

SMi Group Proudly Present the 7th Annual Conference...

Lyophilisation

HOLIDAY INN KENSINGTON FORUM | LONDON, UK

3RD-4TH
JUNE
2019

Applications of Impedance Spectroscopy in the
Determination of In-Vial Phase Behaviour

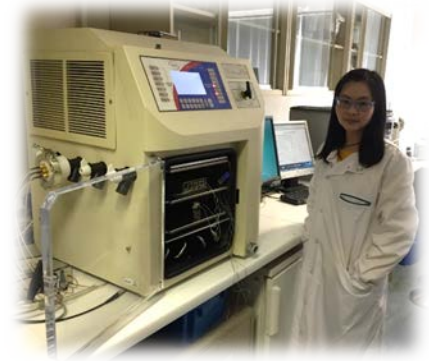
Prof. Geoff Smith & Yowwares Jeeraruangrattana

Leicester School of Pharmacy, De Montfort University,
United Kingdom

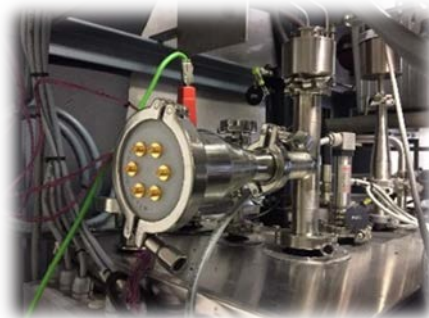
www.lyophilisation-europe.com

Outline

- Through Vial Impedance Spectroscopy
 - Description of measurement system
 - Dielectric loss mechanisms
- TVIS Applications
 - Overview
 - TVIS parameters for different facets of the freezing process (ice nucleation to solidification end point)
 - Determination of in-vial glass transitions
 - Determination of Collapse Phenomena
- Acknowledgements



Yowwares Jeeraruangrattana
GPO Thailand



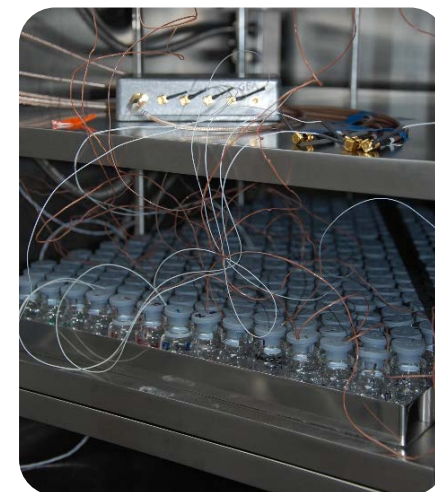
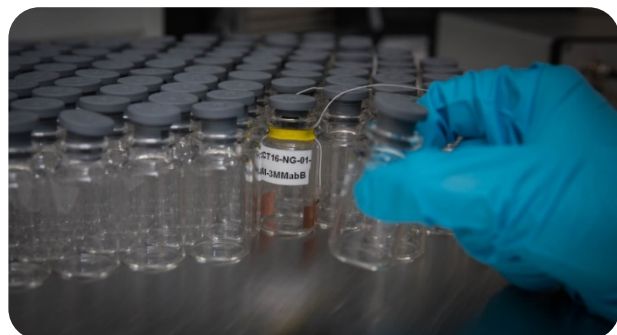
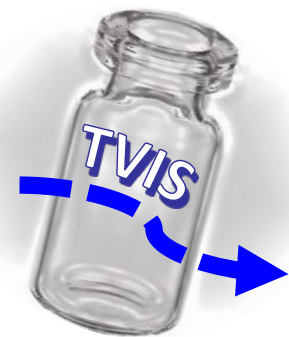
TVIS pass through on
GEA Lyophil dryer, Hurth, Cologne



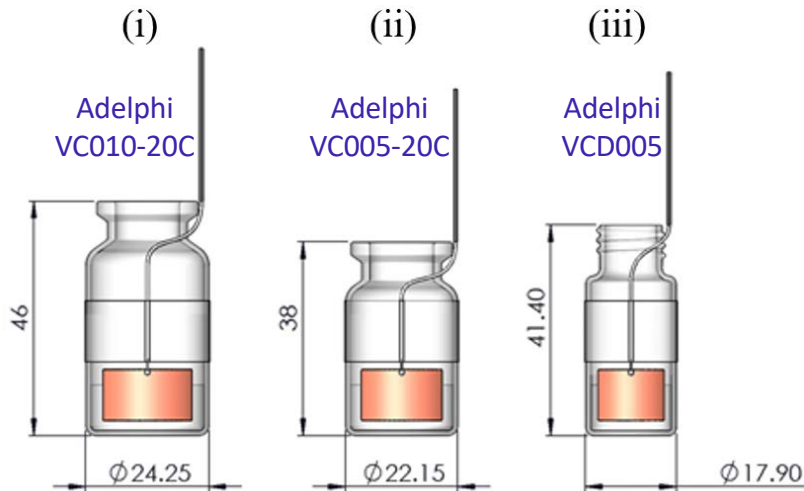
TVIS system connected on Lab Scale
Freeze Dryer LyoBeta15, NIBSC, UK

Introduction to the TVIS System

- Impedance spectroscopy characterizes the ability of materials to conduct electricity under an applied an oscillating voltage (of varying frequency)
- Impedance measurements **across a vial** rather than **within the vial**
- Hence **“Through Vial Impedance Spectroscopy”**
- Features
 - Single vial “non-product invasive”
 - Both freezing and drying characterised in a single technique
 - Non-perturbing to the packing of vials
 - Stopper mechanism unaffected



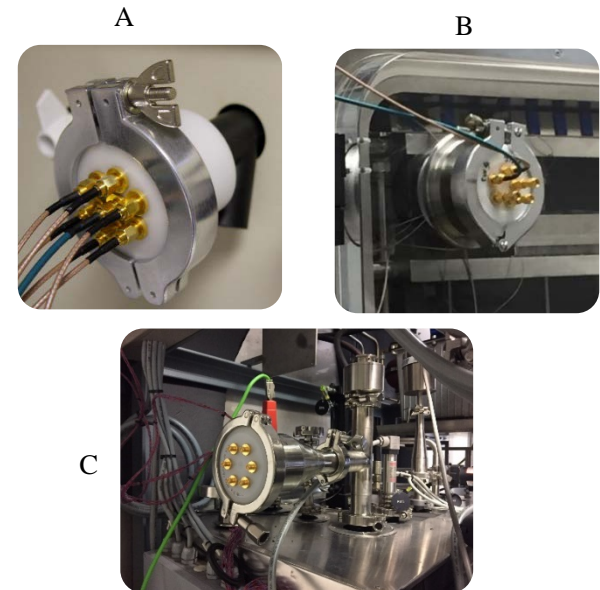
TVIS Measurement System



- The designs of various vials that have been modified with copper foil electrodes (10 mm in height and 3 mm from the base of each container)
 - i. 20 mm crimp-neck vial with 10 ml nominal capacity
 - ii. 20 mm crimp-neck vial with 5 ml nominal capacity
 - iii. screw-neck vial with 5 ml nominal capacity

- The different styles of a bespoke pass-through for TVIS systems

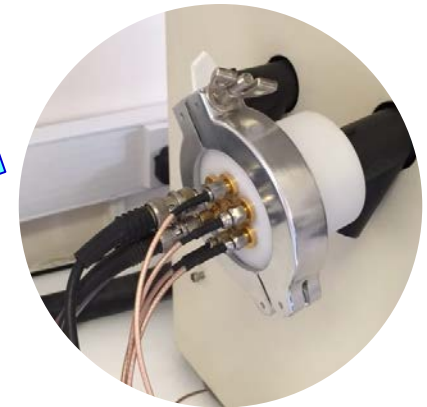
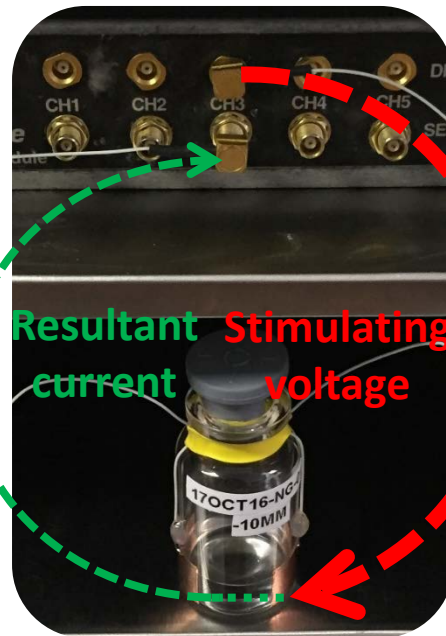
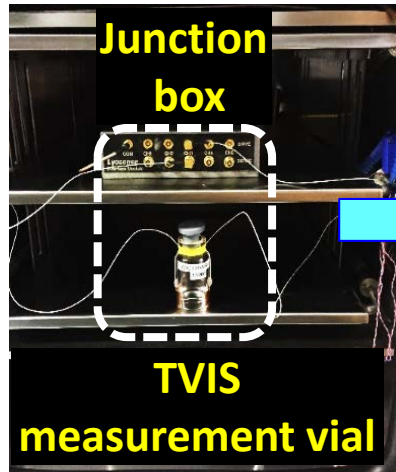
- A. Connected via the manifold hose on the outside of the dryer
- B. Connected via the port on top left side of the door on the dryer
- C. Connected to a port on the top of the drying chamber



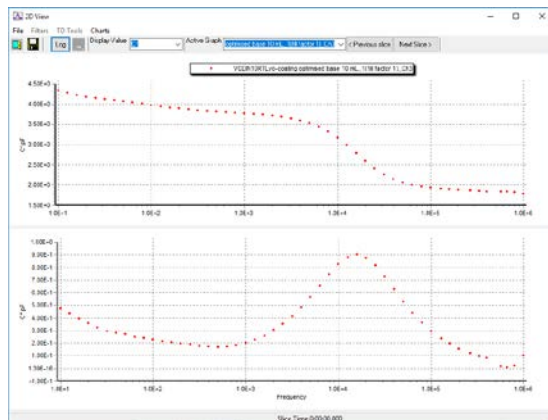
Through Vial Impedance Spectroscopy (TVIS)

Description of Measurement System

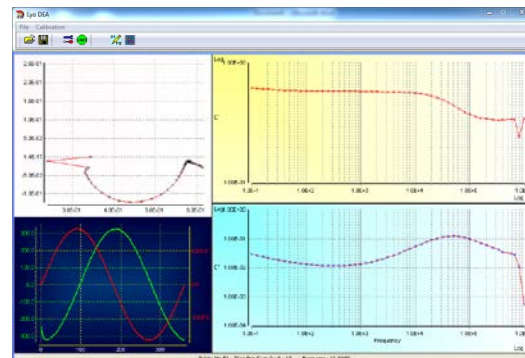
Freeze drying chamber



LyoView™ analysis software



LyoDEA™ measurement software



**TVIS system
(I to V convertor)**



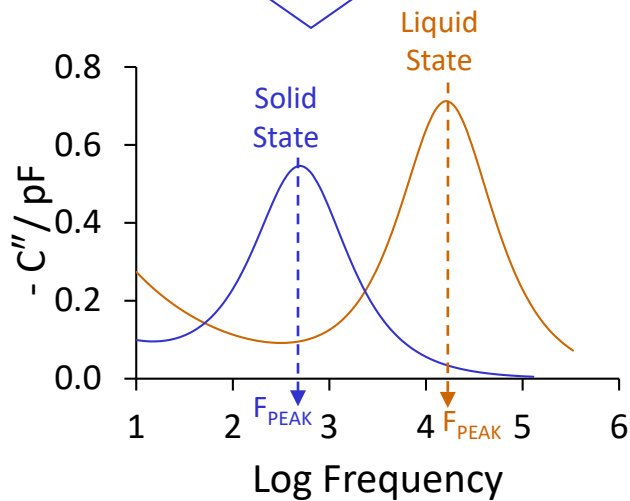
TVIS Applications

Overview

Through Vial Impedance Spectroscopy (TVIS)



Monitoring **Phase Behaviour**
(ice nucleation temperature
and solidification end points
by using F_{PEAK})

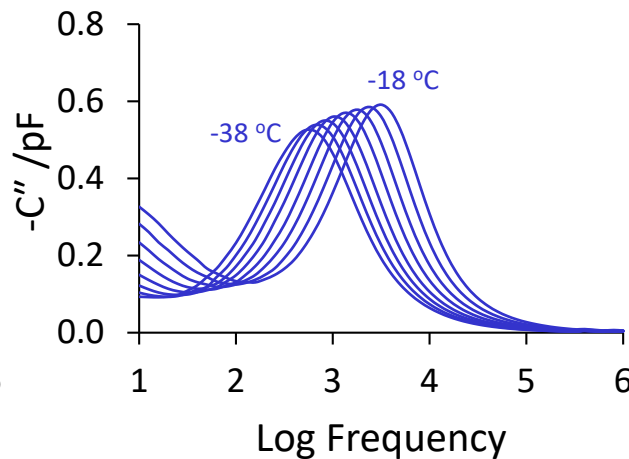
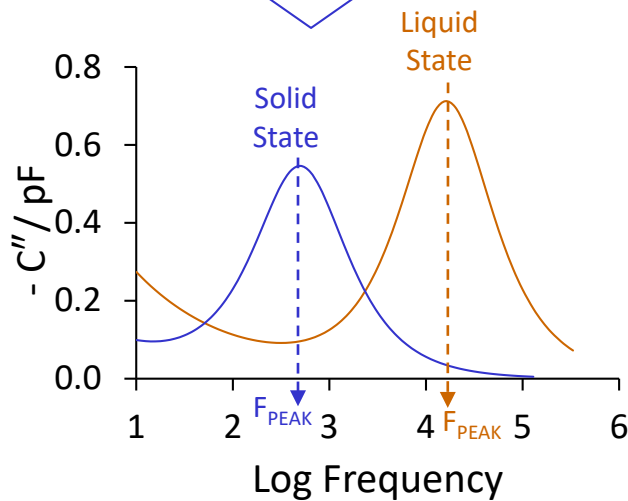


Through Vial Impedance Spectroscopy (TVIS)



Monitoring **Phase Behaviour**
(ice nucleation temperature
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F_{PEAK} temperature calibration
for **predicting temperature**
of the product in primary drying



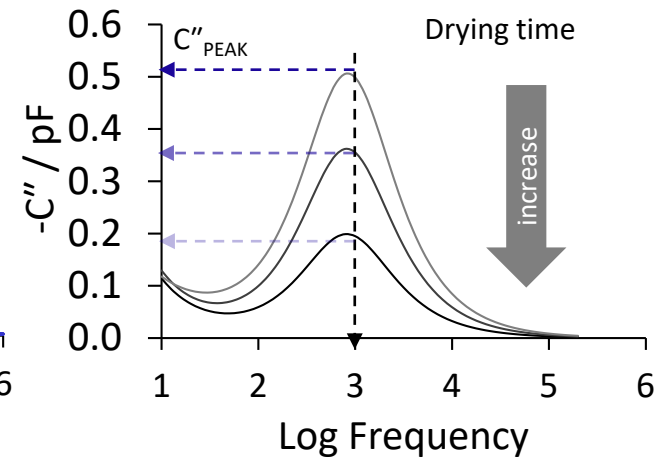
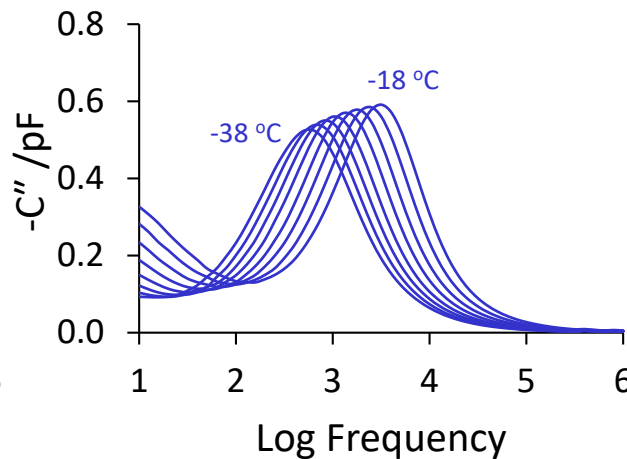
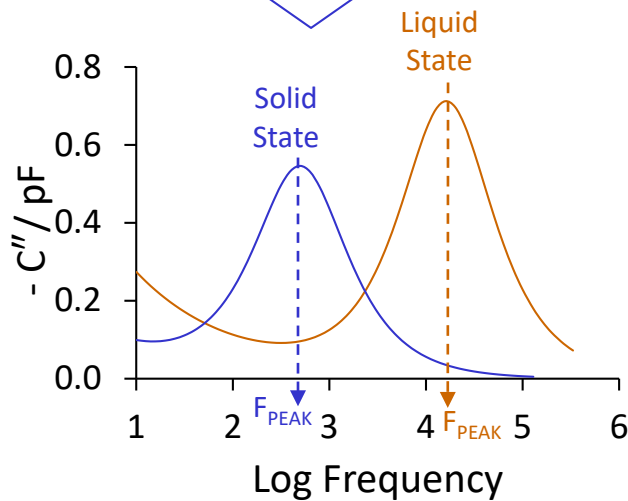
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Monitoring **Phase Behaviour**
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Surrogate **drying rate**
(from $\frac{dC''_{PEAK}}{dt}$)



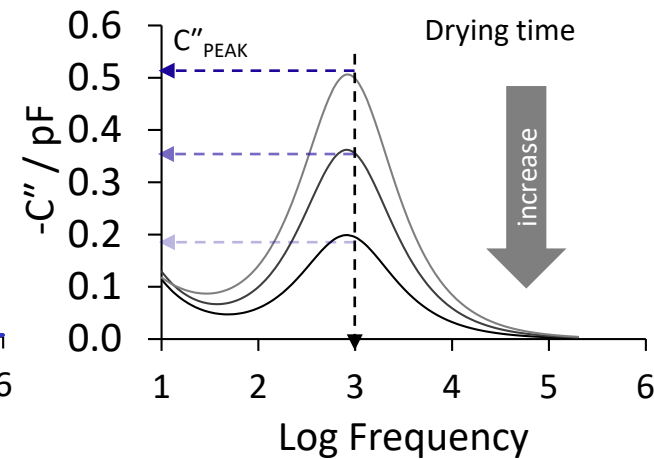
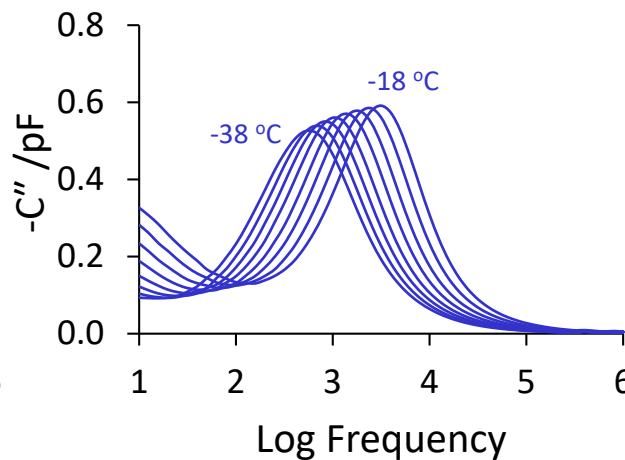
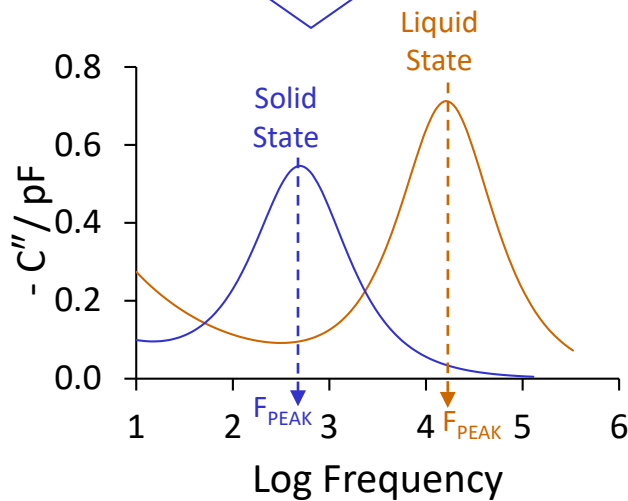
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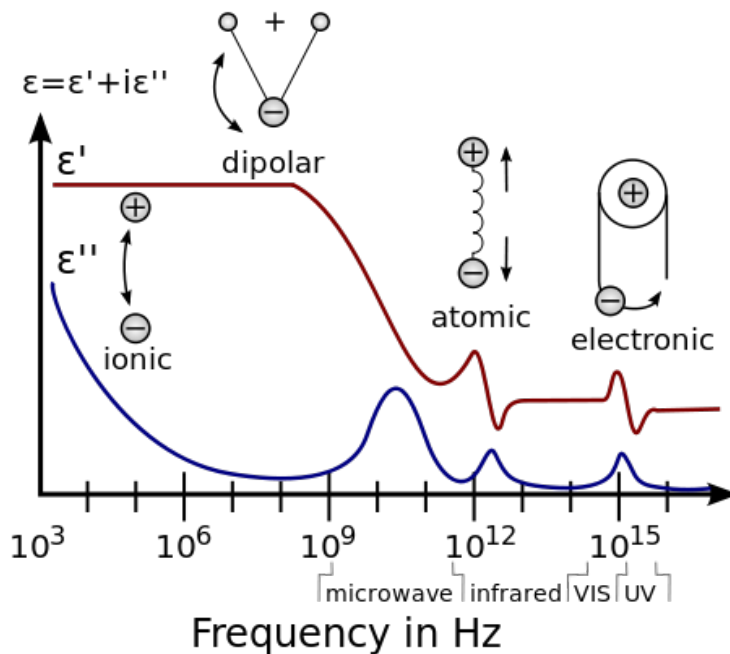
C' (~ 100 kHz) is highly sensitive to low ice volumes; therefore it could be used for determination **end point** of primary drying

Through Vial Impedance Spectroscopy (TVIS)

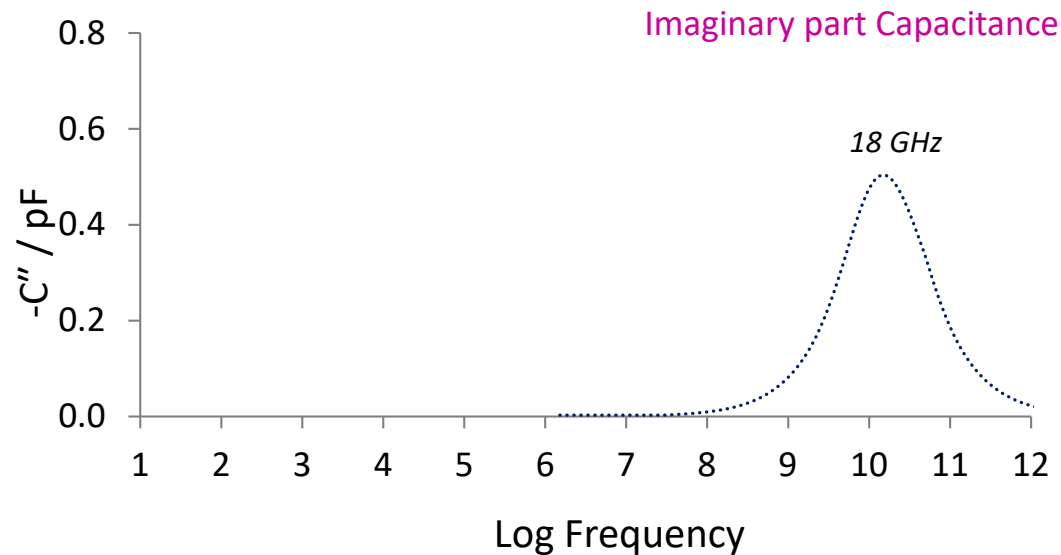
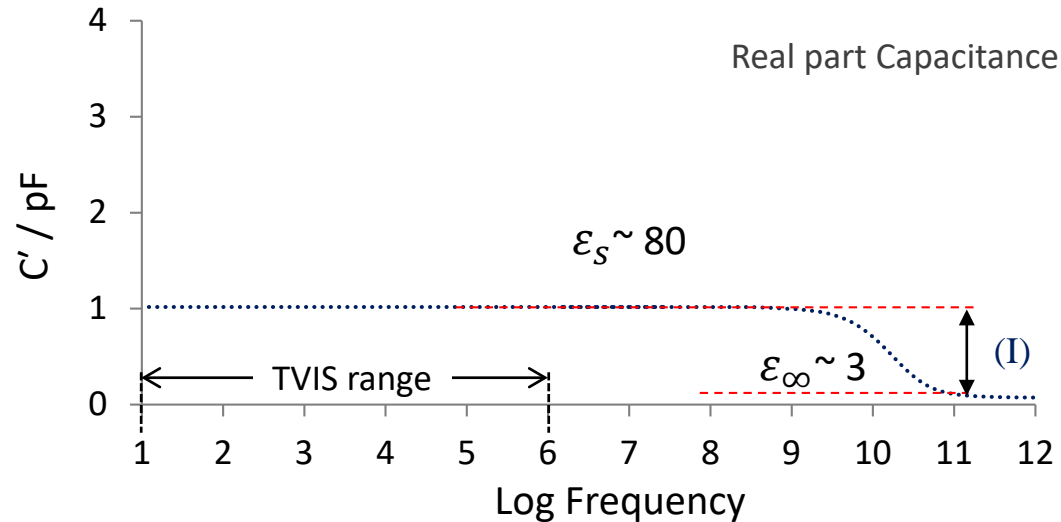
Dielectric Loss Mechanisms

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



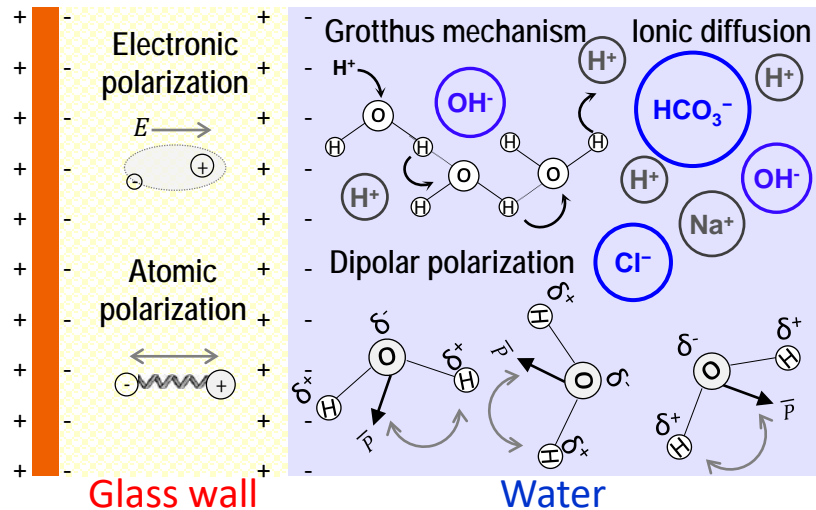
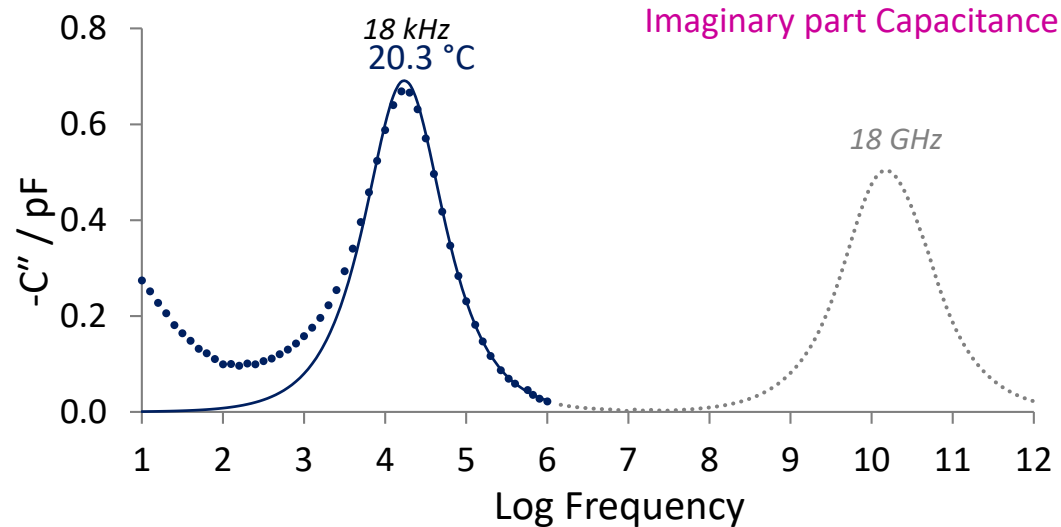
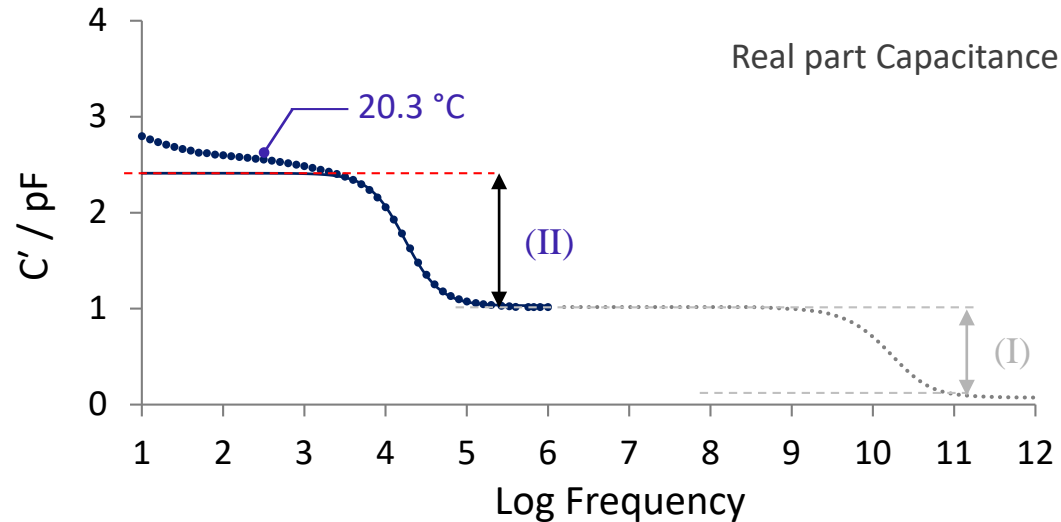
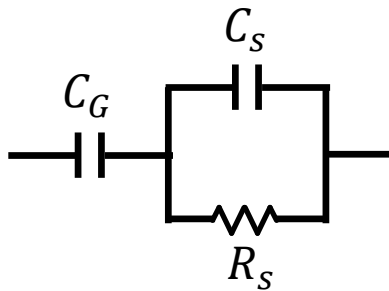
<https://en.wikipedia.org/wiki/Permittivity>



Dielectric Loss Mechanisms

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

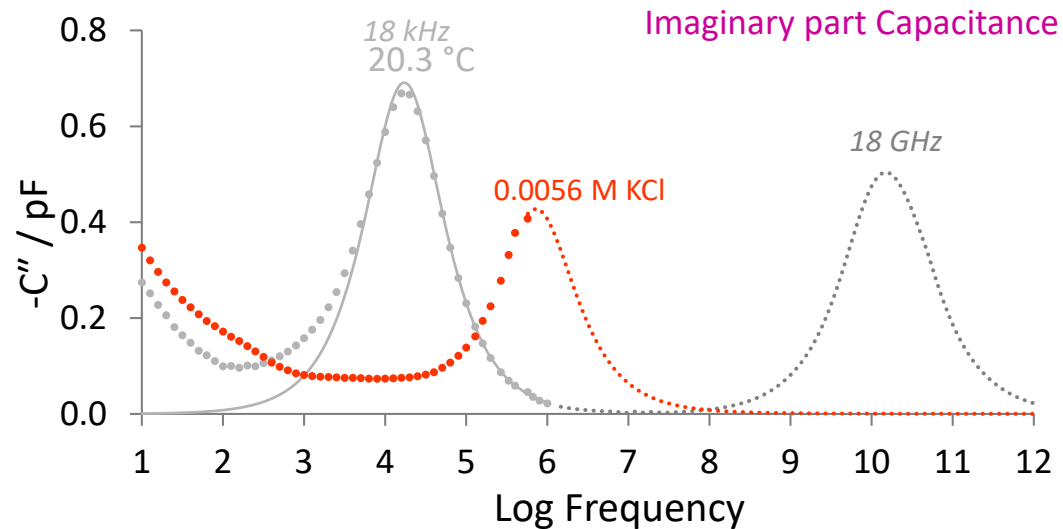
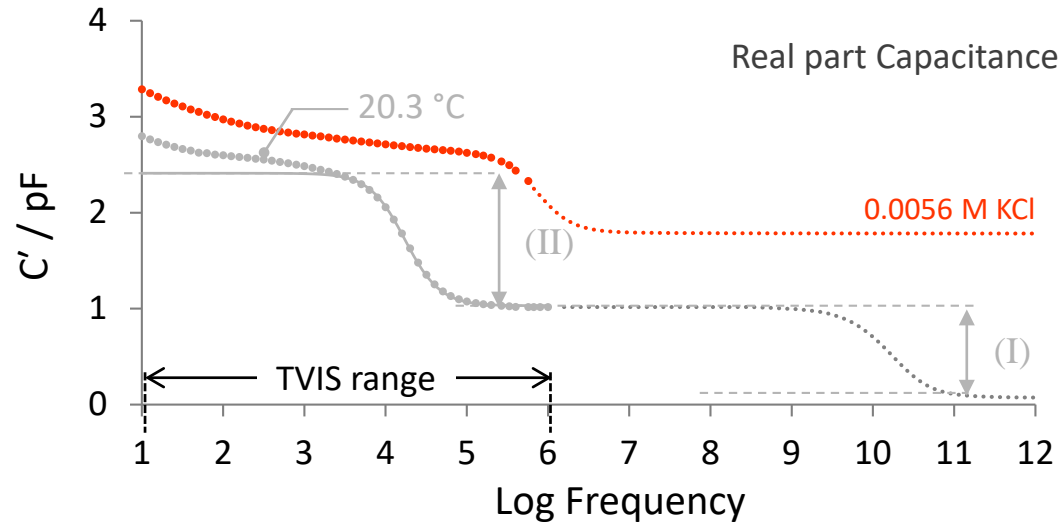
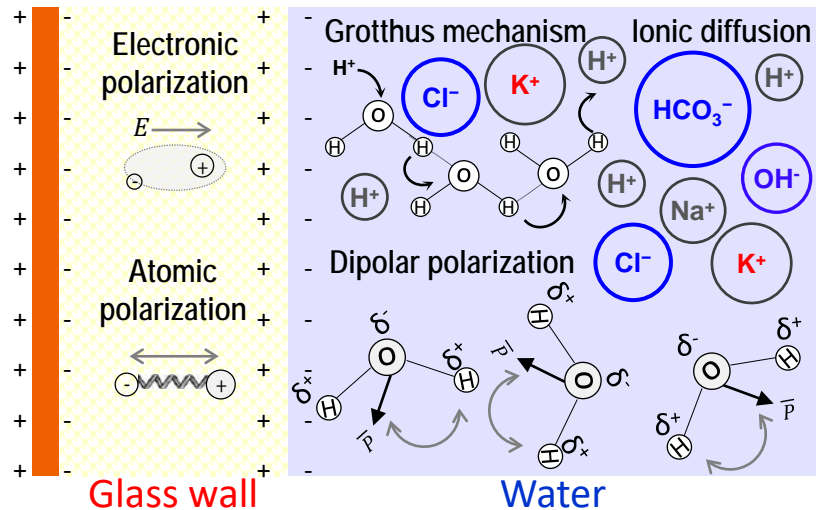
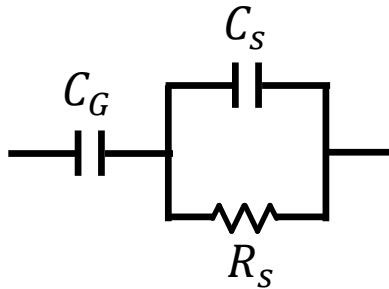
Measurement vial



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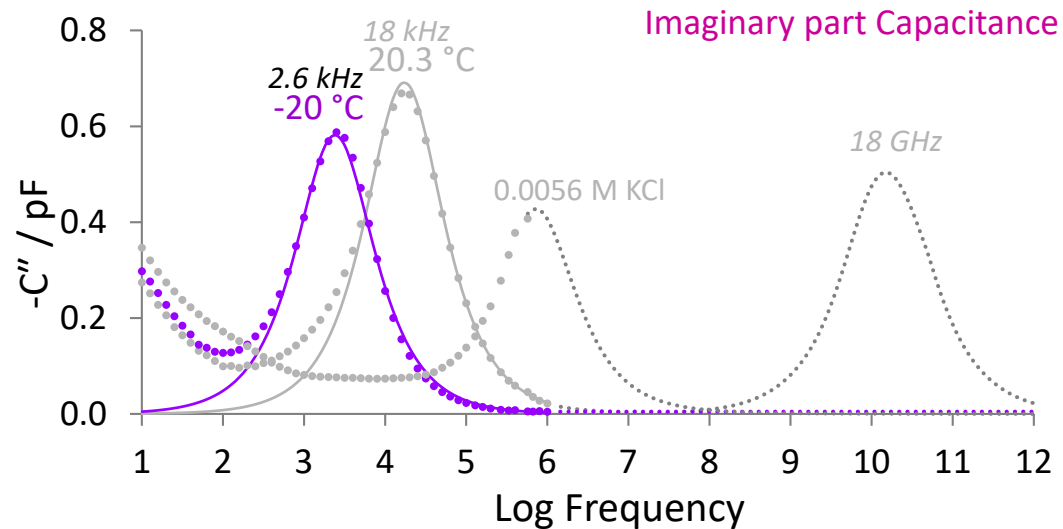
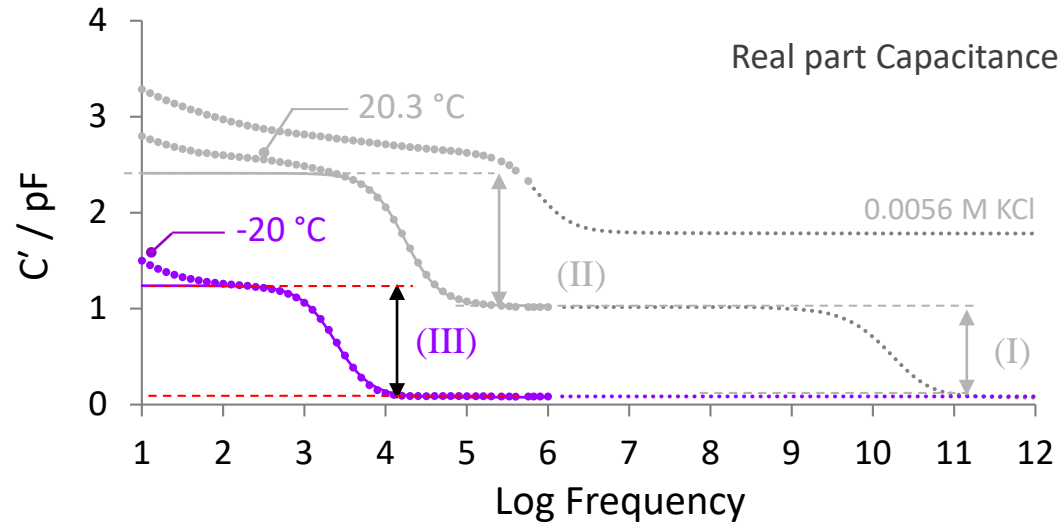
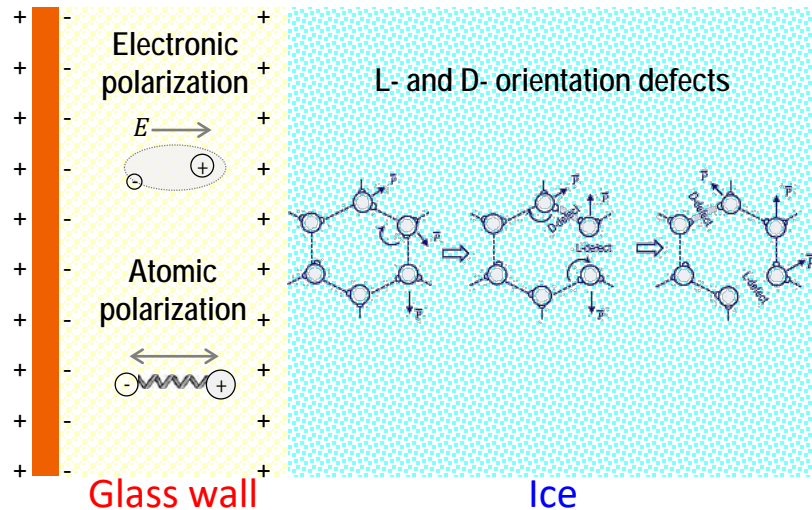
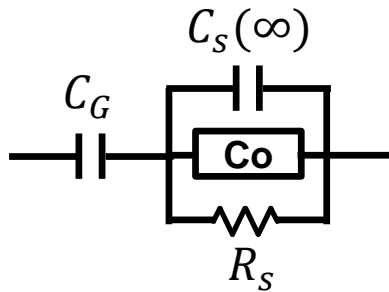
Measurement vial



Dielectric Loss Mechanisms

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

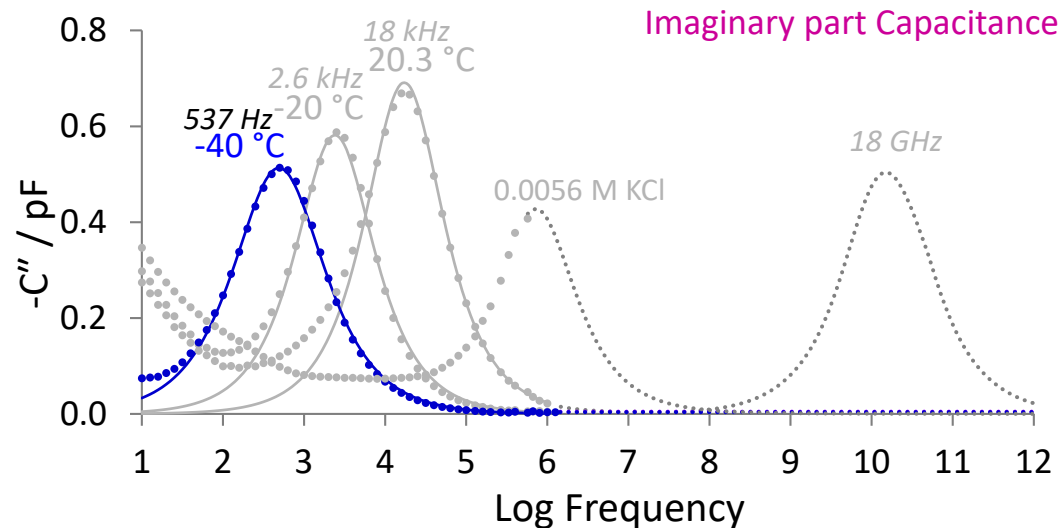
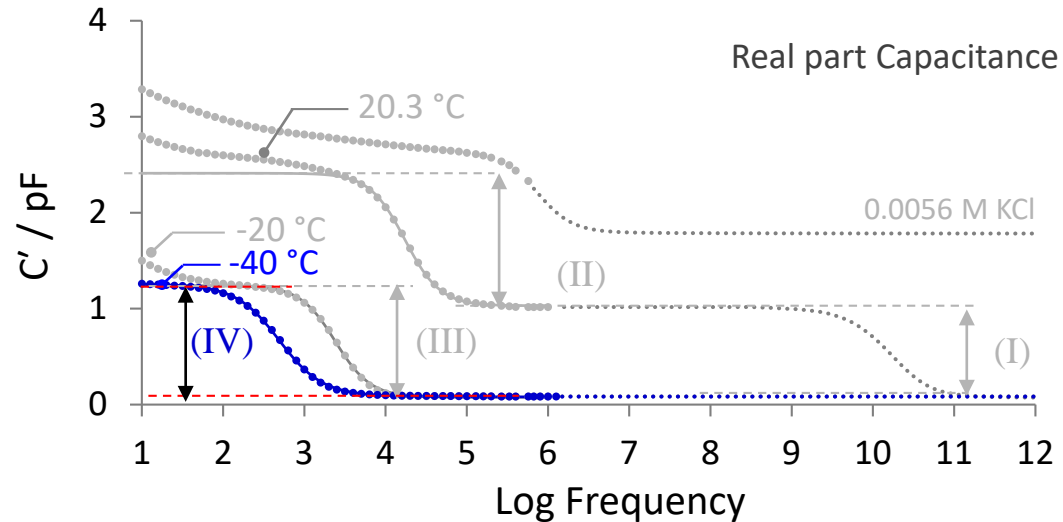
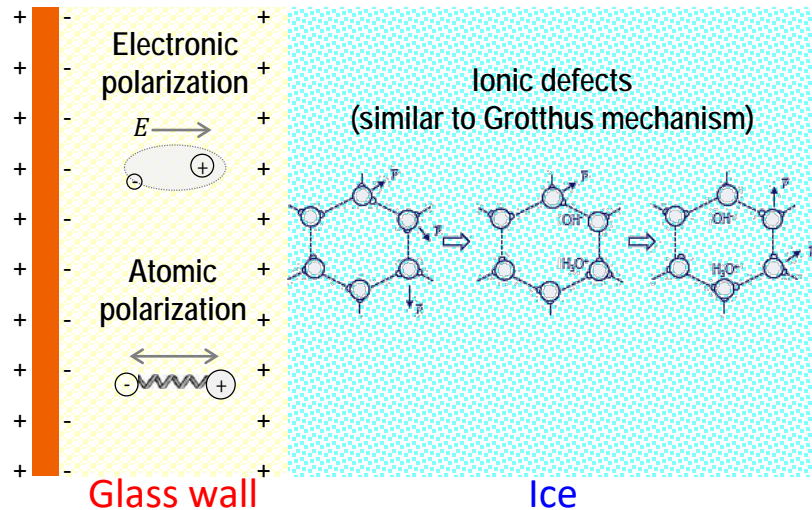
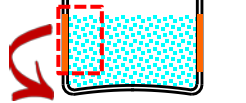
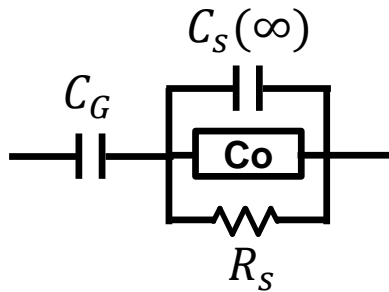
Measurement vial



Dielectric Loss Mechanisms

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

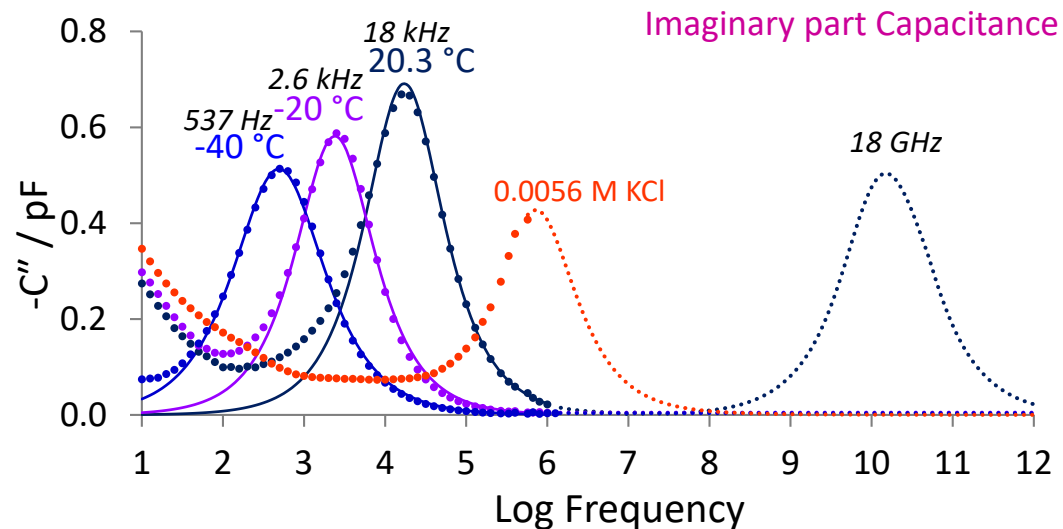
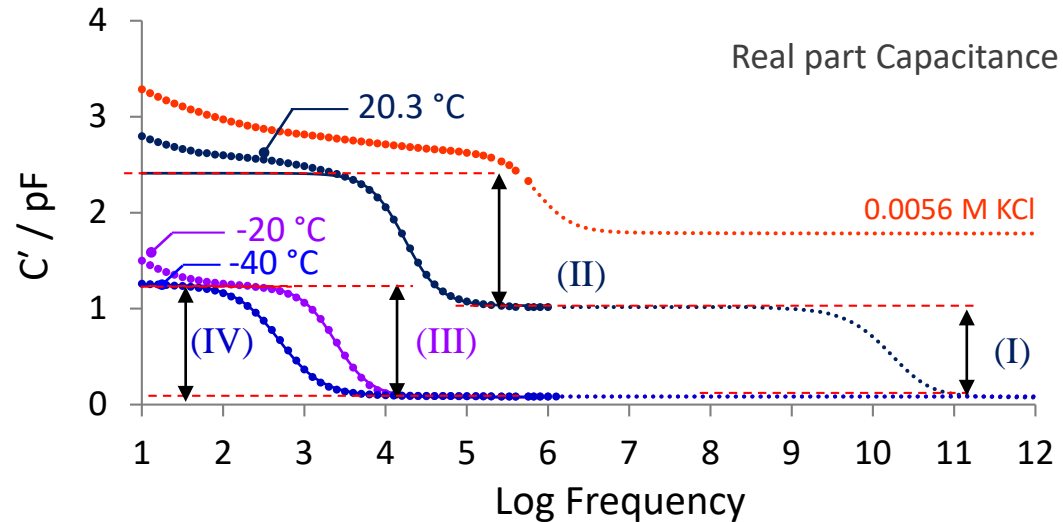
Measurement vial



Dielectric Loss Mechanisms



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- 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.

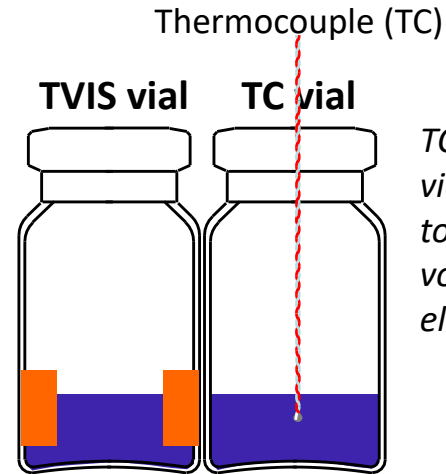
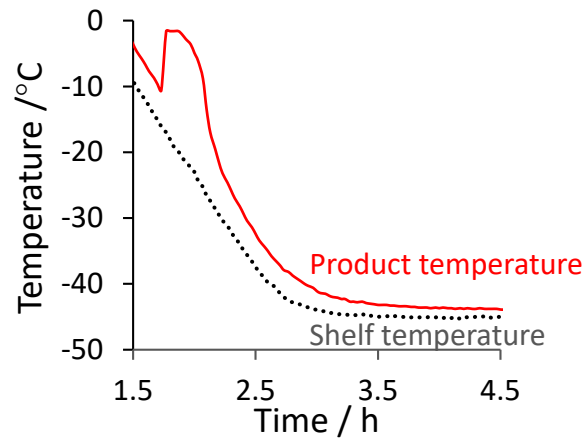


TVIS Applications

TVIS parameters for different facets of the freezing process

Methodology

Freezing step of freeze
drying process

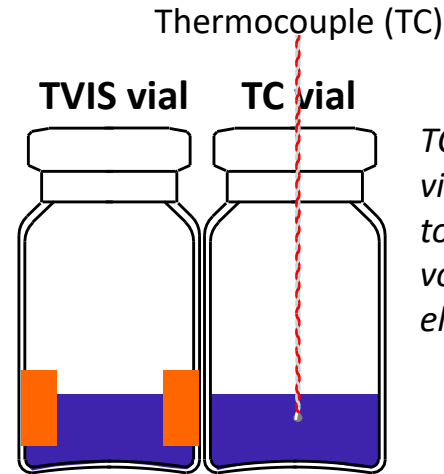
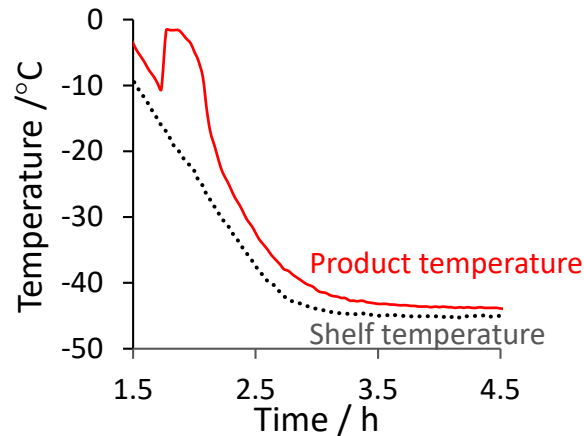


*TC in the nearest neighbour
vial at a height equivalent
to the mid point of the
volume bounded by the
electrodes*

Methodology

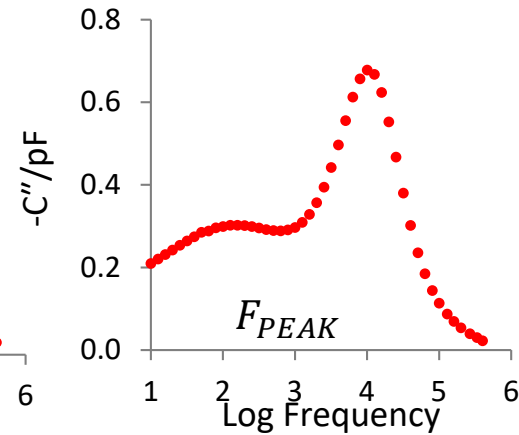
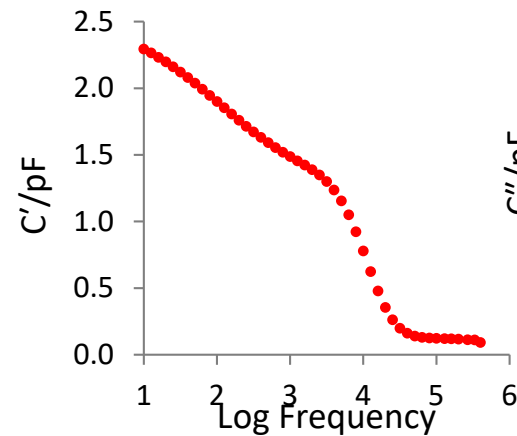


Freezing step of freeze drying process



TC in the nearest neighbour vial at a height equivalent to the mid point of the volume bounded by the electrodes

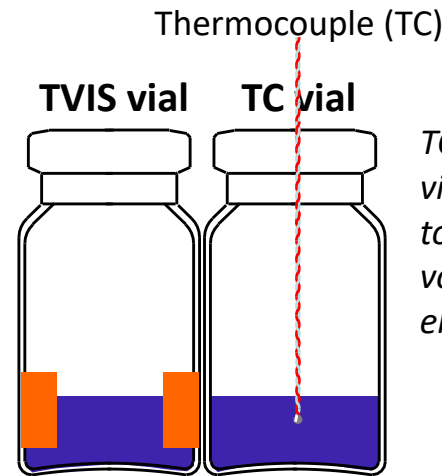
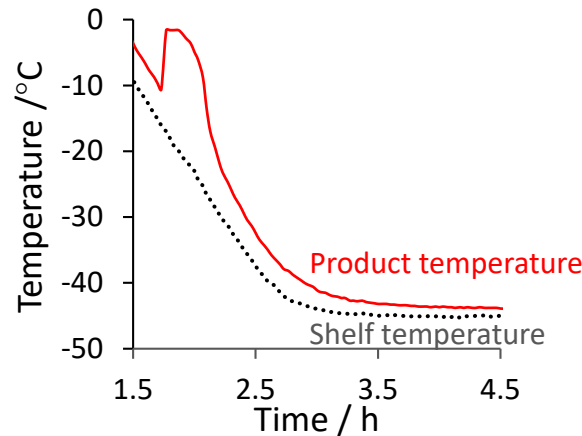
○ In-line TVIS measurement



Methodology



Freezing step of freeze drying process

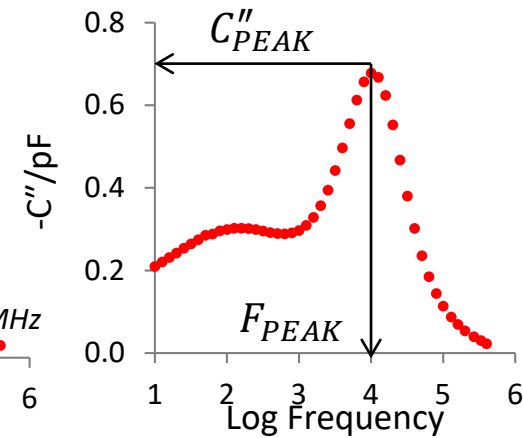
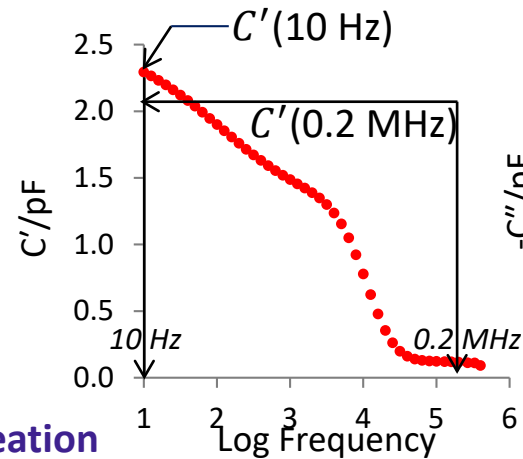


TC in the nearest neighbour vial at a height equivalent to the mid point of the volume bounded by the electrodes

Identifying TVIS parameters using LyoView software

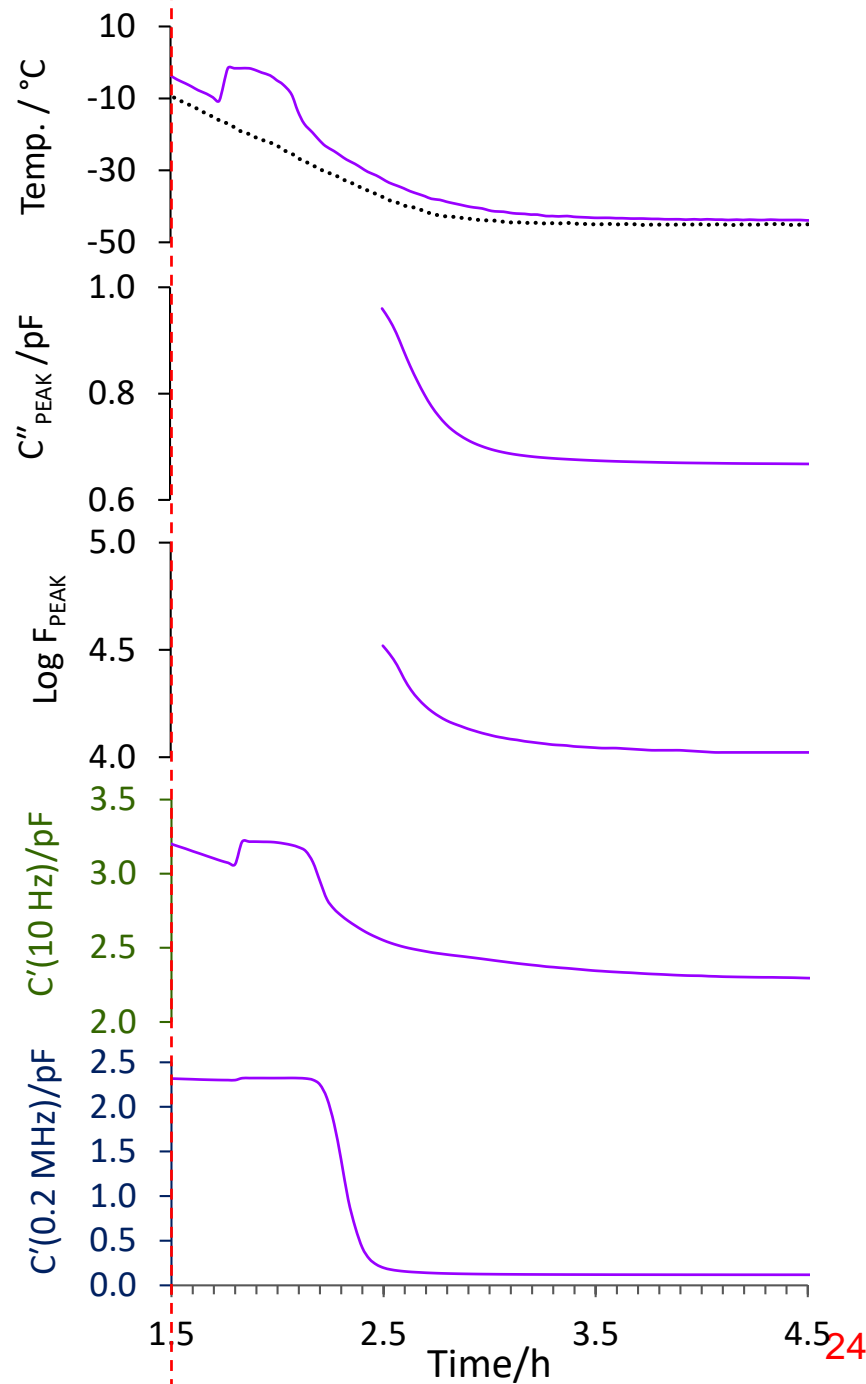
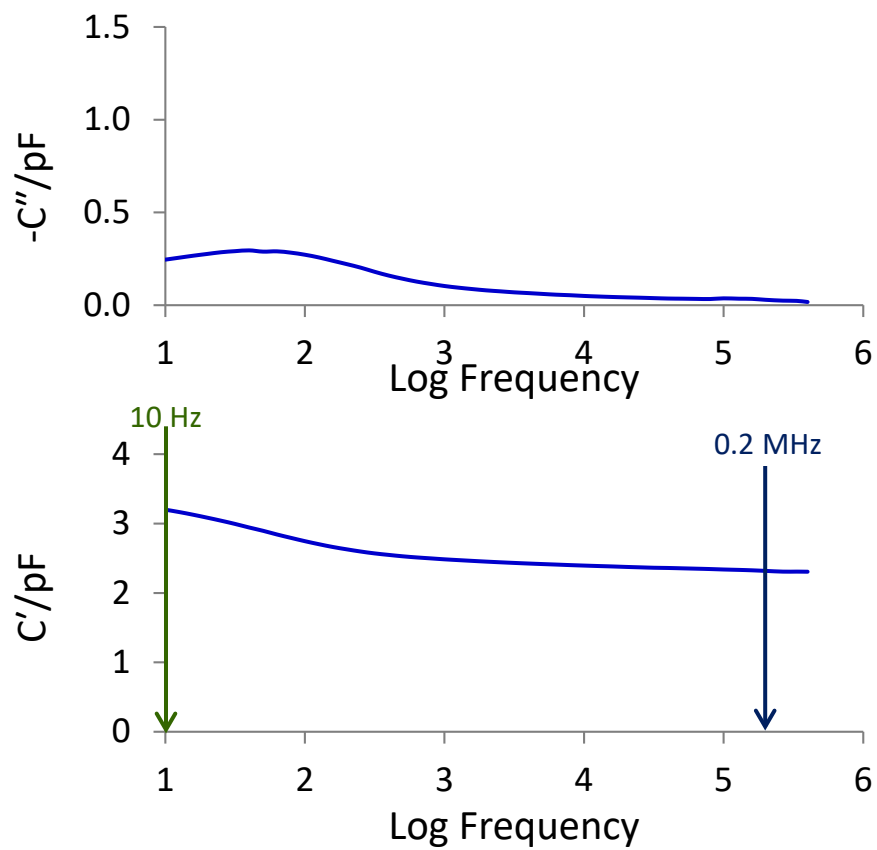
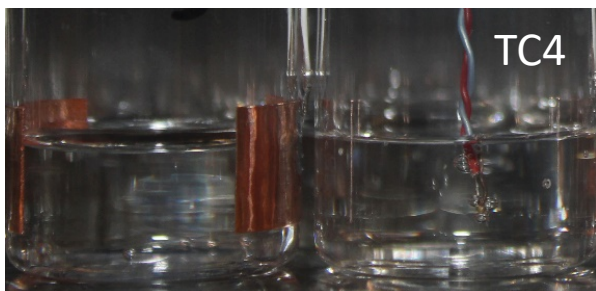
C''_{PEAK}
 F_{PEAK}
 $C'(10\text{ Hz})$
 $C'(0.2\text{ MHz})$

Determination of Ice nucleation event

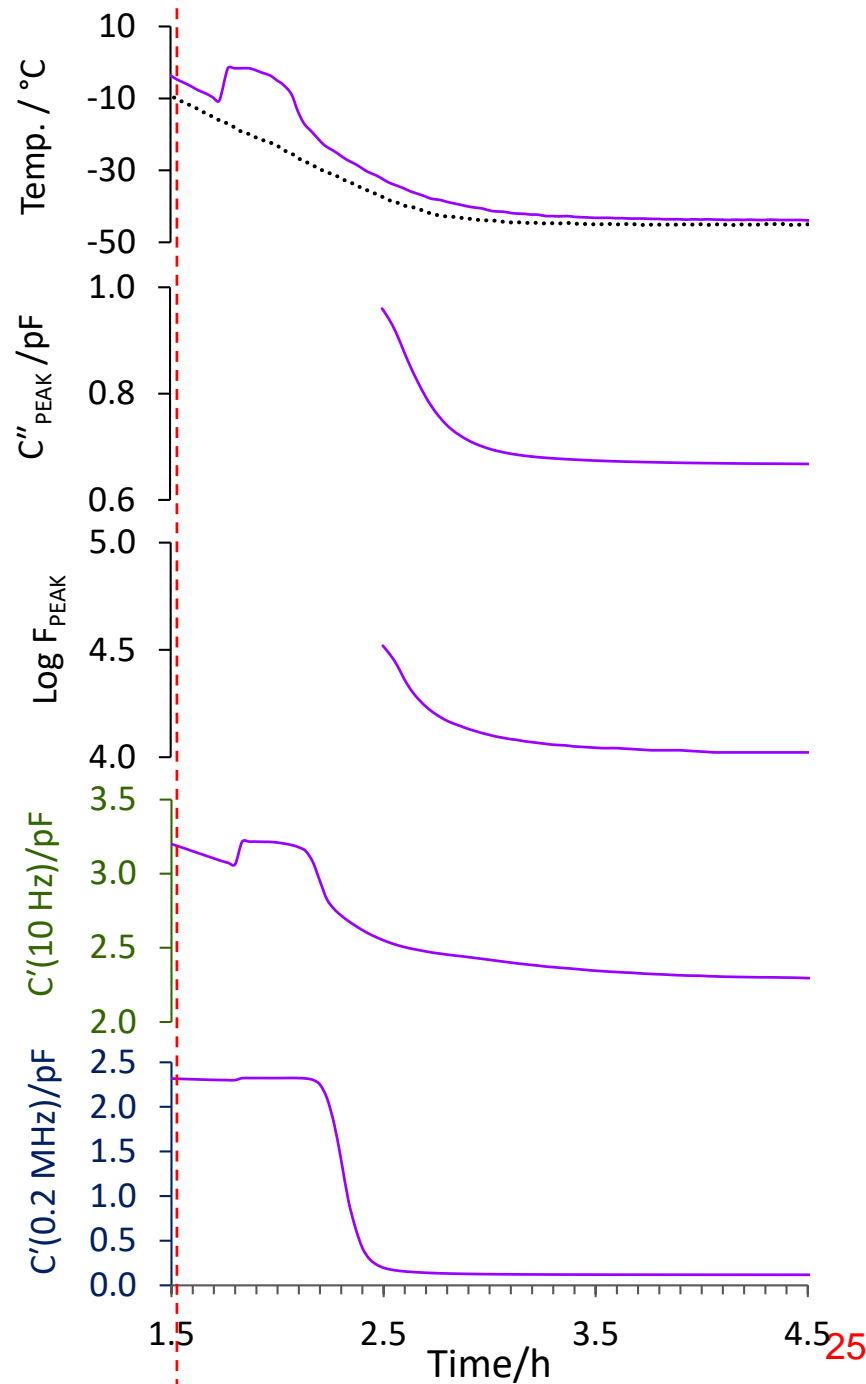
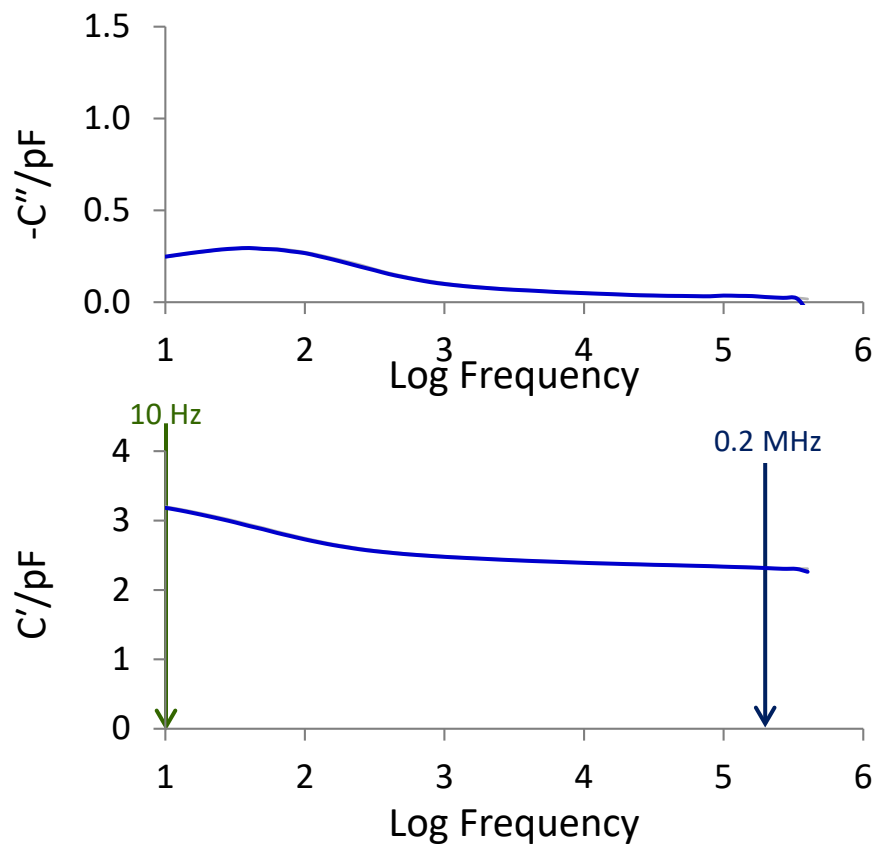
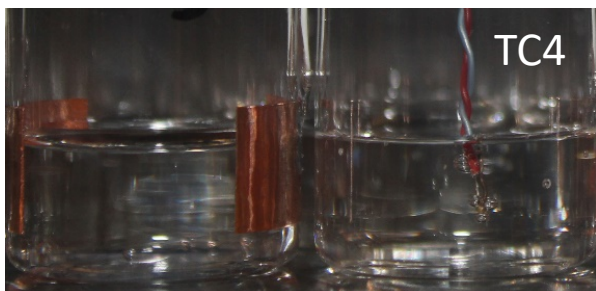


An example : 5 %w/v sucrose in 0.55 %w/v NaCl

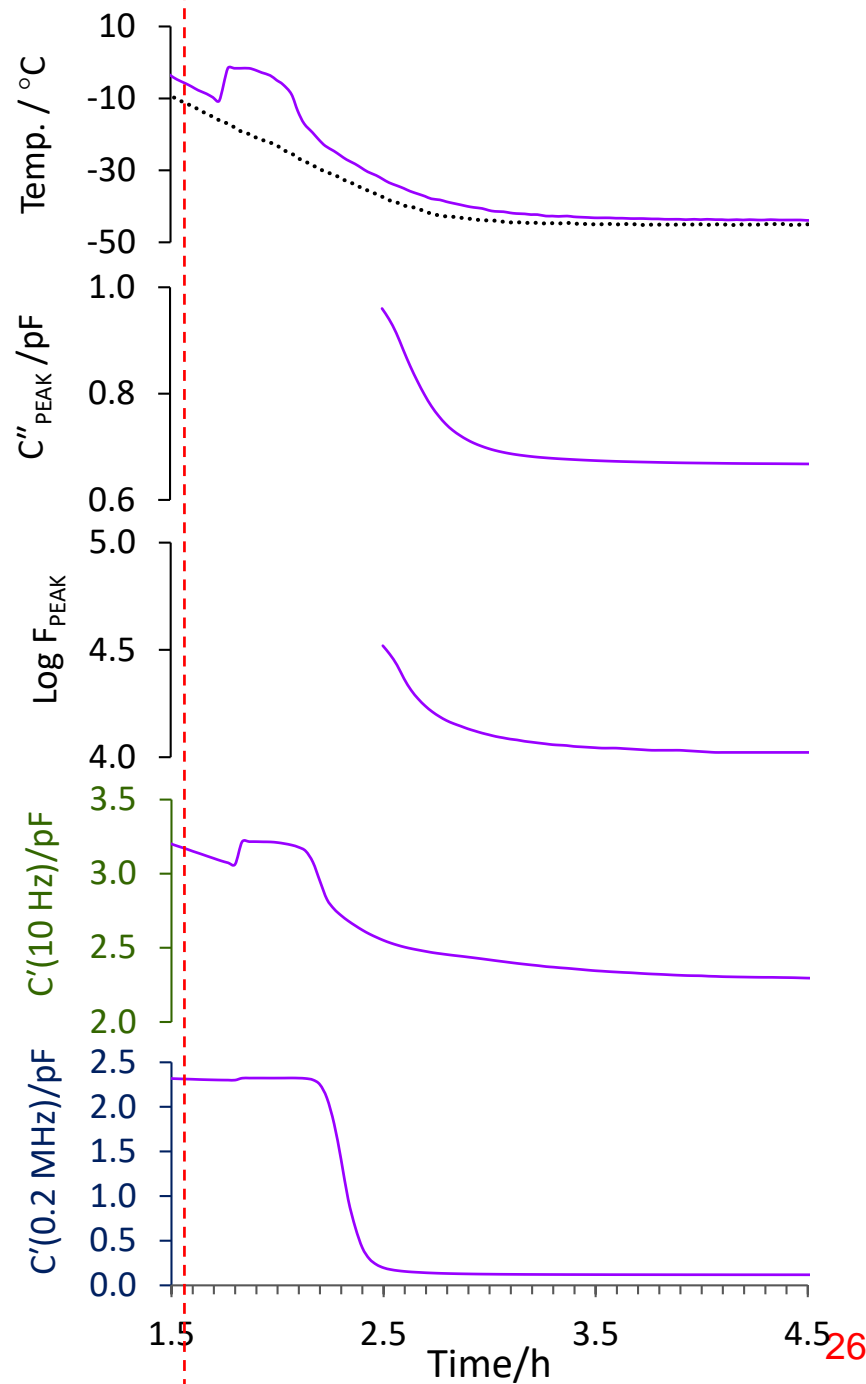
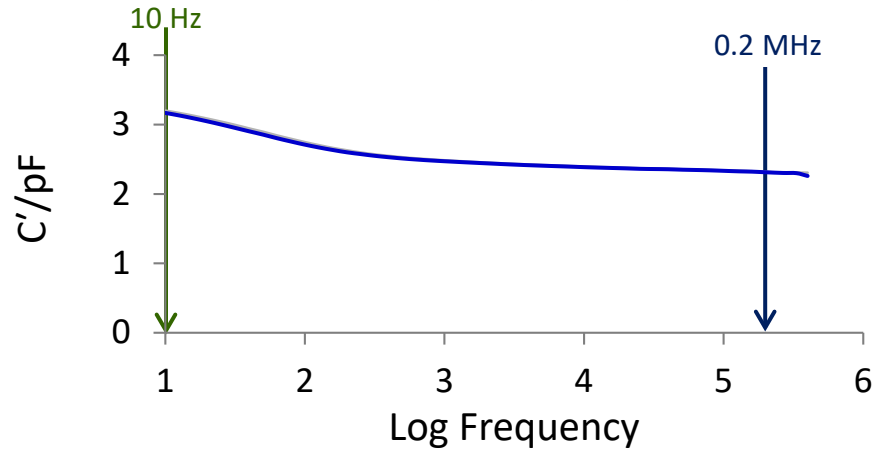
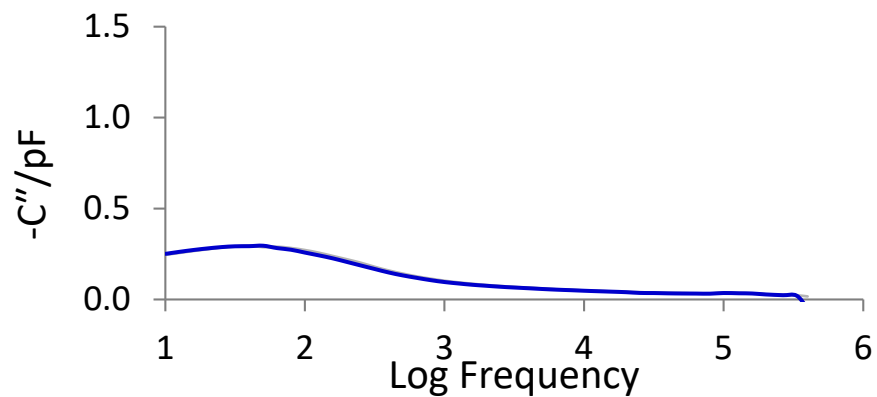
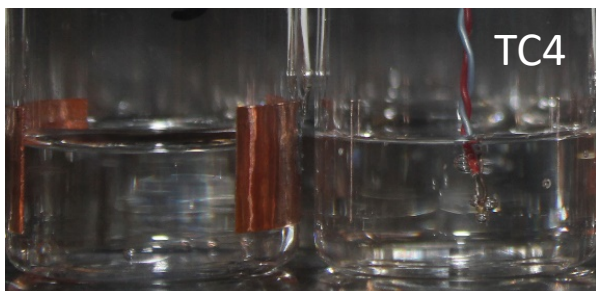
Freezing Step - 5%Sucrose+0.55%NaCl



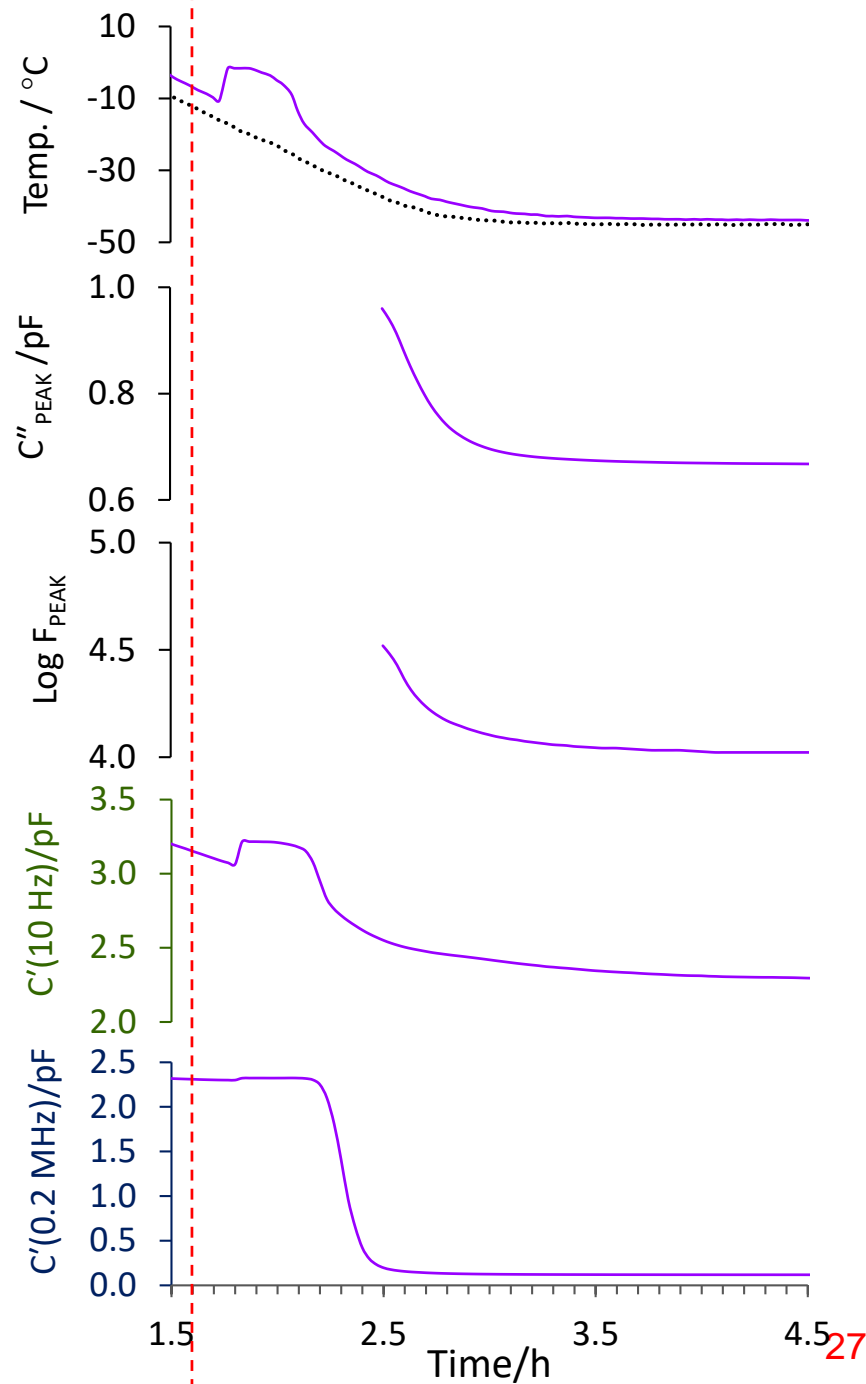
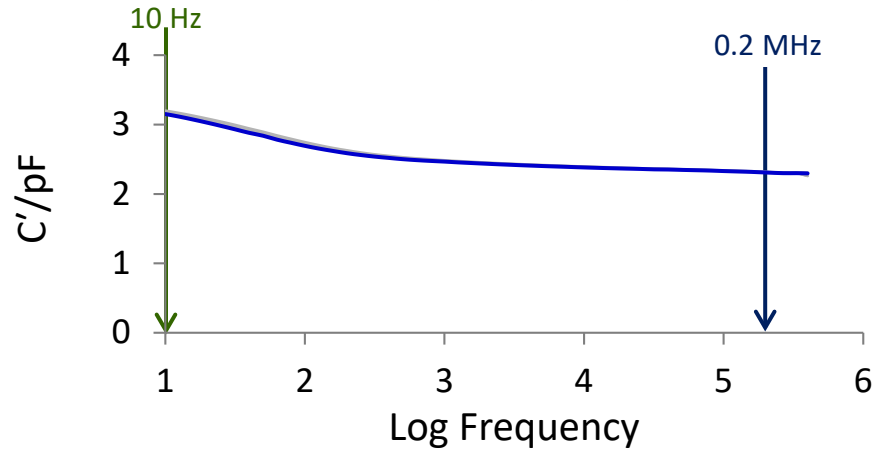
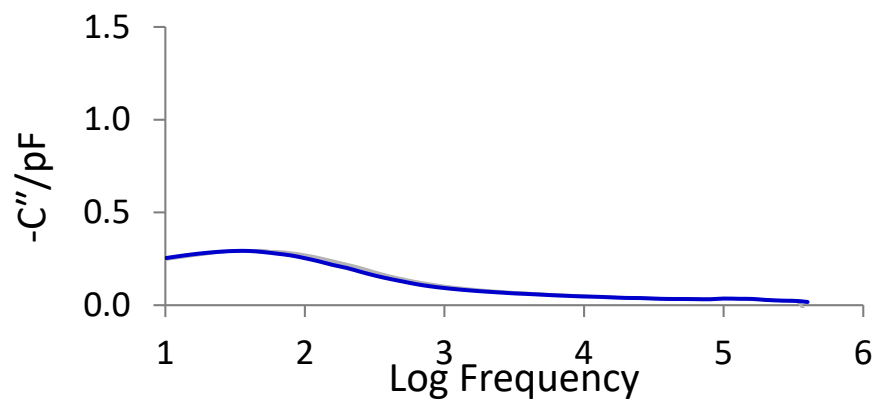
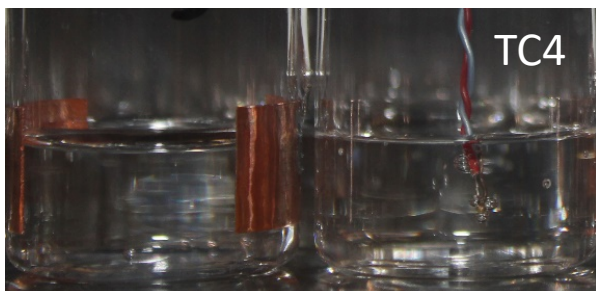
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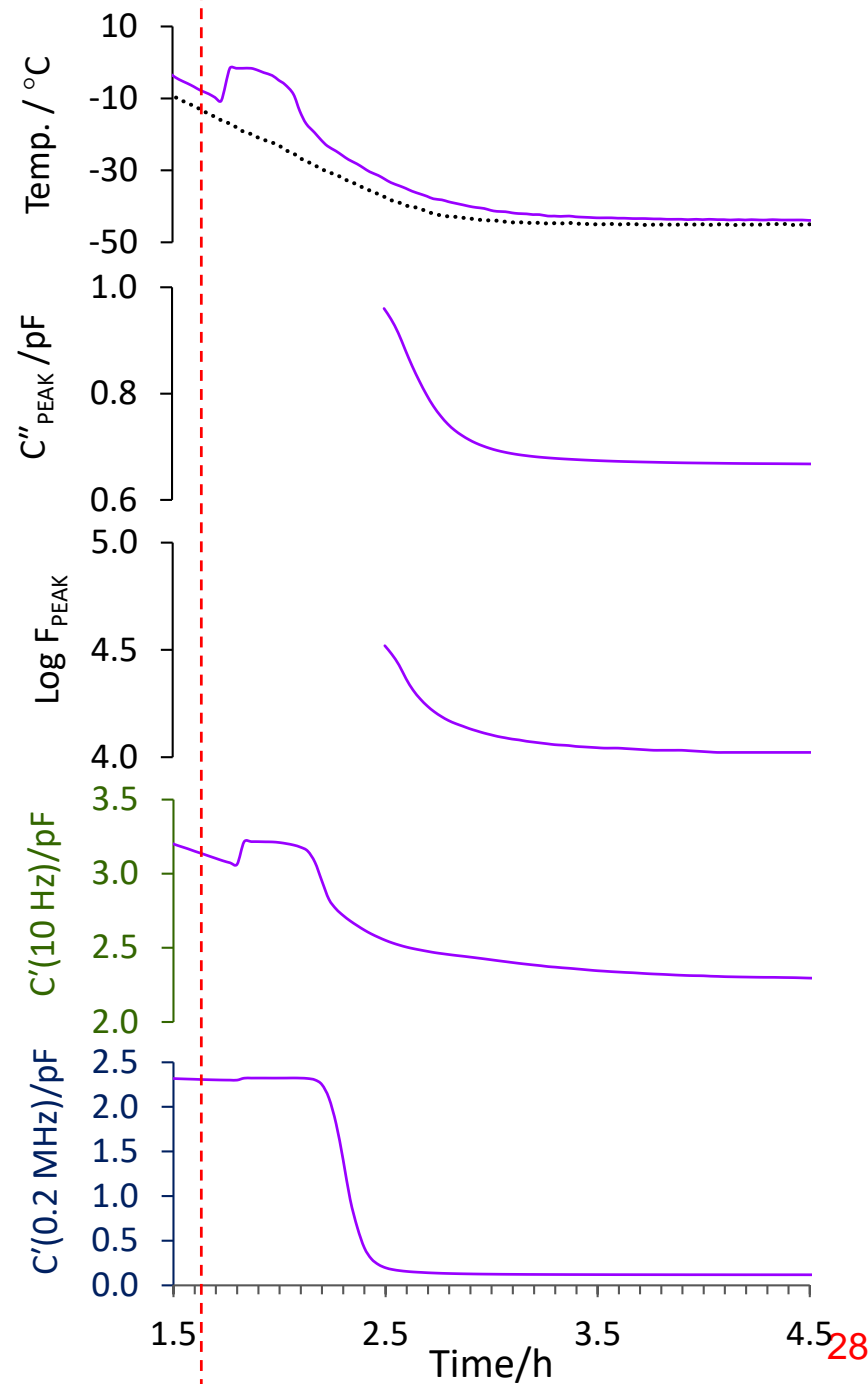
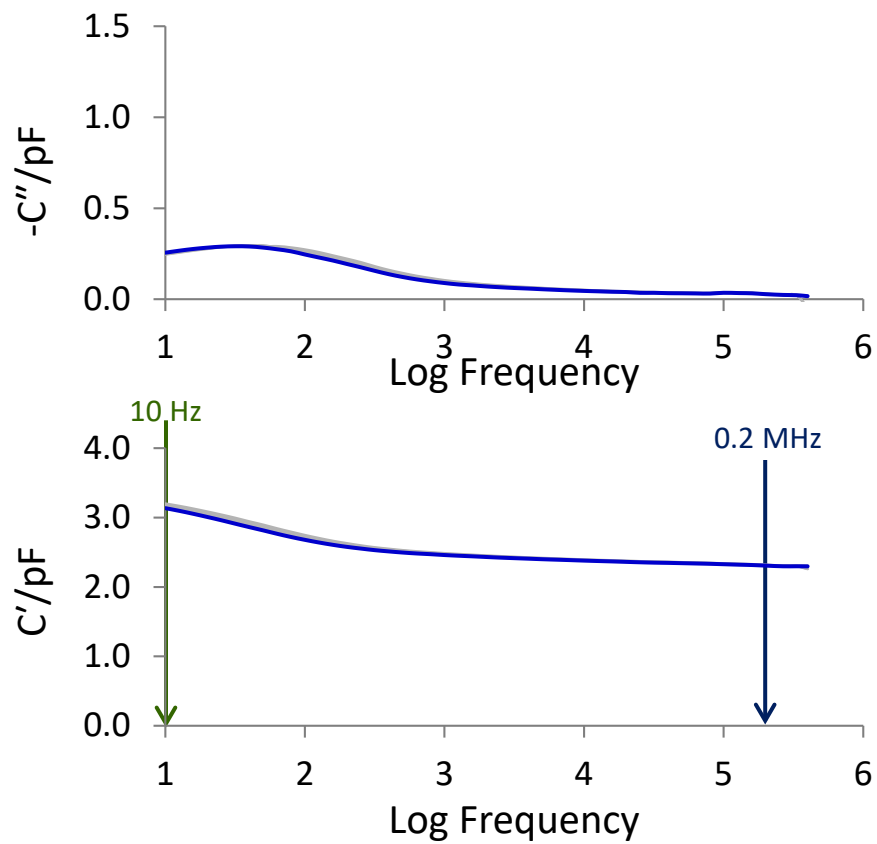
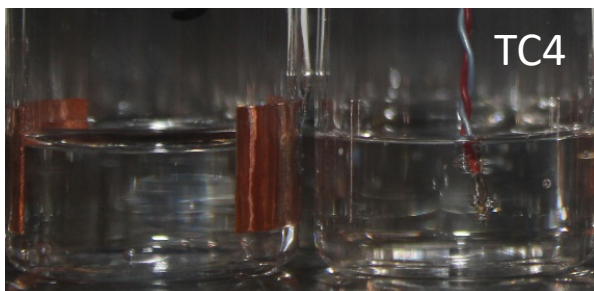
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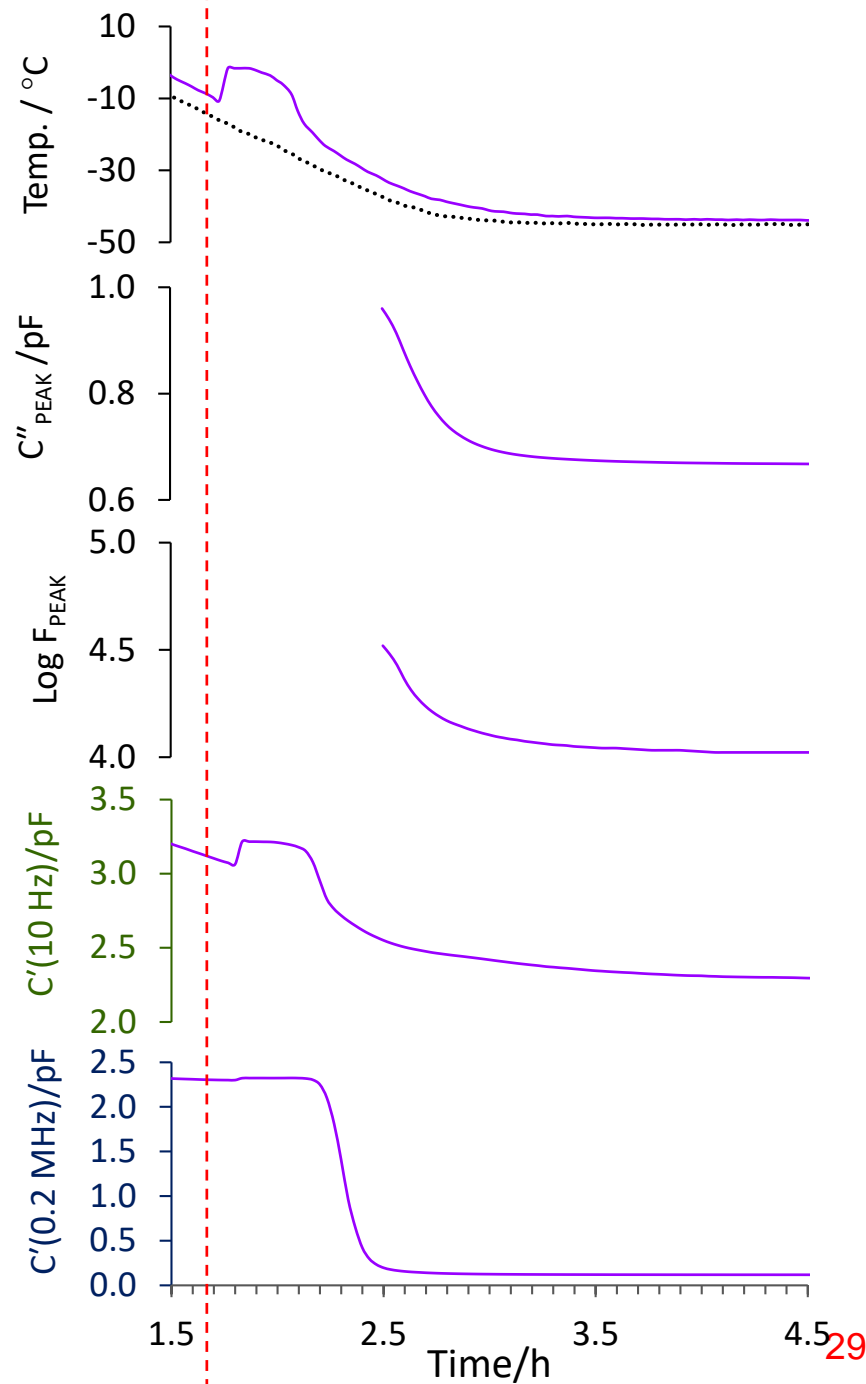
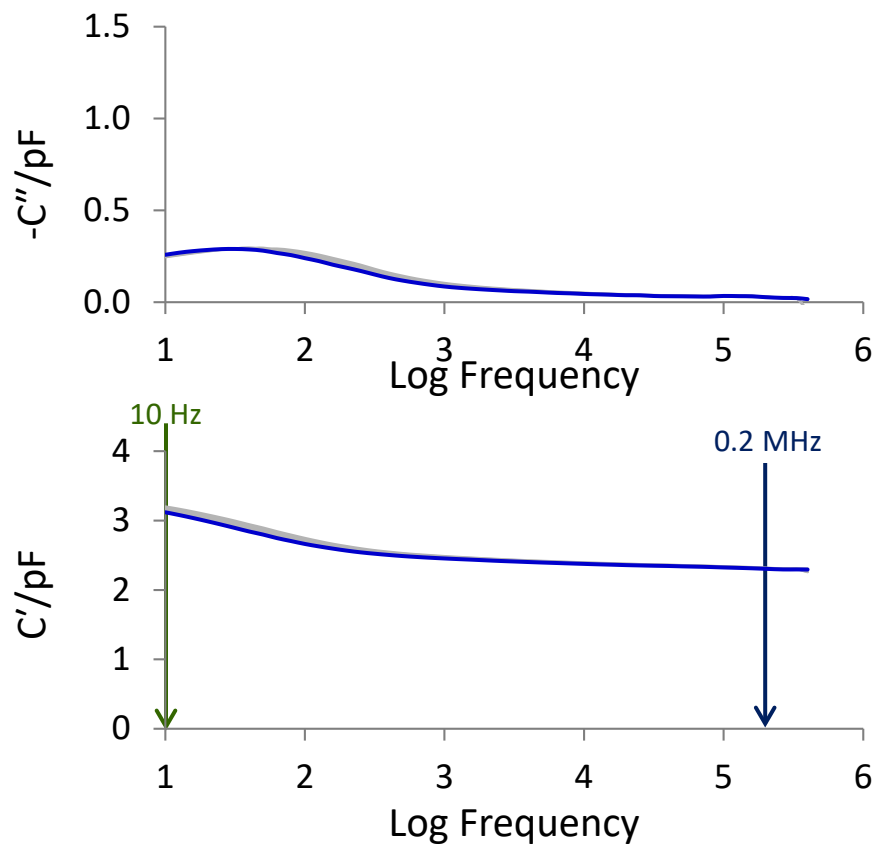
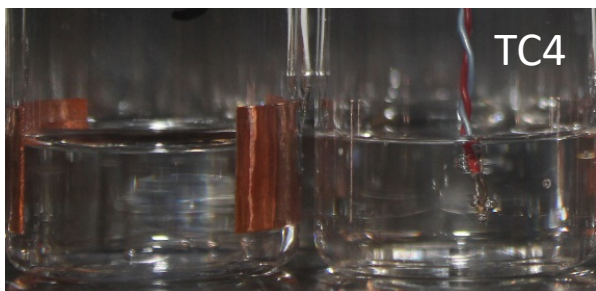
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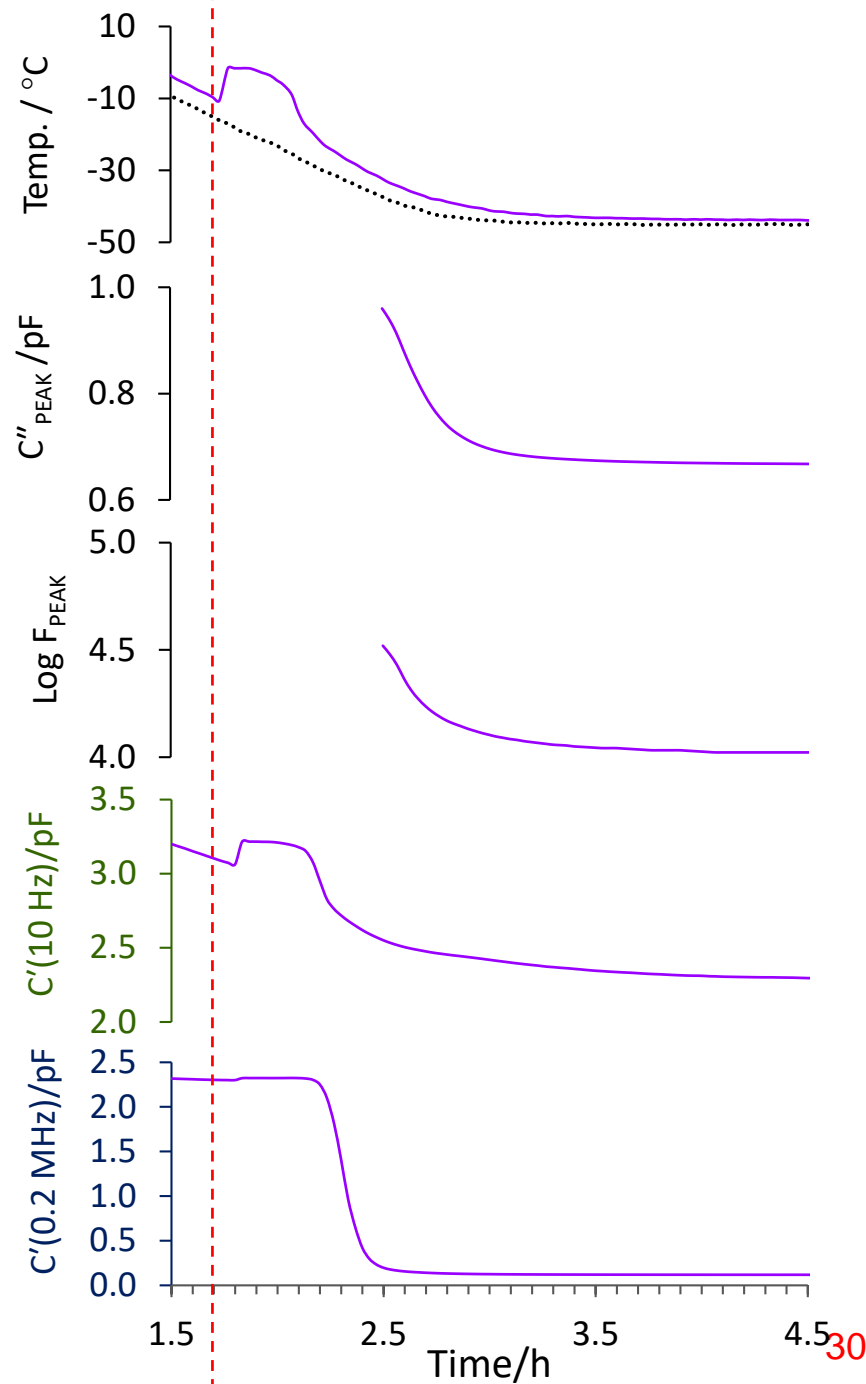
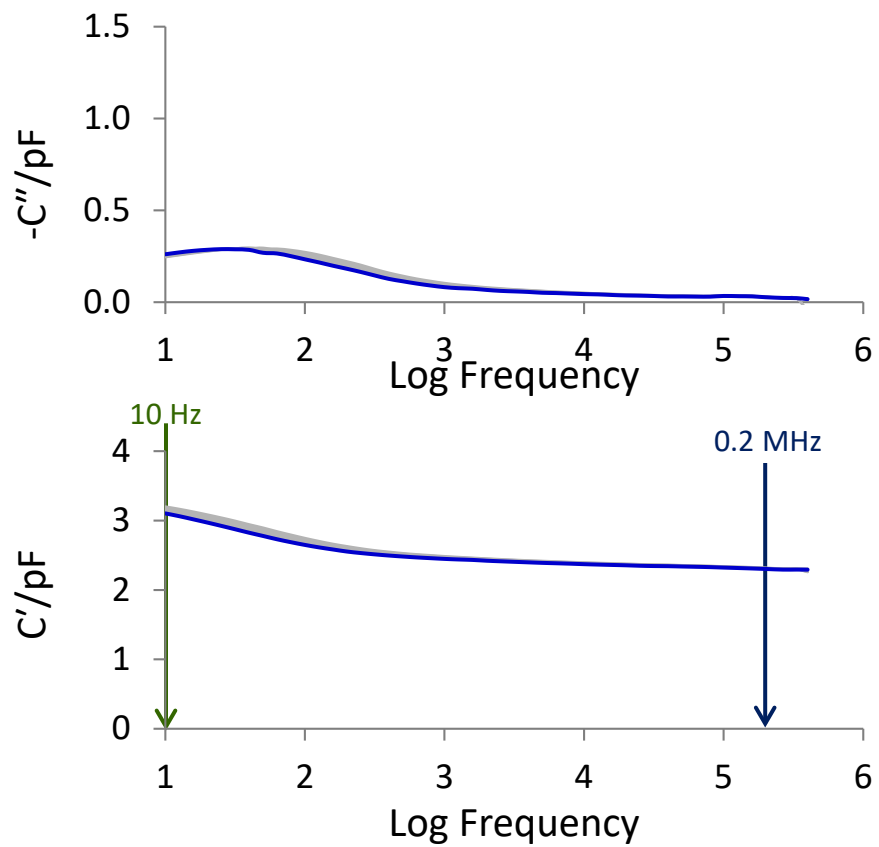
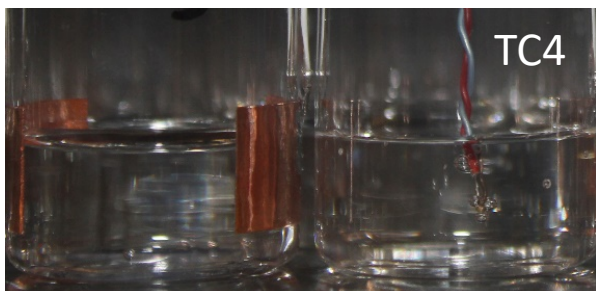
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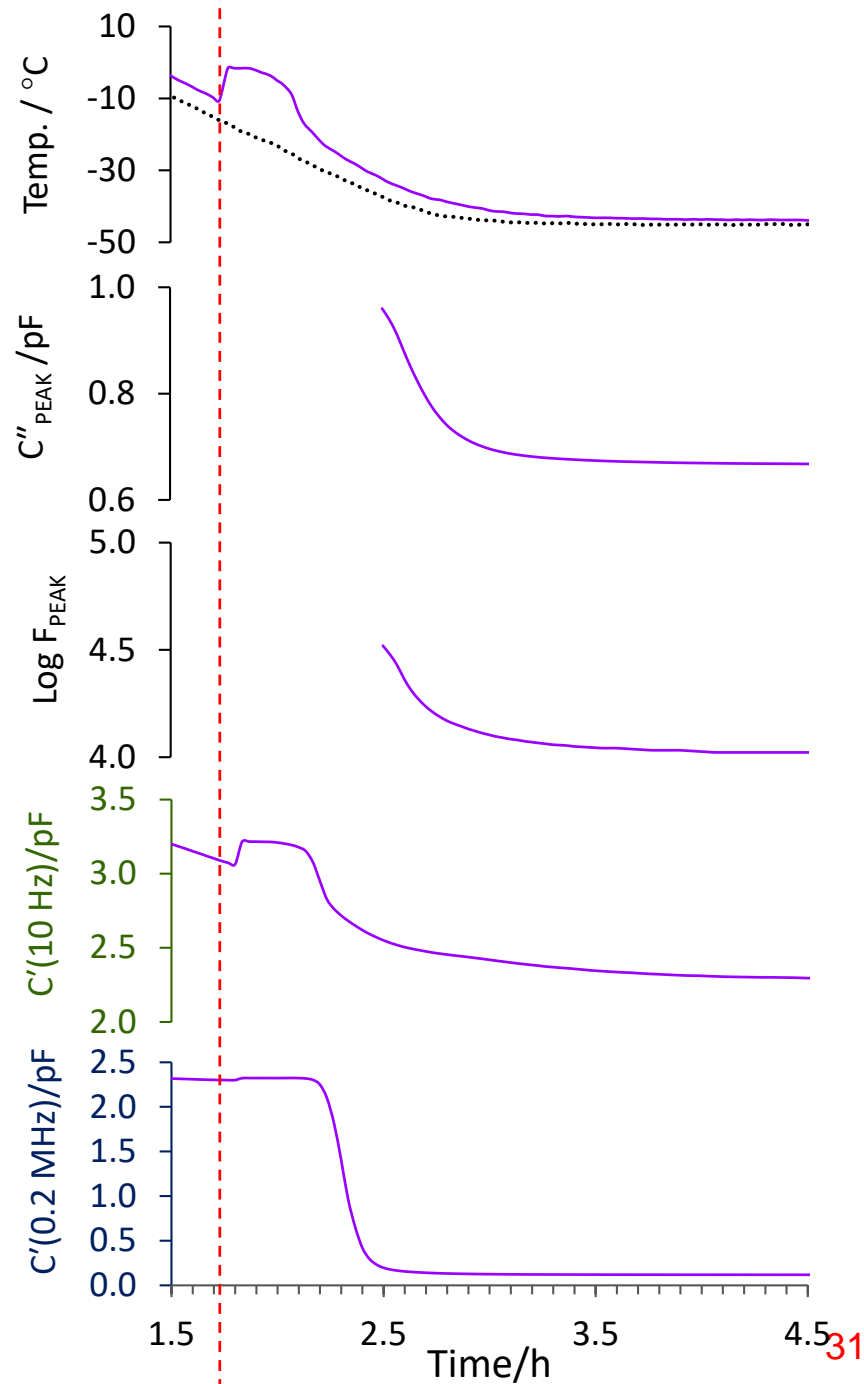
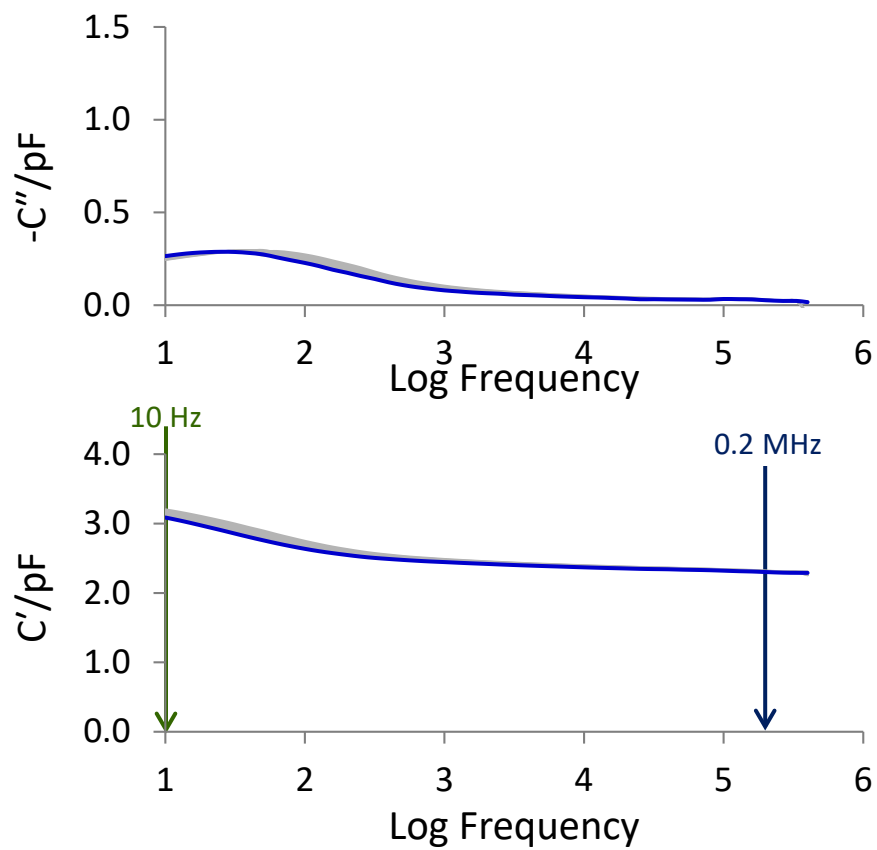
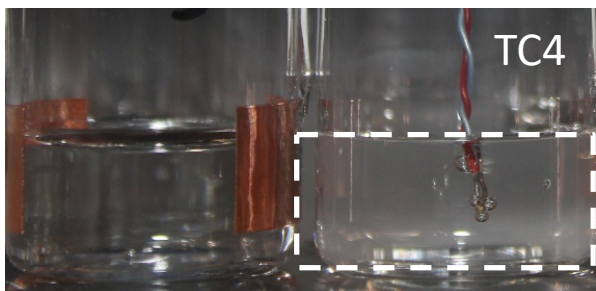
Freezing Step - 5%Sucrose+0.55%NaCl



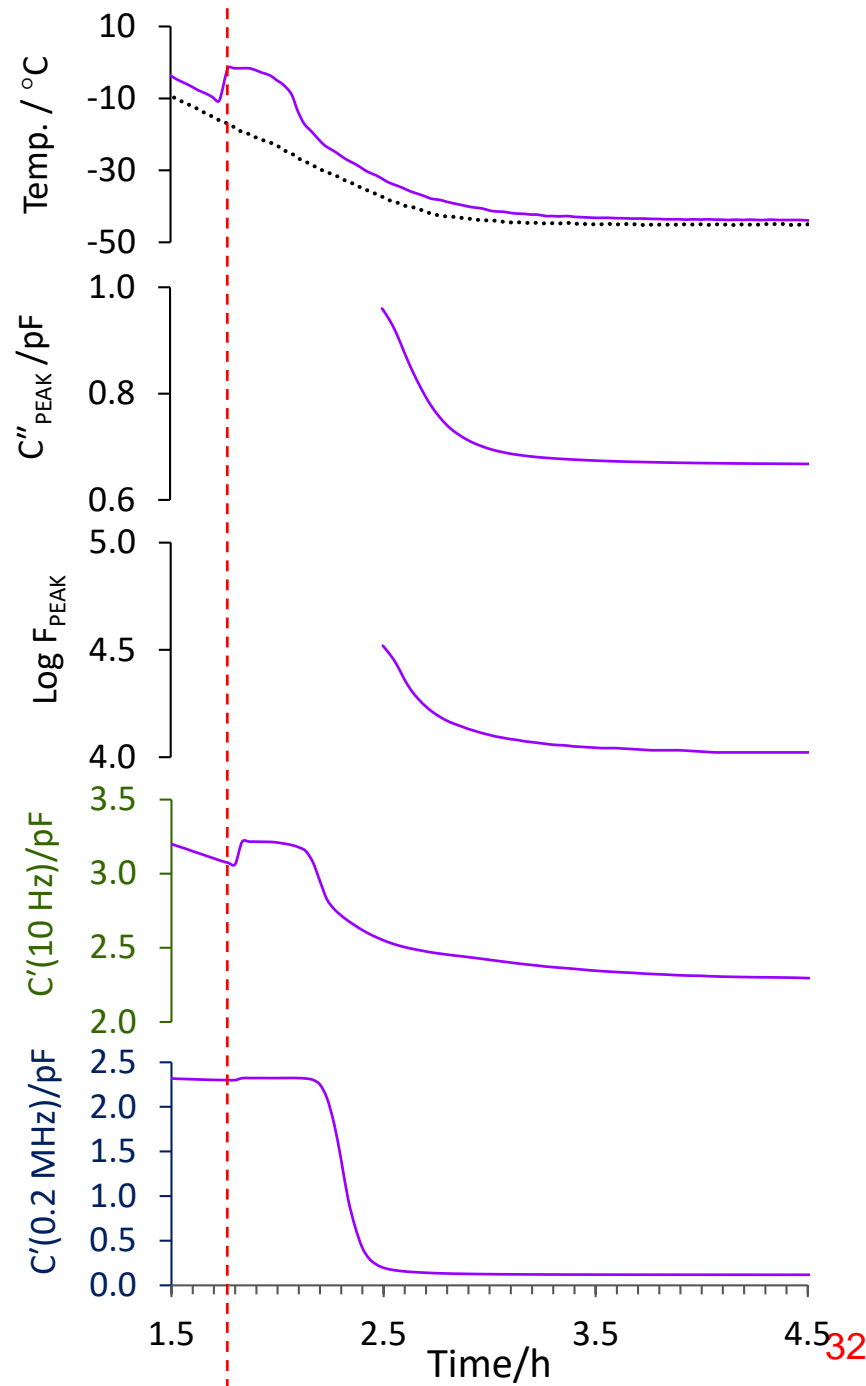
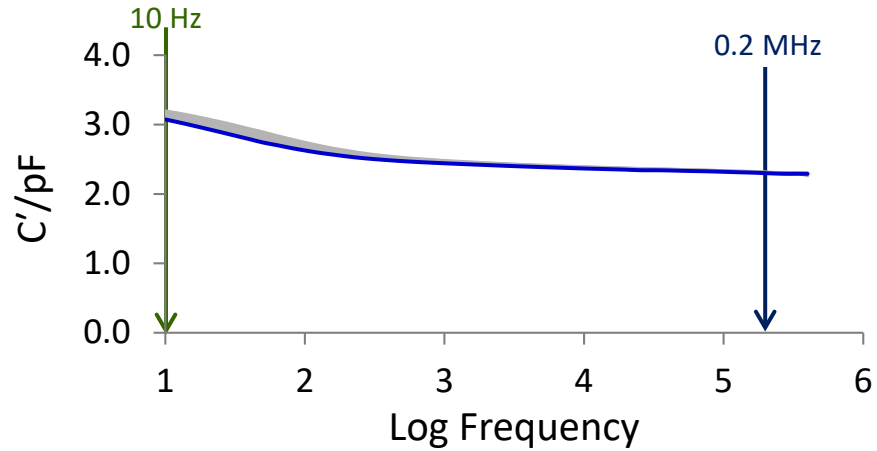
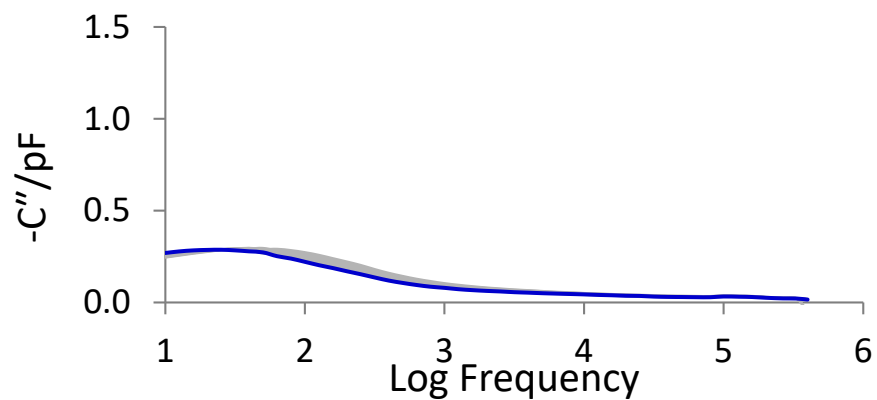
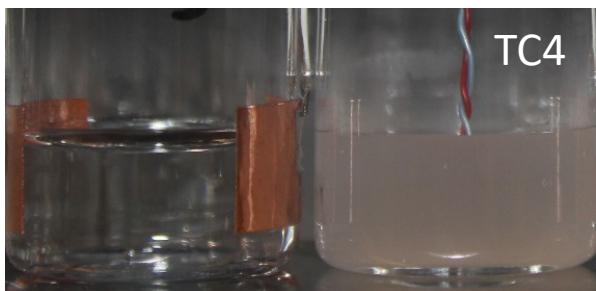
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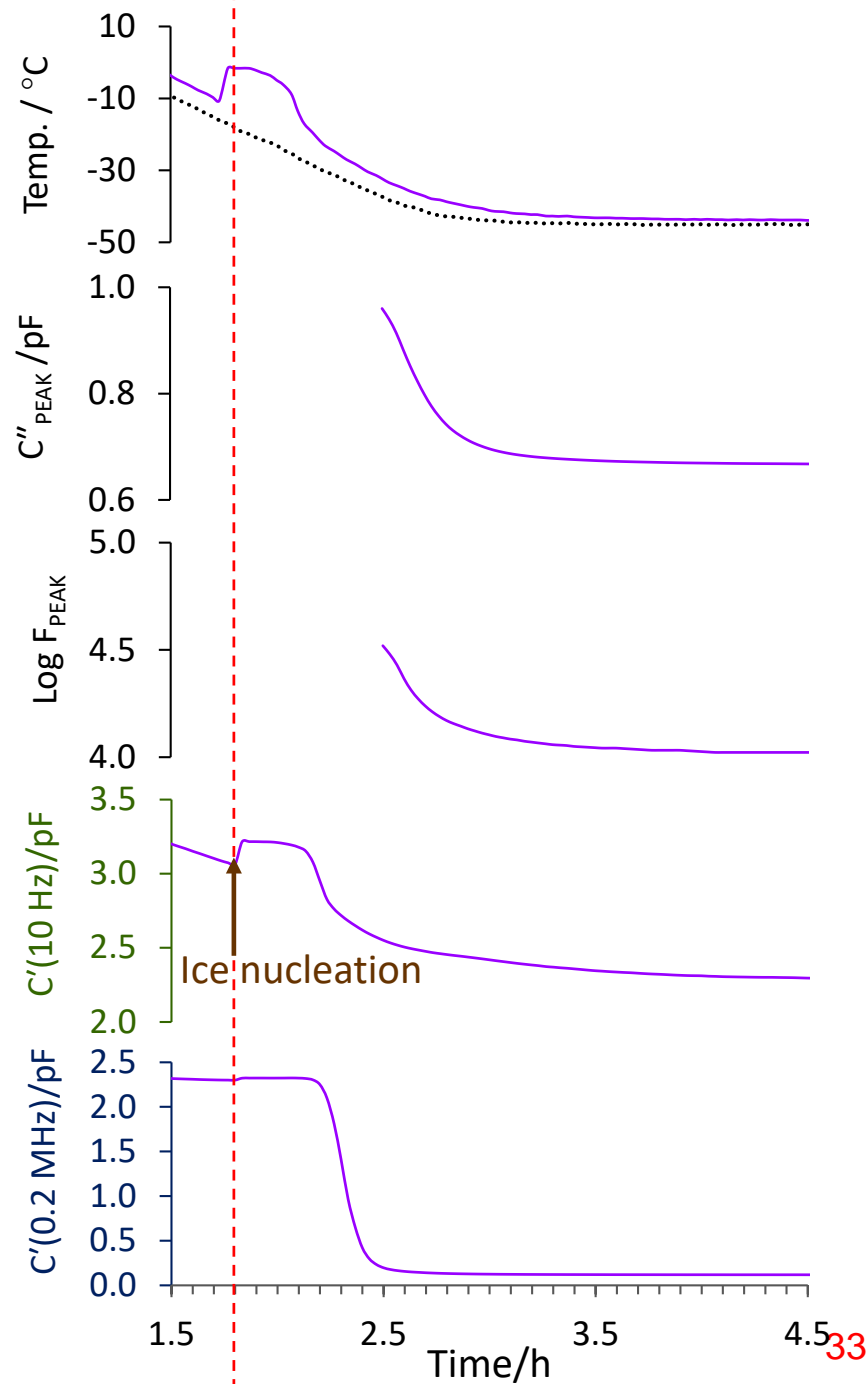
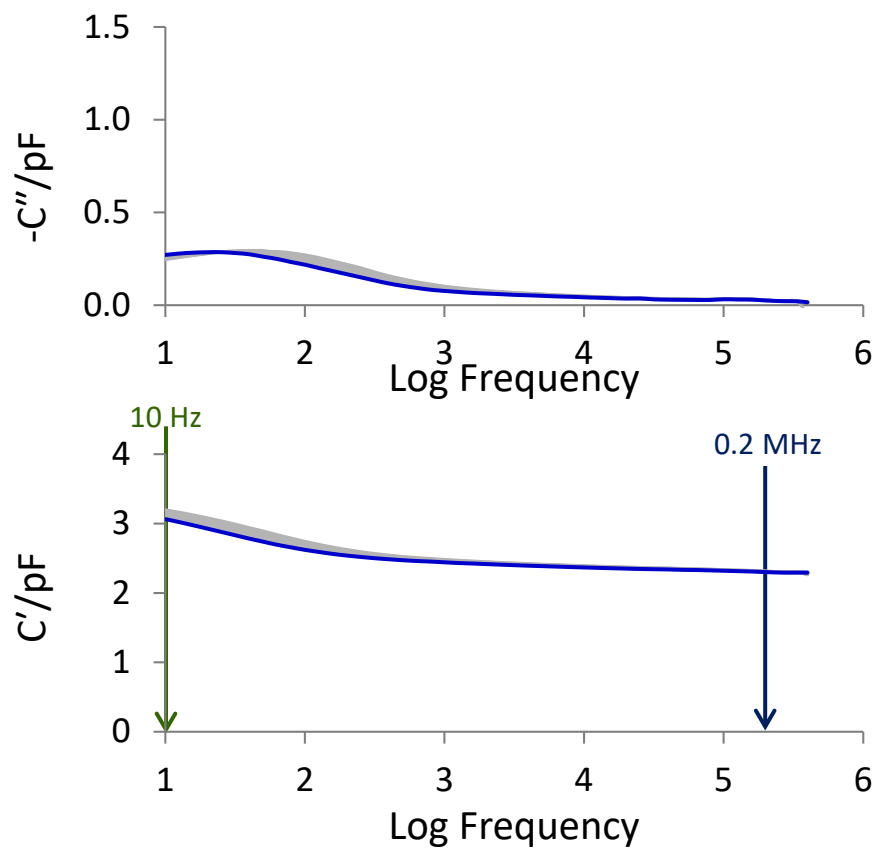
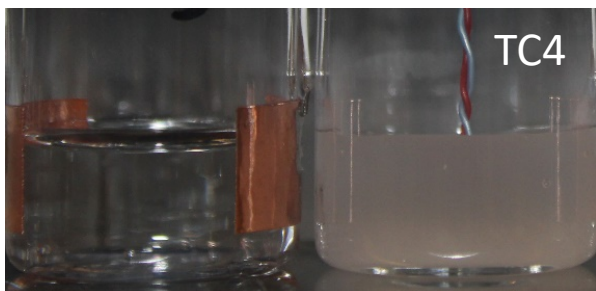
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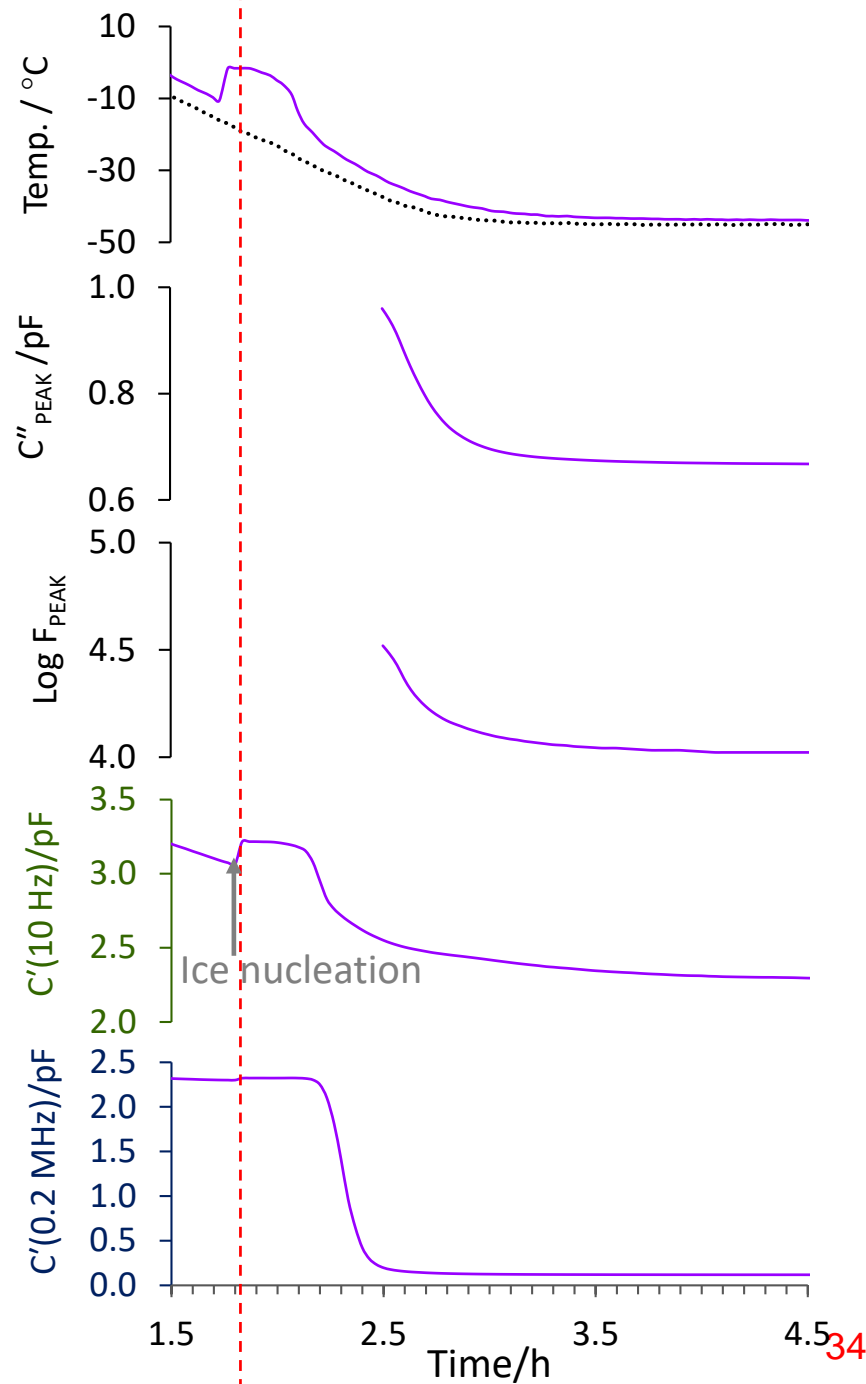
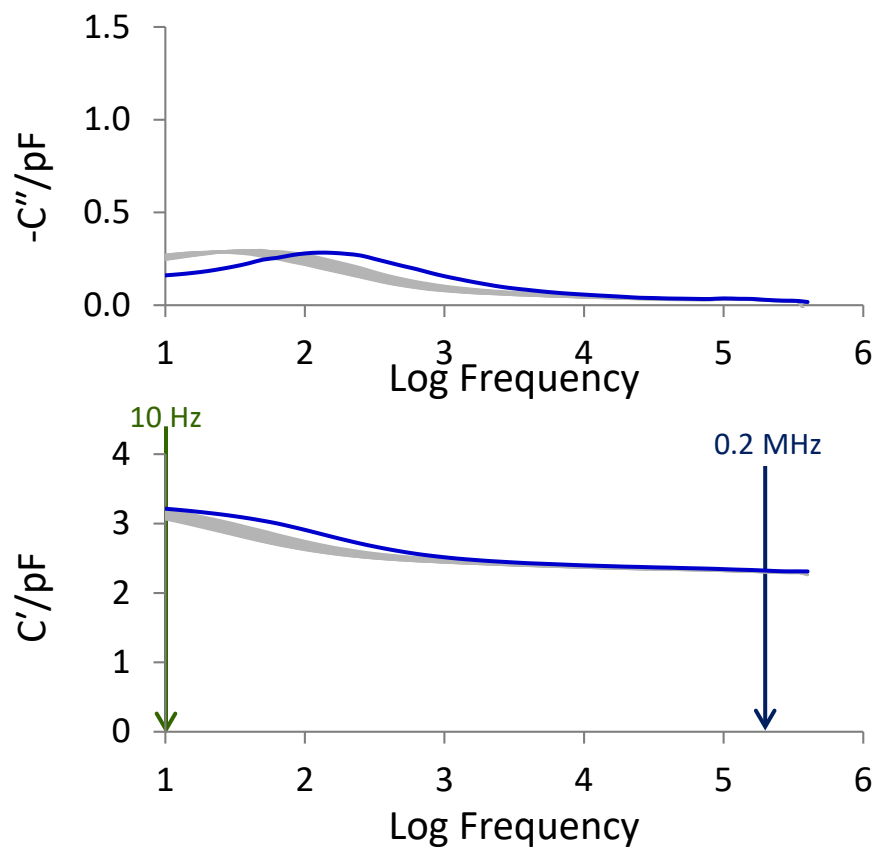
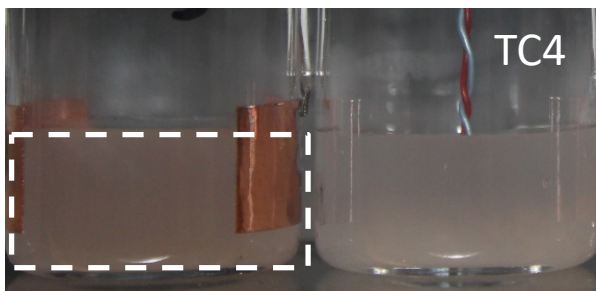
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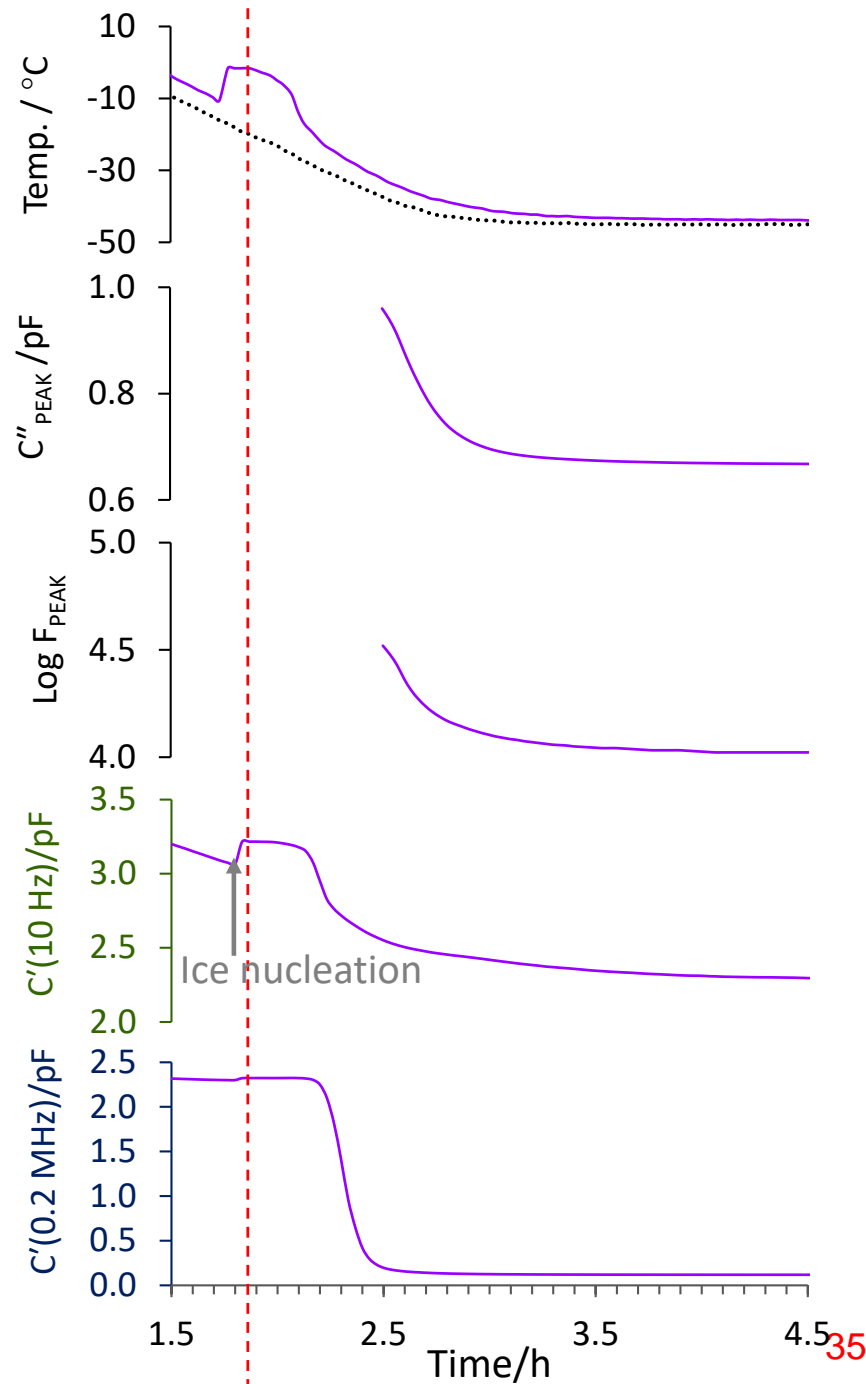
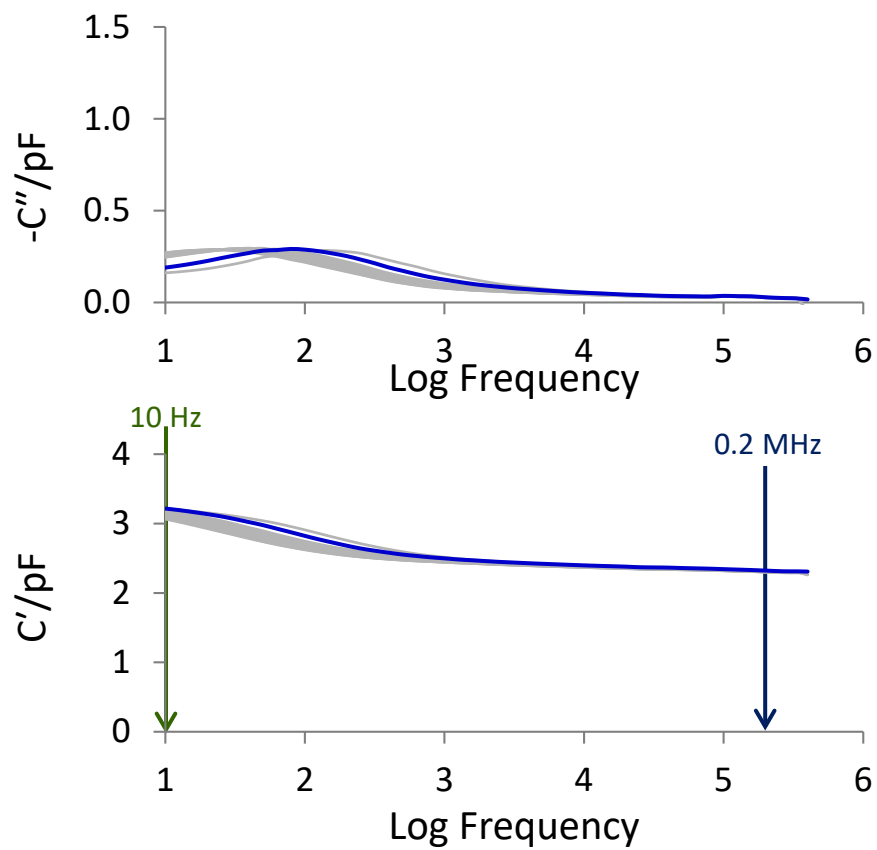
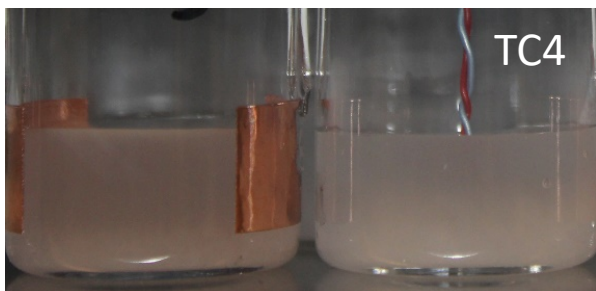
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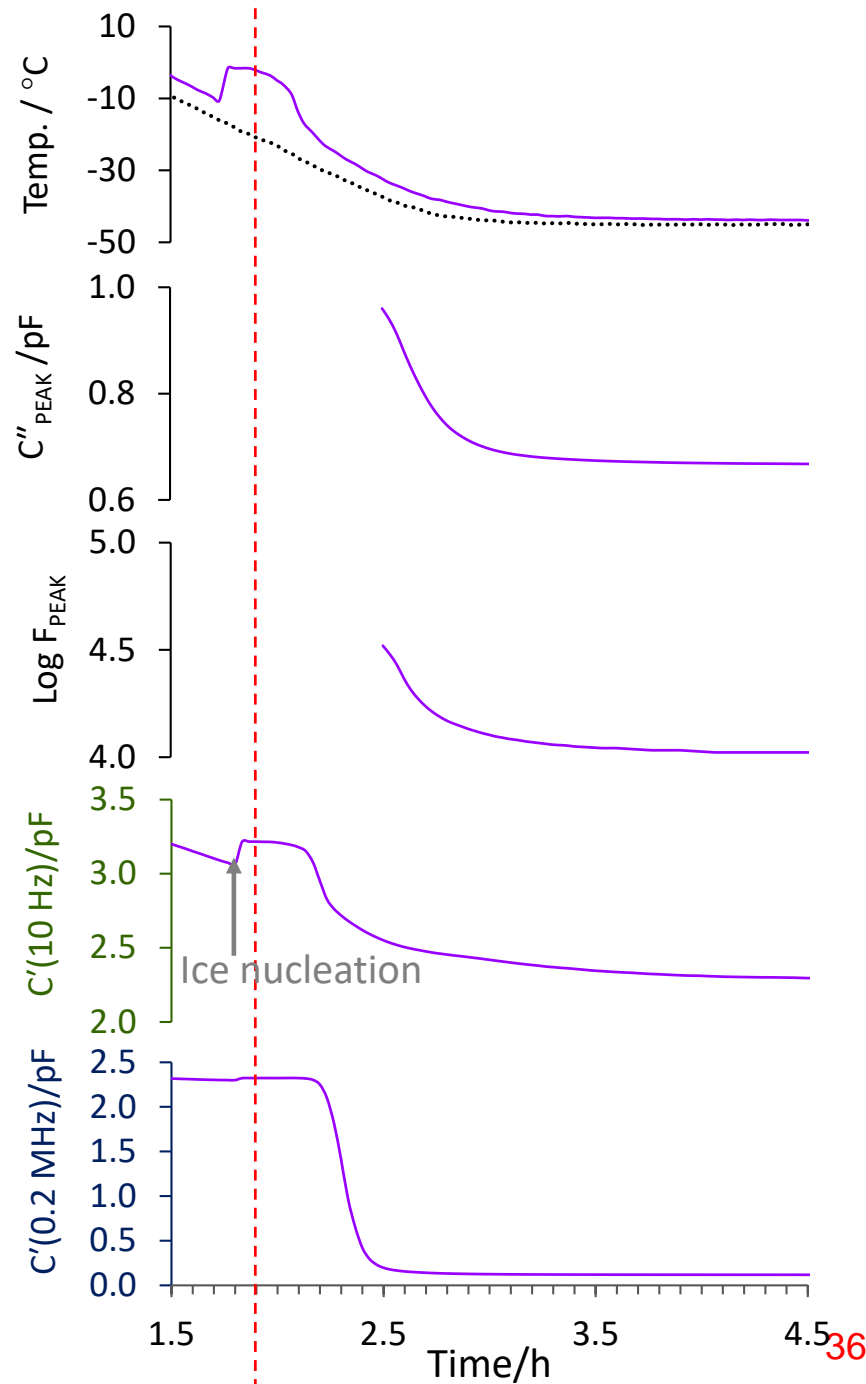
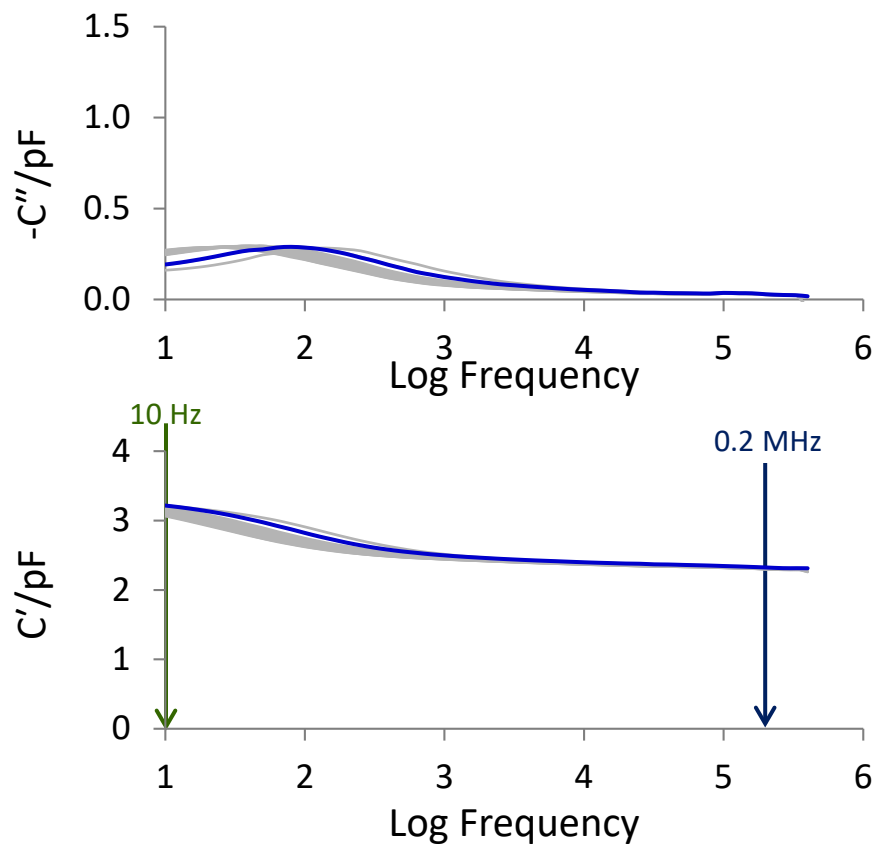
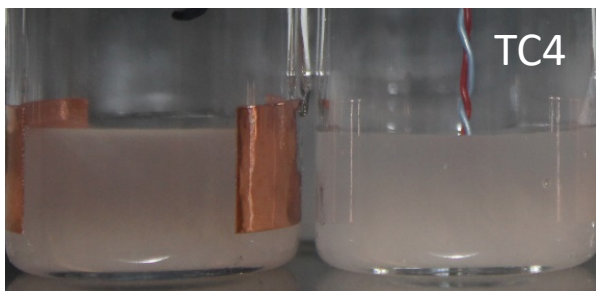
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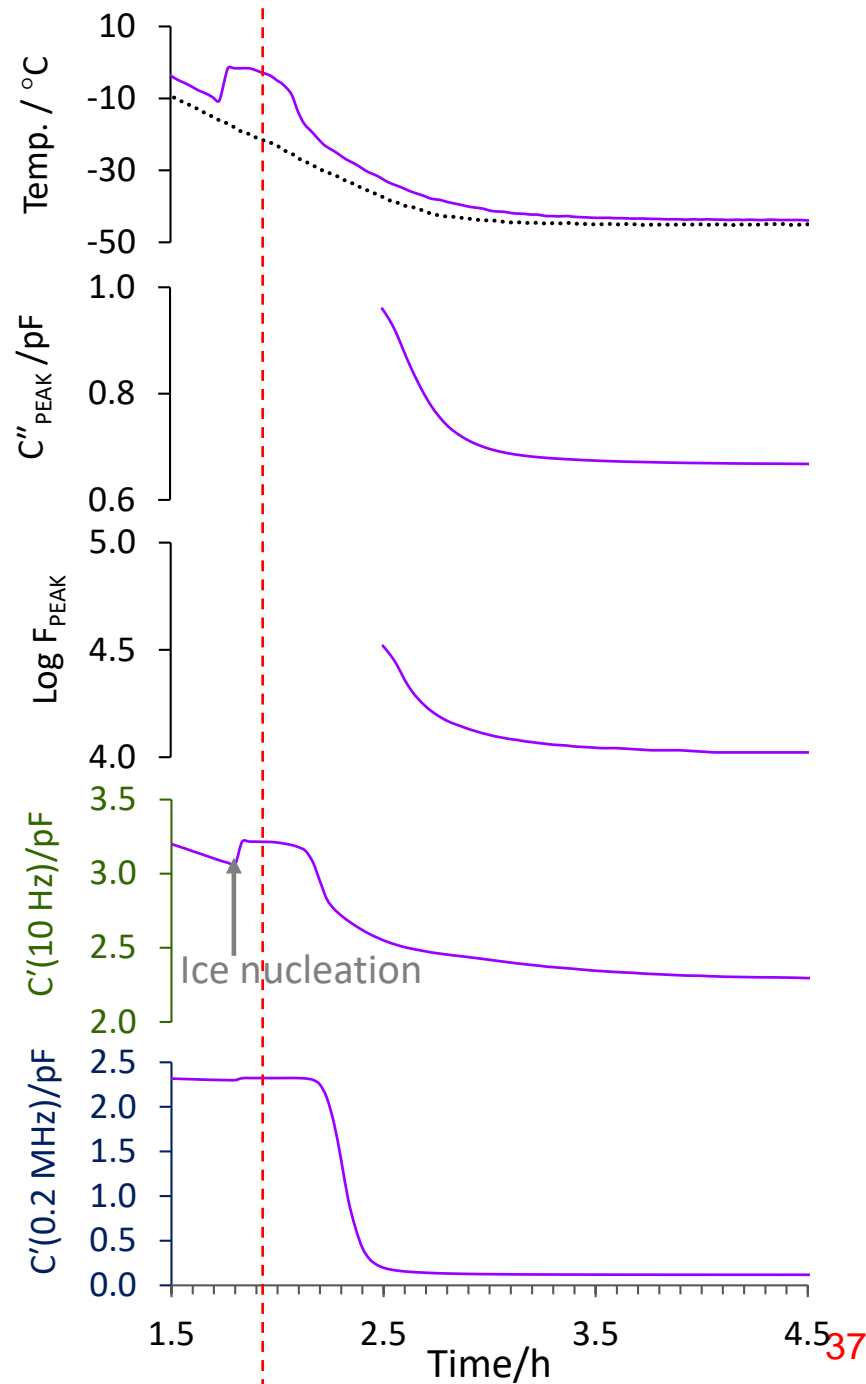
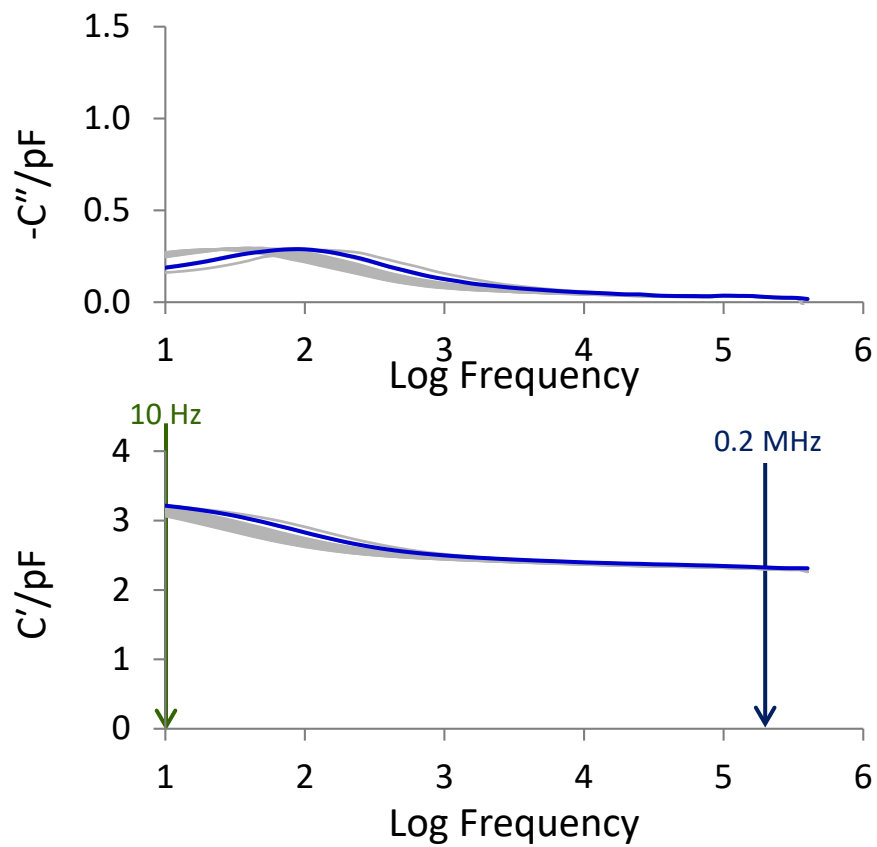
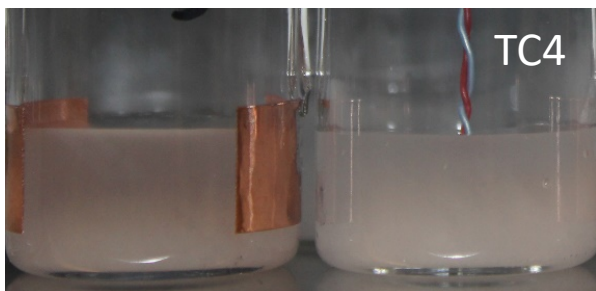
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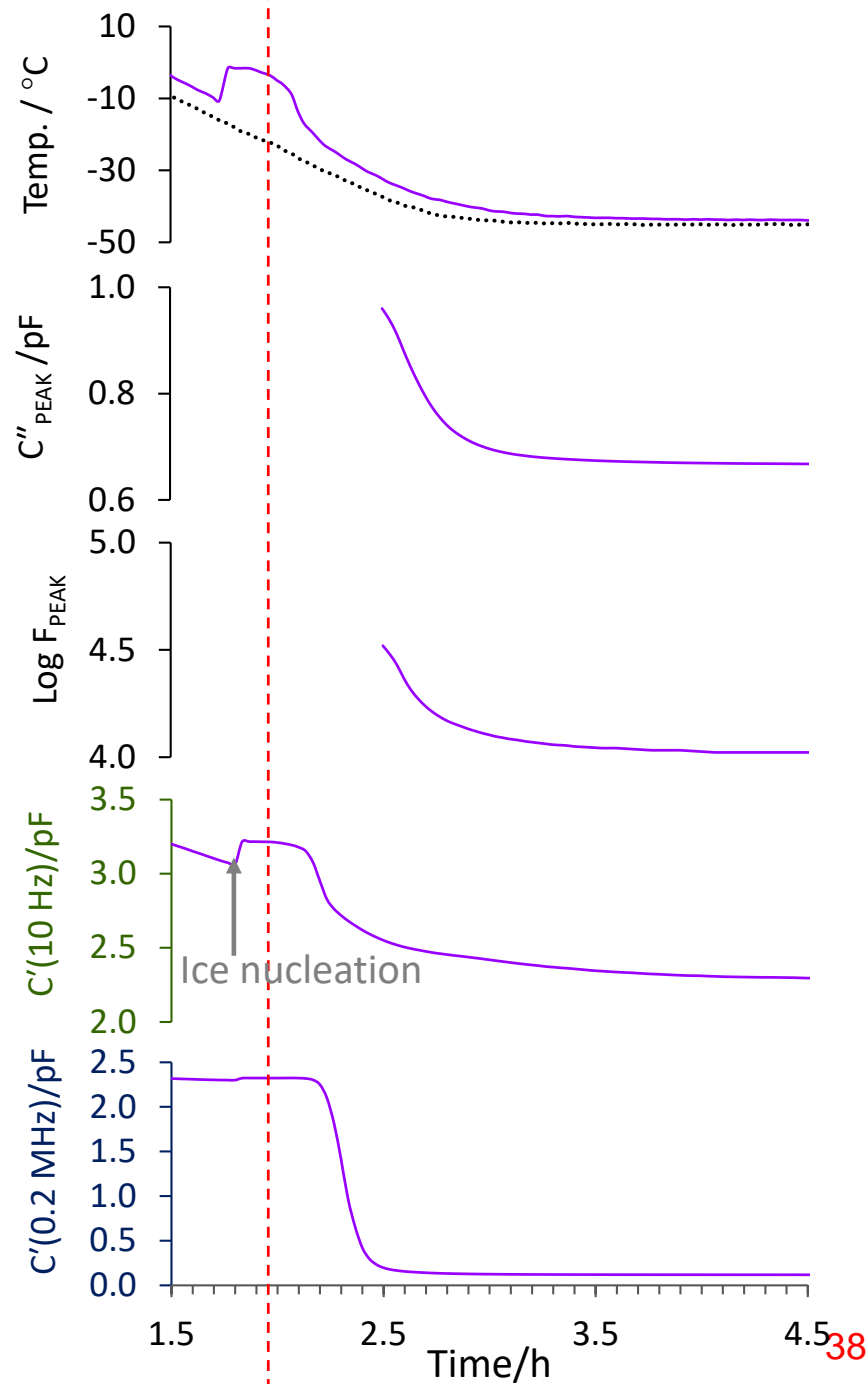
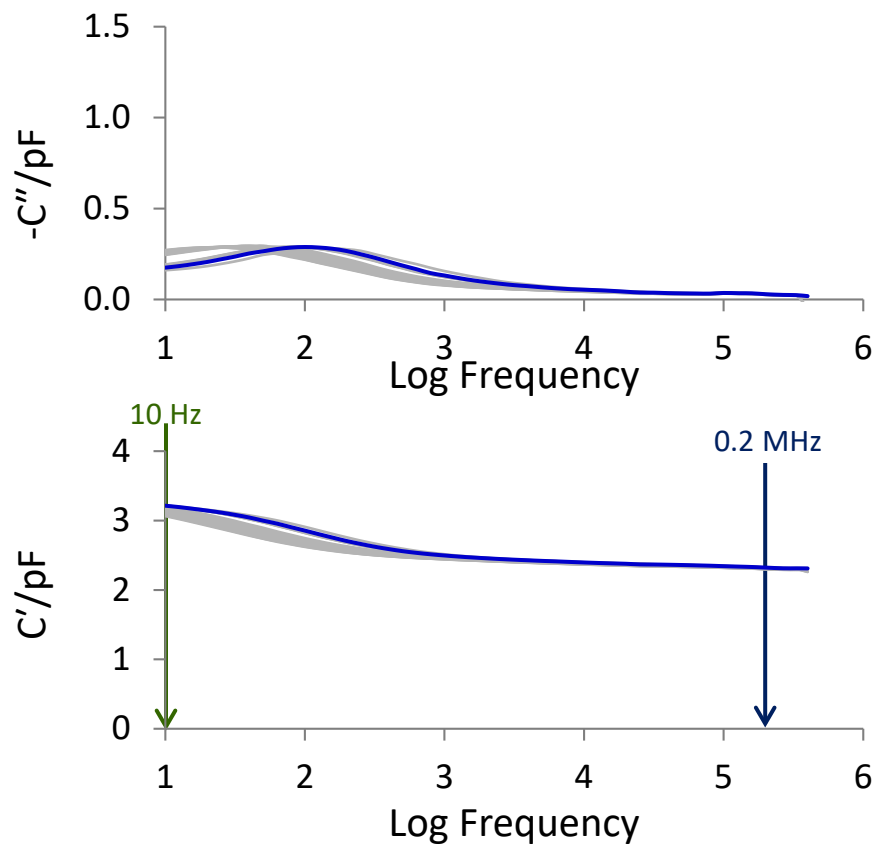
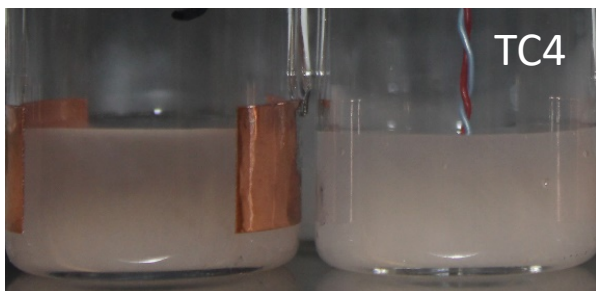
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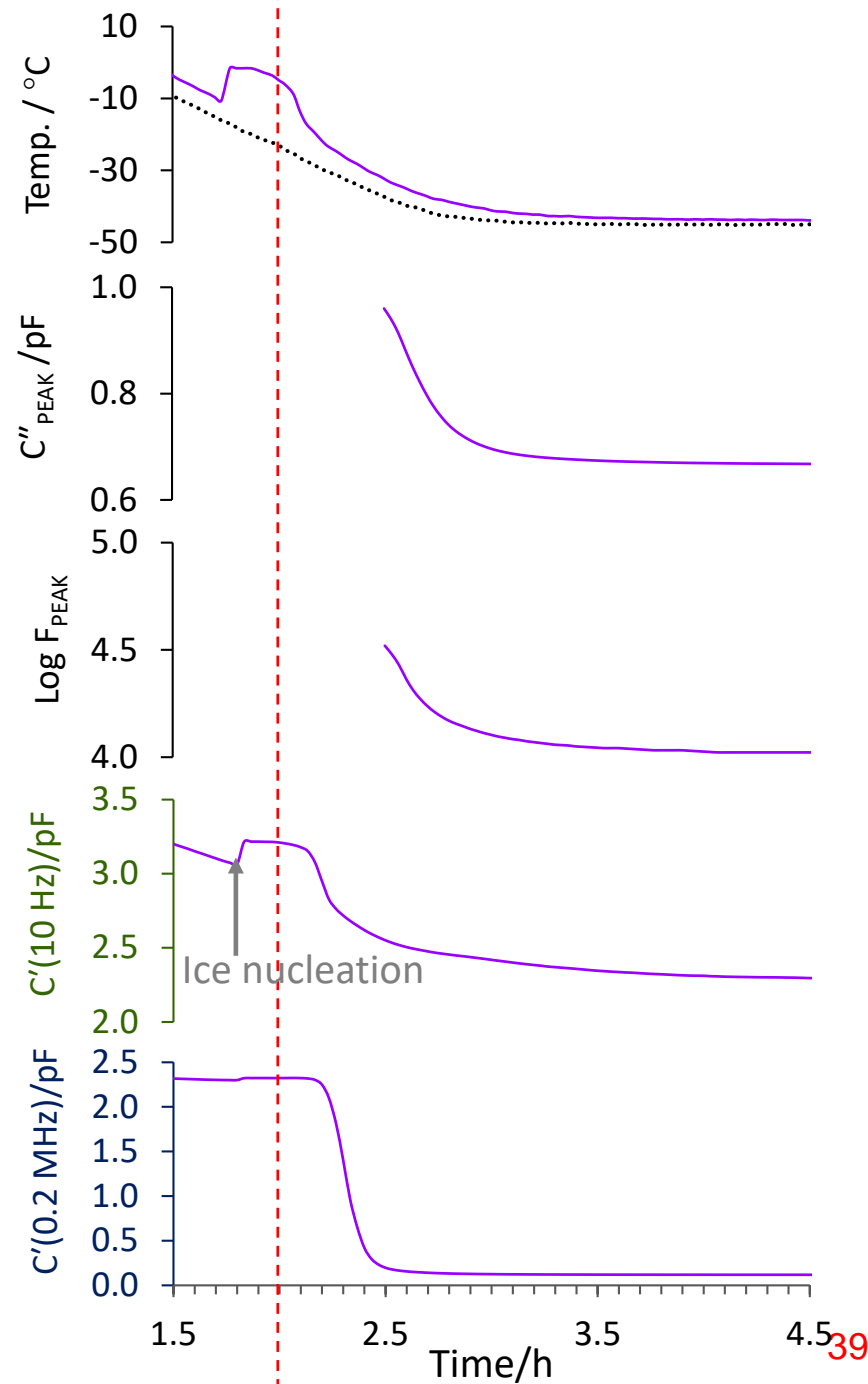
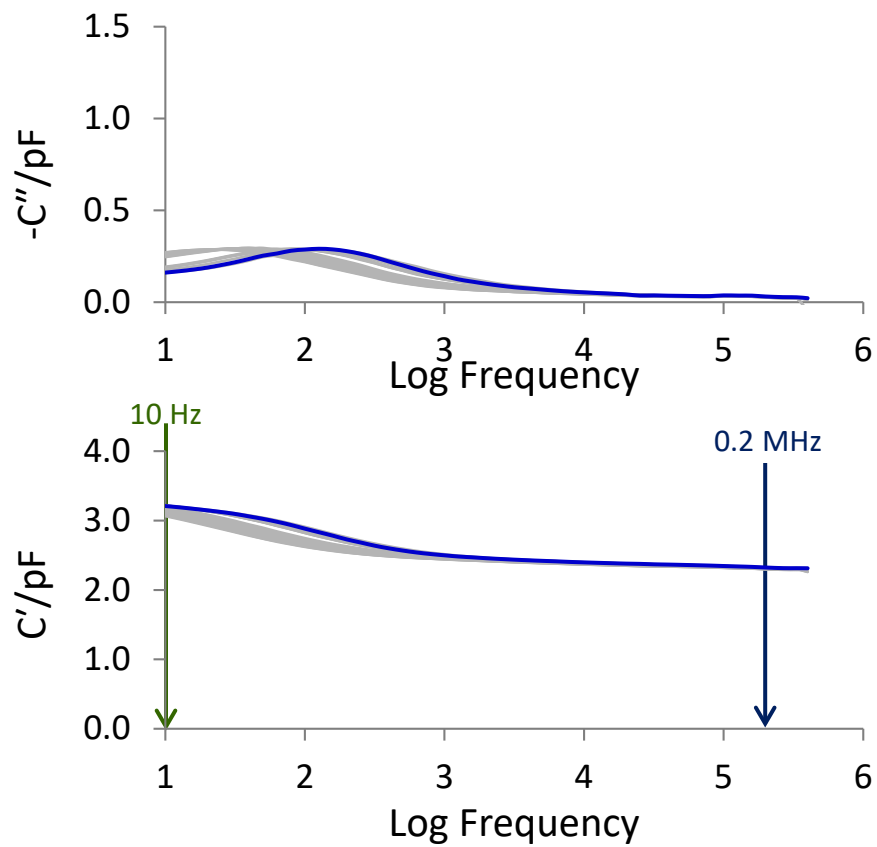
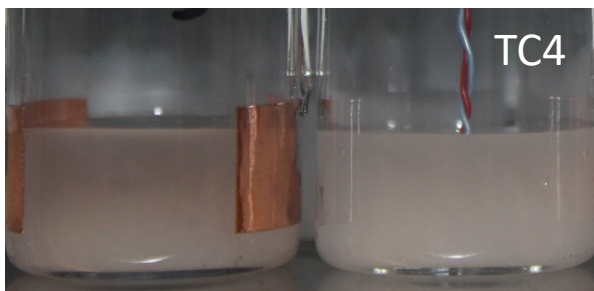
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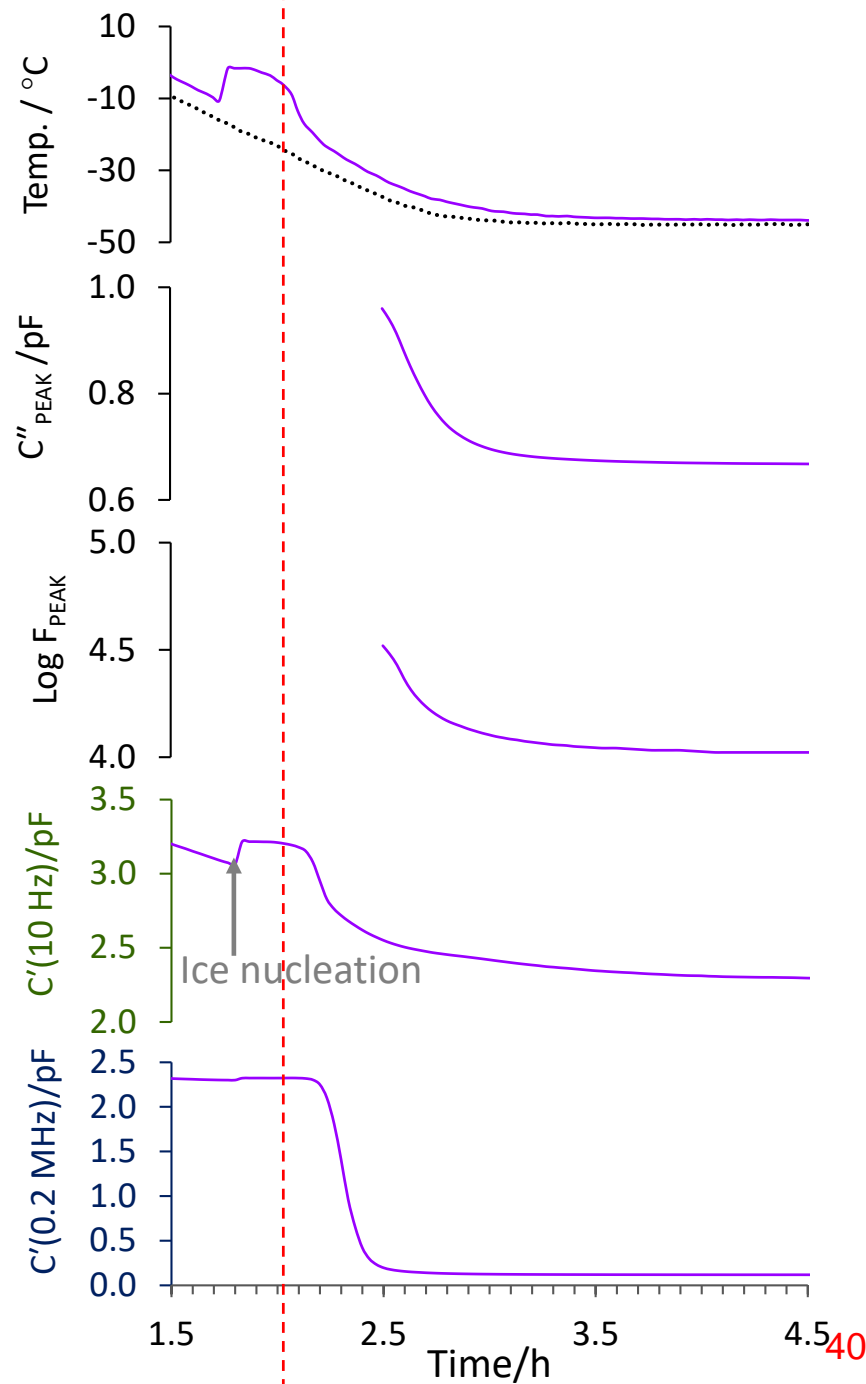
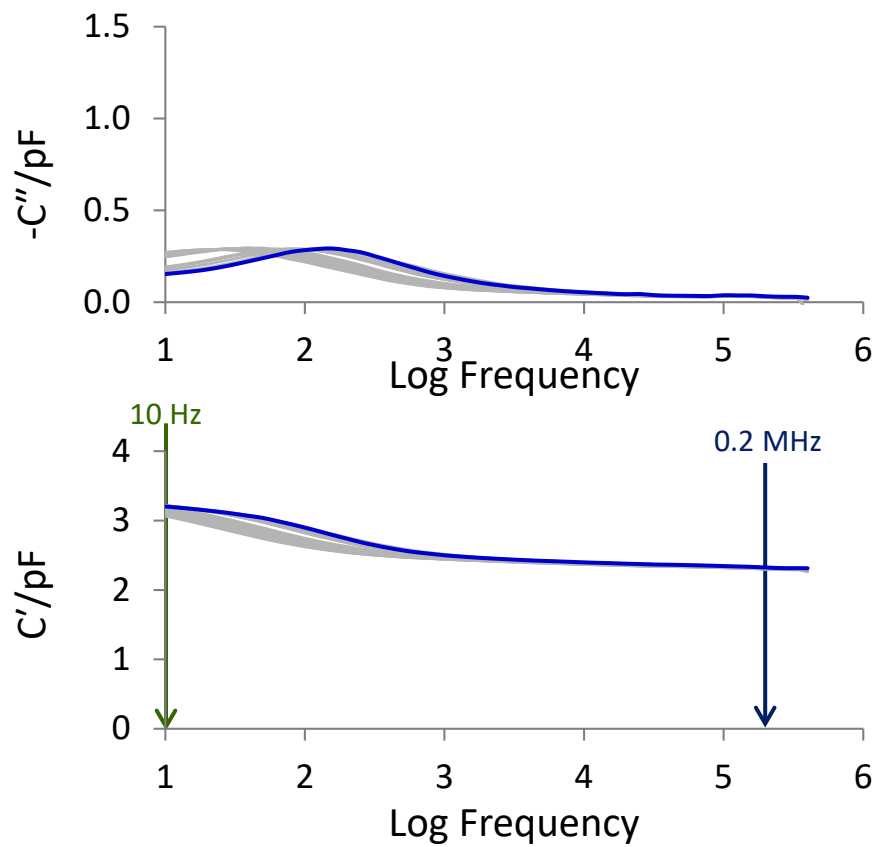
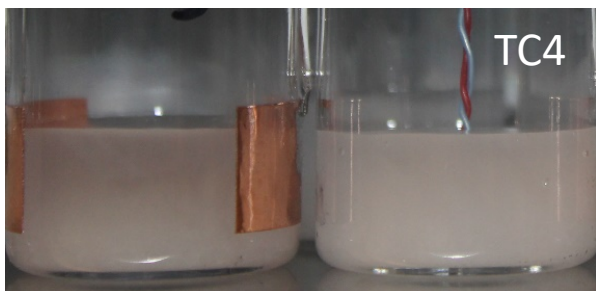
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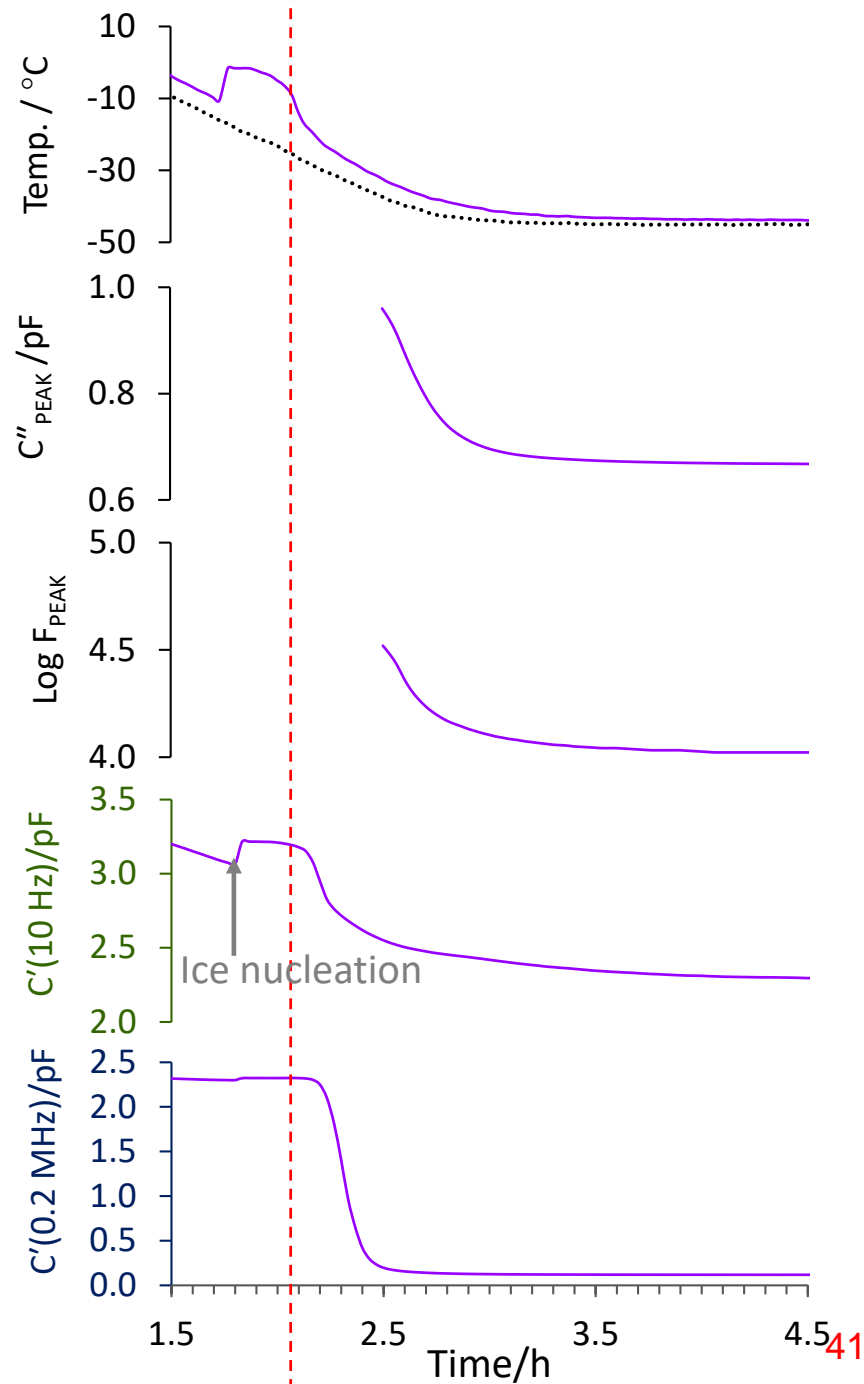
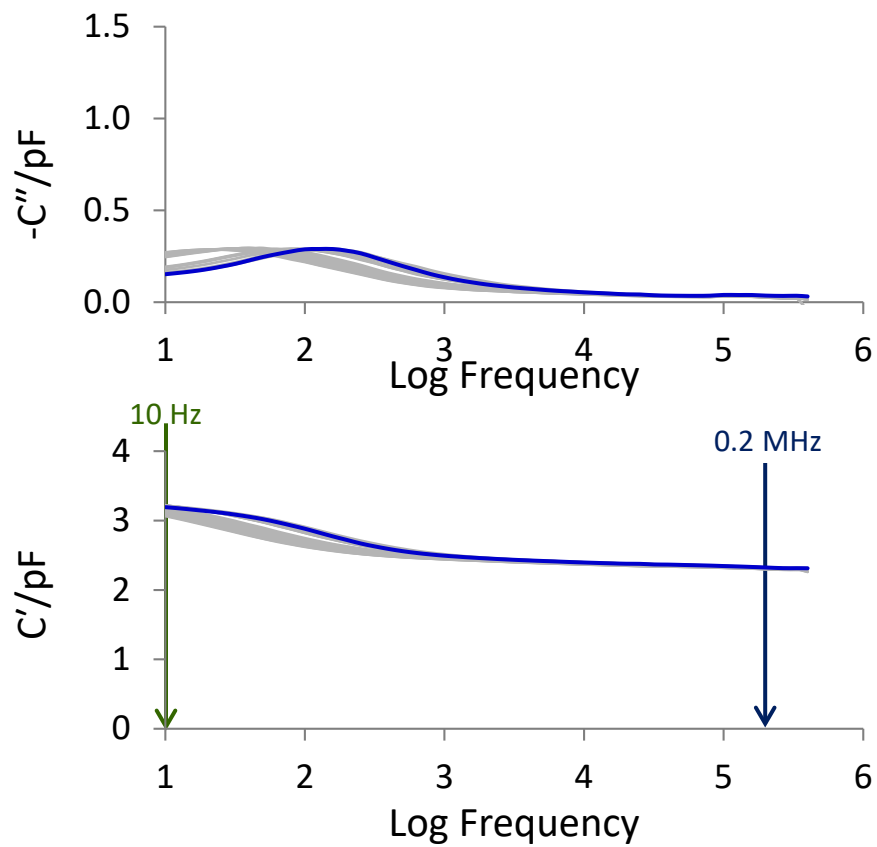
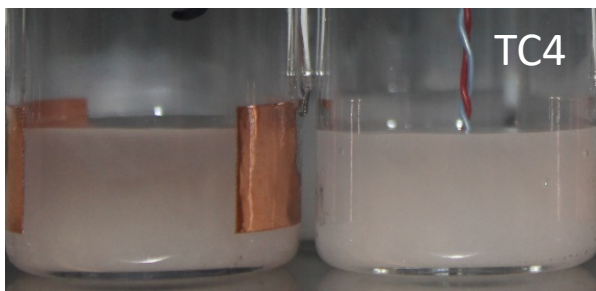
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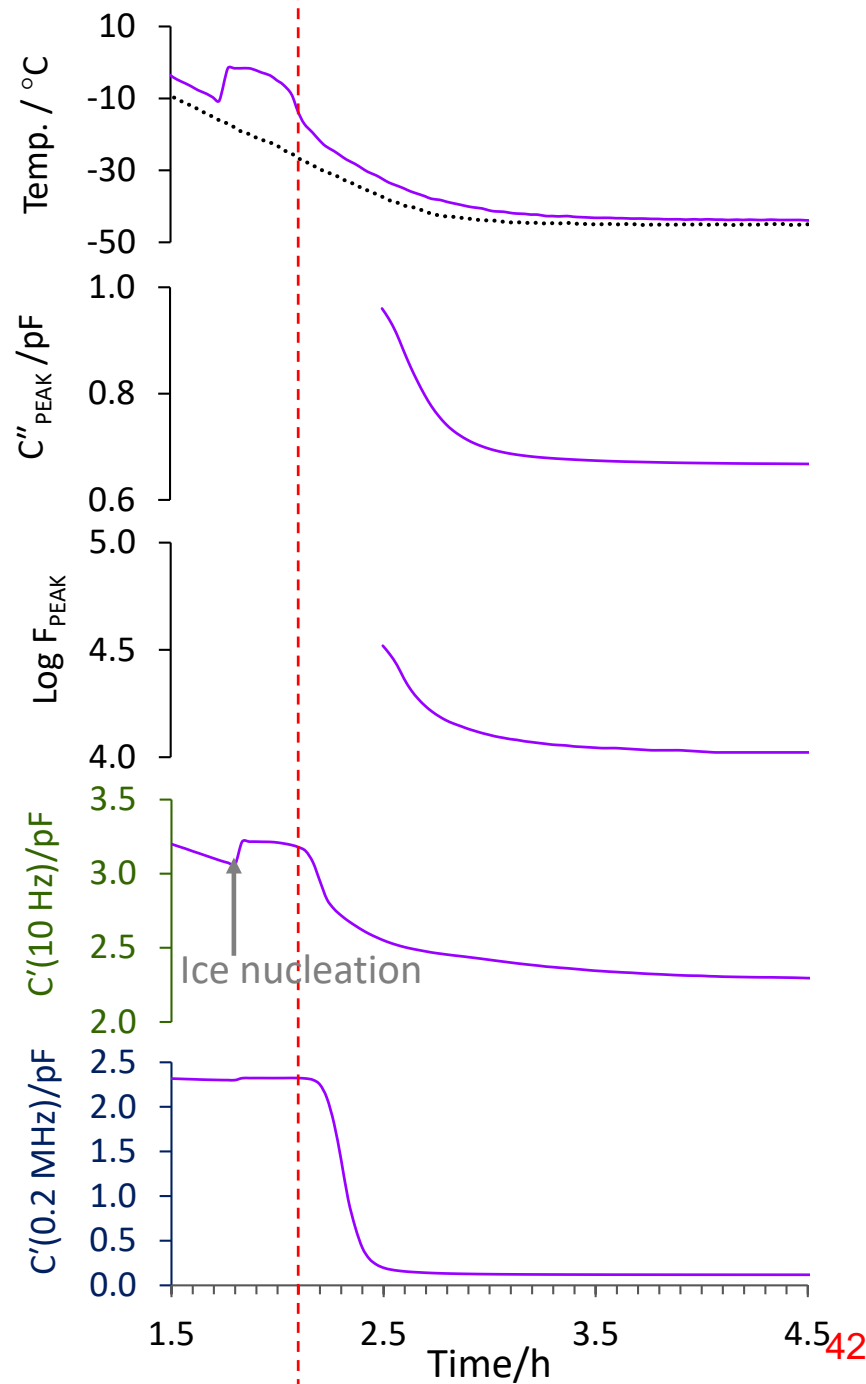
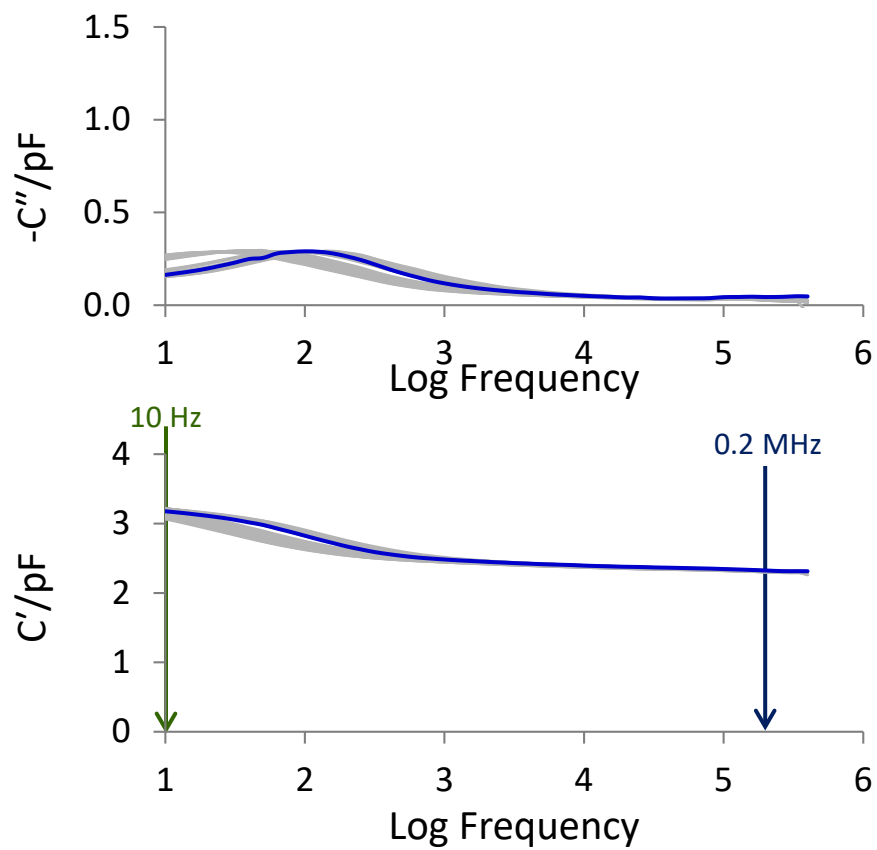
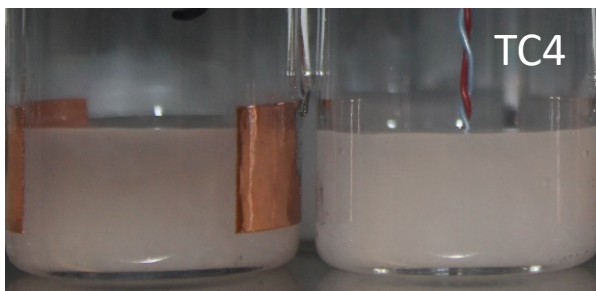
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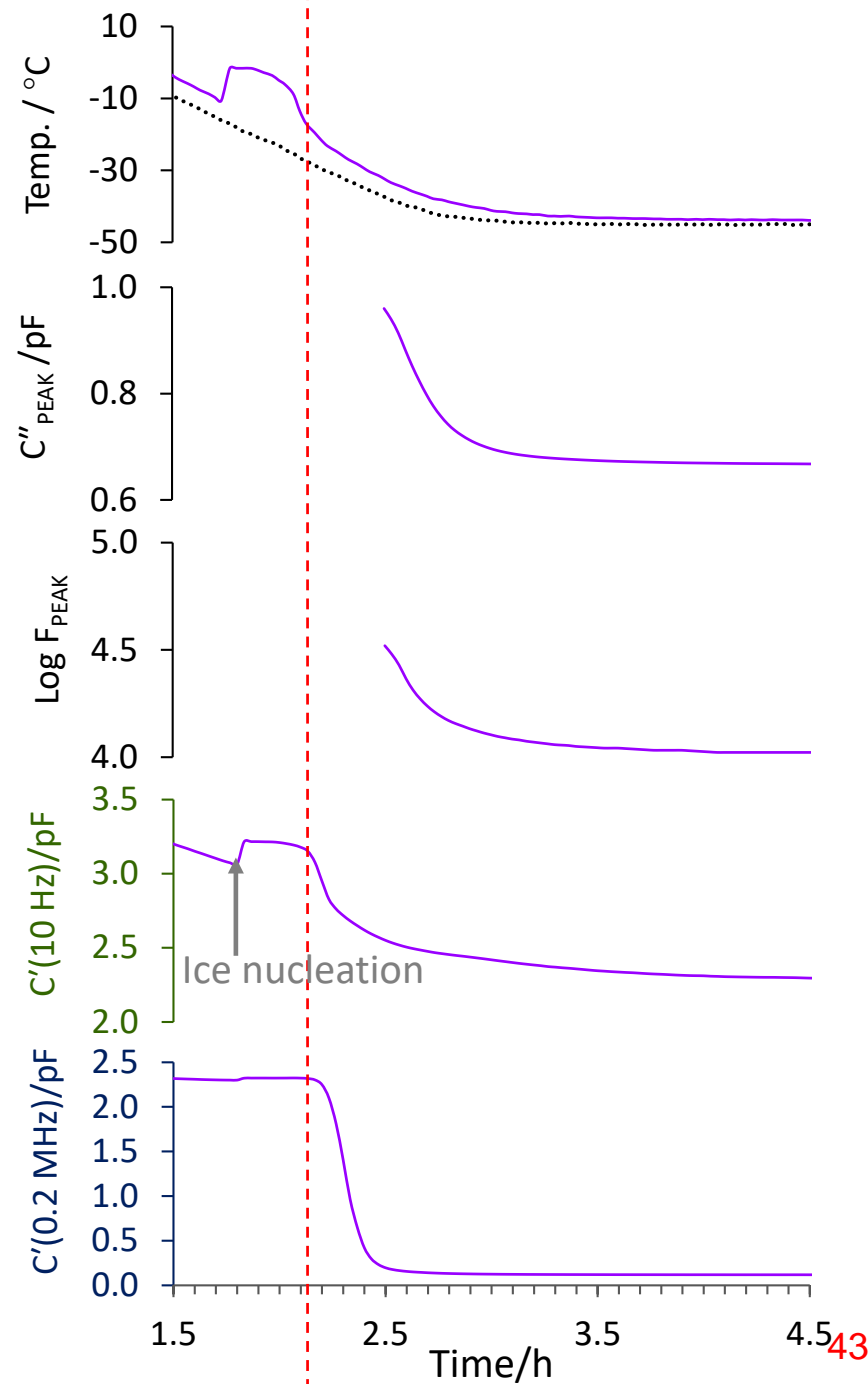
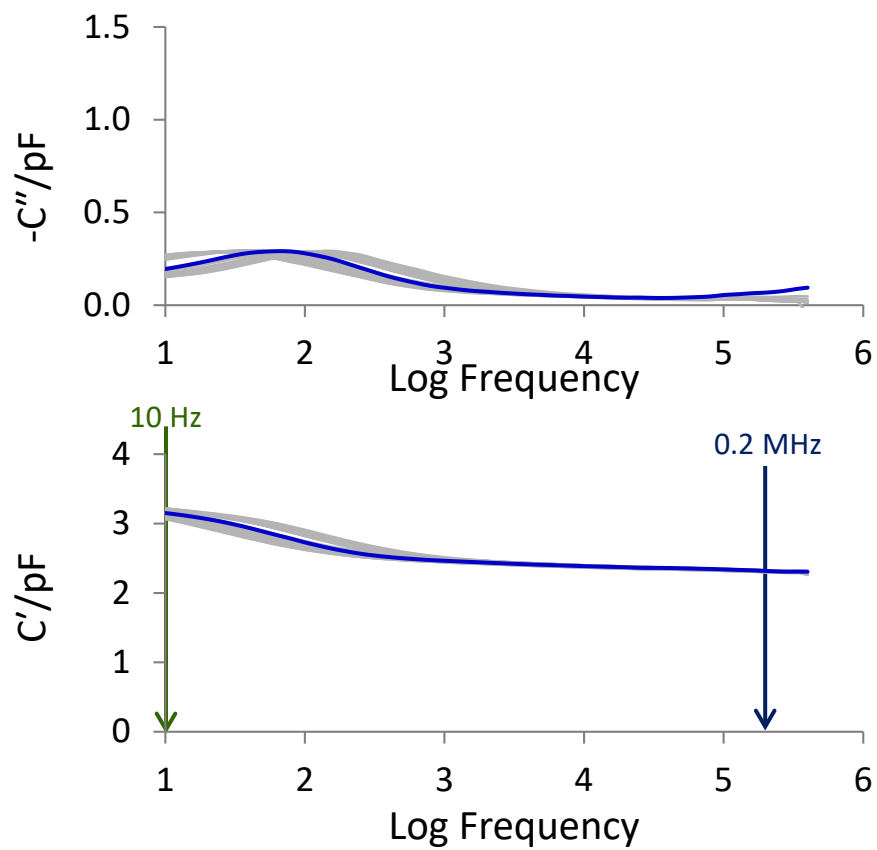
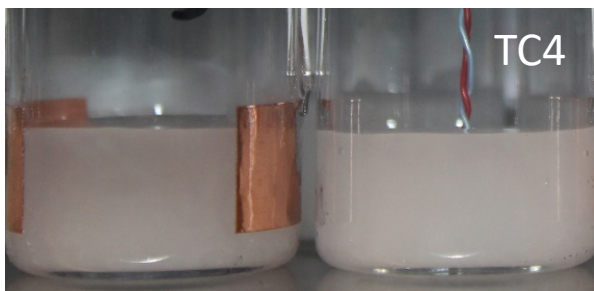
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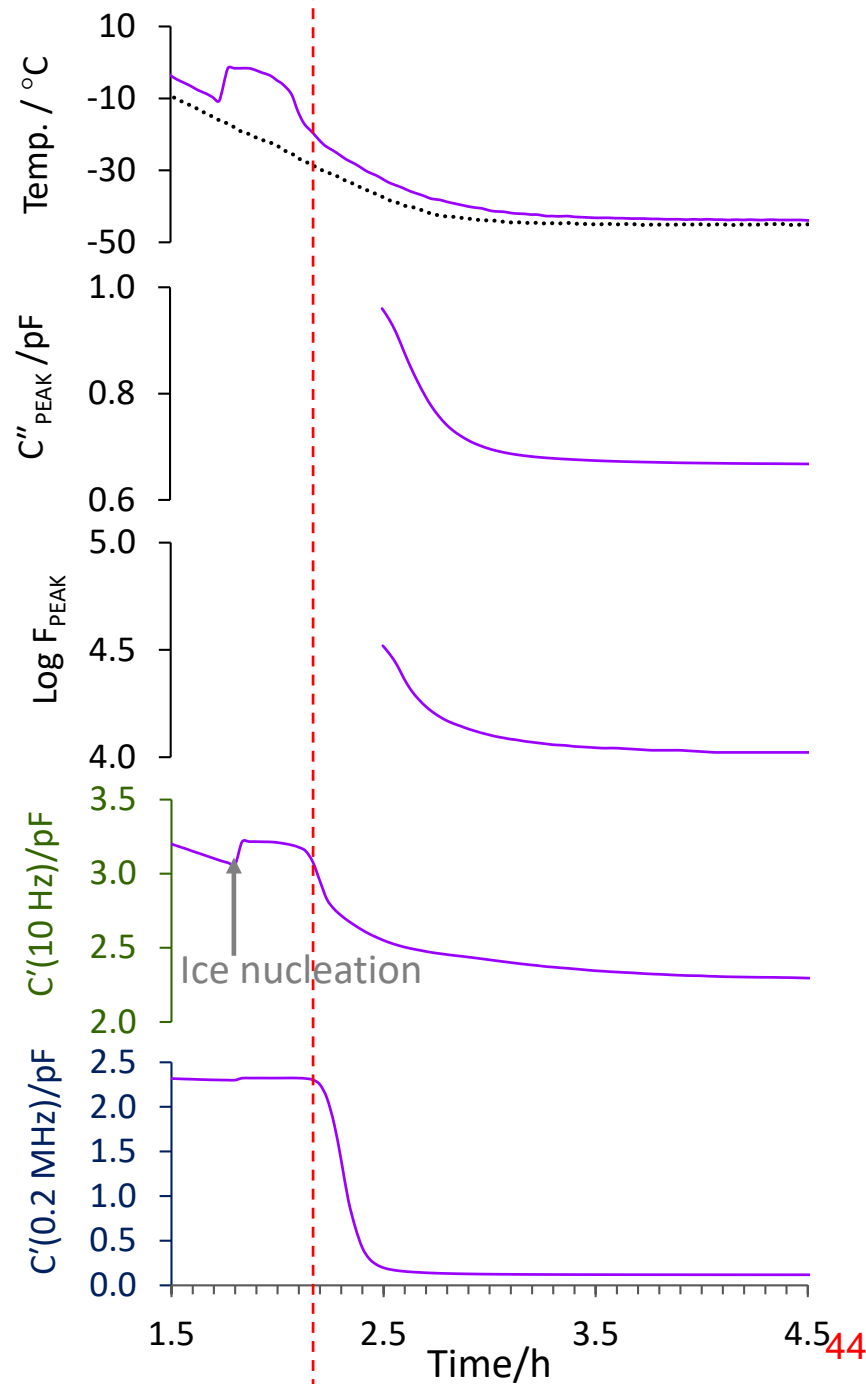
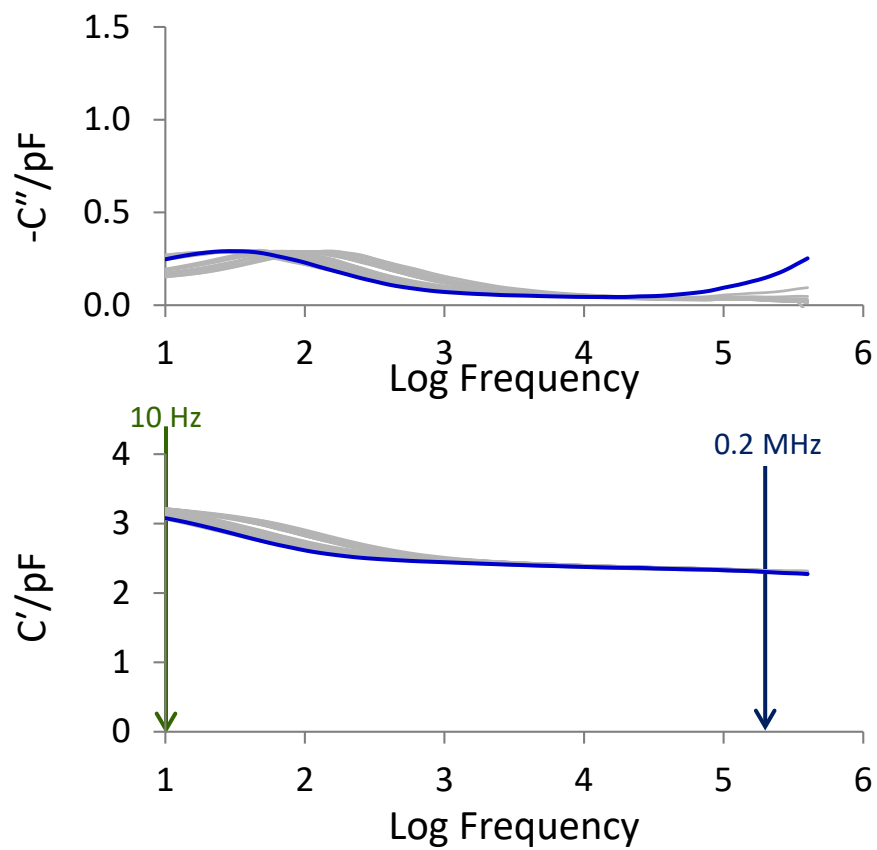
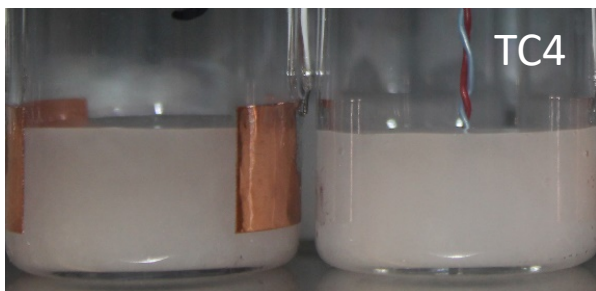
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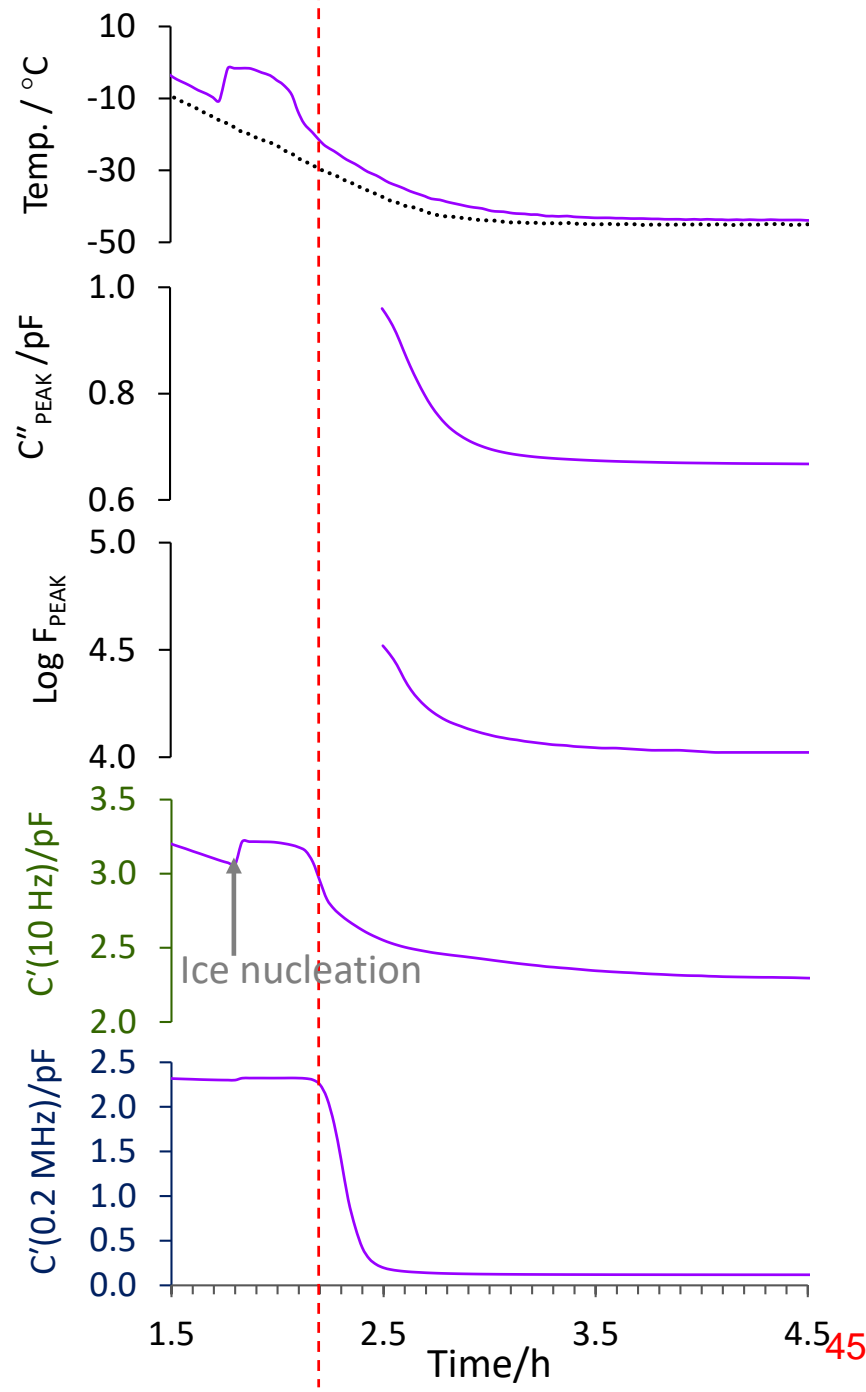
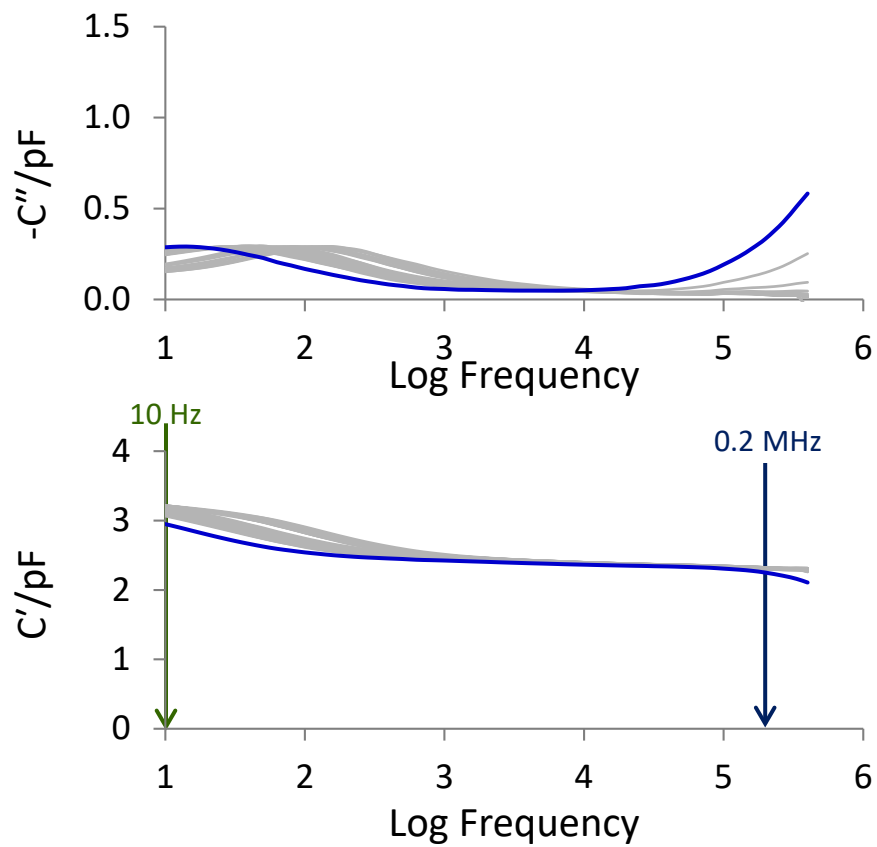
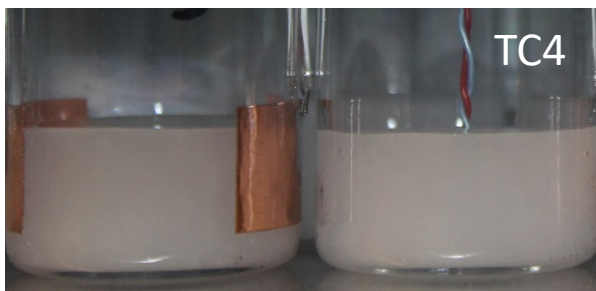
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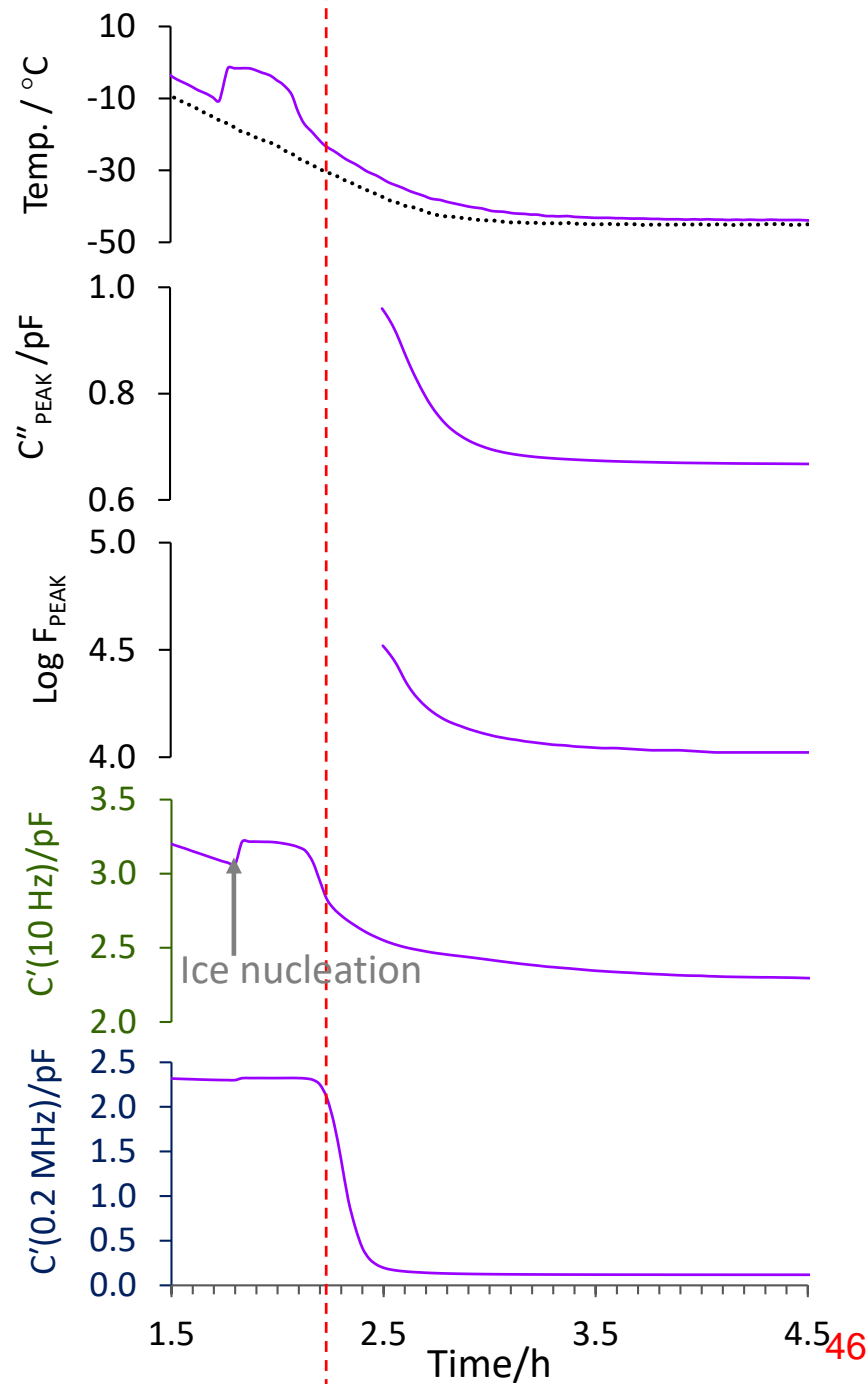
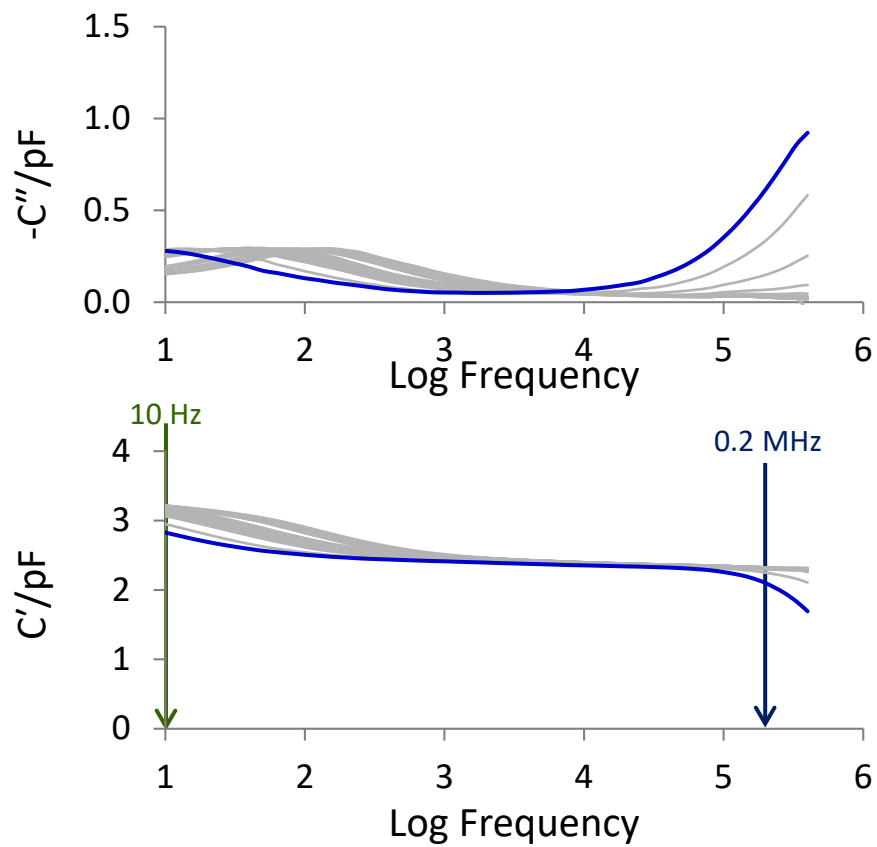
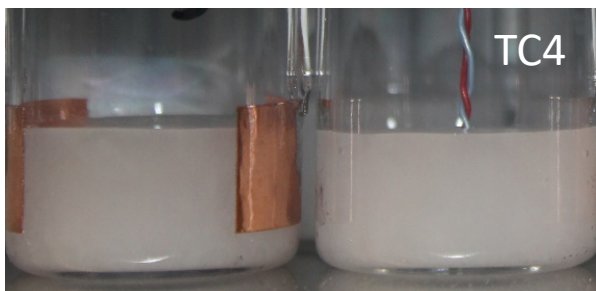
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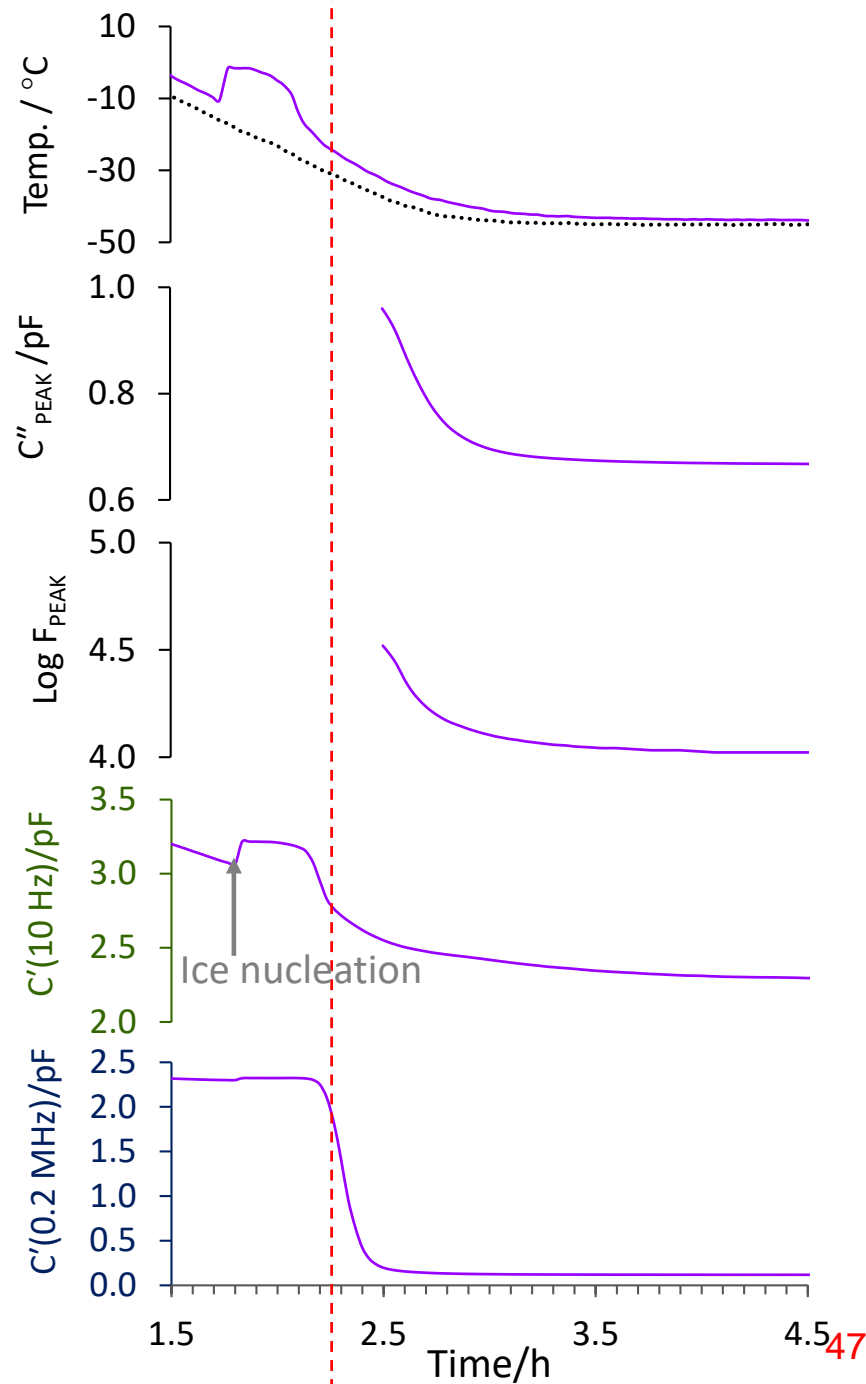
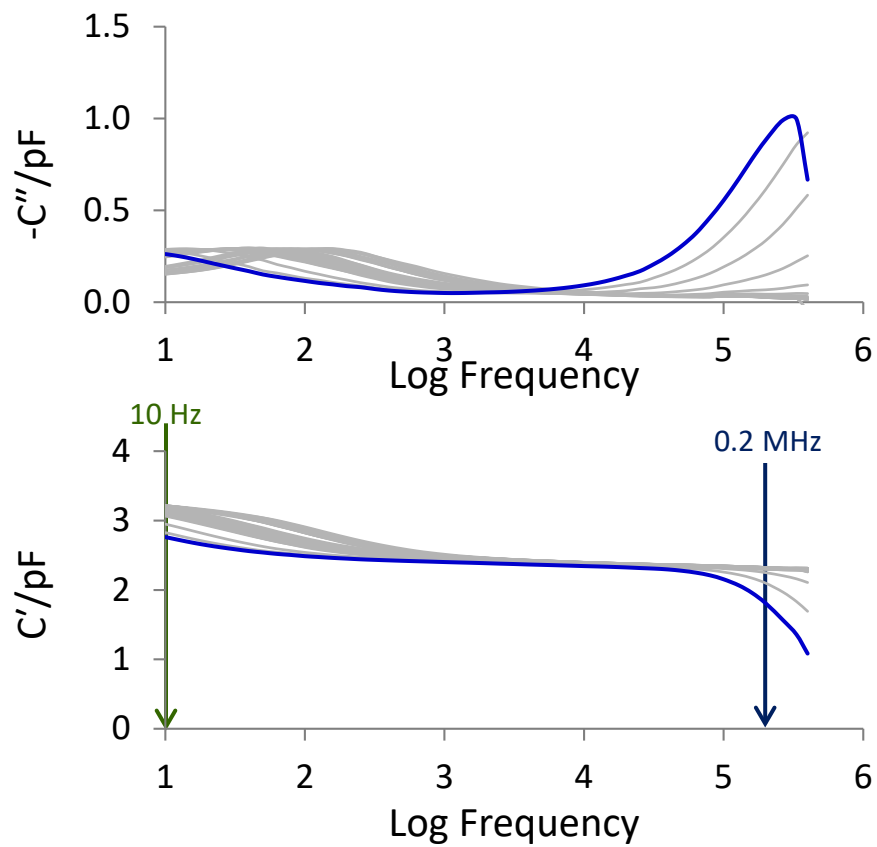
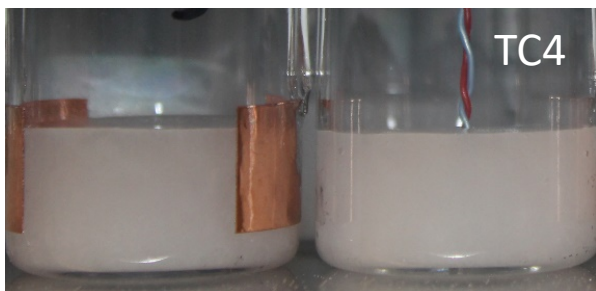
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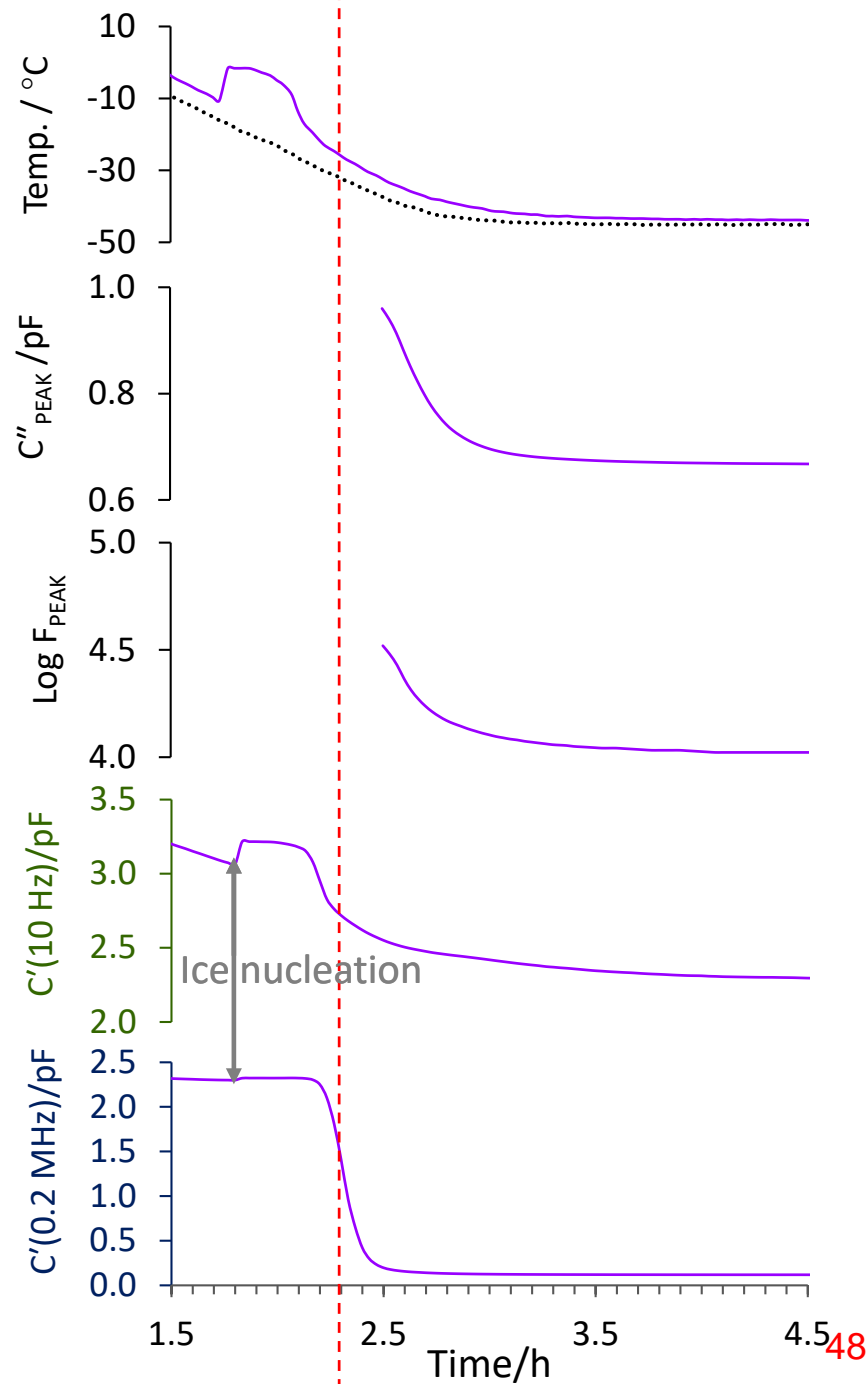
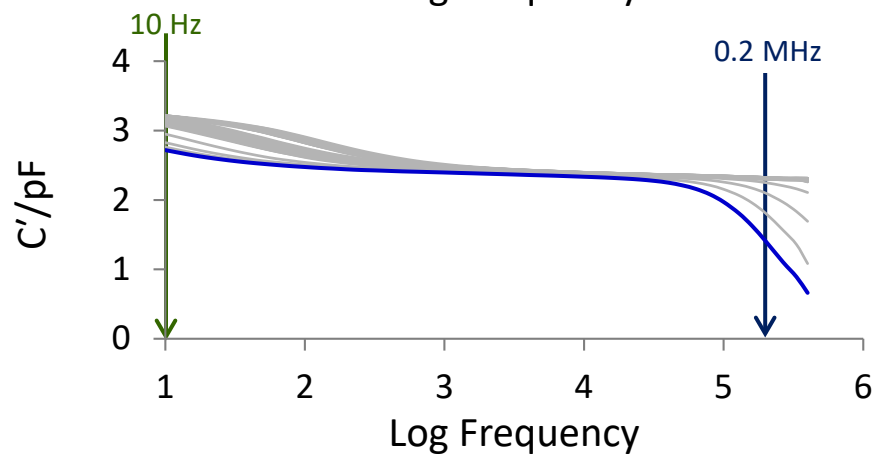
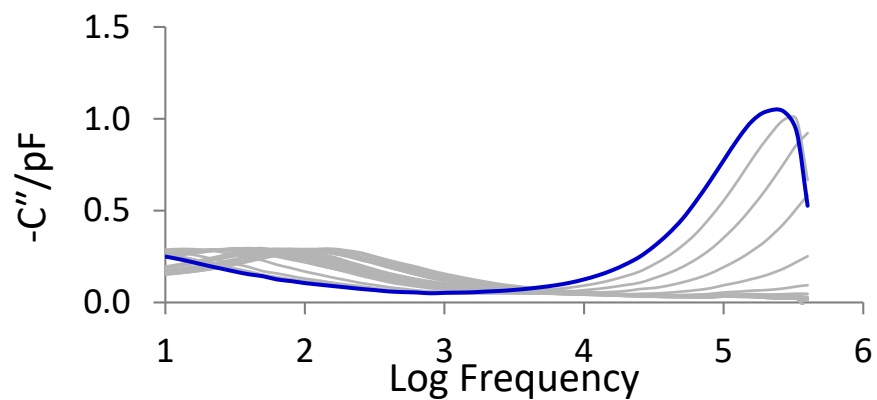
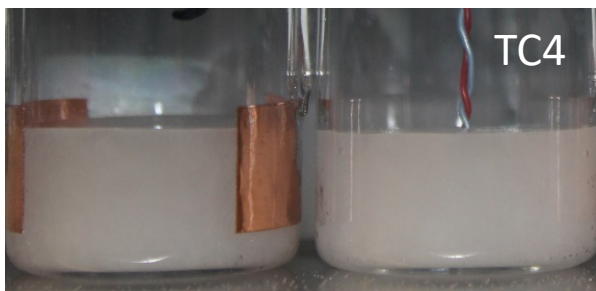
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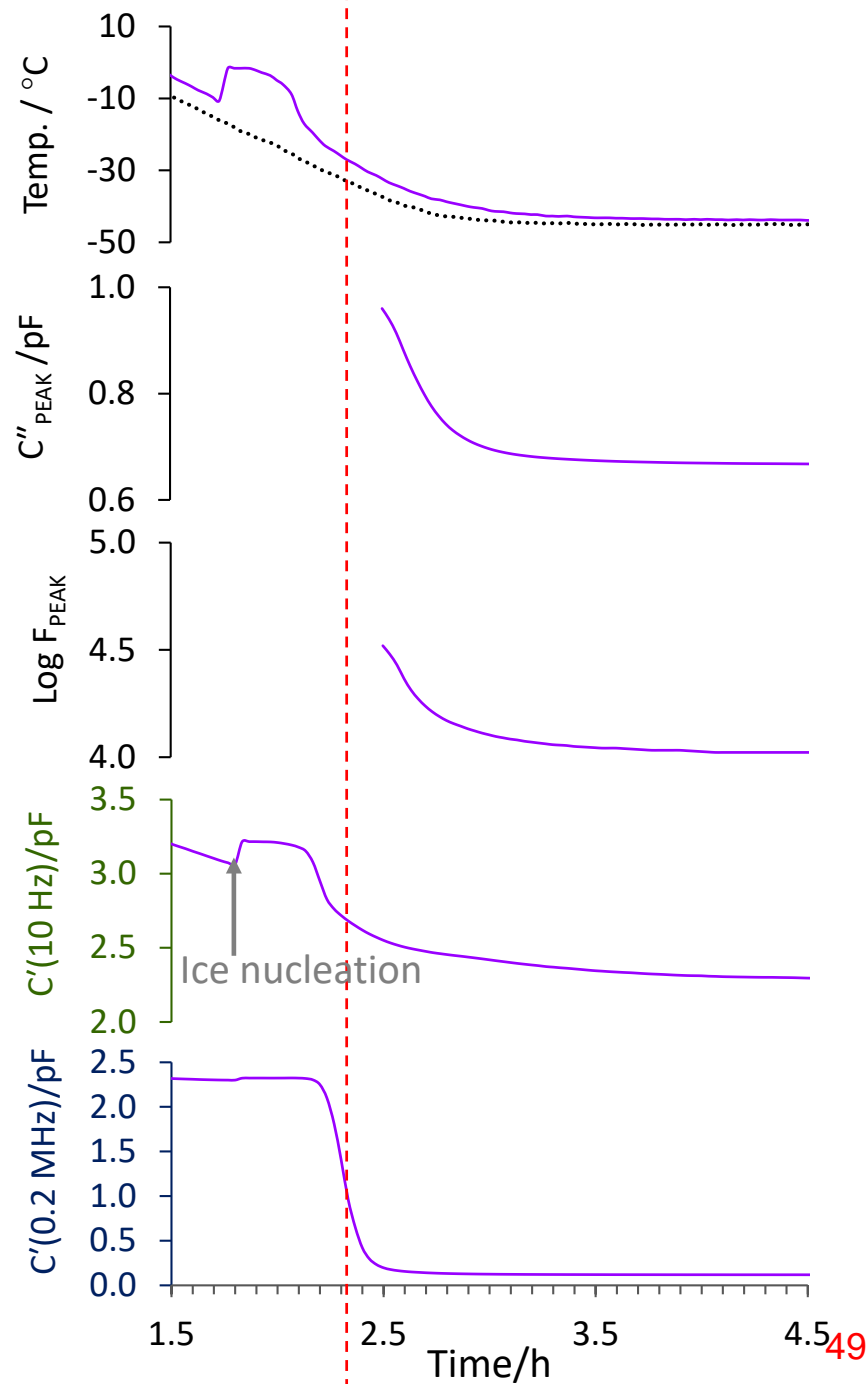
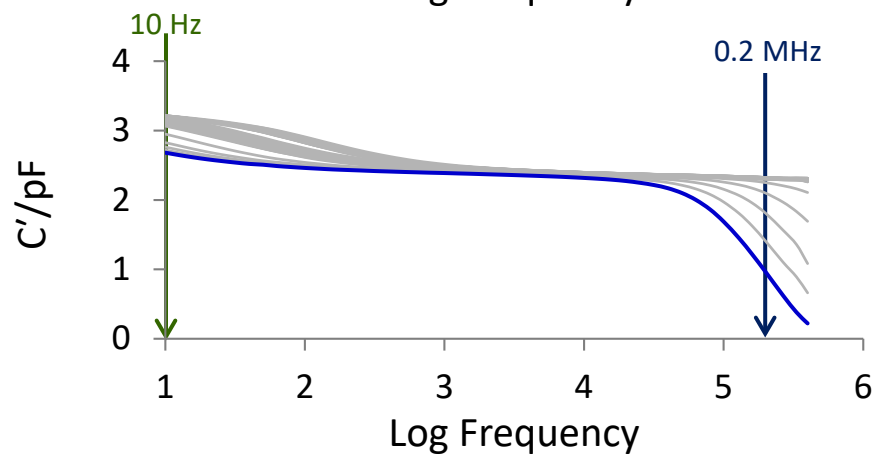
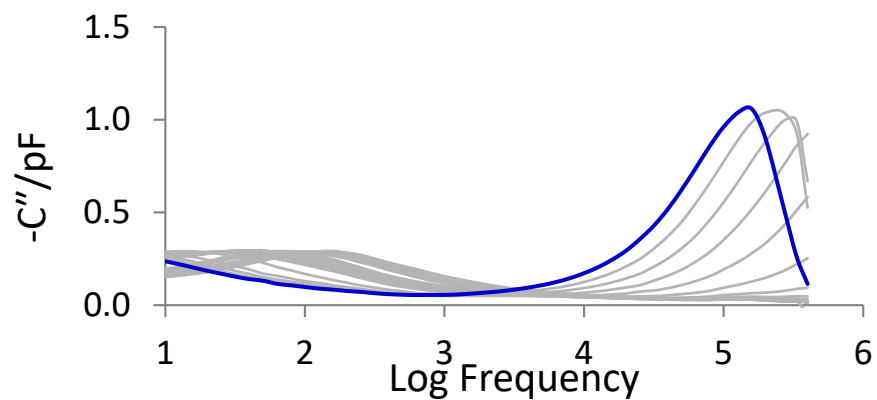
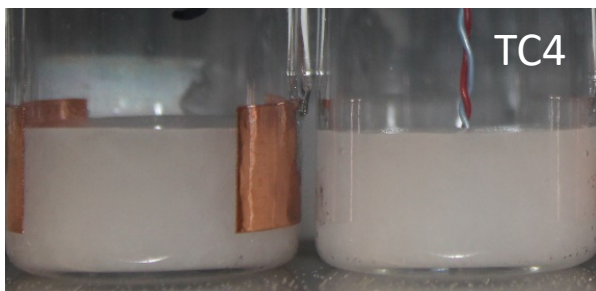
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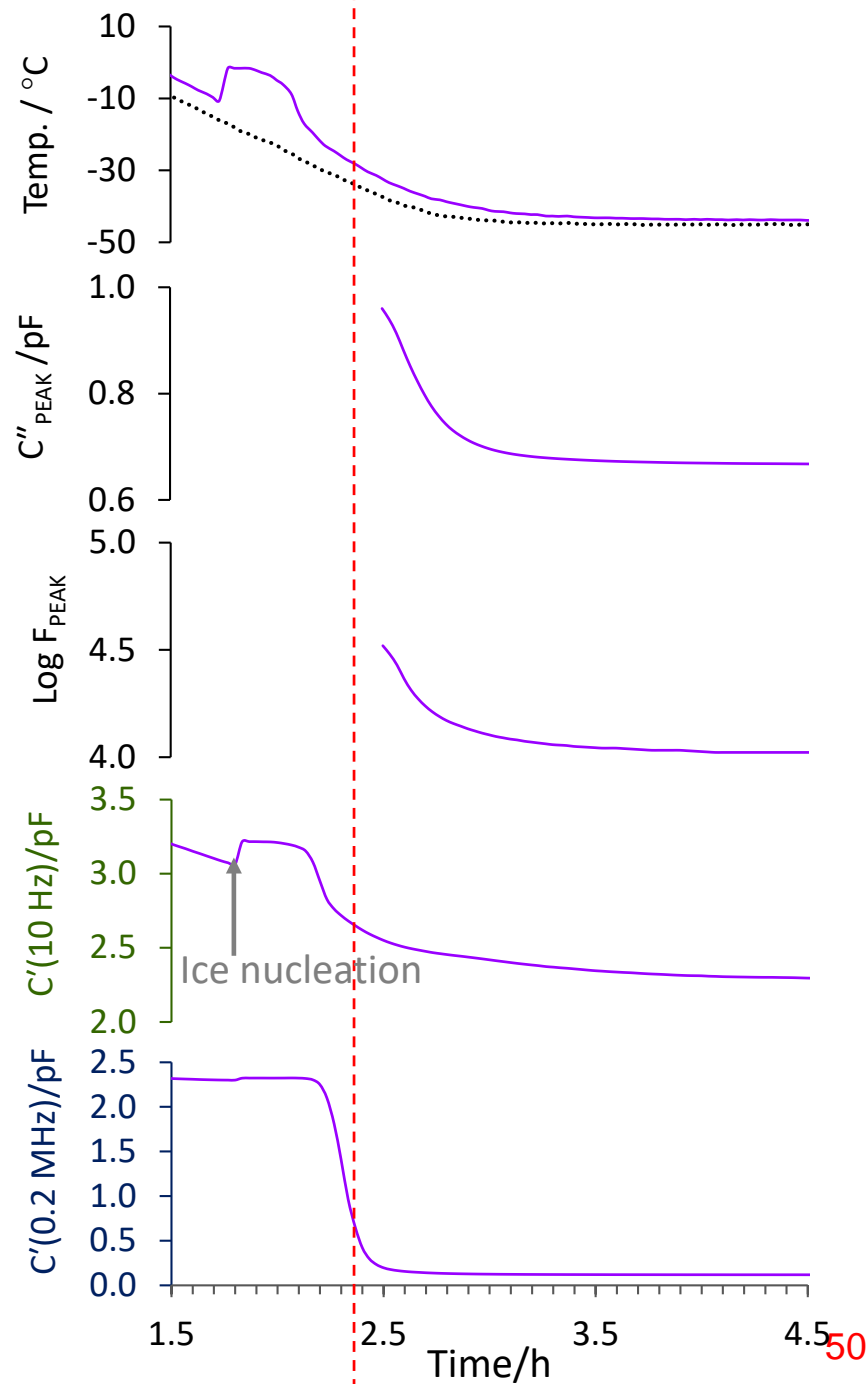
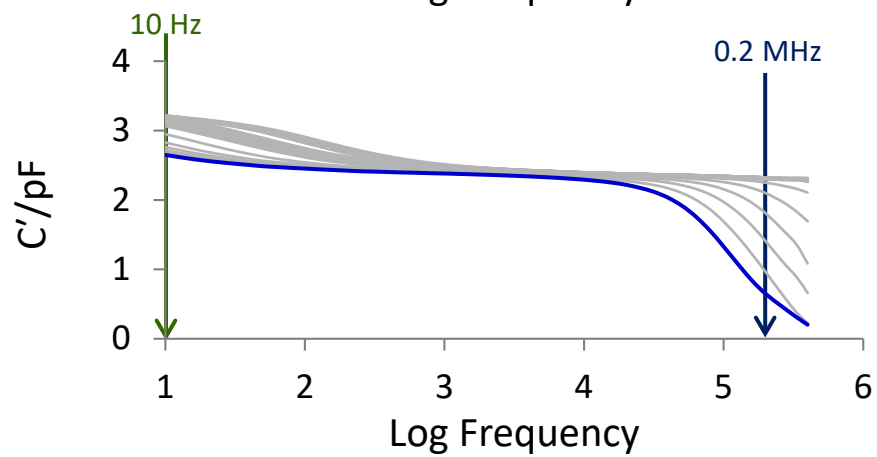
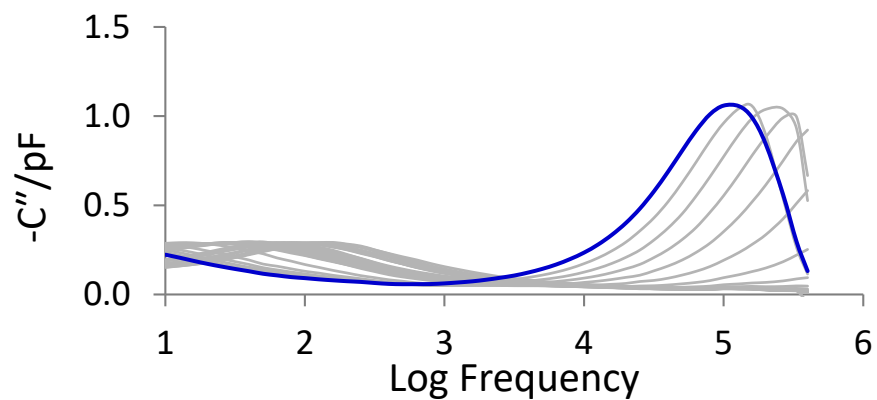
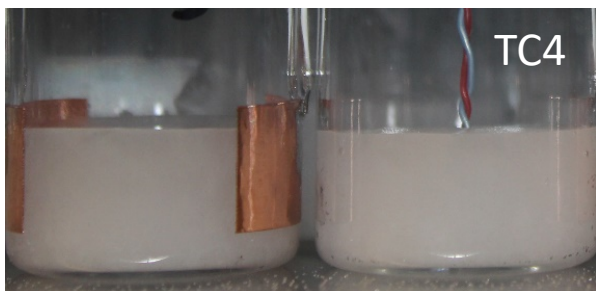
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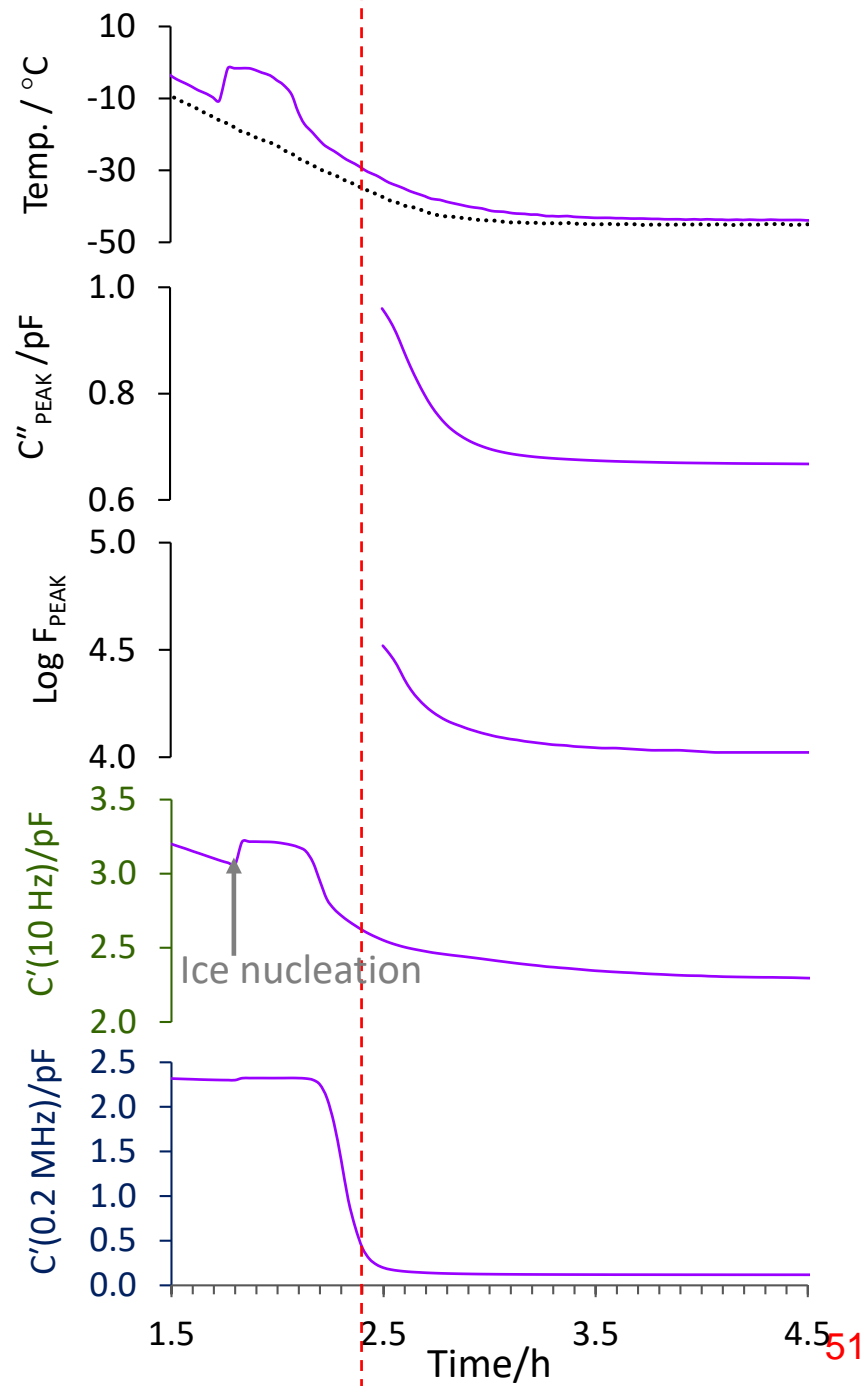
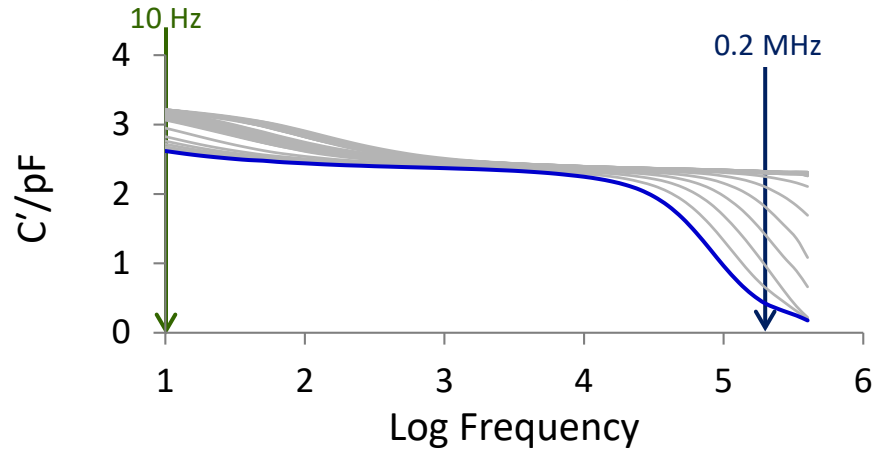
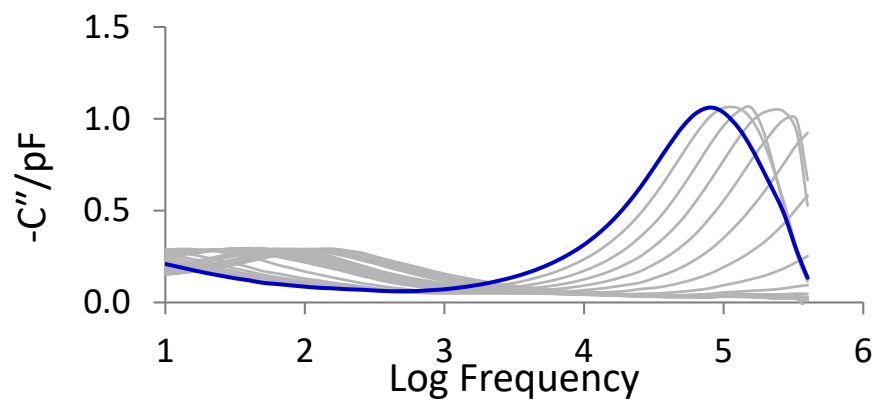
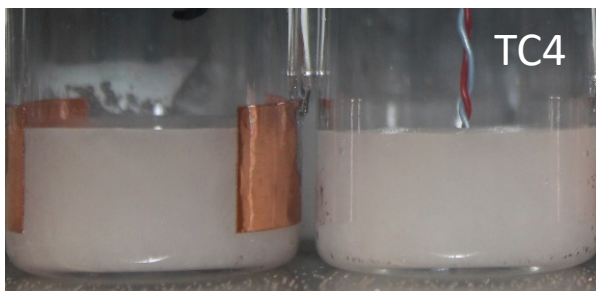
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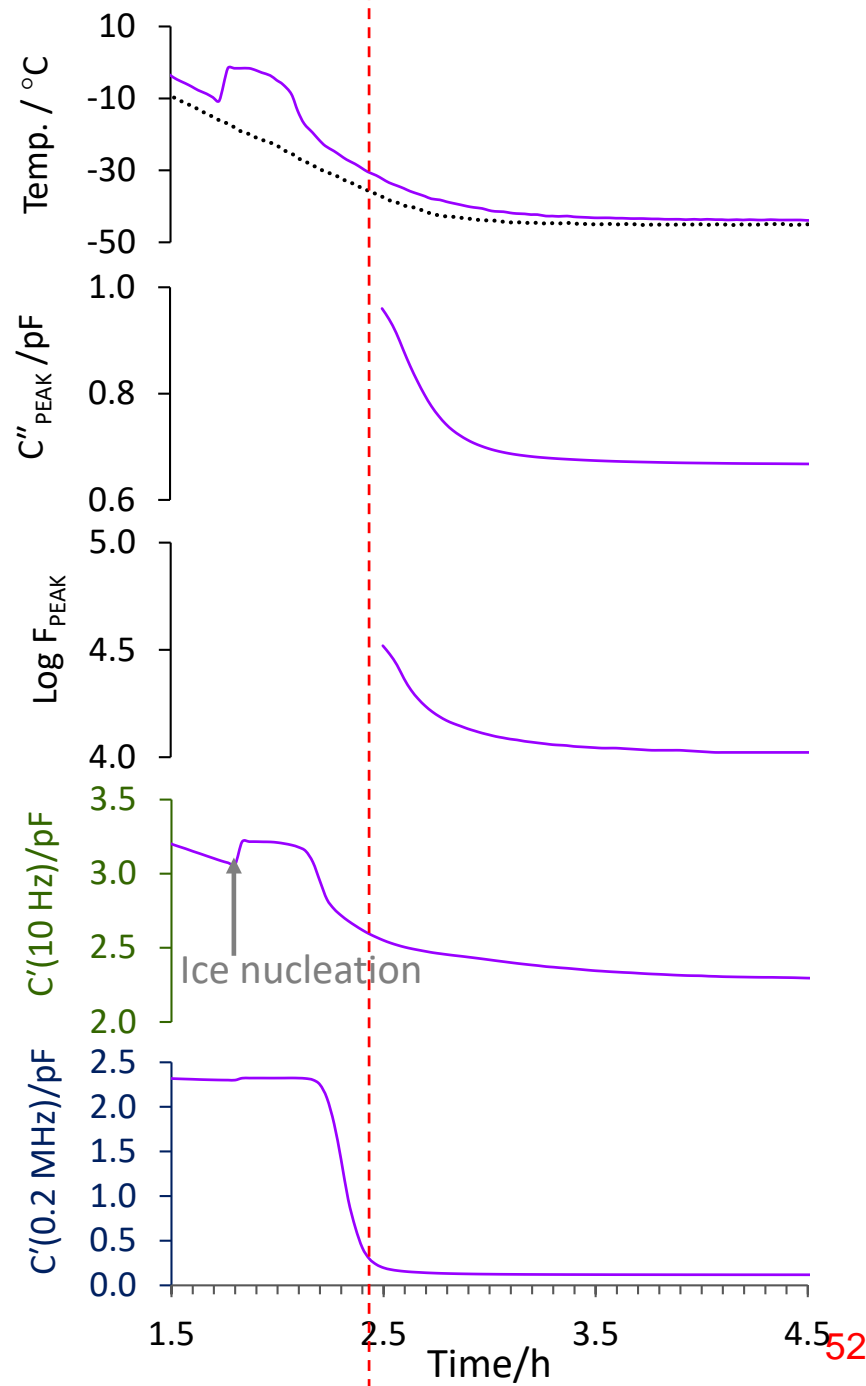
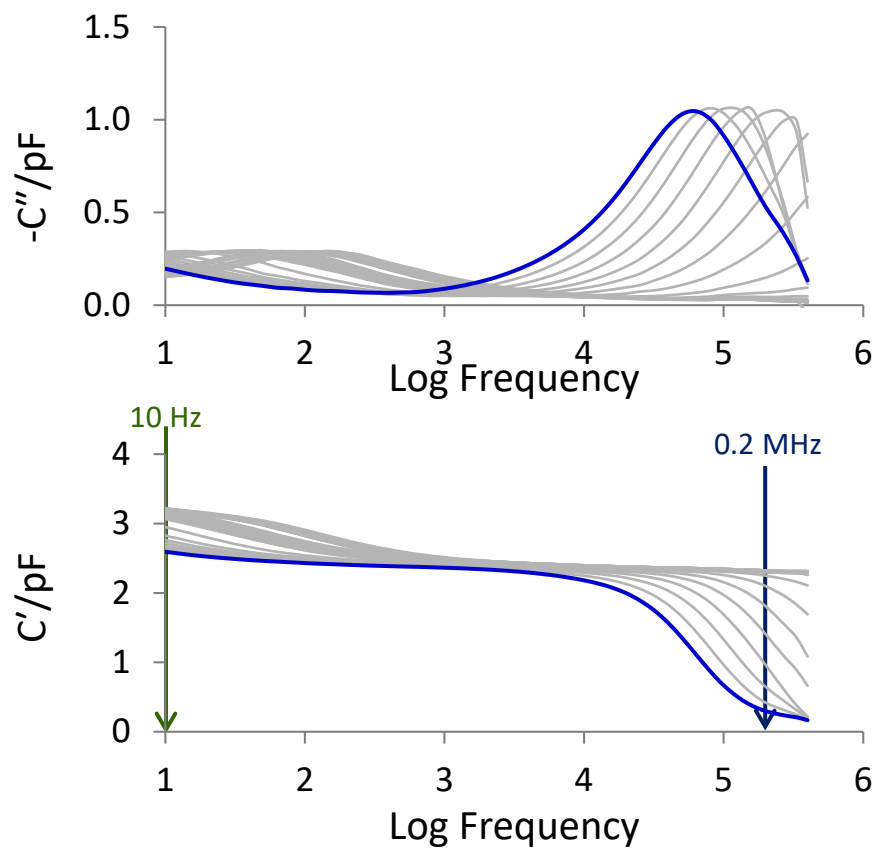
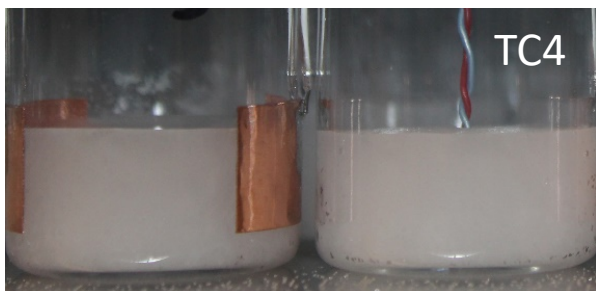
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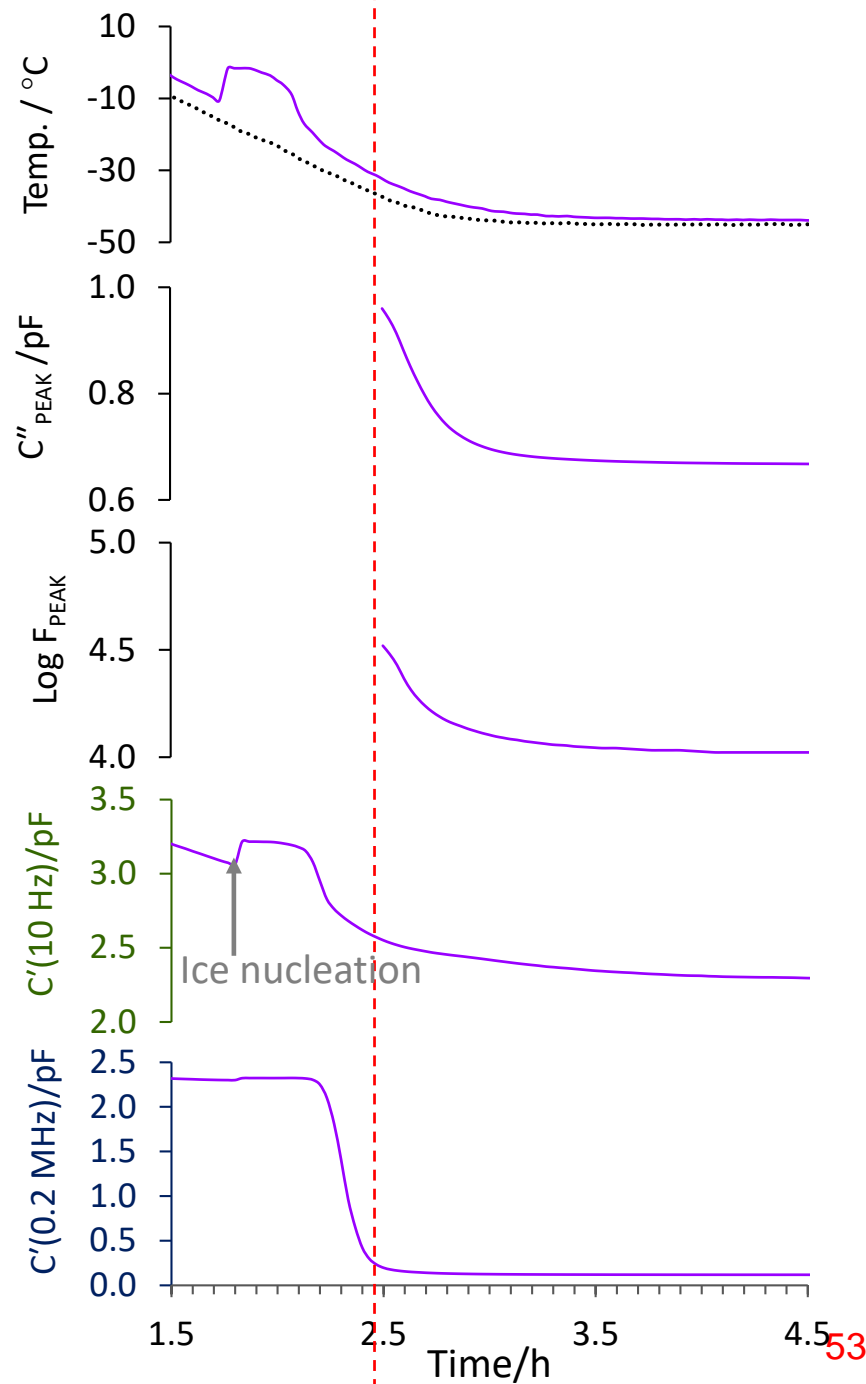
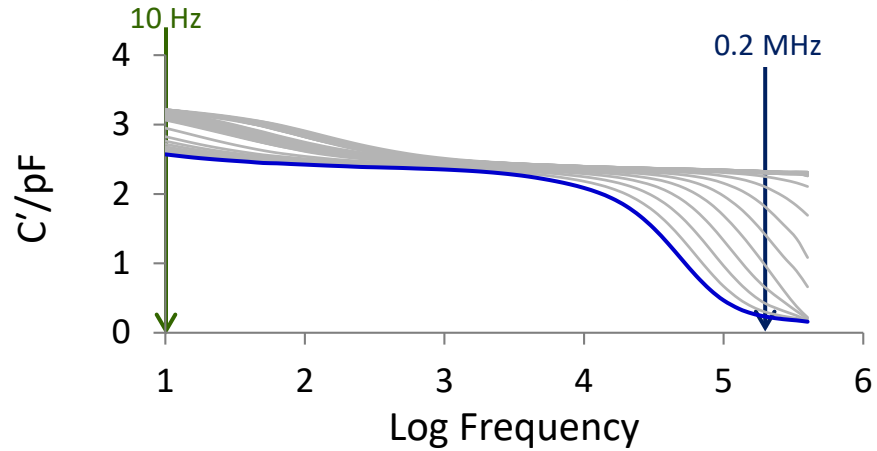
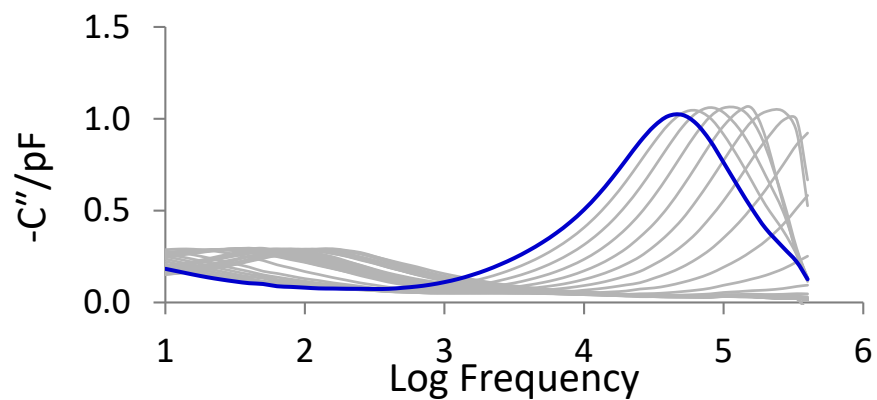
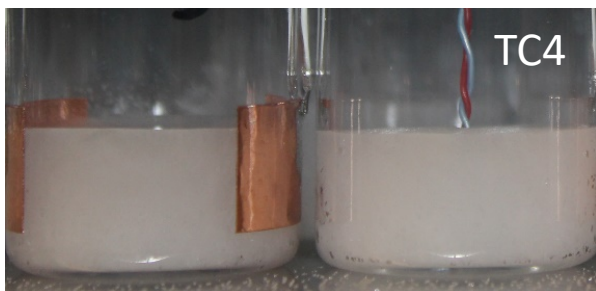
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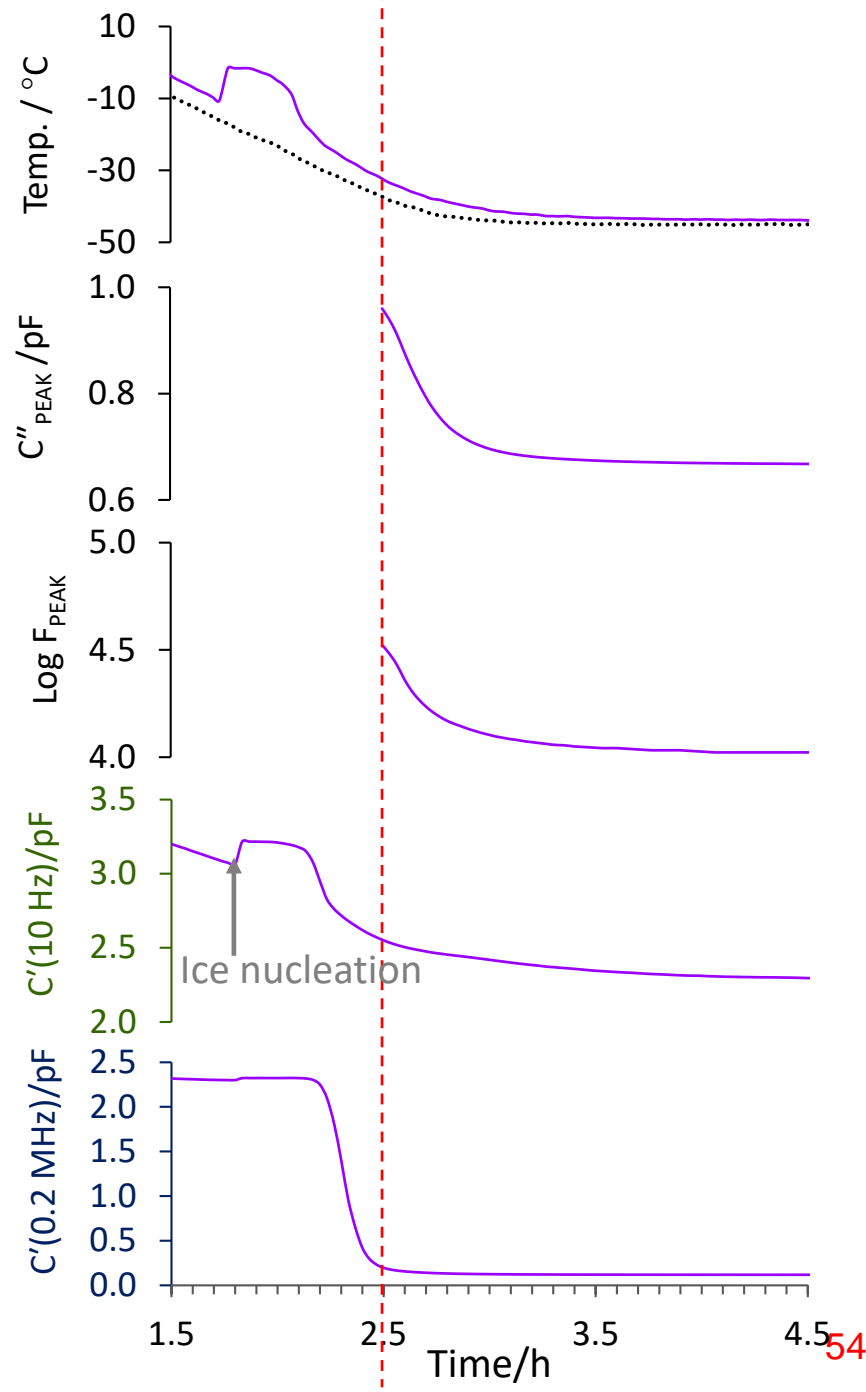
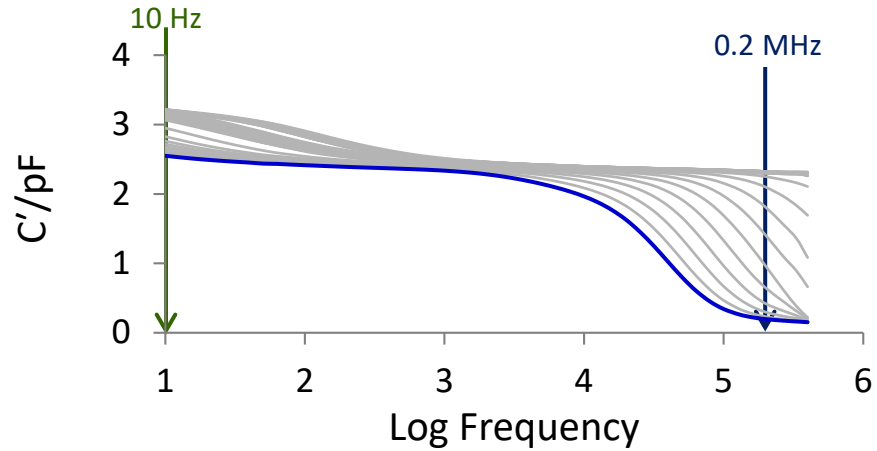
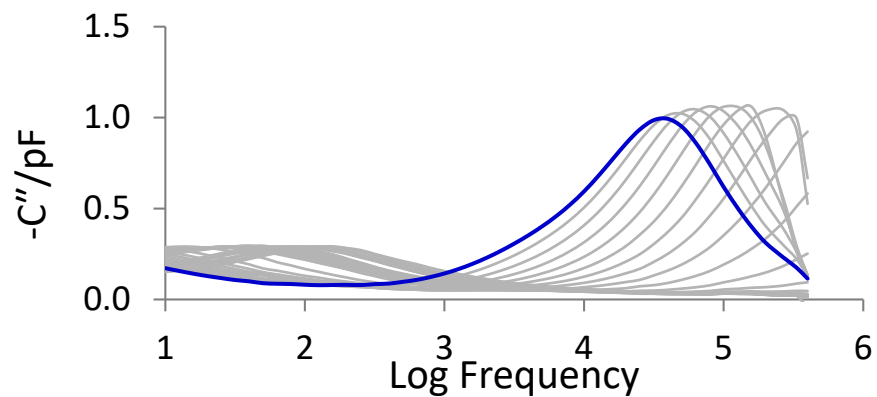
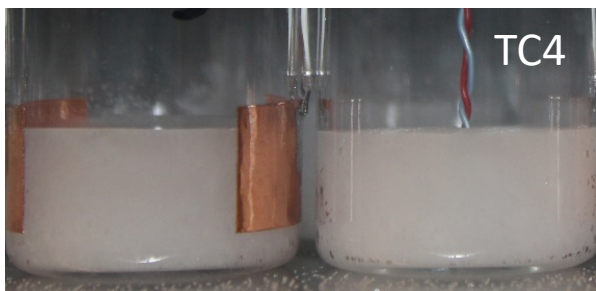
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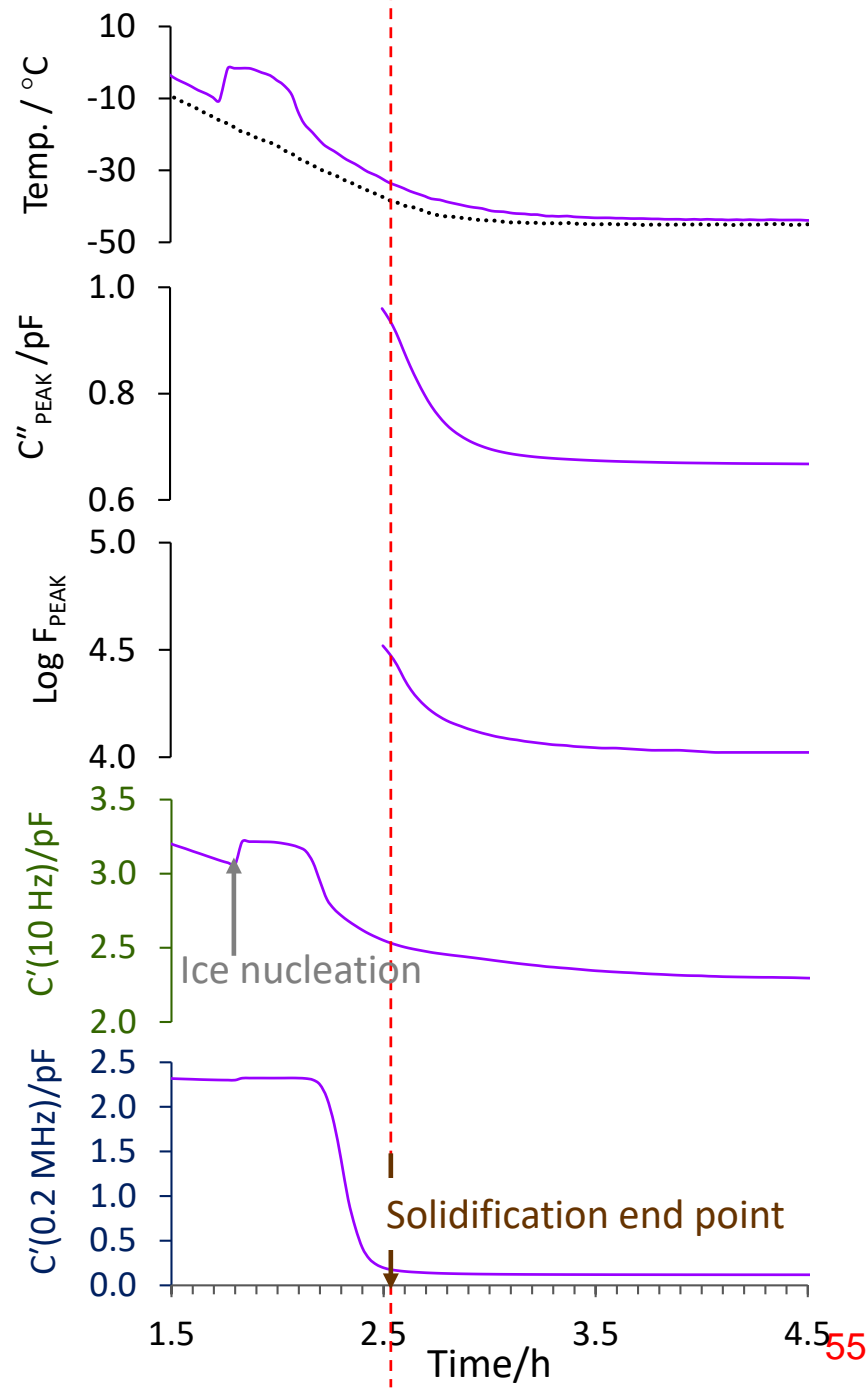
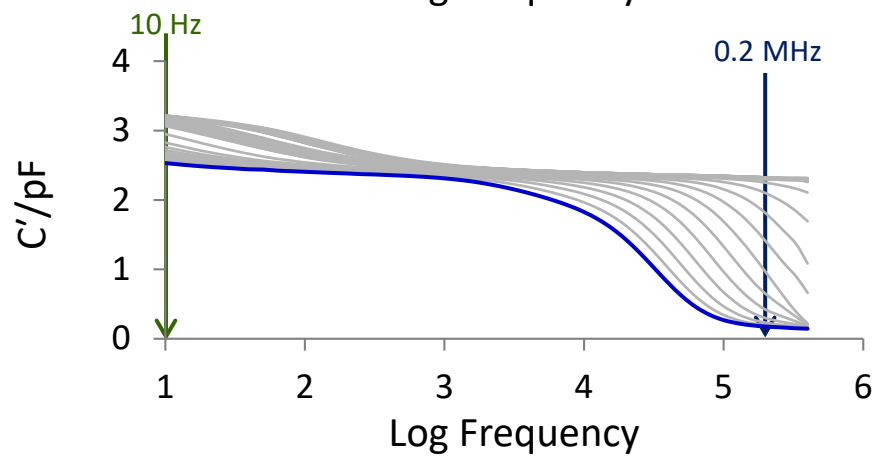
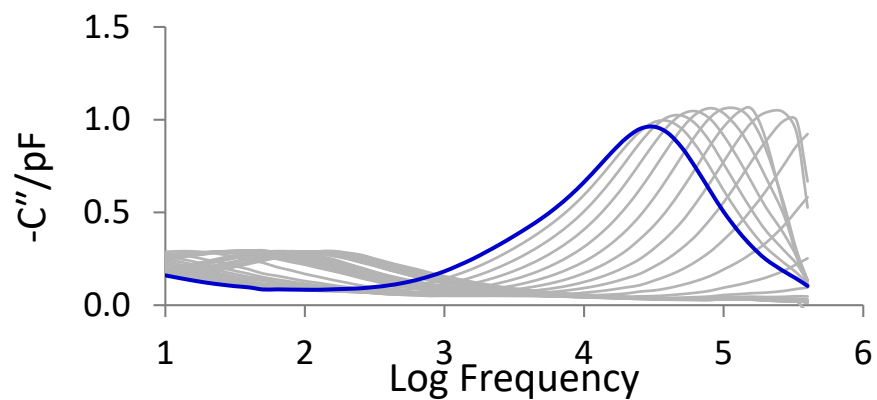
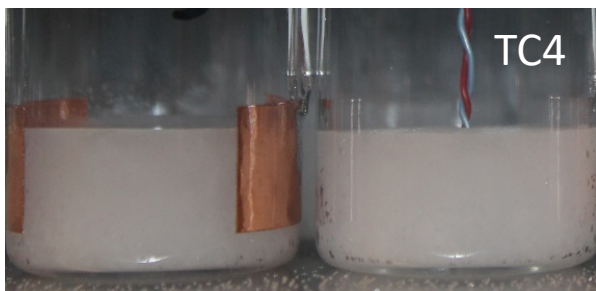
Freezing Step - 5%Sucrose+0.55%NaCl



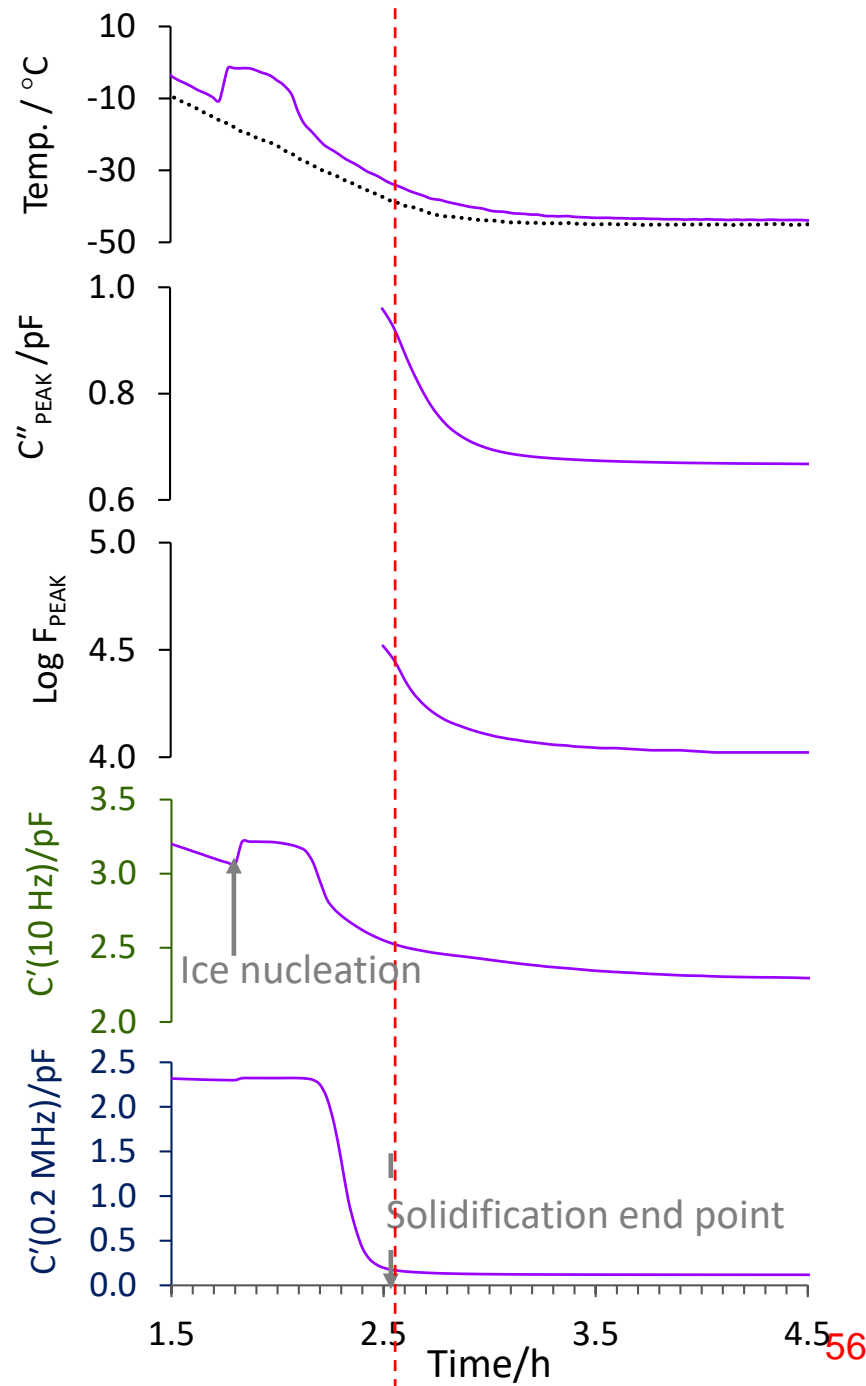
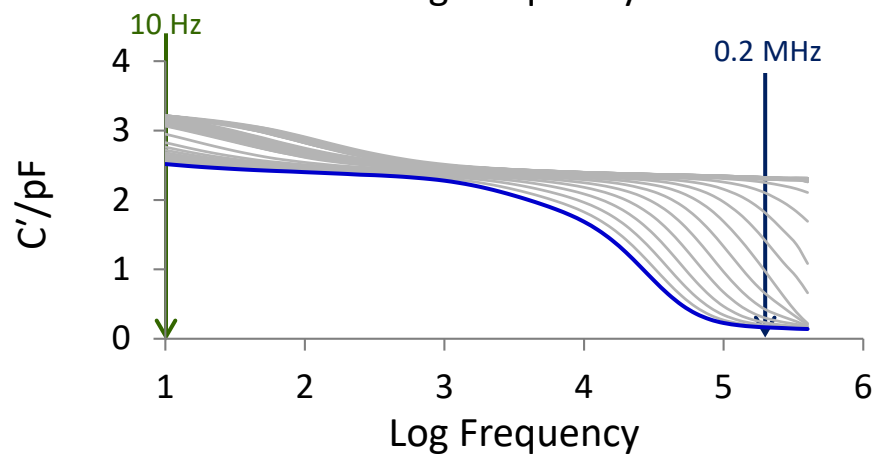
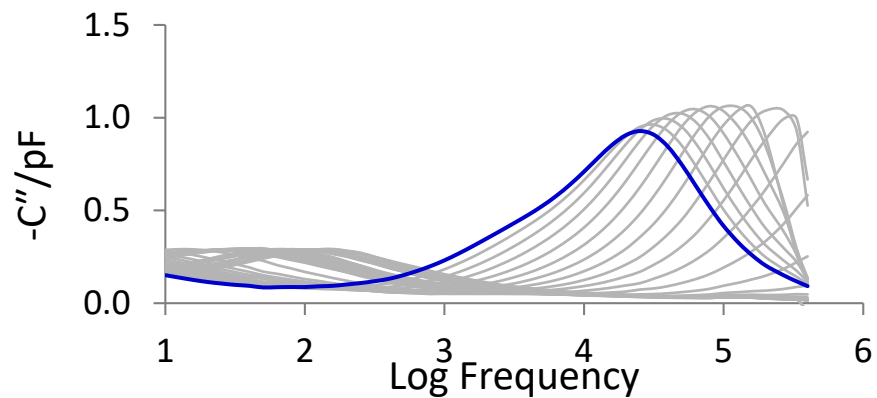
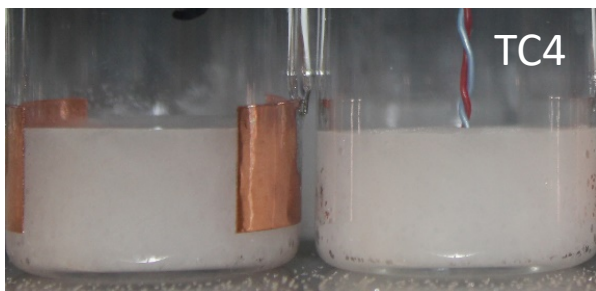
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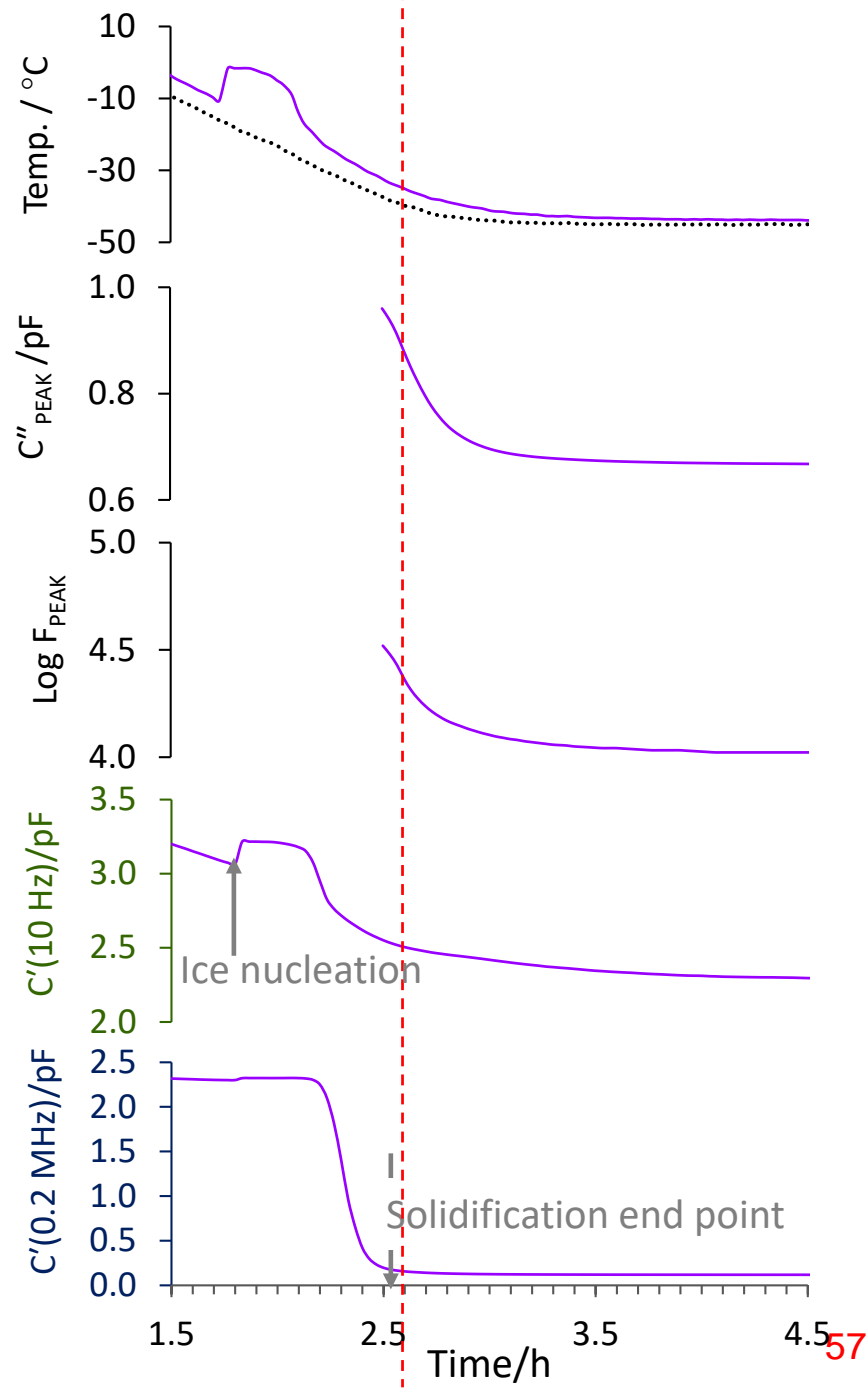
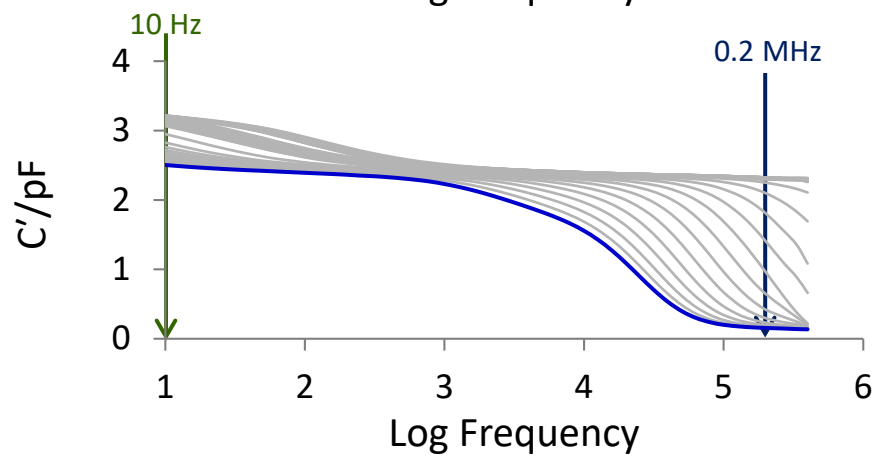
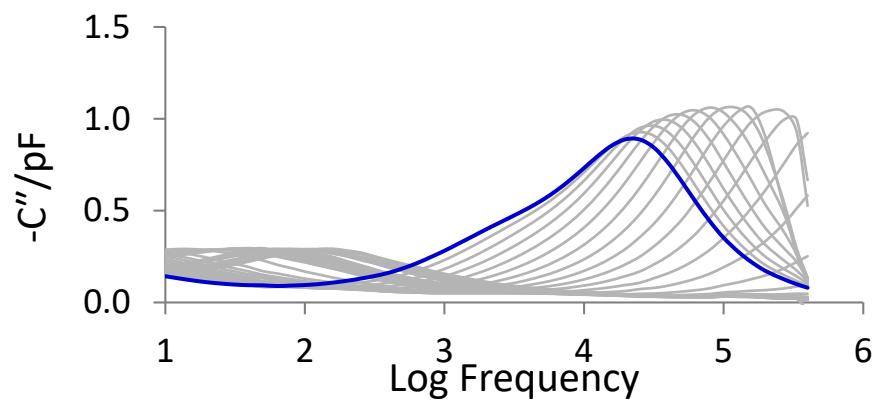
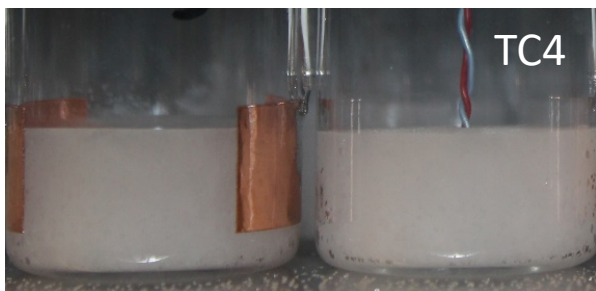
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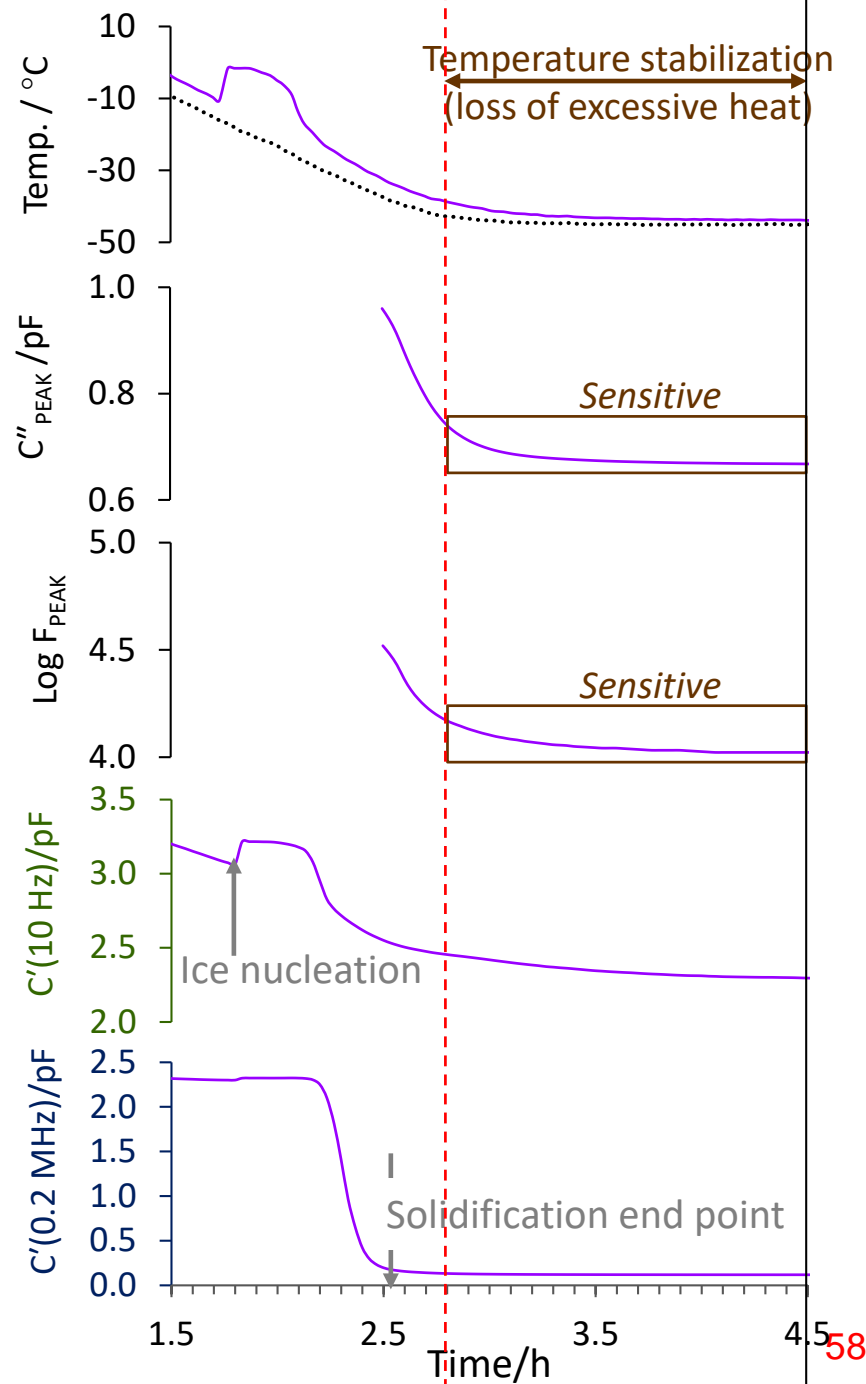
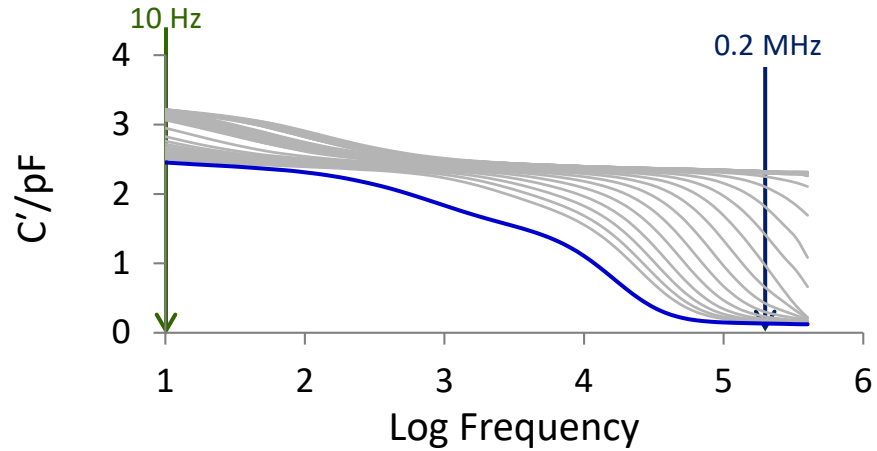
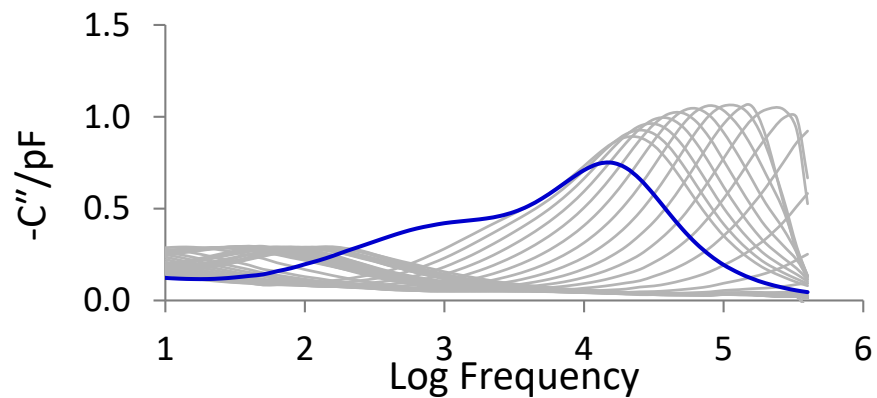
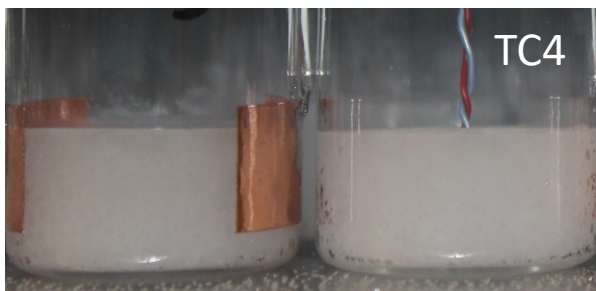
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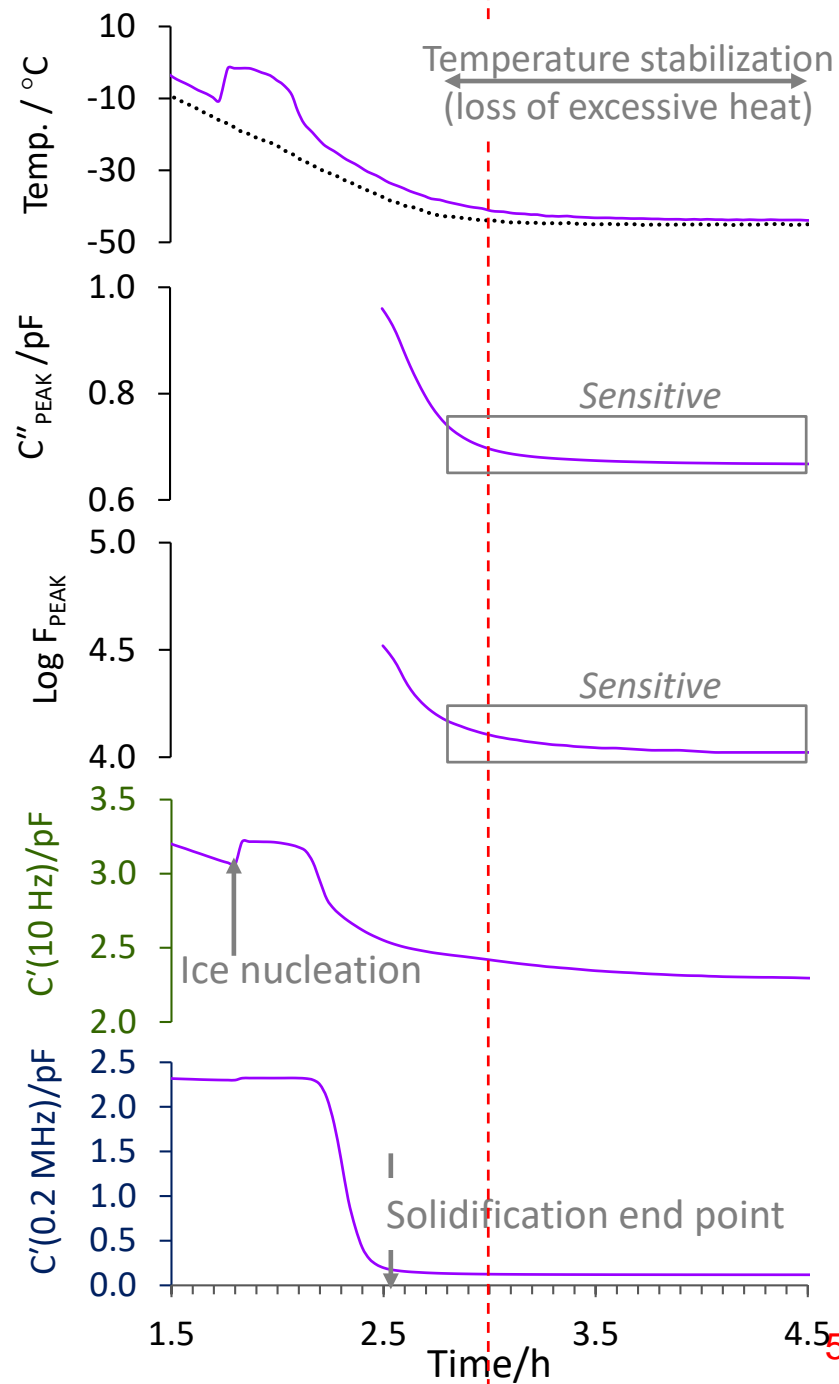
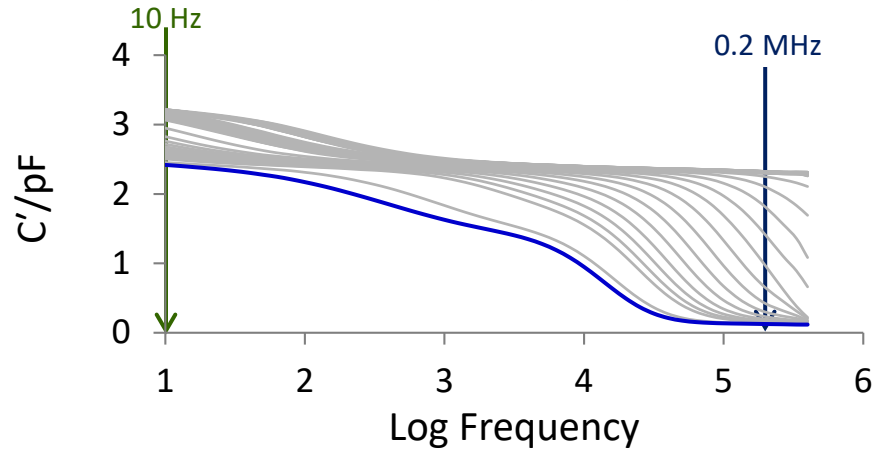
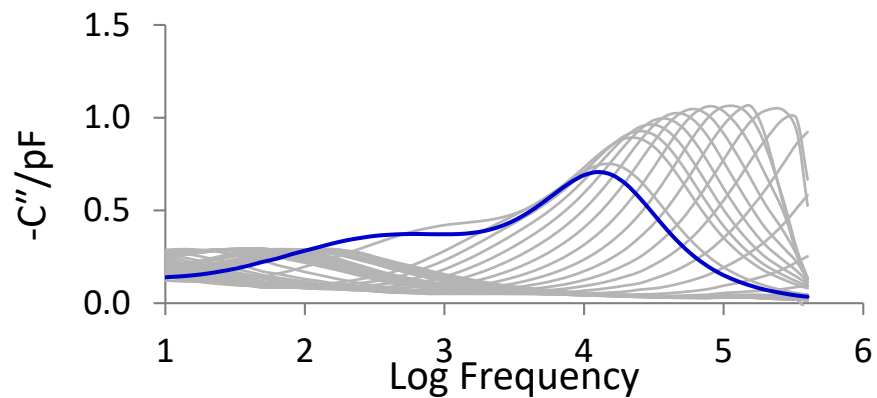
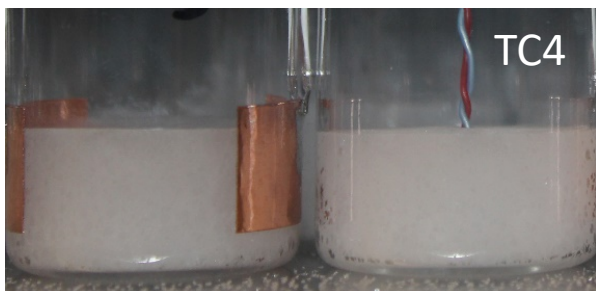
Freezing Step - 5%Sucrose+0.55%NaCl



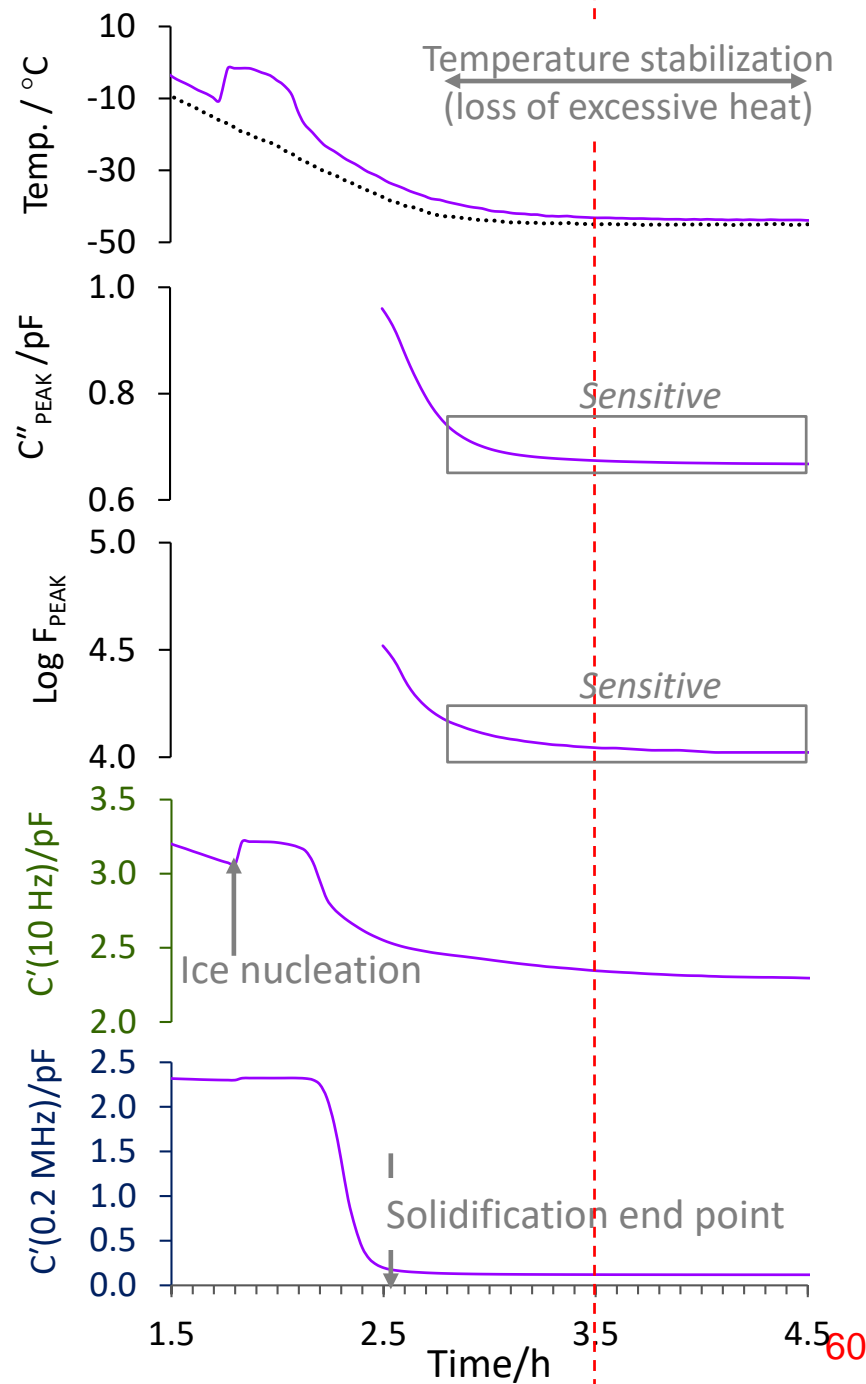
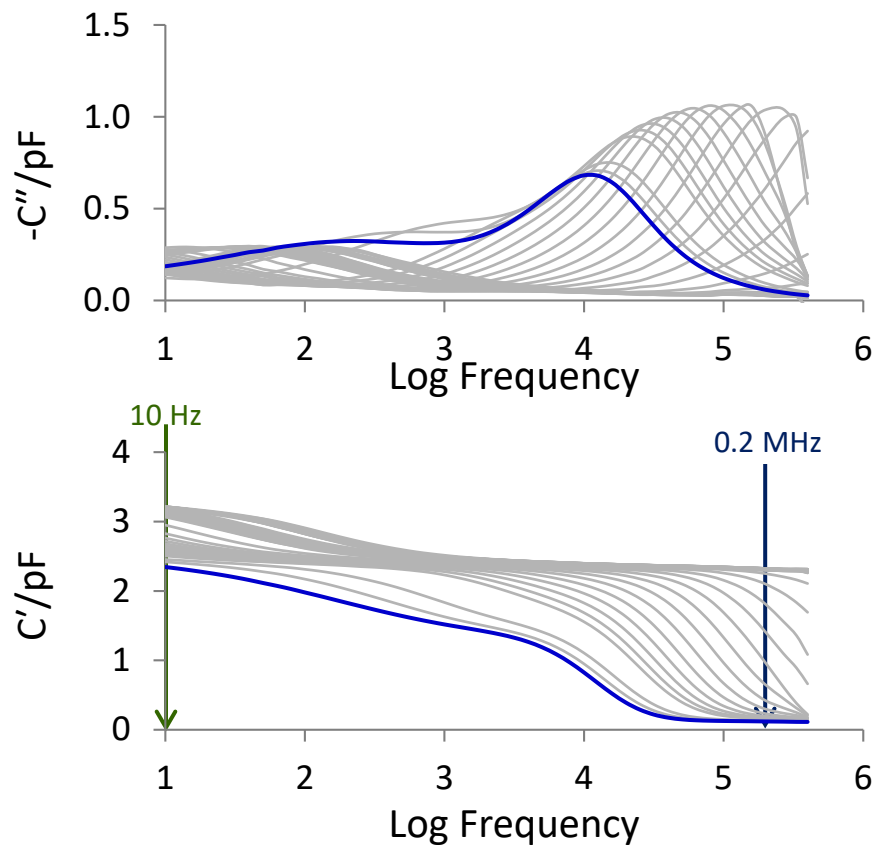
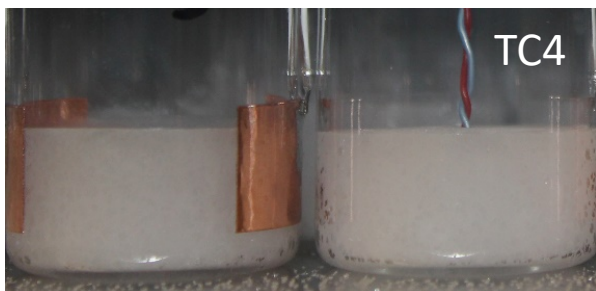
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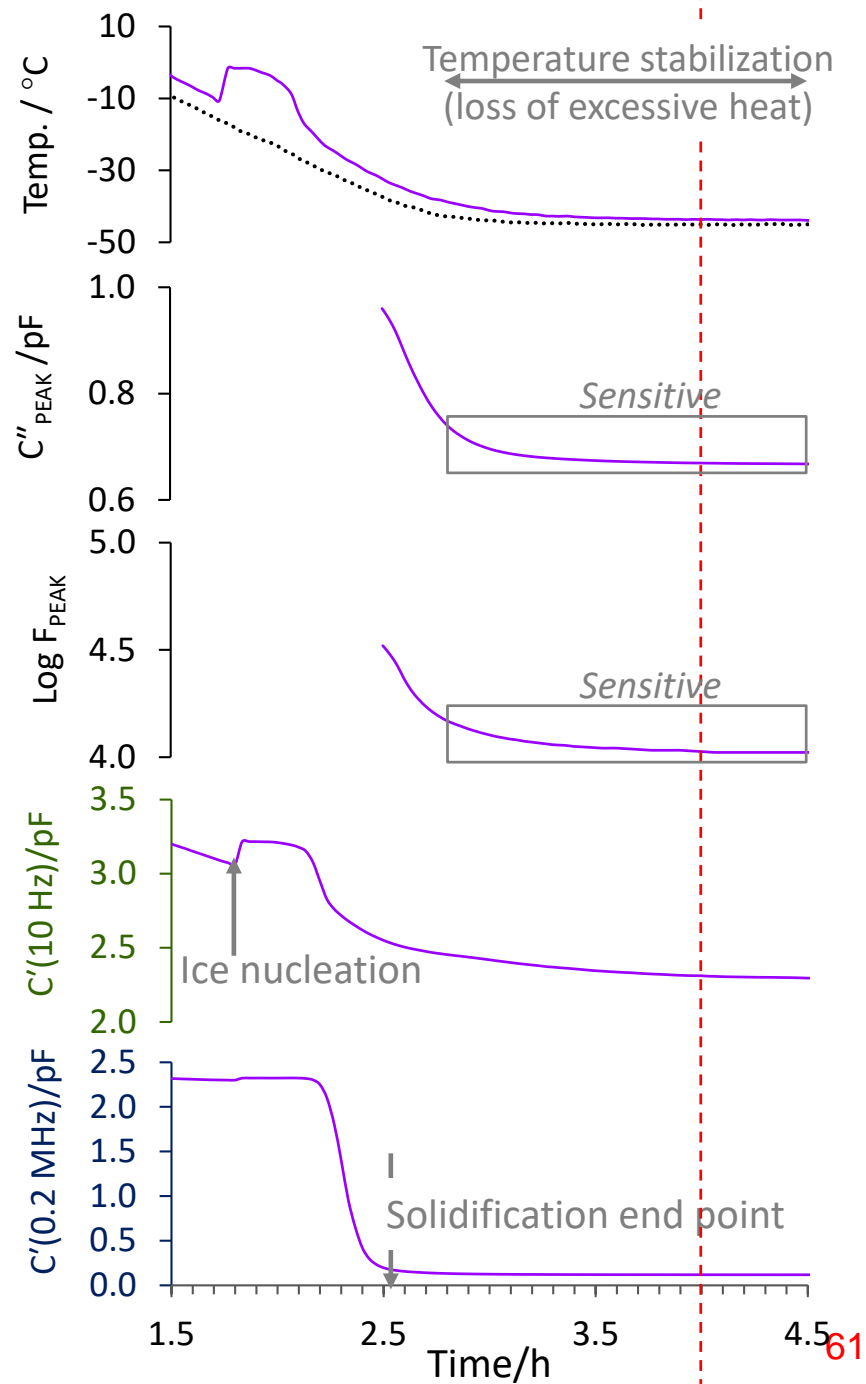
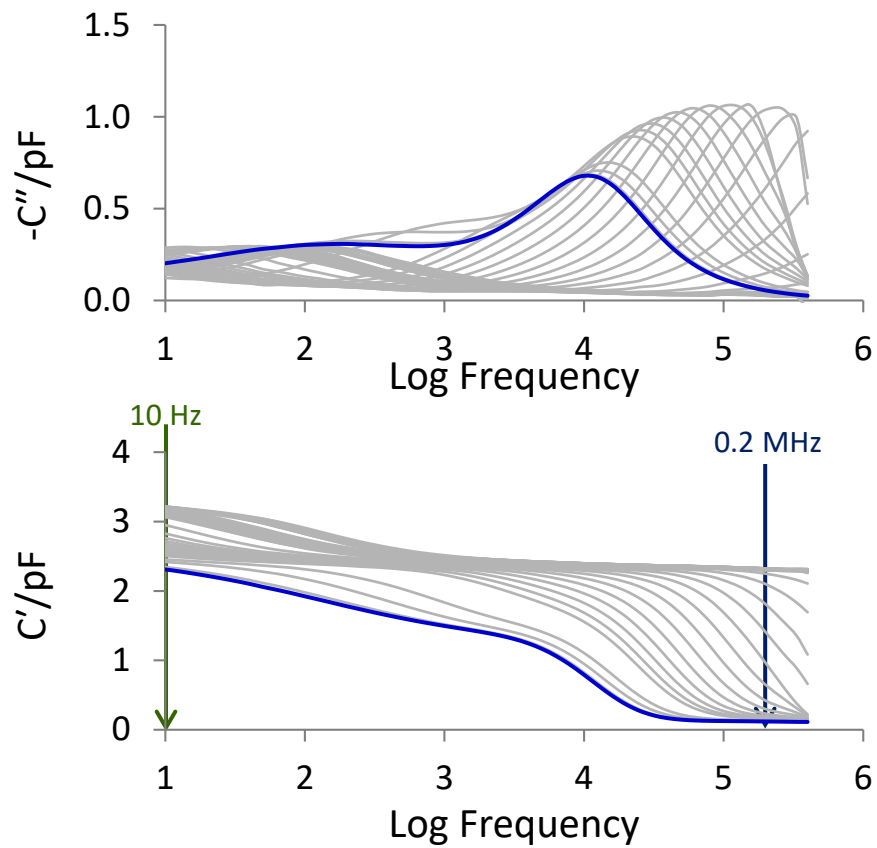
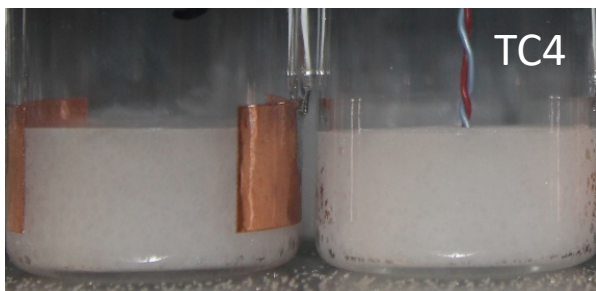
Freezing Step - 5%Sucrose+0.55%NaCl



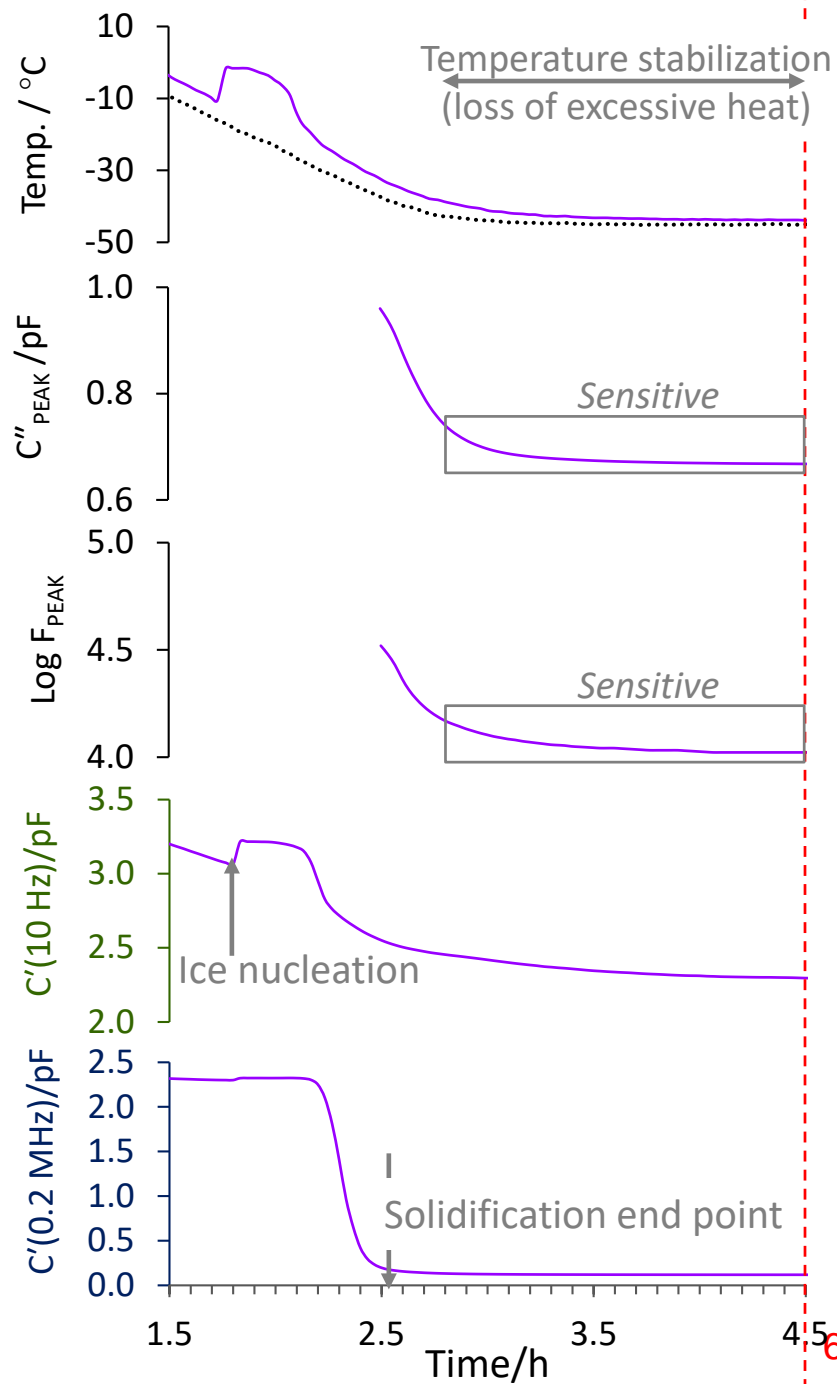
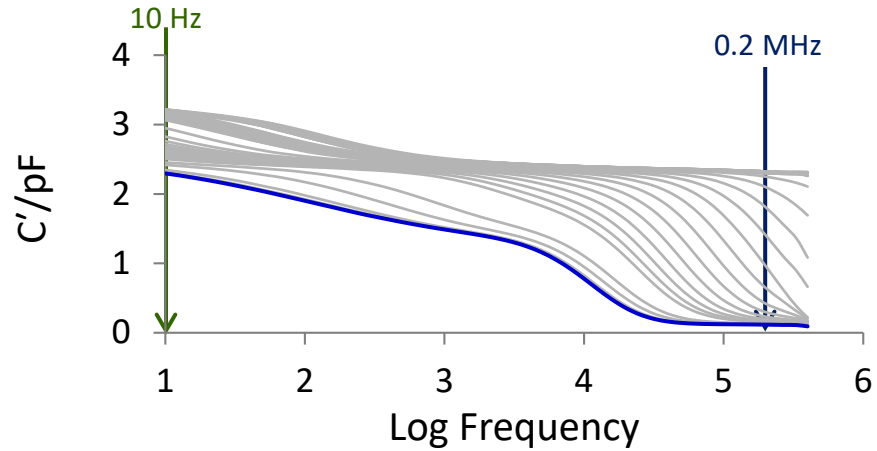
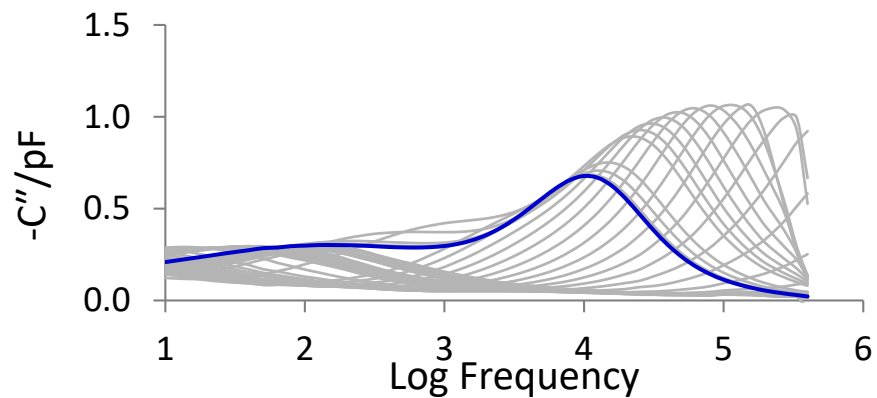
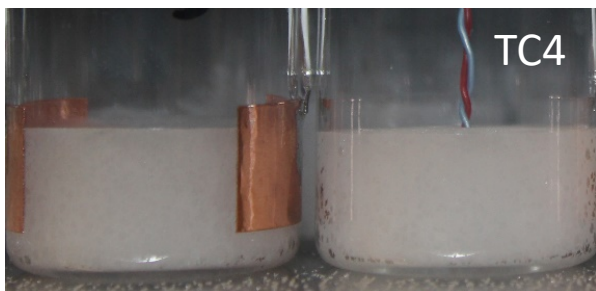
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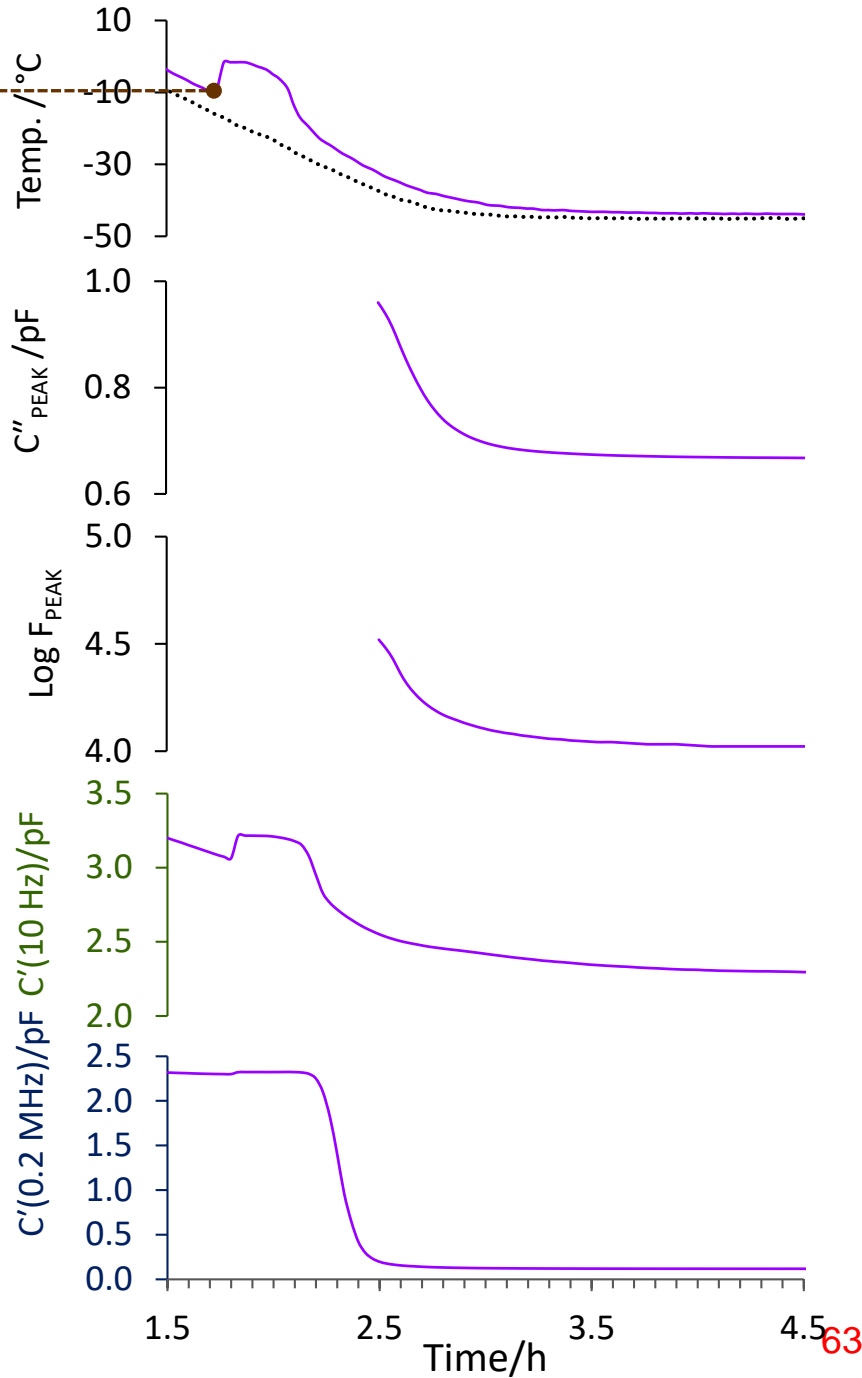


Freezing Step - 5%Sucrose+0.55%NaCl



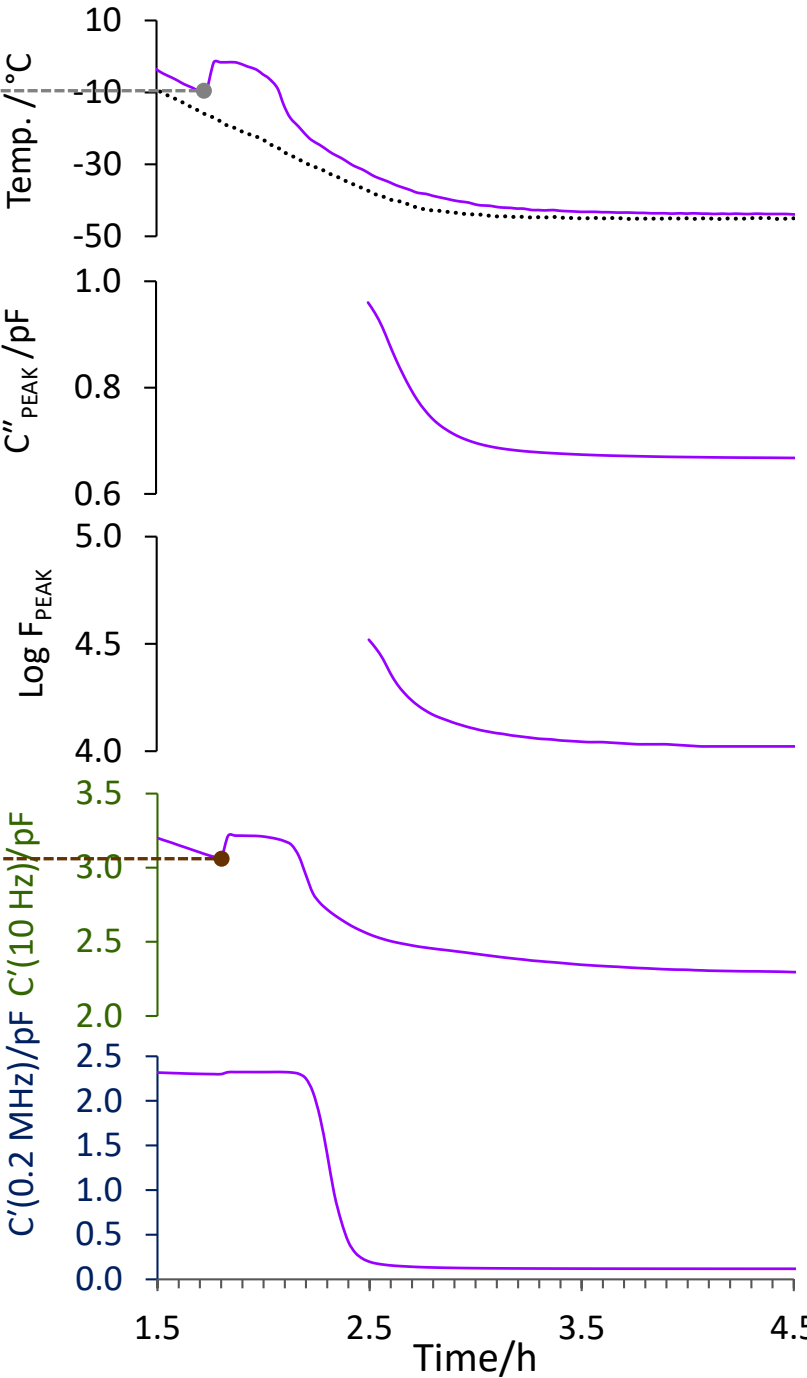
Freezing Step - 5%Sucrose+0.55%NaCl

Nucleation point from TC



Freezing Step - 5%Sucrose+0.55%NaCl

Nucleation point from TC



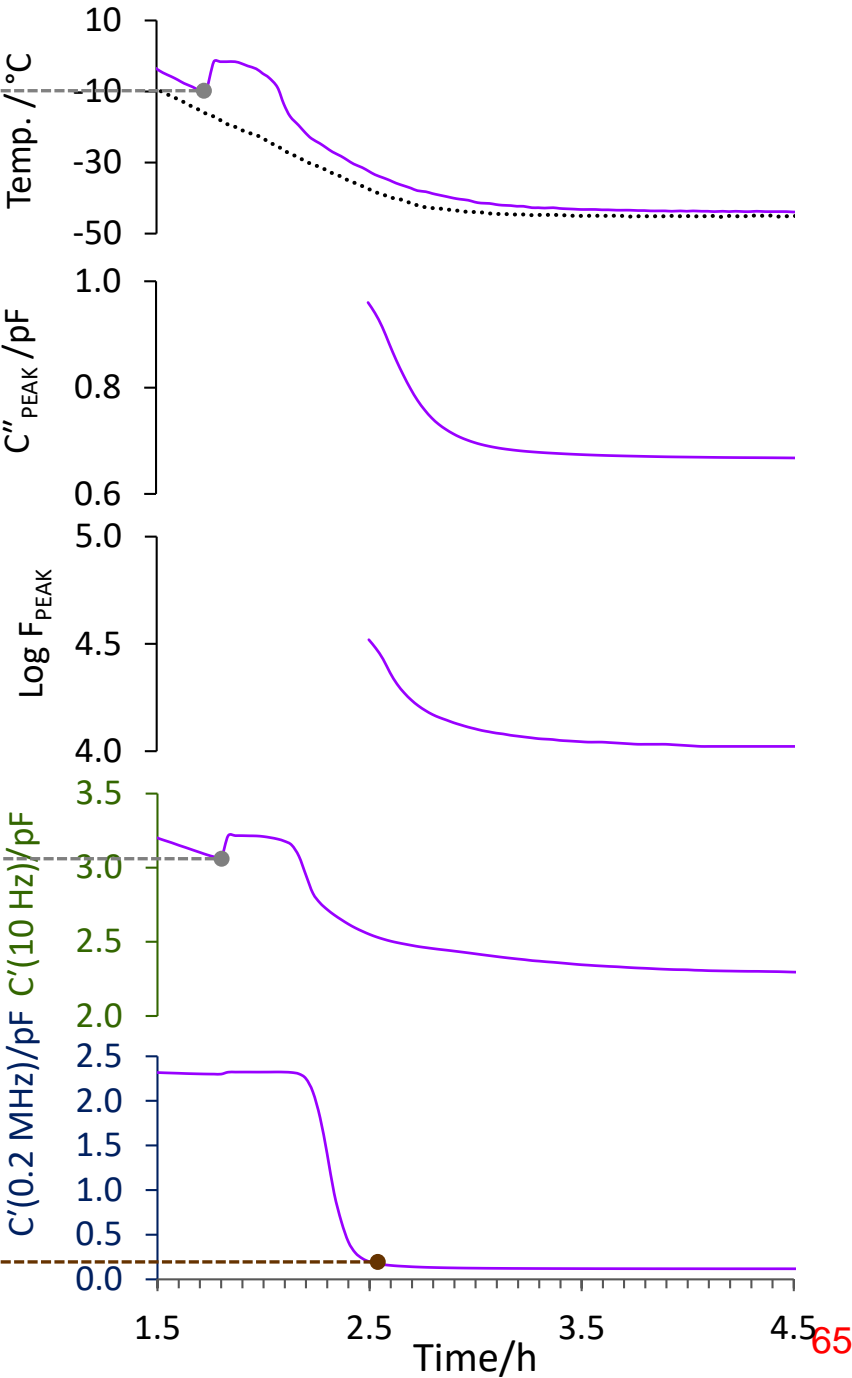
Sensitive to nucleation

Freezing Step - 5%Sucrose+0.55%NaCl

Nucleation point from TC

Sensitive to nucleation

Solidification

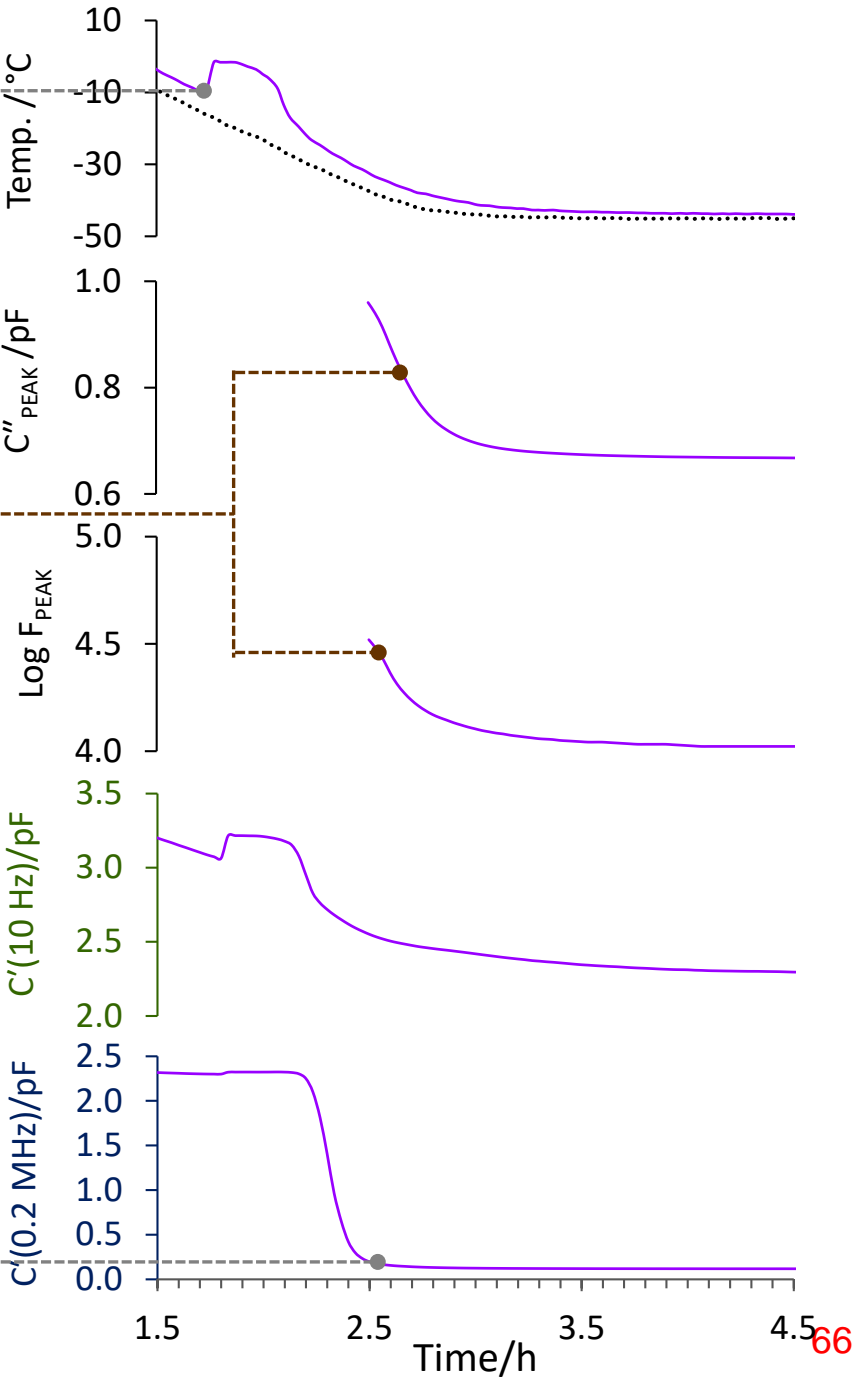


Freezing Step - 5%Sucrose+0.55%NaCl

Nucleation point from TC

Good indication for temperature

Solidification



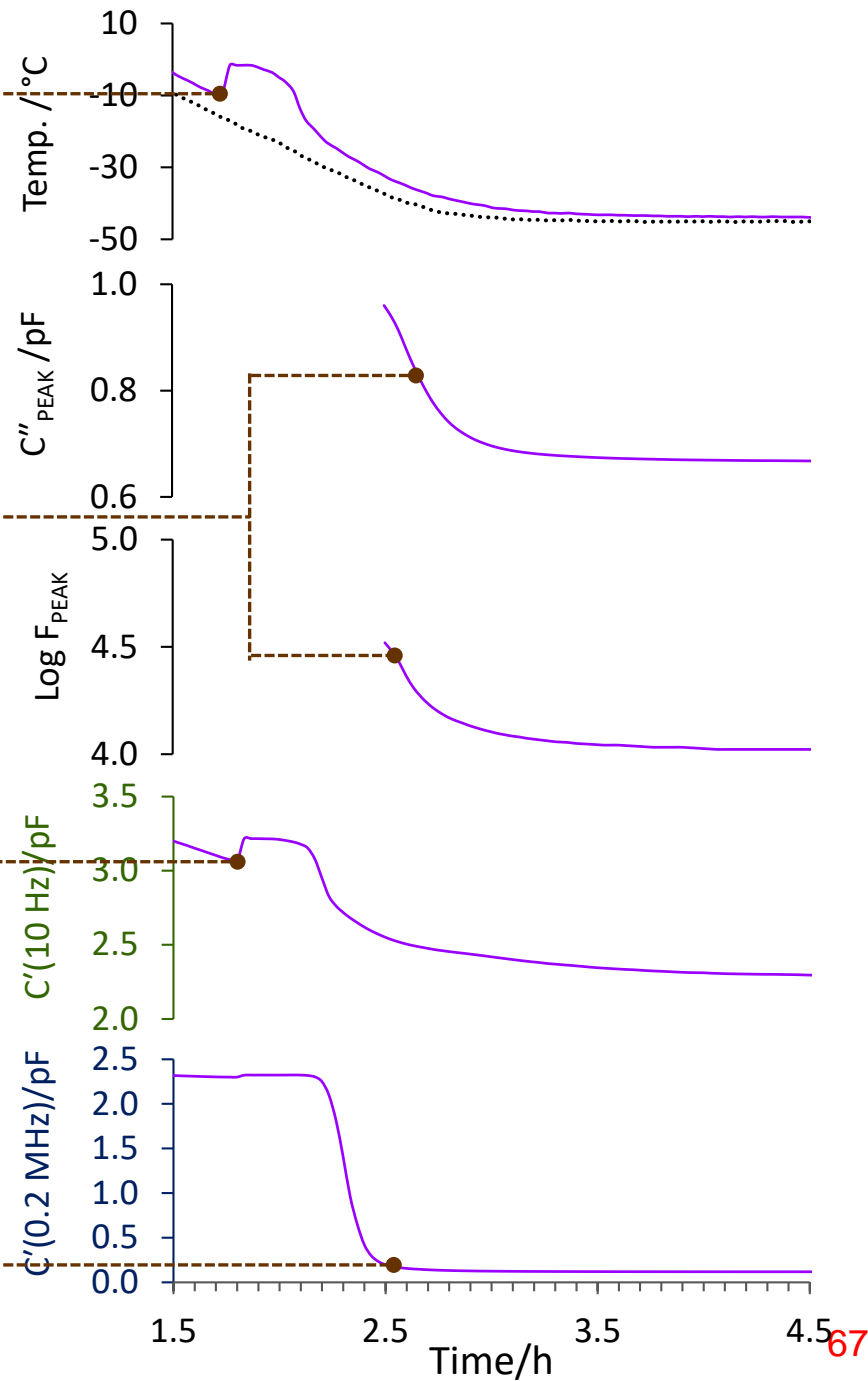
Freezing Step - 5%Sucrose+0.55%NaCl

Nucleation point from TC

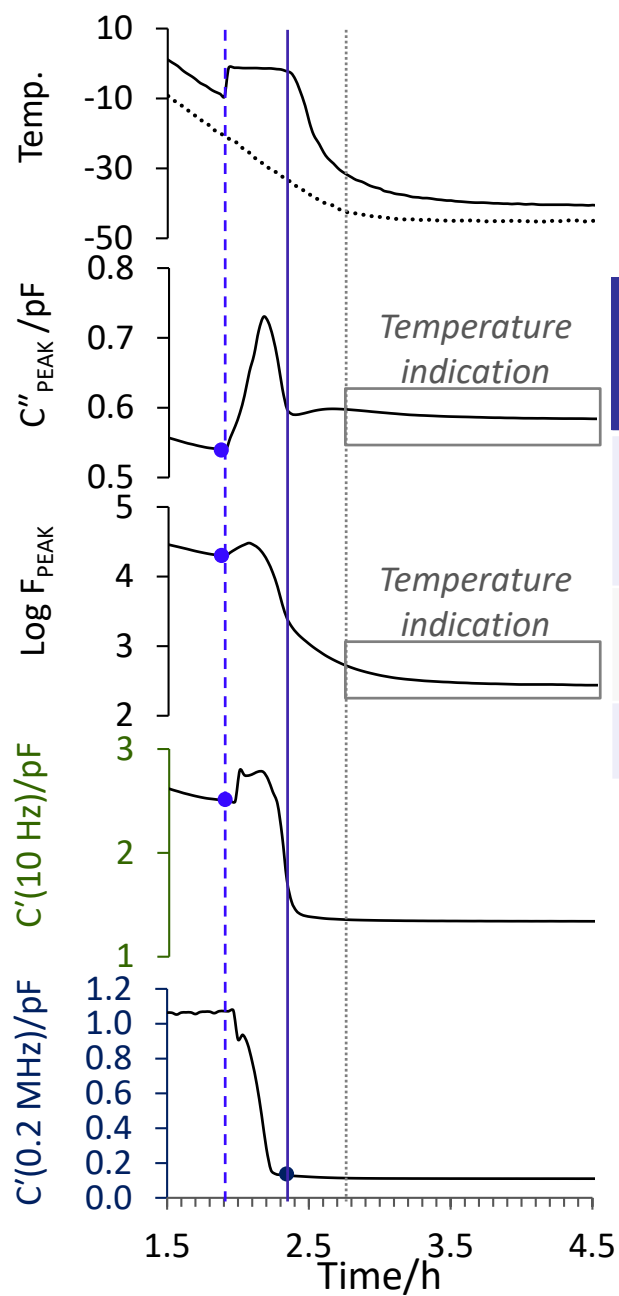
Good indication for temperature

Sensitive to nucleation

Solidification

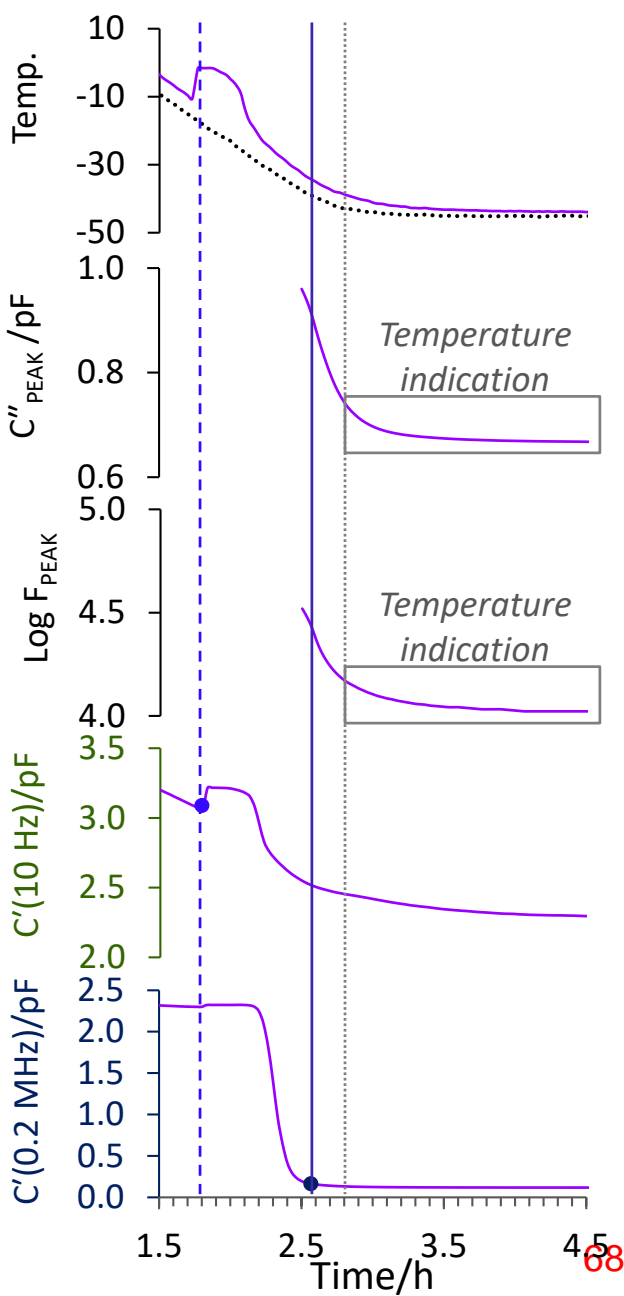


Low conductive sample (LC)



Stage	TVIS Parameters	
	LC	HC
Nucleation	C''_{PEAK} F_{PEAK} $C'(10\text{ Hz})$	$C'(10\text{ Hz})$
Temperature indication	C''_{PEAK} F_{PEAK}	C''_{PEAK} F_{PEAK}
Solidification	$C'(0.2\text{ MHz})$	$C'(0.2\text{ MHz})$

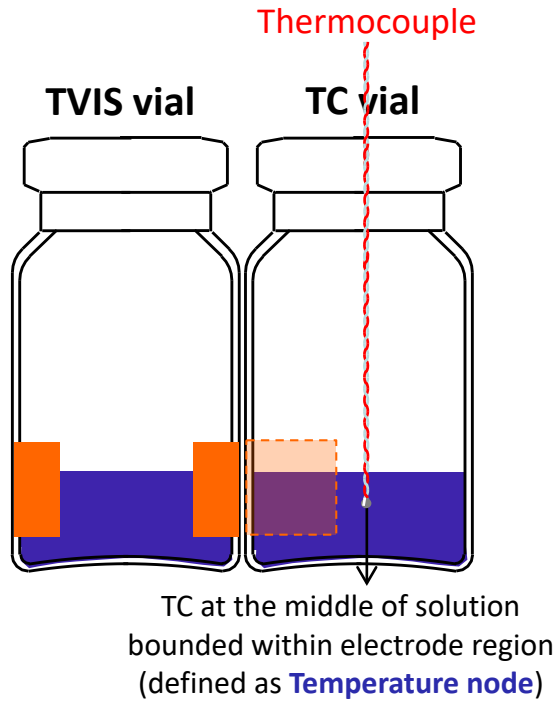
High conductive sample (HC)



TVIS Applications

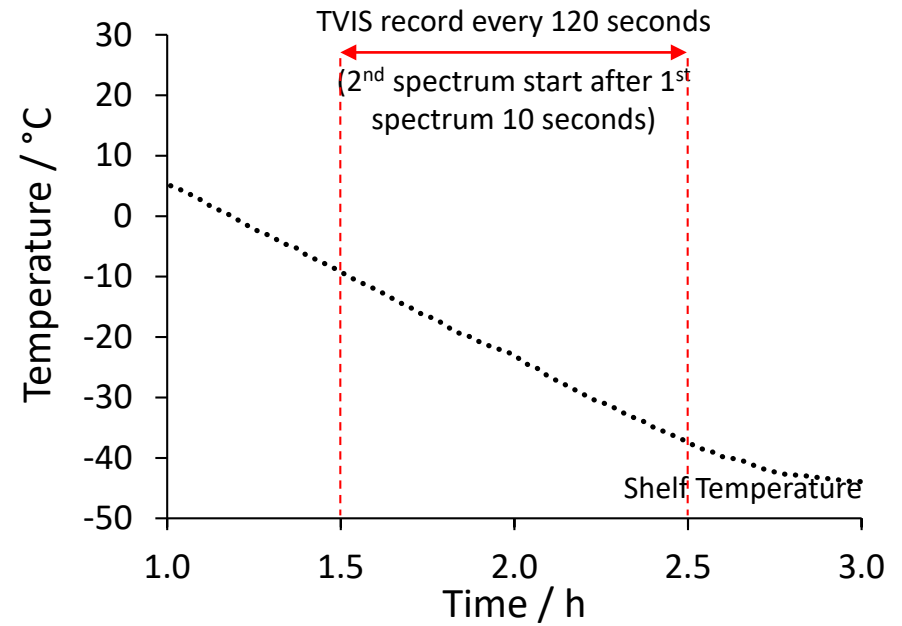
Determination of Ice Nucleation Temperature (T_n)

Ice Nucleation Temperature



Thermocouple position

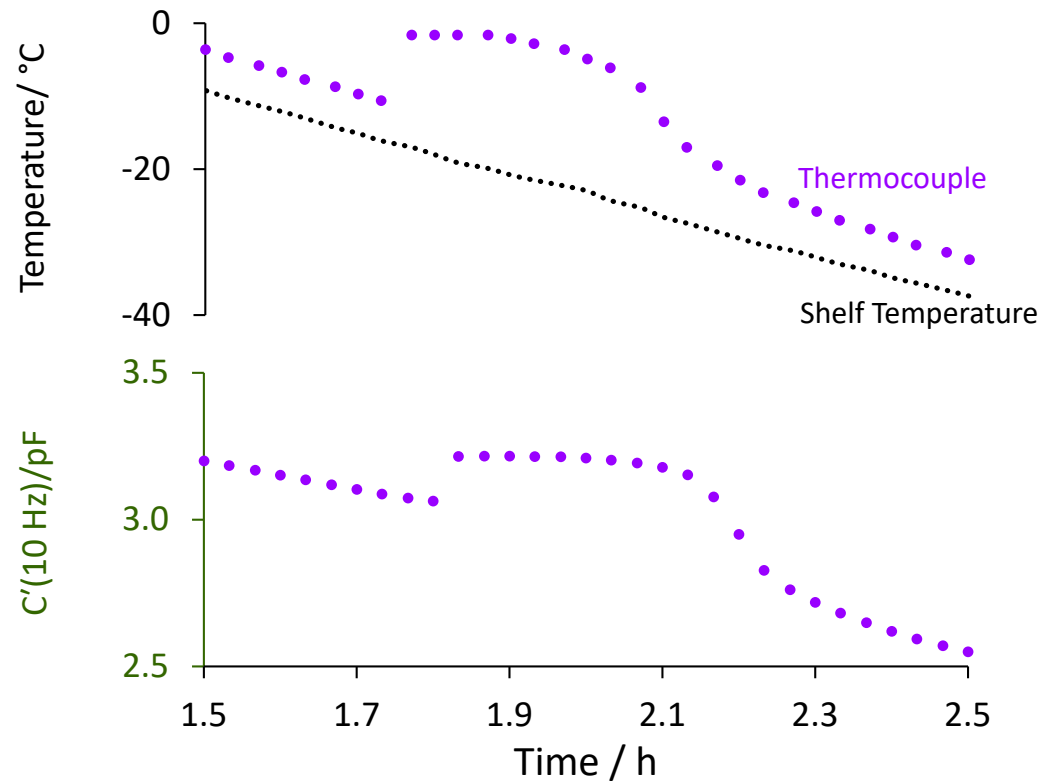
Freezing step



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

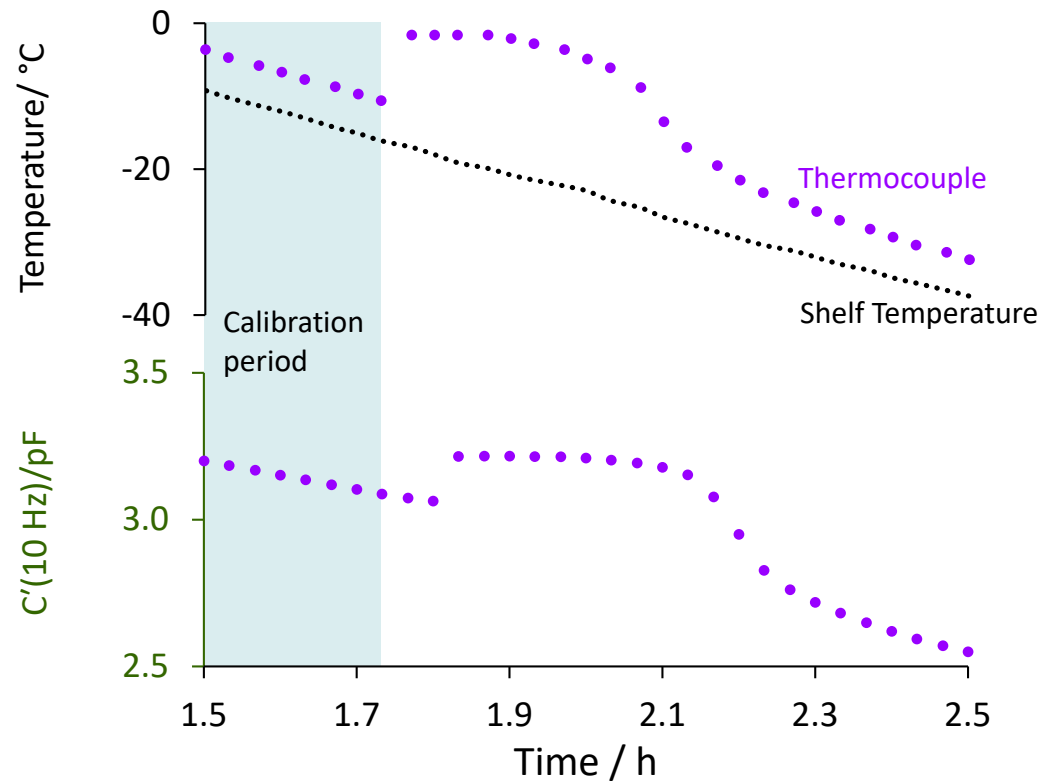
Ice Nucleation Temperature

- In case the TVIS vial nucleates before TC vial, the nucleation temperature in the TVIS vial can be inferred directly from TC temperatures in the nearest neighbor vials
- However, if TVIS vial nucleates later than TC vial, the nucleation temperature can be predicted by fitting a curve to the plot of the average temperature from thermocouple vials against TVIS parameter (i.e. $C'(10\text{ Hz})$)
- In this presentation, the ice nucleation temperature of sample (5 %w/v sucrose in 0.55 %w/v NaCl) extrapolate from fitting because TVIS vials form ice after TC vials



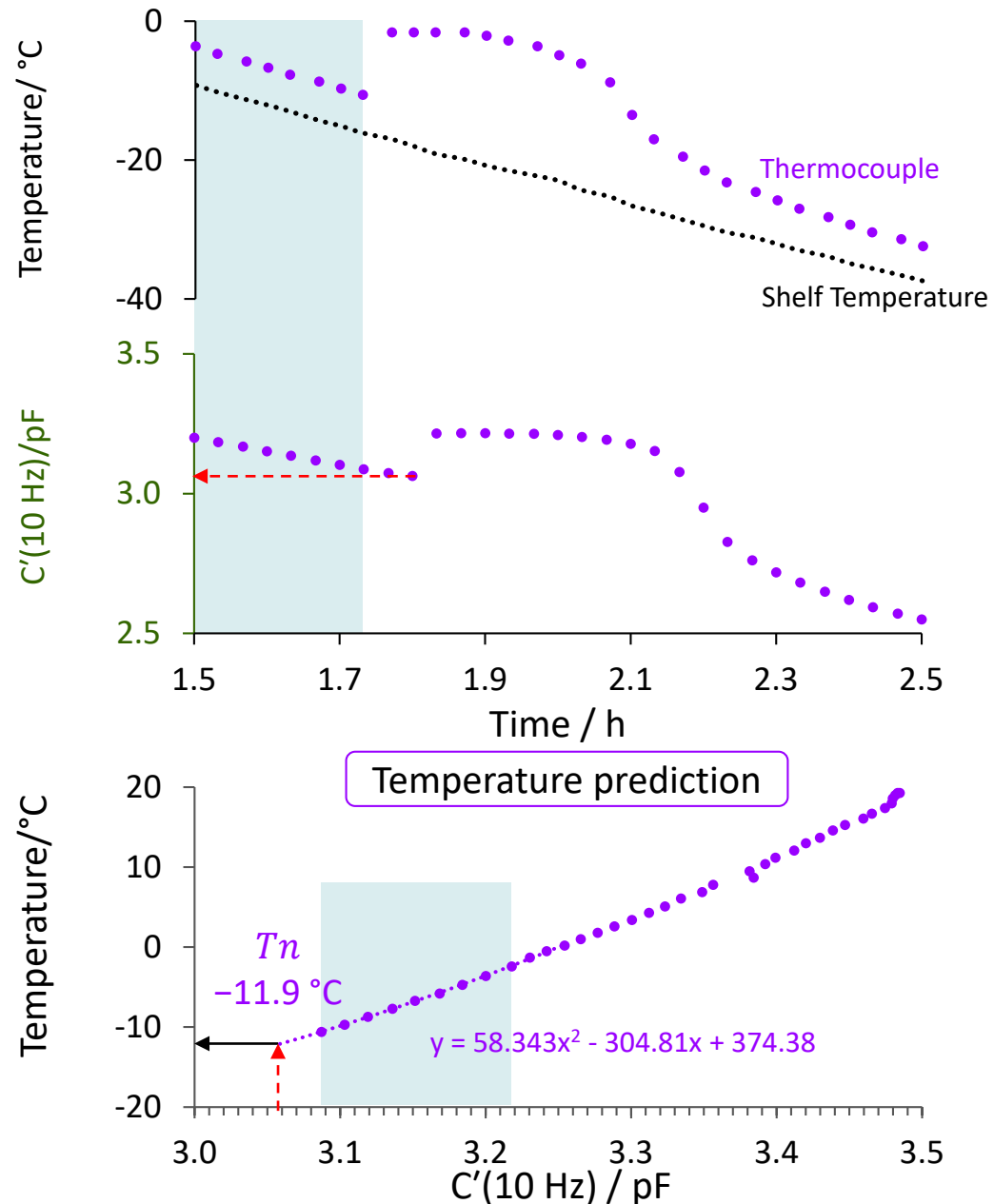
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Ice Nucleation Temperature

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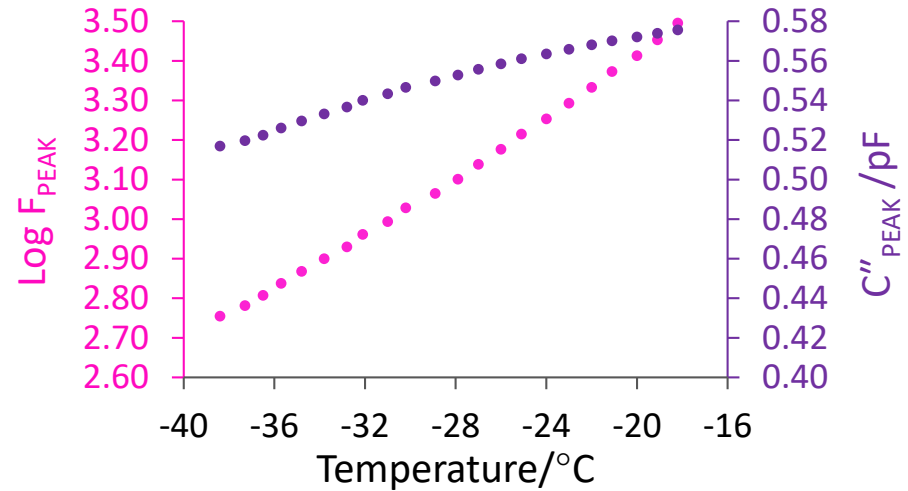
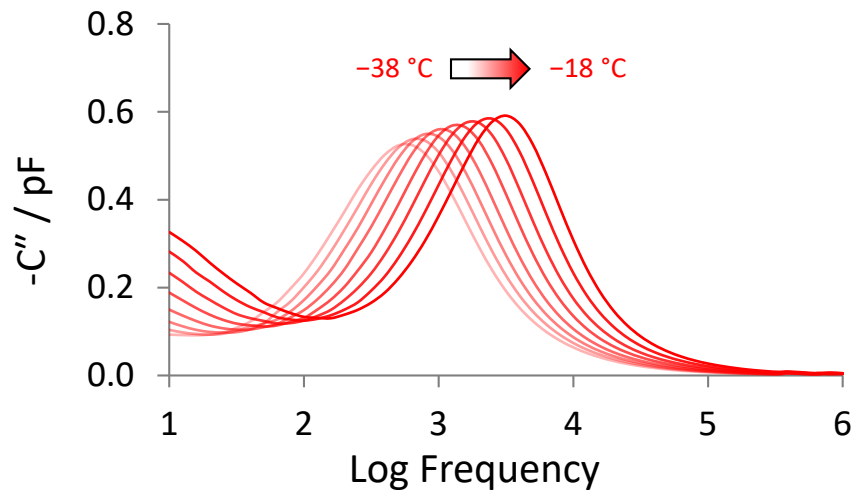
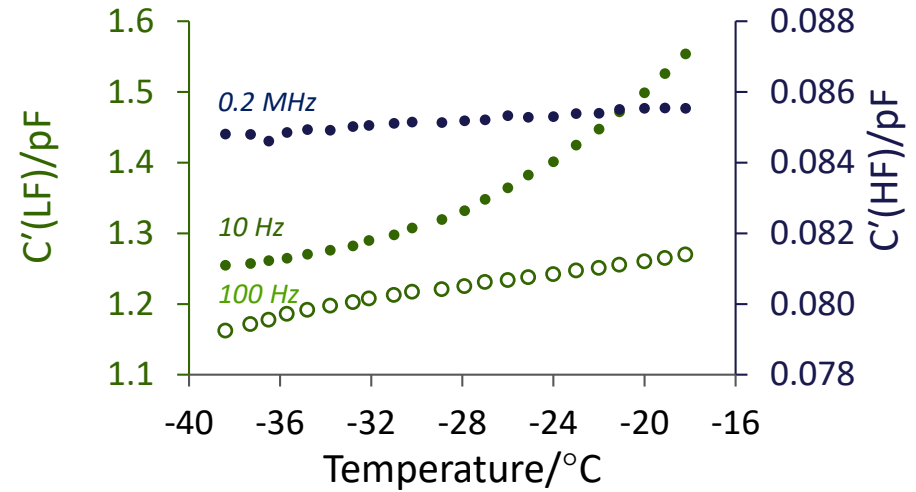
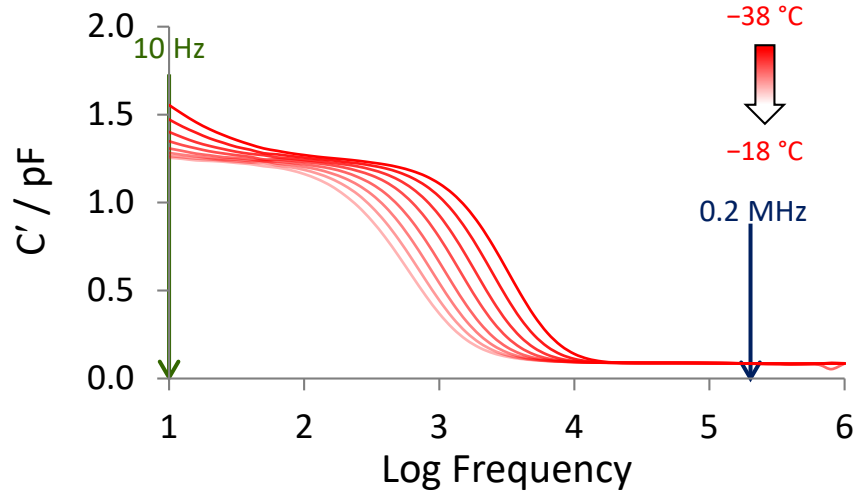


TVIS Applications

Ice crystallization time

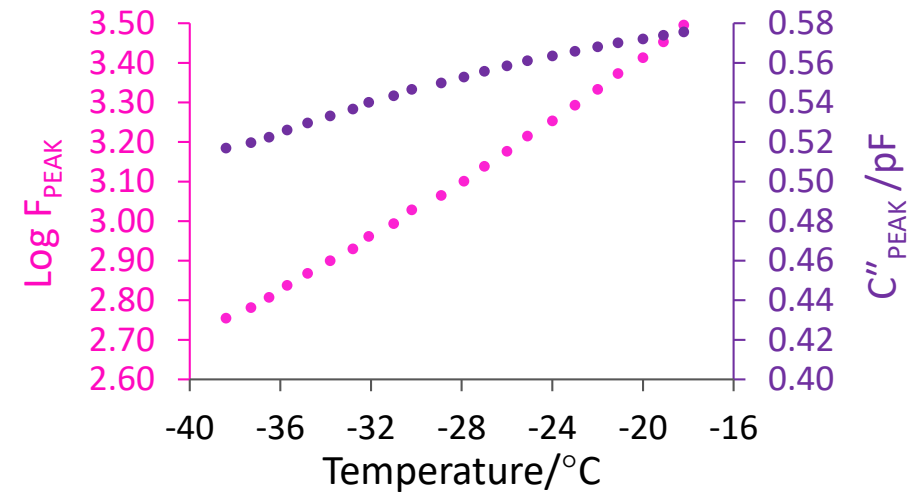
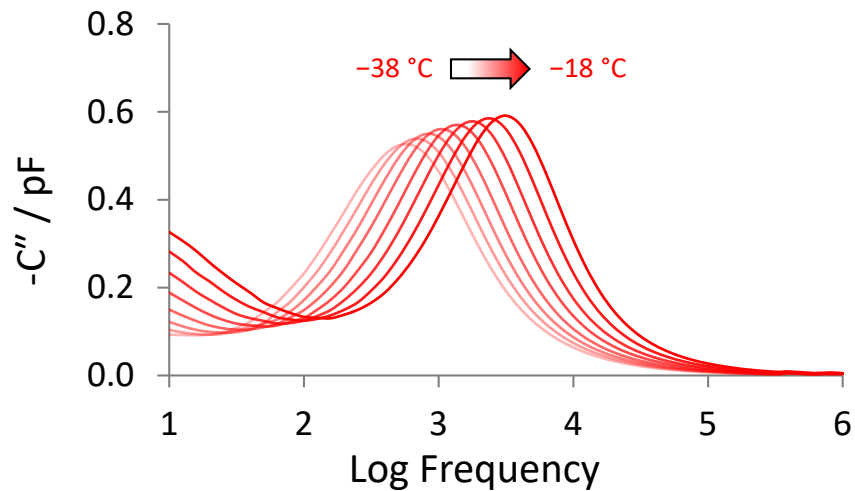
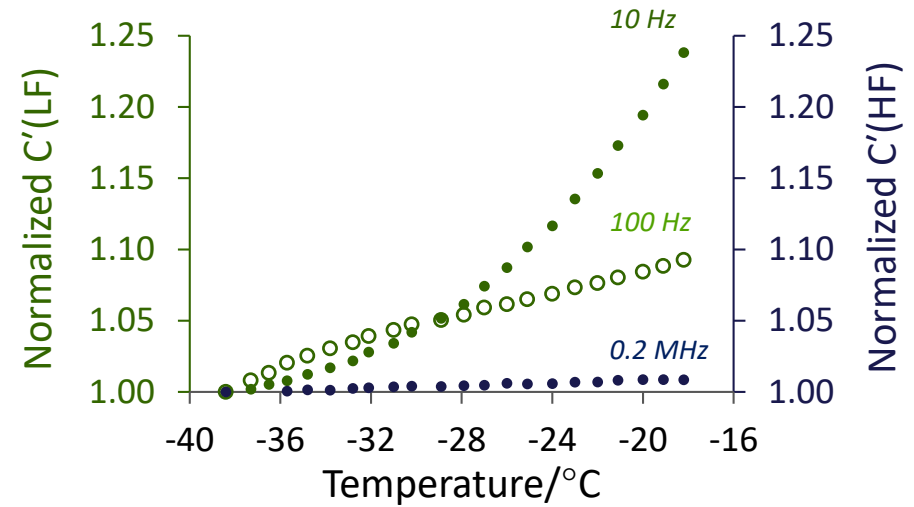
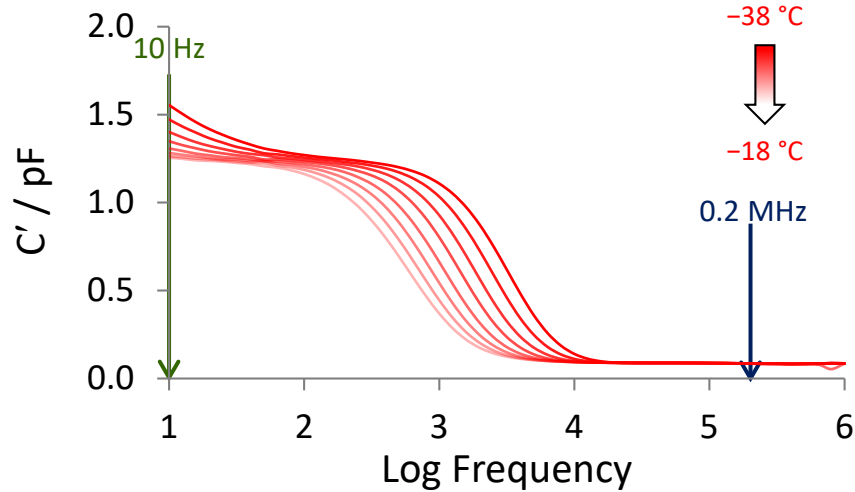
Temperature Profile of Frozen Water

Re-heating



Temperature Profile of Frozen Water

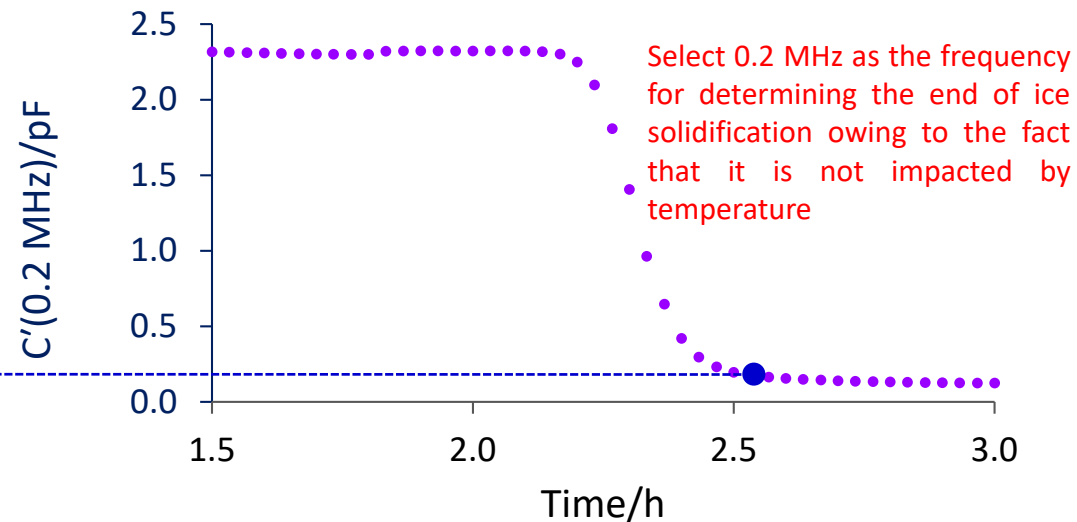
Re-heating



Solidification End Point

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

Solidification end point

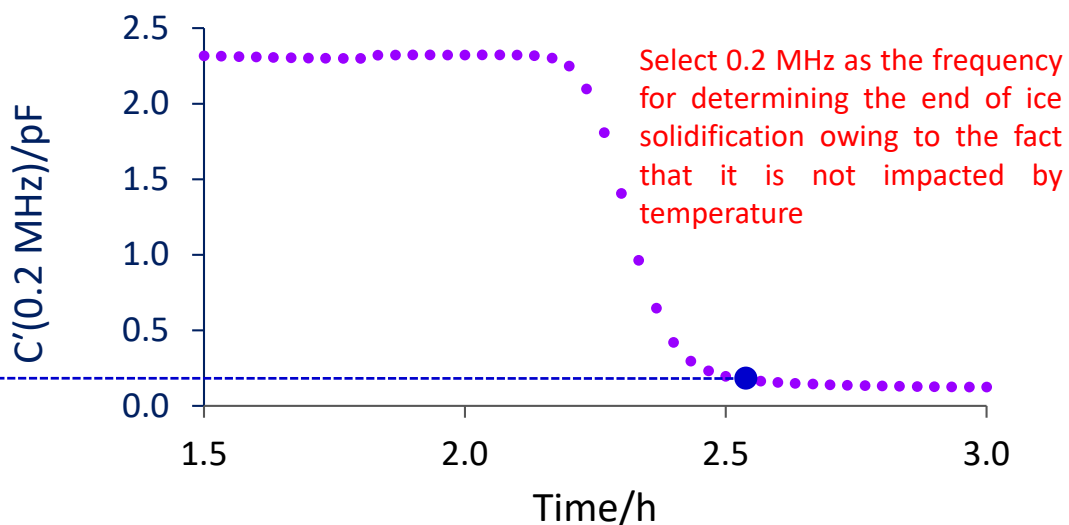
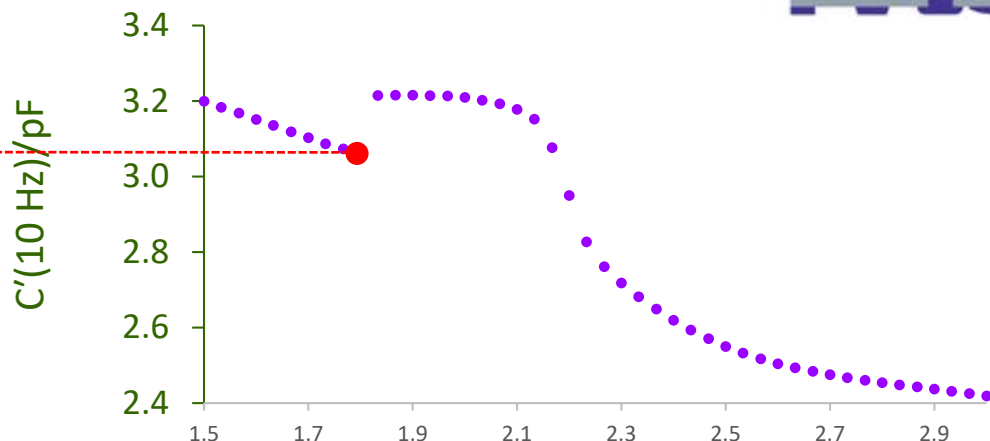


Solidification End Point

Ice nucleation

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

Solidification end point

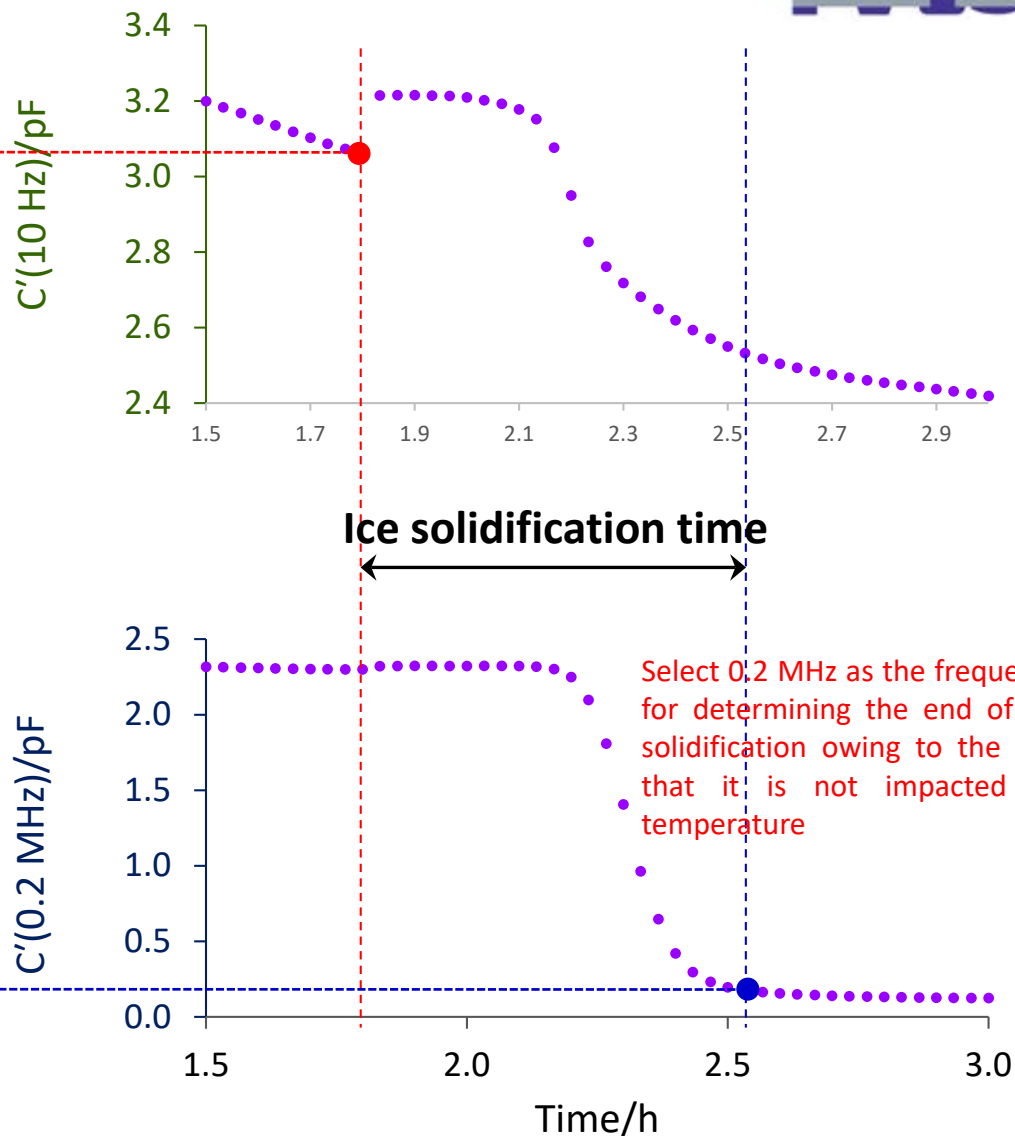


Solidification End Point

Ice nucleation

- The capacitance of ice has almost no temperature dependence at frequency above the relaxation frequency of ice (~ 1 kHz) such as $C'(0.2 \text{ MHz})$.
- Any changes in $C'(0.2 \text{ MHz})$ either with time or temperature, can be associated with the completion of ice formation on freezing or the glass transition (*Next section*)

Solidification end point



Examples

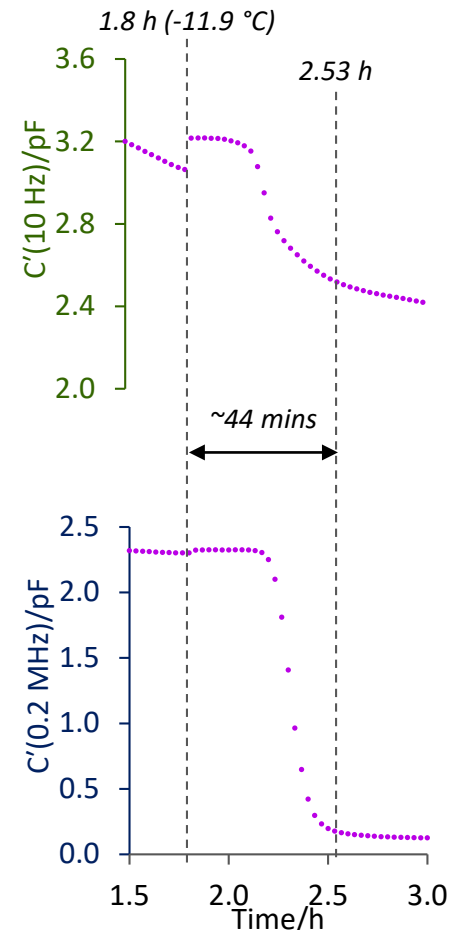
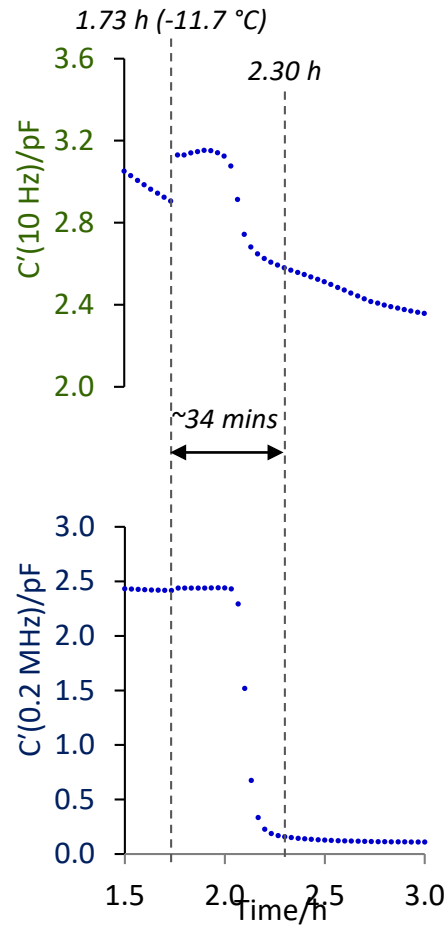
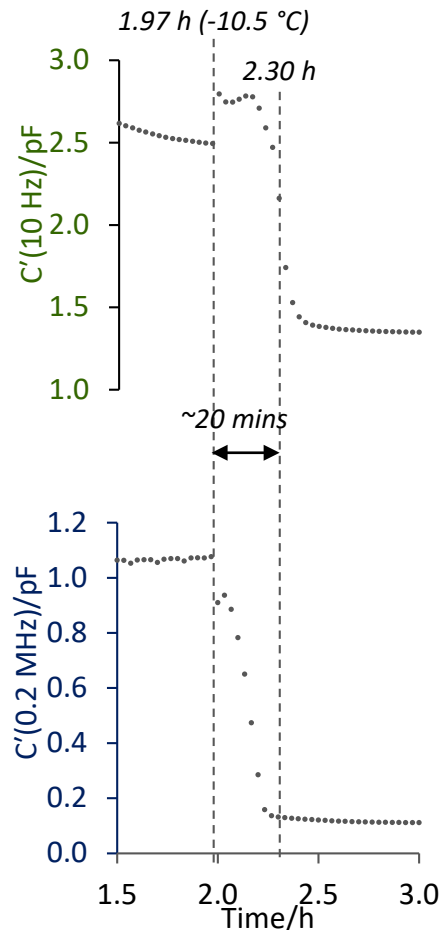
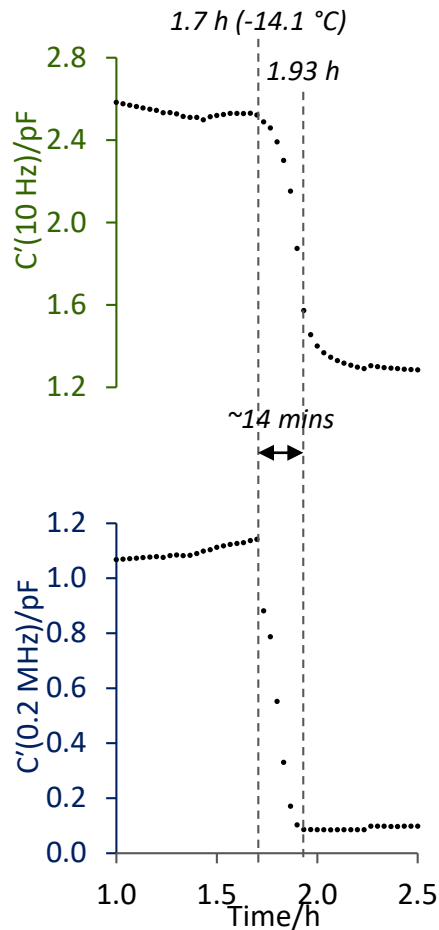
Water

5% Sucrose

5% Sucrose in
0.26% NaCl

5% Sucrose in
0.55% NaCl

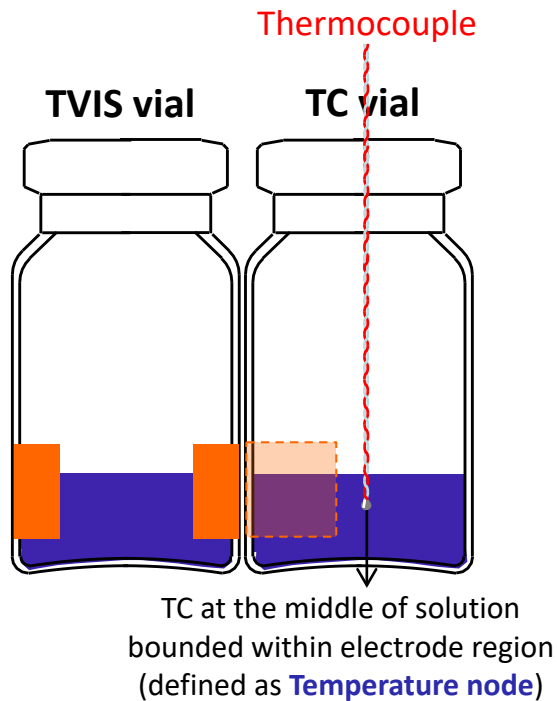
Solidification time for ice formation increases with the salt concentration



TVIS Applications

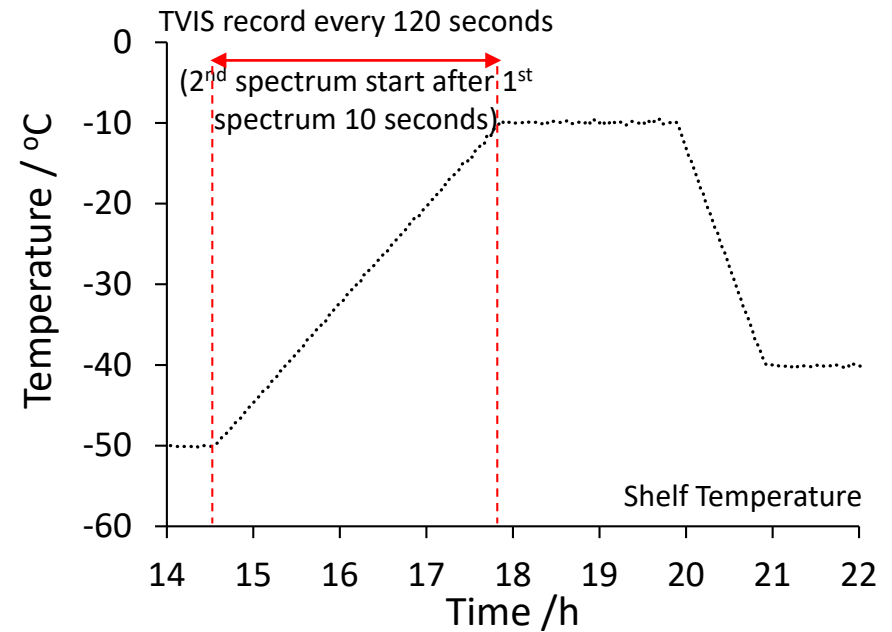
Determination of in-vial Glass Transition temperature (T'_g)

Glass Transition Temperature



Thermocouple position

Annealing



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

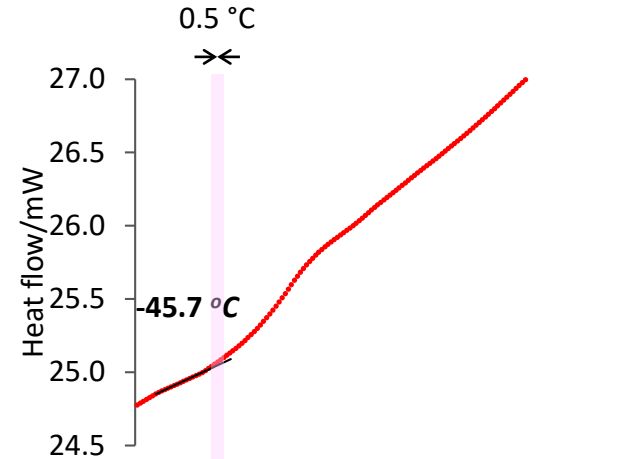
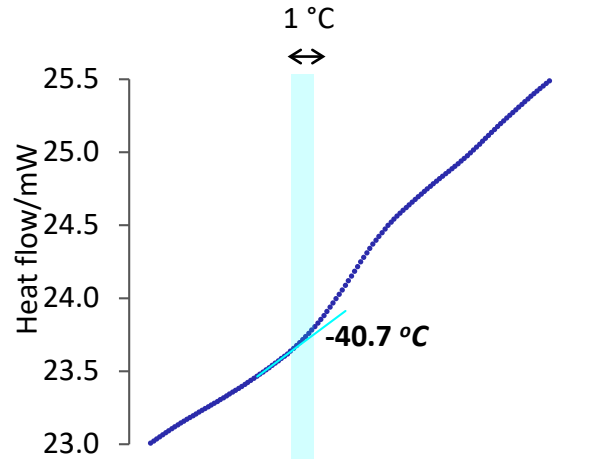
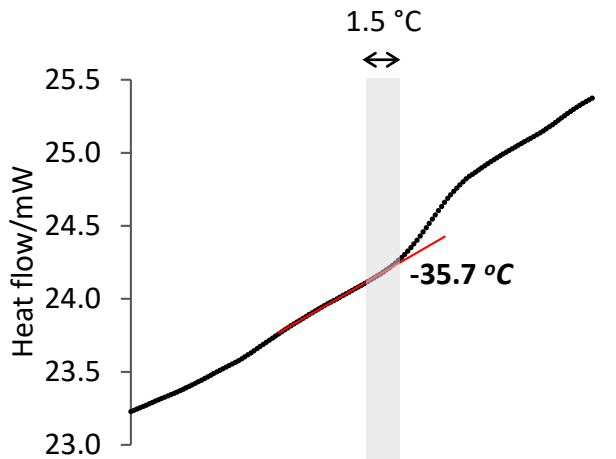
$C'(0.2 \text{ MHz})$ during Re-heating



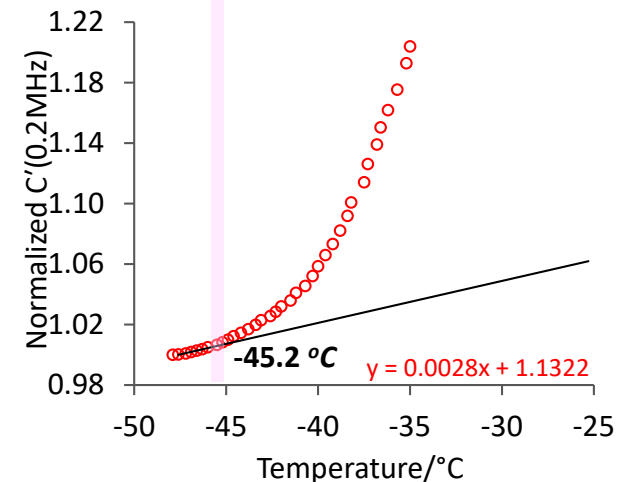
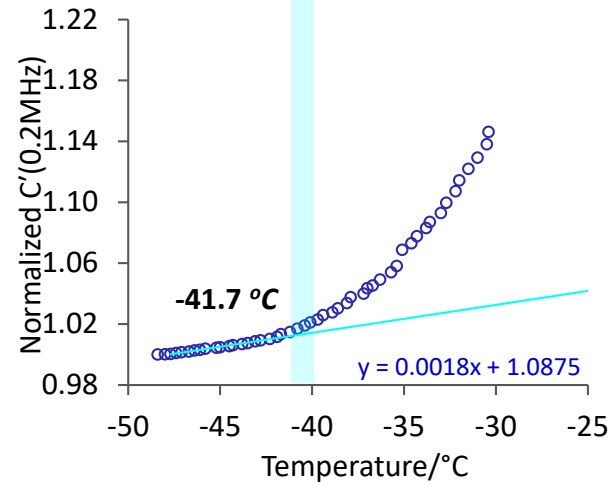
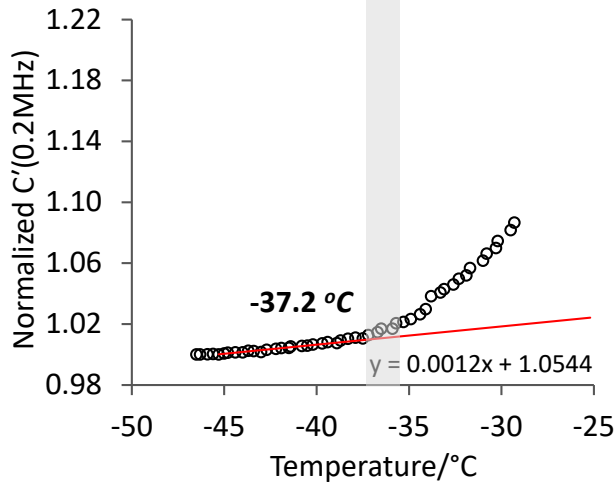
5% Sucrose in
0% NaCl

5% Sucrose in
0.26% NaCl

5% Sucrose in
0.55% NaCl



Onset of glass transition observed by the inflection in the temperature profile of the TVIS parameter $C'(0.2\text{MHz})$



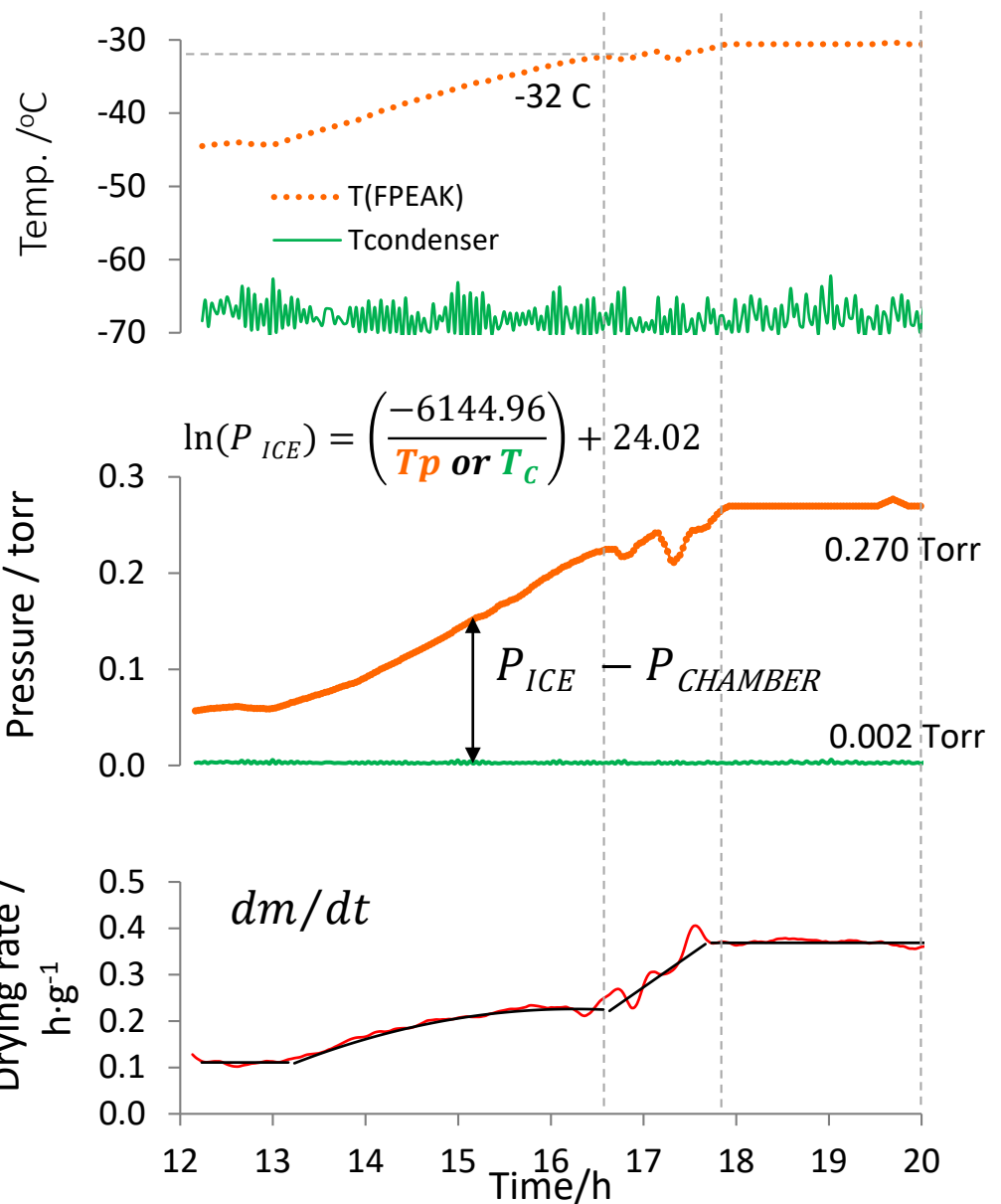
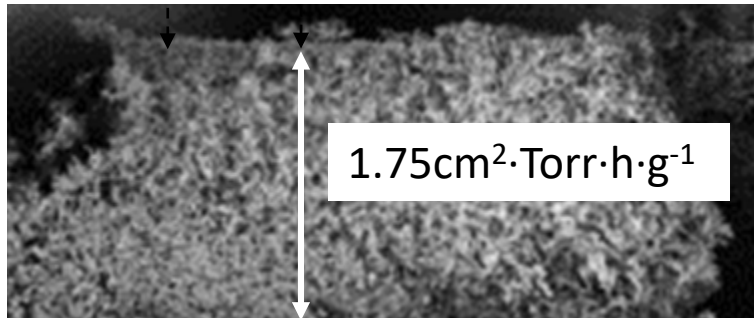
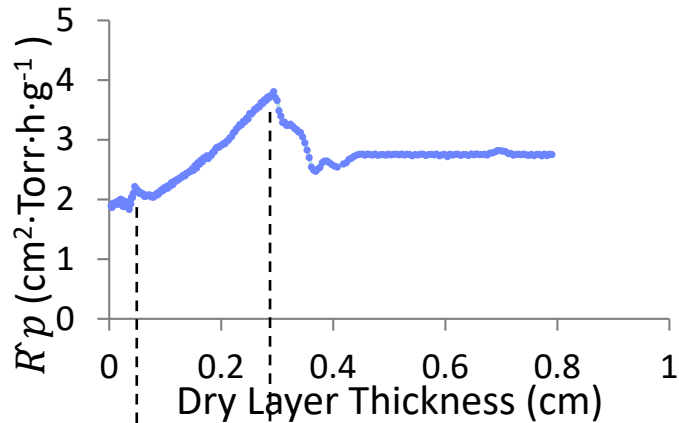
TVIS Applications

Determination of Collapse Phenomena

Collapse Phenomena

5%w/v Lactose in water

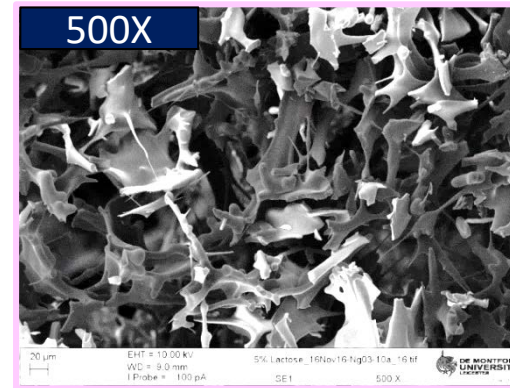
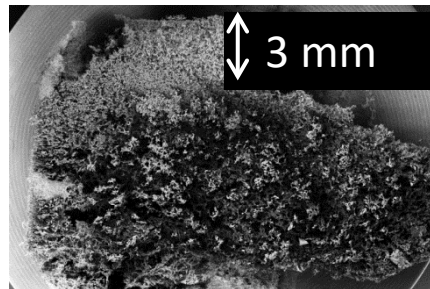
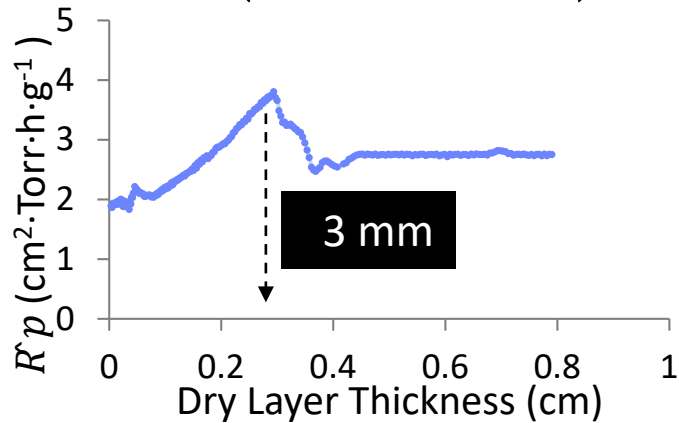
$$\hat{R}p = \left(\frac{P_{ICE} - P_{CHAMBER}}{dm/dt} \right) \cdot A_p$$



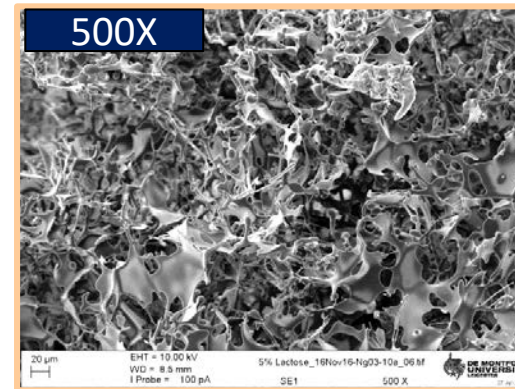
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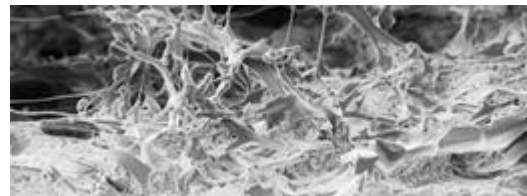


Top layer
Fine pores



Middle layer
Micro-collapse

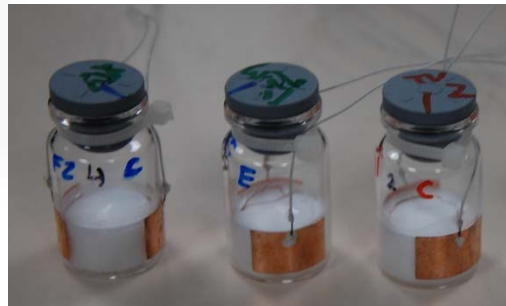
500X



Bottom layer
Full collapse

Future Work

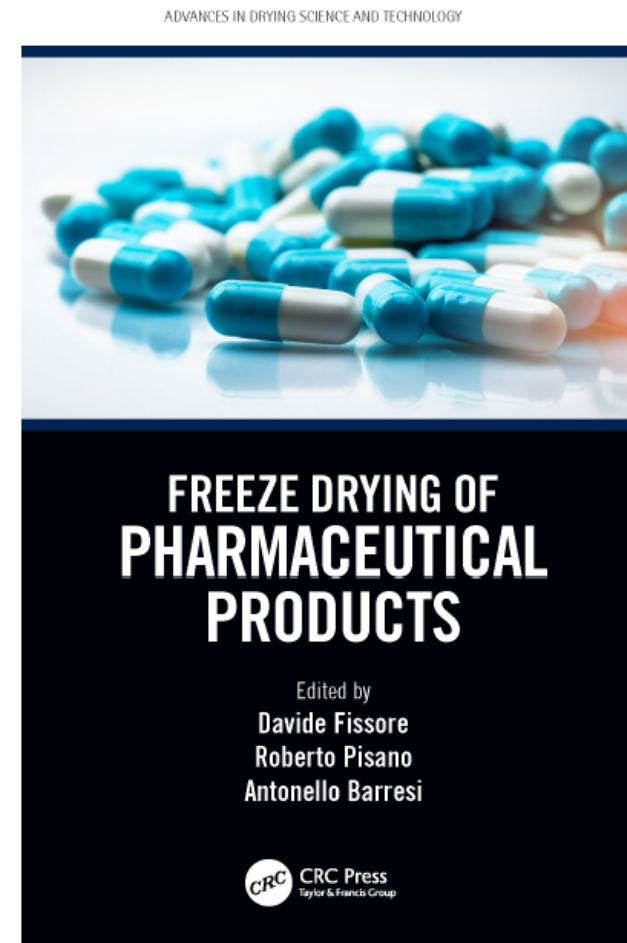
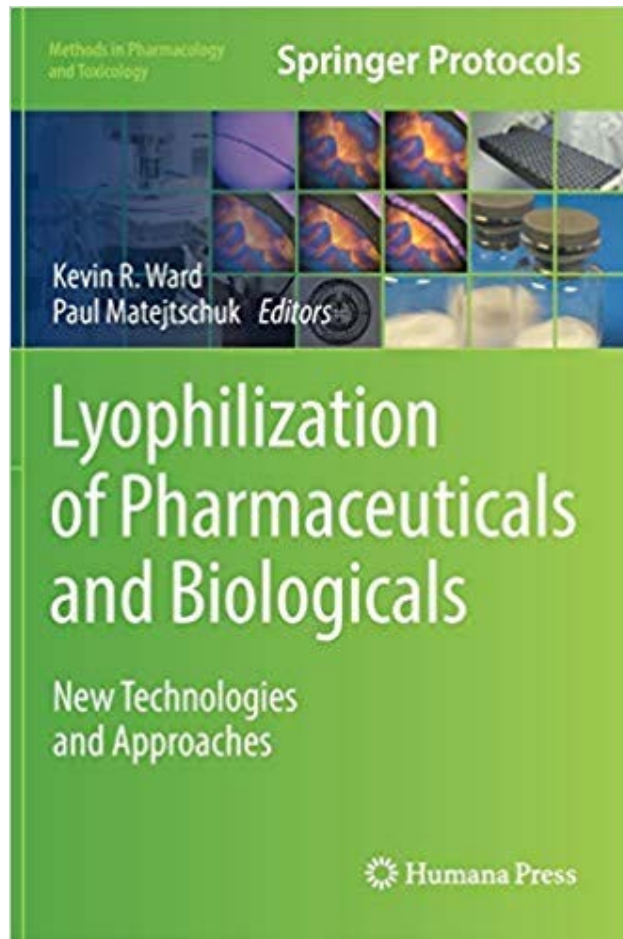
- Development mapping a drying characteristics
 - heat transfer coefficients (K_V)
 - dry layer resistance (R_P)



- Instrument Development
 - Commercial C-TVIS (2018)
 - Non-contact TVIS (2018-19)
 - Micro-well screening
 - Vial clusters in batch FD
 - TVIS - Shuttle (2019-20)

Non-invasive real time information for characterising the freeze drying

Further Reading



New instrumentation development

Z-FDM impedance spectroscopy enabled freeze-drying microscope



Grant Funding:

FASTLyO Innovate UK feasibility study . Completed Feb 28 2019

Application :

- Development of micro-collapsed formulations with fast drying potential



Acknowledgements, Recent Projects & Collaborators

- De Montfort University, School of Pharmacy
 - Evgeny Polygalov: co-inventor of TVIS instrument
 - Yowwares Jeeraruangrattana. PhD student
 - Bhaskar Pandya. PhD student
 - Irina Ermolina. Senior Lecturer



Our data



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GEA Pharma Systems



Innovate UK

Government Support for industry

LyoDEA

Lyophilization process analytics
By dielectric analysis



BIOSTART

Biopharmaceutical Stability at
Room Temperature

AtlasBio



Analytical Technologies for the
Stabilization of Biopharmaceuticals



Through Vial Impedance Spectroscopy