Role of Organic Co-Solvent (t-Butanol) in Frozen and Freeze-Dried Formulations

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April 22nd, 2021

In the last year, more than 40% of drugs approved by the FDA have been lyophilized[1]. Lyophilization stabilizes the drug product and enables a longer shelf life at room temperature with easier transportation.

However, the processing steps (freezing, primary drying and secondary drying) involved in the lyophilization process can expose the proteins to various stress and harsh conditions, leading to denaturation, aggregation and sometimes a loss of functionality. To overcome these stresses experienced by protein throughout freezing, drying and storage use of cryo- or lyo-protectants in certain ratios is a central theme in formulation development.

Recently, Jayesh Sonje, a Ph.D. candidate from Dr. Raj Suryanarayanan's lab at the University of Minnesota, USA presented a webinar that examined the thermal characterization of an organic solvent, tert-Butyl alcohol (TBA) used as an excipient during the freeze-drying process. This tech note summarizes the webinar and includes a selection of questions from the Q&A sessions.

Role of Excipients in Freeze-Drying

Excipients used in freeze-drying include stabilizers, bulking agents, surfactants, buffers, and organic solvents. Alone or in combination they enable retention of the protein in its native state which is responsible for its biological activity. However, given the complex interplay of excipients and protein composition as well as the processing conditions, optimizing the formulation composition and process parameters is challenging. It is important to note that, the physical form of the excipient during the process of freezing, drying as well as storage determines the functionality it provides to the formulation. For example, stabilizers such as sugars prefer to be in an amorphous state whereas bulking agents such as mannitol would rather be in a crystalline state.

tert-Butyl Alcohol (TBA)

Organic solvents such as TBA can provide many advantages to freeze-dried formulations, including increased solubility of hydrophobic drugs, decreased drying time, improved product stability and reconstitution characteristics[2].

TBA has a high freezing point of 24°C which means it is mostly crystalline at room temperature and sublimes during freezing. It can completely crystallize with long needle-shaped ice crystals during the freezing stage and produce a lower cake resistance with a higher specific surface area during the drying stage. These properties help reduce drying times from 100 hours (with 5% w/v sucrose) to 10 hours (with 5% w/v sucrose plus 5% w/v TBA) which will significantly shorten the manufacturing of a drug product[3].

In addition, TBA improves the stability of the drug as was demonstrated for the approved Pfizer drug, Caverject® where the addition of 20% v/v TBA solution increased stabilization of the active ingredient, alprostadil[2].

Thermal Behavior of TBA-Water

When designing and optimizing optimal freezing and drying conditions, the eutectic temperature (the temperature at which water and solute crystallize simultaneously, T_{eu}) can be determined by Differential Scanning Calorimetry (DSC) and X-Ray Diffractometry (XRD). To develop and optimize a freeze-drying cycle, the binary phase diagram for excipients used provides wealth of information. The determination of eutectic temperature aids in identifying the primary drying temperature. Ideally, setting the primary drying temperature lower than the eutectic temperature can prevent melt back.

TBA, as an organic co-solvent, has been explored for its use in freeze-drying for a long time. However, there is still some ambiguity in the phase behavior of TBA with respect to the T_{eu}, composition and solid phases involved.

One of the aims of Dr. Raj Suryanarayanan's lab was to resolve some of this ambiguity and to generate a refined phase diagram of TBA in water.

Eutectic Composition in TBA-Water System

Several concentrations of TBA from 0-25% w/w were assessed under different conditions and analyzed using DSC and XRD techniques[4].

Initial studies revealed complex thermal events during the heating of frozen TBA-water systems with several melting endo-

and exotherms produced on the heating curves at different concentrations making it difficult to unambiguously attribute to phases. A more defined heating curve was produced with an additional annealing step, but DSC experiments alone did not provide a complete understanding of TBA phase behavior.

XRD experiments were performed using the synchrotron at the Argonne National Labs, Illinois, USA that permits a fast scan time of 1 second in transmission mode and enabled data to be close to real-time.

Combining DSC and XRD resulted in identifying different phases dependent on TBA composition. Less than 20% w/w TBA produced three endotherms that corresponded to TBA-dihydrate melting, TBA heptahydrate and ice melting (eutectic composition), and ice melting. However, a TBA concentration above 20% w/w created an exotherm event as TBA-heptahydrate crystallized and an endotherm event as TBA heptahydrate and ice melted (eutectic composition).

The eutectic composition was then confirmed using DSC and this was compared to binary phase diagrams created by other research groups.

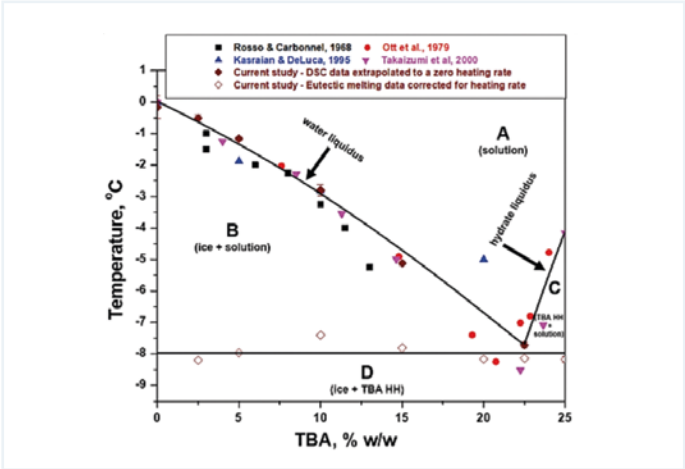

Figure 1: *Refined Phase Diagram. Eutectic Composition 22.5% w/w TBA, Eutectic Temperature - 8 °C, Phases TBA heptahydrate + Ice.*

The eutectic composition was defined as 22.5% w/w TBA at a eutectic temperature of -8°C with phases of TBA heptahydrate and ice[3].

Dual Functionality of Mannitol in TBA Solutions

After determining the phase composition of TBA, mannitol was added to the mixture to characterize its phase behavior in frozen TBA-water systems (5-30% w/w TBA) and evaluate its cryoprotective ability[5].

Mannitol, a popular bulking agent, is widely used in freeze-dried formulations. Aqueous mannitol is well characterized with two glass transition temperatures and partially crystallizes during freezing as a hemihydrate.

DSC heating curves of 5% w/w mannitol in 5-30% w/w TBA demonstrated the glass transition temperatures of mannitol were unaffected by TBA. Interestingly, TBA at certain concentration delayed mannitol crystallization during the freezing stage. As the concentration of TBA increased (at 30% TBA), overlapping endotherms attributable to crystallization of both mannitol and TBA were observed. XRD experiments helped resolve these complex thermal events.

When combined with the XRD data using 22.5% w/w TBA and 2% mannitol, it was evident that mannitol remained amorphous during freezing and heating until -20°C. Mannitol then crystallized into the anhydrous delta form at -20°C in the presence of TBA during heating.

The ability of the amorphous mannitol to act as a cryoprotectant was investigated further using human serum albumin (HSA) as a model protein in the TBA-water system. The formulations were freeze-thawed or freeze-dried and assessed by dynamic light scattering (DLS).

Individually, TBA and mannitol were ineffective as stabilizers. However, when combined with TBA ≤ 22.5% w/w, mannitol remained in an amorphous state and HSA aggregation was minimal. This was comparable to using a commonly used lyoprotectant, such as sucrose.

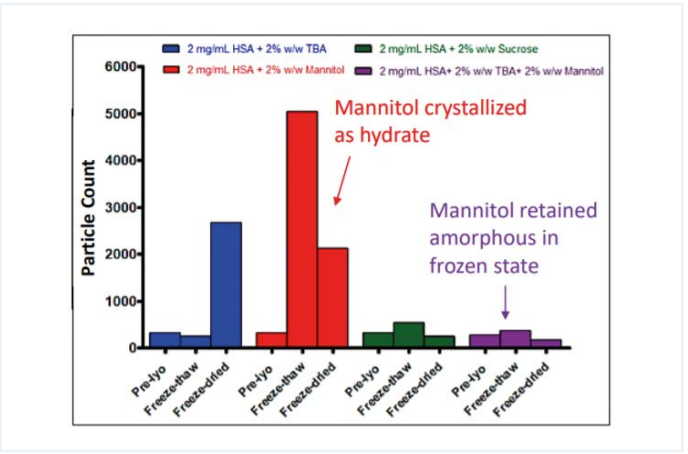

Figure 2: *Results showing particulate count (subvisible particles) measured using dynamic light scattering in prelyophilization, freeze-thawed and freeze-dried samples.*

Without TBA, mannitol crystallized as a hydrate and HSA aggregation occurred[4].

Conclusions

Characterization of TBA as an excipient and in combination with mannitol was described in Jayesh Sonje's webinar. The thermal events observed during warming, and their characterization by DSC and XRD, enabled the generation of well-defined phase boundaries as well as the eutectic temperature and composition of TBA.

It was also evident that mannitol in the presence of ≤ 22.5% w/w TBA could function as both a cryoprotectant during freezing and as a bulking agent in the final lyophile during drying.

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

References

1 BCC research, 2018 - 2026
2 Teagarden, DL *et al*, Eur. J. Pharm. Sci. (2002) 15:115
3 Kasraian K *et al*. Pharm Res. 1995; 12 (4): 491-5
4 Bhatnagar, BS *et al*, Phys. Chem. Chem. Phys. (2020) 22(3):1583-90
5 Sonje, J et al, Mol. Pharm. (2020) 17(8):3075–3086

Q&A Session

1. **How can the residual TBA amounts be quantified post-lyophilization, and what residual amounts are acceptable in an injectable formulation?**

   Gas chromatography (GC) is one of the most common analytical tools used to quantify residual solvent content in the final cake. In the case of TBA, the initial concentration of TBA and presence of other excipients can result in variable residual solvent content. In addition, inclusion of a processing step such as annealing can cause crystallization of any unfrozen solvent and minimize the residual solvent content in the final cake. However, < 0.5 to 1% residual solvent content may be a good target to achieve. As per ICH Q3C R8, the permissible daily exposure for TBA is < 35 mg/day and is recommended to be included as a class 2 organic solvent.

2. **What is the drying behavior of TBA as compared to ethanol during the drying stage? Ethanol sublimes/volatilizes throughout drying, how about TBA?**

   Ethanol has a freezing point of -114°C. During the drying stage, ethanol can be removed to some extent given its high vapor pressure. However, literature reports suggest presence of higher residual solvent content in the final cake. Ethanol does not crystallize during the freezing stage which can result in its retention as unfrozen solute, leading to collapsed cake, making it difficult to dry. On the other hand, TBA has a freezing point of 24 °C. It crystallizes almost completely during freezing (eutectic temperature -8°C). This results in maximum removal of TBA by sublimation during the primary drying stage hence minimum residual solvent content. However, it is critical to optimize the TBA concentration in the initial (prelyophilization) solution.

3. **Are there any other solvents besides TBA that can used for freeze-dried products?**

   A number of organic solvents such as ethanol, IPA, DMSO, acetone etc. have been evaluated for their use in freeze-dried products. Ethanol in some cases has been shown to improve the crystallization of certain drug substances (small molecules) and a minimum concentration of < 5 to 10% can be used. This should be used with caution and process optimization, product characterization (cake elegance) as well as residual solvent content determination should be considered.

4. **Does TBA require the use of explosion-proof lyophilizer?**

   Organic solvents are flammable and proper safety protocols should be followed. Use of explosion proof equipment including electric motors on vacuum pumps as well as special solvent traps may should be considered.

5. **Can you comment on the effect of TBA on drying rate, and specific surface area of the dried cake?**

   TBA in the concentration range of approximately 3 to 20% w/w on freezing has been shown to modify the crystal habit of ice. In the presence of TBA, ice crystallizes in the form of needles as opposed to small sphere-shaped crystals in the absence of TBA. As these needle-shaped ice crystals sublime, they leave behind a much more porous mass (low dry product layer resistance) with higher specific surface area. The drying rates conform to the phase diagram, higher sublimation rates around eutectic TBA composition (Kasraian et al, Phar Res, 1995).

6. **What should be the ideal composition of a lyo drug product with TBA, and how much TBA should be included in the formulation?**

   If the initial concentration of TBA is close to the eutectic composition, the residual concentration in the final lyophile is very low. A eutectic composition confers some unique advantages such as simultaneous and complete crystallization of both TBA and water on freezing which will yield an intimate physical mixture of the two solids, minimizing the risk of intra-vial compositional heterogeneity. Presence of other excipients can influence the freezing behavior of TBA as well. Addition of crystallizing excipients such as mannitol can yield better results.

7. **Can you comment on supercooling? Supercooling is typical for water solutions with variable nucleation vial to vial. Is it similar for TBA/water solutions?**

   Supercooling is observed due to the thermal lag in the product temperature and the shelf temperature of the freeze-dryer. This is also observed in the case of TBA-water solution as well. However, an advantage of using TBA around eutectic composition is its complete and simultaneous crystallization a little below the eutectic temperature. This can minimize the vial to vial heterogeneity to some extent. However, real time monitoring of the product temperature with and without TBA will give a better idea of the supercooling effect.

8. **Do you think TBA + mannitol formulations could have potential applications in cryoperservation and lyopreservation of cells? Or do you mainly see it as being applicable to proteins and small molecules?**

   Literature studies report use of TBA in cases of very few proteins such as mAb and insulin. Retention of mannitol in the amorphous state during freezing provided the necessary cryoprotection during the freezing stage in case of HSA. However, use of TBA has been widely explored in case of small molecules. Use of organic solvents in cryopreservation of cells is not uncommon. Organic solvents such as DMSO are generally used and it would be interesting to see if TBA + mannitol combination can be useful in cell preservation.