

# Formulation Development

## Making the Medicine

by Cheryl Scott

Design and execution of a successful fill/finish and stability project requires careful planning performed by experienced, responsive professionals. Failure to incorporate these activities early in your drug development program can lead to costly delays in meeting milestones essential to the successful launch of your product. (1)

One rule of thumb in drug development is that the larger a molecule is, the more trouble it will be to make, ship/store, and administer to patients. Biotherapeutics include proteins (such as antibodies), some of the smaller peptides (such as hormones), DNA for gene-transfer therapies, vaccines of various types, cells and tissues, and to a lesser extent blood-fractionation products, allergenics, and RNA. The molecules are big, unwieldy things; they are produced in complex mixtures by biological processes; and they face numerous challenges both in storage and within the environment of a human body. Application of cells and tissues presents an entirely different set of problems:

The emergence of cell- and tissue-based technologies and the move toward a global marketplace are creating a demand for new technologies that allow worldwide shipment of such products while maintaining their viability or function—a concept referred to as biological packaging. . . . The principles governing the development of effective biological packaging necessitate an understanding of state-of-the-art hypothermic storage and cryopreservation: the two standard approaches currently used for preserving cells and tissues for extended periods. (2)



Biotech drug formulators have many concerns to juggle in their work, beginning with the physicochemical characteristics of an active molecule and including the reliability, cost, and availability of analytical methods used in formulation work; the array of excipients on the market (and their chemistries); evolving delivery methods and devices; patient preferences and behavior, as well as the biology of diseases being treated; even the concerns of legal, sales, and marketing staff. Formulation is more than science, as a result, but science is its foundation. For years, it has been considered by many as an arena where luck and intuition play a role as well, but just as biotherapeutics have transitioned from the relatively mysterious world of biologics to being thought of as drugs (3), so too is formulation work becoming more methodical and quantifiable.

The basic concerns of biotherapeutic formulation are stability and structural integrity of the active molecule during transit and storage, successful delivery

of the drug to its site of action, and (inevitably) speed and cost-effectiveness of development and the final product. Numerous details come into play including analytical methods and testing protocols, containers and closures, delivery devices and dosage forms, excipients and stabilizers, and compatibility of ingredients. Complicated formulation decision trees necessitate the following types of choices:

- Method of delivery (parenteral, pulmonary, etc.)
- Final product form (solution, lyophilized cake, etc.)
- Single-dose or multidose (necessitating preservatives)
- Ingredients (excipients, stabilizers, preservatives, etc.)
- Dosage details (concentration, frequency, etc.)
- Logistics (transportation, storage conditions, shelf life, etc.)
- Packaging (vials, ampules, etc., even labeling)
- Manufacturing process (scale, equipment, procedures, etc.).

Those decisions cannot be made by formulators alone. Product development and market data are important factors as well as process development experience and regulatory guidance. Intellectual property lawyers help determine whether a device will need to be licensed, for instance, or the formulation infringes a patent. Sales people know what doctors and patients are looking for, marketing specialists understand the final product's eventual placement and financial situation, regulatory affairs and quality personnel see to compliance matters, and other scientists add their

findings along the way. But the formulators must keep all these things in mind. No wonder they've been thought of as "magicians" by their appreciative colleagues over the years!

### STABILITY

Proteins and other large biomolecules are delicate and sensitive to changes in conditions such as pH, osmolality, tonicity, pressure, and temperature. Vaccines may be genes, proteins, pathogen fragments, or whole organisms that have been killed or inactivated (4). Blood-fractionation products and allergens are usually proteins themselves, so they can be treated by formulators the same ways as recombinant antibodies. And peptides are merely amino-acid chains (like proteins, only smaller), which present the least amount of trouble in biotech formulations.

Nucleic acids usually are formulated with gene-transfer vectors such as transformed viruses, lipids, or polymers. Viral vectors can be treated more or less as large proteins in formulation because they are typically protein shells surrounding genes of interest. Synthetic vectors present fewer safety issues than viruses, but they are less efficient transfectors of DNA/RNA (5). They also present more challenges in formulation

because aqueous suspensions of nonviral vectors tend to agglomerate over time. "Some clinical trials have attempted to circumvent the problem . . . by preparing complexes at the bedside immediately prior to injection. Clearly, this method of sample preparation and administration is not practical and leads to significant variations in product quality and gene delivery" (3). It may have contributed to some gene therapy failures in clinical trials so far.

Simply put, stabilization of gene products is also stabilization of their transfer vectors. So issues of lipid/polymer sensitivity to agitation and temperature in solution, for example, must be considered. Transfection efficiency can be lost as a result of freezing and thawing, among other processes. And "it is critical for quality control that vector size be maintained during a process regardless of its effect on transfection rates" (5). Thus, formulation has been the primary challenge to companies developing gene-transfer products, whether as therapies or vaccines. Because chemical degradation of DNA can cause mutation as well as negate therapeutic activity, any kind of destabilization is of great concern.

For those choosing synthetic over viral vectors (and they are growing in number) there appears to be only one option: freezing. Some have quickly frozen samples using liquid nitrogen because slow freezing invites damage to sensitive molecules. But the most promising method of stabilizing

nucleic acid formulations appears to be lyophilization. Because DNA is very sensitive to hydrolytic and oxidative degradation, its half-life in aqueous solutions is measured in hours or days. It appears to fare much better at colder temperatures, and even better in a freeze-dried state. However, much work is yet to be done in nucleic acid formulations before we see confirmed shelf lives approaching the drug-product norm of two years.

**Protein Stability:** Formulators have much more experience with recombinant proteins. They have identified three principal methods of degradation in protein formulations: deamidation, oxidation, and aggregation, all of which are most likely to occur in aqueous solutions (6). Deamidation is mainly associated with hydrolysis of asparagine residues into aspartic acids. Oxidation can be catalyzed by metal ions, directly caused by the presence of hydrogen peroxide and organic solvents or by photochemical reactions involving certain excipients. Aggregation is the final result of many forms of protein denaturation, which can result from changes in temperature, pH, or pressure as well as the presence of denaturing chemicals and exposure to shear forces.

Proteins are amphoteric (their electrical charge depends on the pH of their environment) because they are complex molecules made up of many individually charged elements. They are most stable at their isoelectric point (pI): the pH value at which their net charge is zero, and "opposing

### CAUSES OF INSTABILITY

These conditions encountered during manufacturing and/or storage can present challenges to stability (6):

- freeze-thaw
- heavy metal ions
- interface adsorption
- ionic strength
- light
- organic solvents
- oxygen
- pH extremes
- radiation
- residual moisture
- shear forces
- surfactants
- temperature

### LIQUID OR LYOPHILIZED?

#### LIQUID FORMULATIONS

##### Advantages

- Convenient
- Cost of goods (economical)
- Competitive
- Applicable to many commercially available delivery devices

##### Disadvantages

- Less stable than lyophilized formulations

#### LYOPHILIZED FORMULATION

##### Advantages

- More stable than liquid formulations
- Can achieve higher concentration formulation

##### Disadvantages

- Cost of goods (expensive)
- More complex process and tech transfer
- Limited commercial contract capacity

Source: Chen B, Zapata G, Chamow SM. Strategies for Rapid Development of Liquid and Lyophilized Antibody Formulations: An Integrated Design. *BioProcess International* 2(1) 2004: 48-52.

charges serve to stabilize the protein structure” (7), but that is often the same point at which they are least soluble as well.

Proteins are attracted to interfaces, such as where air and water meet, so they tend to cluster along the walls of their container or clump around bubbles in solution. Simply shaking a vial could destroy the efficacy of the product within. Some denaturation is reversible—if it occurs during processing. In final products, there is no going back.

In the end, protein formulations “are complex systems, and it is often difficult to separate the effects of any single variable that can account for a shift in the equilibrium to favor the denatured protein state. Losses in protein physical stability most likely are due to an interplay between many different stabilizing and destabilizing effects” (7). Thus, it is important to understand a given molecule as well as possible when making decisions about its formulation. Such understanding comes from preformulation, characterization, and dedicated stability testing.

The results from accelerated stability tests, in which product is stored at higher temperatures than are normal for storage and monitored for signs of denaturation and degradation, are commonly used as supporting data in applications for market authorization. However, regulators expect to see real-time testing of final products at normal storage conditions—that is, several months’ intermittent sampling of before- and after-shipment material. Of course, a company’s management may want to rush things. “If it’s stable after three months, isn’t that a good indication of . . . ?” A formulator’s answer must be, “Not necessarily.” After three months, the protocol and results can be reported, but those data should be amended every three months following—until a firm expiration date has been established and confirmed.

### **PREFORMULATION**

The process of formulation development begins with activities collectively known as preformulation, but that doesn’t mean they are limited only to the early phase of development. “Preformulation should be an iterative process that starts early in development and continues through the development life of a molecule . . . . A formulator should be willing to restart preformulation efforts when business and marketing decisions dictate a change or modification of a formulation” (8).

Preformulation work provides a scientific sketch of a drug in development. Later work will fill in the details, e.g., with more detailed characterization. Here, formulators begin to “get a feel” for the molecule’s preferred environment, what sorts of conditions cause it to denature/degrade, and what sorts of excipients/stabilizers might help. The earlier this work can begin, the better. Even as little as a few milligrams of protein are enough for preformulation testing, so it can start as early as the end of drug discovery. Using such material can be problematic, of course, because it will not be as pure as protein from developed processes—which can translate to dramatic differences in later results. But it’s often a good place to start.

“Consequences of a poorly formed preformulation study, or no study, are increased time and cost of product

## **A FORMULATION STUDY PROTOCOL**

The following sample three-phase protocol for a solution formulation comes from McGoff P and Scher DS, Chapter Five: Solution Formulations of Proteins/Peptides in *Protein Formulation and Delivery* (Drugs and the Pharmaceutical Sciences series, Volume 99). McNally EJ, ed. Marcel Dekker, Inc.: New York, NY, 2000; 156–157.

### **Phase 1: Range-Finding Studies**

Protein concentration: 1–10 mg/mL

pH range: 4.0–9.0

Temperature: 2–8 °C

Excipients: NaCl to near isotonic

Time Frame: up to three months

Sampling Interval: weekly at pH extremes, monthly otherwise

### **Phase 2: Narrow It Down**

Results of previous studies help narrow the scope for future testing. Now the focus is on a narrower pH and concentration range while looking at higher temperatures, possibly adding stabilizers, over a longer term (at least a year, sampling at monthly intervals). “Specific buffer ion effects could be investigated during this phase.”

### **Phase 3: Final Parameters**

Address the remaining solution-formulation parameters with an autoclave study, photostability testing, a preervative study (for multidose products), cavitation/shaking tests, materials adsorption testing, and in-depth freeze–thaw studies.

## **THE FREEZE-DRYING PROCESS**

Lyophilization is a three-step process, typically taking many hours to complete.

**1) Freezing:** The product (solution, dispersion, emulsion, or tissue) is solidified by low temperature.

**2) Primary Drying:** Free water is removed by sublimation under a vacuum, ~1 hour/mm thickness (11).

**3) Secondary Drying:** Remaining water (~10–30% present) is removed by desorption.

What’s left at the end is a complex mixture in a stable glassy state, not crystallized as ice. A lyophilized cake “consists of finely divided flakes interlaced with open pores. . . . The channels are the pathways left after sublimation of the ice crystals. . . . The size, shape, and nature (amorphous or crystalline) of the dried product can affect its stability upon dehydration as well as upon storage” (11).

development, insufficient product stability, or the need to register a suboptimal formulation or restrictive storage conditions relative to a competitor’s product” (8). A typical preformulation study examines the general structure and molecular weight of a molecule along with

- solubility and conformation at various pH values
- behavior at air–water interfaces and during freezing/

thawing

- compatibility with organic solvents
- degradation pathways, absorbance spectra, melting point, hydrophobicity, isoelectric point, and general aggregation tendencies
- response to time, light, temperature, and oxygen.

Some of that information may come from other sources—such as analytical work done in product or process development. Commonly used analytical methods include isoelectric focusing (for determining pI and changes in charge), electrophoresis and high-pressure liquid chromatography (for size, conformation, and aggregation states), biological assays (for functionality), ultrafiltration (for solubility), and differential scanning calorimetry (for melting temperature). Although measurements of optical density are possible, a formulator's naked eye can be just as important in making qualitative assessments of a solution's appearance. When stresses are applied (e.g., extreme thermal, pH, and oxidative conditions), some changes will be obvious, but most will require quantitative measurement. And up-to-date knowledge of scientific literature helps put analyses into context.

Initial preformulation studies take several weeks to months of work, and their results provide a basis for real formulations work to start with. "While not absolutely necessary, it is often instructive to identify the type of degradation products being generated" (8). A balance must be struck, however, between the need for information and the supply of purified product. "Drug supply is often limited at this phase of development, so only a few conditions can be studied" (8).

### **PARENTERAL FORMULATIONS**

Not only formulators have been working with the molecule, but also those involved in product and process development. Once process development is making enough purified drug, the sophisticated trial-and-error process of formulation can begin. By now, the drug substance should be well enough understood that

formulations developers know what to look for and how. In addition to the methods listed above for preformulation, other analytical methods may now come into play: polyacrylamide gel electrophoresis (PAGE) and ion-exchange chromatography (IEC) to study charge; circular dichroism, size-exclusion chromatography (SEC), capillary zone electrophoresis, and fluorescence spectroscopy to study molecular conformation; SEC, sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), laser-light scattering, capillary electrophoresis, and matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) mass spectrometry to study molecular size; micellar electrokinetic chromatography and hydrophobic interaction and reversed-phase chromatographies (HIC and RPC) to study hydrophobicity; and cell-based, enzymatic, and enzyme-linked immunosorbent assays (ELISAs) to study biological activity.

Formulation studies should address solubility as a function of pH and salt and the influence of increased temperature, solution pH, buffer ion, salt, protein concentration, and other excipients and preservatives (when necessary) on stability. Other studies to include are photostability, cavitation/shaking, and freeze-thaw cycling. Finally, material compatibility studies should be performed with any storage containers or medical device the molecule/formulation may contact. (9)

The easiest and most economical way to formulate a biomolecule is in solution. Most approved biotherapeutics on the world markets (with the exception of three topicals, a few peptide capsules, and certain tissue products) are parenteral dosage forms intended for injection into patients intravenously, intramuscularly, intracavitarily, and so on (10). About half of them are sold in solution or suspension form. If stability can be achieved this way, it is the first choice of biotech formulators.

Solubility is of primary concern with solution formulations, with a goal of at

least 0.1–5.0 mg/mL (9). Isotonicity contributes to solubility, and it is typically adjusted by changing salt concentrations. By this point, preformulation studies have elucidated the effects of extreme pH, so stability studies now focus on the narrower, usable range of 4–9 (7). Because every biomolecule is different, several of the numerous buffers available on the market will be tested until the most appropriate one is chosen. Other excipients include stabilizers, solubilizers, bulking agents, tonicifiers, vaccine adjuvants, antioxidants, chelating agents, complexants, encapsulators, preservatives, pH adjusters, solvents, surfactants, suspension agents, and polymers for sustained-release formulations (10). Whatever combination is chosen, compatibility must be documented among all the individual components—and the product container and closure system too, not to mention the ultimate delivery device (e.g., hypodermic needles).

Solution formulations are typically refrigerated at 2–8 °C, "with a minimum target shelf life of two years" (9). Most are unstable at or above room temperature. Although normal concentrations are 1–10 mg/mL, several higher-concentration formulations are currently in development, and some sustained-release "depot" formulations are already on the market. An interesting consideration for clinical trials is the need for placebo formulations to be indistinguishable from the actual drug formulation so that intentional or inadvertent unblinding does not occur.

International regulators require every effort to be made for patient safety—not only with the drug itself but also its formulation and packaging. Aseptic processing (see the previous chapter) is vital to the sterility of biologic products. "If a product can be autoclaved, it must be. If the manufacturer claims a product cannot be terminally sterilized, there must be data to support this claim" (9). Excipients used must be classified as GRAS ("generally recognized as safe") by the governing bodies of the intended market—and the criteria are



## ADJUVANTS IN VACCINE FORMULATIONS

Adjuvants are ingredients added to a vaccine to improve the immune response it produces. Researchers are studying many types, but the only type licensed for human use in the United States are so-called “alum” adjuvants composed of aluminum salts. Such compounds bind to the antigens in a vaccine, help retain them at the site of injection, and help deliver them to the lymph nodes. The slowed release of antigens to tissues surrounding the injection site and the improved delivery of antigens to the lymph nodes can produce a stronger antibody response than can the antigen alone. Alum adjuvants are also taken up by monocytes and macrophages to help them better present antigens to lymphocytes.

Some other adjuvants used in the vaccine industry, either outside the United States or in animal vaccines, include liposomes and immunostimulating complexes, the muramyl dipeptide derived from mycobacterial cell walls, certain cytokines (interferons and interleukins), and emulsions of mycobacteria in oil and water.

Source: *Understanding Vaccines: What They Are and How They Work*. US National Institute of Allergy and Infectious Diseases. NIH Publication 03-4219, July 2003; [www.naiad.nih.gov](http://www.naiad.nih.gov).

not the same everywhere.

**Lyophilization:** Solution formulations are not always an option. Many proteins and nucleic acids simply will not remain stable in solution long enough at any temperature. This usually is a result of their extreme sensitivity to interfacial dynamics and oxidation. Removal of water from the formulation is very often the answer. So about half of the biotherapeutics currently being sold are shipped and stored in freeze-dried form—conveniently at ambient temperatures. These products are reconstituted in a “just add water-for-injection” procedure immediately before injection. Methods of reconstitution and use are critical to

maintaining proper bioactivity, of course, so most often the products are administered by health-care professionals. Even so, some variation may be inevitable and should be taken into account by product formulators and package labelers.

As described in the “Freeze-Drying” box, lyophilization is “a process whereby a product in aqueous solution is frozen, producing discrete ice crystals and solute crystals. The solid ice under controlled conditions is sublimed away. Any of the ‘more tightly bound’ water is desorbed by controlled heating. The final product’s solute is relatively undisturbed from that originally in solution and is finely divided, with a large surface area” (12). It is expensive in terms of equipment, time, and power—and often represents a “bottleneck” in manufacturing—so the choice to lyophilize is not made lightly. But it may be the only choice for some products, particularly gene therapies.

Once in the dried state, it is typically assumed that vectors will be stable for indefinite periods of time. This naïve notion has caused leaders in the gene therapy field to respond to questions about the shelf life of their pet vector by stating, “It is lyophilized.” (5)

Of course, biotech formulations offer no easy answers. The keys to successful lyophilization processes are precise control of time, temperature, and pressure—all of which can be determined only through characterization and testing. “Optimization of the drying cycle for a given formulation requires a balanced understanding of the fundamental science of freeze-drying, formulation characteristics, equipment capabilities, and practical risks associated with process parameters” (13). Excipients are compared and chosen as with solution formulations. Adjustments are made to pH, unit dose and overall volume, and containers and closures. Final products may include cryoprotectants, buffers, salts, and/or stabilizers, depending on the effects of freeze-drying on the molecule of interest. A

vial should contain enough product (about 2 cm thick) to work with easily in reconstitution, so bulking agents are common with low-concentration biologics. Finally, lyophilized products are evaluated for their physical characteristics (color, uniformity, and volume, as well as reconstitution rates and clarity), stability, and bioactivity.

There are several types of lyophilizers, from simple laboratory-scale manifold systems to industry-standard chamber freeze-dryers and newer spray-dryers. One recent advance in chamber lyophilizers is replacement of traditional refrigerator compressors with liquid nitrogen condenser systems (14). They are said to provide improved control of freezing temperatures and cooling rates (faster and more constant) as well as simplified operations and maintenance. Another trend is toward use of nonaqueous (organic) solvents such as ethanol for increased sublimation rates and improved product stability.

### OTHER DELIVERY OPTIONS

Biomolecules are “difficult to deliver systemically by noninvasive routes due to their poor bioavailability” (13). Nevertheless, some companies are working toward methods of delivery outside the parenteral norm. The most problematic alternative is also the most attractive: Patients would much rather take a pill than get a shot any day. But one primary function of our digestive systems is to break down incoming proteins into their usable amino acid components, so getting such large molecules through that route intact is a nearly insurmountable challenge. Some smaller peptides, such as insulin, are showing promise however.

**Transdermal delivery** (e.g., “the patch”) is familiar to smokers trying to quit, women on hormone replacement therapy, and people who suffer from severe motion sickness. There have been challenges in its use in delivery of peptides, proteins, and other macromolecules that cannot easily permeate the outer skin layer. “Mechanical abrasion and chemical enhancers increase drug permeation, [but] their effects on the skin’s inherent rate-controlling properties

are difficult to control and they may irritate the skin” (15).

**Transmucosal delivery** encompasses many possible routes of administration including buccal lozenges, nasal sprays, and suppositories. Most patients, I wager, would prefer even a shot to a suppository—and I know of none such biotech products in development. There is little interest in the buccal idea as well, though for more similar reasons to those preventing most companies from following the oral route.

There is a nasal spray vaccine (FluMist) on the market already, and a few other such products in development. These are typically liquid solution formulations either inhaled or swabbed into the nasal cavity, which presents a lot of mucosal surface area through which drugs can interface with the circulatory system. Bioavailability levels reported so far have been low. This is the most common method we become infected with cold and flu viruses, in fact, which may suggest a possible route for gene therapies that use viral vectors.

**Pulmonary Delivery:** The most promising alternative to parenteral

delivery of biomolecular drugs is through the lungs. The alveoli offer a very large surface area with direct access to the bloodstream. Spray-drying (a form of lyophilization) is growing in popularity for creating inhalable powders. But aerosols typically begin as liquid solution formulations that are nebulized by jet, ultrasonic technology, or mechanical means such as liquid atomizers and ultrasonic mesh devices. The metered-dose inhalers familiar to asthmatics are not an option here because of their harsh treatment of products and difficulties with their propellants. In fact, in its development of the recently approved Exubera insulin for inhalation, Pfizer Inc. had to create a new delivery device along with it.

Because all those are newly evolving technologies, formulators working on such products have even more hurdles to overcome than those working on parenteral biomolecules. They can find less information in scientific literature regarding excipient use with these products and these routes of administration. Many excipients that work just fine for injectible products are too strong-



smelling for inhalation, for example, or may cause unacceptable burning sensations in contact with delicate mucosal membranes. Biomolecules can denature from exposure to air-water interfaces during nebulization, and oxidation may result from mixing with air, both of which can drastically reduce the amount of drug that arrives intact to the alveoli. “A delicate balance between physical stability, aerosol performance, and protein stability may have to be struck” (16).

In addition, there are delivery device issues to consider. Development of a nonparenteral formulation can also mean development of a delivery device. This complicates the intellectual property picture for a product and adds regulatory steps as well. Manufacturing issues are raised when bulk product formulations must be stored before being loaded into unit dose or multidose devices. If salt has been used as a tonicifier, it precludes storage of the solution in stainless steel tanks. Frozen storage is a good idea if it doesn't cause product damage.

### MANUFACTURING CONCERNS

Of course, manufacturing efficiencies are always of concern, no matter what delivery method or formulation is chosen. What happens to a product after it has been purified will vary, as does every other aspect of production and processing, according to the nature of the molecule and its formulation. Bulk purified or partially purified product may be frozen until market conditions necessitate a new batch of final product, for example, or until equipment farther down the line in a multiproduct facility has been freed up and cleaned for use. When

## REAL-LIFE PROBLEMS WITH LEACHABLE/EXTRACTABLES

Here are lessons learned from some real-life case studies with extractables and leachables in protein therapeutics:

### Different Form, Same

**Vial?** A process was changed from a lyophilized to a liquid formulation, but the liquid form released a metal cation in contact with the container's rubber stopper. The manufacturer had to add a chelator to the formulation buffer. You can't change the form and assume that the same container system will work.

**Metals and Oxidation:** A prefilled syringe had used a tungsten wire to perforate the syringe barrel, which caused the protein

formulation to oxidize. The tungsten wire was discontinued and replaced with another material.

### Temperature Physics: A

lyophilized formulation was released in a lower dosage form, but after reconstitution at room temperature it became unstable. The smaller amount of material in the same-size vial was affected differently by temperature conditions, so it had to be stored at a lower temperature.

### Take the Time to Do It

**Right:** A solvent (glue) was used for needle attachment, but it caused the protein in solution to oxidize. Analysis revealed that the problem could be solved by giving

the glue at least six hours to dry—rather than assembling, gluing, and filling all at once (while the glue was still a bit tacky).

**Look Upstream:** Gaskets in a filling line absorbed a cleaning agent, which caused the product to show two new phenolic peaks in lot-release testing. The problem may not come from the actual container materials; it could be something leaching down from further upstream.

### Reference

Lee K. Overview of Extractables and Leachables in Protein Therapeutics: Sources, Methods, and Case Studies. *WCBP 2006*. California Separation Science Society: San Francisco, CA, 24–26 January 2006.

**absorbance spectra:** results of absorbance spectroscopy, showing wavelengths of electromagnetic radiation absorbed by a material as dark lines—thus producing a “fingerprint” of that material’s constituents

**aqueous suspension:** solid particles suspended (not dissolved) in a watery solution

**aseptic processing:** drug processing performed under sterile conditions using sterile techniques (e.g., in a cleanroom or with an isolator)

**bioactivity:** effect of a drug or vaccine on a living organism or tissue

**capillary (zone)**

**electrophoresis:** solution- or gel-based electrophoresis performed in tiny glass capillaries; CZE is its simplest form based on differences in the charge-to-mass ratio of analytes

**circular dichroism:** a form of spectroscopy based on differential absorption of circularly polarized light to determine the optical isomerism and secondary structure of molecules

**cleanroom:** a room combining the use of air filters and continuous air circulation to produce levels of air borne particles that are much lower than normal (100,000 particles/ft<sup>3</sup> at most)

**cryoprotectants:** excipients that prevent damage during freezing and thawing processes (usually with high water solubility and low toxicity)

**deamidation:** conversion of an asparagine residue to a mixture of isoaspartate and aspartate—thus altering the structure and peptide arrangement of a protein

**denaturation:** irreversible destruction of a protein’s

structure or amino acid constituents

**desorption:** release from an adsorbed state on a surface to a free gaseous or liquid form (evaporation in secondary drying during lyophilization)

**differential scanning calorimetry:** raising the temperature of a sample and reference standard in increments to compare how much heat energy is required to do so

**electrophoresis:** separation of charged molecules in an electric field across a porous medium

**ELISA:** enzyme-linked immunosorbance assay, an immunochemical method for detecting the presence of antigens or antibodies

**excipients:** bioactively inert substances added to a drug formulation to make it more stable or easier to store/administer

**fluorescence spectroscopy (fluorometry):** analytical technique using a beam of (usually UV) light that excites the electrons in molecules, causes them to emit light (compare the resulting *emission spectra* to *absorbance spectra*)

**HIC:** hydrophobic-interaction liquid chromatography (based on the relative solubility of proteins and matrix materials)

**HPLC:** high-performance liquid chromatography (combining several separation techniques to separate substances at high resolution)

**hydrolysis:** cleavage of a large molecule by the action of an added H<sub>2</sub>O molecule

**hydrophobic:** repelled by water (insoluble in water or other polar liquids)

**IEC:** ion-exchange liquid chromatography (based on the electrical phenomenon of ion

equipment availability allows, then bulk formulations or products will be thawed for further operations. Often, contract organizations take over at that point.

“Fill and finish” describes all the activities involved in turning bulk formulations into finished products for the market: precisely filling vials, cartridges, syringes, or ampules under aseptic conditions; stoppering or otherwise sealing them; manually inspecting the results; labeling and packaging them in boxes and/or cartons with informational inserts; and shipping them out. Class 100 cleanrooms are the norm, but alternative barrier-isolation systems are being used increasingly to create aseptic conditions in smaller-volume areas for more flexible and cost-effective operations.

Another new development has been blow-fill-seal (BFS) systems that create plastic tubes as containers, fill them, and seal them all in one unit

operation. This is showing promise for liquid formulations, which can eliminate use of preservatives because there is no exposure to the air. BFS systems are fast, easy to scale up, and may be more cost-effective when compared with the capital investment of facilities, equipment, and consumables for traditional filling operations. But they involve temperature extremes that may be incompatible with biomolecular formulations.

Leachables/extractables from delivery/packaging materials (and even some processing equipment) can cause proteins to aggregate, among other problems. Even the plastics and other materials used in containers and closures can pose a danger (see the “Real-World Problems” box). Even though plastics have traditionally been a source of concern, they are seeing renewed interest as container materials (17)—but glass is still the standard. Whatever the material, containers

must be thoroughly washed and sterilized (with solutions and methods that won’t compromise container/closure integrity) before use. Gamma irradiation is often used, but it may break down some plastics. Seals are most often made of rubber that may be lubricated by silicone oil, which can have a denaturing effect on proteins (18). Newer materials such as fluoroelastomers are an attempt to mitigate that problem. Many fill and finish operations are outsourced, and the contract organizations that specialize in these activities must keep up-to-date on the latest technology. It is often advisable to include someone from the fill-and-finish company on a formulation development team.

**Postmarket Development:** Formulators begin with timelines and end-product requirements in mind, and just like process development in an ever-evolving technology, their work is never fully complete. As described in my previous chapter,



## GLOSSARY, CONTINUED

exchange; cation exchange binds positively charged molecules, and anion-exchange binds negatively charged ones)

**isoelectric focusing:**

separating proteins based on their relative content of acidic and basic residues

**isoelectric point:** the pH at which a protein or other amphoteric molecule has an equal number of positive and negative charges

**isotonic:** of the same osmotic pressure as blood serum, thus easily mixed with blood

**laser light scattering analysis:**

measuring the amount of light scattered by a solution at some angle relative to the incident laser beam (the intensity is proportional to a protein concentration (in mg/mL) multiplied by its molecular mass)

**MALDI-TOF spectrometry:**

matrix-assisted laser

desorption ionization–time of flight mass spectrometry, which dissociates compounds into their constituent ions (electrons become excited by laser irradiation, which ejects them from the mixture’s surface) to determine molecular weight

**micellar electrokinetic chromatography:**

separating samples by differential partitioning between a pseudostationary micellar phase and an aqueous mobile phase, roughly combining capillary electrophoresis and liquid chromatography methods

**nonaqueous (organic)**

**solvents:** carbon-containing liquids (e.g., ethanol) used to dissolve solutes

**osmolality:** concentration of a solution expressed in osmoles of solute particles per unit of solvent

**oxidative degradation:**

molecular degradation caused by oxidation (removal of electrons by oxygen atoms)

**PAGE/SDS-PAGE:** (sodium dodecyl sulfate)

polyacrylamide gel electrophoresis, by which molecules are separated by migrating at different rates from one electrical pole to another through a gel medium; in SDS-PAGE, a detergent unfolds proteins first to ease their separation

**pH:** (potential of hydrogen) a measure of the activity of hydrogen ions (H<sup>+</sup>) in a solution—its acidity or alkalinity

**PEGylation:** attaching one or more chains of polyethylene glycol (PEG) to a protein molecule

**preservatives:** chemicals added to prevent spoilage, whether from microbial growth or undesirable

chemical changes

**RPC:** reverse-phase liquid chromatography, which separates molecules based on their hydrophobicity

**SEC:** size-exclusion (or gel-filtration) liquid chromatography based on differences in the size (and to a lesser extent, shape) of molecules in a mixture

**sublimation:** phase transition from solid to vapor without melting (water ice sublimates under low relative humidity at temperatures below 0 °C)

**tonicity:** osmotic pressure (i.e., strong enough to prevent osmosis)

**ultrafiltration:** filtration through semipermeable membranes that allow small molecules (e.g., water) to pass through but hold back larger ones (e.g., protein)

business-savvy biotech companies seek ways to extend their products’ lifespans with new formulations, new delivery methods, and new indications. “No single formulation can satisfy all proteins” (12), but neither can a single formulation be considered the only answer for a given drug. So formulators may well find themselves returning to the “drawing board” of preformulation and looking at every variable anew. New questions can arise: Can PEGylation (see the “Controlled and Sustained Delivery” box) increase our product’s in vitro half-life? “The 37 °C temperature and neutral pH conditions of the human body encourage deamidation. In addition, the higher temperature coupled with moisture will promote molecular mobility and thus accelerate degradation reactions” (4). So is controlled or sustained release possible? What about a high-concentration formulation? Market factors and disease behaviors will

## CONTROLLED- AND SUSTAINED-RELEASE PRODUCTS

Many biotherapeutics suffer from a very short half-life upon injection—due to fast excretion or metabolic degradation—and a longer presence in the blood and tissues could improve their therapeutic performance. Some examples include erythropoietin, interleukin, interferons, granulocyte- and granulocyte-macrophage colony stimulating factors, growth factors, leukemia inhibiting factor, and bone morphogenic protein. Controlled-release strategies began with insulin–zinc complexes, which release insulin over at most a few days. Polylactic–polyglycolic-acid (PLGA) based implants and microspheres provide release over a month. Hydrogel technologies have improved release control possibilities and may be preferred by patients. Two approaches currently in clinical trials are hydrogel-based microspheres and in situ (injection site) gelling of viscous solutions or dispersions of protein drugs.

**Polyethylene glycol (PEG) and methoxy-polyethylene glycol (mPEG)** are nontoxic, water-soluble additives approved by the FDA for drug use. They can be used to prolong the circulation half-life of therapeutic proteins as well as to increase their liquid stability. In the process of “PEGylation,” mPEG is fused to a protein molecule, which can increase product heterogeneity, complicating purification and adding characterization work that can raise the overall cost of a drug. It may, however, be a way to increase the lifespan of a marketed product—if there is no loss of biological activity.

dictate the path of a drug once it is on the market, and those forces will present new challenges to the formulation team throughout the lifespan of their product.

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