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Review

The role of formulation in insulin comparability assessments

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Abstract

The concept of comparability can be applied when changes are made to manufacturing processes for biotechnology products subsequent to pivotal clinical trial studies. For many process changes, comparability can be demonstrated based entirely on relevant in vitro data provided that a detailed knowledge of the process/product exists, suitable analytical methodology is employed, and historical data are available for the assessment. Insulin provides an excellent model system to illustrate many important considerations when dealing with comparability exercises for biotechnology products. The physicochemical properties of insulin demonstrate the numerous chemical reactions and physical transformations that are exclusive to proteins. These properties are heavily influenced by formulation conditions and must be carefully evaluated when process changes are made. In addition, physical and chemical testing performed on representative formulations can provide valuable insight when assessing the comparability between pre- and post-change materials. This paper reviews our experience with manufacturing changes involving insulin emphasizing the important role of formulation in the comparability exercise for protein biopharmaceuticals.

Keywords: Comparability; Formulation; Insulin; Manufacturing changes; Physicochemical characterization; Practical significance

1. Introduction

Changes to manufacturing processes for biotechnology products can be implemented subsequent to pivotal clinical trials if product comparability can be adequately demonstrated [1]. Demonstration of comparability requires comparative assay data, as well as reliance on relevant development data from the sponsor. Some examples of relevant information include: assay development/validation data, development formulation data, process validation data, cell line characterization, host cell protein profiles, assessment of related substance characteristics, and effect of post-translational modification on biological response. While the type and extent of data required will depend upon the nature of the change, it is important to realize that the concept of comparability does not mean that the pre- and post-change products will be identical.

A key question that must be considered when contemplating a manufacturing change is under what circumstances can comparability be adequately demonstrated on the basis of physicochemical and in vitro biological data alone? Similarly, when are human clinical studies, particularly efficacy studies, required to demonstrate product comparability? In evaluating these fundamental questions, the following factors must be considered:

- Nature of the process change;
- Extent of process characterization data/knowledge;
- Structural complexity/heterogeneity of the product;
- Sensitivity, specificity, and breadth of analytical tools used to assess comparability; and
- Clinical relevance of the in vitro bioassay.

Each potential manufacturing change must be evaluated on a case-by-case basis with careful evaluation of all these

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factors. Ultimately, good scientific judgment is required to define the data requirements for the comparability exercise. Detailed knowledge and extensive experience with the process and product are key to successful execution of the protocol.

2. Types of manufacturing changes

Fig. 1 shows a representation of a drug product manufacturing process flow diagram illustrating the types of changes that may be encountered at each specific step. Starting with the components of the formulation potential changes might involve the active drug substance, excipients, raw materials, or reagents. Changes may also be implemented in the various manufacturing steps associated with the compounding of the formulation components. Examples in this category include: modifications to operating parameters, equipment, or facilities. Finally, changes to the finished drug product can involve lot size increases or modification of the container closure.

3. Insulin as a model system

Insulin is an excellent biotechnology product to illustrate many important considerations involved in the comparability exercise. The complex physicochemical properties of the molecule are well understood [2,3], and these can be used in a general manner to exemplify various complex structural aspects of concern when dealing with changes to production processes for proteins and their corresponding products. In addition, the insulin drug product line is very diverse with formulations including solutions, microcrystalline suspensions of which there are two distinct crystal forms, biphasic mixtures combining solutions and microcrystalline suspensions, and suspensions composed of microcrystalline and amorphous particles [4]. These various formulations are required to meet both

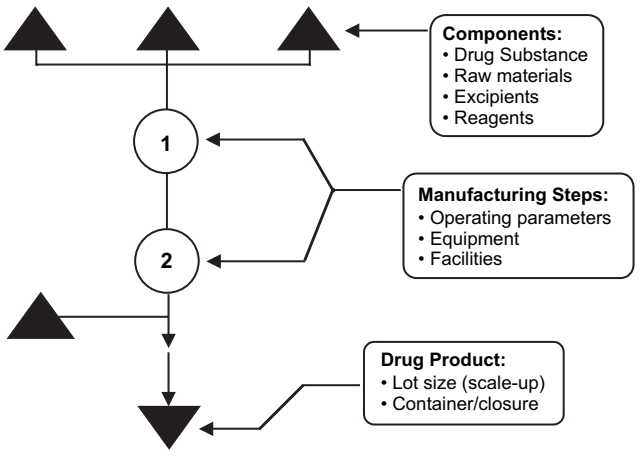

Fig. 1. Representation of a typical pharmaceutical manufacturing process flow diagram. Filled triangles pointing upward indicate components used in the manufacture of the drug product. Open circles with numbers indicate manufacturing steps involved in compounding of the components. Note that only two steps are shown for simplicity. Filled triangles pointing downward indicate the completed drug product. Boxes containing text identify aspects of the process where potential manufacturing changes may occur.

meal-related and basal insulin requirements of people with diabetes. Because the pharmacological properties are a manifestation of the formulation form, changes to manufacturing processes and/or products must be thoroughly analyzed to evaluate potential impact [3]. Insulin products are available in multi-dose vials, with some preparations also supplied in cartridge containers for use in pen delivery devices further adding to the complexity of the comparability assessment [4].

Insulin is a well-characterized protein and there is detailed understanding of the chemical degradation products known to form under a variety of conditions. The primary chemical reactions involved include deamidation and covalent polymerization of which there are three different mechanisms (transamidation, Schiff base and disulfide cross-linking) [2]. While many of these transformations are known to occur in the dry state form of the drug substance, rates of formation are relatively slow provided appropriate storage conditions are maintained. However, in the aqueous insulin formulations, the reaction rates are accelerated and in some cases specific chemical transformations, such as neutral pH deamidation and chain cleavage, are exclusive to the drug products [2]. The specific drug product related chemical considerations for insulin demonstrate the importance of knowing details of the chemistry of the drug substance and highlight the important role of formulation when assessing comparability for process changes. Chemical degradation is an issue with all therapeutic proteins and peptides, and a number of reactions in addition to the degradation pathways observed for insulin are common [5].

The physical properties of insulin also serve to illustrate additional important considerations when engaging in comparability exercises that can be generalized for other protein products. In the absence of divalent metal ions, insulin exhibits a complex pattern of self-association consisting of monomer, dimer, tetramer, hexamer and higher order species all in dynamic equilibrium and influenced by concentration, pH, ionic strength and temperature [6]. Metal ions such as zinc, included in all insulin formulations, will shift the equilibrium towards hexamer [3,7]. The propensity of insulin to self-associate is exploited in formulation. For the solution formulations, zinc is included to induce hexamer formation producing stable preparations [8]. The protracted-acting suspension products are also made possible because of the unique self-association property of insulin that predisposes it to favorably crystallize [3,4]. While insulin has a specific pattern of native assembly, self-association is not unique to this particular protein. Many proteins have a tendency to self-associate as a result of their specific properties, or when exposed to certain solution conditions [9].

Insulin is also known to undergo non-native assembly when exposed to organic solvents, extremes in temperature and pH, or agitation. This phenomenon has been extensively studied and the mechanism appears to be initiated by partial unfolding of the protein followed by aggregation ultimately leading to fibril growth and precipitation [10]. Non-native aggregation is not unique to insulin, as the process has been observed for other therapeutic proteins [11–13]. Protein aggregation is of particular concern in assessing comparability because