Dos & Don'ts For Rapid Throughput Formulation Development: Buffer And Excipient Selection

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The New Reality

Normally, it takes ten years and $1bn to deliver a new drug to market and about one and a half to two years to develop the optimal formulation. With the urgency to manufacture a SARS-CoV-2 drug or vaccine, and to create an annual flu vaccine, there is a need for rapid throughput screening techniques. At the start of this journey is the development of a formulation that will prove to be safe, effective, and stable for at least two years after manufacturing. Although liquids are easier and cheaper to produce, lyophilized products are more stable, therefore the ideal formulation should be capable of being freeze-dried.

Optimal formulation development requires knowledge of not just the protein itself but how the final product will be packaged and used. This will assist the development team in screening appropriate excipients, including buffers, for the target product. To aid this process, high-throughput methods have been developed to rapidly evaluate different conditions and move through the drug development processes quicker.

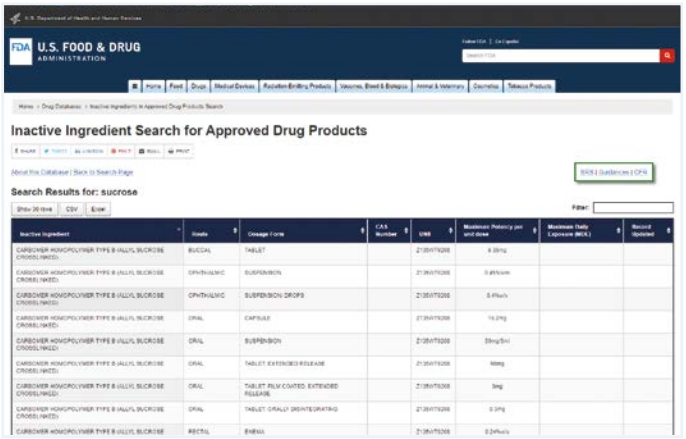
Recently, Dr Jeff Schwegman, CEO at Labyrinth BioPharma, LLC, USA presented a webinar discussing the dos and don'ts of choosing the correct buffer and excipient for high-throughput formulation development. This tech note summarizes the webinar and includes a selection of questions from the Q&A sessions.

Building A Stable Product – Formulation Design

Building a stable product is a methodical process starting with the development stage that characterizes the protein followed by a series of compatibility studies to identify the most stable product that can be lyophilized, before advancing into the manufacturing process.

Sufficient amounts of purified protein, a selection of additional formulation components, and an understanding of dosage, storage and delivery method are all required before this development process can commence. Formulation components come in many guises from buffers to balance the pH to bulking agents, stabilizers, and surfactants that work alongside the active ingredient.

In the early stages of development, even in Phase I Clinical Trials, it is not uncommon to "partially formulate" the product with enough stability to get through the trial. This usually manifests itself as a frozen presentation to be thawed and administered at the clinic. This allows the drug to go through clinical trials while the formulation is being further developed to become more stable, ideally in a lyophilized state.



FDA website (www. fda.gov) offers information about excipients approved for use in drug formulations.

Excipients

Although many of these non-active pharmaceutical agents are important during the manufacturing of a drug, it is worth considering addition of excipients early in the development process as these substances can affect the properties of the active ingredient. A bad choice of excipient can have devastating effects, even causing fatalities. Detailed information about excipients approved for use in previous drug formulations can be found on a number of websites, including the United States Food and Drug Administration (FDA) website, which can provide a useful resource when designing a new drug. In general, keeping the concentration of any excipient to the minimum will help ensure they have minimal effects on the target product, especially during lyophilization.

1. Buffers

During compatibility studies, two or three buffers are initially chosen with a pKa close to the stable pH of the target product. Changes in pH can occur in drug products due to a chemical reaction in the formulation, such as degradation, oxidation, or hydrolysis, but also through the freezing process and in some cases, the purified protein can have some buffering capacity.

2. Surfactants

Products can stick to glass, stainless steel, process tubing, filters, or any product contact surface. Surfactants possess the capability of decreasing surface tension and can block certain surfaces where proteins molecules can stick, or even prevent them from sticking to each other. Polysorbates between 20 and 80 are commonly used in formulations, in particular 20Tween80.

3. Salts

Salts increase the amount of unfrozen water and decrease the collapse temperature during freeze-drying. In doing so, salts can delay or prevent crystallization of other components. The tonicity of a product can be adjusted with mannitol or glycine if it is necessary to achieve an isotonic product.

4. Stabilizers

Biological molecules are especially susceptible to loss of activity due to both the "freezing stress" and the "drying stress" of lyophilization. A good stabilizer is preferentially excluded from the protein surface, remains amorphous, and is capable of hydrogen bonding with the protein. Sucrose and trehalose are both well suited to protect biological molecules from these stresses. Understanding how much stabilizer to add is critical; adding too much can significantly reduce drying time, could collapse the lyophilized cake, or result in destabilization; adding too little will not protect the protein.

5. Bulking Agents

Bulking agents are used when the total amount of active ingredient and other excipients are too small to provide adequate structural support to the cake. Most commonly used in lyophilized products is mannitol but its crystalline behavior can present problems, and vial breakage can be significant.

High-Throughput Screening

During the webinar, Dr Schwegman describes several approaches to study formulation variables. Nano Differential Scanning Calorimetry (DSC) and and Differential Scanning Fluorimetry (DSF) allow the solution to be stressed by temperature in different formulation conditions. The more stable the initial solution condition, the higher the temperature the protein will retain its folded, native state structure. Nano DSC measures the heat that is being absorbed as degradation or unfolding occurs. It is a sensitive method, but testing is time consuming and it needs a lot of material. During DSF, the product temperature is raised until denaturation occurs, changing the florescence due to an extrinsic fluorophore. These methods are compatible with a 96-well format enabling multiple conditions to be tested simultaneously and in high throughput.

Soluble and insoluble aggregates can be measured during freeze-thawing or lyophilization of product using colloidal techniques such as static/dynamic light scattering, Size Exclusion Chromatography (SEC)-HPLC, flow imaging microscopy (Figure 1). After measurements, adjustments can be made to the excipients and the process repeated until aggregation (soluble and insoluble) is minimized or eliminated.

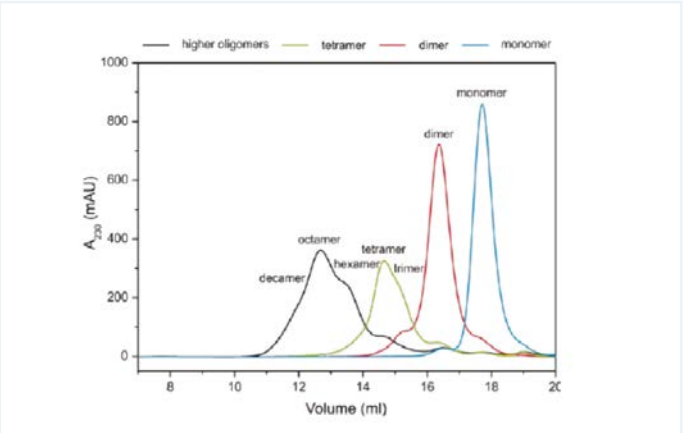


Figure 1. *Size Exclusion Chromatography (Soluble Aggregates) with UV Detection*

Another screening method analyzes protein secondary structure by measuring hydrogen bonding in the different structural elements of a folding protein, including alpha helices, beta sheets, beta turns, etc. Infrared analysis of proteins (FTIR) identifies Amide I vibrational frequency changes caused by C=O backbone stretching vibrations when the oxygen in the carbonyl bonds with hydrogen in the different folded structural elements.

Conclusions

Developing a stable formulation for a biologically-based therapeutic product is an expensive and time consuming process. Excipient selection, including buffer selection, has traditionally been a trial-and-error process involving the preparation of many different formulation variations and placing them on accelerated

stability. High-throughput screening techniques, including but not limited to, nano-scale DSC, DSF, and FTIR are very useful tools for quickly screening excipient variations to determine how they will impact the short/long term stability, and on the effectiveness of stabilizing against the freezing and drying stresses of lyophilization. High-throughput screening techniques, in addition to keeping good lines of communication open between the analytical and manufacturing groups, will help to reduce the time and cost of production. These techniques also enhance the chances of success for producing a stable, safe, and effective product for consumers.

To view the full webinar and download the slides, please go to the archived webinars on our website http://www.sp-scientificproducts.com/Webinars/Archives

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Q&A Session

1. **Can these techniques be easily translated to vaccines or viral vectors?**

   Yes, to both. I have worked with live, modified live, and attenuated viruses, and although I haven't worked with a vector for gene therapy, you still have to preserve the protein shell of the virus.

2. **In your experience, do the best formulants from DSF studies of the liquid state give you benefits in freeze-drying too?**

   Maybe a little in regards to stabilizers (disaccharides), as these will stabilize against both cryo and lyo stresses, but you still need to investigate lyo stresses once you correct for damage from cryo stresses.

3. **Can surfactants reduce sticking due to charge post milling?**

   My expertise is in liquids not solids, but I'm guessing a non-ionic surfactant won't help, but a charged one (phosphatidyl choline) might.

4. **When you have high concentration of protein, does the mechanism of stabilizer still include preferential exclusion?**

   Protein concentration in itself can be a stabilizing mechanism. The higher the concentration the better, but then you start running the risk of aggregation and precipitation. The stabilizing mechanism for freezing stress is preferential exclusion due to surface tension differences between the hydration shell around a protein and the surrounding area. If you super concentrate the protein, that may alter that difference. Drying stress reduction is due to hydrogen bonding of the stabilizer with the protein surface, so you would still need this to occur regardless of concentration.

5. **Do you need a bulking agent when freeze-drying high concentration mAb?**

   If the total solids content of your formulation results in a good cake, then no. If not, then yes. Adding a bulking agent is always the last thing I do with a formulation, and I only add it if there are not enough total solids to give me a good, intact cake after lyo.

6. **Any suggestion on low concentration formulation for lyo?**

   This is done all of the time. I've worked on projects with nanogram levels of active ingredients. You stabilize the formulation as normal and then add a bulking agent to give you a good cake.

7. **How do you compare mannitol to glycine as a bulking agent?**

   In my mind, you can use them interchangeably. They both have about the same thermal properties, give good, white cakes, reconstitute well, and are well tolerated by the body. Mannitol is used in more products than glycine as an excipient, so that gives you more flexibility in using it for your applications, and I would imagine in might be less expensive.