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Evaluation of the In-Use Stability of Monoclonal Antibody IV Admixtures Prepared from Drug Products Containing Polysorbate 20 Degraded by Host-Cell Lipases

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ABSTRACT

Host-cell lipases can be present in monoclonal antibody drug products and can degrade polysorbates present in the formulations as stabilizers. We hypothesized that the in-use stability of the IV admixture prepared from such a drug product might be impacted by decreasing levels of polysorbate 20. Host-cell lipase activity has, in fact, been observed during development of one of our therapeutic monoclonal antibody drug products. Throughout the course of the product shelf life, polysorbate 20 levels decreased but no other quality attributes of the drug product were impacted. An experimental approach was developed to simulate how the prepared IV admixture in-use stability is affected as polysorbate 20 concentration in the drug product decreased over the shelf life, and from that a minimum level of polysorbate 20 required in the drug product was determined to estimate the in-use stability of the IV admixture as the polysorbate 20 in the drug product degrades. The results indicate that although the observed degradation of polysorbate 20 does not affect quality attributes of this drug product, in-use stability of the IV admixture as a function of polysorbate degradation can be impacted and should be assessed to ensure sufficient quality.

Introduction

Intravenous administration of monoclonal antibodies (mAb) is commonly used to introduce such drugs to patients. Formulations of mAbs should be optimized not only for shelf-life stability, but to ensure in-use stability of diluted IV admixtures. Polysorbate 20 and 80 are among the most used non-ionic surfactants in biopharmaceutical protein formulations.[1] They improve liquid drug product (DP) stability and IV admixture in-use stability by protecting proteins from aggregation, reducing interfacial stress, and reducing adsorption onto surfaces.[2,3] Polysorbates however, are known to degrade by two major pathways: auto-oxidation and enzymatic hydrolysis.[4] Enzymatic hydrolysis of polysorbate by residual host-cell lipases during liquid DP storage has been of increasing interest in the biopharmaceutical industry, as polysorbate degradation can lead to protein instability and result in the formation of sparingly soluble compounds such as free fatty acid (FFA) particles.[5,6]

Residual host-cell lipases can be present in a drug product at ppm or even below detectable levels using conventional assays, and their effects on polysorbate degradation may not be meaningful or noticeable for months or years at typical liquid storage conditions (2–8°C).[7] However, new assays are being developed with improved sensitivity.[8] While the effects of polysorbate degradation on drug product quality have been widely studied,[9,10] there is little literature on intravenous (IV) admixture quality when polysorbate degradation is observed in a drug product.[11-13] A study by Sreedhara et al. emphasized the importance of understanding the stability of diluted monoclonal antibody formulations during storage, agitation stress, and in-use conditions and found that the ratio of mAb to polysorbate 20 as well as headspace are important contributing factors for soluble aggregates during IV admixture storage for up to 4 h.[11] Although 4 h is generally accepted as the maximum time a prepared IV admixture can be held before expiring without supporting microbial challenge data,[14] it is important to consider IV admixture storage conditions of ≥4 h to allow maximum flexibility in a clinical or commercial setting.

Abbreviations: CAD, charged aerosol detector; DEHP, di(2-ethyhexyl) phthalate; DP, Drug Product; ECD, equivalent circular diameter; FA, formic acid; FFA, free fatty acid; FI, flow imaging; GMP, good manufacturing practices; IV, intravenous; LO, light obscuration; mAb, monoclonal antibody; MPA, mobile phase A; MPB, mobile phase B; PES, polyether sulfone; PS, polysorbate; PVC, polyvinyl chloride; PVDF, polyvinylidene fluoride; USP, United States Pharmacopeia; UPLC, ultra-performance liquid chromatography.

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Another study by Kannan et al., studied the effect of sodium chloride and polysorbate on the aggregation propensity of a monoclonal antibody during IV administration and found that more than 0.001% (w/v) polysorbate 20 was required to avoid an increase in particulate matter formation.[12] Doshi et al. showed that FFA particles formed in drug products by degradation of polysorbates can dissolve immediately upon dilution into IV diluents.[13] While there is emerging literature working towards understanding the behavior of monoclonal antibody formulations upon dilution into IV admixtures, there is still much left to understand as protein interactions are often complex.

When biopharmaceutical DPs are administered intravenously, they are typically diluted in an IV bag comprised of normal saline (0.9% sodium chloride) or 5% dextrose diluent solution. Biologic drug formulations are optimized to include stabilizers necessary to ensure stability and quality of the drug product. The degradation of polysorbate and the formation of free fatty acid particles in the drug product, however, can lead to particulate matter in the IV admixture. Thus, extensive evaluation and analytical testing must be performed to adequately evaluate the IV dosing materials such as bags, IV sets, catheters, and diluents for admixture compatibility across the concentration of polysorbate present in the DP throughout the lifetime of the drug product shelf life. Although it is possible that free fatty acid particles present in DP can re-solubilize upon dilution into IV diluents, or that they may not cause precipitation or increase in particle size during infusion,[13,15] lowering the concentration of surfactant in DP by enzymatic degradation can increase the risk of mAb instability to physical stresses after dilution in IV infusion bags.[11] Protein aggregates can form in insufficiently stabilized IV admixtures, posing potential immunogenic responses in the body.[12,16] Therefore, it is important to appropriately monitor potential aggregate formation and any other changes in critical quality attributes in the IV admixture. The ability of the remaining polysorbate after degradation by host-cell lipases to stabilize the mAb against the various stresses of IV administration should be evaluated.

Demonstration of DP compatibility with a variety of diluents and IV dosing materials is required as part of the drug development and regulatory submission process (International Conference on Harmonization guideline M4G-Common Technical Document, Quality section 3.2.P.2.6). Biopharmaceutical DPs must be compatible with the various stresses and materials encountered during IV infusion, for example: the effect of agitation stress and exposure to materials present in common IV bags, IV diluents, IV sets, and catheters.[17,18] An array of analytical techniques to monitor the quality attributes in the IV admixture such as mAb concentration, purity, sub-visible particles, and potency are included in these studies.[19] Of these, one of the most important IV admixture quality attributes to monitor is sub-visible particle formation.

There are limitations in the common techniques used to assess insoluble particles that can arise during the IV administration process. Chapter <788> of the United States Pharmacopeia (USP) Particulate Matter in Injections defines that particles do not exceed 6000 particles ≥10 μm and 600 particles ≥25 μm per container.[20] Particle analysis by light obscuration (LO) has generally been the preferred testing method, with microscopic particle count test by flow imaging (FI) often used as an orthogonal method for further characterization and understanding. However, translucent or irregularly shaped particles, or particles that have a similar refractive index as the matrix fluid, are not easily quantified by LO methods and therefore could result in an underestimation of the overall particle count.[21,22] Orthogonal methods such as FI should therefore be considered in IV compatibility and in-use stability studies to gain a deeper understanding of the morphology and number of insoluble aggregates present in solution.

In cases where protein instability is observed, some mitigation strategies can be taken. One strategy to improve protein in-use stability during IV administration is to add a stabilizing solution containing additional polysorbate or other stabilizing agent (e.g., human serum albumin) to the IV admixture to maintain sufficient polysorbate levels upon dilution into the IV bag.[23] However, this strategy may not be feasible in a commercial setting due to increased costs and complexities associated with providing a product-specific stabilizing solution. For cases where polysorbate degradation by host-cell lipases are observed, the preferred pathway for ensuring there is sufficient polysorbate in the DP before dilution into the IV admixture is first by minimizing lipase levels via optimized drug substance (DS) manufacturing processes. A potential secondary mitigation path we explored in this study is ensuring polysorbate levels in the formulation are at a level sufficient to maintain IV admixture quality during the DP lifetime, even if polysorbate degradation occurs.

IV compatibility studies are often performed shortly after a DP is manufactured as well as with aged DP, but generally there is no consistent approach used when selecting aged DP material to mitigate compatibility and in-use stability risks.[14,23] As host-cell lipases can be difficult to remove completely, a robust formulation should allow for sufficient DP stability and IV admixture in-use stability in the presence of potential host-cell lipases throughout the DP shelf life. Variability in residual host-cell lipases between batches of DP may also result in varying amounts of polysorbate degradation between DP lots. Therefore, understanding the minimum required polysorbate levels in a DP to sufficiently stabilize a mAb during IV administration, in addition to understanding how much polysorbate degradation occurs during DP shelf life and ensuring that IV in-use stability is maintained throughout shelf life must be an essential part of the drug product development program.

In this study, an experimental approach was developed to evaluate how the quality of IV admixtures is impacted when prepared from DPs where polysorbate degradation is observed. This paper describes the design and production of a DP, and characterization of the DP quality as polysorbate degradation is simulated. For this case study, the minimum polysorbate 20 concentration required in the DP to maintain product quality in the IV admixture was determined in addition to the minimum concentration of polysorbate 20 required in the IV admixture. This work characterizes the morphology of protein aggregate and particle formation using FI and LO techniques such as MFI and HIAC to count subvisible particles with respect to the concentration of polysorbate 20 in an IV admixture. This approach can be used to minimize protein aggregation and particle formation for protein formulations during IV studies performed during biopharmaceutical drug product development.

Experimental Section

Materials

Monoclonal antibody 1 (mAb1) without polysorbate 20 was produced by Regeneron (Tarrytown, NY) and formulated to 60 mg/mL or 200 mg/mL in histidine pH 6.3. Super-refined polysorbate 20 was purchased from J.T. Baker (Phillipsburg, NJ). 10% (w/v) polysorbate 20 was spiked into mAb1 at varying concentrations to produce mAb1 +polysorbate 20. Diluent polyvinyl chloride (PVC) IV bags were purchased from Baxter International (Deerfield, IL), Hospira (Lake Forest, IL), and B. Braun Medical Inc. (Bethlehem, PA) containing 50 or 100 mL of diluent and were used for IV admixtures (surface area to volume ratios were 2.7–3.9 for 50 mL bags and 1.7–2.5 for 100 mL IV bags). PVC with di(2-ethylhexyl) phthalate (DEHP) IV infusion sets were purchased from BD (Franklin Lakes, NJ) and Baxter International (Deerfield, IL). 20 or 22 G catheters and 0.2 μm polyethersulfone (PES) filters were purchased from BD (Franklin Lakes, NJ) and Pall (Washington, NY), respectively, and were attached to the infusion sets. The Gemini PC-1® pump was purchased from BD (Franklin

Lakes, NJ) and the FloGard® 6201 was purchased from Baxter International (Deerfield, IL) and were used to deliver the contents from the IV bags.

Preparation of mAb1+polysorbate 20 Formulations

Several mAb1+polysorbate 20 formulations were prepared by adding polysorbate 20 ranging from 0% to 0.03% (w/v) polysorbate 20. These levels were chosen to represent polysorbate degradation in the DP formulation nominally containing 0.05% (w/v) polysorbate 20. In all cases, the mAb1+polysorbate 20 formulations were filtered with a 0.22 μm polyvinylidene fluoride (PVDF) filter.

Polysorbate 20 Quantitation

The concentration of polysorbate 20 in the mAb1+polysorbate 20 formulations was determined by ultra-performance liquid chromatography (UPLC) with a charge aerosol detector (CAD). Quantitation was performed using an Acquity H-Class UPLC system purchased from Waters (Milford, MA) with a Corona® Ultra/Veo RSTM Charged Aerosol Detector purchased from Thermo Fisher Scientific (Waltham, MA), and an Oasis Max 30 μm, 2.1×20 mm column purchased from Waters (Milford, MA). Mobile phase A (MPA) consisted of 0.1% formic acid (FA) in water, and mobile phase B (MPB) consisted of 0.1% FA in acetonitrile used in a step gradient at 1.0 mL/min for 8 min. The initial ratio of 90% MPA/10% MPB changed to 80% MPA/20% MPB at 1.0 minute and continued as such until 3.4 min. From 3.5 min to 4.5 min, 100% MPB was used. From 4.6 min through 8 min, the gradient returned to the initial ratio of 90% MPA/10% MPB. A standard curve created with a known polysorbate 20 reference standard was used to quantify the amount of polysorbate 20 present in the test articles. Standards and samples were injected at volumes between 0.3 and 3.0 μL, with protein samples targeting a column load of 0.21 μg. Data analysis was performed with Waters Empower 3 Feature Release 5.

A reference standard containing a known concentration of mAb1 and polysorbate 20 was ran with all mAb1+polysorbate 20 formulations at a column load of 0.21 μg polysorbate 20, and the known polysorbate 20 concentration from that lot was used to normalize the concentration of mAb1+polysorbate 20 formulations over different injection sequences.

IV. Admixture Dose Preparation

IV admixtures were prepared in both 50 mL and 100 mL IV bags. Due to the overfill associated with the IV bags, the final polysorbate 20 concentration was measured and the measured concentration was used. A volume of diluent equal to the volume of mAb1+polysorbate 20 added to the IV bag was removed prior to the addition of mAb1+polysorbate 20. After the addition of mAb1+polysorbate 20, the IV bag was mixed thoroughly. The target protein concentration in the IV admixture of 0.85 mg/mL mAb1 in 100 mL IV bags and 1.6 mg/mL in 50 mL IV bags was verified by RP-UPLC (Method provided in Supplementary Information). All measured protein concentrations were within 5% of the target values. The IV bags were incubated for 24 h at 2–8°C and then for at least an additional 8 h at 25°C. After storage, an infusion set was connected to each IV bag with a catheter and filter, and then primed by gravity and held at ambient room temperature for 60 min. The IV bags were then connected to IV pumps and delivered into a rinsed and cleaned polystyrene container at rates of 50 mL/h for IV admixtures prepared in 50 mL IV bags, and 100 mL/h for IV admixtures prepared in 100 mL IV bags.

Sub-Visible Particle Analysis by Light Obscuration

Particle size was determined by light obscuration with a HIAC 9703+ Liquid Particle Counting System equipped with a HRLD 400 CE/Standard Sensor with a theoretical size range of 2–400 μm and a Tecan 1 mL Sample Syringe obtained from Beckman Coulter Life Sciences (Indianapolis, IN). The instrument performance was verified using 15 μm polystyrene count and size standards purchased from Thermo Fisher Scientific (Waltham, MA). Before particle analysis, samples were vacuum degassed for 15 min. Subvisible particles were reported for ≥ 10 μm and ≥ 25 μm particles per container. Four, 1 mL aliquots were drawn from the samples, and the average of the last three draws was reported.

Sub-Visible Particle Analysis by Flow Imaging

Particles were further characterized by MFI™ 5200 flow microscopy with a Bot1 Autosampler equipped with a 100 μm flow cell 1.6 mm with silane coating (Bio-Techne. Minneapolis, MN; Protein Simple, Inc., Santa Clara, CA). The peristaltic pump was calibrated using water. The flow cell was focused using 10 μm Duke Standard™ size standard, and system suitability was ensured with COUNT-CAL™ 3000/mL, 5 μm concentration standard, both purchased from Thermo Fisher Scientific (Waltham, MA). Following a manual prime of the flow cell with water, 0.86 mL of sample volume was drawn from the sample plate. 0.15 mL was used to flush the flow cell, and 0.1 mL was used to optimize the illumination and subtract the flow cell background. 0.6 mL of each sample was then analyzed. MFI Image Analysis (software version 1.1) was used with remove stuck and remove edge filters to view images of the particles, and the number of particles per container for 2–10 μm, ≥10 μm, and ≥25 μm were reported. Results are reported in maximum feret diameter, which is defined as a measure of diameter expressed in microns along the longest axis.

Raman Microscope

Samples from mAb1 studies showing increased particle formation over time were analyzed with a Single Particle Explorer purchased from rap.ID (Monmouth Junction, NJ) to identify the particles seen with an internal identification database. A Lambda wavelength calibration (520 cm^{-1}) and Raman spectroscopy calibration were performed prior to sample analysis. Samples were prepared with 5 μm gold-coated polycarbonate filters and a glass filter funnel, where 100 μL of sample was pipetted onto the filter with a clean pipette tip and rinsed with cold water at > 10-fold the sample volume. The filtered sample was vacuum dried before analysis.

Curve Fitting and Analysis

Polysorbate 20 degradation kinetics were fit to an exponential decay model shown in Eq. 1.

$$Polysorbate\ 20,\ \%\ (w/v)\ =\ a * Exp(b * time,\ months) \quad (1)$$

In the model, a is the scale, and b is the growth rate. The data were fit to the exponential decay model using nonlinear regression modelling in JMP 16 (JMP Statistical Discovery, LLC).

Results

Identification of Polysorbate 20 Degradation in mAb1 Liquid Drug Product Vials

MAb1 development followed two parallel paths, with development goals having both high-concentration subcutaneous and lower-

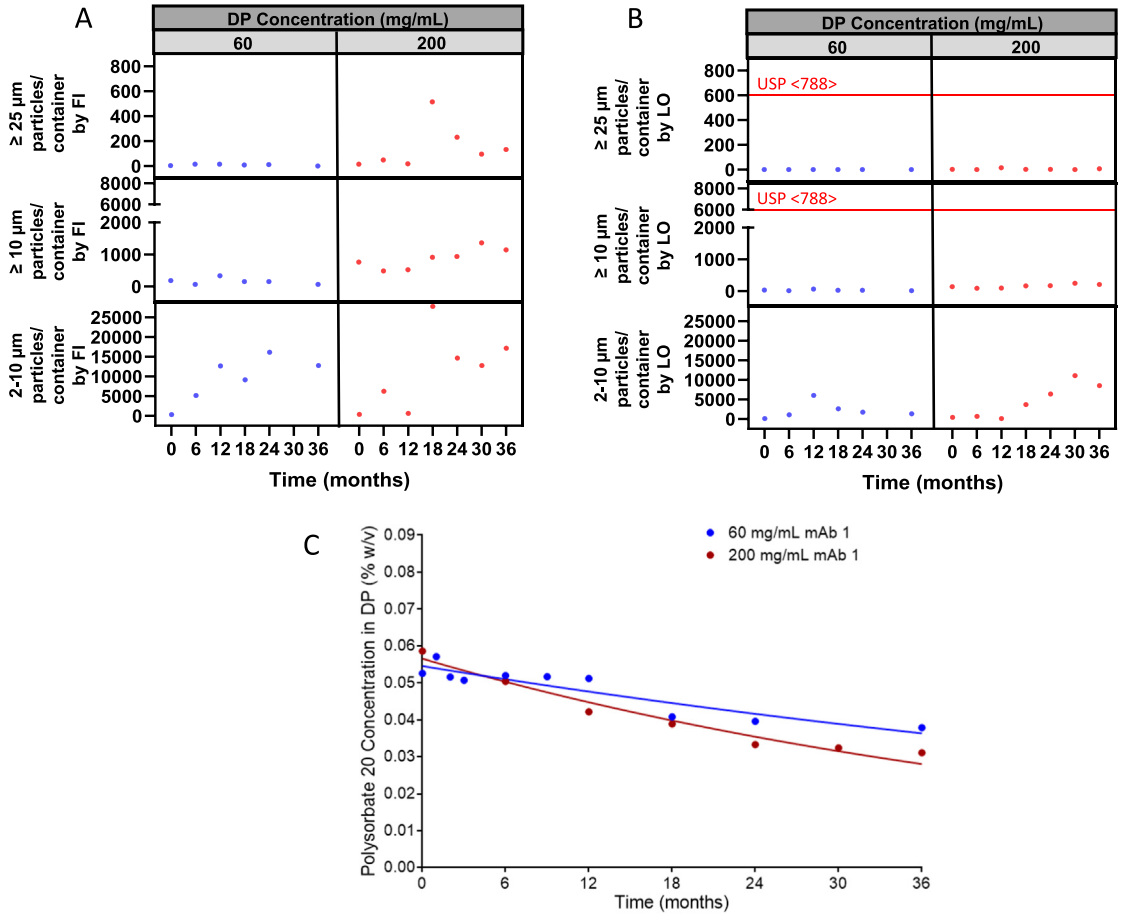

Fig. 1. (A) Particle analysis by FI for high and low DP presentations of mAb1. Increases in particle formation are observed after 18 months of storage at 5°C and continue to be elevated for the duration of the study. (B) Particle analysis by LO shows no meaningful increases in particle counts over the course of the study with the exception of an increase in 2–10 μm. Reference lines indicating the USP<788> limit are shown. (C) Polysorbate 20 levels in mAb1 200 mg/mL and 60 mg/mL DP vials. Data were fit to a single exponential decay model shown in Eq. 1.

concentration IV liquid formulations. The excipient composition for both formulations were identical and the only difference between the two formulations was the concentration of mAb1. Both formulations contained the same concentration of polysorbate 20. Both formulations were stored in glass vials in the upright orientation and were stored at 5°C for up to 36 months. Particle analysis is a standard assay and was included and monitored by FI and LO. Particles were observed by FI during storage at 5°C. The low concentration formulation showed a steady increase for 2–10 μm particles during storage at 5°C. A notable increase in 2–10 μm, ≥10 μm and ≥25 μm particles was observed by FI during storage of the high-concentration formulation at 5°C for 36 months (Fig. 1a). The trend in increasing particles was evident by FI but not observed by LO when assessing particles ≥10 μm or ≥25 μm. A steady increase in 2–10 μm particles was observed by LO during storage of the high concentration formulation at 5°C. All particle concentrations were well within the limits specified in USP<788>, however, USP<788> only has limits defined for particles ≥10 μm or ≥25 μm measured by LO (Fig. 1b). Thus, with respect to particle concentrations the quality of the vialed drug product is considered acceptable and within the limits considered safe.

The observation of increasing particle concentrations by FI prompted an investigation to further understand the nature of the particles and the root cause for their formation. Analysis of the images from FI indicated that the particles that formed in the DP over time were fibrous (Fig. 2 a). Further analysis of the DP by Raman microscopy indicated that the chemical nature of the particles was consistent with fatty acids. The instrument software provided a best match of the sample spectrum to the reference spectrum of myristic acid (Fig. 2 b). Both myristic acid and lauric acid resulted in good fit scores of 966 and 948 (out of 1000), respectively, and was interpreted as a mixture of fatty acids. The samples were assessed for polysorbate 20 levels by CAD-UPLC, and the data were fit to an exponential decay. These results indicated that there was observable polysorbate degradation occurring (Fig. 1c). In the high-concentration formulation, polysorbate 20 degraded by approximately 50% over the course of 36 months of storage at 5°C and in the low concentration formulation, the degradation was approximately 30% relative to initial levels. This suggested that the degradation was related to the concentration of mAb1, since all other formulation components were the same between the two formulations. Our hypothesis was the degradation of polysorbate 20 was not due to auto-oxidation but likely due to enzymatic hydrolysis due to the presence of host-cell lipases. The presence of host-cell lipases was in fact confirmed for this specific antibody and shown to be due to two specific lipases, lysosomal acid lipase and lipoprotein lipase, and has been previously published by Zhang et al.[24] Hydrolytic polysorbate degradation is known to be negligible under the fairly neutral pH 6.3 condition, with the excipients used, and during the 5°C storage temperature tested.[6,25] Apart from the presence of the host-cell lipases, no additional quality attributes were impacted (Supplementary Fig. S1). Size exclusion-UPLC showed no increases in aggregate species. Capillary electrophoresis showed no changes in low molecular weight species. No

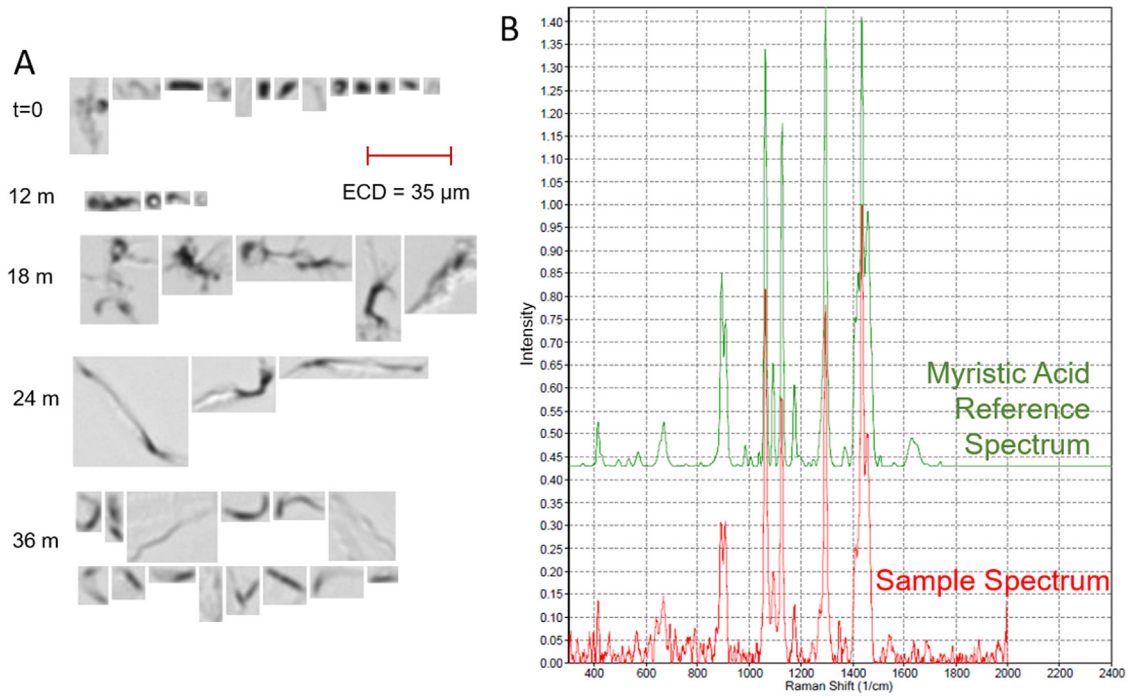

Fig. 2. (A) FI images of particles formed in mAb1 DP indicate a shift to more fibrous particles during storage at 5°C. (B) Raman microscopy of particle from DP at 18 months of storage at 5°C. The raw spectrum of the particle is shown in red, and the closest database match to myristic acid (myristic acid match rank 966 out of 1000) is shown in green. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

changes in the distribution of charge variants were observed. Potency by cell-based bioassay was maintained over the 36-month storage period at 5°C.

In-use stability of mAb1 in the Presence and Absence of Polysorbate 20 in IV Admixtures

Polysorbate 20 is required to stabilize mAb1 to interfacial and handling stresses during manufacturing and handling during product lifecycle. It is also required to stabilize the mAb1 IV admixture to interfacial stresses encountered during preparation and administration (including hold times, mixing, and dilution with diluent) and to reduce antibody adsorption to the surfaces of materials used in the delivery device. Since polysorbate 20 degradation was observed in mAb1 DP samples stored at 5°C, we sought to understand the limit to which polysorbate 20 degradation in mAb1 DP can be tolerated during IV administration by initiating a study to assess the impact of having no polysorbate 20 in IV admixtures. As such, IV admixtures were prepared from mAb1 DP formulated without polysorbate 20. After storage or after delivery of the IV admixture, sub-visible particles were observed, and the morphology of the sub-visible particles was observed by FI to be large and fibrous in nature (Fig. 3a). As no polysorbate 20 was present in this formulation, fatty acid particles were ruled out as the nature of these particles. The observed particles are consistent with the morphology of protein aggregates. Additionally, in one scenario tested for this study, the level of particles exceeded the limits set forth in USP<788> (see Fig. 3b). The protein recovery of all samples tested was within 5% of the starting concentration. Based on these observations, we determined that polysorbate 20 was required to stabilize the IV admixture to the formation of particles. We hypothesized that a minimum level of polysorbate 20 was required in the IV admixture and designed a testing strategy to determine this.

Simulation of Polysorbate 20 Degradation During Shelf-Life and Its Effect on IV Admixtures

We developed an experiment to simulate the levels of polysorbate 20 that might be found in the drug product over the course of its shelf life if host-cell lipases were present and the polysorbate 20 was degrading. The goal was to determine if the product specification was sufficient to ensure not only the quality of the drug product, but also the quality of the IV admixture prepared from a drug product containing trace amounts of host-cell lipases. Bulk formulated drug substance was prepared with all formulation components at target levels but without any polysorbate 20. Different levels of polysorbate 20 were spiked in to simulate the levels that might be found in the drug product over the course of the shelf life if the polysorbate was being degraded by host-cell lipases. The study design is illustrated in Fig. 4.

An increasing trend in subvisible particles was observed by LO (≥10 μm and ≥25 μm) as the levels of polysorbate 20 in the IV admixture decreased (Fig. 5). The trend in particle levels in the sample delivered through the IV infusion set remained stable when the polysorbate 20 concentration was approximately 0.0004% (w/v) or greater. When the polysorbate 20 concentration in the admixture decreased below 0.0004% (w/v), the level of particles in the solution delivered through the infusion set increased. In two cases overall, the level of particles exceeded the limits set forth in USP<788>. The first case was when 0% (w/v) polysorbate was included in the formulation. The other case that failed USP<788> (0.00033% (w/v) polysorbate 20) had more darker particles, compared to some other samples, and these could be distinguished by LO (Supplementary Fig. S2). This sample was delivered through the infusion set and had similar particle counts as other IV admixtures delivered with less than 0.0004% (w/v) polysorbate 20 as seen by FI (Supplementary Table S1). This shows the importance of using FI as an orthogonal method to LO for particle analysis. In most cases, increased particle counts were seen

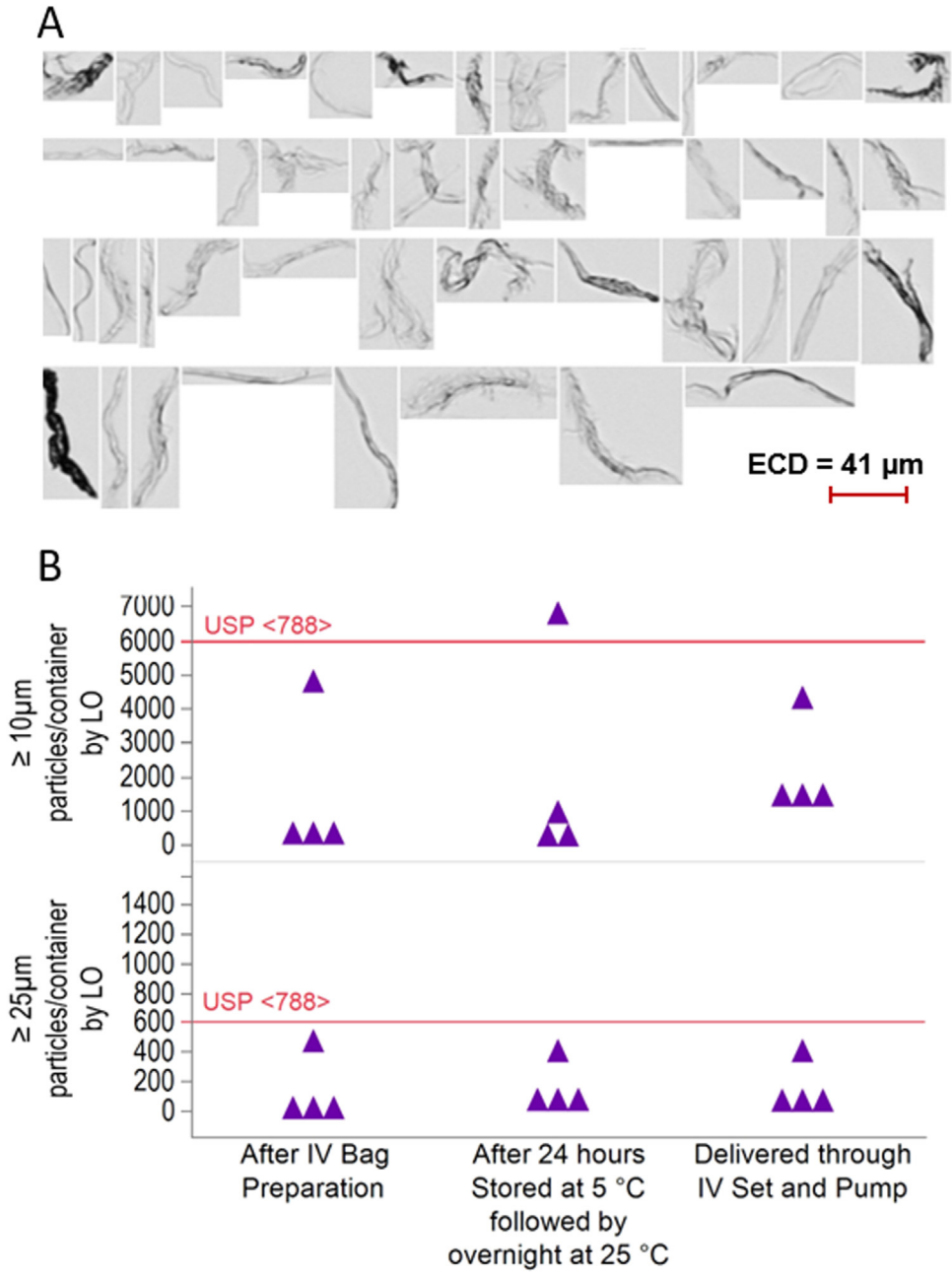

Fig. 3. (A) Particle images after storage of admixtures when polysorbate 20 is not included in the DP formulation, determined by FI. (B) Particle levels in IV admixtures determined by LO for IV admixture solutions containing 0% (w/v) polysorbate 20. USP<788> limits are indicated. 100 mL IV bag sizes were tested.

for the delivered IV admixture and not in the initial (t = 0) sample or the IV admixture storage sample, suggesting that stresses encountered from the infusion process or interactions with the materials of the infusion set contributed to instability in the IV admixture when the polysorbate 20 concentration was lower than 0.0004% (w/v) in the IV admixture. These results indicate that the minimum polysorbate 20 level required to stabilize the IV admixture should be conservatively set at 0.0004% (w/v) to guarantee patient safety. The levels of particles did not seem to be impacted by the final IV bag volume. Regardless of the final volume in the IV bag, the trend in increasing particles holds when crossing the threshold value of 0.0004% (w/v). This suggests that the resulting instability is not a function of surface area to volume ratio within the range tested. Particle formation does not appear to be a function of IV bag or infusion set material, when sufficient polysorbate 20 is present. Additional development studies previously performed with a broad range of materials (including IV bags made from PVC or polyolefin, and IV infusion sets made from polyurethane, PVC containing DEHP, PVC containing TOTM and polyethylene lined PVC) indicated that IV bag and infusion set materials did not impact the in-use stability or quality of IV admixtures of this mAb (supplementary Table S3 and S4). Furthermore, flow rate did not impact the in-use stability of IV admixtures. Flow rate was varied between 25 and 500 mL/h in previous IV studies with mAb1 and was determined not to impact particulate matter formation (Supplementary Table S3 and S4).

LO provides a quantitative measurement of the number and size of particles in solution. USP chapter USP<788> provides guidance on the amount of particulate matter acceptable for injectable products and is a benchmark which many pharmaceutical companies use for IV admixtures.[14] LO however fails to provide any information on the

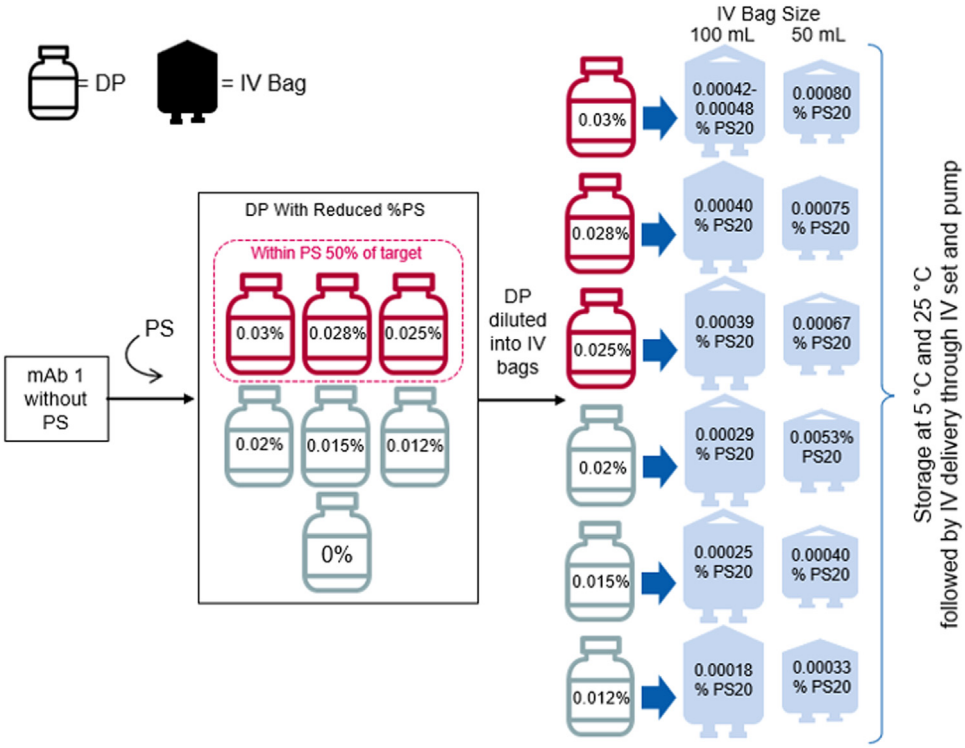

Fig. 4. Study design to simulate polysorbate degradation in IV admixtures. DP with polysorbate 20 levels ranging from 0 to 0.03% (w/v) were prepared and from these DP's, IV admixtures were prepared in 50- or 100-mL IV bags. Final polysorbate 20 levels in IV admixtures are indicated. Three IV admixtures ranging from 0.00042%-0.00048% (w/v) were prepared from the DP containing 0.03% (w/v) polysorbate 20.

morphology of particles. Understanding particle morphology is critical to assess the mechanism for any observed instabilities associated with parenteral products. Flow imaging is a complementary technique to LO in that it provides not only particle size and concentration, but also yields images of individual particles that can aid in understanding the nature of the particles. The same samples analyzed by LO were analyzed by FI. Using images obtained from FI, we determined that the morphology of the particles was fibrous and consistent with the morphology of proteinaceous particles (Fig. 3a). Therefore, the maximum Feret diameter was chosen as a parameter to characterize the trend in particle formation. Fig. 6 (A and B) shows the distribution of particle counts vs. maximum Feret diameter for delivered IV admixtures prepared with decreasing concentrations of

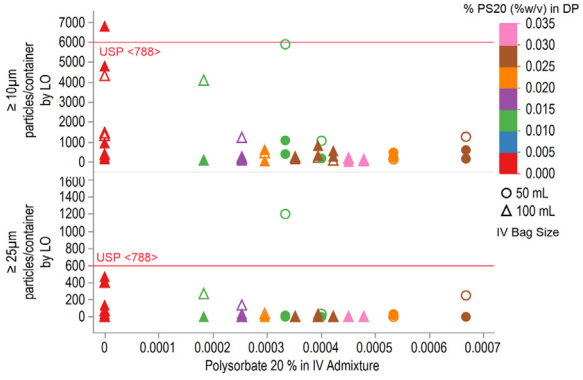

Fig. 5. Particle levels in IV admixtures determined by LO. The samples in this fig. are described in the study design shown in Fig. 4. USP<788> limits are indicated. The IV bag sizes tested are shown by the circles (50 mL) or triangles (100 mL). The solid symbols include the t = 0 and storage samples, while the open symbols show the samples delivered through the IV apparatus. The initial polysorbate 20 levels in the vial DP are indicated by the color key on the top right. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

polysorbate 20. Two sets of admixtures were prepared. Set 1 was prepared in 50 mL IV bags containing diluent, with final mAb1 concentration of approximately 1.6 mg/mL. Set 2 was prepared in 100 mL IV bags containing diluent, with final mAb1 concentration of approximately 0.85 mg/mL. (The surface area to volume ratios were 2.7–3.9 for 50 mL bags and 1.7–2.5 for 100 mL IV bags). In the 50 mL IV bags, the particle distribution remained relatively constant for solutions with polysorbate 20 concentration ≥0.0004% (w/v). At concentrations below 0.0004% (w/v), specifically 0.00034% (w/v), there is a shift towards both more and bigger particles. A similar trend was observed when the admixtures were prepared in 100 mL IV bags. Similar observations were present in IV bag for both t = 0 and storage samples (Supplementary Fig. S3). Admixtures prepared with final polysorbate 20 concentration of 0.0004% (w/v) had comparatively low particle levels with a narrow distribution of particle sizes. When the polysorbate 20 concentration was reduced to 0.0003% (w/v) the particle distribution in delivered samples shifted to higher counts and larger particles. Further reducing the polysorbate 20 concentration to 0% (w/v) resulted in a dramatically shifted distribution to even larger particles and higher counts. These data suggest that particle formation and morphology are due solely to the level of polysorbate 20 and independent of IV bag size. Particle formation and morphology are also independent of protein concentration, at least in the narrow range tested.

Fig. 6c shows representative particle images from FI for IV admixtures containing ≥0.0004% (w/v) polysorbate 20 or ≤0.00033% (w/v) polysorbate 20. It is clear from the images that in admixtures ≥0.0004% (w/v) the particles are small and, in some cases, may be silicone oil or air bubbles. As the polysorbate concentration is reduced to ≤0.00033% (w/v) the morphology clearly shifts to larger, fibrous particles. These particles are likely to be proteinaceous in nature as the morphology is consistent with protein aggregates. It should be emphasized that due to the mechanism of light obscuration used in analysis by LO, measuring fibrous transparent particles may not yield

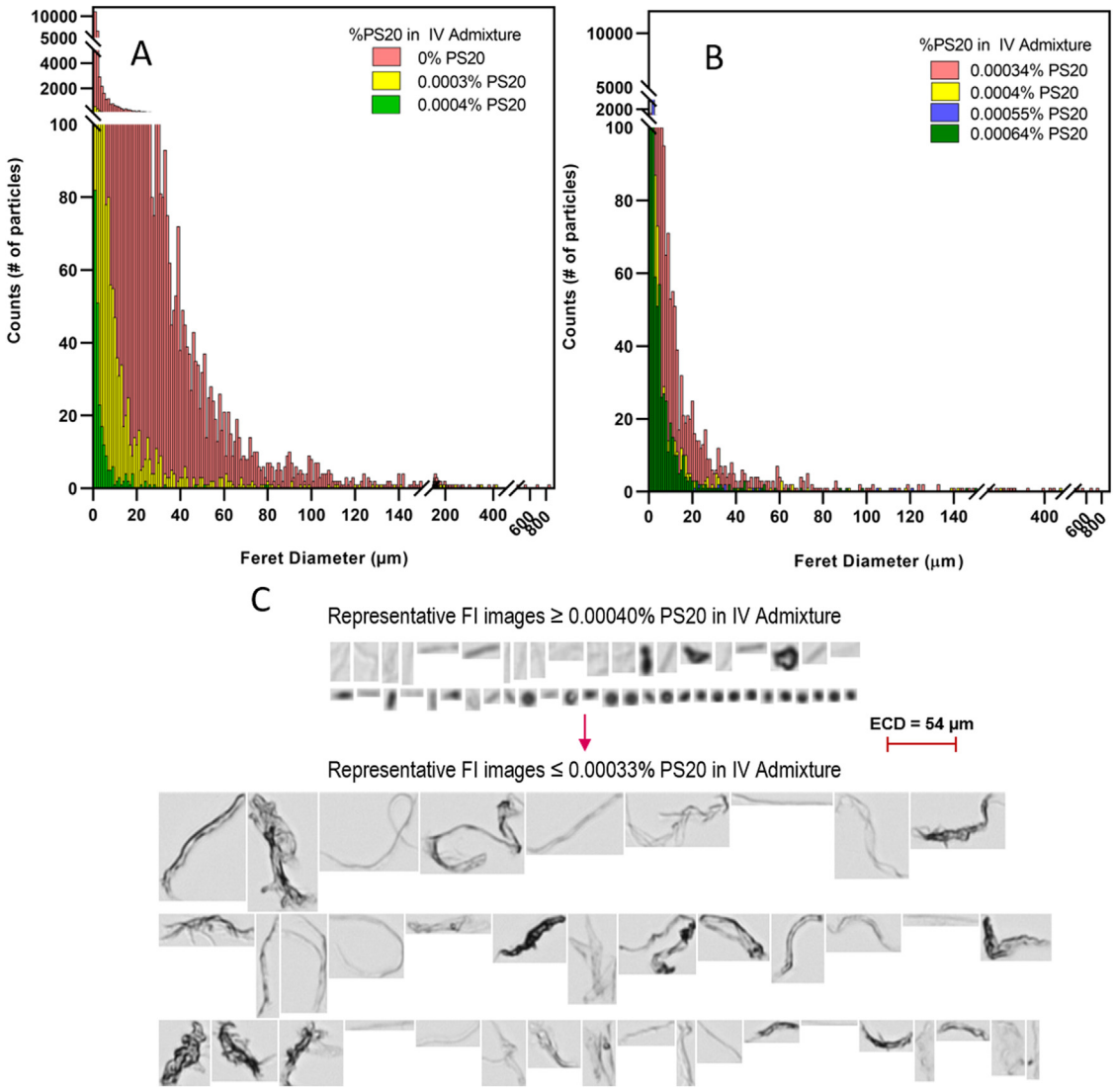

Fig. 6. (A) Particle count by FI for mAb1 admixtures prepared in 100 mL IV bags and (B) 50 mL IV bags after delivery through IV set apparatus. (C) Representative images of mAb1 particles found in IV admixtures with polysorbate 20 levels ≥0.0004% (w/v) or ≤0.00033% (w/v). The figures indicate that the size, number and morphology of the particles shifts to larger, more and more fibrous when polysorbate 20 concentrations are below 0.0004% (w/v).

accurate particle concentration data, and therefore orthogonal methods such as FI are extremely useful at identifying protein instabilities which result in transparent particles.

Fig. 1c demonstrated that polysorbate 20 was degrading over time in a DP lot manufactured from a research lot of drug substance. To better understand the degradation rate in the DP, the polysorbate 20 degradation rates for three lots of DP manufactured using Good Manufacturing Practices (GMP) were measured. The degradation trend and rates were similar between the research and GMP lots (Supplementary Fig. S4 and Table S2). Polysorbate 20 degradation was fit to a single exponential decay model to estimate the concentration of polysorbate 20 remaining at the end of shelf life or at any time during the product shelf life (Eq. 1). Three DP batches from GMP manufactured lots and one lot of non-GMP DP were analyzed and fit to the exponential decay model in Eq. 1. Using this model, two analyses were performed.

The first analysis was a model of the polysorbate 20 levels in the vialed drug product as it degrades over product shelf life based on the starting level of polysorbate 20 (Fig. 7a). We measured the polysorbate levels in available DP lots and fit the data to a two-parameter exponential model (see Eq. 1, Fig. 1c and Supplementary Fig. S4) to obtain the decay rate and scale. The fitted decay rates and scales were averaged for the measured lots. These averaged parameters were used with the exponential model in Eq. 1 to calculate the polysorbate 20 concentration at any point in the DP shelf life, up to 60 months in this case. Different starting concentrations of polysorbate 20, corresponding to the maximum manufacturing variability in DP, were used to derive several curves, each estimating what the values at different points of the shelf life would be based on starting polysorbate 20 concentrations. The number of stability timepoints needed to fit a reliable exponential decay and establish the degradation rate of polysorbate will vary depending on a number of factors including when and if lipase activity is detected, the magnitude of change in polysorbate concentration and the quality and variability of polysorbate quantitation data. In an ideal case, as few as three points can give a good fit. More points may be required if the data are less than ideal. Additionally, shorter or longer times may be required depending on the same factors (i.e., a relatively large change in polysorbate concentration with a good analytical method may require 1 - 3 months of data with a few points for a good fit, whereas a small change measured with an assay with poorer signal to noise will require longer times and more data points). For this analysis, we

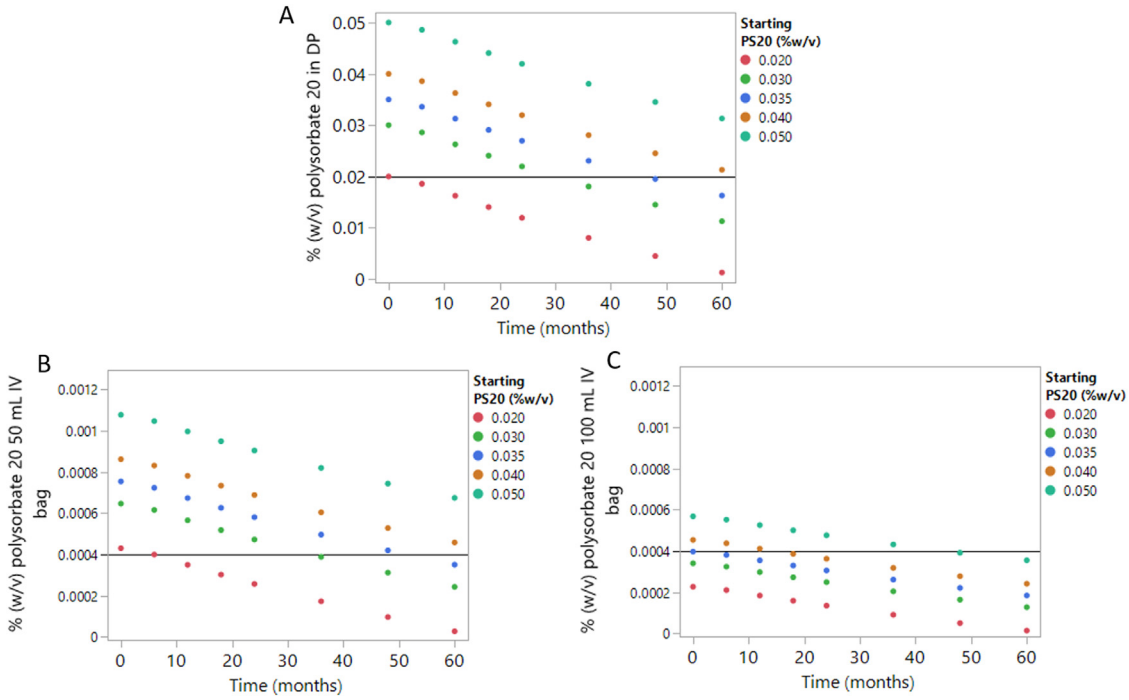

Fig. 7. Prediction of shelf life based on polysorbate 20 levels in DP and in IV admixtures. (A) Predicted polysorbate 20 levels in DP based on a starting level of 0.05% (w/v), calculated from the exponential decay model shown in Eq. 1. The minimum polysorbate 20 concentration required plus a safety margin is indicated by the solid line. Predicted polysorbate 20 concentrations in IV admixtures prepared in 50 mL IV bags (B) or 100 mL IV bags (C). The lines in (B) and (C) represent 0.0004% (w/v), the minimum polysorbate 20 concentration required to maintain a stable IV admixture.

worked under the assumption that the rate of degradation of polysorbate 20 was the same regardless of the starting polysorbate 20 levels in the formulation. The rate we are using was measured for the formulation at the target concentration of 0.05% (w/v). We are modelling the polysorbate concentration in formulations containing lower concentrations of polysorbate 20. Thus, applying the degradation rate for the higher polysorbate 20 concentration to the solutions that contain lower polysorbate 20 levels represents a worst-case scenario and would conservatively overestimate the amount of degradation at the end of shelf life. To maintain stability of the DP to mechanical or interfacial stress and provide a safety margin, polysorbate 20 levels for this mAb should be no less than 0.02% (w/v) in the vial DP. This was determined during the course of surfactant screening and optimization. 0.02% (w/v) polysorbate 20 was the minimum concentration, plus a safety factor, required to see no mAb1 degradation during vigorous agitation. The model was used to predict the shelf life of the DP, assuming the degradation rate is the same regardless of the starting level of polysorbate 20 and that the rate is constant among lots. Based on this model, the starting level of polysorbate 20 must not be less than 0.035% (w/v) in order to meet the minimum 0.02% (w/v) at the end of the 36-month shelf life.

For the second analysis, we wanted to understand how degradation of polysorbate 20 in the vial DP would ultimately impact the in-use stability of the IV admixture. This is important as the amount of polysorbate 20 at the end of shelf life would be less than there would be if no degradation occurred over the course of the shelf life. This is a way to predict if the starting levels in the DP are sufficient to stabilize the DP and the prepared IV admixture, and, if polysorbate 20 degradation occurs due to the presence of lipases, if there is sufficient polysorbate 20 left at the end of shelf life to make a stable IV admixture. Fig. 7b shows the predicted polysorbate 20 concentrations in IV admixtures prepared in 50 mL IV bags based on the predicted polysorbate 20 concentrations in the DP modelled in Fig. 7a. We used the predicted polysorbate 20 concentrations modeled in Fig. 7a to estimate the amount of polysorbate 20 that would be found in subsequently prepared IV admixtures. We assumed the lowest intended clinical dose, which in this case equaled 75 mg (1.25 mL of 60 mg/mL DP). The volume of DP (1.25 mL) multiplied by the polysorbate 20 concentration calculated for a given time point, divided by the IV bag volume yields the estimated polysorbate 20 concentration in the IV bag at any time point and for any starting concentration of polysorbate 20 (Please note that the IV bag volumes used for the calculation are 58 mL and 110 mL for 50 and 100 mL IV bags, respectively. This accounts for the average overfill commonly found in commercial IV bags.). In this case, if the polysorbate 20 concentrations in DP are at the 0.05% (w/v) target concentration upon release, there will be sufficient polysorbate 20 in the IV admixture to maintain the required quality at the minimum dose level. The model also predicts that if DP is released at 0.035% (w/v) polysorbate 20 concentration, then after 36 months there will be sufficient polysorbate 20 in the IV admixture to maintain in-use stability. However, if DP is released at 0.030% (w/v), then there is a risk that the polysorbate 20 concentration in the IV admixture after 36 months will be at or below the threshold 0.0004% (w/v) required to maintain in-use stability. Lot to lot and experimental variability would make this a high-risk scenario. Fig. 7c shows the same analysis, but for IV admixtures prepared in 100 mL IV bags. In this scenario, if DP is released at the target polysorbate 20 level, then the level of polysorbate 20 in the IV bag is predicted to be above the 0.0004% (w/v) in-use stability threshold. However, releasing DP with anything less than 0.05% (w/v) polysorbate 20 will result in the polysorbate 20 concentration in the IV admixture being below the in-use stability threshold and a limitation on the shelf life of the product. These models demonstrate that polysorbate 20 levels in mAb1 should be no less than 0.035% (w/v) in DP to ensure that not only will the DP have sufficient levels of polysorbate 20 for mAb1 stability but will have sufficient polysorbate 20 to stabilize the IV admixture. Additionally, 50 mL IV bags should be used for dosing of this drug. The use of larger-volume IV bags should be avoided for this case study because

of the increased chance that polysorbate levels in the IV admixture will be below the threshold level required for in-use stability.

Discussion

In recent years health authorities have paid an increased level of attention to condition of use studies demonstrating the in-use stability of IV admixtures containing biologic drugs in the interest of assuring patient safety. Among the most important quality attributes to monitor for such studies of biologic drugs are potency, protein concentration and particulate analysis. Both fatty acid particles and protein particles can compromise the quality of parenteral formulations and may pose a safety risk for patients. Protein particles can pose an increased risk for immunogenicity.[26] The presence of fatty acid particles can pose a quality risk, however, the extent of the risk to safety and efficacy is not fully understood. The main products from enzymatic degradation of polysorbate 20 are insoluble particulates composed primarily of lauric, myristic and palmitic acids.[27,5] Although the presence of particles in DP is a quality concern, the risks that fatty acid particles pose to patient safety is unclear. Doshi et al. have shown that the fatty acid particles found in a drug product can dissolve if diluted in saline or dextrose solutions to ≥2x dilution as their concentration falls below the solubility limit.[13] Kim et al. recently demonstrated that human serum albumin can prevent the formation of free fatty acid particles and also reverse fatty acid particles already formed.[28] Kim et al. also showed that fatty acid particles can similarly be reversed using human serum.[28]

We have demonstrated a strategy for assessing the risk of particle formation in IV admixtures for biologic drugs when residual host-cell lipases are present. A challenge associated with biologic drug products purified from Chinese Hamster Ovary cells is the presence of such lipases at levels that may be difficult to detect but that are still sufficient to significantly reduce the level of polysorbate in the drug formulation over the product shelf-life. As new and more sensitive assays are developed, polysorbate degradation can be better understood,[8] however, the testing may be challenging for routine release and stability testing. It is thus important to understand the risk that residual host-cell lipases pose not only to the quality and stability of the drug product, but just as important, the quality and in-use stability of the IV admixture. Degradation of polysorbate may be detected in the drug product if polysorbate analysis is on the release and stability panel, however, a recent survey of companies suggests that polysorbate analysis is not common in IV admixture testing.[14] The same survey indicated that IV admixture testing is typically done in research and development laboratories, and rarely assessed in a GMP environment.[14] It therefore falls under the umbrella of development teams to understand the implications of polysorbate degradation on the in-use stability of IV admixtures. Development groups are responsible for understanding the proper amounts of stabilizers required in the formulation and guarantee that those levels are maintained throughout the shelf life of the drug product. Additionally, as shown in this work, the quality of the drug product may meet all quality attributes, but the extent of polysorbate degradation may be such that impacts to quality are only observed once the biologic drug is diluted in an admixture. Furthermore, degradation of polysorbate and its impact to IV admixture in-use stability should be considered early enough in development to allow for a proper specification setting discussion or adjustment of the formulation excipients to best suit the intended use of the product. A failure to identify polysorbate concentration as a critical quality attribute during development could result in incorrect specifications as well as an incomplete GMP release and stability testing strategy. For accelerated programs that might not have a batch of DP at full shelf life, the approach outlined in this work can provide critical information required to predict safe levels of excipients. In the study reported here, we determined that a threshold level of 0.0004% (w/v) polysorbate 20 must be present in the IV admixture for mAb1 in order to guarantee the solution meets the quality attributes necessary to provide a safe infusion for the intended clinical doses. In order to meet this requirement, the DP must have 0.035% (w/v) polysorbate 20 at release and >0.02% (w/v) at the end of shelf life. This threshold level of polysorbate 20 is not universal and every antibody (or other biologic drug) will require specific testing to determine this minimum level. For example, Kannan et al. studied an antibody that required 0.01% (w/v) polysorbate 20 to stabilize to agitation stres.[12]Sreedhara et al. compared three mAbs and showed that two were stabilized to agitation stress by 0.005% (w/v) polysorbate 20 but the third required higher levels such as 0.04% (w/v).[11] Strickley and Lambert reviewed commercially available antibody formulations and showed that the amount (and type) of surfactant greatly varied among products (from 0.04 −2 mg/mL for polysorbate 20).[29] Degradation of polysorbate (if observed) should be a considering factor in the decision on the level of polysorbate needed for the final formulation. For this study, super-refined polysorbate 20 was used. Use of super-refined polysorbate 20 is the standard practice at Regeneron due to increased quality of the reagent. However, this work may be applicable to any grade or form of polysorbate where degradation is observed on storage stability.

Light obscuration is considered the gold-standard for release and stability testing with respect to particles for drug products, and USP <788> outlines criteria for acceptable levels of particles. Although no industry standard exists, flow imaging is also an important assay for characterizing particle formation in biologic drug solutions because it is generally more sensitive[30] and can yield data on particle morphology which can help understand the nature of the particles and the degradation mechanism.[22] The current work demonstrates how the two techniques can complement each other. LO provided quantitative particle concentrations relative to an industry standard. FI not only provided a quantitative measurement of particles, but also showed how the nature of particle morphology changed with condition or stress. In this work, there was an obvious change in particle morphology as the level of polysorbate in the IV admixture dropped below a threshold level. LO might have some shortcomings as it may be less sensitive to transparent or translucent particles and cannot distinguish between intrinsic protein particles or non-protein particles that may be foreign or result from the components of the infusion set or container.[22,31] This work suggests that orthogonal particle analysis methods should be employed for development studies. This may indeed be common practice in the biopharmaceutical industry. When surveyed about practices regarding particle analysis of IV admixtures, many companies indicated that they use both LO and FI in biologic IV admixture development studies.[14]

IV admixtures should be tested with material that is close to or at the end of proposed expiration date. This is explicitly stated in European Union guidance documents[32,33] but not something explicitly outlined by the US Food and Drug Administration. This practice may be inconsistent among pharmaceutical companies. A survey among companies indicated that less than half of the companies surveyed include aged DP in IV admixture studies.[14] The strategy proposed here can both inform and complement studies on aged DP as we propose a method to simulate aging and the effects of degraded excipients on the quality of IV admixtures. This can be useful to set a product shelf life or a specification on the lower limit of polysorbate needed in the DP.

The study presented describes a way that development groups can determine the minimum amount of polysorbate required to maintain the in-use stability of IV admixtures in advance of the availability of drug product close to or at the end of shelf life. Understanding excipient degradation will be important in developing safe IV drug products.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.xphs.2023.08.020.

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