Effect of Processing Conditions on the Physical State of Solutes in Lyophilized Formulations

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References

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- **Sundaramurthi, P.**, and Suryanarayanan, R. **2011**. *Predicting the crystallization propensity of carboxylic acid buffers in frozen systems – relevance to freeze-drying*. **J Pham Sci. 100,** 1288-1293.
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- **Sundaramurthi, P.**, Patapoff, T.W., and Suryanarayanan, R. **2010**. *Trehalose crystallization in frozen solution and its phase transformation during drying*. **Pharm Res.** 27, 2384-2393.
- **Sundaramurthi, P.**, and Suryanarayanan, R. **2010**. *Influence of crystallizing and non-crystallizing additives on trehalose crystallization during freeze-drying.* **Pharm Res.** 27, 2374-2383.
- **Sundaramurthi, P.** Surayanarayanan, R. **2010**. *Calorimetric and diffractometric evidence for the sequential crystallization of buffer components and the consequent pH swing in frozen solutions.* **J Phys Chem B.** 114, 4915- 4923.
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- **Sundaramurthi, P.,** Shalaev E.Y., Suryanarayanan, R. **2010. '***pH swing' in frozen solution – consequence of sequential crystallization of buffer components.* **J Phys Chem Lett.**1, 265-268.
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Motivation

- Protein drugs and some small molecules
  - solution - chemically & physically unstable
  - frozen solution
  - freeze-dried solid - acceptable shelf life
- Frozen systems
  - highly concentrated and complex
  - not fully characterized
- Excipients - buffers and stabilizers
  - prevent protein denaturation
  - function specific solid-state

Function Specific Solid State

Ingredients	Common examples	Desired solid-state
Small molecules	Antibiotics, oncolytics	Crystalline
Proteins	-	Difficult to crystallize
Bulking agents	Mannitol, glycine	Crystalline
Buffers	Tris, sodium citrate	Amorphous
Lyoprotectants	Sucrose, trehalose	Amorphous

Freeze-Drying

Prelyo solution

Amorphous

Glass transition (Tg`)

Drying

T > Tg`

Cake collapse

Crystalline

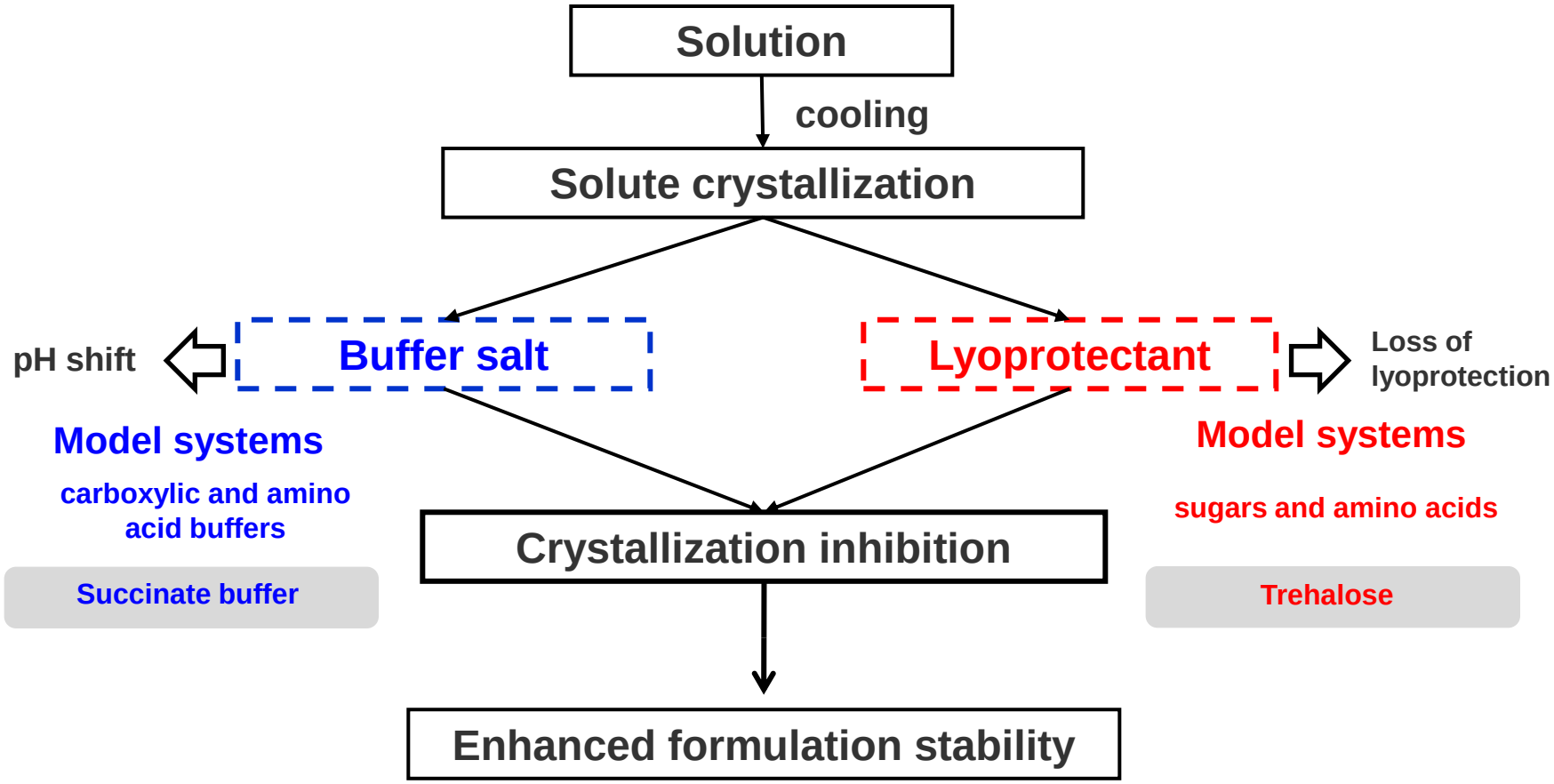
Eutectic (Te)

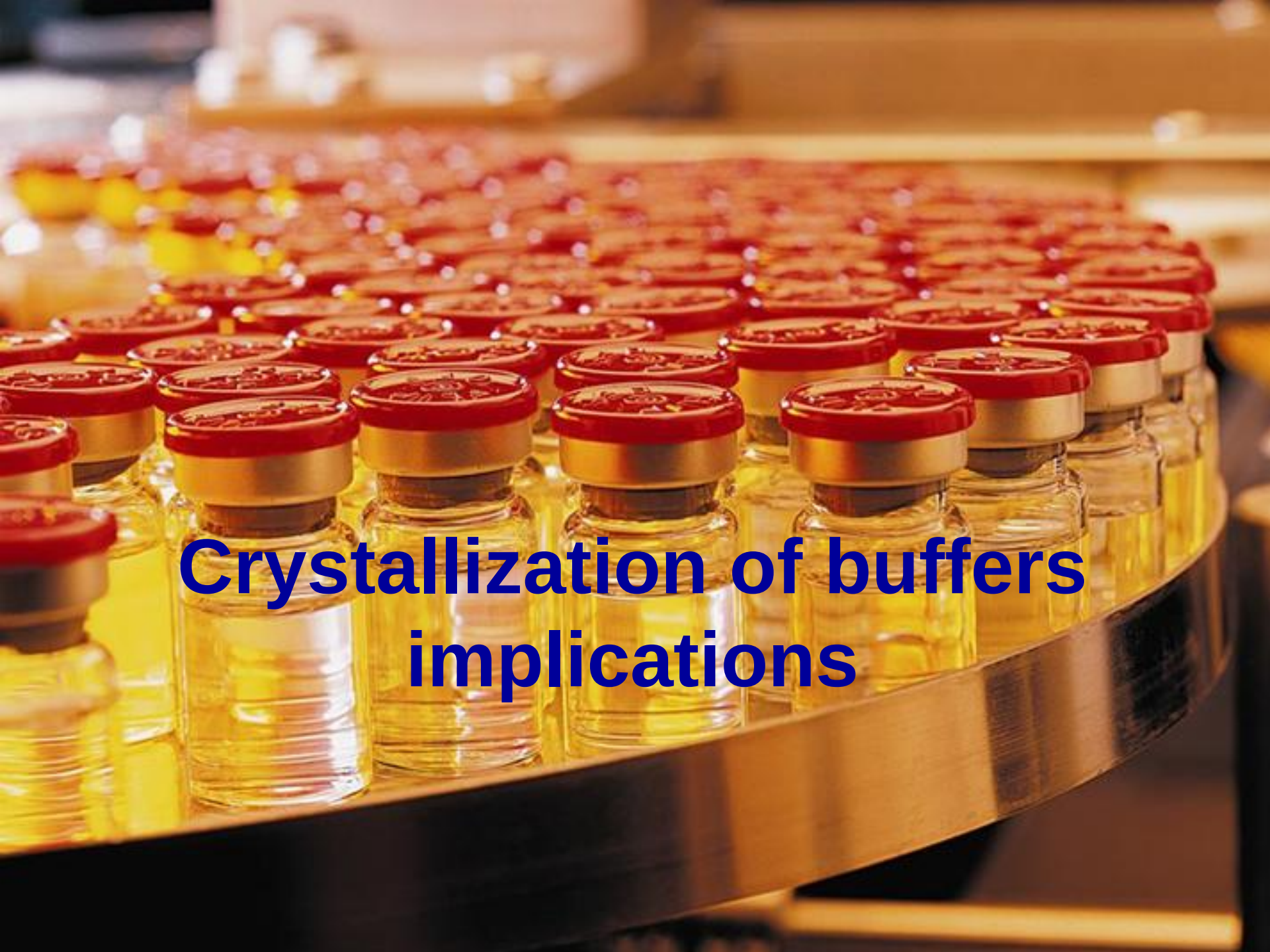
Drying

T > Te

Melt-back

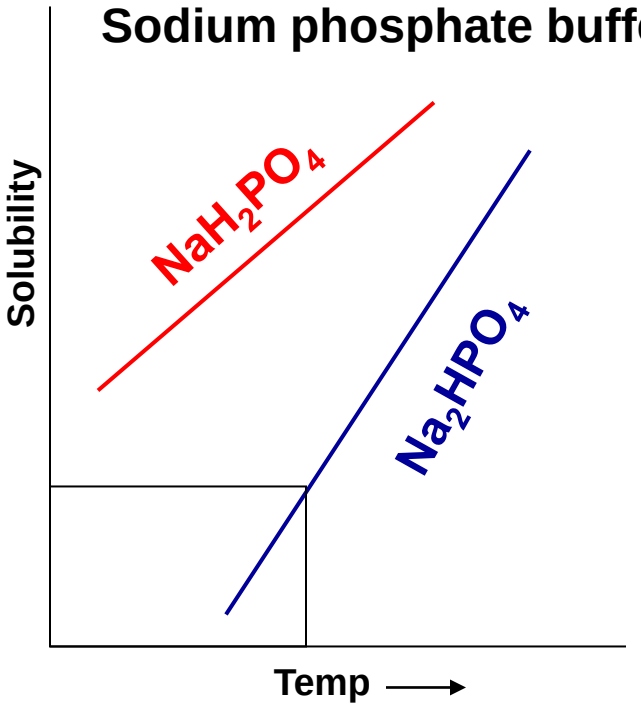
Presentation Overview

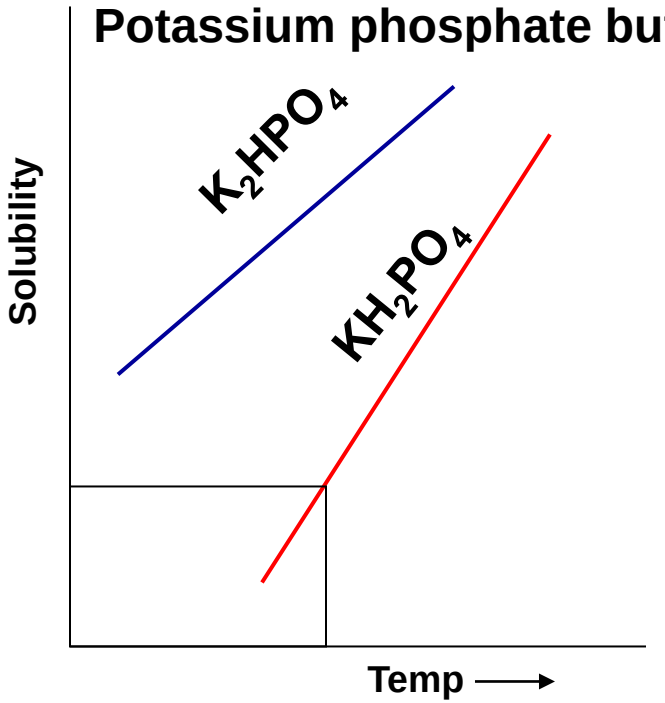




Crystallization of buffers implications

Buffer Crystallization Schematic





acid + salt of acid → buffer

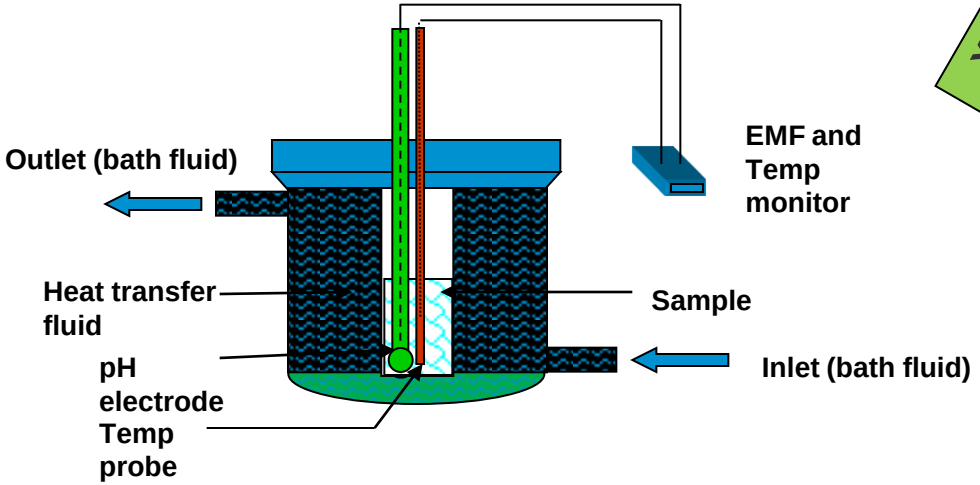
$NaH_2PO_4 + Na_2HPO_4$ → pH 7.4 → pH 3.5

$KH_2PO_4 + K_2HPO_4$ → pH 5.6 → pH 8.5

Methodology

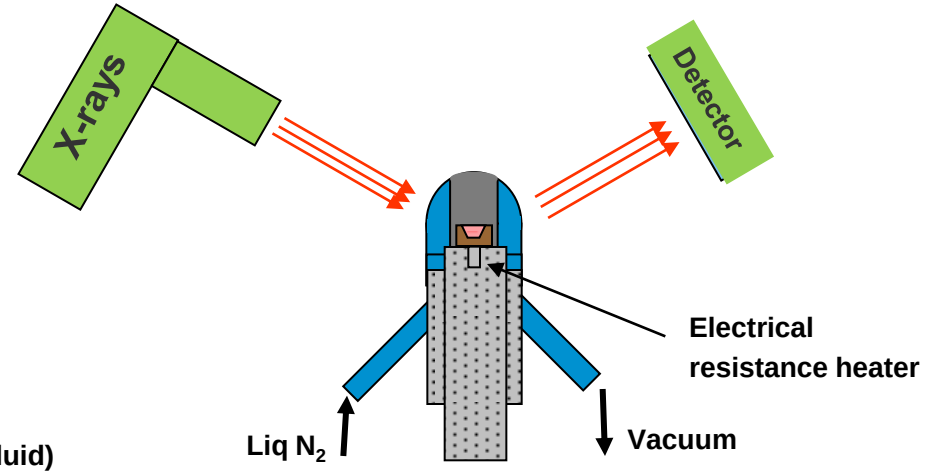
'*Simultaneous monitoring*' of pH and phases crystallizing in the frozen system

Low temperature pH measurements



Measuring the EMF

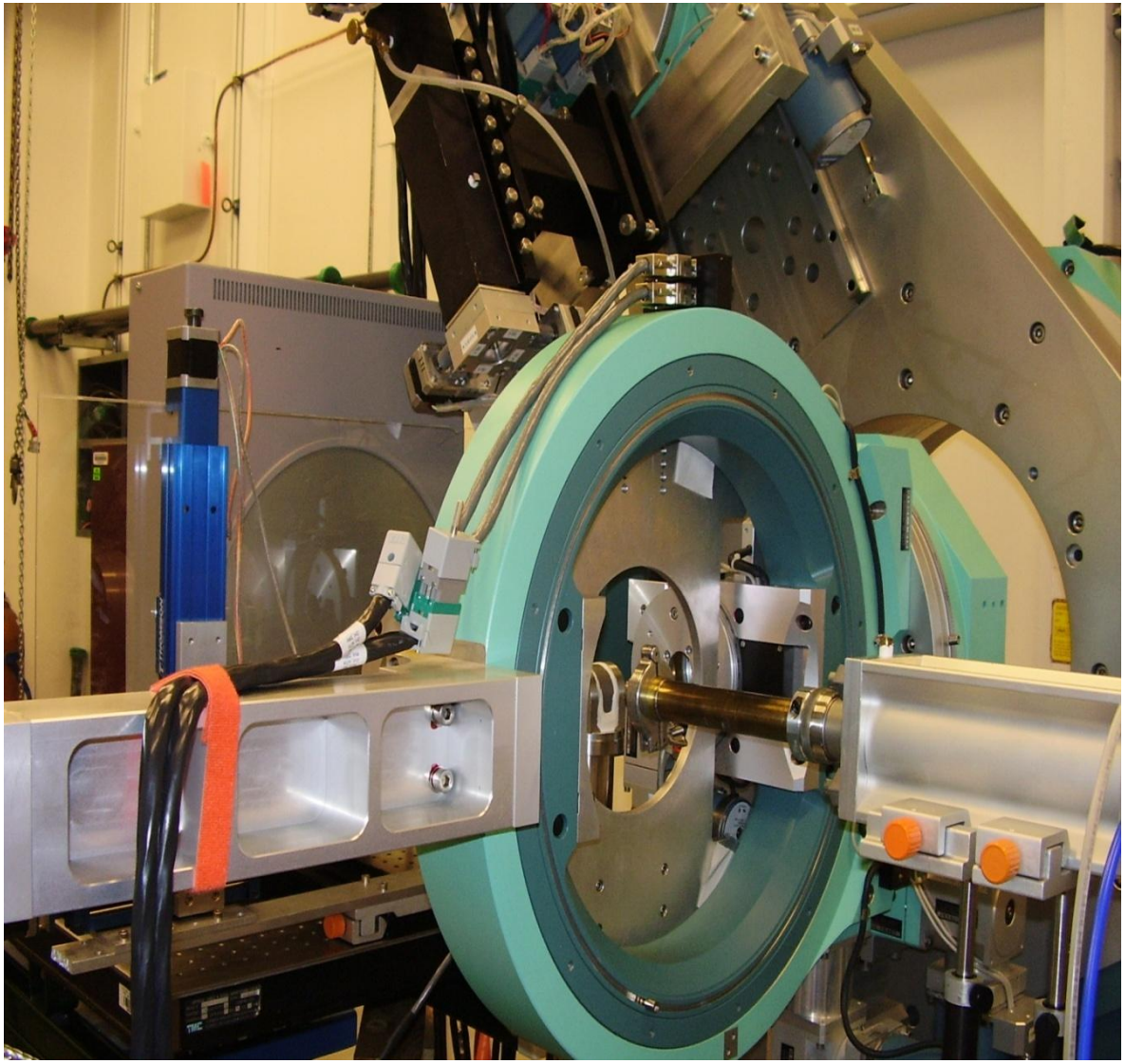
***In situ* freeze-drying in the XRD**



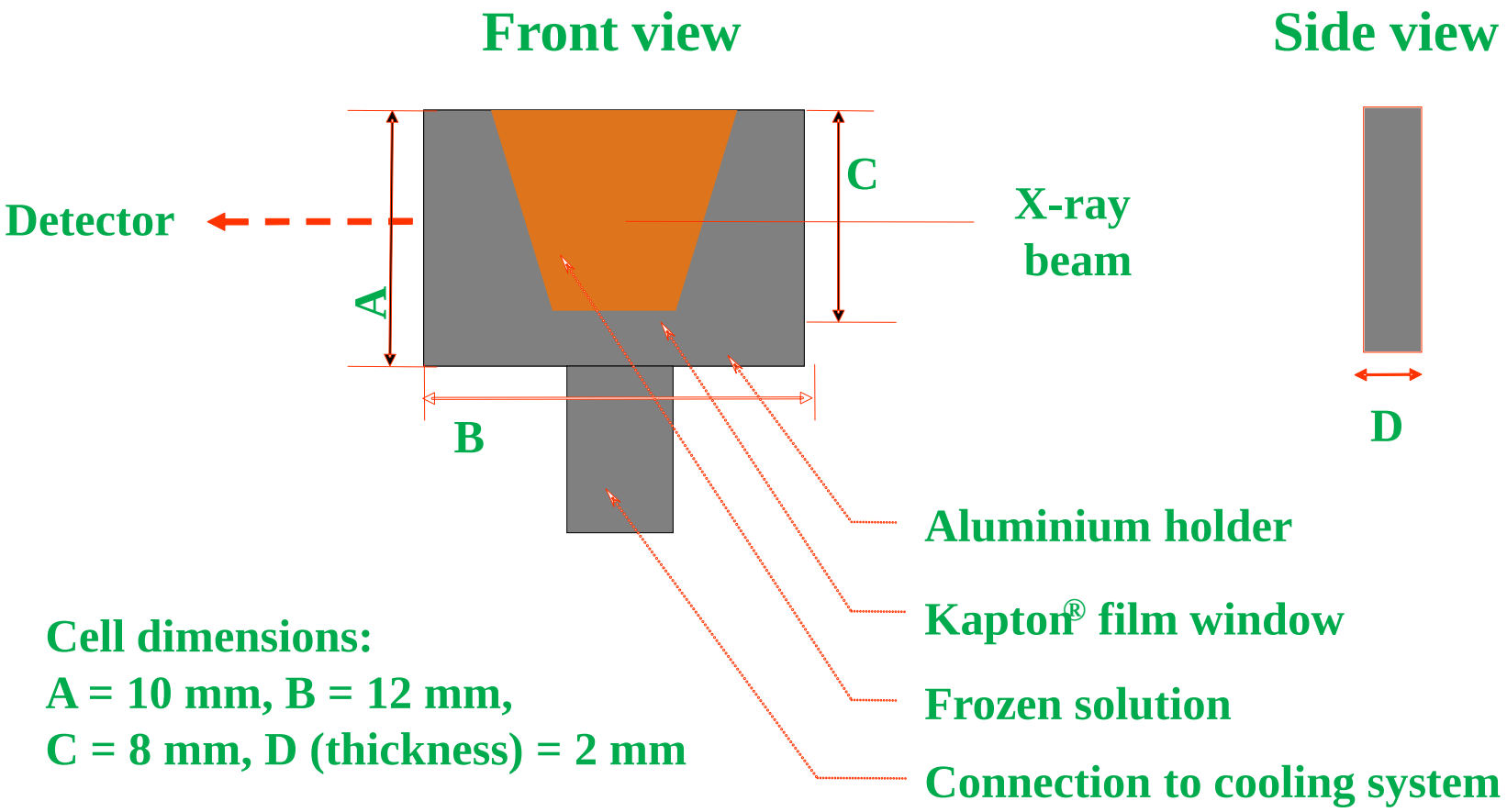
Bragg Brentano geometry (θ – θ) system

Advance Photon Source, Argonne National Lab

Sector 6

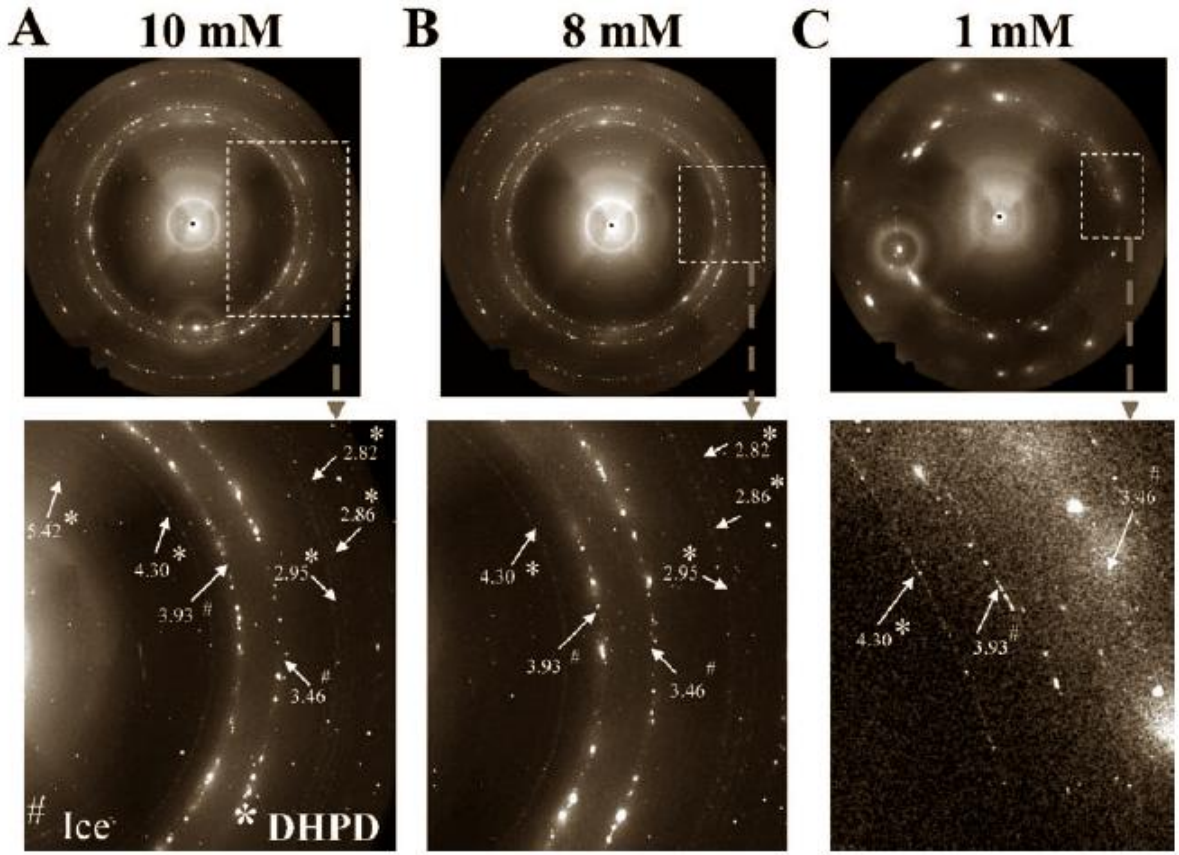


Synchrotron XRD – Sample Holder



Cell dimensions:
A = 10 mm, B = 12 mm,
C = 8 mm, D (thickness) = 2 mm

Sodium Phosphate Buffer

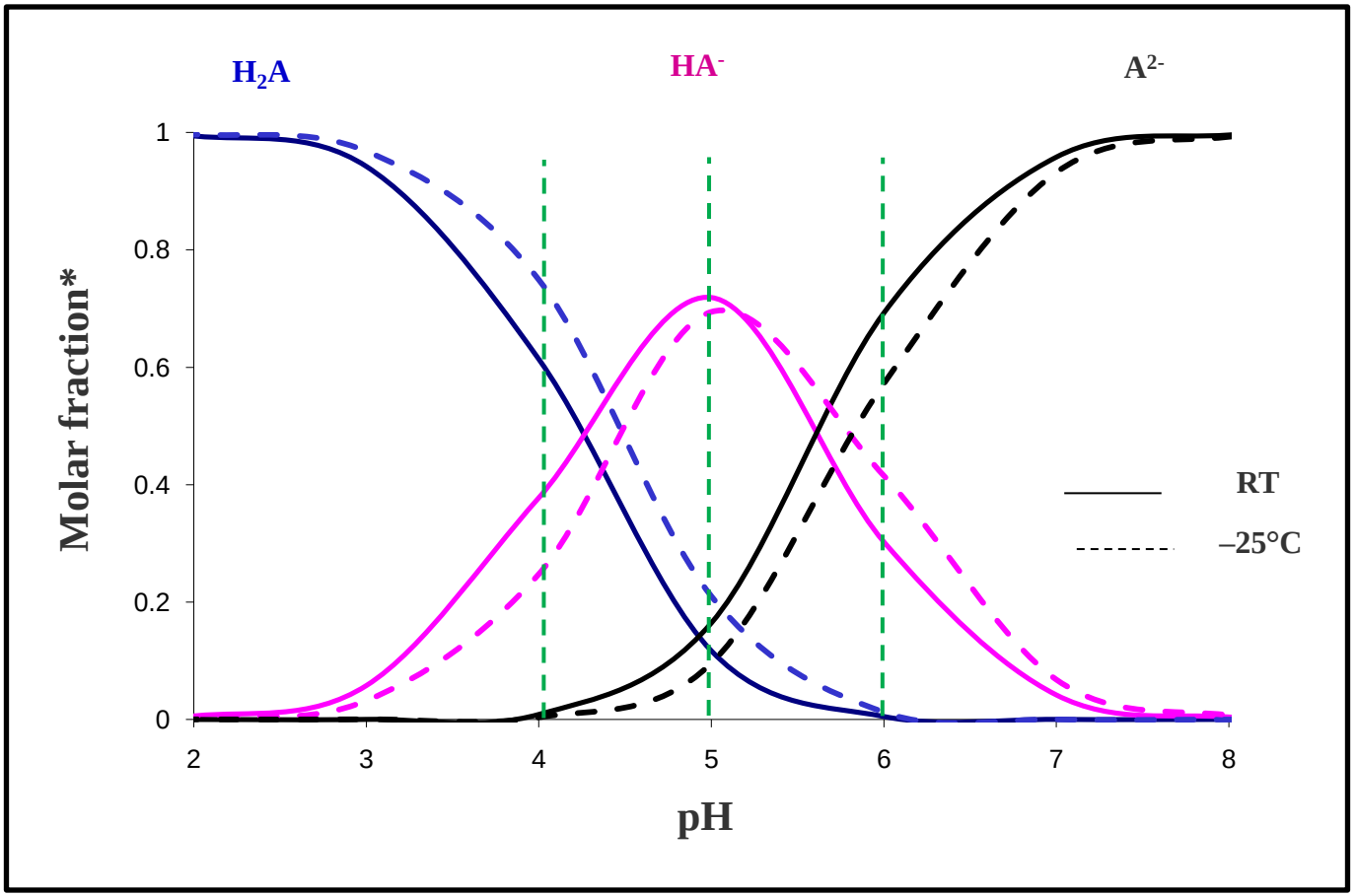


Use of synchrotron radiation to detect crystallization

Varshney et al, Pharm Res, 2006

Sequential Crystallization of buffers components – ‘pH swing’

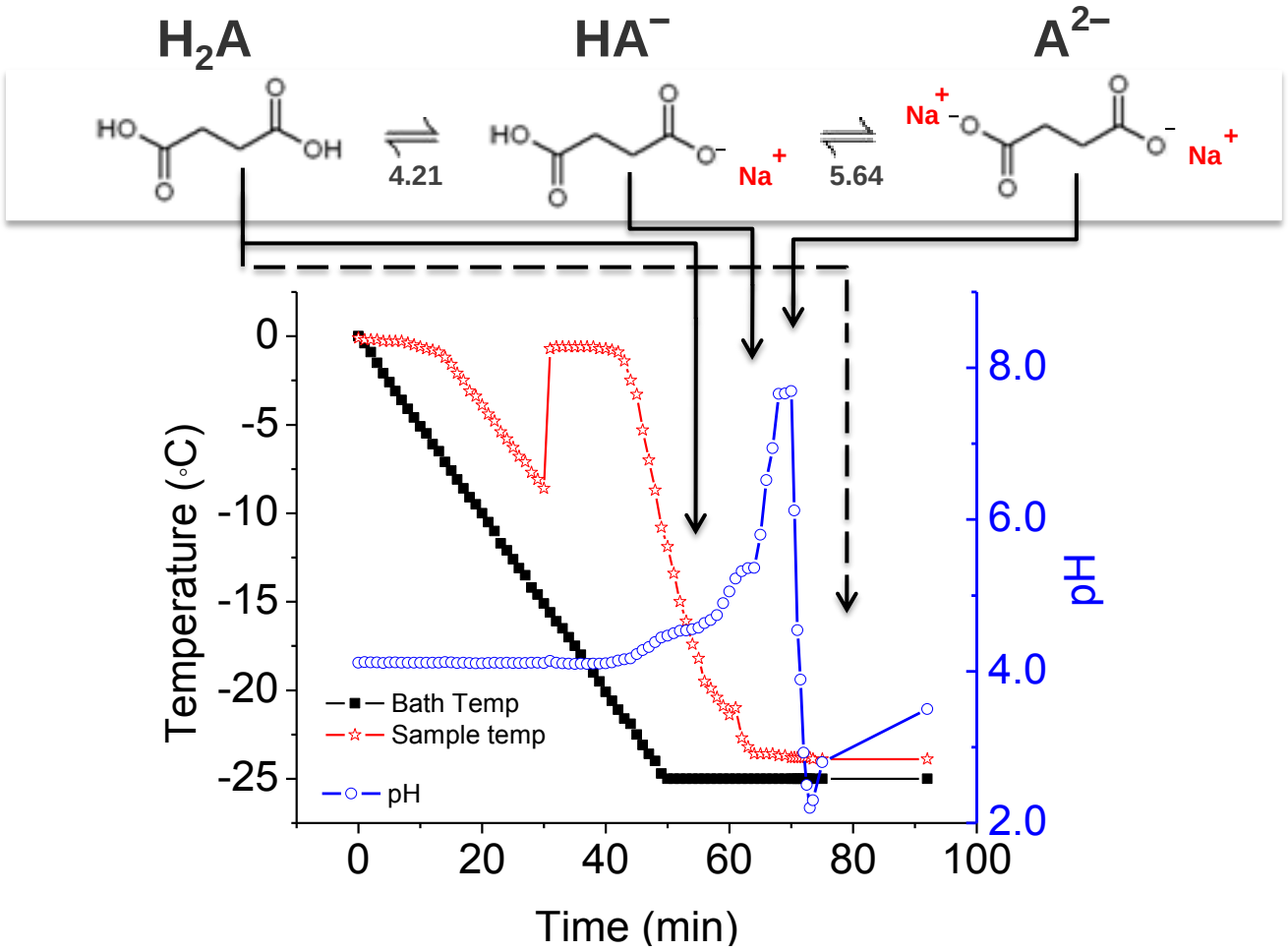
Succinate Buffer



Techniques Used in Frozen Systems

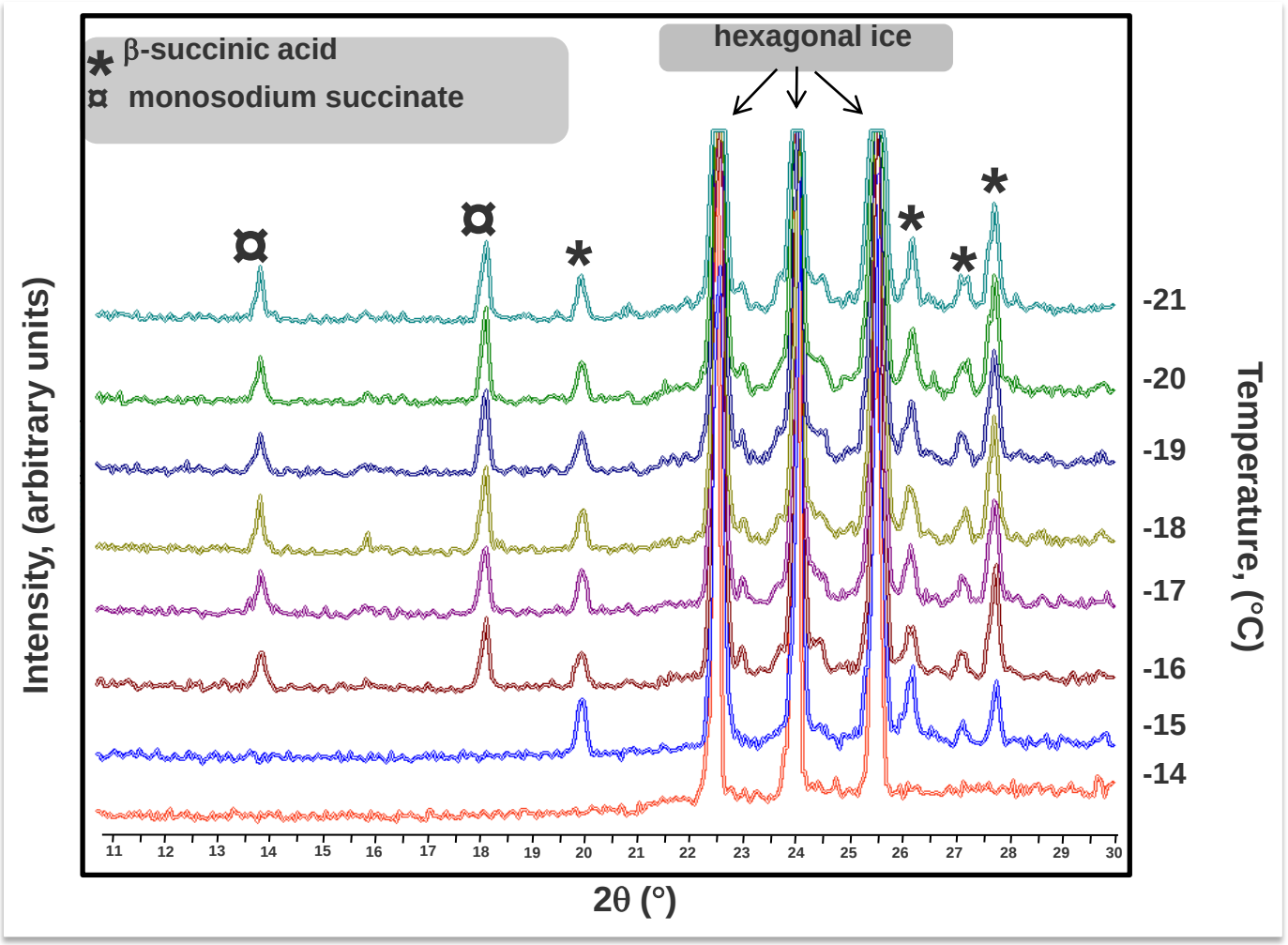
- Differential Scanning Calorimetry (DSC)
  - Limitations
    - Complex to interpret
    - Overlapping thermal events - ice and eutectic melting
- X-ray Diffractometry (XRD)
  - Limitations
    - Laboratory source – lacks sensitivity
    - Synchrotron source
- Low-temperature pH measurements

Low-temp pH Measurements



Sundaramurthi et al., 2010. J. Phys. Chem. Lett.

Low-temp XRD


β-succinic acid
monosodium succinate
hexagonal ice
Intensity, (arbitrary units)
2θ (°)
Temperature, (°C)
-21
-20
-19
-18
-17
-16
-15
-14
11
12
13
14
15
16
17
18
19
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21
22
23
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25
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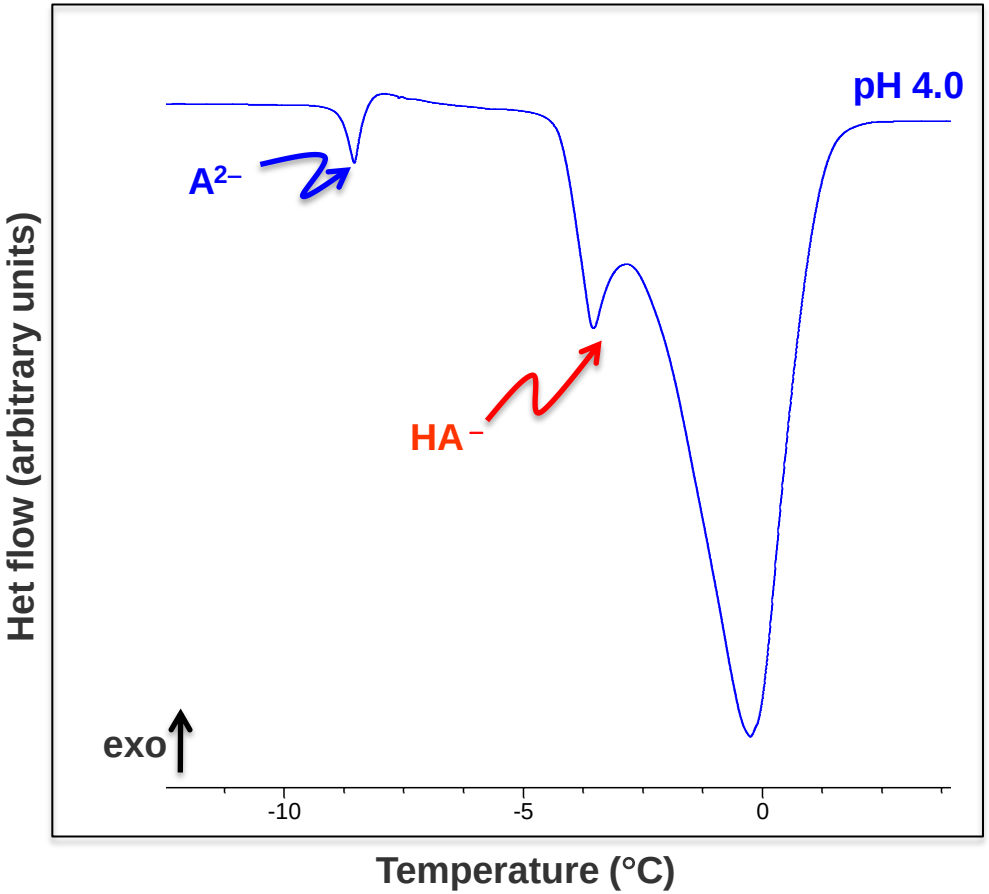
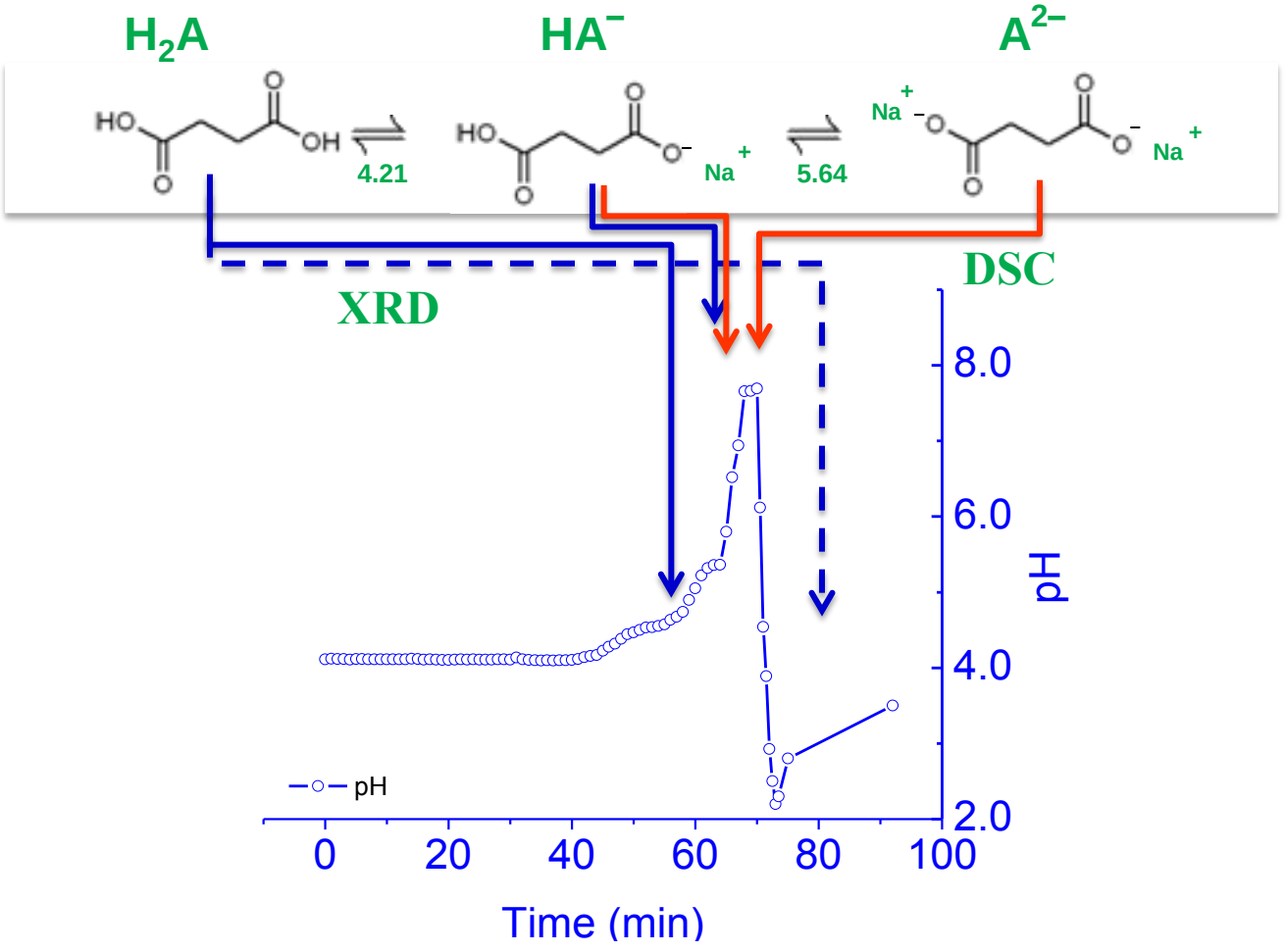
27
28
29
30

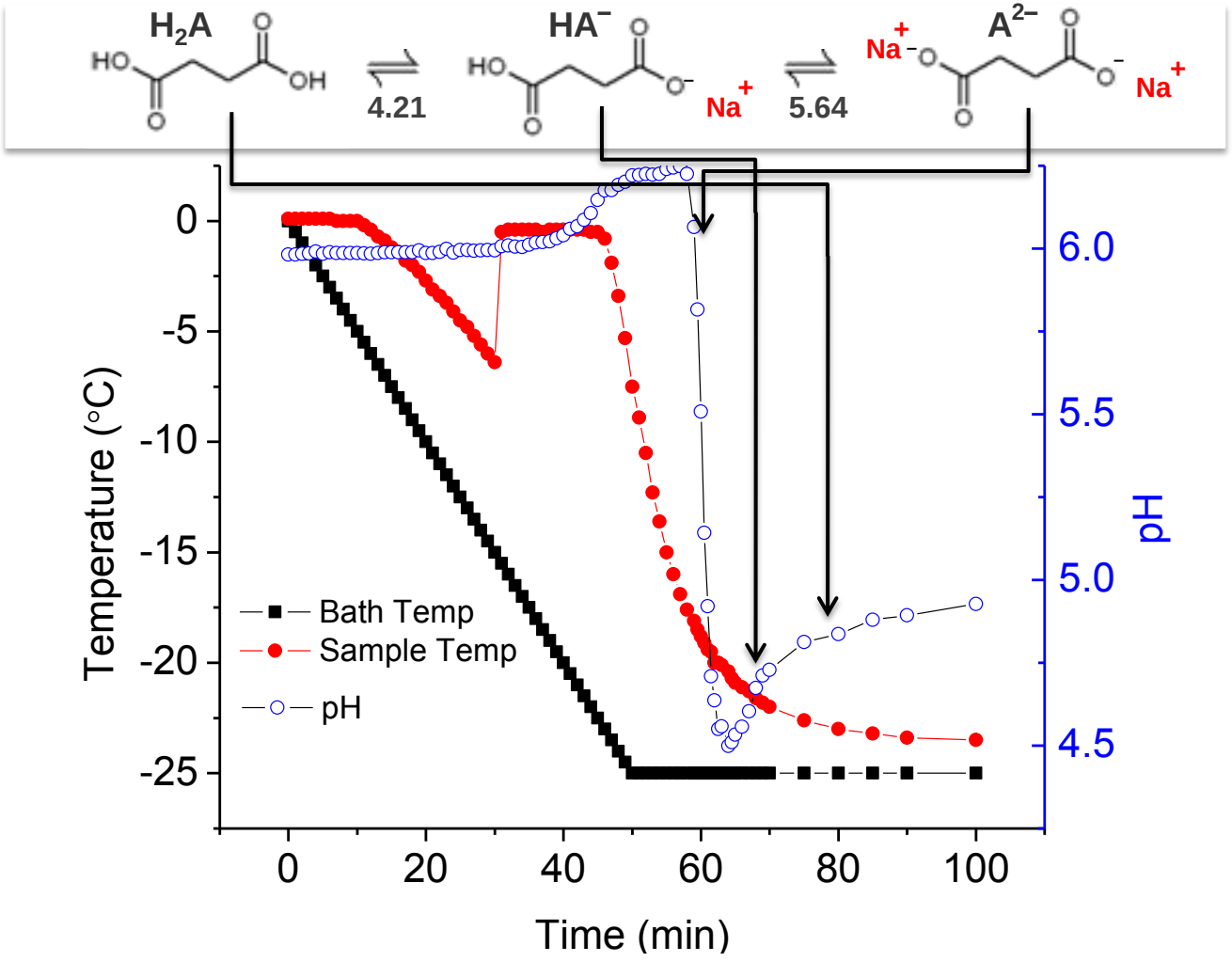
DSC Heating Curve



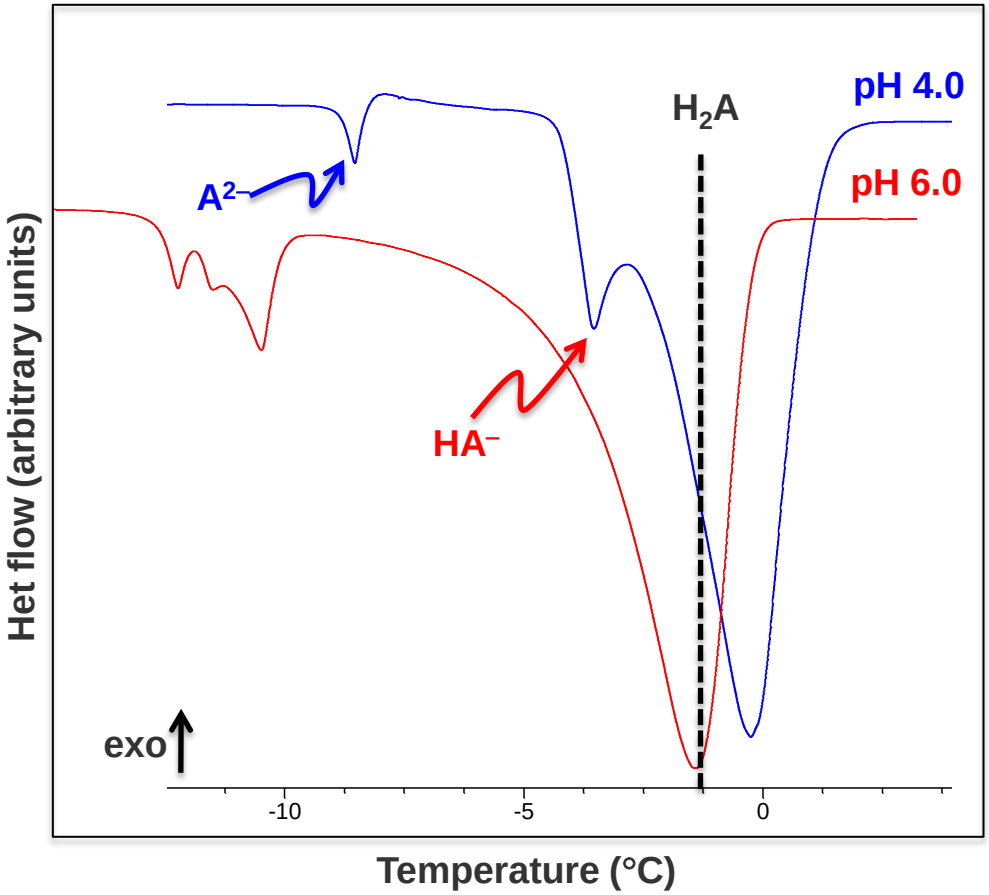
Low-temp pH Measurements



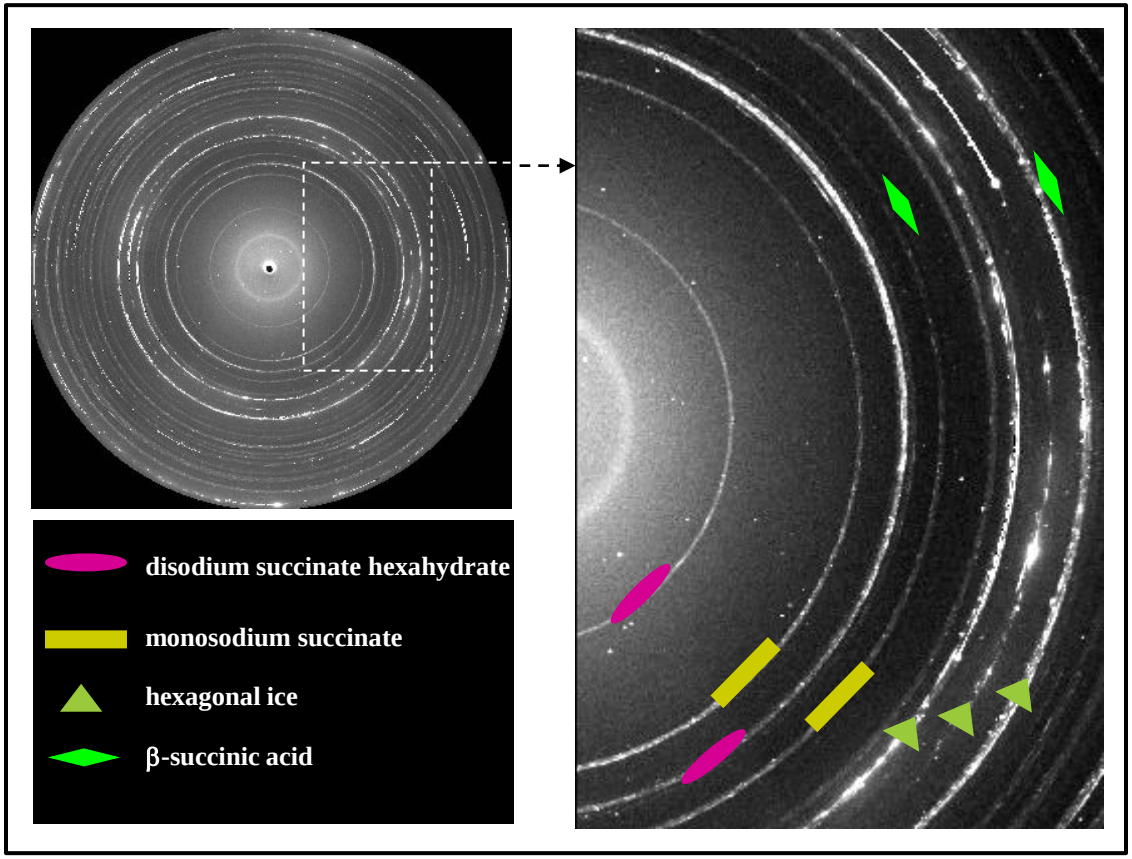
Low-temp pH Measurements



DSC Heating Curve

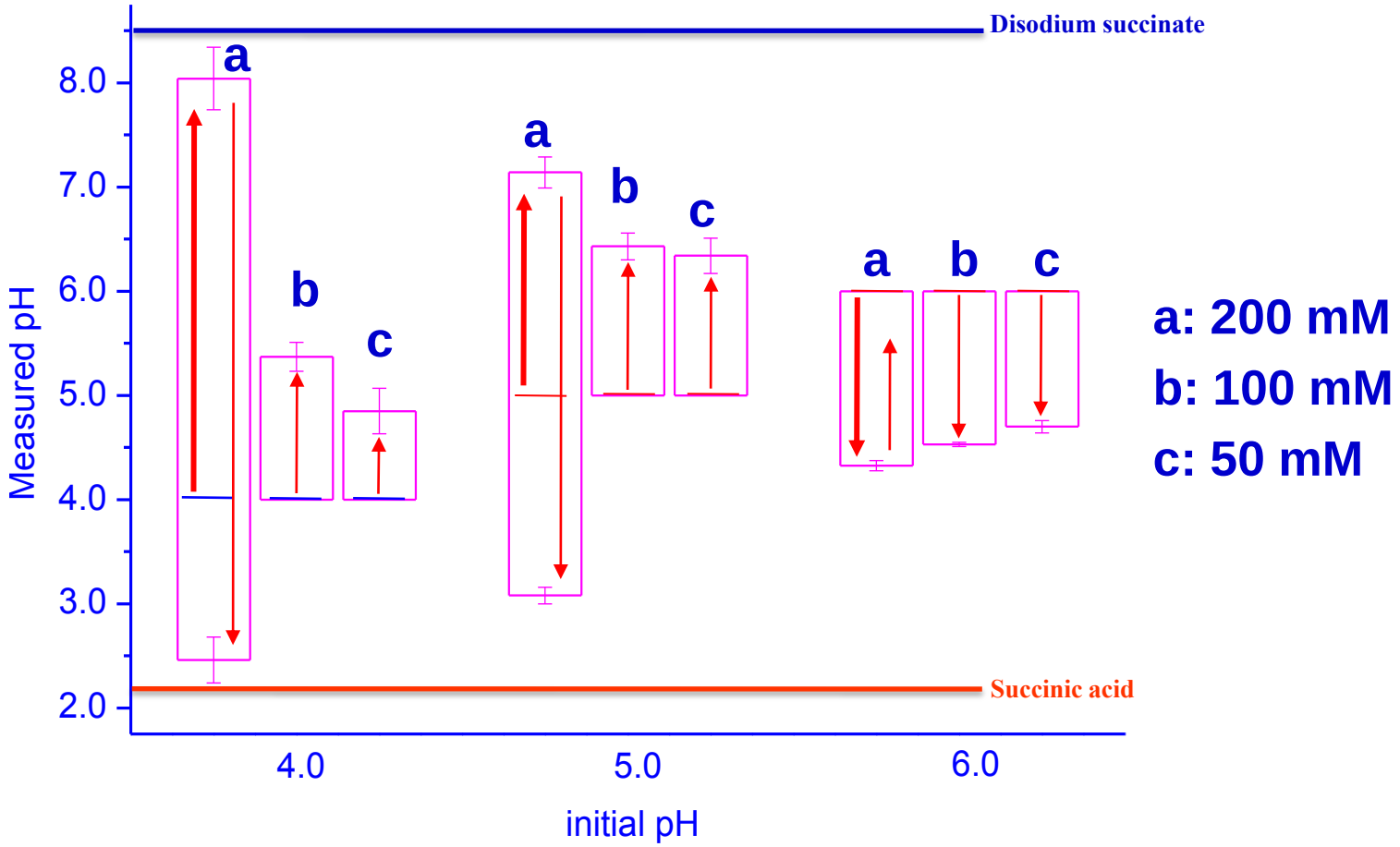


Low-temp Synchrotron XRD

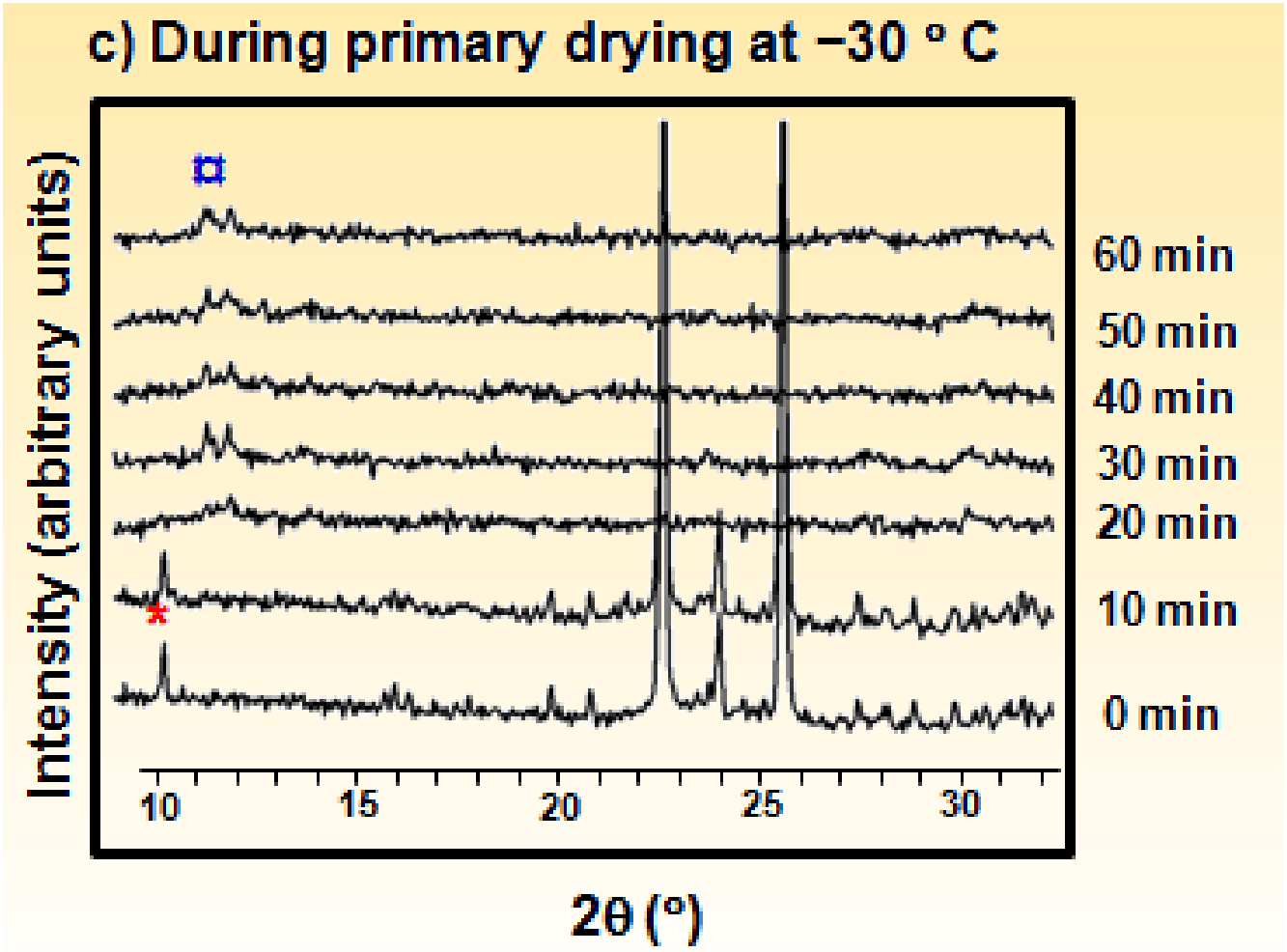


at -25 ºC

Frozen Solution pH Shift



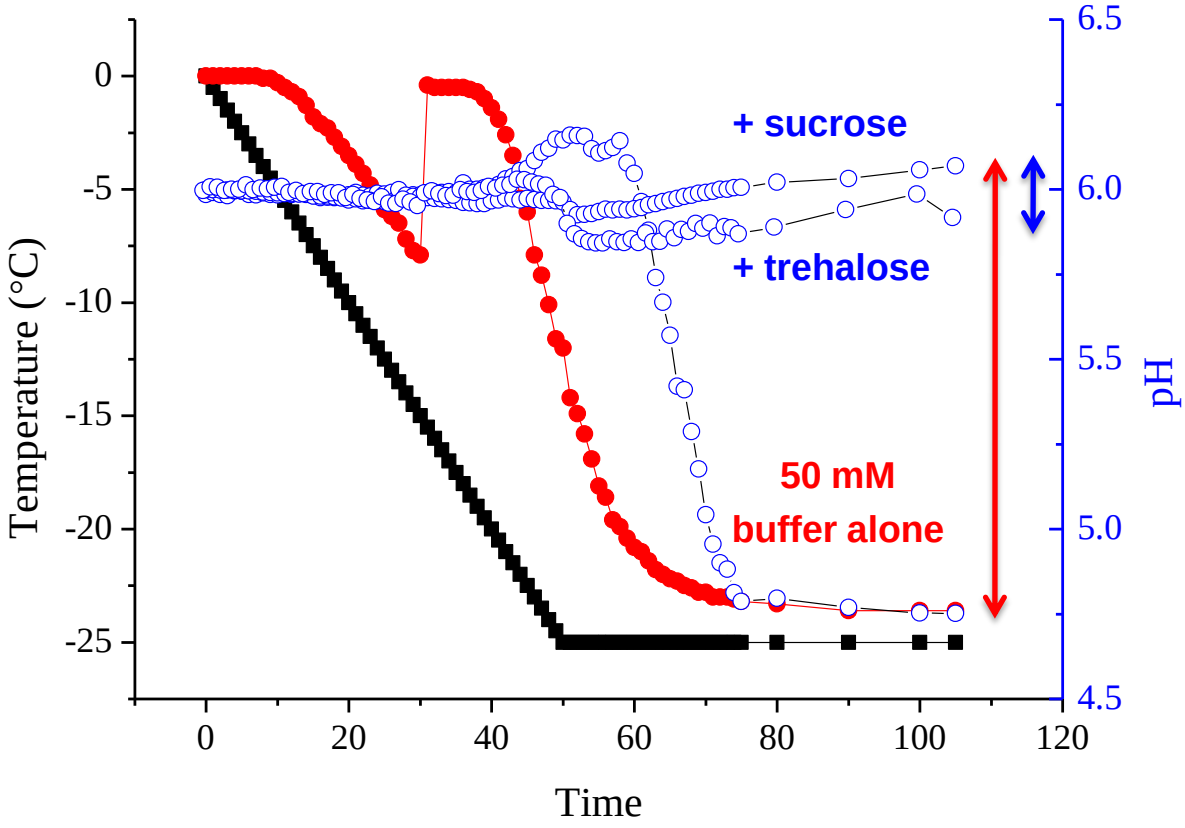
Phase Transformation During Drying



* disodium succinate hexahydrate

¤ disodium succinate anhydrate

Buffer Crystallization Inhibition



Lyoprotectant effectively inhibited buffer crystallization

Summary

- Buffer systems can exhibit “pH swing”
- Initial buffer concentration and pH
- Buffer concentration as low as possible
- Effect of noncrystallizing solute
- Potential additional role of lyoprotectant


Lyoprotectant
Crystallization

Background

- Lyoprotectants
  - prevent protein denaturation - freeze-drying and subsequent storage
  - to be effective – amorphous

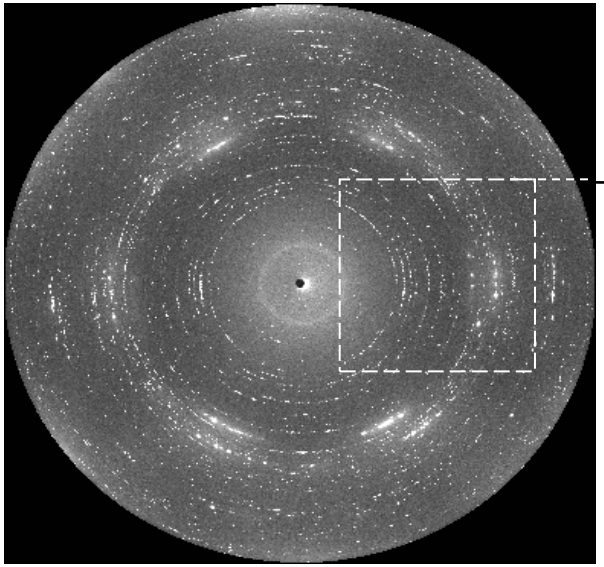
- Trehalose
  - commonly used lyoprotectant
  - Avastin™ (6% w/v), Herceptin® (2% w/v), Lucentis® (10% w/v)*

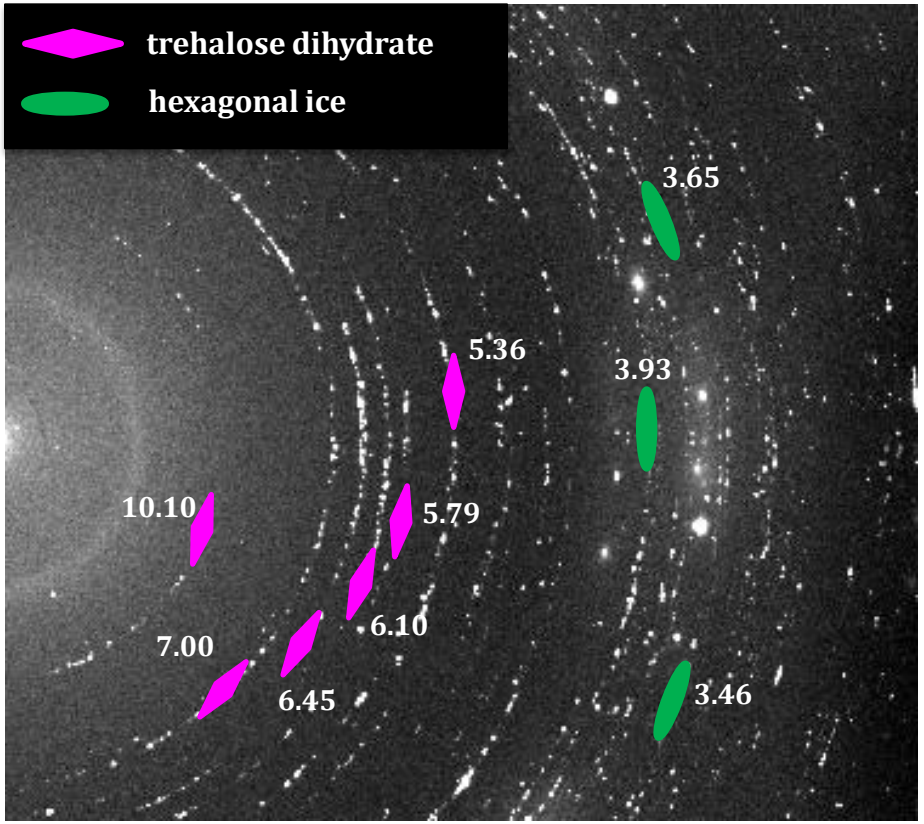
* Physicians Desk Reference, 2006

Trehalose Frozen Solution

4% trehalose

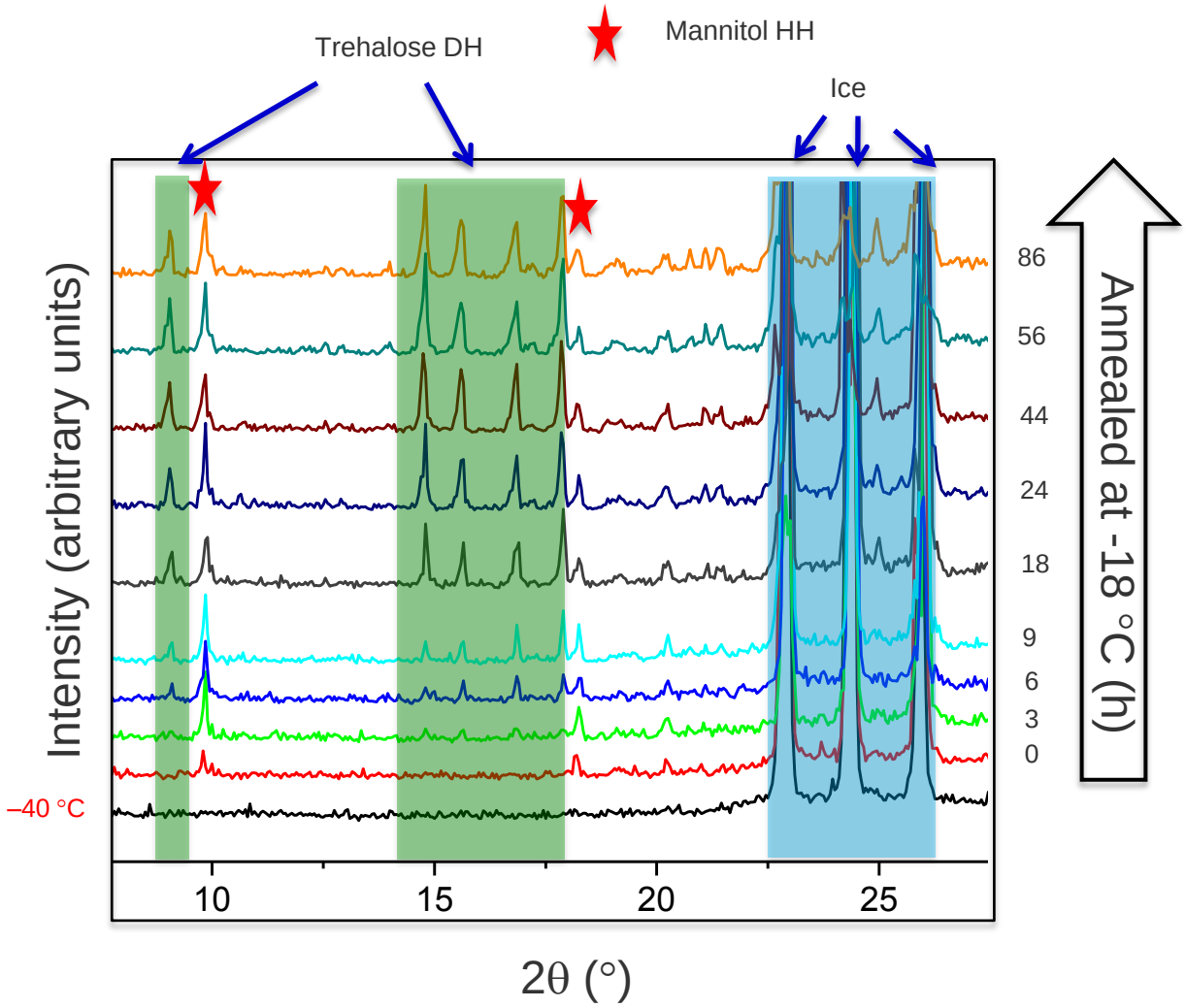
Annealed for 24 h



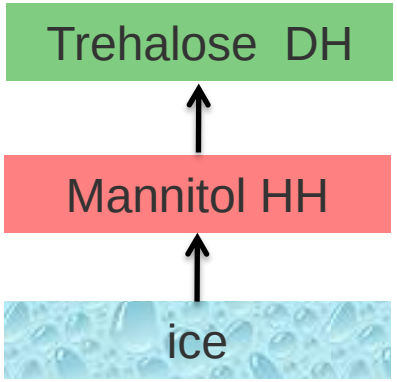


d-spacings, Å

Trehalose & Mannitol Frozen Solution

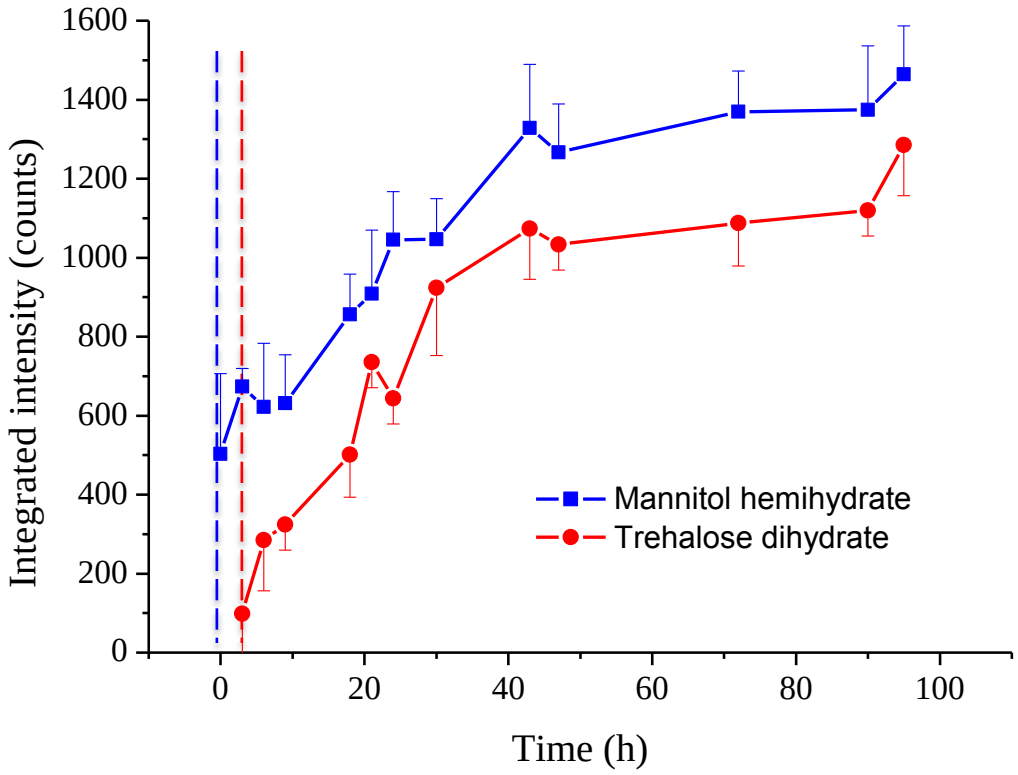


Trehalose (4% w/v)
Mannitol (2% w/v)



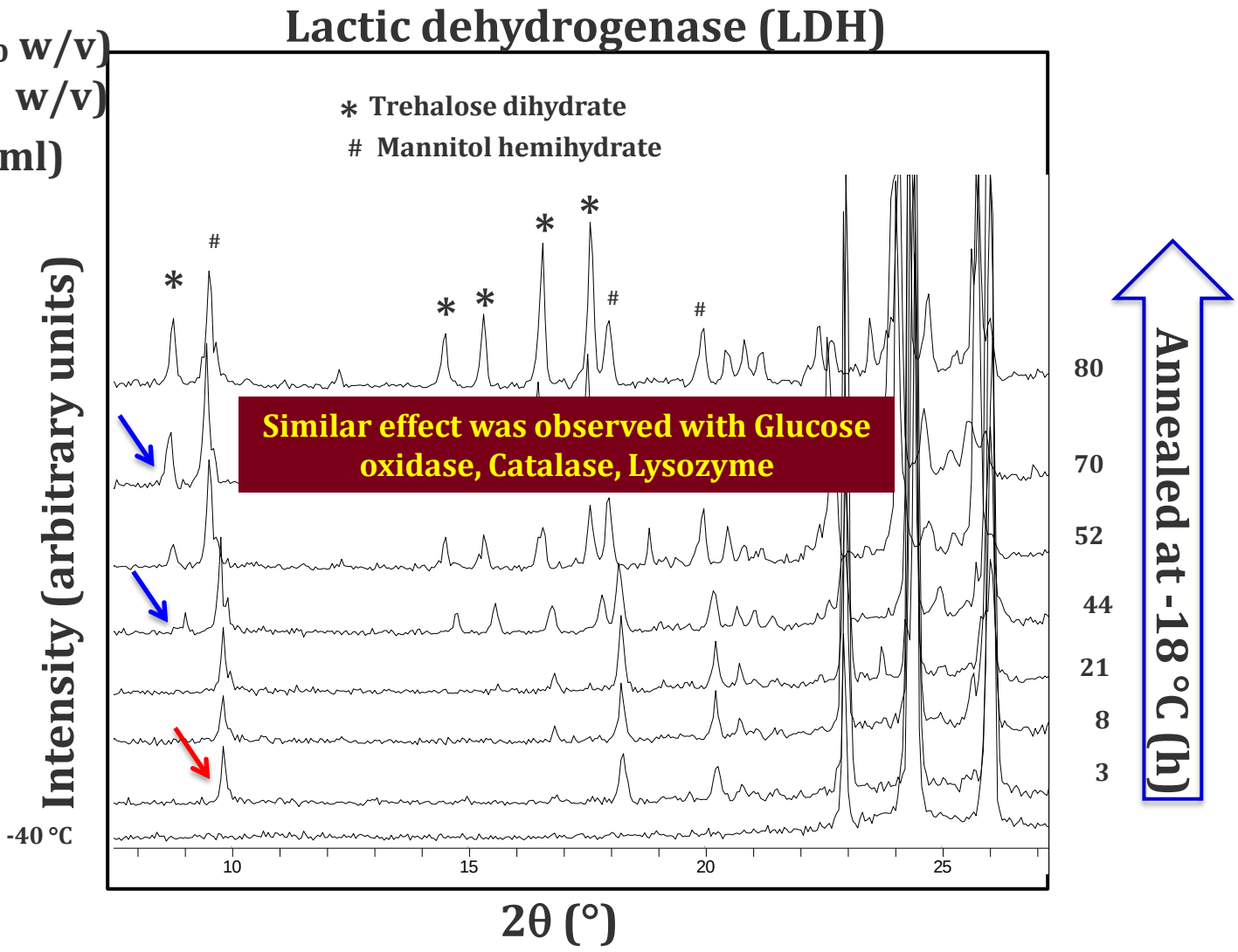
Trehalose & Mannitol Frozen Solution

Trehalose (4% w/v) and mannitol (2% w/v)



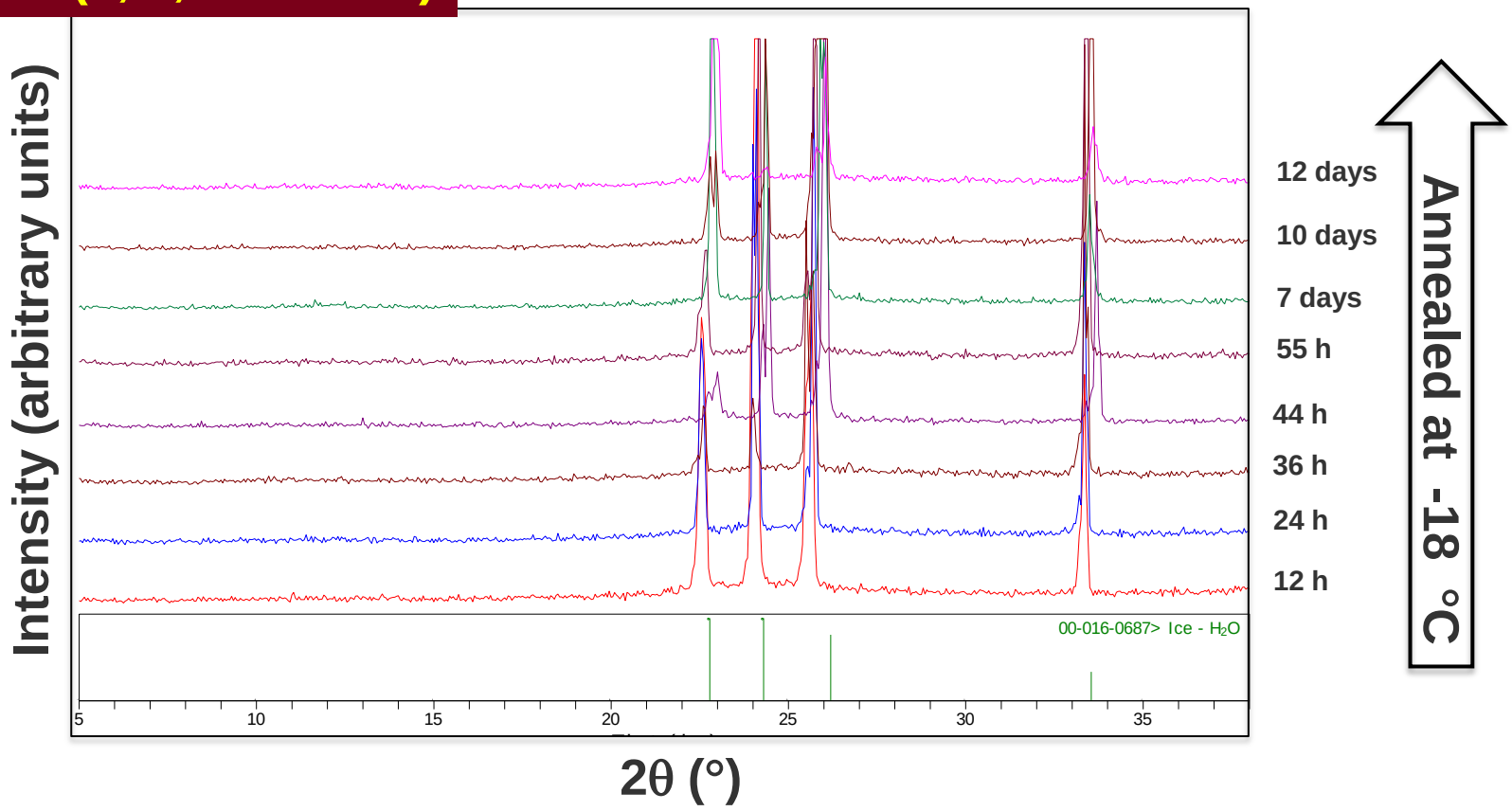
Effect of Protein

Trehalose (4% w/v)
mannitol (2% w/v)
LDH (2 mg/ml)



Effect of Sucrose

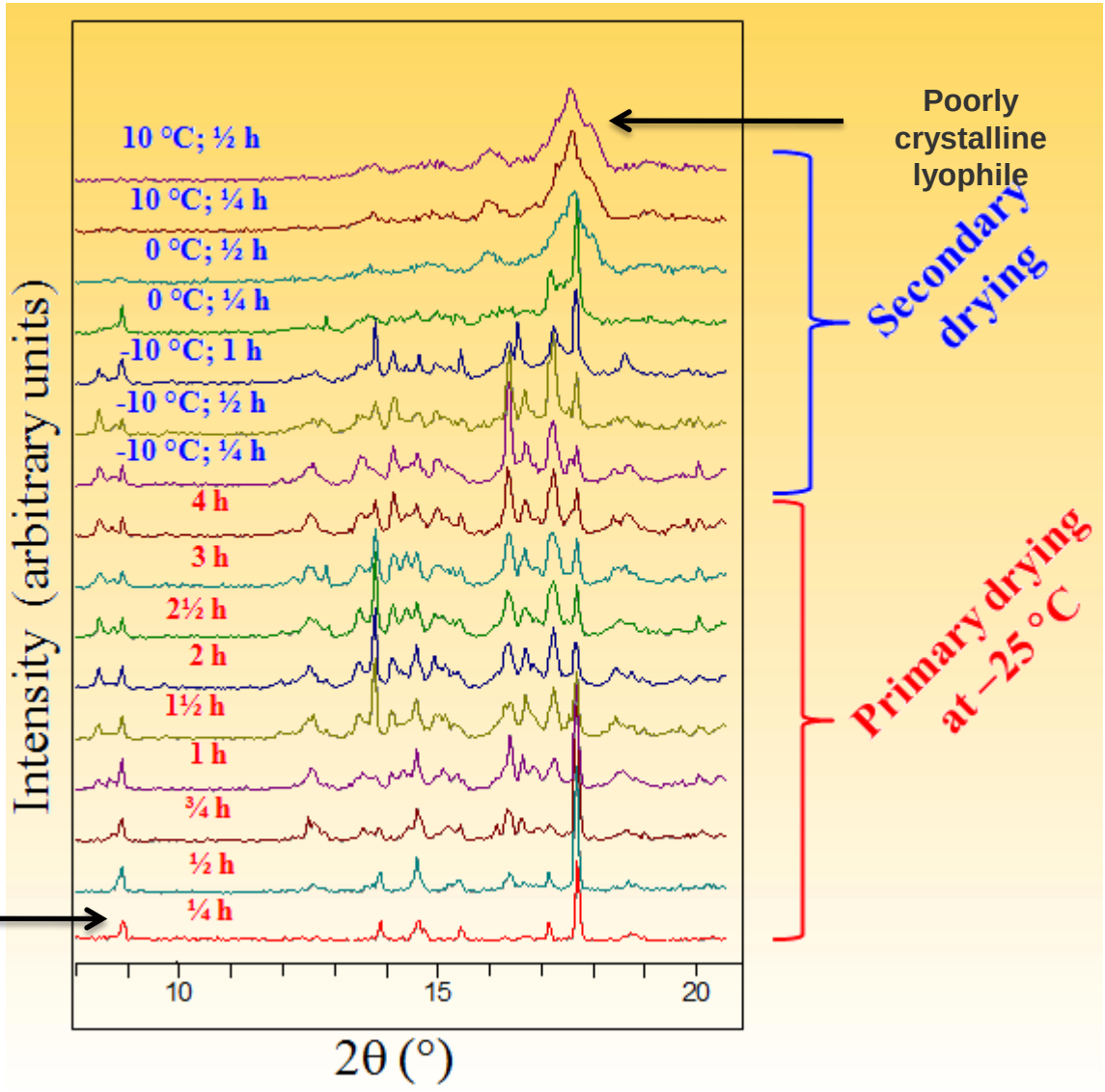
Trehalose (4% w/v)
Sucrose (2, 4, & 8% w/v)



Sucrose completely inhibited trehalose crystallization

Why NO reports of trehalose crystallization during lyophilization?

Phase Transformation During Drying



frozen solution

$(C_{12}H_{22}O_{11}\cdot 2H_2O)$

Crystalline

drying

lyophile

$(C_{12}H_{22}O_{11})$

Amorphous

"Both H-bonding and vitrification are necessary for lyoprotection"

- Crowe J. H. *et al.* 1998

Summary

- Trehalose dihydrate crystallized in frozen solutions. During drying, trehalose dihydrate dehydrated to a predominantly amorphous lyophile

  Lyoprotectant crystallized in model protein formulations - implications on protein stability

- Sucrose completely inhibited trehalose crystallization
- Mannitol accelerated trehalose dihydrate crystallization

Overall Summary

- Potential for complex and multiple phase transitions during freeze drying
  - Both process and formulation variables have profound impact on the physical state of solutes in frozen and freeze-dried product
- Analyzing the final lyophile can be misleading
  - Multiple approaches to characterize these complex systems are required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