Aggregation challenges in the formulation development of multi-dose peptide products

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AGGREGATION CHALLENGES IN THE FORMULATION DEVELOPMENT OF PEPTIDE PRODUCTS: KINETICS AND ANALYTICS

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Peptide Therapeutics Constitutes an Unique Modality

Small molecule drugs < 500 Da

Peptides 5 ~ 40 amino acids

Biologics > 5000 Da (e.g. cytokines, growth factors, mAbs) Injectable

Chemical control and definition

Specificity and half-life

Dosing route

- Peptide products differentiate with other modalities in targets requiring high affinity agonist action, especially those associated with natural hormones.
Peptides Present Unique Challenges for Parenteral Formulation Development

- Formulation goal: bioavailability + **stability** + sterility…
- Peptides typically don’t have well ordered structure and therefore chemical and physical stability could be worse than biologics. Daily use product require >18 month 5°C solution product
- In particular, poor understanding in the developability study for physical stability
- Stability could frequently be the limiting factors in peptide formulation development (e.g. glucagon).
Reversible and Irreversible Aggregation in Synthetic Peptides

Disordered irreversible aggregates

Prefibrillar species

Variable conformation (T, pH, ion, etc)

Monomer

Oligomers

Irreversible fibrils

Nonspecific reversible aggregates

Native conformation

β-sheet conformation
Amyloid Fibrils are Supramolecular Assemblies with Highly Ordered Stacking

Nucleated Polymerization of Fibril Kinetics

LAG PHASE
- Native-state monomers
- Native-state dimerization
- Native-state dimers
- Partial denaturation
- Misfold or Molten Globule Amyloidogenic?
- Complete denaturation
- Denatured state monomers
- Amyloidogenic oligomers
- Unstructured Aggregate
- Non-specific Aggregation
- Small Amyloidogenic oligomers
- Amyloid Seeds
- Polymerization
- Colloidal conversion/folding

GROWTH PHASE
- Protofibrils
- Fibril Bundling
- Mature Fibril
- Fibril Bundling
- Filaments
- Filament elongation
- Protofibril elongation
- Amyloid Seeds
- ThT Fluorescence (FEL)

Graph:
- Soluble Peptides (mg/mL)
- Time (Hour)
- Nucleation
- Fibril Elongation

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Where is the Gap in the Development Knowledge?

• Is it due to the non-ideal behavior in kinetics between accelerated conditions and real time?

\[ k = A \exp\left(-\frac{E_a}{R \times T}\right) \]

High unfolding energy can contribute to the non-Arrhenius behavior.

• Is it because the how representative the materials are (both benchmarks and peptide A)?

  • Purity
  • Manufacturing method
  • Counterions
  • Preexisting seeds
  • ….
Small Peptides are Conformationally Unstable—Unlike Most Proteins

- Helical peptides undergo gradual melting upon heating

Unfolding is of low cooperativity and reversible.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>$T_m$ (°C)</th>
<th>$\Delta H_m$ (kcal/mol)</th>
<th>$\Delta S_m$ (cal/mol/K)</th>
<th>$\Delta G_{20^\circ C}$ (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 6</td>
<td>74.9 ± 1.9</td>
<td>47.7 ± 14.1</td>
<td>137.2 ± 40.5</td>
<td>7.5</td>
</tr>
<tr>
<td>pH 7</td>
<td>75.2 ± 2.4</td>
<td>21.2 ± 4.9</td>
<td>60.9 ± 14.0</td>
<td>3.3</td>
</tr>
<tr>
<td>pH 8</td>
<td>65.2 ± 0.7</td>
<td>26.2 ± 3.9</td>
<td>77.4 ± 11.4</td>
<td>3.5</td>
</tr>
<tr>
<td>pH 9</td>
<td>63.1 ± 1.0</td>
<td>21.5 ± 5.5</td>
<td>64.0 ± 16.4</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Mw 3.8 kDa, 29 residues

Zhang et al. Mol. Pharmaceutics 2018, 15, 5591−5601
Accelerated Study Conditions: Assembly of Peptide Alone Shows Arrhenius Kinetics

Fluorescence (RFU)

Time (min)

50 °C
40 °C
25 °C

k=A*exp(-Ea/R*T)

N=6
Assembly of Peptide Alone Shows Arrhenius Kinetics: Accelerated vs. Real Time

Zhang et al. Mol. Pharmaceutics 2018, 15, 5591–5601
Can “Seeds” Play a Role in the Kinetics?

- Peptide alone demonstrates an aggregation kinetics that are Arrhenius in nature, i.e., accelerated testing at high T and shear could be used to extrapolate to real time.
- Temperature behavior in kinetics cannot explain the observed effects.

- Is it because the how representative the materials are (both benchmarks and peptide 2)?
  - Purity
  - Manufacturing method
  - Counterions
  - Preexisting seeds
  - ....
How can Submicron “Seed” Species Influence Product Quality? Can We Detect Them Quantitatively?

- “nucleated polymerization and growth”
- Submicron species appear to serve as nucleus to physical instability
- Will API history influence DP performance?

![Diagram showing aggregation over time with stages of nucleation and elongation phases, highlighting species like monomer, misfolded monomer, dimer, oligomers, protofibrils, and mature fibrils.](image)

- 5 nm (60%)
- 130 nm
- 2 µm
Methods explored for detecting of the “seeds”

- Conventional aggregation control method did not work for these purpose.

- Use of the ThioT kinetic test (i.e, assume the lag time correlate with presence of amount of seeds)

- Can we leverage the use of submicron tools, although may need to differentiate between active vs inactive seeds.
Peptides are uniquely differentiated from protein and small molecules.

Aggregation is a significant risk for pharmaceutical development of peptide drug products. Understanding of aggregation pathway is highly important to its development.

Fibrillation kinetics are highly sensitive to its preparation method, purity, existing of seeds etc. Seamless integration between DS and DP are required.
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