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Capillary mediated vitrification (CMV) is a user-friendly alternative to lyophilization for the production of thermostable bio-molecules

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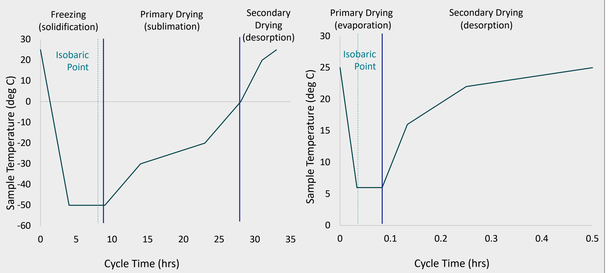
PURPOSE

Lyophilization is the most widely used method for desiccation of biological molecules across the biopharma industry. However, several issues exist with the complexity of the process that impact the thermostability of the products produced.

Capillary Mediated Vitrification (CMV) is a novel method for biomolecule preservation that leverages the process of capillary evaporation to remove solvents from samples without freezing or boiling, producing amorphous thermostable products in less than 1 hour.

In this work, we compared the thermostability of CMV stabilized thermolabile luciferase against catalog equivalent lyophilized products at elevated temperature.



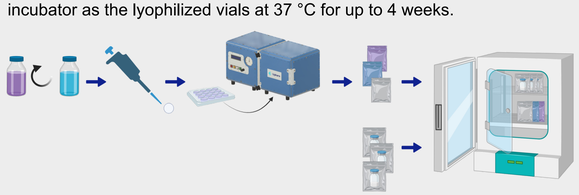
A representation of a typical lyophilization process. *Samples are frozen and subjected to a vacuum, enabling water loss through sublimation. Sample temperature is then raised to further dry the samples through desorption.*

A representation of a typical capillary mediated vitrification process. *Room temperature samples, loaded onto capillary scaffolds are subjected to a vacuum, enabling a controlled loss of moisture, first through evaporation and then through desorption.*

METHOD(S)

Recombinant luciferase was chosen as the model system for this study due to its high sensitivity to thermal stress, and the availability of structurally identical liquid and lyophilized forms from the same vendor.

The commercial lyophilized vials were stored in their original packaging in a 37 °C incubator for up to 4 weeks. The procured liquid luciferase was mixed with an excipient buffer and loaded onto porous scaffolds and placed in a vitrification chamber to perform the CMV process. After a 30-minute cycle, the vitrified scaffolds were packaged in sets of 3 and stored in the same incubator as the lyophilized vials at 37 °C for up to 4 weeks.

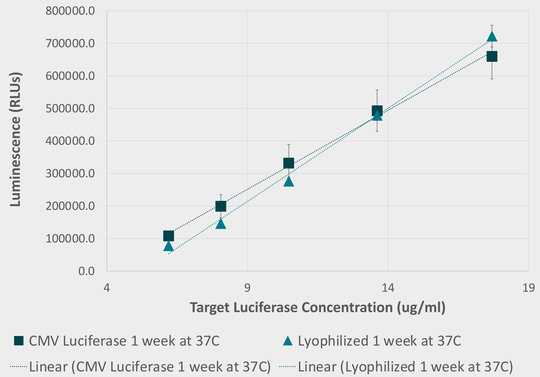


Preparation of samples. *Top row shows the CMV process where liquid luciferase was mixed with an excipient buffer, loaded onto capillary scaffolds, vitrified, packaged and stored in an incubator. Bottom row shows as-received lyophilized luciferase vials stored in the same incubator.*

RESULT(S)

Two independent trials following the procedure outlined in previous section were run and the data reported hereon is the average of the two trials.

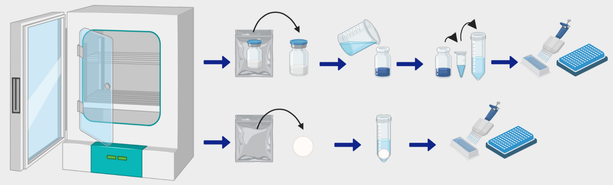
At the end of each week of thermal stress, one lyophilized vial and one set of 3 CMV samples were withdrawn from the incubator. The lyophilized samples were reconstituted as per manufacturer instructions. The CMV samples were reconstituted by depositing each scaffold into one conical tube and eluting with 1 ml of PBS.



Week 1 data as reference. *Raw luminescence data from lyophilized and CMV stabilized samples after 1 week of storage at 37 deg C. This data set was used as the reference to calculate relative activity for weeks 2, 3 and 4.*

The data indicates that lyophilized luciferase degrades at a faster rate than CMV luciferase when stored at elevated temperature. After 4 weeks of storage, CMV luciferase was within 84% of the activity shown after 1 week of storage, while the lyophilized luciferase had dropped to 52% of its week 1 activity. The slope of the lyophilized luciferase activity over time was 2.5 times greater than that of the CMV luciferase samples. The quality of the data was impacted, to an extent, by the variability in activity between the different lyophilized vials from the same lot and the age of the lyophilized samples prior to comparative storage with the CMV samples.

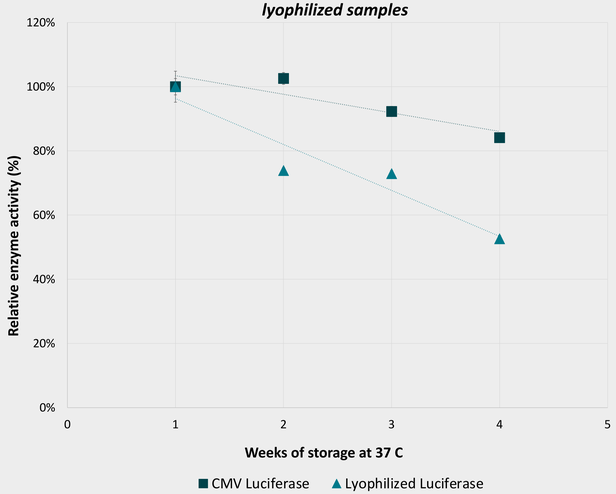
This data set demonstrates the applicability of the CMV process in the production of thermostable products. Both lyophilized and CMV formulations make use of excipients as stabilizers and vacuum to produce amorphous solids. However, the CMV process eliminates the freezing step involved in lyophilization. This greatly simplifies the formulation optimization, the cycle time, the energy burden, and the complexity of the infrastructure required to run the process.



Process for reconstitution of stabilized and stored product*. Top row shows the process followed for the lyophilized product as prescribed by the manufacturer. Bottom row shows the process followed for CMV stabilized samples.*

All eluted and reconstituted samples were diluted 1.3-fold and run in triplicate in a 96 well plate using an in-house luciferase assay. The luminescence values were obtained using a Synergy H1 microplate reader. In the absence of a matched reference for the lyophilized samples, week 1 data from either set was used as a reference standard to analyze the other 3 weeks of data for the respective sample type. The standard curves were analyzed by linear regression and the relative activity for the CMV, and lyophilized sample dilutions was determined by direct interpolation. The relative activity reported was calculated as a percentage of the respective week 1 data.

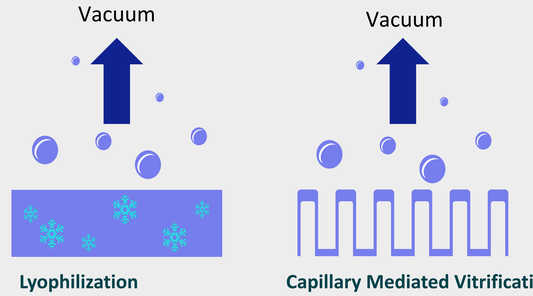
Change in relative activity of luciferase over time in CMV and lyophilized samples



Relative activity of lyophilized and CMV stabilized products. *Data shows the relative activity of each sample set over a 4-week storage period at 37 deg C.*

CONCLUSION(S)

Capillary Mediated Vitrification can be a viable alternative to lyophilization in the production of thermostable biological materials. In addition to producing products that have a comparable thermal stability, the shorter cycle time (30 minutes compared to 24-72 hrs), ease of use (minimal process optimization), and low capital expense requirements could make CMV an attractive process to replace lyophilization in the biopharma industry.



Lyophilization

- Uses freezing and sublimation to prevent boiling
- Cycle time of 12 - 72 hours
- Significant capex
- Signification process optimization

Capillary Mediated Vitrification

- Uses capillary evaporation
- Prevents boiling without freezing
- Cycle time of less than 1 hour
- Reduced capex requirement
- Little to no process optimization

REFERENCES

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5. Figures created with BioRender.com

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