Strategies for Rapid Development of Liquid and Lyophilized Antibody Formulations

An Integrated Design

Bei Chen, Gerardo Zapata, and Steven M. Chamow

As a result of tremendous advances in drug discovery, more drug candidates flow through the pipeline at one time than ever before (1), and this trend will continue. Process development teams are challenged to keep pace with drug discovery. Tight development timelines and limits on material and personnel are other challenging factors faced by process development teams. Therefore, more efficient and systematic strategies need to be created to address the challenges. Particularly, formulation development teams face increasing expectations to develop more stable formulations for more biopharmaceuticals in a shorter period of time with less material and personnel. The strategies described herein are devised to meet such challenges.

PRODUCT: PROTEINS, ANTIBODIES

PROCESS FOCUS: FORMULATION

WHO SHOULD READ: FORMULATION, MANUFACTURING, PROCESS DEVELOPMENT

KEYWORDS: STABILITY, LIQUID, LYOPHILIZATION, EXCIPIENTS, STATISTICAL DESIGN

LEVEL: INTERMEDIATE

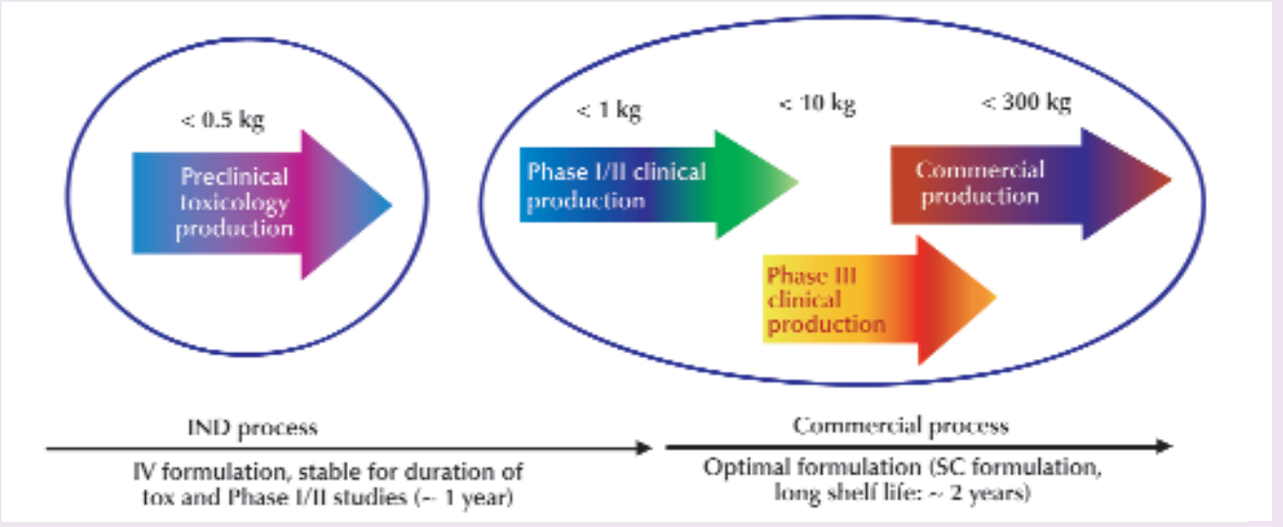
Formulation development teams must address different features and deliverables depending on their stage of product development (see the "Features and Deliverables" box). In early development, a promising drug product must proceed promptly through toxicology studies so that the company can file an Investigational New Drug (IND) application and move into clinical studies. A finalized upstream and downstream process must be defined, and an optimized formulation with a long-term shelf-life (usually two years) is needed to move into pivotal phase III studies.

Although an intravenous (IV) liquid formulation is most commonly used for toxicology and phase I studies, potential target indications and market competition scenarios force the formulation development teams to evaluate different dosage forms. For example, if the intended indication is not life threatening and requires systemic administration, the convenience of a subcutaneous (SC) formulation is preferred to an IV formulation. Similarly, the convenience of administration and improved patient compliance make a stable liquid formulation a better choice than a lyophilized product. Furthermore, less-frequent administration is desirable if the same therapeutic effect can be achieved. Finally, a novel delivery system is more marketable than the conventional vial-and-syringe configuration.

NOVARTIS AG (WWW.NOVARTIS.COM)

Formulation of a drug has important financial ramifications. Any delay in bringing a product to market and/or short shelf-life due to instability of the formulation can potentially cost millions of dollars. As a result, creative strategies for efficiently developing a drug with stability, scalability, and marketability are more critical than

Figure 1: Material and formulation requirements at different development stages



ever before. Using an integrated design based on scientific literature, rationale, and experience combined with a proven statistical design can accelerate the formulation development process and increase productivity.

Degradation Types Inherent to Proteins

Chemical Degradations	Physical Degradations
Deamidation	Unfolding
Isomerization	Aggregation
Oxidation	Precipitation
Proteolysis	Adsorption
Disulfide exchange	

Preventing or Inhibiting Chemical Degradation

The two main types of degradations specific to proteins (2) are chemical degradation and physical degradation (see the "Degradation Types" box). These two degradation pathways are often interrelated; a partially unfolded protein can increase oxidation, for example.

Excipients: Several important approaches have been identified to prevent or inhibit chemical degradation. One such approach is the addition of an effective excipient. A study by dePaz et al at the University of Colorado showed that the addition of sucrose inhibited the conformational mobility of a serine protease, subtilisin, and subsequently inhibited oxidation (3). Similarly, the addition of an antioxidant such as methionine thiosulfate has been shown to significantly reduce the oxidation rate of an anti-HER2 antibody (4).

Optimal pH: Another approach is selection of an optimal pH; most proteins require a slightly acidic to neutral pH for maximum stability. Hydrolytic cleavage of peptide bonds can occur in proteins maintained in an extremely acidic environment. For example, cleavage of the peptide bond at aspartate133–proline134 occurred in interleukin 11 maintained at pH 3 (5). Conversely, deamidation of susceptible residues (Asn and Gln) can occur within a high-pH environment. A study by Goolcharran et al at the University of Kansas compared the rate of deamidation in the model peptide Gly-L-Gln-L-Asn-L-His-L-His (GQNHH), which represent residues 8–12 of rhVEGF, with the rate of deamidation in rhVEGF maintained in pH 5 and 8 at 37 °C. The results showed that the rate of deamidation followed apparent first-order kinetics and increased with increasing pH in both the model peptide and rhVEGF (6).

Lyophilization: The process of freezing and vacuum drying a protein solution is often used in preparing protein products that are not sufficiently stable as aqueous solutions. The "Advantages and Disadvantages" box summarizes contrasting characteristics of liquid and lyophilized formulations.

Minimal stability in a lyophilized protein product requires protecting the protein from unfolding during the freezing and drying stages (7), whereas optimal stability absolutely requires that the glass transition temperature (T_g) of a dried product exceed the planned storage temperature. T_g is a characteristic temperature below which a dried product is in a rigid glass matrix, with molecular mobility greatly retarded. If a dried product is stored at a temperature exceeding T_g, the glassy matrix of the dried product looses its rigidity and becomes a deformable "rubber."

Preventing or Inhibiting Physical Degradations

Strategies to protect proteins from physical degradations have been developed over the years. Thermodynamic stabilizers inhibit unfolding of a protein in the presence of low concentrations of denaturants (8); cryoprotectants

Advantages and Disadvantages: Liquid or Lyophilized?

Liquid Formulation

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 technical transfer

Limited commercial contract capacity

exclude water and protect proteins from freezing stress (**9**); and lyoprotectants substitute for water and protect proteins from drying stress (**10**). Additionally, detergents have been used to prevent surface denaturation of proteins under freezing and agitation stresses.

Sucrose and trehalose, the most commonly used excipients to provide cyroprotection and lyoprotection, inhibit unfolding of proteins induced by freezing and drying. Furthermore, sucrose and trehalose provide a glassy matrix with T_g values higher than proposed storage conditions (**11**). Specifically, the T_g value for trehalose at 1% residual moisture is approximately 100 °C and is higher than the T_g value of 65 °C for sucrose at the same residual moisture. The higher T_g value for trehalose has been credited with better protection and greater stability of some proteins. Other sugars such as glucose, lactose, and maltose are also used for lyoprotectants. However, the utility of those sugars is limited because they are reducing sugars and potentially reactive with proteins, such as by forming covalent adducts of glucose with amino groups on the side chains (Lys and Arg) on the amino terminus of the protein through the Maillard reaction.

Glass Formation: It has also been suggested that glass formation (vitrification) and direct interaction between proteins and additives, most likely by hydrogen bonding to polar residues in dry proteins, are required to maintain the stability of dry proteins. A study by Breen et al at Genentech showed that increased moisture content resulted in a decreased T_g value and, subsequently, decreased stability (**12**). Specifically, the T_g values of an antibody formulation containing sucrose, histidine, and polysorbate 20 varied from 80 °C at 1% moisture to 25 °C at 8% moisture with higher aggregation rates observed in those cakes with higher moisture stored above their T_g values (**11**).

PEG and Tween 20: Although polyethylene glycol (PEG) is an effective cyroprotectant, it is not a good lyoprotectant. Solutions of 1% to 10% (wt/vol) PEG fully protect both lactate dehydrogenase and phosphofructokinase during freezing–thawing, but they do not stabilize the proteins during freeze-drying (**13**).

Another excipient, Tween 20, protects recombinant Factor XIII (rFXIII) against freezing–thawing and agitation-induced aggregation, primarily by competing with stress-induced soluble aggregates for interfaces, thus inhibiting subsequent transition to insoluble aggregates (**14**).

Rational Approaches: Analytical Tools

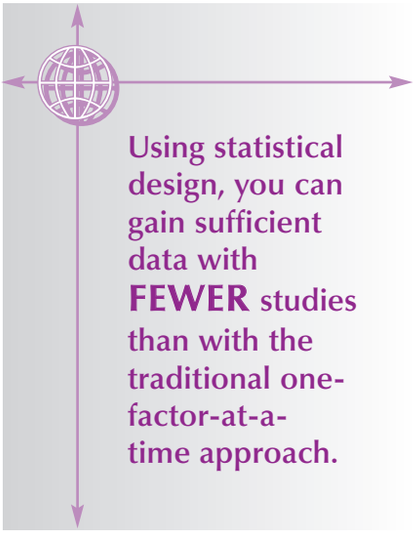
Pharmaceutical scientists have used new analytical tools in a rational approach to formulation development. Remmele et al. at Immunex successfully applied differential scanning calorimetry (DSC) to the liquid formulation development of interleukin-1 receptor (IL–1R) (**15**). Specifically, they showed that increasing the melting temperature (T_m) of a protein enhanced its thermal stability with the minimum aggregation observed at pH 6 to 7, corresponding with the highest T_m. However, considering T_m may not always work, as indicated in a study by Cromwell et al at Genentech in which the extent of aggregation and deamidation of rhDNase correlated with the T_m, but not the precipitation (**16**).

Another example is to predict stability by evaluating the conformational changes in a protein using circular dichroism (CD) or Fourier-transform infrared spectroscopy (FTIR). For many proteins, unfolding during lyophilization leads to clinically unacceptable, nonnative aggregation. That can be minimized by including stabilizing excipients before lyophilization (**17–20**) and/or during rehydration (**21**). Maximizing retention of the native protein structure in a dried solid is essential for optimizing long-term storage stability (**16–19**). A recent study suggests that the strategy of using a mixture of a disaccharide, which preserves the native structure of a protein, and a polymeric carbohydrate such as dextran, which has a high T_g value, can optimize protein storage stability (**22**). It should be noted that maintaining native conformation itself may not be enough for the long-term stability of a protein as evidenced by findings that rhDNase underwent fast deamidation and aggregation upon removal of Ca^{2+} and storage at accelerated temperatures without overall conformational changes (**23**).

Learning from Experience

Scaling Up the Dosage Form During Development: Practical experience is another asset that should be used when developing formulations. Consider the potential issues of

scaling up the dosage form during the development stage. For example, avoid using Cl^- in a low-pH formulation because stainless steel storage tanks for buffers and/or products tend to corrode at a low pH in the presence of Cl^-, compromising protein stability. Special efforts should be made to reduce the viscosity of a liquid formulation containing high concentration of a protein; high viscous formulations are difficult to scale because of poor recovery and instrument constraints. Additionally, it is a good idea to use approved pharmaceutical excipients to avoid any extensive toxicity studies required for a new excipient. Finally, cost of certain excipients should be compared. For example, if sucrose provides sufficient protection for a protein, it may be a better option than trehalose, which is much more expensive and may present supply issues.



Excipient Selection: Additionally, some special concerns about excipient selection are based on dosage forms. Formulations containing sodium phosphate buffer can experience significant pH drop during lyophilization. Precipitation of Na_2HPO_4 can cause abrupt pH decreases after the onset of ice crystallization; therefore, avoid this buffer for a lyophilized formulation if the protein is very sensitive to pH change. A citrate buffer in a SC dosage form may cause injection site pain and should therefore be avoided. Furthermore, a near neutral pH and isotonic formulation is preferred for SC dosage forms. If the protein concentration in a formulation is very low, such as rFXIII (µg/mL), use a detergent such as polysorbate 20 to reduce surface absorption.

Statistical Design

A fractional factorial design can be used to screen many formulation variables in a small number of formulations. The Youden design (**24**) is a modified fractional factorial design matrix in which seven formulation parameters, each at two levels, can be tested in only eight formulations. The Plackett-Burman design is another useful screening tool (**25**). The Youden and Plackett-Burman designs are resolution 3, two-level fractional factorial designs that can quickly pinpoint the critical factors, which then can be further optimized using a classic response surface design such as central composite design. By using statistical design, you can gain sufficient data with fewer studies than with the traditional one-factor-at-a-time approach. Commercially available statistical software such as SAS and JMP can be used effectively for study design and data analysis (www.sas.com and www.jmp.com).

Integrating Design Components

In summary, each component of the integrated design is important, but insufficient by itself. For example, without ample knowledge and expertise in protein formulations, the development of an effective statistical design would be impossible. Therefore, integrating each design component is critical for effective protein formulation development.

References

1 Nayar, R; Manning, MC. High-Throughput Biopharmaceutical Drug Development: Meeting the Coming Challenge. *BioPharm* 2002, 15: 20–28.

2 Manning, MC; Patel, K; Borchardt, T. Stability of Protein Pharmaceuticals. *Pharm. Res.* 1989, 6: 903–918.

3 DePaz, RA; et al. The Excluding Effects of Sucrose on a Protein Chemical Degradation Pathway: Methionine Oxidation in Subtilisin. *Arch. Biochem. Biophys.* 2000, 384: 123–132.

4 Lam, XM; Yang, JY; Cleland, JL. Antioxidants for Prevention of Methionine Oxidation in Recombinant Monoclonal Antibody HER2. *J. Pharm. Sci.* 1997, 86: 1250–1255.

5 Kenley, RA; Warne, NW. Acid-Catalyzed Peptide Bond Hydrolysis of Recombinant Human Interleukin 11. *Pharm. Res.* 1994, 11: 72–76.

6 Goolcharran, C; et al. Comparison of the Rates of Deamidation, Diketopiperazine Formation and Oxidation in Recombinant Human Vascular Endothelial Growth Factor and Model Peptides. *AAPS PharmSci* 2000, 2: E5.

7 Carpenter, JF; Prestrelski, SJ; Arakawa, T. Separation of Freezing- and Drying-Induced Denaturation of Lyophilized Proteins Using Stress-Specific Stabilization, I: Enzyme Activity and Calorimetric Studies. *Arch. Biochem. Biophys.* 1993, 303: 456–464.

8 Kendrick, BS; et al. A Transient Expansion of the Native State Precedes Aggregation of Recombinant Human Interferon-Gamma. *Proc. Natl. Acad. Sci. USA* 1998, 95: 14142–14146.

9 Carpenter, JF; Crowe, JH. The Mechanism of Cryoprotection of Proteins by Solutes. *Cryobiology* 1988, 25: 244–255.

10 Wolkers, WF; van Kilsdonk, MG; Hoekstra, FA. Dehydration-Induced Conformational Changes of Poly-L-Lysine as Influenced by Drying Rate and Carbohydrates. *Biochim. Biophys. Acta*. 1998, 1425: 127–136.

11 Crowe, JH; Carpenter, JF; Crowe, LM. The Role of Vitrification in Anhydrobiosis. *Annu. Rev. Physiol.* 1998, 60: 73–103.

12 Breen, ED; et al. Effect of Moisture on the Stability of a Lyophilized Humanized Monoclonal Antibody Formulation. *Pharm. Res.* 2001, 18: 1345-1353.

13 Kreilgaard, L; et al. Effect of Tween 20 on Freeze–Thawing- and Agitation-Induced Aggregation of Recombinant Human Factor XIII. *J. Pharm. Sci.* 1998, 87: 1597–1603.

14 Carpenter, JF; et al. Rational Design of Stable Lyophilized Protein Formulations: Some Practical Advice. *Pharm. Res.* 1997, 14: 969–975.

15 Remmele, Jr., RL; et al. Interleukin-1 Receptor (IL-1R) Liquid Formulation Development Using Differential Scanning Calorimetry. *Pharm. Res.* 1998, 15: 200–208.

16 Cromwell, ME; Shire, SJ. Correlation of Physical and Chemical Stability of DNase with Melting Temperature. Presented at the AAPS National Biotechnology Conference, AAPS, San Diego, CA, 2002.

17 Prestrelski, SJ; et al. Dehydration-Induced Conformational Transitions in Proteins and Their Inhibition by Stabilizers. *Biophys. J.* 1993, 65: 661–671.

18 Kreilgaard, L; et al. Effects of Additives on the Stability of Recombinant Human Factor XIII During Freeze-Drying and Storage in the Dried Solid. *Arch. Biochem. Biophys.* 1998, 360: 121–134.

19 Kreilgaard, L; et al. Effects of Additives on the Stability of *Humicola lanuginosa* Lipase During Freeze-Drying and Storage in the Dried Solid. *J. Pharm. Sci.* 1999, 88: 281–290.

20 Costantino, HR; et al. Effect of Excipients on the Stability and Structure of Lyophilized Recombinant Human Growth Hormone. *J. Pharm. Sci.* 1998, 87: 1412–1420.

21 Zhang, MZ; et al. The Effect of the Reconstitution Medium on Aggregation of Lyophilized Recombinant Interleukin-2 and Ribonuclease. 1996, *A. Pharm. Res.* 13: 643–646.

22 Allison, SD; et al. Optimization of Storage Stability of Lyophilized Actin Using Combinations of Disaccharides and Dextran. *J. Pharm. Sci.* 2000, 89: 199–214.

23 Chen, B; et al. Influence of Calcium Ions on the Structure and Stability of Recombinant Human Deoxyribonuclease I in the Aqueous and Lyophilized States. *J. Pharm. Sci.* 1999, 88: 477–482.

24 Youden, WJ; Steiner, EH. *Statistical Manual of the Association of Official Analytical Chemists* (AOAC International, 1987), pp. 51–58.

25 Xu, CP; et al. Application of Statistically Based Experimental Designs for the Optimization of Exo-Polysaccharide Production by *Cordyceps militaris* NG3. *Biotechnol. Appl. Biochem.* 2002, 36: 127–131.

Corresponding author ***Bei Chen*** *is scientist, Biopharmaceutics, Process Sciences;* ***Gerardo Zapata*** *is director, Purification, Process Sciences, and Biopharmaceutics; and* ***Steven M. Chamow*** *is vice president, Process Sciences, Abgenix, Inc., 6701 Kaiser Dr. Fremont, CA 94555, 1-510-284-6033; fax 1-510-790-5269, bei.chen@abgenix.com.*