POLYSORBATE HYDROLYSIS AND PROTEIN STABILITY: INVESTIGATING THE ROLE OF FREE FATTY ACID PARTICLES

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Jinghan Li, Veronica F. Blanco, James Humphrey, Sreejit Menon, Stephen Rumbelow

Croda

CONTACT INFORMATION: jinghan.li@croda.com

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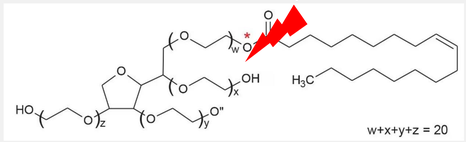
INTRODUCTION

Extreme pH, heat, lipase or esterase



Insoluble Free Fatty Acid (FFA) Particles
Potential Protein Destabilization

PURPOSE

Surfactants are widely used in protein formulations to prevent interfacial stresses. However, as fatty acid esters, PS20 and PS80 can undergo hydrolysis, producing free fatty acid particles. Thus, our study hypothesizes that polysorbate hydrolysis undermines the stability of protein formulations due to (i) the loss of the stabilization function of the surfactants and (ii) interfacial stress resulting from FFA particles.

METHODS

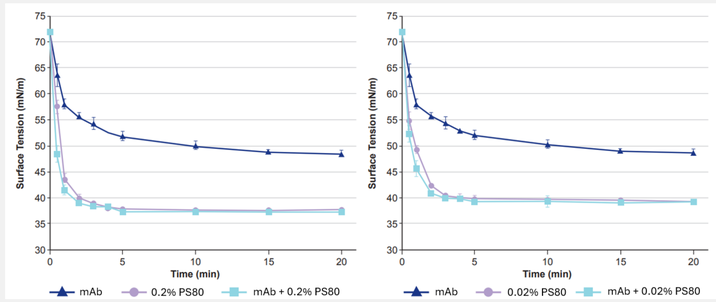
The surface tension of freshly prepared samples was measured using a KRUSS K100 force tensiometer. The mAb solutions were subjected to two stress conditions: (i) incubation at 40 °C in the dark for 4 weeks, and (ii) orbital shaking at room temperature (160 rpm, 72 h).The mAb solutions were characterized by the percentage of high molecular weight species (%HMW) using size exclusion chromatography. The static light scattering intensity and fluorescence spectra of the mAb solutions experiments were performed on a fluorimeter system (UNCLE, Unchained Labs). A regular two-level factorial design was applied to study the synergistic impact of surfactant and free oleic acid on protein stability.

RESULTS

Table 1. mAb solutions studied in the project. 'M' – mAb, 'O' – oleic acid, 'P' – polysorbate 80 and 'S' – sucrose.

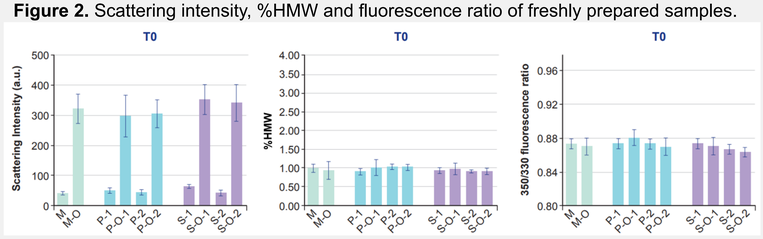
Sample Name	mAb (mg/mL)	Oleic Acid (µg/mL)	Polysorbate 80 (%, w/v)	Sucrose (%, w/v)
M	10			
M-O	10	30		
P-1	10		0.02	
P-O-1	10	30	0.02	
P-2	10		0.2	
P-O-2	10	30	0.2	
S-1	10			7
S-O-1	10	30		7
S-2	10			15
S-O-2	10	30		15

Figure 1. Surface tension of mAb, surfactant and mAb + surfactant solutions.



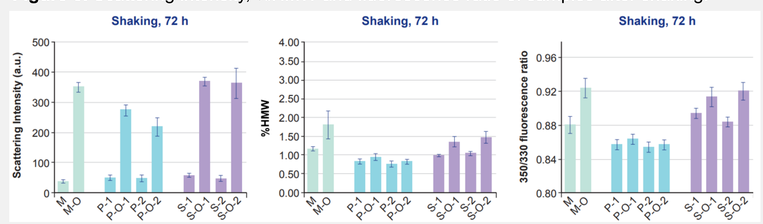
mAb gradually adsorb to liquid surface. PS80 was effective in preventing mAb surface adsorption at > 0.02% w/v.

Figure 2. Scattering intensity, %HMW and fluorescence ratio of freshly prepared samples.



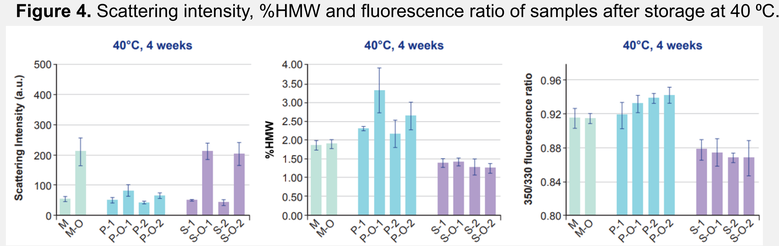
Fresh mAb solutions had a %HMW below 1% and a 350/330 nm fluorescence ratio ranging from 0.85 to 0.89. The addition of free OA resulted in an increase in scattering intensity. However, OA did not affect %HMW or protein structure.

Figure 3. Scattering intensity, %HMW and fluorescence ratio of samples after shaking.



Shaking resulted in mAb aggregation and unfolding, as indicated by an increase in %HMW and the 350/330 ratio. The insoluble OA particles further exacerbated mAb aggregation by introducing additional interfacial stresses. Intact PS80 prevented shaking-induced mAb destabilization. However, sucrose could not stabilize the protein.

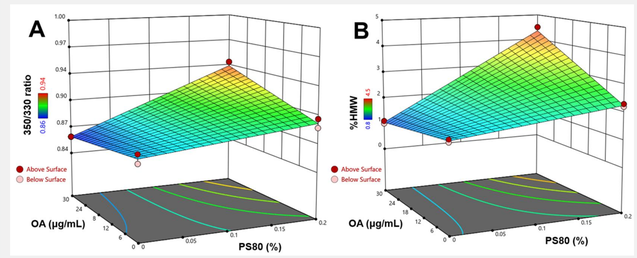
Figure 4. Scattering intensity, %HMW and fluorescence ratio of samples after storage at 40 ºC.



After storage at 40 °C for 4 weeks, PS80 and OA had a synergistic effect, increasing %HMW and promoting protein unfolding. Interestingly, the scattering intensity decreased in solutions containing PS80 and OA, which could be due to the higher solubility of fatty acid at elevated temperatures. On the other hand, sucrose mitigated mAb aggregation and unfolding.

RESULTS

Figure 5. 3D surface of mAb solutions after incubation at 40 ºC. A higher 350/330 ratio or %HMW value indicate more substantial mAb unfolding and aggregation.



The %HMW and 350/330 ratio after the stability test at 40 °C were further applied to study the synergistic effect of PS80 and OA on mAb stability using a two-level factorial design. The 3D surfaces below demonstrate that PS80 and OA exhibited a significant destabilizing effect when used together. However, the adverse effect of each individual component was not pronounced.

CONCLUSIONS

- OA particles destabilized mAb by introducing additional interfacial stresses. Intact surfactant effectively prevented mAb destabilization upon shaking.
- PS80 and OA had a synergistic effect in destabilizing the protein when stored at 40 °C. This adverse effect could be mitigated by sucrose.

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