Mechanisms of Surfactant Degradation: Focus on Enzymatic Hydrolysis

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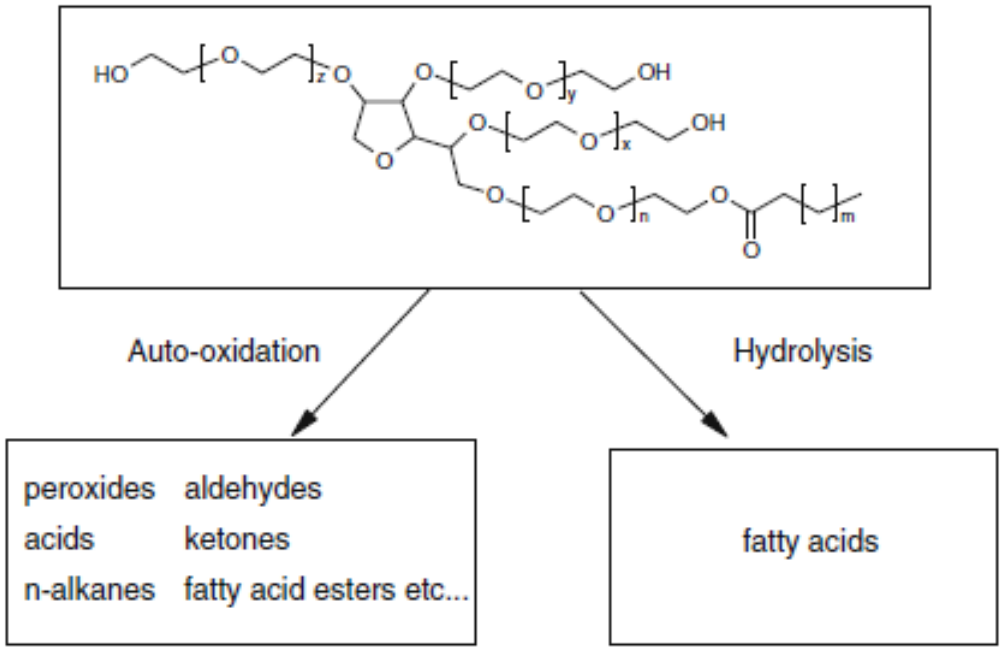
Outline

1. Degradation pathways of polysorbate
2. Specific residual Host Cell Proteins (HCP) for polysorbate hydrolysis
3. Enzymatic polysorbate hydrolysis features
4. Polysorbate stability in commercial mAbs
5. Key challenges and potential solutions

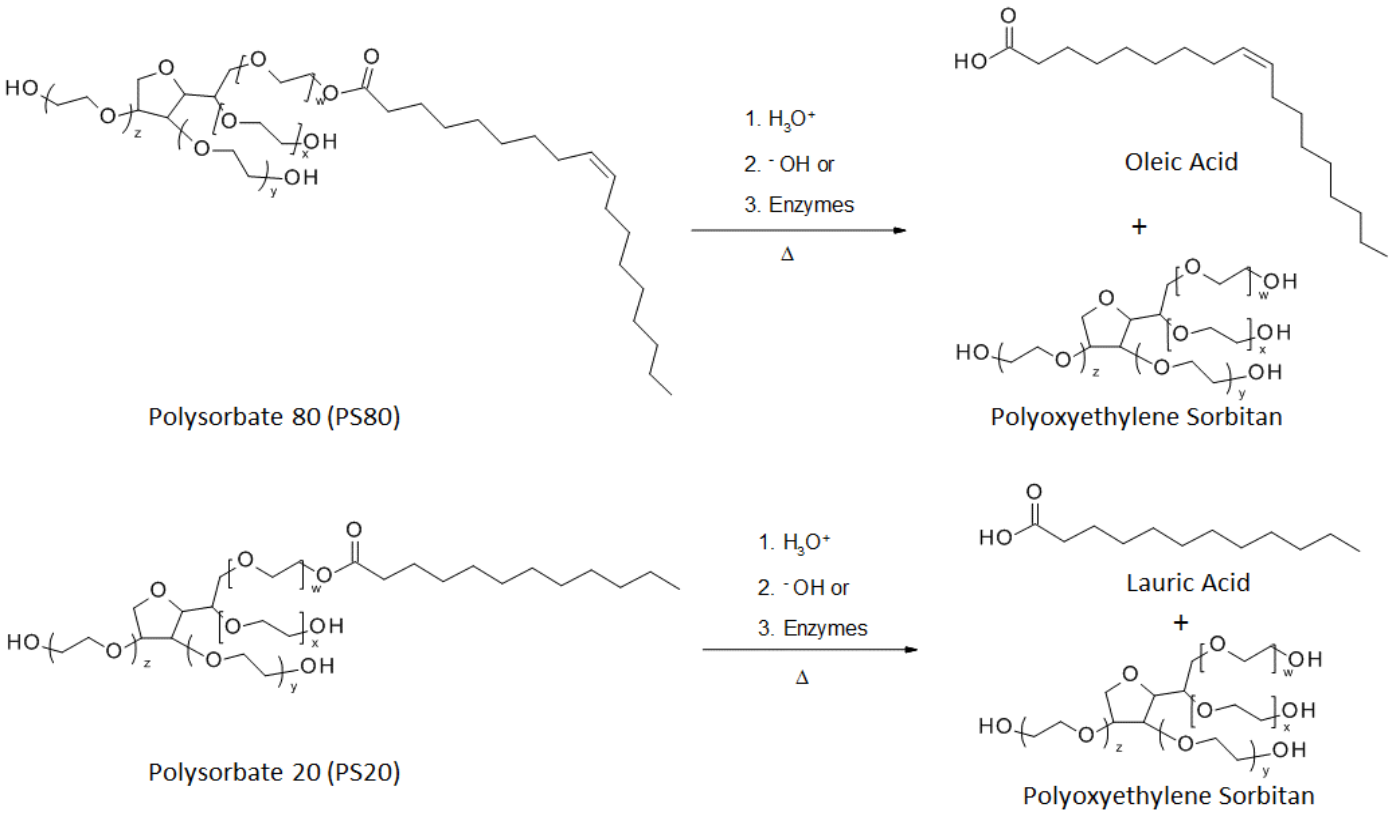
Degradation of Polysorbate

Main Degradation Routes



Kishore, et. al. *Pharm Res* 2011

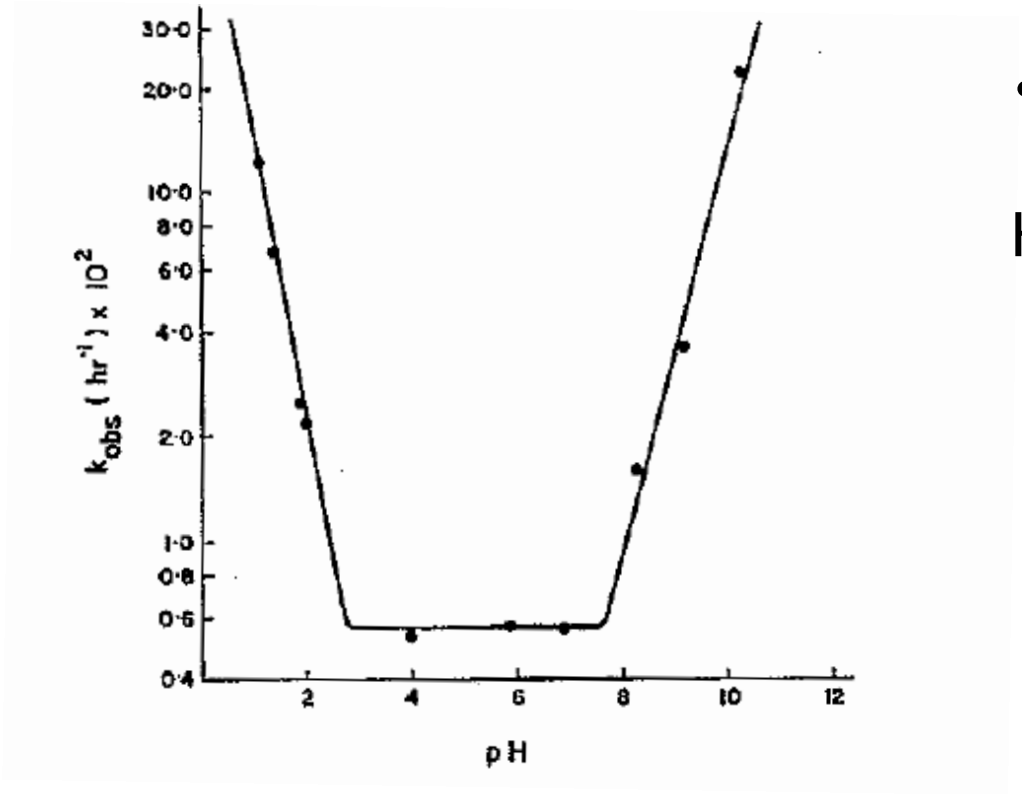
Polysorbate Hydrolysis



Chemical or Enzymatic Hydrolysis Yield Poorly Soluble Free Fatty Acids (FFA)

Chemical Hydrolysis of Polysorbate

Polysorbate Hydrolysis pH Rate Profile



Bates, et. al. *J Pharm Pharmacol* 1973;25

- Stability pH optimum 3.0 – 7.6

Half-life of Polysorbate Hydrolysis (pH 5.5)

Temperature	Time
40 °C	5 months
25 °C	19 months
5 °C	> 760 months

Kishore, et. al. *J Pharm Sci* 2011;100

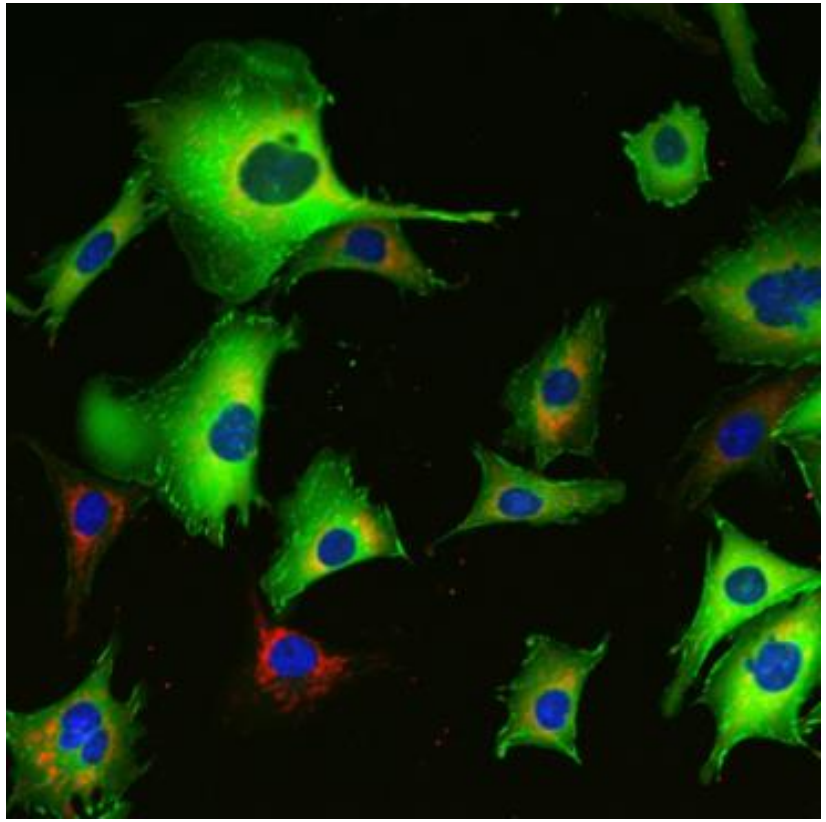
Generally, enzymatic hydrolysis is significantly faster than chemical mechanisms consequently leading to potential impact to product quality at nominal storage conditions

Recent Reports of Visible Particulate Matter & Polysorbate Enzymatic Hydrolysis

1. 2014: Labrenz Ester hydrolysis of polysorbate 80 in mAb drug product: Evidence in support of the hypothesized risk after the observation of visible particulate in mAb formulations. *J Pharm Sci*
2. 2015: Cao et.al. Free Fatty Acid Particles in Protein Formulations, Part 1: Microspectroscopic Identification. *J Pharm Sci*
3. 2015: Siska et.al. Free Fatty Acid Particles in Protein Formulations, Part 2: Contribution of Polysorbate Raw Material. *J Pharm Sci*
4. 2015: Doshi et.al. Understanding particle formation: Solubility of free fatty acids as polysorbate 20 degradation byproducts in therapeutic monoclonal antibody formulations. *Mol. Pharmaceutics*
5. 2015: Tomlinson et.al. Polysorbate 20 Degradation in Biopharmaceutical Formulations: Quantification of Free Fatty Acids, Characterization of Particulates, and Insights into the Degradation Mechanism. *Mol. Pharmaceutics*
6. 2015: Saggu et. al. Identification of Subvisible Particles in Biopharmaceutical Formulations Using Raman Spectroscopy Provides Insight into Polysorbate 20 Degradation Pathway. *Pharm Res*
7. 2016: Dixit et.al. Residual Host Cell Protein Promotes Polysorbate 20 Degradation in a Sulfatase Drug Product Leading to Free Fatty Acid Particles. *J Pharm Sci*
8. 2016: Hall et.al. Polysorbates 20 and 80 Degradation by Group XV Lysosomal Phospholipase A_2 Isomer X1 in Monoclonal Antibody Formulations. *J Pharm Sci*
9. 2017: Chiu et.al. Knockout of a Difficult-to-Remove CHO Host Cell Protein, Lipoprotein Lipase, for Improved Polysorbate Stability in Monoclonal Antibody Formulations. *Biotechnol Bioeng*

Development of Higher Concentration mAbs

Origin of Host Cell Protein

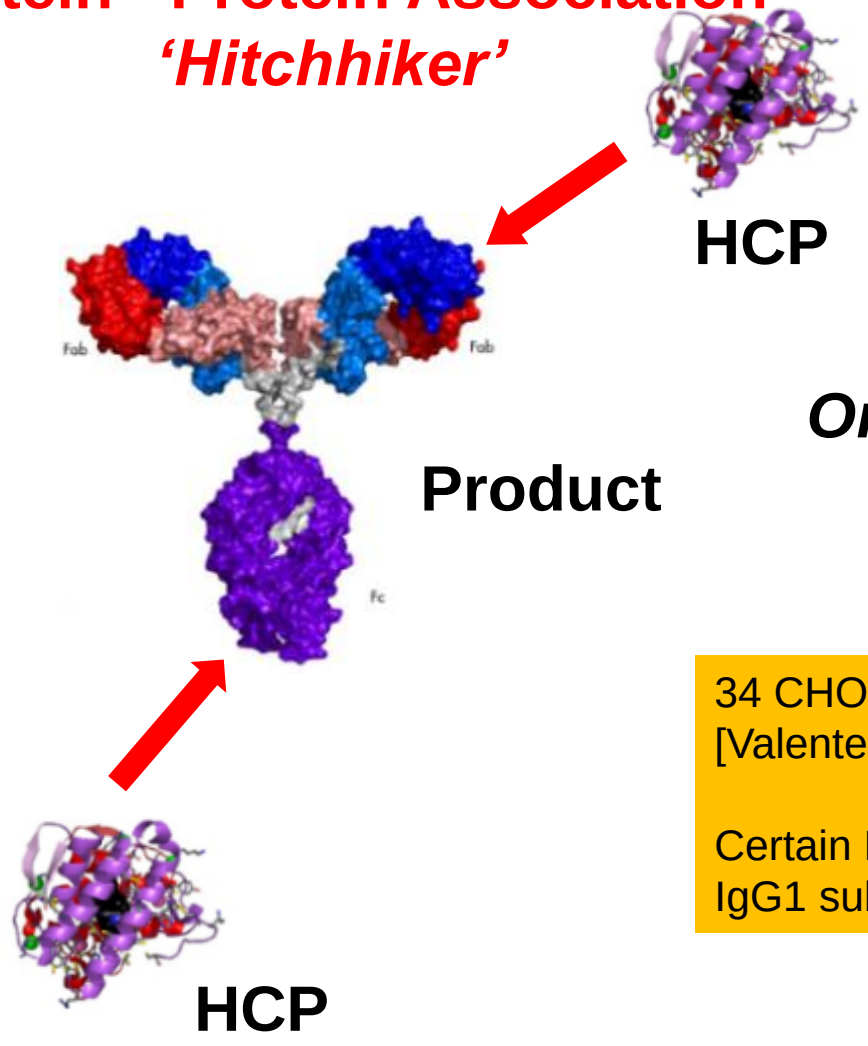


- Transfected cells are used to manufacture biopharmaceuticals
- Cells are producing other Host Cell Proteins as part of normal metabolic processes that are unrelated to the intended product
- Culture media, growth conditions and other process parameters influence HCP expression levels
- HCPs are acknowledged to be part of the product profile (process-related impurities)

Why Are Some HCP Difficult to Remove?

Protein - Protein Association

'Hitchhiker'



Or

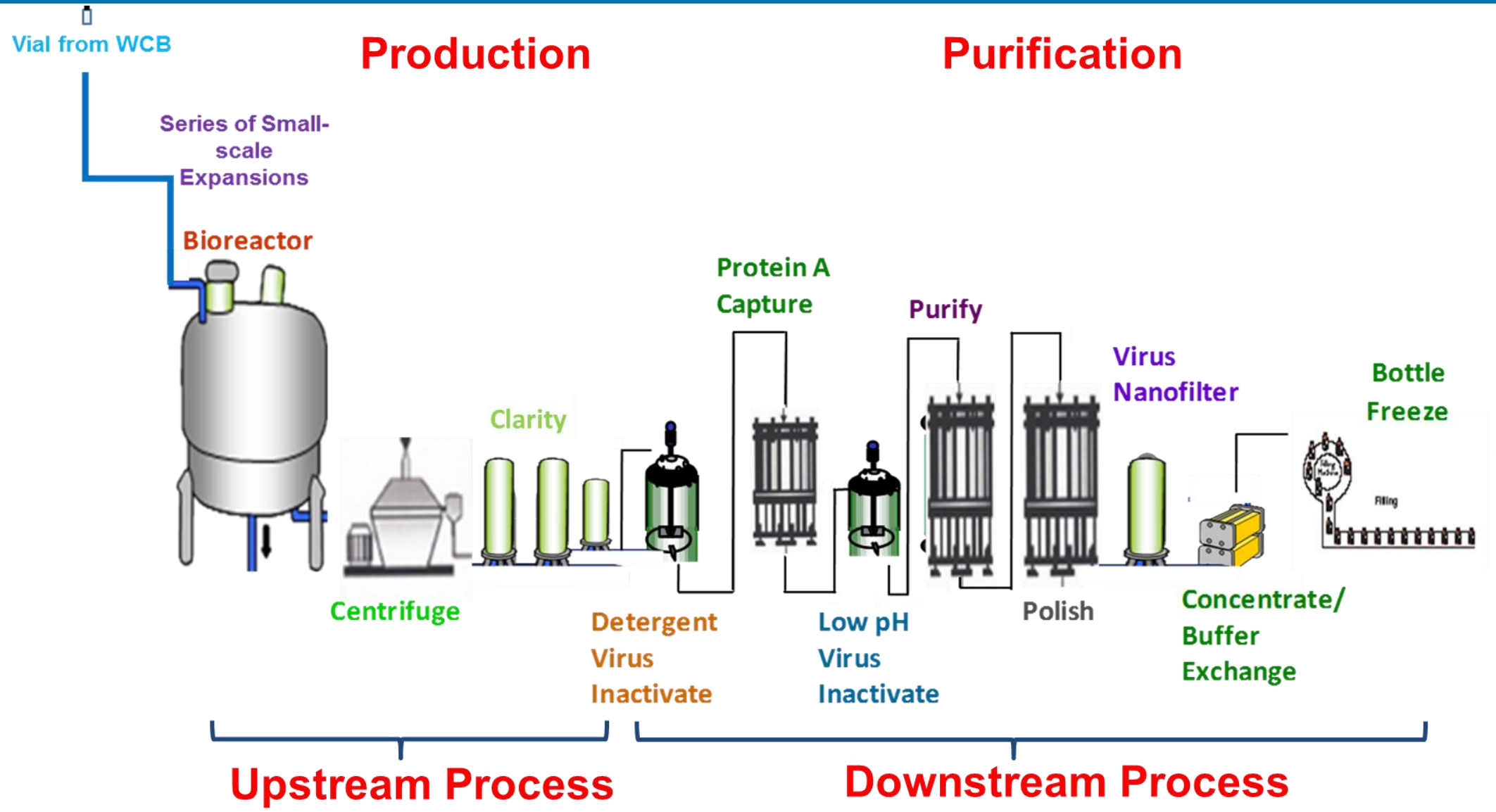
Co-purification

- **Similar molecular properties**
- **Similar retention characteristics**
- **Overlapping elution profile**

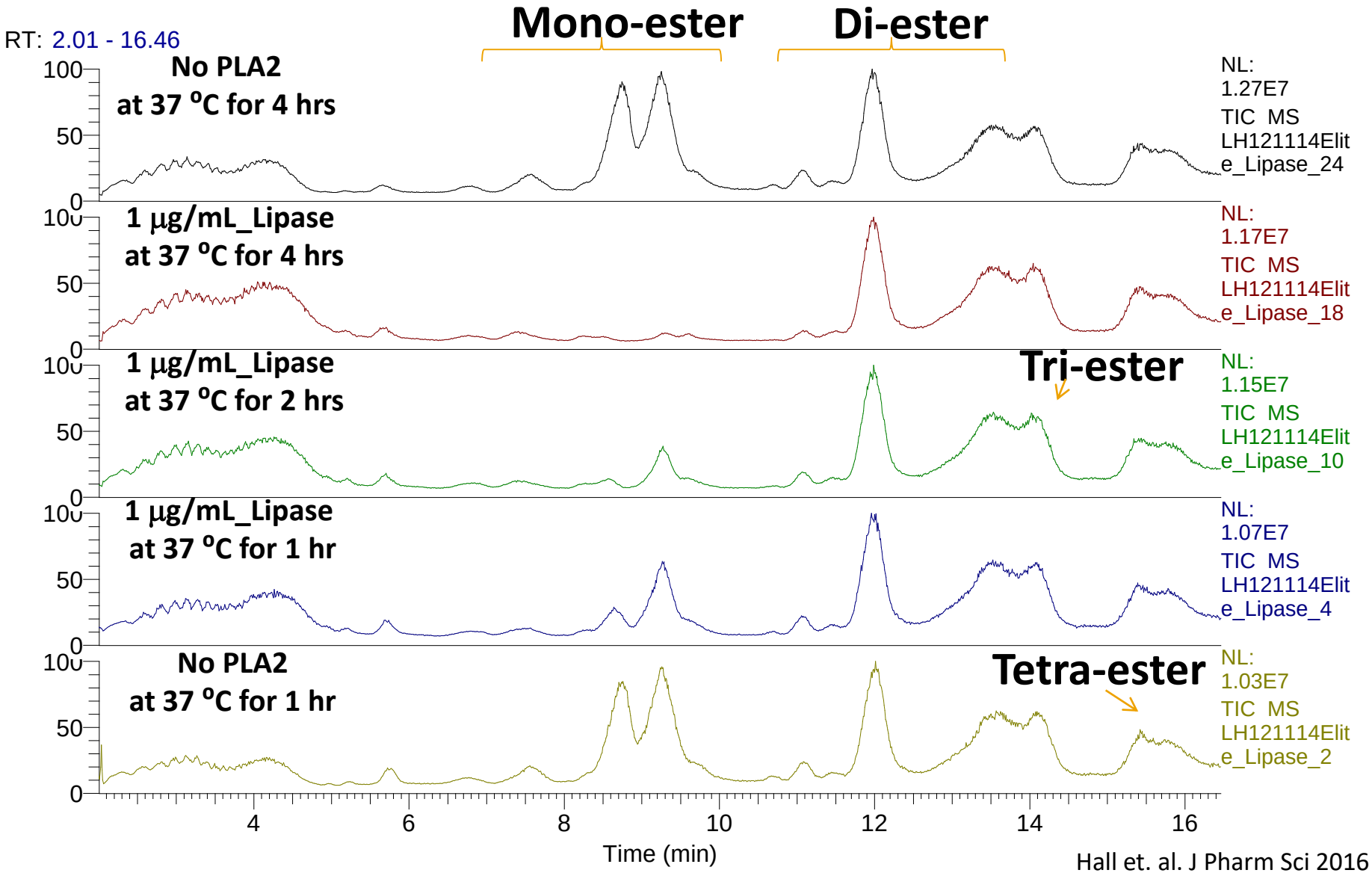
34 CHO HCP previously reported as potential purification challenges [Valente et al. (2015) *Biotechnol Bioeng* 112: 1232–1242]

Certain HCP may have a preference for interactions with IgG4 versus IgG1 subclass [Tran et al. (2016) *J. Chromatogr. A* 1438: 31–38]

Biopharmaceutical Manufacturing Process (Monoclonal Antibody)

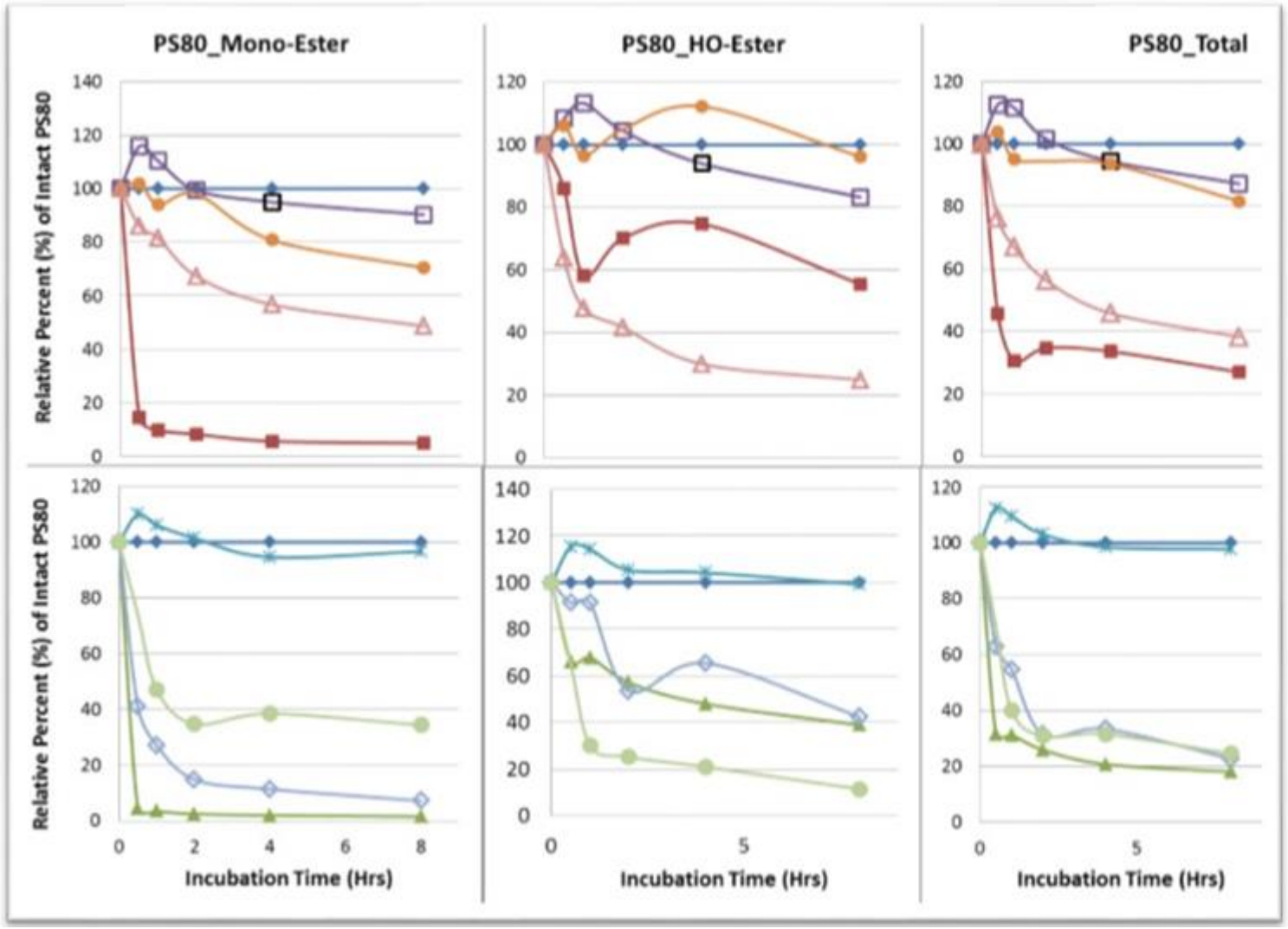


PS-80 Stability in pH6 Solution with or without 1 μg/mL PLA2



Hall et. al. J Pharm Sci 2016

PS80 Hydrolysis Profile With Enzymes

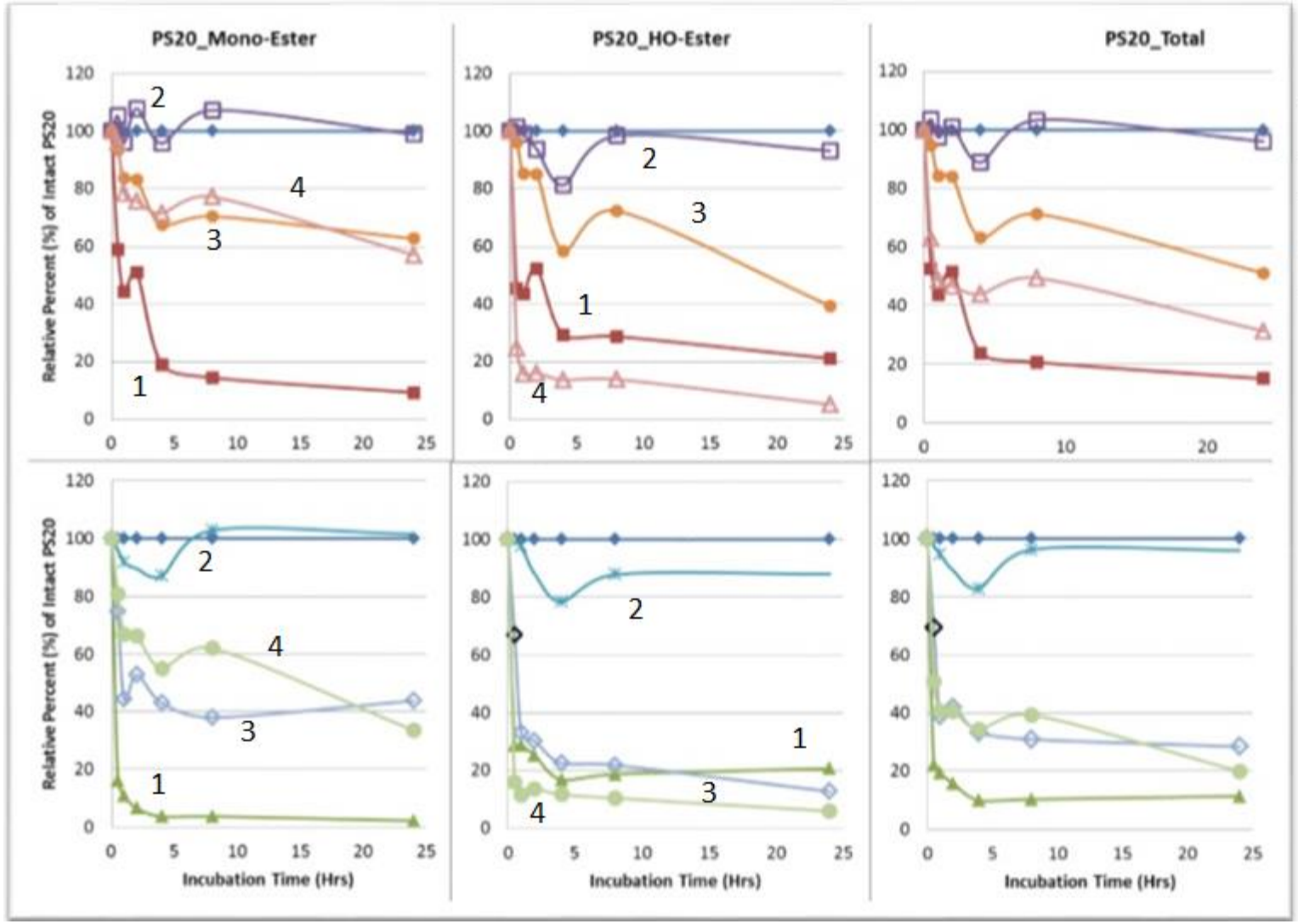


Control
Enzyme 1, 1 ppm
Enzyme 2, 1 ppm
Enzyme 3, 1 ppm
Enzyme 4, 1 ppm

Control
Enzyme 1, 10 ppm
Enzyme 2, 10 ppm
Enzyme 3, 10 ppm
Enzyme 4, 10 ppm

Lipase	Mono	Higher Order (HO)	Lipase Level
Enzyme 1	High	Moderate	Minimal
Enzyme 2	Minimal	Minimal	Minimal
Enzyme 3	Minimal to High	Minimal to Moderate	Dependent
Enzyme 4	Moderate to High	High	Dependent

PS20 Hydrolysis Profile With Enzymes

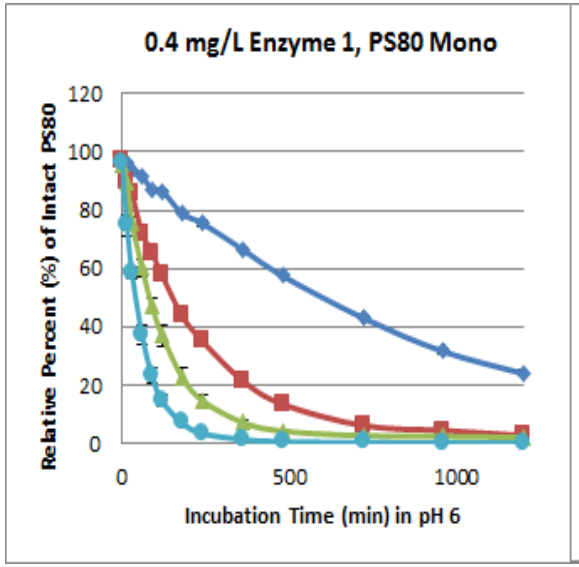


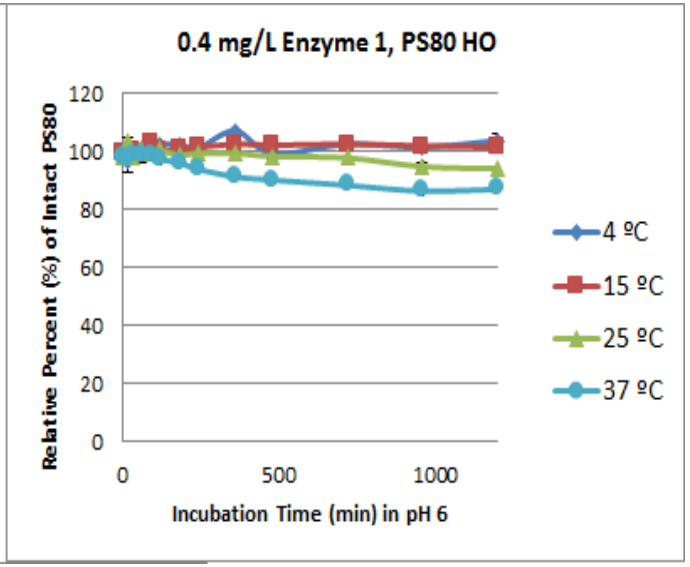
Control
Enzyme 1, 1 ppm
Enzyme 2, 1 ppm
Enzyme 3, 1 ppm
Enzyme 4, 1 ppm

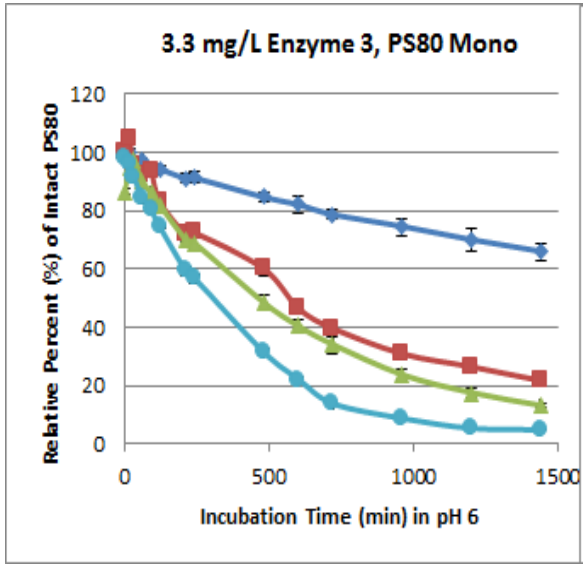
Control
Enzyme 1, 10 ppm
Enzyme 2, 10 ppm
Enzyme 3, 10 ppm
Enzyme 4, 10 ppm

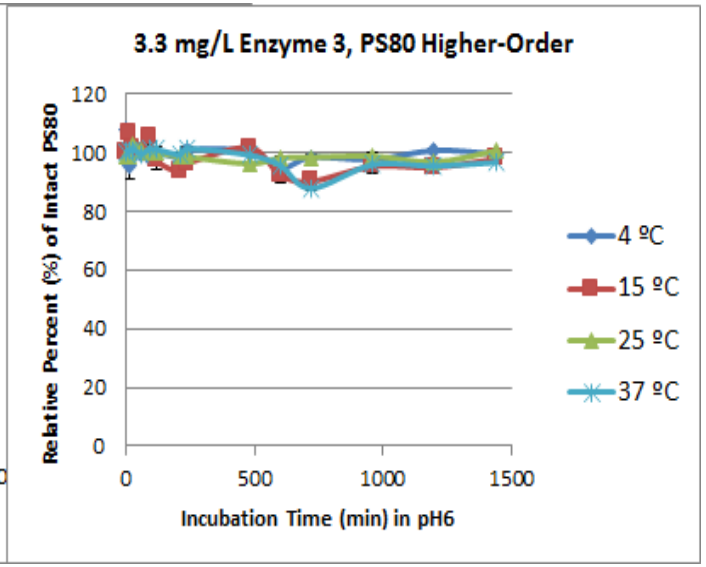
Lipase	Mono	Higher Order (HO)	Lipase Level
Enzyme 1	High	Moderate to high	Minimal
Enzyme 2	Minimal	Minimal	Minimal
Enzyme 3	Moderate to High	Moderate to Moderate	Dependent
Enzyme 4	Moderate	High	Dependent

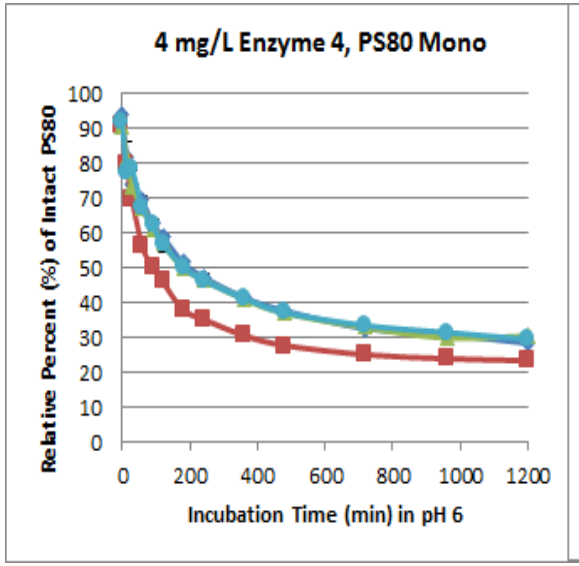
PS80 Hydrolysis Profile with Enzymes

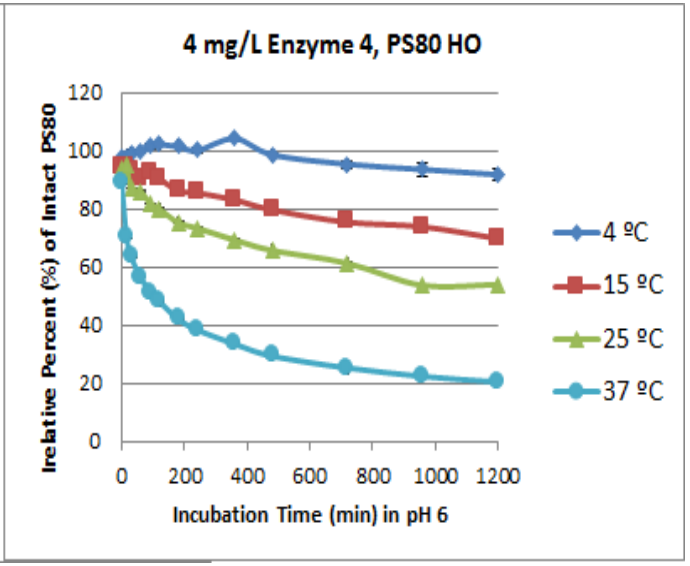


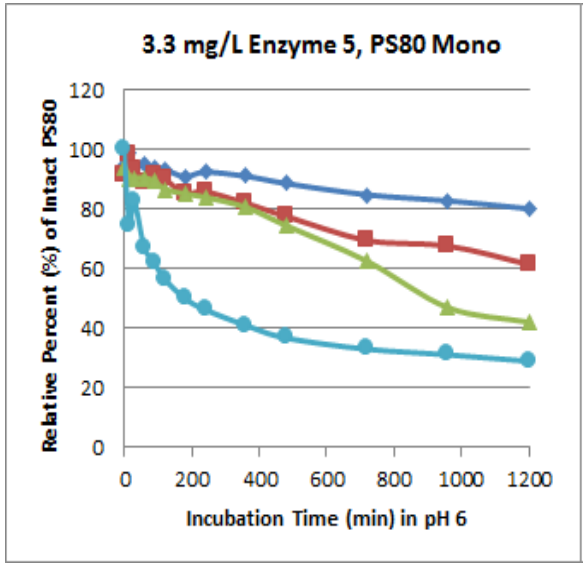


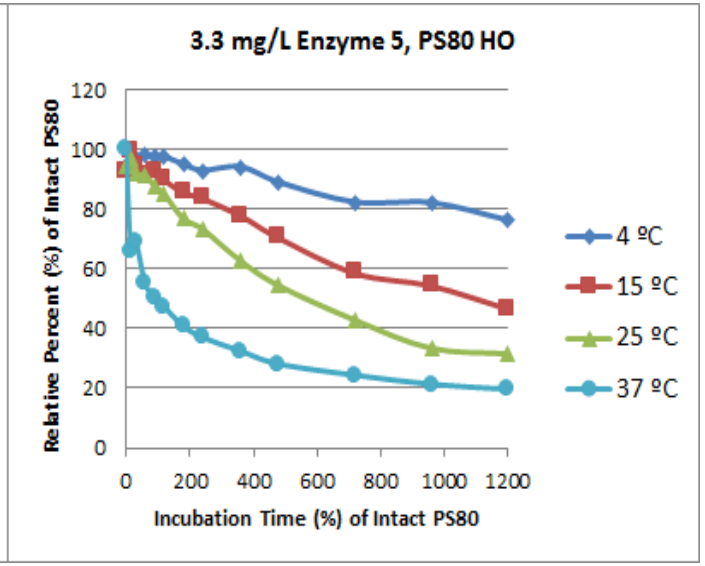




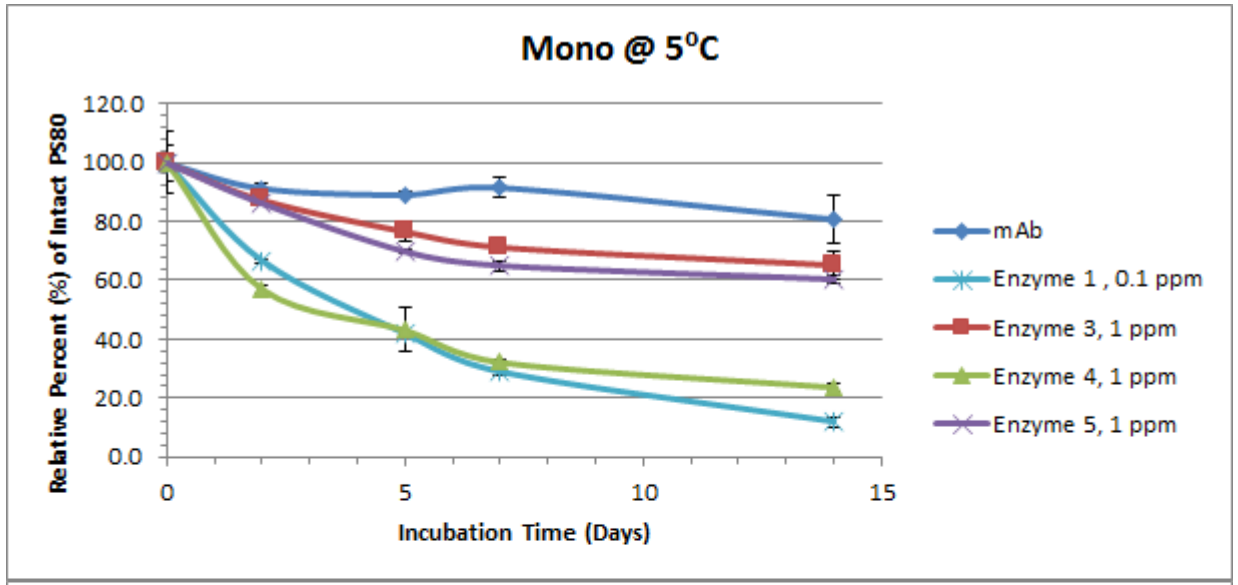


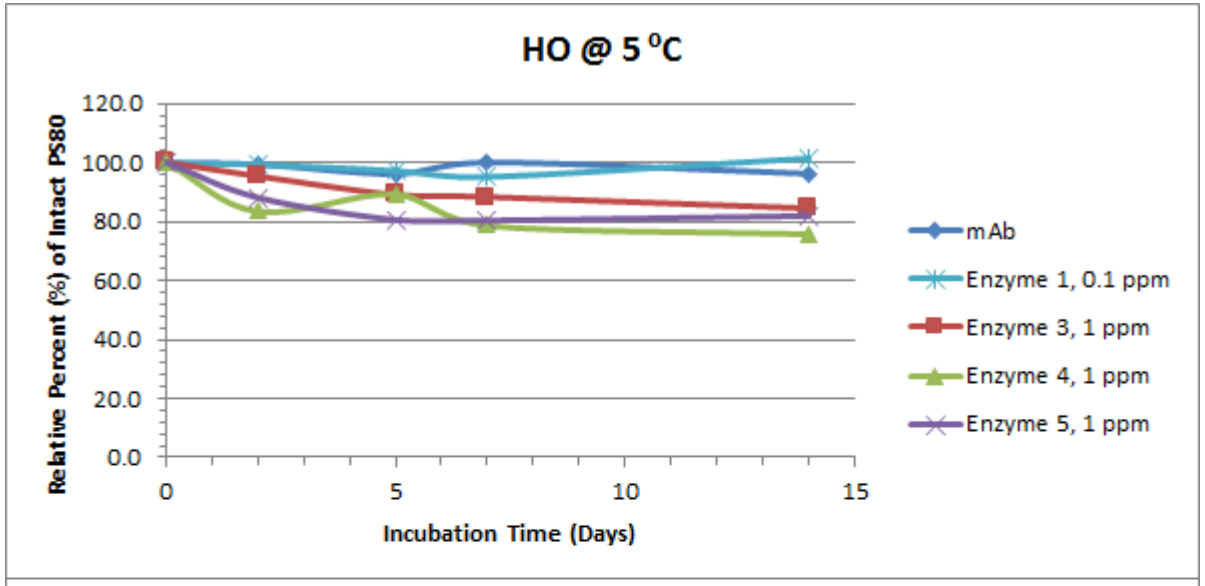


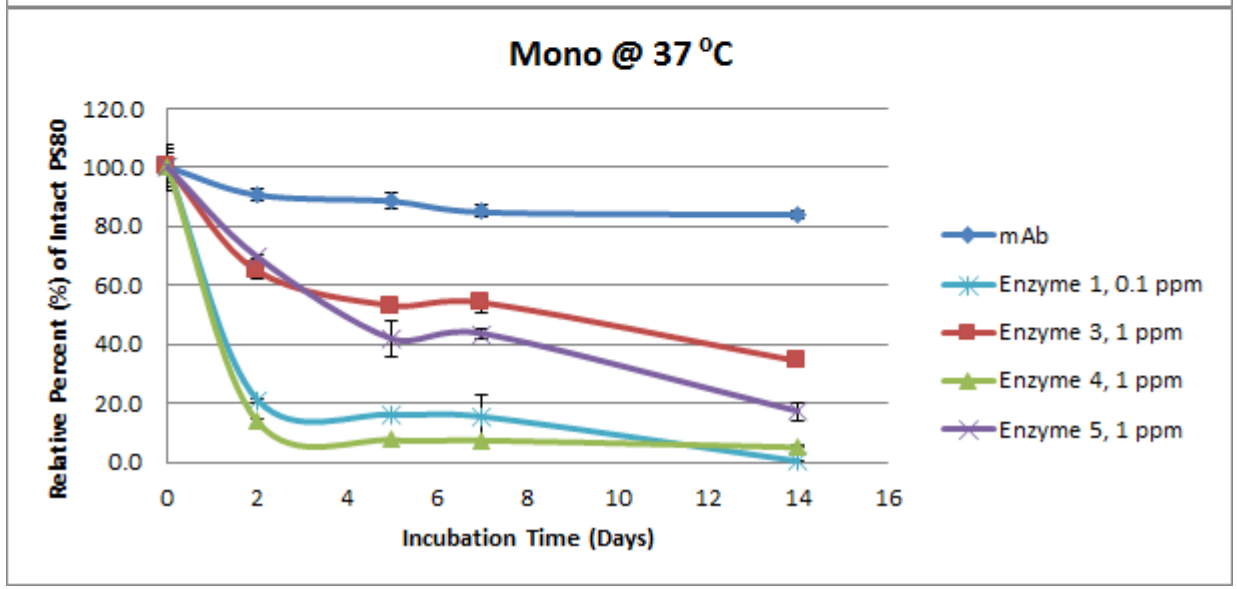


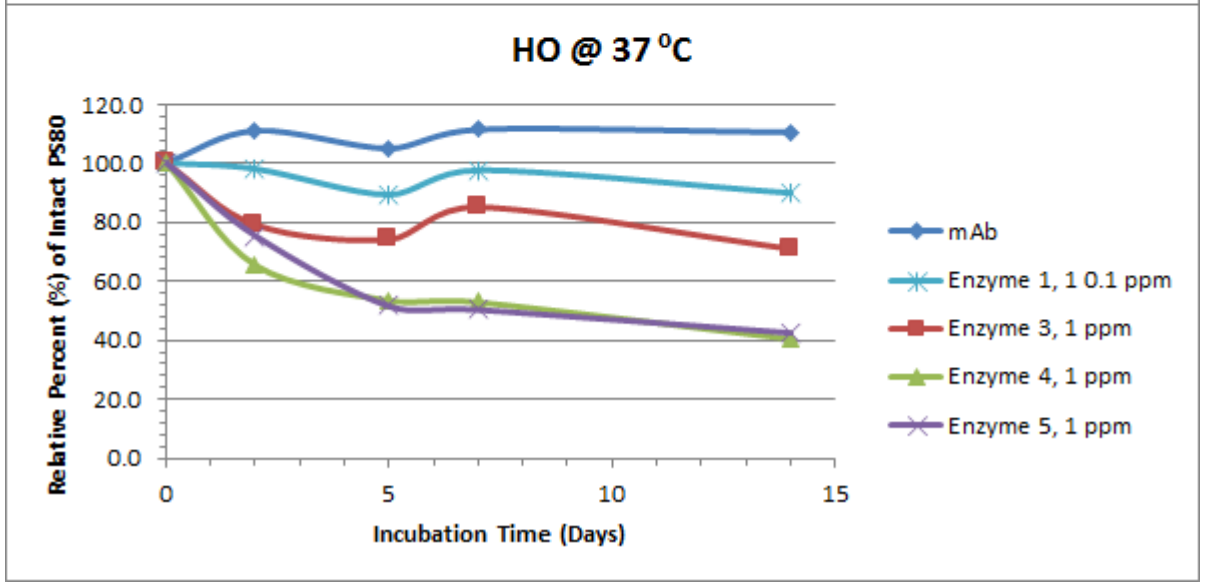


PS80 Hydrolysis Profile in mAb Spiked with Enzymes

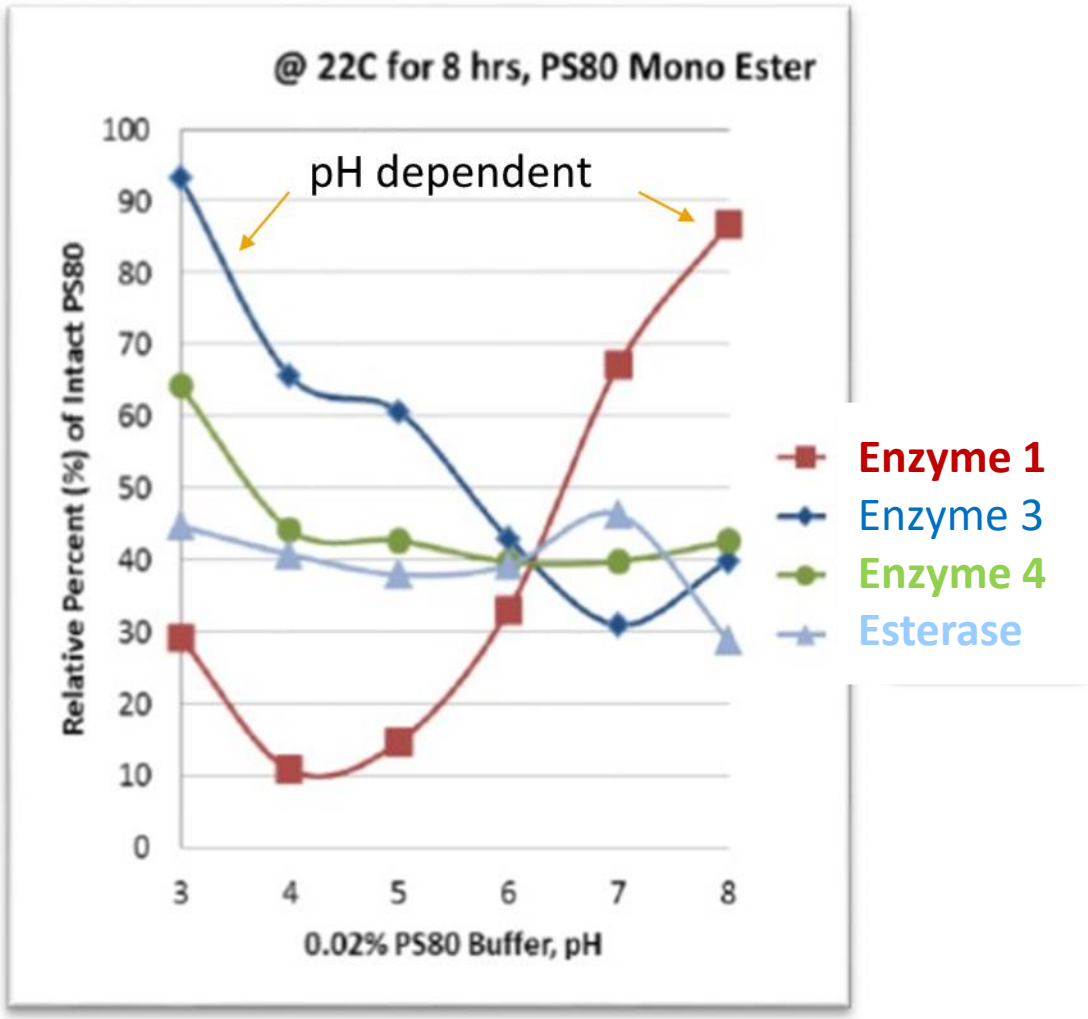


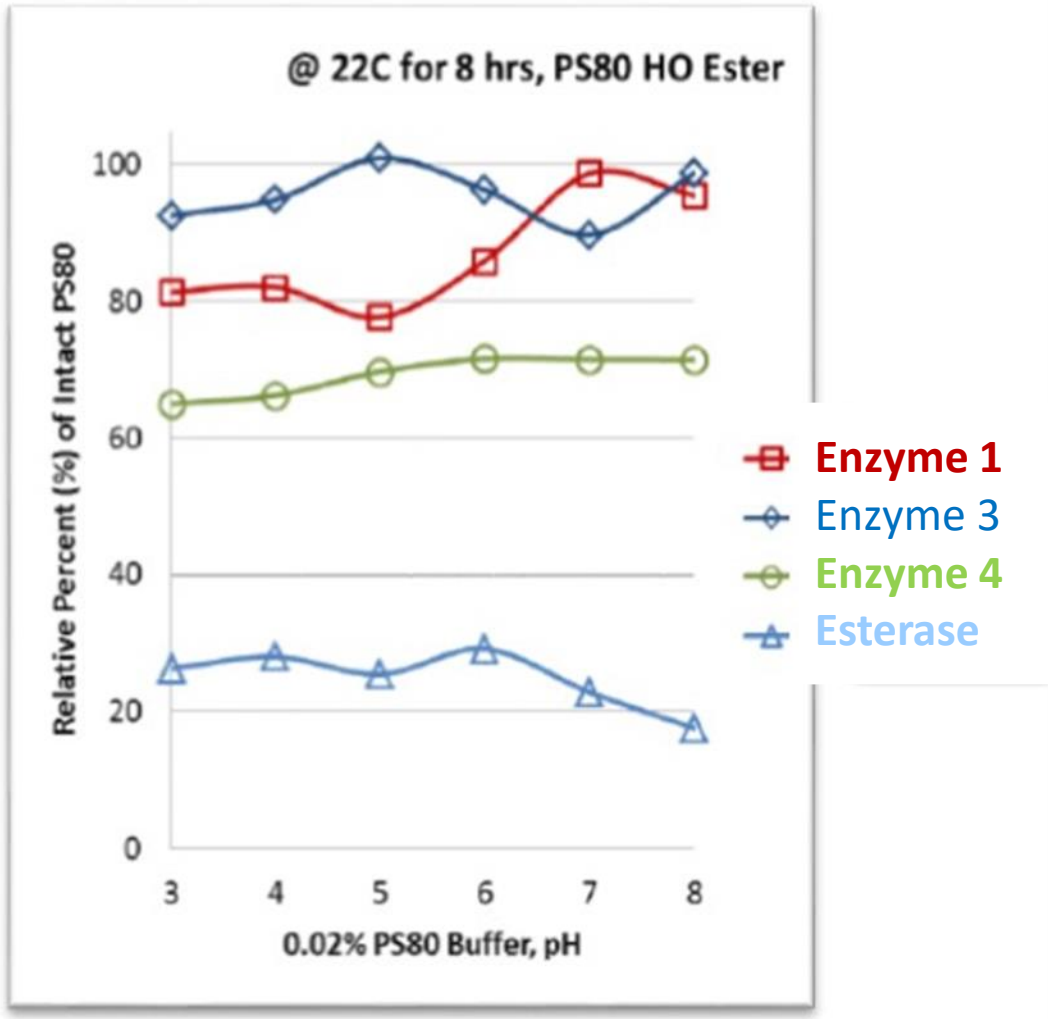




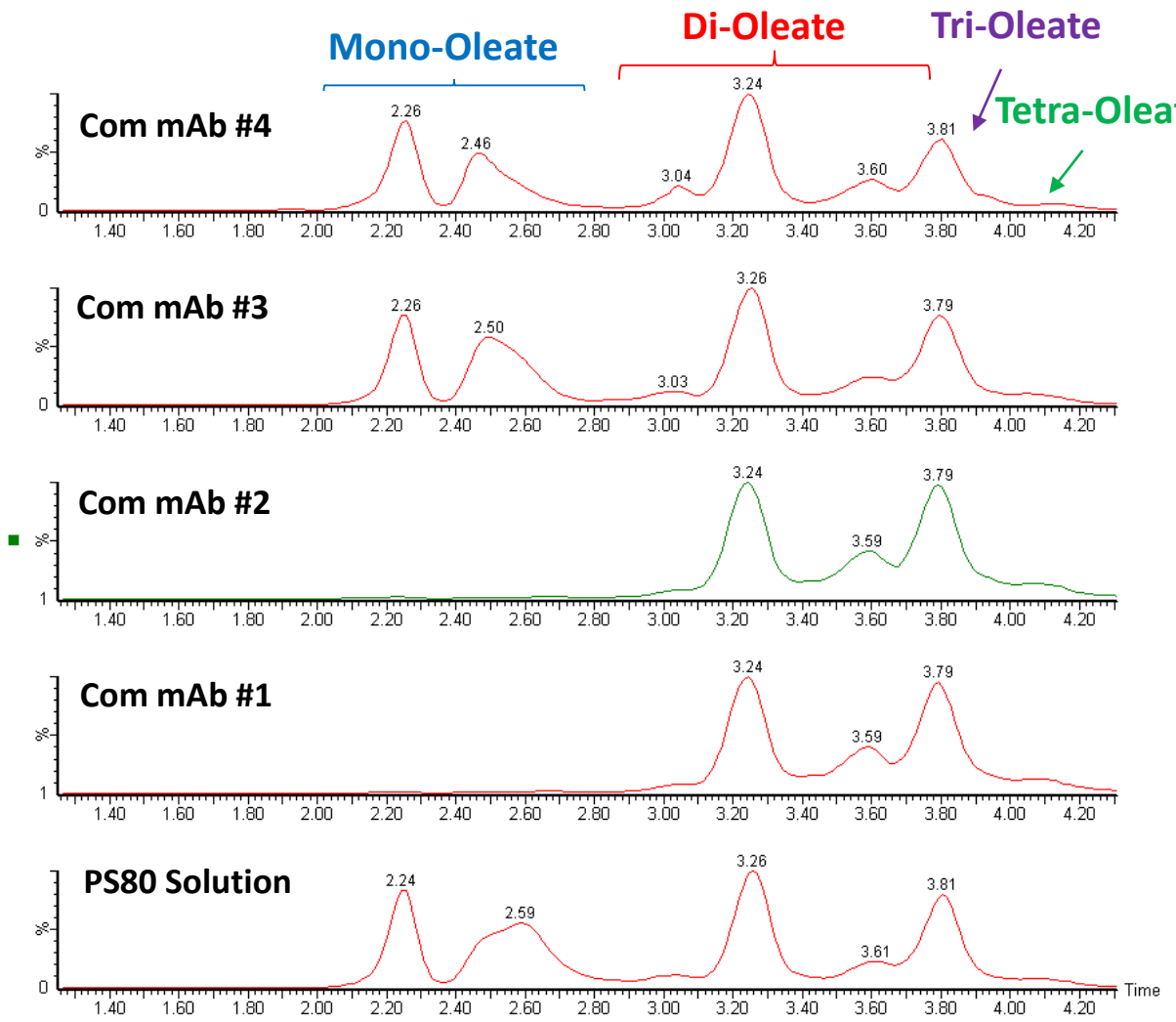


Impact of pH on Enzymatic Activity





Polysorbate Stability in Commercial mAbs



Commercial mAb	Polysorbate		HCP ppm
	Type	Stability	
1	PS80	No	5
2	PS80	No	< 1
3	PS80	Yes	> 500
4	PS80	Yes	< 1
5	PS80	No	27
6	PS80	Yes	3
7	PS20	Yes	2
8	PS20	Yes	17
9	Not Polysorbate		35

Lipase and Esterase Measurement in Commercial Mabs

Commercial mAb	Polysorbate		HCP ppm	ppm or ng/mg mAb				
	Type	Stability		Enzyme 1	Enzyme 2	Enzyme 3	Enzyme 4	Enzyme 5
1	PS80	No	5	0.03	< LOD	< LOD	< LOD	0.11
2	PS80	No	< 1	0.01	0.02	< LOD	< LOD	0.07
3	PS80	Yes	> 500	< LOD	0.02	< LOD	< LOD	< LOD
4	PS80	Yes	< 1	< LOD	< LOD	< LOD	< LOD	< LOD
5	PS80	No	27	0.02	0.19	< LOD	< LOD	< LOD
6	PS80	Yes	3	< LOD	< LOD	< LOD	< LOD	< LOD
7	PS20	Yes	2	< LOD	< LOD	< LOD	< LOD	< LOD
8	PS20	Yes	17	< LOD	< LOD	< LOD	< LOD	< LOD
9	Not Polysorbate		35	< LOD	1.6	< LOD	< LOD	33

Key Challenges and Potential Solutions

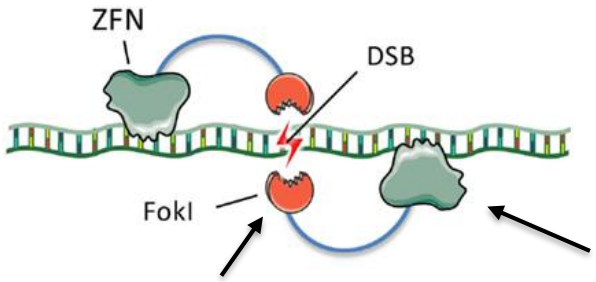
Challenges

- Trace levels present (< 0.1 ppm) making detection, monitoring and removal difficult
- Similar properties to therapeutic protein
- Hitch-hiker via Antibody-HCP association
- Solubility of free fatty acids
- Kinetic process that occurs over shelf-life

Potential solutions that can eliminate and or reduce impact

- Purification process: separate based on charge, size, hydrophobicity, peak fractions
- Siliconized container closure i.e. prefilled syringe
- Storage temperature if rate of hydrolysis is slow enough over shelf-life
- Surfactant alternatives that lack ester bonds
- Cell line engineering

PoC Experimental Flow Scheme



Cell Line Engineering

Parental Cell Lines

- KO (4 enzymes)
- HCP profiling

Generate CDCLs

- Fc Fusion
- mAb (IgG4)
- Bispecific (heteromAb)

Screening & Confirmation

- Shake-Flask Expression

CDCLs = clonally-derived cell lines

DS Manufacture & Formulation

Scale-up (36 L) – mimic historical process

Purification – mimic historical processes

DP set-up:

- Historical API control
- In-expt. control (w.t. cell line) API
- Engineered cell line API

Characterization

HCP profiling

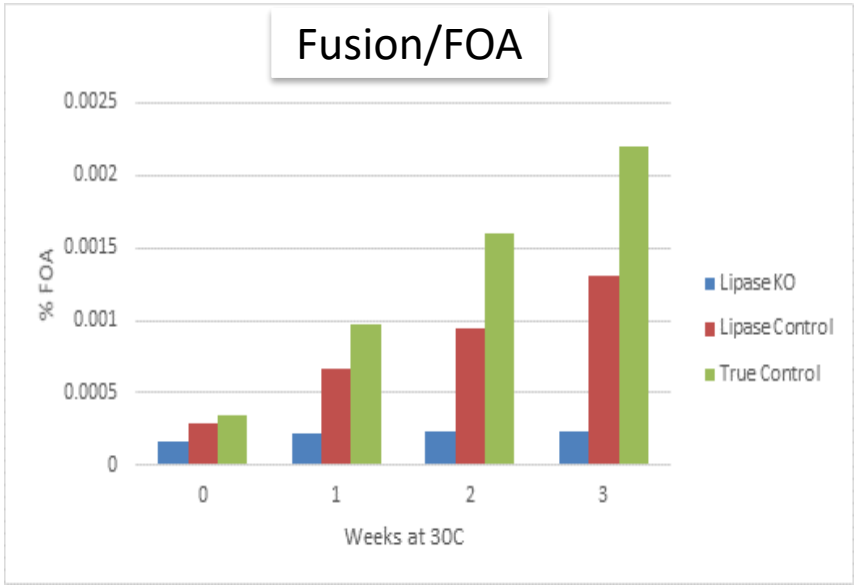
- Cell-Free Medium
- TFF

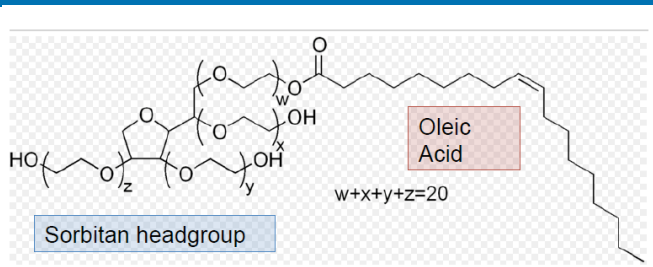
PS-80 analyses

- Cell-Free Medium (Intact)
- DP (Intact and FOA)

Cell Line Engineering Impact on Polysorbate Stability

- Stability samples analyzed for free oleic acid (UPLC-based method)
- PoC Example:





Lipase KO: Engineered cell line API
Lipase control: Non-engineered cell line API (in-experiment control)
True control: Historical API (Historical Production Cell Line)

- **<u>PoC Data</u>:**
  - No polysorbate hydrolysis at 30 ^{o}C at 8 weeks for fusion molecule
  - No hydrolysis observed at 25 ^{o}C at 6 months for bispecific (heteromAb)
  - 7-fold reduction in hydrolysis for IgG4 antibody
- **<u>Platform Data</u>:**
  - <10% hydrolysis observed for 10 programs since implementation of engineered cell line

Engineered Cell Line Performance

- Engineered cell line size is similar to WT parental cell line
- Engineered cell line doubling time is shorter than parental cell line
- Broader HCP profiles not significantly different between engineered and WT parental cell line
- Engineering did not negatively impact productivity of selected bulk cultures
- Highly-Productive CDCLs for Fc fusion protein (>7 g/L/14d) and mAb (>10 g/L/14d)
- Comparable product quality profiles
- Performance demonstrated on IgG1, IgG4, bispecifics, Fc fusions and non-mAb scaffold therapeutic proteins

Questions?