Visible Particles from Polysorbate Degradation
Cases & Perspectives
Particles & Parenterals – not best friends

Particles...

• represent an obligatory cQA for parenteral products

• are often a focus topic in audits and inspections

• are still the major reason for product recalls

• aspiration of zero visible particles is desirable, but unrealistic
Analysis upon gentle agitation (e.g. swirling)
Well trained operators:
  – Periodic eyesight tests
  – Initial and continuous training
  – Performance qualification
  – Periodic performance control

Issue: No clear and harmonized definition of visibility available
Visual Inspection – Process Flow Scheme for Liquid Products

- **Filling into container**
  - **100% Visual Inspection** (sorting according to defect categories)
    - **Accepted part**
    - **Rejected part of the batch (defects)**

  **AQL Visual Inspection** (statistical sampling)
  - **Pass**
  - **Fail a** (Rare random occurrence of particles)
  - **Fail b** (Reflective of systemic failure)

  **Visual Re-Inspection**
  - 100% of accepted part

  **Investigation Mode/Re-Inspection**

  **QC Stability Visual Inspection** (small sample subset)
  - **Pass**
  - **Reject batch**

  **Proposal:** Leverage AQL results for batch release

- **Batch release**

*a* Rare random occurrence of particles

*b* Reflective of systemic failure

Modified from: Mathonet et al., PDA JPST, 2016
Case study: Visible Particles in Biopharmaceutical Development Products
Case Study: Visible Particles in Biopharmaceutical Development Products

Initial optical characterization:
- Large particles
- Translucent to whitish
- Amorphous shape
- Neutral buoyancy
- Freely moving in solution
- Mainly disappear at RT

Initial optical characterization:
- Many very small particles (only under enhanced lighting)
- Highly translucent
- Neutral buoyancy
- Freely moving in solution
- Glittering under stray light

Nature of the particles? Root cause for formation?
Most particles around 300μm
Attached fibred and crystalline structures
Fragile, partially dissolved during preparation

Particle size below 70μm (➔ subvis. methods)
Atypical thin needle like shape, possibly crystalline
Stable at RT and upon filtration/washing

Observed particles are very likely of non-proteinaceous composition
Case Study: Particle Forensics – Chemical Composition

No typical protein spectrum, strong match with long chain fatty acids

Fatty acid composition of particles typical for polysorbate 20
Polysorbates can be degraded via different mechanisms

Fatty acid component | USP42 (%)
--- | ---
C6:0 | ≤1.0
C8:0 | ≤10.0
C10:0 | ≤10.0
C12:0 | 40.0–60.0
C14:0 | 14.0–25.0
C16:0 | 7.0–15.0
C18:0 | ≤11.0
C18:1n-9 | ≤11.0
C18:2n-6,9 | ≤3.0

PS20 is a complex mixture, not a well-defined substance
Case Study: Root Cause within the Manufacturing Process?

- Cell cultivation
- Product purification & Formulation
- Drug Substance
- Aseptic Filling
- Drug Product

Production Cell

- Product
- Host Cell Proteins

Storage/Stability

polysorbate

hydrolysis
Case Study: Mechanism of PS20 Degradation

PS20 degradation (25° C)

Free fatty acid (FFA) release (25° C)

near-complete inhibition of PS hydrolysis by Serine Hydrolase Inhibitor:

confirmation of enzyme as main root cause for polysorbate degradation
Case Study: Visible Particles in Biopharmaceutical Development Products

Nature of the particles:
mainly fatty acids, partly dissolve at RT

Root cause for formation
enzymatic PS20 degradation
Acknowledgements

Ingrid Auernhammer  
Florian Baiz  
Martin Dass  
Thomas Ehrmann  
Beate Eyrich  
Cornelia Gapp  
Patrick Garidel  
Julia Groß-Rother  
Felix Halbach  
Peter Happersberger  
Silke Hövel  
Werner Kliche  
Marius Löffler  
Theresa Müller  
Dagmar Rädel  
Katrin Reinhardt  
Markus Riegger  
Thomas Schwab  
Maik Veelders  
Peter Wanja  
Till Wenger  
Robert Wild  
Justine Witosch  
and many more...
For more information have a look at:
www.boehringer-ingelheim.de
www.boehringer-ingelheim.com