

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

Through Vial Impedance Spectroscopy



Leicester City



- Multicultural
- 330,000 people
- 51.1% voted Remain

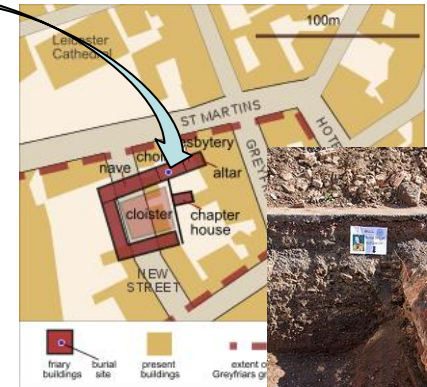
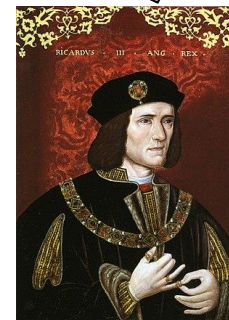
2 May 2016

Leicester champions of the Premier League



King Richard III (1483-1485)

- Last Plantagenet king of the House of York
- Killed at battle of Bosworth, buried without ceremony and then lost until 2012!



found under a car park



- New £139 m Campus
- Student numbers and demographics
- DMUglobal (Berlin, New York)
- LoveInternational

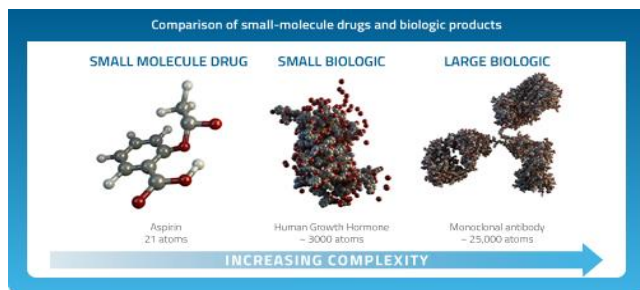


Hawthorn Building:

- 1987 : Arts and Technology
- 1960s: David Bowie; Jimi Hendrix
- Now: Faculty Health & Life Sciences
- 400 FT & PT staff; 5000 students
- Houses School of pharmacy

Pharmaceuticals *(From drugs molecules to products)*

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



Aspirin
21 atoms

Hormone
~3000 atoms

Antibody
25,000 atoms



Production



Product available in the market

- Quality: Safety & Efficacy

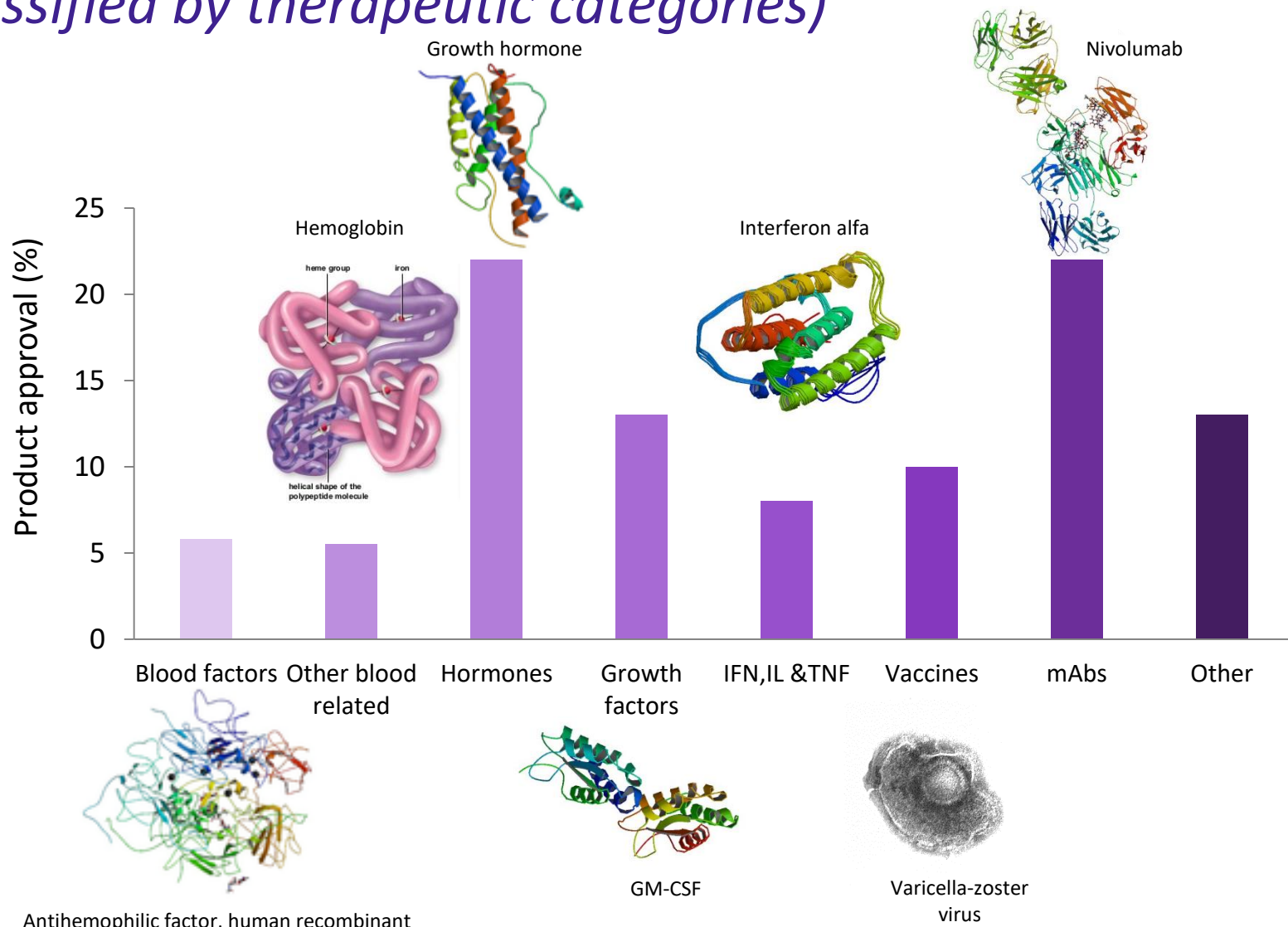


QC Pharmacopoeial tests

Formulation development

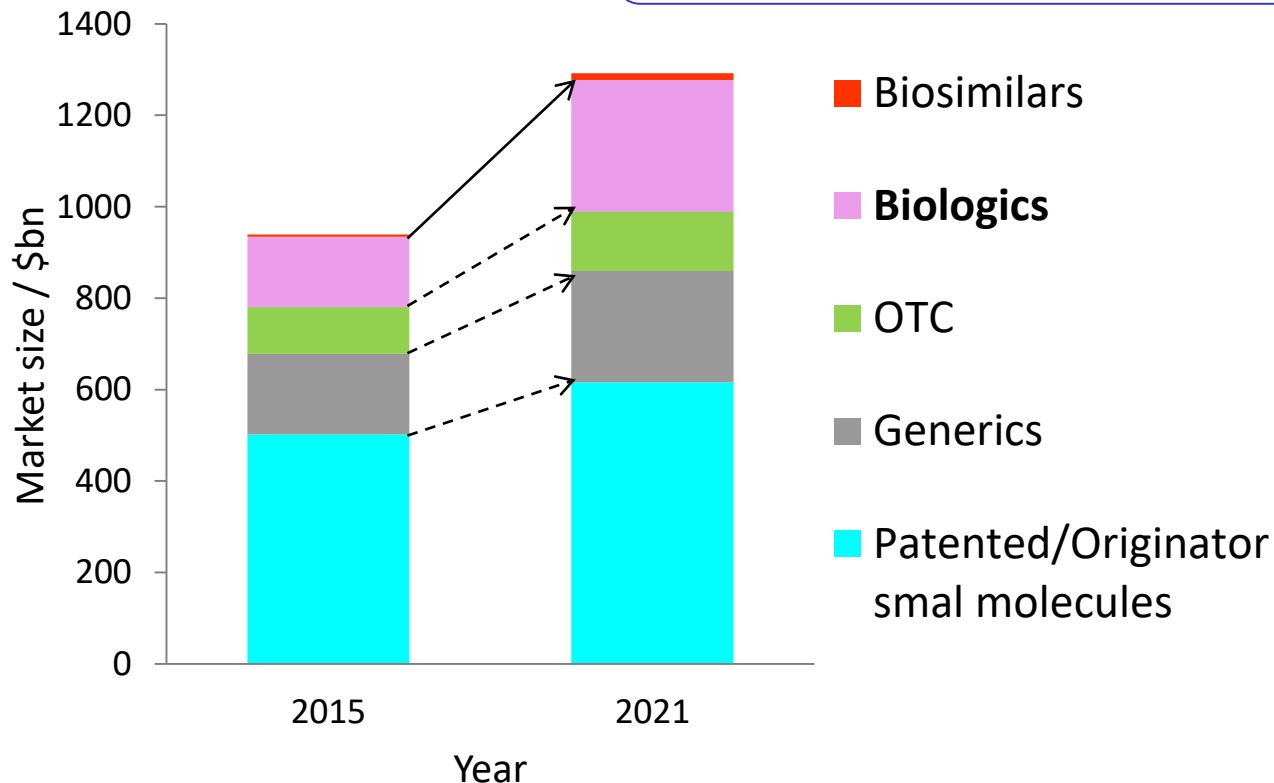
- Drug products (i.e. dosage form: tablets, injections) etc.
- Healthcare and cosmetics product (i.e. nutrition)

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.

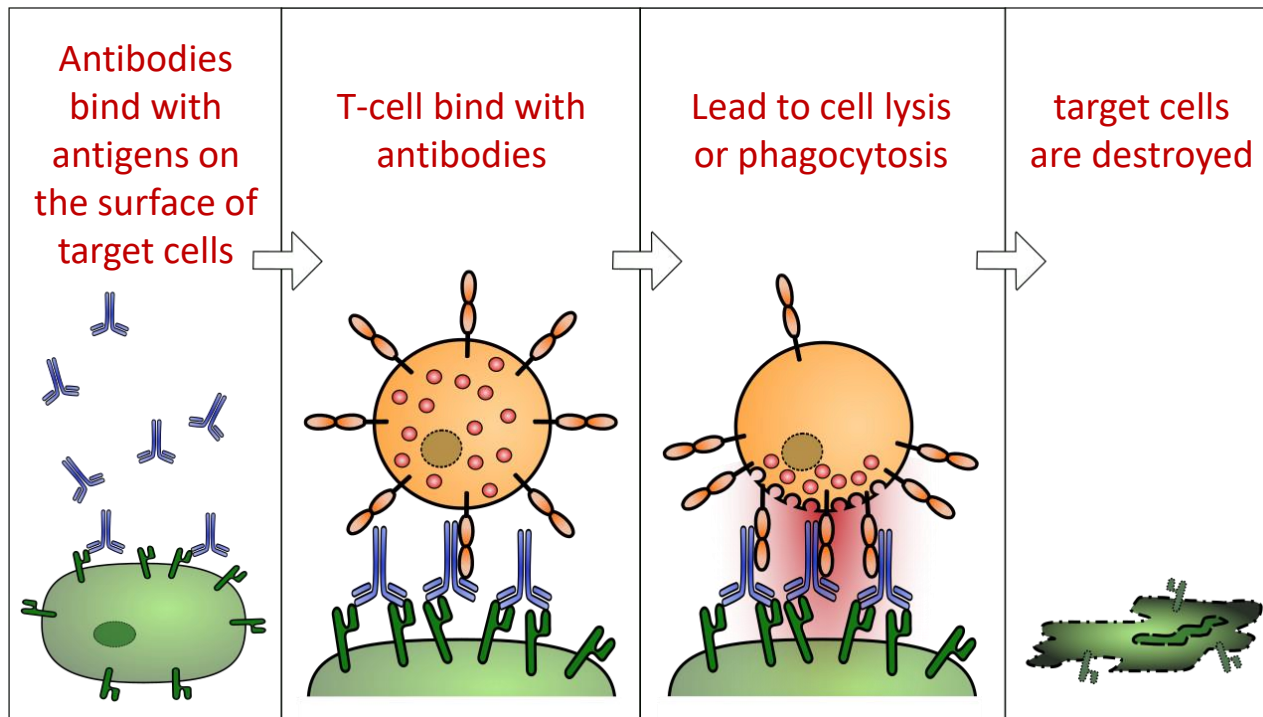


Newrzella A. (2017) Pharma & Biotech 2017 – Review of Outsourced Manufacturing

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

Generic name	Trade name	Antigen	Isotype	Target	Approved indication	First approval	Current status
Muromomab	Orthoclone	Murine, IgG2a	CD3		Allograft rejection in allogeneic renal transplantation	86/06/19	NA
Abciximab ¹	ReoPro	Chimeric, IgG1	GP1Ib/IIIa r		Maintenance of coronary patency	94/12/22	NA
Rituximab ²	Mabthera	Chimeric, IgG1	CD20		CD20-positive B-cell non-Hodgkin's lymphoma	97/11/26	98/06/02
Daclizumab		Humanized, IgG1	CD25 (IL2r)		Allograft rejection	99/02/26	99/02/26
Basiliximab		Chimeric, IgG1	CD25 (IL2r)		Allograft rejection	10/09	10/09
Palivizumab		Humanized, IgG1	Protein F		Respiratory syncytial virus infection in children	97/08/13	97/08/13
Infliximab	Remicade	Chimeric, IgG1	TNF α		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	huFc γ 1/TNFr	TNF α and β		Autoimmune diseases such as ankylosing spondylitis	98/11/02	00/02/03
Gemtuzumab ⁴	Mylotarg	Humanized, IgG1	CD22		Acute myeloid leukemia	00/05/17	NA
Alemtuzumab ⁵	Mabcampath	Humanized, IgG1	CD52		Chronic lymphocytic leukemia	01/05/07	01/07/06
Ibritumomab ⁶	Zevalin ^{90Y}	Mouse, IgG1	CD20		Non-Hodgkin's lymphoma	02/02/19	04/01/16
		Human, IgG1 (PD)	TNF α		Crohn's disease and rheumatoid arthritis	02/12/19	02/12/19
		huFc γ 1/LFA-3	CD2		Chronic plaque psoriasis	03/06/27	03/06/27
		Humanized, IgG1	IgE		Treatment of asthma	03/06/27	03/06/27
Tositumomab ⁷	Bexxar ^{131I}	Murine, IgG2a	CD20		CD20-positive B-cell non-Hodgkin's lymphoma	03/06/27	NA
Efalizumab	Raptiva	Humanized, IgG1	CD11a		Moderate to severe plaque psoriasis	03/10/27	04/09/20
Cetuximab	Erbix	Chimeric, IgG1	EGFR		Metastatic colorectal carcinoma	04/02/12	04/06/29
Bevacizumab	Avastin	Humanized, IgG1	VEGF-A		Metastatic colorectal carcinoma	02/26	05/01/12
Natalizumab ⁹	Tysabri	Humanized, IgG4	Integrin- α 4		Multiple sclerosis	04/11/23	06/06/27
Ranibizumab	Lucentis	Humanized, IgG4	VEGF-A		Wet-type age-related macular degeneration	06/06/30	07/01/22
Panitumumab ¹⁰	Vectibis	Humanized, IgG2	EGFR		Metastatic colorectal carcinoma	06/09/27	07/12/19
Eculizumab ¹¹	Soliris	Humanized, IgG1	C5		Paroxysmal nocturnal haemoglobinuria	07/03/16	07/06/20
Certolizumab ¹²	Cimzia	Humanized, IgG1	TNF α		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 2015⁵

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

Freeze Drying Process



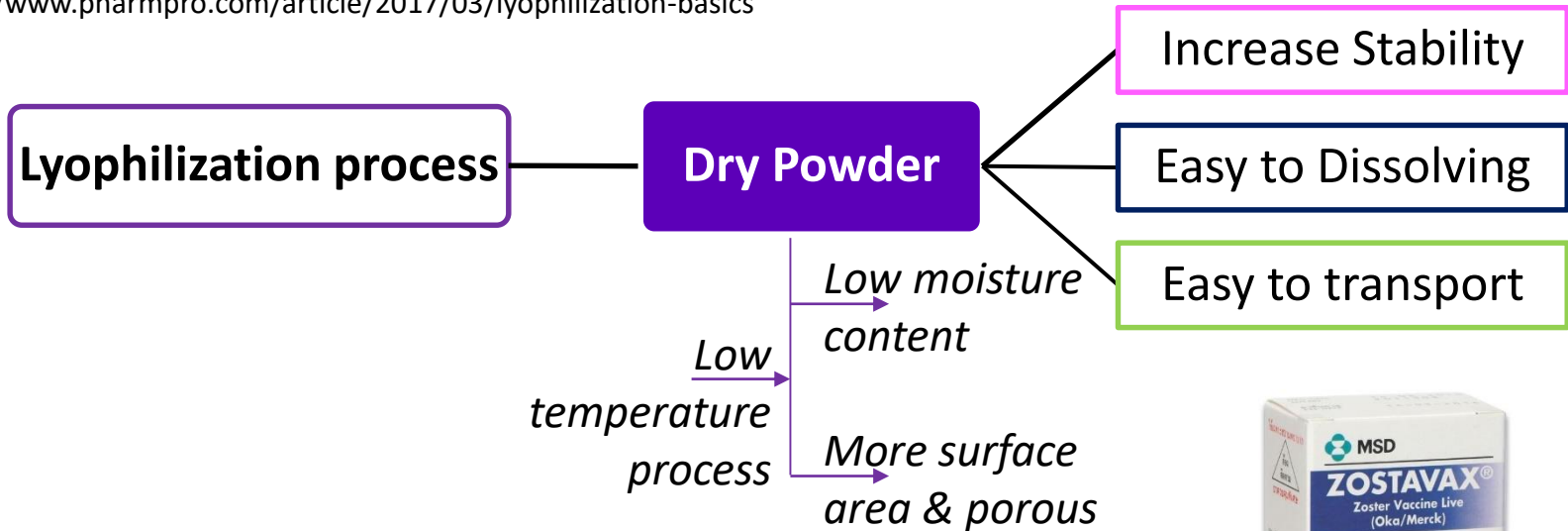
Advantages of Lyophilization

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



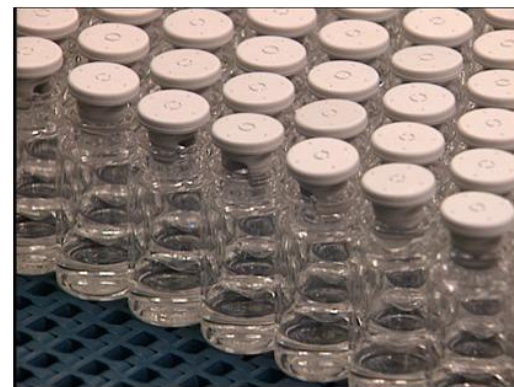
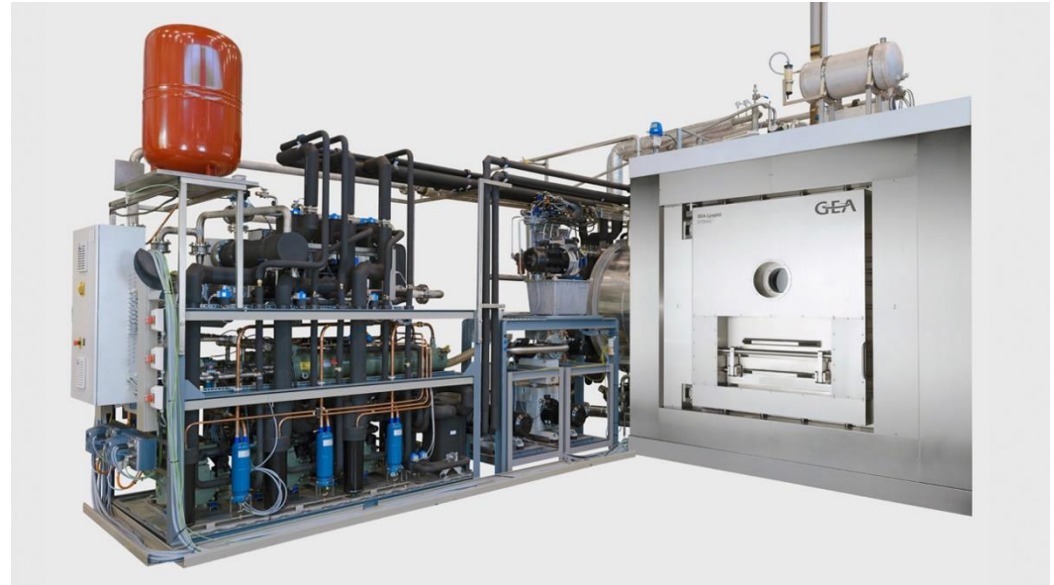
Azithromycin injection.
(Zithromax®)



Zoster vaccine
(Zostavax®)

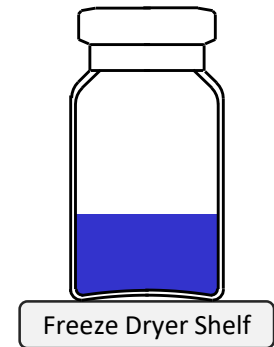
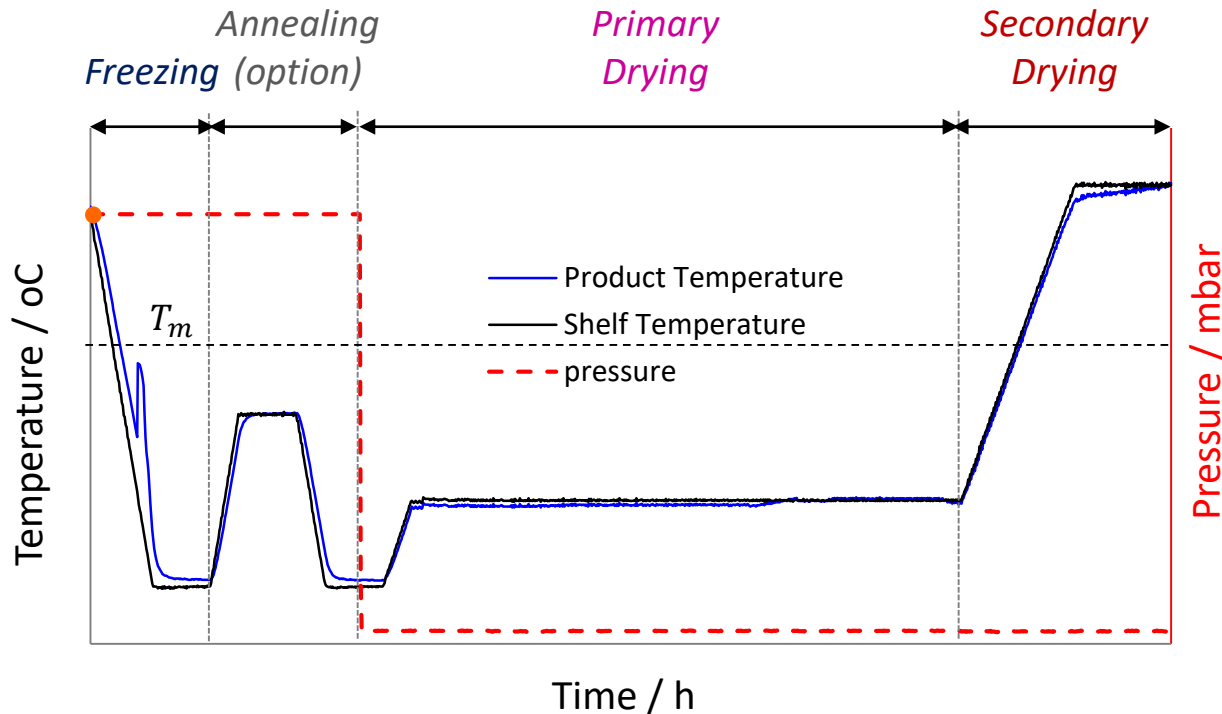
Limitation of Lyophilization Technology

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



Lyophilization or Freeze Drying Process

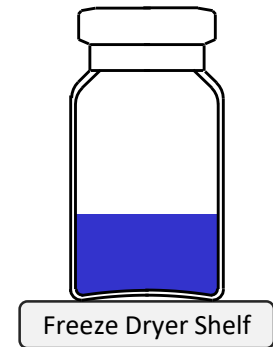
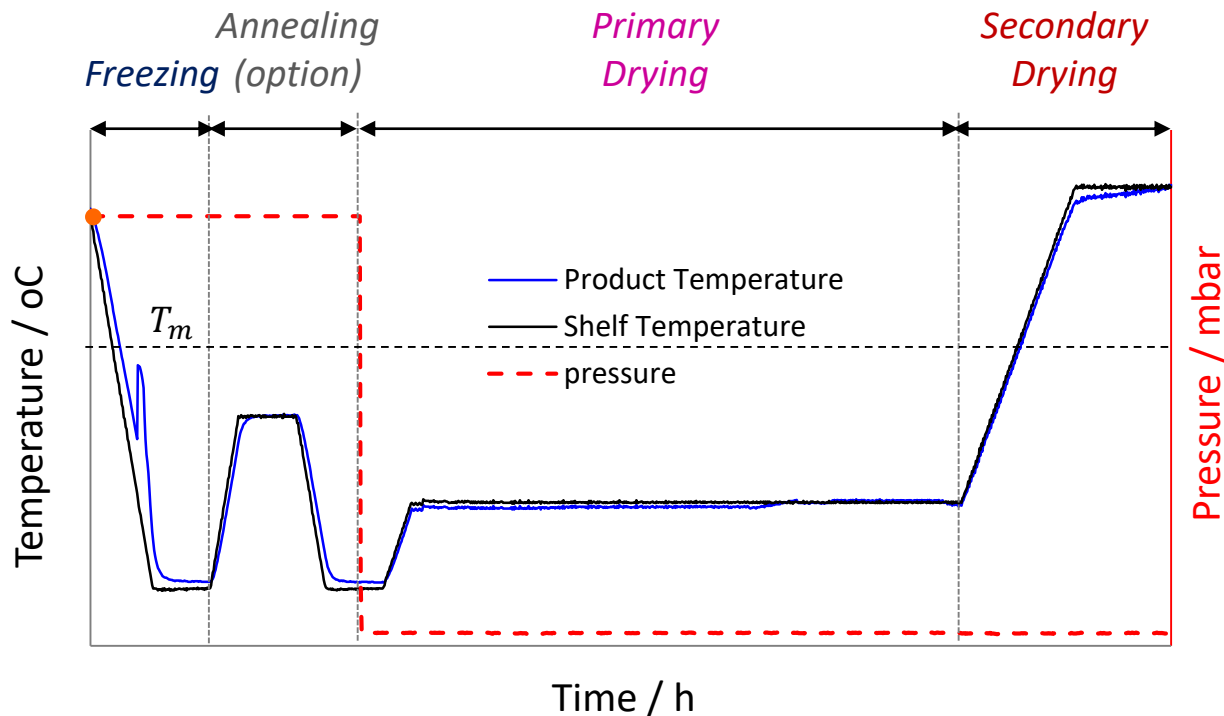
- A technique which dries product at low temperature through sublimation process
- It consists of three main steps : **Freezing**, **Primary drying** and **Secondary drying**



Equilibrium product temperature of all vials

Lyophilization or Freeze Drying Process

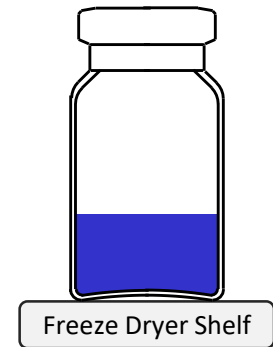
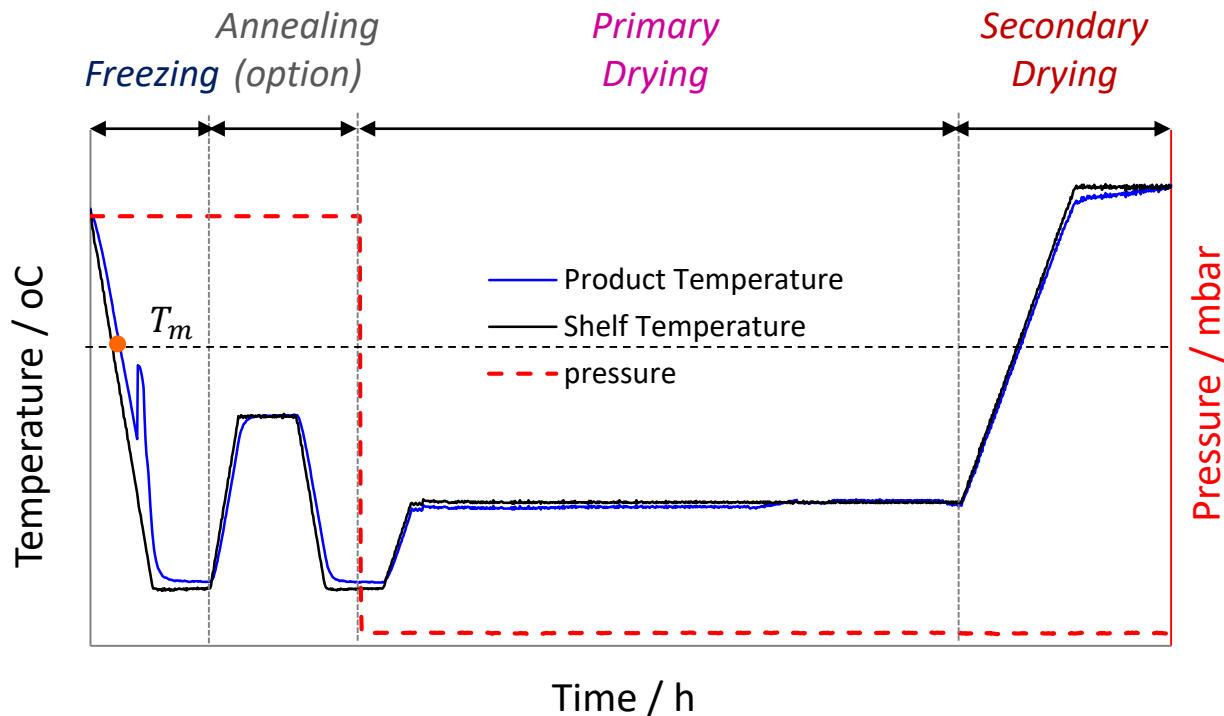
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Temperature of the product (liquid state) decreases (T_p) as shelf temperature decreases (T_s)

Lyophilization or Freeze Drying Process

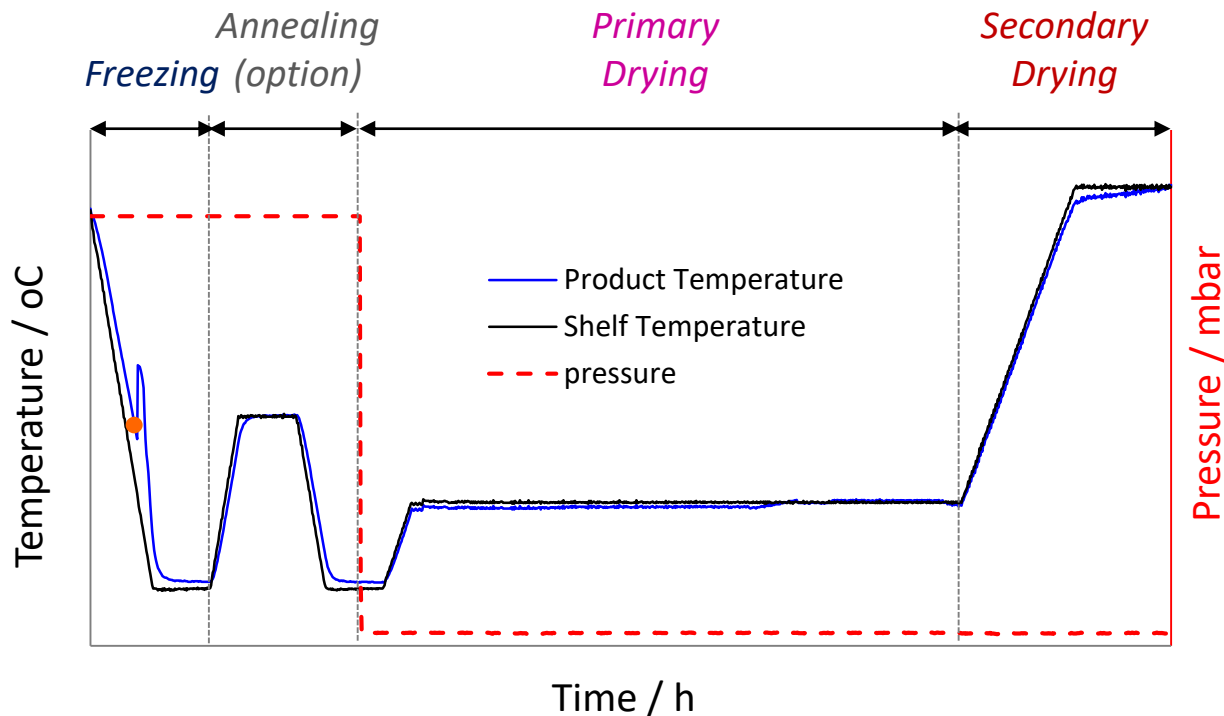
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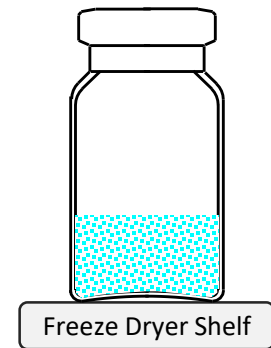
Liquid product supercools below the melting point: Melting temperatures of ice in frozen solution would be less than that of pure water, owing to the freezing point depression of the solutes

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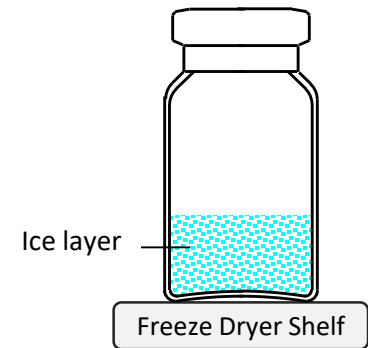
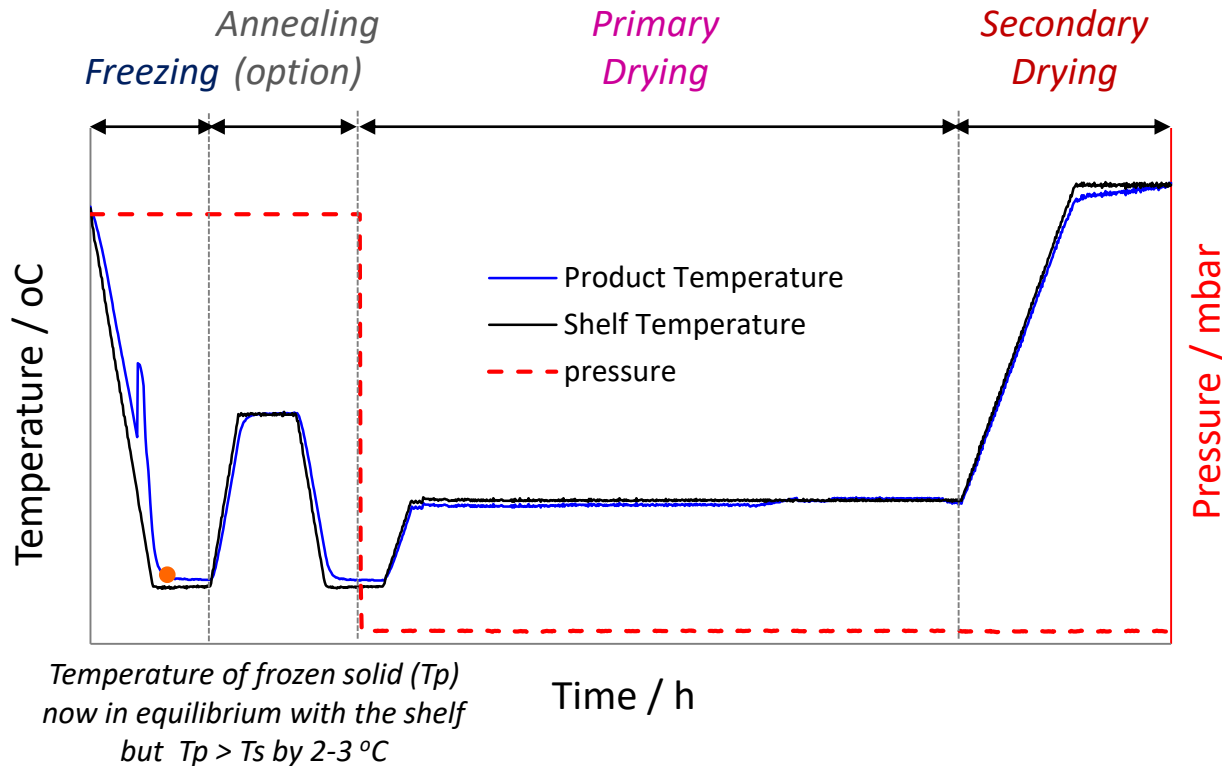
Ice nucleation



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

Lyophilization or Freeze Drying Process

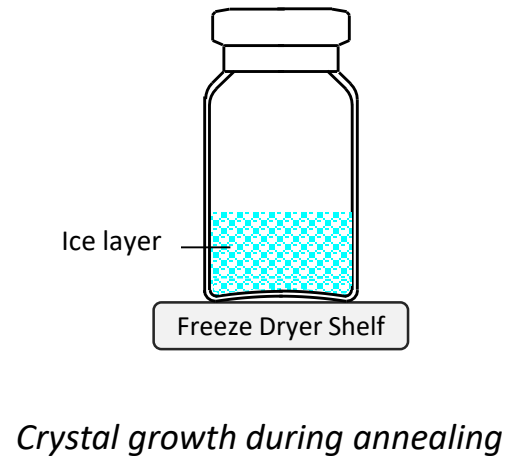
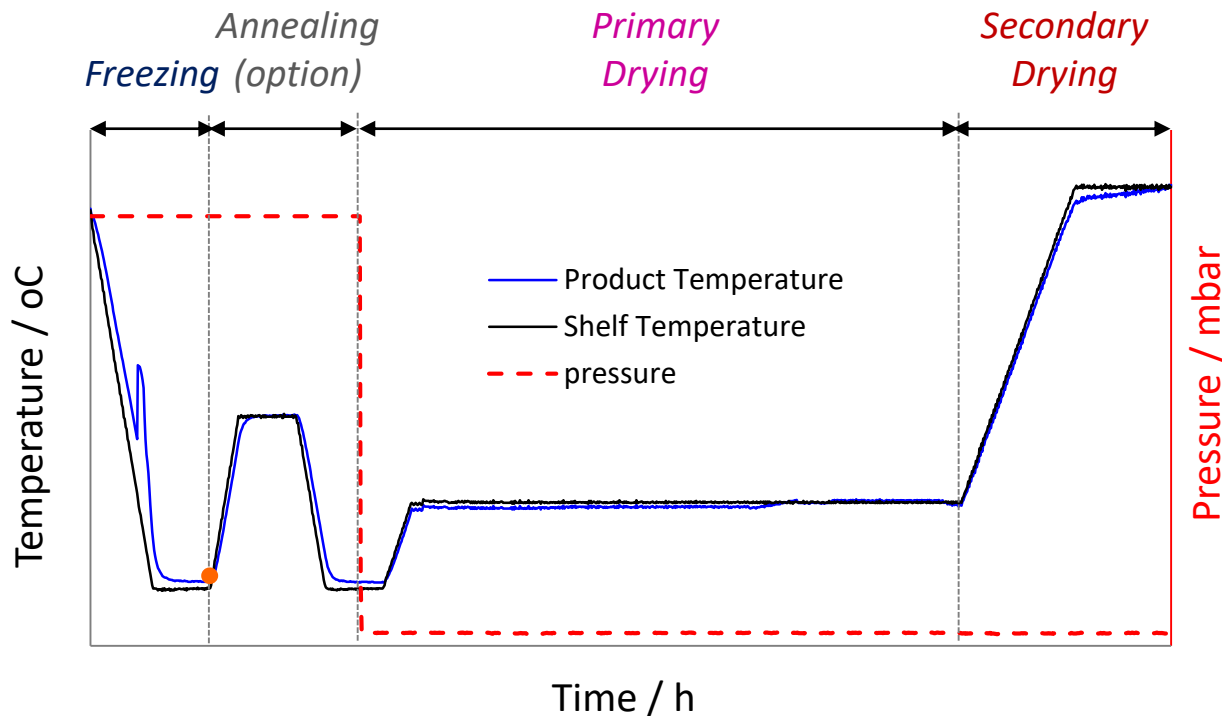
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Temperature hold to ensure complete solidification of ice

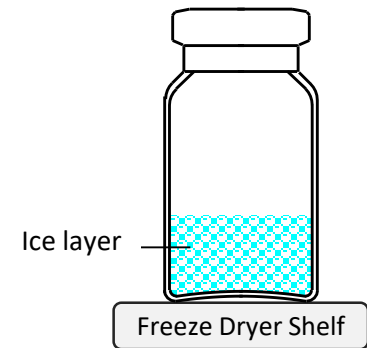
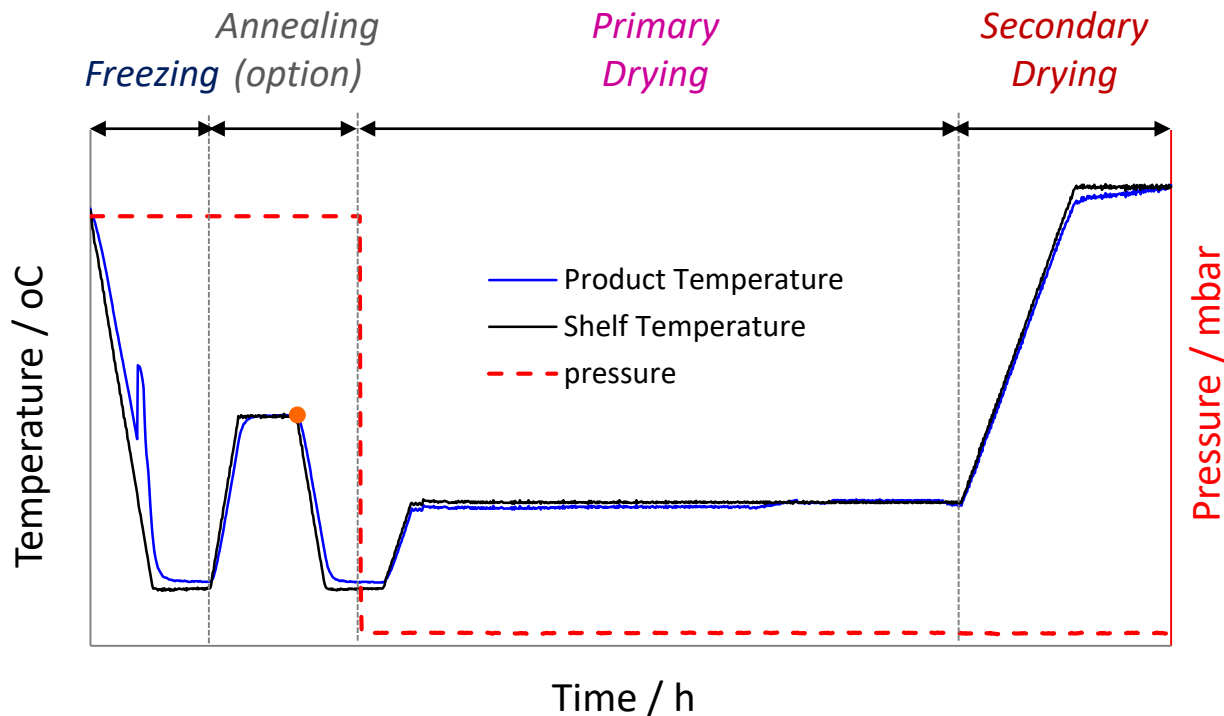
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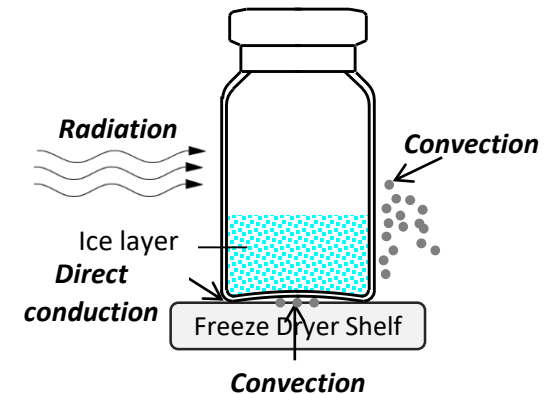
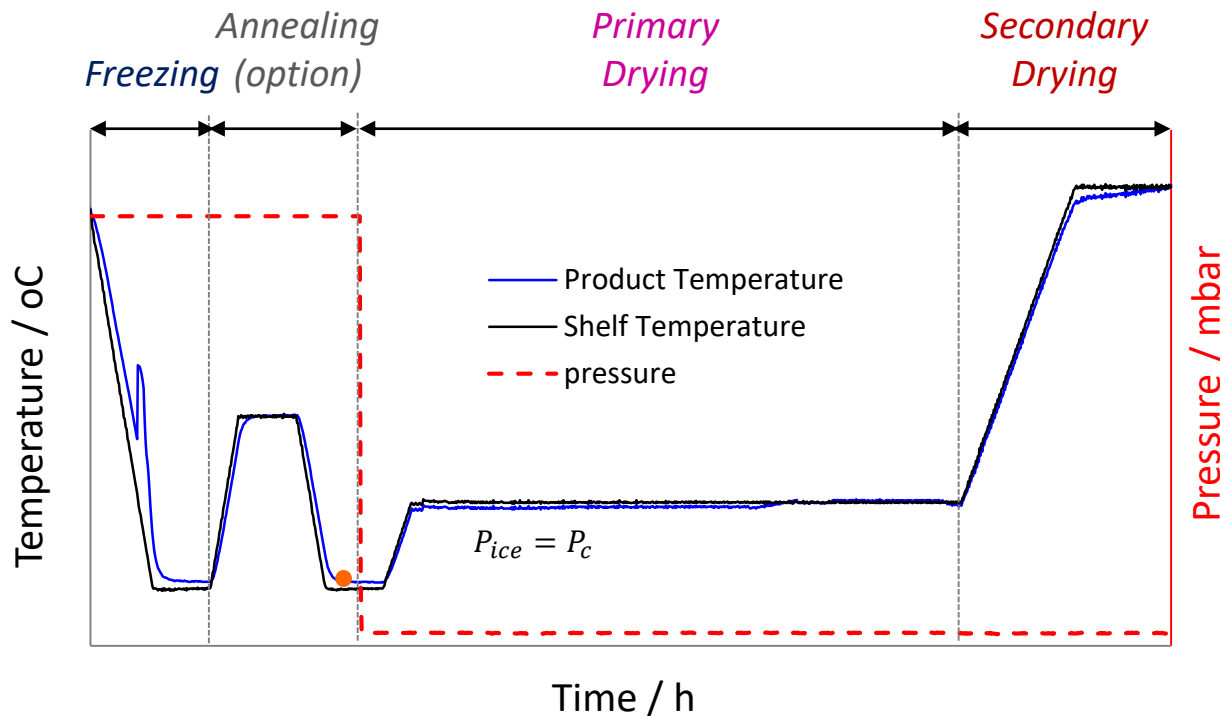
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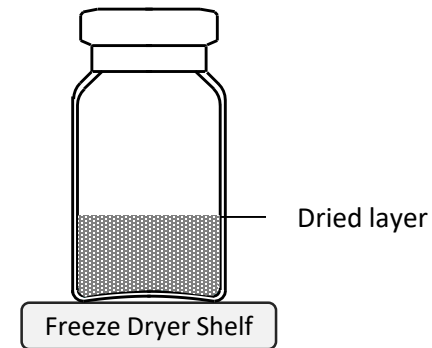
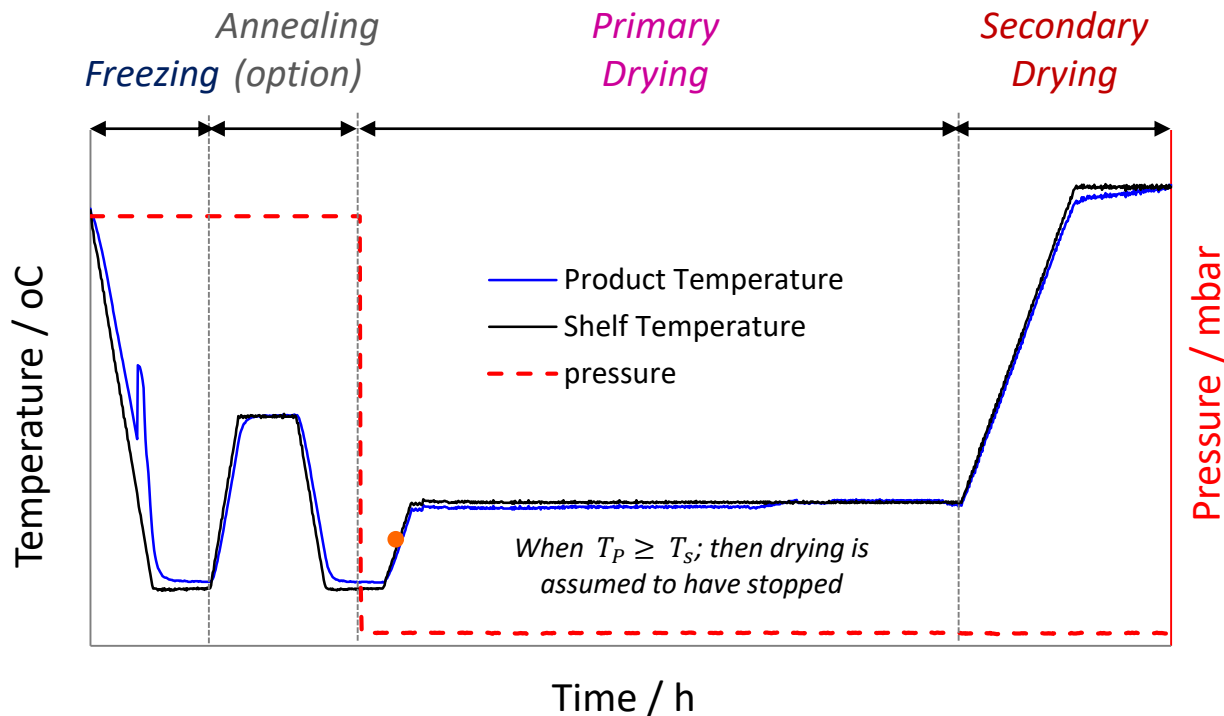
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Increasing shelf temperature (ramp), increases ice temperature and partial pressure until $P_{ice} = P_c$ and drying (sublimation) starts

Lyophilization or Freeze Drying Process

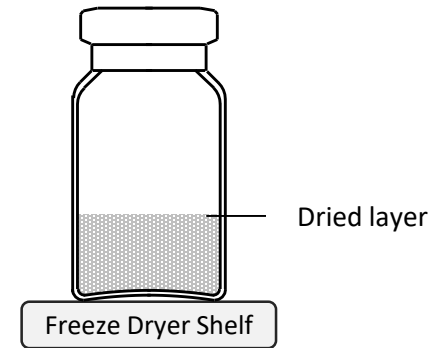
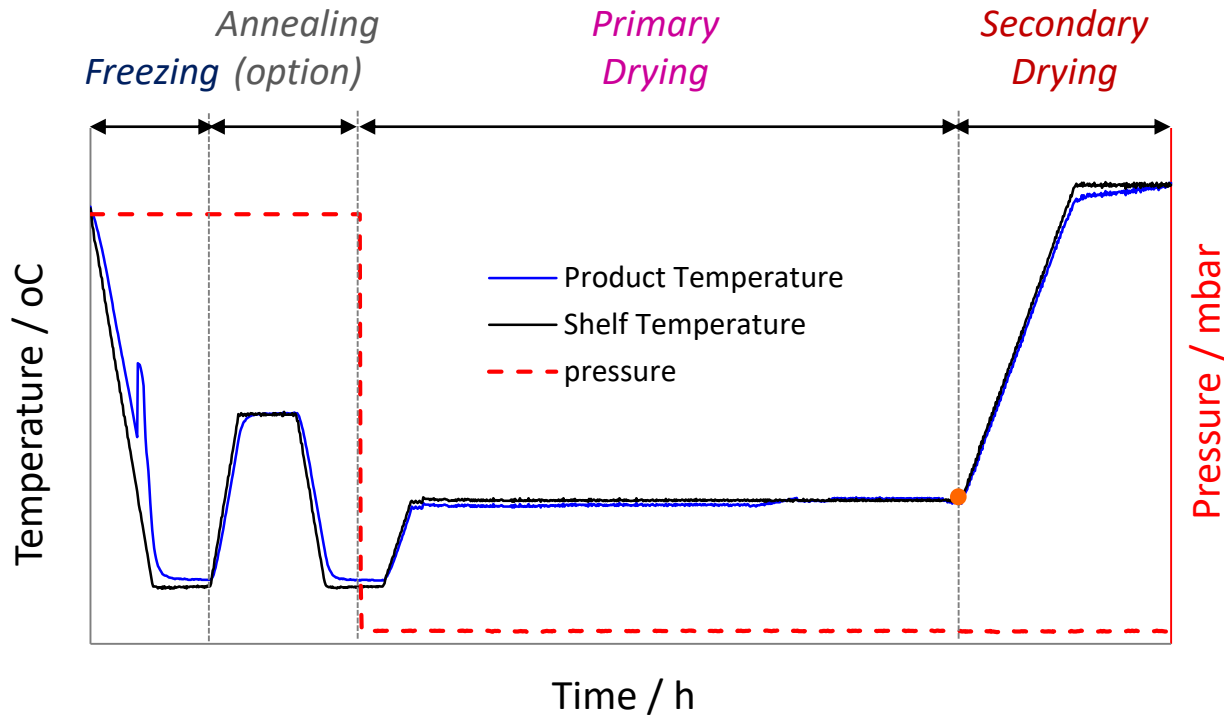
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Once the ice is removed then self cooling stops and the product temperature can now catch up with the shelf temperature.

Lyophilization or Freeze Drying Process

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

Characteristic
of protein
therapeutic
(i.e. unstable)

Regulatory
requirements

Process
variation
(can affect
productivity,
consistency &
repeatability)

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



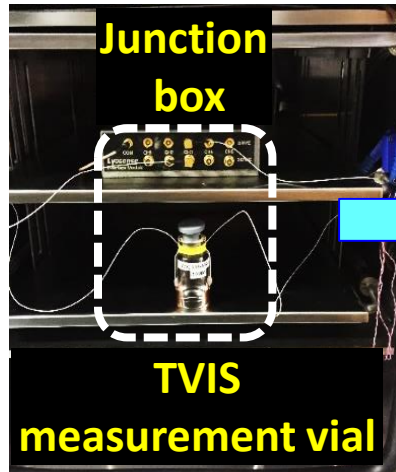
SV product temperature	
SV sublimation rate	
SV end point	



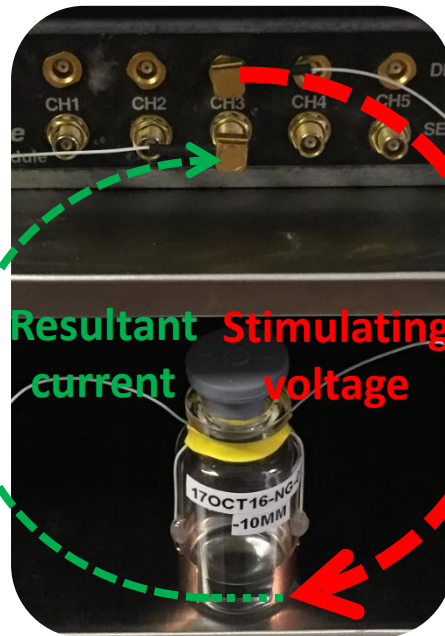
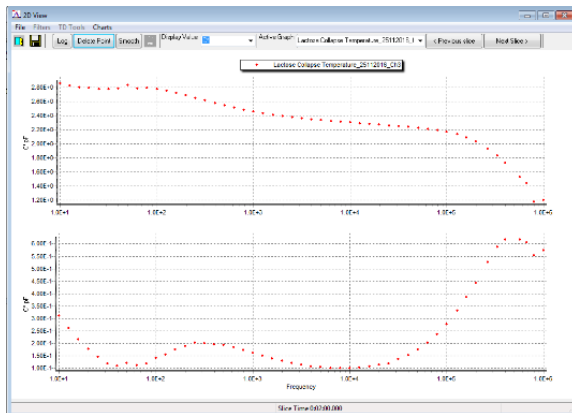
Through Vial Impedance Spectroscopy (TVIS)

Introduction

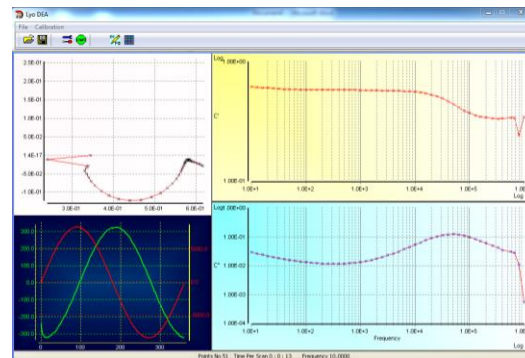
Freeze drying chamber



LyoView™ analysis software

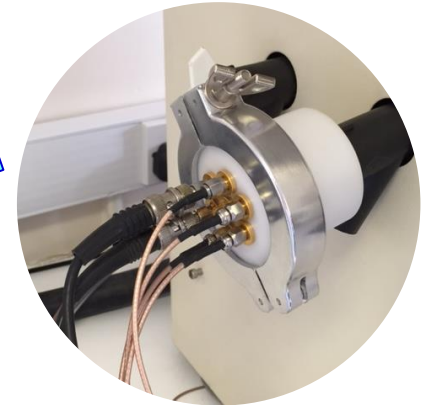


LyoDEA™ measurement software



TVIS

Pass through



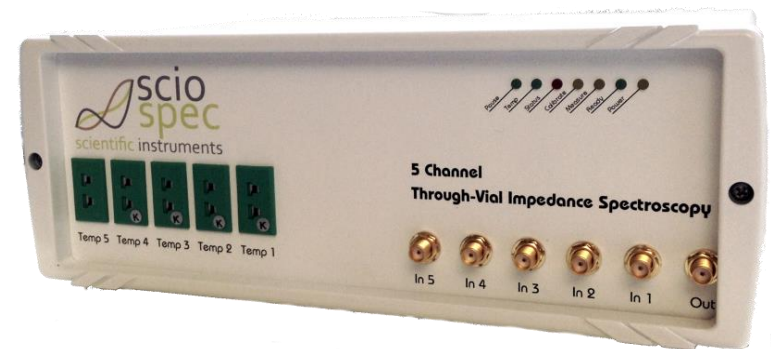
TVIS system
(I to V convertor)



Impedance Analyzer for Lyophilization Process

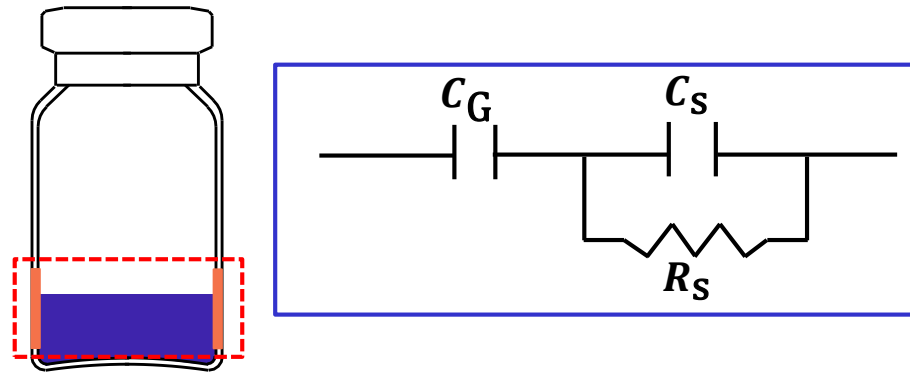


- Through Vial Multi Channel Impedance Analyzer
- Impedance measurement specially optimized for lyophilization experiments (contact method)
- Five sequentially measuring impedance channels
- All five channels share a common excitation signal
- Automatic voltage excitation amplitude adjustment
- Current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



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

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

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

Impedance to Complex Capacitance

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

$$Z_{\text{Total}}^* = Z^*(C_G) + \left[\frac{1}{Z^*(R_S)} + \frac{1}{Z^*(C_S)} \right]$$
$$Z_{\text{Total}}^* = \frac{1}{i\omega C_G} + \frac{R_S}{1 + i\omega R_S C_S}$$

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

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

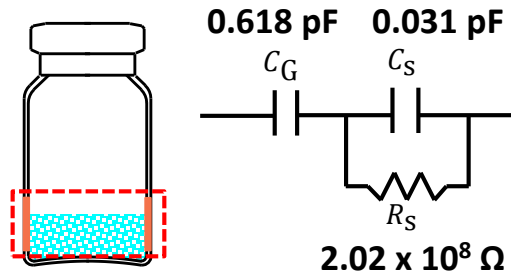
- From the complex capacitance formula, the expressions for real and imaginary capacitance can be calculated to explain the origin of **interfacial polarization peak**. This achieved by multiplying the nominator and denominator by the complex conjugate of the denominator and by grouping the real (C')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

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

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



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

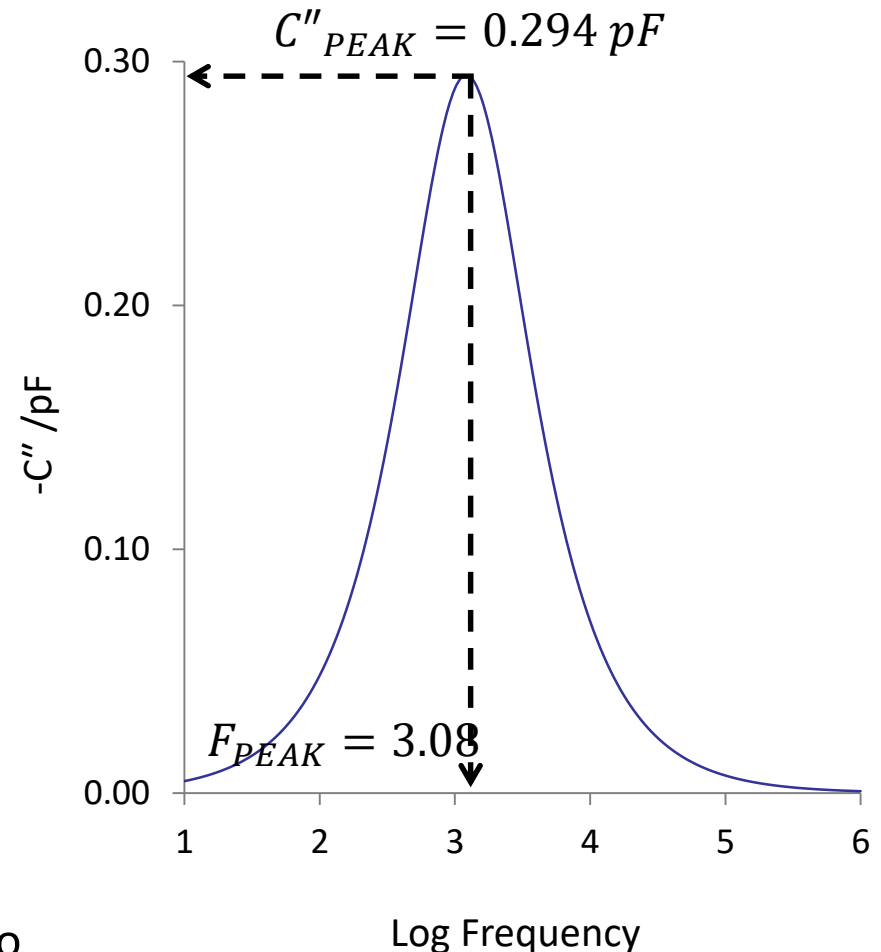
- A frequency of

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

- 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



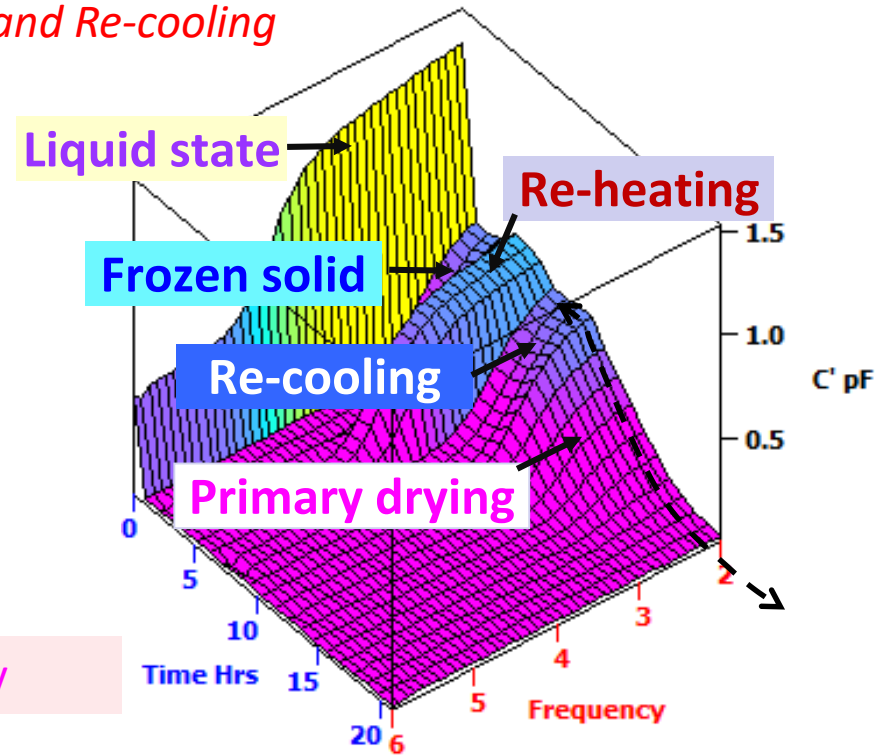
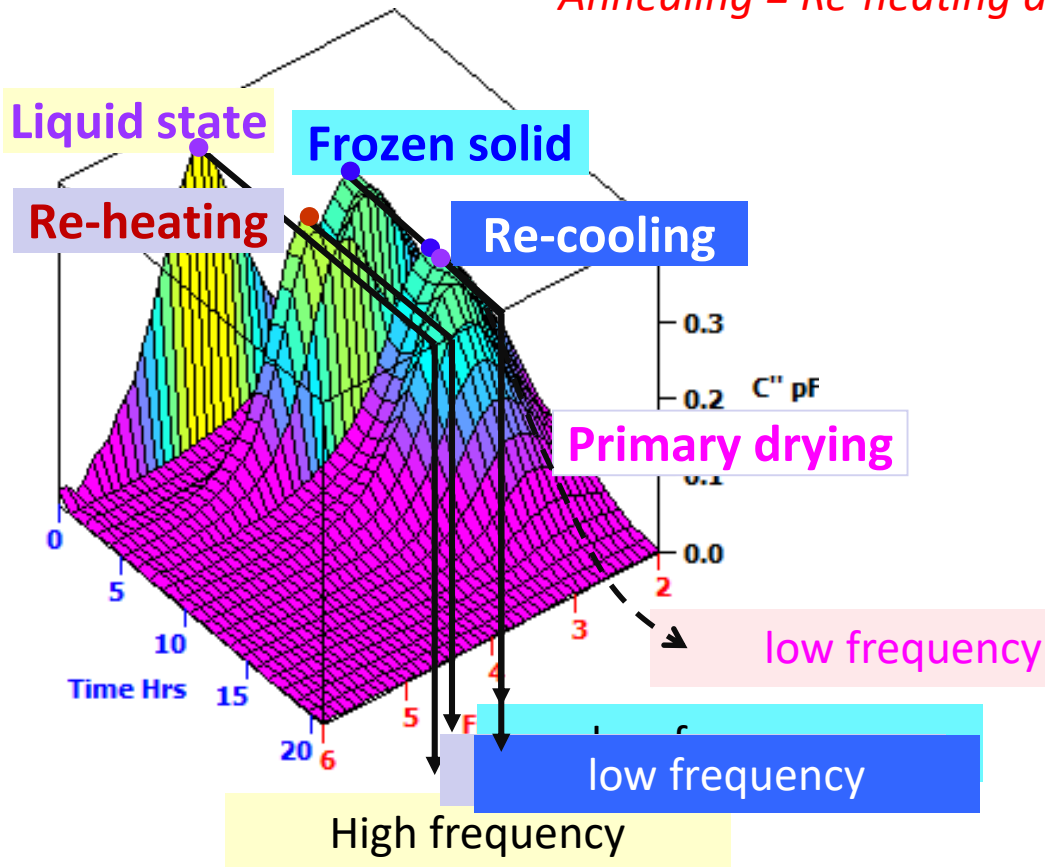
TVIS Response Surface (3D-Plot)



Imaginary Part of Capacitance

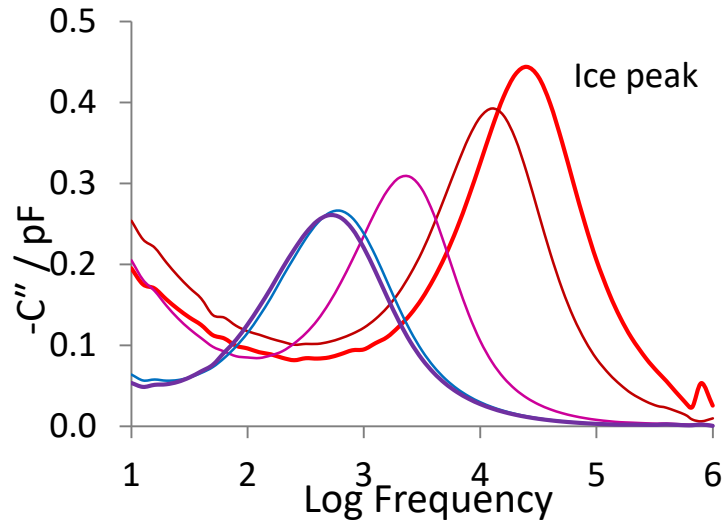
Real Part of Capacitance

Annealing = Re-heating and Re-cooling

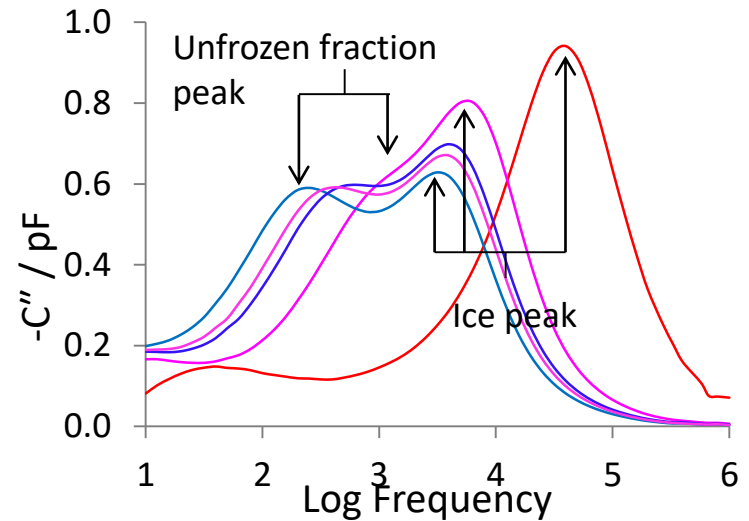


Phase Separation in freezing step

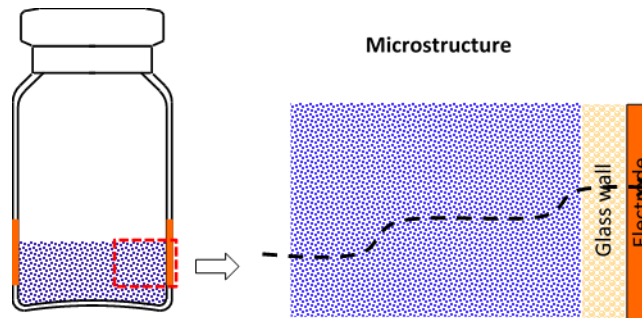
Water (frozen)



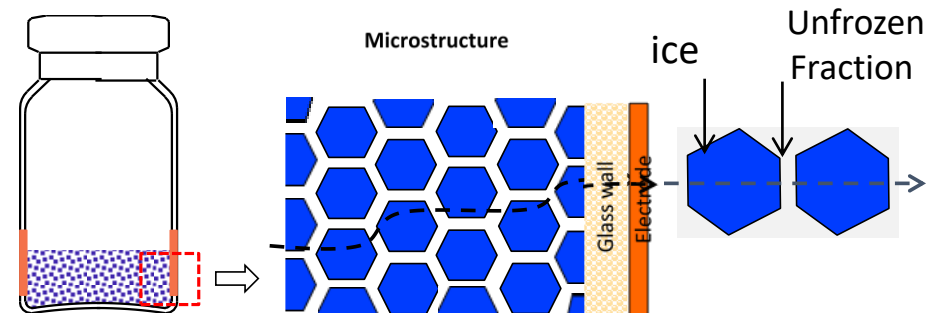
5%w/v Lactose solution (frozen)



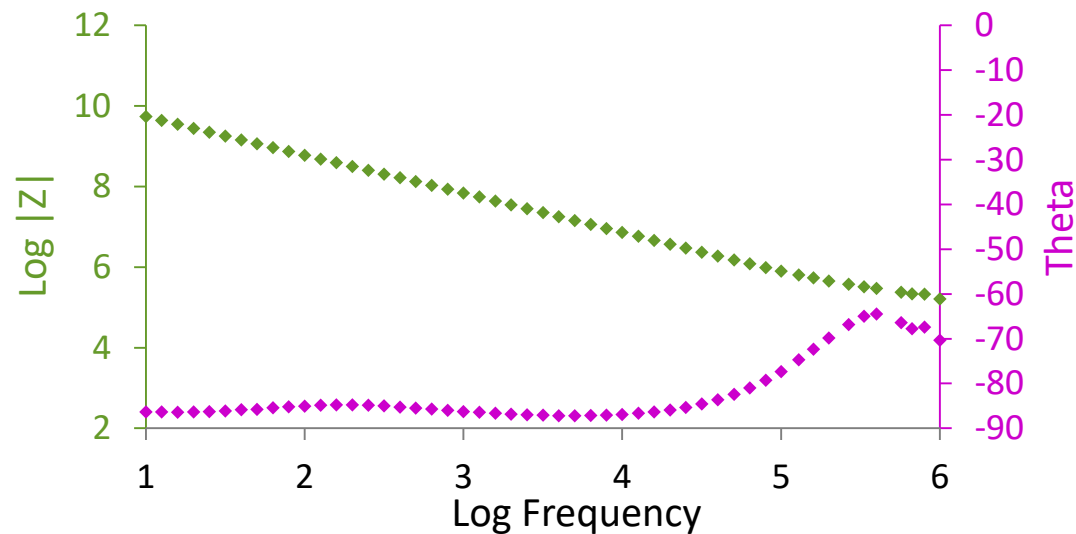
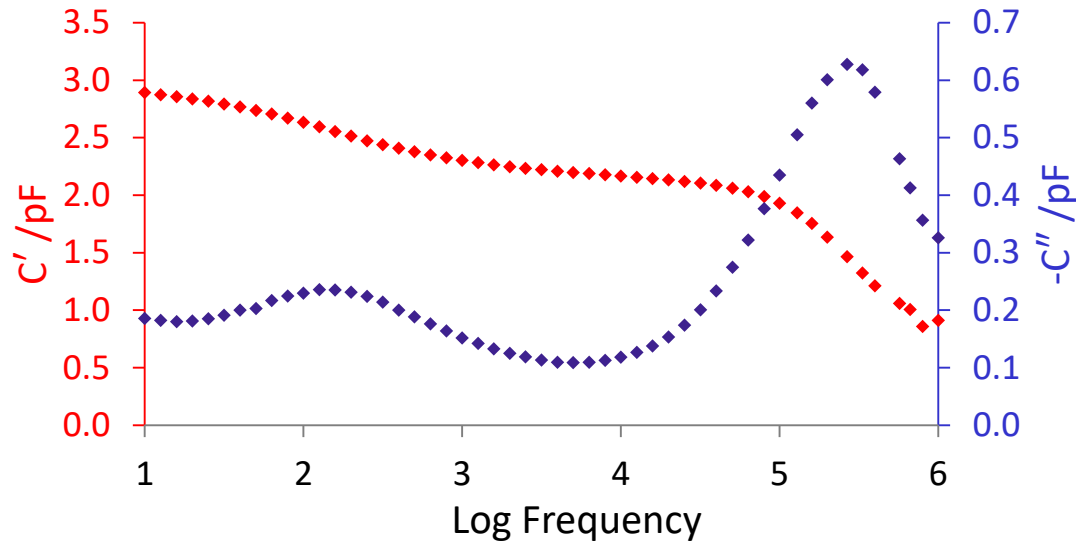
Add the equivalent circuit here



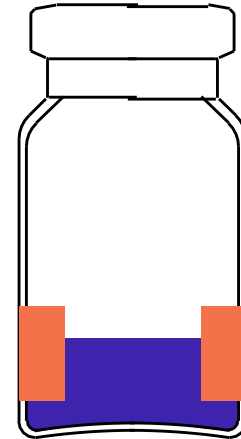
Add the equivalent circuit here



Impedance and Capacitance Spectrum



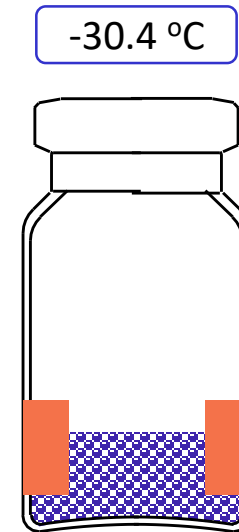
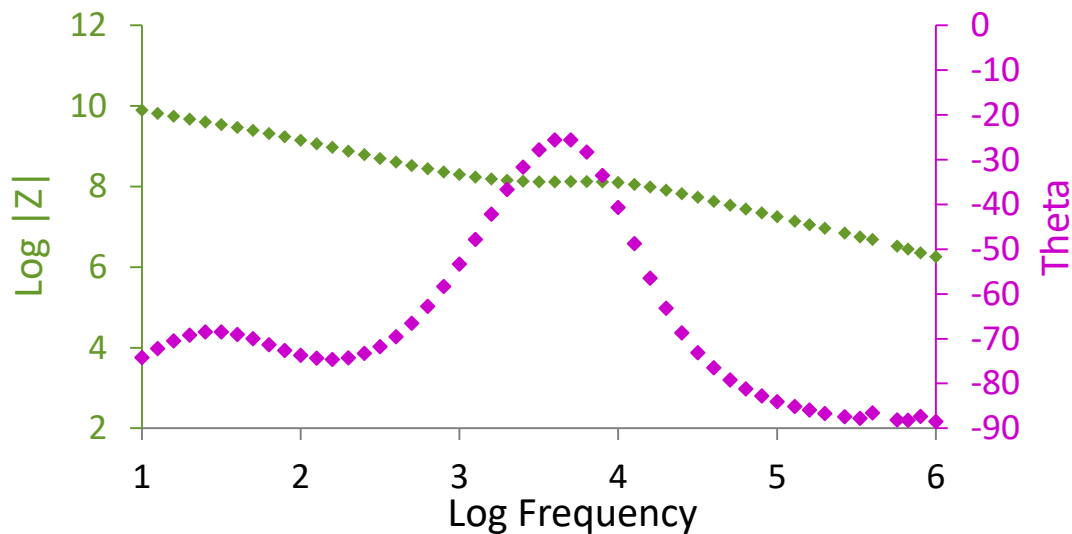
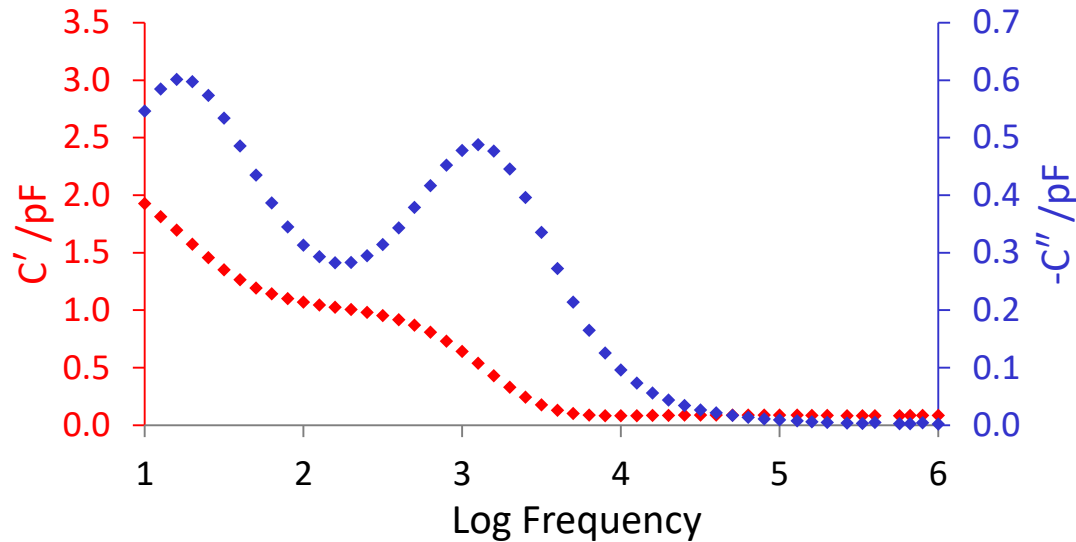
+ 20.3°C



5%w/v Lactose solution

Liquid state

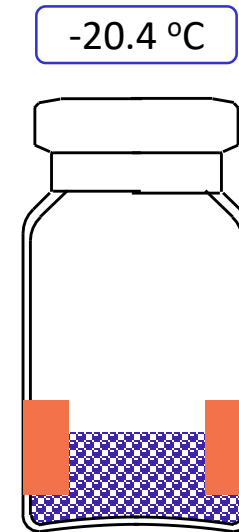
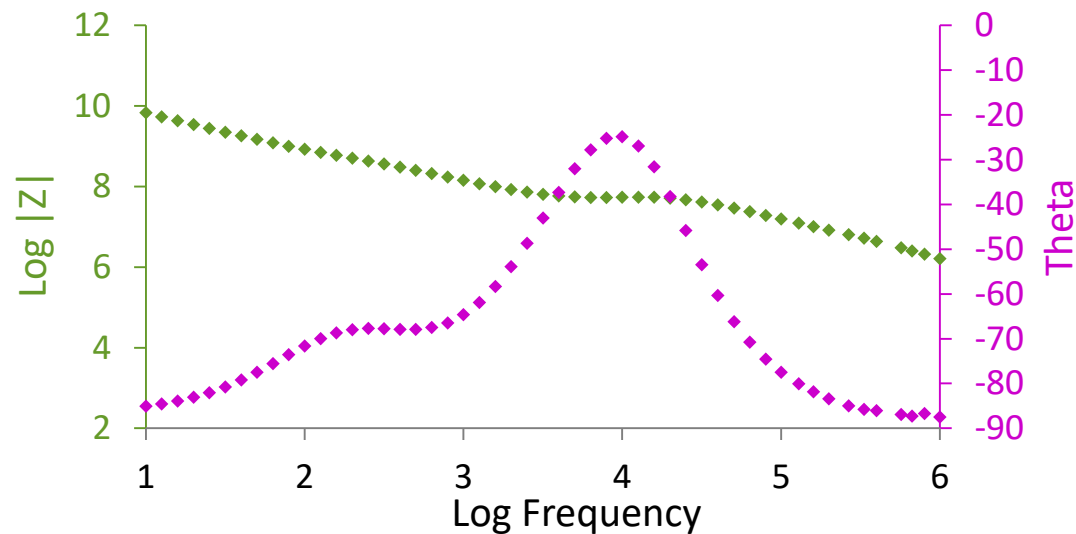
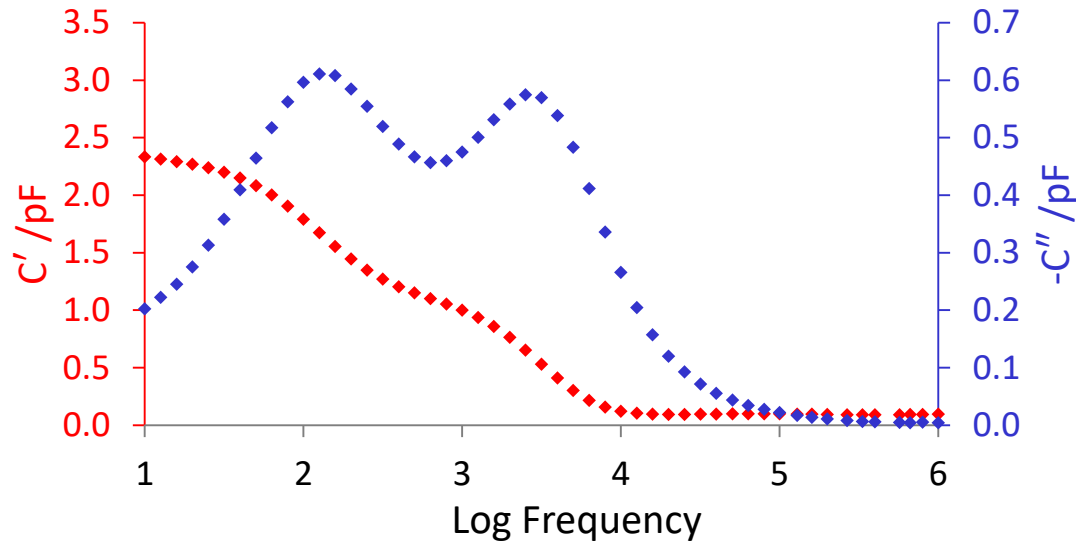
Impedance and Capacitance Spectrum



5%w/v Lactose solution

Solid (frozen state)
lower temp

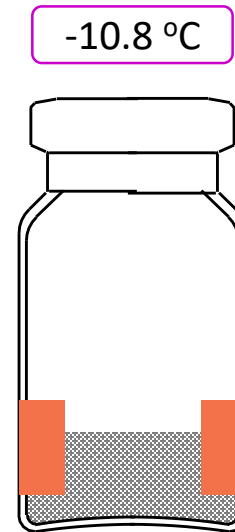
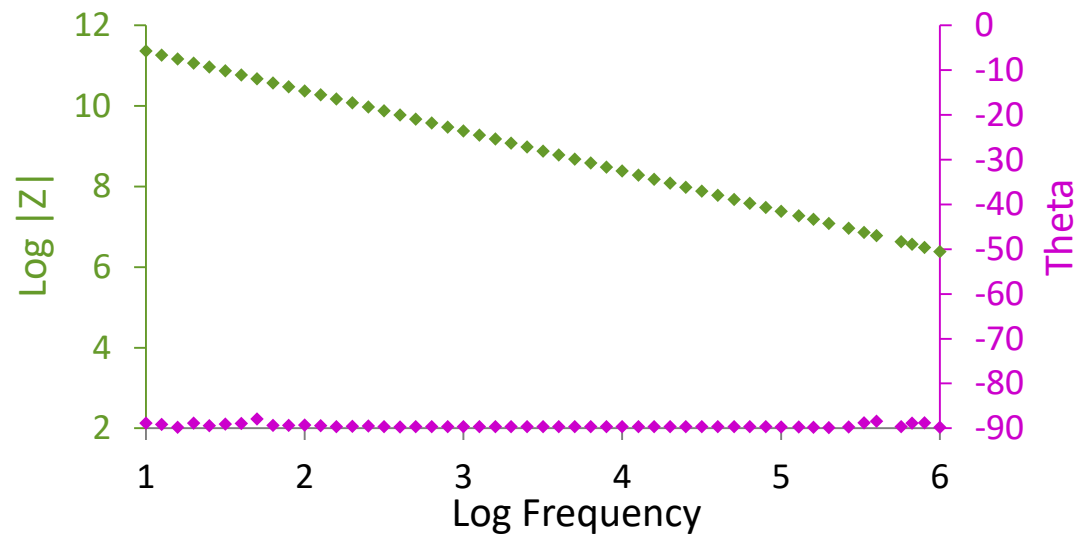
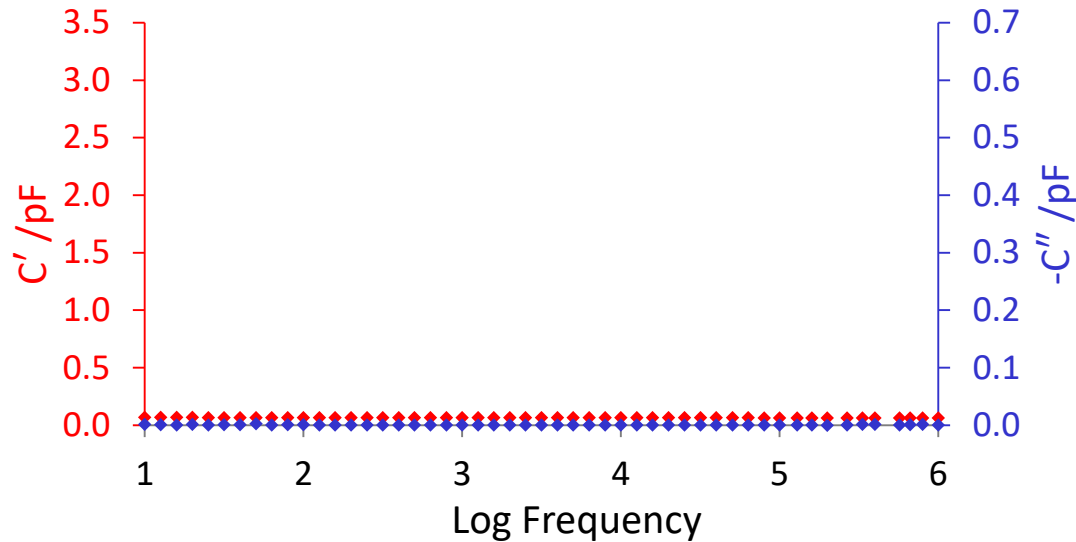
Impedance and Capacitance Spectrum



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)

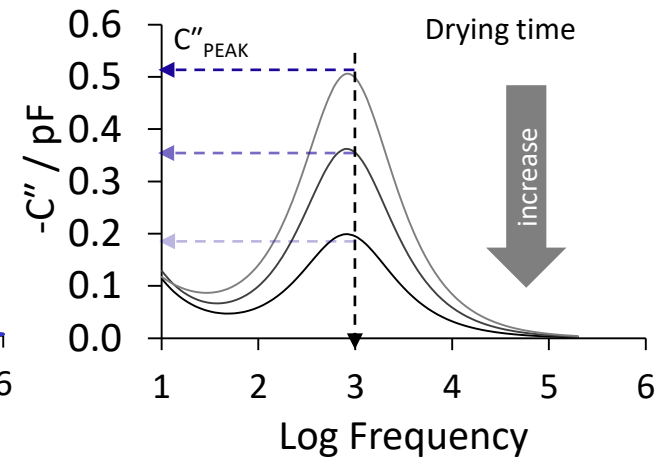
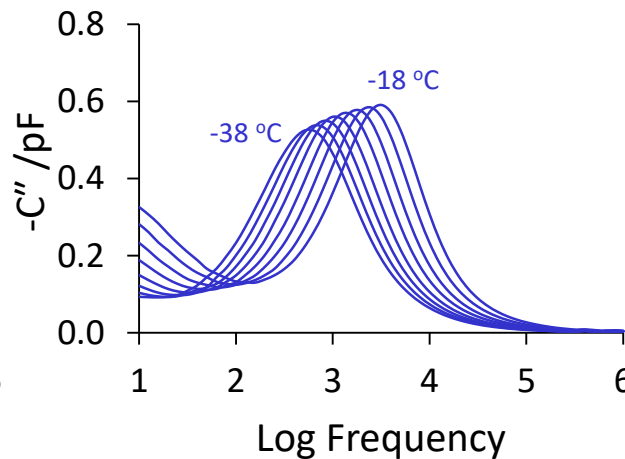
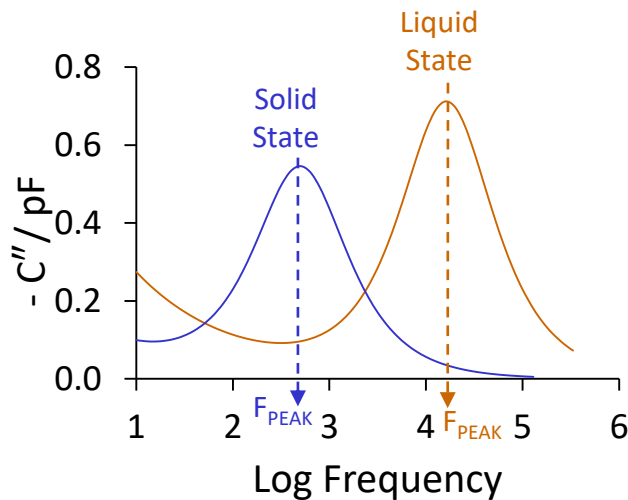


- TVIS measurement relate to both the *electrical resistance* and *electrical capacitance of the vial contents*.

Monitoring Phase Behaviour
(ice nucleation temperature
and solidification end points)

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

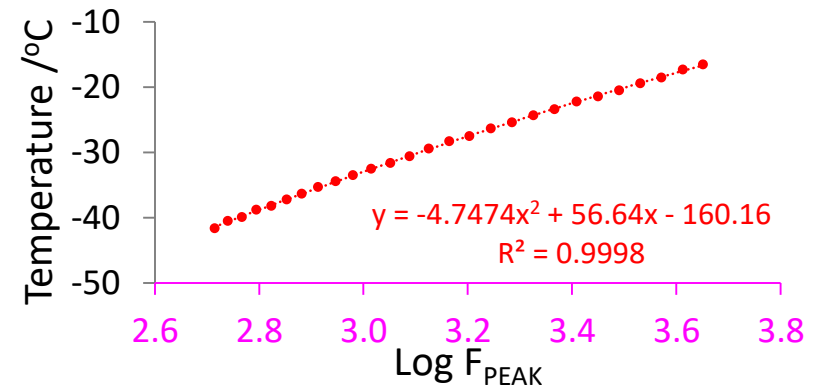
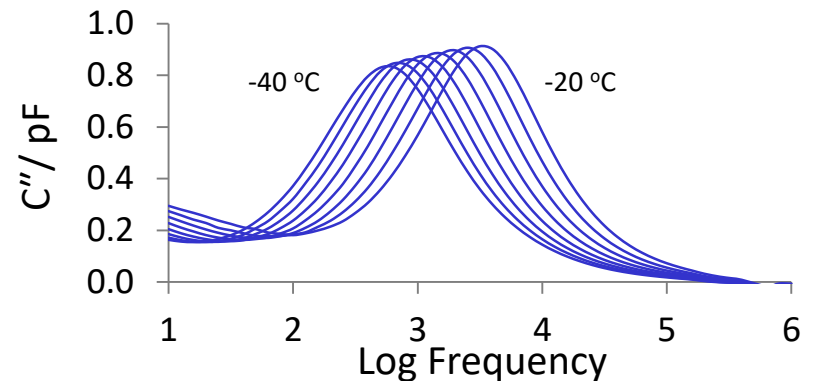
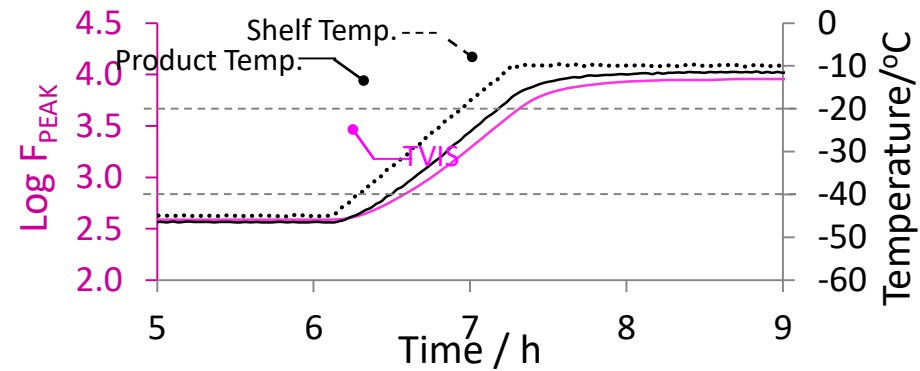
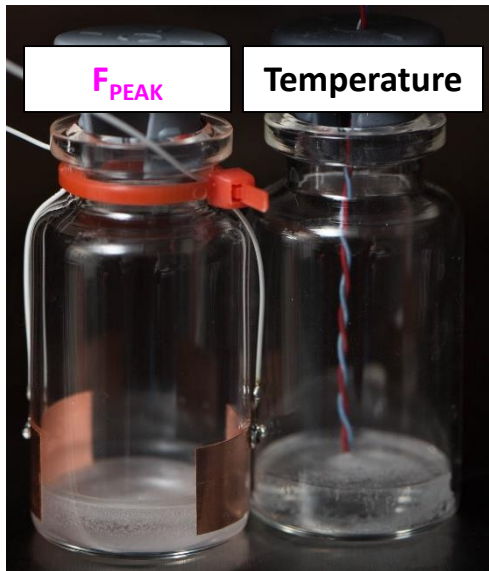
Drying rate surrogate (from
 dC''_{PEAK}/dt)



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

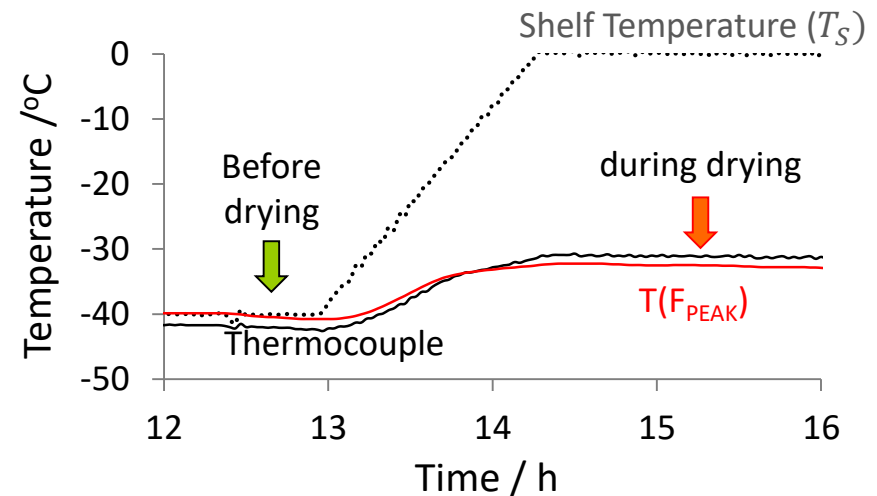
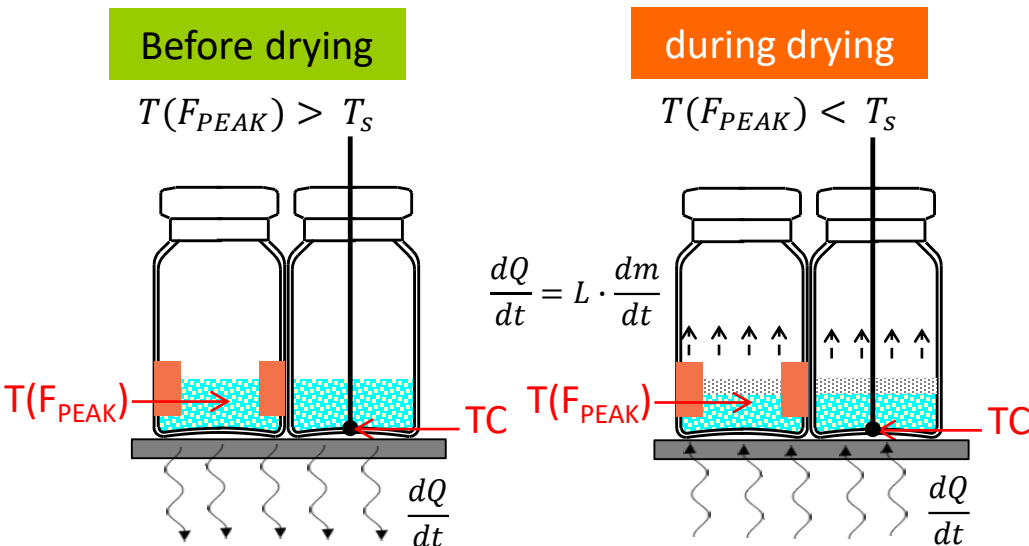
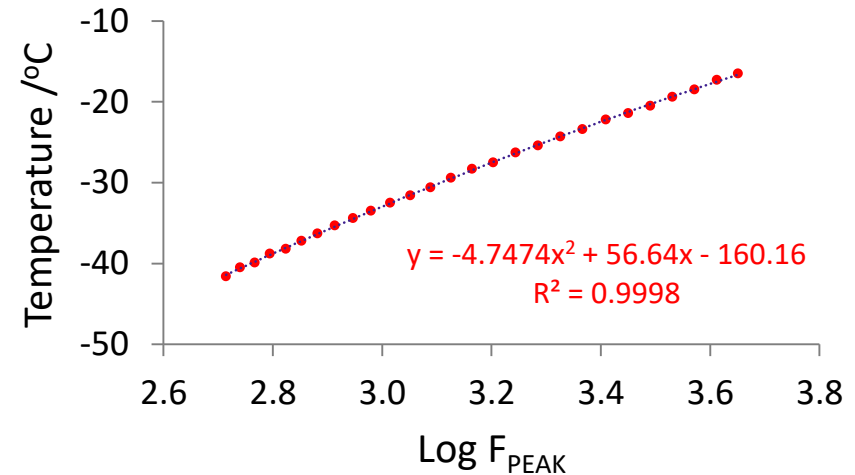
Temperature Calibration

- 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



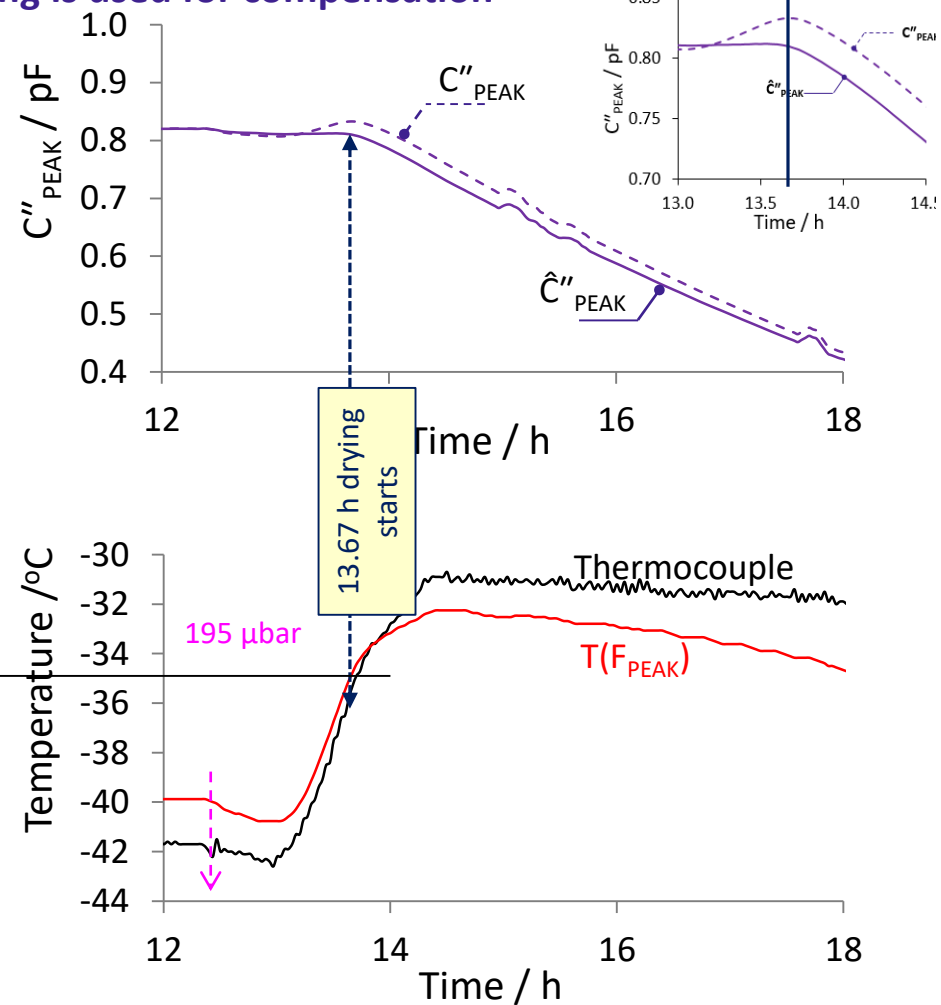
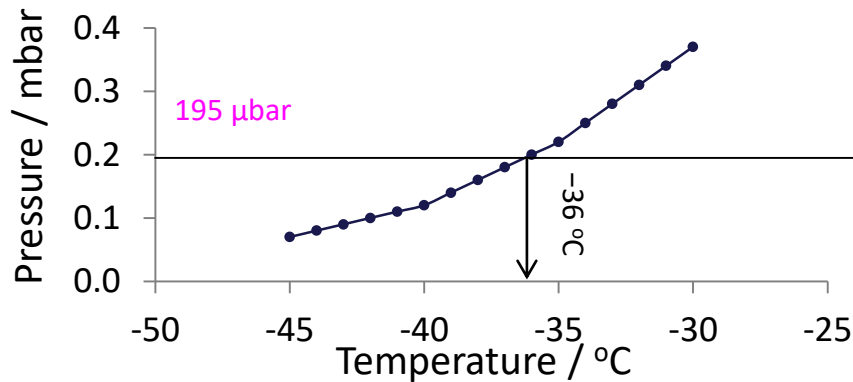
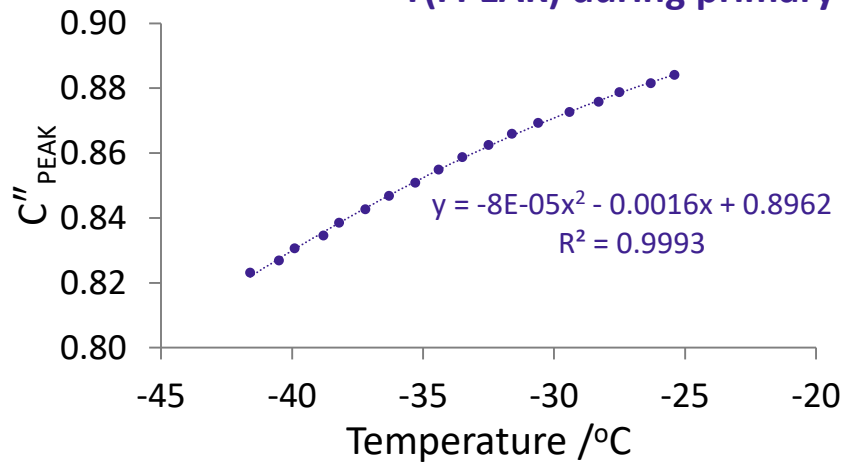
Temperature Prediction in Primary Drying

- Temperature calibration curve selected for temperature prediction in primary drying : $T(F_{PEAK})$
- Good agreement between product temperature (by TC) and $T(F_{PEAK})$

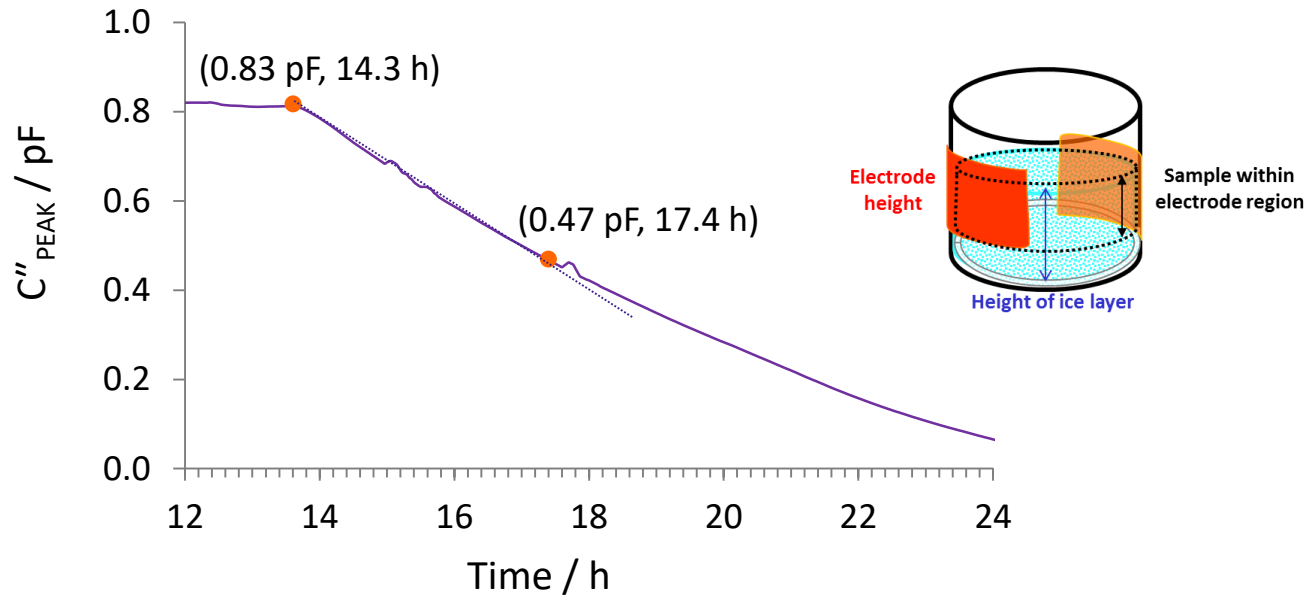


Compensation of C'' PEAK by T(F_{PEAK})

T(F_{PEAK}) during primary drying is used for compensation



Drying rate calculation



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

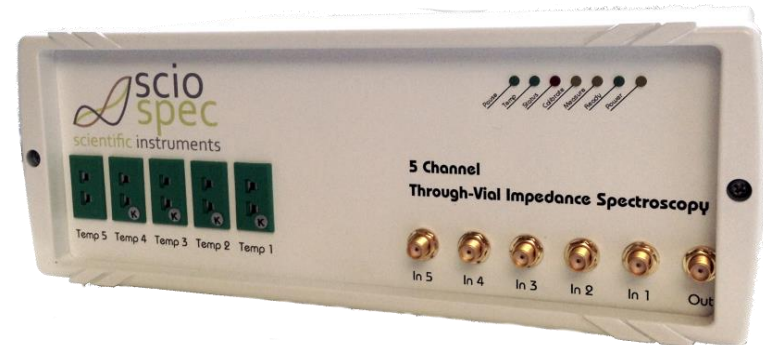
$$\text{Drying rate} = \left(\frac{\hat{C}''_{PEAK(initial)} - \hat{C}''_{PEAK(end)}}{\text{Time}_{(end)} - \text{Time}_{(initial)}} \right) \times \frac{\text{ice mass within electrode region}}{\hat{C}''_{PEAK(initial)}}$$

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

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

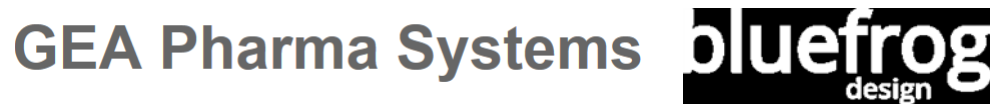
- Development mapping a drying characteristics from lab scale to production
 - Determination of heat transfer coefficients (K_V)
 - Determination of dry layer resistance (R_P) to predict drying efficiency



- 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

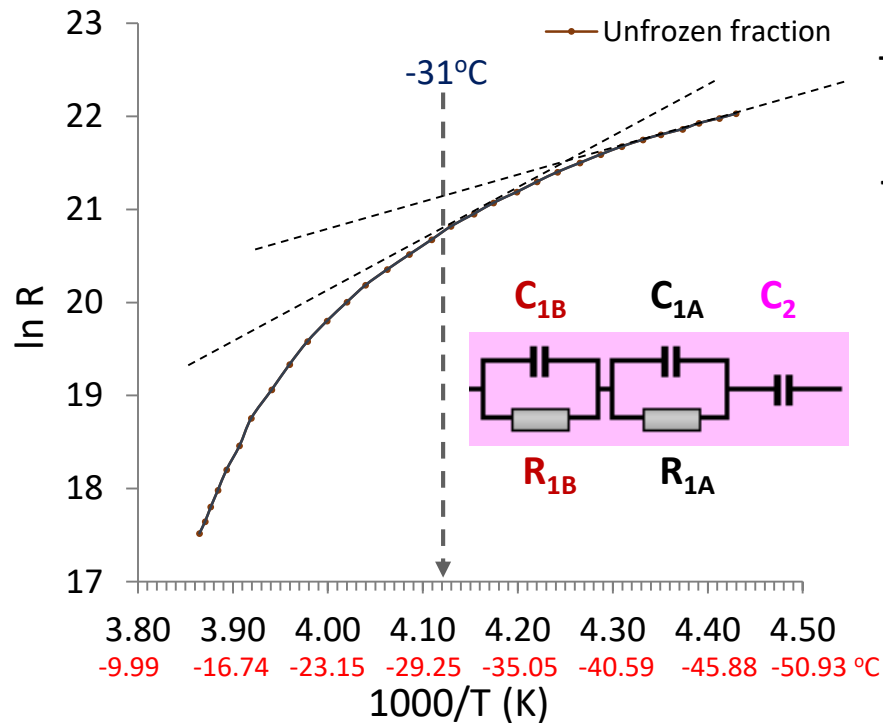
Acknowledgements, Recent Projects & Collaborators

- 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

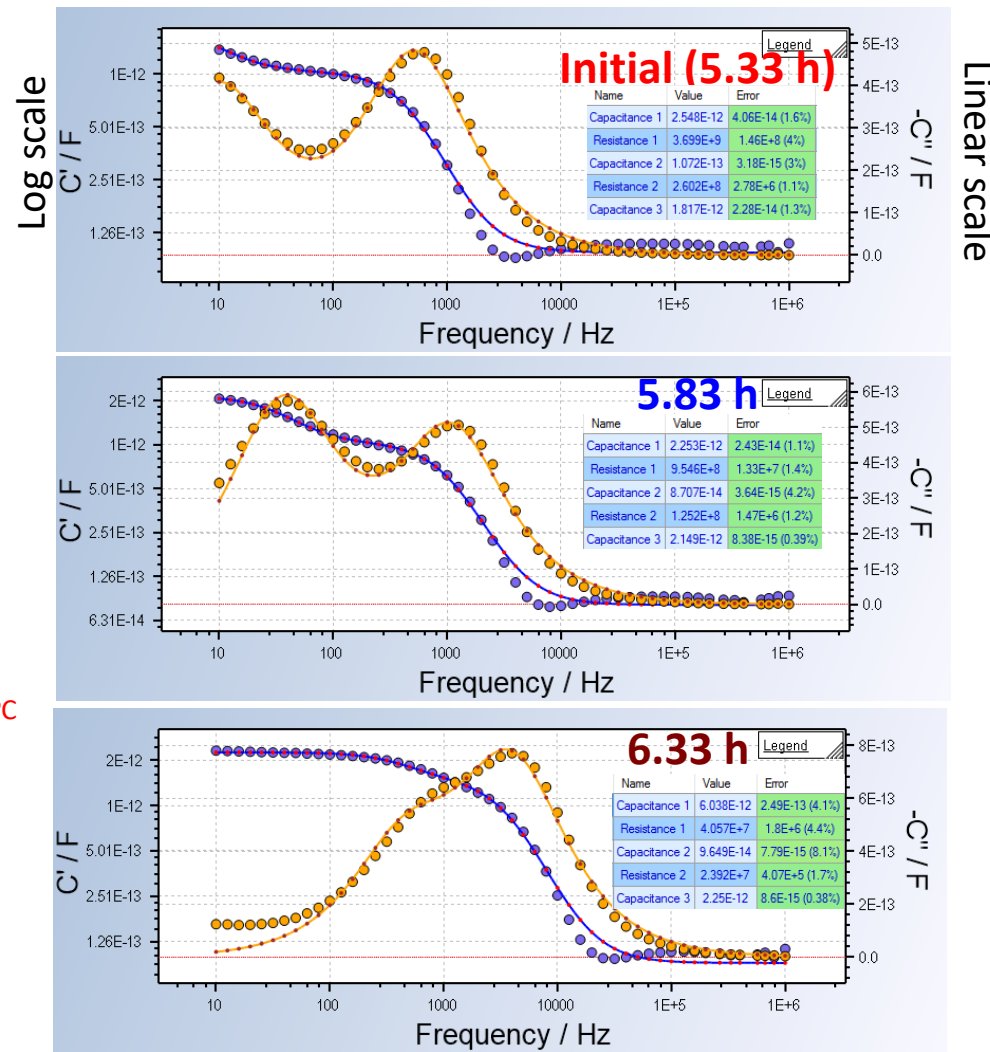


Glass Transition (T_g') Determination

5% w/v Lactose solution

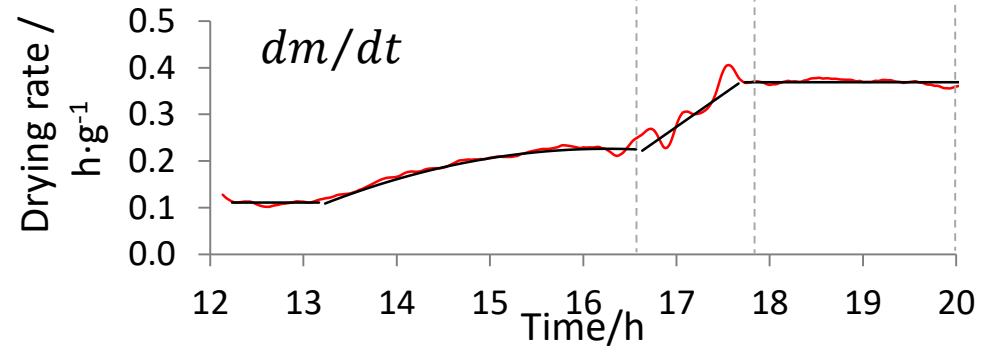
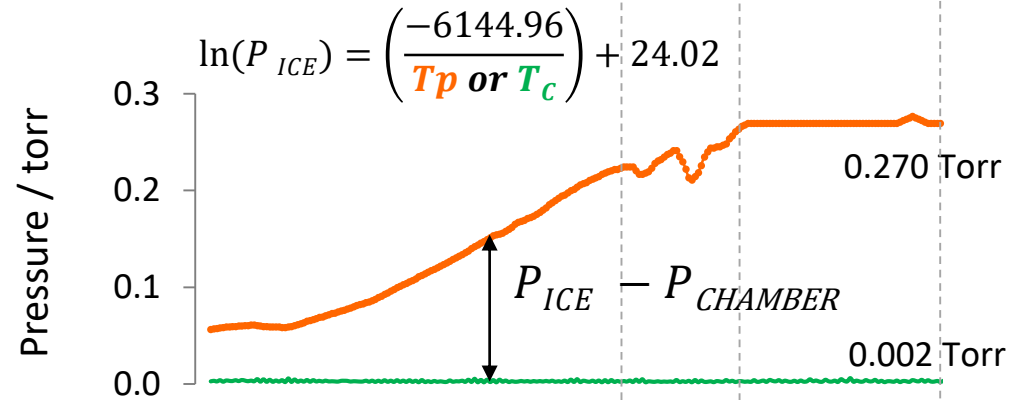
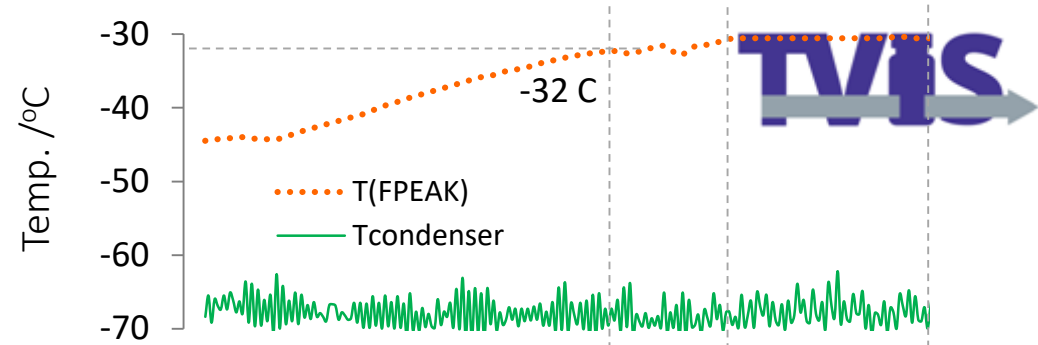
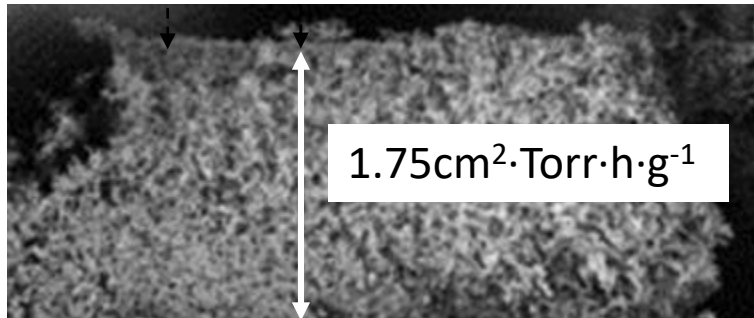
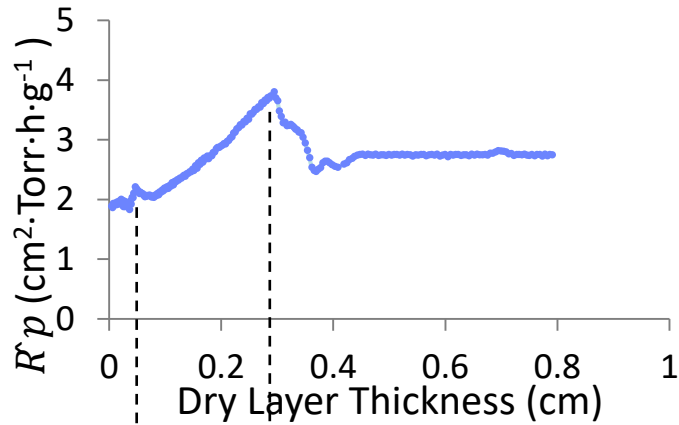


- Below T_g' the changes in product resistance follows Arrhenius
- Above T_g' VTF function models the resistance profile.



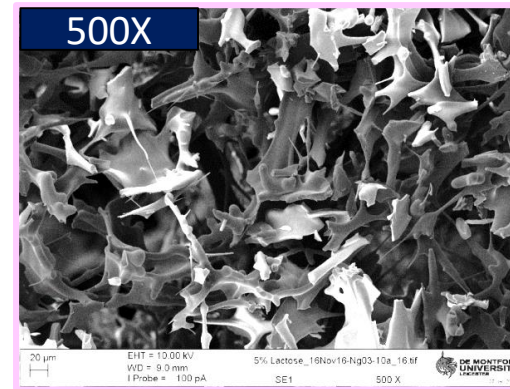
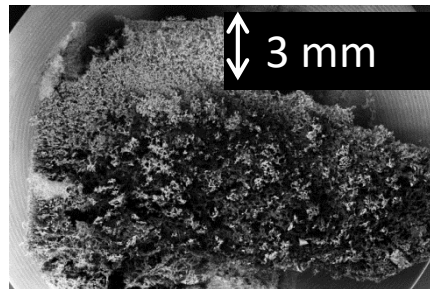
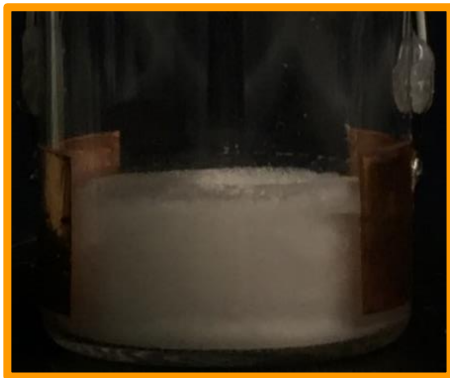
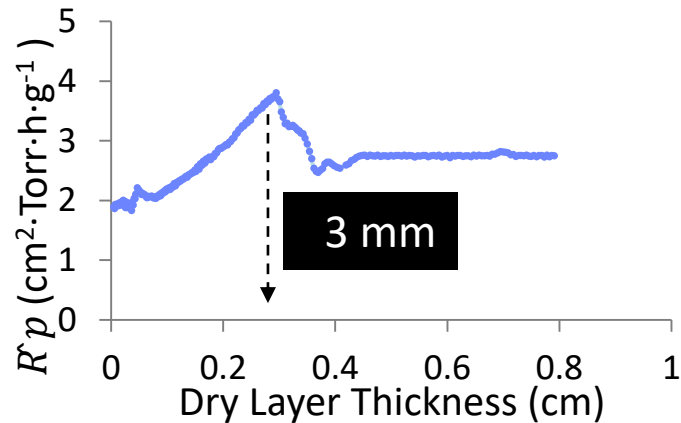
Lactose Dried Product Resistance (R_p)

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



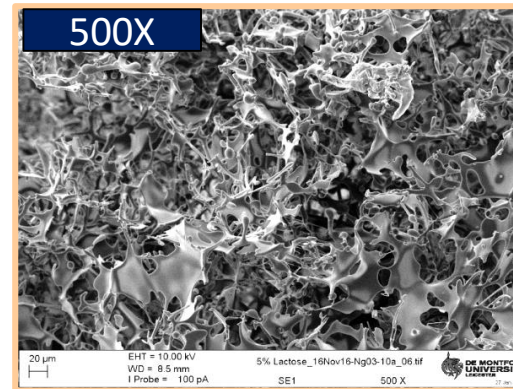
Lactose Dried Product Resistance (R_p)

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



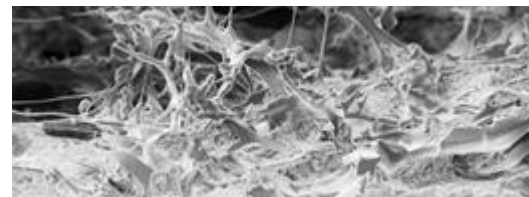
TVIS

Top layer
Fine pores



Middle layer
Micro-collapse

500X



Bottom layer
Full collapse

C''_{PEAK} Criteria & Assumptions

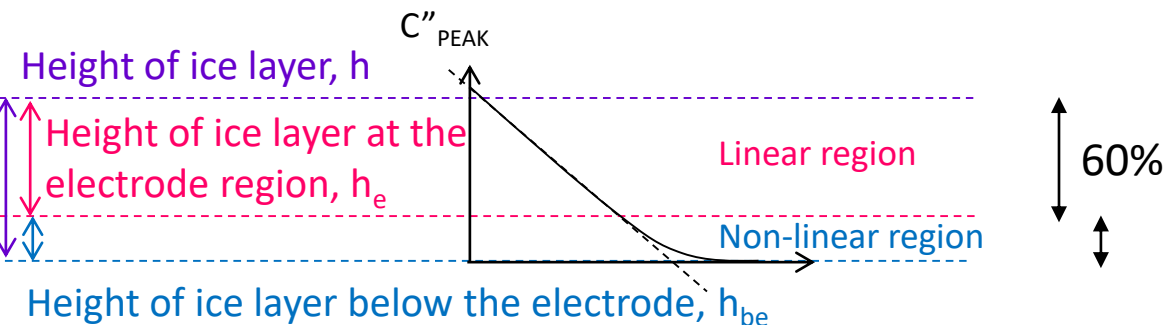
- C''_{PEAK} is proportional to the height of the ice cylinder bounded by the electrode region, through the value of C_G
- Drying rates are based on the assumption of a planar sublimation front
- Below the electrode C''_{PEAK} loses sensitivity to ice layer height (non-linear)

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



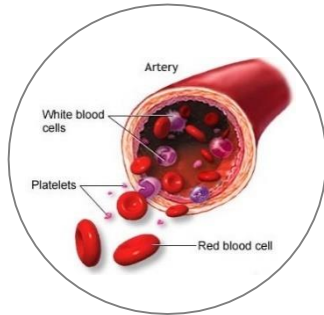
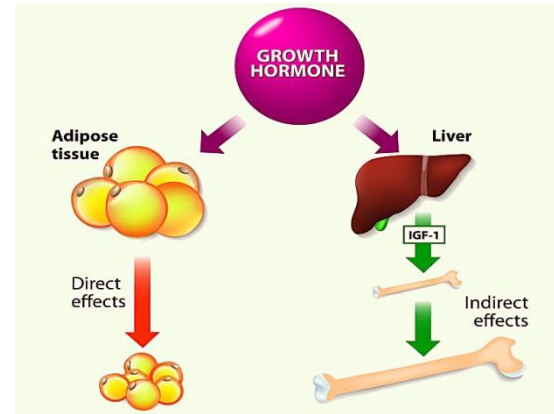
C''_{PEAK} can be considered in three regions

- 1) A linear region (% of C''_{PEAK} est. on water in isolated vial)
- 2) A non-linear region
- 3) An ice cone region – where $C''_{\text{PEAK}} \sim 0$ (use C' @ high freq.)



Examples of Biopharmaceuticals Therapy

Growth hormone



Factor VIII helps blood to clot



Treatment hemophilia

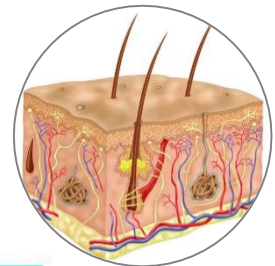
Endocrine system

Cardiovascular system

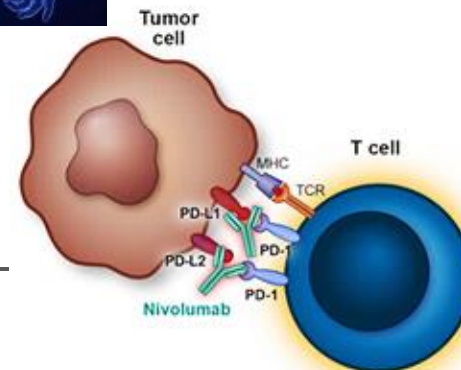


Integumentary system

Nivolumab



PD-1 Blockade (nivolumab)



/ial Impedance

Monoclonal antibodies (mAb)

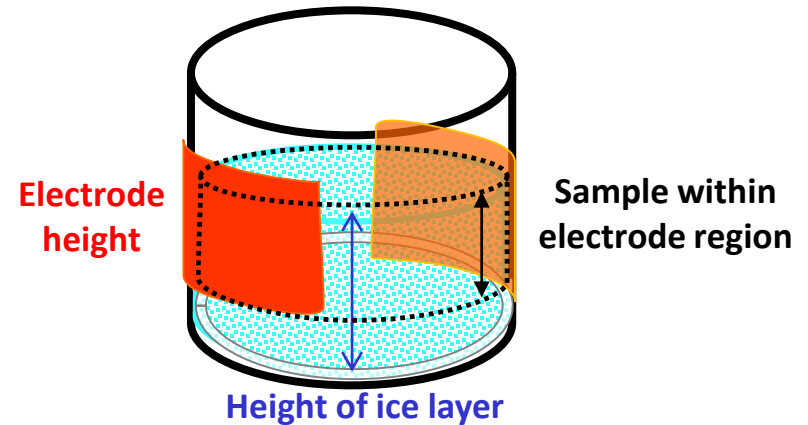
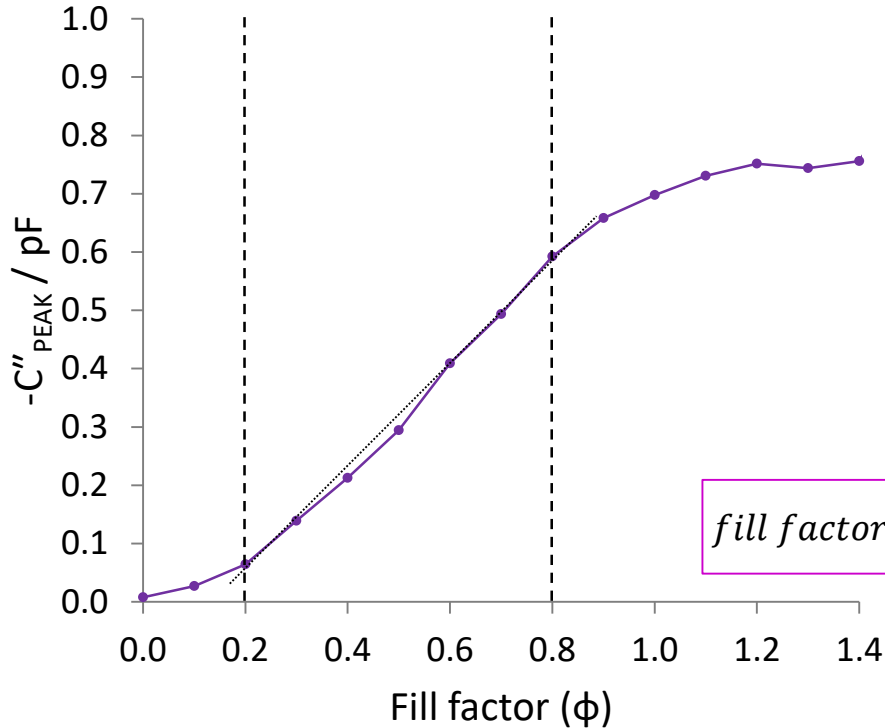
mAbs used for treatment cancer

Table 1 FDA-approved mAbs for use in oncology

Name	Marketed by	Class	Target	First approved indication	Reported mechanisms of action	Approval year
Rituximab (Rituxan)	Biogen Idec/ Genentech	Chimeric IgG1	CD20	Non-Hodgkin's Lymphoma	ADCC, CDC, Induction of Apoptosis ⁴	1997
Trastuzumab (Herceptin)	Genentech	Humanized IgG1	HER2	Breast Cancer	Signal Inhibition, ADCC ⁵	1998
Alemtuzumab (Campath)	Sanofi-Aventis	Humanized IgG1	CD52	B cell Chronic Lymphocytic Leukemia	CDC, Induction of Apoptosis ⁶	2001
Ibritumomab tiuxetan (Zevalin)	Biogen Idec	Murine IgG1	CD20	Non-Hodgkin's Lymphoma	Radioisotope Delivery (⁹⁰ Y)	2002
Tositumomab (Bexxar)	GlaxoSmithKline	Murine IgG2a	CD20	Non-Hodgkin's Lymphoma	Radioisotope Delivery (¹³¹ I), ADCC, CDC, Induction of Apoptosis ⁷	2003
Cetuximab (Erbix)	Bristol-Myers Squibb/Eli Lilly	Chimeric IgG1	EGFR	Squamous Cell Carcinoma of the Head and Neck	Signal Inhibition, ADCC, CDC ⁸	2004
Bevacizumab (Avastin)	Genentech	Humanized IgG1	VEGF	Colorectal Cancer	Signal Inhibition ⁹	2004
Panitumumab (Vectibix)	Amgen	Human IgG2	EGFR	Colorectal Cancer	Signal Inhibition, ADCC ¹⁰	2006
Ofatumumab (Arzerra)	Genmab/GSK	Human IgG1	CD20	Chronic Lymphocytic Leukemia	ADCC, CDC ¹¹	2009
Denosumab (Xgeva)	Amgen	Human IgG2	RANKL	Bone Metastases	Signal Inhibition	2010
Ipilimumab (Yervoy)	Bristol-Myers Squibb	Human IgG1	CTLA-4	Metastatic Melanoma	Signal Inhibition ¹²	2011
Brentuximab vedotin (Adcetris)	Seattle Genetics	Chimeric IgG1	CD30	Hodgkin Lymphoma	ADC	2011
Pertuzumab (Perjeta)	Genentech	Humanized IgG1	HER2	Breast Cancer	Signal Inhibition, ADCC ¹³	2012
Trastuzumab emtansine (Kadcyla)	Genentech	Humanized IgG1	HER2	Breast Cancer	ADC, Signal Inhibition, ADCC ¹⁴	2013

Fill height dependency

Fill factor between 0.2-0.8 (0.7 is typically used)

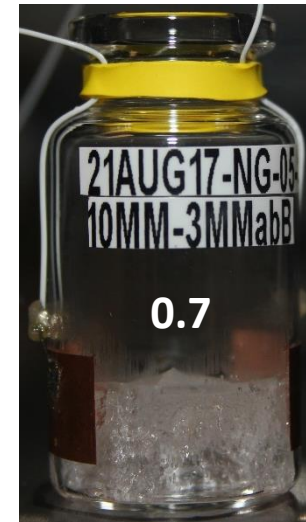
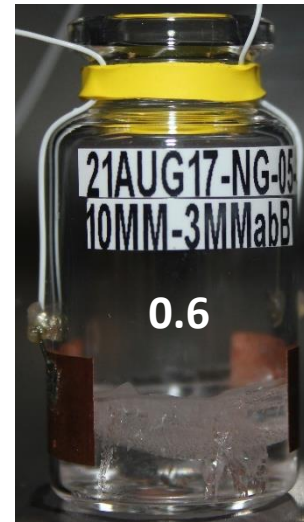
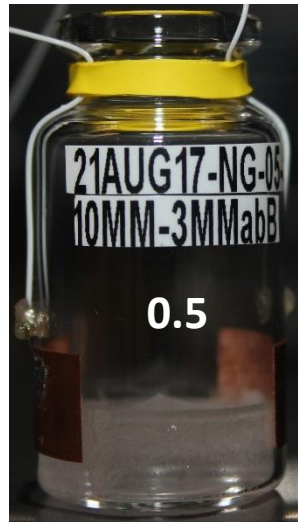
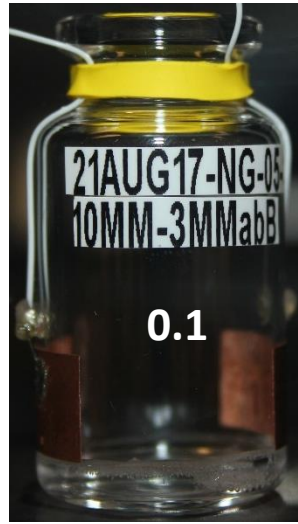


$$\text{fill factor } (\Phi) = \frac{\text{The height of liquid fill within electrode region}}{\text{The height of electrode}}$$

Ice mass within electrode region = Area of vial bottom (inner) $\times$ electrode height $\times \phi \times \text{Density}_{ice}$

$$\text{Ice mass within electrode region} = 0.000382 \text{ m}^2 \times 0.015 \times 0.7 \times 920 \text{ kg} \cdot \text{m}^{-3} = 3.69 \text{ g}$$

Fronzen state



Fronzen state

