Impact of Residual Impurities and Contaminants on Protein Stability

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ABSTRACT: Production of recombinant proteins generates a variety of process-related impurities. The multistep manufacturing processes may introduce many potential contaminants into the final pharmaceutical products. These residual impurities and contaminants can potentially impact the protein stability significantly. In this short review, the authors intend to discuss major sources and types of residual process-related impurities and potential product contaminants, their impact on protein quality/stability, and possible mitigations during product development and manufacturing processes. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1315–1330, 2014

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INTRODUCTION

Recombinant technology has made possible mass production of proteins for different therapeutic purposes. A variety of expression systems can now be selected for production of proteins of interest with desired post-translational modifications. The expressed protein can be isolated and purified to a high degree traditionally through a combination of chromatographic steps. The purified protein is then put into a stable buffered matrix by ultrafiltration/diafiltration (UF/DF), and filled and possibly lyophilized in final drug product containers for commercialization. The multistep manufacturing processes for the protein drug substance (DS) and final drug product generate a variety of process-related impurities and provide many opportunities for potential product contamination.

Product contamination can be classified generally into two categories—microbiological and nonmicrobiological.[1,2] Although microbiological contamination can be lethal, such events should be preventable through quality assurance of manufacturing processes in appropriately controlled facilities. In addition, all injectable drug products have to meet sterility and endotoxin requirements using standardized test methods before product release. In comparison, there is not a standard set of tests to determine the presence of nonmicrobiological contaminants during the manufacturing process. This is mainly because each process may have a unique source of nonmicrobial contamination and the excipients used may differ in type and origin. Therefore, it is almost impossible to establish standardized assays with enough sensitivity to cover all possible contaminants in a biological product. Occasionally, suspicion of a potential product contamination starts with observation of an aberrant release/stability result, an unexpected clinical event, and/or a change in the pattern of efficacy and/or safety of a drug product (DP). For example, around 2008, an acute, rapid onset of serious side effects resembling an allergic-type reaction (hypotension, nausea, and shortness of breath) were observed clinically after administration of certain lots of heparin.[3] Later, it was confirmed that the vials of heparin were adulterated with oversulfated chondroitin sulfate, which was associated with the adverse reactions.[4,5]

The above example highlights the importance of controlling the level of impurities and contaminants in a drug product. In many cases, however, impurities or contaminants are not linked to aberrant release/stability results, and/or changes in clinical observations. The first step in probing any apparent product contamination is to examine the quality of the DS and DP at the time of release. A significant change in product quality at the time of release and/or a change in stability behavior during subsequent storage could be an indication of potential product contamination, which would lead to an investigation and potentially, a corrective action to prevent or minimize future product contamination.

In this short review, the authors intend to discuss major sources and types of residual process-related impurities and potential product contaminants, their impact on quality/stability, and possible mitigations during product development and manufacturing processes. International Conference on Harmonisation (ICH) Q6B defines contaminants as any adventitiously introduced materials (e.g., chemical, biochemical, or microbial species) not intended to be part of the manufacturing process of the DS or DP. Therefore, any nonproduct-related and nonprocess-related impurities or substances can be considered as contaminants, which are either detectable or nondetectable and volatile or nonvolatile, deriving from any manufacturing step, environment, product excipient, or the container/closure system. The goal is to promote awareness of potential product contamination, the origin of these contaminants, and their potential stability impact. The increased awareness would help in successful commercialization of effective and safer drug products. Description and discussion of the residual process-related impurities or different contaminants follow sequential stages of a protein product manufacturing process—(1) DS manufacturing, (2) evaluation and selection of drug product excipients, (3) evaluation and selection of a product container/closure system, and (4) finally, DP manufacturing. The main residual process-related impurities or contaminants are summarized in Table 1.

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Table 1. Summary of Main Residual Process-Related Impurities and Potential Product Contaminants, Possible Sources, Stability Effects, and Possible Mitigations

Impurities/Contaminants (Alphabetical)	Possible Sources	Potential Stability Effects	Possible Mitigations	Representative References
Carbon dioxide	Product contact with dry ice, supercritical fluid	Acidification- and interface-induced protein degradation	Choice of proper packaging systems; avoidance of contact with dry ice and supercritical fluid	6
Cleaning agents (hypochlorous and peracetic acids, etc.)	Use of cleaning agents and/or inadequate rinsing	Cleaning agent-induced protein degradation, for example, oxidation	Establishment of proper cleaning procedure	7,8
Formaldehyde/formic acid	Raw materials, excipients, processing aids	Chemical degradants, adducts, aggregates	Control of the quality of raw materials, excipients, storage conditions	9,10
Fatty acids/lipids	Host cells; expression, harvesting, raw materials, excipients	Interaction-induced instability, for example, aggregation	Optimization of processes, control the quality of raw materials, excipients	11–13
Free radicals	Raw materials, excipients, irradiation	Oxidation, clipping	Control of the quality of raw materials, excipients, lighting conditions, formulation selection	14,15
Metals	Process equipment, raw materials/excipients	Aggregation, oxidation, hydrolysis	Use of disposable containers; control of material quality; use of metal chelators	16–18
Moisture	Inadequate packaging systems	Moisture-induced degradation	Choice of proper containers, closures, processing conditions, formulation selection	19
Non-metallic surface materials or leachables	Resins, filters, tubings, containers, closures, product contact materials	Indirect effect, interaction-induced instability, for example, aggregation	Control of material quality, optimization of process conditions, formulation conditions	20–22
Nucleic acids	Host cells; expression and harvesting	Interaction-induced instability	Optimization of purification processes	23,24
Organic solvents	Processing aids	Aggregation	Minimize use or removal of organic solvents	20,25
Oxygen	Air entrapment, inadequate packaging systems	Oxidation, degradation	Optimization of process conditions, packaging systems	26
Particulates—glass-derived	Glass containers and apparatuses	Protein adsorption, aggregation	Control of container quality, formulation conditions	27,28
Particulates—nonglass-derived	Non-glass containers, product contact surfaces, air	Protein adsorption, aggregation	Control of equipment quality, processing conditions	29–31
Peroxides	Raw materials, excipients	Oxidation, aggregation	Control of the quality of raw materials, storage condition, formulation selection	13,32
Protease/host cell proteins (HCPs)	Host cells; expression and harvesting	Proteolysis, interaction-induced instability	Use of protease inhibitors, optimization of manufacturing processes, formulation selection	33–35
Reducing sugars/polyols	Raw materials, excipients, formulation	Glycation, aggregation	Control the quality of raw materials and excipients, formulation selection	36–38
Silicone oil	Containers, closures, syringes, and so on	Aggregation	Minimize use of silicone oil, use of a surfactant and/or a suitable packaging system	39,40
Vaporized decontamination agents	Clean rooms, isolators	Oxidation, aggregation	Control of the air composition in processing areas	8
Tungsten	Syringes	Aggregation	Optimization of the syringe manufacturing processes, type of syringes	41,42

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