Proteinaceous Visible Particle in Liquid Monoclonal Antibody Formulations

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Proteinaceous Visible Particle in Liquid Monoclonal Antibody Formulations

Agenda

01 Protein & Interfacial Stresses

02 Surfactants

03 Case Studies of effectiveness of PX188

04 Message
Therapeutic proteins are inherently aggregation-prone especially in their unfolded or partially unfolded states

Key Point!
- Fold vs Unfold
- Nature vs Denature

Protein & Interfacial Stresses

Folding of Proteins

All Proteins can be **UNFOLDED** by any type of stresses

*Native Protein/ Folded Protein*  
*Denatured Protein / Unfolded Protein*

- Hydrophilic Residues
- Hydrophobic Residues

Folding  
Unfolding (Stress)
All Proteins can be **DENATURED** by any type of stresses

- Therapeutic Proteins (e.g. Antibody) are the same as Egg Protein (e.g. Albumin)

**Denaturation of Proteins**

**Denatured Protein**

- Temperature
- pH

**Native Protein**

- Pressure
- Interfaces

**Denatured Protein**

*can be HOT SPOT for Aggregation*
All Hydrophobic Interfaces can be “Hot spots for Aggregation”.

**Protein & Interfacial Stresses**

**Hot spot / Interfaces in Therapeutic Protein Drug**

All Hydrophobic Interfaces can be “Hot spots for Aggregation”.

- **“SOLID–liquid Interface”** (Glass)
- **“OIL–liquid Interface”** (ex. Silicone from Siliconized Container)
- **“AIR–liquid Interface”**

**“Hydrophobic Interface”** of molecule (Intermolecular)

**“OIL–liquid Interface”** (ex. Silicone from Siliconized Container)
Importance of Desorption step and Trigger of the step are often discussed in many literatures.
Protein & Interfacial Stresses

Mechanical Stresses / Trigger of particle formation

- Ex. Moving Air bubble, mechanical stress by needle


Protein & Interfacial Stresses

Uncertainty/Complexity/Ambiguity of Mechanism

- All protein can form aggregates, especially on the interface.
- However, a wide variety of worst cases/root causes can be considered.

- It is impossible to remove all interfaces & all stresses.
  ➔ Surfactants can reduce a wide variety of stresses.
Proteinaceous Visible Particle in Liquid Monoclonal Antibody Formulations

Agenda

01. Protein & Interfacial Stresses

02. Surfactants

03. Case Studies of effectiveness of PX188

04. Message
Surfactants are used to stabilize Therapeutic Proteins.

- Polysorbate 20/80, Poloxamer 188

Lukas Bollenbach, Julia Buske, Karsten Mäder, Patrick Garidel, Poloxamer 188 as surfactant in biological formulations – An alternative for polysorbate 20/80?, International Journal of Pharmaceutics, Volume 620, 2022, 121706
Mechanism of Stabilization by surfactants is frequently discussed.
Proteinaceous Visible Particle in Liquid Monoclonal Antibody Formulations

Agenda

01  Protein & Interfacial Stresses

02  Surfactants

03  Case Studies of effectiveness of PX188

04  Message
• A method to characterize the PPO(polypropylene oxide) block length.
Case Studies of effectiveness of PX188

Characterization of PX188

- Surface Tension depends on the Chromatographic characteristics
- Lot-to-lot variability was confirmed.

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Length of PPO block (Late Eluters>17min[area%])

Surface tension (SFT)
## Case Studies of Effectiveness of PX188

### Impact of PX188 Variability for HMWS by SE-HPLC

- No clear impact for HMWS (high molecular species (e.g. dimer))

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<th>Formulation</th>
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</table>
Case Studies of effectiveness of PX188

Impact of PX188 variability for SvP by Flow imaging

- A minor trend but not conclusive.

Protein-like images are counted by filtering (Aspect ratio <0.80, intensity mean <780)

* = Data not available due to preferential use for particle identification
Impact of PX188 variability for VP by visual inspection

- VP occurrence depends on the PX188 characteristics.
  - 5deg, 6 months: No VP observed
  - 25deg, 6 months

<table>
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<th>Proteinaceous VP (1) × (2)</th>
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Note = Raman spectroscopy was used for particle identification
Case Studies of effectiveness of PX188

Conclusion of the Case Study

- Higher hydrophobic PX188 can reduce the risk of Proteinaceous Pericles.
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**Message**

- Protein unfolding/aggregates/particles might be unavoidable.
- However, they can be controlled by appropriate/practical strategies.

Protein Science
Understanding of Product

Regulatory Science
Practically Free/Essentially Free
INNOVATION BEYOND IMAGINATION