Nanostructures

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Pharmaceutical Nano-Cocrystals: Sonochemical Synthesis by Solvent Selection and Use of a Surfactant**

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Crystals of nanometer-scale dimensions are of great interest in synthetic chemistry, materials science, and medicine.[1] Whereas inorganic nanocrystals have experienced utility in a wide range of areas (e.g. semiconductors,[2] medical diagnostics,[3] catalysis[4]), nanocrystals solely comprised of organic components have remained relatively unexplored. This is despite the unique physicochemical properties (e.g. molecular recognition) that emerge from the breadth of functional groups of organic solids.[5] That functionality can be readily incorporated and exploited in organic solids has become more apparent in recent years with cocrystals, which are organic solids comprised of more than one molecular component.[6] The field of pharmaceutics has been a major beneficiary where the properties of pharmaceutical agents (PAs) have been improved using complementary molecules in the form of cocrystal formers (CCFs).[6]

Here, we report an approach to synthesize pharmaceutical cocrystals of nanometer-scale dimensions. A dosage form comprising nano-cocrystals offers promise of enhanced dissolution rates and, thus, improved bioavailability and efficacy of medication.[7] The general instability of organic solids, however, largely prohibits applications of harsh methods (e.g. high temperature) used to prepare inorganic nanocrystalline solids.[8] Sonochemistry has become a means to prepare cocrystals of nanometer-scale dimensions.[9] The technique, which is harsh yet transient,[10] has afforded cocrystals with components comprised of relatively simple organic molecules. We demonstrate the use of sonochemistry to prepare nanometer-scale pharmaceutical cocrystals using a combination of multiple-solvent selection and the surfactant Span-85. The method accounts for a disparity in solubility between a PA and CCF; namely, caffeine and 2,4-dihydroxybenzoic acid, respectively (Figure 1). The method affords pharmaceutical nanococrystals with a narrow size distribution. The majority of pharmaceutical nanocrystals have been prepared using "top-down" media milling.[11]

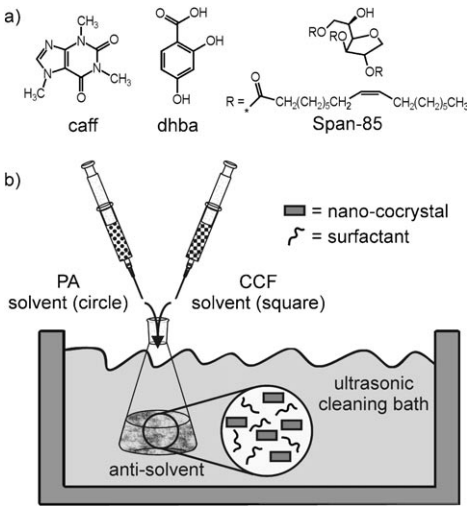

Figure 1. a) Cocrystal components and surfactant. b) Two-solvent method with each component dissolved in a different solvent and injected into an anti-solvent/surfactant mixture under sonication.

Pharmaceutical cocrystals are a viable alternative to polymorphs and salts as solid forms for active pharmaceutical ingredients (APIs).[6] Cocrystals containing a PA can improve the physiochemical properties (i.e. stability,[12] solubility/dissolution rate,[13] and mechanical properties[14]) of a PA. Combining the benefits of pharmaceutical cocrystals with a decrease in particle size to the nanometer scale, one can expect to further improve properties of a PA (e.g. dissolution rate). The enhanced dissolution rate of a nanocrystal will mainly originate from the increased surface area.[7] A slight increase in solubility owing to the curvature and, hence, the high-energy surfaces of nanosized particles will also contribute to faster dissolution.[7] Currently, five marketed medicinal products are formulated using single-component nanocrystalline solids. Four products rely on media milling while the fifth uses high-pressure homogenization.[11] Concerns have been raised over lengthy processing time, high energy input/consumption, contamination, and inadequate particle size

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control.[11,15] In addition, there is an intrinsic inability of such top-down processes to produce organic nanocrystals smaller than 100 nm, which is mainly due to the ductileness and the ease of losing crystallinity.[11,15] Thus, it has been recognized that the development of bottom-up approaches are necessary to facilitate the generation of crystalline particles with sizes on the order of a few hundred nanometers.[11]

A lack of development of pharmaceutical cocrystals of nanodimensions can be ascribed to a diverse tapestry of functional groups in PAs. Although functional groups provide multiple supramolecular synthons[5] for cocrystallization, the groups tend to amplify solubility differences between a CCF and PA. We have reported a method to produce nanosized cocrystals using sonochemistry based on a single high-solubility solvent.[9] The method demonstrated the utility of sonochemistry by forming nano-cocrystals of a cocrystal with simple constituents based on two functional groups. The sonochemistry succeeded when rapid reprecipitation alone failed.[9] The complexity of a PA can reduce the likelihood of finding a common solvent to cosolubilize components of a pharmaceutical cocrystal so as to afford a product of nanometer-sized dimensions with a narrow size distribution appropriate for dosage form development. The size distribution has consequences in terms of long-term stability in that the absence of particles with large size differences will reduce Ostwald ripening.[7]

The cocrystal of focus here is caffeine 2,4-dihydroxybenzoic acid monohydrate (caff)·(dhba)·(H_2O). Caff is one of the most frequently used model compounds in pharmaceutical cocrystal studies[12b,14a,16] while dhba is enlisted in the Everything Added to Food in the United States (EAFUS) list.[17] Improvements to properties of a PA within a cocrystal have been realized with caff.[14a,12b] The components of (caff)·(dhba)·(H_2O) possess four functional groups (that is, −OH, −CO_2H, C=O, N(lone pair)) that occupy six different chemical environments. The components form a two-dimensional (2D) graphite-like structure sustained by a combination of an intramolecular OH(hydroxy)···O(carboxy) and an intermolecular OH(carboxy)···N(imidazole) hydrogen bond (Figure 2).

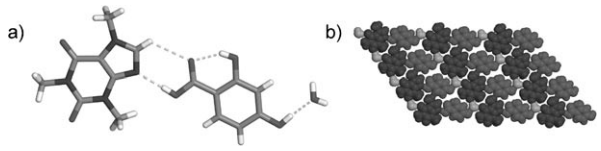

Figure 2. X-ray structure of (caff)·(dhba)·(H_2O): a) hydrogen bonds and b) 2D network.

In (caff)·(dhba)·(H_2O), the water molecule links two components through OH(water)···O(urea), OH-(water)···O(amide) and OH(hydroxy)···O(water) hydrogen bonds. The number and complementary nature of functional groups of a cocrystal will impact differences in solubilities of the components.[18] The prevalence, and impact, of solubility differences in cocrystal formation is reflected in the variety of techniques used to generate cocrystals.[18,19] For (caff)·(dhba)·(H_2O), the CCF and PA exhibit solubility differences of one to two orders of magnitude in common organic solvents, which is expected to preclude rapid assembly of the components to afford the formation of well-defined crystals of nanometer-scale dimensions.

Our first experiment to generate nano-cocrystals of (caff)·(dhba)·(H_2O) involved a single-solvent sonochemical approach[9] using acetone. Acetone was chosen on the basis of the ability of the solvent to dissolve both components while maintaining miscibility with the anti-solvent hexanes. Caff and dhba were separately dissolved in acetone then rapidly injected into a hexanes solution at approximately 0 °C under ultrasonic radiation. After 15 s of irradiation the suspension was filtered and analyzed using powder X-ray diffractometry (PXRD), scanning electron microscopy (SEM), and dynamic light scattering (DLS).

An inspection of the PXRD pattern of the single-solvent precipitate showed an absence of the most intense peak for caff at $2\theta = 12.0°$ and dhba at $2\theta = 13.7°$ (Figure 3). A

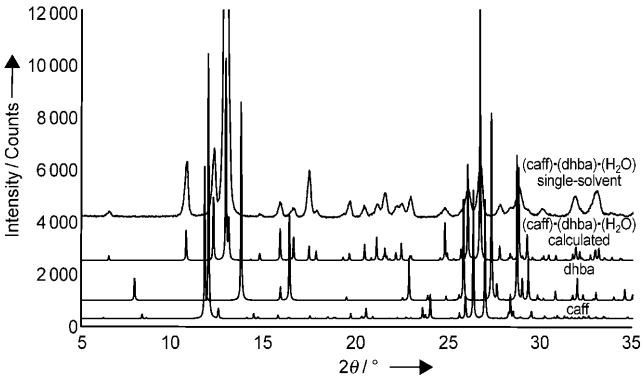

Figure 3. Comparison of PXRD pattern from the single-solvent approach to calculated (caff)·(dhba)·(H_2O) and individual components.

comparison of the diffractogram to the calculated powder pattern of the cocrystal revealed peaks at $2\theta = 10.7°$, 12.9°, 17.4°, 21.5°, and 22.9°, which are consistent with cocrystal formation.[16c]

SEM micrographs of the precipitate confirmed the presence of nanometer-sized crystals of plate morphologies. The smallest crystals exhibited widths of approximately 200 nm and lengths of approximately 200 nm. However, the individual crystal sizes varied from the nanometer-scale to the micrometer scale (i.e. up to 5 μm; Figure 4). The micrographs also displayed agglomeration in the form of stacked, non-fused cocrystals, which is consistent with the formation of a nanosuspension.[15d] DLS measurements were inconclusive and unreliable owing to drifting intensity of scattered light caused by an instability of the auto-correlation function.[20] The results from the DLS experiment were determined to lie outside acceptable values, which suggested a broad particle size distribution and/or presence of large particles caused by an association of nano-cocrystals within the dispersion.[20]

To reduce or eliminate the micrometer-sized particles, we devised a two-solvent approach.[7,15c] More specifically, we

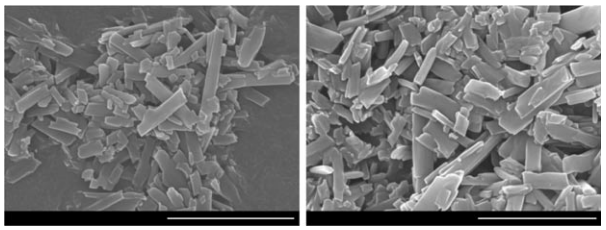

Figure 4. SEM micrographs of size distribution of agglomerated (caff)·(dhba)·(H_2O) from the single-solvent approach. Scale bars: 3 μm (left), 2 μm (right).

turned to modify the injection solvent according to solubility and dielectric constant.[20] In such an experiment, the two solvents in the cocrystallization would be selected based on an ability to provide high solubility (i.e. $>10\,mg\,mL^{-1}$) and miscibility with the anti-solvent. We hypothesized that by cosolubilizing[21] both caff and dhba through the selection of a high-solubility solvent for each component, the cocrystal would be more prone to rapidly nucleate and, thereby, afford particles of nanometer-scale dimensions.[22]

To test our hypothesis, caff and dhba were separately dissolved in chloroform and acetone, respectively, and then rapidly injected into hexanes at approximately 0°C under ultrasonic radiation. After 15 s of sonication the suspension was filtered. PXRD (see Supporting Information) confirmed cocrystal formation.

To determine the particle size, the precipitate was analyzed using SEM. Unlike the single-solvent approach, the individual crystals that formed using the two-solvent method were exclusively of nanometer dimensions. The smallest particles displayed a width of 190 nm and length of 200 nm, while the largest crystals displayed a width of 200 nm and length of 800 nm (Figure 5). Similar to the single-solvent

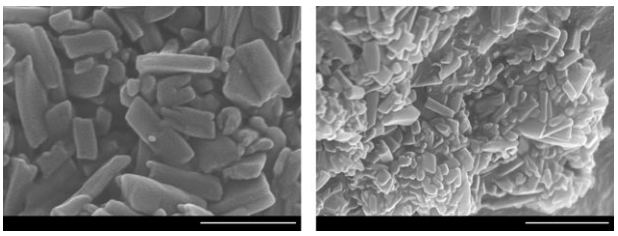

Figure 5. Micrographs of agglomerated (caff)·(dhba)·(H_2O) prepared by the two-solvent approach. Scale bars: 500 nm (left), 1 μm (right).

method, however, the micrographs revealed extensive agglomeration in form of stacked crystals. Agglomeration can be expected owing to the supersaturated nature of the solution used to achieve the nanometer-sized crystals.[23] The agglomeration in the SEM micrographs was again manifested in the DLS experiment that suggested the presence of very large particles. The application of two solvents, however, resulted in a significant improvement in both particle size and distribution.

Although the two-solvent sonochemical method was successful in affording pharmaceutical cocrystals of nanometer-scale dimensions, we sought to reduce or eliminate the agglomeration. Agglomeration can influence solid dosage forms, with negative impacts on quality attributes such as dissolution rate and content uniformity.[15a] We, therefore, turned to study effects of surfactants on the agglomeration, as well as particle size and morphology, on sonochemical preparation.[24] We expected a surfactant to coat the nanococrystals and, thus, provide a steric barrier to agglomeration.[15d] The surfactant could also further reduce the size of the nano-cocrystals,[25,26] with a decrease in surface tension and an increase in nucleation rate.[15c,27] The adsorption of surfactants at a growing solid interface also reduces the interfacial surface energy and inhibits particle growth.[28]

Pharmaceutically acceptable Sorbitan oleate (Span-85) was selected as the surfactant.[29] A single-solvent crystallization was performed by preparing a solution of caff and dhba in acetone followed by rapidly injecting the solutions into hexanes in the presence of 5% (w/v) Span-85 at approximately 0°C under ultrasonic radiation. A PXRD analysis (Supporting Information) confirmed cocrystal formation. An aliquot of the suspension was also analyzed using DLS. In contrast to the experiments without surfactant, DLS measurements of the single-solvent approach resulted in average particle sizes of (280.1 ± 102.2) nm and polydispersity index (PDI) of 0.222 (Figure 6). For the case of two solvents, the

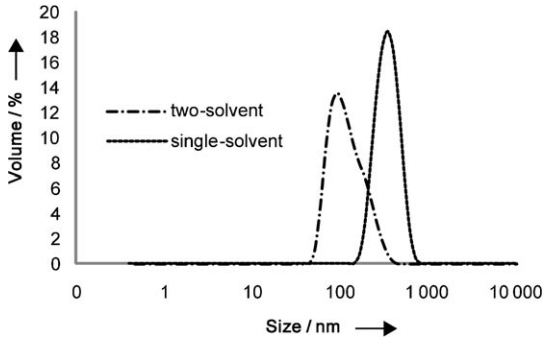

Figure 6. Particle size distribution for (caff)·(dhba)·(H_2O) from single-solvent and two-solvent approaches with Span-85.

DLS revealed an average particle size of (136.4 ± 65.05) nm and PDI of 0.239. The application of the surfactant, thus, provided a means to affect both the agglomeration and crystal size using both the single- and two-solvent sonochemical methods.

SEM micrographs of the cocrystals obtained using the single-solvent sonochemical approach and Span-85 (Figure 7a) revealed morphologies based on spheres and plates. The spherical morphology can be attributed to modified crystal growth due to adhered surfactant on the crystal surface.[20] The crystals appeared dispersed with less stacking compared to those without surfactant. The micrographs using the single solvent also revealed micrometer-sized crystals of widths 200 nm and lengths 5 μm, which were not suggested by the DLS measurements.

Sphere and plate morphologies were also present with two solvents. The average particle sizes of the cocrystals, however, were more comparable to the DLS results with micrometer-

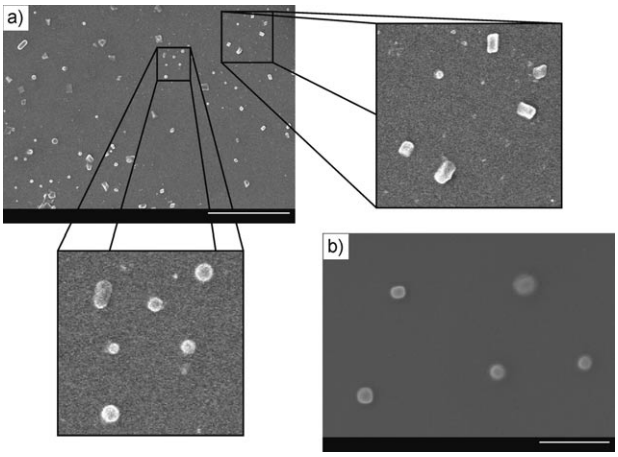

Figure 7. Micrographs of (caff)·(dhba)·(H_2O) prepared with Span-85 (5%): a) single-solvent approach (scale bar 5 μm) and b) two-solvent approach (scale bar 1 μm).

sized crystals not being present (Figure 7b). The use of the surfactant, thus, promoted a general decrease in overall particle size, with a narrowed difference in particle size for the two-solvent approach. Collectively, the results demonstrate the utility of cosolubilizing the cocrystal components using two solvents and incorporating a surfactant to prepare pharmaceutical cocrystals of nanometer-sized dimensions.

To conclude, we have introduced sonochemistry based on solvent selection and use of a surfactant to generate pharmaceutical cocrystals of nanometer-scale dimensions. The method accommodates the inherent solubility difference between the two components of a pharmaceutical cocrystal comprised of increasing numbers of organic functional groups. We are working on PAs with poor dissolution rates and bioavailability whose properties could be improved through cocrystallization and production of nano-cocrystals using sonochemistry and surfactants.

Experimental Section

Nano-cocrystal synthesis: Cocrystals from the single-solvent approach were obtained by separately dissolving 60 mg of caff and 48 mg of dhba in 7 mL and 242 μL of acetone, respectively. The solutions were rapidly injected into 200 mL of hexanes at ca. 0 °C and sonicated for 15 s in a cleaning bath (Branson 2510R-DTM). The two-solvent approach involved the same procedure except 125 mg of caff and 99 mg dhba were separately dissolved in 1 mL of chloroform and 600 μL of acetone, respectively, then rapidly injected into 100 mL of hexanes at ca. 0 °C. The surfactant crystallizations were performed with 5% (w/v) Span-85 added to hexanes.

Characterization: PXRD data was collected using a G3000 diffractometer (Inel Corp., Artenay, France). Particle size measurements for the two-solvent approach required dilution of 250 μL of the original suspension into 3 mL of the 5% (w/v) Span-85 hexanes solution. The single-solvent approach was analyzed without any further dilution. The average particle size of the cocrystal was determined by a Zetasizer Nano ZS (Malvern, Southborough, MA) instrument at 25 °C at a 173 scattering angle. The average particle size and PDI were averaged over a set of three measurements. Further assessment by using SEM (Hitachi S-4800) micrographs operated at a range of 2–5 kV was performed on specimen stubs sputter coated with gold for approximately 3 min.

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