

Optimizing Protein and Peptide Formulations for Drug Delivery

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Emergence of Delivery Systems

- A wide variety of delivery systems have emerged or matured for biomolecules in the last few years
- The formulation scientist is lately confronted with a wide variety of choices and challenges:
 - routes of administration
 - formulations and dosage configurations
 - devices and applications

Alternate Delivery Routes Enhances Compliance

Special **Delivery**

***Alternative methods for
delivering drugs improve
performance, convenience,
and patient compliance.***

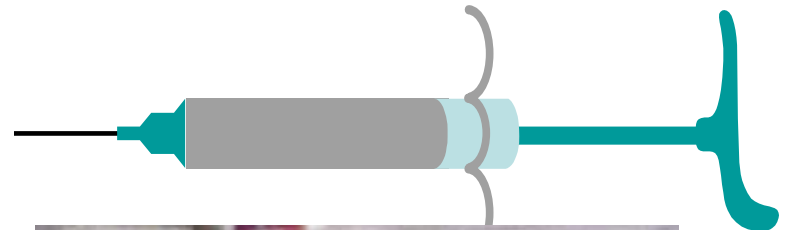


Delivery and Device Formulation Considerations

- Beyond the classical physicochemical stability several practical aspects may also be considered:
 - Functionality tests
 - Compatibility with device or package
 - Local tolerance at site of delivery
 - User friendly and compatible for admixture use
 - Viscosity

Parenteral Delivery Today

- IV administration
- Subcutaneous injection
- Continuous Subcutaneous Infusion (Pumps)
- Continuous Intraperitoneal Infusion
- Subcutaneous Depot (leuprolide, hGH)
 - PLGA microspheres
 - PEG attached peptides
 - Microemulsions
- Intrathecal, Intraparenchymal



Advance in Injectable Delivery

- Auto injectors/ Pen injectors
- Refined needle bore size
 - Novo Nordisk 31-gauge (.28 mm)
- Needleless injectors
- Dual chamber pre-filled syringes
 - Vetter Pharma
- Reconstitution devices
 - West Clip'n'Ject®
- Pre-sterilized low extractible closures
 - Westar®, B2-Teflon Flurotec® coatings

Why Prefillable Syringes?

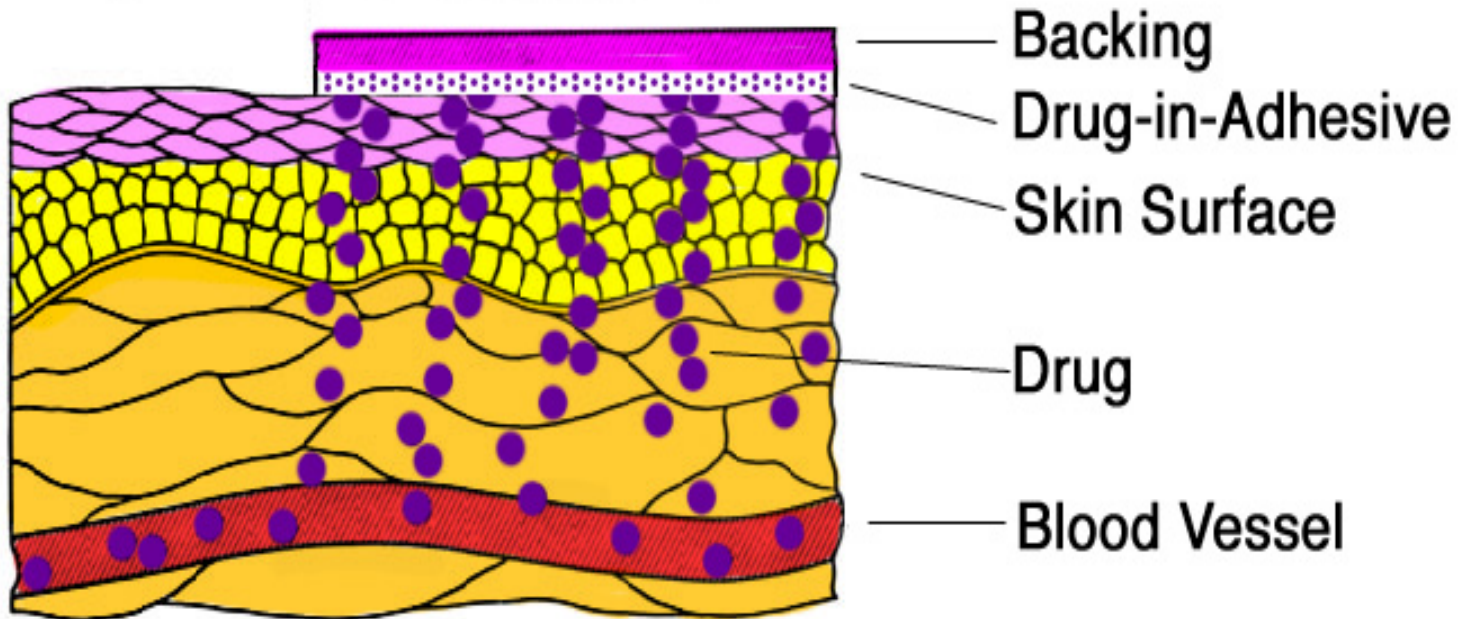
- **Convenience**
- **Safety**
- **Reduce dosing errors**
- **Reduce waste**
- **Cost effective**

Is Transdermal Delivery More Than Skin Deep ?

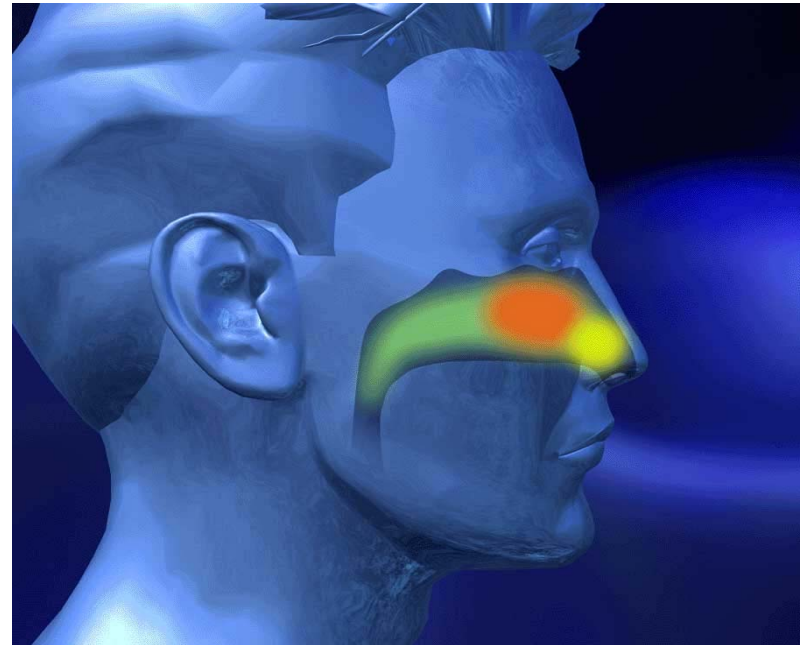
- TDD has evolved as waves of new technology
 - Transdermal patches
 - Iontophoretic patches
 - Electroporation concept
 - Microneedle transdermal systems
- Macromolecular delivery is being attempted
- A few critical issues:
 - Release of in-tact API from patch?
 - Immunogenicity is usually more pronounced

Transdermal Delivery

Drug-in-Adhesive Patch on Skin



Lung versus Nose



Advantages of Pulmonary Delivery

- Suitable for local or systemic agents
- Large absorptive surface area
- Bypasses GI tract and avoids problems of degradation, poor absorption and first-pass metabolism
- Relatively high bioavailability of drug
- Lower doses
- Non invasive; patient friendly
- Cost effective



Advantages Of Nasal Delivery

- Readily accessible
- Good absorptive surface
(large area, good blood supply, and permeable)
- Avoids gastrointestinal complications (first-pass, acid stomach, and protease activity)
- Rapid onset of action
- High patient compliance
- Portable devices
- Cost effective



Preformulation Development

- Preformulation Development Procedure
 - Identify Candidate Preformulation Buffer Conditions
 - Solubility
 - Biophysical Characterization
 - Statistical Design
 - Accelerated Stability
 - Candidate Formulation Buffers

Preformulation

- Characterize the secondary structure
- Determine thermal stability
- Determine solubility (if necessary)
- Narrow down conditions that contribute to a stable formulation
 - pH range
 - Buffer type
 - Excipients

Typical Designs for Preformulation

- Preformulation Workflow:
 - Baseline biophysical studies
 - Linear ranging studies / solubility studies
 - Identify critical categorical factors
 - Construct DOE
 - Prepare summary protocol
 - Buffer exchange & prepare samples for stability
 - Place on stability (time & temperature dictated by biophysical data)
 - Sample and data analysis

pH	Buffer Conc	NaCl Conc	Buffer Type
6	60	0	Phosphate
7	60	0	Histidine
6	10	150	Phosphate
6.5	35	75	Histidine
6.5	35	75	Histidine
7	10	0	Phosphate
7	60	150	Phosphate
6.5	35	75	Phosphate
6.5	35	75	Histidine
6.5	35	75	Phosphate
7	10	150	Histidine
6	60	150	Histidine
6	10	0	Histidine
6.5	35	75	Phosphate

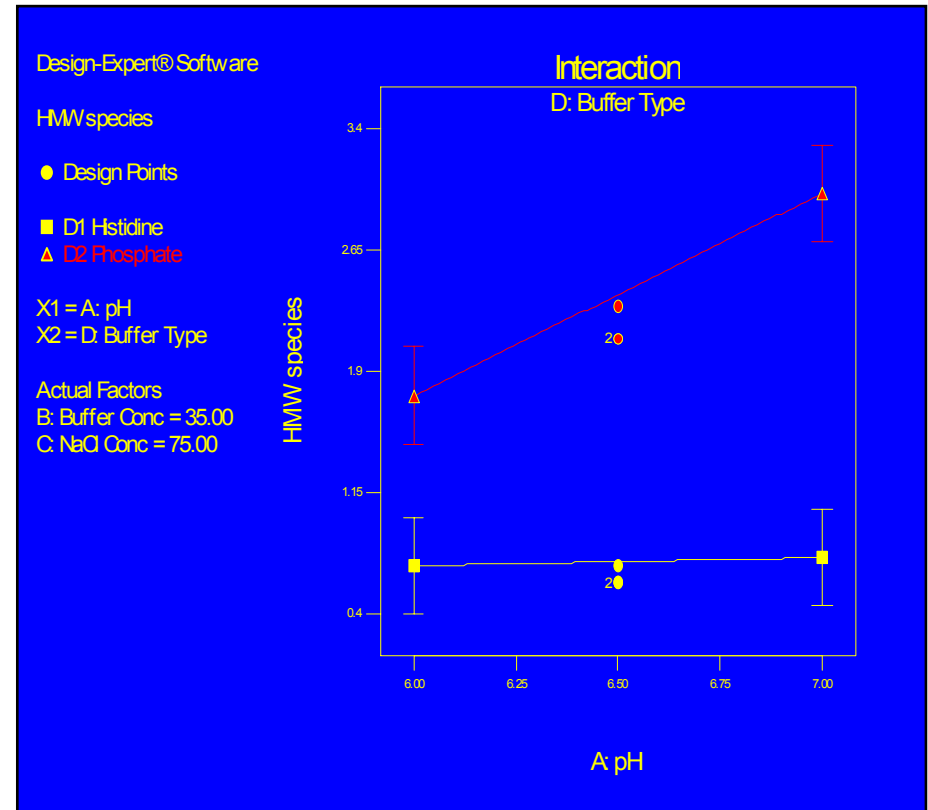
Effect of Buffer Type on MoAb Stability

- DOE Summary

- Two fractional factorial designs
- Selected to span a wide range of pH conditions
- MoAb concentrated to >200 mg/mL, placed at 40°C for 4 weeks

Full panel of analytics performed:

- SEC showed effect of buffer/pH on HMW species
- Phosphate buffer: HMW increases with increasing pH
- Histidine buffer: HMW stable from pH 6 - 7



Considerations for Peptide Formulations

- Physical characteristics may have dramatic effect on formulation
- Drastic solubility changes should be investigated for potential polymorphs
- Inherent surface activity of peptides can form micelles in solution with significant solubility shift and precipitation
- Excessive hydrogen bonding cause gel formation

Preformulation

- pH range
 - Solubility is limited close to the pI
 - Deamidation and oxidation concerns
 - Stability of the protein
- Buffer type
 - Appropriate for freeze-drying?
 - Phosphate and acetate can both experience pH shifts
 - Appropriate for the pH range?
- Excipients
 - Stabilizer
 - Bulking agent
 - Tonicity modifier
 - Other

Formulation Development

- Optimize formulation component concentrations
 - Buffer
 - Stabilizers
 - Other excipients
- Optimize pH
- Perform forced degradation studies
 - Identify degradation pathways
 - Ensure the ability of analytical techniques to detect impurities
- Perform accelerated stability studies

Lyophilization Cycle Development

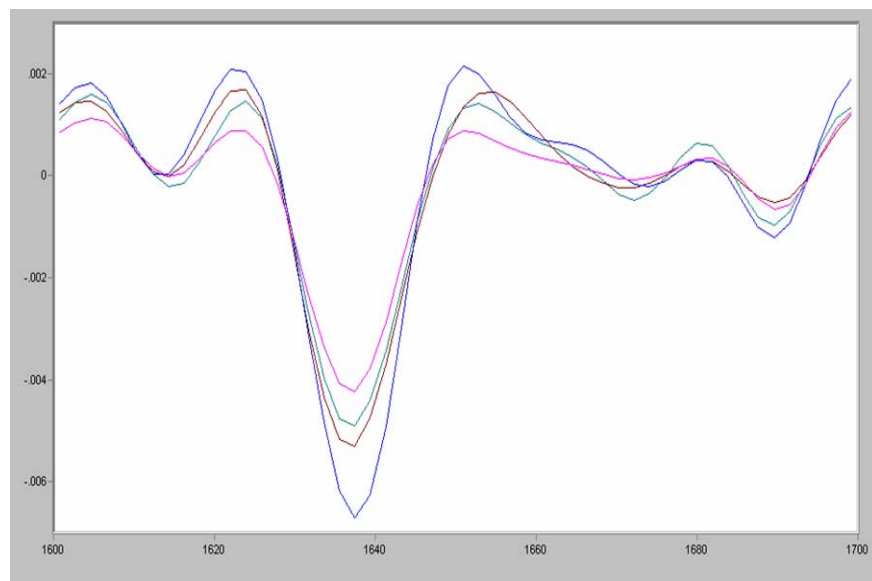
- Determine appropriate product temperatures
 - T_g'
 - Crystallization temperature
 - Eutectic melting point
- Determine appropriate process times
 - Annealing
 - Primary drying
 - Secondary drying

Formulation/Cycle Optimization

- Optimize bulking agent concentration
- Optimize process times, temperatures, and pressures
- Perform cycle robustness studies

Fourier Transform Infrared Spectroscopy

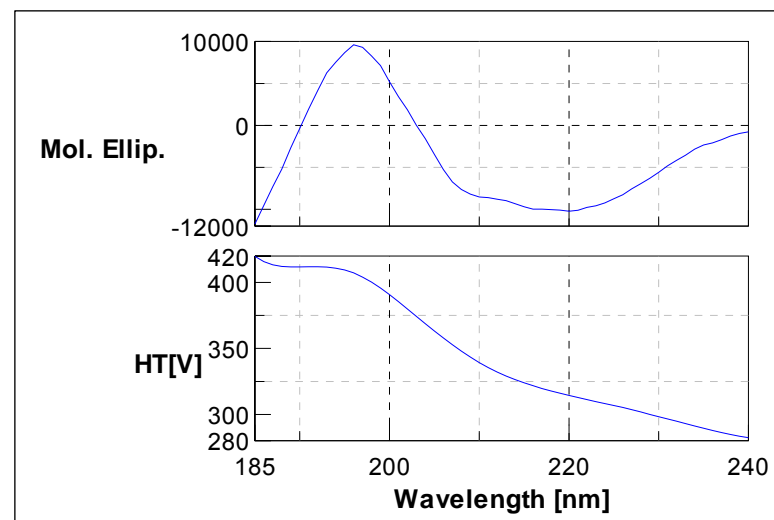
- Determining secondary structures of proteins
- Identifying changes in secondary structure due to processing/stressing



Circular Dichroism

Uses:

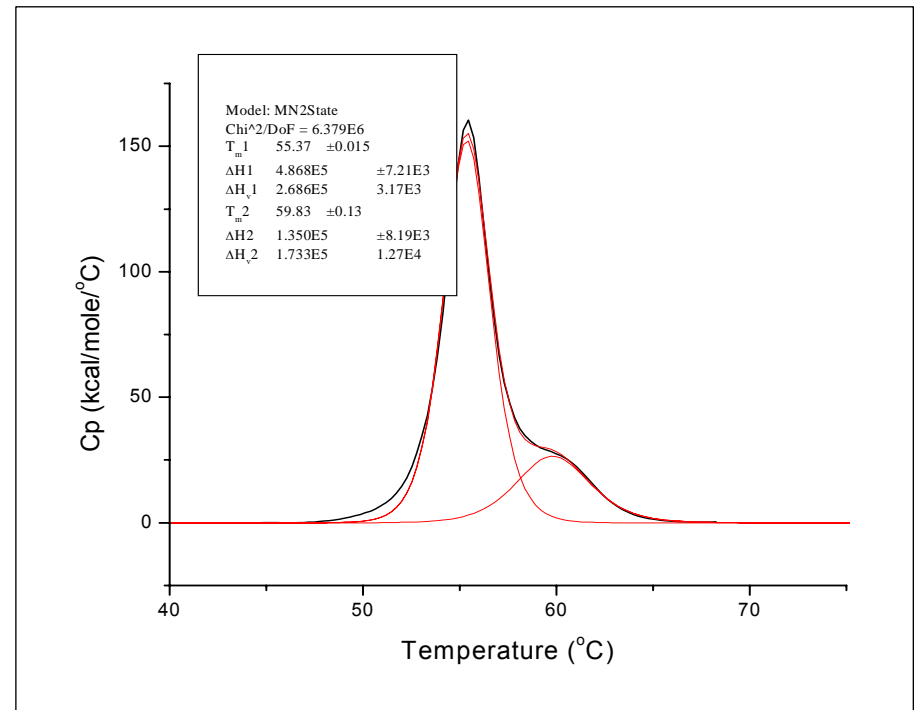
- Analyzing secondary structures
- Identifying changes in secondary structure due to processing/stressing
- Determining melting points of proteins
- Comparing thermal stability of a protein in various formulations



Microcalorimetry

Uses:

- Determining melting points of proteins
- Comparing thermal stability of a protein in various formulations
- Determining reversibility of unfolding



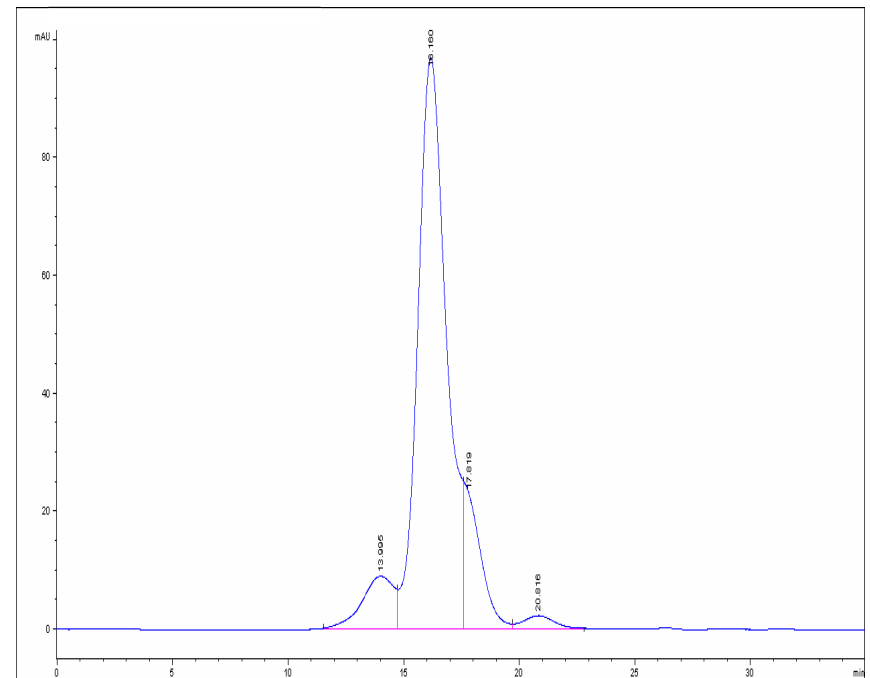
Liquid Chromatography

Uses:

- Identifying low and high molecular weight species
- Identifying physical/chemical changes in the protein

Common types:

- SEC, IEX, Reverse-Phase, HIC



Differential Scanning Calorimetry

- Identify thermal properties of formulations
 - T_g' , maximally concentrated glass transition temperature
 - Crystallization
 - Eutectic melting
 - T_g , glass transition temperature

Other Analytical Tools

- Peptide Map
- SDS-PAGE
- Western Blot
- Iso-Electric Focusing
- Protein Assays
- UV/Vis Spectrophotometry
- Mass Spectroscopy

Challenges to Maintaining Protein Stability

- Buffer exchange and filtration
- Transportation
 - Agitation
- Freeze/thaw
 - Thermodynamic instability
 - Freeze-concentration
 - Phase separation
- Lyophilization process
 - Freezing
 - Drying
- Storage
 - Container/closure integrity
 - Thermal stability
 - Photostability

Buffer Exchange/Filtration

- Be aware of the pH shift and what it will mean for the protein
- Choose appropriate membrane materials and sizes
- Optimize transmembrane pressure and shear when utilizing diafiltration

Freeze/Thaw

- The native state of the protein becomes thermodynamically unfavored at low temperatures
- Freezing causes solute concentration and phase separation
 - pH shifts
 - Increased interaction between solutes
- As the solution thaws the protein can see shifts in its microenvironment

Lyophilization – Freezing

- Heterogeneous supercooling
 - Varying sizes of ice crystals
 - Results in inconsistent drying times
- Inconsistent crystallization
 - Causes problems during drying and storage
- Solutions:
 - Slow cooling rates (limited effect)
 - Annealing

Lyophilization – Drying

- Maintain the product temperature below its T_g' (amorphous solids)
- Maintain the product temperature below its T_{eu} (crystalline solids)
- Must also maintain vacuum and condenser control

Storage

- Must produce a sufficiently dry product
 - Underdrying can lead to product collapse and/or degradation during storage
 - Overdrying can lead to problems during reconstitution and poor recovery
- Container/closure integrity must be maintained
 - Moisture
 - Contamination
- Thermal and Photo- stability must be determined so proper storage conditions are maintained

Formulation and lyophilization cycle development for a labile peptide

Background

- Sponsor API was a labile peptide with a free cysteine and was prone to dimer formation.
- The existing formulation employed mannitol as a bulking agent and utilized an unconventional lyophilization cycle.
- Two API concentrations were used (10 and 100 $\mu\text{g}/\text{mL}$)

Objectives

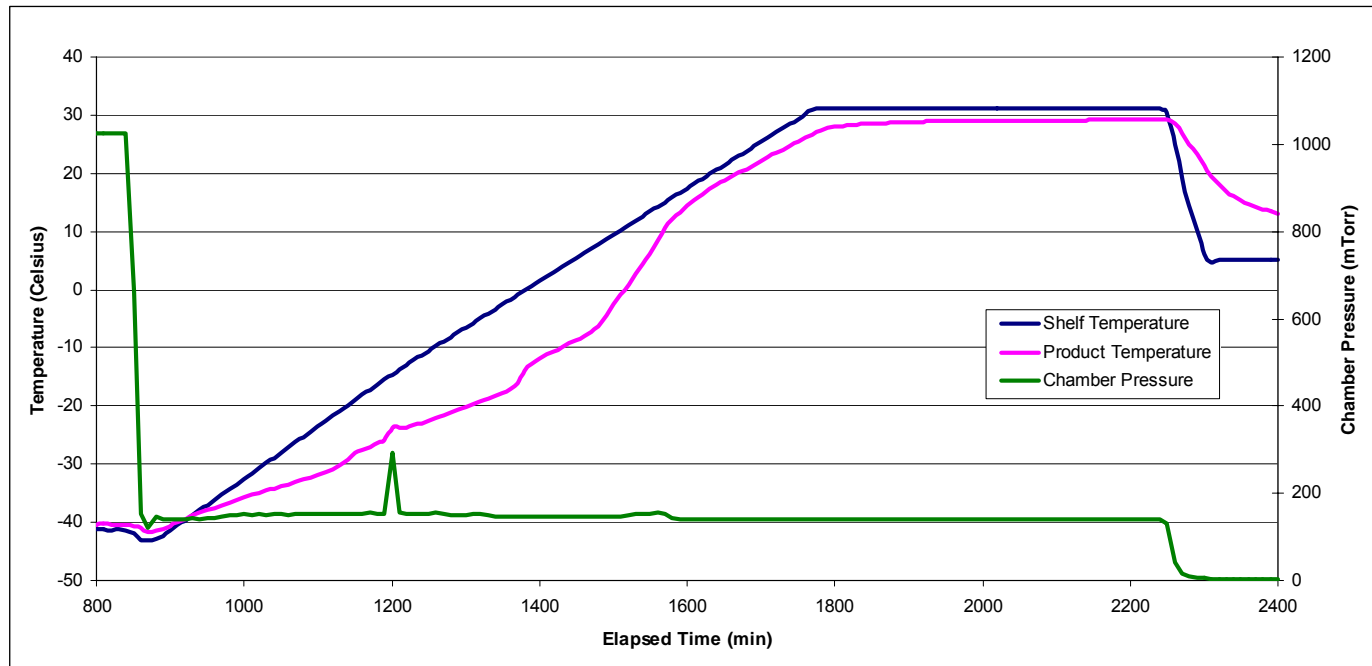
- Identify the pitfalls in the existing formulation and cycle
- Optimize the formulation using statistical analysis
- Develop a lyophilization cycle based on the optimized formulation

Formulation and Lyophilization Cycle Development for a Labile Peptide

- Analysis of existing formulation and lyophilization cycle
- Formulation development
- Lyo cycle development
- Accelerated stability

Background

Original Matrix: 10 or 100 $\mu\text{g}/\text{mL}$ peptide, 10 μM EDTA, and 2% mannitol
Original Lyophilization Cycle:



Freeze-Drying Stability Study

The effects of freezing rate, primary drying, secondary drying, and secondary drying temperature on peptide stability were studied.

Results:

- The peptide was not sensitive to freeze/thaw or freezing rate.
- A significant loss of product purity occurred during the ramp from -15°C to 31°C .
- The final secondary drying temperatures tested did not have a significant effect.

Stability Results for Original Formulation/Cycle

Formulation	Effect of freezing rate on purity	% Purity
100ug/mL Peptide, 10uM EDTA, 2% Mannitol	-80°C fast freeze	99.65
	1°C/min cooling rate to -43°C	99.72
	0.1°C/min cooling rate to -43°C	99.59
	Effect of primary drying on purity	% Purity
	Sample pulled at -15°C during primary drying	99.56
	Effect of secondary drying on purity	% Purity
	20°C secondary drying (7hrs)	97.44
	20°C secondary drying (12hrs)	96.66
	31°C secondary drying	97.91

Even though the peptide purity immediately following lyophilization was high, storage for only 1 week at 40°C/75% relative humidity resulted in a loss in purity of 10-20%.

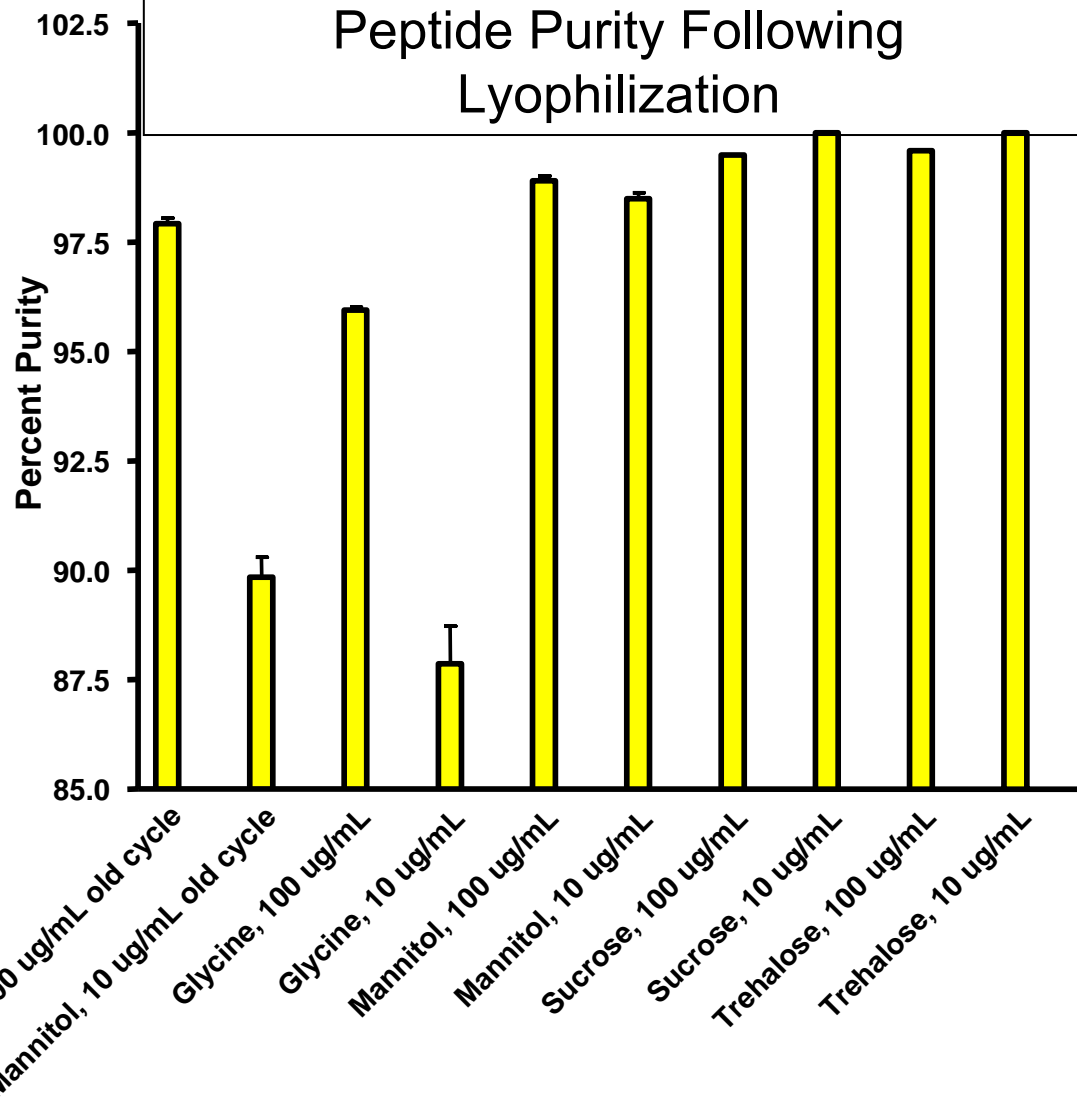
Design of Experiment

Apply a statistical design analyzing the effectiveness of various bulking agents as well as the use of a chelating agent. Also, modify the lyophilization cycle.

Statistical Design:

- Bulking agents screened: mannitol, glycine, sucrose, trehalose
- Treatment/No treatment with a chelating agent
- Peptide concentration: 100 μ g/mL or 10 μ g/mL

Peptide Purity Following Lyophilization



Results of Statistical Design

- Superior post-lyophilization product stability was conferred by amorphous bulking agents (sucrose and trehalose).
- Sucrose containing formulations proved the most stable during an accelerated stability study.
- No statistically significant effect was seen with the chelating agent.
- Peptide concentration was a significant factor only in the glycine formulations.

DESIGN-EXPERT Plot

Percent Purity

X = Excipient

Y = Percent Purity

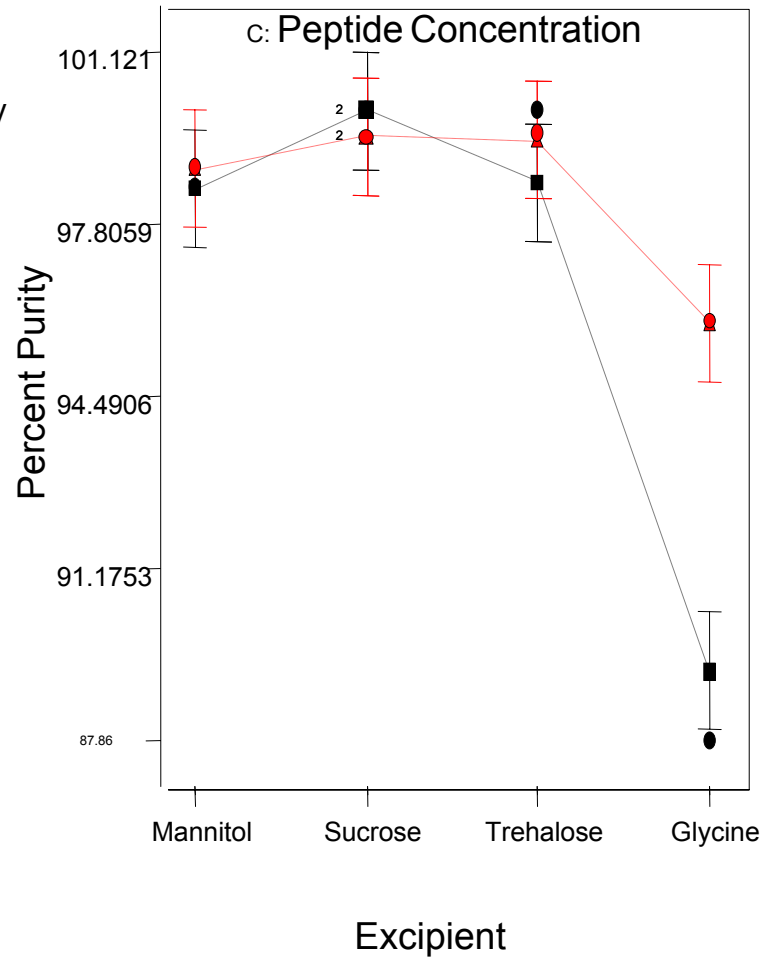
● Design Points

■ C1 10 ug/mL

▲ C2 100 ug/mL

Interaction Graph

c: Peptide Concentration



Final Optimization

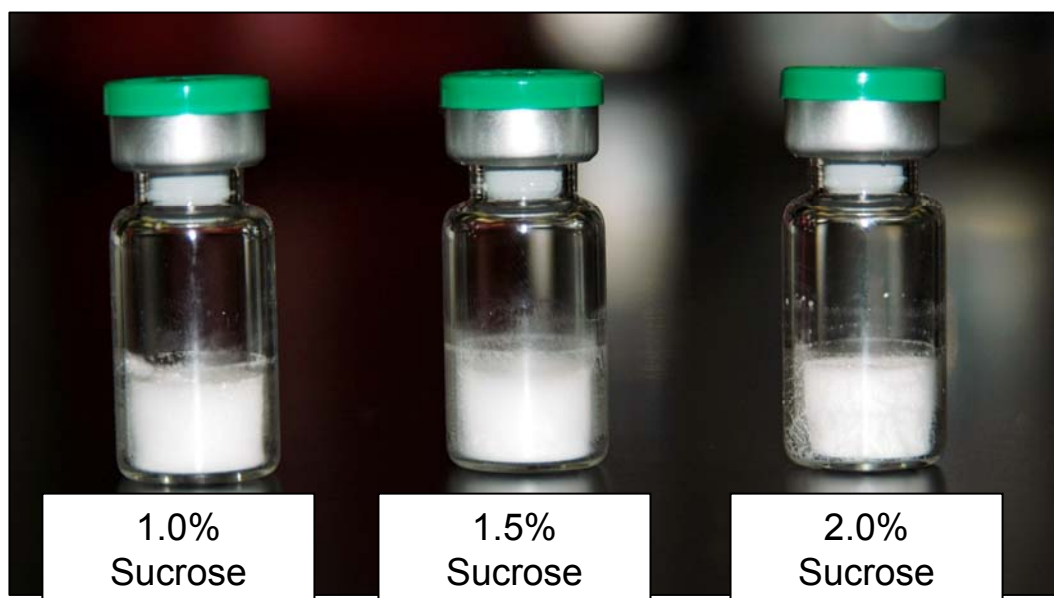
After settling on sucrose as the bulking agent, the concentration of sucrose and the lyophilization cycle were optimized. Sucrose was used at concentrations of 0.5%(w/v), 1.0%, 1.5%, and 2.0%

Final Lyophilization Cycle:

Ramp Rate (°C/min)	Temperature (°C)	Pressure (mTorr)	Hold Time (min)
--	5.0	--	15
0.5	-5.0	--	15
1.0	-43	--	180
0.5	-30	100	1800
0.2	5.0	100	180
1.0	25.0	100	300

Results from Optimized Lyo Formulation and Cycle

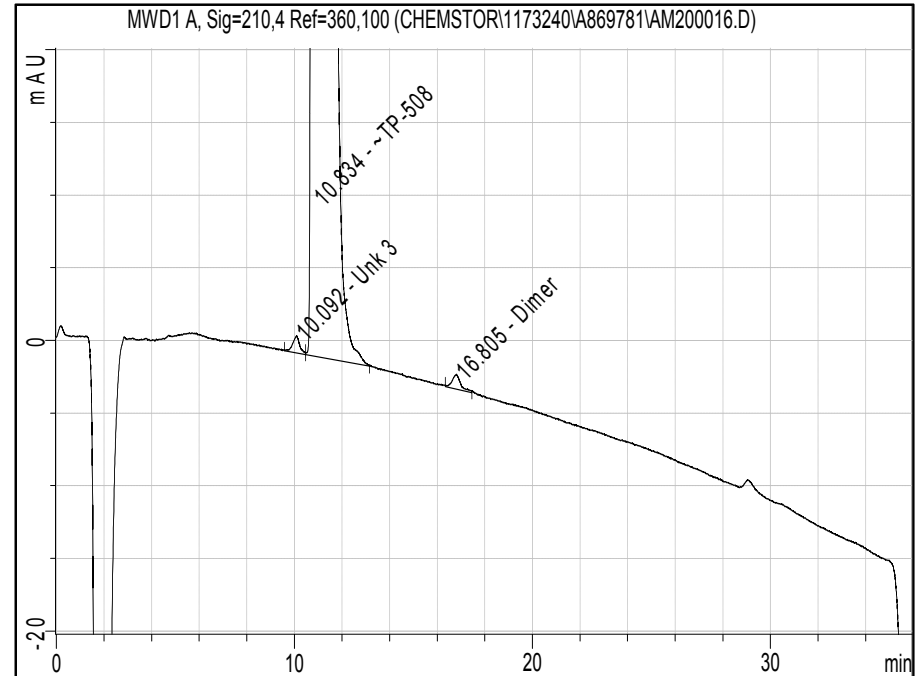
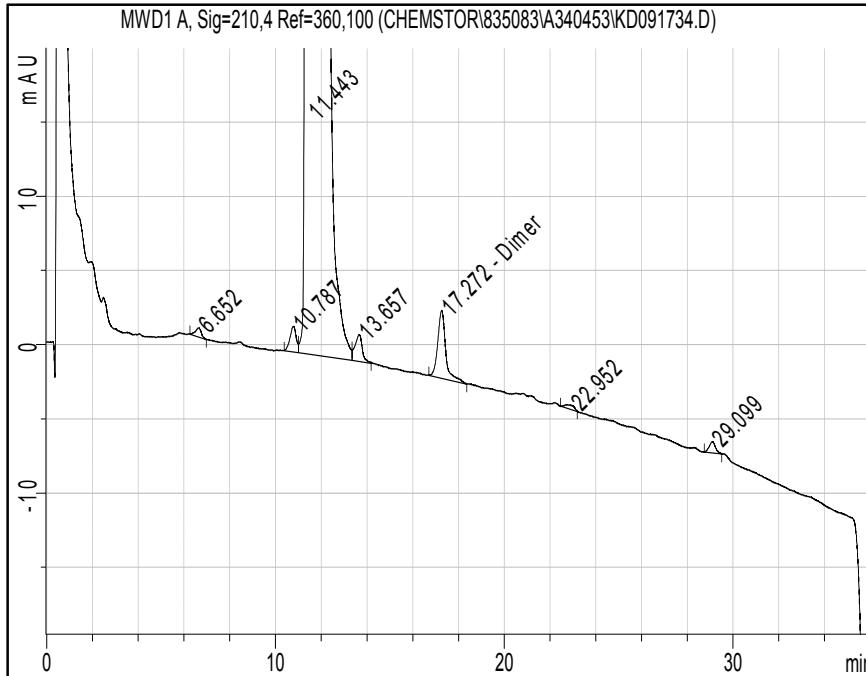
- Peptide purity was maintained at 99% following lyophilization using a sucrose containing formulation along with the optimized lyophilization cycle.
- The lyophilization cycle produced cakes with <math><1.5\%</math> residual moisture.



Comparison

RP-HPLC of original formulation lyophilized with the original lyophilization cycle:
~97% main peak

100µg/mL peptide, 10µM EDTA, 1% sucrose
99% main peak



Results

- An amorphous bulking agent was required for product stability. Sucrose proved to be the optimal choice.
- Primary drying needed to be carried out at a product temperature less than -35°C for the sucrose containing formulation.
- $\geq 1\%$ sucrose was needed for the physical stability of the cake.
- The final lyophilization cycle and formulation maintained peptide purity at 99% for the $100\mu\text{g}/\text{mL}$ peptide samples and 97% for the $10\mu\text{g}/\text{mL}$ peptide samples when stored for 2 weeks at $40^{\circ}\text{C}/75\%$ relative humidity.

Conclusions

- Statistical design of experiment is a valuable screening tool
- The proper excipients are key in maintaining long term product stability
- Lyophilization is not automatically a “safe” process for proteins and peptides

Acknowledgements

- Stephen Cottle
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