

9.9.6.1 Physicochemical Properties

A physicochemical characterization program will generally include the determination of the composition, physical properties, and primary structure of the desired product. In some cases, information regarding the HOS of the desired product (the fidelity of which is generally inferred by its biological activity) may be obtained by appropriate physicochemical methodologies.

An inherent degree of structural heterogeneity occurs in proteins, owing to the biosynthetic processes used by living organisms to produce them; therefore, the desired product can be a mixture of anticipated post-translationally modified forms (e.g., glycoforms). These forms may be active, and their presence may have no deleterious effect on the safety and efficacy of the product. The manufacturer should define the pattern of heterogeneity of the desired product and demonstrate consistency with that of the lots used in preclinical and clinical studies. If a consistent pattern of product heterogeneity is demonstrated, an evaluation of the activity, efficacy, and safety (including immunogenicity) of individual forms may not be necessary.

Heterogeneity can also be produced during manufacture and/or storage of the DS or DP. As the heterogeneity of these products defines their quality, the degree and profile of this heterogeneity should be characterized to ensure lot-to-lot consistency. When these variants of the desired product have properties comparable with those of the desired product with respect to activity, efficacy, and safety, they are considered product-related substances. When process changes and degradation products result in heterogeneity patterns that differ from those observed in the material used during the preclinical and clinical development, the significance of these alterations should be evaluated.

9.9.7 Biological Activity

Assessment of the biological properties constitutes an equally essential step in establishing a complete characterization profile. An important property is the biological activity that describes the specific ability or capacity of a product to achieve a defined biological effect.

A valid biological assay to measure the biological activity should be provided by the manufacturer. Examples of procedures used to measure biological activity include the following:

- Animal-based biological assays, which measure an organism's biological response to the product
- Cell culture-based biological assays, which measure biochemical or physiological response at the cellular level
- Biochemical assays, which measure biological activities, such as enzymatic reaction rates and biological responses induced by immunological interactions

Other procedures, such as ligand- and receptor-binding assays, may be acceptable.

Potency (expressed in units) is the quantitative measure of biological activity, based on the attribute of the product that is linked to the relevant biological properties, whereas quantity (expressed in mass) is a physicochemical measure of protein content.