Lyophilization of Diagnostics: The Challenges of Formulation, Cycle Development and Container-Closure Selection

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In recent years, the freeze-drying of diagnostic reagents and kits has increased which benefits many diagnostic products, particularly those that are sensitive to temperature, light and oxygen. After freeze-drying, these products become more stable and their shelf life is dramatically increased. These freeze-dried materials also boast rapid reconstitution rates accelerating the preparation of diagnostic products for use in the field.

Recently, Dr Kevin Ward, Biopharma Group presented a webinar that discusses the challenges faced with freeze-drying diagnostics. In it, he discusses formulation issues, aspects of cycle development and closure selection (alternatives to the widely used glass for pharmaceuticals). This tech note summarizes the data presented in the webinar and includes a selection of questions from the Q&A session.

Challenges associated with freeze-drying diagnostic products

By the very nature of diagnostics, many kits and reagents need to be reactive quickly, for example drug detection kits at airports, PCR rapid tests or biomolecules and therefore are not stable for long periods of time. Although the complexity of the molecules in diagnostic kits can be similar to pharmaceuticals, their increased reactivity together with the differences in container formats (from plastic 96-well plates to chips or microfluidic devices) make the lyophilization process more complex. Even decisions on whether to freeze-dry independently or within the source container are of great importance.

Heat labile molecules

Small molecules in chemical diagnostics often require rapid cooling as they can be extremely reactive and heat labile. The removal of heat from the sample during the initial stages of freeze-drying is one of the main advantages compared to other forms of drying based on spraying, evaporation or vacuum.

Low volumes

Most diagnostics are dried in low volume aliquots. Although this can be advantageous, there are several challenges associated with freeze-drying low volumes. Loading several hundred samples into a large freeze dryer can result in the first sample evaporating before the last one has been loaded, severely compromising sample consistency. One option to overcome this is to set up the samples on pre-cooled shelves or outside the freeze-dryer.

Biomolecular sensitivity

Biomolecules possess a molecular complexity that is sensitive to biochemical processes. Freeze-drying can minimize or 'quench' this sensitivity, but the process can cause subtle changes in the microenvironment that could cause inactivity. Although rapid cooling can alleviate the freeze-concentration effects (the fluid between ice crystals becoming more concentrated), this can cause interfacial effects by destabilizing the protein molecules at the ice-water interface. Avoidance of these and other issues by changes in formulations can also be worth investigating.

Presence of glycerol

PCR products are commonly part of diagnostic kits, but the glycerol contained in the products can cause major problems during freeze drying. Glycerol does not freeze under typical

lyophilization conditions, persisting as an excluded liquid layer. Controlled nucleation can control the freezing of a sample containing glycerol from the top down which can reduce this issue, alternatively removing/reducing the glycerol from the sample by dilution or dialysis can be successful.

Sealing issues

Container/closure methods for a diagnostic kit or reagent can be even more important in diagnostics than small molecule pharmaceuticals as a small amount of moisture uptake can represent a high percentage of the product mass. A large proportion of diagnostics are housed in plasticware that will likely contain moisture itself (Figure 1). Sealing in a controlled environment that maintains humidity may be key for non-vial-based products (Figure 2), in addition to double wrapping with desiccant to protect them from long term uptake. It is also possible to freeze-dry in specially treated containers where it is possible to 'pick and place' even very small cakes in a controlled environment (Figure 3).

Summary

There are many challenges of the increasing pipeline of diagnostic products, some of which are not encountered for other freeze-dried materials. Fortunately, technology in this area is well-funded and evolving to solve current practical issues.

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

Figure 1. Lyophilization of sample in a 96-well plastic plate

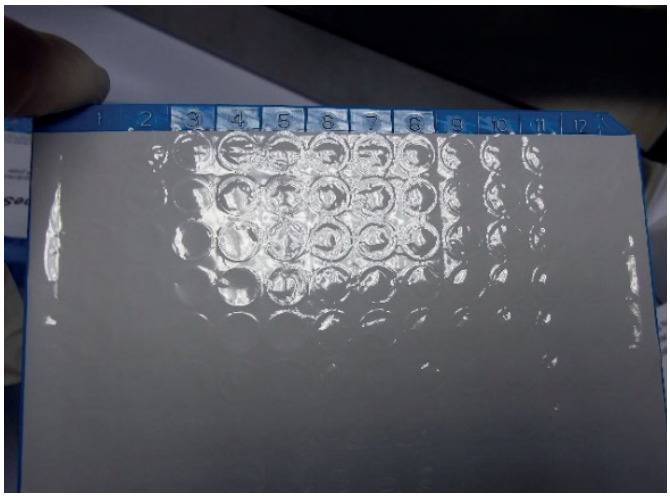

Figure 2. 96-well plate plastic sealant

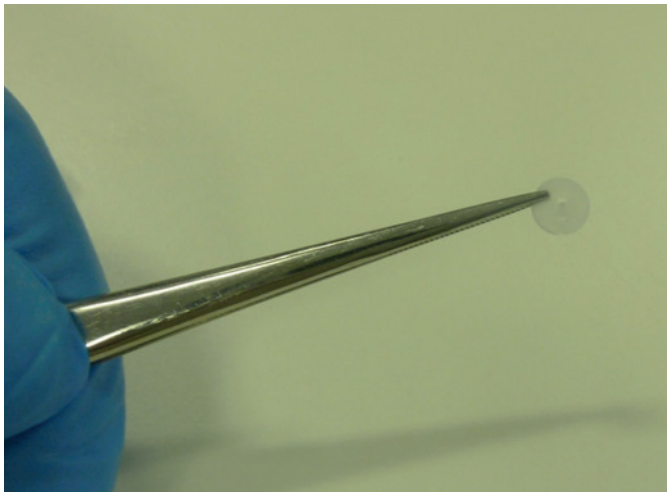

Figure 3. 'Pick and place' very small lyophilized cakes in a controlled environment

Q&A Session

1. **Regarding 'Pick & Place' containers, what materials are used?**
   Usually plastic sheets are coated with a hydrophobic surface to ensure that the product does not stick.
2. **When processing in dryers with an acrylic door, can the radiant heat energy coming in through the door, together with the poor conductive heat transfer of the plastic container, lead to a condition where very low shelf temperatures are needed to keep the product below its collapse temperature?**

   This can be an issue, particularly in small dryers. One idea can be to place aluminium foil over the door in order to reduce the impact of radiative heat transfer. Another can be to avoid placing the product right at the front of the shelf. Alternatively, the most robust solution can be to adjust the formulation.
3. **Can we add something to glycerol to increase the vapor pressure?**

   This should be possible – many organic solvents might work here, although care should be taken that this doesn't lead to additional complicating factors such as phase separation, incomplete freezing, flammability and incompatibility with gaskets and seals.
4. **When you refer to "Rapid Cooling", how do you recommend doing this? LN2 or high ramp rate on lyophilizer?**
   Sometimes quench cooling can be used, depending on the container type, but this can be difficult to scale up reproducibly, so it is often better to use a controlled rate or load onto pre-cooled shelves.
5. **With your experience is it possible to say that any of the following are more sensitive to freeze-drying (in general terms) - primers, probes, DNA, armoured RNA, or enzymes?**

   We often find that the enzymes are the most sensitive component.
6. **Is it possible to anneal out a bulk (over the Tg´) without affecting the ice crystals or will the ice always go along with crystallizing out something else?**

   If you warm the frozen material above Tg', the solute will become more flexible and the ice crystals will have more freedom to grow, so it is difficult to prevent this.
7. **How can you determine the permeability of moisture in a plastic? Any tips on choice of method?**

   We have used DVS (Dynamic Vapor Sorption) to investigate the relationship between various materials and moisture. Our past experience with PCR-based products has also shown that even 96-well plates made from the same polymer but sourced from different manufacturers can have different permeabilities due to the density and thickness of plastic, so we would recommend carrying out some characterization before finalising the container.