

On the mechanochemical formation and “disappearance” of caffeine: citric-acid cocrystal polymorphs

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ABSTRACT

The inability to obtain a crystal form that has previously been reliably prepared is an important concern for industrial solid-state scientists. In the present study, we extend further the evidence that such a phenomenon also applies to cocrystals. Specifically, we report further on the apparent “disappearing polymorphs” of a cocrystal composed of caffeine and citric acid. This study commenced at the University of Cambridge with the initial aim of gaining insight into the mechanisms associated with the mechanochemical formation of the known caffeine: citric-acid cocrystal, namely Form I. A second polymorph, Form II, was soon after prepared mechanochemically through neat grinding, making it seemingly impossible to reproduce Form I in the same laboratory. Subsequent studies also resulted in the mechanochemical formation of another polymorph, Form III, for which no structural characterization has yet been possible. We therefore also focus on the understanding of the factors contributing to the inability to reproduce Form I after another polymorph has been obtained. Experiments that were extended to two other laboratories within different UK universities (De Montfort University Leicester and University College London) revealed that, depending on the “apparent” level of contamination of a specific laboratory, different polymorphs can be obtained, but only one of them at any period of time. The inability to predict, control and understand disappearing polymorphs remains a frustrating experience—even 25 years after Dunitz and Bernstein first stated that “*we are far from being able to present a theory of disappearing polymorphs*”. In this context, we reason that a first step forward would consist of reporting comprehensive and detailed accounts for every example of apparently disappearing polymorphs, with the hope of finding similarities that can subsequently result in a better understanding and possible rationalization of the puzzling phenomenon of disappearing polymorphs. We here report what we know so far of the intriguing caffeine: citric-acid cocrystal system.

1. INTRODUCTION

The synthesis of cocrystals, which in the simplest definition are solids that combine different molecular species in a fixed stoichiometric ratio within the same crystalline lattice is a growing area within organic solid-state chemistry and crystal engineering, and takes the formation of alternate crystal forms to a higher level of refinement and the versatility compared to single-component molecular solids.¹⁻³ Through the identification of reliable supramolecular synthetic strategies,⁴ cocrystallization technology offers numerous avenues for the design and preparation of new solid forms for practical uses in several areas,^{2,5} including pharmaceutical research and development, where cocrystallization has been shown to be successful in modifying relevant pharmacokinetic and mechanical properties of active pharmaceutical ingredients (API).⁶⁻¹¹

The continuing interest in cocrystals has also resulted in a significant improvement of solution- and solid-state-based preparative techniques,¹²⁻¹³ whereby mechanochemical methods have emerged as a particularly promising synthetic solid-state technique, mostly owing to their accessibility, “green” character and high efficiency. Mechanochemical methods also do not require detailed knowledge of the solubility values of the cocrystallizing molecules and allow the exploration of a larger crystallization space (including polymorphs and solids with varying stoichiometric ratios) through the modification of several experimental parameters.¹⁴⁻²¹

The mechanochemical synthesis of cocrystals²² is generally performed using two different methods, the first (and simplest) being neat grinding (NG), which refers to the grinding of two (or more) solids in the absence of any additives (*e.g.* liquids). The second is liquid-assisted grinding (LAG), in which the addition of small amounts of a liquid additive increases the reaction rates and alters the polymorphic outcome.²³⁻²⁵ We have recently also reported that not only the identity but also the amount of the liquid additive can affect the polymorphic outcome of the mechanochemical cocrystallization reaction.²⁶ Such important observations were later supported for a different cocrystal system,²⁷ for which a mechanistic explanation was proposed and subsequently extended.^{28,29} Three

additional methods (namely polymer-assisted grinding (POLAG)^{30,31}, ionic liquid–assisted grinding³² and seeding-assisted grinding (SEAG)³³), have also been successfully applied for the mechanochemical synthesis of different solid forms and polymorphism control.

While the potential of cocrystals³⁴ (for producing functional solids for several applications in materials science) and mechanochemistry³⁵ (as one of the most efficient techniques for obtaining a specific solid form) are obvious, they are also both likely to be susceptible to problems that are commonly encountered during cocrystallization attempts using more conventional preparative methods. For example, mechanochemical cocrystallization has been shown to be susceptible towards some of the less understood aspects of crystallization science, including the apparent impossibility of obtaining a cocrystal without seeding (although its formation is energetically favorable), as reported by Bučar *et al.*³⁶ in the “curious case of the caffeine-benzoic acid cocrystal”.

A further interesting aspect of crystallization science is the issue of disappearing polymorphs, being succinctly described by Dunitz and Bernstein as a scenario wherein “*we did the experiment last week and got this result, and now we cannot repeat it*”.³⁷ This frustrating situation, which results in the loss of control over the crystallization process is perhaps one of the most challenging and feared phenomena in the development of solid oral dosage forms.³⁸ A search within *Scopus*³⁹ of the phrase “disappearing polymorph” produced only 43 outputs, while the number increased to hundreds of thousands if the word “disappearing” was removed. This small fraction of reports of “disappearing polymorphs” may indeed reflect actual experience or (in our opinion) is likely to result from a reluctance to report additional examples, possibly owing to a lack of sufficient and convincing data. This phenomenon is an important issue that can have serious economical repercussions for a corporation, as seen in the well-known case of ritonavir (NORVIR®).⁴⁰ Other relevant examples of disappearing polymorphs in the pharmaceutical industry are summarized in the recent review entitled “Disappearing Polymorphs Revisited” by Bučar, Lancaster and Berstein.⁴¹

In the present study, we report that the phenomenon of disappearing polymorphs extends to cocrystals. Specifically, we report a detailed account of our experiences concerning the disappearing polymorphs of a 1:1 cocrystal composed of caffeine (CAFF) and citric acid (CA).⁴²

Our study started with the aim of gaining insight into the mechanochemical formation of the cocrystal of the 1:1 CAFF-CA cocrystal (also known as “caffeine citrate”), a multicomponent solid of pharmaceutical interest used in the treatment of infant apnea.⁴³ To do so, we performed at the University of Cambridge a series of LAG experiments using 18 different liquids, which we expected might allow us to understand the importance of the chemical nature and amount of the liquid on the formation of the original CAFF-CA form, namely Form I.⁴⁴ Our results, obtained in the period November 2014 – January 2015, did indeed allow us to explore the effects of different liquids on the preparation of Form I of the cocrystal. In June 2017, having become aware the existence of a second polymorph of the cocrystal (Form II, reported by Hagan and Smit⁴⁵) and a suggestion of a third polymorph (Form III, proposed by Mukaida *et al.*⁴²), we set out to determine why initial mechanochemical methods, applied in the Cambridge laboratory in 2007,⁴⁴ yielded only Form I. To our surprise, we now observed Form II in the same laboratory under the same reaction conditions. The second part of the study reported herein was therefore focussed on providing an account of the “disappearance” of Form I that is as detailed as possible. And for this purpose, we also provide further historical background relating to the discussed CAFF-CA cocrystal system.

In 2007, in the first report of a cocrystal of CAFF and CA, Karki *et al.*⁴⁴ reported that NG for 20 min at 30 Hz of anhydrous CAFF and CA produced only a solid physical mixture of the reactants. The same outcome was obtained during neat grinding of anhydrous CAFF with CA hydrate (using the same experimental conditions). Interestingly, NG (again, for 20 min of milling at 30 Hz) of CAFF hydrate with anhydrous CA provided a CAFF-CA cocrystal product, which was confirmed by a comparison of the obtained powder pattern with a simulated one that was derived from single crystal data (gained from a solution-grown crystal). The same cocrystal form was obtained by Karki *et al.*⁴⁴ with anhydrous CAFF and anhydrous CA in LAG experiments involving water as liquid additive.

This form was, in the light of later work (see below) designated Form I. Despite numerous experiments, this was the only cocrystal form obtained mechanochemically at that time.

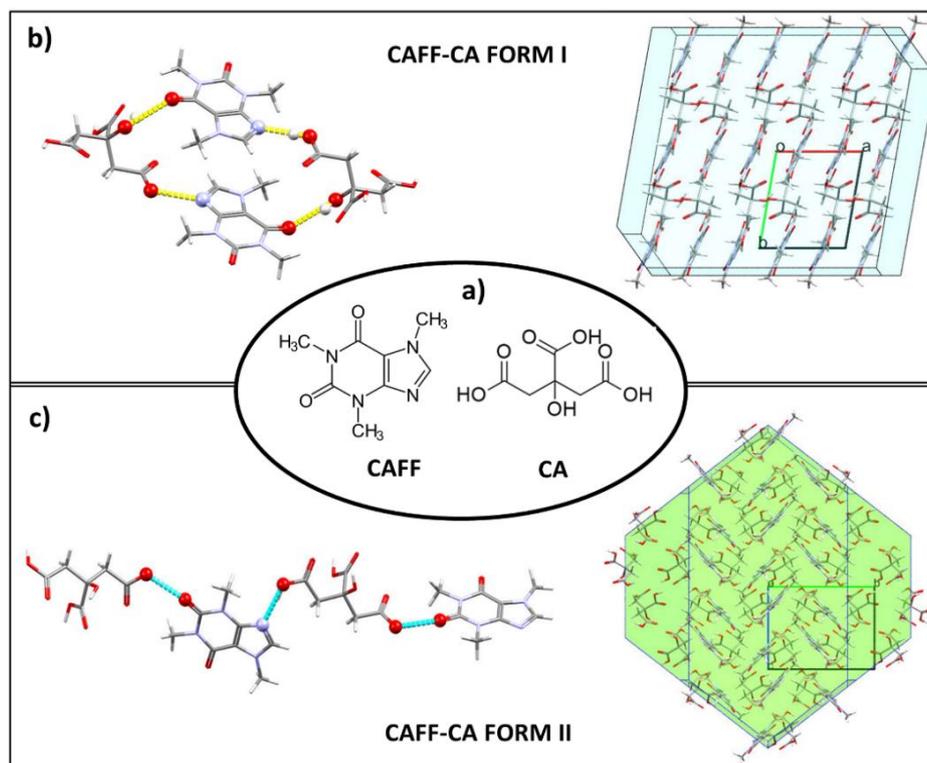


Figure 1. Representations of a) the molecular structures of CAFF and CA, b) the hydrogen-bonded ring existing in Form I (CSD reference code KIGKER⁴⁴) c) the hydrogen bonding between CAFF and CA in the cocrystal Form II (CSD reference code KIGKER01⁴⁵) The BFDH morphologies for both polymorphs were simulated using the CCDC Mercury program.⁴⁶

In 2013, in an attempt to use mechanochemical methods to produce co-amorphous compositions of CAFF and CA in Cambridge, Patel⁴⁷ reported that NG of a 1:1 mixture of anhydrous CAFF and anhydrous CA, a co-amorphous product could be obtained. This amorphous material converted over a period of time into a crystalline solid, which resulted in a diffractogram that was different to that of Form I. Despite numerous attempts, it was not possible to determine the crystal structure of the product from the powder pattern, and thereby unambiguously confirm it was a new polymorph of the cocrystal. This new form, as will be explained below, was subsequently referred to as Form III. Its crystal structure remains unknown.

In 2015, Smit and Hagen reported the single-crystal structure of a new polymorph of the CAFF-CA cocrystal, designated Form II (obtained from solution crystallization experiments).⁴⁵ The authors did not report any attempts to produce this polymorph mechanochemically.

In terms of their crystal structure, Form I exhibits a triclinic unit cell ($a = 7.387 \text{ \AA}$, $b = 8.397 \text{ \AA}$, $c = 13.505 \text{ \AA}$, $\alpha = 91.33^\circ$, $\beta = 99.04^\circ$ and $\gamma = 99.59^\circ$) in the space group $P\bar{1}$, while Form II crystallizes in the monoclinic space group $P2_1/c$ with the following unit cell parameters: $a = 13.778 \text{ \AA}$, $b = 12.315 \text{ \AA}$, $c = 9.659 \text{ \AA}$, $\beta = 92.85^\circ$. In the three dimensional hydrogen-bonding network of CAFF-CA Form I, CAFF and CA interact *via* O–H \cdots N hydrogen bonds between the carboxylic groups of CA and imidazole nitrogen atoms of CAFF, in addition to O–H \cdots O hydrogen bonds between the alcohol and keto functional groups of CA and CAFF, forming centrosymmetric four-component cyclic assemblies (Figure 1).⁴⁴ The hydrogen-bonding network in cocrystal Form II is different and is based on homomeric O–H \cdots O hydrogen bonds, which leads to the formation of infinite zig-zag chains of CA, while the O–H \cdots N hydrogen bonds between the carboxylic group of CA and imidazole nitrogen atom of CAFF do not engage in the formation of a cyclic structure (Figure 1).⁴⁵

Also in 2015, Mukaida and co-authors⁴⁸ published a study where CAFF-CA cocrystal was prepared following a procedure similar to that of Karki *et al.*⁴⁴ and reported that the product was identical to the one reported by Karki *et al.* In a paper published at a later date in 2015,⁴² the same authors acknowledged that the initial product was not actually Form I (nor Form II), but a new crystal form. The authors referred to this product as Form III. Interestingly, the diffractogram of this new form corresponded well to that of the solid obtained by Patel in 2013 (see Figure S1 in the Supplementary Information (SI) document). Notably, Mukaida *et al.* also reported that co-grinding of CAFF and CA yielded cocrystal Form III in several initial batches, whereas after Form I was unexpectedly prepared on one occasion, Form III became unobtainable—in two separate laboratories. Mukaida *et al.*, therefore, appeared initially able to produce Form III and subsequently only Form I by LAG. No explanation is given as to why Form I suddenly appeared.

In their paper, however, Mukaida *et al.* further reported that once they had obtained another form by slurry crystallization methods, namely Form II, then Form I became unobtainable.⁴⁸ The authors astutely referred to the system as a potential example of disappearing polymorphs. Having wisely preserved samples of each polymorph, they were able to determine the order of stability of the three forms, using competitive crystallization experiments and thermal measurements, as Form II > Form I > Form III.

In a separate detailed LAG study (also reported in 2015) another group of researchers (Shimono *et al.*⁴⁹) reported on the kinetics of CAFF-CA cocrystal formation by LAG and appear, despite numerous experiments, to have obtained only Form I. In 2016, Kerr *et al.*⁵⁰ obtained a new cocrystal hydrate in 1:2 molar ratio by grinding anhydrous CAFF and CA monohydrate using a mortar and pestle. Interestingly, the authors reported that NG of anhydrous CAFF and CA monohydrate at a temperature of 20 °C produced the new 1:2 CAFF-CA cocrystal hydrate, while the same process performed in a warmer environment (at about 30 °C) yielded the anhydrous 1:1 cocrystal Form I.

It is therefore evident that all three cocrystal polymorphs can be obtained mechanochemically although, as we will discuss, the precise requirements for their formation are unclear.

2. EXPERIMENTAL SECTION

2.1. Materials.

Anhydrous CAFF (99.5%) and anhydrous CA (95%) were used as starting materials for all the experiments performed in this study and were purchased from *Sigma-Aldrich* for the experiments performed at the University of Cambridge and De Montfort University Leicester, while for the experiments performed at the University College London CAFF was purchased from *Alfa Aesar* (99%), and CA from *Sigma-Aldrich*. All the liquids used during LAG experiments were purchased from *Sigma-Aldrich* ($\geq 98\%$), and used without further purification. The starting materials were

purchased separately for each research laboratory, and there was no exchange of such chemicals among the laboratories.

2.2. Grinding experiments performed at the University of Cambridge and De Montfort University Leicester.

In 2013 Patel undertook grinding experiments using a *Fritsch Pulverisette*.⁴⁷ At this time Cambridge was collaborating with the University of Lille in the formation of amorphous solids by milling. Patel's work there was the only exposure of the Lille laboratory to the combination of CAFF and CA. Samples were ground in zirconia jars using zirconia balls. The grinding experiments were conducted at room temperature or $-8.5\text{ }^{\circ}\text{C}$. The resulting material was amorphous, as determined by powder X-ray diffraction, and has converted to Form III upon heating.

In the period November 2014 – July 2019, all grinding experiments were performed using a *Retsch MM200* ball mill. In the case of NG, 150 mg of physical mixtures composed of equimolar amounts of CAFF and CA (75.4 mg of CAFF and 74.6 mg of CA) were ground at 10, 25 or 30 Hz in a 15 mL steel grinding jar containing two grinding balls ($\varnothing = 7\text{ mm}$). The shortest grinding time used was 20 min, while the longest was 180 min. In the case of milling experiments lasting longer than 60 minutes, a 30 min break for every 60 min of grinding was introduced to avoid a significant temperature increase within the milling jars.

All LAG experiments were performed in a similar manner, but with the addition of different amount of liquids, starting from 20 μL ($\eta = 0.13\text{ }\mu\text{L mg}^{-1}$)²⁴ to 150 μL ($\eta = 1.00\text{ }\mu\text{L mg}^{-1}$)²⁴ of a specific liquid. In the case of seeding experiments (performed prior to the introduction of Form II in the laboratory), different amounts of cocrystal seed (0.75 mg, 1.5 mg, 10.5 mg and 15 mg respectively corresponding to 0.5, 1, 7 and 10 weight percent of the amount of reactants) were added to 150 mg of physical mixtures composed of equimolar amounts of CAFF and CA. The cocrystal seeds of Form I were prepared by LAG with methanol and were used dry (approximately 45 min after their preparation). In the case of LAG experiments using pre-formed cocrystals, approximately 200 mg of

Form I were processed for 20 min at 30 Hz in the presence of 40 μL ($\eta = 0.27 \mu\text{L mg}^{-1}$) of specific liquids.

2.3. Grinding experiments performed at the University College London.

The CAFF-CA cocrystal was prepared by LAG using a *Form-Tech Scientific* FTS1000 shaker mill. In a typical milling experiment, a physical mixture of 75.4 mg of CAFF and 74.6 mg of CA (*Aldrich*, 99.5%) was added to a 15 mL stainless steel milling jar along with 40 μL of a liquid additive ($\eta = 0.27 \mu\text{L mg}^{-1}$) and two 5 mm stainless steel milling balls. The shaker mill was operated at 30 Hz for 30 min.

2.4. Powder X-ray diffraction (PXRD) measurements performed on the samples obtained at the University of Cambridge and De Montfort University Leicester).

PXRD experiments were performed using a *Philips PW3710 X'Pert* diffractometer equipped with an RTMS *X'celerator* detector, with Ni-filtered CuK_α radiation ($\lambda = 1.5406 \text{ \AA}$). A small amount of each sample (15–25 mg) was gently pressed on a glass slide. The data were collected in the 2θ range 3–50° at an ambient temperature of about 20°C.

2.5. PXRD measurements performed on the samples obtained at University College London.

All diffraction data was collected using a *Stoe StadiP* diffractometer in transmission geometry and a monochromatic CuK_α X-ray source ($\lambda = 1.54056 \text{ \AA}$, generated at 40 kV and 30 mA). The samples were placed into a 0.7 mm borosilicate capillary that was subsequently sealed. The sample was aligned with a small aperture collimator using the *Faceit (video) X.view* v2.14 software. All data were collected from the spinning capillary at room temperature (*ca.* 20°C) in the 2–60° 2θ range (in continuous mode: 0.5° step, 20 s/step).

3. RESULTS

Part A: Formation of cocrystal Form I

3.1. LAG experiments using different liquids performed at the University of Cambridge in the period November 2014 – January 2015

In November 2014 (*i.e.* before the publication of the papers by Mukaida *et al.*^{42,48}, Hagen and Smit⁴⁵ and Shimono *et al.*,⁴⁹), at the University of Cambridge, CAFF and CA were milled under NG conditions for 20 min at 30 Hz (see section 2.2 for additional experimental details) to reproducibly yield a mixture of the starting materials (Figure 3A), thus affirming the observation of Karki *et al.*⁴⁴ A series of subsequent LAG experiments involving 18 different liquid additives, using anhydrous CAFF and CA as reactants, showed that not all the liquids under these conditions were equally efficient in promoting the formation of the cocrystal, as evidenced by PXRD analyses. We report here (for the sake of completeness) the outcomes of these experiments that reproducibly gave rise to Form I, along with some conclusions drawn at that time.

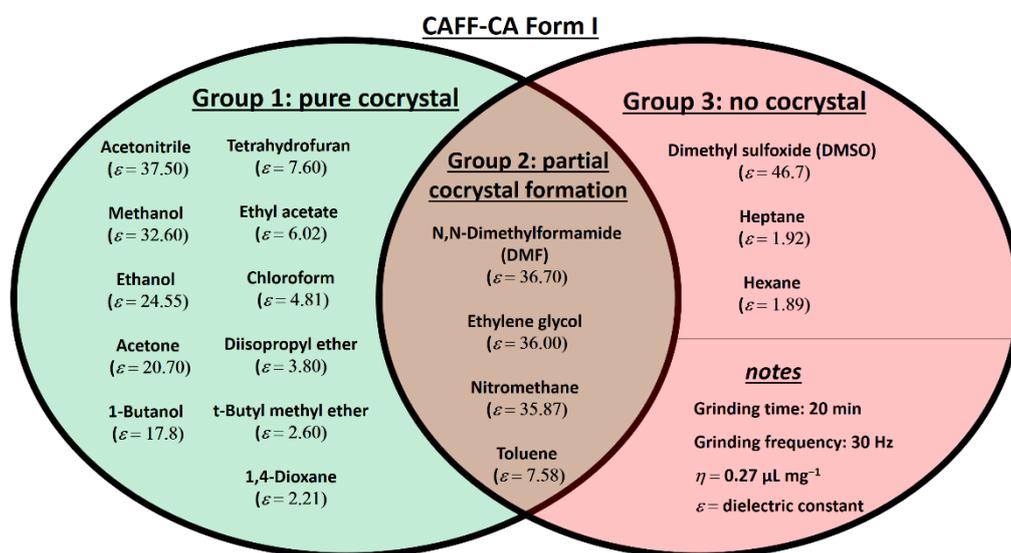


Figure 2. Summary of LAG experiments performed using different liquids, with Form I being the only polymorph observed. Liquids are grouped according to their effectiveness on promoting the formation of the CAFF-CA cocrystal Form I.

These experiments had three different outcomes (Figure 2). Specifically, LAG experiments ($\eta = 0.27 \mu\text{L mg}^{-1}$) with three of these liquids (namely, hexane, heptane and dimethyl sulfoxide; assigned to Group 3) provided solid products with PXRD patterns similar to those of the product obtained by NG, *i.e.* a physical mixture of reactants (Figure 3A). LAG experiments with another series of liquids (named Group 2)) produced a mixture of the cocrystal and starting materials (Figure 3B). The presence of unreacted starting materials in PXRD patterns of the products, obtained by the use of these Group 2 liquids with intermediate catalytic efficiency, was evidenced by the diffraction peak at a 2θ angle of circa 11.8° , which is typical of pure CAFF.⁵¹ It is also worth noting that the intensity of this peak varied with the use of different Group 2 liquids (*i.e.* dimethylformamide, ethylene glycol, nitromethane and toluene). This suggests that each Group 2 liquid facilitates the mechanochemical formation with different yields; hence a different catalytic efficacy. Out of the 18 liquids studied, a phase-pure cocrystal product was obtained in 11 cases using Group 1 liquids (Figure 2; see also the PXRD data shown in Figures S2-S4 in the SI document). An additional Group 1 liquid, namely water, also yielded the Form I cocrystal phase-pure under the same mechanochemical conditions as reported by Karki *et al.*⁴⁴ All PXRD patterns of cocrystals obtained through LAG using Group 1 and Group 2 liquids corresponded to Form I (Figures S2-S4, SI document) and, importantly, despite the range of utilized liquids and milling conditions, no other polymorph of the cocrystal was formed. The use of any of the Group 3 liquids did not result in the formation of a cocrystal.

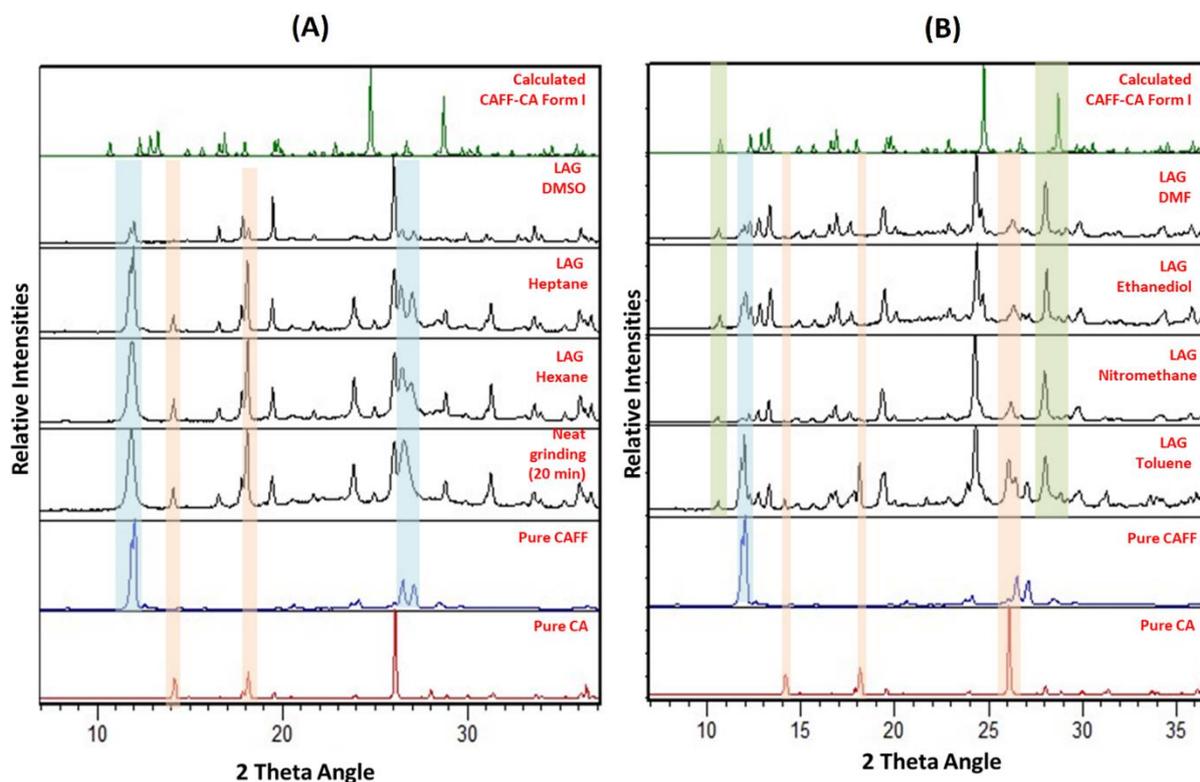


Figure 3. PXRD patterns of the solid products obtained by LAG ($\eta = 0.27 \mu\text{L mg}^{-1}$) with (A) Group 3 liquids and (B) Group 2 liquids (see Figure 2 for a summary of the liquid classification). The calculated pattern of cocrystal Form I² is included for reference. The shifting of the diffraction peaks attributable to cocrystal Form I for the measured PXRD patterns compared to the calculated pattern, is related to the fact that the crystal structure and therefore the calculated pattern corresponds to a temperature of 100 Kelvin, while the experimental PXRD patterns are collected at room temperature (ca 20°C, see Experimental Section 2.4). This is a normal observation for many crystalline structures. Orange, blue and green stripes highlight some diffraction peaks typical of pure CA, pure CAFF and Form I, respectively.

3.2. Seeding and variable-amount LAG mechanochemical experiments, performed at the University of Cambridge in the period November 2014 – January 2015, to possibly improve reaction yields in the case of Group 3 liquids

Seeding: In order to assess whether pre-existing seeds of Form I might enable cocrystallization under NG conditions and in LAG experiments involving Group 3 liquids, we performed a series of milling reactions involving the addition of varying quantities of pre-prepared cocrystal seeds of Form I (see Experimental Section 2.2 for full details). The results shown in Figure 4 suggest that NG for 20 min at 30 Hz of anhydrous CAFF and CA in the presence of 0.5 and 1 weight percent of cocrystal Form I seeds yielded only a physical mixture of the starting materials (Figure 4c and d, left). The same outcome was obtained by LAG ($\eta = 0.27 \mu\text{L mg}^{-1}$) with heptane, a Group 3 liquid (Figure 4c,

d, right). Additional experiments were conducted using higher amounts of cocrystal seeds (up to 10 weight percent, which is above the limit of detection in PXRD) (Figure 4e and f). No additional formation of cocrystals, to that obtained in the absence of seeds, was observed (the PXRD patterns obtained were similar to the previous ones, with the exception of some cocrystal traces that may be associated with the added seeds).

Variable amounts of liquid: Following the observation by Friščić *et al.*,²⁴ that the parameter η can have a significant influence on the rate of cocrystallization, we tried to promote cocrystallization using Group 3 liquids through the modification of the η parameter. We therefore varied the η parameter from 0.27 $\mu\text{L mg}^{-1}$ to 0.13 $\mu\text{L mg}^{-1}$ (and to 1 $\mu\text{L mg}^{-1}$ in the case of hexane). Each LAG experiment, however, yielded a physical mixture of the reactants (*i.e.* a result identical to that obtained from NG experiments, see Figure S5 in the SI document). Finally, three additional LAG experiments involving pre-prepared cocrystals of Form I in the presence of Group 3 liquids showed that Form I is stable in presence of these additives; that is, no phase transformation nor decomposition of the cocrystal into a physical mixture could be observed (Figure S6 in the SI document). In summary, all described experiments showed that only Form I was obtainable in the Cambridge laboratory during the period from November 2014 until January 2015.

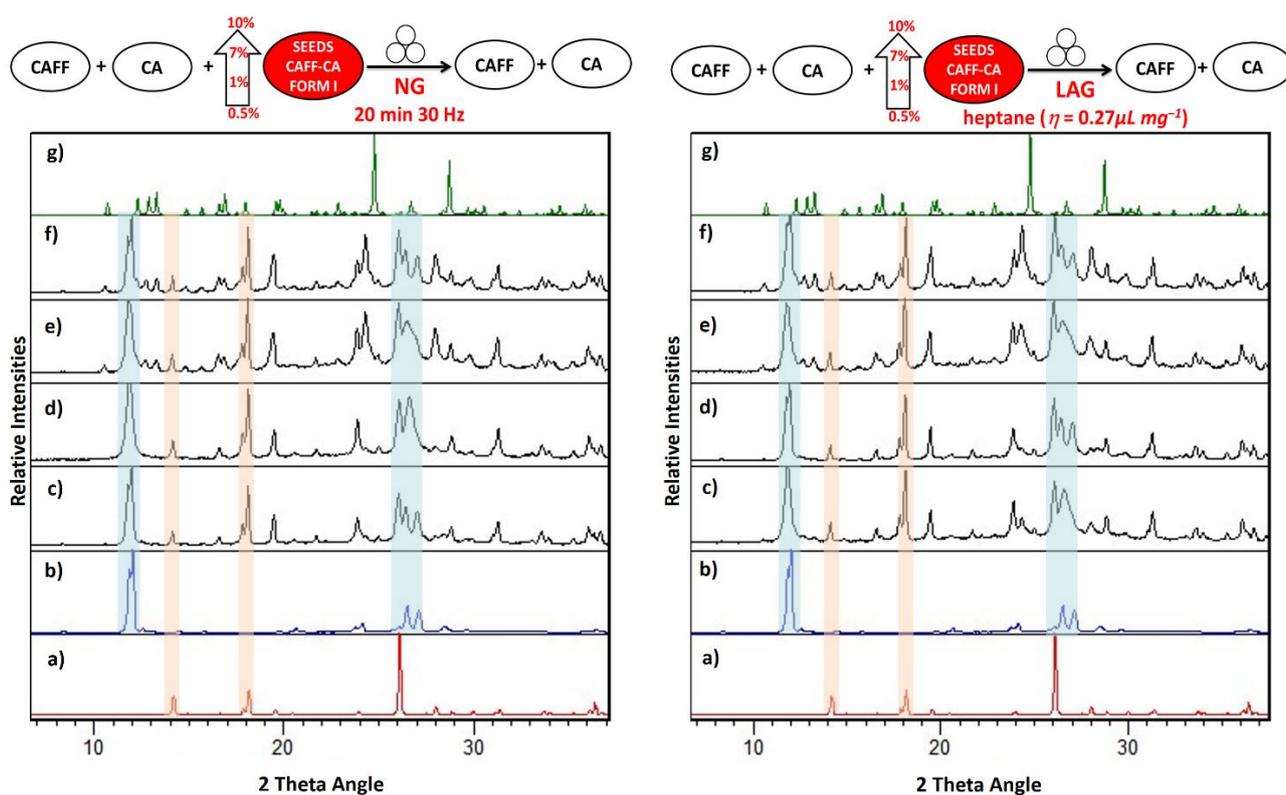


Figure 4. PXRD patterns of a) pure CA b) pure CAFF, c), d), e) and f) grinding experiments using 0.5, 1, 7 and 10 weight percent of crystal seeds of Form I (left under NG conditions, right by LAG with heptane), g) calculated cocrystal Form I. Orange and blue stripes highlight some diffraction peaks typical of pure CA and pure CAFF, respectively.

Part B: Appearance of Forms II and III

3.4. Introduction of Form II to the laboratory of the University of Cambridge in July 2017

All experiments reported in Part A of this report were performed at the Department of Chemistry of the University of Cambridge in the period from November 2014 to January 2015. In March 2015, and independent of our work, Smit and Hagen⁴⁵ had reported the existence of a new polymorph of CAFF-CA cocrystal (Form II) which was prepared by solution crystallization from a saturated chloroform/methanol solvent mixture (12:1 v:v). In September 2015 Mukaida *et al.*, again independent of our work, reported that Form II could also be prepared by slurring a mixture of CAFF and CA in water. In the same study, the authors also reported the existence of a possible third polymorph of CAFF-CA that could be prepared by LAG using chloroform as liquid additive. Mukaida

*et al.*⁴² were not able to determine the structure of this new form (Form III), similar to Patel in 2013 (although in the case of Patel's experiments, Form III was derived from an amorphous mixture of CAFF and CA). Notably, Mukaida *et al.* observed that Form III was repeatedly obtained using LAG until Form I *unexpectedly* emerged. Perhaps equally surprising was their next observation that once they had obtained samples of Form II from their slurry experiments in water, they were no longer able to obtain Form I or III mechanochemically.

In 2017, and now being aware of the work of Mukaida *et al.* and Hagan *et al.*—and motivated by the exclusive LAG formation of Form I in Cambridge—we focused in June of 2017 on a series of additional NG experiments with prolonged milling times of 30, 45, 60, 120 and 180 minutes in the hope to prepare mechanochemically for the first time Forms II and III in Cambridge. During these experiments, a 30 min break for every 60 min of grinding was introduced (to avoid any significant temperature increase⁵²) in the case of milling experiments lasting longer than 60 minutes, while the grinding frequency was kept at 25 Hz (see Experimental Section 2.2 for more details). As shown in Figure 5, Form II indeed appeared after 45 min of milling under NG conditions. Its formation reached near completion within 60 minutes, while a complete transformation was observed after 120 min of milling.

After the formation of Form II by NG, we repeated the series of LAG control experiments using various liquids belonging to all three groups of liquids (Figure 2) that previously (November 2014 – January 2015) facilitated the formation of Form I, as described above in Part A. Remarkably, the PXRD patterns of all the milling products for these LAG experiments now revealed the exclusive formation of Form II, rather than the previously observed Form I (Figure S13 in the SI document). Furthermore, we observed that all previously obtained samples of Form I, having been prepared and stored in capped vials since 2015, transformed into Form II within 72 hours after being exposed to the atmosphere of the laboratory in which Form II had now been produced. We draw the reader's attention to the fact that a similarly swift phase transformation was earlier reported by Bučar *et al.*³⁶.

Previously we had not observed this sensitivity of Form I to convert to Form II. It is also worth noting that, in terms of classification, there was no major difference in the Group classification of the liquids (described in Part A) for effectiveness for cocrystal formation—even though the polymorphic outcome was now Form II, rather than Form I (Table 1). (The only notable difference was for the case of LAG with heptane: while this liquid initially produced a physical mixture (Figure 3A), traces of Form II could be observed in the most recent experiment (Figure S13, SI document)).

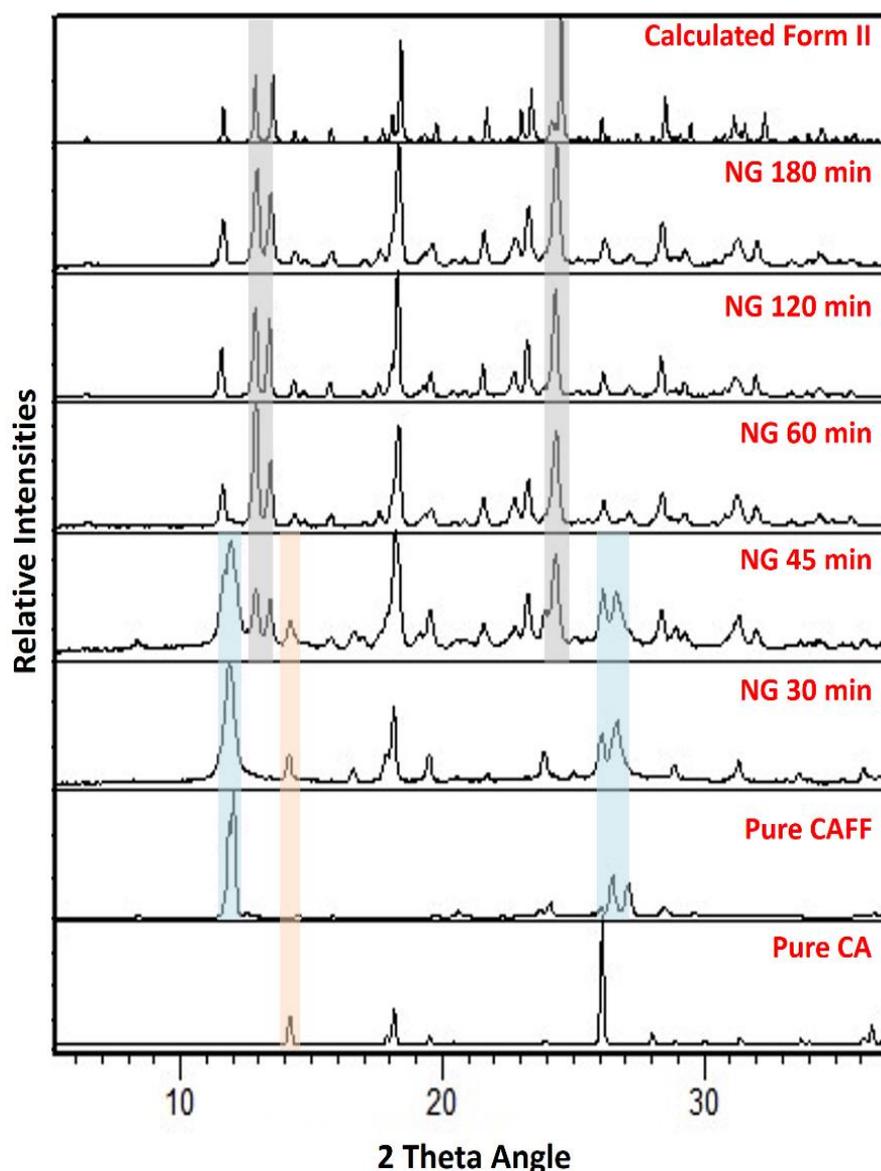


Figure 5. PXRD patterns of solid products obtained by NG of CAFF and CA at 25 Hz for different times (30 min – 180 min). The PXRD patterns of pure CAFF, pure CA and calculated pattern of Form II⁴³ are included for comparison. Orange, blue and grey stripes highlight some diffraction peaks typical of pure CAFF, pure CA and Form II, respectively.

Table 1: Classification of the liquid additives used for LAG reactions before and after the appearance of Form II.

Liquid (ϵ)	Group classification for cocrystal Form I (Figure 2)	Group classification for cocrystal Form II
Heptane (1.92)	3	2
Ethyl acetate (6.02)	1	1
Dichloromethane (8.93)	not assigned to any Group	2
Ethylene glycol (36.00)	2	2
N,N-Dimethylformamide (36.70)	2	2
Dimethyl sulfoxide (46.7)	3	3
Water (80.10)	1*	1

* From the study of Karki *et al.*³⁹

3.5. Experiments performed at De Montfort University Leicester in July 2017

In the same period (July 2017), we also performed similar grinding experiments at De Montfort University Leicester after one of the authors, DH, moved from the University of Cambridge to De Montfort University Leicester, prior to a six month stay in Italy during which no work on the CAFF-CA system was performed. In these experiments, CAFF and CA were milled under NG conditions (30 min at 30 Hz) and LAG conditions using ethyl acetate as liquid additive ($\eta = 0.27 \mu\text{L mg}^{-1}$). The products were brought to the Cambridge laboratory and analyzed by PXRD in Cambridge on the same day of preparation. The PXRD patterns of both products revealed the formation of Form III (Figure S15, SI document). These samples of Form III were again analyzed by PXRD (in Cambridge) after being left exposed to Cambridge laboratory atmosphere for 72 hours. Remarkably, the diffractograms revealed that all the samples of Form III had transformed to Form II (Figure S14, SI document).

3.6. Experiments performed at the University College London in the period September 2016 – July 2017

In an unrelated study commencing in 2015 at University College London (UCL), the synthesis of the CAFF-CA cocrystal was attempted *via* LAG with water as liquid additive ($\eta = 0.33 \mu\text{L mg}^{-1}$). The milling product was amorphous (and possibly similar to the co-amorphous product of Patel prepared in Cambridge in 2013). After discovery that the UCL material was amorphous it was immediately stored in a capped vial. Another analysis of this sample was attempted after 18 months (in 2017) to reveal that the sample had transformed into Form I. It is not known at what time the

amorphous material crystallized as Form I. The analysis of the aged sample prompted a further attempt to prepare the cocrystal by LAG (in the same UCL laboratory, using the same equipment, and ethyl acetate as liquid additive) that, again, yielded directly Form I (Figure S18, SI document).

3.7. Experiments performed at the University College London and De Montfort University Leicester in July 2019

More recently, in July 2019, the UCL group attempted the synthesis of the cocrystal once again under identical conditions by NG and LAG using ethyl acetate and acetonitrile as additives. The NG experiments resulted in the formation of an amorphous and viscous materials that started to crystallize into Form III within two hours. Both LAG experiments also resulted in the crystallization of Form III. At the same time (July 2019) at De Montfort University Leicester, the NG and LAG experiments were repeated and the product was now Form II (we emphasise again that the first Leicester experiments yielding Form III were performed in 2017). Several other subsequent LAG and slurry experiments yielded either Form II or a physical mixture of the reactants (in cases where liquids of the Group 3 were used as additives) (see Figures S16-S17 in the SI document).

Table 2: summary of the reported experiments on the formation of CAFF-CA cocrystal.

Location		Approximate date of work	Reactants	Experimental conditions	Outcome	Reference
1	Cambridge, UK	October 2006	CAFF + CA	LAG vibrational mill with 2 drops of water: 20 min at 30 Hz	Form I	Karki <i>et al.</i> ⁴⁴
			CAFF + CA hydrate	Neat grinding: 20 min at 30 Hz	No reaction	
			CAFF + CA		No reaction	
			CAFF hydrate + CA		Form I	
			CAFF hydrate + CA hydrate		Form I	
		2013	CAFF + CA	Neat grinding in planetary ball mill: 400 rpm. Cycled to minimise temperature rise.	Amorphous, crystallised in 1 day to Form I. Thermal treatment gave Form III	Patel ⁴⁷
CAFF + CA	Neat grinding in vibrational mill: 30 min at 30 Hz		Amorphous + residual citric acid			
CAFF + CA	LAG with different liquids vibrational mill: 30 min at 30 Hz		Form I			
2	West Lafayette, USA	Submitted February 2014	CAFF + CA	Solution crystallization in chloroform/methanol mixture	Form II	Smit and Hagen ⁴⁵
3	Funabashi, Japan	Submitted June 2014	CAFF + CA	LAG: liquid not reported	Form III	Mukaida <i>et al.</i> ⁴⁸
		Submitted July 2015	CAFF + CA	LAG with 10 ml chloroform: 26 mmol mixture. Mill TI-100 high speed 60 min of milling.	Initially several lots of Form III, subsequently Form I, eventually Form II. Afterwards, Forms I and III became unobtainable.	Mukaida <i>et al.</i> ⁴²
			CAFF + CA	Slurry in water	Form II	
		Submitted February 2015	CAFF + CA	LAG with water in planetary ball mill: variable time and rpm	Form I	Shimono <i>et al.</i> ⁴⁹
			CAFF + CA	Neat Grinding in planetary ball mill: variable time and rpm	Form I	
4	Cambridge, UK	November 2014- January 2015	CAFF + CA	Neat, seeding-assisted grinding, LAG with various liquids and variable-amount LAG experiments: 20 min at 30 Hz	Form I	This study
		Submitted Feb 19 2015	CAFF + CA	Polymer-assisted grinding in vibrational mill: various amounts of different polymers, 20 min at 30 Hz	Form I	Hasa <i>et al.</i> ³¹
5	London, UK	January 2015	CAFF + CA	LAG in a mixer mill using water as liquid additive	Glassy material that crystallized into Form I after 18 month	This study
		September 2016	CAFF + CA	LAG in a mixer mill using ethyl acetate as liquid additive	Form I	
6	Durham, UK	Submitted 27 June 2016	CAFF + CA hydrate	Neat grinding of CAFF and CA hydrate in 1:2 molar ratio using a mortar and pestle at 20°C and 30°C	At 20° new cocrystal hydrate of CAFF and CA in 1:2 molar ratio, while at 30° the 1:1 cocrystal Form I was obtained	Kerr <i>et al.</i> ⁵⁰
7	Leicester, UK	July 2017	CAFF + CA	Neat and LAG with ethyl acetate in vibrational mill: 30 min 30 Hz	Form III	This study
8	Cambridge, UK	July - August 2017	CAFF + CA	Various conditions	Form II	This study
9	Leicester, UK	July 2019	CAFF + CA	LAG and neat grinding in vibrational mill: 25 Hz, different milling times. Slurry with different liquids	Form II	This study
10	London, UK	July 2019	CAFF + CA	LAG using a mixer mill using ethyl acetate and acetonitrile as liquid additive	Form III	This study
		July 2019	CAFF + CA	Neat grinding using a mixer mill	Amorphous product crystallizing to Form III	

4. DISCUSSION

We present in Table 2 a summary of all reported studies that concern the CAFF-CA cocrystal polymorphs, including the experiments performed in this work, to provide the reader with a timeline experimental location for the disappearing cocrystal polymorphs.

The disappearing polymorph phenomenon is well known in the community of crystallographers and solid-state chemists,³⁷ yet poorly studied and understood—despite the gravity of concerns and consequences that solid-state scientists face when a relevant crystal form cannot be obtained although it has been earlier routinely prepared.⁴¹ The scientific community has, to date, no clear mechanistic explanations for the disappearance of a crystal form, other than the possible existence of “invisible seeds”. Dunitz and Bernstein mentioned in their seminal review that every new form introduced is potentially more stable than the previous (disappeared) polymorph.³⁷ Additionally, the authors mentioned that, once discovered, crystal forms do not disappear permanently but can potentially still be reproduced under appropriate experimental conditions. One relevant phenomenon that potentially impedes such reproducibility is the possible aggressive and unintentional seeding, by presumed and undetectable micro-(or nano)-particles, persisting in the laboratory for months or years after the last experiment was performed. Aggressive seeding can also “infect” other laboratories that are geographically distant from the “source of infection”.⁴¹

We reason that such “invisible seeds” contributed to the disappearance of the studied CAFF-CA cocrystal polymorphs in our laboratories. Studies in the laboratories at De Montfort University in Leicester initially afforded Form III. One of the authors of this study (DH of De Montfort University Leicester) frequently visited the laboratory in Cambridge at a time when Form II was already introduced to this facility. It is remarkable that Form III could not be reproduced in the Leicester laboratory upon return from Cambridge and that only Form II could be obtained. These events and observations are consistent with those reported by Mukaida *et al.*, namely that once Form II was present in a laboratory, Forms I and III apparently disappeared.⁴² Interestingly, the UCL group

was to this date not able to produce Form II and it is necessary to note that the students who performed the experiments at UCL never visited the laboratories in Cambridge or Leicester during this period, and could therefore not have been “contaminated” with Form II. Furthermore, no samples were ever exchanged between UCL and the laboratories in Leicester and Cambridge.

There is another another important factor that should not be neglected during mechanochemical reactions, namely the atmospheric moisture in the laboratory. A recent study Tumanov *et al.*⁵³ demonstrated that the neat mechanochemical cocrystal formation between alpha-glycine and malonic acid is driven by atmospheric moisture, which is absorbed into the reaction mixture owing to the high hygroscopicity of the acid. It is therefore clear that, although there was no intentional addition of water to the system, NG may in reality have “inadvertently” been a LAG reaction. The study of Tumanov *et al.* does, however, not provide any evidence that the atmospheric humidity influences the polymorphic outcome of the mechanochemical cocrystal synthesis. A related outcome has recently been observed in our studies of the theophylline: citric-acid cocrystal, in which the degree of dryness of the citric acid drives the reaction towards the formation of either the anhydrous cocrystal or the cocrystal hydrate.⁵⁴ The mechanisms by which moisture facilitates the formation of cocrystals have also been discussed earlier by Jayasankar *et al.*⁵⁵

What can we conclude from these results? It is clear that, as stated by Bernstein and Dunitz,³⁷ a disappeared polymorph will be obtainable again if the correct conditions for its formation are found. In the case of the CAFF-CA cocrystal, there is no doubt that the three polymorphs can be obtained mechanochemically—but not at the same time or place, even if the experiments are performed under apparently similar conditions. Further studies concerning this cocrystal system are underway but, as in many cases similar events reported in the literature, the studies are impeded by the fact that once a laboratory is “contaminated” with one polymorph, the other polymorphs become unobtainable (at least for the time being). This would also render difficult proposing a reliable mechanism of cocrystal formation. A few observations, however, can be discussed.

The results summarized in Figures 2 and 3 imply that the formation of CAFF-CA by LAG cannot be simply the consequence of the physical role of the liquid, and that the chemical nature of the liquid is relevant to the preparation of a particular polymorph. Of all polar liquids, protic ones seem to have a greater catalytic effect than aprotic ones (*e.g.* DMSO, DMF), although this distinction is not valid for all the liquids considered; other aprotic liquids such as acetone and ethyl acetate provided a pure cocrystal product. As for the effect of the solubility of the starting materials in the liquid additives, CA is practically insoluble in chloroform and hexane. The outcomes of LAG reactions using chloroform or hexane, however, were completely different: in one case a quantitative cocrystal was obtained (chloroform), while in the other it was not possible to obtain the cocrystal at all (hexane). Moreover, both CAFF and CA are soluble in dimethyl sulfoxide, although the LAG reaction using this liquid did not result in cocrystal formation. It is reasonable to suggest that if the liquids actively participate during the nucleation and/or growth phases of CAFF-CA (for all polymorphs), then the chemical properties of the added liquid will be relevant. If this was not the case, a plausible hypothesis for the failure of cocrystal formation LAG with Group 3 liquids would be that cocrystal formation under such conditions is hindered by a kinetic barrier. The seeding method represents a typical strategy for overcoming such high kinetic barriers and promoting the formation of the desired product.³⁶ Seeding experiments performed in this study (Figure 4) suggest that in the case of CAFF-CA system, the presence of preformed cocrystal seeds do not accelerate the formation of CAFF-CA in the absence of Group 1 liquids, if the milling time is less than 45 min.

Belenguer *et al.*⁵⁶ reported that the stability of a polymorphic system under several LAG conditions is directly correlated to the solvation energies of the crystal surfaces of the possible polymorphs, and that such energy differs with both the amount and nature of the liquid. Such a hypothesis can also be applied in our case: in the presence of a specific liquid, the formation of the cocrystal phase is a consequence of favorable solvation conditions, which can lead to the formation of the first cocrystal clusters. The crystal surface solvation effect, however, does not have the ability of preventing the disappearance of Form I, *i.e.* controlling polymorphism in this cocrystal system.

For the cocrystal growth phase, we suggest that it may occur via agglomeration of the smaller cocrystal clusters rather than the addition of coformer molecules to the already existing cocrystal. The outcome of the seeding experiments would seem to confirm this hypothesis. Additionally, the fact that dimethyl sulfoxide did not promote cocrystallization even after the appearance of Form II (Figure S13, SI document) would be another confirmation that nucleation and growth of the multicomponent phase in LAG conditions is related to certain intermolecular interactions between the reactants and specific liquids. In contrast, the mechanism of formation of CAFF-CA after prolonged milling under neat conditions should be different, as in that case the mechanical energy is the lone factor affecting the reaction equilibrium. It is therefore likely that under neat conditions, the formation of CAFF-CA would occur through the formation of an amorphous phase and/or through a liquid intermediate phase.⁵⁷ Furthermore, we observed that after 60 min (or longer) of grinding, the solid product was hard to remove from the jar, with a typical appearance of a solidified liquid. Such hardness increased with longer milling times. Similar observations were reported for the experiments performed at University College London, and from Patel at the University of Cambridge. We also performed a series of additional NG experiments where the samples were milled for several 30 min cycles (see Figures S7-S12 in the SI document), whereby the samples were left to cool at ambient conditions or in a refrigerator for 60 min inbetween milling cycles. These experiments also generated exclusively Form II. The purity of this sample was, however, not comparable to the one obtained by NG in experiments that were conducted with prolonged milling times and in continuous mode.

5. CONCLUSIONS

To the best of our knowledge, CAFF-CA represents the first cocrystal system exhibiting evidence of disappearing polymorphs, suggesting, perhaps not surprisingly, that cocrystals may also be susceptible to this significant phenomenon, along with metal-organic frameworks for which a disappearing polymorph has recently been reported.⁵⁸ Additionally, this is the first trimorphic system

where two polymorphs disappear (other reported systems featuring disappearing polymorphs have been only dimorphic). It was observed, however, that the mechanisms of formation for different polymorphs are similar. Indeed, during LAG conditions only certain liquids promote the formation of both Form I (before disappearing) and Form II. It is also possible to obtain Form III by neat grinding, if neither Form I or Form II apparently have been introduced into the laboratory.

The disappearing-polymorph phenomenon remains one of the most poorly understood events in organic solid-state chemistry; and raises two important questions. The first is related to the time during which a laboratory exists in a “contaminated” state, and whether the duration of this period depends on the nature of the polymorphic system; *i.e.* will some seeds persist (remain effective) for longer time than others? The second question pertains to the varying behavior of distinct polymorphic systems: why do polymorphs disappear in some systems after a new form has been produced, and in other cases not (leaving all known polymorphs accessible at any given time)? What are the thermodynamic and kinetic factors that become so imperative in such cases that cause “total” disappearance for a certain amount of time of a solid form? It is currently very difficult to provide an answer to these questions (or at least a more specific hypothesis), other than the possible seeding effect. Is the disappearing polymorph phenomenon so rare, or is it simply that there are many other examples not reported because they are difficult to rationalize, or as in this case so dependent on time and location and the requirement of a prolonged study? We believe that the reporting and collating of very detailed accounts would significantly improve our understanding of this fascinating topic. We therefore hope that other examples and similar accounts will follow in the coming years.

Finally, we would hope that Professor Joel Bernstein would have found that the results discussed herein present an interesting system concerned with disappearing polymorphs and worthy of further studies.

ACKNOWLEDGEMENTS

W.J. and B.P. thank the EU INTERREG IVA 2 Mers Seas Zeeen Cross-border Cooperation Programme. D.H. thanks the University of Trieste for partially supporting this study (FRA 2013). Professor Kenneth D.M. Harris and Professor Graeme M. Day are thanked for their efforts in our attempt to solve the structure of Form III from the PXRD data.

ASSOCIATED CONTENT

Supporting Information. Experimental PXRD patterns of several NG and LAG experiments and other studies are reported.

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Notes. The authors declare no competing financial interest.

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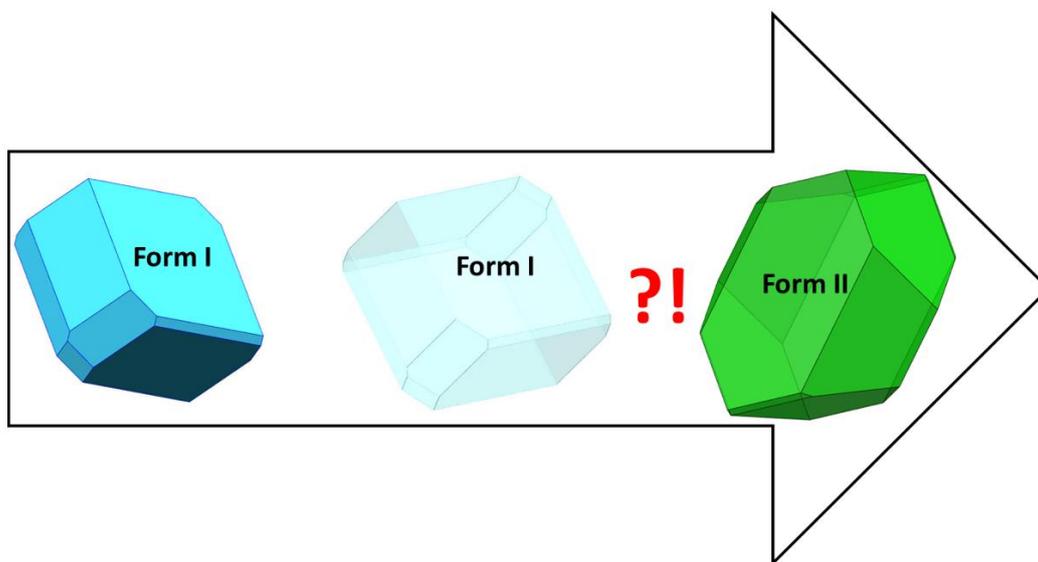
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*Submitted as part of the Crystal Growth and Design virtual special issue
in memory of Professor Joel Bernstein*

For Table of Contents Use Only

**On the mechanochemical formation and “disappearance” of caffeine: citric-acid
cocrystal polymorphs**

**Dritan Hasa, Marina Marosa, Dejan-Krešimir Bučar, Merina K. Corpinot, Divya Amin,
Bhavnita Patel and William Jones**



Brief Synopsis. The disappearing-polymorph phenomenon remains one of the most poorly understood events in organic solid-state chemistry. In this study, we report the first example a trimorphic cocrystal system where two polymorphs disappear. We believe that the reporting and collating of very detailed accounts would significantly improve our understanding of this fascinating topic.