Phase Transformations During Freeze-Drying: Potential Implications on Drug Product Performance

Raj Suryanarayanan (Sury), Ph.D.
University of Minnesota
Minneapolis, MN 55455
surya001@umn.edu

Acknowledgements

Graduate students and post-doctoral fellows

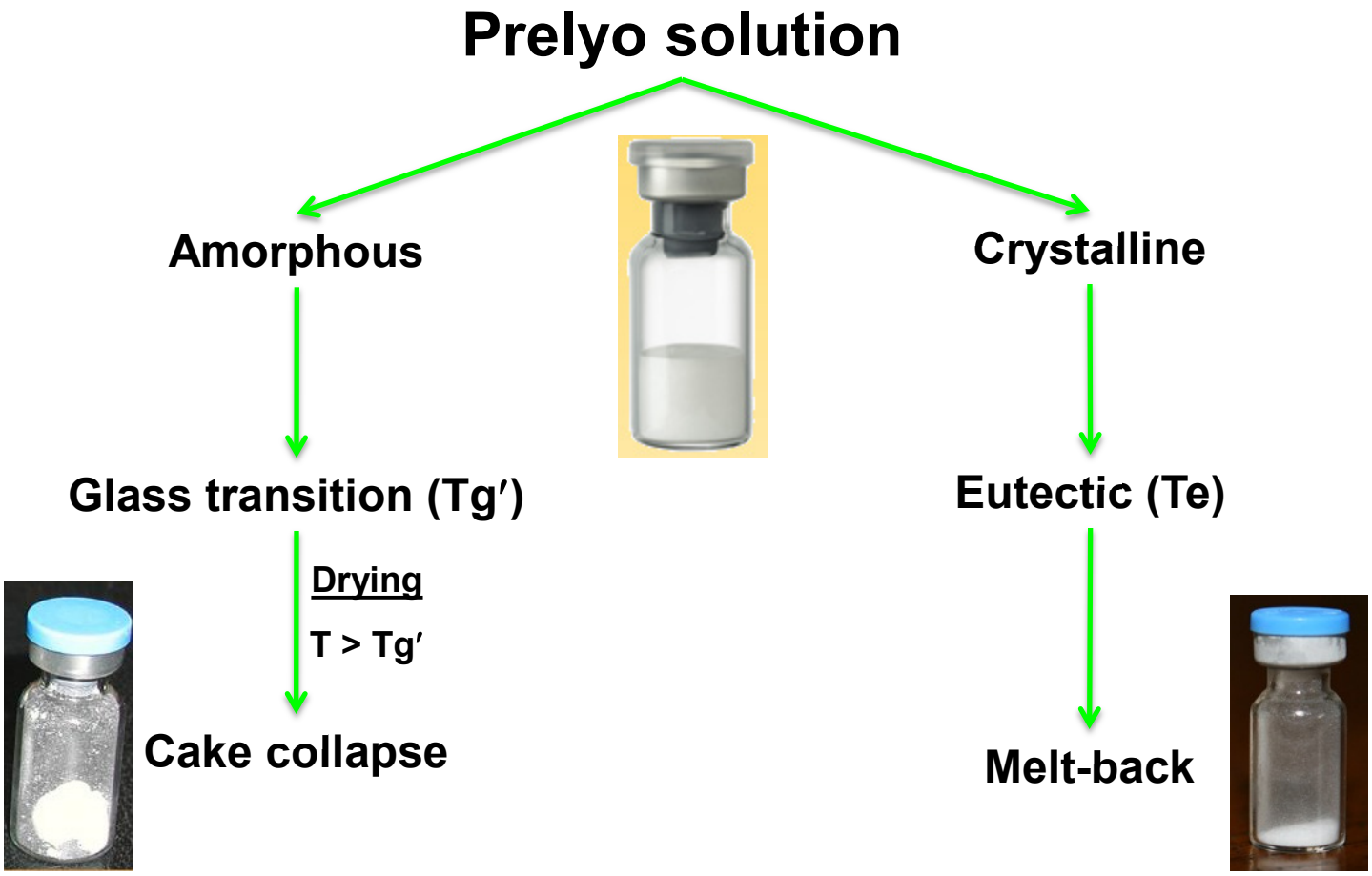
Prakash Sundaramurthi, Ph.D.
Dushyant Varshney, Ph.D.
Mehak Mehta, Ph.D.
Seema Thakral, Ph.D.
Sampada Koranne

Acknowledgements

Funding

William and Mildred Peters Endowment Fund
International Students Work Opportunities Program, University of Minnesota
US Department of Energy

Pharmaceutical freeze-drying



In situ phase transition

- Monitoring them can be a challenge but valuable
- Multiple analytical techniques may be needed
- Excipients – use judiciously; more is NOT better
- Potential for interaction between formulation components
  - Influence the physical form

Function-specific solid-state

Ingredient	Common examples	Desired solid-state
Small molecules	Antibiotics, oncolytics	Crystalline
Proteins	--	*Difficult to crystallize*
Bulking agents	Mannitol, glycine	Crystalline
Buffers	Phosphate, histidine, citrate	Amorphous
Lyoprotectants	Sucrose, trehalose	Amorphous

Case studies

- **Trehalose crystallization**
- Mannitol hemihydrate
- In situ salt formation
- Salt disproportionation

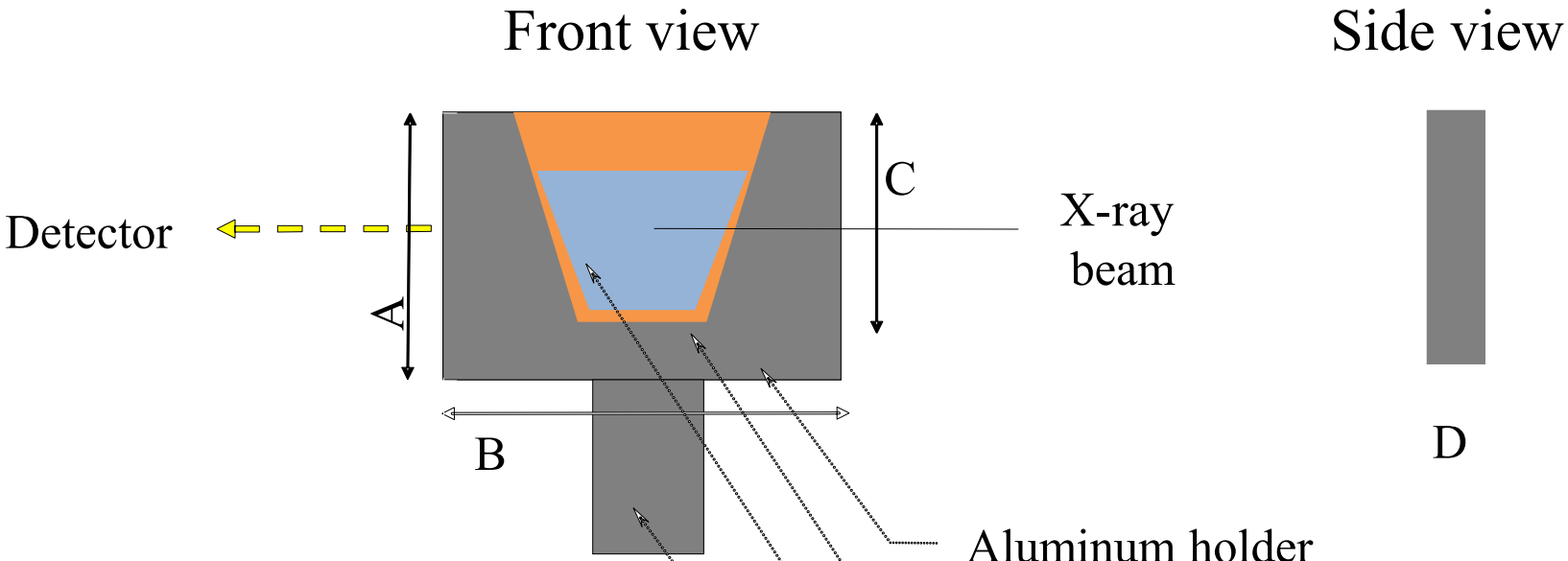
Protein stabilization

Lyoprotectant hydrogen bonds to protein – prevents denaturation ← **Conventional wisdom**

↓ Lyoprotectant crystallizes

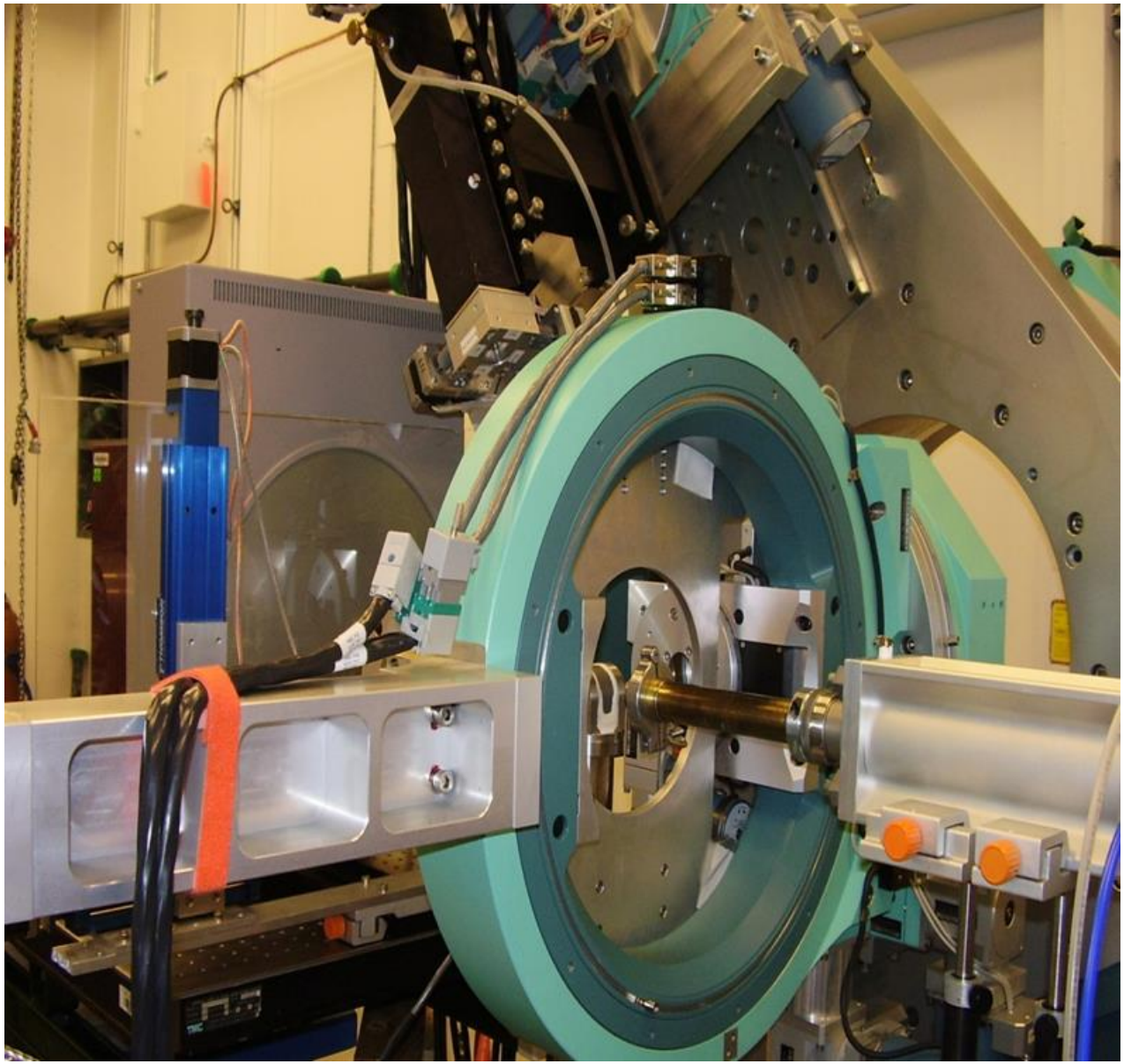
Ability to hydrogen bond with protein? → **Protein stable?**

www.anl.gov



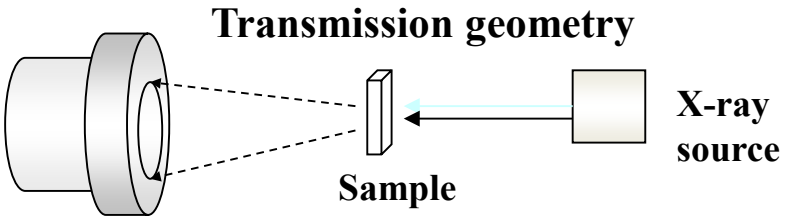
Cell dimensions:
A = 10 mm, B = 12 mm,
C = 8 mm, D (thickness) = 2 mm

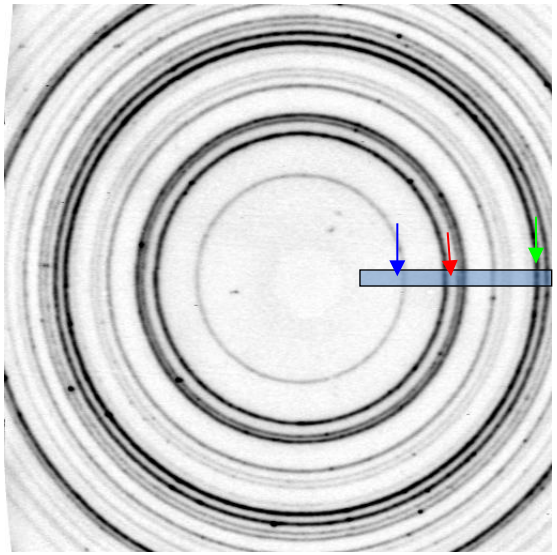
Varshney et al, Pharm Res, 2009



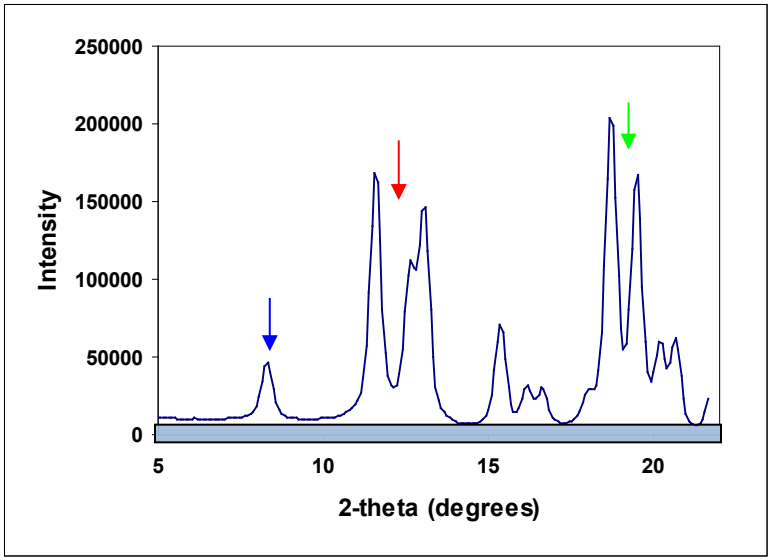
Varshney et al, Pharm Res, 2009

2-Dimensional XRD using high intensity sources





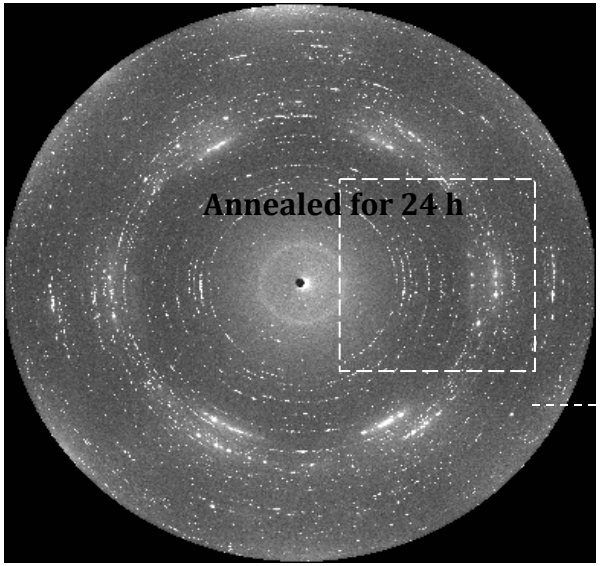
2-D X-ray pattern of crystalline sucrose

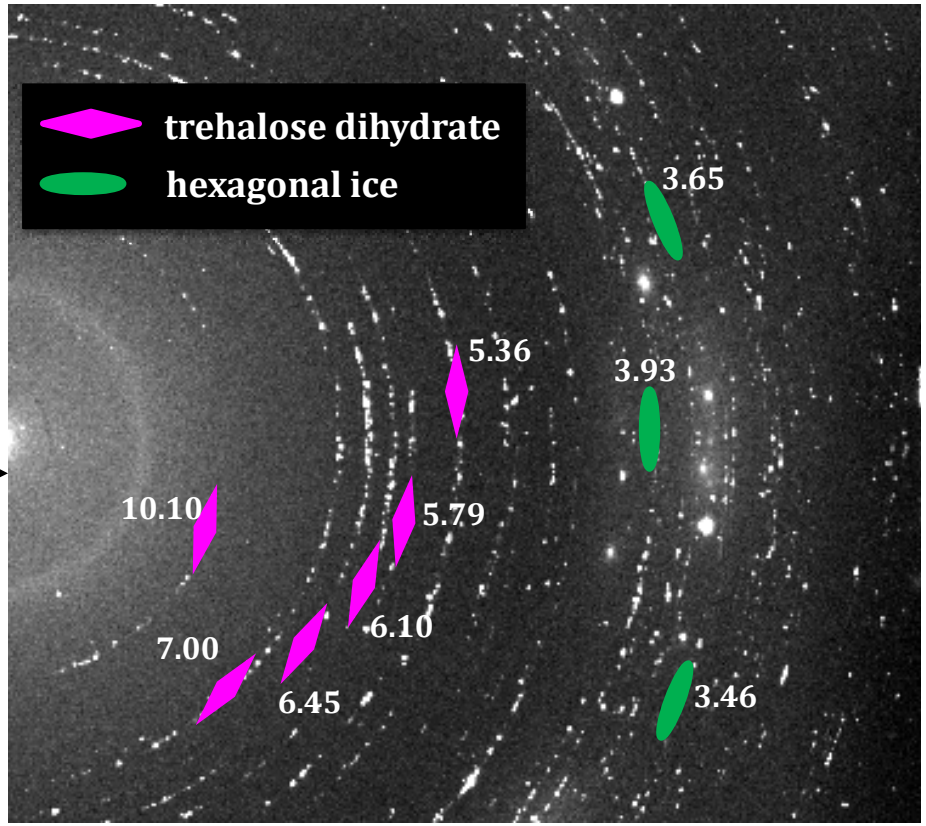


C. Nunes, Ph.D. Dissertation, Univ of MN

Trehalose frozen solution

4% Trehalose



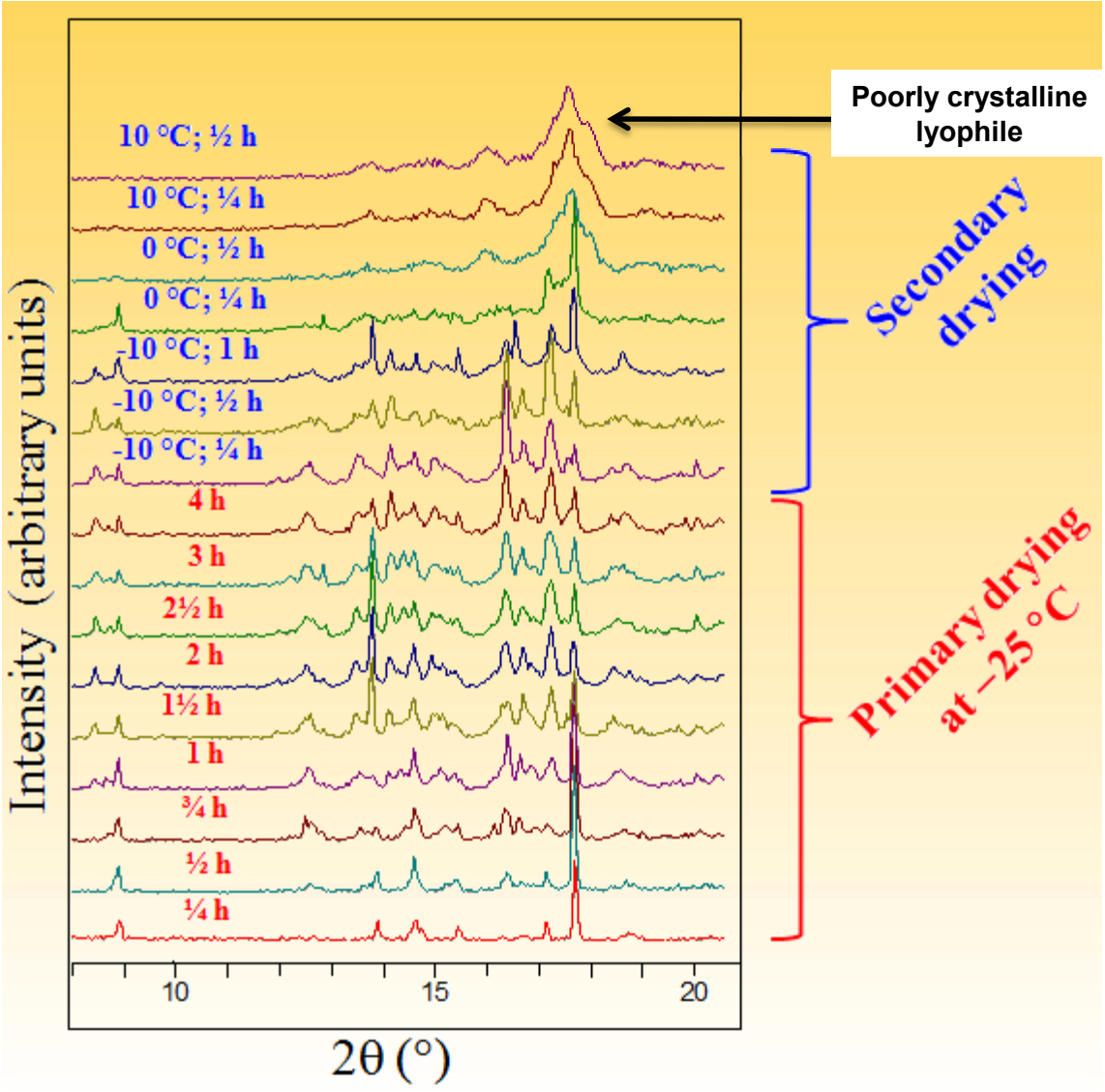


Evidence of crystallization of trehalose dihydrate in frozen solutions

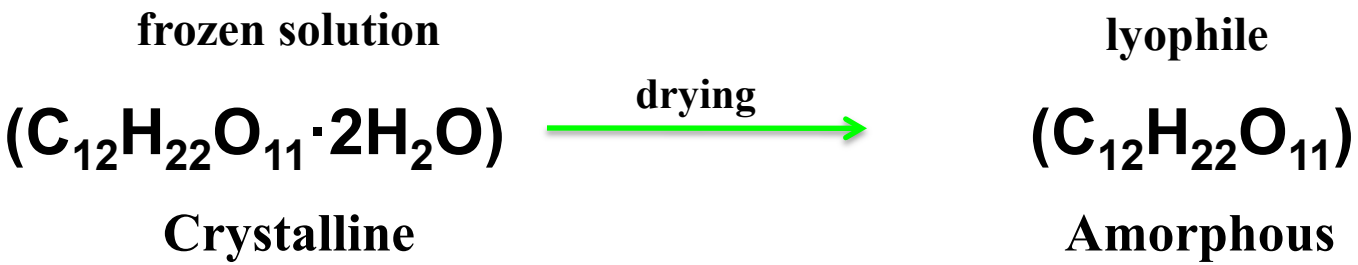
Sundaramurthi and Suryanarayanan, J Phys Chem Lett, 1 (2010) 510

Why was trehalose crystallization not reported in the literature?

Phase transition during drying



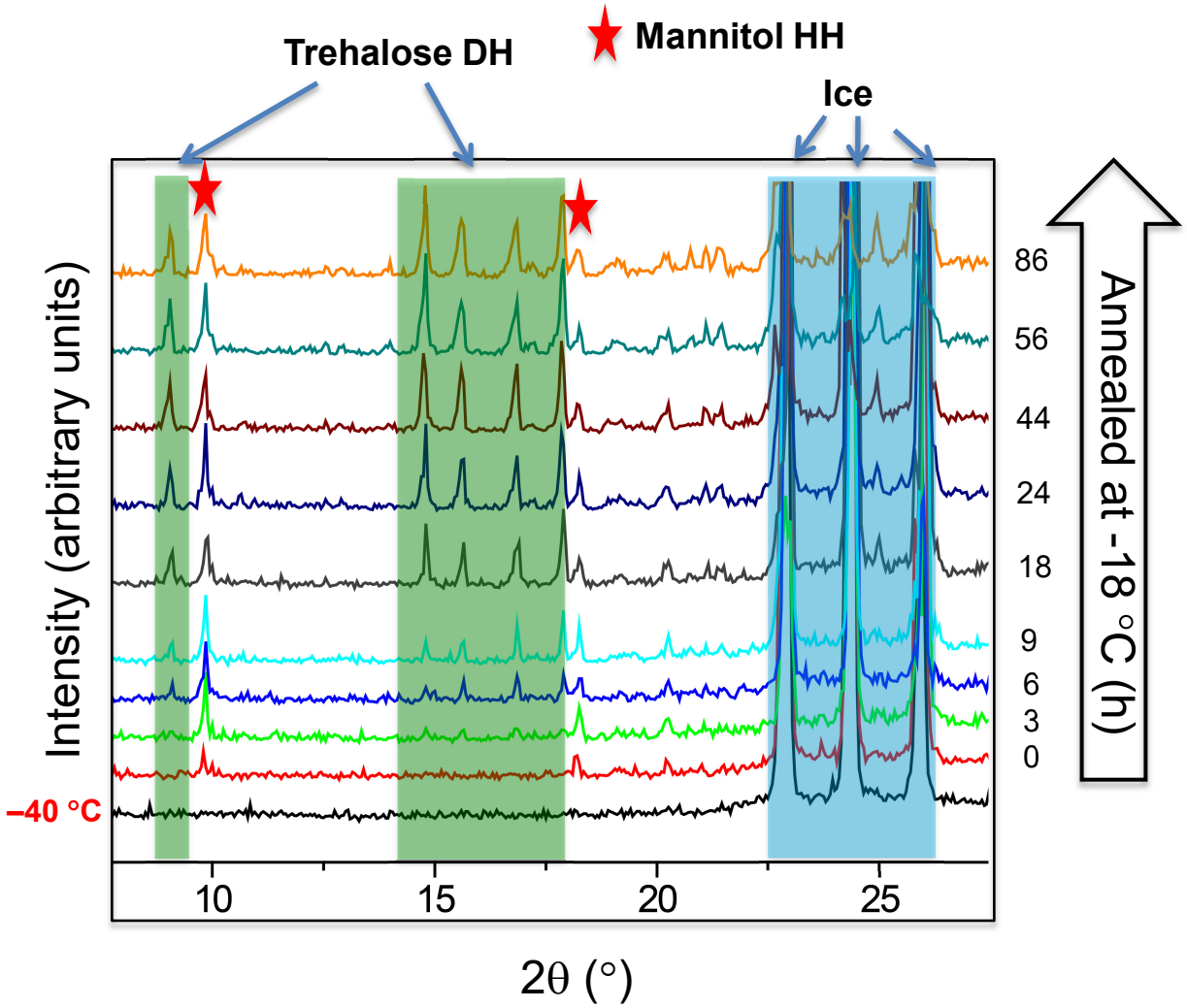
Sundaramurthi and Suryanarayanan, J Phys Chem Lett, 2010



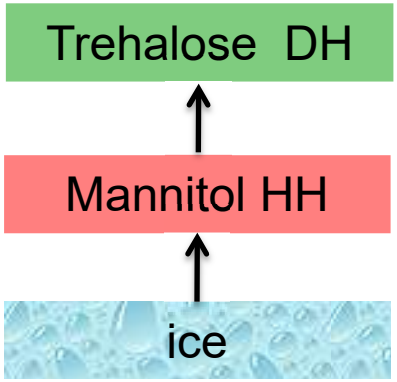
Dehydration

Crystaline → amorphous transition

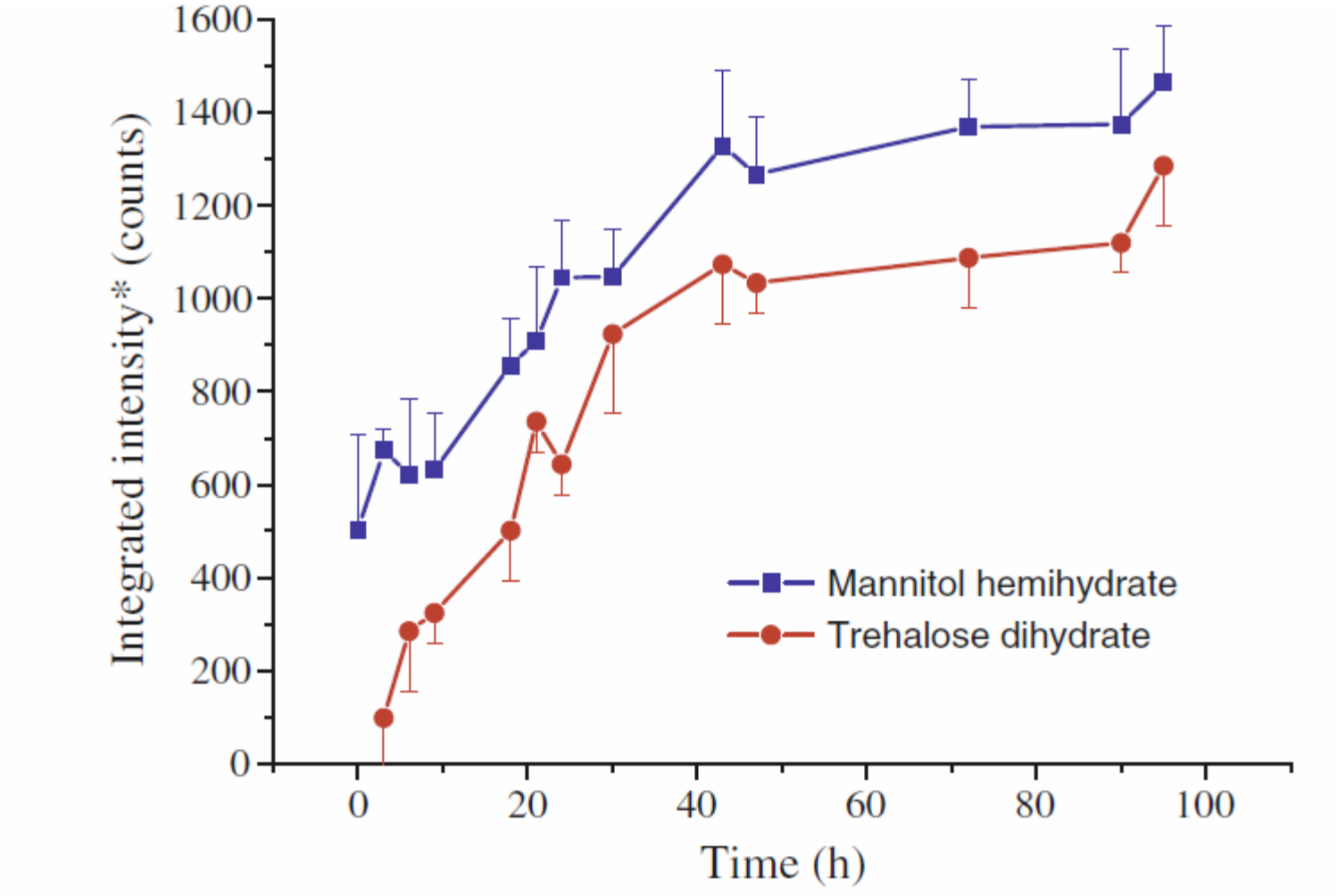
Trehalose & mannitol frozen solution



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



Sundaramurthi and Suryanarayanan, Pharm Res 27 (2010) 2384

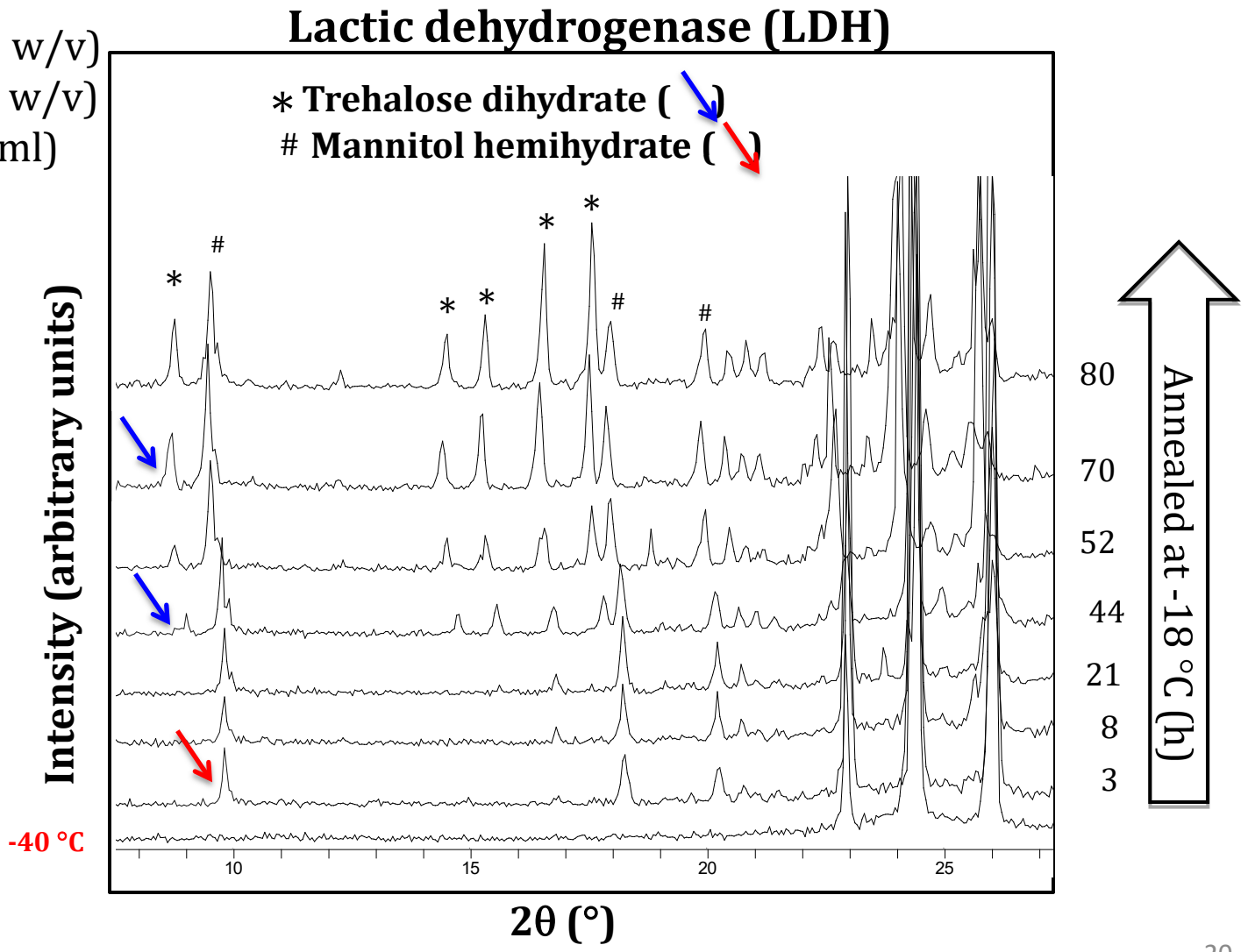


Sundaramurthi and Suryanarayanan, Pharm Res, 27 (2010) 2384

Role of the protein?

Effect of proteins

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



Sundaramurthi and Suryanarayanan, Pharm Res, 27 (2010) 2384

- Protein inhibits trehalose crystallization

- This effect is concentration dependent

Sequence of events

Protein inhibits trehalose crystallization

Trehalose is retained amorphous

Amorphous trehalose functions as an effective lyoprotectant and stabilizes the protein

Case studies

- Trehalose crystallization
- **Mannitol hemihydrate**
- In situ salt formation
- Salt disproportionation

Mannitol

- Bulking agent

- Advantages
  - Readily crystallizes
  - High eutectic temperature

- **Potential issue**
  - Formation of mannitol hemihydrate (MHH) during lyophilization

Mehta et al, Eur J Pharm Biopharm 85 (2013) 207

MHH – effect on product stability

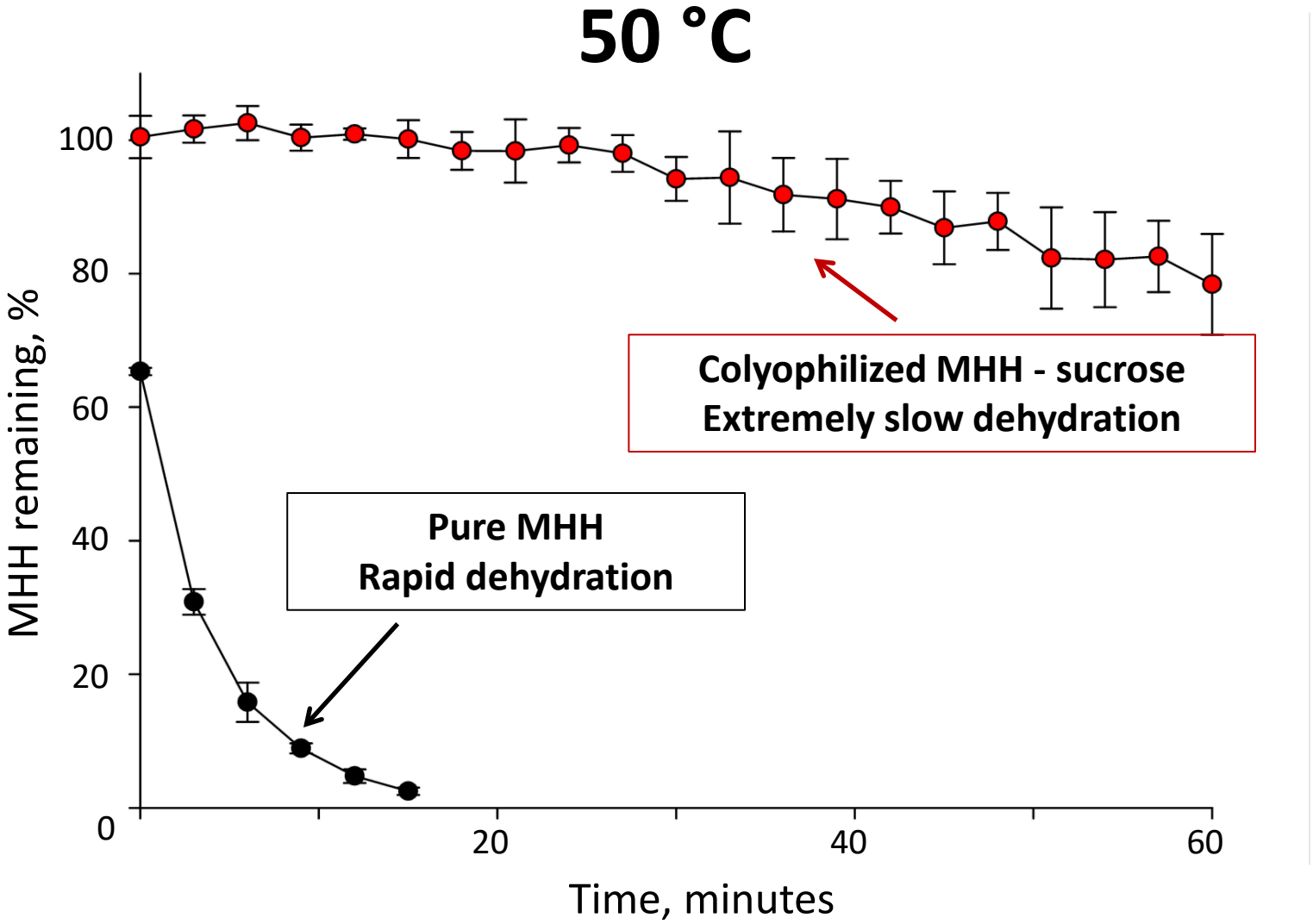
Stoichiometric water content - 4.7% w/w

Dehydration and Release of water during storage

- API – hydrolysis
- Moisture-induced protein aggregation
- Plasticize amorphous components – crystallization. For example – sucrose*

*Bhatnagar et al, unpublished work

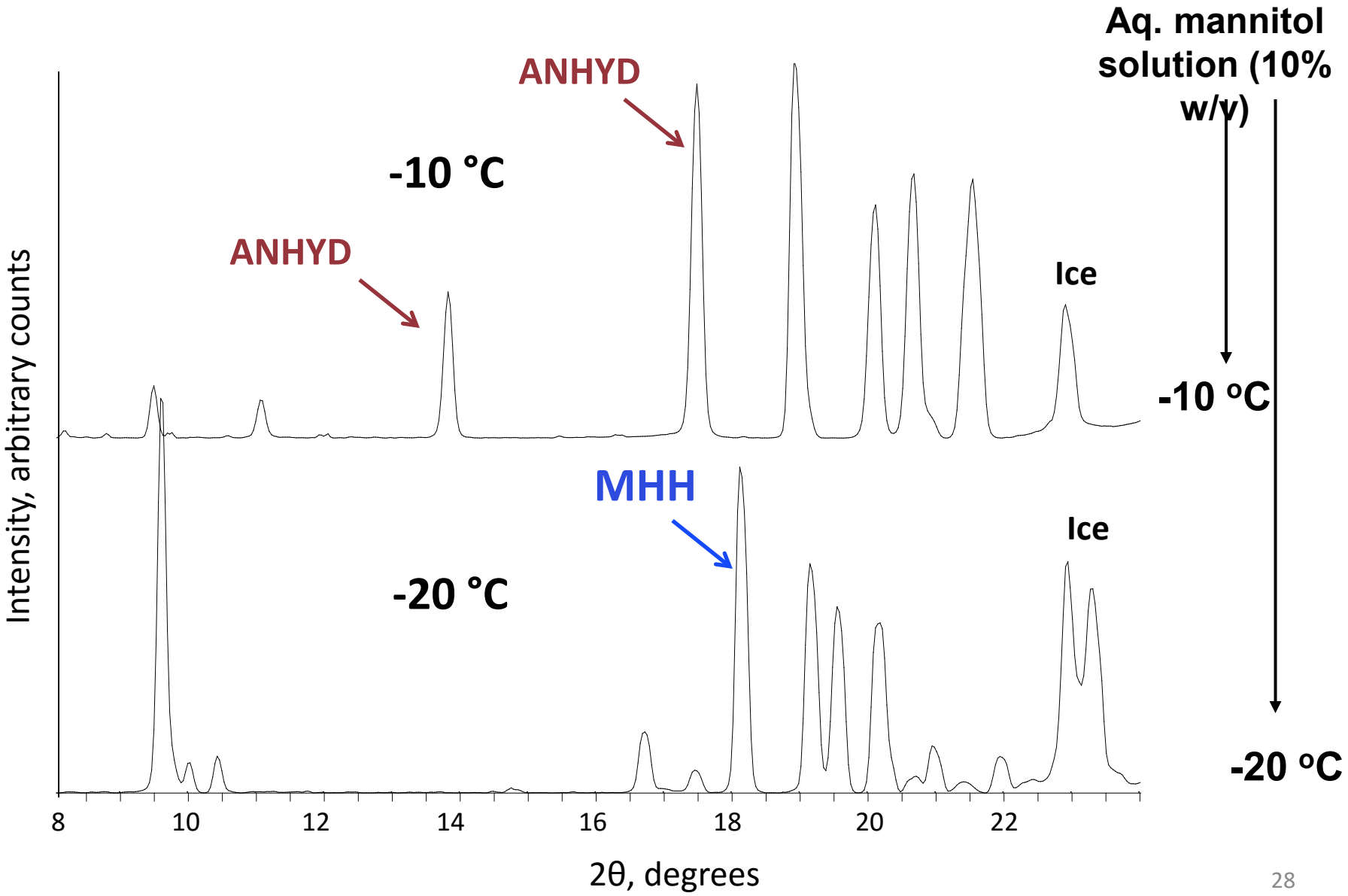
Once formed, MHH is difficult to dehydrate



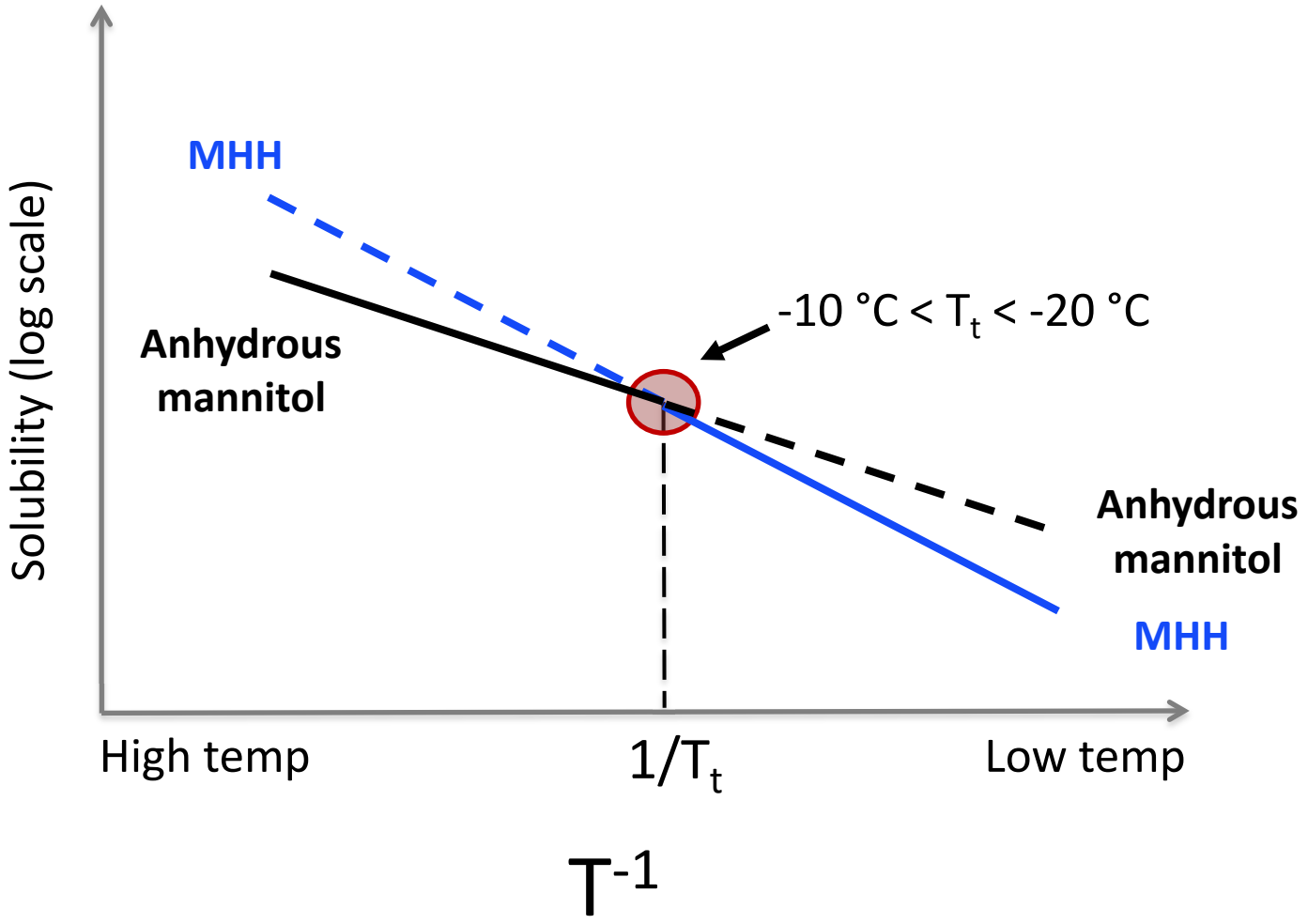
Strategy: Avoid MHH formation during lyophilization.

How?

Mannitol phase formed – appeared to depend on the temperature of crystallization



Hypothesis - The temperature of crystallization (during cooling) governs the mannitol phase crystallizing from solution

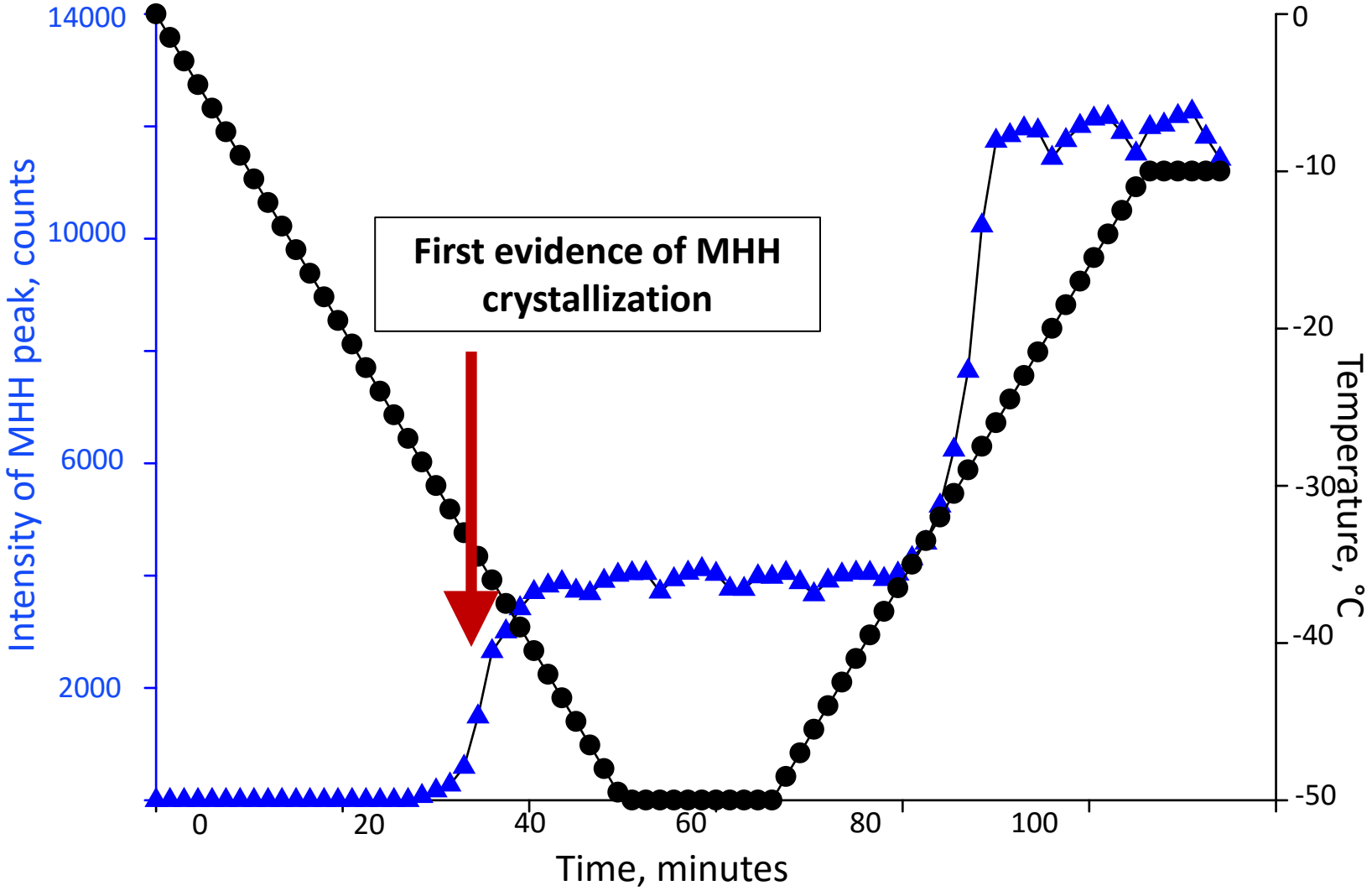


Frozen state characterization

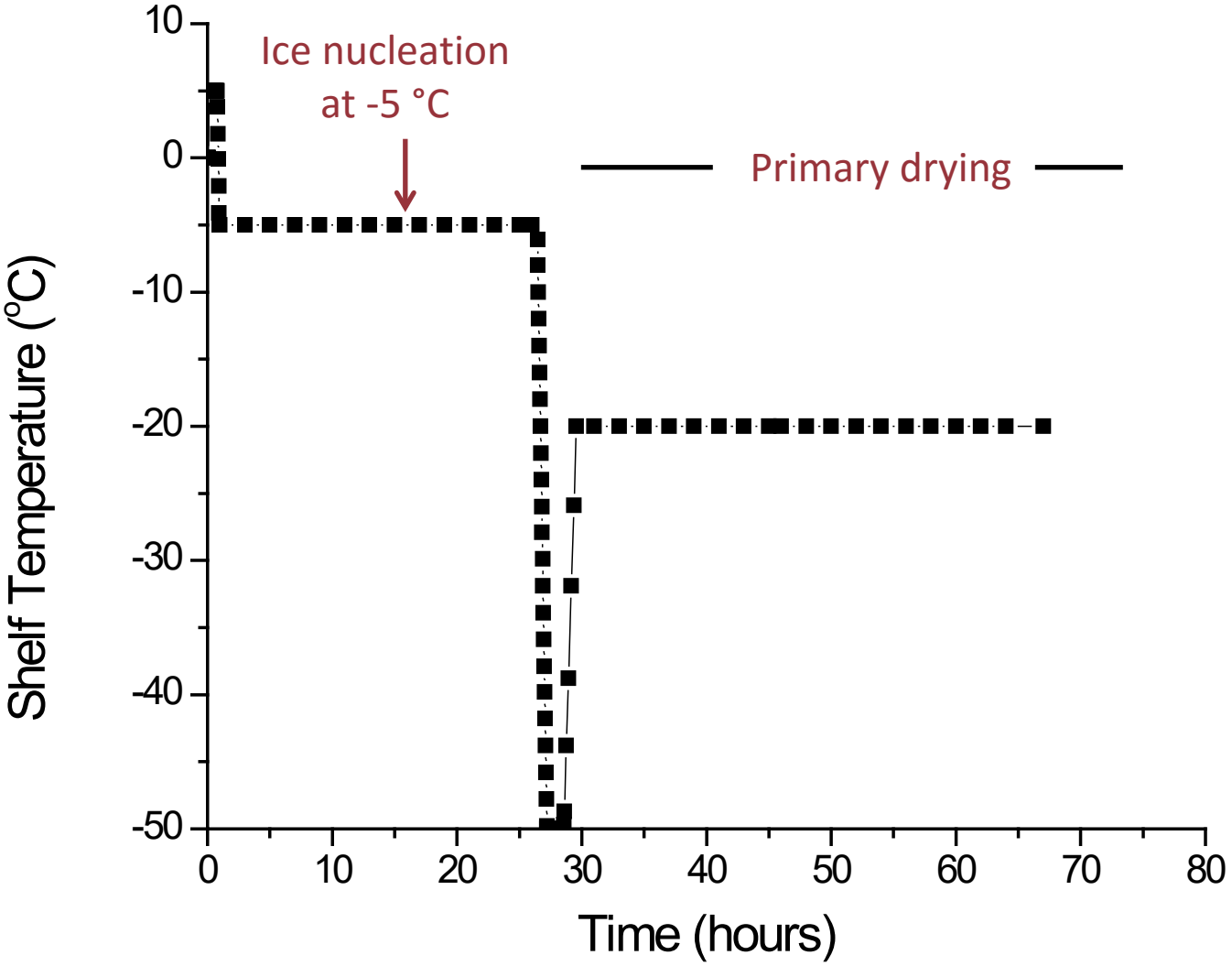
Synchrotron XRD – Argonne National Labs

- Real time monitoring during freezing
- High sensitivity

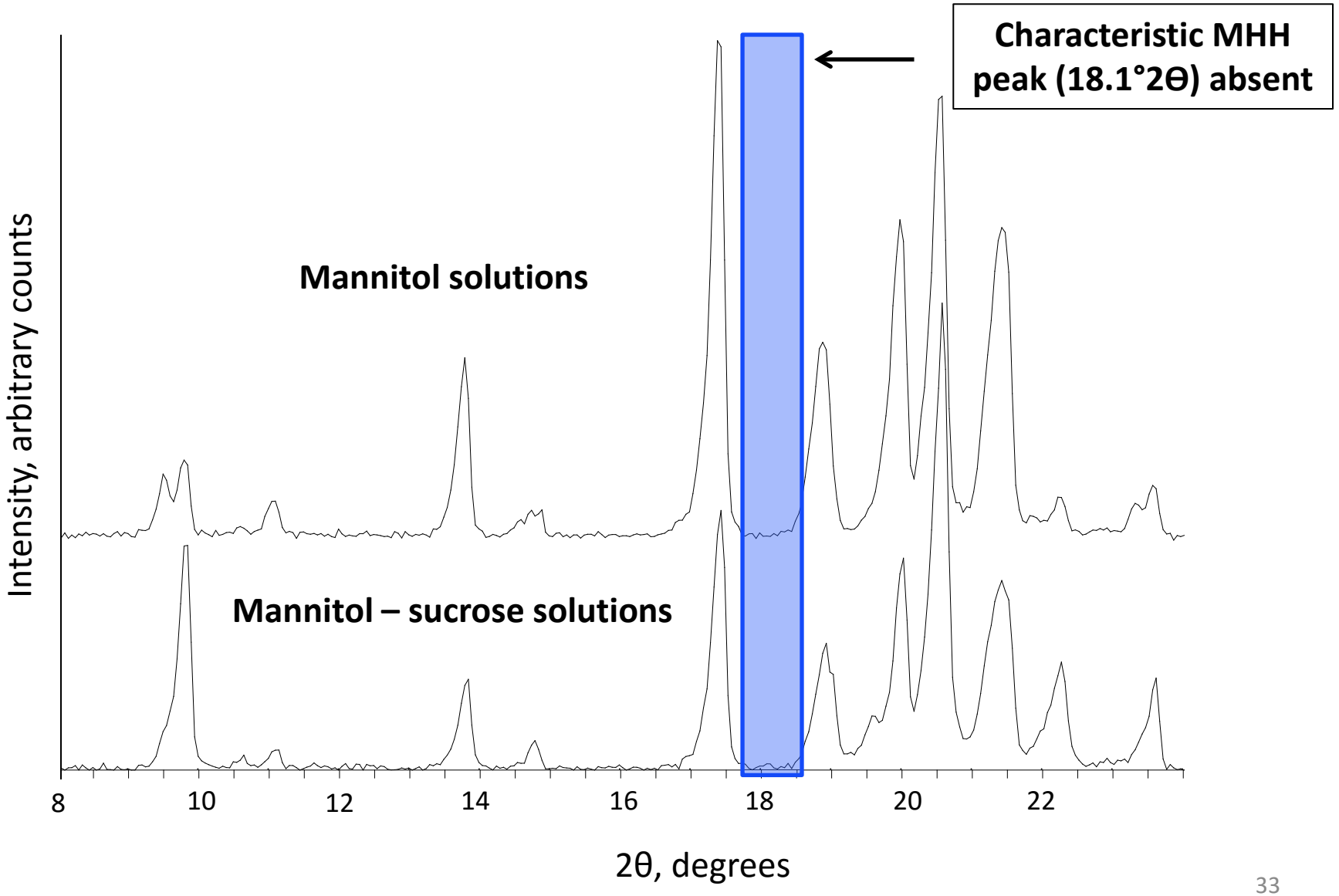
MHH first forms during the freezing step



Prevent MHH formation in lyo product by modifying cycle using ControLyo™ technology



No MHH in the final lyo product



Controlling the physical form of mannitol..

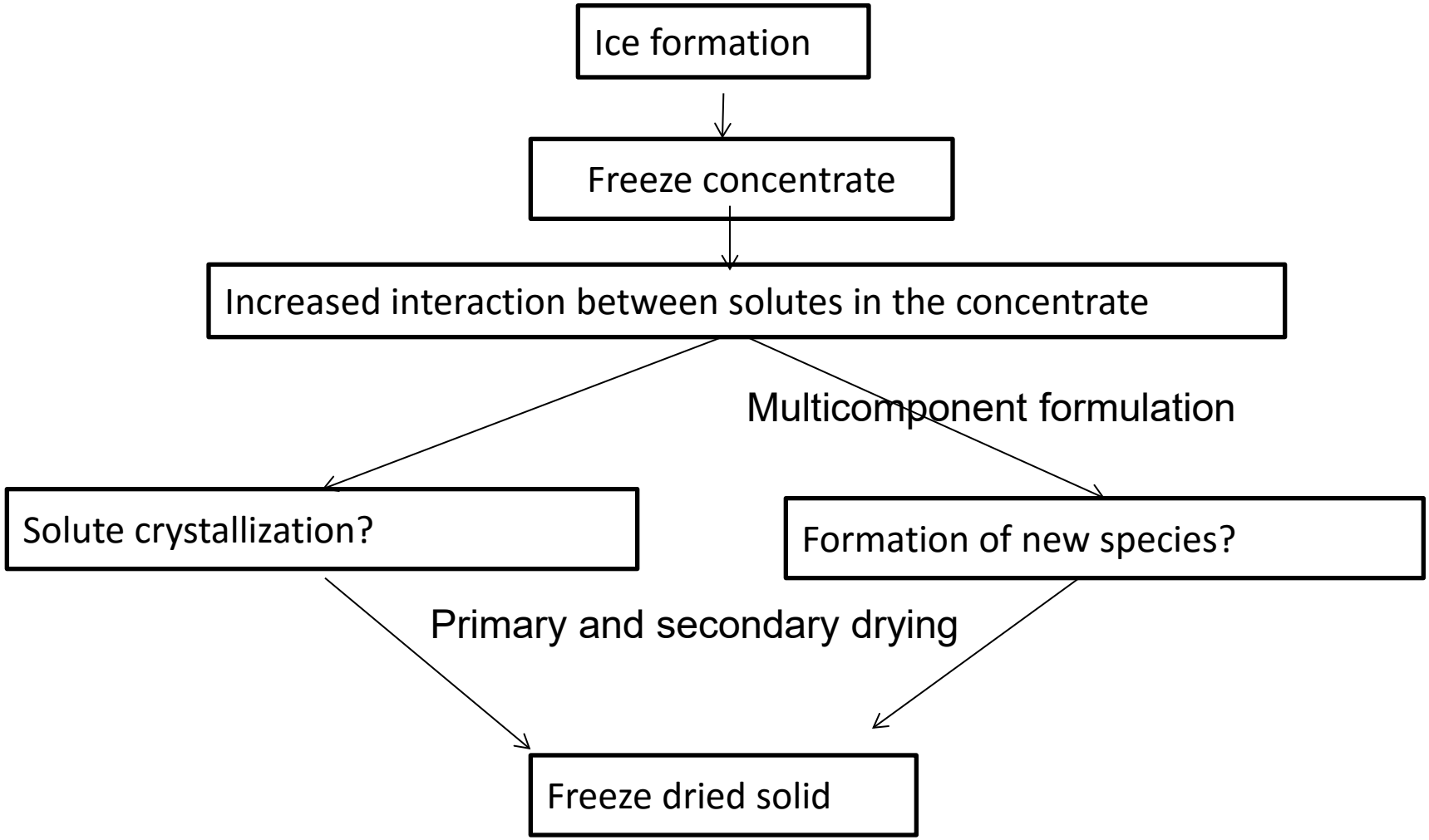
- Temperature of mannitol crystallization
- Governed by the ice crystallization temperature

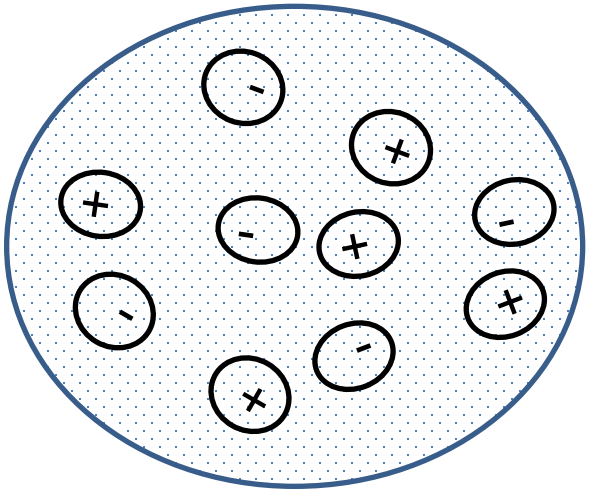
Monitoring the frozen solution

Case studies

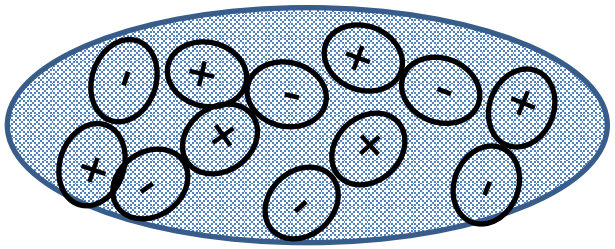
- Trehalose crystallization
- Mannitol hemihydrate
- **In situ salt formation**
- Salt disproportionation

Sequence of events during freeze drying



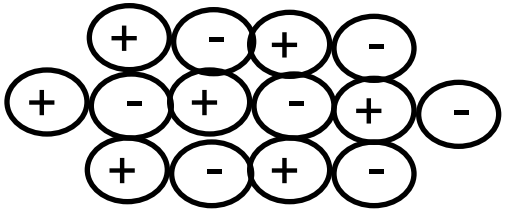


Ions in solution



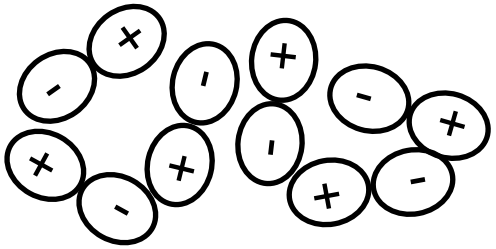
Ions in freeze concentrate

Freeze drying



Crystalline salt

Annealing



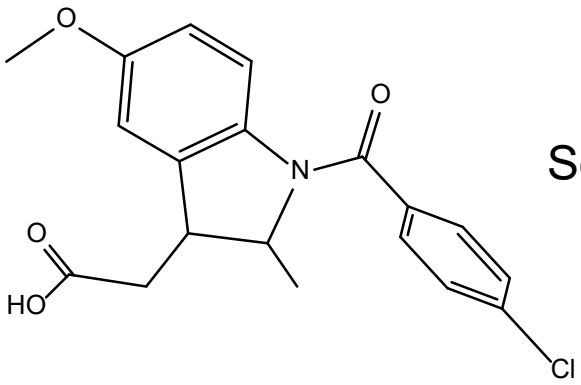
Amorphous salt

Model system

Indomethacin (IMC) with tris as counter ion

0.1 M IMC in 0.15 M tris

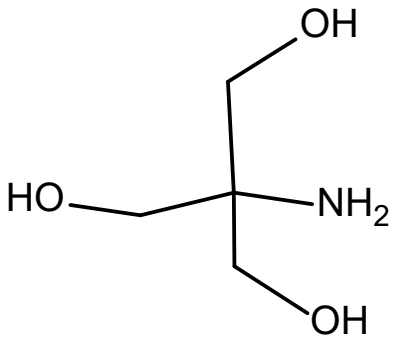
pH of the final solution ~7.7



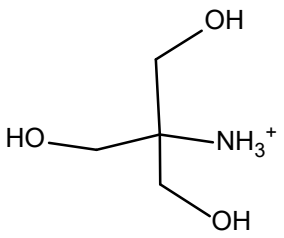
IMC (0.1 M)
pKa = 4.0
Complete ionization

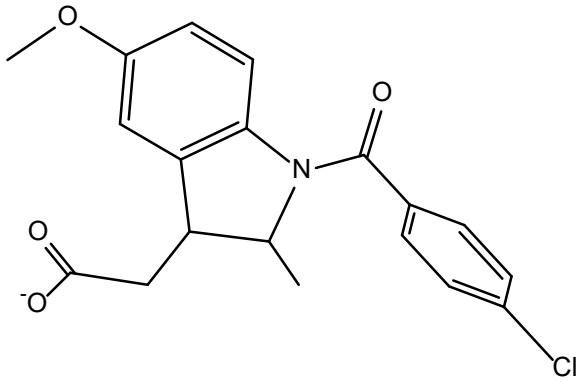
Solution pH = ~7.7

+



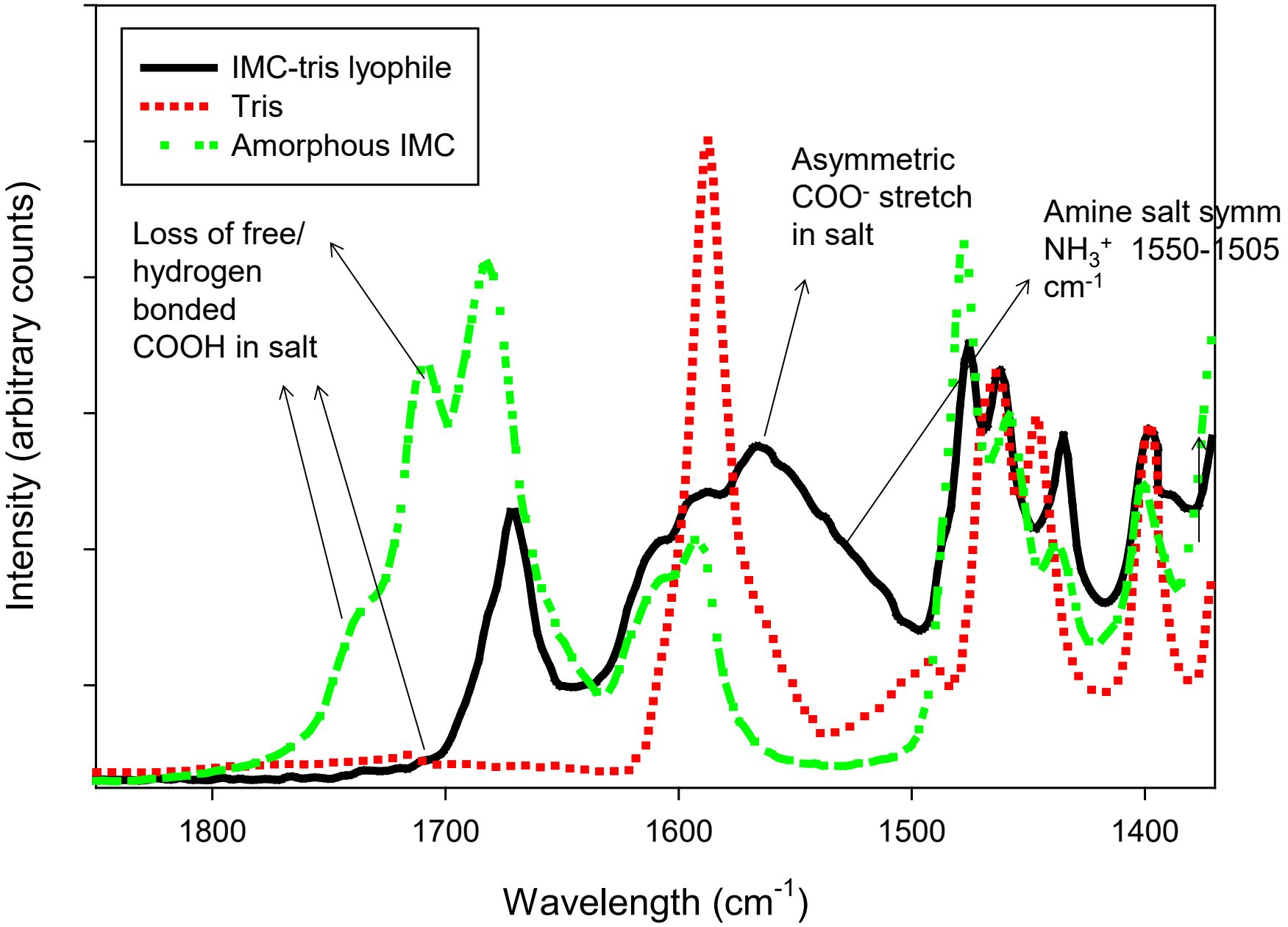
Tris (0.15 M)
pKa = 8.0
~65% ionized
(≡0.0975M)



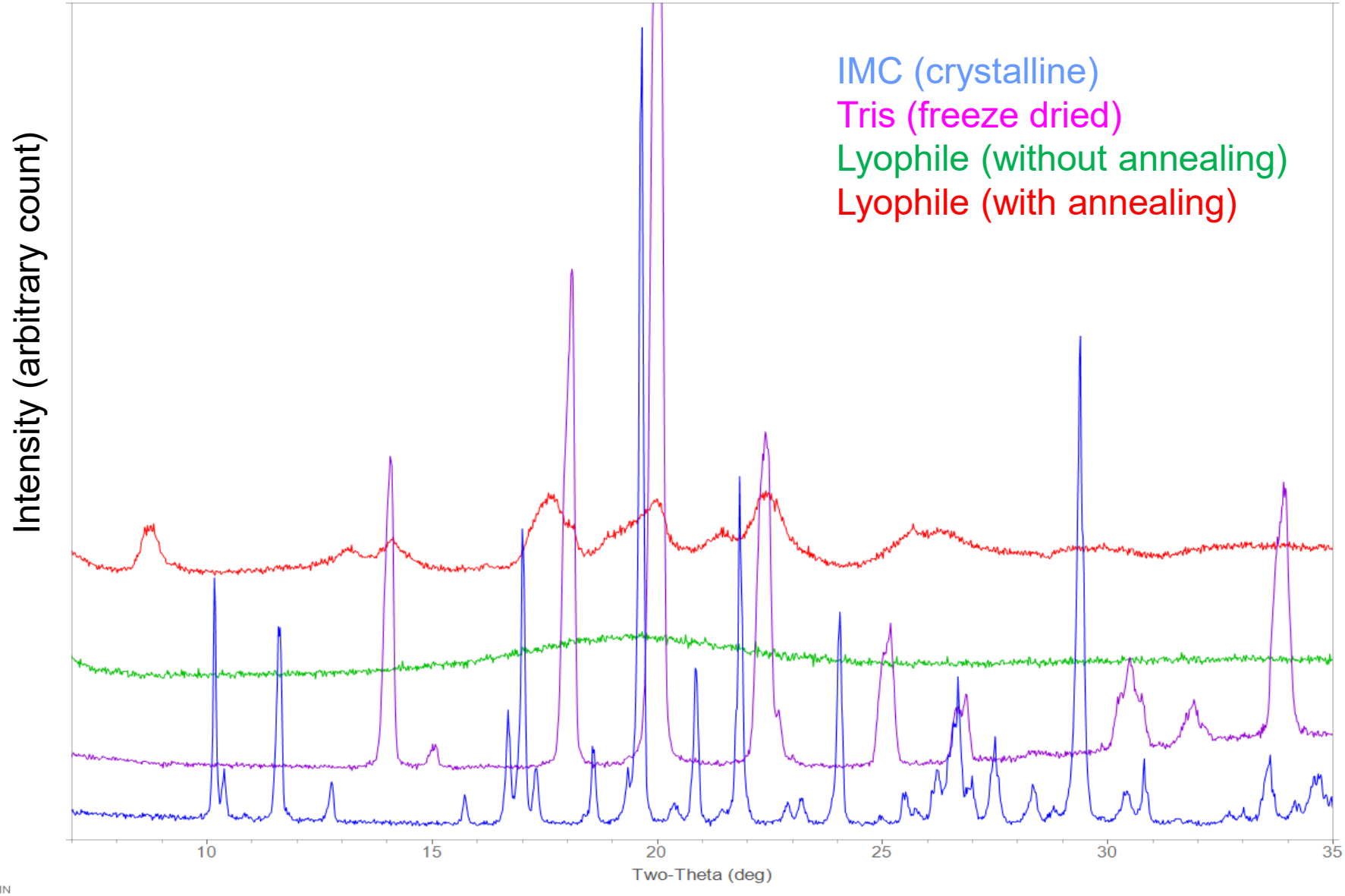


Formation of IMC-tris salt

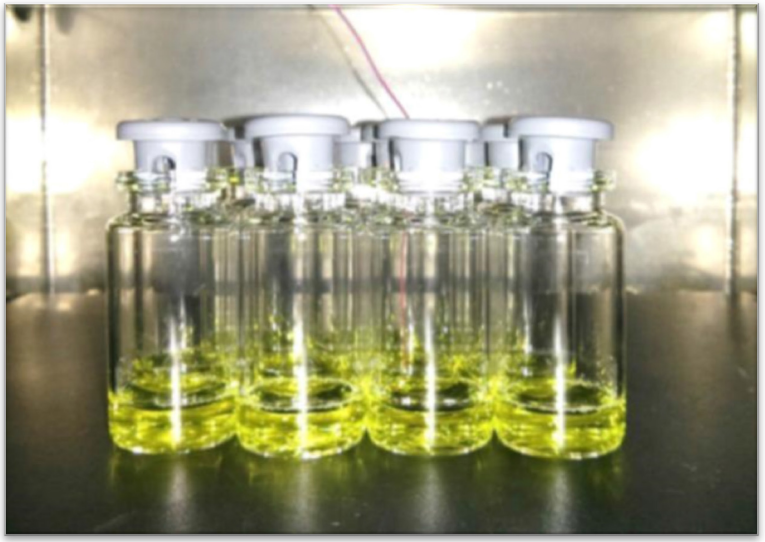
IR spectra


IMC-tris lyophile
Tris
Amorphous IMC
Loss of free/ hydrogen bonded COOH in salt
Asymmetric COO- stretch in salt
Amine salt symm NH3+ 1550-1505 cm-1
Intensity (arbitrary counts)
1800
1700
1600
1500
1400
Wavelength (cm-1)

X-ray powder diffraction patterns



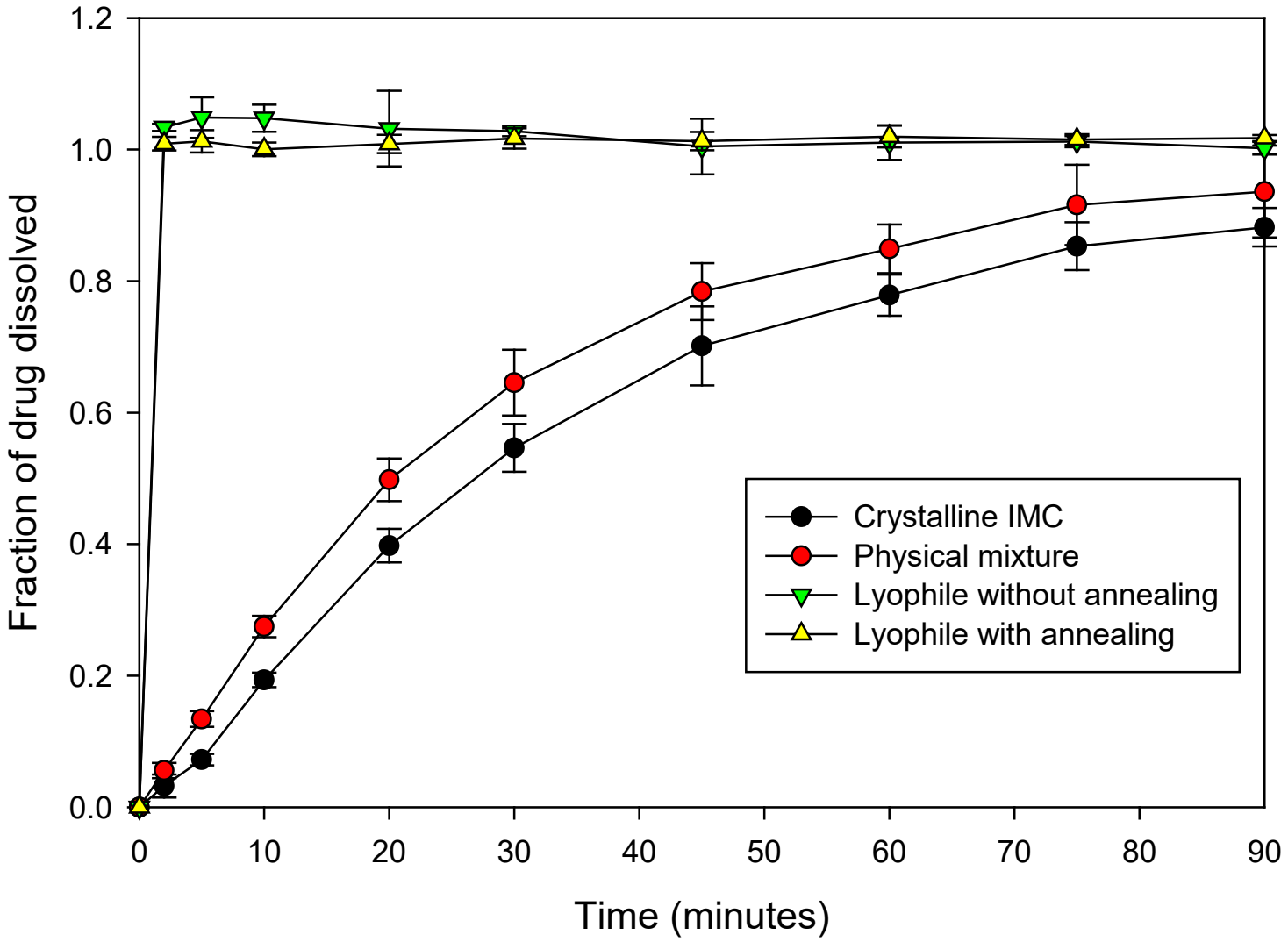
Vials loaded in lyophilizer



Before annealing (frozen)

After annealing (frozen)

Dissolution profiles



Summary

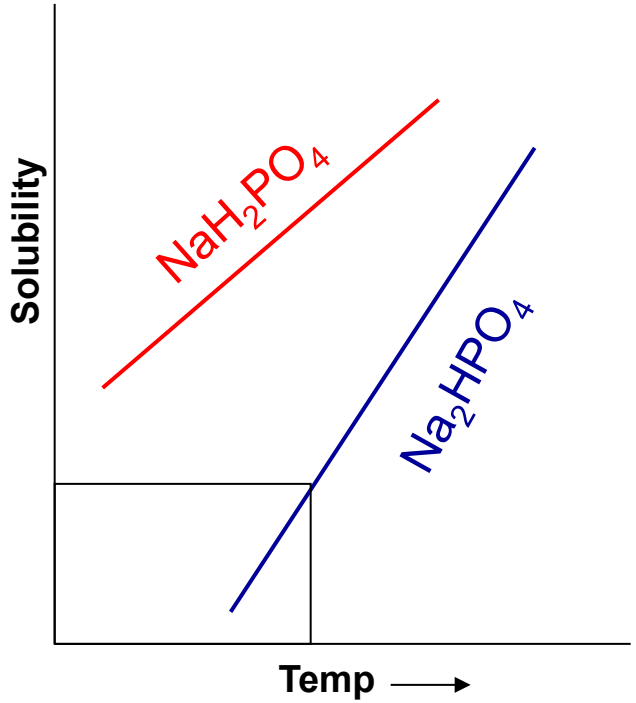
- Salt formation during freeze-drying
- Enhanced dissolution
- Annealing - control the physical form of the lyophile

Case studies

- Trehalose crystallization
- Mannitol hemihydrate
- In situ salt formation
- **Salt disproportionation**

Buffer Crystallization - Schematic

Sodium phosphate buffer



acid + salt of acid → buffer

NaH_2PO_4 + Na_2HPO_4 → pH 7.4

pH 3.5

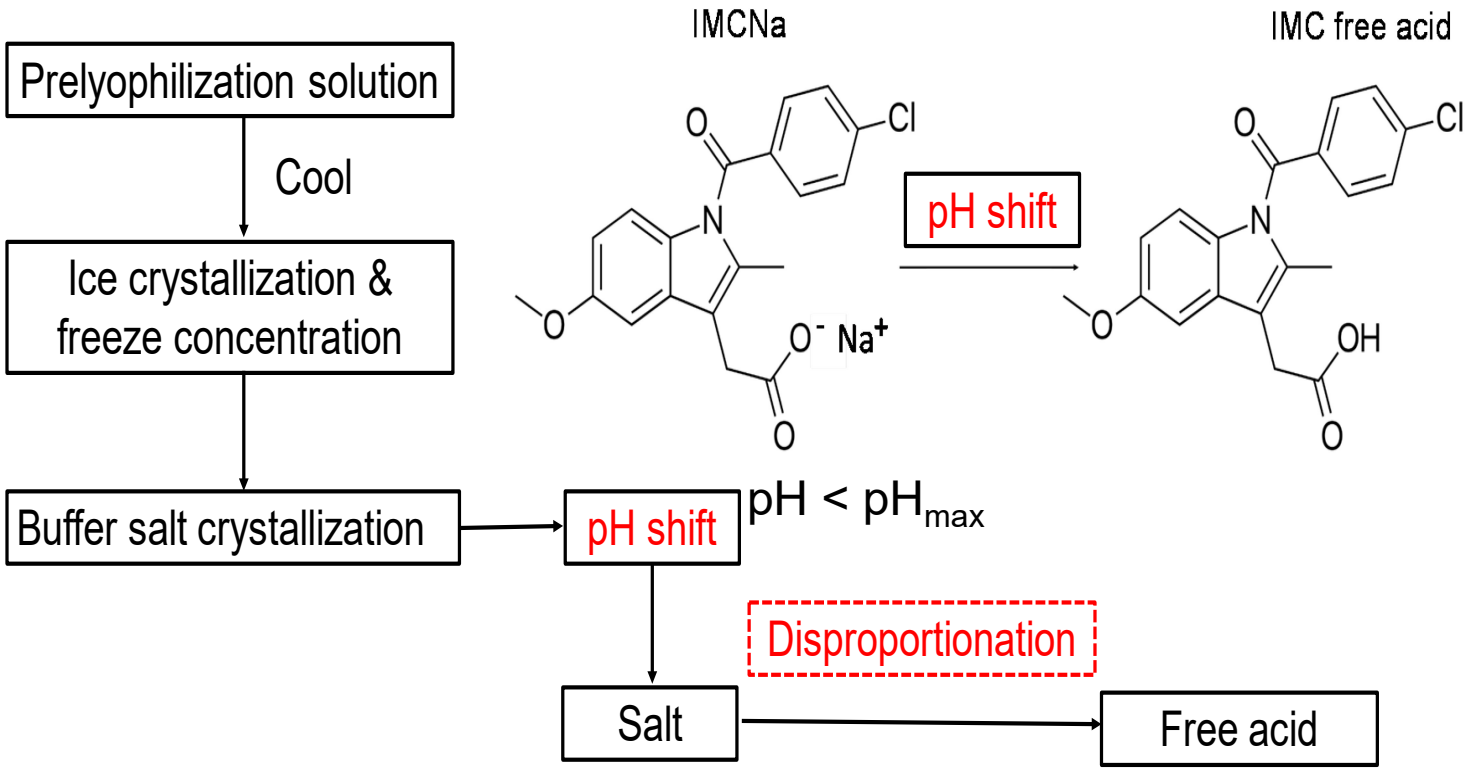
Working Hypothesis

In indomethacin sodium/sodium phosphate buffer system

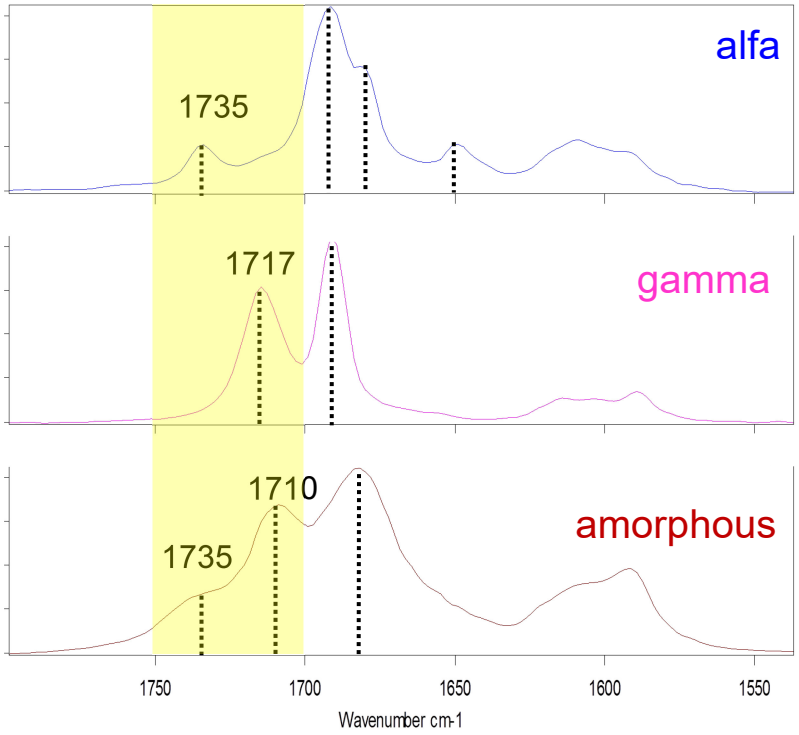
Selective crystallization of buffer component and the consequent pH shift causes disproportionation of indomethacin sodium salt resulting in formation of poorly soluble indomethacin free acid.

Koranne et al, unpublished results

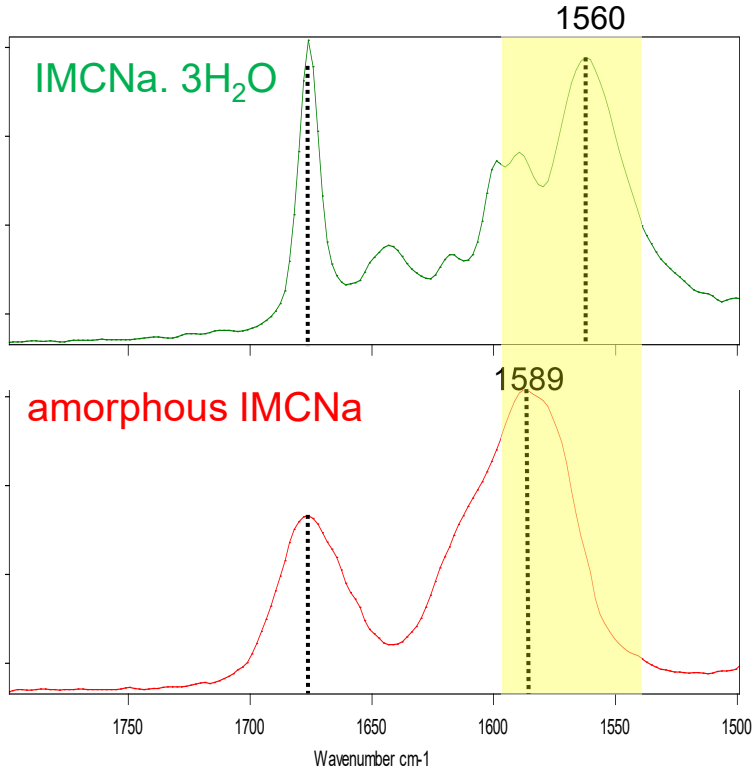
Buffer crystallization & pH shift



FTIR IMC free acid



FTIR IMCNa salt



Lyophiles: FTIR Summary

NaP concentration, mM	IMCNa trihydrate concentration		
	15 mg/ml (34.6 mM)	10 mg/ml (23 mM)	5 mg/ml (11.5 mM)
100	D	D	D
50	D	D	D
35	ND	D	D
20	ND	ND	D
10	ND	ND	ND

D: Disproportionation (IMC acid formation) observed
ND: No disproportionation (no IMC acid formation) observed

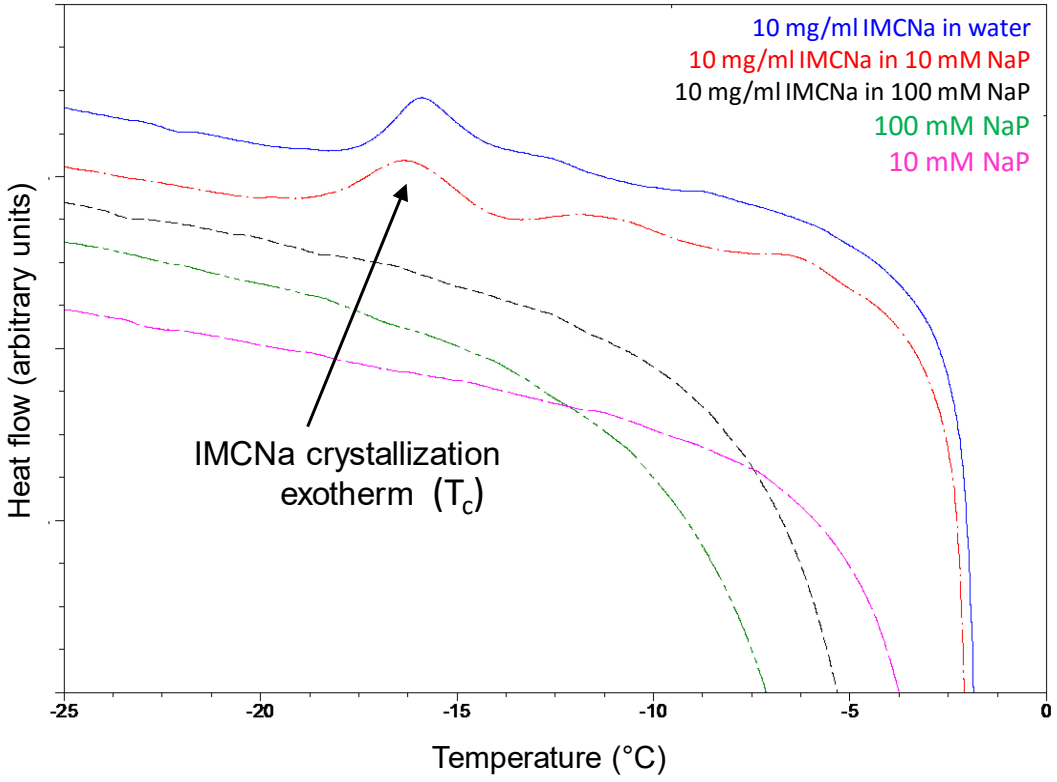
Summary: Low temperature pH measurements

NaP concentration, mM	IMCNa · $3H_2O$ concentration, mg/ml (mM)	pH at 20 °C[a]	pH at -25 °C[b]	ΔpH[c]	
100	-	7.1	2.9	4.2	
100	10 (23.0)	7.1	2.8	4.3	D
35	-	7.2	3.1	4.1	
35	15 (11.5)	7.2	6.7	0.5	
35	10 (23.0)	7.2	5.2	2.0	D
35	5 (34.6)	7.1	5.6	1.5	
10	-	7.2	3.5	3.7	
10	10 (23.0)	7.2	7.4	-0.2	

Initial pH [a]
pH after the buffer solution was cooled to -25 °C and held for 2 hours[b]
$\Delta pH^{(c)} = pH^{(a)} - pH^{(b)}$
Maximum error in pH measurements is ±0.1

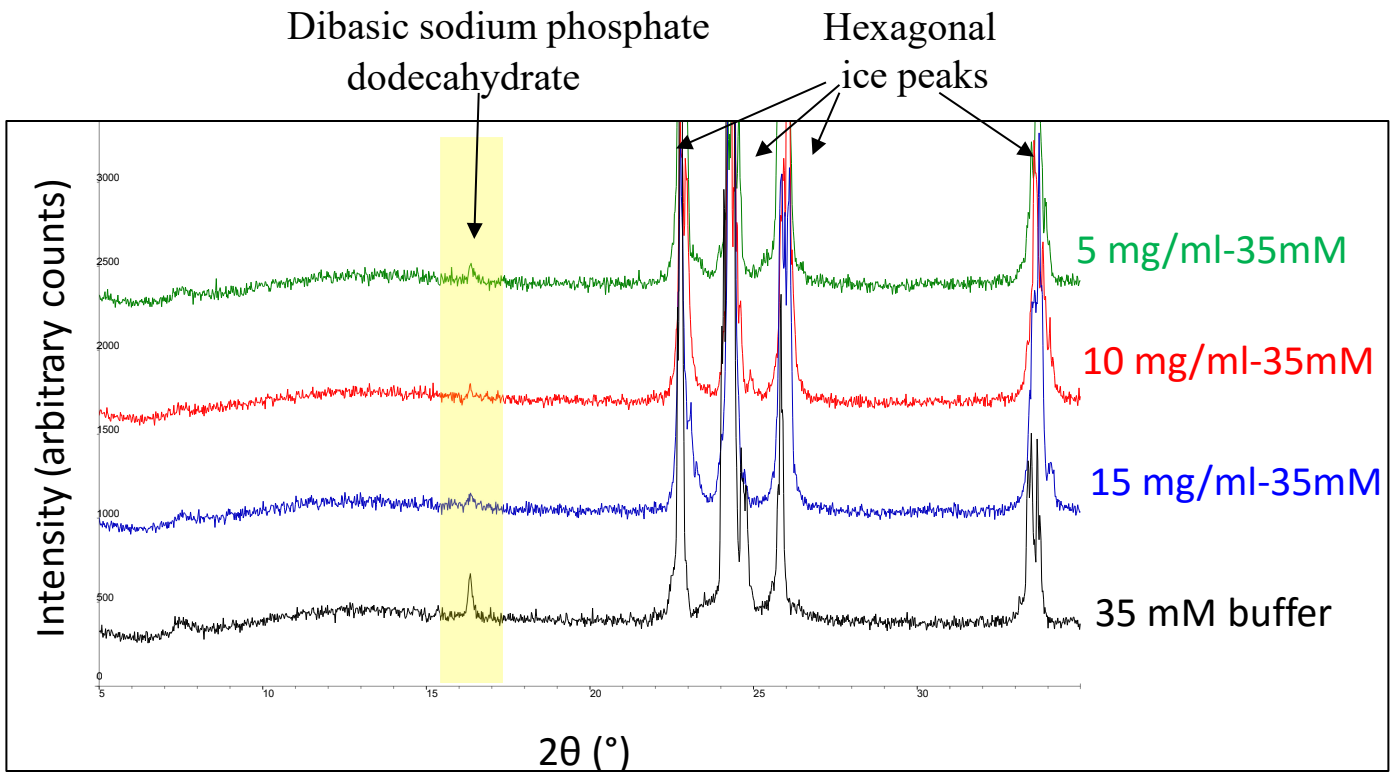
- pH shift during freezing causes disproportionation

DSC - Prelyophilization Solutions



- Absence of IMCNa crystallization exotherm in systems that undergo disproportionation

Low temperature XRD - Prelyophilization Solutions



Conclusions

- Disproportionation of IMCNa due to selective crystallization of buffer component ($Na_2HPO_4 \cdot 12H_2O$) and the consequent pH shift
- Disproportionation is dependent on concentration of buffer and IMCNa
- The absence of IMCNa crystallization event in DSC heating curves indicates disproportionation

Summary

- Selection of excipient
  - Product stability hinges on excipient functionality
  - Physical form of the excipient can be critical
- Excipient concentration – select judiciously
  - More is NOT better
- Excipient with multiple functionalities
  - Has the potential to simplify the formulation

Summary

- Complex interplay of drug and excipients
  - API can influence excipient behavior
  - One excipient can influence the behavior of a second excipient
- Numerous processing steps
  - Potential for phase transitions
  - Monitoring them can be a challenge but very valuable
    - Multiple analytical techniques may be needed