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Polysorbate degradation case studies: characterization, mechanism elucidation, mitigation measures and implications for control strategy

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Lonza Drug Product Services

27 January 2020 | CMC Strategy Forum | Washington DC

Contents

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- **PS heterogeneity** – scope of the challenge
- What **analytical tools** are available and how to use them?
- How to elucidate the **PS degradation mechanism(s)**?
- What are the **potential consequences** of PS degradation and how to mitigate the risks?
- How to setup a **sound control strategy**?

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PS heterogeneity

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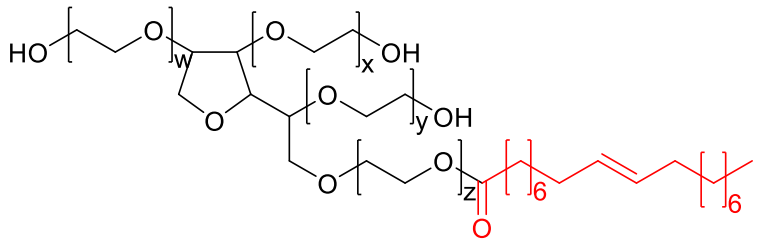
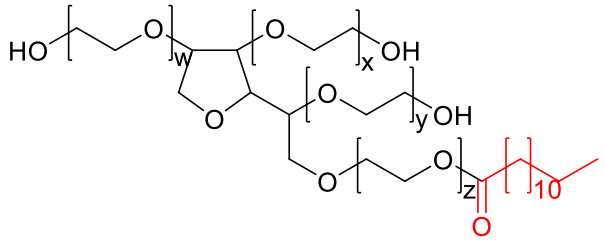
Polysorbate as a pharmaceutical excipient

Complex and heterogeneous mixtures



PS80

w+x+y+z=20



PS20

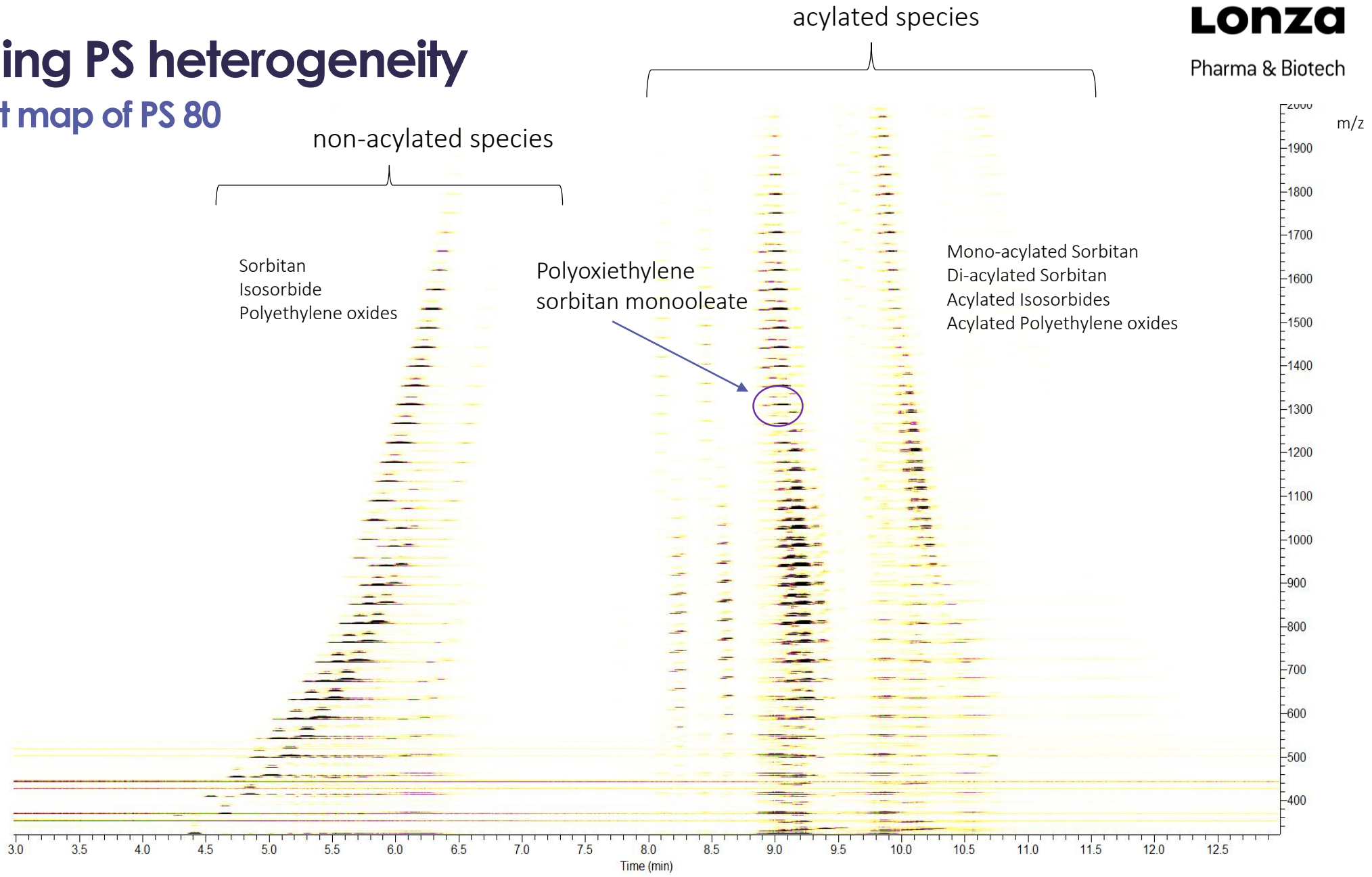
- PS are complex and heterogeneous mixtures (synthesis uses precursors from natural products)
- Manufacturing processes may vary/ change

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

Understanding PS heterogeneity

APCI LC-MS heat map of PS 80



Degradation increases PS heterogeneity

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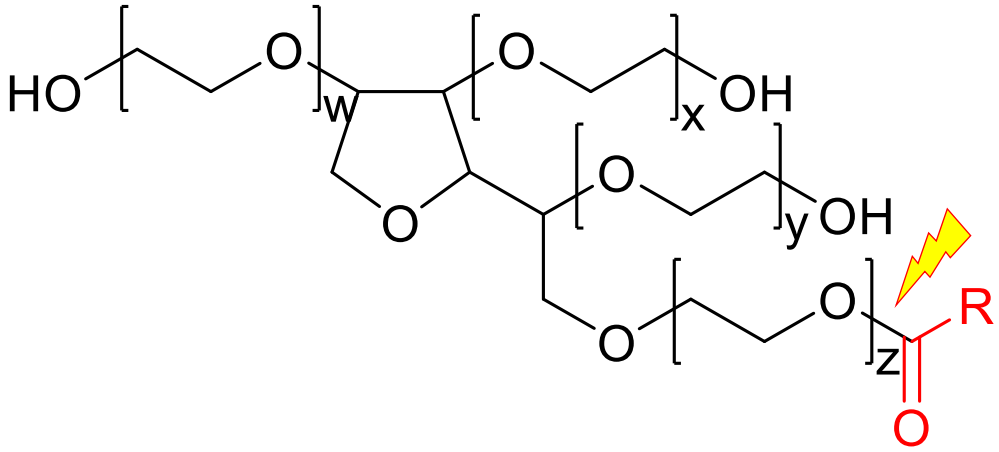
- Hydrolytic
  - Non-enzymatic

Bates et al., 1973. J Pharm Pharmacol 25:470–477
Kishore et al., J Pharm Sci, 2011, 100:2, 721-731

Insignificant at pharmaceutically-relevant conditions

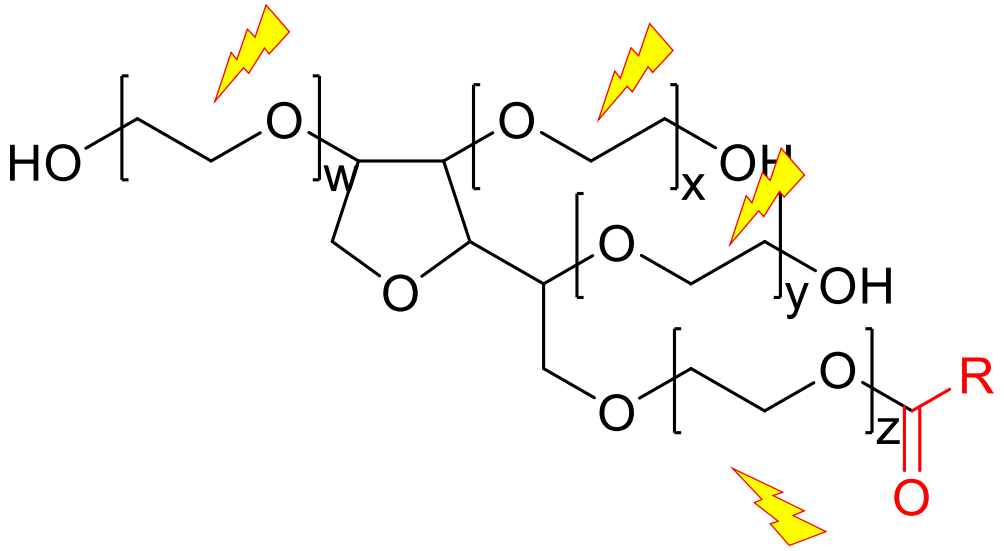


  - Enzymatic

LaBrenz, 2014, J Pharm Sci,103:2268–2277
Hall et al., J Pharm Sci. 2016 ,105(5):1633-42
Dixit et al., J Pharm Sci. 2016,105(5):1657-66

- Oxidative

Donbrow et al., 1978, J Pharm Sci 67:1676–1681
Borisov et al., J Pharm Sci, 104(3),1005–1018;
Porter et al., 1995, Lipids 30: 277–290;
Yin and Porter, 2005, Antioxid Redox Signal 7:170–184;
Kerwin BA 2008. J Pharm Sci 97(8):2924-2935
Kishore et al., J Pharm Sci, 2011, 100:2, 721-731



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Analytical toolbox

How to use the available analytical tools – routine monitoring vs. characterization?

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Analytical Toolbox for Characterization and Control of Surfactants in Biopharmaceuticals

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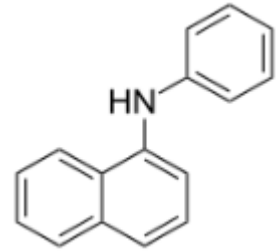
- Surfactant quantity and quality has to be monitored / controlled throughout the shelf life of the product
- Analytics are challenging
  - Due to the complexity of the composition of surfactants
  - Due to high molecular weight species
  - Due to restrictions of analytical methods / instrumentation in QC environment
- Necessity of implementing analytical methods for different purposes
  - Routine methods for monitoring the content / quantity of the surfactant
    - Routine methods for monitoring surfactant degradation (stability indicating methods)
  - Special characterization methods for e.g. investigational support

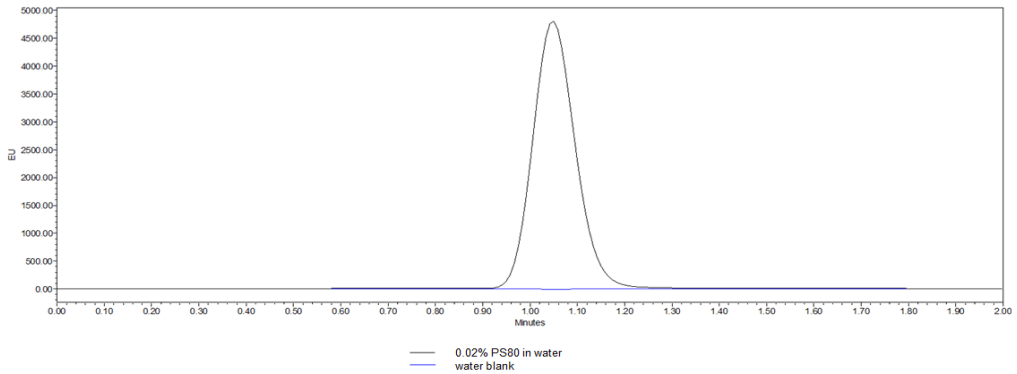
Routine methods for monitoring PS content

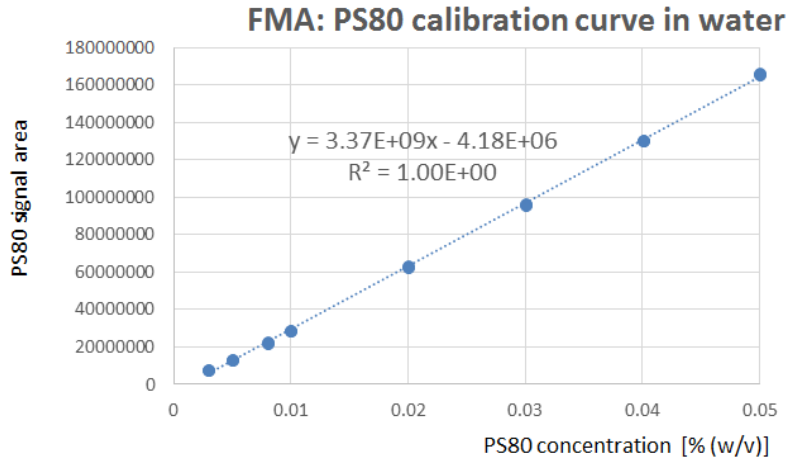
Fluorescence micelle assay (FMA) for quantification of PS20 / PS80

- Fluorescence quantum yield of N-phenyl-1-naphthylamine (NPN) increases in hydrophobic environment
- Fluorescence (emission) intensity increases with micelle concentration, i.e. with polysorbate concentration
- FMA used for quantification of PS20 / PS80 (HPLC (reaction coil) or a plate reader configuration)
- Often samples can be directly measured without interference of other DS / DP constituents
- Check for offset of intercept and accuracy (calibration curves in water and in reformulated DS)
- In case of interference samples have to be worked up
  - Protein precipitation with organic solvent (acetonitrile, acetone, etc.)
  - Removal of organic solvent
- Additional matrix effects can be addressed with standard calibration curve in formulated DS



N-phenyl-1-naphthylamine

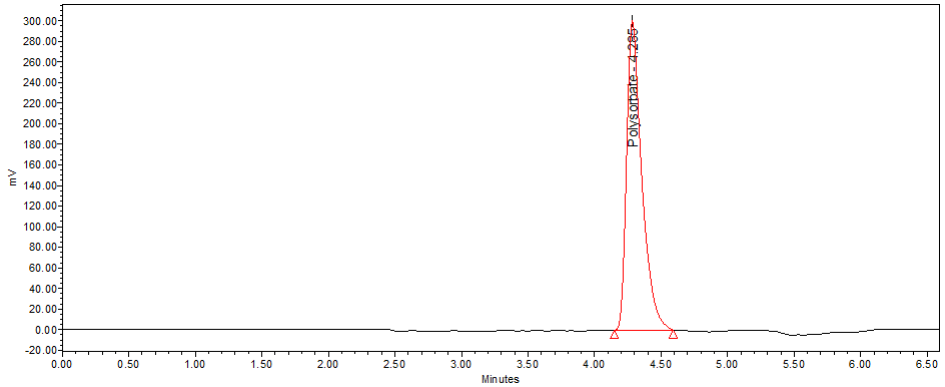




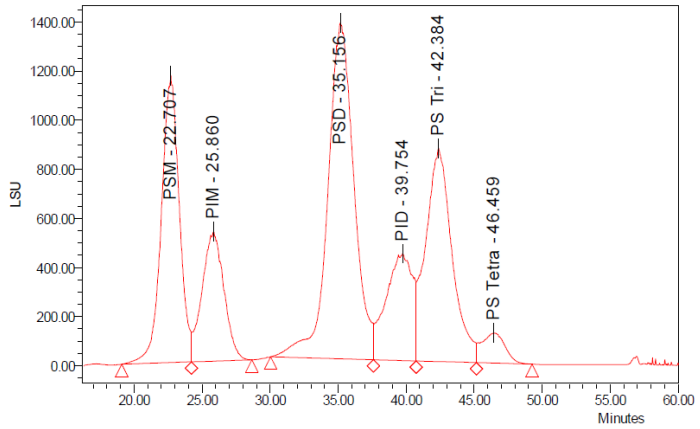
Routine methods for monitoring PS content

HPLC ELSD/CAD methods

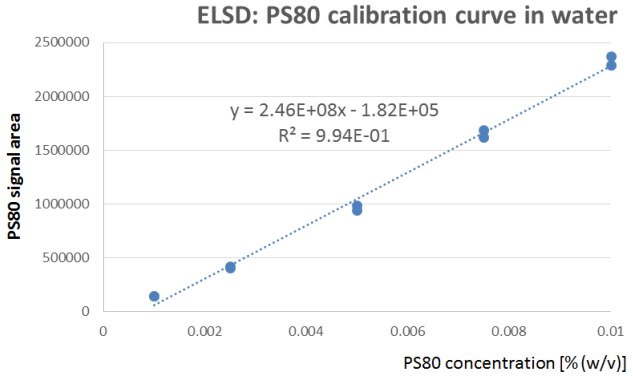
- Separation by mixed mode chromatography – short vs. long gradients
- RP HPLC eluent nebulized by inert gas and volatile constituents are evaporated form the droplets; Non-volatile components are detected by light scattering (ELSD) or ionized by positively charged nitrogen gas from a high-voltage platinum corona and quantified by an electrometer (CAD); Universal detection – i.e. separation of surfactant from polar excipients / protein is necessary



Short gradient
(typically used for content)



Long gradient
(typically used for characterization)



Polysorbate 80 species	Abbreviation
Polyoxyethylene sorbitan monooleate	PSM
Polyoxyethylene isosorbide monooleate	PIM
Polyoxyethylene sorbitan dioleate	PSD
Polyoxyethylene isosorbide dioleate	PID
Polyoxyethylene sorbitan trioleate	PS Tri
Polyoxyethylene sorbitan tetraoleate	PS Tetra

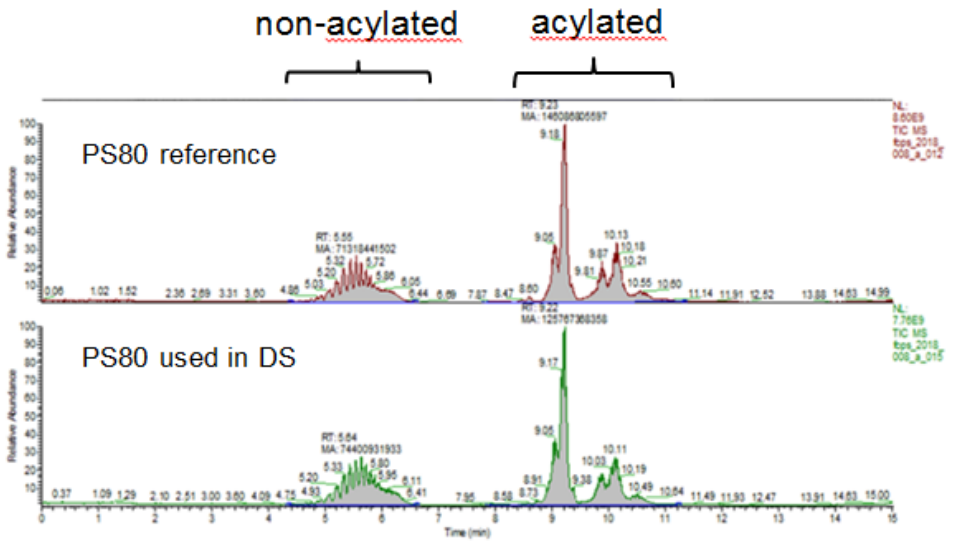
Hewitt D. et al, J. Chromatogr. A, 2008, 1215 (1-2),156-160;
Zhang R. et al, J. Chromatogr. Sci., 2012, 50 (7), 598-607

Routine methods for monitoring PS content

Method stability indicating properties

- Case study:
  - In DS measured by HPLC-ELSD (short gradient): ~100% (of target)
  - In DP measured by HPLC-FMA: ~85% (of target)
  - Additional analysis of PS80 formulated into DS by LC-MS
  - Ratio (acylated / non-acylated): ~82% in PS80 compared to reference
  - Degraded PS80 raw material

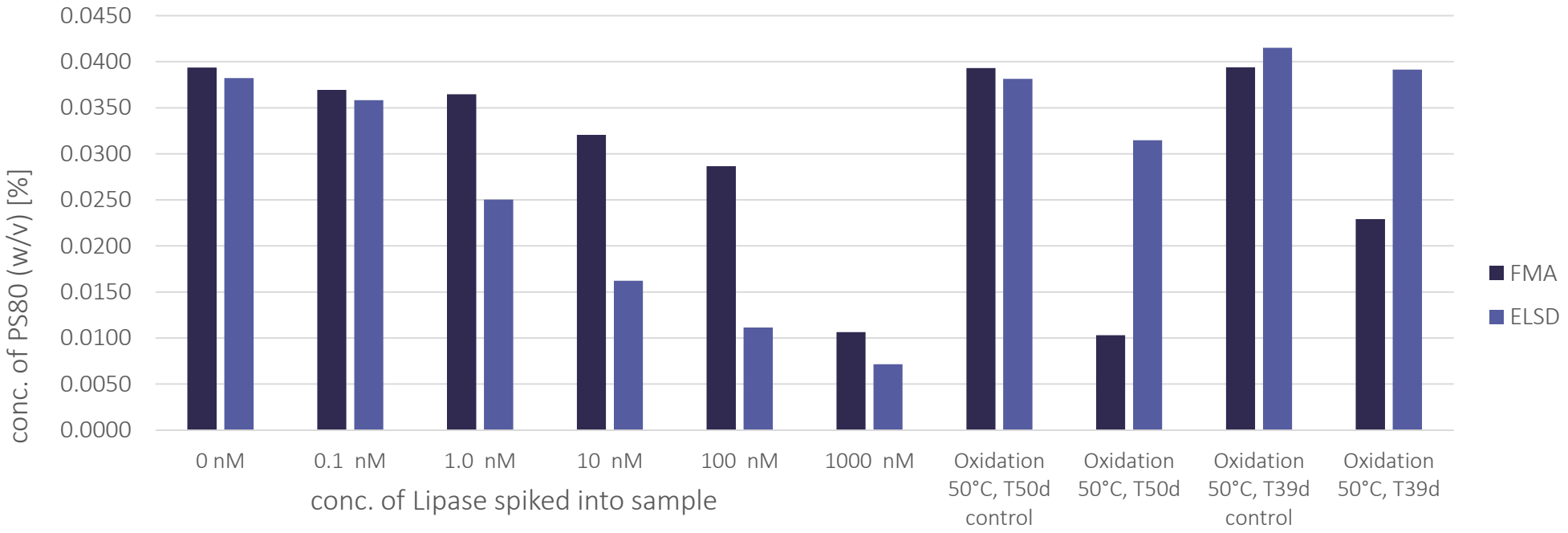
  => FMA method shows better stability indicating properties



Routine methods for monitoring PS content

Method stability indicating properties

Difference in measured PS80 concentration by HPLC-FMA and HPLC-ELSD after application of hydrolytic and oxidative stress



- FMA appears to be a better “generic” method to monitor PS degradation (both oxidative and hydrolytic)
- HPLC-ELSD typically more sensitive to hydrolytic degradation of PS80, but partially “blind” to oxidative degradation

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PS degradation

What are the potential consequences?

How to elucidate PS degradation mechanism(s)?

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Potential consequences of PS degradation

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- Product stability
  - Loss of surfactant may lead to insufficient stabilization against interfacial stress
  - Protein modifications due to the presence of oxidative species (oxidative surfactant degradation)
  - Possible impact on protein stability by e.g. free fatty acids (FFA) and FFA particles
- Compliance to current DP requirements
  - Formation of visible and sub-visible particles on stability
- Potential safety concerns
  - Some concerns raised regarding side effects (various reports of anaphylactoid systemic reactions, hypotension, hypersensitivity, dermatitis, injection site reactions,); potential of PS related species to act as haptens and adjuvants; *references available upon request*
  - Different PS components have very different safety profile

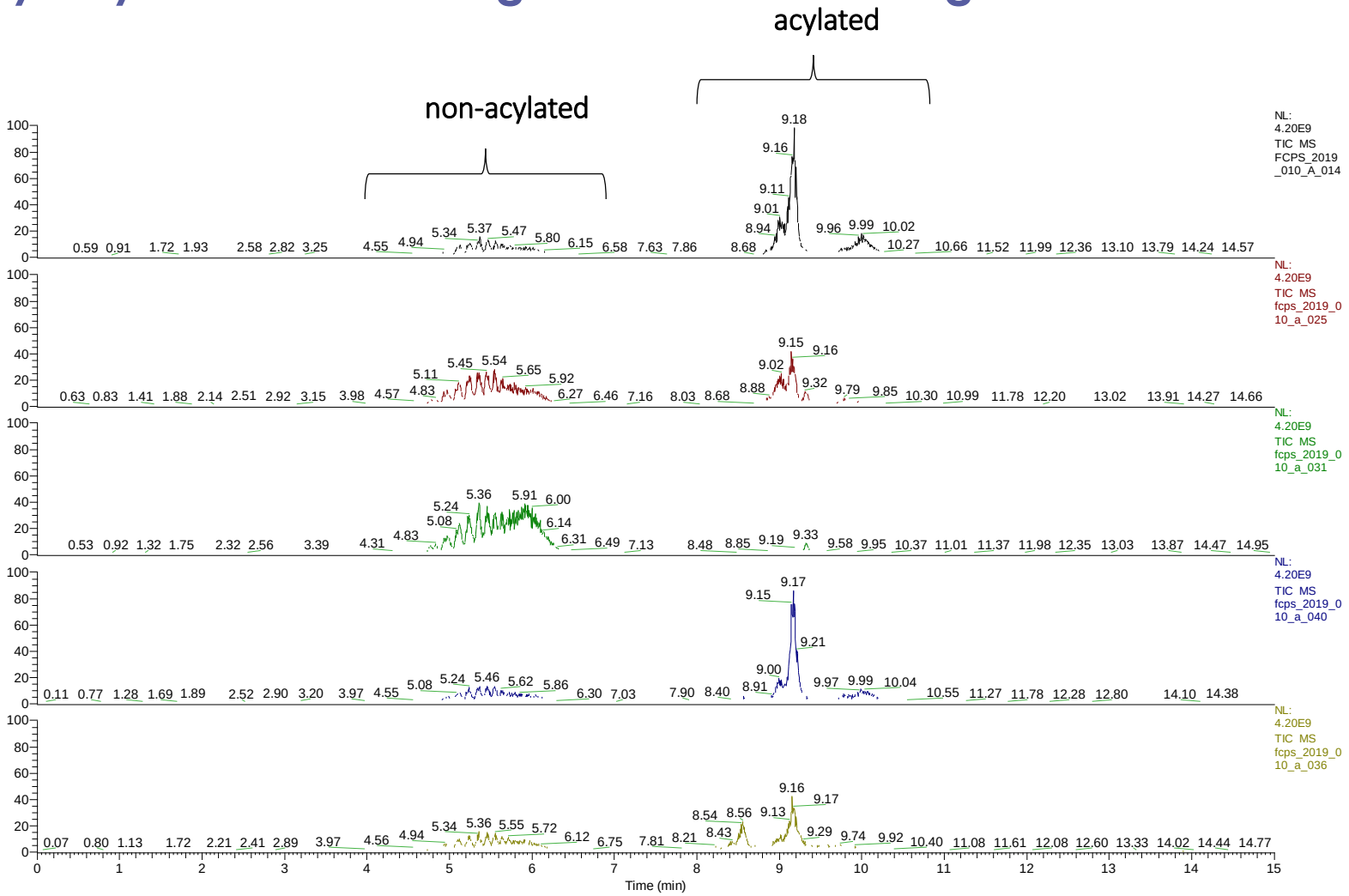
Mechanisms of PS degradation

Oxidative vs. hydrolytic

- Oxidation
  - Auto oxidation via radical mechanism:
    a) Initiation (hydrogen abstraction produces free radicals),
    b) Formation of peroxy radicals (reaction with molecular oxigen),
    c) Propagation – intra- or intermolecular hydrogen abstraction
  - Light or transition metals may accelerate these reactions
  - Temperature dependent, though significant degradation can happen at 2-8°C as well
  - Can happen in formulation (during storage)
  - Can happen in placebo
  - Likely concomitant protein oxidation
- Hydrolysis
  - Polysorbates (esters) are susceptible to hydrolysis (proteins have aqueous formulations)
  - Largely not relevant under DP storage conditions (2-8°C)
- Enzyme-catalyzed hydrolysis
  - May be caused by co-purified trace quantities of HCPs (lipases)

Mechanisms of PS degradation

Hydrolytic vs oxidative degradation – how to diagnose?



Reference 0.04% (w/v) PS80

0.04% (w/v) PS80 + 1 nM lipase/ 2 days/ RT

0.04% (w/v) PS80 + 1000 nM lipase/ 2 days/ RT

0.04% (w/v) PS80 + oxidative stress (high)

0.04% (w/v) PS80 + oxidative stress (extreme)

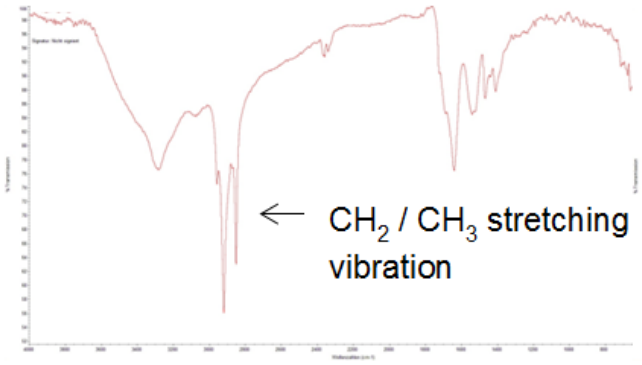
Hydrolytic stress reduces the amount of acylated species, whereas oxidative stress results in new acylated species

Particle formation

Formation of VPs and SvPs may result in incompliant DP

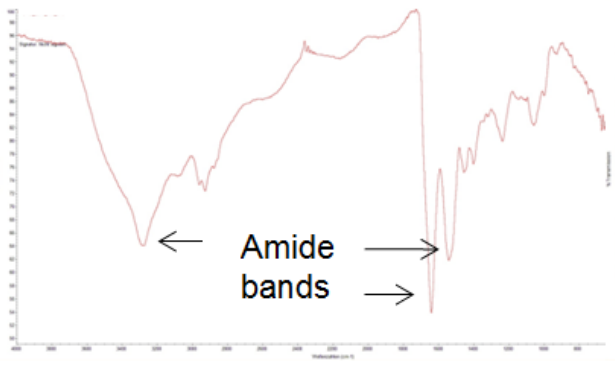
- Case study: Visible particles detected during visual inspection of DP samples (mAb, PS20 formulation) stored for 6 months at 5°C and at 25°C
  - FTIR microspectroscopy analysis of filter residues

filtered visible particles



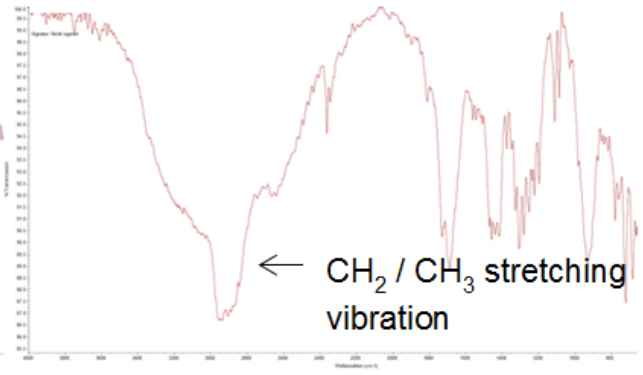
FFA and protein

filtrate – negative control

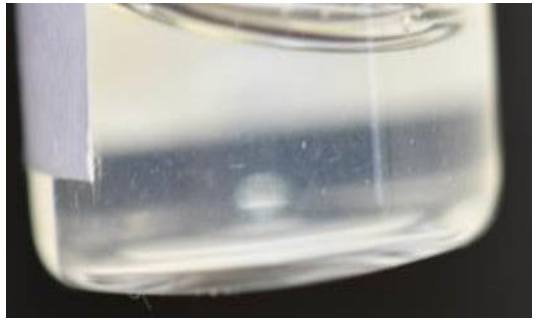


protein

filtrate – negative control spiked with FFA



FFA and protein



- Proteinaceous particles are a common analytical artifact – requires appropriate controls

Particle formation

FFA distribution in particles – LC-UV/MS

- FFA have different solubilities – preferential enrichment of long-chain unsaturated FFA

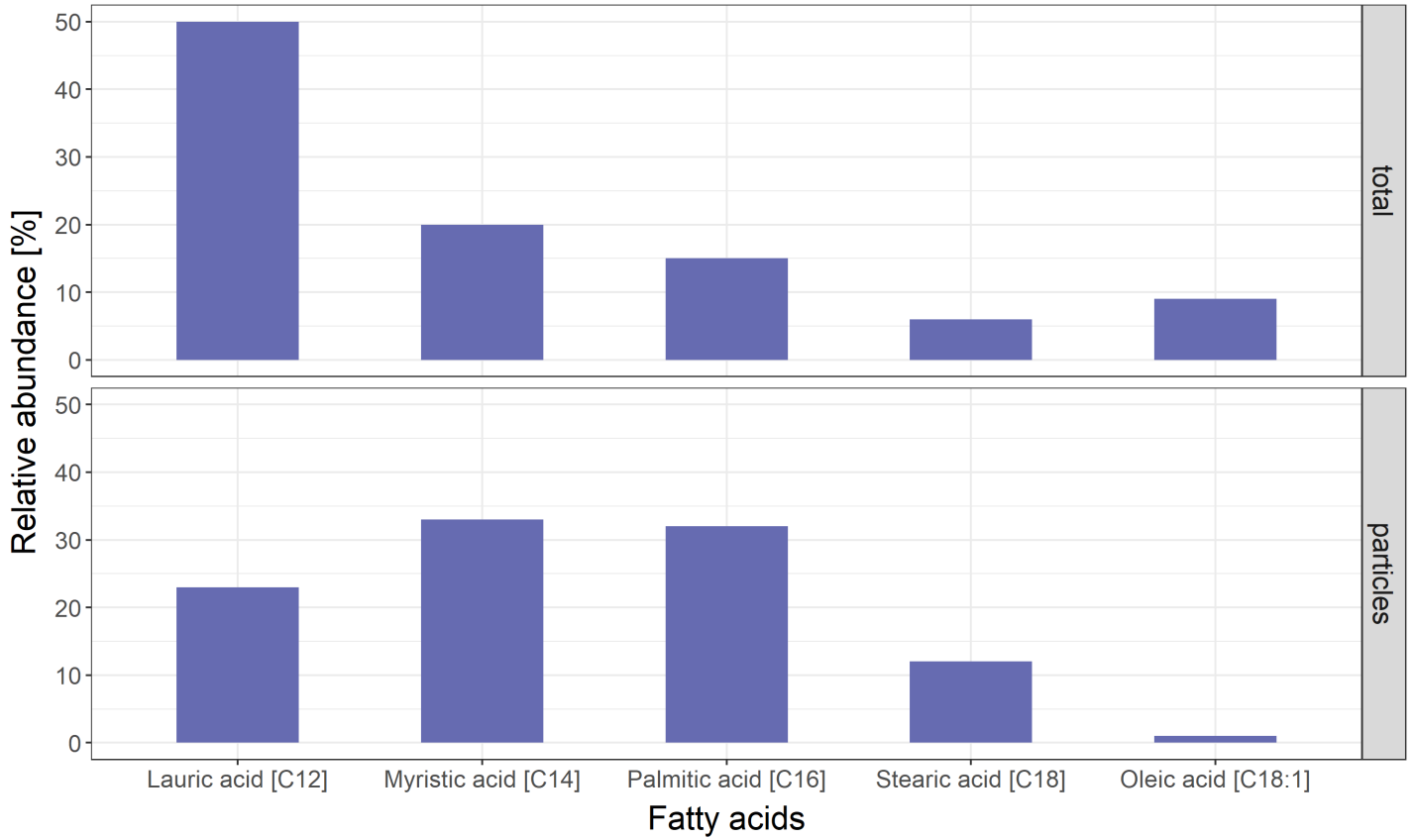


Table 4. FFA Solubilities of Lauric, Myristic, and Palmitic Acid in mAb-A and mAb-B Buffers and Active Formulations after 1 Month Storage at 2–8 °C

FFA	solubility limit (μg/mL)			
	in mAb-A formulation buffer	in mAb-B formulation buffer	in 60 mg/mL mAb-A active	in 30 mg/mL mAb-B active
lauric (C12)	15 ± 1	23 ± 1	17 ± 1	> 22
myristic (C14)	3 ± 1	3 ± 1	3 ± 1	3 ± 1
palmitic (C16)	1.5 ± 0.5	0.75 ± 0.25	1.5 ± 0.5	0.75 ± 0.25

Doshi et al, Mol Pharm. 2015 12(11):3792-804

Additional causes of particles reported in the literature:

free fatty acids (Siska et al., 2015, J Pharm Sci, 104:447–456) and other impurities e.g. **12-tricosanone** (Hampl, V. et al, J. Pharm. Sci., 2018, 107(6), 1552-1561) to particle formation

Summary

Analytical Toolbox

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- Multiple complementary analytical technologies are available. Right tool for the right job?
- Measuring content vs. characterization.
  - Content: FMA and HPLC-ELSD / CAD have pro's and con's; Be aware of stability indicating properties
- Additional analytical technologies may be required If polysorbate degradation is observed
  - Mass spectrometry, Microspectroscopy, peroxide assays, etc.
  - Be aware of what is being measured – ionization and detection methods, analyte solubilities etc.

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PS Control Strategy

How to build a holistic PS control system

What are the potential consequences?

How could we mitigate related risks?

New tools for PS stabilization and process development support

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Risk mitigation of surfactant degradation requires a holistic approach

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A good control strategy is comprised of:

- Raw materials testing/ qualification
- Product characterization throughout development
- Adherence to GMP
- Manufacturing process validation
- In-process control
- Specifications (release, stability)
- Stability testing

"Quality should be built into the product, and testing alone cannot be relied on to ensure product quality"

FDA Guidance for Industry: Quality Systems Approach to Pharmaceutical CGMP Regulations)

Raw materials testing, qualification and control

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Measures

- Sourcing

ENSURE:

  - Supplier qualification
  - Batch control

- Handling and storage recommendations

ENSURE:

  - Store at 2-8 °C
  - Protect from air (e.g. N_2 overlay)
  - Protect from light
  - Single-use containers (avoid re-use after opening)

Potential impact

- Batch-to-batch and supplier variability
  - Impact of impurities present in the raw material
    - Free fatty acids present in PS – presumably unreacted starting material (Siska et al., 2015, J Pharm Sci, 104:447–456)
    - Other impurities – e.g. 12-tricosanone (Hampl V, et al, J Pharm Sci. 2018, 107(6):1552-1561)
    - Presence of peroxides in PS
      (Singh et al., AAPS PharmSciTech, Vol. 13, No. 2, June 2012, Wasylaschuk et al., 2007, J Pharm Sci, 96(1) Ha et al., J Pharm Sci. 2002 Oct;91(10):2252-64.)
- Improper storage and handling can result in oxidative degradation of PS.
  - Trp oxidation as a result of improper storage and handling of PS (Lam et al., Pharm Res. 2011, 28(10):2543-55)

Stabilization of PS raw material

Stabilization of PS80 against oxidation with BHT/BHA (0.2%, w/w)

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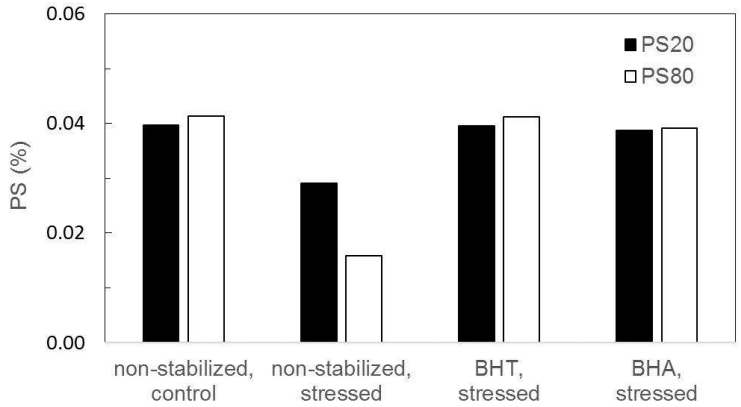
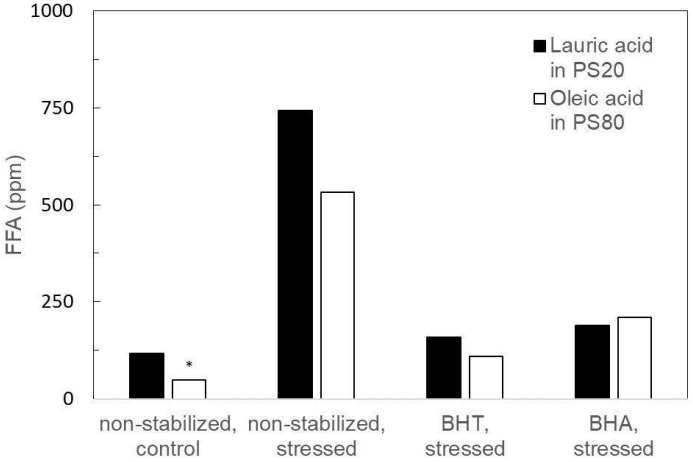
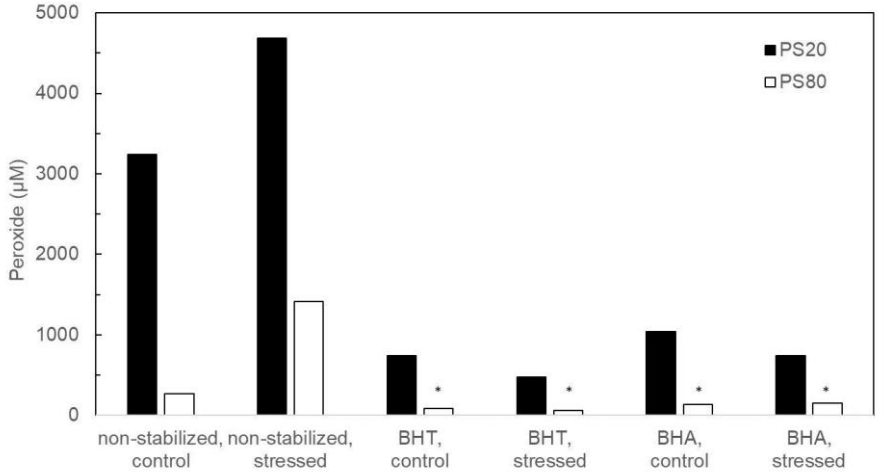
- BHT/BHA additive protects PS raw material against oxidative degradation
- BHT/BHA additive inhibits the liberation of FFAs in PS raw material under oxidative stress
- BHT/BHA additive inhibits peroxide formation occurs in PS raw material



PS content by HPLC-FMA (10% PS80, 7 weeks, 40°C, air contact) and controls (-20°C)



FFA content by LS-MS negative mode (10% PS80, 7 weeks, 40°C, air contact) and controls (-20°C)



Peroxide content by FOX assay (7 weeks, 40°C, air contact) and controls (-20°C)

Lonza proprietary – patent pending

Product characterization in development

Know your product

Measures

- Decrease in surfactant content during storage
  TEST IT:
  -> monitor surfactant content throughout development using appropriate methods
  -> characterize the predominant surfactant degradation pathways throughout development
  -> assess protection against interfacial stress at EOSL
  -> assess the presence of lipase activity
- Purify out lipases of present
  - Potential loss of protection against interfacial stress, BUT, some degradation products are also surface active
    TEST IT: e.g. agitation studies at end of shelf life
- Effect of surfactant degradation products on the product
  - Protein oxidation – the presence of oxidative species may result in oxidation of e.g. Met, Trp
    TEST IT: protein characterization studies (incl. antioxidants)
  - Possible impact on protein stability by e.g. free fatty acids
    TEST IT: careful monitoring of particles (VPs and SvPs) throughout

Potential impact

- Decrease in surfactant content during storage
  - Potential loss of protection against interfacial stress, BUT, some degradation products are also surface active

Degradation products of PS still show surface activity even after 60% loss of content

(Kishore et al., 2011, Pharm Res., 28:1194)

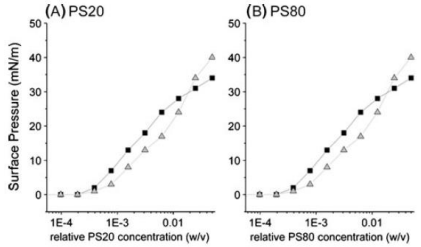


Fig. 4 Surface pressure plots of polysorbate 20 and 80 solutions measured by a modified Du Nuoy method over a concentration range up to 0.05% (w/v) polysorbate for samples at t0 (■) and after 12 months (▲).

- <u>FFA particle formation may result in incompliant DP</u>
- Effect of surfactant degradation products on the protein
  - Protein oxidation – the presence of oxidative species may result in oxidation of e.g. Met, Trp
  - Possible impact on protein stability by e.g. free fatty acids

Product characterization in development

Can we monitor lipolytic activity in DS and DP?

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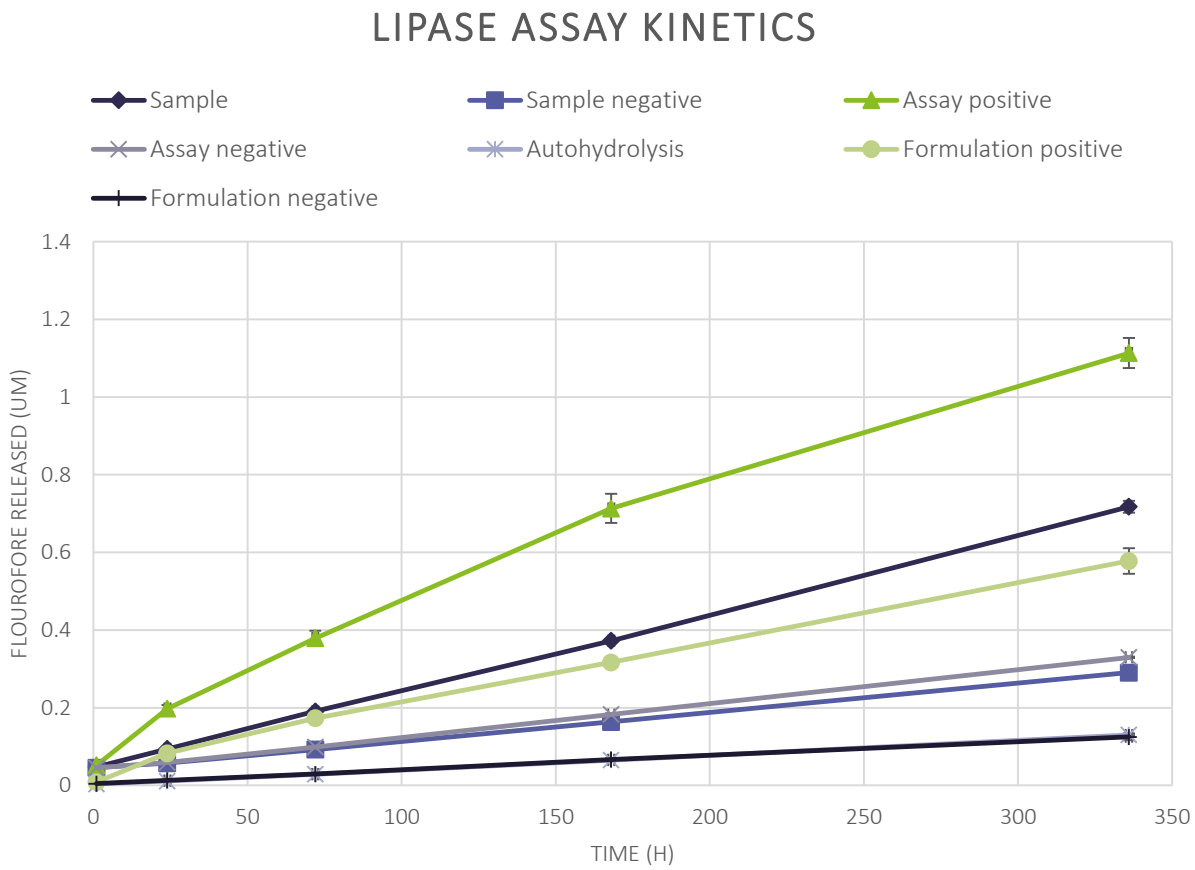
- One root cause for polysorbate degradation is enzymatic hydrolysis due to residual lipase activity, where the lipase(s) are host cell proteins (HCPs)
- Lipolytic activity in DS and DP should be monitored
- Strongly recommended: application of lipase assay to:
  - identify lipolytic activity as root cause for polysorbate degradation
  - improve the downstream purification process,
    i.e. to efficiently remove lipases.

Lonza proprietary – patent pending



Process development, validation and in-process control

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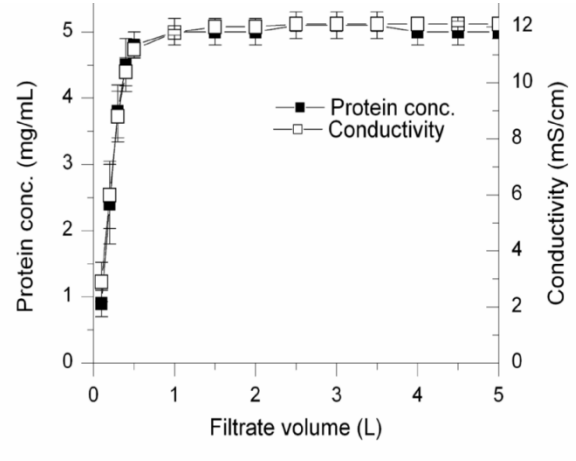
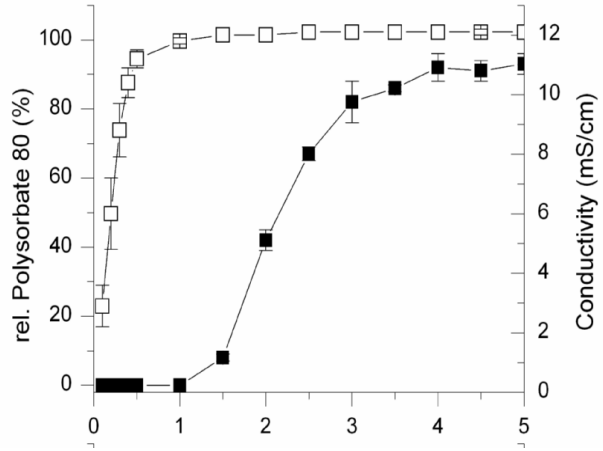
Measures

TEST IT: Monitor surfactant content and qualify critical unit operations during process development

Potential impact

- Surfactants can adsorb to contact surfaces e.g. manufacturing equipment (filters, tubing, etc.), leading to significant losses or product imhomogeneity





Mahler et al., (2010) J. Pharm. Sci.

Specifications

Release and stability

Pharm Res (2018) 35:148
https://doi.org/10.1007/s11095-018-2430-5

RESEARCH PAPER

Considerations for the Use of Polysorbates in Biopharmaceuticals

Michael T. Jones[1] • Hanns-Christian Mahler[2] • Sandeep Yadav[3] • Dilbir Bindra[4] • Vincent Corvari[5] • R. Matthew Fesinmeyer[6] • Kapil Gupta[7] • Alexander M. Harmon[8] • Kenneth D. Hinds[8] • Atanas Koulov[2] • Wei Liu[8] • Kevin Maloney[7] • John Wang[3] • Ping Y. Yeh[6] • Satish K. Singh[2]

Recent PS consortium paper distinguishes 3 cases:

1. No significant change in the polysorbate level over the shelf-life. No impact to product quality related to polysorbate performance.
2. Significant change in polysorbate level over the shelf-life, BUT surfactant functionality remains intact. No correlated impact to the product critical quality attributes.
3. Significant change in polysorbate level over the shelf-life AND one or more product quality attributes are impacted.

Specifications

Release and stability

Pharm Res (2018) 35:148
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RESEARCH PAPER

Considerations for the Use of Polysorbates in Biopharmaceuticals

Michael T. Jones[1] • Hanns-Christian Mahler[2] • Sandeep Yadav[3] • Dilbir Bindra[4] • Vincent Corvari[5] • R. Matthew Fesinmeyer[6] • Kapil Gupta[7] • Alexander M. Harmon[8] • Kenneth D. Hinds[8] • Atanas Koulov[2] • Wei Liu[8] • Kevin Maloney[7] • John Wang[3] • Ping Y. Yeh[6] • Satish K. Singh[2]

Surfactant testing for release and stability typically done in the "extended characterization" assay panel provided that:

- Raw material qualification and control is performed
- Proper procedures for raw material storage and handling are implemented
- Behavior of PS during the development process is characterized, including:
  - Degradation of surfactant measured appropriately
  - Degradation pathways understood
- Careful and sound drug product and manufacturing process development and characterization has been done
  - Potential influence of surfactant degradation on product stability and CQAs understood

Specifications

Release and stability

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Recent PS consortium paper distinguishes 3 cases:

1. No significant change in the polysorbate level over the shelf-life. No impact to product quality related to polysorbate performance.
2. Significant change in polysorbate level over the shelf-life, BUT surfactant functionality remains intact. No correlated impact to the product critical quality attributes.
3. Significant change in polysorbate level over the shelf-life AND one or more product quality attributes are impacted.

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Thank you