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Abstract

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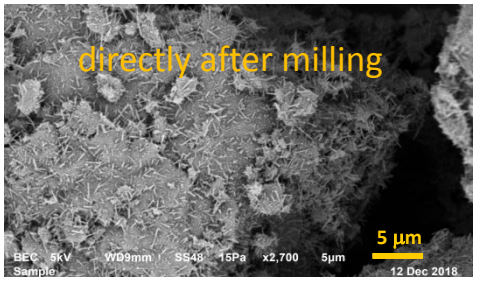

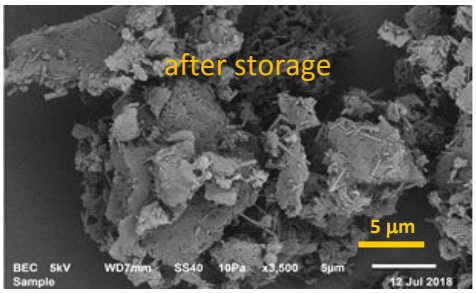

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Introduction

Integrating solid-state reactions with crystallization techniques is an attractive means of adapting 'Green Chemistry' to industrial manufacturing, as one of the twelve principles of Green Chemistry is the reduction and/or possible removal of solvents from a chemical reaction.[1,2] Solvent-free crystallization reduces waste, cost, environmental and health impacts, as well as batch to batch variation due to solvents sourced from different suppliers with different impurity profiles. Co-crystallization allows the combination of complementary drugs e.g. the combination of drugs known to cause nausea with anti-nausea drugs, into a single crystalline phase and the optimisation of chemical, physical, and pharmacological properties.

Solid-state reactions are becoming a growing trend in organic synthesis.[3] They often require relatively high energy inputs *via* manual grinding (mortar and pestle),[4] high impact milling (mechanical rotational or oscillating milling),[5] or continuous grinding (twin screw extrusion, hot melt extrusion).[6] Recently, resonant acoustic mixing was shown to produce co-crystals under rather "soft" mixing conditions.[7-10] On the other hand it is known that solids with a low vapour pressure can spontaneously undergo solid-state reactions forming salts, solvates, or co-crystals.[11-14] Solid-state co-crystallization *via* manual grinding has been known for over a century. In 1893 Ling and Baker reacted an equimolar mixture of metadichloroquinone and metadichloroquinol to produce the metadichloroquinone-metadichloroquinol co-crystal.[15] Currently solid-state co-crystallization is routinely used in many fields of solid-state chemistry and pharmaceuticals.[16] Recent work has shown the adoption of high impact milling as a method to screen for co-crystals and co-amorphous systems.[17-19]

Spontaneous solid-state reactions can involve a chemical reaction or a change of the crystal structure.[20,21] Spontaneous solid-state reactions are well documented where surface contact with a template or another solid form induces a solid-state transformation of the material. This phenomenon is well known for mono-component systems that transform to different polymorphs.[22] Multi-component solid-state reactions can occur spontaneously when two different compounds come into contact and are most often observed as solvate or hydrate formation, but rarely as co-crystal formation.[11-13,23] If the reaction is thermodynamically and kinetically favoured, spontaneous co-crystal formation can be induced by physically mixing stoichiometric ratios of the powdered co-formers. Ibrahim *et al*. monitored the effects of humidity when mixing samples of 2-methoxybenzamide and urea, and of caffeine and

malonic acid. Humidity was shown to increase the rate of co-crystallization in both cases and a minimum level of humidity was needed to induce the reaction.[14] Maheshwari *et al.* used free energy calculations to predict thermodynamically favoured co-crystals of carbamazepine (carbamazepine–nicotinamide and carbamazepine–saccharin) and investigated the influence of the storage conditions on the spontaneous co-crystallization. They found that humidity and temperature play a role in co-crystal formation. A new co-crystal polymorph of carbamazepine–saccharin was produced from milled samples stored at high temperatures and humidity.[23] Ervasti *et al.* investigated the effects of storage conditions, starting material particle size, and the use of anhydrous theophylline or its hydrate on the formation of theophylline and nicotinamide co-crystals.[12] The rate of co-crystal formation increased with humidity, temperature, and particle size reduction. Using theophylline hydrate hindered the transformation to the co-crystal. Although all studies observe partial or almost complete transformation of powdered samples to the co-crystal at high temperature, no examples could be found of a system fully transforming to a co-crystal at ambient temperature and relatively low humidity.

There are several possible mechanisms for spontaneous solid-state reactions including vapour diffusion, moisture sorption, sunken eutectic-liquid phases, amorphization, solid dispersion, and long range anisotropic molecular migration.[24] Rastogi *et al*. and others attributed the spontaneous co-crystallization of naphthalene and picric acid to a vapour diffusion mechanism.[25-29] Davey and coworkers investigated the co-crystallization of benzophenone and diphenylamine and observed a submerged eutectic temperature of 13.3 °C which allowed the spontaneous reaction of two particles at room temperature.[30] Transient amorphous phases are well documented during grinding[31-33] and milling-induced co-crystallization.[34] Kaupp used atomic force microscopy to identify a topotactic reaction for a number of solid-state reactions.[24]

This work investigates the co-crystallization of caffeine and urea *via* a spontaneous solid-state reaction. Caffeine is a widely used additive in food and pharmaceuticals and has been shown to interact with urea in solution.[35] Urea is well known to form supramolecular assemblies. These include the urea inclusion compounds with guest molecules occupying tunnels in the urea host structure[36] and hydrogen-bonded co-crystals. Because of its special H bond donor and acceptor capabilities, a large number of urea co-crystals have been described, *e.g.* with carboxylic acids,[37-46] amides,[47] α,ω-dihydroxyalkanes,[48-50] phenols[51] and carbohydrates,[52] various of which were obtained by mechanochemical techniques. Honer *et*

al., for example, reported the mechanochemical synthesis of urea ionic co-crystals with Mg and Ca salts[53] and Casali *et al.* prepared two polymorphs of the ionic co-crystal urea·$ZnCl_2$·KCl by solution crystallization and mechanochemistry.[54] Zhou *et al*. synthesized mechanochemically polymorphs of 1:2 co-crystals of urea with 1,6-dihydroxyhexane and 1,8-dihydroxyoctane.[48] In the present work an in-house mixer was designed to slowly mix the physical mixture of caffeine and urea, allowing particle-particle interactions with little external energy input. Infrared spectroscopy, X-ray diffraction, Raman microscopy, differential scanning calorimetry and scanning electron microscopy were used to monitor the solid-state reaction and to characterize the co-crystal obtained. Solution crystallization experiments were carried out in order to obtain single crystals for structure determination.

Materials & Methods

Materials

Caffeine (≥ 99.0 %) and urea (≥ 98.0 %) were supplied by Sigma-Aldrich (Saint Louis, Missouri, USA). Acetonitrile (≥ 99.9 %) was purchased from Honeywell **(**Wabash, Indiana, USA). Diethyl ether (≥ 99.5 %), methanol (≥ 99.9 %), and ethyl acetate (≥ 99.5 %) were supplied by Merck (Kenilworth, New Jersey, USA). Acetone (≥ 99%) and ethanol (≥ 99 %) were supplied by Fisher (Pittsburgh, Pennsylvania, USA).

Methods

Solid-State Analysis

IR spectra were recorded using a PerkinElmer Spectrum 400 (Waltham, Massachusetts) equipped with a DATR 1 bounce Diamond/ZnSe Universal ATR sampling accessory. Spectra were measured in the range from 4000 to 650 cm^{-1} with 8 accumulations and a resolution of 4 cm^{-1}.

Thermal analysis was performed on a Rheometric Scientific STA625 thermal analyser (Piscataway, New Jersey) with a constant heating rate of 10 °C/min. The measurements were made in open aluminium crucibles, nitrogen was purged in ambient mode (40 mL/min) and calibration was performed using an indium standard.

Raman microscopy was conducted using a Renishaw inVia confocal microscope with a ×50 optical lens and the WiRE 3.4 software. Powdered samples were lightly dispersed manually on a glass slide using a spatula. Individual particles were focused and selected using a XYZ

sample stage. Spectra were collected from 200 to 3200 cm^{-1} using 600/cm grating (785 nm laser at 10% power; 3 acquisitions, 3 s exposure time).

In-situ SEM-Raman spectroscopy was performed on an inVIA Reflex micro-spectrometer (Renishaw, Wotton under Edge, UK) coupled to a DM2500 Leica microscope and a JSM-6510LV SEM (JEOL) equipped with secondary electron (SE (high vacuum (HV)) and backscatter electron (BSE (low vacuum (LV)) detectors. The 785 nm excitation laser (diffraction grating groove density 1800 grooves/mm) was used throughout and instrument calibration was performed using the Si (100) peak (520.5 ± 1 cm^{-1}) (50× objective, laser power 10 mW, acquisition time 10 s, 1 accumulation).

SEM was performed by dispersing powder samples on carbon disks (Agar, Oxford Instruments) attached to SEM stubs, placed in an SEM stub holder and a montage of the whole stub collected using the optical microscope. Following inspection of the dispersed powders on the optical microscope a region of interest was selected and spectra collected by point mapping (20x objective, laser power 1 mW, acquisition time ≥ 10 s, ≥ 5 accumulations). In the SEM the region of interest was identified using optical images of the whole stub montage and the region of interest. The particles were then examined in BSE mode (3 - 5 kV, 10 - 20 Pa) and the co-ordinates of the particles of interest and images recorded. Following focussing of the Raman laser at each point of interest the acquisition time and the number of acquisitions for each spectrum was varied to improve the signal-to-noise ratio (laser power 0.1 - 6 mW, acquisition time ≥ 30 secs, ≥ 5 accumulations), over a narrow spectral range of interest. Following spectral acquisition the samples were gold-coated (SI50B, Edwards) and the particles of interest re-examined by SEM (JSM-6510LV (JEOL)) to obtain high resolution images of the position from which spectra had been collected.

Single crystal X-ray diffraction was carried out on an Oxford Diffraction Xcalibur system (Oxfordshire, UK) at room temperature. The crystal structure was solved by direct methods using SHELXT and refined using SHELXL 2018/3 within the Oscail package.[55-57] Crystallographic data and details of refinement are reported in Table 1. The cif file can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, Cambridge, UK with the REF code 1935528.

Powder X-ray diffraction (PXRD) patterns were collected on an Inel Equinox 3000 powder diffractometer (Artenay, France), fitted with a curved position sensitive detector calibrated

using Y_2O_3. Data were collected between 5 and 90° (2θ) using Cu K_α radiation (λ = 1.54178 Å, 35 kV, 25 mA). Theoretical powder patterns were calculated using the Oscail software package.[57]

Co-Crystallization

Milling:

1 g of an equimolar mixture of caffeine and urea was milled at room temperature using an oscillating ball mill (Mixer Mill MM400; Retsch GmbH, Haan, Germany) at 25 Hz in a 25 mL stainless steel jar using a 15 mm stainless steel ball. After 30 min the milling was interrupted for 15 min to avoid overheating of the sample. The milling was continued for another 30 min so that the total milling time was 60 min. The sample was stored overnight at room temperature and 30 % relative humidity (RH). The formation of the co-crystal was confirmed by PXRD.

To confirm the 1:1 interaction between the components 1:3 and 3:1 mixtures of caffeine and urea were milled at 25 Hz for 60 min (using the same procedure as above with a 15 min break after the first 30 min.), kept at room temperature for 1 d and analyzed by PXRD.

Mixing using an in-house low energy mixer:

Caffeine and urea (1 g each) were milled separately for 60 min. with a 15 min. break after the first 30 min interval. The milled caffeine and urea were mixed in a 1:1 molar ratio (1 g in total) in a 28 mL vial (60 mm height × 20 mm diameter). The vial was attached to a bespoke low energy mixer designed to rotate at 50 rpm, 3 clockwise rotations followed by 3 counter clockwise rotations as previously described.[58] The mixer was assembled using an Arduino Uno R2 single board microcontroller (Ivera, Italy) to control a stepped motor spinning the vial at a *ca.* 15º horizontal tilt.

Mixing using a magnetic stirrer:

1 g of a 1:1 mixture of caffeine and urea, individually milled as described above, was placed in a 10 mL glass vial. The sample was mixed at 100 rpm using a magnetic stirring plate and a 8 × 3 mm stirring bar. Mixing was done at room temperature and <30% RH.

Crystallization from solution:

Under-saturated solutions of caffeine and urea (1:1 molar ratio) were prepared in duplicate using the following solvents; methanol, ethanol, isopropanol, water, acetonitrile, and ethyl acetate. The solutions were divided into two sample sets. One sample set was seeded with the

co-crystal prepared by milling and storage, the other was allowed to crystallize unseeded. Both sample sets were left to evaporate in a fume-hood at room temperature (22 – 26 °C). IR spectra of the powders/crystals were collected and processed using Principal Component Analysis (PCA) and Unscrambler 10.1 (CAMO) to find the optimum conditions for the crystallization of the caffeine-urea cocrystal. The IR spectra were pre-treated using Standard Normal Variate (SNV) transformation on ranges containing characteristic peaks of caffeine, urea and the co-crystal and were then processed by singular value decomposition PCA with seven latent variables. Solvents from which the co-crystal crystallized after evaporation were further investigated to grow X-ray suitable single crystals by cooling crystallization.

A Thermo Fisher Scientific oven (UT 6420) coupled with a Heraeus (Thermicon P) temperature controller was used for controlled cooling crystallization. A solution containing 600 mg of an equimolar caffeine and urea mixture in 15 mL of acetonitrile was held at 70 °C for 12 h, then cooled to 25 °C with temperature cycling over the course of several days.

Results

Caffeine-Urea Co-crystal Formation in Milled Samples

An equimolar mixture of caffeine and urea was milled for 60 min. and the milled sample was analyzed by PXRD and IR spectroscopy (Figure 1). Immediately after milling peaks in the PXRD pattern match the peaks of commercial urea and caffeine, indicating that no new crystalline material was formed. Although there are no changes in peak position, changes in intensity are observed. The peaks of caffeine are slightly reduced, whereas all peaks associated with urea are reduced by a factor of five (Figure S1, SI). It is unlikely that preferred orientation effects are the reason for the intensity decrease, as preferred orientation would only affect certain peaks and not all. The loss of intensity is likely caused by a reduction in particle size and crystallinity during milling.[59] Although no new crystalline material was detected immediately after milling, when the milled sample was stored at room temperature new peaks were observed at 8.64°, 10.82°, 13.89°, 24.30°, 25.08°, 25.46°, and 28.07° (2θ) in the PXRD pattern. This new pattern was compared to the calculated patterns of the urea and caffeine structures reported in the CSD (Figure S2, SI) ruling out a simple polymorphic transformation. Instead the new peaks can be attributed to a spontaneous co-crystal formation during storage as confirmed by comparing the experimental PXRD pattern with the simulated PXRD pattern calculated from the single crystal data of the urea-caffeine

co-crystal obtained by solution crystallization (see below). Figure 2 shows the change in the normalized peak heights of the caffeine peaks at 11.7 and 26.5° (2θ) and of the urea peak at 22.7° (2θ) *vs.* storage time.

The solid-state transformation during storage of milled caffeine and urea was also monitored using IR spectroscopy. Figure 1a shows the calculated spectrum of an equimolar physical mixture of caffeine and urea overlaid with the spectrum of caffeine and urea immediately after milling. Immediately after milling slight changes in the IR spectrum are seen. Most notably the caffeine ν(C=O) band at 1677 cm^{-1} is shifted to 1682 cm^{-1} and the urea ν_s(N-H) band is shifted from 3341 cm^{-1} to 3335 cm^{-1} with a new shoulder at 3347 cm^{-1}. When the milled sample is stored overnight at room temperature more significant changes are observed indicating changes in H-bonding interactions and the formation of the co-crystal. The caffeine ν(C=O) peak at 1682 cm^{-1} is shifted to 1707 cm^{-1} and the ν_s(N-H) urea peak is shifted to 3185 cm^{-1}. Similar, large shifts of ~200 cm^{-1} were reported for the ν_s(N-H) band in other urea co-crystals.[60] The spectrum also shows the convolution of several peaks throughout the spectrum and the appearance of a new peak at 809 cm^{-1} unique to the co-crystal. The discrepancy between the IR and PXRD data of the samples analyzed directly after milling may be due to the fact that ATR-FTIR spectroscopy is a surface-biased technique. A Diamond/ ZnSe ATR-IR has a penetration depth of 1.66 μm, resulting in an overemphasis of changes on the particle surface.

To investigate the co-crystallization of caffeine and urea further, mixtures of different stoichiometric ratios, 1:3 and 3:1, were milled for 60 min. and analyzed 24 h after preparation (Figure S3, SI). The PXRD patterns and IR spectra of both ratios show mixtures of the co-crystal and excess starting material. This indicates a 1:1 interaction between caffeine and urea with no other transformation taking place.

Mixing of Caffeine and Urea Using a Low Energy Mixer

Caffeine and urea were milled separately for 60 min. at 25 Hz. No solid-state changes were observed after milling by IR spectroscopy or PXRD. The milled caffeine and urea were then mixed in a 1:1 molar ratio in an in-house low energy mixer[58] at room temperature and <30 % relative humidity. Direct transformation of the caffeine and urea physical mixture to the co-crystal was observed and monitored using IR spectroscopy (Figure 3). Transformation was detected within three days. Peaks specific to the co-crystal (1707 and 809 cm^{-1}) as well as the

characteristic shift of the ν_s(N-H) urea band to 3185 cm^{-1} were observed. At the same time the caffeine and urea bands at 1677 and 3341 cm^{-1} decreased in intensity. By contrast, when an equimolar mixture of un-milled caffeine and urea was mixed using the low energy mixer, a small broad peak at 809 cm^{-1} in the IR spectrum indicated the formation of only a small amount of the caffeine-urea co-crystal after 10 weeks (Figure S4, SI).

More aggressive mixing of unmilled caffeine-urea using a magnetic stirrer increased the rate of transformation to the co-crystal when compared to the low energy mixing. IR and PXRD peaks characteristic to the co-crystal appeared within five weeks (Figure S5).

Equimolar physical mixtures of separately milled caffeine and urea and of un-milled caffeine and urea were left without mixing as controls for two months. No changes were observed in the IR spectra or PXRD patterns.

DSC Analysis

Figure 4 compares the DSC thermograms of an equimolar physical mixture of caffeine and urea before milling, immediately after milling for 60 min. and after storing the milled sample for 24 h. Immediately after milling the caffeine-urea sample shows an exotherm at 91.3 °C and a sharp endotherm at 132.7 °C, slightly lower than the melting point of urea (135.3 °C). The former indicates the transformation into the co-crystal and the latter is assigned to the melting of the co-crystal. A melting endotherm at 132.7 °C is also observed in the thermogram of the caffeine-urea co-crystal prepared by cooling crystallization from acetonitrile (see below). After storing the milled sample for 24 h at room temperature the exotherm at 91.3 °C is no longer observed. This corroborates that the exotherm at 91.3 °C is due to the transformation of the caffeine-urea sample (still present as a physical mixture directly after milling) to the co-crystal. This transformation is not seen in the DSC plot of the un-milled physical mixture and this is due to the lack of contact between caffeine and urea particles in this sample (see SEM images discussed below).

Crystal Growth and Single Crystal X-ray Structure

Various solvents were investigated in order to obtain single crystals of the caffeine-urea co-crystal; methanol (MeOH), ethanol (EtOH), water, acetone (Ace), acetonitrile (Atrn), ethyl acetate (EtA), and isopropanol (IPA). Undersaturated solutions of caffeine and urea (1:1)

were prepared in duplicate. One sample set was left to evaporate unseeded using each solvent (xxxx_U, where xxxx is the solvent and U indicates the sample was unseeded). The other sample set was seeded using the co-crystal prepared by milling and left to slowly evaporate at room temperature (xxxx_S, with S indicating seeding).

Figure 5 shows the 3D PCA scores plot explaining 95 % of the information of the 3650 – 2825 cm^{-1}, 1755 – 1325 cm^{-1}, and 940 – 750 cm^{-1} ranges of the SNV pre-processed IR spectra of the evaporated samples. Caffeine, urea, and the co-crystal are well separated, with most solvents (seeded and unseeded) giving mixtures of the starting materials and the co-crystal. From unseeded acetone solutions only the co-crystal crystallized, while seeding gave mixtures of the co-crystal, urea and caffeine which crystallized in different regions of the evaporation dish. Both seeded and unseeded crystallization from acetonitrile gave the pure co-crystal. Hence acetonitrile was chosen as the solvent to produce single crystals for X-ray analysis *via* cooling crystallization.

Initial samples from cooling crystallization using acetonitrile produced thin, aggregated needles unsuitable for single crystal XRD. Because of this a slower cooling rate was used with temperature cycling at critical points of crystal growth where aggregation/agglomeration was expected. Cooling from 70 °C to 25 °C at a rate of ~0.3 °C h^{-1}, with an increase of 1 °C followed by a decrease of 2 °C between 60 and 55 °C enabled crystal growth to the size and quality where single crystal XRD was feasible.

The caffeine-urea cocrystals grew as needles extended along the c axis (Figure S6). In the crystal structure the caffeine molecules were disordered over two positions with 75 and 25 % occupancies. The principal component of the disorder is shown in Figure 6. The hydrogen bonding scheme has hydrogen bonded urea ladders running parallel to the *c* axis with the caffeine molecules hydrogen bonded to these chains, Figures 6b and 6c and Table S1. Adjacent chains along *b* have the hydrogen bonded caffeine molecules alternating in the vdW contact stacks along *c*. The crystal structure is well packed with a 69 % packing coefficient and the caffeine molecule stacking along *c* has 80 % of the atoms in each molecule in vdW contact with their stack neighbours. This vdW contact stacking is probably the reason for the needle growth along *c*.[61] Figure S7 compares the experimental PXRD patterns of the co-crystals formed spontaneously during storage of the milled mixture, the co-crystal isolated from solution and the theoretical pattern calculated from the single crystal data. All peaks align within 0.25° (2θ). The PXRD pattern of the sample crystallized from solution shows

preferred orientation effects. It would be interesting to see whether the co-crystals that were obtained by solid-state transformation of milled or low-energy mixed samples have the same disorder as the crystals formed in solution. However, the calculated PXRD patterns for the major and the minor component of the single-crystal structure are identical without any significant differences in the relative intensities (Figure S8) so that the experimental diffractograms of the mechanochemically prepared samples cannot give further insight into their disorder.

Raman Microscopy and SEM

Raman microscopy was performed on multiple regions of several crystals obtained by milling and storage. The spectra were compared with those of caffeine, urea, and the co-crystal produced by cooling crystallization (Figure 7). Raman spectra taken throughout the length of multiple crystals of the co-crystal have consistently peaks specific to the co-crystal at 1710.8, 505.3, and 108.6 cm^{-1}.

SEM micrographs of the as-received and milled urea and caffeine samples confirmed that milling results in a reduction in the primary particle size as shown in Figure 8 (additional micrographs are included in the Supporting Information, Figure S9). Before milling the urea particles show no distinct particulate features and vary in the length of their longest axis between 200 and 800 μm, whereas particles of milled urea show a rough corrugated surface and are agglomerated particles of approximately 2 μm. The reduction in the primary particle size of the milled urea may explain why PXRD indicates a reduction in the relative crystallinity of the milled urea whereas no significant differences are observed in the IR spectra before and after milling. A comparison of the micrographs of unmilled and milled caffeine shows that the aggregated needle like caffeine particles of 5 – 30 μm size (Figure 8c) break up into aggregates of about half the size with thinner needles with higher aspect ratios (Figure 8d).

SEM micrographs of the freshly milled caffeine and urea mixture (1:1) and of the mixture stored for one week allowing the transformation to the co-crystal are compared in Figures 8e and 8f. Immediately after milling the SEM images show larger particles (urea) engrained and covered by smaller and finer crystals (caffeine). These samples transformed to the co-crystal within hours. Aged samples had converted to needles of a fairly uniform size with a length of approximately 2 μm and an aspect ratio intermediary of that of the particles of the unmilled

and milled caffeine samples. It is proposed that the spontaneous co-crystal formation in the milled sample is due to surface effects induced by milling, as milled samples show direct contact of smaller caffeine particles spread over the surface of urea particles. By contrast, the SEM images of caffeine and urea milled separately and mixed using the low energy mixer revealed aggregates of caffeine needles assembled onto urea (Figure S10).

Effects of Surface Contact on Co-Crystallization

As described in the introduction, various mechanisms have been proposed for solid-state co-crystallization. One of the mechanisms proposed for the spontaneous transformation of a physical mixture to a co-crystal is vapour diffusion of the two solids. However, this seems unlikely in this case. Caffeine and urea have vapour pressures of 9.0×10^{-7} mm Hg and 1.2×10^{-5} mm Hg respectively,[62,63] while in most spontaneous solid-state reactions that take place *via* vapour diffusion at least one solid has a vapour pressure in the 10^{-1} - 10^{-4} mm Hg range. A eutectic melt-mediated mechanism can also be excluded, as the thermal analysis of a physical caffeine-urea mixture did not show any endothermic event below 132.7 °C. Grinding-induced defects and lattice distortions, *i.e*. mechanical activation, can play a role in milling-mediated solid-state transformations.[64] Mechanical activation by milling the two components individually was also described for carbamazepine co-crystals with nicotinamide and saccharin.[23] In addition, surface contact seems to be an important factor here as the effect on the rate of spontaneous co-crystallization is most prominent when caffeine and urea are milled together. These samples undergo the fastest transformation to the co-crystal on storage and the co-crystal formation can be observed in the DSC plot as an exothermic event at 91.3 °C. As seen in the SEM images immediately after milling, caffeine-urea samples contain small particles of caffeine ingrained onto urea particles giving tight particle-particle contact. Conversely, the transformation is slowest, when (un-milled) urea and caffeine are subjected to low energy mixing. This mixture has the largest particles and the least particle-particle surface contact. When caffeine and urea are pre-milled separately, the transformation rate under low energy mixing is increased compared to the un-milled physical mixture, as the larger surface-to-volume ratio leads to enhanced particle-particle surface contact during mixing. These observations are in agreement with the work by Ibrahim *et al*. who showed that the rate of spontaneous formation of the caffeine-malonic acid and urea-2-methoxybenzamide co-crystals during low-energy convection mixing increased with

decreasing particle size of the pre-milled components.[14] It was proposed that inter-particle contact and increase in contact areas are the main factors in the accelerated spontaneous co-crystal formation. In our case the different co-crystallization kinetics suggest that while the particle size matters in the spontaneous co-crystallization of caffeine and urea, tight particle-particle contact is more important in this system.

Conclusions

The formation of the 1:1 co-crystal of caffeine and urea readily occurs in the solid state. Little energy input is needed to transform a physical mixture of the two components to the co-crystal at room temperature and <30% RH. Milling caffeine and urea together greatly increases the rate of co-crystallization during storage and this can be attributed to enhanced inter-particle surface contact.

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Supporting Information

Hydrogen bonding interactions, additional XRPD patterns, IR spectra and SEM images.

References

(1) Cue, B. W.; Zhang, J. Green process chemistry in the pharmaceutical industry. *Green Chem. Lett. Rev.* **2009**, *2*, 193– 211.

(2) Anastas, P. T.; Warner, J. C. *Green Chemistry: Theory and Practice*. ed.; Oxford University Press Oxford: 2000; Vol. 30.

(3) Kaupp, G. Organic Solid-State Reactions. *Encyclopedia of Physical Organic Chemistry* **2016**, 1–80.

(4) Cheung, E. Y.; Kitchin, S. J.; Harris, K. D. M.; Imai, Y.; Tajima, N.; Kuroda, R. Direct Structure Determination of a Multicomponent Molecular Crystal Prepared by a Solid-State Grinding Procedure. *J. Am. Chem. Soc.* **2003,** *125*, 14658–14659.

(5) Jones, W.; Eddleston, M. D. Introductory Lecture: Mechanochemistry, a versatile synthesis strategy for new materials. *Faraday Discuss.* **2014**, *170*, 9– 34.

(6) Crawford, D. E.; Casaban, J. Recent Developments in Mechanochemical Materials Synthesis by Extrusion. *Adv. Mater.* **2016**, *28*, 5747–5754.

(7) Michalchuk, A. A. L.; Hope, K. S.; Kennedy, S. R.; Blanco, M. V.; Boldyreva, E. V.; Pulham, C. R. Ball-free mechanochemistry: in situ real-time monitoring of pharmaceutical co-crystal formation by resonant acoustic mixing. *Chem. Commun.* **2018**, *54*, 4033– 4036.

(8) Anderson, S. R.; Am Ende, D. J.; Salan, J. S.; Samuels, P. Preparation of an Energetic-Energetic Cocrystal using Resonant Acoustic Mixing. *Propellants, Explos., Pyrotech*. **2014**, *39*, 637–640.

(9) Am Ende, D. J.; Anderson, S. R.; Salan, J. S. Development and Scale-Up of Cocrystals Using Resonant Acoustic Mixing. *Org. Process Res. Dev*. **2014**, *18*, 331–341.

(10) Nagapudi, K.; Umanzor, E. Y.; Masui, C. High-throughput screening and scale-up of cocrystals using resonant acoustic mixing. *Int. J. Pharm*. **2017**, *521*, 337–345.

(11) Sarcevica, I.; Orola, L.; Belyakov, S.; Veidis, M. V. Spontaneous cocrystal hydrate formation in the solid state: crystal structure aspects and kinetics. *New J. Chem.* **2013**, *37*, 2978–2982.

(12) Ervasti, T.; Aaltonen, J.; Ketolainen, J. Theophylline–nicotinamide cocrystal formation in physical mixture during storage. *Int. J. Pharm.* **2015**, *486*, 121–130.

(13) Arora, K. K.; Tayade, N. G.; Suryanarayanan, R. Unintended Water Mediated Cocrystal Formation in Carbamazepine and Aspirin Tablets. *Mol. Pharm.* **2011**, *8*, 982–989.

(14) Ibrahim, A. Y.; Forbes, R. T.; Blagden, N. Spontaneous crystal growth of co-crystals: the contribution of particle size reduction and convection mixing of the co-formers. *CrystEngComm* **2011**, *13*, 1141–1152.

(15) Ling, A. R.; Baker, J. L. XCVI.—Halogen derivatives of quinone. Part III. Derivatives of quinhydrone. *J. Chem. Soc., Trans.* **1893,** *63*, 1314–1327.

(16) Kavanagh, O. N.; Croker, D. M.; Walker, G. M.; Zaworotko, M. J. Pharmaceutical Cocrystals: from Serendipity to Design to Application. *Drug Discov. Today* **2019**, *24*, 796–804.

(17) Kasten, G.; Grohganz, H.; Rades, T.; Lobmann, K. Development of a screening method for co-amorphous formulations of drugs and amino acids. *Eur. J. Pharm. Sci*. **2016**, *95*, 28–35.

(18) Walker, G.; Römann, P.; Poller, B.; Löbmann, K.; Grohganz, H.; Rooney, J. S.; Huff, G. S.; Smith, G. P. S.; Rades, T.; Gordon, K. C.; Strachan, C. J.; Fraser-Miller, S. J. Probing Pharmaceutical Mixtures during Milling: The Potency of Low-Frequency Raman Spectroscopy in Identifying Disorder. *Mol. Pharm.* **2017**, *14*, 4675–4684.

(19) Gniado, K.; MacFhionnghaile, P.; McArdle, P.; Erxleben, A. The natural bile acid surfactant sodium taurocholate (NaTC) as a coformer in coamorphous systems: Enhanced physical stability and dissolution behavior of coamorphous drug-NaTc systems. *Int. J. Pharm.* **2018**, *535*, 132–139.

(20) Vittal, J. J.; Quah, H. S. Engineering solid state structural transformations of metal complexes. *Coord. Chem. Rev.* **2017,** *342*, 1–18.

(21) Byrn, S. R.; Pfeiffer, R. R.; Stephenson, G.; Grant, D. J. W.; Gleason, W. B. Solid-State Pharmaceutical Chemistry. *Chem. Mater.* **1994,** *6*, 1148–1158.

(22) Bučar, D.-K.; Lancaster, R. W.; Bernstein, J. Disappearing Polymorphs Revisited. *Angew. Chem. Int. Ed.* **2015**, *54*, 6972–6993.

(23) Maheshwari, C.; Jayasankar, A.; Khan, N. A.; Amidon, G. E.; Rodríguez-Hornedo, N. Factors that influence the spontaneous formation of pharmaceutical cocrystals by simply mixing solid reactants. *CrystEngComm* **2009**, *11*, 493–500.

(24) Kaupp, G. Solid-state molecular syntheses: complete reactions without auxiliaries based on the new solid-state mechanism. *CrystEngComm* **2003**, *5*, 117–133.

(25) Rastogi, R. P.; Bassi, P. S.; Chadha, S. L. Kinetics of reaction between naphthalene and picric acid in the solid state. *J. Phys. Chem.* **1962**, *66*, 2707–2708.

(26) Rastogi, R. P.; Singh, N. B.; Singh, N. B. Chemistry of organic eutectics: II. The naphthalene-p-chloronitrobenzene eutectic system. *J. Cryst. Growth* **1977**, *37*, 329–333.

(27) Ross, S. D.; Kuntz, I. Molecular Compounds. II. Picric Acid-Naphthalene, Picric Acid-m-Dinitrobenzene and Picric Acid-1,3,5-Trinitrobenzene in Chloroform. *J. Am. Chem. Soc.* **1954**, *76*, 74–76.

(28) Rastogi, R. P.; Bassi, P. S.; Chadha, S. L. Mechanism of the reaction between hydrocarbons and picric acid in the solid state. *J. Phys. Chem.* **1963,** *67*, 2569–2573.

(29) Banerjee, A.; Brown, C. J. Picric acid-naphthalene 1/1 [pi] complex, $C_6H_3N_3O_7 \cdot C_{10}H_8$. A disordered structure. *Acta Crystallogr. C* **1985**, *41*, 82–84.

(30) Chadwick, K.; Davey, R.; Cross, W. How does grinding produce co-crystals? Insights from the case of benzophenone and diphenylamine. *CrystEngComm* **2007**, *9*, 732–734.

(31) MacFhionnghaile, P.; Hu, Y.; Gniado, K.; Curran, S.; McArdle, P.; Erxleben, A. Effects of Ball-Milling and Cryomilling on Sulfamerazine Polymorphs: A Quantitative Study. *J. Pharm. Sci.* **2014**, *103*, 1766–1778.

(32) Hu, Y.; MacFhionnghaile, P.; Caron, V.; Tajber, L.; Healy, A. M.; Erxleben, A.; McArdle, P. Formation, Physical Stability, and Quantification of Process-Induced Disorder in Cryomilled Samples of a Model Polymorphic Drug. *J. Pharm. Sci.* **2013**, *102*, 93–103.

(33) Caron, V.; Hu, Y.; Tajber, L.; Erxleben, A.; Corrigan, O. I.; McArdle, P.; Healy, A. M. Amorphous solid dispersions of sulfonamide/soluplus® and sulfonamide/PVP prepared by ball milling. *AAPS PharmSciTech* **2013**, *14*, 464–474.

(34) Healy, A. M.; Worku, Z. A.; Kumar, D.; Madi, A. M. Pharmaceutical solvates, hydrates and amorphous forms: A special emphasis on cocrystals. *Adv. Drug Deliv. Rev.* **2017**, *117*, 25–46.

(35) Tavagnacco, L.; Schnupf, U.; Mason, P. E.; Saboungi, M.-L.; Cesàro, A.; Brady, J. W. Molecular Dynamics Simulation Studies of Caffeine Aggregation in Aqueous Solution. *J. Phys. Chem. B* **2011**, *115*, 10957–10966.

(36) Harris, K. D. M. Meldola Lecture: understanding the properties of urea and thiourea inclusion compounds. *Chem. Soc. Rev*. **1997**, *26*, 279–289.

(37) Jyothi, K. L.; Gautam, R.; Swain, D.; Guru Row, T. N.; Lokanath, N. K. Cocrystals of gallic acid with urea and propionamide: supramolecular structures, Hirshfeld surface analysis, and DFT studies. *Cryst. Res. Technol*. **2019**, *54*, 1900016.

(38) Harkema, S.; ter Brake, J. H. M.; Helmholdt, R. B. Structure of urea-oxalic acid (1/1), $CH_4N_2O.C_2H_2O_4$, determined by neutron diffraction. *Acta Crystallogr. C* **1984**, *40*, 1733–1734.

(39) Colman, P. M.; Medlin, E. H. The crystal structure of urea parabanic acid. *Acta Crystallogr. B* **1970**, *26*, 1547– 1553.

(40) Emsley, J.; Reza, N. M.; Kuroda, R. J. Hydrogen bonding of urea-salicylic acid, U·SA. *Crystallogr. Spectrosc. Res*. **1986**, *16*, 57–69.

(41) Videnova Adrabinska, V.; Etter, M. C. J. Urea-glutaric acid (2∶1) structural aggregates as building blocks for crystal engineering. *Chem. Crystallogr*. **1995**, *25*, 823–829.

(42) Smith, G.; Baldry, K. E.; Byriel, K. A.; Kennard, C. H. L. Molecular cocrystals of carboxylic acids. XXV The utility of urea in structure making with carboxylic acids and the crystal structures of a set of six adducts with aromatic acids. *Aust. J. Chem*. **1997**, *50*, 727–736.

(43) Smith, G.; Kennard, C. H. L.; Byriel, K. A. The preparation and crystal structures of a series of urea adducts: with fumaric acid (2 : 1), with itaconic scid (1 : 1) and with cyanuric acid (1 : 1). *Aust. J. Chem.* **1997**, *50*, 1021–1025.

(44) Smith, G.; Baldry, K. E.; Kennard, C. H. L.; Byriel, K. A. The preparation and crystal structure of the 1 : 1 adduct of glycine with urea. *Aust. J. Chem*. **1997**, *50*, 737–739.

(45) Cruz-Cabeza, A. J.; Day, G. M.; Jones, W. Towards prediction of stoichiometry in crystalline multicomponent complexes. *Chem.—Eur. J.* **2008**, *14*, 8830–8836.

(46) Chadwick, K.; Davey, R.; Sadiq, G.; Cross, W.; Pritchard, R. The utility of a ternary phase diagram in the discovery of new co-crystal forms. *CrystEngComm* **2009**, *11*, 412–414.

(47) Cysewski, P.; Przybylek, M.; Ziolkowska, D.; Mrocynska, K. Exploring the cocrystallization potential of urea and benzamide. *J. Mol. Model* **2016**, *22*, 103–103.

(48) Zhou, Y.; Guo, F.; Hughes, C. E.; Browne, D. L.; Peskett, T. R.; Harris, K. D. M. Discovery of new metastable polymorphs in a family of urea co-crystals by solid-state mechanochemistry. *Cryst. Growth Des*. **2015**, *15*, 2901–2907.

(49) Lee, S.-O.; Harris, K. D. M.; Kariuki, B. M. Hydrogen-bonded chains of α,ω-diaminoalkane and α,ω-dihydroxyalkane guest molecules lead to disrupted tunnel structures in urea inclusion compounds. *New J. Chem*. **2005**, *29*, 1266–1271.

(50) Martí-Rujas, J.; Kariuki, B. M.; Hughes, C. E.; Morte-Ródenas, A.; Guo, F.; Glavcheva-Laleva, Z.; Taştemür, K.; Ooi, L.-L.; Yeo, L.; Harris, K. D. M. Structural diversity, but no polymorphism, in a homologous family of co-crystals of urea and α,ω-dihydroxyalkanes. *New J. Chem*. **2011**, *35*, 1515–1521.

(51) Pickering, M.; Small, R. W. H. Structure of the 1:1 complex of resorcinol and urea. *Acta Crystallogr. B* **1982**, *38*, 3161– 3163.

(52) Snyder, R. L.; Rosenstein, R. D. The crystal and molecular structure of the 1:1 hydrogen bond complex between [alpha]-D-glucose and urea. *Acta Crystallogr*. *B* **1971**, *27*, 1969–1975.

(53) Honer, K.; Pico, C.; Baltrusaitis, J. Reactive mechanosynthesis of urea ionic cocrystal fertilizer materials from abundant low solubility magnesium- and calcium-containing minerals. *ACS Sustainable Chem. Eng*. **2018**, *6*, 4680–4687.

(54) Casali, L.; Mazzei, L.; Shemchuk, O.; Honer, K.; Grepioni, F.; Ciurli, S.; Braga, D.; Baltrusaitis, J. Smart urea ionic co-crystals with enhanced urease inhibition activity for improved nitrogen cycle management. *Chem. Commun.* **2018**, *54*, 7637–7640.

(55) Sheldrick, G. M. SHELXT - Integrated space-group and crystal-structure determination. *Acta Cryst*. **2015**, *A71*, 3–8.

(56) Sheldrick, G. M. Crystal structure refinement with SHELXL. *Acta Cryst*. **2015**, *C71*, 3–8.

(57) McArdle, P. Oscail, a program package for small-molecule single-crystal crystallography with crystal morphology prediction and molecular modelling. *J. Appl. Crystallogr*. **2017**, *50*, 320–326.

(58) Civati, F.; Erxleben, A.; Kellehan, S.; McArdle, P. Conversion of gel-forming crystal needles to easily processable more equant crystals using high-shear-ultra-low-attrition

agitation: Accelerated Ostwald ripening without crystal attrition. *Cryst. Growth Des*. **2019**, *19*, 1502–1504.

(59) Chikhalia, V.; Forbes, R. T.; Storey, R. A.; Ticehurst, M. The effect of crystal morphology and mill type on milling induced crystal disorder. *Eur. J. Pharm. Sci.* **2006**, *27*, 19–26.

(60) Tothadi, S. Polymorphism in cocrystals of urea:4,4′-bipyridine and salicylic acid:4,4′-bipyridine. *CrystEngComm* **2014**, *16*, 7587–7597.

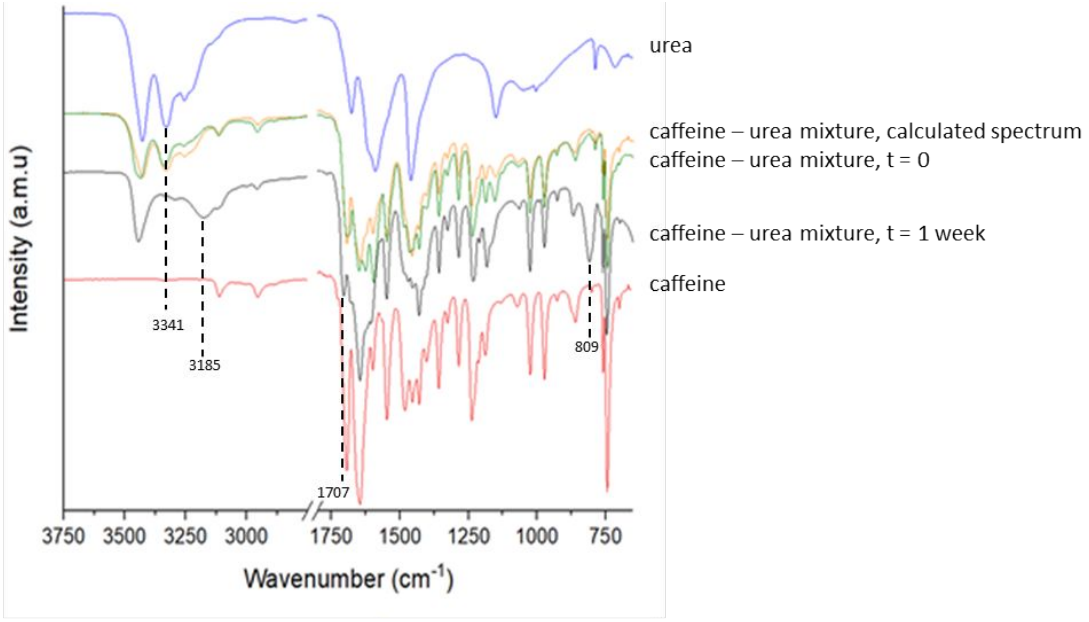
(61) Walshe, N.; Crushell, M.; Karpinska, J.; Erxleben, A.; McArdle, P. Anisotropic crystal growth in flat and non-flat systems: The important influence of vdW contact molecular stacking on crystal growth and dissolution. *Cryst. Growth Des.,* **2015**, *15*, 3235–3248.

(62) Jones, A. H. Sublimation Pressure Data for Organic Compounds. *J. Chem. Eng. Data* **1960**, *5*, 196–200.

(63) Emel'yanenko, V. N.; Verevkin, S. P. Thermodynamic properties of caffeine: Reconciliation of available experimental data. *J. Chem. Thermodyn.* **2008,** *40*, 1661–1665.

(64) De Gusseme, A.; Neves, C.; Willart, J. F.; Rameau, A.; Descamps, M. Ordering and disordering of molecular solids upon mechanical milling: the case of fananserine. *J. Pharm. Sci.* **2008**, *97*, 5000–5012.

(a)



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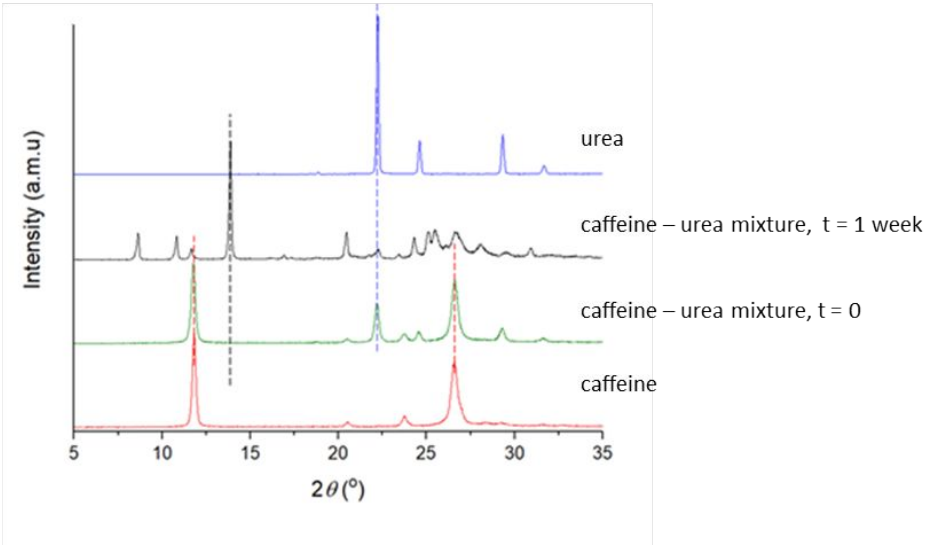


Figure 1. (a) IR spectra and (b) PXRD patterns of urea (blue), caffeine (red), a physical 1:1 mixture of urea and caffeine immediately after milling (green), a physical 1:1 mixture of urea and caffeine after milling and storage for one week (black) and the calculated IR spectrum of a physical 1:1 mixture of urea and caffeine (yellow).

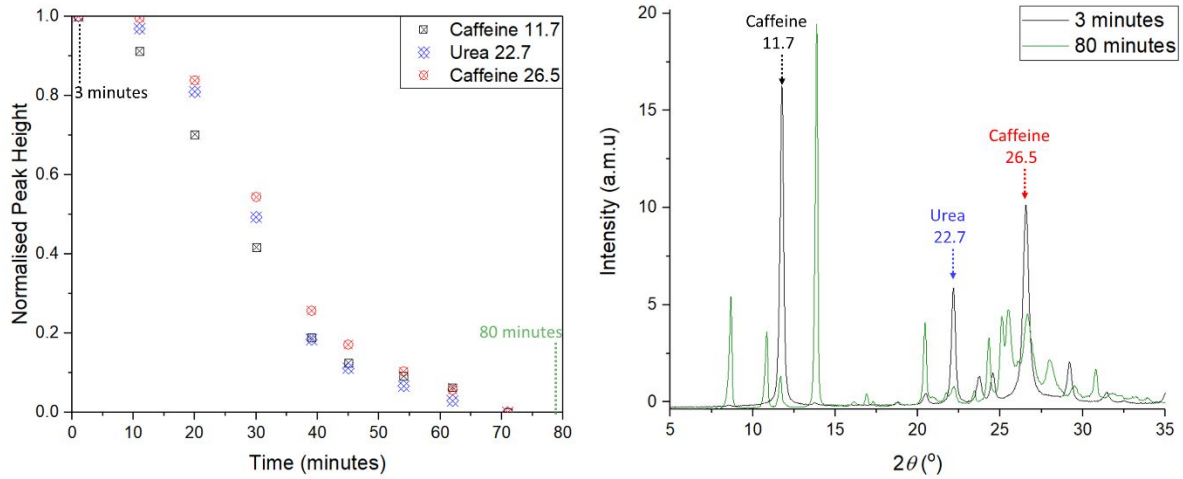


Figure 2. Transformation of caffeine and urea to the co-crystal during storage of milled 1:1 mixtures over time using PXRD. (a) Plot of the normalized peak heights of the caffeine peaks at 11.7 and 26.5 ° (2θ) and of the urea peak at 22.7 ° (2θ). (b) PXRD patterns after storage for 3 (black) and 80 (green) min.

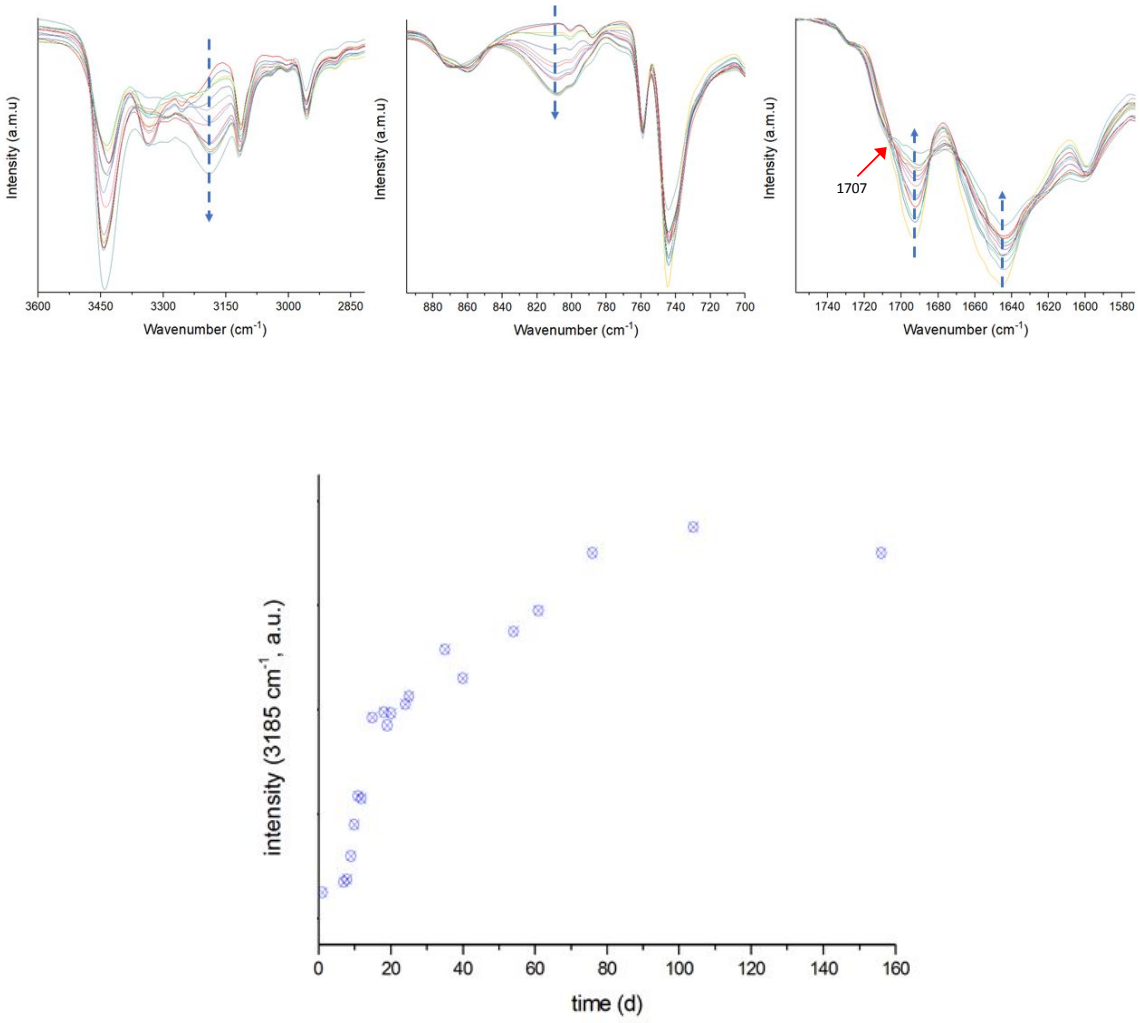


Figure 3. Time-dependent changes in the 2850 – 3600 cm^{-1}, 700 – 880 cm^{-1} and 1580 – 1740 cm^{-1} ranges of the IR spectrum of a 1:1 mixture of separately pre-milled caffeine and urea during low energy mixing over 23 weeks (top). Increase of the intensity of the co-crystal peak at 3185 cm^{-1} from SNV treated data (bottom).

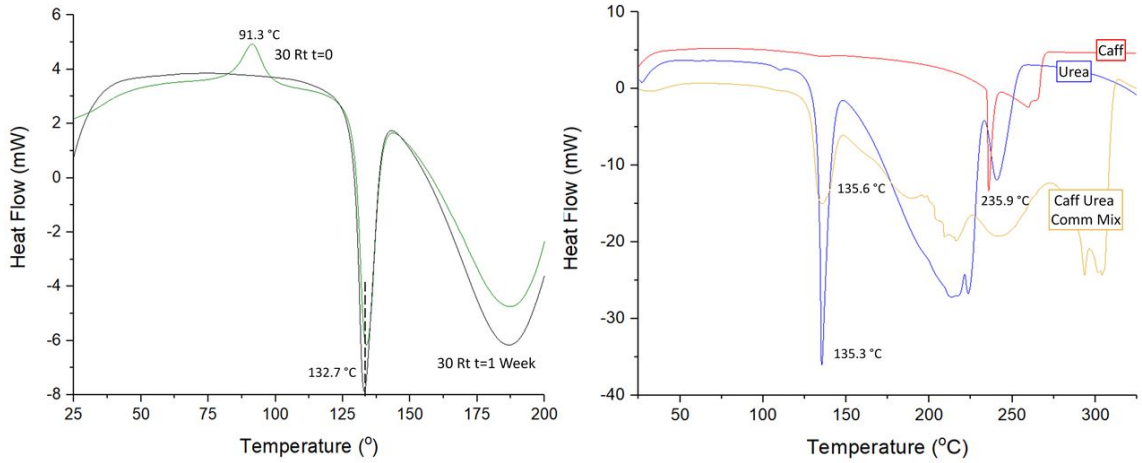


Figure 4. DSC plots of samples of caffeine (red), urea (blue), a 1:1 caffeine-urea mixture immediately after milling (green), a 1:1 caffeine-urea mixture after milling and storage for 24 h (black), and a physical mixture of (un-milled) caffeine and urea (yellow).

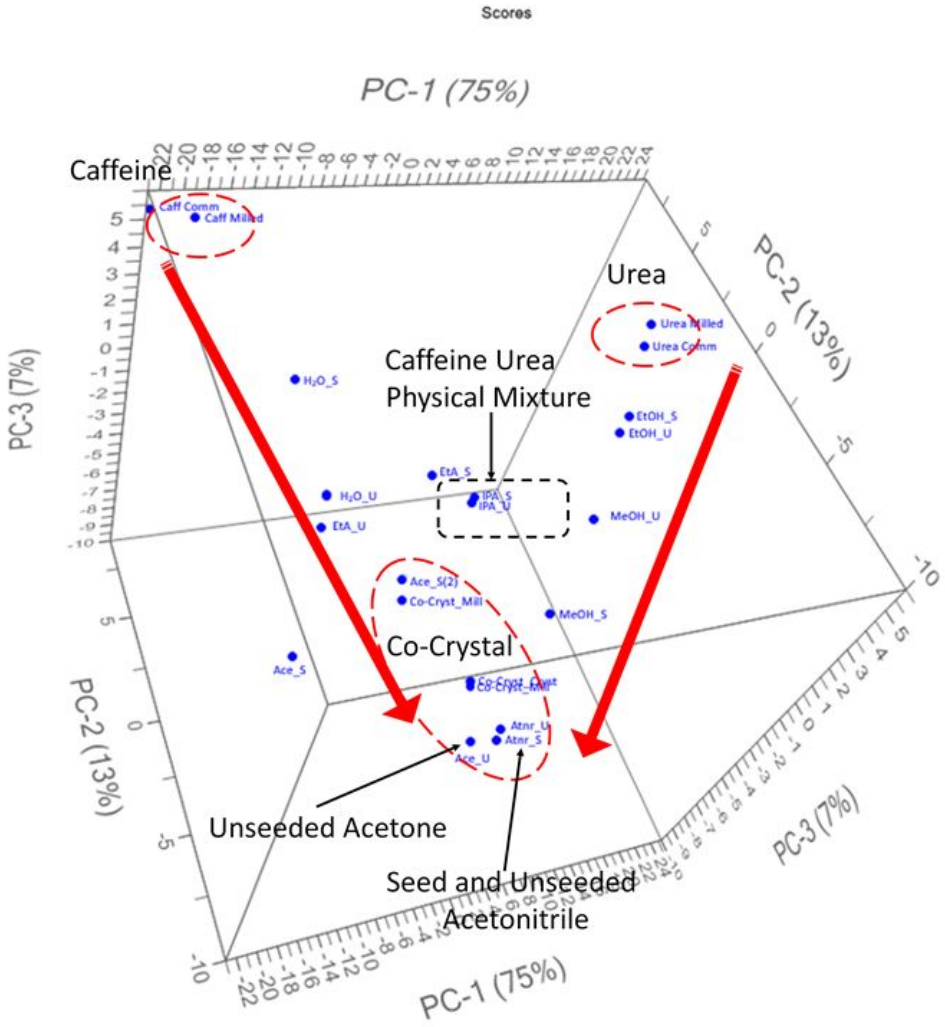
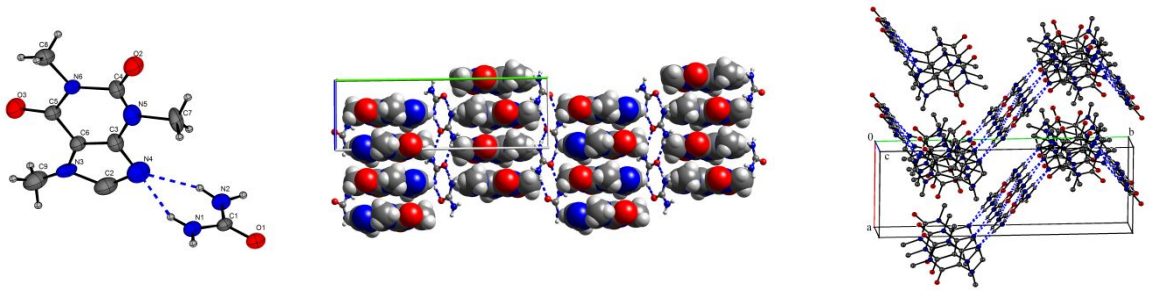


Figure 5. PCA scores plot of the IR spectra of caffeine-urea co-crystallization by solvent evaporation.



(a) (b) (c)

Figure 6. (a) Asymmetric unit of the caffeine-urea co-crystal with the principal component of the caffeine disorder shown. (b) Hydrogen bonded urea chains running parallel to *c* with hydrogen bonded caffeine molecules and (c) urea chains and caffeine stacking along the *c* direction (H atoms not involved in H-bonding not drawn for clarity).

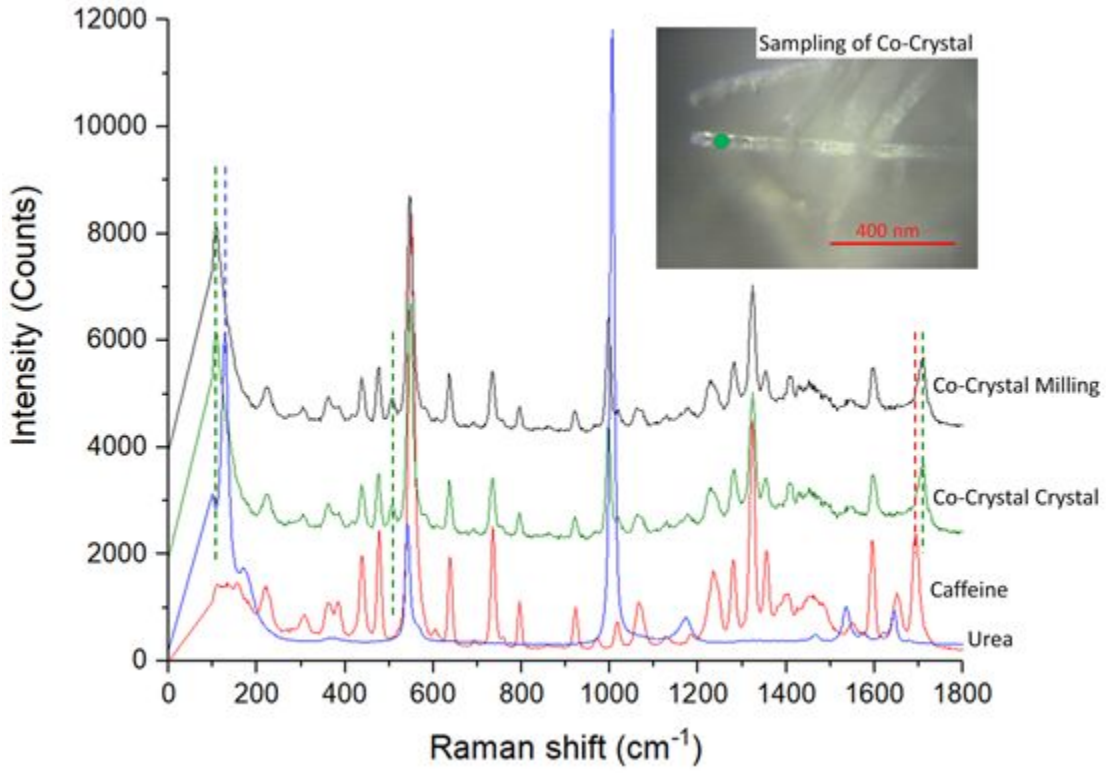


Figure 7. Raman microscopy spectrum of a single co-crystal (green), compared to spectra of caffeine (red) and urea (blue), and the spectrum of the co-crystal formed after milling and storage (black).

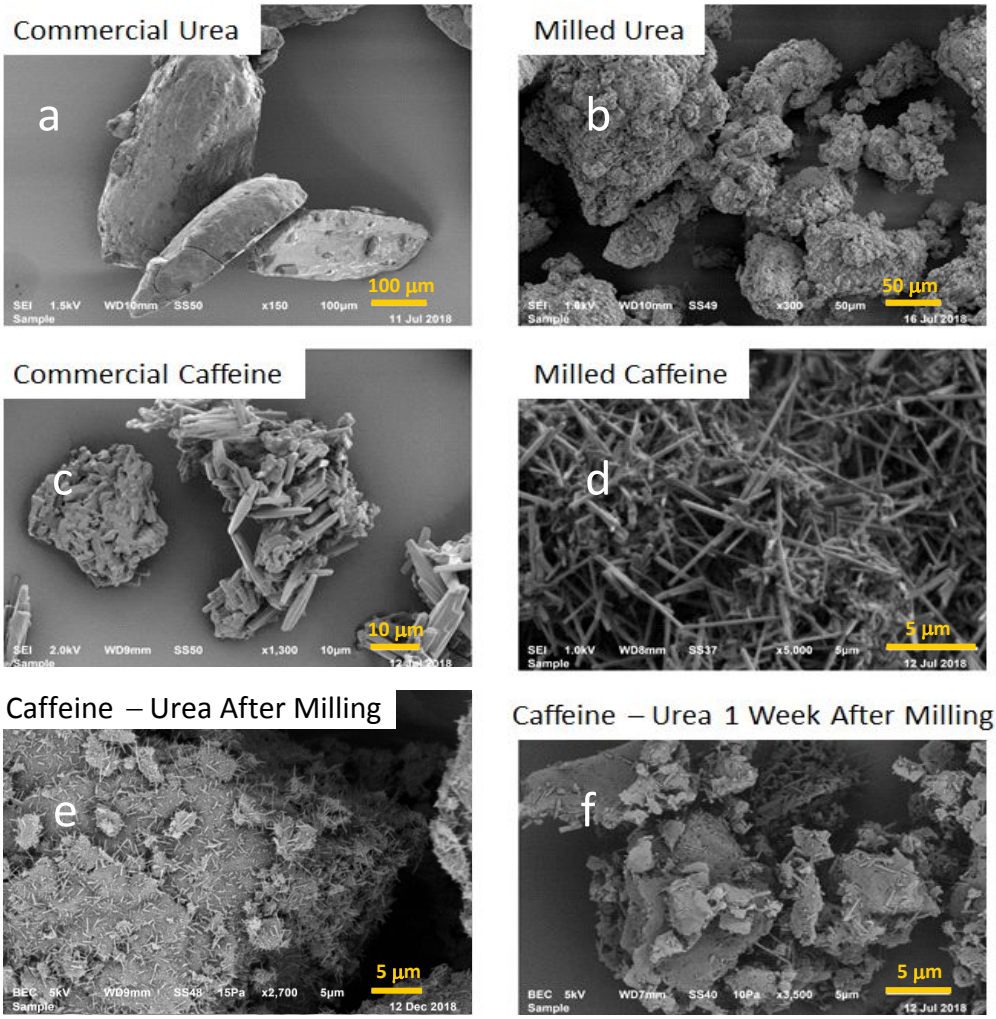


Figure 8. SEM images of urea and caffeine before and after milling (a-d) and milled 1:1 mixtures of caffeine and urea directly after milling (e) and after storage for one week at room temperature (f).

Table 1. Crystal data of the urea-caffeine cocrystal.

Formula	$C_9H_{13}N_6O_3$
M_r	253.25
Crystal colour and habit	colourless block
Crystal size (mm)	0.50 × 0.40 × 0.20
Crystal system	monoclinic
Space group	$P2_1/c$
Unit cell dimensions	
a [Å]	8.6741(8)
b [Å]	20.421(2)
c [Å]	7.1057(7)
β [°]	110.300(10)
V [Å^3]	1180.5(2)
Z	4
D_{calc} (g cm^{-3})	1.425
No. measd. reflections	4786
no. unique reflections (R_{int})	2628 (0.0165)
No. obs. reflections	1600
Final R_1, wR_2 (obs. refl.)	0.1021, 0.2825
Goodness-of-fit (obs. refl.)	1.094

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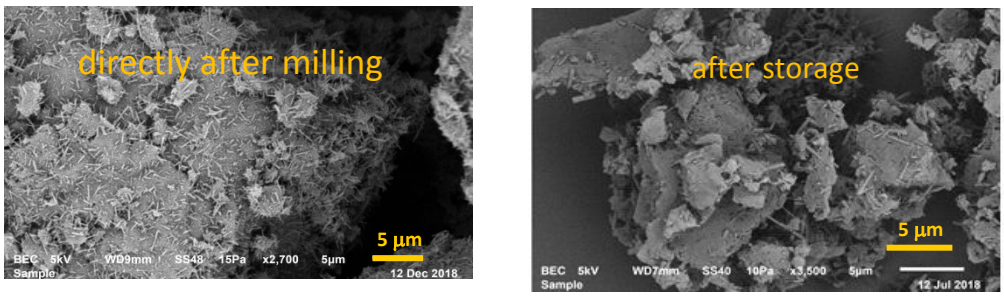
Spontaneous Solid-State Co-Crystallization of Caffeine and Urea

Pól MacFhionnghaile, Clare M. Crowley, Patrick McArdle,* and Andrea Erxleben*



Enhanced inter-particle surface contact in a milled physical mixture of caffeine and urea leads to the spontaneous transformation to the caffeine-urea cocrystal on storage at room temperature and <30 % RH.