Therapeutic Proteins: Solution Behavior During Pre-Formulation and Formulation Development

2007 Current Trends in Calorimetry
Boston, MA

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Calorimetry in Pharmaceutical Protein Development

- Academic vs. pharmaceutical development: different emphasis
- Needs to be rapid
  - Shorter development timelines
  - Formulation development time "squeezed"
- Needs not be absolute
- Needs predictive power

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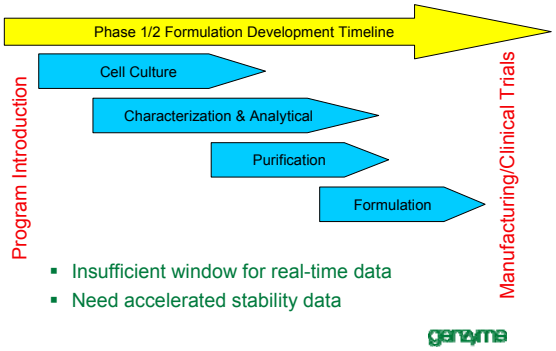
Product Development Timeline



- Insufficient window for real-time data
- Need accelerated stability data

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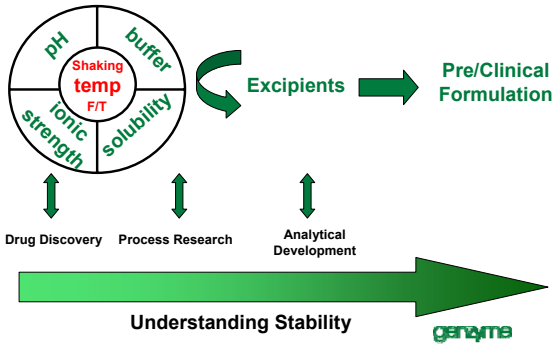
Formulation Development Overview



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Protein Degradation and Characterization

- Common modes of degradation
  - Physical
    - 2º and 3º structural changes
    - Aggregation/self-association
    - Fragmentation
  - Biochemical
    - Oxidation
    - Deamidation
    - Isomerization
- Characterization methods
  - DSC
  - CD
  - Fluorescence
  - FTIR
  - DLS
  - SEC-MALS-HPLC
  - Peptide mapping
  - IEF-PAGE
  - IEC-HPLC
  - SDS-PAGE

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Thermodynamics and Predicting Structural Stability

- Common mechanism linked to structural instability
  - Partial unfolding → aggregation
  - Preferential exclusion → aggregation prevention
  - Ligand effects → structural stabilization
- Potential pitfalls
  - Non-ideal system → apparent parameters
  - No direct link to kinetics
  - Degradation pathway dependant

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DSC, Thermodynamics, and Formulation Development

- Applications
  - Preformulation development
    - Solution behavior characterization
    - Storage temperature identification
    - Buffer selection
  - Interface with other groups
    - Characterization
    - Drug discovery
    - Purification/process development
  - Formulation development
    - Excipient screening
    - Ligand screening
    - Search for synergistic effects
- Considerations
  - Material constraints
    - Interface with drug discovery
    - Orthogonal techniques-spectroscopy utilizing SVD analysis
  - Complexity of deg path
  - Multi-domain unfolding
  - Scan rate issues and kinetics

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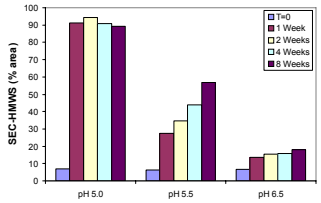
Preformulation Development: Appropriate Accelerated Storage Temperatures

- Need accelerated stability data
  - Trends must be representative
  - Avoid irrelevant degradation pathways



- pH screening
- 37°C storage
- 90% HMWS after 1 week at pH 5

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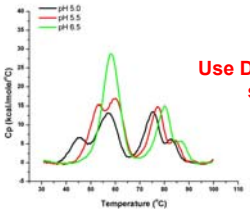
Preformulation Development: Appropriate Accelerated Storage Temperatures



Use DSC to help set appropriate storage temperatures

- Low melting temperatures
- Strong pH effect
- Lower pH → shift in unfolding equilibrium below 40°C

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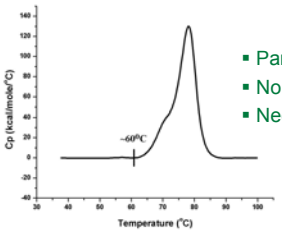
Preformulation Development: Appropriate Accelerated Storage Temperatures



- Particularly stable molecule
- No changes at 30°C
- Needed additional stress

Increased storage temperature to 40°C

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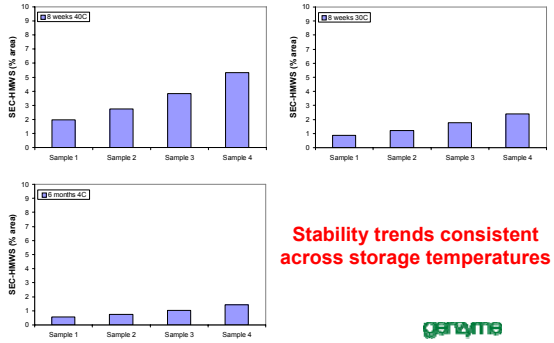
Preformulation Development: Appropriate Accelerated Storage Temperatures



Stability trends consistent across storage temperatures

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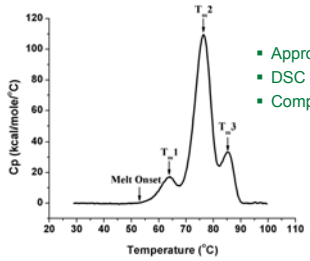
Preformulation Development: pH Identification and Buffer Effects



- Appropriate pH is critical
- DSC + accelerated temperature
- Complex multi-domain unfolding

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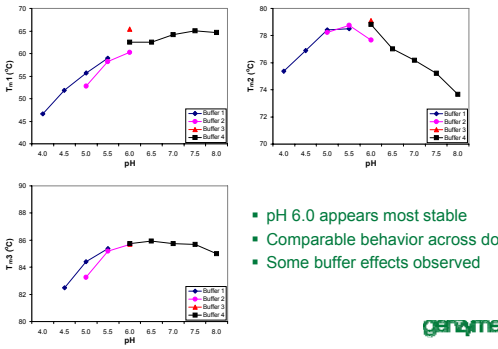
Preformulation Development: pH Identification and Buffer Effects



- pH 6.0 appears most stable
- Comparable behavior across domains
- Some buffer effects observed

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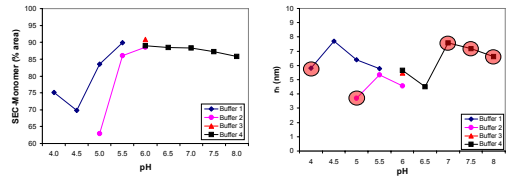
Preformulation Development: pH Identification and Buffer Effects

Samples stored 8 weeks @ 37°C



=samples also show large aggregate (>100 nm)

Size-exclusion chromatography
- Most monomer retained pH 5.5-6.5

Dynamic light scattering
- pH 5.5-6.5 shows decreased aggregation/self-association

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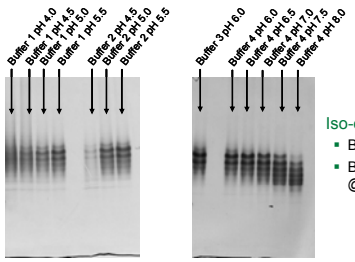
Preformulation Development: pH Identification and Buffer Effects

Samples stored 8 weeks @ 37°C



Iso-electric focusing
- Buffer 2 degradation
- Biochemical instability @ higher pH

Cannot consider structural stability only!

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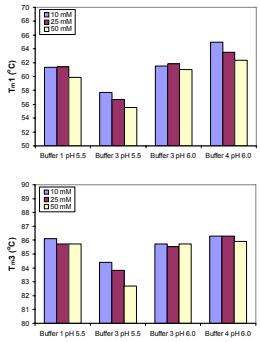
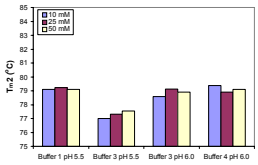
Preformulation Development: Buffer Species and Concentration Identification





- pH trend consistent with previous data
- Variable behavior across domains
- Higher concentrations → less stable?

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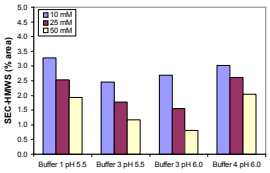
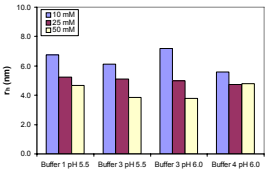
Preformulation Development: Buffer Species and Concentration Identification

Samples stored 10 weeks @ 37°C





Size-exclusion chromatography
- Both buffer and concentration effects observed

Dynamic light scattering
- Higher concentration → more compact molecule

Stability trend not predicted by DSC

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Preformulation Development Summary

- Storage temperature identification
  - Consistency of trends is critical
  - DSC helps identity accelerated storage temperature
- pH identification
  - Good correlation between thermal stability and degradation
  - Thermodynamics cannot predict biochemical stability
  - DSC helps identify some buffer effects
- Buffer identification
  - Multiple transition make prediction more difficult → variable behavior
  - DSC did not predict concentration or buffer effects

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DSC, Thermodynamics, and Formulation Development

- Applications
  - Preformulation development
  - Purification/process development interface
  - Formulation development
- Considerations
  - Orthogonal techniques
  - Complexity of degradation pathways
  - Scan rate

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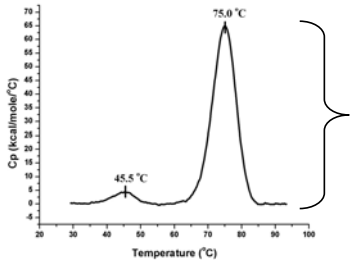
Purification/Process Development Interface: Low pH Incubation

- Low pH incubation → 60-90 minutes
  - Decreased stability post incubation
- Identify post incubation storage conditions



pH 3.8 thermogram

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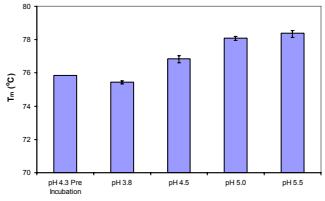
Purification/Process Development Interface: Low pH Incubation



Post low pH incubation

- Increased pH → increased thermal stability

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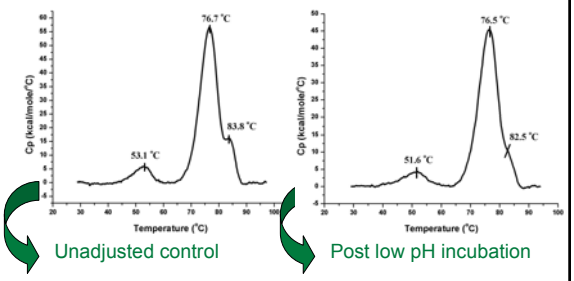
Purification/Process Development Interface: Low pH Incubation



Unadjusted control

Post low pH incubation

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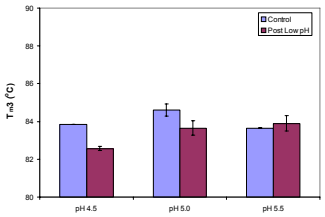
Purification/Process Development Interface: Low pH Incubation



Low pH incubation yields less thermally stable molecule

- Partial unfolding confirmed by CD and AUC at low pH

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DSC, Thermodynamics, and Formulation Development

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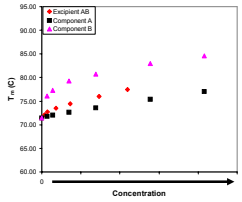
Formulation Development: Stabilization Mechanisms

- Identified excipient with significant impact on thermal stability
- Excipient → 2 components
- Investigate mechanism of stabilization



Greatest stabilization from component B

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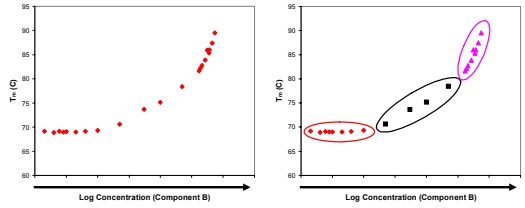
Formulation Development: Stabilization Mechanisms



- Investigated wide range of component B concentrations
- Large thermal stabilization (up to 20°C)
- Multiple stabilization trends

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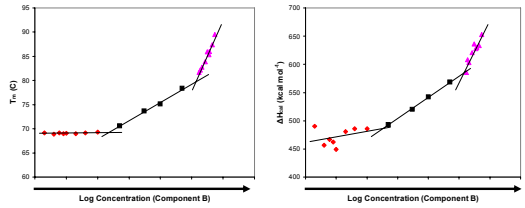
Formulation Development: Stabilization Mechanisms



- Apparent T_m and calorimetric enthalpy display similar trends
- May imply multiple stabilization mechanisms

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DSC, Thermodynamics, and Formulation Development

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DSC Limitations and Considerations

- Pros
  - High throughput
  - Reproducible
  - First principle
  - Virtually unlimited buffer compatibility
- Cons
  - Some buffers are favored
  - DSC material requirement are not trivial
    - 400µL at 1 mg/mL

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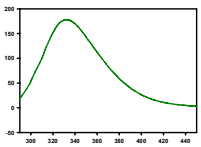
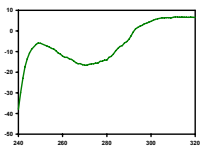
DSC Limitations and Considerations: Orthogonal Techniques

Fluorescence



Near UV-CD



- Fluorescence
  - Low sample requirements (500 µL at 50 µg/mL)
  - Relies on distribution of tryptophans
- Near UV-CD
  - Global probe → includes all aromatic residues
  - Larger sample requirement (300 µL at 100 µg/mL)

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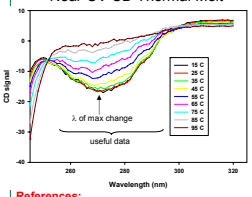
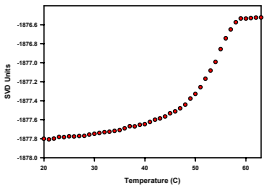
DSC Limitations and Considerations: Orthogonal Techniques

- Single-Value Decomposition (SVD) analysis
  - Generate single unfolding curve from all unfolding data (i.e. multiple CD wavelengths)
  - Model independent
  - Improve signal:noise compared to single wavelength

Near-UV CD Thermal Melt



SVD Unfolding Curve



References:
Henry, E.R. and Hofrichter, *J. Methods Enzymol*. 1992, 210 (1), 129-192.
Ionescu, et al. *Biochemistry* 2000, 39 (31), 9540-9550.

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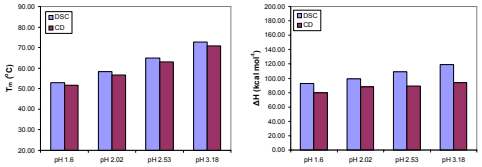
DSC Limitations and Considerations: Orthogonal Techniques

Lysozyme Unfolding



DSC and CD+SVD in good agreement for T_m and ΔH

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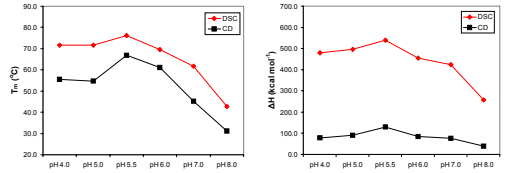
DSC Limitations and Considerations: Orthogonal Techniques

Protein Unfolding



Absolute values for T_m and ΔH differ but trends are similar

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DSC, Thermodynamics, and Formulation Development

- Applications
  - Preformulation development
  - Purification/process development interface
  - Formulation development
- Considerations
  - Orthogonal techniques
  - Complexity of degradation pathways
  - Scan rate

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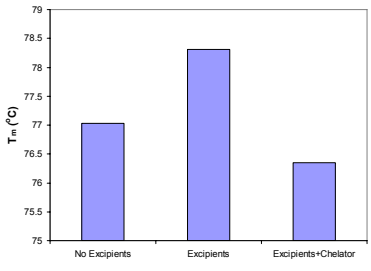
DSC Limitations and Considerations: Complexity of Degradation Pathway

- pH and buffer selection complete
- Excipient screening



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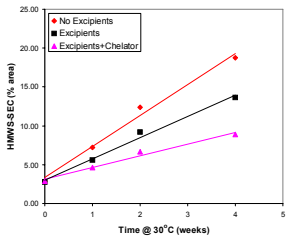
DSC Limitations and Considerations: Complexity of Degradation Pathway



- Addition of excipients
  - Increased thermal stability
  - Decreased degradation
- Addition of chelator
  - Decreased thermal stability
  - Decreased degradation

Need to understand degradation pathways to fully utilize predictive power of DSC

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DSC, Thermodynamics, and Formulation Development

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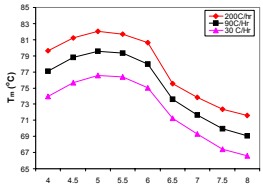
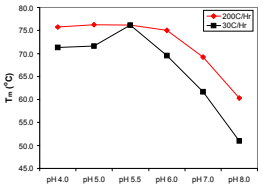
DSC Limitations and Considerations: Scan Rate Issue

Non-ideal systems → thermodynamic parameters are scan rate dependent



Trends are consistent



Stability trend is compromised

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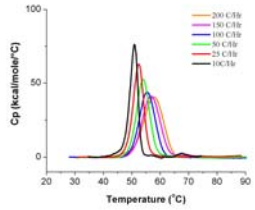
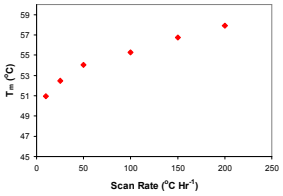
DSC Limitations and Considerations: Scan Rate Issue

Investigation into scan rate effects





Apparent T_m follows predictable trend as function of scan rate

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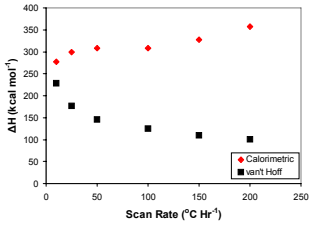
DSC Limitations and Considerations: Scan Rate Issue



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DSC Limitations and Considerations: Scan Rate Issue

- Practical considerations
  - Potential loss of predictive power
  - Throughput
- Potential applications
  - Thermodynamics vs. kinetics
  - Potential link to kinetics of unfolding
  - Extrapolation of degradation kinetics???

Reference:
Sanchez-Ruiz, J.M., Biochemistry. 1988, 27, 1648-1652.

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Summary

- DSC and thermodynamics are critical for preformulation/formulation development
  - Identification of storage temperature
  - pH and buffer identification
  - Excipient and ligand characterization
  - DSC provides value to process development
- DSC and thermodynamics have limitations
  - Material requirements (DSC)
  - Knowledge of degradation pathway is essential
  - Non-ideal behavior → norm for biopharmaceutical proteins

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