STABILISATION OF ANTIBODY DRUG CONJUGATES BY FREEZE DRYING

Antibody-Drug Conjugates (ADCs) are a class of therapeutic compounds designed to target cancer cells. They combine the cancer-killing (oncolytic) properties of potent cytotoxic drugs with the targeting capabilities of monoclonal antibodies, meaning that the drug can be delivered to diseased tissue and not to healthy cells, limiting the customary side effects that are traditionally associated with anti-cancer therapies while also extending the therapeutic window.

Due to the complex and sensitive nature of antibodies and the potent nature of cytotoxic drugs, together with the requirement to maintain the structural 'linker' between these 2 components when they are conjugated together in an ADC, it is easy to understand how such a chemical entity may not possess significant levels of stability in an aqueous solution for injection, or during the traditional (and usually aggressive) drying methods that are often used to create a powder suitable for tableting.

Freeze drying has long since been used on antibodies and cytotoxic drugs, and can be used to stabilise ADCs in order to provide a longer shelf life; however, with the freeze-concentration effects that occur during lyophilisation, and the potential sensitivity of molecules to dehydration, it is unlikely that a suitable combination of formulation and process conditions will be hit upon by trial and error.

A number of challenges need to be met for freeze drying to be successful:

- Antibodies (or even antibody fragments) may need to be protected from the potentially damaging effects of freezing and the removal of water by the use of 'lyoprotectants' in order to maintain their 3D structure and function,
- The cytotoxic element of the ADC means that special handling precautions are necessary, particularly when dealing with the freeze dried material, which may be friable unless formulated appropriately to give a cohesive product,
- The linker between the antibody and the drug may also be sensitive to processing; some ADCs are designed with cleavable linkers to work on a broad range of targets, while others comprise linkers that are designed to be uncleavable in order for the drug to remain more specifically targeted. It is essential that the lyophilisation process does not adversely affect the nature of the linker molecule.

+ CASE STUDY: Formulation and Lyo Cycle Development for an ADC

Biopharma Group was asked to assist a customer with the formulation and cycle development for a novel ADC. After screening a number of candidate bulking agents, buffers and stabilisers, a promising formulation was developed (Table 1) which was then analysed by freeze-drying microscopy (FDM) for collapse temperature. Figure 1 shows the formulation at -33.1°C after the collapse event became evident (onset was -37.5°C).

	Excipient 1	Excipient 2	Excipient 3	Excipient 4	Water grade
Formulation	L-Histidine	Sucrose	Glycine	Polysorbate 80	WFI* Top up to 1ml

Table 1: Excipients selected for the formulation

Additionally, as part of the R&D process, Biopharma Group was asked to evaluate 3 different fill volumes to maximise throughput. Volumes of 2mL, 6mL and 10mL were evaluated, with the smaller volume being a higher concentration to give the same total amount (20mg) of API per vial overall.

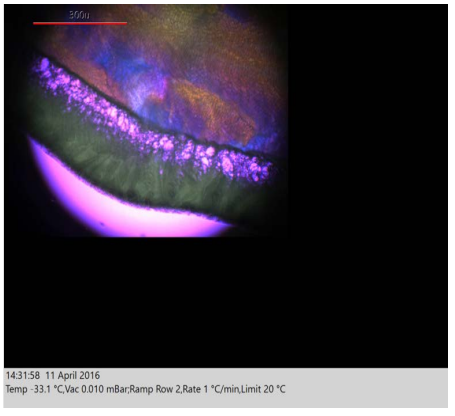

Figure 1: FDM image of formulation at -33.1°C

Biopharma Group
btl@biopharma.co.uk | www.intelligentfreezedrying.com

Analysis	Event	Temperature	Description of events/general comments
Impedance (ZSinϕ)	Z1	-38.5°C to -37.5°C	Step change in the impedance indicative of a **possible glass transition** with a midpoint at -38.0°C
	Z2	-28.5°C	Increase in downward gradient of the impedance curve indicative of a **softening** within the frozen material
	Z3	-3.0°C	Minimum impedance indicative of **full mobility** within the frozen material
DTA	D1	-42.0°C to -36.5°C	Baseline shift with a midpoint at -40.0°C indicative of a **glass transition** of the frozen material.
	D2	-1.0°C	**Ice melt** endotherm

Table 2: Description of events observed in Figure 2

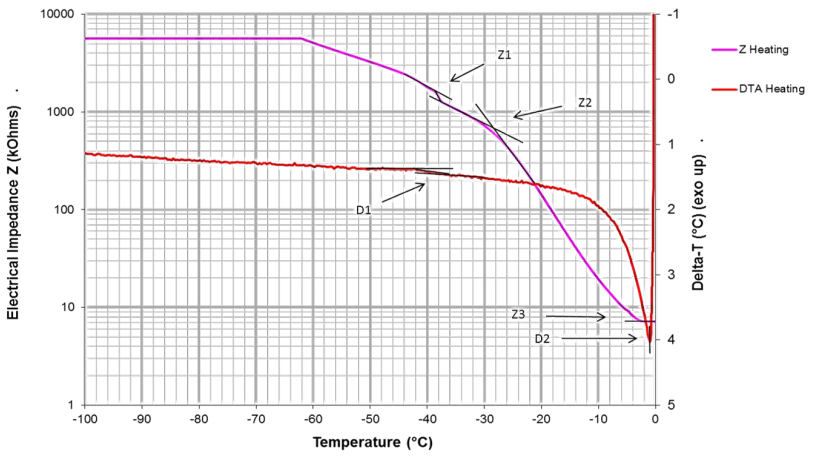

Figure 2: Lyotherm3 graph showing events in DTA curve (in red) and impedance curve (in pink)

A number of developmental freeze drying cycles were carried out, using mostly placebo but with some vials containing the API, so that the effect of freeze drying on the API could be established. A cycle 96 hours in length (Figure 3) was established as being the best balance between efficiency and product quality, providing cakes with good to excellent appearance (Figure 4) and a mean residual moisture content of <0.3%, giving good stability.

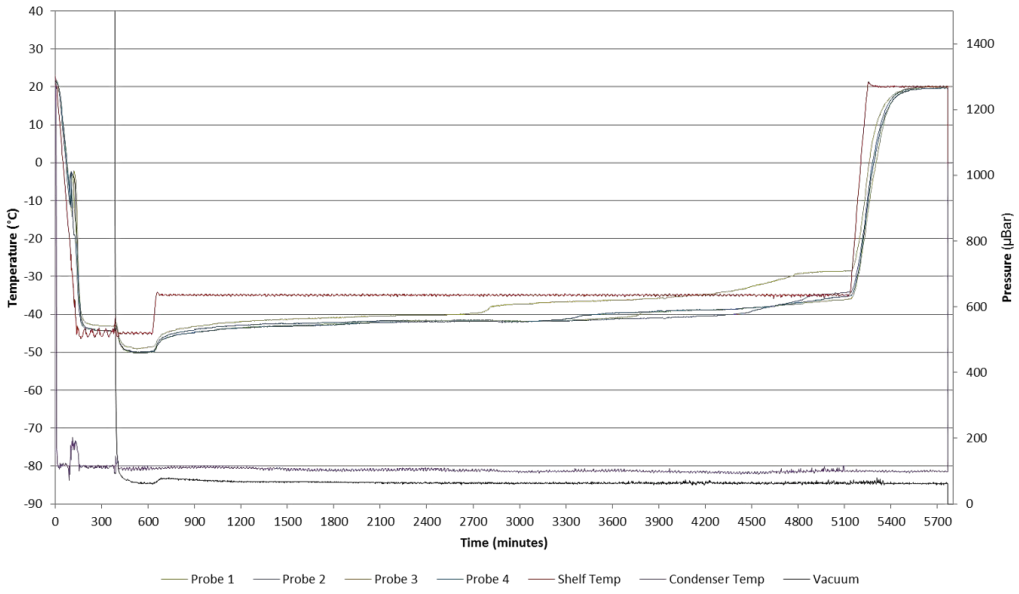

Figure 3: Final (96 hour) freeze-drying cycle for ADC

Figure 4: Vials of lyophilised product

Biopharma Group has worked on more than 2,000 projects since 1997, not just on therapeutic materials but also vaccines and medical diagnostics. Through its extensive knowledge of protein / antibody stabilisation, its dedicated cytotoxic freeze-drying R&D lab and its range of cutting-edge characterisation technologies, Biopharma Group possesses the know-how and the technical capability to provide clients with a high degree of assurance that its logical and scientific approach will lead to a successfully formulated, lyophilised and shelf-stable ADC product.

To discover more, please contact us today:
btl@biopharma.co.uk, or +44 (0) 1962 841092