Electronic Supplementary Material (ESI) for Reaction Chemistry & Engineering.

Supplementary Information

Automated Self-Optimization of Continuous Crystallization of Nirmatrelvir API

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Continuous Crystallization Experimental Setup

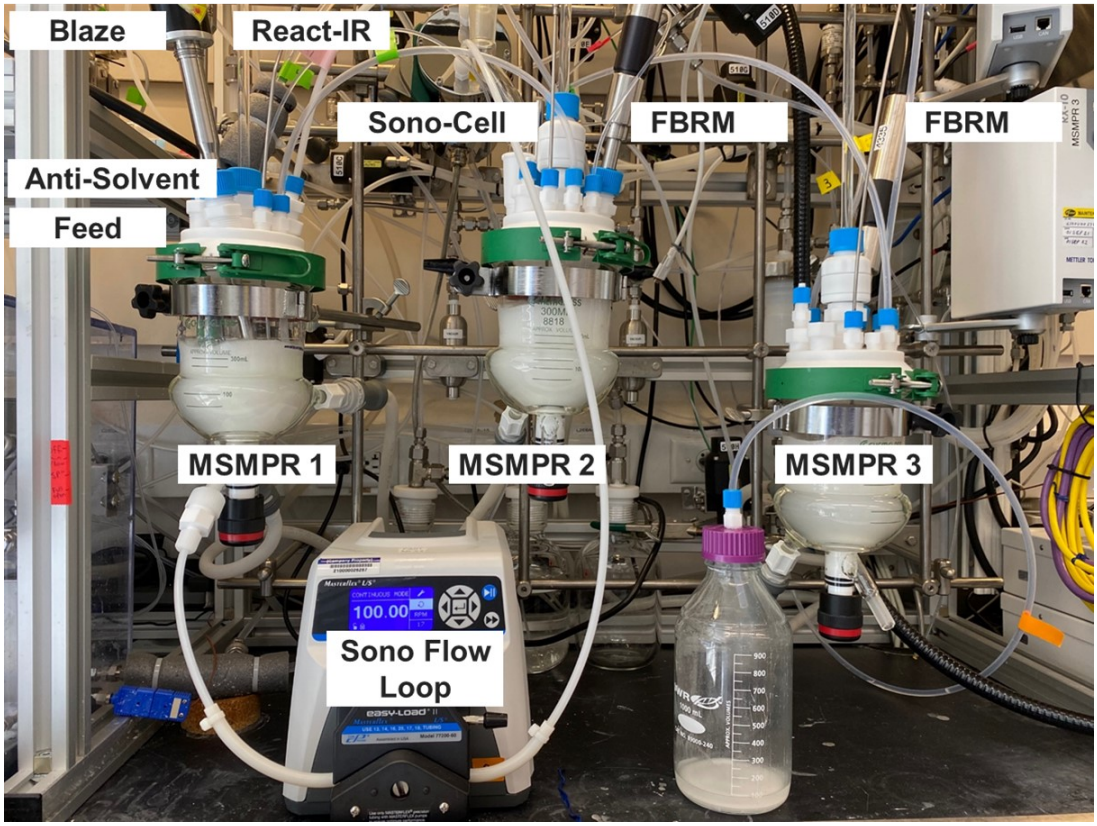

Fig. S1 Continuous crystallization experimental platform.

The experimental setup of continuous crystallization with various PATs is shown in Fig. S1.

Key components of the MSMPR crystallizer platform: positive displacement pump (VICI, Model M50 pump), peristaltic pump (Cole-Parmer Masterflex^{TM}), Bronkhorst M13 flow meter, Julabo (FP50 or FP32) temperature control units (TCUs), overhead stirrers (IKA EUROSTAR power control-visc), 20 kHz Ultrasonic Flow Cell (model FC150-20, flow sonication), valves (Swagelok 153 series pneumatic spring return actuator), pressure transducer (Swagelok PTI-S-NC60-12AQ), and solenoids (SMC SY5000 series).

Process analytical technologies (PATs): Blaze™ probe (model 900), Mettler Toledo React-IR™ (model 702), and Mettler Toledo ParticleTrack™ G400 FBRM® (Focused Beam Reflectance Measurement) technology.

Automation of the Continuous Crystallization Platform

The automation of the continuous crystallization platform is achieved by an in-house designed

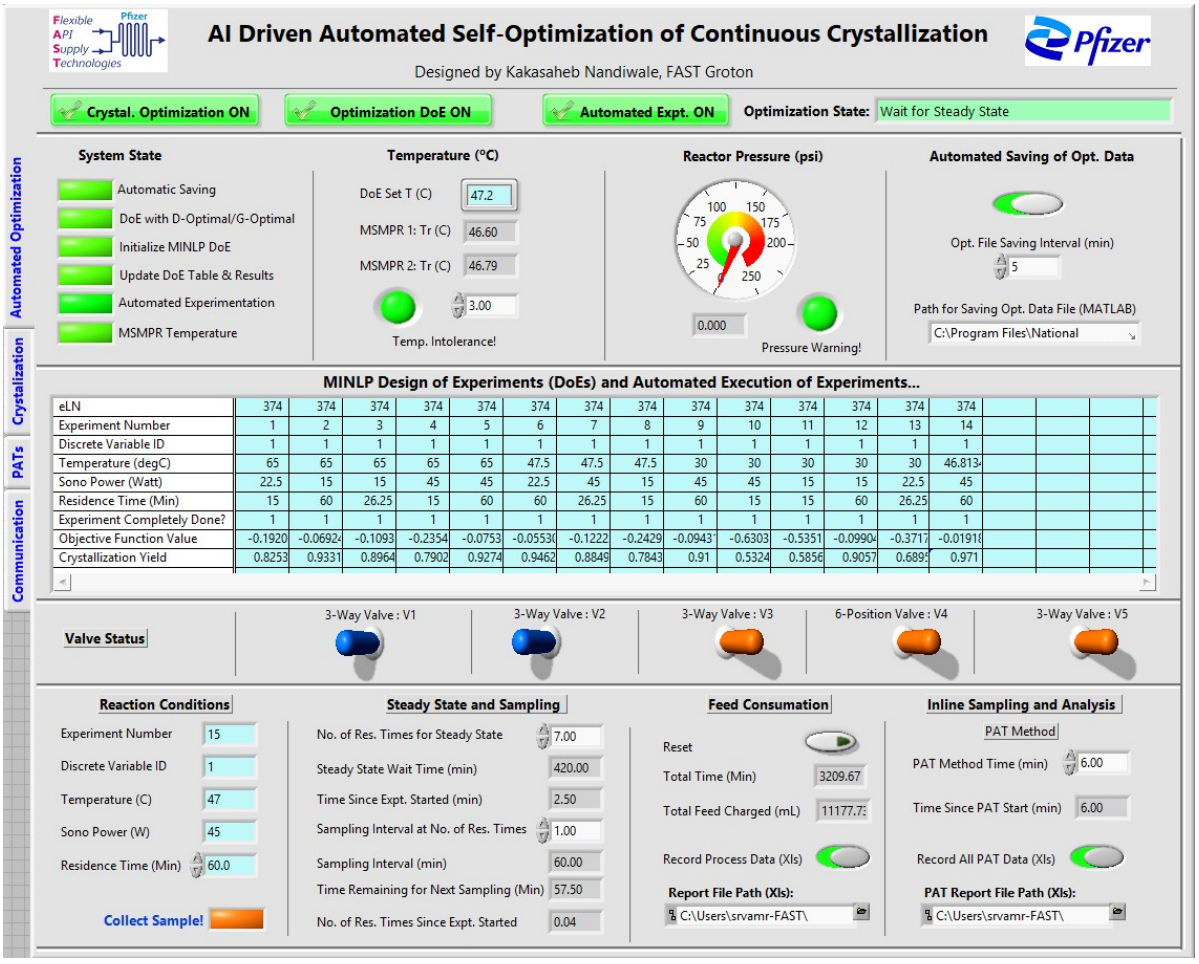

Fig. S2 LabVIEW™ VI user interface for the DoE driven automated self-optimization of the continuous crystallization of APIs.

virtual instrument (VI) in LabVIEW™ (National Instrument, NI ver. 21.0). The LabVIEW™

automation VI consists of several tabs (user interfaces) to achieve seamless integration of lab

equipment, PATs, and optimization algorithms (Fig. S2-S4). Fig. S2 shows the LabVIEW™ VI

user interface including the automated design of experiment (DoE) table, optimization variables,

the experimental progress, warning indicators, data paths, and automated experimentation time.

The automated DoE is achieved by the integration of optimization algorithms in the MATLAB®

& SIMULINK® (MathWorks, Inc., ver. R2021b) with the MATLAB-Script node in

LabVIEW™.[1] Fig. S3 shows the LabVIEW™ user interface for the continuous crystallization

including schematic of the setup. This LabVIEW™ VI allows control including, the flow rates, stirrer RPM, power of flow-sono cell, and temperatures. The user interface is designed for a dual purpose: (1) to allow "fully" automated execution of experiments in an automated DoE during the optimization campaign, and (2) to allow user inputs (manual interventions) for the continuous crystallization experiment without automated DoE. The user controls with on/off switches on the LabVIEW™ interface used to prime the pumps and prefill the feed and antisolvent lines. In addition, this interface is also used for setting the desired temperature setpoints to allow time for temperature equilibration, before starting the "fully" automated self-optimization campaign.

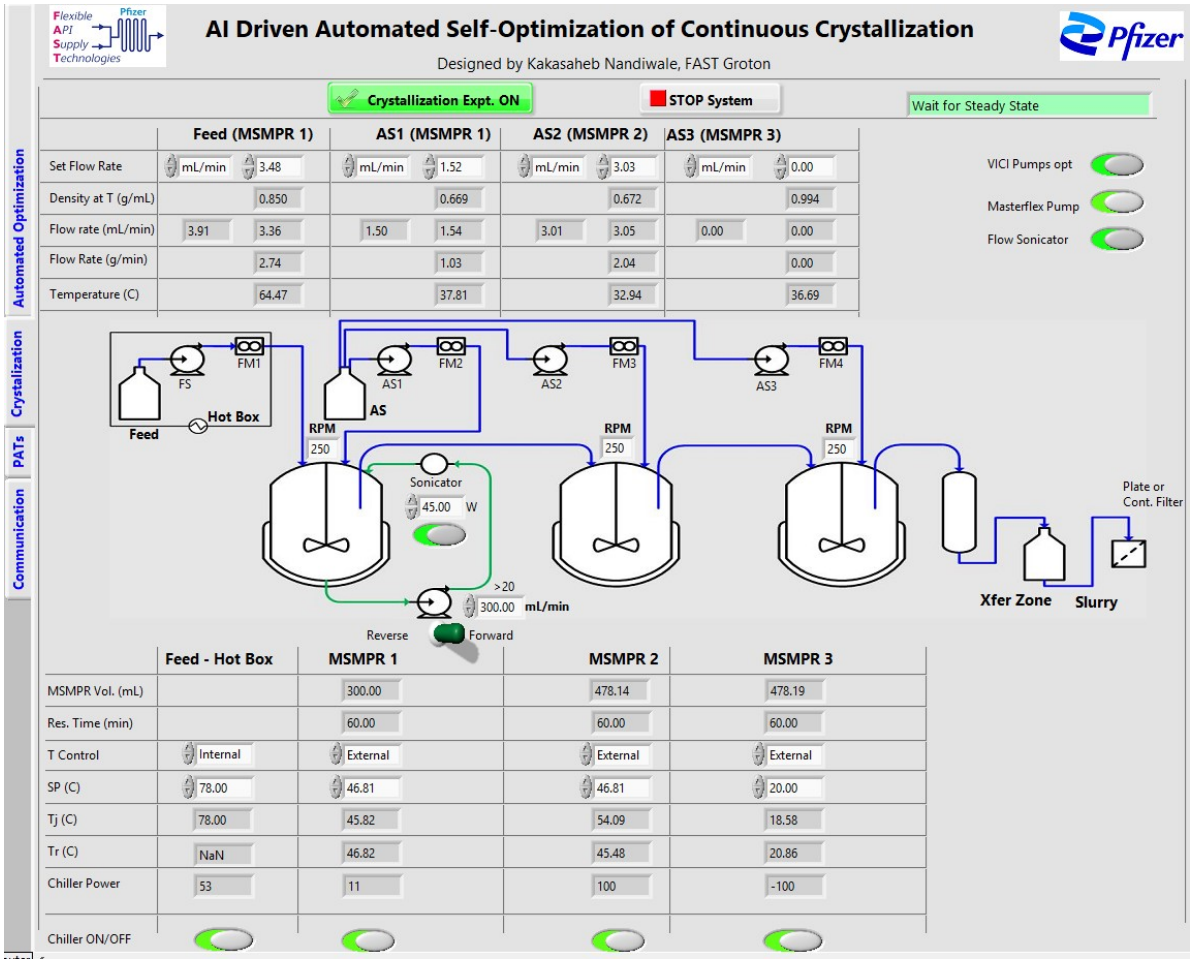

Fig. S3 LabVIEW™ VI for lab equipment controls of continuous crystallization platform.

During the execution of the "fully" automated self-optimization campaign, the DoE setpoints for the flow rates (calculated based on the residence time in the DoE), process temperatures, power of flow-sonication cell etc. are automatically adjusted by the LabVIEW™, without requiring any human interventions. Fig. S4 indicates the LabVIEW™ VI tab with plots of data from the PATs obtained by OPC UA and process parameters (e.g., pump flow rates, temperatures etc.). The FBRM® plots also include the process variables, e.g., process temperature, to allow real-time understanding of the effect of each process variable on the particle trends (Fig. S4).

Moreover, this VI tab is also used to observe any undesired variations in the flow rates, pressure spikes etc., in the event of clogging or pump failure (Fig. S4).

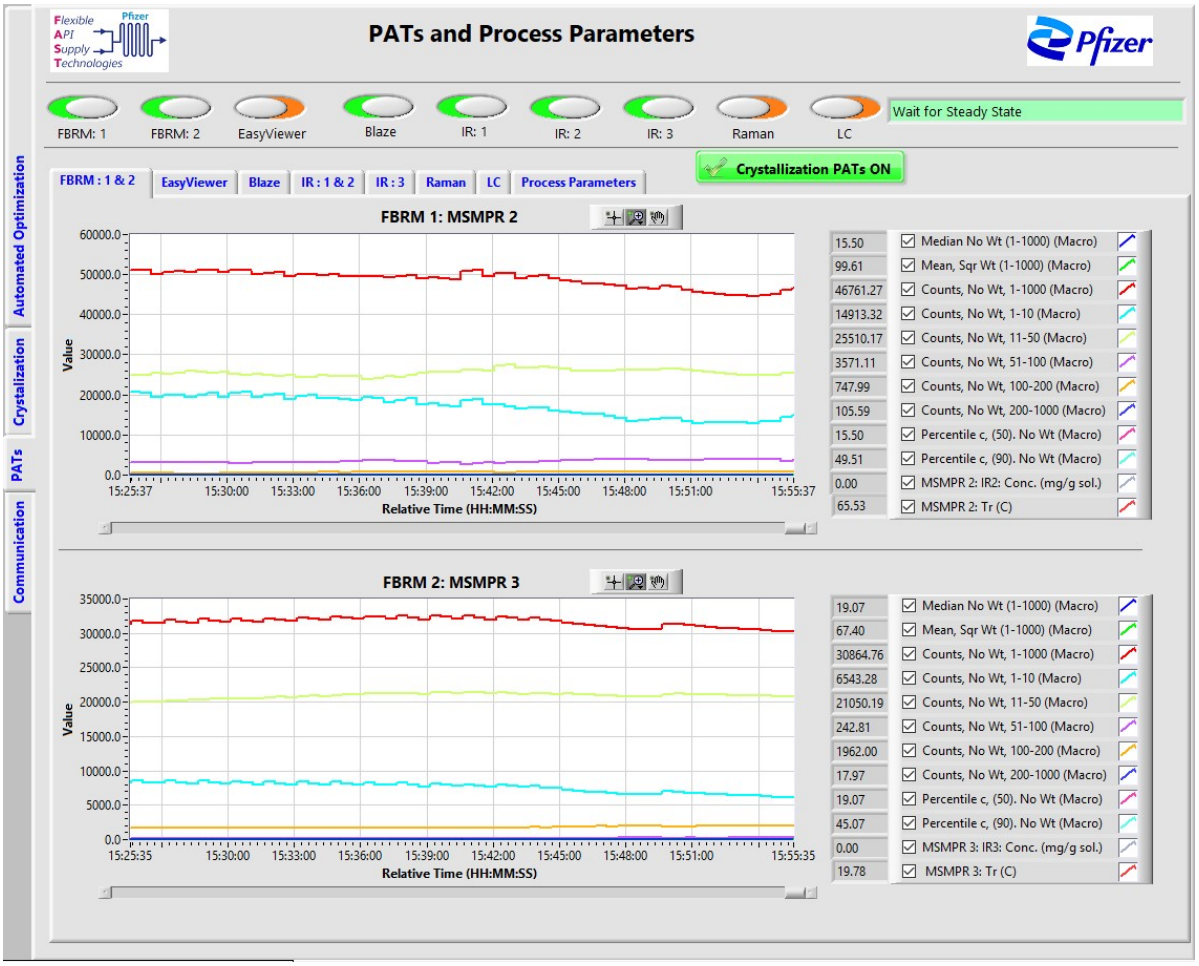

Fig. S4 LabVIEW™ VI showing the real-time data from the PATs obtained via OPC UA.

Design of Continuous Crystallization Process

The batch crystallization process involves an antisolvent and cooling crystallization. This process takes MTBE (Methyl Tertiary Butyl Ether) solvate of the API and through the recrystallization, the free form of nirmatrelvir API is isolated. The antisolvent (heptane) is dosed in at 65 °C and then after reaching a final solvent composition of 53.6 wt% heptane the reaction content is cooled to the final isolation temperature of 20 °C.

The initial approach for the continuous crystallization using three MSMPRs is to employ all three vessels for the equal amount of desaturating the solution. However, the batch crystallization analysis revealed that the crystal growth is slow at the cooler temperatures (~20 °C).

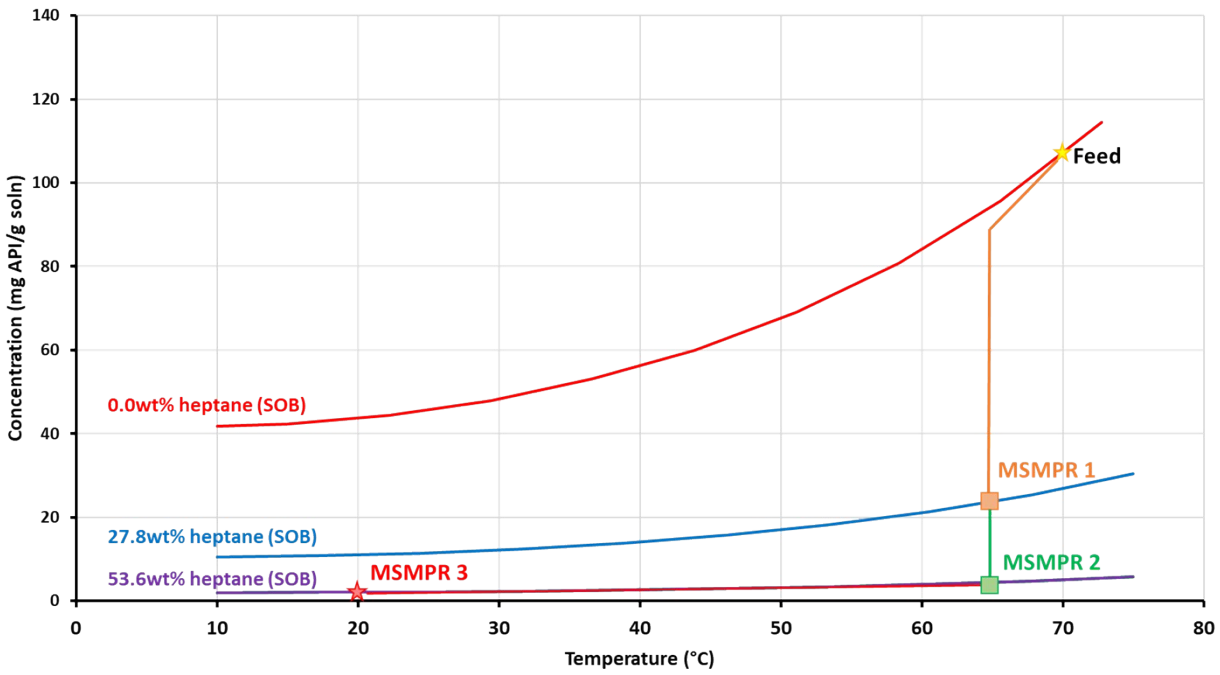

Fig. S5 Solubility based design of center point conditions for the continuous crystallization of nirmatrelvir API.

Therefore, all antisolvent to the continuous crystallization was added while still at high temperature (e.g., 65 °C in MSMPR-1 and MSMPR-2) to facilitate the fastest crystal growth

(Fig. S5). The final reactor (MSMPR-3) would quickly reduce to the final isolation temperature of 20 °C and finish off the desaturation of the solution.

Equation 1 is used to find the flow rate required for the feed. Equation 1 uses the volume of the first MSMPR (v), the set residence time (τ), the density of the feed stream (at temperature), the wt% of the API in the feed stream, and the mass ratio of antisolvent to API to determine the required feed flow rate. An API usage rate is calculated by multiplying the feed flow rate by the wt% of the API in the feed to know how many g/min of API is being fed. The API usage rate is used to calculate the flow rate for the antisolvent to control the MSMPR at the desired wt% of antisolvent by multiplying the API usage rate by the grams of antisolvent per gram of API required.

$$Feed\ flow\ rate\ (g/min) = \frac{V}{\tau} \times \left(\frac{1}{\frac{1}{\rho_{Feed}} \times \frac{wt\%\ API_{Feed} \times \frac{g_{AS}}{g_{API}}}{\rho_{Antisolvent}}} \right) \quad \text{(Equation 1)}$$

The continuous crystallization design of experiments (DoEs) based on mixed-integer nonlinear programming (MINLP) algorithms involves the crystallization temperature (C), the flow sono cell power (W), and the residence time (min). The MSMPR-1 and MSMPR-2 were held at the same crystallization temperature based on the DoE, while the MSMPR-3 was maintained at 20 °C all the time. All three MSMPRs use same DoE residence time. The continuous crystallization pathways that were analyzed during the DoE can be viewed in Fig. S6. Residence time was controlled by adjusting the flow rates of the feed and antisolvent, while keeping them proportional to keep the composition of each reactor the same through all DoE experiments.

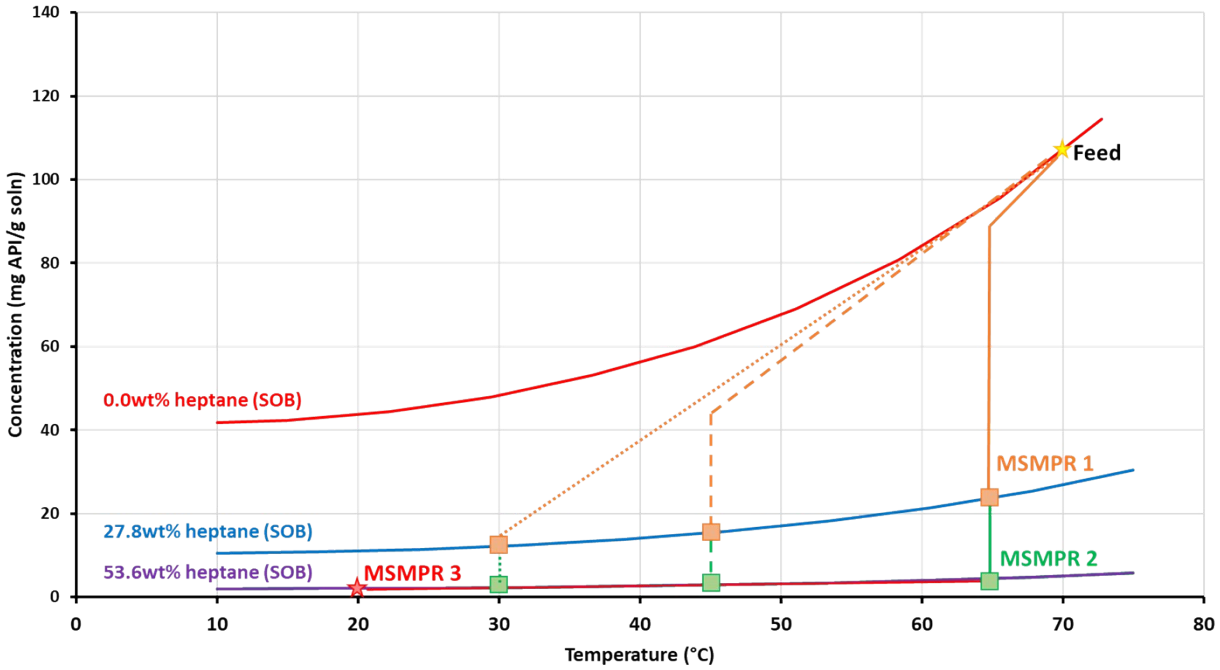

Fig. S6 Solid line- Process path for highest temperature of the DoE = 65 °C. Dashed line – Process path for the center temperature of the DoE = 47.5 °C. Dotted line – Process path for the lowest temperature = 30 °C.

System Startup and Operation: Automated Self-Optimization of Continuous Crystallization of Nirmatrelvir API

The day before the run: To ensure that the run goes smoothly, we did some prep work the day before the run. All the equipment is thoroughly tested to ensure that they are communicating with the LabVIEW™ automation user interface. Each MSMPR top is fitted with the PATs that will be used during the run. The continuous crystallization system is checked for potential leaks by applying vacuum/pressure cycles. We made sure there is no potential leak, which may hinder the transfer of slurry between the reactors. After all the PATs are in their proper position, each MSMPR is filled to the desired operational volume with the main solvent (isopropyl acetate) of the process. This is to mark the liquid level while under agitation to know where to place the transfer dip tube to keep the MSMPR at the proper volume after each transfer. After the reactor is

marked, the reactor is rained and dried overnight. The TCUs are turned on and set to the desired temperatures (jacket temperature control) and left on overnight for the temperature equilibration.

The startup solution for each of the MSMPRs is prepared ahead of time. This is done by combining the appropriate amount of solvent (isopropyl acetate), and antisolvent (heptane) to start each reactor as closely to steady state as possible based on the design of the process for each MSMPR. Likewise, the appropriate mass of desired form of the API are portioned out based on the expected amount in the reactor based on the concentration of the feed solution. Since this process is taking an MTBE solvate and recrystallizing it into a freeform solid, additional MTBE is added to the prep solution per mole of API to match the expected composition as closely as possible while at steady state. These solutions are prepared with 10% excess mass so that when the transfer pumps are started, material will transfer immediately right away.

On the day of the run: On the day of the run, the first activity is to prepare the feed solution for the process. The feed solution is prepared in a 2 L pressure rated glass vessel with a Teflon lid fitted with a thermocouple and transfer fitting. For this process, the feed solution is a combination of 160 g of the MTBE solvate form of the API with 1113 g of the solvent (isopropyl acetate). This solution is then placed on a hot plate, stirred with a magnetic stir bar, and then heated up to the process temperature of 65 °C. A total of 11 feeds were required for the entirety of the run (~55 hours). Once the desired temperature is reached and the solids are dissolved, the solution is transferred into the 2 L pressure vessel in the hot box. To prevent opening the hot box the solution is transferred using pressure transfer through a heated line. This is performed by connecting a nitrogen line and the heated transfer line to the transfer fitting on the Teflon lid. Nitrogen pressure is then applied to the top of the solution forcing it through the transfer tube into the hot box.

The prep solution and solids for each MSMPR are combined in the respective reactor. Each of the PATs were initiated including iC FBRM®, iC ReactIR™, and Blaze™ analytics. LabVIEW™ program is then opened, and the autonomous DoE program is started up. The LabVIEW™ interface records results of all PATs for real time process understanding via OPC UA. Controls of the TCUs are switched from jacket control to reactor control, and they are automatically controlled by the LabVIEW™ to adjust the DoE setpoints. The LabVIEW™ automation interface is used to set agitation for each of the MSMPRs to 250 rpm. The flow sono loop is then initiated by LabVIEW™ and the peristaltic pump is set at 270 mL/min allowing slurry to flow though the sono cell. The power of the sono cell is controlled by the LabVIEW™ and automatically set to the DoE condition.

Before starting the feed and antisolvent pumps the vacuum transfer between each of the MSMPRs is started. The vacuum transfers are controlled by the LabVIEW™ interface by entering in the vacuum pressure and cycle times. These are fixed values throughout the entire run. Using a vacuum pressure of 10 psi and a blowback pressure of 17 psi is enough to transfer the slurry between the MSMPRs and clear the lines after the transfer. The cycle time between transfers is typically 1/10 of the residence time (or less), which has been shown to approximate the behavior of a truly continuous process.[2] The transfers are then started beginning with the transfer from MSMPR-3 to the product collection vessel first. After 30 seconds delay, the transfer from MSMPR-2 to MSMPR-3 is started. Another 30 seconds delay was used for the transfer from MSMPR-1 to MSMPR-2. Then, the LabVIEW™ automation interface was used to automatically set the desired flow rates of feed pump and antisolvent pumps based on the DoE residence time.

Thus, the LabVIEW™ routine with central virtual instrument (VI) was used to execute simultaneous loops including automated DoE based on the MINLP algorithms, temperature control, flow rate manipulation, and PATs (Blaze™ and FBRM®).

The slurry sample after five residence times (DoE residence) was collected manually from the MSMPR-3. The sample was filtered and analyzed on the offline high performance liquid chromatography (HPLC) to determine the liquid phase concentration of the API based on an external standard calibration. The crystallization yield in MSMPR-3 was calculated according to equation 5, and the results were submitted back to the MINLP DoE optimization campaign. Table S1 shows the DoE design based on MINLP algorithms and experimental results for the self-optimization of continuous crystallization of nirmatrelvir API.

Table S1. DoE and experimental results for self-optimization of continuous crystallization of nirmatrelvir API.

Experiment Number	Temperature (MSMPR-1 & MSMPR-2) (ºC)	Sono Power (W)	Residence Time (All MSMPRs) (Min)	Yield (MSMPR-3) (%)
1	65	22.5	15	**82.5**
2	65	15	60	**93.3**
3	65	15	26.25	**89.6**
4	65	45	15	**79.0**
5	65	45	60	**92.7**
6	47.5	22.5	60	**94.6**
7	47.5	45	26.25	**88.5**
8	47.5	15	15	**78.4**
9	30	45	60	**91.0**
10	30	45	15	**53.2**
11	30	15	15	**58.6**
12	30	15	60	**90.6**
13	30	22.5	26.25	**69.0**
14	46.81	45	60	**97.1**
15 (optimal)	47	45	60	**97.0**

Quadratic response surface model parameters:

The quadratic response surface model is given by,

$$\ln Y = y_1\left(\theta_1 + \theta_2\hat{T}\right) + \theta_3\hat{t}_{res} + \theta_4\hat{P} + \theta_5\hat{t}_{res}\hat{T} + \theta_6\hat{t}_{res}\hat{P} + \theta_7\hat{P}\hat{T} + \theta_8\hat{t}_{res}^{\,2} + \theta_9\hat{T}^2 + \theta_{10}\hat{P}^2$$

The terms are defined in Table S2.

Table S2. Definitions of terms in quadratic response surface model.

Term	Definition
Y	Yield expressed as a fraction (between 0-1)
y_i	Discrete variable term, (set to 1 for Flow Sonication device used in this study, so that this model can be expanded to screen different nucleator devices)
$\hat{T}$	Transformed and scaled temperature variable (between -1 and 1): $\hat{T} = 2\left(\frac{T^{-1} - T_{min}^{-1}}{T_{max}^{-1} - T_{min}^{-1}}\right) - 1$ where T is temperature in Kelvin.
$\hat{t}_{res}$	Transformed and scaled residence time variable (between -1 and 1): $\hat{t}_{res} = 2\left(\frac{\ln t_{res} - \ln t_{res,\,min}}{\ln t_{res,\,max} - \ln t_{res,\,min}}\right) - 1$ where t_{res} is residence time in minutes.
$\hat{P}$	Transformed and scaled sono power variable (between -1 and 1): $\hat{P} = 2\left(\frac{\ln P - \ln P_{min}}{\ln P_{max} - \ln P_{min}}\right) - 1$ where P is sono power in W.

θ_j are the model parameters fitted to the experimental data using weighted (by crystallization yield) least squares regression whose values and uncertainties are given in Table S3.

Table S3. Values and uncertainties of model parameters.

Parameter	Coefficient for term	Value	Standard Error (±)
θ_1	Flow Sonication -specific constant	-0.144	0.0268
θ_2	Flow Sonication-specific temperature	0.102	0.0100
θ_3	Residence time	0.1436	0.0097
θ_4	Sono Power	-0.0180	0.0096
θ_5	Residence time × Temperature	0.0920	0.0113
θ_6	Residence time × Sono Power	0.0022	0.0112
θ_7	Sono Power × Temperature	0.0186	0.0107
θ_8	Residence time squared	-0.084	0.0187
θ_9	Temperature squared	-0.045	0.0217
θ_{10}	Sono Power squared	0.0353	0.0223

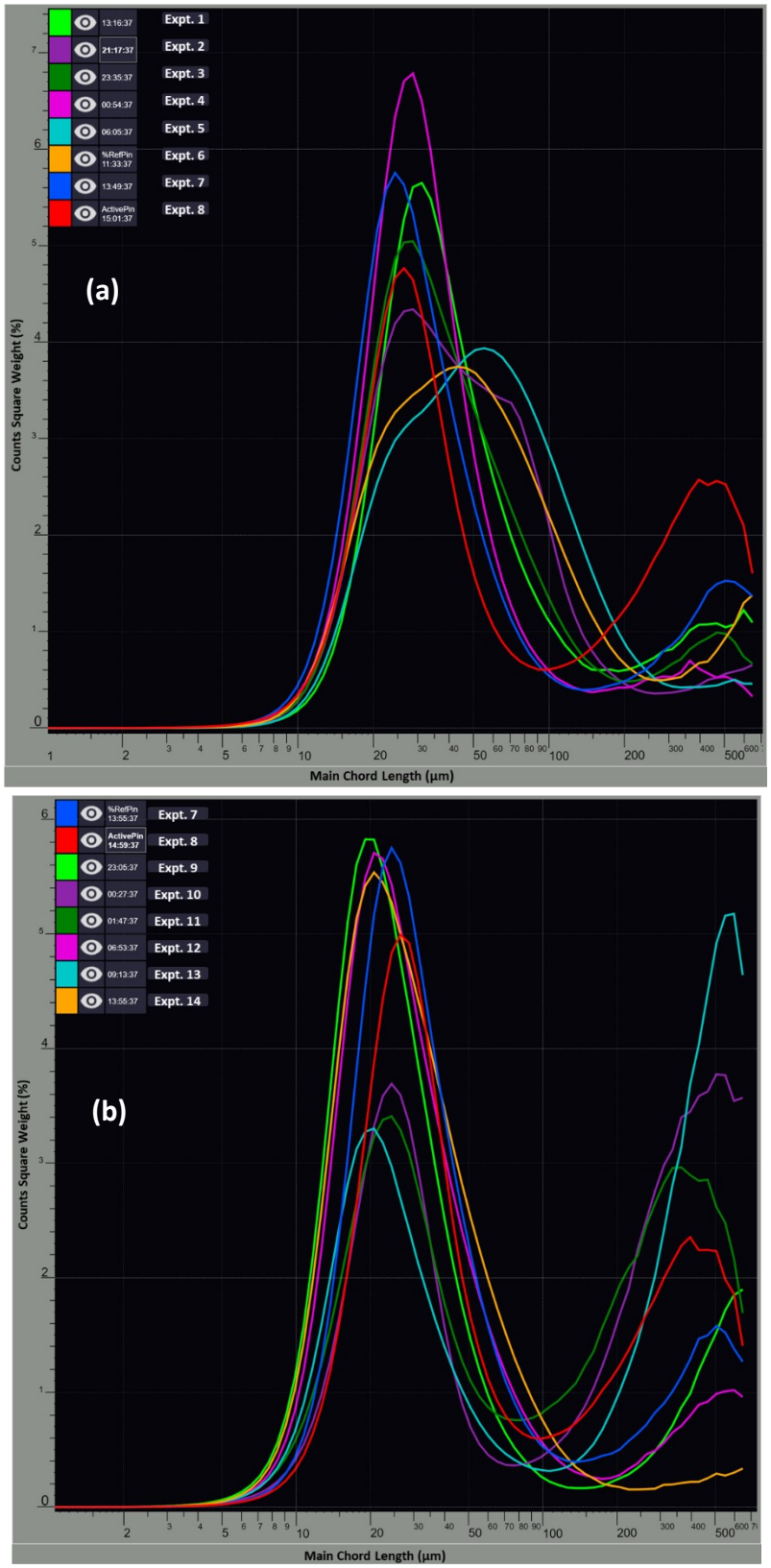

Fig. S7 Blaze™ image derived chord length distributions (ID-CLD) particle size and counts in MSMPR-1: (a) experiments 1 to 8 and (b) experiments 7 to 14.

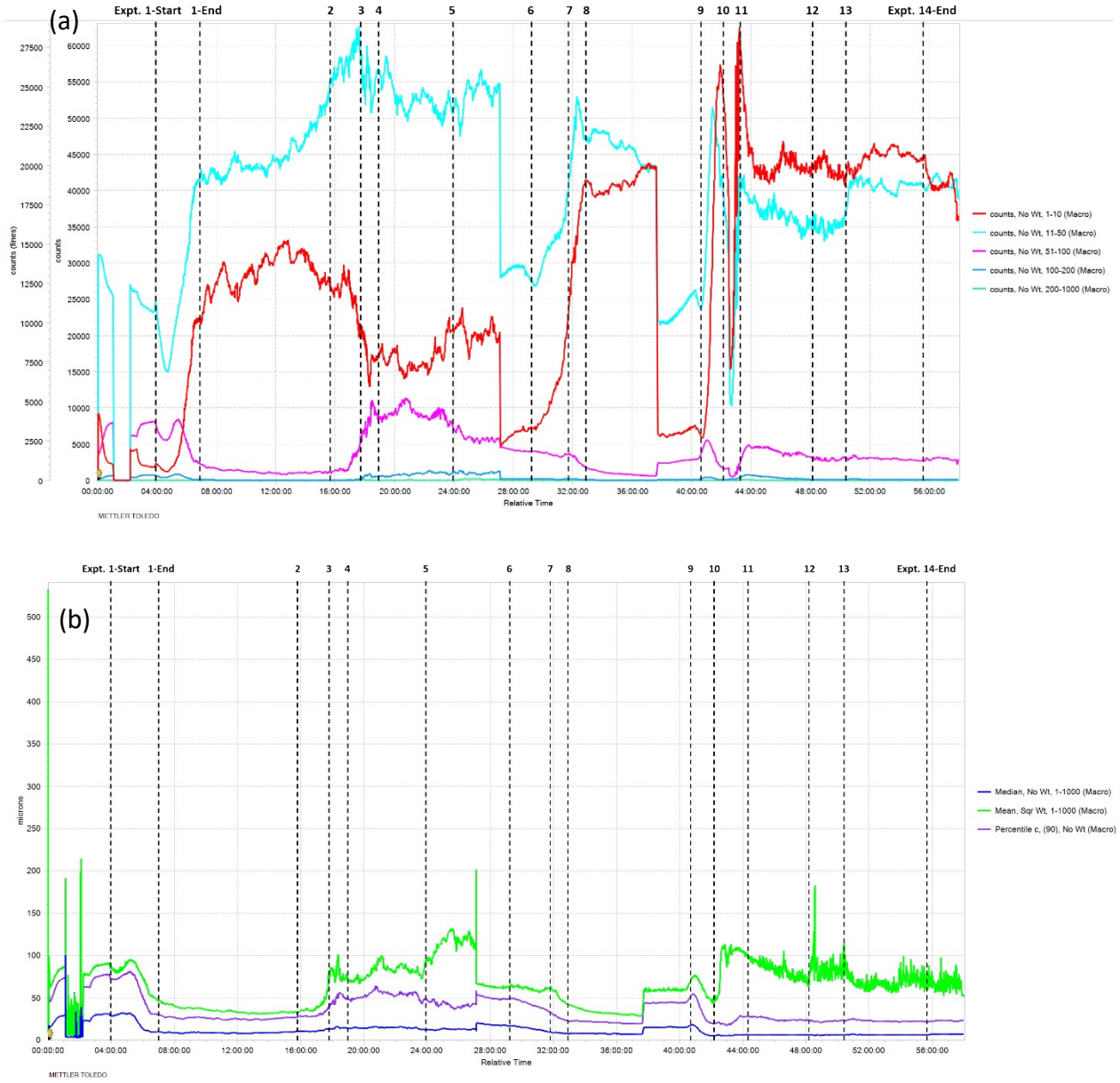

Fig. S8 (a-b) FBRM® particle trends in MSMPR-2 for experiments 1 to 14.

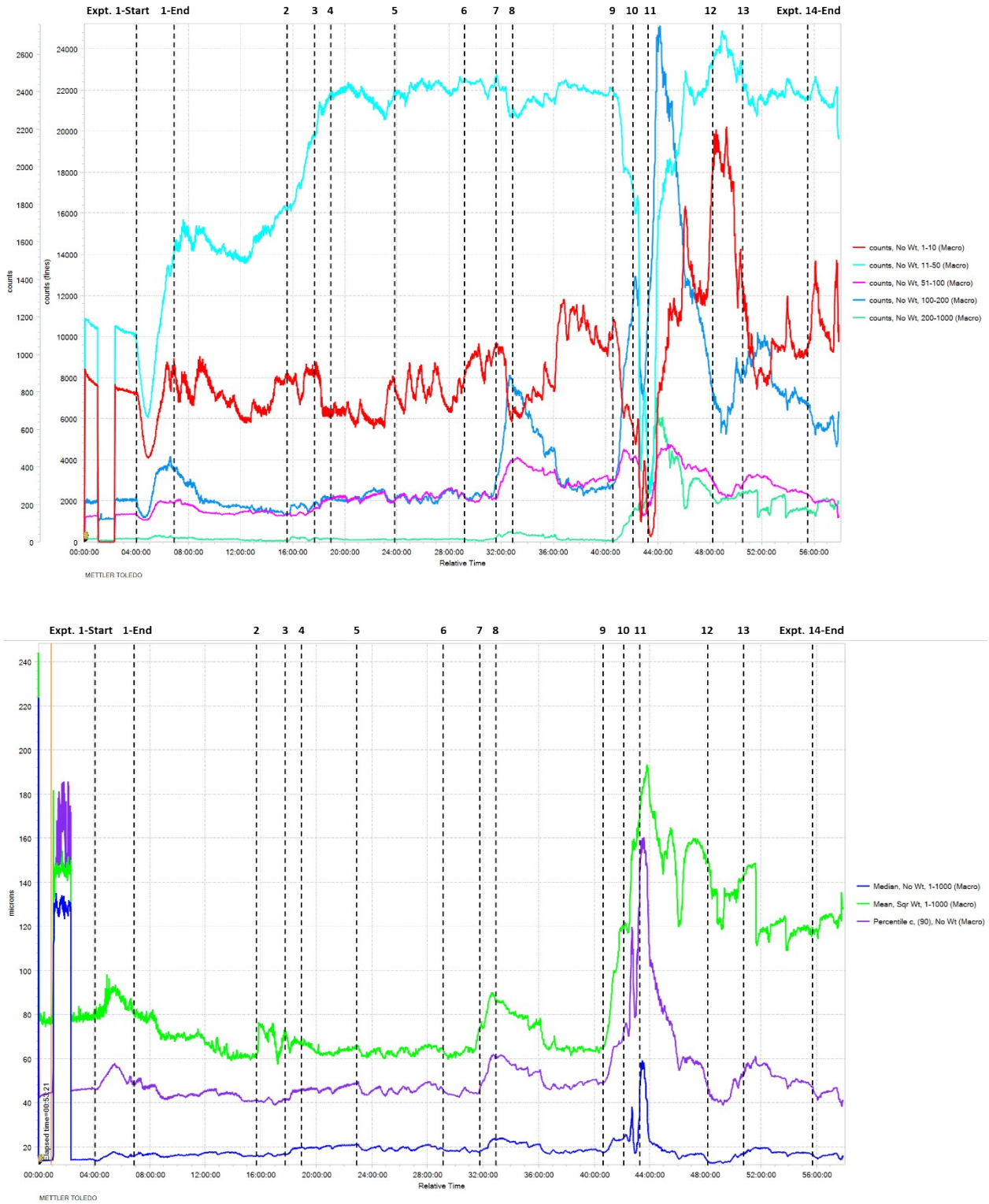

Fig. S9 (a-b) FBRM® particle trends in MSMPR-3 for experiments 1 to 14.

HPLC Analysis

Throughout the DoE, the crystallization yield in the MSMPR-3 was analyzed during each experiment after five residence times by sampling of the slurry content. By filtering the collected samples, the filtrate was analyzed using HPLC to determine the concentration. The yield of solids in the MSMPR-3 was calculated by using the concentration of the filtrate. To get accurate concentration data for the DoE an external standard was prepared using a single lot of freebase solids with a known potency. For the calibration, a small number of solids were dissolved using methanol and then diluted up to 10 mL. One milliliter of the dissolved standard was further diluted using 90:10 acetonitrile and water to 10 mL to get a sample with a concentration of about 1.5 mg/mL. the solution was then injected in the HPLC at 1.0 µL, 0.8 µL, 0.6 µL, 0.4 µL, and 0.2 µL injection volumes each three times to ensure consistency. The data is analyzed with 210 nm wavelength. The data from each standard injected was exported to excel where a calibration curve was generated which can be seen in Fig. S10.

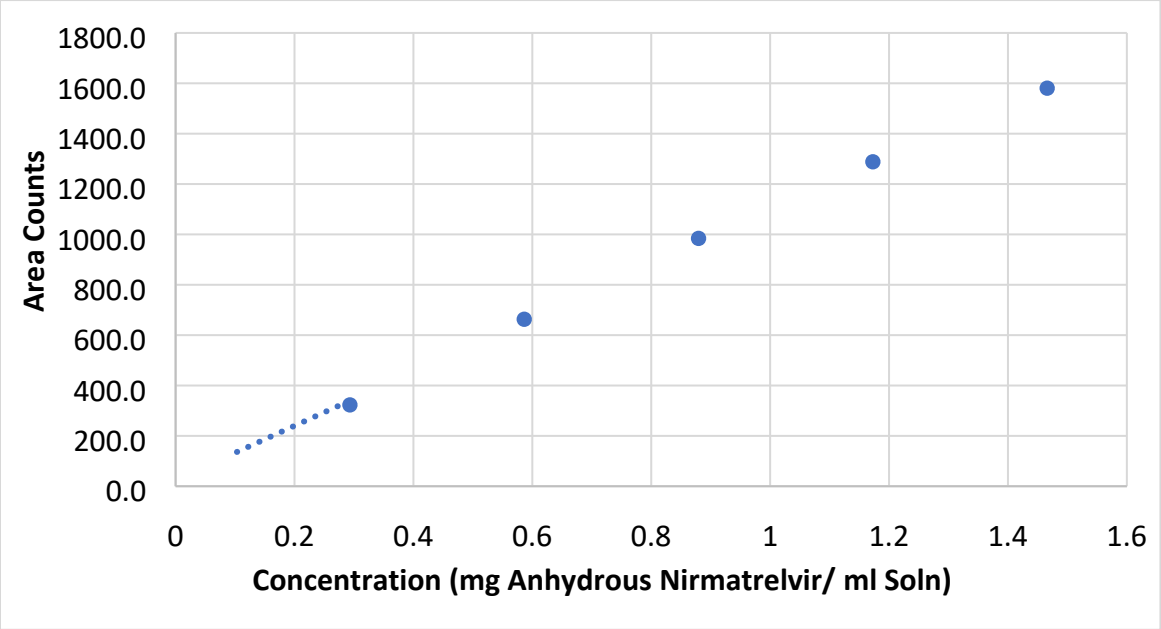

Fig. S10 HPLC calibration by using external standard.

Th samples from MSMPR-3 were taken by using a large pipette to collect ~10 mL of the slurry and filtered it using a pop-it filter with a 5 µm filter mesh. The filtered solution was then diluted using acetonitrile 1:20. The sample was then injected into the HPLC at 1.0 µl. Each sample was analyzed for the area count for the API and then using equation 2 the number of micrograms on the column was calculated based on the external standard calibration curve (Fig. S10). Then, the equation 3 was used to calculate the concentration of API in the MSMPR-1. Knowing how many volumes (mL/g) were in the MSMPR-1, by using the concentration we could calculate the amount of API lost to the mother liquor per each gram of API using equation 4. The yield can then be calculated based on how much API is being lost to the mother liquor using equation 5.

$$\mu g\ on\ column = \frac{(Area\ count - \ y\ intercept)}{Slope} \quad \text{(Equation 2)}$$

$$Conc.\frac{mg}{ml} = \frac{\frac{\mu g\ on\ column}{1000}}{\left(\frac{sample\ size\ (\mu L)}{1000} \times \frac{injection\ volume\ (\mu L)}{1000}\right)} \times Dillutent\ factor \quad \text{(Equation 3)}$$

$$Loss\ to\ mother\ liquor\left(\frac{mg}{g}\right) = \left(Conc.\frac{mg}{ml}\right) \times \left(Volumes\frac{ml}{g}\ in\ MSMPR\right) \quad \text{(Equation 4)}$$

$$Yield\ \% = \frac{1000\left(\frac{mg}{g}\right) - Loss\ to\ mother\ liquor\left(\frac{mg}{g}\right)}{1000\left(\frac{mg}{g}\right)} \quad \text{(Equation 5)}$$

References

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