Key Interactions of Polysorbates in Protein Formulations

Hanns-Christian Mahler & Kishore Ravuri
Pharmaceutical Development & Supplies, PTD Biologics EU
Hoffmann-La Roche AG, Basel, Switzerland

Molecular Interactions in Biopharmaceutical Formulations:
Can stability be rationalised and predicted?
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Surfactants are potent Stabilizers for Proteins against Interfacial Stresses

- "Interfacial stresses" are encountered during many stages of production, shipment and use e.g.
  - Air/liquid interfaces (e.g. shaking)
  - Ice/Liquid interfaces (e.g. freezing and thawing)
  - Surface interactions (e.g. manufacturing equipment)
- Interfacial stresses may lead to "protein instabilities" such as **adsorption**, **aggregation** or **precipitation** ("particle formation") *Kiese et al, J. Pharm Sci. 2008, 2009*
- Surfactants (such as the non-ionic **Polysorbate 20 or 80**) effectively **protect** Proteins against aggregation caused by interface-induced stresses and adsorption
  Carpenter, Arakawa et al. 1992; Kendrick et al. 1996; Kerwin, Heller et al. 1998; Randolph and Jones 2002; Mahler, Mueller et al. 2005; Kiese et al., 2008/2009

Polysorbate 20 and 80 are the most widely used Surfactants in Protein Formulations

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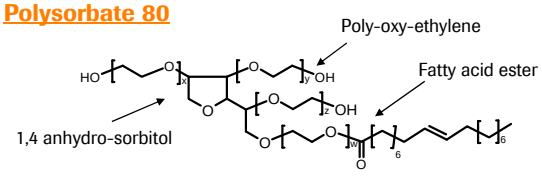
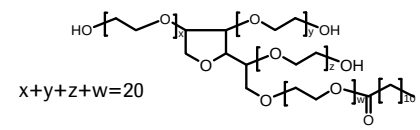
Polysorbate 80

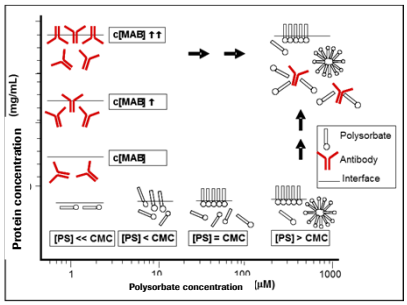


Polysorbate 20



Polysorbate 80	Polysorbate 20
• Campath • Humira • Orthoclone • Zenapax • ReoPro • Rituxan • Tysabri • Remicade • Erypro	• Avastin • Lucentis • Raptiva • Xolair • Herceptin

70% of marketed antibodies are formulated with Polysorbates
Typical concentrations of PS20/PS80 range from 0.001-0.1% (w/v)



Effective **protection** of proteins against aggregation caused by interface-induced stresses and adsorption

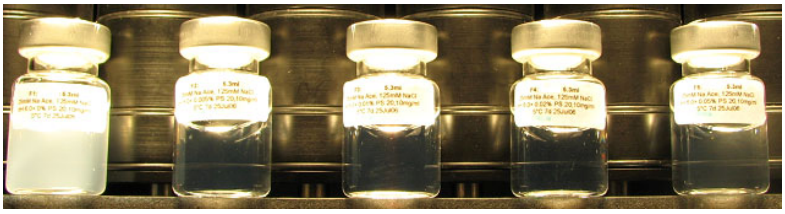
Roche

How Polysorbates stabilize Proteins

Polysorbate stabilize (many) Proteins against shaking-induced aggregation/precipitation

Kiese, S., Pappenberger, A. Friess, W., Mahler, H.-C. (2008) J. Pharm. Sci 97(10):4347-4366

Effect of mechanical stress on Mab T in the presence and absence of Polysorbate



0 % 0.005 % 0.01 % 0.02 % 0.05 % PS20

- Note: the CMC of Polysorbates does not unequivocally correlate to the stabilization behaviour

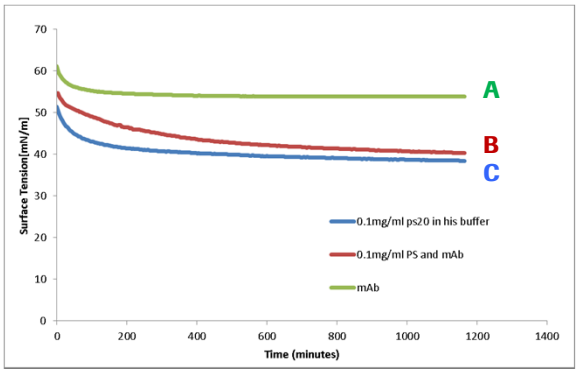
  Mahler, H.-C., Senner, F., Mäder, K., Müller, R. (2009) J.Pharm.Sci. 98(12):4525-33

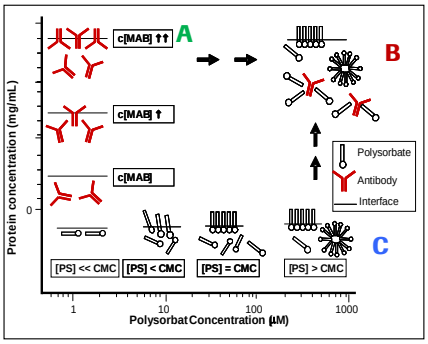
Mechanism of Polysorbate Function(s)

Adsorption of polysorbate to interface: a surface tension treatment

Kishore Ravuri, yet unpublished results

Stronger adsorption of polysorbate 20 observed in surface tension isotherm demonstrating that the stabilizing ability of the surfactant is surface energy driven.



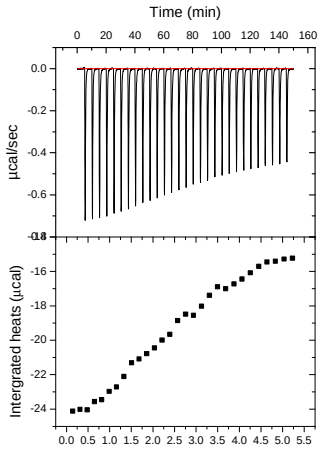


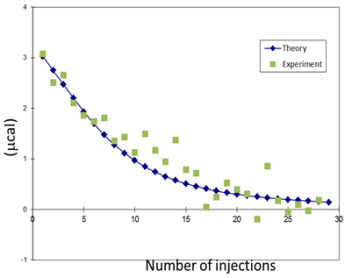
Langmuir adsorption isotherm $KC = \frac{\Gamma}{\Gamma_\infty - \Gamma}$

adsorption equation $\Pi_S = k_B T \Gamma_\infty \ln\left(\frac{\Gamma_\infty}{\Gamma_\infty - \Gamma}\right)$

Interaction of non-ionic surfactants with proteins: Studies with ITC

Kishore Ravuri, yet unpublished results





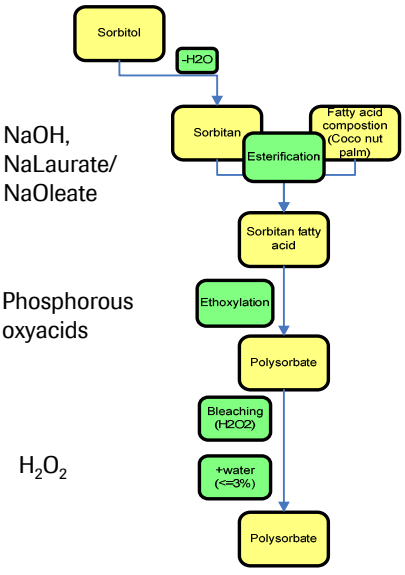
ITC titration of a non-ionic surfactant into a 10mg/mL mAb formulation, 20mM His/HCl, pH6, 25 °C

- Low binding constants (K= 10^2-10^3 M^{-1})
- Comparable to literature e.g. Garidel et.al.

- The above results confirms that interactions between surfactant and protein are *non specific*.
- Stabilizing effect of non-ionic surfactants is NOT interaction driven.

Synthesis of Polysorbates and Polysorbate Heterogeneity

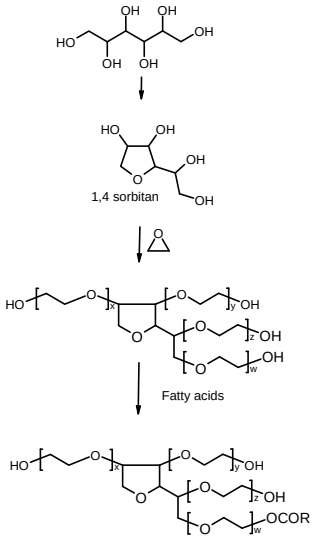
Industrial Synthesis of Polysorbates



NaOH, NaLaurate/ NaOleate

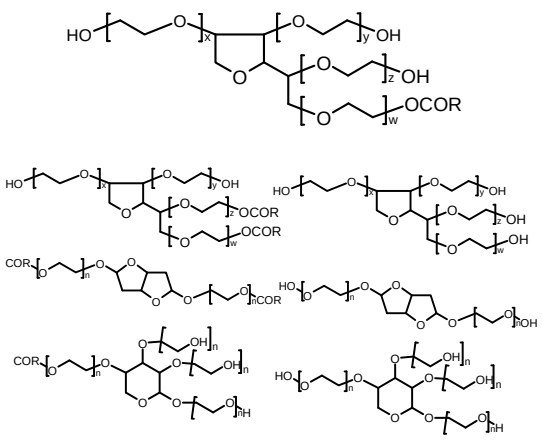
Phosphorous oxyacids

H_2O_2



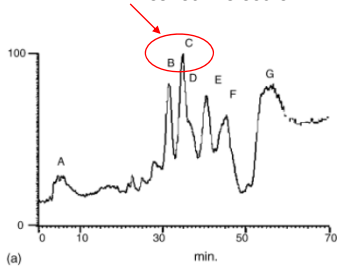
H. Vu Dang et al. / J. Pharm. Biomed. Anal. 40 (2006) 1155–1165

Side Products in Synthesis of Polysorbates



PEG, Fatty acid/salts

Desired molecule!



LC-MS TIC of polysorbate

Peaks	Identities
A	Sorbitan-POE (20–32)
B	Sorbitan-POE (18–34)-monopalmitates
C	Sorbitan-POE (15–34)-monostearates
D	Isosorbide-POE (11–15)-monopalmitates
E	Isosorbide-POE (11–15)-monostearates
F	Sorbitan-POE (20–30)-palmitate/stearates
G	Isosorbide-POE (9–15)-palmitate/stearates

H. Vu Dang et al. J. Pharm. Biomed. Anal. 40 (2006) 1155–1165

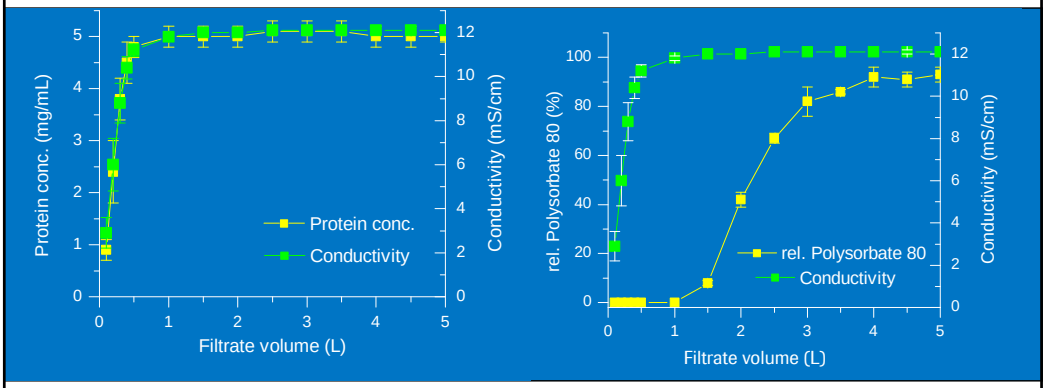
- High batch to batch & supplier variability

Some challenges in the context of the use of Polysorbates in Protein Formulations

1. Adsorption of Polysorbate

Polysorbates can significantly adsorb to some filters

Mahler, H.-C., Huber, F., Ravuri, S.K.K., Reindl, J., Rückert, P., Müller, R. (2010) J. Pharm. Sci.



- Dilution effects in filters (e.g. due to residual water), observed via parallel change of protein concentration and conductivity
- Adsorption of Polysorbate 80 to filters (>80% recovery after 3L pre-rinse with Product)

Some challenges in the context of the use of Polysorbates in Protein Formulations

2. Polysorbates in Formulations used for preclinical animal (Dog) studies

Pseudoallergenic reaction in dogs after administration of Polysorbate

- Polysorbate 20 (Tween 20) used for i.v. formulation of protein (stabilizer)
- A tox study was performed with rats and dogs: also placebo vehicle showed a pseudoallergic reaction in the dogs (well tolerated in rats and monkeys – as well as humans)

⇒ dogs to be excluded for tox studies with formulations containing polysorbates because hypersensitive compared to other species

- **Potential mechanisms:**
  - **Histamine-releasing properties of polysorbates** Masini et al. (1985)
    10 mg/kg of Polysorbate 80* caused severe hypotension after first administration and increase in plasma histamine
  - **Haemolytic properties of polysorbates** Krantz et al. (1948!)
    haemolysis of red blood cells in vitro due to the solubilizing activity of Tween 20 at concentrations > 100 mg/ 100 ml

Some challenges in the context of the use of Polysorbates in Protein Formulations

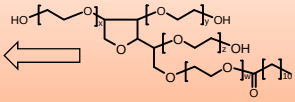
3. Degradation of Polysorbates in Bulk and/or aqueous formulation

Polysorbates Can Undergo Degradation

Temperature

Light, O_2, metals

Auto-oxidation



Peroxides, formic and acetic acid

Hydrolysis

Fatty acids

pH, Temperature

J. Pharm. Pharmac., 1973, 25, 470–477 Received January 15, 1973

Kinetics of hydrolysis of polyoxyethylene (20) sorbitan fatty acid ester surfactants 1973

THEODORE R. BATES,† CHARLES H. NIGHTINGALE AND EDWARD DIXON

Development of Acidity in Non-ionic Surfactants: Formic and Acetic Acid 1978

M. Donbrow, R. Hamburger, E. Azaz and A. Pillersdorf

Pharmacy Department, School of Pharmacy, Hebrew University of Jerusalem, P.O. Box 12065, Jerusalem, Israel

Autoxidation of Polysorbates 1978

M. DONBROW *, E. AZAZ, and A. PILLERSDORF

Received September 6, 1977, from the Pharmacy Department, School of Pharmacy, , publication March 31, 1978.

Polysorbates 20 and 80 Used in the Formulation of Protein Biotherapeutics: Structure and Degradation Pathways 2007

Peroxide Formation in Polysorbate 80 and Protein Stability 2002

EMILY HA, WEI WANG, Y. JOHN WANG

Analytics & Formulation Department, Process Sciences, Bayer Biotechnology, 800 Dwight Way, Berkeley, California 94701

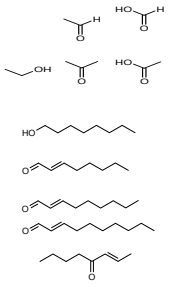
Received 30 January 2002; revised 11 April 2002; accepted 1 May 2002

Mechanistic picture

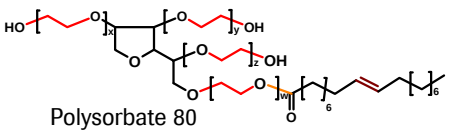
Findings from investigations:

Degradants isolated from placebo formulations stored at 25°C for 20 months

Small molecules

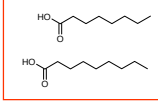


Major degradants

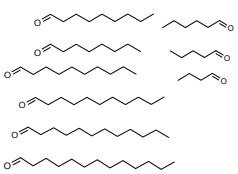


Polysorbate 80

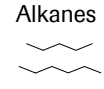
Fatty acids



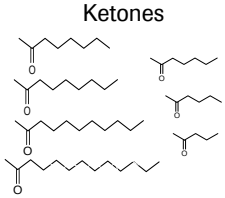
Aldehydes



Alkanes



Ketones

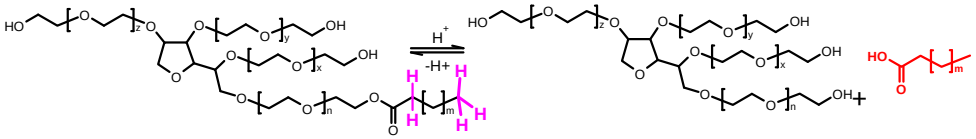


Minor degradants

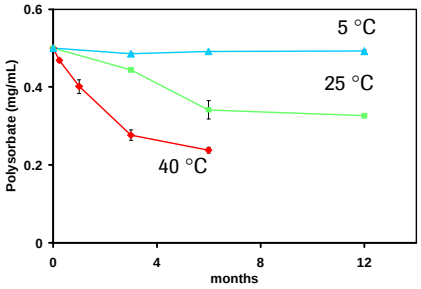
Dominant Mechanisms In Polysorbate Degradation

Kishore, R.S.K., Pappenberger, A., Bauer Dauphin, I., Ross, A., Buergi, B., Staempfli, A., Mahler, H.-C. J. Pharm Sci., 2011, 100: 721

Ester hydrolysis



Profile of NMR signals of fatty acid in PS20



	Temp (°C)	k (h^{-1})	$t_{1/2}$ (h)
PS20	5	9e-07	5.50e05
	25	5e-05	1.39e04
	40	2e-04	3.47e03
PS80	5	4e-07	1.73e06
	25	5e-05	1.39e04

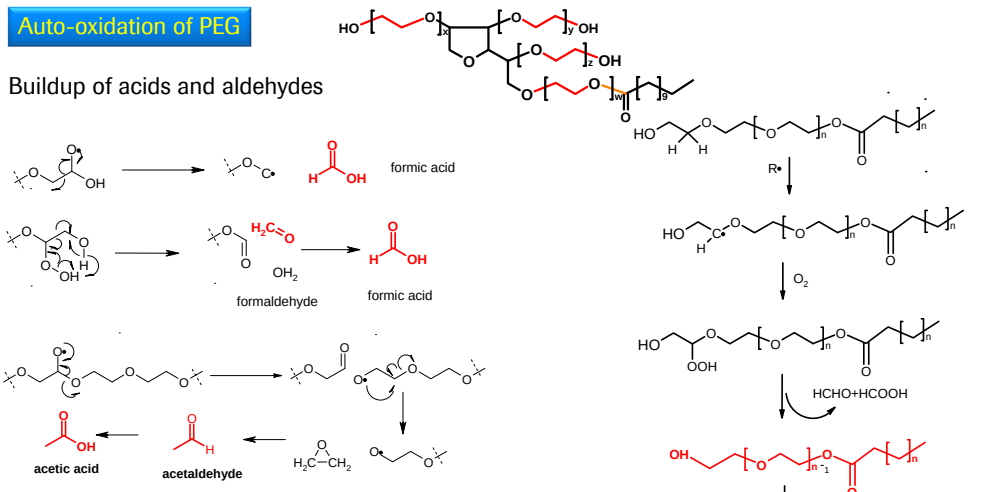
- *t1/2* of Polysorbate20 hydrolysis at 40°C was about 5 months
- *t1/2* of Polsyorbate20 hydrolysis at 5°C was negligible

Dominant Mechanisms In Polysorbate Degradation

Kishore R. S. K., Kiese S., Fischer S., Pappenberger A., Grauschopf U., Mahler H.-C. Pharm Res. 2011, 28:1194

Auto-oxidation of PEG

Buildup of acids and aldehydes



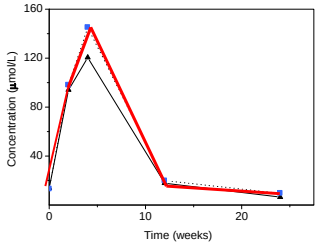
Decker et al, *Die Makromolekulare Chemie, 1974, 3531-3540*

Storck et al, *Die Makromolekulare Chemie, 1966, 50-73*

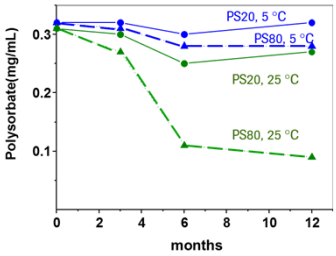
Mechanistic Picture

Summary

- Auto-oxidation plays a dominant role in degradation of polysorbates. The rate of **Hydrolysis is negligible** at pharmaceutically relevant conditions (drug product storage of 5 °C & 25 °C)
- Along with rupture of PEG chains, there also occurs rampant degradation at the **olefinic sites.**
- It is likely that the radical initiation occurs first at the olefin site and then spreads to the PEG chains.



Peroxide formation in placebos containing 0.02% PS20 and PS80 at 40 °C



Changes in PS20 and PS80 concentration measurement by micelle method over 1 year at 25 and 5 °C

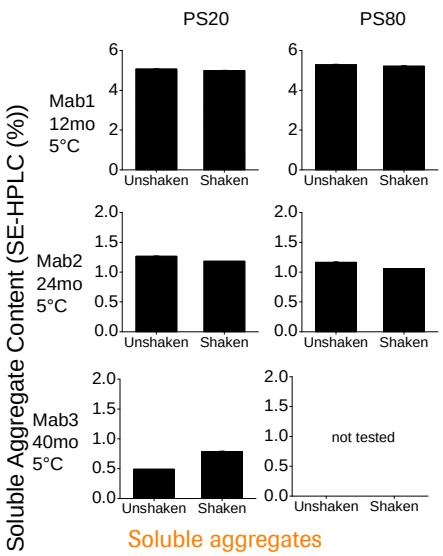
Impact of Polysorbate degradation on protein formulations

Overall considerations

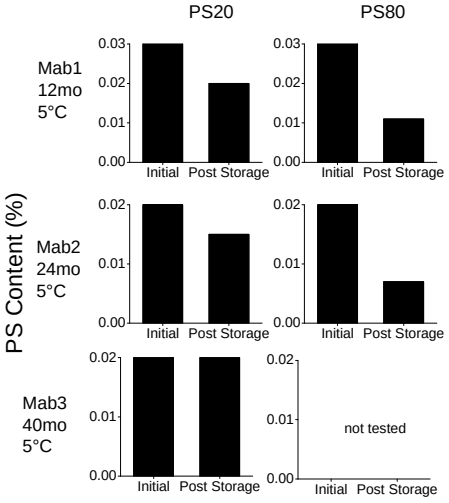
Effect of decreased surfactant content	▪ What is the impact of decreased surfactant content on the stability of the protein? Not enough stabilizer??
Effect of insoluble degradants from hydrolysis	▪ What do the degradants do to the protein? ▪ Do degradants impact product quality other than interacting with protein?
Effect of peroxides from auto-oxidation	▪ What influence does auto-oxidation of PS have on the mAb?

Impact of Polysorbate degradation on protein formulations

End of shelf life shake stress



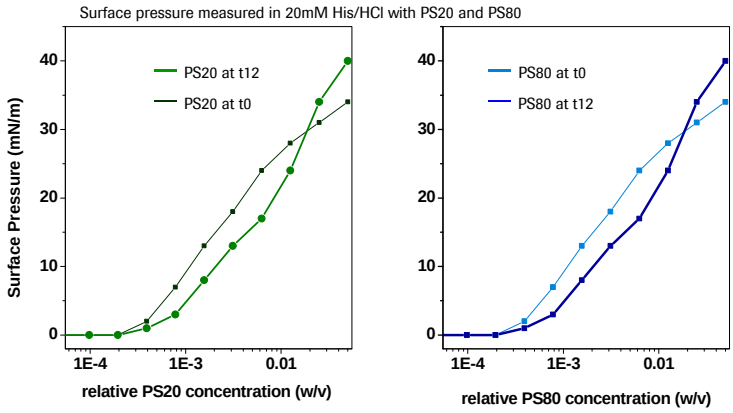
Soluble aggregates



Polysorbate content (by micelle assay)

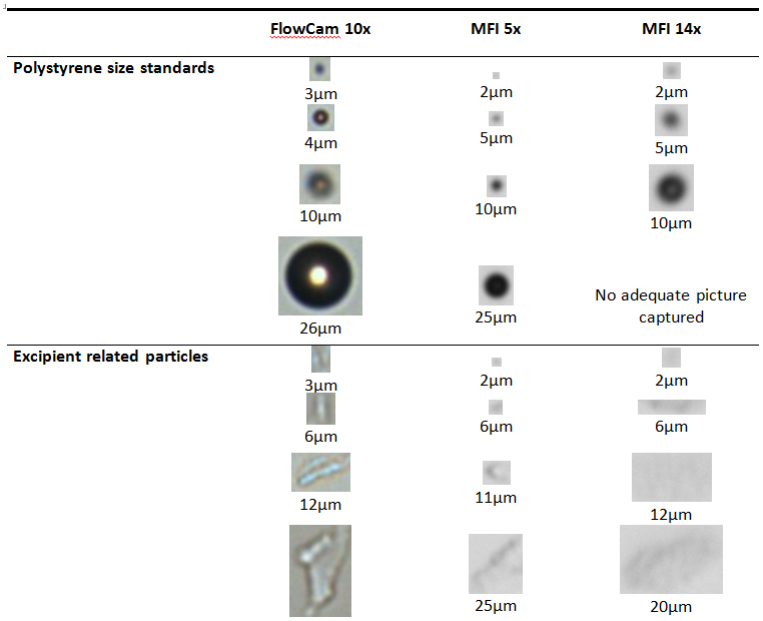
Impact of Polysorbate degradation on protein formulations

Surface pressure in aged formulations



- Degradation products of PS still show surface activity even after 60% loss of content by micelle assay

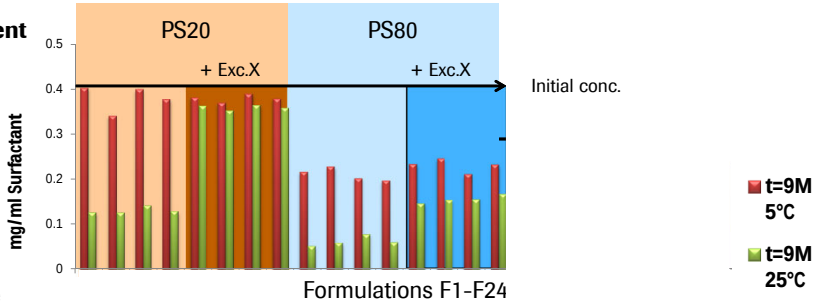
Insoluble degradants



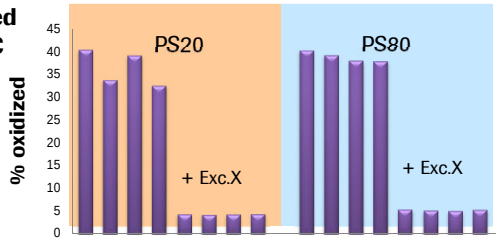
Impact of Polysorbate degradation on protein formulations

Oxidation

Surfactant content determination



% Fc Methionine Oxidation observed in protein at 25 °C



Impact of Polysorbate degradation on protein formulations

Summary

Effect of decreased surfactant content	▪ Surface pressure experiments show comparable surface tension ▪ End of shelf life studies show effective protection even with decreased surfactant content
Effect of insoluble degradants from hydrolysis	▪ Fatty acids can appear as visible or subvisible particles ▪ Fatty acids may induce aggregation, above a threshold concentration
Effect of peroxides from auto-oxidation	▪ Degradation of polysorbates correlates to extent of oxidation in mAbs ▪ It is possible that peroxides generated from PS degradation causes mAb oxidation

Overall Summary and Conclusions

- Polysorbates are a complex mixture of Sorbitan-POE-fattyacid esters
- Polysorbates stabilize (most) Proteins (against interfacial stress-induced aggregation) and can minimize protein adsorption to interfaces
- Polysorbates can adsorb to e.g. filters and other material
- Degradation may occur in bulk and/or pharmaceutical formulations via hydrolysis or by auto-oxidation (major pathway)
- Polysorbates can degrade and this requires sufficient attention during formulation development

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