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General Commentary

Postproduction Handling and Administration of Protein Pharmaceuticals and Potential Instability Issues

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ABSTRACT

The safety and efficacy of protein pharmaceuticals depend not only on biological activity but also on purity levels. Impurities may be process related because of limitations in manufacturing or product related because of protein degradation occurring throughout the life history of a product. Although the pharmaceutical biotechnology industry has made great progress in improving bulk and drug product manufacturing as well as company-controlled storage and transportation conditions to minimize the level of degradation, there is less control over the many factors that may subsequently affect product quality after the protein pharmaceuticals are released and shipped by the manufacturer. Routine handling or unintentional mishandling of therapeutic protein products may cause protein degradation that remains unnoticed but can potentially compromise the clinical safety and efficacy of the product. In this commentary, we address some potential risks associated with (mis)handling of protein pharmaceuticals after release by the manufacturer. We summarize the environmental stress factors that have been shown to cause protein degradation and that may be encountered during typical handling procedures of protein pharmaceuticals in a hospital setting or during self-administration by patients. Moreover, we provide recommendations for improvements in product handling to help ensure the quality of protein pharmaceuticals during use.

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Introduction

In the past 2 decades, protein pharmaceuticals have become the fastest growing class of therapeutics because of their beneficial impacts in the treatment of severe and life-threatening conditions and diseases.[1] Development and manufacturing of protein pharmaceuticals is, however, challenging and requires overcoming various manufacturing hurdles such as issues with the purity of the protein product. Common impurities include protein product–related degradants (e.g., protein aggregates, fragments, and chemical degradants) and nonproduct, manufacturing process–related materials (such as process residuals; host-cell proteins/DNA; and particulates such as silicone oil droplets, glass particles, and delaminated primary packing materials or those derived from polysorbate degradation byproducts) as well as chemically degraded excipients.[2-5] Impurities within therapeutic protein products can cause severe adverse drug reactions (ADRs) in patients. ADRs can be acute[6-10] or more long term, as it is usually the case for unwanted immunogenicity,[11,12] and may result in compromised safety and efficacy.[13-15]

Aggregation and chemical degradation of proteins have been reported to enhance their immunogenicity upon administration.[16-29] Neutralizing antibodies can reduce the efficacy of therapeutic proteins[7,30-33] and sometimes cross-react with essential endogenous proteins to cause severe ADRs.[34] Physical aggregation and chemical degradation can occur throughout the life of a protein product, and even modest environmental stresses can cause extensive damage. The pharmaceutical biotechnology industry has made great strides in improving bulk and drug product manufacturing processes, and the cumulative outcome of these efforts has been significant and

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continues to improve/preserve the quality of protein products in all steps of production, storage, and transportation. Despite these improvements, once the protein product is released and shipped by the manufacturer, there is potentially little control over the many factors that may affect the structural integrity and quality of these environmentally sensitive protein products. Therefore, they may be damaged by various types of (mis)handling. It is plausible that even accepted routine handling of protein drugs in a clinical setting or in patients' hands may cause product degradation that remains unnoticed but potentially compromises the safety and efficacy of the product. For example, when preparing protein products for intravenous (IV) administration, protein particles can result from the numerous stresses generated during routine handling, and foreign materials can contribute to the particle loads delivered to patients.[35,36] Recent reports on the handling of protein drugs in hospital pharmacies and patients' hands suggest that these handling practices can potentially compromise the stability of protein pharmaceuticals.[37,38] In recognition of such problems, a conference session entitled "Fragile—handle with care" at the 2017 American Association of Pharmaceutical Scientists Annual Meeting was devoted to discussing the gap between the drug developer's understanding of real-world conditions and handling of their protein pharmaceuticals and the end user's understanding of the importance of proper handling of these drugs and the reasons behind it.

This commentary aims to address the potential risks associated with handling of protein pharmaceuticals after release and shipping by the manufacturer, that is, at the hospital pharmacies, in trained health care personnel's hands, or during patient self-administration. In this context, we first briefly discuss the different environmental stress factors that could be experienced as part of routine handling and administration procedures and how they could lead to compromised protein stability. We focus on the environmental stress factors that have been widely investigated in the past decade and proven to be detrimental to protein structural integrity and stability. The second part of the commentary contains remarks and recommendations for different units of the involved community for improvements in routine product handling, which may lead to a more reliable, controlled, and safer use of protein pharmaceuticals immediately before administration.

Handling of Protein Pharmaceuticals, Stress Factors, and Stability Concerns

During normal handling in clinical settings, protein therapeutics that are formulated for IV administration are typically prepared in the pharmacy (e.g., reconstituted from a lyophilized formulation, drawn into a syringe, injected into a bag of IV solution, and mixed), transported to the patient's floor (e.g., via a pneumatic tube or traditional hand-carried hospital trolleys), placed in temporary storage, and eventually administered to the patient. Alternatively, protein therapeutics that are formulated for subcutaneous injections are often handed over to a patient at the pharmacy, transported by patients to their homes, stored (e.g., in a refrigerator), and eventually injected subcutaneously by the patients themselves or by a caregiver. The pharmacist and a health care worker generally give recommendations to patients for proper storage and use. Furthermore, patients have access to the patient information leaflet[39] that includes descriptions of the optimal conditions for storage and use.

Each of the aforementioned steps, even when followed strictly, can expose proteins to various stresses that may cause product degradation as well as contamination. Contaminants (e.g., cellulose, dust, bacteria, virus) are of extraneous origin and, in principle, should not be present in a product or introduced during routine handling. However, extreme care should be taken to protect the product during handling at steps where it may become exposed to the external environment, such as during transfer from a vial to an IV bag. This commentary focuses on protein product–related impurities rather than these foreign matter contaminants.

It is noteworthy that in general, there appears to be a lack or an insufficient level of strict procedures for handling of protein pharmaceuticals in hospital pharmacies from a protein stability point of view. There is also more obviously a relative lack of control over the patients' handling and treatment of the protein product during at-home use. In a recent observational study performed in a hospital, some of authors have documented several incidents during the process of compounding at the pharmacy and transport of the drug to patients which could jeopardize the quality of protein pharmaceuticals. For instance, observations included vigorous manual agitation of a vial that contained a liquid formulation of a protein pharmaceutical causing formation of foam in the vial, repeated back and forth movement of a syringe plunger resulting in foaming in a syringe, nonuniform processes for injection of drug into infusion bags, and careless handling of IV bags to the patient's section by a nurse who was unaware of the contents of the IV bags.[37] In other observations in a different hospital, some of the authors identified the transportation of the protein pharmaceutical–containing IV bags with a pneumatic tube system to be of concern with respect to the stability of the proteins. In addition, discussions with pharmacists from a number of hospitals corroborate the abovementioned observations, the general lack of awareness, and the lack of suitable procedures for handling of protein pharmaceuticals in hospital pharmacies (let alone situations where a cold supply chain is not available or properly maintained in both developed countries and many developing parts of the world, a topic that has been widely reviewed with temperature-sensitive vaccines[40]). Furthermore, special cases such as issues with repackaging of a drug product for off-label use (as for an antivascular endothelial growth factor drug, Avastin) and complications with the effects of handling on aggregation and potential contamination have been reported and deserve further attention.[41,42]

One can envision that such concerns are similarly valid for protein pharmaceuticals provided to patients for self-administration at home. The scientific community has raised this concern, and a few publications have reported that home storage temperatures often deviate from the recommended temperature range.[38,43] Obviously, it is more difficult to gather information about other stress parameters, and it is not known how patients may treat protein pharmaceuticals with respect to mechanical shocks, light exposure, and various combinations of these stress factors.

In this next section of the commentary, we summarize the environmental stress factors that have been shown to cause protein degradation and could be encountered during typical handling procedures and mishandling of protein pharmaceuticals in a hospital setting or in patients' or caregivers' hands.

Mechanical Stresses and Contact With Interfaces

Mechanical stress is arguably the most common type of stress that a protein drug product may be exposed to in the compounding and transportation processes in a hospital or in a patient's hands during at-home use. A certain level of stability against mechanical stresses is a must-have property for protein pharmaceutical products. To this end, great care is taken in formulation design to add excipients (e.g., nonionic surfactants) that decrease a protein's susceptibility to mechanical stresses[44] as well as in the design of manufacturing processes to reduce protein damage that can arise during formulation/filling processes and during transportation of containers holding protein solutions. In hospital and home environments, however, generated mechanical forces and surface

exposures can have a different nature and magnitude compared with the stresses generally screened for during protein drug product development. Some examples of these mechanical forces include transport of IV bags and syringes in pneumatic tube systems, aspiration of formulations into syringes, accidental dropping of containers/delivery devices (e.g., vials, syringes, and IV bags), transfer of solutions from a large IV bag into smaller containers with peristaltic pumps, and operation of IV infusion pumps. Cavitation can occur when dropping a drug container, during aspiration of formulations into syringes, or when an IV bag or syringe hits the receptacle in a pneumatic tube system. Cavitation is a pressure wave–induced formation of bubbles, which, upon collapse, creates microscopic local hotspots where temperature and pressure may reach extreme values,[45,46] generating hydrogen and hydroxyl radicals[47] and initiating protein aggregation and particle formation.[48,49] Recent studies reveal that the mechanical shock caused by dropping of a vial can lead to formation and collapse of cavitation bubbles, which in turn can cause formation of particles in protein pharmaceutical products.[48] Recent studies have corroborated and expanded these results and suggest that the detrimental effects of such stresses can be increased synergistically if 2 or more types of mechanical stresses act together. For example, mechanical shock followed by agitation generates high levels of protein particles.[50]

Agitation (as in shaking, stirring, and pumping) of protein solutions has been shown to exert stresses that may contribute to protein aggregation and particle formation. Such stresses are generally a combination of mechanical stress and exposure of the protein to interfaces. This may occur during the different stages of routine product handling, as described previously. Several studies have shown that shaking of prefilled syringes and vials containing liquid or even lyophilized protein drug products can result in physical instability and particle formation.[51-58] It is well established that the various interfaces to which proteins may be exposed (e.g., during pumping, mixing, shipping, and storage in primary container closure and drug administration systems) can cause proteins to aggregate and form particles. These interfaces include liquid-air interfaces (e.g., in vials and infusion bags), liquid-solid interfaces (e.g., in vials, IV bags, and lines), and liquid-liquid interfaces (e.g., silicone oil-water interfaces found in prefilled glass syringes as well as in disposable plastic syringes used for transfer of protein solutions).[54,59-61] Upon adsorption to an interface, protein molecules readily become structurally altered and aggregate, forming interfacial films that, upon mechanical disruption (e.g., shear forces inherent to syringe operation), generate and transfer particles into the solutions that are administered to patients. Importantly, compounding of protein pharmaceuticals can involve introduction of proteins to interfaces as well as disruption of these interfaces. Recent studies show that this combination can lead to formation of large amounts of protein aggregates and potentially a significant decrease in the monomer content, as observed in a model stirring process.[62] Disturbance (or any touching) of adsorbed layers of protein at solid-liquid interfaces such as those found in vials and infusion bags or at air-liquid interface which can be present throughout the handling process may lead to this process of aggregate formation.[62-65]

In addition, each of the solid surfaces that protein pharmaceuticals encounter can potentially shed nonproteinaceous microparticulate and nanoparticulate materials into the solution. Examples of such particles include delaminated glass from syringe or vial walls; silicone oil droplets from lubricants added to facilitate plunger movement in syringes; and polymeric nanoparticles found in IV solutions, bags, filter sets, and administration tubing.[41,66-68] Protein molecules readily adsorb to these materials, which may act as adjuvants (and lead to protein denaturation and aggregation) to enhance adverse immune responses to the therapeutic protein and provoke infusion reactions.[69-71] In-line filters in IV setups are typically insufficient to reduce particle levels reaching patients during IV administration. In fact, they do not remove the majority of nanoparticles and may even expose proteins to new interfaces that generate additional particles downstream from the filter membrane. Here, again, mechanical stresses imposed on the materials and interfaces during the handling have been shown to enhance the shedding of particles.[72]

With respect to the abovementioned cases, it must be realized that the protective action of excipients, such as nonionic surfactants that are typically included in a protein formulation to inhibit the degradation of proteins caused by interfaces, may be largely diminished upon dilution of the pharmaceutical (and thus dilution of the stabilizing excipients) in an IV infusion bag. Also, even with optimal concentrations of stabilizing excipients, therapeutic proteins may still form substantial levels of nanoparticles and microparticles upon exposure to interfaces in combination with mechanical stresses.

Temperature Excursions and Freeze-Thaw Cycles

Proteins are naturally less stable at elevated temperatures where they readily undergo structural alterations often leading to aggregation. Generally speaking, proteins can degrade because of high temperature exposure in 2 ways: exposure above a threshold temperature that causes protein unfolding (e.g., above the thermal onset and thermal melting temperatures, T_{onset} and T_m, respectively) or exposure to elevated temperatures below the T_{onset}/T_m values but over a certain extended time period. From a practical perspective, temperatures above 40°C are considered detrimental to protein pharmaceuticals, but even prolonged exposure to room temperature can cause damage. Protein pharmaceutical products may be exposed to a wide range of temperatures that greatly exceed the typical recommended storage temperatures of 2°C-8°C. In some climates and circumstances (e.g., in a hot car in the summer), exposure to temperatures approaching 50°C are possible, unless care is taken to keep protein products in a temperature-controlled environment. Also, there are unpublished reports of patients warming their drug products in microwave ovens.

Not only “too hot,” but also “too cold” may be detrimental to proteins. Freezing of a liquid protein formulation or cycles of freeze-thawing can lead to destabilization and have frequently been reported to result in protein aggregation and particle formation.[73-76] Although the magnitude of such degradation may not always be large enough to significantly reduce the total monomer content, measurable levels of protein particles can be formed even during a single freeze-thawing cycle of a well-formulated product. In addition, results from preclinical studies suggest that very small amounts of such protein particles (i.e., low microgram range) may increase immunogenicity risk.[77] Therapeutic protein products may accidently freeze during shipment from the pharmacies to patients when subzero (e.g., −20°C) polymer packs are included in the shipping container. Also, transportation by patients from the pharmacy in winter can lead to product freezing, for example, if the patient stops at another location on the way home and the interior of his or her vehicle cools to subzero temperature. And, of course, storing a protein product in the home freezer instead of the refrigerator can lead to unintended freezing. In addition, certain “frost-free” freezers may subject frozen formulations to multiple freeze-thaw cycles.

The effect of such freeze-thaw stresses on protein pharmaceuticals is typically tested during drug product formulation development, and measures for stabilization are taken if needed, such as the addition of stabilizing excipients. However, it is important to note that during freeze-thaw stress studies, not all company

scientists are using sensitive analytical methods that are capable of reproducibly detecting protein nanoparticles and microparticles. For example, if no methods for detection of subvisible particles are used or if particles are only measured with light obscuration, the actual levels of protein particles formed during the stress may be much higher than those detected,[78,79] especially if only particles of sizes $\geq$10 μm are reported. In such cases, company scientists would judge their formulations as robust enough to withstand stresses, whereas in fact, they are sensitive to stresses that are routinely encountered in the field when the product is no longer necessarily being handled under well-controlled conditions.

One can expect near-optimal and perhaps suboptimal storage conditions for the protein drug at a pharmacy and in patients' hands, respectively. Although there are no published reports that address the former, it has been shown that the storage conditions of liquid protein drugs by patients can substantially deviate from those that are recommended. Vlieland et al.[38] monitored the storage temperature at patients' homes using a validated temperature logger. They reported that the majority of the patients do not store their biopharmaceutical within the manufacturer's recommended temperature range (2°C-8°C), whereas more than a quarter stored the drug at temperatures below 0°C or above 25°C for more than 2 h. The same study revealed that the unfavorable storage includes several (sometimes up to 10 or 100) cycles of −5 to 5°C.[38] It is unclear whether all protein drug products are designed to remain stable under such severe stress conditions. In a follow-up study, a few commercial protein pharmaceuticals in prefilled syringes were exposed to similar freeze-thaw cycles as those that were experienced in patients' refrigerators; the authors observed that the applied stress caused measurable increases in particle levels.[80] Whether such changes in the impurity profile have an impact on the overall safety and efficacy of the products remains to be studied, but potentially, they could contribute to immunogenicity in the patients receiving a product mishandled in such a way.

Exposure to Light

To avoid photodegradation, protein pharmaceutical products are protected from light by secondary packaging. However, in hospital settings and during at-home use, normal exposure to light (e.g., fluorescent lights found in hospital settings or ambient light in home care settings and infusion clinics) can chemically damage protein molecules and cause color formation and generation of aggregates and particles.[81-84] It has been suggested that light at wavelengths typical of fluorescent lighting in clinics can induce a variety of oxidative reactions as well as protein particle formation and aggregation.[85] In another example, the exposure of an octreotide-containing solution to light from a fluorescent desk lamp led to the generation of oxidative modifications similar to those induced by stress conditions involving ultraviolet light, albeit at lower yields.[86] The combination of chemical degradation and aggregation caused by light exposure has been shown in preclinical models to result in protein products that are particularly immunogenic.[85]

Many protein pharmaceuticals should be allowed to warm up to room temperature immediately before injection, and there are anecdotal accounts of patients placing prefilled syringes in sunlight to speed up this warming. Furthermore, it is important to realize that in clinical settings, infusion bags are often placed on a hanger, thereby exposed to ambient light that could amplify the photodegradation risks. In some hospitals, patients are even allowed to walk outside while carrying their IV bag on a hanger with them, where the bags and their contents are exposed to direct sunlight. Moreover, protein pharmaceuticals that are provided to patients for at-home use may be exposed to direct or indirect sunlight while being transported, for example, laying in the passenger cabin in a car, or before use by the patients, for example, when left on the table before injection. These exemplary conditions can expose the protein pharmaceutical to 2 or potentially 3 different types of stresses, that is, light exposure, temperature excursions, and agitation by shaking. To the best of our knowledge, such combined stress conditions are not routinely tested during protein drug product formulation development. Systematic studies are necessary to determine the magnitude of the damage a protein product may undergo for each of such cases.

Summarizing Discussion and Recommendations

It is noteworthy that this commentary is not the first one addressing the challenges that arise with handling of protein pharmaceuticals. Some of the authors of this commentary, among others, have raised concerns about the instability of such drugs in the manufacturing, supply, and use chain previously around the time when the commercial market of protein pharmaceuticals was rapidly growing.[87-89] The advancements in pharmaceutical development of protein drugs in the past 2 decades have brought a significant growth of knowledge and understanding of the influential stress factors, resulting in good control over the critical stability parameters during the manufacturing and distribution of protein pharmaceuticals. It is, therefore, an opportune time for (re) addressing the last piece of the protein drug product supply chain, that is, the postproduction handling of protein drugs immediately before administration. We realize there are challenges to tackle these potential problems with handling of protein drugs at the hospital setting and at patient's self-administration level and wish to propose a set of recommendations for the involved parties to consider to improve the situation for a safer and more effective handling and use of protein drugs (see Table 1 for a summary of the recommendations). After all, improvements in this area are in the best interest of all the parties, including manufacturers, health care systems, regulatory agencies, and—above all—patients.

Expansion of Research on Effects of Real-World Handling on Quality of Protein Pharmaceuticals

There is a clear need to perform and publish more research to better understand the extent of the severity of the risk associated with postmanufacturing handling of protein pharmaceuticals (for various classes as well as specific examples of protein drugs, e.g., monoclonal antibodies in general vs. a specific monoclonal antibody drug product). Studies should be focused on breaking down the handling steps and extracting the characteristics of the environmental stress factors in each and every step (as well as the effects of various combinations of such stresses). Such investigations must span a wide range of common handling procedures from the moment the drug arrives at the hospital setting until it is administered into the patient. Moreover, analysis of protein products under conditions that are experienced when injected by patients in the home situation should be performed to shed more light on the quality of protein products used by patients. Studies should ideally address 2 questions: (1) does postproduction (mis)handling of protein pharmaceuticals cause protein degradation and to what extent? and (2) do potential protein degradation byproducts interfere with safety and efficacy of the drug? Setting up a database on the subject that is shared between public and private sectors (ideally in a global framework) would be of great value and should be considered.

Table 1
Summary of Recommendations for the Multiple Involved Parties to Improve the Situation for a Safer and More Effective Use of Protein Pharmaceuticals During Routine Handling in a Hospital Setting and During Patient Self-Administration at Home

Recommendation	Potential Contributing Party					Comments
	Manufacturers	Hospitals and Pharmacies	Patients	Regulatory Bodies	Academia and Research Institutions	
Improved understanding of potentially stressful handling steps and their effects on protein quality	X	X		X	X	Prerequisite to most efforts; in progress, but much more research and publications needed
Development of clear and detailed product handling guidelines for use at hospital pharmacies	X	X		X		Must have, regardless of other efforts
Introduction and use of a coding and labeling system for protein pharmaceuticals in hospitals		X		X		May help to improve end user awareness
Inclusion of training courses on product handling in the pharmacy curricula					X	Must have; in place in several academic institutions but not yet implemented in every curriculum
Offering postacademic training courses for pharmacists and health care workers	X	X			X	Must have for pharmacists and health care workers dealing with protein pharmaceuticals
Better communication between manufacturers and end users for understanding of critical handling parameters	X	X	X	X		Would help improve the robustness of the supply chain in hospital settings and training of patients for use at home

Development of Improved Protein Product–Handling Guidelines

Pharmacists and other health care professionals face questions related to the optimum handling procedures for protein pharmaceuticals. There must be a collaboration between the clinicians, manufacturers, and regulators to better define detailed and clear guidelines for storage, reconstitution (if applicable), transfer to IV bags (if applicable), transporting, and all other steps of handling in the hospital setting. These guidelines can be developed as general guidelines for a typical hospital as a first step but will eventually have to be adjusted for each and every hospital based on the characteristics and conditions of that particular hospital. To make such an effort feasible, a protein pharmaceutical supervisor who can be assigned within the hospital pharmacy, after receiving the proper training, can review and assess the risk factors and arrange mitigation plans within his/her health center.

Introduction and Use of a Coding and Labeling System in Hospitals

Definition of a coding and labeling system containing minimum handling requirements can have a significant influence. Such coding will allow for designating a separate handling unit with its own standards, allowing only trained personnel to handle the drug (and over time perhaps development of specialized tools and packaging), ensuring a safer treatment of protein pharmaceuticals. Dedicated stickers on the IV bags and syringes that contain protein pharmaceuticals will allow the transporters and nurses in other departments to immediately recognize the presence of this class of drugs and help them to follow the appropriate handling guidelines. Such a coding and labeling system may be considered costly and difficult to implement globally, but it can be regarded similar to the guidelines used for the aseptic treatment of pharmaceuticals and the preparation of IV bags, which have been shown to be effective in decreasing the contamination rate of pharmacy-compounded preparations.[90] Such a system could be an effective vehicle for introducing a set of standards with minimum handling requirements for protein pharmaceuticals which may eventually save money because of more effective treatments with fewer adverse effects.

Enhancement of Training on the Appropriate Handling of Protein Pharmaceuticals

Training on the appropriate handling of protein pharmaceuticals during real-world conditions of administration should be an integral part of pharmacy curricula. Moreover, postacademic training on this topic for retail pharmacists and hospital pharmacists should be available. The latter is particularly important because many of the currently practicing pharmacists may not have received such training, let alone specific training on properly instructing medical personnel and patients. Academic and health institutions and regulatory agencies should work toward implementation of such training. The previously mentioned protein pharmaceutical expert in a hospital setting can play an active part here and aim for internal training of the involved personnel. This should involve repeated training with updates on the handling of protein pharmaceuticals and reporting of problems that resulted from mishandling. Materials for such updates and special training sessions can be provided regularly by appropriate authorities and scientists from academia. It should be pointed out that the awareness of the need for such training has become increasingly well recognized for the use and administration of vaccines; in this case, improved education on the proper handling of vaccines on the day of administration is being addressed as part of the “last mile” in the vaccine cold chain.[40,91]

Improvements in Communication Between Manufacturers and End Users

There must be good communication between developers of protein pharmaceuticals and hospital pharmacies, allowing developers to obtain a better understanding of real-world conditions

and handling of their protein drug products. Efforts for obtaining such understanding would be a rewarding practice for the manufacturer. Considerations regarding the stresses or combination of stresses that can occur during (mis)handling of the drug after it leaves the factory can become an integral part of the in-use studies in the drug product development process. Such practice would allow for identification of the main risk factors and can lead to putting the right emphasis on critical stress factors that matter the most in the guidelines for pharmacists and patients. As one example, although the common technical document format for the quality section of regulatory filings for commercial approval of protein drugs includes a section on compatibility (ICH M4Q(R1), section 3.2.P.2.6), the guidance is relatively high level and does not necessarily focus on the real-world conditions discussed in this commentary. In addition, it is well known that large groups of patients misinterpret package inserts, if they read those at all,[92,93] and forget verbal instructions.[94] Therefore, perhaps the greatest effort that should be made is finding novel ways to effectively obtain information concerning the real handling practices and to train and inform the pharmacists, other involved hospital personnel, and patients. It is expected that raising awareness with clear examples and case reports would be of great influence. For instance, if a pharmacist sees how particles are introduced upon mishandling of a drug product, he or she may be more cautious when preparing the drug for a patient. In this context, modern technologies can be invaluable tools on the one hand to gather and document data on what a drug product experiences beyond the release by the manufacturer and on the other one to deliver more effective trainings and communication to end users. For instance, sensors may be used to record the drug product conditions, such as temperature, light exposure, and movement; videos recorded by hospital pharmacies and patients would provide a wealth of information regarding the steps taken to prepare and administer the drug. Moreover, potentially more effective training tools could include online resources (e.g., short training videos and information forums) and webinars. Such tools, alongside the design and implementation of visual aids such as abovementioned labels; pictograms; and brief written instructions, such as “do not drop,” “do not leave in sunlight,” “do not shake,” and so forth, may help improving the awareness of patients—and end users in general—that protein pharmaceutical products are delicate and must be handled with care.

References

1. Mitragotri S, Burke PA, Langer R. Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies. *Nat Rev Drug Discov*. 2014;13(9):655-672.
2. Manning MC, Chou DK, Murphy BM, Payne RW, Katayama DS. Stability of protein pharmaceuticals: an update. *Pharm Res*. 2010;27(4):544-575.
3. Wang W, Singh S, Zeng DL, King K, Nema S. Antibody structure, instability, and formulation. *J Pharm Sci*. 2007;96(1):1-26.
4. Martos A, Koch W, Jiskoot W, et al. Trends on analytical characterization of polysorbates and their degradation products in biopharmaceutical formulations. *J Pharm Sci*. 2017;106(7):1722-1735.
5. Kishore RS, Kiese S, Fischer S, Pappenberger A, Grauschopf U, Mahler HC. The degradation of polysorbates 20 and 80 and its potential impact on the stability of biotherapeutics. *Pharm Res*. 2011;28(5):1194-1210.
6. Yanai H, Hanauer SB. Assessing response and loss of response to biological therapies in IBD. *Am J Gastroenterol*. 2011;106(4):685-698.
7. Goodin DS, Frohman EM, Hurwitz B, et al. Neutralizing antibodies to interferon beta: assessment of their clinical and radiographic impact: an evidence report: report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology*. 2007;68(13):977-984.
8. Hemmer B, Stuve O, Kieseier B, Schellekens H, Hartung HP. Immune response to immunotherapy: the role of neutralising antibodies to interferon beta in the treatment of multiple sclerosis. *Lancet Neurol*. 2005;4(7):403-412.
9. Varela H, Villamanan E, Plasencia C, et al. Safety of antitumour necrosis factor treatments in chronic rheumatic diseases: therapy discontinuations related to side effects. *J Clin Pharm Ther*. 2016;41(3):306-309.
10. Nagai Y, Yokogawa N, Shimada K, Sugii S. Severe thrombocytopenia induced by first infliximab administration for rheumatoid arthritis. *Am J Ther*. 2016;23(6):e1933-e1937.
11. Schellekens H. Immunogenicity of therapeutic proteins: clinical implications and future prospects. *Clin Ther*. 2002;24(11):1720-1740. discussion 1719.
12. Schellekens H. Immunogenicity of therapeutic proteins. *Nephrol Dial Transplant*. 2003;18(7):1257-1259.
13. De Groot AS, Scott DW. Immunogenicity of protein therapeutics. *Trends Immunol*. 2007;28(11):482-490.
14. Rosenberg AS, Sauna ZE. Immunogenicity assessment during the development of protein therapeutics. *J Pharm Pharmacol*. 2018;70(5):584-594.
15. Tamilvanan S, Raja NL, Sa B, Basu SK. Clinical concerns of immunogenicity produced at cellular levels by biopharmaceuticals following their parenteral administration into human body. *J Drug Target*. 2010;18(7):489-498.
16. Brinks V, Jiskoot W, Schellekens H. Immunogenicity of therapeutic proteins: the use of animal models. *Pharm Res*. 2011;28(10):2379-2385.
17. Alexander J, Oseroff C, Dahlberg C, et al. A decaepitope polypeptide primes for multiple CD8+ IFN-gamma and Th lymphocyte responses: evaluation of multiepitope polypeptides as a mode for vaccine delivery. *J Immunol*. 2002;168(12):6189-6198.
18. Valdes I, Bernardo L, Gil L, et al. A novel fusion protein domain III-capsid from dengue-2, in a highly aggregated form, induces a functional immune response and protection in mice. *Virology*. 2009;394(2):249-258.
19. Jiskoot W, Randolph TW, Volkin DB, et al. Protein instability and immunogenicity: roadblocks to clinical application of injectable protein delivery systems for sustained release. *J Pharm Sci*. 2012;101(3):946-954.
20. Bessa J, Boeckle S, Beck H, et al. The immunogenicity of antibody aggregates in a novel transgenic mouse model. *Pharm Res*. 2015;32(7):2344-2359.
21. Boll B, Bessa J, Folzer E, et al. Extensive chemical modifications in the primary protein structure of IgG1 subvisible particles are necessary for breaking immune tolerance. *Mol Pharm*. 2017;14(4):1292-1299.
22. Ryff JC, Schellekens H. Immunogenicity of rDNA-derived pharmaceuticals. *Trends Pharmacol Sci*. 2002;23(6):254-256.
23. Hochuli E. Interferon immunogenicity: technical evaluation of interferon-alpha 2a. *J Interferon Cytokine Res*. 1997;17 Suppl 1:S15-21.
24. Ling ZD, Ziltener HJ, Webb BT, Matheson DS. Aggregated immunoglobulin and Fc fragment of IgG induce IL-6 release from human monocytes. *Cell Immunol*. 1990;129(1):95-103.
25. Barandun S, Kistler P, Jeunet F, Isliker H. Intravenous administration of human gamma-globulin. *Vox Sang*. 1962;7:157-174.
26. Moore WV, Leppert P. Role of aggregated human growth hormone (hGH) in development of antibodies to hGH. *J Clin Endocrinol Metab*. 1980;51(4):691-697.
27. Ring J, Stephan W, Brendel W. Anaphylactoid reactions to infusions of plasma protein and human serum albumin. Role of aggregated proteins and of stabilizers added during production. *Clin Allergy*. 1979;9(1):89-97.
28. Rosenberg AS. Effects of protein aggregates: an immunologic perspective. *AAPS J*. 2006;8(3):E501-507.
29. Prummer O. Treatment-induced antibodies to interleukin-2. *Biotherapy*. 1997;10(1):15-24.
30. Runkel L, Meier W, Pepinsky RB, et al. Structural and functional differences between glycosylated and non-glycosylated forms of human interferon-beta (IFN-beta). *Pharm Res*. 1998;15(4):641-649.
31. Malucchi S, Sala A, Gilli F, et al. Neutralizing antibodies reduce the efficacy of betaIFN during treatment of multiple sclerosis. *Neurology*. 2004;62(11):2031-2037.
32. Reipert BM, van Helden PM, Schwarz HP, Hausl C. Mechanisms of action of immune tolerance induction against factor VIII in patients with congenital haemophilia A and factor VIII inhibitors. *Br J Haematol*. 2007;136(1):12-25.
33. Neumann TA, Foote M. Megakaryocyte growth and development factor (MGDF): an Mpl ligand and cytokine that regulates thrombopoiesis. *Cytokines Cell Mol Ther*. 2000;6(1):47-56.
34. Casadevall N. Antibodies against rHuEPO: native and recombinant. *Nephrol Dial Transplant*. 2002;17 Suppl 5:42-47.
35. Pardeshi NN, Qi W, Dahl K, Caplan L, Carpenter JF. Microparticles and nanoparticles delivered in intravenous saline and in an intravenous solution of a therapeutic antibody product. *J Pharm Sci*. 2017;106(2):511-520.
36. Kumru OS, Liu J, Ji JA, et al. Compatibility, physical stability, and characterization of an IgG4 monoclonal antibody after dilution into different intravenous administration bags. *J Pharm Sci*. 2012;101(10):3636-3650.
37. Jiskoot W, Nejadnik MR, Sediq AS. Potential issues with the handling of biologicals in a hospital. *J Pharm Sci*. 2017;106(6):1688-1689.
38. Vlieland ND, Gardarsdottir H, Bouvy ML, Egberts TC, van den Bemt BJ. The majority of patients do not store their biologic disease-modifying antirheumatic drugs within the recommended temperature range. *Rheumatology (Oxford)*. 2016;55(4):704-709.
39. Kitching JB. Patient information leaflets—the state of the art. *J R Soc Med*. 1990;83(5):298-300.
40. Kumru OS, Joshi SB, Smith DE, Middaugh CR, Prusik T, Volkin DB. Vaccine instability in the cold chain: mechanisms, analysis and formulation strategies. *Biologicals*. 2014;42(5):237-259.
41. Liu L, Ammar DA, Ross LA, Mandava N, Kahook MY, Carpenter JF. Silicone oil microdroplets and protein aggregates in repackaged bevacizumab and ranibizumab: effects of long-term storage and product mishandling. *Invest Ophthalmol Vis Sci*. 2011;52(2):1023-1034.

42. Kahook MY, Liu L, Ruzycki P, et al. High-molecular-weight aggregates in repackaged bevacizumab. *Retina*. 2010;30(6):887-892.
43. Cuellar MJ, Marco JL, Perez-Castello I, Castello Escriva A. Quality of storage of thermolabile drugs in patients' homes. *Rev Calid Asist*. 2010;25(2):64-69.
44. Kamerzell TJ, Esfandiary R, Joshi SB, Middaugh CR, Volkin DB. Protein-excipient interactions: mechanisms and biophysical characterization applied to protein formulation development. *Adv Drug Deliv Rev*. 2011;63(13):1118-1159.
45. Didenko YT, Suslick KS. The energy efficiency of formation of photons, radicals and ions during single-bubble cavitation. *Nature*. 2002;418(6896):394-397.
46. Flannigan DJ, Suslick KS. Plasma formation and temperature measurement during single-bubble cavitation. *Nature*. 2005;434(7029):52-55.
47. McLean JR, Mortimer AJ. A cavitation and free radical dosimeter for ultrasound. *Ultrasound Med Biol*. 1988;14(1):59-64.
48. Randolph TW, Schiltz E, Sederstrom D, et al. Do not drop: mechanical shock in vials causes cavitation, protein aggregation, and particle formation. *J Pharm Sci*. 2015;104(2):602-611.
49. Nakajima K, Ogi H, Adachi K, et al. Nucleus factory on cavitation bubble for amyloid beta fibril. *Sci Rep*. 2016;6:22015.
50. Torisu T, Maruno T, Hamaji Y, Ohkubo T, Uchiyama S. Synergistic effect of cavitation and agitation on protein aggregation. *J Pharm Sci*. 2017;106(2):521-529.
51. Kiese S, Pappenberger A, Friess W, Mahler HC. Shaken, not stirred: mechanical stress testing of an IgG1 antibody. *J Pharm Sci*. 2008;97(10):4347-4366.
52. Mahler HC, Friess W, Grauschopf U, Kiese S. Protein aggregation: pathways, induction factors and analysis. *J Pharm Sci*. 2009;98(9):2909-2934.
53. Telikepalli S, Kumru OS, Kim JH, et al. Characterization of the physical stability of a lyophilized IgG1 mAb after accelerated shipping-like stress. *J Pharm Sci*. 2015;104(2):495-507.
54. Krayukhina E, Tsumoto K, Uchiyama S, Fukui K. Effects of syringe material and silicone oil lubrication on the stability of pharmaceutical proteins. *J Pharm Sci*. 2015;104(2):527-535.
55. Fleischman ML, Chung J, Paul EP, Lewus RA. Shipping-induced aggregation in therapeutic antibodies: utilization of a scale-down model to assess degradation in monoclonal antibodies. *J Pharm Sci*. 2017;106(4):994-1000.
56. Weinbuch D, Zolls S, Wiggenhorn M, et al. Micro-flow imaging and resonant mass measurement (Archimedes)—complementary methods to quantitatively differentiate protein particles and silicone oil droplets. *J Pharm Sci*. 2013;102(7):2152-2165.
57. Liu L, Qi W, Schwartz DK, Randolph TW, Carpenter JF. The effects of excipients on protein aggregation during agitation: an interfacial shear rheology study. *J Pharm Sci*. 2013;102(8):2460-2470.
58. Peters BJ, Capelle MA, Arvinte T, van de Garde EM. Validation of an automated method for compounding monoclonal antibody patient doses: case studies of Avastin (bevacizumab), Remicade (infliximab) and Herceptin (trastuzumab). *MAbs*. 2013;5(1):162-170.
59. Gerhardt A, McGraw NR, Schwartz DK, Bee JS, Carpenter JF, Randolph TW. Protein aggregation and particle formation in prefilled glass syringes. *J Pharm Sci*. 2014;103(6):1601-1612.
60. Tyagi AK, Randolph TW, Dong A, Maloney KM, Hitscherich Jr C, Carpenter JF. IgG particle formation during filling pump operation: a case study of heterogeneous nucleation on stainless steel nanoparticles. *J Pharm Sci*. 2009;98(1):94-104.
61. Cromwell ME, Hilario E, Jacobson F. Protein aggregation and bioprocessing. *AAPS J*. 2006;8(3):E572-579.
62. Sediq AS, van Duijvenvoorde RB, Jiskoot W, Nejadnik MR. No touching! Abrasion of adsorbed protein is the root cause of subvisible particle formation during stirring. *J Pharm Sci*. 2016;105(2):519-529.
63. Bruckl L, Hahn R, Sergi M, Scheler S. A systematic evaluation of mechanisms, material effects, and protein-dependent differences on friction-related protein particle formation in formulation and filling steps. *Int J Pharm*. 2016;511(2):931-945.
64. Mehta SB, Lewus R, Bee JS, Randolph TW, Carpenter JF. Gelation of a monoclonal antibody at the silicone oil-water interface and subsequent rupture of the interfacial gel results in aggregation and particle formation. *J Pharm Sci*. 2015;104(4):1282-1290.
65. Ghazvini S, Kalonia C, Volkin DB, Dhar P. Evaluating the role of the air-solution interface on the mechanism of subvisible particle formation caused by mechanical agitation for an IgG1 mAb. *J Pharm Sci*. 2016;105(5):1643-1656.
66. Bernstein RK. Clouding and deactivation of clear (regular) human insulin: association with silicone oil from disposable syringes? *Diabetes Care*. 1987;10(6):786-787.
67. Ennis RD, Pritchard R, Nakamura C, et al. Glass vials for small volume parenterals: influence of drug and manufacturing processes on glass delamination. *Pharm Dev Technol*. 2001;6(3):393-405.
68. Liu L, Randolph TW, Carpenter JF. Particles shed from syringe filters and their effects on agitation-induced protein aggregation. *J Pharm Sci*. 2012;101(8):2952-2959.
69. Van Beers MM, Gilli F, Schellekens H, Randolph TW, Jiskoot W. Immunogenicity of recombinant human interferon beta interacting with particles of glass, metal, and polystyrene. *J Pharm Sci*. 2012;101(1):187-199.
70. Fradkin AH, Carpenter JF, Randolph TW. Glass particles as an adjuvant: a model for adverse immunogenicity of therapeutic proteins. *J Pharm Sci*. 2011;100(11):4953-4964.
71. Nejadnik MR, Jiskoot W. Measurement of the average mass of proteins adsorbed to a nanoparticle by using a suspended microchannel resonator. *J Pharm Sci*. 2015;104(2):698-704.
72. Callahan DJ, Stanley B, Li Y. Control of protein particle formation during ultrafiltration/diafiltration through interfacial protection. *J Pharm Sci*. 2014;103(3):862-869.
73. Desai KG, Pruett WA, Martin PJ, Colandene JD, Nesta DP. Impact of manufacturing-scale freeze-thaw conditions on a mAb solution. *BioPharm Int*. 2017;30(2):30-36.
74. Schwegman JJ, Carpenter JF, Nail SL. Evidence of partial unfolding of proteins at the ice/freeze-concentrate interface by infrared microscopy. *J Pharm Sci*. 2009;98(9):3239-3246.
75. Kerwin BA, Heller MC, Levin SH, Randolph TW. Effects of Tween 80 and sucrose on acute short-term stability and long-term storage at −20 degrees C of a recombinant hemoglobin. *J Pharm Sci*. 1998;87(9):1062-1068.
76. Hawe A, Kasper JC, Friess W, Jiskoot W. Structural properties of monoclonal antibody aggregates induced by freeze-thawing and thermal stress. *Eur J Pharm Sci*. 2009;38(2):79-87.
77. Jiskoot W, Kijanka G, Randolph TW, et al. Mouse models for assessing protein immunogenicity: lessons and challenges. *J Pharm Sci*. 2016;105(5):1567-1575.
78. Wuchner K, Buchler J, Spycher R, Dalmonte P, Volkin DB. Development of a microflow digital imaging assay to characterize protein particulates during storage of a high concentration IgG1 monoclonal antibody formulation. *J Pharm Sci*. 2010;99(8):3343-3361.
79. Huang CT, Sharma D, Oma P, Krishnamurthy R. Quantitation of protein particles in parenteral solutions using micro-flow imaging. *J Pharm Sci*. 2009;98(9):3058-3071.
80. Vlieland ND, Nejadnik MR, Gardarsdottir H, et al. The impact of inadequate temperature storage conditions on aggregate and particle formation in drugs containing tumor necrosis factor-alpha inhibitors. *Pharm Res*. 2018;35(2):42.
81. Kerwin BA, Remmele Jr RL. Protect from light: photodegradation and protein biologics. *J Pharm Sci*. 2007;96(6):1468-1479.
82. Mason BD, Schoneich C, Kerwin BA. Effect of pH and light on aggregation and conformation of an IgG1 mAb. *Mol Pharm*. 2012;9(4):774-790.
83. Sreedhara A, Yin J, Joyce M, et al. Effect of ambient light on IgG1 monoclonal antibodies during drug product processing and development. *Eur J Pharm Biopharm*. 2016;100:38-46.
84. Qi P, Volkin DB, Zhao H, et al. Characterization of the photodegradation of a human IgG1 monoclonal antibody formulated as a high-concentration liquid dosage form. *J Pharm Sci*. 2009;98(9):3117-3130.
85. Fradkin AH, Mozziconacci O, Schoneich C, Carpenter JF, Randolph TW. UV photodegradation of murine growth hormone: chemical analysis and immunogenicity consequences. *Eur J Pharm Biopharm*. 2014;87(2):395-402.
86. Mozziconacci O, Schoneich C. Effect of conformation on the photodegradation of Trp- and cystine-containing cyclic peptides: octreotide and somatostatin. *Mol Pharm*. 2014;11(10):3537-3546.
87. Crommelin DJ, Storm G, Verrijk R, de Leede L, Jiskoot W, Hennink WE. Shifting paradigms: biopharmaceuticals versus low molecular weight drugs. *Int J Pharm*. 2003;266(1-2):3-16.
88. Jiskoot W, Crommelin DJA. What makes protein drugs different? pharmaceutical aspects. *Eur J Hosp Pharm*. 2006;12(5):20-21.
89. Crommelin DJA, Bissig M, Gouveia W, Tredree R. Storage and handling of biopharmaceuticals: problems and solutions - a workshop discussion. *Eur J Hosp Pharm*. 2003;8:100-105.
90. Trissel LA, Gentempo JA, Saenz LM, Woodard MY, Angeles CH. Effect of two work practice changes on the microbial contamination rates of pharmacy-compounded sterile preparations. *Am J Health Syst Pharm*. 2007;64(8):837-841.
91. Prosser W, Sampath S. *Keeping the Cold Chain Cold. Vaccine Supply Chains: Reaching the Final 20 Policy Paper Series*. Seattle: VillageReach; 2014.
92. van Beusekom MM, Grootens-Wiegers P, Bos MJ, Guchelaar HJ, van den Broek JM. Low literacy and written drug information: information-seeking, leaflet evaluation and preferences, and roles for images. *Int J Clin Pharm*. 2016;38(6):1372-1379.
93. Wolf MS, Davis TC, Shrank W, et al. To err is human: patient misinterpretations of prescription drug label instructions. *Patient Educ Couns*. 2007;67(3):293-300.
94. Kessels RP. Patients' memory for medical information. *J R Soc Med*. 2003;96(5):219-222.