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PHARMARON

From Blobs to Rods
Navigating Liquid-Liquid Phase Separation

Alice McNelly

Technobis Webinar

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What Is Liquid-Liquid Phase Separation?

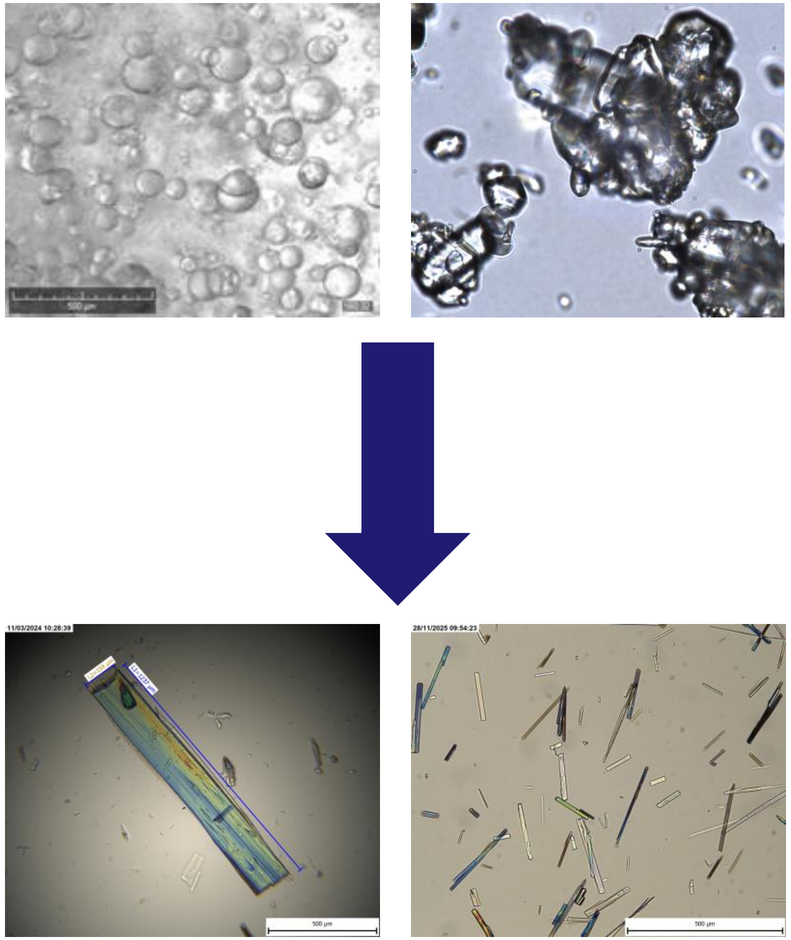
- How Can We Spot LLPS?
- Why Do We Care About Avoiding LLPS?

How Do We Avoid LLPS?

- How Do We Plot LLPS Diagrams?
- How Can LLPS Diagrams Help?

Case Studies

- Reducing Deposition
- Choosing Seeding Parameters
- Solvent Choice



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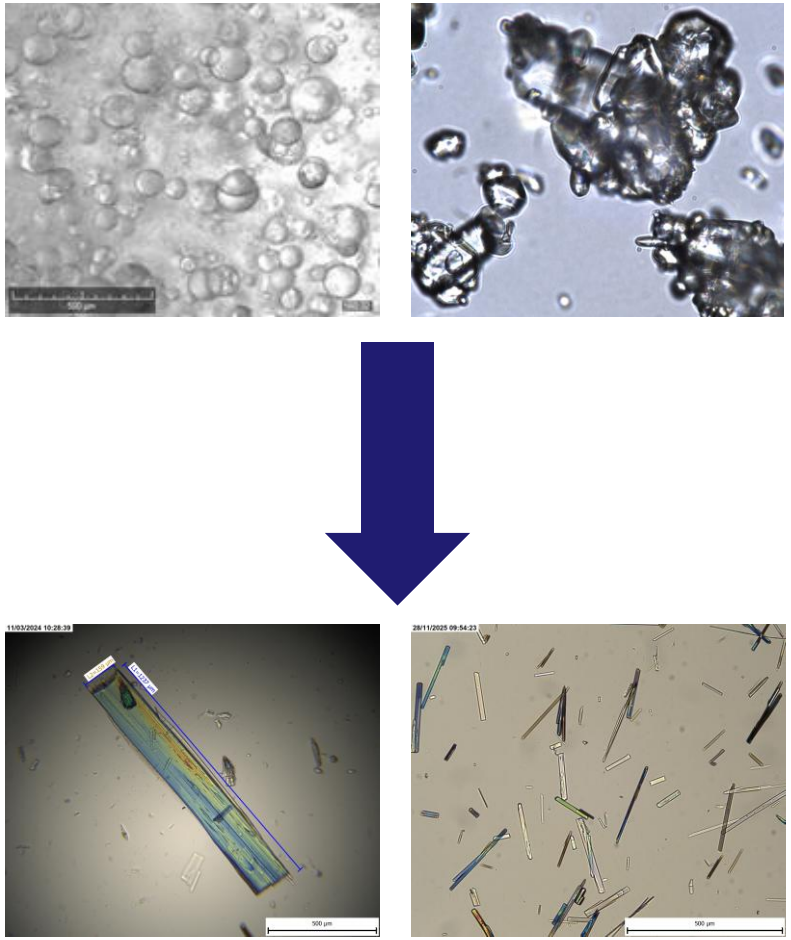

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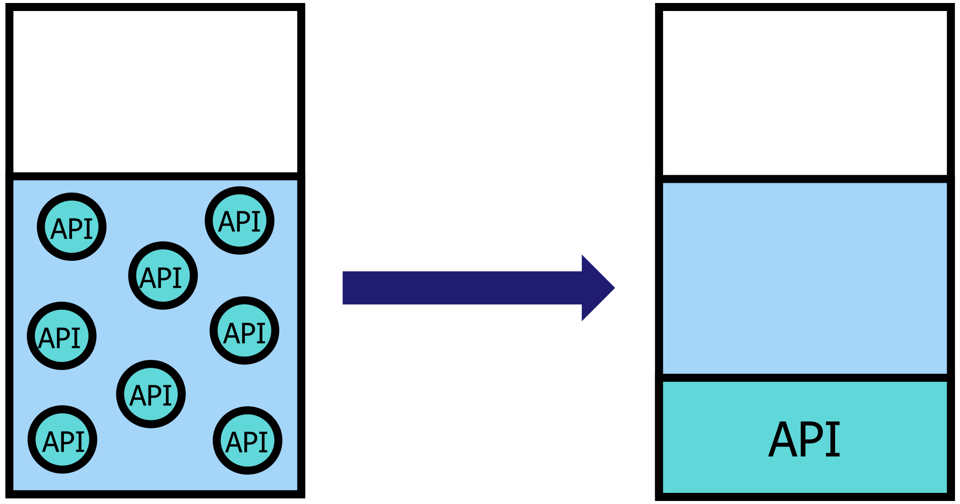
- These oil droplets are rich in solute (active pharmaceutical ingredient, API) and can separate into a distinct layer if left undisturbed.



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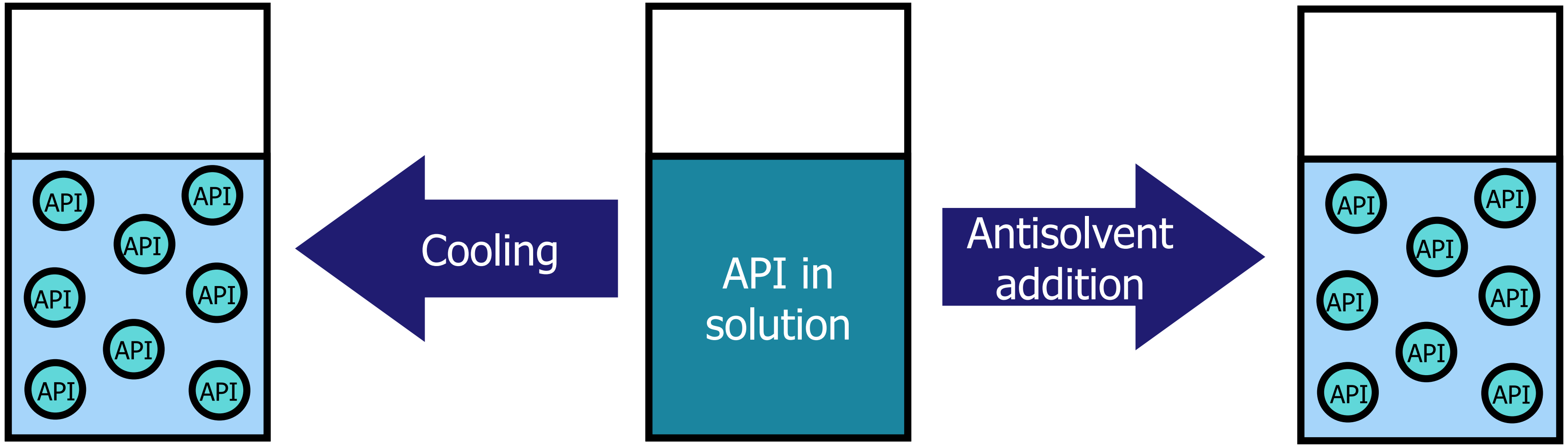
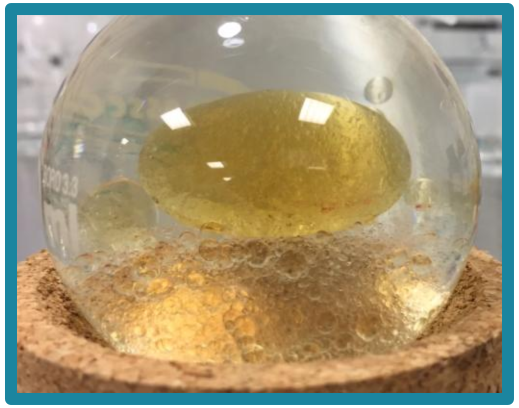
- Liquid-Liquid phase separation (LLPS) '**oiling out**' refers to the emergence of oil like droplets in solutions.
- These oil droplets are rich in solute (active pharmaceutical ingredient, API) and can separate into a distinct layer if left undisturbed.
- LLPS can be triggered by cooling or antisolvent addition.

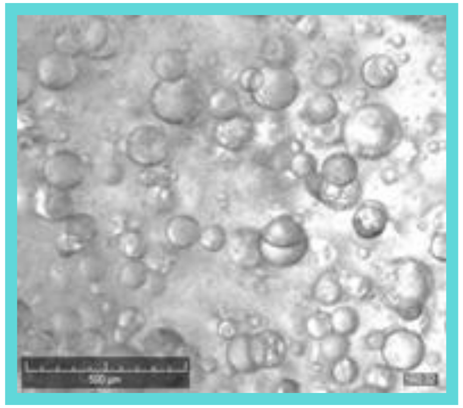


How Can We Spot LLPS?

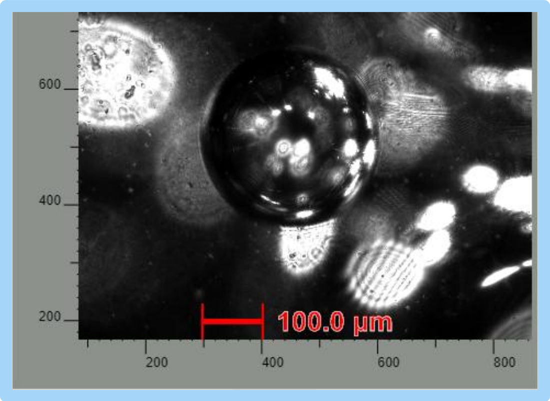
Visual LLPS



Crystalline



Blaze Probe

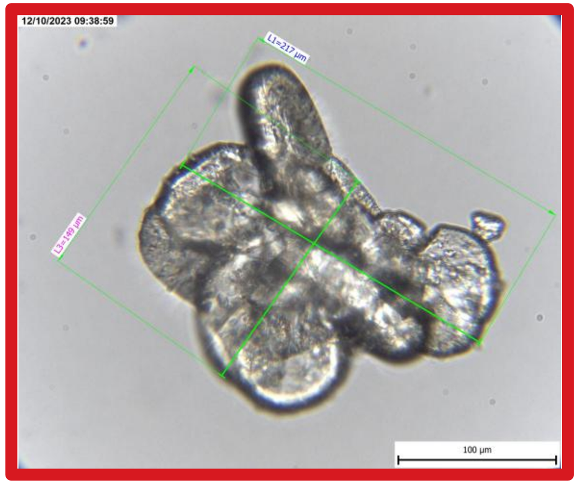


How Can We Spot LLPS?

- You do not need a Blaze Probe or Crystalline instrument to identify LLPS, there are other **factors that indicate oiling out**:
  - Consistent high concentration of sampled aliquots compared to the theoretical concentration.
  - Cloud points coinciding with clear points on phase diagrams.
  - Boulder-like solid.
  - Crowning and deposition on reactor vessel walls.
  - Fused crystals.

Isolation of boulder/rock like solids

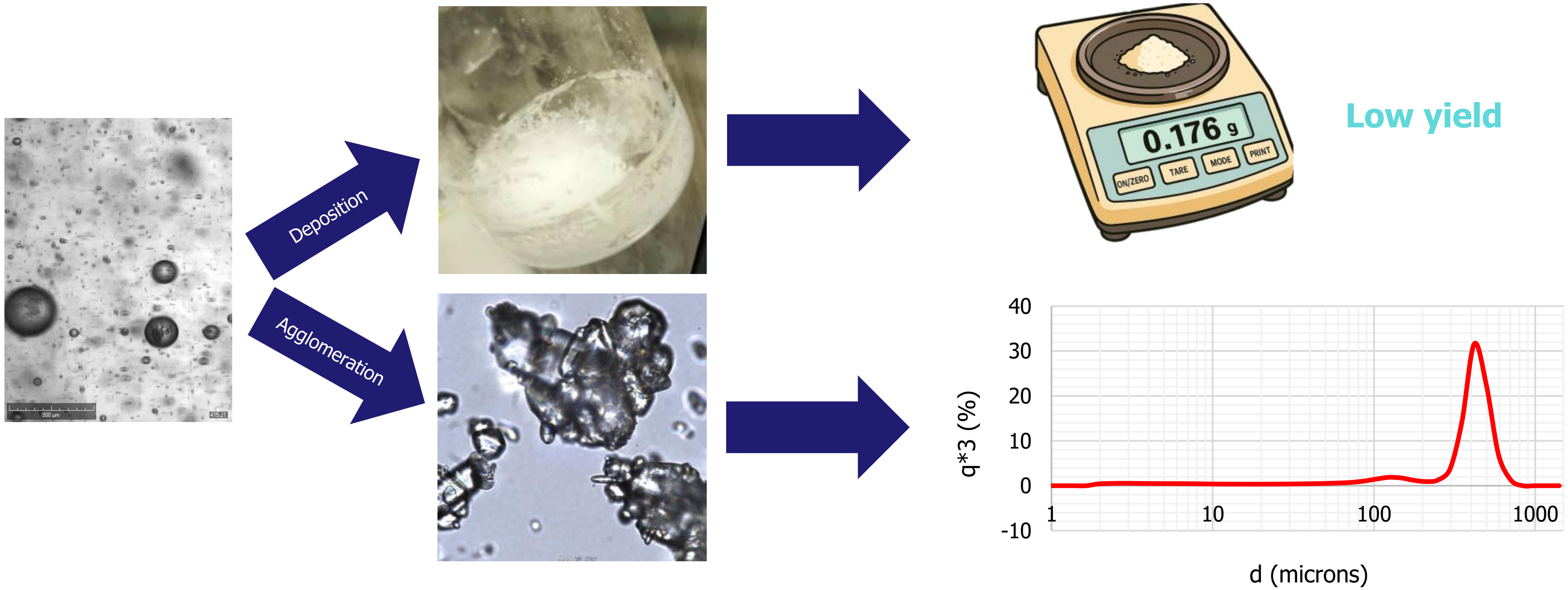
Crowning and deposition on reactor wall or propeller



Fused crystals

Why Do We Care About Avoiding Oiling?

- LLPS creates a different local environment for nucleation and crystal growth.
- This significantly impacts the final product quality including impurity profile, particle morphology and size, as well as the isolated polymorph.
- Oiling can also result in deposition and agglomerated crystals.



Large PSD

How Do We Avoid LLPS?

Solvent Switch

- According to regular solution model, solvent-solute interaction relative to solute-solute and solvent-solvent interactions plays a critical role in determining the mixing behaviour.
- Switching to different solvent is a common strategy to avoid LLPS.

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- Adding a solution of API to an antisolvent rich phase with seed can result in avoiding LLPS.
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- Ultrasound can be used as an alternative to adding a seed.
- Ultrasound results in the creation of a small nuclei at relatively low supersaturation levels by insonation.

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Plotting LLPS Diagrams

- LLPS diagrams identify conditions an oil phase is likely to be stable though identification of clear and cloud points.

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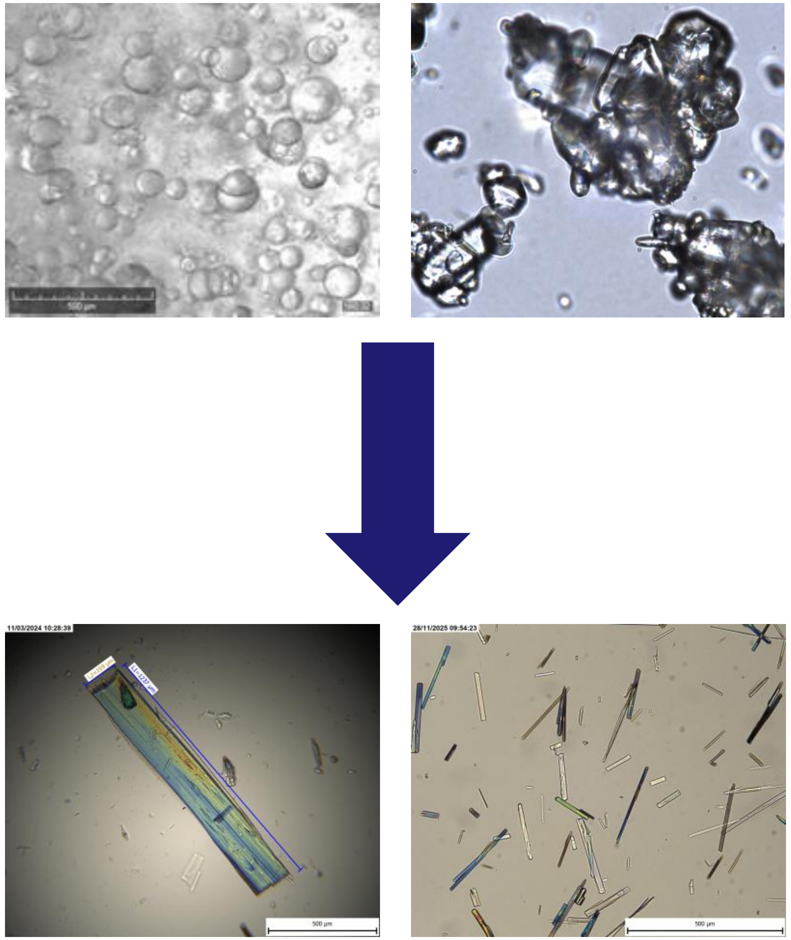
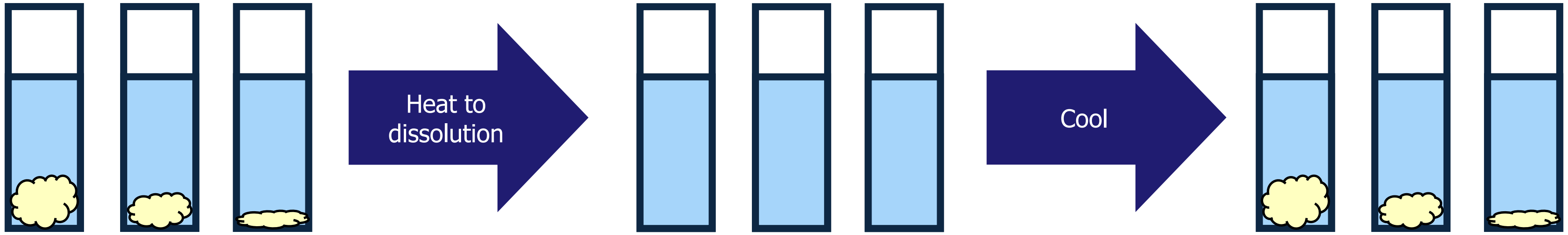


How Do We Plot LLPS Diagrams?

- The **Technobis Crystalline** is used to plot LLPS Diagrams
- It allows 8 vials to be individually agitated and temperature controlled
- It has in-situ cameras which take images of the vials throughout an experiment to provide information about clear and cloud points
- It has lasers to monitor transmissivity throughout an experiment

How Do We Plot LLPS Diagrams?

1. Prepare suspensions of different concentrations in a chosen solvent system (concentrations which you are confident will fully dissolve on heating to the chosen high temperature).
2. Use the **Crystalline** to slowly heat the vials.
3. Slowly cool the vials to room temperature.
4. Images are taken of the vials frequently throughout experiments.



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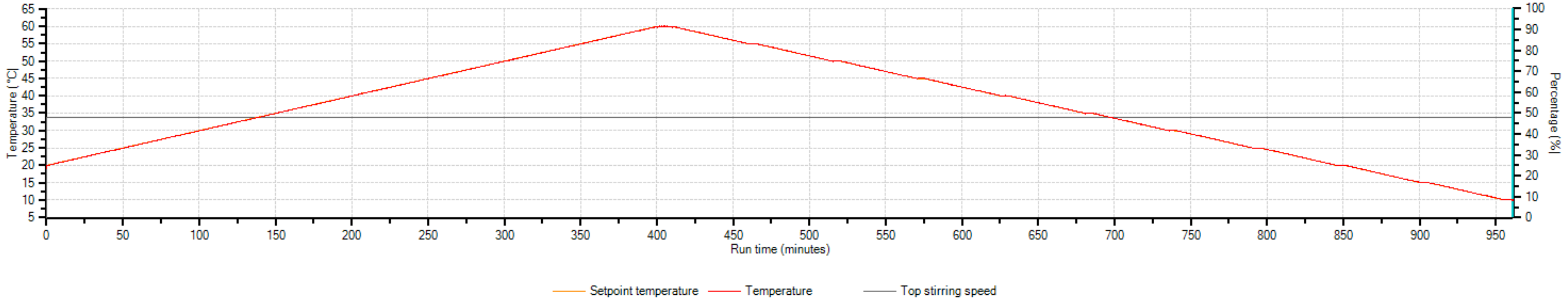

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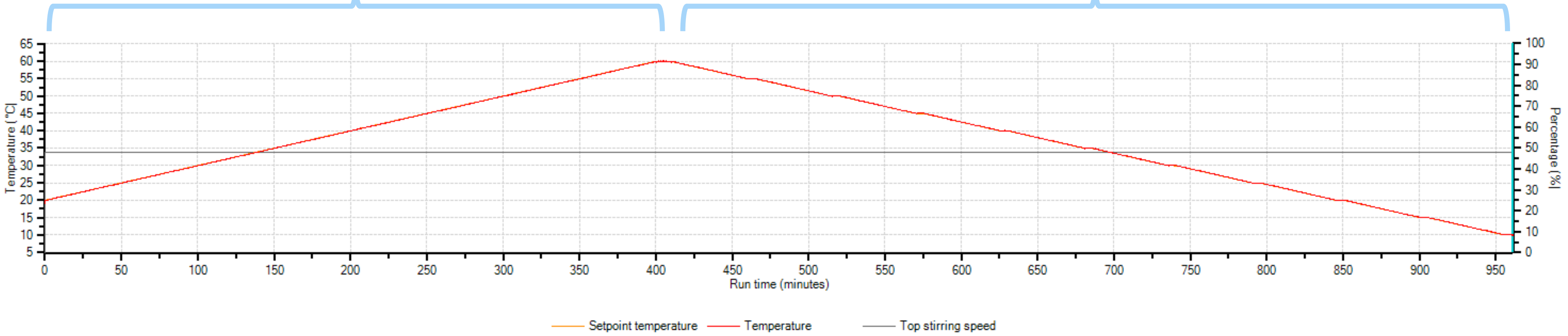
Clear points are identified through the heating step

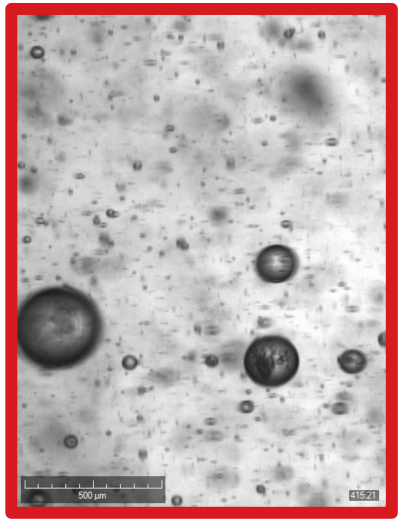
Cloud points are identified through the cooling step



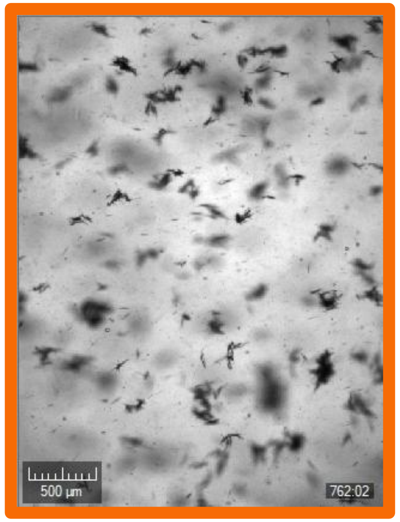
How Do We Plot LLPS Diagrams?

- *In-situ* images are collected to identify the following:
  - *Clear points* - temperatures at which all solid had dissolved, the solution becomes a single-phase liquid.
  - *Cloud points due to LLPS* – temperature at which oil is first observed.
  - *Cloud points due to nucleation* – temperature at which solid first nucleated.

Liquid

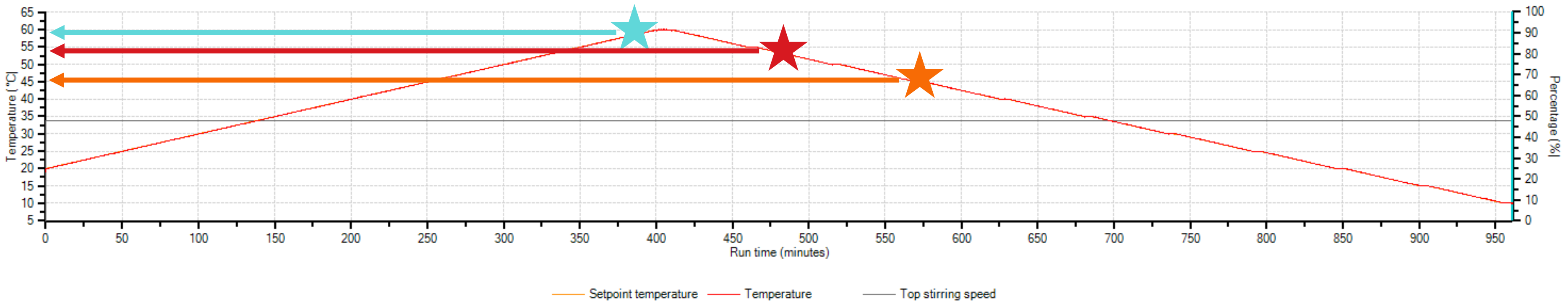


Oil

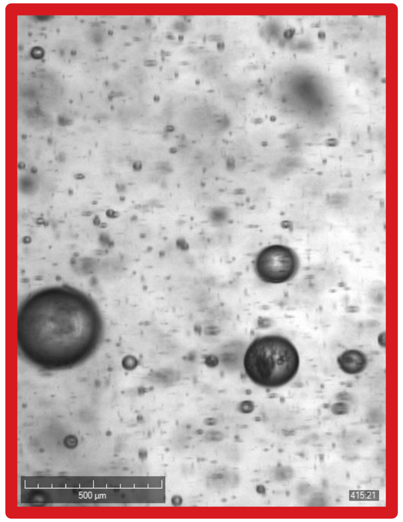


Solid

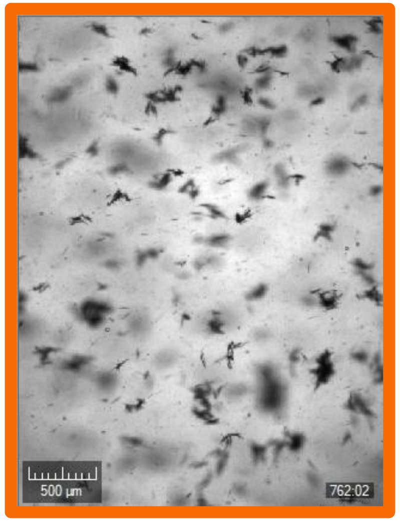
How Do We Plot LLPS Diagrams?



59.7 °C



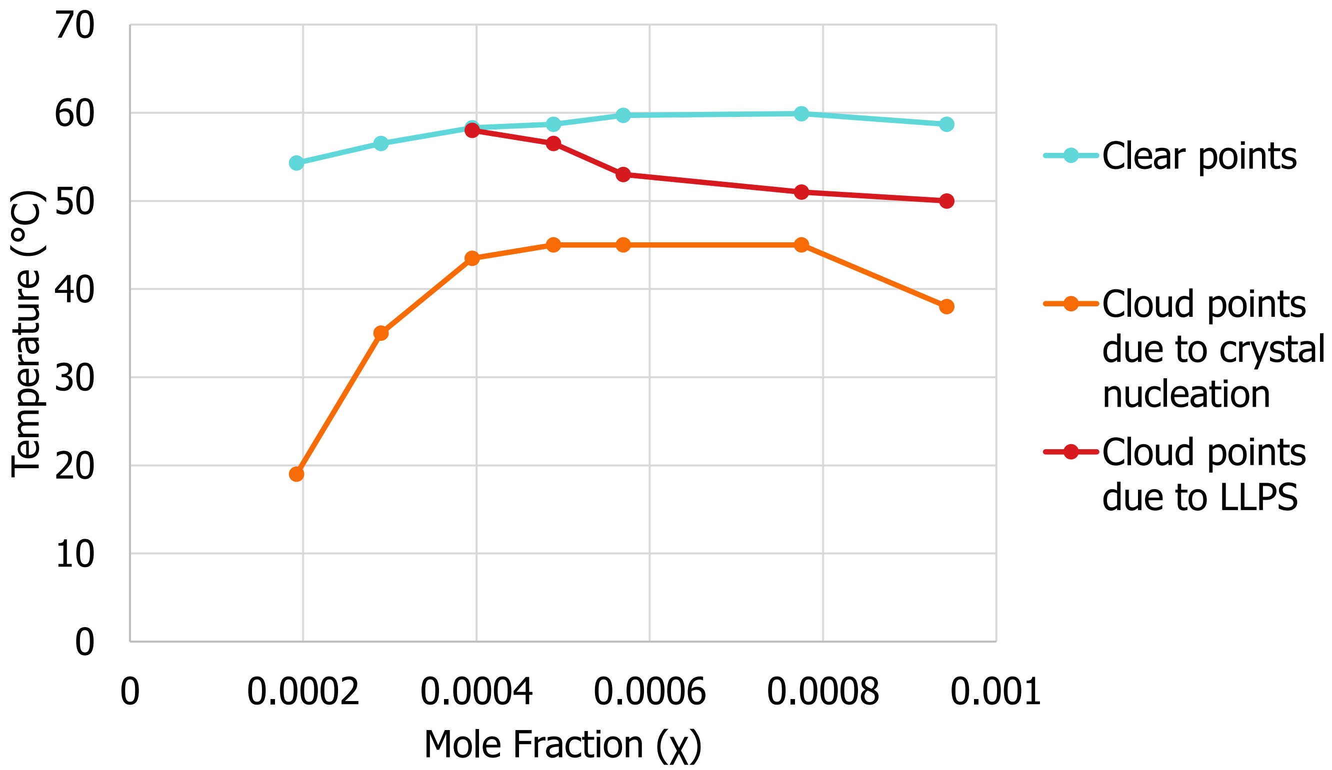
53.0 °C



45.0 °C

How Do We Plot LLPS Diagrams?

1. Plot clear and cloud points.



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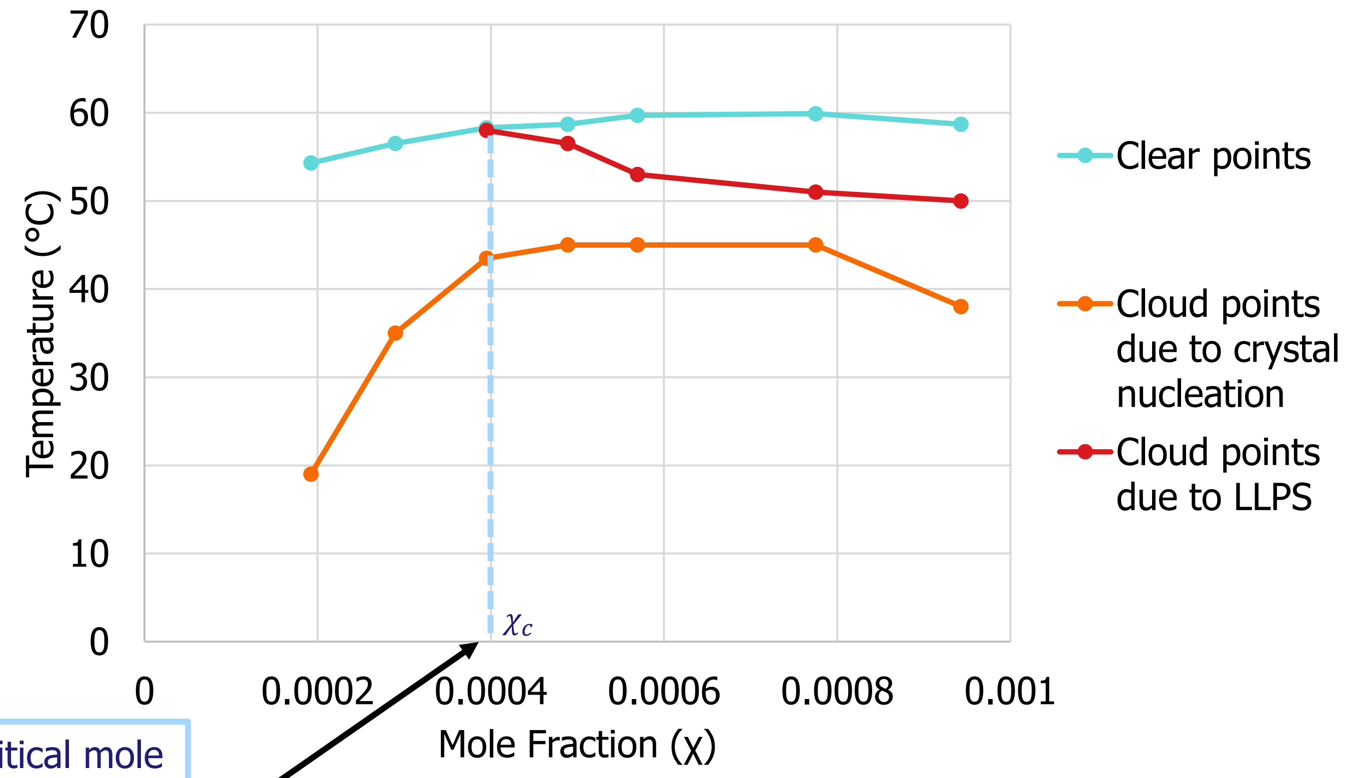
How Do We Plot LLPS Diagrams?

2. Identify any critical mole fractions (χ) – mole fractions where clear and cloud points overlap.

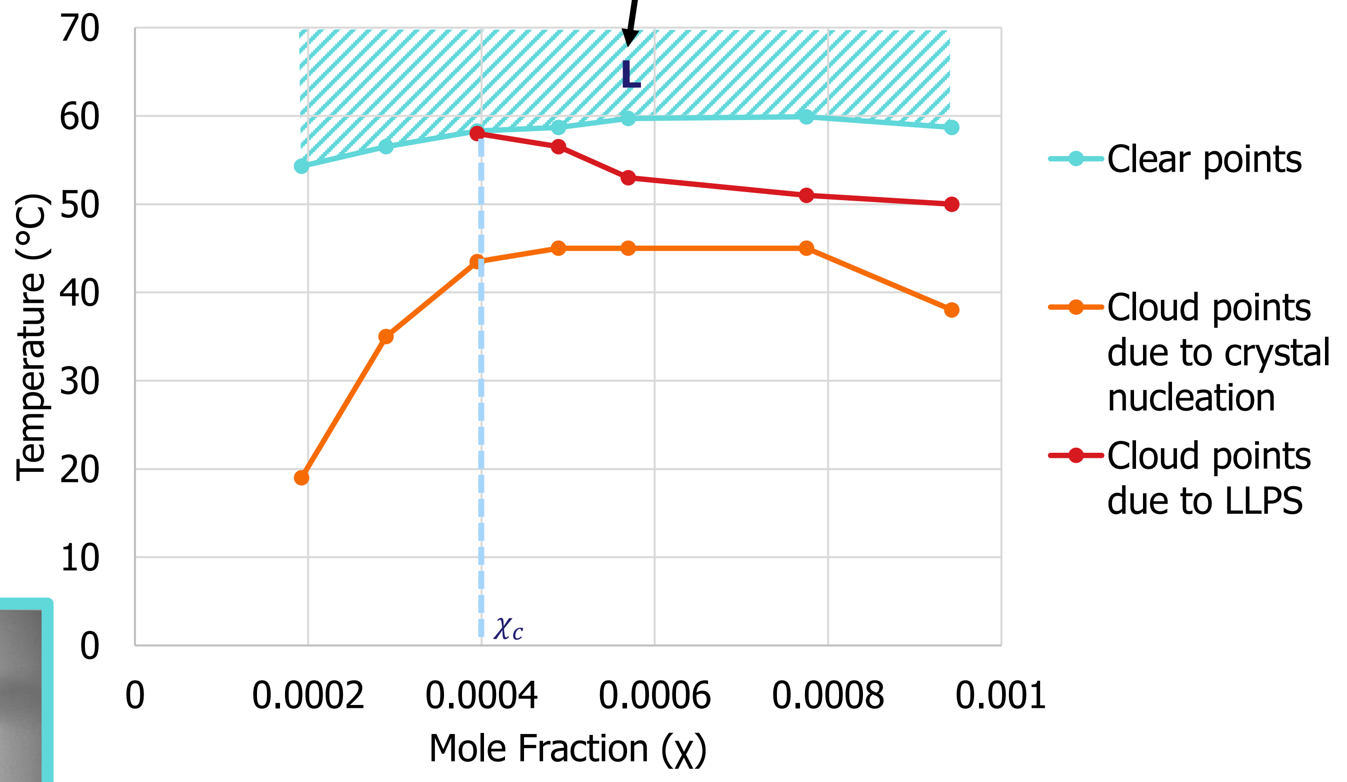


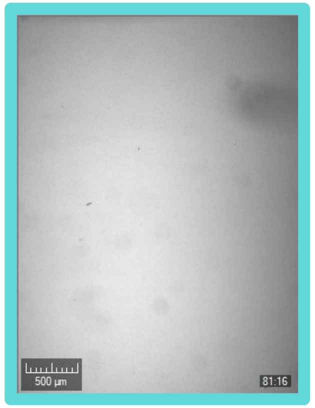
χ_c is the critical mole fraction, the mole fraction at which clear points and cloud points overlap

How Do We Plot LLPS Diagrams?

3. Identify phase regions.

L represents a liquid phase, the API is in solution

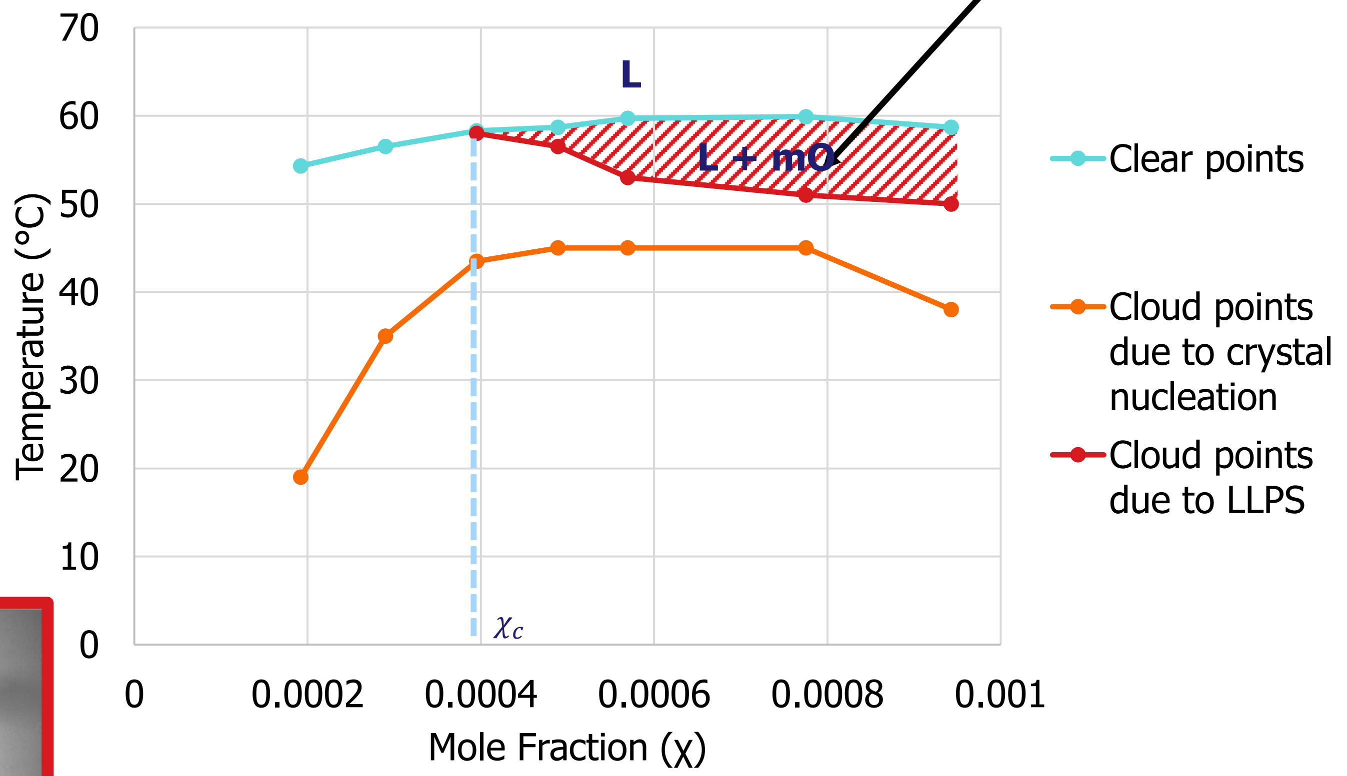


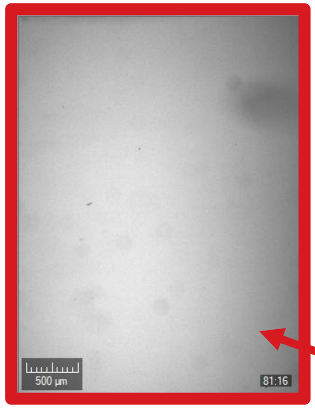


How Do We Plot LLPS Diagrams?

3. Identify phase regions.

L+mO represents an equilibrium between a liquid and metastable oil phase – the API may oil out in this region



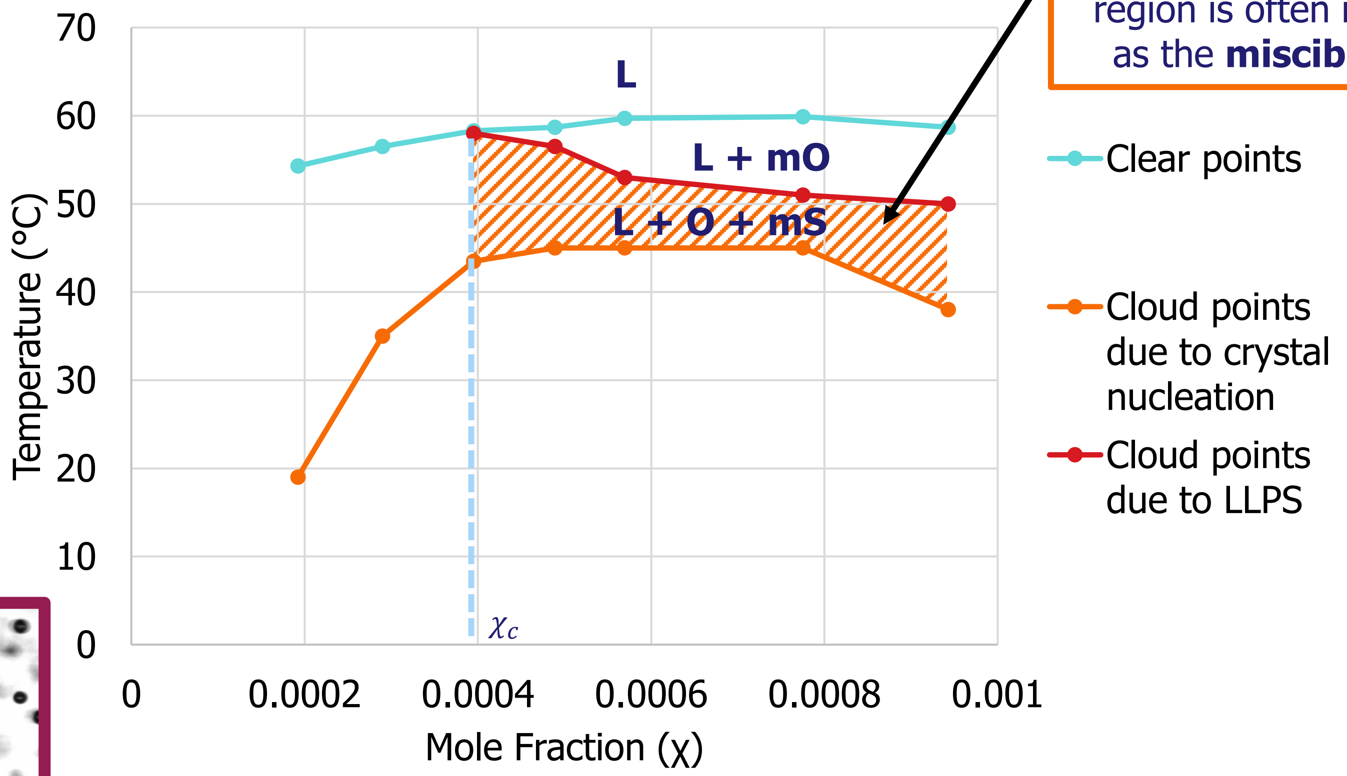


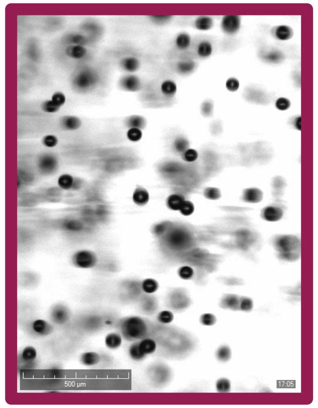
Will still appear liquid in this region unless seeded as the oil is metastable

How Do We Plot LLPS Diagrams?

3. Identify phase regions.

L+O+mS represents an equilibrium between a liquid, oil and metastable solid phase. The oiling region is often referred to as the **miscibility gap**

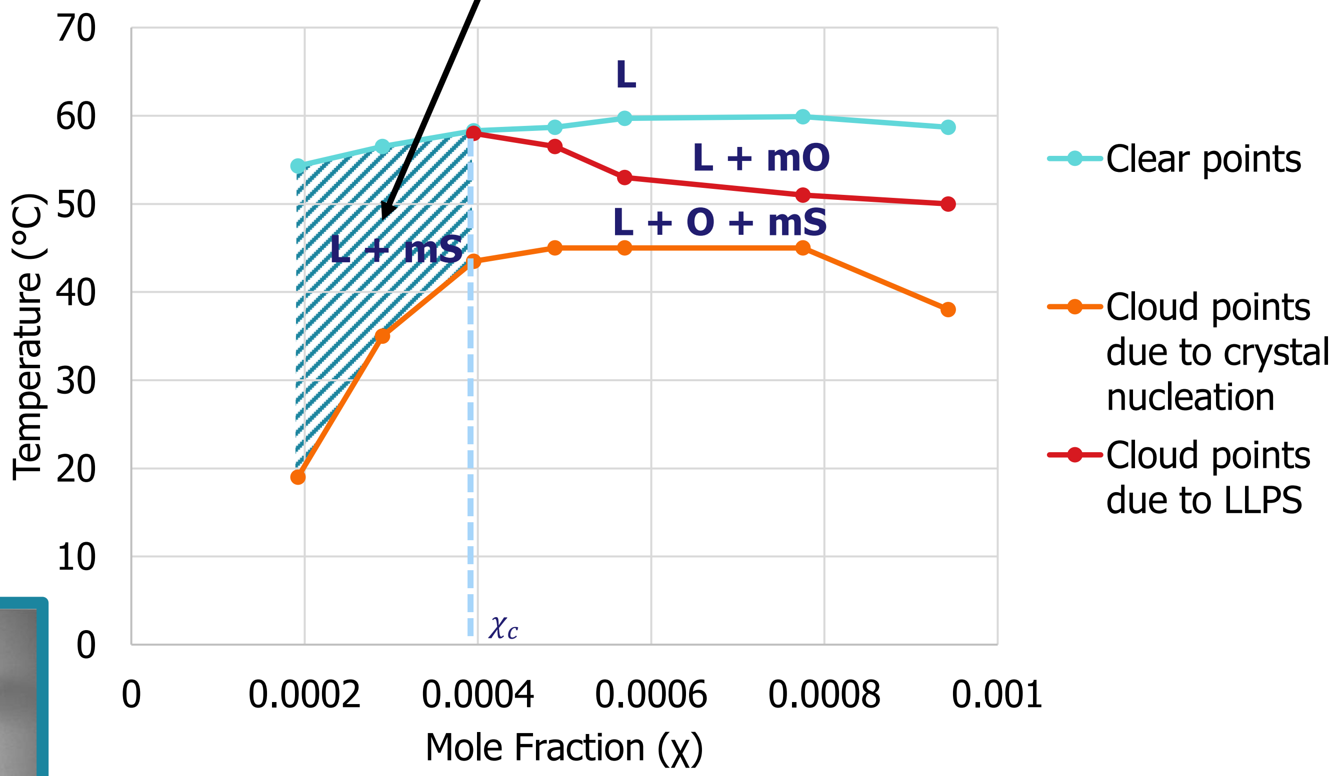


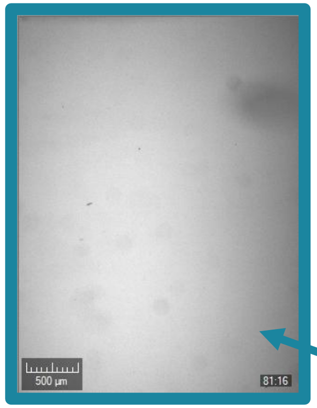


How Do We Plot LLPS Diagrams?

3. Identify phase regions.

L+mS represents an equilibrium between a liquid and metastable solid phase – usually referred to as the **metastable zone width**





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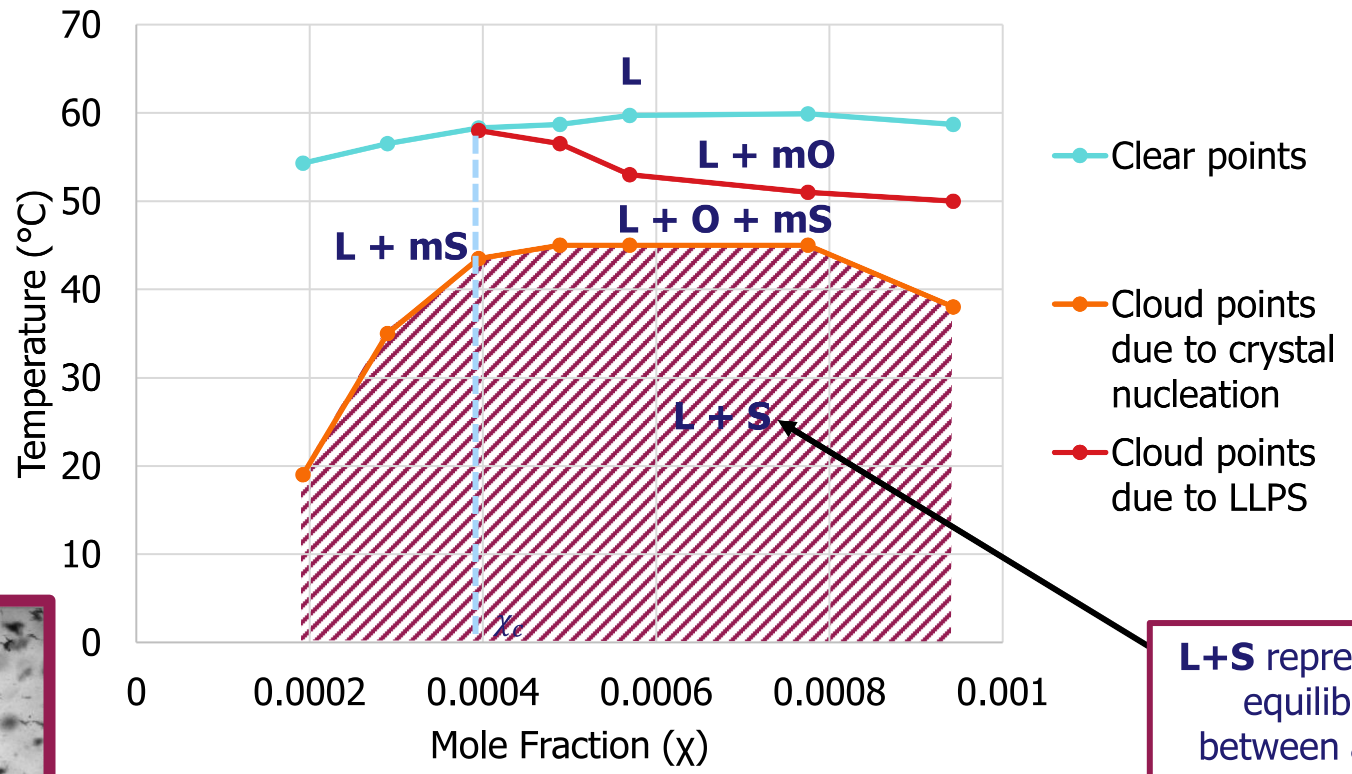
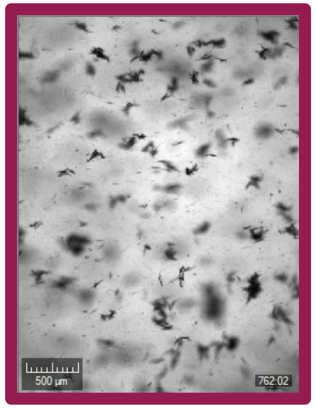
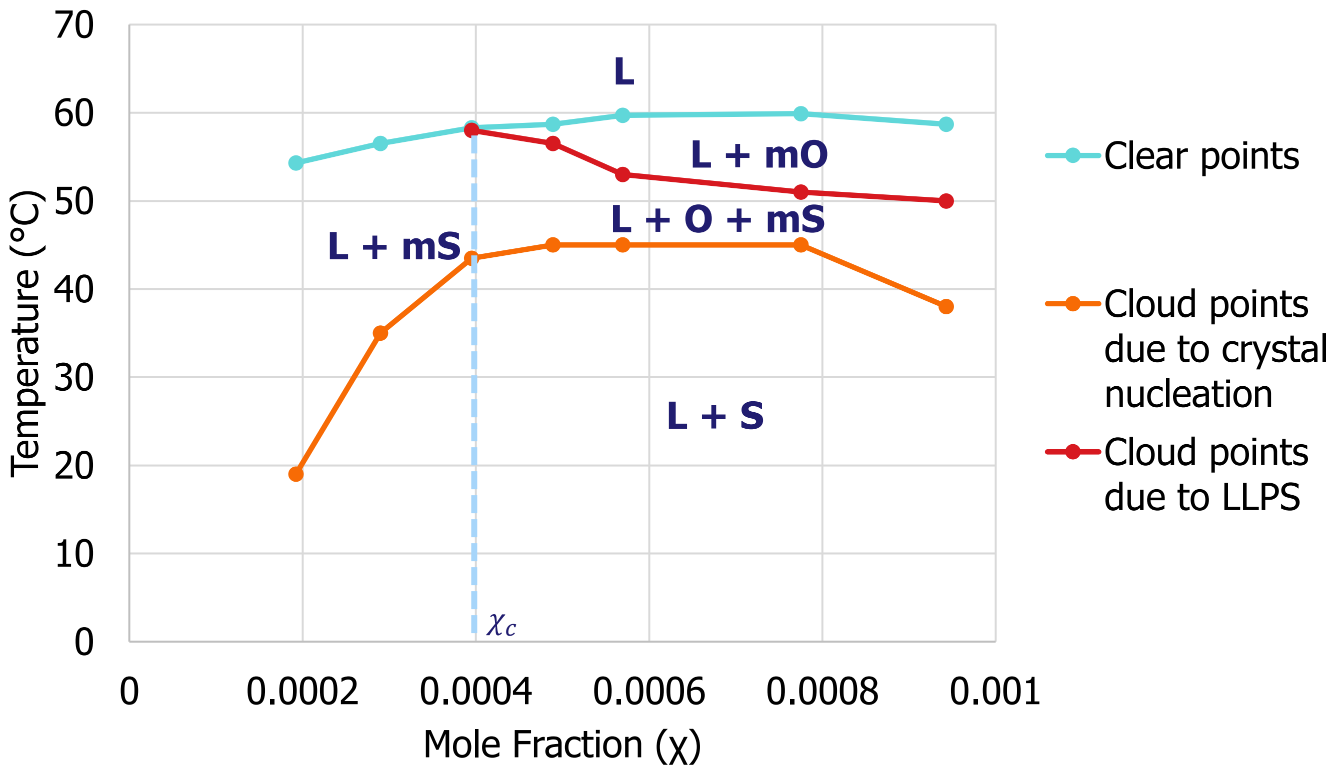


L+S represents an equilibrium between a liquid and a solid phase - a slurry



How Do We Plot LLPS Diagrams?

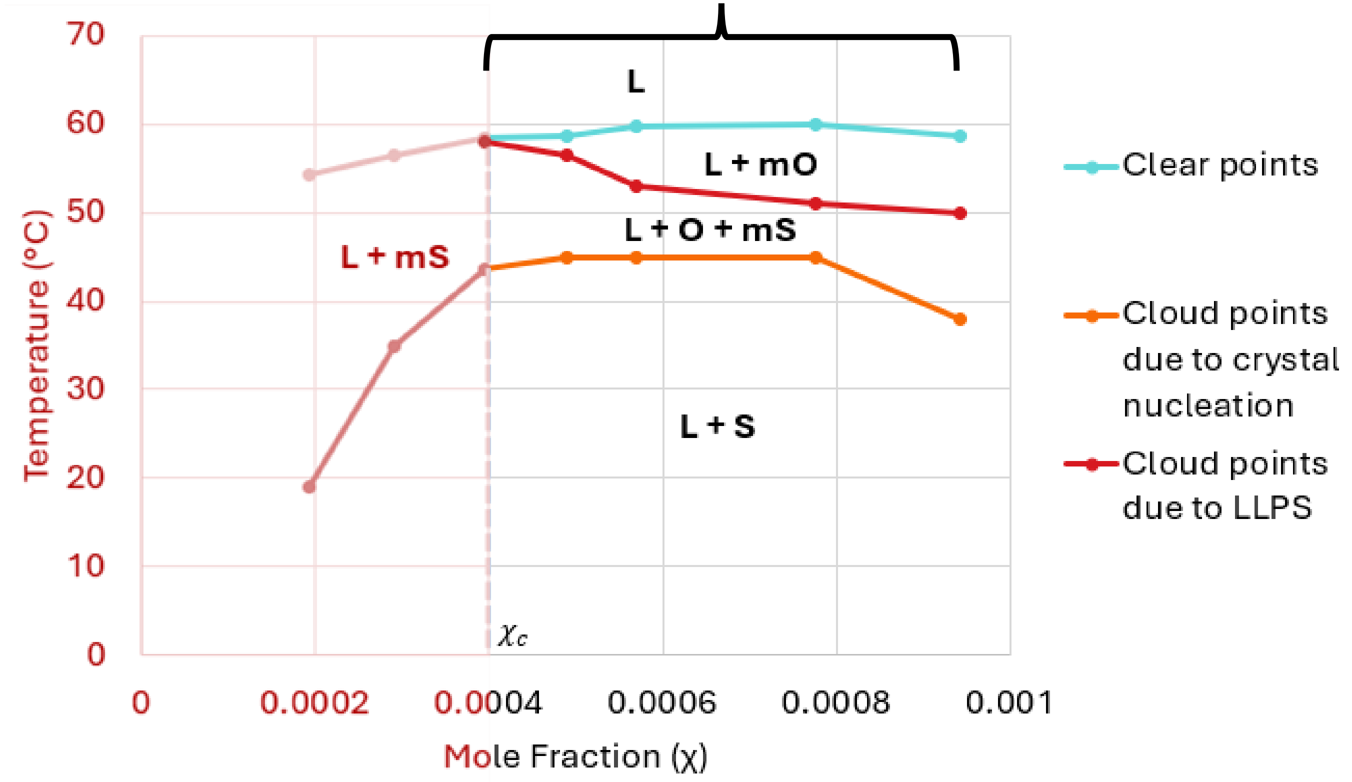
4. Identify a safe crystallisation pathway.



How Do We Plot LLPS Diagrams?

4. Identify a safe crystallisation pathway.

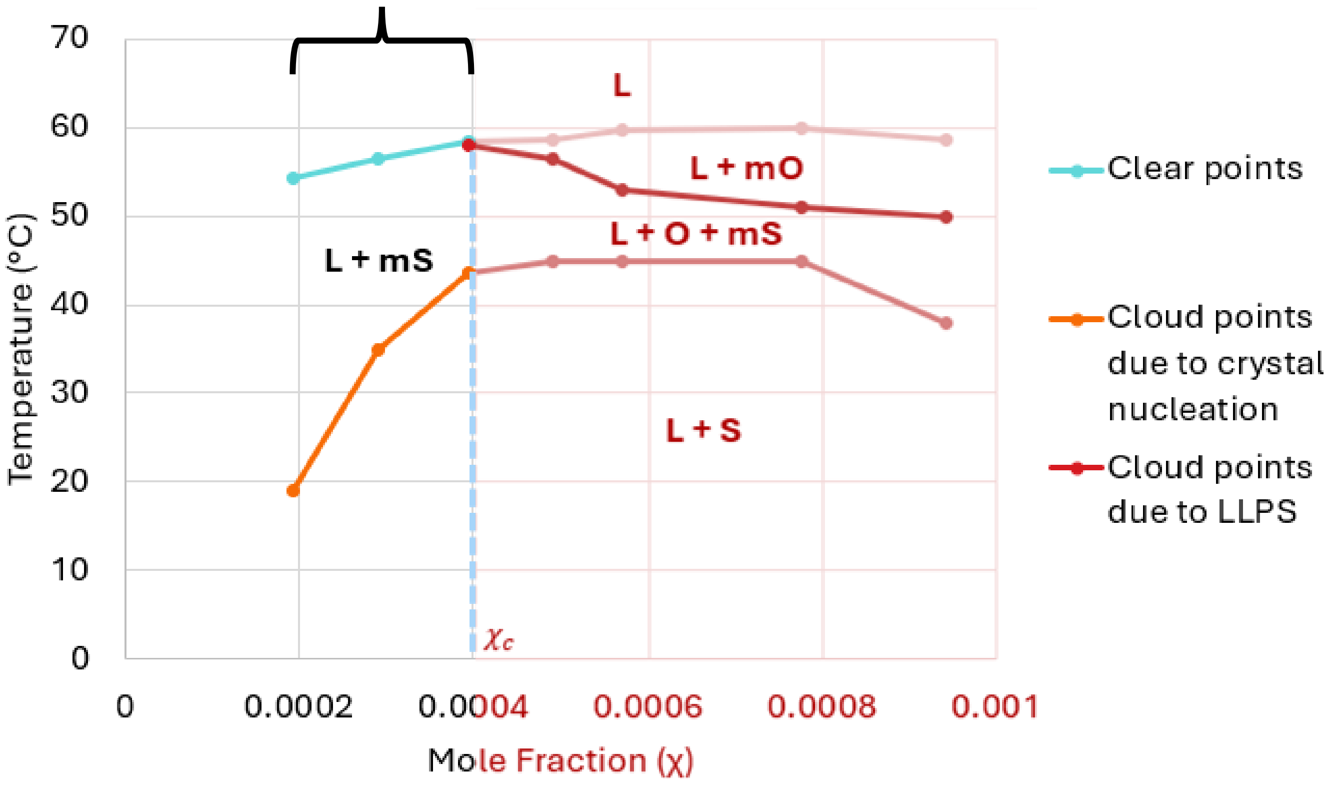
Operating in this region will result in passing through an oil phase



How Do We Plot LLPS Diagrams?

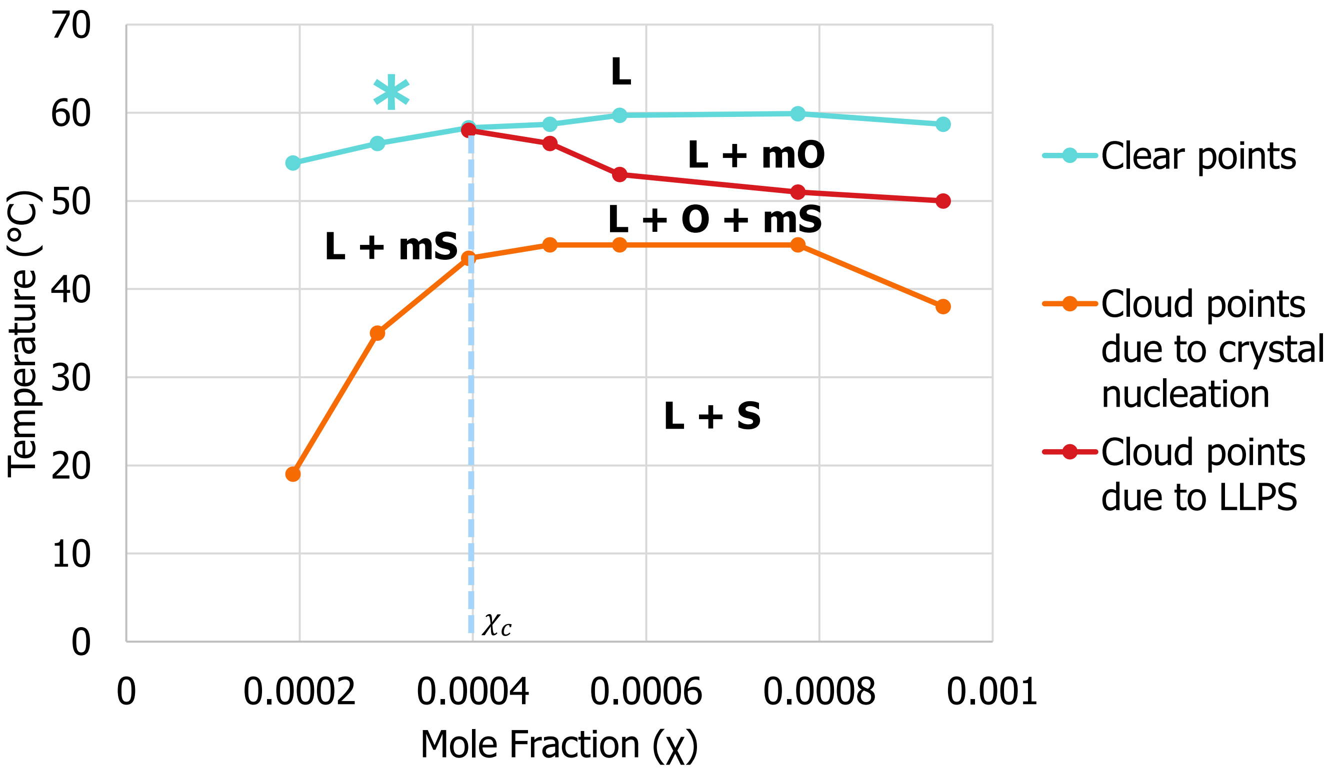
4. Identify a safe crystallisation pathway.

Operating in this region will avoid the oiling phase – the crystallisation should be conducted within this range



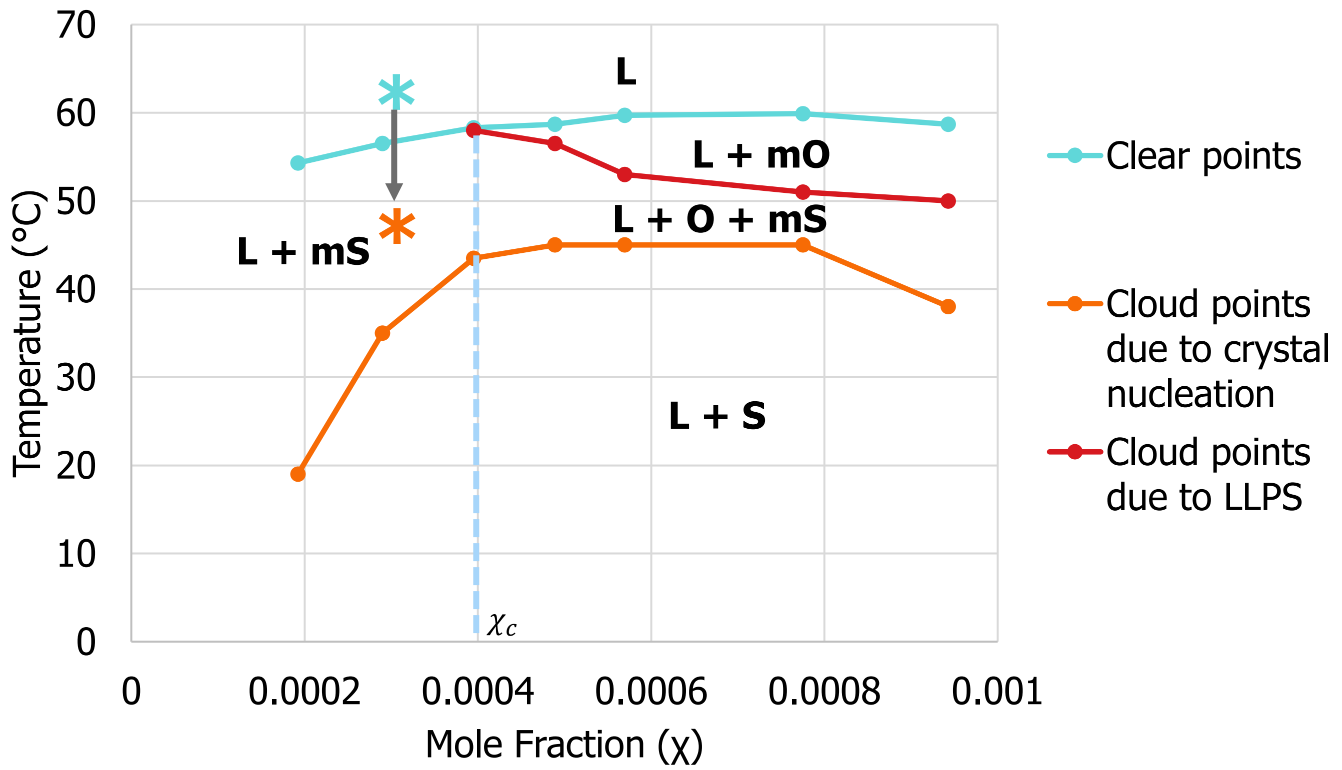
How Do We Plot LLPS Diagrams?

4. Identify a safe crystallisation pathway.
   – Dissolve material in the liquid phase



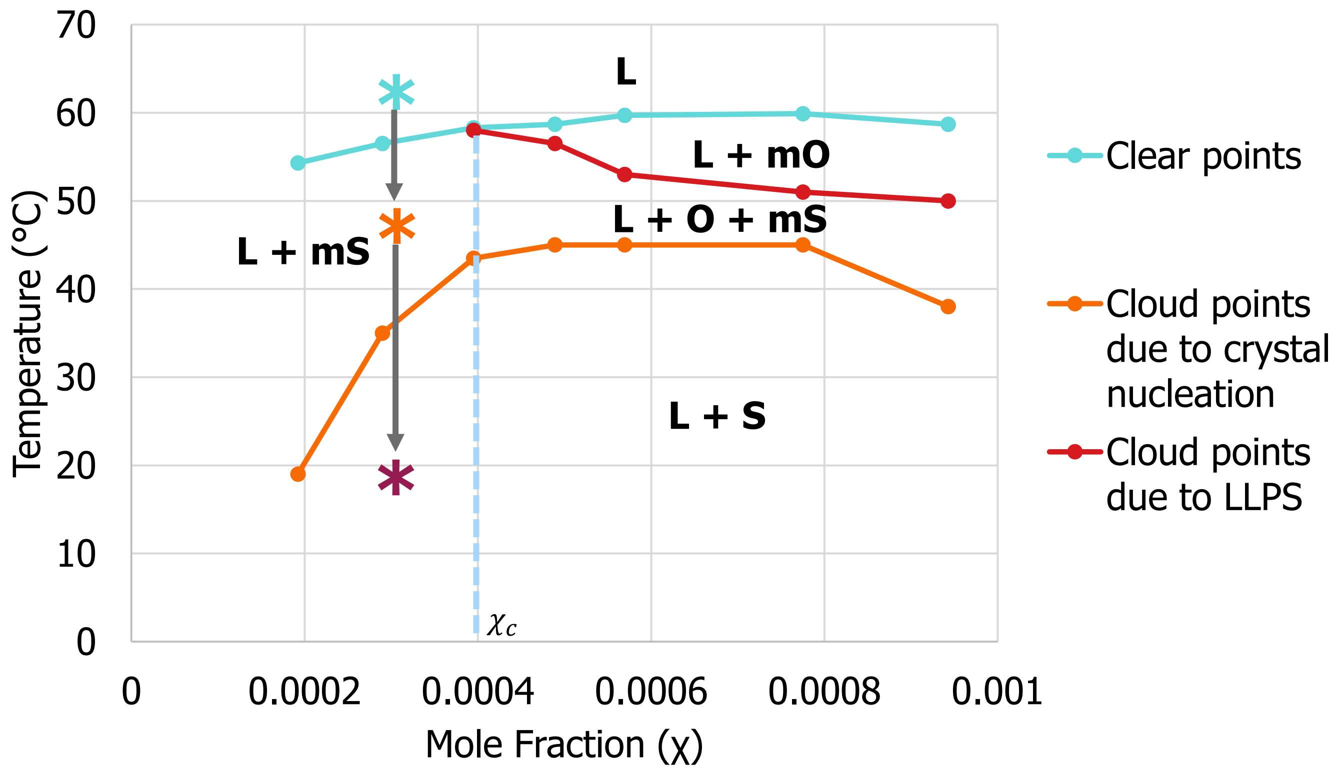
How Do We Plot LLPS Diagrams?

4. Identify a safe crystallisation pathway.
   - Cool to the metastable solid phase and seed – the seed will hold as we are in the metastable solid region.



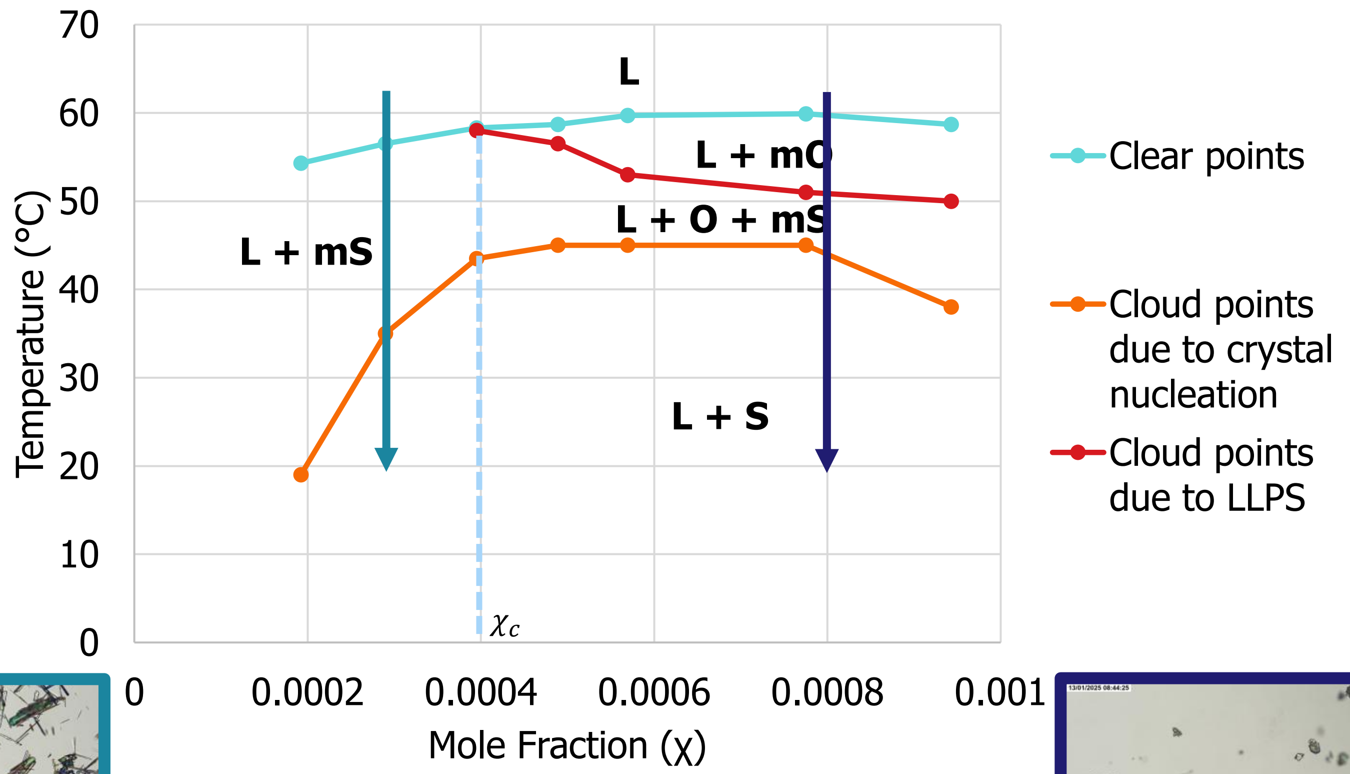
How Do We Plot LLPS Diagrams?

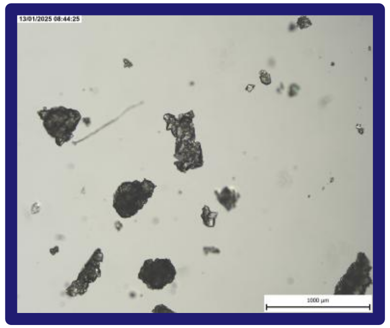
4. Identify a safe crystallisation pathway.
   - Slowly cool to room temperature for controlled growth



How Do We Plot LLPS Diagrams?

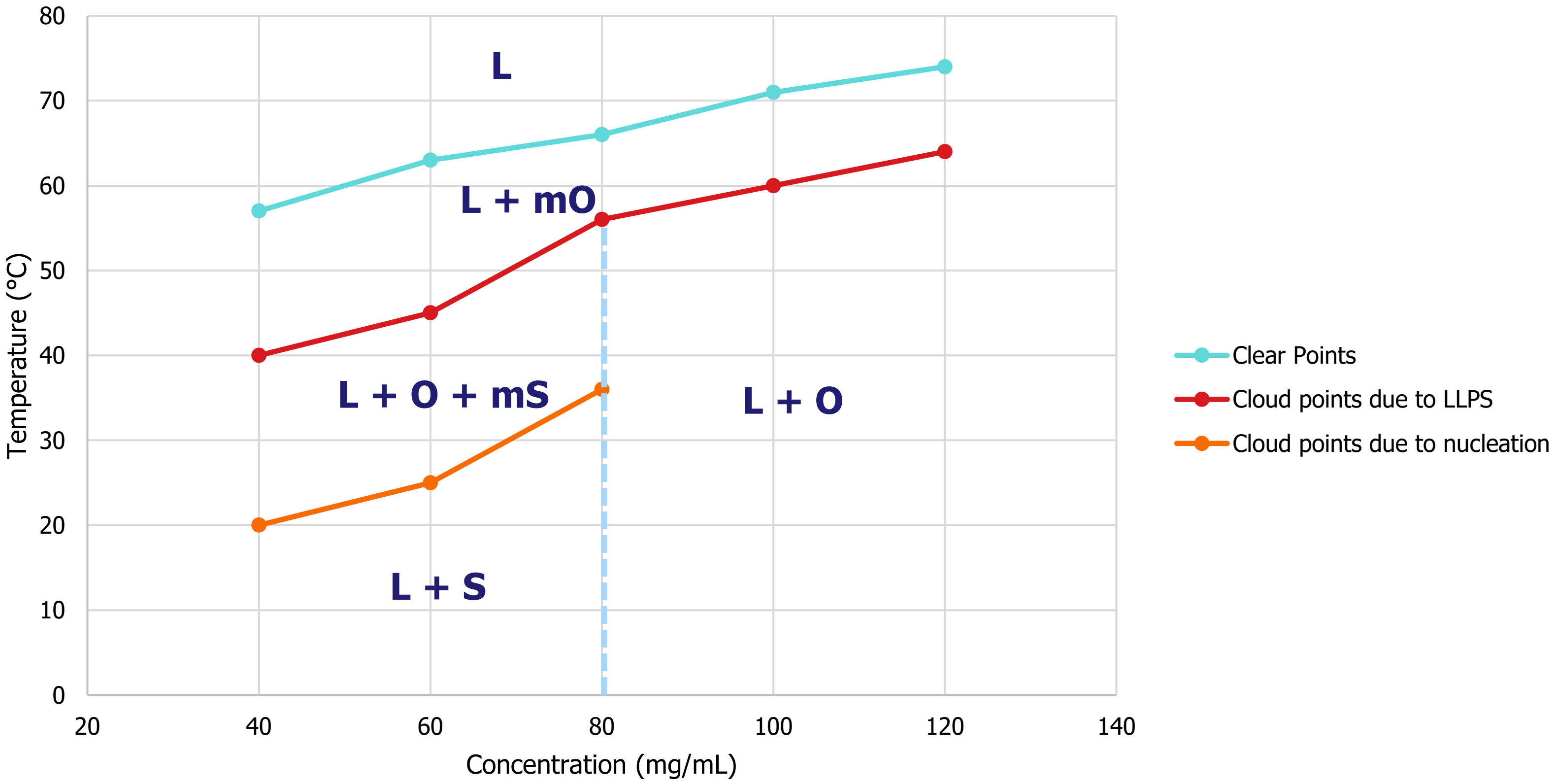
- Traversing through the **L+mS** phase, avoiding the oil, will result in single crystals.
- Going through the oil phase will likely result in aggregated crystals/clusters.





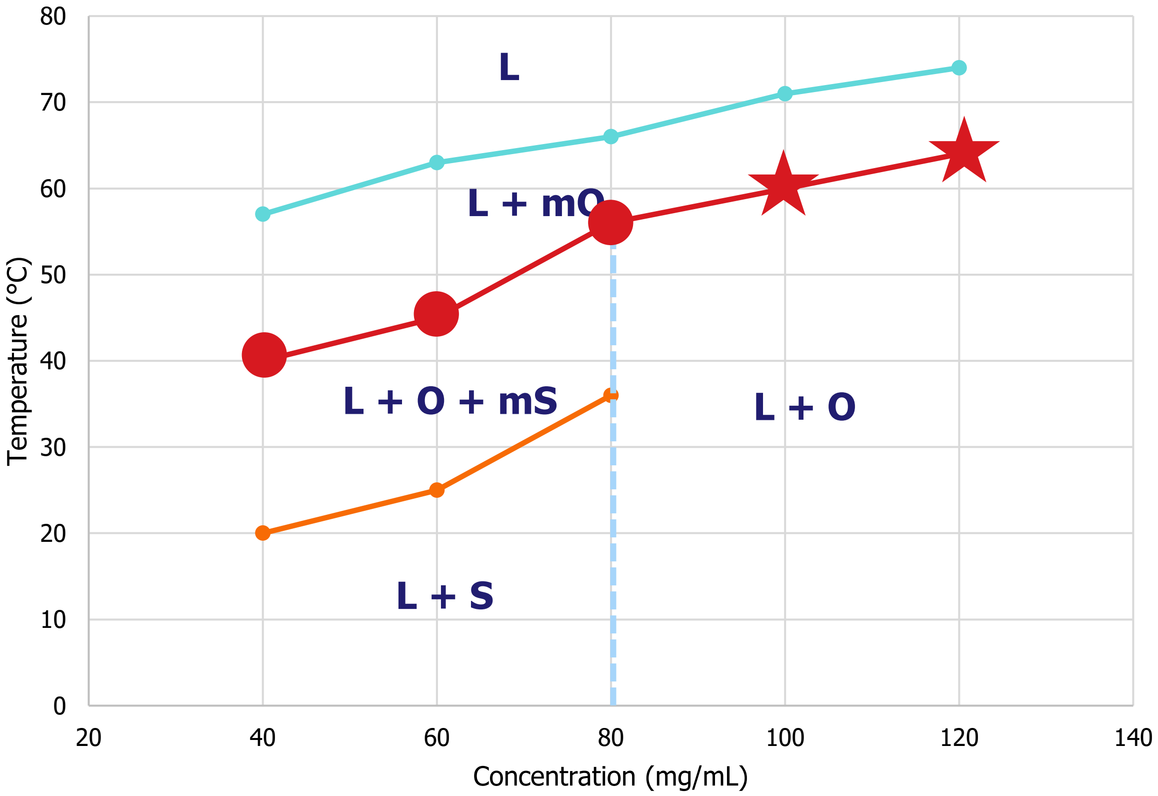
Spinodal vs. Nucleation and Growth

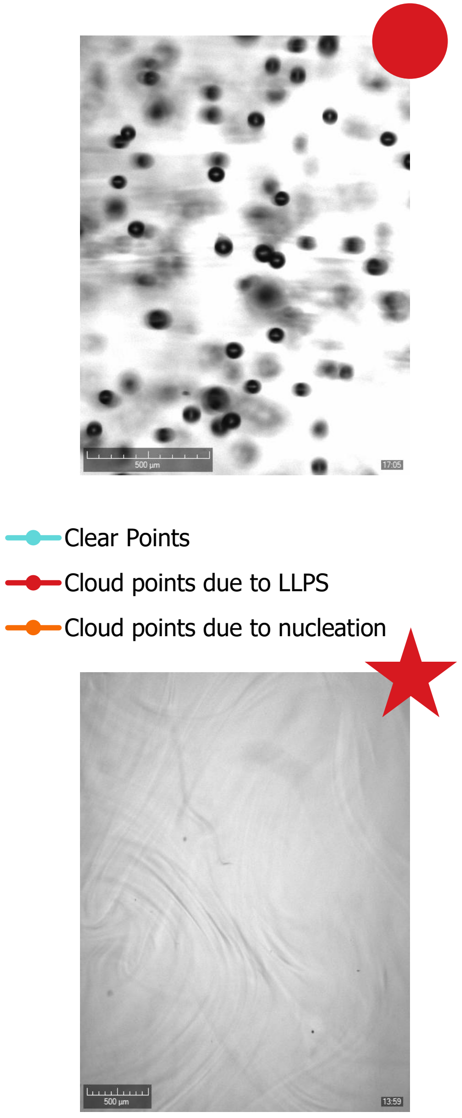
- LLPS occurs by two mechanisms, both of which can be plotted separately on a LLPS diagram to provide a more detailed explanation of the system.



Spinodal vs. Nucleation and Growth

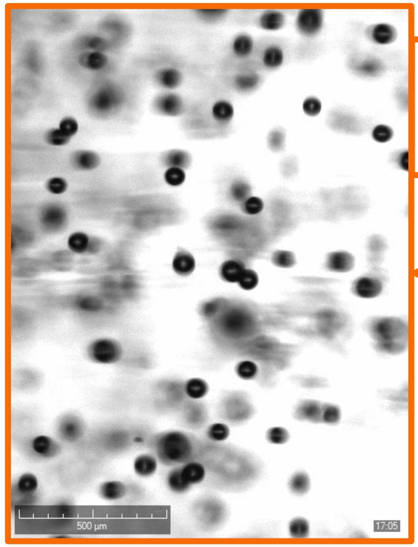
- Below 80 mg/mL, oil droplets are observed.
- Above 80 mg/mL, oil layers are observed.





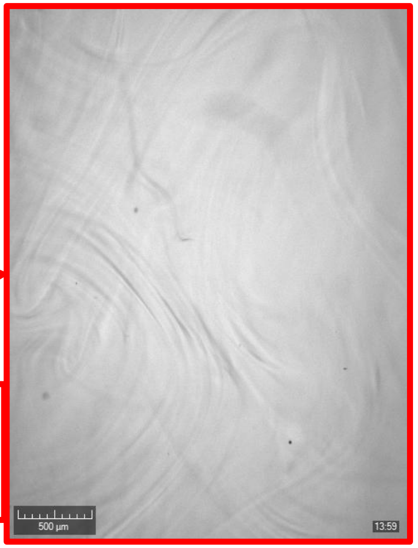
Spinodal vs. Nucleation and Growth

- There are two mechanisms of oil phase formation: **Spinodal decomposition** mechanism or **nucleation and growth** mechanism.
- The second derivative relationship between the Gibbs free energy (ΔG^{mix}) and molar fraction (χ) of solute determines the mechanism for the investigated LLPS.
  - $\frac{\partial^2(\Delta G^{mix})}{\partial \chi^2} < 0$ → Spinodal decomposition mechanism.
  - $\frac{\partial^2(\Delta G^{mix})}{\partial \chi^2} > 0$ → Nucleation and growth mechanism.



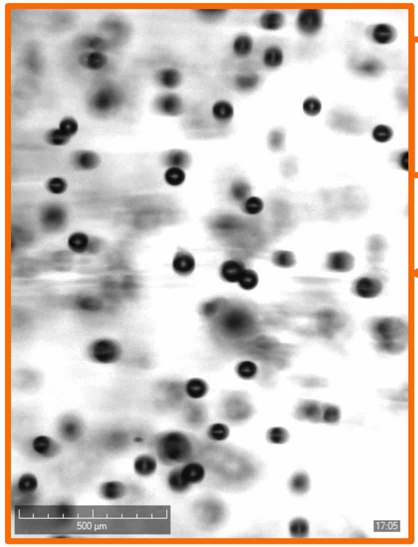
Cloud point nucleation and growth

Cloud point spinodal decomposition



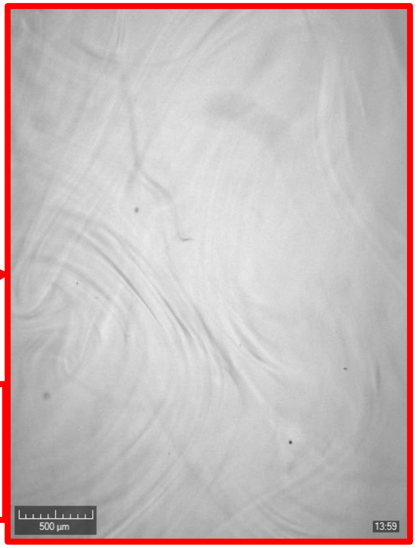
Spinodal vs. Nucleation and Growth

- Oiling by the **nucleation and growth** mechanism is seen as oil droplets – if this oiling is observed, it can be reversed.
- Oiling by **spinodal decomposition** is seen by lines of oil through the system – this type of oiling is spontaneous so should be avoided.
- If an oil phase can't be avoided but there is an option to either go through a nucleation and growth or spinodal decomposition region, going through the nucleation and growth region in advised.



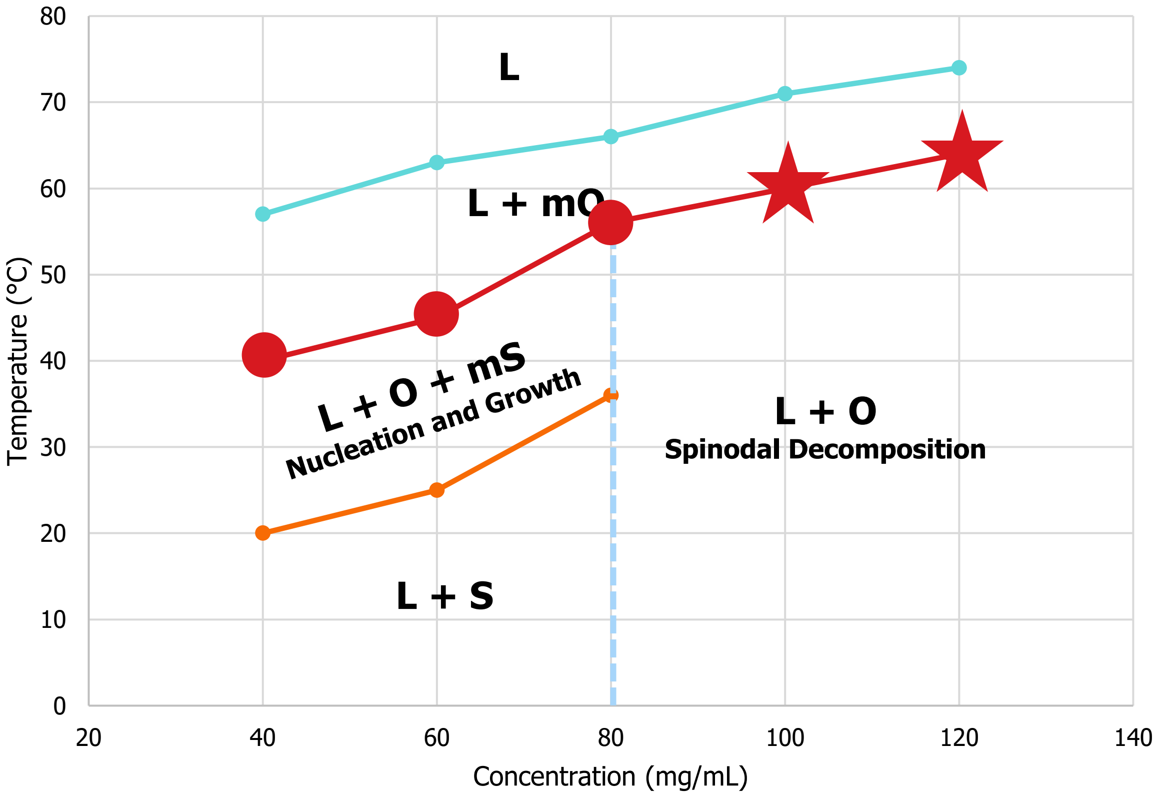
Cloud point nucleation and growth

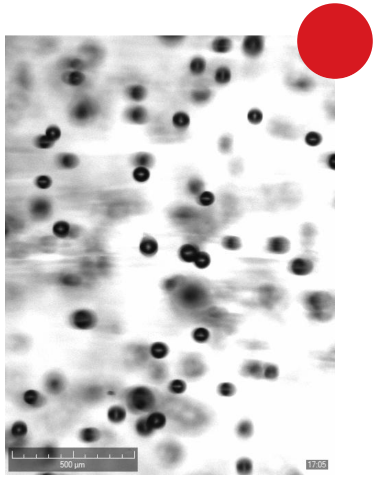
Cloud point spinodal decomposition



Spinodal vs. Nucleation and Growth

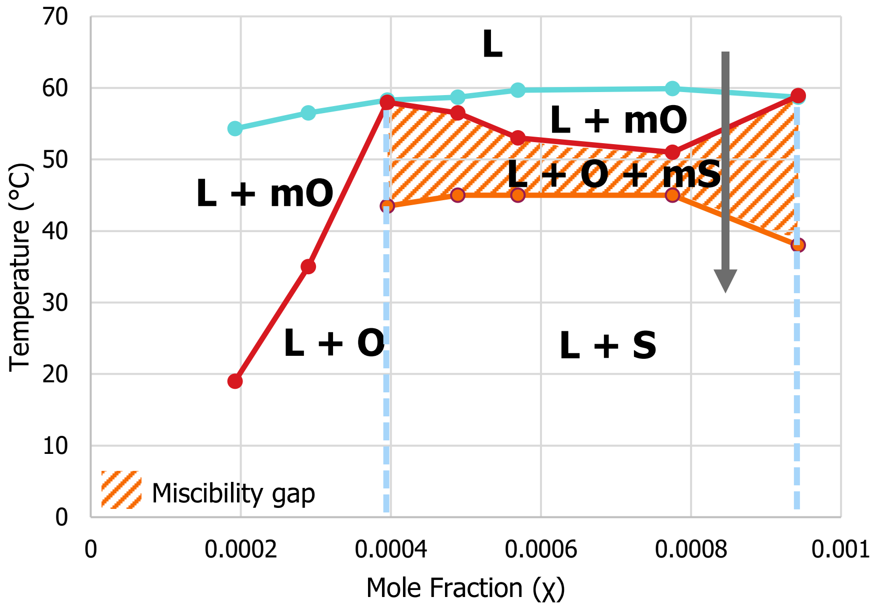
- As the oil region spinodal decomposition region is spontaneous, is should be avoided.
- The crystallisation should occur below 80 mg/mL in this example.





Process Transversing Miscibility Gap

- Sometimes, traversing the oiling region (miscibility gap) may be the only option.
- This may be the case if the yield will be very low if starting concentration is reduced.



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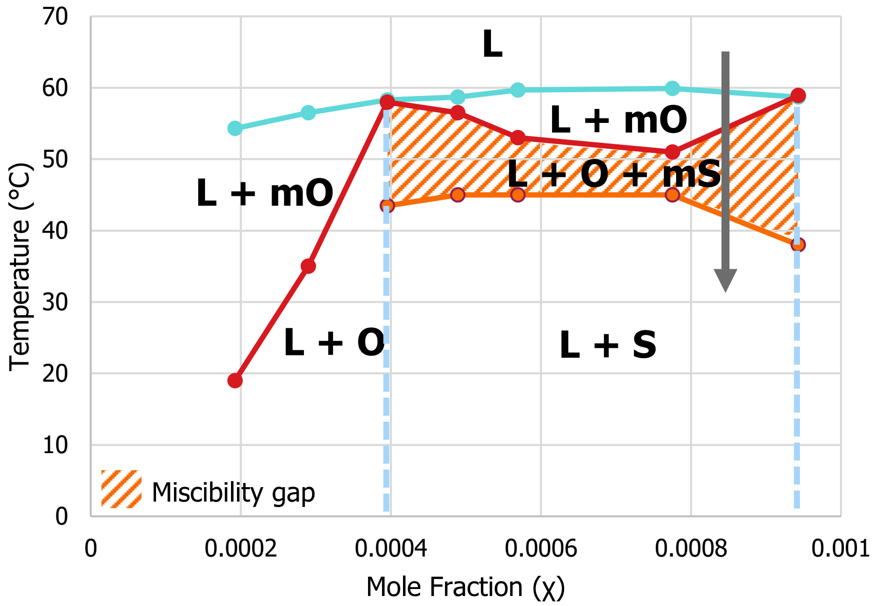

[1] de Albuquerque, I. and Mazzotti, M. (2017), Influence of Liquid-Liquid Phase Separation on the Crystallization of L-Menthol from Water. Chem. Eng. Technol., 40: 1339-1346. https://doi.org/10.1002/ceat.201700032

Process Transversing Miscibility Gap

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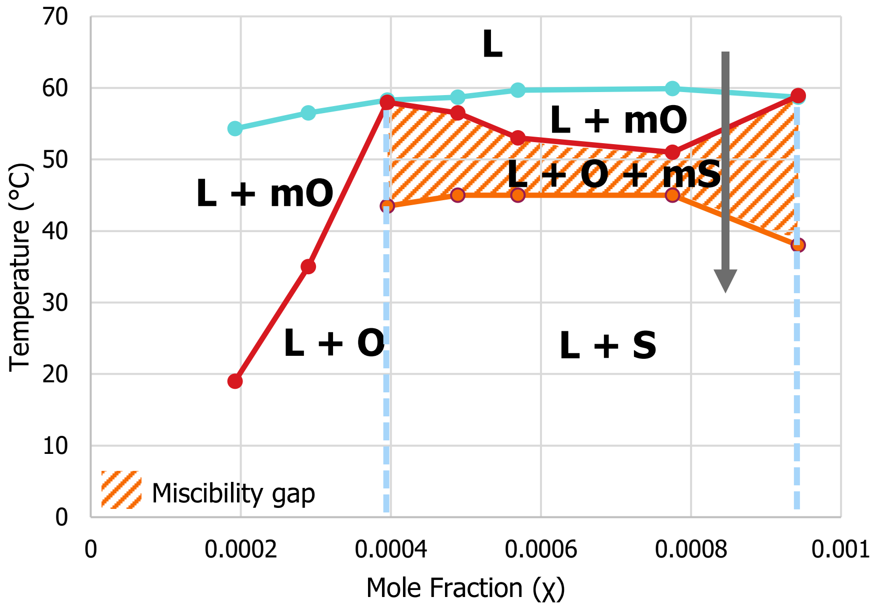

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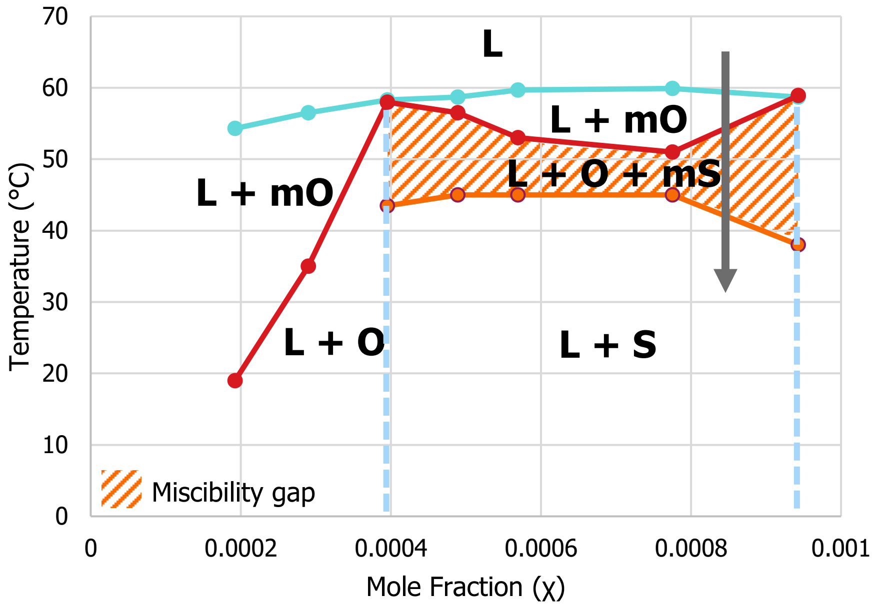
- Overall, crystal habits are severely affected by crystallisation transversing the miscibility gap and can result in interesting morphologies but also in trapping solvents.



'Desert rose' crystals

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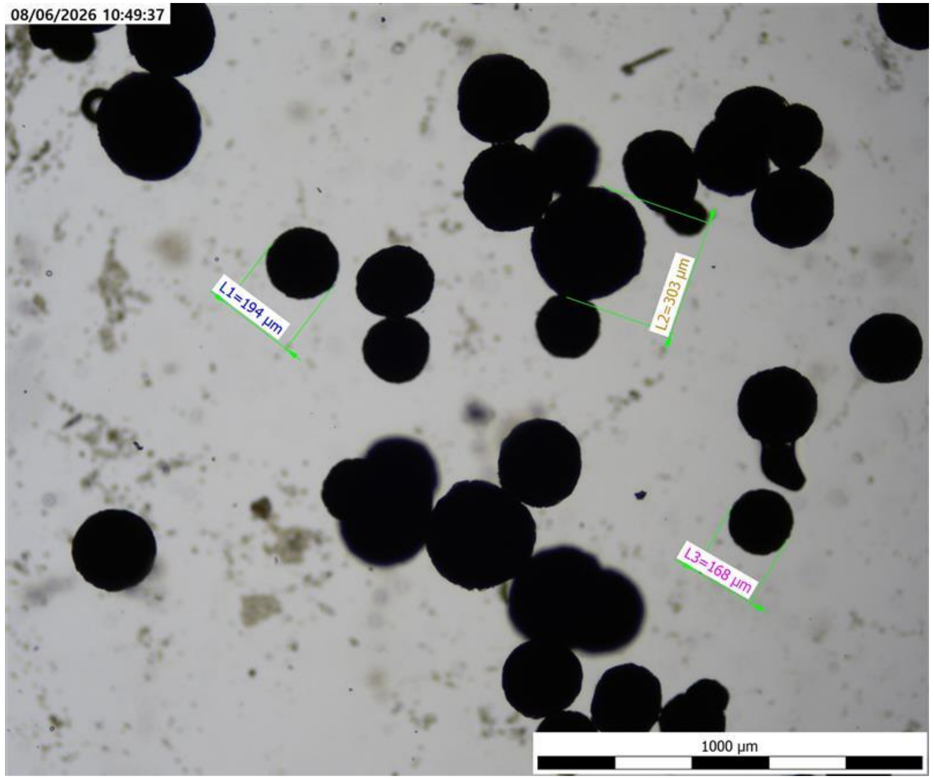
Process Transversing Miscibility Gap

- Occasionally, traversing the miscibility gap is beneficial, if wanting to produce spherical crystals for their good flowability and compatibility performance when tableting.



Contents

What Is Liquid-Liquid Phase Separation?

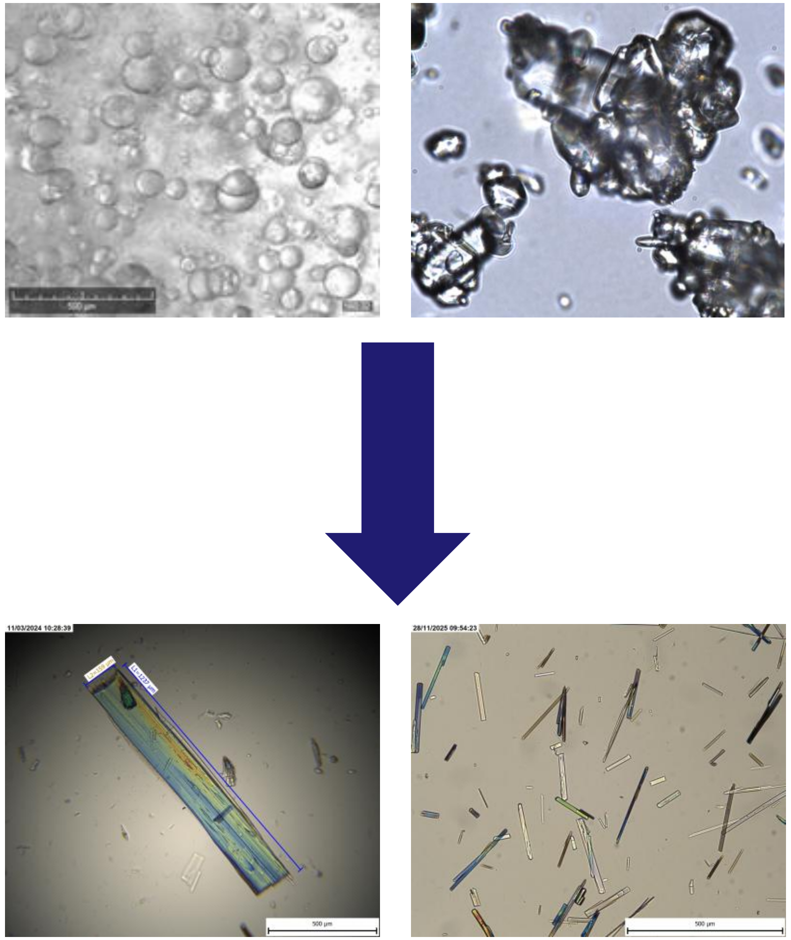
- How Can We Spot LLPS?
- Why Do We Care About Avoiding LLPS?

How Do We Avoid Oiling?

- How Do We Plot LLPS Diagrams?
- How Can LLPS Diagrams Help?

Case Studies

- **Reducing Deposition**
- **Choosing Seeding Parameters**
- **Solvent Choice**



Case Study I – Reducing Deposition

Project aim: Aqueous crystallisation development of an API with the aim of improving crystal shape and reducing agglomeration.

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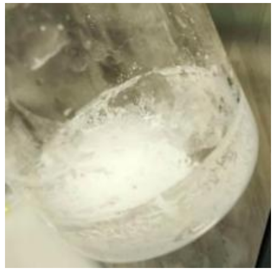

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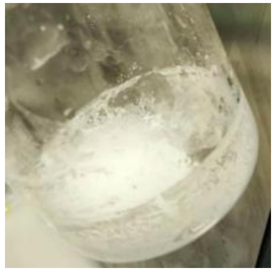

Low isolated yields (theoretical yields by HPLC of >90% but isolated yields of <50%).

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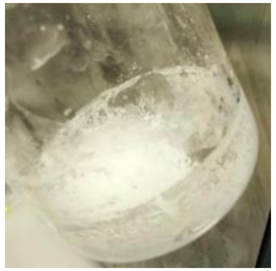
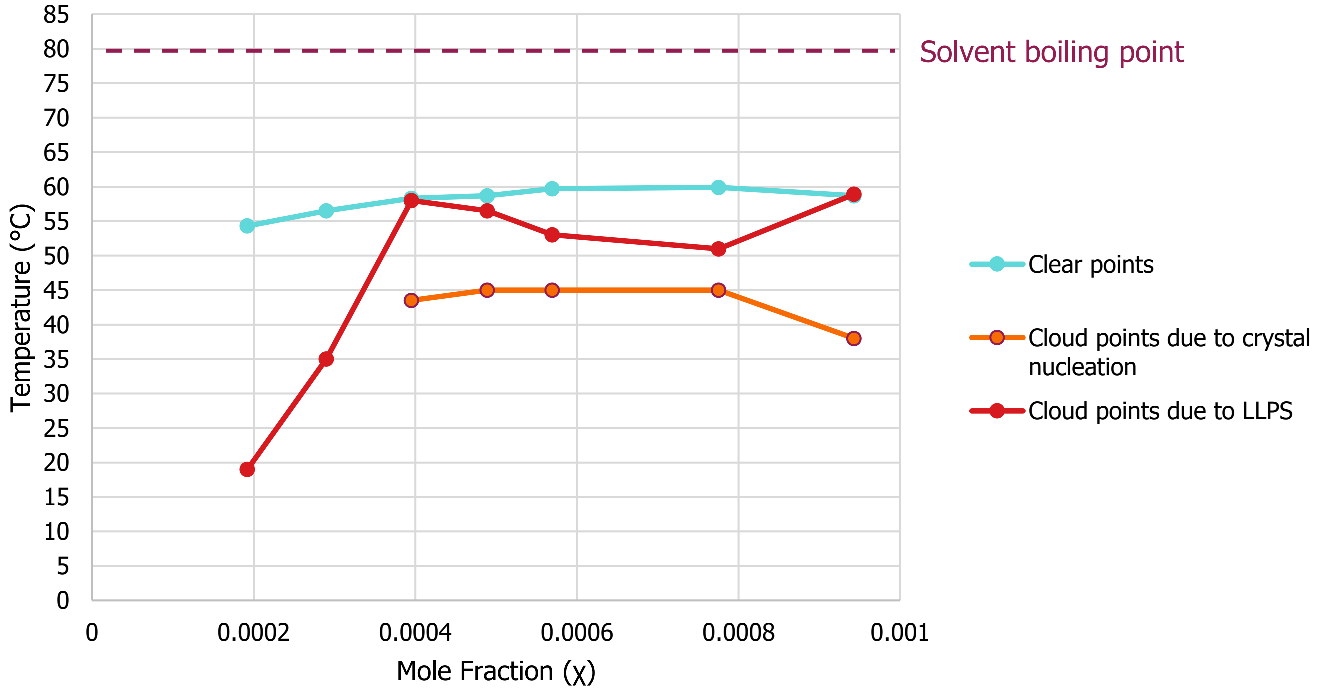


Low isolated yields (theoretical yields by HPLC of >90% but isolated yields of <50%).

Hypothesis: Suspected LLPS

Case Study I – Reducing Deposition

- Suspensions of varying concentrations were slowly heated to 70 °C, clear points were noted.
- Solutions were then slowly cooled to 20 °C and cloud points due to LLPS and nucleation were noted.



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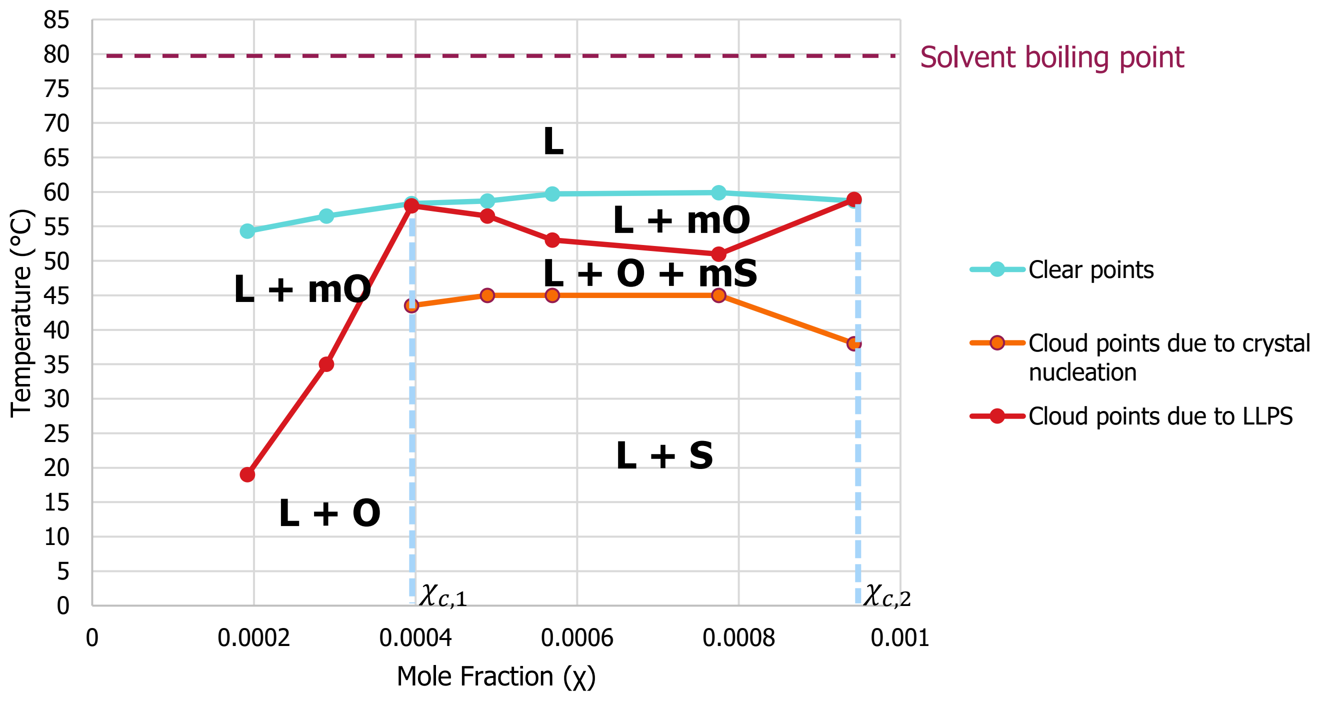

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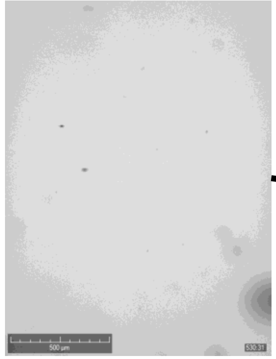

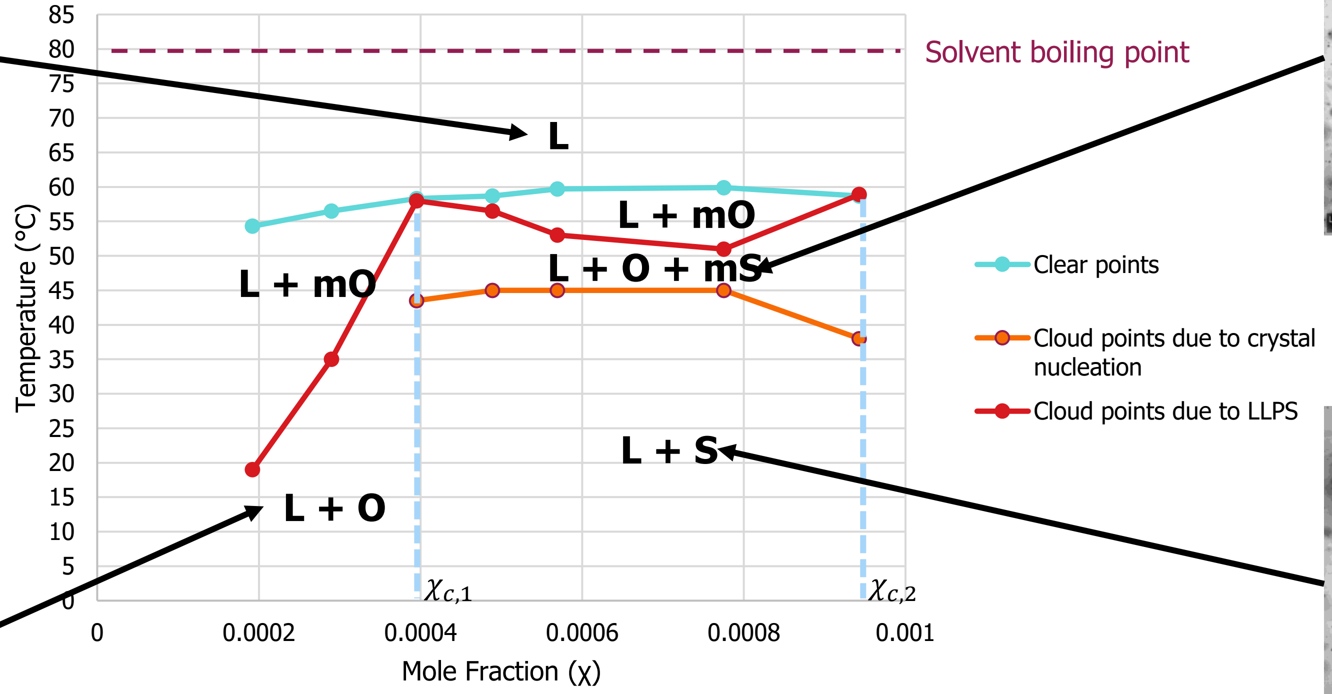

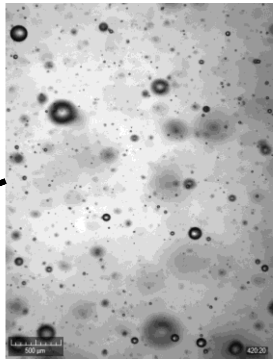

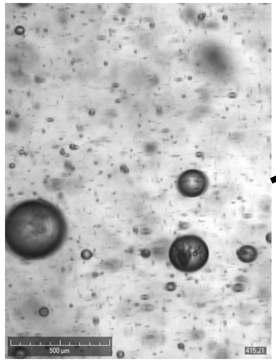

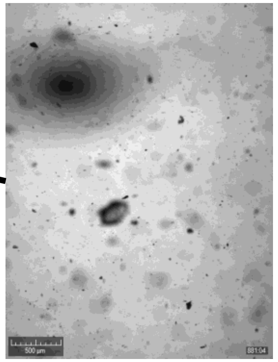

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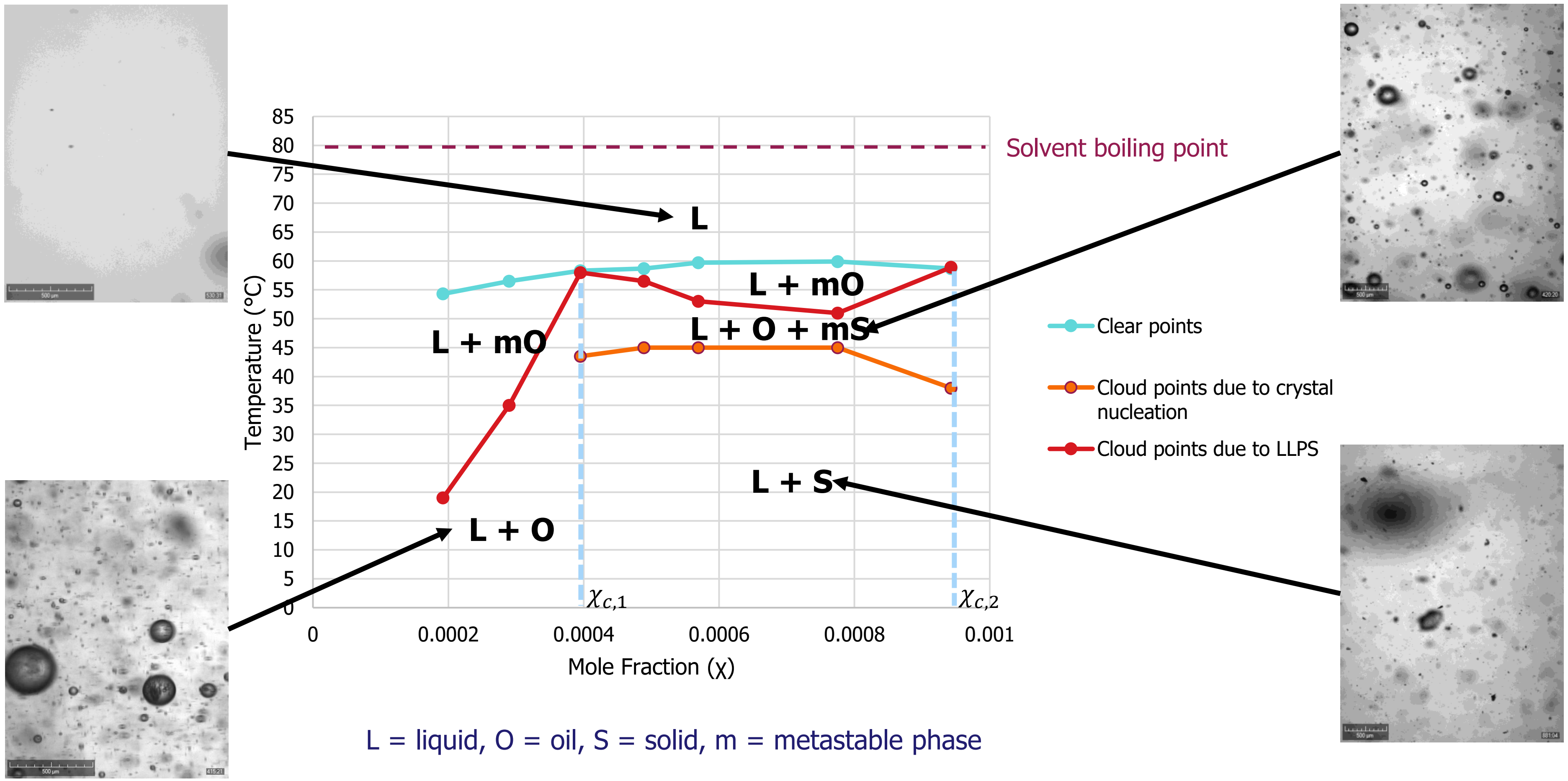
Case Study I – Reducing Deposition

- The diagram shows that in the region investigated, no oil-free mole fraction was identified in the solvent system.



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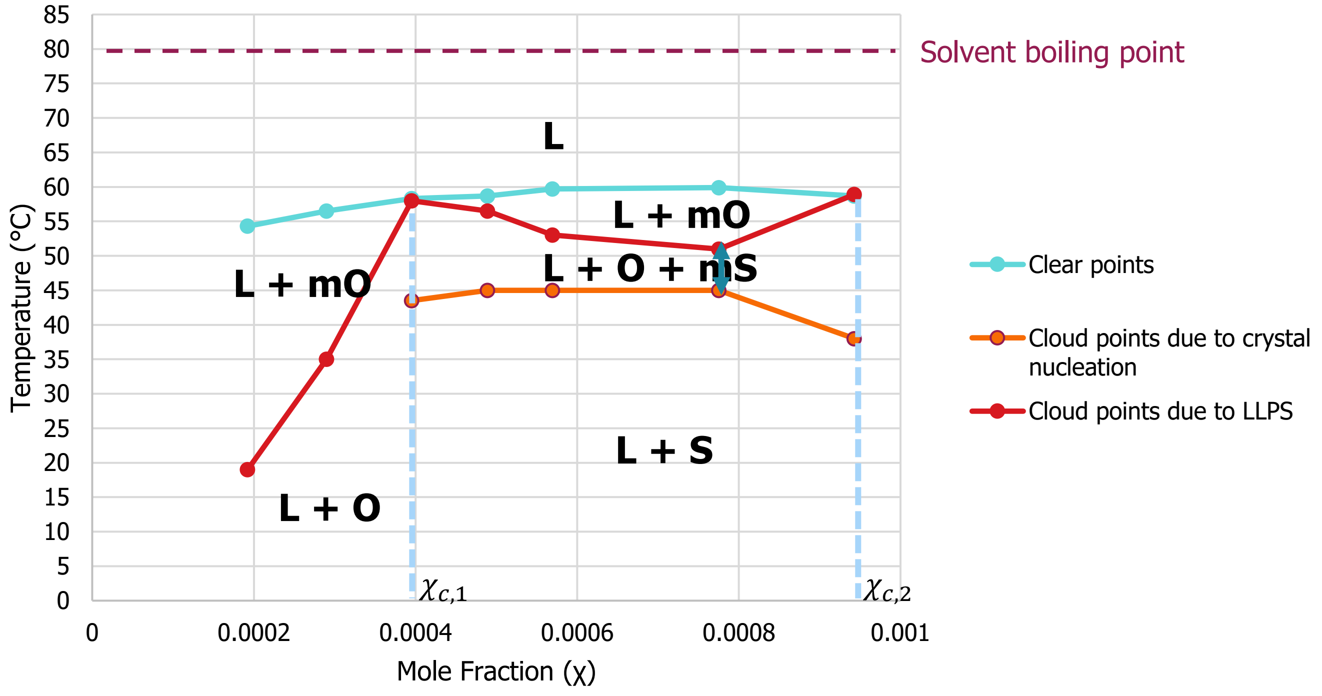
Case Study I – Reducing Deposition

- The diagram shows that in the region investigated, no oil-free mole fraction was identified in the solvent system.
- The narrowest stable oil region **(miscibility gap)** was at 0.00077 mole fraction (20 mg/mL) – this would be the 'safest' region to operate within this this solvent system.



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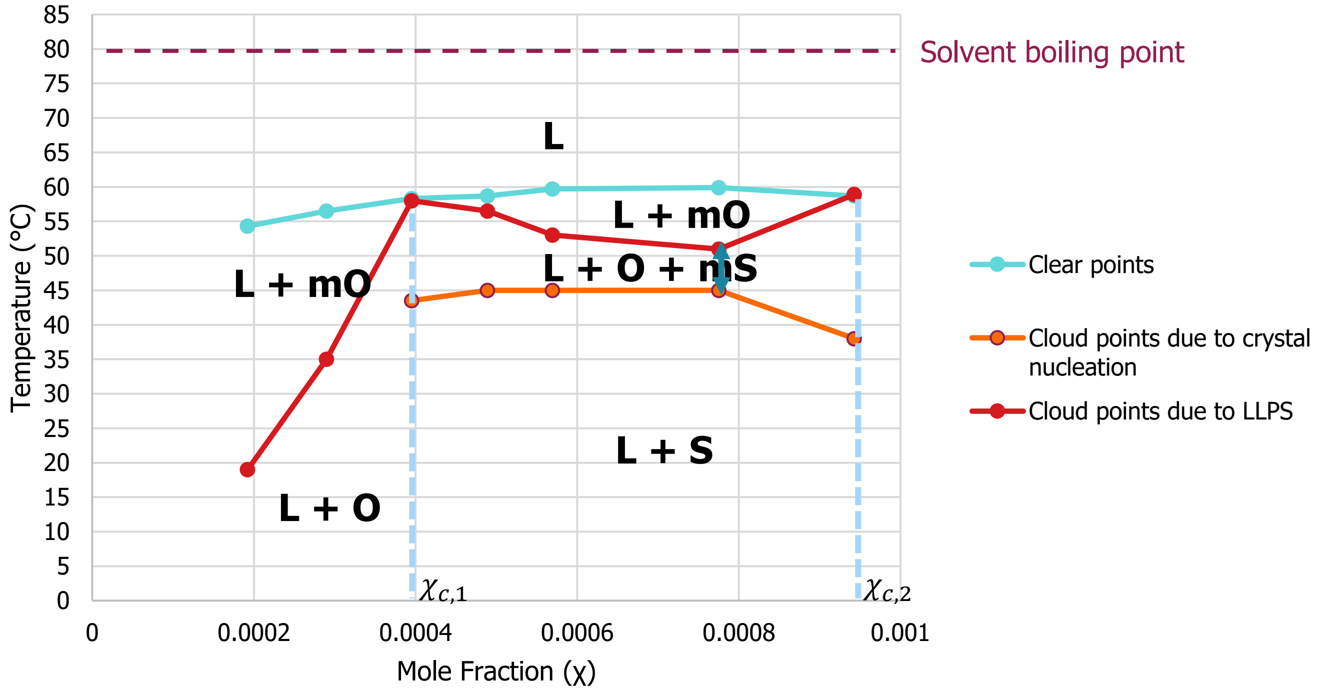
Case Study I – Reducing Deposition

- Changing the solvent system would be advised in this situation.
- Plotting of LLPS diagrams saved time and avoided choosing a solvent system by trial and error.



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Case Study II – Identifying Seeding Conditions

Project aim: Crystallisation development of an API in THF:Water system.

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Deposition observed after seeding.

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Deposition observed after seeding.

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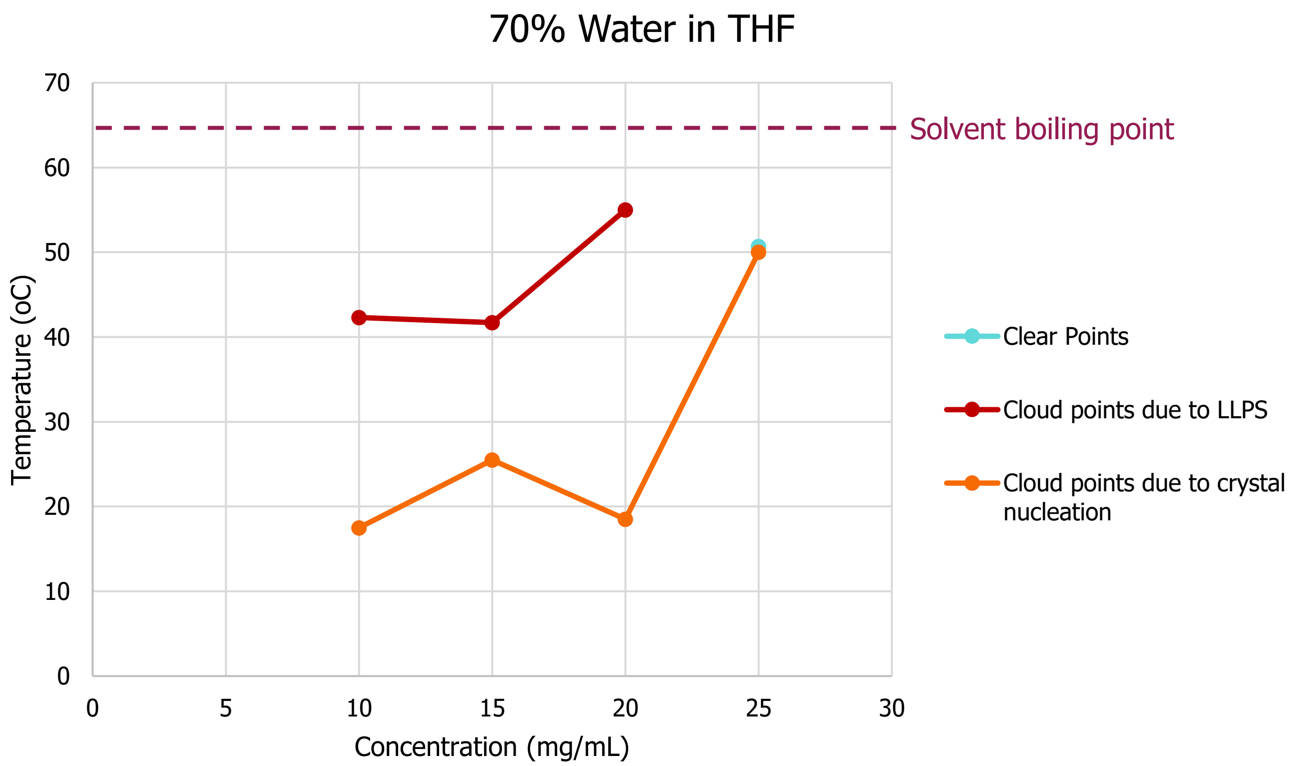
↓

Solution: Plot LLPS diagrams in different THF:Water ratios to find a safe region for seeding

Case Study II – Identifying Seeding Conditions

- Suspensions of varying concentrations were slowly heated to 60 °C, clear points were noted.
- Solutions were then slowly cooled to 20 °C and cloud points due to LLPS and nucleation were noted.



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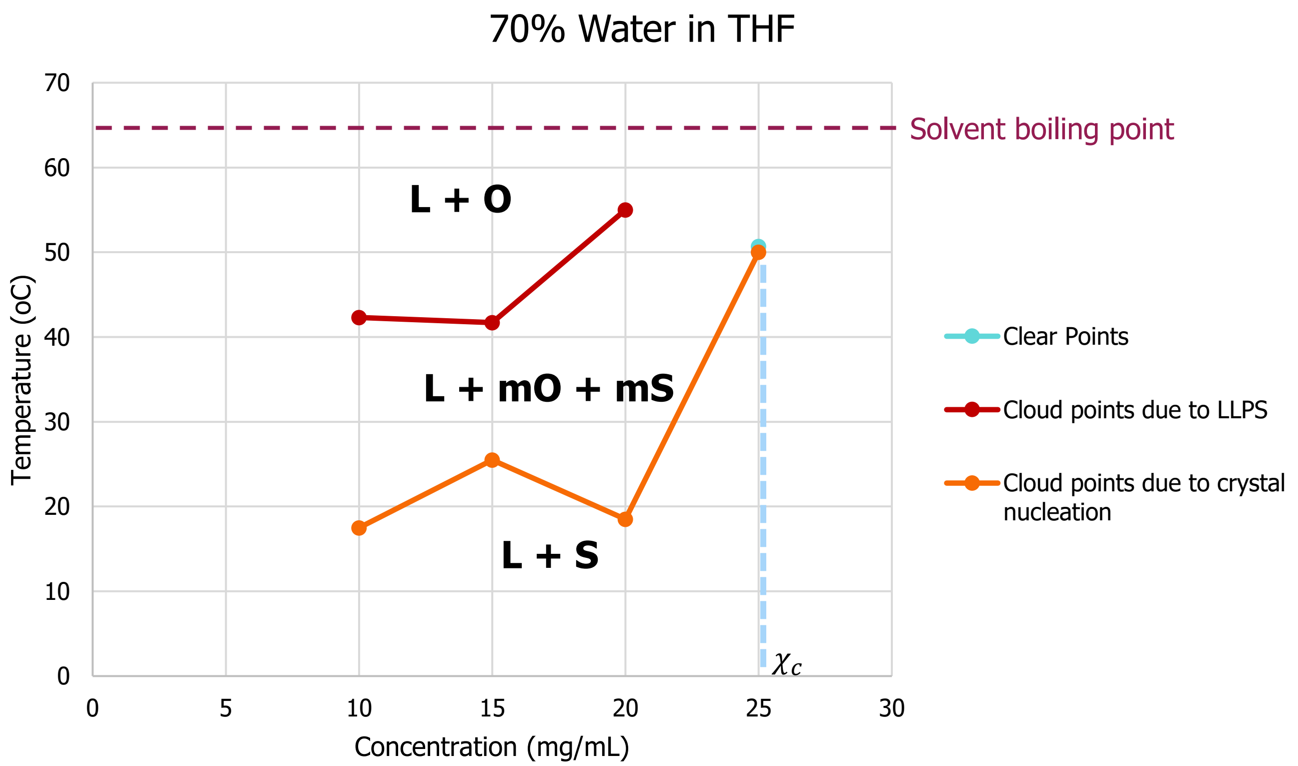

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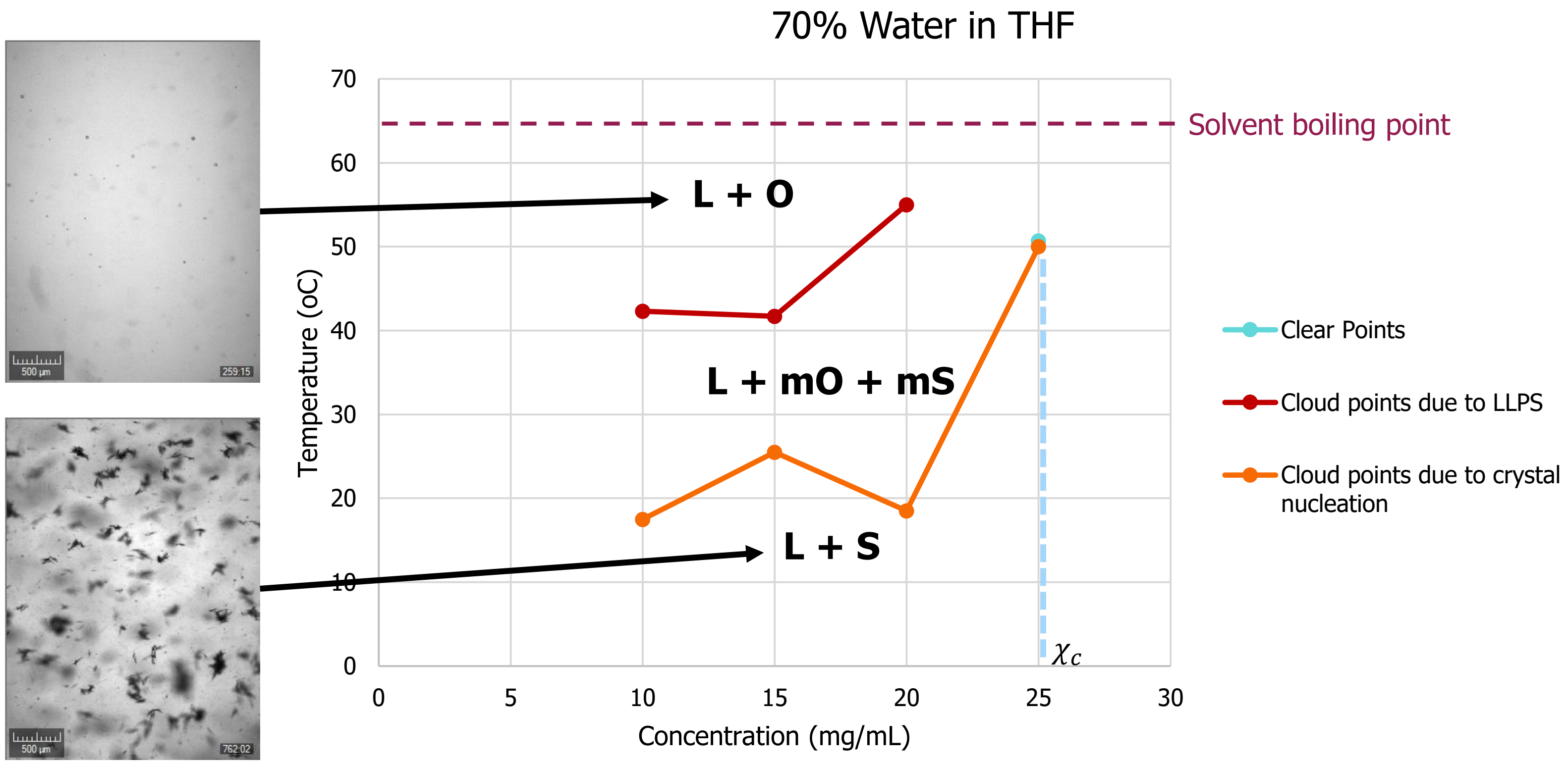

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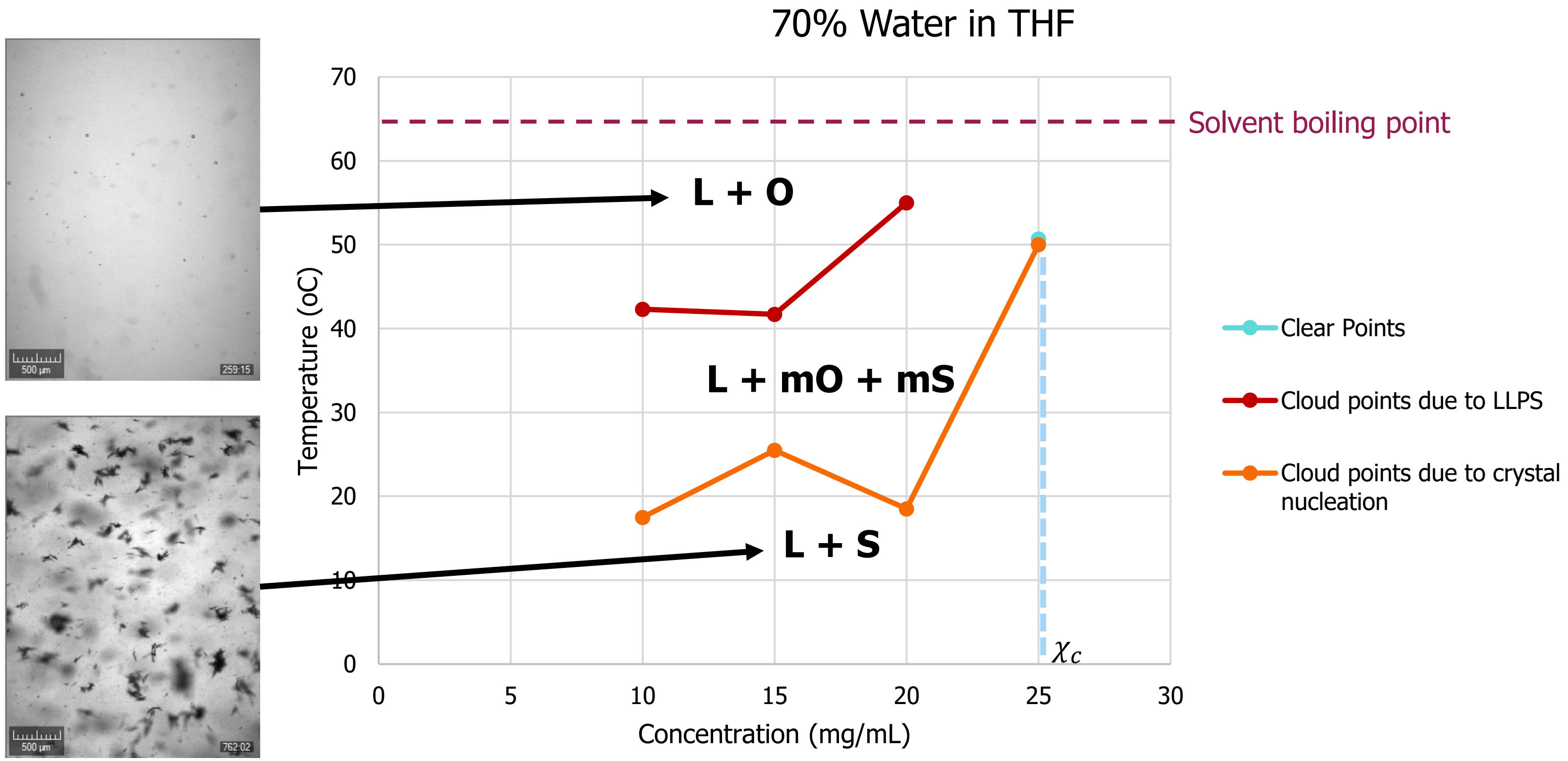
Case Study II – Identifying Seeding Conditions

- On heating the 10 mg/mL, 15 mg/mL and 20 mg/mL suspensions, no clear points were observed, instead oiling was observed at high temperatures, resulting in a high temperature **L + O** phase.
- To observe clear points for all concentrations, in theory the suspensions would need to be heated to higher temperatures, but this is not possible due to the boiling point of THF being 66 °C.



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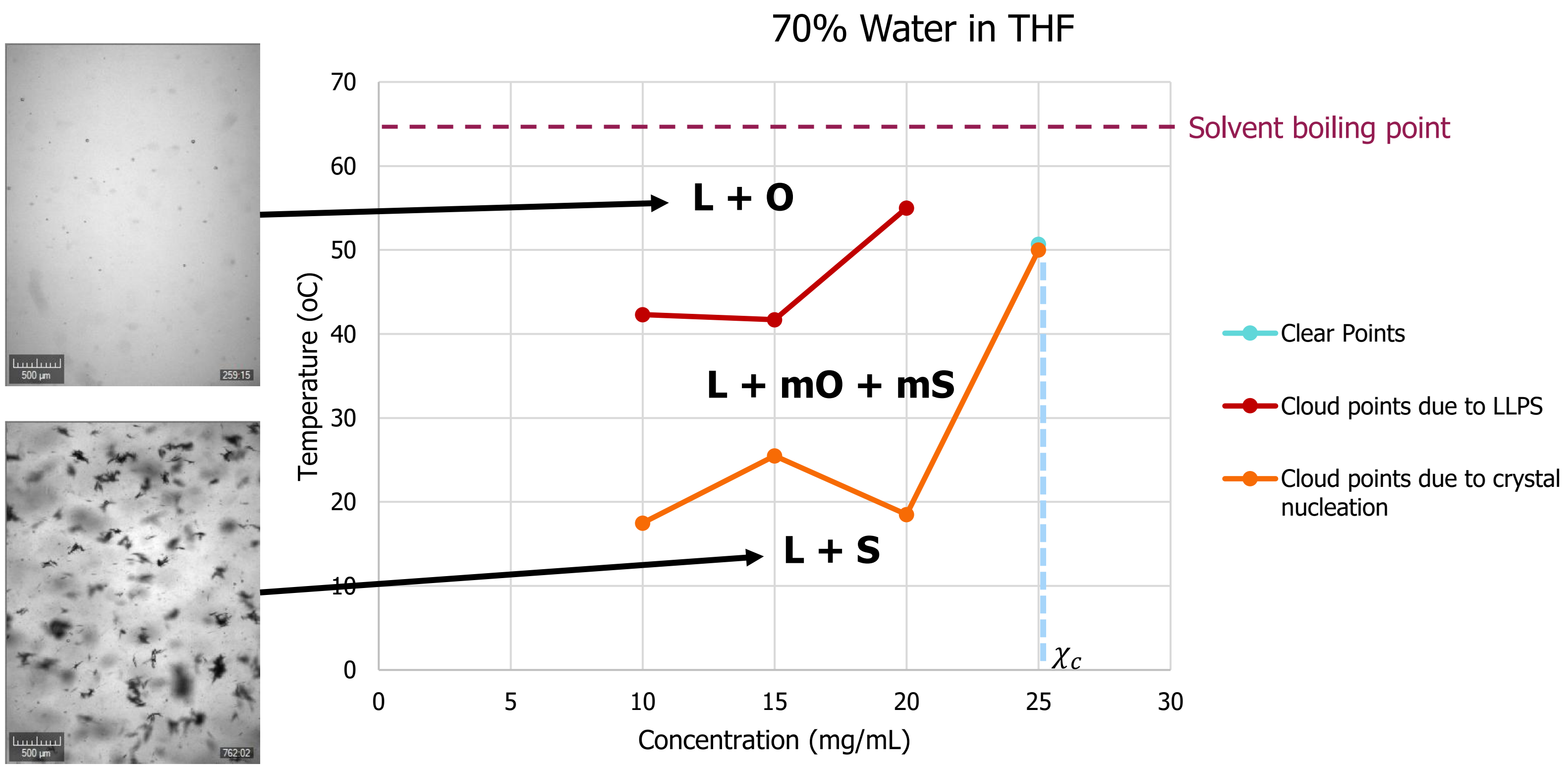
Case Study II – Identifying Seeding Conditions

- A critical concentration was observed at 25 mg/mL.
- Above this concentration there is likely to be no oil region.



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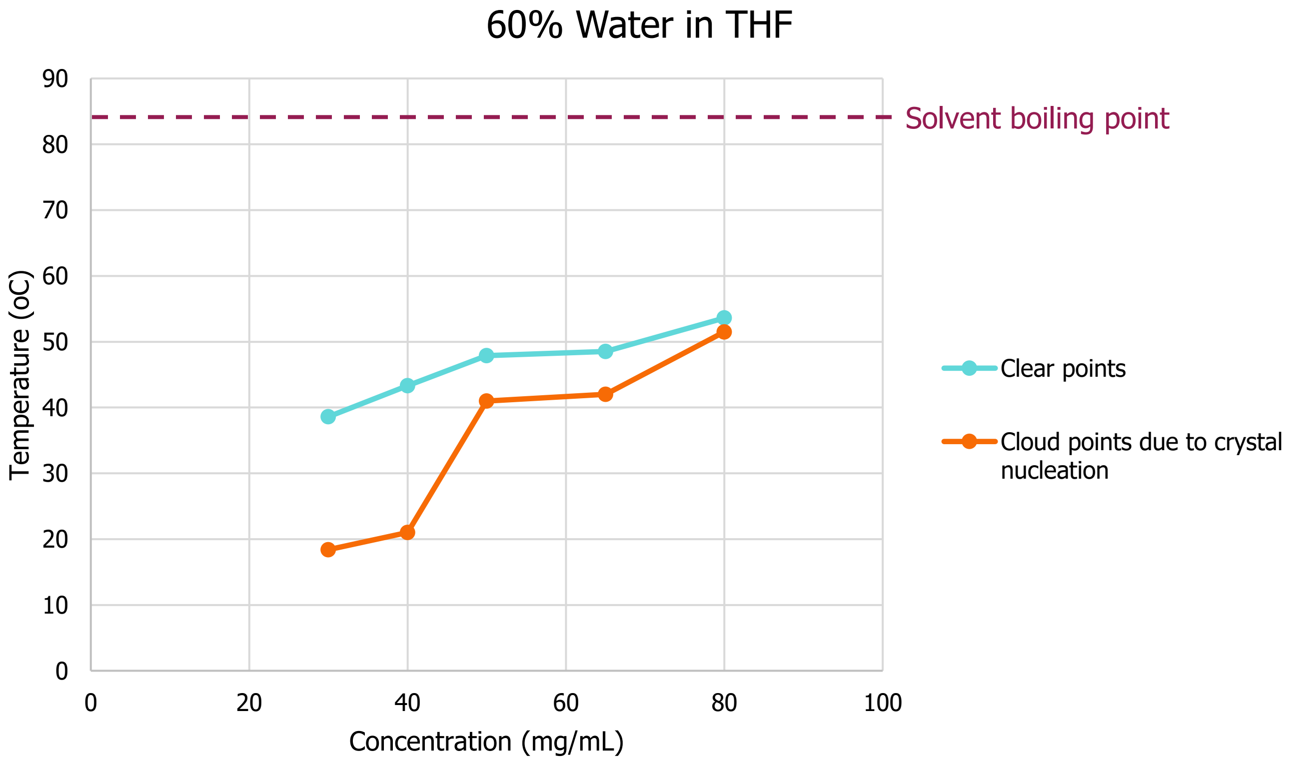
Case Study II – Identifying Seeding Conditions

- Water content was reduced to see if this would reduce the tendency for the API to oil out.



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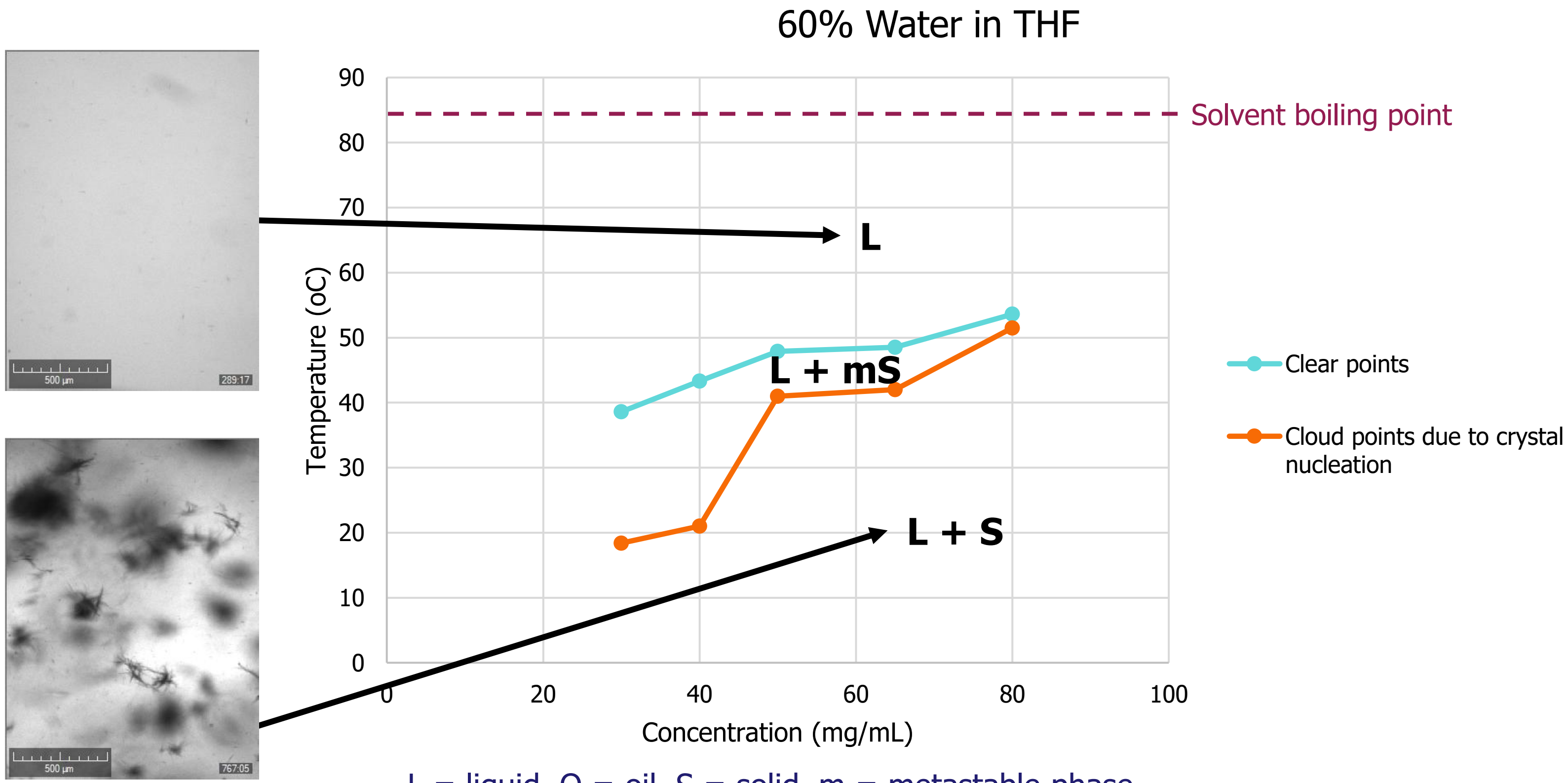
Case Study II – Identifying Seeding Conditions

- Water content was reduced to see if this would reduce the tendency for the API to oil out.
- No oiling was observed in the concentration range investigated.
- As the concentration increases, the clear and cloud points begin to converge – an indication at higher concentrations, an oil phase may be observed.



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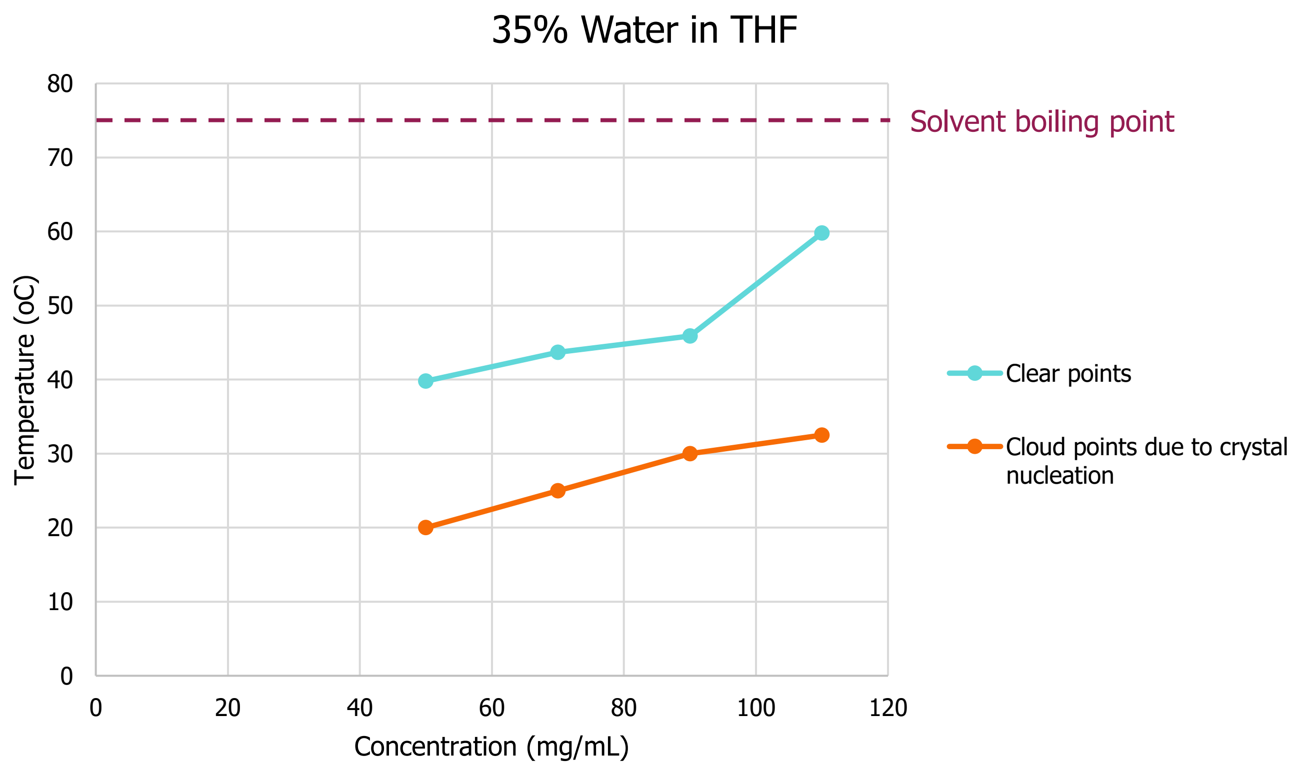
Case Study II – Identifying Seeding Conditions

- Water content was further reduced to see the effect on the oil phase.



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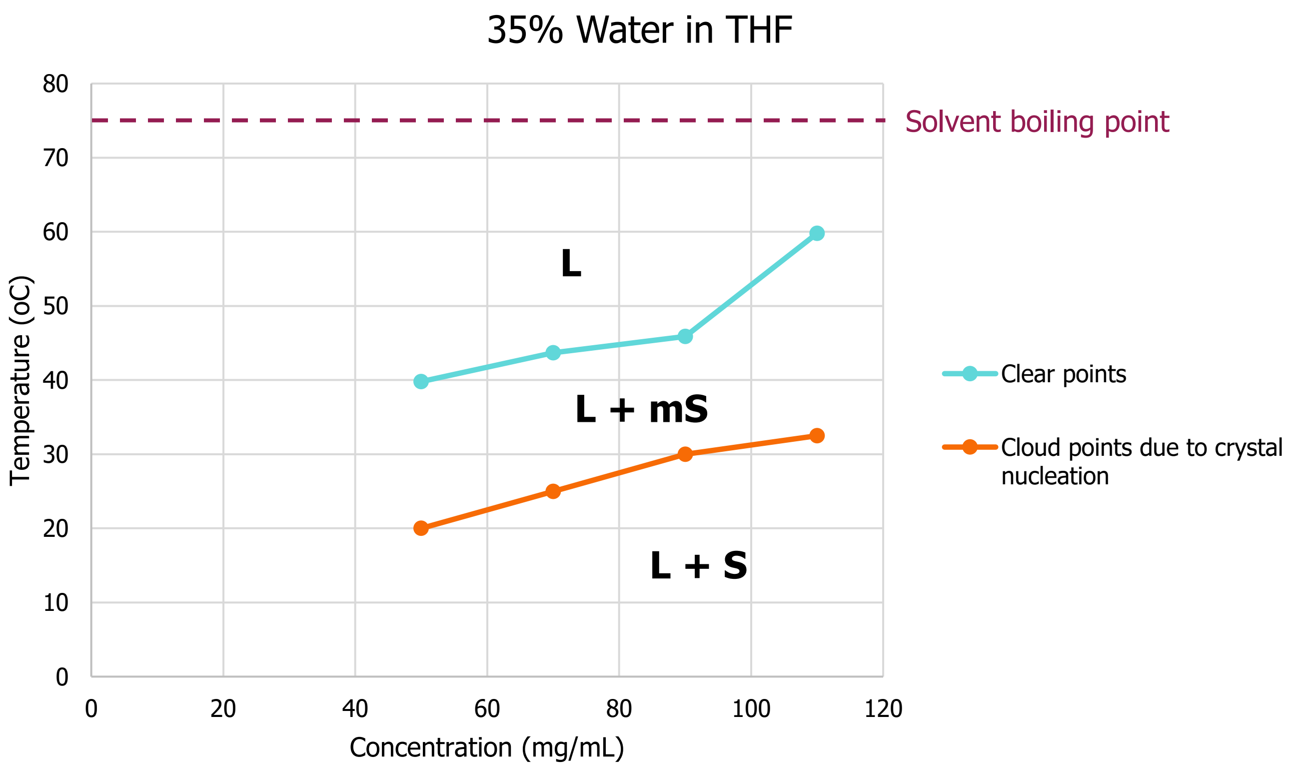
Case Study II – Identifying Seeding Conditions

- Water content was further reduced to see the effect on the oil phase.
- No oiling was observed in the concentration range investigated.
- No convergence of clear and cloud points.
- **Conclusion:** It was recommended to seed in 35% water in THF.



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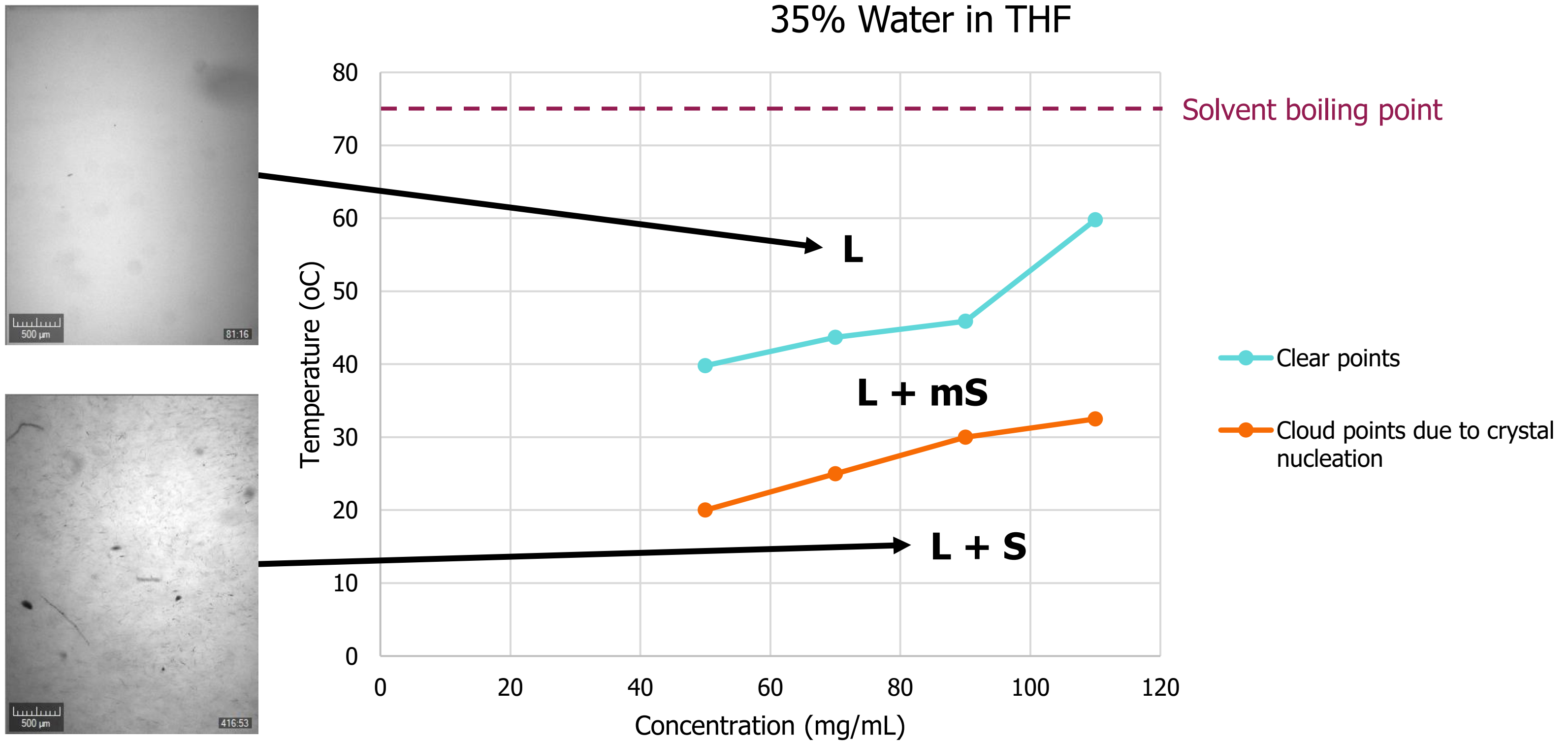

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Case Study III – Solvent Choice to Avoid LLPS

Project aim: Crystallisation development in various solvent systems to target a specific particle size distribution (PSD).

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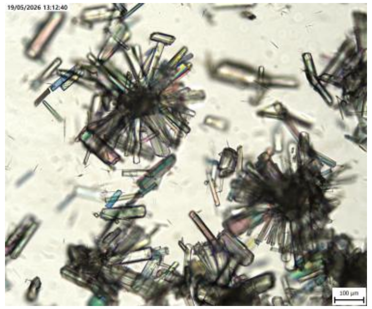

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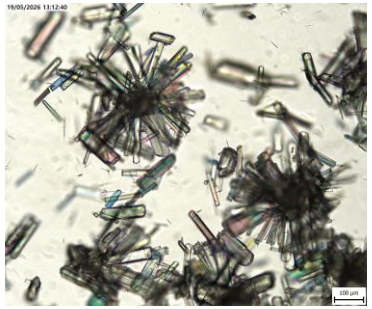

Clusters indicated oiling and resulted in PSD too large to meet the specification
Computational calculations indicated polarity of solvent may be utilised to reduce oiling.

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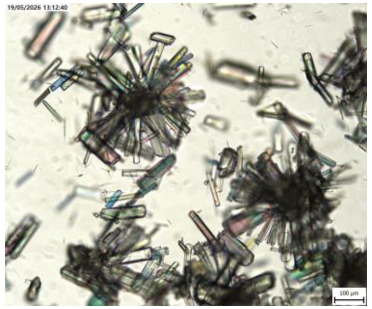

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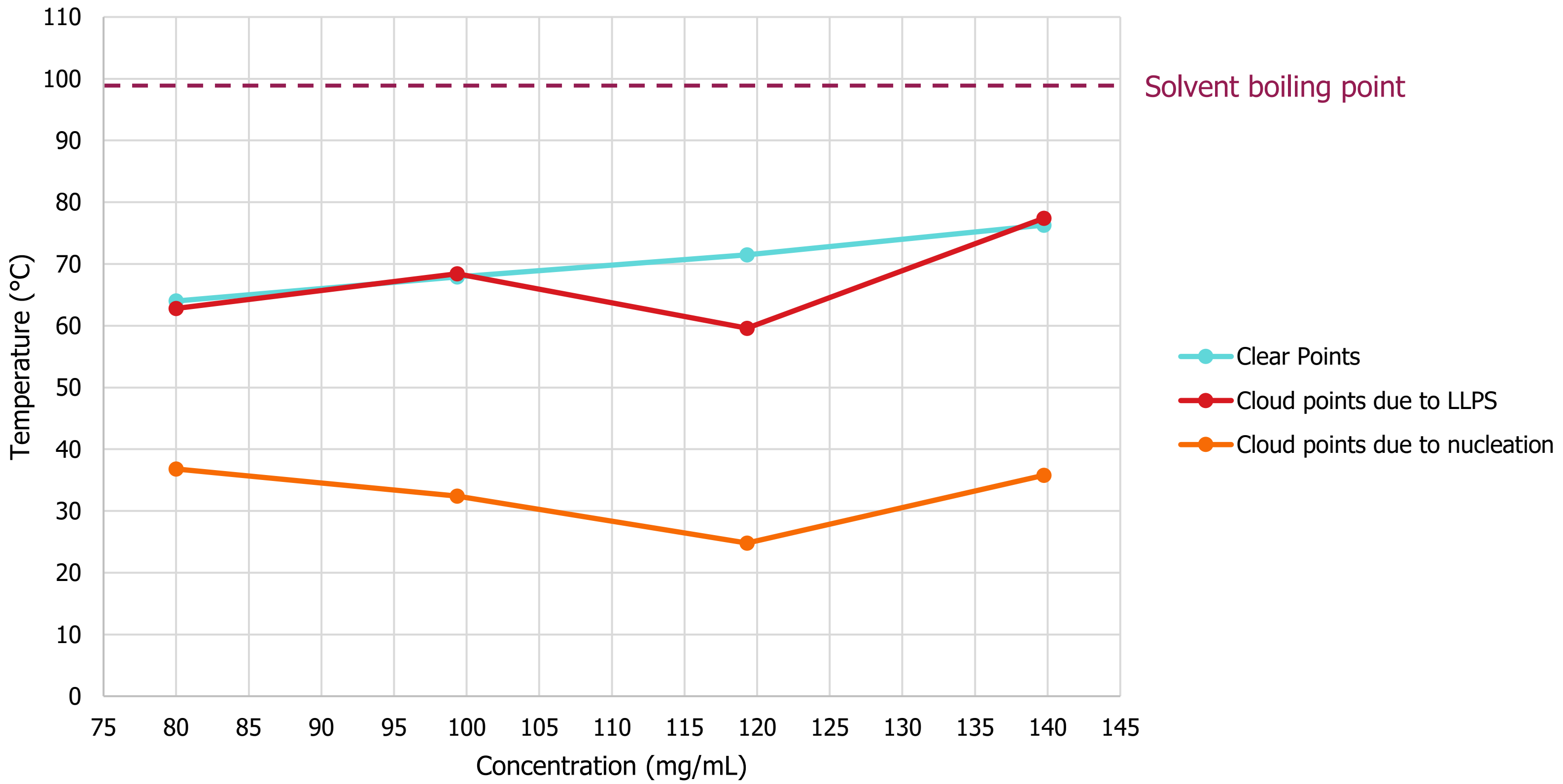
Solution: Plotting of LLPS diagrams

Case Study III – Solvent Choice to Avoid LLPS

- Initially, a LLPS diagram was plotted in 35:65 2-Butanol:Heptane, the solvent system that seeding was occurring in.
- Suspensions of varying concentrations were slowly heated to 90 °C, clear points were noted.
- Solutions were then slowly cooled to 20 °C and cloud points due to LLPS and nucleation were noted.



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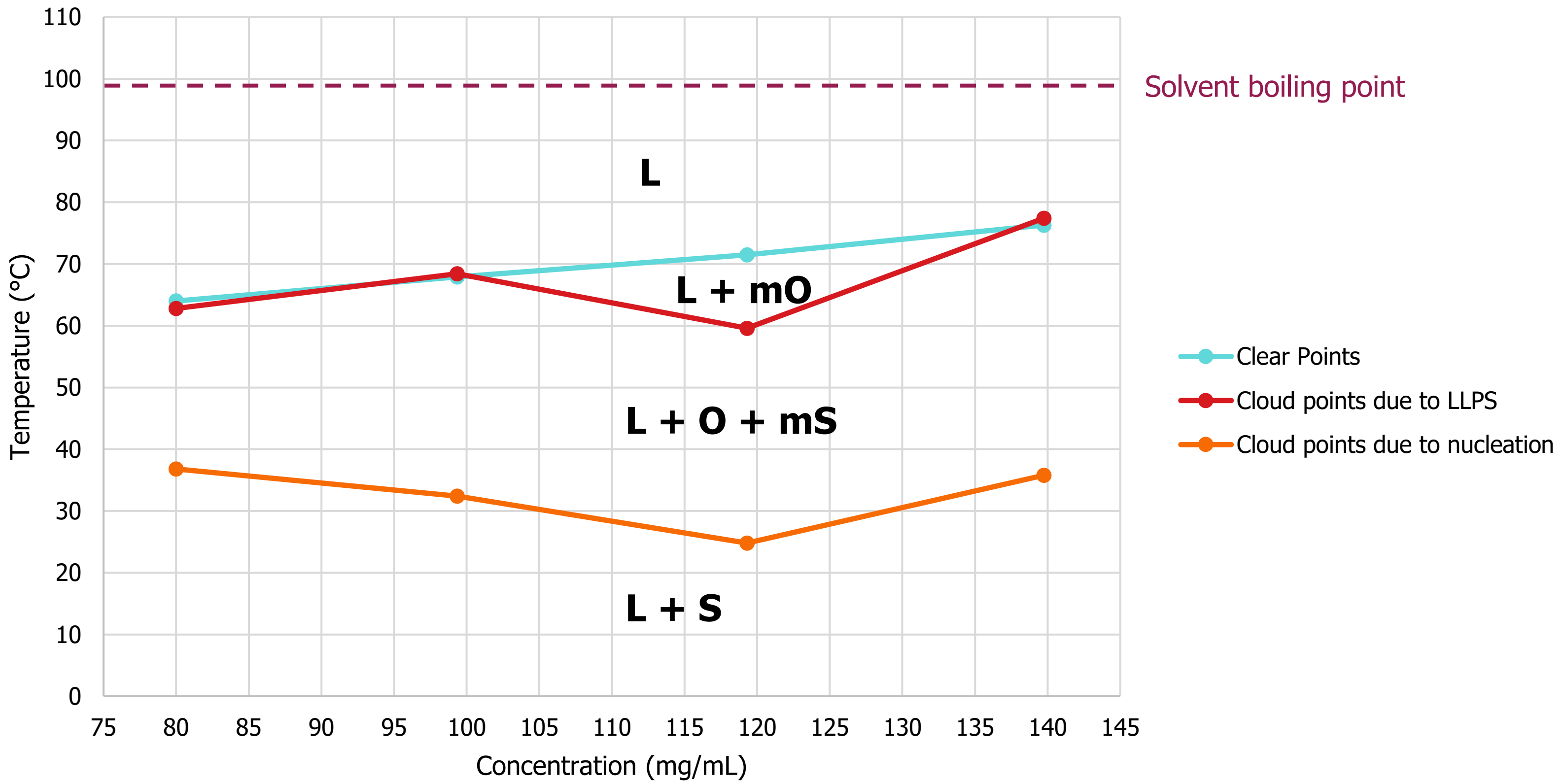

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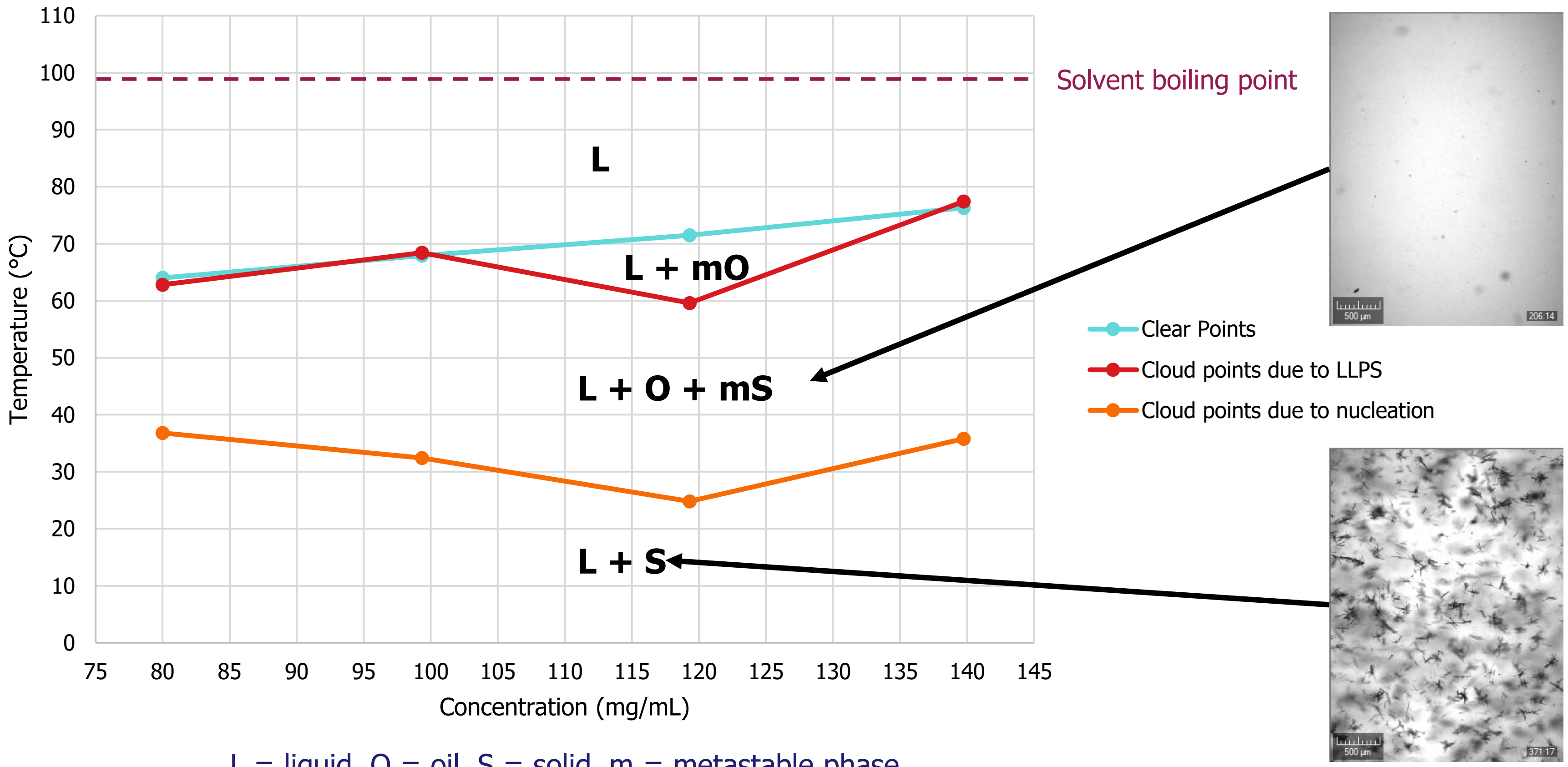

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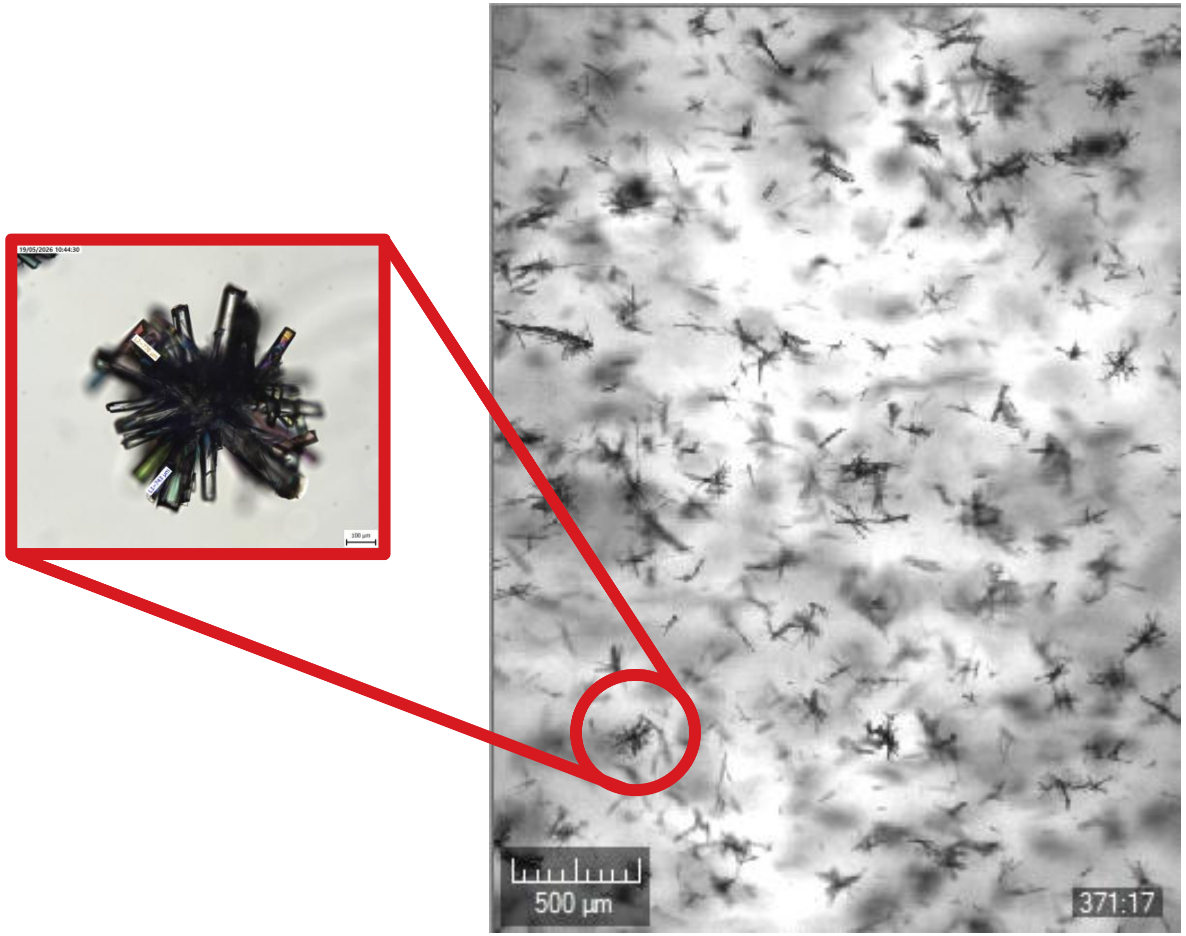
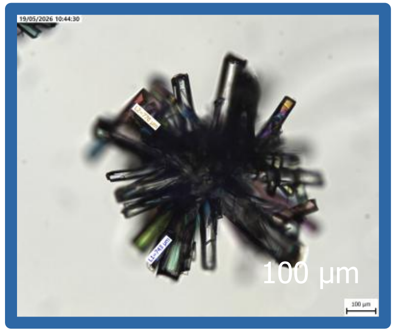
Case Study III – Solvent Choice to Avoid LLPS

- We saw clusters forming in the **L+S** phase as they had grown from oil droplets



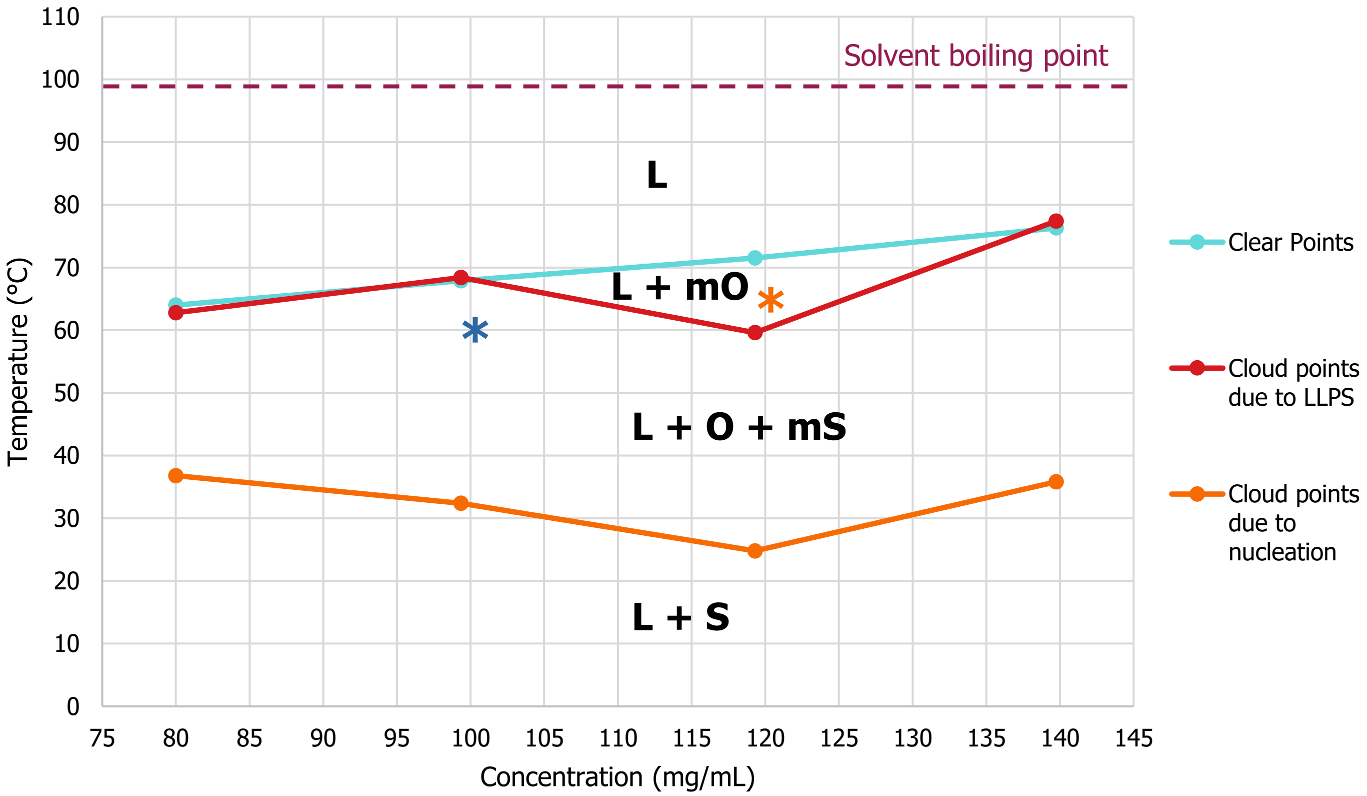
Case Study III – Solvent Choice to Avoid LLPS

- Two seeding point experiments were conducted in 35:65 2-Butanol:Heptane:
  - Seeding at 60 °C in a 100 mg/mL solution
    - To investigate if oiling could be avoided by seeding close to the liquid phase boundary.
  - Seeding at 65 °C in a 120 mg/mL solution
    - To investigate if oiling could be avoided by seeding in the metastable oil region.
- Clustering was seen in both experiments.
- No good seeding conditions were found.



100 mg/mL, seeding at 60 °C

120 mg/mL, seeding at 65 °C



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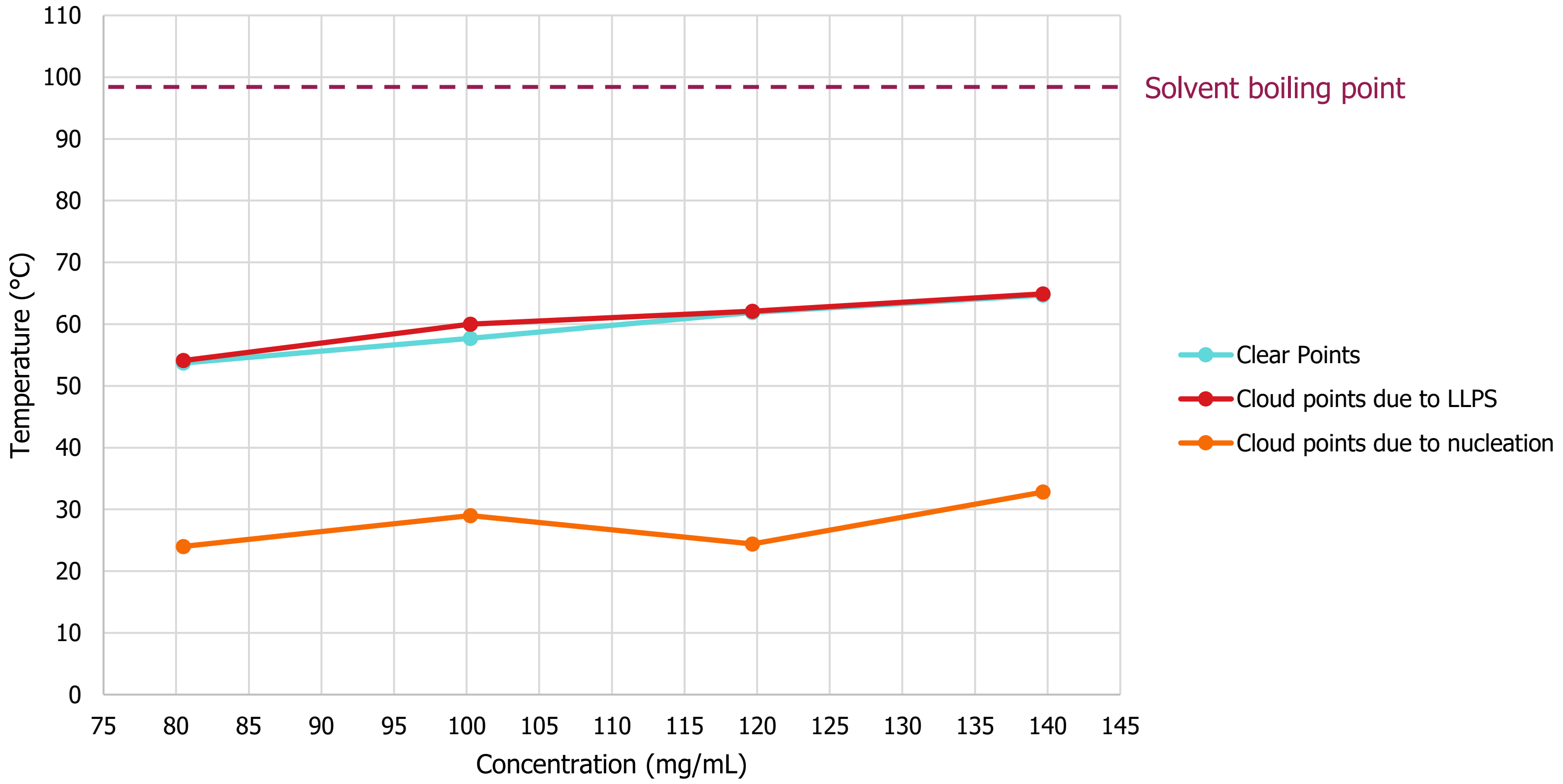
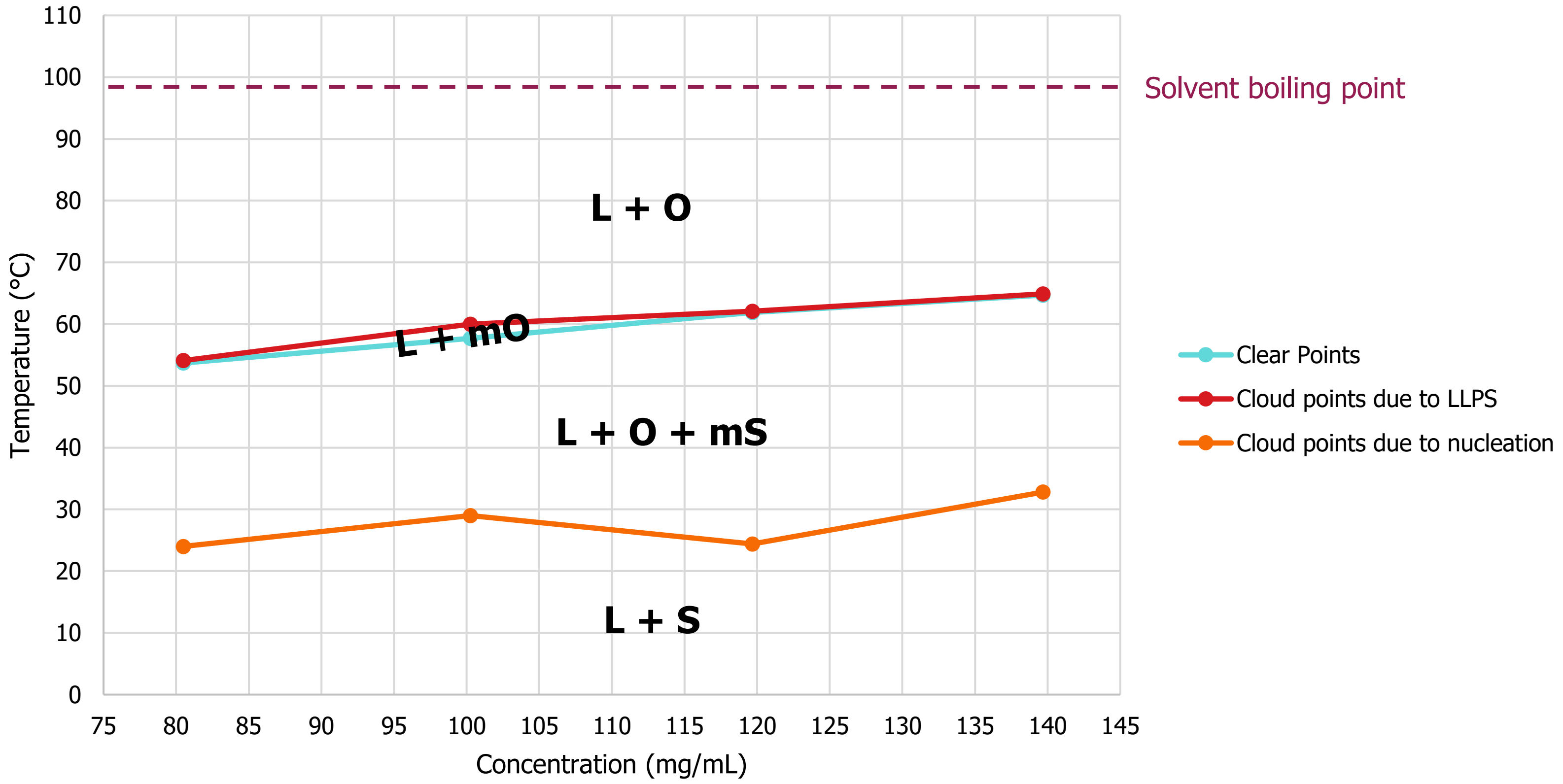
Case Study III – Solvent Choice to Avoid LLPS

- It was decided to increase the 2-Butanol percentage to see if this reduced the oiling.
- A LLPS diagram was plotted in **45:55 2-Butanol:Heptane**.



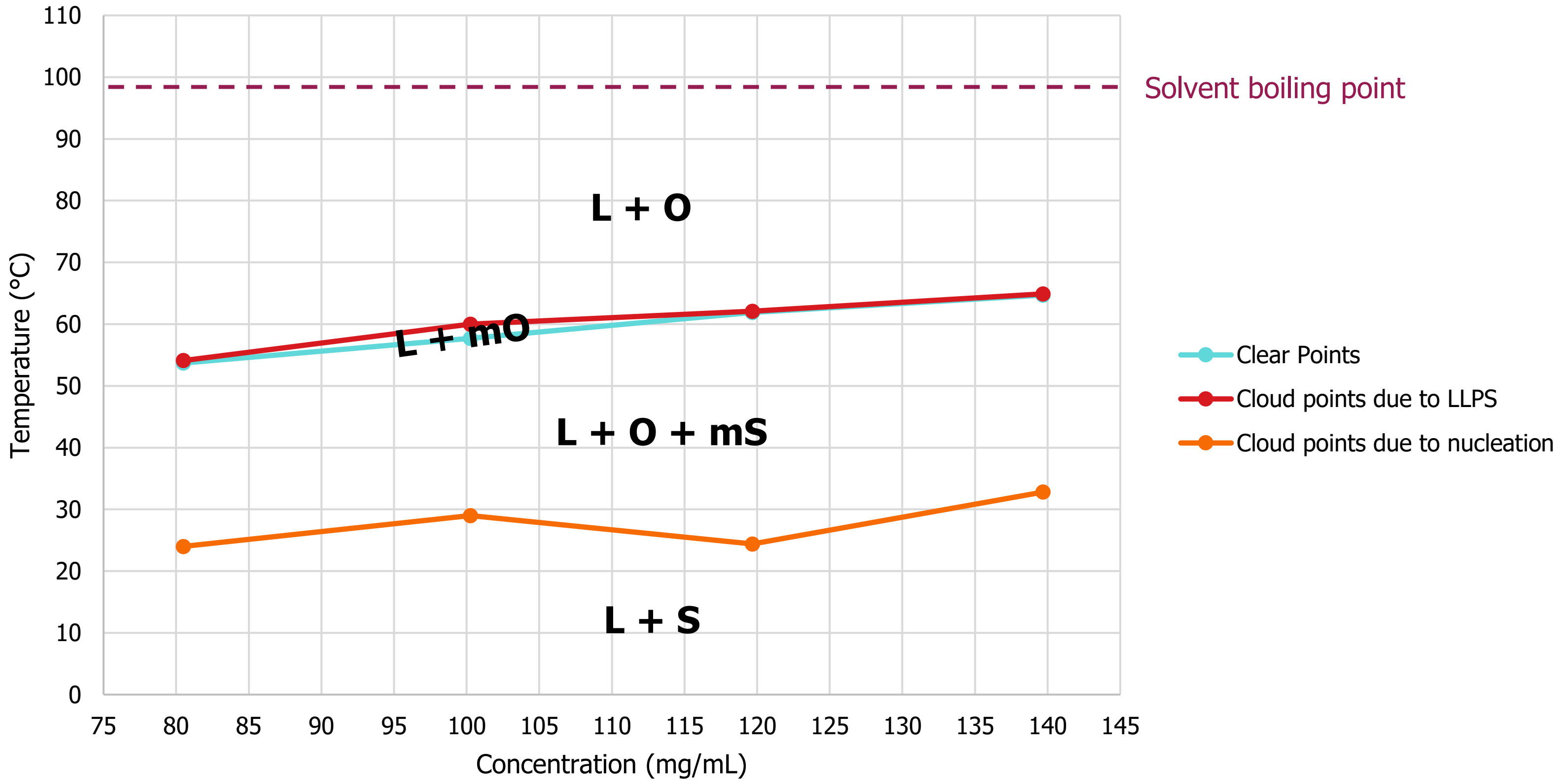
Case Study III – Solvent Choice to Avoid LLPS

- On heating each suspension, solid dissolved (represented by the clear points).
- Almost as soon as dissolution was observed, oil droplets were seen, these persisted to the maximum temperature and were observed on cooling, until nucleation was observed.



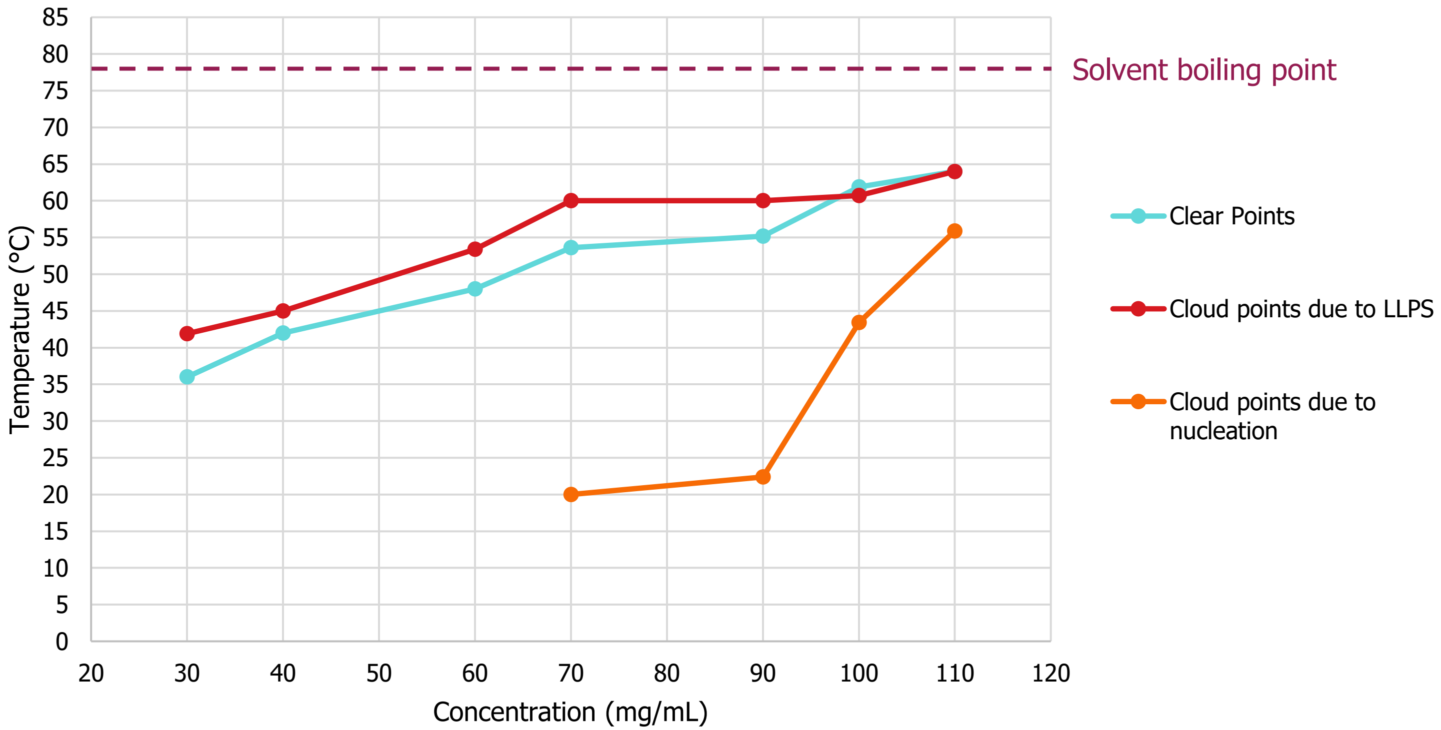
Case Study III – Solvent Choice to Avoid LLPS

- No true clear point was identified when the system existed as a single liquid phase – it is not possible to generate supersaturation without the oil phase being present.
- This system thus had no seeding opportunity.



Case Study III – Solvent Choice to Avoid LLPS

- EtOAc was investigated as a solvent system.



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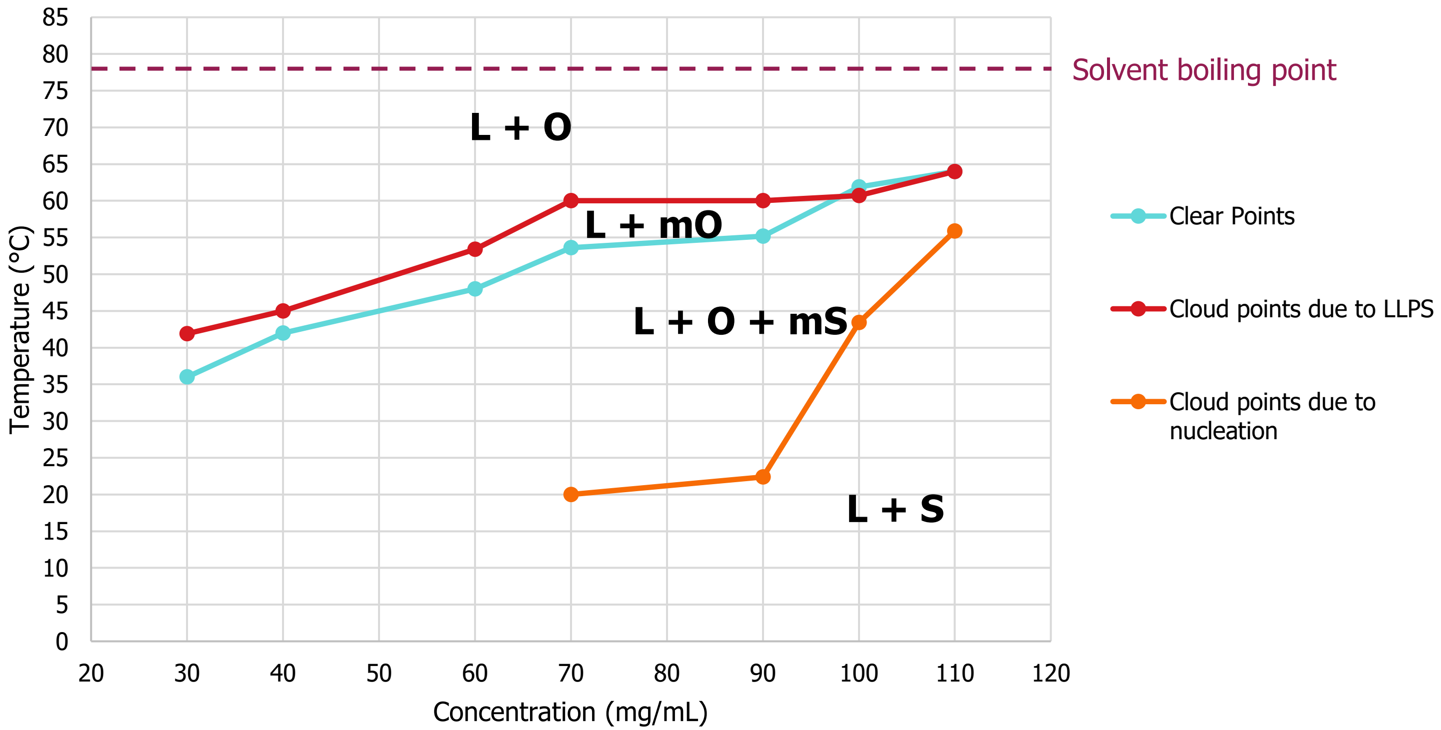
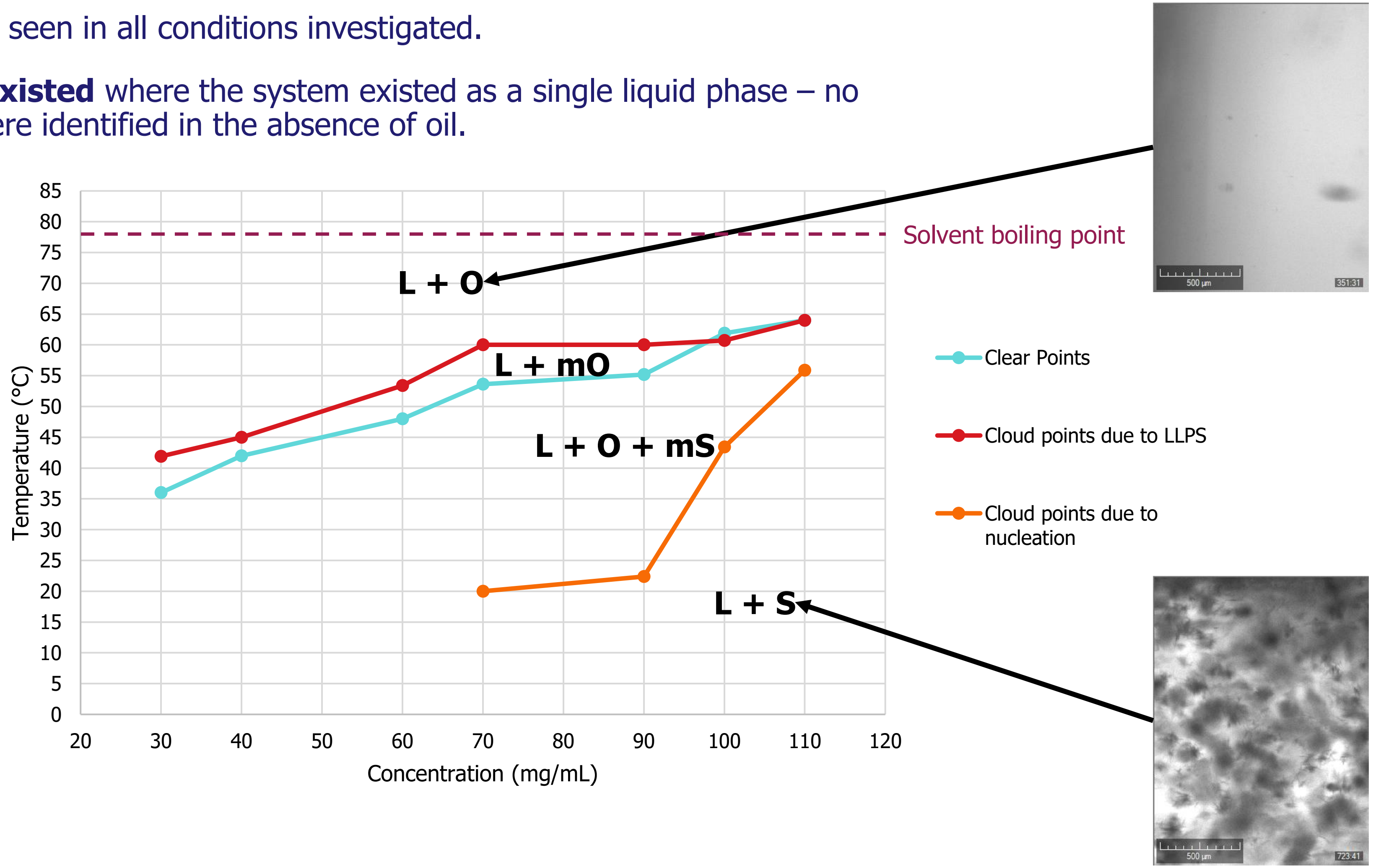


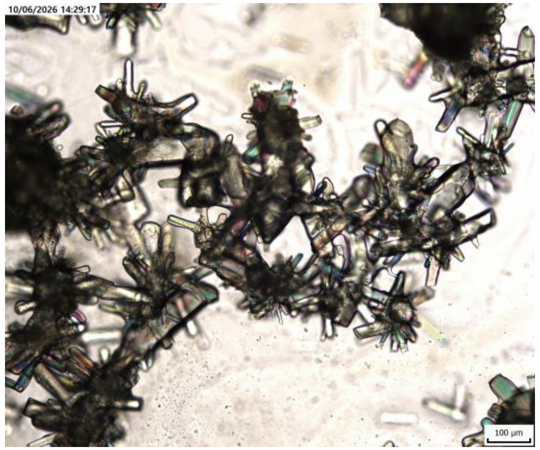
Case Study III – Solvent Choice to Avoid LLPS

- EtOAc was investigated as a solvent system.
- Unfortunately, oiling was seen in all conditions investigated.
- **No 'true clear point' existed** where the system existed as a single liquid phase – no seeding opportunities were identified in the absence of oil.



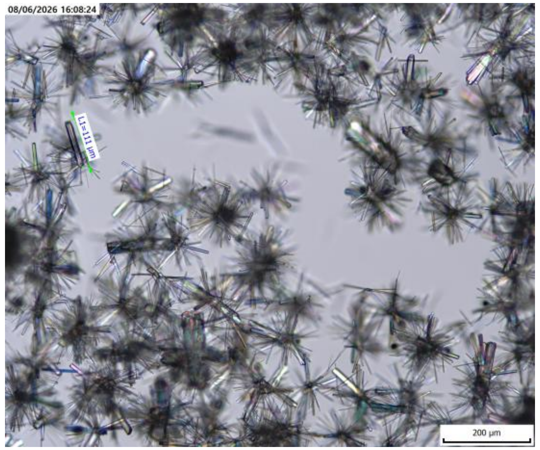
Case Study III – Solvent Choice to Avoid LLPS

- LLPS plots of range of solvents showed that a true clear point with seeding opportunity could not be found, hence a change in approach was required to minimising effects of oiling.
- We had to find a 'best case scenario'.
- Throughout the project, crystallisations had been conducted with seeding to observe crystal habit in different solvent systems – despite seeding not being able to occur in a clear point.
- A few examples are shown below:

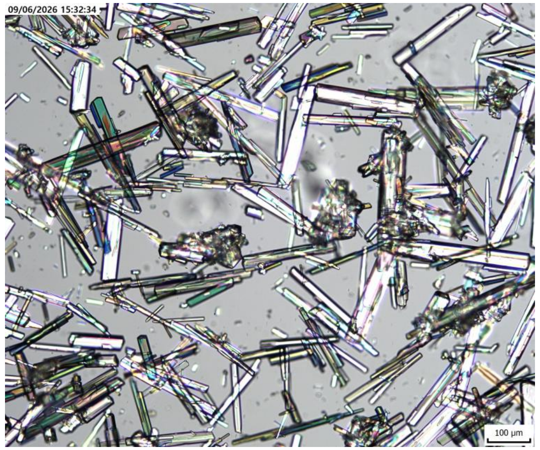


50:50 MeCN:Toluene

75:25 2-Methyl-2-butanol:Heptane



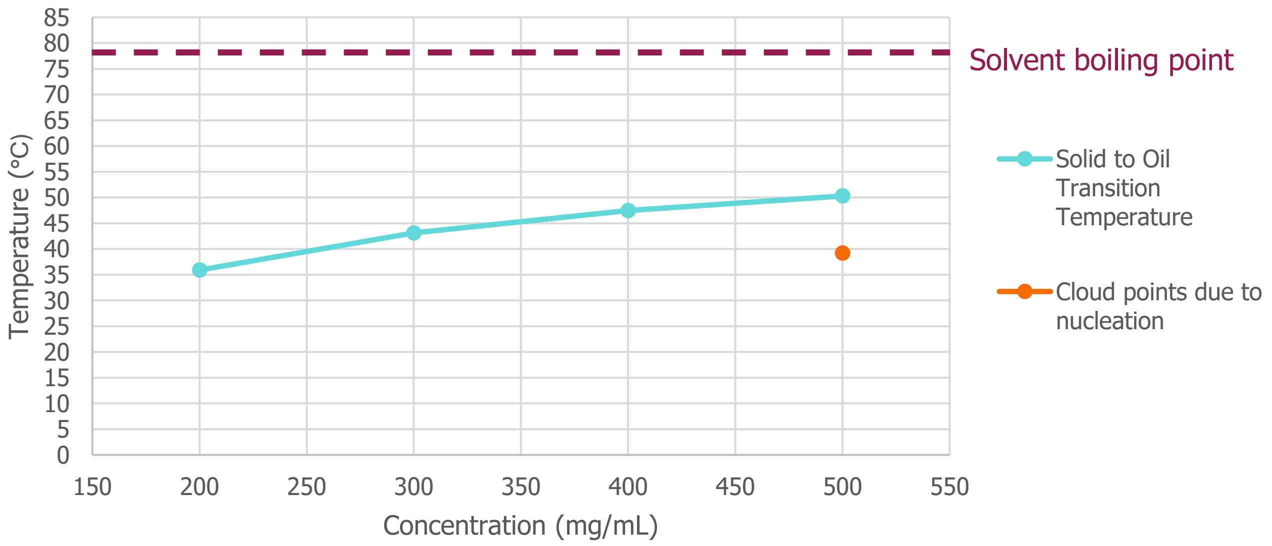
IPA



75:25 EtOH:Heptane

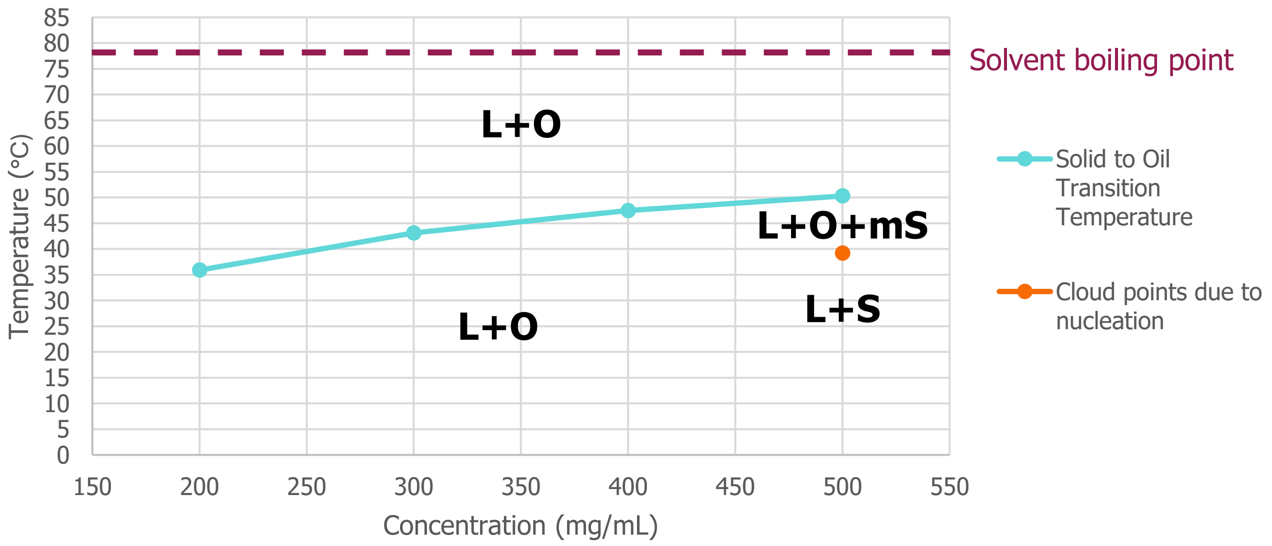
Case Study III – Solvent Choice to Avoid LLPS

- It was decided to investigate EtOH as a solvent.
- It was thought a more polar solvent would help by coordinating to the solvent, presenting it from cyclising on itself.



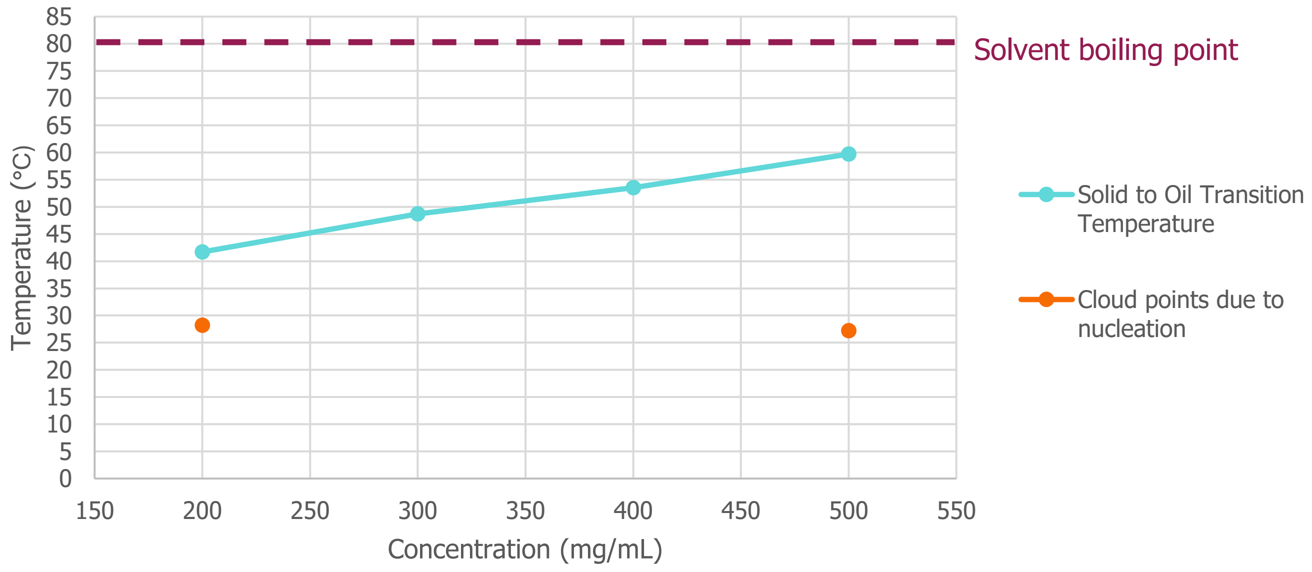
Case Study III – Solvent Choice to Avoid LLPS

- It was decided to investigate EtOH as a solvent.
- It was thought a more polar solvent would help by coordinating to the solvent, presenting it from cyclising on itself.
- Unfortunately, oiling was seen throughout in the 200 – 500 mg/mL concentration range investigated.
- Crowning was observed in 200, 300, 400 mg/mL – no cloud point due to nucleation observed.



Case Study III – Solvent Choice to Avoid LLPS

- As no clear point was found in pure EtOH, it was decided to plot an LLPS diagram in 75:25 EtOH:Heptane.
- It was theorised having 25% Heptane would increase the boiling point of the system and allow higher temperatures to be reach where a clear point may exist.



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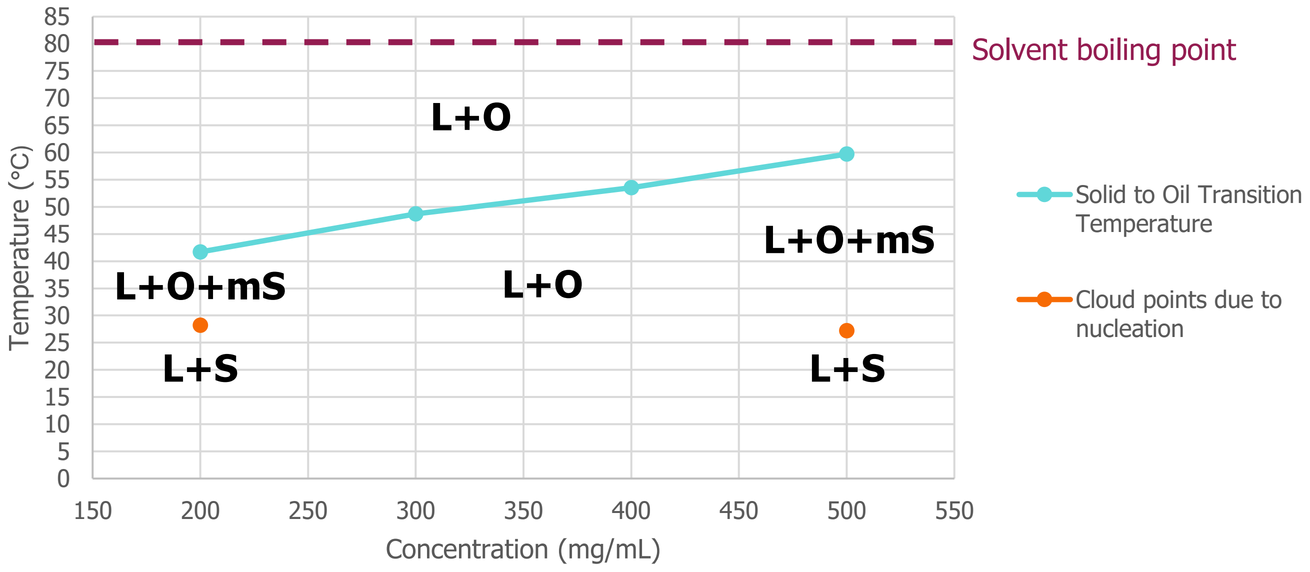
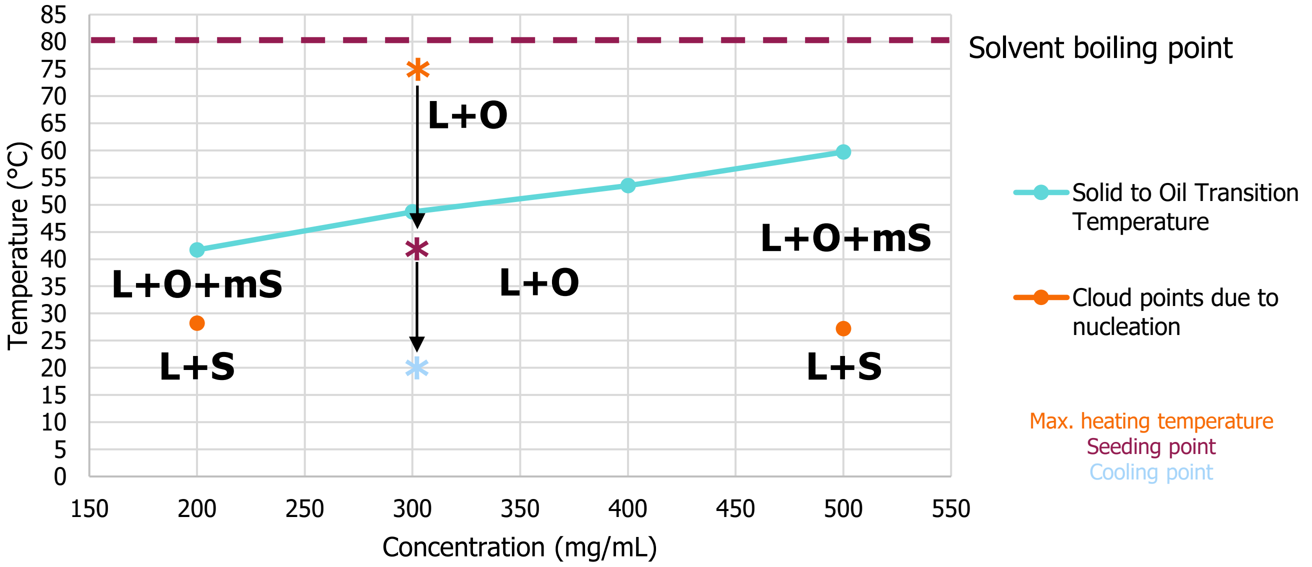
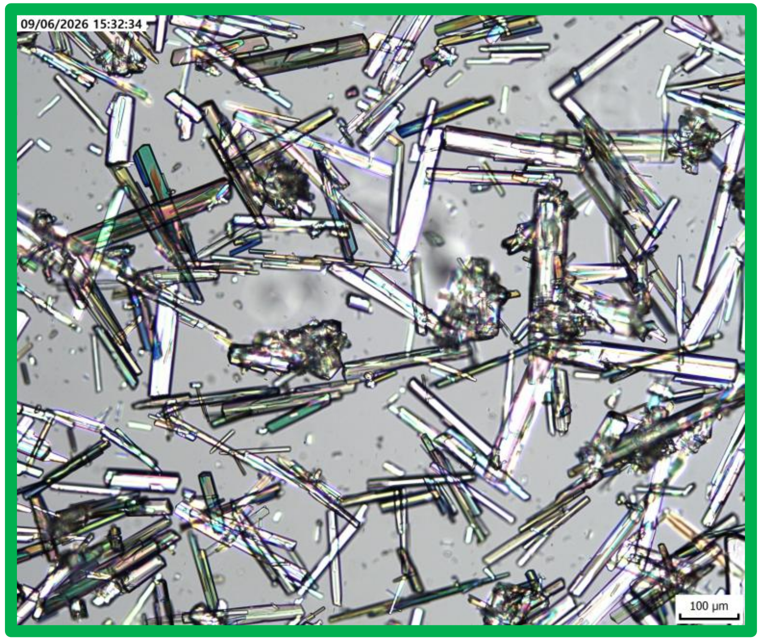


Case Study III – Solvent Choice to Avoid LLPS

- The best result was from a crystallisation in 75:25 EtOH:Heptane.
- Although using this solvent system meant seeding in an oiling region, it gave solid that was the least clustered.
- As no oil free region was identified, this was the best particle morphology that could be obtained with this API.
- Temperature plays an important role.
- No clear point on LLPS.





75:25 EtOH:Heptane

Conclusions

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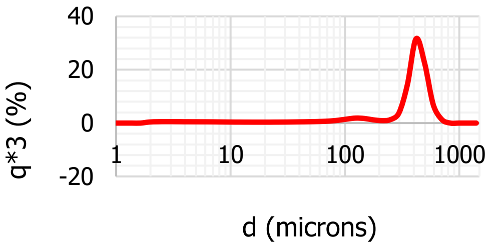
  - Product purity
  - Yield
  - Particle properties (size, morphology, agglomeration)



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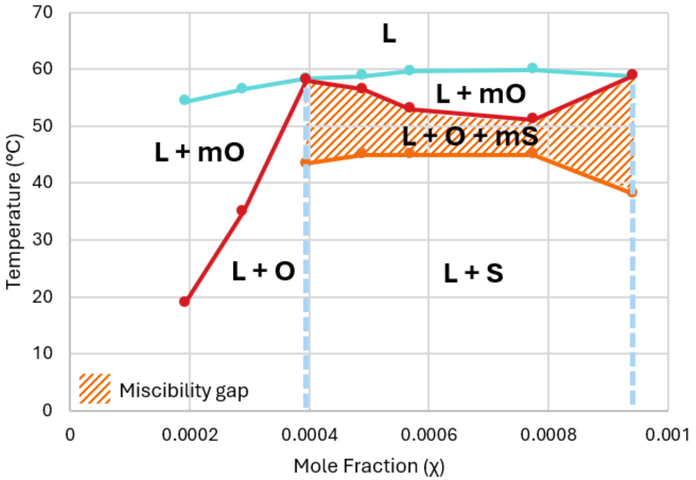

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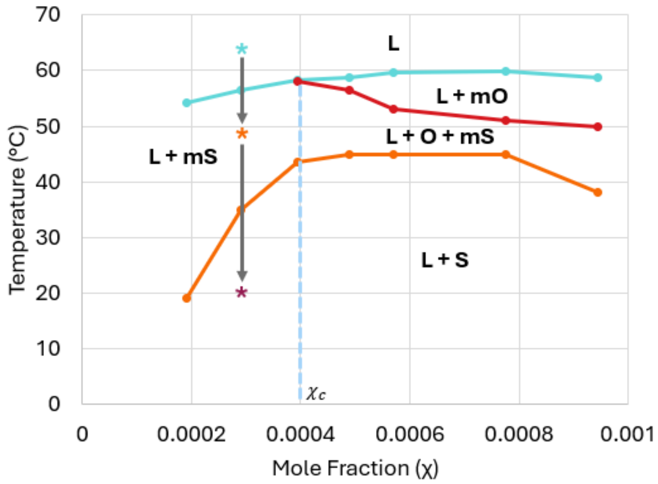

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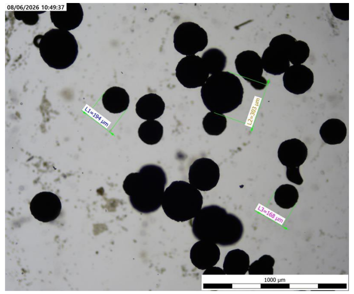

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