



# Electrical Impedance Methods for Developing a Lyophilization Cycle

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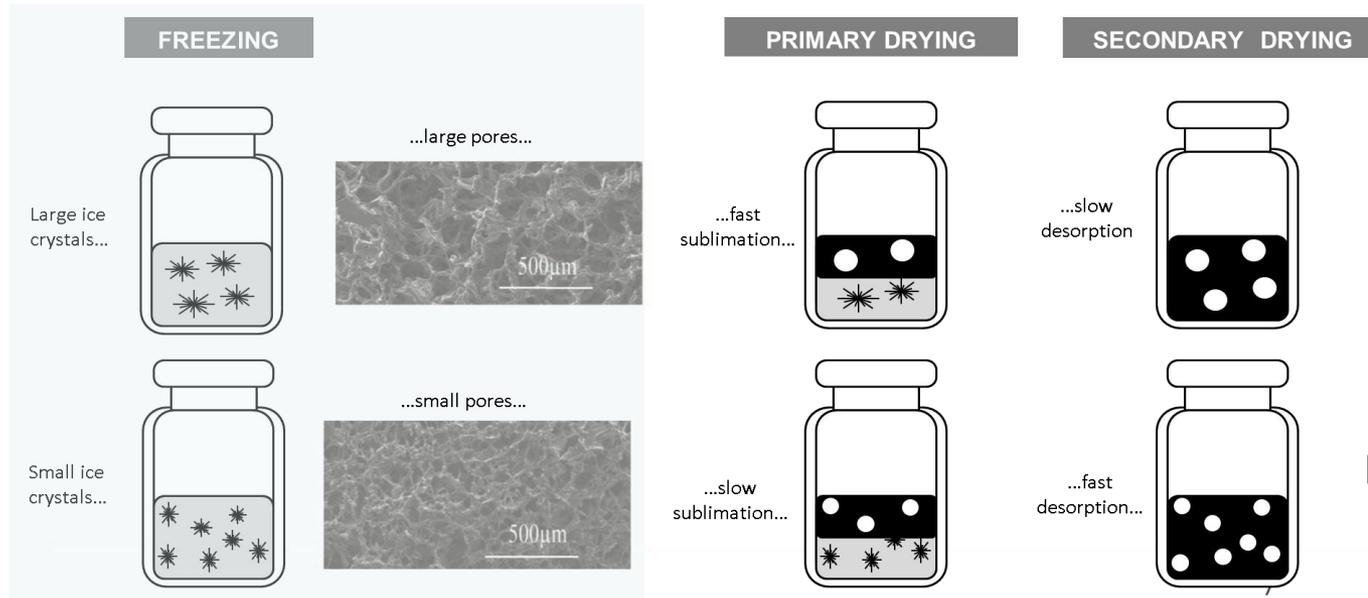
ISLFD 2019 – 9<sup>th</sup> International Symposium on Lyophilization of Pharmaceuticals  
Ghent, Belgium, 2-6 September 2019

# Overview

- Critical parameters in freezing
- On-line impedance spectroscopy (TVIS)
- Dielectric loss / dielectric relaxation processes (liquid to frozen)
- Dielectric loss or dielectric permittivity analysis?
- Dielectric permittivity spectrum: What frequency?
- In-vial determination of .....
  - Ice solidification rate
  - Ice nucleation temperature ( $T_n$ )
  - Eutectic melting ( $T_{eu}$ ) or glass transition temperature ( $T'_g$ )

# Critical Parameters

- Ice crystal structure (defined by freezing process and formulation)
  - Dry layer resistance impacting primary drying rate
  - Surface area of dry later impacting secondary drying rate



B Scutella

- High temperature nucleation and slow cooling favours larger crystals
- Low temperature nucleation and fast cooling favours smaller crystals

# Through Vial Impedance Spectroscopy

## Single Vial PAT



Non-perturbing to packing of vials



Temperature calibration

- using nearest neighbour vial(s)

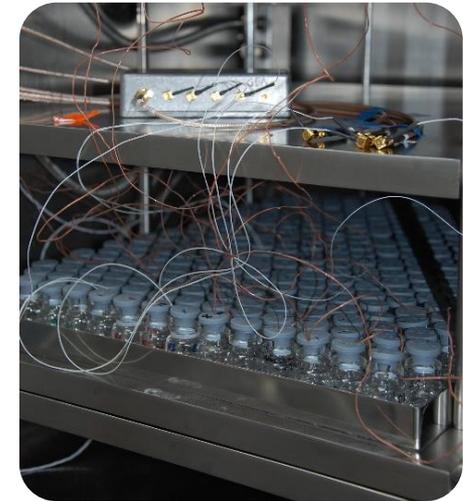


Low thermal mass of electrodes

- no interference with heat transfer & drying rates

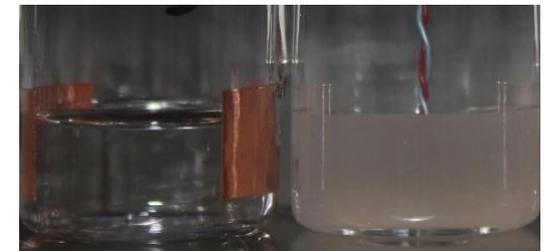


## Multichannel



Thin flexible cables  
(0.5 - 2 m)

- Stoppering unaffected



Non-sample invasive

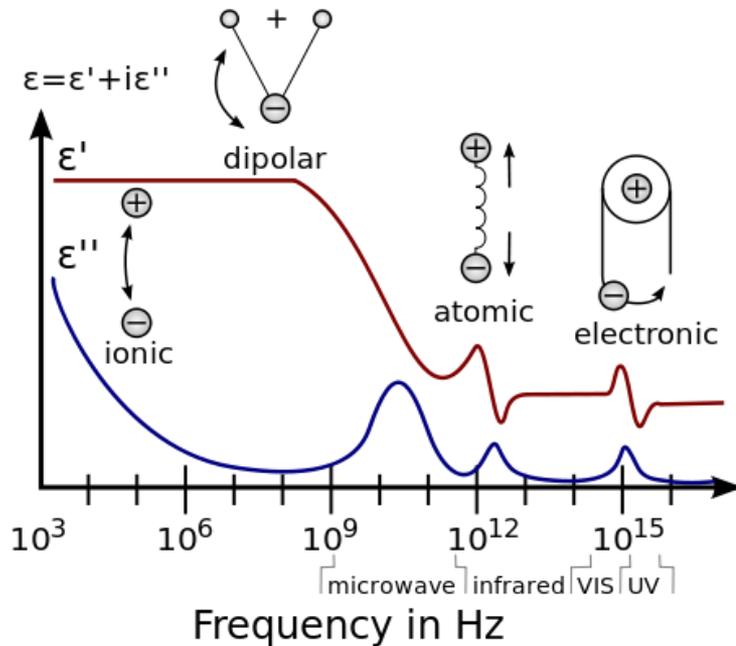
- no impact on ice nucleation

# Through Vial Impedance Spectroscopy (TVIS)

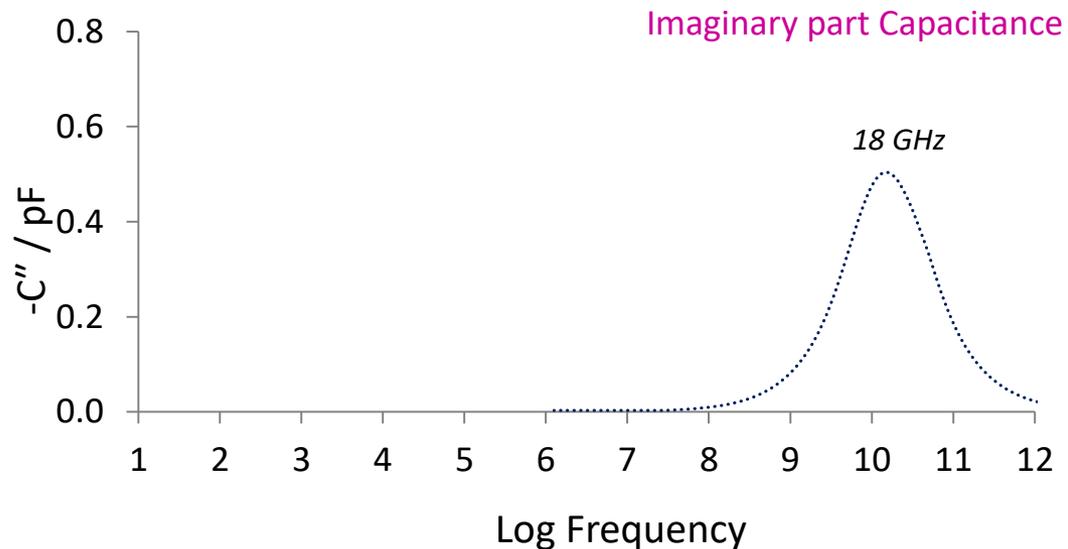
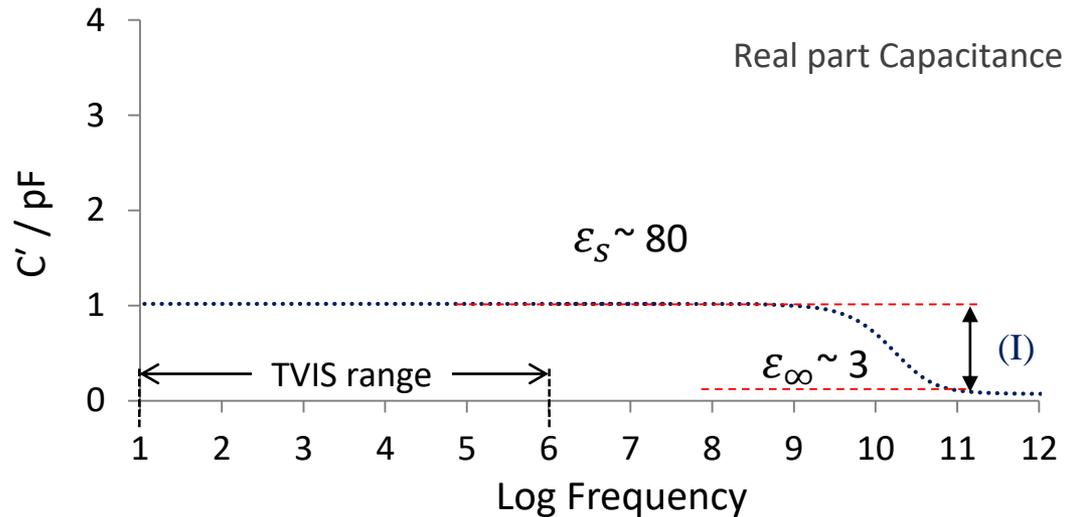
## *Dielectric Loss/Relaxation Mechanisms*

# Dielectric Loss Mechanisms

- The polarization of the water dipole in liquid water at 20 °C, with a dielectric loss peak frequency of ~ 18 GHz



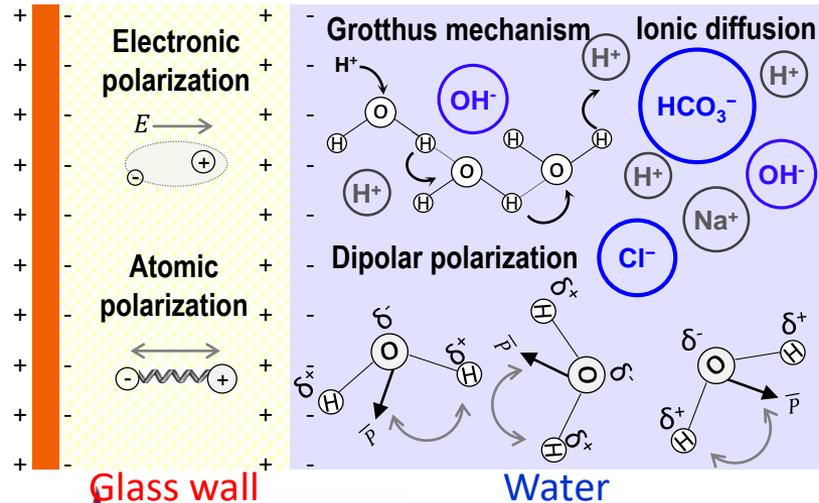
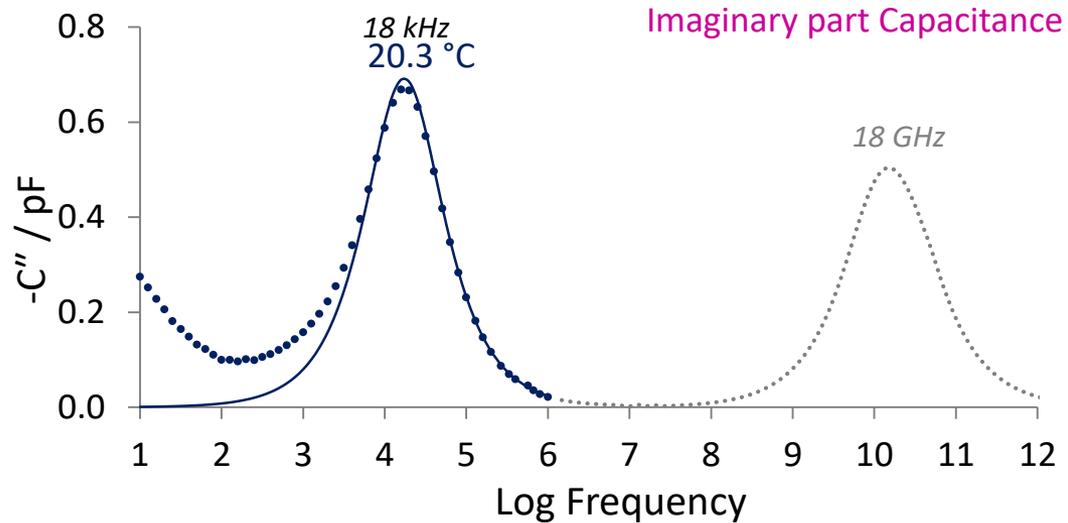
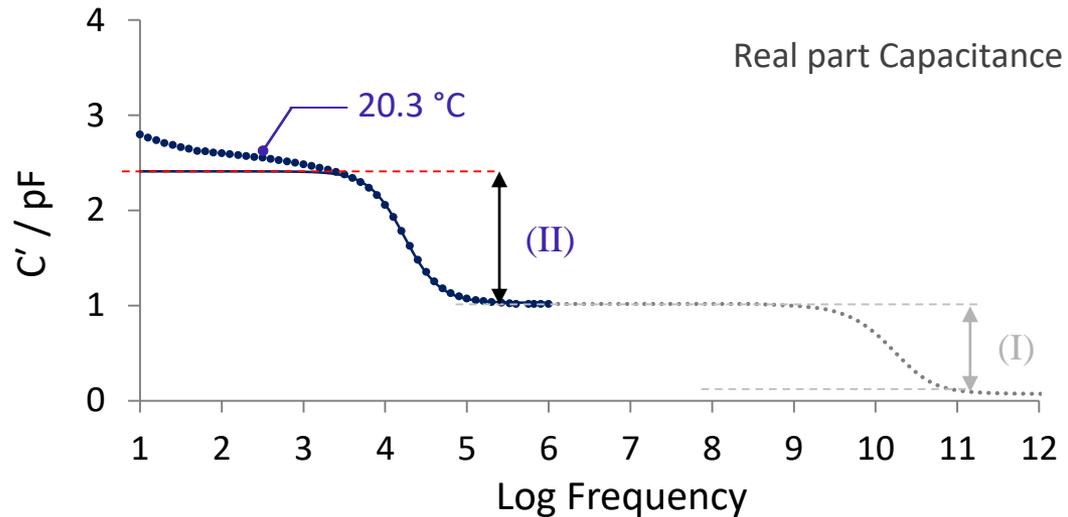
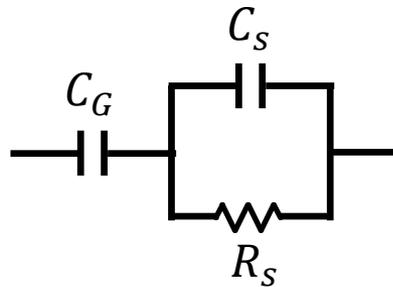
<https://en.wikipedia.org/wiki/Permittivity>



# Dielectric Loss Mechanisms

II. Maxwell-Wagner (MW) polarization of the glass wall of the TVIS vial at +20 °C, with a dielectric loss peak frequency of 17.8 kHz

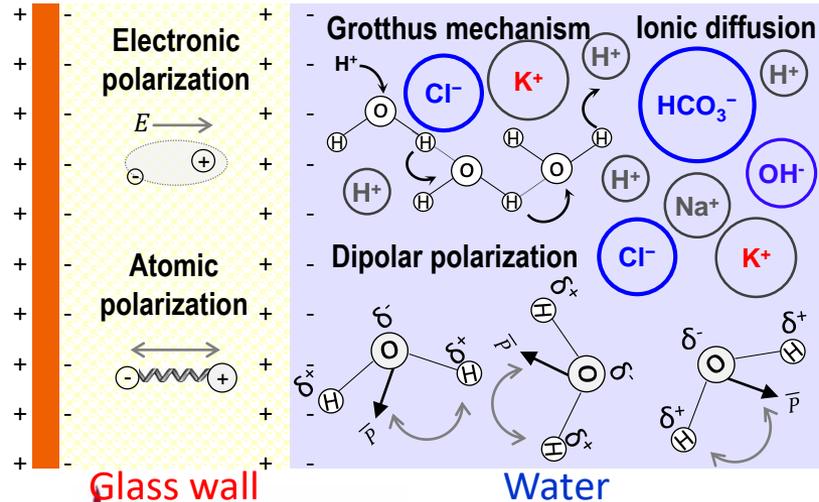
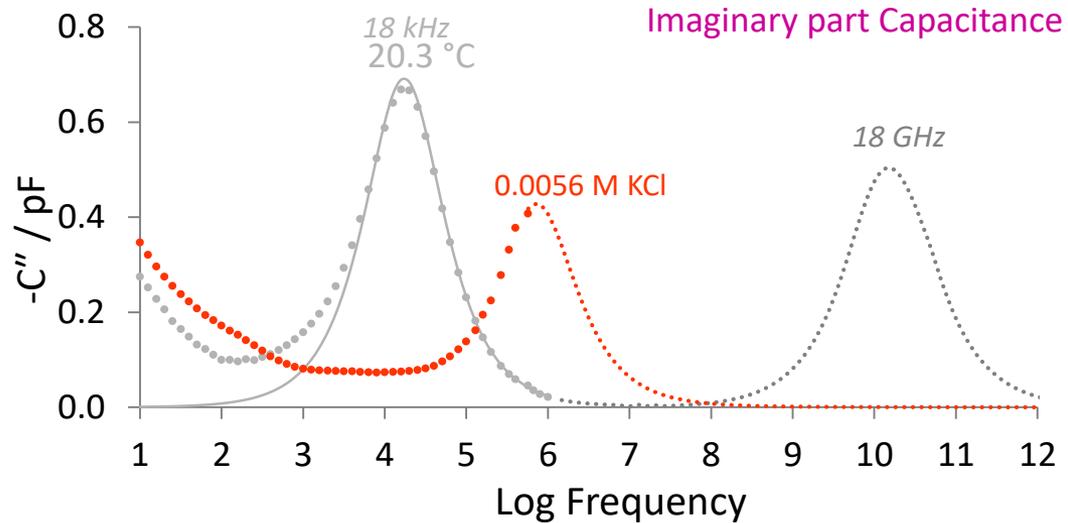
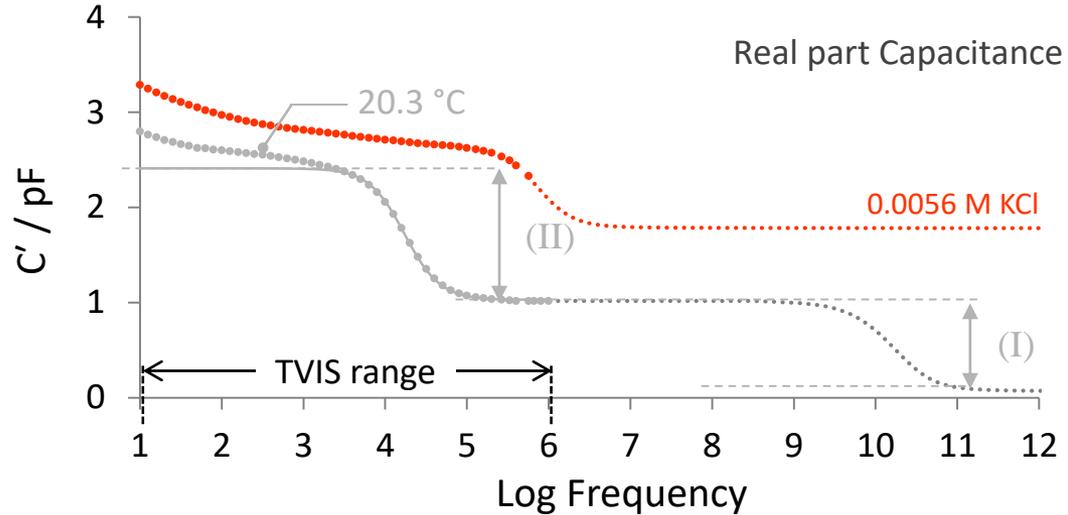
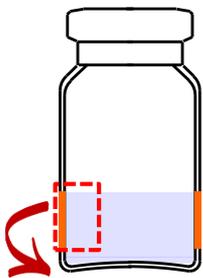
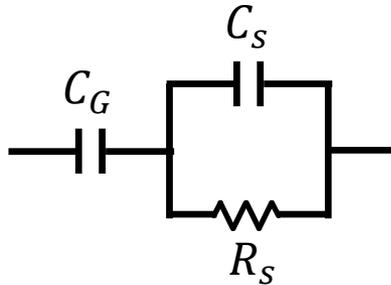
Measurement vial



# Dielectric Loss Mechanisms

II. Maxwell-Wagner (MW) polarization of the glass wall of the TVIS vial at +20 °C, with a dielectric loss peak frequency of 17.8 kHz

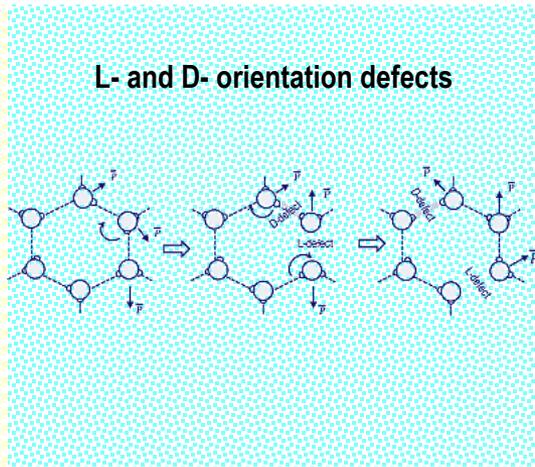
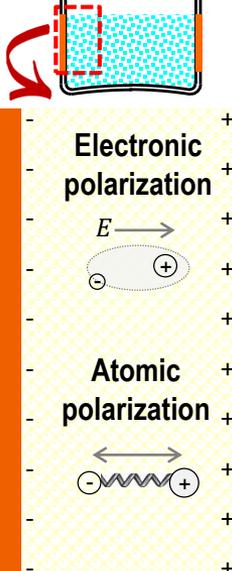
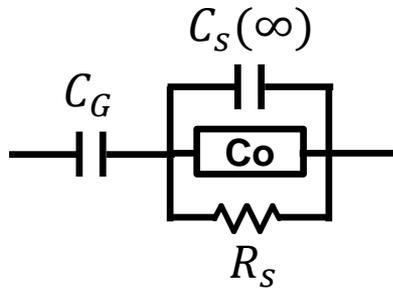
Measurement vial



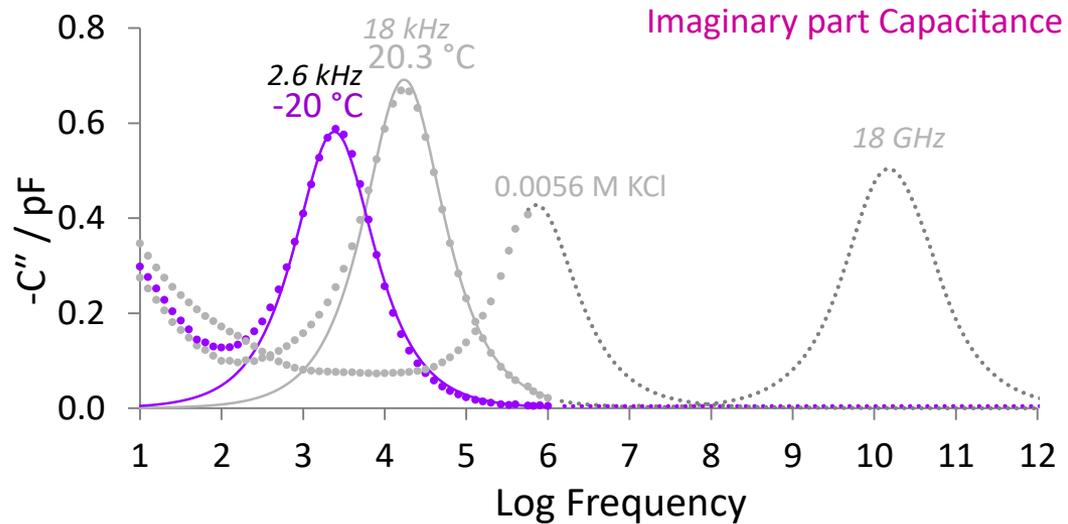
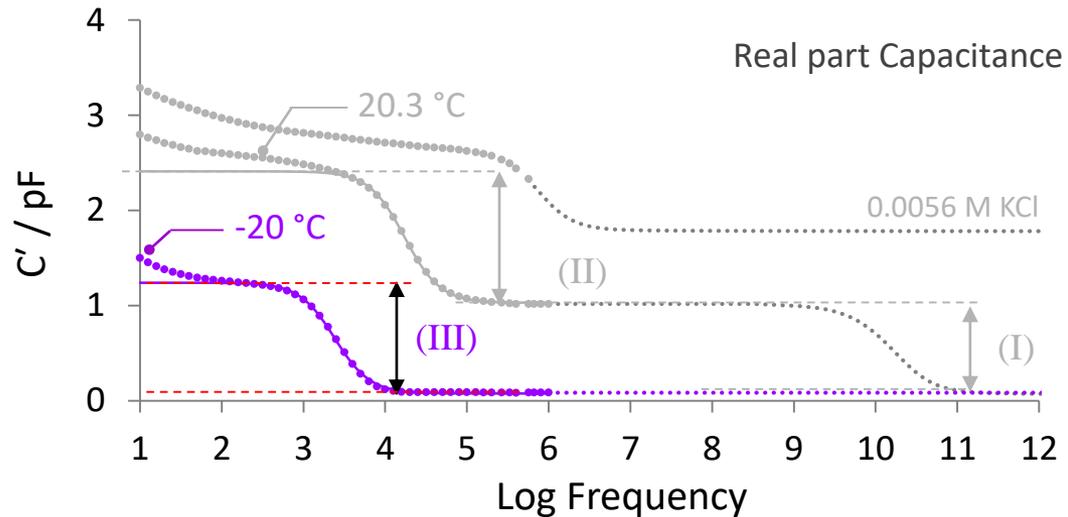
# Dielectric Loss Mechanisms

III. The dielectric polarization of ice at  $-20\text{ }^{\circ}\text{C}$ , with a dielectric loss peak frequencies of 2.57 kHz

Measurement vial



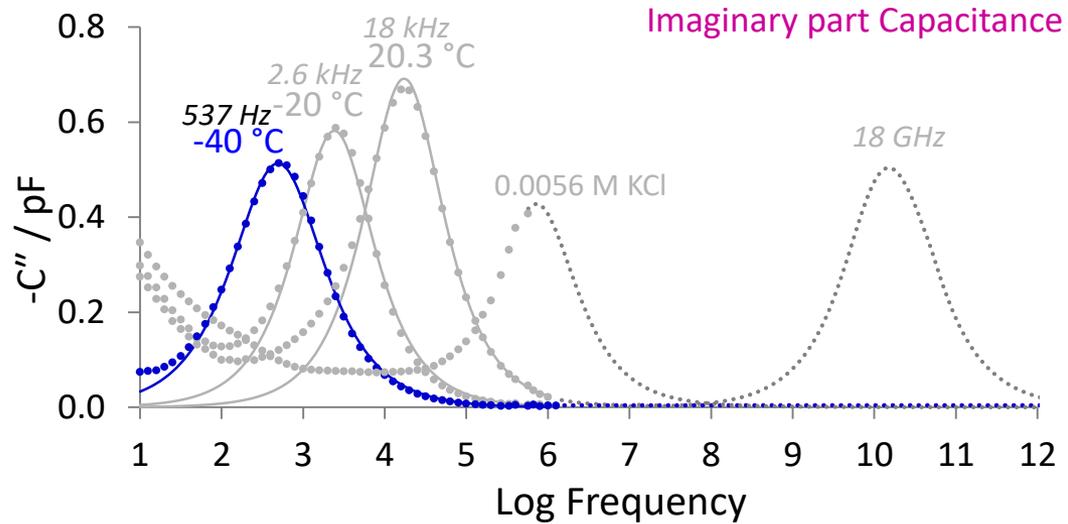
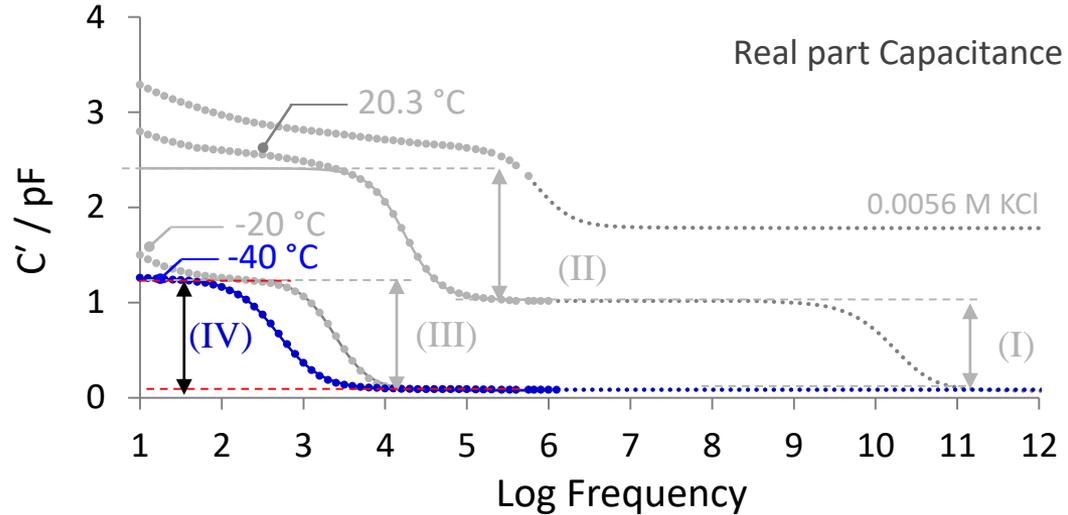
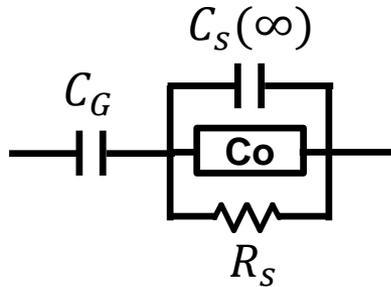
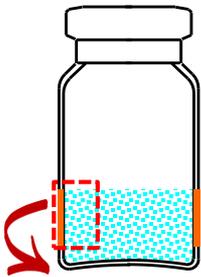
Ice



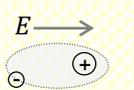
# Dielectric Loss Mechanisms

IV. The dielectric polarization of ice at  $-40\text{ }^{\circ}\text{C}$  with a dielectric loss peak frequencies of 537 Hz.

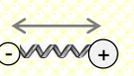
Measurement vial



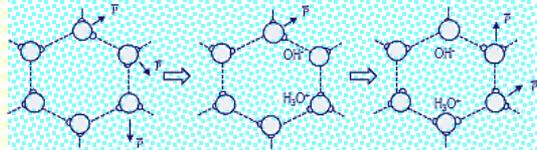
Electronic polarization



Atomic polarization



Ionic defects (similar to Grotthius mechanism)

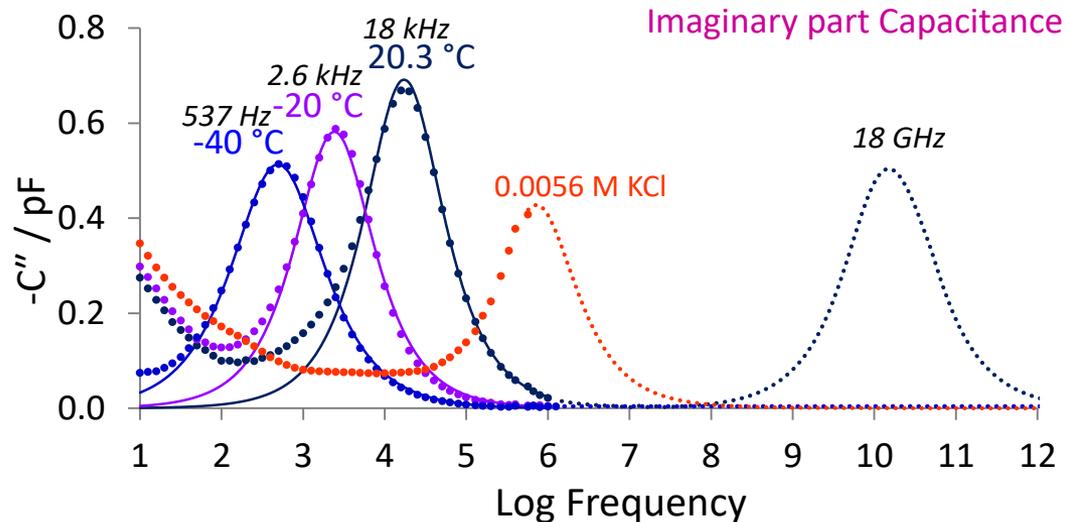
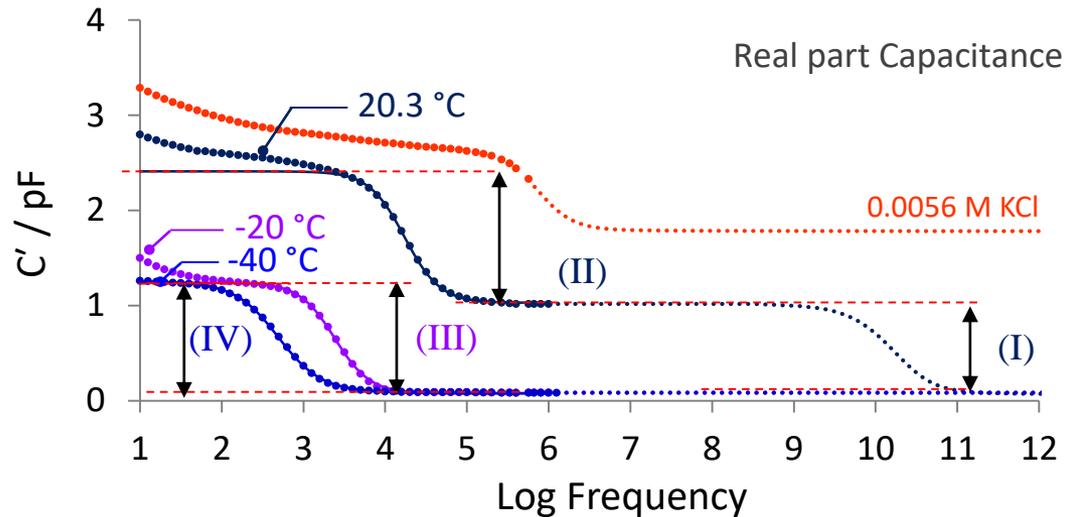


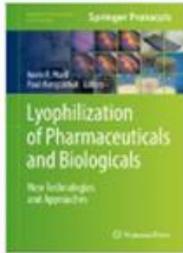
Glass wall

Ice

# Dielectric Loss Mechanisms

- I. The polarization of the water dipole in liquid water at 20 °C, with a dielectric loss peak frequency of ~ 18 GHz
- II. Maxwell-Wagner (MW) polarization of the glass wall of the TVIS vial at +20 °C, with a dielectric loss peak frequency of 17.8 kHz
- III. The dielectric polarization of ice at -20 °C, with a dielectric loss peak frequencies of 2.57 kHz
- IV. The dielectric polarization of ice at -40 °C with a dielectric loss peak frequencies of 537 Hz.





[Lyophilization of Pharmaceuticals and Biologicals](#) pp 241-290 | [Cite as](#)

## Through Vial Impedance Spectroscopy (TVIS): A Novel Approach to Process Understanding for Freeze-Drying Cycle Development

Authors

[Authors and affiliations](#)

Geoff Smith , Evgeny Polygalov

- Introduction to TVIS theory
- Description of the measurement principles
- Dielectric loss and relaxations mechanisms (liquid and frozen states)

# Through Vial Impedance Spectroscopy (TVIS)

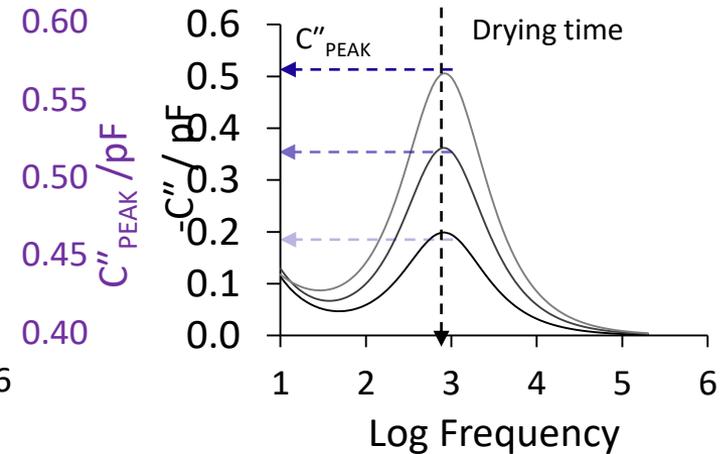
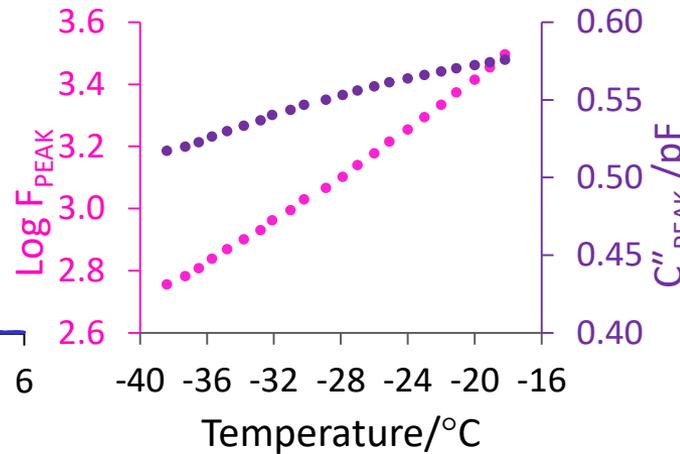
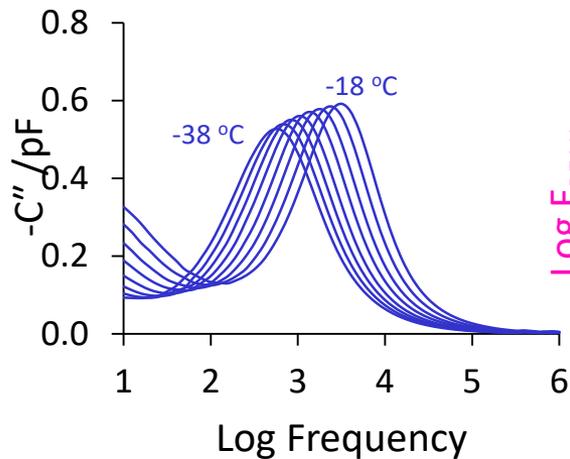
*Dielectric loss or dielectric permittivity analysis?*

# Applications for the dielectric loss spectrum

$F_{PEAK}$  temperature calibration for **predicting temperature** of the product in primary drying

Temperature compensation for  $C''_{PEAK}$  prior to determination of drying rate

**drying rate** from  $\frac{dC''_{PEAK}}{dt}$



These concepts were used in our recent paper :

Smith, G., Jeeraruangrattana, Y., Ermolina, I. (2018). The application of dual-electrode through vial impedance spectroscopy for the determination of **ice interface temperatures, primary drying rate** and **vial heat transfer coefficient** in lyophilization process development. European Journal of Pharmaceutics and Biopharmaceutics.

# Applications for dielectric permittivity spectrum

- $C'$  (~ 100 kHz) is highly sensitive to low ice volumes
- To date we have been using that for the determination **end point** of primary drying. # See Conference Poster 11 (Bhaskar et al)
- More recently we have started using the dielectric permittivity spectrum for
  - Ice nucleation temperatures
  - Ice crystallization end-point
  - Glass transition temperature.....” **The focus for the rest of the presentation**”



**Multiplexing Through Vial Impedance Spectroscopy (TVIS) with Comparative Pressure Measurement**  
for the Determination of the Primary Drying Endpoint of Immunoglobulin (IgG)

ISLFD 2019 – 9<sup>th</sup> International Symposium on Lyophilization of Pharmaceuticals, September 2-6, 2019, Ghent, Belgium

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<sup>2</sup>National Institute of Biological Standards and Control (NIBSC), Pottery Dar, United Kingdom



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**Introduction**

- Attaining a long-term stability by freeze-drying can be attractive as it can eliminate the use of cold chain for biopharmaceutical products (e.g. proteins).
- By the end of the primary drying stage, when all the ice is removed by sublimation, the shelf temperature is raised above the critical product temperature ( $T_c$  or  $T_L$ ) to allow desorption of unfrozen water, termed secondary drying. However, if the shelf temperature is raised before all the ice is removed, it can lead to collapse or eutectic melt of the product. A precise determination of this primary drying endpoint has been one of the strategies for freeze-drying process control.<sup>1</sup>
- The Pirani pressure sensor, which is more sensitive to the water vapour escaping from the vials, is used with a capacitance manometer (CM) that controls the absolute pressure of the chamber. In practice, one needs to judge by trial and error the point at which the Pirani pressure approaches that of CM, which is taken as the endpoint.<sup>2</sup>
- Single vial techniques (e.g. thermocouples, resistance temperature detectors, etc.) are relatively less expensive and involve inserting some probe into the product to measure the product temperature but it is known that probe containing vials dry faster than the vials without the invasive probes.<sup>3</sup>
- With Through Vial Impedance Spectroscopy (TVIS), which is a process analytical technology that senses the amount of ice in the vial by employing a pair of electrodes externally attached to a single vial (i.e. non-product invasive), it has been possible to determine the endpoint of a sucrose formulation using the imaginary capacitance at 1 kHz.<sup>4</sup>

**Aim and Objectives**

The aim of this study is to develop an impedance-based methodology to determine the primary drying endpoint with the following objectives:

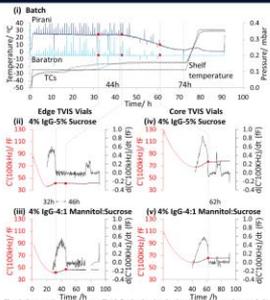
- to use the time-line of the real capacitance at 100 kHz, i.e.  $C'(100\text{kHz})$  for a complex protein formulation located at the edge and the core of the batch
- to compare the endpoint from TVIS with the endpoint given by the comparative pressure measurement

**Materials and Methods**

- A batch of 308 x 5 mL vials (Adephi VC-005-20C) were filled with 3 g of 20 mM Histidine Buffer and 0.01% Tween 20 pH 6.5, containing either (i) 4% IgG with 5% sucrose; (ii) 4% IgG with 5% of a 4:1 mannitol:sucrose mixture; or (iii) their placebo equivalents.
- Two vials from (i) and two vials from (ii) were modified with copper electrodes (19 mm by 10 mm; copper adhesive tape 1381 3M) attached externally to the glass wall at a distance of 3 mm from the vial baseline.
- One TVIS vial from each IgG containing formulation were placed in the middle of the first row of the edge vials facing the dryer door and the other two TVIS vials were placed in the core. Each TVIS vial was accompanied by two Type T thermocouples placed in the immediate neighbour vials.
- Freeze drying was carried out at NIBSC in a Telstar Lyobeta 15 dryer equipped with Pirani and Baratron<sup>®</sup> Capacitance Manometer pressure sensors and a 5-channel TVIS system (Scopec, Germany).
- The lyo cycle consisted of a freezing ramp from 20 °C to -50 °C at 2 °C/min, two annealing steps (to -15 °C and -28 °C), followed by a 72 h primary drying step at a shelf temperature of -25 °C and finally a secondary drying step at a shelf temperature of 30 °C. The total cycle time for the recipe was approx. 92 h




**Results and Discussion**



- Fig. 3(i) shows the time-lines of the Pirani and Baratron<sup>®</sup> sensors, the shelf temperature and the temperature from the thermocouples during the primary drying and the secondary drying stages.
- Fig. 3(ii) to (iv) show the time-line of  $C'(100\text{kHz})$  on the primary Y-axis for the TVIS vials containing the IgG-sucrose rich and the IgG-mannitol rich formulations that were placed at the edge and the core. Note the values of  $C'(100\text{kHz})$  were averaged over 30 min from the lowest point (referred to as the dip in the time-line) to the end of the plateau is shown as black dotted lines. The first derivative of this rolling average, i.e.  $d[C'(100\text{kHz})/dt]$ , is shown on the secondary Y-axis for each case.
- Pure ice experiments (unpublished data) have shown that the magnitude of  $C'(100\text{kHz})$  decreases as the height of the ice cylinder in intimate contact with the glass wall bounded by the electrodes decreases planar to the vial base. Photographs of the sublimation front of pure ice in an edge vial during primary drying have shown that the point at which  $C'(100\text{kHz})$  reaches its characteristic ‘dip’ is when the ice block has transformed from a cylinder into an ice dome with its diameter approximately equal to that of the vial base. As the sublimation proceeds,  $C'(100\text{kHz})$  then starts to recover as it continues to sense the ice dome which gradually retracts towards the centre of the vial base. Finally, the point at which all the ice has sublimated corresponds to the point when  $C'(100\text{kHz})$  has recovered fully and has reached a plateau, called the TVIS endpoint.
- In this study, it was possible to determine the TVIS endpoint for the complex formulations since we know that  $C'(100\text{kHz})$  is sensitive to the amount of ice in the TVIS vial regardless of other components of the frozen matrix and the vial location. The profile of  $d[C'(100\text{kHz})/dt]$  which changes much more during the recovery compared to the point when  $C'(100\text{kHz})$  reaches a plateau, could then be used as an indicator of the sublimation endpoint.
- The TVIS endpoint for the sucrose-IgG edge vial occurred 12 h earlier than the onset of the reduction in the Pirani vapour pressure (44 h). In addition, the TVIS endpoint for both core TVIS vials occurred at 62 h and yet the Pirani was still active until 73 h. This may be due to one or both of two factors: (i) other core vials were still drying (most probable) and/or (ii) the Pirani continues to sense water vapour in the dryer even when all the ice has sublimated (least probable).

**Table 1. TVIS endpoints for complex formulations at edge and core**

Vial Location	4% IgG with 5% Sucrose	4% IgG with 4:1 Mannitol-Sucrose
Front Edge	15 h	45 h
Core	62 h	62 h

**Conclusion**

TVIS could be used in conjunction with batch measurement sensors to further the understanding of the hold and cold spots of the shelf

**References**

[1] S.M. Patel, T. Doen, M.J. Pihaj, Determination of End Point of Primary Drying in Freeze-Drying Process Control, *Appl Pharmaceut* 11 (2010) 73-84.  
 [2] S.L. Nair, W. Johnson, Methodology for in-process determination of residual water in freeze-dried products, *Dev Biol. Stand.* 74 (1992) 137.  
 [3] S. Nair, S. Thiruvalluvar, E. Sridharan, E. Ramesh, P. Dimarzio, M. Phal, G. Sacha, A. Alessandro, T.R. Thompson, C. Rettec, J. Searles, P. Colucci, Recommended Best Practices for Process Monitoring Instrumentation in Pharmaceutical Freeze Drying –2017, *Appl Pharmaceut* 18 (2017) 2379-2393.  
 [4] G. Smith, E. Polygalov, M.S. Arshad, T. Page, J. Taylor, I. Ermolina, An impedance-based process analytical technology for monitoring the lyophilisation process (2013).

**Acknowledgements**

This project is part of the Atalioo Project. Special thanks to Paul Matejtschuk and team for the freeze-drying run at NIBSC.

# *Dielectric Permittivity Spectrum: What ?*

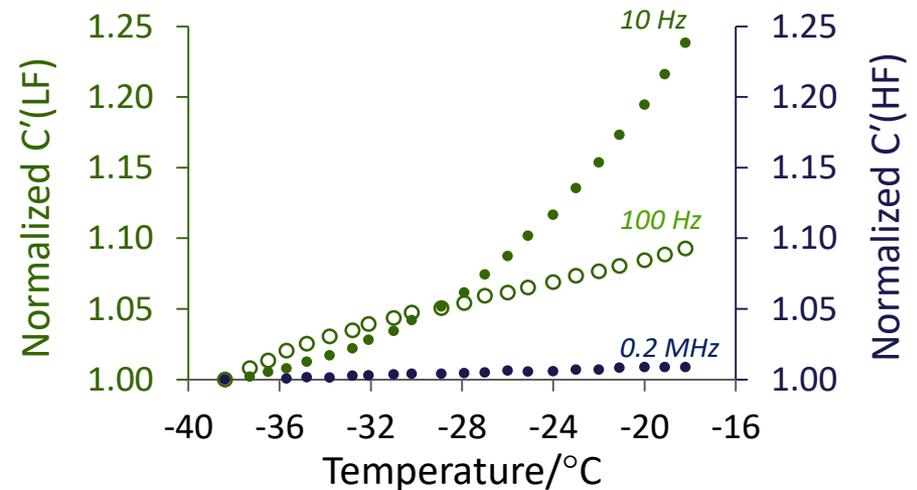
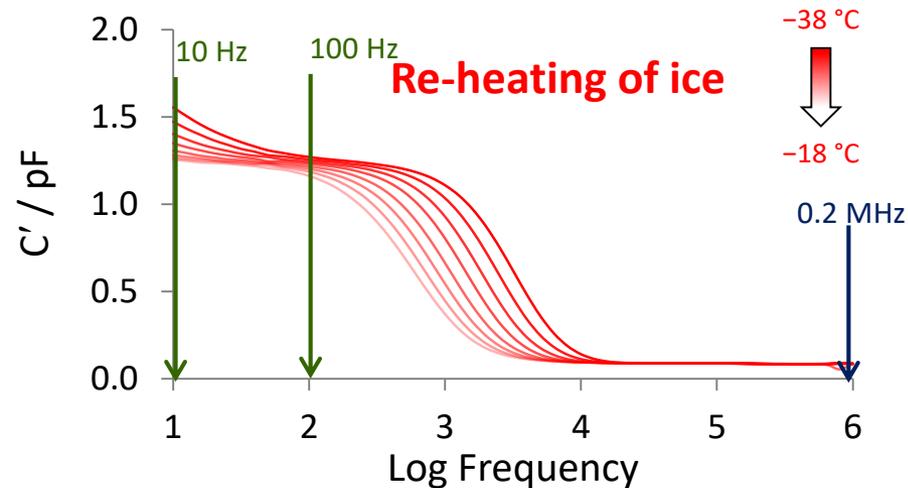
## **Through Vial Impedance Spectroscopy (TVIS)**

*Dielectric Permittivity Spectrum: What frequency?*

# Applications for dielectric permittivity spectrum



- Temperature sensitivity of the real part capacitance (dielectric storage or dielectric permittivity) of the TVIS vial (containing ice) depends on the measurement frequency
- The **low frequency** capacitance is strongly temperature dependent
- The **high frequency** capacitance is weakly temperature dependent



**Significance:** *Optimization of ice crystal structures with larger interconnected crystals increases the porosity of the dry layer, which is the layer that is restricting the diffusion of water vapour from the ice interface*

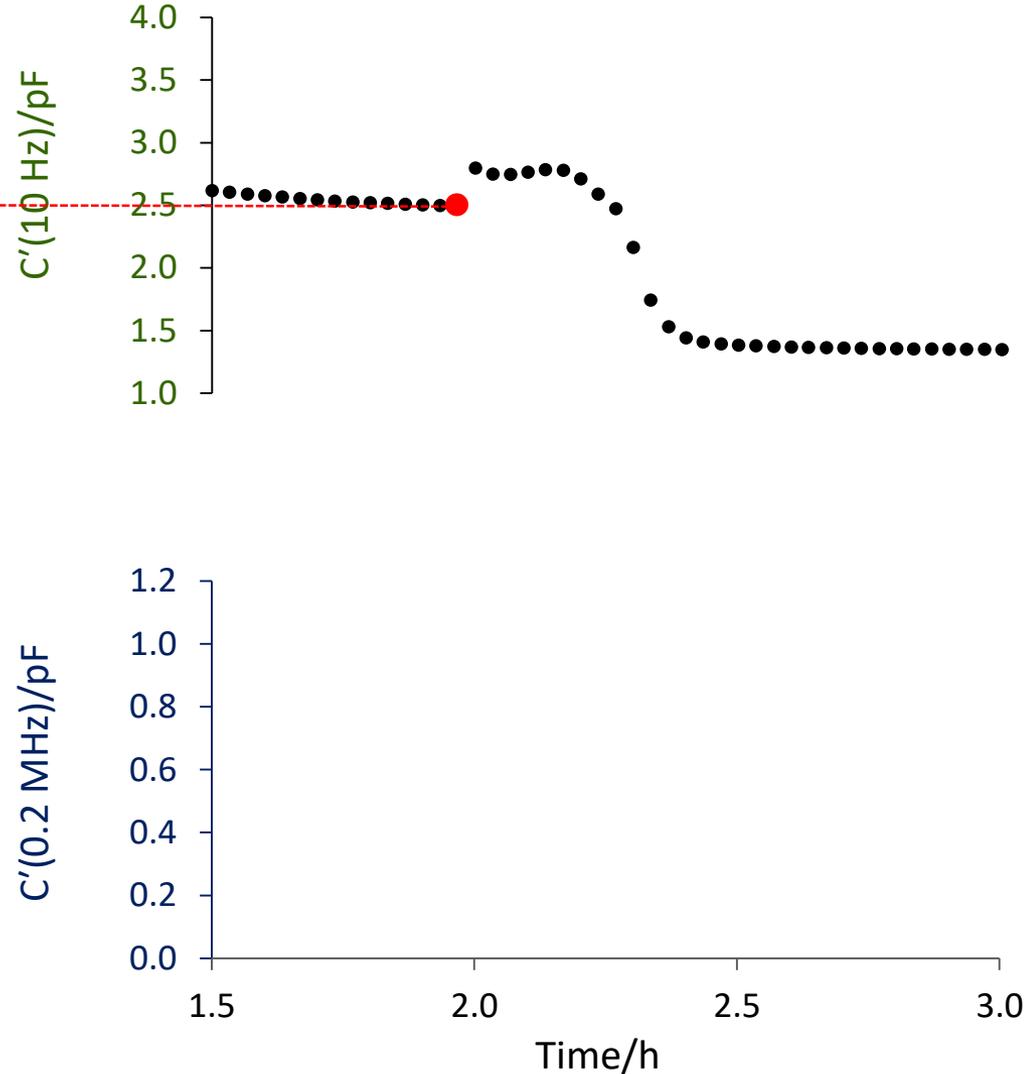
## TVIS Applications

*Ice solidification rate*

# Nucleation onset

Ice nucleation

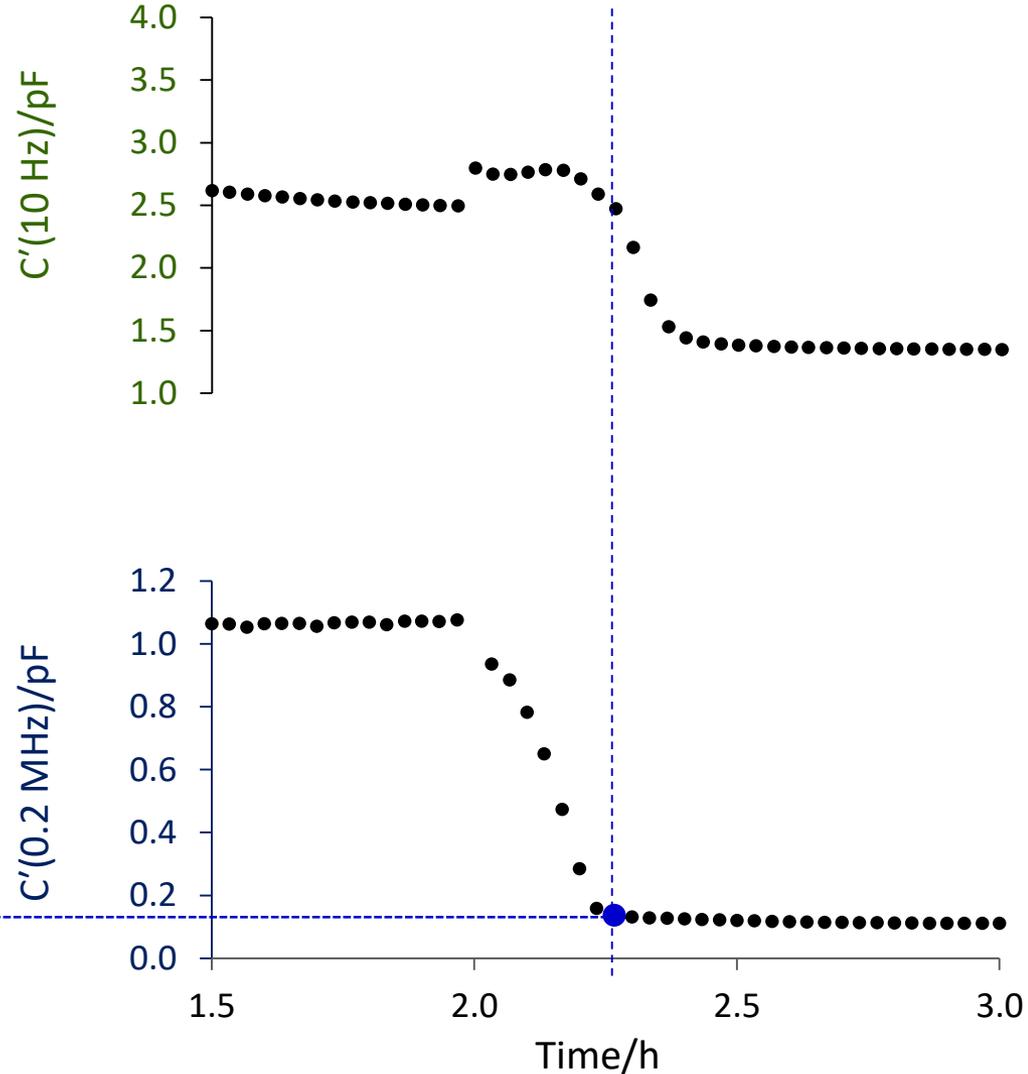
- The capacitance of ice at frequencies below the relaxation frequency of ice (e.g. 10 Hz) is strongly dependent on temperature
- Any changes in  $C'$  @ 10 Hz, either with time or temperature, can be associated with the onset of ice nucleation (which is an exothermic event)



# Ice formation end point

- The capacitance of ice has almost no temperature dependence at frequencies above the relaxation frequency of ice ( $\sim 1$  kHz) such as  $C'(0.2$  MHz).
- Any changes in  $C'$  (0.2 MHz) either with time or temperature, can be associated with the completion of ice formation on freezing

Ice solidification end-point

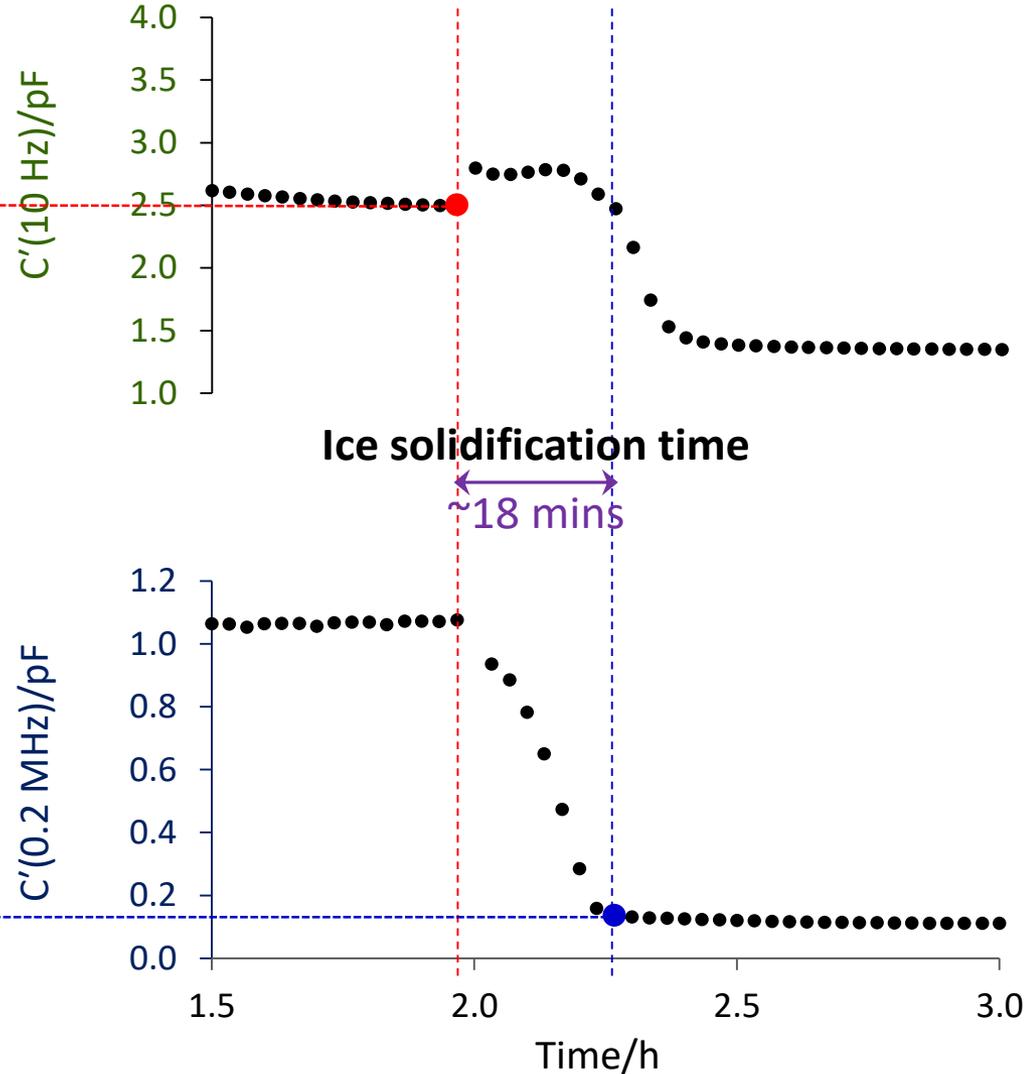


# Ice crystallization period

Ice nucleation

- The difference between these two times if the ice solidification time
- Knowing the height of the product in the vial one can then estimate an average solidification rate

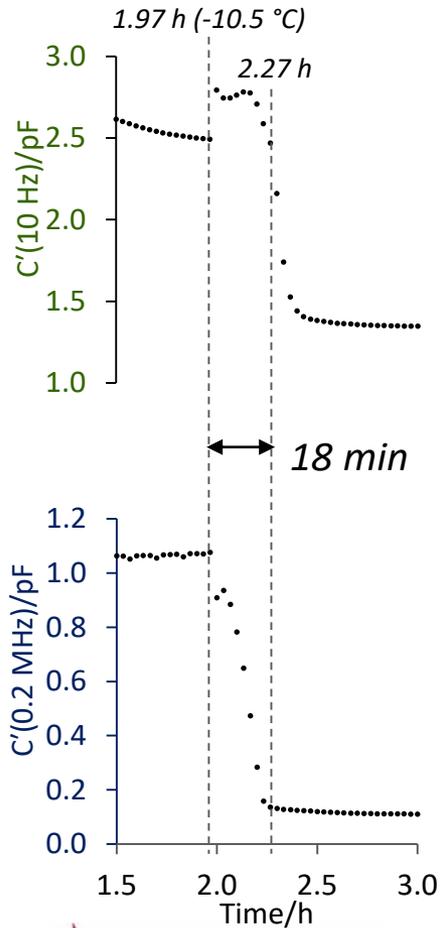
Solidification end point



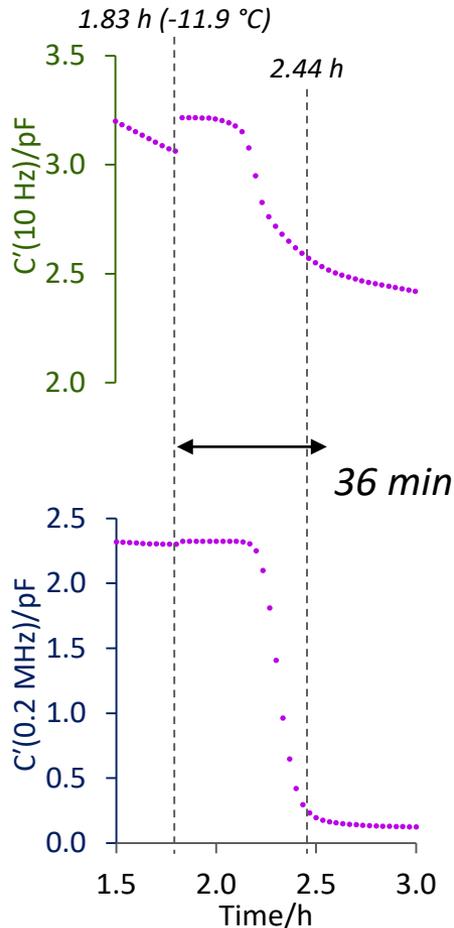
$$\text{Average solidification rate } (R_{av}) = \frac{\text{Ice height } (L)}{\text{solidification time } \Delta t}$$

# Examples (Edge Vials)

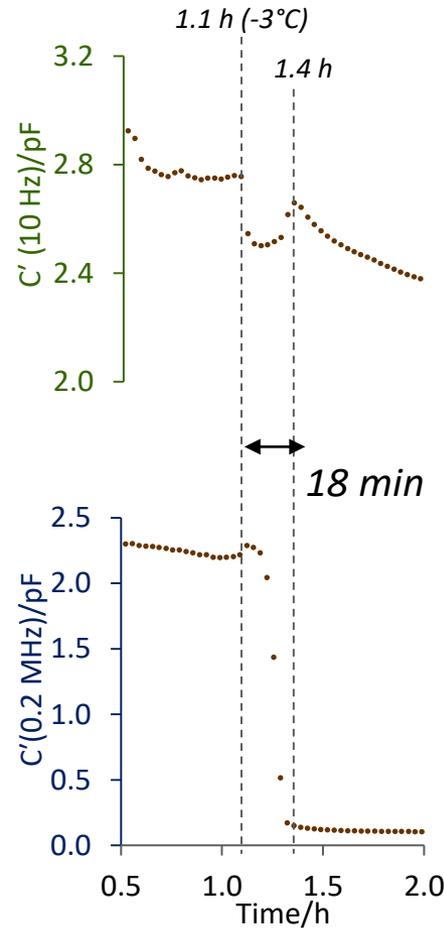
5% Sucrose



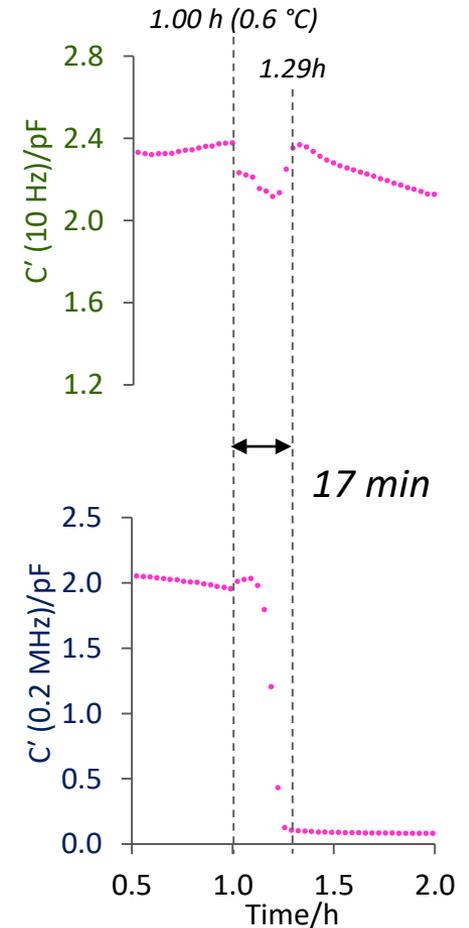
5% Sucrose in  
0.55% NaCl



IgG in  
Mannitol and  
Sucrose based  
formulation



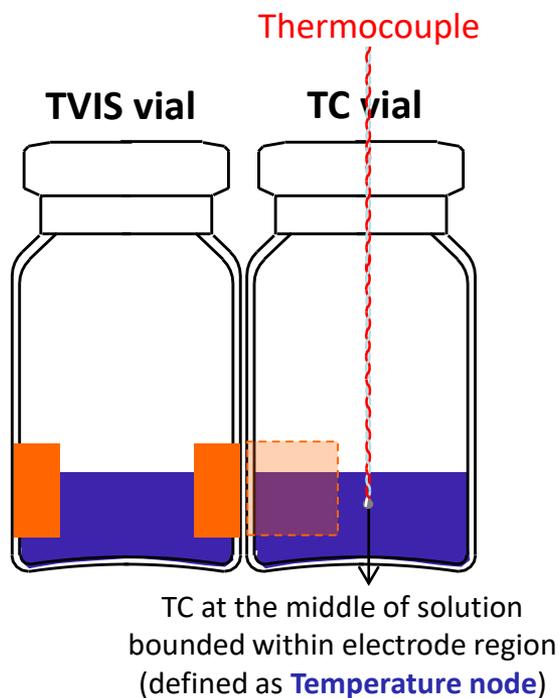
IgG in Sucrose  
based  
formulation



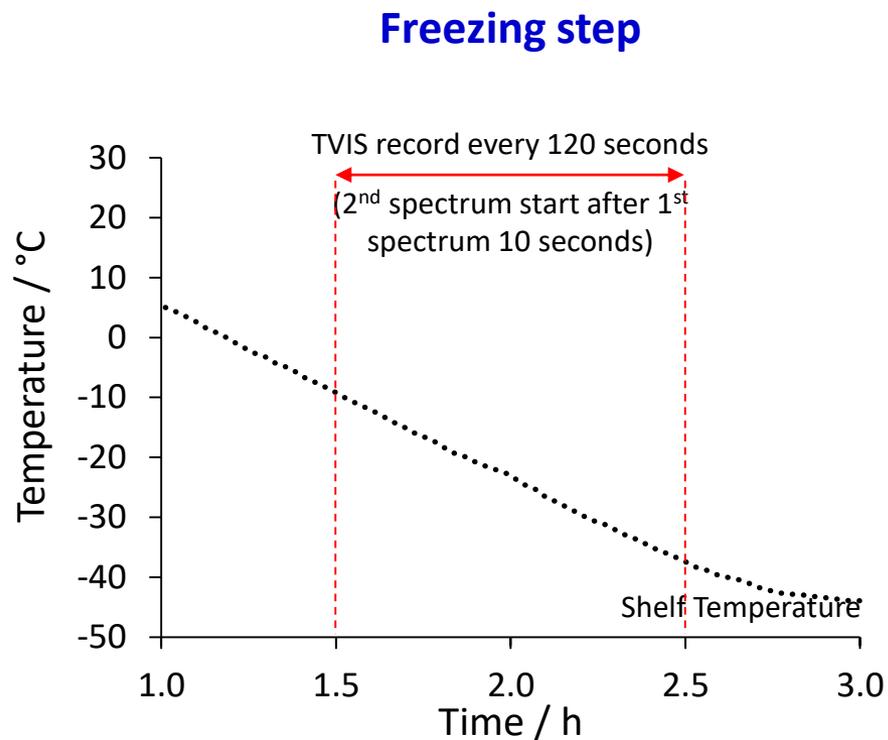
# TVIS Applications

*Determination of Ice Nucleation Temperature ( $T_n$ )*

# Ice Nucleation Temperature



Thermocouple position



Freezing from 20 °C to -45 °C with 0.5 °C/min

## Ice Nucleation Temperature

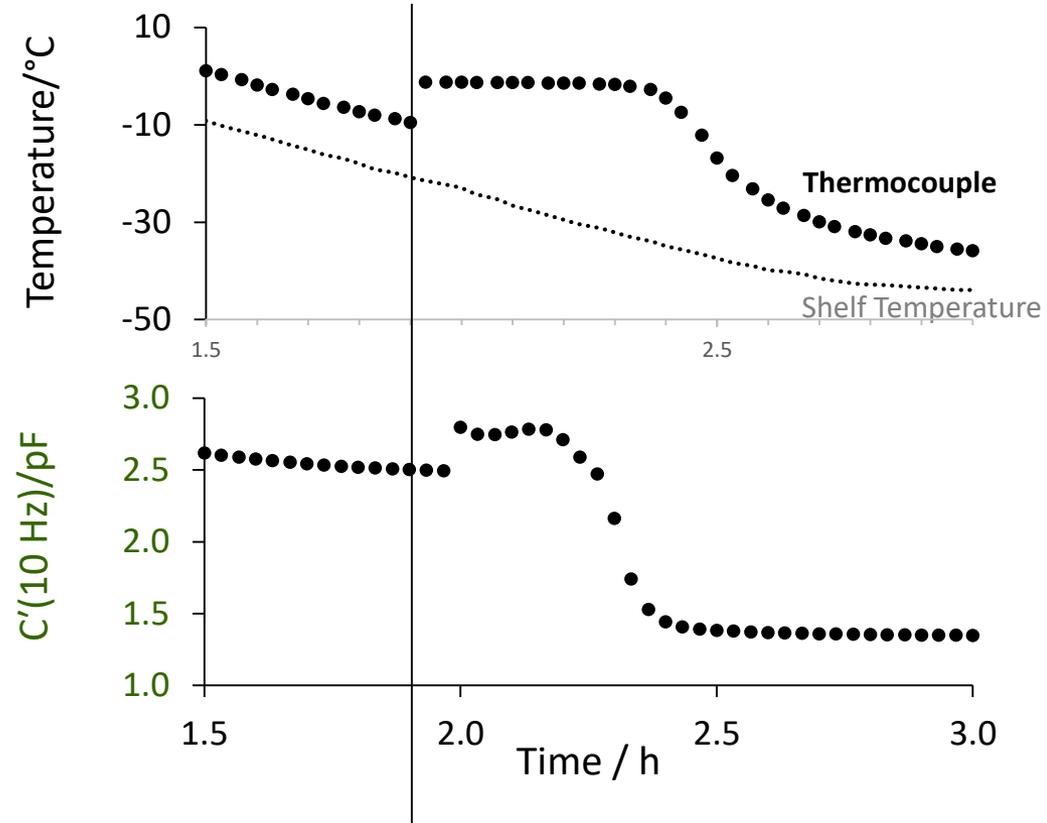
- In case the TVIS vial nucleates before TC vial, the nucleation temperature in the TVIS vial can be inferred directly from TC temperatures in the nearest neighbor vials

Thermocouple

Shelf Temperature

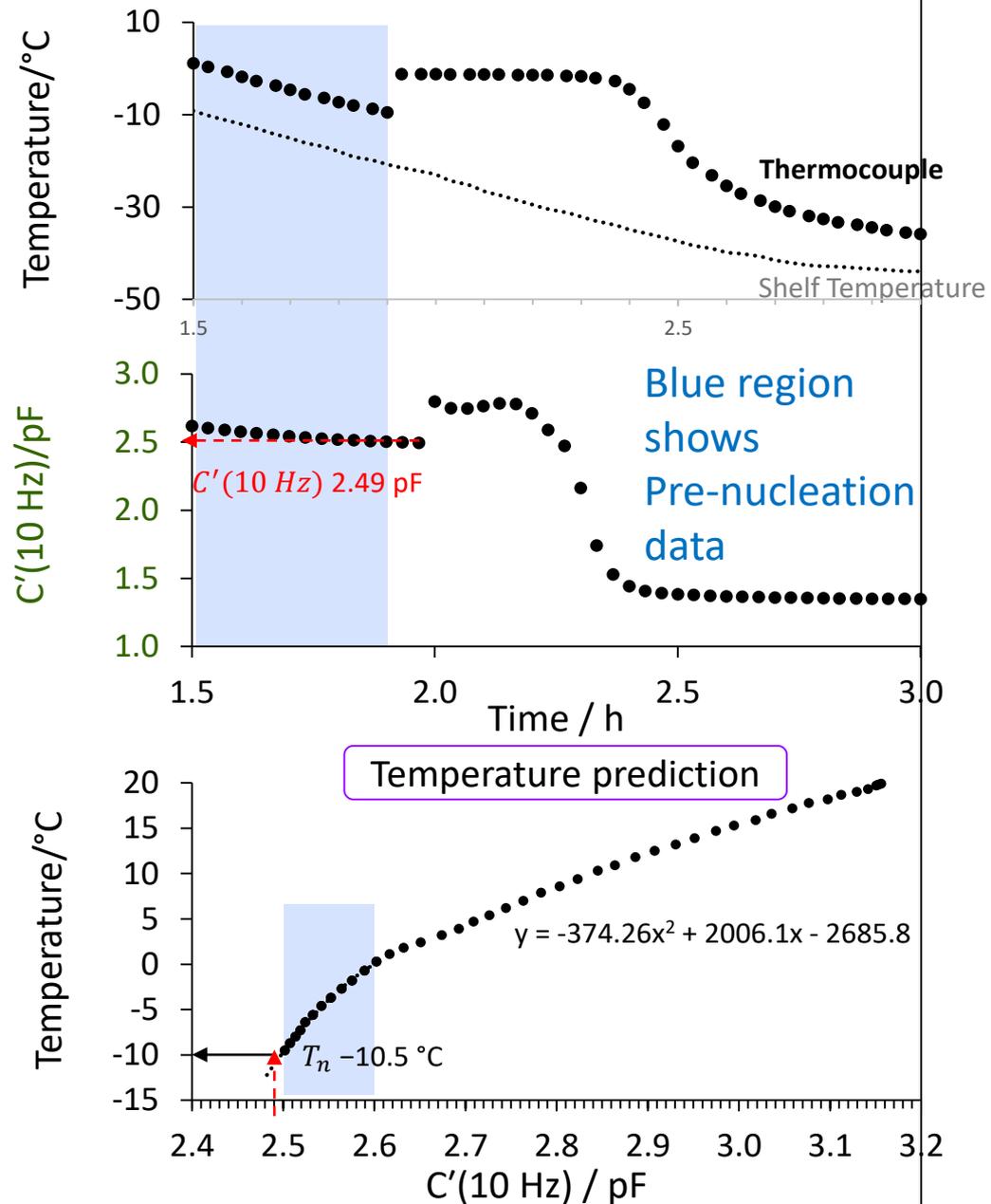
## Ice Nucleation Temperature

- In case the TVIS vial nucleates before TC vial, the nucleation temperature in the TVIS vial can be inferred directly from TC temperatures in the nearest neighbor vials
- However, if TVIS vial nucleates later than TC vial,



## Ice Nucleation Temperature

- In case the TVIS vial nucleates before TC vial, the nucleation temperature in the TVIS vial can be inferred directly from TC temperatures in the nearest neighbor vials
- However, if TVIS vial nucleates later than TC vial, the nucleation temperature can be predicted by fitting a curve to the plot of the average temperature from thermocouple vials against TVIS parameter (i.e.  $C'(10\text{ Hz})$ )
- The ice nucleation temperature of sample (5 %w/v sucrose) was found to be  $-10.5\text{ C}$  in the case of this particular TVIS vial (other vials will differ owing to the stochastic nature of ice formation).



# Conclusions : Ice formation stage

- Ice nucleation onset ( $t_n$ )
  - determined at low frequency (e.g. 100 Hz)
- Ice solidification end point ( $t_s$ )
  - determined at high frequency (e.g. 100 kHz)
- Ice solidification time ( $\Delta t$ ) is the difference between  $t_s$  and  $t_n$
- Average ice growth rate determined by

$$\text{Average solidification rate } (R_{av}) = \frac{\text{Ice height } (L)}{\text{solidification time } \Delta t}$$

- Nucleation temperature ( $T_n$ )
  - determined from extrapolation of pre-nucleation data

# New Book

## Chapter 5 Through Vial Impedance Spectroscopy (TVIS) A New Method for Determining the Ice Nucleation Temperature and the Solidification End point



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# Through-Vial Impedance Spectroscopy (TVIS): A New Approach to Characterizing Phase Transition of Sugar-Salt Solutions

Yowares Jeeraruangrattana, Eugene Polygalov, Irina Ermolina, Geoff Smith  
DMU LyoGroup, School of Pharmacy, De Montfort University, UK  
ISL-FD's 9th International Conference, 2-6 September 2019, Ghent University, Belgium



## INTRODUCTION

The development of a robust freeze-drying product and processes necessitates an understanding of the in-vial characteristics during processing especially freezing stage. The majority of techniques up to date for determining ice nucleation are restricted to the off-line instrument. Through-vial impedance spectroscopy (TVIS) is a relatively new technique which could explore the different facets of the in-situ material behaviour under freezing process (i.e. ice nucleation to solidification end points); however, an TVIS applications for the determination ice nucleation process have been recently restricted to the low-conductivity solutions such as pure water [1].

## AIM

In this study, other features of TVIS system were explored to develop a new approach for determining nucleation process of conductive samples.

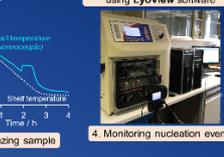
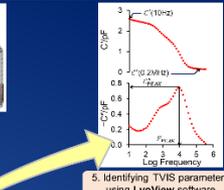
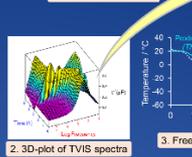
## EQUIPMENTS

Instrument / Sensor	Measurement / Process
TVIS	Electrical capacitance of TVIS vial containing sample measured every 2 min during freezing
Thermocouple	Thermocouple temperature in a nearest neighbour vial provides predictive temperature of TVIS vial (calibration)
VirTis Advantage Plus Freeze-dryer	Freezing from +20 to -45 °C at 0.5 °C min <sup>-1</sup>
Digital camera	Photographic image for observation of ice nucleation event

## MATERIALS & METHODS



Sample	Sucrose (%)	NaCl (%)
S-1	5.00	0.00
S-2	5.00	0.26
S-3	5.00	0.55



1. In-vial measurement, 2. 3D-plot of TVIS spectra, 3. Freezing sample, 4. Monitoring nucleation event, 5. Identifying TVIS parameters using LyoView software

## RESULTS & DISCUSSIONS

Inflections in the time profiles of TVIS parameters [ $C_{TC}^{(10Hz)}$ ,  $F_{Peak}$  and  $C^{(10Hz)}$ ] corresponded with the onset of ice nucleation of 5% sucrose (as confirmed by images) as demonstrate in Fig 1b - 1e.

However, samples having the higher conductivity (5% sucrose with either 0.26% or 0.55% NaCl), the relaxation process before frozen could not be detected by TVIS system (Fig 1i - 1j and Fig 1p - 1q, for 0.26% and 0.55% NaCl respectively). This could be exemplified by the spectrum of liquid state of sugar-salts solution (Fig 1n & Fig 1u) and pure sugar (Fig 1g). Hence, only real part capacitance at 10 Hz was used to indicate the onset of ice formation in a high conductive solution (Fig 1k & 1r).

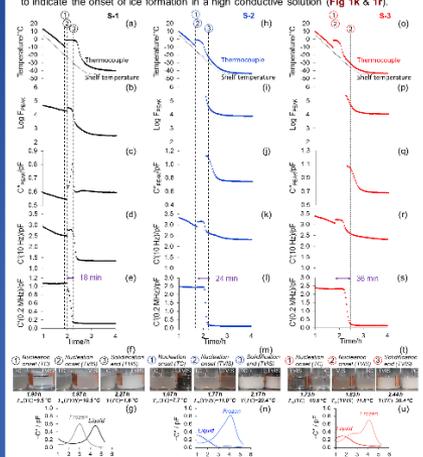


Fig.1 TVIS parameters of 5% sucrose solution with different salt concentrations and images during freezing: (a-g) 0% NaCl, (h-n) 0.26% NaCl, (o-u) 0.55% NaCl

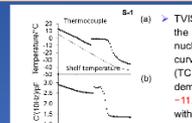


Fig.2 Nucleation temperature of 5% sucrose predicted by TVIS  $C^{(10Hz)}$  [ $T_n$ (TVIS)]

TVIS vial generally nucleate later than TC vial due to the impact of thermocouple probe. In this case, the nucleation temperature can be predicted by fitting a curve to the plot of temperature from neighboring vial (TC vial) against TVIS parameter [i.e.  $C^{(10Hz)}$ ] as demonstrated in Fig 2c, which are -10.5, -11.0 and -11.8 °C respectively for the solutions of 5% sucrose with 0, 0.26 and 0.55% NaCl (Fig 3).

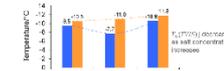


Fig.3 Nucleation temperature of sugar-salt solutions predicted by TVIS parameter  $C^{(10Hz)}$

At 200 kHz or 0.2MHz (which is well above the ice relaxation frequency of 1 kHz), the capacitance of ice has almost no temperature dependence and so any changes in  $C^{(0.2MHz)}$  either with time or temperature, can be associated with the completion of ice formation on freezing (Fig 4b). Here, the end point of solidification for 5% sucrose with 0, 0.26 and 0.55% NaCl were 2.27, 2.17 and 2.43 h, respectively.

By using the time different between nucleation point (Fig 4a) and solidification end point (Fig 4b), ice forming duration was obtained. The results were reported in Fig 5, and also demonstrated a twofold increase in the solidification time as salt concentration increases from 0 to 0.55 %.

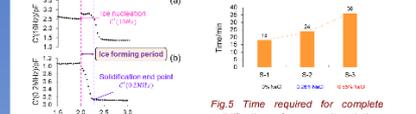


Fig.4 Determination of nucleation period of 5% sucrose by TVIS

## CONCLUSION

TVIS creates a new opportunities to detect phase change during freezing process including the nucleation onset and the solidification end point.

## REFERENCE

[1] Smith, G., Polygalov, E., Arshad, M.S., Page, T., Taylor, J., Ermolina, I., 2013. An impedance-based process analytical technology for monitoring the lyophilisation process. Int. J. Pharm., 449, 72-83.

# Jeeraruangrattana et al # Poster 10

ice formation time and nucleation temperature for Sucrose + NaCl solutions



<https://doi.org/10.21253/DMU.9771512>

**Significance:**  $T'_g$  or  $T_{eu}$  underpins/defines the collapse temperature which in turn defines the highest permissible product temperature during primary drying and therefore impacts the maximal achievable drying rate

## TVIS Applications

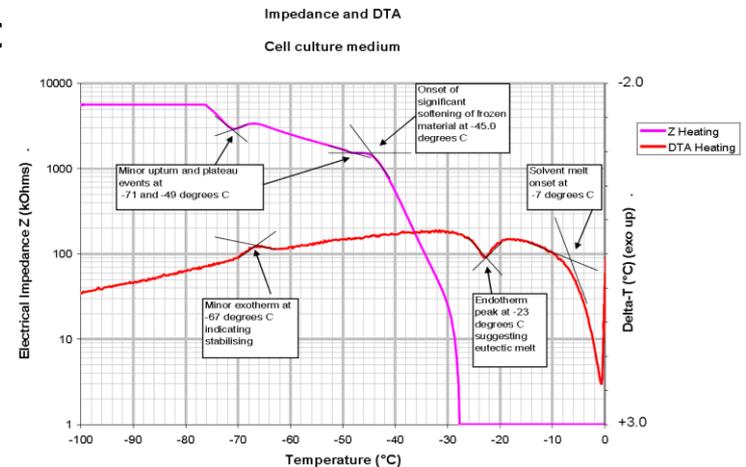
*Determination of in-vial  
Eutectic melting ( $T_{eu}$ ) or  
Glass Transition temperature ( $T'_g$ )*

# PAT for critical temperature determination $T_m$ , $T_e$ , $T_g$

- Collapse temperature (defined by formulation and related to  $T_{eu}$ ,  $T_g$ )
  - Maximal permissible temperature avoiding structural changes to the product

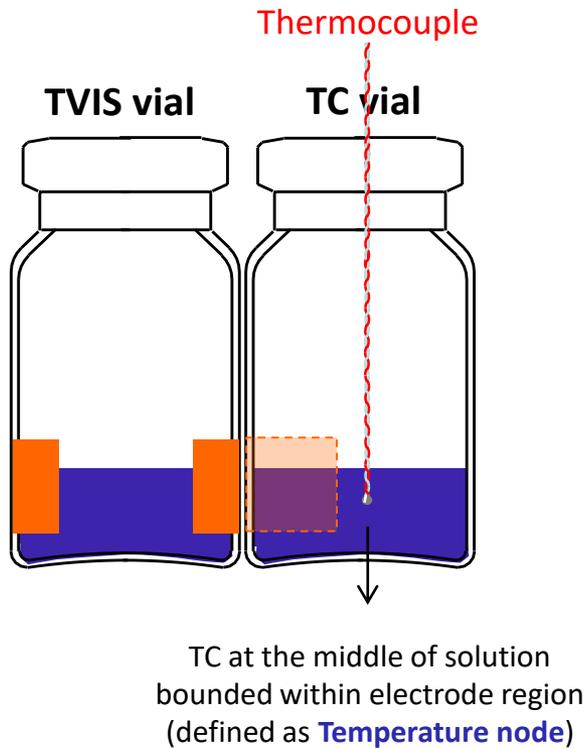
## Lyotherm – integrated electrical Impedance ( $Z_{sin\phi}$ ) and DTA

designed to measure glass transition ( $T_g'$ ), eutectic ( $T_{eu}$ ) and melting ( $T_m$ ) temperatures relevant to freeze-drying



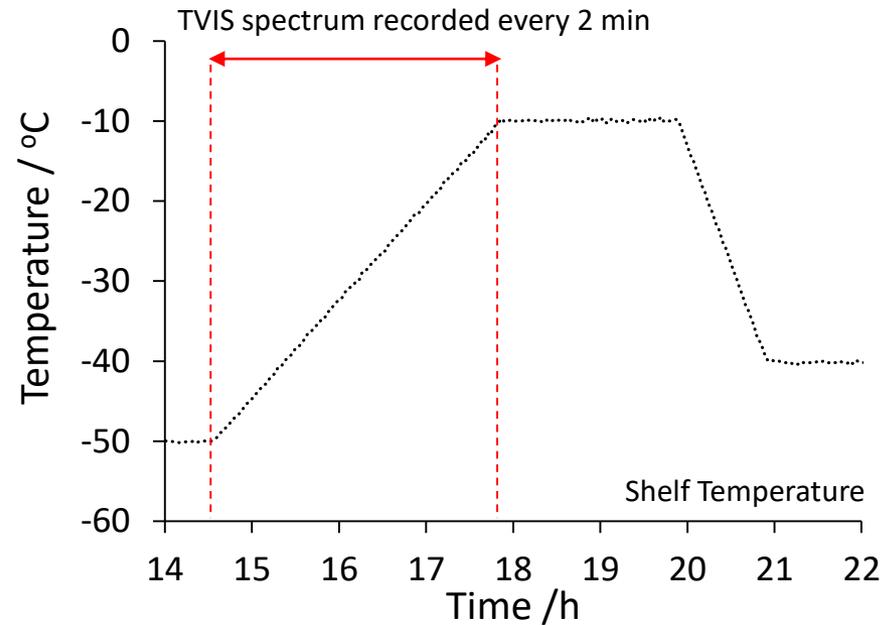
Ward & Matejtschuk , 2010 in *Freeze Drying/ Lyophilization of Pharmaceutical & Biological Products 3<sup>rd</sup>* ed. Rey,L & May JC eds, Informa Press, New York

# Glass Transition Temperature



Thermocouple position

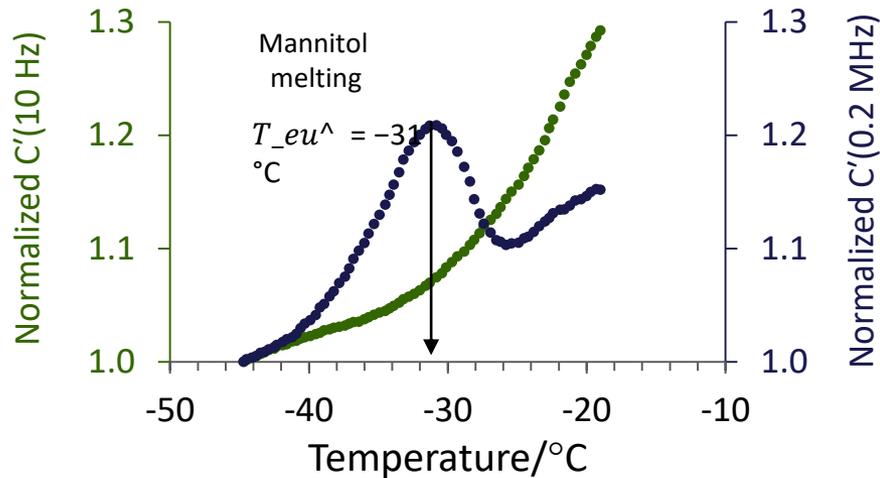
## Re-heating of pre-frozen solution



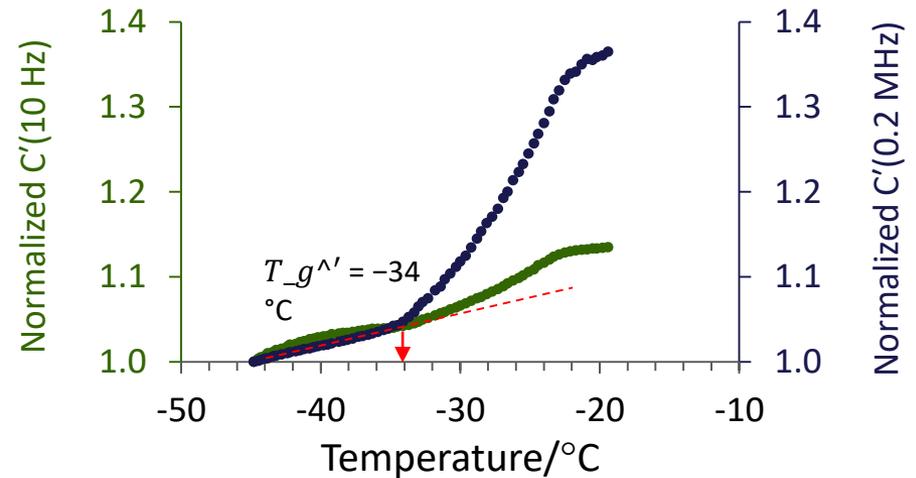
Ramp from -50 °C to -10 °C at 0.2 /min

# IgG formulations : melt back vs glass transition

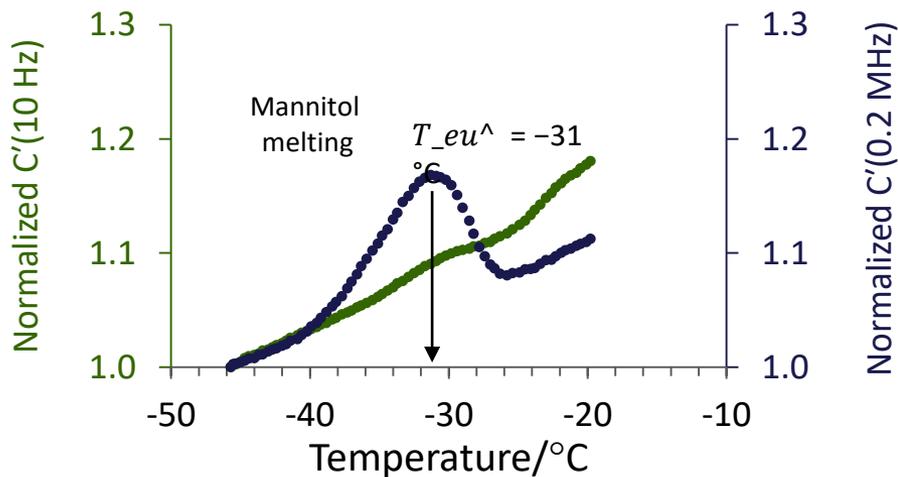
Mannitol+Sucrose based formulation  
at Edge



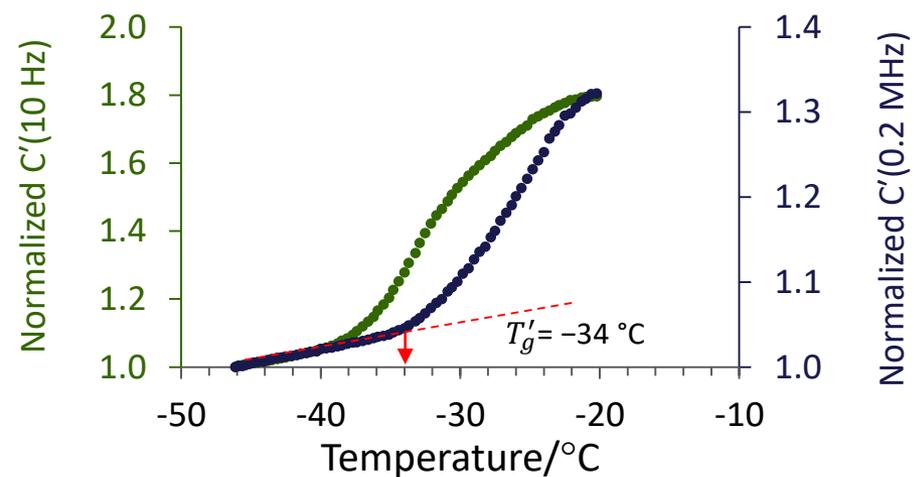
Sucrose based formulation  
at Edge



Mannitol+Sucrose based formulation  
at Core



Sucrose based formulation  
at Core



# Conclusions concerning real part capacitance spectrum

- Low frequency dielectric properties of ice
  - Pronounced temperature dependency
  - Determination of the **onset of ice formation**
  - (and time point when excess thermal energy has dissipated from the system – use in defining start of annealing phase)
- High frequency dielectric properties of ice
  - Negligible temperature dependency
  - Determination of **end point of ice crystallization**
  - Mono-tonic changes with product temperature reflect changes in viscosity.
  - Discontinuity with product temperature reflect phase changes in the unfrozen fraction. Exploit in a study of the glass transition and/or eutectic melt of the unfrozen fraction.
- **Onset** and **end point of ice crystallization** gives rate of ice formation ( $dm/dt$ )
- Pre-nucleation data (MW relaxation) predicts the nucleation temperature ( $T_n$ )
- $dm/dt$  and  $T_n$  (+ soln visc.) control the size distribution of ice crystals and  $R_p$ .

# Longinus et al # Poster 18

## Mannitol crystallization & melt back

**Through Vial Impedance Spectroscopy (TVIS) determination of ice nucleation, growth and crystallization of mannitol during lyophilisation**

International Society for Lyophilisation and Freeze-Drying (ISL-FD) 9<sup>th</sup> International Conference, Ghent, Belgium 2-6<sup>th</sup> sept 2019

Longinus Ogugua, Geoff Smith, Ahmet Orun, Muiyiwa Oyinlola

### 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 invasive and online 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

TCV = Vitris TC vial  
TVIS = TVIS vial  
TCD = Data logger TC vial

● = Mannitol solution  
● = Deionized water

Vial arrangement in the drying chamber

- Fill factor, 0.07 used equivalent to 3.5g of solution
- Vitris Advantage Plus Benchtop Freeze dryer
- 5%w/v of 98% D(+)-Mannitol
- TVIS Multi-channel Sciospec

Fig.1 Freeze drying instrument and methodology

Step	Temp (°C)		Time (min)	Rate °C/min	Pressure (ubar)
	Start	End			
Equilibrium phase	20	20	30	-	-
Freezing ramp	20	-45	120	0.1	-
Freezing hold	-40	-45	120	-	-
Re-heating ramp	-40	-30	100	0.2	-
Re-heating hold	-20	-30	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

### 3. RESULTS

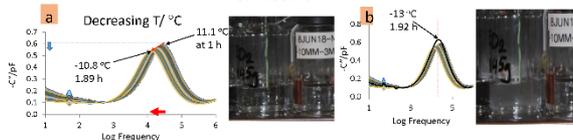


Fig.2 a) demonstrates  $C''_{PEAK}$  response to decreasing temperature with time by moving in two directions as a time: 1) lower frequencies (red arrow), and 2) downwards, reducing peak height (blue arrow). b) shows the event at the onset of ice growth depicted by sudden spike of  $C''_{PEAK}$  at 1.92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

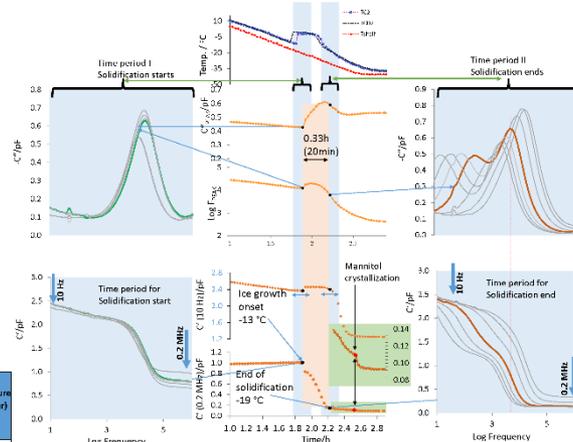


Fig.3 Log  $F_{PEAK}$  and  $C''_{PEAK}$  with respect to time depict the events that happened 6 min before and after ice growth onset and during the solidification end point. Spectra around the two major events in the freezing process could assist for more understanding of the happenings during freezing 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

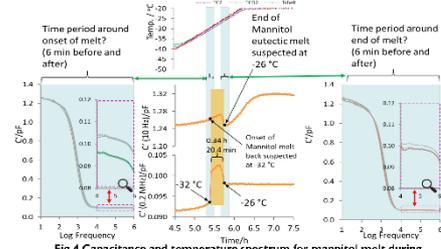


Fig.4 Capacitance and temperature spectrum for mannitol melt during re-heating period

- 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 endpoint 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

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- De Montfort University, School of Pharmacy
  - Evgeny Polygalov: co-inventor of TVIS instrument
  - PhD students : Yowwares Jeeraruangrattana, Bhaskar Pandya, Longinus Odogua
  - Irina Ermolina. Senior Lecturer
- National Institute for Biological Standards and Control
  - Paul Matejtschuk (IgG TVIS data)



Our data



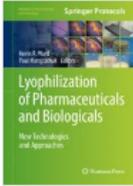
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## Through Vial Impedance Spectroscopy (TVIS): A Novel Approach to Process Understanding for Freeze-Drying Cycle Development

Authors

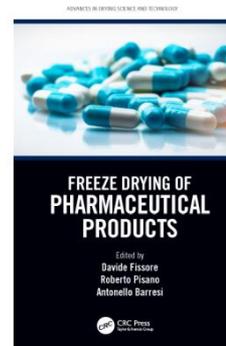
[Authors and affiliations](#)

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Research paper

Through-vial impedance spectroscopy of critical events during the freezing stage of the lyophilization cycle: The example of the impact of sucrose on the crystallization of mannitol

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