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

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Abstract: Studies of the freezing behavior of aqueous solutions of mannitol are highly relevant to lyophilization process development given the prevalent use of this excipient. Here, through-vial impedance spectroscopy (TVIS) was used to study the behaviour of an aqueous solution of 5%w/v mannitol during a freezing and re-heating cycle. Temperature calibration of the TVIS parameter $\boldsymbol{F_{PEAK}}$ enabled the determination of the ice nucleation temperature $\boldsymbol{T_n}$ at −13 °C while the ice solidification end point was observed based on the time profile of $\boldsymbol{C'(0.2MHz)}$, i.e. the real part capacitance at 0.2 MHz. A time difference of 20 min between the onset and end point then defines the ice solidification time $\boldsymbol{t_i}$. A later step in $\boldsymbol{C'(0.2MHz)}$ indicated that mannitol crystallized at -32 °C and 20 minutes from end of ice solidification. Upon reheating at 0.2 °C/min, a large increase in $\boldsymbol{C'(0.2MHz)}$was seen at −32 °C indicating the onset of melting of mannitol crystals which then lasted 20 min.

Introduction: The initial freezing step, and any subsequent thermal treatment (annealing) of mannitol-containing formulations are critical to the efficiency and outcomes of a lyo cycle; given that the effective use of mannitol (as a bulking agent that improves the mechanical strength of the cake) necessitates the crystallization of this compound during the initial freezing or subsequent annealing stages. Laboratory studies of aqueous mannitol solutions (DSC, CSM and XRD) observed ice formation and mannitol crystalization at −20 °C and −30 °C respectively, and a melting endotherm of mannitol at −30 °C on re-heating[1]. Whether this behavior is replicated in the larger volume of a freeze-drying vial is the focus of this study.

Method: Through-vial impedance spectroscopy recorded the real and imaginary capacitance spectra of a 10 mL glass tubing vial, with a pair of 10x18 mm electrodes at 3 mm from the base, and containing 3.5 g of a 5% w/v aqueous mannitol solution. Thermocouple temperatures were recorded in a near neighbor vial. Measurements were taken at intervals of 2 min during a 0.5 °C min^{-1} freezing ramp to -40 °C followed by a 0.2 °C min^{-1} annealing ramp to -20 °C.

Results and Conclusion:

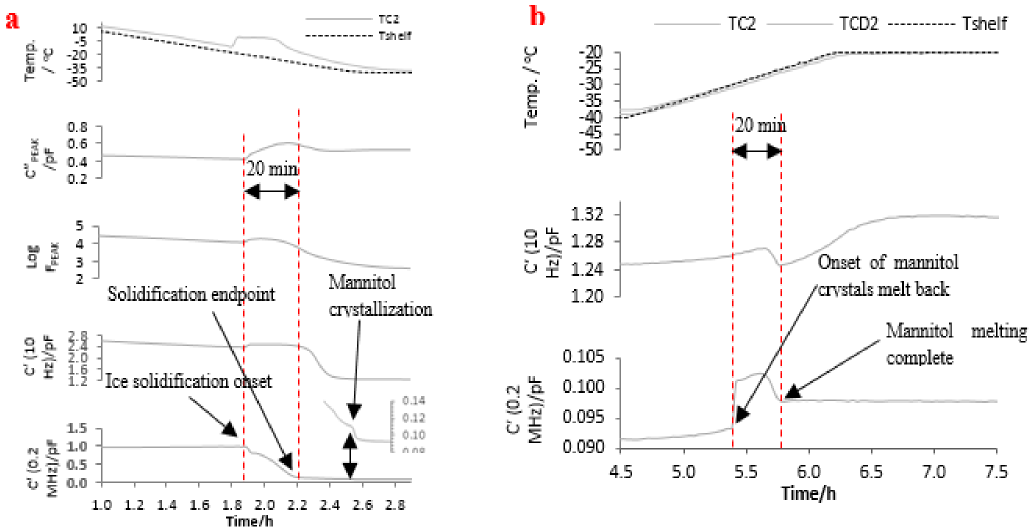

Fig.1 *a) Shows how C″$_{PEAK}$, Log F$_{PEAK}$ and Temperature change with time; events that occur with time. b) Demonstrates C″$_{PEAK}$ response to the melting mannitol crystals during re-heating period.*

Fig. 1a demonstrates that the classic spike in temperature, which is associated with the onset of ice formation, is mimicked by all four TVIS parameters: $\boldsymbol{F_{PEAK}}$, $\boldsymbol{C''_{PEAK}}$, $\boldsymbol{C'(10Hz)}$, and $\boldsymbol{C'(0.2MHz)}$. The explanation is partly due to the temperature spike but mainly due to the dielectric relaxation mechanism changing from the liquid-state Maxwell-Wagner polarization to the solid-state dielectric relaxation of ice. Once ice formation is complete there will be no contribution from the MW process and therefore any further change in temperature (from the loss of the excess latent heat of ice crystallization and the progressive cooling of the shelf) will be reflected in the dielectric parameters of ice and the unfrozen fraction. However, given that the high frequency dielectric properties of ice are temperature independent then $\boldsymbol{C'(0.2MHz)}$ turns out to be the best TVIS parameter for determining the ice solidification end point (ISE) and any subsequent events, such as mannitol crystallization (observed 20 min after the ISE) and mannitol melt back on re-heating (Fig. 1b). Calibration of the TVIS parameter $\boldsymbol{F_{PEAK}}$ then allows for the temperatures of these events to be recorded, i.e. −13 °C for ice nucleation temperature, and – 32 °C for both the crystallization and the subsequent re-melt of mannitol. $\boldsymbol{F_{PEAK}}$is also a good indicator of the point at which the product achieves thermal equilibrium with the shelf.

References:

[1] V.L. Kett, S. Fitzpatrick, B. Cooper, D.Q.M Craig. An Investigation Into the Subambient Behavior of Aqueous Mannitol Solutions Using Differential Scanning Calorimetry, Cold Stage Microscopy, and X-Ray Diffractometry. J Pharm Sci 92 (2003) 1919–1929.