DE MONTFORT UNIVERSITY LEICESTER

# Through Vial Impedance Spectroscopy (TVIS)

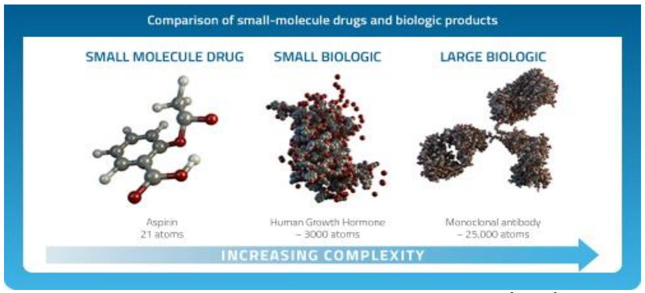
*A new method for the development of manufacturing processes for injectable drug product*

Prof. Geoff Smith

10th International Workshop on Impedance Spectroscopy
28-29th September 2017, Chemnitz, Germany

# **Pharmaceuticals** *(From drugs molecules to products)*

Man-made drugs – small molecules (chemical synthesis) to large molecules (biotechnology)




| Aspirin | Hormone | Antibody |
|---|---|---|
| 21 atoms | ~3000 atoms | 25,000 atoms |


**Production**

Product available in the market

- Quality: Safety & Efficacy

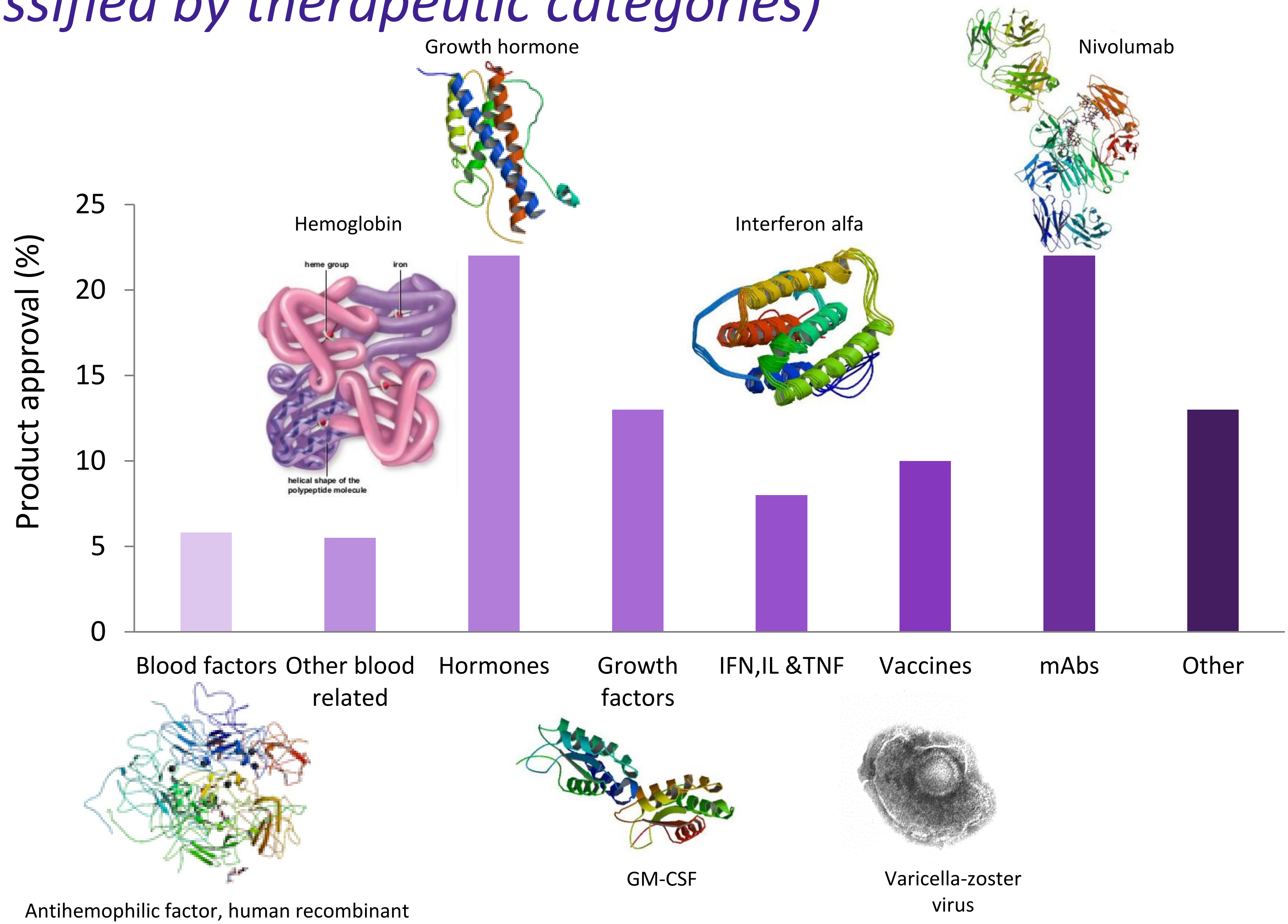
**QC** Pharmacopoeial tests

Formulation development

- Drug products (i.e. dosage form: tablets, injections) etc.
- Healthcare and cosmetics product (i.e. nutrition)

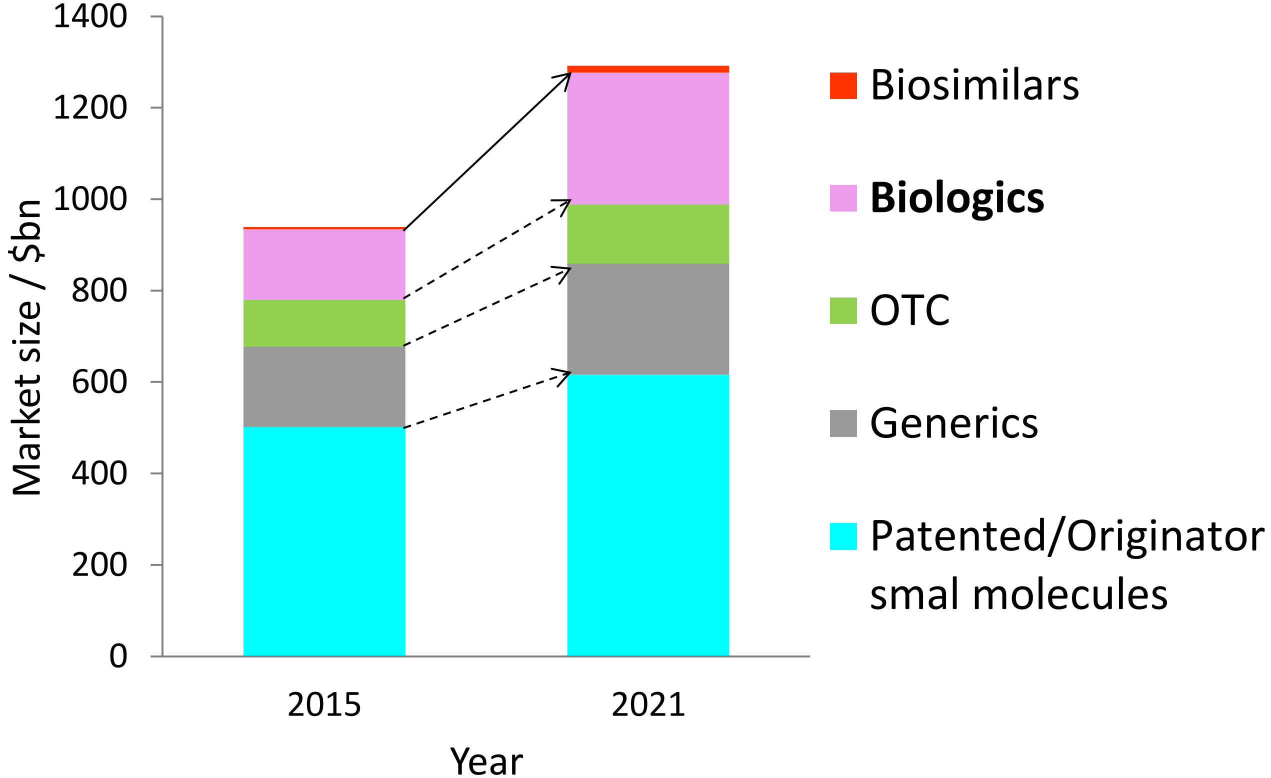
# Biopharmaceutical in Market from 1982-2014

## *(classified by therapeutic categories)*



# Global Pharmaceutical Market 2015 and 2021

The biologics market increases rapidly from ***16.6%*** in 2015 to ***22.2%*** in 2021.

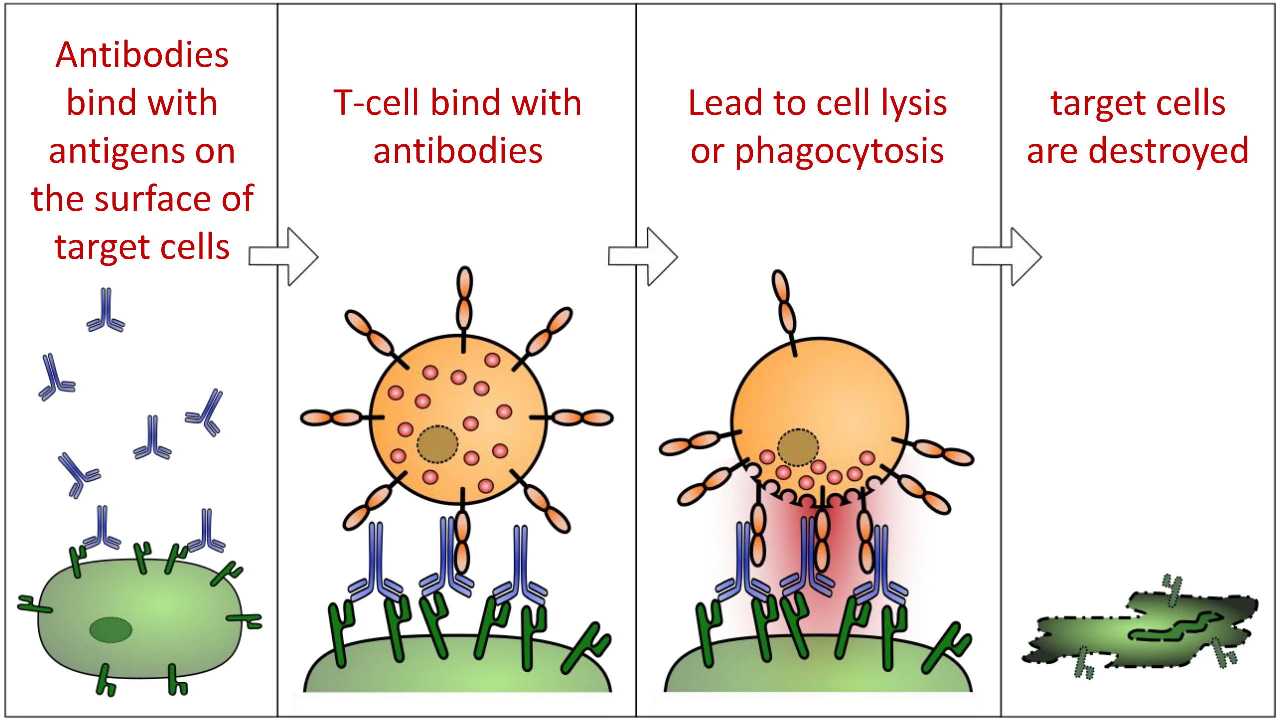



Newrzella A. (2017) Pharma & Biotech 2017 – Review of Outsourced Manufacturing

# Monoclonal antibodies (mAbs)

- A monospecific immunoglobulin
- Medicinal application of mAbs
  - Diagnostic application (i.e. immunoassay, immunoscintigraphy), e.g. Prof.Abdelhamid
  - Therapeutic applications (i.e. Cancer, Transplantation, Immune disease etc.)

Example of mAbs mechanism of action



# Monoclonal antibodies (mAb)

## The growing role of antibodies in therapy

Rheumatoid arthritis

Breast cancer

Leukaemia

Organ Transplantation

Colorectal cancer

Crohn's disease

Psoriasis

Multiple Sclerosis

Lung cancer

| Generic name | Tr[illegible] | [illegible] | [illegible] | Approved indication | [illegible] | [illegible] |
|---|---|---|---|---|---|---|
| Muromomab | Orthoclone | Murine, IgG2a | CD3 | Allograft rejection in allogeneic renal transplantation | 86/06/19 | NA |
| Abciximab[1] | ReoPro | Chimeric, IgG1 | GPIIb/IIIa r | Maintenance of coronary patency | 94/12/22 | NA |
| Rituximab[2] | Mabthera | Chimeric, IgG1 | CD20 | CD20-positive B-cell non-Hodgkin's lymphoma | [illegible]7/11/26 | 98/06/02 |
| Dacliz[illegible] | [illegible] | [illegible]umanized, IgG1 | CD25 (Il2r) | Allograft[illegible] | [illegible] | 99/02/26 |
| Basili[illegible] | [illegible] | [illegible]eric, IgG1 | CD25 (Il2r) | A[illegible] | [illegible] | [illegible]0/09 |
| Palivizu[illegible] | [illegible] | [illegible]manized, IgG1 | Protein F | Re[illegible] children | [illegible] | [illegible]08/13 |
| Infliximab | Remicade | Chimeric, IgG1 | TNFα | Crohn's disease and rheumatoid arthritis | 98/08/24 | 99/08/13 |
| Trastuzumab | Herceptin | Humanized, IgG1 | HER2/Neu | Metastatic breast cancer | 98/09/25 | 00/08/28 |
| Etanercept[3] | Enbrel | huFcγ1/TNFr | TNFα and β | Autoimmune diseases such as ankylosing [illegible] | 98/11/02 | 00/02/03 |
| Gemtuzumab[4] | Mylotarg | Humanized, I[illegible] | [illegible] | [illegible]oid leukemia | 00/05/17 | NA |
| Alemtuzumab[5] | Mabcampath | Humanized, [illegible] | [illegible] | [illegible]leukemia | 01/05/07 | 01/07/06 |
| Ibritomomab[6] | Zevalin $^{90}Y$ | Mouse, IgG1 | [illegible] | [illegible]ymphoma | 02/02/19 | 04/01/16 |
| [illegible] | [illegible] | Human, IgG1 (PD) | TNFα | Crohn's disease and rheumatoid arthritis | 02/1[illegible] | [illegible] |
| [illegible] | [illegible] | [illegible]Fcγ1/LFA-3 | CD2 | Chronic plaque psoriasis | 03/[illegible] | [illegible] |
| [illegible] | [illegible] | Humanized, IgG1 | IgE | Treatment of asthma | 03/06[illegible] | [illegible] |
| Tositumomab | Bexxar $^{131}I$ | Murine, IgG2a | CD20 | CD20-positive B-cell non-Hodgkin's lymphoma | [illegible]3/06/27 | NA |
| Efalizumab | Raptiva | Humanized, IgG1 | CD11a | Moderate to severe plaque psoriasis | 03/10/27 | 04/09/20 |
| Cetuximab | Erbitux | Chimeric, IgG1 | EGFR | Metastatic colo[illegible] | 04/02/12 | 04/06/29 |
| Bevacizumab | Avastin | Humanized, IgG1 | VEGF-A | [illegible] ca[illegible] | [illegible]2/26 | 05/01/12 |
| Natalizumab[9] | Tysabri | Humanized, IgG4 | Integrin-α4 | Multiple sclerosis | 04/11/23 | 06/06/27 |
| Ranibizumab | Lucentis | [illegible] | [illegible]F-A | Wet-type age-related macular degeneration | 06/06/30 | 07/01/22 |
| Panitumumab[10] | Vectibis | [illegible] | [illegible] | Metastatic colorectal carcinoma | 06/09/27 | 07/12/19 |
| Eculizumab[11] | Soliris | [illegible] | [illegible] | Paroxysmal nocturnal haemoglobinuria | 07/03/16 | 07/06/20 |
| Certolizumab[12] | Cimzia | Humanized, IgG1 | TNFα | Crohn's disease | 08/04/18 | NA |

⬅ Immune disorder

⬅ Oncology disease

Chames P et al Br J Pharmacol 157: 220 (2009)

# Drug Product Development

## DEVELOPMENT COSTS

Average cost to develop a drug (including the cost of failures):[2]

2000s–early 2010s = **$2.6 billion**

1990s–early 2000s = **$1.0 billion***

1980s = **$413 million**

1970s = **$179 million**

## PERCENTAGE OF SALES THAT WENT TO R&D IN 2015[5]

Domestic R&D as a percentage of domestic sales = **24.8%**

Total R&D as a percentage of total sales = **19.8%**

## MEDICINES IN DEVELOPMENT

Medicines in development globally = **7,000**[14]

Potential first-in-class medicines** across the pipeline = an average of **70%**[15]

Medicines in development to treat rare diseases = more than **450**[16]

## RESEARCH AND DEVELOPMENT (R&D)[1]

Average time to develop a drug = **10 to 15 years**

Percentage of drugs entering clinical trials resulting in an approved medicine = less than **12%**

## VALUE OF MEDICINES

**Cancer:** Since peaking in the 1990s, cancer death rates have declined **23%**.[17] Approximately **83%** of survival gains in cancer are attributable to new treatments, including medicines.[18]

http://phrma-docs.phrma.org/sites/default/files/pdf/biopharmaceutical-industry-profile.pdf

# Freeze Drying Process

# Advantages of Lyophilization

*"40% of biologically based products have to be freeze dried"*
http://www.genengnews.com/gen-articles/lyophilization-growing-with-biotechnology/1083

*"80% of the available products lyophilized in vial"*
https://www.pharmpro.com/article/2017/03/lyophilization-basics

**Lyophilization process** → **Dry Powder** →
- Increase Stability
- Easy to Dissolving
- Easy to transport

*Low temperature process* → *Low moisture content*; *More surface area & porous*

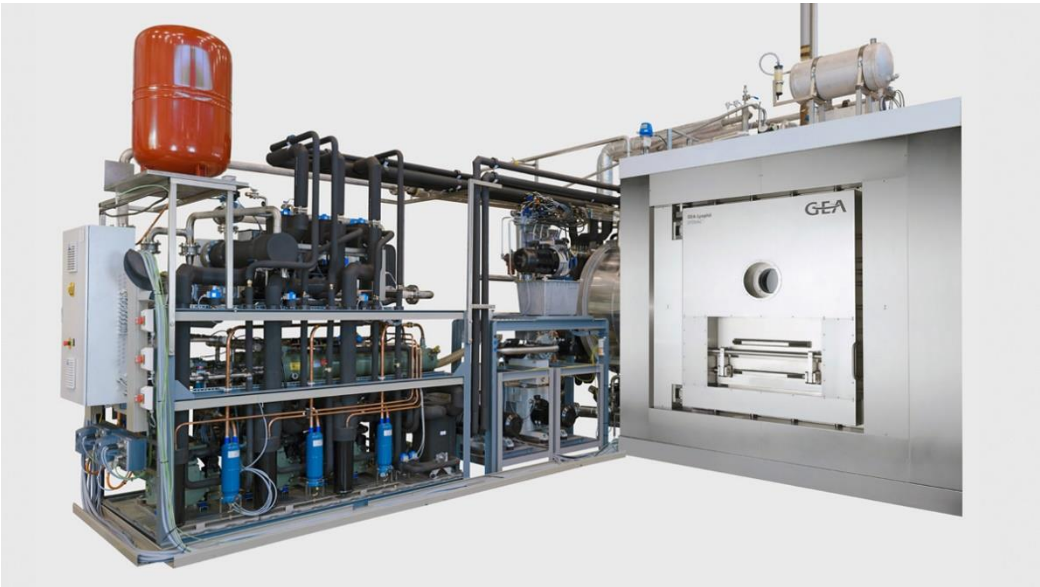
- Lyophilization commonly used for
  - Large Molecule Drugs (e.g. proteins, DNA)
  - Small Molecules Drugs (e.g. penicillin)
  - Microorganisms (e.g. bacteria, virusus)
  - Blood products


Azithromycin injection. (Zithromax®)

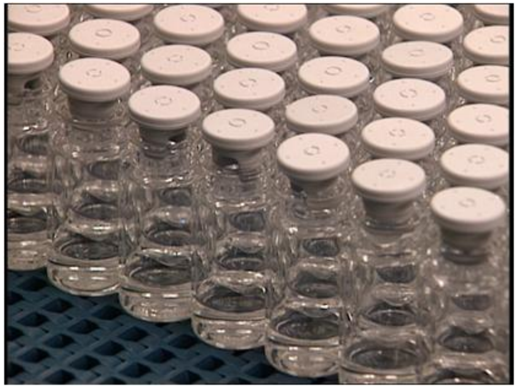

Zoster vaccine (Zostavax®)

# Limitation of Lyophilization Technology

- Complicate
- Costly
- Long process
- Difficult to scale up
- Variation between batch

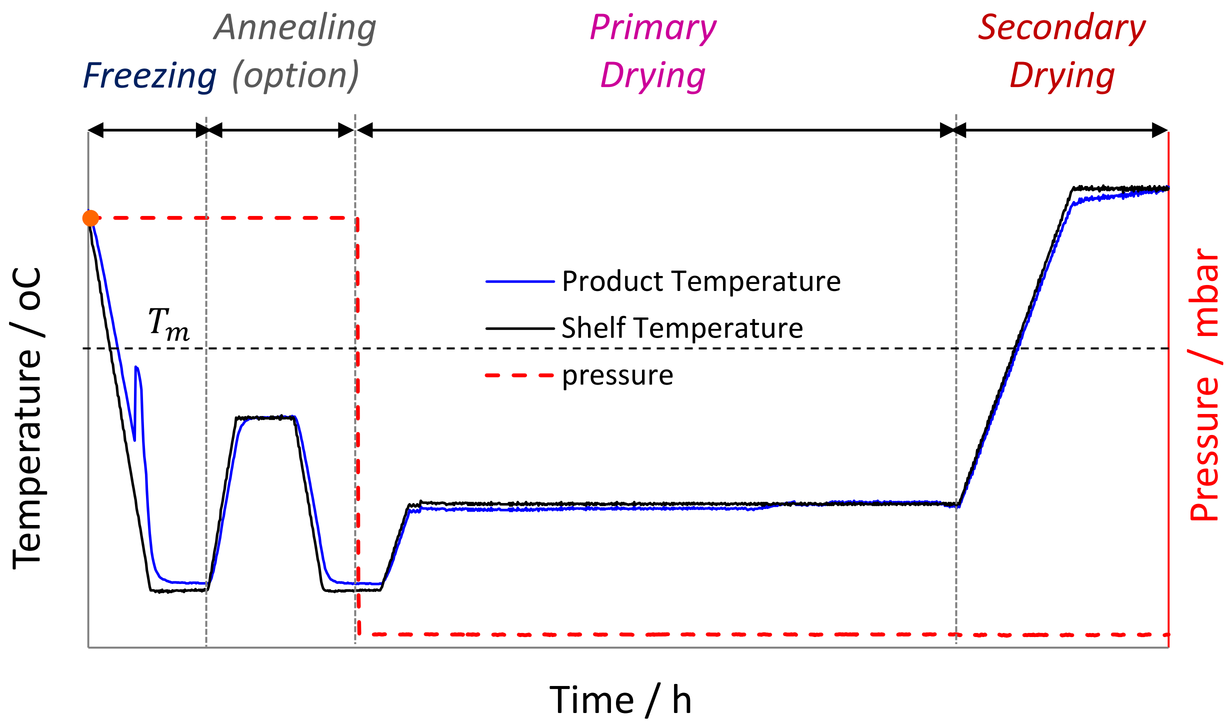


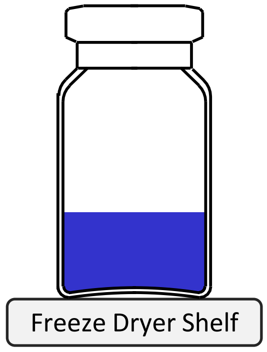




# Lyophillization or Freeze Drying Process

- A technique which dries product at low temperature through sublimation process
- It consists of three main steps : **Freezing**, **Primary drying** and **Secondary drying**




*Equilibrium product temperature of all vials*

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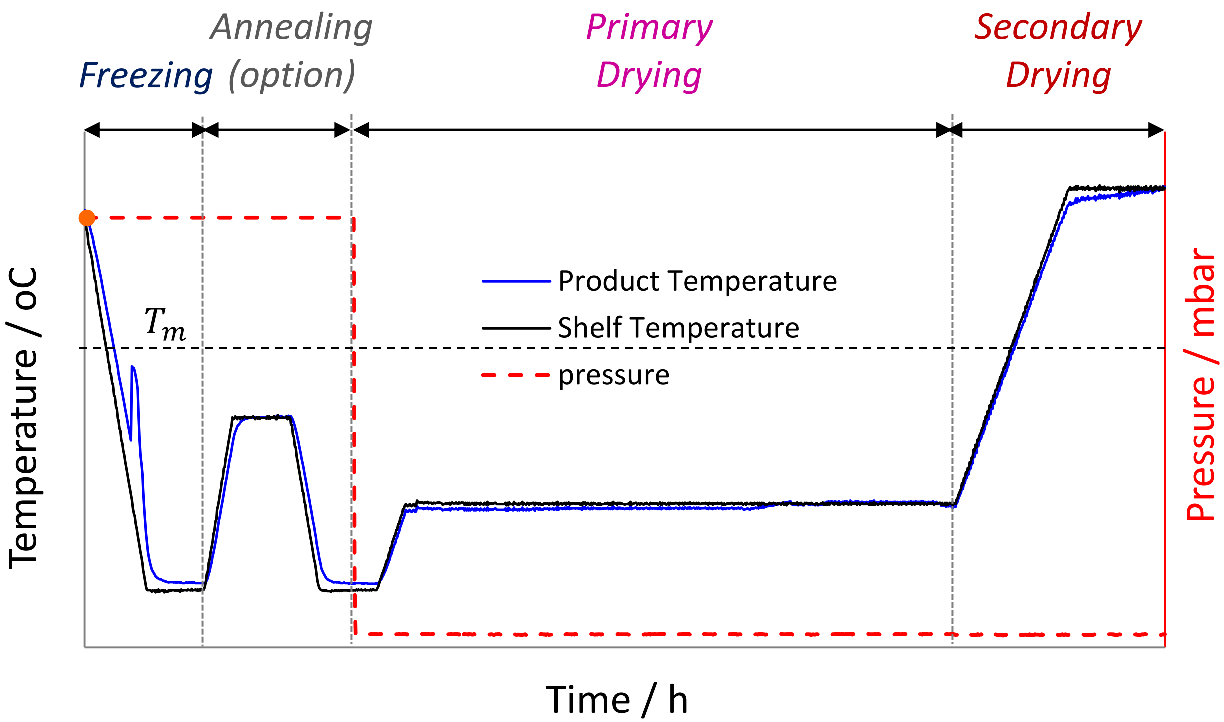


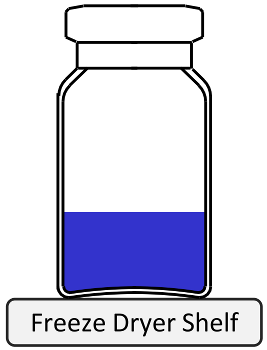


*Temperature of the product (liquid state)  decreases (Tp) as shelf temperature decreases (Ts)*

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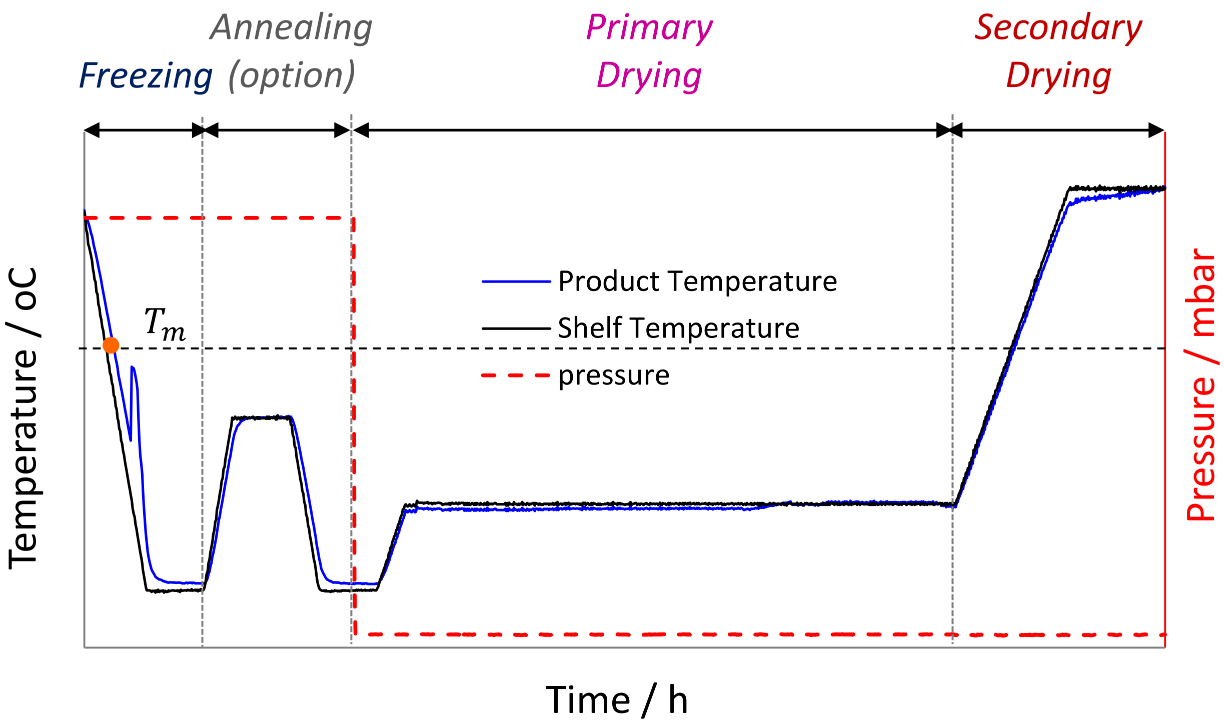


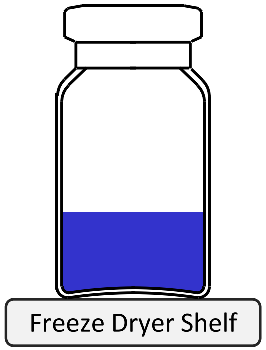


*Liquid product supercools below the melting point: Melting temperatures of ice in frozen solution would be less than that of pure water, owing to the freezing point depression of the solutes*

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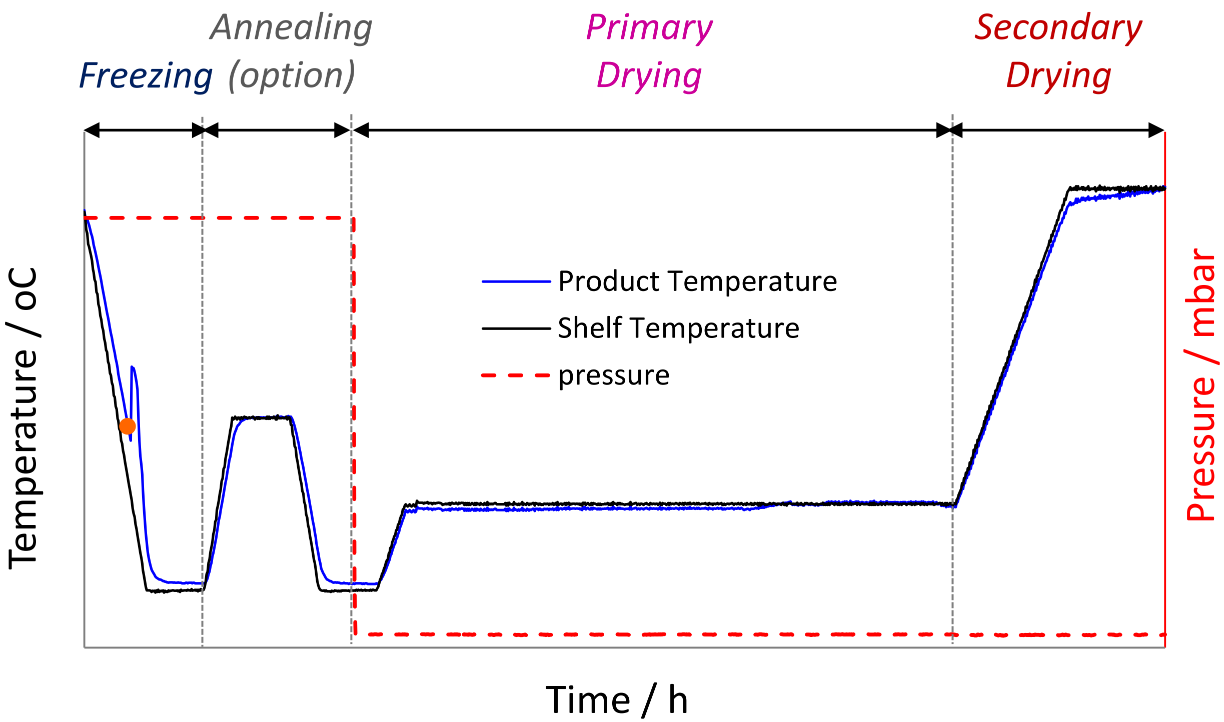
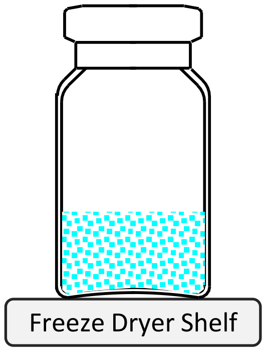



Ice nucleation




*Ice crystal growth from the bottom of the vial (typically takes less than 2 min). Release of heat causes a spike in the product temperature*

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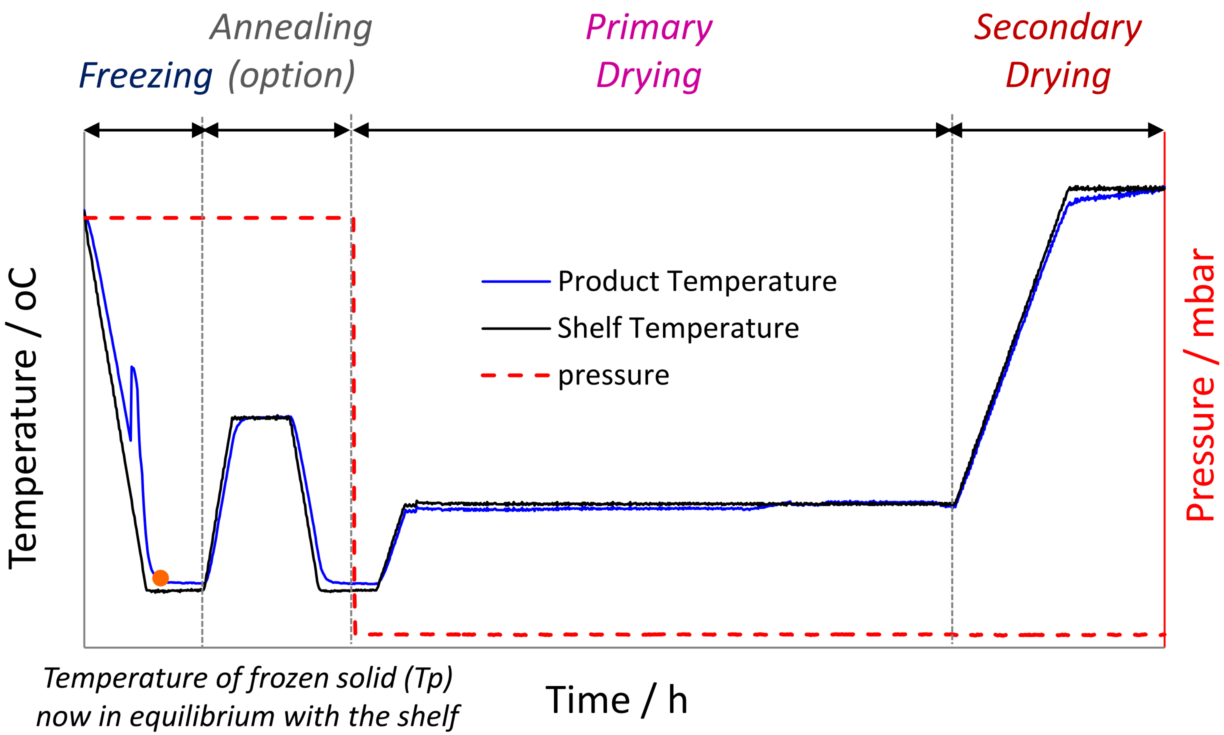
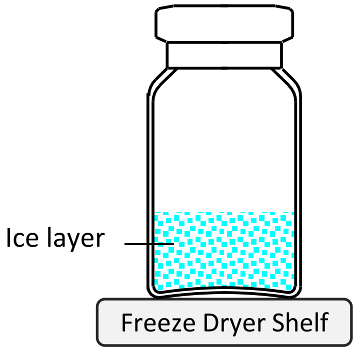



*Temperature of frozen solid (Tp) now in equilibrium with the shelf but  Tp > Ts by 2-3 °C*




*Temperature hold to ensure complete solidification of ice*

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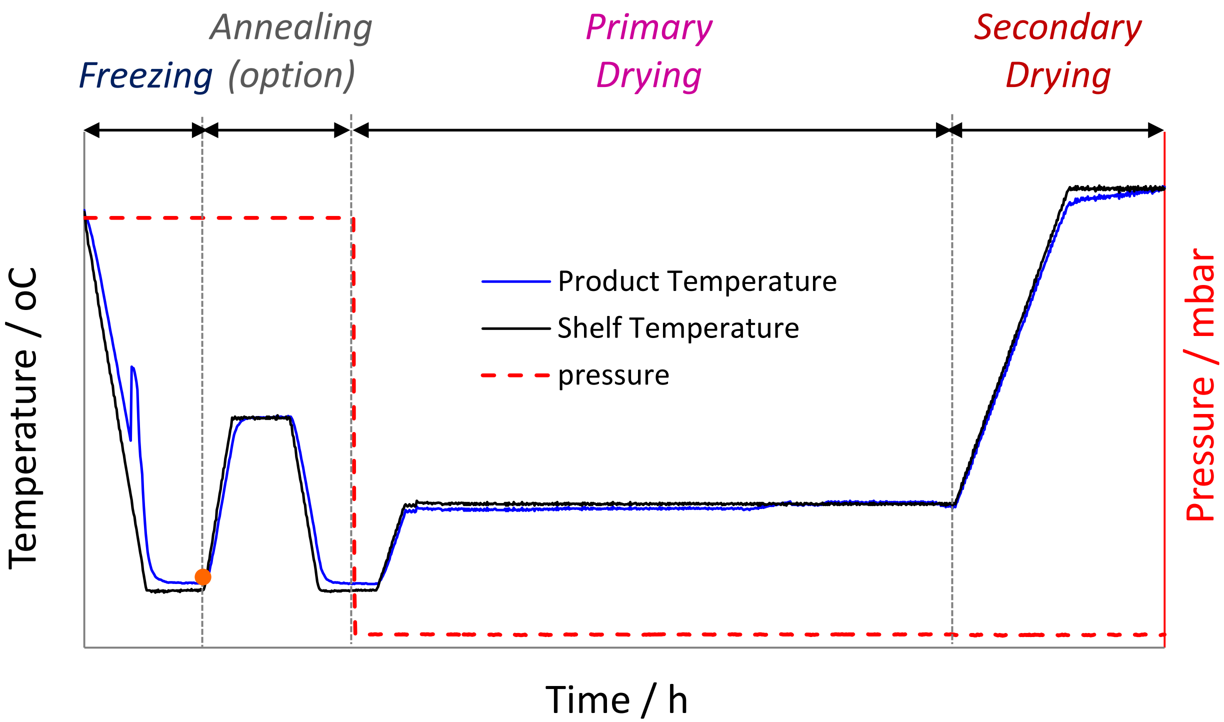


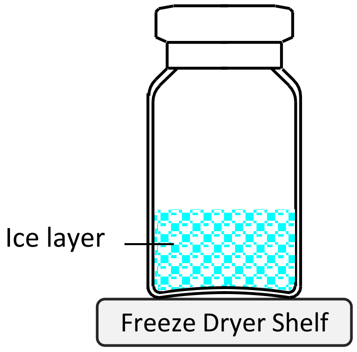


*Crystal growth during annealing*

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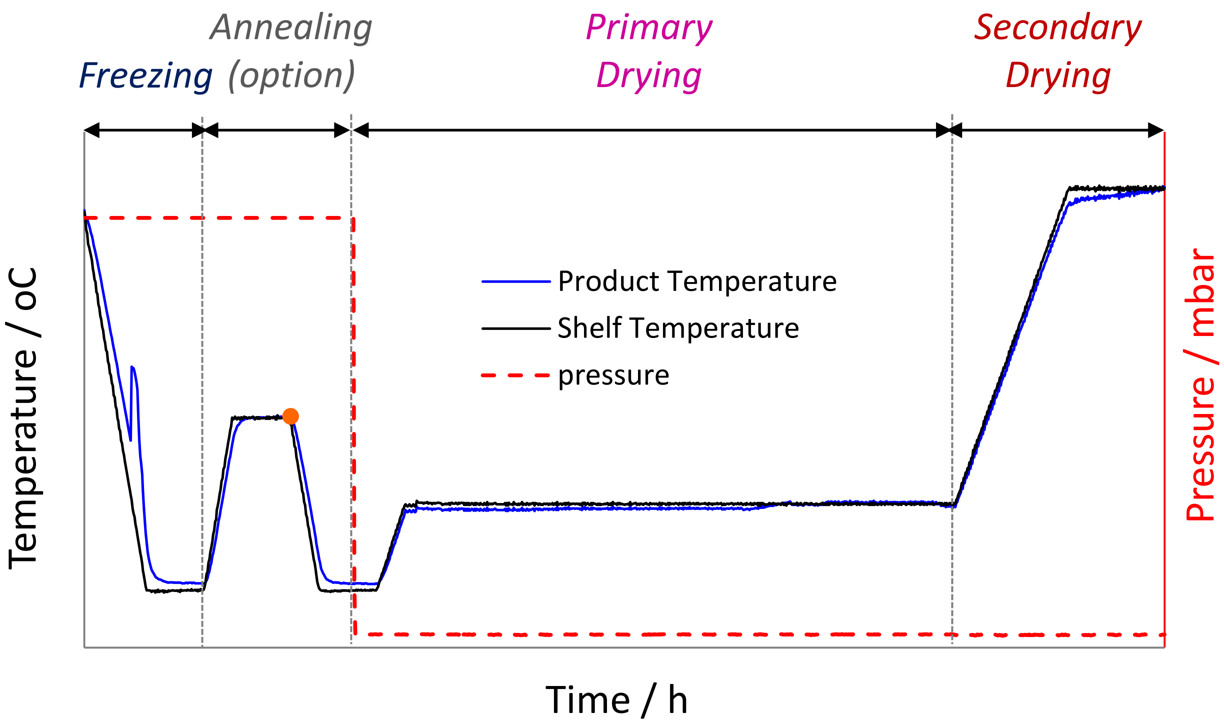


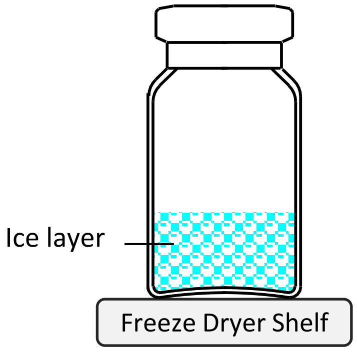

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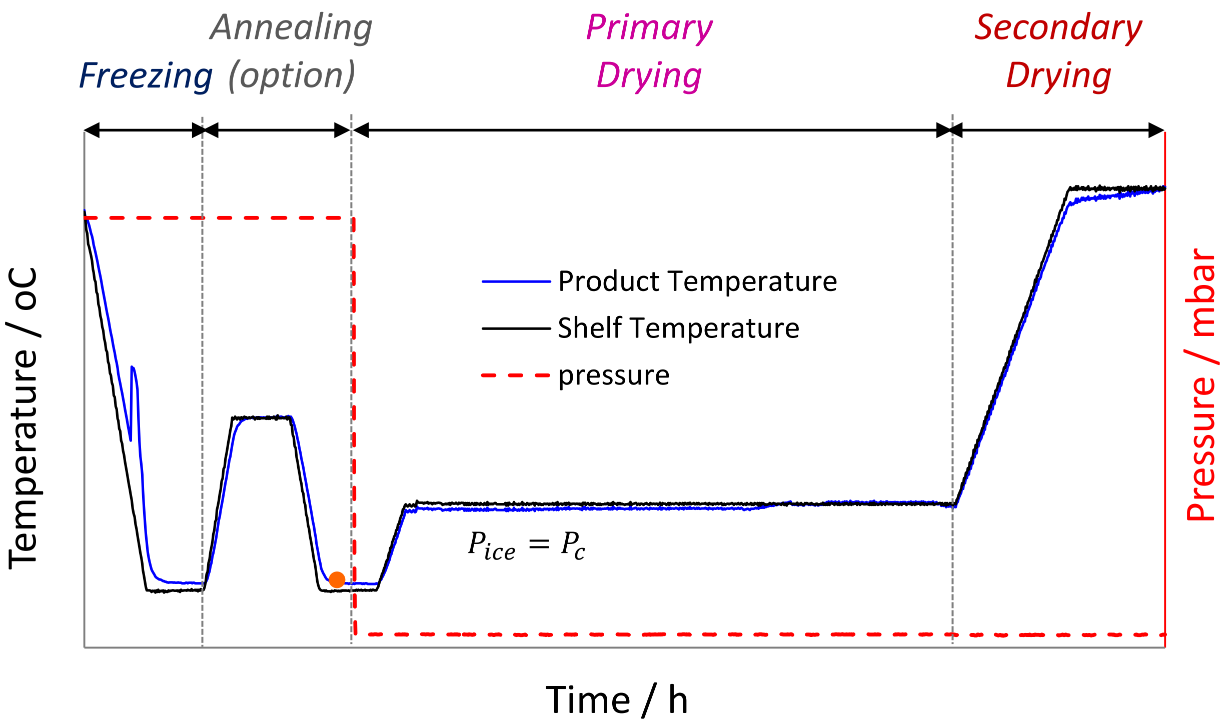


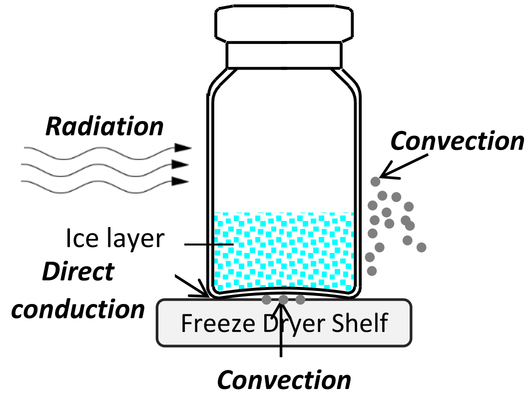


*Increasing shelf temperature (ramp), increases ice temperature and partial pressure until $P_{ice}$ = $P_c$ and drying (sublimation) starts*

# Lyophillization or Freeze Drying  Process

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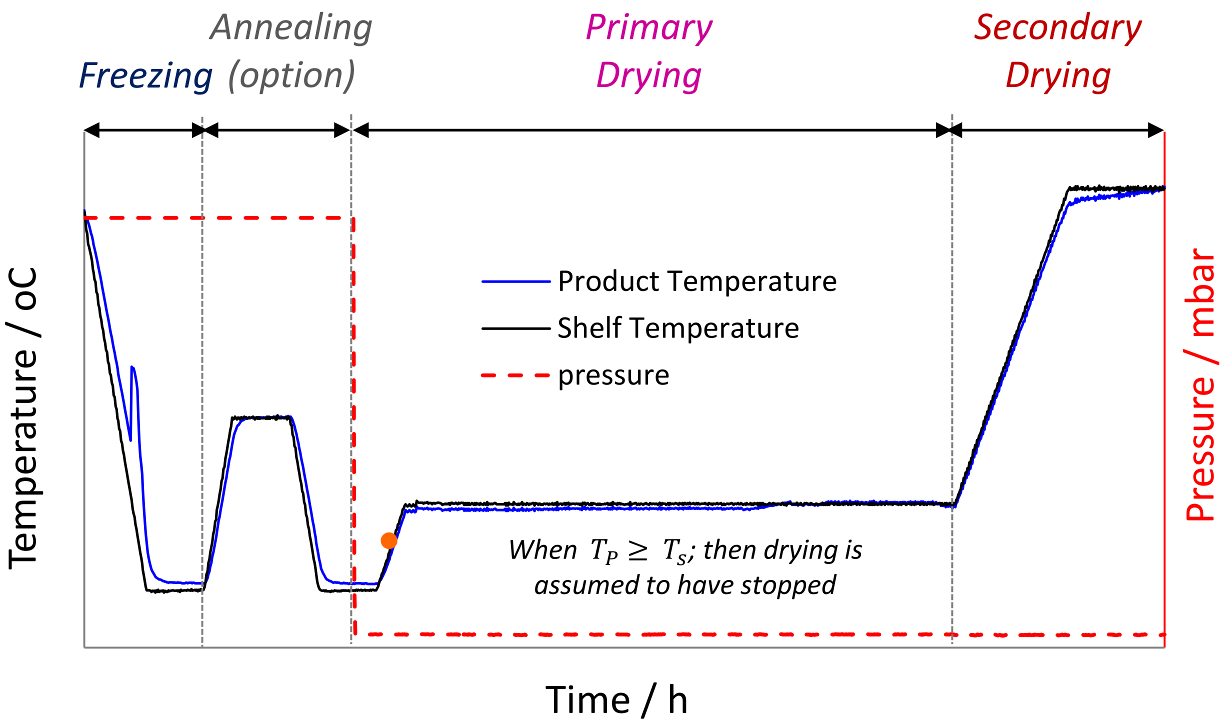


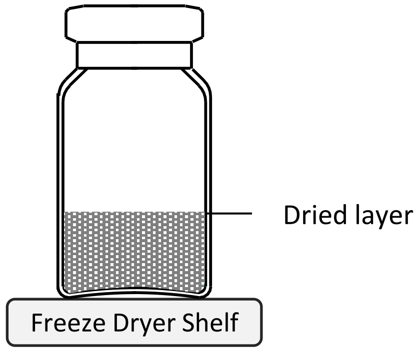


*Once the ice is removed then self cooling stops and the product temperature can now catch up with the shelf temperature.*

# Lyophillization or Freeze Drying  Process

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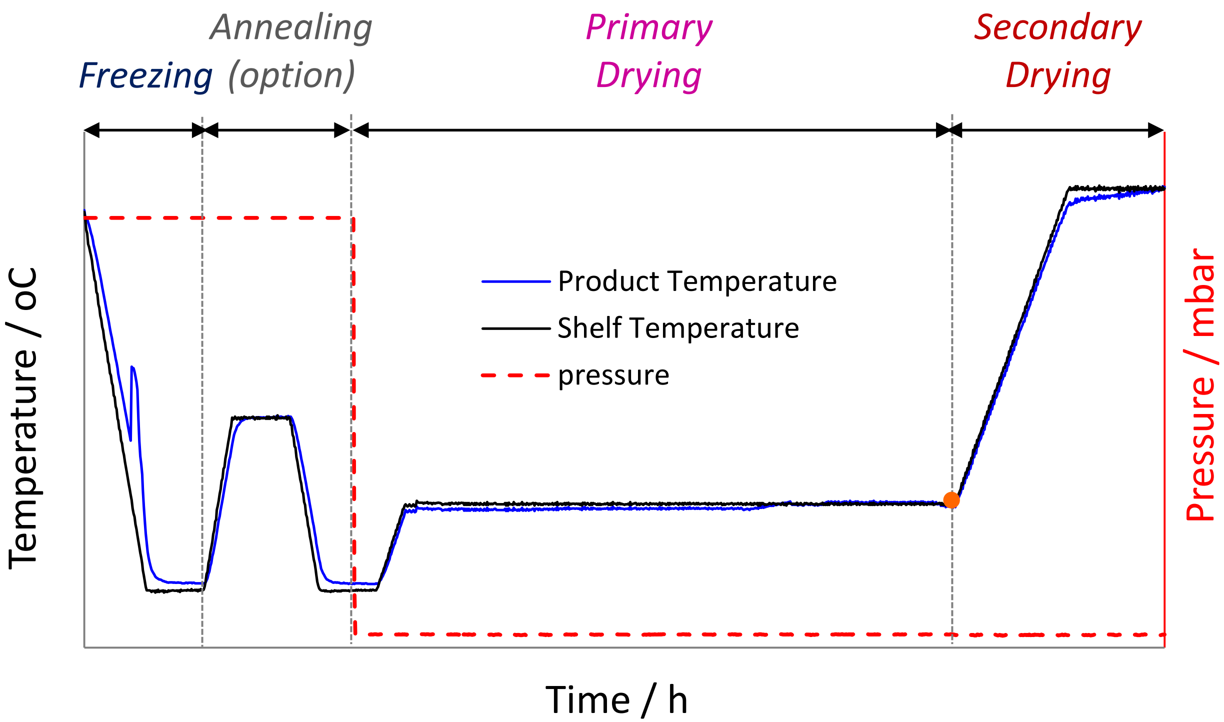
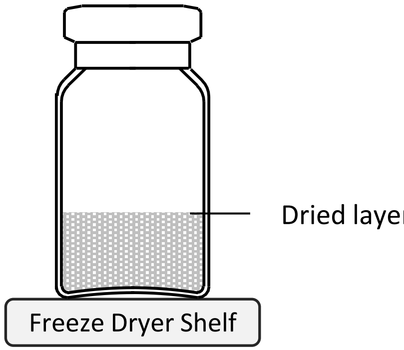
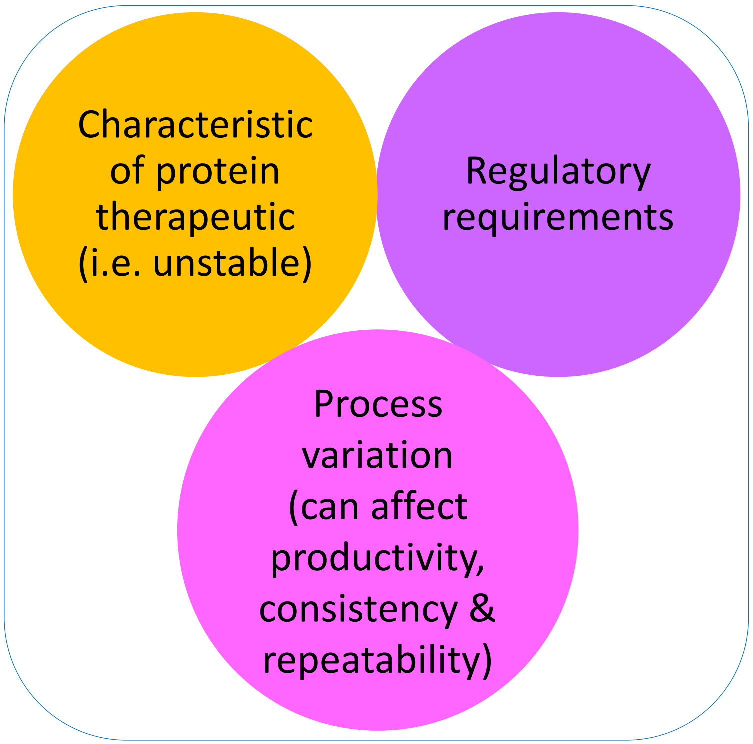


# Process Analytical Technologies

**Challenging in development and manufacture of freeze-dried biopharmaceuticals**




**Process Analytical Technology (PAT)**

Definition by US FDA:

A mechanism to design, analyze and control pharmaceutical manufacturing process through the measurement of Critical Process Parameters (CPP) which affect Critical Quality Attributes (CQA)

- Manometric Temperature Measurement (MTM)
- Tunable Diode Laser Absorption Spectroscopy (TDLAS)

*Limitation :*

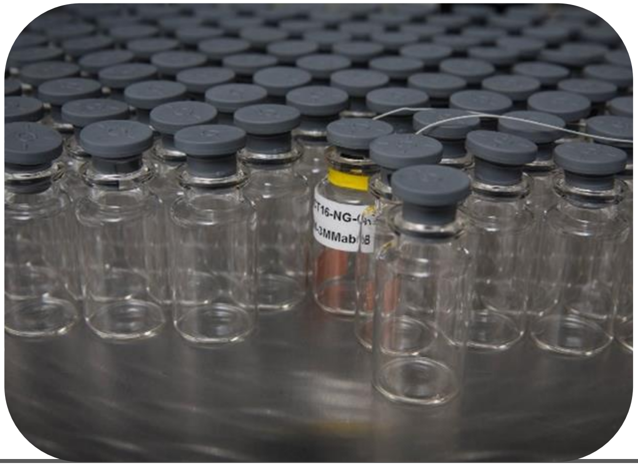
- *Batch method (representative parameter) → not suitable for high variation batch (e.g. edge effect)*
- *TDLAS is difficult to calibrate and costly*

# 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**"

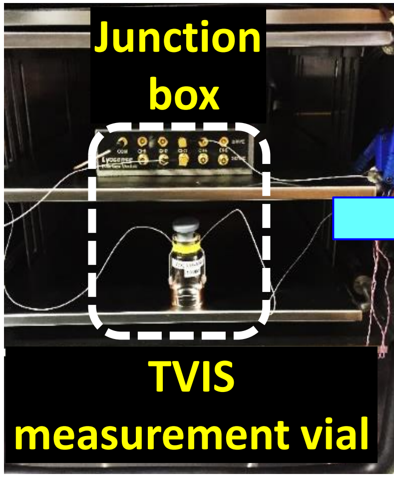

- Features
  - Single vial "non-product invasive"
  - Both freezing and drying characterised in a single technique
  - Non-perturbing to the packing of vials
  - Stopper mechanism unaffected

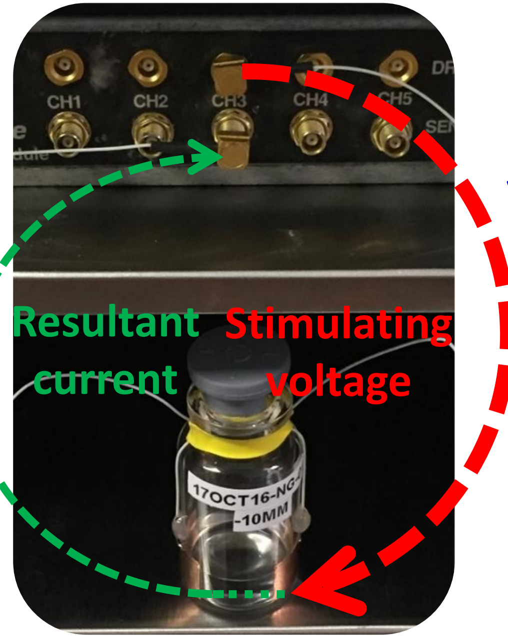
| | |
|---|---|
| SV product temperature | |
| SV sublimation rate | |
| SV end point | |



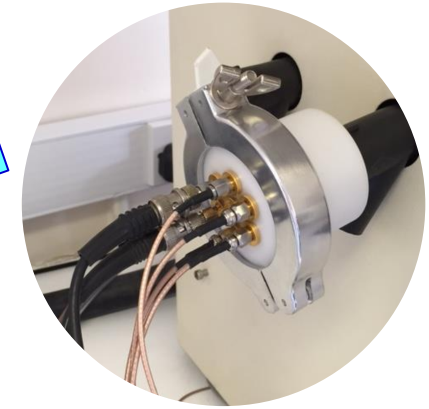
## Through Vial Impedance Spectroscopy (TVIS)
## *Introduction*

## Freeze drying chamber

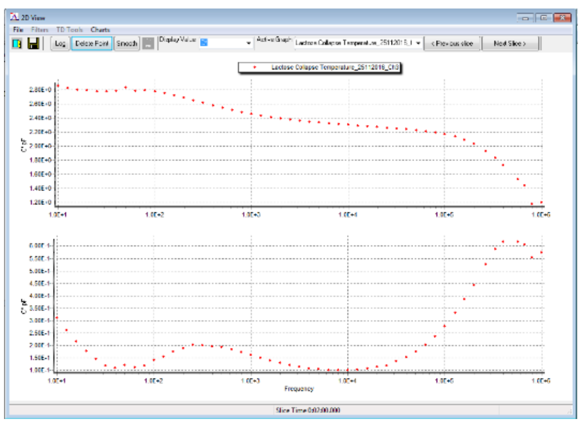


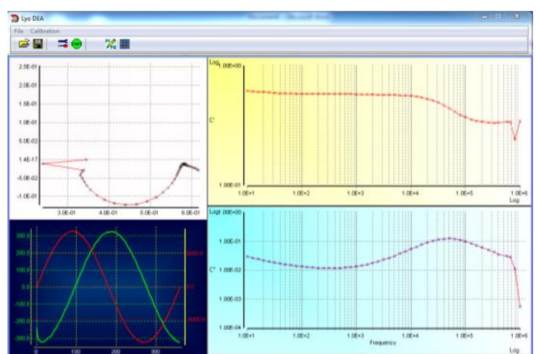

## Pass through



## LyoView™ analysis software



## LyoDEA™ measurement software



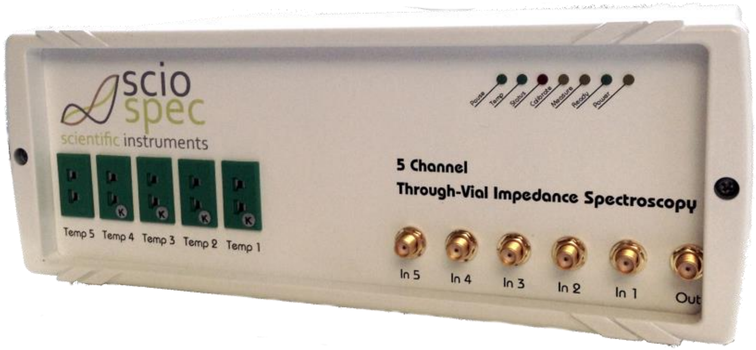
## TVIS system (I to V convertor)

# Impedance Analyzer for Lyophilization Process

TVIS

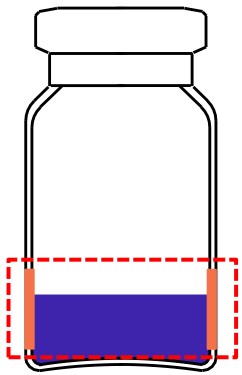
- Through Vial Multi Channel Impedance Analyzer
- Impedance measurement specially optimized for lyophilization experiments (contact method)
- Five sequentially measuring impedance channels
- All five channels share a common excitation signal
- Automatic voltage excitation amplitude adjustment
- Current Gain $10^9$ (1 Gigaohm trans impedance amplifier gain)
- Five synchronized type K thermocouple measuring ports

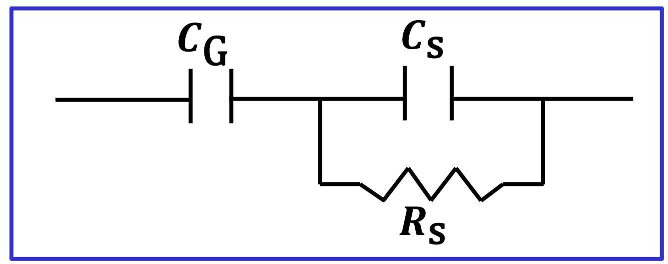
scio spec scientific instruments



# Equivalent electrical circuit model

- An equivalent electrical circuit model is created by combining the circuit elements which includes the solution resistance ($R_s$) and the capacitances of the glass-solution interface ($C_G$) and the solution ($C_s$) in an appropriate configuration of series and parallel elements.






$C_G$ is the capacitance of the glass-solution interface,
$C_S$ and $R_S$ are the capacitance and resistance of the solution

$$Z_{Total} = Z(C_G) + Z(R_s = C_s)$$

$$Z_{Total} = Z(C_G) + [\frac{1}{Z(R_s)} + \frac{1}{Z(C_s)}]$$

# Impedance to Complex Capacitance

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

$$Z^*_{\text{Total}} = Z^*(C_G) + [\frac{1}{Z^*(R_S)} + \frac{1}{Z^*(C_S)}]$$

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

which re-arranges to

$$Z^*_{\text{Total}} = \frac{1 + i\omega R_S(C_G + C_S)}{i\omega C_G + i\omega^2 R_S C_G C_S}$$

- Impedance can be expressed in terms of a complex capacitance

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

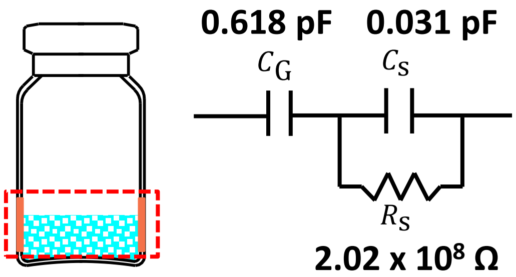
- The complex capacitance can also be expressed in form of real part and imaginary part

$$C^* = C' + iC''$$

- 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

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

# Dielectric loss spectrum of frozen water at -27 °C




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

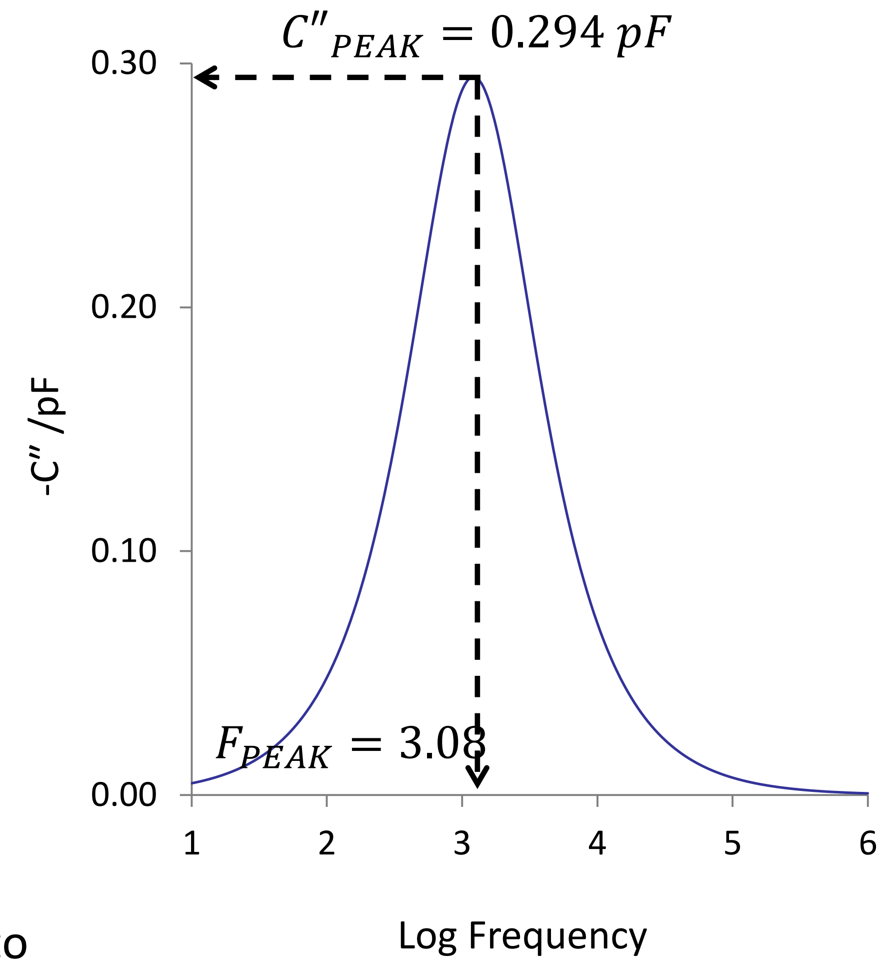
- A frequency of

$$F_{PEAK} = \frac{1}{2\pi R_S (C_S + C_G)}$$

- If $C_G > C_S$ then

$$C''_{PEAK} \cong C_G$$

- Which explains the sensitivity of $C''_{PEAK}$ to the height of the ice layer

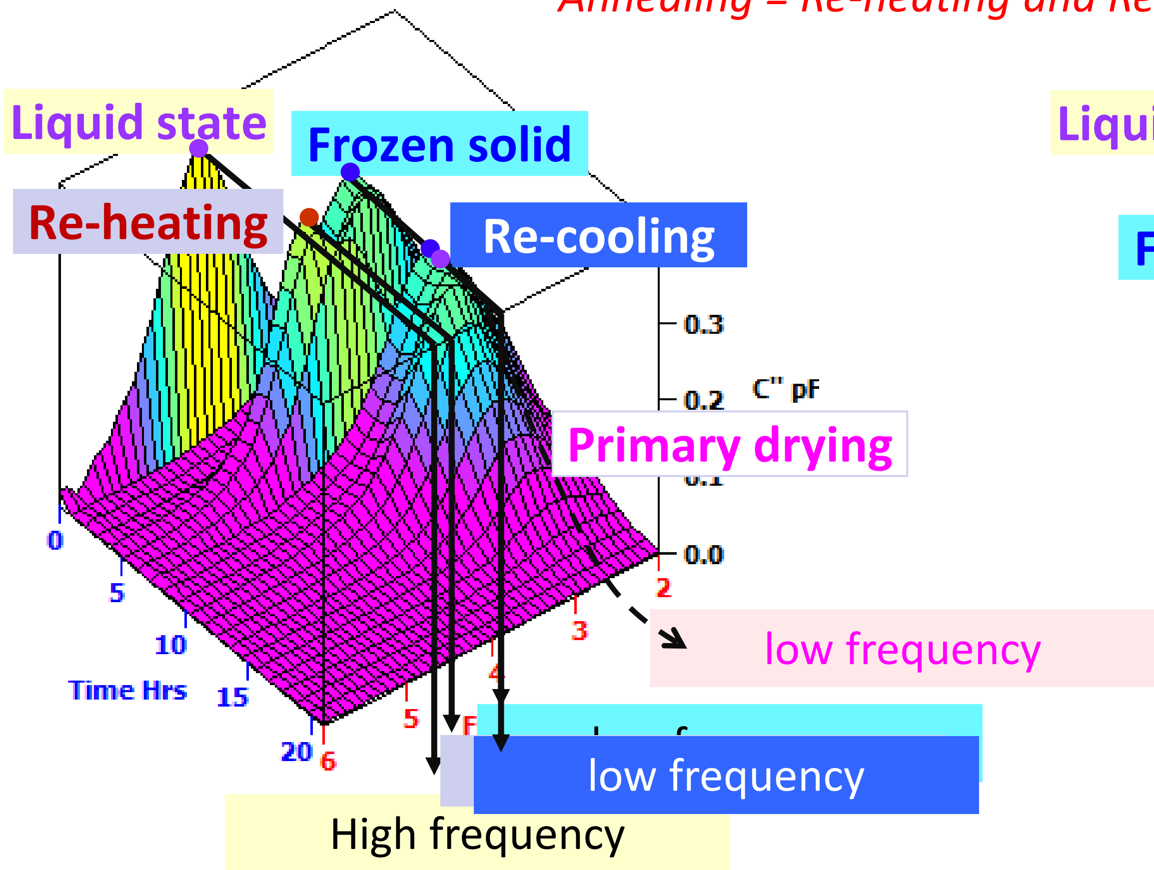


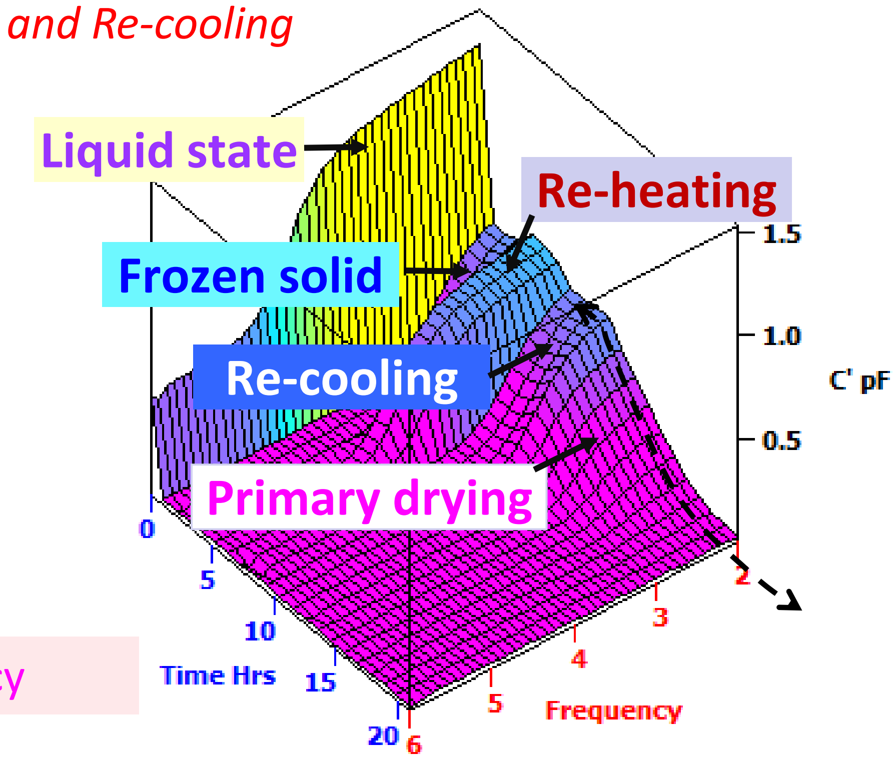
# TVIS Response Surface (3D-Plot)

Imaginary Part of Capacitance

Real Part of Capacitance

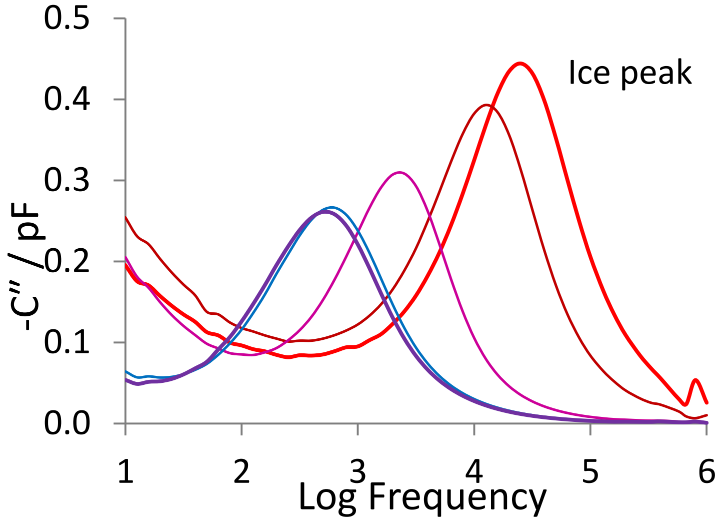
*Annealing = Re-heating and Re-cooling*



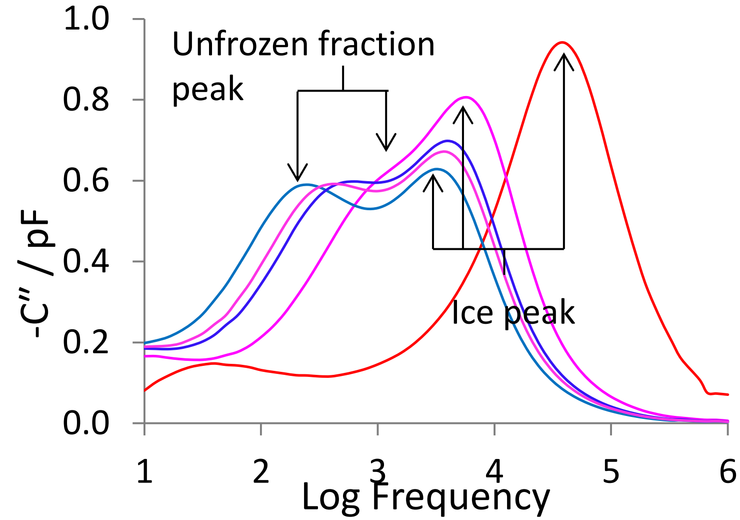



# Phase Separation in freezing step

## Water (frozen)

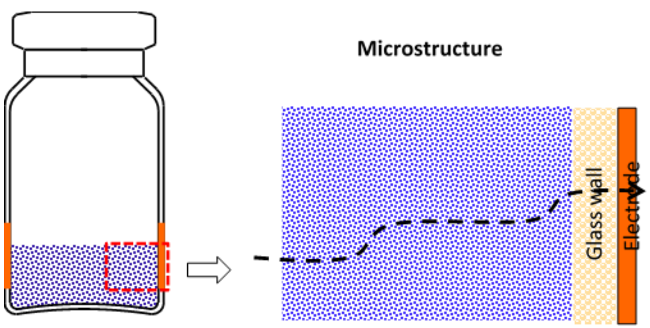


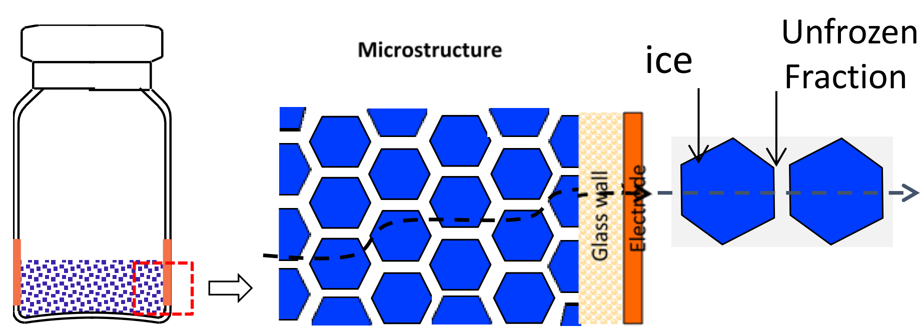

## 5%w/v Lactose solution (frozen)



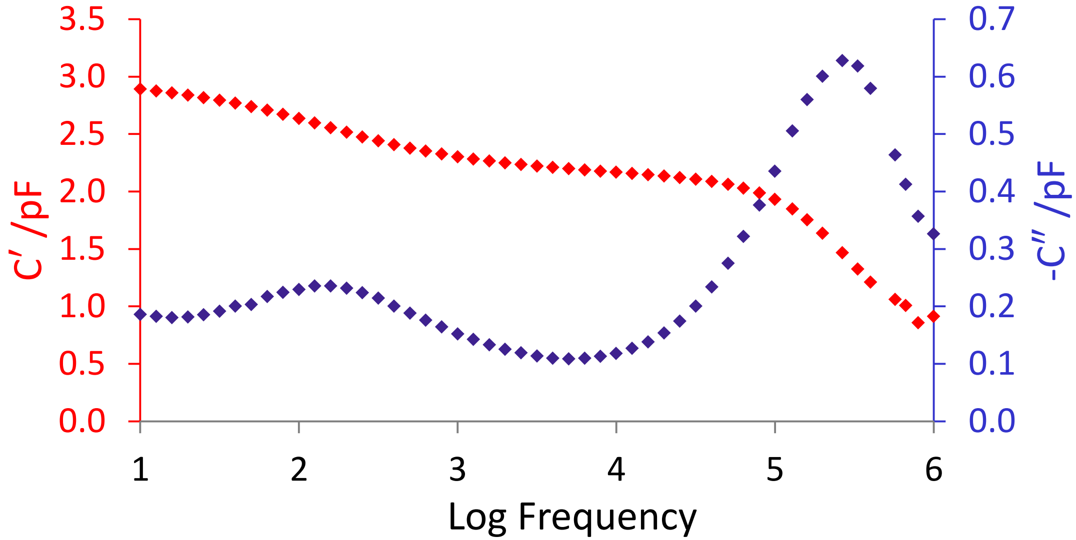

Add the equivalent circuit here

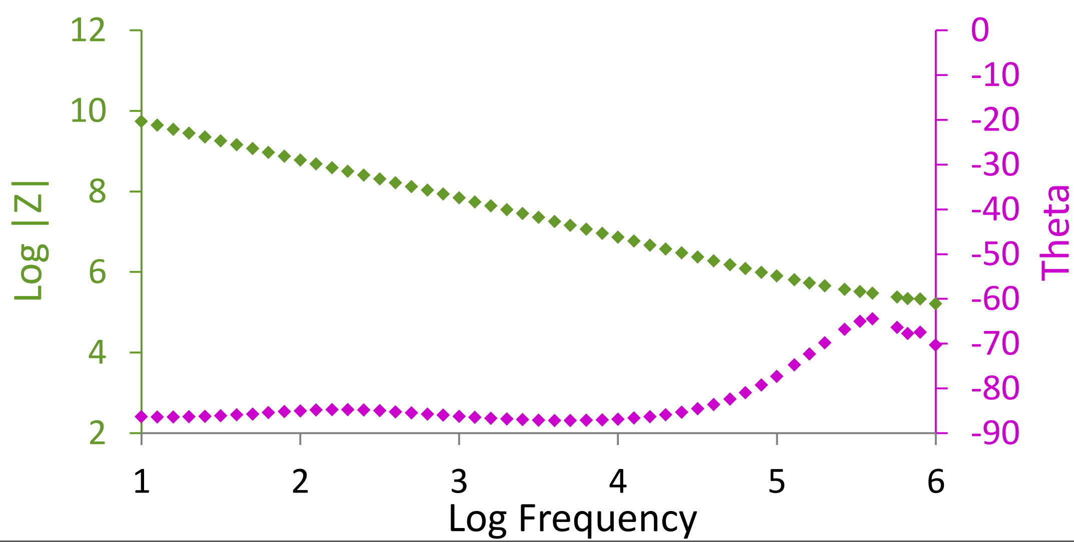
Add the equivalent circuit here

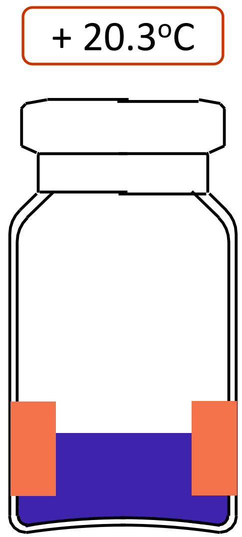


# Impedance and Capacitance Spectrum



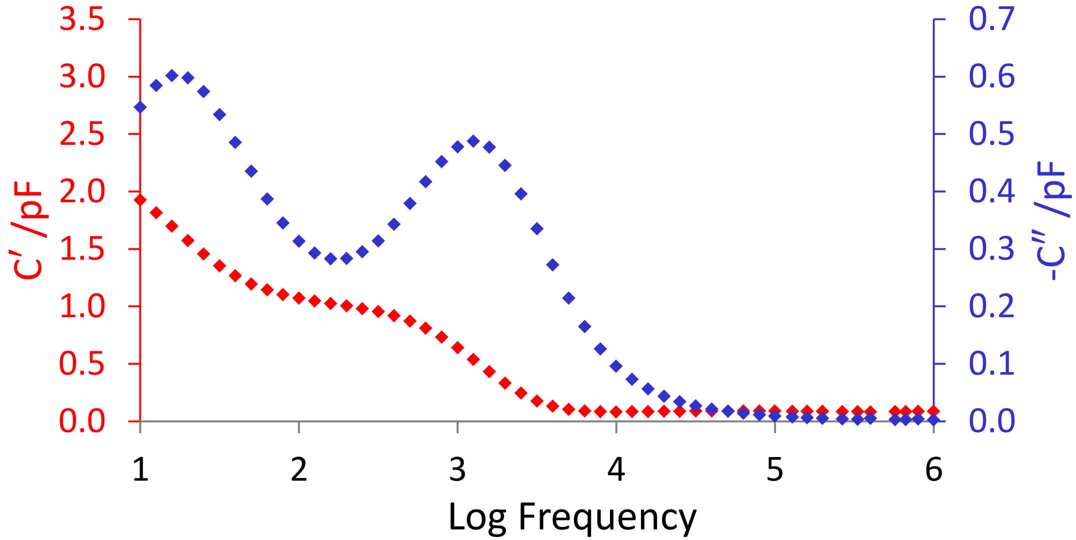



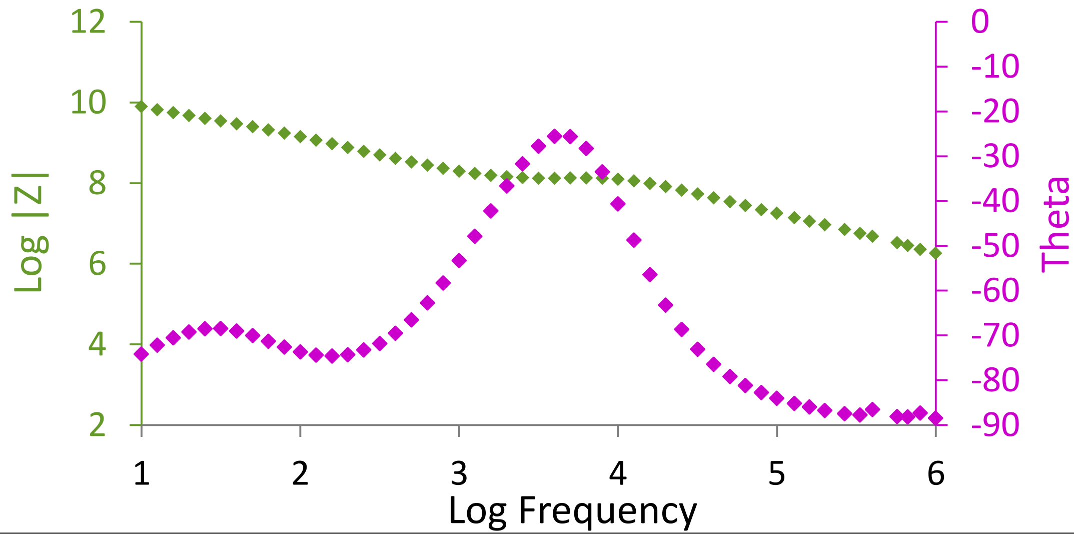



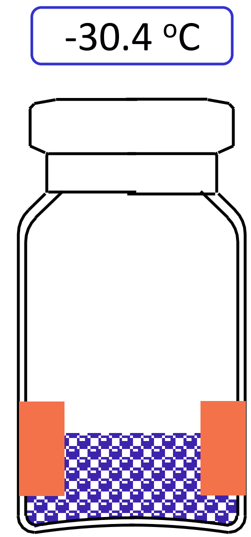

5%w/v Lactose solution

Liquid state

# Impedance and Capacitance Spectrum



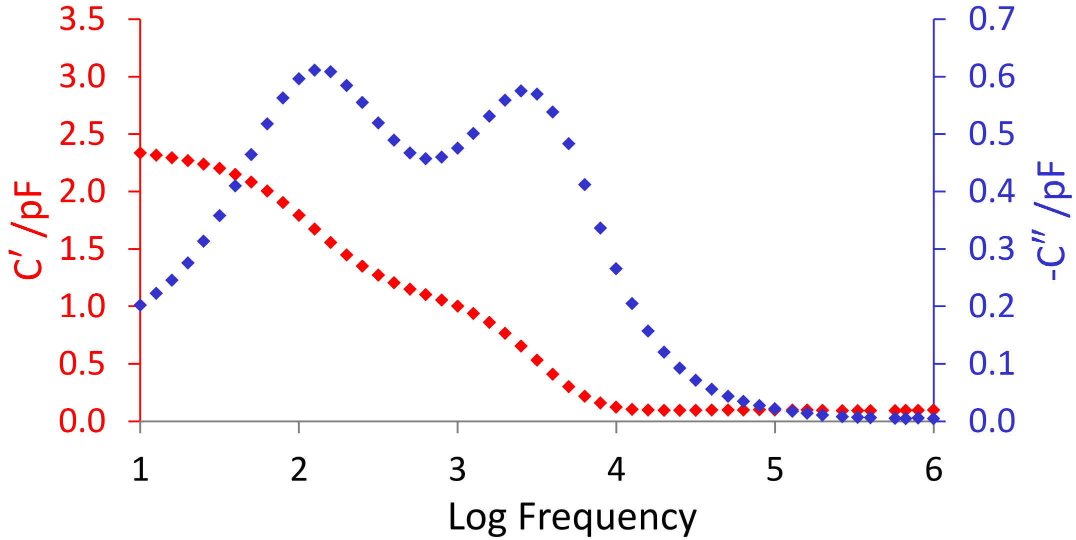



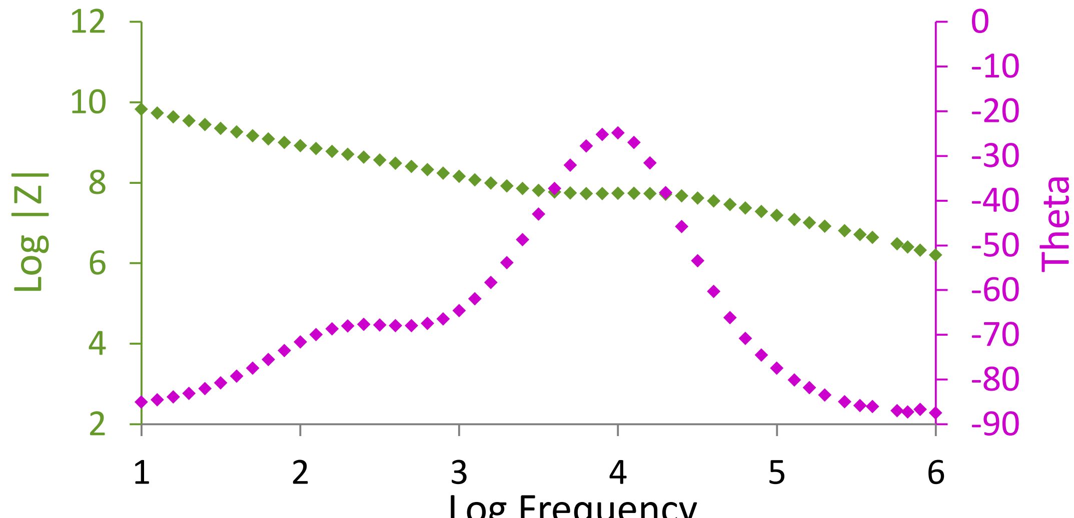




5%w/v Lactose solution

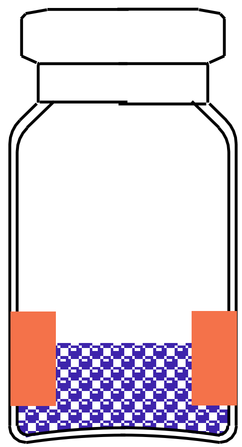
Solid (frozen state)
*lower temp*

# Impedance and Capacitance Spectrum



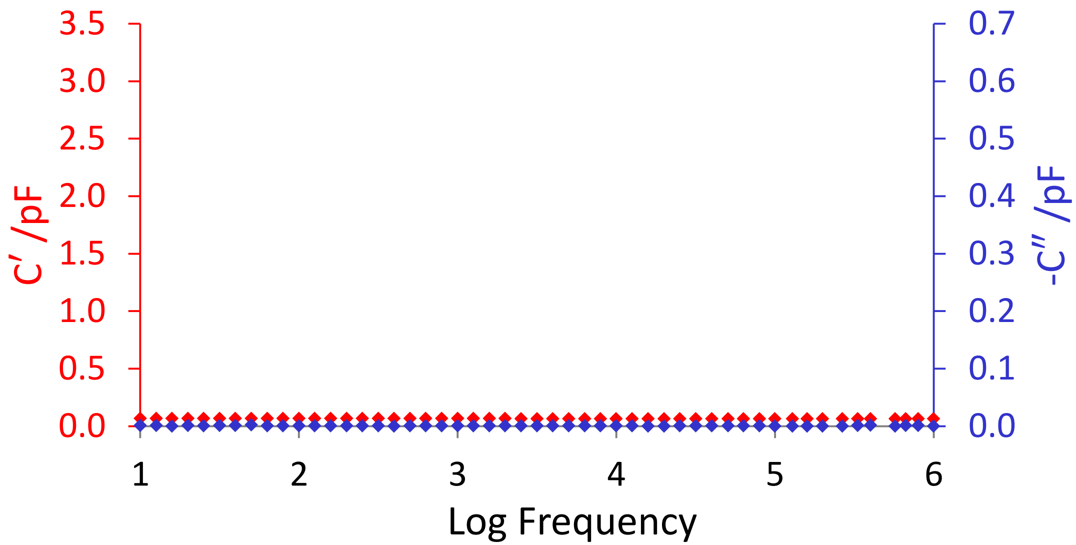

-20.4 °C

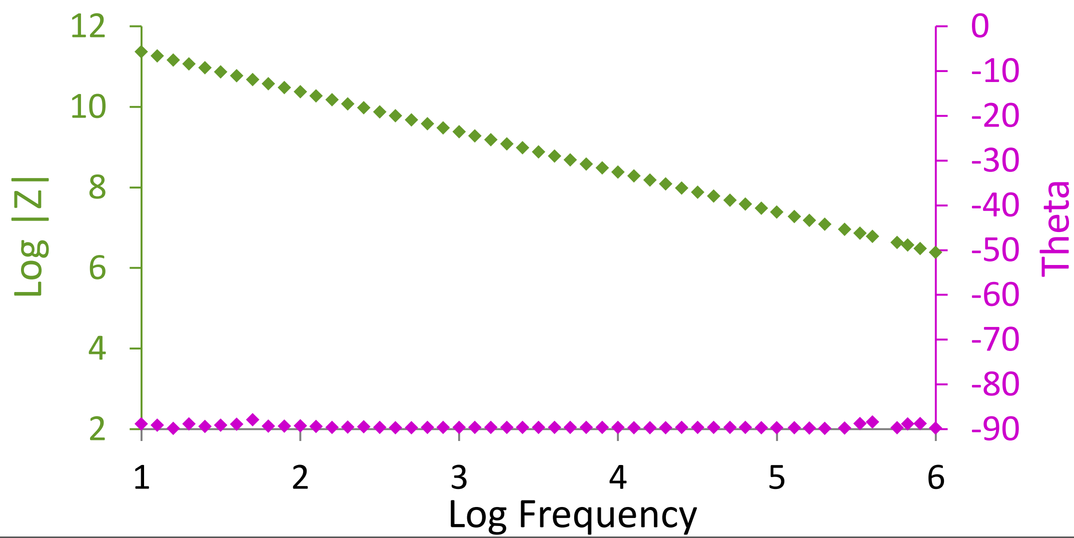


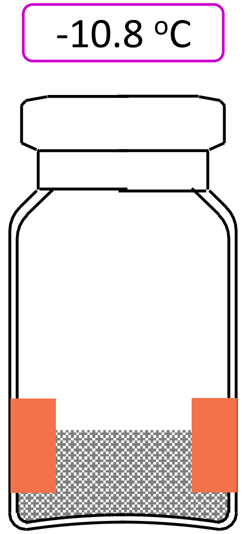
5%w/v Lactose solution

Solid (frozen state)
*high temp*

# Impedance and Capacitance Spectrum




5%w/v Lactose solution

Dry state

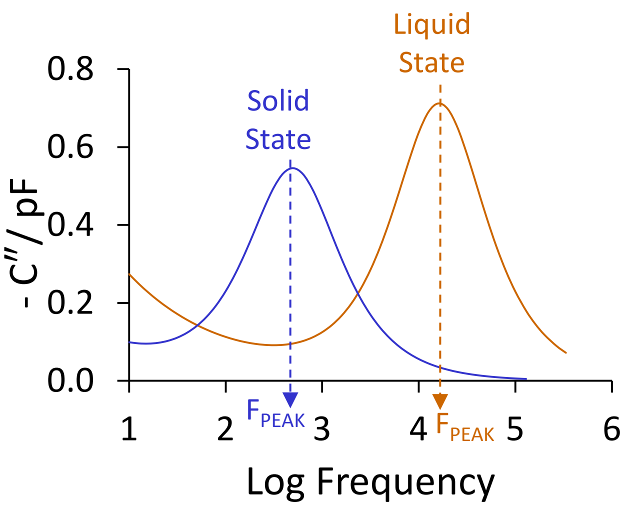
# Through Vial Impedance Spectroscopy (TVIS)

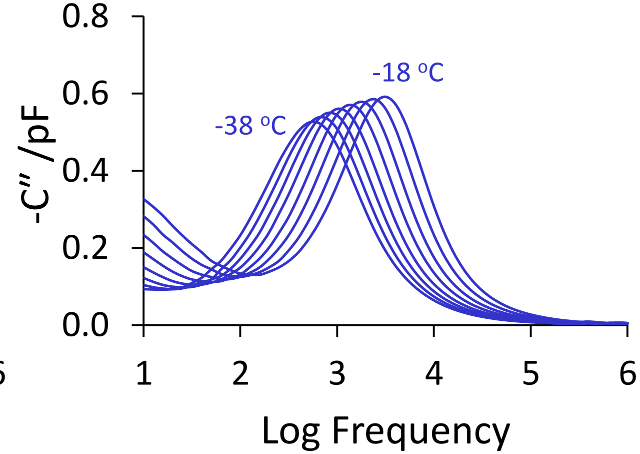
- TVIS measurement relate to both the *electrical resistance* and *electrical capacitance of the vial contents.*

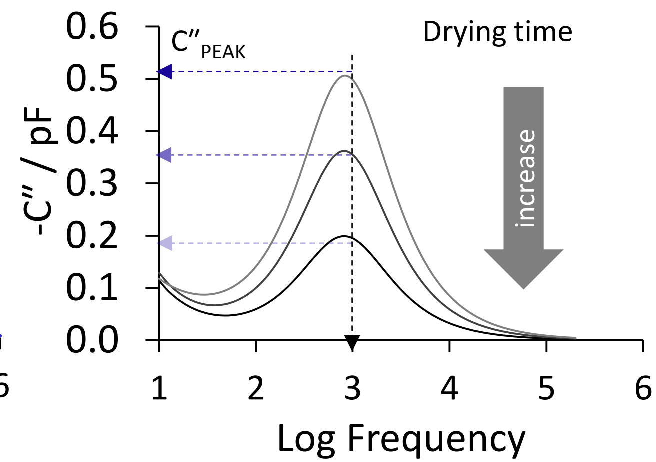
Monitoring Phase Behaviour (ice nucleation temperature and solidification end points

FPEAK temperature calibration for predicting temperature of the product in primary drying

Drying rate surrogate (from $dC''_{PEAK}$ /dt )



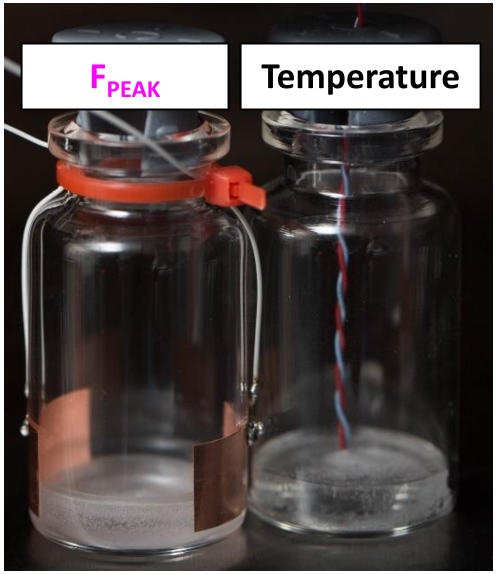



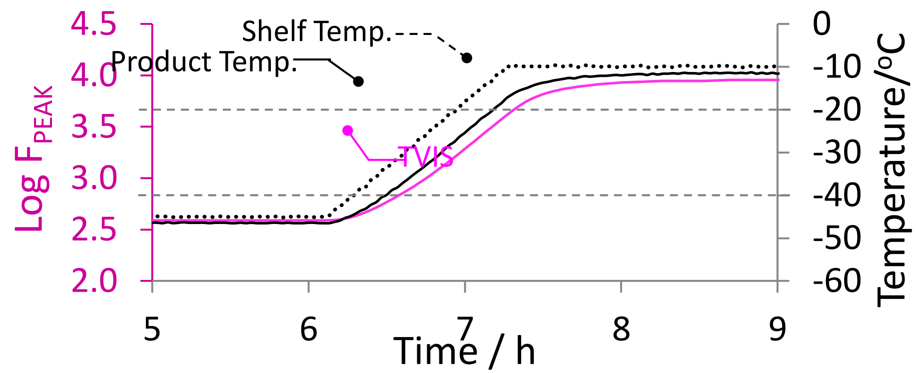



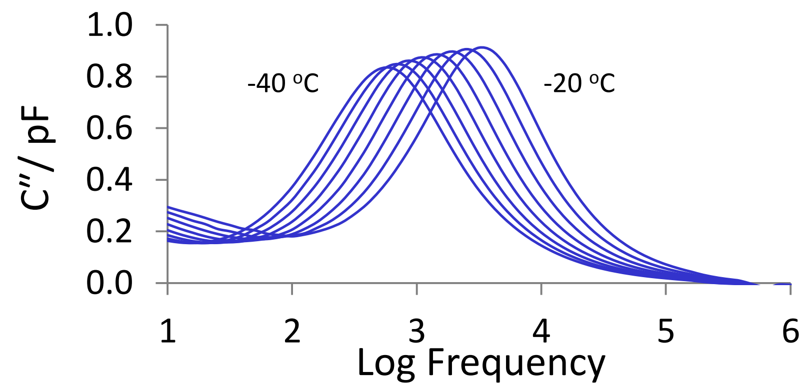

C′ (real part of the complex capacitance) is highly sensitive to low ice volumes; therefore it could be used for determination end point of primary drying

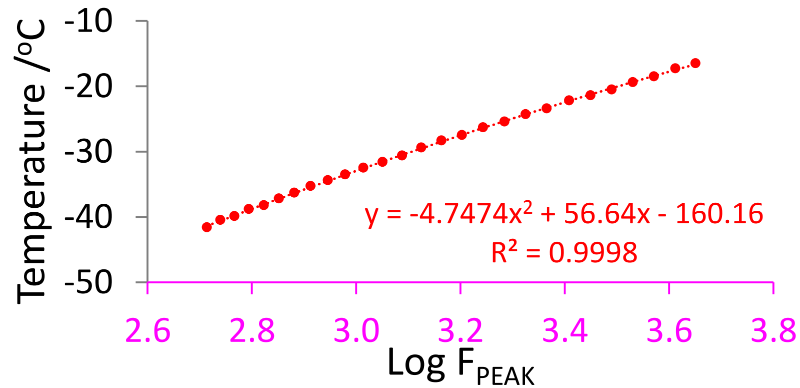
# Temperature Calibration

- $F_{PEAK}$ profile during annealing has ‘similar’ profile with product temperature.
- Assuming thermal equivalence between the thermocouple (TC) vial and TVIS vial, then the temperature calibration from annealing might be employed for the prediction of temperature during primary drying



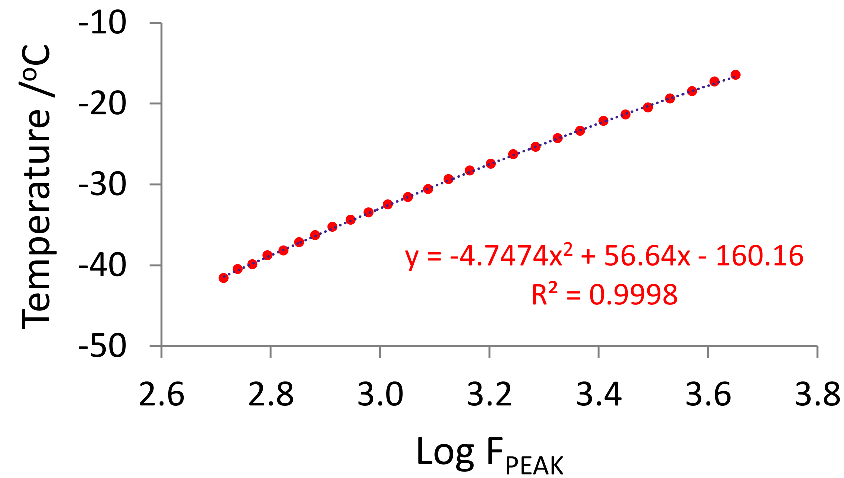



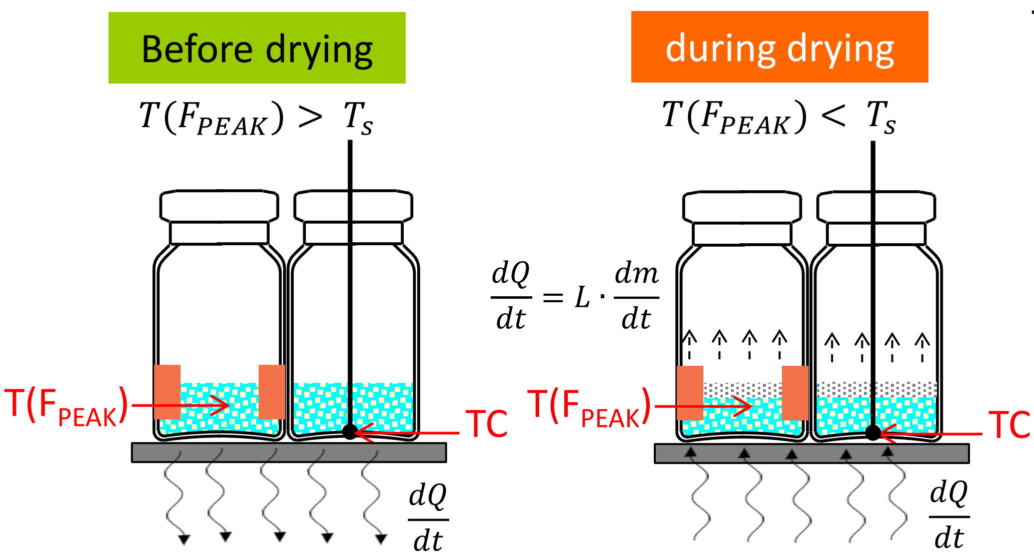



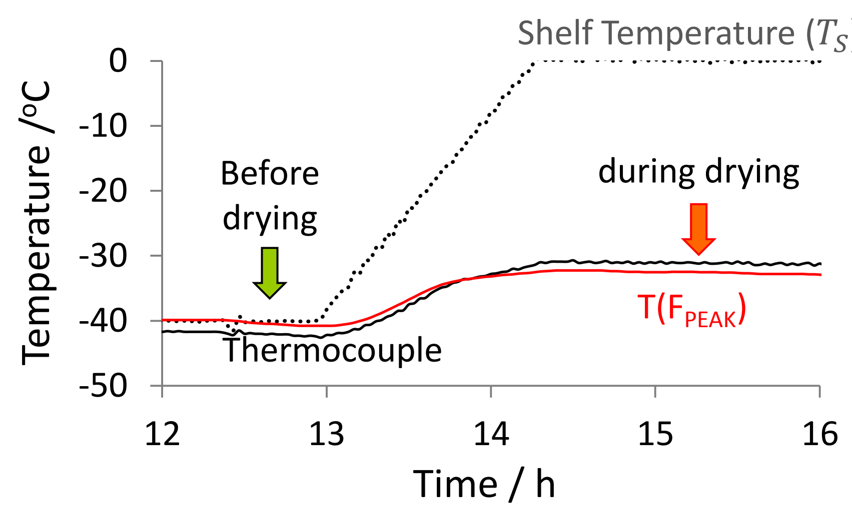
# Temperature Prediction in Primary Drying

- Temperature calibration curve selected for temperature prediction in primary drying : $T(F_{PEAK})$



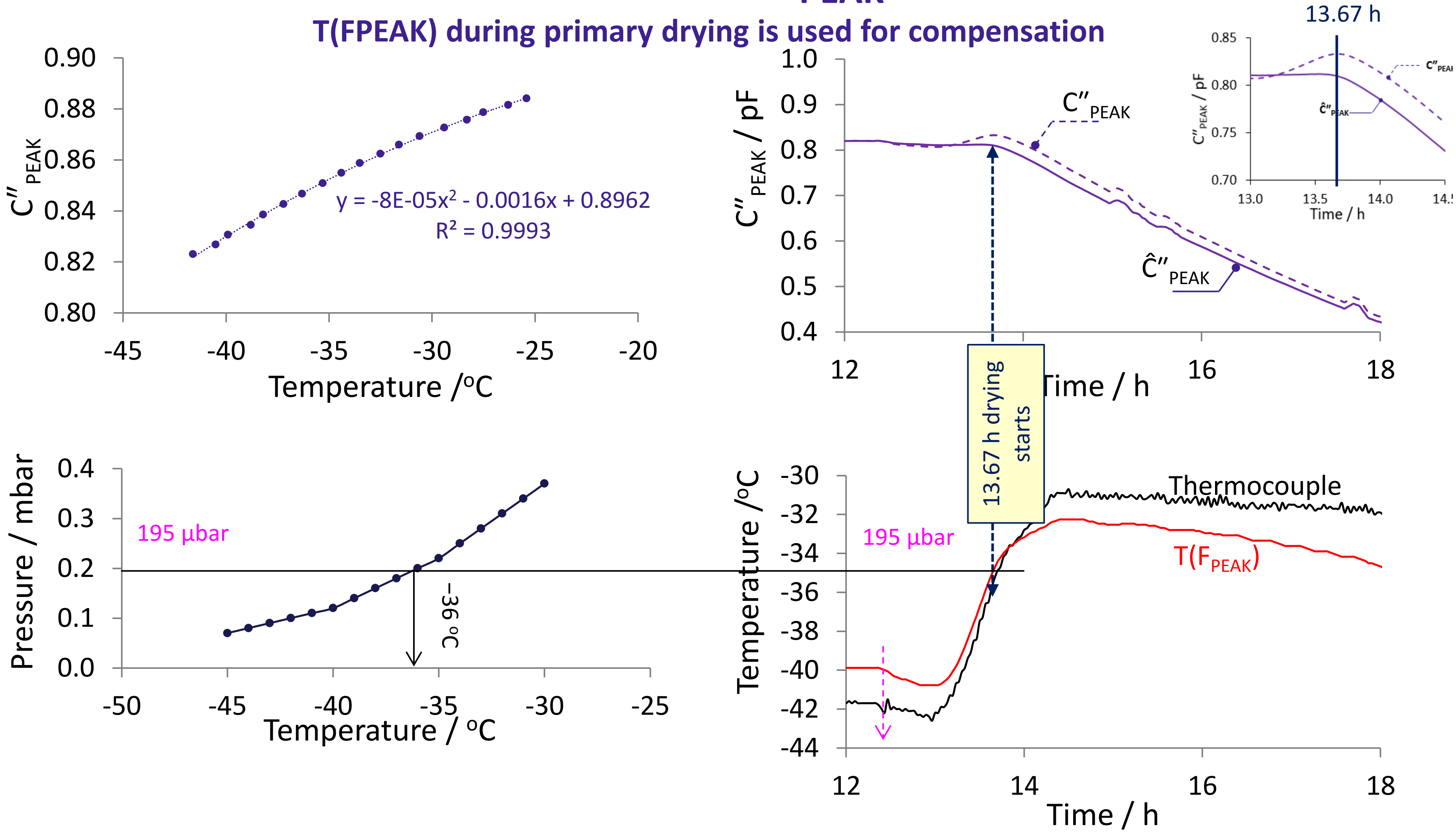

- Good agreement between product temperature (by TC) and $T(F_{PEAK})$



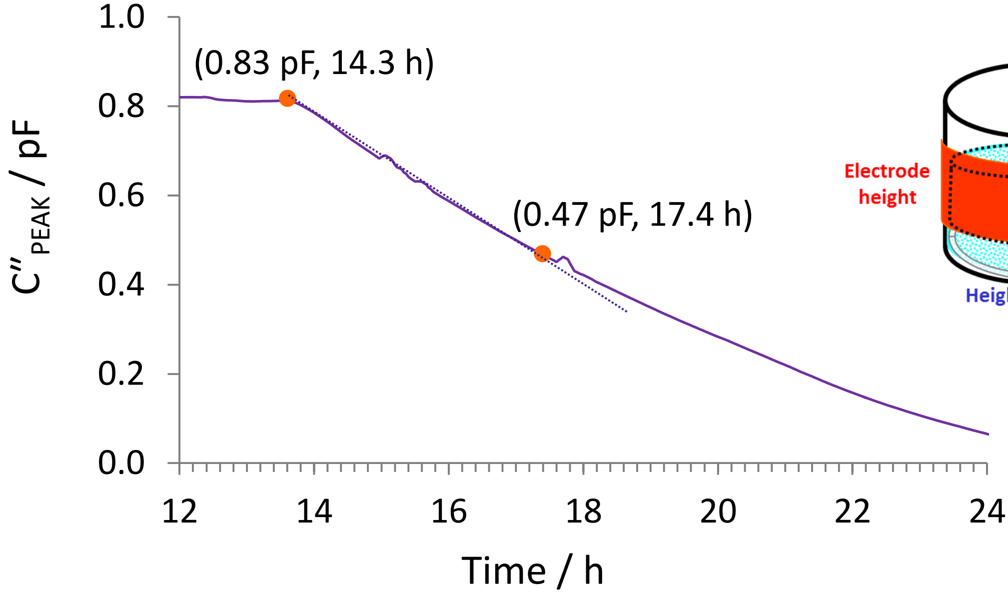



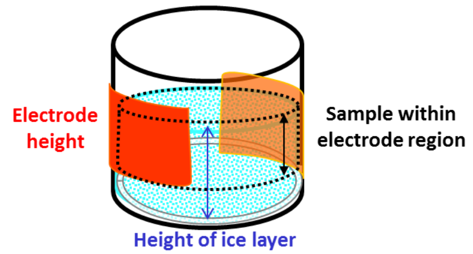
# Compensation of C"PEAK by T($F_{PEAK}$)

T(FPEAK) during primary drying is used for compensation



# Drying rate calculation




- Drying rate (g/h) for $\hat{C}''_{PEAK}$

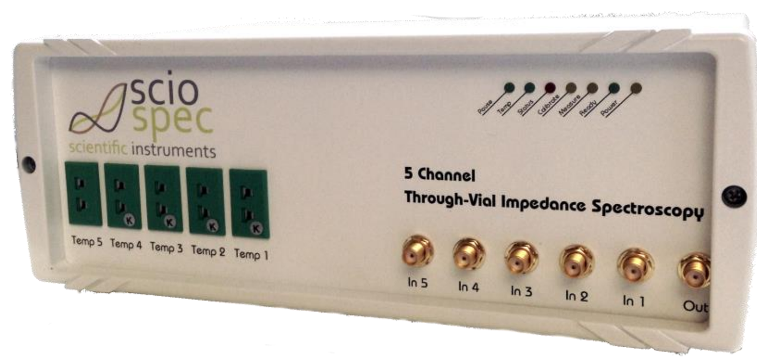
$$Drying\ rate = \left(\frac{\hat{C}''_{PEAK(initial)} - \hat{C}''_{PEAK(end)}}{Time_{(end)} - Time_{(initial)}}\right) \times \frac{ice\ mass\ within\ electrode\ region}{\hat{C}''_{PEAK(initial)}}$$

$$Drying\ rate = \left(\frac{0.83 - 0.47}{17.4 - 14.3}\right) \times \frac{3.69}{0.83} = 0.52\ g \cdot h^{-1}$$

# Summary

- Temperature calibration of the TVIS parameter ($F_{PEAK}$) for ice during an additional temperature cycling stage applied to a prediction of ice temperatures during the initial (few hours) of primary drying
- Temperature compensation of TVIS parameter ($C''_{PEAK}$) allows for an accurate estimation of ice mass during primary drying as evidenced by comparable results of drying rate between the determined by TVIS and that determined (gravimetrically) by loss weight

Non-invasive real time information for characterising the freeze drying



# Future Work

- Development mapping a drying characteristics from lab scale to production
  - Determination of heat transfer coefficients ($K_V$)
  - Determination of dry layer resistance ($R_P$) to predict drying efficiency


- Investigation the molecular dynamic of the unfrozen fraction
  - Monitoring product stability
  - Examine the mechanical strength of the freeze dried product (i.e. collapse behaviour)

- Develop (new) continuous drying technologies

# Acknowledgements, Recent Projects & Collaborators


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

- Sciospec Scientific Instruments

  Commercial Development of TVIS instrument
  - Martin Bulst
  - Sebastien Wegner

GEA Pharma Systems bluefrog design NIBSC SANOFI

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