Purification and Isolation Technologies

Ricardo Mendonça & Rui Loureiro
Hovione In it for life
Who we are and what we do?

Drug Substance | Particle Engineering | Drug Product
---|---|---
Off Patent API | Control of API physical properties | Formulations Services
Custom Synthesis | Licensing DPI Inhalers | 

1.307 Manufacturing Facilities - m³

1.215 Hovione Team Members

Customers
Medium, specialty and large pharma
Biotech
Generics pharma

CMO Leadership Awards 2016
FDA 26
Rui Loureiro

Rui Loureiro joined Hovione in 2008 as a Process Chemist and after several positions he is currently the Director of the R&D Process Chemistry area where is responsible for the development and scale-up of processes to produce active pharmaceutical ingredients under development.

Rui Loureiro has a degree in Physics and Chemistry from the University of Algarve, Portugal and a Ph.D. in organic chemistry from the University of Liverpool, UK.

After completing his Ph. D. Rui continued in the University of Liverpool as a post-doc fellow before joining Lintfield Ltd as a process chemist where he stayed between 2004 and 2008.

In his work Rui Loureiro as published ten papers in peer review magazines and is author of four patents. He has also carried out several oral (8) and poster communications (10).

Currently his interests are scaling-up of processes to produce and purify APIs under a quality by design approach, flow chemistry and process modelling.
Ricardo Mendonça joined Hovione in 2007 as a process chemist and is currently a Group Leader in the R&D Process Chemistry Development area where he is responsible for the development and scale-up of processes to produce Active Pharmaceutical Ingredients (APIs) under development.

Ricardo has a degree in Biochemistry at the University of Algarve in 1998. Afterwards he moved to Liverpool UK to carry out a Ph.D. in Organic Chemistry. After completing his Ph.D. Ricardo moved to Tempe, Arizona as a post-doc fellow working at the Cancer Research Institute, under the supervision of Prof. George R. Pettit, in the area of Natural Products Synthesis. After moving to Portugal Ricardo worked between 2005 and 2007 at the New University of Lisbon working in the development of new asymmetric brominating reagents.

Ricardo Mendonça has published nine scientific papers and is the author of one patent. He has also carried out several oral and poster communications. Ricardo also contributes to the scientific community as a reviewer of scientific papers and as an invited lecturer of Industrial Synthesis of APIs at the Faculty of Pharmacy (University of Lisbon).

Currently his interests are biocatalysis, carbohydrate chemistry, peptide synthesis and large-scale chromatography.
Agenda

Introduction

Purification Technologies

Purification by IEX – Case Study

Isolation technologies

Integrated Purification & Isolation

QbD applied to purification and isolation
Introduction – Complexity increasing

New chemical entities are becoming more complex and more difficult to purify and isolate

Bryostatin 1

Dolastatin 10

Telavancin

Enfuvirtide
Introduction – Complexity increasing

- Long synthesis
- Enantiomeric separation
- Purification of complex mixtures
- Removal of specific impurities such as GTI’s
- Particle Design

Purification and Isolation Challenges
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Introduction

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Introduction – Purification: Crystallization

Crystallization has been at the forefront of purification technologies for a long time:

- From the melt
  - Not widely used

- From solution
  - Temperature controlled
  - Evaporation controlled
  - Anti solvent addition

New trends:

- Sonocrystallization
  - The Use of Ultrasound for Improved Industrial Crystallization
  - The most important effect of ultrasound on crystallization is the induction of nucleation and the principal benefits derived from an ability to manipulate this effect.

- Continuous Flow
  - Emerging technique
  - Offers several benefits
    - Cost reduction
    - Faster scale-up
    - Controllable quality
Introduction – Purification: Chromatography

Chromatography is a common technology used industrially to purify a whole lot of compounds. In the pharmaceutical arena it’s mostly used industrially to purify complex compounds and pure enantiomers.

Several (LC) types: Low-pressure (up to 3 bar), Medium pressure (up to 240 bar), High-pressure.

By mode of separation:

- Specific binding interactions (affinity chromatography)
- Charge (ion exchange chromatography)
- Size (size exclusion chromatography/gel filtration chromatography)
- Hydrophobic surface area (hydrophobic interaction chromatography and reverse phase chromatography)
- Multiple properties (multimodal or mixed-mode chromatography)

Simulated moving bed (SMB)

- Multiple smaller columns containing the solid adsorbent (beds) rather than one large column, and to “move” the beds in the opposite direction of the fluid to achieve a countercurrent flow, rather than flowing fluids through one static bed. **Allows much higher productivity (20x) relative to batch (single-column) methods.**

Centrifugal Partition Chromatography (CPC)

- Liquid-liquid chromatography technique based on partition of compounds between two immiscible liquid phases.
- Has been used for the purification of oligonucleotides (WO2013030263 A1)

Supercritical fluid chromatography (SFC)

Drugs purified using SMB:
- Prozac
- Citaprolam
Introduction – Purification: Membrane separation processes

Extremely useful technique for the removal of water from solutions of APIs.

Can be used to remove dissolved gases, salts, impurities etc.

Depending on the size of compounds present and their difference several types of membranes are available for:

- Reverse osmosis
- Nanofiltration
- Ultra filtration
- Microfiltration
- Particle filtration
Organic solvent nanofiltration (OSN) is emerging as a novel method to remove organic impurities from APIs. Use of molecularly imprinted polymers (MIPs) and synergistic combinations of these two technologies is also a hot research field.

These technologies allow reduced energy consumption, improved process capability, low maintenance, increased automation, modularity and reduced footprint in the purification of APIs.

NEMOPUR (New Molecular Purification Technology for Pharmaceutical Production)

European Commission FP7-funded Marie Curie Initial Training Network of nine partner organisations from industry and academia

Organic solvent nanofiltration: A platform for removal genotoxins from active pharmaceutical ingredients; Journal of Membrane Science 381 (2011), 21-33

A hybrid approach to reach stringent low genotoxic impurity contents in active pharmaceutical ingredients: Combining molecularly imprinted polymers and organic solvent nanofiltration for removal of 1,3-diisopropylurea; Separation and Purification Technology 86 (2012), 78-87

Design, preparation and characterization of novel molecularly imprinted polymers for removal of potential genotoxic 1,3-diisopropylurea from API solutions; Separation and Purification Technology 86 (2012), 190-198
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Introduction

Purification Technologies

**Purification by IEX – Case Study**

Isolation technologies

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QbD applied to purification and isolation
Purification – IEX case study
Scaling-up

Chromatographic processes:
✓ Efficient product purification
✓ Resins can be re-used
✓ Different types of resins can be used
✓ Elution monitored by PAT tools;
✓ QbD approach

✗ Packing may be challenging;
✗ Uses large volumes of solvent;

Crude API: ~85-90% purity
Final API: ~99.5% purity
Purification – IEX case study

• Chromatography scaling-up challenges:
  • Column size
  • Stationary phase used
  • Wall effect
  • Pressure
  • Mobile phase flow
  • Gradient
  • Elution rate variation from small scale to large scale
  • Column packing – ensuring it is properly packed
Purification – IEX case study
Laboratory scale

• Starting point!
  • Preparative chromatography system
  • Binary gradient pumping system (2.5 to 250 ml/min)
  • Pressure max 50 bar
  • Fraction collector
  • UV detector (200-840 nm) with 4 simultaneous channels
  • Controlled via SepacoreControl software
Purification – IEX case study
Resin selection

Ion exchange resins are **highly ionic, covalently cross-linked, insoluble polymers supplied as beads**. The beads have either a dense internal structure with no discrete pores (gel resins, also called microporous resins) or a porous, multichannelled structure (macroporous or macroreticular resins).
Purification – IEX case study
Resin selection

Ion exchange resins are **highly ionic, covalently cross-linked, insoluble polymers supplied as beads**. The beads have either a dense internal structure with no discrete pores (gel resins, also called **microporous resins**) or a porous, multichannelled structure (**macroporous or macroreticular resins**).

- Several **types of resins available**:
  - Strongly acidic (*i.e.* sulfonic acid groups)
  - Weakly acidic (*i.e.* carboxylic acid groups)
  - Strongly basic (*i.e.* quaternary amino groups)
  - Weakly basic (*i.e.* primary, secondary and ternary amino groups)
  - Ampholytic containing acidic and basic groups
  - Selective and chelating resins
Purification – IEX case study
Scale challenges

Scale available:

Each scale presents its own challenges!
Purification – IEX case study
Filling properties

Wall effect
Purification – IEX case study
Resin properties

Pressure Flow curves

At small scale the pressure in the column increases in a linear manner

- At large scale the pressure in the column increases in a linear fashion to a point and then it is exponential, indicative that there is no wall effect at this scale.
Purification – IEX case study
Resin properties

Pressure Flow curves

At smale scale the pressure in the column increases in a linear manner

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Purification – IEX case study
Resin properties

Pressure Flow curves

At small scale the pressure in the column increases in a linear manner

- At large scale the pressure in the column increases in a linear fashion to a point and then it is exponential, indicative that there is no wall effect at this scale.
Purification – IEX case study

Packing

Column packing

Is one of the most important aspects that should be taken in account when scaling up a chromatographic process

Irregularities may cause:

• Uneven flow within the bed
• Band broadening (resolution lost)
• Zone mixing
• Changes in flow rates
Purification – IEX case study
Packing

How to check column packing?

• Visual inspection (i.e. dye)

• Determining plate numbers/HETP, Asymmetry (Efficiency)
Purification – IEX case study

Packing

Pulse experiments using NaCl

Replicate #1

Replicate #2

<table>
<thead>
<tr>
<th>Results</th>
<th>Recommended values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vmaximum</td>
<td>0.5973</td>
</tr>
<tr>
<td>W half height</td>
<td>0.04629</td>
</tr>
<tr>
<td>Assymetry factor</td>
<td>0.58</td>
</tr>
<tr>
<td>Number plates</td>
<td>922</td>
</tr>
<tr>
<td>HETP</td>
<td>0.06</td>
</tr>
<tr>
<td>RPH</td>
<td>3.05 (&lt;3.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Results</th>
<th>Recommended values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vmaximum</td>
<td>0.5855</td>
</tr>
<tr>
<td>W half height</td>
<td>0.04748</td>
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<tr>
<td>Assymetry factor</td>
<td>0.49</td>
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<tr>
<td>Number plates</td>
<td>842</td>
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<tr>
<td>HETP</td>
<td>0.07</td>
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<tr>
<td>RPH</td>
<td>3.34 (&lt;3.5)</td>
</tr>
</tbody>
</table>
Purification – IEX case study

Packing

Pulse experiments using Ammonium Trifluoroacetate

Preference to use same salt formed upon adsorption
Purification – IEX case study

Packing

How to check resin packing and regeneration?

- Determining plate numbers/HETP, Asymmetry (Efficiency);
  - PAT used to monitor transitions.

\[
* \text{HETP}_N = \frac{L \sigma^2}{M_1^2} \text{M}_0
\]

- \( L \) – column length
- \( \sigma \) – variance
- \( M_1 \) – first moment
- \( M_0 \) – zeroth moment


Purification – IEX case study

Packing Summary

<table>
<thead>
<tr>
<th></th>
<th>Results</th>
<th>Recommended values</th>
</tr>
</thead>
<tbody>
<tr>
<td>V max</td>
<td>0.5531</td>
<td>0.5</td>
</tr>
<tr>
<td>W half height</td>
<td>0.0387/75</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Asymmetry factor</td>
<td>0.17</td>
<td>0.15 – 1.5</td>
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<tr>
<td>Number plates</td>
<td>700.75</td>
<td>700 ± 10</td>
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<tr>
<td>HETP</td>
<td>0.07</td>
<td>&lt; 3.5</td>
</tr>
<tr>
<td>RPH</td>
<td>2.82</td>
<td>&lt; 3.5</td>
</tr>
</tbody>
</table>

- Transition Analysis
- Salt pulse
- Right resolution every time!
Agenda

Introduction

Purification Technologies

Purification by IEX – Case Study

Isolation technologies

Integrated Purification & Isolation

QbD applied to purification and isolation
Isolation Technologies

- Filtration
- Liophylization
- Spray drying
# Isolation Technologies

<table>
<thead>
<tr>
<th>Technology</th>
<th>Lyophilization</th>
<th>Filtration/drying</th>
<th>Spray drying</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantages</strong></td>
<td>• Very mild method</td>
<td>• Standard equipment</td>
<td>• Continuous process</td>
</tr>
<tr>
<td></td>
<td>• Thermolabile products can be dried</td>
<td>• Low cost</td>
<td>• Fewer unit operations</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Easily scalable</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Ability to manipulate CQAs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Improved solubility without physical milling</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>• Batch process</td>
<td>• Potential stability issues</td>
<td>• High CAPEX</td>
</tr>
<tr>
<td></td>
<td>• High CAPEX</td>
<td>• Designed for batch operations</td>
<td>• Can have low thermal efficiency</td>
</tr>
<tr>
<td></td>
<td>• Several unit operations</td>
<td>• Drying may be non-uniform</td>
<td>• High energy and pressure requirements</td>
</tr>
<tr>
<td></td>
<td>• Long times</td>
<td></td>
<td>• Maintenance</td>
</tr>
<tr>
<td></td>
<td>• Hygroscopic products</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Isolation – Spray drying

- Spray drying

Atomization of the feed creates small droplets with a target particle size distribution.

Droplet evaporation takes place within the drying chamber.

Hot drying gas.

Cyclone

Dry powder is collected.
Isolation – Spray drying

Spray drying:

- Ability to **handle thermo labile compounds**
- **Control over final particle morphology**
- Continuous process
- Can handle solutions, suspensions and emulsions
- Product with low residual solvent content with improved product stability and shelf life
- Reduced risk of microbial growth
Isolation – Spray Drying

Allows the isolation of

- Amorphous products
- Encapsulated products
- Spray-dried dispersions
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QbD applied to purification and isolation
Integrated purification & isolation

Complex APIs are difficult to access using standard techniques and typically require a mix of SMART synthesis and ADVANCED purification/isolation processes.

Chromatography generates dilute solutions which are typically aqueous solutions and frequently the molecules are thermally unstable.
Integrated purification & isolation

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Chromatography generates dilute solutions which are typically aqueous solutions and frequently the molecules are thermally unstable.
Integrated purification & isolation

Membrane Purification Advantages:

- Easily controlled temperature
- Removal of inorganic and organic impurities (even GTIs!)
- Fast and energetically efficient water removal method
- Low impact on overall process cost, due to re-usability
- Lower waste treatment cost (water)

Sepa CF unit: scale-up
MetCell: screening
Total: 267 cm²
Integrated purification & isolation

Synthesis Work-up
- Crude product is a salt

Ion-exchange Chromatography
- Product binds to resin and releases salt
- Water wash to remove salts
- Product elutes with ammonia

Concentration by RO
- Dilute solution concentrated prior to charcoal

Charcoal
- Clarification

Spray drying
- Product isolated as amorphous solid
Integrated purification & isolation

- **Synthesis Work-up**
  - Crude product is a salt

- **Ion-exchange Chromatography**
  - Product binds to resin and releases salt
  - Water wash to remove salts
  - Product elutes with ammonia

- **Diafiltration**
  - Ammonia removed by diafiltration

- **Concentration by RO**
  - Dilute solution concentrated prior to charcoal

- **Charcoal**
  - Clarification

- **Spray drying**
  - Product isolated as amorphous solid
Agenda

Introduction

Purification Technologies

Purification by IEX – Case Study

Isolation technologies

Integrated Purification & Isolation

QbD applied to purification and isolation
QbD methodology
Overview

Target profile
(quality, safety, efficacy)

CQA definition
(critical quality attributes)

Risk assessment I
(rank process parameters)

Process Development
(statistical, mechanistic)

Design space & NOR
(feasible & preferable)

Risk assessment II
(process FMEA)

Regulatory filing & approval

Change control & implementation

Process control strategy

PAT Implementation

Criticality analysis

PAT Strategy

QbD methodology
Overview
QbD methodology - Statistical models
QbD methodology - Statistical models

Knowledge space

Screening stage
- Control variables (initial set)
- Quantify criticality & Check the space
  - Average resolution
  - Wide space

Optimization stage
- Control variables (reduced set)
- Refine quantification & Locate optimality
  - High resolution
  - Average space

Robustness stage
- "Fixed" variables (emulate deviations)
- Assume optimal point & Check the stability
  - Low resolution
  - Narrow space
QbD applied to chromatography

Key steps & tools: Risk assessment

Risk assessment is used to reduce the number of parameters to study.
QbD applied to Spray-drying

Key steps & tools: Risk assessment

Atomization of the feed creates small droplets with a target particle size distribution

Droplet evaporation takes place within drying chamber

Risk assessment is used to reduce the number of parameters to study

Feed Tank (solution, suspension or emulsion)

F_feed
P_feed
T_feed

Hot drying gas
F_dry
T_in

Drying chamber

T_out

Cyclone

Gas recycling unit

T_cond

Nozzle
Type & Prop.

Product

Dry powder is collected
## QbD applied to Spray-drying

### Key steps & tools: Risk assessment

**RAM (Risk-assessment matrix):** useful to systematize prior knowledge and obtain a ranking of "perception of criticality" on which a subsequent pareto analysis can be based.

<table>
<thead>
<tr>
<th>Process step</th>
<th>Critical Quality Attributes (CQA's)</th>
<th>Potentially Critical Process Parameters (pCCP's)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BD</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>BD</td>
<td></td>
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</table>

### Process step: Spray Drying

<table>
<thead>
<tr>
<th>(#)</th>
<th>(description)</th>
<th>(#)</th>
<th>(tag)</th>
<th>(description)</th>
<th>(#)</th>
<th>(tag)</th>
<th>(description)</th>
<th>Criticality perception</th>
<th>Study?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T_out</td>
<td>4</td>
<td>3</td>
<td>Outlet temperature of the drying gas</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>3.7</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>T_cond</td>
<td>2</td>
<td>1</td>
<td>Temperature of the condenser</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1.7</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>F_feed</td>
<td>3</td>
<td>2</td>
<td>Feed solution flowrate</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2.7</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>C_feed</td>
<td>4</td>
<td>5</td>
<td>Solids content in the feed solution</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4.3</td>
<td>N</td>
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<tr>
<td>5</td>
<td>R_atom</td>
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<td>5</td>
<td>Atomization pressure</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5.0</td>
<td>Y</td>
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<tr>
<td>6</td>
<td>D_noz</td>
<td>3</td>
<td>4</td>
<td>Nozzle orifice diameter</td>
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<td>4</td>
<td>5</td>
<td>4.0</td>
<td>Y</td>
</tr>
<tr>
<td>7</td>
<td>T_feed</td>
<td>1</td>
<td>2</td>
<td>Feed solution temperature</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1.3</td>
<td>N</td>
</tr>
</tbody>
</table>

**Criticality perception (1-5):**

- **User #1:** 4
- **User #2:** 3
- **User #3:** 4

**Study?**

- **Y:** Yes
- **N:** No
Conclusions

• The complexity of new chemical entities is increasing;

• Purification and isolation are becoming more challenging;

• Technologies like membrane purification, spray drying, chromatography are increasingly being used;

• Quality by design principles applied to these technologies greatly contributes to speed-up its development/scale-up;

• The current know-how enables the scale-up of this processes and combinations thereof.
Thank you for your attention.