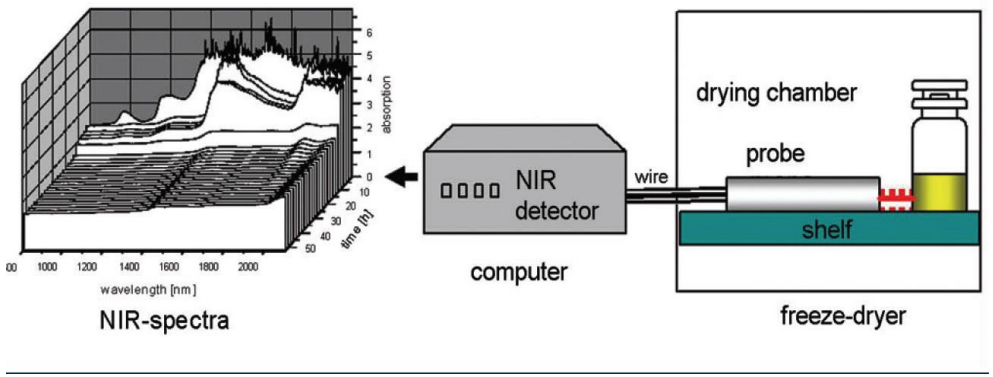
Removing a vial



Sampling Pattern



Other methods of Individual Sample Monitoring



NIR – Surface scan
Fourier – Scans across spectrum
Raman – Laser excitation

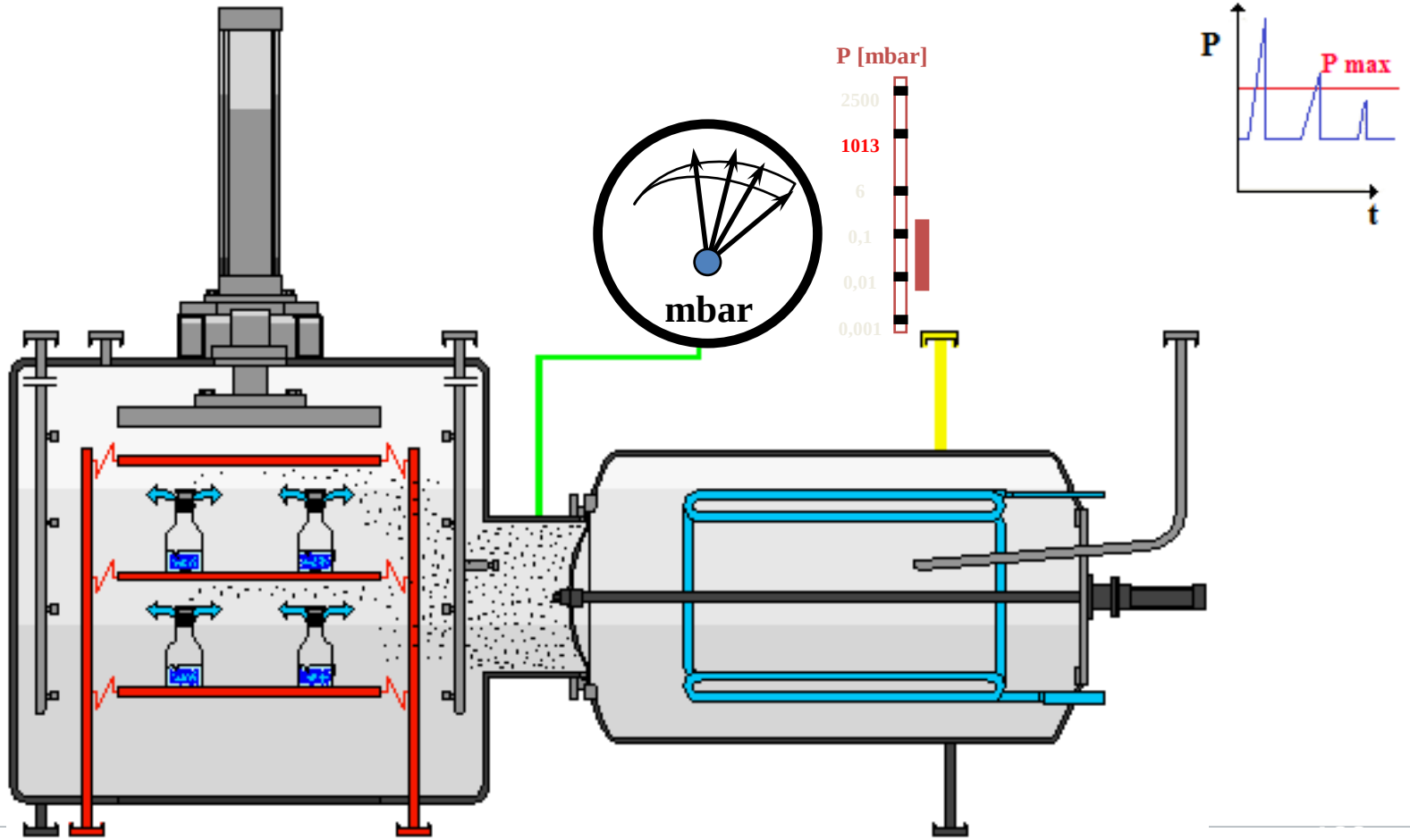
Microbalance measures vial weight periodically
Limited to small number of samples / locations
Vials that are monitored can be considered atypical

Batch Determinations

The whole batch is monitored and an average response is returned. Batch methods cannot be invasive and edge effects are not as pronounced. Accurate methods take heed of edge effects.

- **PRT**
- **Windmills**
- **Moisture gauges**
- **Mass spectrometry**
- **Hot wire v capacitance manometer gauges**
- **Barometric / Manometric temperature measurement**
- **Tuneable diode laser absorption spectroscopy**

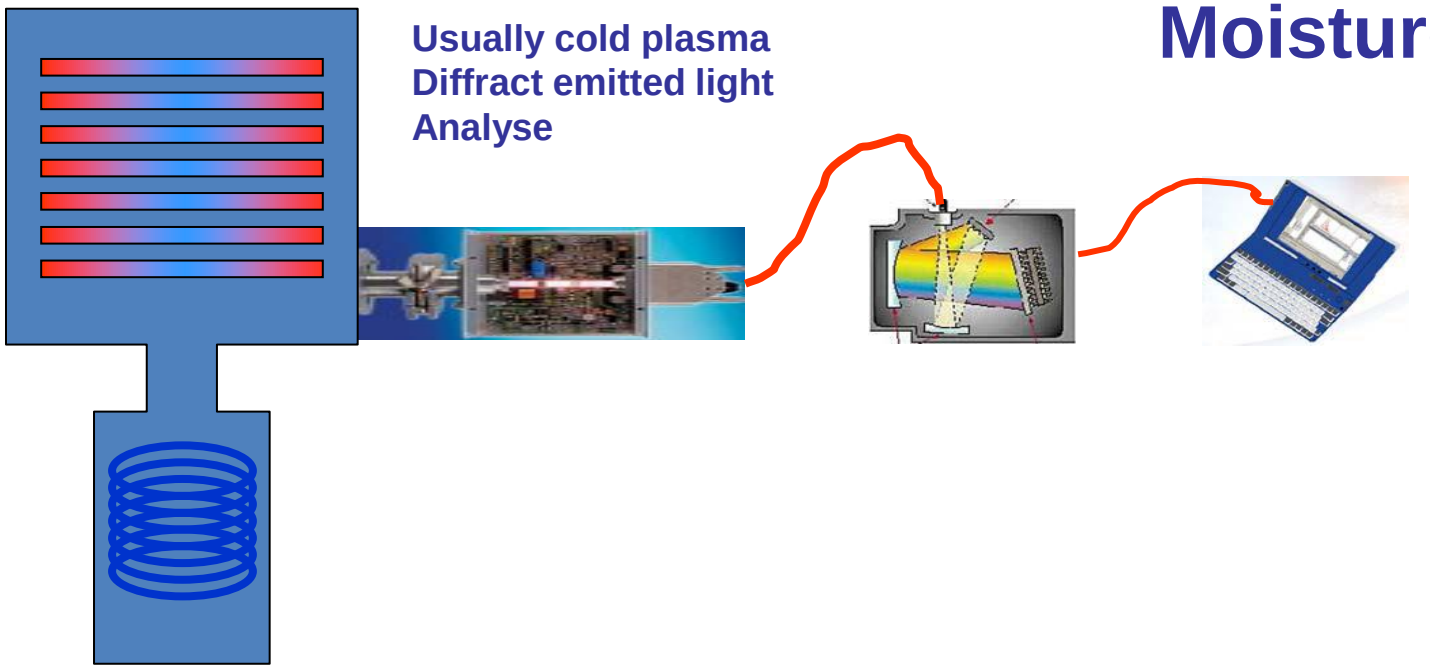
Endpoint Check – Pressure Rise Test



Windmills

- Simple idea
- ‘Indicative’ rather than accurate
- Particle shedding a concern in sterile production

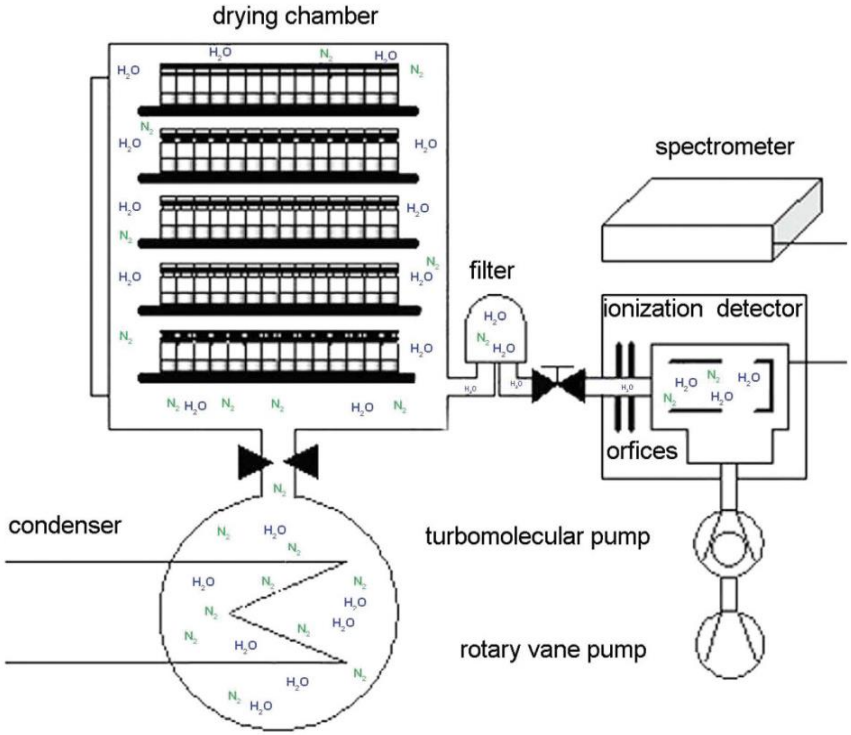
Moisture gauges



- Often not fully understood
- Often just get to an arbitrary value
- Batch measurement
- Sterilisable – yes/no?
- Validation?

Adixen “Lyotrack”

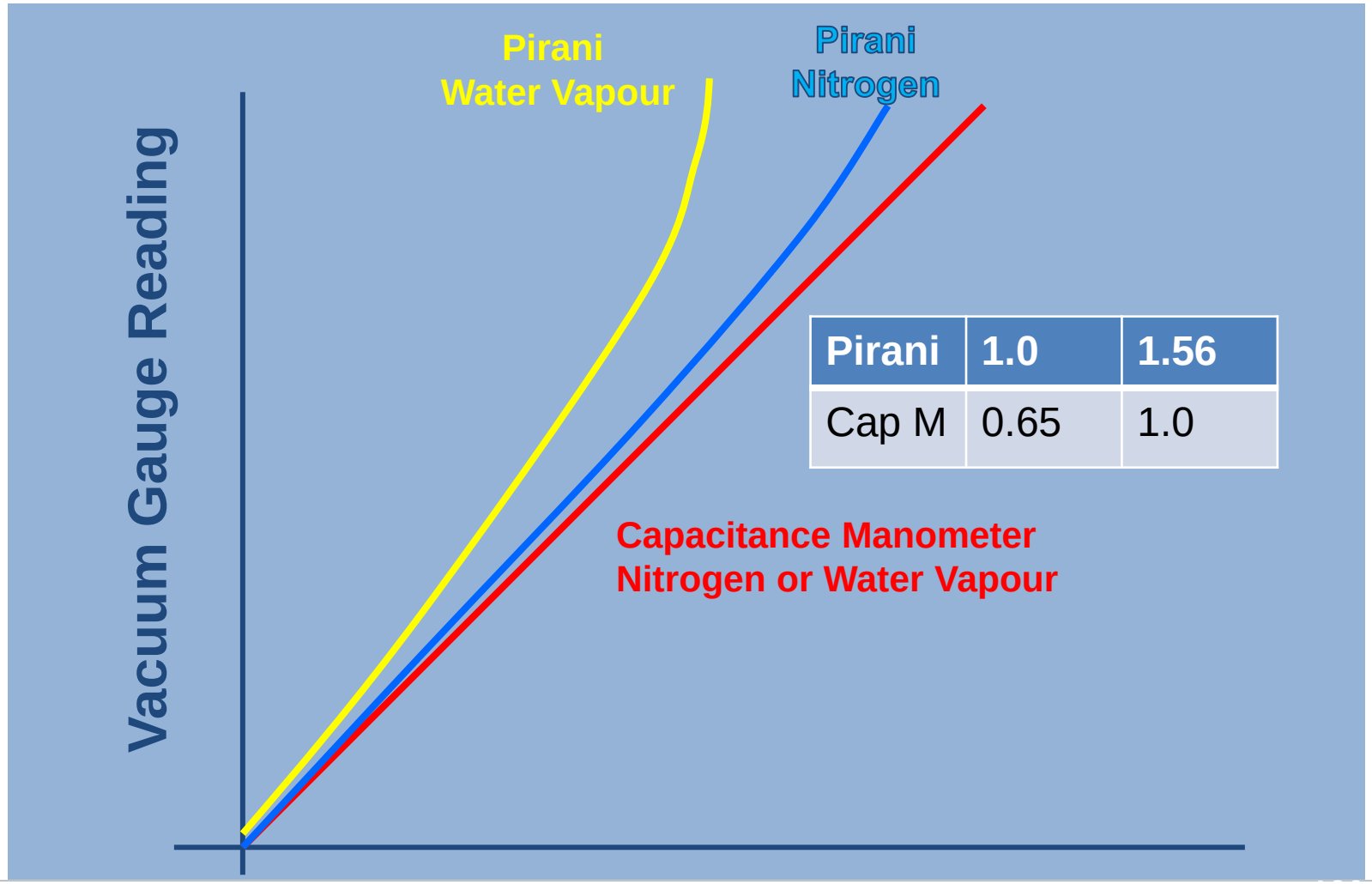
Mass Spectrometry



Measures moisture in process chamber

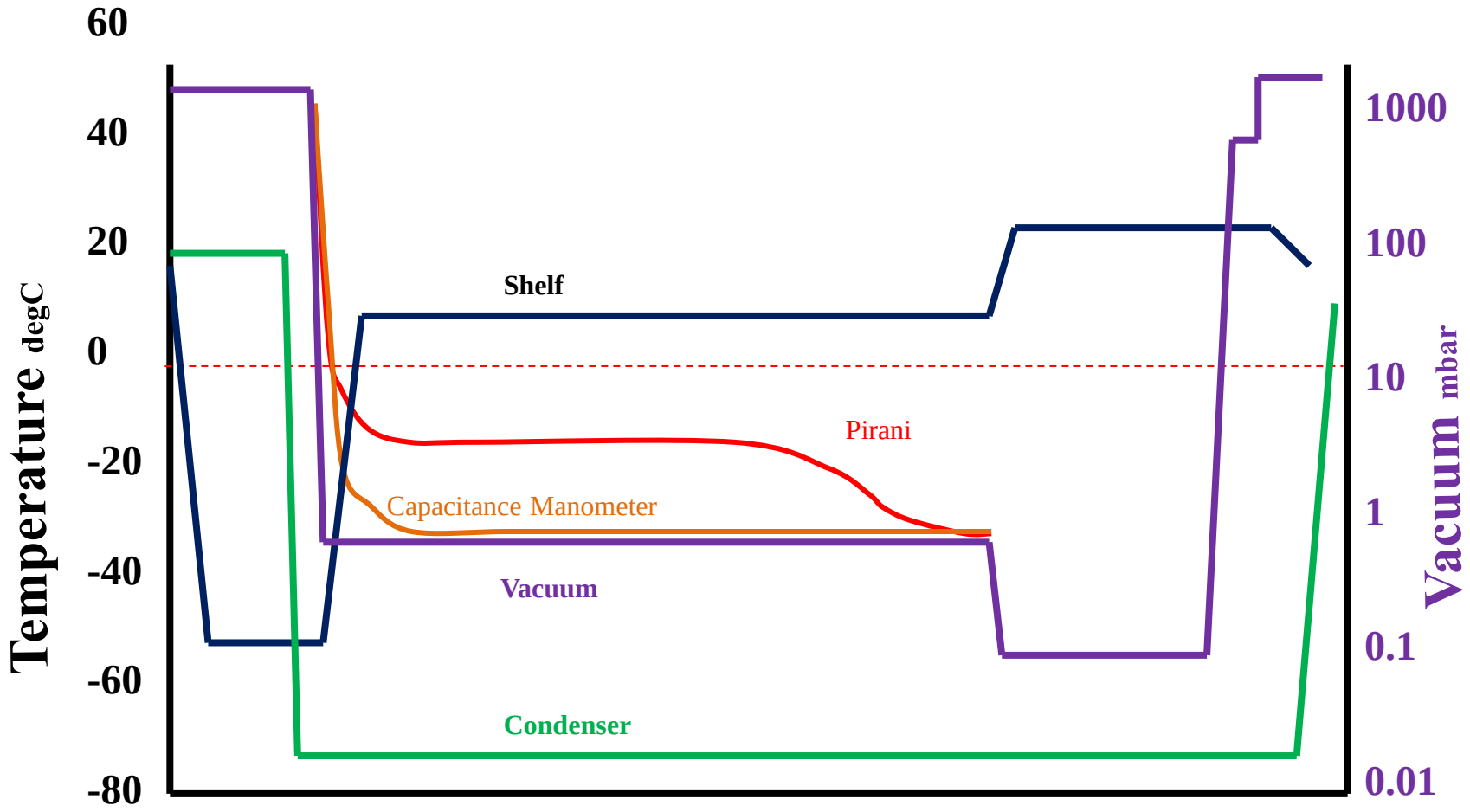
- Old technology
- Never really adopted
- Where do you sample?
- Cost!
- Sterilisable?
- Validation?

Pirani v Capacitance Manometer



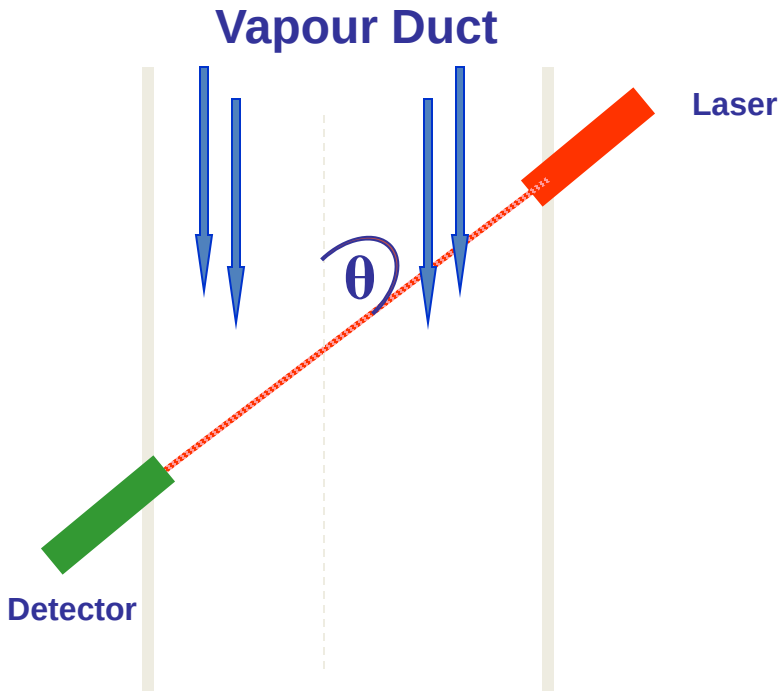
Pirani	1.0	1.56
Cap M	0.65	1.0

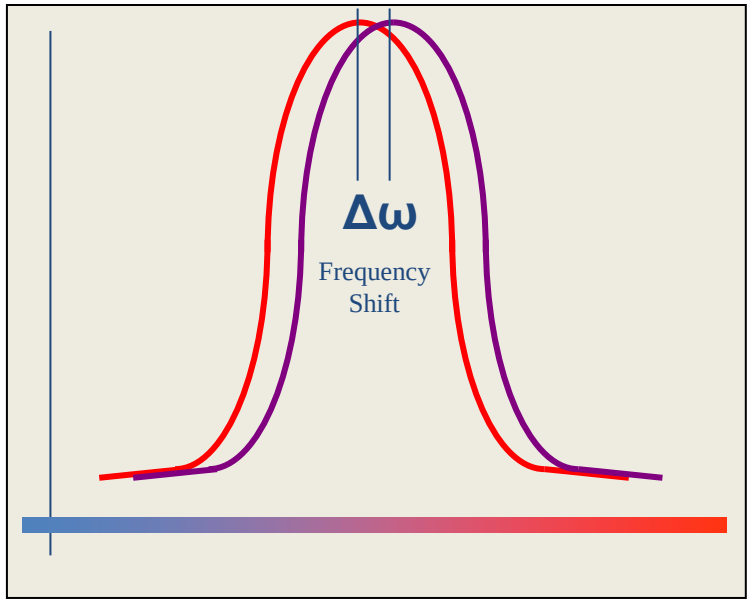
Pirani v Capacitance Manometer - example



End Point Determination

Tuneable Diode Laser Absorption Spectroscopy





End Point Determination

Tuneable Diode Laser Absorption Spectroscopy

Velocity

$$U = \frac{\Delta\omega C}{\omega_o \ \mathrm{Cos}\ \theta} \mathrm{cms}^{-1}$$

Density

$$\rho = \frac{\int_o^{\omega} \ln(I/I_o)d\omega}{S\ L} \ \mathrm{g/cm}^3$$

Mass Flux

$$Dm/dt = U\ p\ A\ \ \mathrm{gs}^{-1}$$

Where

C = speed of light
Δω =frequency shift
ω_o = absorption peak
θ = path angle

L = path length
I = transmitted laser intensity
I_o = initial laser intensity
S = Absorption line strength

A = cross sectional area of vapour duct

Mass Flux *should be a truly scalable parameter!*

Compatibility of PAT methods with GMP Sterile Manufacturing

Mayeresse et al (2007)

A Temperature probe
B Wireless temperature probe
C Conductivity probe
D Microbalance
E FTNIR product probe
F Pirani/capacitance differential
G Moisture probe
H Pressure rise measurement
I Mass spectrometry
J TDLAS
K Cold plasma

Success	**A**	**B**	**C**	**D**	**E**	***F***	**G**	**H**	**I**	**J**	**K**
Monitor global load	No	No	No	No	No	***Yes***	Yes	Yes	Yes	Yes	Yes
Auto loading	No	No	No	No	No	***Yes***	Yes	Yes	Yes	Yes	Yes
CIP	No	No	No	No	No	***Yes***	Yes	Yes	Yes	Yes	Yes
Aseptic handling	+/-	+/-	No	No	No	***Yes***	Yes	Yes	Yes	Yes	Yes
Steam sterilisable	Yes	Yes	Yes	No	Yes	+/-	No	Yes	No	Yes	Yes
Leak rate control	Yes	Yes	Yes	+/-	Yes	***Yes***	Yes	No	No	Yes	Yes
Simple integration	+/-	+/-	Yes	No	No	***Yes***	Yes	Yes	no	no	Yes
calibration	yes	yes	Yes	yes	yes	***yes***	Yes	no			

Conclusions

To some extent temperature measurement in freeze drying is still evolving - particularly with product temperature measurement.

Accurate, reliable and robust temperature measurement, accurate recording and data logging form an integral part of the R&D and production process for many products

PAT methodologies offer additional information in-process and may be helpful in R&D and production

Some PAT methods should be more scalable than empirical data

Cycle Development and Scale up: Use of the Iterative Approach and Use of SMART Software

(45 Minutes)

Starting Point for Cycle Development

- Defined list of Critical Quality Attributes or Target Product Profile? (see QbD)
- Formulation candidate(s) *fully characterised*:
  - FDM analysis to examine freezing and drying characteristics of formulation, Tc, Teu
  - Thermal analysis for Tg', Teu, crystallisation, need for / effect of annealing
- Prior knowledge about active ingredient(s):
  - sensitive to slow cooling / fast cooling? (usually proteins and biologicals)
  - pH stability plot
- Container type / size / fill volume may be assessed as part of cycle development
- Formulation may also be developed / selected as the process is refined

Some factors affecting freezing & drying…

The traditional control parameters in a freeze-dryer are **Shelf Temperature** and **Chamber Pressure** (and to some extent, time) but there are many other factors associated with the formulation, container and dryer design that have an impact…

Heat transfer coefficient of product container: depends on material, thickness, flatness…

CONVECTION **CONDUCTION**
RADIATION

Shelf spacing

Pressure

Degree of thermal contact between shelf and container

Fill depth of Product

Cooling rate of shelves

Critical temperature of formulation

Viscosity of solution at the point of ice nucleation

Filtered liquid? (supercooling)

Devising a cycle from scratch

There are many points to consider, including:

- Filling and Loading conditions
- Thermal Treatment (Freezing)
  - Target temperature
  - Cooling rate and holding time
  - Annealing (Yes / No / Conditions)
- Primary Drying conditions:
  - T_{shelf}
  - $P_{chamber}$
  - time
  - Dynamics
- Secondary Drying Conditions
- Backfilling and stoppering

Devising a cycle from scratch

There are many points to consider, including:

- Filling and Loading conditions
- Thermal Treatment (Freezing)
  - Target temperature
  - Cooling rate and holding time
  - Annealing (Yes / No / Conditions)
- Primary Drying conditions:
  - T_{shelf}
  - $P_{chamber}$
  - time
  - Dynamics
- Secondary Drying Conditions
- Backfilling and stoppering

- The default for most products is loading at ambient temperature, but this can vary and affect freezing
- Some products loaded onto sub- 0°C shelves
  - Suspensions that may sediment
  - Heat-labile formulations
  - Biological materials if small ice crystals needed
- Practical issues with loading onto shelves sub- 0°C
  - Condensation / frost formation on shelves
  - This can induce variability in ice and solute behaviour across a batch (and between batches)
- Loading at 4-5°C can be more practically achievable, while also maintaining control over the freezing process

Devising a cycle from scratch

There are many points to consider, including:

- Filling and Loading conditions
- Thermal Treatment (Freezing)
  - Target temperature
  - Cooling rate and holding time
  - Annealing (Yes / No / Conditions)
- Primary Drying conditions:
  - T_{shelf}
  - $P_{chamber}$
  - time
  - Dynamics
- Secondary Drying Conditions
- Backfilling and stoppering

- **Target product temperature** will be based on its critical temperature, plus a safety margin
  - Usually a safety margin of 2-7°C is adopted
- Typical default **cooling rate** is 0.5°C/minute unless product is known to require slower or faster rate
  - This is something we often explore during the initial freeze-drying cycle for a new product
- **Holding time** generally based on real-time measurements in production equipment
- The use of **annealing** can be investigated using FDM and thermal analysis – case by case basis
- Finally, we may also consider the potential benefits or risks of using **controlled nucleation** at this point

Devising a cycle from scratch

There are many points to consider, including:

- Filling and Loading conditions
- Thermal Treatment (Freezing)
  - Target temperature
  - Cooling rate and holding time
  - Annealing (Yes / No / Conditions)
- Primary Drying conditions:
  - T_{shelf}
  - $P_{chamber}$
  - time
  - Dynamics
- Secondary Drying Conditions
- Backfilling and stoppering

- **Target product temperature** will once again be based on the critical temperature plus a safety margin
- The standard approach is to maintain constant shelf temperature during the chamber evacuation phase
- Select a chamber pressure between ⅓ - ½ VP at Tp
- Wait (30 – 60 minutes) to see where Tp stabilises
- Increase Ts and/or Pc accordingly to increase heat input *via* convection / conduction / radiative transfer
- Reassess Tp periodically to ensure it is not at risk of exceeding critical temperature while ice still present
- Remember that Rp increases and conduction becomes more effective even at constant Ts and Pc!
- Endpoint of primary drying can be evaluated using a variety of different methodologies…

As a general rule, we typically add 10% extra primary drying time because containers with no probes usually take longer to dry than containers with probes

Devising a cycle from scratch

There are many points to consider, including:

- Filling and Loading conditions
- Thermal Treatment (Freezing)
  - Target temperature
  - Cooling rate and holding time
  - Annealing (Yes / No / Conditions)
- Primary Drying conditions:
  - T_{shelf}
  - $P_{chamber}$
  - time
  - Dynamics
- Secondary Drying Conditions
- Backfilling and stoppering

- Default usually +20°C to +25°C unless product is unstable at these temperatures at this point
- At the start of secondary drying, the product will likely contain several % moisture
  - Tg may be below +20°C at this point
  - Go stepwise or gradually up to +20°C?
- 'Endpoint' of secondary drying is harder to detect:
  - There is less moisture in the chamber
  - The endpoint itself may not be clearly defined
  - Use of sample thief / offline methods / PAT?
  - Balance time against final moisture level needed

Devising a cycle from scratch

There are many points to consider, including:

- Filling and Loading conditions
- Thermal Treatment (Freezing)
  - Target temperature
  - Cooling rate and holding time
  - Annealing (Yes / No / Conditions)
- Primary Drying conditions:
  - T_{shelf}
  - $P_{chamber}$
  - time
  - Dynamics
- Secondary Drying Conditions
- Backfilling and stoppering

Only really an issue for vials…

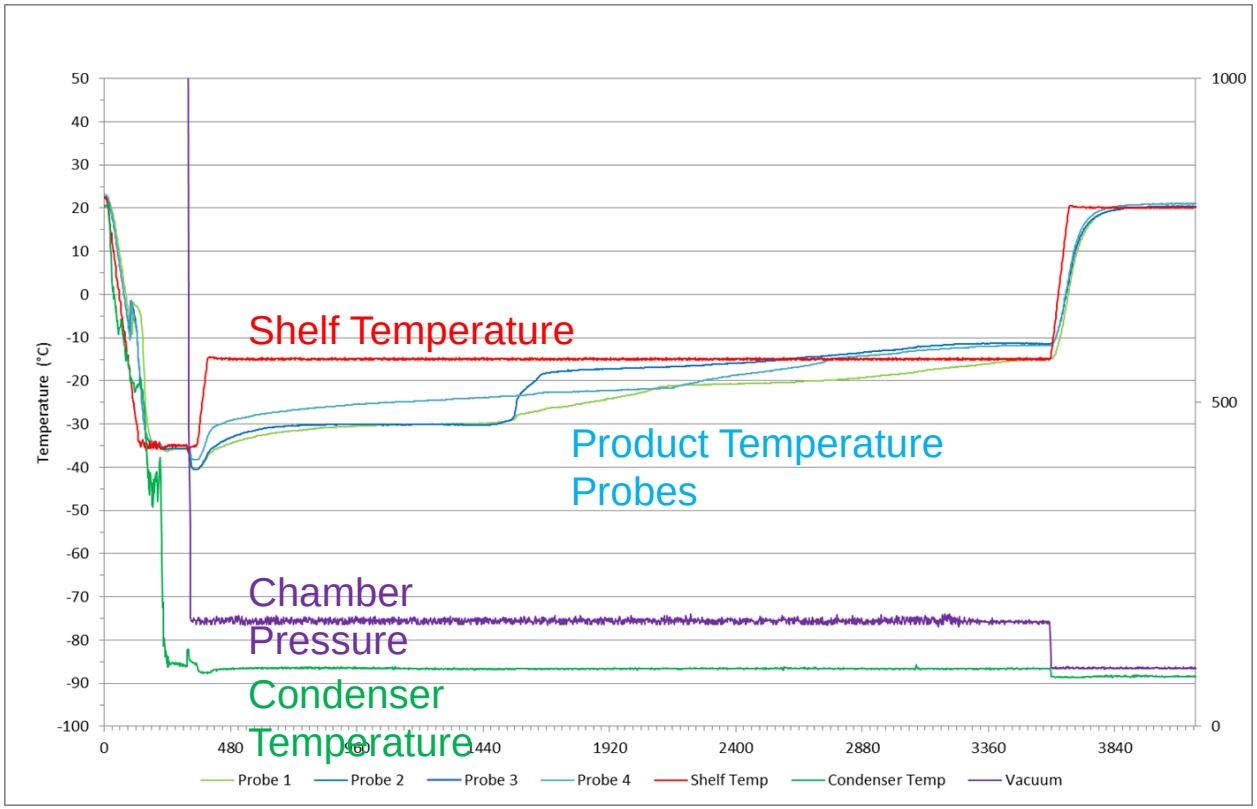
- To backfill or not to backfill (with nitrogen / argon)?
- For injectables containing surfactant, foaming may be avoided / minimised by partial backfill
- Argument that a smaller pressure differential inside and outside the vial will lead to less moisture ingress
  - Maybe only significant for products of low mass

For non-vial formats…

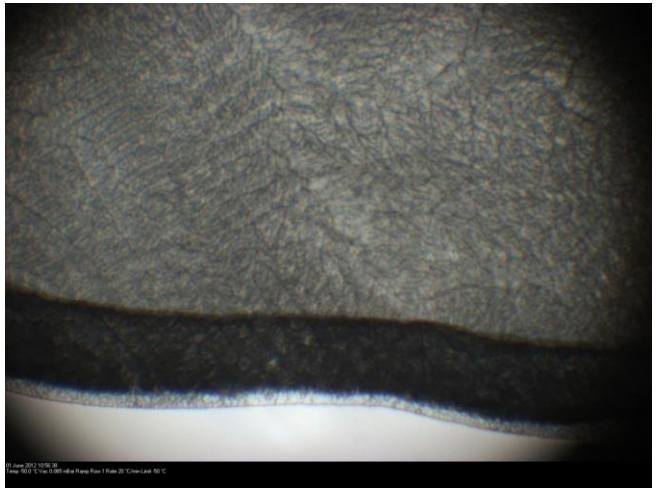
- Flushing lyo chamber with nitrogen before unloading may be advisable to minimise moisture uptake
- Unloading into nitrogen box / tent / dry box?
- Secondary packaging carried out in same box / tent

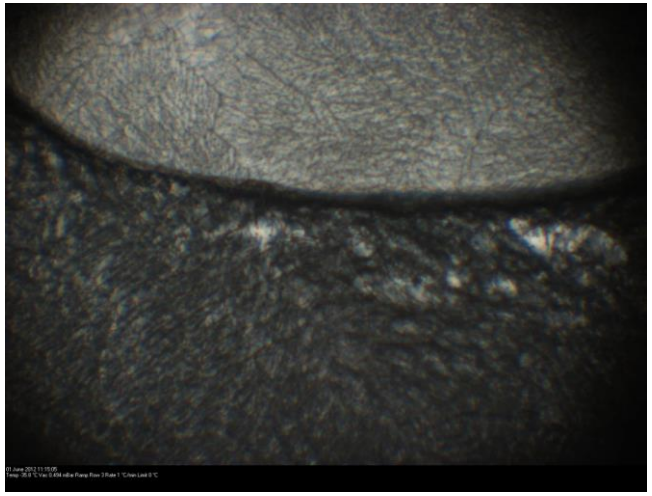
Putting all the information together…

- Most people use an iterative approach to cycle development, but this approach can be scientifically sound, rational and evidence-led!
- Data from formulation characterisation and equipment qualification provide a solid basis for the first cycle parameters
- Feedback from the first cycle allows conditions to be refined in subsequent cycles…



Case Study – Blood Product – FDM analysis

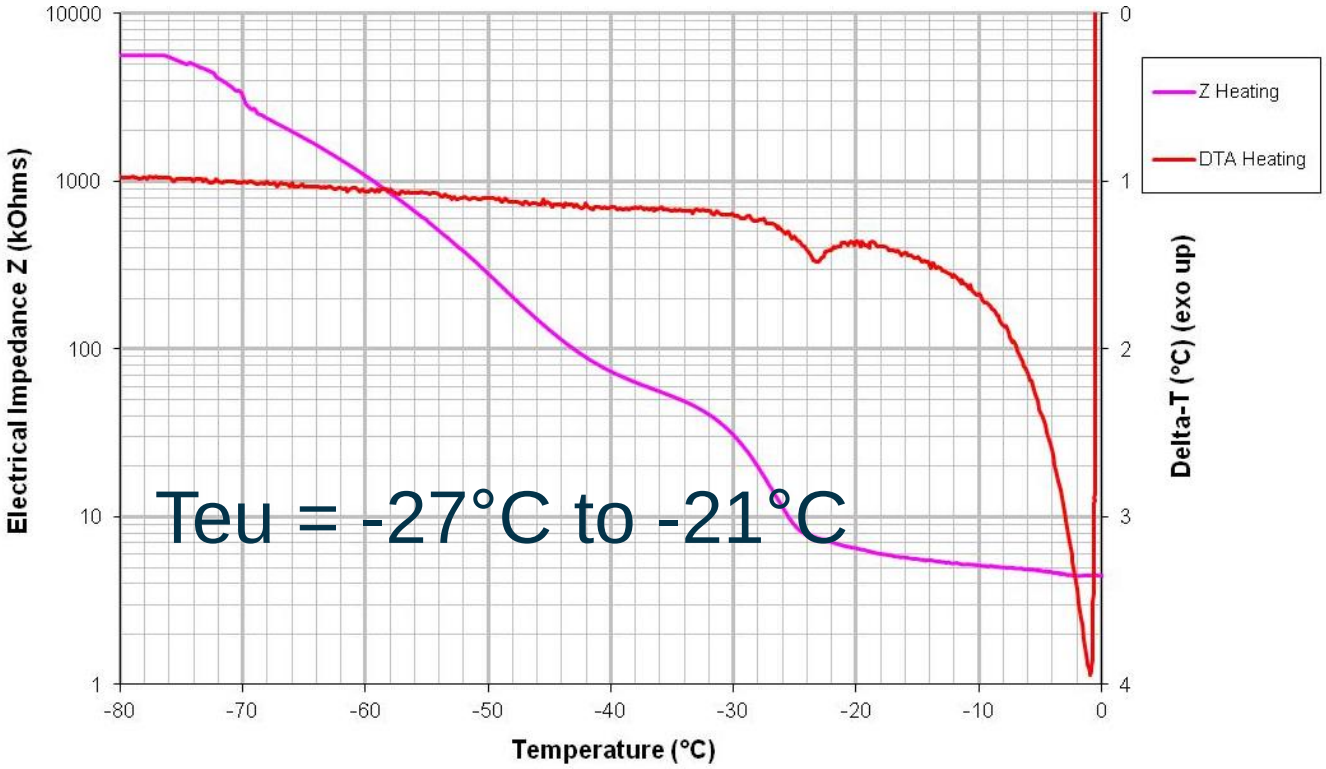




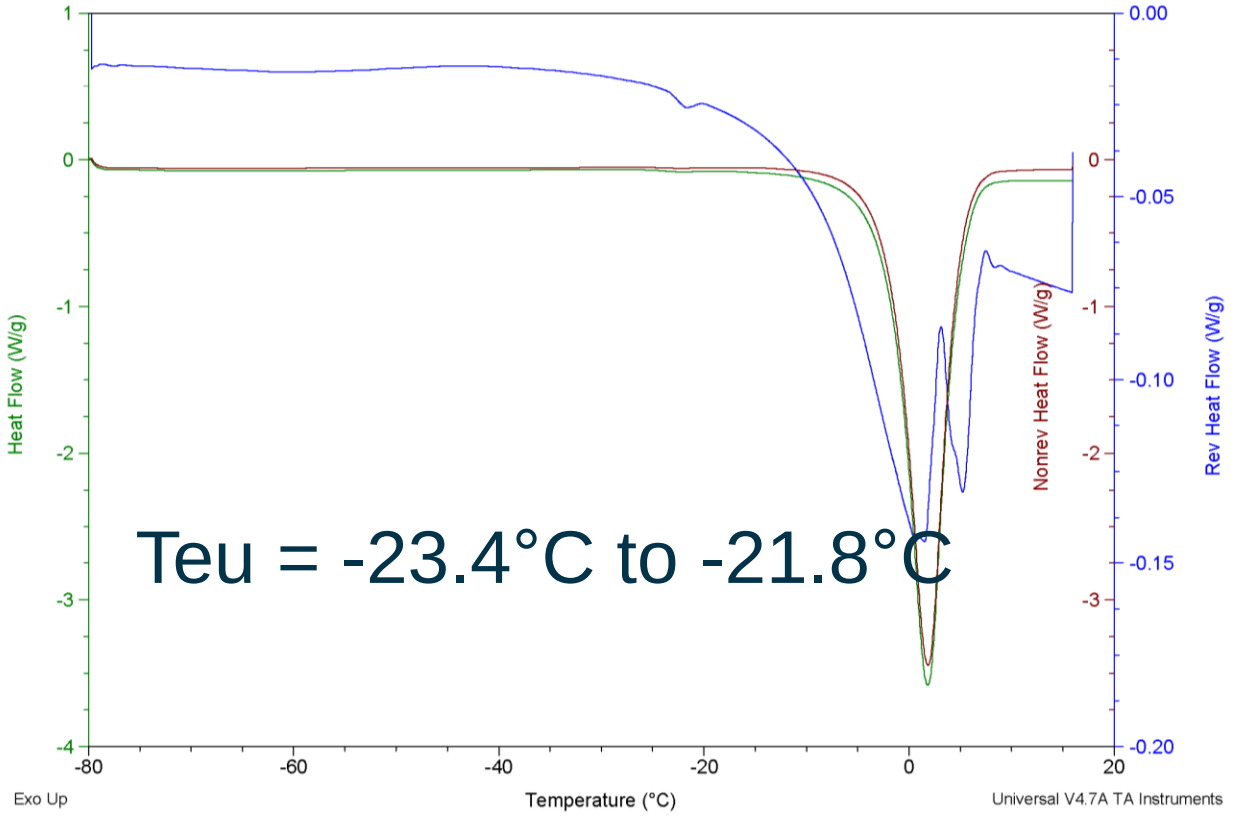
Collapse onset = -39.6°C

Annealed Collapse onset = -25.6°C

Case Study – Blood Product – DTA/Impedance Analysis



Case Study – Blood Product – MDSC analysis



Case Study – Blood Product – Customer original cycle

Thermal Treatment Steps

	Temp	Time	Ramp/Hold
Step # 1	20	10	H
Step # 2	-45	130	R
Step # 3	-45	180	H
Step # 4	0	0	H
Step # 5	0	0	H
Step # 6	0	0	H
Step # 7	0	0	H
Step # 8	0	0	
Step # 9	0	0	
Step # 10	0	0	
Step # 11	0	0	
Step # 12	0	0	

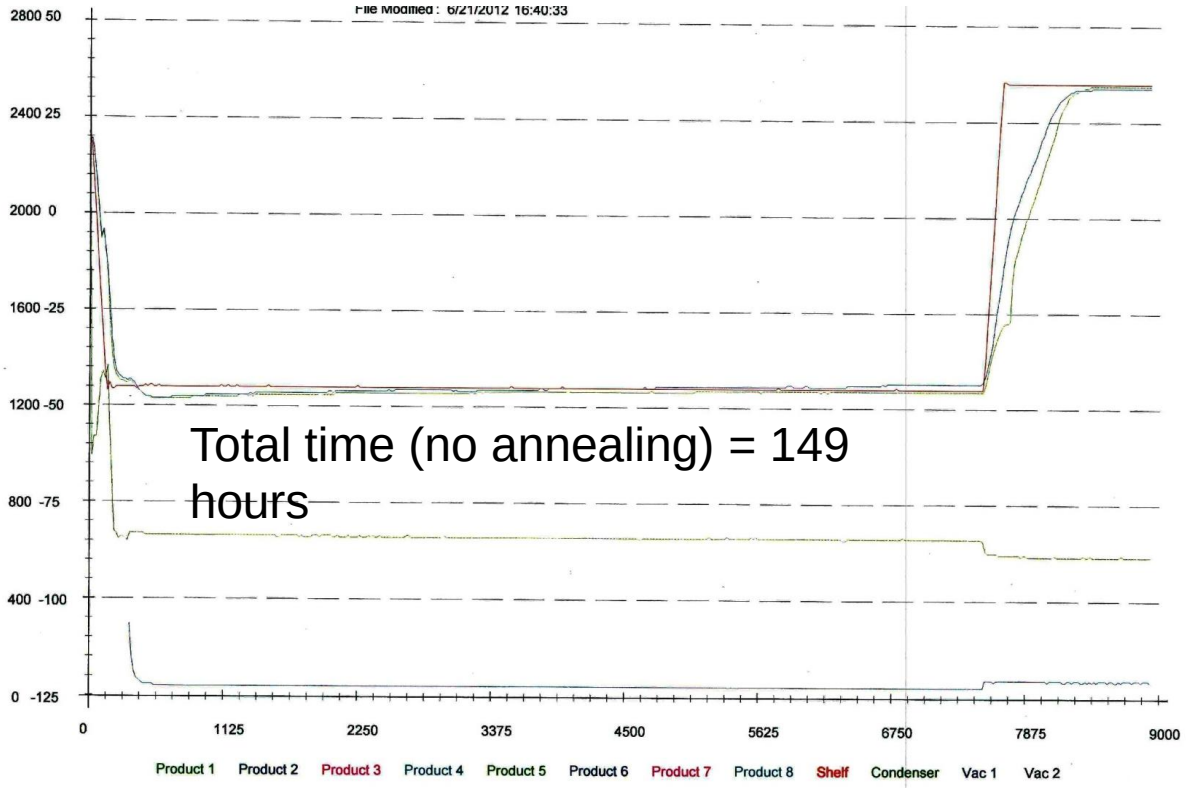
Freeze Temp	-45 °C
Additional Freeze	0 min
Condenser Setpoint	-60 °C
Vacuum Setpoint	600 mTorr

Primary Drying Steps

	Temp	Time	Vac	Ramp/Hold
Step # 1	-45	1200	30	H
Step # 2	-45	1200	30	H
Step # 3	-45	1200	30	H
Step # 4	-45	1200	30	H
Step # 5	-45	1200	30	H
Step # 6	-45	1190	30	H
Step # 7	35	160	60	R
Step # 8	35	1200	60	H
Step # 9	35	60	60	H
Step # 10	0	0	0	H
Step # 11	0	0	0	R
Step # 12	0	0	0	H
Step # 13	0	0	0	
Step # 14	0	0	0	
Step # 15	0	0	0	
Step # 16	0	0	0	

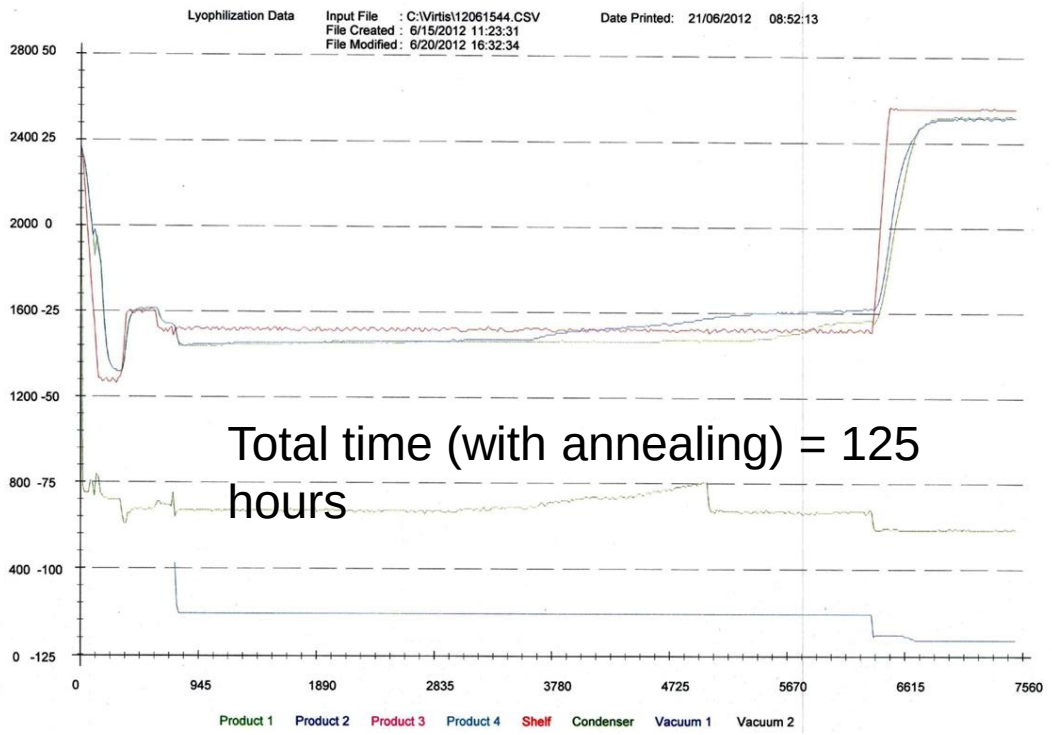
Case Study – Blood Product – Customer original cycle

Vial	Average Moisture Content % (w/w)	Standard Deviation	No. Of Replicates
1	1.54	0.93	4
2	1.19	0.57	4



Case Study – Blood Product – Development Run 1

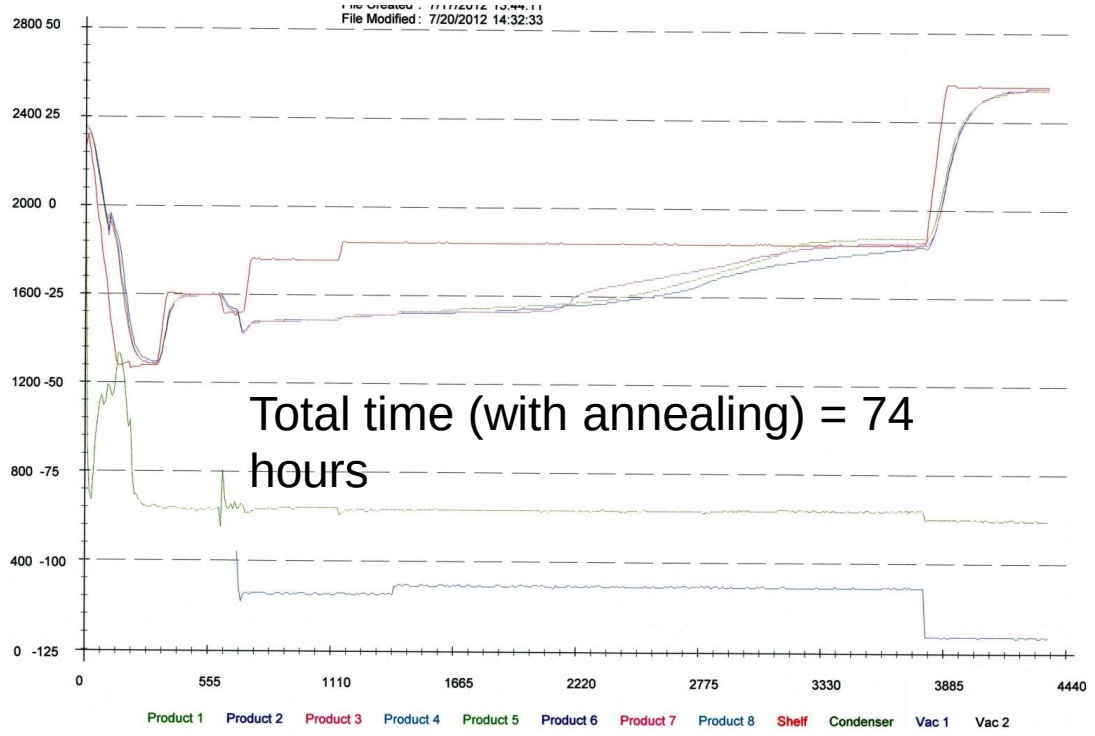
Vial	Average Moisture Content % (w/w)	Standard Deviation	No. Of Replicates
1	1.54	0.52	2
2	1.64	0.70	4



Total time (with annealing) = 125 hours

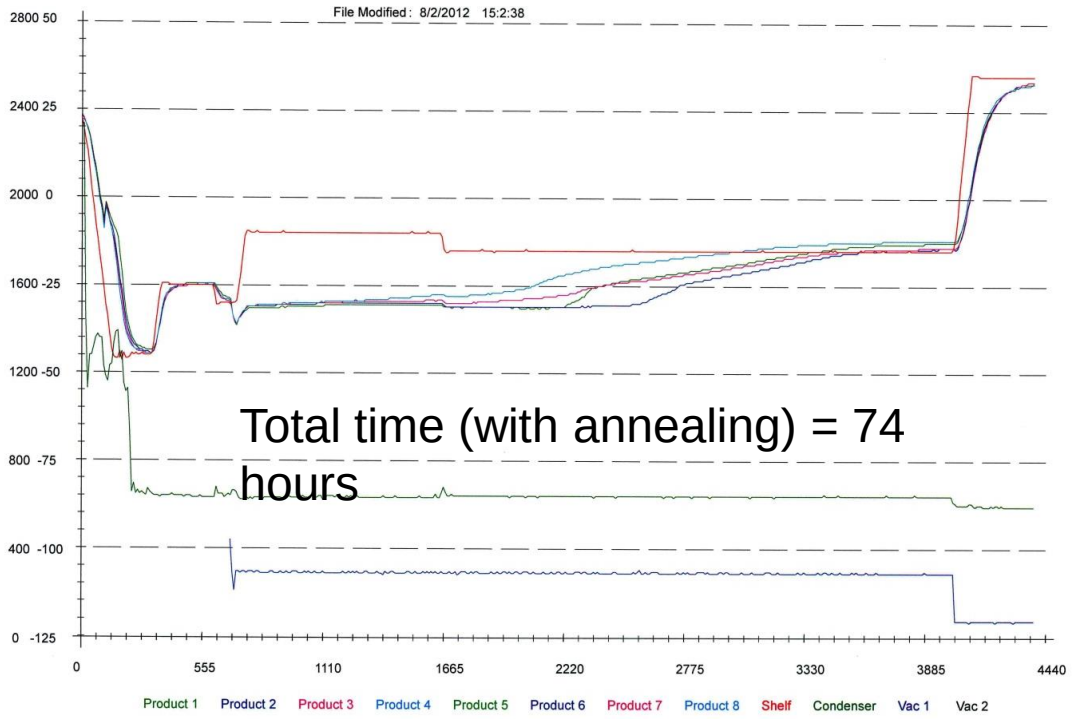
Case Study – Blood Product – Development Run 2

Vial	Average Moisture Content % (w/w)	Standard Deviation	No. Of Replicates
1	1.10	0.40	4
2	1.39	0.50	4
3	1.09	0.44	4



Case Study – Blood Product – Development Run 3

Vial	Average Moisture Content % (w/w)	Standard Deviation	No. Of Replicates
1	2.05	0.92	4
2	1.73	1.19	4
3	1.39	0.73	4

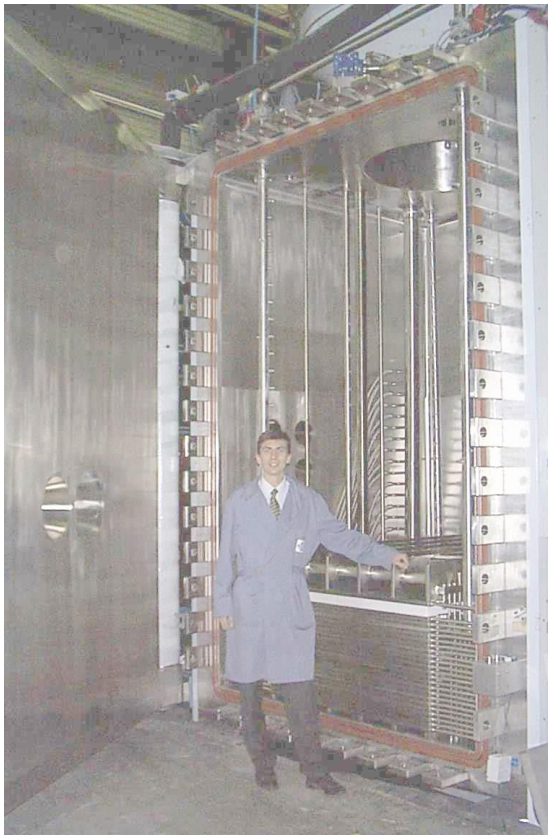


Total time (with annealing) = 74 hours

Case Study - Summary

- Original customer cycle was 149 hours with good appearance and 1.4% moisture
- This cycle used low temperature because without annealing, the critical temperature of the formulation was close to -40°C
- Further analysis of the formulation by FDM / DTA / Zsinφ / MDSC showed that after annealing, the critical temperature was **between -27°C and -21°C**
- Three developmental runs enabled us to explore efficiency measures including looking at shelf temperature, time of each step, and the use of annealing
- Final result: cycle was 74 hours with excellent appearance and 1.7% moisture

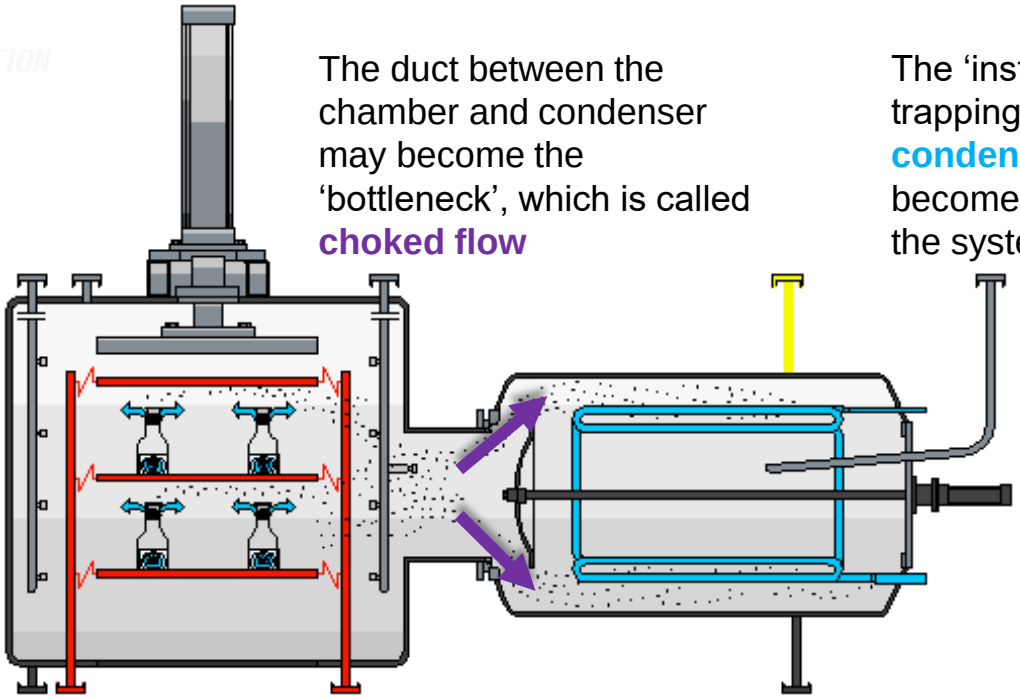
Scale-Up Parameters and Cycle Robustness Testing



What are the Main Scale-Up Factors in Freeze-Drying?

- **Equipment Factors** (design differences and practical limitations)
  - Ultimate shelf temperature achievable
  - Shelf cooling / warming rates (*under load?)*
    - Dimensions of shelves (area and thickness)
    - Flow rate of silicone oil through shelves
    - Where is shelf temperature measured?
    - Shelf mapping important!
  - Relative trapping rates of condensers and valve / duct diameters (may lead to 'choked flow' phenomenon)
  - Removal of residual heat from freeze-dryer prior to loading and/or during freezing, related to mass of machine itself
  - Different vacuum gauges (*e.g.* CM / MKS vs. Pirani)
  - Different control systems / units of measurement
- **Operational Factors** (especially upstream) and differences in timings of various steps

Trapping rate issue and choked flow



The duct between the chamber and condenser may become the 'bottleneck', which is called **choked flow**

The 'instantaneous trapping rate' of the **condenser itself** may also become the bottleneck in the system

What are the Main Scale-Up Factors in Freeze-Drying?

- **Equipment Factors** (design differences and practical limitations)
  - Ultimate shelf temperature achievable
  - Shelf cooling / warming rates (*under load?)*
    - Dimensions of shelves (area and thickness)
    - Flow rate of silicone oil through shelves
    - Where is shelf temperature measured?
    - Shelf mapping important!
  - Relative trapping rates of condensers and valve / duct diameters (may lead to 'choked flow' phenomenon)
  - Removal of residual heat from freeze-dryer prior to loading and/or during freezing, related to mass of machine itself
  - Different vacuum gauges (*e.g.* CM / MKS vs. Pirani)
  - Different control systems / units of measurement
- **Operational Factors** (especially upstream) and differences in timings of various steps

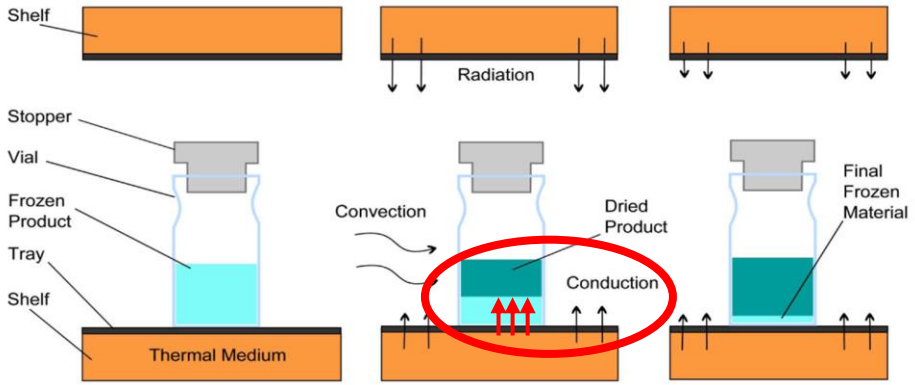
Conductive and *Radiative* heating

Effectiveness of **conductive** heat transfer improves throughout the process, due to the reducing thickness of the (insulating) ice layer. It will also be affected by:

- Degree of contact between container and shelf
- Heat transfer coefficient of the container itself (Kv)

***Radiative heating effectiveness** is governed partly by shelf spacing, which may or may not be adjustable, depending on the design of the dryer*

Heat Entry into Product



***Radiative heating** can also come from the walls or door of the dryer, which can lead to 'edge effects'*

What are the Main Scale-Up Factors in Freeze-Drying?

- **Equipment Factors** (design differences and practical limitations)
  - Ultimate shelf temperature achievable
  - Shelf cooling / warming rates (*under load?)*
    - Dimensions of shelves (area and thickness)
    - Flow rate of silicone oil through shelves
    - Where is shelf temperature measured?
    - Shelf mapping important!
  - Relative trapping rates of condensers and valve / duct diameters (may lead to 'choked flow' phenomenon)
  - Removal of residual heat from freeze-dryer prior to loading and/or during freezing, related to mass of machine itself
  - Different vacuum gauges (*e.g.* CM / MKS vs. Pirani)
  - Different control systems / units of measurement
- **Operational Factors** (especially upstream) and differences in timings of various steps

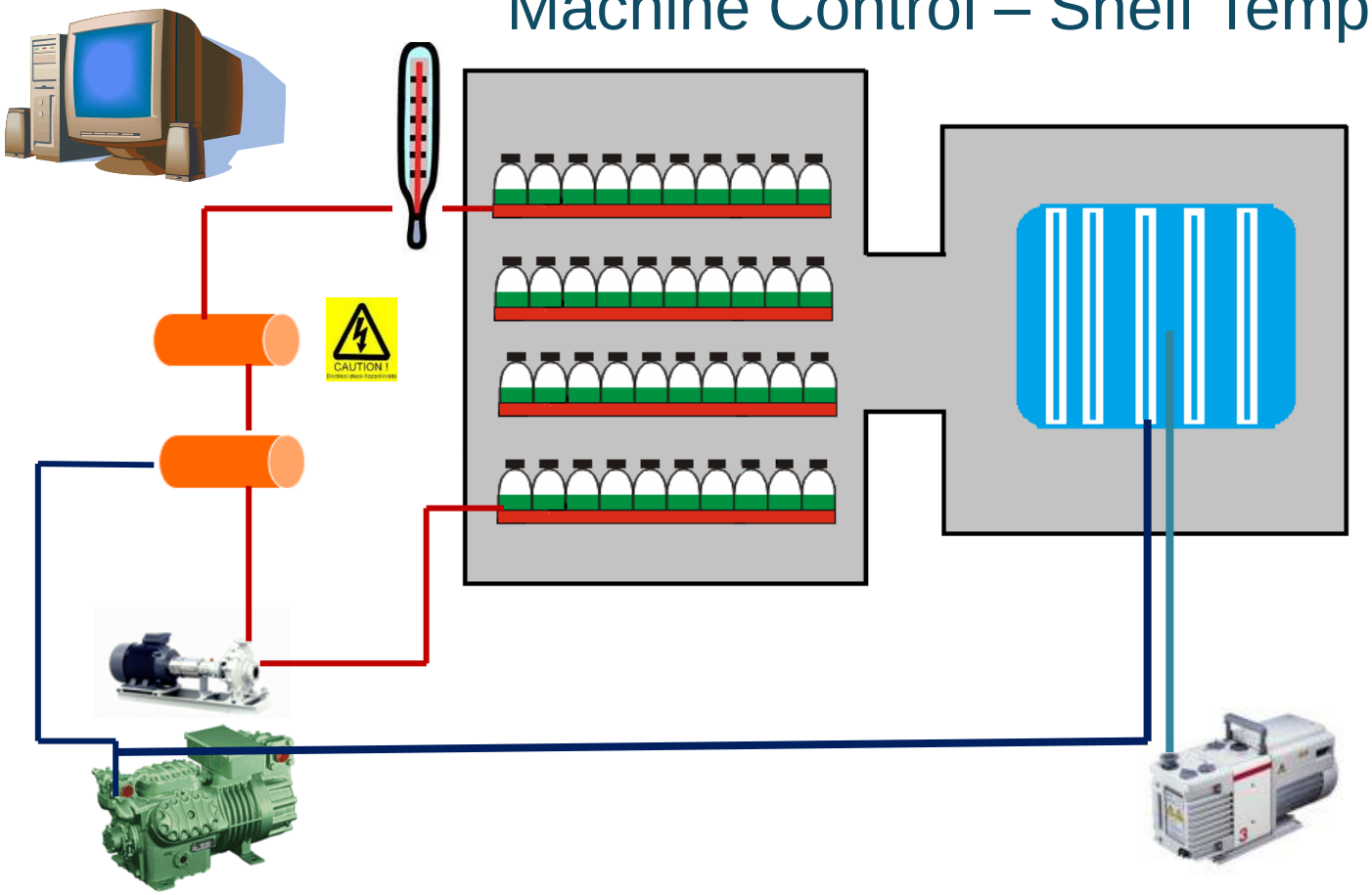
Equipment: *Vacuum gauge type, design & tolerance*

- Different vacuum readings can be expected from:
  - Capacitance Manometer (CM) gauge, and
  - Thermocouple gauge such as a Pirani gauge (PVG)
- This is because the gauges work differently, which is a result of their design and construction
- The PVG is influenced by water, whereas the CM gauge reading is independent of gas type
- We exploit this difference when we use the gauge comparison method for endpoint determination
  - *See next presentation on PAT!*
- PVG gauge typically has 10-15% error, while the CM gauge is typically <<1% error

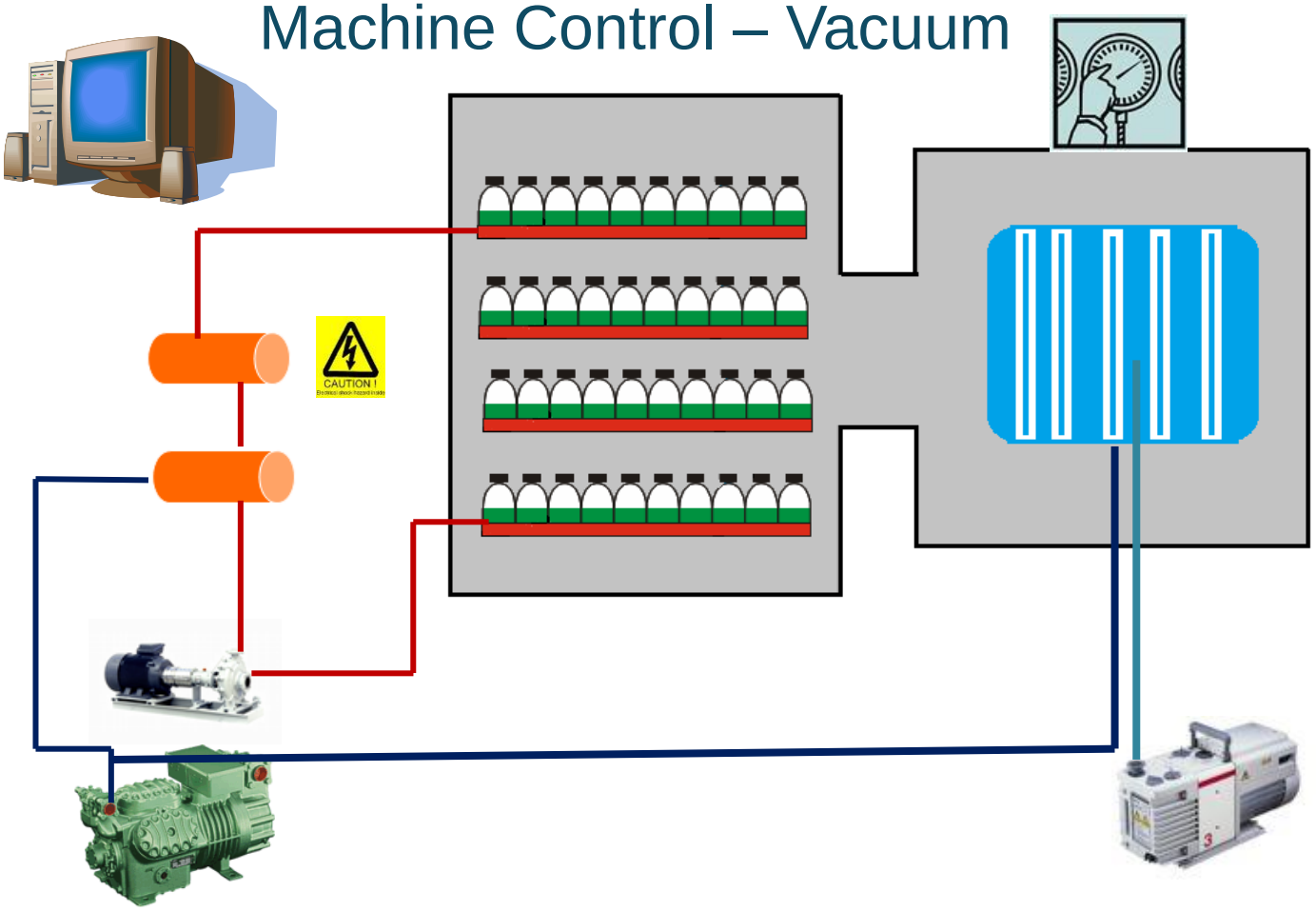
Equipment: *Control System Differences*

- Number of steps available in freezing and drying
- Chamber evacuation time?
- Other ‘safety checks’ and alarm settings?
- “Rounding Errors” and pressure measurement
  - 300mTorr = 400ubar, which is simple, but
  - 200mTorr = 266.6666ubar (*use 270ubar?)*
- Shelf ramping calculations (time or gradient?)
  - Ramping time e.g. +20°C to -30 °C in 60 minutes
  - This translates to 0.8333°C per minute (*round up to 0.85?)*

Machine Control – Shelf Temperature



Machine Control – Vacuum



What are the Main Scale-Up Factors in Freeze-Drying?

- **Equipment Factors** (design differences and practical limitations)
- **Operational Factors** (especially upstream) and differences in timings of various steps

- It's best to predict what the upstream operations will be in production, and try to match them in the lab as far as practicable:
  - Filling / loading operation (time, temperature)
  - Sterile Filtration (can affect ice nucleation and solute crystallisation)
  - Primary packaging: dimensions and material(s) →
    - Heat transfer coefficient (Kv)
    - Permeability to moisture post-lyo
  - Secondary packaging configuration

What are the Main Scale-Up Factors in Freeze-Drying?

- **Equipment Factors** (design differences and practical limitations)
- **Operational Factors** (especially upstream) and differences in timings of various steps

- It's best to predict what the upstream operations will be in production, and try to match them in the lab as far as practicable:
  - Filling / loading operation (time, temperature)
  - Sterile Filtration (can affect ice nucleation and solute crystallisation)
  - Primary packaging: dimensions and material(s) →
    - Heat transfer coefficient (Kv)
    - Permeability to moisture post-lyo
  - Secondary packaging configuration

What are the Main Scale-Up Factors in Freeze-Drying?

- **Equipment Factors** (design differences and practical limitations)
- **Operational Factors** (especially upstream) and differences in timings of various steps

- It's best to predict what the upstream operations will be in production, and try to match them in the lab as far as practicable:
  - Filling / loading operation (time, temperature)
  - Sterile Filtration (can affect ice nucleation and solute crystallisation)
  - Primary packaging: dimensions and material(s) →
    - Heat transfer coefficient (Kv)
    - Permeability to moisture post-lyo
  - Secondary packaging configuration

Cycle “Robustness”

There are many approaches to test the robustness of a freeze-drying cycle, such as:

1. Varying temperature and pressure throughout the cycle
   - High temperature + High pressure
   - High temperature + Low pressure
   - Low temperature + High pressure
   - Low temperature + Low pressure

This would require 4 freeze-drying runs, but some people also vary the time of the steps, which can require more runs… e.g. 16 runs

2. Deliberately implementing temperature or pressure excursions to represent what might realistically happen in a production environment (based on risk analysis or actual experience)
3. Using a Quality by Design (QbD) approach and creating a Design Space (see later)

Summary: Scale-Up

- Many scale-up issues to consider:
  - Effects of equipment differences – size, geometries, capacities, control systems, vials
  - Response of products to differences in upstream processing (e.g. filtering, filling, loading)
- Scale-up issues can be reduced by using a suitable temperature safety margin and increasing the time of many of the ‘hold’ steps
- Repeatability is not the same as robustness:
  - Robustness means we need to **challenge** the process or product
  - The simplest robustness test typically involves varying Ts and Pc to give 4 cycles
  - More complex robustness testing can involve 16 or more cycles, maybe by DoE approach
  - Another realistic test can be to include brief temperature or pressure excursions to represent power failures, temporary loss of refrigeration power or small vacuum leaks

Summary

- We have reviewed the three main steps int eh freeze drying process and how these can effect the rate of drying and speed at which the process can be completed.
- Review commonly used analytical techniques to determining the critical temperature of the formulations in the frozen state.
- Reviewed common excipients and how the compositions of the formulation will impact the critical temperature of the product during freeze drying.
- Reviewed a case study of how to optimize a freeze-drying cycle for a product based upon characterization of the product in the frozen state.
- Discussed the steps and thought processes to develop and optimize the freeze-drying process for a product.
- Reviewed the use of PAT methodology for determining the end of primary drying.

Round-up Discussion and Questions

(15 Minutes)