The effect of controlled mixing on ROY-polymorphism

Margot Van Nerom, Dominique Maes, Quentin Galand
Structural Biology Brussels, Vrije Universiteit Brussels, Belgium

James F. Lutsko
Center for Nonlinear Phenomena and Complex Systems, Université Livre de Bruxelles, Brussels, Belgium

Importance of polymorphism

Importance for Pharmaceutical Field: Production of Active Pharmaceutical Compounds

- Importance of size distribution and polymorph selection
- Polymorphs: different stability, dissolvability, physiological activity and/or bioavailability

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This study focuses on polymorphism in flow which has its importance in the pharmaceutic field. Indeed, when producing active compounds on a large scale in fermenters, selection is crucial as different polymorphs show differences in stability, dissolvability, physiological activity and/or bioavailability.

1. ROY as model system for studying polymorphism

2. Supersaturation protocol

3. Controlled mixing
   - Confinement
   - Microfluidic chip
   - Acoustic and diffusive mixing within microfluidic channel

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1. **ROY as model system for studying polymorphism**

2. Supersaturation protocol

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   - Confinement
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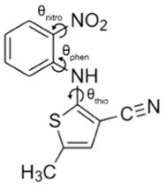
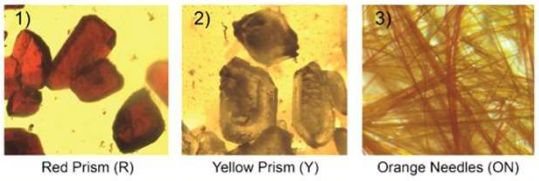
   - Acoustic and diffusive mixing within microfluidic channel

1. ROY as model system for studying polymorphism



ROY (5-methyl-2- [(2-nitrophenyl)amino] -3- thiophen carbonitrile)



- Six stable polymorphs at room temperature
- Visual distinction
- Acetone as solvent
- Water as antisolvent

From Ziemecka et al., 2019

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For studying polymorphism, ROY is often used as a model system due to its ability to form at least 9 distinct polymorphs, of which 6 are stable at room temperature: red prisms, yellow prisms, orange needles, orange plates, yellow needles and orange red plates. These are formed by increasing antisolvent to solvent ratio which are water and acetone respectively

As the polymorph names suggest, distinct polymorphs of ROY are easily detected according to their color, which is also represented in the name of the compound, ROY, an acronym for Red, Orange and Yellow.

(3 other polymorphs are RPL, Y04 and YT04: red plates (RPL) crystallize from vapor on succinic acid. Y04 is a metastable polymorph obtained from a melt

crystallization and it transforms to YT04.)

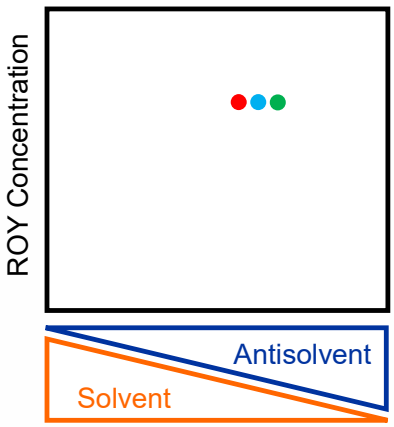
1. ROY as model system for studying polymorphism

2. **Supersaturation protocol**

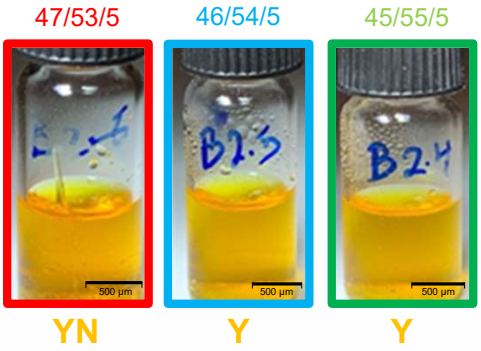
3. Controlled mixing
   - Confinement
   - Microfluidic chip
   - Acoustic and diffusive mixing within microfluidic channel

2. Supersaturation protocol

Phase diagram ROY polymorphism



Acetone (Vol%) / Water (Vol%) / ROY (mg/mL)



YN Y Y

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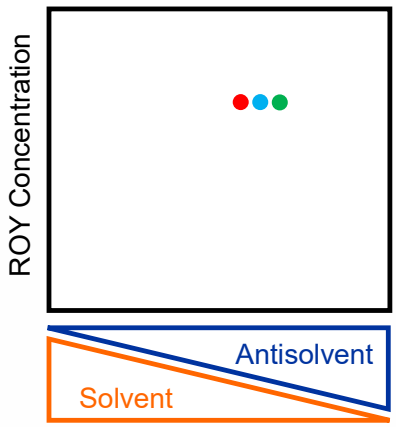
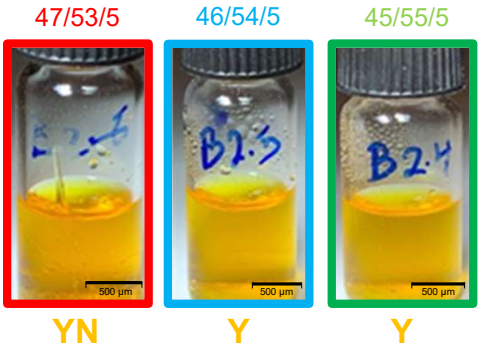
Which polymorphs are favored obviously depend on conditions such as concentration of ROY and of solvent to antisolvent ratio. This can be represented in a phase diagram. Here are shown three different conditions after mixing solvent and antisolvent with only slight changes in the final ratio. However, these slight changes can already induce different behaviors of ROY polymorphism.

2. Supersaturation protocol

Phase diagram ROY polymorphism



Acetone (Vol%) / Water (Vol%) / ROY (mg/mL)



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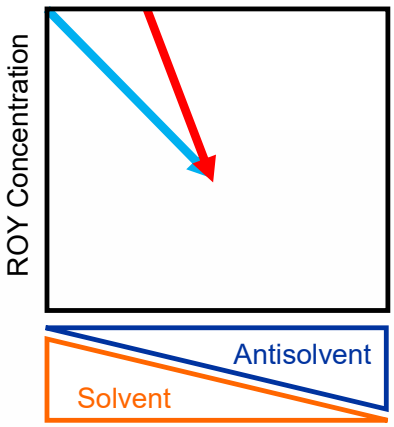
Very slight changes in solvent to antisolvent ratio induce drastic change in ROY polymorphism.

This showcases the sensitivity of ROY polymorphism and the requirement of a more controlled mixing system.

2. Supersaturation protocol

Phase diagram ROY polymorphism



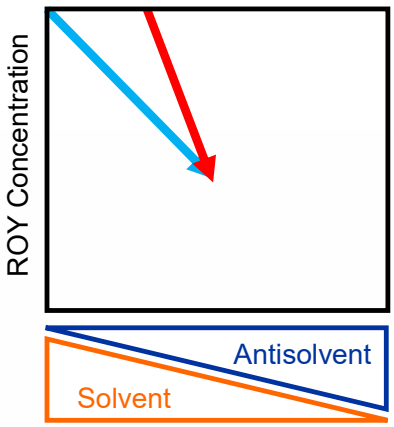
Supersaturation protocol

1. Initial concentration

Moreover, we observed that despite the same final position within the phase diagram, the supersaturation protocol to this position can also alter ROY polymorphism.
A first way to alter this protocol is by changing initial concentrations.

2. Supersaturation protocol

Phase diagram ROY polymorphism



Supersaturation protocol

1. Initial concentration

- 10 minutes
- 1mL
- 100 rpm

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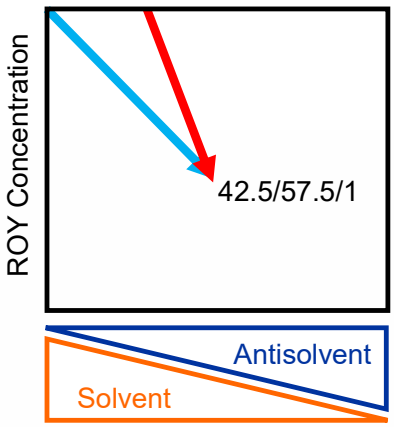
Indeed, we set up a controlled environment in which antisolvent is consistently added to solvent with syringe pumps to obtain reproducible results.

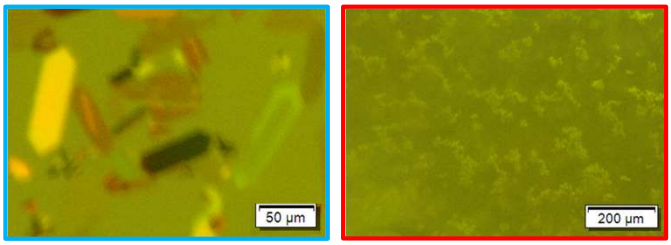
2. Supersaturation protocol

Phase diagram ROY polymorphism



Supersaturation protocol

1. Initial concentration



OP - ORP **Y**

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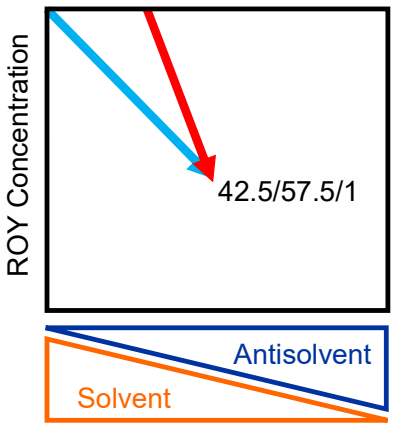
When we altered solvent to antisolvent ratios in the initial solutions, different polymorphs were formed.

BLUE – A (syringe) = 100% Water - 0% Acetone
RED – A (syringe) = 70 % Water - 30% Acetone

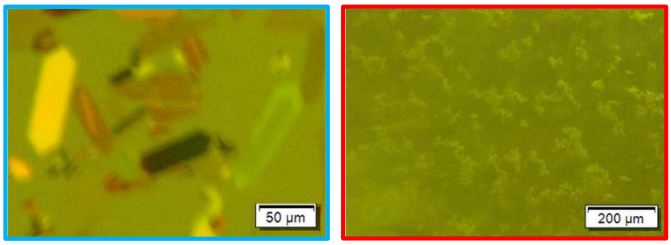
2. Supersaturation protocol

Phase diagram ROY polymorphism



Supersaturation protocol

1. Initial concentration



OP - ORP **Y**

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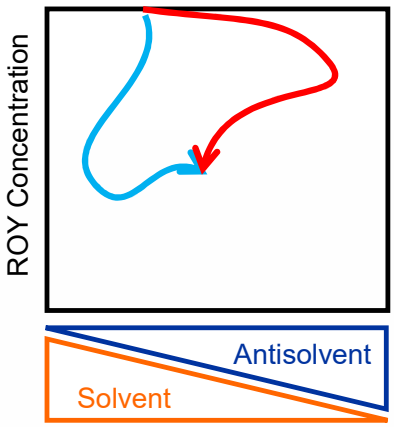
Initial condition of supersaturation protocol affects ROY polymorphism

This means, that not only the concentrations of ROY, solvent and antisolvent play a role in polymorphism, but also the supersaturation protocol.

2. Supersaturation protocol

Phase diagram ROY polymorphism



Supersaturation protocol

1. Initial concentration
2. Mixing method

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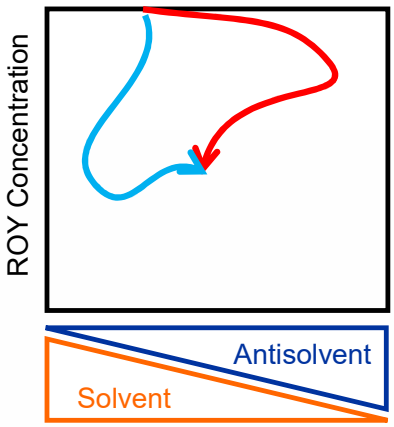
Next to altering the supersaturation protocol by changing the initial conditions, the protocol can also be changed by using different mixing methods.

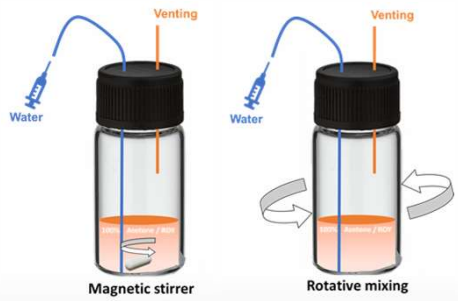
2. Supersaturation protocol

Phase diagram ROY polymorphism



Supersaturation protocol

1. Initial concentration
2. Mixing method



- 10 minutes
- 1mL
- 100 rpm/400rpm

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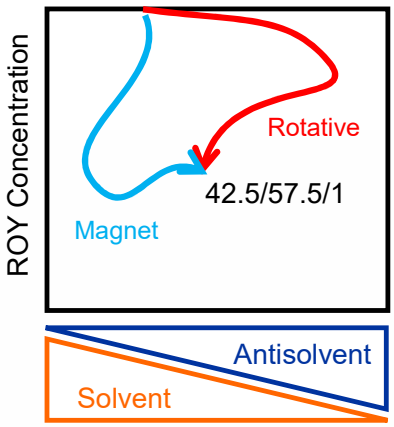
We opted for two distinct mixing methods. A first method consists of a magnetic stirrer (100 rpm), while a second method was performed by shaking the mixture while injecting (400 rpm).

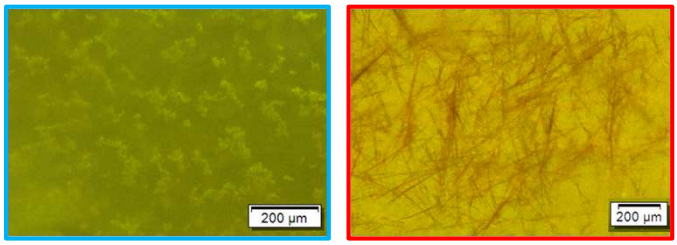
2. Supersaturation protocol

Phase diagram ROY polymorphism



Supersaturation protocol

1. Initial concentration
2. Mixing method



Y ON - YN

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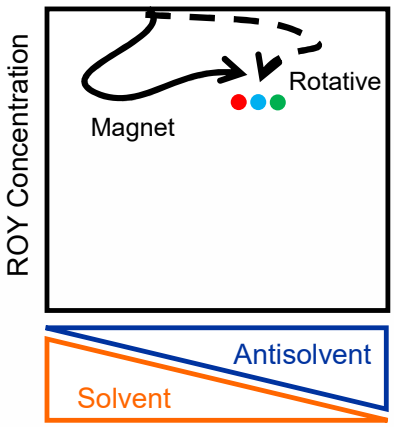
Again, different polymorphs were obtained despite identical initial and final conditions: yellow prisms when mixing with a magnet and orange needles and yellow needles when mixing by rotation.

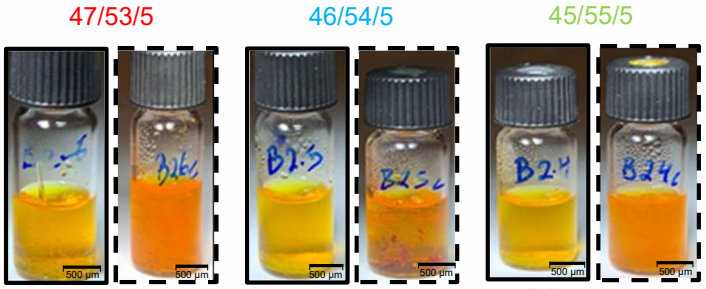
2. Supersaturation protocol

Phase diagram ROY polymorphism



Supersaturation protocol

1. Initial concentration
2. Mixing method



YN ORP OP ON YN

Y ORP OP ON YN

Y ORP OP ON YN

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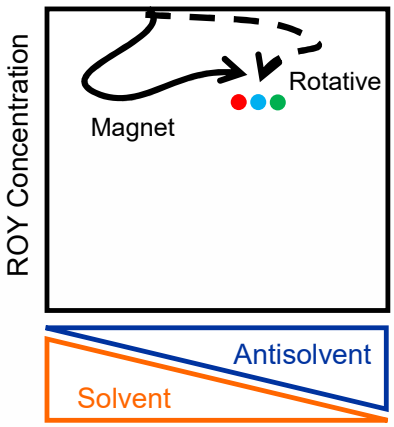
This phenomenon was observed in different regions within the phase diagram.

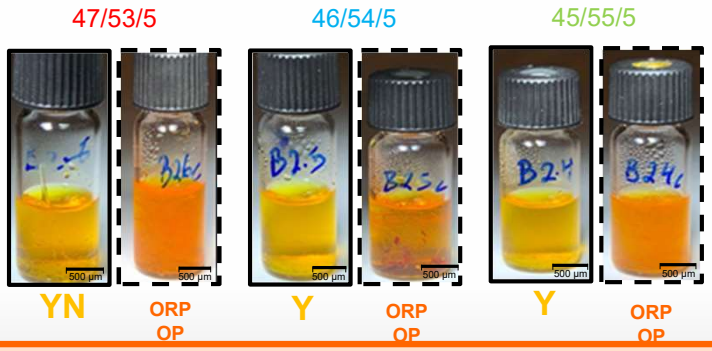
2. Supersaturation protocol

Phase diagram ROY polymorphism



Supersaturation protocol

1. Initial concentration
2. Mixing method



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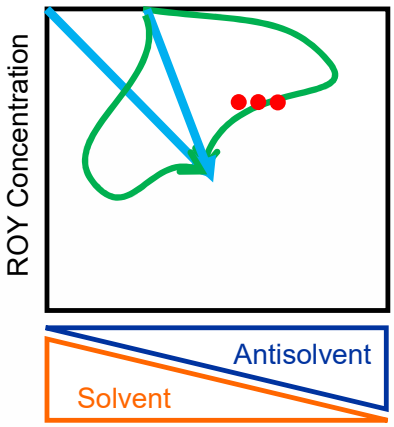
Mixing method affects ROY polymorphism

2. Supersaturation protocol

Phase diagram ROY polymorphism



1. Phase diagram
2. Start concentration
3. Mixing method

- Road to supersaturation affects ROY Polymorphism
- Limited reproducibility
- Controlled mixing required

➡ **Microfluidic approach**

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In conclusion, the supersaturation protocol affects ROY polymorphism. Despite having identical initial and final conditions, the differences in samples might be explained by different energy barriers that are crossed when travelling different paths throughout the phase diagram.
Additionally, reproducibility was not achieved for each point within the phase diagram. These observations led to the realization that a controlled mixing method is required, leading to the implementation of several mixing methods in microfluidic setup.

1. ROY as model system for studying polymorphism
2. Supersaturation protocol
3. **Controlled mixing**
   - **Confinement**
   - Microfluidic chip
   - Acoustic and diffusive mixing within microfluidic channel

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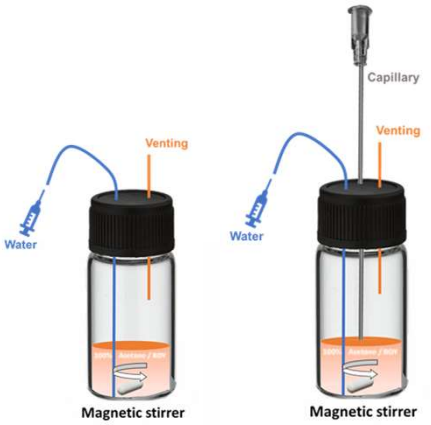
However, going from relatively big volumes (1 ml) to a microfluidic environment, raised the question whether confinement is also to be considered when looking at ROY polymorphism within the microchannel.

3. Controlled mixing - Confinement



BULK

Confinement

After mixing, the mixture is confined in a 100µm capillary.

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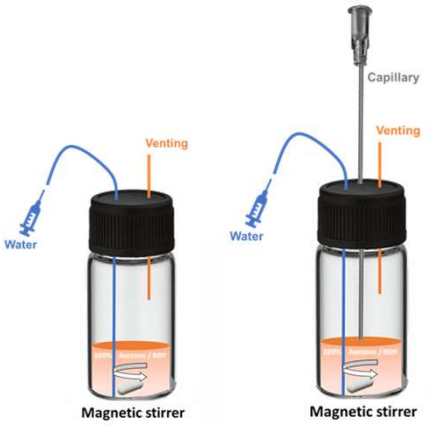
To analyze this effect, we compared the obtained polymorphs in identical samples where crystallization happens in bulk or in confinement.

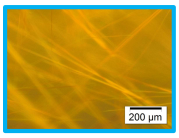
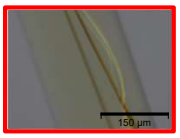
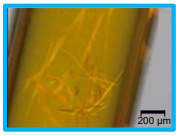
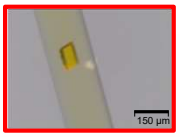
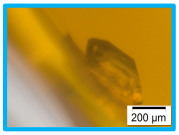
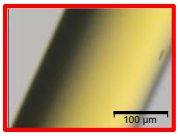
3. Controlled mixing - Confinement



BULK

Confinement

After mixing, the mixture is confined in a 100µm capillary.

	Bulk	Confined
40% Acetone – 1mg/mL ROY		
	ON - YN	ON - YN
42.5% Acetone – 1mg/mL ROY		
	ON - YN	OP
45% Acetone – 1mg/mL ROY		
	OP	OP

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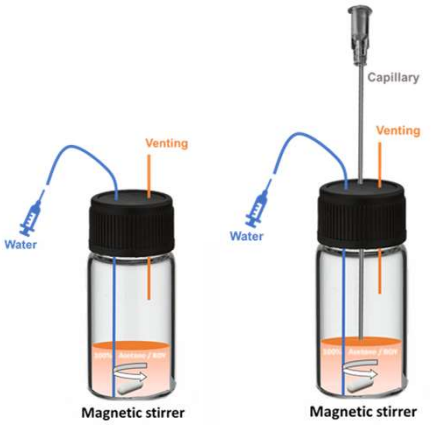
No major differences were found except for one condition: 42.5% solvent and 1mg/mL ROY. Orange needles and yellow needles were obtained in bulk, while orange plates were observed in the capillary.

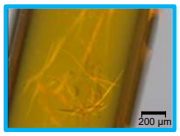
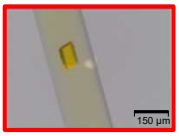
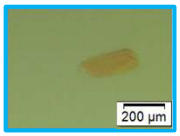
3. Controlled mixing - Confinement



BULK

Confinement

After mixing, the mixture is confined in a 100µm capillary.

	Bulk	Confined
42.5% Acetone – **1mg/mL** ROY		
	ON - YN	**OP**
42.5% Acetone – **0.8 mg/mL** ROY		
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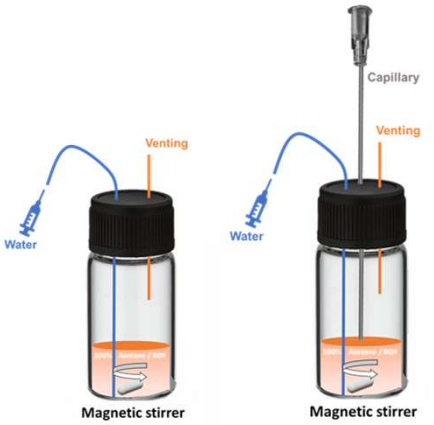
However, when lowering ROY concentration in bulk we obtained the same polymorph.

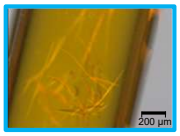
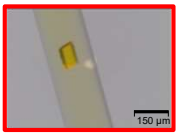
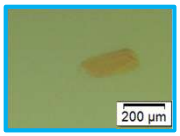
3. Controlled mixing - Confinement



BULK

Confinement

After mixing, the mixture is confined in a 100µm capillary.

	Bulk	Confined
42.5% Acetone – **1mg/mL** ROY		
	ON - YN	**OP**
42.5% Acetone – **0.8 mg/mL** ROY		
	OP	

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Effect of confinement can be explained by decrease in ROY availability (depletion)

In conclusion, the effect of confinement is minimal and can be explained by the depletion in ROY molecules that can nucleate within a confined space such as a capillary.

1. ROY as model system for studying polymorphism

2. Supersaturation protocol

3. **Controlled mixing**
   - Confinement
   - **Microfluidic chip**
   - Acoustic and diffusive mixing within microfluidic channel

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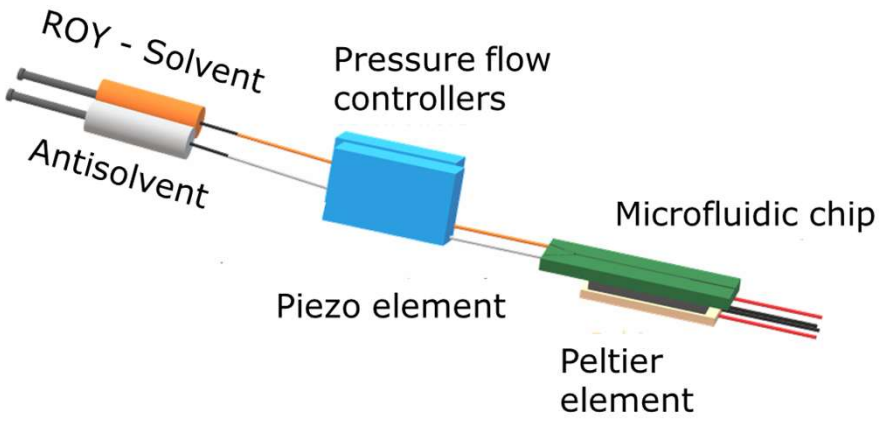
3. Controlled mixing – microfluidic chip

Experimental setup



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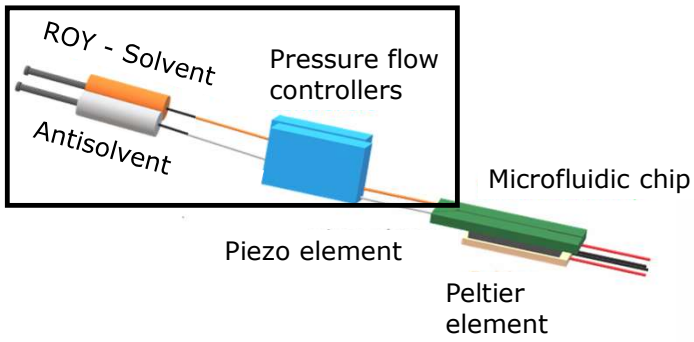
The experimental setup of the controlled mixing within a microfluidic channel is systematically explained in the following slides.

3. Controlled mixing – microfluidic chip

Experimental setup



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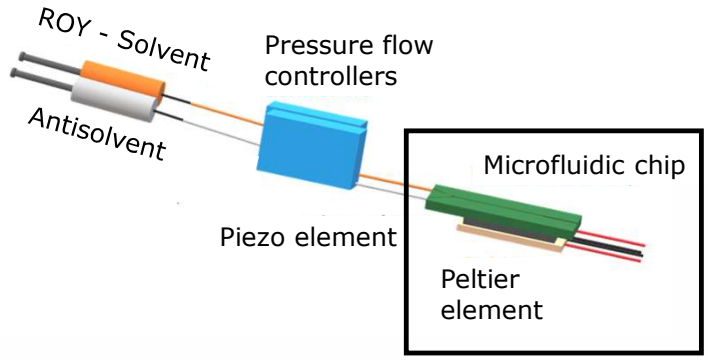
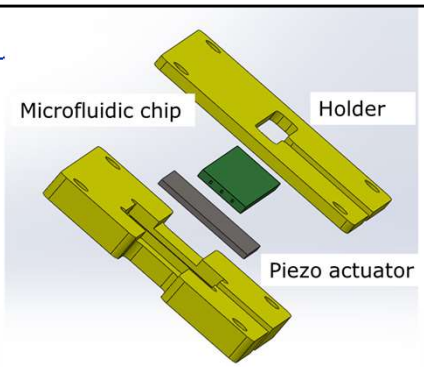
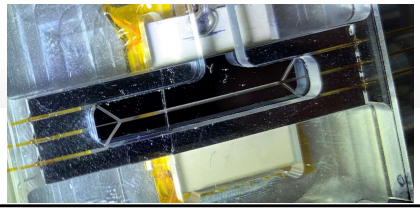
Parallel flows of solvent and antisolvent are injected in the microchannel by pressure flow controllers.

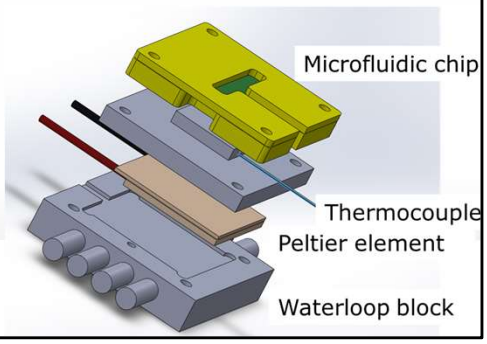
3. Controlled mixing – microfluidic chip

Experimental setup









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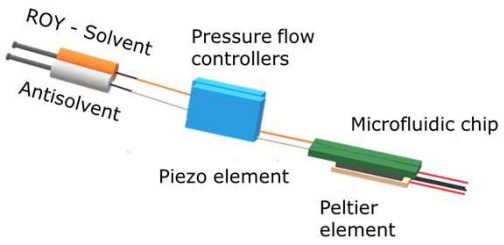
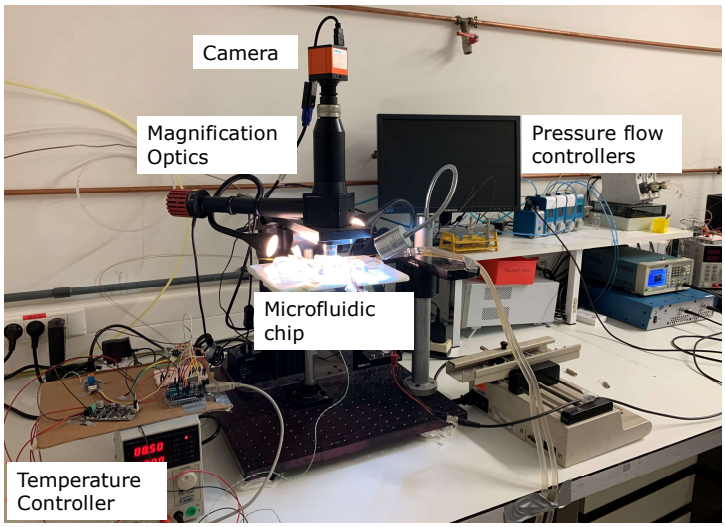
The chip is controlled in temperature and the piezo element is used to implement acoustic mixing.

3. Controlled mixing – microfluidic chip

Experimental setup





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Eventually, the complete experimental setup includes magnification optics and a camera focused on the microfluidic chip, a pressure controller and a temperature controller.

1. ROY as model system for studying polymorphism

2. Supersaturation protocol

3. **Controlled mixing**
   - Confinement
   - Microfluidic chip
   - **Acoustic and diffusive mixing within microfluidic channel**

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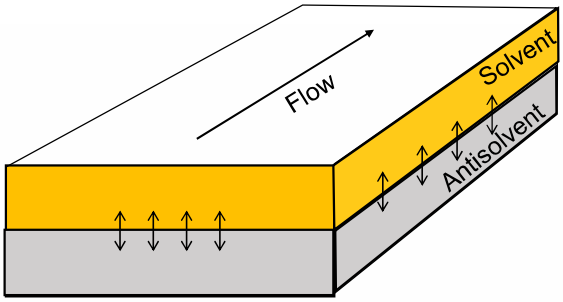
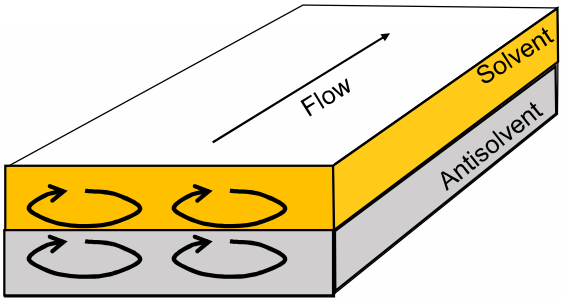
3. Controlled mixing – acoustic and diffusive mixing within microfluidic channel

Mixing within microfluidic channel

1. Diffusive mixing



2. Acoustic mixing



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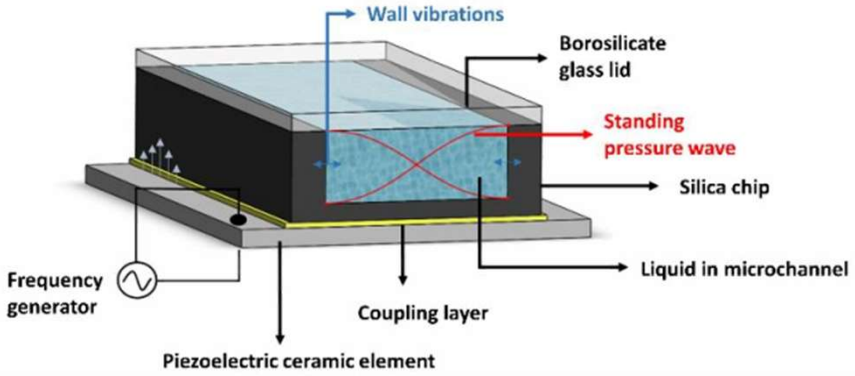
Mixing flows within the microfluidic chip not only allows controlled mixing, but also a range of mixing methods. We tested two mixing methods. In the first one we stop the flows and spontaneous diffusive mixing occurs. In a second method, forced mixing is achieved with acoustic waves.

3. Controlled mixing – acoustic and diffusive mixing within microfluidic channel

Mixing within microfluidic channel

2. Acoustic mixing

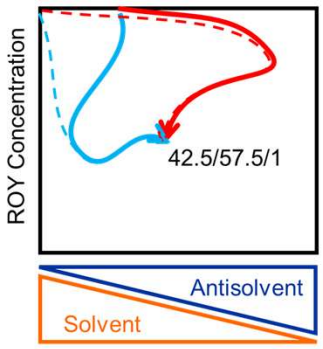


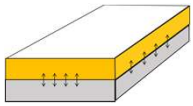
From Gelin et al., 2019

In acoustic mixing, a frequency generator produces the vibration of a piezoelectric element. These vibrations travel along the channel walls and produce a pressure wave within the fluid resulting in vortices.

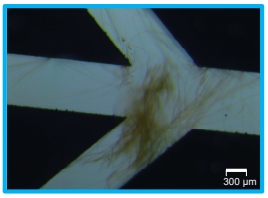
3. Controlled mixing – acoustic and diffusive mixing within microfluidic channel

Mixing within microfluidic channel

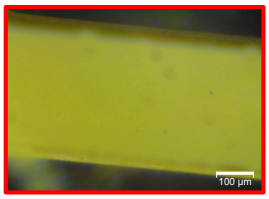




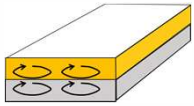
Diffusive mixing



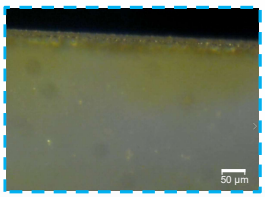
ON - YN



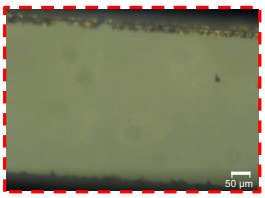
Y



Acoustic mixing



OP - Y



Y

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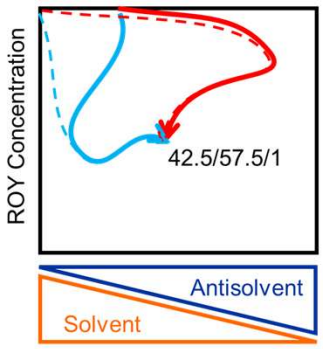
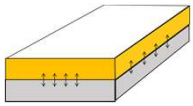
We applied both diffusive mixing and acoustic mixing for two different initial concentrations.
The top half represents the results of diffusive mixing, the lower half of acoustic mixing.
Blue represents what happens when we add 100% antisolvent, red when 70% antisolvent is added.

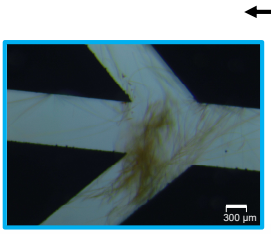
3. Controlled mixing – acoustic and diffusive mixing within microfluidic channel

Mixing within microfluidic channel

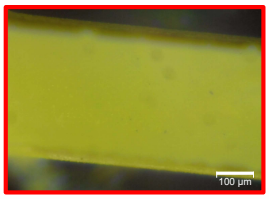




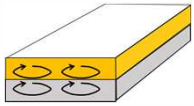
Diffusive mixing



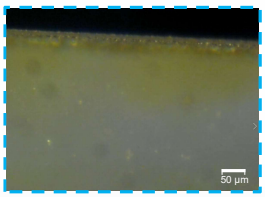
ON - YN



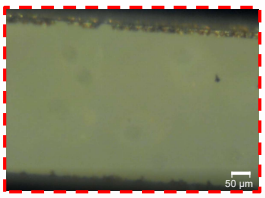
Y



Acoustic mixing



OP - Y



Y

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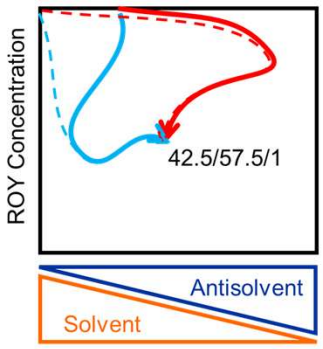
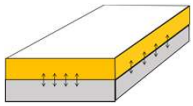
Supersaturation protocol initial conditions also affect ROY polymorphism in microfluidic channel.

Same as with the bulk experiments, altering the supersaturation protocol by altering initial concentrations, changes the behavior of ROY polymorphism, both in diffusive mixing and in acoustic mixing environments.

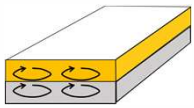
3. Controlled mixing – acoustic and diffusive mixing within microfluidic channel

Mixing within microfluidic channel

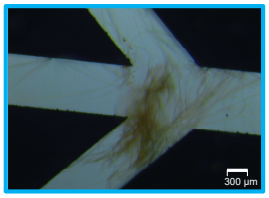




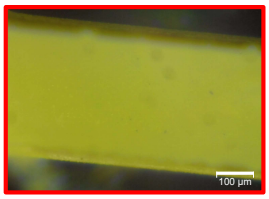
Diffusive mixing



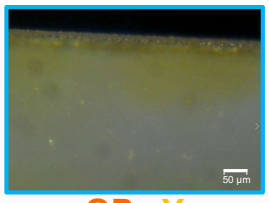
Acoustic mixing



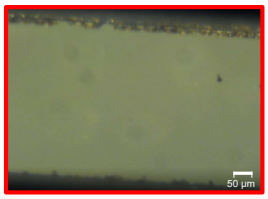
ON - YN



Y



OP - Y



Y

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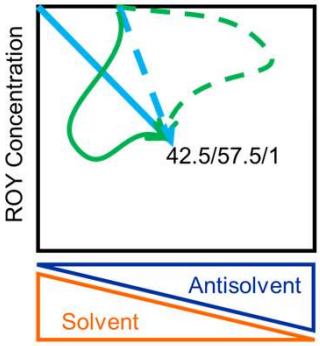
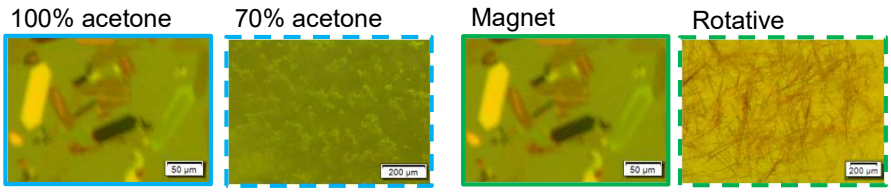
Mixing method also affects ROY Polymorphism in microfluidic channel.

Comparing diffusive mixing with acoustic mixing results in different polymorphs when 100% antisolvent is used as one flow, not when 70% antisolvent is used as one flows.

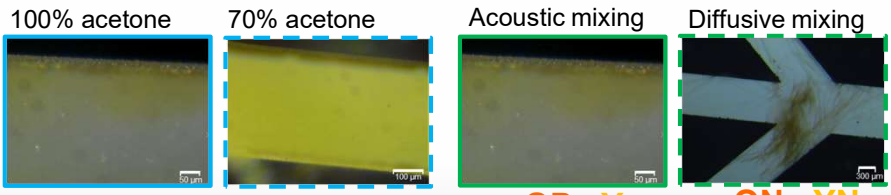
4. Conclusion



Bulk



Microfluidic chip



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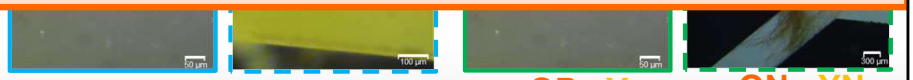
4. Conclusion

Bulk

100% acetone | 70% acetone | Magnet | Rotative

- Many parameters influence ROY polymorphism
  - Both in bulk and in microfluidic channel
- Comparison between bulk and microfluidic channel
  - Magnetic stirrer in bulk ↔ Acoustic mixing in microfluidic channel
  - Rotation in bulk ↔ Diffusive mixing in microfluidic channel

  } Shear stress?
- First step towards tuning a controlled mixing regime favoring one polymorph



OP - Y | Y | OP - Y | ON - YN

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In conclusion, many parameters play a role in ROY polymorphism, raising the need for a controlled mixing setup. Within this controlled mixing setup, the supersaturation protocol can still influence polymorphism. This might be explained by the crossing of different energy barriers and different shear stress regimes. This study can be seen as a first step in the design of controlled mixing regimes to favor one polymorph, very important for industrial applications.

Acknowledgements

Pierre Gelin, Wim de Malsche
Chemical Engineering Department, Vrije Universiteit Brussels, Belgium

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Apart from my co-authors I would like to thank the group of Wim De Malsche of the Chemical Engineering Department at the VUB and in particular Pierre Gelin who helped with the microfluidic experiments. This work was supported by the research department of both universities and the European Space Agency via Belspo.