Whitepaper

Steriles

Early-phase injectable formulation development

Proven strategies for success

Executive summary

Developing formulations for injectable therapeutics can be met with unexpected challenges. Many molecules, both large and small, have poor solubility, especially at the high concentrations used for injectables. They may also have high viscosity, which is undesirable. Additionally, many molecules are sensitive to light, heat, oxidation, and other exposures. This white paper summarizes a discussion with three subject matter experts from Thermo Fisher Scientific, Alessandro Chreim, Christy Eatmon, and Liji Joseph, on their approach and best practices for formulation development. This paper will explore tools like DOE modeling, analytical characterization, and risk-based approaches.

We cover:

- **General approaches to formulation development:** Strategies and methodologies for developing effective injectable formulations.
- **Addressing core challenges in formulation development:** Tackling issues such as poor solubility, high viscosity, and sensitivity to environmental factors.
- **Complex, late-stage formulation development including the development of higher concentrations:** Advanced techniques for developing formulations at higher concentrations.
- **Bridging the gap from formulation to GMP:** Ensuring a smooth transition from formulation development to Good Manufacturing Practice (GMP) compliance.

Approach

Selecting the correct formulation for your active pharmaceutical ingredient (API) can be intimidating. Intuitively, the choice may be to leave it in the standard buffer system that was used for development. However, these systems may contain components that are not suitable or compatible with therapeutics. The formulation may also fail stability studies or other stressors. It is also important to keep in mind that stability studies can be rate-limiting for product development, so it may be beneficial to put accelerated temperature conditions in the formulation development stability studies.

According to Liji Joseph, process development lead for biologics, we start at the desired endpoint. The target product profile, which is usually provided by the client, typically includes information such as:

- Dosage form
- Route of administration
- Concentration
- pH
- Osmolality
- Viscosity
- Storage conditions (for drug substance and drug product)
- Shelf life

Some attributes may not be available at the time of formulation development stage, but it is advised to start with end goals in mind. A design of experiments (DOE) approach is used for formulation screening, starting with pH that is determined by buffer salts. Then, excipients including tonicity modifiers, stabilizing agents, and surfactants are studied. For antibodies and antibody-derived modalities, since they are biochemically similar, a platform approach is used where up to 46 known formulations are screened. Within this process, proteins are characterized to determine their thermal and colloidal stability within different formulations. High throughput screening technologies like nano differential scanning fluorimetry (nanoDSF) and dynamic light scattering (DLS) are used to evaluate thermal and colloidal stability by measuring thermal unfolding, aggregation, and particle sizing in a single thermal ramp [1]. This is followed by a confirmational stability study to confirm the final formulation.

Formulation development for small molecules

For small molecules, a common challenge is solubility. Similarly to large molecules, target doses and routes of administration must be considered. Christy Eatmon, global subject matter expert for steriles, emphasizes, “When it comes to small molecules, we have to take a few things into account. What is the aqueous solubility and how much further do we need to go to achieve the target doses? To do that, we might start with questions like what is the target dose? What is the route of administration? Because for IV administration, the concentration (mg/mL) can be much lower than a subcutaneous product since we can inject a larger volume for IV than subcutaneously.” Early-phase development may require enhancing API solubility without a well-defined target product profile or information about the proper dose in humans. Benchtop experiments can be conducted using excipients such as cyclodextrins, chromophores, PEGs, and polysorbates to improve solubility. These excipients are selected based on their GRAS (generally recognized as safe) status, ensuring they are already approved for use in other injectable products. It is also important to stay within the range that is approved for the desired route of administration.

A DOE approach can also be used with various concentrations of different excipients in a single formulation. If a formulation shows promise early in development, it may be placed on short-term stability to assess whether we are on the right track to achieving a viable formulation. A DOE approach may include a matrix of solubility-enhancing excipients and buffers at various pH to optimize the API stability. If excipients are ineffective, energy inputs such as heat, sonication, or rotor-stator mixing can be employed. Initial approaches prioritize well-understood methods with no regulatory implications, using known excipients and straightforward solutions.

Strategies for excipient selection

When selecting excipients in the early phases of drug formulation, it is crucial to consider not only their functional properties but also proactively design a formulation suitable for the final administration dosage form. Potential interactions with the API and primary packaging must be considered. Alessandro Chreim, global subject matter expert for steriles, emphasizes, “It is a fundamental aspect to create a robust formulation, because usually in early phases, the goal is always trying to create the simplest possible formulation that ensures the drug is stable, it’s safe and deliverable without adding accessory complexity that could create issues down the process.” This approach involves leveraging prior knowledge and starting with well-characterized excipients that have a long history of safe use in parenteral products, such as common buffers like citrate or phosphate, tonicity modifiers like sodium chloride, and surfactants like polysorbates.

To ensure the quality and compatibility of excipients, impurity profiles are assessed, as impurities like peroxides in polysorbates can cause oxidation in sensitive APIs. High purity and low peroxide grades of critical excipients are sourced from the beginning. Experimental compatibility studies are conducted under various stress conditions to evaluate interactions between the formulated API and excipients with the primary packaging material. Analytical techniques such as HPLC are used to detect degradation or loss of API, indicating incompatibility. Additionally, potential aggregation caused by lubricants in packaging materials is assessed, and alternative materials like polymeric containers may be considered. Understanding the molecule's intrinsic liabilities, such as susceptibility to oxidation, guides the inclusion of protective antioxidants like ascorbic acid or sodium metabisulfite in the formulation. Excipient selection often requires a multifaceted approach and multi-step workflow.

Solving common formulation challenges

Despite planning and experience, each molecule presents unique challenges. Liji Joseph explains, "Biologic drugs are inherently complex when you think about the physical chemical properties such as isoelectric point (pI), molecular weight, glycosylation, or any other post-translation modifications and overall amino acid compositions are uniquely specific for each drug candidate." While platform approaches for pre-formulation development exist, the unique characteristics of each biologic drug can make the process challenging. Common degradation pathways, such as deamidation and oxidation, pose significant hurdles. For instance, surfactants are essential for protecting against freeze-thaw stress and aggregation but can introduce oxidative impurities. Lowering pH to mitigate deamidation can adversely affect solubility, creating a need to balance competing factors. Customized studies are often necessary to address specific stability issues identified during preformulation, ensuring a tailored approach to mitigate these challenges.

When should lyophilization be considered?

Sometimes a molecule is not stable or soluble enough in solution, so lyophilization, or freeze-drying, is the best choice. Other factors like storage or shipping conditions may also influence the decision to lyophilize. Determining whether a molecule needs to be lyophilized involves balancing scientific necessity with practical considerations, such as phase I timelines where speed is crucial. The primary scientific triggers for lyophilization are instability in liquid form, even at refrigerated or frozen temperatures, and poor solubility. Lyophilization can also provide dosing flexibility, allowing a stable solid-state product to be reconstituted to various concentrations on demand at clinical sites, which is particularly advantageous for dose escalation studies. This approach enables the manufacture of a single stable batch that can serve the entire trial, improving the shelf life and handling of sensitive APIs.

However, lyophilization development is a complex and resource-intensive process, adding time to overall development timelines. Developing a robust lyophilization cycle involves understanding the thermal characteristics of the liquid formulation using techniques like differential scanning calorimetry (DSC) and freeze-drying microscopy to establish the scientific foundation. With this data, pilot lab-scale cycle development (~30 vials) and optimization runs are conducted to monitor parameters in real-time such as capacitance versus Pirani gauges. Cycles can be adjusted as needed, and the softening of the frozen matrix can be monitored. Ultimately, the decision to pursue lyophilization in early-phase development is strategic, weighing the benefits of clinical dosing flexibility against the impact on project timelines.

Additional considerations for small molecules

Many molecules show promising clinical responses but have poor water solubility. Additionally, small molecules can be hygroscopic, sensitive to light, heat, or oxygen, necessitating thorough investigation. Rapid development for Phase I trials requires concurrent evaluation of these factors to identify and address potential issues. While animal PK studies often involve direct injection of solubilized API, translating this to first-in-human studies requires longer-term stability studies, which can span several months or years.

Lyophilization can serve as a backup when solution stability is inadequate. Managing programs with tight windows from API addition to product freezing is crucial to ensure manufacturing processes meet human use standards. Christy Eatmon states, "As a formulator, I've had situations where I thought I cracked the code on solubility and had achieved a nice clear solution, only to come back to the lab on day three or four and find a vial of beautiful crystals instead of a clear solution." GMP production involves extended compounding, filtration, and filling times, requiring careful consideration of insoluble materials that may clog commonly used 0.22 µm filters. Laboratory studies are essential to identifying and mitigating these issues, supporting the formulation of small molecules for first-in-human trials.

Solubility and equipment considerations

Formulating small molecules for clinical use involves significant challenges, with solubility being a major consideration. Alessandro Chreim notes, “Solubility is the primary hurdle, but we also see some often-underestimated stability challenges for small molecules, especially early in development.” These include physical instability against the equipment train that can be used during the manufacturing process and moving from pilot scales into the GMP environment at a larger scale. Proactive strategies are essential to address these issues and prevent costly delays later in development. Hydrolysis, oxidation, and physical instability are common challenges. Conducting a pH rate profile study helps identify the optimal pH for formulation, guiding the selection of buffering agents and potentially pivoting to nonaqueous formulations or lyophilization for extremely labile compounds.

Oxidation, catalyzed by metal ions or residual peroxides after VHP decontamination, requires proactive assessment by stressing the formulation with strong oxidizing agents like hydrogen peroxide. Mitigation strategies include nitrogen overlay during filling and prolonged decontamination cycles. Physical instability, such as surface absorption and aggregation, also needs attention. For instance, peptide molecules may undergo conformational changes leading to fibril formation, which is pH-dependent.

Complex late-stage formulation development

High concentration formulation development is a critical area, requiring tailored strategies to address solubility and viscosity challenges. Liji Joseph explains, “In the higher concentration space, the critical challenge is solubility and viscosity.” Two primary strategies are employed based on client needs. The first involves reformulating existing early-phase formulations to achieve higher concentrations for later phases and commercialization. This approach maintains the same excipients while evaluating concentration-dependent viscosity to determine the highest feasible concentration. Strategies such as pH screening and the use of salts or viscosity-modifying agents are employed to manage protein-protein and hydrophobic interactions, ensuring the formulation remains stable and processable.

The second strategy is for clients to start with high concentration formulations from the outset. This involves identifying a base formulation and assessing its developability by focusing on viscosity and concentration dependence. Similar to the first approach, excipients are tweaked using pH, salts, or viscosity-modifying agents to achieve a stable and acceptable formulation. Collaboration with the process development team is crucial to address potential issues like filter clogging during processing. Both strategies culminate in confirmational stability studies to finalize the development work, ensuring the formulation meets the required stability and physical property standards.

Formulation considerations for subcutaneous administration

For subcutaneous formulations, the target concentration is typically quite high, often in the range of 150 to 200 mg/mL. At these concentrations, there is often an inflection point where viscosity increases dramatically, which must be managed to ensure the formulation remains below this threshold. Most peristaltic pump-driven filling lines can handle viscosities up to ~50 cP, but injectability is also a concern. Advances in syringe technology have allowed for higher viscosity formulations, but biologics also pose the risk of aggregation. Therefore, it is crucial to handle these molecules gently during the drug product fill-finish process, using peristaltic pumps and slow, steady filtration to avoid shear forces that could induce aggregation.

Freeze-thaw studies are essential to ensure stability, as aggregation can occur upon thawing the drug substance. Subcutaneous formulations are typically filled into syringes, requiring careful consideration of the transition from vials to syringes. Syringes are often siliconized, which can cause large molecules to aggregate. Therefore, spiking studies with silicone are necessary to ensure no adverse impact on the molecule. Despite these challenges, extensive experience in filling high-concentration biologics into both vials and syringes provides confidence in achieving a stable formulation and a robust drug product process that minimizes shear stress and stability issues.

In summary, developing high-concentration subcutaneous formulations involves managing solubility, viscosity, and aggregation risks. Proactive strategies, such as gentle handling during fill-finish, freeze-thaw stability testing, and careful evaluation of syringe interactions, are essential to ensure the stability and efficacy of the final product.

Bridging the gap from formulation to GMP

Addressing challenges prior to GMP production can save significant time and resources. Designing non-GMP process studies for complex modalities like peptides or oligonucleotides requires a proactive approach to uncover hidden liabilities before they impact GMP manufacturing. Alessandro Chreim explains, “For those complex modalities, the standard development playbook doesn’t always apply. Our entire approach is designed to proactively uncover what we call hidden liabilities before they can impact workflow when we are in GMP space.” These studies are not just simple confirmations but are carefully scoped to include stress tests at critical manufacturing steps, such as mixing, sterile filtration, and the fill-finish process. This is particularly important for modalities known to be sticky or sensitive, where issues like shear sensitivity, oxidation, filter binding, and process-induced aggregation need to be addressed.

To effectively investigate these risks, a DOE approach is employed. This approach not only identifies normal operating ranges (NORs) but also intentionally pushes process boundaries to map robustness margins (PARs). For example, varying mixing speeds or filtration flow rates helps determine when and how the product starts to degrade or bind to surfaces. This is coupled with the development of sensitive analytical methods to detect minor degradants or changes, allowing early identification of potential failures. Whenever possible, pilot-scale equipment and components that closely mimic the commercial process are used to ensure the data is meaningful and translatable to full-scale manufacturing. This strategy of proactive risk identification and the use of representative studies and equipment builds a comprehensive process understanding, informing control strategies and reducing the risk of expensive failures during GMP production.

Identifying risks for biologics with force degradation and accelerated stability studies

Forced degradation and accelerated stability studies play a crucial role in defining formulation boundaries before filing an Investigational New Drug (IND) application. Liji explains, “Forced degradation and accelerated stability studies definitely help to understand the degradation pathways. Knowing the degradation pathways can help us find out the potential risks during the formulation development work and can be mitigated as well.” These studies expose the drug and formulation to extreme conditions to reveal stability issues and potential degradation pathways, providing valuable information on the product’s shelf life and stability under intended conditions.

Typically, forced degradation studies involve scenarios such as freeze-thaw cycles, agitation, pH stress (both low and high), and oxidation. These tests challenge the formulation to identify failure points, helping to understand the gaps in the process and potential mitigation strategies to avoid issues during the lifecycle management of the product. While some clients prefer to conduct these studies early in formulation development to proactively address potential risks, others opt to perform them in later phases, closer to regulatory filings like the Biologics License Application (BLA). Regulatory agencies often require data from forced degradation studies to ensure that the analytical methods used can identify degradation products, making these studies critical at various stages of development.

Choose the right partner

By working with over 810 biologics batches and over 100 small molecule commercial products, our teams have the knowledge, equipment, and experience to help you develop the right formulation that will take you from preclinical work through commercial manufacturing.

Our seven state-of-the-art steriles manufacturing sites feature the latest equipment, and many also have dedicated PDS lines—typically for the production of smaller clinical batches.

Our expertise in aseptic processing, fill-finish operations, and lyophilization—combined with robust regulatory support and quality control—ensures the highest standards of product quality and compliance.

Reference

1. Kim SH, Yoo HJ, Park EJ, Na DH. Nano Differential Scanning Fluorimetry-Based Thermal Stability Screening and Optimal Buffer Selection for Immunoglobulin G. Pharmaceuticals (Basel). 2021 Dec 25;15(1):29. doi: 10.3390/ph15010029. PMID: 35056086; PMCID: PMC8778976.

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