Polysorbate 20 degradation in biotherapeutic formulations and its impact on protein quality

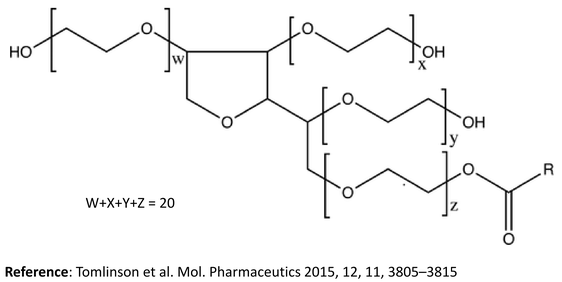
Baikuntha Aryal, Mari Lehtimaki, and Ashutosh Rao
FDA/CDER/OPQ/OBP/DBRRIII

Abstract

Polysorbate 80 (PS80) and polysorbate 20 (PS20) are the most commonly used surfactants in formulations to stabilize therapeutic proteins against interfacial stresses. Polysorbates can undergo oxidative or enzyme-mediated hydrolytic degradation to produce subvisible particles in formulations. The impact of fatty acid composition on the stability of polysorbate in formulation and subsequently on protein product quality has not been clearly understood. In the present study we compared hydrolysis of compendial and super refined grade PS20 using two enzymes for the (i) release of free fatty acid, (ii) formation of subvisible particles, and (iii) impact on protein quality. Two therapeutic protein formulations with or without 1 mg/mL therapeutic proteins were prepared using matching formulations containing compendial or super refined grade of PS20 at pH 5.0 and 6.2. Formulations were spiked with either esterase or lipase enzymes, and the release of free fatty acids in formulation buffers and formation of particles were monitored after incubation at 4°C or 37°C. Protein quality was monitored via changes in secondary structures, formation of high molecular weight species, and biological activity. Our results indicate that addition of hydrolytic enzymes in PS20 containing formulations increases free fatty acid concentration and number of subvisible particles both at 4°C or 37°C. The free fatty acid concentration remains stable at 4°C over a month but decreases after 2 hours of incubation at 37°C. Release of free fatty acid and formation of sub-visible particles were found to be temperature- and pH-dependent with relatively greater number of particles at acidic pH compared to near neutral pH. Degradation of PS20 and formation of sub-visible particles did not show significant impact on biological activity of protein or stability against degradation or aggregation to form high molecular weight species. Overall, our results indicate that hydrolysis of polysorbate is one of the sources for the formation of subvisible particles in therapeutic protein formulation, but additional studies are needed to better understand the safety and quality impact of particles originated from free fatty acids.

Introduction

PS20 and PS80 are commonly used as surfactants in drug formulation.
Polysorbates undergo chemical or enzymatic hydrolysis in biopharmaceutical formulations to form free fatty acid (FFA) particles.
Residual host cell proteins can hydrolyze polysorbates.
Polysorbates are also degraded by oxidation, temperature, light, and metal ions.
There has been growing quality and safety concern for FFA particles; however the impact of polysorbate degradation on the quality and safety of biotherapeutic products is not clearly understood.



Reference: Tomlinson et al. Mol. Pharmaceutics 2015, 12, 11, 3805–3815

Materials and Methods

- Compendial PS20 (PS20 NF) and super refined PS20 (PS20 SR) were used to prepare formulations with and without therapeutic proteins.
- Bevacizumab and Trastuzumab emtansine (T-DM1) were used in our study at concentration of 1mg/mL.
- Samples were spiked with 1U/mL esterase (E) or 250 U/mL lipase (L), and stored at 4°C or 37°C for up to 4 weeks.
- Degradation or hydrolysis of PS20 was estimated by measuring free fatty acid (FFA) concentration in formulations.
- FFA was quantified using NEFFA kit.
- Subvisible particles were measured using microfluidic imaging (MFI).
- ADCC activity for bevacizumab and T-DM1 was determined using Promega kit.
- Protein stability for aggregation or degradation was analyzed by SDS-PAGE and SEC-UPLC under reducing and non-reducing conditions.
- Secondary structures of bevacizumab and T-DM1 after enzyme-induced hydrolysis of PS20 were determined by Circular dichroism (CD) spectroscopy.

Results and Discussion

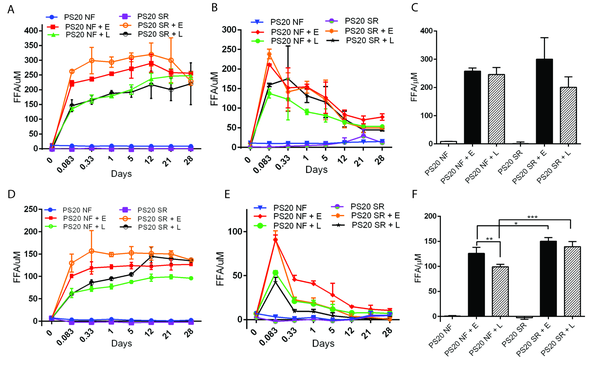


Figure 1. Effect of enzymes and fatty acid composition in PS20 for the release of FFA. Release of FFA was monitored at 4°C and 37°C for 4 weeks for bevacizumab (A and B) and T-DM1 (D and E). Panels C and F represent FFA levels for bevacizumab and T-DM1 respectively at 4°C after 4 weeks. E = esterase, L = Lipase (*** p<0.001, ** p<0.01, *p<0.05)

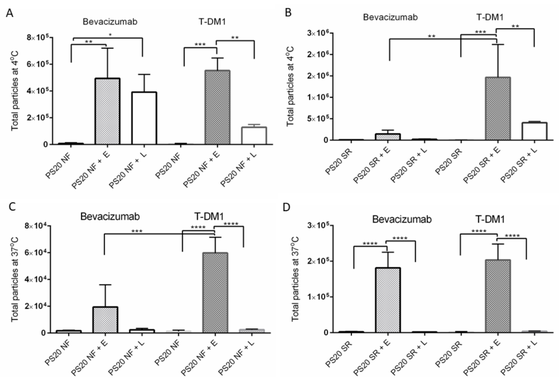


Figure 2. Comparison of PS20 NF and PS20 SR degradation to form subvisible particles in bevacizumab and T-DM1 formulations at 4°C or 37°C in presence of esterase or lipase.

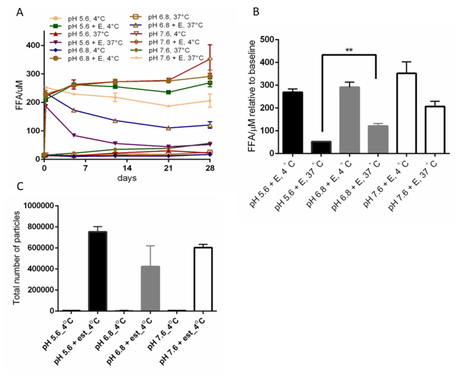


Figure 3. pH dependent release of FFA and formation of particles at pH 5.6, 6.8 and 7.6 at 4°C and 37°C after 4 weeks.

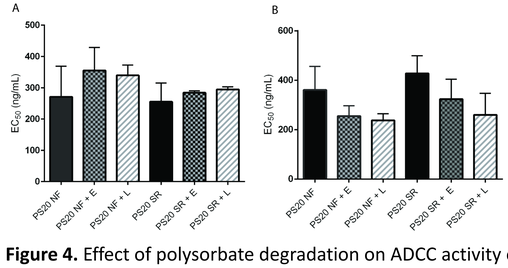


Figure 4. Effect of polysorbate degradation on ADCC activity of T-DM1 (A) and bevacizumab (B) after 4 weeks of enzyme treatment at 37°C. No significant differences in ADCC activity was observed between control and enzyme treated samples.

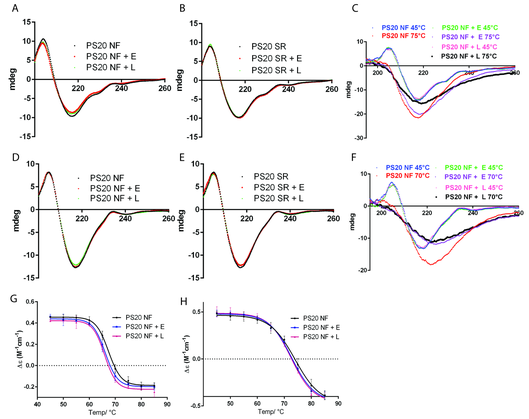


Figure 5. Effect of polysorbate degradation on the secondary structures. Representative CD spectra of T-DM1 in PS20 NF (A), and PS20 SR (B) containing formulations. Representative CD spectra of bevacizumab in PS20 NF (D) and PS20 SR (E) containing formulation at 20°C. Representative CD spectra for T-DM1 (C) and bevacizumab at 45 °C and 70 °C (F). Molar circular dichroism (Δε) as a function of temperature at 204.6 nm for T-DM1 (G) and at 205.4 nm bevacizumab (H) were plotted to determine changes in protein folding.

Conclusions

- Traces of hydrolytic enzymes in the therapeutic protein formulations cause hydrolytic degradation of PS20 leading to formation of FFA particles.
- pH, temperature, and fatty acid composition of PS20 impact the hydrolysis of PS20 and formation of subvisible particles.
- Degradation of PS20 and formation of FFA particles did not show significant impact on the biological activity or stability of bevacizumab and T-DM1 under our experimental conditions.
- Results from our work will provide better understanding for risk assessment to make a regulatory decision for products containing PS20 in formulations.

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