BIOPHARMA GROUP APPLICATION NOTE

Optimisation and Evaluation of a Lyophilized Immunoassay in Lyobead Format

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INTRODUCTION

Lyobeads have recently gained attention as an adaptable, cost-effective, and efficient format, especially for their suitability for high-throughput production environments and other advantages. Formulation development for diagnostic assay is crucial to ensure product stability. For lyobead applications, formulation development is especially important to ensure that the lyobeads are mechanically robust to withstand various pick-and-place operations. Leveraging both practical experience and Design of Experiments (DoE) in formulation development can enhance project success while potentially saving time and resources.

This application note explores the main steps to develop a successful lyobead product through a case study:

- The selection of stabilisers will be based on excipient compatibility data provided by customer.
- Enhancing the formulation's collapse temperature by incorporating stabilisers and optimising the lyophilization process for greater efficiency.
- As some excipients can interfere with assays, this requires a case-by-case approach.
- Using practical experience (a non-DoE method) and data from the customer's analysis of the liquid format, Biopharma Group and the customer agreed on 3-5 candidates to be tested in screening cycles.

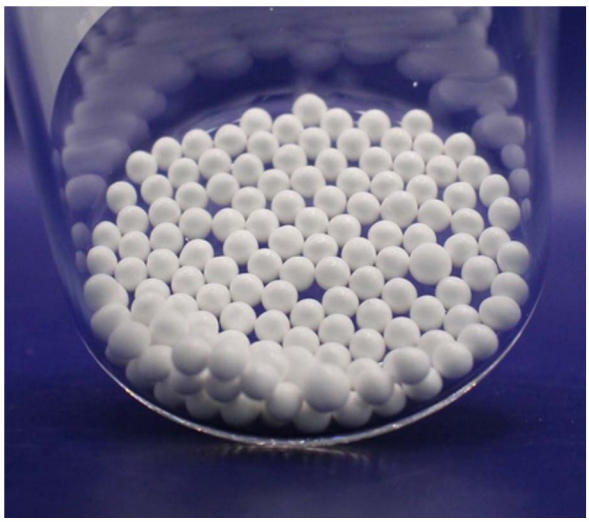

Figure 1: Having the correct formulation is crucial for optimal assay performance in lyobeads. These lyobeads will be placed into the final container and then placed into a reader.

Figure 2: Frozen beads produced using Biopharma Group's LyobeadPRO™

- Once a lead candidate is selected, Biopharma Group suggests implementing an optimised lyophilization cycle.
- A Design of Experiments (DoE) approach identifies key excipients, assesses their interactions and effects, and predicts outcomes when formulation components are adjusted.

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WHY LYOPHILIZATION?

The customer had developed an immunoassay targeting a specific protein, with the goal of producing approximately 500,000 to 1,000,000 units per year. However, the liquid format of the assay was unstable at 30°C for more than two days. The solid format presented additional challenges, including hygroscopicity and poor stability under ambient storage conditions. Biopharma Group was commissioned to improve the dry-state stability in the lyobead format and provide further support with product development and manufacturing.

IS THE FORMULATION SUITABLE FOR LYOPHILIZING?

A few excipients were suggested for the customer to test. Using Biopharma Group's data and experience, we predicted which formulation will show the lowest collapse temperature. After this, the formulation was analysed using Biopharma Group's Lyostat5 freeze-drying microscope (FDM). This device allowed us to design a cycle that mitigated the risk of collapse in formulations.

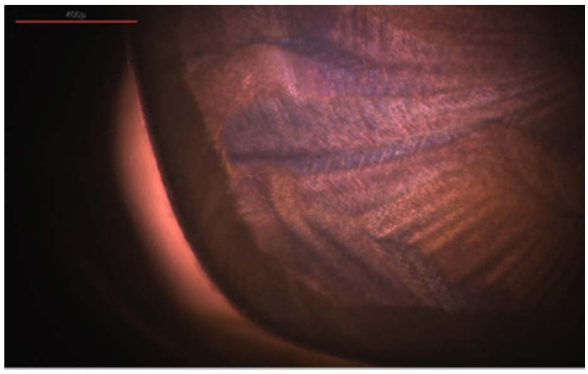

Figure 3: First signs of collapse onset observed as the temperature of the sample reached -37,2°C

WHAT FACTORS NEED TO BE CONSIDERED WHEN SELECTING A FORMULATION?

Selecting the correct formulation in the initial stages can help prevent major issues, such as breakage during shipment. To design the formulation, the customer requirements are key. In this case study, the target criteria were fast reconstitution, robust structure and stability at 30 °C for 2-6 months. After assessing the products from the first trial cycle, the following observations were noted:

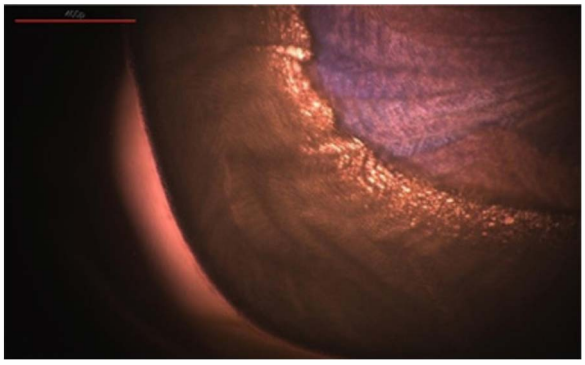

Figure 4: The loss of drying structure became more apparent as the temperature reached -20.2 °C

A

B

Figure 5: Formulation A showed no signs of incomplete drying. After refinement, no signs of collapse or incomplete drying were observed in Formulation B.

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IS THE FORMULATION STABLE ENOUGH?

After assessing the appearance of the formulations, 3 candidate formulations were selected, and the glass transition temperature and moisture content of the candidate formulation were assessed using modulated differential scanning calorimetry (MDSC) and Karl Fischer (KF) analysis, respectively.

Formulation	Onset of glass transition (Tg) event	Average residual moisture content % (w/w)
2 (CRP Mix 2 + 5% excipient A)	65.36°C	1.78
3 (CRP Mix 2 + 5% excipient A + 2% excipient B)	70.32°C	1.19
4 (CRP Mix 2 + 5% excipient A + 2% excipient C)	81.72°C	1.06

Table 1: mDSC and KF Analysis results. Formulation 4 shows the highest Tg onset at 82°C and the lowest moisture content of 1.06 % (w/w), reflecting the positive impact of increasing the solid content and adding excipient C.

CAN WE PICK AND PLACE THE LYOBEADS EASILY?

MicroPress analysis was performed to assess the mechanical properties of the candidate formulation, revealing that slight differences in excipient type impacted the mechanical properties of the dried material. Table 1 on the next page, shows the average Young's modulus and stress at the fracture point.

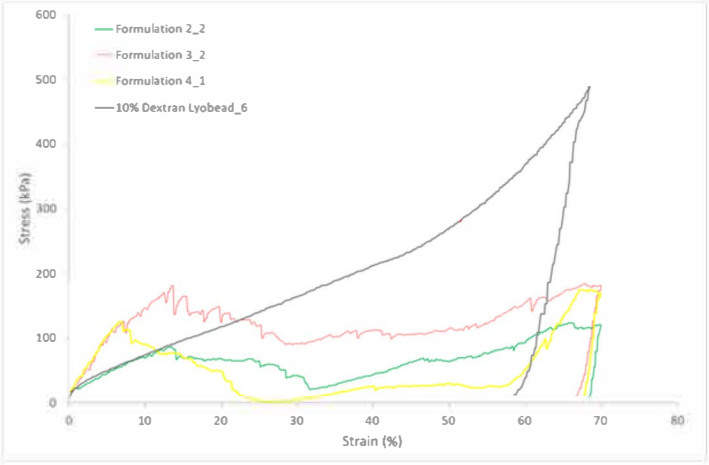

Figure 6: Micropress data

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Formulation	Mean Young's Modulus (kPa)	Stress at Fracture (kPa)	Mean Max Stress at Fracture (kPa)
2	13.8	70.00	78.60
		87.30	
		78.60	
3	15.8	126.0	104.5
		82.00	
		105.6	
4	15.2	125.6	136.8
		151.7	
		133.1	

Table 1: MicroPress analysis results

Formulation 2 was brittle, with microfractures occurring at ~78 kPa stress. Formulations 3 and 4 showed better robustness, with maximum stress at fracture recorded at 100 kPa and 137 kPa, respectively.

To confirm findings from MicroPress analysis, friability testing also showed slight mass losses in formulations 2 and 3 but no mass loss in formulation 4.

Based on these data findings, formulation 4 was selected due to its highest Tg onset, lowest RMC, and greater mechanical strength.

DOES THE FORMULATION REQUIRE FURTHER CHANGES?

During the initial screening cycle, formulations were assessed for their compatibility. The main objective after this was to increase the robustness of the product and develop a more stable product while not impacting the compatibility of the formulation.

In the second cycle, the same sugar (excipient C) was selected to preserve the enzyme activity of the product, and a range of new polymers were investigated in combination with excipient C. Studies proved the positive impact of polymers in increasing the mechanical robustness of the product. The potential negative aspects of using polymers can be increasing the reconstitution time and inducing static behaviour.

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HOW MUCH DOES CHANGING AN EXCIPIENT IMPACT THE PRODUCT'S OVERALL STABILITY?

Varying the polymer used in formulation 4, while keeping the solid content constant, showed improved robustness with reduced microfractures and a max stress at fracture of ~123.67 kPa. However, all formulations exhibited electrostatic behaviour.

Formulation 4 also had a suitable reconstitution profile and the onset Tg value suggested that storage at 2-8°C should be acceptable.

WHAT ARE THE NEXT STEPS AFTER CONFIRMING THE FORMULATION?

This series of screening cycles has identified promising formulations that could significantly enhance the stability and performance of CRP mix 2 in lyobead format. After this step, a suitable cycle was developed for the candidate formulation. The total cycle time was reduced to 4 days. This cycle was developed to handle scale-up to over 30,000 assays per cycle. This significant efficiency increase will result in cost savings for future production runs.

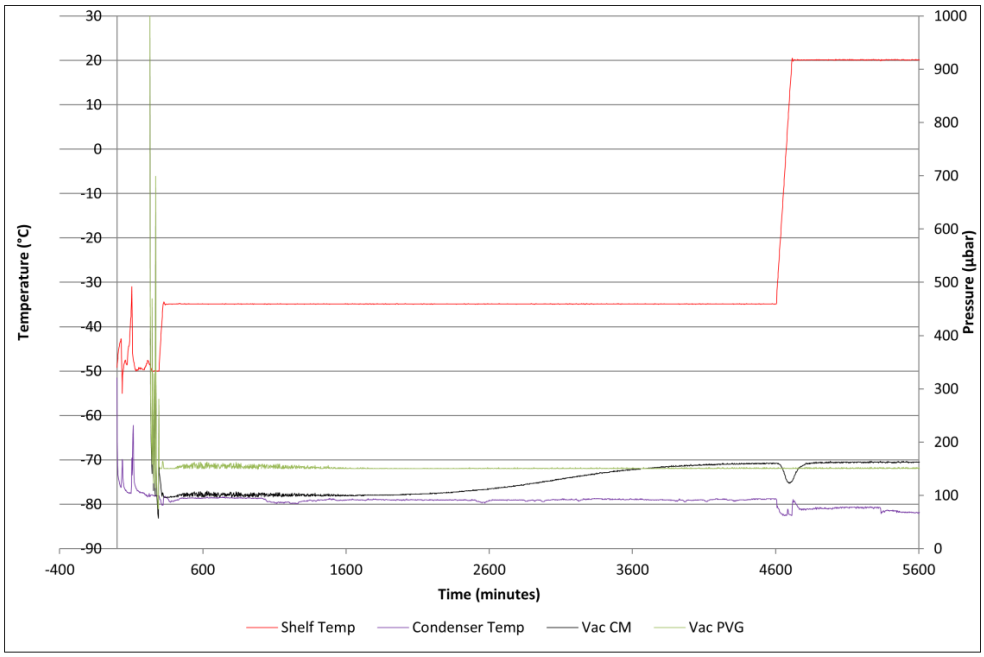

Figure 8: Cycle plot of the final developed formulation. The pressure during this cycle was monitored by the Pirani gauge (PVG), represented by the green line in the plot. The dryer used for this cycle was also equipped with a capacitance manometer (CM) gauge. As shown in the graph, the endpoints of both the primary and secondary drying phases are indicated by the convergence of the CM and PVG gauge readings.

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The importance of formulation is evident in various stages of lyophilized product development. It is arguably the most fundamental aspect that determines the success of the final product. To ensure this, it is essential to first perform compatibility testing and identify which excipient works best with the assay.

Following this, freeze-drying microscopy (FDM) analysis is critical to understanding the formulation's collapse temperature. Assessing the thermal behaviour of the solid-state product and its physical robustness is another key step, where adjustments to the excipient or its proportion in the formulation may be necessary. However, it is crucial to select the optimal concentration, as increasing the solid content will also increase Rp (product resistance to vapour flow) and may unnecessarily extend the cycle length. Although methods such as Design of Experiments (DoE) offer structured approaches for developing a successful formulation, a strong foundational knowledge of excipients and their impact is invaluable. This can greatly reduce time spent on trial and error, leading to more efficient development.

In summary, robust lyobead formulation relies on several reasons such as high molecular weight polymers. Balancing excipient concentration is crucial to avoiding extended lyophilising cycles, while methods like DoE and foundational excipient knowledge help to streamline development, leading to more efficient and effective products.