SECTION 1

MANUFACTURING SPECIALTIES
1.1

BIOTECHNOLOGY-DERIVED DRUG PRODUCT DEVELOPMENT

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INTRODUCTION

Although the origins of the first biological and/or protein therapeutics can be traced to insulin in 1922, the first biotechnology-derived pharmaceutical drug product approved in the United States was Humulin in 1982. In the early stages of pharmaceutical biotechnology, companies that specialized primarily in the development of biologicals were the greatest source of research and development in this area. Recent advances in molecular and cellular biological techniques and the potential clinical benefits of biotechnology drug products have led to a substantial increase in their development by biotechnology and traditional pharmaceutical companies. In terms of pharmaceuticals, the International Conference on Harmonization (ICH) loosely defines biotechnology-derived products with biological origin products as those that are “well-characterized proteins and polypeptides, their derivatives and products of which they are components, and which are isolated from tissues, body fluids, cell cultures, or produced using rDNA technology” [1]. In practical terms, biological and biotechnology-derived pharmaceutical agents encompass a number of therapeutic classes, including cytokines, erythropoietins, plasminogen activators, blood plasma factors, growth hormones and growth factors, insulins, monoclonal antibodies, and vaccines [1]. Additionally, short interfering and short hairpin ribonucleic acids (siRNA, shRNA) and antisense oligonucleotide therapies are generally characterized as biotechnology-derived products.

According to the biotechnology advocacy group, The Biotechnology Industry Organization (BIO), pharmaceutical-based biotechnology represents over a $30 billion dollar a year industry and is directly responsible for the production of greater than 160 drug therapeutics and vaccines [2]. Furthermore, there are more than 370 biotechnology-derived drug products and vaccines currently in clinical trials around the world, targeting more than 200 diseases, including various cancers, Alzheimer’s disease, heart disease, diabetes, multiple sclerosis, acquired immunodeficiency syndrome (AIDS), and arthritis. While the clinical value of these products is well recognized, a far greater number of biotechnology-derived drug products with therapeutic potential for life-altering diseases have failed in development.

As the appreciation of the clinical importance and commercial potential for biological products grows, new challenges are arising based on the many technological limitations related to the development and marketing of these complex agents. Additionally, the intellectual property protection of an associated agent might not
provide a sufficient window to market and regain the costs associated with the discovery, research, development, and scale-up of these products. Therefore, to properly estimate the potential return on investment, a clear assessment of potential therapeutic advantages and disadvantages, such as the technological limitations in the rigorous characterization required of these complex therapeutic agents to gain Food and Drug Administration (FDA) approval, is needed prior to initiating research. Clearly, research focused on developing methodologies to minimize these technological limitations is needed. In doing so we hypothesize the attrition rate can be reduced and the number of companies engaged in the development of biotechnology-derived products and diversity of products will continue to expand.

Technological limitations have limited the development of follow-on, or generic biopharmaceutical products that have lost patent protection. In fact, the potential pitfalls associated with developing these compounds are so diverse that regulatory guidance concerning follow-on biologics is relatively obscure and essentially notes that products will be assessed on a case-by-case basis. The reader is encouraged to see Chapter 1.2 for a more detailed discussion concerning regulatory perspectives pertaining to follow-on biologics.

Many of the greatest challenges in producing biotechnology-derived pharmaceuticals are encountered in evaluating and validating the chemical and physical nature of the host expression system and the subsequent active pharmaceutical ingredient (API) as they are transferred from discovery through to the development and marketing stages. Although this area is currently a hotbed of research and is progressing steadily, limitations in analytical technologies are responsible for a high degree of attrition of these compounds. The problem is primarily associated with limited resolution of the analytical technologies utilized for product characterization. For example, without the ability to resolve small differences in secondary or tertiary structure, linking changes to product performance or clinical response is impossible. The biological activity of traditional small molecules is related directly to their structure and can be determined readily by nuclear magnetic resonance (NMR), X-Ray crystallography (X-ray), mass spectrometry (MS), and/or a combination of other spectroscopic techniques. However, methodologies utilized for characterizing biological agents are limited by resolution and reproducibility. For instance, circular dichroism (CD) is generally considered a good method to determine secondary structural elements and provides some information on the folding patterns (tertiary structure) of proteins. However, CD suffers from several limitations, including a lower resolution that is due in part to the sequence libraries used to deconvolute the spectra. To improve the reliability of determining the secondary and tertiary structural elements, these databases need to be developed further. An additional example is the utility of two-dimensional NMR (2D-NMR) for structural determination. While combining homonuclear and heteronuclear experimental techniques can prove useful in structural determination, there are challenges in that 2D-NMR for a protein could potentially generate thousands of signals. The ability to assign specific signals to each atom and their respective interactions is a daunting task. Resolution between the different amino acids in the primary sequence and their positioning in the covalent and folded structures become limited with increasing molecular weight. Higher dimensional techniques can be used to improve resolution; however, the resolution of these methods remains limited as the number of amino acids is increased.
Understand the limitations of the analytical methodologies utilized for product characterization has led to the development of new experimental techniques as well as the refined application of well-established techniques to this emerging field. Only through application of a number of complementary techniques will development scientists be able to accurately characterize and develop clinically useful products. Unfortunately, much of the technology is still in its infancy and does not allow for a more in-depth understanding of the subtleties of peptide and protein processing and manufacturing. For instance, many of the analytical techniques utilized for characterization will evaluate changes to product conformation on the macroscopic level, such as potential denaturation or changes in folding, as observed with CD. However, these techniques do not afford the resolution to identify subtle changes in conformation that may either induce chemical or physical instabilities or unmask antigenic epitopes.

Further limiting successful product development is a lack of basic understanding as to critical manufacturing processes that have the potential to affect the structural integrity and activity of biopharmaceuticals. As with traditional small molecules, stresses associated with the different unit operations may affect biopharmaceutical products differently. In contrast to traditional small molecules, there is considerable difficulty in identifying potentially adverse affects, if any, that a particular unit operation may have on the clinically critical structural elements of a drug. Considering that many proteins exhibit a greater potential for degradation from shear stress, it is particularly important to assess any negative effects of mixing, transport through tubing, filtration, and filling operations. Essentially all unit operations for a given manufacturing process could create enough shear stress to induce minor structural changes that could lead to product failure. The difficulty is establishing what degree of change will have an impact on the stability, bioactivity, or immunogenic potential of the compound. Unfortunately, unless exhaustive formulation development studies are conducted, coupled with a comprehensive spectrum of analytical methodologies, these effects may not be readily evident until after scale-up of the manufacturing process or, worse yet, in the clinical setting. Moreover, modeling shear and stress using fluid dynamic structurally diverse molecules is a foreboding task. Extending these models to validate process analytical technologies (PAT) and incorporate critical quality by design (QbD) elements in the development process for a collection of biopharmaceuticals would be largely hindered by the daunting nature of the task at hand.

The use of biological systems to produce these agents results in additional variability. Slight changes in nutrient profile could affect growth patterns and protein expression of cultured cells. Furthermore, microbial contamination in the form of viruses, bacteria, fungi, and mycoplasma can be introduced during establishment of cell lines, cell culture/fermentation, capture and downstream processing steps, formulation and filling operations, or drug delivery [3]. Therefore, establishing the useful life span of purification media and separation columns remains a critical issue for consistently producing intermediates and final products that meet the defined quality and safety attributes of the product [4]. In short, understanding the proper processability and manufacturing controls needed has been a major hurdle that has kept broader development of biopharmaceutical products relatively limited.

Notwithstanding the many technological hurdles to successfully develop a pharmaceutically active biotechnology product, they offer many advantages in terms of
therapeutic potency, specificity, and target design (not generally limited to a particular class or series of compounds). This is an iterative approach, whereby every new approved compound, new lessons, and applications to ensure successful product development are realized, thereby adding to our knowledge base and facilitating the development of future products. This chapter will discuss some of the fundamental issues associated with successful biopharmaceutical drug product development and aims to provide an understanding of the subtleties associated with their characterization, processing, and manufacturing.

1.1.2 FORMULATION ASSESSMENT

In order to select the most appropriate formulation and route of administration for a drug product, one must first assess the properties of the API, the proposed therapeutic indication, and the requirements/limitations of the drug and the target patient population. Development teams are interdisciplinary comprised of individuals with broad expertise, for example, chemistry, biochemistry, bioengineering, and pharmaceutics, that can provide insight into the challenges facing successful product development. As such, knowledge gained through refinement of the API manufacturing process through to lead optimization is vital to providing an initial starting point for success. Information acquired, for example, in the way of analytical development and API characterization, during drug discovery or early preclinical development that can be applied to final drug product development may contribute to shorter development times of successful products.

The host system utilized for API production is critical to the production of the final product and will determine the basic and higher order physicochemical characteristics of the drug. Typically biopharmaceuticals are manufactured in *Escherichia coli* as prokaryotic and yeast and Chinese hamster ovary (CHO) cells as eukaryotic expression systems [5]. While general procedures for growth condition optimization and processing and purification paradigms have emerged, differences in posttranslational modifications and host–system related impurities can exist even with relatively minor processing changes within a single production cell line [5]. Such changes have the potential to alter the biopharmaceutical properties of the active compound, its bioactivity, and its potential to elicit adverse events such as immunogenic reactions. These properties will be a common theme as they could potentially play a major role in both analytical and formulation development activities.

During the process of lead optimization, characterization work is performed that would include a number of parameters that are critical to formulation and analytical development scientists. The following information is a minimalist look at what information should be available to support product development scientists:

- Color
- Particle size and morphology (for solid isolates)
- Thermoanalytical profile (e.g., $T_g$ for lyophiles)
- Hygroscopicity
- Solubility with respect to pH
- Apparent solution pH
• Number and pKₐ of ionizable groups
• Amino acid sequence
• Secondary and tertiary structural characteristics
• Some stability parameters with respect to
  - pH
  - Temperature
  - Humidity
  - Light
  - Mechanical stress
  - Oxygen sensitivity
• Impurity profile
  - Misfolded/misaligned active
  - Potential isoforms
  - Expression system impurities
• Potency [median inhibitory concentration (IC₅₀)]
• Animal Pharmacokinetic/Pharmacodynamic (PK/PD) and Tₗox profiles

All of the above information will prove invaluable in determining the potential methods for rational drug delivery. Particular attention should be paid to the relative hygroscopicity of the API, of course, any stability information, as well as the impurity profile and ADMET (absorption, distribution, metabolism, excretion, and toxicity) information. In short, the more information that is available when development activities are initiated, the easier it is to avoid common pitfalls and make development decisions more rationally.

1.1.2.1 Route of Administration and Dosage

Biologics are traditionally very potent molecules that may require only picomolar blood concentrations to elicit a therapeutic effect. Given that the amount of drug required per dosage will be commensurate with the relative potency of the molecule, small concentrations are generally required for any unit dose. Biopharmaceuticals typically have large molecular weights relative to conventional pharmaceutical agents, which may be increased further by posttranslational modifications. The pharmacokinetics (ADMET) of biotechnology products have been reviewed elsewhere [6], but generally they have short circulating half-lives [7]. As such, biological products are most often delivered parenterally and formulated as solutions, suspensions, or lyophilized products for reconstitution [8, 9]. However, one must first ascertain the potential physiological barriers to drug delivery and efficacy before assessing potential routes of administration. These barriers may include actual physical barriers, such as a cell membrane, that could restrict the drug from reaching its site of action or chemical barriers, including pH or enzymatic degradation. Based on current drug delivery approaches, the proteinaceous nature of biological products limits their peroral delivery due to their susceptibility to proteases and peptidases present in the gastrointestinal tract as well as size limitations for permeating through absorptive enterocytes [10].
Difficulties in peroral delivery have stimulated researchers to explore alternate delivery mechanisms for biologics, such as through the lungs or nasal mucosa [11, 12]. Further, advances in technology and our understanding of the mechanisms limiting oral delivery of biotechnology products have led to innovative drug delivery approaches to achieve sufficient oral bioavailability. However, no viable products have successfully reached the market [13]. As a result of the technological limitations inherent in biopharmaceutical delivery, these compounds are largely delivered parenterally through an injection or implant.

When assessing the potential routes of administration, one must consider the physicochemical properties of the drug, its ADMET properties, the therapeutic indication, and the patient population, some of which are discussed below. Table 1 provides a list of some of those factors that must be addressed when determining the most favorable route of administration and the subsequent formulation for delivery. Ideally the route of administration and subsequent formulation will be optimized after identifying critical design parameters to satisfy the needs of patients and health care professionals alike while maintaining the safety and efficacy of the product.

Parenteral administration is the primary route of delivering biopharmaceutical agents (e.g., insulin); however, issues associated with patient compliance with administration of short-acting molecules are a challenge. Yet, the risk-to-benefit ratio must be weighed when determining such fundamental characteristics of the final dosage form. For instance, a number of biopharmaceutical compounds are administered subcutaneously, but this route of parenteral administration exhibits the highest potential for immunogenic adverse events due to the presence of Langerhans cells [14]. A compound’s immunogenic potential is related to a host of factors, both

<table>
<thead>
<tr>
<th><strong>TABLE 1</strong> Factors That Determine Route of Administration</th>
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<tbody>
<tr>
<td>Site of action</td>
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<tr>
<td>Therapeutic indication</td>
</tr>
<tr>
<td>Dosage</td>
</tr>
<tr>
<td>Potency/biological activity</td>
</tr>
<tr>
<td>Pharmacokinetic profile</td>
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<tr>
<td>Absorption time from tissue vs. IV</td>
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<tr>
<td>Circulating half-life</td>
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<tr>
<td>Distribution and elimination kinetics</td>
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<tr>
<td>Toxicological profile</td>
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<tr>
<td>Immunogenic potential</td>
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<tr>
<td>Patient population characteristics</td>
</tr>
<tr>
<td>Disease state</td>
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<tr>
<td>Pathophysiology</td>
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<tr>
<td>Age</td>
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<tr>
<td>Pharmacodynamic profile</td>
</tr>
<tr>
<td>Onset and duration of action</td>
</tr>
<tr>
<td>Required clinical effect</td>
</tr>
<tr>
<td>Formulation considerations</td>
</tr>
<tr>
<td>Stability</td>
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<tr>
<td>Impurity profile</td>
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</table>
patient and treatment related; however, if an alternate, potentially safer route of administration is available, it may be prudent to consider it. Other factors, such as the frequency of dosing (especially into an immune organ such as the skin) and the duration of treatment, can also dramatically increase the potential for immunogenic reactions [14]. Many of the factors that contribute to the immunogenic potential of biopharmaceuticals, such as impurities, degradation products, and native antigenic epitopes, can be mitigated through altering the physicochemical properties of the drug (e.g., pegylation [15, 16], acylation [17, 18], increased glycosylation to mask epitopes [19]) or changing the characteristics of the formulation [20, 21]. In reality, the pharmaceutical industry has done a good job of recognizing the potential implications of immunogenic reactions and readily embraced technologies that can either mask or eliminate potential antigenic epitopes. However, additional research is needed to further identify and remove immunogenic epitopes.

1.1.2.2 Pharmacokinetic Implications to Dosage Form Design

Biological agents are generally eliminated by metabolism into di- and tripeptides, amino acids, and smaller components for subsequent absorption as nutrients or clearance by the kidney, liver, or other routes. Renal elimination of peptides and proteins occur primarily via three distinct mechanisms. The first involves the glomerular filtration of low-molecular-weight proteins followed by reabsorption into endocytic vesicles in the proximal tubule and subsequent hydrolysis into small peptide fragments and amino acids [22]. Interleukin 11 (IL-11) [23], IL-2 [24], insulin [25], and growth hormone [26] have been shown to be eliminated by this method. The second involves hydrolysis of the compound at the brush border of the lumen and subsequent reabsorption of the resulting metabolites [6]. This route of elimination applies to small linear peptides such as angiotensin I and II, bradykinin, glucagons, and leutinizing hormone releasing hormone (LHRH) [6, 27, 28]. The third route of renal elimination involves peritubular extraction from postglomerular capillaries and intracellular metabolism [6]. Hepatic elimination may also play a major role in the metabolism of peptides and proteins; however, reticuloendothelial elimination is by far the primary elimination route for large macromolecular compounds [29].

Biopharmaceutical drug products are subject to the same principles of pharmacokinetics and exposure/response correlations as conventional small molecules [6]. However, these products are subject to numerous pitfalls due to their similarity to nutrients and endogenous proteins and the evolutionary mechanisms to break them down or prevent absorption. The types of pharmacokinetic-related problems that a biotechnology drug development team may encounter range from lack of specificity and sensitivity of bioanalytical assays to low bioavailability and rapid drug elimination from the system [6]. For example, most peptides have hormone activity and usually short elimination half-lives which can be desirable for close regulation of their endogenous levels and function. On the other hand, some proteins such as albumin or antibodies have half-lives of several days and formulation strategies must be designed to account for these extended elimination times [6]. For example, the reported terminal half-life for SB209763, a humanized monoclonal antibody against respiratory syncytial virus, was reported as 22–50 days [30]. Furthermore, some peptide and protein products that persist in the bloodstream exhibit the potential for idiosyncratic adverse affects as well as increased immunogenic poten-
tial. Therefore, the indication and formulation strategy can prove crucial design parameters simply based on clearance mechanisms.

1.1.2.3 Controlled-Release Delivery Systems

Given that the majority of biopharmaceutical products are indicated for chronic conditions and may require repeated administrations, products may be amenable to controlled-release drug delivery systems. Examples include Lupron Depot (leuprolide acetate), which is delivered subcutaneously in microspheres [31], and Viadur, which is implanted subcutaneously [32]. Various peptide/protein controlled delivery systems have been reviewed recently by Degim and Celebi and include biodegradable and nondegradable microspheres, microcapsules, nanocapsules, injectable implants, diffusion-controlled hydrogels and other hydrophilic systems, microemulsions and multiple emulsions, and the use of iontophoresis or electroporation [33]. These systems offer specific advantages over traditional delivery mechanisms when the drug is highly potent and if prolonged administration greater than one week is required [5, 33]. However, each of these systems has its own unique processing and manufacturing hurdles that must be addressed on a case-by-case basis. These factors, coupled with the difficulties of maintaining product stability, limit the widespread application of these technologies. However, the introduction of postapproval extended-release formulations may also provide the innovator company extended patent/commercial utility life and, as such, remains a viable option for postmarketing development. A current example of this is observed in the development of a long-acting release formulation of Amylin and Eli Lilly’s co-marketed Byetta product.

1.1.3 ANALYTICAL METHOD DEVELOPMENT

The physical and chemical characterization of any pharmaceutical product is only as reliable as the quality of the analytical methodologies utilized to assess it. Without question, the role of analytical services to the overall drug product development process is invaluable. Good analytical testing with proper controls could mean the difference between a marketable product and one that is eliminated from development. Analytical methodologies intended for characterization and/or assessment of marketed pharmaceutical products must be relevant, validatable, and transferable to manufacturing/quality assurance laboratories.

1.1.3.1 Traditional and Biophysical Analytical Methodologies

Typically, there are a handful of traditional analytical methodologies that are utilized to assess the physical, chemical, and microbiological attributes of small-molecule pharmaceutical products. While many of these testing paradigms can still be utilized to assess biopharmaceuticals, these molecules require additional biophysical, microbiological, and immunogenic characterization as well. In brief, analytical methodologies should evaluate the purity and bioactivity of the product and must also be suitable to assess potential contaminants from expression systems as well as different isoforms and degradation products of the active. Biophysical
methodologies allow for assessment of the structural elements of the product with respect to its activity. Such assessments include structural elements, such as the folding of the molecule, and also encompass potential posttranslational modifications and their impact on structure. A list of typical analytical parameters and methodologies utilized to assess those parameters can be found in Table 2.

The impact of a molecule’s biophysical characteristics on its clinical efficacy should be readily quantifiable. With respect to rational drug design, it is also extremely important to minimize external factors that may influence the formation of any adverse response. One such factor is the presence of degradation products and drug-related impurities that may be responsible for an immune response. One such industrial example is granulocyte-macrophage colony-stimulating factor [GM-CSF, or Leukine (sargramostim), by Berlex Co.], which is produced as a recombinant protein synthesized and purified from a yeast culture, Saccharomyces cerevisiae. As expected, the expression system has an impact on the final product: sargramostim, manufactured from S. cerevisiae, yields an O-glycosylated protein, while molgramostim (Leucomax), synthesized using an E. coli expression system, is nonglycosylated [34]. The E. coli-derived product exhibited a higher incidence of adverse reactions in clinical trials and never made it to the market. With respect to the drug product, the immunogenic reactions included [34, 35]:

<table>
<thead>
<tr>
<th>Parameter Assessed</th>
<th>Methodologies</th>
<th>Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Visual appearance, colorimetric assays, turbidity</td>
<td>Simple determination of physical stability, i.e., are there particles in solution, is the solution the correct color/turbidity? Is the container closure system seemingly intact?</td>
</tr>
<tr>
<td>Purity, degradation products and related substances</td>
<td>GPC/SEC-HPLC, RP-HPLC, gel electrophoresis, immunoassays, IEF, MS, CD, CE</td>
<td>Gives a general idea of the relative purity of the API and the drug product. Are there impurities related to the expression system? Are there alternate API isoforms present? Can degradation products be distinguished from the active component(s)?</td>
</tr>
<tr>
<td>Molecular weight determination</td>
<td>GPC/SEC-HPLC, gel electrophoresis, multiangle laser light scattering (MALLS), laser diffraction</td>
<td>Is the product a single molecular weight or polydisperse? Is the molecular weight dependent on posttranslational modifications?</td>
</tr>
<tr>
<td>Potency</td>
<td>Biological activity (direct or indirect)</td>
<td>Does the compound have reproducible in vitro activity and can this be correlated to in vivo?</td>
</tr>
<tr>
<td>pH</td>
<td>Potentiometric assays</td>
<td>Is the product pH labile or do pH changes affect potency is such ways that are not evident in other assays, i.e., minimal degradation and/or unfolding?</td>
</tr>
</tbody>
</table>
### TABLE 2  Continued

<table>
<thead>
<tr>
<th>Parameter Assessed</th>
<th>Methodologies</th>
<th>Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary structural elements</td>
<td>Protein sequencing, N-term degradation (Edman degradation), peptide mapping, amino acid composition, 2D-NMR</td>
<td>Verifies primary amino acid sequence and gives preliminary insight into activity.</td>
</tr>
<tr>
<td>Secondary structural elements</td>
<td>CD, 2D-NMR, in silico modeling from AA sequence</td>
<td>Secondary structural elements result from the primary sequence and help define the overall conformation (3D folding) of the compound.</td>
</tr>
<tr>
<td>Tertiary structural elements</td>
<td>Disulfide content/position, CD</td>
<td>Determines correct folding and overall integrity of the 3D product. Qualitative determination for denaturation potential. Also correlates to immunogenic potential.</td>
</tr>
<tr>
<td>Agglomeration/ aggregation</td>
<td>Subvisual and visual Particle size analysis, immunogenicity</td>
<td>Indicator of physical instability. Also gives an indication of immunogenic potential.</td>
</tr>
<tr>
<td>Carbohydrate analysis</td>
<td>RP-HPLC, gel electrophoresis, AE-HPLC, CE, MALDI-MS, ES-MS, enzyme arrays</td>
<td>Ensures proper posttranslational modifications and carbohydrate content.</td>
</tr>
<tr>
<td>Water content (lyophilized products)</td>
<td>Karl Fischer, TGA, NIR</td>
<td>Indicator of hydrolytic potential and process efficiency.</td>
</tr>
<tr>
<td>Immunogenic potential</td>
<td>Surface plasmon resonance, ELISA, immunoprecipitation</td>
<td>Methodologies generally only give positive/negative indicators of immunogenic potential. In vitro methodologies do not always correlate to in vivo.</td>
</tr>
<tr>
<td>Sterility</td>
<td>Membrane filtration</td>
<td>Indicator of microbial contaminants from manufacturing operations.</td>
</tr>
<tr>
<td>Bacterial endotoxins</td>
<td>Limulus amebocyte lysate (LAL)</td>
<td>Gives an idea of processing contaminants and potentially host organism contaminants.</td>
</tr>
<tr>
<td>Container closure integrity</td>
<td>Dye immersion, NIR, microbial ingress/sterility</td>
<td>Demonstrates viability of container closure system over the life of the product.</td>
</tr>
</tbody>
</table>

*Abbreviations:* gel permeation chromatography (GPC), size exclusion chromatography (SEC), high-performance, or high-pressure, liquid chromatography (HPLC), reverse phase (RP), isoelectric focusing (IEF), mass spectrometry (MS), circular dichroism (CD), capillary electrophoresis (CE), nuclear magnetic resonance (NMR), anion exchange (AE), matrix-assisted laser desorption ionization (MALDI), electrospray ionization (ES), thermogravimetric analysis (TGA), near infrared (NIR), enzyme-linked immunosorbent assay (ELISA)
1. Formation of antibodies which bind and neutralize the GM-CSF
2. Formation of antibodies which bind but do not affect the efficacy of GM-CSF
3. Antibody formation against proteins not related to GM-CSF, but to proteins from the expression system (E. coli)
4. Antibodies formed against both product- and non-product-related proteins
5. No antibody formation

This example clearly illustrates not only the range of clinical manifestations with respect to antibody formation to drug therapy but also how the choice of an expression system can affect the final product. In this example, the expression system was responsible for the adverse events reported. This finding is certainly clinically relevant considering the homologous product, sargramostim, has been on the U.S. market for quite some time.

The above example also gives an indication of the relative importance of carbohydrate analysis. Without question, protein glycosylation is the most complex of all posttranslational modifications made in eukaryotic cells, the importance of which cannot be underestimated. For many compounds, glycosylation can readily affect protein solubility (as influenced by folding), protease resistance, immunogenicity, and pharmacokinetic/pharmacodynamic profiles (i.e., clearance and efficacy) [36]. Typical analytical methodologies used to assess carbohydrate content are also listed in Table 2.

### 1.1.3.2 Stability-Indicating Methodologies

Analytical methodologies that are specific to the major analyte that are also capable of separating and quantifying potential degradation products and impurities, while simultaneously maintaining specificity and accuracy, are deemed stability indicating. Traditional stability-indicating high-performance liquid chromatography (HPLC) methodologies for small molecules are developed and validated with relative ease. Typically, the stability-indicating nature of an analytical method can be demonstrated by subjecting the product to forced degradation in the presence of heat, acid, alkali, light, or peroxide [37]. If degradation products are sufficiently well resolved from the active while maintaining specificity and accuracy, the method is suitable. In contrast to small molecules, there is no one “gold standard” analytical methodology that can be utilized to determine the potential degradation products and impurities in the milieu that may constitute a biopharmaceutical drug product. Furthermore, a one-dimensional structure assessment (e.g., in terms of an absorption spectrum) does not give any indication of the overall activity of the product, as is the case with traditional small molecules. Thus, the stability assessment of biopharmaceuticals will typically comprise a multitude of methodologies that when taken together give an indication of the stability of the product. The overall goal is to assess the structural elements of the compound as well as attempt to determine the relative quantities of potential degradation products, as well as product isoforms and impurities, that are inherent to the expression systems utilized for API manufacture. However, it is still advised that bioactivity determinations are made at appropriate intervals throughout the stability program, as discussed below. Furthermore, any biopharma-
ceutical stability program should also minimally include an evaluation of the in vitro immunogenicity profile of the product with respect to time, temperature, and other potential degradative conditions.

1.1.3.3 Method Validation and Transfer

Analytical method validation is the process by which scientists prove that the analytical method is suitable for its intended use. Guidances available on validation procedures for some traditional analytical methodologies [38] can be adapted to nontraditional methodologies. The United States Pharmacopeia (USP) and National Formulary (NF) do provide some guidance on designing and assessing biological assays [39], as does the U.S. FDA [40]. Essentially, validation determines the acceptable working ranges of a method and the limitations of that method. At a minimum the robustness, precision, and accuracy of quantitative methodologies should be determined during support of API iteration and refinement, while at the very least the robustness of qualitative methodologies should be assessed. Of particular importance for successful analytical method validation is ensuring that the proper standards and system suitability compounds have been chosen and are representative or analogous to the compound to be analyzed and traceable to a known origin standard, such as the National Institute of Standards and Technology (NIST) or USP/NF. If a reference standard from an “official” source is not available, in-house standards may be used provided they are of the highest purity that can be reasonably obtained and are thoroughly characterized to ensure its identity, strength, quality, purity, and potency.

Methods developed and validated during the product development phase are routinely transferred to quality control or contract laboratories to facilitate release and in-process testing of production batches. Ensuring that method transfer is executed properly, with well-defined and reproducible system suitability and acceptance criteria, is the responsibility of both laboratories. Experiments should consist of all those parameters assessed during method validation and should include an evaluation of laboratory-to-laboratory variation. This information will give an idea of the reliability of the methodology and equipment used under the rigors of large-scale manufacturing.

1.1.4 FORMULATION DEVELOPMENT

The previous sections have highlighted some of the limitations and difficulties in developing biotechnology-derived pharmaceuticals. Although there are major technological limitations in working with these products, their synthesis and manufacturing are significantly more reproducible compared to naturally derived biologics. Determining the most appropriate route of administration and subsequent formulation is dependent on a number of factors, including the product’s indication, duration of action, pharmacokinetic parameters, stability profile, and toxicity. As mentioned previously, biopharmaceuticals are typically delivered parenterally, and thus we will focus on those studies required to successfully develop a parenteral formulation of a biopharmaceutical agent. The goal of formulation development is to design a dosage form that ensures the safety and efficacy of the product through-
out its shelf life while simultaneously addressing the clinical needs of both the patient and caregivers to ensure compliance. Formulation development is truly a balancing act, attempting to emphasize the benefits of the therapy and patient compliance while maximizing drug efficacy and minimizing toxicity. As such, a number of studies are required to properly design and develop a formulation, many of which are discussed below.

1.1.4.1 Processing Materials and Equipment

An important factor in the quality and reproducibility of any formulation development activity is the materials utilized for formulating and processing studies. In addition, the choice of container closure systems for the API and the formulation needs to be considered carefully to provide maximum product protection and optimal stability. Variability between small- and larger scale development stages may also be significant depending on the API and materials involved during process scale-up. It is important to conduct process development studies utilizing equipment representative of what will be used for large-scale production, if possible. Implementing this design approach will enable at least some limited dimensional analysis, allowing for early identification of critical design parameters, thereby facilitating scale-up or permitting earlier attrition decisions and cost savings. Regardless, it is important to consider the chemical composition and material properties of every manufacturing component that may contact the drug product. For instance, processing vessels may be made of glass, glass-lined steel, or bare steel, while stir paddles used to ensure homogeneity may be manufactured of a number of different materials. In short, any manufacturing unit that could potentially come into intimate contact with either the formulation or the API should be demonstrated to be compatible with the product, including sampling instruments, sample vials, analytical and processing tubing, and so forth. Material incompatibility could result in something as simple as unexplained analytical variability due to a loss of drug through adsorptive mechanisms to something as serious as a loss of bioactivity or an increase in immunogenic potential. Therefore, equipment design and materials would ideally be consistent from formulation development through to scale-up and process validation; however, this may not be readily feasible. As such, determining the chemical and physical compatibility of each piece of processing equipment with the API is critical to maintaining the physical and chemical attributes of the product. Furthermore, such studies help eliminate potential sources of experimental variability and give a better indicator as to the relative technological hurdles to successful product development.

Material compatibility protocols must be clearly defined and require that analytical methodologies be suitable for their intended use. Typically, product purity methods and cleaning methodologies utilized to determine residual contaminating product on processing equipment are used for compatibility studies as they are sufficiently sensitive and rugged to accurately determine product content in the presence of a multitude of potential confounding factors. This is particularly important when assessing potential metal, glass, and tubing compatibilities. Compatibility is a function not only of the product’s intimate contact with surrounding materials but also of the contact time and surface area with these equipment. As such, protocols should be designed to incorporate expected real-world conditions the product will
see when in contact with the material. For instance, temperature, light, and mechanical stimulation should mimic usage conditions, although study duration should include time intervals that surpass expectations to estimate a potential worst case. These factors should all be considered when examining potential process-related stability.

1.1.4.2 Container Closure Systems

The ICH guideline for pharmaceutical development outlines requirements for container closure systems for drugs and biologics [41]. The concept paper prepared for this guidance specifically states that “the choice of materials for primary packaging should be justified. The discussion should describe studies performed to demonstrate the integrity of the container and closure. A possible interaction between product and container or label should be considered” [42]. In essence, this indicates that the container closure system should maintain the integrity of the formulation throughout the shelf life of the product. In order to maintain integrity, the container closure system should be chosen to afford protection from degradation induced by external sources, such as light and oxygen. In addition to the primary container, the stability of the product should also be examined in the presence of IV administration components if the product could be exposed to these conditions (see Section 1.1.5.6). Understanding the potential impact of product-to-container interactions is integral to maintaining stability and ensuring a uniform dosage. For example, adsorption of insulin and some small molecules has been demonstrated to readily occur in polyvinyl chloride (PVC) bags and tubing when these drugs were present as additives in intravenous (IV) admixtures [43].

In addition to their use in large-volume parenterals and IV sets, thermoplastic polymers have also recently found utility as packaging materials for ophthalmic solutions and some small-volume parenterals [43]. However, there are many potential issues with using these polymers as primary packaging components that are not major concerns with traditional glass container closure systems, including [44]:

1. Permeation of vapors and other molecules in either direction through the wall of the plastic container
2. Leaching of constituents from the plastic into the product
3. Sorption (absorption and/or adsorption) or drug molecules or ions on the plastic material

These concerns largely preclude the utility of thermoplastic polymers as the primary choice of container closure system for protein and peptide therapeutics, although the formulation scientist should be aware of the potential advantages of these systems, such as the ease of manufacturability and their cost. These systems are also finding greater utility in intranasal and pulmonary delivery systems.

Parenterally formulated biopharmaceuticals are typically packaged in glass containers with rubber/synthetic elastomeric closures. Pharmaceutical glass is composed primarily of silicon dioxide tetrahedron which is modified with oxides such as sodium, potassium, calcium, magnesium, aluminum, boron, and iron [45]. The USP classifies glass formulations as follows:
Type I, a borosilicate glass  
Type II, a soda–lime treated glass  
Type III, a soda–lime glass  
NP, a soda–lime glass not suitable for containers for parenterals

The tendency of peptides to adsorb onto glass surfaces is well known and a major concern in the pharmaceutical industry. This is especially important when the dose of the active ingredient is relatively small and a significant amount of drug is adsorbed to these surfaces. In addition, the leaching of atoms or elements in the glass's silicate network into solution is also a potential issue. This is especially important for terminally heat sterilized products where oxide additives included in the silicate network are relatively free to migrate/leach, resulting in increased solution pH, reaction catalysis, and so on [45]. As such, only type 1 treated glass is traditionally used for parenterally administered formulations, where these alkaline-rich phases in the glass have been eliminated, thus decreasing the potential for container closure system interactions. Additional approaches, including surface treatment with silicone (siliconization), have also been developed to minimize the interaction of biotechnology products with free silanols (Si–OH) [46].

Elastomeric closures are typically used for syringe and vial plungers and closures. For vials, elastomers provide a soft and elastic material that can permit the entry of a hypodermic needle without loss of the integrity [45]. For syringes, the closures not only provide a permeation barrier but also allow for a soft gliding surface facilitating plunger movement and drug delivery. Elastomeric polymers, however, are very complex materials composed of multiple ingredients in addition to the basic polymers, such as vulcanizing agents, accelerators, activators, antioxidants, fillers, lubricating agents, and pigments [45]. As leaching of these components into solution is a potential issue, the compatibility of the drug formulation with the closures must be studied early during the formulation development process. The choice and type of elastomeric closure depends on the pH and buffer, if any preservatives are present, the sterilization method, moisture vapor/gas protection, and active compatibility [47]. In addition, the problem of the additives in rubber leaching into the product can be reduced by the coating with specific polymers such as Teflon [48].

Container closure systems required for implantable devices are further restricted by the fact that they are required to be compatible with the formulation over the intended shelf life and therapeutic application time as well as being biocompatible. This means that the system not only must afford protection to and contain the formulation but also cannot cause any potential adverse effects, such as allergy. Typically, implantable systems are composed of biocompatible metals, such as titanium or polymers such as polyethylene glycol or polyactic-co-glycolic acid.

### 1.1.4.3 Sterility Assurance

Maintaining the sterility of biopharmaceutical products is especially important due to the relative potency and their innate potential for immunogenic reactions. Further, the biochemical nature of these compounds enables them to serve as potential nutrients for invading organisms. Methods for sterilizing small molecules include
heat terminal sterilization, terminal filtration coupled with aseptic processing techniques, ultraviolet (UV) and gamma irradiation, ethylene oxide exposure (for containers and packaging only), and electron beam irradiation. While terminal heat sterilization is by far the most common sterilization technique, it normally cannot readily be utilized for peptide or protein formulations due to the potential effects of heat and pressure on the compound’s structure [48]. Furthermore, irradiation can affect protein stability by cross-linking the sulfur-containing and aromatic residues, resulting in protein aggregation [49].

To overcome these issues, sterile filtration coupled with aseptic processing and filling is the preferred manufacturing procedure for biopharmaceuticals. Garfinkle et al. refer to aseptic processing as “those operations performed between the sterilization of an object or preparation and the final sealing of its package. These operations are, by definition, carried out in the complete absence of microorganisms” [50]. This highlights the importance of manufacturing controls and bioburden monitoring during aseptic processes. Newer technologies such as isolator technology have been developed to reduce human intervention, thereby increasing the sterility assurance. These technologies have the added benefit of facilitating aseptic processing without construction of large processing areas, sterile suites, or gowning areas [50].

Even the most robust monitoring programs do not ensure the sterility of the final formulation. As such, aseptically processed formulations are traditionally filtered through a retentive final filter, which ensures sterility. Coupled with proper component sterilization, traditionally by autoclaving, these processes ensure product sterility. However, filtration is a complex unit operation that can adversely affect the drug product through increased pressure, shear, or material incompatibility. Therefore, filtration compatibility must be assessed thoroughly to demonstrate both product compatibility, and sufficient contaminant retention [51]. Parenteral Drug Association (PDA) technical report 26 provides a thorough systematic approach to selecting and validating the most appropriate filter for a sterilizing filtration application [51].

### 1.1.4.4 Excipient Selection

Pharmaceutical products are typically formulated to contain selected nonactive ingredients (excipients) whose function is to promote product stability and enable delivery of the active pharmaceutical ingredient(s) to the target site. These substances include but are not limited to solubilizers, antioxidants, chelating agents, buffers, tonicity contributors, antibacterial agents, antifungal agents, hydrolysis inhibitors, bulking agents, and antifoaming agents [45]. The ICH states that “the excipients chosen, their concentration, and the characteristics that can influence the drug product performance (e.g. stability, bioavailability) or manufacturability should be discussed relative to the respective function of each excipient” [42]. Excipients must be nontoxic and compatible with the formulation while remaining stable throughout the life of the product. Excipients require thorough evaluation and optimization studies for compatibility with the other formulation constituents as well as the container/closure system [52]. Furthermore, excipient purity may be required to be greater than that listed in the pharmacopeial monograph if a specific impurity is implicated in potential degradation reactions (e.g., presence of trace metals) [48].
One of the critical factors in excipient selection and concentration is the effect on preferential hydration of the biopharmaceutical product [53, 54]. Preferential hydration refers to the hydration layers on the outer surface of the protein and can be utilized to thermodynamically explain both stability enhancement and denaturation. Typical excipients used in protein formulations include albumin, amino acids, carbohydrates, chelating and reducing agents, cyclodextrins, polyhydric alcohols, polyethylene glycol, salts, and surfactants. Several of these excipients increase the preferential hydration of the protein and thus enhance its stability. Cosolvents need to be added in a concentration that will ensure their exclusion from the protein surface and enhance stability [54]. A more comprehensive review of excipients utilized for biopharmaceutical drug products is available elsewhere [48].

**Buffer Selection** In addition to maintaining solution pH, buffers serve a multitude of functions in pharmaceutical formulations, such as contributing toward overall isotonicity, preferential hydration of proteins and peptides, and serving as bulking agents in lyophilized formulations. The buffer system chosen is especially important for peptide and proteins that have sensitive secondary, tertiary, and quaternary structures, as the overall mechanisms contributing to conformational stabilization are extremely complex [48]. Furthermore, a protein’s propensity for deamidation at a particular pH can be significant, as illustrated by Wakankar and Borchardt [55]. This study illustrated stability concerns with peptides and proteins at physiological pH in terms of asparagine (Asn) deamidation and aspartate (Asp) isomerization, which can be a major issue with respect to circulating half-life and potential in vivo degradation. This study and others also provide insight into predicting potential degradative mechanisms based on primary and secondary structural elements allowing for formulation design with these pathways in mind.

Selecting the appropriate buffer primarily depends on the desired pH range and buffer capacity required for the individual formulation; however, other factors, including concentration, effective range, chemical compatibility, and isotonicity contribution, should be considered [56]. Some acceptable buffers include phosphate (pH 6.2–8.2), acetate (pH 3.8–5.8), citrate (pH 2.1–6.2, pK 3.15, 4.8, and 6.4), succinate (pH 3.2–6.6, pK 4.2 and 5.6), histidine (pK 1.8, 6.0, and 9.0), glycine (pK 2.35 and 9.8), arginine (pK 2.18 and 9.1), triethanolamine (pH 7.0–9.0), tris-hydroxymethylaminomethane (THAM, pK 8.1), and maleate buffer [48]. Additionally, excipients utilized solely for tonicity adjustment, such as sodium chloride and glycerin, may not only differ in ionic strength but also could afford some buffering effects that should be considered [52].

**Preservatives** In addition to those processing controls mentioned above (Section 3.1.4.3), the sterility of a product may be maintained through the addition of antimicrobial preservatives. Preservation against microbial growth is an important aspect of multidose parenteral preparations as well as other formulations that require preservatives to minimize the risk of patient infection upon administration, such as infusion products [52]. Aqueous liquid products are prone to microbial contamination because water in combination with excipients derived from natural sources (e.g., polypeptides, carbohydrates) and proteinaceous active ingredients may serve as excellent media for the growth [57]. The major criteria for the selection of an appropriate preservative include efficiency against a wide spectrum of micro-
organisms, stability (shelf life), toxicity, sensitizing effects, and compatibility with other ingredients in the dosage form [57]. Typical antimicrobial preservatives include \( m \)-cresol, phenol, parabens, thimerosal, sorbic acid, potassium sorbate, benzoic acid, chlorocresol, and benzalkonium chloride. Cationic agents such as benzalkonium chloride are typically not utilized for peptide and protein formulations because they may be inactivated by other formulation components and their respective charges may induce conformational changes and lead to physical instability of the API. Further, excipients intended for other applications, such as chelating agents, may exhibit some antimicrobial activity. For instance, the chelating agent ethylenediaminetetraacetic acid (EDTA) may exhibit antimicrobial activity, as calcium is required for bacterial growth.

Identifying an optimal antimicrobial preservative is based largely on the effectiveness of that preservative at the concentration chosen. In short, it is not enough to assess the compatibility of the preservative of choice with the API and formulation and processing components. There also needs to be a determination of whether the preservative concentration is sufficient to kill certain standard test organisms. The USP presents standard protocols for assessing the relative efficacy of a preservative in a formulation using the antimicrobial effectiveness test (AET) [58]. Briefly, by comparing the relative kill efficiency of the formulation containing varying concentrations of the preservative, the formulator can determine the minimal concentration required for preservative efficacy and design the formulation accordingly.

1.1.5 DRUG PRODUCT STABILITY

1.1.5.1 Defining Drug Product Storage Conditions

From a regulatory standpoint, the primary objective of formulation development is to enable the delivery of a safe and efficacious drug product to treat and/or mitigate a disease state throughout its proposed shelf life. The efficacy and in many cases the safety of a product are directly related to the stability of the API, both neat and in the proposed formulation under processing, storage, and shipping conditions as well as during administration. As such, the concept of drug stability for biotechnology-derived products does not change substantially from that of small molecules, although the level of complexity increases commensurate with the increased complexity of the APIs in question and the formulation systems utilized for their delivery.

Stability study conditions for biotechnology-derived APIs and their respective drug products are not substantially different from those studies conducted for small molecules. Temperature and humidity conditions under which to conduct said studies are outlined in ICH Q1A(R2), which incorporates ICH Q1F, stability study conditions for zones III and IV climactic conditions [59]. Additional guidance specific to conducting stability studies on biopharmaceutical drug products is given in ICH Q5C [1]. However, the intention of ICH Q5C is not to outline alternate temperature and humidity conditions to conduct primary stability studies; rather it provides guidance with respect to the fact that the recommended storage conditions and expiration dating for biopharmaceutical products will be different from product to product and provides the necessary flexibility in letting the applicant determine
the proper storage conditions for their respective product. Furthermore, this document provides general guidance in directing applicants in the types of analytical methodologies that may be used and direction on how to properly assess the stability of these complex molecules [1]:

Assays for biological activity, where applicable, should be part of the pivotal stability studies. Appropriate physicochemical, biochemical and immunochemical methods for the analysis of the molecular entity and the quantitative detection of degradation products should also be part of the stability program whenever purity and molecular characteristics of the product permit use of these methodologies.

One recent approach to aid in defining the design space for protein and peptide therapeutics has been to create empirical phase diagrams indicating the relative stability of compounds based on altering conditions and assessing conformational changes via a compilation of analytical techniques (Figure 1) [60–62]. These empirical phase diagrams can be generated based on pH, temperature, salt concentration, and so on, and, although seemingly laborious at first glance, could provide invaluable information in defining the extremes to which a compound may be subjected without altering its conformation. For instance, if an empirical phase diagram determines the safe temperature range for a compound is up to 35°C and an excursion occurs to 33°C, this information would give the stability scientist a guideline as to the appropriate course of action. Under the traditional testing paradigm of ICH Q1A, where stability testing is limited to 25, 30, and 40°C, one may not know the compound’s upper transition temperature to induce conformational changes. If the information is not already available, then additional excursion studies may need to be conducted to assimilate this information and take the appropriate course of action.

1.1.5.2 Mechanisms of Protein and Peptide Degradation

The inherent heterogeneity of peptide and protein drug substances results in their relative sensitivity to processing, storage, and handling conditions as well as a mul-

![FIGURE 1 Empirical phase diagram for ricin toxin A-chain generated using CD molar ellipticity at 208nm, ANS fluorescence, and intrinsic Trp fluorescence intensity data. Labels indicate the state of the protein within the same region of color based on evaluation of a compilation of data sets. (Reproduced with permission from ref. 62.)](image-url)
attitude of other factors. Most importantly, this heterogeneity results in a whole host of potential degradative mechanisms, some of which are compiled in Table 3 and include chemical instability pathways such as oxidation, hydrolysis of side chains and potentially the peptide backbone, and deamidation of Asn and Gln side chains. Also, physical instability manifesting in the form of protein unfolding, formation of intermediate structures, aggregation, and adsorption to the surfaces of containers and other equipment can be a major technical hurdle in developing any biopharmaceutical and may or may not be related to chemical instability [63]. Further complicating matters is that instability can potentially manifest in various ways and may or may not be detectable by any one method. Taken together, however, the compilation of methodologies utilized for stability assessment should give a good approximation as to the degradative mechanisms of the compound in its respective formulation. Further, bioactivity and immunogenicity assays should play integral roles in assessing the relative stability of any biopharmaceutical compound. Briefly stated, the chemical and physical stability of products is extraordinarily difficult to assess and will not be belabored here as good reviews on this topic are readily available in the literature [63, 64].

### 1.1.5.3 Photostability

In certain cases, exposure of pharmaceutical compounds to UV and visible light could result in electronic excitation, termed vertical transition, that could ultimately result in light-induced degradation. The ICH guideline Q1B [65] is a reference on how to conduct photostability stress testing for pharmaceutical compounds. In brief, compounds are exposed to an overall illumination of not less than 1.2 million lux hours and an integrated near-UV energy of not less than 200Wh/m² [65]. These requirements are in addition to normal stability stress testing and require the additional caveat that analytical methodologies are suitable to also detect photolytic degradation products, as discussed above. A comprehensive discussion of small-molecule photolytic degradative mechanisms is available for further review [66].

### TABLE 3 Potential Degradative Mechanisms of Peptides and Proteins

<table>
<thead>
<tr>
<th>Degradative Mechanism</th>
<th>Site of Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical degradative mechanisms</strong></td>
<td></td>
</tr>
<tr>
<td>Oxidation</td>
<td>Intrachain disulfide linkages Met, Trp, Tyr</td>
</tr>
<tr>
<td>Peptide bond hydrolysis</td>
<td>AA backbone</td>
</tr>
<tr>
<td>N-to-O migration</td>
<td>Ser and Thr</td>
</tr>
<tr>
<td>α- to β-Carboxy migration</td>
<td>Asp and Asn</td>
</tr>
<tr>
<td>Deamidation</td>
<td>Asn and Gln</td>
</tr>
<tr>
<td>Acylation</td>
<td>α-Amino and ε-amino group</td>
</tr>
<tr>
<td>Esterification/carboxylation</td>
<td>Glu, Asp, and C-term</td>
</tr>
<tr>
<td><strong>Physical degradative mechanisms</strong></td>
<td></td>
</tr>
<tr>
<td>Unfolding</td>
<td>Partial unfolding of tertiary structure</td>
</tr>
<tr>
<td>Aggregation</td>
<td>Aggregation of subunits could result in precipitation</td>
</tr>
<tr>
<td>Adsorption</td>
<td>Adsorption to processing equipment and container closure systems</td>
</tr>
</tbody>
</table>

*Source: Modified from Crommelin et al. [5].
1.1.5.4  Mechanical Stress

Regulatory guidance on appropriate methods to evaluate the effect of shear stress and process-handling stability studies is not available. However, these studies are integral in determining the relative stability of the product with respect to mechanical stresses introduced during development and manufacturing. Although not typically recognized as a major degradative pathway for most small-molecule dosage forms, the introduction of mechanical stress is recognized as a major challenge in the formulation of semisolids and can potentially induce physical instability of biopharmaceuticals, although the extent of this effect is currently unknown. For example, processing shear may influence the protein’s outer hydration shell, altering the stabilizing energy provided from preferential hydration and resulting in the exposure of internal, nonpolar residues. This may facilitate aggregation if enough shear force is provided. Alternately, the shear energy required to force unfolding has been studied but has not been related to the fluid dynamic shear experienced during processing. Therefore, stress studies should include meticulous controls in the form of temperature, light and humidity, and fluid dynamic shear as a function of time. Data from these studies could be incorporated into empirical phase diagrams, and/or response surfaces, to help further define the design space for the active and finished drug product. Understanding the effects of stress introduced during the manufacturing processing of biopharmaceutical products could facilitate the selection of appropriate PAT tools and QbD incorporation in the development of these products. Clearly, there is a considerable need for research in this area, and until the extent of the possible effects are understood, this lack of knowledge poses an unknown risk and prevents adequate risk assessment for biopharmaceutical development activities consistent with ICH Q9.

1.1.5.5  Freeze–Thaw Considerations and Cryopreservation

The rapid or continuous freezing and thawing of protein products could contribute significantly to instability of the API. Such studies are typically designed to assess the implications of potential transport and handling conditions. These conditions include not only the manufacturing processing, storage, and shipment to warehouses and pharmacies but also subsequent pharmacy storage and patient handling [52]. Unpredictable and somewhat modest temperature fluctuations could easily induce degradation or conformational changes that may reduce bioactivity or expose antigenic epitopes [5]. These effects could also be a result of altered preferential hydration at the surface of the peptide or protein through salting-out effects induced by rapid freezing, which could easily denature the product [67].

1.1.5.6  Use Studies

Stability of biopharmaceutical compounds should also be determined under conditions that mimic their normal usage. For instance, the stability of reconstituted lyophilized products should be assessed with respect to time and temperature and, if applicable, light and mechanical stimuli. Likewise, the stability of a compound included in implantable devices and controlled-release microsphere formulations should be determined over the course of its required use, under conditions which mimic the heat, moisture, light, and enzymatic physiological conditions to which it
will be implanted. Such studies should also determine the release profile of the compound over these specified conditions.

Drug products intended for IV administration are generally dosed as an initial bolus followed by a slow infusion. Consequently, admixture studies of the compound in potential IV fluids, such as 0.9% (w/v) saline, 5% (w/v) dextrose, and Ringer’s solution, should also be assessed to determine the relative stability of the compound in this new environment. These studies are critical as the formulation dynamic that protected and stabilized the compound has now been altered dramatically with dilution. This environmental change could potentially impact the preferential hydration of the compound as well as directly induce conformational changes based on the diluent chosen and the compound’s potential degradative mechanism(s). Additional contributing factors to instability in admixture solutions could be due to changes in pH, mechanical mixing of the compound in the IV bag, adsorption of the compound to the bag itself (which is typically polymeric), or IV sets used for administration, as well as an increased potential for oxidative degradation. The suitability of analytical methodologies should also be determined in the presence of these additional analytes.

1.1.5.7 Container Closure Integrity and Microbiological Assessment

Ensuring that parenteral pharmaceuticals maintain their sterility over the course of their shelf life is an integral part of any stability assessment [68]. Parenteral dosage forms must be free from microbiological contamination, bacterial endotoxins, and foreign particulate matter. Selection of the adequate sterile manufacturing process has been briefly discussed above. Determining the microbiological integrity of the product over its shelf life also gives an indication of the relative quality of the container closure system chosen for the formulation. Compendial sterility and endotoxin testing are often used for this purpose; however, sampling is dependent on a statistical evaluation of the batch size, unit fill volume, and method of product sterilization [68]. Additionally, since these tests are destructive, it would be impossible to test an entire stability batch to ensure viability of a container closure system. Other nondestructive tests have been developed to determine the integrity of a container’s closure system [69]. These tests could also serve as a surrogate indicator of product manufacturing quality over time.

1.1.5.8 Data Interpretation and Assessment

Interpretation of primary stability data for determining expiration dating and primary storage conditions has been outlined by ICH Q1E [70]. This guidance document delineates broad methodologies for interpreting primary and accelerated stability data and extrapolation of said data for determining expiry dating. Of course, expiry dating cannot be made without reference to specifications for those primary stability-indicating parameters assessed, which is discussed below. Traditionally, stability assessments performed during preformulation will give an indication of the potential storage conditions as well as allow for extrapolation of accelerated stability studies to kinetic degradation rates. Typically this is done through Arrhenius manipulations. However, as one would expect, these analyses are not readily useful for biopharmaceutical products, as there is rarely a linear correlation between
temperature and the compound’s degradative rate. This is primarily due to the complex and often competing degradative mechanisms as well as the potential for so-called molten globule intermediate phases. In spite of these limitations, ICH Q5C does provide relevant guidance in illustrating the flexibility required for determining storage conditions, as these products usually require a very narrow temperature condition to maintain optimal stability. Further guidances may be needed to enhance uniformity in testing methodology and enable the utilization of validated PAT methodologies.

1.1.6 QUALITY BY DESIGN AND SCALE-UP

1.1.6.1 Unit Operations

Unit operations are defined as the individual basic steps in a process that when linked together define the process train and result in the final product. In practical terms, a unit operation is often defined as an individual step that is carried out on one piece of equipment. Typical biopharmaceutical API unit operations may include fermentation or bioreactor processes, cell separation through centrifugation or microfiltration, virus removal or inactivation, cell lysis and inclusion body precipitation, product refolding, and purification steps [71]. Conversely, those unit operations for drug product manufacturing procedures would be similar to those seen in the manufacture of a small molecule of comparable dosage form, namely mixing, fluid transfer, sterile filtration, dose filling, lyophilization, and so on. Of course, unit operations will be dependent on the manufacturing process for the specific dosage form, but careful preformulation and characterization studies will enable relatively straightforward process design and ease subsequent scale-up activities. Modeling of unit operations for both small and large molecules is a recognized gap in our ability to achieve QbD. The application of accepted engineering methods to the problem is the subject of active research.

1.1.6.2 Bioburden Considerations

Bioburden refers to the amount of microbial flora that can be detected on an item, on a surface, or in a solution [68]. As mentioned previously, microbial contamination and bioburden are especially important for biotechnology-derived parenteral products since these products are typically capable of supporting microbial growth. Special care should be taken to ensure not only that the final packaged product does not contain microbial contamination but also that manufacturing equipment is also free from contamination. Monitoring bioburden and determining potential levels of microbial contamination on equipment surfaces are particularly important with respect to the material being evaluated.

In general, bioburden counts in parenteral solutions are obtained by conducting the total aerobic counts and total yeast and mold counts as specified in the USP microbial limits test (61) or an equivalent test [72]. In addition, membrane filtration of larger than specified volumes may also be used to detect any microbial contamination when sample results are expected to contain a negligible number of microbial flora or in the presence of potential confounding factors, such as antimicrobial
preservatives [68, 72]. It is important to note that the presence of a high bioburden count can present an endotoxin contamination problem, as whole microbial cells and spores can be removed by sterilizing grade filtration (0.2 μm), while endotoxins are not [68]. These issues also underscore the importance of cleaning methods and their respective validation as well as assessing relevant product contamination on manufacturing equipment.

1.1.6.3 Scale-Up and Process Changes

The FDA defines process validation as “establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined quality attributes” [73]. While validation studies are typically performed at full scale, in most cases scale-down or laboratory-scale models were used to initially develop the manufacturing process. Consequently, scale-down process precharacterization and characterization studies are considered crucial to successful process validation for both API and drug product manufacturing schemes [74]. Although they do require qualification work and a significant commitment of time and resources, characterization studies provide significant insight into the critical process and control parameters for each unit operation as well as improved success rates for process validation due to a better, more complete understanding of the process [74]. In engineering terms, characterization studies identify the critical parameters useful for dimensional analysis that enable successful process scale-up.

While the above explanation attempts to simplify the scale-up process, it is not meant to trivialize it. In fact, scale-up is probably the most difficult manufacturing challenge for traditional small molecules, let alone biopharmaceuticals. Issues such as homogeneous mixing, bulk product holding and transfer, and sterile filtration could all be potentially compounded due to the increased scale and introduced stress. However, a QbD approach to rational drug design should enable simplified process scale-up and validation. This is only true if experimental design approaches have been utilized to identify the design space for the processes involved in the production of the molecule. This is also where the greatest benefit of developing empirical phase diagrams early in development could materialize. Essentially, the QbD approach identifies the quality attributes of the product based on scientific rationale as opposed to attempting to fit the proverbial square peg into a round hole through a trial-and-error approach. This rational design approach goes further to identify the limiting factors of each unit operation and provides the means of attempting to correlate how each unit operation affects the final product quality attributes.

In order to initiate a successful QbD program, the first step is to identify those process parameters that are essential to product quality and develop well-validated analytical methodologies to monitor those parameters. In short, the process involves identification of the potential design space for production of the molecule and confirmation that design space through rational, deliberate experimentation. Ideally, process monitoring should be done in real time to minimize production time and if possible online; however, this may not always be the case or even necessary depending upon the relative duration of the process to the test. Recognizing potential quality metrics earlier in the development process could also potentially facilitate
greater flexibility during product development and subsequent process characterization [74]. Certainly, manufacturing site-specific differences could also potentially introduce variability into processes. It is for this reason that site-specific personnel training, process/technology transfer and validation, and stability assessments are required to ensure product quality.

By definition, a process designed under the auspices of QbD should enable a degree of process knowledge that allows for controlled process changes without affecting the final product or requiring regulatory approval. For immediate- and controlled-release solid dosage products, SUPAC guidelines provide direction on the studies to conduct to determine the impact of a process change. Although there is some regulatory guidance available for biological products (e.g., “Changes to an Approved Application for Specified Biotechnology and Specified Synthetic Biological Products” or “FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-Derived Products”), process changes need to be evaluated on a case-by-case basis. The comparative analysis of process changes should also be evaluated with respect to defined product specifications. PAT will be invaluable in determining the potential impact of process changes. While stability is often the main metric for small-molecule drug product, bioactivity and immunogenicity will need to be added metrics for biopharmaceuticals. Therefore, any process change should be approached subjectively and care should be taken to validate the relative impact on the safety and efficacy of the product.

1.1.7 CONCLUDING REMARKS

Although the goals are the same, developing biotechnology molecules presents challenges that are unique compared to the development of conventional small molecules. The innate complexity of the molecular and macromolecular structures requires three dimensionally viable stability assays and understanding. The complexity of possible physiological responses and interactions requires an enhanced understanding of the formulation and processing stresses to identify the minor but critical changes that result in product unacceptability. A key to addressing these challenges is the development of analytical techniques with the sensitivity and reliability to detect and monitor such changes and to provide data to another gap-closing activity—modeling unit operations. Also the need to develop meaningful kinetic models is obvious to everyone involved in the development of both large and small molecules. Linking this type of information to the major efforts in the discovery arena is a necessary step to bringing the products of the future to market.

The use of biotechnology products is increasing exponentially and many opportunities exist to improve their development. The first step may be defining rational biotechnology-derived drug “developability” standards that can be assessed during preclinical/early development testing. Such a tiered approach based upon the potential risk, the confidence in methodology, and benefit has of course been a proven strategy for small molecules, and a preliminary version applicable to biotechnology drug products is likely possible today given the topics discussed in this chapter.
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REFERENCES


32. Prescribing information for Viadur®. Manufactured by Alza Corporation, Mountain View, CA 94043.


58. U.S. Pharmacopeia (USP 26 2003), Chapter (51) Antimicrobial effectiveness testing, Rockville, MD.
72. U.S. Pharmacopeia (USP 26 2003), Chapter (61) Microbial limits test, USP, Rockville, MD.
73. Center for Drug Evaluation and Research, FDA (1987, May), Guideline on general principles of process validation.