Prof. Geoff Smith
Leicester School of Pharmacy
gsmith02@dmu.ac.uk

TVIS
Through Vial Impedance Spectroscopy

SMi Lyophilisation Europe

4th July to 5th July 2016,
Kensington, London, United Kingdom

BIOPHARMACEUTICAL QUALITY BY DESIGN

Through Vial Impedance Spectroscopy

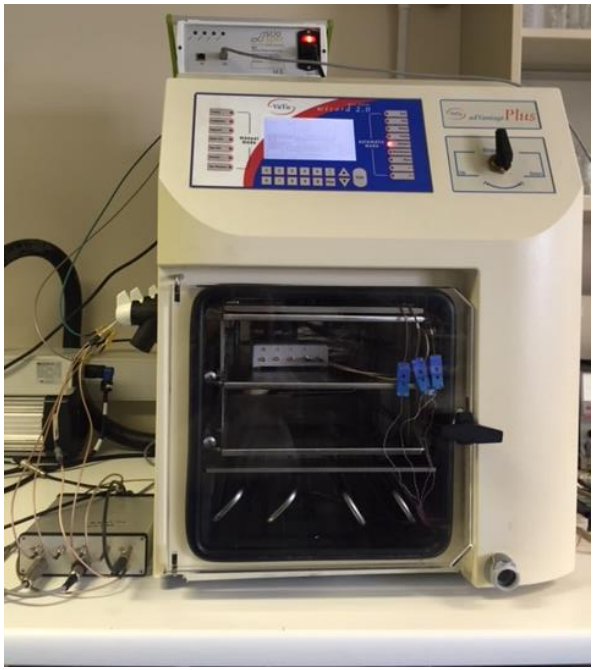
- Impedance measurements across a vial rather than within the vial
- Hence the term Through Vial Impedance Spectroscopy
- Impedance is a frequency dependent parameter largely because the impedance of a capacitance is dependent on the frequency of the applied field (whereas an ideal resistor has zero frequency dependence)
- By fitting the impedance spectrum one can extract the sample resistance and capacitance

TVIS could be deployed at the scales of

1. Product (micro-vial, microplate, or single)
2. Mini-Pilot (Clusters of vials)
3. Batch (Large population /cluster of vials)

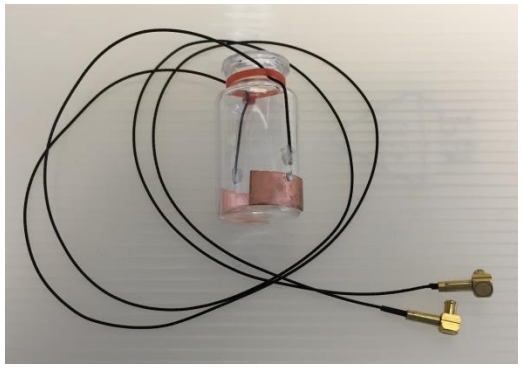
The mesoscale is accessible by assessing the temperature dependence of the impedance

Freeze Dryer and TVIS System



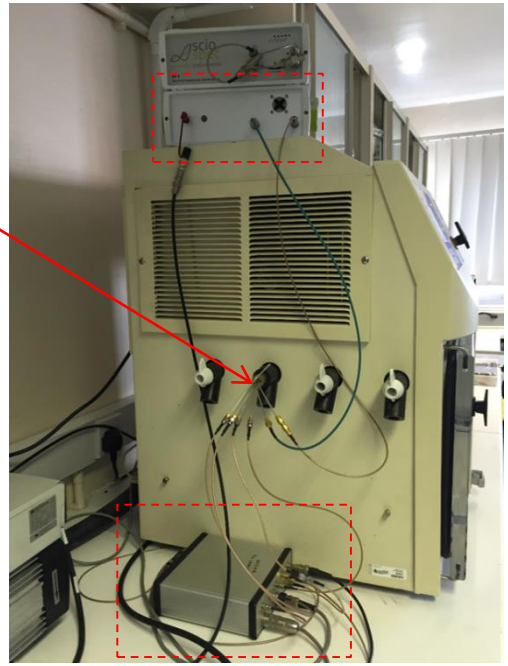
Virtis Advantage Pro
Freeze Dryer

Impedance test vial



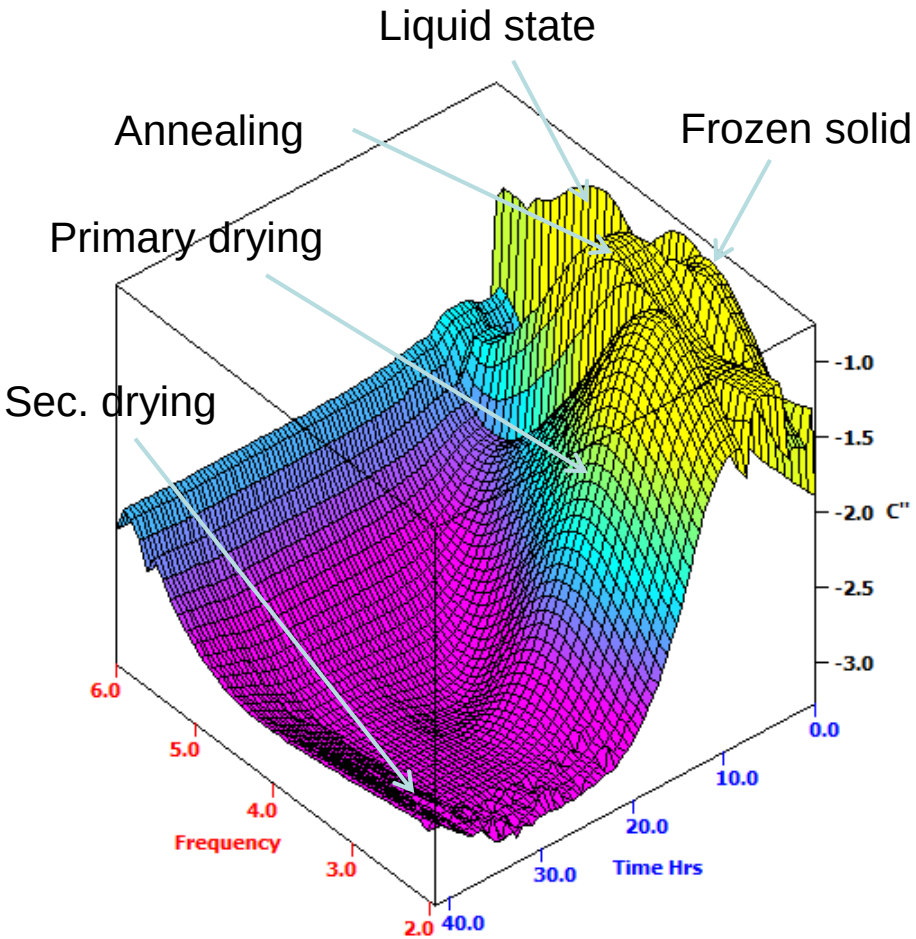
TVIS Systems

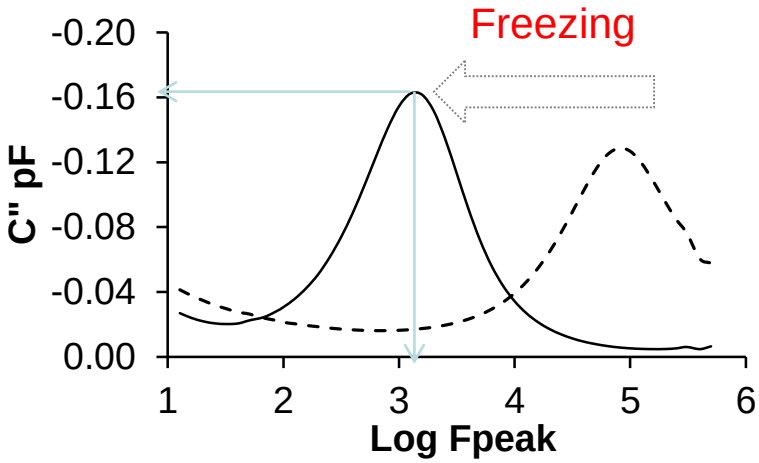
Pass through

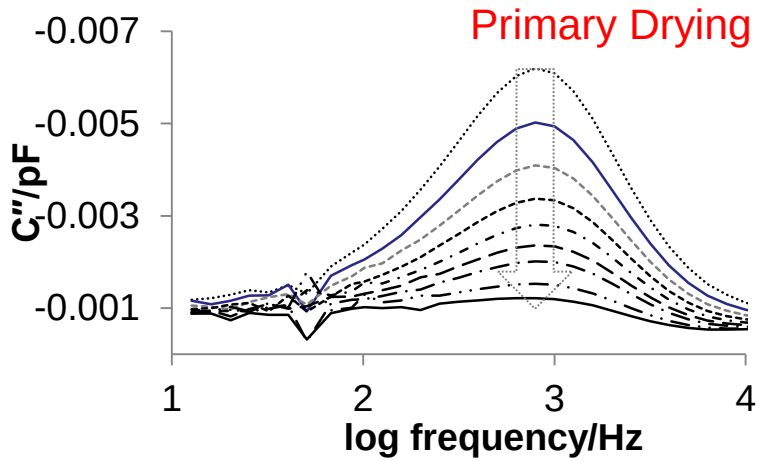


DMU 'LyoDEA'

TVIS response surface

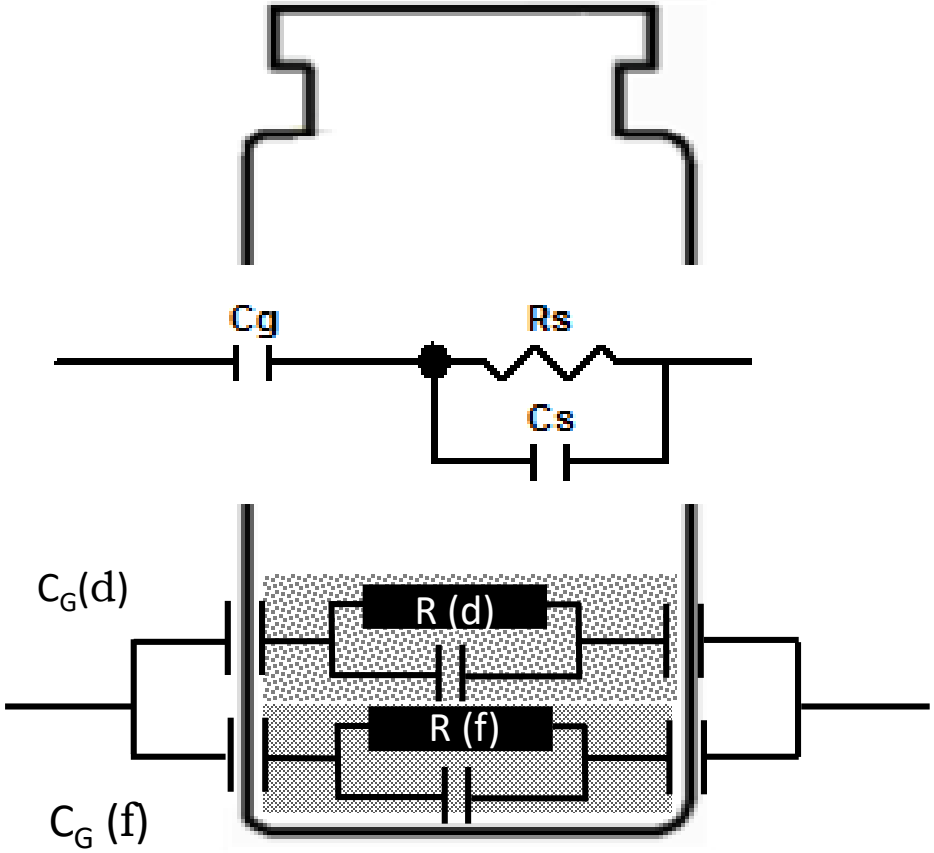






Through Vial Impedance Spectroscopy (TVIS)

- Electrodes attached to the external surface of a vial (either across the vial or on one side) or placed above the vial
- The sample has both resistive and capacitive properties whereas the container and any air space between the sample and the electrodes is predominantly capacitive in nature
- A typical circuit model would therefore be a capacitor modelling the glass wall (and any air space) and a parallel combination of a resistor and a capacitor modelling the electrical properties of the sample.



f : frozen layer; d : dry layer

The impedance of the model can be calculated from the following equation

$$Z^* = \frac{1}{i\omega C*} = \frac{1}{i\omega C_G} + \frac{1}{\frac{1}{R_S} + i\omega C_S}$$

which re-arranges to

$$Z^* = \frac{1}{i\omega C_G} + \frac{R_S}{1 + i\omega R_S C_S} = \frac{1 + i\omega R_S(C_G + C_S)}{i\omega C_G - \omega^2 R C_G C_S}$$

Impedance can also be expressed in terms of a complex capacitance

$$C^* = C' + C'' = \frac{1}{i\omega Z^*} = \frac{C_G + i\omega R_S C_G C_S}{1 + i\omega R_S(C_G + C_S)}$$

From the complex capacitance formula, the expressions for real and imaginary capacitance can be calculated to explain the origin of interfacial polarization peak. This achieved by multiplying the nominator and denominator by the complex conjugate of the denominator and by grouping the real (C') and imaginary (C'') parts

$$C^* = \frac{1}{i\omega Z^*} = \frac{(C_G + i\omega R_S C_2 C_G)(1 - i\omega R_S(C_S + C_G))}{(1 + i\omega R(C_S + C_G))(1 - i\omega R_S(C_S + C_G))} = \frac{C_G + \omega^2 R_S^2 C_{2G} C_S(C_S + C_G) - i\omega R_S C_G^2}{1 + (\omega R_S((C_S + C_G))^2}$$

To obtain

$$C' = \frac{C_G + \omega^2 R_S^2 C_G C_S(C_S + C_G)}{1 + \left(\omega R_S((C_S + C_G)\right)^2} \quad \text{and} \quad C'' = -\frac{\omega R_S C_G^2}{1 + (\omega R_S((C_S + C_G))^2}$$

Real Part Capacitance

- The value of real part of capacitance at ω→0 is

$$C' = C_{G(fl)} = f(v_{ice})$$

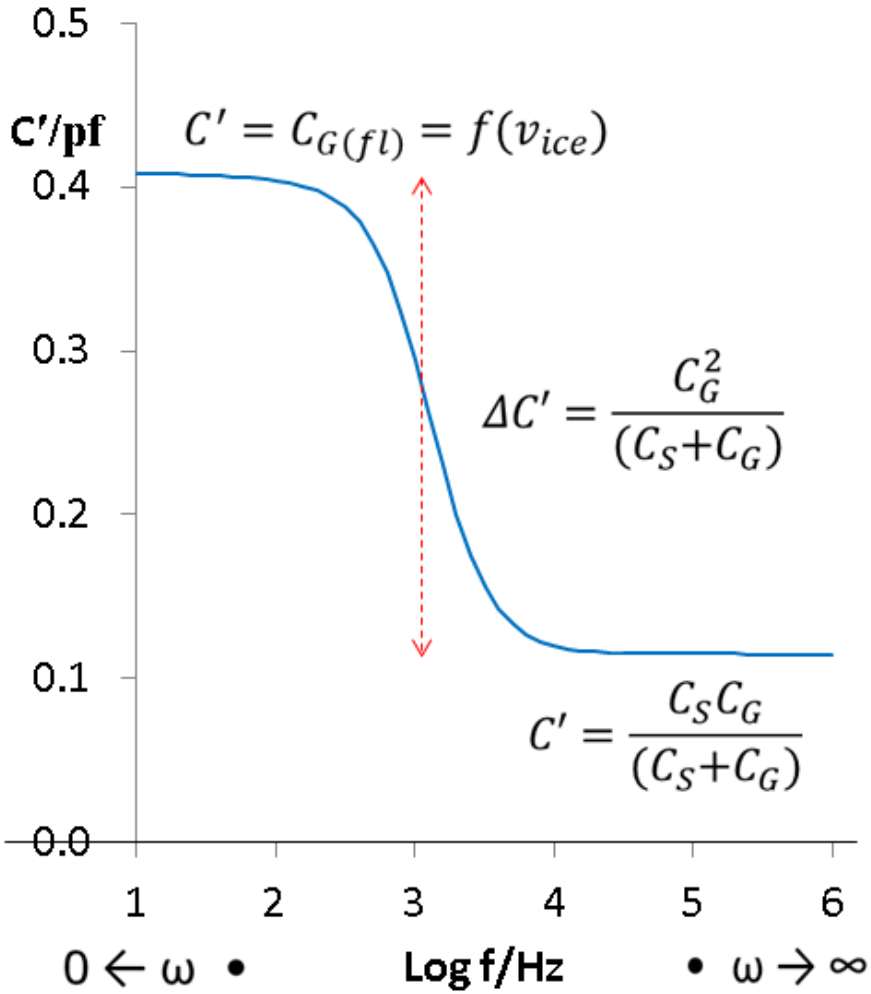
- and value at ω → ∞

$$C' = \frac{C_S C_G}{(C_S + C_G)}$$

- It follows that the step change in capacitance is

$$\Delta C' = C_G - \frac{C_S C_G}{(C_S + C_G)}$$

$$\Delta C' = \frac{C_G^2}{(C_S + C_G)}$$



Real Part Capacitance

Value at ω → ∞

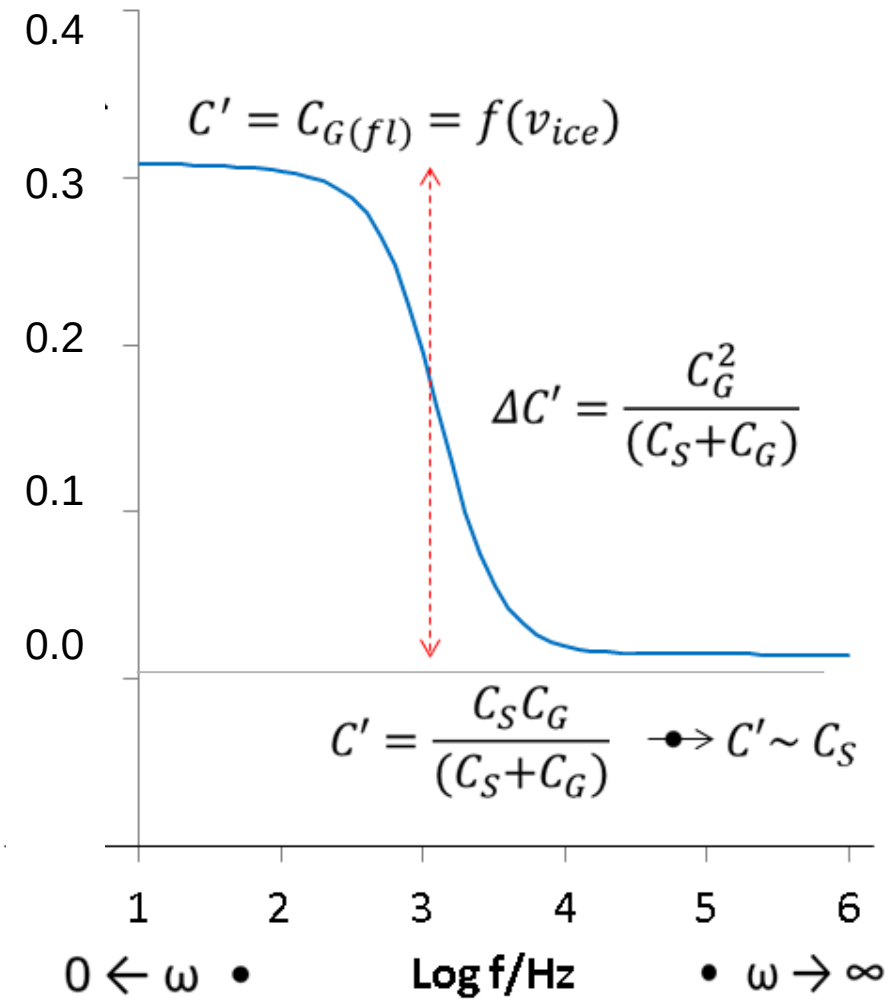
$$C' = \frac{C_S C_G}{(C_S + C_G)}$$

- If $C_S << C_G$ then

$$C' = \frac{C_S C_G}{(\cancel{C_S} + C_G)}$$

$$C' = \frac{C_S \cancel{C_G}}{\cancel{C_G}}$$

$$C'(\omega \to \infty) \sim C_S$$



Imaginary Part Capacitance

At ω → 0, C″ = 0. As the frequency increases, C″ increases to a maximum (C''_{max}) then decreases to 0 as the frequency ω → ∞

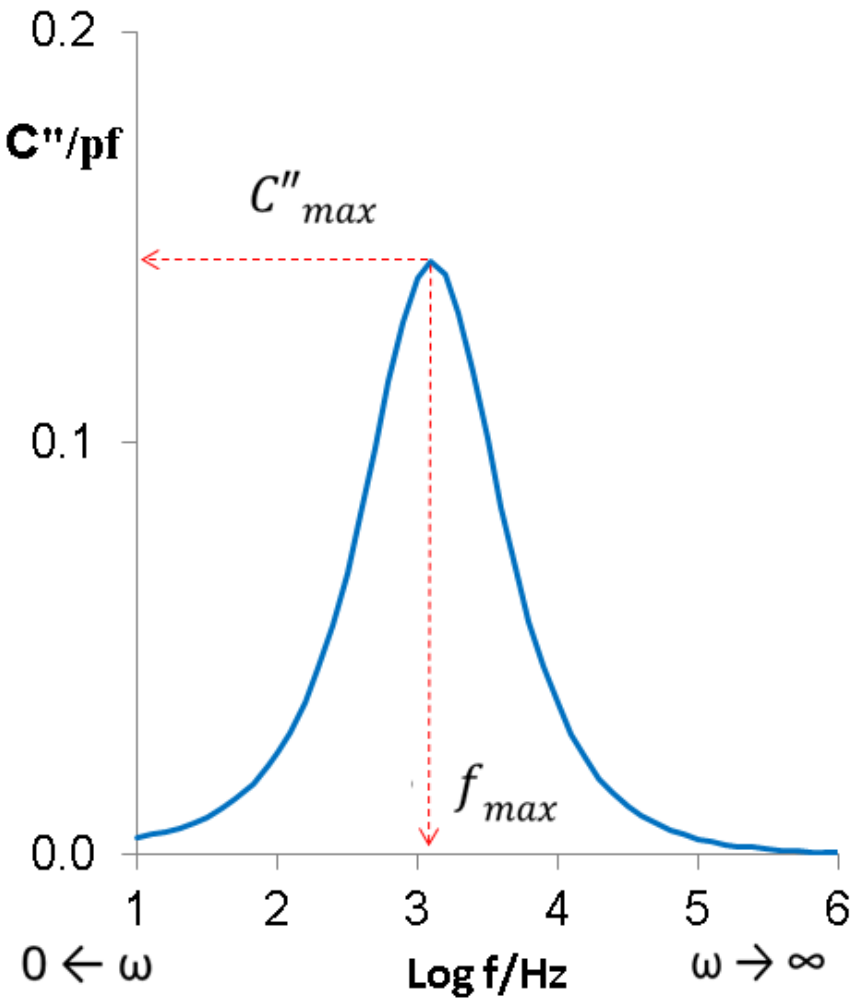
$$C''_{max} = \frac{C_G^2}{2(C_S+C_G)}$$

at a frequency of

$$\omega_{max} = \frac{1}{R(C_S+C_G)} \text{ in radians}$$

Or

$$f_{max} = \frac{1}{2\pi R(C_S+C_G)} \text{ in cycles per second}$$



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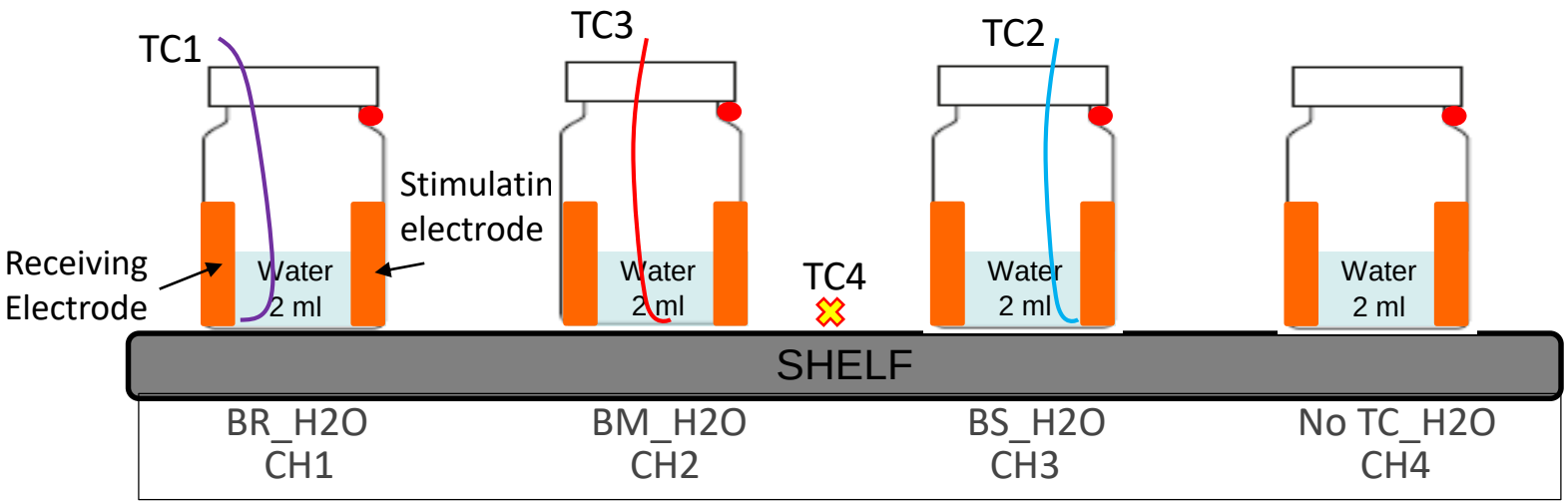
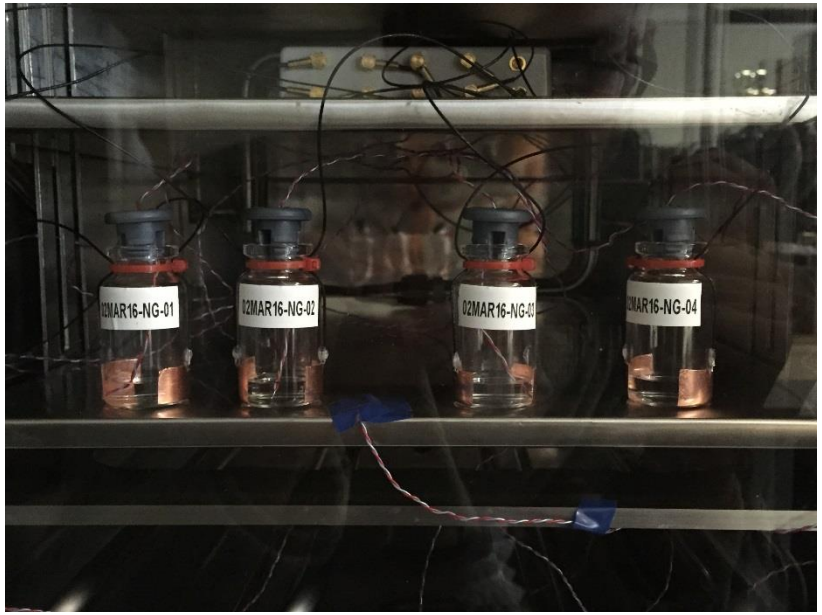
Single Vial Measurements

Freeze-Drying of Water (End point Determination)

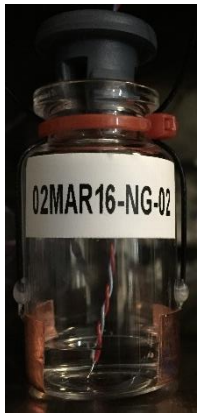
BIOPHARMACEUTICAL QUALITY BY DESIGN

Vial positions in Dryer

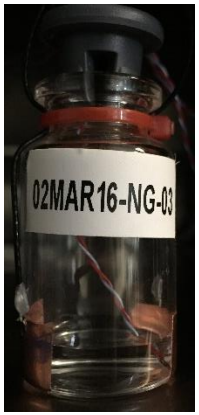




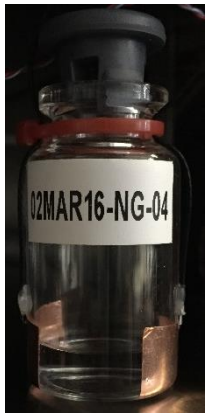
TC1_BR_H2O



TC3_BM_H2O



TC2_BS_H2O

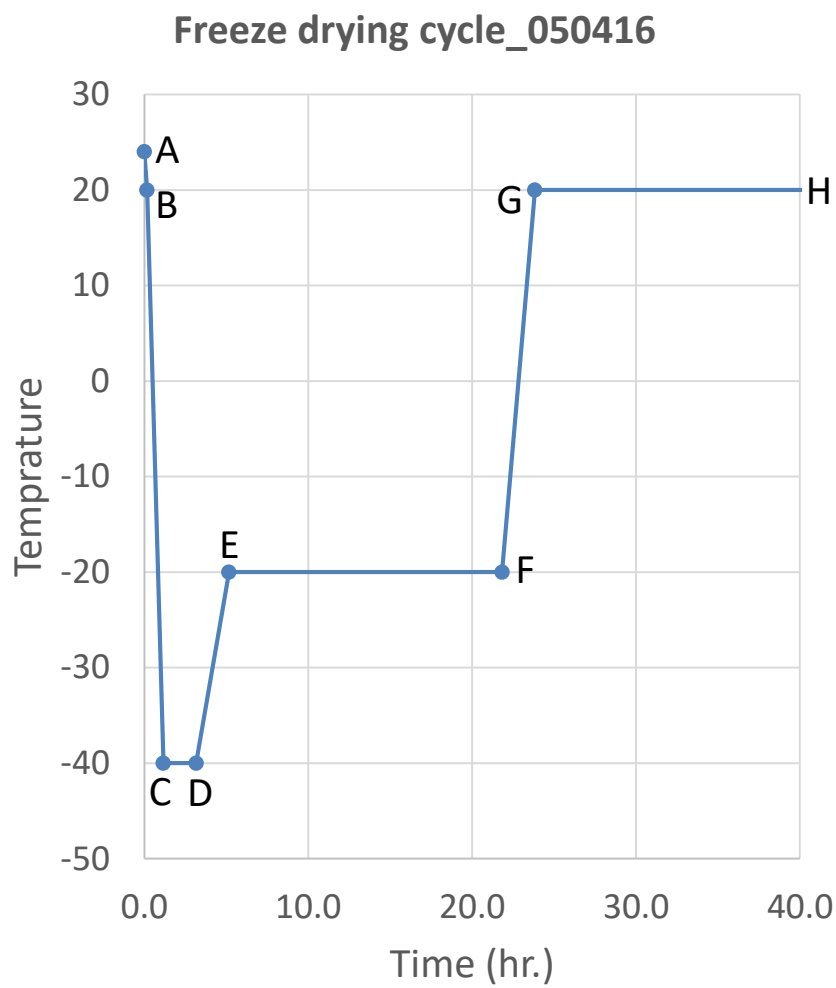


No TC_H2O

Process Cycle Design: Virtis Advantage Plus

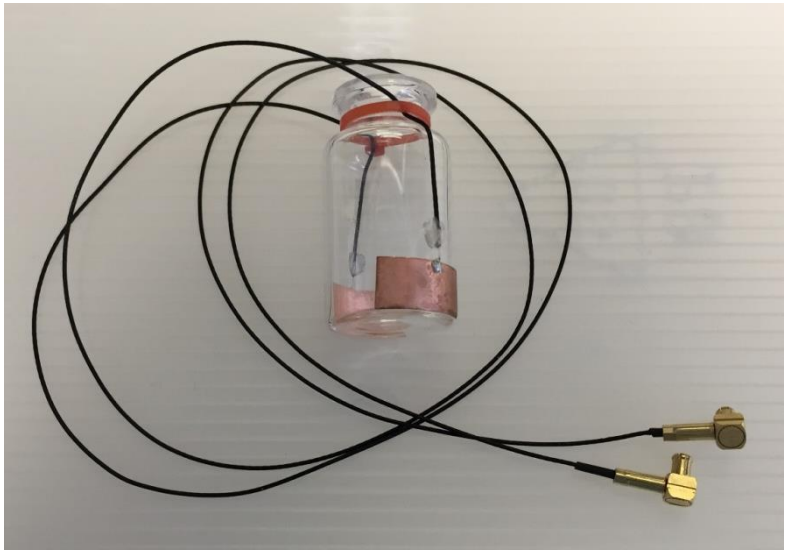
- **Methodology (Freeze Drying Recipe):**
  - Sample : Double Distilled Water

	Time/ min	Time/ hour	Temp/ °C	Ramp /Hold
Initial Condition (A)	0	0	24	-
Incubation (B)	10	0.17	24 to 20	Ramp
Freezing (B-D)	60 (B-C)	1	20 to -40	Ramp
Equilibrium Phase	120 (C-D)	2	-40	Hold
Annealing (D-E)	120	2	-40 to -20	Ramp
Primary Drying (100 µbar) (E-F)	1000	16.6	-20	Hold
Secondary Drying (100 µbar) (F-H)	120	2	-20 to 20	Ramp
	1000	16.6	20	Hold

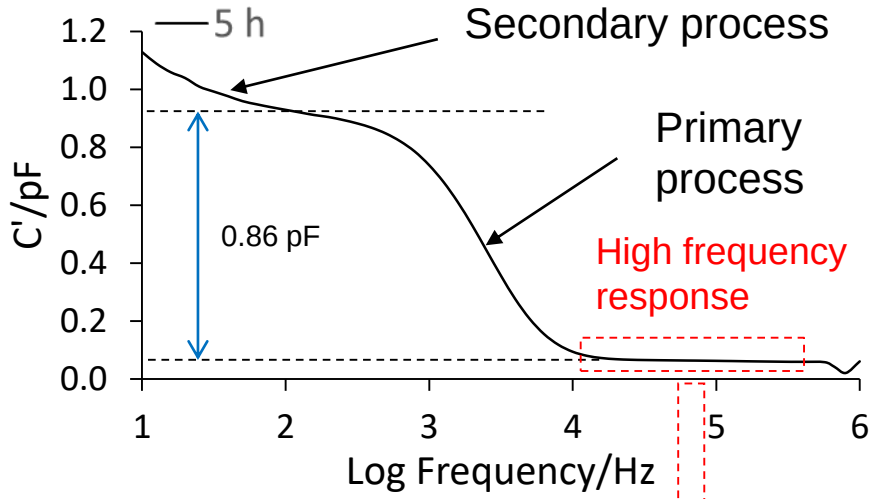


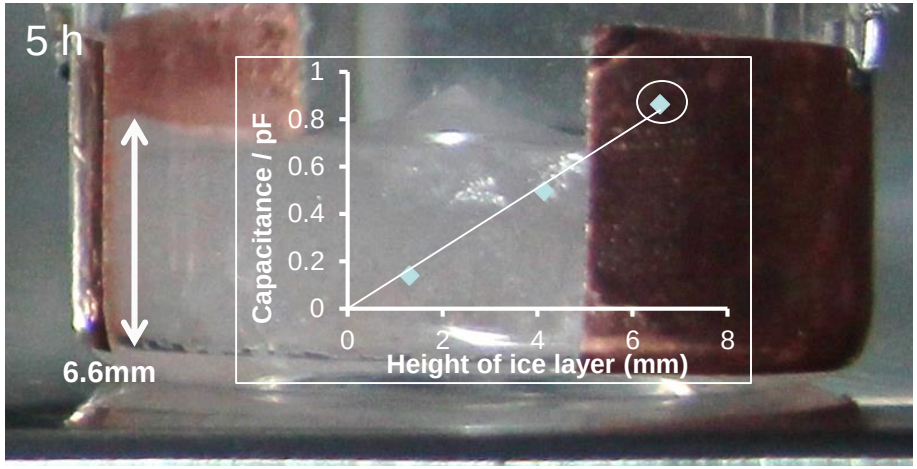
TVIS Parameters

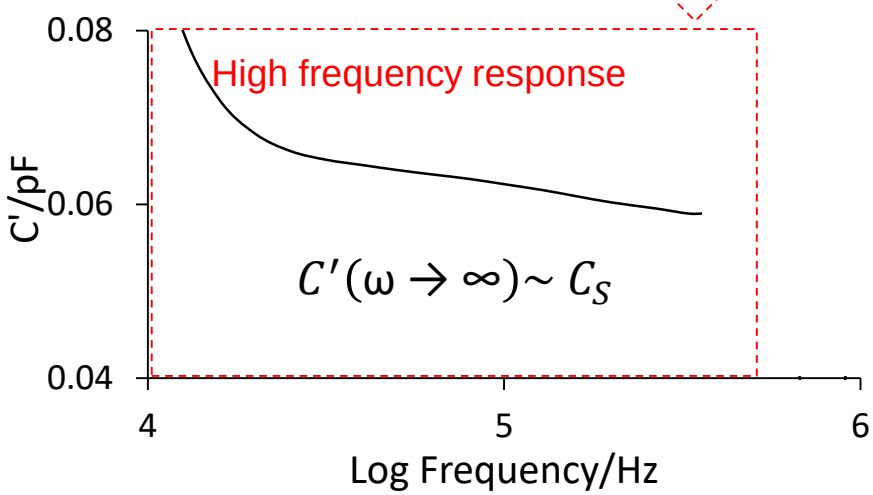
Frequency range	10 Hz to 1MHz
AC Voltage	0.2 to 8 V
Measurement time or Scan Interval	1 spectrum every 2 min
Electrode Design	No Guard Electrode
Electrode Dimensions	Approx. 20 mm (width) 10 mm (height)

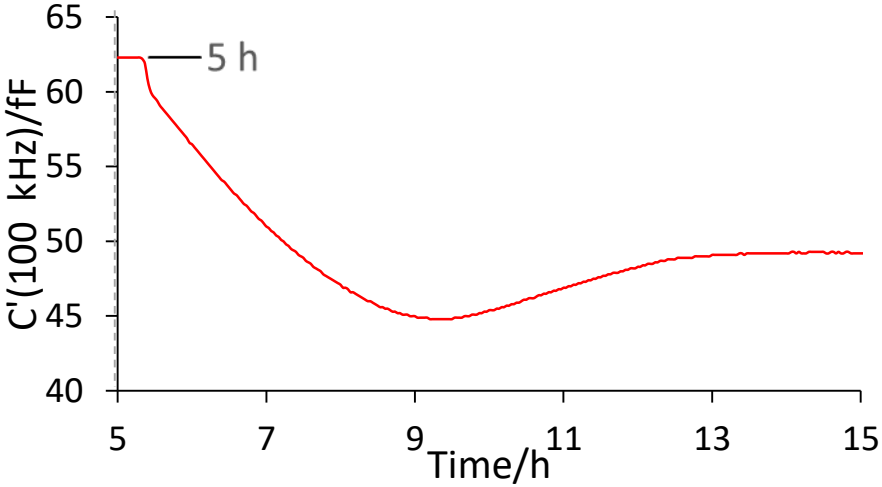


Pre-Primary Drying

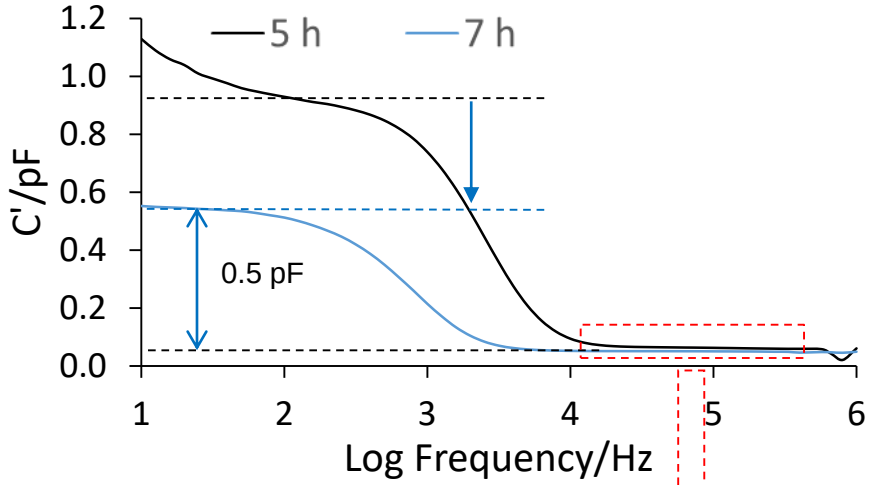


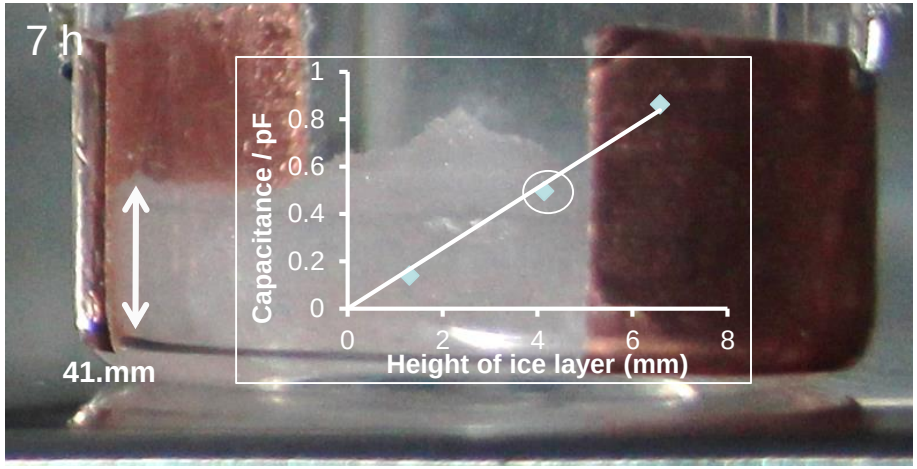


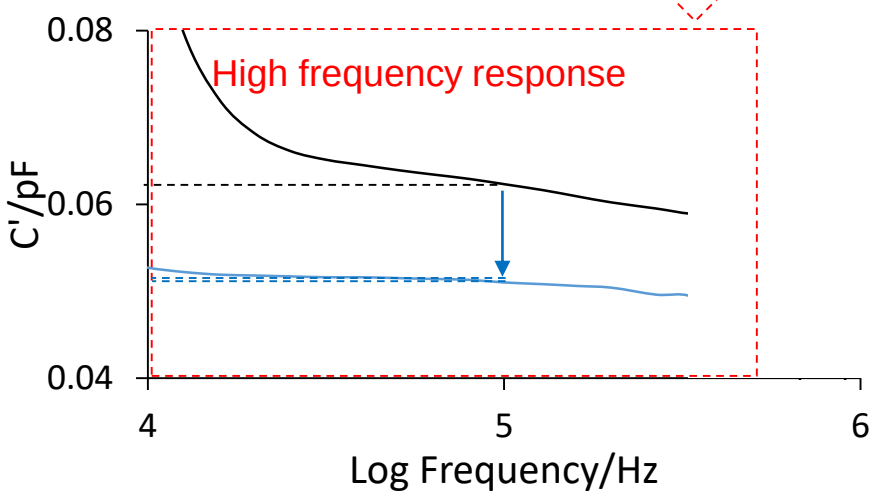


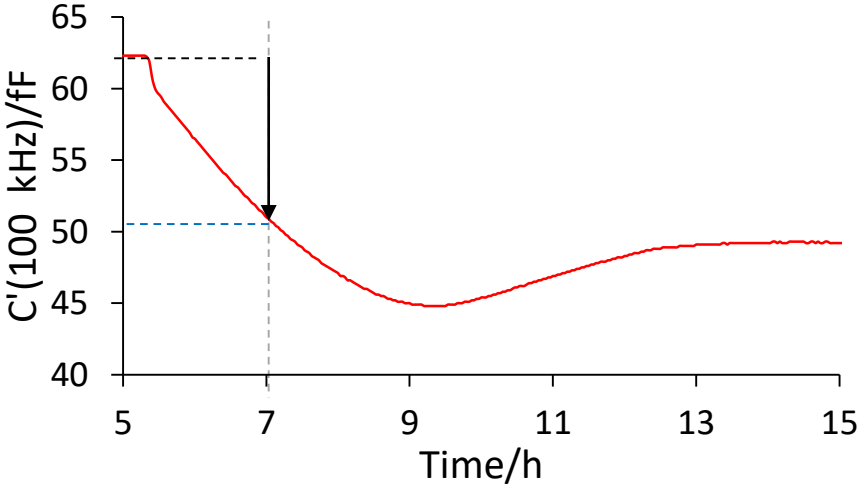


2h of Primary Drying

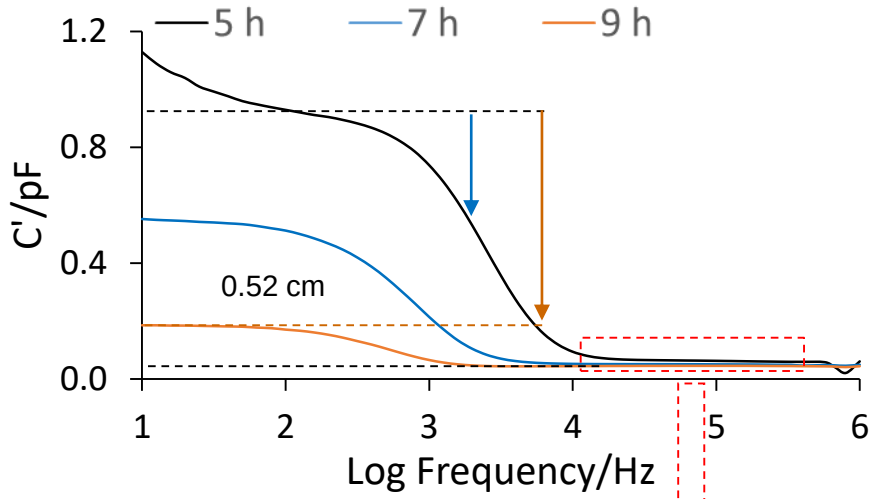


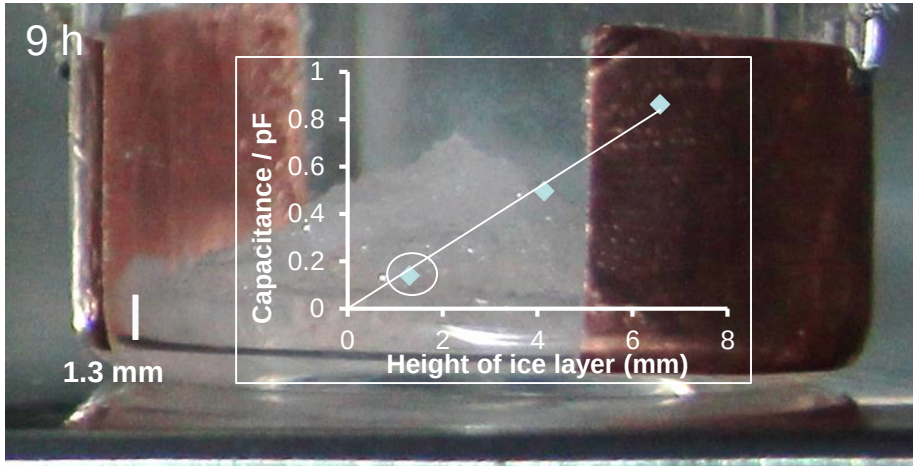


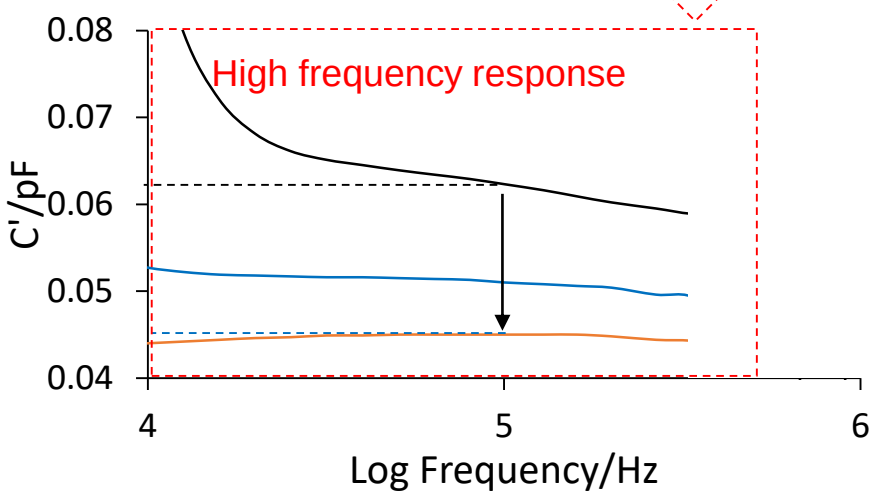


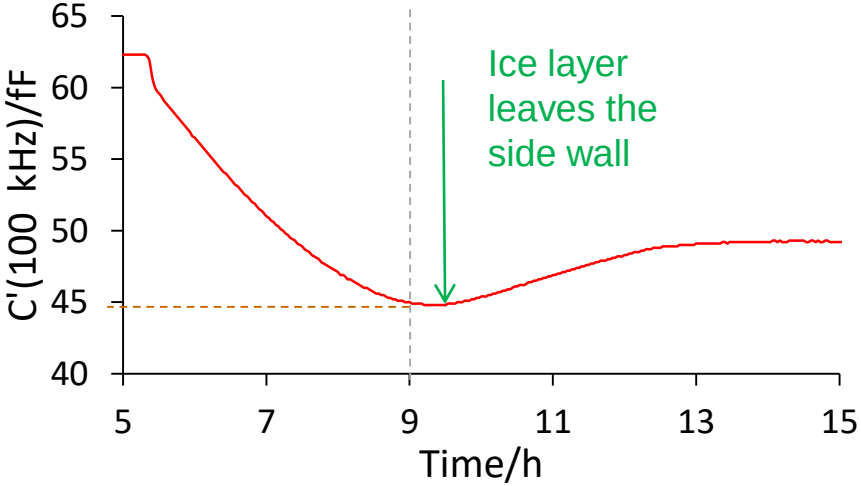


Endpoint

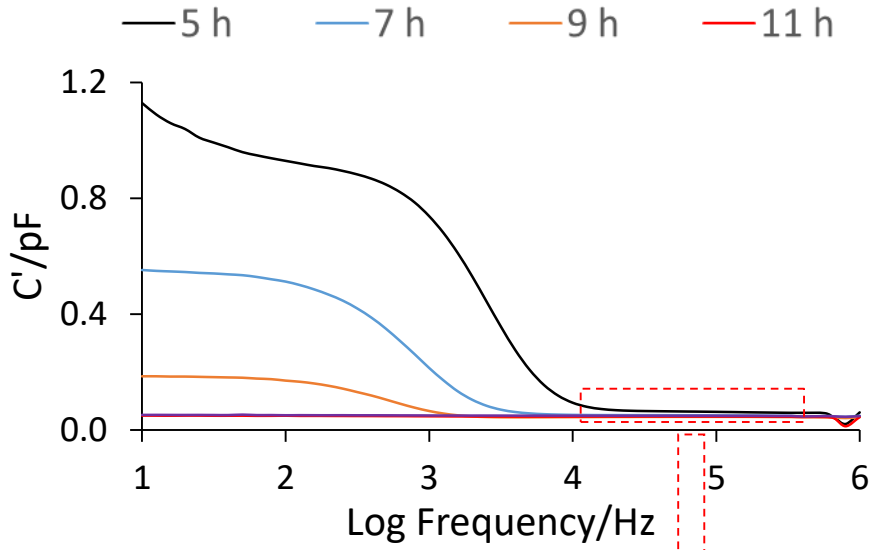

5 h
7 h
9 h
1.2
0.8
0.4
0.0
C'/pF
0.52 cm
1
2
3
4
5
6
Log Frequency/Hz

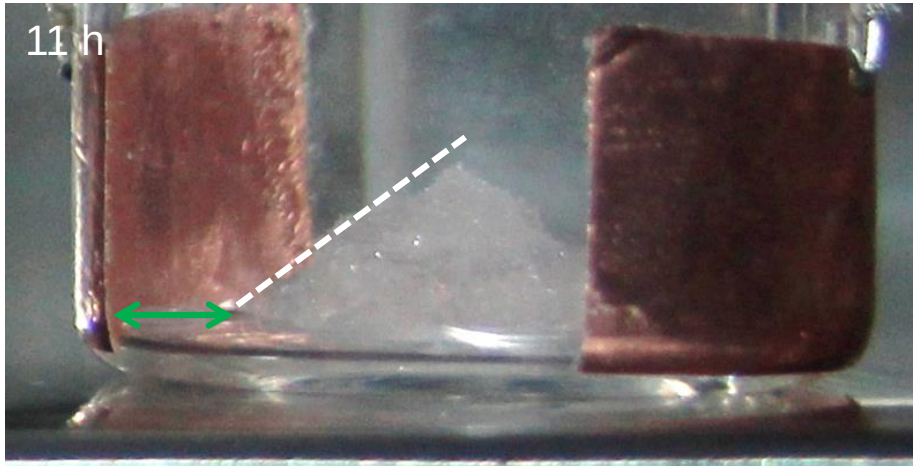

9 h
1.3 mm
Capacitance / pF
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
Height of ice layer (mm)

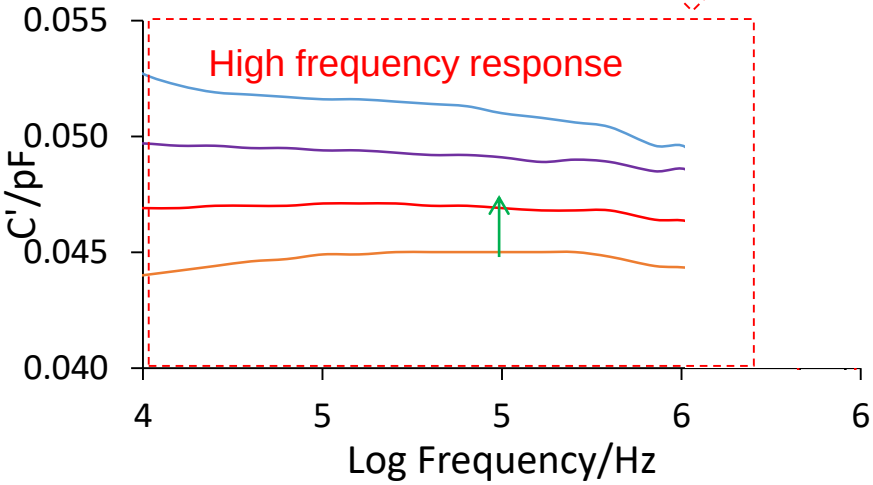

High frequency response
0.08
0.07
0.06
0.05
0.04
C'/pF
4
5
6
Log Frequency/Hz

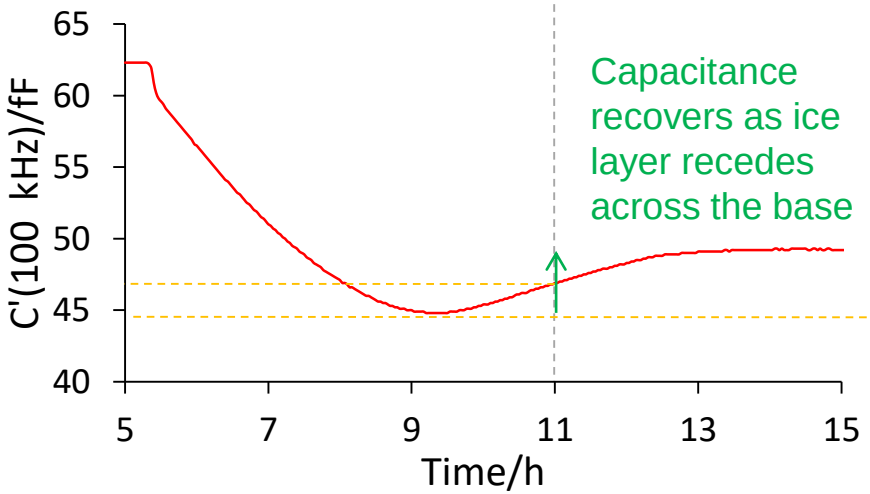

Ice layer
leaves the
side wall
65
60
55
50
45
40
C'(100 kHz)/fF
5
7
9
11
13
15
Time/h

Endpoint

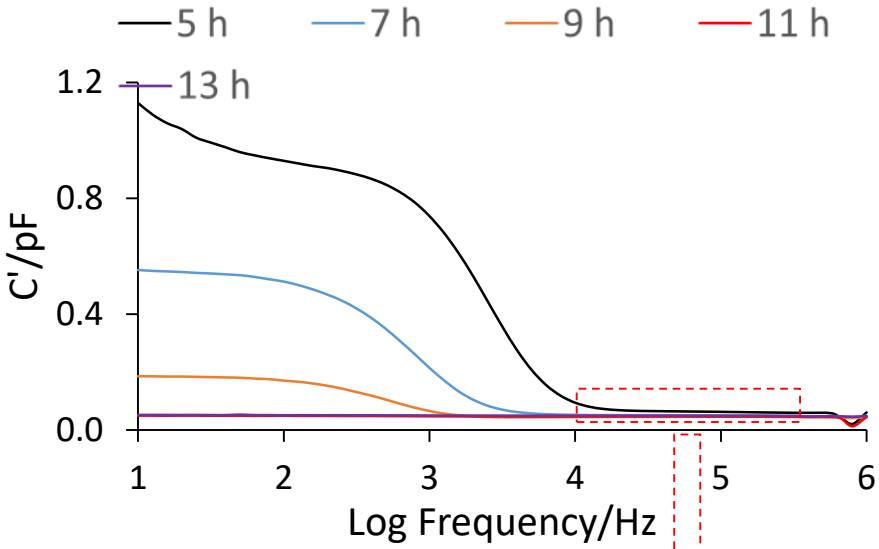

5 h
7 h
9 h
11 h
1.2
0.8
0.4
0.0
C'/pF
1
2
3
4
5
6
Log Frequency/Hz

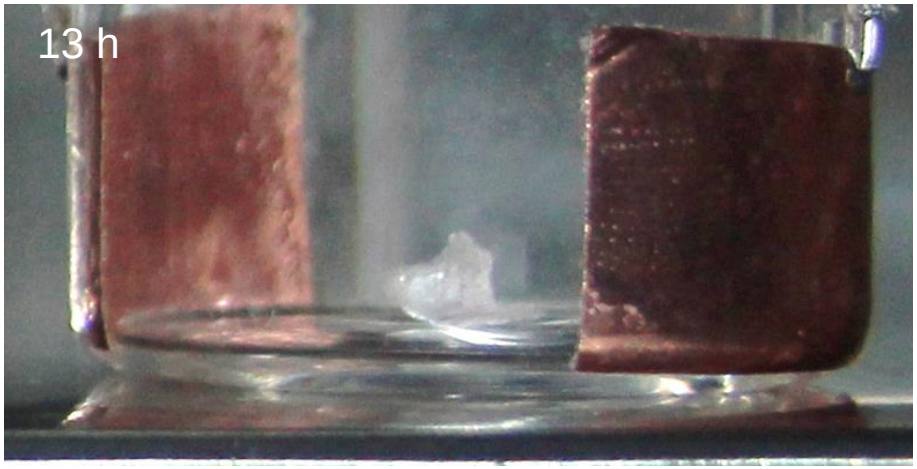

11 h

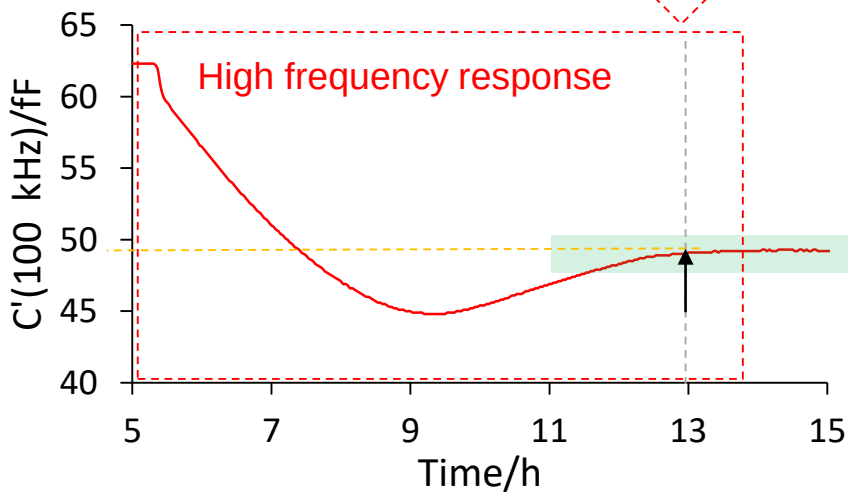

High frequency response
0.055
0.050
0.045
0.040
C'/pF
4
5
5
6
6
Log Frequency/Hz

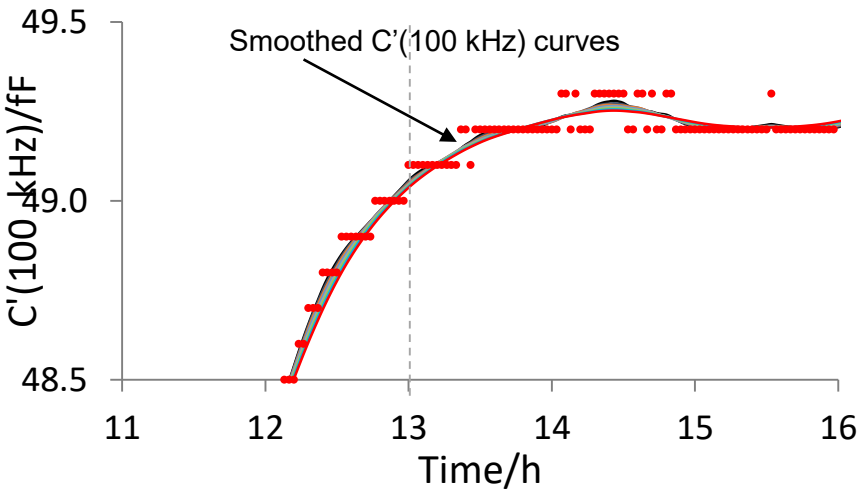

Capacitance recovers as ice layer recedes across the base
65
60
55
50
45
40
C'(100 kHz)/fF
5
7
9
11
13
15
Time/h

Endpoint

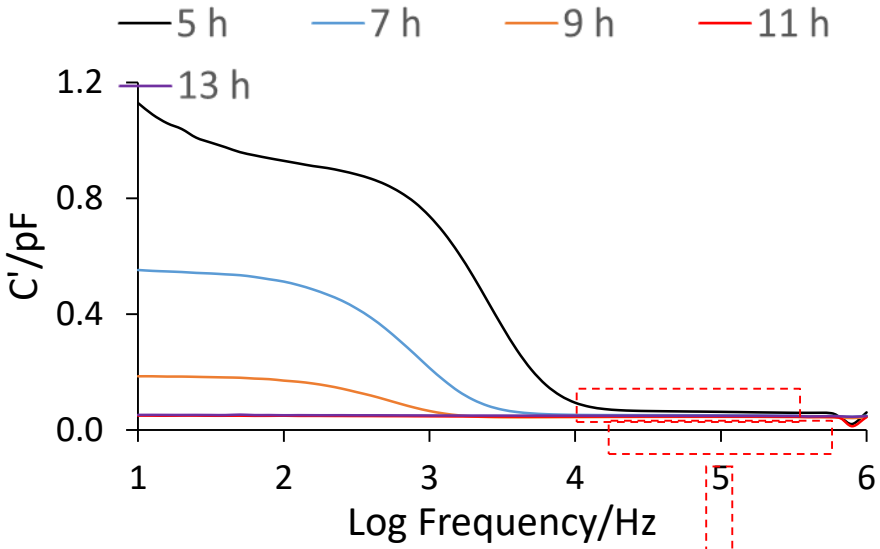

5 h
7 h
9 h
11 h
13 h
1.2
0.8
0.4
0.0
C'/pF
1
2
3
4
5
6
Log Frequency/Hz

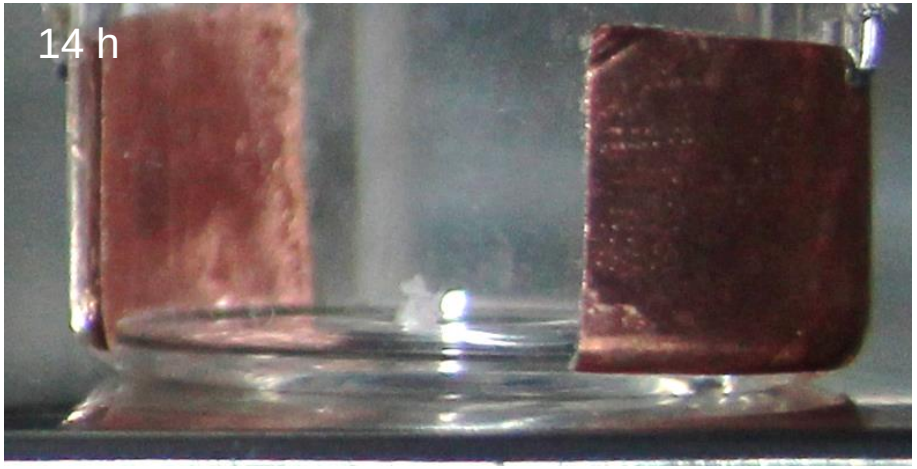

13 h

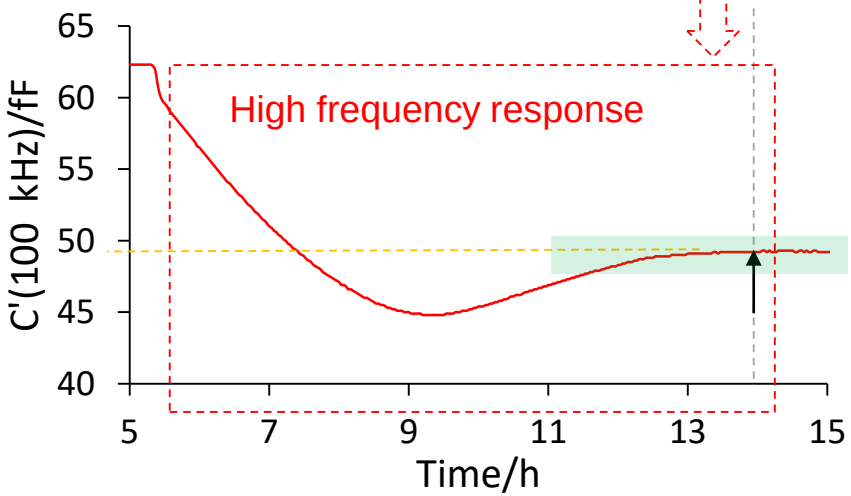

High frequency response
65
60
55
50
45
40
C'(100 kHz)/fF
5
7
9
11
13
15
Time/h

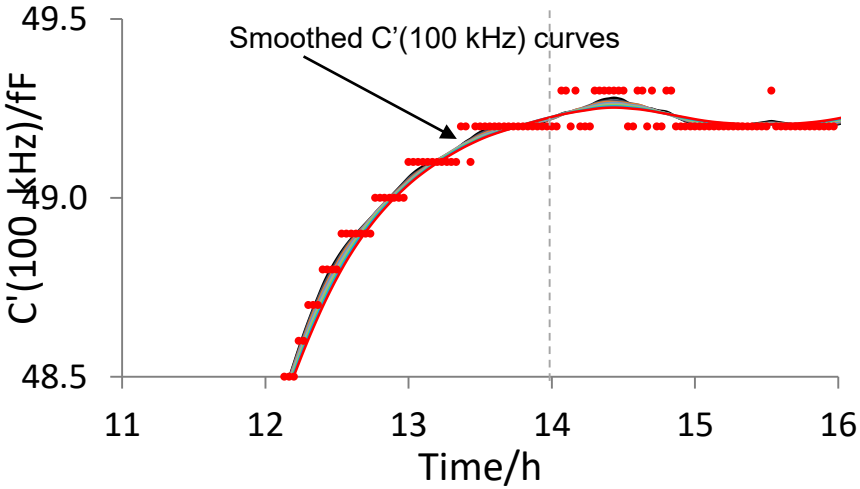

Smoothed C'(100 kHz) curves
49.5
49.0
48.5
C'(100 kHz)/fF
11
12
13
14
15
16
Time/h

Endpoint

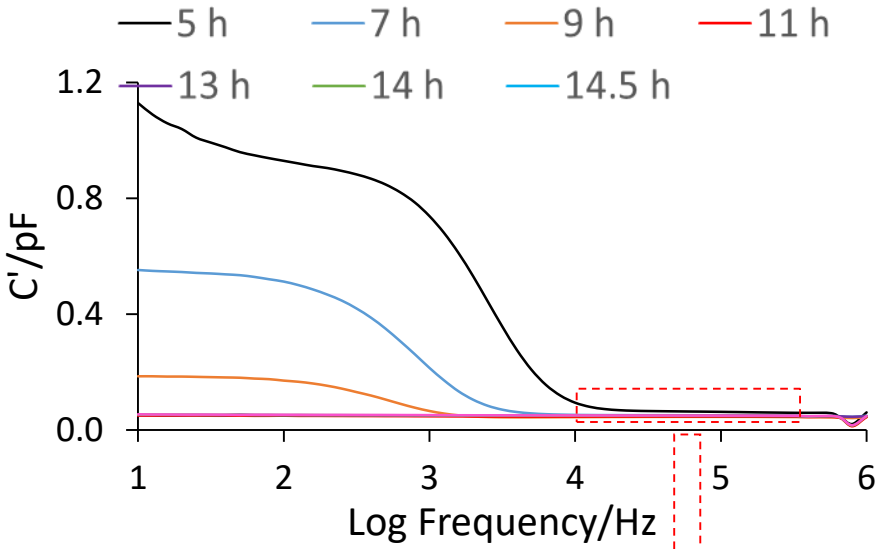

5 h
7 h
9 h
11 h
13 h
1.2
0.8
0.4
0.0
C'/pF
1
2
3
4
5
6
Log Frequency/Hz

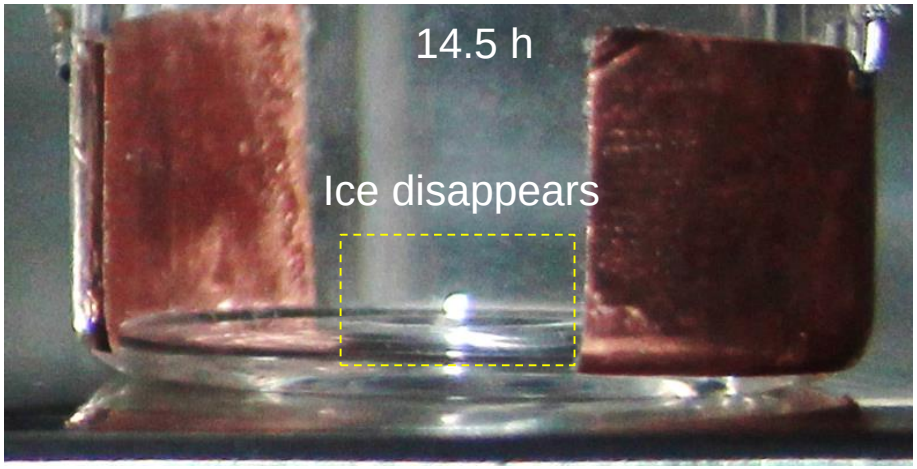

14 h

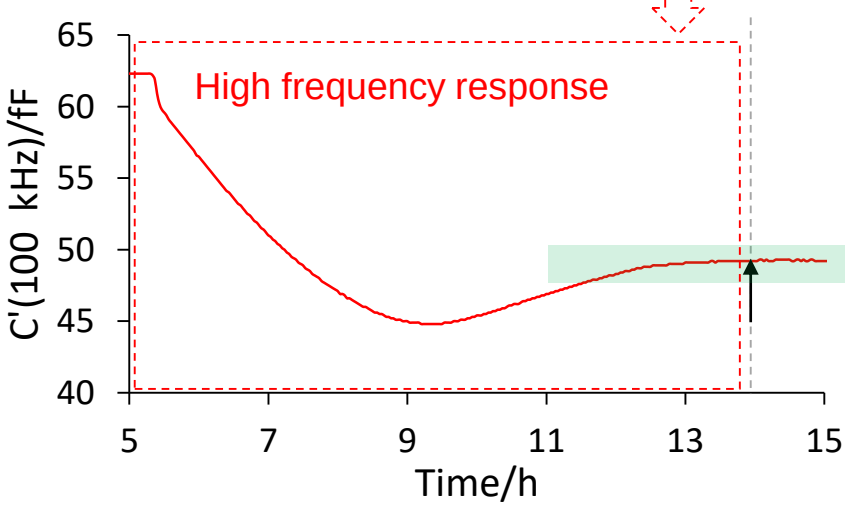

65
60
55
50
45
40
C'(100 kHz)/fF
High frequency response
5
7
9
11
13
15
Time/h

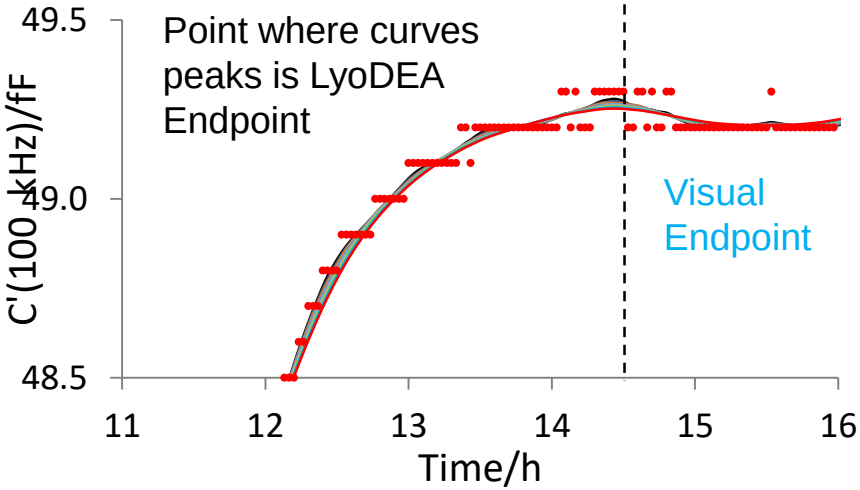

49.5
49.0
48.5
C'(100 kHz)/fF
Smoothed C'(100 kHz) curves
11
12
13
14
15
16
Time/h

Endpoint


5 h
7 h
9 h
11 h
13 h
14 h
14.5 h
C'/pF
1.2
0.8
0.4
0.0
1
2
3
4
5
6
Log Frequency/Hz


14.5 h
Ice disappears


High frequency response
C'(100 kHz)/fF
65
60
55
50
45
40
5
7
9
11
13
15
Time/h


Point where curves peaks is LyoDEA Endpoint
Visual Endpoint
C'(100 kHz)/fF
49.5
49.0
48.5
11
12
13
14
15
16
Time/h

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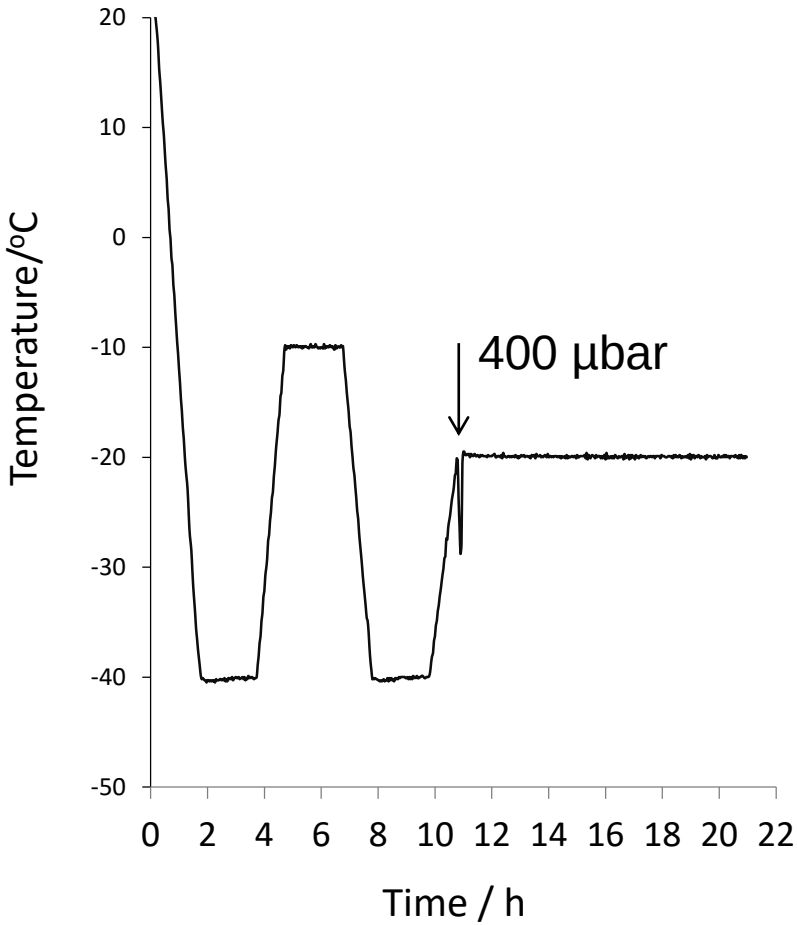
Leicester School of Pharmacy

Multi-Vial Measurements

Product Temperature, Drying Rate and K_v Determination

BIOPHARMACEUTICAL QUALITY BY DESIGN

Freeze Drying Protocol & Sample Arrangement



Sample arrangement

Imaginary Part Capacitance

At ω → 0, C″ = 0. As the frequency increases, C″ increases to a maximum (C''_{max}) then decreases to 0 as the frequency ω → ∞

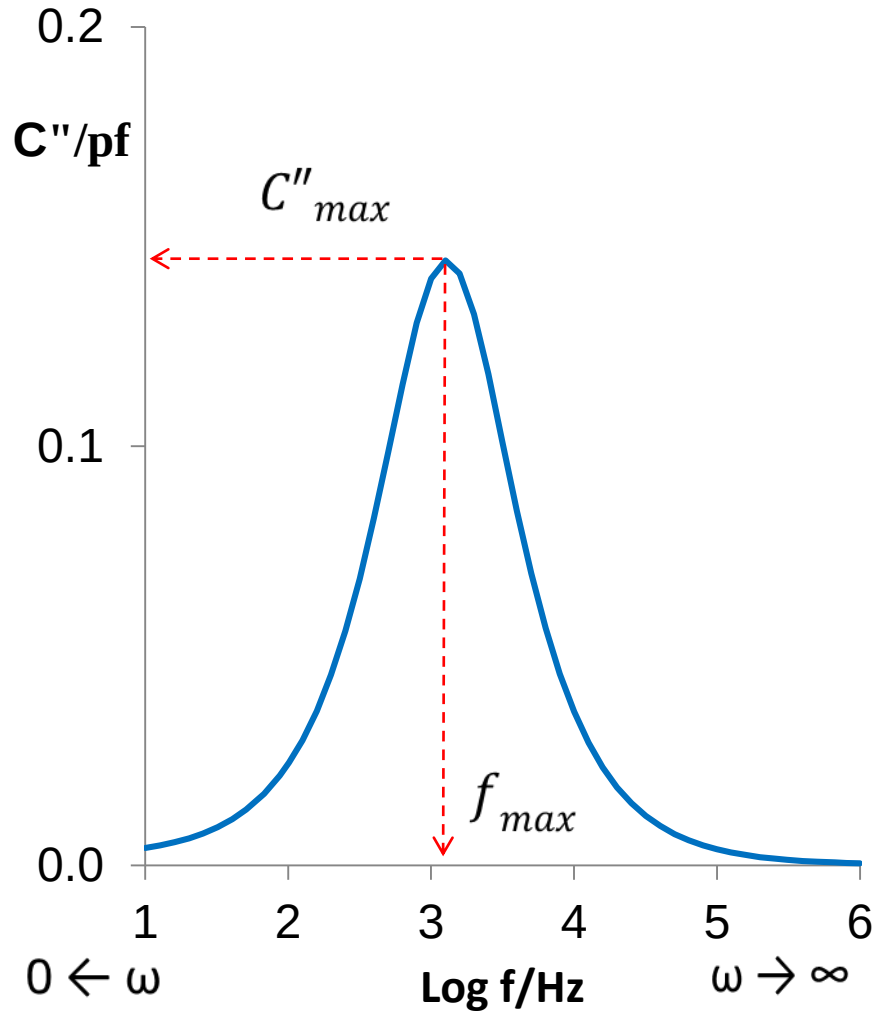
$$C''_{max} = \frac{C_G^2}{2(C_S+C_G)}$$

at a frequency of

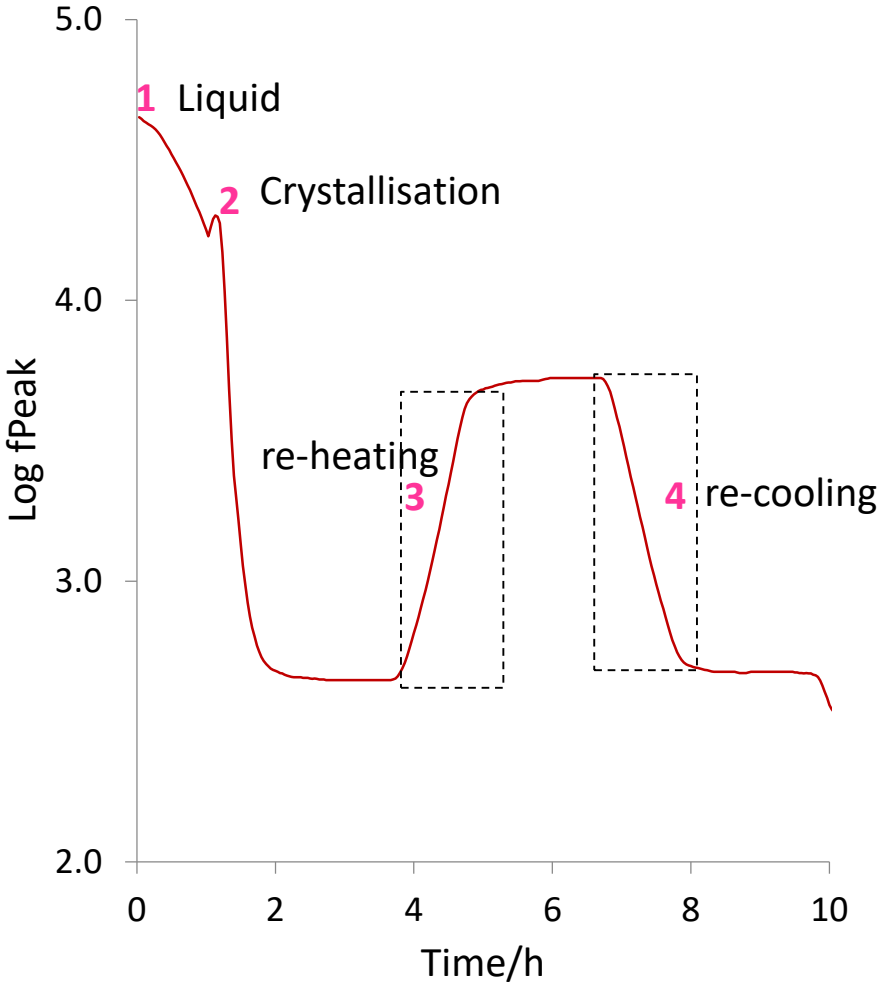
$$\omega_{max} = \frac{1}{R(C_S+C_G)} \text{ in radians}$$

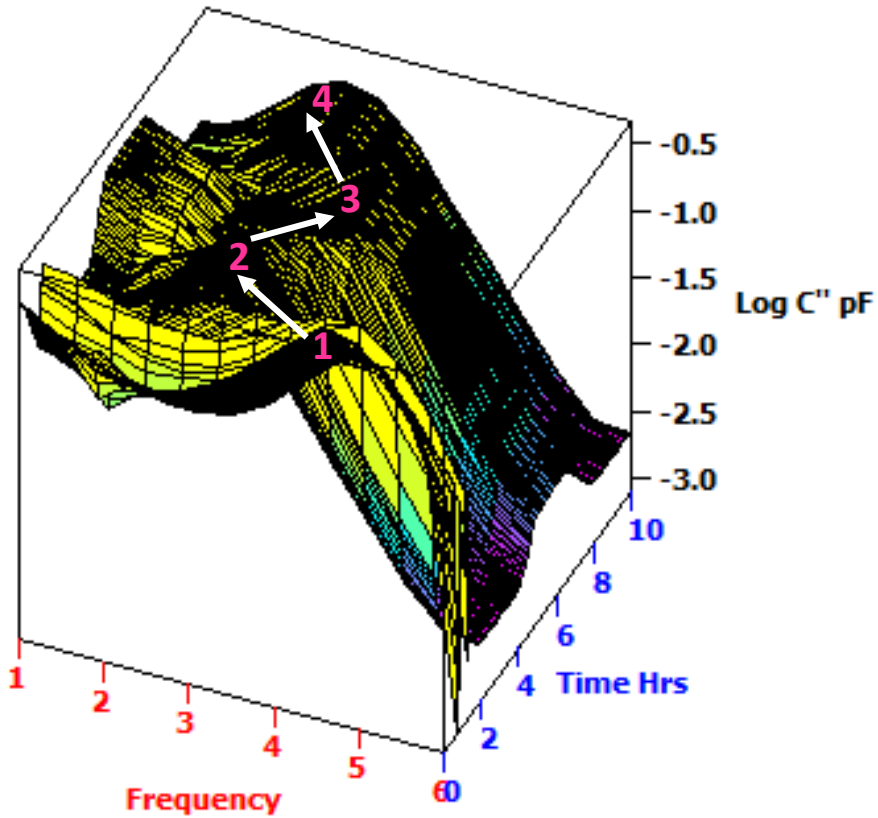
Or

$$f_{max} = \frac{1}{2\pi R(C_S+C_G)} \text{ in cycles per second}$$

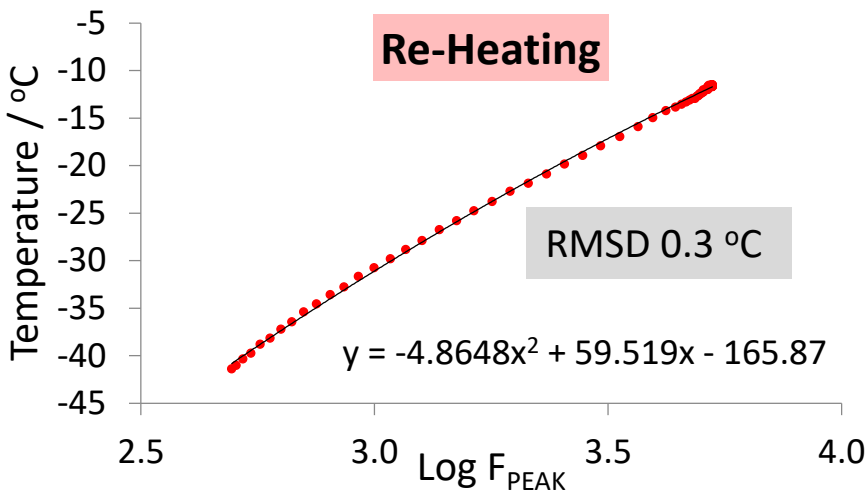


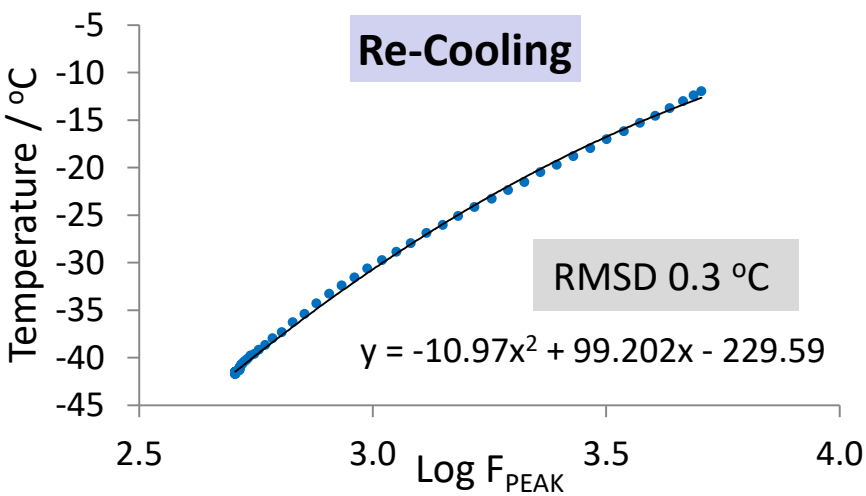
Dependency of F_{PEAK} on temperature

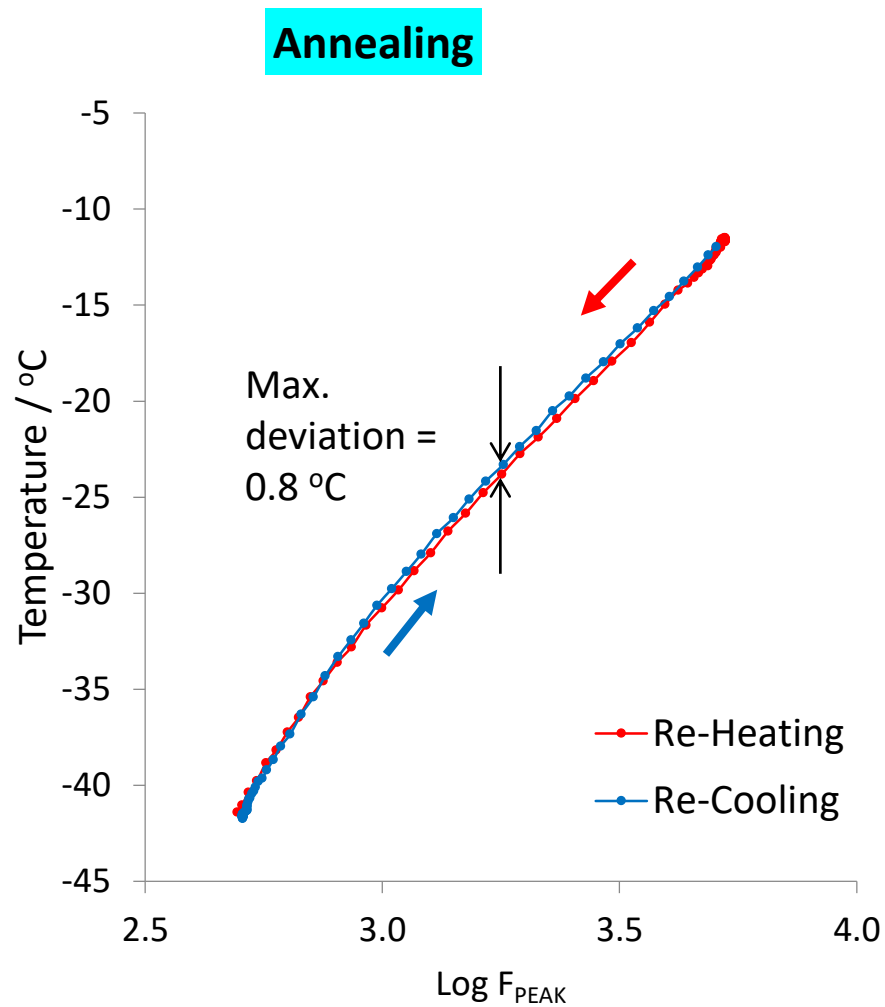




Temperature Calibration

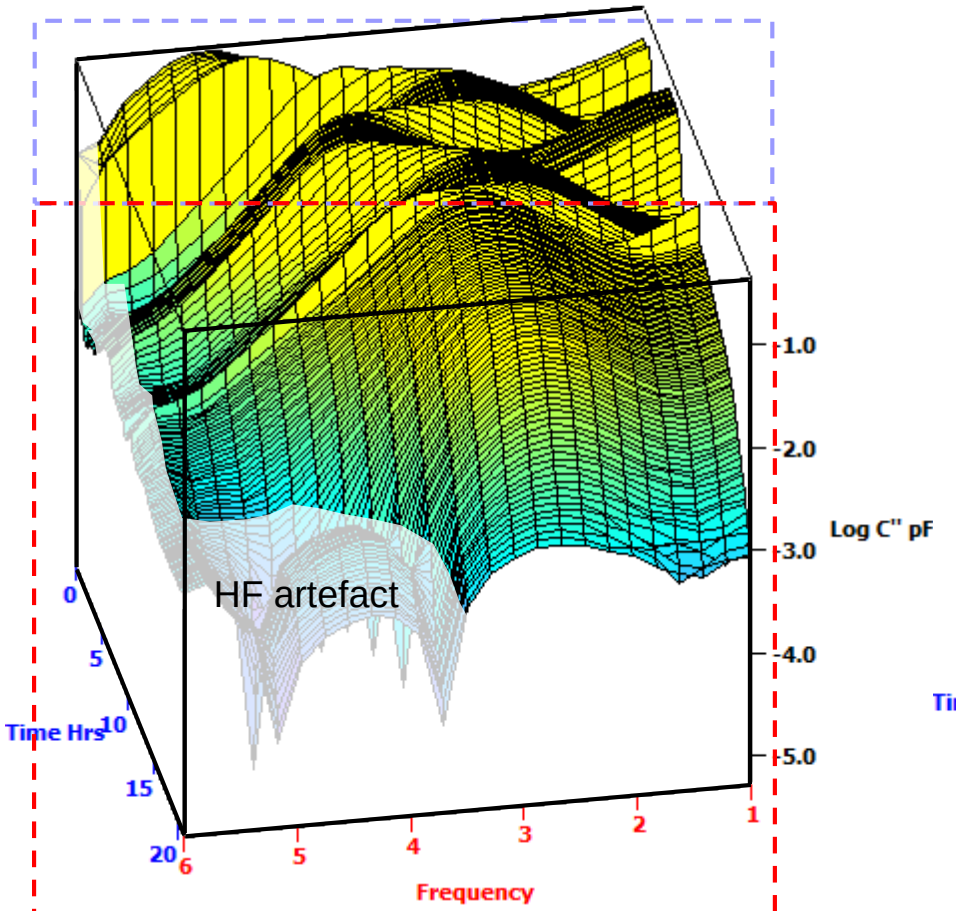




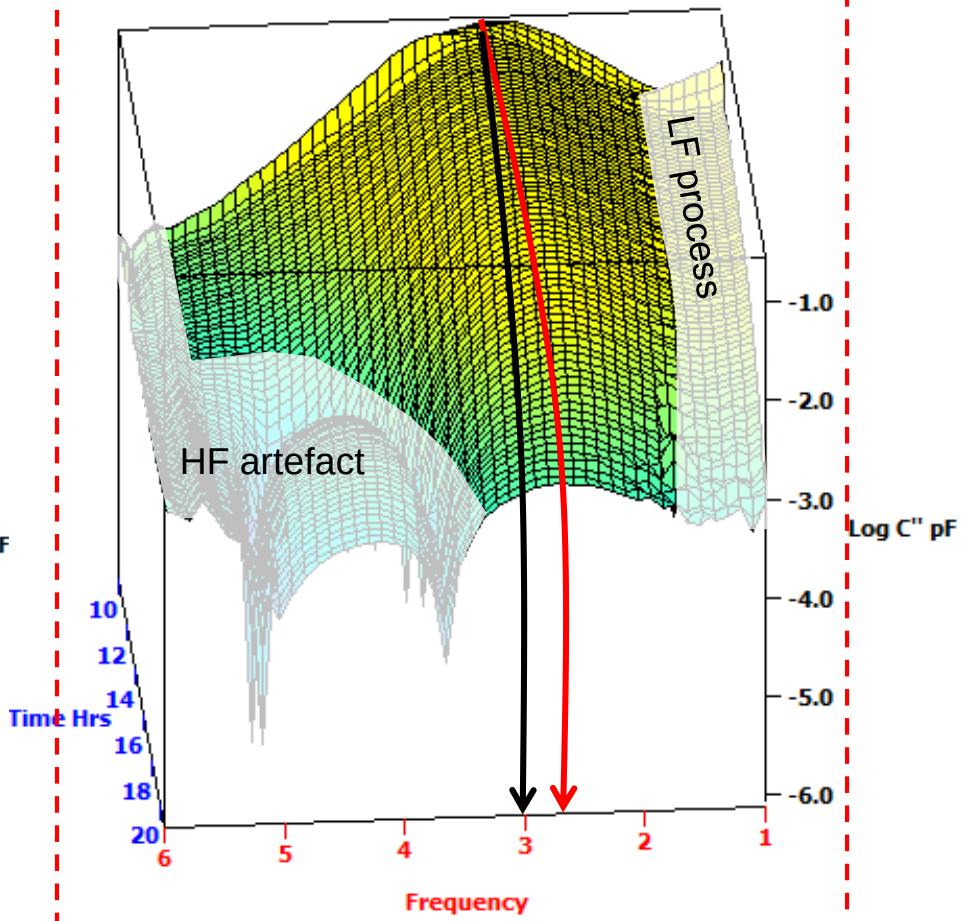


Freeze-drying of water

Freezing and annealing stages

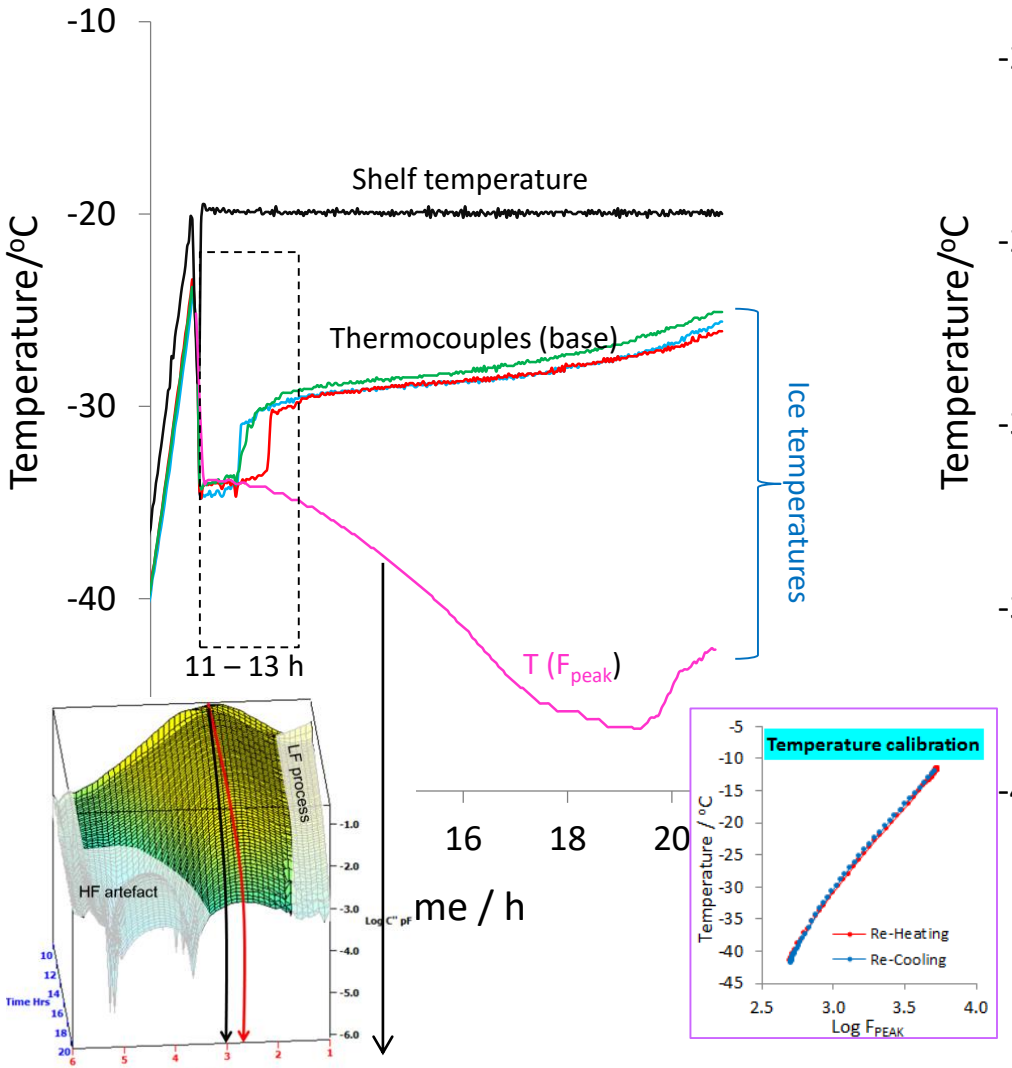


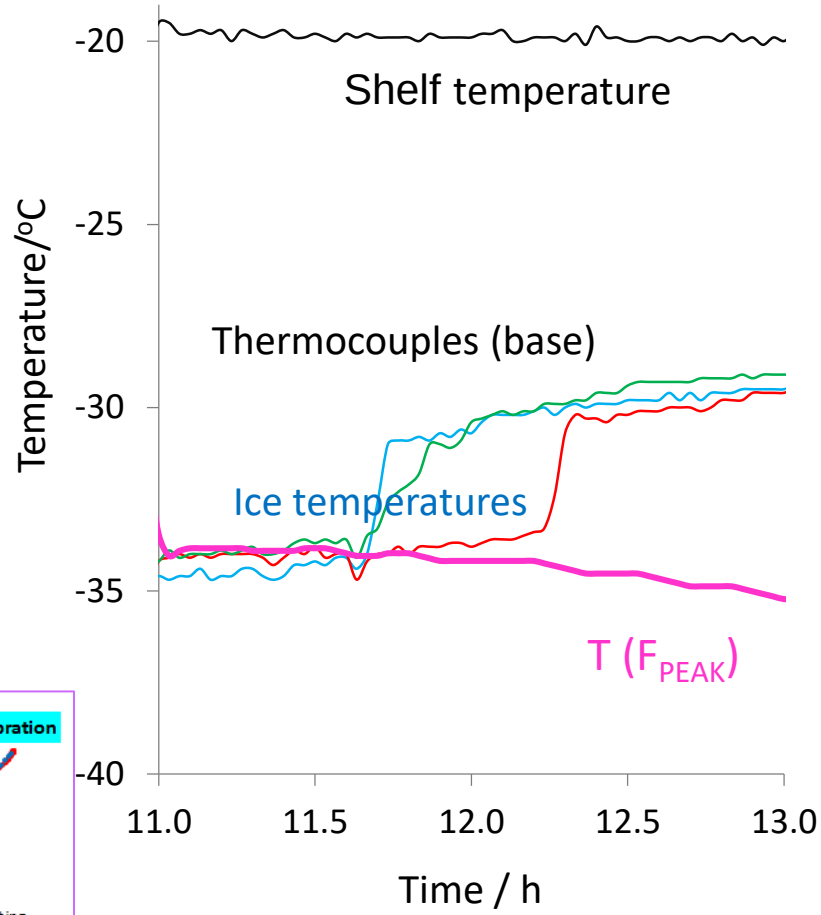
Freeze Drying Process



Primary Drying Process

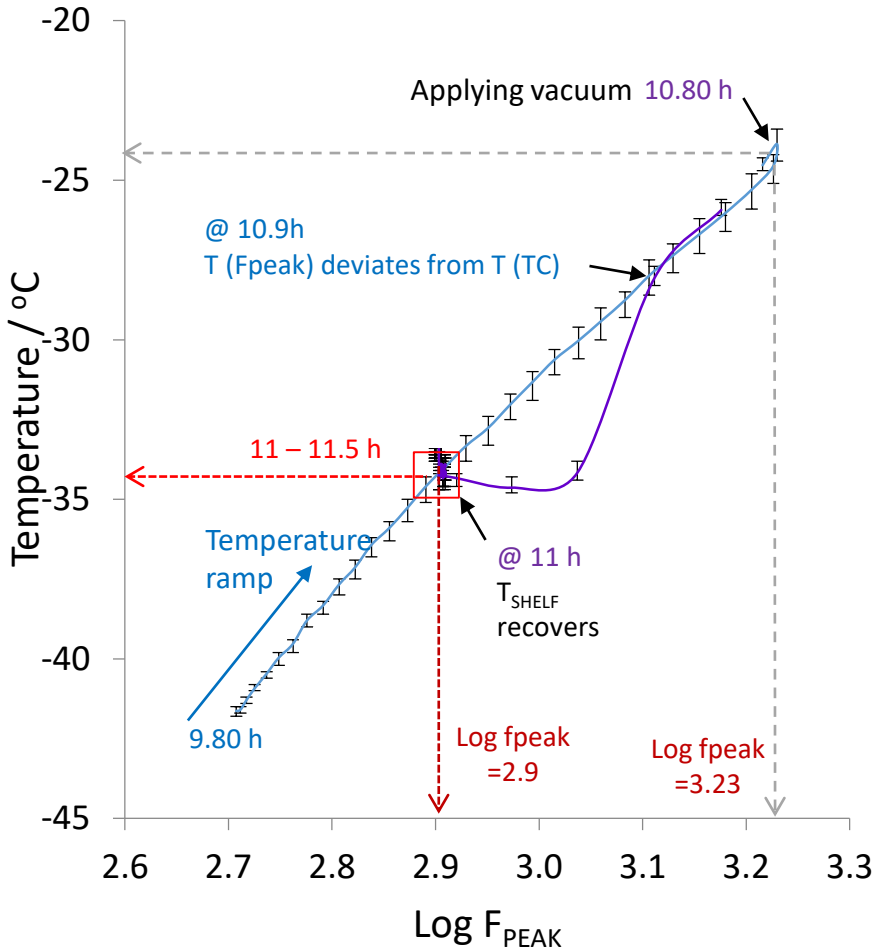
Primary Drying

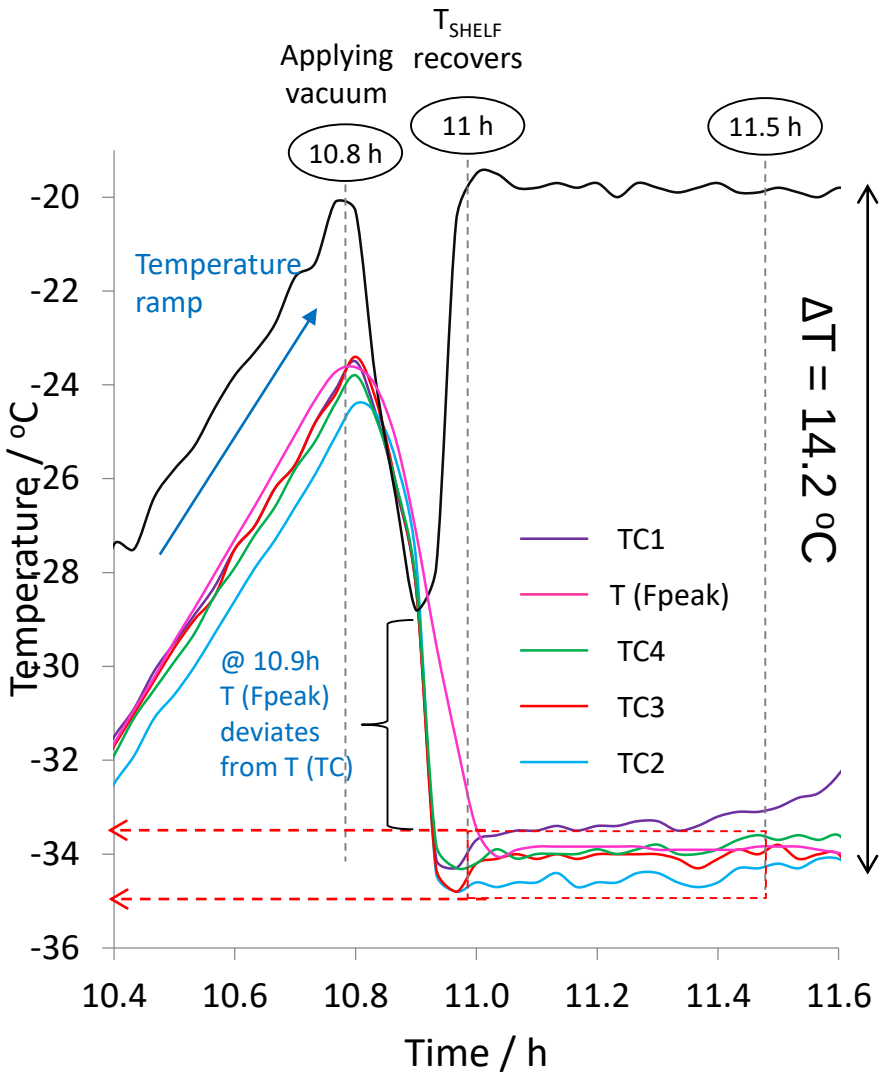




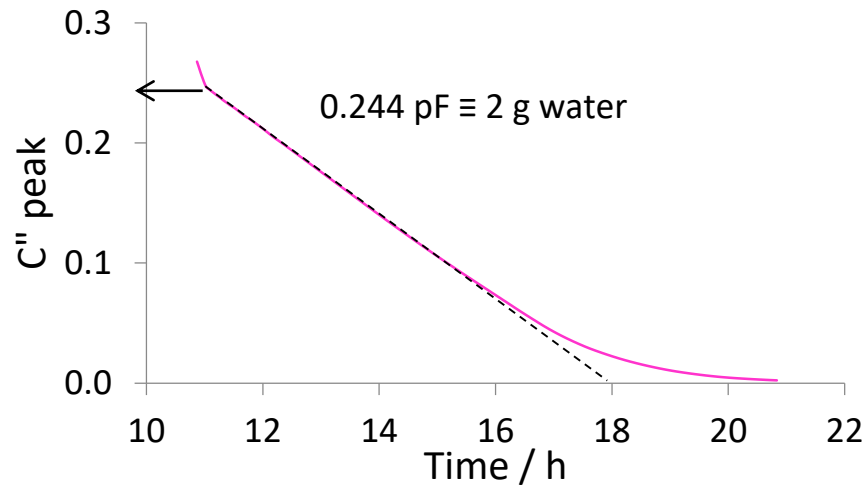
Decrease in T(Fpeak) due to change in spectrum shape

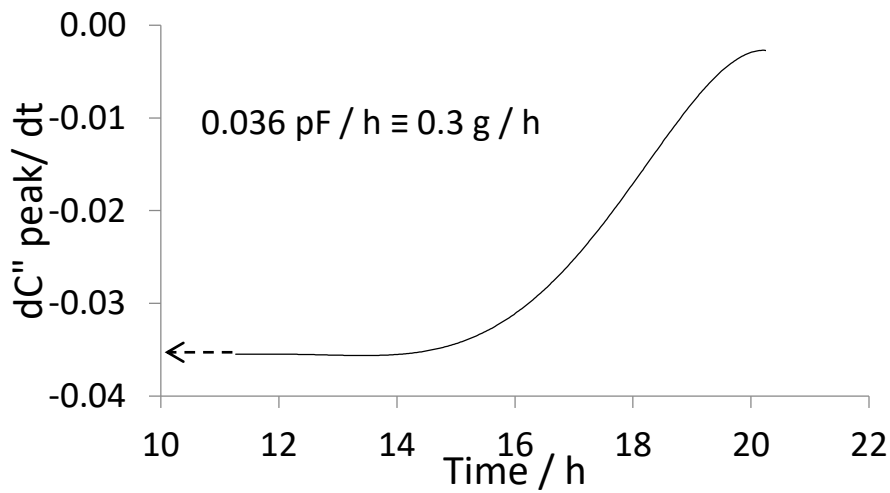
Temperature (before and after start of primary drying)

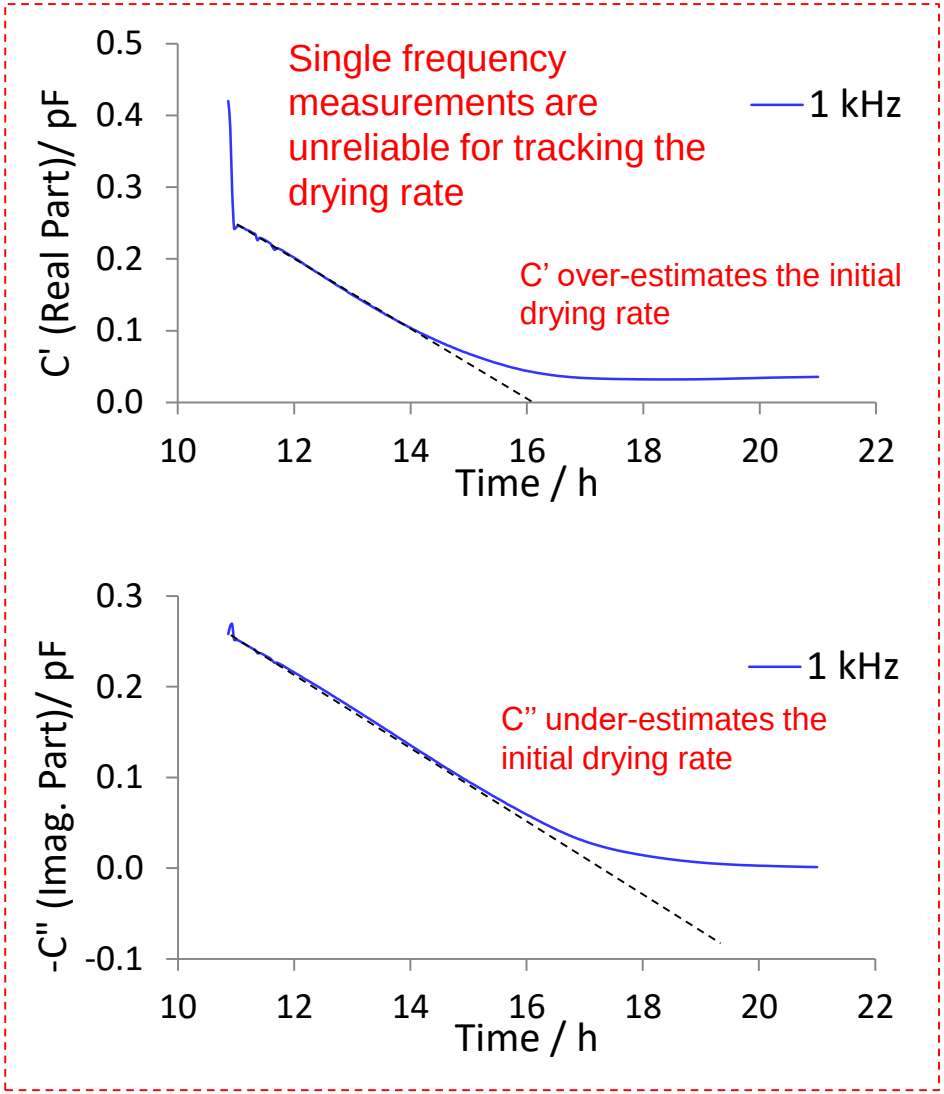




Primary Drying Rate







Primary Drying Modelling : Heat and Mass Transfer

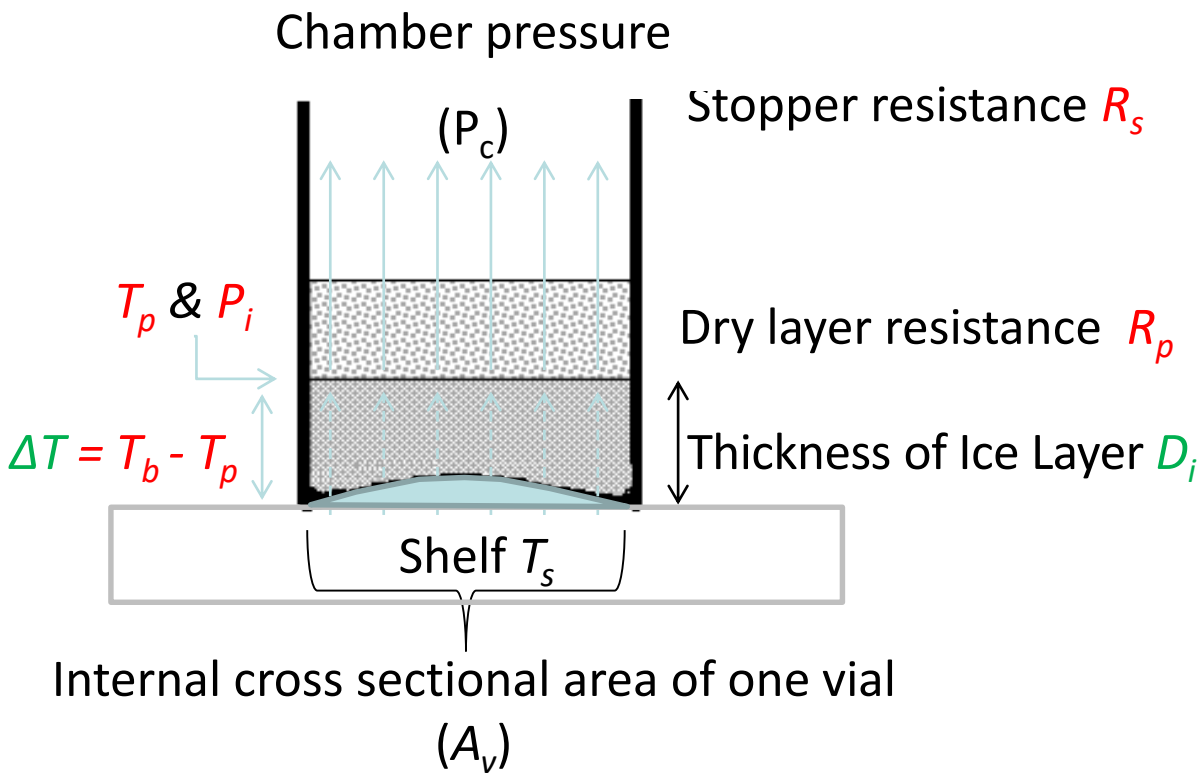
$$dm/dt = \frac{A_v(P_i - P_c)}{R_{ps}}$$

$$dq/dt = L\, dm/dt$$

K_v determination

$$dq/dt = A_v K_v (T_s - T_p)$$

$$\ln P_i = -145/T_p + 24.02$$



Heat Transfer Coefficient (K_v) Determination

First convert dm/dt to dq/dt using the latent heat of sublimation

$$L\,\frac{dm}{dt} = \frac{dq}{dt}$$

dm/dt = 0.3 g / h

L = 2844 J g^{-1}

dq/dt = 853 J / h

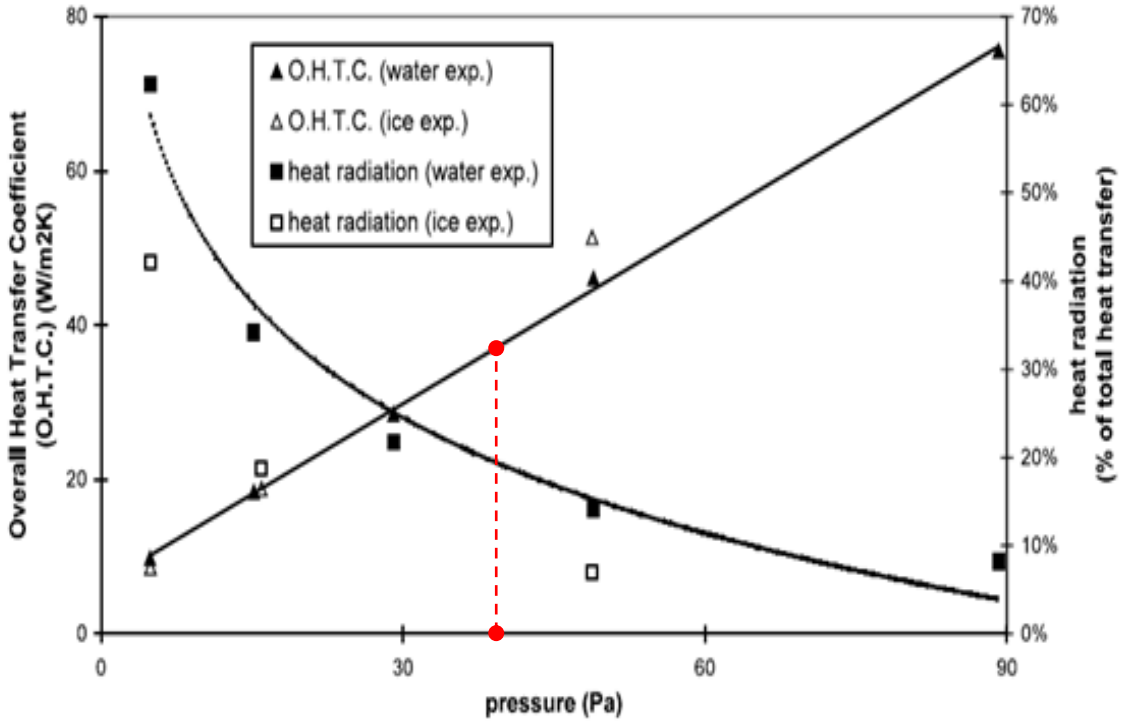
$$dq/dt = A_v K_v (T_s - T_p)$$

T_S = − 20 °C; T_P = − 34.2 °C

A_v = 0.0045 m^2 (10 ml tubing vial)

K_v = 37 W m^{-2} K^{-1} (@ 40 Pa, 400 µBar)

K_v values for 10 mL tubing vials (2 mL fill volume)



Brülls, M., and Ramusson, A. (2002) Heat Transfer in Lyophilization. Int J Pharm 10;246(1-2):1-16.

Protein Solutions

50% v/v dilution of clot serum in PBS (protein ~ 3-4 %w/v)

Conductive Liquids!!!

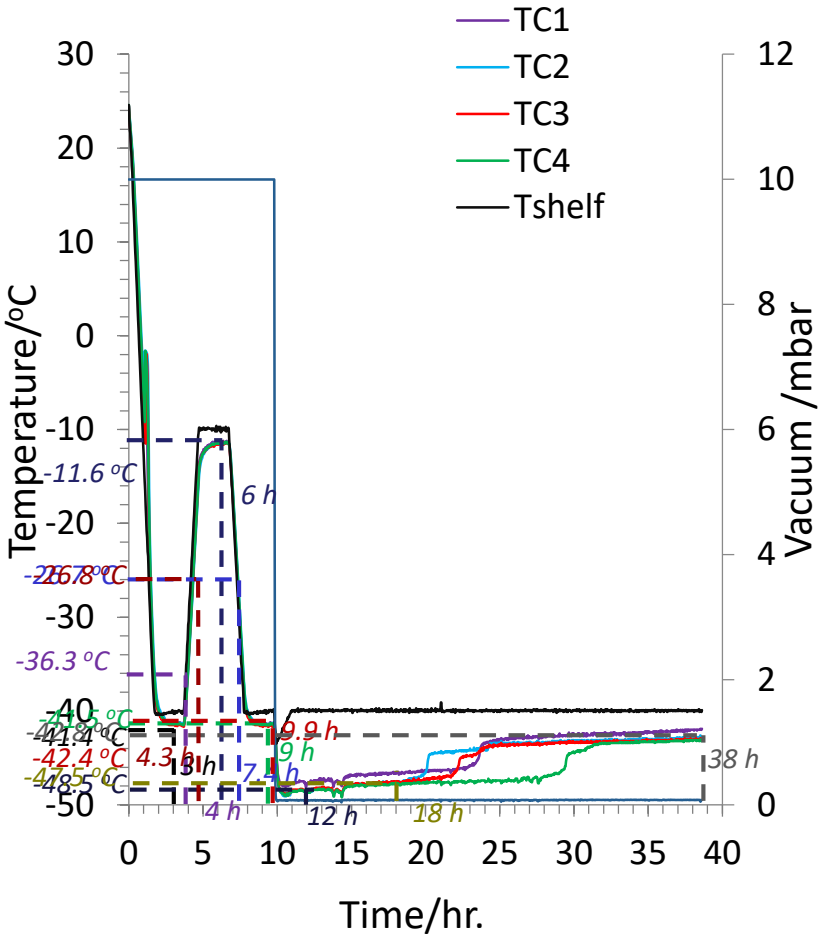
Freeze Drying Protocol & Sample Arrangement

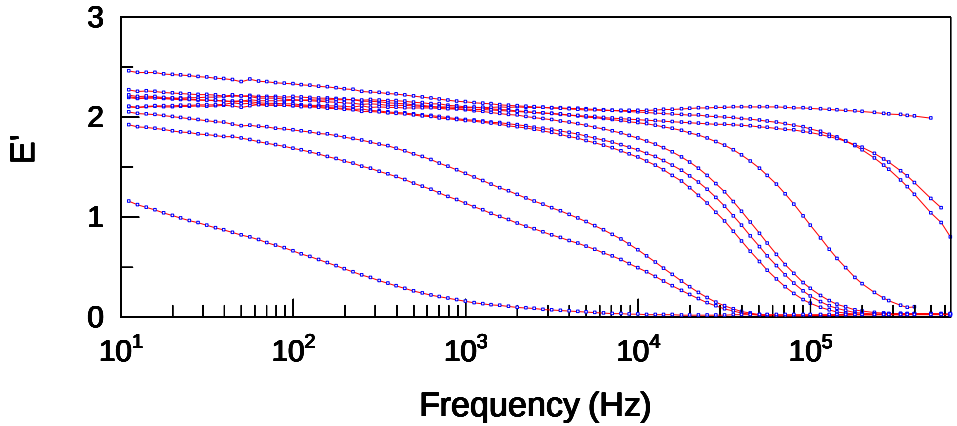
Freeze Drying: Virtis Advantage Plus

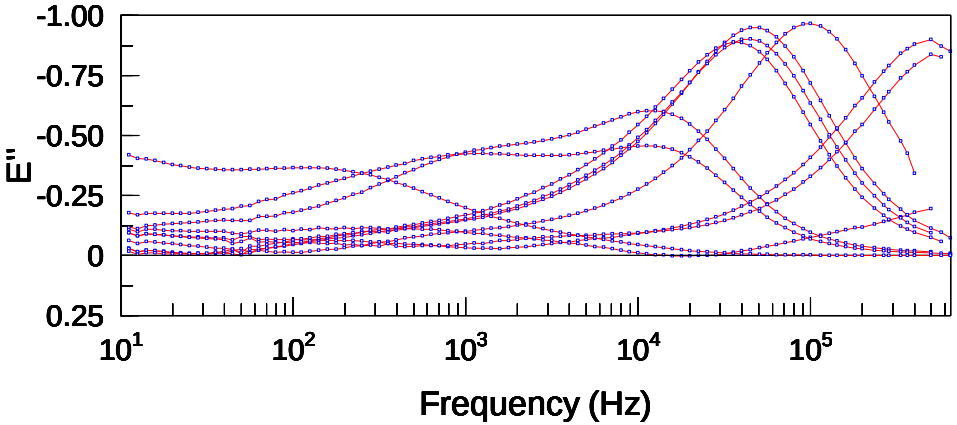
Step	Temp (°C)	Time (min)	Pressure (µbar)	Ramp/ Hold
Equilibrium phase	20	10	-	Ramp
Freezing	-40	90	-	Ramp
	-40	120	-	Hold
Annealing	-10	60	-	Ramp
	-10	120	-	Hold
Re-Freezing	-40	60	-	Ramp
	-40	120	-	Hold
Primary drying	-40	60	100	Ramp
	-40	1920	100	Hold

Sample arrangement

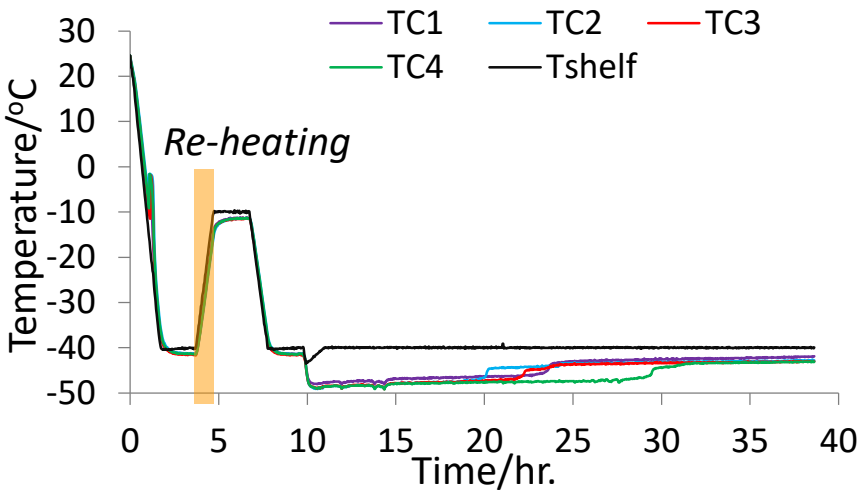
Impedance Spectra of Freeze Drying Process

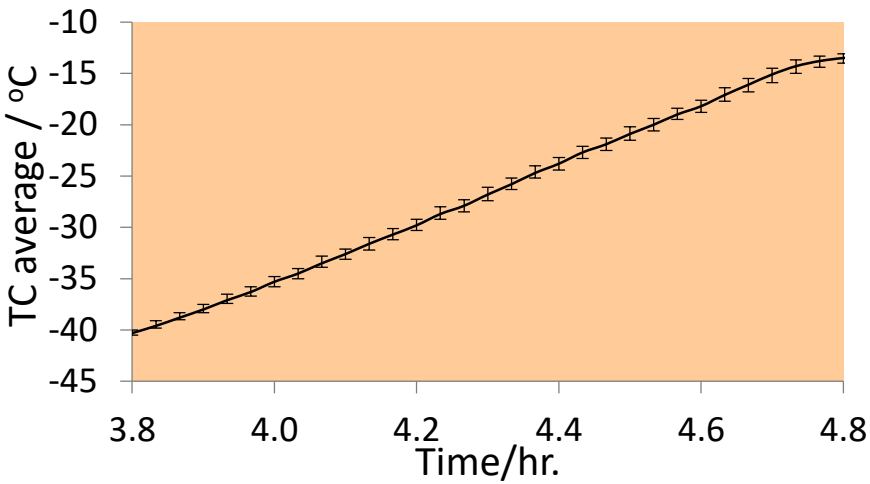


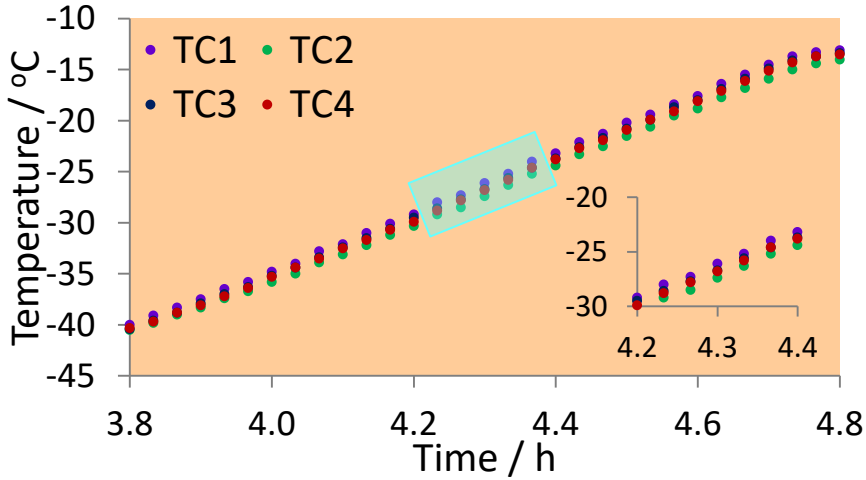


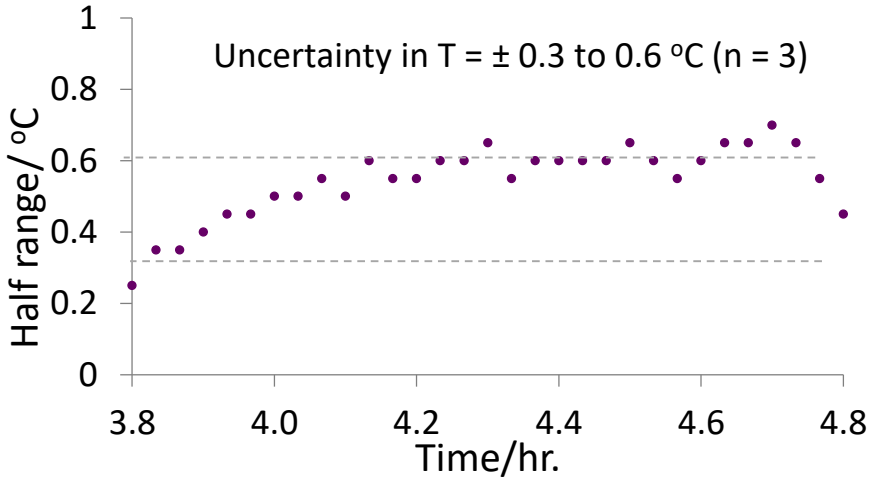


Temperature Data during Annealing Process

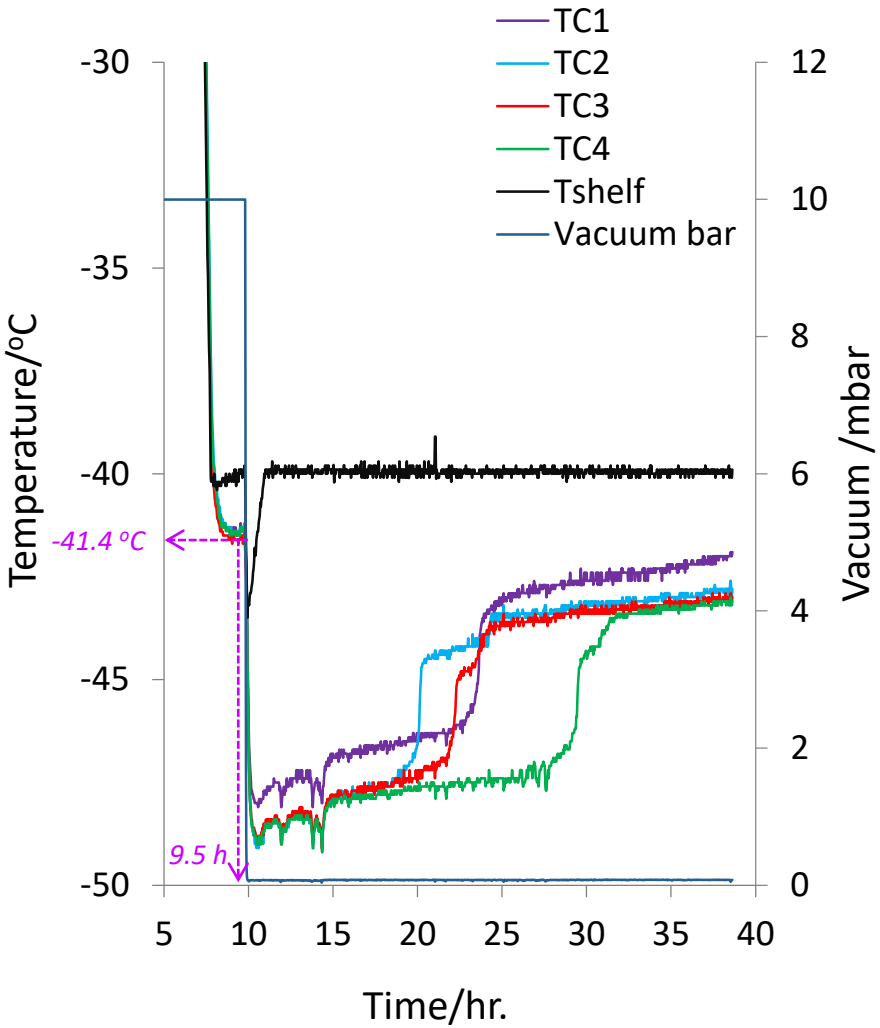


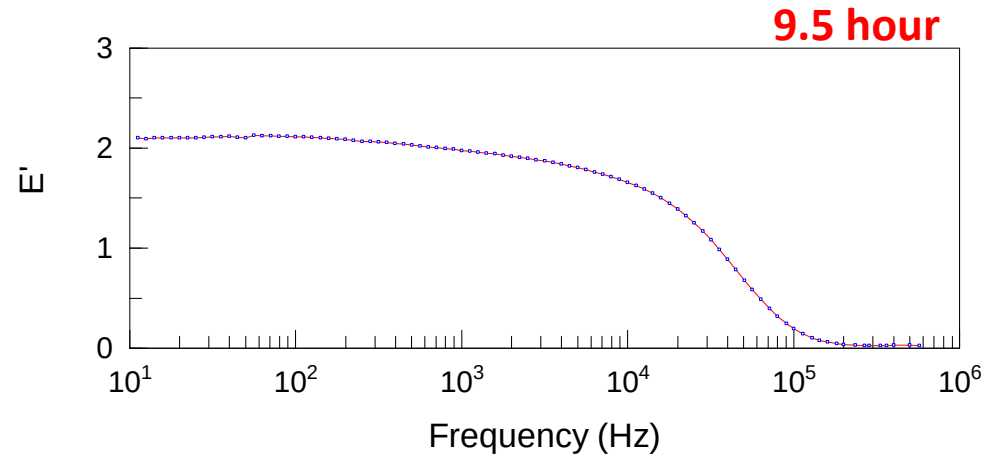


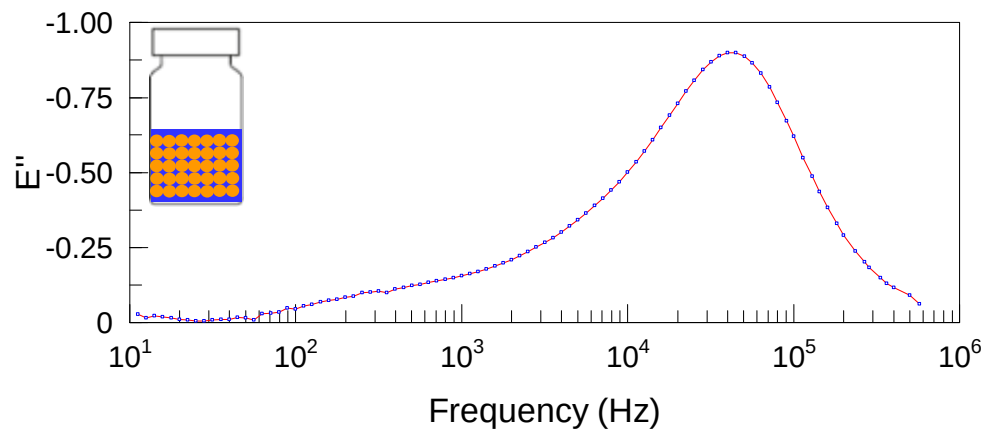




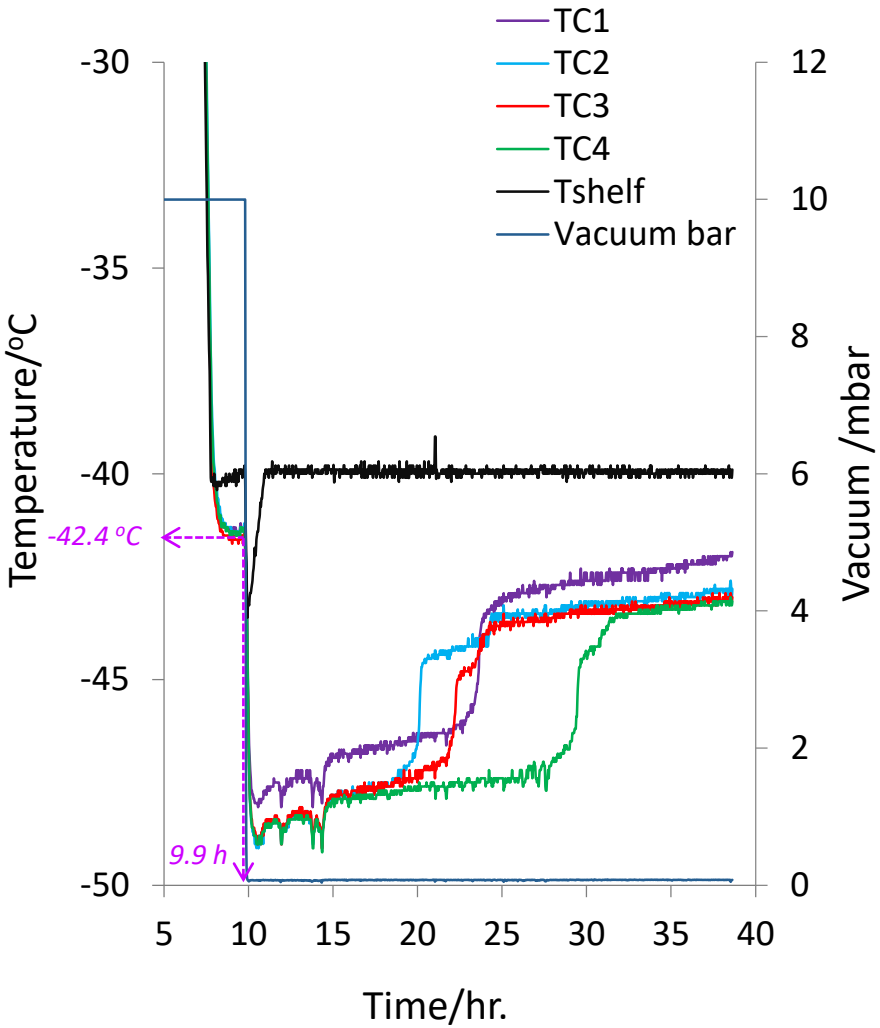
Impedance Spectra of Primary Drying Process

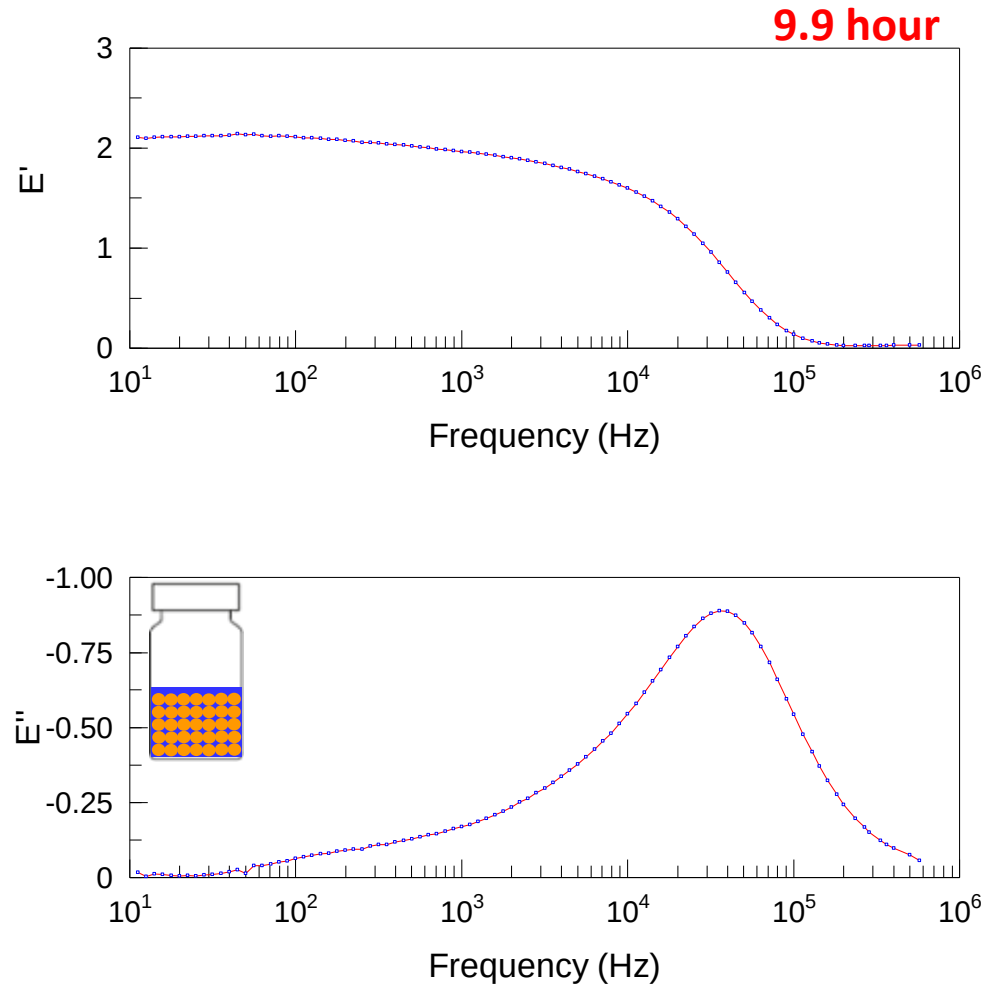




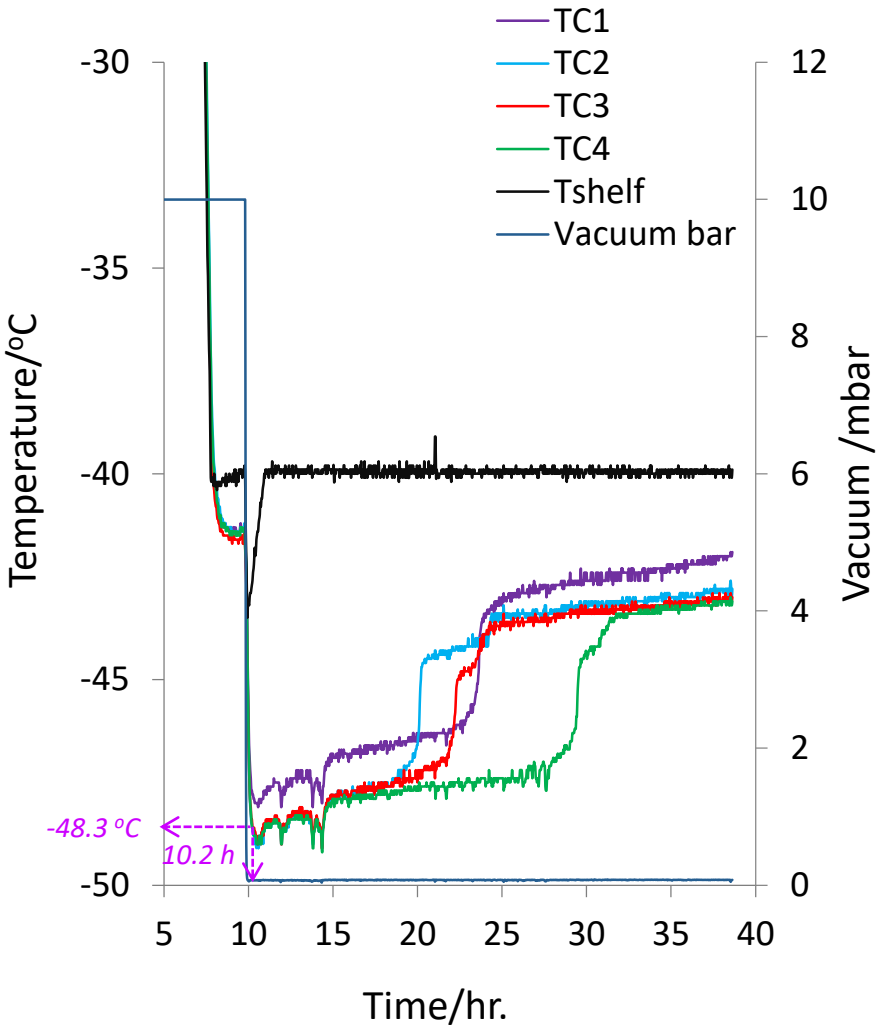


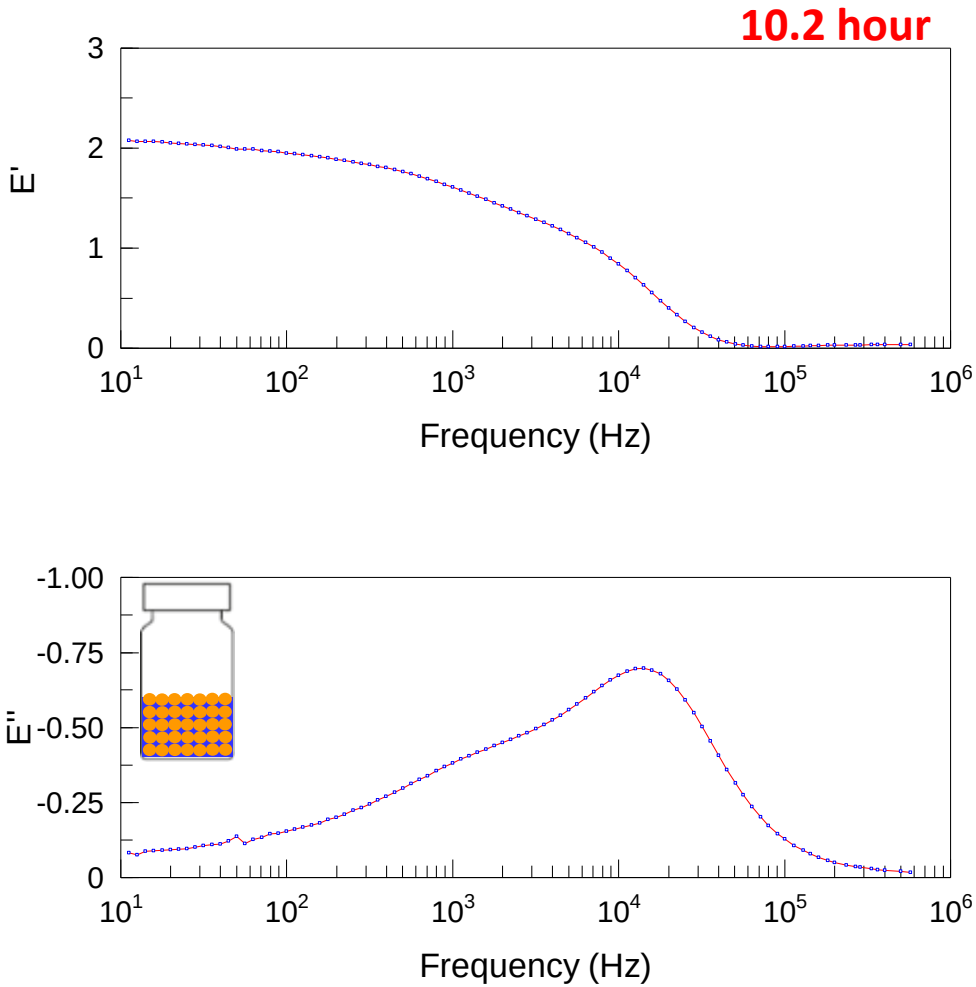
Impedance Spectra of Primary Drying Process



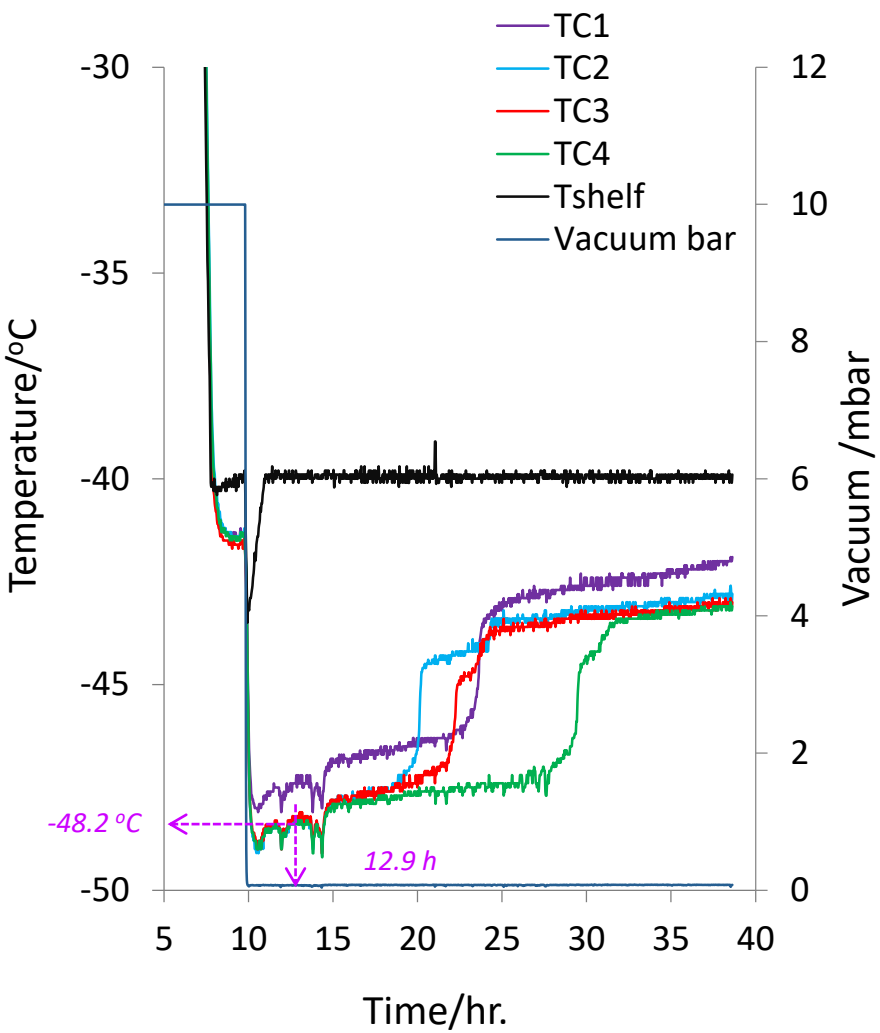


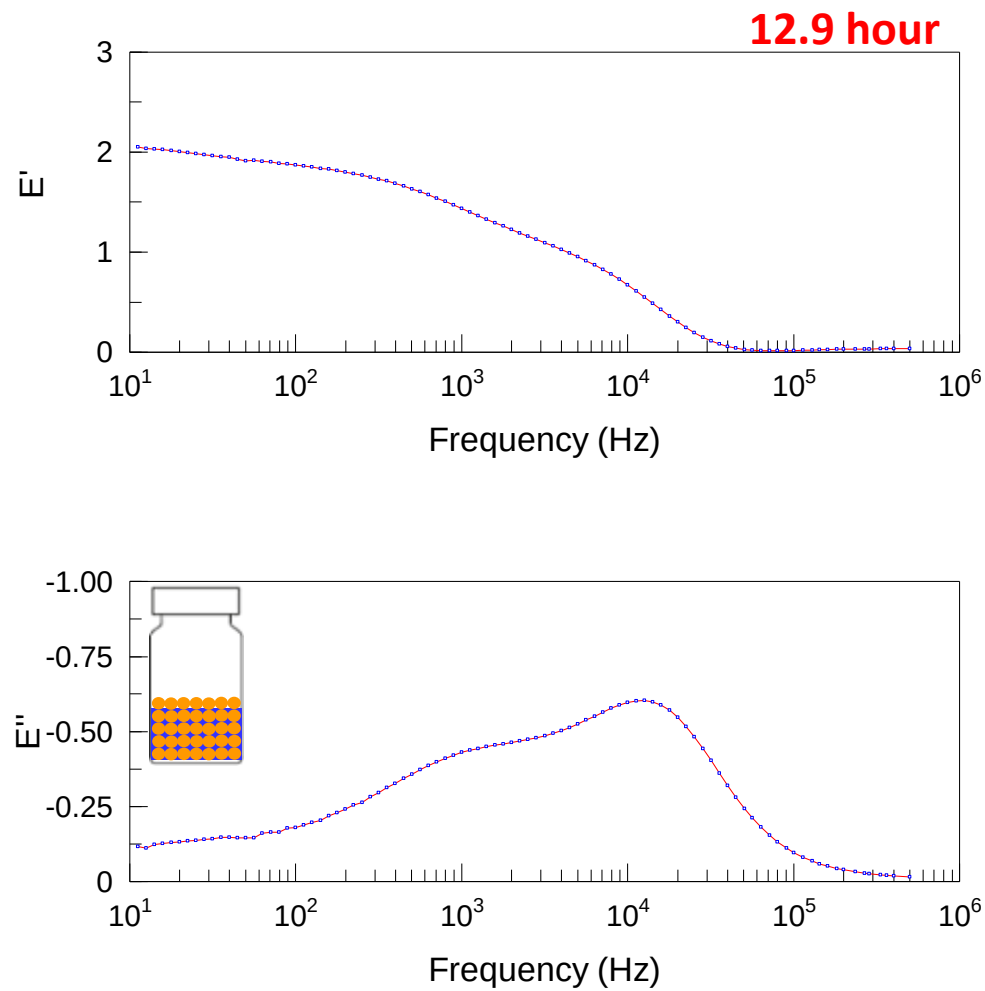
Impedance Spectra of Primary Drying Process





Impedance Spectra of Primary Drying Process





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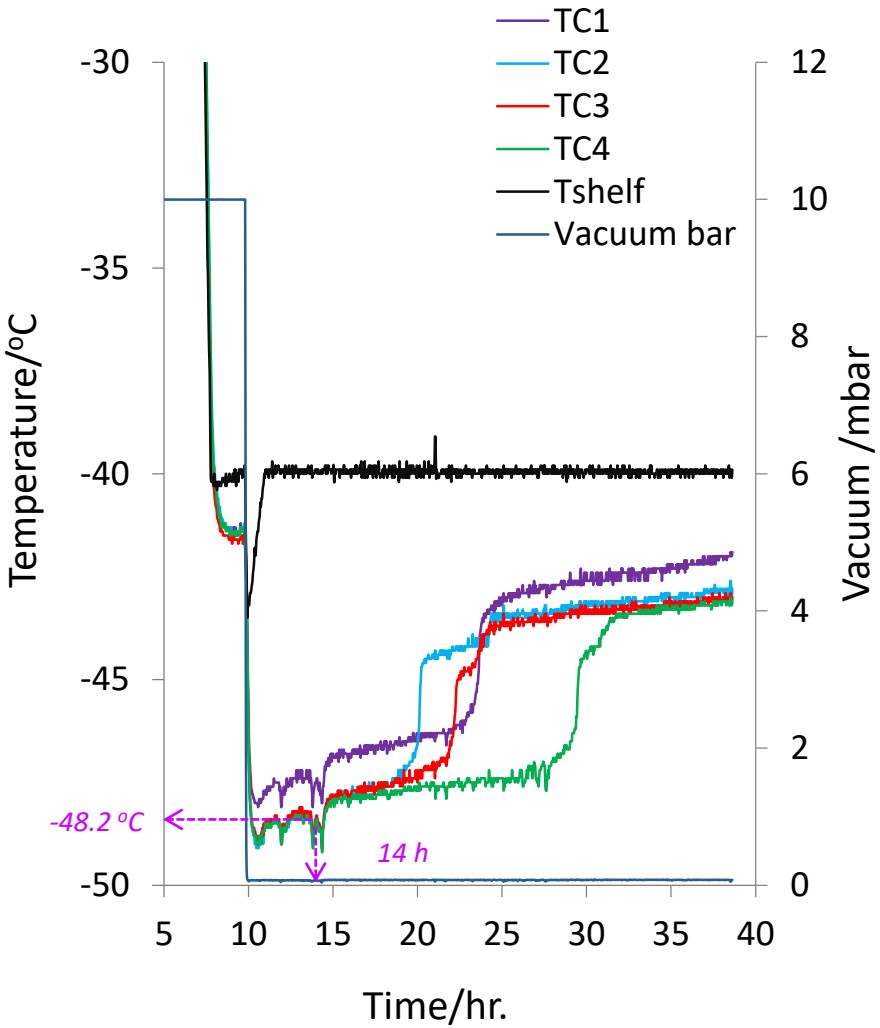
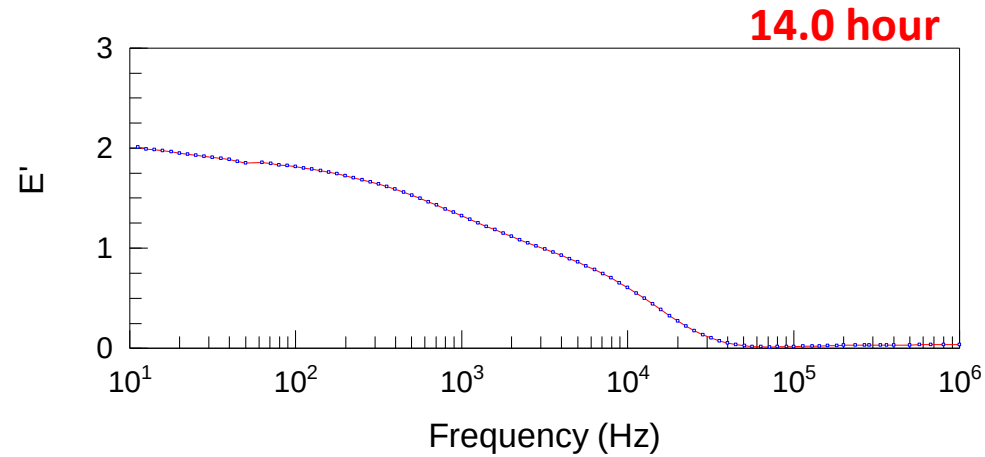
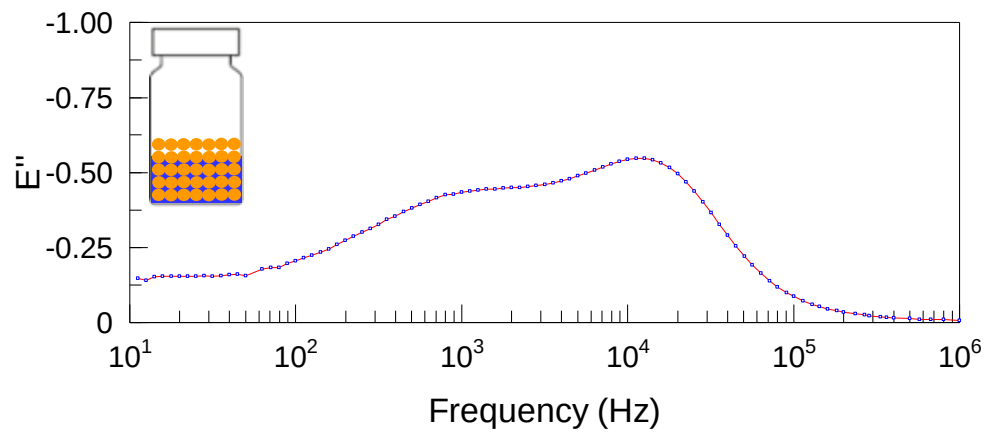
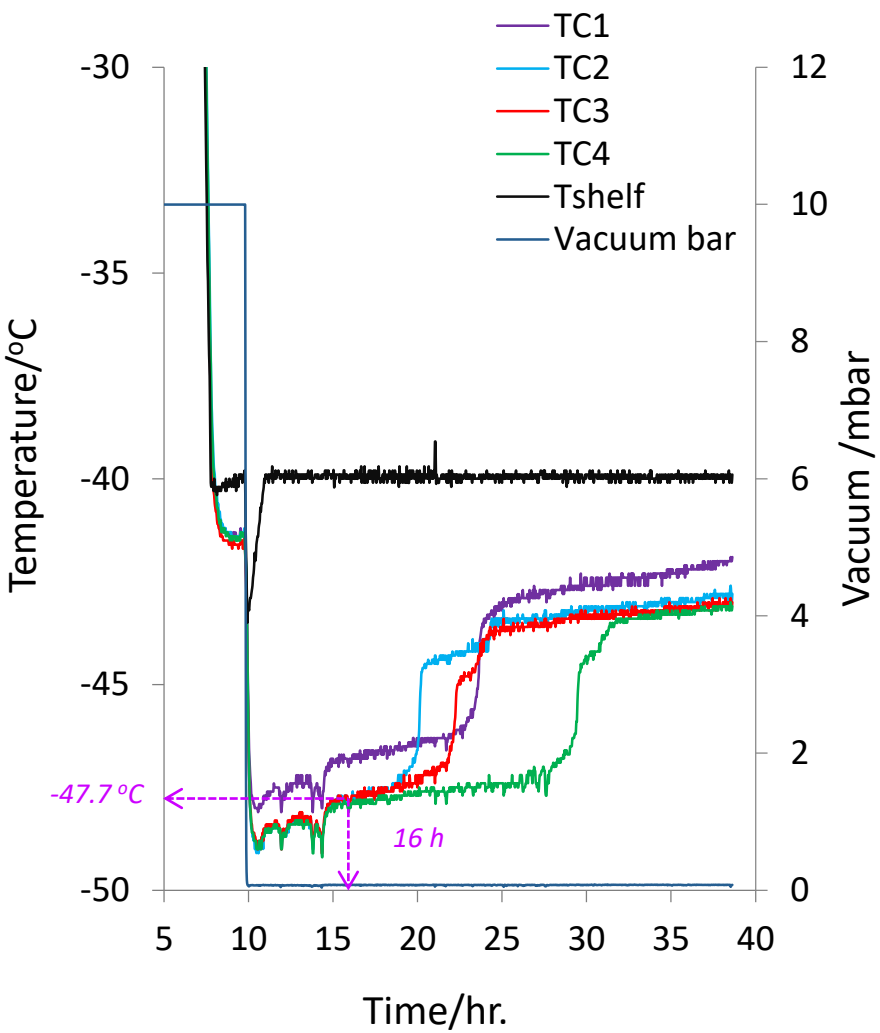
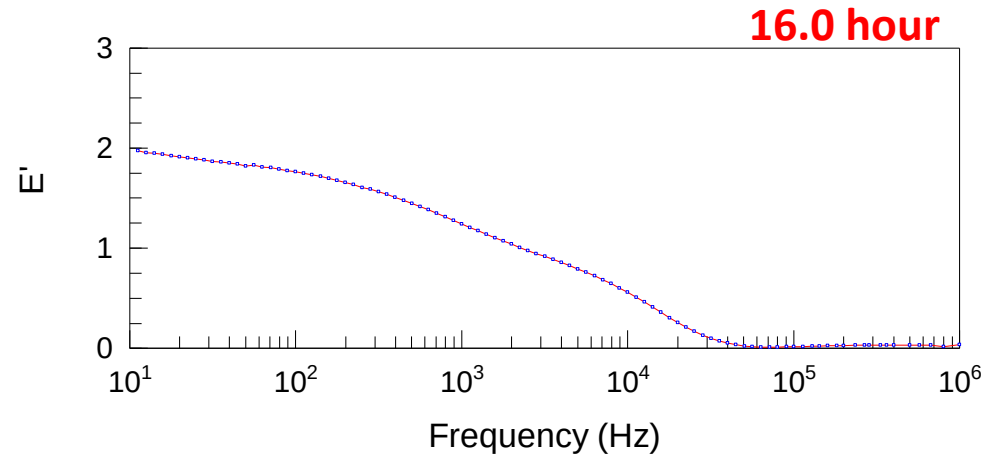


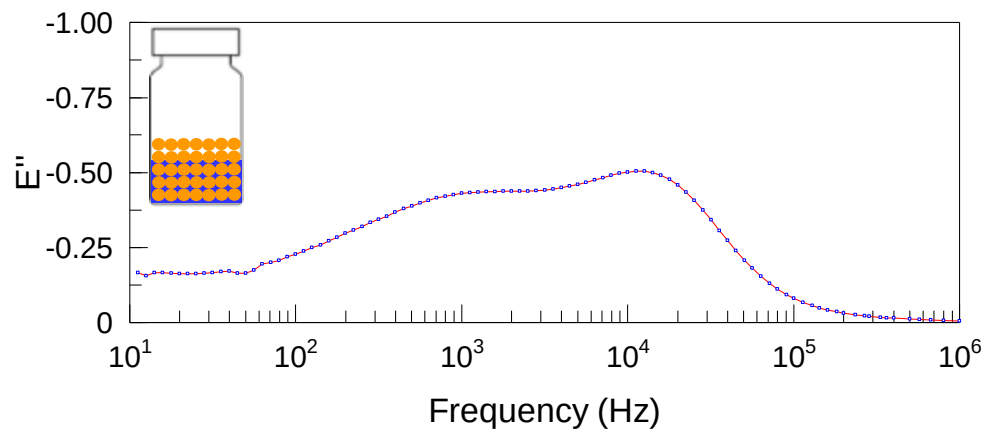




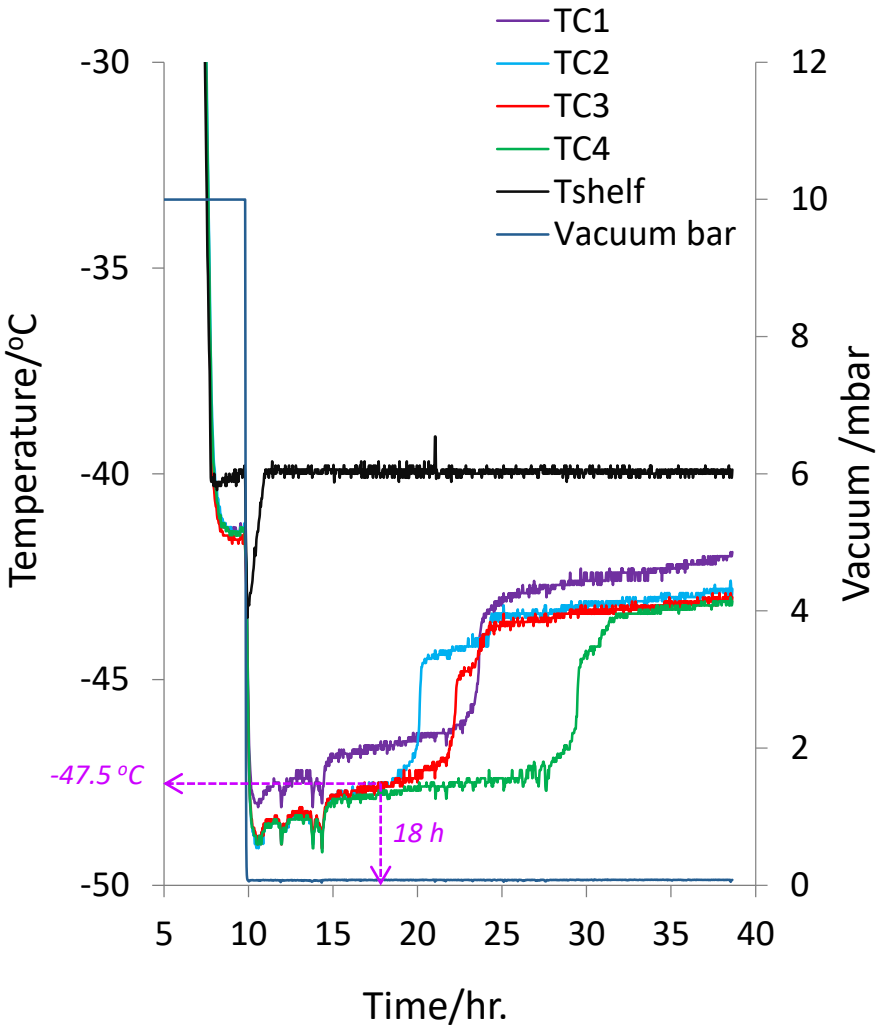
Impedance Spectra of Primary Drying Process

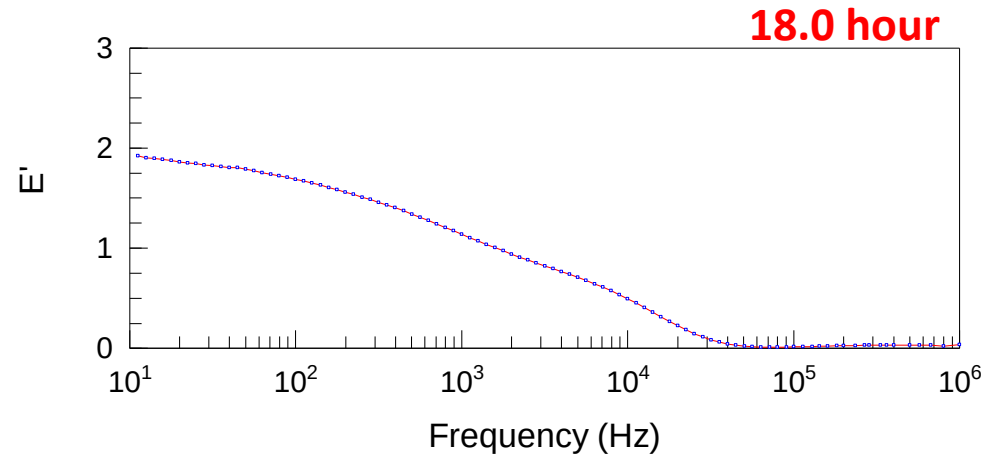


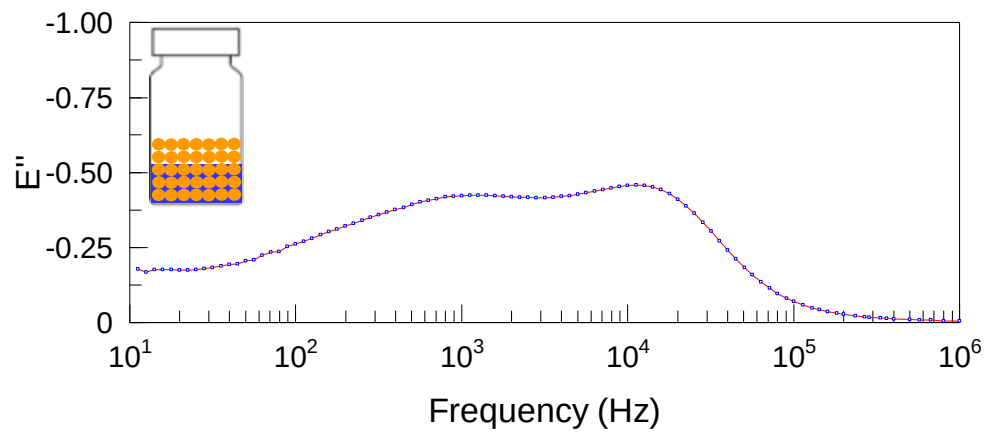




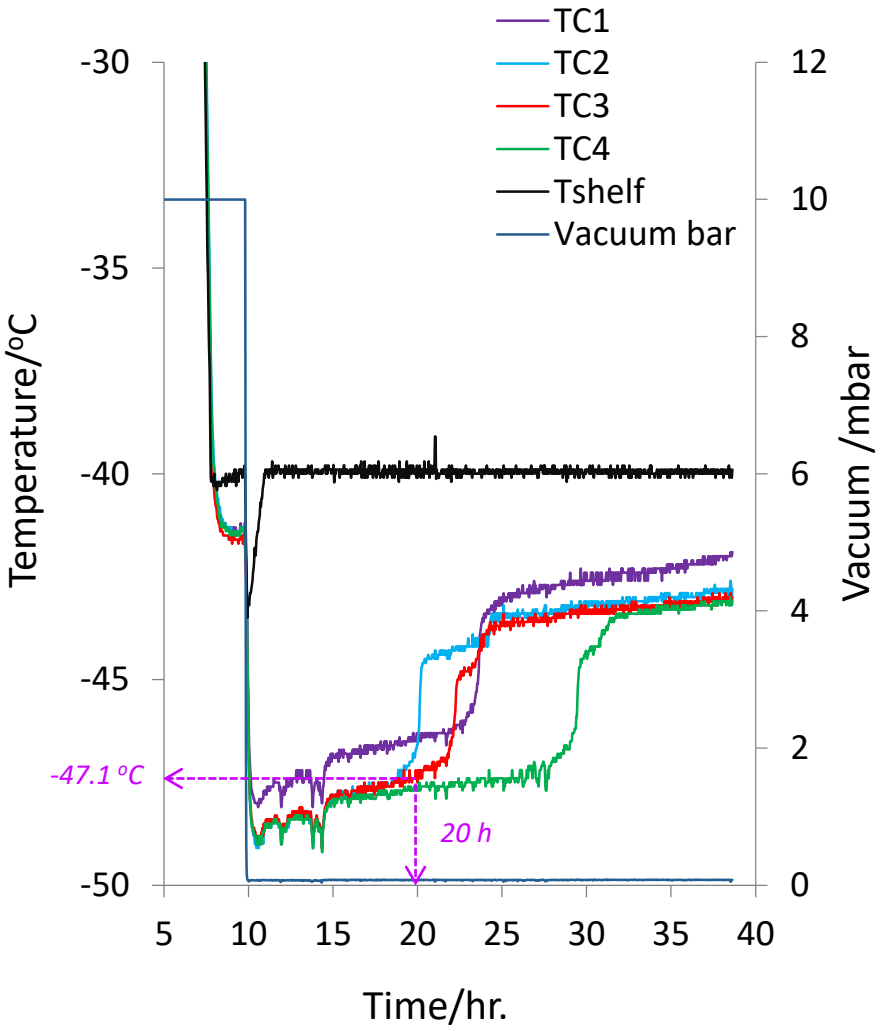
Impedance Spectra of Primary Drying Process

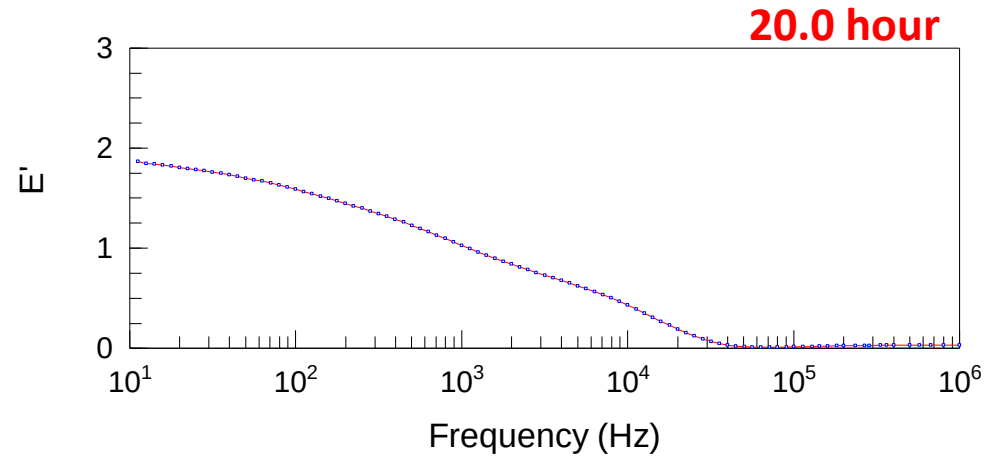


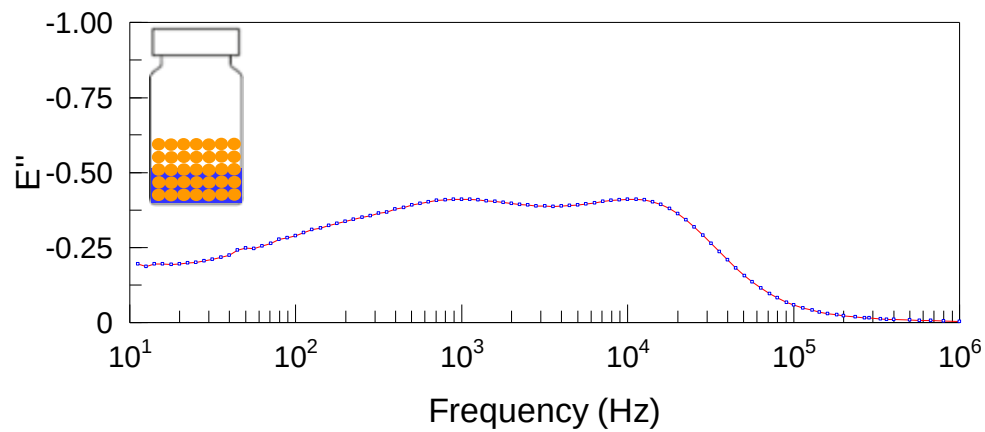




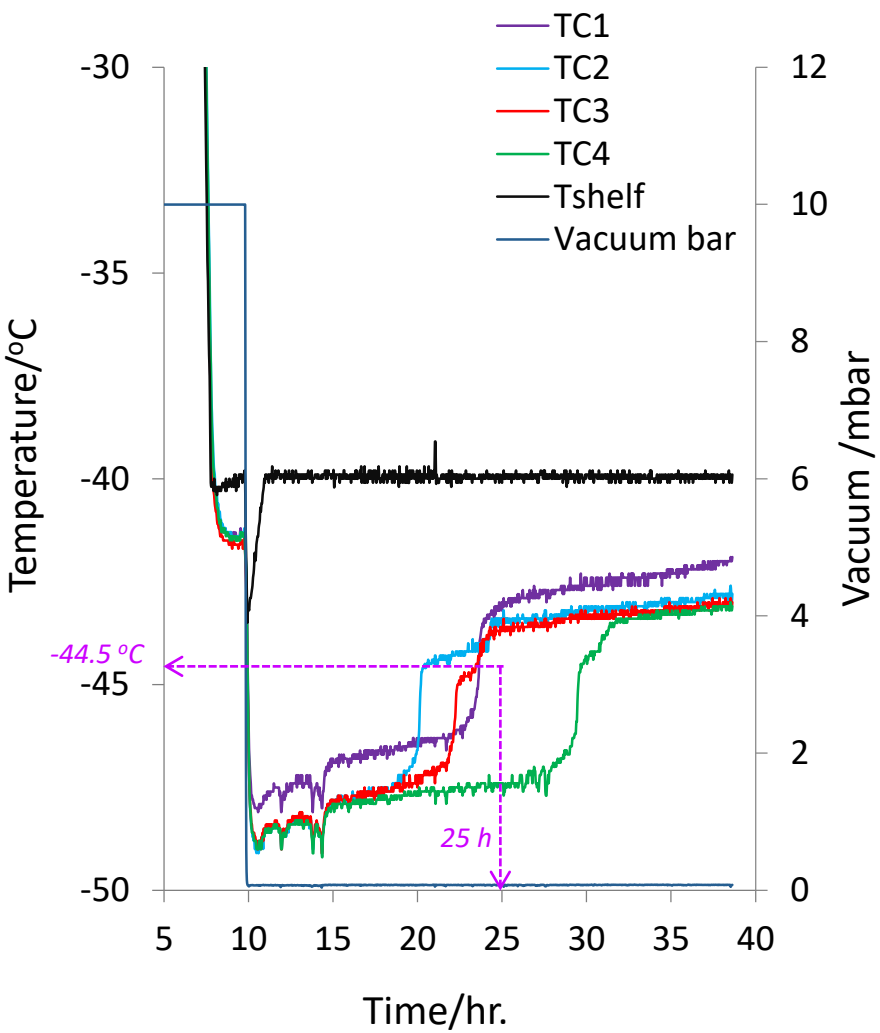
Impedance Spectra of Primary Drying Process

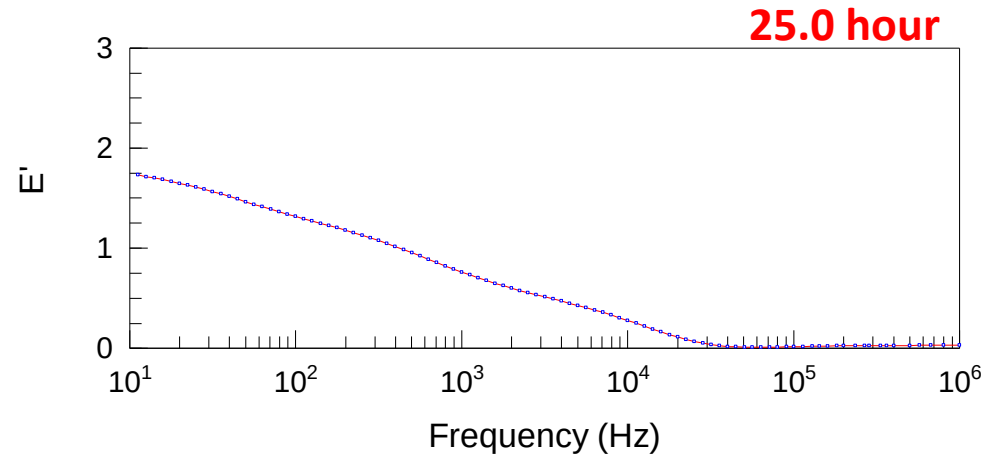


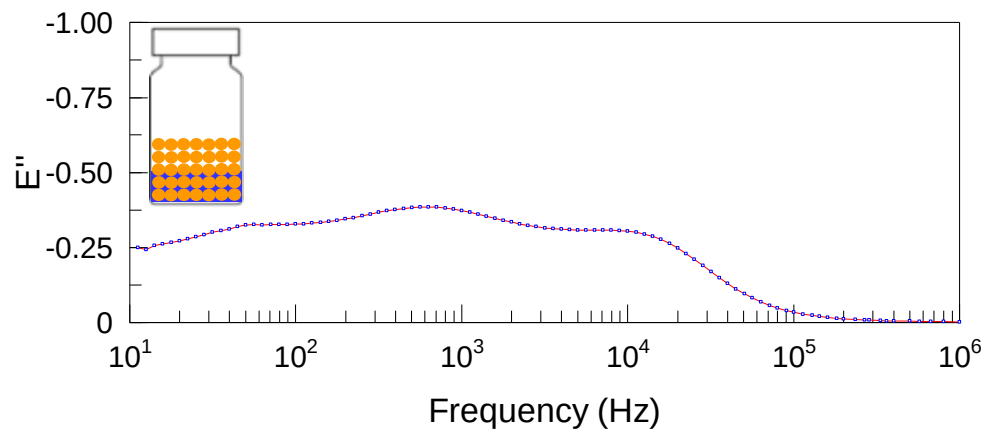




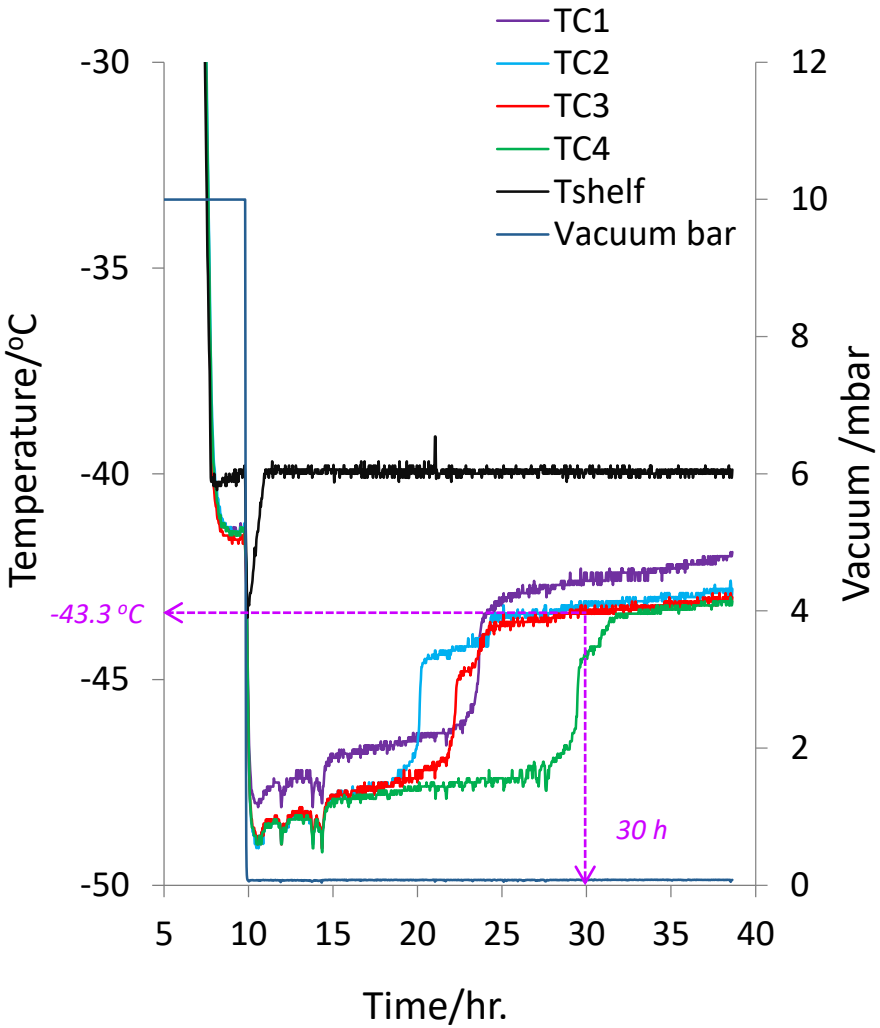
Impedance Spectra of Primary Drying Process

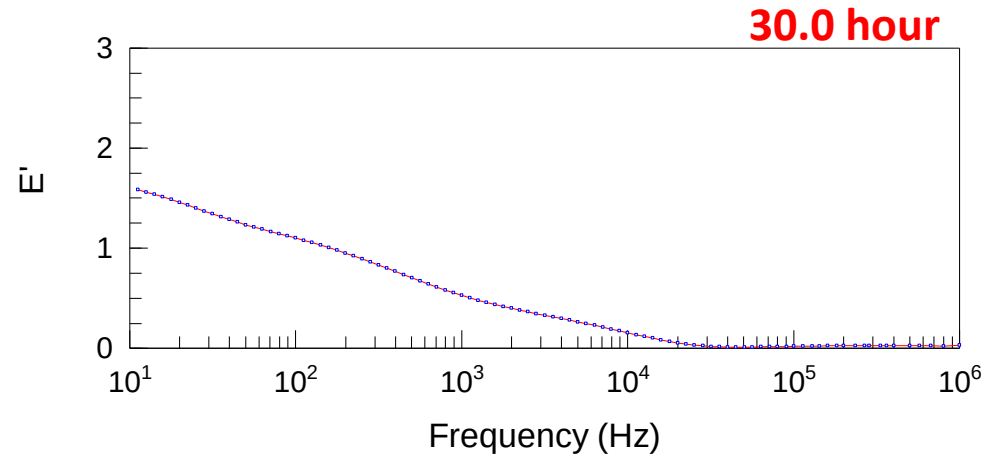


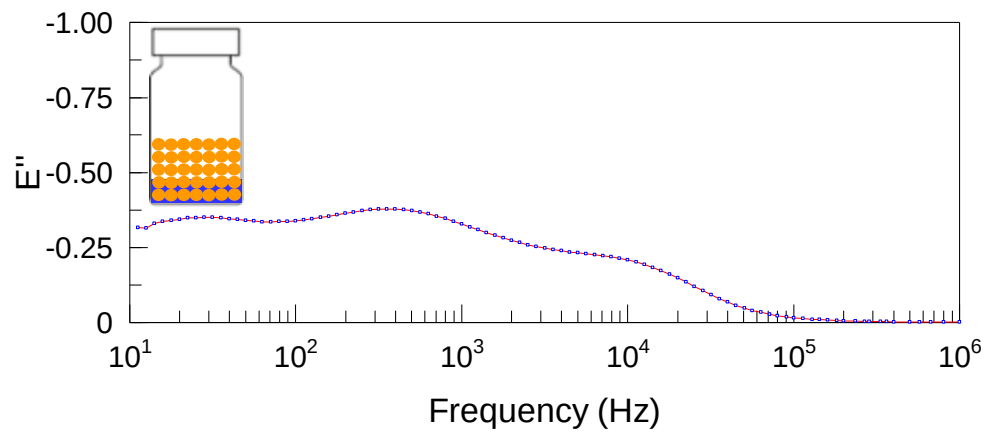




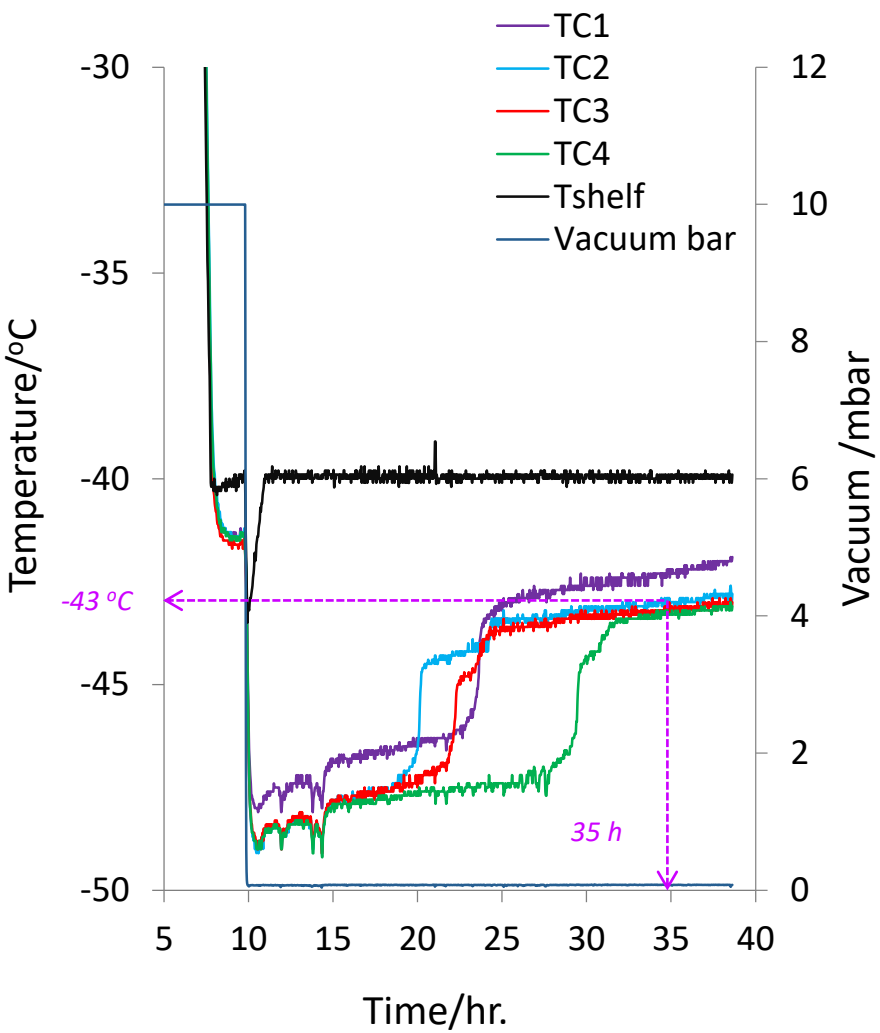
Impedance Spectra of Primary Drying Process

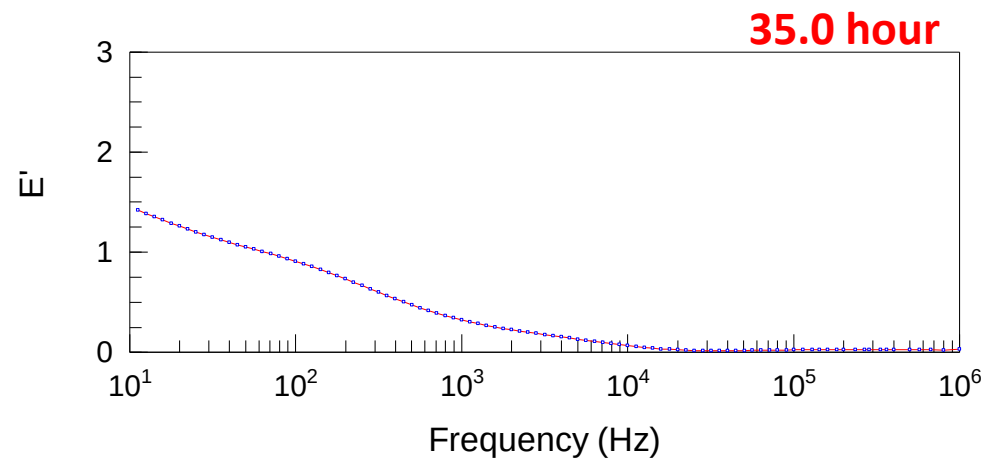


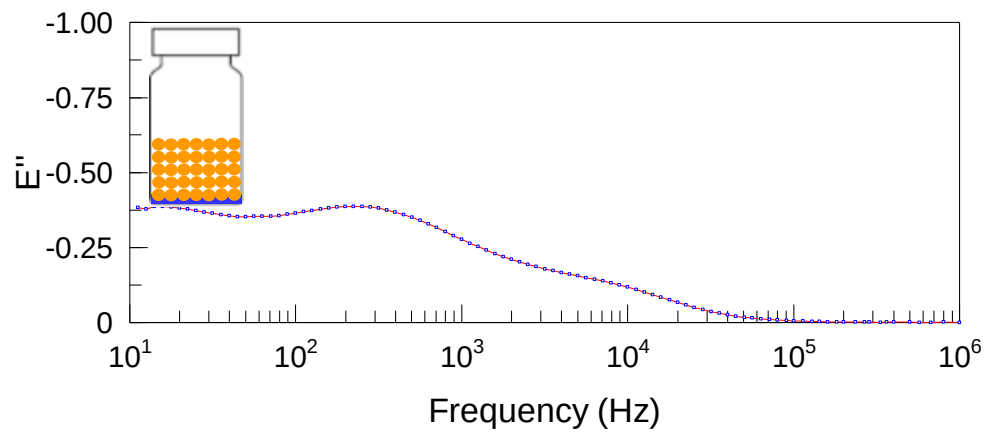




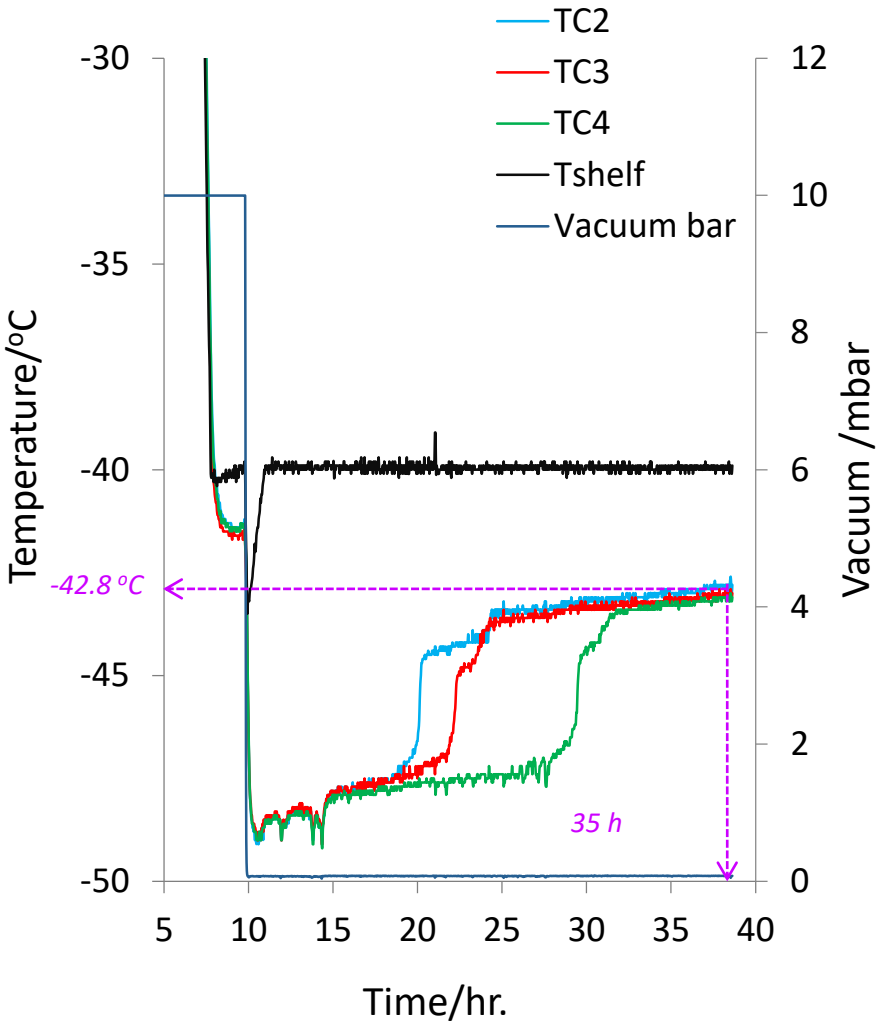
Impedance Spectra of Primary Drying Process

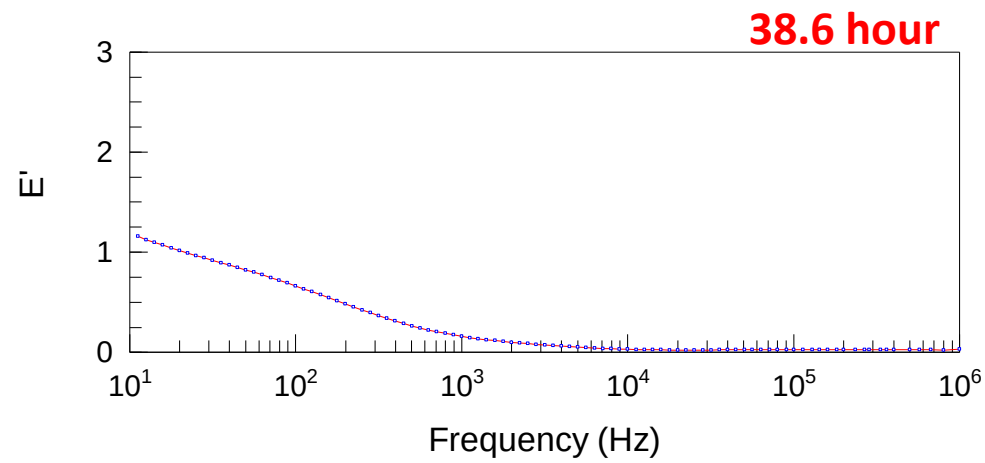


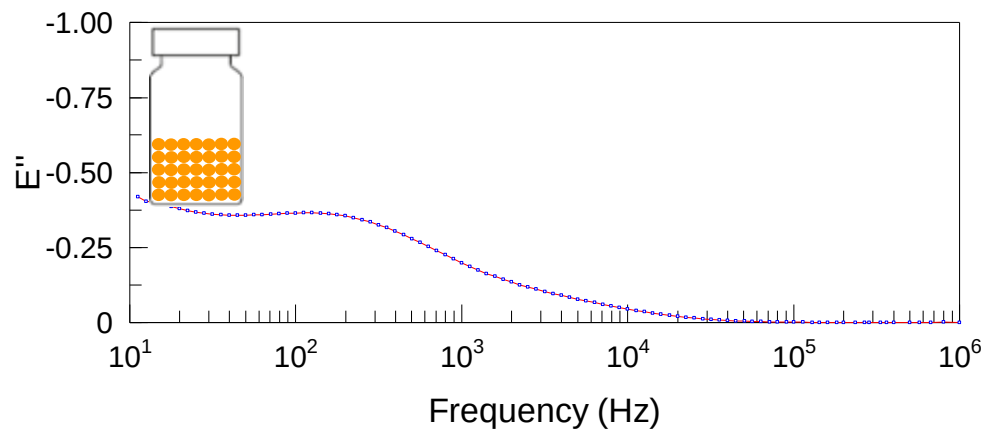




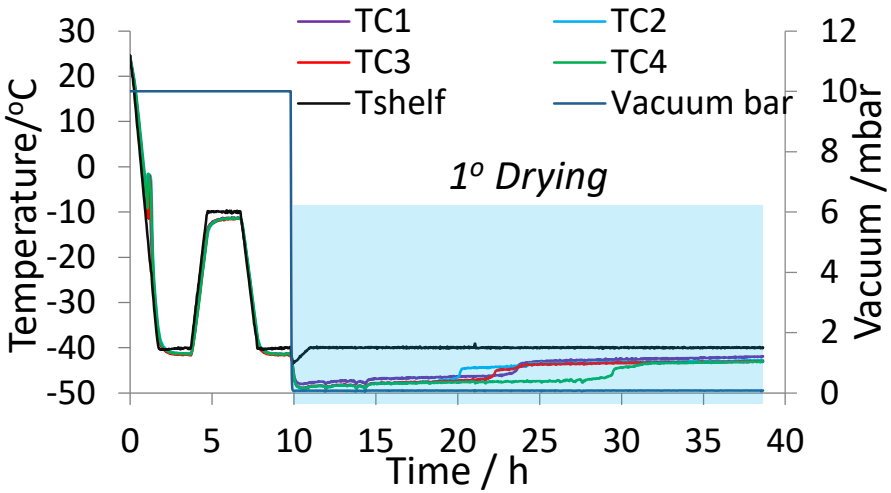
Impedance Spectra of Primary Drying Process

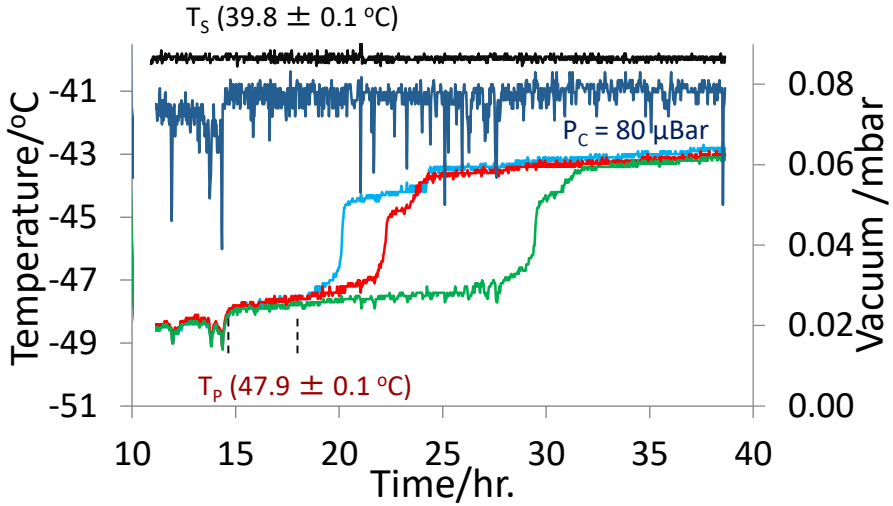


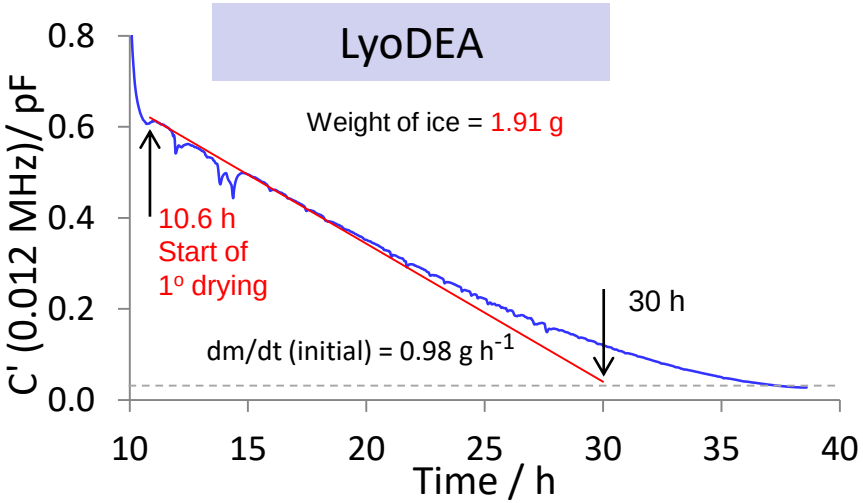




Evaluation of Drying Rate



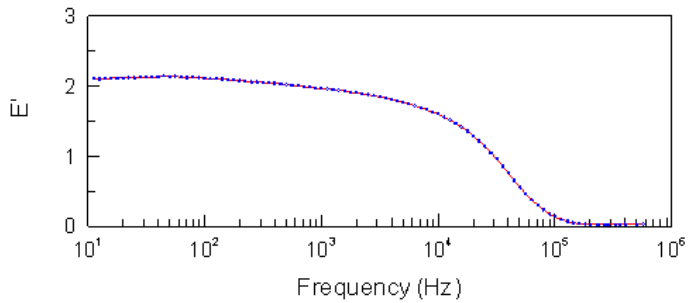


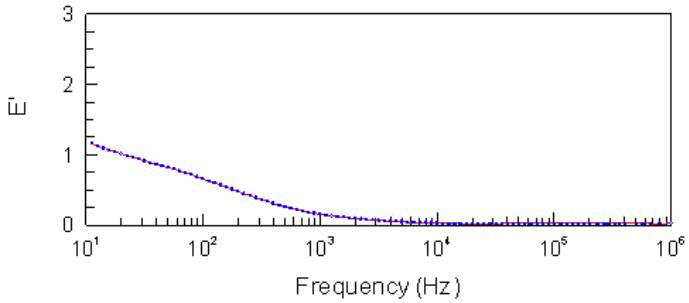


Parameter	Value	ΔT & ΔP
Product Temperature	– 47.9 ± 0.1 ºC	8.0 ± 0.2 ºC
Shelf Temperature	– 39.9 ± 0.1 ºC	
Drying Rate (dm/dt)	0.98 g h^{-1}	

Opportunities

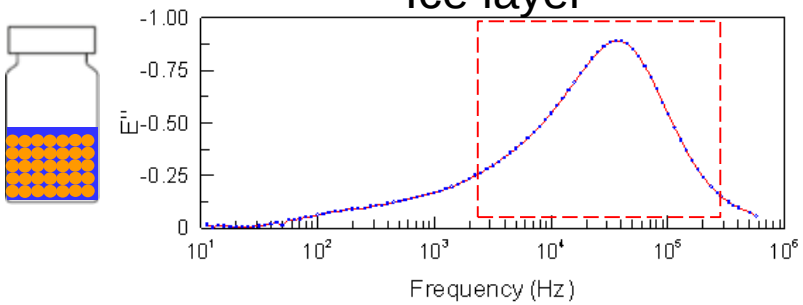
- Calculation of dry layer resistance (R_P) as a function of time
- Low frequency process may hold information on the state/properties of the protein layer, inc. progression and end point of secondary drying

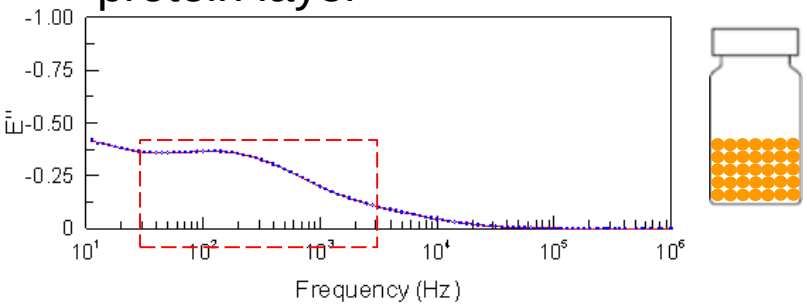




Ice layer

protein layer





High frequency process (start of primary drying)

Low frequency process (end of primary drying)

DE MONTFORT UNIVERSITY LEICESTER

Leicester School of Pharmacy

Other Application

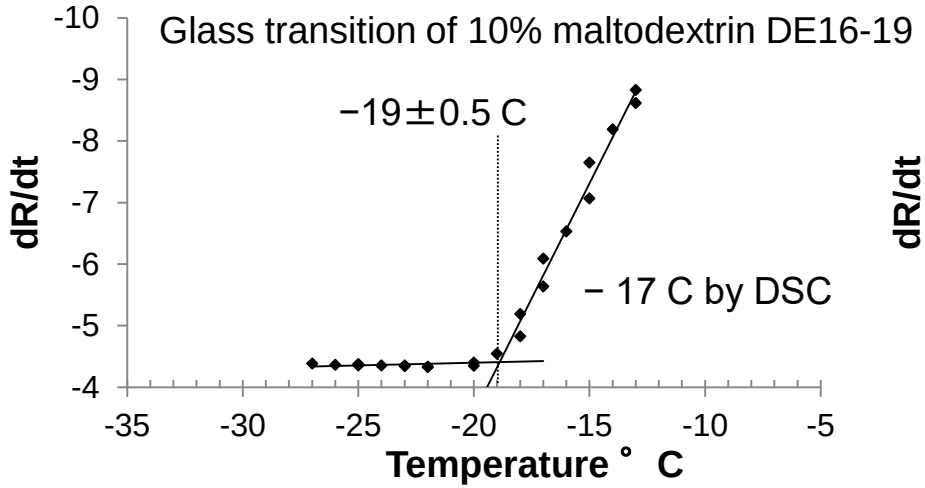
Product Collapse

Glass Transition

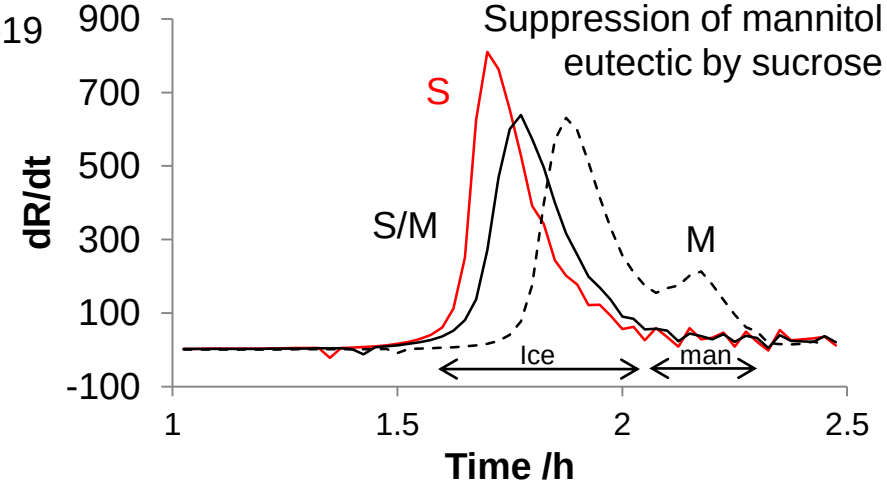
Eutectic Formation

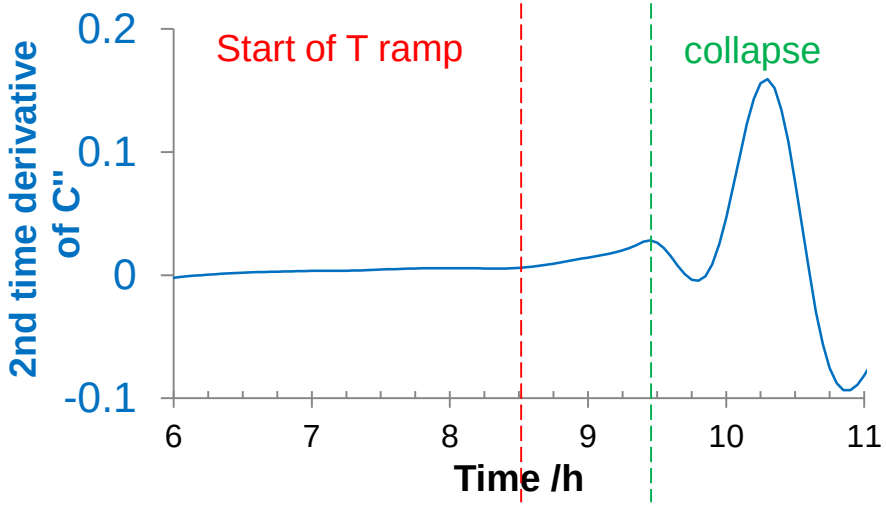
Product Characterization : Glass transition, Eutectic Crystallization, Collapse

Smith G et al (2013) Eur J Pharm Biopharm 85 (3)1130-40



Arshad MS et al (2014) Eur J Pharm Biopharm 87(3):598-605





Smith G et al (2014) Pharmaceutical Technology 38(4)

Challenges!!

- What should be classed as critical process parameters ?
  - Chamber pressure and shelf temperature

  →Product Temperature (relative to the collapse temperature) – how is T_P monitored and/or controlled?
- What are the challenges inherent to pre-formulation (e.g. phases transitions and phase separation of drug and/or excipients during lyophilisation)?
  - FDM used routinely for Tc measurement but evidence exists that Tc in vial is not always the same as Tc by FDM
- What happens on microscale-down and scale up ?
  - Container size / geometry and dryer design and construction impact the process
- Developing the toolbox-overlapping PAT from single vial to batch monitoring
  - Requires multiplexed PAT (Raman, NIR & Impedance)
  - Combine data – exploit synergies
  - Cross validate/calibrate technologies (best for batch / best for continuous)

Opportunities

- TVIS registers thermal events through changes in the sample resistance associated with
  - Discontinuous changes in viscosity (glass transition, collapse)
  - Change of state (e.g ice formation and eutectic formation)
- Temperature control might be possible through monitoring of log f_{peak}
  - Requires improved modelling and calibration (Equivalent ciruits)
- Primary drying (loss of ice) is monitored through changes in the strength of the dielectric loss peak (or step in the real part capacitance)
  - Enables drying rate determinations for Kv and Rp calculations

Other (not reported here)

- Meso-structural information was extracted through the (non-Arrhenius) temperature dependence of the resistance
- Mechanisms of annealing where elucidated from changes in resistance with time (during the heating-hold phase) and from the absence of any changes in T_G

Future (Next Steps)

- Opportunities to track the physical characteristics from micro-titre plates to of collections of vials is being researched

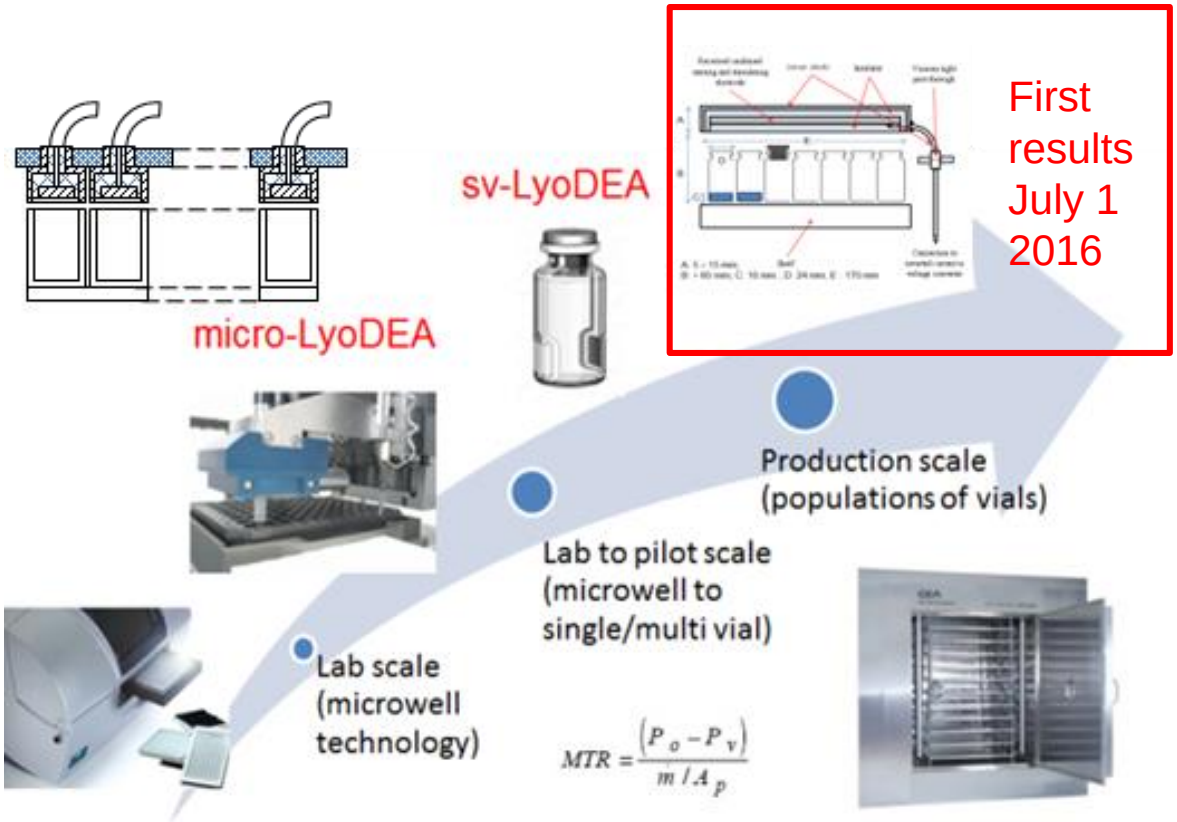
BIOSTART : Scale Down to Scale Up

Collaborative R&D project

- GEA Pharma Systems, BlueFrog,
- National Institute for Biological Standards and Control,
- Genzyme Ireland,
- De Montfort University

3 year project to implement TVIS across different scales:

- microtitre plates up to pilot scale



Supported by UK government Innovate UK

Acknowledgements

- **Evgeny Polygalov**. Senior Research Fellow. Leicester School of Pharmacy. De Montfort University (designer of the original LyoDEA system)
- **Yowwares Jeeraruangrattana**[1] and **Bhaskar Pandya**[2]. PhD students at Leicester School of Pharmacy, De Montfort University
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- **Tim McCoy** Genzyme
- **Paul Matejtschuk** NIBSC
- Technology Strategy Board and Innovate UK

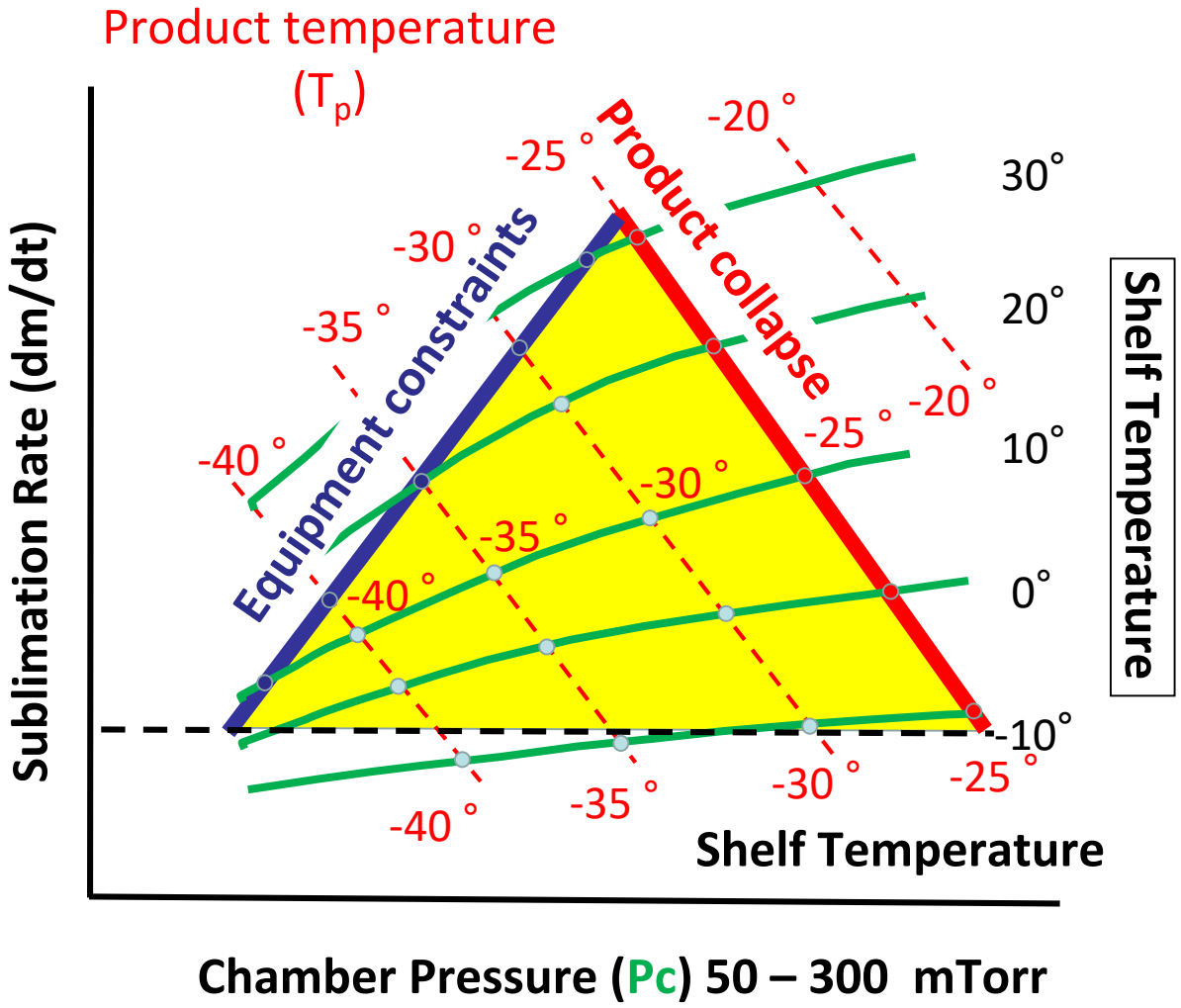
(1) sponsored by the Government Pharmaceutical Organization of Thailand
(2) Sponsored by De Montfort University's High Flyers PhD scholarship scheme.

Future Work : Design Space

Use a range of chamber pressures and shelf temperatures to establish the limits of the equipment

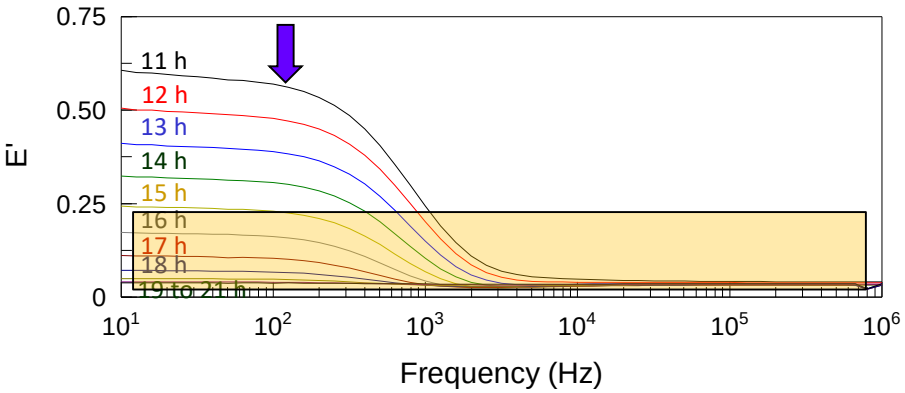
The design space is shown by the yellow triangle

Operate at the apex of the triangle to drive process efficiencies

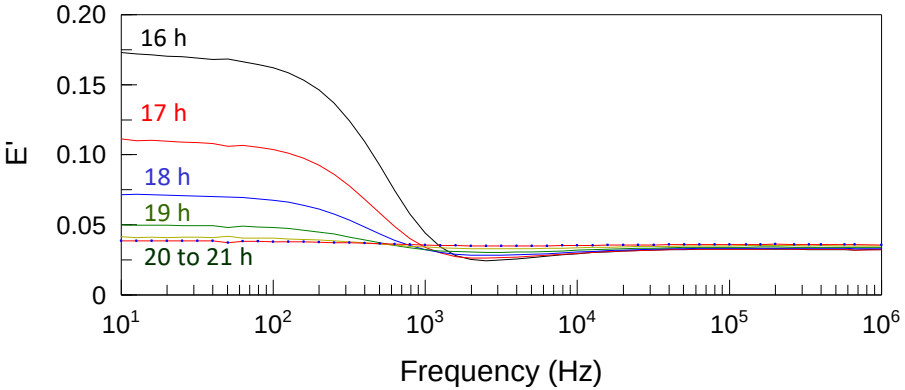


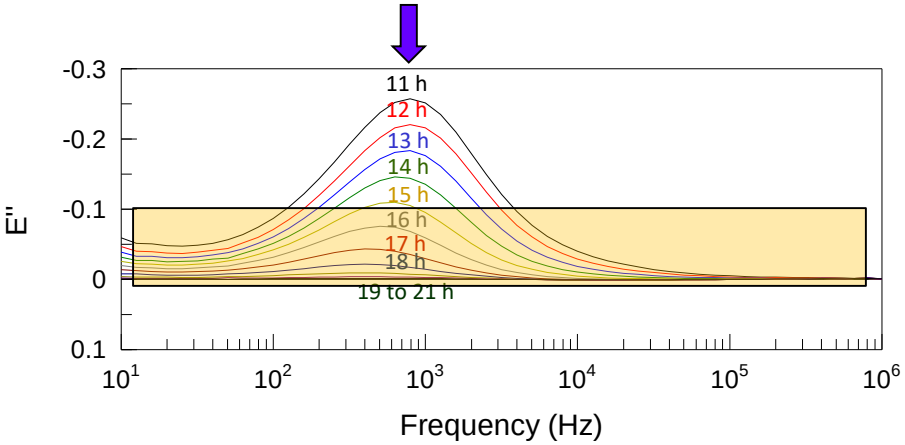
Impedance Spectrum during Primary Drying

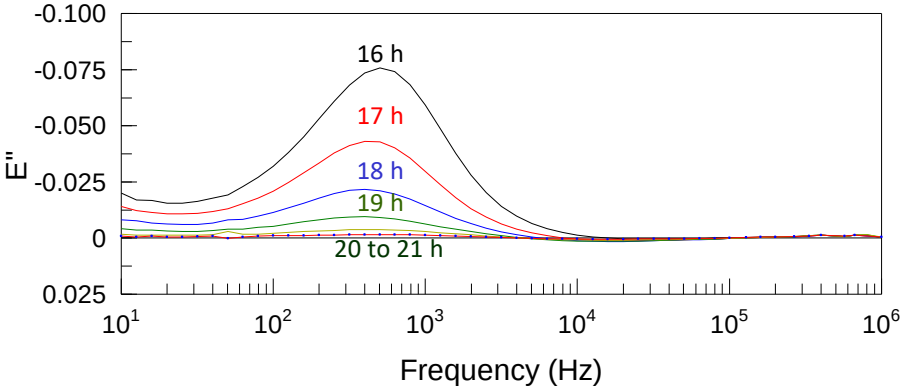
11 to 21 h



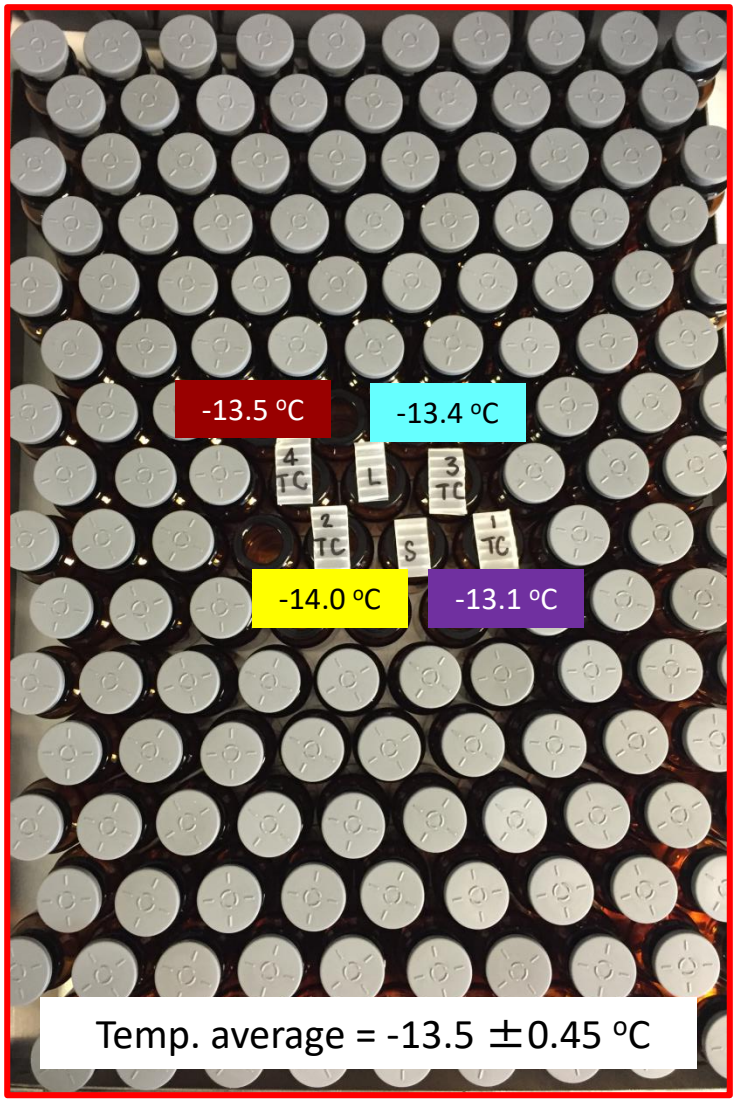
16 to 21 h

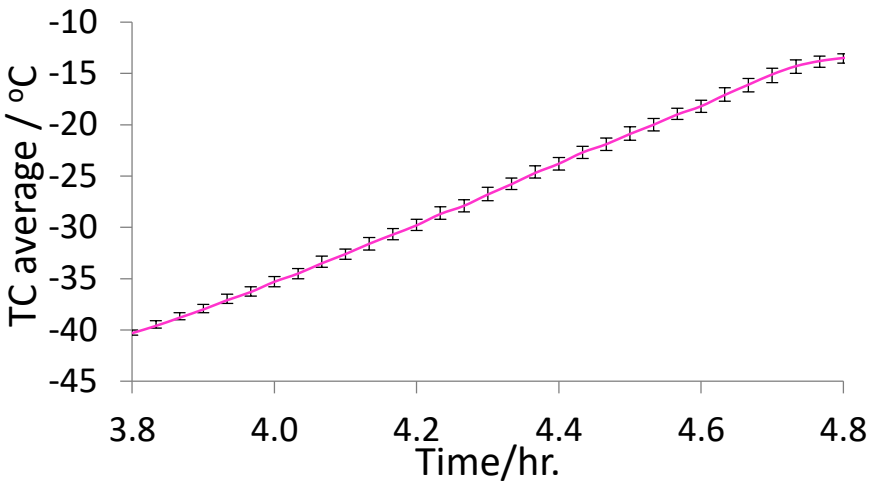


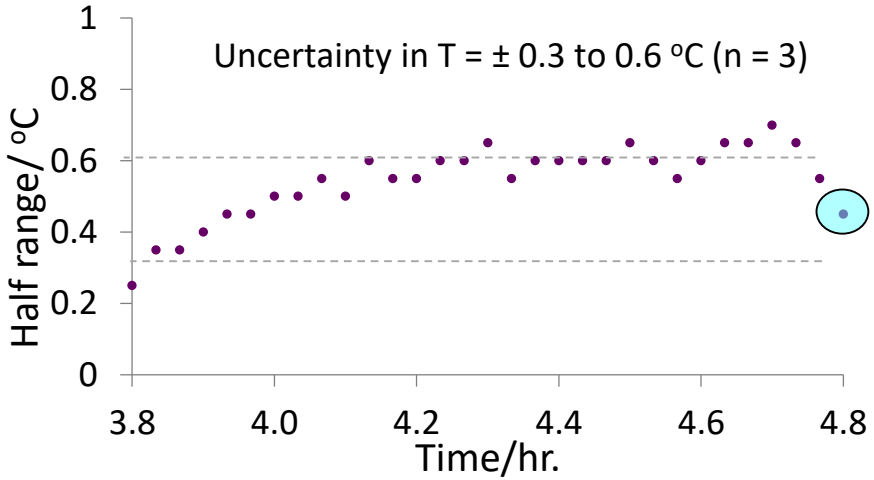




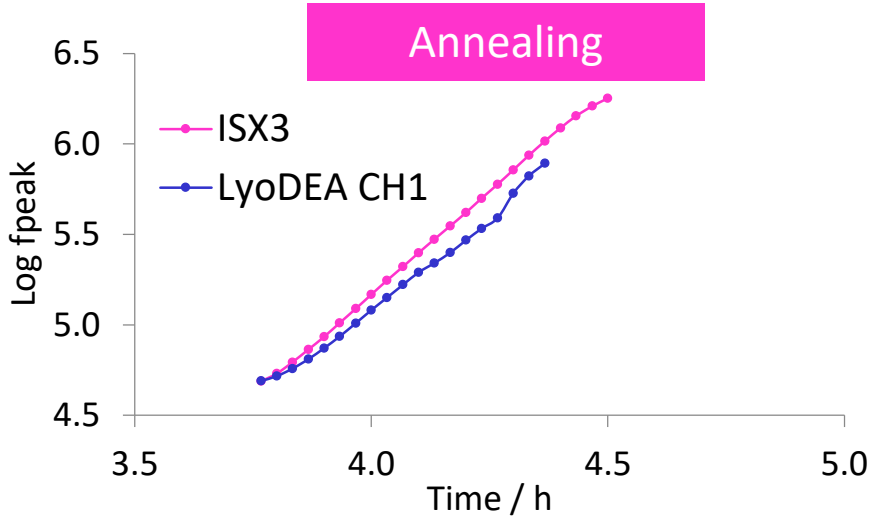
Temperature Data during Annealing Process

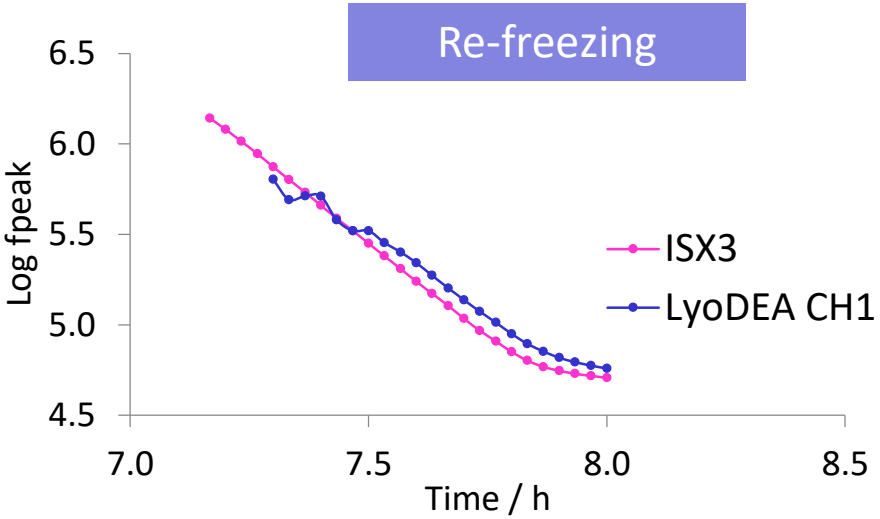


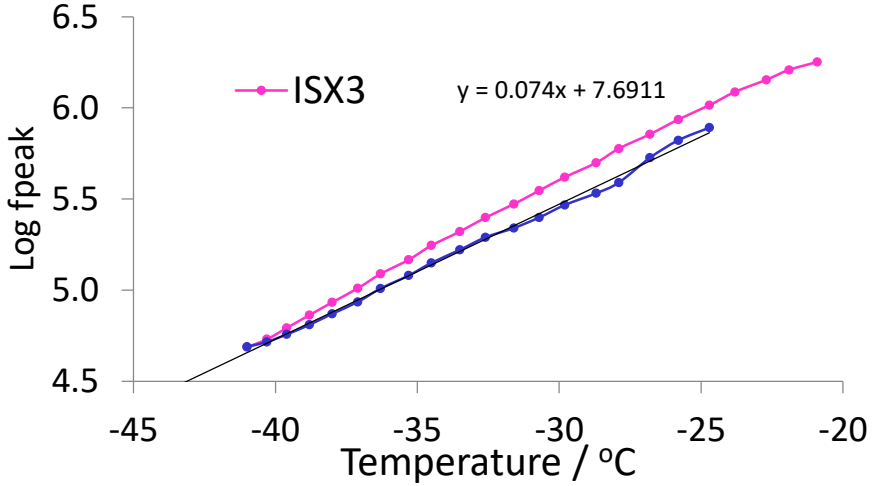


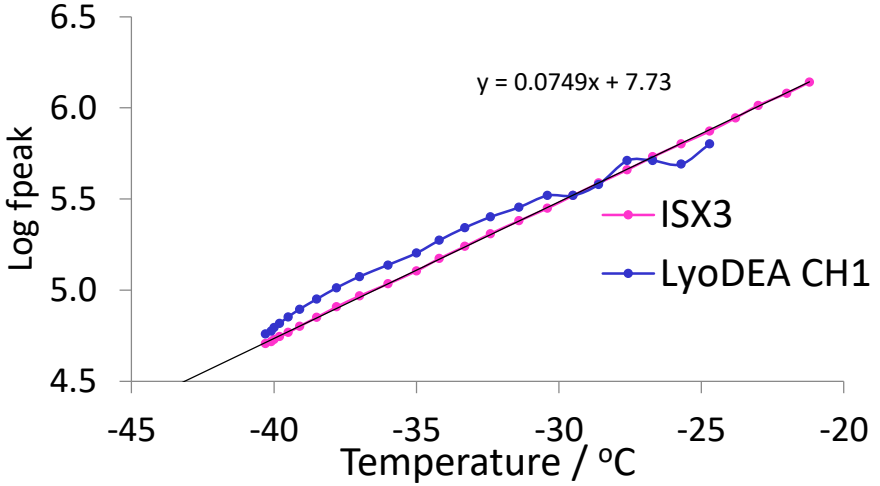


Time and Temperature Profile of Log fpeak

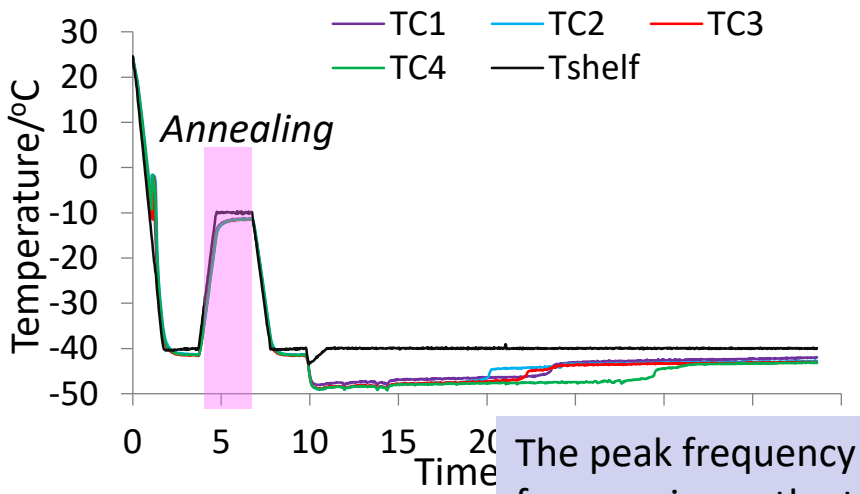


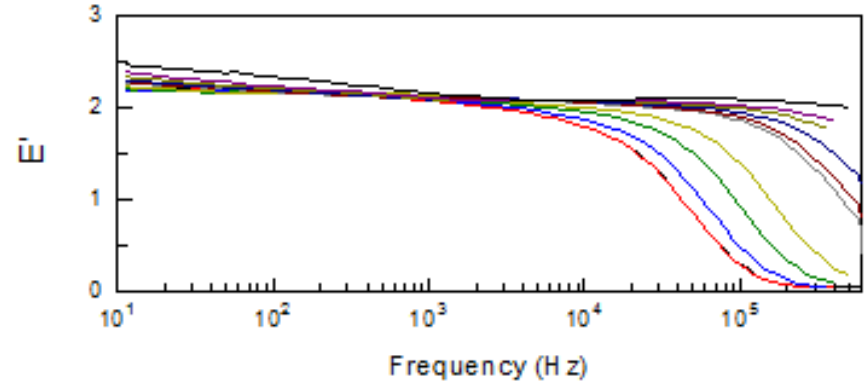


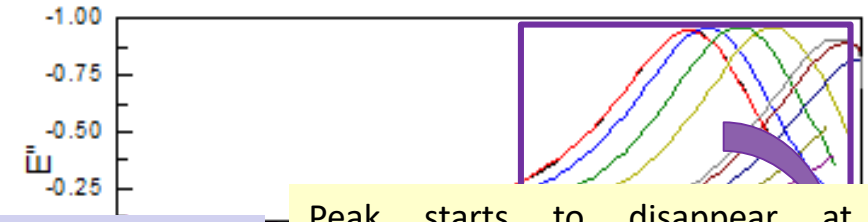




Limit of peak detection
(LyoDEA)

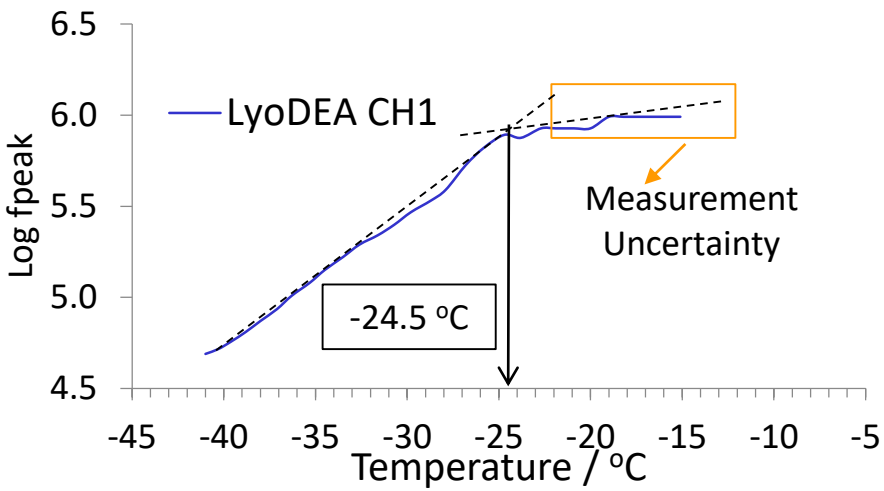


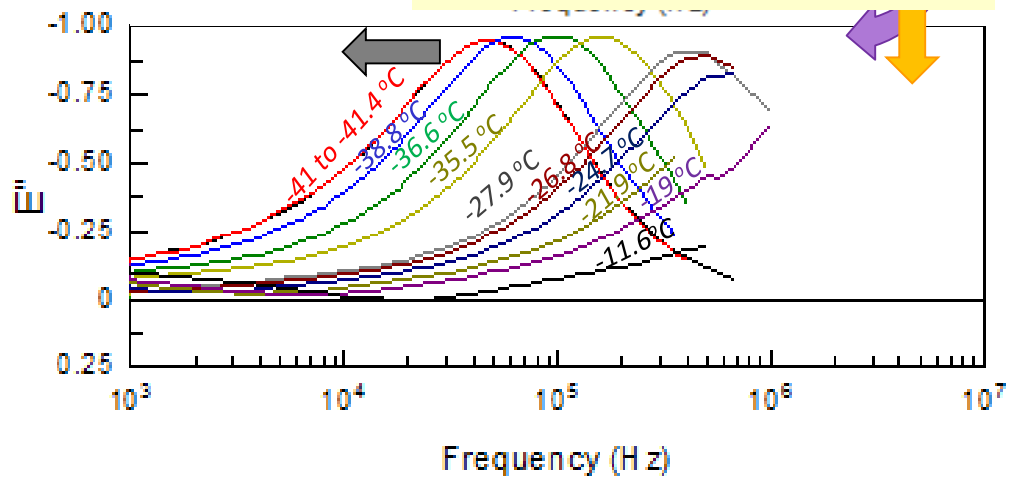




The peak frequency shifts to lower frequencies as the temperature decreases

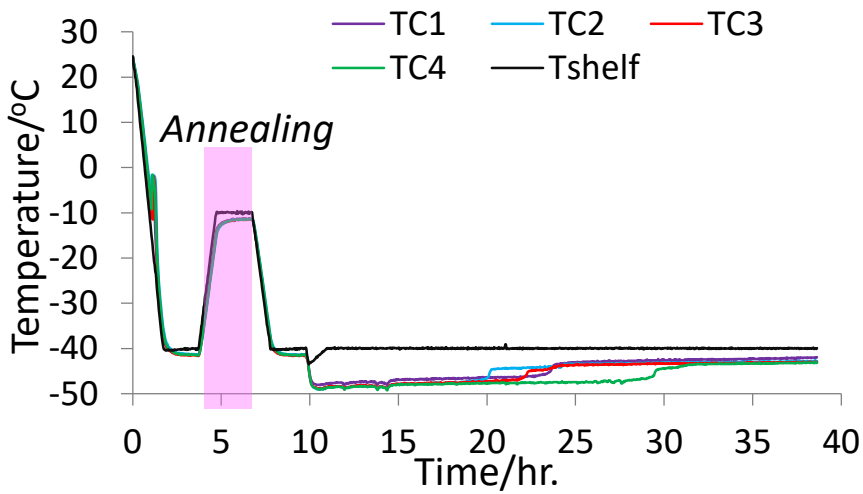
Peak starts to disappear at temperature higher than this point (-24.7°C), therefore the upper limit of detection is around -25°C

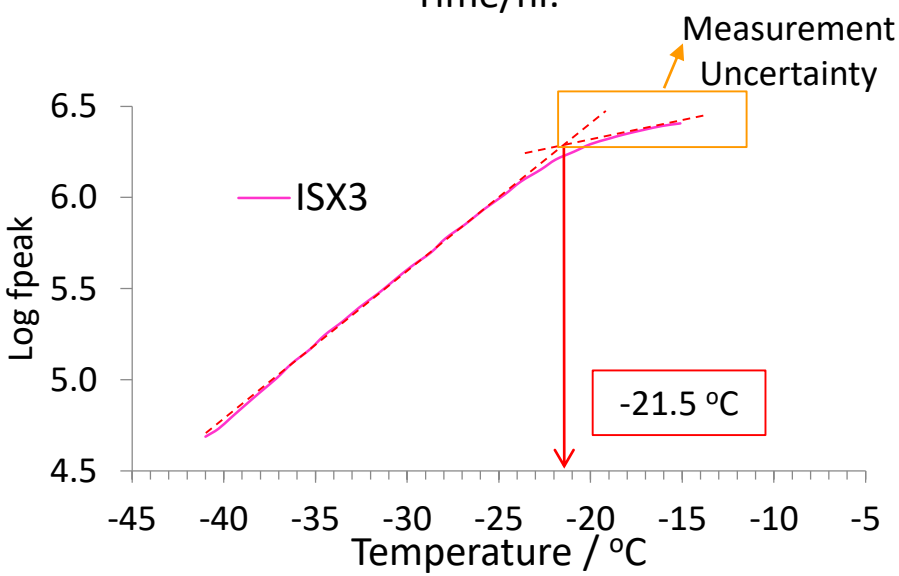


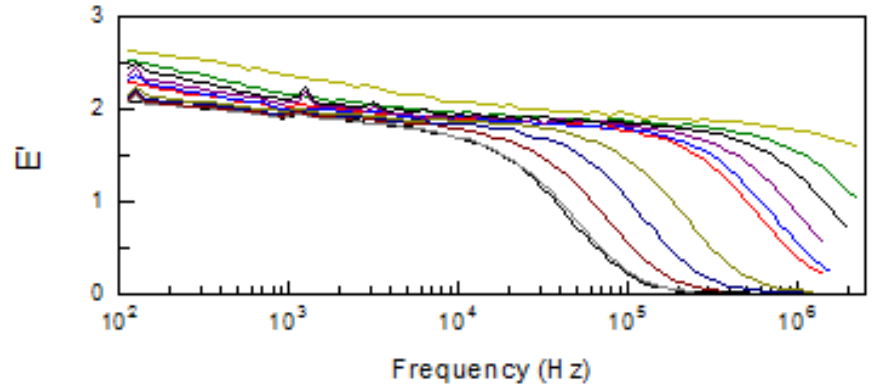


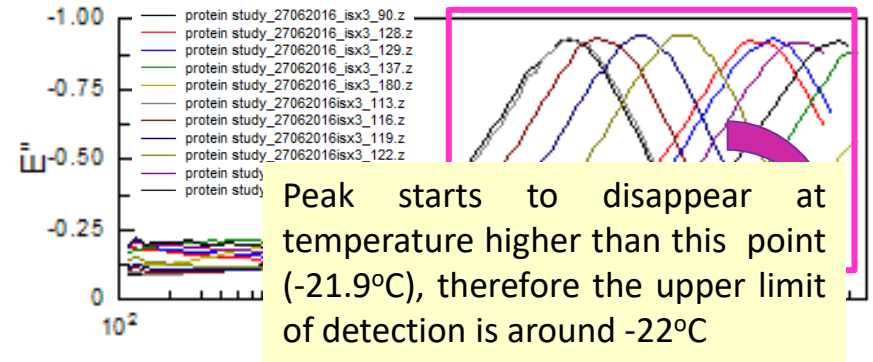
Limit of peak detection

(Sciospec)

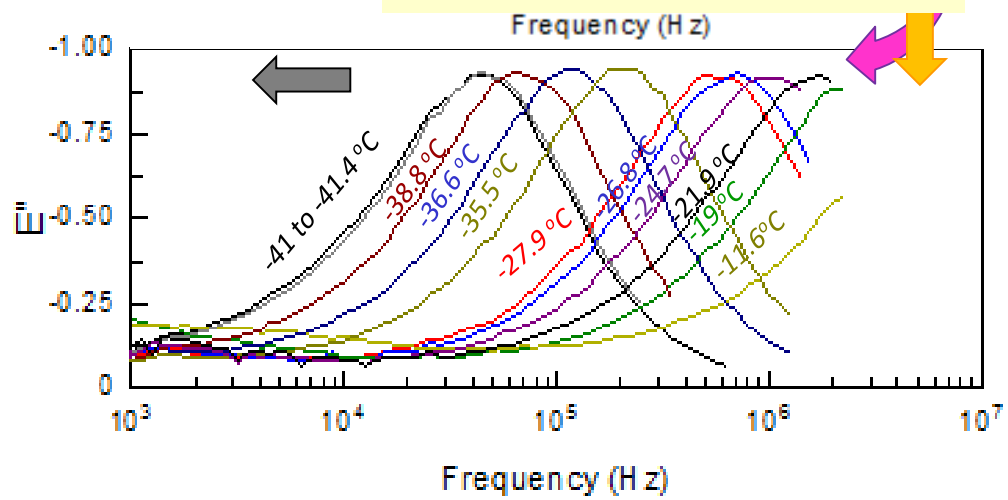




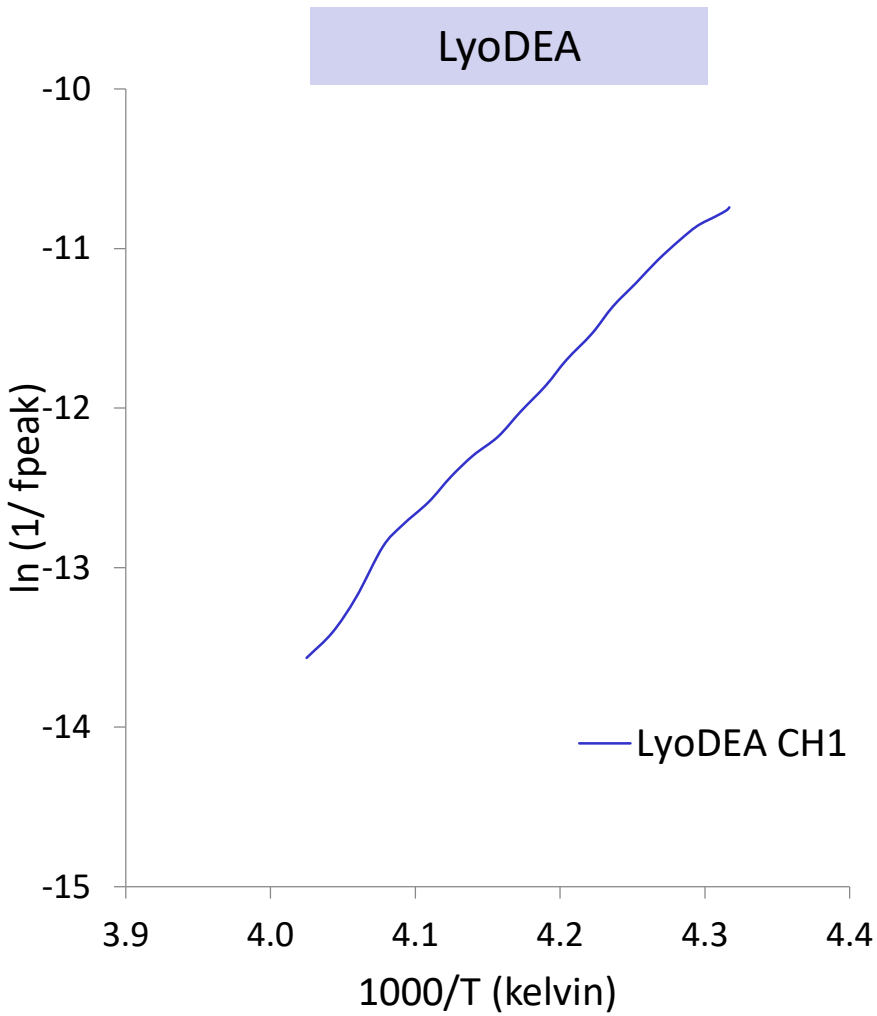


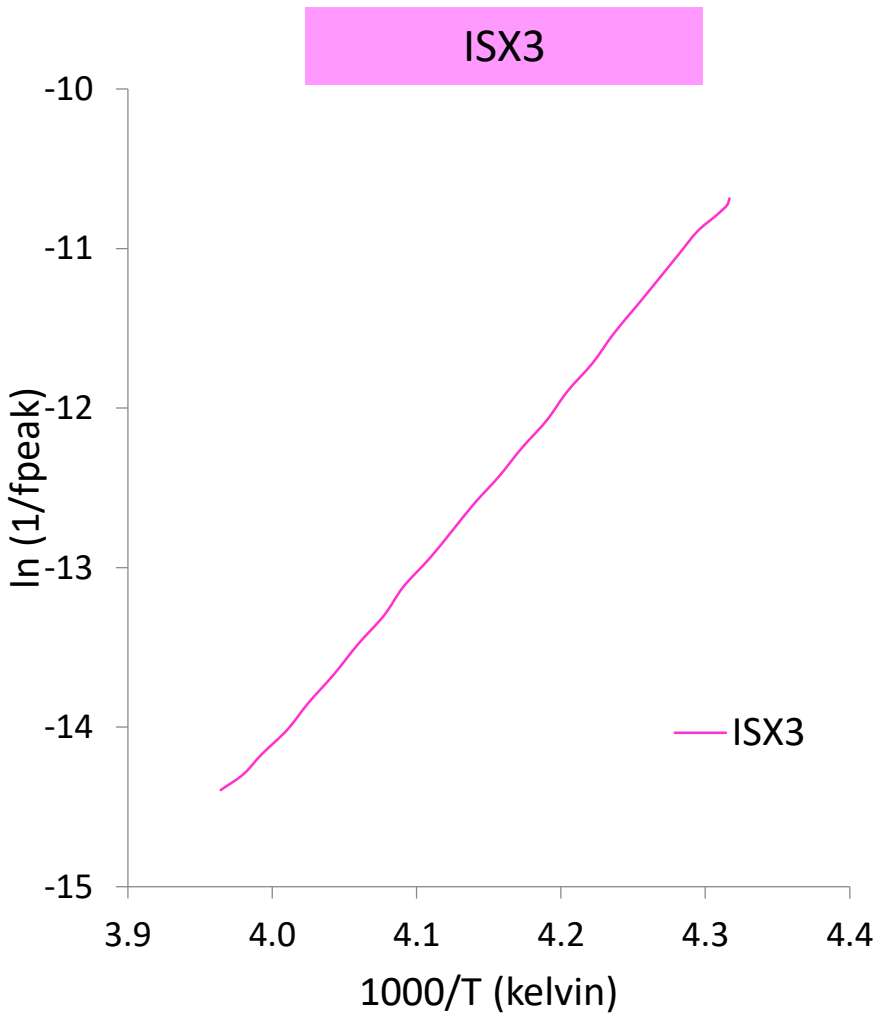


Peak starts to disappear at temperature higher than this point (-21.9°C), therefore the upper limit of detection is around -22°C

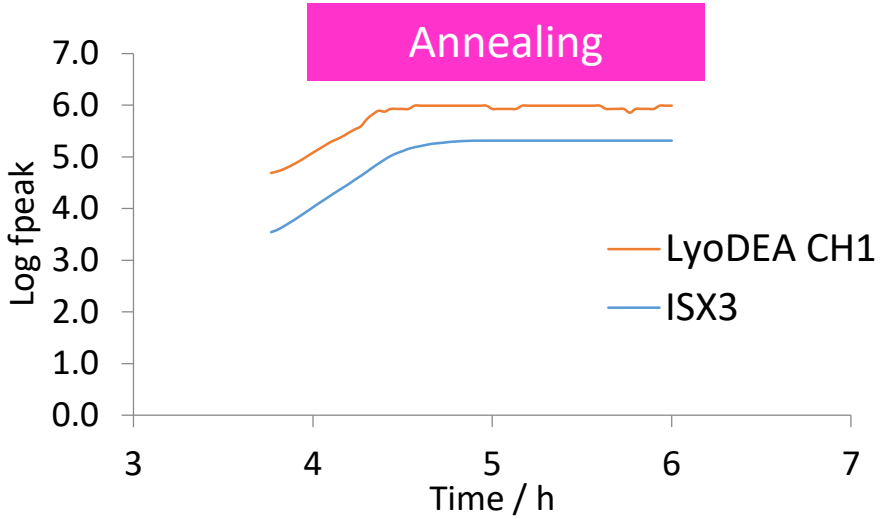


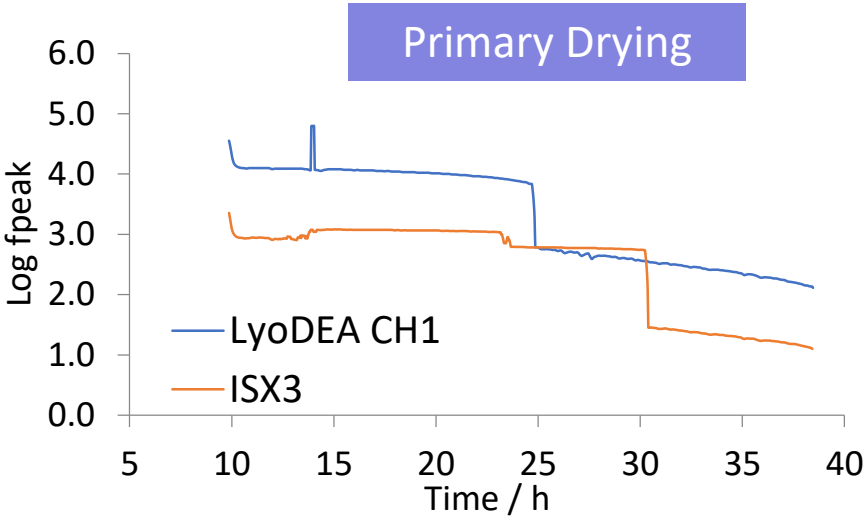
Temperature profile of ln (1/fpeak)-Annealing Step

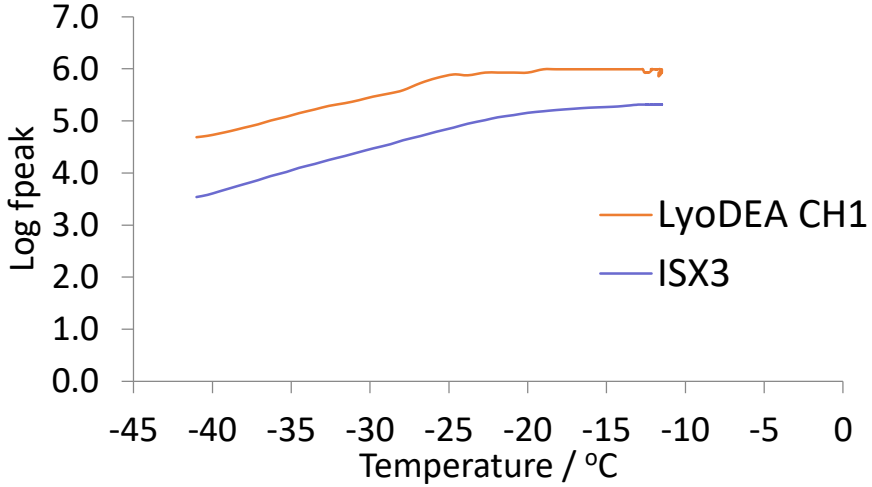


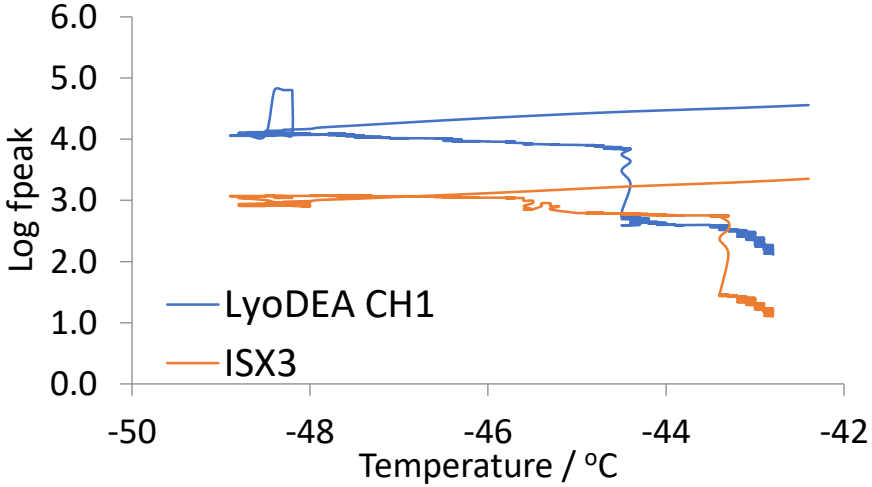


Time and Temperature Profile of Log fpeak

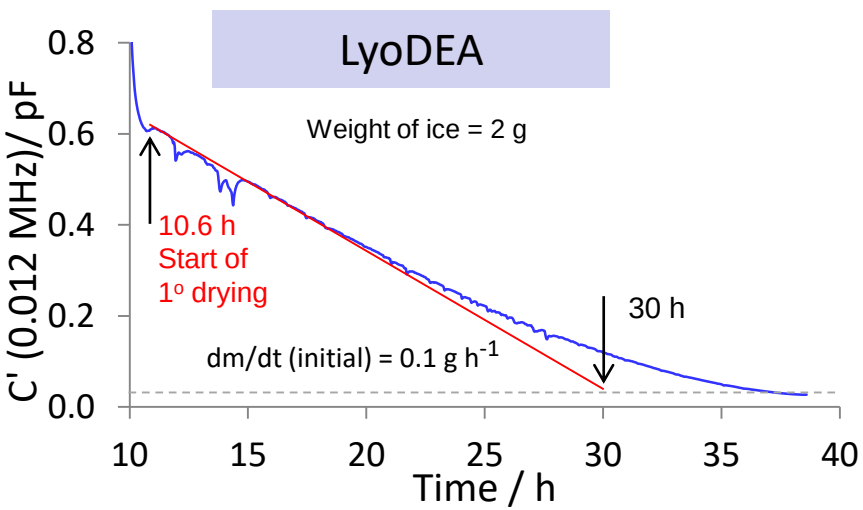


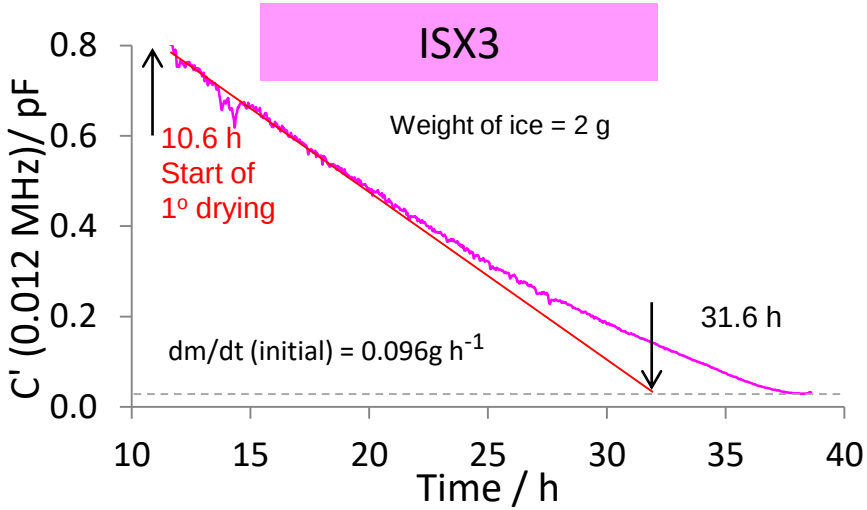


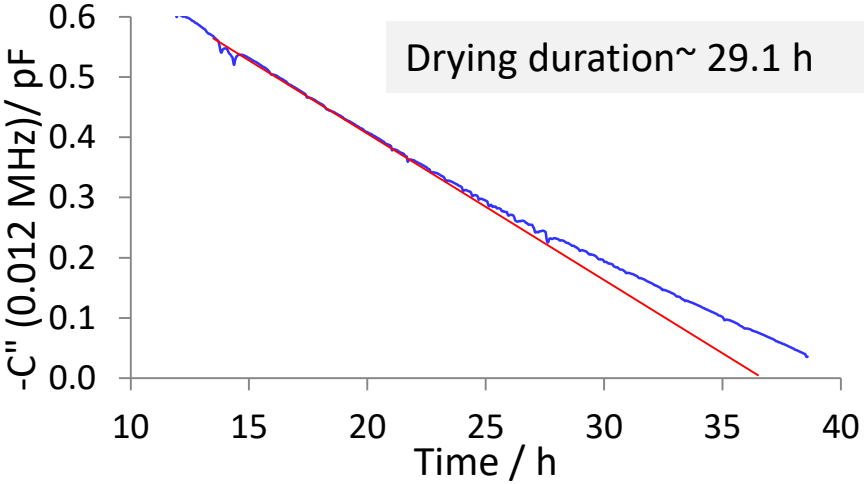


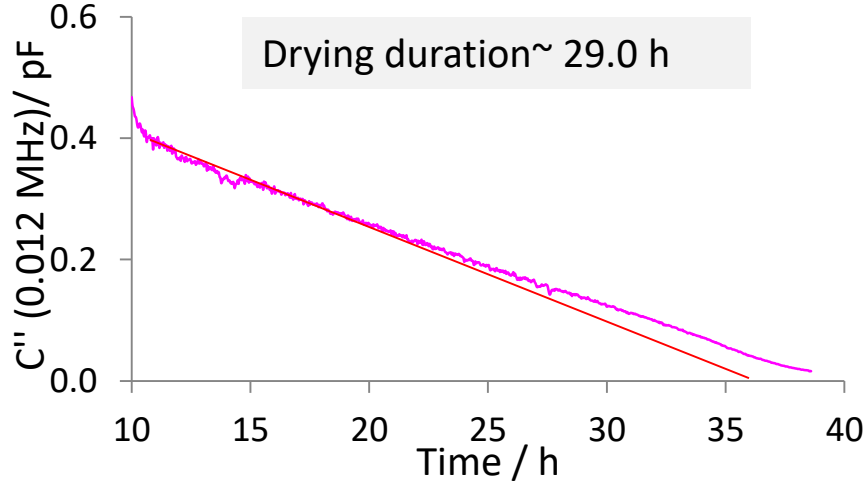


Evaluation of Drying Rate









Through-vial Impedance Spectroscopy Instruments

Instrument	Parameter
LyoDEA	
- Frequency range	10^1-10^6 Hz
- Scan interval	Every 2 min
- Point/Decade	20
ISX3	
- Frequency range	10^2-10^7 Hz
- Scan interval	Every 2 min
- Point/Decade	20

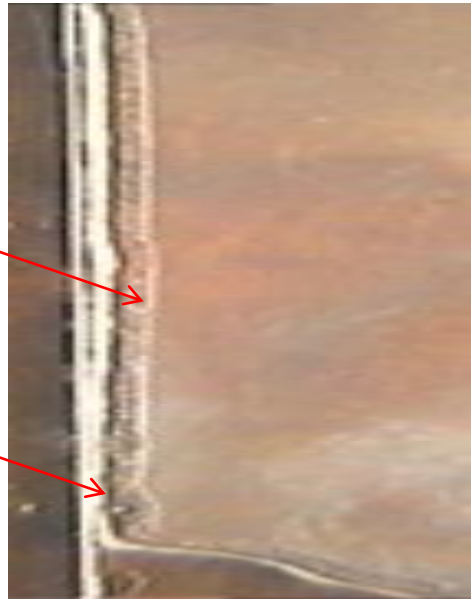
Analysis software : ZView

Product Collapse

- Conventionally measured by cryo-microscopic images

Sublimation front

Collapsed product



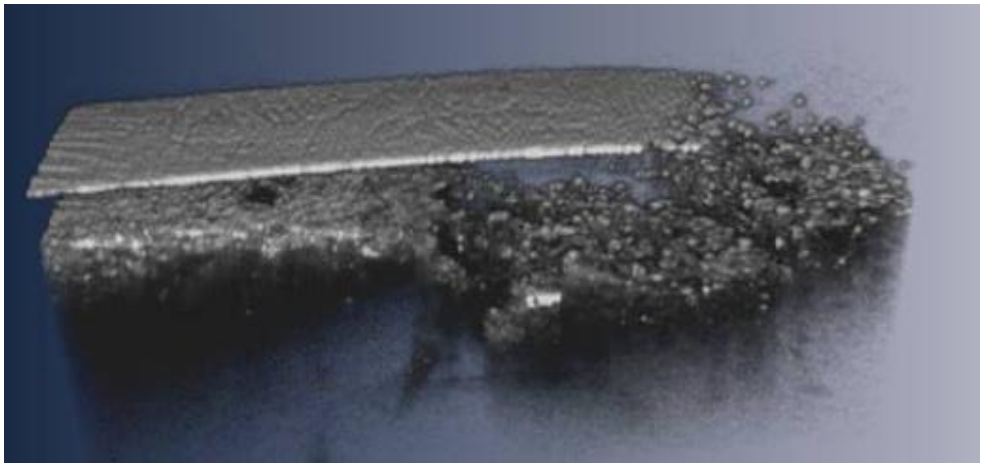
Microscopic images may not account for increase in the vapor pressure at sublimation front ,following increased resistance to vapor flow during the later stage of primary drying; potentially vulnerable to collapse

Collapse measurement within the real conditions may provide such information

Sample lactose 10% w/v

Collapse in situ imaging of collapse

- **Optical coherence tomography-based freeze-drying microscopy provides in situ assessment of the collapse temperature**
- T_C can be a few degrees different to T_C measured by conventional FDM
- Possible consequence of the differences in T_G prime that results from the freezing process impacting the amount of ice that forms.



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Phase Behaviour

Glass formation

- Linked to collapse temperature (T_c)
- T_c is ~ 1-5 ^oC above T_g for many small molecular weight compounds and excipients but almost equal to Tg for polymeric compounds

Product Collapse

- Freeze-drying above T_c results in collapse which could prolong drying
- In some circumstances collapse is considered desirable, given that it improves stability (enthalpic relaxation)?
- Is pre-collapse, e.g. T_g', detectable?

Eutectic formation

- Crystallization of excipients, e.g. mannitol
- Depends on the characteristics of the freezing process.
  - Metastable states can be generated which crystallise on storage
- Annealing used to 'guarantee' eutectic formation
- Do how to now to know if excipients have crystallised?

Critical Process Parameters

- What are the critical process parameters?
- Product temperature
  - should be as high as possible to accelerate drying
  - A limit is imposed by the collapse temperature
- Shelf Temperature (possibly but only if considered in relation to the chamber pressure)
  - Interplay between self-cooling of the product (dependent on the rate of sublimation and the thermal insulation by the glass vial)
- Chamber pressure
  - also important in order to improve heat transfer to the vial (improves efficiency)

Critical Process Parameter (CPP): A process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired quality.

Examples can include end points.

What happens on microscale-down and scale up ?

- Can scale down 'formulation' screening approaches be guaranteed to translate to the product scale (vial) and through scale-up into production
- Ice formation
  - depends on formulation variables coupled to freezing rate, container geometry and composition (surfaces acting as nucleation sites)
  - Is a stochastic process (inherently variable) and difficult to model.
  - Ice fog or pressure reduction used to regulate ice formation process.

- PAT for linking microscale phenomenon to product scale (vial)
  - NIR and Raman suitable for continuous processing (accessibility of the vial)
- PAT for quality attributes (e.g. protein stability) determination in development
- And/or process parameter determination in scale up and production (e.g. product temperature)