Application of a novel impedance-based freeze-drying microscope for formulation development

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INTRODUCTION

Over the past few decades, a number of analytical instruments have been developed for the characterization of product formulations intended for Lyophilisation. Freeze-drying microscopy (FDM) is now used routinely to determine the critical temperature (Tc) at which the product may collapse during primary drying whereas a combination of differential thermal analysis (DTA) with electrical impedance analysis has been used to study the critical temperatures of a sample in a frozen state (i.e. the glass transition, ice crystallisation and eutectic melting temperatures). In this study, a combination of impedance spectroscopy with freeze-drying microscopy (Z-FDM, G. Smith, 2019, *doi.org/10.21253/DMU.7980071.v1*) has been reported for the first time.

MATERIALS, INSTRUMENTS AND METHODS

Materials/Instruments

A. 5% w/v solution of sucrose (Sigma-Aldrich)
B. Lyostat 5 freeze drying microscope (Biopharma)
C. ED-IDE1-AU gold interdigitated electrode (Micrux)
D. ISX3-mini Impedance analyser (Sciospec)

Z-FDM Experimental parameters

Experiment 1: Freezing and drying study of 5% sucrose solution

Step	Rate (°C/min)	Limit (°C)	Time (hh:mm:ss)	Vacuum (mbar)	Image capture delay (s)
1	10	-42	00:05:00	1E+3	0.1
2	1	-42	01:40:00	1E-3	30
3	10	+20	00:00:00	1E-3	0.1

Impedance measured at set frequency at 1.6 kHz

Experiment 2: Collapse study of 5% sucrose solution

Step	Rate (°C/min)	Limit (°C)	Time (hh:mm:ss)	Vacuum (mbar)	Image capture delay (s)
1	10	-60	00:05:00	1E+3	1
2	5	-50	00:00:00	1E-3	1
3	1	+20	00:00:00	1E-3	1

Impedance measured at frequency range 10 Hz - 100 kHz

Z-FDM ***Gold electrode integrated into the FDM stage*** ***Interdigitated electrode***

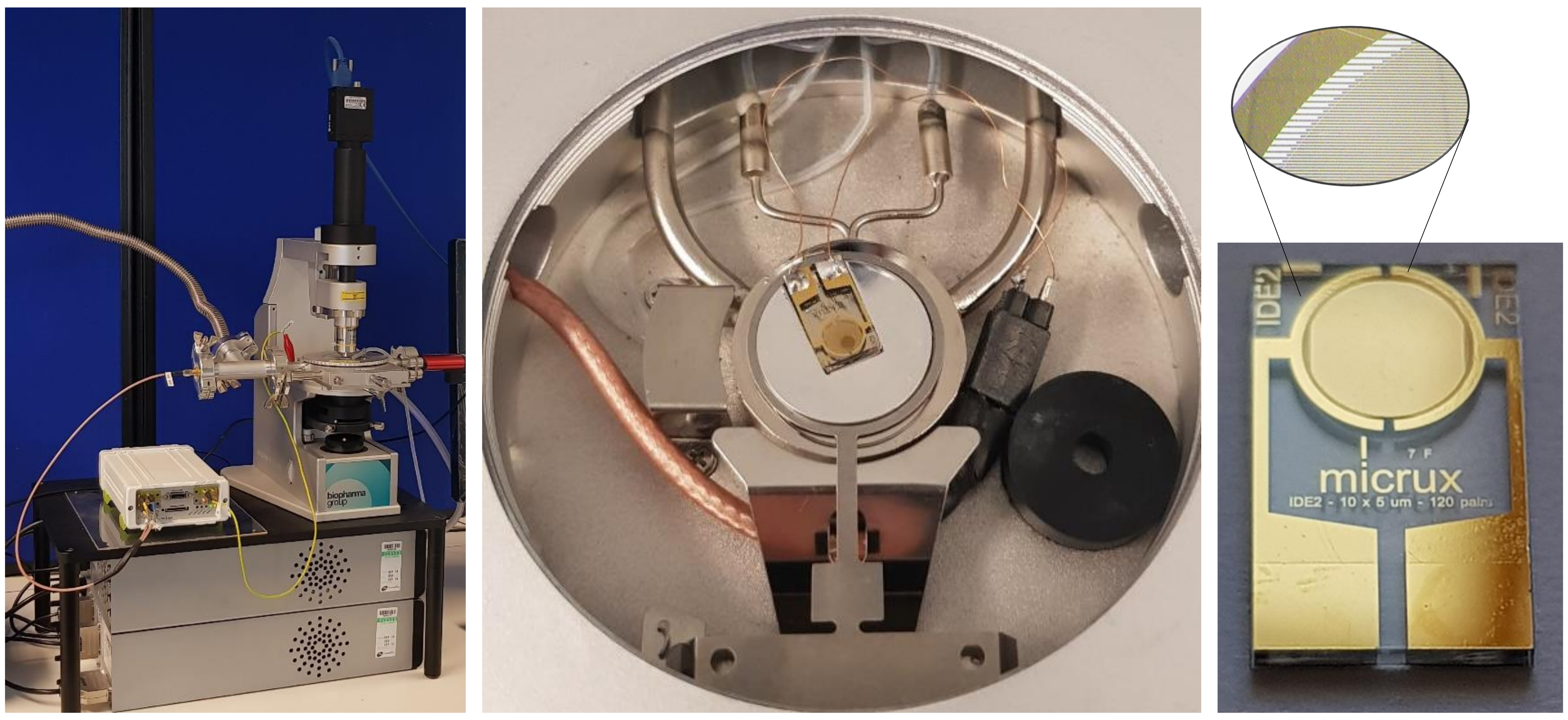


Figure 1: Key components of the Z-FDM study. From left to right: Z-FDM system, integrated FDM stage and Z-FDM electrode

RESULTS

Experiment 1: ***Freezing & drying*** of 5% w/v sucrose solution simulated on Z-FDM (figure 2) enabled to analyse both optically and with impedance method using 0.5 μL of the sample.

Key: Freezing and Nucleation | Drying | Ramp to RT

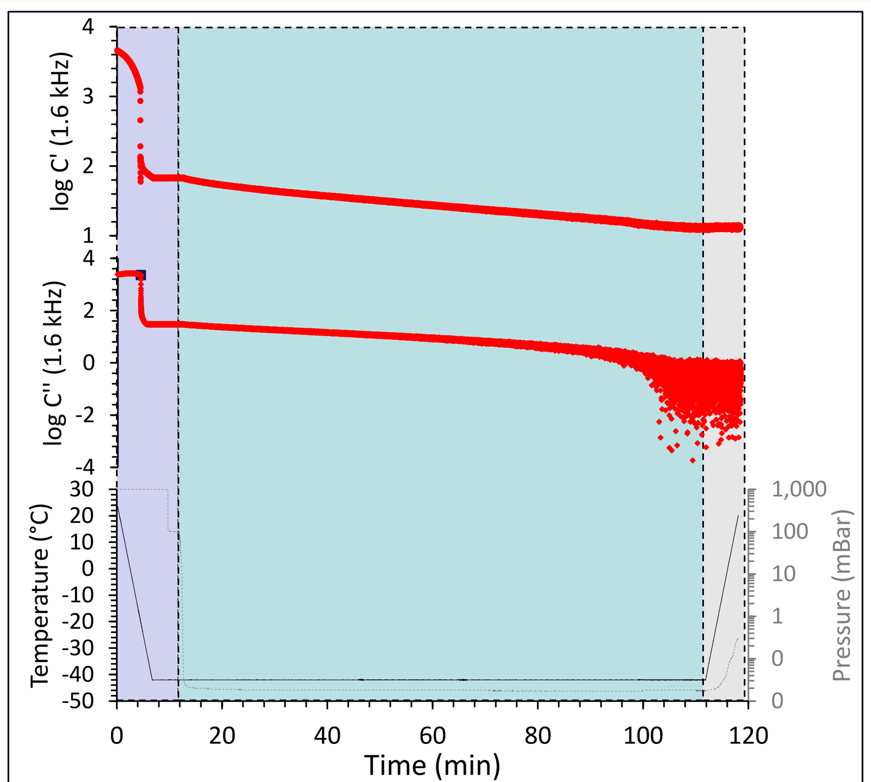


Figure 2: Real and imaginary capacitance of 5% w/v sucrose solution freeze-drying experiment measured at fixed frequency of 1.6 kHz.

Nucleation (below): Z-FDM is sensitive and captures minute changes in the freezing step. Impedance records the change in liquid, i.e. onset and end point of freezing when these two processes are outside the view of the microscope (figure 3).

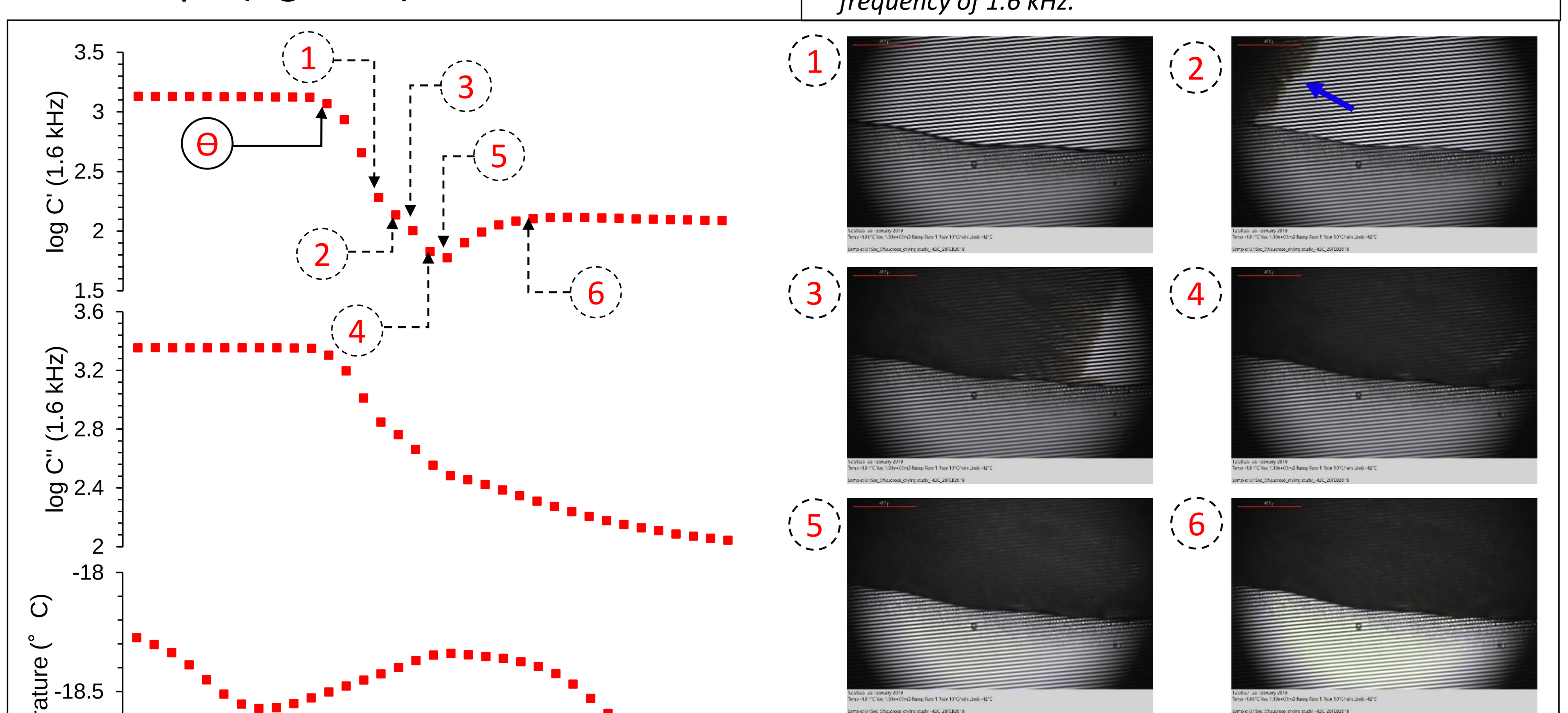
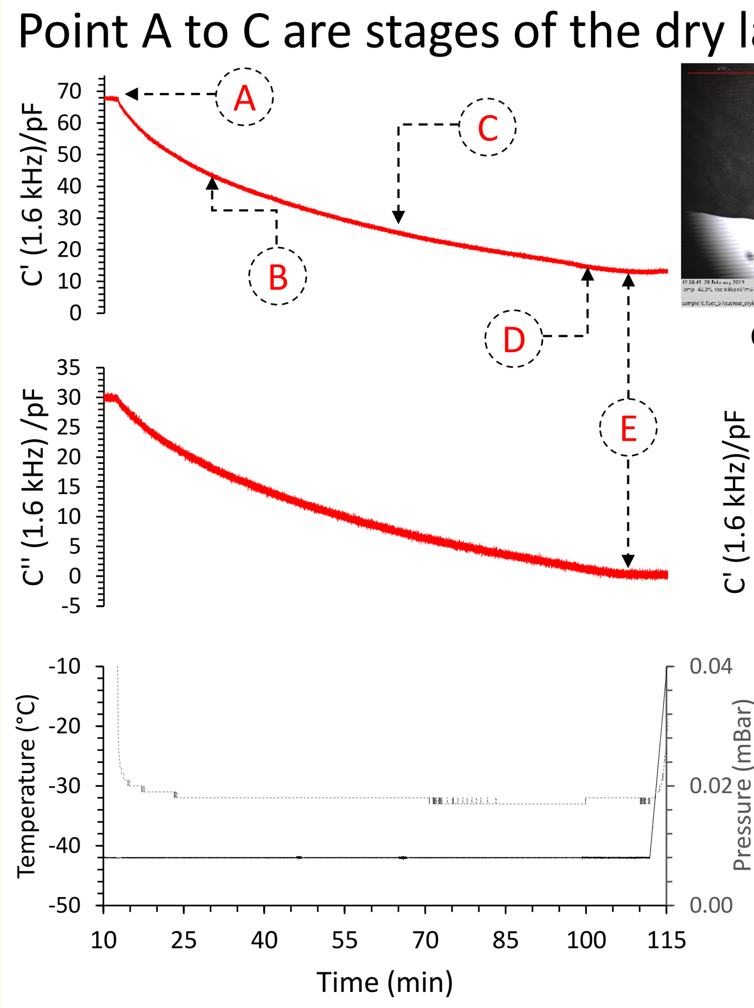
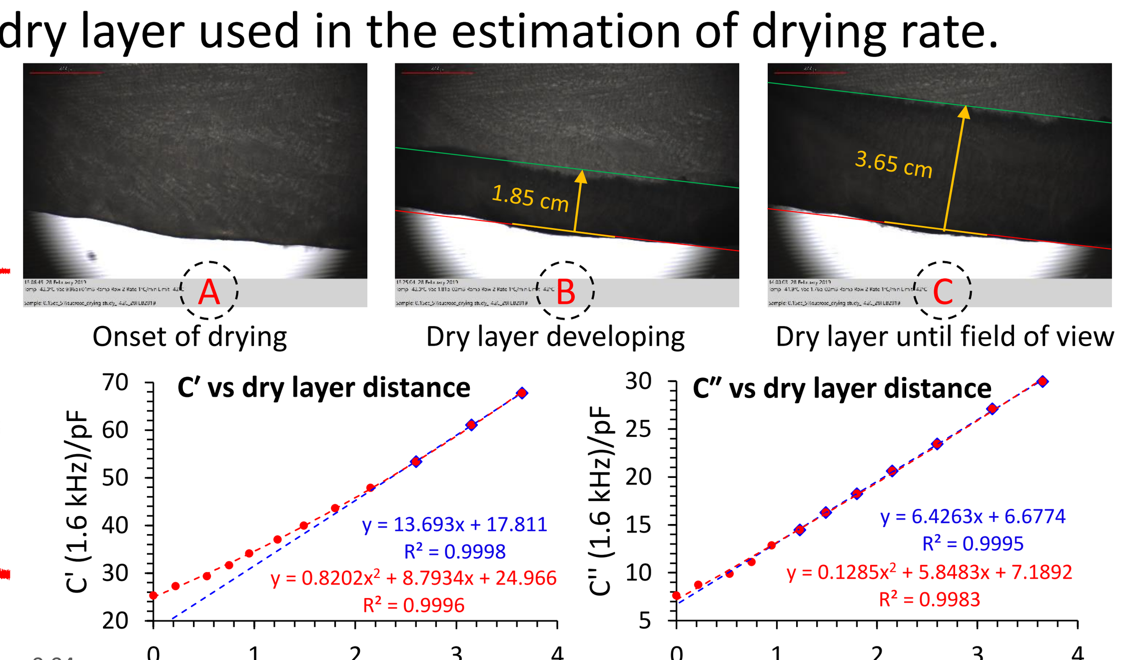


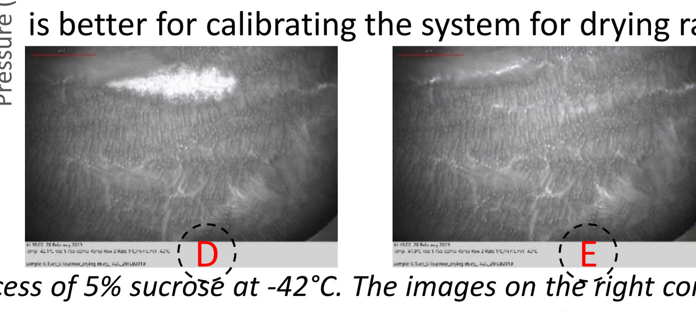
Figure 3: The images (1-6) corresponds to the individual data point on the left impedance profile. Ice crystal growth front apparent at point 2 (see blue arrow on image 2) but nucleation had already been detected by impedance signal at Point ϴ. At point 5, there is a change in the slope of log C′ as this is the end of ice formation & at point 6 is solidification end-point.

During drying, C′ and C′′ decrease continuously until the ice disappears (point E). Point A to C are stages of the dry layer used in the estimation of drying rate.



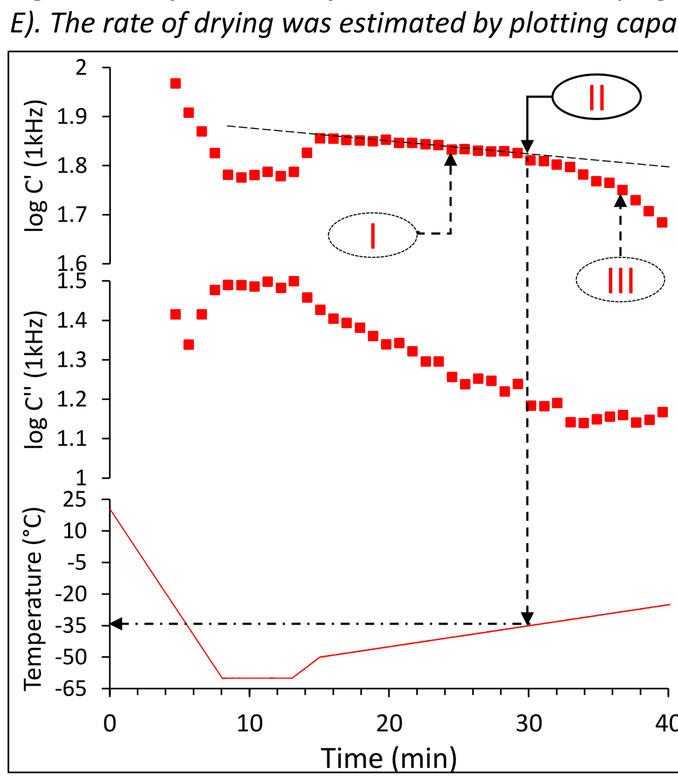


NB: Distance is an arbitrary unit. The parameter C″ has a linear relationship with distance – possibility indicating that this parameter is better for calibrating the system for drying rate.



Ice interface disappears from view but impedance continuous to record the rate of drying.

Figure 4: Profile on the left demonstrates the drying process of 5% sucrose at -42°C. The images on the right correspond to date point (A-E). The rate of drying was estimated by plotting capacitance vs dry layer measured using FDM image from point A to C.



Experiment 2:

In a separate study (table 2, fig. 5), the onset of ***collapse*** for the 5% sucrose solution was recorded at -34.5°C by a change in gradient of the real capacitance at 1kHz (point II).

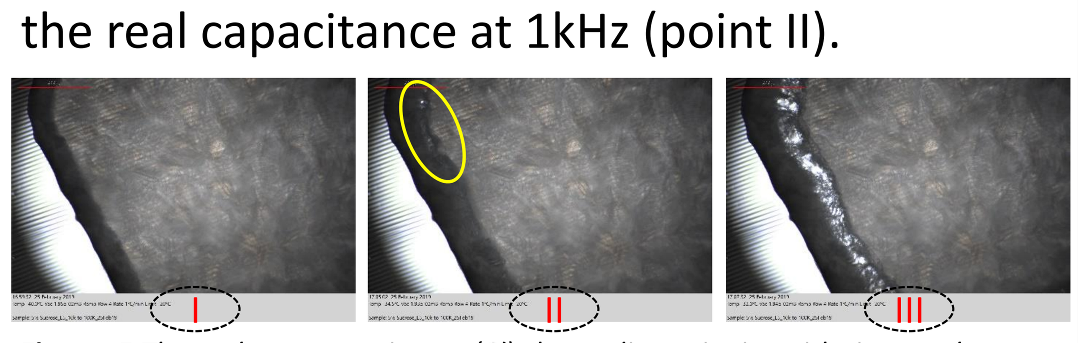


Figure :5 The real part capacitance (C′) shows discontinuity with time at the onset of collapse. [Tc (°C) by FDM = -32.0°C reported by K. R. Morries et al,. PDA J Pharm Sci Technol. 1994, ***48****(6), 318]*

CONCLUSION

The addition of impedance spectroscopy to FDM has the potential to make the determination of **Tc** less subjective while expanding its application to other critical freeze-drying such as the nucleation temperature (T_n), freezing rate & sublimation rate.

ACKNOWLEDGMENT

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