Through Vial Impedance Spectroscopy (TVIS)

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

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Pharmaceuticals (From drugs molecules to products)

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

Product available in the market
• Quality: Safety & Efficacy

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

Through Vial Impedance Spectroscopy
Biopharmaceutical in Market from 1982-2014 (classified by therapeutic categories)

- Blood factors
- Other blood related
- Antihemophilic factor, human recombinant
- Hemoglobin
- Growth hormone
- GM-CSF

- Hormones
- IFN, IL & TNF
- Interferon alfa

- Growth factors
- Vaccines
- Varicella-zoster virus

- IFN, IL & TNF
- Interferon alfa
- Varicella-zoster virus

- mAbs
- Nivolumab

- Other
Global Pharmaceutical Market 2015 and 2021

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

![Market Size Graph]

- Red: Biosimilars
- Pink: Biologics
- Green: OTC
- Gray: Generics
- Cyan: Patented/Originator small molecules

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

- Antibodies bind with antigens on the surface of target cells
- T-cell bind with antibodies
- Lead to cell lysis or phagocytosis
- Target cells are destroyed
Monoclonal antibodies (mAb)

The growing role of antibodies in therapy

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Target</th>
<th>Approved indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muromomab</td>
<td>Orathec</td>
<td>CD3</td>
</tr>
<tr>
<td>Abciximab</td>
<td>Reopro</td>
<td>GPIb-IIa</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Mabthera</td>
<td>CD20</td>
</tr>
<tr>
<td>Daclizumab</td>
<td>Chimeric, IgG1</td>
<td>CD25 (IL2r)</td>
</tr>
<tr>
<td>Basiliximab</td>
<td>Chimeric, IgG1</td>
<td>CD25 (IL2r)</td>
</tr>
<tr>
<td>Palivizumab</td>
<td>Humanized, IgG1</td>
<td>Protein F</td>
</tr>
<tr>
<td>Infliximab</td>
<td>Remicade</td>
<td>TNFα</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>Herceptin</td>
<td>HER2/Neu</td>
</tr>
<tr>
<td>Etanercept</td>
<td>Enbrel</td>
<td>TNFα and β</td>
</tr>
<tr>
<td>Gemtuzumab</td>
<td>Mylotarg</td>
<td>Humanized, IgG1</td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td>Mabcampath</td>
<td>Humanized, IgG1</td>
</tr>
<tr>
<td>Infliximab</td>
<td>Remicade</td>
<td>TNFα</td>
</tr>
<tr>
<td>Tositumomab</td>
<td>Bexxar</td>
<td>Humanized, IgG1 (PD)</td>
</tr>
<tr>
<td>Ofatumumab</td>
<td>Astara</td>
<td>Humanized, IgG1</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>Avastin</td>
<td>VEGF-A</td>
</tr>
<tr>
<td>Natalizumab</td>
<td>Tysabri</td>
<td>Integrin-α4</td>
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<tr>
<td>Ranibizumab</td>
<td>Lucentis</td>
<td>VEGF-A</td>
</tr>
<tr>
<td>Panitumumab</td>
<td>Vectibis</td>
<td>Humanized, IgG1</td>
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<tr>
<td>Eculizumab</td>
<td>Soliris</td>
<td>Humanized, IgG1</td>
</tr>
<tr>
<td>Certolizumab</td>
<td>Cimzia</td>
<td>Humanized, IgG1</td>
</tr>
</tbody>
</table>

Immune disorder: Rheumatoid arthritis, Psoriasis, Multiple Sclerosis
Oncology disease: Breast cancer, Leukaemia, Colorectal cancer, Lung cancer, Crohn’s disease, Psoriasis, Multiple Sclerosis, Organ Transplantation, Oncology disease

**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**
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

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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, virusus)
  - Blood products

Lyophilization process → Dry Powder
  - Increase Stability
  - Easy to Dissolving
  - Easy to transport
  - Low moisture content
  - Low temperature process
  - More surface area & porous

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**

![Image of freeze drying process](image.png)

**Equilibrium product temperature of all vials**

**Diagram showing**
- Annealing
- Freezing (option)
- Primary Drying
- Secondary Drying

**Graph showing**
- Product Temperature
- Shelf Temperature
- Pressure (mbar)

**Table showing**
- Temperature / °C
- Time / h
- Freeze Dryer Shelf

**Equilibrium product temperature of all vials**
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

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

Through Vial Impedance Spectroscopy
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.
Lyophillization or Freeze Drying Process

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![Diagram of Freeze Drying Process]

**Freeze Drying Process Flow Chart:**
- **Freezing**: Ice crystal growth from the bottom of the vial (typically takes less than 2 min).
- **Primary Drying**: Release of heat causes a spike in the product temperature.
- **Secondary Drying**: Ice nucleation

**Through Vial Impedance Spectroscopy**
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### Diagram:

- **Annealing**
- **Freezing** (option)
- **Primary Drying**
- **Secondary Drying**

**Physical Variables:**
- **Temperature / °C**
- **Pressure / mbar**

**Graph:**
- **Product Temperature**
- **Shelf Temperature**
- **Pressure**

**Equation:**

\[ P_{\text{ice}} = P_{\text{c}} \]

**Legend:**
- **Radiation**
- **Convection**
- **Ice layer**
- **Direct conduction**
- **Freeze Dryer Shelf**

**Text:**

- Increasing shelf temperature (ramp), increases ice temperature and partial pressure until \( P_{\text{ice}} = P_{\text{c}} \) and drying (sublimation) starts.
Lyophilization or Freeze Drying Process

- A technique which dries product at low temperature through sublimation process
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### Pressure / mbar

### Temperature / oC

### Time / h

**Freezing**

- **Annealing** (option)

**Primary Drying**

**Secondary Drying**

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

When $T_P \geq T_s$; then drying is assumed to have stopped
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**

![Graph showing the process of lyophilization](image)

**Annealing**
- *Freezing* (option)

**Primary Drying**
- **Product Temperature**
- **Shelf Temperature**
- **Pressure**

**Secondary Drying**
- Dried layer

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**Through Vial Impedance Spectroscopy**

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Process Analytical Technologies

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

Through Vial Impedance Spectroscopy
Introduction to the TVIS System

• Impedance spectroscopy characterizes the ability of materials to conduct electricity under an applied 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

<table>
<thead>
<tr>
<th>SV product temperature</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SV sublimation rate</td>
<td></td>
</tr>
<tr>
<td>SV end point</td>
<td></td>
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</table>
Through Vial Impedance Spectroscopy (TVIS)

Introduction
Through Vial Impedance Spectroscopy

Freeze drying chamber

Junction box

TVIS measurement vial

LyoView™ analysis software

LyoDEA™ measurement software

Pass through

TVIS system (I to V convertor)

Resultant current

Stimulating voltage
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

\[ C' = \frac{C_G + \omega^2 R_s^2 C_G C_S (C_S + C_G)}{1 + (\omega R_s ((C_S + C_G))^2} \]

\[ C'' = -\frac{\omega R_s C_G^2}{1 + (\omega R_s ((C_S + C_G))^2} \]
Dielectric loss spectrum of frozen water at -27 °C

A frequency of

\[ F_{\text{PEAK}} = \frac{1}{2\pi R_s (C_s + C_G)} \]

If \( C_G > C_S \) then

\[ C''_{\text{PEAK}} \approx C_G \]

Which explains the sensitivity of \( C''_{\text{PEAK}} \) to the height of the ice layer
Imaginary Part of Capacitance

Real Part of Capacitance

Annealing = Re-heating and Re-cooling

TVIS Response Surface (3D-Plot)

Liquid state

Frozen solid

Re-heating

Re-cooling

Primary drying

Low frequency

High frequency

Through Vial Impedance Spectroscopy
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
- + 20.3°C
- Liquid state

Through Vial Impedance Spectroscopy
Impedance and Capacitance Spectrum

Through Vial Impedance Spectroscopy

Solid (frozen state) lower temp

5%w/v Lactose solution

-30.4 °C
Impedance and Capacitance Spectrum

-20.4 °C

5%w/v Lactose solution

Solid (frozen state)

high temp

Through Vial Impedance Spectroscopy
Impedance and Capacitance Spectrum

Through Vial Impedance Spectroscopy
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 $dC''_{\text{PEAK}}/dt$)

*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

- $F_{\text{PEAK}}$ 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 \( T(F_{PEAK}) \)

\[
\begin{align*}
\text{Before drying} \quad & T(F_{PEAK}) > T_S \\
\text{during drying} \quad & T(F_{PEAK}) < T_S
\end{align*}
\]

\[
\frac{dQ}{dt} = L \cdot \frac{dm}{dt}
\]

\[
y = -4.7474x^2 + 56.64x - 160.16 \quad \text{R}^2 = 0.9998
\]

\[
\begin{align*}
\text{Shelf Temperature (}\ T_S \text{)}
\end{align*}
\]

\[
\begin{align*}
\text{Before drying} \quad & \text{Thermocouple} \\
\text{during drying} \quad & T(F_{PEAK})
\end{align*}
\]

\[
\begin{align*}
\text{Time / h}
\end{align*}
\]
Compensation of $C''_{\text{PEAK}}$ by $T(F_{\text{PEAK}})$

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

\[ y = -8 \times 10^{-5}x^2 - 0.0016x + 0.8962 \]

\[ R^2 = 0.9993 \]

Through Vial Impedance Spectroscopy
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
  o Determination of heat transfer coefficients ($K_V$)
  o Determination of dry layer resistance ($R_P$) to predict drying efficiency

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

• Develop (new) continuous drying technologies
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