Industry perspective on polysorbate degradation & control strategies for biopharmaceutical products - *A view of EFPIA Working Group*

5th December 2022

CMC Strategy Forum Japan 2022

MQEG PS Working Group

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BTDS | Janssen Research & Development, LLC

Karoline Bechtold-Peters
(Novartis)

Disclaimer: *The perspectives and opinions of this talk are those of the presenter*

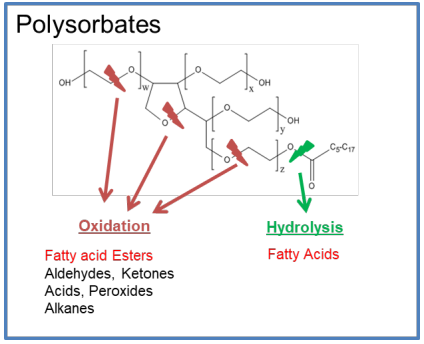
Problem statement

Protein formulations are exposed to **various stresses and interfaces** during processing, transport and application

Polysorbate 20 or Polysorbate 80 (“PS”) are used as excipients to:

- **Prevent protein adsorption**
- **Protect protein against interfacial stress**, surface-induced aggregation and particle formation

Polysorbates form **poorly soluble PS-related degradation products** and can also contain sparingly soluble impurities resulting in sub-visible and visible particles



Cross Industry Workstream formed under the umbrella of EFPIA’s Biomanufacturing Working Group

Meet the Team

EFPIA MQEG PS

21 active team members (16 Companies)

- **Klaus Wuchner**, J&J (Lead)
- **Linda Yi**, Biogen (Co-Lead)
- **Shawn Cao**, Amgen
- **Cyrille Chery**, UCB
- **George Crotts**, GSK
- **Karoline Bechtold-Peters**, Novartis
- **Rien de Ruiter**, Byondis
- **Patrick Garidel**, Boehringer Ingelheim
- **Sylvain Huille**, Sanofi
- **Michael Jahn**, Lonza
- **Friederike Junge**, AbbVie
- **Virginie LeBrun**, Lonza
- **Michael Leiss**, Roche
- **Felix Nikels**, Boehringer Ingelheim
- **Sebastian Peuker**, Bayer
- **Sarah M Richer**, Lilly
- **Gianluca Rinaldi**, Merckgroup
- **Sonal Saluja**, Biogen
- **Melissa Shuman**, GSK
- **Jason Starkey**, Pfizer
- **Tingting Wang**, Lilly

From industry survey to position paper

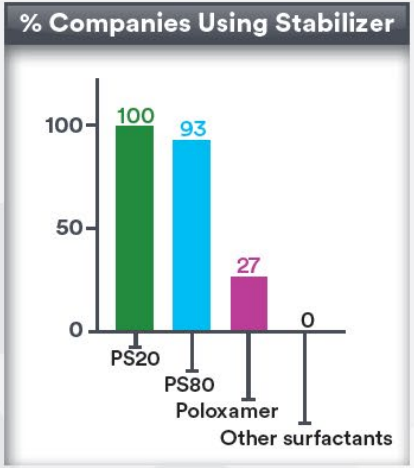
PS Industry Survey *(137 questions, 27-page survey)*

- Use of surfactants for biological products, incl. new grade PS – general aspects
- Polysorbate raw material for cGMP use
- PS handling during cGMP manufacture
- Degradation of PS in biological products *(including proteins and synthetic peptides)* and placebos
- Analytical methods for of PS in products
- Mechanistic Understanding of PS degradation and detectability
- Model systems/predictive models
- Mitigation strategies
- Safety / toxicology
- Regulatory interactions related to PS / PS degradation / particle formation / specifications

Deep dive of survey results, literature review, lots of team discussion

- **Manuscript:** Industry perspective on the use and characterization of polysorbates for biopharmaceutical products
  - **Part 1:** Survey report on current state and common practices for handling and control of polysorbates
  - **Part 2:** Survey report on control strategy preparing for the future

Industry Survey Results on Use of Surfactant as Stabilizer



100% companies use PS20

93% of participants PS80;

27% Poloxamers

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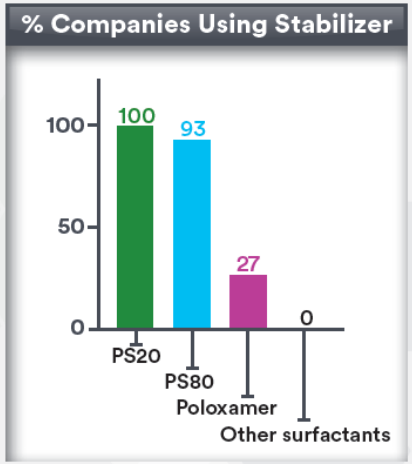

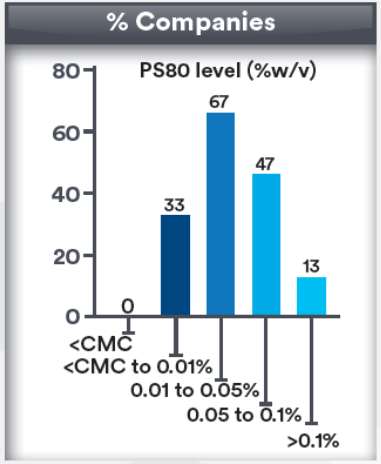

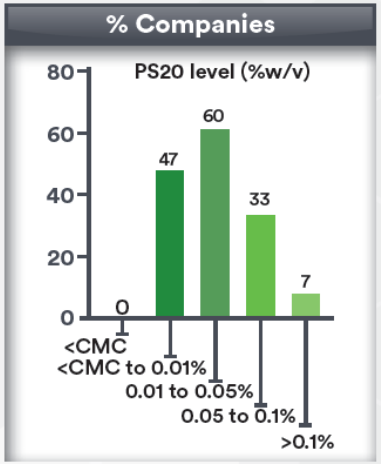

100% companies use PS20

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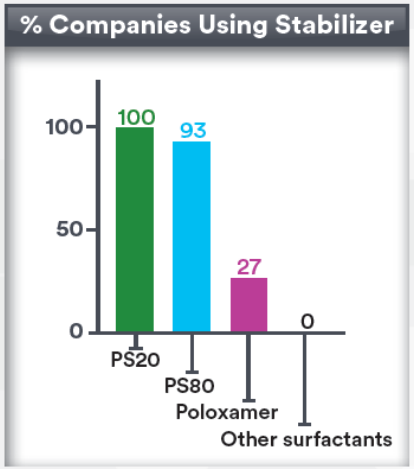
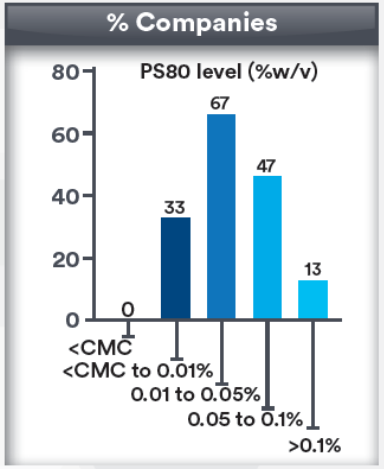
PS80 and PS20 levels are mostly in the **0.01 to 0.05% range**

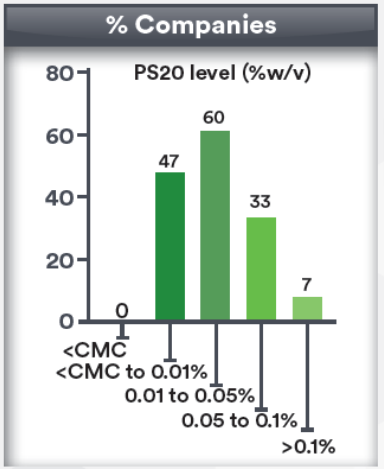
CMC of PS 20 0.0060 %, CMC of PS80 0.0014 %
(L Wan et al., JPharmSci, 1974)

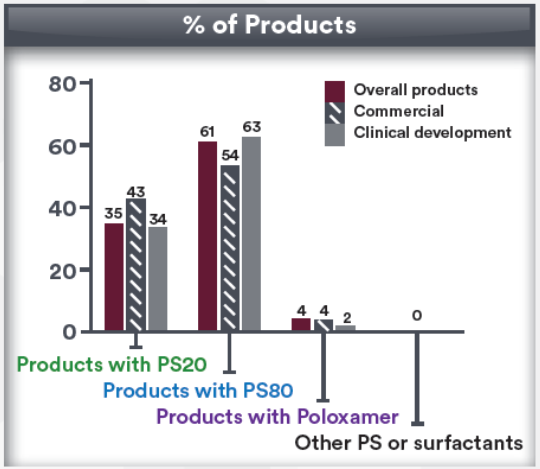
Industry Survey Results on Use of Surfactant as Stabilizer

efpia janssen Pharmaceutical Companies of Johnson & Johnson









100% companies use PS20

93% of participants PS80;

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PS80 and **PS20** levels are mostly in the **0.01 to 0.05% range**

PS80 is **more often formulated in biopharmaceutical products** compared to other surfactants

No other types of surfactants than PS20, PS80 and Poloxamer are currently **in clinical development for biopharmaceutical parenteral products** (based on this survey)

CMC of PS 20 0.0060 %, CMC of PS80 0.0014 % (L Wan et al., JPharmSci, 1974)

Supply of parenteral grade PS products

100%

source PS as standard, multicompendial (MC) polysorbate (USP, Ph. Eur, JP)

7 companies

also use higher purity or customized polysorbates for pharmaceutical use

CRODA

SEPPIC

NOF CORPORATION

KLK OLEO

KLK Kob

南京威尔药业股份有限公司

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PS products are purchased at various sizes ranging from **<0.1kg (13% companies)** with most **69% at 0.1 to 1kg size** and still **38% of companies at >4kg size**

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with most **69% at 0.1 to 1kg size** and still 38% of companies at >4kg size

For PS20

10 companies use 1 PS manufacturer, all others ≥2

For PS80

8 companies use 1 PS manufacturer all others ≥2

some have additional suppliers

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KLK OLEO

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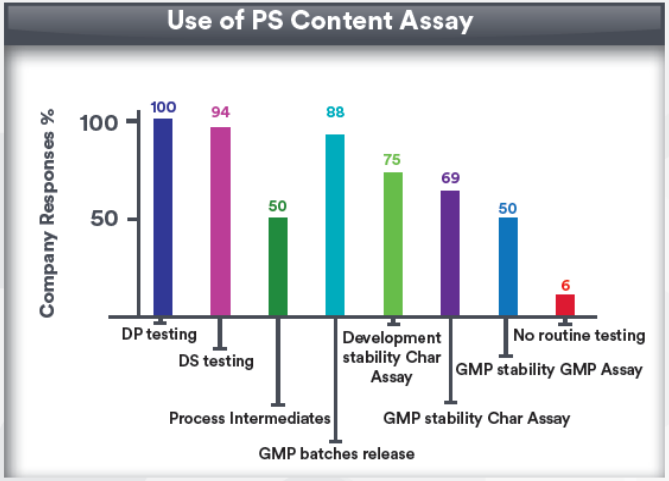
wei wei 威尔药业 威尔化工 南京威尔药业股份有限公司

Industry Survey Results on Routine Testing of PS



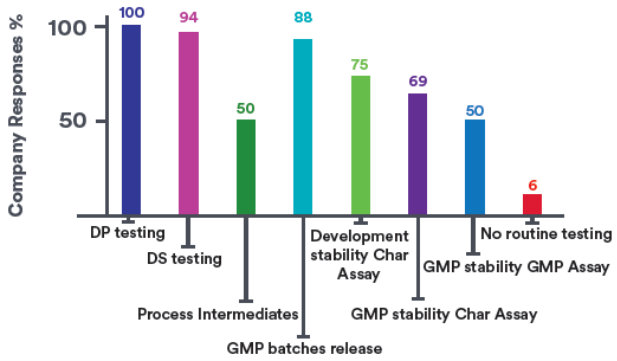
PS content assay is **implemented by 100%** of companies and is considered being standard practice

PS content assay is **platform method across multiple products** and was validated by **88% companies**

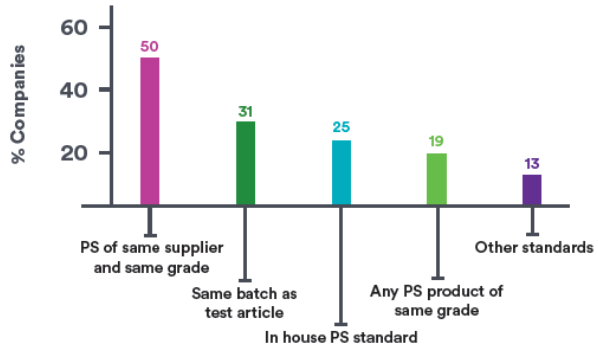
Chromatography based PS content assay is used by **94% of companies** for **81% without additional sample preparation** *(mixed mode "single-peak" method at least for certain products)*

Industry Survey Results on Routine Testing of PS

Use of PS Content Assay



PS Standard for PS Content Assay



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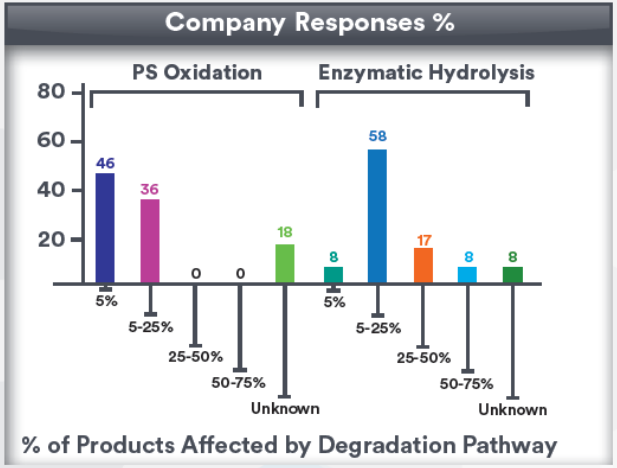
Use of PS of same grade and from same supplier **as analytical standard allows counterbalance inherent variability of PS**

Relevant Polysorbate Degradation Routes

Residual host cell proteins (hydrolases) can cleave fatty acid ester bond

Air, light, transition metals cause PS oxidation through peroxides/ reactive oxygen species

Industry Survey Results on PS degradation in biological products



Enzymatic hydrolysis

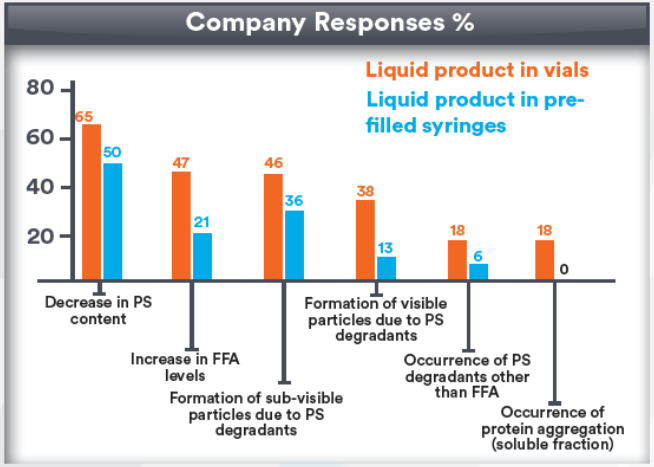
is affecting more

biotech products

than

oxidative pathway

Industry Survey Results on PS degradation in biological products

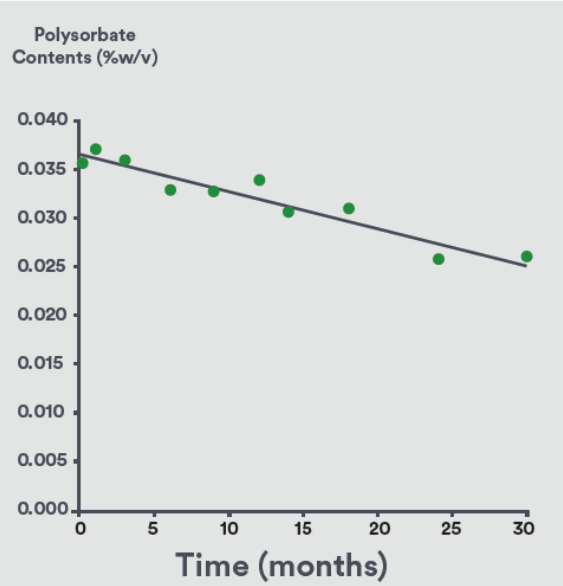


"Decrease in PS content" was found to be the **first indicator for liquid protein-based products** *(both vials and pre-filled syringe presentation)*

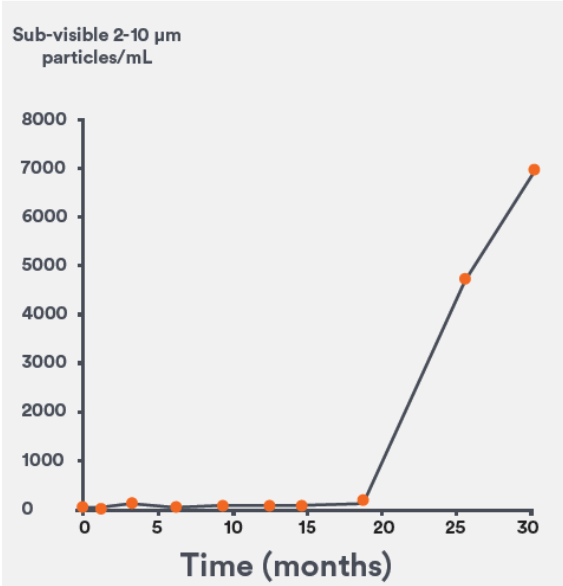
This was followed by **increase in levels of free fatty acids (FFAs)** and the formation of sub-visible or visible particles related to PS degradants (FFAs)

Protein aggregation/protein particle formation **not prominent indicator for PS degradation**

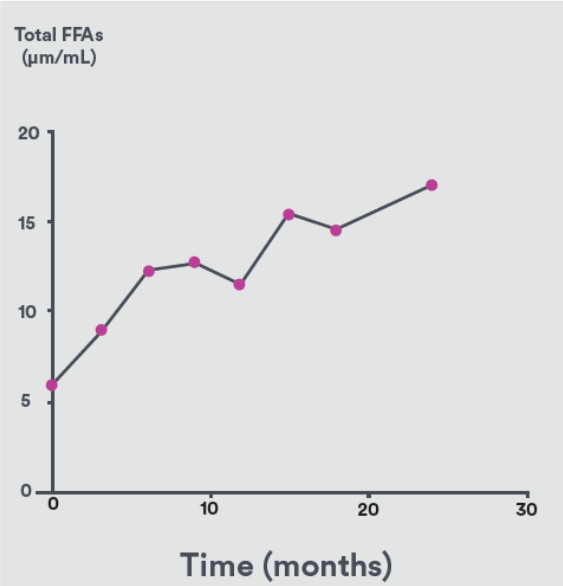
Example for PS degradation in vialed protein product



Polysorbate content decreases with increasing storage time of protein formulation



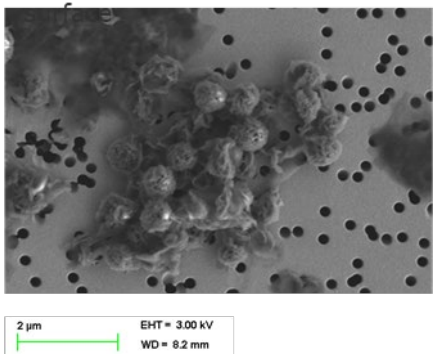
Sub-visible particle levels increase after lag-time

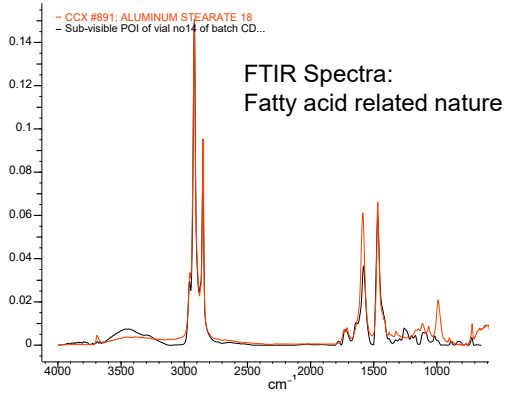


FFAs increase with incrasing storage time (frozen retains)

Visible and subvisible particle appearance and nature

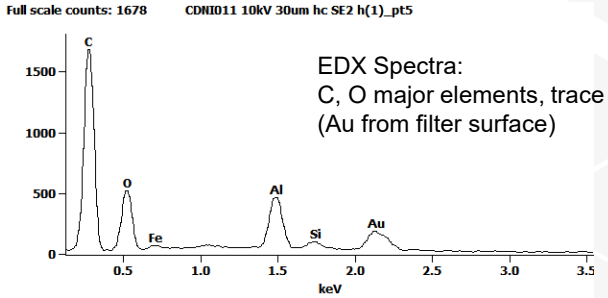
SEM image on filter





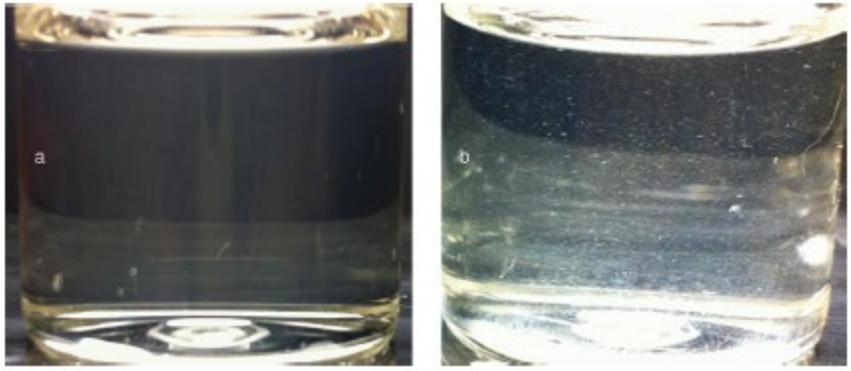
FTIR Spectra:
Fatty acid related nature

Sub-visible particles were of fatty acid related nature



EDX Spectra:
C, O major elements, trace metals
(Au from filter surface)

Visible and subvisible particle appearance and nature



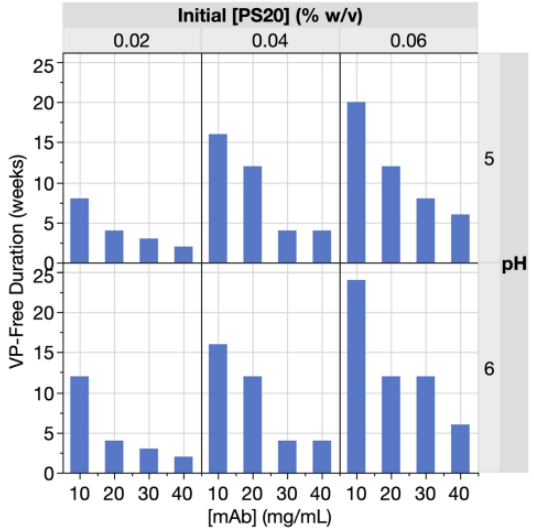
Photos of mAb formulated without PS20 (left) and with PS20 (right) during storage at 4°C for 2 years

Table 1. Visual Inspection Data of mAb-I Formulated with and without PS20 during Storage at 4°C

	T0	1w	2w	3w	4w	5w	6w	7w	8w	9w	12w
– PS20	–	–	–	–	–	–	–	–	–	–	–
+ PS20	–	–	–	–	–	–	+	++	++	++	++

Samples were scored as "–" no granular particles, "+" low levels of granular particles, and "++" high levels of granular particles.

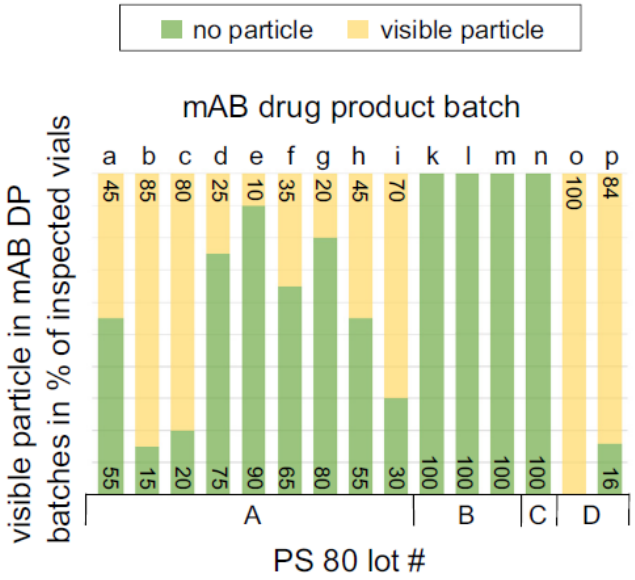
Siska et al. JPharmSci, 2015



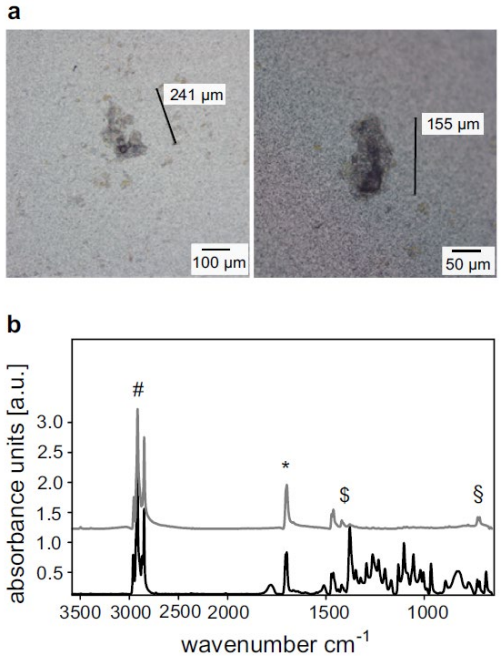
Storage duration without Visible Particles for mAb X DP as a function of mAb concentration, initial PS20 concentration and pH during a 24 weeks' stability study at 5°C

Yuk et al, AAPS Open, 2022

...however not in every case consequence of storage, but impurity



Visual inspection of mAb DP batches that revealed that visible particle formation depends on PS80 raw material lot



Light microscopy pictures of representative pictures found (upper photo) and FTIR spectra of particles indicating identity (ketone 12-tricosanone) (lower graph)

Hampl et al, JPharmSci 2018

Survey Study Results on Accelerated PS degradation studies
(predictive PS degradation models)

efpia janssen Pharmaceutical Companies of Johnson & Johnson

Degradation studies at accelerated and stressed temperatures

are considered appropriate model systems

(88% companies)

Accelerated PS degradation studies
(model systems)
are used by

67%

of surveyed companies

63%

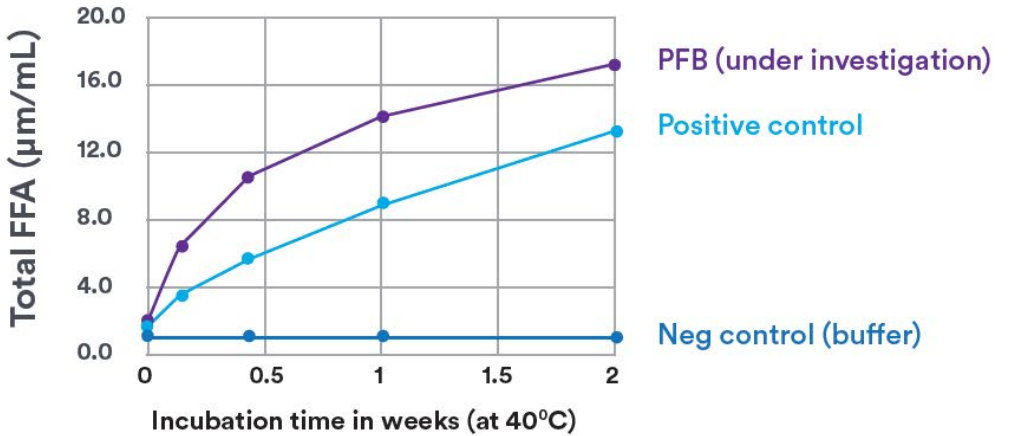
use spiking studies
(E.g., with oxidative agents) or
enzyme incubation studies
(E.g., neat PS spiked into PS free down-stream sample)

Other model systems assess functional properties of PS at different levels
(E.g., end of shelf-life shaking studies, reduced PS levels)

or impact of PS degradants on CQAs

Such studies enhance mechanistic understanding but study results are usually not filed in regulatory documents *(unless requested)* **due to concerns about reliability and predictability**
(more work needed)

Example for short term stability studies
(E.g., PS spiking or enzyme incubation studies)



Increase in FFAs indicates enzyme-mediated PS degradation for pre-formulated bulk (PFB) under investigation

Drivers for investigations and mitigation measures to reduce/minimize PS degradation

Triggers

Formation of **unacceptable levels of sub-visible** ***(87%)**** or **visible** ***(100%)**** particles

Decrease of the PS content below certain threshold ***(71%)****

Formation of **visible particles at any level** (one would be sufficient) ***(60%)****

PS degradation at any level/rate ***(36%)****

Formation of sub-visible particles at any level **if increase is meaningful** ***(29%)****

Drivers for investigations and mitigation measures to reduce/minimize PS degradation

Mitigations

Highly effective mitigation strategies are

available for PS oxidation with simpler changes in formulation

(E.g., addition of chelator, antioxidant)

Effective mitigation strategies for

enzyme-mediated PS hydrolysis are more labor intensive

and may involve more dramatic changes

(e.g., changes in DS purification process)

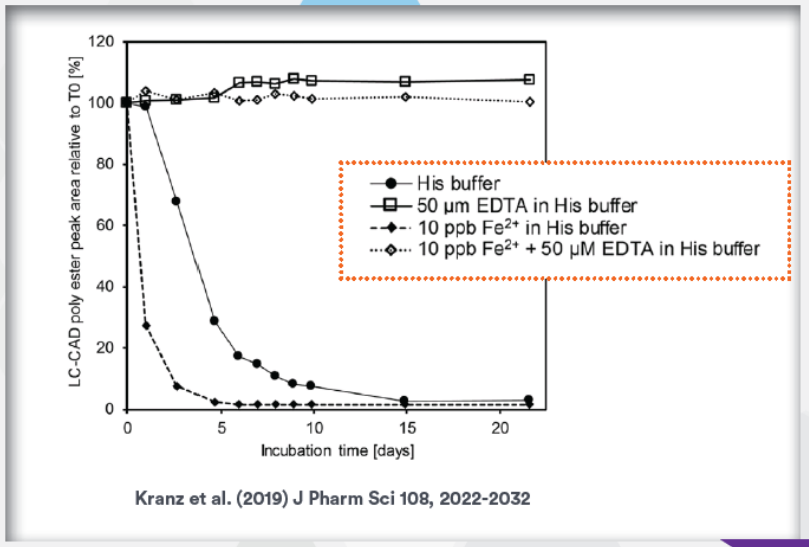
FFAs / PS degradant related particle formation may be

acceptable to a certain extend

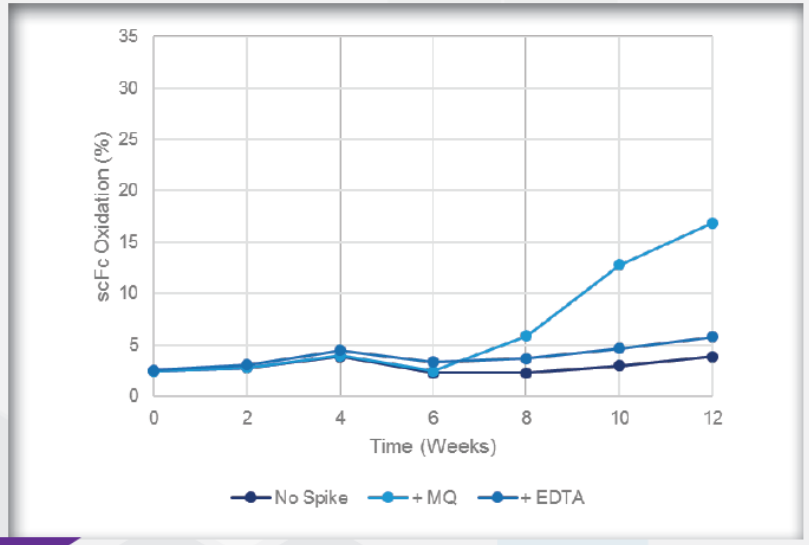
(no tox/safety concerns)

if no other CQA impacted

Successful mitigation strategy to reduce oxidative degradation



Kranz et al. (2019) J Pharm Sci 108, 2022-2032



EDTA protects PS and protein against oxidative degradation

Survey results on mitigation/control strategies to reduce/minimize PS degradation

67%

start to develop mitigation strategies for PS degradation as soon as such event is detected, this is done on

a case-by-case basis

Change in DP storage temperature *(frozen storage conditions)* or lyophilization is

considered being effective measure

●●●

however **not wanted for other reasons**

Mitigate enzyme-mediated PS hydrolysis:

5 companies reported successful downstream process changes with at least some success

1 company did evaluate clone selection approach but with limited success only

4 companies tried DP formulation changes (3 of them with no or <25% of success)

Rational to justify **acceptability of PS degradation were a maintenance of product quality over shelf-life regardless of PS level changes (100%*)** or based on demonstration of minimum effective PS to maintain product quality **(78%*)** or development studies (model systems, **63%*)**

*% based on 9 company responses

Survey study results on control strategies

Control strategies are in place and are further developed to prevent unacceptable consequences of PS degradation

Maintenance of consistent quality of PS product is important: using smaller PS container size, consistent protection from light and oxygen and reduced sampling and testing are advantageous *(avoid opening of PS containers until addition to product stream)*

FFAs and other low soluble impurities and FA-esters beyond compendial requirements
(e.g., C20 FA esters)
reduced and controlled by PS supplier/manufacturer

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If no PS content decrease is observed during long term storage conditions, PS content will be controlled at release, but not during stability once sufficient batch history is available

If PS degradation is observed, its degradation is acceptable if no other CQA is impacted
(PS content to be monitored during release and stability)

End of shelf-life characterization stress studies and PS boundary studies may help to justify PS degradation and stability specifications

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Alternative surfactants *(e.g., not prone to enzymatic degradation)* **are explored but there is still a lack of well characterized and acceptable suitable stabilizers for parenteral use**

Requirements for alternative protein stabilizers and related regulatory hurdles

Requirements for «alternative protein stabilizer»

- Low toxicity / high biocompatibility (comparable to polysorbates), i.e. not hemolytic at relevant concentrations, not immunogenic, no allergic or pseudoallergic reaction, suitable for frequent & life-long treatment,...
- High efficiency to stabilize therapeutic proteins at interfaces and to avoid protein adsorption to surfaces
- Fast mode of action
- Not degraded via enzymes nor oxidized
- Controlled manufacturing process
- Available in GMP grade
- ...and ideally already registered globally for a relevant mode of administration at relevant concentration

Regulatory hurdles

- Alternative stabilizer cannot be tested stand-alone in a clinical trial
- Qualification in clinical trials together with therapeutic protein potentially increasing risk and time for drug program
- Requirements for preclinical testing program for the purpose not clear (it is not an API!) and may vary from drug application to drug application
- DMF system not available in all geographies, and Excipient DMF only reviewed when used in a clinical trial

A specific HA program to support new surfactants/excipients and/or an industry consortium desirable (similar to IPACT I/II for alternative propellants)?

4 important take aways

Polysorbates (PS)
are a very effective protein stabilizer for biopharmaceutical products

Challenges are mainly
the enzyme-mediated hydrolysis
of PS potentially generating free fatty acid related particles and
oxidative degradation
of both **PS and the therapeutic protein**

In the past years, **industry** *(with help of academic research)* has
gained thorough understanding of PS degradation routes
and **developed appropriate analytical tools and effective mitigation measures to minimize PS degradation**

Despite the drawbacks,
PS will continue to be broadly used in our product formulations
as at least we know the **weak points and as alternative efficient protein stabilizers are missing**

Thank you

Q&A

Janssen
PHARMACEUTICAL COMPANIES OF
Johnson & Johnson

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