



BioPharma
Solutions

 white paper edition

Pharmaceutical Development for ADCs:

Not as Simple as ABC

Baxter

Pharmaceutical Development for ADCs

Formulation, process, and analytical development for antibody-drug conjugates, or ADCs, is complex. While the development of an aqueous solution formulation may be preferred over a lyophilized product, it is useful to initially pursue a dual path and develop drug product formulations for both a sterile solution and a freeze-dried solid presentation, ultimately choosing the most successful candidate for final development.

This white paper provides an overview of:

- ADC structure and function
- The development process
- Lyo cycle optimization

ADC Structure and function

An antibody-drug conjugate, or ADC, consists of a monoclonal antibody (mAb) joined to a highly potent or cytotoxic drug by a stable, chemical linker with labile bonds. The antibody is targeted to attach to an antigen on the surface of a tumor cell. Once the connection is made between surface antigen and antibody, the antibody and linked drug are incorporated into the tumor cell. The drug is then cleaved from the antibody, and goes to work to destroy the tumor cell. Once internalized, the amount of “payload drug” required to cause cell death is relatively small. For that reason, ADC’s are generally better tolerated by patients than more traditional forms of chemotherapy.

Development Process

An optimal formulation for an ADC protects the stability of the mAb, the linker, and the drug product. As a protein, the antibody may be subject to deamidation, aggregation, and/or fragmentation. Formulation ingredients designed to protect the protein may be sufficient to allow the final product to remain in the liquid state, but most often a careful freeze-drying of the product is also required to maintain the stability of the antibody. Although freeze-drying adds to the manufacturing cost, it often pays off in less complicated cold chain management of the finished drug product, since the lower required storage temperatures associated with a liquid formulation (often -80°C) are no longer a factor.

By pursuing a dual path strategy, where initial formulation development focuses on ingredient mixtures that are suitable for both liquid and freeze-dried formulations, an ADC can be evaluated in both configurations, and the best path forward can be chosen.

Common Analytical Methods for ADCs

Concentration	Aggregation	Degradation
<ul style="list-style-type: none"> • UV-Vis • Nano-Drop 	<ul style="list-style-type: none"> • SEC • DLS 	<ul style="list-style-type: none"> • iCE • Peptide Mapping
Fragmentation	Particulates	Compendial
<ul style="list-style-type: none"> • SDS-PAGE • CE-PAGE 	<ul style="list-style-type: none"> • MFI • HIAC 	<ul style="list-style-type: none"> • pH • Appearance
Drug Antibody Ratio	Linker Stability	Lyo
<ul style="list-style-type: none"> • DAR-HIC 	<ul style="list-style-type: none"> • Free-Drug by RP-HPLC 	<ul style="list-style-type: none"> • Residual Moisture • Recon Time

Basic steps for the dual-path process:

- Establish Analytical Methods
- Study the Effect of pH, Buffer, Ionic Strength, and Surfactants on Chemical and Physical Stability
- Biophysical Characterization by FTIR and Calorimetry
- Screening of Candidate Formulations under Stressed Conditions
- Long Term Stability studies on Selected Formulations, concurrently in solution and freeze-dried solid

Physical stability can be assessed through the use of size exclusion chromatography (SEC), and by HIAC or microflow imaging (MFI). FTIR is used to evaluate the amide region of the protein, to evaluate formulation and processing effects.

During stability studies, protein aggregation is monitored by Dynamic Light Scattering (DLS), SEC, and MFI or HIAC. In addition, changes in charge heterogeneity are tracked with imaging capillary electrophoresis (iCE). Note that the specific characteristics of each ADC require that methods be optimized for each ADC studied.

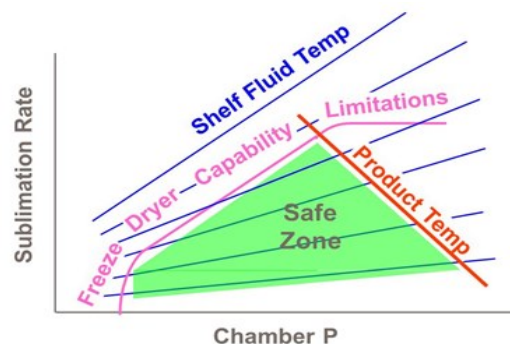
Lyophilized ADCs

If a freeze-dried formulation is ultimately required, additional studies are necessary to optimize the process. These include low

temperature thermal analysis of the formulation by DSC and freeze dry microscopy, design space development to identify all acceptable as well as the optimal primary drying conditions, and optimization of secondary drying by analyzing the effect of residual moisture on stability of the product.

To construct a design space for primary drying, tunable diode laser absorption spectroscopy (TDLAS), an in-process water vapor flow meter, is used with laboratory development freeze dryers to measure the heat transfer properties of the glass vial and the resistance of the dried product layer to the flow of water vapor. These variables are used in well-established equations for heat and mass transfer to determine the impact of shelf temperature and chamber pressure on product temperature and sublimation rate. Once the capabilities of the freeze-drying equipment are factored in, boundaries are established so that any processing combination within the “safe zone” is considered acceptable for the specific ADC being freeze-dried.

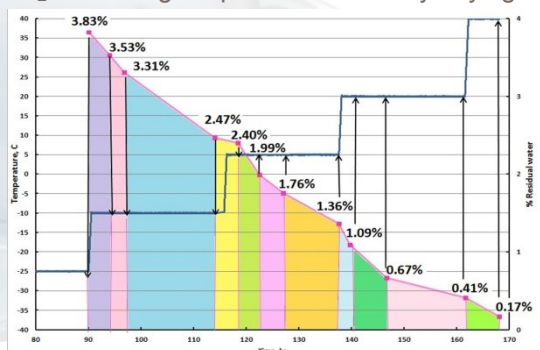
Primary Drying Design Space



During freeze-drying, protein-containing drugs like ADCs are often dependent upon retaining a specific amount of water to protect the conformation of the mAb. During primary drying, sublimation removes all water that freezes as ice. By warming the shelf for secondary drying, the remaining water is removed by evaporation. Parameters must be developed to remove the optimal amount of water, while keeping the product at a safe temperature. Trial cycles are run, and samples with varying levels of residual water are placed on stability.

To generate sufficient samples for each test condition to be analyzed, vials of the ADC are frozen and primary dried. Instead of using traditional secondary drying, which slowly ramps then holds the shelf at a warmer temperature until most of the unfrozen water evaporates quickly when the product reaches a specific temperature; the shelf is instead stepped through progressively warmer temperatures. This allows for a more gradual reduction of water and better uniformity between all vials. Samples are collected as the cycle progresses and are analyzed for water content.

H₂O% during Step-wise Secondary Drying



Once the water content of the lyo cake is determined progressively through this step-wise cycle, a second identical cycle is run, with sufficient samples pulled at the proper time for each water content level that will be studied on stability. If a non-destructive method, such as near IR spectroscopy, is used to measure water content of the cake, the influence of water content on results for stability indicating assays can be monitored over the course of the study.

Also influencing the behavior of the lyophilized product is the differential between the glass transition temperature (T_g) of the dried solid and the temperature at which the product is stored on stability. A storage temperature warmer than the T_g of the product can increase molecular mobility, and lead to possible protein aggregation, collapse of the cake, and crystallization of amorphous, protein-protecting ingredients. The T_g increases as water content decreases, so a drier product can withstand a higher storage temperature. The design challenge then for the secondary drying stage of the lyo cycle is to maximize the allowable safe storage temperature by finding the lowest residual water content for the product that will still support optimal protein conformation, and therefore optimal efficacy of the drug.

The design and analysis of ADCs can prove to be a complicated process; however, the wide variety of available research tools and methods help to bring the very best drugs from the laboratory to the market.



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