eurofins | CDMO

Strategic Isolation of API Synthetic Intermediates Using Crystal16 and Crystalline

Martin Glavinović • Bahareh Khalili

Our Growth Story

Start

2003

Company Founded

Process Chemistry Expertise
cGMP Analytical Capabilities
Prep-Chromatography

2008

1st cGMP Pilot Plant Operational

1 x 200 L Reactor
1 x 500 L Reactor

2017

Acquired by Eurofins

Purchased new facilities to expand capabilities

2018

Solid State Lab & Pre-Formulation Lab

Established

2nd cGMP API Manufacturing Suite

1 x 200 L Reactor
1 x 500 L Reactor

2019

DS & DP Technology Development

Integrated

3rd cGMP API Manufacturing Suite

2 x 500 L Reactors

2020

Fully Functional Drug Product Facility

Expanded Spray Drying capabilities
(Q3 2021)

2022

Expanded Labs and Office Support

R&D Lab, Analytical Services, HPAPI expansion and Office Support

Expandable to 210,000 sqft.

2023

4th cGMP Future API Manufacturing

2 X 2000 L Reactor

Expandable to 160,000 sqft.

Alphora's Experience in Numbers

Strong **Analytical to Process Chemistry Team**
1:1 Ratio

Over **200 Employees**
79% Scientists
25% PhD
25% MSc
29% BSc

3 Self-held Drug Master File across a range of countries i.e. US, Canada, China, Japan, EU

Experience with **Complex Chemistry**
One molecule with **75 conversions and 19 chiral centers**

Developed 100s of compounds, for pre-clinical and clinical

Technology transfer of **over 500 unique processes**

Over **1200 Batches**,
Over **70 APIs**

Validated processes for **6 Commercial APIs; 7 remain on the market; 6 active**

6 FDA Approvals
2 Breakthrough/ Fast Track Programs

Our science is your success.

Solid State R&D Overview

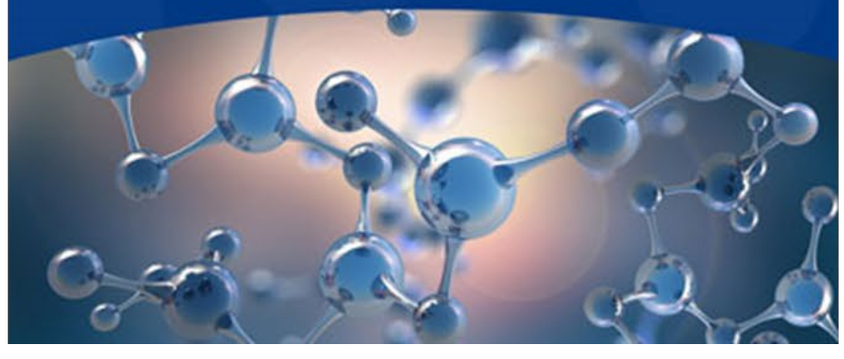
Provide expertise and services in physicochemical characterization of API, intermediates as well as screening for different API solid forms and salts. Offer quality-by-design approach for small molecule APIs at different phases of pharmaceutical development.

High-Throughput Screening

Screening for discovery of new polymorphs, pharmaceutical salts, solvates, co-crystals, etc. using minimal amount of API

Characterization

Complete physicochemical characterization of APIs and advanced intermediates utilizing state-of-the-art instrumentation.



Crystallization Engineering

Optimized crystallization and scale-up through crystal engineering and inline monitoring techniques.

Solid Form in Pharma Industry

- Polymorphs are crystalline materials composed of the same molecules, but with a different packing structure.

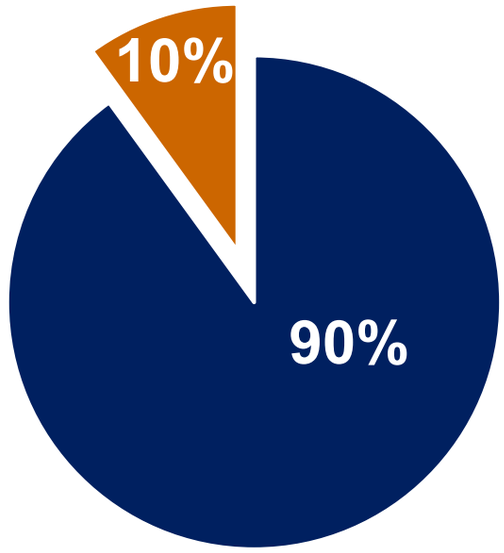
- 90% of active pharmaceutical ingredients (APIs) show polymorphism

  Acetaminophen – 3 polymorphic forms

  Atorvastatin – 60 solid forms

  Ritonavir – 5 polymorphic forms

  Axitinib – 60 solid forms



- Polymorphism (APIs/ intermediates) directly affect the manufacturing process and final product:

  Solubility – Reactivity and yield

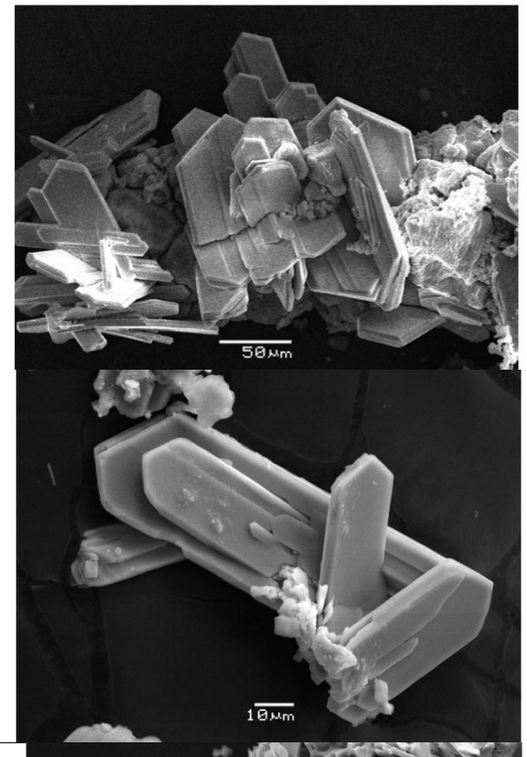
  Morphology & particle size – Filtration and drying

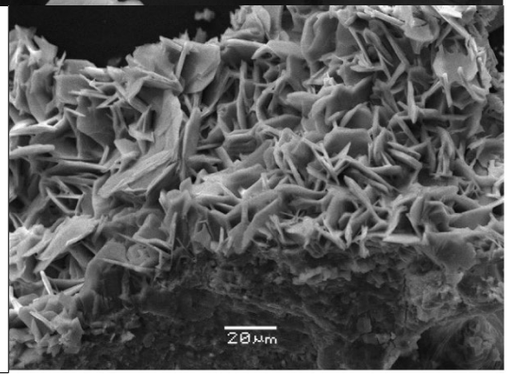
  Occlusion & inclusion – Impurity purge and solvent levels

  Hygroscopicity – Stability and storage

Effect of Process Parameters on Polymorphism and Particle Size

- Solvent composition.
- Isolation temperature. Cooling/heating rates.
- Seeding
- Mechanical activation of the solid substance
- Exposure to vapor at high or low humidity
- Exposure to organic vapor



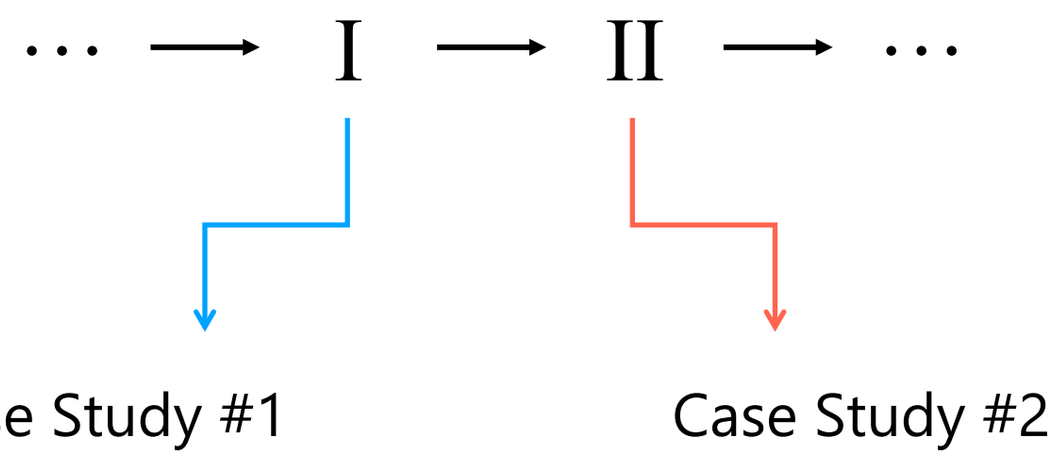


Introduction

- PhD Chemist with experience in porous solids and gas separations.
- Worked on this project between May 2023-Sep 2023.
- First time using Crystal 16 and more recently, Crystalline.
    - Instruments are easy to use.
    - Intuitive workflows.
    - In short: Someone who's relatively inexperienced can use these instruments and get a lot of useful information.

Introduction

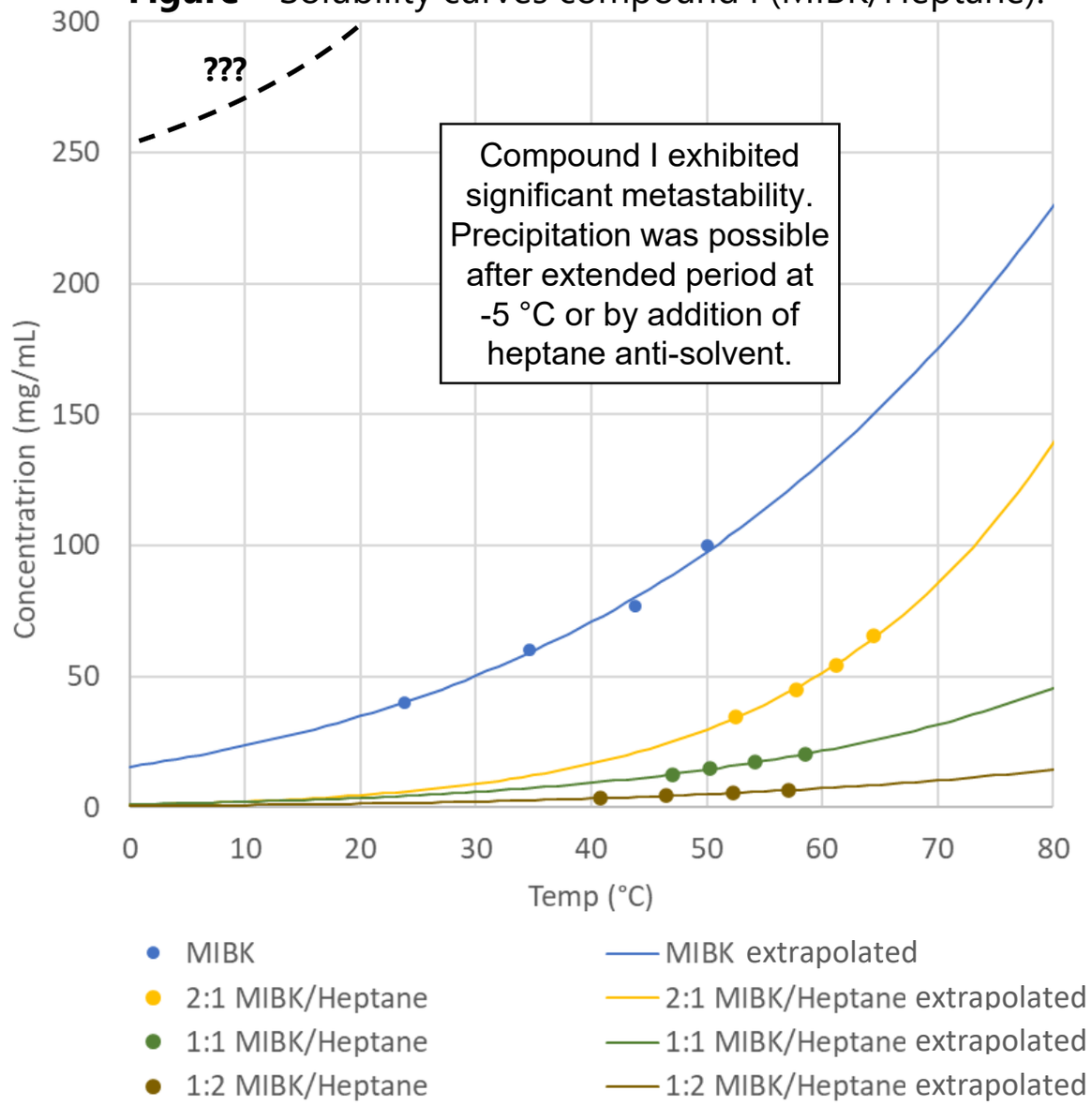
- Multi-step synthesis of API.
- Compounds I and II are approx. 500-600 g/mol, $C_xH_yCl_zN_\alpha O_\beta$, couple aryl rings, flexible molecule.
- Challenge: Optimize crystallization protocols to isolate the desired products from their respective reaction mixtures.
- Targeted high yields, good crystal quality (large crystals, high purity), simple procedure.



Case Study I: Part I MIBK/Heptane

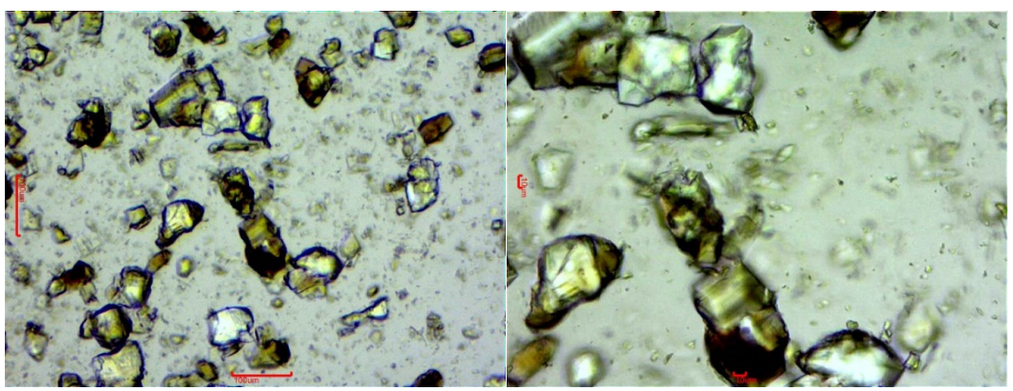
- One solid form of Compound I was discovered during development process.
- Isolation from various solvents was possible. Methyl iso-butyl ketone (MIBK) with heptane anti-solvent was a reasonable choice.
- Solubility curves of crystalline Compound I collected using Crystal 16. Compounds heated from 20 to 70 °C at 0.5 °C/min, held for 15 minutes, cooled to -5 °C at 0.1 °C/min. Held for 12 hours.
- Note: curves extrapolated from experimental data using Van't Hoff equation.

Figure – Solubility curves compound I (MIBK/Heptane).



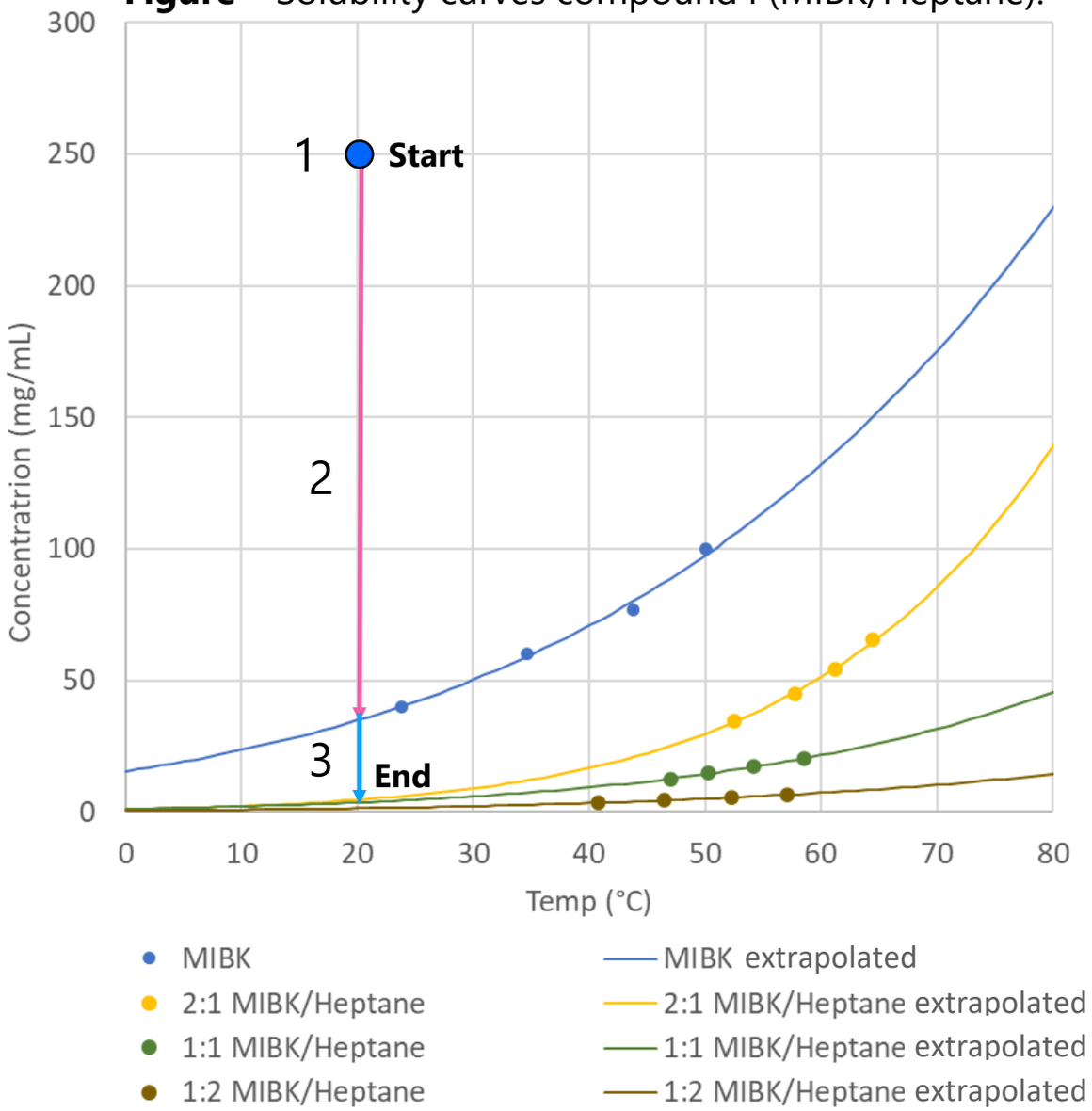
Case Study I: Part I MIBK/Heptane

- Input:
  1. Supersaturated organic fraction with approximately 5g of product dissolved in 20 mL MIBK.
- The first plan:
  2. Seed with crystalline compound I.
  3. Add heptane anti-solvent.
- Theoretical yield: 96%



Polarized Microscope Images: 10x and 40x magnification

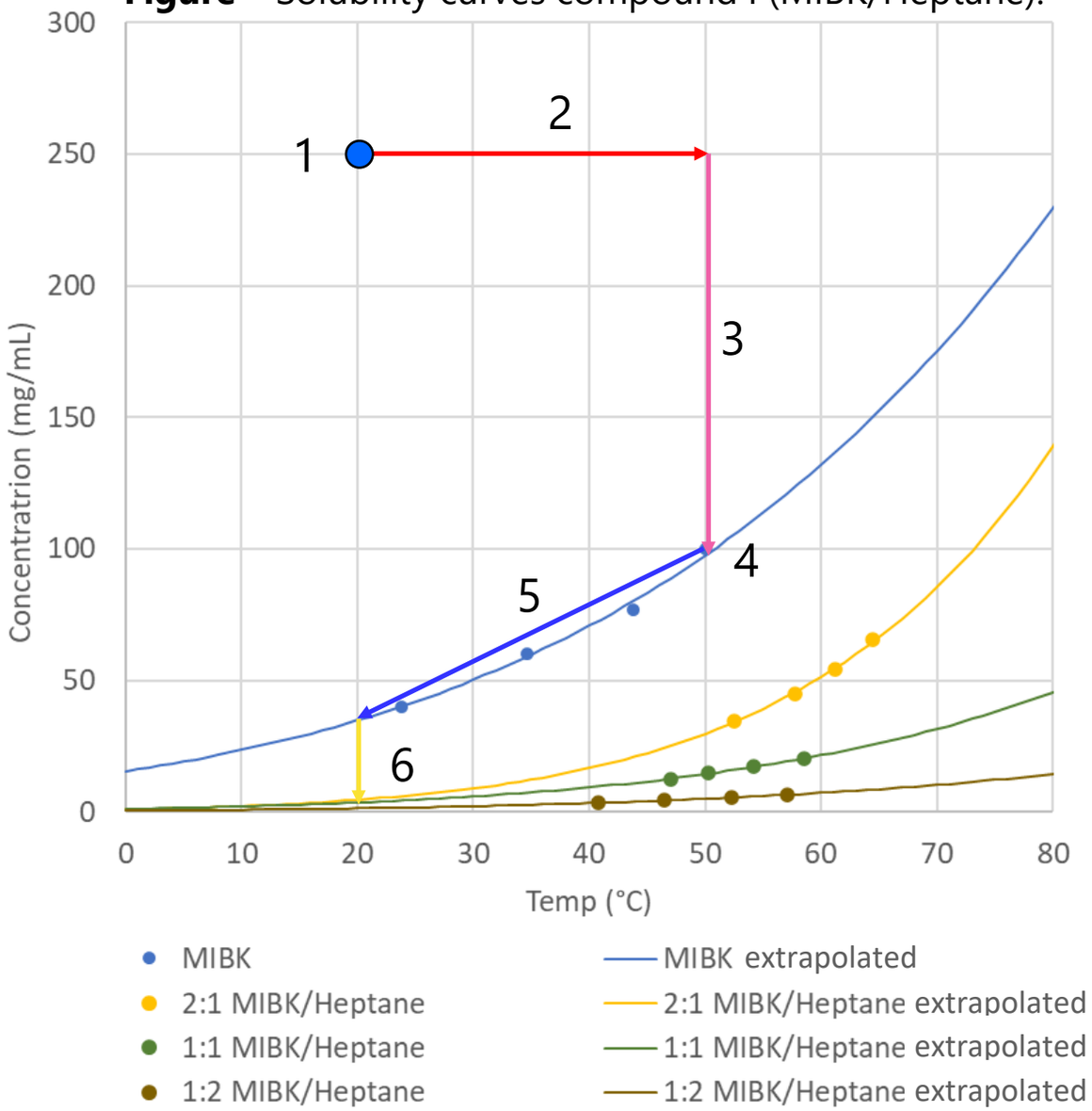
Figure – Solubility curves compound I (MIBK/Heptane).



Case Study I: Part I MIBK/Heptane

- Input:
  1. Supersaturated organic fraction with approximately 5g of solid dissolved in 20 mL MIBK.
- The second plan:
  2. Heat to 50 °C.
  3. Seed with crystalline compound I to induce crystallization.
  4. Wait for product to desaturate from solution. Perform crystal growth at warm temperature. Promotes larger/higher quality crystals.
  5. Cool slowly (0.5 °C/min)
  6. Add anti-solvent portionwise.

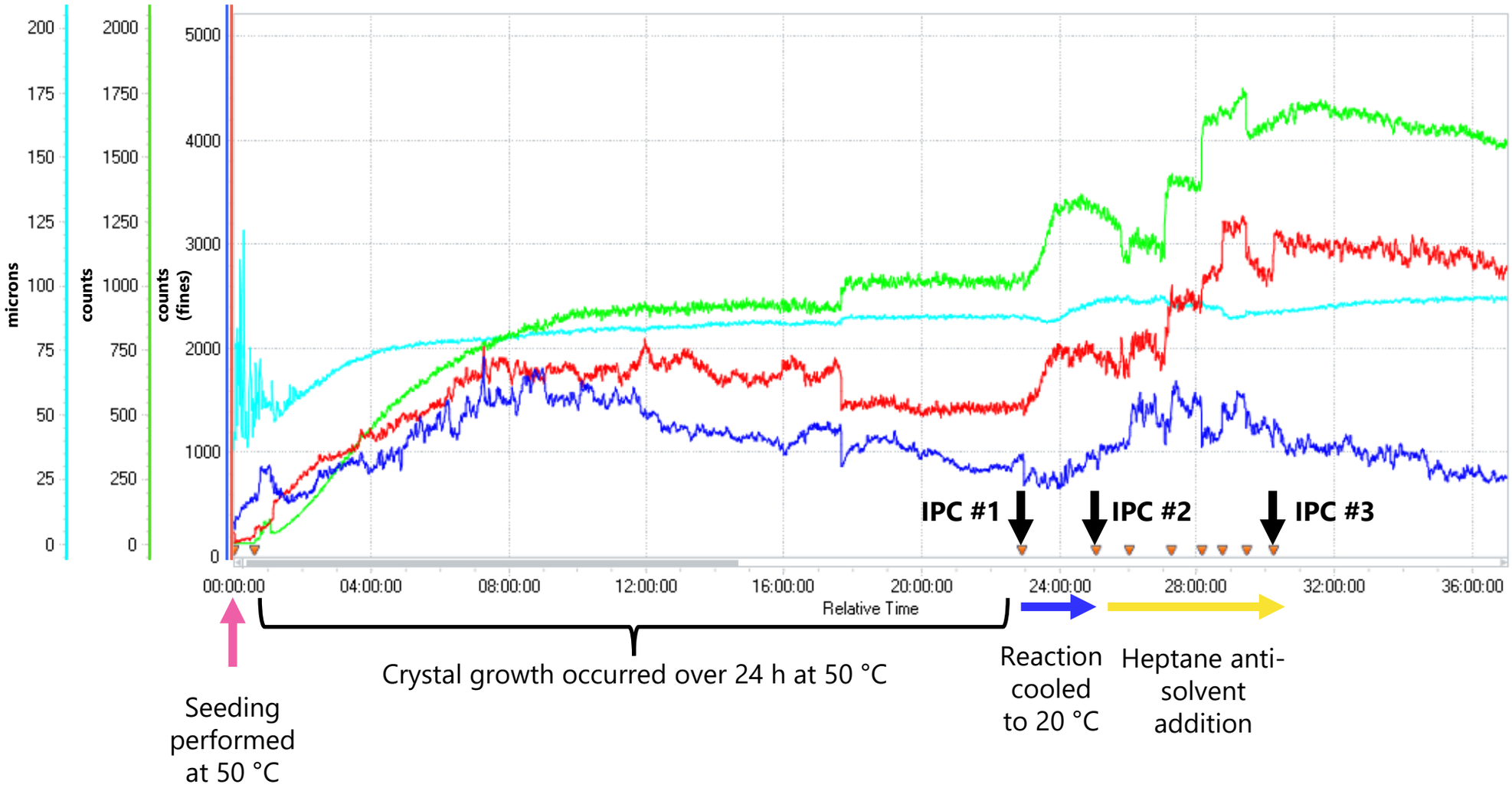
Figure – Solubility curves compound I (MIBK/Heptane).

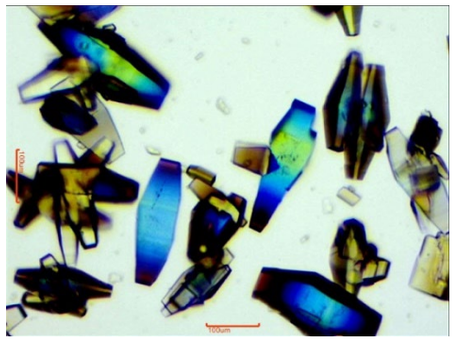


Case Study I: Part I MIBK/Heptane

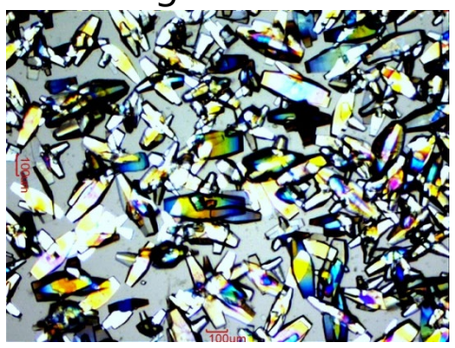
In-line FBRM probe and supplementary PLM

Figure – FBRM monitored crystallization of compound I. Measuring chord length vs Time.
<10 µm | **10-50 µm** | **50-1000 µm** | **Mean sqr.**

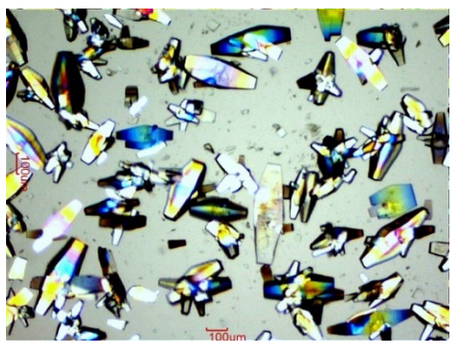




IPC #1
10 x magnification



IPC #2
4 x magnification



IPC #3
4 x magnification

Purity
UPLC: 99.9% pure
qNMR: 100 ±5% pure

Theoretical yield: 96%
Input: Approximately 5g in 20 mL MIBK
Isolated yield: 5.32 g

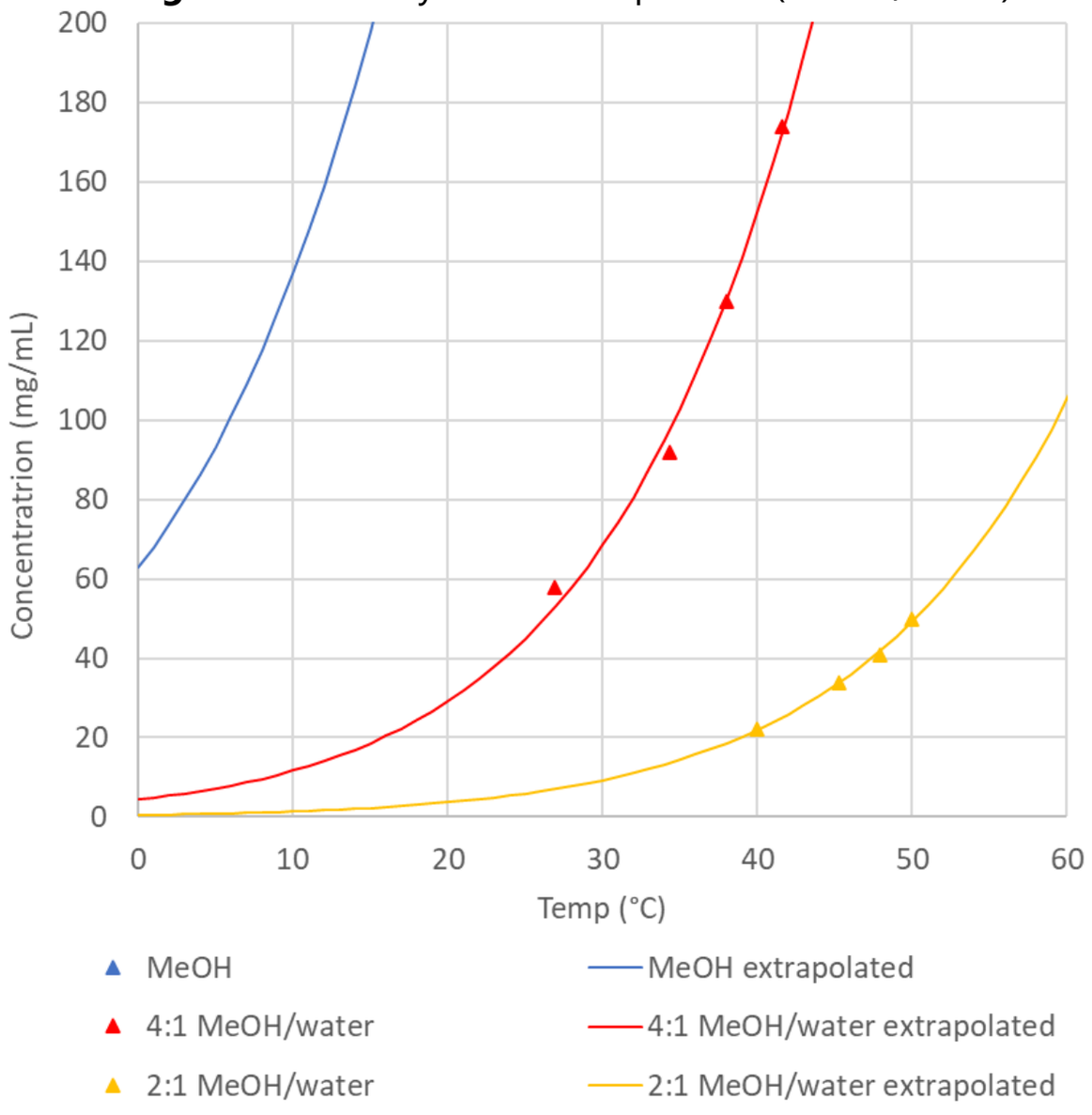
Case Study I: Part 2 MeOH/water

- Isolation in MIBK/Heptane was successful. Possible to improve?
- Isolation requires a solvent swap to MIBK. Is it possible to isolate from reaction solvent?
- Reaction was performed in MeOH.
- Quenching with aqueous solution of acetic acid resulted in the precipitation of the product in high purity.
- Challenge: Optimize the recrystallization directly from reaction mixture.

Case Study I: Part 2 MeOH/water

- Challenge: Optimize the recrystallization directly from reaction mixture.
- Solubility curves of crystalline Compound I collected using Crystal 16. Compounds heated from 20 to 50 °C at 0.5 °C/min, held for 15 minutes, cooled to 5 °C at 0.1 °C/min. Held for 12 hours.
- Note: curves extrapolated using Van't Hoff equation.

Figure – Solubility curves compound I (MeOH/water).



Case Study I: Part 2 MeOH/water

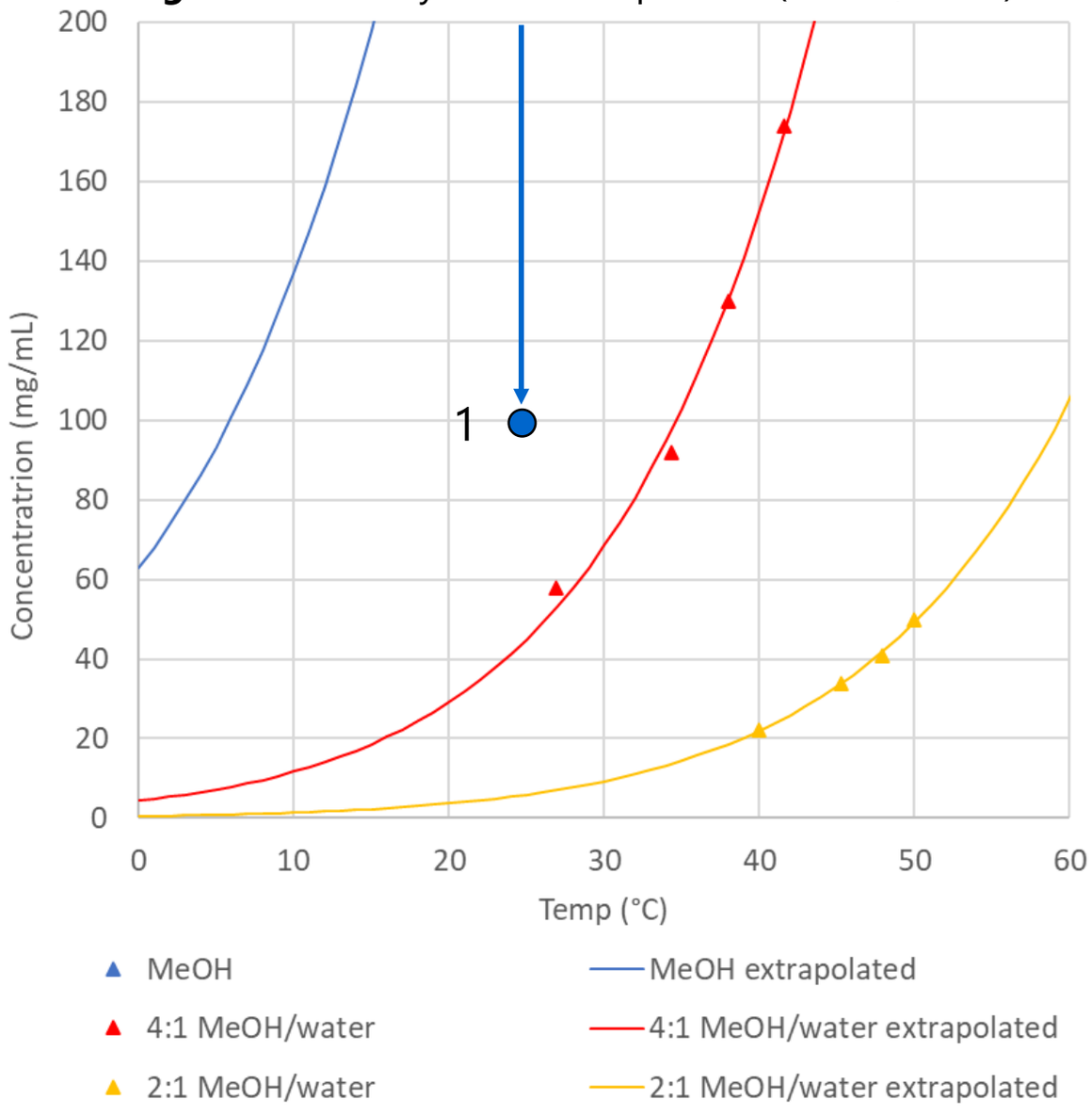
- Input:

  1. Organic fraction with 5g of product in 50 mL MeOH.

Solubility of Compound I is high. Need to add water anti-solvent first to crystallize product.

Addition of water has a significant oiling out risk. This was monitored in-situ using Crystalline.

Figure – Solubility curves compound I (MeOH/water).

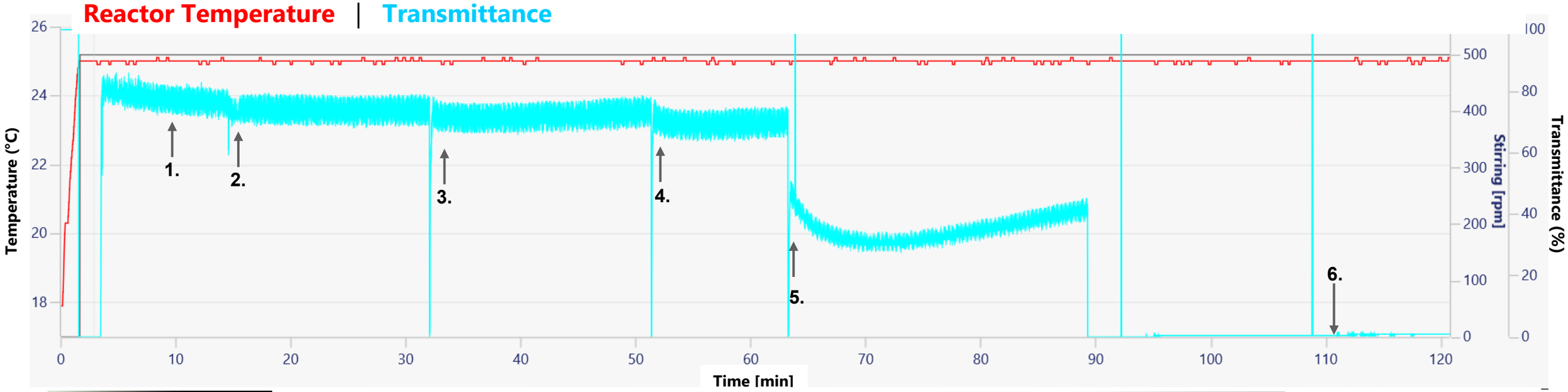


Case Study I: Part 2 MeOH/water

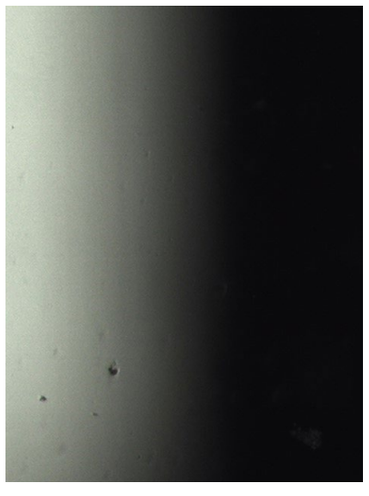
Monitoring anti-solvent additions with Crystalline

Figure – "Crystalline" monitored crystallization of compound I.

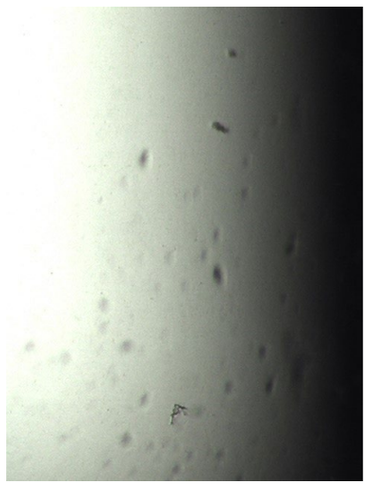
Reactor Temperature | **Transmittance**



1. +0.75 mL (50 %) (0h 9m)

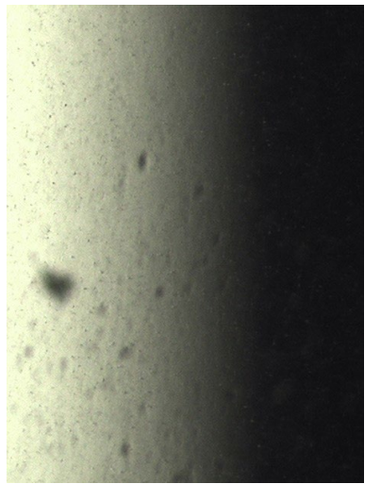


2. +0.075 mL (5%) (0h 15m)

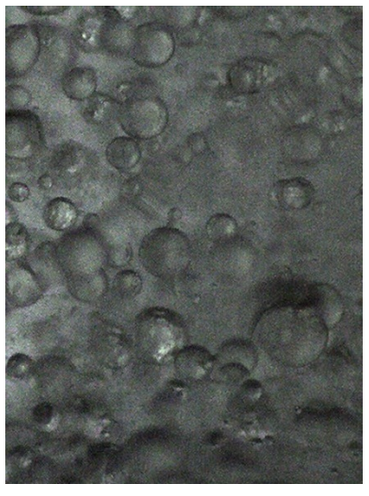


3. +0.113 mL (7.5%) (0h 33m)

4. +0.150 mL (10%) (0h 52m)



5. +0.188 mL (12.5%) (1h 04m)



6. +0.225 mL (15%) (1h 53m)

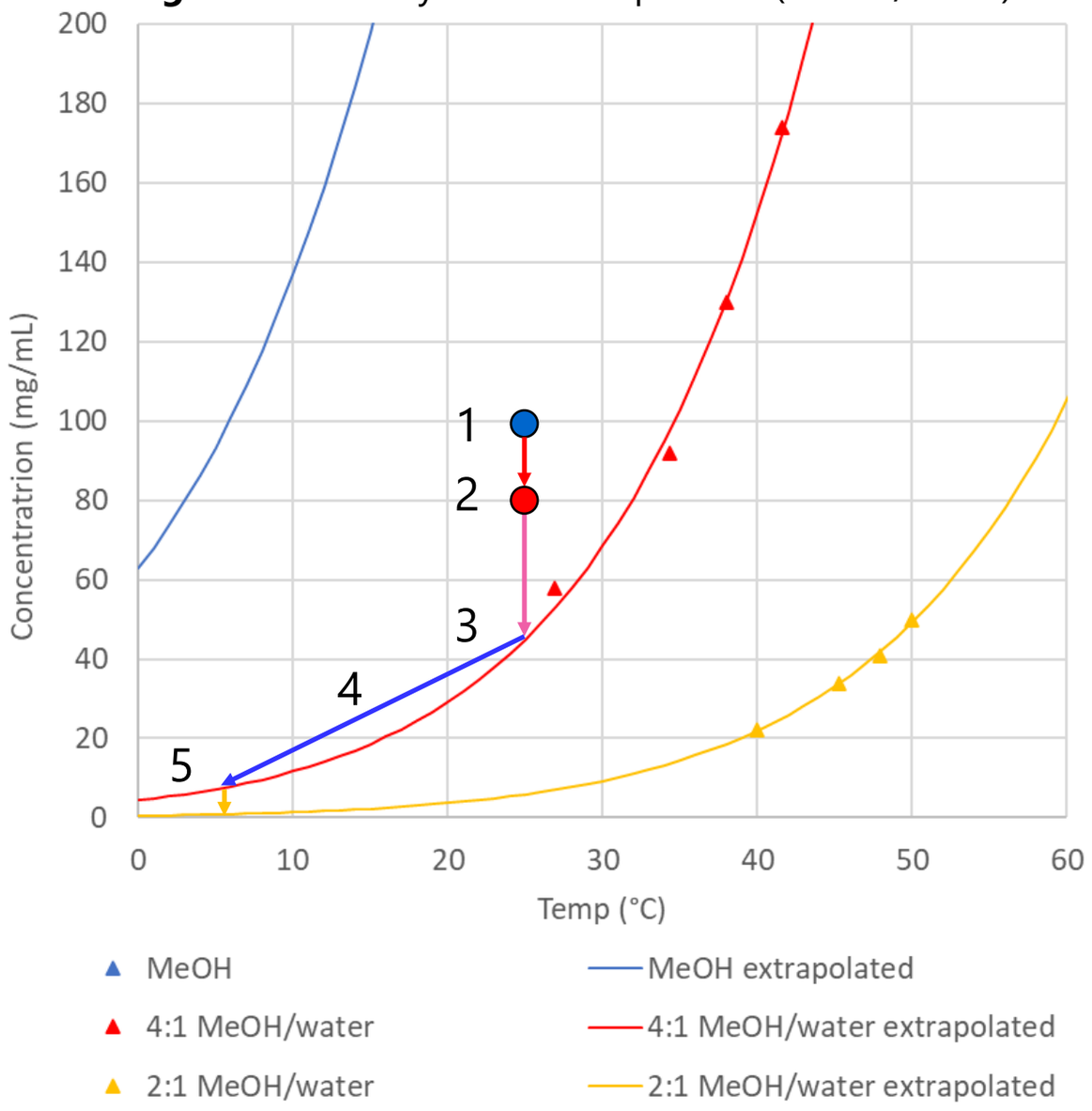
4:1 MeOH/water

2:1 MeOH/water

Case Study I: Part 2 MeOH/water

- Input:
  1. Organic fraction with 5g of product dissolved in 50 mL MeOH.
- The first plan:
  2. Add water to obtain a metastable 4:1 MeOH/water mixture.
  3. Seed at 25 °C
  4. Cool slowly to 5 °C (0.5 °C/min)
  5. Add water to obtain a 2:1 MeOH/water mixture.

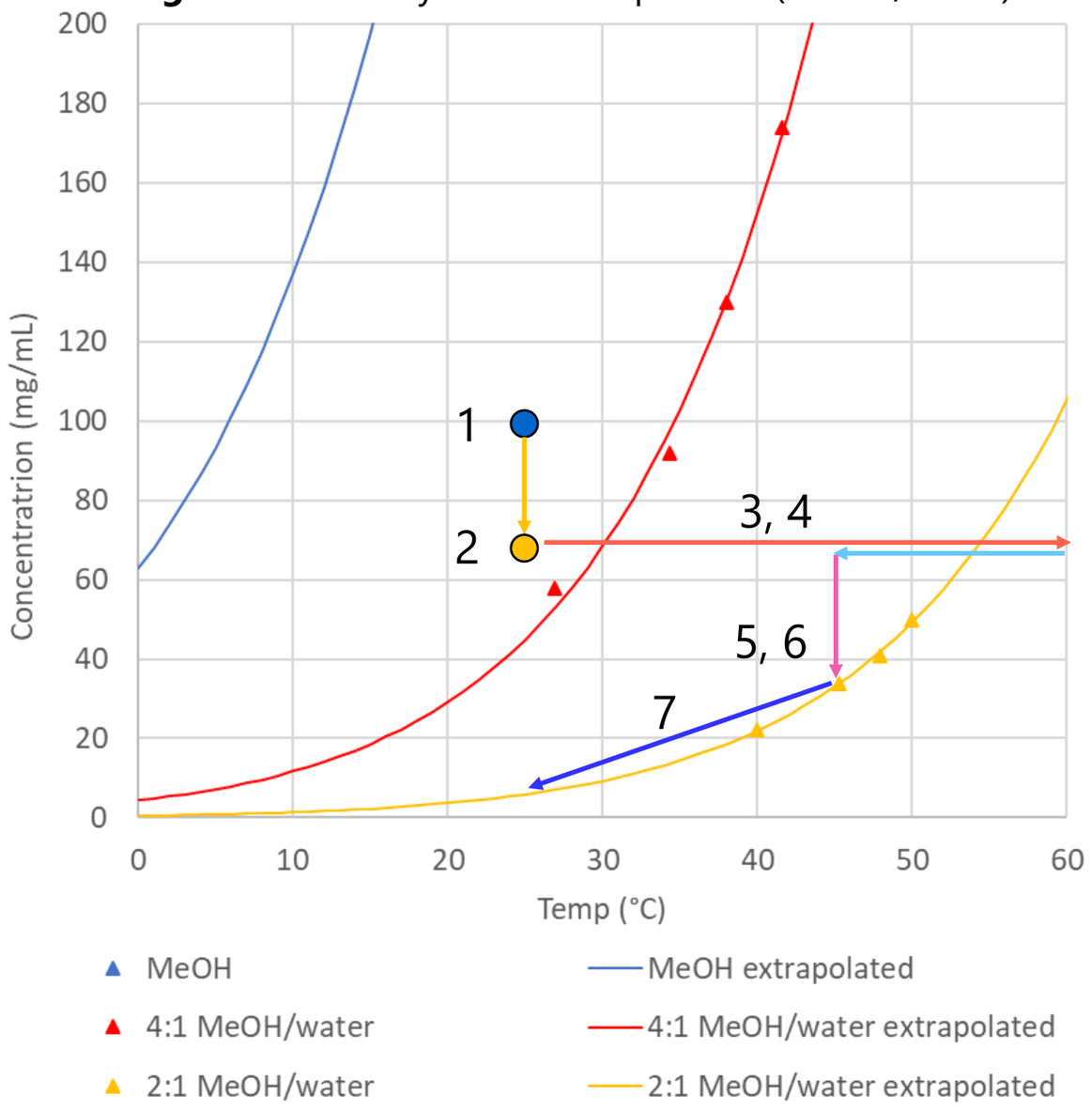
Figure – Solubility curves compound I (MeOH/water).



Case Study I: Part 2 MeOH/water

- Input:
  1. Organic fraction with 5g of product dissolved in 50 mL MeOH.
- The second plan:
  2. Add water to obtain an oiled out 2:1 MeOH/water mixture.
  3. Heat until complete dissolution (60 °C)
  4. Cool slowly to 45 °C to obtain a metastable solution.
  5. Seed at 45 °C.
  6. Wait for product to desaturate from solution. Perform crystal growth at warm temperature.
  7. Cool slowly to 25 °C (0.5 °C/min)

Figure – Solubility curves compound I (MeOH/water).

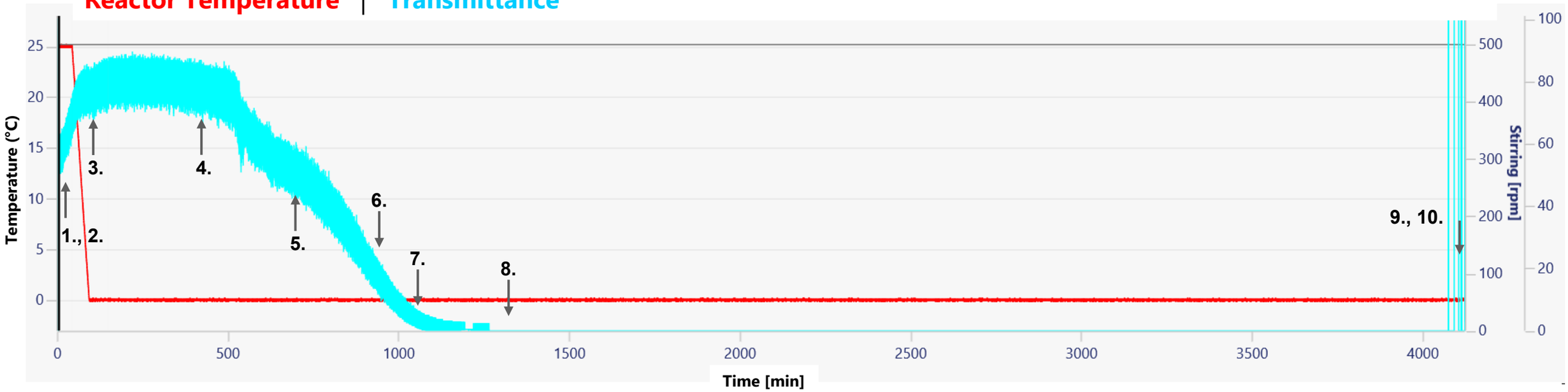


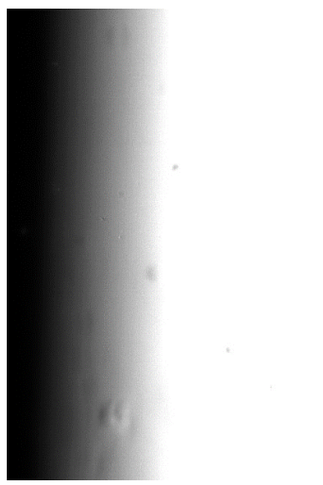
Case Study I: Part 2 MeOH/water

Monitoring seeded 4:1 MeOH/water with Crystalline

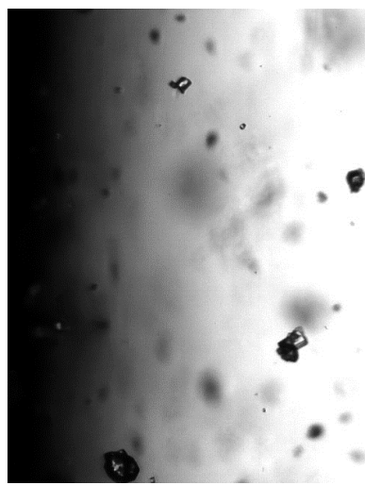
Figure – "Crystalline" monitored crystallization of compound I.

Reactor Temperature | **Transmittance**

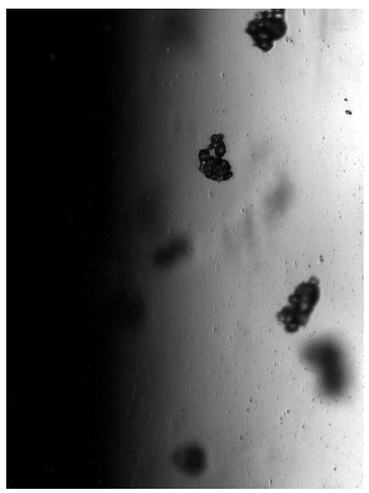




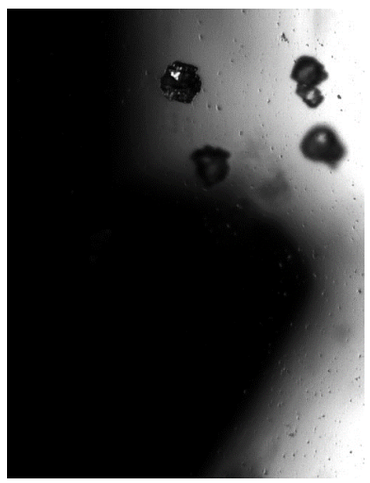
1. 4:1 MeOH/water 25 °C (0h 3m)



2. Seeding 25 °C (0h 5m)

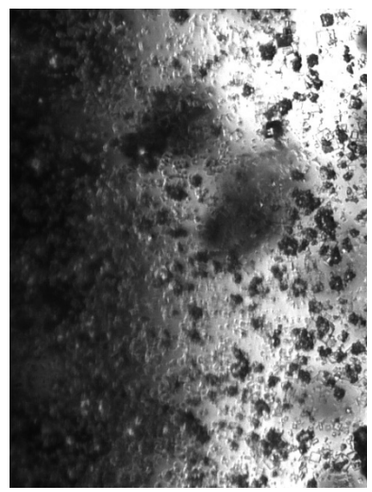


3. Cooled to 0 °C (1h 36m)



4. Crystal growth 0 °C (6h 59m)

5. Crystal growth 0 °C (11h 25m)



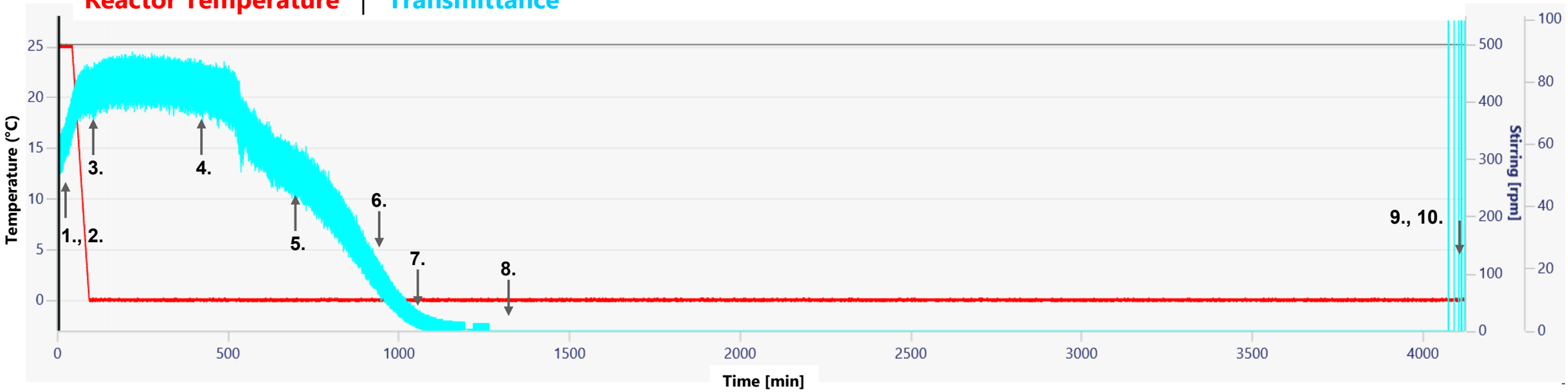
6. Crystal growth 0 °C (15h 25m)

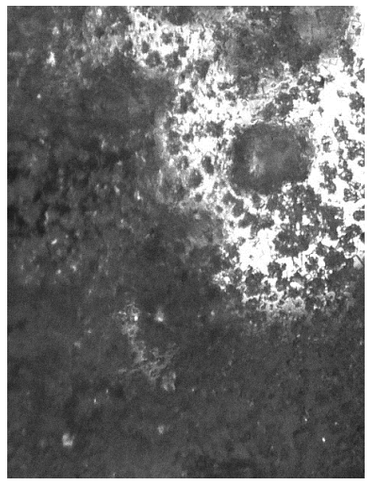
Case Study I: Part 2 MeOH/water

Monitoring seeded 4:1 MeOH/water with Crystalline

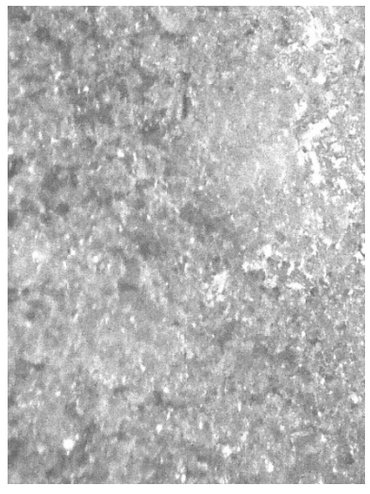
Figure – "Crystalline" monitored crystallization of compound I.

Reactor Temperature | **Transmittance**

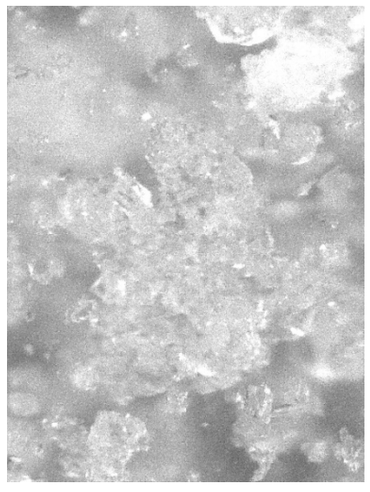




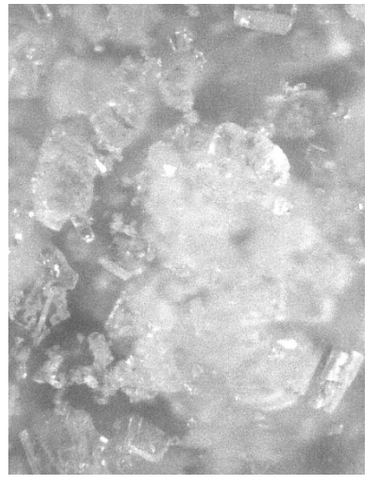
7. Crystal growth 0 °C (17h 18m)



8. Crystal growth 0 °C (21h 40m)



9. Crystal growth 0 °C (68h 27m)



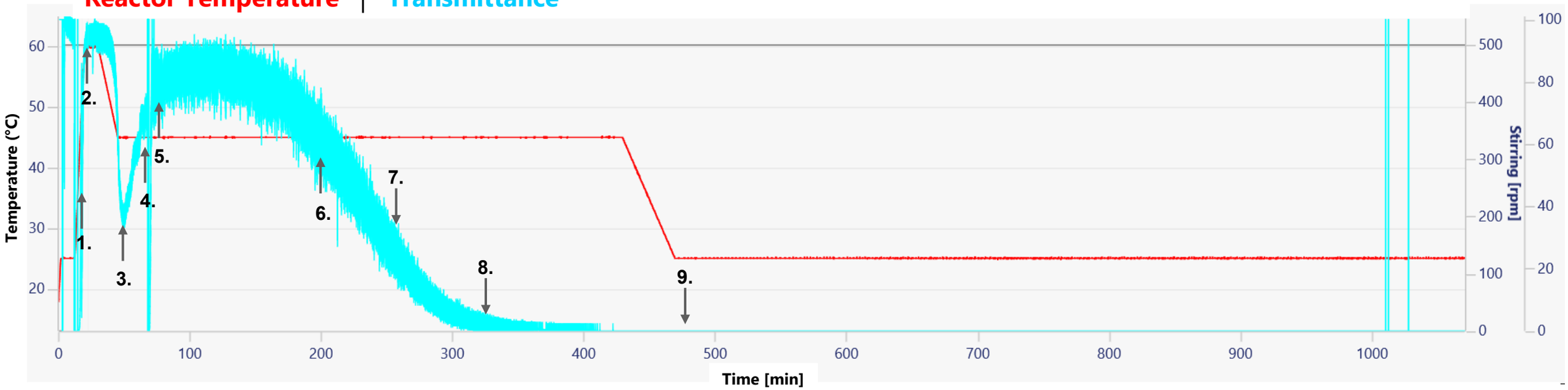
10. Addition of water (68h 33m)

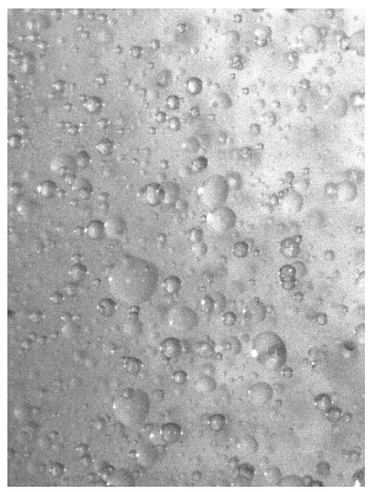
Case Study I: Part 2 MeOH/water

Monitoring seeded 2:1 MeOH/water with Crystalline

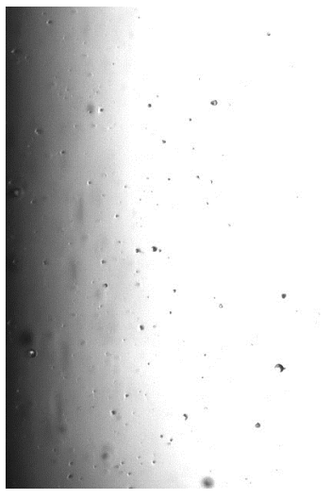
Figure – "Crystalline" monitored crystallization of compound I.

Reactor Temperature | **Transmittance**





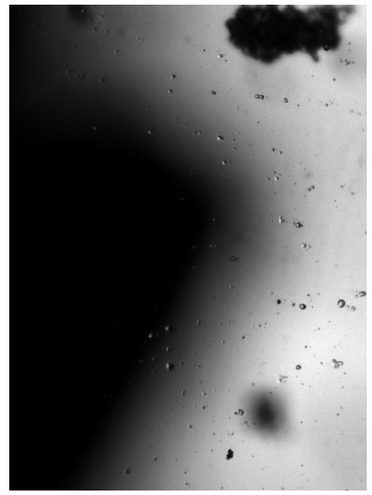
1. Warming to 60 °C (0h 15m)



2. At 60 °C (0h 20m)

3. Partial oiling out (0h 50m)

4. Reheat to 45 °C (1h 11m)



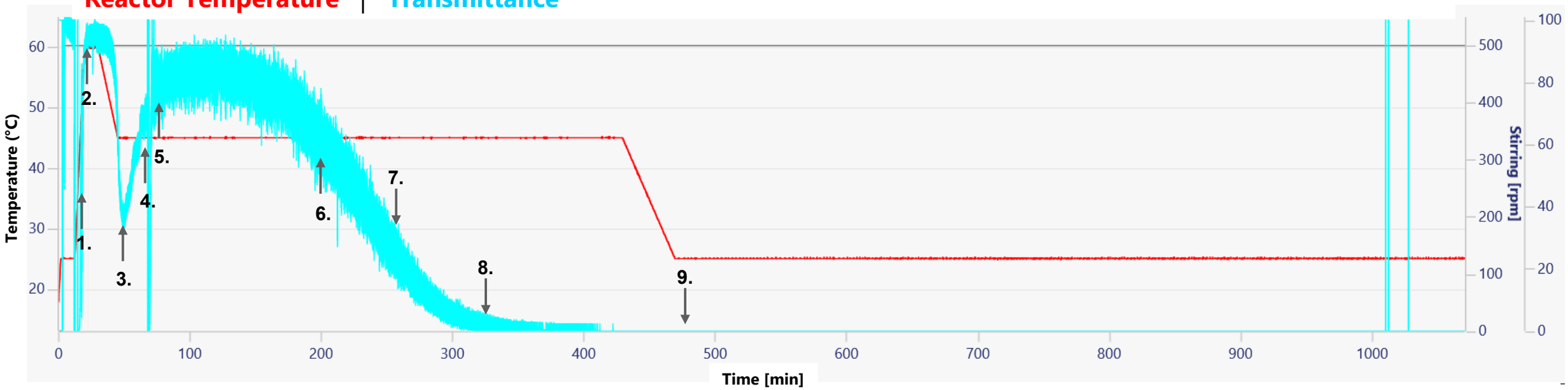
5. Seeding 45 °C (1h 16m)

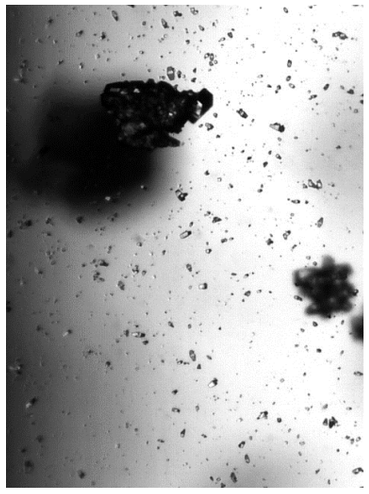
Case Study I: Part 2 MeOH/water

Monitoring seeded 2:1 MeOH/water with Crystalline

Figure – "Crystalline" monitored crystallization of compound I.

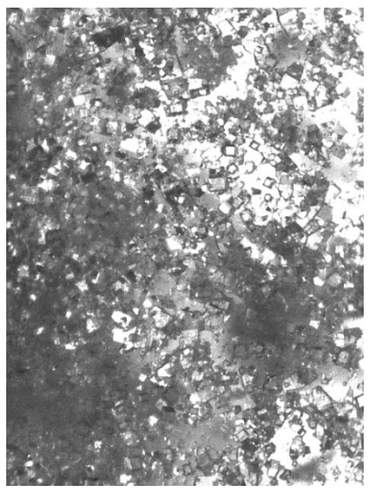
Reactor Temperature | **Transmittance**





6. Crystal growth 45 °C (3h 20m)

7. Crystal growth 45 °C (4h 16m)

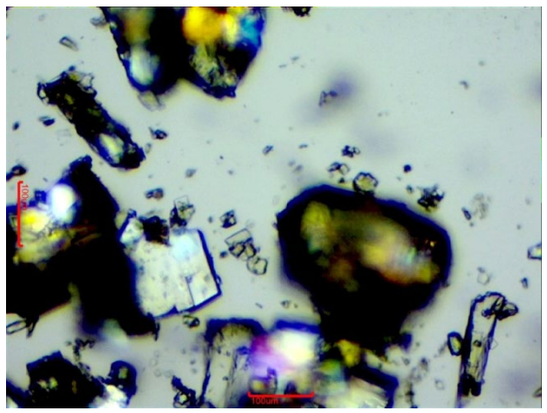


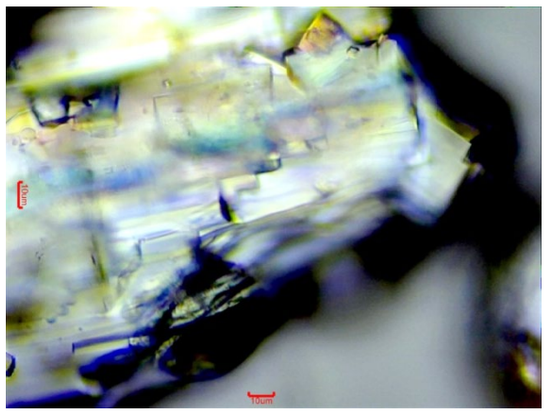
8. Crystal growth 45 °C (5h 24m)

9. At 25 °C (7h 56m)

Case Study I

Comparison of 4:1 and 2:1 MeOH/water crystallizations

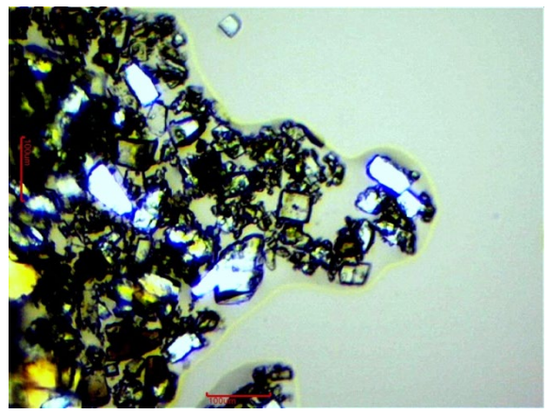


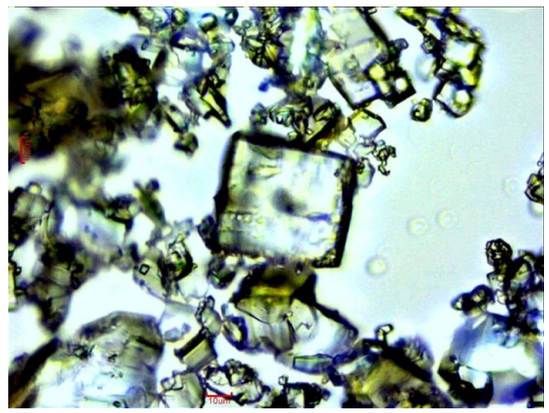


4:1 MeOH/water isolated product

PLM images

10x and 40x magnification

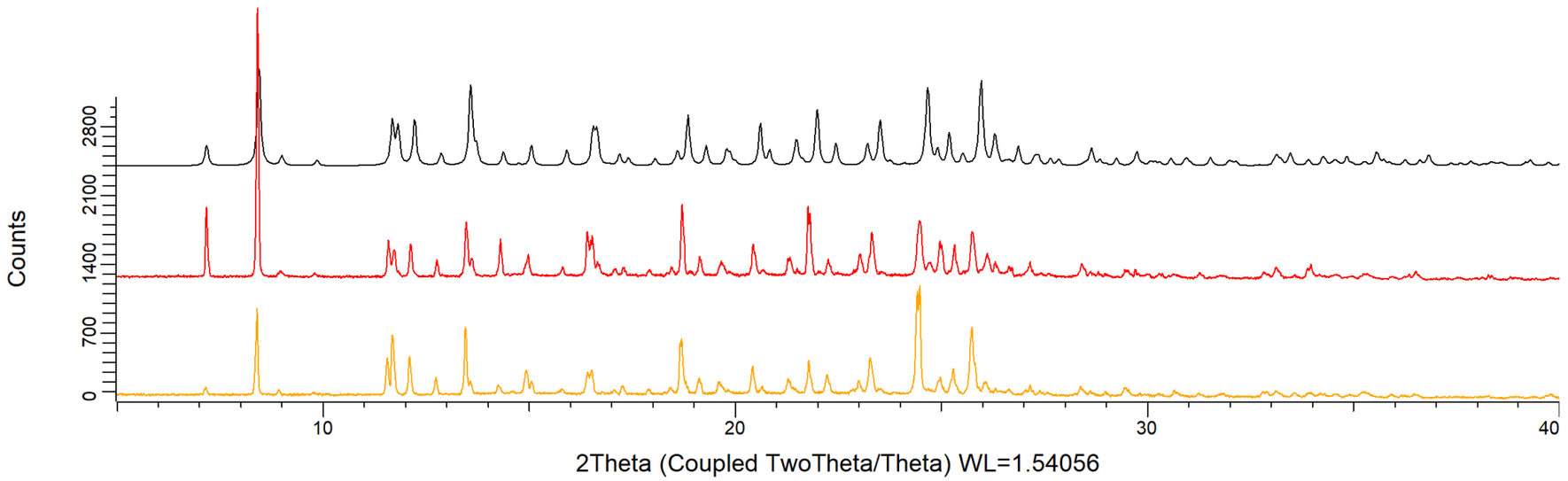




2:1 MeOH/water isolated product

PLM images

10x and 40x magnification



4:1 MeOH/water
UPLC: 100.0% product
Isolated yield: 77.7%

2:1 MeOH/water
UPLC: 100.0% product
Isolated yield: 80.9%

Figure – PXRD patterns of compound I.
Simulated from SCXRD | **4:1 MeOH/water recrystallization** | **2:1 MeOH/water recrystallization**

Case Study I
Conclusions

- MIBK/Heptane: Much higher quality crystals of compound I were grown using the higher temperature crystallization,

- 100 kg scale synthesis of compound I was performed. Using the MIBK/Heptane crystallization protocol developed at Eurofins Alphora resulted in a yield of 85%.

- MeOH/water: In-situ monitoring using Crystalline revealed the significant oiling risk of Compound I from MeOH/water. The solubility curves from Crystal 16, coupled with in-situ monitoring using Crystalline, allowed us to rapidly develop two crystallizations procedures in which we could maneuver around this hazard.

- Optimization of MeOH/water isolation will be the focal point of the next campaign.

- In-house we performed a 100g scale reaction using the 2:1 MeOH/water isolation, obtaining a yield of 80%.

Case Study II

- Two solid forms (Form A and B) of Compound II were discovered and structurally characterized during development.

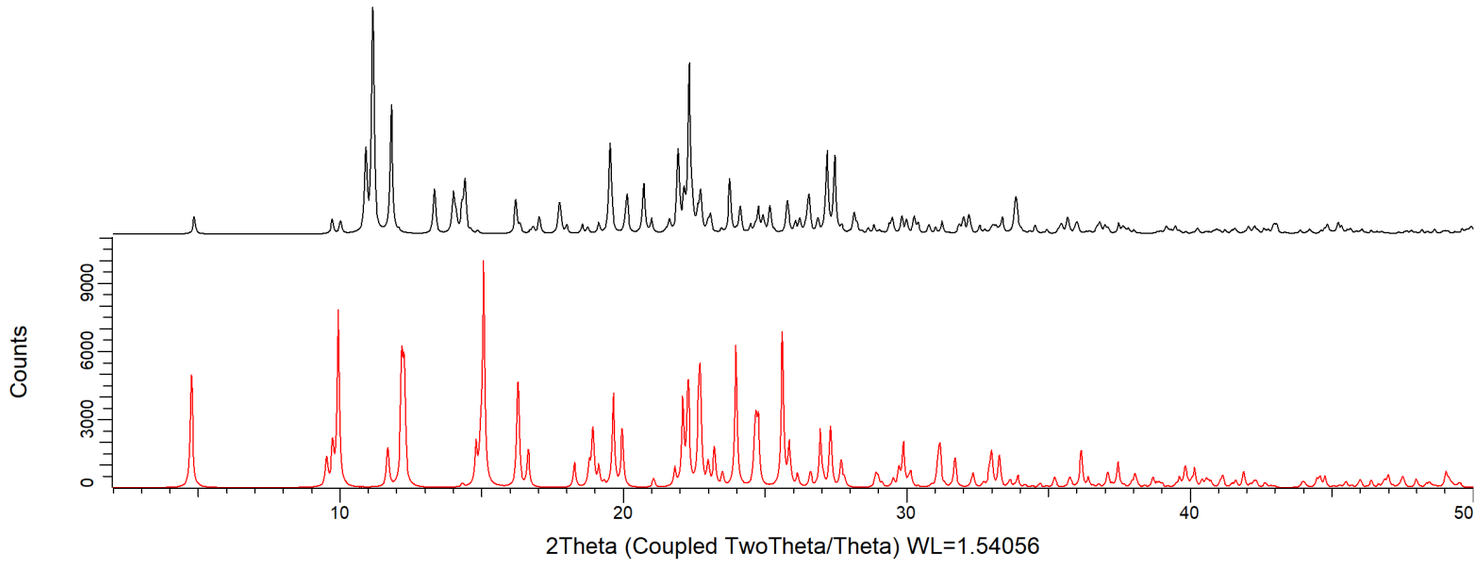


Figure – Simulated PXRD patterns of Compound II
Form A | **Form B**

- DSC reveals that Form B has slightly higher stability.

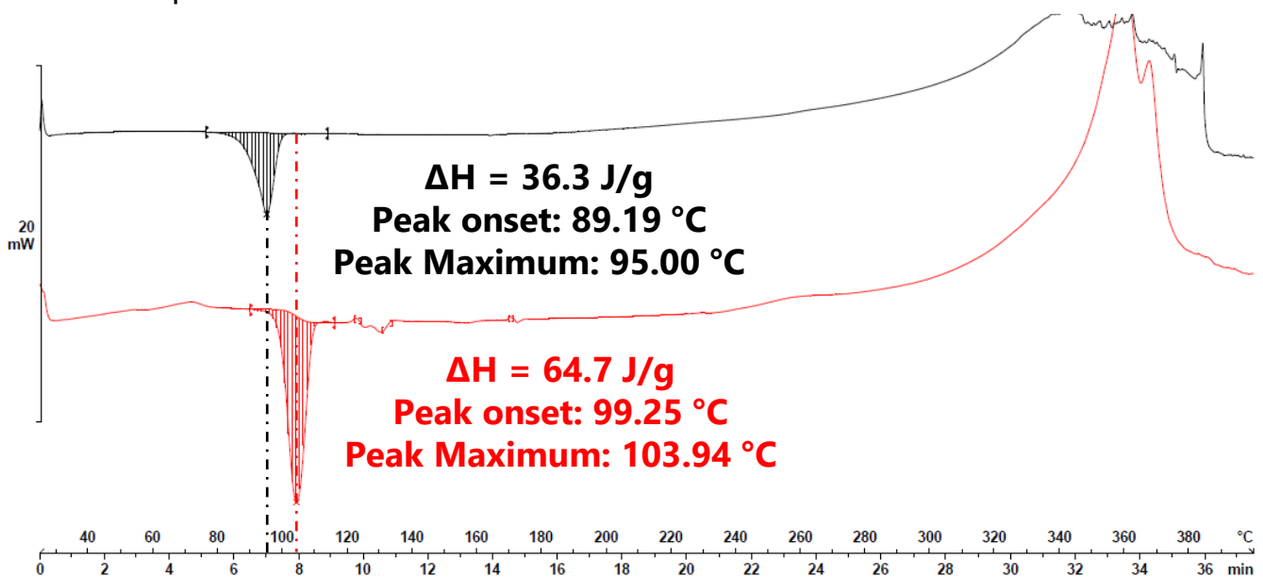
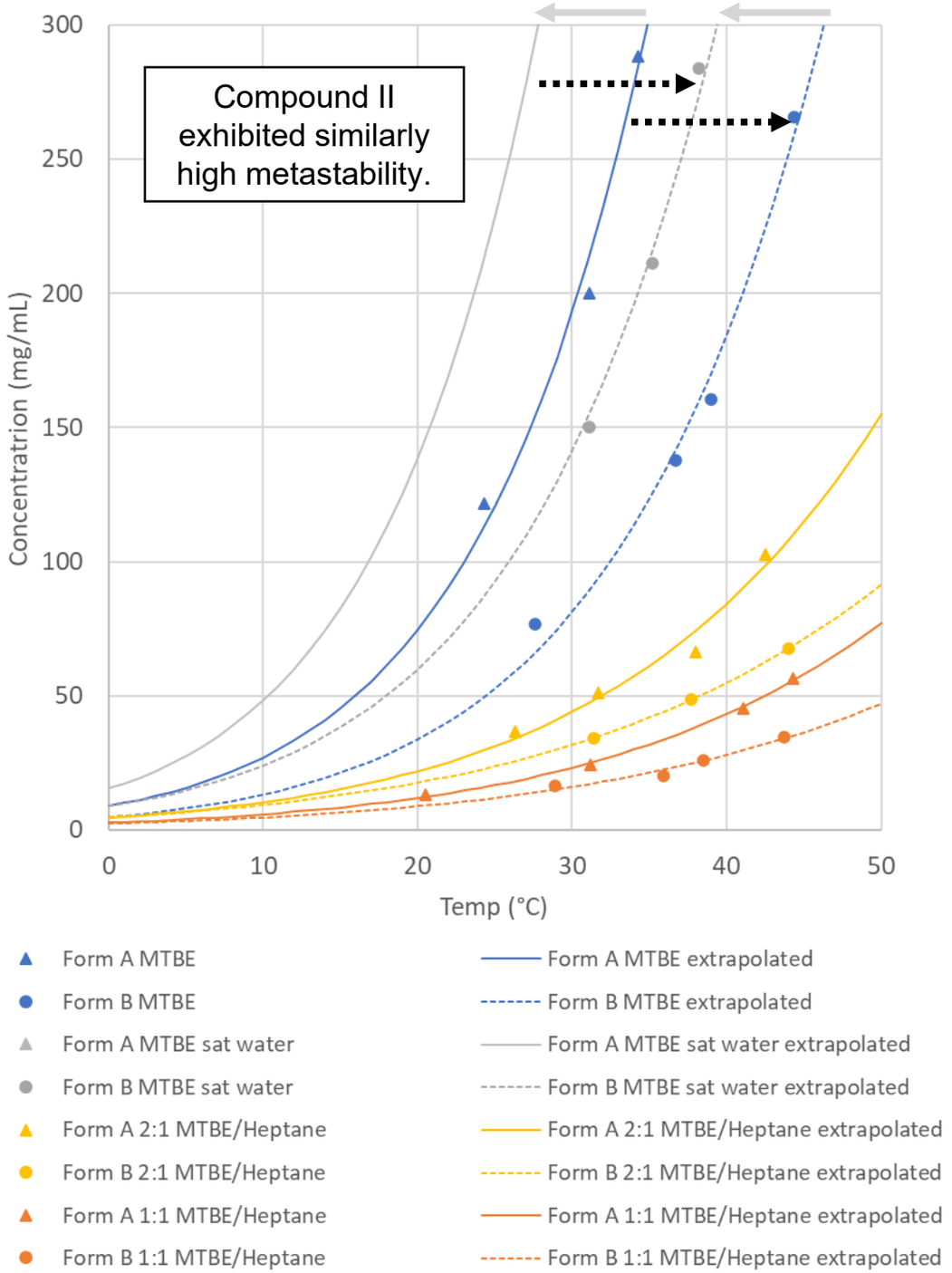


Figure – DSC thermograms of Compound II
Form A | **Form B**

Case Study II: MTBE/Heptane

- Solubility curves of crystalline Compound II Form A and B were collected using Crystal 16. Compounds heated from 20 to 70 °C at 0.5 °C/min, held for 15 minutes, cooled to -5 °C at 0.1 °C/min. Held for 12 hours.
- Trace water increases solubility.
- Form B is consistently less soluble.
- Isolation was attempted by seeding with different forms.

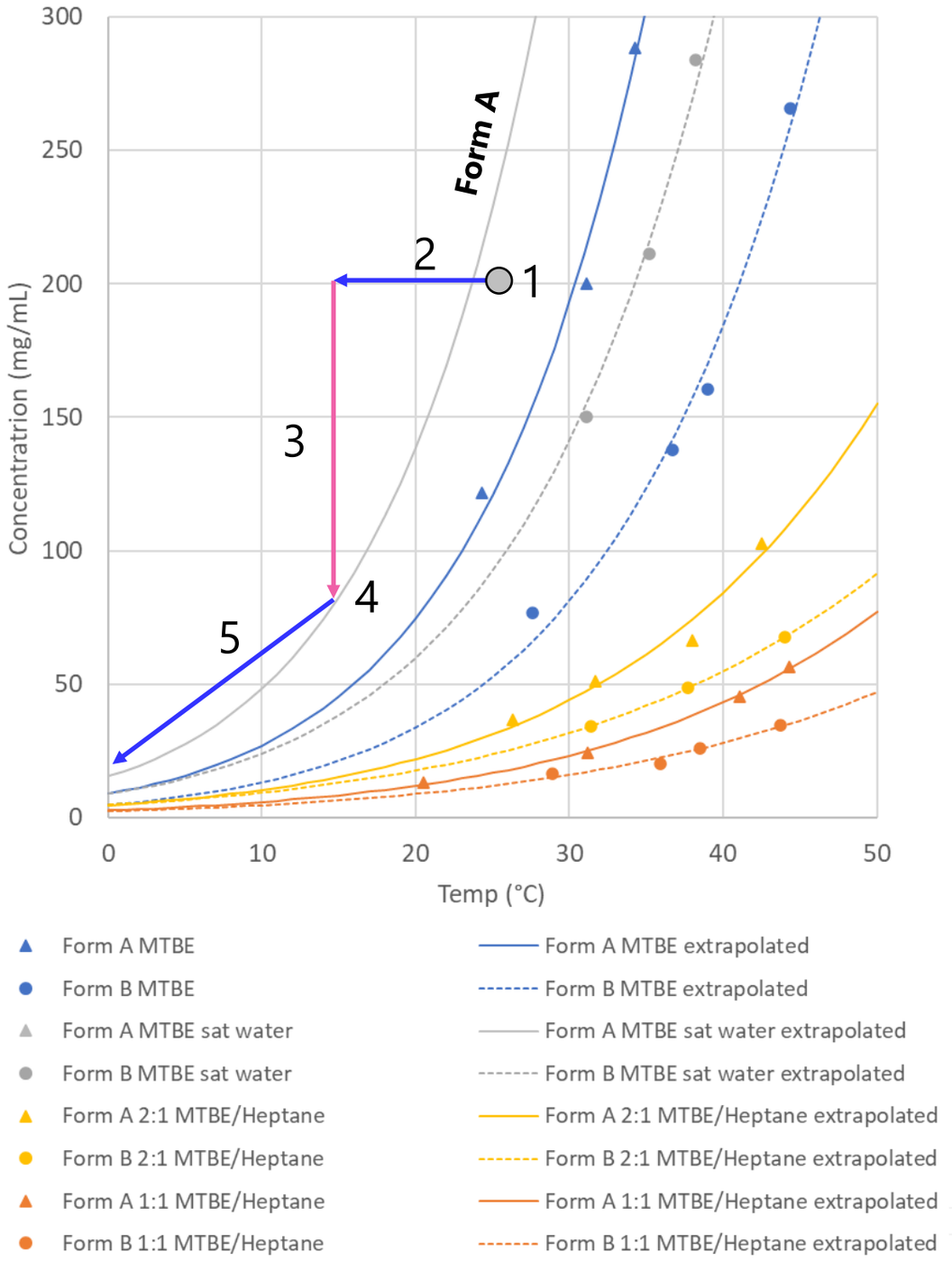
Figure – Solubility curves compound II (MTBE/heptane).



Case Study II: MTBE/Heptane
Seeding Form A

- Input:
  1. Organic fraction with 0.6 g of product dissolved in 3 mL MTBE saturated with water.
- Plan to seed form A:
  2. Cool to 15 °C to make a metastable solution.
  3. Seed at 15 °C.
  4. Grow crystals at 15 °C.
  5. Cool slowly to 0 °C (0.5 °C/min)

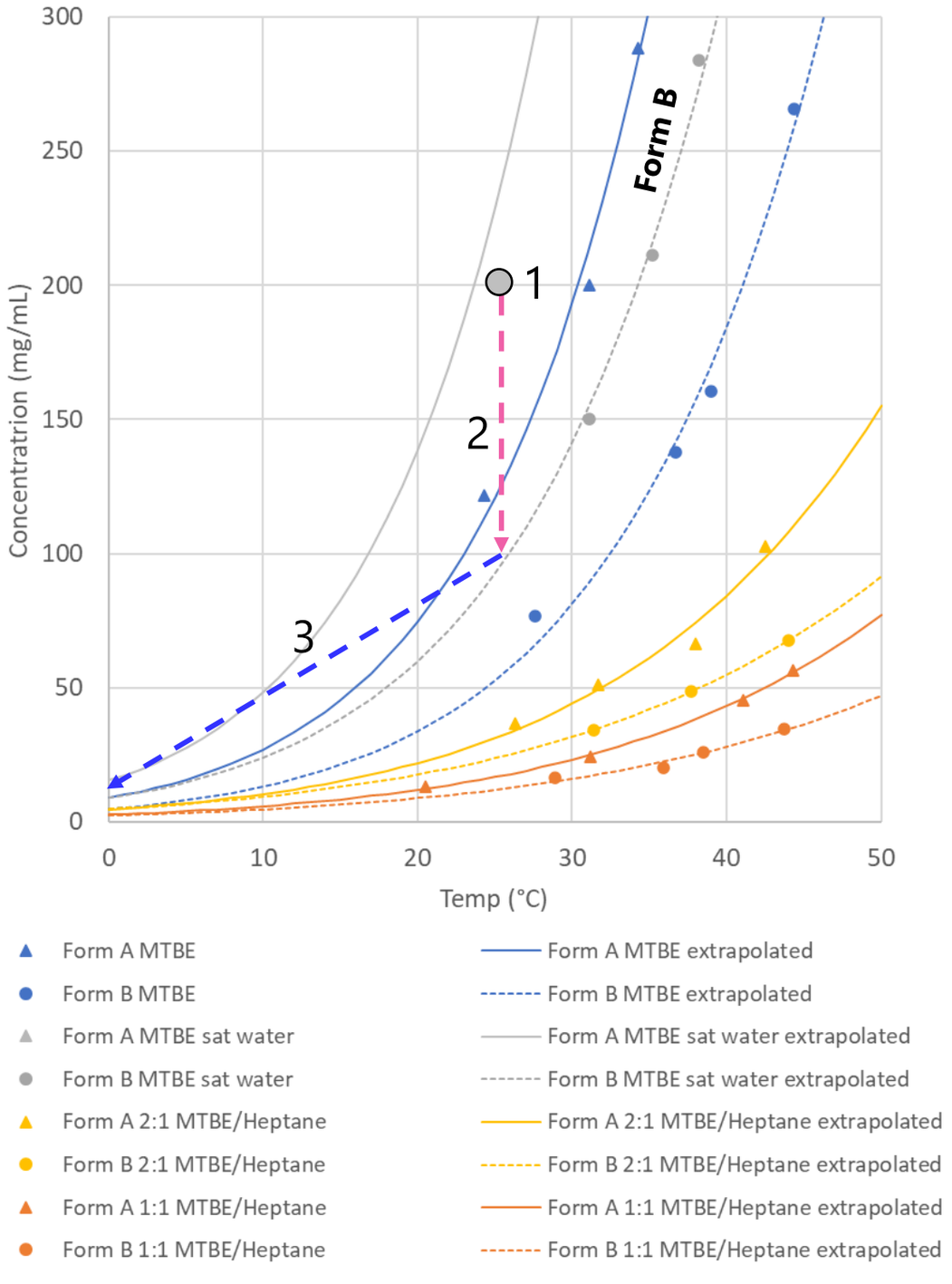
Figure – Solubility curves compound II (MTBE/heptane).



Case Study II: MTBE/Heptane
Seeding Form B

- Input:
  1. Organic fraction with 0.6 g of product dissolved in 3 mL MTBE saturated with water.
- Plan to seed form B:
  2. Seed at 25 °C.
  3. Cool slowly to 0 °C (0.5 °C/min)

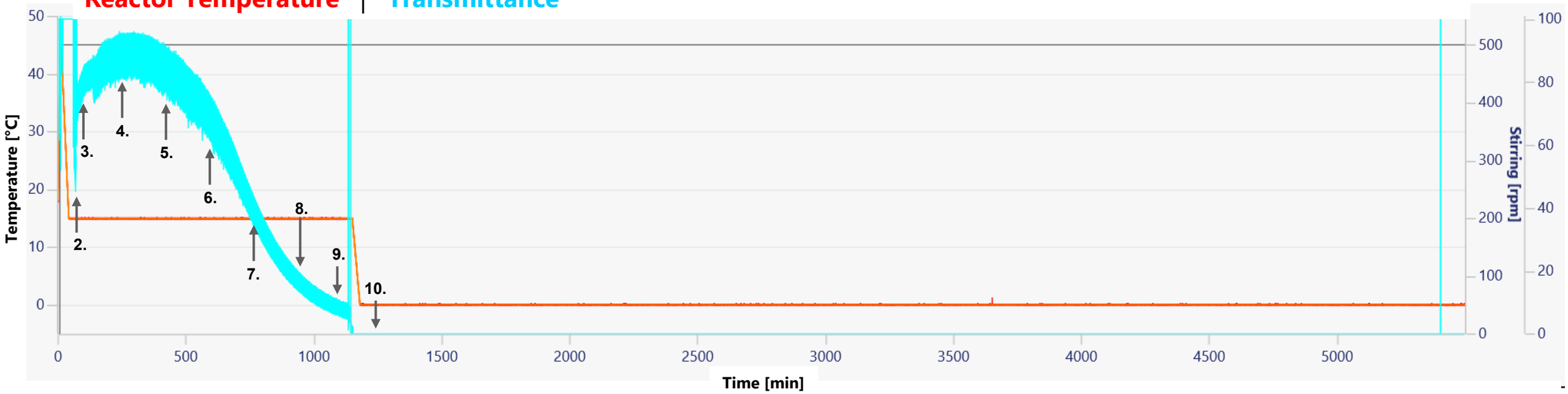
Figure – Solubility curves compound II (MTBE/heptane).



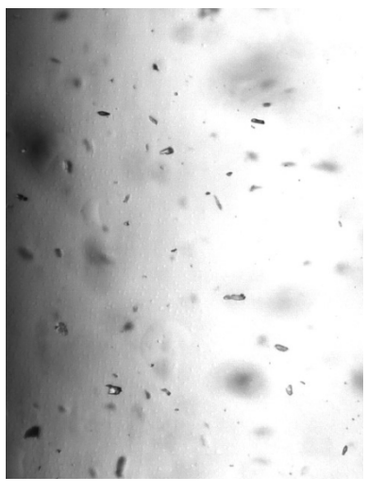
Case Study II: MTBE/Heptane
Monitoring Form A seeding with Crystalline

Figure – "Crystalline" monitored crystallization of compound II.

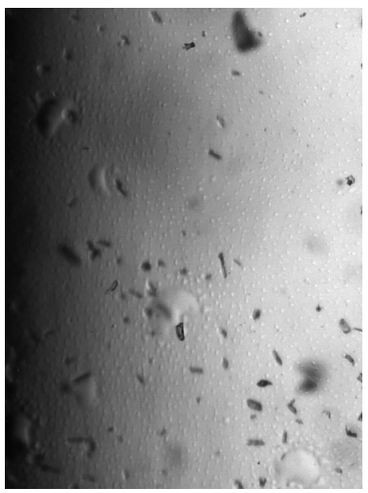
Reactor Temperature | **Transmittance**



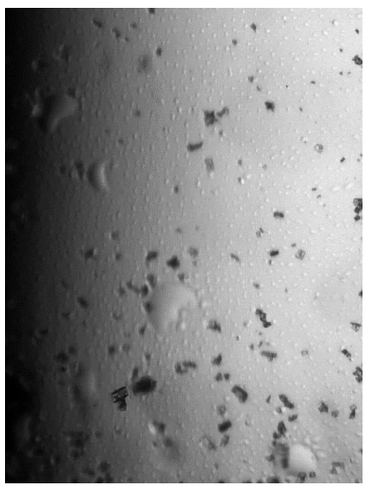
1. Pre-heating to 45 °C (0h 50m)



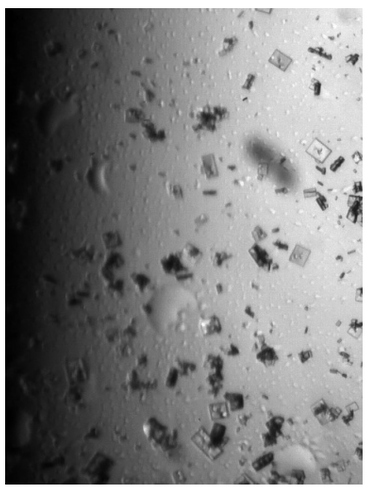
2. Seeding at 15 °C (1h 10m)



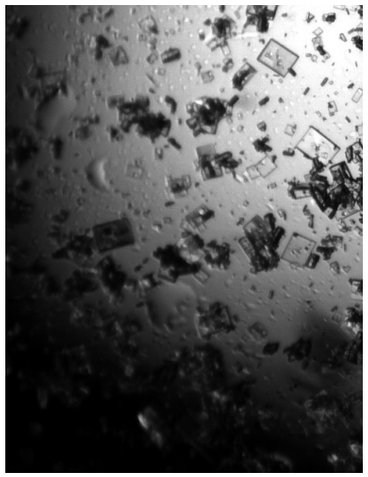
3. 1h after seeding (2h 10m)



4. 3h after seeding (4h 10m)



5. 6h after seeding (7h 10m)

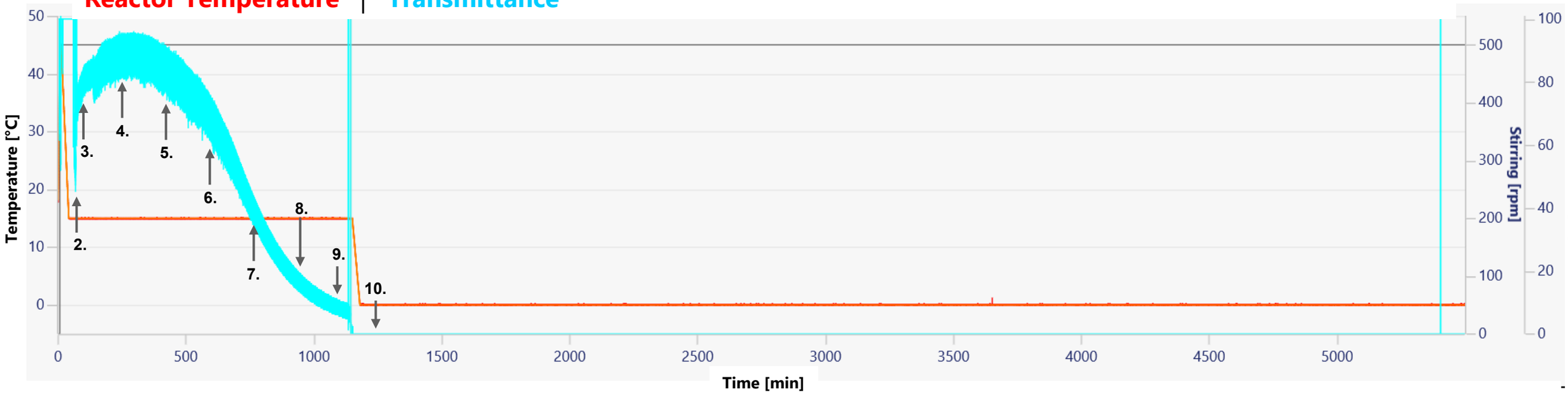


6. 9h after seeding (10h 10m)

Case Study II: MTBE/Heptane
Monitoring Form A seeding with Crystalline

Figure – "Crystalline" monitored crystallization of compound II.

Reactor Temperature | **Transmittance**



7. 12h after seeding (13h 10m)

8. 15h after seeding (16h 10m)

9. 17h after seeding just before cooling (18h 10m)

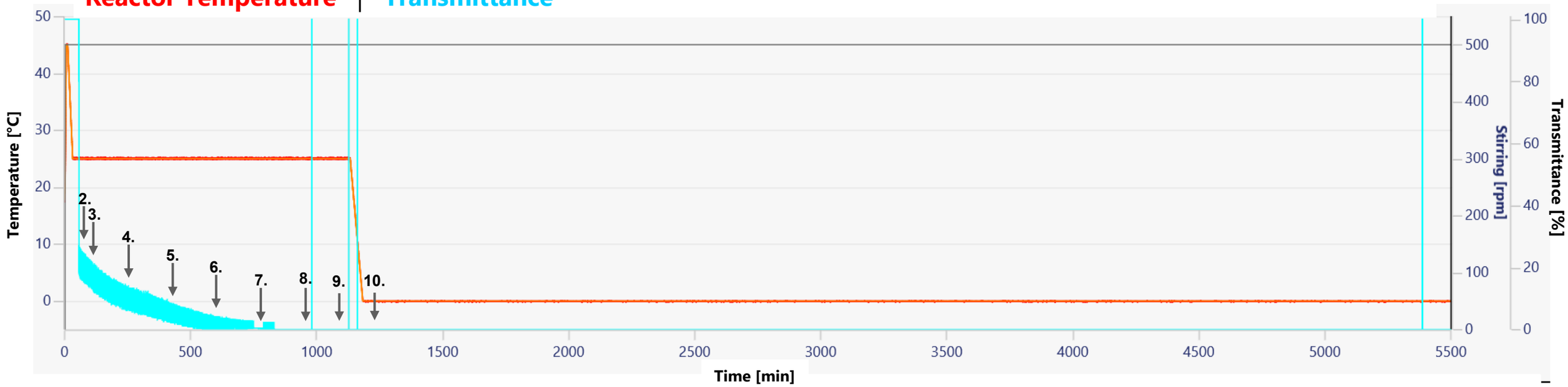
10. 18h after seeding just after cooling (19h 10m)

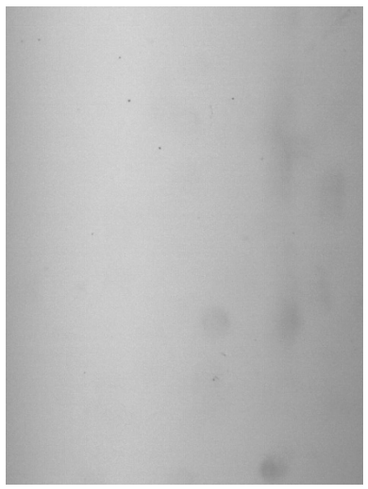
Case Study II: MTBE/Heptane

Monitoring Form B seeding with Crystalline

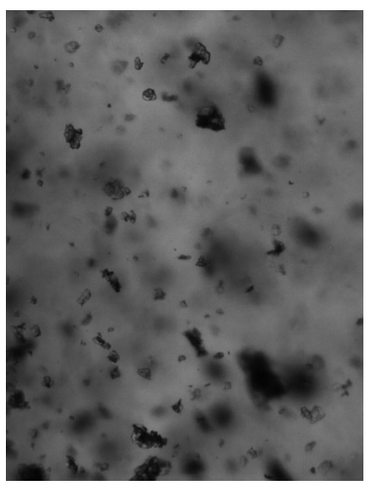
Figure – "Crystalline" monitored crystallization of compound II.

Reactor Temperature | **Transmittance**

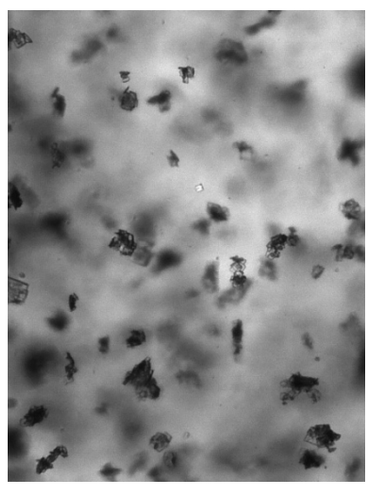




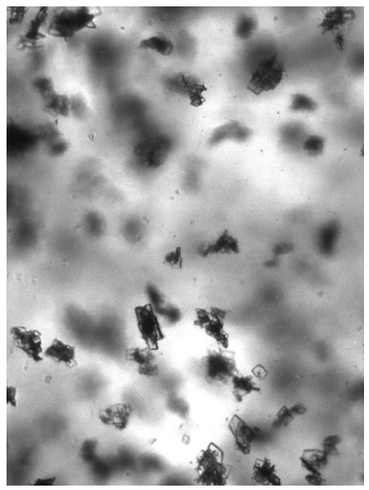
1. Pre-heated to 45 °C (0h 55m)



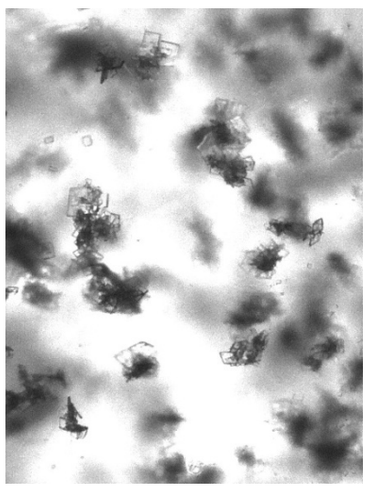
2. Seeding at 25 °C (0h 57m)



3. 1h after seeding (1h 57m)



4. 3h after seeding (3h 57m)



5. 6h after seeding (6h 57m)

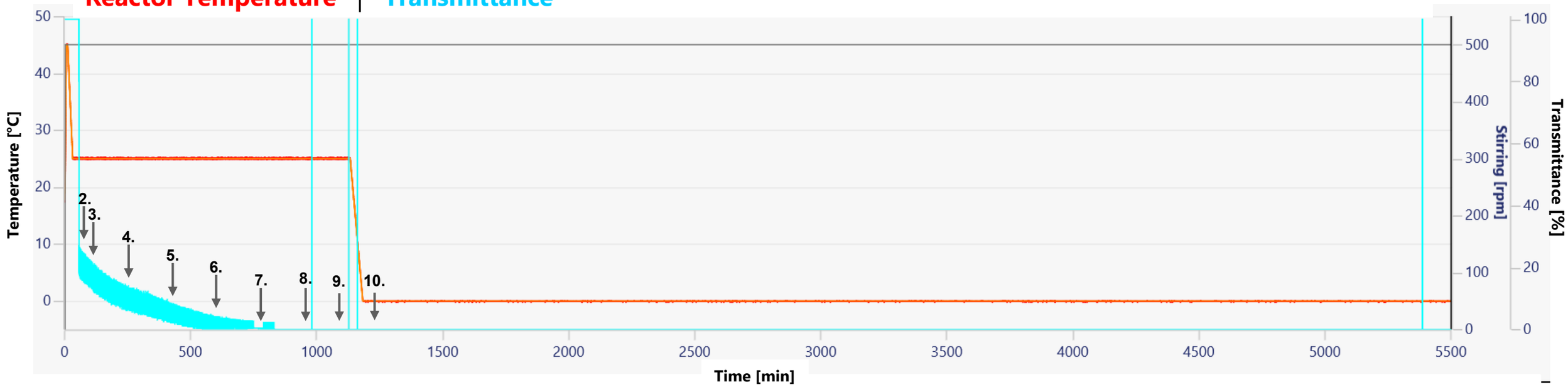
6. 9h after seeding (9h 57m)

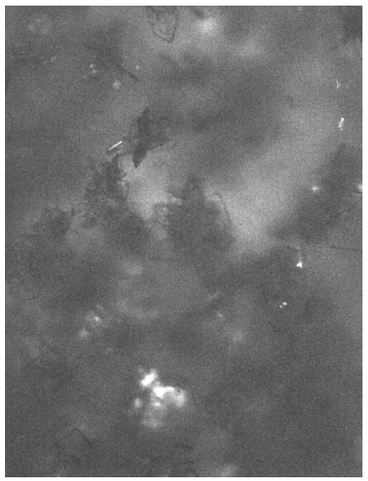
Case Study II: MTBE/Heptane

Monitoring Form B seeding with Crystalline

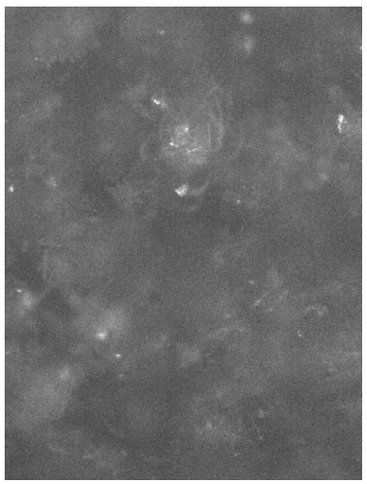
Figure – "Crystalline" monitored crystallization of compound II.

Reactor Temperature | **Transmittance**

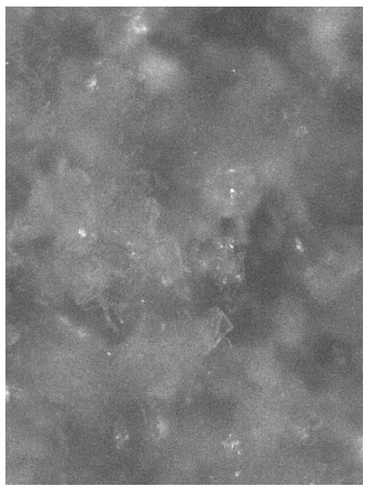




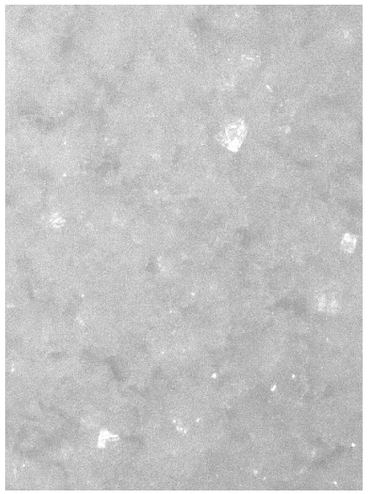
7. 12h after seeding (12h 57m)



8. 15h after seeding (15h 57m)

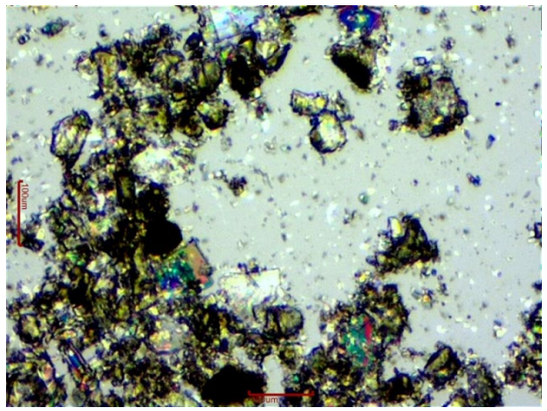


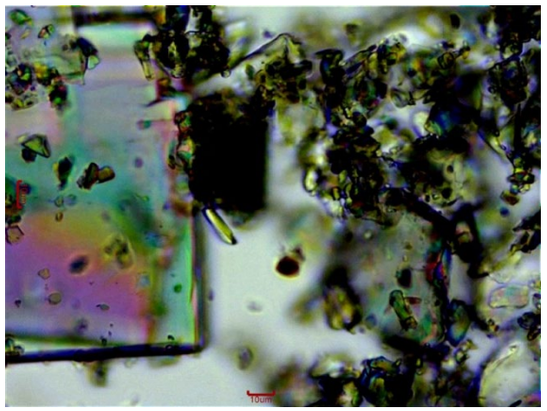
9. 17h after seeding (17h 57m)



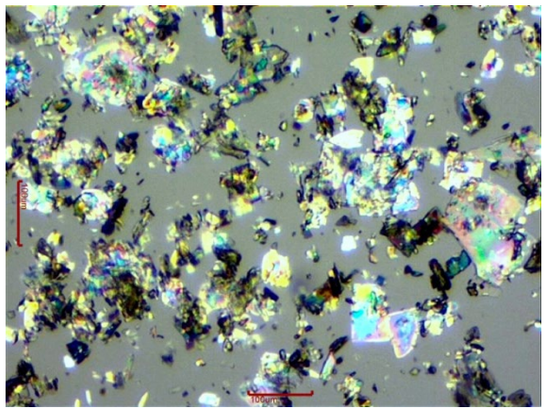
10. 19h after seeding (19h 57m)

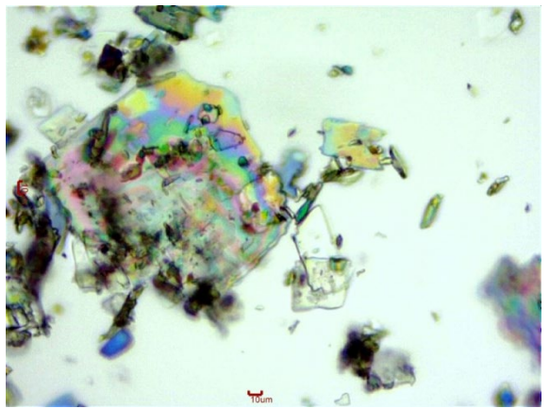
Case Study II
Comparison of Form A and B seeding



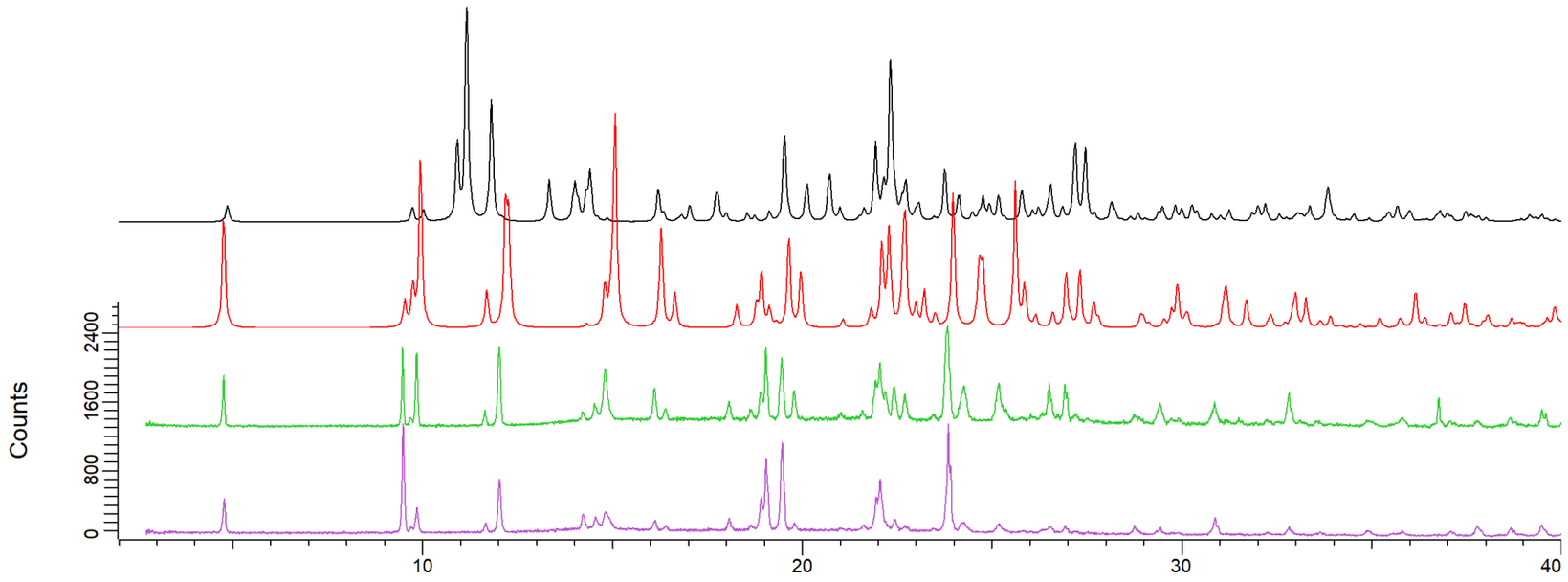


Form A seeded isolated product
PLM images
10x and 40x magnification





Form B seeded isolated product
PLM images
10x and 40x magnification



Form A seeded
UPLC: 99.83% product
Isolated yield: 83.2%
Isolated form: Form B

Form B seeded
UPLC: 99.74% product
Isolated yield: 97.0%
Isolated form: Form B

Figure – PXRD patterns of compound II.
Form A Simulated from SCXRD | **Form B Simulated from SCXRD** | **Form A seeded product** | **Form B seeded product**

Case Study II
Comparison of Form A and B seeding

Figure – "Crystalline" monitored crystallization of compound II (Form A seeding).
Reactor Temperature | **Transmittance**

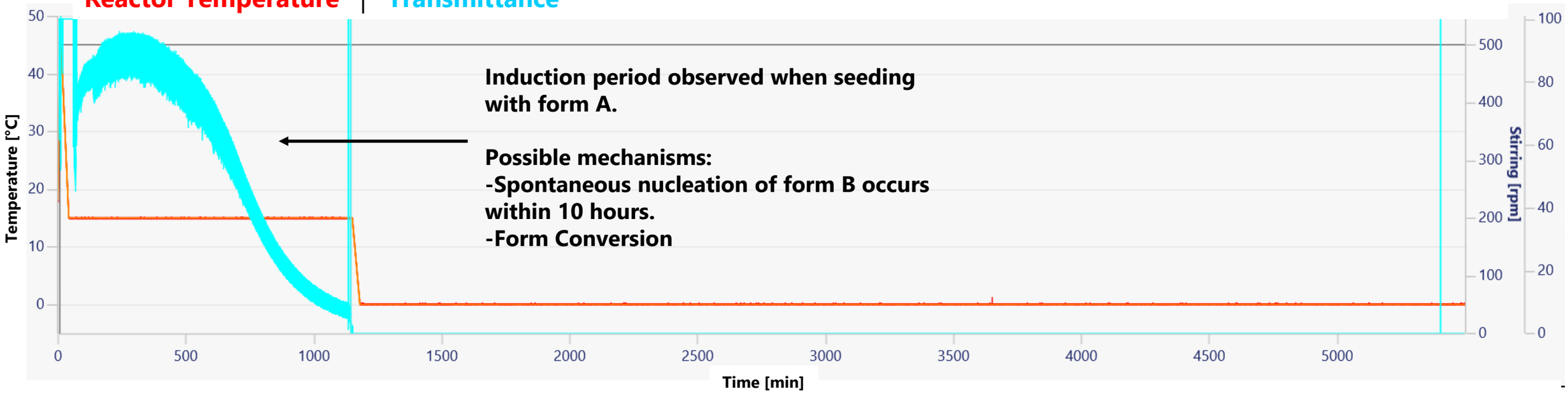
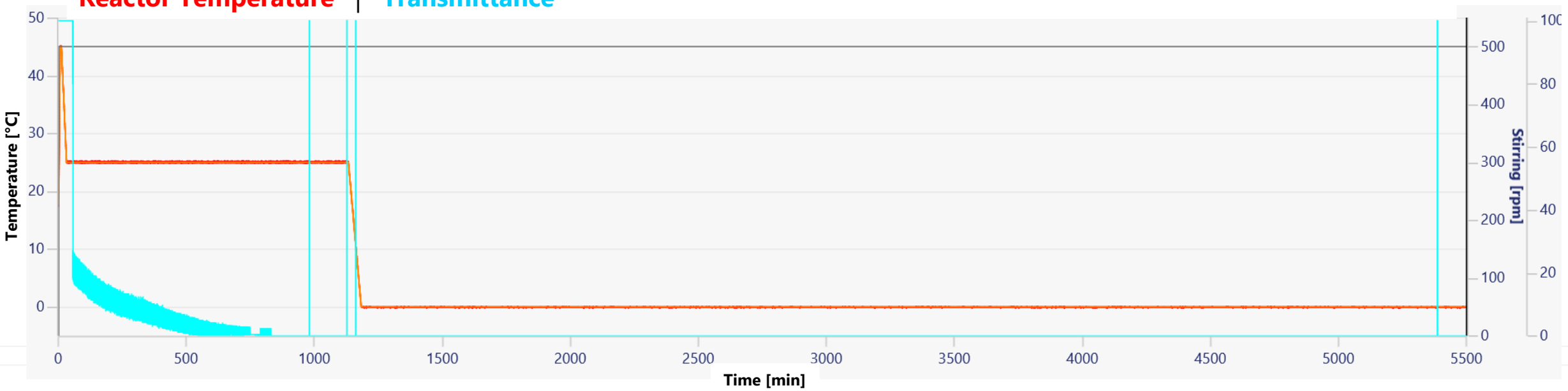
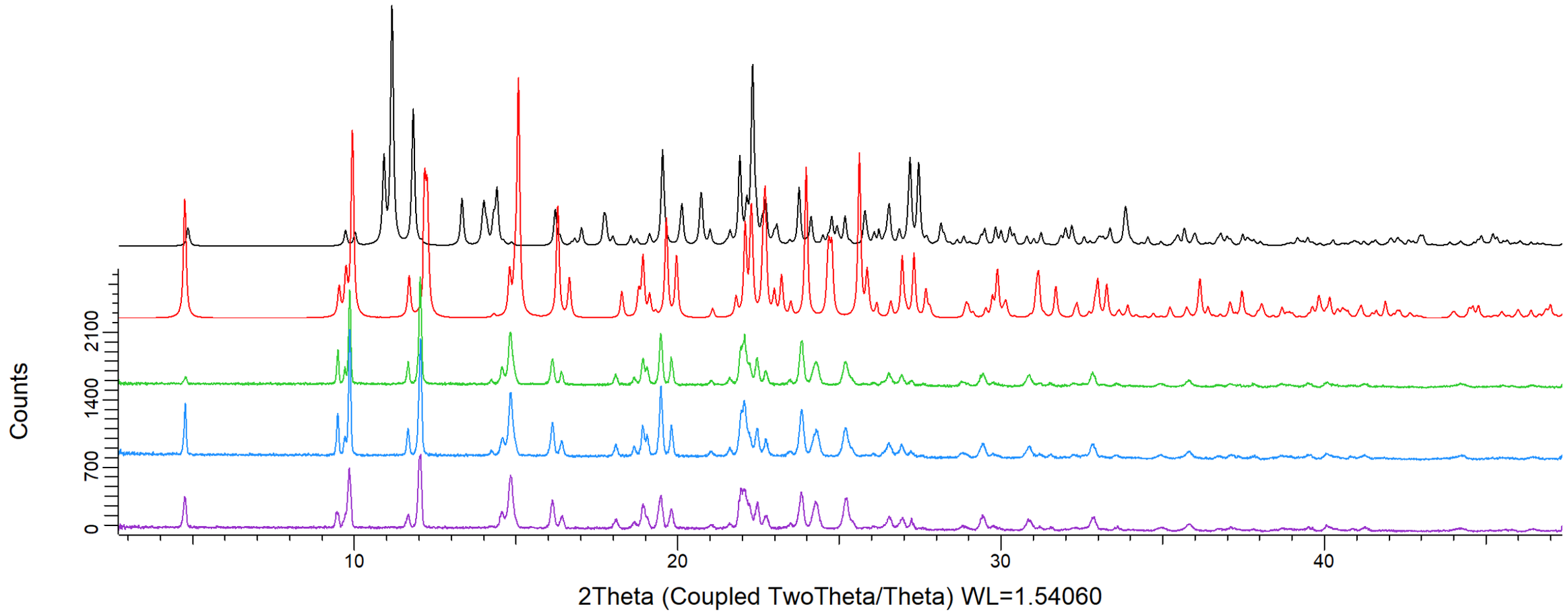


Figure – "Crystalline" monitored crystallization of compound II (Form B seeding).
Reactor Temperature | **Transmittance**



Case Study II
Competitive slurry Form A and B

Figure – PXRD of competitive slurry experiments.
Form A Simulated from SCXRD | **Form B Simulated from SCXRD** | **50: 50 Form A and B competitive slurry** | **10: 90 Form A and B competitive slurry** | **90: 10 Form A and B competitive slurry**



Mixture of solid Form A and B were stirred for 72 hours in MTBE saturated with water and Compound II

Case Study II
Conclusions

- Crystal 16 revealed varying solubilities of Form A and B with and without trace water present. This informs the resulting crystallization protocol.

- In-situ monitoring using Crystalline revealed the different rates of crystal growth upon seeding with either form A or B.

- Induction period for crystal growth when seeding with Form A is likely due to slow growth rate of form A crystals. Spontaneous nucleation of Form B coupled with form conversion to Form B was observed.

- Isolation of Form A from this solvent system may not be possible and is risky.

- Currently still trying to find the right solvent system for large-scale. Many other systems are being explored.

  IPrOAc/Heptane
  IPA/water
  MeCN/water
  etc.

Conclusions

- Solubility curves from Crystal 16 provide a backbone from which crystallization protocols can be developed.
- Crystalline provides detailed monitoring of the crystallization which helps elucidate the notable events in crystallization, enabling rapid optimization.
- Coupling Crystal 16, and Crystalline, with other inline and offline characterizations enabled rapid crystallization process development
- Much left to learn (particle size distribution, kinetic modelling, etc.)
- Looking forward to the workshop!

Crystal 16

Crystalline

eurofins | CDMO

Thank You

www.eurofins.com/cdmo

CONTACT US