THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

Stabilization of the Amorphous Structure of Spray-Dried Drug Nanoparticles

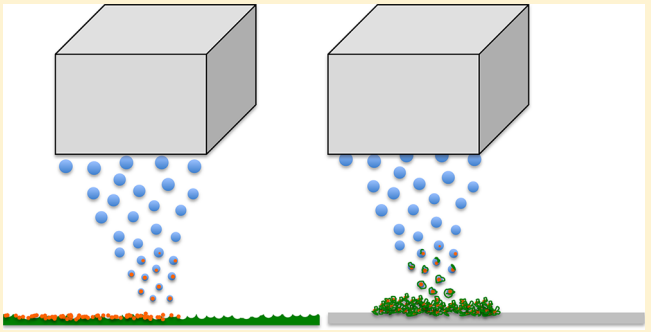
Esther Amstad,*[,†,§] Frans Spaepen,[†] and David A. Weitz[†,‡]

[†]School of Engineering and Applied Sciences and [‡]Department of Physics, Harvard University, Cambridge, Massachusetts 02138, United States

[§]Institute of Materials, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne CH-1015, Switzerland

Supporting Information

ABSTRACT: The bioavailability of hydrophobic drugs strongly increases if they are formulated as amorphous materials because the solubility of the amorphous phase is much higher than that of the crystal. Moreover, the stability of these particles against crystallization during storage increases with decreasing particle size. Hence, it is advantageous to formulate poorly water soluble drugs as amorphous nanoparticles. The formulation of an amorphous structure is often difficult because many of these drugs have a high propensity to crystallize. This difficulty can be overcome if drugs are spray-dried using a microfluidic nebulator we recently developed. However, these nanoparticles agglomerate when they come in contact with each other, and this compromises the stability of their amorphous structure through crystallization. To improve their stability, we coat the nanoparticles with a sterically stabilizing polymer layer; this can be accomplished by co-spraying them with an excipient. However, this excipient must meet strict solubility limits, which severely limit the choice of polymers. Alternatively, the nanoparticles can be sterically stabilized by spraying them directly into a polymeric matrix; this enables a much wider choice of stabilizing polymers.



INTRODUCTION

Many newly developed drugs are strongly hydrophobic and thus poorly water soluble; this limits their bioavailability and severely restricts their use as drugs.[1] Their bioavailability, and hence their potential use as drugs, increases if they are formulated as amorphous particles (e.g. without crystalline long-range order), because the solubility of amorphous materials is much higher than that of the corresponding crystal.[1−6] Moreover, their stability against crystallization during storage increases with decreasing particle size.[3] Hence, it is advantageous if these drugs are formulated as amorphous nanoparticles. One method that enables the production of sub-20 nm diameter amorphous drug nanoparticles is spray-drying with a microfluidic nebulator.[3] However, these spray-dried nanoparticles aggregate if they come in contact with each other. To prevent aggregation, spray-dried drug particles are usually stabilized by co-spray-drying them with excipients. In addition, these excipients are essential to prevent crystallization. By contrast, the microfluidic nebulator uses a completely different principle: an amorphous structure is produced because the solvent is dried so quickly that molecules have no time to arrange into a crystalline structure;[3] hence, no excipient of any kind is required to prevent crystallization. Unfortunately, however, the resultant nanoparticles are not sterically stabilized and hence any agglomeration increases their size, thereby reducing the stability of the amorphous structure and leading to crystallization.[4] Because the production mechanism is so different from traditional spray-drying, it is not clear whether the usual routes toward stabilization, for example co-spray-drying drugs with excipients, extend to the nebulator. Therefore, it may be important to develop new design principles to achieve steric stabilization. However, these principles have never been investigated.

In this article, we develop methods to ensure steric stabilization of amorphous nanoparticles produced by the microfluidic nebulator. We sterically stabilize amorphous drug nanoparticles during their production in the microfluidic nebulator by co-spray-drying with an excipient, which, however, must be carefully selected to ensure that it does not induce crystallization of the drug during the spray-drying process. This severely limits the choice of the polymer. To broaden this choice, we show that the amorphous structure of the nanoparticles can also be stabilized by spraying them directly into a polymeric matrix. If particles are spray-dried into any of the polymer matrices tested, their amorphous structures retain their stabilities for an extended period of time, even if they are stored above their glass transition temperatures, T_g's.

Received: May 30, 2016
Revised: July 21, 2016

ACS Publications © XXXX American Chemical Society

DOI: 10.1021/acs.jpcb.6b05417
J. Phys. Chem. B XXXX, XXX, XXX−XXX

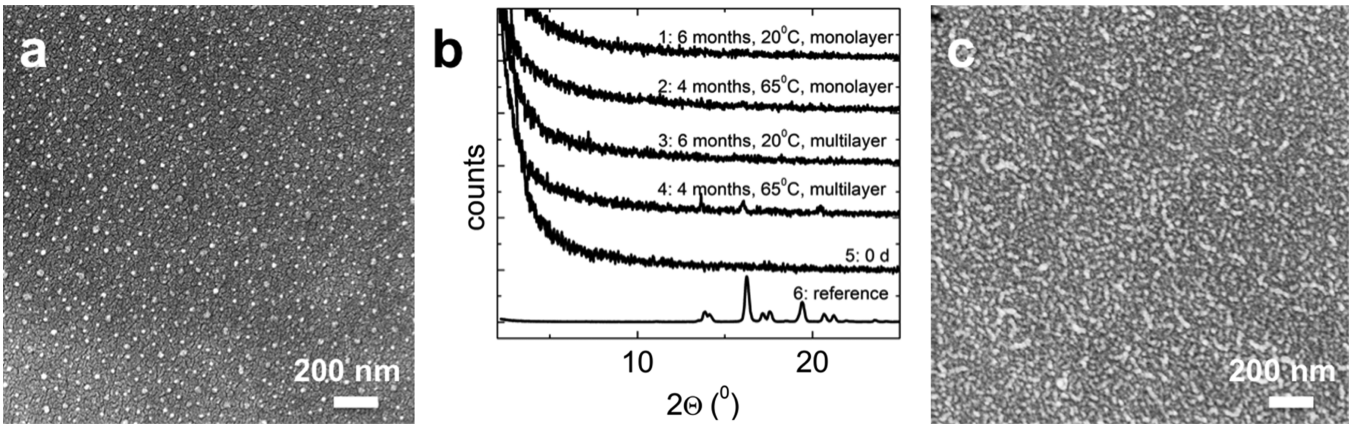


Figure 1. Stability of spray-dried danazol. (a) Scanning electron micrograph of well-separated spray-dried danazol nanoparticles. (b) XRD traces of spray-dried danazol nanoparticles stored at a constant temperature for the time indicated on the graph. The reference trace is for bulk danazol. (c) Scanning electron micrograph of a multilayer of spray-dried danazol nanoparticles.

EXPERIMENTAL SECTION

Spray-Drying. The microfluidic nebulator is produced from poly(dimethylsiloxane) using soft lithography. It contains two inlets for liquids and six inlets for air that lead into a main channel that is 80 μm wide and 100 μm tall, as schematically shown in Figure S1. We fabricate the nozzle of the device by cutting the main channel with a razor blade.[3] We control the flux of air through the channel by tuning its pressure at the air inlets; we keep this pressure constant at 0.28 MPa at all the air inlets. We dissolve 5 mg/mL of a drug and an optional excipient in ethanol and inject this solution through one liquid inlet at 1 mL/h, using volume-controlled pumps. The second liquid inlet, which is included for flexibility, is blocked to avoid leakage of liquid and air. We collect the dried particles 9 cm away from the nozzle on a polished Si wafer. These nanoparticles are stored at temperatures between 20 and 65 °C and a relative humidity around 20%.

Characterization of Nanoparticles. We characterize the size of the nanoparticles using a field emission scanning electron microscope (Zeiss Supra 55VP) operated at an acceleration voltage of 2 kV. Images are acquired using the InLens detector. To examine the structure of the nanoparticles, we use X-ray diffraction (XRD; Scintag XDS 2000 Powder Diffractometer) and vary the angle between the Cu Kα X-ray source and the detector from 2θ = 2 to 25° at a rate of 1 °C/min. We quantify the amount of nanoparticles contained in a polymer matrix using X-ray photoelectron spectroscopy (XPS; Thermoscientific) using a monochromatic Al Kα source operated at an acceleration voltage of 12 kV. To acquire a depth profile, we sequentially removed 78 nm of the sample using an Ar gun 13 times.

RESULTS AND DISCUSSION

The solubility of amorphous drugs, such as fenofibrate, can be more than 10 times as high as that of their crystalline counterparts, as exemplified for fenofibrate in the Supporting Information. Hence, it is advantageous to formulate poorly soluble drugs as amorphous particles. However, these particles are truly useful only if they retain their amorphous phase during storage. To explore the stability of the amorphous structure of drugs we use three different model drugs, danazol, clotrimazole, and fenofibrate; all these drugs belong to the biopharmaceutics classification system (BCS) class II and are thus poorly soluble in water but have a high permeability. Moreover, they all have a high propensity to crystallize, yet they have very different glass transition temperatures, T_g. We produce amorphous drug nanoparticles by dissolving the drug in ethanol, which is a good solvent for all of them, and spray-drying this solution using the microfluidic nebulator.

Many drugs have a very high propensity to crystallize. Thus, even if produced as amorphous particles, they usually crystallize during storage. First, we spray-dry danazol, which has a glass transition temperature, T_g, of 88 °C;[7] hence, if amorphous, it is a glass at room temperature. The spray-dried nanoparticles remain well separated if collected on a Si wafer for up to 5 h, as shown in the scanning electron microscopy (SEM) image in Figure 1a. These particles do not show any sign of crystallinity if stored at 20 °C for 6 months, as confirmed by the absence of any peaks in XRD, as shown in trace 1 of Figure 1b. Even if stored at 65 °C for as much as 4 months, no signs of crystallinity are observed, as shown in XRD trace 2 of Figure 1b. We attribute this excellent stability against crystallization to the small particle size: the nucleation rate is very low for glasses, as the molecules are kinetically arrested and hence have a very low mobility. Moreover, even if crystallization were to occur, only a single nanoparticle would crystallize. This is in sharp contrast with the bulk, where crystal nucleation can lead to growth of much larger crystals, which can spread through the whole sample. Thus, these samples are stabilized against crystallization by the small size of nanoparticles.

The probability for a crystal nucleus to form in an amorphous particle is proportional to its volume. However, this volume may increase during storage due to coalescence, which reduces the surface area. This will occur if the particles are touching one another, for example, when they are deposited as multilayers, and stored at temperatures where the molecular mobility is sufficiently high. In this case, we expect their stability against crystallization to decrease. To test this, we form multilayers of danazol nanoparticles by collecting the spray-dried samples for at least 10 h. Because these nanoparticles are glasses at room temperature, they do not coalesce but instead form aggregates, as shown in the scanning electron micrograph in Figure 1c. Nevertheless, they do not display any sign of crystallinity, even if stored as multilayers for as long as 6 months at 25 °C, as shown in XRD trace 3 in Figure 1b. However, if stored at 65 °C, a temperature closer to the T_g of danazol where molecules have a higher mobility, the particles

DOI: 10.1021/acs.jpcb.6b05417
J. Phys. Chem. B XXXX, XXX, XXX−XXX

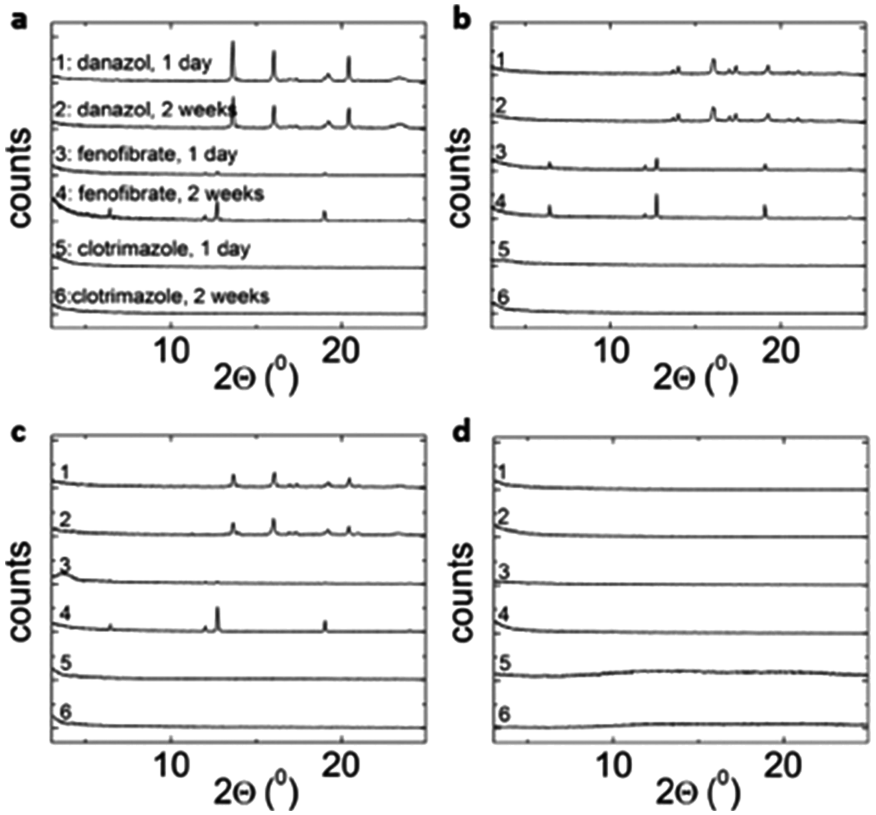


Figure 2. XRD traces of (1−2) danazol, (3−4) fenofibrate, and (5−6) clotrimazole nanoparticles co-spray-dried with (a) Pluronics F68, (b) Pluronics P84, (c) Pluronics P104, and (d) poly(vinylpyrrolidone) (PVP). The samples are stored at room temperature for (1, 3, 5) 1 day and (2, 4, 6) 2 weeks.

coalesce and start to crystallize after 4 months, as indicated by the diffraction peaks in XRD trace 4 in Figure 1b.

To ensure good stability of the amorphous structure during storage, coalescence must be prevented. This can be achieved by coating the particles with a polymer. If produced in commercial spray driers, this must be accomplished by using polymers that inhibit crystallization. Hence, polymers must fulfill two tasks: They must inhibit crystallization and impart steric stability to the particles. By contrast, the microfluidic nebulator enables production of amorphous drugs without the use of any crystallization inhibitors. Hence, the polymers must only fulfill a single task: They must impart steric stability to the particles. This should significantly facilitate the choice of an appropriate polymer. Hence, we expect that many more polymers can sterically stabilize amorphous drug nanoparticles if formulated in the nebulator than if produced with commercially available spray driers. To test this expectation, we co-spray-dry danazol with Pluronics, an amphiphilic block-copolymer composed of a poly(ethylene glycol) (PEG) block covalently linked to a poly(propylene) (PP) block. We dissolve 5 mg/mL danazol and 5 mg/mL Pluronics F68 in ethanol and spray-dry this solution. Surprisingly, a large fraction of the resulting nanoparticles is crystalline 1 day after production, as shown in XRD trace 1 of Figure 2a, and this fraction of crystalline particles remains nearly constant for 2 weeks, as shown by XRD trace 2 in Figure 2a.

To produce amorphous nanoparticles, we must ensure that the probability for crystal nuclei to form during the drying process is close to zero because once a crystal nucleus forms the entire spray-dried nanoparticle will be crystalline; this probability is much higher if particles are produced in the presence of heterogeneous nucleation sites, such as precipitated Pluronics. Hence, to obtain amorphous nanoparticles, we must minimize the formation of heterogeneous nucleation sites. Pluronics F68 has a limited solubility in ethanol because 80% of this block-copolymer consists of PEG, which is poorly soluble in ethanol. Therefore, it likely precipitates earlier in the drying process of the drops than danazol, which could result in the formation of amorphous particles that can act as heterogeneous nucleation sites for danazol crystals. To test if the crystalline structure of danazol co-spray-dried with Pluronics F68 is related to the poor solubility of the polymer, we co-spray-dry danazol with two other block-copolymers that have a higher solubility in ethanol, Pluronics P84 and Pluronics P104, each of which are only 40% PEG, with Pluronics P104 having a higher molecular weight. Again, in both cases, a large fraction of the danazol nanoparticles is crystalline, as indicated by XRD traces 1 in Figure 2b,c. This fraction does not significantly change within 2 weeks of storage, as shown in traces 2 in Figure 2b,c.

The fraction of spray-dried particles that is crystalline scales with the probability of crystal nucleus formation during the drying process. Danazol nanoparticles produced in the absence of Pluronics are amorphous, as shown in Figure 1, indicating that this probability is close to 0. By contrast, danazol nanoparticles produced in the presence of Pluronics are crystalline, as shown in Figure 2, indicating that, in this case, this probability is close to 1. The probability for crystal nuclei to form during the drying process depends on the time during which the drops are supersaturated, but not fully dried. To reduce this probability, we decrease the crystallization time by increasing drug solubility. Therefore, we employ fenofibrate, a hydrophobic drug with a higher solubility in ethanol. In this

DOI: 10.1021/acs.jpcb.6b05417
J. Phys. Chem. B XXXX, XXX, XXX−XXX

case, only a small fraction of the nanoparticles are crystalline, as shown by the small XRD peaks in traces 3 in Figure 2a–c. However, this drug has a T_g of −20 °C and thus, if amorphous, is an undercooled liquid at room temperature. As a result, the small crystals rapidly grow and a large fraction of the fenofibrate nanoparticles crystallize within 2 weeks,[4] as indicated by the strong peaks in the XRD traces 4 of Figure 2a–c. To further decrease the probability for drug crystals to form, we increase the drug solubility even more. Therefore, we co-spray-dry Pluronics with clotrimazole, a hydrophobic drug with an even higher solubility in ethanol. The resulting composite nanoparticles do not display any sign of crystallinity, as shown by the absence of any peaks in XRD traces 5 in Figure 2a–c. Moreover, they remain amorphous for at least 2 weeks, as shown in traces 6 in Figure 2a–c. Hence, polymer-stabilized amorphous drug nanoparticles can be produced by co-spray-drying with the microfluidic nebulator if the solubility of the drug is sufficiently high.

Pluronics promotes crystallization of spray-dried drugs because it probably precipitates at an early stage of the drying process, thereby forming heterogeneous nucleation sites. This behavior limits the number of drugs that can be processed into polymer-stabilized amorphous nanoparticles. To overcome this limitation, we should select stabilizers that precipitate later in the drying process. Polymers with greater solubility in ethanol are obvious candidates. If heterogeneous nucleation of the crystalline drug on the precipitated polymer is avoided, the drying process can produce either a two-phase amorphous drug/amorphous polymer particle or an amorphous polymer–drug solution, both of which are desirable forms. A polymer with a very high solubility in ethanol is poly(vinyl pyrrolidone) (PVP). To ensure good stabilization of the drugs and because the solubility of all of the tested drugs is more than 5 times lower than that of PVP, we dissolve 5 mg/mL of danazol and 25 mg/mL PVP in ethanol and spray-dry this solution. Indeed, PVP-stabilized danazol nanoparticles are amorphous, as shown in XRD trace 1 in Figure 2d. They retain their structure for at least 2 weeks if stored at room temperature, as shown in XRD trace 2 in Figure 2d. Similarly, fenofibrate and clotrimazole nanoparticles co-spray-dried with PVP are amorphous and retain their structure for at least 2 weeks, as shown in XRD traces 3–6 in Figure 2d. Thus, if the compositions are carefully selected, amorphous drug nanoparticles can be stabilized with a polymer layer during the spray-drying process.

Drugs formulated with commercial spray driers are crystalline unless they are co-spray-dried with a polymer that prevents their crystallization. Hence, these polymers must fulfill two functions: they must prevent both crystallization and agglomeration of the particles. The number of polymers that simultaneously fulfill both functions is limited, which makes an appropriate selection difficult. By contrast, the microfluidic nebulator enables production of amorphous nanoparticles without the use of any crystallization inhibitor. Hence, in this case, polymers must fulfill only one function: they must prevent particle agglomeration. This reduction in the function of the polymers should significantly facilitate their selection. To demonstrate this feature, we stabilize amorphous drug nanoparticles only after they are produced by directly depositing them into a polymer matrix. We produce this matrix by dropping an ethanol solution containing a dissolved polymer on a polished silicon wafer and evaporating the solvent. We use PVP as a model matrix, spray-dry amorphous danazol particles into it, and characterize their structure over time. These particles do not show any sign of crystallinity, even though they are deposited at a high density and stored at 65 °C for 4 months, as shown by the absence of any diffraction peaks in the top XRD trace in Figure 3a. To test if they also remain

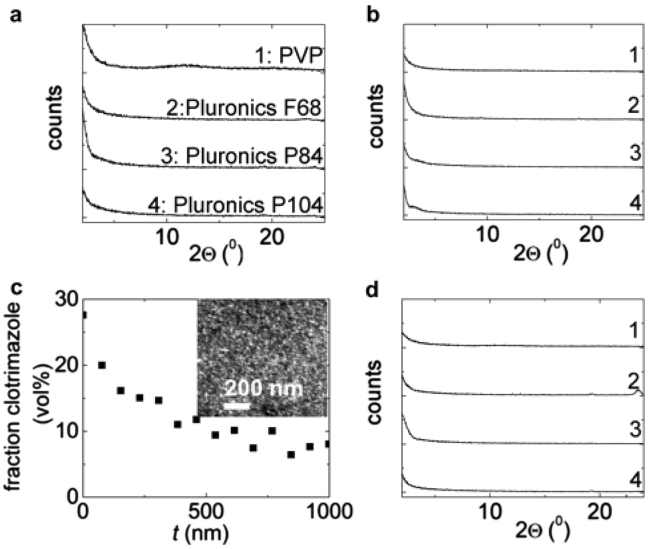


Figure 3. XRD traces of (a) danazol, (b) clotrimazole, and (d) fenofibrate that are sprayed onto matrices of (1) PVP, (2) Pluronics F68, (3) Pluronics P84, and (4) Pluronics P104. Traces are acquired after storing samples for 4 months at 65 °C. (c) Volume fraction of clotrimazole in the PVP matrix as a function of the matrix depth, *t*. We determine the volume fraction with XPS by removing 78 nm of the sample using an ion gun 13 times and measuring the Cl 2p and N 1s peaks after each removal. The inset is a SEM image of the clotrimazole–PVP composite.

amorphous if sprayed onto a matrix that does not inhibit crystallization in co-spraying, we deposit them into Pluronics. These nanoparticles remain again amorphous for at least 4 months if stored at 65 °C, as indicated by the absence of any reflection peak in XRD trace 2 in Figure 3a. Similarly, they remain amorphous if sprayed into Pluronics P84 and P104, which are pastes at room temperature, as shown by XRD traces 3 and 4 in Figure 3a.

Amorphous drugs that are produced with spray driers typically have particle diameters above 1 μm. These large particles have a large volume; therefore, they tend to crystallize during storage, strongly limiting their use. Their propensity to crystallize during storage depends on the molecular mobility, which increases with decreasing difference in the storage temperature, T_s, and glass transition temperature, $\Delta T = T_s - T_g$. Danazol has a high T_g and thus a low propensity to crystallize during storage, even if the volume of these particles is large. To test if the small size of nanoparticles produced with the nebulator reduces their propensity to crystallize during storage, we spray-dry a drug with a lower T_g onto polymer matrices. Therefore, we spray-dry clotrimazole nanoparticles into polymer matrices, which have a T_g of 29 °C.[8] Even though clotrimazole has a higher propensity to crystallize, spray-dried nanoparticles remain amorphous for at least 4 months even if stored at 65 °C, a temperature well above their T_g, if sprayed into any of the tested polymer matrices, as shown by the XRD traces in Figure 3b. We attribute their excellent stability to their good dispersion within the matrix: clotrimazole nanoparticles sprayed into a PVP matrix are spatially well separated from each

DOI: 10.1021/acs.jpcb.6b05417
J. Phys. Chem. B XXXX, XXX, XXX–XXX

other, even though they are deposited at a high density, as shown in the SEM image in the inset of Figure 3c. We quantify the clotrimazole loading by measuring the molar ratio of Cl to N using XPS. Because Cl is contained only in clotrimazole and N is contained in the clotrimazole and Pluronics, we can use this molar ratio to quantify the amount of drug contained in the matrix. We acquire XPS depth profiles by removing 78 nm of the sample using an Ar ion gun 13 times. The clotrimazole loading is as high as 30 vol % in the top 78 nm and decreases to 6 vol % at a depth of 1 μm, as summarized in Figure 3c. This result indicates that spray-dried nanoparticles have a sufficiently high kinetic energy to penetrate into the polymer matrix, thereby enabling storage of these nanoparticles at a higher density without risking aggregation.

The mobility of molecules is much higher in liquids, including undercooled liquids, than it is in glasses; hence, undercooled liquids crystallize much faster than their glassy counterparts. To test if even drugs that are undercooled liquids at room temperature can be stabilized, we spray-dry fenofibrate into different matrices. Remarkably, even this drug displays an excellent stability against crystallization if sprayed into any of the tested excipient matrices: fenofibrate nanoparticles show no sign of crystallinity whatsoever, even after they have been stored at 65 °C for 4 months, as shown by the XRD traces in Figure 3d. These results demonstrate the potential of the nebulator to produce polymer-coated amorphous drug nanoparticles that are much more stable against crystallization than drug particles produced using bulk methods or commercially available spray driers.

CONCLUSIONS

Amorphous nanoparticles produced in the microfluidic nebulator can be sterically stabilized with a polymer during their production through co-spray-drying. However, to prevent the polymer stabilizer from acting as a heterogeneous nucleation site, thereby inducing drug crystallization, it must precipitate later during the drying process than the drug. This requirement limits the choice of polymeric stabilizers to those with a high solubility in the employed solvent. Amorphous nanoparticles can also be sterically stabilized after their production if deposited onto a polymer matrix. In this case, polymers do not have to fulfill any prerequisites, which significantly facilitates their selection. Therefore, drugs could even be stabilized with polymers containing other functionalities such as DNA fragments, to make them selectively bind to certain locations. However, a continuous production of sterically stabilized amorphous nanoparticles would require additional infrastructure as the polymer matrix must be continuously moved relative to the spray-dry nozzle to ensure a homogeneous drug deposition. Hence, the most suitable method to sterically stabilize nanoparticles produced in the nebulator depends on the requirements of each application.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.6b05417.

Schematic illustration of the nebulator and the calculations of the solubility of amorphous fenofibrate (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: esther.amstad@epfl.ch.

Notes

The authors declare the following competing financial interest(s): The content of this paper has been patented.

ACKNOWLEDGMENTS

This work was supported by the NSF (DMR-1310266) and the Harvard MRSEC (DMR-1420570). Part of this work was performed at the Center for Nanoscale Systems (CNS), a member of the National Nanotechnology Infrastructure Network (NNIN), which is supported by the National Science Foundation under NSF award no. ECS-0335765. CNS is part of Harvard University.

REFERENCES

(1) Kawabata, Y.; Wada, K.; Nakatani, M.; Yamada, S.; Onoue, S. Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: Basic approaches and practical applications. *Int. J. Pharm.* **2011**, *420*, 1−10.

(2) Möschwitzer, J. P. Drug nanocrystals in the commercial pharmaceutical development process. *Int. J. Pharm.* **2013**, *453*, 142−156.

(3) Amstad, E.; Gopinadhan, M.; Holtze, C.; Osuji, C. O.; Brenner, M. P.; Spaepen, F.; Weitz, D. A. Production of amorphous nanoparticles by supersonic spray-drying with a microfluidic nebulator. *Science* **2015**, *349*, 956−960.

(4) Amstad, E.; Spaepen, F.; Weitz, D. A. Crystallization of undercooled liquid fenofibrate. *Phys. Chem. Chem. Phys.* **2015**, *17*, 30158−30161.

(5) Brough, C.; Williams, R. O., III Amorphous solid dispersions and nano-crystal technologies for poorly water-soluble drug delivery. *Int. J. Pharm.* **2013**, *453*, 157−166.

(6) Laitinen, R.; Lobmann, K.; Strachan, C. J.; Grohganz, H.; Rades, T. Emerging trends in the stabilization of amorphous drugs. *Int. J. Pharm.* **2013**, *453*, 65−79.

(7) Hu, J. H.; Johnston, K. P.; Williams, R. O. Stable amorphous danazol nanostructured powders with rapid dissolution rates produced by spray freezing into liquid. *Drug Dev. Ind. Pharm.* **2004**, *30*, 695−704.

(8) Benmore, C. J.; Weber, J. K. R.; Tailor, A. N.; Cherry, B. R.; Yarger, J. L.; Mou, Q.; Weber, W.; Neuefeind, J.; Byrn, S. R. Structural characterization and aging of glassy pharmaceuticals made using acoustic levitation. *J. Pharm. Sci.* **2013**, *102*, 1290−1300.

DOI: 10.1021/acs.jpcb.6b05417
J. Phys. Chem. B XXXX, XXX, XXX−XXX