Novel electrical impedance methods in formulation and process development

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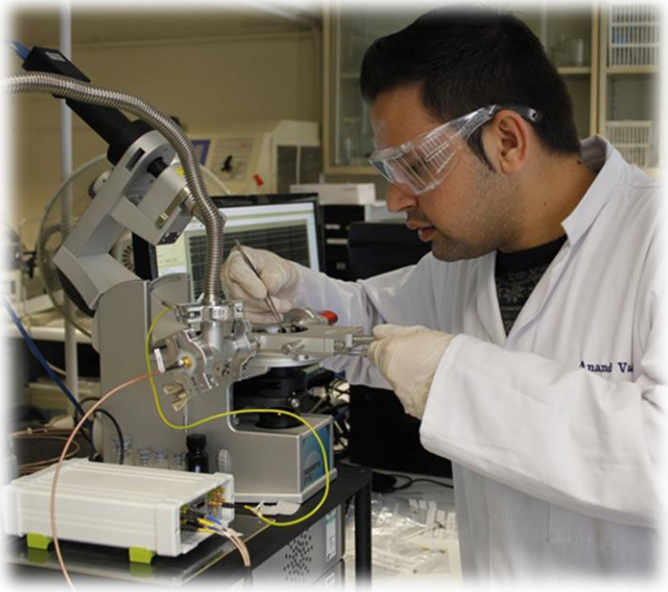
Overview – Vienna

- Introduction to electrical impedance and dielectric relaxation spectroscopy
- Micro-scale measurements in a freeze-drying microscope; applications for formulation screening
- In-vial measurements in a freeze-dryer: applications for process development

Acknowledgements

Innovate UK for Funding for Z-FDM development (FastLyo Project 133425)

In collaboration with ….



Anand Vadesa (PhD 2018)
Funded by UKRI – EPSRC

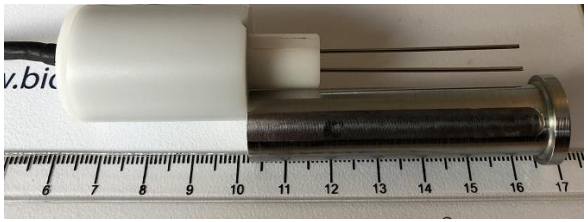
Electrical Impedance Spectroscopy and Dielectric Relaxation Spectroscopy Techniques

Single frequency (1 kHz) Electrical Impedance Analysis

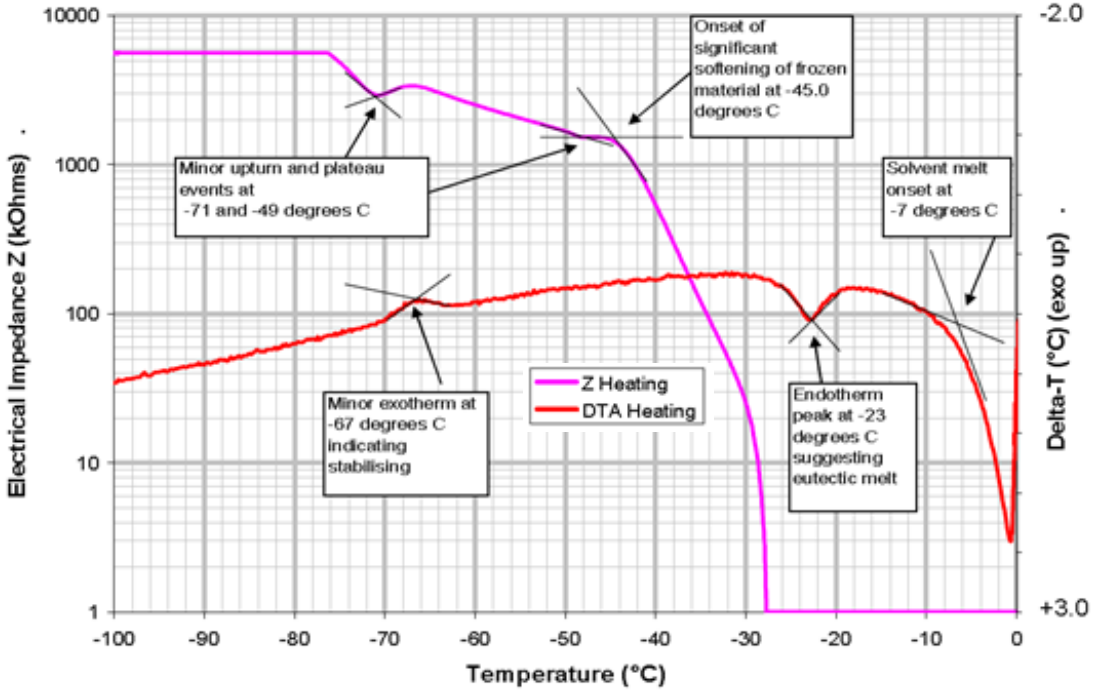
Lyotherm – Integrated impedance analysis (Zsinϕ) and DTA

designed to measure glass transition (Tg'), eutectic (Teu) and melting (Tm) temperatures relevant to freeze-dried formulations

- Pin electrode (pair)



- Integrated within cryostat



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

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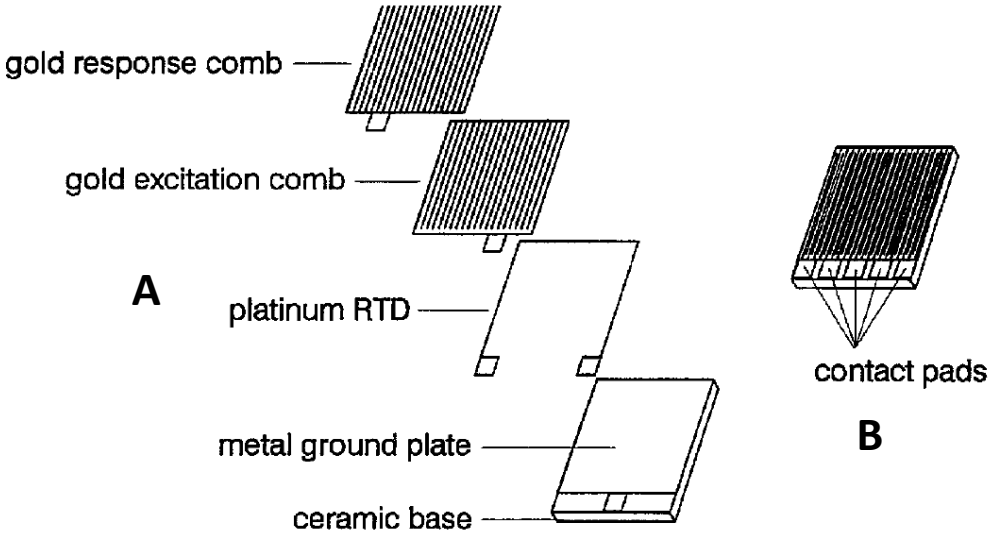
Interdigitated electrodes

- Interdigitated electrodes have been used in past for the prediction of lyophile collapse temperature



A: Showing individual components of a single surface, co-planer, interdigitated-comb sensor and **B:** the complete sensor

RESEARCH ARTICLE

Prediction of Lyophile Collapse Temperature by Dielectric Analysis

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ABSTRACT: A new method for predicting lyophile collapse temperatures based upon dielectric analysis (DEA) of frozen two component systems is presented. The method, called the take off frequency model (TOF), relies both on the inherent ability of DEA to detect molecular motion and on the abrupt change in viscosity experienced by a frozen sample undergoing a glass-liquid transition. Collapse temperatures for binary glass forming systems (an antibiotic, sucrose, trehalose, or sorbitol, with water) were in good agreement with the values reported in the literature. DEA was easily able to detect glass transitions poorly defined by differential scanning calorimetry (DSC). Conservative lyophilization cycles for simple systems can be quickly determined on the basis of the TOF model.

Introduction

Dielectric analysis (DEA) has been used extensively in polymer science for determining the characteristics of polymer films (1). There is also a considerable history of DEA in the study of molecular properties including those of biological molecules (2–6). With the advent of commercially available instruments (see Experimental), some preliminary pharmaceutical applications have been explored in our lab. The current work summarizes efforts to characterize representative frozen aqueous systems intended for lyophilization for the purpose of determining the highest allowable temperature for primary drying without collapse.

Pikal (7) has shown that there is a correlation between collapse temperature (Tc) and the glass transition temperature (Tg′) of glass forming systems. There are, however, difficulties in the determination of Tg′ by the common methods such as differential scanning calorimetry (DSC), conductivity, etc. DSC may require relatively high concentrations in systems with very low energy transitions and direct current or single frequency resistivity measurements depend on ionic content and do not easily distinguish first order from higher order transitions. It has also been documented that the Tg′ may precede the observed Tc by varying intervals up to several degrees C (7). It was thought that DEA might provide a more sensitive and accurate measure of Tg′ for reasons described below. As with most techniques, we have come to view DEA as complementary to classical thermal techniques for the complete characterization of such transitions. This report will present the basis of our "model" for predicting the Tc based on DEA results. This is a new application of the technique and the development of our model may provide an approach that will prove useful in the study of other pharmaceutical processes and systems.

Background

Basically, DEA involves the construction of a capacitor in which the sample to be examined is the dielectric material between the capacitor plates. A sinusoidal voltage of fixed amplitude and known frequency is impressed across the capacitor and the resulting current is followed with time. Changes in the phase of the current relative to that of the applied voltage are then used to calculate the dielectric constant (ε). Since ε is ultimately a function of frequency and temperature, it is not a constant and is simply referred to as permittivity or relative permittivity. This concept may be described mathematically in terms of the force that the dielectric material experiences in the capacitor. For a static field Maxwell's relationship (cgs system) (8) for a non polar dielectric is

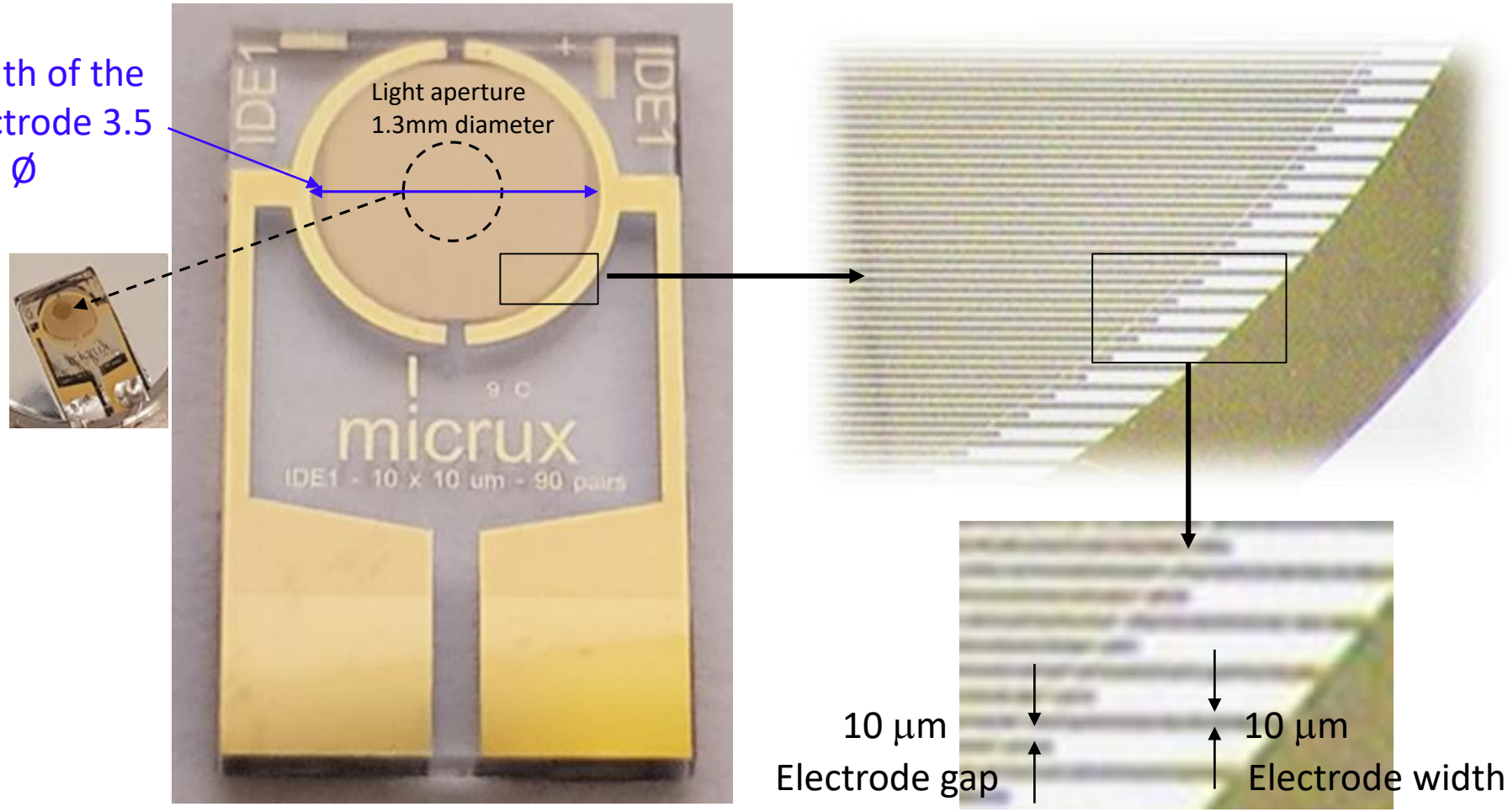
$$\vec{D} = \epsilon_r \vec{E} \qquad (1)$$

where D is the displacement force, E is the electric field inside the capacitor ($D = E$ in vacuo), and ϵ_r is the

Received September 15, 1993. Accepted for publication May 18, 1994.
This work was presented in part on 5/14/92 in Newark, DE at the Spring Thermal Analysis Symposium & Exhibition on Applications in the Food, Pharmaceutical and Cosmetic Industries sponsored by the Thermal Analysis Forum of Delaware Valley and as a poster at the Eastern Regional AAPS Meeting on 6/2/92 in New Brunswick, NJ.
Author to whom correspondence should be addressed: Dr. Sean A. Evans, ImmuLogic Pharmaceutical Corp., 610 Lincoln Street, Waltham, MA, 02154.

Mackenzie, A. P., Evans, S. A. and Morris,. Prediction of Lyophile Collapse Temperature by Dielectric Analysis Prediction of Lyophile Collapse Temperature by Dielectric Analysis, *PDA J Pharm Sci and Tech* **1994**, *48* 318-329.

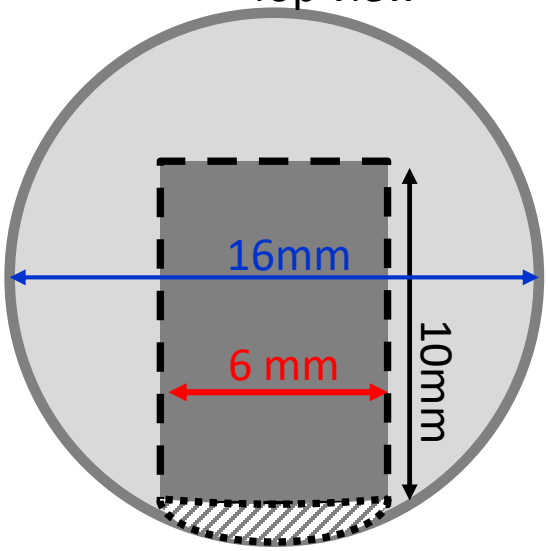
Example interdigitated electrode (gold on glass)



Commercial IDE – Micrux™

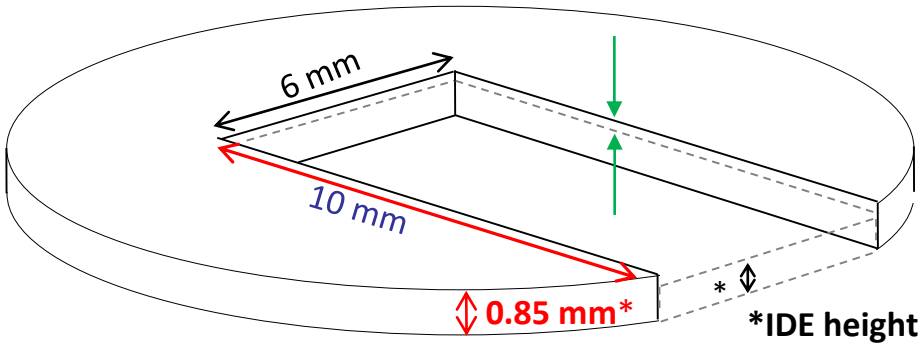
Design of IDE holder

Top view



IDE dimensions:
10 x 6 x 0.75 mm

Theoretical sample height



***IDE height 0.75 mm**

* different height of IDE adaptors used for initial assessment:

- 0.85 mm
- 0.90 mm
- 0.95 mm
- 1.00 mm

IDE is lower than the adapter and sample height derived between difference between then IDE adaptor size

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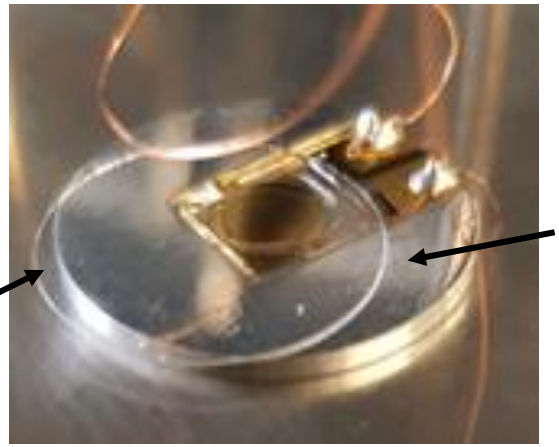
Spectroscopy Systems : BDS (sub Hz to 10 MHz)

- Inter-digitated electrode

- Integrated within the BDS cryostat



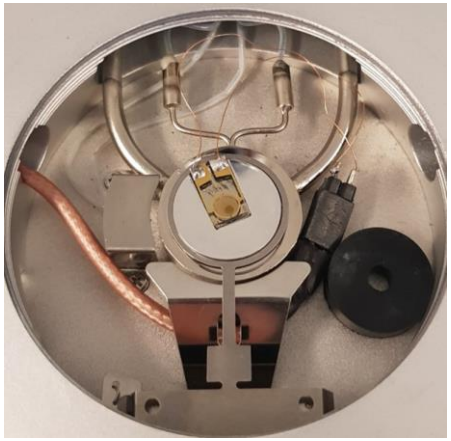
Commercial state of the art broad-band dielectric spectrometer (BDS) from Novocontrol GmbH (mHz to 10 MHz)

Novocontrol BDS system

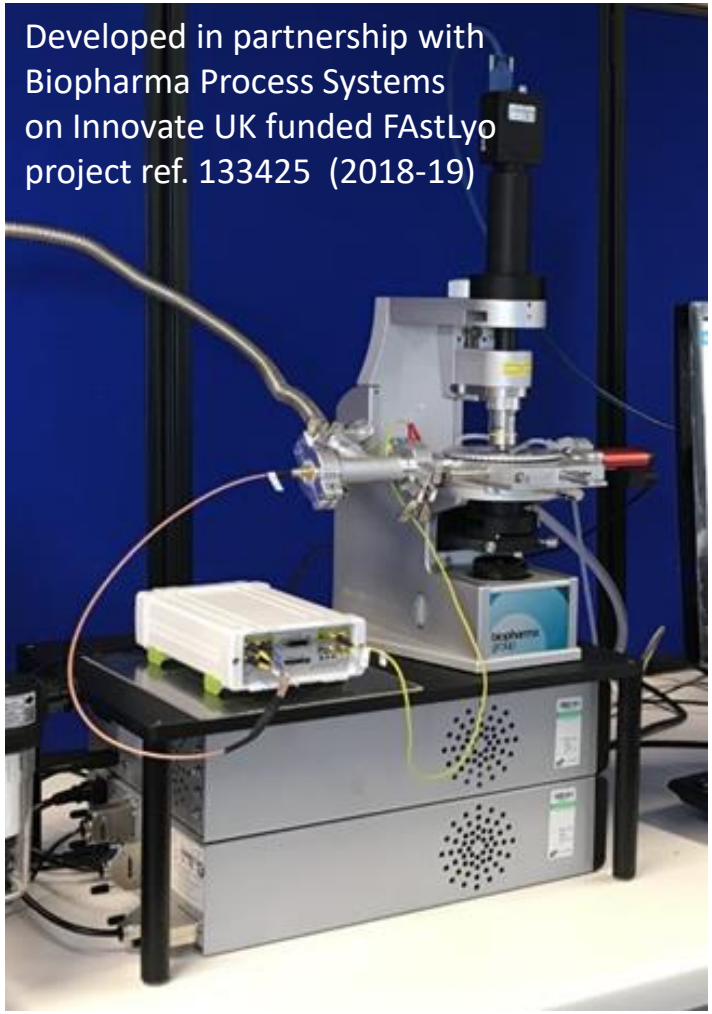
Spectroscopy Systems : Z-FDM

- Inter-digitated electrode

- Integrated within the FDM stage



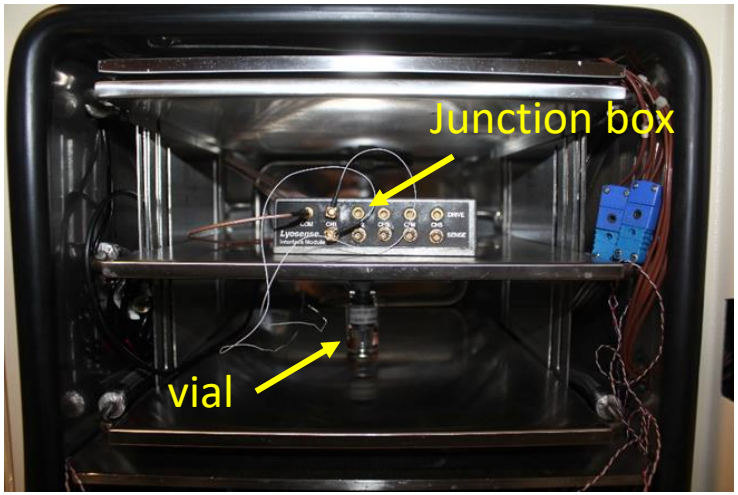
Developed in partnership with Biopharma Process Systems on Innovate UK funded FAstLyo project ref. 133425 (2018-19)



Spectroscopy Systems : TVIS

- Modified glass vial

- Integrated within the dryer



TVIS (Through-Vial Impedance Spectroscopy) was developed in partnership with GEA Pharma Systems on Innovate UK funded LyoDEA project (2010-13)

Dielectric Loss Mechanisms

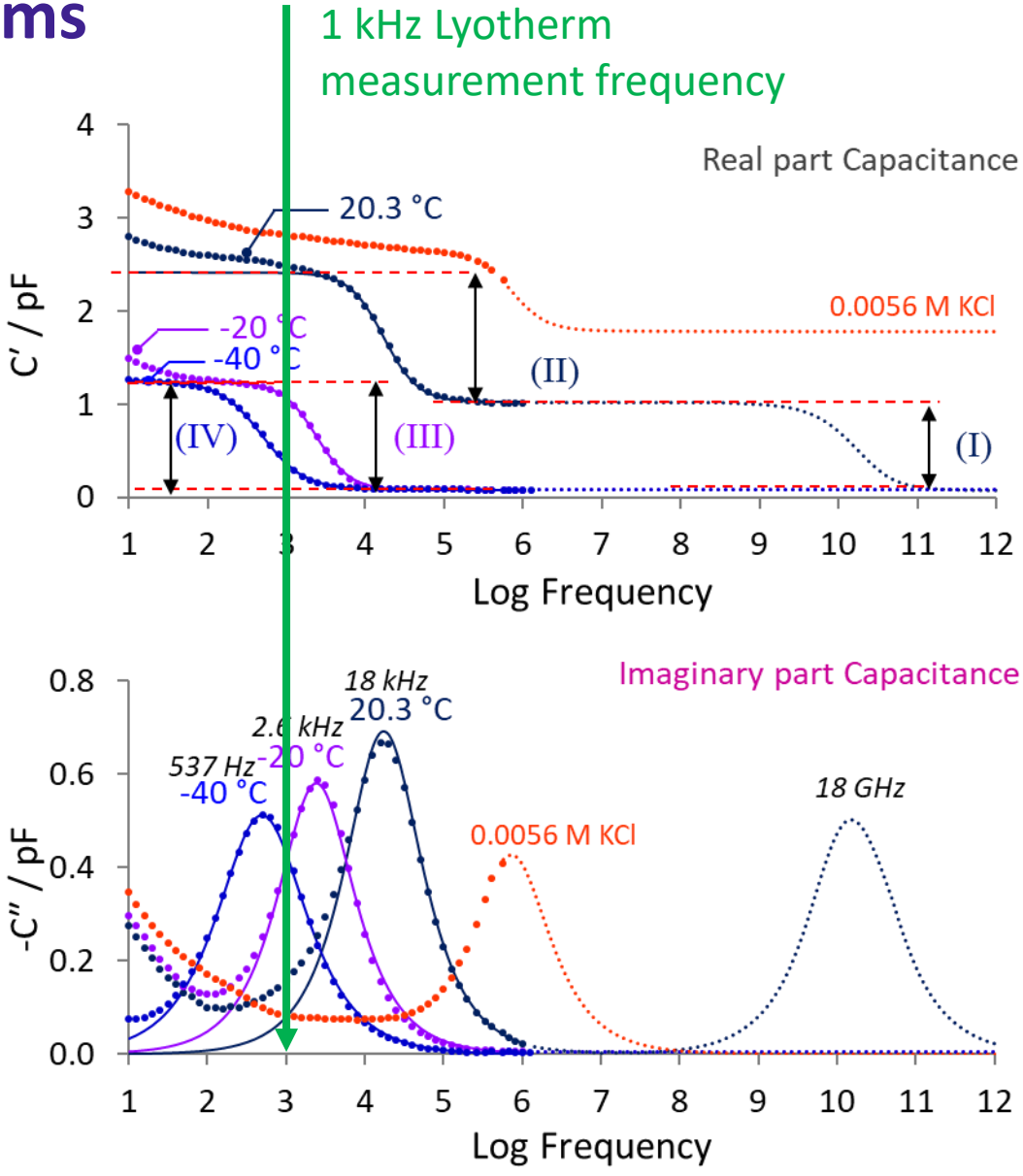
I. The polarization of the water dipole in liquid water at 20 ˚C, with a dielectric loss peak frequency of ~ 18 GHz

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

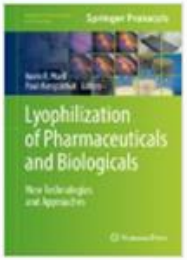
III. The dielectric polarization of ice at −20 ˚C, with a dielectric loss peak frequencies of 2.57 kHz

IV. The dielectric polarization of ice at −40 ˚C with a dielectric loss peak frequencies of 537 Hz.

Note: Process II only seen in TVIS vial; in Z-FDM process II is replaced by electrode polarization impedance)



Further Reading



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)

Further Reading

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

CRC Press
Taylor & Francis Group

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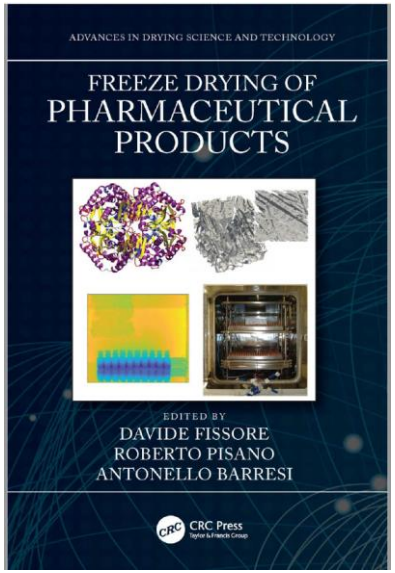

Freeze Drying of Pharmaceutical Products

1st Edition

Davide Fissore, Roberto Pisano, Antonello Barresi

Hardback
£118.00

CRC Press
November 13, 2019 **Forthcoming**
Reference - 214 Pages - 4 Color & 66 B/W Illustrations
ISBN 9780367076801 - CAT# K405807
Series: Advances in Drying Science and Technology

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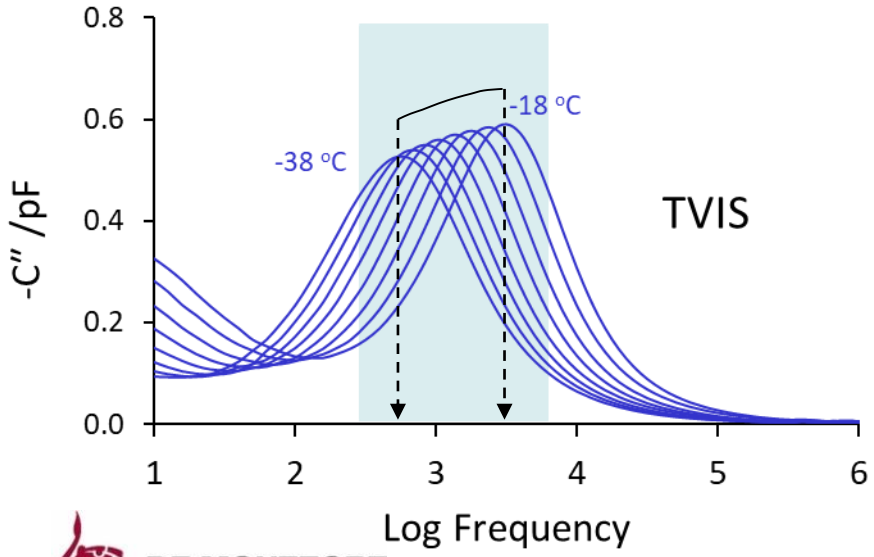
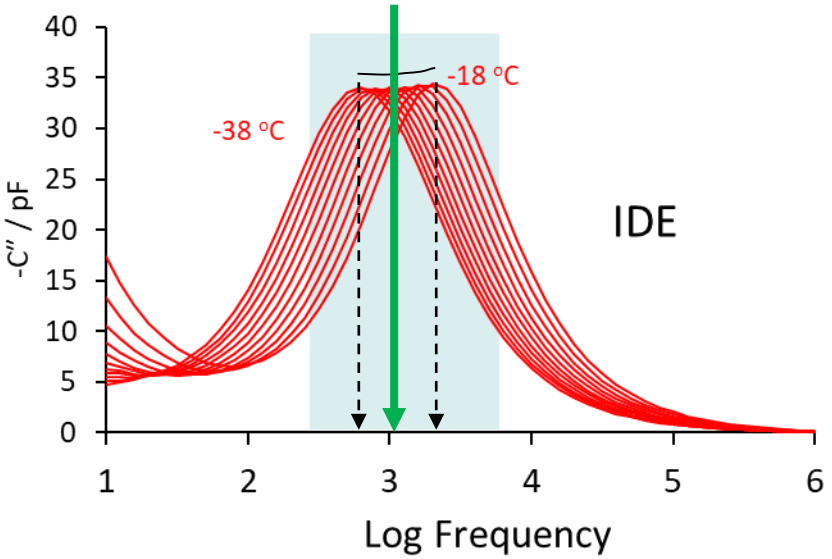
Dielectric relaxation of ice

5 mL water in
10 mL glass TVIS vial
(1 pair of 10/19 mm
height/width electrodes)

2 µL water over IDE
(90 pairs of gold interdigitated electrodes)
1.6 kHz temperature sensitive





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Micro-scale measurements in a freeze-drying microscope; applications for formulation screening

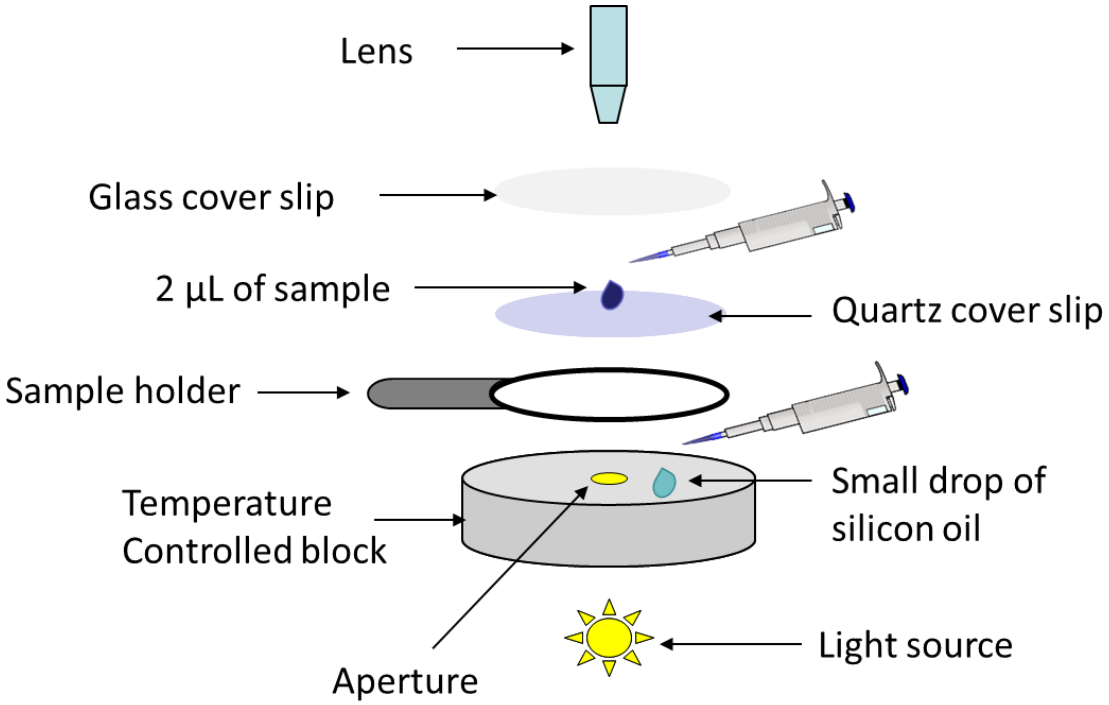
Z-FDM : A description of the new measurement system

Freeze drying microscopy

Real-time observation of the behavior of formulations during freeze drying and typically used for study Critical Temperatures of a formulation

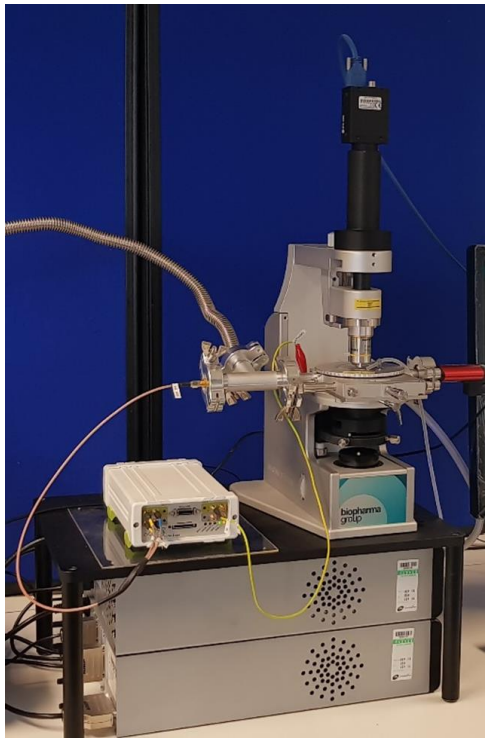
- ✓ In amorphous products this is the collapse temperature (T_c)
- ✓ In crystalline solutions this is the eutectic point (T_{eu})

Biopharma Lyostat5 FDM

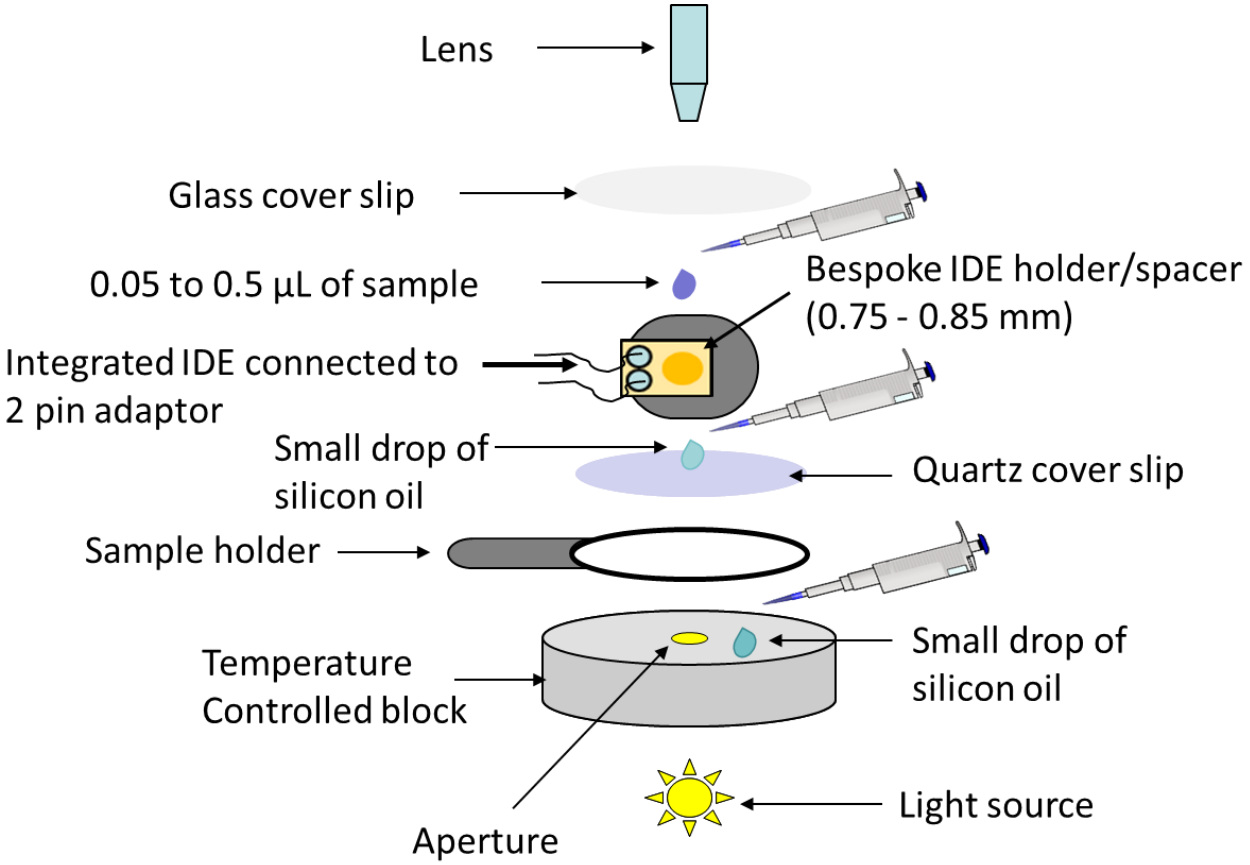


Impedance enabled Freeze drying microscopy

- ✓ Impedance analyzer connected to the FDM with bespoke adapters
- ✓ FDM stage remains intact, and IDE sit above the quartz cell
- ✓ Gold IDE does not affect the optical application of the FDM



Z-FDM



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Z-FDM with TASC and Image Analysis

- Combined approaches provides comprehensive analysis of critical parameters

Event	Visual assessment	TASC/pixel analysis	Impedance
Collapse	Yes (Subjective)	Yes	Yes*
Eutectic melt	Yes (Subjective)	Yes	??
Glass transition (T_g')	No	??	TBC
Ice nucleation	Yes	Yes**	Yes*
Solidification end	Yes	Yes**	Yes*
Annealing	No	??	TBC
Drying rate	No	Yes	TBC

TBC : To be confirmed in this presentation.

*Vadesa A, Smith G, Horley N, Ward K, Dalby P. Application of a novel impedance-based freeze drying microscopy for formulation development. Podium & Poster presentation at ISL-FD 9th International Conference 2-6th September, 2019, Ghent, Belgium. February 2020. doi:10.21253/DMU.9767048

**Requires all of the sample to be within view of the camera

Publications suggestive of Z-FDM application

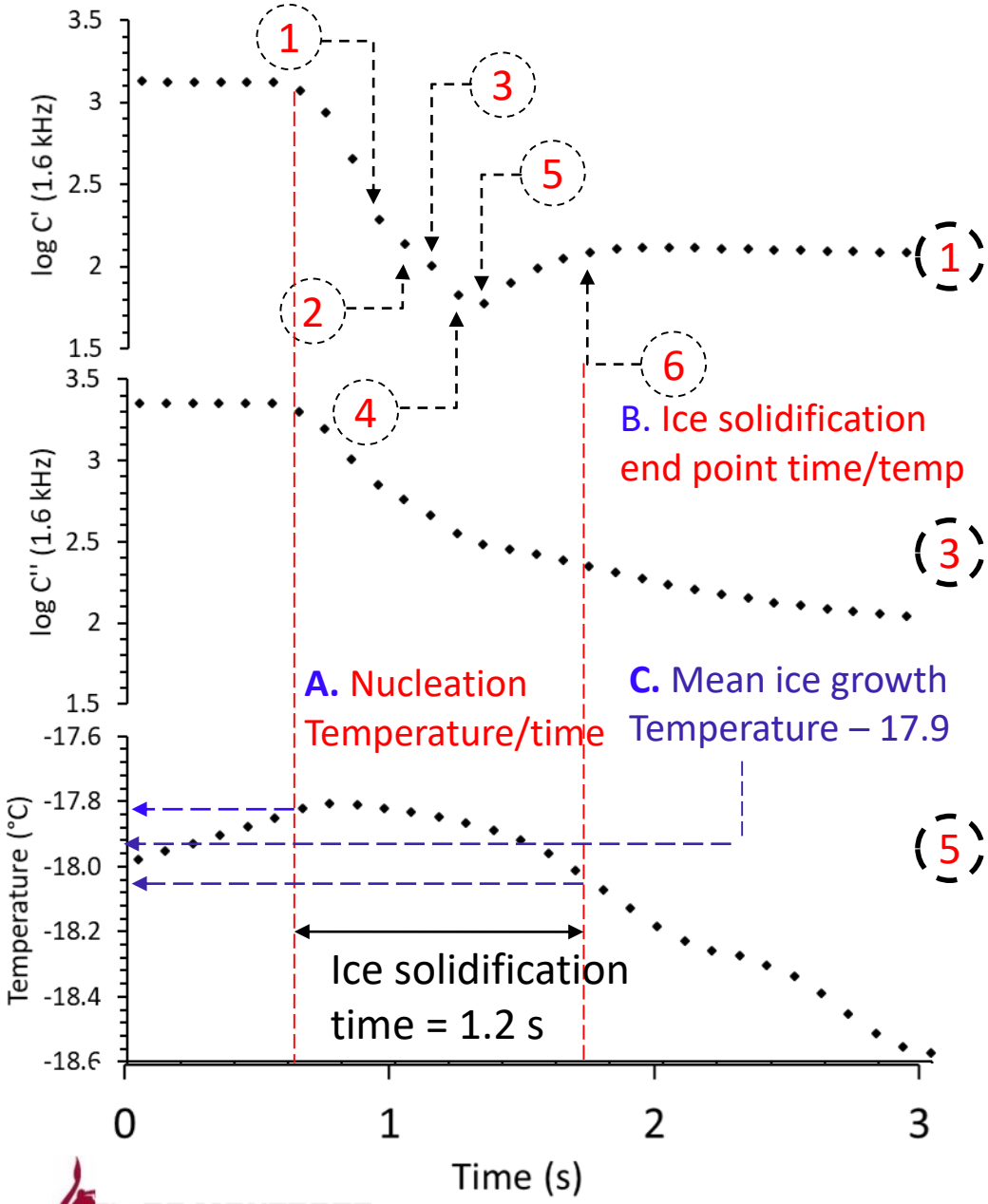
- Smith, G., Jeeraruangrattana, Y., Ermolina, I. (2018). The application of dual-electrode through vial impedance spectroscopy for the determination of ice interface temperatures, **primary drying rate** and vial heat transfer coefficient in lyophilization process development. European Journal of Pharmaceutics and Biopharmaceutics
- Smith, G., Arshad, M.S., Polygalov, E., Ermolina, I., McCoy, T.R., Matejtschuk, P. (2017). Process Understanding in Freeze-Drying Cycle Development: Applications for Through-Vial Impedance Spectroscopy (TVIS) in Mini-pilot Studies. Journal of Pharmaceutical Innovation, 12 (1), pp. 26-40 **Key observation was the potential to measure temperature non-invasively**
- Arshad, M.S., Smith, G., Polygalov, E., Ermolina, I. (2014). 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**. European Journal of Pharmaceutics and Biopharmaceutics, 87 (3), pp. 598-605
- Smith, G., Arshad, M.S., Polygalov, E., Ermolina, I. (2014). Through-Vial Impedance Spectroscopy of the **Mechanisms of Annealing** in the Freeze-Drying of Maltodextrin: The Impact of Annealing Hold Time and Temperature on the Primary Drying Rate. Journal of Pharmaceutical Sciences, 103 (6), pp. 1799-1810
- Smith, G., Arshad, M.S., Nazari, K., Polygalov, E., Ermolina, I. ; Taylor, J., Page, T. (2014) Through-Vial Impedance Spectroscopy: A New In-Line Process Analytical Technology for Freeze Drying. Pharmaceutical Technology, 38 (4), pp. 38-46
- Smith, G., Arshad, M.S., Polygalov, E., Irina Ermolina, I. (2014) Factors Affecting the Use of Impedance Spectroscopy in the Characterisation of the Freezing Stage of the Lyophilisation Process: the Impact of Liquid Fill Height in Relation to Electrode Geometry. AAPS PharmSciTech, 15 (2), pp 261–269
- Smith, G., Arshad, M.S., Polygalov, E. and Ermolina, I. (2013) An application for impedance spectroscopy in the characterisation of the **glass transition** during the lyophilization cycle: The example of a 10% w/v maltodextrin solution. European Journal of Pharmaceutics and Biopharmaceutics, 86 (3 Part B), pp. 1130-1140.
- 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. International Journal of Pharmaceutics, 449 (1-2), pp. 72-83

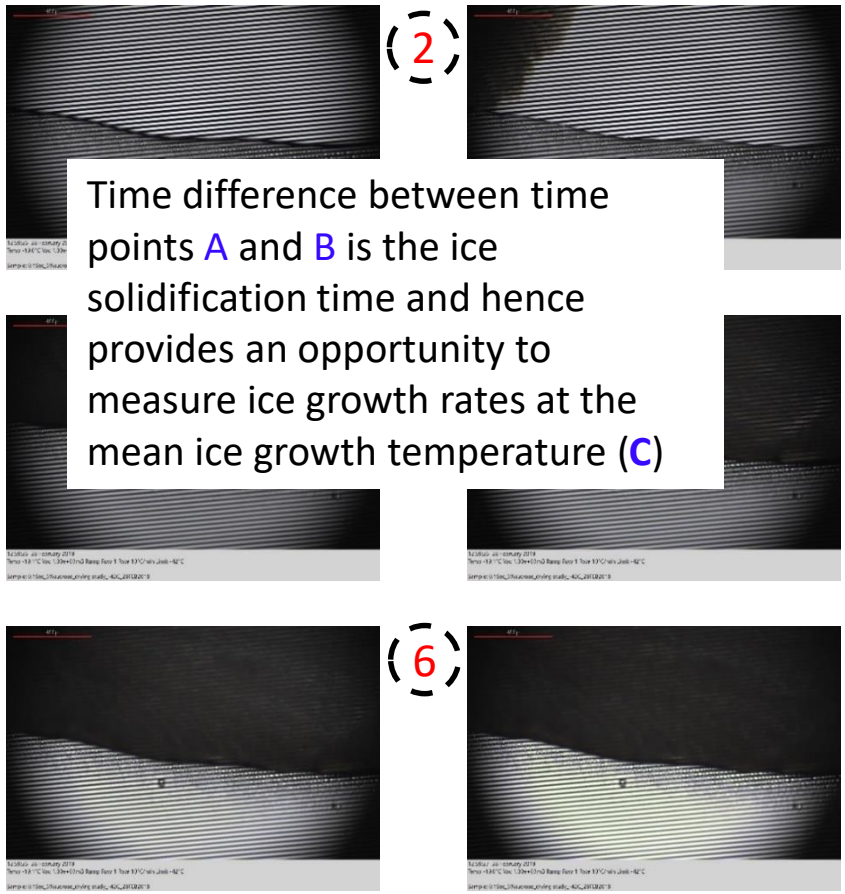
Applications in freezing (nucleation temperature, ice growth rates, solidification end point)

Observations on Sample Size

Case study of 5% w/v Sucrose Solution

Nucleation of 0.5µL of 5% Sucrose





Time difference between time points A and B is the ice solidification time and hence provides an opportunity to measure ice growth rates at the mean ice growth temperature (C)

Ice growth rates

- 1 mL of 5% w/w sucrose has 0.95 g water
- Assumption: unfrozen fraction comprises 80:20 ratio of sucrose to water
- It follows that 0.0125 g (0.05 x 20/80) is bound and produces 0.9375 g ice

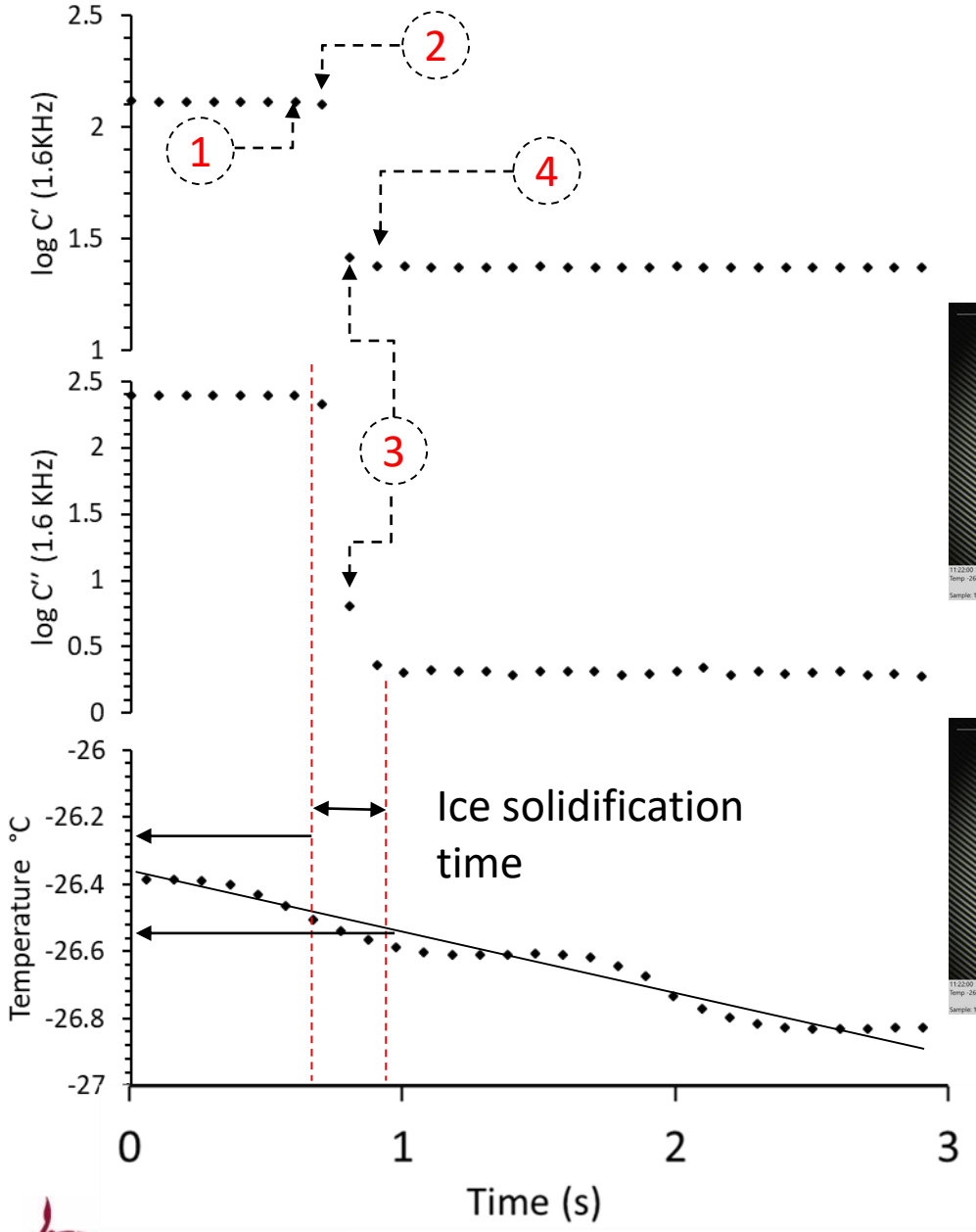
Estimated from:

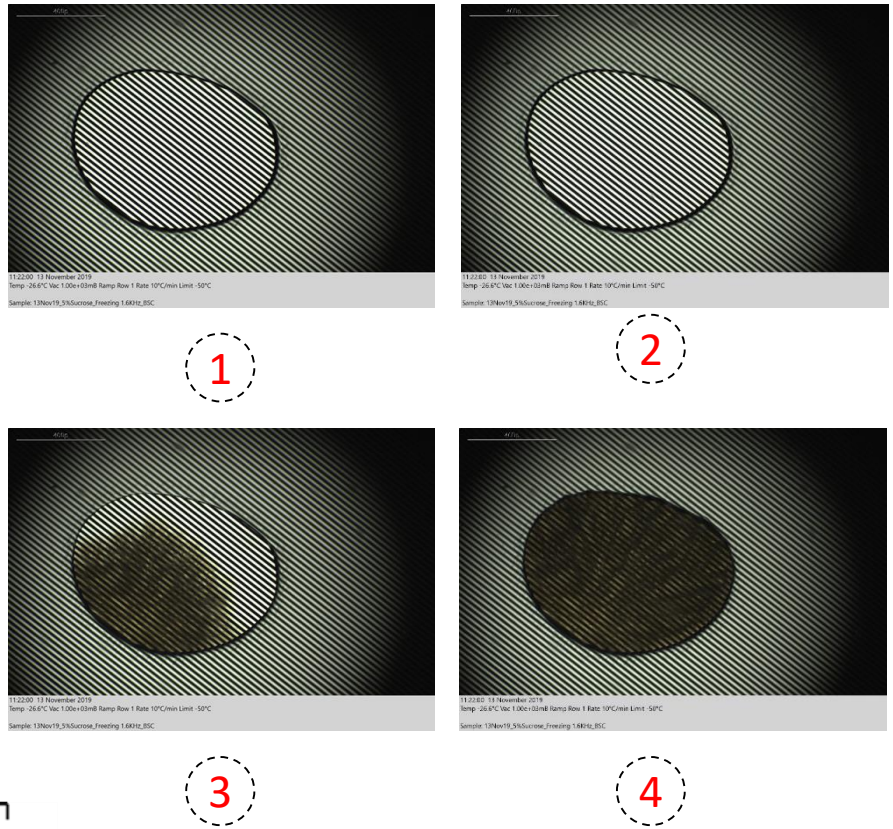
Larger sample : 0.5 µL of 5% sucrose (produces 4.688E-04 g ice)

- Ice formation time = 1.2 s (12 data points – more accurate)
- Ice growth rate: 4.688E-04 / 1.2 = 0.39 mg/s

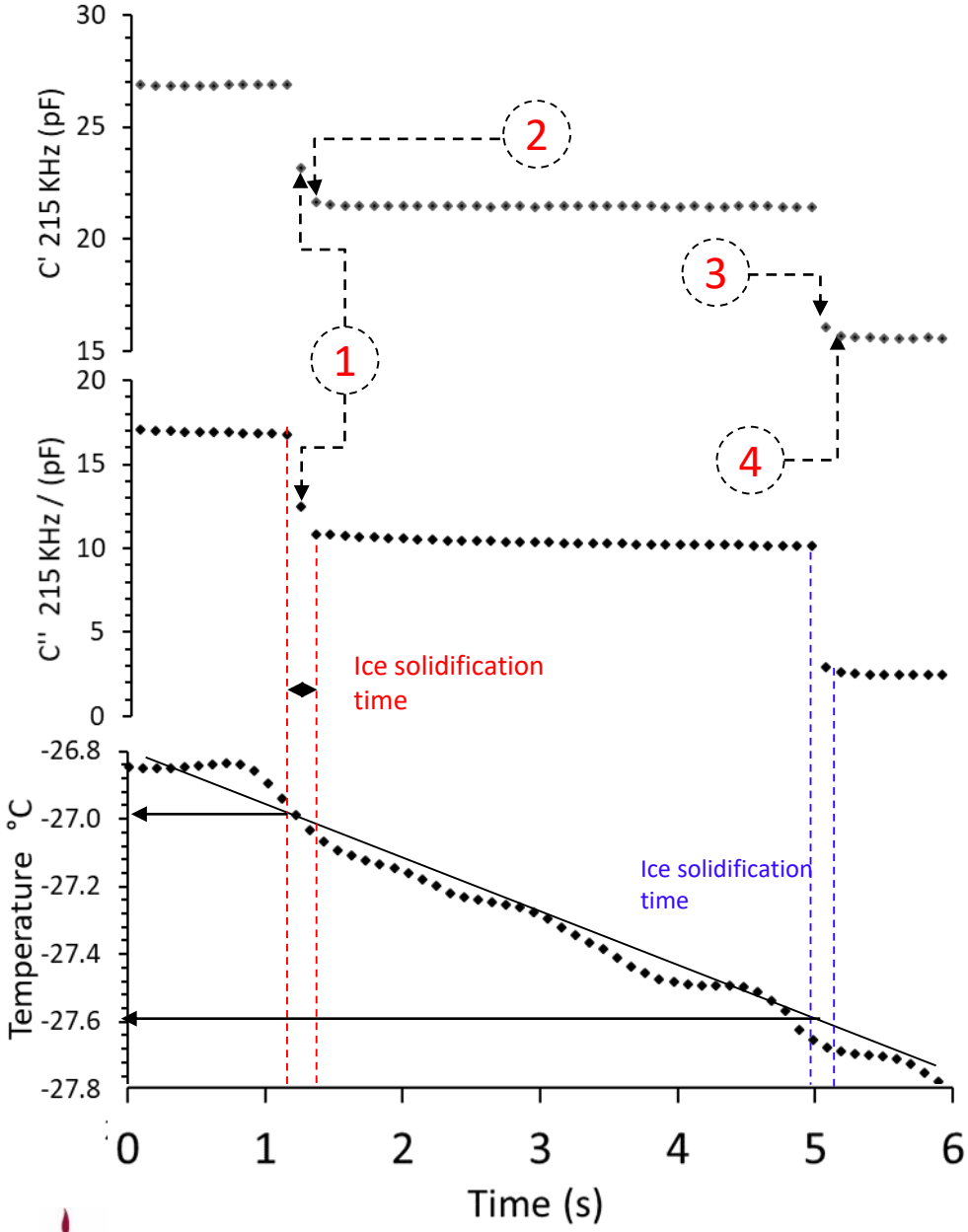
Relevance : ice crystal size?

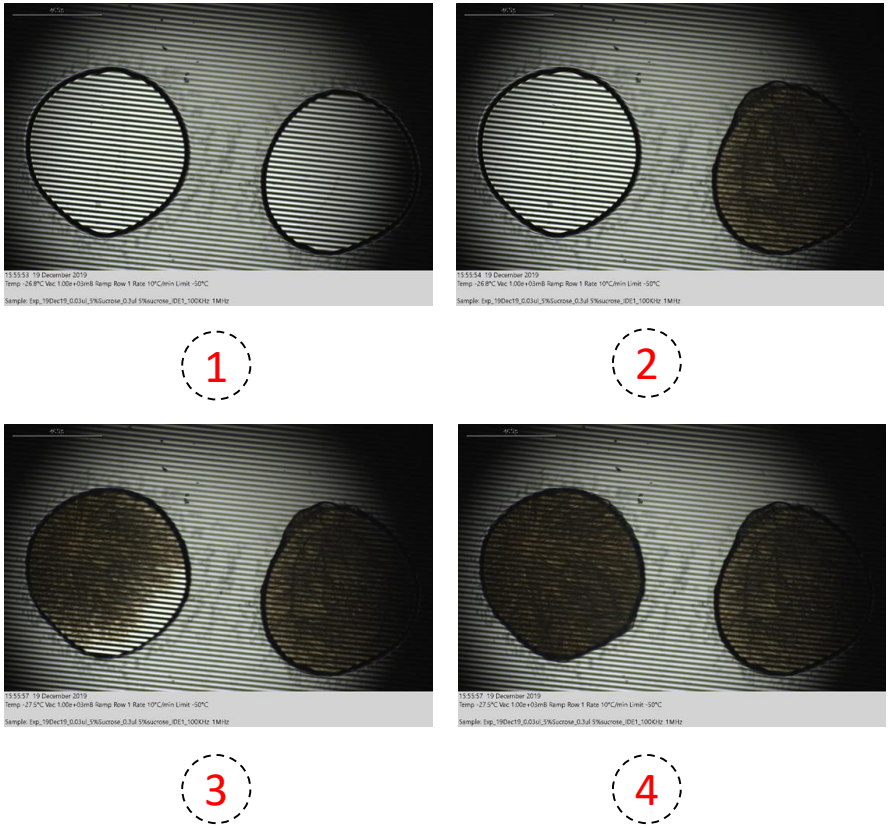
Nucleation of 0.05 µL of 5% Sucrose





Nucleation of 2 x 0.03 µL of 5% sucrose



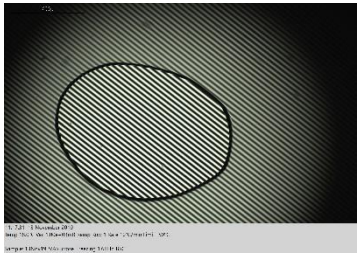


Applications in primary drying
(drying rate, product collapse)

Freeze drying of 5% sucrose (0.05µL)

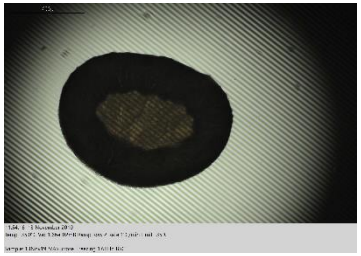
Studied by Image analysis

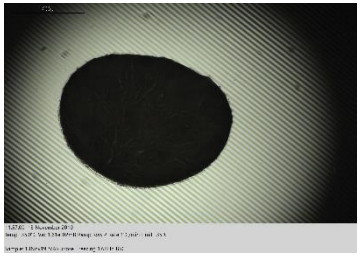
FDM protocol


Liquid state

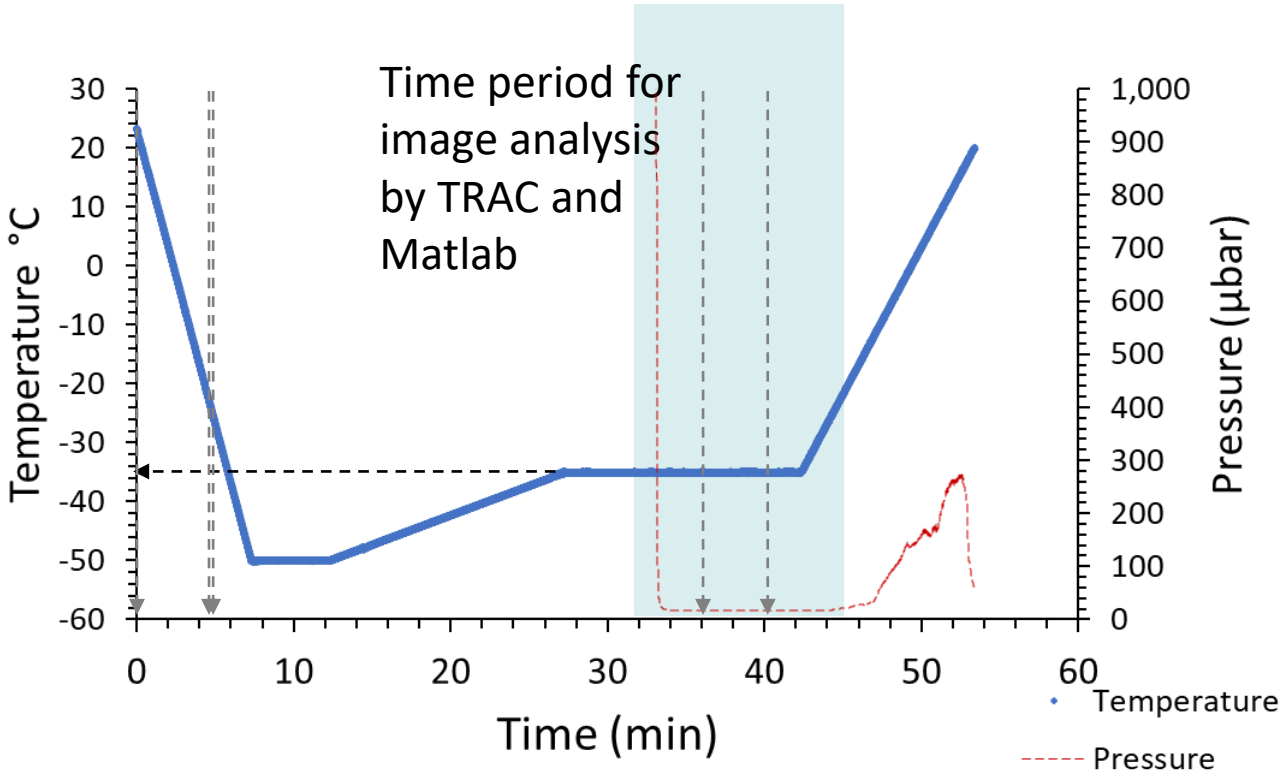
ice growth

ice solidification


sublimation


end of primary drying

FDM freeze-drying of 5% sucrose solution (0.05 µL)



NB: Temperature and pressure measured on FDM every 100 ms

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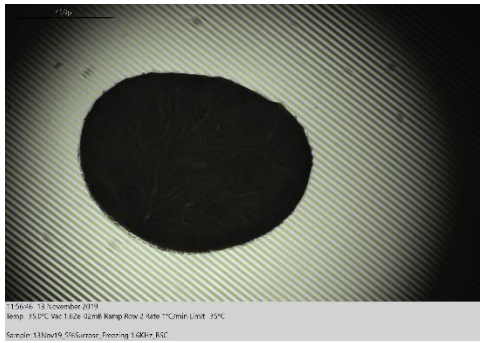
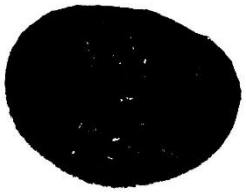
MATLAB® Image analysis (pixel counting)

FDM primary-drying of 5% sucrose soln (0.05 µL) at -35˚C

1. **Global templet generation** – dried product image



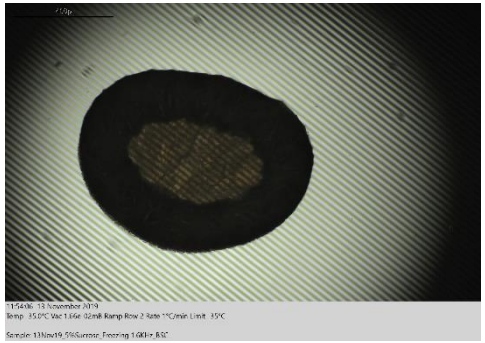
MATLAB®



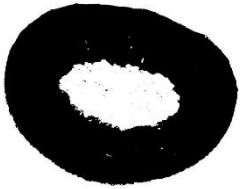
Global pixel : 527515

Dried product image

2. **Threshold test image** – frozen image or drying stage image



MATLAB®



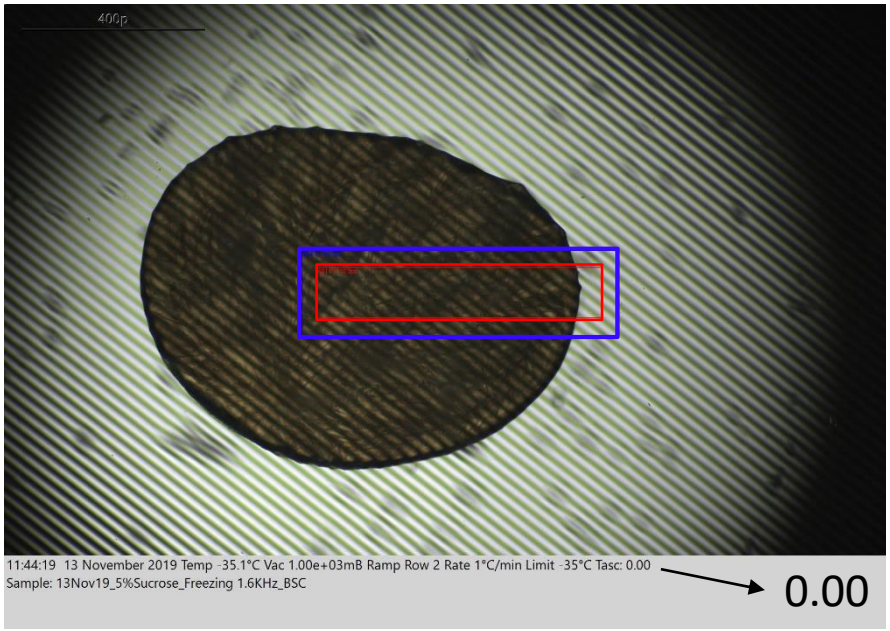
Pixel for central area: 86317

Product during drying

TASC - description

Thermal Analysis by Surface Characterisation (TASC) works by analyzing a sequence of images and tracking the changes from one to another

- First, select the range of analytical run (e.g. drying period)
- Select a ‘region of interest’ that need needs to be tracked (red box)
- Then select a ‘region to be scanned’ (blue box)



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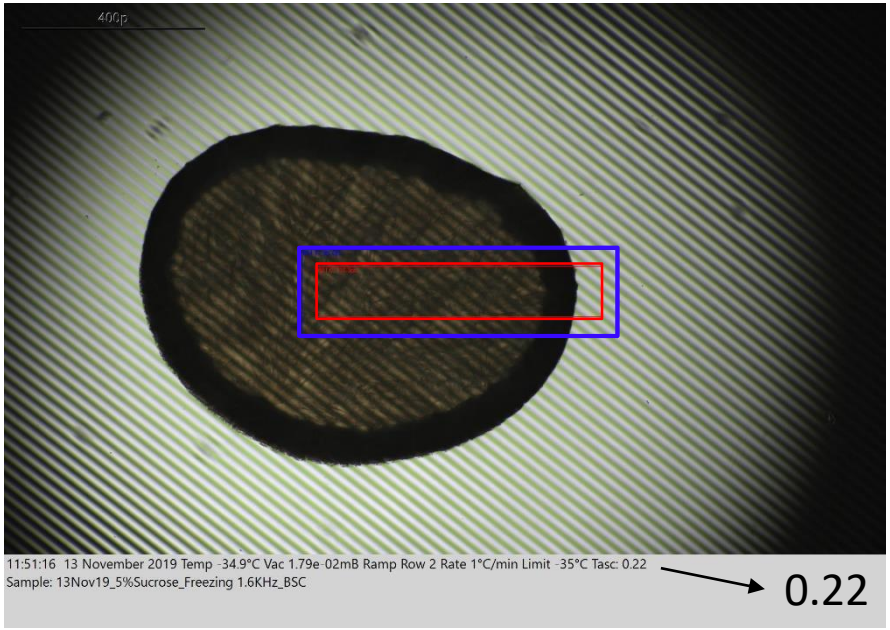

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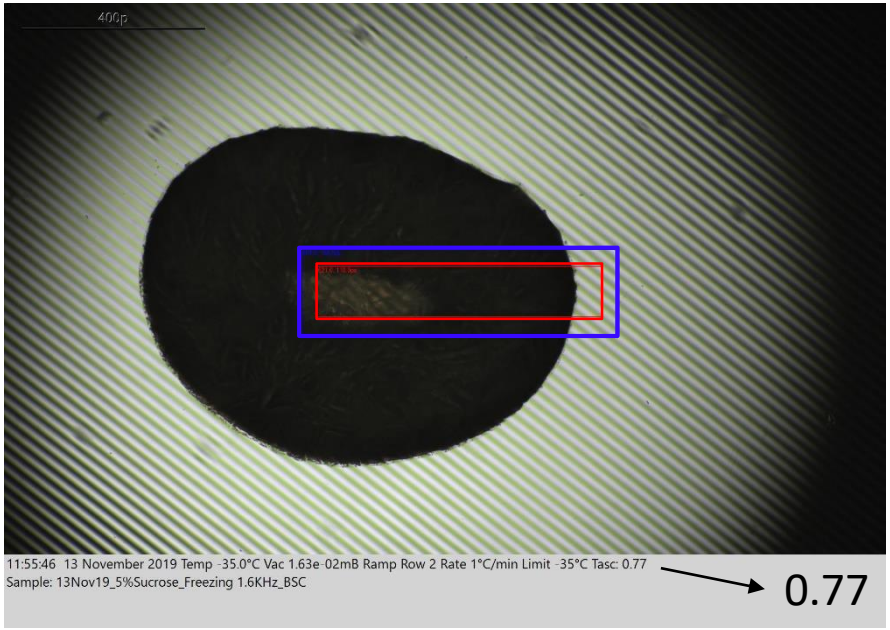

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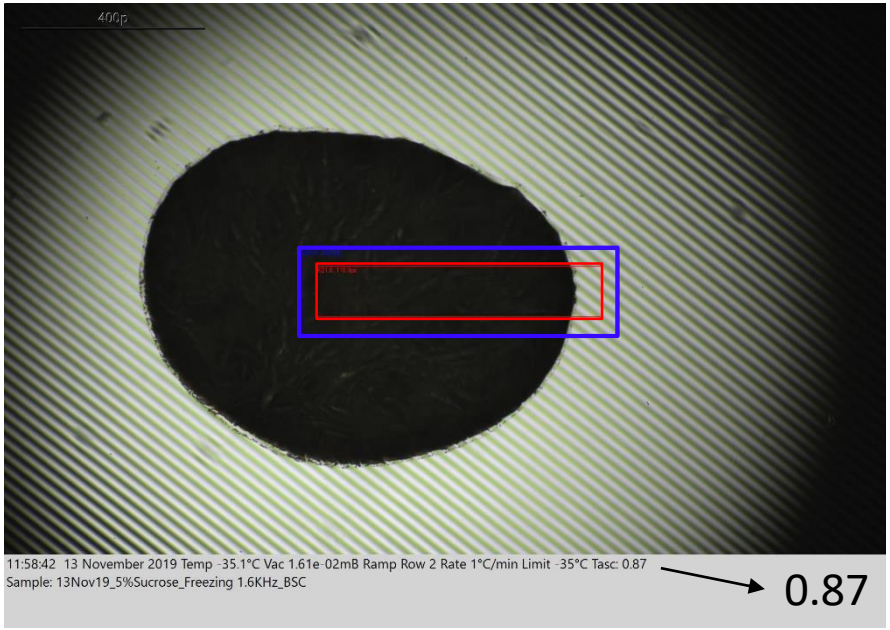

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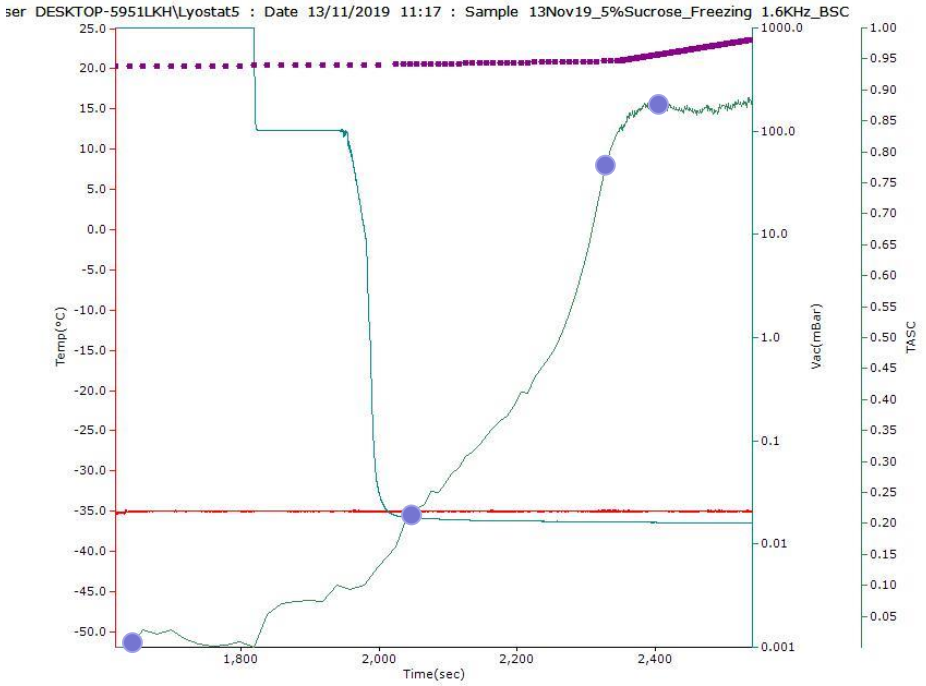
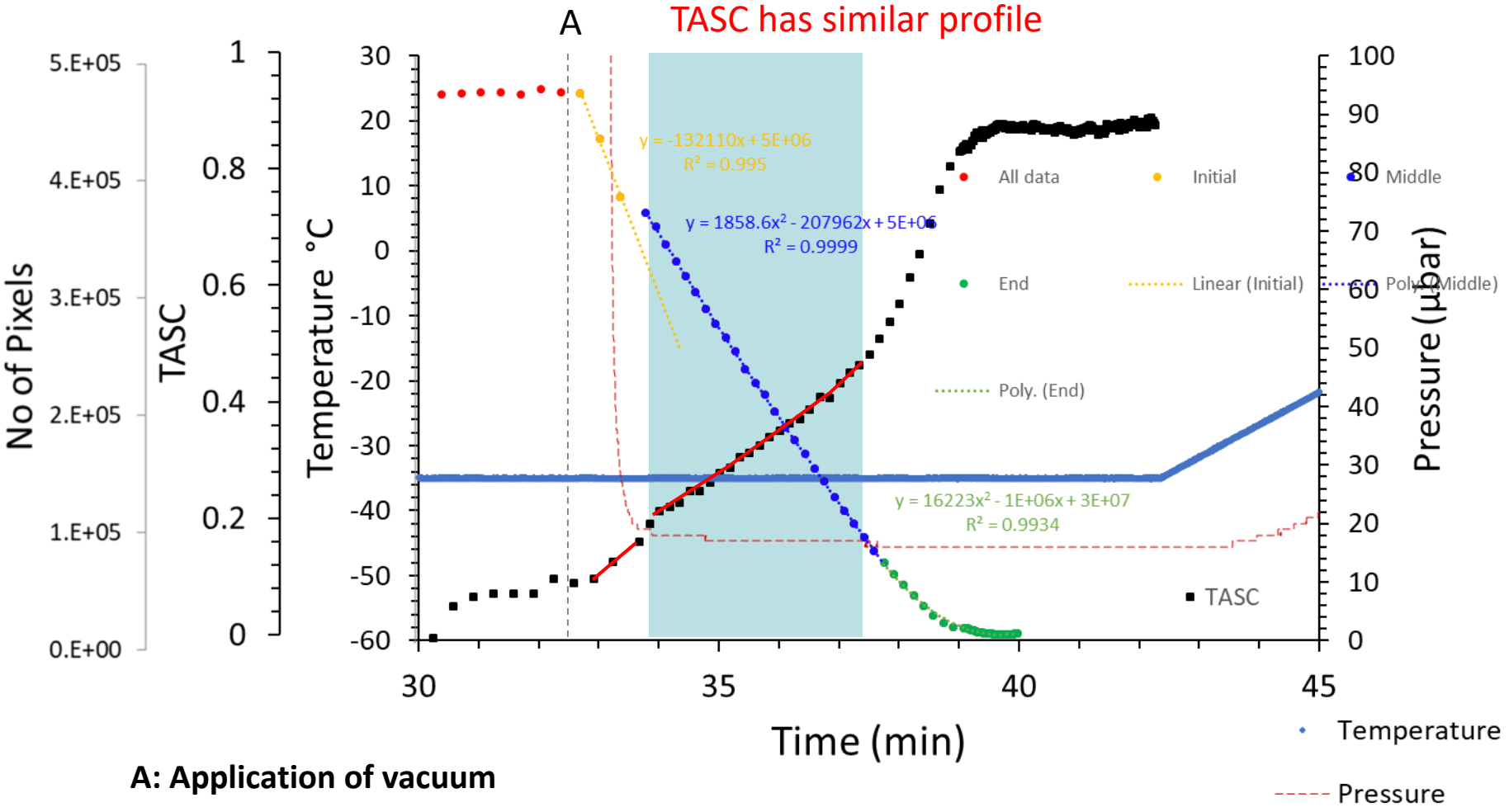


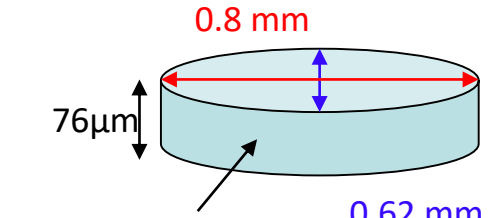
Comparison of TASC and Pixel analysis



A: Application of vacuum

NB: Temperature and pressure measured every 100 ms

Drying rate determination



Area of perimeter
0.00342 cm²

Weight fraction of sucrose	0.05	(5% Sucrose)
Weight fraction of water	0.95	i.e. 95 % water
Weight fraction of bound water (1)	0.0125	
Weight fraction of freezable water	0.9375	

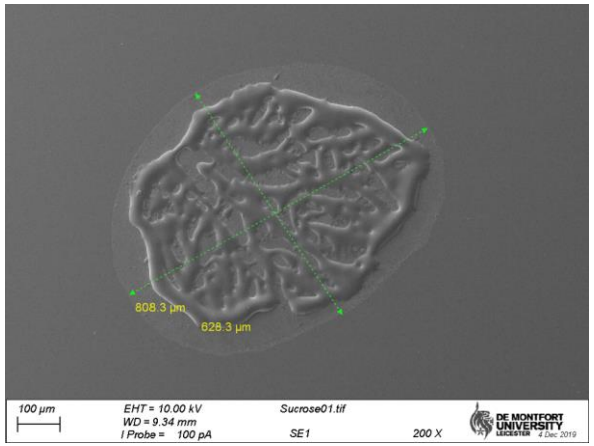
(1) based on 80:20 ratio of sugar to water in freeze-concentrated solution

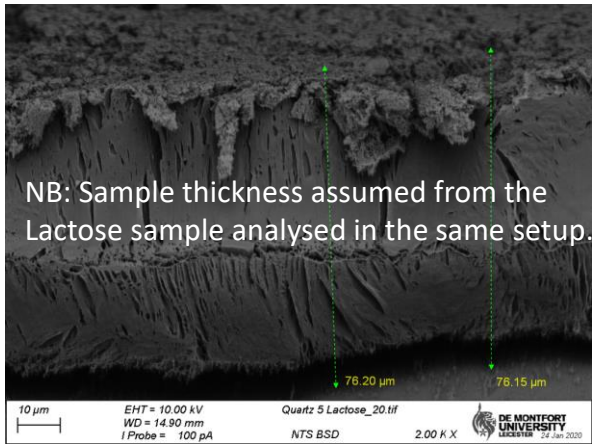
Sample volume	0.05	µL
Freezable water	0.0469	µL
Freezable water in mg	0.0469	mg of ice

Total pix before drying starts	466873	
1 pixel (a)	1.004E-07	mg of ice

Gradient of linear part (b)	132110	pixel per min (yellow line)
Drying rate (a x b)	0.0133	mg min^{-1}
Drying rate (B)	**0.00080**	**g h^{-1}**

Area of perimeter of sample (A)	0.00342	cm^2
Specific drying rate (B/A)	**0.233**	**g h^{-1} cm^{-2}**





Example drying rate from a 10 mL glass tubing vial is 0.25 g h^{-1}
Vial diameter : 22 mm Internal area : 3.8 cm^{-1}
Specific drying rate : 0.065 g h^{-1} cm^{-2}
Difference due to differences in heat transfer etc.

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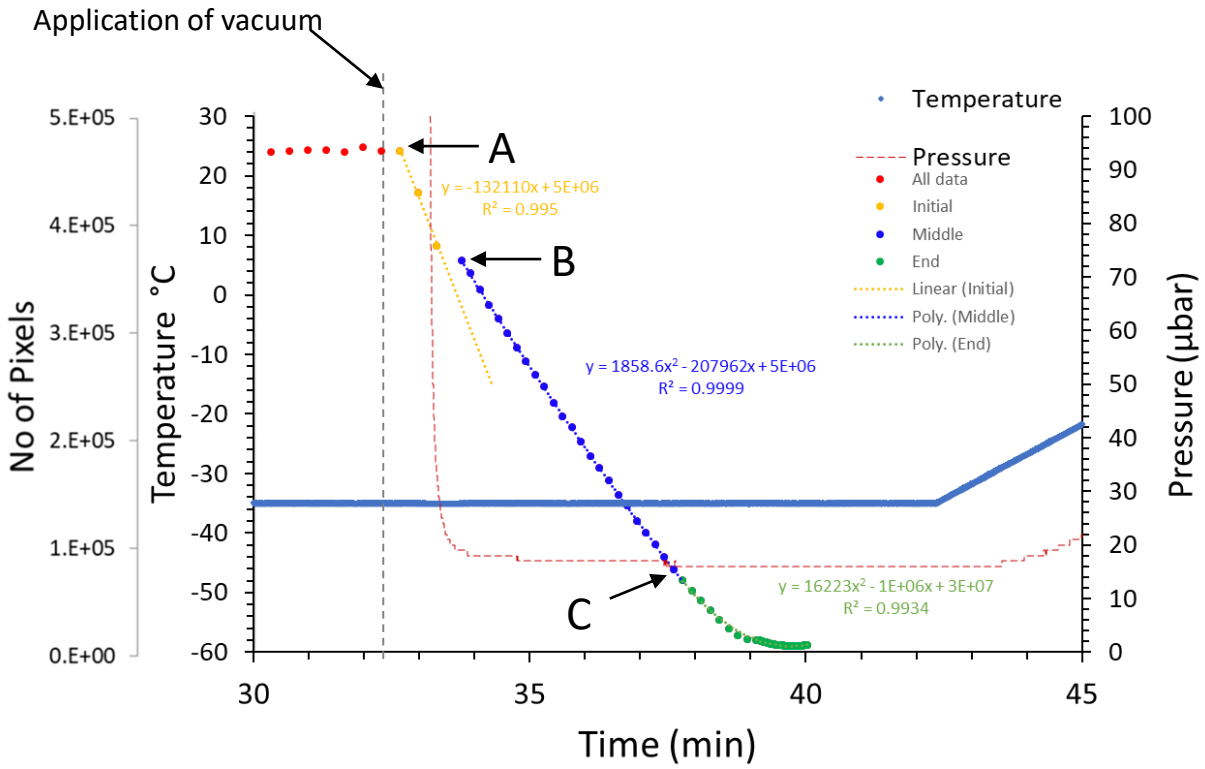
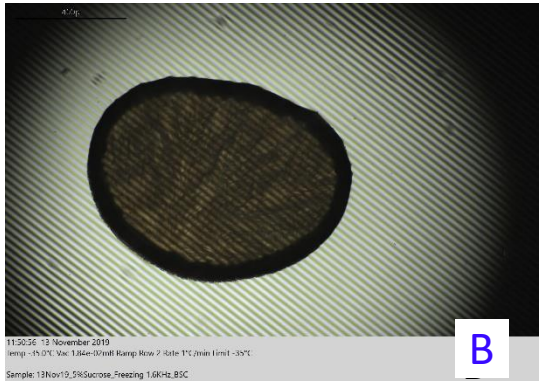
Drying rate at different gradient





Drying Rates

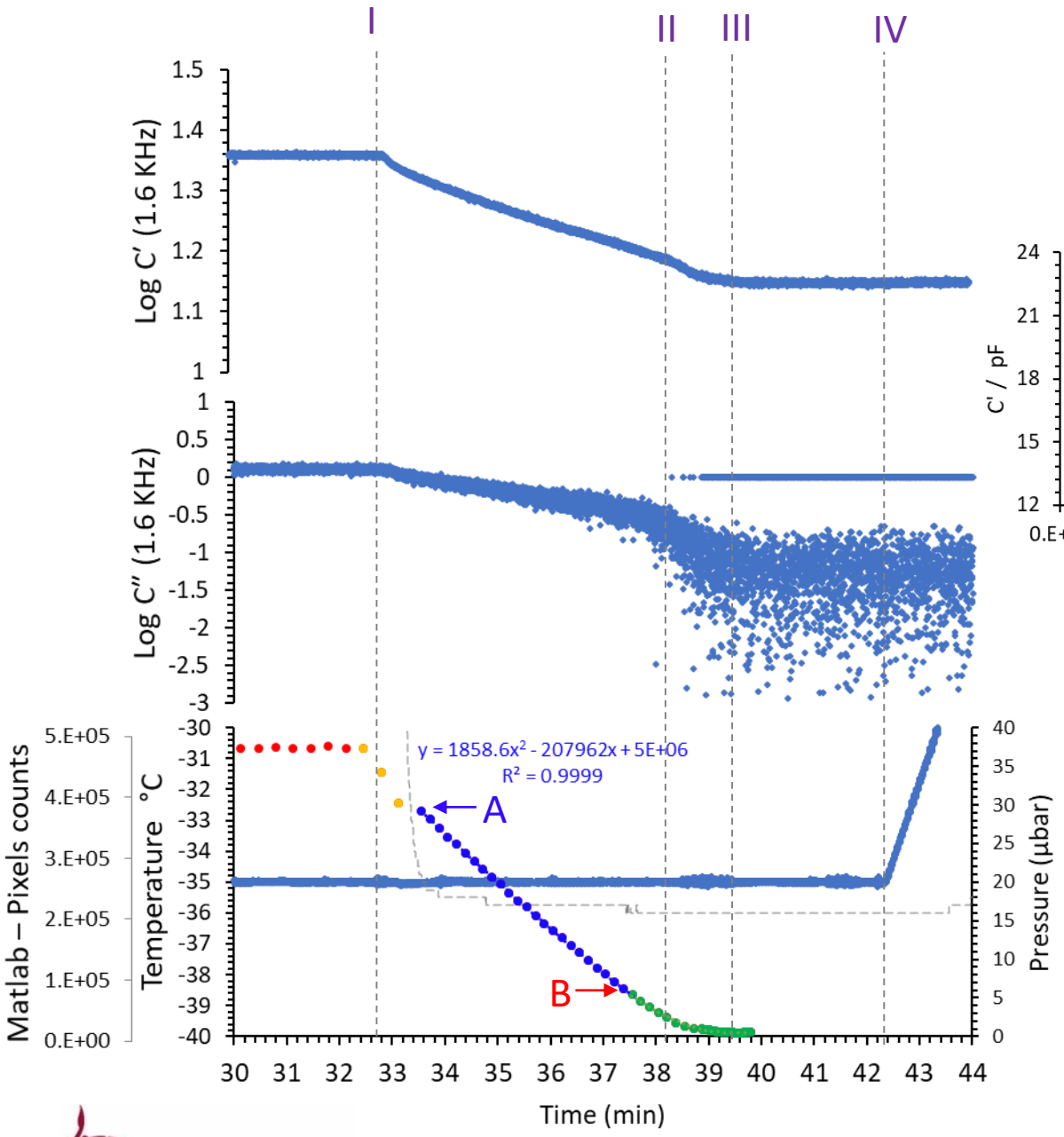
- At A Initial – 0.00080g h^{-1}
- At B: Middle – 0.00050 g h^{-1}
- At C: Middle – 0.00041 g h^{-1}

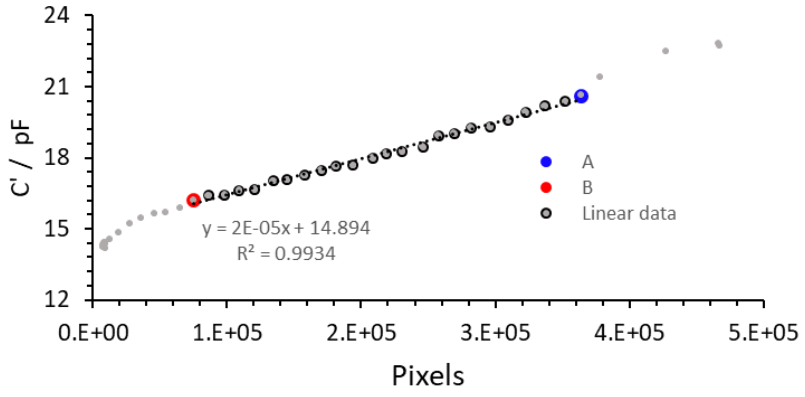
NB: Temperature and pressure measured every 100 ms

Freeze drying of 5% sucrose (0.05µL)

Studied by Z-FDM

Drying: Image analysis vs Capacitance during drying of 0.05 µL 5%sucrose





Linear relationship between real part capacitance C' and pixel count (ice content) confirms the opportunity to use Z-FDM for drying rate estimation

I: Application of vacuum. Primary drying starts
II: Both gradients of imaginary and real part capacitance change towards the end of drying
II : end of primary drying
IV: Ramped to RT and capacitance remains unchanged with temperature

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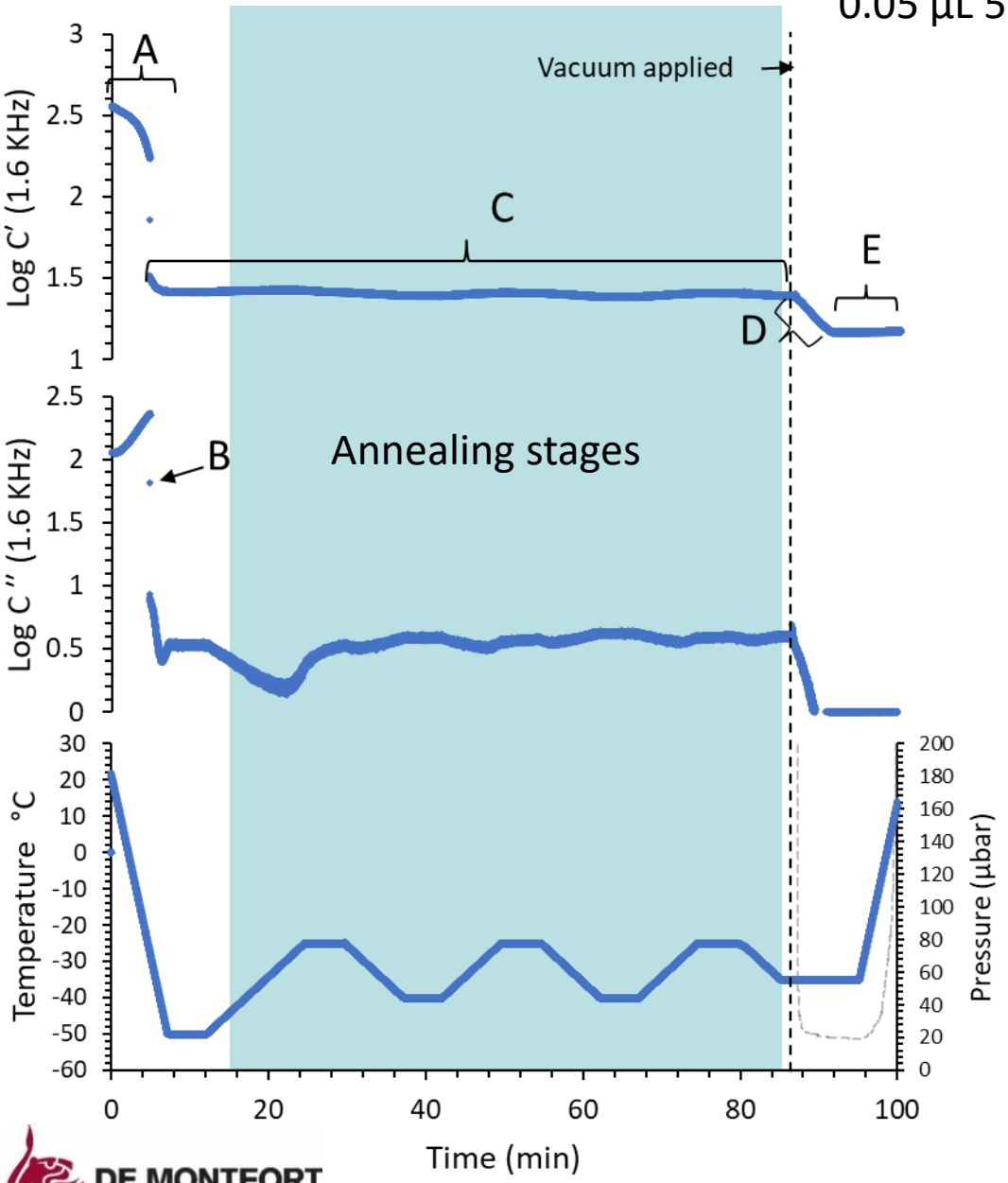
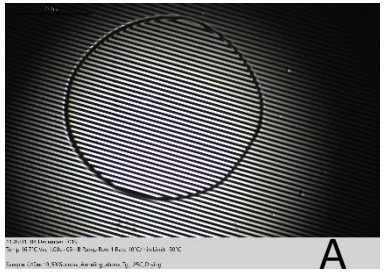
Annealing of 5% sucrose (0.05µL)

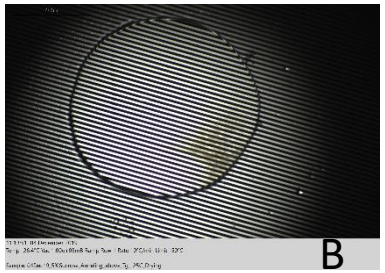
Studied by Z-FDM

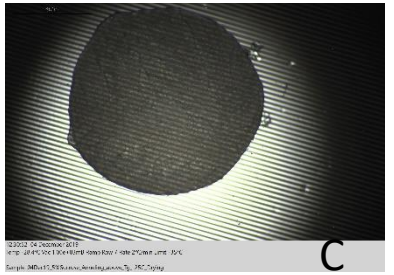
0.05 µL 5%sucrose

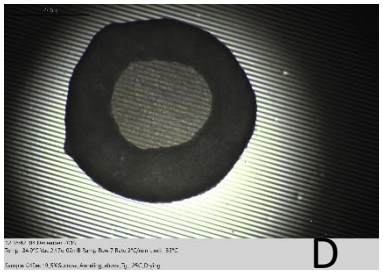
Full process

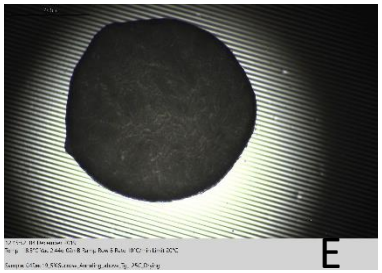












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Capacitance data measured at 1.6KHz

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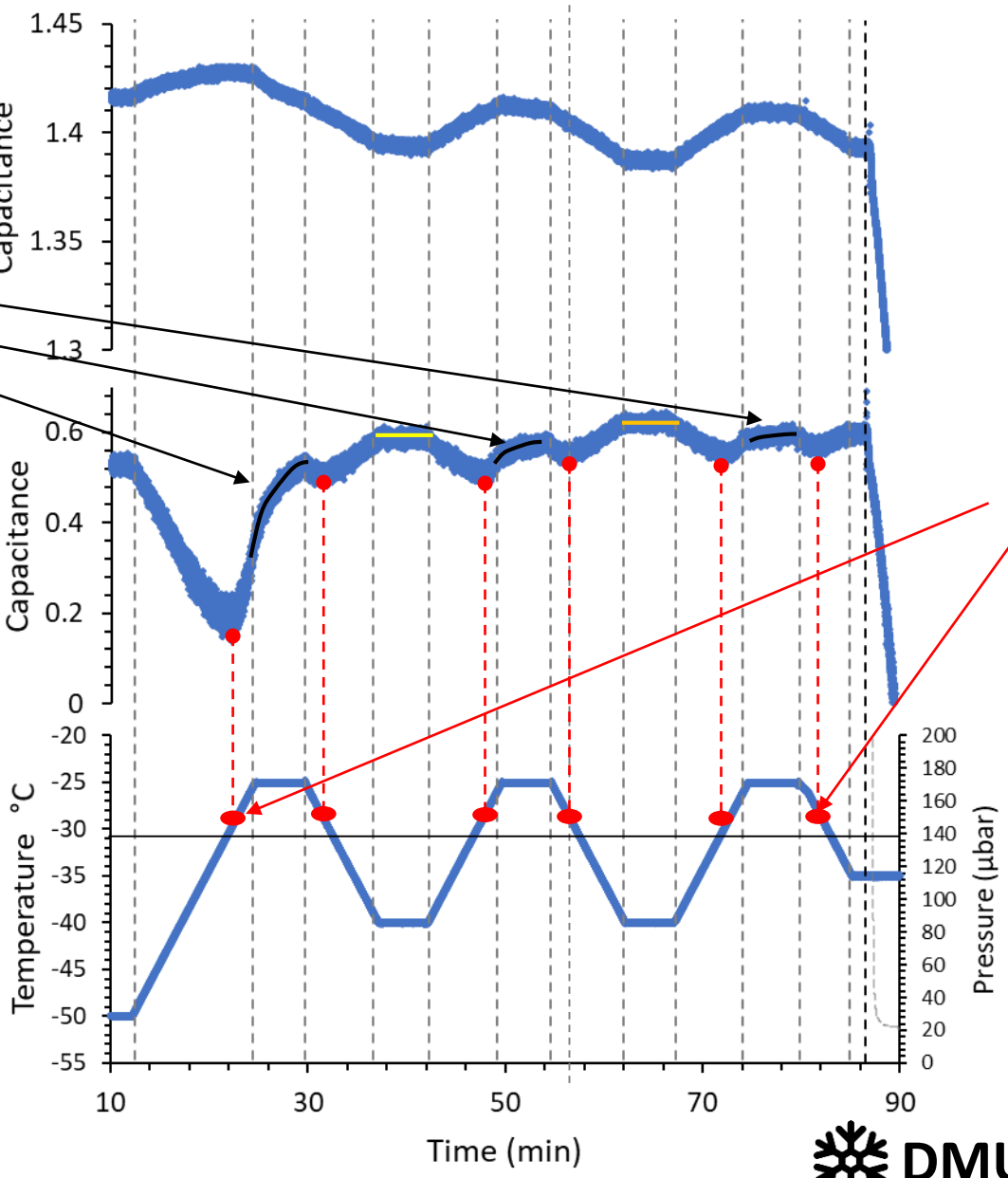
Annealing (1.6KHz)

0.05 µL 5%sucrose

Structural changes on re-heating decrease with each annealing



Excursions in temperature just above the glass transition

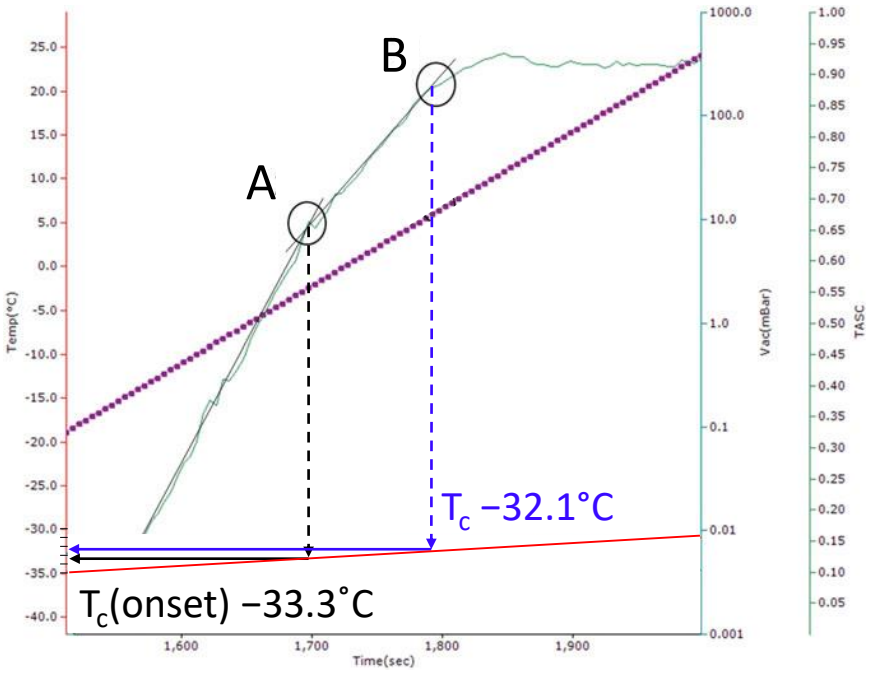
Applications in primary drying : product collapse

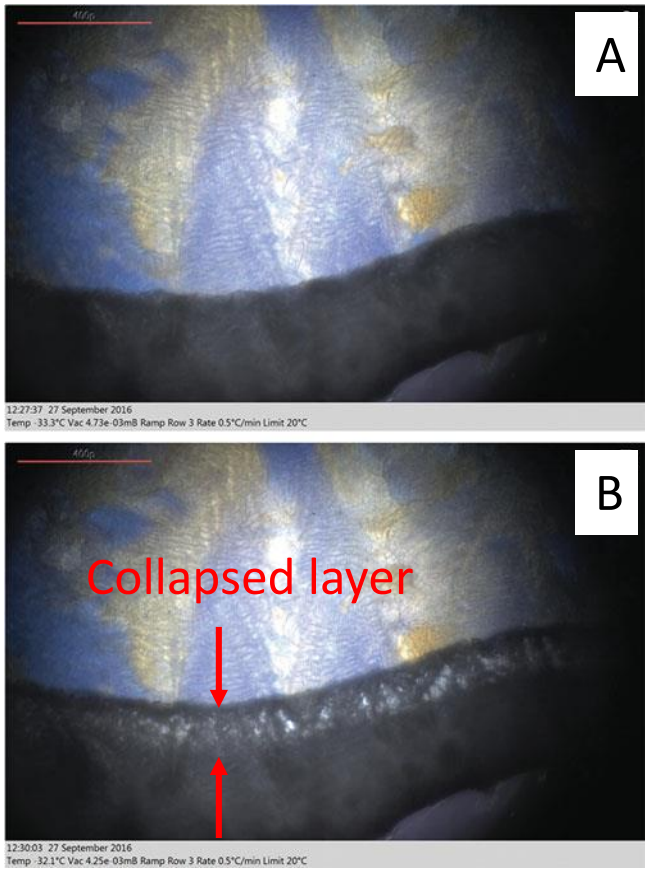
Collapse of 5% sucrose (0.5 µL)

Studied by Z-FDM

TASC – image analysis of sucrose solution

Reduces operator error in the analysis of the the collapse temperature and can use for drying rate.





Adapted from: Ward, K. and Matejtschuk, P., 2019. Chapter 1 Characterization of Formulations for Freeze-Drying In: K. R. WARD and P. MATEJTSCHUK, eds, Lyophilization of Pharmaceuticals and Biologicals: New Technologies and Approaches. 1 edn. New York: Humana Press, pp. 1-33.

Images coinciding with TASC features
(A) onset of collapse at −33.3°C, and
(B) full collapse occurring at −32.1°C

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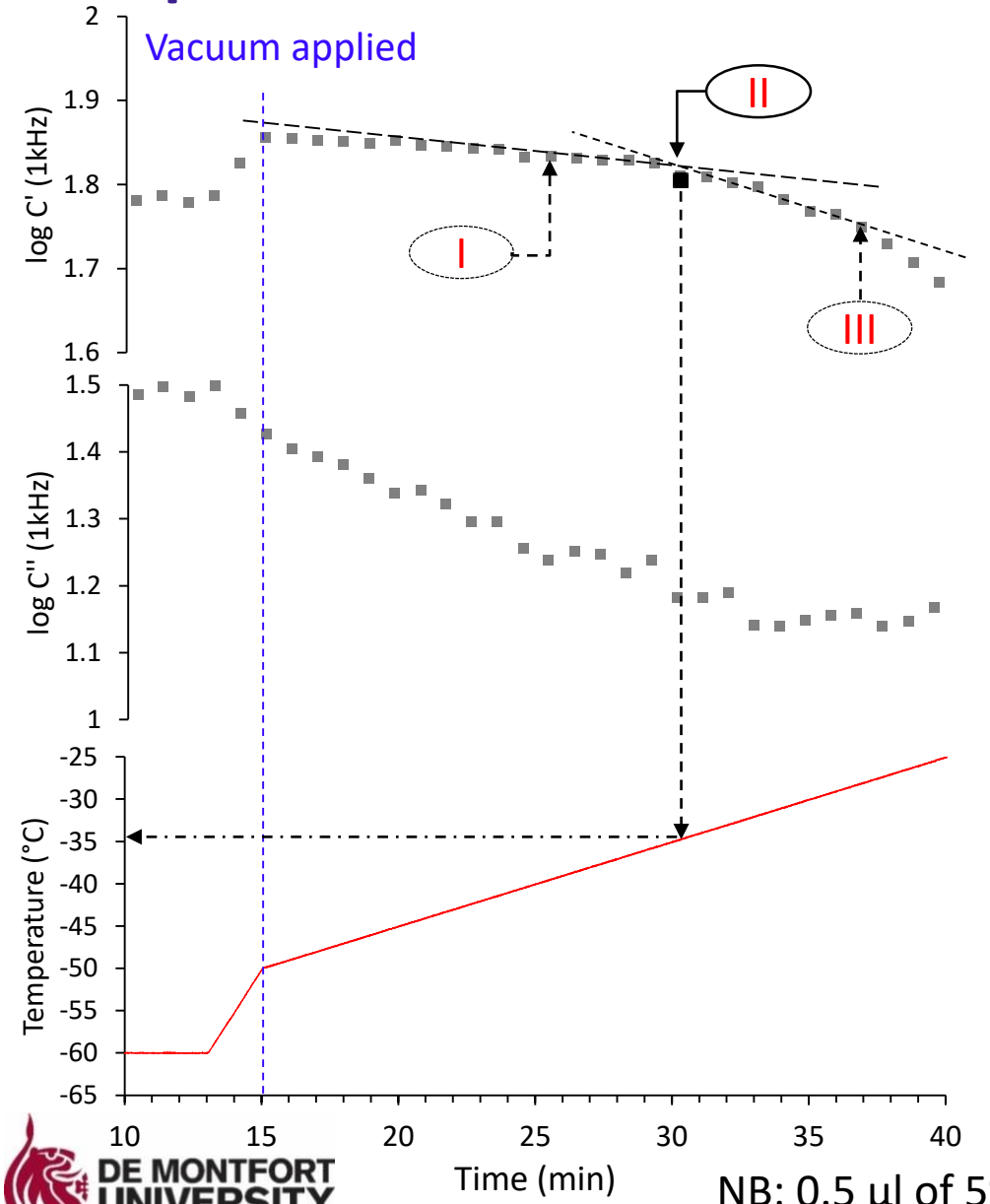
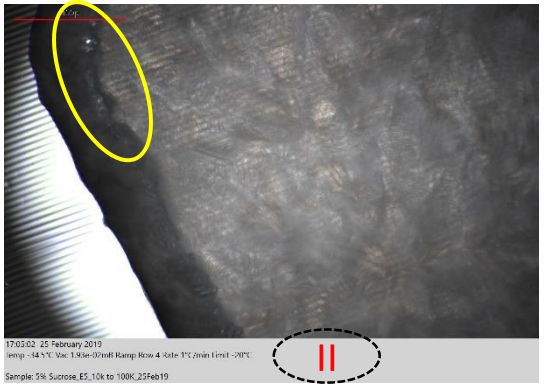
Collapse Observation at 1 kHz





NB: 0.5 µl of 5% Sucrose soluiton

Take home messages (from measurements at 1.6 KHz)

- Real and imaginary part capacitances can be used for the determination of ice nucleation and ice growth rates
- Pixel analysis works for drying rate determination (TASC can also be used)
- Real capacitance has a linear relationship with pixel count, and hence ice mass, so can be used for drying rate determination
- Imaginary part capacitance can be used to study the annealing process but requires further work in order to be able to determine the glass transition temperature.
  - Selection of a higher measurement frequency is likely to provide the answer to the glass transition temperature assessment
- Step changes in drying rate (observed from real part capacitance) can be used to determine the collapse temperature (in a similar way to TASC)
- Relevance of the results is questionable because of differences in sample size, heat transfer etc. to product container

Acknowledgements, Recent Projects & Collaborators

- De Montfort University, School of Pharmacy
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  - Neill Horley. Senior Lecturer
  - Glen McCann: Lecturer
- University College London
  - Prof. Paul Dalby
- Biopharma Process Systems
  - Kevin Ward

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