Lyobead Technology in the Diagnostics and Pharma Sectors: Why, How, What?

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May 26th, 2021

Biological reagents are particularly sensitive to changes in temperature and require specific storage conditions to remain bioactive in the long term. Dehydrating the reagents reduces this sensitivity and is the main driver for the growing lyophilized bead technology in both the diagnostic and pharmaceutical industries.

Recently, Dr Mattia Cassanelli, Technical Business Manager at Biopharma Group, UK presented a webinar on the use of Lyobead technology in the diagnostic and pharmaceutical sectors and described how these beads have been used for different applications. This tech note summarizes the webinar and includes a selection of questions from the Q&A sessions.

What are Lyobeads?

Lyophilized beads (Lyobeads) are spheres of customizable lyophilized material that contain a specific volume of material per unit.

One of the most common uses of these beads in the pharmaceutical and diagnostic industry is for encapsulating reagents in PCR assays to enable longer-term storage at room temperature. They are also used in microfluidics, drug delivery, and encapsulation of bacteria, especially to meet the increases in demands dictated by the market.

Why use Lyobeads?

The main benefit of using Lyobeads is the ability to develop one formulation that can be easily modified for several applications at different temperatures or in different containers thereby reducing R&D investment. Production can also be increased by high throughput loading into the lyophilizer and bulk storage before packaging, both due to the stable nature of these Lyobeads.

After lyophilization, the time for reconstitution can also be shortened due to the spherical nature of the Lyobeads maximizing the surface area.

How are Lyobeads made?

Lyobeads can be obtained through snap freezing of the liquid formulation of reagents in liquid nitrogen and then dehydrating in a freeze dryer, or through polymeric gelation in which a gelling polymer, such as anionic alginate is placed into a solution containing calcium ions. When alginate encounters the divalent cations, it creates a stable polymer that forms a membrane protecting the reagents in the solution.

Use of Lyobeads in R&D

When selecting the liquid formulation of excipients for lyophilization into Lyobeads, there are three main testing stages: compatibility, shortlisting and screening.

As part of the compatibility step, a selection of up to thirty excipients is proposed based on experience and initial thermal analysis results. These excipients are then tested to check the compatibility in the liquid state and ensure no test interference occurred.

From these initial tests, a shortlist of promising candidates in the liquid state are chosen to be lyophilized as part of the screening process. All formulation candidates are processed using the same single freeze-drying cycle. This allows drying all samples under the same process conditions for a true comparison. The most promising dried candidates are then characterized, and the combined results allow the identification of the lead formulations.

In some cases, a statistical analysis approach (Design of Experiment (DoE)) expands the formulation candidate list to identify the best formulation based on specific parameters, such as glass transition temperature (T_g) of the active pharmaceutical ingredient (API), mechanical properties of the lyophilized beads, and product activity post-lyophilization. This also enables a design space to be defined which identifies optimal lyophilization conditions for each batch and supports regulatory compliance.

Once the Lyobead formulation has been developed then it can be scaled up for manufacturing.

Case studies

1. Diagnostics - PCR assays

This study aimed to increase the shelf life of a liquid formulation of a COVID19 detection PCR assay already developed. The reagents needed to be stored at room temperature in high throughput (96 well format) and without their reaction quality being affected. There was also a need to consider ramping up manufacturing to millions of reactions a month in a short timeline.

The thermal behavior of the liquid formulation was initially analyzed using a freeze-drying microscope with a gradual increase in temperatures to visually identify the point of collapse. Further analysis using a Lyotherm (Biopharma Group), which combines electrical impedance and Differential Thermal analysis (DTA), determined the glass transition temperature of the formulation.

Based on these results, it was decided to reformulate the product into a new Lyobead mastermix capable of containing all formulation components (enzymes, buffer, primers, and probes).

As part of the process of compatibility, shortlisting and screening, Biopharma conducted several tests to identify the most promising candidates.

Visual assessment assessed the morphology and elegance of the bead and was followed by modified differential scanning calorimetry (DSC) which further characterized the thermal properties of the product.

To understand how the product behaves during storage, water

Figure 1: *R&D - Visual assessment of PCR assay lyobeads*

absorption and mechanical stability were assessed. Dynamic vapor sorption (DVS) measures how water is absorbed by a sample at a given temperature and will indicate how long a product can be stored without absorbing an irreversible amount of water vapor.

Lyophilized products can undergo mechanical stresses during storage and transport. Applying controlled pressure on the freeze-dried cake using a MicroPress (Biopharma Group) actuator can measure the percentage of strain that the Lyobeads can withstand without regaining shape. Graphically this is illustrated by breaks in the curve which suggest a friable and brittle product.

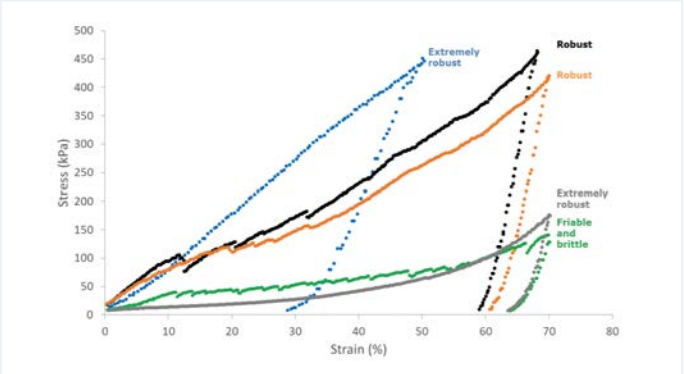

Figure 2: *R&D - Mechanical properties of lyobeads*

Reconstitution of the most robust candidates is then assessed. Freeze-dried products can be reconstituted much more quickly and easily because the process leaves microscopic pores. The size, distribution and interconnection of these pores can be examined by electron microscopy to optimize candidates that will reconstitute in a short time.

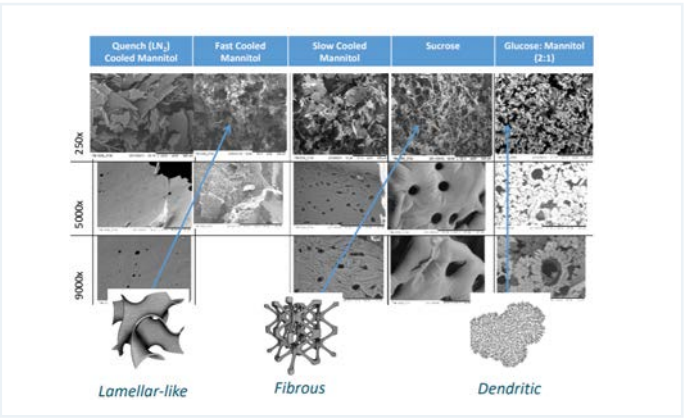

Figure 3: *R&D Analyses – Microstructure*

Overall, several candidates for the COVID19 PCR assay were tested and compared according to the studies described above. After identifying lead candidates, and optimizing the cycle conditions, validation with several batches confirmed the optimal Lyobead products that could be manufactured for commercial use.

2. Biologics - Bacterial Vaccines

In this study, the goal was to develop a suitable formulation containing an inactivated gram-negative bacterium for an oral vaccine that was stable long-term and could be reconstituted rapidly.

Investigation of different formats and methods demonstrated Lyobeads to be an ideal option for this product. This study also provided support with the technology transfer and set up for a new production line.

Initial studies revealed that snap freezing the Lyobeads did not damage the bacteria and was chosen as the method of choice to synthesize the Lyobeads. The compatibility testing and post-process analysis after the first screening cycle identified suitable cryo- or lyo- protectants to be encapsulated within the Lyobeads.

Finally, Biopharma assisted with transferring this technology to the client's location and helped to create an efficiently run process at this site including recommendations on the selection of a suitable freeze dryer.

3. Pharmaceuticals - Oral tablets

Oral tablets require milling and tableting before being placed in blister packs. In this study, the goal was to trial the use of Lyobeads to prepare a pharmaceutical product for oral administration. The design of the Lyobeads negated the need for milling and tableting which is beneficial both technically and commercially. The Lyobeads were then placed into the blister packs.

Analyzing and optimizing the Lyobeads required a DoE approach to maximize the parameters. The process analyzed the activity of the API, speed of dissolution, thermal stability in a frozen state, and the mechanical properties of the dried sphere using several techniques described earlier. DoE was based on the characterization of 19 candidates formulations that were processed in both screening and refinement cycles. The mechanical properties of combining several excipients can be demonstrated on a heat plot which can identify the limitations of product stability.

Calculating residual moisture content together with modulated Differential Scanning Calorimetry (MDSC) analysis identified the most appropriate time out of the freeze dryer.

These studies led to the development of the efficient production of Lyobeads which, when compared to milled and tableted products, absorbed less moisture and were quicker and more economical to produce.

4. Nutraceuticals - Probiotics

This case study regards orally administering probiotics. The final formulation requires millions of probiotic bacteria to remain viable after processing. Long shelf life and short reconstitution time were also essential goals.

Lyobeads was the chosen optimal format, but these 2 mm beads were not snap-frozen but were created as polymers from alginate in a buffer containing calcium ions.

Suitable cryo- or lyo- protectants and optimal freezing rates were identified in compatibility studies with several freeze-drying cycles. Any physical clumping or loss of shape of the Lyobeads was visualized on a freeze-drying microscope.

Changing the pressure and freeze-drying temperatures in the freeze-drying cycles also enabled the appropriate design space for this product to be created.

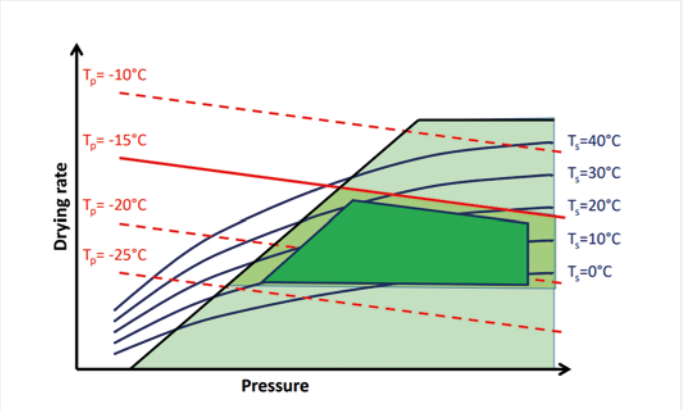

Figure 4: *Four additional cycles to work out a suitable design space*

Conclusions

In the pharmaceutical and diagnostic field, Lyobeads are a simple solution to creating stable products that can be stored long term. Once manufactured, the beads can be placed into any container for different product changes and can be stored in bulk before packaging. However, there are different methods to create these Lyobeads and conditions that require optimizing through compatibility testing, shortlisting, and screening which requires an initial investment of time.

Lyobeads can be used in many different areas, several of which are described in the case studies above. One of the most significant applications of these beads is in PCR, where a stable lyophilized mastermix stored at room temperature can speed up and increase throughput in PCR screening assays.

With multiple benefits including cost savings and reduced environmental impact, companies are moving toward lyophilized beads in their product lines.

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

Q&A Session

1. **How do you dispense beads?**

   Beads are dispensed by using a pump-syringe system or an encapsulator. It is important to calibrate the pump correctly, select the correct syringe type and have the right distance from the nozzle to the surface of liquid N2.

2. **Regarding product temperature or sublimation temperature front in lyophilization, what is your approach in cycle development/optimization?**

   Considering the low volume involved, probing the beads with thermocouples could be challenging. Therefore, we also rely on differential pressure by using a capacitance manometer and a Pirani gauge.

3. **How do you determine maximum stress that product will experience?**

   We use our MicroPress instrument, a proprietary instrument that we designed and currently provide worldwide. This consists of an indenter that compresses the bead and generates a stress/strain curve.

4. **What are the different attributes of the formulation or cake and/or difference in the standard attributes of a formulation you're looking for when developing a lyobead product, as opposed to a standard vial filling and cake process?**

   Talking about beads, mechanical properties and friability become key parameters. The excipients are selected in order to enhance these properties too.

5. **How does dispensing to a cold plate to freeze a reagent liquid compare to dispensing into liquid nitrogen?**

   The cooling rate will be much faster, creating very small ice crystals. Although this may increase the product resistance to sublimation, considering that the surface area of beads compared to volume is much higher than standard vials, the overall sublimation rate will be still very fast.

6. **When lyophilizing lyocakes in 96 well plates, do the plates degrade when subject to the temperatures and pressures inside the freeze dryer?**

   Considering the standard operation parameters during freeze-drying, there is no significant degradation of the container material.

7. **How are electrostatics controlled for the Lyobeads?**

   By using appropriate excipients and containers you could minimize the electrostatic issues.

8. **Have you found that clients are interested in process robustness, with regard to method transfer to several sites, with quite often very different ambient environments?**

   We have had several projects that require support in defining a design space for both formulation and process. Considering that companies may have several sites all around the world, having a tolerance range for each parameter would be very useful.

9. **What manufacturing control measures are effective in preventing bead damage / breakage?**

   A suitable dispensing method for the dried beads will help minimize cracking and damaging. We use vacuum pen solutions, powder fillers, magnetic pens and bulk dispensers. Based on the product characteristics, one method is more suitable than others.

10. **What happens if two beads freeze together during dispensing into liquid nitrogen?**

    This could be minimized by the correct set up of the bead generator. However, if this happens, they can be manually removed.