Chiral Resolution Crystallizations
Fundamentals and Applications

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Definitions and Principles
Resolution Types and Examples
Use of Lasentec FBRM/PVM in development of a classical resolution
Louis Pasteur, 1848
Sorbonne, Paris

HOOC-CH-CH-COOH $\rightarrow$ Na$^+$[OOC-CH-CH-COO]$_2$$^\cdot$NH$_4^+$

tartaric acid  sodium ammonium tartrate

Pasteur found two different crystals.
“I carefully separated the crystals hemihedral to the right and the crystals hemihedral to the left; I observed separately their solutions in the polarizing apparatus. I then saw with no less surprise than pleasure that the crystals hemihedral to the right deviated the plane of polarisation to the right, and that those hemihedral to the left deviated it to the left; and when I took an equal weight of each of the two crystals, the mixed solution was indifferent towards the light in consequence of the neutralization of the two equal and opposite individual deviations”

Louis Pasteur

Importance of Enantiomerically Pure Compounds in the Pharma Industry

(R)-Thalidomide is a mild sedative
(S)-Thalidomide is a teratogen

- FDA published a 1992 guidance document concluding that new chemical entities required quantitative pharmacological and toxicological effects of each enantiomer
Racemates
Three Physical Types

**Conglomerate**
A two phase physical (mechanical) mixture of enantiomers

**Racemic Compound**
Contains an equal number of molecules of each enantiomer in the unit cell of the crystal
Most common type of crystalline racemate

**Pseudoracemate**
A solid solution - material with equimolar enantiomeric composition
Conglomerates

- A two phase mechanical mixture
- Mixture acts as a eutectic - melts as if it were a pure substance
- Approximately 250-300 enantiomeric organic compounds are known in the literature to be conglomerates
- Examples:
  - hydrobenzoin, o-chloromandelic acid, phenylhydracrylic acid, sodium ammonium tartrate

\[ \Delta H_{fp} = \text{Heat of Fusion – Pure Isomer} \]
\[ R = \text{gas constant} \]
\[ T_P = \text{Melt Temp of Pure Isomer} \]
\[ T = \text{Melt Temp of mixture} \]
Racemic Compounds

Crystals contain same number of both enantiomers in the unit cell

Examples: Mandelic acid, malic acid, benzylidenecamphor

Prigogine and Fay Equation for liquidus curve

\[
\ln[4x(1 - x)] = \frac{2\Delta H_{fR}}{R} \left[ \frac{1}{T_R} - \frac{1}{T} \right]
\]

\(\Delta H_{fR}\) = Heat of Fusion – Racemate
R = gas constant
\(T_R\) = Melt Temp of Racemate
T = Melt Temp of mixture
Pseudoracemates (Solid Solutions)

Mixtures of two enantiomers of a given compound that form mixed crystals

Examples:
- camphor (ideal)
- carvoxime (maximum melting)
- 2-amyl carbamate (minimum melting)

Roozeboom Type I (Ideal Solid Solution)
Roozeboom Type II (Maximum Melting Point)
Roozeboom Type III (Minimum Melting Point)
Resolution of Enantiomers by Direct Crystallization

Manual Sorting of the Conglomerate
   The “Pasteur” Method

Simultaneous and Separate Crystallization
   Localization of crystallization – seed crystals disposed within a racemic supersaturation solution

Simultaneous and Differentiated Crystallization
   Seeding using large crystals of one enantiomer and small crystals of the other

Jacques, Collet, Wilen
“Enantiomers, Racemates, and Resolutions
1994
Krieger Publishing
Resolution by Entrainment

Example: hyrobenzoin

Resolution by Formation of Diastereomers

- Pasteur discovered this method in 1853
- Diastereomers can have significantly different physical properties
- Formation of diastereomeric salts between racemic substrates and optically active resolving agents

\[
\begin{align*}
dA & \rightarrow dBdA + lBdA \\
dBIB & \\
lA & \rightarrow dBIA + lBIA
\end{align*}
\]
Diasteriomer Resolution Methods

Marckwald Principle (reciprocal resolutions)

The two enantiomers of a resolving agent give access to both enantiomers of the resolution substrate

dAlA + dB

<table>
<thead>
<tr>
<th>dAdB</th>
<th>lAdB</th>
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<tbody>
<tr>
<td>mirror</td>
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<tr>
<td>less soluble</td>
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dAlA + lB

<table>
<thead>
<tr>
<th>dAlB</th>
<th>lAlB</th>
</tr>
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<tbody>
<tr>
<td>less soluble</td>
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</tbody>
</table>
Diasteriomer Resolution Methods

Non-stoichiometric Quantities

Use of “half quantities” of resolving agent

\[
\text{dAI} + \frac{1}{2} \text{dB} \rightarrow \text{dAdB (solid)} + \text{lA}
\]

Method of Pope and Peachey

Use of “half quantities” of resolving agent with neutralization of the racemate by addition of an achiral substrate

\[
\text{dlB} + \frac{1}{2} \text{dA} + \frac{1}{2} \text{HCl} \rightarrow \text{dAI} + \text{lA} + \text{H}^+, \text{Cl}^-
\]

Jacques, Collet, Wilen
“Enantiomers, Racemates, and Resolutions
1994
Krieger Publishing
How to improve your chances:

Thermal Analysis methods for selecting best resolving agent for diastereomeric salts have been developed

DSC approaches using:

- Schroeder-van Laar and Prigogine-Defay Equations

\[
\ln(x) = \frac{\Delta H_{fP}}{R} \left[ \frac{1}{T_p} - \frac{1}{T} \right]
\]

\[
\ln[4x(1-x)] = \frac{2\Delta H_{fR}}{R} \left[ \frac{1}{T_R} - \frac{1}{T} \right]
\]

References:

Dyer, Henderson, and Mitchell; OPRD, 1999, 3, 161-165
Solvent Choice in Diastereomeric Resolutions

• Polar Solvents tend to be better for resolutions of diastereomeric salts

• A statistical analysis of resolutions and solvent choice showed 90% used mixture of solvents containing an alcohol

Source data “Tables of Resolving Agends and Optical Resolutions”, S.H. Wilen, University of Notre Dame Press, 1972

• Anhydrous vs aqueous solvents systems often see differing course of resolutions
Development of a Chiral Resolution using Lasentec FBRM/PVM

**Process:**
- Resolution of a racemic amine with an organic chiral acid
- Specification <2% undesired isomer

**Issues:**
- 10 g Lab scale - results gave <2% undesired isomer
- 10-20 kg Pilot Plant scale > 3 to 5% undesired isomer in product
- Pilot Plant filtrations 8-18 hr

**Goal:**
- Minimization of undesired isomer
- Robust Processing
Experimental Conditions

• Parameters
  – Cooling Profile
  – Isolation Temperature
  – Concentration
  – Stoichiometry

• Jacketed vessel, 250 mL working volume
• Cooling crystallization in aqueous methanol
• Lasentec M400L/ D600 FBRM and PVM to monitor crystallization
• HPLC to monitor isomer levels
Formation of the Undesired Isomer

Stirring overnight

Stirring overnight

---Desired Isomer

---Undesired Isomer

2.5%

35.2%
Baseline Experiment

- Linear Cooling Ramp 65 to 20 °C at 0.7 °C/min
- 20 °C isolation temperature
- 1.03 eq chiral acid
- HPLC & FBRM show growth of undesired isomer on stirring at 20 °C
Chord Length Distribution for Baseline

Linear Cooling at 0.7 °C/min 1.03 eq Acid

Linear Cooling (0.7 °C/min) with 1.03 eq Acid

Chord Length Distribution (Chords/sec)  Chord Length Distribution (Percentage)
Isolation at 30 °C

- Linear cooling ramp 65-30 °C at 0.7 °C/min
- 30 °C isolation temperature
- Stir time results in minimal growth of undesired isomer
- Yield impacted at 30 °C
Chord Length Distribution for 30 °C

Chord Length Distribution (Chords/sec) Chord Length Distribution (Percentage)
0.9 eq Overview –
No Secondary Nucleation

FBRM Trend Data

Median, No Weight, 1-1000 µm
Mean, Square Weight, 1-1000 µm
Counts/sec, 1-5 µm
Counts/sec, 30-85 µm
Counts/sec, 292-1000 µm

Primary nucleation
0.9 eq Overview – Quantifying Needle Growth

**FBRM Distribution Data**

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<th>2</th>
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<tbody>
<tr>
<td>Median, No Wt (1 - 1000)</td>
<td>26.49</td>
<td>19.45</td>
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<tr>
<td>Median, No Wt, dt (1 - 1000)</td>
<td>NaN</td>
<td>NaN</td>
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<tr>
<td>Mean, Square Wt (1 - 1000)</td>
<td>169.64</td>
<td>279.52</td>
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<tr>
<td>Counts/sec, 1-5 µm</td>
<td>619.30</td>
<td>349.82</td>
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<tr>
<td>Counts/sec, 10-25 µm</td>
<td>1574.78</td>
<td>1488.57</td>
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<td>Counts/sec, 29-95 µm</td>
<td>2231.51</td>
<td>1499.25</td>
</tr>
<tr>
<td>Counts/sec, 292-1000 µm</td>
<td>1054.77</td>
<td>1004.37</td>
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<tr>
<td>Counts/sec, 29-1000 µm</td>
<td>31.21</td>
<td>184.51</td>
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</tbody>
</table>

**FBRM Trend Data**
- Median, No Weight, 1-1000 µm
- Mean, Square Weight, 1-1000 µm
- Counts/sec, 1-5 µm
- Counts/sec, 30-85 µm
- Counts/sec, 292-1000 µm

**Graphs**
- Chord Length
- Counts/Sec
- Time
- Relative Magnitude

**Legend**
- Fines increase
- Midrange decrease
- Coarse increase
1.1 eq Overview – Secondary Nucleation Event

FBRM Trend Data

- Median, No Weight, 1-1000 µm
- Mean, Square Weight, 1-1000 µm
- Counts/sec, 1-5 µm
- Counts/sec, 30-85 µm
- Counts/sec, 292-1000 µm

Time

Relative Magnitude

Primary nucleation

Secondary nucleation event
1.1 eq Initial Growth –
Similar Growth Kinetics to 0.9 eq
1.1 eq Secondary Nucleation – Quantifying the Increase in Fines
Cooling Profile modification to improve Filtration Rate

- Original process - Poor filtration on scale up
- Uncontrolled crystallization by cooling as fast as possible
  - Result: Slow Filtration ~8 to 17 hours on PP scale

Process modifications to enable control of crystallization
- Water addition to process to give sub-saturated phase
- Cooling profiles
- Lasentec/ HPLC monitoring
Cooling Rate Effect

Crystallization using linear 0.7 °C/min cooling

Crystallization using linear 0.125 °C/min cooling
Comparison of Chord Length Trend Maps
Concluding Remarks

- FBRM can measure crystallization of undesired isomer in chiral resolution crystallization without sampling.
- On Line monitoring of undesired isomer formation observed.
- Chiral Crystallization Process improved to control to specifications by reducing resolving agent levels and controlling temperature profile.
- Understanding the type of racemic mixture can help determine best method of resolution.