crystals

MDPI

Communication

Metastable Crystallization by Drop Impact

Akari Nishigaki [1], Mihoko Maruyama [1,2,3], Shun-ichi Tanaka [1], Hiroshi Y. Yoshikawa [2], Masayuki Imanishi [2], Masashi Yoshimura [4], Yusuke Mori [2] and Kazufumi Takano [1,*]

[1] Department of Biomolecular Chemistry, Kyoto Prefectural University, Sakyo-ku, Kyoto 606-8522, Japan
[2] Graduate School of Engineering, Osaka University, 2-1, Yamadaoka, Osaka 565-0871, Japan
[3] Institute for Advanced Co-Creation Studies, Osaka University, 2-1, Yamadaoka, Osaka 565-0871, Japan
[4] Institute of Laser Engineering, Osaka University, 2-6, Yamadaoka, Osaka 565-0871, Japan
* Correspondence: takano@kpu.ac.jp

Abstract: It has been reported that cavitation bubbles (air–liquid interface) by femtosecond laser and ultrasonic irradiations are effective for metastable phase crystallization in polymorph control. It has also been noted that cavitation bubbles are generated by mechanical shock when dropping a vial. Here we describe the crystallization of acetaminophen by drop impact. In the condition where spontaneous nucleation did not occur, the drop impact produced the metastable form (form II) and trihydrate. This supports the potency of the air–liquid interface in metastable phase formation. Furthermore, crystallization by drop impact is a completely new phenomenon, and new developments are expected in the future.

Keywords: crystal engineering; crystal growth; crystallization; formulation; phase transition; polymorphism; supersaturation

Citation: Nishigaki, A.; Maruyama, M.; Tanaka, S.-i.; Yoshikawa, H.Y.; Imanishi, M.; Yoshimura, M.; Mori, Y.; Takano, K. Metastable Crystallization by Drop Impact. *Crystals* **2022**, *12*, 1104. https://doi.org/10.3390/cryst12081104

Academic Editor: Sławomir Grabowski

Received: 15 July 2022
Accepted: 4 August 2022
Published: 6 August 2022

1. Introduction

Polymorphism, a phenomenon of crystallography where one chemical compound has different possible crystal structures, is a very important issue in the pharmaceutical industry due to the different physical and chemical properties, solubility, stability, and the bioavailability of drug compounds [1]. Polymorph crystals are classified into the most thermodynamically stable phase (form) and unstable phases, including metastable forms. The stable form crystallizes more easily and has lower solubility than the unstable forms [2]. In drug development, a stable form is generally used in commercial formulations, but a search for unstable phases is essential because of a patent strategy and the need to collect information on functional and cost-effective properties [3,4].

In practical terms, it is a challenge to obtain unstable crystals [5]. From an energy perspective, the unstable phases form crystal nuclei more readily than those in the stable phase. In contrast, in normal crystallization operations of solution mixing, the unstable forms that appear first undergo a phase transition to the stable form that appears later [6–9]. One solution to this problem is to quickly grow an unstable form before the stable form appears and then reduce the solution concentration to a condition where the stable form is not able to nucleate. In accordance with this strategy, we have developed selective crystallization methods for metastable phases using forced nucleation techniques, femtosecond laser irradiation, and ultrasonic irradiation [10–14]. Laser and ultrasonic irradiations into the solution generate cavitation bubbles (air–liquid interface), which increase the solute concentration temporarily and locally around the bubble interface, resulting in crystal nucleation [15,16]. After the bubble disappearance, only the nucleated metastable form grows. In this way, cavitation bubbles are effective in producing the metastable phases. Recently, it has been reported that cavitation bubbles are generated by mechanical shock due to dropping a vial of protein solution, resulting in protein aggregation [17,18]. This indicates that a polymorphic search is possible merely by drop impact, generating cavitation bubbles without machinery.

Acetaminophen (paracetamol) is a widely used antipyretic and analgesic drug. Various polymorphs of acetaminophen have been reported, form I (stable phase) [19], form II (metastable phase) [20], and form III (metastable phase) [21]. Three hydrates (monohydrate [22], dihydrate [23], and trihydrate [24]) have also been reported. Form I is used in commercial formulations. To date, it has been shown that acetaminophen crystal nucleation is promoted by a microflow system [25]. Furthermore, crystallization of the unstable phases by femtosecond laser and ultrasound irradiations has been reported [11,12,26]. Here, we tried to crystallize acetaminophen by dropping a vial. It is known that cavitation bubbles cause protein aggregation when a vial containing a protein solution falls [17,18]. Therefore, the crystallization of acetaminophen by drop impact can be expected. To our knowledge, this is the first study to apply crystal growth by drop impact. When a vial contacts a solid surface after being dropped from a height of 1-m, cavitation bubbles appear within about 30 μs and disappear at about several hundred μs [17]. Computational fluid dynamic calculations show that drop impact generates a low-pressure region at the bottom of a vial, causing cavitation bubbles [17].

2. Materials and Methods

Acetaminophen was purchased from Acros with a purity of 98%. This material was previously confirmed [27] as being form I by powder X-ray diffraction (PXRD) [28,29]. Acetaminophen solution with a concentration of 30 mg/mL was prepared by dissolving acetaminophen form I in ultrapure water. The solution was heated at 60 °C for 3 h in a drying oven. After filtration (0.22 μm), 0.5, 1.0, and 4.0 mL aliquots of the solution were dispensed into 5 mL glass vials. These samples were placed in an incubator at 55 °C for 1 h and then cooled to 0 °C at a constant rate of 3 °C/h. After the constant cooling, they were maintained at that temperature for one day. The supersaturation of form x, σ_x, was calculated using the formula $\sigma_x = (C - C_x)/C_x$, where C is the acetaminophen concentration, and C_x is the solubility of form x. The supersaturation of the form I solution prepared here at 0 °C was $\sigma_I = 3.4$. C_I, C_{II} and C_{tri} at 0 °C were 6.8, 8.4 and 7.1 mg/mL, respectively [11,30]. Under this solution condition, no spontaneous crystallization occurred. Figure 1 shows a schematic drawing of the vial dropping method in this research. The vial was dropped onto a metal surface through a 1- or 2-m long polyvinyl chloride pipe. As a control without dropping, the vial was turned over once to move the solution and allowed to stand. The sample prepared at 0 °C was taken out from the incubator to room temperature, immediately dropped, and returned to 0 °C again. After incubation at 0 °C for 1 h, the sample was left at room temperature. The obtained crystals were observed under an inverted microscope. The polymorphs were identified by PXRD measurements, as described previously [27,28]. For the PXRD measurements, the obtained crystals were collected using filter paper, dried, and powdered with a mortar and pestle.

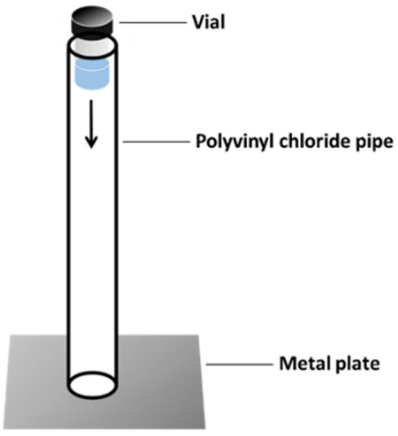

Figure 1. Experimental setup of vial dropping.

3. Results and Discussion

When the 0.5 mL solution vials were dropped from a height of 1-m and 2-m, in some vials, the crystals appeared immediately after dropping. No crystals were observed in the control vials. As shown in Figure 2, a visual inspection of the obtained crystals revealed two shapes, prism-like and needle-like. As a result of PXRD (Figure 3), the prism-like crystals were found to be form II [29], and they remained stable at room temperature for over a month. The needle-like crystals immediately transitioned to form II (prism-like crystals) at room temperature. Based on polymorphism control research with acetaminophen to date, it is speculated that the needle-like crystals obtained were trihydrate [30,31]. At room temperature, the supersaturations of form II (σ_{II}) and trihydrate (σ_{tri}) of the sample solutions were $\sigma_{II} = 0.75$ and $\sigma_{tri} = -0.12$ (unsaturation), so the trihydrate crystals were phase-transferred to form II. The present results indicate that the drop impact is effective for the selective crystallization of metastable phases.

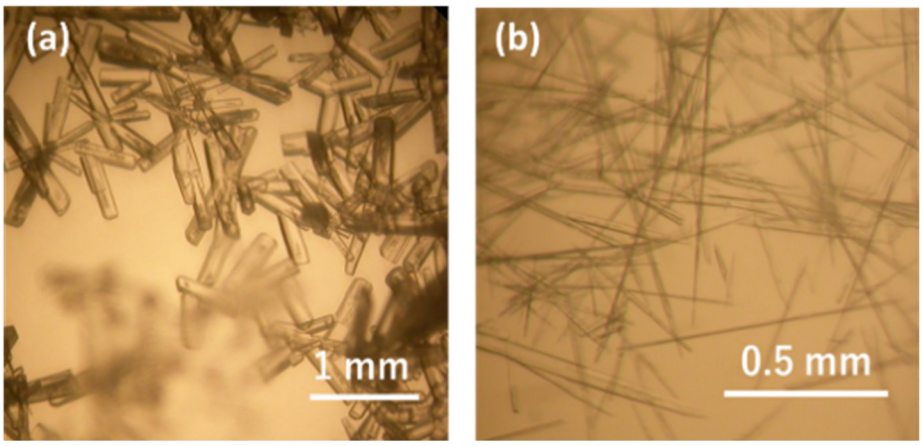

Figure 2. Photographs of acetaminophen crystals obtained: (**a**) prism-like and (**b**) needle-like crystals.

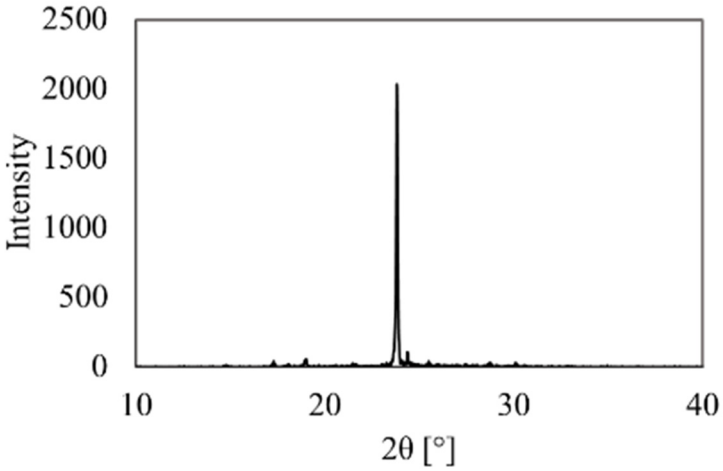

Figure 3. PXRD pattern of the prism-like acetaminophen crystals.

The supersaturations of form II (σ_{II}) and trihydrate (σ_{tri}) of the sample solutions at 0 °C were $\sigma_{II} = 2.6$ and $\sigma_{tri} = 3.2$. Since the supersaturations of form I, form II, and trihydrate of the sample solutions are sufficiently low, no spontaneous nucleation occurs at 0 °C, as shown in this experiment. The appearance of form II crystals with supersaturation lower than for trihydrates at 0 °C suggests that the drop impact formed a high local concentration region necessary for the nucleation of form II. The lack of transition of form II to form I indicates that both trihydrate and form II nucleated, and form I did not crystallize by drop impact. This is because the nucleation rate of form I is slower than that of form II and

trihydrate [9]. After the drop impact, the high concentration region disappeared before the nucleation of form I, and then only nucleated form II and trihydrate crystals grew.

The crystallization probability of the 0.5 mL solution vials is shown in Figure 4a. The crystallization probability was calculated as $(n_{cry}/n_{total}) \times 100$, where n_{cry} and n_{total} are the number of samples crystallized, and the number of total samples examined, respectively. Crystallization was promoted more in the fall from the height of 2-m than from 1-m. This means that a stronger drop impact, that is, a condition favorable for the generation of cavitation bubbles (air–liquid interface), contributes to the crystallization of metastable phases. In protein solution vial dropping, higher fall has been reported to produce more aggregates [17]. In the present results, crystallization did not necessarily occur in all vials, and the crystallization probability was somewhat low. The cause is that this condition is quite severe for the nucleation and subsequent growth of acetaminophen crystals because it is performed under conditions where natural nucleation does not occur to obtain the effect of drop impact. Moreover, the configuration of bubbles is not uniform for each experiment because the contact state between the vial and the interface at the time of dropping is not constant [17]. In the future, research is required to increase the crystallization efficiency due to the drop impact.

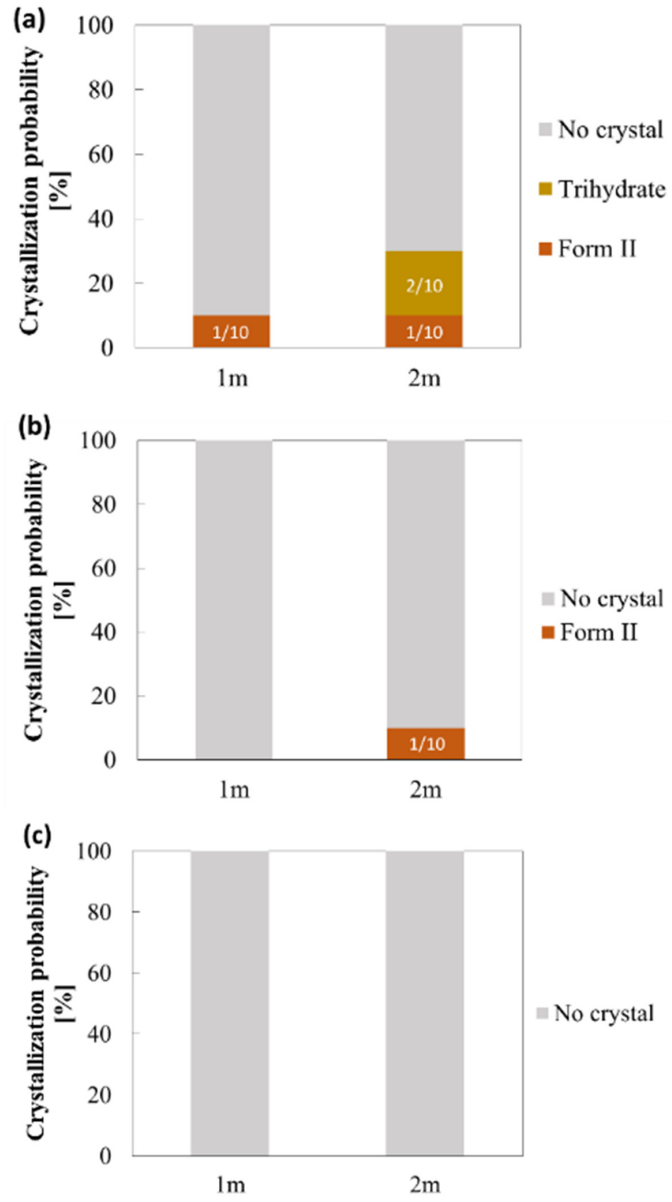

Figure 4. Crystallization probability for each case: (**a**) 0.5 mL, (**b**) 1.0 mL, and (**c**) 4.0 mL solution vials.

Next, the same experiments were performed by increasing the solution volume to 1.0 mL and 4.0 mL. When the solution volume was 1.0 mL, form II was crystallized after a 2-m drop, but no crystallization occurred with a 1-m drop (Figure 4b). This indicates that a strong drop impact is more effective in generating the metastable phase, whereas the crystallization probability is lower than for the 0.5 mL solution. Furthermore, no crystal formation was observed in the 4.0 mL solution (Figure 4c).

As in the cases of laser or ultrasonic irradiations, the cavitation bubbles generated by drop impact are considered to be involved in the production of metastable phases. In this work, however, the crystallization probability decreased as the amount of solution increased (Figure 4). It is known that cavitation bubbles are generated near the bottom of a vial by a drop impact regardless of the amount of protein solution in the vial [17]. It has also been found that cavitation bubbles near the air–liquid interface by laser irradiation shrank asymmetrically, then collapsed toward the interface [10]. After that, many bubbles (the second bubbles) appeared and then reached a region along the meniscus of the solution. With the second bubbles, evaporation occurred efficiently, and high supersaturation was realized locally, resulting in the metastable phase growth of indomethacin [10]. In the present study, cavitation bubbles alone might not be sufficient for the generation of metastable phases since the crystallization condition was severe, as noted. The second bubbles, arising from cavitation bubbles generated at the air–liquid interface with a small amount of solution, could be crystallization points of the unstable forms. In contrast, when the amount of solution increased, the cavitation bubbles generated at the bottom of a vial were far from the air–liquid interface, so the second bubbles were not generated, and the crystallization conditions could not be obtained. Therefore, a drop impact with a small amount of solution can trigger crystal nucleation. A detailed analysis of the second bubble formation and nucleation is anticipated in the future.

4. Conclusions

In this study, it was shown that the vial drop impact is interesting for the crystallization of the metastable phase of acetaminophen. This presents a simple method for polymorph search and contributes to the elucidation of the crystallization mechanism by cavitation bubbles. In addition, if a vial falls accidentally during experiments, it suggests that the sample should be excluded from further testing due to unexpected molecular association in the solution.

Author Contributions: Conceptualization, K.T.; methodology, K.T. and M.M.; investigation, A.N.; validation, S.-i.T. and H.Y.Y.; resources, M.I., M.Y. and Y.M.; writing—original draft preparation, K.T.; writing—review & editing, K.T.; visualization, A.N.; supervision, K.T.; project administration, K.T.; funding acquisition, M.M. and Y.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Grant-in-Aid for Scientific Research (B) No. 26286042, and Grain-in-Aid for Challenging Exploratory Research Nos. 15K13380 and 17H02774 for Y.M., Research Fellowships of JSPS, KAKENHI Grant number JP 18J40134 and Shiseido Female Researcher Science Grant for M.M., and Caterpillar STEM Grant for A.N. and M.M.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bernstein, J. *Polymorphism in Molecular Crystals*; Oxford University Press: New York, NY, USA, 2002.
2. Bhattachar, S.N.; Deschenes, L.A.; Wesley, J.A. Solubility: It's not just for physical chemists. *Drug Discov. Today* **2006**, *11*, 1012–1018. [CrossRef] [PubMed]
3. Gupta, H.; Kumar, S.; Roy, S.; Gaud, R. Patent protection strategies. *J. Pharm. Bioallied. Sci.* **2010**, *2*, 2–7. [CrossRef] [PubMed]

4. Thayer, A.M. Centering on chirality. *Chem. Eng. News* **2007**, *85*, 11–19.
5. Bučar, D.K.; Lancaster, R.W.; Bernstein, J. Disappearing polymorphs revisited. *Angew. Chem. Int. Ed.* **2015**, *54*, 6972–6993. [CrossRef] [PubMed]
6. Nichols, G.; Frampton, C.S. Physicochemical characterization of the orthorhombic polymorph of paracetamol crystallized from solution. *J. Pharm. Sci.* **1998**, *87*, 684–693. [CrossRef] [PubMed]
7. Gu, C.H.; Young, V., Jr.; Grant, D.J. Polymorph screening: Influence of solvents on the rate of solvent-mediated polymorphic transformation. *J. Pharm. Sci.* **2001**, *90*, 1878–1890. [CrossRef] [PubMed]
8. Greco, K.; Bogner, R. Solution-mediated phase transformation: Significance during dissolution and implications for bioavailability. *J. Pharm. Sci.* **2012**, *101*, 2996–3018. [CrossRef]
9. Maruyama, M.; Yoshimura, M.; Takano, K.; Mori, Y. Development of Polymorphic Control Technology for Pharmaceutical Compounds. In *Kinetic Control in Synthetic and Self-Assembly*; Numata, M., Yagai, S., Hamura, T., Eds.; Elsevier: Amsterdam, The Netherlands, 2019; pp. 269–291.
10. Ikeda, K.; Maruyama, M.; Takahashi, Y.; Mori, Y.; Yoshikawa, H.; Okada, S.; Adachi, H.; Sugiyama, S.; Takano, K.; Murakami, S.; et al. Selective crystallization of the metastable phase of indomethacin on the interface of liquid/air bubble induced by the femtosecond laser irradiation. *Appl. Phys. Express* **2015**, *8*, 045501. [CrossRef]
11. Mori, Y.; Maruyama, M.; Takahashi, Y.; Ikeda, K.; Fukukita, S.; Yoshikawa, H.; Okada, S.; Adachi, H.; Sugiyama, S.; Takano, K.; et al. Selective crystallization of metastable phase of acetaminophen by ultrasonic irradiation. *Appl. Phys. Express* **2015**, *8*, 065501. [CrossRef]
12. Mori, Y.; Maruyama, M.; Takahashi, Y.; Yoshikawa, H.; Okada, S.; Adachi, H.; Sugiyama, S.; Takano, K.; Murakami, S.; Matsumura, H.; et al. Crystallization of acetaminophen form II by plastic-ball-assisted ultrasonic irradiation. *Appl. Phys. Express* **2017**, *10*, 025501. [CrossRef]
13. Kobayashi, N.; Maruyama, M.; Mori, Y.; Fukukita, S.; Adachi, H.; Takano, K.; Murakami, S.; Matsumura, H.; Inoue, T.; Yoshimura, M.; et al. Atomic-scale imaging of surface and hydration structures of stable and metastable acetaminophen crystals by frequency modulation atomic force microscopy. *J. Phys. Chem. C* **2018**, *122*, 21983–21990. [CrossRef]
14. Tsuri, Y.; Maruyama, M.; Fujimoto, R.; Okada, S.; Adachi, H.; Yoshikawa, H.; Takano, K.; Murakami, S.; Matsumura, H.; Inoue, T.; et al. Crystallization of aspirin form II by femtosecond laser irradiation. *Appl. Phys. Express* **2019**, *12*, 015507. [CrossRef]
15. Yoshikawa, H.Y.; Murai, R.; Adachi, H.; Sugiyama, S.; Maruyama, M.; Takahashi, Y.; Takano, K.; Matsumura, H.; Inoue, T.; Murakami, S.; et al. Laser ablation for protein crystal nucleation and seeding. *Chem. Soc. Rev.* **2014**, *43*, 2147–2158. [CrossRef]
16. Iefuji, N.; Murai, R.; Maruyama, M.; Takahashi, Y.; Sugiyama, S.; Adachi, H.; Matsumura, H.; Murakami, S.; Inoue, T.; Mori, Y.; et al. Laser-induced nucleation in protein crystallization: Local increase in protein concentration induced by femtosecond laser irradiation. *J. Cryst. Growth* **2011**, *318*, 741–744. [CrossRef]
17. Randolph, W.T.; Schiltz, E.; Sederstrom, D.; Steinmann, D.; Mozziconacci, O.; Schöneich, C.; Freund, E.; Ricci, M.S.; Carpenter, J.F.; Lengsfeld, C.S. Do not drop: Mechanical shock in vials causes cavitation, protein aggregation, and particle formation. *J. Pharm. Sci.* **2015**, *104*, 602–611. [CrossRef] [PubMed]
18. Torisu, T.; Maruno, T.; Hamaji, Y.; Ohkubo, T.; Uchiyama, S. Synergistic effect of cavitation and agitation on protein aggregation. *J. Pharm. Sci.* **2017**, *106*, 521–529. [CrossRef] [PubMed]
19. Haisa, M.; Kashino, S.; Kawai, R.; Maeda, H. The monoclinic form of p-hydroxyacetanilide. *Acta Crystallogr. Sect. B* **1976**, *32*, 1283–1285. [CrossRef]
20. Haisa, M.; Kashino, S.; Maeda, H. The orthorhombic form of p-hydroxyacetanilide. *Acta Crystallogr. Sect. B* **1974**, *30*, 2510–2512. [CrossRef]
21. Burger, A. Interpretation of polymorphism studies. *Acta Pharm. Technol.* **1982**, *28*, 1–20.
22. Parkin, A.; Parsons, S.; Pulham, C.R. Paracetamol monohydrate at 150 K. *Acta Crystallogr. Sect. E* **2002**, *58*, o1345–o1347. [CrossRef]
23. Fabbiani, F.P.A.; Allan, D.R.; David, W.I.F.; Moggach, S.A.; Parsons, S.; Pulham, C.R. High-pressure recrystallisation—A route to new polymorphs and solvates. *Cryst. Eng. Comm.* **2004**, *6*, 505–511. [CrossRef]
24. McGregor, P.A.; Allan, D.R.; Parsons, S.; Pulham, C.R. Preparation and crystal structure of a trihydrate of paracetamol. *J. Pharm. Sci.* **2002**, *91*, 1308–1311. [CrossRef] [PubMed]
25. Nishigaki, A.; Maruyama, M.; Numata, M.; Kanzaki, C.; Tanaka, S.-I.; Yoshikawa, H.; Imanishi, M.; Yoshimura, M.; Mori, Y.; Takano, K. Microflow system promotes acetaminophen crystal nucleation. *Eng. Life Sci.* **2020**, *20*, 395–401. [CrossRef] [PubMed]
26. Wang, S.; Wang, S.; Jiang, L.; Wang, M.; Wei, Y.; Sun, J.; Zhan, S.; Li, X.; Qu, L. Polymorph-controlled crystallization of acetaminophen through femtosecond laser irradiation. *Cryst. Growth Des.* **2019**, *19*, 3265–3271. [CrossRef]
27. Nishigaki, A.; Maruyama, M.; Tanaka, S.-i.; Yoshikawa, H.Y.; Imanishi, M.; Yoshimura, M.; Mori, Y.; Takano, K. Growth of acetaminophen polymorphic crystals and solution-mediated phase transition from trihydrate to form II in agarose gel. *Crystals* **2021**, *11*, 1069. [CrossRef]
28. Mori, Y.; Maruyama, M.; Takahashi, Y.; Yoshikawa, H.; Okada, S.; Adachi, H.; Sugiyama, S.; Takano, K.; Murakami, S.; Matsumura, H.; et al. Metastable crystal growth of acetaminophen using solution-mediated phase transformation. *Appl. Phys. Express* **2017**, *10*, 015501. [CrossRef]
29. Fujimoto, R.; Maruyama, M.; Mori, Y.; Okada, S.; Adachi, H.; Yoshikawa, H.; Takano, K.; Murakami, S.; Matsumura, H.; Inoue, T.; et al. Growth of high-quality metastable crystal of acetaminophen using solution-mediated phase transformation at low supersaturation. *J. Cryst. Growth* **2018**, *502*, 76–82. [CrossRef]

30. Fujimoto, R.; Maruyama, M.; Okada, S.; Adachi, H.; Yoshikawa, H.; Takano, K.; Imanishi, M.; Tsukamoto, K.; Yoshimura, M.; Mori, Y. Large-scale crystallization of acetaminophen trihydrate by a novel stirring technique. *Appl. Phys. Express* **2019**, *12*, 045503. [CrossRef]
31. Nii, K.; Maruyama, M.; Okada, S.; Adachi, H.; Takano, K.; Murakami, S.; Yoshikawa, H.; Matsumura, H.; Inoue, T.; Imanishi, M.; et al. Improvement of metastable crystal of acetaminophen by control of crystal growth rate. *Appl. Phys. Express* **2018**, *11*, 035501. [CrossRef]