White Paper

Agilent
Trusted Answers

Ensuring Product Stability: The Necessity of Polysorbate Degradation Analysis in Biopharma

Abstract

Polysorbates are essential surfactants in biotherapeutic formulations, crucial for maintaining protein stability and drug efficacy. However, polysorbate degradation through hydrolysis and oxidation poses significant risks, including particle formation and protein instability. The current regulatory landscape for polysorbates varies across regions, and future regulations regarding polysorbate degradation are anticipated. This white paper emphasizes the need for innovative analytical methods to detect and mitigate polysorbate degradation early in the development process. The Agilent AdvanceBio Surfactant Profiling HPLC column is introduced as a promising solution for facilitating rapid and more comprehensive analysis of polysorbate degradation products.

Introduction

Polysorbates are crucial surfactants in the biotherapeutics industry, playing a vital role in stabilizing biotherapeutic proteins for the maintenance of drug product safety and efficacy. However, polysorbate degradation can lead to adverse effects, including the production of proteinaceous or free fatty acid particles through hydrolysis or the oxidation of formulation components.

Although there is widespread awareness of the risks and effects of polysorbate degradation, the regulatory landscape for polysorbates in biologics varies significantly across compounds and countries. Furthermore, future regulations to address polysorbate degradation are anticipated.

Given the known risks and anticipated regulatory changes, there is a need for innovation in polysorbate degradation analysis. By adopting robust analytical methods and proactive strategies, manufacturers can help to ensure biologic compliance and quality. This white paper explores the current regulations imposed on polysorbates as well as methods of polysorbate analysis. New methods of analysis, although not yet widely adopted, are able to provide more comprehensive information than the current gold standard using mixed-mode liquid chromatography (LC). Notably, the Agilent AdvanceBio Surfactant profiling HPLC column has an alternate bonded phase selectivity, which allows for high resolution in a short analysis time.

Current regulatory status of polysorbates in biotherapeutics

Polysorbates, particularly polysorbates 20 and 80, are widely used as surfactants in biotherapeutic formulations. These surfactants stabilize the biotherapeutic protein by preventing aggregation and denaturation, which is essential for maintaining the efficacy and safety of the drug product. Polysorbates are highly biocompatible and have low toxicity, further demonstrating their versatility and effectiveness.

Current regulations regarding surfactants in biologics vary by compound and country. The Chinese Pharmacopoeia[1] imposes the strictest regulations on the fatty acid composition of polysorbate 80:

- Oleic acid composition is required to be at least 98%.
- The remaining fatty acids – linoleic, palmitic, palmitoleic, stearic, myristic, and linolenic acid – must be no more than 0.5% each.

The European[2], Japanese[3], and U.S. Pharmacopeias[4] have aligned their regulations:

- Oleic acid must be at least 58% of the composition.
- The remaining fatty acids – linoleic, palmitic, palmitoleic, stearic, myristic, and linolenic acid – must be less than or equal to 18, 16, 8, 6, 5, and 4%, respectively.

Polysorbate 20 fatty acid composition regulations exhibit even greater variability among countries. The Japanese Pharmacopeia does not have a specific monograph for polysorbate 20.[3]

The European and Chinese Pharmacopeias have identical regulations:

- Lauric acid composition should range between 40 and 60%, myristic acid between 14 and 25%, and palmitic acid between 7 and 15%.
- The remaining fatty acids must be within the following limits: oleic acid ≤ 11%, capric and caprylic acids ≤ 10%, stearic acid ≤ 7%, linoleic acid ≤ 3%, and caproic acid ≤ 1%.[1,2]

The U.S. Pharmacopoeia (USP) has similar regulations to the European and Chinese Pharmacopoeias but allow a higher stearic acid content of ≤ 11%.[4,5]

The U.S. Food and Drug Administration's (FDA) guidance for industry regarding Investigational New Drugs recommends listing all components used in the drug product.[6] This includes specifically calling out compendial excipients as well as referencing quality standards from the USP or the National Formulary. While updates on the degradation profile of the drug product are required, this does not extend to excipients or surfactants. However, data are requested for particle size distribution, an attribute that can directly be affected by polysorbate hydrolysis.[6]

The European Medicines Agency (EMA) guidance mandates constituents of any excipients used, including quantitative information, the nature of the excipient, and an explanation covering the function and choice of composition.[7] The EMA also requires an identification test, and more recent guidance also includes warnings and risks on the package leaflet.[8]

Although the USP, FDA, and EMA acknowledge the adverse effects of polysorbate degradation, there are no formal statements or regulations that require polysorbate degradation testing to date. Because lipases are known to hydrolyze polysorbates, the USP has started offering stable isotope-labeled peptides for lipoprotein lipase to aid in host cell protein analysis.[9] At the 2023 FDA Science Forum, the FDA featured a poster on the hydrolytic degradation

of polysorbate 20 and later published a journal on the same topic.[10,11] These publications noted that polysorbate hydrolysis can form subvisible proteinaceous or free fatty acid particles, which can negatively impact the biologic and are considered critical quality attributes. The EMA references the tendency of polysorbates to auto-oxidize, which may cause protein instability.[8] With regulatory agencies aware of the risks and effects of polysorbate degradation, future regulations are anticipated by the biopharmaceutical community.

Accelerate innovation: the critical need for polysorbate degradation analysis

The gold standard method for meeting current regulatory requirements is a widely adopted surfactant quantitation method, which uses a mixed-mode LC column and evaporative light scattering detection (ELSD) or charged aerosol detection (CAD).[12] This method is usually quick, requiring less than 10 minutes, and straightforward to quantify, as surfactants elute as a single peak. Degradation products elute in the void and do not interfere with quantitation.

A growing number of scientists are aware of polysorbate degradation through hydrolysis or oxidation and have adopted a second surfactant assay. This secondary assay is a reversed-phase method, which, although longer, provides more comprehensive information than the mixed-mode method by retaining and separating polysorbate degradation products. Hydrolysis is detected by resolving the free fatty acid from the polysorbate monoester peak using ELSD, CAD, or mass spectrometry (MS). Oxidation is detected through various means, including identifying esters, epoxy esters, formic acid, aldehydes such as formaldehyde, acetic acid, and peroxyl radicals, among others. However, because oxidation can occur at the polyoxyethylene (POE) moieties and unsaturated sites of fatty acids on surfactants, MS detection is often preferred for clearer identification.

Determining the degradation pathway of polysorbates is crucial to the development of an effective mitigation strategy. If hydrolysis is occurring, the risk of particle formation is significant and the mitigation strategy will likely involve improved purification to remove host cell proteins, particularly lipases or esterases. Oxidation may result from residual peroxides, transition metals, or light exposure and can also oxidize the biologic. Both forms of degradation will also reduce the effectiveness of the polysorbate itself.

An extensive study of polysorbate degradation was conducted with 16 major biopharmaceutical companies. In this study, both hydrolysis (69%) and oxidation (63%) were observed by approximately two-thirds of the companies in at least one of their drug products.[13,14] Approximately 90% of companies reported hydrolysis, indicating that this is a prevalent issue. However, although oxidation is less frequent, this degradation process can still cause significant problems.

Given the potential negative effects of polysorbate degradation, it is important to adopt a polysorbate degradation characterization assay during earlier stages of the development pipeline to detect and mitigate issues promptly. The reversed-phase method, typically using a C8 or C18 column, is effective but time-consuming, often taking 40 to 60 minutes to resolve impurities from the full product peaks.

The Agilent AdvanceBio Surfactant Profiling column specifically addresses surfactant degradation. This LC column, designed for robustness, has an alternate bonded-phase selectivity, allowing for high resolution of impurities with shorter columns in less time.

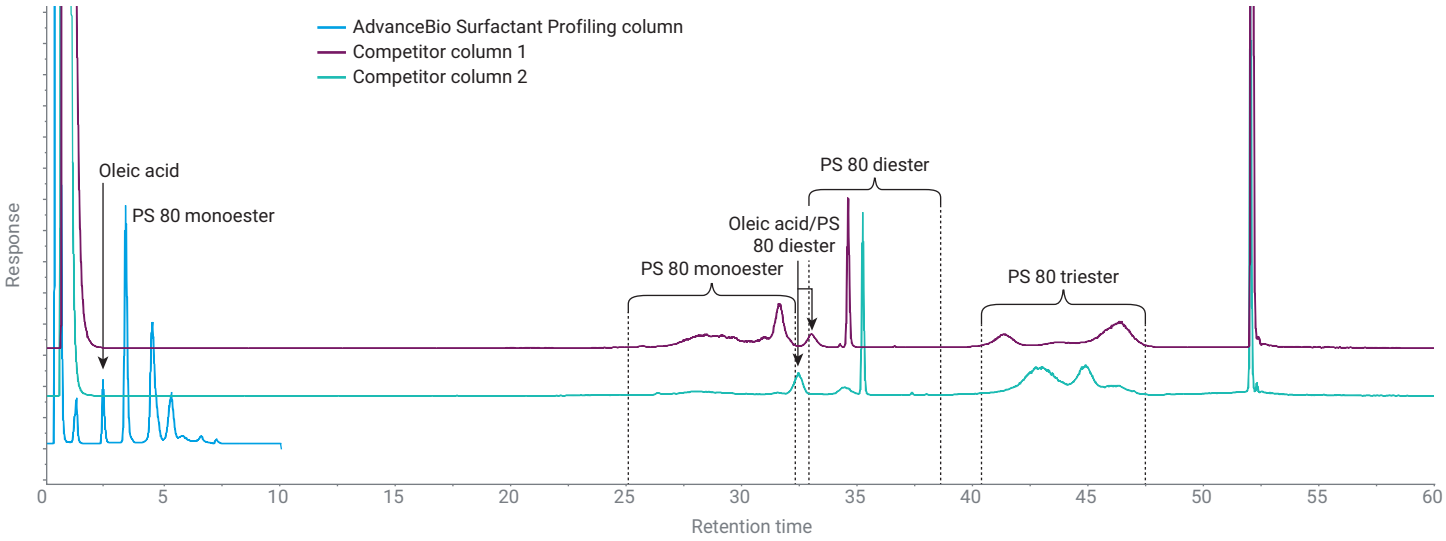

Figure 1. Traditional reversed-phase methods are longer, requiring up to 60 minutes, as shown in this example. The Agilent AdvanceBio Surfactant Profiling column requires only 10 minutes and produces higher resolution.

The risks and potential consequences of neglecting polysorbate degradation analysis

Although it may be tempting to defer the implementation of this more comprehensive assay until it is mandated by regulatory bodies, doing so only saves time and money in the short term. Strategically, for any therapeutics containing surfactants, establishing this assay in a development or formulation laboratory as early as possible is more prudent.

Neglecting polysorbate degradation can lead to compromised product quality, safety risks, regulatory noncompliance, increased costs, and reduced therapeutic efficacy. Therefore, it is essential for manufacturers to implement robust monitoring and control strategies to ensure the integrity and safety of their biologic products. Although this white paper has focused on polysorbates, it is important to note that other surfactants, such as poloxamer, can also degrade through oxidation.

Addressing a simple quality issue can cost approximately $10,000 USD, whereas more complex issues can escalate to $100,000 USD or even millions if product recalls are involved.[15] By proactively adopting these assays, laboratories will not only be prepared for future regulatory requirements but will also be able to address quality issues early in their development process.

Conclusion

Polysorbate degradation poses risks to the stability, safety, and efficacy of biotherapeutic products. Current regulations across compounds and countries are variable, and future regulations to address polysorbate degradation are anticipated. Robust and innovative analytical methods for polysorbate analysis are needed to help develop proper, effective mitigation strategies and ensure product compliance.

The widely adopted surfactant quantitation method using mixed-mode LC meets current regulatory requirements but does not offer comprehensive information on polysorbate degradation products formed by hydrolysis or oxidation. To obtain more detailed polysorbate data, some labs have adopted a reversed-phase LC method. This method is effective at retaining and separating polysorbate degradation products but is longer, taking 40 to 60 minutes.

The Agilent AdvanceBio Surfactant Profiling column is an innovative and effective solution, offering a promising approach to achieving high-resolution polysorbate degradation analysis in less time. By implementing these solutions sooner, laboratories and manufacturers can address potential quality issues earlier in their development process, ultimately helping to safeguard the integrity of their products.

To learn more about the AdvanceBio Surfactant Profiling HPLC column and how it can help your lab, visit the product page.

References

1. Ministry of Health of the People's Republic of China. The Pharmacopeia of the People's Republic of China; 2020 Edition - English Version. https://store.usp.org/product/3124465.
2. European Directorate for the Quality of Medicines & HealthCare. European Pharmacopeia. https://pheur.edqm.eu/home.
3. Pharmaceuticals and Medical Devices Agency. Japanese Pharmacopeia. https://www.pmda.go.jp/english/rs-sb-std/standards-development/jp/0029.html.
4. United States Pharmacopeial Convention. United States Pharmacopeia. https://www.uspnf.com.
5. Weber, J.; Buske, J.; Mäder, K.; Garidel, P.; Diederichs, T. Oxidation of Polysorbates: An Underestimated Degradation Pathway? *Int. J. Pharm. X* **2023**, *6*, 100202–100232. DOI: 10.1016/j.ijpx.2023.100202.
6. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for Industry INDs for Phase 2 and Phase 3 Studies, Chemistry, Manufacturing, and Controls Information; rev. 05.2003, last revised February 2018. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/inds-phase-2-and-phase-3-studies-chemistry-manufacturing-and-controls-information.
7. Official Journal of the European Communities. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community Code Relating to Medicinal Products for Human Use; rev. 11.2001. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:311:0067:0128:en:PDF.
8. Committee for Medicinal Products for Human Use, European Medicines Agency. Information for the Package Leaflet Regarding Polysorbates Used as Excipients in Medicinal Products for Human Use; EMA/CHMP/190743/2016, last revised December 2023. https://www.ema.europa.eu/en/documents/scientific-guideline/information-package-leaflet-regarding-polysorbates-used-excipients-medicinal-products-human-use_en.pdf.
9. Host Cell Protein Contaminants in mAb and Protein Therapy Manufacturing. https://www.usp.org/biologics/host-cell-proteins.
10. Aryal, B.; Lehtimaki, M.; Rao, V. A. Stress-Mediated Polysorbate 20 Degradation and Its Potential Impact on Therapeutic Proteins. *Pharm. Res.* **2024**, *41*, 1217–1232. DOI: 10.1007/s11095-024-03700-7.
11. 2023 FDA Science Forum: Polysorbate 20 Degradation in Biotherapeutic Formulations and Its Impact on Protein Quality. https://www.fda.gov/science-research/fda-science-forum/polysorbate-20-degradation-biotherapeutic-formulations-and-its-impact-protein-quality.
12. Koppolu, V.; Vemulapalli, B.; Thomas, J.; Wang, S.C.; Borman, J. A Universal Method for the Determination of Polysorbate 80 in Monoclonal Antibodies and Novel Protein Therapeutic Formulations. *Anal. Methods* **2018**, *10*, 1296–1304. DOI: 10.1039/c7ay02537h.
13. Wuchner, K.; Yi, L.; Chery, C.; Nikels, F.; Junge, F.; Crotts, G.; Rinaldi, G.; Starkey, J.; Bechtold-Peters, K.; Shuman, M.; *et al.* Industry Perspective on the Use and Characterization of Polysorbates for Biopharmaceutical Products Part 1: Survey Report on Current State and Common Practices for Handling and Control of Polysorbates. *J. Pharm. Sci.* **2022**, *111*, 1280–1291. DOI: 10.1016/j.xphs.2022.02.009.
14. Wuchner, K.; Yi, L.; Chery, C.; Nikels, F.; Junge, F.; Crotts, G.; Rinaldi, G.; Starkey, J.; Bechtold-Peters, K.; Shuman, M.; *et al.* Industry Perspective on the Use and Characterization of Polysorbates for Biopharmaceutical Products Part 2: Survey Report on Control Strategy Preparing for the Future. *J. Pharm. Sci.* **2022**, *111*, 2955–2967. DOI: 10.1016/j.xphs.2022.08.021.
15. Barnett, A. The Cost of Quality: Can We Really Afford to Ignore It?; *NSF International*: York, UK, **2017**.

www.agilent.com

DE-003898

Printed in the USA, January 31, 2025
5994-8110EN

Agilent
Trusted Answers