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Controlled growth of MOF crystals under microgravity mimicking conditions using microfluidic environments.

Creixement controlat de cristalls de MOF en condicions que simulen microgravetat mitjançant chips microfluídics.

Martí Sanz Mir

Gener 2021

Universitat de Barcelona

B:KC Barcelona Knowledge Campus
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L'experimentador que no sap el que està buscant no comprendrà el que troba.

Claude Bernard

Voldria agrair a tots els membres de ChemInFlow Group, que han sigut molt bons amb mi i han permès que la realització de la part experimental d'aquest TFG es fes de manera molt agradable i sense problemes. Agraeixo als meus dos tutors, l'Alessandro i en Josep, que m'han sabut guiar i ajudar-me a treballar de manera més sistemàtica. A més a més de supervisar les meves activitats al laboratori dia rere dia, m'han ajudat molt a l'hora d'escriure aquesta memòria. També cal mencionar a en Michele, que no era tutor però m'ha ajudat molt durant tots aquests mesos, i a en James, que em va preparar un muntatge per poder escalfar o refredar els chips de PDMS emprats.

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REPORT

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1. SUMMARY

The classical nucleation theory states that crystallization processes generally occur via monomer-by-monomer addition, a mechanism that leads to the most stable solid phase, i.e. to the thermodynamic product. However, there are systems that prefer to form less stable phases that do not lay in the global minima of Gibbs energy. These phases require less energy to form, but at the end, they can be also guided to the most stable phase via successive phase transformations that can be triggered, for example, by changes in the solvent composition. This research theory, also known as mesoscale assembly, is expected to provide new insights for a better understanding of crystallization processes; a result that would enable unprecedented control during crystals growth.

The main goal of this project is to study the mesoscale assembly of soft porous crystalline networks such as peptide-based metal-organic frameworks (MOFs). We focus on these materials as their crystallization pathway is especially dependent on the solvent composition. Additionally, the host group has recently demonstrated the control synthesis of peptide-based MOFs in microfluidic devices due to a control diffusion of reagents[1]. Accordingly, herein, we will study the mesoscale assembly of a MOF formed by the coordination of the tripeptide glycine-*L*-histidine-glycine (GHG) to Cu^{2+}, hereafter called CuGHG, via the control diffusion of a solvent and a non-solvent across a polymeric membrane. To achieve this goal, we have used a double-layer microfluidic device in our investigations fabricated with polydimethylsiloxane (PDMS), *vide infra.*

I observed that after doing a controlled diffusion of a solvent (e.g. water) and a non-solvent (e.g. ethanol) through the PDMS membrane of the double-layer microfluidic device and into a microreaction chamber containing an aqueous solution of GHG and Cu^{2+}, a phase separation process occurs that leads to the formation of highly concentrated droplets. Eventually, these droplets coalesce resulting after sometime into a CuGHG crystal. This result is of particular importance as it demonstrates that MOFs can also follow mesoscale assembly before its crystallization occurs.

Keywords: Microfluidic conditions, controlled diffusion, mesoscale assembly, crystallization, metal-organic frameworks (MOFs).

2. RESUM

La teoria clàssica de la nucleació explica que els processos de cristal·lització succeeixen generalment per addició monòmer-per-monòmer, un mecanisme que permet obtenir la fase més estable, es a dir, el producte termodinàmic. Però hi ha sistemes que prefereixen formar fases menys estables que no estan en el mínim global d'energia de Gibbs. Aquestes fases requereixen menys energia per formar-se, però es poden guiar a la fase més estable via transformacions de fase successives, les quals es poden donar mitjançant canvis en la composició del solvent. Aquesta teoria, anomenada assemblatge a la mesoescala, és d'esperar que proporcioni una nova visió per a un millor enteniment dels processos de cristal·lització, un resultat que podria permetre el control durant el creixement dels cristalls.

L'objectiu principal d'aquest projecte és estudiar l'assemblatge a la mesoescala de xarxes cristal·lines poroses toves com els metal-organic frameworks (MOFs) basats en pèptids. Ens centrem en aquests materials perquè tenen un procés de cristal·lització depenent especialment de la composició del solvent. Addicionalment, el grup amfitrió ha demostrat recentment la síntesi controlada del MOF basats en pèptids en dispositius microfluídics degut a la difusió controlada dels reactius[1]. D'acord amb això, aquí estudiarem l'assemblatge a la mesoescala d'un MOF format per la coordinació del tripètid glicina-*L*-histidina-glicina (GHG) amb Cu^{2+}, més endavant anomenat CuGHG, via la difusió controlada de un solvent i un no-solvent a través d'una membrana polimèrica. Per aconseguir aquest objectiu, hem utilitzat dispositius microfluídics de doble-capa fabricats amb polidimetilsiloxà (PDMS), *vide infra*.

He observat que després de fer una difusió controlada de un solvent (per exemple aigua) i un no-solvent (per exemple etanol) a través de la membrana de PDMS del dispositiu microfluídic de doble-capa, i a dintre d'una cambra de microreacció la qual conté una solució aquosa de GHG i Cu^{2+}, succeeix una separació de fases que dona lloc a la formació de gotes altament concentrades. Eventualment, aquestes gotes coalesceixen després d'un cert temps per donar a

lloc a un cristall de CuGHG. Aquest resultat és de particular importància, ja que demostra que els MOFs poden seguir assemblatge a la mesoescala abans de cristal·litzar-se.

Paraules clau: Condicions microfluídiques, difusió controlada, assemblatge a la mesoescala, cristal·lització, metal-organic frameworks (MOFs).

3. INTRODUCTION

3.1. CRYSTALLIZATION BY PARTICLE ATTACHMENT[2]

Geological, biological and synthetic materials systems have motivated the study of crystal nucleation and growth. Since mid-90s, the results obtained have been interpreted through the classical nucleation theory, which is based on monomer addition of chemical species. But there are a lot of crystal formation phenomena that cannot be explained via this classical theory. It has been demonstrated that some crystalline materials are generated from amorphous precursors, and hence, they do not follow the classical nucleation theory. For example, mineralization of sea urchin embryonic spicules occurs by accumulation of amorphous calcium carbonate, which transforms into calcite under certain environmental conditions.

Another nonclassical crystal formation is the oriented-attachment (OA), which implies attachment events of particles on specific lattice-matched crystal faces. For example, precursors of tooth enamel assemble into single-crystal rods with orientation given by structured protein oligomers.

As we can see in the examples above, crystallization can occur via attachment of more complex species than simple ions, which is called crystallization by particles attachment (CPA), and that gives “birth” to the mesoscale assembly.

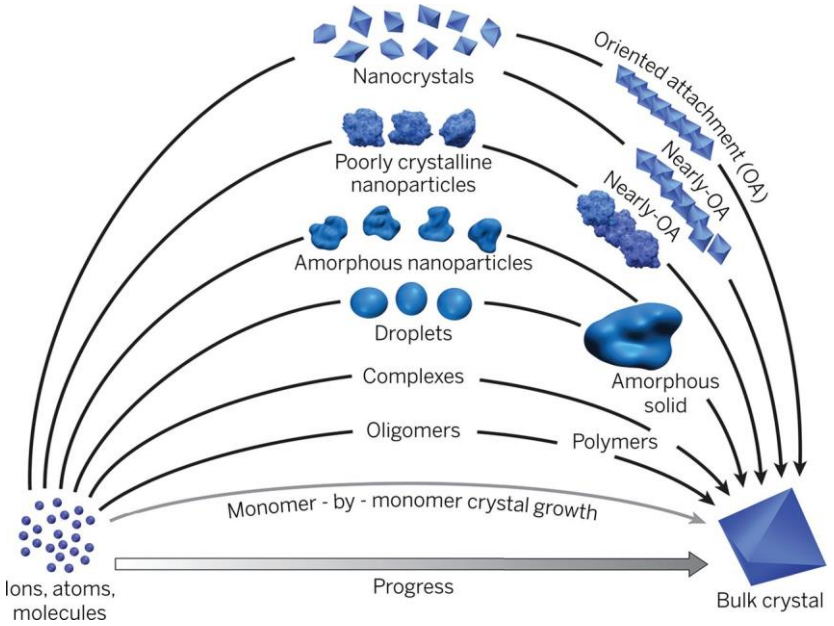

Figure 1. *Classical vs nonclassical (mesoscale) crystallization*.
*(image extracted from De Yoreo et al. Science **2015**, 349 (6247), aaa6760-2)*

In Figure 1, we can see the difference between the classical nucleation theory (grey curve) which occurs from the addition of ions, atoms and molecules, and the non-classical pathways, which occur from the aggregation of higher-ordered species, like complexes, droplets, nanocrystals...

3.1.1. Interplay of thermodynamics and kinetics[2]

Monomers in solution can aggregate via different crystallization pathways, which can be understood thanks to the interplay of free-energy landscapes and reaction dynamics. The first one determines the thermodynamic preferences, and the second one determines whether these occur or another alternate and kinetically controlled is traversed. As I commented in Figure 1, these pathways can be simple like monomer-by-monomer addition to incipient nuclei that display a single structure, or can be complex, involving higher-ordered species that can be structurally different from the thermodynamically stable bulk phase.

It is very important to consider the magnitude of the free-energy barrier to achieve nucleation with respect to the thermal energy, because is a decisive factor in determining the number and nature of the particles produced.

At low concentrations, the free-energy barrier is large. It is a rare event to generate a critical nucleus, so it is unlikely that nucleated particles see other ones in their immediate vicinity. In this scenario, the most common pathway followed by many systems is the monomer-by-monomer nucleation where crystal growth is described by the classical nucleation theory.

As concentration increases, the free-energy barrier its reduced, particles are generated in greater numbers, and they grow via monomer exchange, collision or coalescence events. If the concentration is increased until the free-energy barrier is comparable to the thermal energy, the solution undergoes spinodal decomposition, particles are generated in very large numbers, and they grow by direct collision and coalescence.

In these examples, the free-energy landscape displays a barrier, so the system should prefer to crystallize as one large particle. However, in some situations, the particles do not reside in the global minimum of energy but in a local energy minimum, and hence, it becomes thermodynamically stable the formation of particles of different sizes or morphologies (i.e. kinetically trapped or metastable states are favored). In this context, the system can undergo different crystallization pathways where metastable precursors are formed before obtaining the thermodynamically stable bulk crystal. For example, a metastable solid phase is formed because the barrier of its nucleation is lower than the more stable phase's one. The more stable phase nucleates eventually, via, for example, changes in the solvent composition or through dissolution-reprecipitation processes. The latter pathway is commonly referred to as the Ostwald's rule of stages.

3.1.2. Ostwald's rule of stages[3]

Wilhelm Ostwald, stated that in a system about to undergo phase transition and form multiple phases, both stable and metastable, it does not form directly the most stable one. Instead, the system prefers to transition into the less stable phases, which have free-energy barriers and structures closer to the parent phase (e.g., the solution).

But this has been restated by other scientists. Stranski and Totomanov indicated that the phase which the system transitions into first (should be a dense liquid phase) is the one separated from the parent phase by the smallest free energy barrier. Ogino et al., stated that the metastable phases formed after the dense liquid phase may undergo consecutive phase transformation (via dissolution-reprecipitation or solid-state transformation) until the most stable phase is formed, i.e. the thermodynamic state. This multi-step precipitation sequence is called Ostwald's rule of stages.

For example, in the $CaCO_3 \cdot H_2O$ system, before forming the thermodynamically stable phase, it undergoes multiple phase transformations, in this order: dense liquid precursor ⟶ amorphous calcium carbonate (ACC) ⟶ ikaite⟶ monohydrocalcite ⟶vaterite ⟶ aragonite.

4. OBJECTIVES

The overall objective of this project is to study if peptide-based MOFs can undergo mesoscale assembly before crystallization with the help of a controlled diffusion of a solvent and a non-solvent inside a double-layer microfluidic device. To achieve this overall objective, some specific tasks need to be fulfilled:

- Fabrication of the PDMS-based double-layer microfluidic devices.
- Learn how to use syringe pump device, inverse microscopes, and their respective software.
- Optimize the conditions for a systematic investigation. For example, the concentration of reagents, solvent switching, and/or temperature, among other variables. All these features need to be optimized to avoid a rapid crystallization of CuGHG to its thermodynamic product.
- Understand the diffusion of a solvent (H_2O) and a no-solvent (EtOH) through the PDMS membrane.
- Perform image analysis to study crystal growth and crystallization kinetics via time-lapse optical imaging .
- Physico-chemical characterization of the crystals obtained.

5. EXPERIMENTAL SECTION

Double-layered microfluidic devices with square-shaped or U-shaped pneumatic valves that act as a clamp were employed in this experiments. The devices consisted of two layers of PDMS and a glass slide as a support, see Figure 2. The bottom layer (fluidic layer) was designed to accommodate the main channel where the solvent and non-solvent are flowed with the help of a syringe pump system. As a second layer, the device has a set of channels that terminate with a square-shaped or a U-shaped channel. These end shapes of the second layer act as pneumatic clamps deflecting the PDMS membrane located between the first and second layer when nitrogen is introduced in the inlets of the channels located in the second layer.

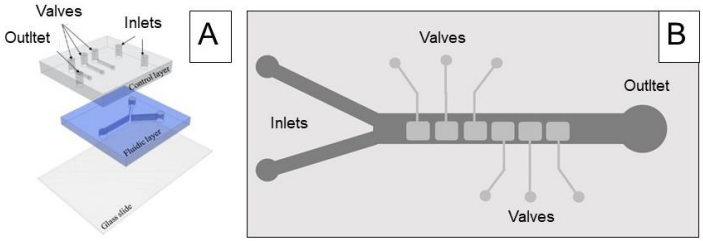

Figure 2. Expanded view of the PDMS-based double-layered microfluidic devices used in our experiments (A) and schematic top view (B).
(*image A extracted from J. Puigmartí-Luis et al. ACS Nano,* ***2014****, 8, 818-826*)

The precursor reactants of the peptide-based MOF under investigation are $Cu(CO_2CH_3)_2 \cdot H_2O$ and GHG. Once the CuGHG aqueous solution is prepared (see Appendix 2), it is injected to the pneumatic valves and pressurized with N_2 or Ar.

To better understand the experiments below, I will define two parameters, the total flow rate (TFR) and the flow rate ratio (FRR). The TFR is the sum of the solvent and non-solvent flow rates used in an experiment. The FRR is the ratio between the non-solvent flow rate and solvent flow rate.

First of all, I started doing some preliminary experiments, where I tested different conditions (Figure 3):

- Co-flowing: Thanks to the microfluidic environment achieved inside microfluidic devices, it is possible to co-flow two different fluids, one next to the other, without mixing them. This condition is known as laminar flow where mixing is only achieved via

diffusion. With this in mind, I decided to do some experiments co-flowing EtOH and H_2O inside a microfluidic channel. To determine an optimal laminar flow, I studied flow rate ratio (EtOH flow rate/H_2O flow rate) changes maintaining the total flow rate. After that I observed that a FRR of 2 was optimal to achieve an evident crystallization process inside the valve. Moreover, I studied changes in the TRF maintaining the FRR at 2. I observed that a TFR of 400 µL/min was an ideal value to accomplish a higher number of precipitation events inside the valve.

- Solvent mixture: With the FRR and TFR studies, I decided to try flowing a 2:1 EtOH/H_2O solution to see if the precipitation of the droplets changed, but nothing happened, i.e. no precipitations events were observed.
- Solvent switching: Apart from these conditions, I tested EtOH to H_2O solvent switch.

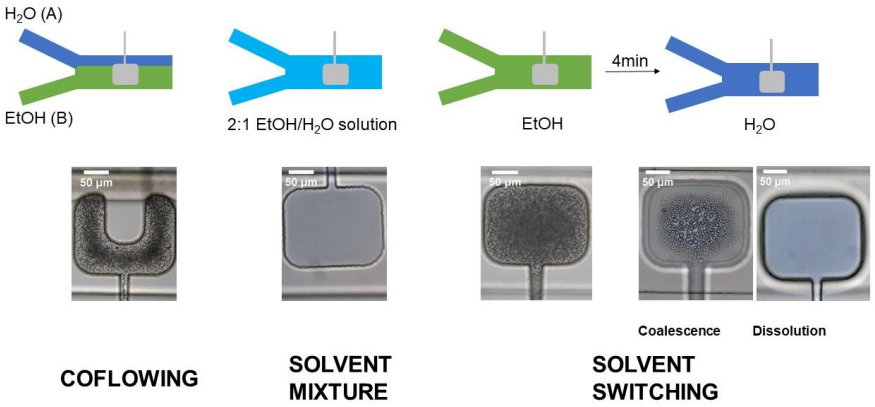

Figure 3. Different conditions tested during preliminary experiments.

During the coflowing experiments, I noticed that the position of the laminar flow affects the precipitation, swapping the A and B flow positions leads to no precipitation. Also, the precipitates near the EtOH and H_2O separation are bigger. From the images acquired of the larger precipitates, it seems clear that this precipitation event leads to the formation of droplets.

In the solvent switching experiments, whenever I had a precipitation in EtOH and swapped to H_2O flow, it was observed a coalescence process of the droplets being generated (Figure 4) and their subsequent dissolution.

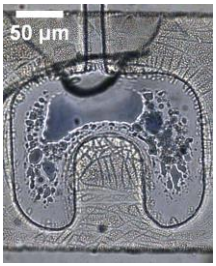

Figure 4. Example of a coalescence process observed inside a U-shaped valve.

5.1. SOLVENT SWITCHING STUDIES

The coalescence observed during the preliminary experiments was a very exciting proof towards mesoscale assembly, so I decided to make experiments with a few precipitations and dissolutions of the droplets by flowing first EtOH and then switching to H_2O a couple of times, varying the frequency of the switch (Table 1).

Solution	EtOH time (min)	H_2O time (min)	Flow rate (µL/min)	Pressure (bar)
Fresh	10	10	200	1.4
Fresh	10	3	200	1.4
Fresh	10	1.5	200	1.4
Fresh	9	1.75	200	1.4
Fresh	5	1.75	200	1.4
Fresh	9	2	200	1.4
Fresh	2.5	2.5	200	1.4
1day	9	3	200	1.4
1day	9	2	200	1.4

Table 1. The frequencies of the solvent and non-solvent switch studied in our investigations with the double-layer microfluidic device.

First, I focused the experiments on studying fresh solutions to confirm the precipitation-coalescence/dissolution-precipitation behavior.

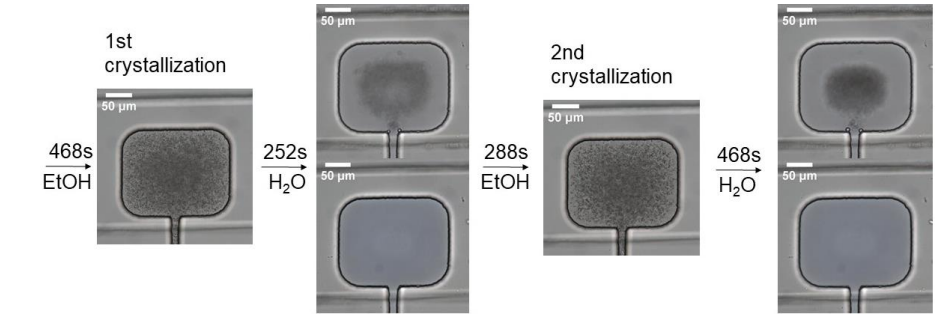

Figure 5. Precipitation-coalescence/dissolution-precipitation behavior on a fresh solution

Interestingly, and as shown in Figure 5, the time of the second precipitation is lower than the first one; on the other hand, the first coalescence and dissolution takes less time than the second one. We attribute this behavior to the increase of EtOH into the valve via its diffusion through the PDMS membrane. When the amount of EtOH increases it is easier to generate droplets and it gets more time to coalesce them.

While doing these experiments, I observed that leaving the valve under pressure overnight leads to the generation of a darker blue color solution inside the microfluidic valve. This change of color indicates a concentration increase of the precursor solution located inside the valve. It should be noted here that the concentration of the fresh precursor solution inside the vale is saturated, and hence, it cannot be further concentrated in bulk without leading to crystal formation and crystal precipitation. Conversely, inside the microfluidic device, the fresh precursor solution can be obviously further concentrated avoiding crystallization and crystal precipitation. Interestingly, I observed that doing the solvent and non-solvent switching with this highly concentrated phase the precipitation-coalescence/dissolution-precipitation behavior reported above turned to a precipitation-coalescence-crystallization pathway, see Figure 6.

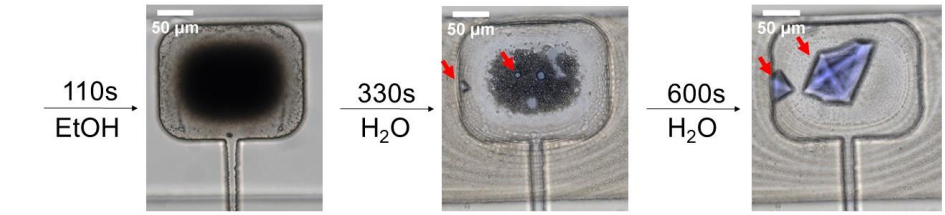

Figure 6. Precipitation-coalescence-crystallization behavior stablished in a concentrated precursor solution aged 1-day.

In figure 6, in the middle micrograph, there are two red arrows that indicate two crystallization points that have their "birth" from the droplets generated after a precipitation-coalescence step accomplished by flowing EtOH (110 seconds) and H_2O (330 seconds) in the fluidic layer (i.e. the first and bottom microfluidic layer) and onto a concentrated precursor solution located inside the valve (i.e. the second and top microfluidic layer). The last optical microscope image shows the CuGHG crystals generated from the two nucleation points indicated by the red arrows in the middle micrograph.

5.2. REVERSE OSMOSIS

Regarding the concentration increase that I noticed during solvent switching experiments, I had two hypotheses of what was happening on the valve:

- First, I thought that it was due to solution aging, but UV-vis did not show any changes in the absorbance spectrum, see Figure 12.
- Then, I thought about reverse osmosis. To confirm this hypothesis, I left a microfluidic valve with fresh precursor solution under pressure (1.4 bars) and against H_2O during ca. 17h. The H_2O was located in the first fluidic layer and with time the blue solution located on the second fluidic layer, i.e. the microfluidic valve, started to darken into a deeper blue color (Figure 7). The change of color can be quantified by image analysis, see Figure 13.

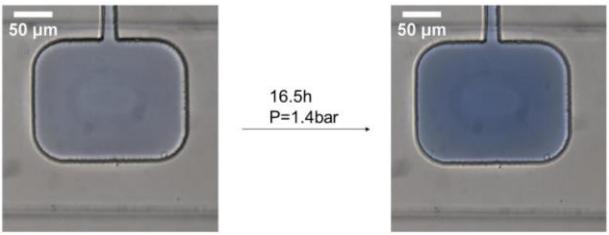

Figure 7. Optical microscope images of a square-shaped valve where a reverse osmosis process was investigated overnight.

5.3. TEMPERATURE STUDIES

I did some temperature studies to check what happened to the EtOH droplet precipitation whenever I increased or decreased temperature (T), see Figure 8.

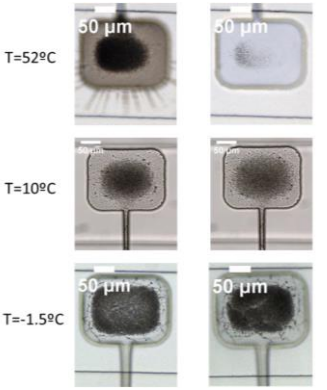

Figure 8. Optical microscope images showing the effect of changes in T over the precipitates generated with EtOH diffusion through the PDMS membrane.

In summary, one can observe that increasing the temperature leads to coalescence and posterior dissolution of the droplets. However, decreasing T does not affect the droplets generated.

5.4. BULK STUDIES

After all these data were obtain, a key question emerged: Can this phase separation be achieved in bulk experimentation? To answer this intriguing question, I prepared multiple solutions where the ratio of MOF precursors (i.e. GHG, Cu^{2+}) in water and EtOH was systematically varied (Table 2).

Solution (Cu-GHG(aq)/EtOH)	Evolution		
	Fresh	1 day after	4 days after
1/0.25	A few blue porous crystals	Still blue crystals	Still blue crystals
1/0.50	A few blue porous crystals	Still blue crystals	Still blue crystals
1/0.75	A few blue porous crystals	Platelet shaped pink crystals observed	The same amount of pink crystals
1/1	A few blue porous crystals	Platelet shaped pink crystals observed	Mostly pink crystals
1/2	A few blue porous crystals	Platelet shaped pink crystals observed	Almost everything is pink crystal

Table 2 Solution ratio and the effect observed

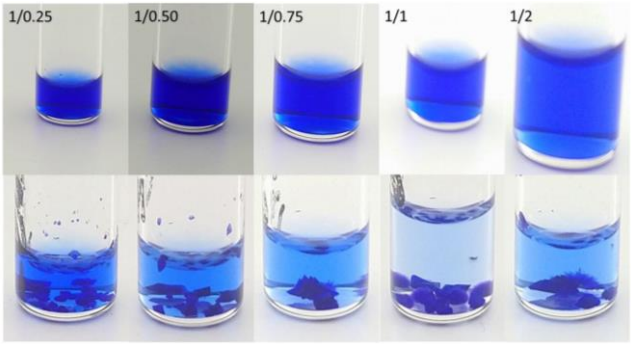

Figure 9. Photos of the evolution of the fresh solution mixtures presented in Table 2. The photos above were taken when the fresh solution was prepared. On the other hand, the lower ones were taken 24h after.

As can be seen in Figure 9, the solutions are homogeneous at the beginning and at the end of the experiment, thus, demonstrating that no phase separation is achieved in bulk crystallization studies.

5.5. ADDITIONAL EXPERIMENTS

I also tried to cool down a highly concentrated precursor solution after a reverse osmosis process was accomplished inside a microfluidic valve. As shown in Figure 10, a temperature decrease to ca.-1ºC lead to the formation of multiple small CuGHG crystallites inside the valve, as it can be clearly observed in the crossed polarized micrograph. Interestingly, flowing EtOH in the first microfluidic layer while a highly concentrated precursor solution is present in the valve, also enabled the generation of multiple small CuGHG crystallites inside the valve (Figure 11). On-going research in the group is looking at controlling these nucleation crystallization processes inside the valve and from previously concentrated samples via reverse osmosis. The main objective will be to control the mesoscale assembly unveiled for first time for a MOF crystallization to generate MOF single crystals with a rationalize size and shape, just like Nature does.

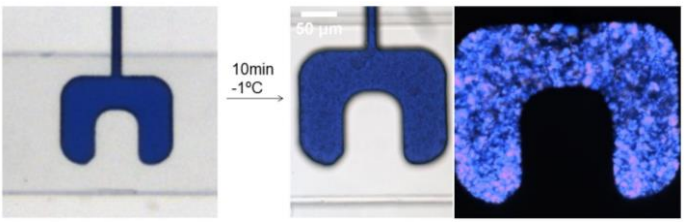

Figure 10. Cooling down a highly concentrated solution.

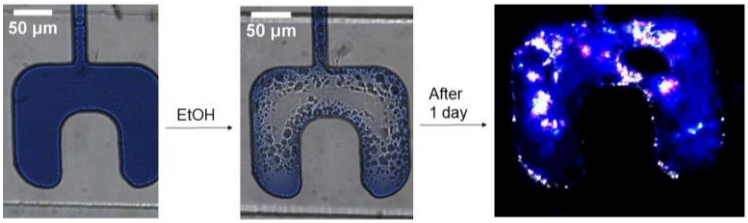

Figure 11. Coalescence-crystallization behavior observed in a highly concentrated solution.

6. REVERSE OSMOSIS

Via UV-vis, it was demonstrated that solution aging was not the cause of the concentration increase mentioned earlier (Figure 12). This can be observed by having a constant absorbance over 70h, which implies that the concentration is constant.

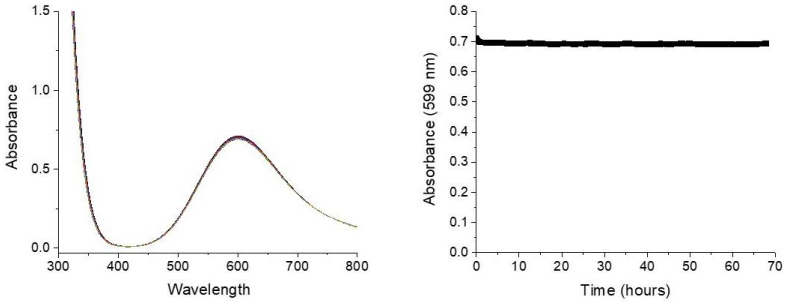

Figure 12. Absorption spectra of a solution of CuGHG in water during 70h. Conc=150mmol, l=0.1cm

Via image analysis, it was quantified the increase of concentration shown when I left the valve under pressure against water by reverse osmosis (Figure 13).

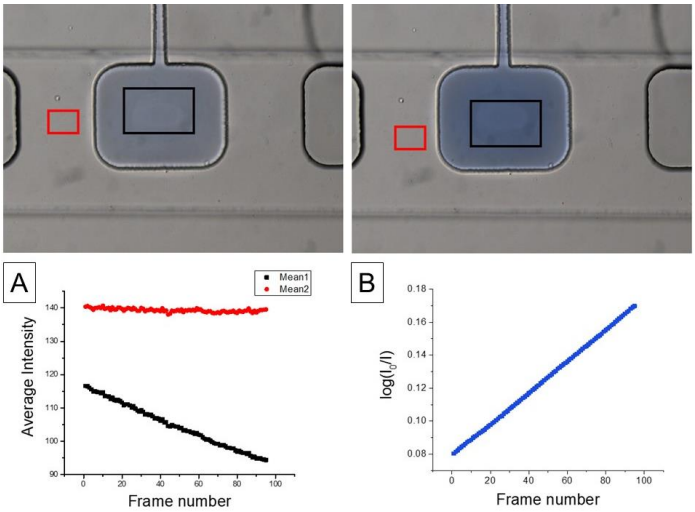

Figure 13. Image analysis to prove the reverse osmosis occurring in the microfluidic valve. Mean 1 (black) is the average intensity decrease inside the valve, Mean 2 (red) is the constant average intensity outside the valve.

To demonstrate this, I plotted the logarithm of I_0(intensity outside)/I(intensity inside) vs the frame number of the video to obtain a representation that follows the Lambert-Beer law. When looking at the concentration outside the valve, A graph shows that is constant all over the analyzed time (Mean 2, red). The change of the intensity inside the valve (Mean 1, black) must be a direct consequence of the concentration increase inside the valve. This can be seen in B graph, where the logarithm increases with the frame number. This increase means that the concentration inside also increases, due to pressuring the valve against water by reverse osmosis overnight. The ratio final concentration/initial concentration is 2.13. Note that this increase of concentration cannot be accomplished in bulk experimentation without crystal precipitation.

7. BULK STUDIES

As you can see in Table 2 and Figure 9, the H_2O/EtOH ratio makes the blue CuGHG MOF crystals (Figure 14 (A)) transform into a platelet-like pink phase (Figure 14 (B)). While the blue

CuGHG MOF crystals correspond to the tetragonal porous phase[4], the platelet-like pink crystals correspond to a non-porous form of CuGHG, a reported monoclinic phase[5]. Therefore, the platelet-like pink crystals cannot be considered as a MOF because they are not porous.

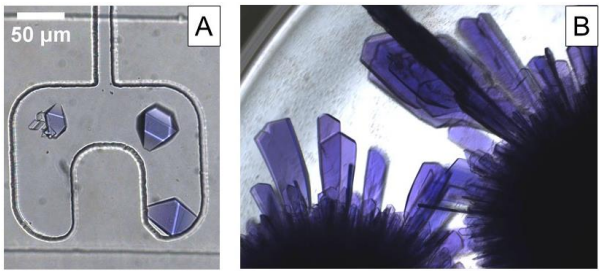

Figure 14. Porous blue MOF crystals (A) and non-porous platelet-like pink phase (B)

Surprisingly, we demonstrated that the transformation from the blue CuGHG MOF crystals to the non-porous platelet-like pink crystals happens via dissolution-reprecipitation, following a Ostwald's rule of stages. As shown in Figure 15, this process consists in the dissolution of the blue phase crystals and the reprecipitation into the platelet-like pink crystals.

Figure 15. Optical microscope images of the transformation of the porous blue MOF crystals to the platelet-like pink phase via dissolution reprecipitation.

To clearly confirm the formation of the porous and non-porous phase of CuGHG, we conducted x-ray diffraction studies (XRD) on the porous blue MOF crystals and the platelet-like pink crystals. Next, the spectra obtained for both crystal types were compared with the reported structures present in the Cambridge Data Base (Figure 16 (A)), thus corroborating their porous and non-porous nature. Additionally, we observe that also Raman spectroscopy can be effectively used to confirm the formation of the porous and non-porous phase of CuGHG (Figure 16 (B)).

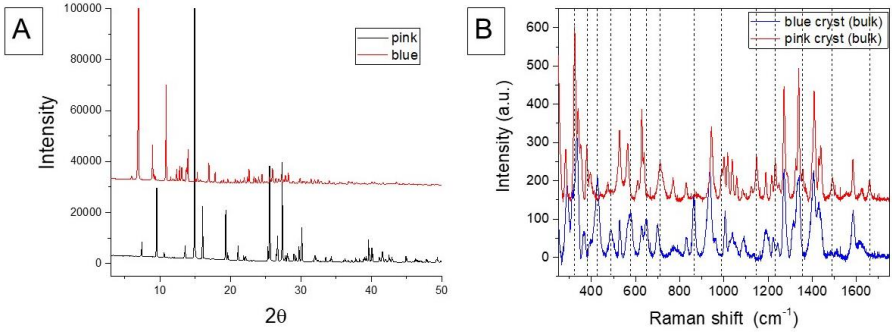

Figure 16. PXRD (A) and Raman shift (B) from the blue and pink phases.

8. CHARACTERIZATION OF THE CRYSTALS OBTAINED INSIDE THE VALVES

After flowing EtOH and H_2O to a 4 day concentrated solution in the valve, a crystal was obtained (Figure 17).

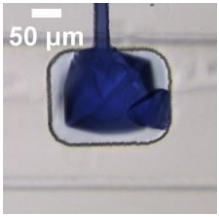

Figure 17. Crystal obtained via solvent switching

This crystal obtained is the same as the blue porous phase of the MOF, the most stable one in water. To demonstrate that, we compared its Raman spectra with the blue porous phase that I acquired in the bulk studies (Section 7).

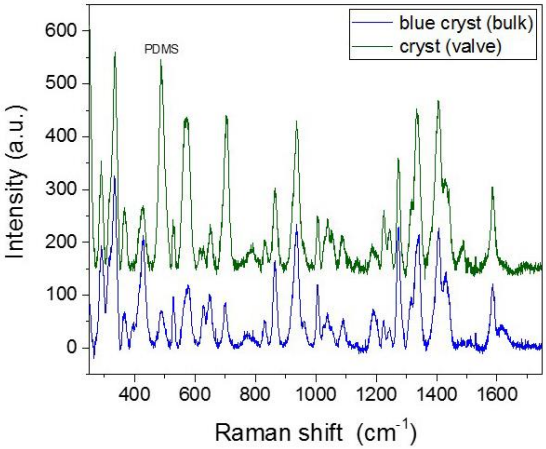

Figure 18. Raman spectra of the blue CuGHG crystal generated in bulk and the blue CuGHG crystal obtained inside the valve.

As shown in Figure 18, the Raman spectra of the two CuGHG crystal match perfectly to each other, which clearly demonstrates the generation of the porous CuGHG phase inside the microfluidic valve.

9. CONCLUSIONS

The conclusions of the current study are:

- Coflowing is not an optimal condition to induce a phase separation along the CuGHG crystallization pathway, it depends on where the flows of EtOH and H_2O are located with respect of the valve.
- When working with solvent switching, there is a clear difference depending on the state of the precursor solution. If it is fresh, it shows precipitation-coalescence/dissolution-precipitation behavior. On the other hand, if the solution has been concentrated one or more days, it shows a precipitation-coalescence-crystallization pathway.
- This concentration increase happens by reverse osmosis, when the valve is under pressure and against water. As mentioned above, concentrating the solution helps the crystallization, but highly concentrated solutions do not crystallize into single crystals, they crystallize into multiple small crystallites as shown in the additional experiments section.
- In bulk conditions, it is possible to obtain the pink phase of CuGHG stable in EtOH depending on the EtOH/water ratio of the solution. With ratios above 1:1, the blue porous phase that it is stable in water transforms into the pink phase via dissolution-reprecipitation, this is a clear example of Ostwald's rule of stages.

10. REFERENCES AND NOTES

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11. ACRONYMS

ACC: amorphous calcium carbonate

CPA: crystallization by particle attachment

CuGHG: Copper-glycine-L-histidine-glycine

MOF: Metal-organic framework

OA: oriented attachment

PDMS: polydimethylsiloxane

PXRD: Powder X-ray diffraction

UV-vis: Ultraviolet-visible

APPENDICES

APPENDIX 1: PDMS DEVICES FABRICATION

Prepare two PDMS:catalyst mixtures: 20:1 for the fluid layer (Y shaped main channel) and 5:1 for the control layer (valves). Mix both well from 5 to 10min. Pour the 20:1 mixture on the wafer and spin-coat it 10s at 500rpm and 50s 1200rpm. Curate the 5:1 mixture 30min in the 70ºC oven without spin-coating. After being spin-coated, curate the 20:1 mixture 30min in the same oven. Punch the inlets of the control layer. Thermal bond the two layers overnight in the oven at 70ºC. Next morning punch the inlets and outlets of the main channel.

After that, it is needed to plasma-bond the PDMS device to a glass slide. Before doing it, clean properly the glass slide with isopropanol and clean the dust over the PDMS device with scotch tape. With O_2 plasma, the SiOH groups of the PDMS of the device and the glass are activated. After that, if you put the PDMS over the glass slide (the bottom of the fluid layer must be in contact with the glass), Si-O-Si bonds are formed between the device and the glass, creating an irreversible seal.

APPENDIX 2: PREPARATION OF CUGHG SOLUTION

To prepare a 500µL 150mmol 1:1 CuGHG solution, we must put 20.195mg of the GHG peptide on an Eppendorf tube. After that, pour 500µL $Cu(CO_2CH_3)_2 \cdot H_2O$ solution previously prepared with 89.84mg of $Cu(CO_2CH_3)_2 \cdot H_2O$ and 500µL of Milli-Q water. Vortex the content of the tube for 3s and gently mix it until the solution is completely homogeneous.