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Journal of Pharmaceutical Sciences 111 (2022) 887–902

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Special Topic Cluster

Stress Factors in Primary Packaging, Transportation and Handling of Protein Drug Products and Their Impact on Product Quality

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ARTICLE INFO

Article history:
Received 18 October 2021
Revised 15 January 2022
Accepted 16 January 2022
Available online 23 January 2022

Editor: Dr K Audus

Keywords:
Autoinjector
Biologics
Clinical administration
Foreign particle
Glass vial
Leachable
Protein aggregation
Primary packaging
Transportation
Syringe

ABSTRACT

Protein-based biologic drugs encounter a variety of stress factors during drug substance (DS) and drug product (DP) manufacturing, and the subsequent steps that result in clinical administration by the end user. This article is the third in a series of commentaries on these stress factors and their effects on biotherapeutics. It focuses on assessing the potential negative impact from primary packaging, transportation, and handling on the quality of the DP. The risk factors include ingress of hazardous materials such as oxidizing residuals from the sterilization process, delamination- or rubber stopper-derived particles, silicone oil droplets, and leachables into the formulation, as well as surface interactions between the protein and packaging materials, all of which may cause protein degradation. The type of primary packaging container used (such as vials and prefilled syringes) may substantially influence the impact of transportation and handling stresses on DP Critical Quality Attributes (CQAs). Mitigations via process development and robustness studies as well as control strategies for DP CQAs are discussed, along with current industry best practices for scale-down and in-use stability studies. We conclude that more research is needed on postproduction transportation and handling practices and their implications for protein DP quality.

Introduction

A vast majority of protein-based biologics are produced using recombinant DNA technologies in cell culture processes, and encounter stresses throughout the drug substance and drug product production.[1,2] Developing a stable, safe, and efficacious product starts with target identification in early discovery and progresses through all phases of development and manufacturing, finally resulting in administration of the commercial product to the patient. This manuscript is the last in a series of 3 papers describing the stresses to

Abbreviations: CQA, critical quality attribute; DP, drug product; DS, drug substance; EtO, ethylene oxide; FDA, US Food and Drug Administration; HSA, human serum albumin; IV, intravenous; PFS, prefilled syringe(s); PQA, product quality attribute; SC, subcutaneous; QCM-D, Quartz Crystal Microbalance with Dissipation; TPP, target product profile.

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[1] In Memoriam of Wim Jiskoot & Lloyd Waxman, colleagues, friends, and contributors to this manuscript who passed away during the revision phase

https://doi.org/10.1016/j.xphs.2022.01.011
0022-3549/

which a biotherapeutic is exposed throughout production. The first 2 focused on stresses encountered during the manufacturing of the drug substance, and those involved in the manipulation of drug substance (DS) into the drug product (DP) formulation and dosage form at the manufacturing facility.[1,2] In the final steps for getting drug to patients, the DP must be packaged (fill and finish) into a primary container or device as appropriate, stored, and distributed to the patient (either in the clinic or at their home), and then administered; this is the focus of the current manuscript. DP quality including potency may be impacted by external factors such as primary device material, container closure, product viscosity, and stress during transportation, and these should be taken into consideration during DP design. The drug manufacturer is responsible for the safety and efficacy of the final drug product throughout manufacture, distribution, and use. This includes ensuring safety and efficacy of the active ingredient (drug substance, DS), all DP formulation components, and the container or device used to deliver the drug to patient, whether it is something as common as a vial, or as complicated as an electromechanical injector.

Multiple factors during this process can result in changes to the critical quality attributes (CQAs), including protein modification and aggregation. Interactions between the protein drug and the surface of the container, or with leachables from the container, are among the known sources of protein modification and aggregate formation. If there is an air-liquid interface present, the protein can unfold at this hydrophobic surface, and then act as a nucleation site for the formation of protein aggregates as it is released from the interface back to the bulk solution. Transportation-related stresses such as agitation and bubble formation provide many opportunities for the drug to be exposed to these interfacial interactions, hence transportation studies should be included as part of the development process, as well as taken into consideration when developing formulations for commercial use. [3-6]

The broad set of primary container solutions include vials, syringes, cartridges, and ampules among others. The most common primary containers for protein-based biologics are vials and prefilled syringes (PFS). Vials and PFS are available as glass or polymer, however vials used for commercial products remain predominantly glass. Each of these container types have their own contributions to formation of protein aggregates/particles and other protein CQA modifications. Glass delamination through various routes has been shown to result in visible particles, which can sometimes contain protein, while protein oxidation can occur with time in air-permeable polymer containers.[7] PFSs commonly use silicone oil as a lubricant to allow the plunger to glide through the barrel for smooth delivery of the correct drug dosage, and this component can occasionally induce protein particle formation, or form droplets on its own.[8-10] Protein formulations usually include surfactant to prevent the formation of protein aggregates, and interactions of protein with these hydrophobic surfaces and components.

The formation of protein aggregates is highly dependent on the physicochemical and structural properties of the protein. In a few instances, the protein forms aggregate in the presence of silicone oil droplets.[10-12] In the majority of cases there is no evidence of increased protein aggregates, and any increase in particles can be attributed to an increase in silicone oil droplets.[13-16] If the device being used to deliver a protein therapeutic involves potential exposure to silicone oil, then the effect of silicone oil on the formation of protein aggregates should be assessed.

While a device can affect the product quality of the therapeutic, the characteristics of the therapeutic protein can affect the functioning of the device, and this also needs to be considered during development. For example, a very viscous formulated therapeutic could result in failure of the device to deliver the required therapeutic dose within specified time or conditions of use.

The downstream stress factors experienced by the finished product include protein destabilization during shipment and handling/manipulation/dose administration at the clinic,[17] as well as potential challenges due to interactions with leachables, and any exposed surfaces. To evaluate the stresses involved and the associated stability risk factors, the packaging materials as well as the steps involved in transportation, storage, handling and clinical administration need to be examined thoroughly as part of process development, including using scale-down and in-use stability studies.

General Background

Analytical methods to identify and monitor DP product quality attributes (PQAs), particularly the key leading indicators of aggregation and particle formation, throughout the product life cycle from manufacture to administration to patients are critical to ensuring safe and effective drugs. Potency assays should also be included, but are not the focus of this review. Examples of PQAs during development include: visible and subvisible particles, protein modification, clarity/opalescence, color, pH, protein concentration, purity, and potency. Many of these attributes are monitored using compendial methods, with detailed guidance and instructions available in the Pharmacopeia. Appropriate analytical methods for assessing the effect of the stresses of different manufacturing processes are discussed in United States Pharmacopeia (USP) chapters <1787>, <1788>, <1790>, and several excellent review articles.[2,18-22] For subvisible particle analysis, it is highly advisable to apply analytical methods that are sensitive to protein particles, which are often translucent and not well detected by the compendial methods (i.e., light obscuration). The panel of analytical methods used in development should include those that can also differentiate proteinaceous particles from other types of particles which may originate from the device or the formulation components.

The viscosity of the product should be measured at ambient temperature and at intended storage temperature, with careful consideration of the relevant shear rate, as these can affect the choice of both the device and the route of administration. It is also crucial for the development of the proper product handling procedure prior to administration by the end user at the clinic or at home. In addition to the effect on the CQAs of the therapeutic molecule, the functionality of the device used in the final DP should be assessed. The syringeability of the drug-device combination should be evaluated by measuring the force profile associated with expelling the formulated product from the syringe barrel using tests such as breakaway and glide force. Breakaway force measures the initial force required to begin plunger movement and the glide force is the average force required to continue depressing the plunger until the end of the injection.

Scope

The focus of this article is to critically assess the impact of primary packaging, transportation, and handling on DP quality. Container closure integrity, as well as a detailed discussion of analytical procedures including potency assays are out of scope, as is the manufacturing process and formulation considerations which were covered in the previous 2 manuscripts.[1,2] This paper captures the major risk factors to protein DP related to packaging, transportation, handling and administration. The discussions include potential negative impact of these risk factors on PQAs, and appropriate mitigations through process development and robustness studies.

Primary Packaging

The primary packaging materials used in protein DPs fall into three broad categories based on the container type: syringe, vial, and

Table 1
Materials of construction and leachables for different types of primary packaging.

Primary Packaging	Common Materials of Construction (Product contact)						Potential Leachables				
	Borosilicate Glass (type 1)	Halobutyl Rubber	Fluoroelastomer	Olefin Polymer	Silicone Oil	Stainless Steel	Wash Residues	Plasticizers	Polymer Fragment or Droplets	Tungsten Process Residues	Metal Ion Colorant (eg manganese and iron oxides)
Glass Vial	X	X	X				X	X	X		
Amber vial	X	X	X				X	X	X		X
Glass Pre-Filled Syringe – Silicone Lubricated*	X	X	X		X	X	X	X	X	X	
Glass Pre-Filled Syringe – Silicone Free	X	X	X			X	X	X	X	X	
Polymer Pre-Filled Syringe – Silicone Free		X	X	X		X	X	X	X		
Glass Cartridge*	X	X	X		X		X	X	X		
Polymer Cartridge		X	X	X			X	X	X		

* Note also that dual-chamber configurations may also be available for applications such as reconstitution of lyophilized product.

cartridge (Table 1). Another key component of primary packaging is the closure system, most commonly rubber stoppers. Both container and closure systems come into contact with the protein formulation. Therefore, selecting an appropriate material of construction of primary packaging components (Table 1) is an important consideration for product quality and stability. Stability of the combination of formulated drug product and container and closure systems during filling, shipping, storage and administration should also be assessed. This should include interactions that occur during shipping and storage between the therapeutic, the formulation components, and any packaging materials or leachates from the container/closure (CC) system they contact, as well as interactions at the air-liquid interface.

Table 1 contains a summary of materials of construction and leachables for different types of primary packaging. Some leachables are common across the packaging types shown, while others are associated with specific packaging types. Wash residues, plasticizers, and polymer residues are generally common to the systems shown in the table. These leachables can lead to degradation or aggregation of the drug product.[23] Fluroelastomer films within the primary packaging system can act as a barrier between drug product and source of leachables.[24] Examples include film coatings on plungers or stoppers to minimize drug product contact with plasticizers within the formulated synthetic rubber component. Latex is a potential allergen that can be present in rubber components of the system.[25,26] In order to minimize the potential allergenic effects of latex, efforts by manufacturers and trends within industry have favored reduction in latex systems. Detailed information and product examples for various configurations are available from the manufacturers such as Becton Dickinson, Schott, Stevanato, West Pharma, Daikyo, Dow Corning, and Gore.

This section describes the types of primary packaging used in injectable protein DPs, including glass vials, the most commonly used type of primary packaging, prefilled-syringes/vials (both glass and polymer-based, with and without silicone oil as a lubricant), on body devices, and other specialty containers. The major drivers for selection of the primary packaging to be used include assurance of container closure integrity, time available for development prior to commercialization, and the Target Product Profile (TPP). The TPP includes disease indication, product shelf life, and profile of anticipated users of the product. For each packaging type, the major stress factors on product quality are highlighted considering both historical knowledge and emerging data. Each section also covers recommendations during product and process development to mitigate these risks.

Sterilization

Before any primary packaging can be filled it must first be sterilized. Empty primary drug containers can be sterilized by one of several methods, including steam sterilization, Ethylene oxide (EtO)-mediated sterilization, nitrogen dioxide (NO_2), and radiation (electron beam [e-beam] and gamma irradiation).[27,28] Although it is not directly used to sterilize primary packaging materials, the vaporized hydrogen peroxide (VHP) biodecontamination process is a common sterilization method for isolated filling lines, and the residual VHP may adsorb to the outer layer of primary packaging materials (e.g., vials and stoppers). The VHP also can get into the product in the open vial prior to stoppering and have negative impact on the stability of the drug product (e.g., oxidation). The risk of VHP on drug product quality and corresponding mitigation strategies were covered extensively in part II of this series.[2]

It has been shown that the method of sterilization of polymer components can influence the respective extractables/leachables (E/L) profile quite significantly. Thus, we can expect that the amount and the kind of leachables from the primary packaging to vary according to how it is sterilized.[29]

Autoclave/steam Sterilization

Autoclave, or steam, sterilization is a widely used method for medical device and final packaging that can be performed with relatively low-cost equipment. Through this process, saturated steam is forced into a pressure chamber at a temperature range of 121-148°C (250-300°F) at 15psi for a predetermined period of time sufficient to provide sterilization. Although terminal steam sterilization is frequently used for low molecular weight drugs after filling into primary containers, biotherapeutics are generally sensitive to denaturation by heat, and are not compatible with this method of sterilization. Therefore, steam sterilization can only be applied to the packaging material (i.e., prefillable syringes and vials) prior to actual filling of a protein pharmaceutical.

A potential issue with steam sterilization is that it can alter the mechanical and chemical properties of the material being sterilized (e.g., softening). Therefore, high heat resistant material must be used with this process. High heat resistant plastic, such as polypropylene (PP), is ideal as the packaging can undergo the autoclave process multiple times before material properties are compromised. This is the reason that PP is the most widely used material for commodity plastics and parts that require autoclave sterilization. The autoclave cycle and its compatibility with a packaging material depends on the nature of the material, part geometry, residual stress of the plastics part and autoclave sterilization settings. One advantage of steam sterilization is that it results in lower levels of extractables in comparison with gamma sterilization (see below).

Ethylene Oxide Sterilization (EtO)

Ethylene Oxide (EtO) sterilization is another method that is often used, especially for plastic syringes. EtO sterilization is a gaseous method involving the highly diffusive, permeable and toxic EtO gas. EtO is processed at a low temperature and is generally mixed with other substances such as carbon dioxide (CO_2) or steam to destroy bacteria and other micro-organisms. EtO is frequently used to sterilize materials that are otherwise sensitive to heat or radiation sterilization. Due to the nature of the process, it is particularly suitable for medical devices containing electronic components and typical cycle times are between 24 and 48 hours. This technique requires thorough process control to eliminate any residuals on the sterilized components. Typical EtO sterilization processes involve several stages of gas removal; humidification, EtO exposure and air washes. Early studies showed that EtO has the capability of forming adducts with histidine, cysteine, or methionine residues.[30] The stability of therapeutic protein formulations in EtO-sterilized plastic vials was investigated and the authors found that residual EtO rapidly formed adducts with methionine residues in pegylated granulocyte colony stimulating factor and caused similar chemical modifications to human serum albumin (HSA).[31] Based on other studies, these types of changes in the protein CQAs could potentially have adverse effects in patients. For example, it was found that EtO adducts of HSA that were formed in dialysis machines at residual EtO levels below the limits set by the International Organization for Standardization could cause anaphylaxis.[32,33] EtO adducts, similar to other types of chemical modifications to proteins, may also promote immunogenicity in patients treated with protein therapeutics.[34-36]

More recently, EtO and steam-sterilized polymer syringes were compared to determine the effect of residual EtO on HSA degradation.[37] Although the amount of residual EtO in the EtO-sterilized syringes was below the International Organization for Standardization limit, EtO adducts to cysteine and methionine residues in HSA could be readily detected by liquid chromatography and mass spectrometry. The EtO adduct ratio of HSA stored for 2 weeks in EtO-sterilized syringes was about 45%, but no chemical degradation was observed in HSA stored in steam-sterilized syringes. Because of the reactivity of EtO with proteins, an alternative to EtO should be utilized to sterilize polymer-based prefillable syringes for use with therapeutic protein products.[37]

Nitrogen Dioxide (NO_2) Sterilization

Nitrogen dioxide is a sterilant gas that is frequently used in the terminal sterilization of medical instruments. Recently, the use of nitrogen dioxide (NO_2) has been shown to have several advantages over the traditional methods for sterilization of polymeric syringes,[38] including operation at room temperature, a relatively low sterilant concentration, and rapid anti-microbicidal activity. Another advantage of NO_2 sterilization is that it does not leave harmful residuals on the surface of the device being sterilized. Additionally, the NO_2 process is compatible with most medical device materials, including fluoropolymers. polypropylene, and cyclic olefins, which are frequently used in polymeric syringes.[39] The NO_2 sterilization process has been validated for the terminal sterilization of medical devices using typical sterilization chamber-based systems. However, due to the fact that it is a newer technology, NO_2 sterilization has not yet been implemented as part of most commercial biologic manufacturing processes.

Radiation Sterilization

Radiation sterilization can be performed using two types of radiation - non-ionizing radiation and ionizing radiation. Non-ionizing radiation uses a longer wavelength and lower energy. As a result, non-ionizing radiation loses the ability to penetrate materials, and can only be used for sterilizing surfaces.[40] The most common form of non-ionizing radiation is ultraviolet light which, due to aforementioned limitations, is not used to sterilize primary packaging for pharmaceuticals. Ionizing radiation commonly utilizes Gamma, X-rays, or E-beam. Gamma sterilization is an ionizing sterilization technique that involves exposing materials to gamma rays, most commonly Cobalt-60. Gamma irradiation is a popular sterilization method; it is estimated that more than 40% of all single-use medical devices are sterilized using gamma irradiation technique.

Electron Beam (E-beam) sterilization is a process that utilizes an electron beam to sterilize the product through a uniform dose of ionizing radiation. E-beam radiation is generally characterized by its relatively low penetration and high dose rates. In comparison, gamma radiation has high penetration and low dose rate.[41] Materials that can be gamma sterilized can also be E-beam sterilized and both technologies can give a reproducible and highly effective irradiation process.

Radiation sterilization is frequently employed for polymeric containers, with widespread use to sterilize disposable plastic syringes. However, it is well-established that irradiation of plastic generates free radicals. The exposure of a plastic to high-energy electrons results in a cascade of electrons through the material that interact with molecules within the polymer, ejecting electrons from their orbits and generating free radicals. It is primarily the reactions of these species that are responsible for cross-linking polymers to improve the stability of the device. However, residual free radicals from radiation sterilization could cause both protein oxidation and aggregation of protein therapeutics stored in PFSs. In contrast, no such damage was observed in the same syringes that were steam sterilized.[42] When the e-beam irradiation was replaced with steam sterilization the oxidation of the erythropoietin stored in syringes was abrogated.[43] Electron spin resonance analysis indicated a high level of free radicals in e-beam-sterilized syringes, which was not detected in steam-sterilized syringes; additional analyses confirmed changes in the chemical composition of the e-beam-sterilized syringes.[43] The half-life of the radicals generated by e-beam sterilization of the polymer syringes was not determined in this study, but it may be practical to store syringes prior to filling with DP to allow the free radicals to dissipate.

Summary and Risk Mitigation

As discussed above, each sterilization process has its strengths and liabilities. Heat sterilization can potentially affect the integrity and functioning of the device. EtO and irradiation can potentially change the CQAs of the DP, and might affect the stability, safety, or efficacy of the specific biotherapeutic. Understanding of the sensitivities of the therapeutic, and of the device required to deliver to the needs of the specific patient population, can be used to inform the choice of sterilization process. Studies such as testing in small scale models, and CQA and functionality assessment after exposure to the sterilization process chosen, should be considered during development in order to minimize risks to the product, and the patient to whom it is administered. Importantly, each protein should be evaluated on a case-by-case basis to identify the appropriate packaging system and method of sterilization to ensure stability of the biotherapeutic.

Primary Packaging: Glass Vials

Glass vials (see Fig. 1) are mechanically the simplest primary container in use, and also the one with the longest history of use for biotherapeutics. Formulated proteins do not usually interact with the glass surface, and the manufacturing process does not involve exposure to tungsten and other materials as discussed for the syringes below. The primary areas of concern for glass vials are glass delamination, and leachables/extractables, both arising from the glass

Figure 1. Glass vials in nest with tub.[44]

material itself. The stress introduced by sterilization of these containers was discussed above.

Delamination in vials Made of Glass

Glass particles or fragments can be generated as a consequence of handling during manufacture, washing or filling, as well as from glass-to-glass contact and from damage during shipping. Relatively large fragments (> 100 μm) are easily detected by current inspection methods for visible particles. It has been demonstrated, at least in mice, that glass micro-particles with adsorbed protein molecules have the potential to serve as adjuvants and enhance immune responses to proteins.[45]

While glass delamination is well-described in the literature, it is still not completely understood.[46,47] Glass delamination occurs when the top layers of a glass surface separate and flake off, typically as a glass ribbon-like particle (Lamellae) as thin as one micron and ranging in length from 10 to 1000 micron. This is at a scale nearly invisible to the unaided eye, which makes the detection of delamination difficult. However, changes to the glass surface occur well before the appearance of the lamellae, taking place over a period ranging from months to years.

Numerous factors can contribute to the tendency of glass to delaminate including the aggressiveness of the product formulation: basic pH (>7.0), high ionic strength (>100 mM NaCl), and buffers containing citrate can all contribute to this process.[48] Choosing a formulation that is suitable for glass can mitigate glass delamination. The length of time the DP is exposed to the inner surface of the container has a direct correlation with the potential to form lamellae during the product shelf-life as does storage at room temperature rather than at colder temperatures. Terminal sterilization can also affect glass stability,[48] and freezing protein products at -70°C was found to generate lamellae in the solution after thawing.[7] Conventional converting processes lead to inhomogeneities in the composition of glass on the surface particularly near the inner bottom region of the vial, and this region is highly sensitive to delamination.[49] However, processing the glass at lower temperatures during cane production and vial forming can reduce susceptibility to delamination.[49,50] By using enhanced processing techniques Schott has been able to reduce the tendency of delamination in a line of vials, Schott Vials DC (delamination controlled).

Glass Leachables

In addition to delamination, glass has the potential to release alkali-based substances into the DP particularly at high pH.[51] Among the major leachables are silicon, boron, and sodium. The minor ones include potassium, barium, calcium, and aluminum, depending on the specific glass formulation. The presence of phosphate anions in the formulation is particularly problematic because of their ability to form insoluble complexes with divalent metal cations present at the inner glass surface. The amount of such extractable ions generally depends on how the glass was manufactured and the temperature as well as exposure to high temperatures during sterilization.[51] Adsorption of the protein to such particles or binding of metal ions to the protein both have the potential to compromise the efficacy of the drug. The leachable/extractable profile of the vials when exposed to the product specific formulation (placebo) and the effect of these molecule on the DP, should be determined during development.

Summary and Risk Mitigation

Glass vials are the simplest of the primary containers, with a long history of successful use for biotherapeutics. Glass delamination can be minimized by avoiding formulations that are at neutral pH or contain citrate and other buffer components known to contribute to this process. If conditions favoring delamination must be used in order to ensure stable DP than use of specialized glass vials is an option.

Studies to determine the leachable/extractables profiles of the glass in the formulation can be used to determine if the specific DP is sensitive to interactions or modifications from any of the leachables found. If the leachables could potentially modify or lead to aggregation of the protein then use of another primary container, or adjustment in the formulation, should be considered. Understanding of the CQAs of your product, the TPP, including the route and method of administration, coupled with prior knowledge of the properties of the vial, should be used during selection of the primary container. This can also help determine any studies that need to be done to support the choice of glass vials for a specific DP.

Primary Packaging: Prefilled Syringes

The PFS serves as a convenient, dual-purpose primary packaging and parenteral delivery device option for many DPs, and has been the fastest growing choice for protein based therapeutics for several years with many advantages over vials.[51] However, there are a growing number of reports related to leachables observed in products stored in PFSs, and materials used in the manufacture of PFS systems including steel, silicone oil, tungsten, glass, plastic, and rubber have been shown to cause therapeutic protein aggregation and particle formation, and in some cases chemical degradation. For example, it is well established that silicone oil lubricants on the syringe barrel as well as on the rubber stopper can cause protein aggregation and particle formation (see below for a fuller discussion).[14,23,52] Furthermore, residual tungsten oxide species from the process used to form the hole to insert the needle for glass syringes can cause protein aggregation.[53,54] Also, chemical degradation has been shown to be promoted by residual radicals from light-cured glues used to fix needles in glass syringes,[55] as well as by leachables from rubber needle shields and syringe pistons.[56,57] More recently, concerns have been raised about the effects of residual components and byproducts from syringe sterilization methods on protein stability.[42,43] Three major categories of PFSs based on materials of construction are discussed below, highlighting their advantages and limitations related to impact on product quality.

Glass Prefilled Syringes

Silicone coated glass prefilled syringes (PFS, see Fig. 2) have been on the market for many years. This section will explore silicone oil –

Figure 2. Syringe with flange extender, plunger rod, and rigid needle shield.[58]

protein interactions, as well as the impact of formulation, extractables/ leachables, and sterilization on protein stability in glass PFS. It is important to note that delamination has not been observed in PFS made of glass.[59] This is due in part to the use of Type IB glass, which allows for lower manufacturing temperatures, and the silicone oil coating on the inner surface of the PFS, which protects the glass from long term exposure to formulation components.

Needles are also coated with silicone oil to reduce frictional resistance when penetrating tissues.[60] This helps to decrease the pain typically associated with injections.[61] However, whether the small amount of silicone from the needle could have an impact on drug stability has not been adequately addressed.

Silicone Oil Interactions with Protein. Silicone oil (polydimethylsiloxane, PDMS) is used as a coating on the interior of glass PFS due to its low surface tension and hydrophobicity that improves plunger glide forces, allowing smooth and complete delivery of a protein DP. Medical grade silicone oil or emulsion (e.g., Dow Corning™360, 365 and 366) is coated onto the syringe barrel and rubber stopper to maximize lubricity. The coating is typically applied through spray-on methods and either simply allowed to dry or can be baked on.

It is important to consider the potential impact of the silicone coating on the DP. Silicone can detach from the syringe surface and move into the formulation, causing formation of droplets in the DP, particularly under stress conditions such as agitation or freeze thaw. A high background of silicone oil droplets can make it difficult to quantitate foreign particles and protein aggregates, especially using the compendial light obscuration technique for subvisible particle (approximately 2-100 micrometers).[13,62,63] When evaluating particles, in addition to counting them it is important to understand their etiology, and to be able to differentiate between protein particles, silicone oil droplets, and other types of particulates. The particles must be differentiated by type to truly determine if silicone release is impacting protein stability. Multiple reviews, and several USP informational chapters <1787>, <1788>, <1790>[19,64] have described the various analytical methods available for particle identification and characterization. Proteins are amphiphilic and tend to interact with surfaces, including hydrophobic silicone, providing an opportunity for conformational changes and aggregation.[10-12] Aside from removing therapeutic protein from the formulation and losing functionality, these protein aggregates also form particulates and have been associated with immunogenicity which can be a safety concern.[12,63] Many protein DPs, however, are packaged in silicone-coated PFSs and have been on the market for years without significant incidents occurring that have been attributed to the presence of silicone oil.[10] Furthermore, recent studies did not show enhanced immunogenicity of a monoclonal antibody in the presence of silicone oil droplets.[14,15,65] However, additional work is needed to understand the effect of silicone oil on proteins and immunogenicity.

Visible and subvisible particulate levels in therapeutics must be kept within regulatory guidelines (e.g., USP <787>, USP <788>, and USP <790> and the EP2.9.19, and other pharmacopeia chapters). Silicone oil droplets in solution are most often detected as subvisible particles between 1 and 25 μm,[13,15] but could potentially reach sizes large enough to be seen during visual inspection. A recent study found silicone oil does not form a significant number of submicron particles in PFSs[66] in the various therapeutics tested. Regardless of the size, minimizing silicone desorption and protein aggregation associated with hydrophobic surface interactions is critical to reducing particle formation.

Additionally, the appearance of particles in protein formulations is often related to applied stress factors such as storage temperature, agitation and freeze-thaw cycles.[15] Agitation increases the forces that can desorb silicone from surfaces as well as protein contact with the air/water/surface interfaces, causing unfolding. Other parameters impacting the number of subvisible and submicron particles in each formulation include DP formulation ingredients, air-water interface, freeze-thaw, and characteristics of the specific protein therapeutic, as well as the concentration and volume of the silicone oil coating, and the coating application method.

As the volume and concentration of silicone coating increases, the number of particles released into the formulation also increases.[13,15] The method of application to the syringe is also critical. In one study, spray-on, baked-on and crosslinked silicone coated syringes were evaluated.[15] Baked-on and crosslinked silicone released fewer particles than the spray-on silicone coating. It is hypothesized that the increased molecular weight, and hence viscosity, of the silicone from baking or crosslinking reduced the ability to slough off into the DP.

While interaction of protein with silicone oil may affect protein stability, other formulation ingredients may also affect stability. The addition of surfactant (e.g., polysorbate 80) that is used to stabilize proteins against interfacial interactions that can lead to aggregation has been shown to increase the number of silicone oil particles in a formulation, suggesting surfactants emulsify the silicone and pull it into suspension.[15] The concentration of protein particles was reported to decrease in the presence of polysorbate 80 after drop shock treatment, while the silicone oil particle concentration increased.[67] In this case, the surfactants reduced protein aggregation even while liberating silicone into the formulation.[67] Other studies, several using quartz crystal microbalance with dissipation (QCM-D), have shown that the presence of surfactant reduces the adsorption of protein to silicone-coated surfaces,[15,68-72] suggesting surfactant

protects the protein from adsorption. In these studies, the surfactant was only effective if added prior to or concurrent with the protein. Protein pre-adsorbed to the siliconized surface was not significantly displaced by surfactant. The surfactant presumably preferentially interacts with the hydrophobic siliconized surface and protects the protein from binding. Surfactant addition must be balanced with the potential to liberate silicone from the surface via emulsification.

To reduce particle formation, whether due to silicone droplets or protein aggregation, in siliconized PFSs, it is recommended to:

- Reduce the volume and concentration of silicone loading onto the syringe.
- Increase silicone molecular weight and substantivity by baking or crosslinking.
- Include surfactants to improve protein colloidal stability and compete with the protein for silicone surface interactions.
- Reduce head space to decrease the air-water interface during agitation and shipping.

Tungsten and adhesives. In addition to silicone oil, other materials present on the glass PFS can affect product CQAs. The process generally used to fix the needle into a glass syringe requires heating a tungsten pin to high temperature (>1200°C) to form the channel for insertion of the needle. Tungsten readily oxidizes above 400°C, and this can result in the deposition of various tungsten polyanionic species primarily in the funnel region of the syringe barrel.[54] If not properly washed from the syringe these tungsten species can result in aggregation of some sensitive proteins, particularly at pH below 5.[53,73] This was implicated in an increase in visible particles seen with one product.[54,73] In another example, the increased immunogenicity of an epoetin biosimilar, and the development of pure red blood cell aplasia at the end of a clinical trial, was attributed to tungsten-mediated protein denaturation and aggregation in a small number of individual syringes.[74]

Several suppliers of glass syringes have minimized this problem by offering prefillable syringes that have been extensively rinsed with water to remove tungsten-associated residues. Other strategies such as replacing tungsten pins with other materials or forming the needle hole in the presence of decreased atmospheric oxygen have also been used. Although tungsten-induced aggregation appears to be protein specific, it is important to leverage prior knowledge or to test for protein-tungsten interactions at an early stage of drug development.

UV activated adhesives are frequently used to fix the stainless-steel hypodermic needle to the glass barrel. Acrylic acid used in some adhesives has been detected as a leachable and shown to react with proteins stored in PFSs.[75] In other studies, an incomplete UV curing process led to an unexpected impurity found in a new staked-in needle PFS presentation for a biological product which had not been previously observed when a luer cone PFS was used.[76] Process improvements aimed at controlling the adhesive formulation, application, activation, and curing as well as cleanup have been identified as potentially important steps to prevent these materials from leaching into the drug formulation.

Polymer-based Prefilled Syringes

Polymer-based syringes (see Fig. 3) offer an alternative to glass. A plastic prefillable syringe system can eliminate the need for silicone, tungsten, and adhesive, depending on the quality attributes of the entire system. The CZ insert needle system, for instance, uses no silicone for syringe functionality, no tungsten (used during the glass syringe forming process), and no adhesive (used to fix the needle in place in glass syringes).

Generally molded from cyclic olefin copolymer (COC), these syringe systems can offer attractive structural properties including avoidance of fracture and breakage, but may require additional considerations to ensure compatibility and stability of drug product. Permeability of the resin (and subsequent oxygen exposure of DP) and secondary effects of sterilization are among those considerations.

When the degradation of the oxygen-sensitive protein erythropoietin in polymer-based syringes was examined two sources of protein oxidation were identified.[35,43] The first originated from dissolved oxygen due to the higher permeability to gases in these syringes than in ones made of glass. This could be mitigated by leveraging the permeability characteristics of the polymer syringe with oxygen-scavenging technology available from several manufacturers. A deoxygenated packaging system was used consisting of an oxygen absorber inside the secondary packaging, such as a pouch or bag, along with the filled syringe. In the presence of the oxygen absorber, the concentration of dissolved oxygen decreased rapidly just after packaging and continued to drop over time; after eight weeks, the concentration of dissolved oxygen was close to zero. The combination of polymer syringe, the deoxygenated package system and oxygen absorber were shown to be effective in preventing protein oxidation.[35] The osteoporosis drug calcitonin has been successfully marketed in Japan since 2002 in a prefillable syringe made of Daikyo CZ polymer wrapped in a light-protected package containing an oxygen scavenger, and polymer syringes stored in nitrogen-filled aluminum pouches were shown to present a promising alternative for the storage of oxidation-sensitive biopharmaceuticals.[36]

Figure 3. Pre-filled syringe with flange extender, plunger rod, and rigid needle shield.[77]

Silicone-free Prefilled Syringes

The potential negative impact of silicone oil droplets on the DP, including the CQAs of the therapeutic, were discussed in section 2.3.1.1. Several strategies to avoid protein degradation caused by silicone oil include the use of alternative materials and/or modifying the surfaces of the plunger or syringe barrel to eliminate the need for oil-based lubrication to function. Currently, there are two silicone oil-free, commercially available syringes for biologics. Both consist of polymer-based, plastic syringe barrels: PLAJEX syringes (Terumo Medical Corp., Somerset, NJ) use a plunger with a polymerized silicone coating and Crystal Zenith syringes (cyclic olefin polymer) use FluroTec coated plungers (Daikyo Seiko, Ltd., Tokyo, Japan).[78] Silicone free glass syringes are available but as of yet there are no commercial products using these primary containers. A comparison of the pharmaceutical compatibility of two protein therapeutics with PLAJEX plastic syringes, silicone oil-free glass syringes, and siliconized glass syringes found the rates of aggregate and subvisible particle formation of an Fc-fusion protein and a monoclonal antibody during agitation were much slower in both the bare glass and PLAJEX plastic syringes than in the siliconized syringes.[8]

Another study tested a novel fluoropolymer surface developed for use in glass syringes that are not siliconized to understand the impact of the fluoropolymer and glass container/closure system on the aggregation of therapeutic proteins in PFSs and vials during quiescent incubation and during agitation stress.[79] The fluoropolymer surface of the syringe stopper is designed to cover the rubber and directly contacts the pharmaceutical formulation. Additionally, the fluoropolymer surface provides solid-phase lubrication to facilitate the movement of the syringe plunger against an unsiliconized glass syringe barrel to deliver the drug to the patient. Aggregation and particle formation of intravenous (IV) immunoglobulin during agitation were compared to those obtained in typical siliconized glass PFSs and shown to be much lower during agitation with the fluoropolymer surface than with the siliconized surface.[79]

The development of prefillable syringes that do not need silicone oil for lubrication offers advantages because potential problems with oil droplets shed into the formulation are avoided. As the latter study showed, agitation of protein formulations in contact with the fluoropolymer surface promotes less aggregation than when the surface is siliconized and does not contribute silicone particles to the product. However increased permeability of these materials could result in increased exposure of the DP to oxygen and other atmospheric components. As these technologies are newer, more studies will be needed to determine if there is any long-term impact on protein stability.

Summary and Risk Mitigation

The use of PFSs is convenient for patients and clinicians. While silicone coated glass syringes have been safely used for many years, the impact of silicone oil on protein stability needs to be evaluated for each drug product. The process to coat with silicone (baked instead of sprayed) and formulation ingredients (e.g., surfactants) may be useful in mitigating protein aggregation.

Advances in pre-filled syringe systems to address known shortcomings have provided drug manufacturers with options to fit the TPP and the needs of both users and product. Advances in manufacture and materials of construction have resulted in more optimal control or elimination of destabilizing stressors from materials (such as tungsten oxides or silicone oil) enabling administration of a broader set of drug product. These options do require risk benefit consideration such as the potential destabilizing stressors of traditional lubricated glass syringe systems, novel potential stressors such as permeability and free-radicals, and overall fit and performance of each system in the intended fill network and final drug product delivery system where appropriate. Mitigation of risk includes consideration of the device to be used as early as feasible, and early vendor engagement, to enable predictive analysis or experimental characterization of performance. It is vital to be familiar with all of the steps in the device manufacturing process of vendors, and to consider how these could impact the DP.

Primary Packaging in Handheld and on Body Devices

As the industry has evolved to meet patient needs, devices such as autoinjectors (see Fig. 4 for an example) have been developed that allow patients to self-administer their drug. Self-administration and use of autoinjectors can introduce additional stresses to the drug product during distribution and drug delivery. For example, to ensure usability by the patient, health care provider, or caregiver, autoinjectors commonly complete delivery within less than 15 seconds while held against the abdomen or thigh. Delivery of the drug product within this time limit may require elevated forces and can therefore

Figure 4. Hand-held autoinjector.[80]

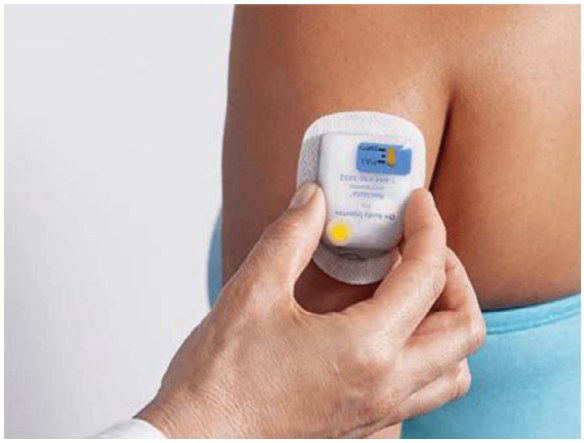

Figure 5. On-body device.[81]

result in elevated impact loading or fluid shear stress of up to 150,000 sec^{-1} on the drug product during injection. Distribution can introduce other stresses associated with protein aggregation including subjecting drug product to a wider range of temperatures or increased light exposure as they are placed in the patient's home or daily life environment. The most challenging of these are the on-body devices that involve multiple surfaces for protein interaction, can include positive-displacement pumps and other features for delivering the drug, and prolonged exposure to physiological temperature. Two commercial examples are shown in Fig. 5, the autoinjector YpsoMate and the Neulasta OnPro on body device. This complicated array of conditions and its potential impact on product quality will be discussed in the following section.

Physical Design

On-body devices represent a unique set of environments for use, storage, and delivery of the DP. On-body devices provide a more enduring interaction with the patient and the patient's body. Whereas PFS and pen-style injectors are configured to be held and operated by hand with a pen-like or similar grip, on-body devices are configured for discretion when worn on the patient's body. This generally leads to a final configuration which is more closely fitting to the patient's body. Commonly these are designed to be adhered directly to the body at the abdomen or on the back of the patient's arm, where placement and removal are convenient, and where the device can be worn comfortably and discretely during the activities of daily living. For example, targeting a device profile which conforms to the body provides a lower likelihood of striking the worn device on other articles of clothing or stationary objects which are encountered throughout the day.

Syringe or syringe-based pen-style injectors generally integrate the injection site cannula directly into or onto the container by a bonding or assembly process. This usually involves both a cannula with a short length with reduced ullage, and a minimal number of fluid path connections between the reservoir and the injection site. In contrast, for the on-body type device, the cartridge or syringe reservoir and the cannula at the site of injection are frequently located at a substantial distance from each other, and connected by a configuration of hypodermic or polymeric tubing. While this achieves the desired conformed profile, it also lengthens the fluid path between reservoir and injection site. This may also introduce additional materials of construction such as select polymers, and likely introduces additional fluid connections. These additional lengths, materials, and fluid connections can be thought of as additional surfaces of interaction between the device and the DP, and may introduce additional surface properties and surface energies for consideration when assessing compatibility of DP and the delivery system.

Prolonged Environmental Exposure to Temperature and Agitation

Body-worn devices are designed to have a body-conforming profile, are commonly adhered directly to the body, and are generally in contact with the body for a longer duration than pre-filled syringes or pen-style injectors. Whereas the common time a syringe or pen-style injector is held may be measured in seconds, on-body style injectors are frequently in contact with the body and exposed to environmental conditions for a period of minutes, hours, or even days.

Therapeutic proteins commonly have time-dependent sensitivity to increasing temperature.[82,83] Adhering on-body injectors directly to the body creates a more intimate thermal connection between body and device, and the potential addition of clothing isolates the device from ambient environment. Thus, the on-body device and the medication inside can have prolonged exposure to temperatures closer to that of the body as durations extend. Where sensitivity to thermal exposure is high, selection of materials for thermal conductivity and configuring materials for response to radiant heating can favorably influence the time to achieve equilibrium with the body.

Proteins are known to have sensitivity to agitation as well as temperature.[84] When body-worn, increased environmental stresses include increased agitation as the wearer goes about their activities of daily living. As the length of time the device is worn increases, so does the magnitude of total exposure to these stresses. These differences, when compared to vials, PFS, and pens, should be considered during product development and selection of device for administration of the drug, and included in the comprehensive characterization of product quality.

Summary and Risk Mitigation

The development of handheld and body-worn autoinjectors have brought improvements which increase the use of self-administration and continue to optimize user experience, delivering to the TPP and increasing patient compliance. The benefits gained by these injection systems bring additional considerations to ensure product quality. Product quality characterization must include all contacting materials in the fluid path and primary container, the lengthened duration of product contact with materials of construction, and consideration of extended environmental exposure to physiological temperature, and agitation of the DP. The sooner the TPP and patient population for the drug is identified, the more device material compatibility assessment can become part of the candidate selection process, and device options and potential incompatibilities should be considered during early development studies. This allows the requirements for the proposed device to be included in studies on protein compatibility and stability, and to be considered in the selection of the final molecule to move into the clinic.

Primary Packaging: Closure Systems

Container closure systems refer to the sum of packaging components that together contain and protect the dosage form. The primary purpose of a closure system is to keep the drug product isolated from potential external contaminants. Closure systems typically include screw caps and rubber stoppers, or the plungers for PFS.

Rubber formulations are composed of the elastomer and typically contain a wide variety of low molecular weight chemical components including a curing agent, an activator, a filler, and additional compounds to control cure rate, color, and resistance properties.[51] All of these will contribute to the physical and chemical characteristics of the final product. Leaching of these ingredients can occur when the DP contacts the rubber closure. Some examples of rubber

components which have been found to leach into the DP include 2-mercaptobenzothiazole, aluminum, nitrosamines and zinc.[51] Metal ion interactions with monoclonal antibody therapeutics has been shown to cause fragmentation.[85-88] To reduce the problems due to leachables, laminates or coatings can be applied to the product contact surfaces of closures. A coated rubber closure consists of monomers applied directly to the rubber, which are then polymerized and bonded during processing. A laminated rubber closure consists of a polymeric coating applied to part or all of the closure as a laminated film. However, the two terms are frequently used interchangeably to describe rubber closures that do not require siliconization. Coatings can perform one or two primary functions, (a) to serve as a barrier between the stopper and the DP to reduce leachables and extractables, (b) to eliminate the requirement of silicone for processing, lubricity and machinability.

Other coatings are also available that provide a barrier between the product and the stopper formulation but still require siliconization or may contain silicone. These stoppers do not require additional siliconization for processing and reduce particle and extractable silicone in the finished product. An inner Flurotec® coating can also be used.

In addition to leachables, coring of rubber stoppers[89] is a concern for injectable products packaged in vials with a rubber stopper width of 2 mm or 4 mm. To extract product from a vial, the rubber stopper is punctured using a needle or a canula at an angle of $45^\circ - 90^\circ$; to reduce the particle formation as a result of stopper coring, using a 45° puncture angle and a blunt needle is recommended.[89] Coring is also observed when closed system transfer devices (CSTDs) are used for drug product extraction. Coring occurs when the penetration of the rubber closure system, for example inserting a needle through the vial stopper to withdraw drug into the syringe, pushes a plug of material into the container, and the DP. Coring can introduce extrinsic visible particles into the drug product vial during clinical administration and may pose potential risks to patient safety.[90-92] Stopper push-in has been demonstrated to be dependent on multiple factors including but not limited to spike design, spike surface, stopper properties and stopper design.[92]

Summary and Risk Mitigation

During the biologic drug-device development process, it is important to demonstrate compatibility between the formulated drug and the container-closure system by conducting scaled-down studies to mimic direct contact between the product and the container/delivery device. A key part of these studies is the development and implementation of benchtop models along with standard analytical methods and other appropriate, fit-for-purpose assays to monitor physical and chemical instabilities of the protein as well as protein adsorption to the primary container.

In recent years, the industry has become more aware of the role of 'spiking' studies, where the formulated drug substance is exposed to a full range of direct and indirect product contact materials inherent in a container-closure system. The purpose of these studies is to see whether any components of the selected containers/devices that have potential to contact the product can affect product quality and CQAs through unwanted interactions or by leaching substances into the solution. A comprehensive characterization of leachable profiles and coring behavior during drug product development will inform on potential incompatibilities with the biologic and will facilitate a data-driven decision on the container closure system configuration.

In addition to the above scaled down and spiking studies, ICH Q5C (reference: ICH Topic Q5C Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products) also recommends that stability studies should include samples that are maintained in the inverted or horizontal position in full contact with the closure. Data generated from these samples along with the stability studies from upright vials are used to understand the impact of closure systems on the CQAs and to support shelf life.

Since it is imperative to understand the container closure and DP interactions prior to market authorization, several studies such as leachables, stability and impact to product quality are conducted during later stages of development, i.e., during pivotal clinical trials. It is hence advisable that container closure configurations are not changed after this point, as that could lead to significant delay in filings due to the lack of relevant data packages.

Table 2
Attributes to be considered for deviceability and route of administration for candidate selection

Parameters	Criteria
Indication	Oncology, metabolic, etc.
Modality	mAb, ADC, bispecific, multispecific, siRNA, cell and gene therapy, synthetic
Dose	0.5 to 10 mg/kg
Presentation	Liquid, Lyophilized, Frozen, Suspension, Gel etc.
Protein concentration	0.5 to 200 mg/mL
Route of administration	IV, IM, Subcutaneous, Oral
Patient population	Pediatric, Adolescents, Adults
User population	Healthcare providers, caregivers, adult patients, adolescent patients
Drug Delivery Devices	Hypodermic needle, pen/autoinjector, nebulizer, large volume and high viscosity injectors

Combination Products

The definition of a combination product is a therapeutic that combines two or more products that is regulated and sold as a single entity. For biotherapeutics this is usually a combination of therapeutic protein and delivery device, to form the final DP. Successful development of a combination product requires a cooperative effort by a team of drug product, medical device, combination product, quality, manufacturing, clinical, and regulatory experts. Proper integration of drug and device relies on understanding the intricate relationship between the biologic and the device, clear understanding of the TPP and patients' needs, accurately defining CQAs and product requirements, and early establishment of clinical and regulatory strategies, as well as careful consideration of inherent variations in a device due to its unique manufacturing process.

The deviceability (Table 2) of a new development candidate can be assessed as early as during the candidate selection stage provided the dose is locked in to define specific areas of risk and how they can impact product quality, suitability for use with specific devices, and patient satisfaction.

In order to ensure the proper-functioning of all the elements of a combination product (e.g., drug, primary container, and injection device), the interdependence of the device, biologic molecule, formulation, and patient and/or end user, including any physical and chemical incompatibilities, needs to be characterized as soon as target product concentration, fill volume, and container-closure candidates are chosen. This should involve the use of the appropriate benchtop models and analytical tools. Therefore, it is critical to study the interactive effects of the molecule and the delivery system as early as possible in the development process in order to define the design space for the combination product and characterize its manufacturing and delivery process. Table 2 lists some of the attributes to consider for different devices and routes of administration. Some examples of the key parameters for evaluation include: physical/chemical stability of the biologic, required delivery volume, viscosity of the drug product, dosing regimen, and product contact materials. Generally speaking, benchtop risk evaluation during device development involves the following four experimental categories: deviceability, leachables

and lubricants, physical instabilities, and material-of-construction compatibility.[93]

Given the fact that a combination product is both a drug and a device, any effects that the drug product may have on the drug delivery function of the device should also be evaluated. Just as importantly, any successful benchtop evaluations should be followed by an assessment of effect of the fill-finish process on the characteristics of the drug product as part of an overall risk mitigation strategy during biopharmaceutical device development. For these studies, the ability to access lab scale or pilot scale fill-finish equipment is a significant advantage; given that they provide the opportunity to develop the product and the manufacturing process simultaneously.

Transportation and Handling of Primary Packaging

The final steps in delivering drug to patients involves filling into vials or PFSs, which are labeled and packaged for transportation to various depots across the globe (depending on where the product has been approved for use). Liquid DP vials typically contain significant headspace, and the protein is susceptible to interfacial stress and related physical instabilities during transportation.[94] The American Society for Testing and Materials (ASTM) document "Standard Practice for Performance Testing of Shipping Containers and Systems" (ASTM D4169-98) is recommended by the USP general chapter <1079> for the evaluation of transportation risks and performance of DPs. Transportation risks such as vibration, shock and changes in pressure and temperature for liquid protein DPs have also been discussed previously.

Several strategies are used by various pharmaceutical companies to understand transportation risk. Representative DP development strategies include conducting small-scale agitation studies to understand the physical instability issues at the interface.[95,96] While proteins are surface active and can unfold at the air-liquid interface, non-ionic surfactants such as polysorbate 20, polysorbate 80 or poloxamer 188 are widely used formulation excipients to overcome these interfacial challenges.[97] These surfactants also protect protein products during transportation where they undergo some amount of agitation. Product development studies to understand the minimum amount of surfactant required to protect the protein against interfacial stresses also need to consider the fact that surfactant degradation can occur over the shelf life of the product[98] and that the remaining surfactant may not provide ample protection during transportation. It is thus important to understand the mechanisms of surfactant degradation and its impact on protein product quality throughout the product shelf life (described in the previous manuscript in this series).[2]

PFS carry unique challenges during transportation. Typical syringe products contain silicone oil as the lubricant and several reports have indicated interactions between the protein and silicone oil, as discussed above. Additional risks to PFS include pressure induced changes during air transport that could impact the plunger leading to sterility challenges. Piston movement during air shipment in both glass and plastic syringes have been evaluated (West Pharma report[99]). This study indicates piston movement in both glass and plastic syringes occur during non-pressurized air transportation, although they happen under different pressure conditions. However, the results from this study indicate that piston movement could result in microbial ingress in DP PFSs and needs to be carefully evaluated.

A recently published article regarding transportation induced shock and particle formation in various parcel shipments of protein DPs reported that shock ranging from 8 to 36G could lead to particle formation in some products[4]. A clear interaction between shock and vibration was reported in this study, emphasizing the need to understand physical stability of protein DPs as a result of shipment stresses. Interestingly, it was previously shown that mechanical shock of glass vials led to cavitation, protein aggregation and particle formation.[5] These studies and related literature clearly indicate that there is a greater need to understand various mechanisms of shock related instability of protein DPs. Small scale models that mimic such stresses are needed since it is difficult to conduct all these studies at scale for all products, and more importantly to come up with mitigation strategies to ensure that DPs supplied to clinical sites and pharmacies are safe and efficacious.

Generally, it is believed that lyophilized DPs are more stable and have longer shelf life than their liquid counterparts. However, lyophilized monoclonal antibody formulations have also been shown to be susceptible to shake stress.[100] The lyophilized product was susceptible to cake collapse prior to reconstitution, and subvisible particles and increased turbidity after the stressed material was reconstituted. This study highlights that certain mechanical stresses potentially encountered during shipment of lyophilized DPs can result in physical instability of the protein post-reconstitution.

Lastly, product impact due to potential X-ray irradiation during and after transportation is a possible concern to protein DPs. X-rays have high energy due to their shorter wavelength and can ionize water molecules and generate radicals that are able to break covalent bonds. The impact of X-ray radiation on small molecules has been reported.[101] The X-ray doses used in this study included 0.34 mGy (corresponding to thrice the dose from typical scanning in X-ray inspection equipment), 0.1 Gy (the limit specified by the food sanitation law in Japan), 0.5 Gy (a dose that has severe effects on blood cells) and 300 Gy (the maximum dose from our X-ray equipment). Exposure to X-rays did not affect the product quality of the drug (chemical modification or aggregate formation). The samples exposed to X-rays exhibited almost the same profile in formulation tests (dissolution test, disintegrating test, and hardness test) as control samples (0 Gy). The combination of X-ray exposure with accelerated temperature and humidity tests (six months) also did not affect the pharmaceutical quality. The color change of light-sensitive drugs (nifedipine and furosemide tablets) after X-ray exposure was negligible (< 1.0). In contrast, tablet color was remarkably changed by light from a D65 lamp. Impact to a lyophilized and room temperature-stable rFVIIa formulation due to air transport and X-ray radiation exposure through airport security was reported, although no negative impact was noticed in this study at the two doses tested (400 microSv and 2000 microSv). Limited data is available on proteins, but they are expected to show similar stability to X-ray exposure.

Summary and Risk Mitigation

DP products are constantly shipped from manufacturing sites to several countries across the globe. DP faces significant stability challenges during production, storage and shipping and it is the role of development scientists to understand various routes of degradation and risk mitigation steps. Mechanistic understanding of the various degradation routes that can occur during shipping is critical to ensure that the highest quality DP is available to the patient.

Surfactant degradation can occur during real time storage of biologics DP. Depending on the mechanism by which surfactants degrade in the DP vials (i.e., hydrolytic or oxidative), the impact to protein product quality can be as varied as aggregation or oxidation to sensitive amino acid residues and needs to be evaluated on a case by case basis. Since surfactants are primarily added to protect the protein against interfacial stress, especially during transportation, it is rather critical to understand the impact of the remaining surfactant and its degradation products on the CQAs of the biotherapeutic. Studies involving surfactant degradation products and small-scale stress models could potentially be a useful tool during development studies and be employed for risk mitigation. This is discussed in more detail in the second paper in this series.[2]

While literature is limited, no negative impact of the X-ray inspection on liquid biological DP has been reported so far. This may be due to the low-energetic X-rays and very short exposure (around 250 ms) duration as the common standard during inspections,[102] indicating that X-ray exposure may not be a high stress condition for protein DPs. Similar to the small-scale models to understand product quality impact of shock and vibration, we believe that teams should also consider studies to understand exposure of the DP to limited amounts of radiation. Such models are currently lacking and potentially useful for a mechanistic understanding of radiation mediated DP degradation.

In summary, transportation stress for protein DPs in their primary packaging (such as vials and pre-filled syringes) need to be carefully considered during clinical and commercial production. Although small scale models are not a complete replacement of at-scale transportation studies, especially as a product moves into the commercial space, they are immensely useful for helping to increase our understanding of the impact of shock, vibration and radiation on product degradation since not every study can be conducted at scale.

Drug Product Handling in the Clinic

Protein DPs are typically presented as a lyophilized or liquid formulation with either an IV or subcutaneous (SC) route of administration. Biologics are susceptible to degradation during dose preparation and administration due to several factors,[17] including (but are not limited to) material incompatibility with the drug compounding and administration components, interfacial and agitation stresses during administration, and thermal stress due to potential mishandling of the final DP in a clinical setting. Table 3 lists a summary of issues (not an exhaustive list) observed during dose preparation and administration of DP for IV and subcutaneous routes of delivery.

Intravenous (IV) Administration

For an IV route of administration the biologic can be injected neat or diluted with a vehicle to achieve the final clinical dose.[103] A typical protein DP development cycle will include an extensive compatibility evaluation with the anticipated administration components such as syringes, needles, IV bags and infusion lines (with or without filters), and IV diluent (saline, dextrose etc.) with the intent of ensuring stability of the biologic over the course of administration;[104] stability to ambient temperature during the length of IV delivery should be part of these studies. The IV administration bag and lines are made of various plastics including polyethylene, PVC, EVA (Ethyl Vinyl Acetate) etc. In-line filters can be used to filter out any particles that are formed (and are invisible to the naked eye) due to DP incompatibility with the materials of administration. In-line filter materials are varied as well including cellulose acetate (CA), regenerated cellulose (RC), polyethersulfone (PES), polypropylene (PP), polytetrafluoroethylene (PTFE), nylon and polyvinylidene fluoride (PVDF), and others. In-line filters are highly effective at particle removal especially for multi-drug infusions.[105] Usage of incorrect in-line filter with the DP can often result in adsorption of DP on filter membranes and the possibility of incorrect dosing especially for low dose infusions.[106] The chemical composition, chemical nature (cationic, anionic, non-ionic), electrostatic forces (hydrophilic, hydrophobic), filter pore size and surface area of the filter membranes should be considered along with DP formulation components (buffer, pH, ionic strength) before choosing an in-line filter. Health care professionals should carefully consider the manufacturer's instructions before deciding on the use of in line filtration during IV administration. The filtration process itself is also a source of stress to the biologic, and could result in aggregation or modification of CQAs. Before using an in line filter the effect of the filtering on the DP that the patients receive should be studied, to demonstrate no adverse effect on CQAs, dosage amount, components, etc.[107]

Biologics may also be susceptible to shear and agitation stresses during administration in pharmacies and in clinical settings. Many sites prepare IV infusion bags at a centralized facility and then typically transport them to local centers for administration. Because of the dilution of the formulation components, especially surfactants that protect against agitation stress, sensitive biologic DPs could undergo physical degradation such as aggregation during transportation.[108] Additionally, several clinical sites and hospitals also transport prepared IV bags via pneumatic tubes between floors.[17] DP stability under these in-use and administration conditions should be understood and risk mitigations should be documented so that sensitive

Table 3
Summary of representative issues (not an exhaustive list) observed during dose preparation and administration of DP

Clinical aspect	Components	Issues	Mitigation
Dose Preparation	Needle and syringe	appropriate dosage	follow manufacturer's instructions for dose preparation
	Closed system transfer devices (CSTDs) e.g., vial adaptors, IV bag spike, disposable syringes	Hold up volume & stopper coring due to use of CSTD vial adaptors, particle formulation due to CSTD vial adaptors and/or syringe lubricants	CSTD usage only if the dose form allows, perform risk-based assessment and/or compatibility study during drug product development
Dose administration	Dose range	Especially for low dose loss of protein to material of construction	Optimize surfactant concentration including potential addition of IV solution stabilizer
	IV bag spike, IV lines, IV-line filter	Drug product compatibility issues with material of construction	Perform compatibility studies during drug product development
	Closed system transfer devices e.g., IV-line luers/adaptors	Drug product compatibility issues with material of construction	CSTD usage only if the dose form allows, perform risk-based assessment and/or compatibility study during drug product development
	Pumps	Drug product degradation due to mechanical stress	Evaluate compatibility of drug product and addition of excipients to minimize stress on drug product
	Duration of infusion (modality dependent)	Microbial growth	Addition of anti-microbial agent
		Light Exposure	Protect from extended exposure to light; use of amber sleeves to protect infusion bags
		Particles from syringes	Pre-filled syringe
		Needle clogging	Ensure syringes are not left uncapped during dose preparation/administration. Reduction of temperature and pressure fluctuations for the PFS. [115]
	Take home devices e.g., autoinjector for high volume high viscosity products	Needle clogging as take-home devices have finer needles compared to PFS	Ensure take-home devices are not left uncapped and manufacturer's instructions for use are followed
		Device issues (mechanical or electronic)	human factor studies to avoid expected mechanical and electronic issues.

biologic drug products are not unintentionally mishandled in the clinical settings after the DP has been manufactured.

While microbial stability during clinical in-use is out of scope for this article, the reader is directed to a recent review article.[109] Another stress factor for IV infusion bags that is routinely missed is ambient light exposure for extended periods of time. Many biotechnology products have been shown to be light sensitive, even to ambient light that is devoid of significant UV wavelengths.[110,111] Protecting the infusion bags using amber sleeves and/or limiting light exposure should be considered as a part of clinical in use studies.

Closed System Transfer Devices (CSTDs)

CSTDs have seen an increase in usage recently to compound and administer biologics classified as hazardous by the National Institute for Occupational Safety and Health (NIOSH) (USP <800>). These devices are meant to protect healthcare personnel from accidental exposure to the drug. However, there are several challenges associated with CSTD usage including material incompatibility with the biologic, large holdup volumes resulting in inaccurate dosing and stopper coring leading to the introduction of rubber particles in the DP vial.[90-92,112] CSTD should only be used if absolutely necessary to ensure safety of the healthcare personnel handling the drug, and then only if data has been collected to demonstrate no impact on DP safety, efficacy, and dosage delivered.

Subcutaneous (SC) Administration

Biologic DP face challenges not only during dilution into IV bags as mentioned above but potentially also during SC administration. High concentration drugs injected SC could be subjected to high mechanical shear rates when pushed through a needle ultimately causing aggregation of the biologic. These effects can often be mitigated by varying the surfactant concentration to decrease the interfacial stress and by adding excipients to modulate viscosity.[113] A detailed understanding of the correlation between injection forces, viscosity and time factor for a SC injection is critical to minimize protein aggregation.

Temperature control is a critical factor in ensuring stability of the DP. Although temperature excursions are evaluated during formulation development, care must be taken to store the DP at the recommended storage temperature to avoid potential degradation. Elevated temperature exposure can lead to protein unfolding which may trigger protein aggregation and compromise the quality of the DP. This is especially important for lyophilized biologics that are typically less stable after reconstitution.

Proteins that are unstable in the liquid form are often manufactured as lyophilized products. Reconstitution of the drug product is an additional step for lyophilized products which requires access to sterile diluent. Proper dose preparation requires implementation of the procedure described by the manufacturer. Depending on the concentration of the DP, reconstitution time of the product can be anywhere from thirty seconds to 15 – 30 minutes. The DP vial should be swirled gently to ensure complete reconstitution for a clear liquid before dose administration.

Summary and Risk Mitigation

DP handling and compatibility guidance are typically captured in the pharmacy instruction manual for DPs in clinical trials, and in the package insert for commercial DPs. Strict adherence to the instructions provided on DP handling during preparation and administration in these documents are crucial to ensure the delivery of a safe DP and dosage to the patient.

It is imperative to understand DP stability during administration conditions, either during IV infusion or SC administration. The risk of protein aggregation due to agitation stresses that the DP faces during transportation (e.g., transportation or pneumatic tubes) especially due to dilution of key formulation excipients (such as surfactants) to levels below those needed to prevent aggregation need to be well understood. Stress studies used to screen for protein aggregation intended for IV administration should be performed in conditions representative of their intended route of administration.[114] Such studies and models are much needed to understand the potential mishandling of sensitive biologics DP in the clinical settings. For low concentration biologics, minimizing loss of the DP due to adsorption on administration container surfaces is a key aspect of the compatibility evaluation. A holistic testing strategy during drug development can support a formal recommendation on what materials are compatible with the DP, based on the route of administration. Typical assays used to monitor the drug product stability include SEC, visual inspection and protein recovery. Biologics administered via continuous IV infusion over multiple days have the added requirement of being able to withstand the elevated temperature, light and agitation stresses presented in the environment of an ambulatory patient. Most stress conditions are typically simulated during DP development to ensure stability of the biologic in a clinical or home use setting.

Concluding Remarks

The present review article completes a trilogy, following two previous review papers about stress factors in protein DS manufacturing[1] and protein DP manufacturing.[2] With this trilogy we have addressed the potential impact on product quality of the numerous stress factors a therapeutic protein may be exposed to from the moment it is expressed in a production cell up until the moment it is administered to a patient. Compromised protein DP quality, including changes to its CQAs, resulting from these stress factors potentially has a negative effect on its safety and efficacy. This includes an increased risk of immunogenicity, as has been shown in numerous preclinical and clinical studies and addressed extensively in several review papers and references cited therein.[116-125] Therefore, it is crucially important for stakeholders to be aware of the susceptibility of therapeutic proteins to common stress factors that may occur in their daily practice and to understand how to mitigate risk associated with these stress factors. Stakeholders may include representatives from the pharmaceutical biotechnology industry and regulatory agencies, (hospital) pharmacists and other healthcare professionals as well as patients (e.g., in the case of home storage, self-administration and use via on-body devices). In an attempt to create and improve potentially life-saving awareness of these stress factors and associated measures to mitigate risk, we (representatives of the Biopharmaceutical Product Attributes and Biological Consequences Community of the American Association of Pharmaceutical Scientists (AAPS)) set out to write this series of review papers.

With this third article we have covered the impact of stress factors on protein DP quality related to primary packaging, transportation and handling. Although primary packaging can be considered an inherent part of DP manufacturing, the large number of options and associated stress factors involved in this important step led us to address primary packing issues separately, in order to accommodate all the information and enhance the focus on this aspect of DP manufacturing and delivery. Primary packaging-related risk factors may include ingress of hazardous materials (e.g., oxidizing residuals from the sterilization process, delamination- or rubber stopper-derived particles, silicone oil droplets, leachables, surfactant stability) into the formulation as well as interactions between the protein and

primary packaging material components (e.g., their surface, silicone oil droplets, tungsten oxides) or the gas-liquid interface (depending on the headspace volume, the formulation composition and external factors, such as mechanical stress), all of which may cause protein degradation.

Importantly, the primary packaging material, in addition to the type of therapeutic molecule and the DP formulation, may substantially influence the impact of transportation and handling stresses on CQAs. Moreover, the type of primary packaging material determines to a large extent the way a product is handled and administered. For instance, DP formulations in PFS, vials, or on-body devices undergo distinct handling and administration practices, which affect the types and extents of the stresses involved. Thus, the impact on DP quality of container closure system, transportation and handling are interconnected, and the interplay between these may significantly impact the quality of the therapeutic protein that eventually is administered to a patient.

The longest section in the present paper is devoted to primary packaging-related stress factors, including sterilization methods used for container closure systems, types (e.g., vials with stoppers, prefillable syringes, devices) and materials of construction. We by no means want to suggest that transportation and handling are less important. Rather, this is the result of stress and risk factors related with container closure systems being better documented and controlled than those related to transportation and handling. The latter are much less controlled and knowledge gaps with regard to postproduction practices exist, as discussed elsewhere.[17] For instance, the manufacturing of DS and DP are under control and responsibility of the manufacturer, and general in-house knowledge can be applied to thoughtfully design product specific formulation-primary packaging combinations. In contrast, much less is known about local transportation and handling practices (including in-use storage conditions), which in addition may substantially vary between countries, or even within a country. The latter applies, for instance, to local (often site-specific) compounding protocols, type(s) of infusion lines and bags used, and the use (or not) of in-line filters, filter types, CSTDs and pneumatic tubes or other means of transportation. Moreover, the knowledge levels of health care providers and patients most likely differ substantially.

From the above, it is apparent that more research is needed on postproduction transportation and handling practices and their implications for protein DP quality. This does not mean that everything is understood with respect to stress factor risk management related to primary packaging materials. The impact of primary packaging-related stress on DP quality not only depends on the materials involved but also on the specific biotherapeutic molecule selected as the drug candidate, and the DP formulation, and therefore should be tested during (accelerated) storage, transportation and use for each specific product. Moreover, with the advent of novel packaging materials and sophisticated devices, new questions will arise with respect to their compatibility with each DP formulation. We hope that this paper begins to address these questions, with the aim to support the development of safe and efficacious protein DPs.

Declaration of Competing Interests

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.

Acknowledgments

The authors thank AAPS (American Association of Pharmaceutical Scientists) for bringing them together to brainstorm and contribute to this article through the AAPS Biopharmaceutical Product Attributes and Biological Consequences Community's Mechanisms of Protein Aggregation Working Group. The authors also thank Dr. Vibha Jawa for robust discussions on the content.

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