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Review

Industry Perspective on the Use and Characterization of Polysorbates for Biopharmaceutical Products Part 2: Survey Report on Control Strategy Preparing for the Future

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ABSTRACT

Polysorbate (PS) 20 and 80 are the main surfactants used to stabilize biopharmaceutical products. Industry practices on various aspects of PS based on a confidential survey and following discussions by 16 globally acting major biotechnology companies is presented in two publications. Part 1 summarizes the current practice and use of PS during manufacture in addition to aspects like current understanding of the (in)stability of PS, the routine QC testing and control of PS, and selected regulatory aspects of PS.[1] The current part 2 of the survey focusses on understanding, monitoring, prediction, and mitigation of PS degradation pathways in order to propose an effective control strategy. The results of the survey and extensive cross-company discussions are put into relation with currently available scientific literature.

Introduction

The body of research and publications related to polysorbate (PS) in the past few decades have significantly improved our understanding of PS structure, its degradation pathway(s), and consequences of PS degradation on critical quality attributes of biopharmaceuticals.

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This has contributed to our current state and common practice for handling and control of polysorbates within the biopharmaceutical industry, as presented extensively in Part 1 of this survey report.[1]

As discussed in a recent USP Polysorbate stimuli article[2] and at the CASSS CMC Strategy Forum 2020,[3] there are still numerous challenges to overcome in the biopharmaceutical industry to achieve a thorough understanding of PS composition, structure-function relationship and degradation mechanisms to achieve a robust control strategy. These challenges can be summarized into 4 main categories. 1.) *PS structure diversity*; as discussed in detail in our Part 1 report, commercially available polysorbates are chemically diverse mixtures - the composition and distribution of PS subspecies and impurities vary between manufacturers, grades, as well as lots. This makes PS product sourcing, sample handling, and quality control testing important aspects of the PS control strategy as an excipient in biopharmaceutical formulations. 2.) *PS analytical characterization*; multiple analytical challenges arise from the fact that PS lacks strong chromophores for commonly used detection by UV-Vis or fluorescence. The universal detectors such as charged aerosol detector (CAD) or evaporative light scattering detector (ELSD) used for PS quantification have shortcomings, e.g., lack of specificity for individual PS components and non-linear response. In addition, no single method can provide a holistic view of PS composition due to its chemically diverse nature. 3.) *PS degradation*; PS as a protein stabilizer can also degrade via two major pathways. As discussed in Part 1,[1] PS degradation is a complex issue, and a clear and simple root cause may not always be identified. In addition, an increasing number of publications indicate that host cell derived hydrolytic enzymes are causing PS degradation in biopharmaceutical solutions.[4-8] Enzymatic cleavage of ester-bonds in PS can increase the level of free fatty acids (FFAs) in the drug product, which may eventually agglomerate and form FFA particles. Hydrolytic enzymes often represent only a small fraction of the total amount of host cell protein (HCP) contaminants typically present in biopharmaceutical drug products which makes their identification and control a challenging task. 4.) *PS protein stabilizing mechanism*; we do not yet have complete knowledge and understanding on structure-function relation of this excipient. For example, it is not clear if/how the heterogeneous nature of PS contributes to its properties of being a highly effective surfactant and protein stabilizer. In order to proactively mitigate negative effects of PS degradation and its potential impact on product quality, an end-to-end control strategy encompassing PS product sourcing and handling, manufacturing process control, and drug substance (DS) and/or drug product (DP) release and stability monitoring is needed. Current state and common practices for handling of PS as an outcome of an industry survey were summarized in Part 1.[1]

Part 2 builds on Part 1 survey report and focuses on: 1.) current state of mechanistic understanding of polysorbate degradation pathways; 2.) analytical methods for comprehensive polysorbate characterization; 3.) predictive PS degradation models; and 4.) strategies to control and mitigate PS degradation. It is expected that the control and mitigation strategies will continue to evolve with increasing knowledge of PS degradation, predictive modeling, new and improved analytical methods, as well as the availability of PS alternatives.

Current state of Mechanistic Understanding of PS Degradation

Two main polysorbate degradation pathways are known and have been reported in the literature[4,9–11]: 1.) oxidation and 2.) enzyme- or chemical-mediated hydrolysis. Chemical hydrolysis under pH conditions commonly used for most liquid biopharmaceutical formulations (pH 5-7) has been found to be negligible.[12] According to our present knowledge, oxidation and enzyme-mediated hydrolysis are the main drivers for PS instability. The progress in mechanistic understanding of PS degradation for each pathway is summarized and discussed below.

Oxidative PS Degradation

Oxidation can occur to PS in biopharmaceutical formulations (usually as diluted PS solution) as well as in neat PS (raw) products, in particular, where containers are not properly closed and/or stored. Exposure to air, light and transition metals are known factors to cause PS oxidation, resulting in undesired levels of peroxides or other related reactive oxygen species (ROS) which could negatively impact product quality (based on spiking studies but not necessarily under pharmaceutical relevant conditions).[13]

Mechanistic understanding of PS oxidative degradation has been studied in a few model systems, such as spiking trace levels of transition metals,[14-17] H_2O_2,[14,17] AAPH (2,2′-Azobis(2-amidinopropane) dihydrochloride),[18,19] or under heat-stress.[10,15,20,21] The PS oxidative degradation pathway is complex and has been reported to occur at both the polyoxyethylene (POE) chain and fatty acid chain,[15,20] generating POE esters and short chain alkanes, ketones, aldehydes, free fatty acids (FFA), peroxides, and acids as degradants.[10] The extent of FFA generation because of PS oxidation is much lower than that of the hydrolytic (enzyme-mediated) degradation pathway.[10] Oxidation on the fatty acid chain preferentially occurs on unsaturated fatty acids,[16,18] which is consistent with a higher measured oxidation rate of PS80 (containing high levels of unsaturated FA-esters) compared to PS20 (containing low levels of unsaturated FA-esters).[19] In addition, the degree of homogeneity of the fatty acid ester composition can impact the susceptibility to oxidation, e.g., all-oleate PS80 are more prone to oxidation compared to the more heterogeneous PS80 counterpart.[14,22] However, PS oxidation mechanisms are not yet fully understood as all-laurate PS20 (with virtually no unsaturated FA-esters) appeared to be similarly susceptible to oxidation as all-oleate PS80 (with approx. 100% unsaturated FA-esters).[15] Formation and/or accumulation of ROS in micelle cores of physico-chemical properties and morphologies depending on e.g., the PS FA-ester composition may play a role in the oxidative mechanism.[15,16,23,24]

Consistent with published research, approximately half of the survey participants noted that additional factors such as buffer agents might also play a role in oxidative degradation. Specifically, histidine was found to play a complex role in PS oxidation.[25] Histidine buffer can suppress PS oxidation induced by H_2O_2[14] or AAPH[25] but does not inhibit the oxidation induced by transitional metals. In contrast, citrate buffer and Ethylenediaminetetraacetic acid (EDTA) have demonstrated effective suppression of transition metal-induced PS oxidation due to their cation chelating properties.[14,22,26] In a recent study[27] citrate buffer, in the presence of iron, was reported to undergo photo-degradation upon exposure to near UV and visible light, and the free radicals generated led to PS80 oxidation. In the additional presence of disulfide containing peptides and insulin, cis/trans isomerization of PS80 oleic acid esters and protein disulfide cleavage were observed via various radical reactions.[27]

According to a recent study,[26] temperature does not appear to play a role in initiating PS80 oxidation in a histidine or citrate buffer, it may play a role in a phosphate buffer system under otherwise the same conditions. Protein concentration was also found to play a role since PS80 oxidation was not observed once the protein concentration exceeds a certain threshold concentration. More specifically, data indicates that increasing protein concentrations may have a protective effect through its chelating properties of iron or by stabilization or quenching of free radicals, which not only reduces PS oxidation but also protein oxidation.[20,22]

Accelerated degradation studies provide insights into the mechanism as well as into interdependent factors contributing to PS degradation. These degradation studies not only provide support in

devising an investigational strategy of PS stability but are also indicative of a potential PS mitigation strategy that will be discussed later in this manuscript.

Enzyme-Mediated PS Hydrolysis

Enzyme-mediated PS hydrolysis is believed to be the primary root cause of PS hydrolytic degradation observed in biopharmaceutical formulations. Enzyme-mediated hydrolytic degradation is caused by trace amounts of host cell proteins (HCPs) that are co-purified with the therapeutic protein. Various studies have shown hydrolytic degradation of PS in biopharmaceutical formulations due to insufficient HCP removal during bioprocessing steps.

Interestingly, PS 80 hydrolysing esterases were already described and purified in the 1983 although not in a biopharmaceutical context.[28,29] Due to the nature of their substrate Bhargava et al.[30] have further sub-classified these enzymes as carboxylic ester hydrolases. Recent studies indicate that relevant assays to monitor enzyme activity under biopharmaceutical relevant conditions as well as to identify active enzymes can be established.[8,31-33] While enzymatic PS hydrolysis pathway is less complex than the oxidation pathway, the consequence to the final product can be more severe. Ester bonds are cleaved, and the hydrophilic head group and fatty acids are released.[4,11,34,35] The released FFAs may eventually agglomerate and form subvisible and visible FFA particles, depending on solution conditions (e.g., pH, temperature) and the solubility of the released FAs.[4,12,34,36-39] Other aspects like nucleation or interfacial stresses have also been attributed to the formation of FFA particles.[40]

HCPs that have been reported to be associated with PS degradation are provided in Table 1. A wide variety of different hydrolases have been identified as a potential root cause of PS hydrolysis and it is very likely that that multiple HCPs can play a role in PS degradation.

Analytical Methods for Comprehensive Characterization of Polysorbates

Polysorbate is inherently heterogeneous, consisting of thousands of structural variants. Its degradation during storage and use further increases this complexity. In addition, PS is typically formulated at relatively low concentrations (0.01 to 0.05%, w/v) in a complex

Table 1
Host cell proteins (HCP) reported to be associated with PS hydrolytic degradation.

Hydrolytic enzyme	Additional Study information and comments	References
CA (acid ceramidase)	CA was identified in a mAb product; recombinant CA thereof however showed no relevant PS degradation. It remains unknown whether the observed lack of CA activity towards PS is related to an unintended loss of enzyme activity during manufacturing of the recombinant enzyme version. CA is not a lipase.	7
CES-B1L (liver carboxylesterase B-1-like protein) CES-1L (liver carboxylesterase 1-like protein)	Rapid PS80 degradation observed in mAb (18 h/ 5°C) with a low level of HCP (<20 ppm; ELISA), suggesting that trace amounts of unknown HCP(s) with strong enzymatic activity on PS degradation was present in this drug substance. Using an activity-based protein profiling (ABPP) method 2 hydrolases were identified: liver carboxylesterase B-1-like protein (CES-B1L) and liver carboxylesterase 1-like protein (CES-1L). Depletion of both enzymes resulted in stable level of PS80 in DS confirming the 2 hydrolases were responsible for the rapid PS80 degradation. The PS80 degradation pattern was found to be equivalent to that generated by a human liver carboxylesterase-1 (hCES-1) and a rabbit liver esterase (rLES). CES-B1L and CES-1L are the primary cause of PS80 degradation in this mAb product.	6
LAL (lysosomal acid lipase) LPL (lipoprotein lipase)	Both LAL and LPL were identified in mAb drug formulations showing PS20 degradation. LAL, instead of LPL, was found to be associated with a higher risk of PS20 degradation based on 1) matching PS20 degradation pattern as recombinant LAL, 2) positive correlation of LAL amount on PS20 loss, and 3) effectiveness in preventing PS20 degradation by using LAL inhibitors.	41
LPL (lipoprotein lipase)	Identified in a mAb product, induces PS20 degradation. An HCP enrichment approach is described; HCP characterization by mass spectrometry.	7
	Recombinant LPL was found to have enzymatic activity against PS80 and PS20 in various solution conditions that are typical of mAb formulations. LPL knockout CHO cells were created; resulting cell culture harvest fluid demonstrated significantly reduced PS degradation without significant impact on cell viability when compared to wild-type samples.	42
LIPA (lysosomal acid lipase)	Identified in a mAb product and induces PS20 degradation. LIPA have been shown to bind to analogs of Orlistat. An HCP enrichment approach is described; HCP characterization by mass spectrometry.	7
LPLA2 (lysosomal phospholipase A2)	XV lysosomal phospholipase A2 and isomer X1(LPLA2) identified in a mAb product. Induces PS80/20 degradation. Incubation of recombinant produced LPLA2 with PS20 and PS80: hydrolysis of PS20 and PS80 monoester but a much slower rate was observed for multiester PS80. Endogenous LPLA2 detected and quantitated at less than 1 ppm in 3 mAbs while LPLA2 was not detected (or less than 0.1 ppm) in a fourth mAb. mAbs with detectable quantities of endogenous LPLA2 demonstrated PS hydrolysis while in contrast the mAb without detectable LPLA2 did not show polysorbate hydrolysis. Study suggests that LPLA2 may play a key role in PS degradation in some mAb preparations.	5
PLA2G7 (phospho-lipase A2, group VII from LPLA2 family)	An optimized activity-based protein profiling (ABPP) approach for the global profile of active serine hydrolases in bioprocess samples can identify low-abundance HCP and fills the gap between lipase abundance and activity. Among the 8 lipases, only PLA2G7 is identified as a novel lipase.	32
PLBL2 (phospho-lipase B-like 2)	Investigation of the root cause behind an observed FFA particle formation and resulting PS20 loss for a sulfatase DP upon long-term storage at 5°C. PLBL2 identified as a residual HCP impurity in DS with reported hydrolase activity. First published evidence where residual HCP present in DS was identified and experimentally shown to catalyze the degradation of PS20 in a protein formulation over time, resulting in FFA particles and PS20 loss.	43
PLBD2	Verification of PLBD2 role in PS degradation of DPs purified from recombinant CHO cells. Genetic knock-out and immuno-depletion of PLBD2 demonstrated that degradation of PS20 or PS80 was neither diminished nor reduced. Quantitative analysis of PLBD2 and PS20 degradation in multiple formulated mAb products did not establish a correlation between the amount of PLBD2 and the level of PS20 degradation.	44
PPT1 (palmitoyl-protein thio esterase 1)	Identified in a mAb product; induces PS20 degradation. PPT1 have been shown to bind to analogs of Orlistat. An HCP enrichment approach is described; HCP characterization by mass spectrometry. PPT1 is not a lipase, it is the first reported case of a thioester hydrolase degrading PS.	7
SIAE (sialate O-acetylesterase)	Unique degradation pattern distinct from the other published PS20 degrading enzymes observed in multiple formulated mAbs. Subsequent investigation revealed sialate O-acetylesterase (SIAE) as responsible HCP for a strong PS20 degradation at concentrations of less than 5 ppm. SIAE was found to selectively hydrolyze PS20 but not PS80, and only PS20 monoester species while leaving di- and tri-esters intact.	45

Table 2
Analytical toolbox for PS characterization and use within the biopharmaceutical industry.

Purpose of analytical method	Targeted species of analytical method	Use for DS analysis[a]	Use for DP analysis[a]
Routine PS Content	Sum of all PS subspecies (100%, 16 of 16)	15 of 16	16 of 16
PS characterization assays	PS subspecies (63%, 10 of 16)	7 of 10	9 of 10
	PS degradants (69%, 11 of 16)	5 of 11	10 of 11
	FFA (56%, 9 of 16)	4 of 9	8 of 9
Supportive assays to characterize PS degradation for			
Oxidative pathway	Peroxides (44%, 7 of 16)	1 of 7	3 of 7
	Trace metals (63%, 10 of 16)	6 of 10	7 of 10
Hydrolytic pathway	HCP-Hydrolases (profiling) (75%, 12 of 16)	10 of 12	3 of 12
	Enzyme activity (38%, 6 of 16)	6 of 6	4 of 6

[a] Number of survey responses relative to total number of companies using the stated analytical method.

matrix containing the active biopharmaceutical ingredient and other excipients like sugars, amino acids, and buffer components typically present at much higher concentrations than the PS. Consequently, an analytical toolbox with multiple tailored methods is required for a comprehensive characterization of PS and its degradants throughout the product life-cycle (Table 2). PS content assays, used by all companies for routine testing of formulated PS, were already described in detail in part 1 of this survey report[1]. A chromatography-based PS content assay is the most widely used method for routine PS quantitation. The following sections of this survey report focus on non-routine methods that are used for a more in-depth characterization of various PS species during product development, investigations, root cause analysis, and evaluation of mitigation effectiveness. We also provide a holistic strategy for how these methods are applied to address PS related challenges.

We propose the following terminologies to facilitate a common understanding for PS related compounds in PS products, intermediates, or when formulated drug products containing PS:

- *PS subspecies* are PS-related molecules or structural variants which are "controlled and intentionally present" (e.g., polyoxyethylene (20) sorbitan monolaureate, polyoxyethylene (20) sorbitan monooleate, polyoxyethylene (19) isosorbitan monolaureate. . ..). Different PS products may contain different concentration and composition of PS subspecies due to differences in synthetic processes (e.g., between PS manufacturers) or raw materials (e.g., between raw material batches used for PS synthesis). Despite these differences, these PS products are compliant in its qualitative and quantitative distribution of major subspecies with USP, EP, JP and ChP (*standard multi-compendial (MC) grade*, "as protein stabilizer") or *more chemically defined PS grades* like all-oleate PS80 (e.g., ChP PS 80 grade II; "for injection").
- *PS impurities* are species not intentionally present in PS product and may be controlled within PS product specification. These include impurities related to PS (e.g., polyoxyethylene (20) sorbitan monoeicosanoate (C20), unreacted FFA such as oleic acid. . ..), or unrelated to PS such as peroxides, metals, and ketones. Different PS products may contain different concentration and composition of these impurities due to differences in synthetic and purification processes (e.g., between PS manufacturers) or raw materials (e.g., between raw material batches used for PS synthesis). *Enhanced pharma purity grade PS* may contain lower levels of PS impurities compared to standard MC grade.
- *PS degradants* are structurally altered PS-related components e.g., free fatty acids, oxidized PS species, aldehydes, short chain ketones. PS degradation may also result in increase of certain PS subspecies e.g., free POEs. The presence and / or increase of these PS degradants are markers for PS degradation.

It is well understood that, under different circumstances, the same chemical entity may be classified as both, a PS subspecies and also a PS degradant (e.g., monoesters originating from enzyme-mediated hydrolysis of diesters) or be a PS impurity and a PS degradant (e.g., free fatty acids). Consequently, the same analytical toolbox may be used for various purposes such as degradation investigations or comprehensive PS product characterization.

Analytical Methods to Characterize PS Subspecies

63% (10 of 16) of the companies (Table 2) indicated that they characterize PS subspecies in drug substance (7 of 10) or drug product (9 of 10), process intermediates (4 of 10) being characterized less frequently. This analysis is used to support investigations (8 of 10) or product development (5 of 10). Consequently, platform type qualitative methods suitable for their intended use are applied as non-routine assays. No company operates these assays for GMP release or stability purposes, although one company implemented the assay for a commercial product.

Usually, (ultra-)high performance liquid chromatography-based methods target the main classes of PS subspecies e.g., free POE-sorbitan/isoborbides, POE Mono-ester or POE multi-ester fractions of PS[14], or with more resolution at the FA-ester levels e.g., POE sorbitan monolaurate and POE sorbitan monomyristate etc. [11,46,47] For even further granularity to detect single PS species, LC methods are coupled with high-resolution mass spectrometry,[15,16,48-50] and/or use 2-dimensional chromatography approaches.[51] The MS-based high resolution methods developed to assess PS subspecies in formulated product samples are generally also suitable to evaluate PS degradants and will be discussed in more detail hereafter.

Analytical Methods to Characterize PS Degradants

PS degradation is generally first detected by a decrease in the PS content or the formation of subvisible or visible FFA particles[1]. Further characterization of the relevant PS degradation pathway (if applicable) requires different methods (Fig. 1) tailored to identify and/or quantify relevant PS degradants. Observations such as increased/changed levels of FFAs (see next section) may indicate a hydrolytic PS degradation pathway, while markers for an oxidative PS pathway include POE sorbitan hydroxy oleate form,[21] POE sorbitan glycolic acid derivatives[15] or PEG sorbitan 2-decenedioic acid esters[16]. The majority (69%, 11 of 16) of the companies have implemented platform type non-routine PS degradant related methods to support PS degradation investigation. Such methods are applied to evaluate changes of PS subspecies patterns or emergence of PS degradants in DP (10 of 11) or in DS (5 of 11). These methods are used for investigational purposes or during product development and are typically not validated. Multiple methods may be needed to confirm a specific degradation pathway, as shown in Fig. 1. The separation and identification of the different PS degradant species is mainly performed by liquid chromatography methods (LC) coupled to mass spectrometry (LC-MS), followed by LC-CAD (charged aerosol

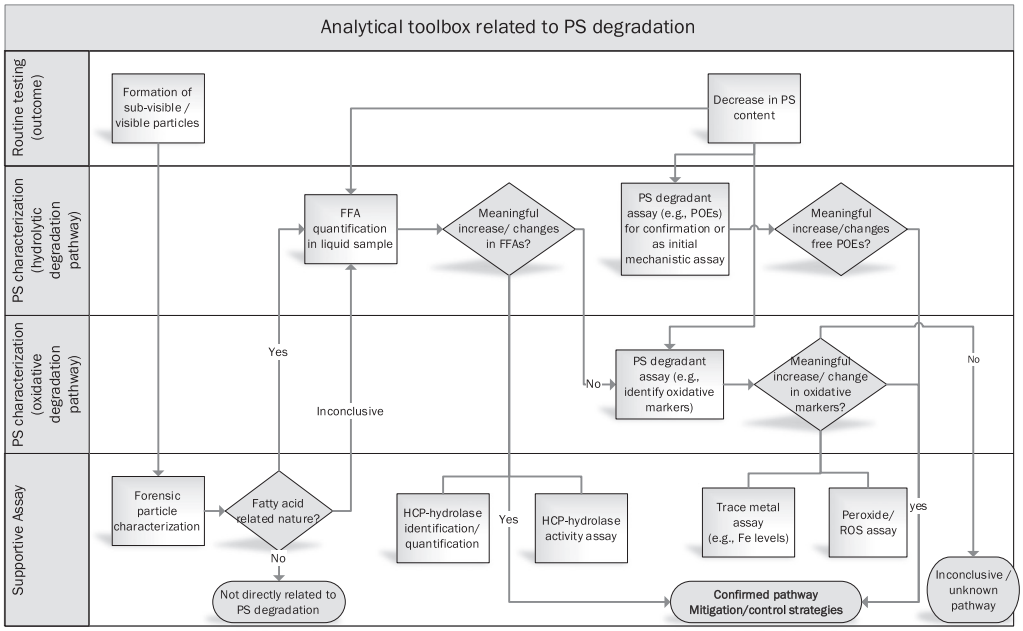

Figure 1. Analytical strategy for monitoring and investigating the primary route of PS degradation.

detection) and LC-ELSD (evaporative light scattering detection), as depicted in Fig. 2. Gas chromatography-based methods (GC-MS) or LC separation methods coupled to other detectors are less frequently used, which is consistent with current literature characterizing PS degradation pathways.[15,16,18,21,25,52–55] Protein removal in DS or DP samples is an essential step prior to PS characterization. All companies (11 of 11) running a PS degradant assay reported removing the protein either by precipitation with organic solvent (6 of 11), by solid phase extraction (SPE; 5 of 11), derivatization (2 of 11) or other approaches (3 of 11) which were not specified. Identification of PS degradants utilizes MS and putative structures; with the exception of FAs, there is a clear lack of suitable isolated, well-characterized PS degradation products which could be used as analytical standards.

Analytical Methods to Characterize Free Fatty Acids (FFA)

FFAs are one prominent PS degradant family resulting from hydrolytic PS degradation in formulated products and may also be regarded as a PS impurity when present in the neat PS product. Due to potential nucleation/precipitation and ultimately the formation of FFA particles, they require special attention. An increase in FFA levels

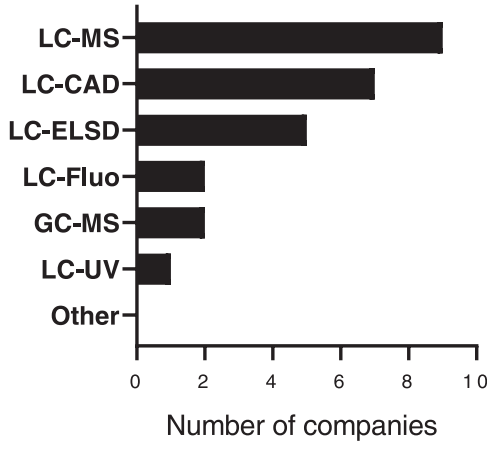

Figure 2. Analytical technologies used for PS degradant characterization within industry.

with concomitant decrease of PS content is a strong indicator for the hydrolytic (enzymatic) PS degradation pathway (Fig. 1). FFA assays are used by 56% (9 of 16) companies (Table 2) mainly at the DP stage (8 of 9) and less frequently for DS (4 of 9), process intermediate (1 of 9), or formulation screening (5 of 9) samples. FFA methods are usually platform assays (8 of 9) to predominantly support investigations (7 of 9) and less frequently used in a more routine way for product development (2 of 9) or product stability (e.g., at least for 1 stability batch, one company). One company implemented the assay for GMP release and stability purposes based on internal requirements as a validated platform assay, 2 of 9 validated the FFA assay, 1 of 9 qualified, and 5 of 9 companies indicated research method status. Table 3 summarizes additional information related to FFA assays based on the survey responses. It should be noted that one company considered switching from a derivatization LC method to a column-switching LC-MS approach. Recent literature suggests that this may indicate a trend, as one of the initial methods published for FFA quantification in biopharmaceutical samples used Phenyldiazomethane (PDAM) derivatization and LC-UV detection,[56] whereas more recent approaches rely on LC-MS methods.[53,55,57,58]

Supportive Assay to Characterize Oxidative PS Degradation

Peroxide/ROS Assay

Oxidation by atmospheric oxygen under increased temperatures or light results in reactive oxygen species (ROS) including peroxides. Consequently, many companies (88%, 14 of 16) determine peroxide levels in the PS product as an incoming test[1]. In addition, peroxides are characterized by non-routine impurity assays by 7 companies in DS (1 of 7), DP (3 of 7) or other samples mainly for investigational purposes (6 of 7). Assays are platform research methods (3 of 7) or qualified assays (2 of 7) using H_2O_2 as a standard (6 of 7). No method targeting lipophilic peroxides is currently implemented per survey responses. The lipid-compatible peroxide assay kit (ferrous oxidation-xylenol orange (FOX-2)) can be used for detection of hydroperoxide species (HPOs) being a marker for auto-oxidation[23]. A differentiation between organic hydroperoxides (ROOH) and H_2O_2 requires a catalase reaction to selectively convert H_2O_2 into oxygen and water.[59] The formation of peroxides in PS products is minimized by appropriate handling or additives (see later section).

Table 3
Technical details on FFA assays.

Assay feature	Related parameter	Number of responses of 9 companies that implemented a FFA assay
Analytical technology	LC-MS	6
	RP-(U)HPLC after derivatization	5
	RP-(U)HPLC without derivatization	2
	UV/VIS/Fluorescence assay after derivatization	1
	Other	1
	NMR-based assay	0
Sample preparation approach	Organic solvent precipitation	5
	SPE	2
	Both	1
	Other	1
Nature of assay	Qualitative	1
	Quantitative	7
	Both	1
Use of FFA standards	Yes	9 (2 without internal STD)
	Internal standards	6 (1 only internal STD)

LC-MS: Liquid chromatography hyphenated with mass spectrometry, RP-(U)HPLC: reversed phase (ultra) high performance liquid chromatography; NMR: nuclear magnetic resonance, SPE: solid phase extraction STD: standard.

Trace Metal Assay

Besides heat and light, metals (particularly iron) even at ppb levels may cause PS auto-oxidation (see oxidative PS degradation section). Ten of 16 companies use platform trace metal assays for investigational purposes (7 of 10) or during product development (4 of 10), for measurements of DS (6 of 10) or DP samples (7 of 10). The assay(s) have either a research status (3 of 10) or are qualified (4 of 10) or even validated (3 of 10). In many cases, a quantitative (9 of 10) or at least a semi-quantitative assay is in place. Assays should allow determinations of metals at trace levels since iron was shown to accelerate PS oxidation at levels as low as 10 to 20 ppb.[15,22]

Supportive Assay to Characterize Hydrolytic PS Degradation

Despite the availability of ultra-sensitive mass spectrometry-based HCP profiling approaches, it remains a challenge to establish an unambiguously positive correlation between specific enzymes and PS degradation due to several reasons: 1.) enzymes can be extremely active and may play a role in PS hydrolysis even at a level below the detection limit of the current state of the art HCP MS methods, 2.) multiple enzymes at low levels may be detected at the same time, 3.) conventional HCP MS methods alone cannot differentiate active enzyme from inactive enzyme; furthermore, many enzymes also depend on co-factors, and 4.) enzyme names may be misleading as they are usually focussing on the main function of the respective enzyme, thereby potentially neglecting either further known or unknown additional functionality. Both, HCP-hydrolase profiling methods and enzyme activity assays will be briefly discussed hereafter.

HCP-Hydrolase Profiling (Identification and/or Quantitation)

Traditional ELISA-based HCP assays feature a target-agnostic design to provide a comprehensive assessment of the total rather than individual levels of the HCPs present in a biopharmaceutical sample. Due to their inherent lack of specificity towards individual HCP species, these assays are not considered suitable to track the levels of specific enzyme contaminants. Most companies (75%, 12 of 16) participating in this survey indicated that they have addressed this gap by developing dedicated assays that enable specific assessment of hydrolytic enzyme levels, but these assays are typically performed on selected products rather than on every product.

In general, monitoring of PS degrading enzymes is applied to cases where investigational analyses require support (11 of 12) or for product development (4 of 12). In one case, testing of commercial products has been reported. Alignment of internal requirements with agency requests seem to be the main drivers for performing enzyme content assays in the industry. Testing on DS levels is clearly the focus, 8 of 12 companies reported testing of DS bioprocess intermediate purification pools, 10 of 12 on DS, and 3 of 12 on DP samples.

Generic platform-type HCP/hydrolase content assays (11 of 12) have been reported to be the most used assay type, of which 7 of 12 are applied as research type assay, while around 4 of 12 have been qualified and 2 of 12 validated for certain product characterization purposes. The survey results did not show a clear preference of target-specific over target-unspecific assays that are implemented at the various companies. With respect to the assay methodology that is used for hydrolase content determination, 10 of 12 companies indicated the application of MS-based and 6 of 12 companies of an ELISA-based HCP/hydrolase method with 5 of 12 companies using both analytical approaches. Two of 12 companies use another type of assay with no details on the applied methodology. Only 2 of 12 companies reported a successful purification and isolation of individual hydrolytic enzymes, and 8 of 12 indicated the use of commercially available enzymes as standard for analytical purposes.

Although the knowledge surrounding the identity of PS degrading enzymes is increasing (Table 1), the tracing of specific enzymes in a given product and process remains a very challenging task. In fact, reports on successfully identified hydrolytic enzymes indicate the requirement to apply extremely sensitive MS-based methods and innovative sample preparation procedures as a means to enable the detection of hydrolytic enzymes that are often present only at sub-ppm levels.

Enzyme Activity Assay

Activity-based protein profiling approaches (ABPP) will become more and more important in the future[32] as the identification and quantitative determination of pertinent enzymes yield necessary but, in some cases, incomplete information with respect to the root cause of PS degradation. Differing selectivity of the respective enzymes towards PS is conceivable due to their proposed catalytic activity which is also modulated by the specific enzymes' concentration levels, potentially by cofactors, substrate (PS subspecies) concentration and possibly enzyme inhibitors. Six of 16 companies reported the use of enzymatic activity assays for investigational purposes, mainly for the DS (5 of 6); 4 of 6 companies also analyse DS process intermediates or DP samples. The obtained data is predominantly generated for internal information. Five of 6 companies responded that their enzyme activity assay is a platform (generic) method. In contrast to

the more broadly leveraged platform assay concept, there was a single response stating that their assay is specifically applied and qualified for one product.

The questions arise as to how enzyme activity is determined. Is it a pragmatic and holistic test for enzymatic hydrolytic activity leading to PS degradation by the entirety of HCPs? Or is it specifically tailored towards the relevant hydrolase(s)? The former can be assessed with a rather straight forward approach (e.g., *PS-spiking studies* by combining intact PS with e.g., downstream samples and exposing at higher temperatures, see next section), either by determination of the decrease rate of intact PS, or the rate of increase of FFAs.[53,57] The later uses surrogate substrates to measure the lipolytic activity,[30,31] or specific enrichment of hydrolases and synthetic probes containing tag and reactive group (ABPP approach[32]). Another promising enrichment method could be the use of aptamers.[60] Further development of appropriate enzyme activity assays would certainly be useful and expand their use during product development.

Accelerated PS Degradation Studies (Predictive PS Degradation Models)

Accelerated PS degradation studies (model systems), specifically designed to assess the risk and extend of potential PS degradation under biopharmaceutically relevant conditions, are used by 67% (10 of 16) of surveyed companies. These model systems are often employed to assess degradation behavior in a shorter amount of time since the intended shelf life of a liquid biopharmaceutical product is typically 2 years or more. Such model systems should be representative for the impact of inherent impurities of the DS upstream process and/or conditions (e.g., interfacial stresses, leachables) encountered during manufacturing, storage, and handling. Degradation studies at accelerated and stressed temperatures are considered appropriate model systems (88%, 14 of 16 companies) although they may not be predictive of actual product quality issues. Indeed, PS degradation at lower temperatures, e.g. 25°C and 5°C may be dominated by autooxidation, and at a rate typically negligible compared to those at 40°C. Whereas PS degradation at 40°C can also be driven by hydrolysis.[10] Degradation studies may be used to test the stabilizing properties of (different) surfactants,[13,61-63] determine the potential risk of early PS degradation, and investigate potential root cause of degradation. Short term stability studies (e.g., *PS spiking or enzyme incubation studies*) conducted under stressed temperature conditions help to determine the influence of residual host cell proteins on PS degradation.[5,41,43] ICH photostability of antibody formulations has also been studied by Singh et al.,[64] who showed protein stability being affected by the quality of PS 80 generating peroxide.

Sixty-three percent of surveyed companies (10 of 16) considered models involving spiking studies with artificial (recombinant) enzymes and/or hydrolysis inhibitors[4,7,31] or oxidative agents as representative systems to enhance the understanding of PS degradation. Fractions of PS degraded either by oxidation or by hydrolysis (including FFAs) were spiked into biopharmaceutical formulations to investigate protein stability under long term storage conditions and to determine the ability of the remaining PS and FAA fractions to protect the protein during mechanical stress.[13,65-67] These model systems should be used with precaution and care, as they can present some pitfalls. For example, the oxidizing agents used for preparation of degraded PS may also trigger protein (e.g., iron catalyzed fragmentation[68]) and excipients to oxidize/degrade, leading to wrong conclusions or inconclusive results. Additionally, enzymes have different specificities and may degrade the PS in a manner that is not representative of the process. Hall et al.[5] observed a more pronounced hydrolysis of PS 80 than PS 20 in antibody formulations containing a lysosomal phospholipase A2 isomer X1, whereas McShan et al.[69] showed PS hydrolysis was dependent on the order of esters, the identity of the hydrophilic head group, the identity of the fatty ester tail, and the identity of the enzyme. This is in accordance with Glücklich et al.[33] who used different surrogate lipases and showed that depending on the lipase tested, a different PS degradation fingerprint is observed.

Forty-four percent (7 of 16) of the companies have a strategy for assessing the propensity of PS degradation as part of formulation development, 44% (7 of 16) indicated that they evaluate oxidation (e.g., by metal spiking, peroxide, photostability), 38% (6 of 16) assess enzymatic degradation, and 19% (3 of 16) assess degradation by chemical hydrolysis (e.g., pH, temperature, light). As model systems are employed to understand mechanistic degradation with varying levels of fidelity to actual conditions, data from such studies are considered in regulatory filings when asked by regulators by 25% of the companies (4 of 16).

Considerations for PS Control and Mitigation Strategy

As discussed in part 1 survey report, the importance of PS as a protein stabilizer in biopharmaceutical formulations was recognized by all participating companies. Active PS control and preventive measures have been taken by each company to assure its quality and functionality remain throughout processing, storage, and clinical/patient use. Survey results also indicate certain differences in control strategies used at different companies, likely due to the diversity of companies' product portfolios, prior knowledge, and supply chain challenges. Nevertheless, participants' overall PS control strategies share many common elements, similar to those discussed by Jones, et al.[70], and Katz et al.[71] An end-to-end control strategy typically includes control of PS products, DS/DP manufacturing process and in-process controls, release analytics and stability studies, as summarized in Table 4. In regard to PS grades, we differentiate between *standard multi-compendial (MC) grade PS* (with heterogeneous nature of intended species such as FA-ester distribution conforming to USP/EP/JP/ChP) vs. *enhanced pharma purity grade PS* (standard MC grade PS containing reduced and better controlled impurities such as residual trace metals, peroxides). More *chemically defined PS grades* (e.g., all-oleate PS80, all-laurate PS20) are not considered enhanced pharma purity grades per se.

At the time of the survey, most (63%, 10 of 16) pharmaceutical companies did not have a prospective mitigation strategy applicable to the entire portfolio. Instead, product specific observations either at recommended or long-term storage conditions, such as formation of subvisible or visible particles above a certain defined threshold, trigger the development and implementation of mitigation strategies for PS degradation of participating companies (Table 5). Conceptually, there are a number of mitigation strategies at one's disposal e.g., selection of enhanced pharma purity grade PS products, addition of antioxidants and/or chelating agents to the DS/DP formulation, reducing HCP level during DS purification, and/or alternate surfactant/formulation/storage conditions. Which of these strategies to use depends on the nature and severity of the problem, and the effectiveness of the mitigation as summarized in Fig. 3 and discussed for the main PS degradation pathways in more details below.

Control and Mitigation Strategy for PS Oxidative Degradation

The survey results in Part 1 indicates that PS oxidative degradation does not affect the biopharmaceutical industry as extensively as enzyme-mediated PS hydrolytic degradation; PS oxidation is observed in less than 25% of survey participant's products.[1] One reason may be that effective mitigation measures, discussed below, are already in place.

The first stage to minimize PS oxidative degradation is a thoughtful choice of PS product (e.g., with respect to impurities like

Table 4
High level summary of end-to-end PS control strategy.

Stage of PS control	Sub-elements	Intended purpose	Recommended/desired practice/state
PS product (raw material for biopharmaceutical products)	Sourcing consideration	PS grade with appropriately specified purity (e.g., low peroxide), sustained and consistent quality provided by the supplier	Transparency of communication (manufacturer/supplier/customer) Enhanced pharma purity grade for sensitive products PS grade controlled beyond pharmacopeia requirements e.g., with minimum levels of FFAs (minimize particle formation) and of non-compendial FA-esters
	Package size	Easier raw material storage and handling, number of openings minimized	Smaller size is preferred (via direct supply or repackaging into smaller containers under inert gas)
	Package container type	Minimize PS raw material degradation during storage	Container that protects PS from light, such as brown glass, and from oxygen ingress or leachates; best is single-use of containers/packs
	Storage	Minimize PS raw material degradation during storage	Protection from light, application of an inert gas overlay, storage according to supplier recommendation for unopened PS products; storage at sub-ambient temperature[a] once opened or alternatively opening in an inert gas atmosphere in case of multi-use (both minimize oxidative degradation)
	Incoming quality control testing	Consistent raw material quality	Reliance on supplier certificate (requires appropriate quality system) - wherever possible Discard the containers opened for incoming quality testing (e.g., for annual release testing); container identity testing may be performed using left-overs, but other quality measures would be preferred
	Shelf life assignment	Consistent quality during storage	Single-use container; or multi-use container with an appropriate (shortened) expiry date, maintenance of low levels of peroxides is critical (constitutes main risk for deterioration of PS quality)
Manufacturing process and in-process control	PS stock solution preparation	Accurate PS addition	Target PS stock solution 4-10% (w/v) is most common, "just in time" use[b] is preferred
	PS stock solution storage and shelf life	Minimize PS stock solution degradation	"Just in time" use is preferred, light protection and refrigeration during intermediate storage for longer storage periods (frozen storage of dilution might be considered)
	Filtration steps	Ensure consistent levels of PS by minimizing impact of PS adsorption losses	Pre-wetting, recirculation steps to saturate PS adsorption sites, process characterization studies
	Residual hydrolytic enzymes	Minimize hydrolytic PS degradation at DS level	Optimized platform purification process steps to remove hydrolytic enzymes to acceptable low levels
	Light exposure, elevated temperature of formulated PS; excipients, materials	Minimize formation of PS oxidation degradants at DS/DP level	Protection from light, reduce RT exposure time periods (but still allow wide enough window for robust manufacturing); select excipients with very low levels of transition metals, product contact surfaces with minimum metal leachables
DS or DP release	PS level	To assure PS content at expected level	PS content as release (preferred at DP level) or characterization based on clinical phase
DP stability	PS level	To assure PS content at expected level	PS content monitored without acceptance criteria[c], unless degradation of PS impacts other CQAs; PS range robustness study during formulation development; end of shelf life functionality tested to confirm functional properties of PS (e.g., if decrease of PS level during storage)

[a] PS products stored at 2 to 8°C may show cloudiness due to precipitation of e.g., salts of FFA residues, and upon room temperature equilibration, the liquid reclarifies.
[b] Use within 24 h is generally preferred.
[c] most companies rely on a product-specific polysorbate specification if PS content decreases during long-term storage conditions.

peroxides). Commercially available PS grades differing in subspecies composition can affect the stability of a given biopharmaceutical formulation dramatically. The different physicochemical properties and functional effects of different PS subspecies can ultimately determine the performances of this compound as a stabilizer within the formulation. For example, the more chemically defined grade of PS80 was found to be more prone to oxidative degradation compared to the standard MC grade. One possible hypothesis for the higher propensity of oxidative degradation in the chemically defined PS80 grades is the higher amount of oleate esters compared to the standard MC grade.[14,16,22,39]

Choice of the formulation matrix (including buffer type, excipients, antioxidant) can be an additional measure to effectively control PS oxidative degradation. The type of buffer (histidine vs acetate vs citrate) plays a role in the mechanistic aspect of PS oxidation (see previous section). However, no clear recommendation for a single best choice can be provided, as specific factors (pH, light exposure, metal vs peroxide induced oxidation) may influence the antioxidant vs. pro-oxidant role of a given buffer and one has to consider the overall protein stability. Multiple studies[14,15,39,54,72] demonstrated that EDTA (formulated as disodium edetate) or DTPA (diethylenetriaminepentaacetic acid) exhibited a protective effect on PS20 and PS80 against oxidative degradation. EDTA and similar chelating agents are thought to inactivate potential traces of iron, which are known to elicit an oxidative degradation mechanism. PS20 and PS80 solution containing antioxidants such as butylhydroxytoluene

Table 5
Survey results on triggers for PS degradation related mitigation strategies for a specific project/product.

Potential triggers	Number of companies confirming relevance of trigger[a]	Temperature conditions for a relevant trigger (number of companies)	
		Recommended condition	Accelerated condition
Formation of visible particles above a certain defined threshold	14 of 14	13	7
Formation of subvisible particles above a certain defined threshold	13 of 15	13	8
Occurrence of PS degradation with PS content below a certain defined threshold	10 of 14	8	9
Formation of visible particles at any level (one would be sufficient)	9 of 15	8	5
Occurrence of PS degradation at any level/ rate	5 of 14	5	3
Formation of subvisible particles at any level (meaningful increase)	4 of 14	4	2
Other product independent, general triggers			
Agency Feedback	8 of 13	NA	
Mitigation strategy by default/ based on prior knowledge	6 of 14	NA	
HCPs (presence of "active" enzyme with hydrolytic activity)	3 of 13	NA	

[a] Not all 16 participating companies provided a response; some considered that their current product portfolio does not require a mitigation strategy, or that PS degradation was not (yet) observed.
NA = not applicable.

(BHT) and butylhydroxyanisole (BHA) were found to be more stable against oxidative degradation than those without.[73] The use of methionine represents another possibility to reduce oxidative degradation.[15]

Another approach is to consider the use of alternative surfactants that are less susceptible to oxidization. Although poloxamer 188 (P188) can be oxidized in histidine buffer,[74] a recent head-to-head comparison with PS80 showed that P188 is more resistant to oxidation compared to PS80 under pharmaceutically relevant conditions.[75] The characteristics of poloxamers as protein stabilizer still need further research, particularly as (auto) oxidation of polyethylene and polypropylene oxide chains remains a concern[76] and protein-PDMS particles in P188 formulations were observed after long-term storage at 2-8°C for some protein formulations.[61]

The oxidative PS degradation represents a complex pathway with many interdependent factors (e.g., buffer type, pH, purity and composition of PS, protein concentration) and different root causes (transition metals, peroxides, light). Even so, a skilled product developer can proactively devise an effective control and mitigation strategy by applying sound knowledge of the mechanisms to prevent oxidative degradation.

Control and mitigation strategy for enzymatic (HCP)-induced PS hydrolysis

Storage temperature and storage time were ranked as the highest influencing factors (see part 1 survey report), reflecting the enzymatic kinetics of HCP-induced PS degradation. Protein concentration and HCP concentration were ranked the second highest influencing factors as both may be related to total amount of PS-degrading HCP (s) in the drug product. This may form the basis of potential mitigation strategies for enzymatic-induced PS hydrolysis at DS or DP stages as discussed in more detail below.

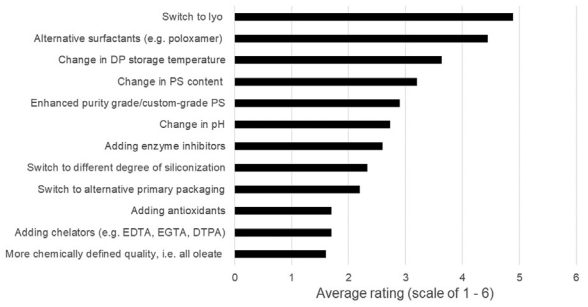

Figure 3. Rating of effectiveness of DP mitigation strategies in case of observed enzymatic polysorbate 20 degradation. Scale of 1 to 6 with 1 = least effective and 6 = highly effective (similar mitigation strategies scoring is also reported for Polysorbate 80 by survey participants)

Strategies to control and mitigate enzymatic PS degradation with potential formation of related particles were surveyed with exemplary responses for PS20 related degradation shown in Fig. 3. These strategies are equally valid for PS80 degradation and are discussed further below.

Amongst all mitigation strategies, a switch from liquid formulation to a freeze-dried preparation is ranked as the most effective. This is supported by the survey results in Part 1 that no decrease in PS content was reported in lyophilized drug product. Lyophilization removes the majority of the water (the reaction medium for enzyme-mediated PS hydrolysis), thereby preventing PS hydrolysis (and on a side note also mitigates PS oxidation). However, due to supply chain challenges, complexity in clinical handling like reconstitution, reduced patient and health care professional convenience, scale-up issues, difficulties to apply for devices, etc., lyophilization may not be the leading option in practice.

DS strategies to mitigate PS degradation by enzyme-mediated hydrolysis may include the knockout of pertinent enzymes in CHO cells using CRISPR and TALEN technologies,[42] as well as improving HCP/hydrolase clearance in downstream process. One company attempted to change the cell line with limited success, none of the companies participating in the survey had considered changing the cell line or clone so far. However, 3 of 16 companies successfully removed troublesome enzymes (and 2 of 16 with some success) and hence solving the issue by changing the purification process.

The use of a PS alternative that is resistant to enzyme hydrolysis, such as poloxamer, can be an effective mitigation and is being considered by some companies. The majority (9 out of 15) of companies are also considering alternative surfactants other than PS and P188. Alternative candidate strategies are looking into replacing the ester bond by more stable bonds as well as into alternatives to the hydrophilic parts of the polysorbate molecule.[71,77,78]. Six of 9 companies indicated that alternatives may be able to mitigate the risk of PS degradation from a scientific perspective. Two of 9 companies suggested that alternatives may also help to mitigate the regulatory risk. P188 has demonstrated both advantages[74] and disadvantages[61,76,79] in biopharmaceutical formulations, thus it might not be a universal replacement for PS. Developing alternative surfactants is not a trivial task. All participating companies in the survey acknowledged at least one hurdle to developing alternative surfactants, including material availability, budget, time needed, as well as regulatory acceptance. The top-ranking hurdle is the time requirement, followed by anticipated difficulties to obtain regulatory acceptance. Limited material

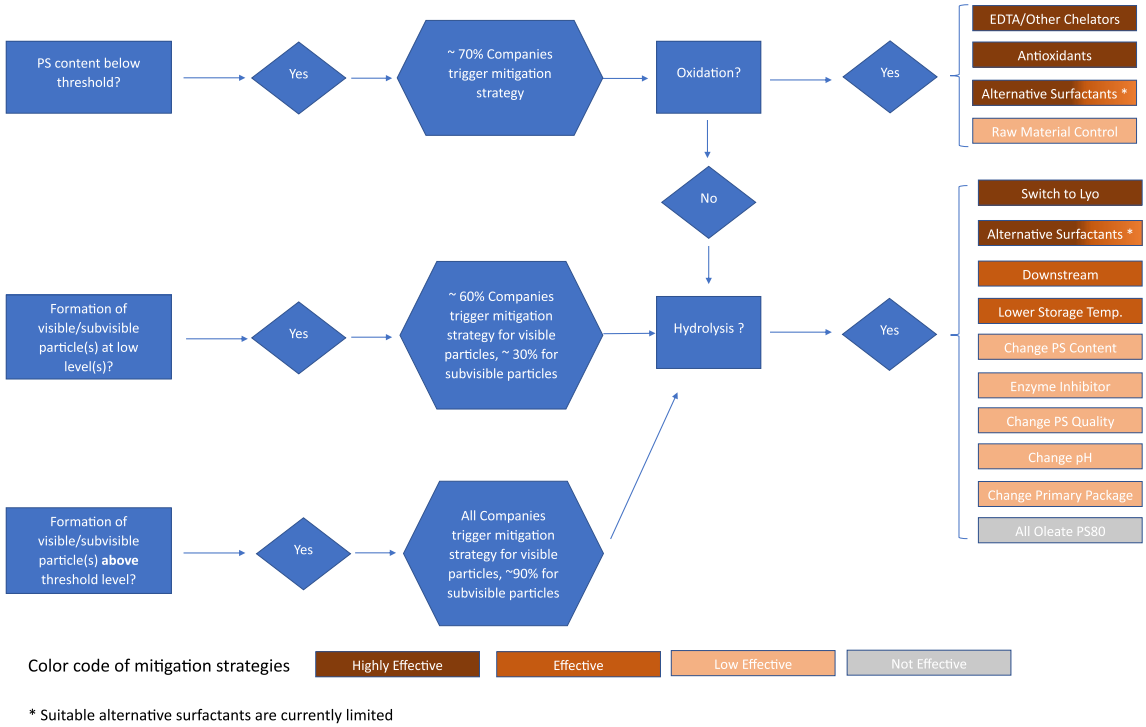

Figure 4. Mitigation strategies based on PS degradation.

availability and budget are also stated by many companies. It is understandable that developing any novel excipient will require a significant amount of time to confirm stabilizing properties under various conditions (e.g., processing, shipment, freeze-thaw), drug product stability and compatibility, major efforts to generate toxicology and medical safety data packages particularly for parenteral use, and extensive communication with regulatory agencies. Toxicology data packages can be very expensive, depending on the clinical study stage at hand. Even though the question which alternatives those companies have explored was not surveyed, existing publications discussed the possibility for the use of novel excipient classes (e.g., FM1000[77]), and application of existing classes such as cyclodextrins,[80] polyoxyethylene ethers[62,81] and maltosides.[82] Out of those alternatives, only cyclodextrins have been used in FDA approved parenteral drug products. However, the protective effect of cyclodextrins may vary case by case, depending on the properties of the proteins they are meant to stabilize and on the choice of cyclodextrin derivative selected.[80] Thus, cyclodextrins cannot be considered a universal replacement for PS, and a significant amount of development work remains before the industry can find a true alternative for PS.

A further mitigation measure for enzyme-mediated PS degradation raised in the survey is a change of the drug product storage temperature, as lowering of the storage temperature can decrease the rate of enzyme kinetics of PS hydrolysis. This approach has been brought up by a few respondents, although for the most part, this would result in frozen state storage. The feasibility of this mitigation measure is, however, highly questionable, as storing drug product in a frozen state often leads to complex supply chain challenges at the late clinical and commercial stages.

The use of enzyme inhibitors to inhibit hydrolytic activity of PS degrading HCPs may be another option in mitigating enzyme-mediated PS degradation.[32,62] However, the feasibility of this approach is highly dependent on the definitive identification of the causal enzyme as well as the availability and safety of corresponding inhibitors.

Based on the discussion above as well as survey results, mitigation measures against PS oxidation and enzyme-mediated hydrolysis are summarized in the decision tree in Fig. 4. The effectiveness of each approach is color coded and ranked from high success rate to low. Choice of each approach needs to be evaluated holistically based on the overall drug product development strategy and other potential risk factors.

Discussion and Outlook

Polysorbates are an essential component to warrant the stability of biopharmaceutics during manufacturing and to maintain its quality during storage until use. For injectable biopharmaceutics, PS is not used as a solubilizer, but as a stabilizer, and therefore is present at low concentration, usually in the range between 0.1 to 0.5 mg/mL. In the past, reports have shown that PS are prone to both chemical and enzymatic degradation. Depending on whether the chemical (i.e., oxidative) degradation pathway or the hydrolytic degradation pathway (i.e., enzymatic) is most prominent, the various mitigation measures have a different probability of success. A control strategy and related mitigation measures as outlined in Table 4 and Fig. 4 represent possible approaches to keep the levels of visible and subvisible particles below the pharmacopeial thresholds and ensure sufficient residual levels of functional PS. However, the prevailing degradation pathway must first be determined to identify the mitigation(s) with the greatest probability of success. Appropriate tools to characterize the PS degradation pathway are fingerprint analysis of the polysorbate degradation products by LC-MS and/or by FFA determinations, either on stability samples (trend in FFA levels) or in short-term stability studies at elevated temperatures (model systems). These analytical characterization methods complement a robust, well developed, and validated PS content assay. The participating survey companies agree that a PS content assay should be used routinely for DP GMP release testing and monitoring DP stability for characterization purposes.

While polysorbate is a highly functional excipient, it is also chemically and enzymatically labile, and recognition of these characteristics within the biopharmaceutical industry is paramount. PS oxidation can happen but, with the appropriate handling procedures (e.g., inert atmosphere overlay, light-protection of PS products until first time of use, just in time use of intermediate dilutions), the oxidative degradation pathway is rather unlikely to become a significant issue. The selection of an adequate supplier and appropriate quality of the respective PS also helps to avoid out of control situations. Peroxide, for instance, a potent trigger for PS oxidation, is assessed at PS receipt stage by most of the companies. Additionally, there is a trend to use single-use, small volume containers, enhanced pharma purity grade PS qualities or standard MC PS grades with a lower peroxide specification than described in the pharmacopeias. Minimizing the presence of transition metals (particularly iron) in the DS and DP formulations even at ppb levels through careful selection of excipients and processing materials also reduces the risk for oxidative PS degradation. In cases where additional measures are needed, the addition of antioxidants/chelators to the formulation can be considered as an option. It is typically a matter of a case-by-case assessment to determine if the oxidation of PS and radical formation is accompanied with the oxidation of the therapeutic protein in a critical domain, which may compromise the functionality or stability of the protein.

Enzyme-mediated PS hydrolysis has been identified as the predominant and most challenging PS degradation pathway in participating companies' products. The challenge starts with the question of how to determine if an enzymatic degradation issue is present or not. A decrease of PS content during storage of liquid product in vials and pre-filled syringes, accompanied by an increase in FFA levels is a distinctive initial indicator of hydrolytically driven PS degradation via host cell-derived enzymes. In order to probe the degradation, it has become more and more obvious that specialized analytics using highly sensitive mass spectrometry is a prerequisite to enable the identification of the critical HCP species (i.e., the hydrolytic enzyme (s) responsible for the observed degradation). The HCP profiling, approaches should be complemented by assays monitoring the enzymatic activities in DS related samples. MS-based and activity-based profiling enables the differentiation of inactive enzymes from active ones, and – according to the authors unanimous view – will increasingly gain importance. All participating companies agree that a holistic and collaborative approach is needed preferably at a program's early development stage to proactively prevent HCP-mediated PS degradation. Specifically, this approach requires thoughtfully cell line and clone selection, targeted purification process optimization, vigilant HCP profiling and PS degradation monitoring, and balanced formulation development that possibly is supportive of a manageable speed of hydrolysis and provide a sound justification for a degree of degradation that can be considered acceptable. While these cross-functional activities describe the ideal state, and rather sketch out an iterative strategy to prevent risks from the beginning of a program or mitigate as first signs of degradation are observed, industry professionals in daily practice are often confronted with the task to eliminate a prevailing risk in ongoing programs particularly with accelerated product development timelines. Depending on the product development phase, lyophilization or a switch to an alternative surfactant such as poloxamer are currently considered promising options to remediate the issue. Alternatively, some companies have also been working on optimization of the DS purification process to mitigate enzyme-mediated PS degradation. A rationale to justify an acceptable degree of PS degradation and presence of PS degradants (including FFA related particulate matter) is considered by all companies. Important success factors are besides regulatory acceptance primarily overall acceptable product quality attributes, followed by the determination of minimal effective surfactant levels by specific supportive development studies for some companies, while shelf-life restriction as appropriate measure is only considered being a temporary solution.

Even though most companies are thinking of alternative surfactants, all companies face the challenge of time needed, regulatory burden and delays in approval, material availability and budget. More importantly, no new alternative besides poloxamer P188 (by far not being a universal "trouble-free" alternative) has been developed so far. Recent experience of mixed success with initially compelling alternatives e.g., by more chemically defined ("higher purity") PS, such as all-oleate PS80, illustrate well that a better alternative to the heterogeneous standard multi-compendial grade PS to stabilize proteins is not easily found under real-world conditions. The use of all-oleate PS80, reflected in the 2015 Chinese Pharmacopeia as requirement for injectables, urged many companies to initiate evaluation studies to assess the applicability of this more chemically defined PS80 grade. In the end, study results did not make a compelling case in favour of more chemically defined PS grades, and thus hardly any company considered switching to all-oleate PS80 as they tend to be more prone to oxidative break-down than the standard MC grade equivalent. Nonetheless, it remains an important topic to be taken on by the biopharmaceutical industry to identify approaches to overcome this limited choice of appropriate alternatives to PS.

The present work has outlined the current state of PS degradation understanding. The authors acknowledge that there is yet, but by no means an exhaustive list of substantial questions that remain open: 1.) Which PS subspecies are most effective protein stabilizers and which are not (structure-function relation for thousands of PS subspecies)? 2.) What would an ideal, affordable, highly consistent and functional, enhanced pharma purity grade PS product look like? 3.) Which PS degradants and at what levels are acceptable, and how to best set related, justifiable threshold for impacted products? 4.) From which data basis (study design) should acceptance criteria (AC) be derived of, and is there a reasonable phase-appropriate approach for AC setting accepted by regulators in various regions? 5.) Is there a functional assay allowing to demonstrate and justify the effectiveness of degraded PS avoiding "end of shelf life" studies? 6.) What is the best sequence of mitigation actions to follow in case substantial PS degradation is observed? and 7.) What can be done from an industry position to expedite the establishment of an alternative surfactant to PS? The authors support the possibility of individual sponsors building the rationale of how much degradation is acceptable by taking into account the development history, including definition of design space.

Future work will help to shed light on these and many other open questions in this area. The authors remain committed to contributing to this effort.

Conflict of Competing Interest

The authors declare that they have no competing interests.

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References

1. Wuchner K, Yi L, Chery C, 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(5):1280–1291.

2. Moore DC, Houck K, Sun H, et al. Understanding the composition and quality of polysorbates to strengthen USP– NF compendial standards. *USP PF Stimuli Art*. 2021;47(1).
3. Rao A. *Excipients and Biotechnology Product Quality Attributes: A Regulator's Perspective on Reducing the Interfacial Tension*. Washington: CASSS CMC Strategy Forum 2020; 2020. ed..
4. Labrenz SR. 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*. 2014;103(8):2268–2277.
5. Hall T, Sandefur SL, Frye CC, Tuley TL, Huang L. polysorbates 20 and 80 degradation by Group XV lysosomal phospholipase A2 Isomer X1 in monoclonal antibody formulations. *J Pharm Sci*. 2016;105(5):1633–1642.
6. Zhang S, Xiao H, Molden R, Qiu H, Li N. Rapid polysorbate 80 degradation by liver carboxylesterase in a monoclonal antibody formulated drug substance at early stage development. *J Pharm Sci*. 2020;109(11):3300–3307.
7. Graf T, Tomlinson A, Yuk IH, et al. Identification and characterization of polysorbate-degrading enzymes in a monoclonal antibody formulation. *J Pharm Sci*. 2021;110(11):3558–3567.
8. Li X, Wang F, Li H, Richardson DD, Roush DJ. The measurement and control of high-risk host cell proteins for polysorbate degradation in biologics formulation. *Antib Ther*. 2022;5(1):42–54.
9. Kerwin BA. Polysorbates 20 and 80 used in the formulation of protein biotherapeutics: structure and degradation pathways. *J Pharm Sci*. 2008;97(8):2924–2935.
10. Kishore RS, Pappenberger A, Dauphin IB, et al. Degradation of polysorbates 20 and 80: studies on thermal autoxidation and hydrolysis. *J Pharm Sci*. 2011;100(2):721–731.
11. Dwivedi M, Blech M, Presser I, Garidel P. Polysorbate degradation in biotherapeutic formulations: identification and discussion of current root causes. *Int J Pharm*. 2018;552(1-2):422–436.
12. Dwivedi M, Buske J, Haemmerling F, Blech M, Garidel P. Acidic and alkaline hydrolysis of polysorbates under aqueous conditions: towards understanding polysorbate degradation in biopharmaceutical formulations. *Eur J Pharm Sci*. 2020;144: 105211.
13. Kishore RS, Kiese S, Fischer S, Pappenberger A, Grauschopf U, Mahler HC. The degradation of polysorbates 20 and 80 and its potential impact on the stability of biotherapeutics. *Pharm Res*. 2011;28(5):1194–1210.
14. Kranz W, Wuchner K, Corradini E, Berger M, Hawe A. Factors influencing polysorbate's sensitivity against enzymatic hydrolysis and oxidative degradation. *J Pharm Sci*. 2019;108(6):2022–2032.
15. Kranz W, Wuchner K, Corradini E, Menzen T, Hawe A. Micelle driven oxidation mechansim and novel oxidation markers for different grades of polysorbate 20 and 80. *J Pharm Sci*. 2020;109(10):3064–3077.
16. Liu H, Jin Y, Menon R, et al. Characterization of polysorbate 80 by liquid chromatography-mass spectrometry to understand its susceptibility to degradation and its oxidative degradation pathway. *J Pharm Sci*. 2022;111(2):323–334.
17. Schroter A, Koulov AV, Huwyler J, Mahler HC, Jahn M. 4-Hydroxynonenal is an oxidative degradation product of polysorbate 80. *J Pharm Sci*. 2021;110(6):2524–2530.
18. Borisov OV, Ji JA, John Wang Y. Oxidative degradation of polysorbate surfactants studied by liquid chromatography–mass spectrometry. *J Pharm Sci*. 2015;104(3): 1005–1018.
19. Yao J, Dokuru DK, Noestheden M, et al. A quantitative kinetic study of polysorbate autoxidation: the role of unsaturated fatty acid ester substituents. *Pharm Res*. 2009;26(10):2303–2313.
20. Gopalrathnam G, Sharma AN, Dodd SW, Huang L. Impact of stainless steel exposure on the oxidation of polysorbate 80 in histidine placebo and active monoclonal antibody formulation. *PDA J Pharm Sci Technol*. 2018;72(2):163–175.
21. Hvattum E, Yip WL, Grace D, Dyrstad K. Characterization of polysorbate 80 with liquid chromatography mass spectrometry and nuclear magnetic resonance spectroscopy: specific determination of oxidation products of thermally oxidized polysorbate 80. *J Pharm Biomed Anal*. 2012;62:7–16.
22. Bensaid F, Dagallier C, Authelin JR, et al. Mechanistic understanding of metal-catalyzed oxidation of polysorbate 80 and monoclonal antibody in biotherapeutic formulations. *Int J Pharm*. 2022;615: 121496.
23. David Friedl J, Wibel R, Burcu Akkus-Dagdeviren Z, Bernkop-Schnurch A. Reactive oxygen species (ROS) in colloidal systems: are "PEG-free" surfactants the answer? *J Colloid Interface Sci*. 2022;616:571–583.
24. Nayem J, Zhang Z, Tomlinson A, Zarraga IE, Wagner NJ, Liu Y. Micellar morphology of polysorbate 20 and 80 and their ester fractions in solution via small-angle neutron scattering. *J Pharm Sci*. 2020;109(4):1498–1508.
25. Zhang L, Yadav S, John Wang Y, Mozziconacci O, Schneich C. Dual effect of histidine on polysorbate 20 stability: mechanistic studies. *Pharm Res*. 2018;35(2):33.
26. Doyle Drbohlav LM, Sharma AN, Gopalrathnam G, Huang L, Bradley S. a mechanistic understanding of polysorbate 80 oxidation in histidine and citrate buffer systems-Part 2. *PDA J Pharm Sci Technol*. 2019;73(4):320–330.
27. Prajapati I, Subelzu N, Zhang Y, Wu Y, Schoneich C. Near UV and visible light photodegradation mechanisms in citrate buffer: one-electron reduction of peptide and protein disulfides promotes oxidation and cis/trans isomerization of unsaturated fatty acids of polysorbate 80. *J Pharm Sci*. 2022;111(4):991–1003.
28. Saito H, Tomioka H, Watanabe T, Yoneyama T. Mycobacteriocins produced by rapidly growing mycobacteria are Tween-hydrolyzing esterases. *J Bacteriol*. 1983;153 (3):1294–1300.
29. Tomioka H. Purification and characterization of the tween-hydrolyzing esterase of Mycobacterium smegmatis. *J Bacteriol*. 1983;155(3):1249–1259.
30. Bhargava AC, Mains K, Siu A, et al. High-throughput, fluorescence-based esterase activity assay for assessing polysorbate degradation risk during biopharmaceutical development. *Pharm Res*. 2021;38(3):397–413.
31. Jahn M, Zerr A, Fedorowicz FM, Brigger F, Koulov A, Mahler HC. Measuring lipolytic activity to support process improvements to manage lipase-mediated polysorbate degradation. *Pharm Res*. 2020;37(6):118.
32. Li X, Chandra D, Letarte S, et al. Profiling active enzymes for polysorbate degradation in biotherapeutics by activity-based protein profiling. *Anal Chem*. 2021;93 (23):8161–8169.
33. Glücklich N, Carle S, Buske J, Mader K, Garidel P. Assessing the polysorbate degradation fingerprints and kinetics of lipases - how the activity of polysorbate degrading hydrolases is influenced by the assay and assay conditions. *Eur J Pharm Sci*. 2021 105980.
34. Cao X, Fesinmeyer RM, Pierini CJ, et al. Free fatty acid particles in protein formulations, Part 1: microspectroscopic identification. *J Pharm Sci*. 2015;104(2):433–446.
35. Doshi N, Ritchie K, Shobha T, et al. Evaluating a modified high purity polysorbate 20 designed to reduce the risk of free fatty acid particle formation. *Pharm Res*. 2021;38(9):1563–1583.
36. Glücklich N, Dwivedi M, Carle S, Buske J, Mader K, Garidel P. An in-depth examination of fatty acid solubility limits in biotherapeutic protein formulations containing polysorbate 20 and polysorbate 80. *Int J Pharm*. 2020;591: 119934.
37. Doshi N, Demeule B, Yadav S. Understanding particle formation: solubility of free fatty acids as polysorbate 20 degradation byproducts in therapeutic monoclonal antibody formulations. *Mol Pharm*. 2015;12(11):3792–3804.
38. Doshi N, Martin J, Tomlinson A. Improving prediction of free fatty acid particle formation in biopharmaceutical drug products: incorporating ester distribution during polysorbate 20 degradation. *Mol Pharm*. 2020;17(11):4354–4363.
39. Doshi N, Giddings J, Luis L, et al. A comprehensive assessment of all-oleate polysorbate 80: free fatty acid particle formation, interfacial protection and oxidative degradation. *Pharm Res*. 2021;38(3):531–548.
40. Allmendinger A, Lebouc V, Bonati L, Woehr A, Kishore RSK, Abstiens K. Glass leachables as a nucleation factor for free fatty acid particle formation in biopharmaceutical formulations. *J Pharm Sci*. 2020.
41. Zhang S, Riccardi C, Kamen D, et al. Identification of the specific causes of polysorbate 20 degradation in monoclonal antibody formulations containing multiple lipases. *Pharm Res*. 2022;39(1):75–87.
42. Chiu J, Valente KN, Levy NE, Min L, Lenhoff AM, Lee KH. Knockout of a difficult-to-remove CHO host cell protein, lipoprotein lipase, for improved polysorbate stability in monoclonal antibody formulations. *Biotechnol Bioeng*. 2017;114(5):1006–1015.
43. Dixit N, Salamat-Miller N, Salinas PA, Taylor KD, Basu SK. Residual host cell protein promotes polysorbate 20 degradation in a sulfatase drug product leading to free fatty acid particles. *J Pharm Sci*. 2016;105(5):1657–1666.
44. Zhang S, Xiao H, Goren M, et al. Putative phospholipase B-like 2 is not responsible for polysorbate degradation in monoclonal antibody drug products. *J Pharm Sci*. 2020;109(9):2710–2718.
45. Zhang S, Xiao H, Li N. Degradation of polysorbate 20 by sialate O-acetylesterase in monoclonal antibody formulations. *J Pharm Sci*. 2021;110(12):3866–3873.
46. Lippold S, Koshari SHS, Kopf R, et al. Impact of mono- and poly-ester fractions on polysorbate quantitation using mixed-mode HPLC-CAD/ELSD and the fluorescence micelle assay. *J Pharm Biomed Anal*. 2017;132:24–34.
47. Evers DH, Schultz-Fademrecht T, Garidel P, Buske J. Development and validation of a selective marker-based quantification of polysorbate 20 in biopharmaceutical formulations using UPLC QDa detection. *J Chromatogr B*. 2020;1157: 122287.
48. Borisov OV, Ji JA, Wang YJ, Vega F, Ling VT. Toward understanding molecular heterogeneity of polysorbates by application of liquid chromatography-mass spectrometry with computer-aided data analysis. *Anal Chem*. 2011;83(10):3934–3942.
49. Christiansen A, Backensfeld T, Kuhn S, Weitschies W. Stability of the non-ionic surfactant polysorbate 80 investigated by HPLC-MS and charged aerosol detector. *Pharmazie*. 2011;66(9):666–671.
50. Hewitt D, Alvarez M, Robinson K, et al. Mixed-mode and reversed-phase liquid chromatography-tandem mass spectrometry methodologies to study composition and base hydrolysis of polysorbate 20 and 80. *J Chromatogr A*. 2011;1218 (15):2138–2145.
51. Li Y, Hewitt D, Lentz YK, Ji JA, Zhang TY, Zhang K. Characterization and stability study of polysorbate 20 in therapeutic monoclonal antibody formulation by multi-dimensional ultrahigh-performance liquid chromatography-charged aerosol detection-mass spectrometry. *Anal Chem*. 2014;86(10):5150–5157.
52. Zhang L, Yadav S, Demeule B, Wang YJ, Mozziconacci O, Schneich C. Degradation mechanisms of polysorbate 20 differentiated by (18)O-labeling and mass spectrometry. *Pharm Res*. 2017;34(1):84–100.
53. Honemann MN, Wendler J, Graf T, Bathke A, Bell CH. Monitoring polysorbate hydrolysis in biopharmaceuticals using a QC-ready free fatty acid quantification method. *J Chromatogr B*. 2019;1116:1–8.
54. Brovc EV, Mravljak J, Sink R, Pajk S. Degradation of polysorbates 20 and 80 catalysed by histidine chloride buffer. *Eur J Pharm Biopharm*. 2020;154:236–245.
55. Evers DH, Carle S, Lakatos D, Hammerling F, Garidel P, Buske J. Hydrolytic polysorbate 20 degradation - sensitive detection of free fatty acids in biopharmaceuticals via UPLC-QDa analytics with isolator column. *J Chromatogr B*. 2021;1174: 122717.
56. Tomlinson A, Demeule B, Lin B, Yadav S. Polysorbate 20 degradation in biopharmaceutical formulations: quantification of free fatty acids, characterization of particulates, and insights into the degradation mechanism. *Mol Pharm*. 2015;12 (11):3805–3815.
57. Cheng Y, Hu M, Zamiri C, et al. A rapid high-sensitivity reversed-phase ultra high performance liquid chromatography mass spectrometry method for assessing

polysorbate 20 degradation in protein therapeutics. *J Pharm Sci*. 2019;108(9): 2880–2886.
58. Zhang S, Riccardi C, Kamen D, Xiao H, Li N. Monitoring polysorbate hydrolysis in therapeutic proteins using an ultrasensitive extraction-free fatty acid quantitation method. *Anal Biochem*. 2022;637: 114472.
59. Wasylaschuk WR, Harmon PA, Wagner G, Harman AB, Templeton AC, Xu H, Reed RA. Evaluation of hydroperoxides in common pharmaceutical excipients. *J Pharm Sci*. 2007;96(1):106–116.
60. McGivney JBt, Csordas AT, Walker FM, et al. Strategy for generating sequence-defined aptamer reagent sets for detecting protein contaminants in biotherapeutics. *Anal Chem*. 2018;90(5):3262–3269.
61. Grapentin C, Muller C, Kishore RSK, et al. Protein-polydimethylsiloxane particles in liquid vial monoclonal antibody formulations containing poloxamer 188. *J Pharm Sci*. 2020;109(8):2393–2404.
62. Roy I, Patel A, Kumar V, et al. Polysorbate degradation and particle formation in a high concentration mAb: formulation strategies to minimize effect of enzymatic polysorbate degradation. *J Pharm Sci*. 2021;110(9):3313–3323.
63. Doshi N, Fish R, Padilla K, Yadav S. Evaluation of super refined polysorbate 20 with respect to polysorbate degradation, particle formation and protein stability. *J Pharm Sci*. 2020;109(10):2986–2995.
64. Singh SR, Zhang J, O'Dell C, et al. Effect of polysorbate 80 quality on photostability of a monoclonal antibody. *AAPS PharmSciTech*. 2012;13(2):422–430.
65. Larson NR, Wei Y, Prajapati I, et al. Comparison of polysorbate 80 hydrolysis and oxidation on the aggregation of a monoclonal antibody. *J Pharm Sci*. 2020;109(1): 633–639.
66. Grabarek AD, Bozic U, Rousel J, et al. What makes polysorbate functional? impact of polysorbate 80 grade and quality on IgG stability during mechanical stress. *J Pharm Sci*. 2020;109(1):871–880.
67. Zhang J, He J, Smith KJ. Fatty acids can induce the formation of proteinaceous particles in monoclonal antibody formulations. *J Pharm Sci*. 2022;111(3):655–662.
68. Nowak C, KC J, MD S, et al. Forced degradation of recombinant monoclonal antibodies: a practical guide. *MAbs*. 2017;9(8):1217–1230.
69. McShan AC, Kei P, Ji JA, Kim DC, Wang YJ. Hydrolysis of polysorbate 20 and 80 by a range of carboxylester hydrolases. *PDA J Pharm Sci Technol*. 2016;70(4):332–345.
70. Jones MT, Mahler HC, Yadav S, Bindra D, Corvari V, Fesinmeyer RM, Gupta K, Harmon AM, Hinds KD, Koulov A, Liu W, Maloney K, Wang J, Yeh PY, Singh SK. Considerations for the use of polysorbates in biopharmaceuticals. *Pharm Res*. 2018;35(8): 148.
71. Katz JS, Chou DK, Christian TR, et al. Emerging challenges and innovations in surfactant-mediated stabilization of biologic formulations. *J Pharm Sci*. 2022;111(4): 919–932.
72. Yarbrough M, Hodge T, Menard D, et al. Edetate disodium as a polysorbate degradation and monoclonal antibody oxidation stabilizer. *J Pharm Sci*. 2019;108(4): 1631–1635.
73. Schmidt A, Koulov A, Huwyler J, Mahler HC, Jahn M. Stabilizing polysorbate 20 and 80 against oxidative degradation. *J Pharm Sci*. 2020;109(6):1924–1932.
74. Wang T, Markham A, Thomas SJ, et al. Solution stability of poloxamer 188 under stress conditions. *J Pharm Sci*. 2019;108(3):1264–1271.
75. Fang L, Richard CA, Shi GH, Dong X, Rase M, Wang T. Physicochemical excipient-container interactions in prefilled syringes and their impact on syringe functionality. *PDA J Pharm Sci Technol*. 2021;75(4):317–331.
76. Bollenbach L, Buske J, Mader K, Garidel P. Poloxamer 188 as surfactant in biological formulations - an alternative for polysorbate 20/80? *Int J Pharm*. 2022;620: 121706.
77. Katz JS, Nolin A, Yezer BA, Jordan S. Dynamic properties of novel excipient suggest mechanism for improved performance in liquid stabilization of protein biologics. *Mol Pharm*. 2019;16(1):282–291.
78. Dubey S, Giovannini R. Stability of biologics and the quest for polysorbate alternatives. *Trends Biotechnol*. 2021;39(6):546–549.
79. Soeda K, Fukuda M, Takahashi M, et al. Impact of poloxamer 188 material attributes on proteinaceous visible particle formation in liquid monoclonal antibody formulations. *J Pharm Sci*. 2022.
80. Serno T, Hartl E, Besheer A, Miller R, Winter G. The role of polysorbate 80 and HPbetaCD at the air-water interface of IgG solutions. *Pharm Res*. 2013;30(1):117–130.
81. Yue L, Yan Z, Li H, Liu X, Sun P. Brij-58, a potential injectable protein-stabilizer used in therapeutic protein formulation. *Eur J Pharm Biopharm*. 2020;146:73–83.
82. Haji Abdolvahab M, Fazeli A, Fazeli MR, Brinks V, Schellekens H. The effects of dodecyl maltoside and sodium dodecyl sulfate surfactants on the stability and aggregation of recombinant interferon Beta-1b. *J Interf Cytok Res*. 2014;34(11): 894–901.