LyoDEA™ An impedance based approach to the development of optimized freeze-drying cycles

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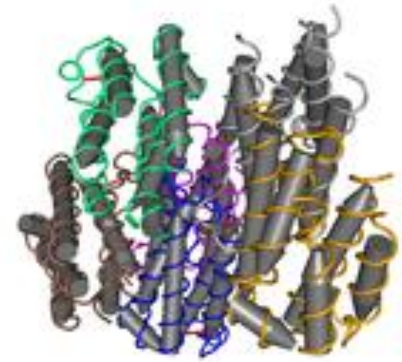
PHARMACEUTICAL QUALITY BY DESIGN

BACKGROUND Freeze-Drying of Biotherapeutics



> 130 products on the market worth $50bn

fastest growing segment amongst novel pharmaceutical entities today[1, 2]

market for lyophilization of biotherapeutics ~ 200 m units[3]

- **Without lyophilisation, 60% of biotherapeutics**, including recombinant proteins, plasma, vaccines and antibodies, **would not be available commercially[3]**

[1] Matejschuk, P. et al., (2009). *American Pharmaceutical Review*, March, pp. 54-58.
[2] Bobst, C.E. et al., (2009). *Analytical Chemistry*, 80 (19), pp. 7473-7481.
[3] Genetic Engineering & Biotechnology News (2005). *Lyophilization: growing with biotechnology*.

Freeze drying cycle Development

1. Product characterization

Time consuming &
Costly techniques

critical product temperatures

($T_{g'}$, T_{eu} or T_c)

offline techniques DSC, Electrical resistivity, cryomicrospy

2. Process development

Trial and error approach

Set up freeze drier shelf below the critical temperatures for sufficient times to allow complete freezing and primary drying

3. Monitoring process

Existing PAT are either invasive or perturb heat flow and vial array

Apply PAT to

- Ascertain that product temperature does not exceed critical temperatures (Thermocouple)
- define end of primary drying (Thermocouple, microbalance)

Existing PATs can not measure events associated with T_g', product collapse and ice recrystallization during annealing

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    - An alternative to thermocouples for macro-homogeneous materials
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    - Drying end points; sublimation rates?
6. Annealing mechanisms
    - Process development: Optimization of ice crystal structure
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    - Is it to late to change?
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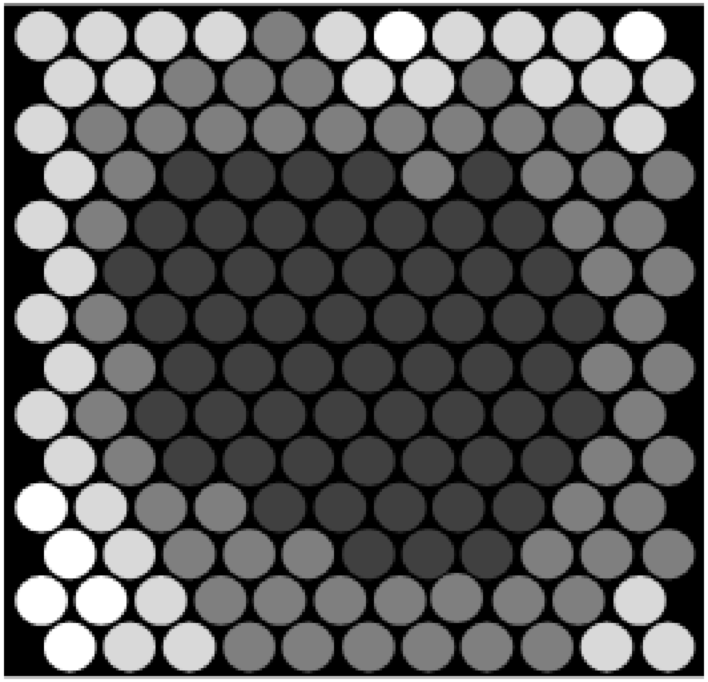
1. Shelf temperature distribution: Spatial mapping

- The temperature variation measurement during freezing stage.
- Thermocouple measurements of vials filled with oil.
- Gray scale shows minimum-maximum during freezing.
- ΔT ~ 1-2 °C across shelf can affect ice formation (already stochastic) and impact drying time
- 1 °C increase in 1° drying T can shorten drying time by ~13%



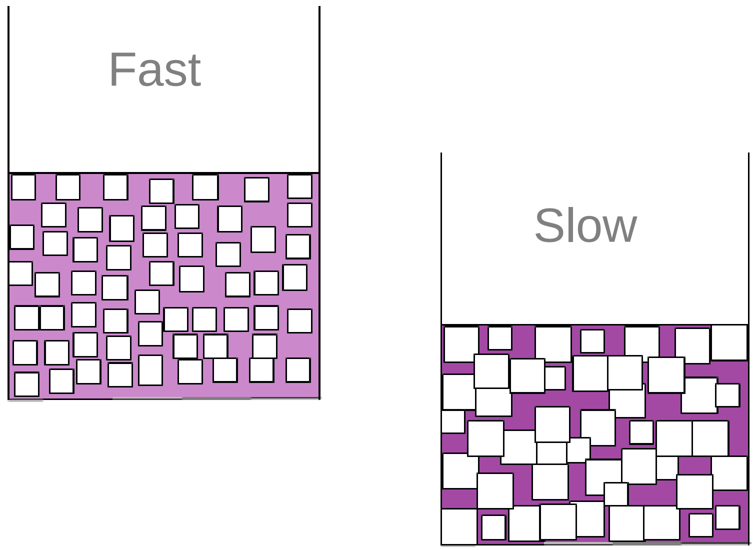
1. Impact of Spatial Temperature Map on Ice Formation

- Ice crystallization rates can impact the amount of ice and the particle size

F Rate	Ice Crystal Size	R_p	Unfrozen fraction
Fast	Small	High	High
Slow	Large	Low	Low

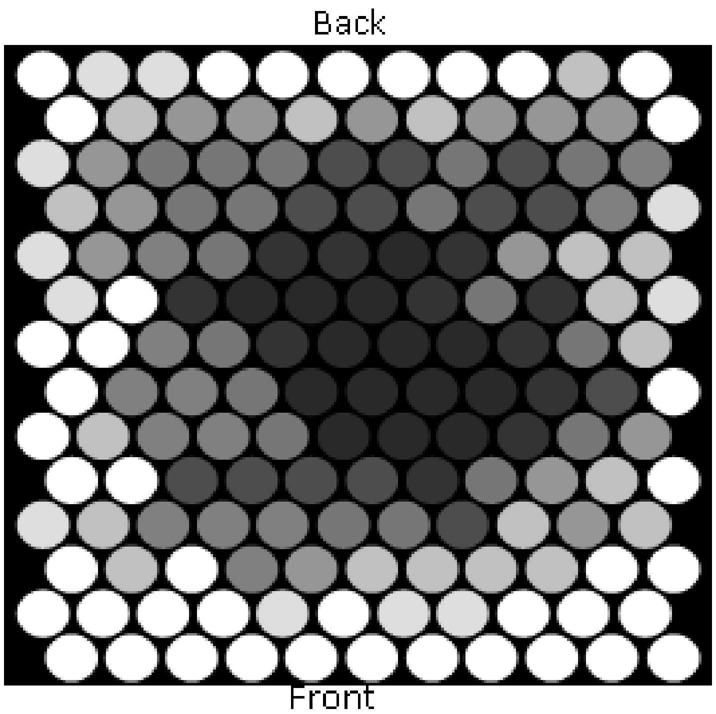
Leading to variations in:

1. drying rates (because of the impact on the resistance to vapour flow, R_p)
2. concentration on solutes in the unfrozen fraction, which impacts T_g which impacts the primary drying temperature.



1. Distribution of Drying Rates : truncated trial sublimation runs

- Impact of spatial temperature variation and variations in heat transfer rates have a significant impact on drying rates.
- By truncating the primary drying phase one can assess the variation in rates of drying across the drier.
- The variation is more pronounced than one could expect from the sole impact of shelf temperature.
- Confirms that the impact of variations in heat transfer rates is significant!



1. A Complex Picture!

- Some advantage in combining several techniques to follow:

1. nucleation and growth
2. ice sublimation rates
3. crystallization changes in excipient
4. monitoring primary and secondary drying endpoints .

- Single Vial Measurements

1. Thermocouple
2. Resistance temperature detector (RTD)
3. Microbalance

- Batch Measurements

1. Manometric temperature measurement (MTM)
2. Pirani Pressure gauge
3. Capacitance manometer
4. Tuneable diode laser absorption spectroscopy
5. Cold Plasma ionization device

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2. LyoDEA Brief Description

- Freeze-Driers:
  - GEA LyoPhil SMART Lyo®2
- Arrangement of vials:
  - Hexagonal arrays & Full shelf
- Range of freeze-drying cycles and placebo formulations:
  - lactose
  - sucrose
  - mannitol
  - maltodextrin

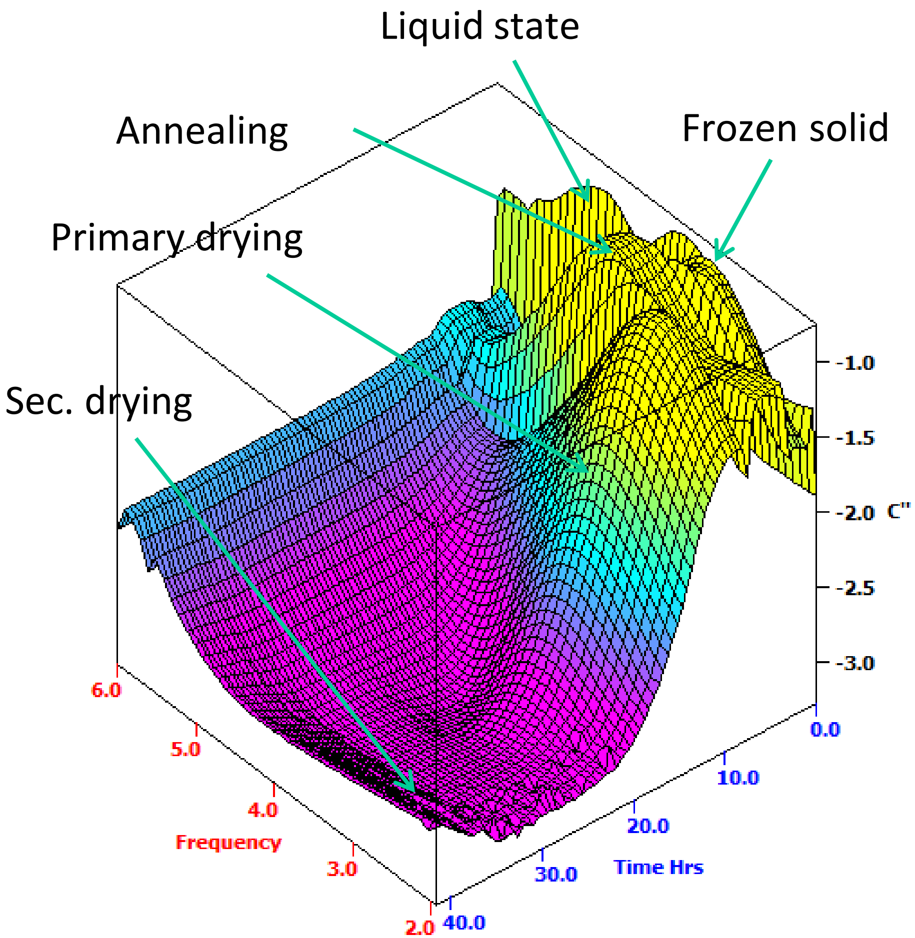
GEA LyoPhil SMART Lyo®2

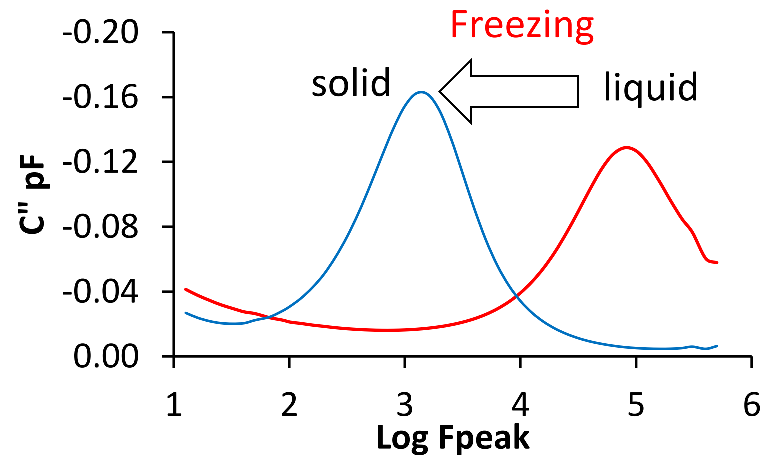
2. LyoDEA Brief Description

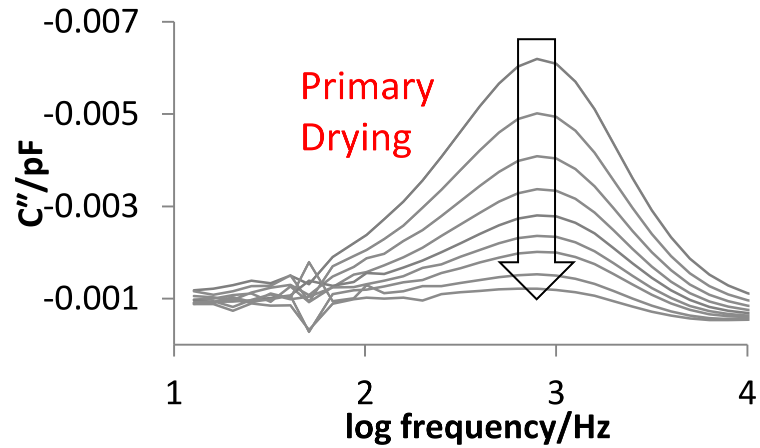
- The system connects via a junction box to 5 individual **LyoDEA™** test vials positioned around the freeze-drier shelf.
- Frequency scans (10 Hz – 1 MHz) of the LyoDEA™ test vial impedance were recorded every 3-5 minutes throughout a freeze-drying cycle (20 s for each spectrum)
- The **LyoDEA™** measurement and control software saves the spectra from each time point
- Spectral data were analyzed by Z-View® and LyoView™

LyoDEA™ Measurement Vial

2. LyoDEA™ response surface

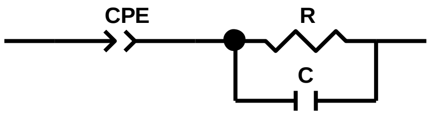


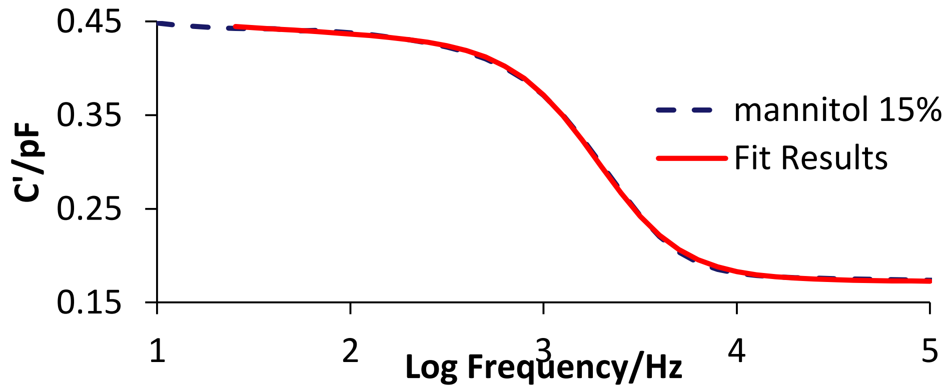


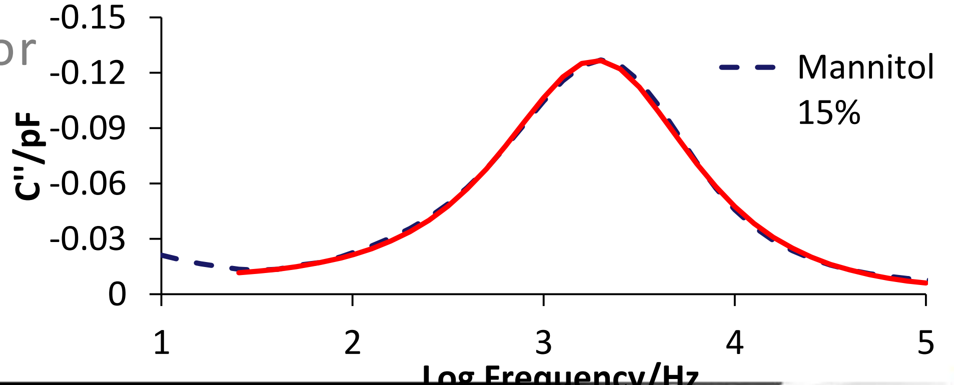


2. Impedance Modelling

- CPE explains the interfacial impedance of the glass wall of the vial.
- Resistance element records conductivity of ions
- Capacitor element defines dielectric properties of the product.
- The circuit element R was shown to be a sensitive indicator of the phase behavior of the solution, i.e. ice formation and solute crystallization during the freezing cycle







2. Application of LyoDEA in freeze drying cycle development

	LyoDEA	Conventional Methodology
Thermal characterization	‘Realistic’ in line measurement of critical events within the vial. Set point established by ‘event’ rather than ‘temperature’	Non-representative, off-line temperature measurements by DSC, electrical impedance (off-line) and freeze drying microscopy
Trials	**1º Drying End point** (determined by product non-invasive measurement) **Rate of sublimation** (potential to predict end point)	**End point + time overage** from the thermocouple response (inserted into the product) **Rate of sublimation** (Requires additional technology : microbalance)
Scale up	Fewer cycles with realistic process time	Potentially more cycles with extended time (owing to process uncertainty/higher risk)
Freeze drying cycle optimization	Real time information (fewer cycles)	Retrospective characterization (numerous cycles)
Finished product moisture content	End point of secondary drying in line (after calibration)	Always required (Off line end of process)
Cost of cycle development	Minimised	High

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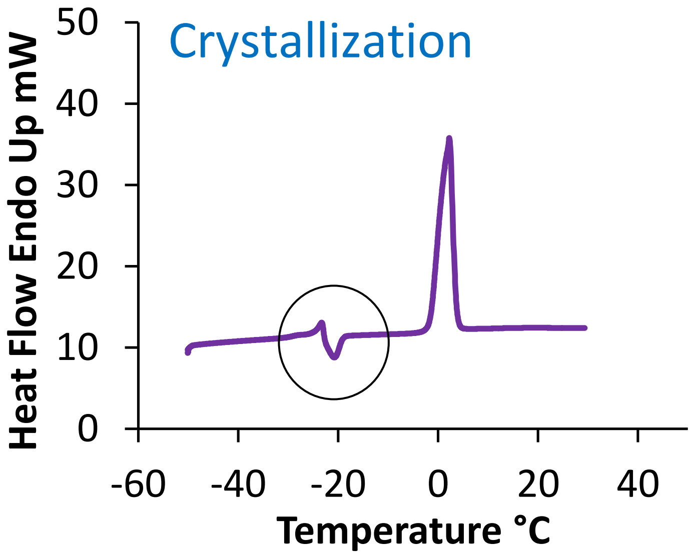
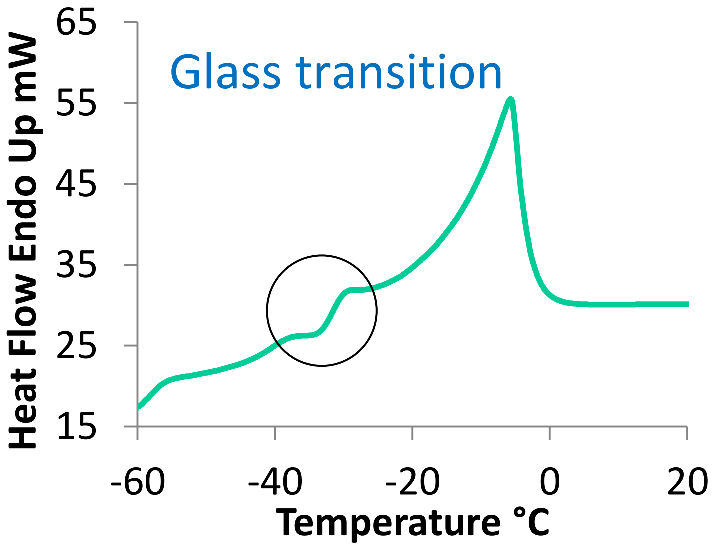
3. Product Characterization: Critical Temperatures

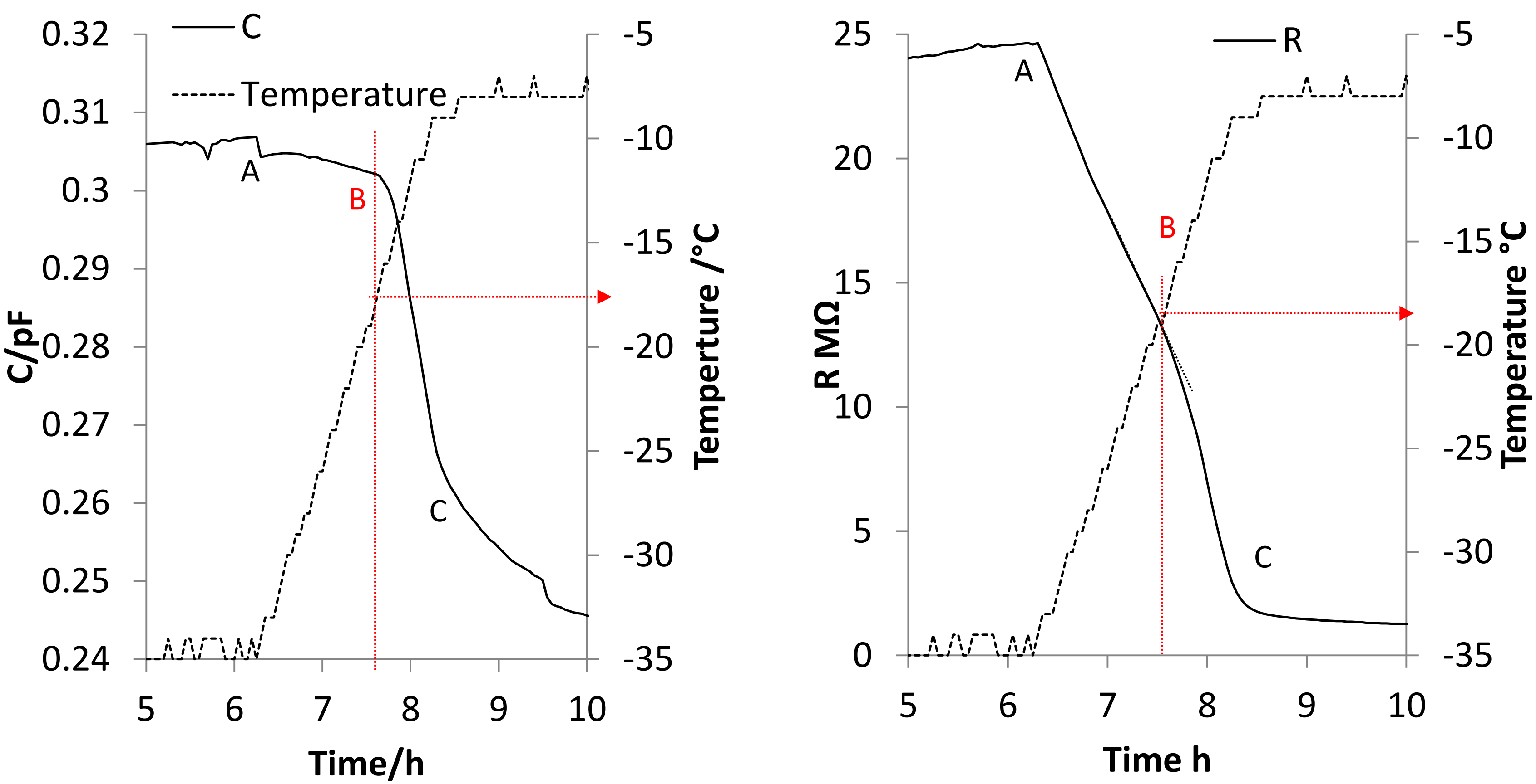
- Critical thermal events such as eutectic melting and glass transition are frequently measured by DSC.





- Off-line measurements differ from the vial conditions in many regards e.g. Sample geometry, heat flow coefficient, freezing patterns and heat transfer rates.
- **Time** at which critical changes take place (glass transition, eutectic crystallization) is indirectly **estimated** from product temperature data to ascertain the completion of freezing stage.

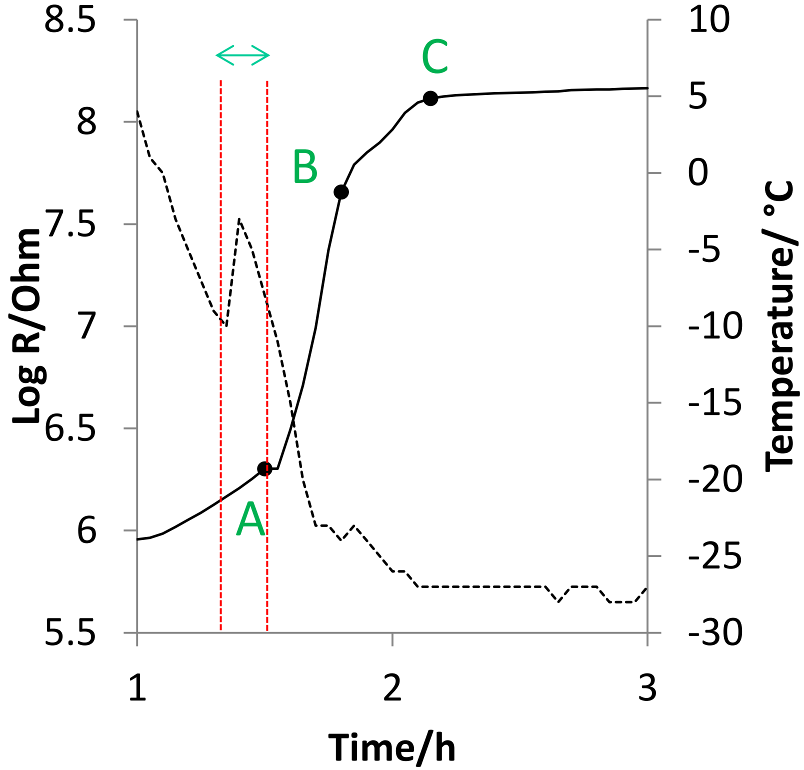
3. LyoDEA: Identifying the glass transition temperature



An inflection (Point B) the product capacitance and resistance profile during the thermal scans signifies second order transitions which are seldom observed by thermocouple; The capacitance decreases more abruptly following the glass transition.

3. LyoDEA: Identifying eutectic crystallization

- Ice (A-B) and mannitol crystallization (B-C) in the solution were observed by LyoDEA as steps in the product resistance during freezing.
- The small time delay between LyoDEA defined event and that defined by the thermocouple may be attributed to additional nucleation sites provided by the invasive probe.



Crystallisation/Eutectic temperature measurement for 15% w/v mannitol

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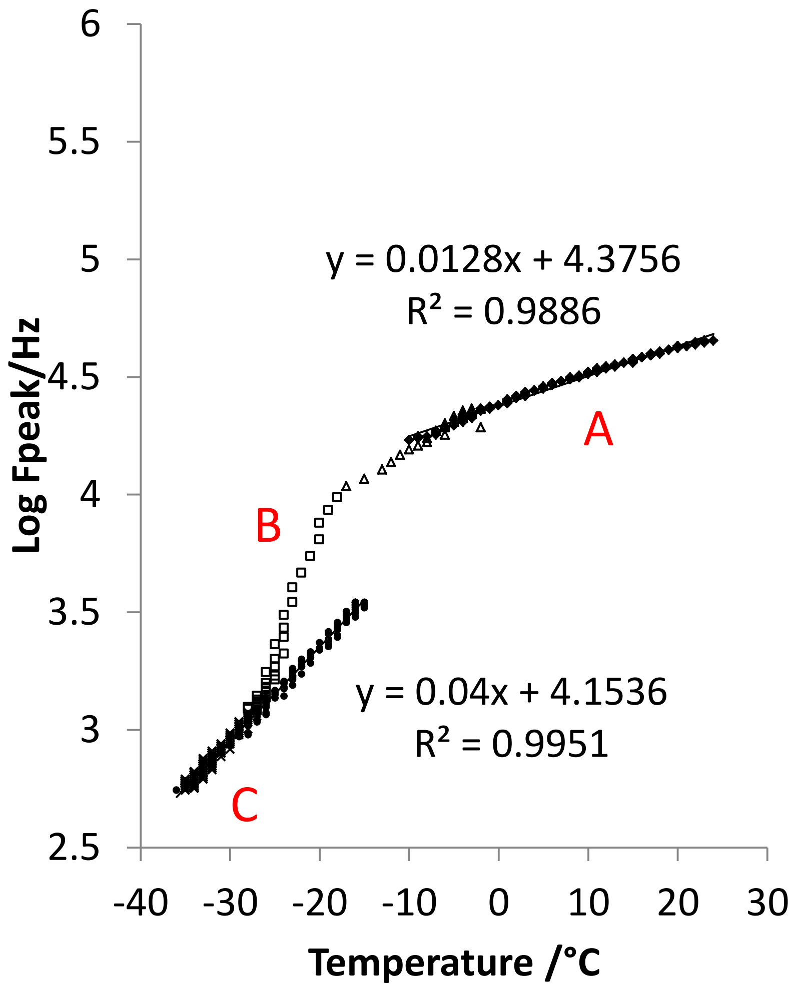
4. Product Characterization : Temperature

1. The f_{peak} parameter is strongly dependent on the product temperature during product cooling (A), freezing (B) and thawing (C)
2. Provided there is no change in phase, then a quasi-linear correlation exists between Log F and temperature (A, C-D)



4. Product Characterization : Temperature

- Application for LyoDEA as a non-invasive tool to measure the degree of super-cooling and the onset of freezing
  - which defines the rate of ice crystallization and hence the ice crystal size
  - which in turn impacts the vapour flow resistance during primary drying.

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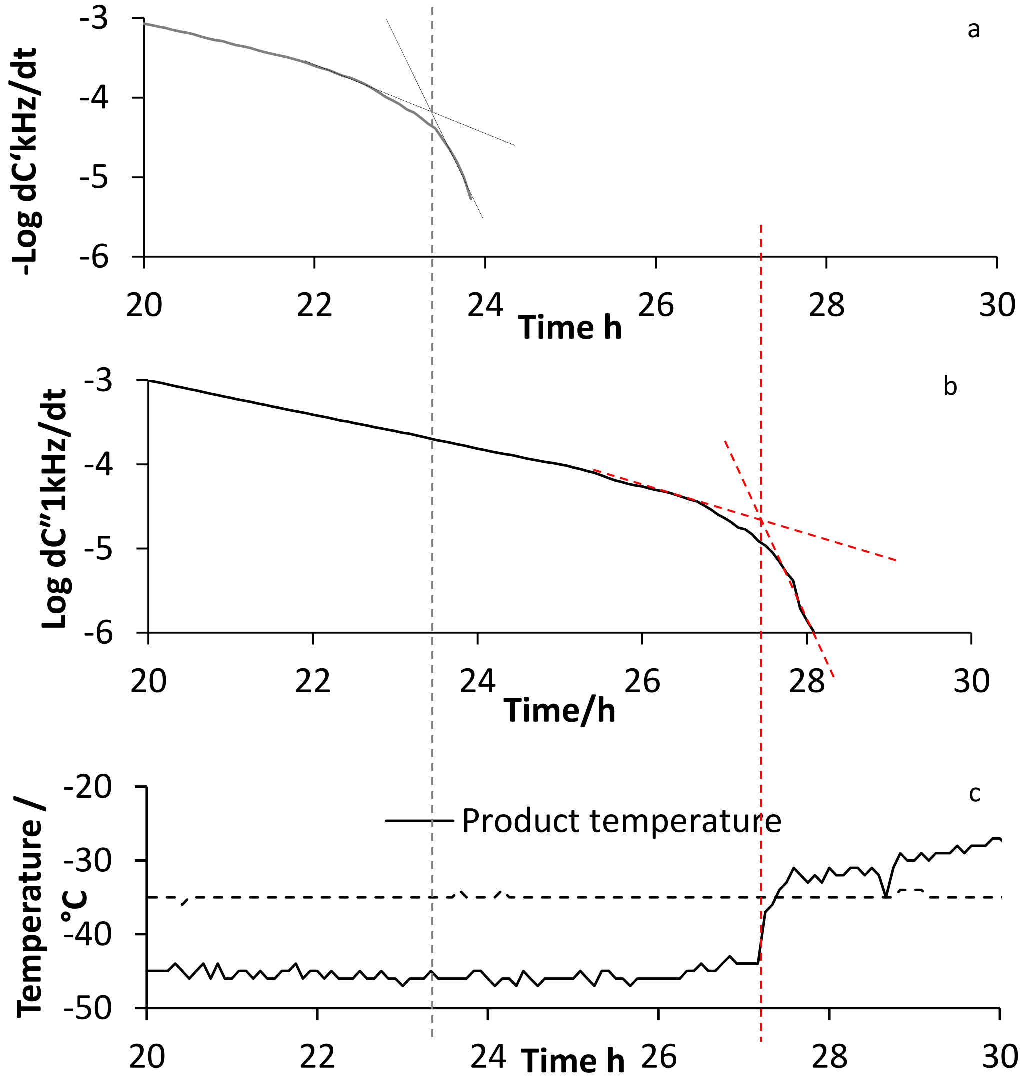
5. Defining the End of Primary Drying - Results

LyoDEA offers a non invasive measurement of primary drying time which is in good agreement with the thermocouple.



5. End of Primary Drying

- The reason why the imaginary capacitance has an end point which more closely mirrors the end point determined by the thermocouple is that it is sensitive to the electrical conductivity of the system.
- The shunt conductance provided by the ice layer dominates the response, so while there is still a layer of ice at the base of the vial then the imaginary capacitance will continue to change as the ice layer shrinks to a negligible thickness.
- The real capacitance, by contrast, is simply the sum of the contributions from the electrical capacitance of the ice layer and the dry layer.
- And while the capacitance of ice is approx. 50-100 times that of the dry layer, the conductivity of the ice layer compared to the dry layer is likely to be orders of magnitude higher.

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6. Annealing Mechanisms

- Annealing step is generally included in the freeze drying cycles to Ostwald ripening **" growth of the bigger ice crystals at the expense of smaller ones."**
- **Thermocouple** cannot measure the impact of annealing on the primary drying time due to potential errors linked to positioning of probe, additional vapour flow paths and heat input.
- Evidence of such increase in the ice crystals are made retrospectively
  - **SEM analysis** of the **Freeze dried products** show bigger pore size in the annealed product matrices.
  - **MTM studies** report decreased resistance to vapour flow **during primary drying**
- **Changes in the product Impedance during annealing may exhibit a relationship with the drying time**

6. Annealing mechanisms: Capacitance vs ice crystal volume

Increase in capacitance has been previously linked to the ice crystal volume

Similar growth in the capacitance was observed during annealing

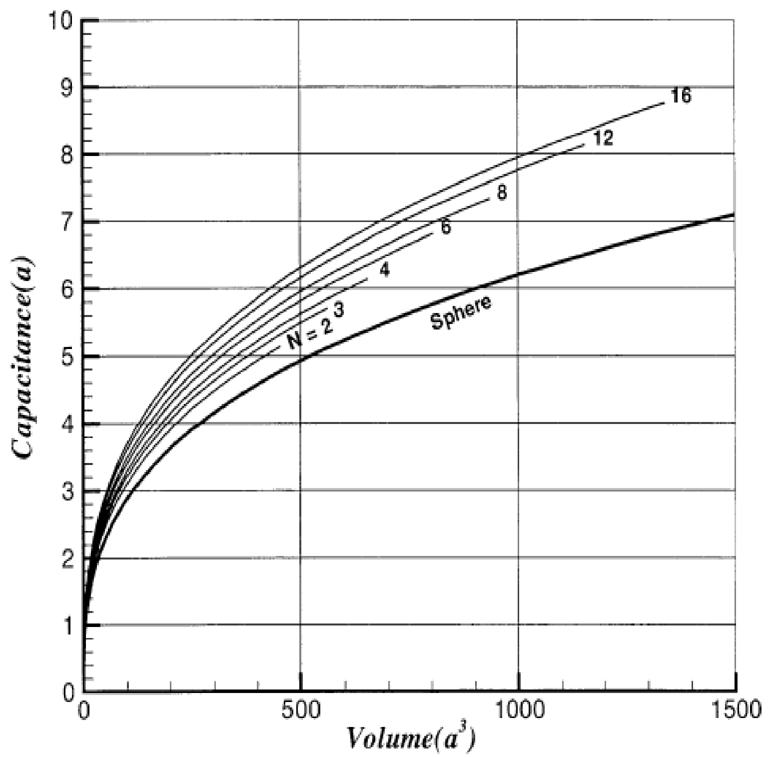
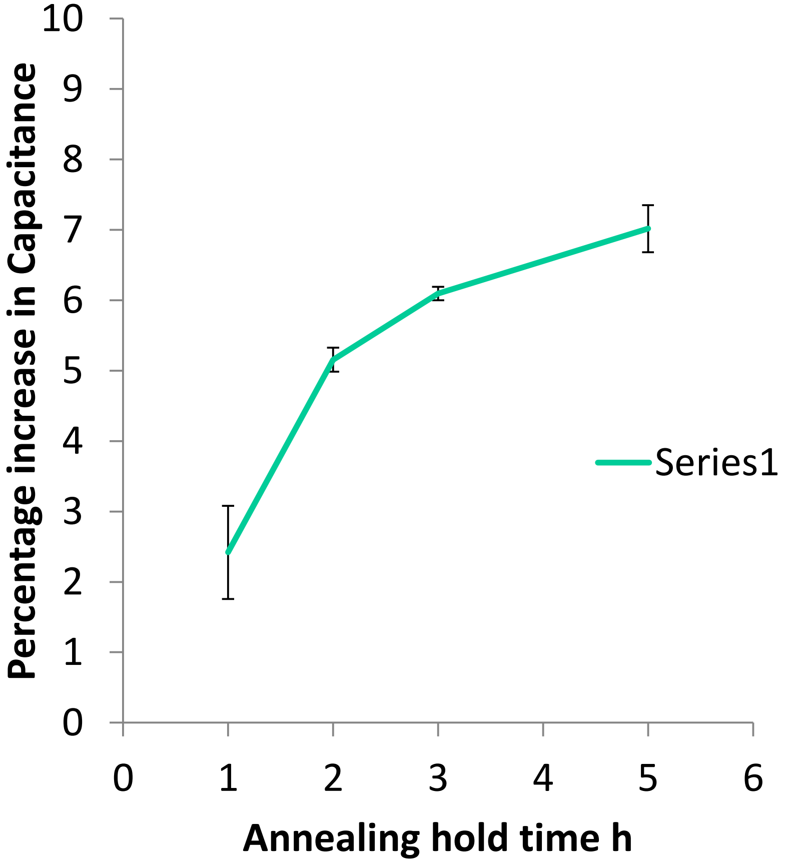


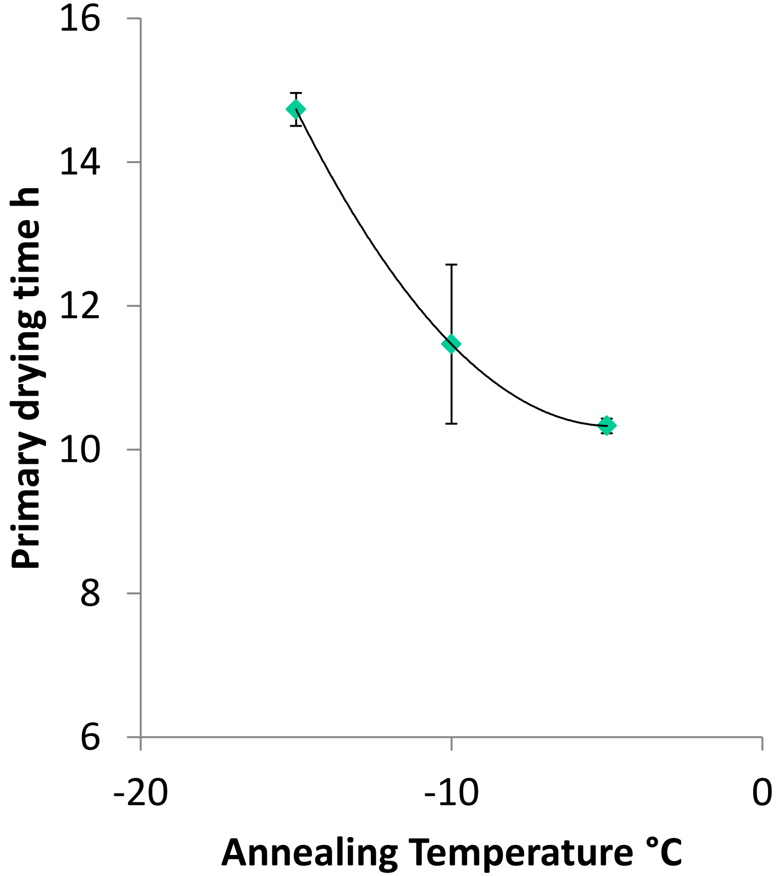
FIG. 12. Variation of capacitance with rosette volume. Here N represents the number of lobes of the rosette. The thick curve is for spheres.



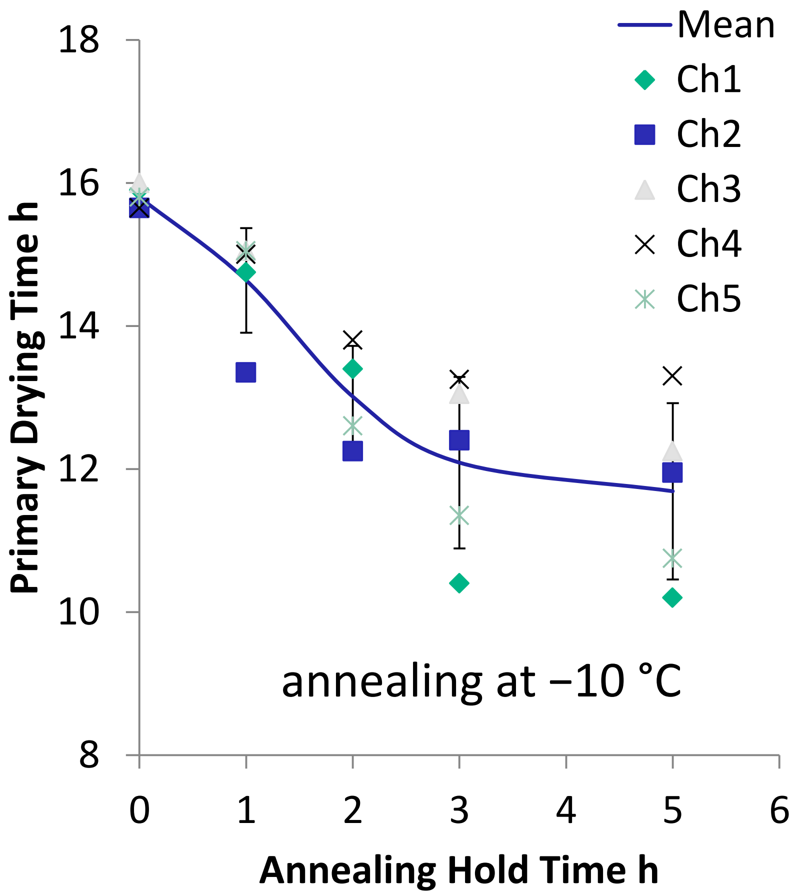
Chiruta, M., Wang, P.K., 2003. Journal of the Atmospheric Sciences 60, 836-846

6. LyoDEA: Optimizing the annealing step (Maltodextrin 10% w/v)

Drying time decreases exponentially with the elevation in annealing temperatures



3h hold time provides an optimum drying time

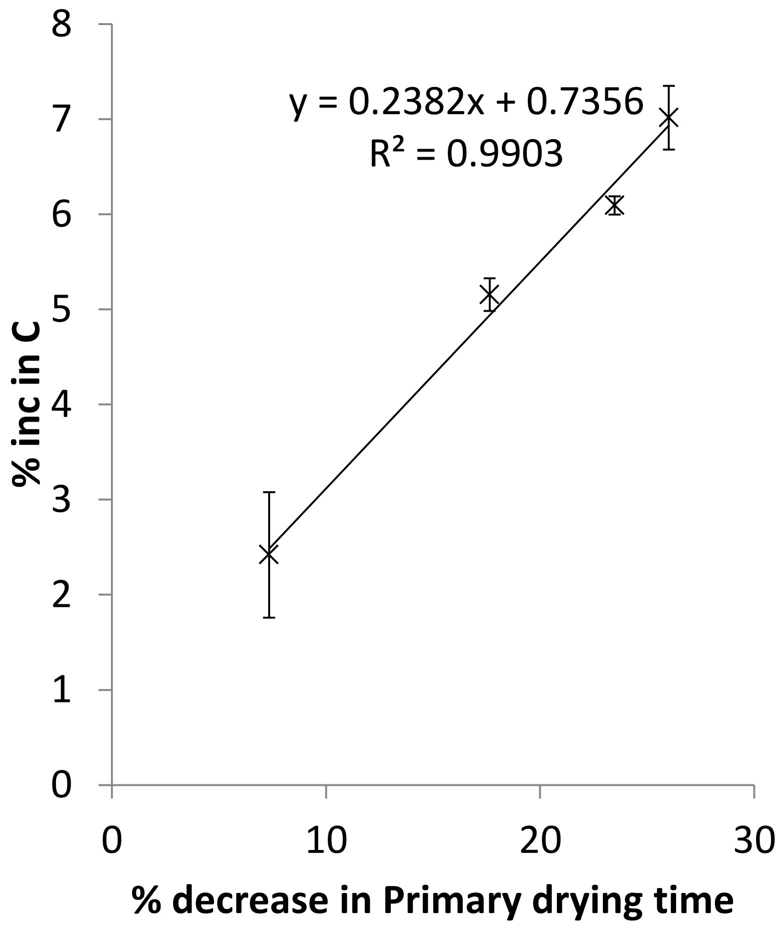


6. Predictive control of the primary drying time

- Increase in the capacitance correlates well with the decrease in the primary drying time.
- Changes in the Product capacitance during the annealing can be applies as a predictive parameter which define the primary drying time.

For every ~2% increase in capacitance the primary drying decreases by ~8%

QUESTION: Is there a universal rule? Could this be used to predict time savings in primary drying?



6. Optimization of the Annealing stage

	LyoDEA	Conventional Methodologies (TC)
Annealing with different hold time (5 time periods) and temperature (3 levels)	Monitor changes in the impedance after one 'extended' annealing at each temperature (No. of cycles = 3)	Record drying time after individual freeze-drying cycles with a discrete hold time and temperature. (No. of cycles=15)
Process Time (cycle time: 50 h)	~3 weeks	~4 months
Sample Quantities (5 Vial per run)	15 vials	75 Vials
Energy consumption	x	5x
Results	precise	Error prone (measured end point is sensitive to the placement of the probe with thin vials)

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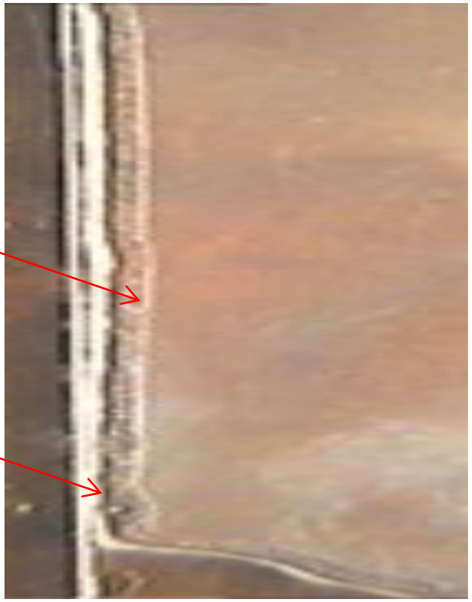
7. Product Collapse witnessed by FDM

- Conventionally measured by cryo-microscopic images

Sublimation front

Collapsed product

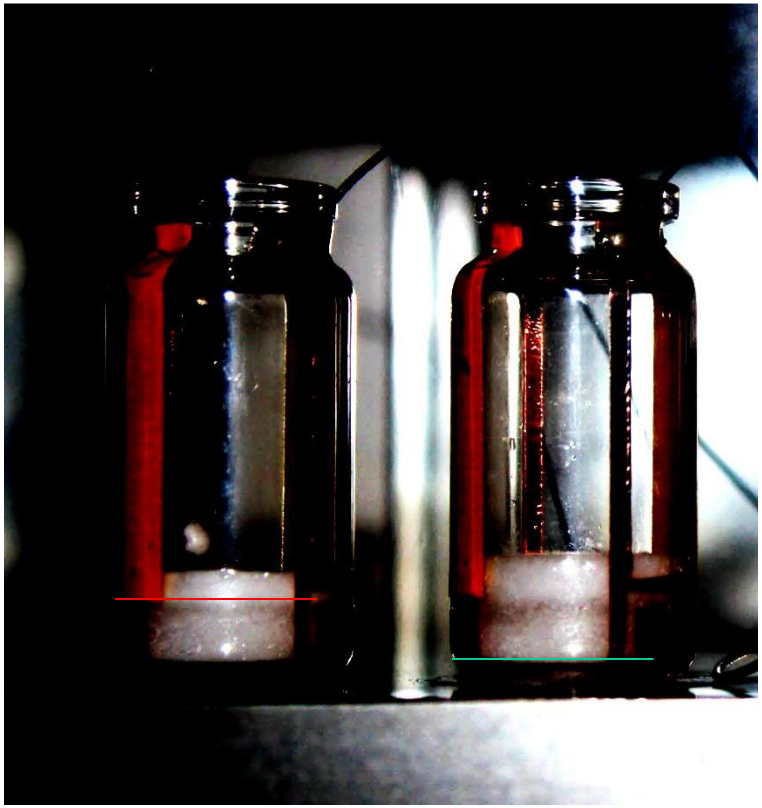


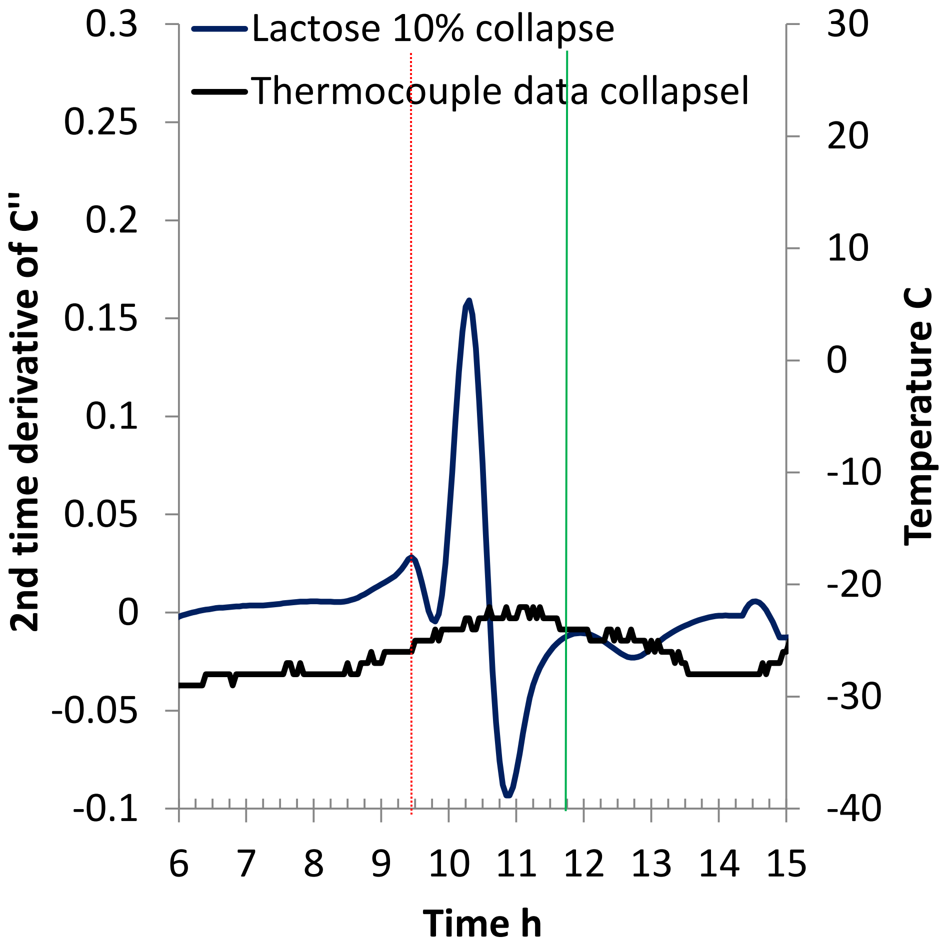
Microscopic images may not account for increase in the vapor pressure at sublimation front ,following increased resistance to vapor flow during the later stage of primary drying; potentially vulnerable to collapse

Collapse measurement within the real conditions may provide such information

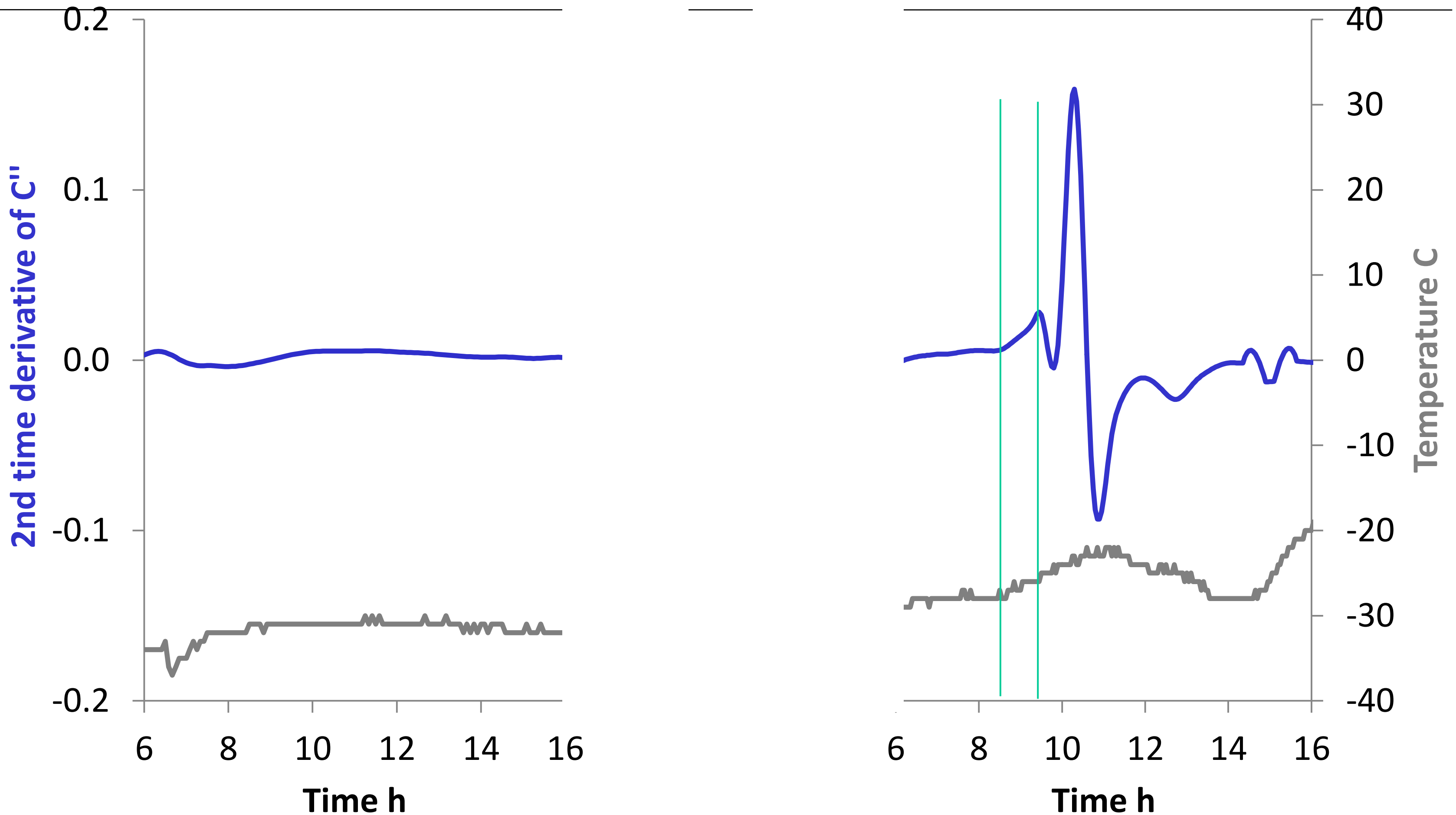
7. Product collapse witnessed by TVIS

A sharp spike in the impedance profile at 1 kHz characterizes the product collapse





7. Product Collapse Lactose 10% : 2nd derivative



The capacitance profile of collapse free product (LEFT) was seen to be fairly uniform, un-like the collapsed product (RIGHT)

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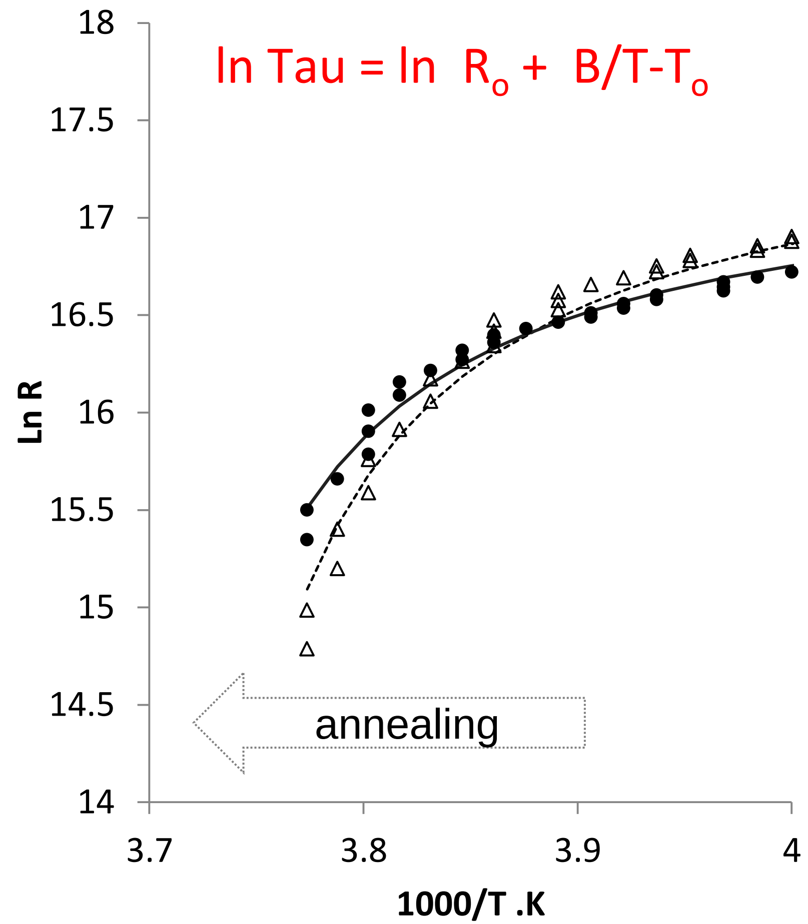
VTF Fit to describe the above Tg' resistance

- Above T_g the temperature dependence of the product resistance follows the Vogel-Tammann-Fulcher function.

- The curvature of the resistance plot decreases following annealing which relates to the increased strength of the glassy material.

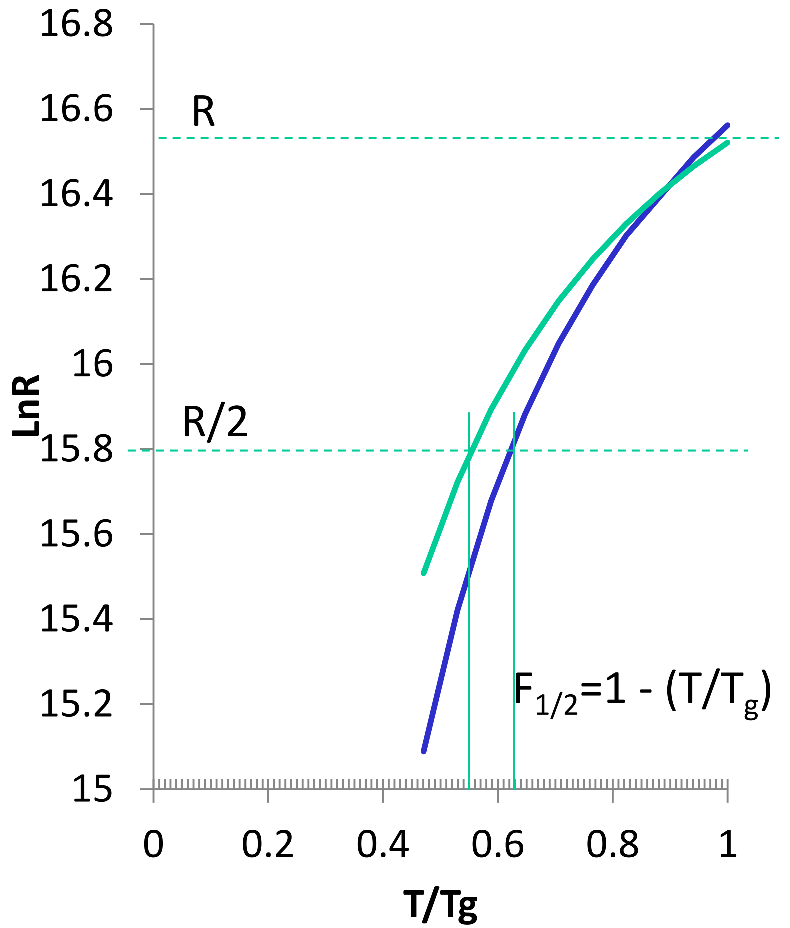


Annealing impact on glassy matrix

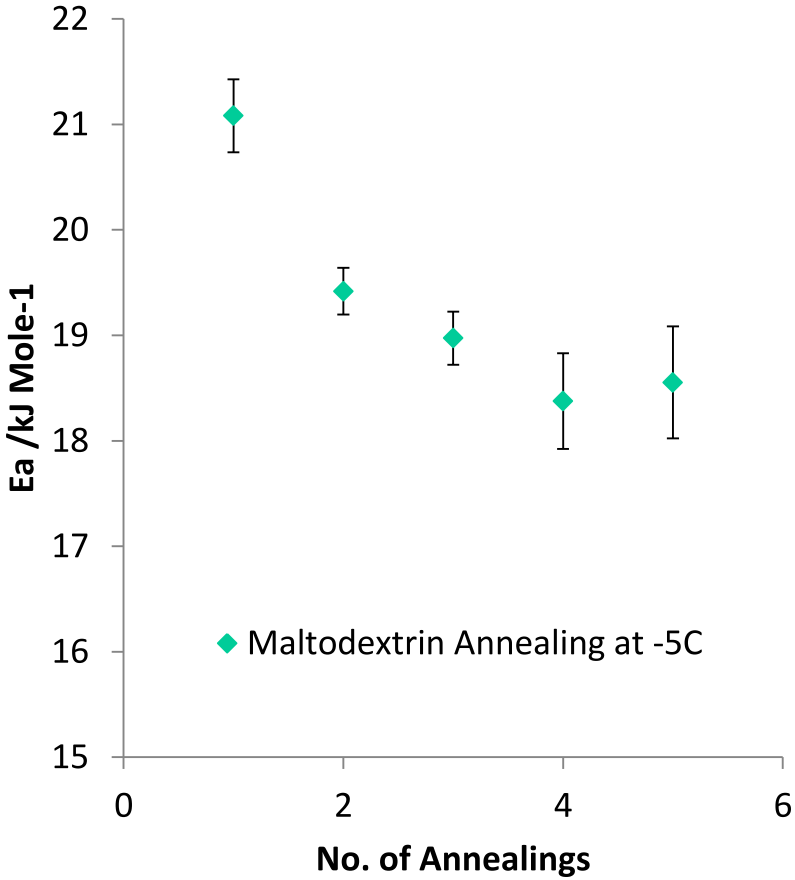
- The fragility is a dimensionless parameter employed to explain the strength of a glassy material, it is calculate from

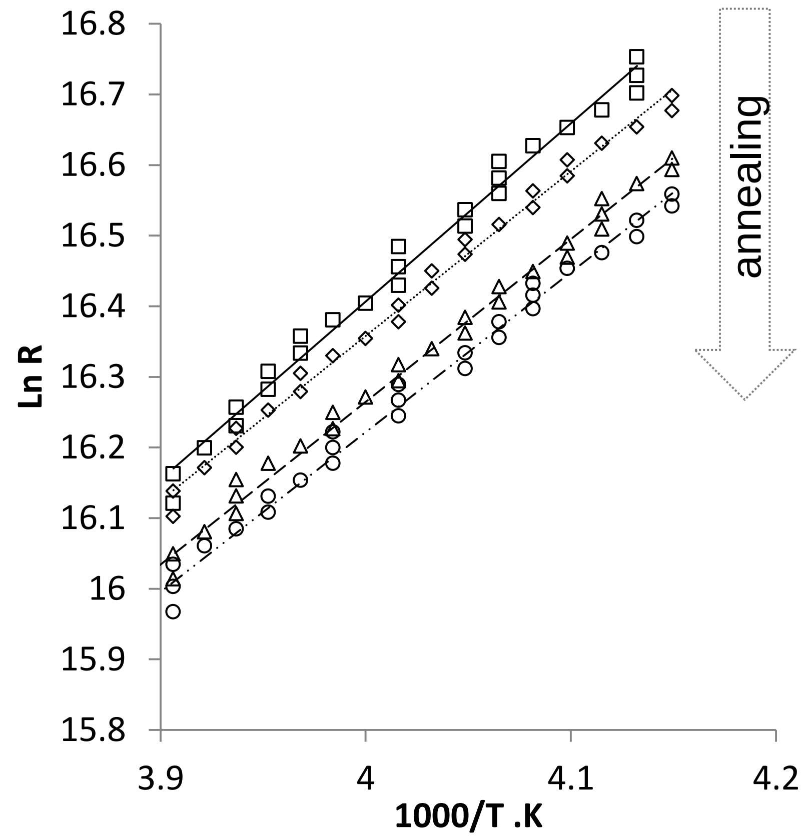
$$F_{1/2}=1 - (T/T_g)$$

- Fragility index range from 0-1 in the increasing order of strength.
- The fragility (or the steepness index) of the glassy material increase from 0.38 to 0.44 after annealing.
- Link b/w fragility and (i) moisture content of the unfrozen phase ?, (ii) ease of moisture desorption during 2° drying ??



Arrhenius Fit to describe the below Tg’ resistance





The product resistance and the activation energy for charge transport (E_a) in the sub-Tg’ temperature region decreases following annealing. The explanation is that the unfrozen fraction has a super high viscosity and that the ice structure now dominates the resistance

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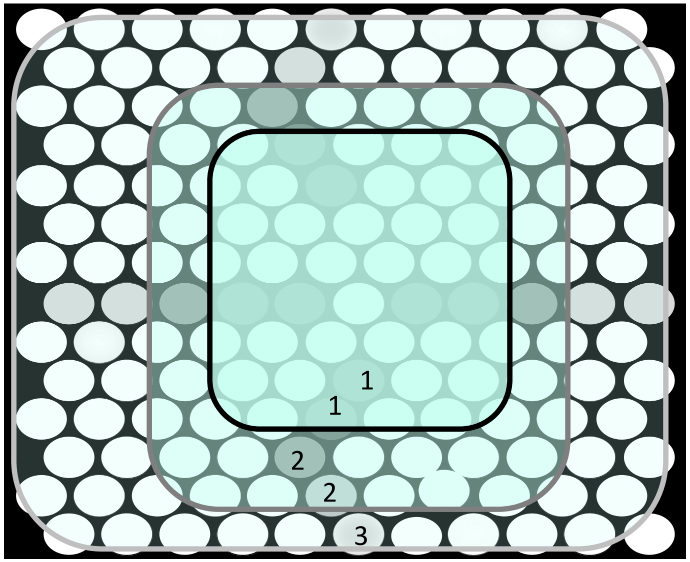
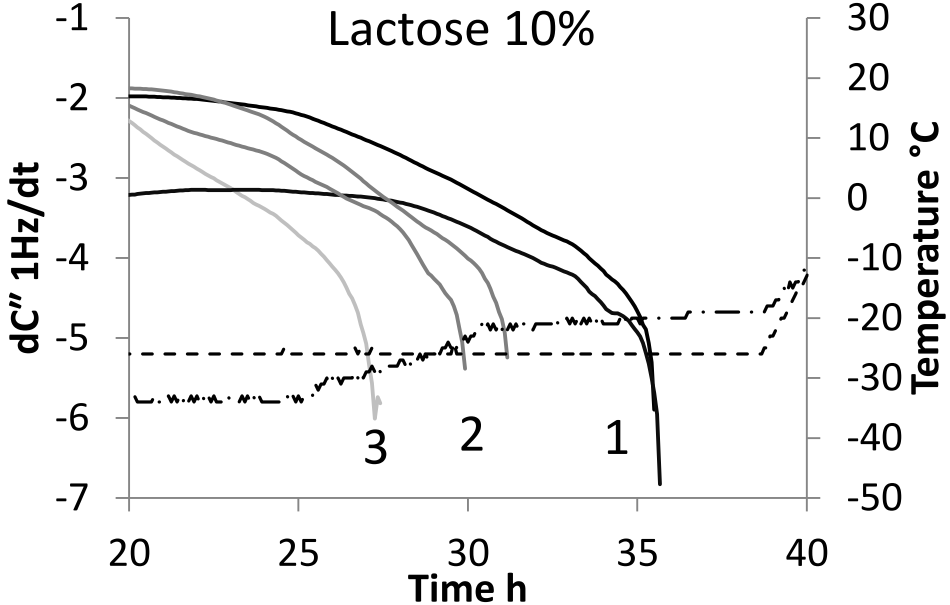
   - Mapping of shelf: Freeze-drier operation qualification

8. LyoDEA Spatial mapping: Primary drying times





1. Primary drying time distribution across the shelf identifies three distinct spatial regions characteristic of thermal variations in the shelf.
2. Edge effects – may extend across three vials around the periphery of the shelf

Conclusion

LyoDEA can effectively characterize:

The product

- ice formation, collapse, glass transition, and eutectic formation

The process parameters

- temperature, primary drying end point, and annealing

- LyoDEA™ provides some information on spatial mapping of the shelves in a freeze-drier and contributes to the isolation of hot and cold spots that may impact individual vial drying rates.

- LyoDEA™ can accelerate the product development phase by reducing the no. of trial runs needed to optimize the process.

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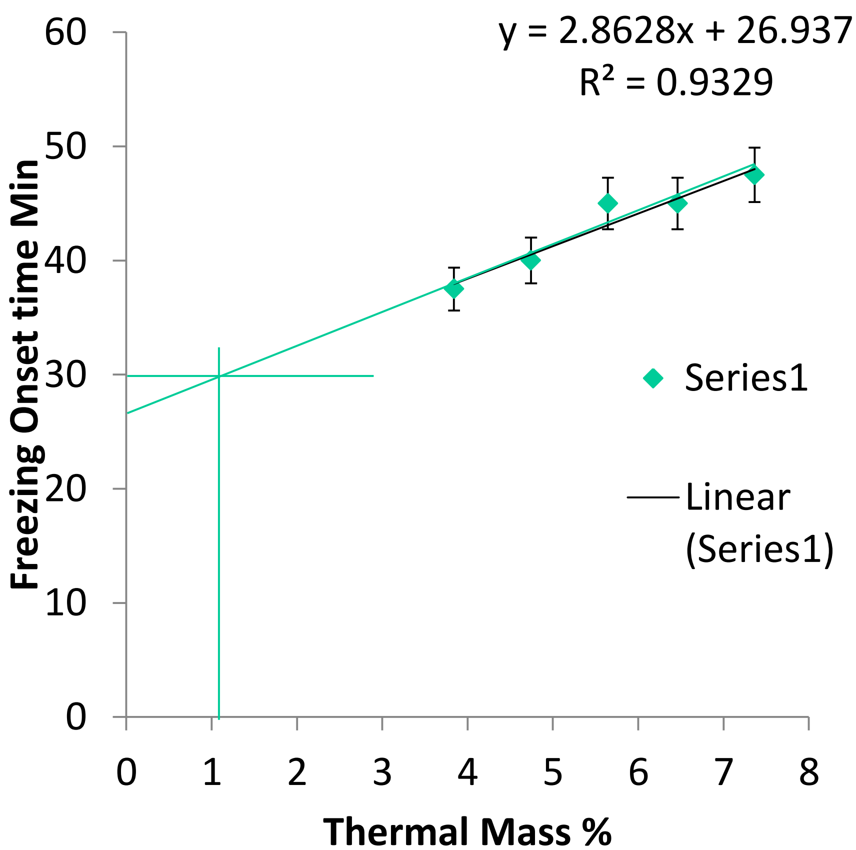
Annex – Miscellaneous Observations

Thermal Mass

- Each 1 % of extra weight, due to the mass of the electrodes and connection strips, extends the freezing onset time for 1 minute.
- The back extrapolation of the trend line suggest that thermal mass less than 1% of the vial weight will have negligible impact on the freezing onset time (the solution in the control vial, i.e. without electrodes, freezes at 30 min)



Secondary Drying (malto-dextrin 10%)

- Time slice of the C” at 10 kHz demonstrates an increase in the capacitance during the secondary drying ramp temperature.
- Non-linear increase in the capacitance may suggest a potential correlation with the loss of bound water during secondary drying.
- Future studies may be performed to confirm this observation.