The use of through vial impedance spectroscopy (TVIS) for determination of ice nucleation, solidification end point, and mannitol crystallization during freezing and re-heating

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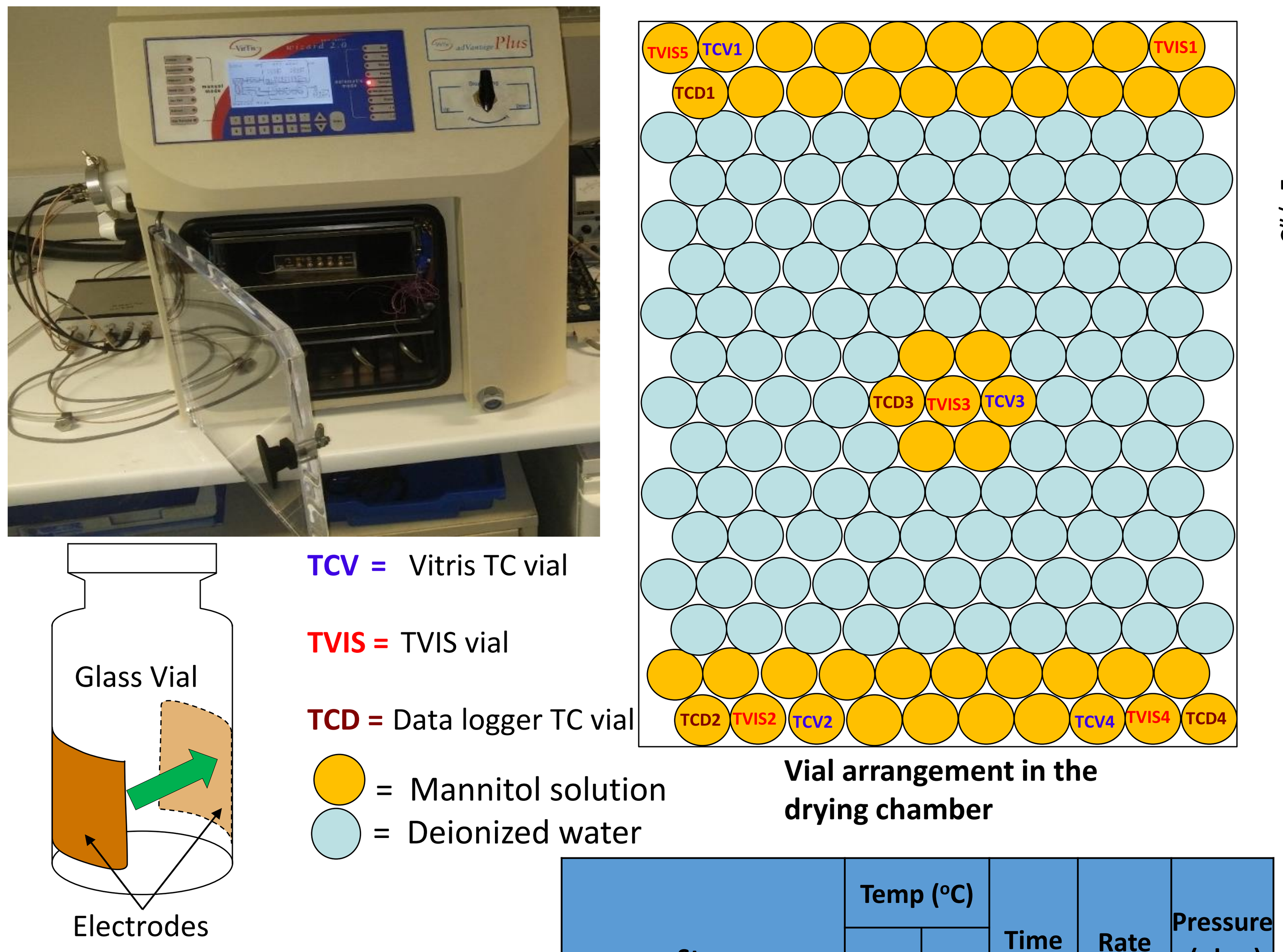
1. INTRODUCTION

- Mannitol improves mechanical strength of lyophilised product cake and thereby presents with elegant cake structure.
- Primary drying of mannitol-containing formulation must be performed below its critical temperature to avoid melt-back which would result to increase in primary drying time.
- Previous study (Kett et al. 2003) performed offline using DSC, CSM, and XRD showed mannitol crystallises and melts at -30 °C.
- Online study during actual freeze-drying process may be required to ascertain this behavior in a continuous freeze drying condition.
- TVIS measures material charges across a vial rather than within the vial. It may be used to perform both non-invasive and real-time measurement of aqueous frozen mannitol.

AIM: To demonstrate the use of TVIS for online study of thermal transition events including ice growth, crystallization and melting-back of mannitol in aqueous solution during lyophilization process.

2. METHOD



- Fill factor, Ø 0.7 used equivalent to 3.5g of solution
- Virtis Advantage Plus Benchtop Freeze dryer
- 5%w/v of 98% D(+)-Mannitol
- TVIS Multi-channel Sciospec

Step	Temp (°C) Start	Temp (°C) End	Time (min)	Rate (°C/min)	Pressure (µbar)
Equilibrium phase	20	20	30		
Freezing ramp	20	-45	120	0.1	
Freezing hold	-40	-45	120	-	
Re-heating ramp	-40	-10	100	0.2	
Re-heating hold	-20	-10	120	-	
Re-cooling ramp	-20	-45	40	0.2	
Re-cooling hold	-40	-45	120	-	
Primary drying equil.	-40	-40	30	-	400
Primary drying ramp	-40	-25	30	0.5	400
Primary drying hold	-25	-25	2500	-	400
Secondary drying ramp	-25	20	225	0.2	400
Secondary drying hold	20	20	480	-	400

Fig.1 Freeze drying instrument and methodology

3. RESULTS

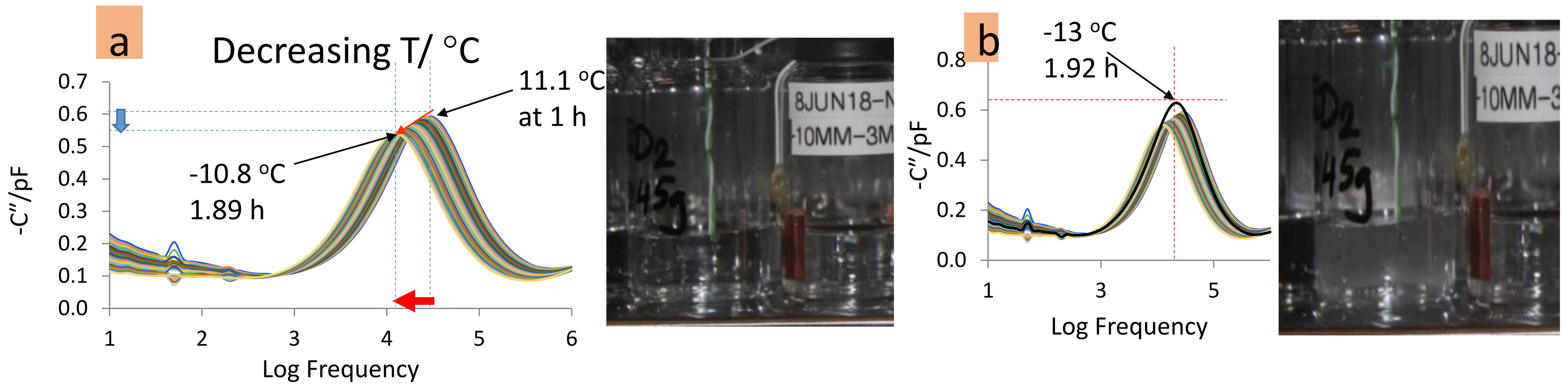


Fig.2 a) demonstrates C''_{PEAK} response to decreasing temperature with time by moving in two directions at a time: 1) lower frequencies (red arrow), and 2) downwards, reducing peak height (blue arrow). b) shows the event at the onset of ice growth depicted by sudden spike of C''_{PEAK} at 1.92 h when product temperature was -13 °C as determined by the temperature calibration of the F_{PEAK}. Evident pictures of the physical process shows that the peak upward spike was accompanied by a change of solution in vials to a cloudy ice matrix from clear solution 3 minutes before the solidification onset.

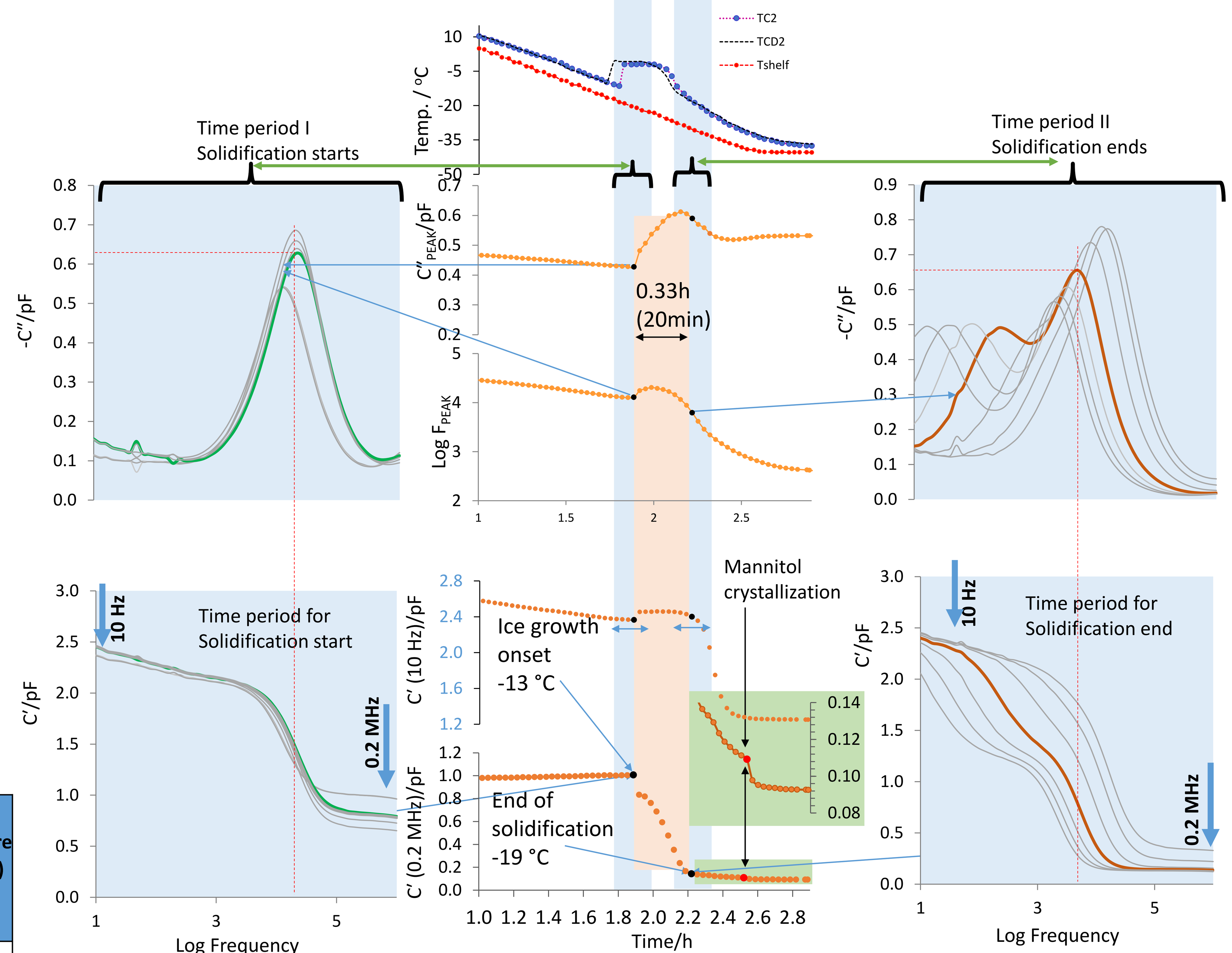


Fig.3 Log F_{PEAK} and C''_{PEAK} with respect to time depict the events that happened 6 min before and after ice growth onset and during the solidification end point. Spectra around the two major events in the freezing process could assist for more understanding of the happenings during freezing process. In addition, capacitance spectra at lower frequency (10 Hz) and higher frequency (0.2 MHz) show the temperature dependence in the lower frequencies.

- Real part capacitance shows response due to ice solidification from its onset to the end of the solidification period
- Lower frequencies are temperature dependent
- Unfrozen concentrate continued to respond to electric current (see fig.3 gradient from 2.2h-2.52h) until mannitol crystallised at 2.52 h

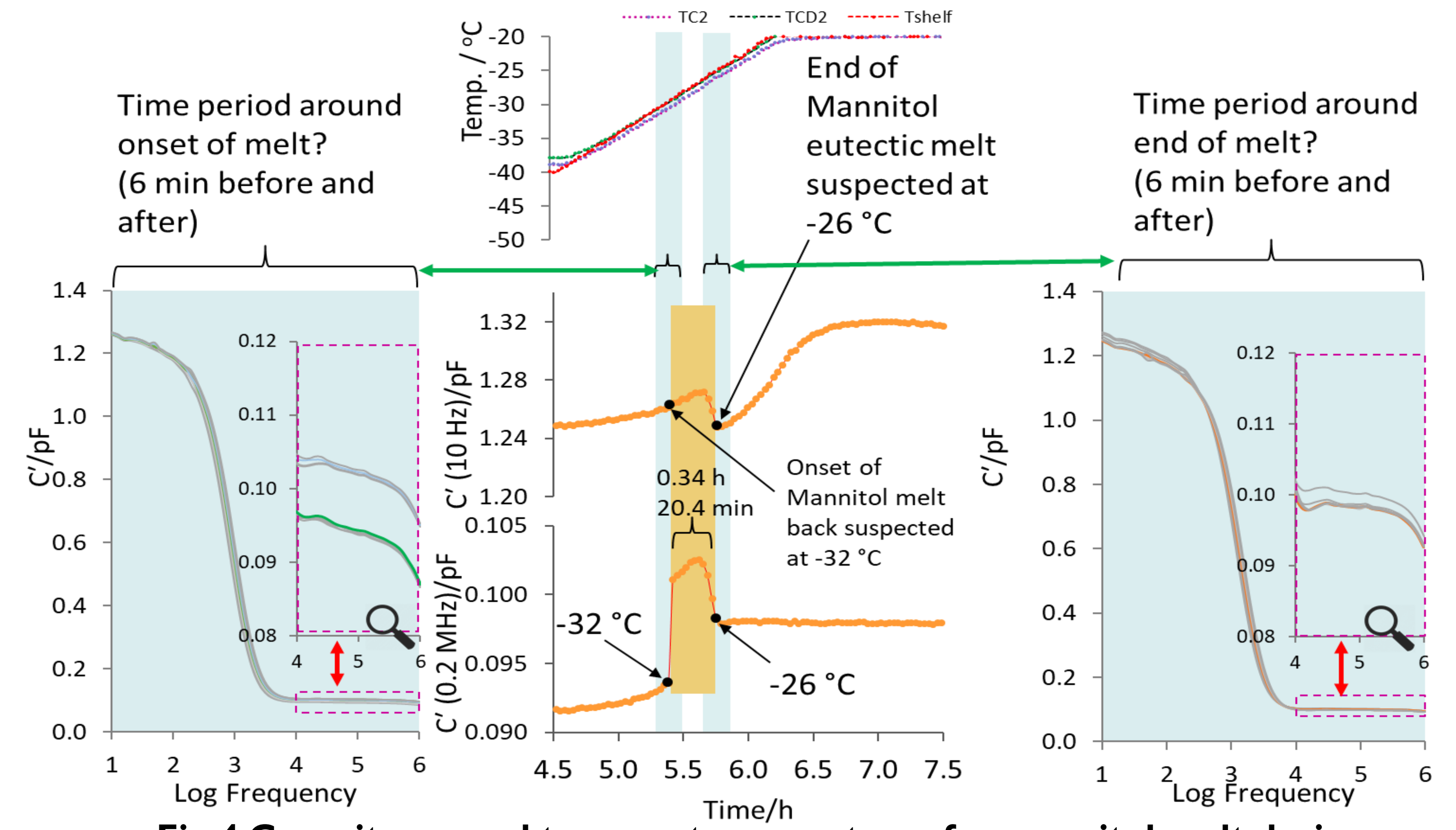


Fig.4 Capacitance and temperature spectrum for mannitol melt during re-heating period

- Fig.3 shows crystallization of mannitol. The black dotted line in fig.3 at 2.52 h sits on the point of crystallization where system experience exotherm. Temperature at this point was -32 °C.
- The change in gradient with time/ temperature after the end of solidification and before crystallization point supports the idea of TVIS response to events due to unfrozen fraction.
- Mannitol crystallization set in at 2.52 h evidenced by a step down in capacitance just 40 min from ice formation onset as shown in fig.3.
- Fig.4 shows TVIS response to the phase behavior of mannitol during re-heating process.
- Melting onset was detected in high frequency at -32 °C, but both the low and high frequencies agreed to the melt-back endpoint at -26 °C.
- Dielectric property of the TVIS vial and contents at 10 Hz is temp. dependent, the frequency is good for demonstrating the changes in temperature during freezing.
- But the dielectric properties at 0.2 MHz are dominated by the properties of the solution and insensitive to ice temperatures, hence good for determining the end of ice formation.
- Duration between the onset of ice growth and the solidification end-point is 20 min while the ice growth onset temperature is -13 °C.

4. CONCLUSIONS

- TVIS has demonstrated ability as an efficient non-invasive and real time PAT tool for determination of ice growth, crystallization and melting back of mannitol in aqueous solution during lyophilization.

5. SIGNIFICANCE

- In process development, freezing characteristics of materials are important as it impact process outcome
- Prediction of freeze drying parameters at the early stage of the process can inform decision making for production
- This investigation employed TVIS system to confirm thermal transformation events of mannitol in sub-ambient condition

REFERENCE

Smith et al (2018) Eur J Pharm & Biopharma Vol 130, pp 224-235