# PRACTICAL CONSIDERATIONS FOR LYOPHILIZED FORMULATION AND CYCLE DEVELOPMENT

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# Introduction

- **Lyophilization is commonly used in the pharmaceutical and biotech industries to improve stability and shelf life of parenteral dosage forms.**
- **The scope of molecules that are typically lyophilized include proteins, peptides, oligonucleotides and liposomes.**

# Introduction (cont.)

- **Challenges to the pharmaceutical scientist in developing a lyophilized dosage form include:**
  - **Excipient selection**
  - **Container/closure system**
  - **Biocompatibility**
  - **Preformulation and formulation development studies**
  - **Reconstitution must result in a biocompatible solution suitable for IV infusion, IM injection or SC injection**

# Introduction (cont.)

- Lyophilization cycle development
  - Robust
  - Reproducible
  - Scale of manufacture, clinical? commercial?
  - QbD
- Analytical testing
- Specification setting
- Stability studies

# Lyophilization or Freeze Drying

- **Definition**
  - **A process where water is removed from a product after it is frozen and placed under vacuum**
  - **Ice "sublimates" from solid to vapor without forming a liquid**
  - **"Three" steps**
    - **Freezing**
    - **Primary drying**
    - **Secondary drying**

# Attributes of "Elegant" Cakes

- Sterile
- Low endotoxin
- Uniform appearance
- Intact cake
  - Remains intact after shipping/handling
- Low moisture content
- Minimal particulates
- Reasonable reconstitution time

# Typical Excipients

- **Bulking Agent**
  - **Build structure upon sublimation**
    - **Sucrose**
    - **Mannitol**
    - **Maltose**
    - **Trehalose (non reducing, avoid Maillard Reaction with certain APIs)**
    - **Dextrose**
    - **Lactose**

# Typical Excipients

- Stabilizers
  - Cryoprotectants (during freezing)
    - PEG
  - Lyoprotectants (during drying)
    - Disaccharides
  - Protective effect may be concentration dependent

# Typical Excipients

- **Buffers**
  - **Citrate**
  - **Histidine**
- **Avoid salts that undergo pH change during freezing such as**
  - **Phosphates**

# Typical Excipients

- **Tonicity Adjusters**
  - **NaCl**
  - **Mannitol**
  - **Sucrose(caution using in some patient populations)**
  - **Glycine**
  - **Glycerol**

# Glass Transition $T_g$ and Collapse $T_c$ Temperatures

- **In the great majority of cases the solutes do not crystallise at the solubility limit, ice continues to form as the temperature is decreased and the solution continues to concentrate until it is so viscous it turns to glass. This it does at a characteristic and reproducible temperature, the glass transition temperature Tg'. The "prime" mark is used to denote this is the glass formed by freeze concentration, a glass which may still contain 20-50% by weight of unfrozen water.**

- **This water is not bound in any way but diffusion is limited by the extremely high viscosity of the glass. For products that behave like this, Tg' is the temperature at which the ice starts to melt back and the product starts to collapse. (i.e. Tc=Tg′). The glass transition and the eutectic melt have quite different profiles in a DSC scan, the glass transition is a step like transition whereas a eutectic melt appears as a peak, or trough depending upon the set up of the DSC.**

**TABLE 29.1. Glass Transition Temperatures of Freeze Concentrate ($T_{g'}$) and Collapse Temperatures ($T_C$) of Selected Proteins and Excipients Commonly Used in Freeze-Dried Pharmaceutical Products**[a]

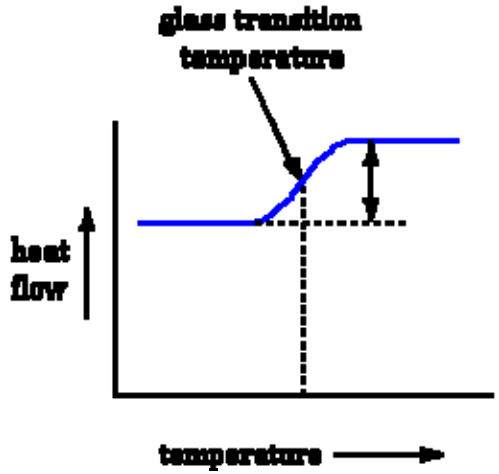
| Components | | $T_{g'}$ (°C) | $T_C$ (°C) |
|---|---|---|---|
| Proteins | Galactosidase | −29 | −15 |
| | Ovalbumin | −11 | −10 |
| | rhuMAB HER | — | −20 |
| Sugars | Sucrose | −32 | −31 |
| | Trehalose | −29 | −28.5 |
| | Lactose | −28 | −30.5 |
| | Maltose | −30 | — |
| Polyols | Glycerol | −65 | — |
| | Sorbitol | −46 | — |
| | Mannitol | −35 | −1.4[b] |
| Polymers | Dextran (70 K) | −10 | −10 |
| | Polyvinylpyrrolidone (PVP) (40 KD) | −21 | −24 |
| | Ficoll | −19 | −20 |
| | Gelatin | −9 | −8 |
| | Hydroxyethyl starch | −12 | >−5 |
| Amino acids | Glycine | −62 | −3.5 |
| | Alanine | −65 | — |
| | Histidine | −33 | — |
| Salts | Sodium acetate | −64 | — |
| | Sodium citrate | −41 | — |
| | $KH_2PO_4$ | −55 | — |
| | $K_2HPO_4$ | −65 | — |
| | NaCl | −60 | — |
| | $CaCl_2$ | −95 | — |
| | $ZnCl_2$ | −88 | — |

[a] From (2,11–13).
[b] In crystalline state.

# Preformulation Studies

- Solubility
- pH stability
- DSC thermograms
  - Glass transition temperature
- Counterion
- Buffer salt
- Polymorphism

# DSC Thermogram

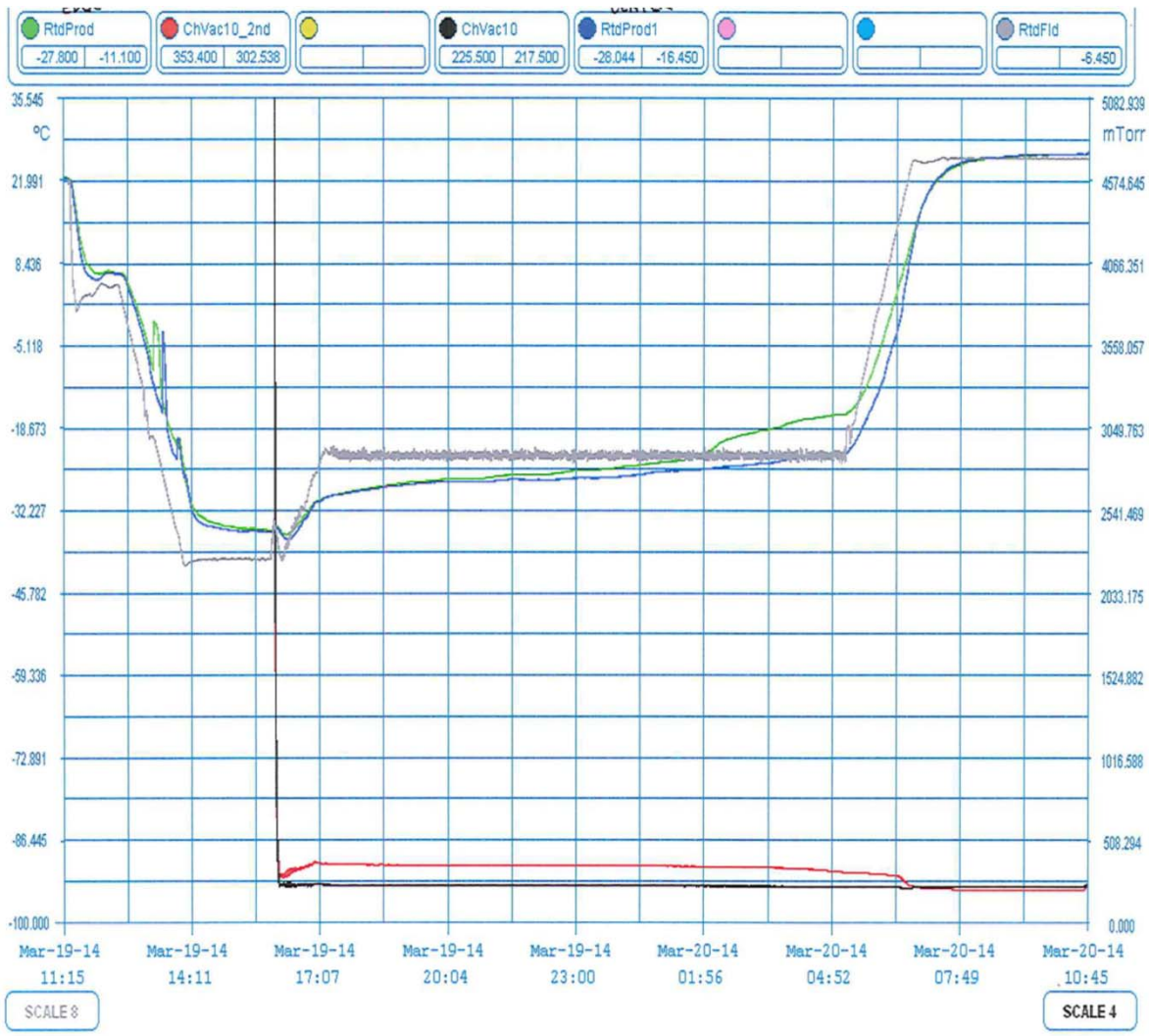


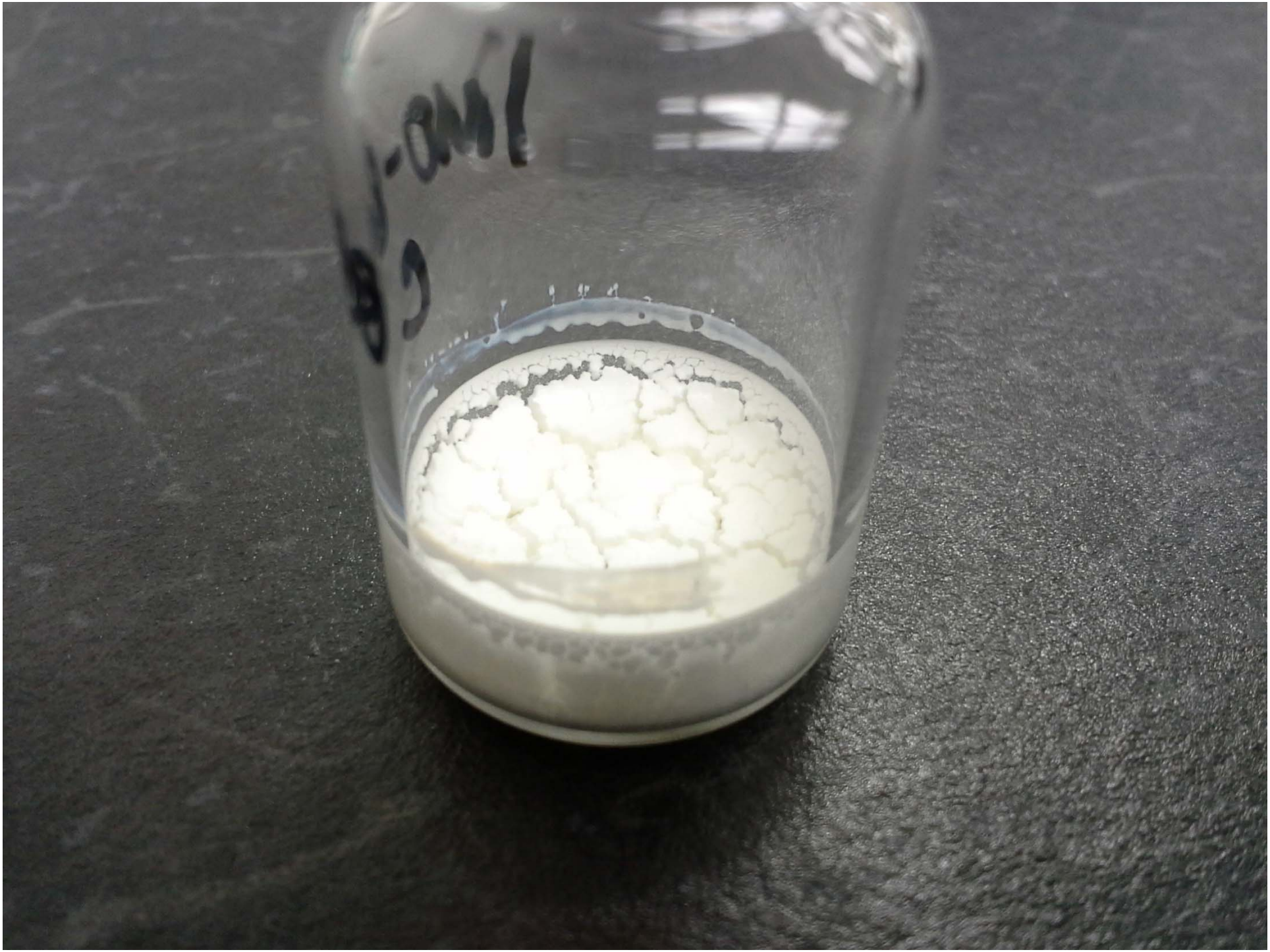
# Formulation Process Studies

- Aspetic process design
- Sterile filter selection
  - Compatibility
  - Formulation
  - Microbial retention
  - Filter validation
- Compatibility with compounding vessels, tubing, etc.
- Bulk solution stability
- Bioburden
- Fill volume

# Cycle Development Studies

- **Pre-freeze**
  - **Shelf temp**
- **Freeze**
  - **Pore structure determined by freezing rate**
  - **Desired crystalline structure**
  - **Maintain well below $T_g$**
- **Anneal**
- **Primary Drying**
  - **Sublimation under vacuum**
  - **Avoid meltback while achieving reasonable cycle time**
- **Secondary Drying**
  - **Isothermal desorption of bound water**
- **Post Cycle**
  - **Stoppering**


RtdProd
-27.800
-11.100
ChVac10_2nd
353.400
302.538
ChVac10
225.500
217.500
RtdProd1
-26.044
-16.450
RtdFld
-6.450
35.545
°C
21.991
8.436
-5.118
-18.673
-32.227
-45.782
-59.336
-72.891
-86.445
-100.000
5082.939
mTorr
4574.645
4066.351
3558.057
3049.763
2541.469
2033.175
1524.882
1016.588
508.294
0.000
Mar-19-14 11:15
Mar-19-14 14:11
Mar-19-14 17:07
Mar-19-14 20:04
Mar-19-14 23:00
Mar-20-14 01:56
Mar-20-14 04:52
Mar-20-14 07:49
Mar-20-14 10:45
SCALE 8
SCALE 4

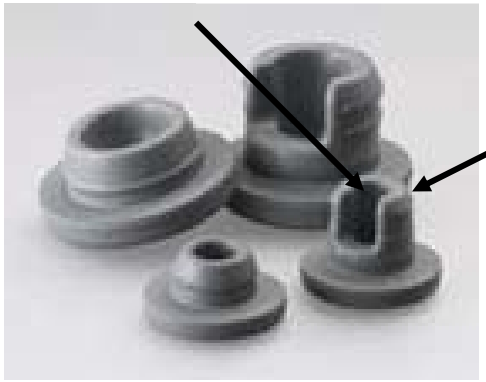



A
C
E
F

# Container Closure Selection

- Vial size
  - 2 mL, 5 mL, 10 mL, 20 mL, >20 mL
- Vial type
  - Glass
  - Other
- Stopper
  - Material
  - Coating
  - Size
    - 13 mm, 20 mm
- Crimp seal
  - Color
  - Type

Fluorotech inside plug only



No Fluorotech

# Stability Testing

- **Appearance**
- **Reconstitution time**
- **Potency**
- **Purity**
- **Water content**
- **pH**
- **Sterility**
- **Endotoxin**

# Specification Setting

- **In-process**
  - **Potency**
  - **pH**
  - **Osmolality**
  - **Density**
  - **Bioburden**
  - **Appearance**
- **Fill weight**

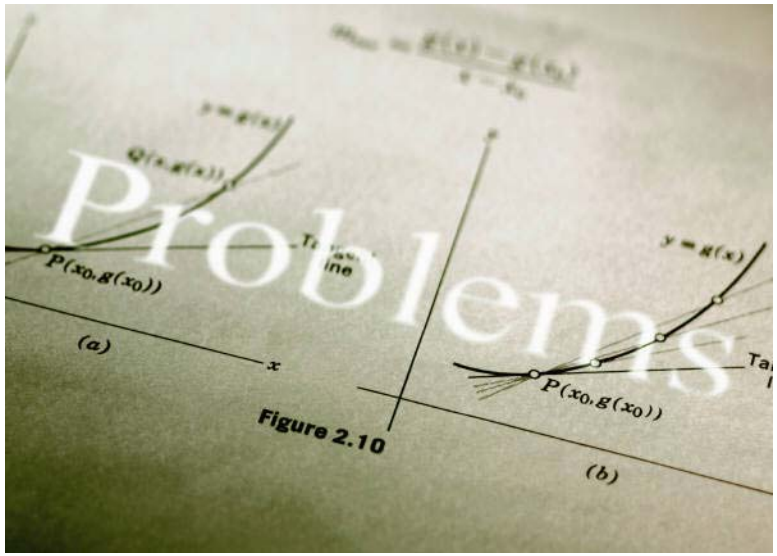
# Specification Setting

- **Finished product**
  - **Potency**
  - **Purity**
  - **Reconstitution time**
  - **Appearance**
  - **Sterility**
  - **Endotoxin**

# Scale Up

- **Development lyophilizer to Commercial Scale**
  - **Cycle modification needed?**
- **Batch size?**
- **Hold time stability?**

# CASE STUDY



# OBJECTIVES

- **Sponsor wishes to develop a lyophilized dosage form containing their large peptide, which upon reconstitution and dilution would be suitable for SC administration.**

- **The Sponsor requests a 5 or 10 mg/mL peptide concentration in the bulk drug product solution to be filled as 5 mL volumes into 10 mL vials then lyophilized yielding 25 or 50 mg of peptide per vial.**

# OBJECTIVES (cont.)

- **The bulk drug product solution should exhibit at least 24 hours of physical stability to facilitate drug product manufacturing (compounding, filtration, filling and lyophilization) and the reconstituted solutions should exhibit sufficient physical stability to permit convenient administration of the dose in the clinic or hospital.**

# TECHNICAL CHALLENGES

- **To determine suitable candidate formulations of a peptide that possessed at least 24 hours of physicochemical solution stability (freedom from precipitation) at room temperature**

- **To carry out MDSC analysis of the candidate formulations that have the desired stability characteristics in preparation for lyophilization cycle development**

- **To develop a lyophilization cycle (or cycles) that would result in elegant lyophilized cakes and reconstituted drug product solutions that would possess the desired physicochemical stability.**

# TECHNICAL CHALLENGES

- **Studies demonstrated that the solubility (and subsequent physical stability) of the peptide is dependent upon the solution pH with adequate solubility and physical stability observed in the pH 1 - 4 range. The peptide in aqueous solution tends to aggregate at varying rates depending on the pH and excipients present.**

# TECHNICAL CHALLENGES

- **Parenteral formulations for SC administration should be isotonic and in the pH 4 – 9 range to avoid tissue damage and pain upon injection.**

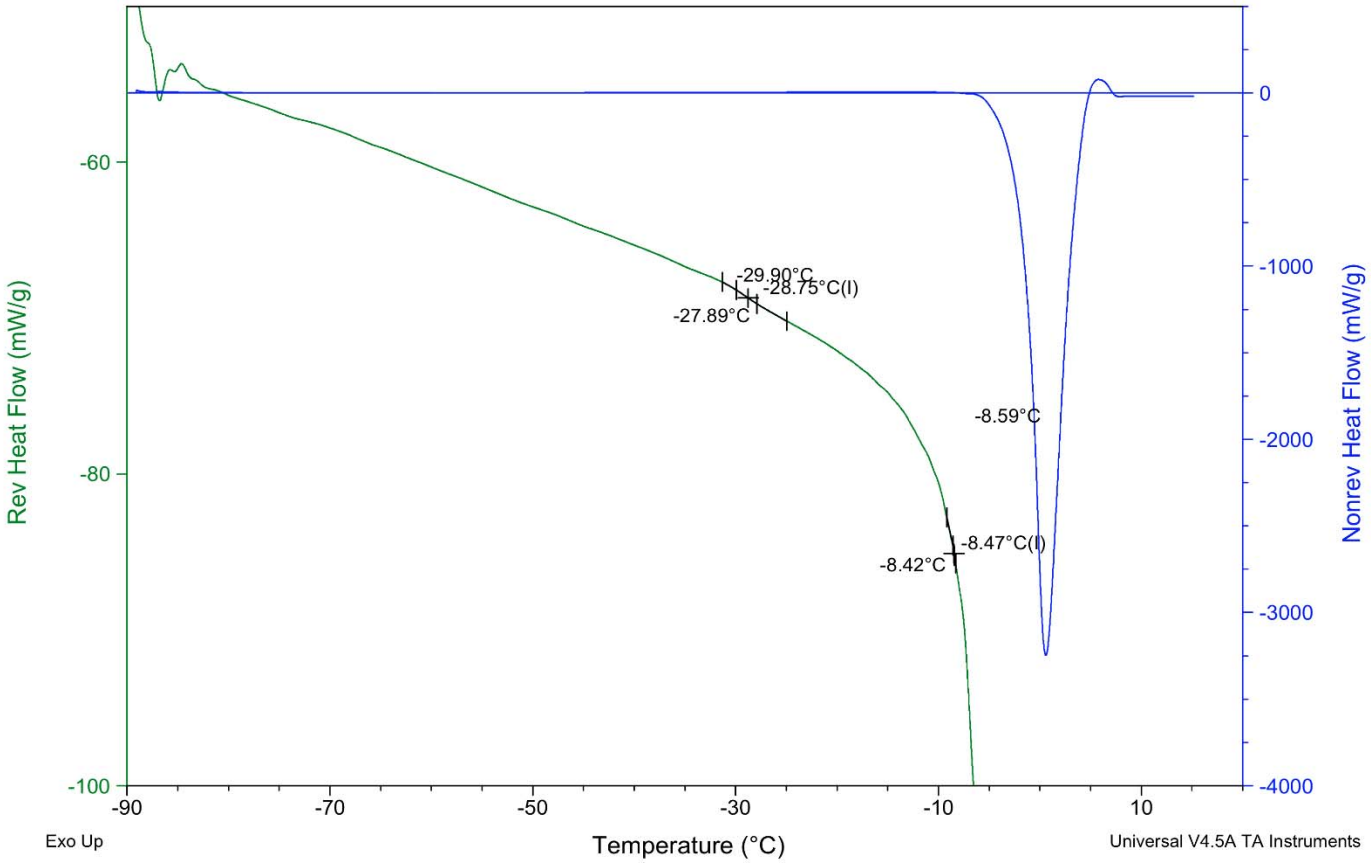
# PREFORMULATION STUDIES

- Studies were undertaken to enhance peptide physical stability beyond 24 hours for the pH-adjusted formulations.
- It appeared that the precipitation phenomena observed in aqueous solutions of peptide are driven predominately by intermolecular ionic charge interactions.
- In an effort to minimize these peptide aggregating interactions, experiments were performed to evaluate physical stability enhancement using different excipients to optimize formulation physicochemical stability.

# LYOPHILIZED FORMULATION DEVELOPMENT

- Counterion effects
- pH
- Surfactants
- Bulking Agents
- Co-solvents
- Cryoprotectants
- Bulk Solution Stability

# LYOPHILIZATION CYCLE DEVELOPMENT



# LYOPHILIZATION CYCLE DEVELOPMENT

- Pre-freeze
- Freeze
- Anneal
- Primary Drying
- Secondary Drying
- Post Cycle

# ASEPTIC FILL/FINISH

- Filter selection/compatibility studies
- Compatibility with manufacturing components
- Engineering/filling studies
- Vial/stopper selection
- Container closure integrity

# STABILITY TESTING

- Potency/purity
- Cake appearance
- Residual moisture content
- Osmolality
- Reconstitution time
- Sterility
- Endotoxin

# SUCCESS-OBJECTIVES MET!

- Formulation developed that exceeded solubility target enabling lower dosing volume
- Bulk formulation stability improved to enable large-scale manufacture
- Robust lyophilization cycle
- Reconstitution stability improved to eliminate aggregation and shorten reconstitution time
- Client licensed formulation and technology to large pharma

# CLINICAL FORMULATION DEVELOPED!

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