

Freeze drying of 5% Mannitol in deionised water

- Freezing step
- Re-heating period

Through Vial Impedance Spectroscopy



Aim



To demonstrate the use of Through Vial Impedance Spectroscopy (TVIS) as a PAT tool for determination of:

- ice growth onset and end point
- crystallization temperature and time for mannitol in aqueous solution during lyophilization
- Mannitol crystal melt-back temperature

Introduction: lyophilisation/mannitol



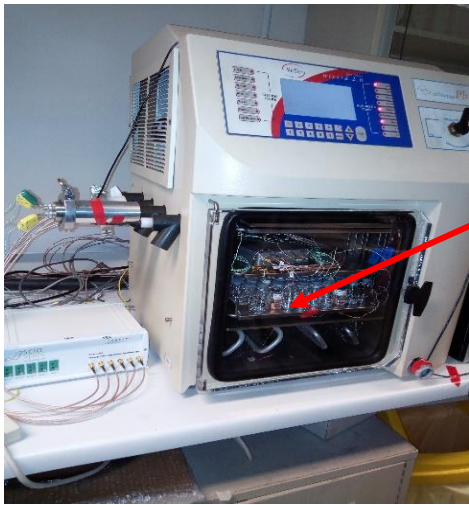
- The term “freeze-drying” is quite descriptive: a process where a product is initially frozen, and then dried from the frozen state.
- Mannitol is one of the excipients used in drug formulation before lyophilization.
- Rationale behind the usage of mannitol includes:
 - formation of an elegant and stable product
 - efficient freeze-drying due to the high melting temperature
 - cryoprotective properties (Williams et al. 1986)
- Some drawback in the use of mannitol in the formulations:
 - Crystallization upon reheating may crack vials due to product expansion (Williams and Dean, 1991),
 - Reduction of cryoprotectant potency in protein formulations (Izutsu and Yoshioka (1994)
- Mannitol is believed to exist in three polymorphic forms as α , β and, δ . (Walter-Levy L. 1968), Hulse et al. (2009)
- The differences have been attributed to freezing rate: while α and β form are due to slow freezing rate, δ form is due to fast freezing rate (Kim et al. 1998)
- Kett et al. 2003, used DSC, CSM and XRD to identify the thermal transitions and it was found that ice formed at -20°C while a further exotherm was seen $\sim -30^{\circ}\text{C}$, reheating gave an endotherm at -30°C followed by an exotherm at -25°C .

Introduction: impedance /TVIS



- Impedance spectroscopy measures the electric properties of a material as a function of frequency when voltage is applied
- It is based on the interaction of an external field with the electric dipole moments of the material
- Through vial impedance spectroscopy (TVIS) refers to when mobile charges conduct current across a vial rather than within the vial
- TVIS has been used to predict important lyophilization process parameters including ice interface temperatures and drying rates Smith et al (2018)
- There have been a limited and inconsistent information about the thermal transition events of mannitol in frozen condition, this investigation will employ TVIS system to confirm transformation events of mannitol in sub-ambient condition

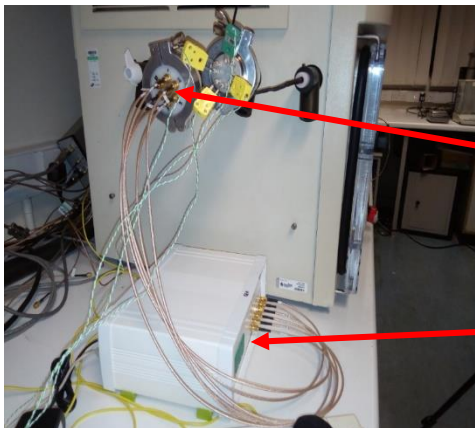
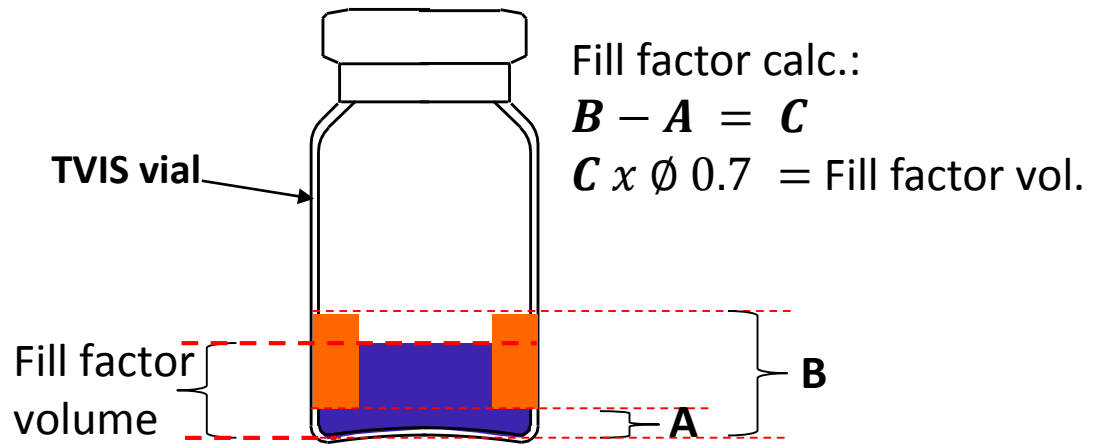
Materials/Instrument



Vials on the shelf

Virtis Advantage Plus
Benchtop Freeze dryer

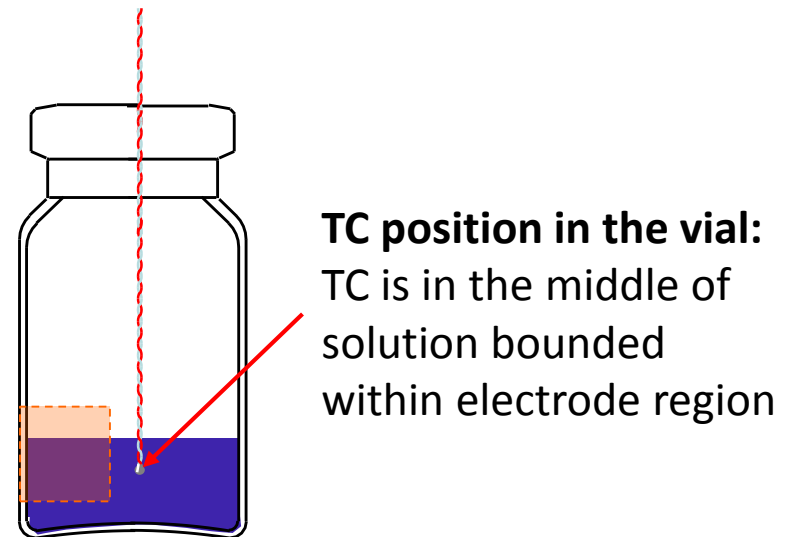
- 5%w/v of 98% D(+)-Mannitol
- Fill factor, ϕ 0.7 used equivalent to 3.5g of solution



Pass-through

5-channel
Sciospec

TVIS Multi-
channel Sciospec

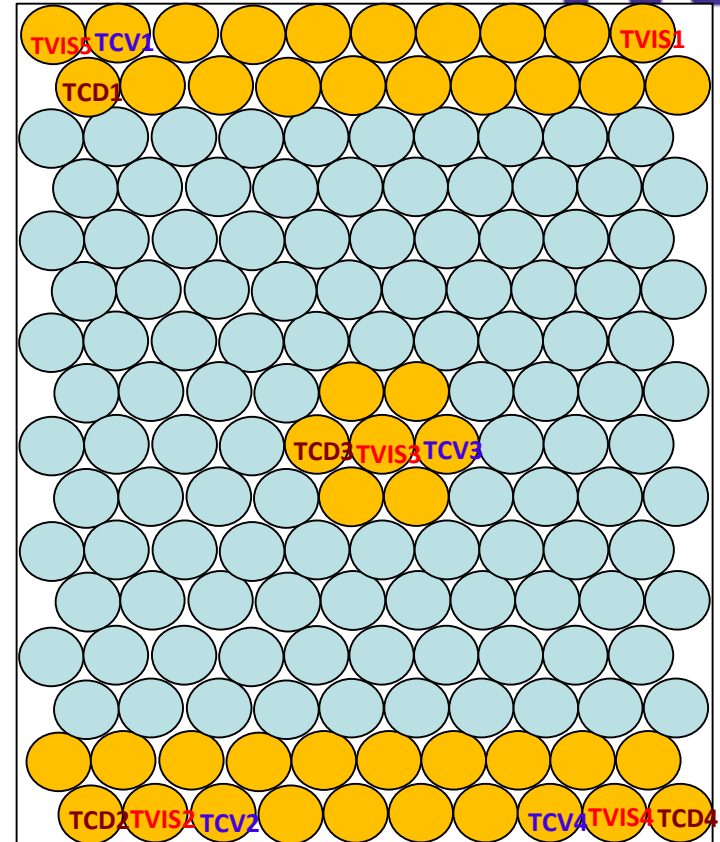


Method



| Step | Period | | Time (min) | Rate (°C/min) | Pressure (μbar) |
|-----------------------|--------|-----|------------|---------------|-----------------|
| | Start | End | | | |
| Equilibrium phase | 20 | 20 | 30 | | |
| Freezing ramp | 20 | -40 | 120 | 0.5 | |
| Freezing hold | -40 | -40 | 120 | - | |
| Re-heating ramp | -40 | -20 | 100 | 0.2 | |
| Re-heating hold | -20 | -20 | 120 | - | |
| Re-cooling ramp | -20 | -40 | 40 | 0.5 | |
| Re-cooling hold | -40 | -40 | 120 | - | |
| Primary drying equil. | -40 | -40 | 30 | - | 400 |
| Primary drying ramp | -40 | -25 | 30 | 0.5 | 400 |
| Primary drying hold | -25 | -25 | 2500 | - | 400 |
| Secondary drying ramp | -25 | 20 | 225 | 0.2 | 400 |
| Secondary drying hold | 20 | 20 | 480 | - | 400 |

Freeze drying process protocol



TCV = Vitris TC vial

TVIS = TVIS vial

TCD = Data logger TC vial

 = Mannitol solution

 = Deionized water

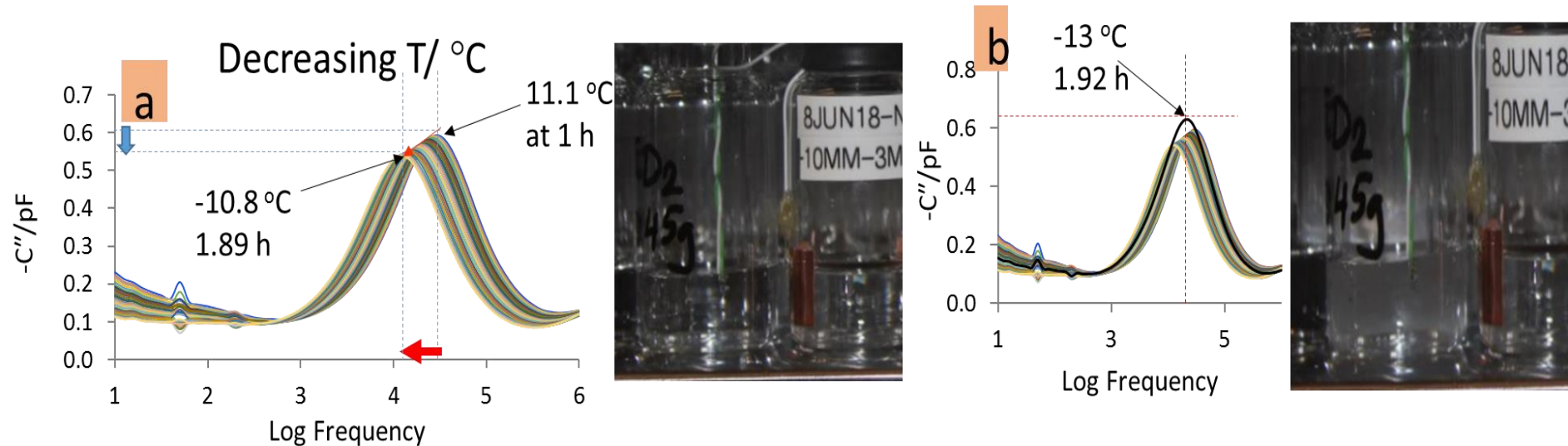
Vial arrangement on the shelf plate

Method description



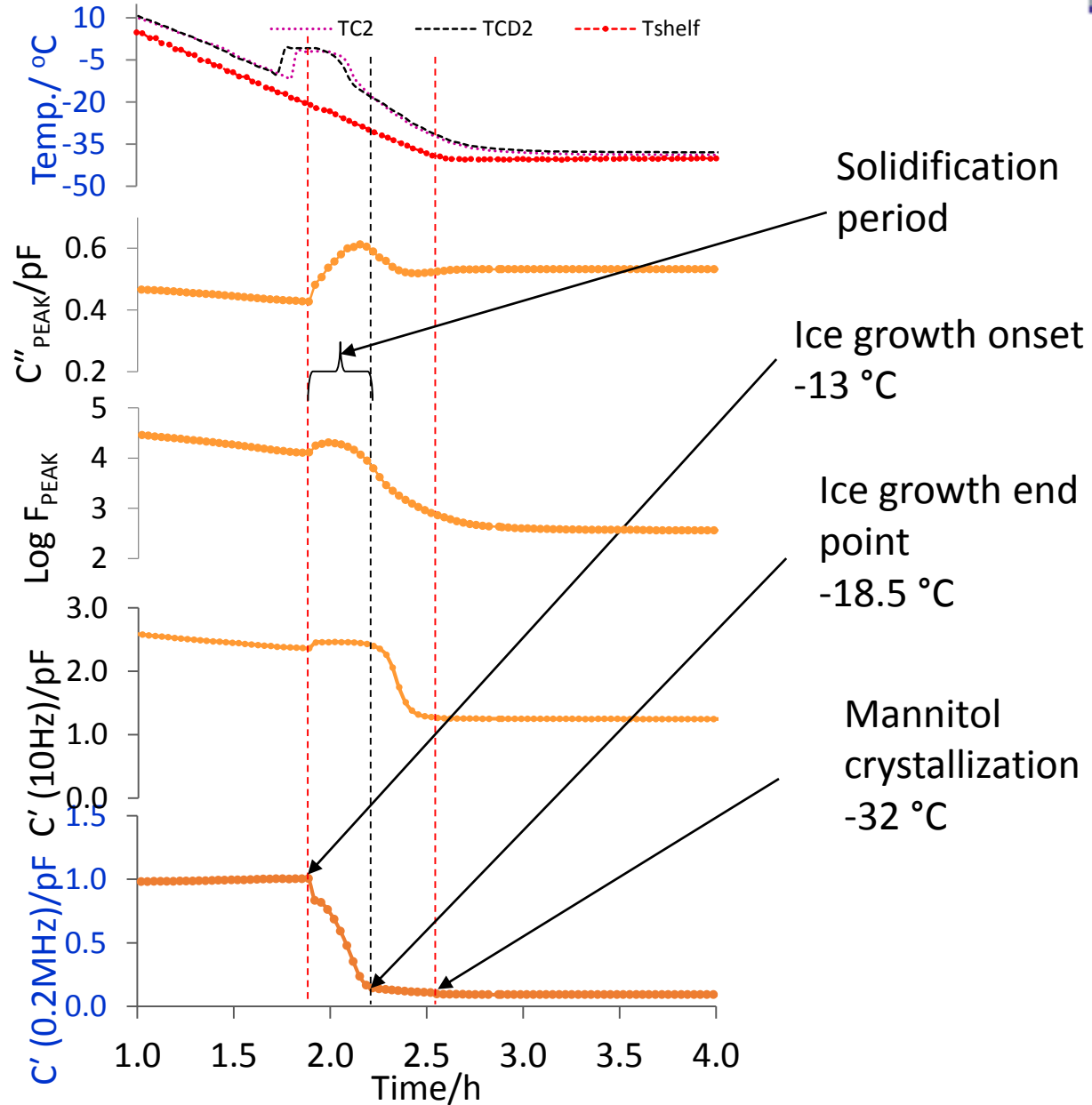
- **Formulation:** Formulation of 5%w/v was prepared from 98% D(+)-Mannitol from which a fill factor 0.7 was calculated to 3.5 g fill volume. One full shelf of 160 vials was loaded with the sensor vials (TVIS and TC vials) placed in the four corners and the Centre of the shelf plate as shown in fig?.
- **Protocol:** Shelf was equilibrated for 30 min followed by freezing ramp to -40 °C at 0.5 °C/min. reheating was performed at low rate 0.2 °C/min to -20 °C and hold for 2 h before recoiling (annealing). The process was continued to primary and secondary drying step.
- **Measurements:** Impedance measurements were performed over a frequency range of 10^1 - 10^6 Hz at the interval of 2 min and temperature measurements were recorded using type K thermocouple at concurrent intervals with impedance measurement. Data from the sensor vials were collected in TVIS Multi-channel Sciospec software via a passthrough system attached to the Virtis Advantage Plus Bench top Freeze dryer.
- **Data analysis:** LyoView and Relaxls software were deployed for data analysis

FREEZING STEP: peak response to temperature

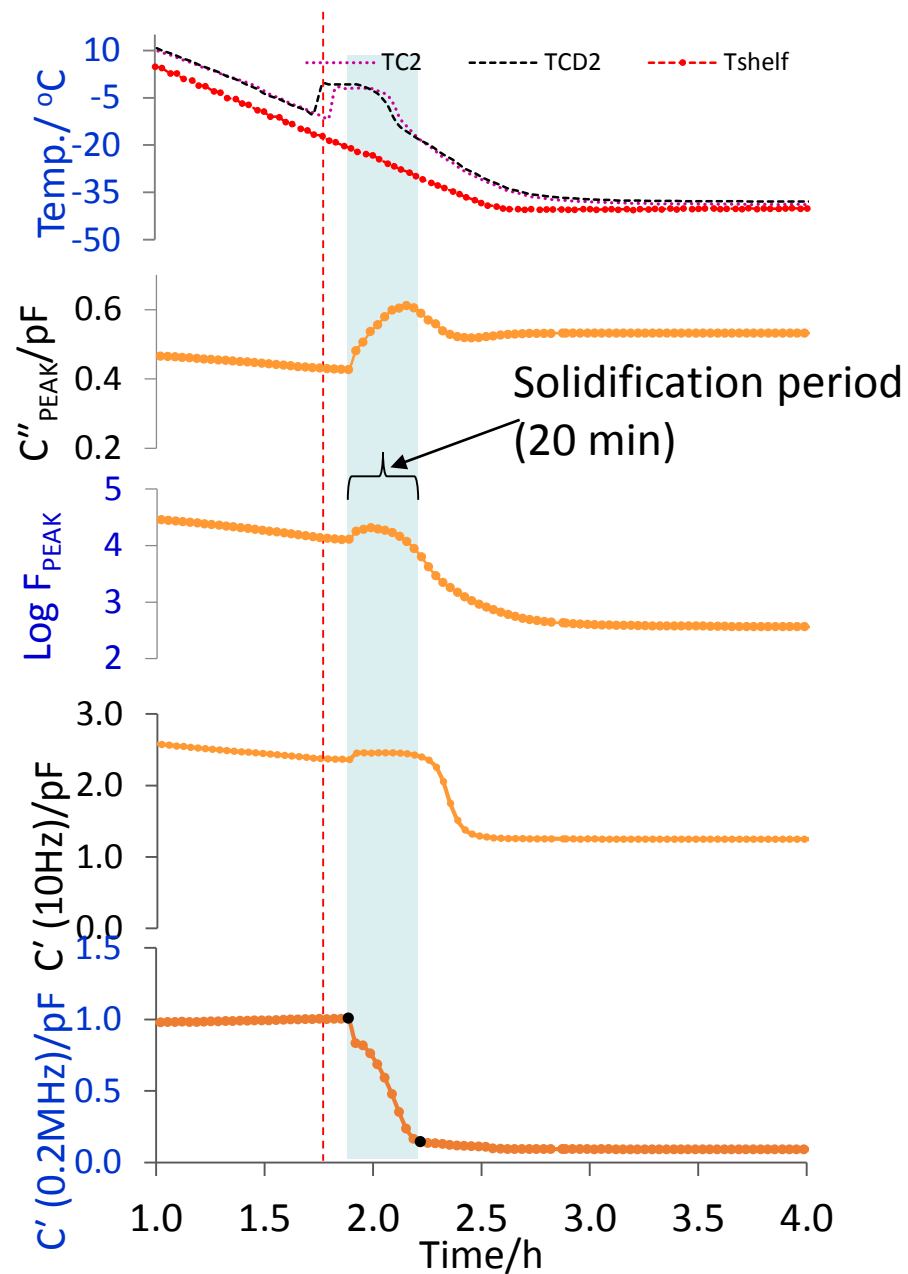
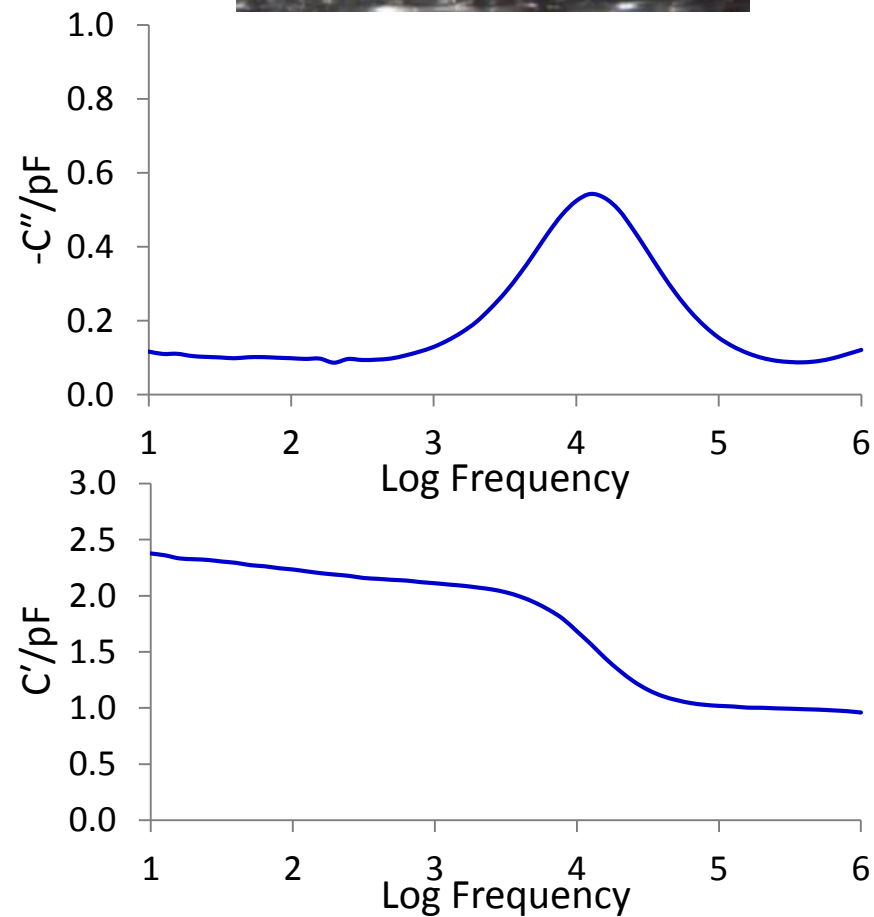


a) demonstrates C''_{PEAK} response to decreasing temperature with time by moving in two directions at a time: 1) lower frequencies (red arrow), and 2) downwards, reducing peak height (blue arrow). b) shows the event at the onset of ice growth depicted by sudden spike of C''_{PEAK} at 1.92 h when product temperature was -13°C . Evident pictures of the physical process shows that the peak upward spike was accompanied by a change of solution in vials to a cloud ice matrix from clear solution 3 minutes before the solidification onset.

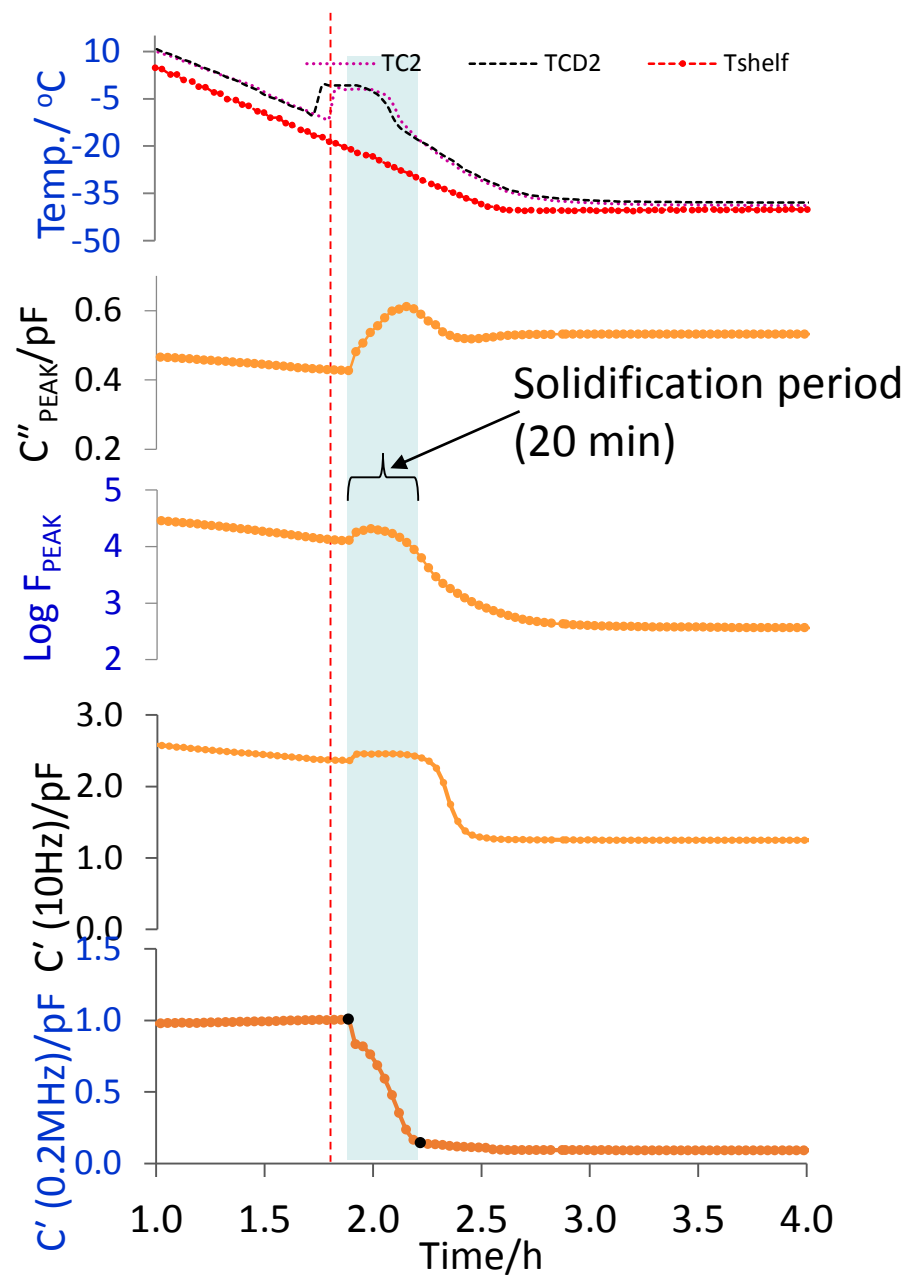
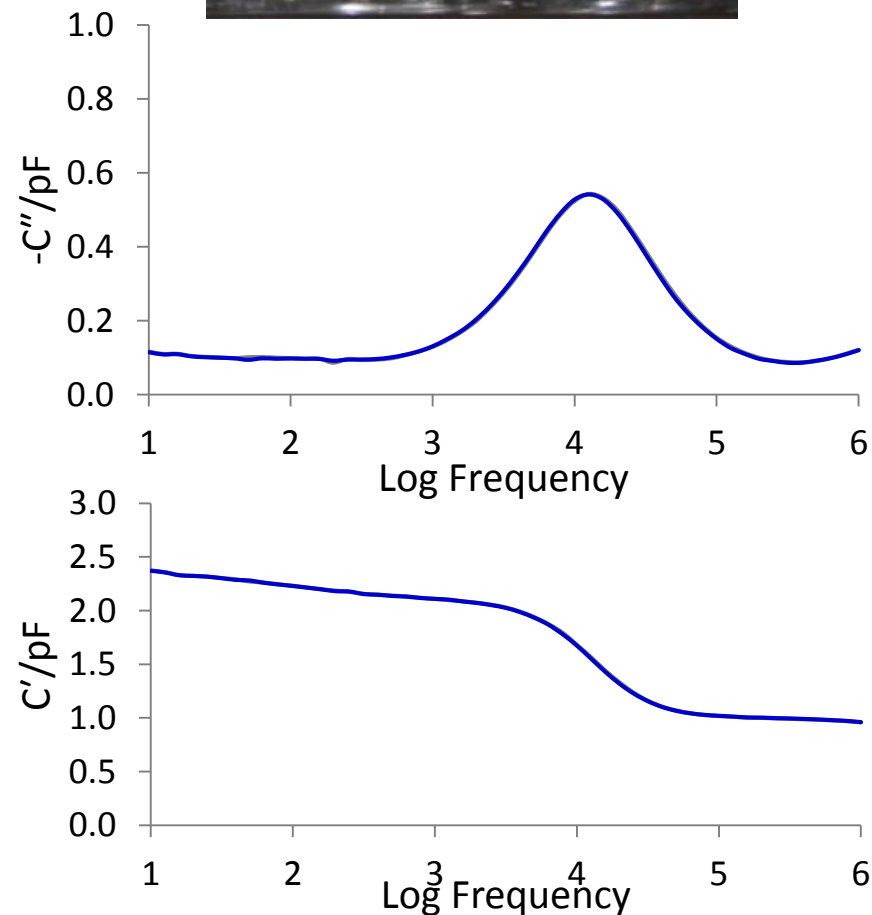
TVIS event identification with temperatures



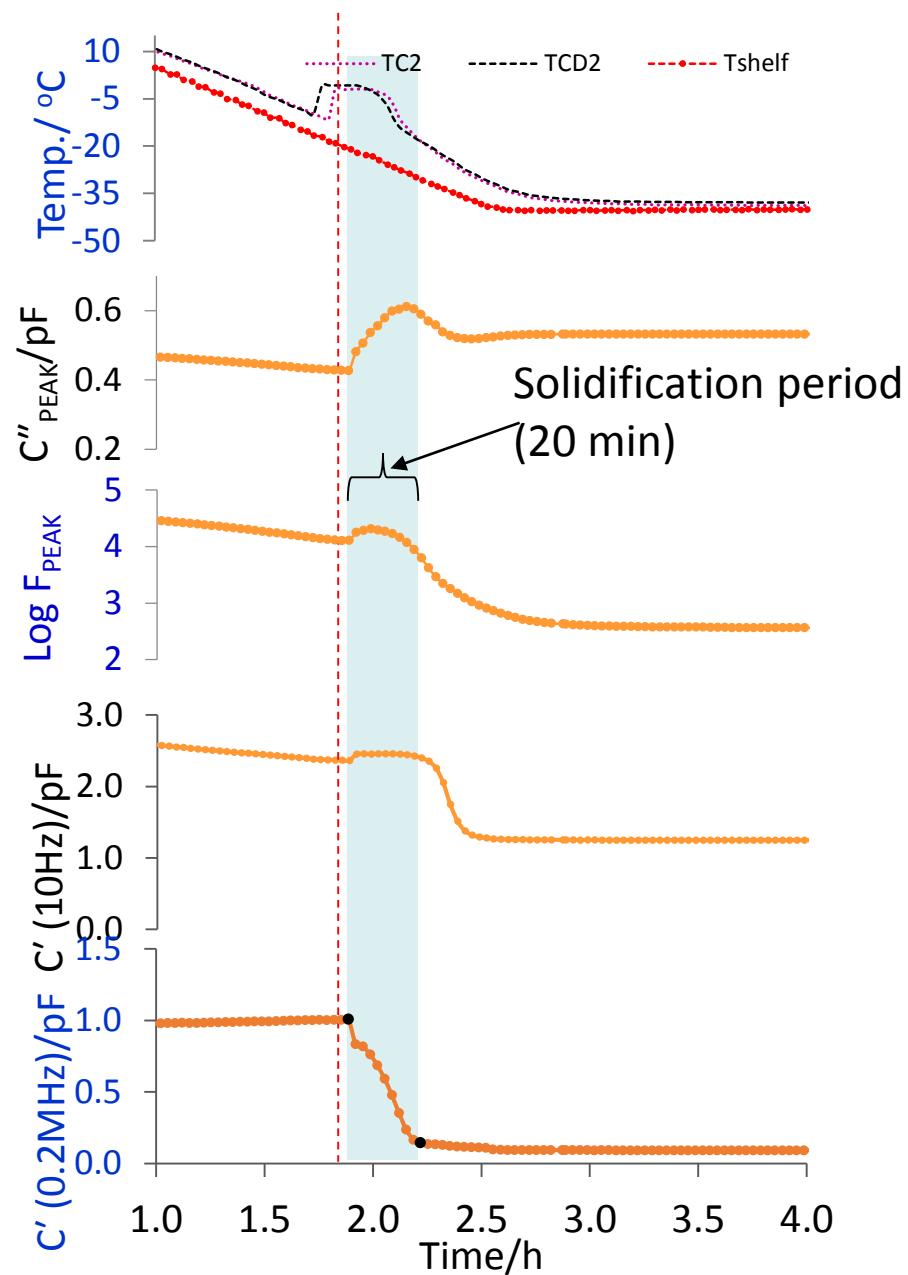
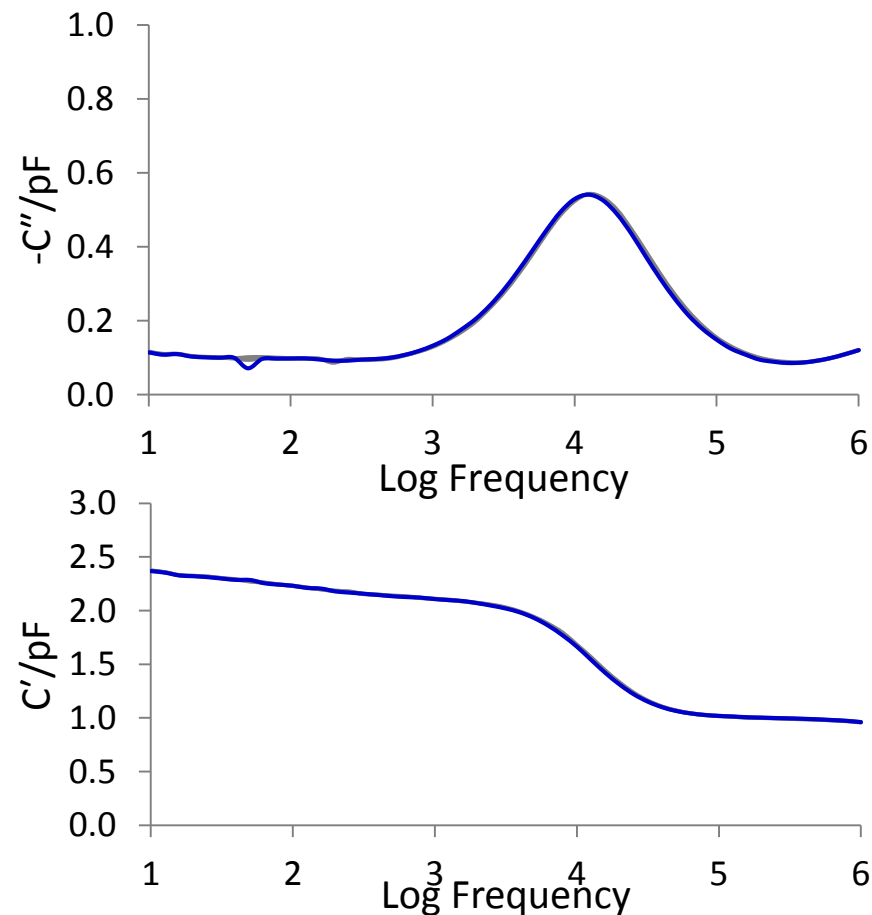
TVIS event identification with temperatures



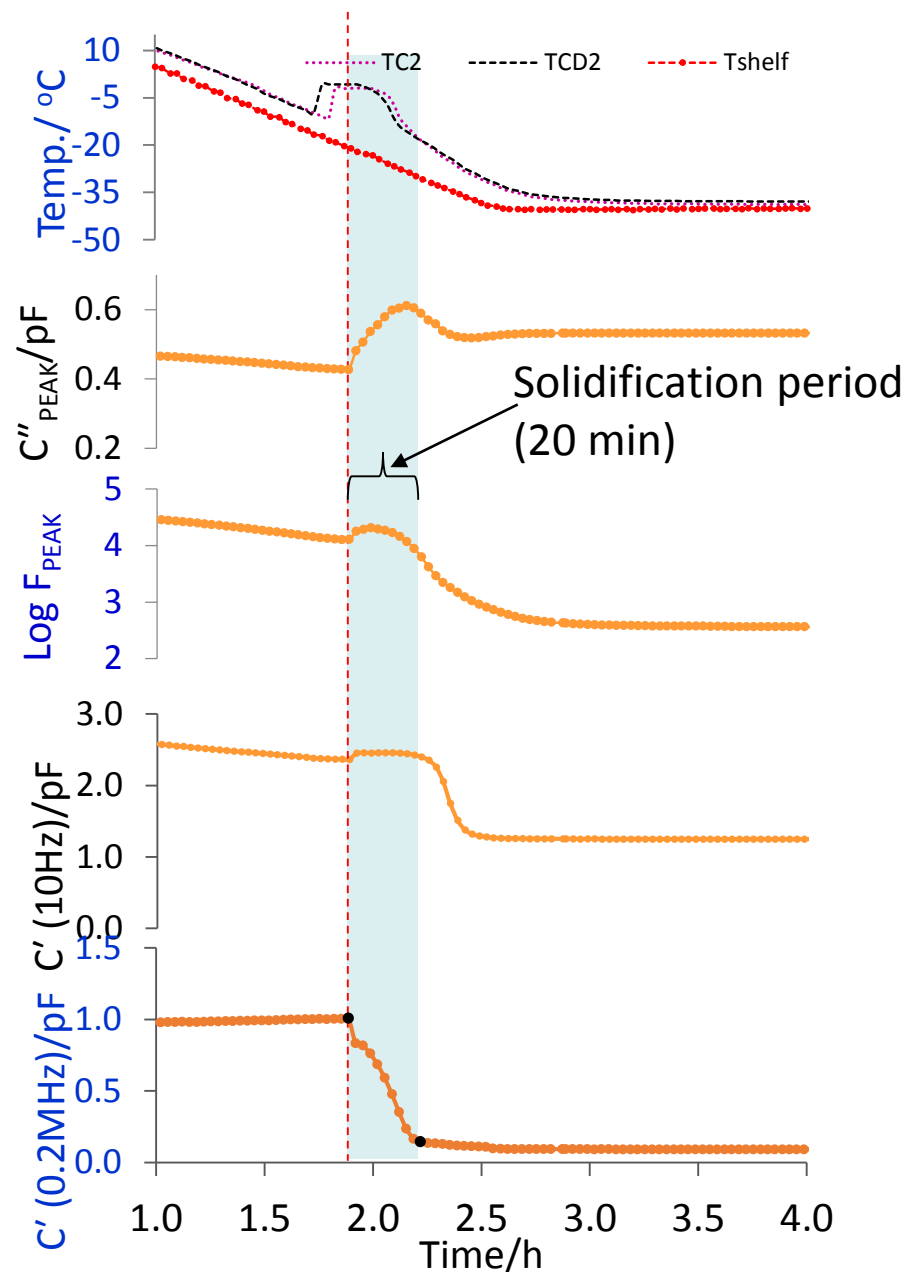
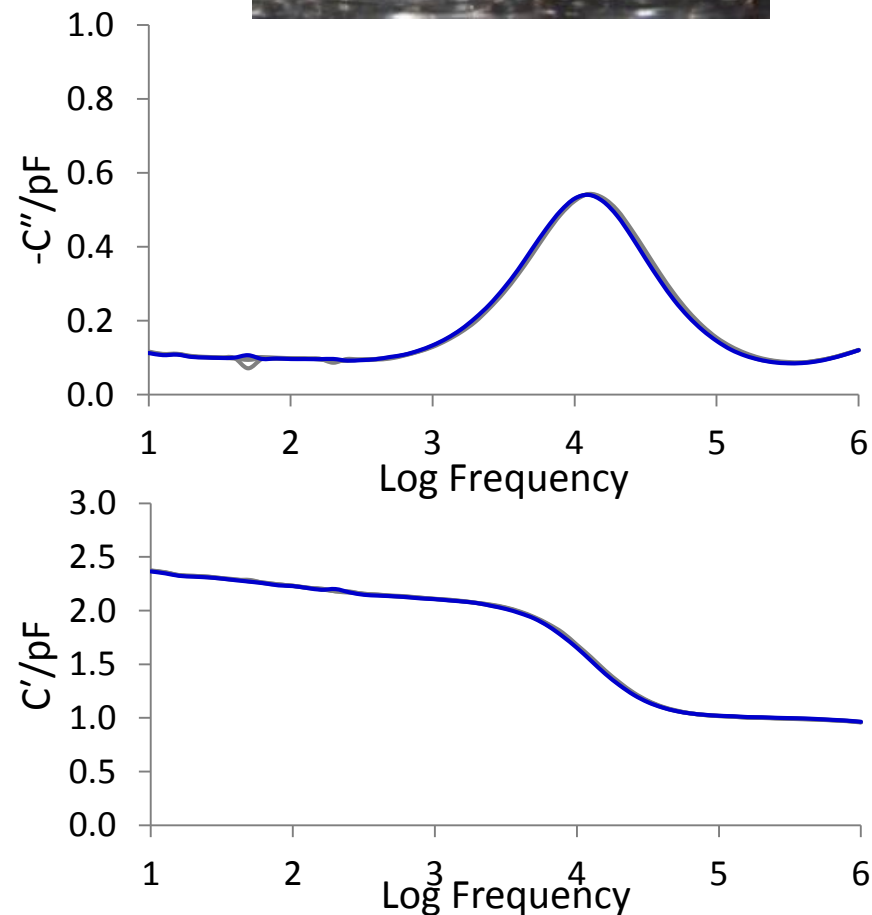
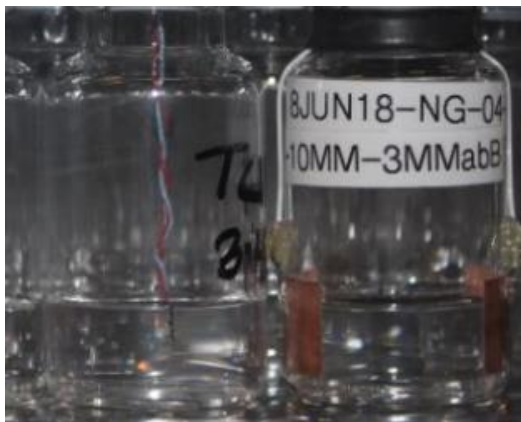
TVIS event identification with temperatures



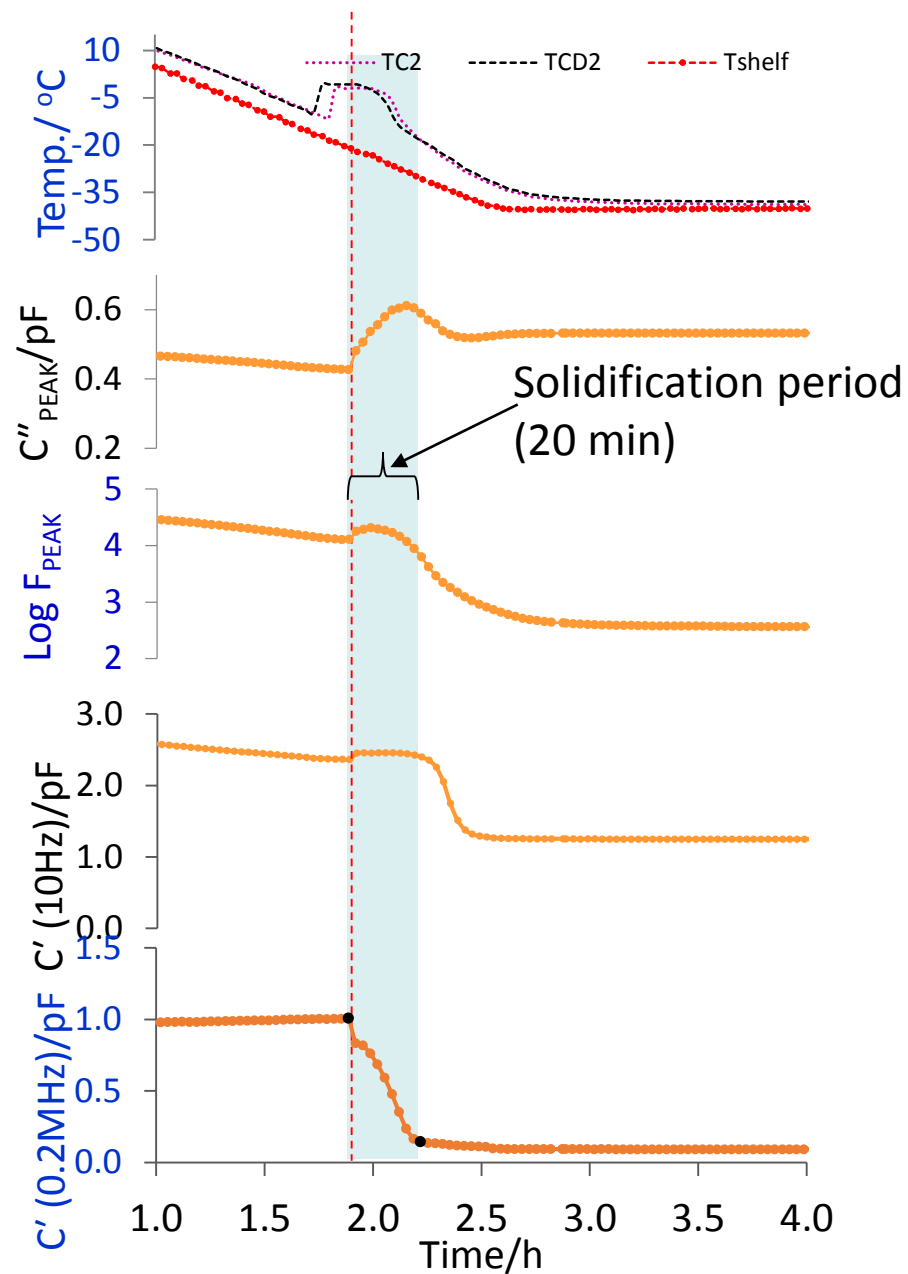
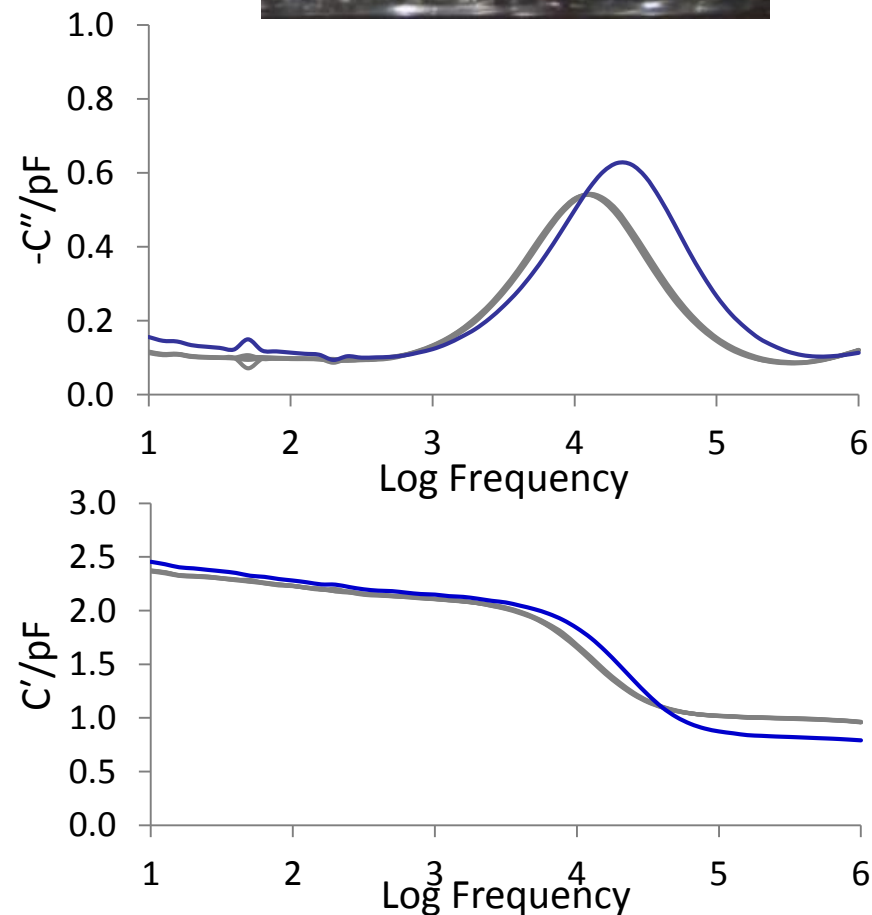
TVIS event identification with temperatures



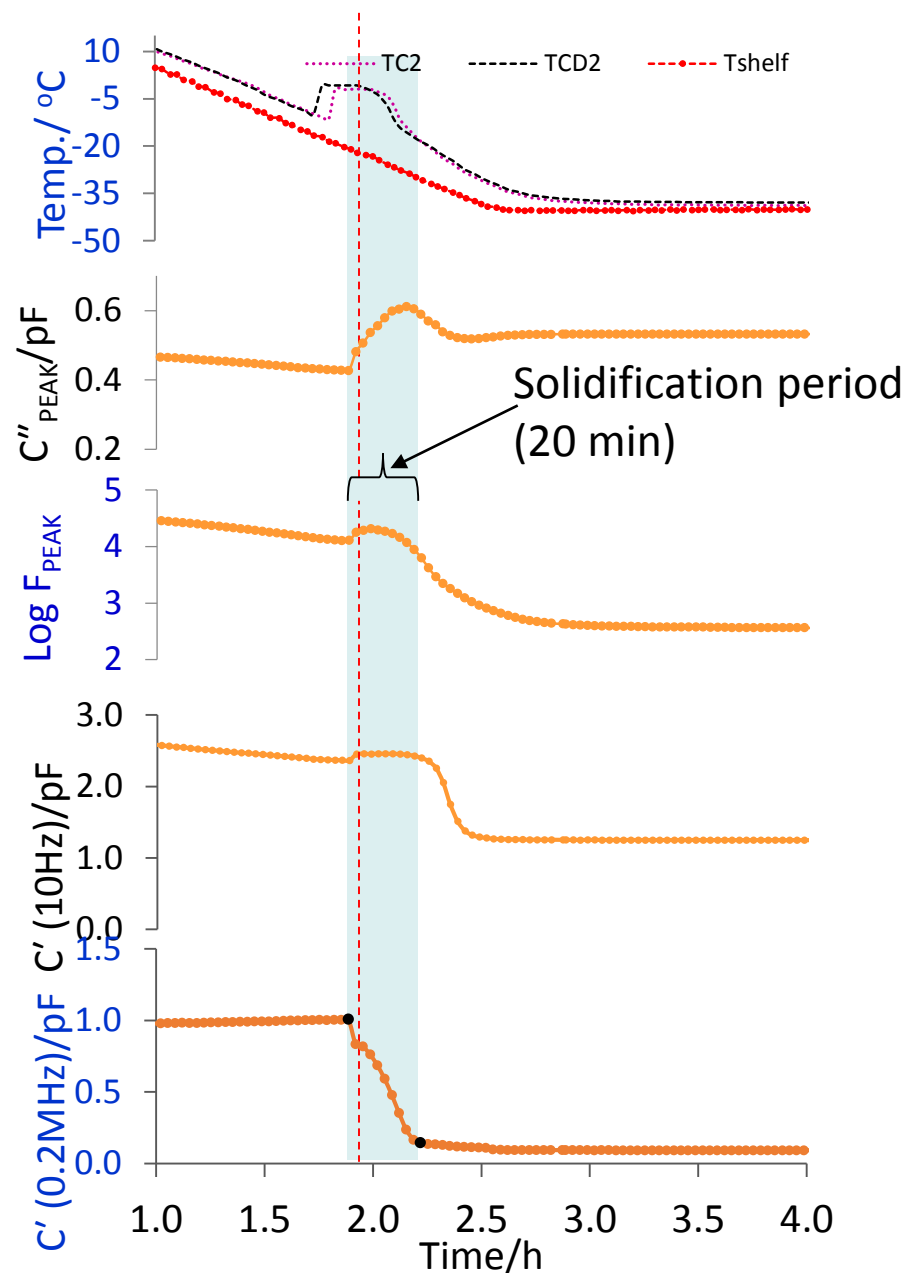
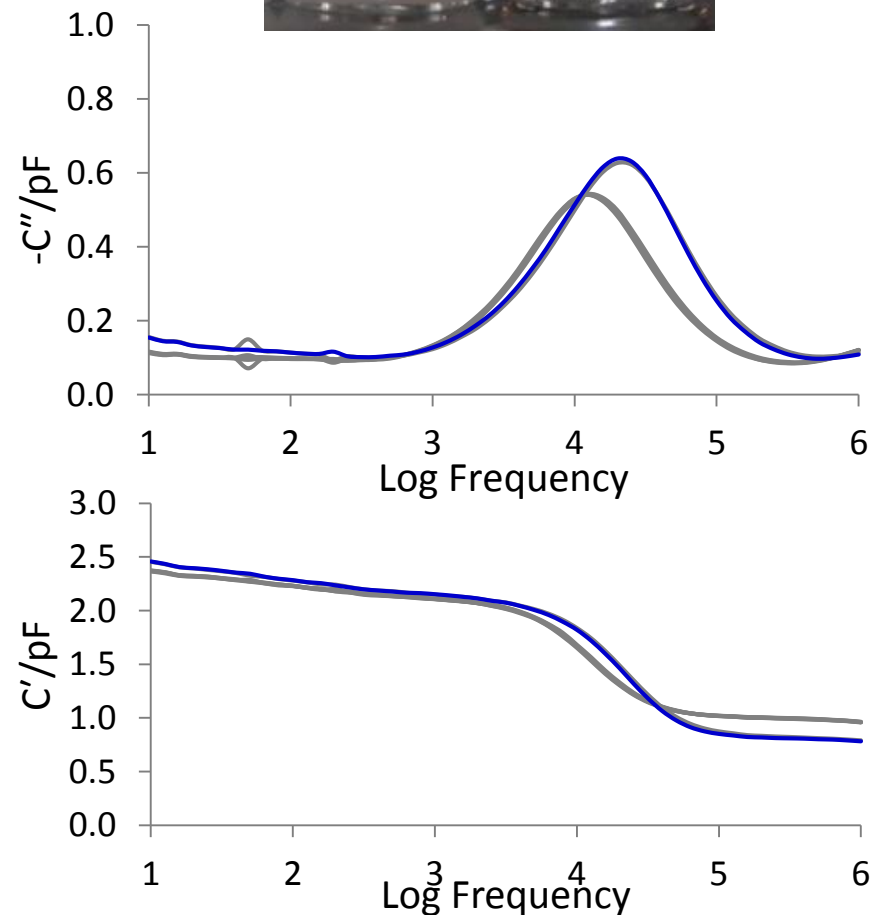
TVIS event identification with temperatures



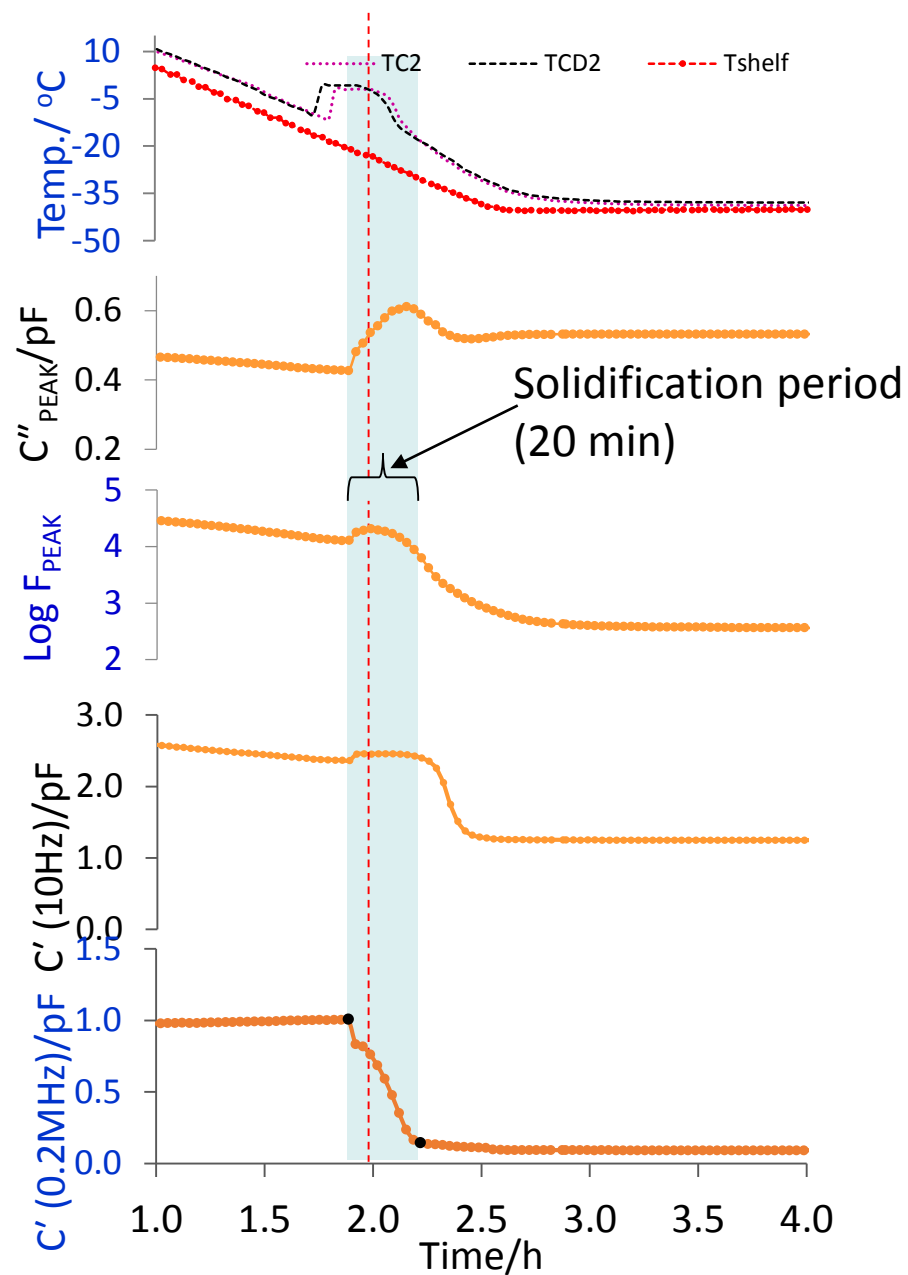
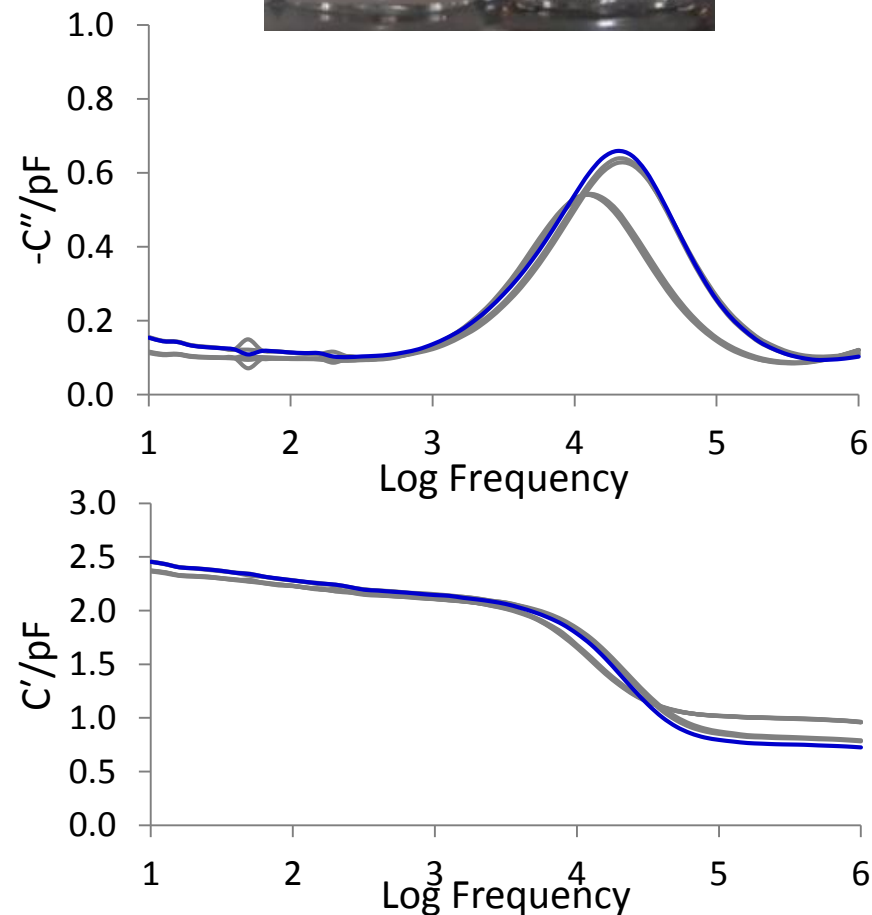
TVIS event identification with temperatures



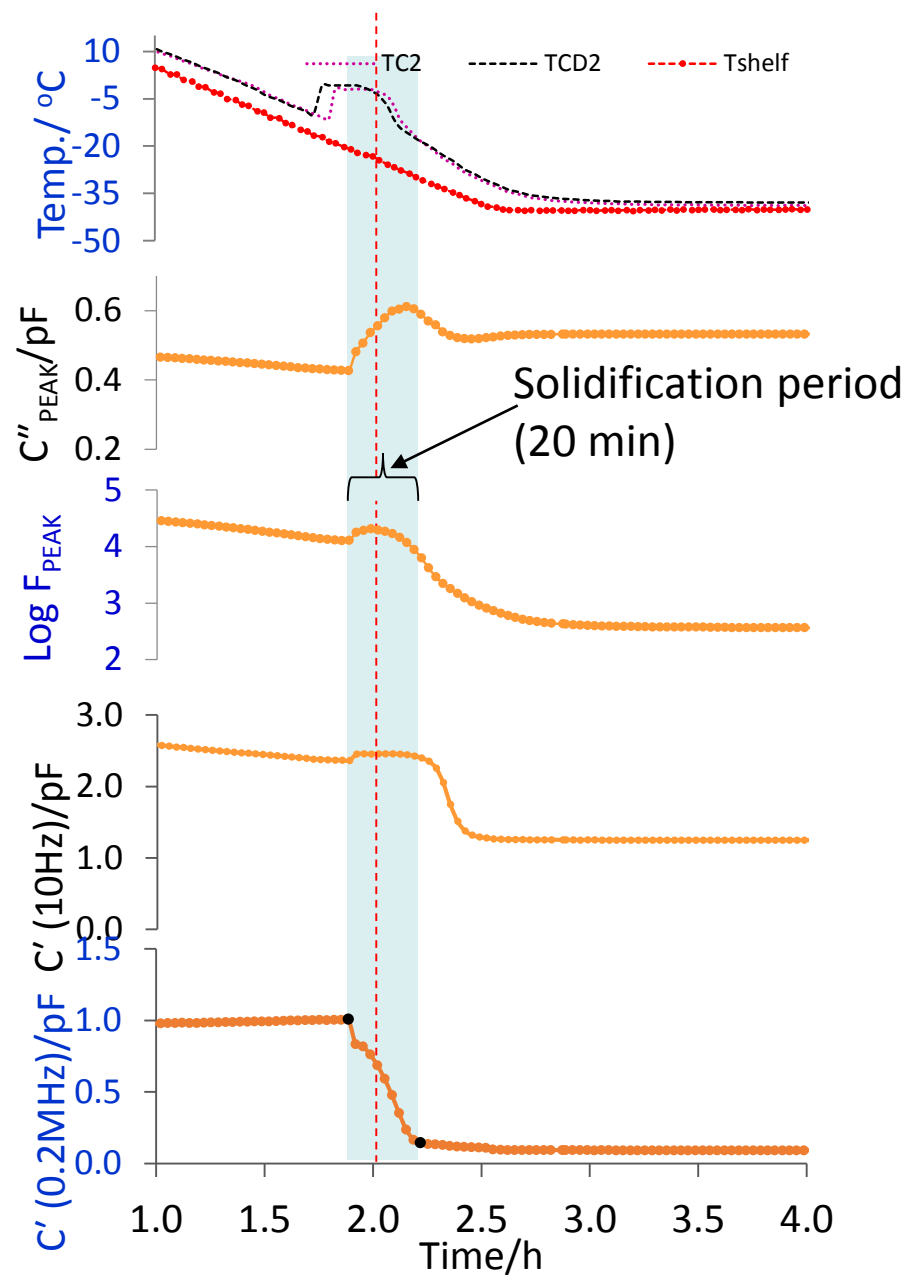
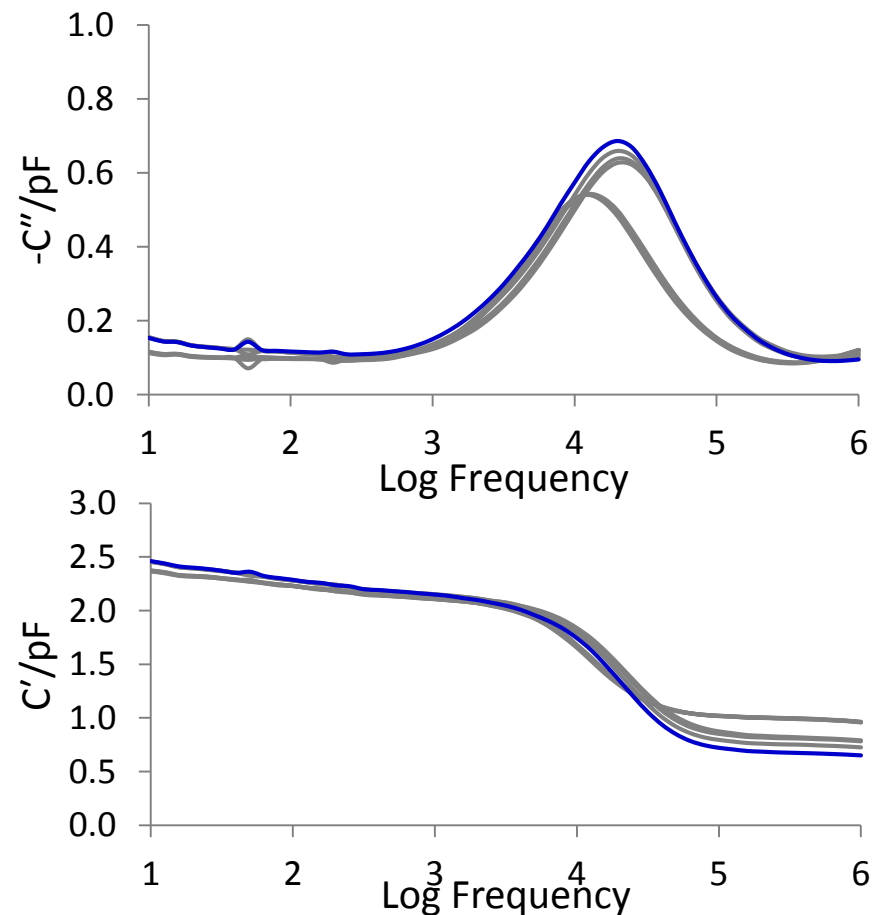
TVIS event identification with temperatures



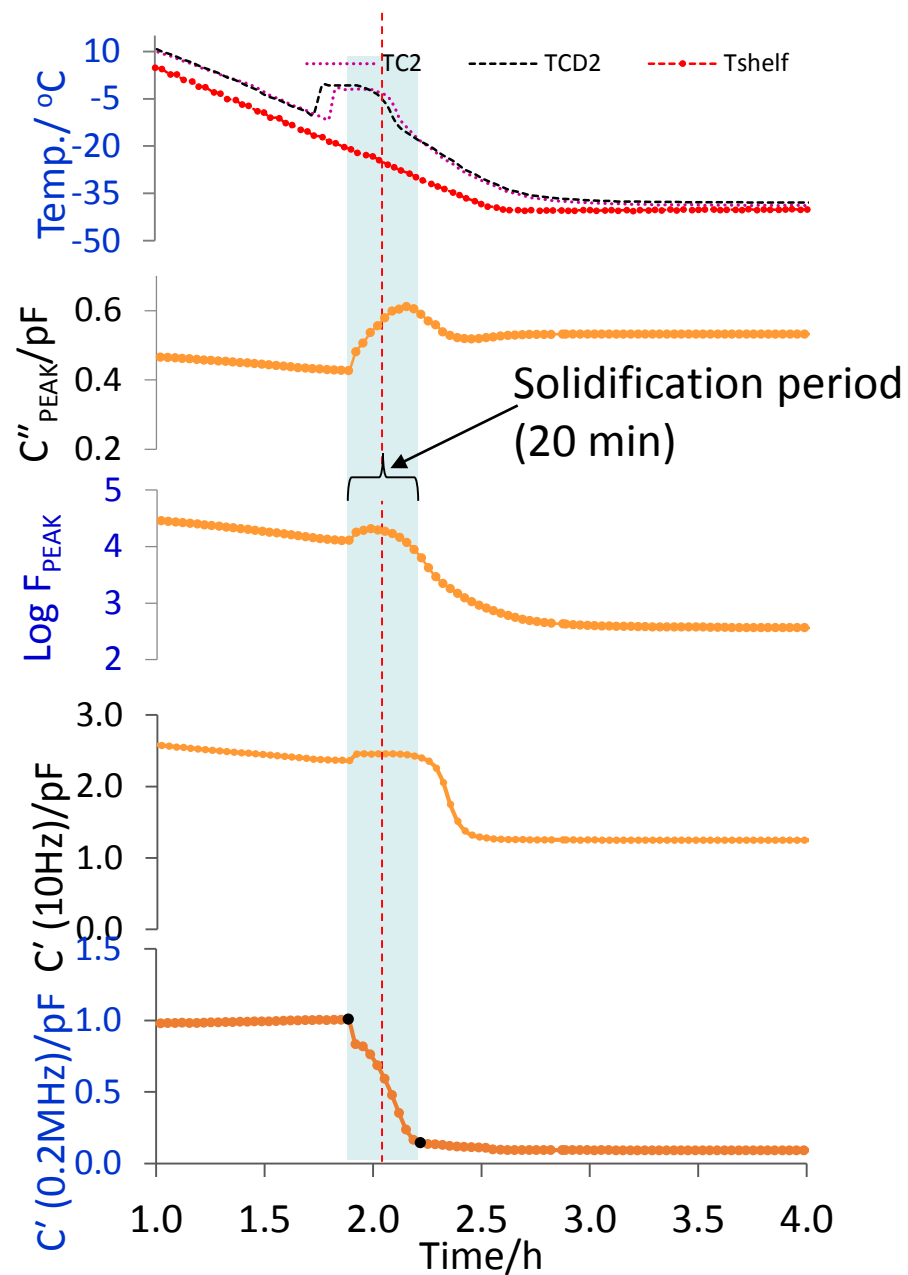
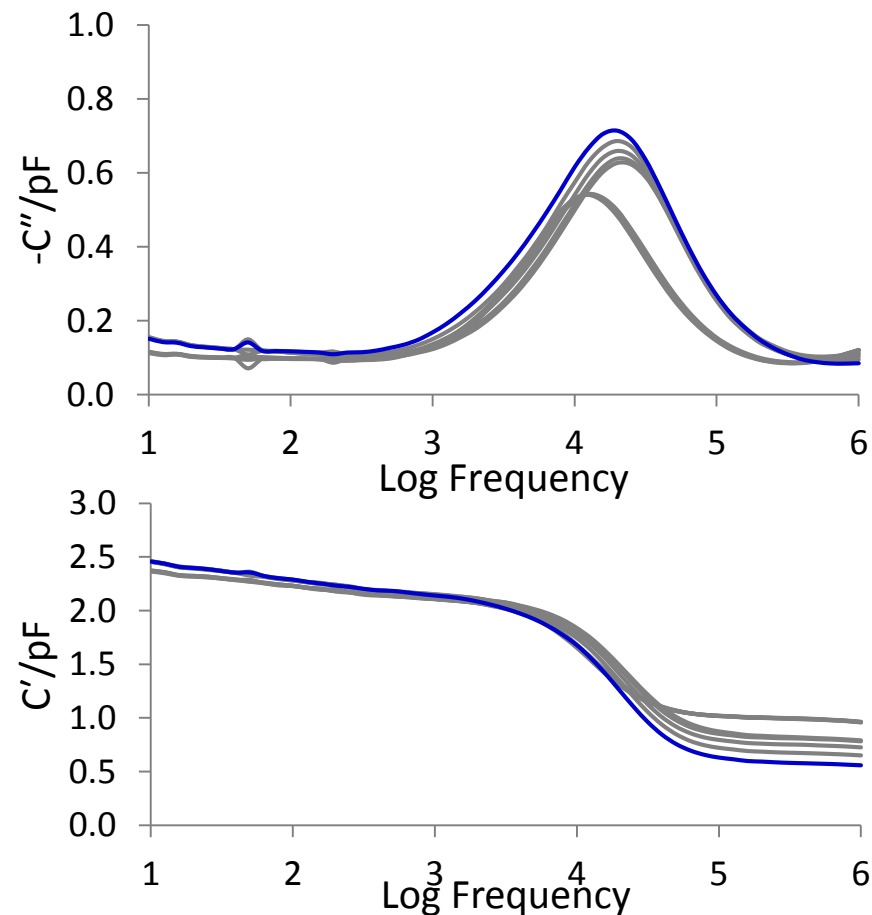
TVIS event identification with temperatures



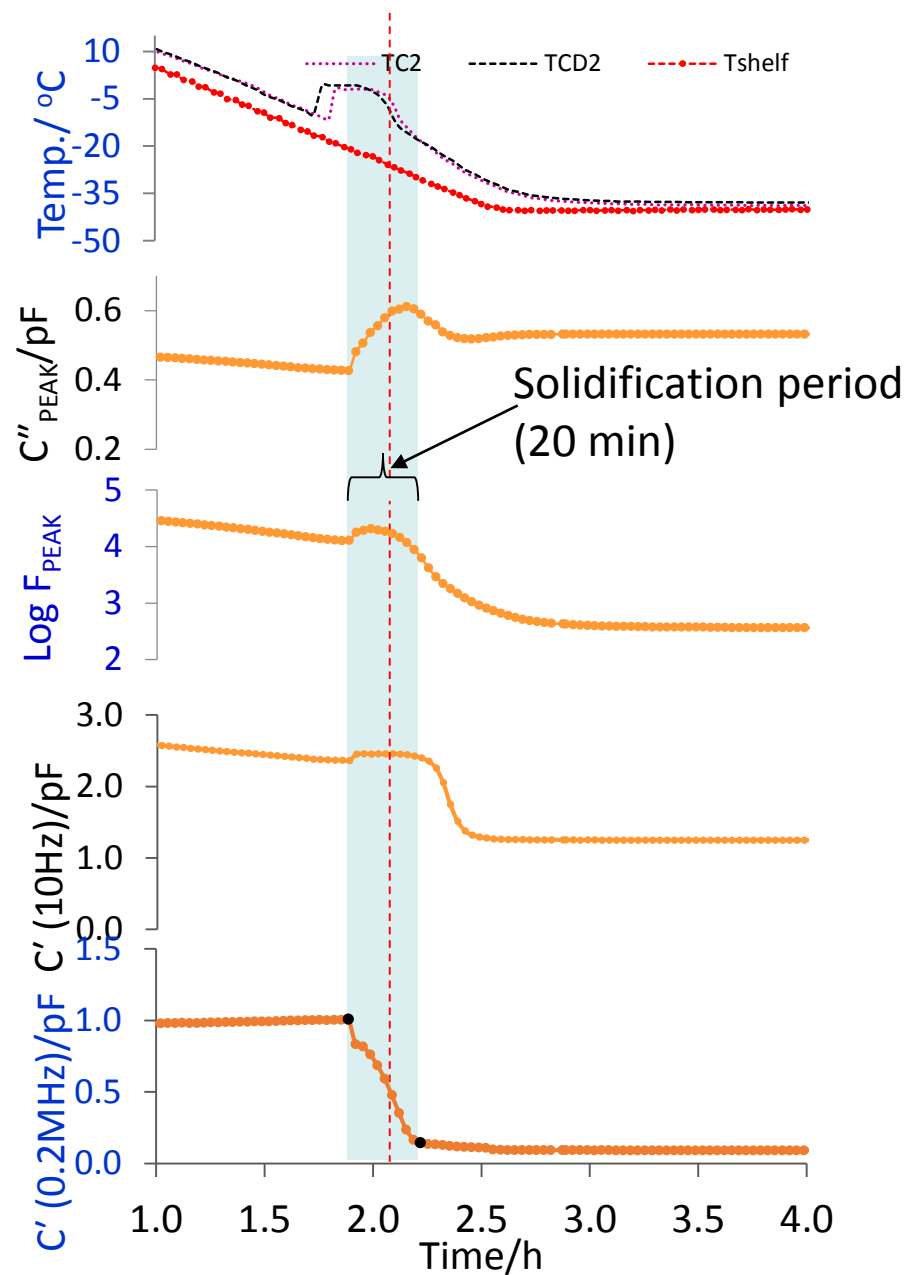
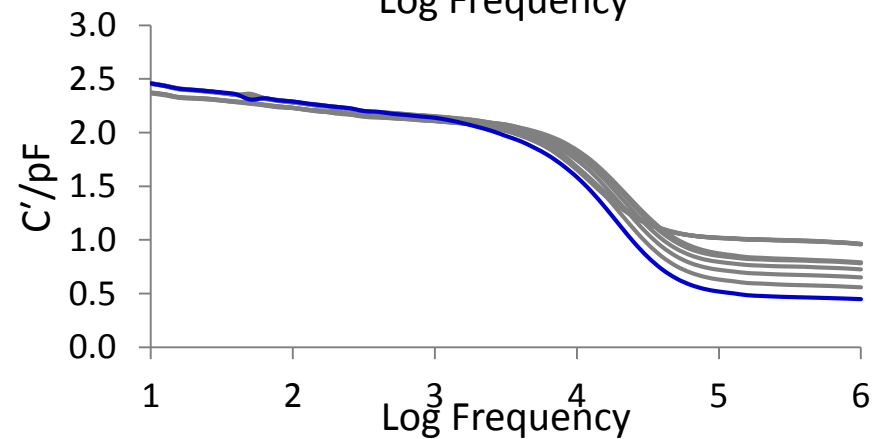
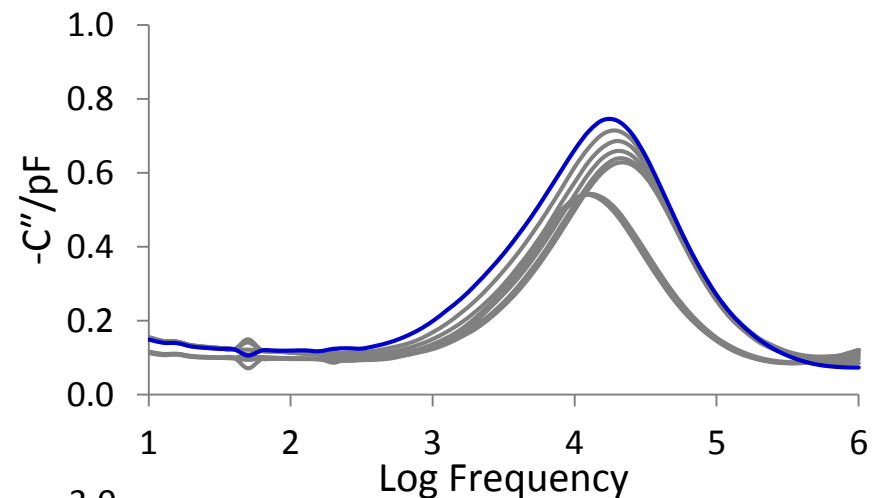
TVIS event identification with temperatures



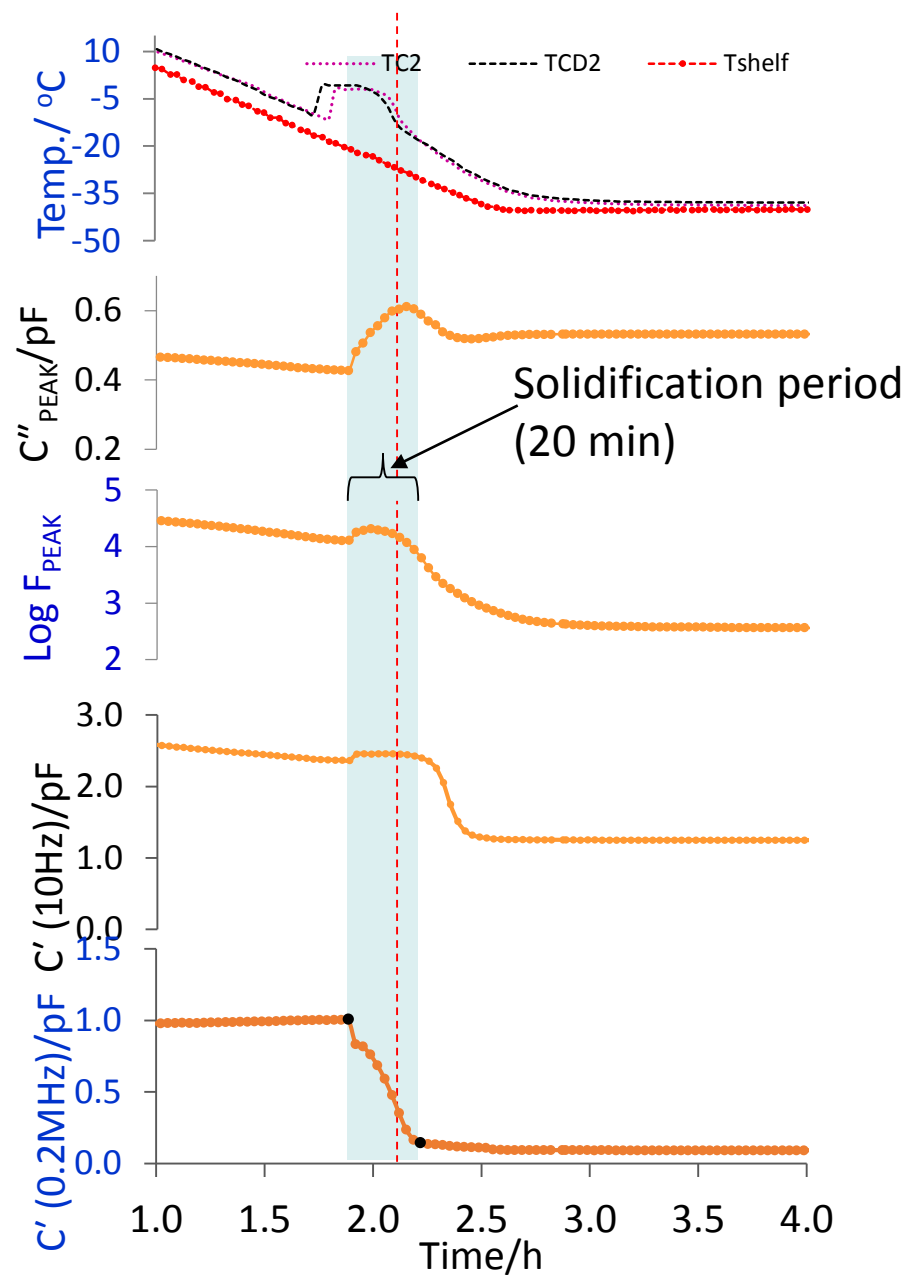
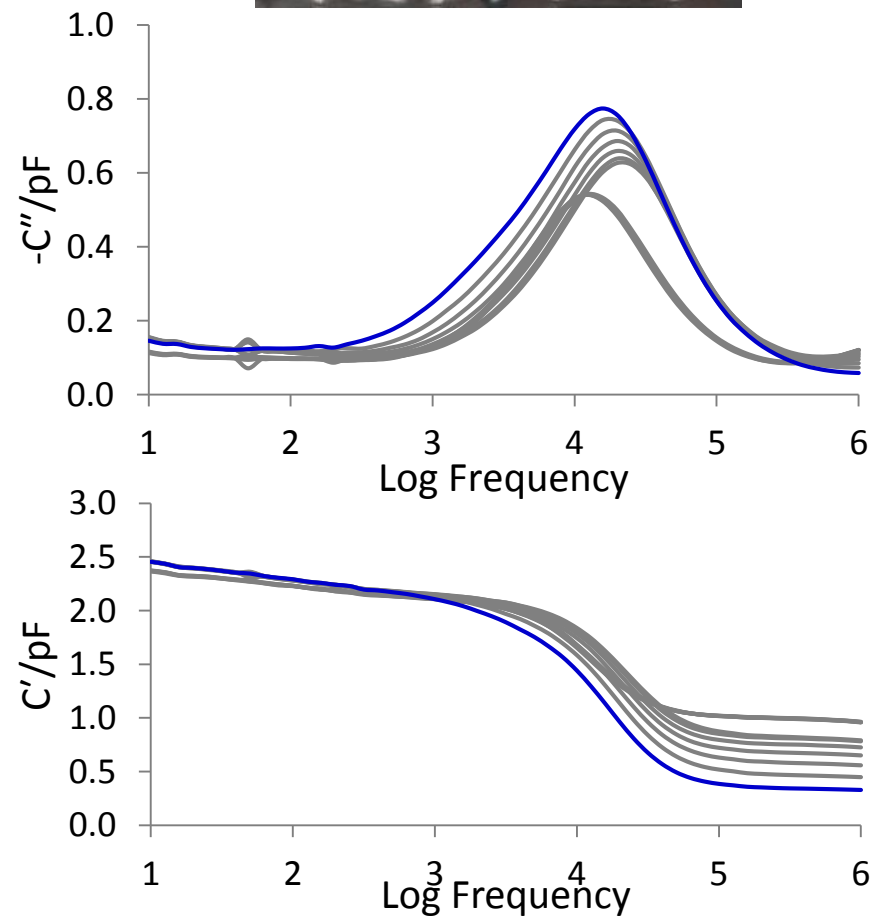
TVIS event identification with temperatures



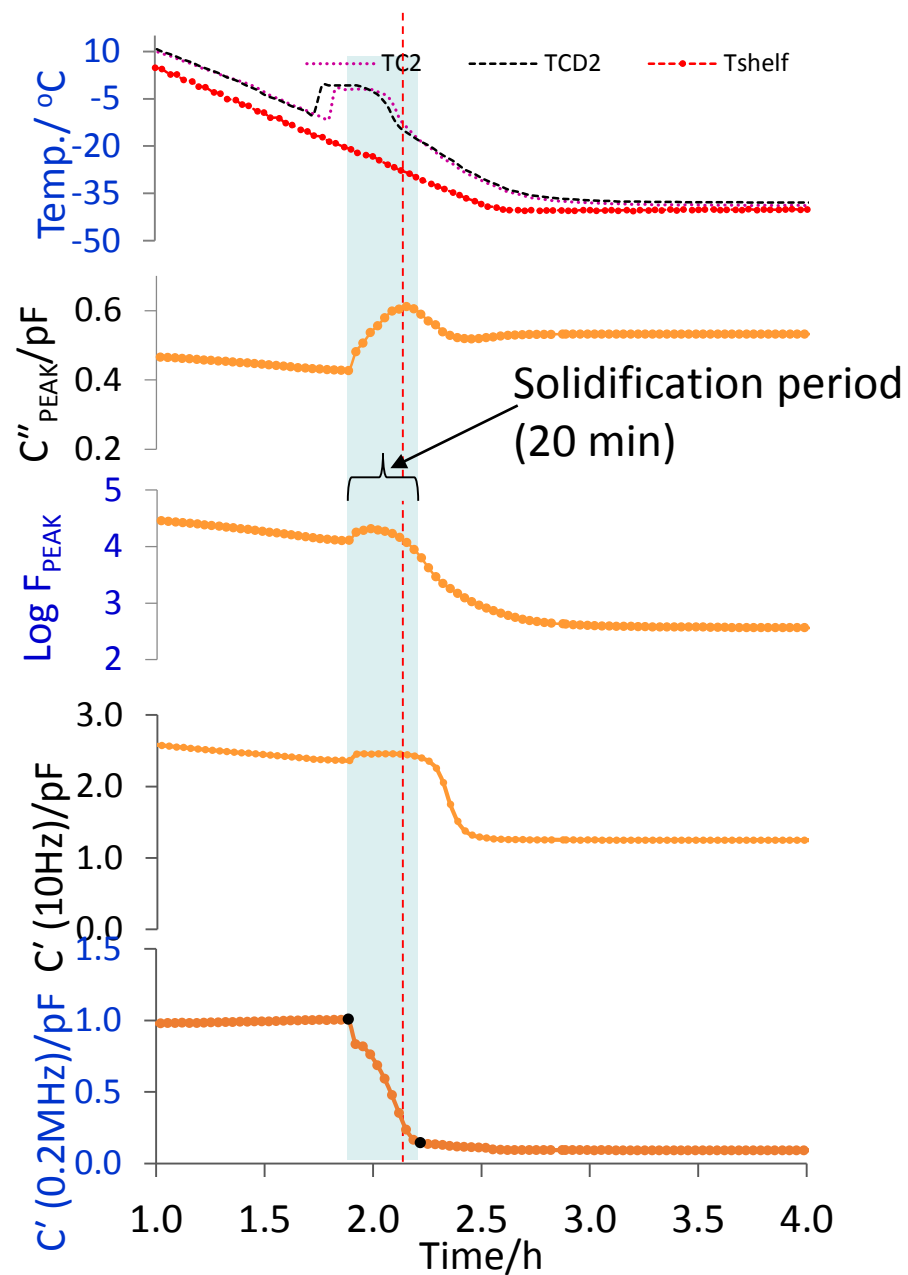
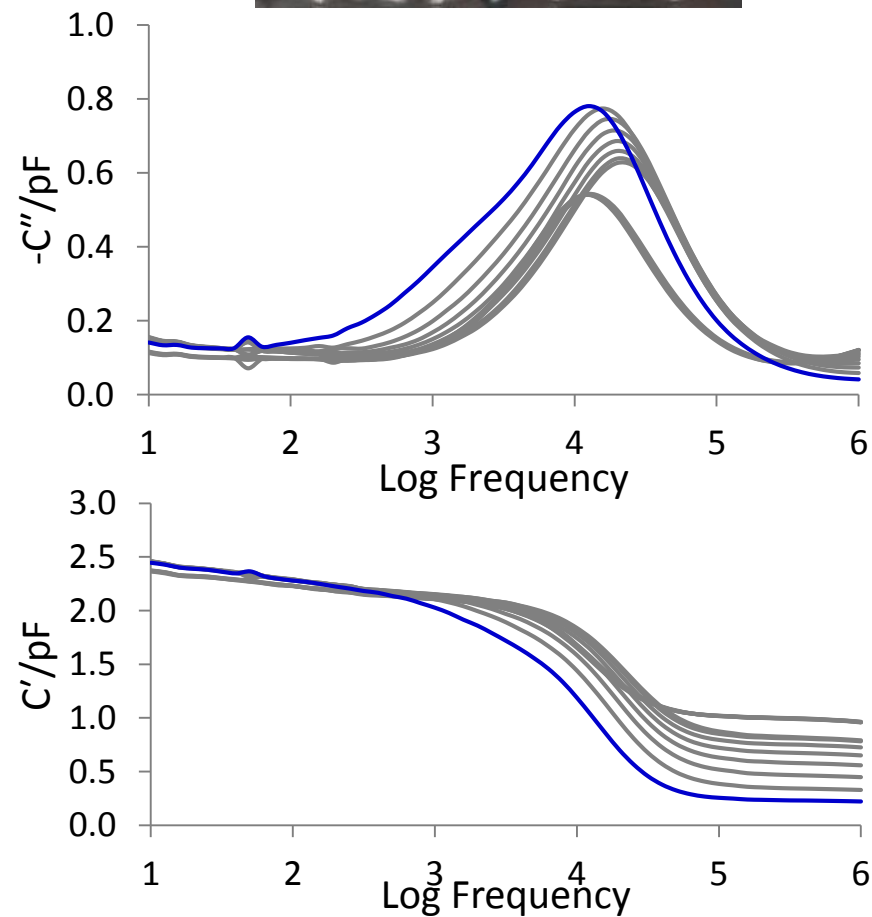
TVIS event identification with temperatures



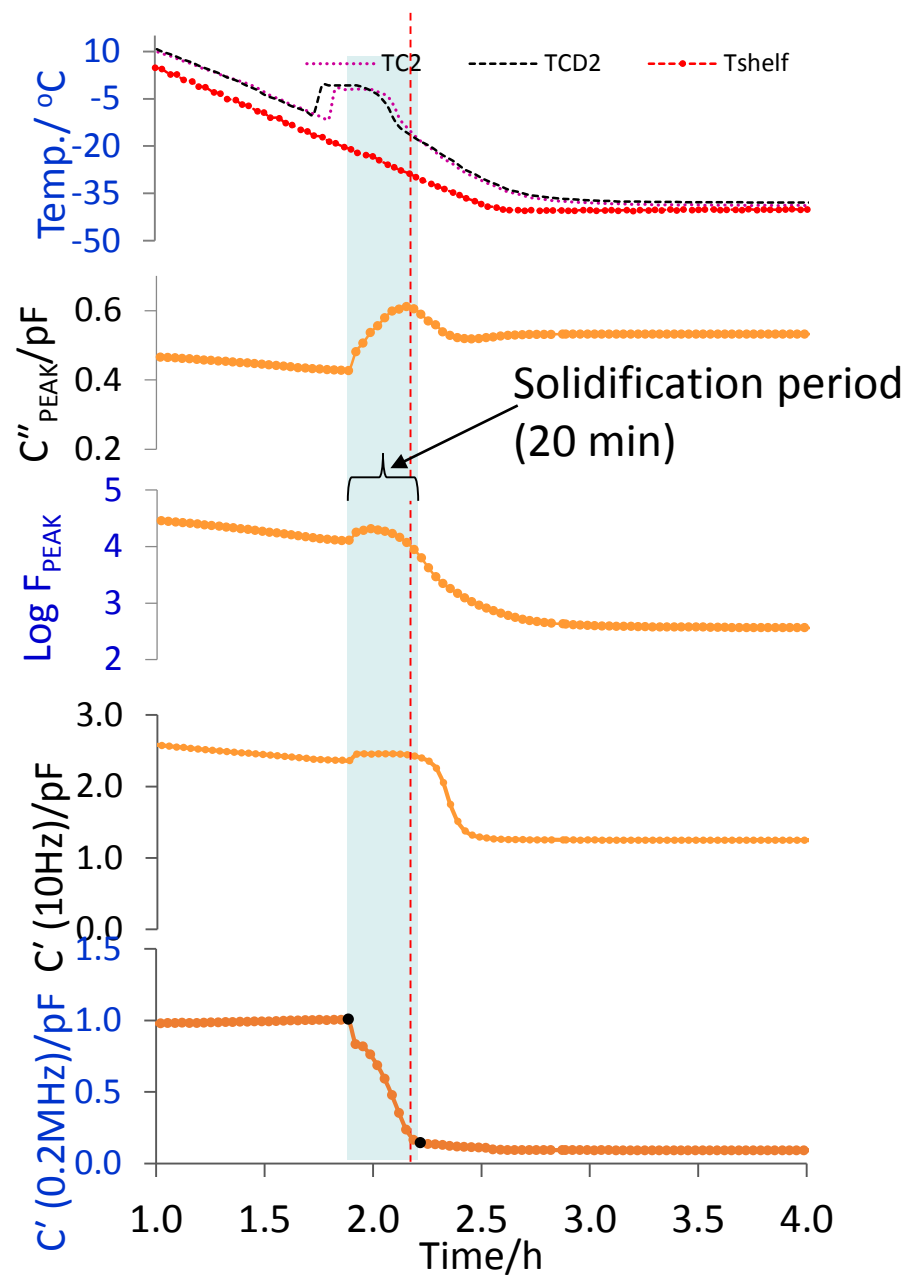
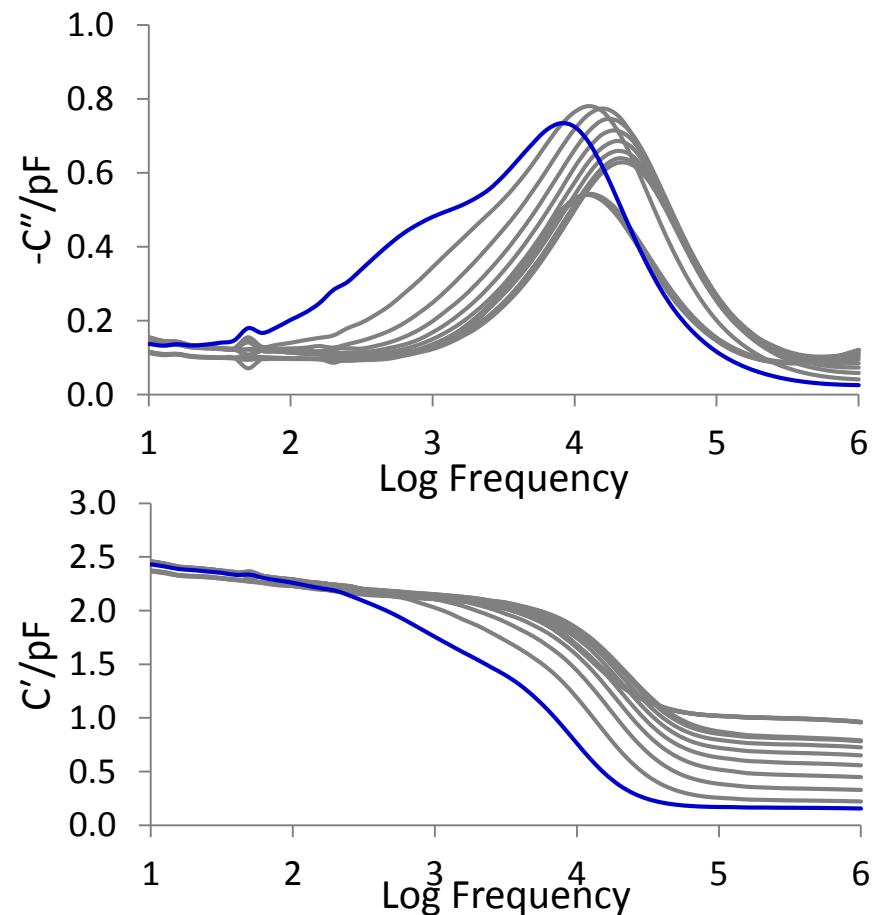
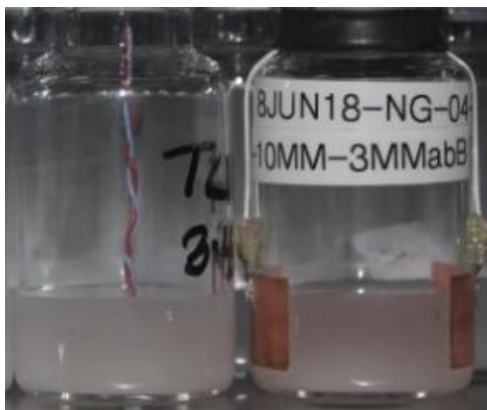
TVIS event identification with temperatures



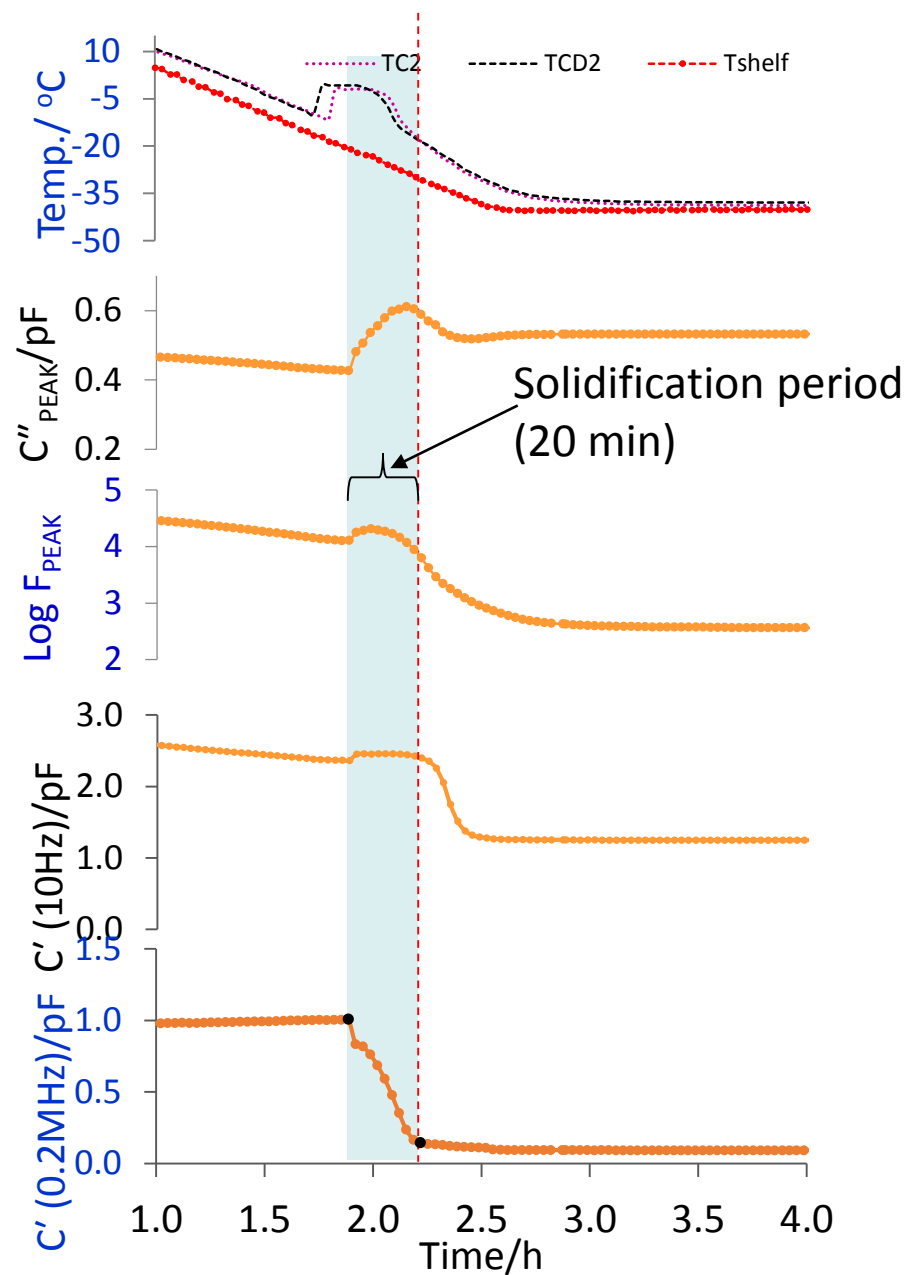
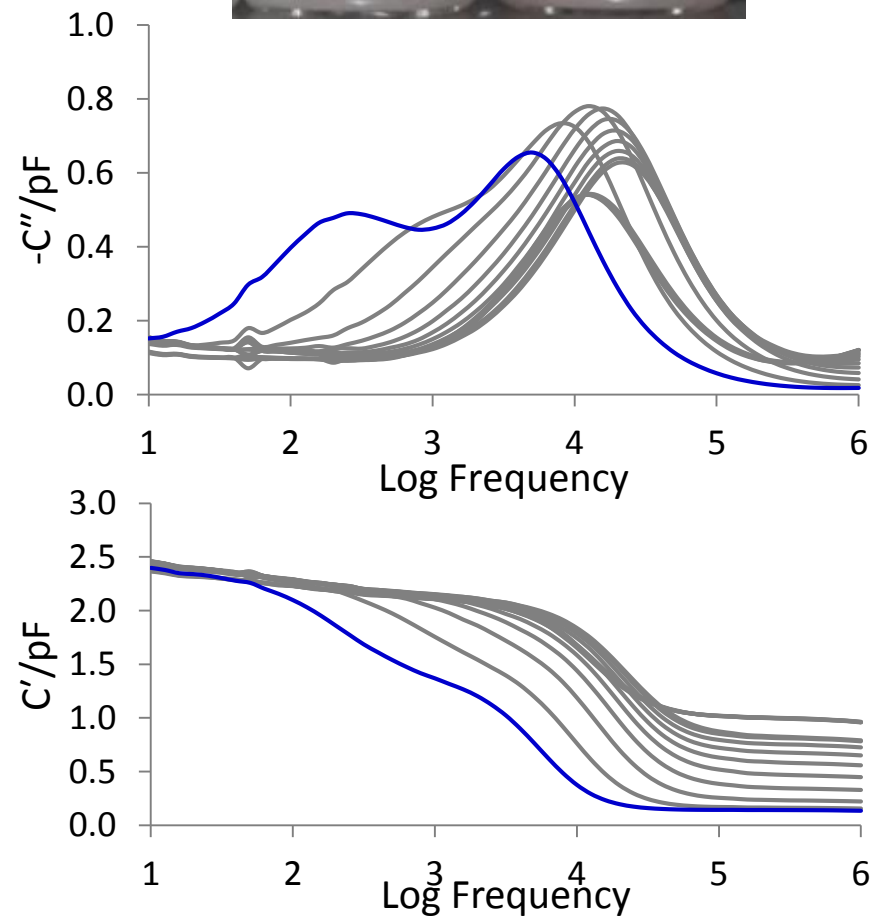
TVIS event identification with temperatures



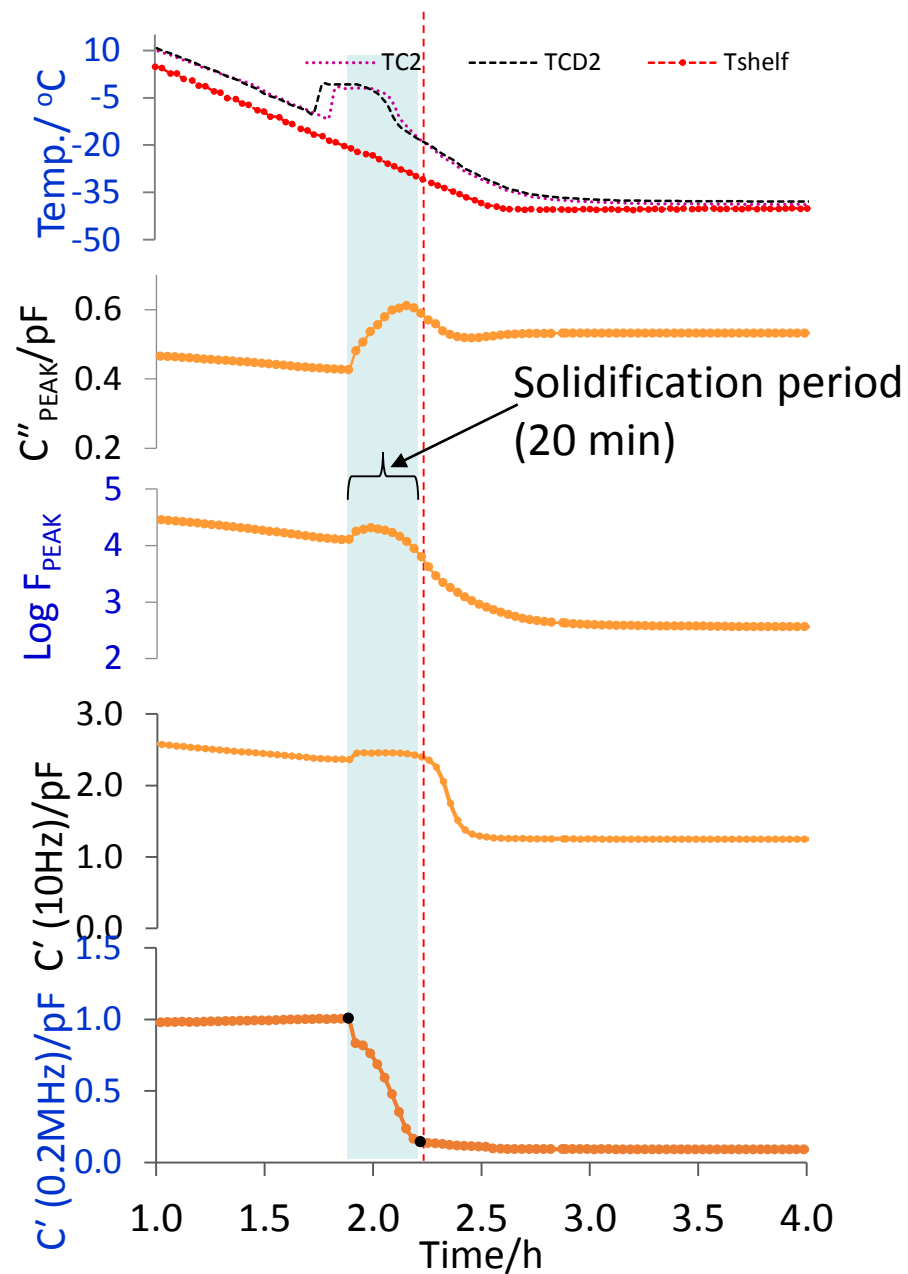
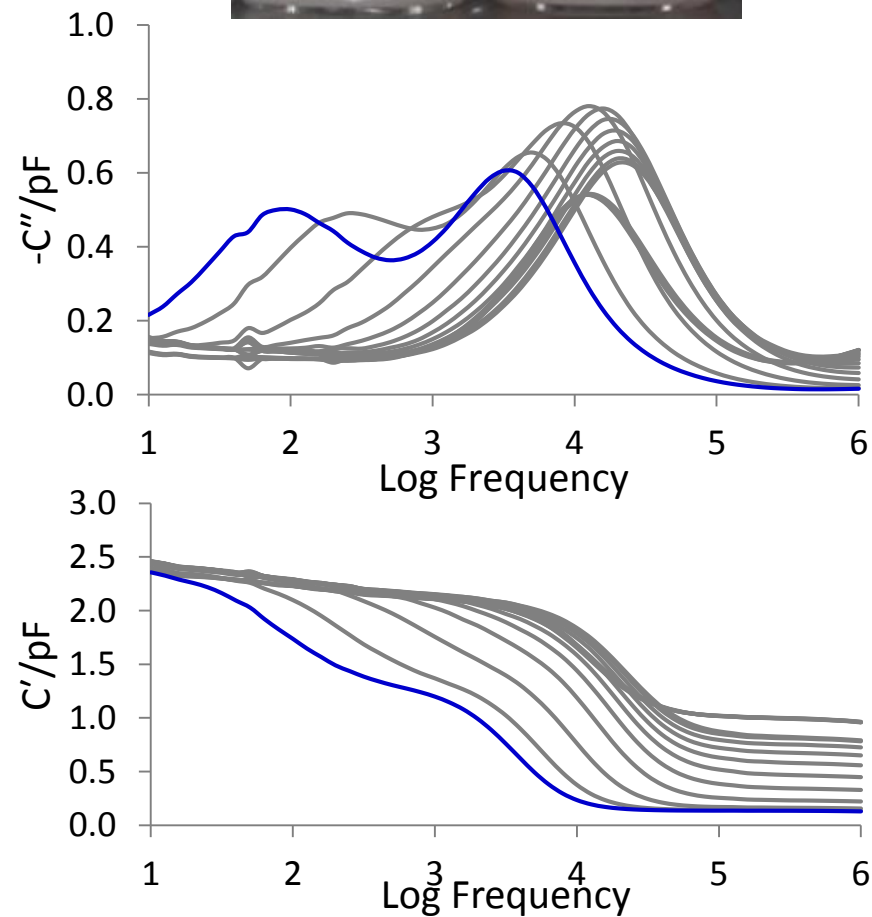
TVIS event identification with temperatures



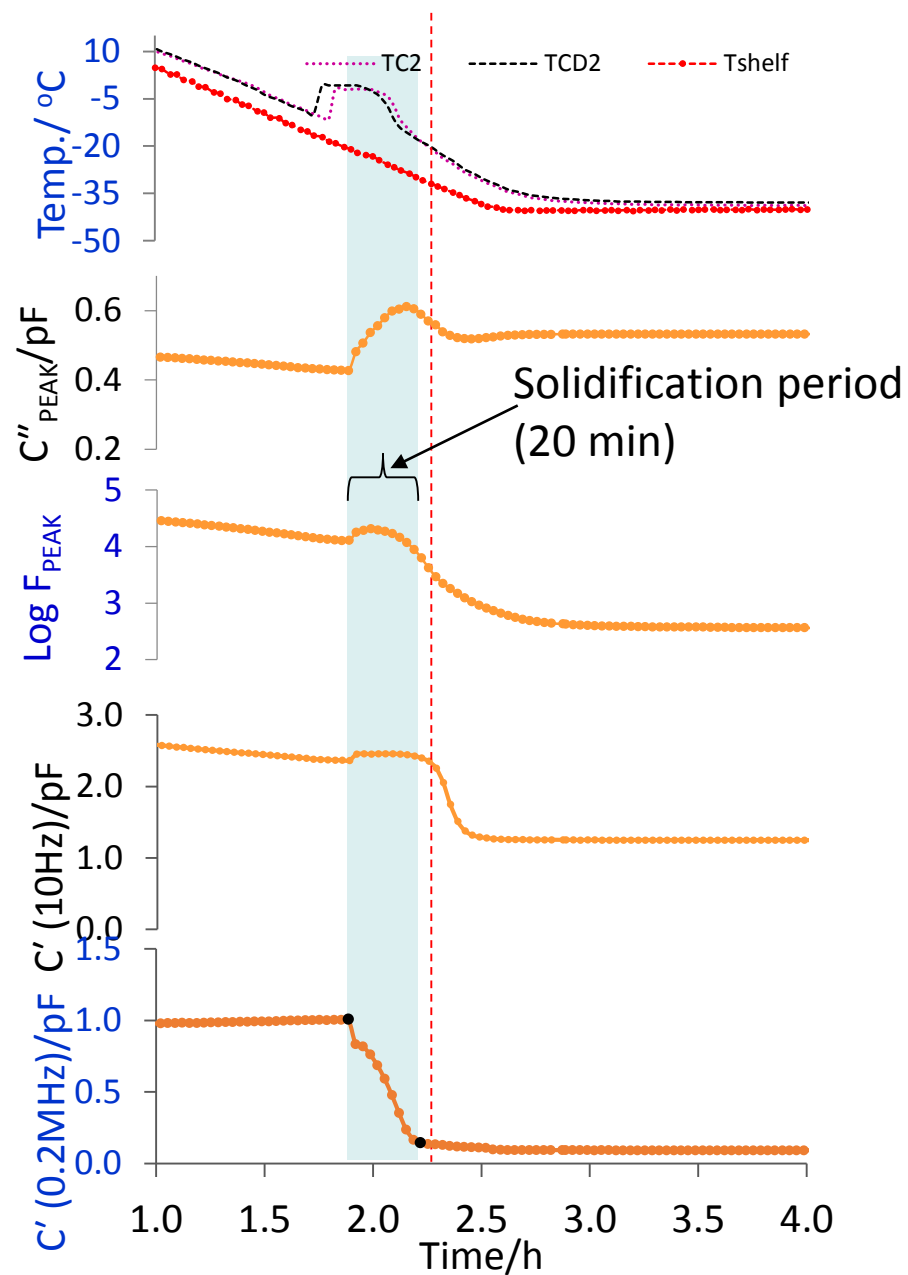
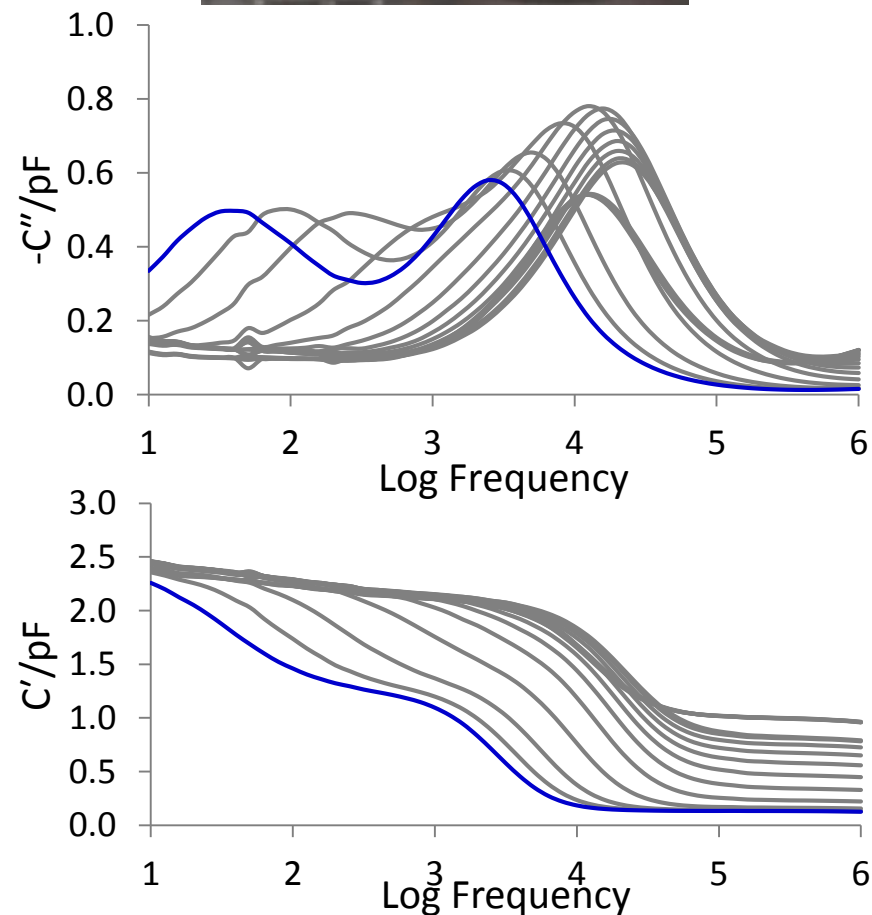
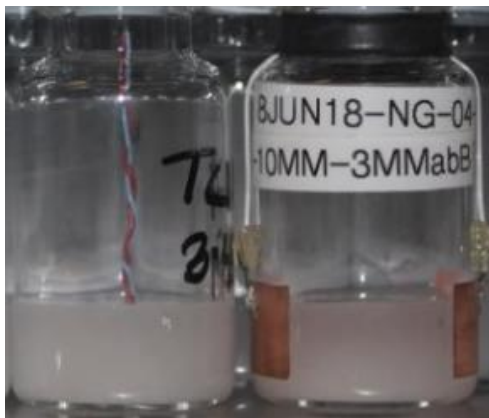
TVIS event identification with temperatures



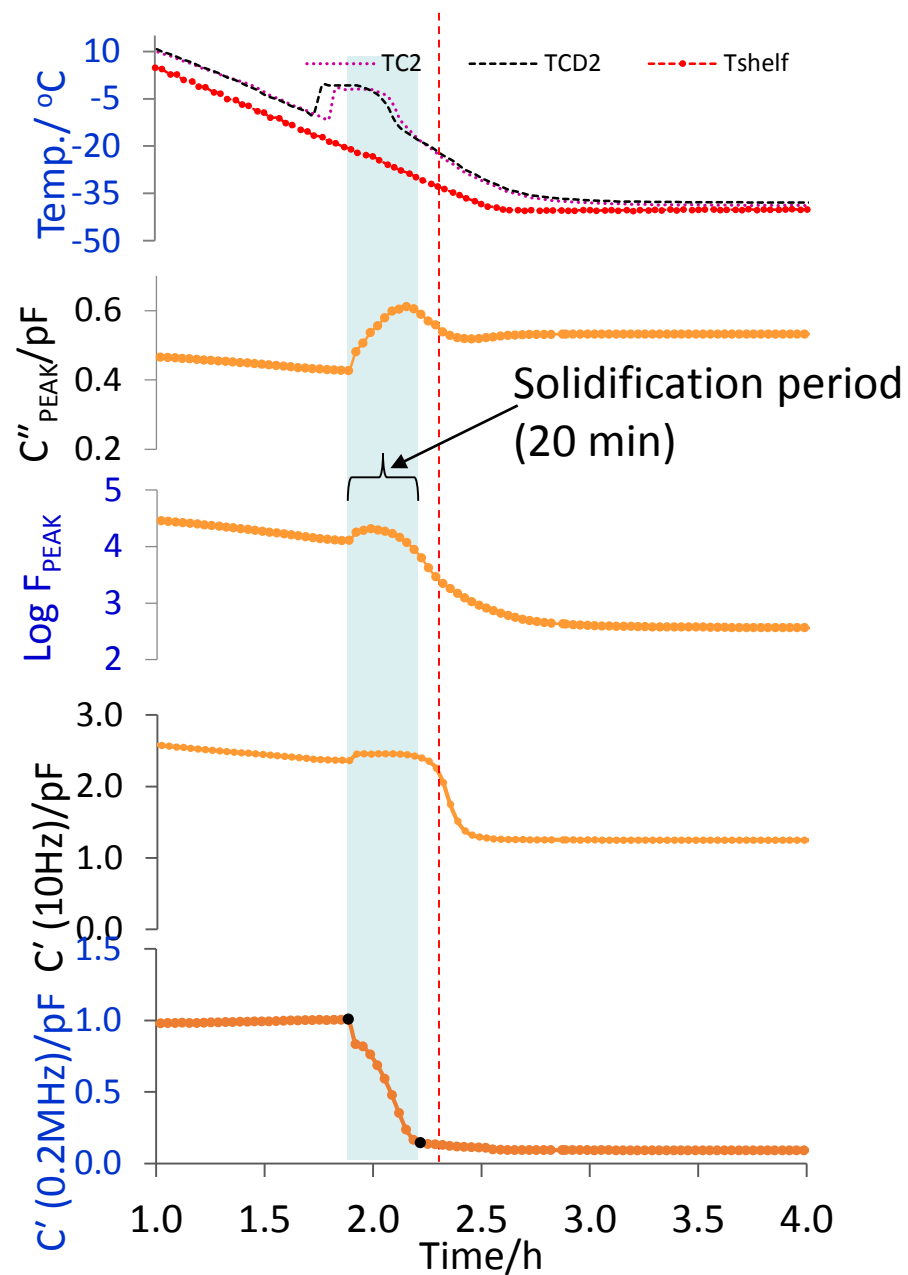
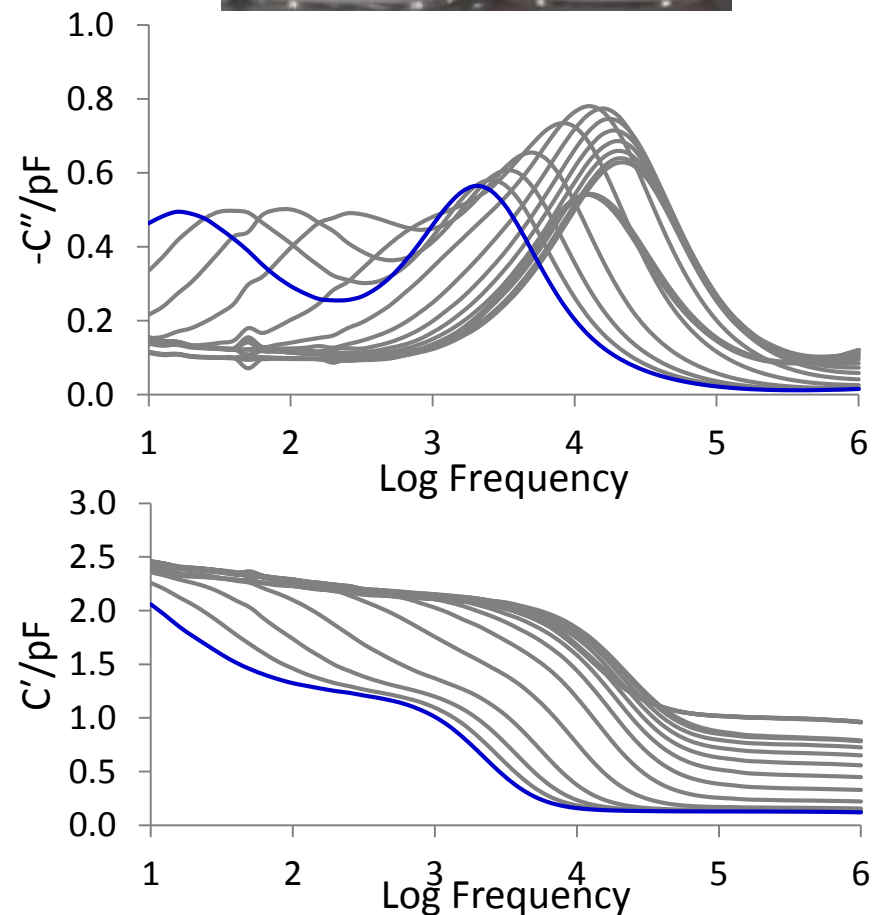
TVIS event identification with temperatures



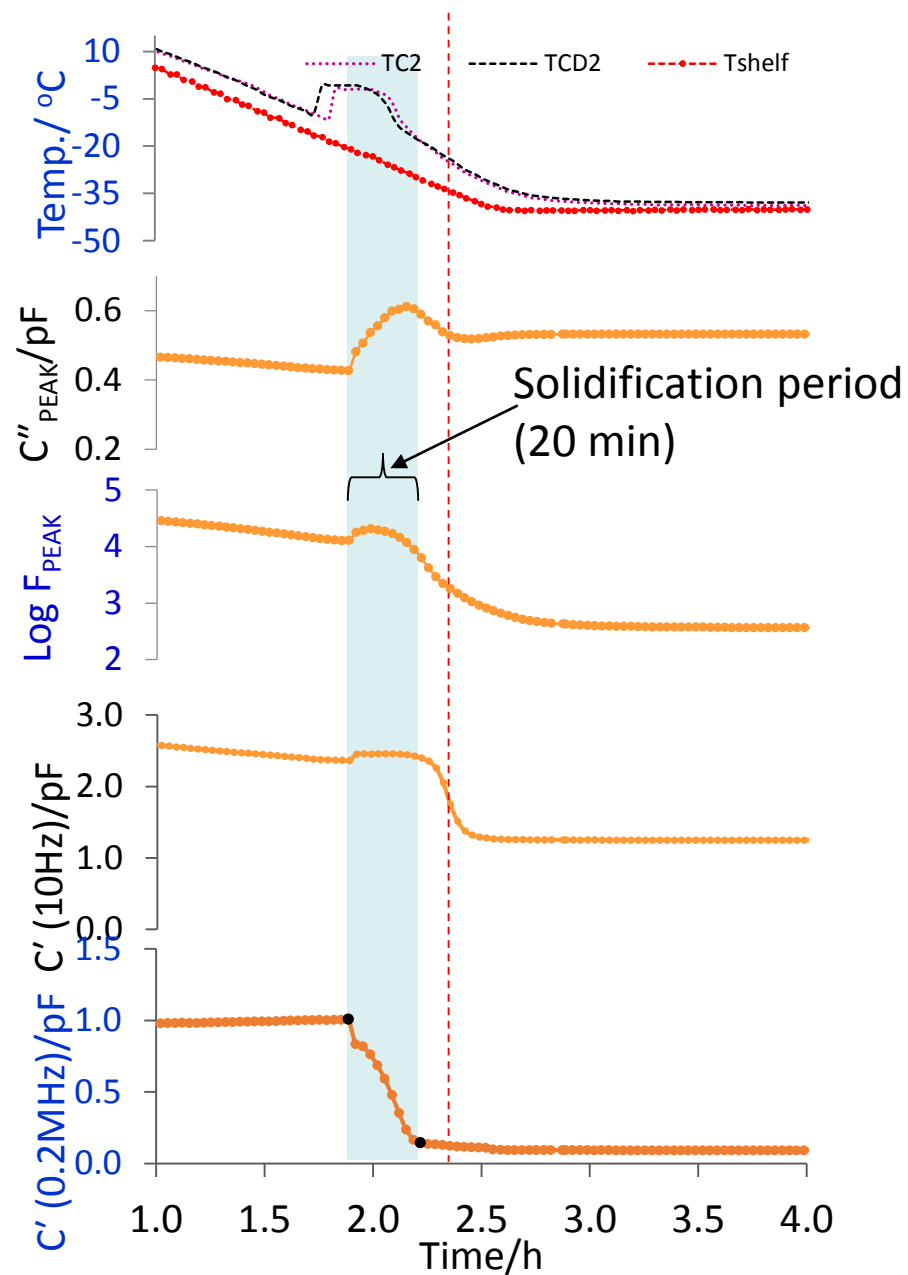
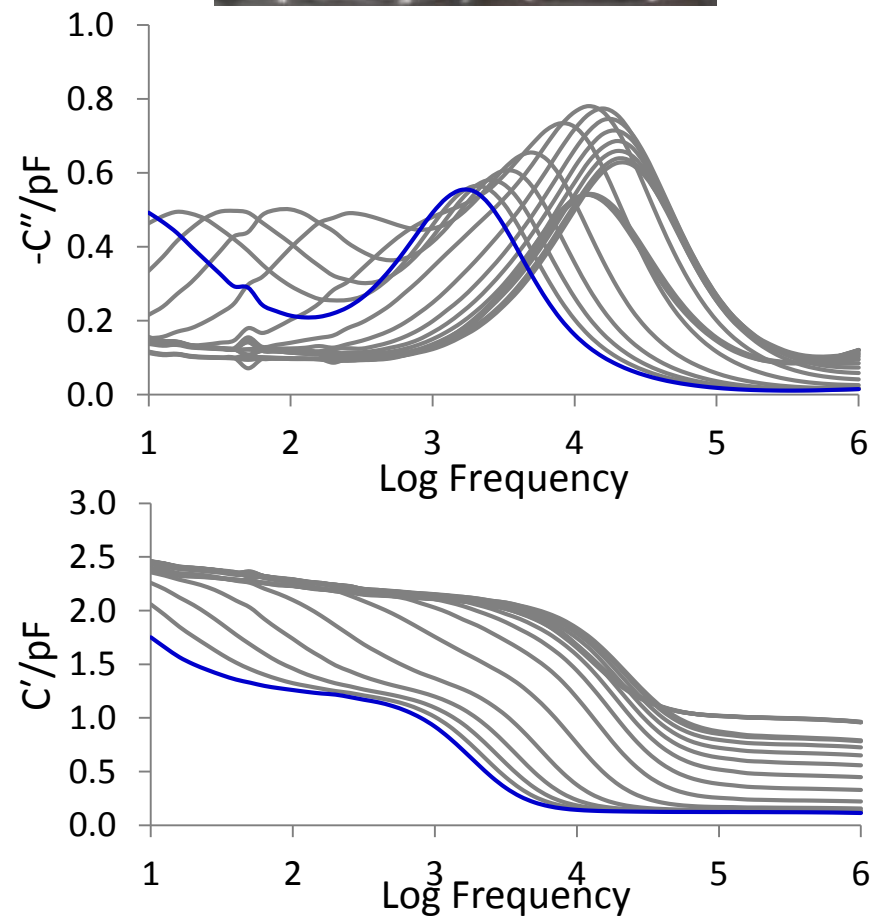
TVIS event identification with temperatures



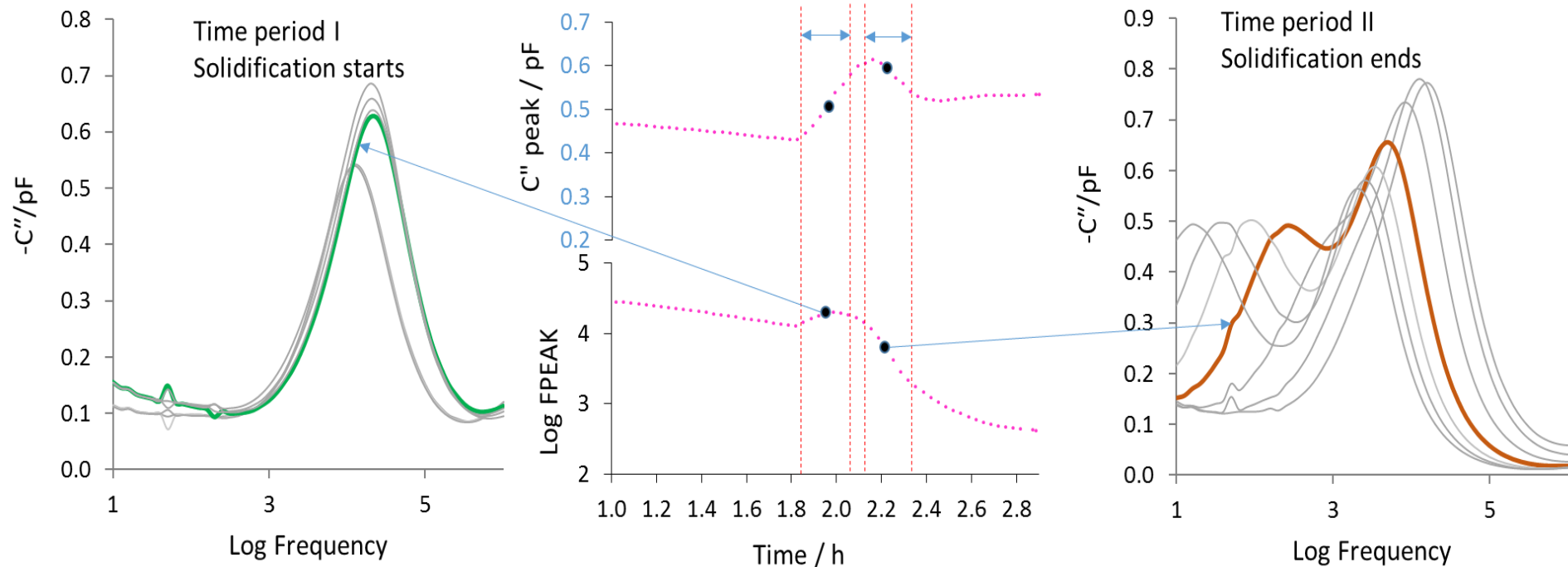
TVIS event identification with temperatures



TVIS event identification with temperatures

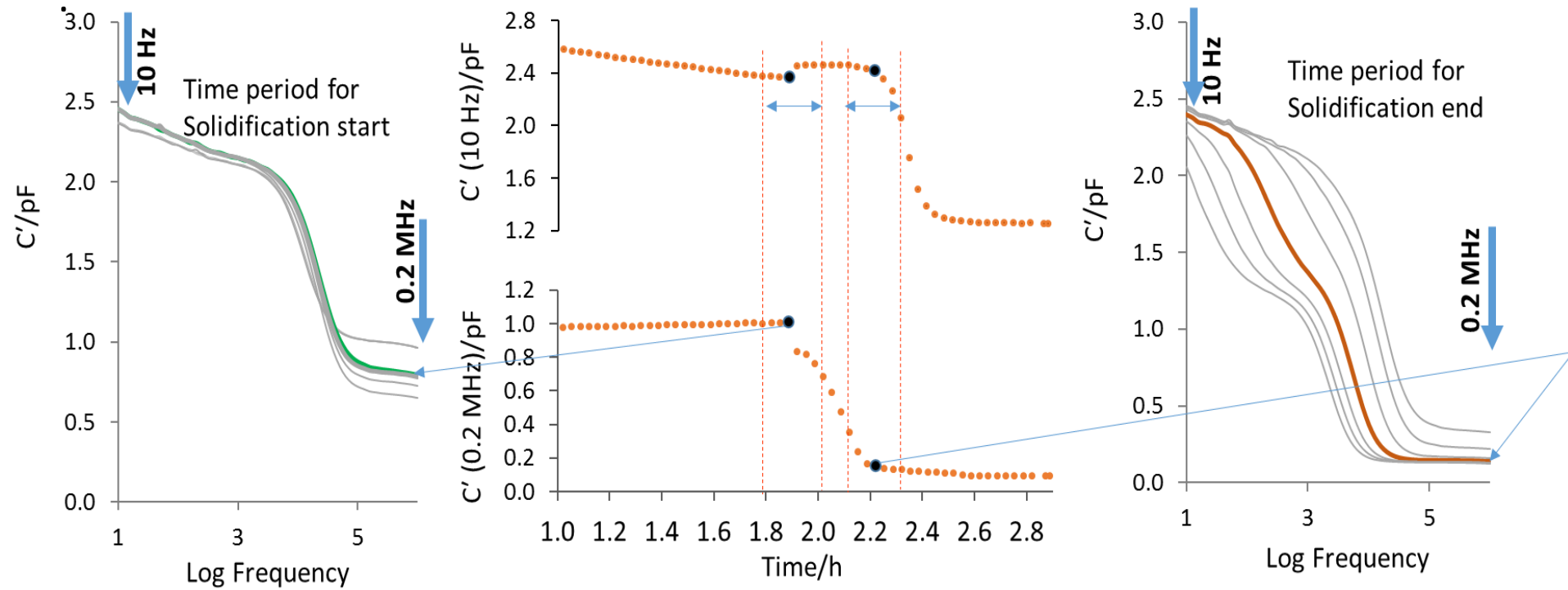


FREEZING STEP: events during solidification onset/end point



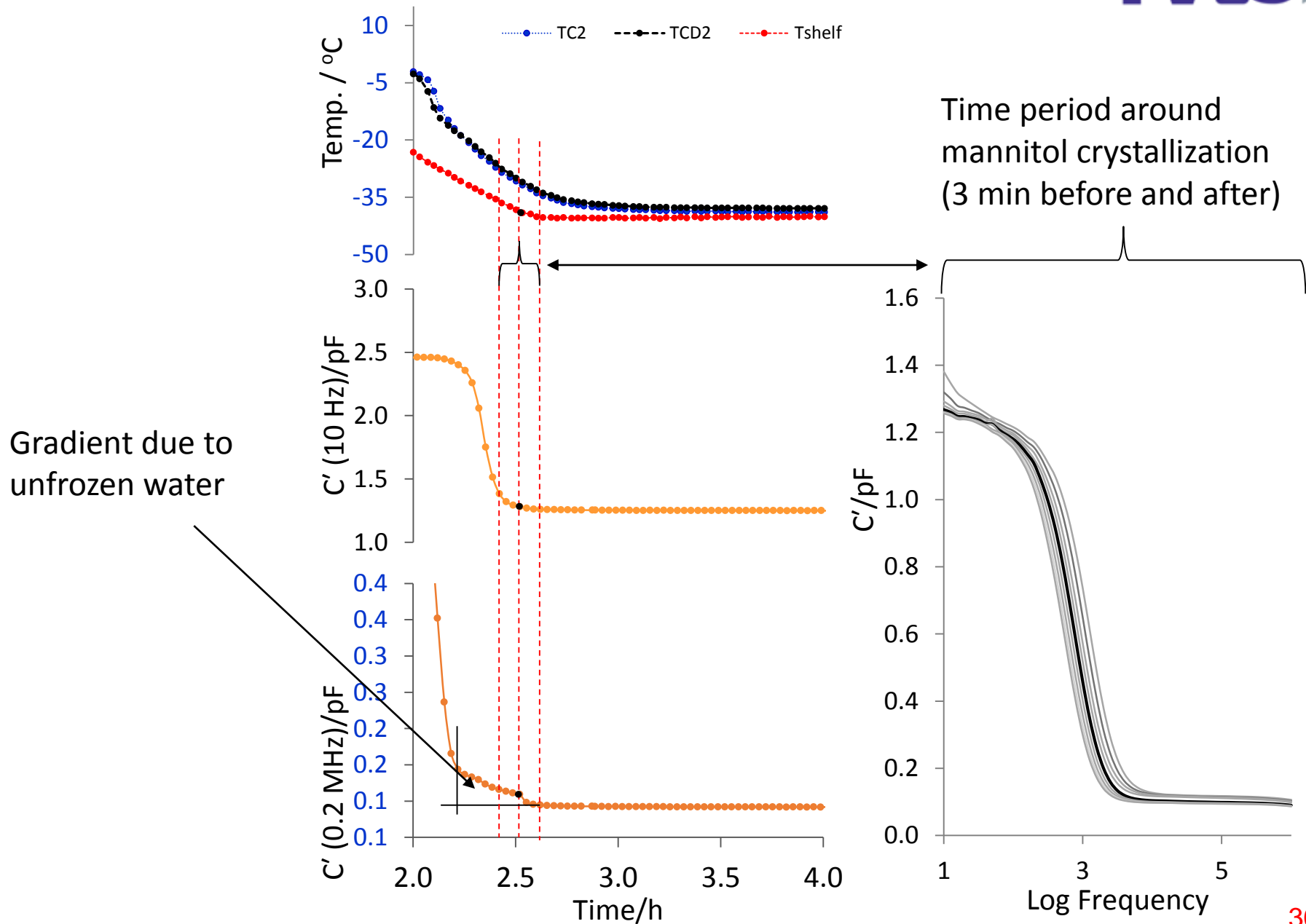
Log F_{PEAK} and C''_{PEAK} with respect to time depict the events that happened 6 min before and after ice growth onset and during the solidification end point. Spectra around the two major event in the freezing process could assist more understanding of the happenings during freezing process

FREEZING STEP: frequency and temperature dependent events during solidification



In addition to demonstrating the events that happened before and after ice growth onset and during the solidification end point, capacitance spectra at lower frequency (10 Hz) and higher frequency (0.2 MHz) show the temperature dependence in the lower frequencies

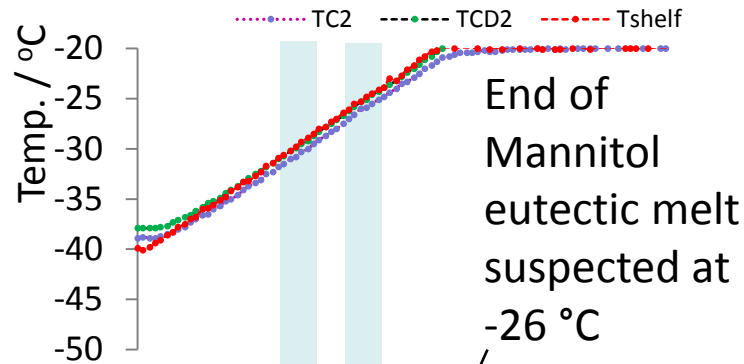
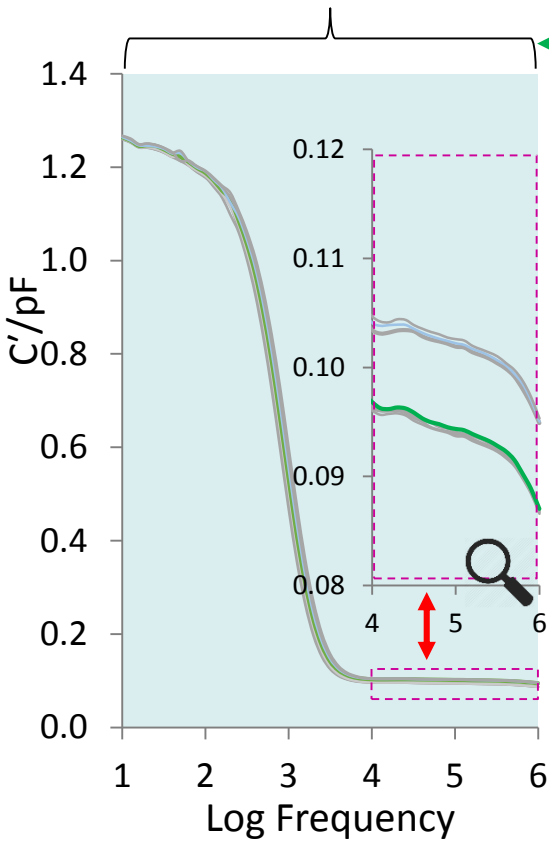
FREEZING STEP: Mannitol crystallization?



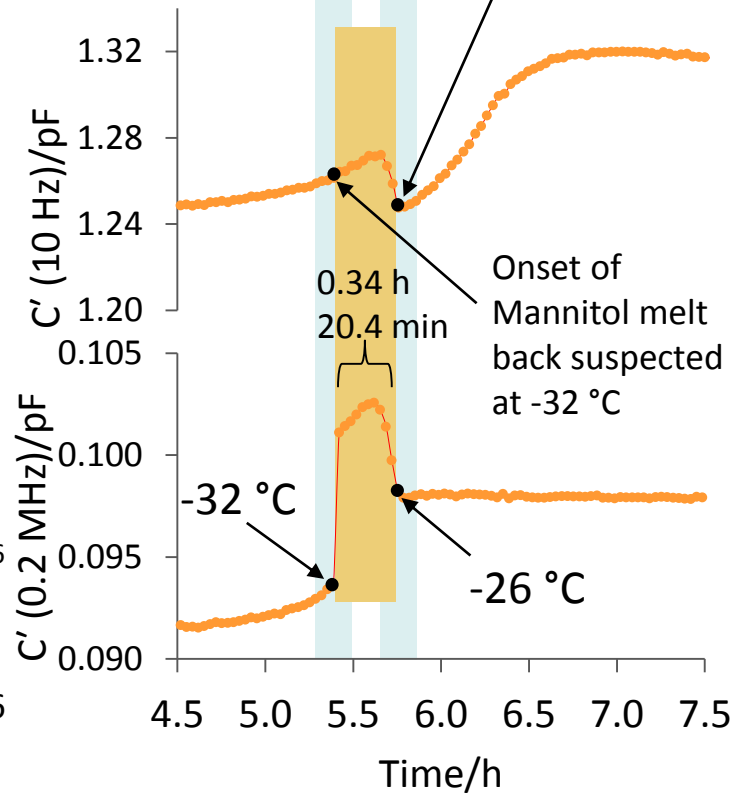
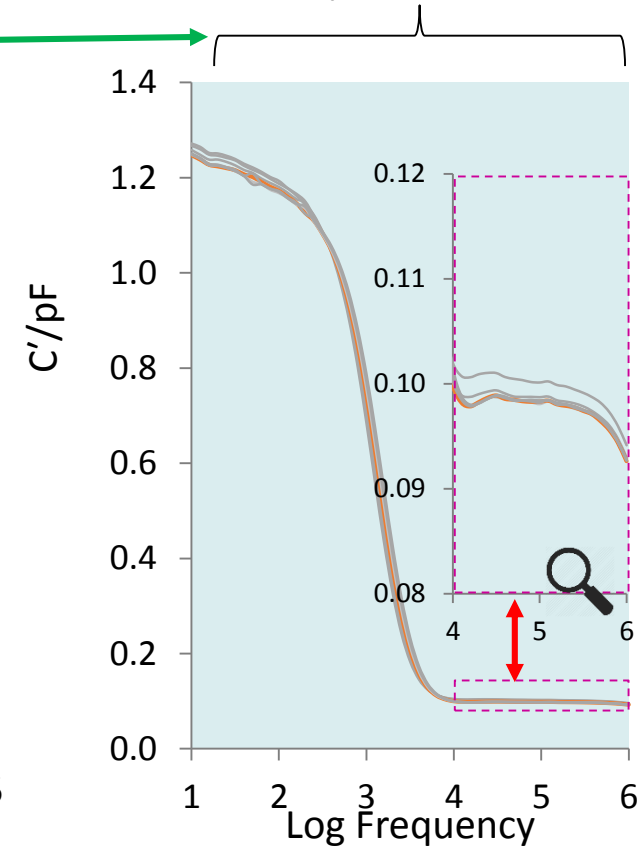
Re-heating period



Time period around onset of melt?
(6 min before and after)



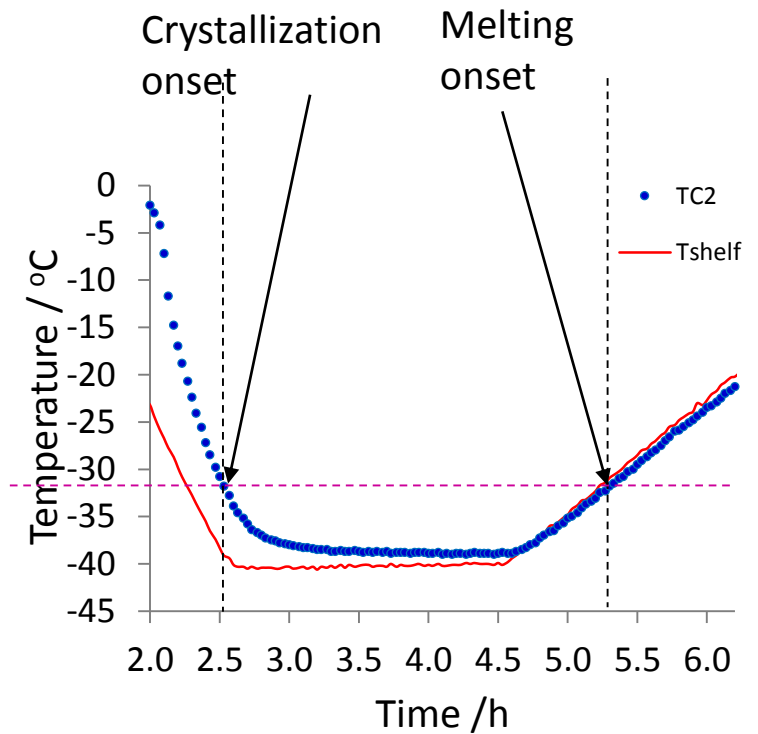
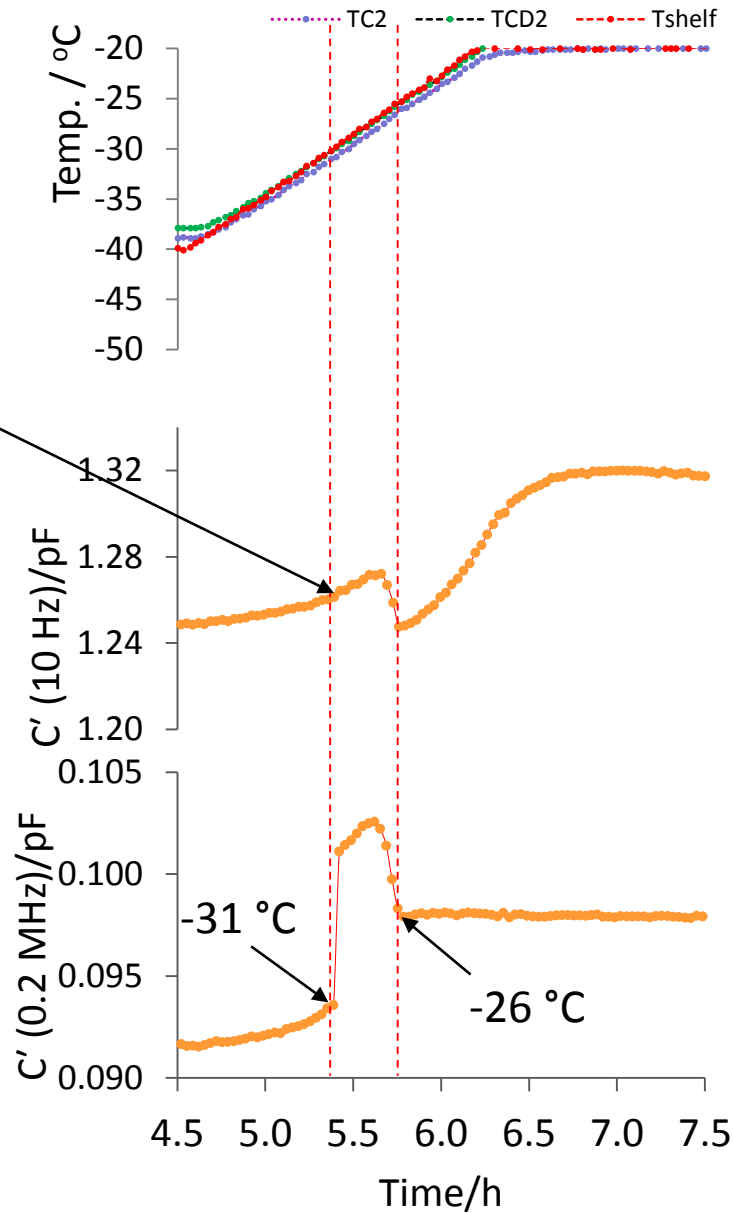
Time period around end of melt?
(6 min before and after)



Re-heating period



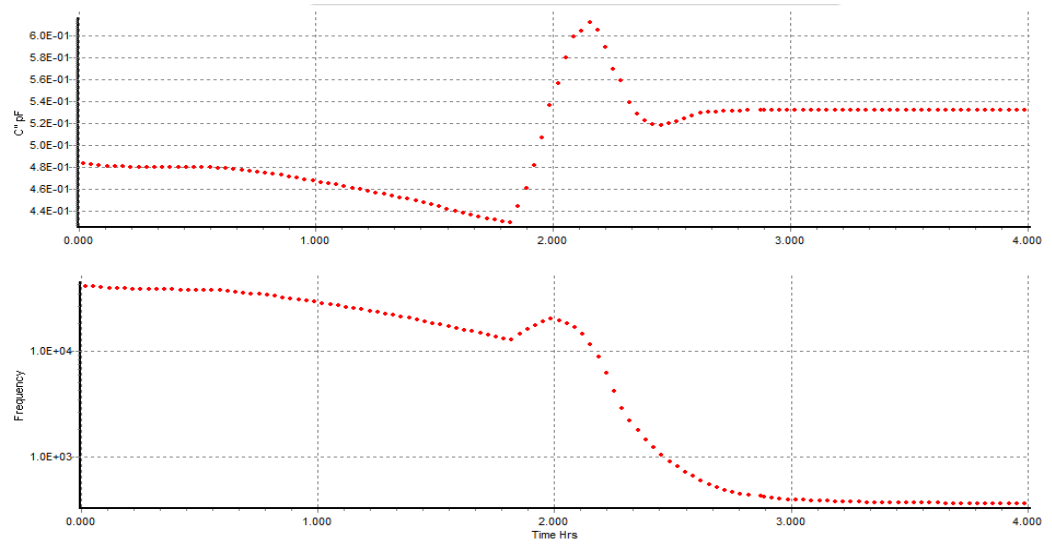
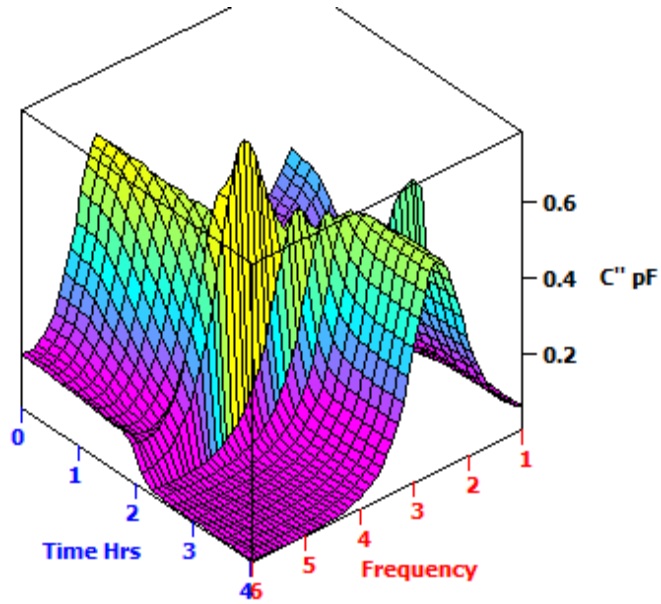
Onset of Mannitol melt back
suspected at -31 °C



LyoView™ Data Presentation

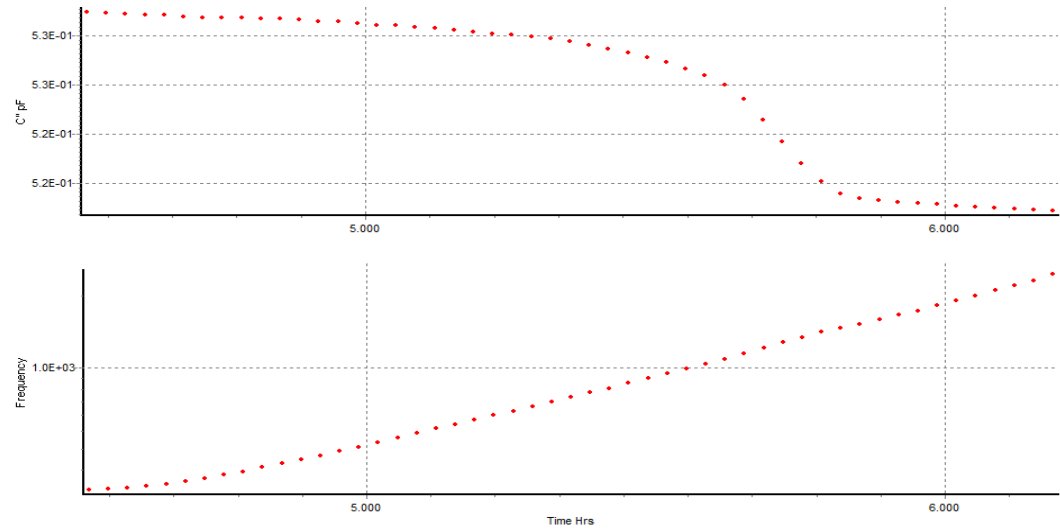
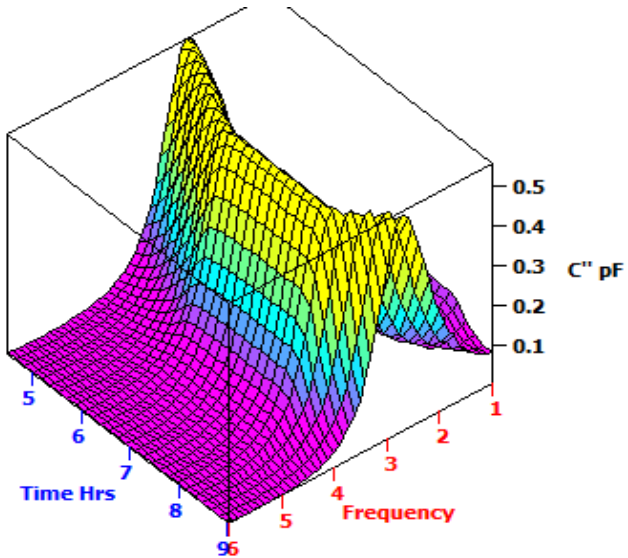


Freezing step



Freezing step

Annealing step



Re-heating period

slops

