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Tackling the Polysorbate Degradation Challenge: Control Strategies and Case Studies

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Polysorbates – complex mixtures of compounds

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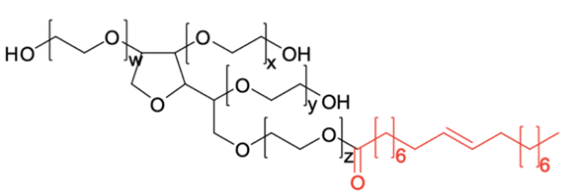
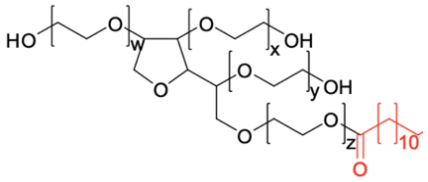


PS80



PS20

w+x+y+z=20

- PS are complex and heterogeneous mixtures (synthesis uses precursors from natural products)
- PS manufacturing processes can vary

Fatty acid ester	PS20	PS80
Caproic $CH_3(CH_2)_4COOH$	≤1%	-
Caprylic $CH_3(CH_2)_6COOH$	≤10%	-
Capric $CH_3(CH_2)_8COOH$	≤10%	-
Lauric $CH_3(CH_2)_{10}COOH$	40-60%	-
Myristic $CH_3(CH_2)_{12}COOH$	14-25%	≤5%
Palmitic $CH_3(CH_2)_{14}COOH$	7-15%	≤16%
Palmitoleic $CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	-	≤8%
Stearic $CH_3(CH_2)_{16}COOH$	≤7%	≤6%
Oleic $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	≤11%	58-85%
Linoleic $CH_3(CH_2)_3(CH_2CH=CH)_2(CH_2)_7COOH$	≤3%	≤18%
Linolenic CH3(CH2CH=CH)3(CH2)7COOH	-	≤4%

European Pharmacopoeia

Polysorbates are critical excipients for biologics

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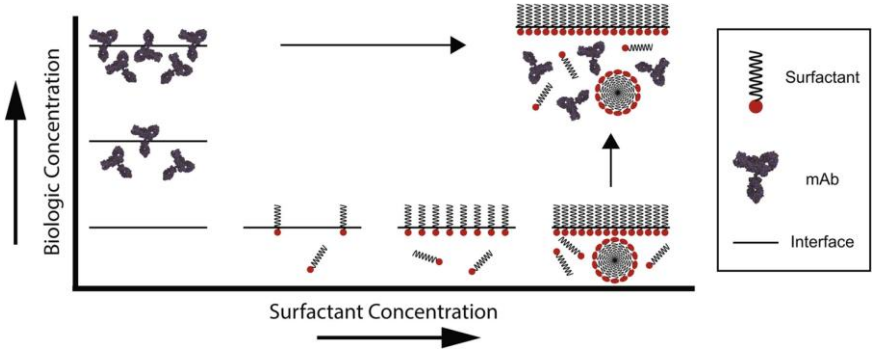
- Polysorbates are critical excipients, widely used in biologics - highly effective in protecting proteins against interfacial stress
- 56% of all marketed biotherapeutics and ~93% of all marketed mAbs contain a surfactant



Khan TA, et al, E J Pharm Biopharm, (97A), 2015, 60-67

- Polysorbates can degrade and present liabilities too:
  - Liberation of FFA → Particles → **Incompliant drug product**
  - Loss of protection against interfacial stress → **Unstable drug product / reduced shelf life**

Polysorbate Degradation - Two Distinct Relevant Mechanisms

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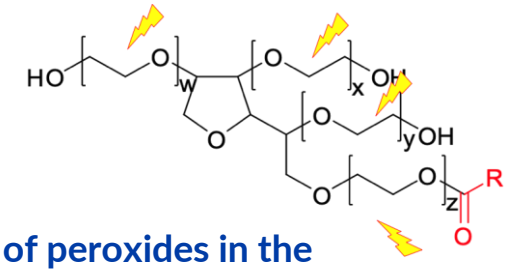
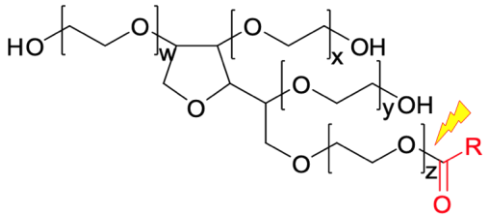


- **OXIDATION**
  - Presence of peroxides in the PS raw material
  - Exposure to light
  - Exposure to oxygen (air)
  - Exposure to transition metals

- **Mitigation Strategies:**
  - Raw material qualification, storage and handling best practices

- **HYDROLYSIS (ENZYMATIC)**
  - Traces of contaminating lipases from the host cell (CAUTION: common occurrence!)



  - **Downstream process improvements to eliminate co-purifying enzymes**

- **Ascertaining the degradation mechanism is of critical importance to implement the correct mitigation measures**

Impact of Polysorbate Degradation - Potentially Severe Consequences

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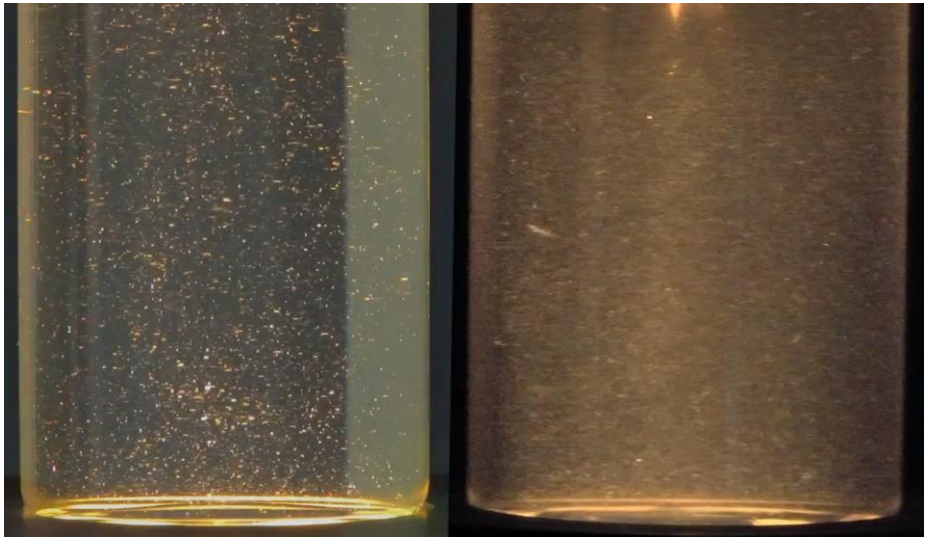
1. **Reduction of PS Content**
   - Reduction in protection against interfacial stress
     - Instability on storage / transportation
     - Instability during processing
2. **Oxidative damage of the API**

   (in the case of oxidative degradation)
3. **Liberation of poorly soluble free fatty acids (FFA)**
   - Particle formation
   - Potential co-precipitation of protein in biopharmaceuticals



Potential impact on

SAFETY
QUALITY
STABILITY
SHELF LIFE
COMPLIANCE

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Analytical Methods for Polysorbate Characterization and Quantification

Analytical Toolbox – PS Quantification

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- Quantification of Polysorbate is typically achieved by one of the following (single peak) methods:
  - Fluorescent Micelle Assay (FMA)
  - HPLC(MAX)-ELSD/ CAD
- Quantification methods need to be stability-indicating
- Quantification assays have different selectivities:
  - FMA more sensitive to oxidative degradation
  - HPLC-ELSD/ CAD - more sensitive to lipolytic degradation

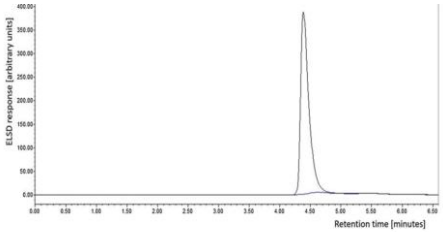

Figure 6.3 HPLC-ELSD applying a step gradient: Chromatogram of a 0.02% (w/v) PS80 solution.

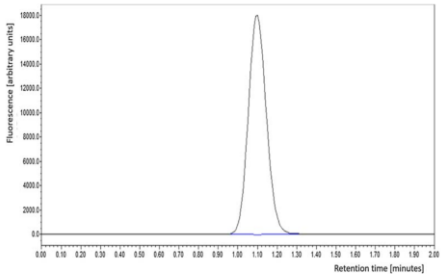

Figure 6.2 HPLC-FMA: Chromatogram of a 0.02% (w/v) PS80 solution.

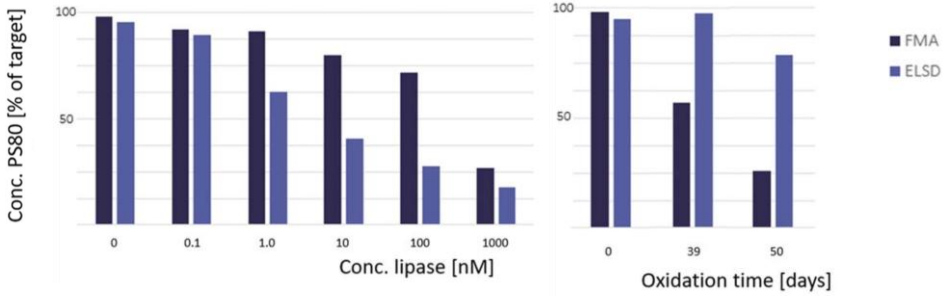

Figure 6.4 Polysorbate stability indication with HPLC-FMA versus HPLC-ELSD: Analysis of 0.04% (w/v) PS80 subjected to hydrolytic stress at different lipase concentrations (top) and oxidative stress at elevated temperatures for different durations (bottom).

Jahn, Ch 6, Ed(s): Koulov AV, Singh SK, Surfactants in Biopharmaceutical Development, Academic Press, 2023, 119-136

Analytical Toolbox – PS Characterization

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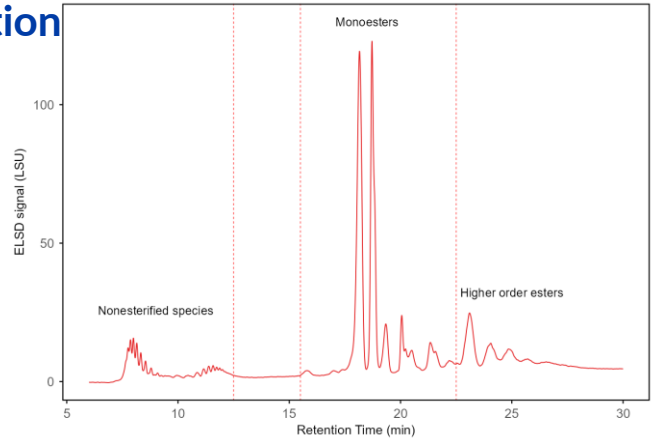
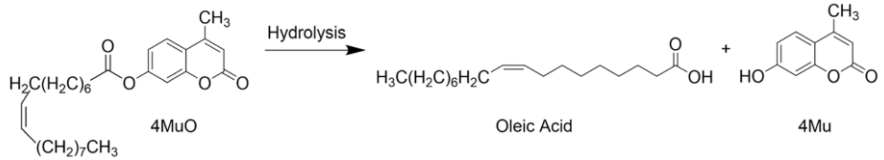
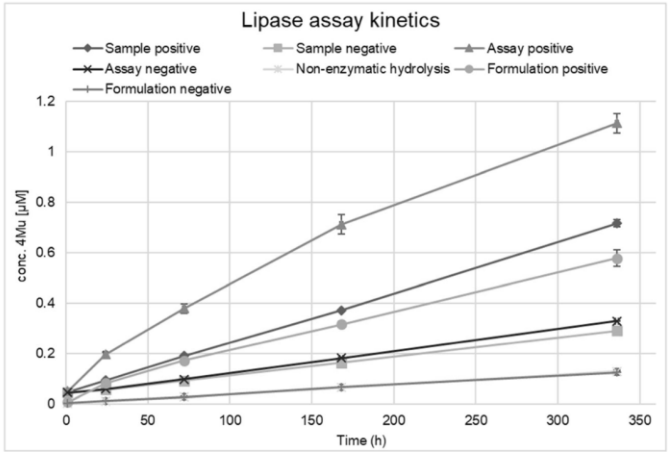
- Polysorbate profiling by reversed phase shallow gradient HPLC (ELSD/ CAD/ MS - detection)



- Proteomic approaches to ID HCP lipases/ esterases
- **Enzyme activity assays**
  - PS degradation mechanism diagnosis
  - Downstream process development support





Jahn, M., Zerr, A., Fedorowicz, F.M. et al.. Pharm Res 37, 118 (2020)

Analytical Toolbox – Particle Characterization

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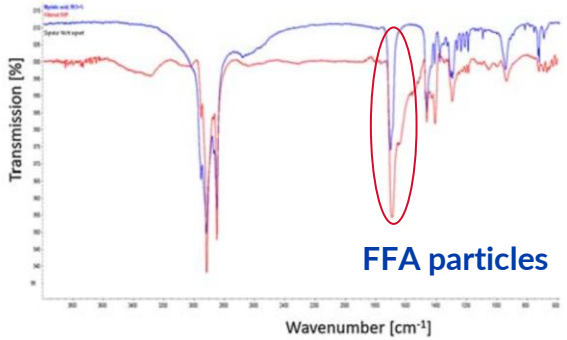
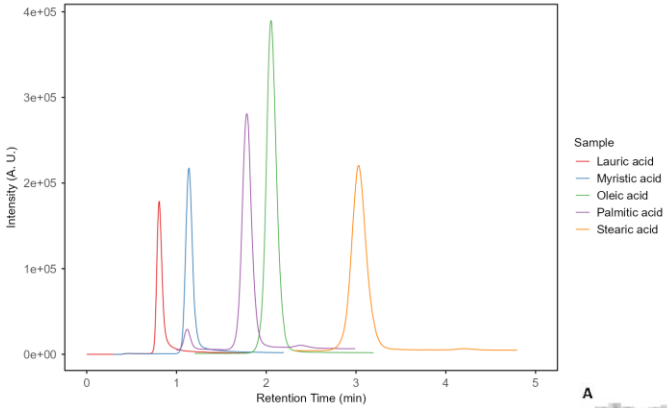
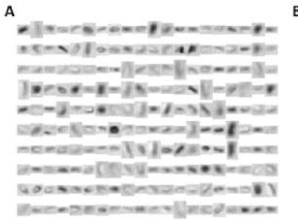
- Visible particle isolation and characterization by FTIR/ Raman microspectroscopy



- FFA target quantification by LC-MS



- Discrimination of FFA Particles by AI-aided Flow Imaging Microscopy



Polysorbate Characterization Toolbox

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- PS quantification
- Polysorbate Profiling
- Lipase activity assay
- Particle isolation and ID

THE "MUST-HAVE" ANALYTICAL TOOLBOX

to ensure adequate characterization

(product quality and process control)

PS Control Strategy Components - Analytical Toolbox

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CONTROL STRATEGY COMPONENT	ANALYTICAL TESTS NEEDED
Raw materials testing/ qualification	• Compendial tests • Additional characterization (profiling, FFAs)
Manufacturing process development (DS and DP), validation and in-process control	• Quantitation (PS content) • Additional characterization (profiling, lipase assays) - DS, DP
Product characterization throughout development: Stability testing - PS quality and quantity Particles	• Quantitation (PS content) • Additional characterization (profiling, FFAs, lipase assays, particle characterization)
Control system: Specifications (release, stability)	• Quantitation (PS content; stability-indicating method)

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CASE STUDIES

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CASE STUDY 1

Detection of Particles

CASE STUDY I: Appearance of Free Fatty Acid (FFA) Particles

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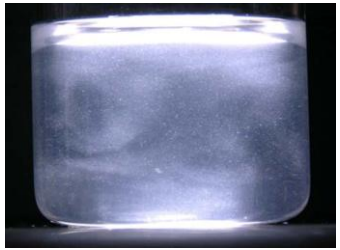
Observation:

Visible particles on stability



Question:

Particle composition → **FFA only** or **FFA + API**?

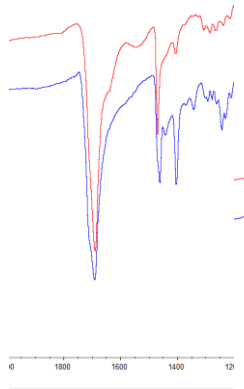
Solution:

1. Particle isolation

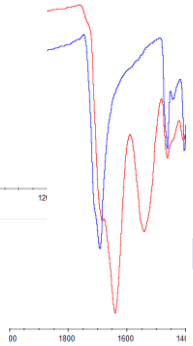
2. Particle ID

a. ✕

b.



FFA only



FFA + API

Appearance of Free Fatty Acid (FFA) Particles - Points to Consider

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- **Think ahead** (*better safe than sorry*):
  - Always **monitor PS levels on stability** (every clinical batch).
    Note: do you have the right method?
  - **SvP increase** typically precedes the appearance of Visible Particles. Measuring SvPs with orthogonal methods (FIM) is a good practice
  - Consider using an **in-line administration filter** whenever possible in early clinical development
    Note: Not a solution, only a proactive risk mitigation measure
- Ensure **detailed particle characterization** (stability time-course) to exclude potential protein aggregation
- Consider timing for clinical safety assessment
- Particle isolation & characterization can be technically complex - develop internal procedures OR consider a competent partner

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CASE STUDY 2

Quantification Method

CASE STUDY II: PS Quantification Methods

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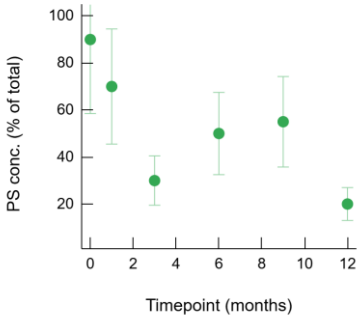
Observation:

-No PS quantification method available OR
- Method suitability unclear



Question:

Can a robust and stability-indicating method be developed/ implemented?

Solution:

1. Review of method performance Validation/Qualification report

2. CSL Platform PS method evaluation with the product:
a) profiling, b) quantification

3. Bespoke method development

4. Method Qualification

5. Method Transfer & Troubleshooting support

PS Quantification Method Development, Qualification and Transfer

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- Applied Quantification method must be stability indicating
- Especially for high concentration protein formulation the API often interferes with the PS quantification method
- Protein removal e.g. by protein precipitation could be necessary
- A robust qualification/ validation data set needs to demonstrate that the method is fit for purpose

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CASE STUDY 3

Reduction of PS Content on Stability

CASE STUDY III: Elucidation of PS Degradation Mechanism

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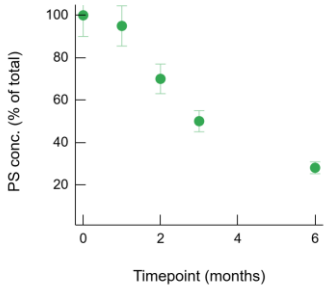
OBSERVATION:

Significant reduction of PS content on stability

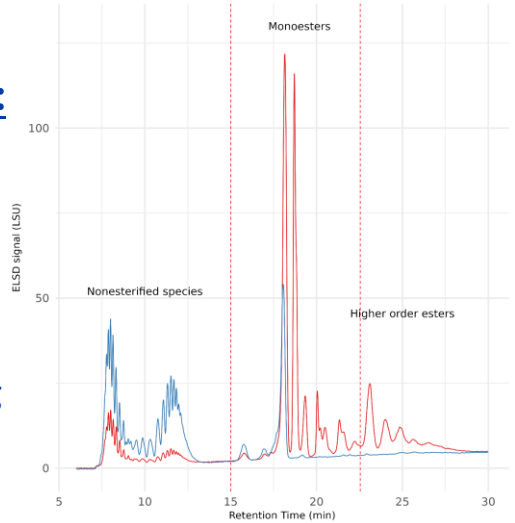


QUESTION:

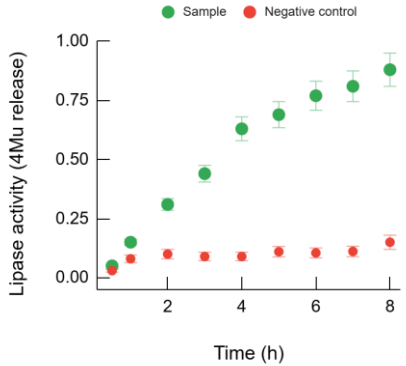
What is the degradation mechanism?

SOLUTION:

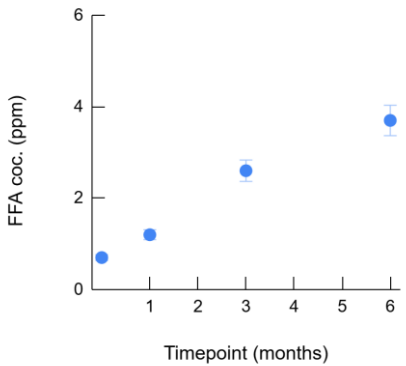
1. PS profiling



2. Lipase activity assay



3. FFA quantification



PS Degradation Mechanism - Points to Consider

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- Understand **the extent of the issue** (relevance of analytical results; interference)
  - Always use of **well-characterized & qualified analytical methods**
  - Do you have sufficient shelf life?
- Always use **orthogonal methods** to ensure complete picture
- Always **think about the next step** - do you have the **right analytical toolbox**?
- Consider different mitigation pathways:
  - Re-formulation - amount and type of surfactant (*be aware of pitfalls!*)
  - Shorten shelf life
  - DSP optimization

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CASE STUDY 4

Lipolytic PS Degradation

CASE STUDY IV: Mitigation of Lipolytic PS Degradation

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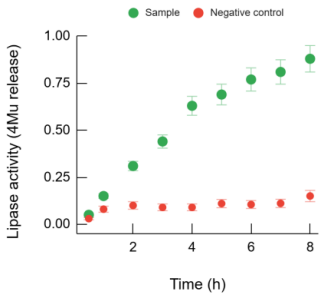
OBSERVATION:

- PS rapidly degraded
- Presence of lipolytic activity

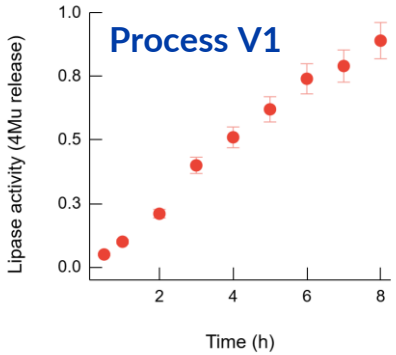


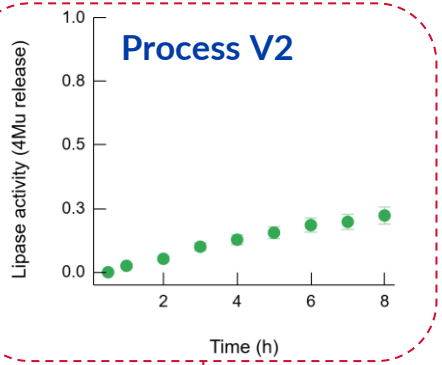
QUESTION:

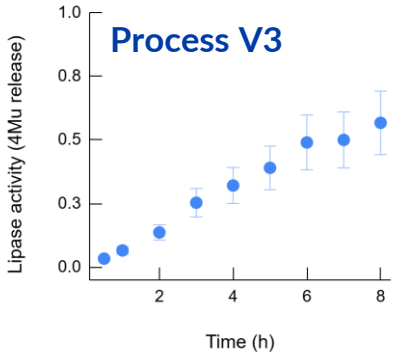
Can DSP be optimized to remove HC lipases?

SOLUTION:

Protein A optimization







1. Enzyme activity assay IPC testing to support DSP optimization

2. Select low-enzymatic-activity process version

DSP Optimization to Remove Enzymatic Activity - Points to Consider

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- Consider optimizing different DSP steps
  - **Protein A, HIC, MM** typically give the highest probability of success
- Often multiple enzymes are present in DS (trace amounts)
- Ballance removal of lipases with other attributes
- It does not have to be perfect
- Apply all characterization tools to stability assessments of the new process version - PS profiling, PS content, SvP and VP characterization

Conclusions

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- Polysorbate degradation can present **significant challenges** to biologics development and commercial programs, with **potentially severe impact on safety and quality**
- Setting up a **comprehensive control strategy** requires:
  - An innovative, fit-for purpose analytical toolbox
  - **Use the right tool for the right job → each question requires dedicated analytical tools**

Wuchner, K et al., Do we worry too much about polysorbate degradation? An industry-wide perspective with real-life case studies, J Pharm Sci, 2026 (in press)

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Thank you!