Fundamentals of Freeze Drying

Lyo-Hub Summer School

Part 5 – Formulation Considerations

Critical Quality Attributes

- Sterility
- Meets USP Criteria for Endotoxin
- Meets USP Criteria for Extraneous Particulate Matter
- Complete Recovery of Activity
- Rapid, Complete Reconstitution
- [Isotonicity]
- [Physiological pH]
- [Free from Preservatives?]

Preformulation Considerations

- Dose
- Chemical Stability
- Physical Stability
- Solubility
- Ionization Constant (weak acids and bases)
- Vapor pressure?

Be Aware of Intended Route of Administration

Route	Volume	Isotonic?	Phys. pH?
IV Bolus	About 10 ml	No	No
IV Infusion	no limit	No	No
I.M.	2-5 ml	No	No
SubQ	about 2 ml	No	No
Intrathecal	about 10 ml	Yes	Yes
Epidural	about 5 ml	Yes	Yes
Intra articular	about 2 ml	No	No

Selection of Formulation Components

- Keep it simple!
- Have a rationale for inclusion of every formulation component
- Document the rationale

Excipients in Freeze Dried Products

- Buffers
- Bulking Agents
  - Crystalline (Mannitol, Glycine)
  - Amorphous (Sucrose, Trehalose, Lactose, HSA)
- Tonicity Modifier
  - Commonly NaCl
- Surfactant
  - Particularly for protein formulations
  - Polysorbates 20 and 80 are common. Poloxamer less common.
- Preservative
  - Rarely used in freeze-dried formulations, but they do exist

Buffers

- There is no rule that says you have to have one
- More is not necessarily better
- Keep in mind that many times the API can act as a buffer
- Keep in mind that buffers in frozen systems can be paradoxical. Beware of the possibility of pH shifts.

Buffers in Approved Products

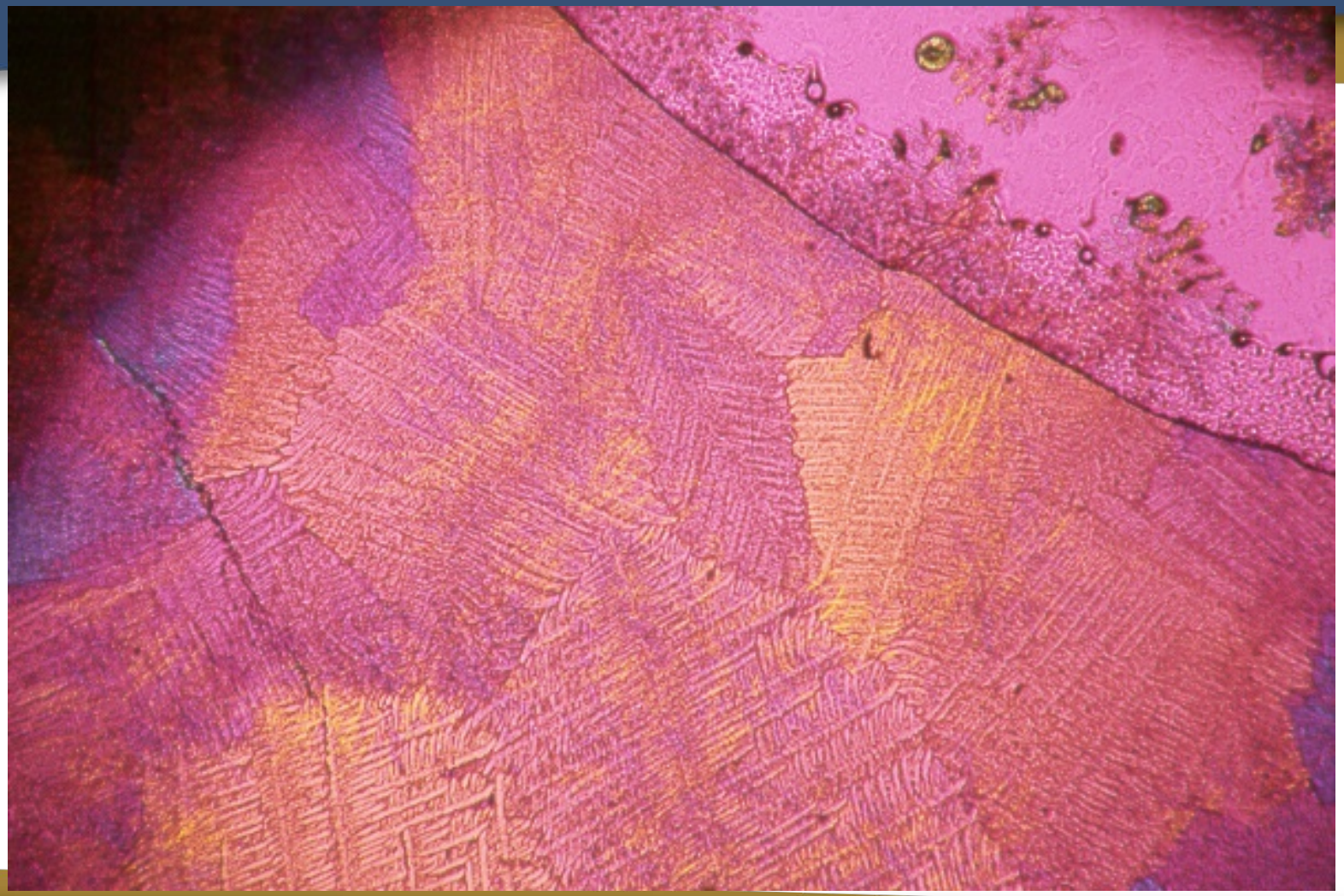
Phosphate (pKa = 2.1, 7.2, 12.7)
Citrate (pKa = 3.2, 4.8, 6.4)
Tris (pKa = 8.1)
Succinate (pKa = 4.2, 5.6)
Histidine (pKa = 1.8, 6.0, 9.0)
Glycine (pKa = 2.4, 9.8)
Arginine (pKa = 2.2, 9.1)
Malic (pKa = 3.4, 5.1)
Tartaric (pKa = 3.2, 4.8)

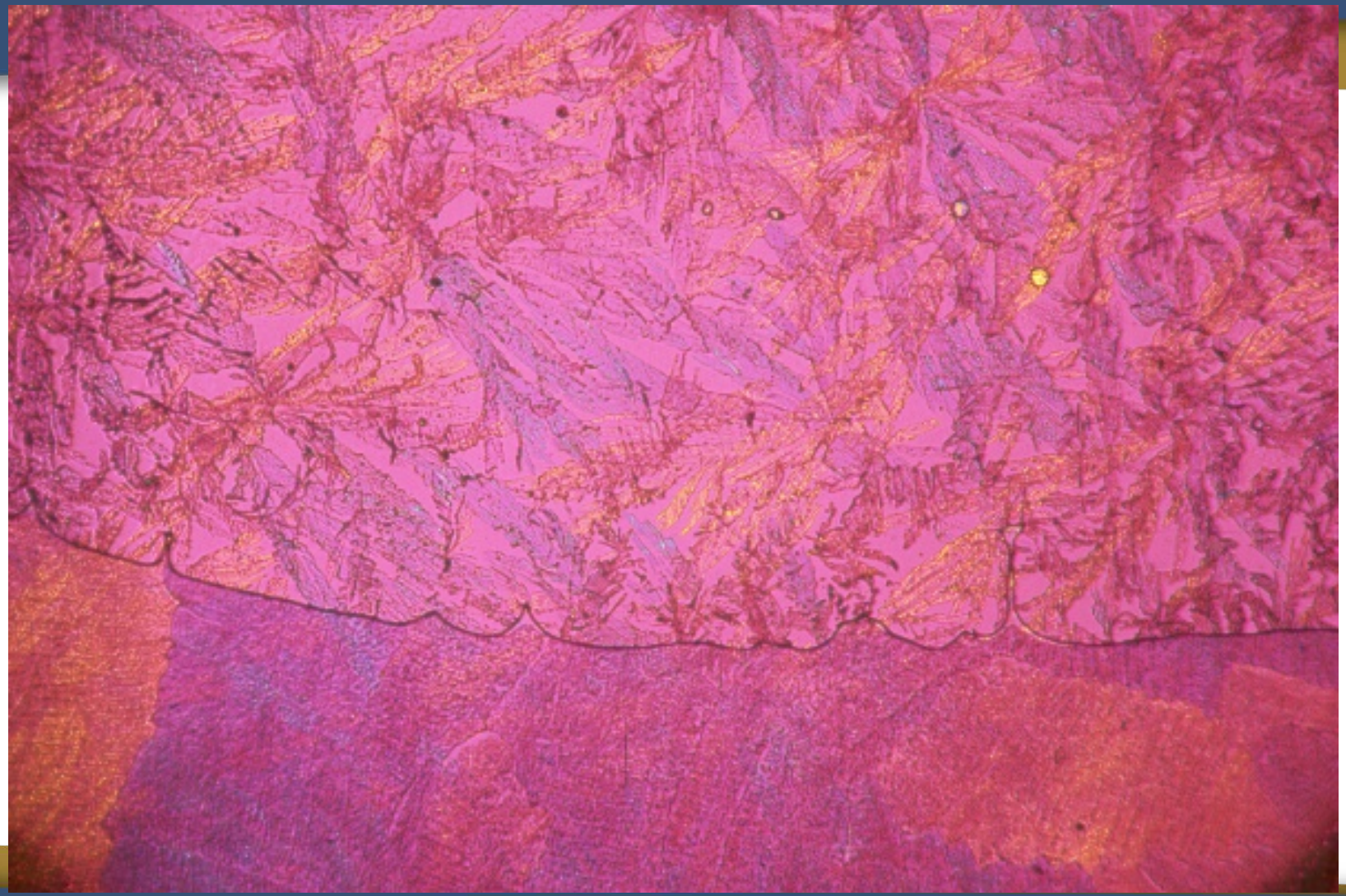

Citrate


Tris

Bulking Agent

- The need for a bulking agent depends very much on the dose of drug. Remember that there are plenty of freeze dried drug product, usually small molecules, that consist ONLY of the API.
- Make sure that the total concentration of dissolved solids is neither too high nor too low. Total dissolved solids in rough range of 30 to 120 mg/ml is a reasonable estimate.
- We think it is good practice to include both a crystalline and an amorphous bulking agent/protectant
- Keep in mind that an amorphous excipient, such as sucrose or trehalose, can serve as both a bulking agent and a stabilizer.





Mannitol

- Most commonly used of all bulking agents in freeze-dried products
- Vial breakage can be significant
- Exhibits polymorphism: unknown significance
- The rate of freezing may influence the extent of mannitol crystallization in the freeze dried product
  - An annealing step may be needed to promote crystallization

Vial Breakage in Freeze-Drying

- Note: This is of particular concern for oncolytic agents

Shattering

"Lensing"

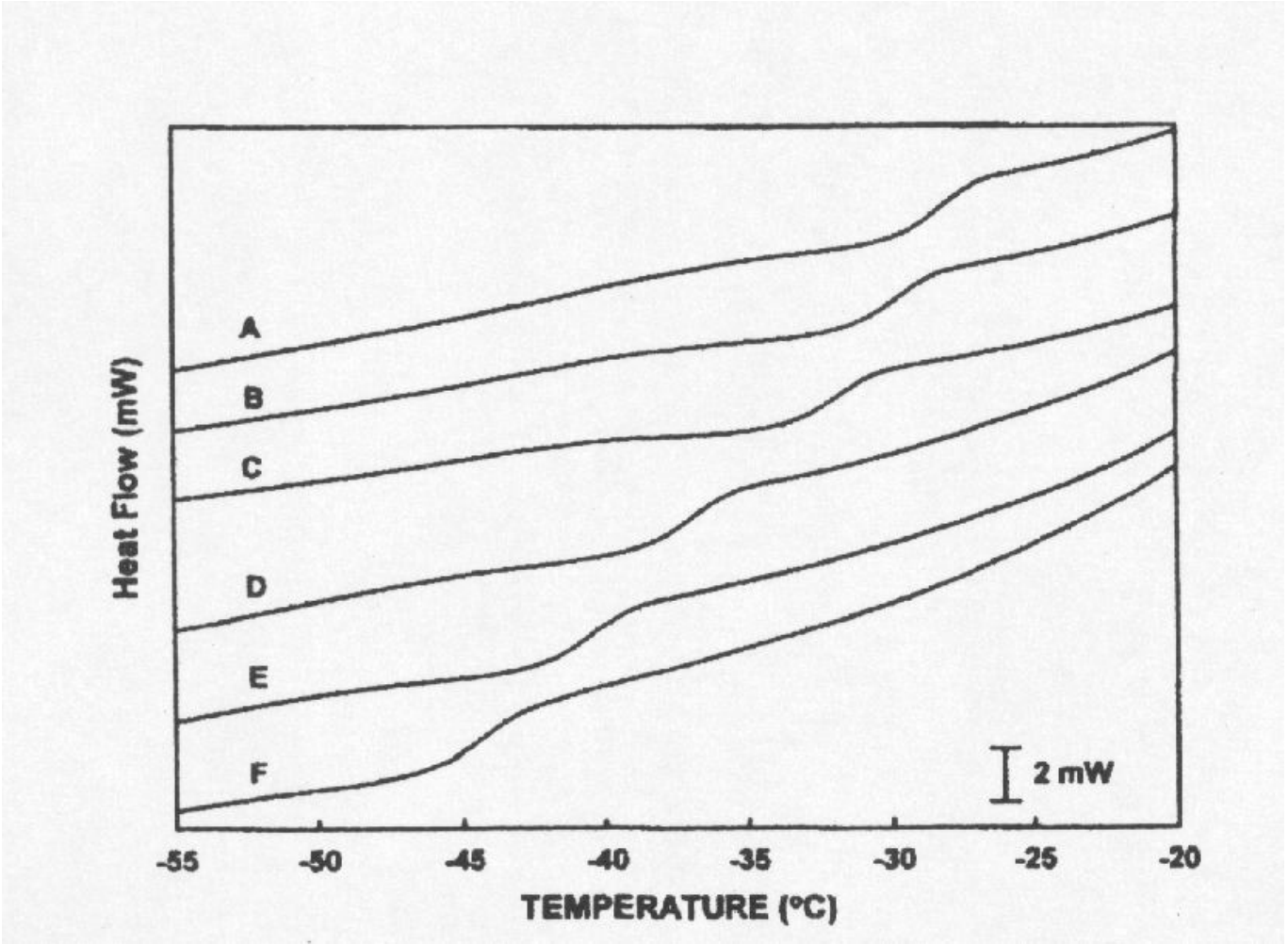
Important Factors in Vial Breakage

- The relative fill volume – keep under about 30%
- The relative amount of crystallizing solute
- The heel radius of the vial
- The thermal history of freezing

Added Salts – Keep to a Minimum Because. . .

- They tend to decrease the collapse temperature of a formulation
- They can inhibit crystallization of formulation components that you want to crystallize
- The increased ionic strength associated with freeze concentration of salts can cause damage to biologicals.

Effect of Sodium Chloride on T_g' of Lactose



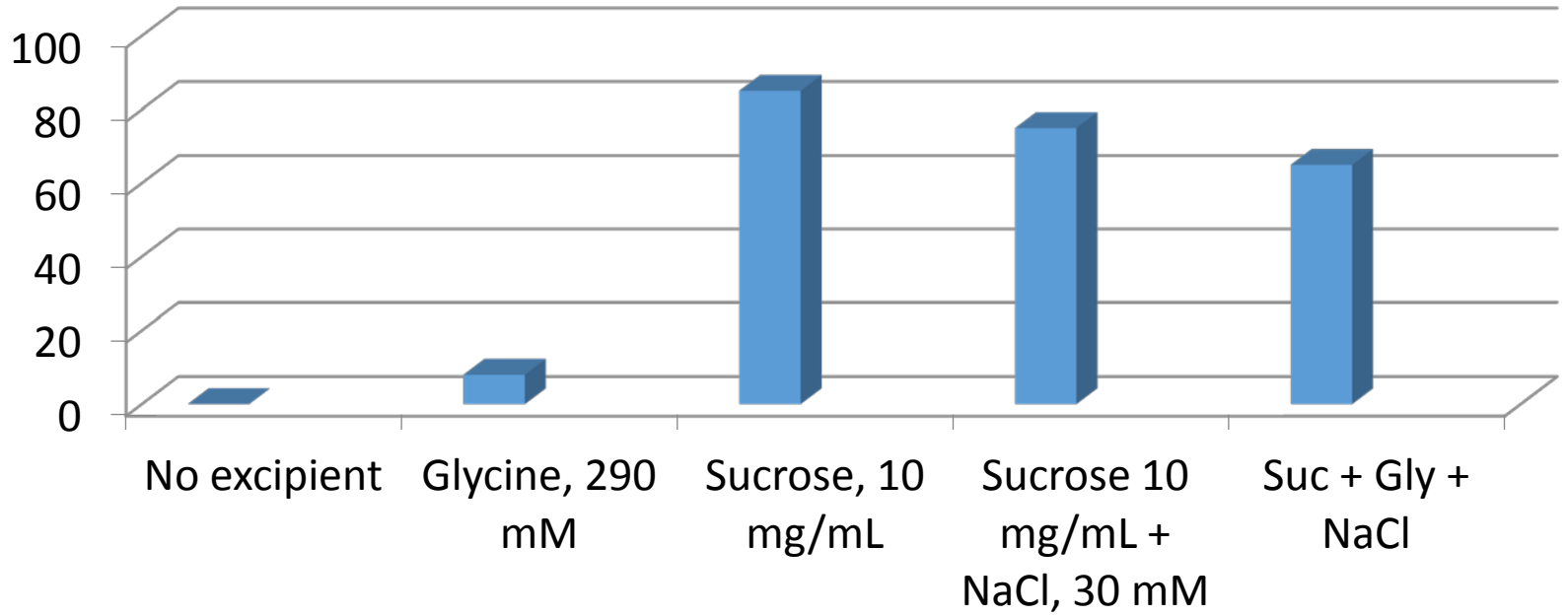
Formulation of Freeze Dried Proteins

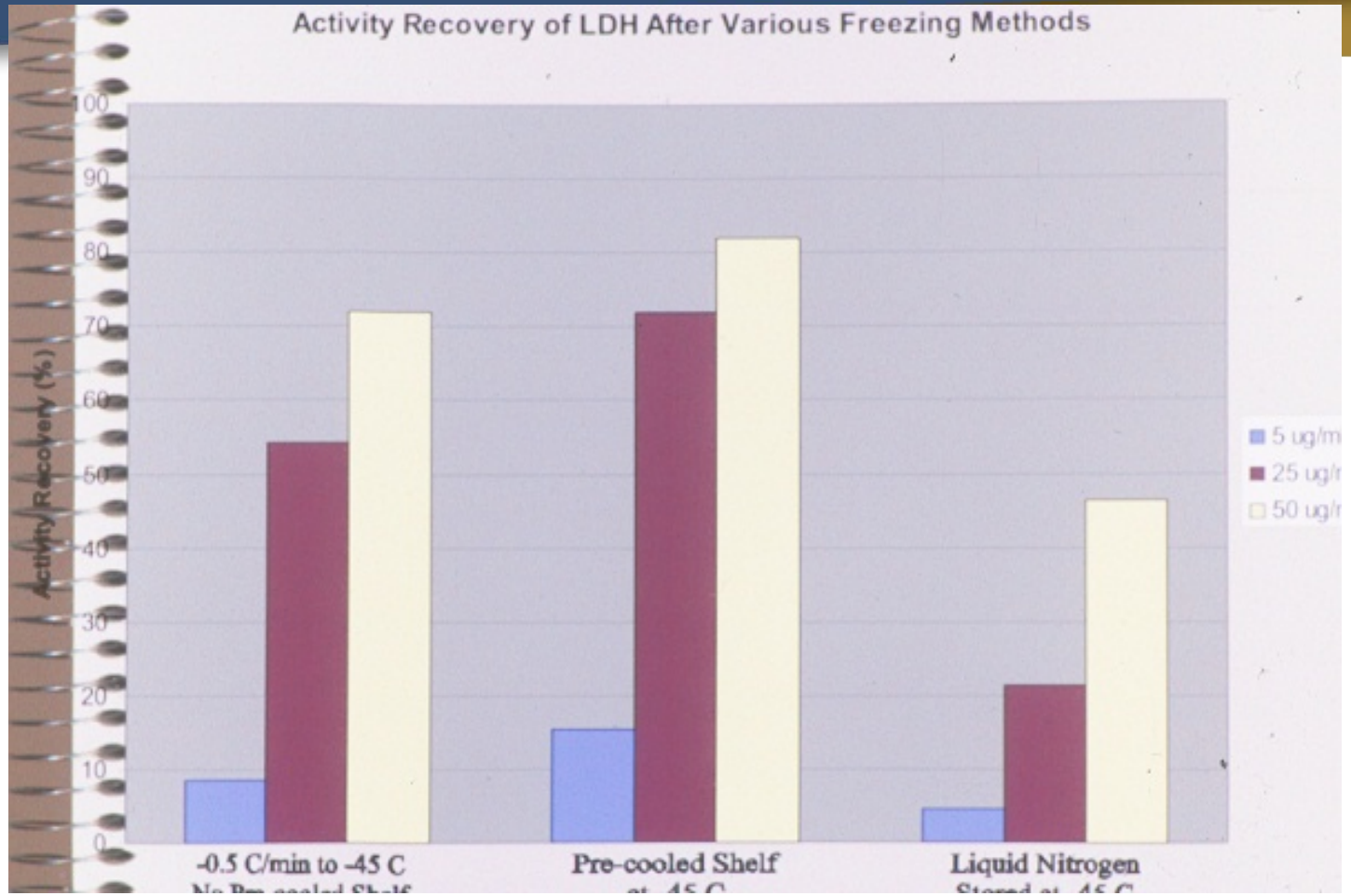
- What Makes Them Different from Small Molecules?
  - Both short-term and long-term stabilities are strongly affected by formulation composition. Generally speaking, a stabilizer is needed.
  - Integrity of the protein can be affected by differences in the thermal history of freezing.
  - Long-term stability may be affected by seemingly small differences in residual moisture level
  - Inclusion of a surfactant is generally considered good formulation practice. This may be because of the tendency of proteins to adsorb at the ice/freeze-concentrate interface.

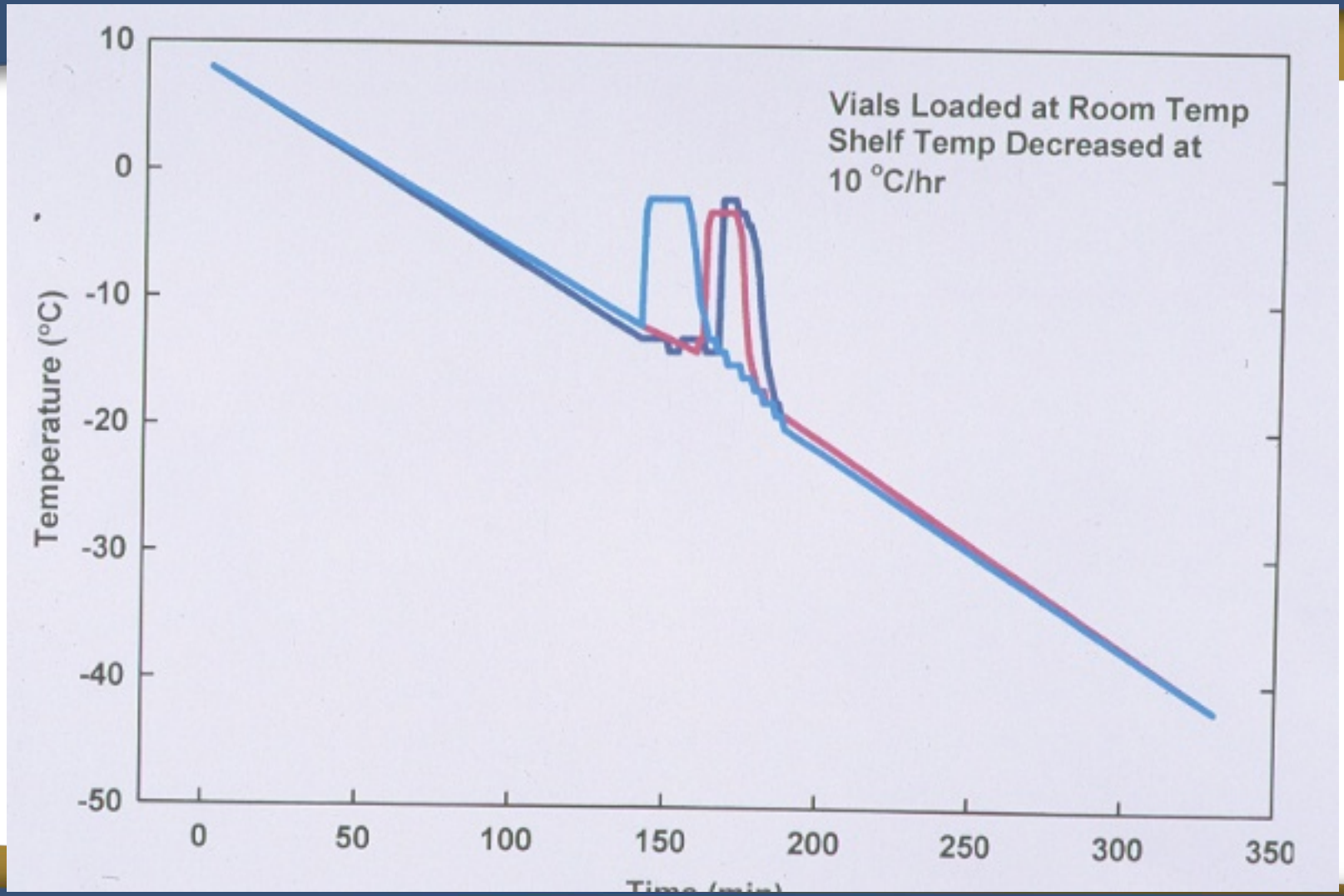
Adsorption of Protein at the Ice/Freeze Concentrate Interface

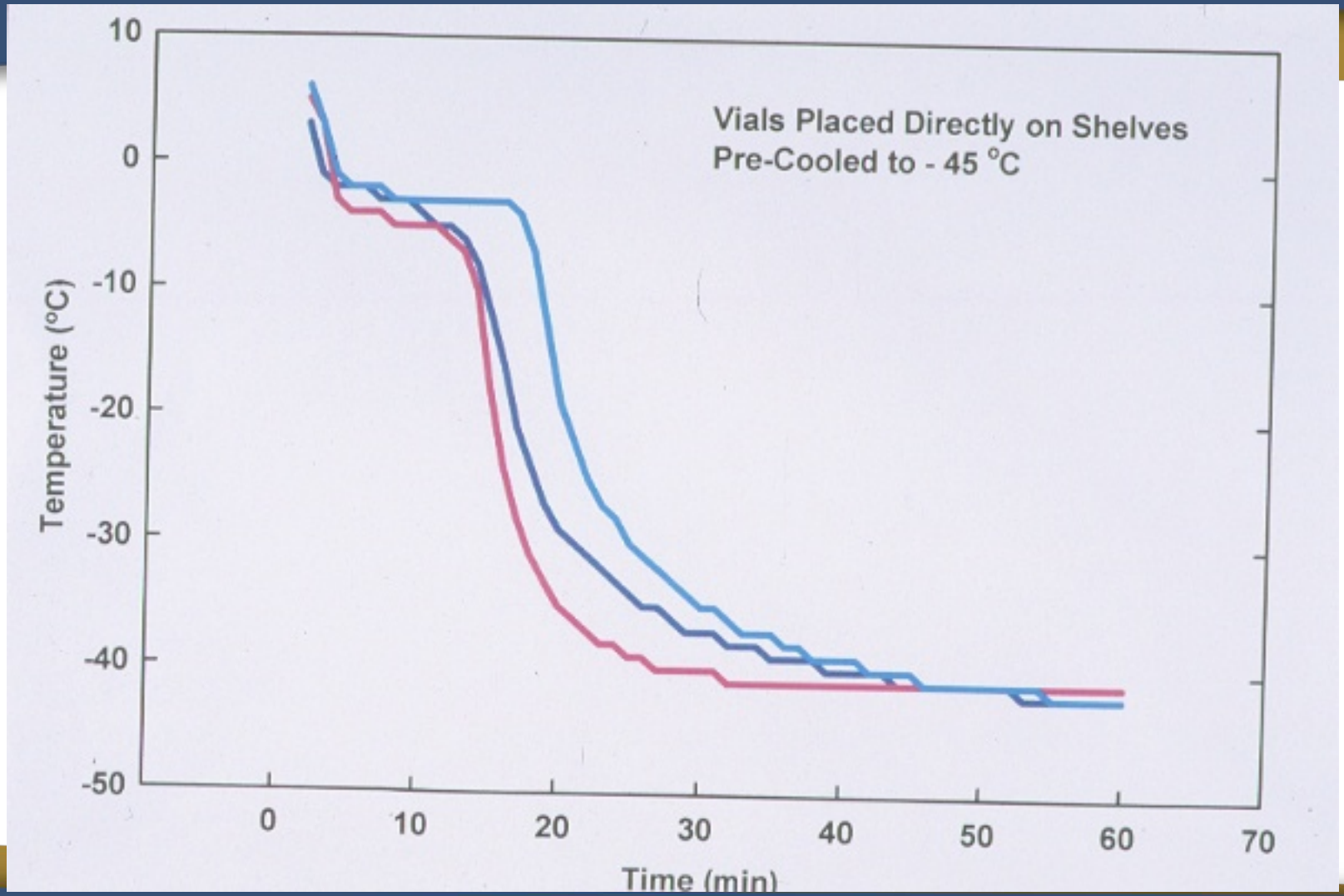
- The idea is that protein tends to adsorb at the ice/freeze concentrate interface and partially unfold. While this process is largely reversible upon freeze/thawing, it may not be completely reversible upon freeze drying.
- This mechanism explains much of the phenomenology of freeze drying of proteins, such as:
  - Concentration dependence
  - Perhaps sensitivity to differences in thermal history of freezing
  - The stabilizing influence of surfactants

Example: Short-Term Stability of Different Formulations of a Protein

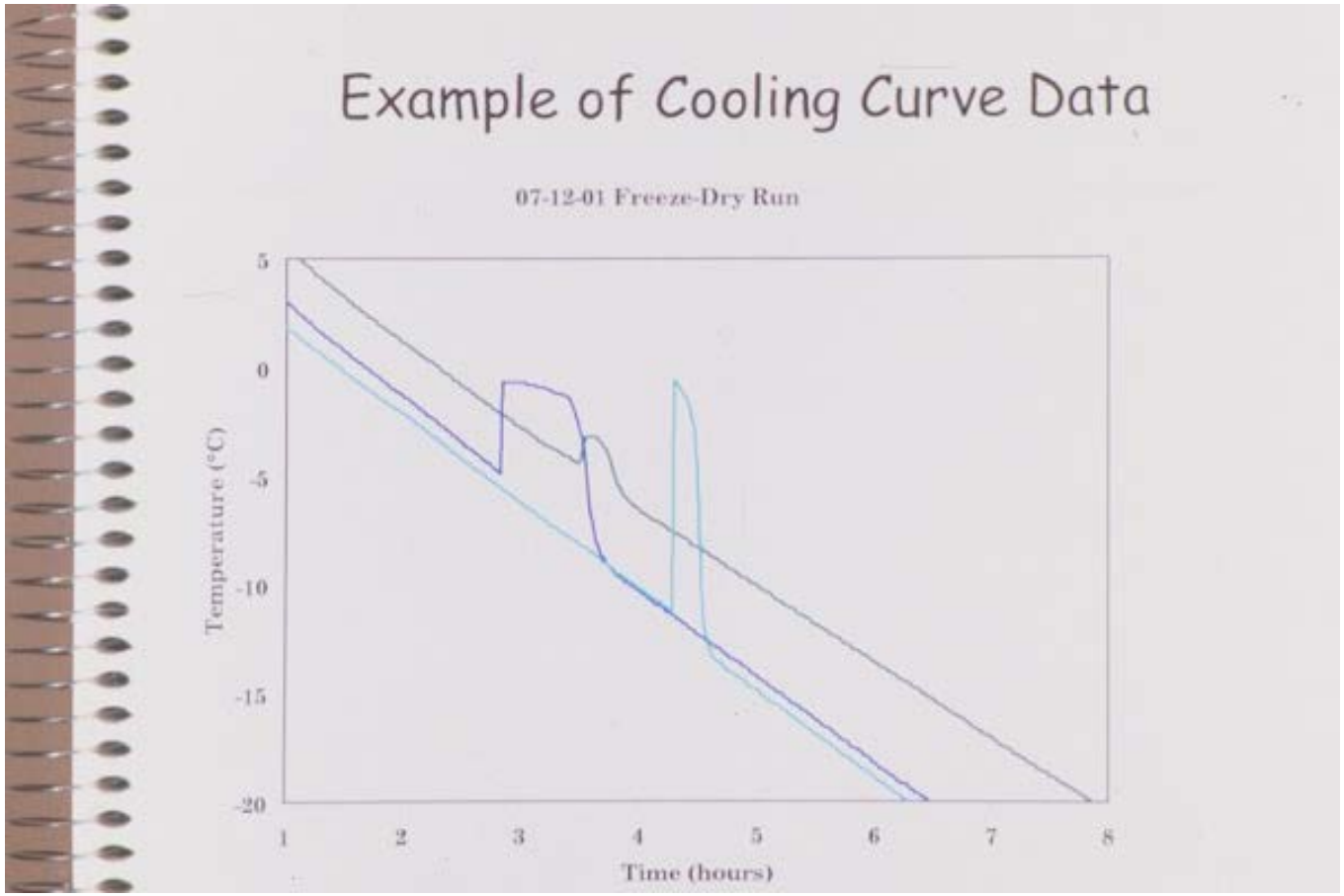



Activity Recovery of LDH After Various Freezing Methods
Activity Recovery (%)
100
90
80
70
60
50
40
30
20
10
0
5 ug/m
25 ug/
50 ug/
-0.5 C/min to -45 C
Pre-cooled Shelf
Liquid Nitrogen

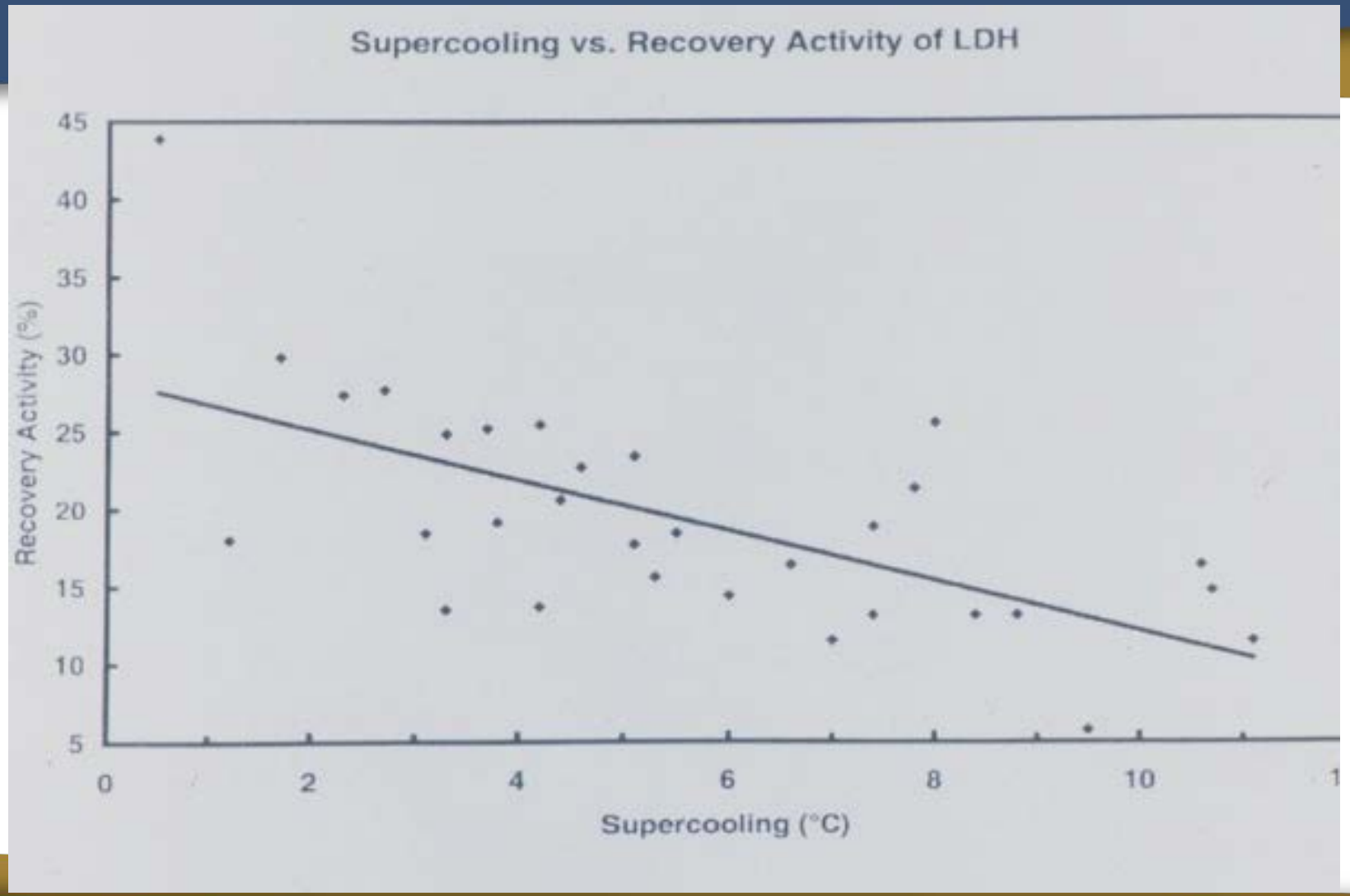

Vials Loaded at Room Temp
Shelf Temp Decreased at
10 °C/hr
Temperature (°C)
10
0
-10
-20
-30
-40
-50
0
50
100
150
200
250
300
350


Vials Placed Directly on Shelves
Pre-Cooled to - 45 °C
Temperature (°C)
10
0
-10
-20
-30
-40
-50
0
10
20
30
40
50
60
70
Time (min)

Dependence of Activity Recovery on Supercooling



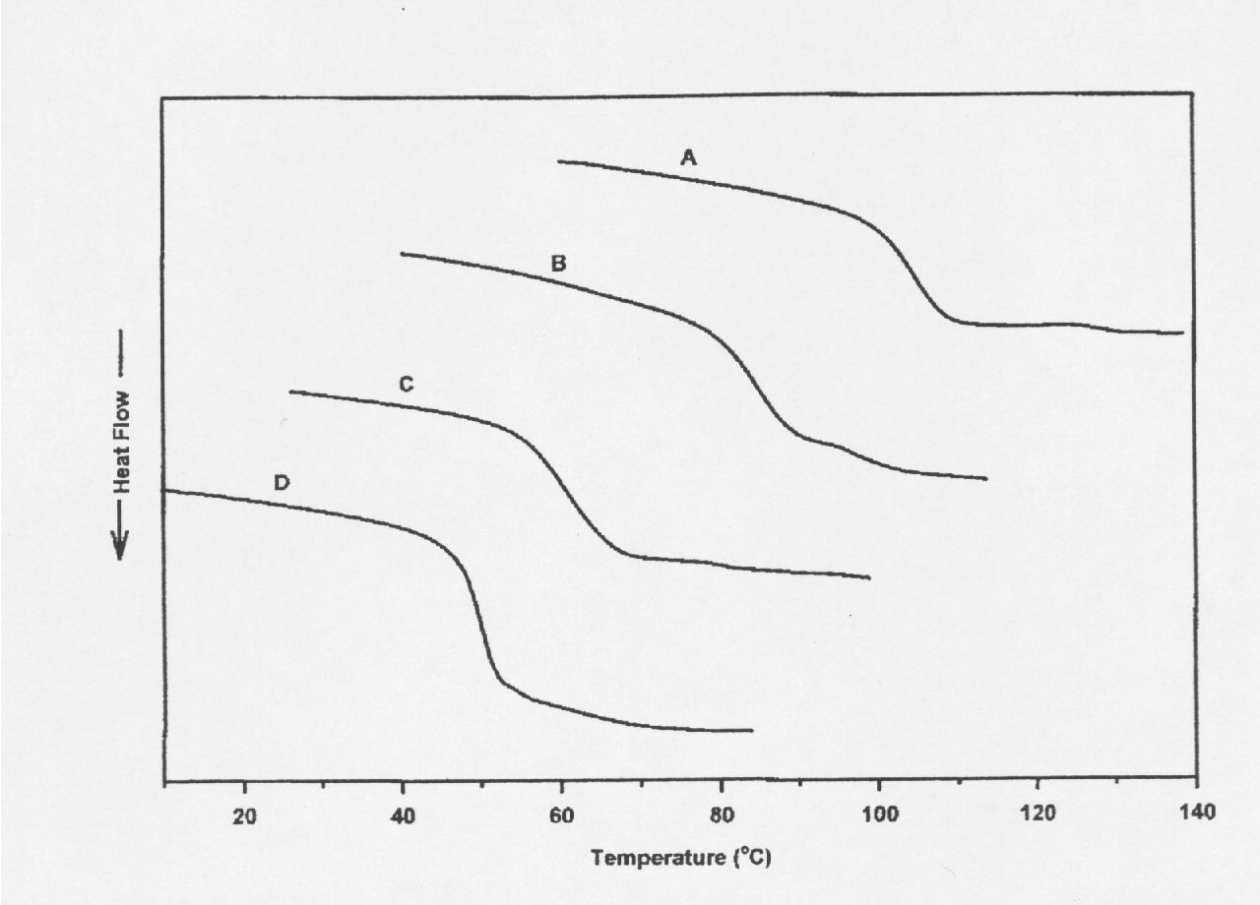

FTS LyoStar


Supercooling vs. Recovery Activity of LDH
Recovery Activity (%)
45
40
35
30
25
20
15
10
5
0
2
4
6
8
10
Supercooling (°C)

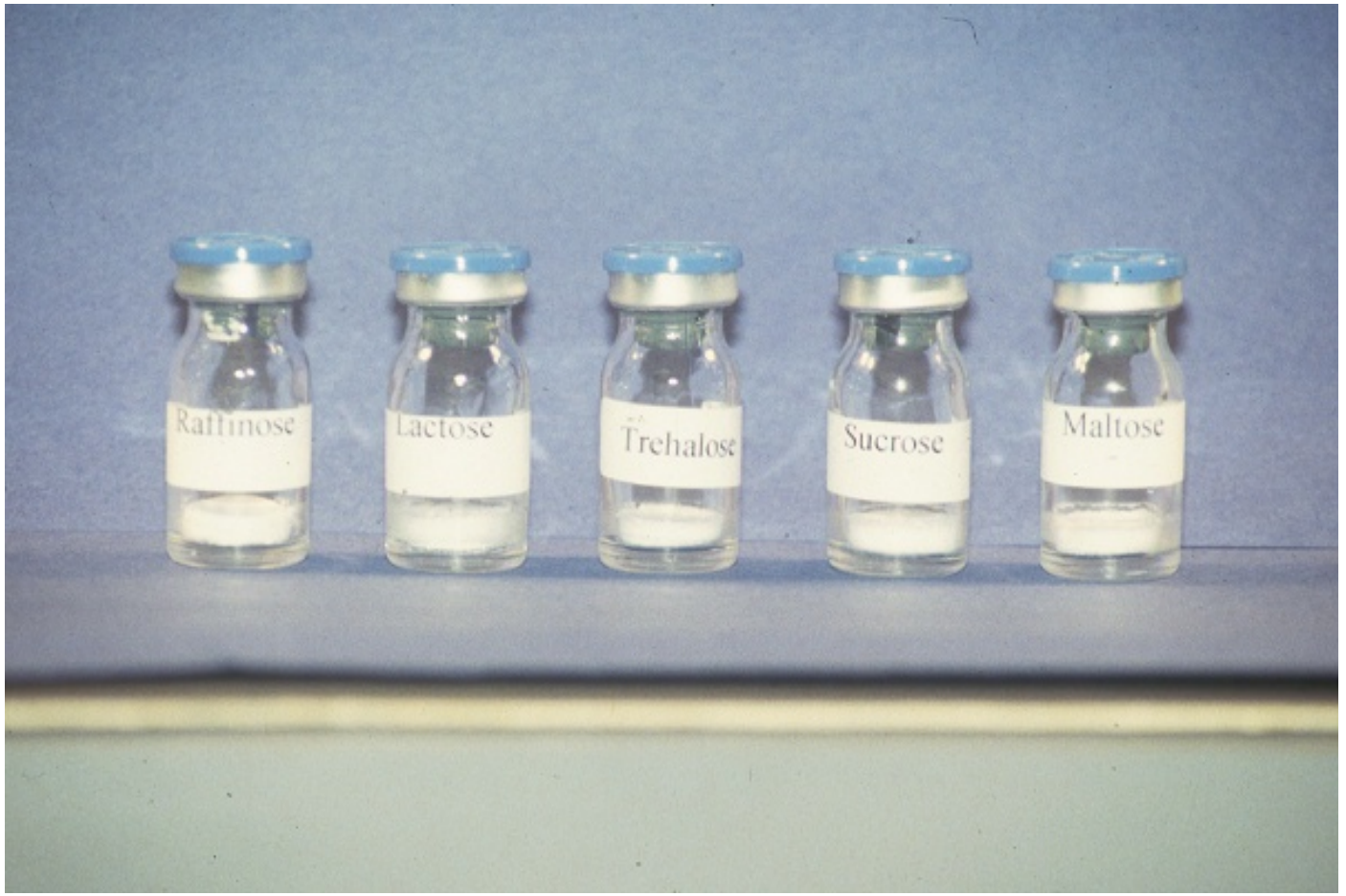
Don’t Forget to Characterize the Dried Product

- Thermal Analysis
  - Amorphous: glass transition of solid
    - How is the glass transition temperature affected by increased residual moisture?
  - Crystalline: which crystal form?
- X-Ray Diffraction

Small differences in formulation composition can significantly affect glass transition temperature of solid



Glass Transition Temperature May be an Important Determinant of Physical Stability

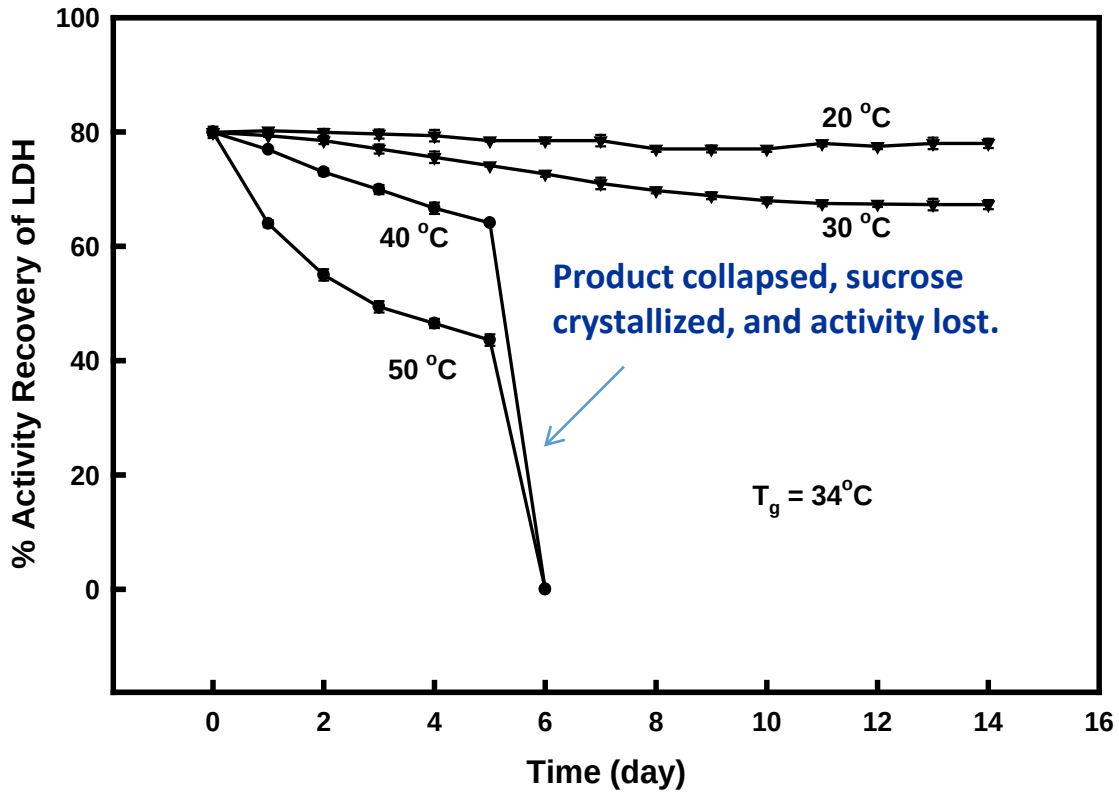



Sucrose
Maltose
Trehalose
Lactose
Raffinose

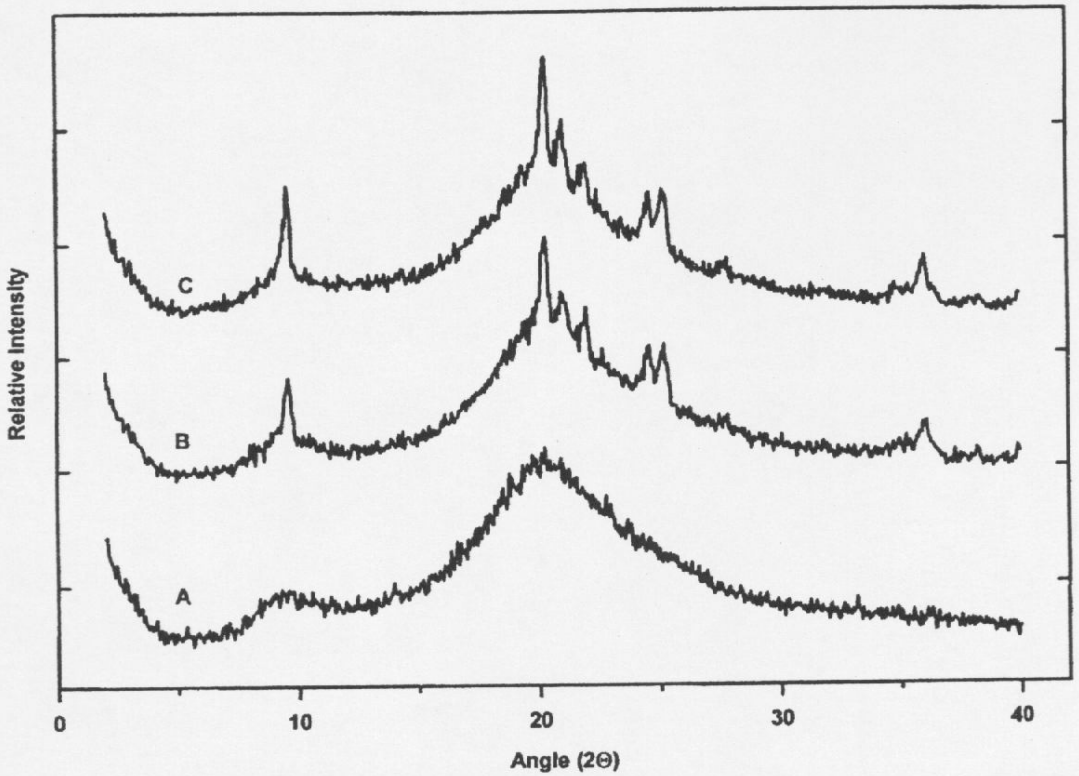

Sucrose
Maltose
Trehalose
Lactose
Raffinose

Example: Long-Term Stability of a Protein

Accelerated Stability Testing of Protein Formulation – 50C



Beware of Crystallization of Components of the Formulation During Storage – Particularly Mannitol

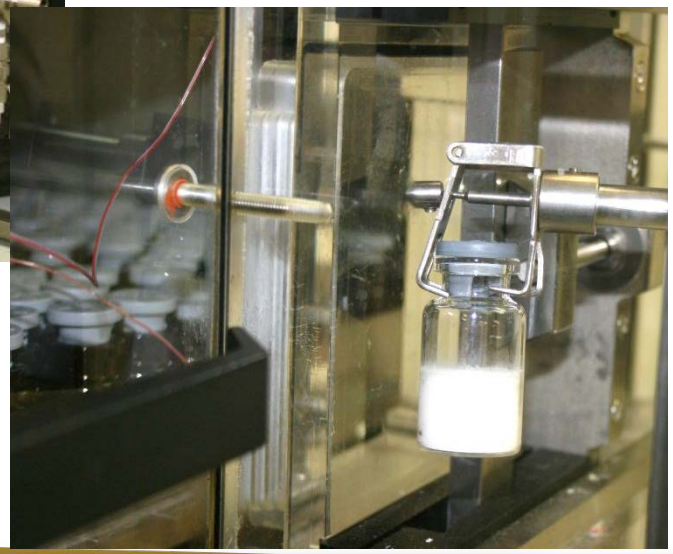


X-ray Powder Diffractograms of Lysozyme in Mannitol Initially (a) and After 3 Weeks (b) and 5 Weeks (c) Storage at 40°C

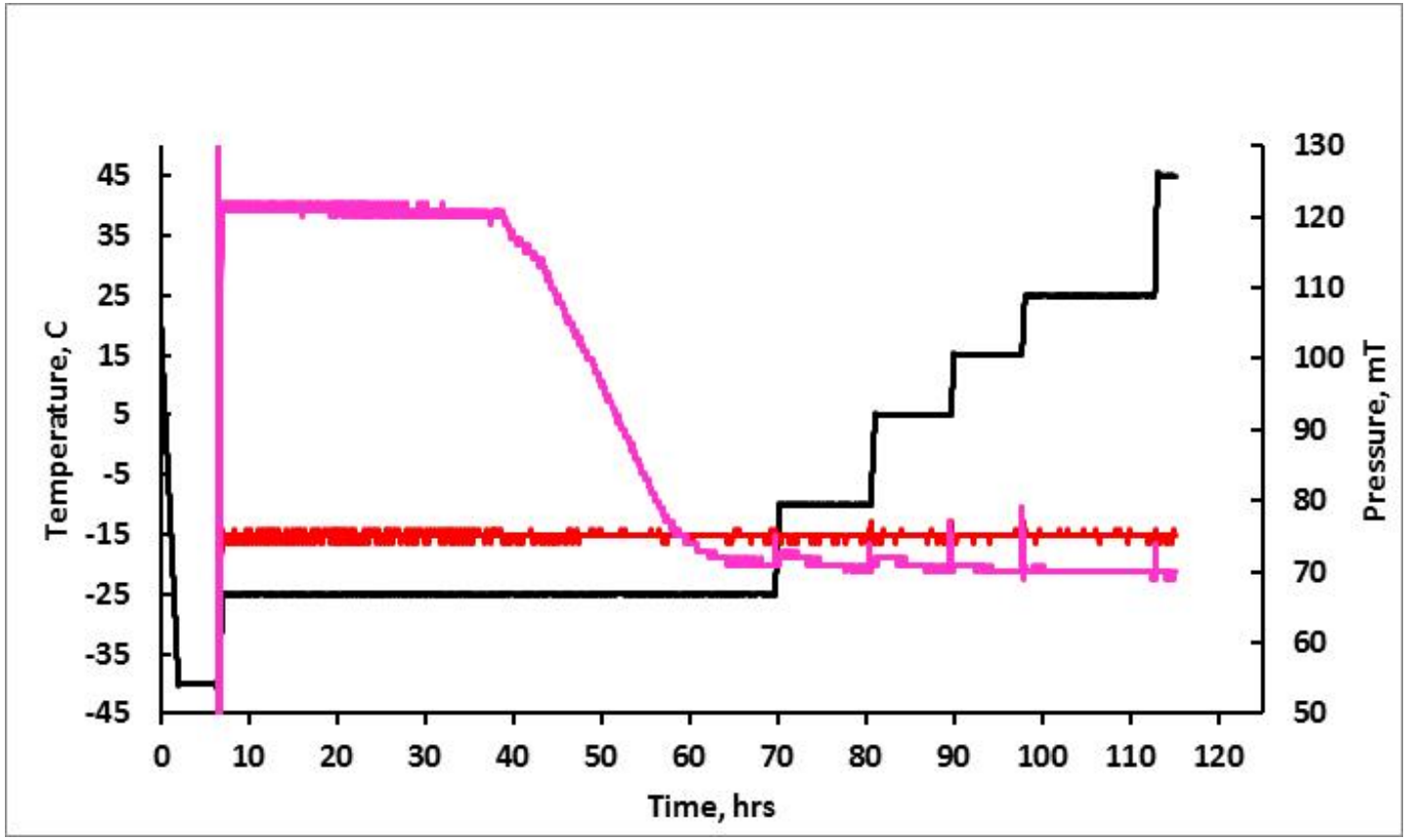
An Example of "Traditional" Approach vs. QbD Approach: Residual Moisture Specification

- "Traditional"
  - Run trial batches during development and test for residual moisture to determine process capability.
  - Residual moisture specification based on these data and lots placed on stability
- Quality by Design
  - Measure how stability of drug product is affected by residual moisture level over a reasonably wide range of residual moisture.
  - Look for the "edge of failure" in establishing a residual moisture specification.

Thief Sampling During Secondary Drying



Lyo Cycle with “Step-wise” Secondary Drying



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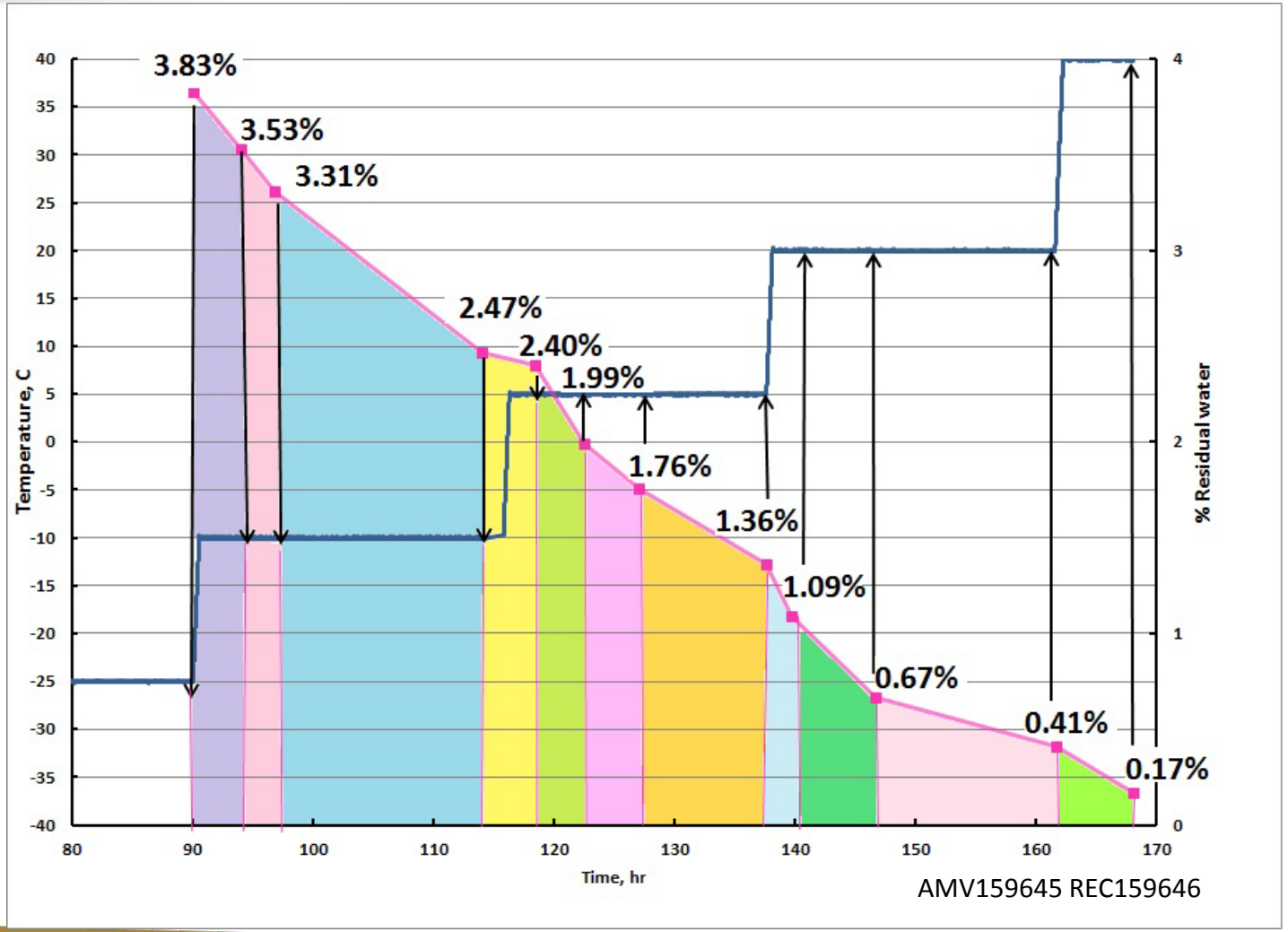
NIR

Water Content as Secondary Drying Progresses



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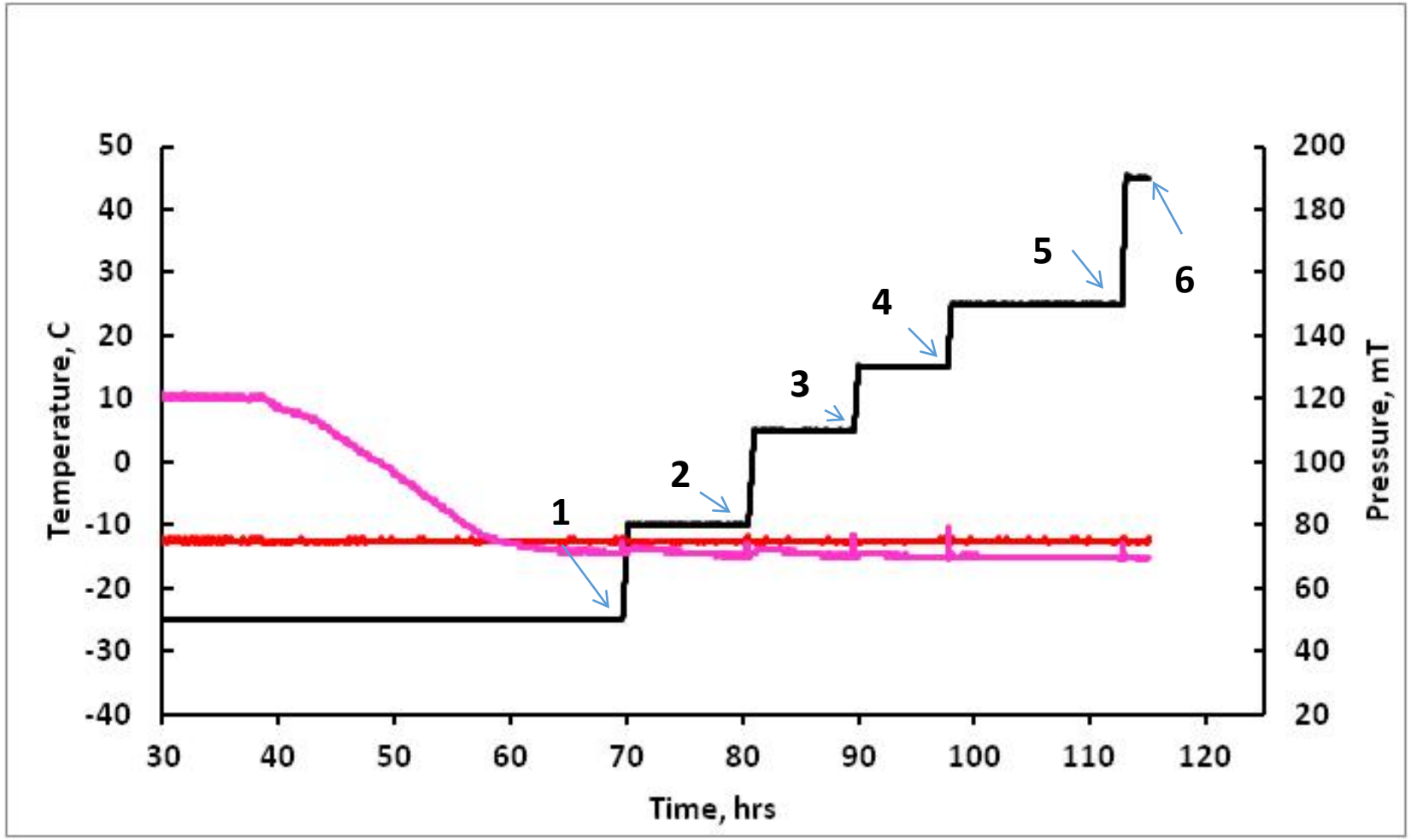
Example for an Antibody-Drug Conjugate



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Residual Moisture for Thief Samples

Sampling Point	Average H_2O	Range
1	4.79	4.46 – 5.36
2	3.10	2.90 – 3.34
3	1.91	1.48 – 2.20
4	0.96	0.85 – 1.17
5	0.27	0.27 – 0.41
6	0.17	0.08 – 0.19

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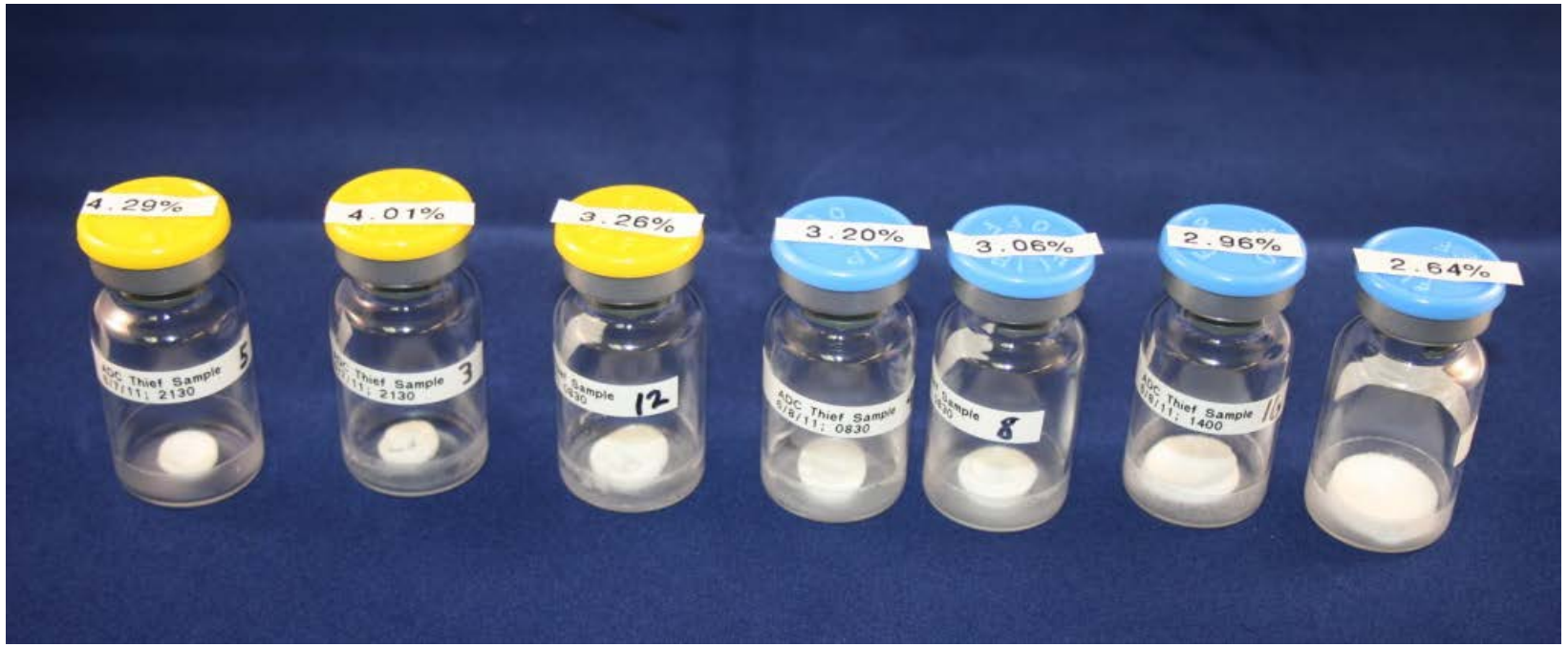
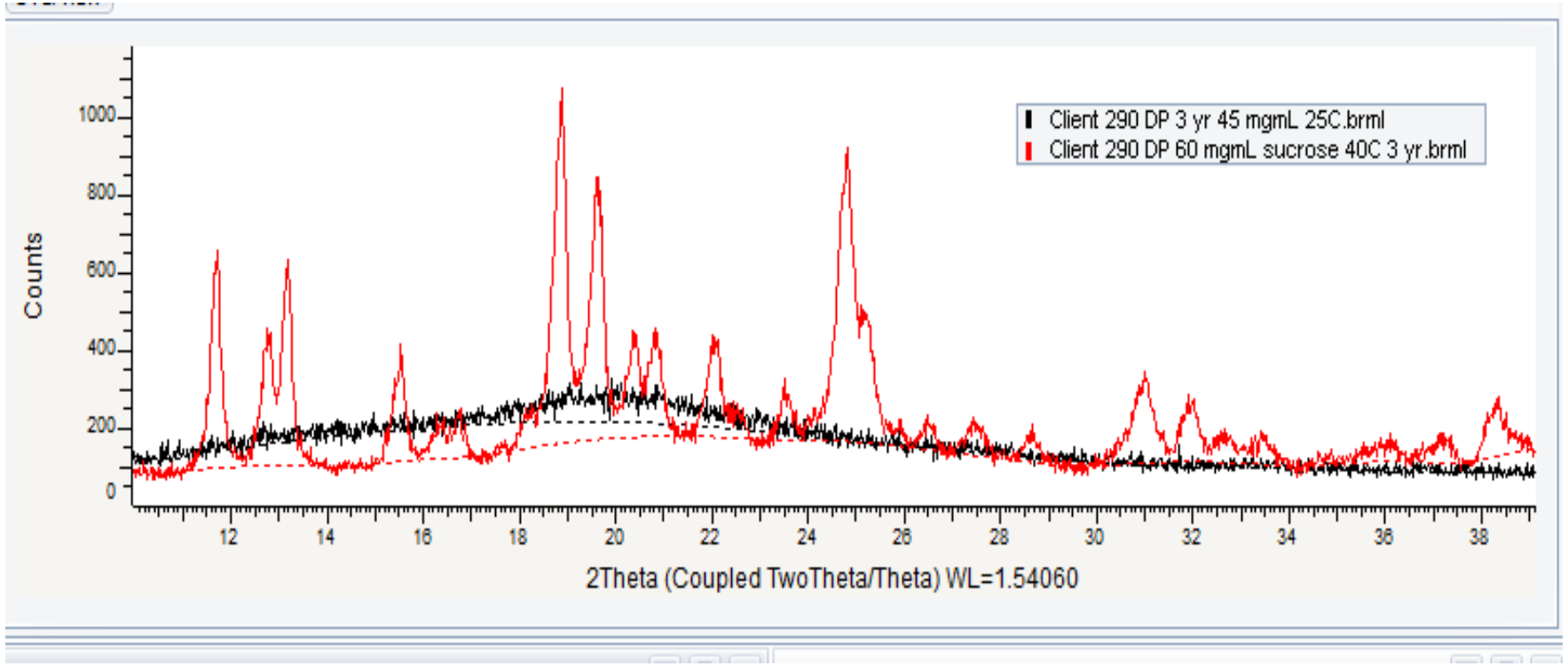
Various Levels of Collapse During Stressed Stability Testing at 50°C



X-Ray Powder Diffraction of Collapsed vs. Normal Cakes



Representative Stability Data

Sample	Recon Time	% H_2O	8 Days at 50°C	
			% Aggregate	% Fragment
Pre-Freeze-Dry			1.57	1.29
Initial FD	< 30 sec		1.58	1.20
Vial # 1	> 3 min	4.01	8.65	4.09
Vial # 2	> 3 min	3.68	1.89	1.36
Vial # 3	< 30 sec	2.96	1.70	1.25

- In this case, the physical stability of the cake and the chemical stability are connected. When the residual moisture is high enough that the cake collapses at the storage condition chosen, this results in crystallization of the sucrose, loss of protective activity, and relatively rapid aggregation of the protein. In this case, as long as the cake is physically stable, drug product stability appears to be independent of residual moisture level.

Some General Formulation "Rules"

- Minimize the amount of buffer
- Add other salts only if needed (for example, for solubility)
- Try to maximize Tg' for a robust freeze dry process. A crystallizing excipient, such as glycine or mannitol, can help as a physical stabilizer of the cake.
- Try to keep the protein concentration as high as practical
- Pay attention to residual moisture level. Generally speaking, dryer is more stable, but not always.