Organic Process Research & Development

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A Cheap, Easy Microfluidic Crystallization Device Ensuring Universal Solvent Compatibility

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ABSTRACT: Microfluidic devices are increasingly used for the screening of crystallization conditions. Their advantage is the generation of droplets, every droplet being an independent crystallizer with volumes in the nanoliter range. This enables a large number of experiments to be carried out under identical conditions necessitating only small quantities of materials. However, classic microfluidic crystallization devices are made of poly(dimethylsiloxane), only compatible with an aqueous medium. In addition, they generally involve very complicated setups, often inaccessible to nonmicrofluidics specialists. In this paper, we overcome these drawbacks, presenting a cheap and universally applicable microfluidic crystallization tool. This thermostatted device makes it possible to study nucleation in both aqueous and organic solvents, rendering microfluidic devices applicable to organic molecules such as APIs, explosives, and metal oxide nanoparticles.

1. INTRODUCTION

The crystallization of novel materials requires experimental strategies for the rapid screening of crystallization conditions. When only small quantities of materials are available for preliminary studies, new crystallization tools are vital. In the past decade, an interest in microfluidic crystallization has increased.[1−4] Microfluidic devices allow a large number of experiments under identical conditions, every droplet being an independent crystallizer with volumes in the nanoliter range. Most studies use poly(dimethylsiloxane) (PDMS) devices and focus on complicated setups, multilayer PDMS chips with multiinputs that are generally inaccessible to nonmicrofluidics specialists.[5−8] PDMS is used because this building material is cheap and convenient, but it has disadvantages, the main one being its poor solvent compatibility. However, some groups have recently started to develop simple microfluidic devices[10−12] in which plugs are generated via a T-junction coupled to PVC or Teflon tubing. Our aim here was to address the problem of solvent-compatibility, building on these recent attempts at simplification, with a view to developing a laboratory-scale tool. Therefore, the key points of this work are an easy-to-use, universally solvent-compatible and cheap microfluidic setup which avoids the use of surfactant and is easily transferable to the laboratory for crystallisation studies. To prove the viability of our setup, we apply simple methods previously developed for measuring metastable zone width[13] and nucleation kinetics[14] for aqueous media. The results presented here concern nucleation of a biological molecule and active pharmaceutical ingredients (APIs) in different crystallization media, including organic solvents.

2. EXPERIMENTAL SECTION

2.1. Solutions. *2.1.1. Protein Solutions.* Hen-egg white lysozyme (14600 Da, pI = 11.2) was purchased from Sigma (batch 057K7013 L 2879) and used without further purification. Lysozyme purity was checked by molecular sieving. A suitable amount of lysozyme was dissolved in pure water (ELGA UHQ reverse osmosis system) to obtain the stock solutions required. A 1.4 M NaCl solution was also prepared. The different solutions were buffered with 80 mM acetic acid, adjusted to pH = 4.5 with NaOH (1 M), and filtered through 0.22 μm Millipore filters. The pH was checked with a pH meter (Schott Instrument, Prolab 1000) equipped with a pH microelectrode. Lysozyme concentrations were checked by optical density measurements (Biochrom, Libra S22) using an extinction coefficient of 2.64 mL cm^{-1} mg^{-1} at 280 nm. In all the crystallization experiments hereafter, the NaCl concentration was fixed to 0.7 M at pH = 4.5.

2.1.2. Organic Solutions. Caffeine was purchased from Sigma (batch A0283756) and used without further purification. A suitable amount of caffeine was dissolved in ethanol 99% (batch V0D770190D) to obtain the stock solutions required. Isonicotinamide was purchased from Sigma (batch BCBD6627) and used without further purification. A suitable amount of isonicotinamide was dissolved in ethanol 99% and nitrobenzene (batch STBB8950 V).

2.2. Devices. The basic design and construction of our microfluidic devices were as described by Laval et al.[9] In our previous adaptation[13] of this classic microfluidic device, chips are divided into two parts: the plug-factory zone for the mixing and generation of droplets and the droplet-storage zone for the storage and observation of droplets. The channel size of the plug-factory determines droplet size. Using this setup, we generate droplets of the same volume at a given concentration, and we fill storage chips that we store at different temperatures. Here, we present the chronology of our experimental approach. we constructed two devices based on our previous adaptation, which used the same droplet method but chips built from different materials, namely varying combinations of PDMS, Teflon, and polyether ether ketone (PEEK). During generation and storage, the devices and the feeding syringes were thermostatted (see details hereafter). Precision syringe pumps (Bioseb BS-8000)

Received: October 18, 2011
Published: February 28, 2012

were used to control the flow rates (100−600 μL/h) of drops and continuous phase, and the temperature of the pumps was controlled by an incubator. Drops were observed under a stereomicroscope (Wild Makroskop) equipped with a CCD camera (imaging source, DFK 31BF03).

2.2.1. Pure PDMS Device. Both plug-factory and storage chips are made of PDMS. During the experiment, the device is thermostatted by Peltier elements (Anacrismat, France). Teflon tubing (SCI, BB311-24), of 584 μm inner diameter, connects plug-factory and storage chips. Each droplet has a volume of 250 nL with a volume polydispersity of a few percent.[15,16] This device can be used to store droplets for 24 h, although longer storage will lead to extensive evaporation due to the porosity of PDMS.[17]

2.2.2. Mixed PDMS/Teflon Device. While in the previous setup, Teflon tubing connects plug-factory and storage chips, this device uses Teflon tubes as storage chips. There are two major advantages: no detectable evaporation after 1 week at room temperature (evaporation being estimated from the evolution of the size of the droplet) and universal applicability to organic solvents. However, the plug factory is still in PDMS thermostatted by Peltier elements. In order to reach a good wettability of the oil for both PDMS and Teflon wall, we use FMS Oil (Poly-3,3,3-trifluoropropylmethylsiloxane, Hampton Research). Hence, we avoid the risk of forming oil droplets in the aqueous phase. After filling, the tube entrance is heated and pinched to seal the tube. An incubator controls temperature. Note that, during observation, the storage tube is immersed in a temperature-controlled water vessel, allowing direct observation through the transparent Teflon wall.

2.2.3. Mixed PEEK/Teflon Device. In this device the plug-factory is a T-junction made of PEEK (IDEX P-727 PEEK Tee) as proposed by Dombrowski,[12] the output tube being a Teflon storage tube. The T-junction (Figure 1) has an inner diameter

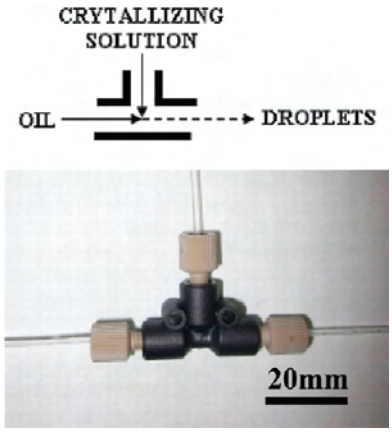

Figure 1. Scheme and photo of plug-factory T-junction made of PEEK.

of either 508 μm (250 nL droplets) or 152 μm (2 nL droplets) and is connected to Teflon tubing of a diameter of 508 or 150 μm, respectively. The advantage of both Teflon and PEEK polymers is their compatibility with all solvents, the sole exception being concentrated acid (sulfuric or nitric acid). In order to ensure maximum versatility, we used fluorinated oil FC-70 (Hampton Research), which has no or very low miscibility with solvents and good wettability with Teflon. In addition, the device being thermostatted, we can store droplets for several weeks without significant evaporation.

2.3. Methods. *2.3.1. Crystallization Strategy.* The way supersaturation is achieved in chips or tubes defines the crystallization process. An easy way to achieve supersaturation consists of using temperature, making it possible to control supersaturation at constant composition with a reversible effect.[18] This is the method used here. When the temperature dependence of solubility is too low, another method consists of using a solvent mixture where the solubility of the solute is high in the first solvent and (very) low in the second. Thus, the supersaturated stage is rapidly reached. This method can be carried out easily with our setup by adding a second T-junction at the entrance to the crystallizing solution (Figure 1).

2.3.2. Determining Metastable Zone. The metastable zone represents a kinetic limit in the supersaturated region of the phase diagram below which no nucleation occurs for a given time in a given volume. Note that crystallization of an organic compound such as an active pharmaceutical ingredient (API) is different from crystallization of protein, because organic molecules have a higher nucleation rate than proteins. Hence, the nucleation time chosen in this work is 4 h for caffeine compared to 20 h for lysozyme. The experimental procedure to determine the metastable zone was as previously described.[13] To summarize, four chips or tubes are filled with the same solution and stored at different temperatures. Then, nucleated crystals are counted, and the temperature at which there is no crystal, T_{MZ}, is extrapolated (interpolation) from a plot of the average number of crystals per droplet versus temperature.

2.3.3. Nucleation Rate: Double Pulse Technique.[19−21] The method and the data treatment were as presented in a previous paper.[14] To summarize, crystals are nucleated at a nucleation temperature, outside the metastable zone (below T_{MZ}) and for a given nucleation time. Then, the temperature is raised until it reaches the metastable zone (growth temperature), where the crystals already formed grow to detectable dimensions and are counted. The use of this method allows nucleation to be separated from the ensuing growth. Thus, the steady-state nucleation rate is given by the slope of the average number of crystals nucleated as a function of the nucleation time. For all experimental conditions, four devices were filled with the same solution, allowing four nucleation times to be tested simultaneously. Here nucleation and growth temperatures are 20 and 40 °C, respectively, the choice of these temperatures being clearly explained by Ildefonso et al.[14]

3. RESULTS AND DISCUSSION

In this contribution, we present the results of nucleation experiments on a biological molecule and two APIs in different crystallization media including organic solvents. Teflon tubing is used as storage chip with two different plug-factories, as described above (part 2.2).

3.1. Nucleation of Protein in the Mixed PDMS/Teflon Device. In order to prove the viability of our Teflon storage devices, we compare nucleation of our model system, lysozyme, in the classic PDMS storage chip and the Teflon storage chip, with the plug-factory in PDMS in both cases. Results are summarized in Table 1. Figure 2a shows the time dependence of the average number of crystals nucleated per droplet using the double pulse technique; the slope of the straight line gives us the primary nucleation rate using the Teflon chip. In Figure 2b, we plot the nucleation rate versus supersaturation obtained in the Teflon storage chip and in the classic PDMS chip.[14] We used the same protein batch, the same concentration range, and the same temperature conditions. The plug-factory is identical

dx.doi.org/10.1021/op200291z | Org. Process Res. Dev. 2012, 16, 556−560

Table 1. Average number of lysozyme crystals nucleated per droplet[a] for a given protein concentration at a given nucleation time

protein concentrations (mg/mL)	Nucleation times								
	0 min	20 min	30 min	40 min	1 h	1.33 h	1.5 h	2 h	3 h
40	0		0 (−)		0.023 (2.5)		0.051 (2.5)	0.012 (5.4)	0.058 (1.5)
45	0	0.117 (1.6)		0.329 (0.8)	0.381 (0.7)	0.472 (1.0)		0.665 (0.7)	
50	0		0.541 (0.6)		1.166 (0.4)				

[a]The deviation in % is given in parentheses.

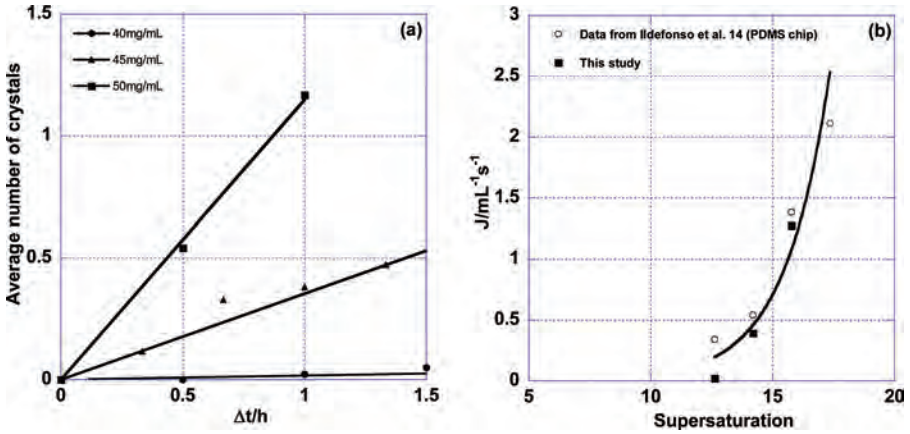

Figure 2. (a) Average number of lysozyme crystals nucleated per droplet versus nucleation time (NaCl = 0.7 M and pH = 4.5). (b) Primary nucleation rate vs supersaturation, at 20 °C (the line is a guide for the eye).

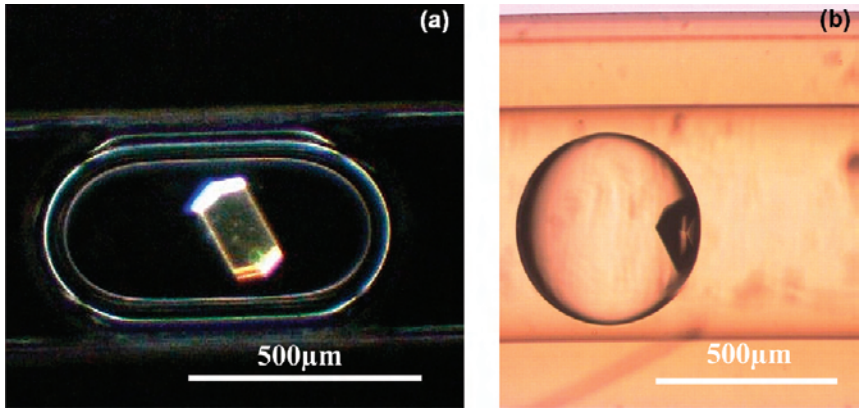

Figure 3. Images of lysozyme crystals in a 250 nL droplet (20 mg/mL, NaCl = 0.7 M, pH = 4.5 at 20 °C) in (a) PDMS chip and (b) in Teflon chip.

in both devices, with a droplet volume of 250 nL. However, the tubing has a diameter of 584 μm while PDMS chips have a diameter of 500 μm; thus, the shape of the droplets differs, as shown in Figure 3. We also changed both the oil, from silicone (Sigma oil AP 100) to FMS Oil, and channel material, inducing a modification of the surface tension of droplets. Moreover, the droplet evaporation rate in Teflon tubing is lower than in PDMS. Despite these differences, we measure nucleation rates in good agreement with those obtained in pure PDMS chips. To sum up, this Teflon storage device is versatile, cheap, and permits nucleation experiments in droplets over a longer period than PDMS chips.

3.2. Nucleation in the Mixed PEEK/Teflon Device. One of the limitations of the mixed PDMS/Teflon device tested in part 3.1. is the poor solvent compatibility of the PDMS plug-factory. Here we used a T-junction made of PEEK as plug-factory.

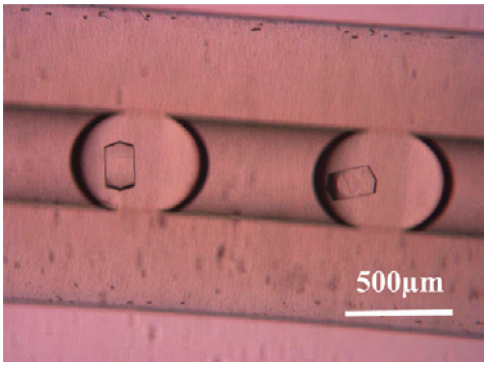

Figure 4. Images of lysozyme crystals in droplets (35 mg/mL; NaCl = 0.7 M; pH = 4.5; T = 20 °C) in a mixed PEEK/Teflon device.

3.2.1. Generating Aqueous-Phase Droplets. We checked the validity of the ensuing device for generation of aqueous-phase

dx.doi.org/10.1021/op200291z | Org. Process Res. Dev. 2012, 16, 556−560

droplets. Dombrovsky et al. showed for lactose that a PEEK T-junction is able to generate aqueous droplets with a volume polydispersity of a few percent.[12] Here, we generated droplets of lysozyme and stored them in Teflon tubing. We obtained regular droplets in which lysozyme crystals appear after a few hours (Figure 4).

3.2.2. Generating Organic Solvent Droplets. To ascertain the universal solvent compatibility of this device, we tested it with different organic solvents common in industrial crystallization studies, namely ethanol, acetone, ethyl acetate, as well as less conventional solvents such as nitrobenzene or acetonitrile. In every solvent, the PEEK/Teflon device generates droplets which can be stored for more than 1 week without significant evaporation (Figure 5a). It is noteworthy that no droplet coalescence is observed. We also tested the ability of crystals to nucleate in this new device using three different solutions, solutions of caffeine and isonicotinamide in ethanol (Figure 5b,c) and a solution of isonicotinamide in nitrobenzene (Figure 5d). In all cases, crystals with totally different habits were obtained. To sum up, the PEEK/Teflon device is versatile, cheap, and universally compatible with organic solvents, as well as being suitable for crystallization experiments.

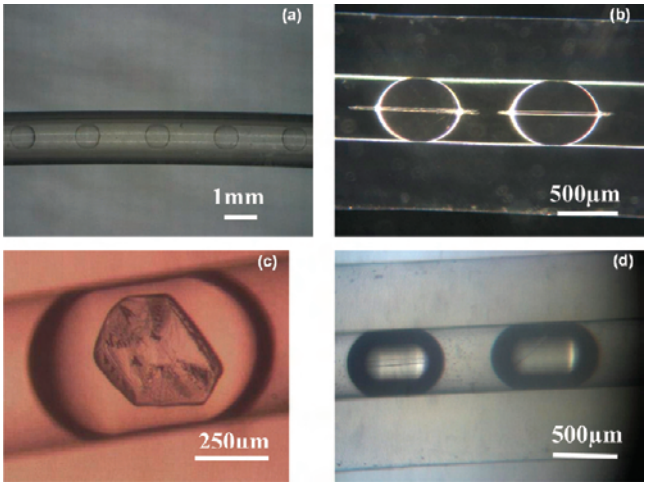

Figure 5. (a) Acetone droplets in fluorinated oil after 3 months storage. (b) Caffeine crystals in ethanol droplets (10 mg/mL; T from 30 to 10 at 0.5 °C/min). (c) Isonicotinamide crystal in an ethanol droplet (143 mg/mL; T = 10 °C). (d) Isonicotinamide crystals in nitrobenzene droplets (7 mg/mL; T = 10 °C).

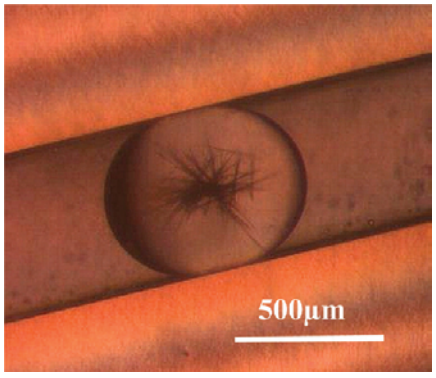

Figure 6. Caffeine crystals in an ethanol droplet (7 mg/mL − T = 20 °C).

3.2.3. Application to the Nucleation of Caffeine. The control of nucleation is a real challenge. For instance, in the pharmaceutical industry nucleation will control the phase and the crystal size distribution, which are key parameters of APIs. Here, we carried out a preliminary caffeine nucleation study using the PEEK/Teflon device and obtained reliable data on nucleation of caffeine in ethanol. The high anisotropy of the API crystal structure frequently involves the production of needle-shaped or acicular crystals.[22] This is confirmed with caffeine, which crystallizes with an acicular habit (Figure 5b). Moreover, for higher supersaturation, activated secondary nucleation appears, leading to a random nucleation of needles[23] (crystalline agglomerates) (Figure 6). This is a limitation for nucleation studies using the double-pulse method because it is impossible to count crystals. Therefore, we adapted our method to measure the metastable zone width; the knowledge of the metastable zone width presents an interest in the control of industrial crystallization. Instead of counting crystals and extrapolating the temperature T_{MZ} at which there is no crystal, we counted the number of droplets that nucleated after 4 h. T_{MZ} is extrapolated from a plot of the percentage of nucleated droplets versus temperature. In practice, T_{MZ} is bracketed according to the following formula:

$$T_{0\%} - 5 < T_{MZ} < T_{0\%} \quad (1)$$

with $T_{0\%}$ the lowest nucleation temperature in °C yielding no crystals.

We tested four concentrations from 4 to 8 mg/mL with temperature varying from 5 to 30 °C (Figure 7a). For each condition 30−110 droplets were generated. Using this method, we obtained the metastable zone limit of caffeine in ethanol, shown on the phase diagram (Figure 7b). To sum up, this PEEK/Teflon device is a cheap and universally applicable crystallization tool.

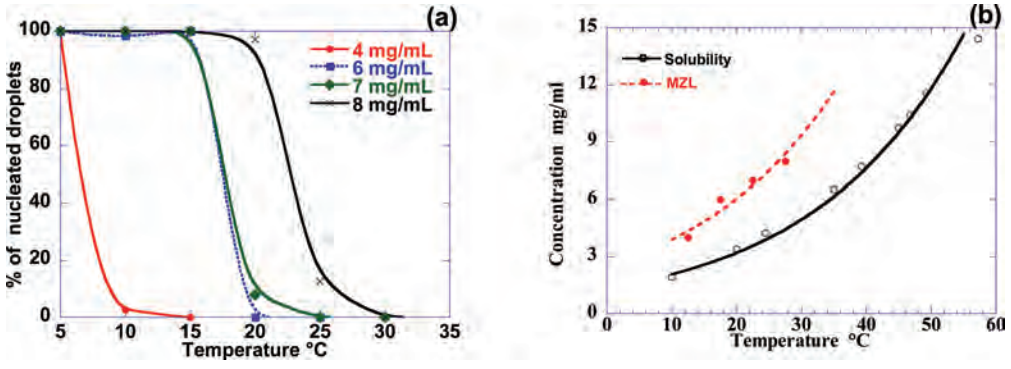

Figure 7. (a) Percentage of nucleated droplets of caffeine vs temperature and (b) caffeine solubility in ethanol versus temperature (our data) and MZ limit, lines improve legibility.

dx.doi.org/10.1021/op200291z | Org. Process Res. Dev. 2012, 16, 556−560

4. CONCLUSION

In this paper we present a cheap and universally applicable crystallization tool, in the form of a PEEK/Teflon microfluidic device. This thermostatted device allows droplets to be formed in different aqueous and organic solvents and stored for several weeks without significant evaporation. Thus, our device makes it possible to study nucleation in both aqueous and organic solvents, rendering microfluidic devices applicable to organic molecules such as APIs, explosive metal oxide nanoparticles. However, for systems leading to a random nucleation of acicular crystalline agglomerates, the double-pulse method cannot be directly applied to measure nucleation rate. Future studies will examine whether using the induction time method[24,25] could provide a solution. Lastly, it should be noted, as pointed out by Yadav et al.[11a], that Teflon tubing is suitable for in situ screening X-ray diffraction studies, thus opening interesting perspectives in the field of crystallization screening for which solid-phase characterization is vital.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank T. Bactivelane (CINaM) and M. Audiffren (Anacrismat) for their help in the development of the setup. We thank M. Sweetko for English revision.

ADDITIONAL NOTE

[a]In their paper Yadav et al. centered a water plug in a Teflon capillary in the beam of a Rigaku MSC R-AXIS IV X-ray diffractometer; after 10 min collection the data were suitable for indexing crystal.

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