- Lower eukaryotes such as yeasts and fungi are able to make relatively simple PTMs such as glycoproteins. Since yeasts produce *N*-glycosylations rich in mannose residues, which are strongly immunogenic for humans, the choice may be limited.
- Higher eukaryotes such as mammalian cells and insect or plant cells provide much lower yields and are more difficult to manage. Mammalian cells such as ovarian cells of Chinese hamsters (CHO cells) are commonly used to produce complex glycoproteins.
- Plant and transgenic animals produce therapeutic proteins in tissue (for the plant) or in a fluid (most often milk for transgenic animals) in large quantities and are highly practical sources of future biological products.

Given that living entities are involved in the expression of proteins, it is easy to see how a change in bioprocessing conditions can readily alter the structure of proteins. More difficult to manage are the variance in the glycan patterns, the antibody dependent cellular toxicity (ADCC), and other similar variations that might not necessarily be clinically meaningful, yet to prove it otherwise may be impossible, resulting in a biosimilar product developer to modify the bioprocessing to assure that the structure of protein expressed is as close to the originator molecule as possible.

## 9.7 Preformulation Considerations

An early characterization of biopharmaceuticals (proteins) is needed to evaluate the comparability of materials, which is more complicated owing to the inherently heterogeneous nature of many biologicals. This includes factors such as microheterogeneity of glycosylation, differential proteolytic processing during cellular production, and variations in PTMs, factors that are not common to small-molecule characterization and evaluation for interaction. This requires the availability of highly specific discriminating methodologies, such as spectrophotometric, chromatographic, electrophoretic methods, and mass spectroscopy (MS), often combined with liquid chromatography (LC).

Unlike small-molecule drugs, there are 3D and four-dimensional (4D) considerations (aggregates) with almost endless variations of polypeptides and proteins that make them a challenge to develop into products. Whereas in small-molecule drugs, there can be classes of drugs with common elements, this is not the case with protein drugs, as each one of them offers a unique structure, requiring techniques of production and purification specific to the protein. The same holds true for the stability profile of these compounds. Specification of biopharmaceutical drugs also includes elements not found in small molecules such as virus clearance, aggregate formation, and so on. As a result, the regulatory authorities worldwide treat biopharmaceutical drugs under separate administration, wherein a high level of expertise is inducted to evaluate these products.

Marketing authorization approvals for biological products are subject to a similar process as adopted for chemical drugs; however, the nature of these products mandates special evaluation and monitoring techniques. As a result, historically, the U.S. FDA has established separate sections for these products. Title 21 of the Code of Federal