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High-throughput screening and scale-up of cocrystals using resonant acoustic mixing

Karthik Nagapudi*, Evelyn Yanez Umanzor, Colin Masui

Small Molecule Pharmaceutical Sciences, One DNA way, South San Francisco, CA, United States

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ABSTRACT

This paper explores the effectiveness of resonant acoustic mixing RAM for screening and scale up of cocrystals. 16 cocrystal systems were selected as test cases based on previous literature precedent. A 96 well plate set up in conjunction with zirconia beads was used for cocrystal screening using RAM. A success rate of 80% was obtained in the screen for plates containing solvent or solvent plus Zirconia beads. A proof of concept production of hydrated and anhydrous cocrystals of 1:1 Theophylline Citric acid system at a 400 mg scale was demonstrated using solvent and bead assisted RAM. Finally the parameters affecting the scale up of 2:1 Theophylline Oxalic acid cocrystals was explored in a systematic fashion using a Design of Experiments DOE approach. The RAM parameters of acceleration and mixing time were optimized using the DOE approach. A quantitative XRPD method was developed to determine the extent of conversion to the cocrystal when using RAM Mixing time of 2 h and an acceleration of 60 G were determined to be optimal. The optimized parameters were used to demonstrate scale up of 2:1 Theophylline Oxalic acid cocrystals at an 80 gram scale with a net yield of 94%. RAM is thus established as an environmentally friendly mechanochemical technique for both high throughput screening and scaled up production of cocrystals.

1. Introduction

Poorly soluble crystalline small molecules pose a challenge during drug development as they may have limited oral bioavailability (Lipinski et al., 2012). Increased number of such poorly soluble compounds in development has forced pharmaceutical scientists to look for ways to improve oral bioavailability. Cocrystals have emerged as one of the strategies to overcome low bioavailability (Bak et al., 2008; McNamara et al., 2006; Stavropoulos et al., 2015; Variankaval et al., 2006; Wang et al., 2016). In addition cocrystallization has also been shown to improve physical stability (Trask et al., 2005; Trask et al., 2006) and mechanical properties (Karki et al., 2009; Sun and Hou, 2008) of the active molecule thereby making them more amenable to traditional solid dosage form development.

Cocrystals have been defined as solids that are comprised of two or more components in the unit cell of the crystalline structure which are solids at room temperature are held together by non-covalent forces (Bond, 2007; Desiraju, 2003; Duggirala et al., 2016; Dunitz, 2003). This definition distinguishes cocrystals from solvates. These components in the cocrystal are typically held together by hydrogen bonding as opposed to salts in which proton transfer takes place between the constituents. In the last decade or so there has been intense activity in the field of cocrystals in the pharmaceutical industry and several papers have been published about identifying and making cocrystals. However there are still challenges associated with screening methodologies and manufacturing of cocrystals.

Traditionally cocrystals have been prepared using solution or slurry crystallization. The use of solution crystallization poses a challenge as the components of the cocrystal may have different solubilities in the solvent used for crystallization thereby making it complicated to ensure the production of the right form of the cocrystal (Chiarella et al., 2007). At small scale mechanochemical methods such as dry grinding and liquid assisted grinding using ball mills have been demonstrated as efficient methods to generate cocrystals and have been shown to circumvent the issues seen with solution crystallization (Friščić et al., 2006). However, the ball milling process is difficult to scale up. Recently scale up of cocrystallization by mechanochemical means was demonstrated using Twin Screw Extrusion (TSE) (Daurio et al., 2011; Daurio et al., 2014; Dhumal et al., 2010; Kulkarni et al., 2015; Li et al., 2016) and Resonant Acoustic Mixing (RAM) (am Ende et al., 2014; Anderson et al., 2014) technologies, both of which are conducive to

* Corresponding author.
E-mail address: nagapudi.karthik@gene.com (K. Nagapudi).

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making cocrystals at large scales. In contrast to ball milling and TSE, RAM is a low shear approach where low frequency high intensity acoustic energy is used to agitate samples. The energy is uniformly distributed across the sample and the mixing elements do not come into contact with the sample.

Screening of cocrystals using traditional solution-based high throughput techniques (Morissette et al., 2004) suffers from problems similar to that outlined for manufacturing of cocrystals from solution and have low success rates. In order to improve the success of screening methods, slurry and mechanochemical based high throughput screening have been reported (Bysouth et al., 2011; Kojima et al., 2010; Luu et al., 2013). Luu et al. (2013) have reported high throughput screening using a combination of sonication, vortexing and centrifugation while Kojima et al. (2010) have reported slurry based high throughput screening. On a different note Leung et al. (2014) have demonstrated a high throughput screening procedure for nanosuspension formulations using a 96-well plate in the RAM apparatus. They achieved nanosizing using small zirconia beads in the 96-well plate. The use of RAM for low throughput screening of cocrystals was already demonstrated by am Ende et al. (2014) in the same paper where they demonstrated scale up of cocrystals.

In this paper we have combined the methodology of Leung et al. and am Ende et al. and demonstrate for the first time high throughput 96-well plate cocrystal screening using RAM. For the screening Caffeine, Carbamazepine, and Theophylline were used as model cocrystal formers as they have been shown in literature to form cocrystals with a variety of acids (Childs et al., 2008; Trask et al., 2005, 2006). Following screening we describe the scale up of both hydrated and anhydrous form of Theophylline-citric acid cocrystal system using RAM. Finally we have also systematically studied the parameters that affect the scale up of Theophylline-oxalic acid cocrystal system produced by RAM by using a design of experiments approach.

2. Materials and methods

Caffeine, Theophylline and Carbamazepine were used in the screening and scale up studies. All three APIs (Active Pharmaceutical ingredients) were sourced from Sigma–Aldrich Co (St. Louis, MO) and were used as received. The following cocrystal formers were used in the screening: oxalic acid, glutaric acid, malic acid, maleic acid, succinic acid, malonic acid, benzoic acid, adipic acid, and citric acid. All cocrystal formers and solvents used in the study were sourced from Sigma–Aldrich Co (St. Louis, MO) and were used as received. All sourced APIs and cocrystal formers had >98% purity.

2.1. Procedure for high throughput screening of cocrystals using RAM

All reagents and Zirconium beads were dosed to an aluminum vial block designed to hold 96-individually sealed wells (Unchained Labs, Part# S141937) (Fig. 1) using a solids dosing system (SV powder dispense system, Unchained labs *freeslate* robotic platform). APIs were dispensed at a target of 6 mg per well and the coformers at 1:1 equivalents to the corresponding API unless noted (Fig. 2). The 0.5 mm Zirconium beads (Glenmills, Clifton, NJ) were dispensed at a target of 6 mg weight per well. If the well condition required water or ethanol, it was dispensed at a 6 μL level using a manual pipette. Total concentration in each well where solvent was used was kept at 1000 mg/mL. The plate design used is shown in Fig. 2. The composition of each well is represented as a colored pie chart based on both mole and weight fraction of components added. For example well A1 contains Caffeine (red) and Oxalic acid (green)

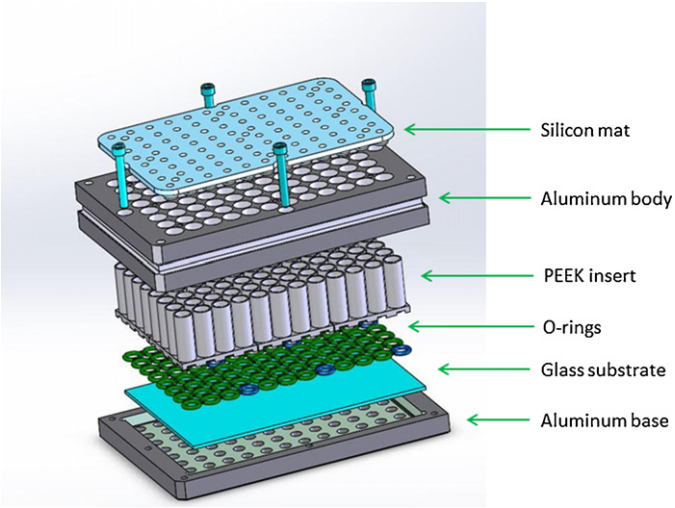

Fig. 1. 96-well high throughput assembly.

at a mole ratio of 2:1. The actual composition of the components by weight in each well is provided in the Supplementary information section.

After all the dispensing was completed the aluminum block was covered with a silicon sealing mat and placed into the RAM (LabRAM II, Resodyn Inc., Butte, Montana). A mixing program with four consecutive 35 min intervals was used. In each 35-min interval, 30 min of acoustic mixing was performed at an acceleration set point of 60 g with 5-min stop time at the end of the mixing period. After the mixing program was completed, the plate was unsealed and allowed to rest overnight. XRD was performed on all the samples using a Bruker D8 X-Ray diffraction system set in reflection mode.

2.2. Procedure for making cocrystals using RAM

Proof-of-concept scale up experiments at a 400 mg scale using RAM was conducted with Theophylline-citric acid cocrystal system. Theophylline and citric acid (at a 1:1 mole ratio) were weighed in 20 mL plastic vials. Five different conditions were tested: (i) solids with no beads, (ii) solids with water, (iii) solids with beads and water, (iv) solids with ethanol, (v) solids with beads and ethanol. 50 μL of solvent and 3 mm stainless steel beads were used. The five vials were placed in a sample holder and subjected to Acoustic mixing at an acceleration of 60 g. The mixer was stopped for 10 min after a mixing time of 30 min for a total mixing time of 2 h.

A design of experiments (DOE) approach was used to study the parameters that affected the scale up of Theophylline-Oxalic acid cocrystal system in the RAM. Two designs were conducted to assess the effect of acceleration and mixing time on cocrystal conversion. The main difference between the designs was in the range for the levels of the two factors investigated. For Design 1, 200 μL of water was used with a total of 400 mg of solids (2:1 molar ratio) and 3-mm steel beads (1× of solids loading) were used. For Design 1, the solid reactants were individually weighed into the vials for each experimental condition. At the end of the experiment the samples were dried for 3 h under vacuum at 60 °C. Parameters investigated in Design 1 are presented in Table 1. For Design 2, 600 μL of water was used with a total of 1.2 g of solids and 3-mm Zirconia beads (1× of solids loading) was used. For Design 2, 30 g blend of Theophylline and Oxalic acid was prepared at 2:1 mole ratio using Turbula blender (Glenmills, Clifton, NJ) operated for 10 min. This blend was then subdivided into 1.2 gram samples for the different experiments. At the end of the experiment the samples

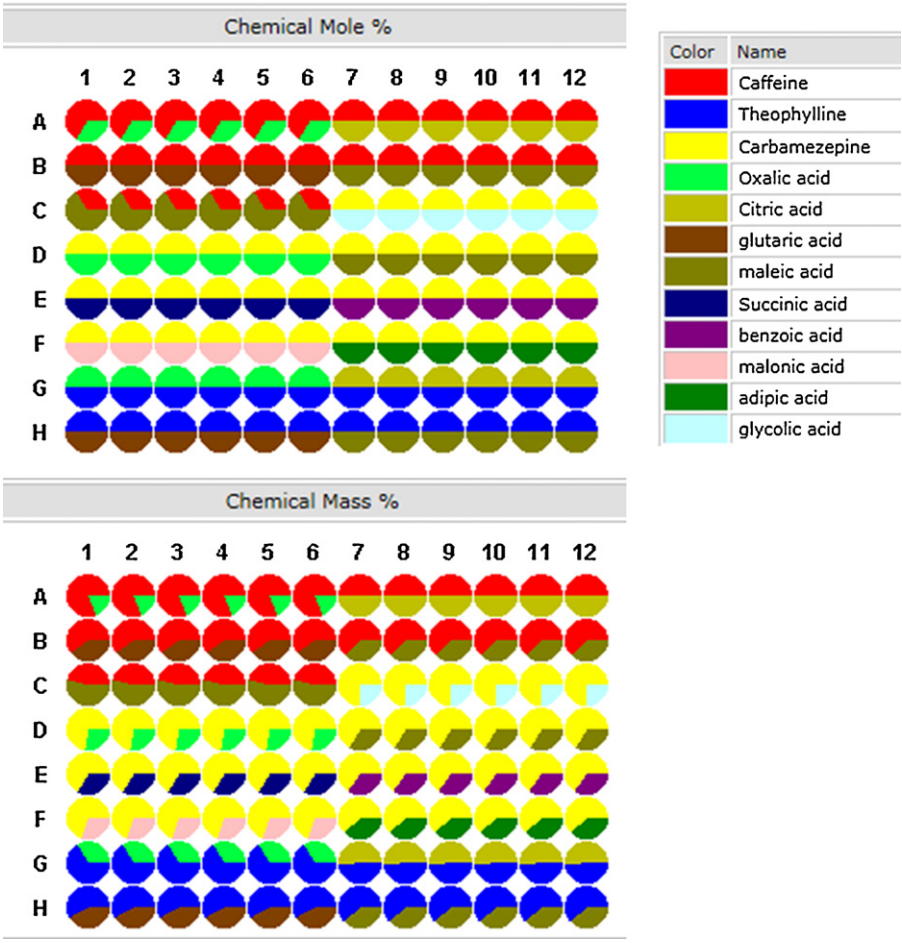

Fig. 2. 96-well high throughput screen design.

were dried for 3 h under vacuum at 60 °C. Parameters investigated in Design 2 are presented in Table 2. The results from the two DOEs were analyzed using JMP software (SAS, Cary, NC). Sample from each experiment in both the DOEs were analyzed by XRPD in the transmittance mode to determine the extent of conversion to the cocrystal using the quantitative method developed (c.f. XRPD methods section).

For the 25 gram scale up run, the mixture of solid reactants at a 2:1 mole ratio was prepared using a Turbula blender operated for 10 min. The solids were transferred to a plastic container and 12.5 mL of water and 1× by weight of Zirconia beads (25 g) was added to solids. The solids were subjected to Acoustic mixing at an acceleration of 60 g for 2 h. After the experiment, the solid in the plastic vial was dried for 8 h under vacuum at 60 °C and was analyzed using the quantitative XRPD method.

For the 80 gram scale up lot, the mixture of solid reactants at a 2:1 mole ratio was prepared using a Turbula blender operated for 10 min. The solids were transferred to a plastic container and 40 mL of water and 5× by weight of Zirconia beads (400 g) was added to solids. The solids were subjected to Acoustic mixing at an acceleration of 60 g for varying lengths of time from 0 to 10 h. Samples were drawn at each time point and were analyzed by XRPD in the transmittance mode to follow the kinetics of conversion to the cocrystal using the quantitative method developed. After the experiment, the solid in the plastic vial was dried for 8 h under vacuum at 60 °C. After drying the vial was transferred to the RAM and acoustic mixing was performed for 5 min at 60 g in order to ensure that the powder did not agglomerate on the beads. Then the solids were sieved using a 710 μm sieve to remove the Zirconia beads from the sample. The net yield for the process was around 94%.

Table 1
Design of experiment 1.

Experiment#	Acceleration (g)	Time (min)
1	60	15
2	70	30
3	80	60
4	80	90
5	60	120
6	60	180
7	70	15
8	80	30
9	50	60
10	70	90
11	50	120
12	50	180

Table 2
Design of experiment 2.

DOE	Acceleration (g)	Time (h)
1	45	4
2	30	6
3	30	2
4	60	2
5	60	6
6	45	4

2.3. X-ray powder diffraction (XRPD)

Powder X-ray diffraction was performed on 96-well plates using a Bruker D8 Discover diffractometer equipped with a Hi-Star two-dimensional detector. Cu K_α radiation was used at a tube voltage and current of 40 kV and 40 mA, respectively. Data were collected from 5–40° 2θ in reflection geometry. Plates were oscillated in the *XY* plane over a 3 × 3 mm area. The sample-to-detector distance was 30 cm, and two frames were collected in step mode to cover the desired 2θ range. Counting time for each frame was 240 s. The incident slit and collimator were 0.3 mm. Samples were at ambient temperature.

XRPD data was obtained using a PANalytical Empyrean diffractometer operating in Bragg-Brentano geometry. The radiation used was Cu K_α with tube voltage and current of 45 kV and 40 mA. The incident beam path was equipped with a 0.02° soller slit, a fixed 1° anti scatter slit, a fixed incident beam mask of 10 mm, and a programmable divergence slit in automatic mode. A beam knife for linear detectors was used. The diffracted beam was equipped with a 0.02° soller slit, a programmable anti scatter slit in automatic mode, and a nickel K_β filter. A PIXcel 1D detector was used in the scanning line detector (1D) mode. For data collected in the reflection mode, zero background Si sample holder and a rotation time of 2.0 sec was used. Data were collected at ambient temperature from 3.0 to 40.0° 2θ using a step size of 0.0263°. For data collected in the transmission mode, a Kapton film holder and a rotation time of 2.0 s was used. Data were collected at ambient temperature from 8.0 to 14.0° 2θ using a step size of 0.00656°.

A quantitative XRPD method was developed using data collected in the transmission. The ratio of the peak at 12.7° 2θ (characteristic of theophylline) to the peak at 10.6° 2θ (characteristic of the cocrystal) was used to develop the quantitative method. Three standard samples containing approximately 1%, 5% and 10% Theophylline in the cocrystal were prepared in triplicate. Each sample was prepared at a 100 mg scale and was mixed for 10 min in a Turbula blender to ensure uniform mixing. The calibration curve developed is shown in Fig. 3.

2.4. Differential scanning calorimetry (DSC)

DSC analysis was conducted on a TA Instruments Q2000 instrument. A sample size of approximately 5–15 mg was weighed out into a Tzero aluminum DSC pan and the pan was crimped. The sample was heated at 10 °C/min under dry nitrogen flow at 50 mL/min.

2.5. Scanning electron microscopy (SEM)

A samples of approximately 1–2 mg was mounted on aluminum stub with carbon adhesive tab and coated for 3 min using Emtech K550X sputter coater to form a thin layer of gold with about 20 nm thickness. SEM images were acquired under high vacuum on a Phenom scanning electron microscope.

2.6. Particle size distribution (PSD)

Particle size analysis was performed using a Malvern Mastersizer 2000 instrument equipped with a Hydros 2000SM wet dispersion attachment. A pump speed of 1500 RPM was used. About 20 mg of sample was weighed into a vial and 1 mL of 0.1% Span 85 in heptane was added. The vial was mixed by hand for 2 s, and the suspension was added to the sampler until an obscuration of about 10–20% was achieved. The sample was then analyzed 3 times and the average results are reported.

3. Results

3.1. High-throughput cocrystal screening using RAM

The results of the high throughput cocrystal screening using RAM are shown in Fig. 4. The percentage of hits is highest (13 out 16 systems) when solvent was used in the screening wells and is the lowest when no beads or solvents (3 out of 16 systems) were used. The use of beads without solvent resulted in only a moderate increase in the number of hits (5 out of 16 systems). Thus its seems that use of solvent is essential to improve chances of finding the cocrystal in the screen. When beads were used in conjunction with solvent the hit percentage was similar to what was obtained with just the use of solvents. These results are consistent with the reported effectiveness of liquid assisted grinding in producing cocrystals (Childs et al., 2008; Weyna et al., 2009). A complete table of results from the HTS screen is provided in the supplementary data available with this paper.

It should be noted that in only three cases: Caffeine-citric acid, Caffeine-malic acid and Theophyilline-glutaric acid systems, solvent-based RAM screens were unable to produce the cocrystal. Among these cases, it was observed for Caffeine-citric acid and Caffeine-malic acid systems that there was no material available for analysis. This is presumably due to the solubility of the system

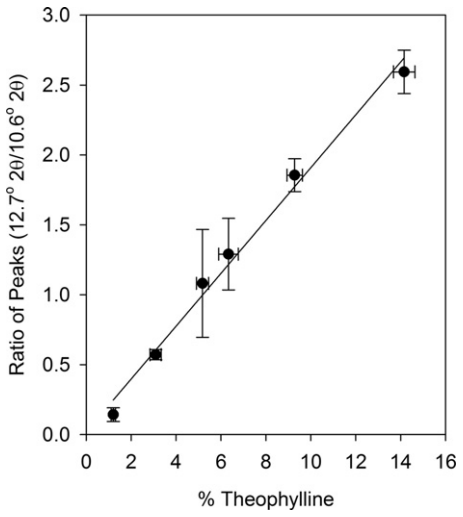

Fig. 3. Calibration curve of ratio of intensity versus percentage theophylline in the sample.

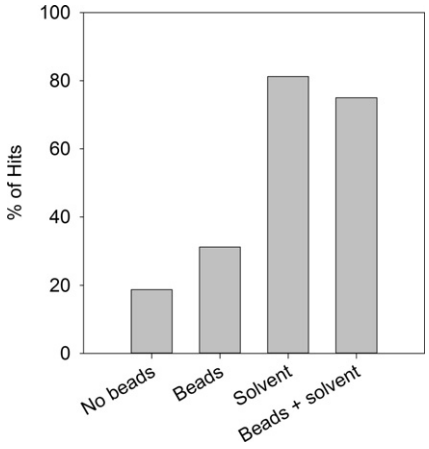

Fig. 4. Outcome of HTS screening reported as the percentage of hits obtained from the screen under various screening conditions.

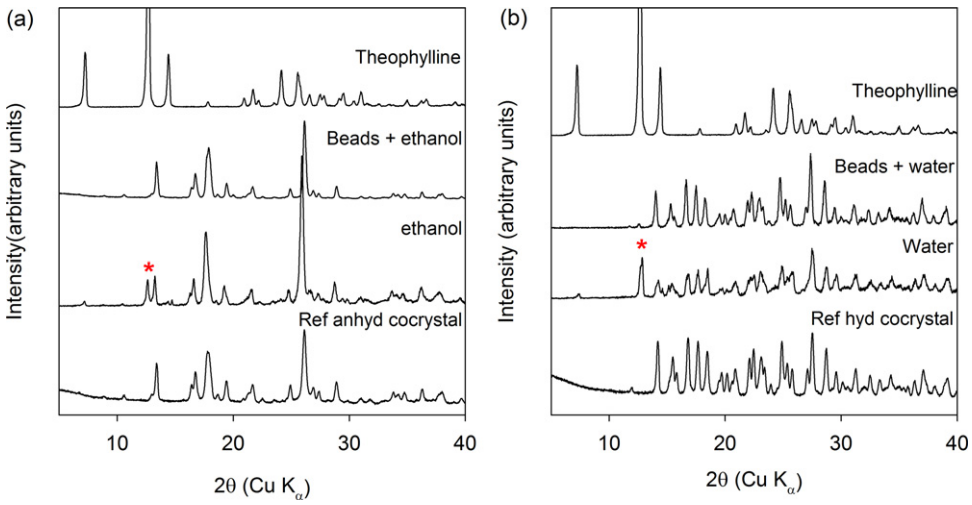

Fig. 5. XRPD data from RAM of theophylline-citric acid system: (a) RAM conducted with ethanol and beads, (b) RAM conducted with water and beads. "*": unreacted theophylline, Ref anhyd cocrystal: Reference anhydrous cocrystal, and Ref hyd cocrystal: Reference hydrated cocrystal.

in the selected solvents, ethanol and water. Only in the case of Theophylline-Glutaric acid system, the well was composed of mainly starting materials. The formation of Theophylline-glutaric acid cocrystal by ball milling has been demonstrated (Trask et al., 2006). The inability of RAM screening to reproduce this result may be attributed to one of two reasons (i) choice of wrong solvent and/or (ii) insufficient energy imparted in the RAM to effect the cocrystal conversion. The liquid assisted high-throughput screening using RAM was thus found to be an effective method to generate cocrystals.

3.2. Demonstration of proof of concept for scale up using theophylline-citric acid system

In order to demonstrate that different phases of a cocrystal system could be scaled up, Theophylline-Citric acid system was used. It has been reported in literature that Theophylline-Citric acid cocrystal can be formed using neat grinding or liquid-assisted grinding either as an anhydrate or a hydrate phase (Karki et al., 2007). The outcome of the grinding experiment depends on the nature of the reactants and medium used for grinding. If one or both of the reactants are hydrates or if water is used to assist the grinding, a hydrated phase is obtained. However if both reactants are anhydrous or if an organic solvent is used to assist the grinding, an anhydrous phase is obtained. To evaluate whether the hydrate and anhydrous phase of the cocrystal could be produced by RAM, experiments were carried out with 400 mg of total solids (1:1 molar ratio) with 50 μL of either ethanol or water. Experiments were also conducted with and without stainless steel beads. The outcome of these experiments is presented in Fig. 5.

The procedure for making the reference hydrated and anhydrous cocrystals and their corresponding XRPD patterns have been reported elsewhere (Daurio et al., 2011; Karki et al., 2007). Based on comparison with the reference XRPD patterns, the data shows that RAM is capable of producing both the anhydrous and the hydrated phase of the Theophylline-Citric acid cocrystal. It was observed that when beads were used in conjunction with the solvent complete conversion to the cocrystal was observed. However, when only solvent was employed, unreacted theophylline was observed (marked by an "*" in Fig. 5). Thus the use of beads accelerates the kinetics of conversion under the experimental set up used. It should be noted that it may be possible to optimize the RAM parameters to produce the cocrystal without necessitating the use of beads as previously demonstrated by am Ende et al. (2014). However based on this set of data, it was concluded that use of beads in conjunction with solvent will help with cocrystal production especially at larger scales.

3.3. The effect of solvent amount on the outcome of cocrystallization by RAM

There are four parameters that affect the outcome of cocrystallization by RAM: (i) nature and amount of solvent, (ii) amount of beads, (iii) acceleration and (iv) mixing time. It was decided to investigate the amount of solvent and beads required by "one factor at a time" approach and acceleration and mixing time using a DOE approach. Water was selected as the solvent of choice based on the high throughput screening results. To make appropriate comparison to published data, we have adopted the nomenclature of Friscic et al. (2009). They have defined an empirical parameter η as the ratio of the solvent used (in μL) to a fixed weight of the reaction mixture (in mg). In order to study the effect of amount of solvent on cocrystal formation, increasing amount of solvent (η = 0 to 2) was added to approximately 400 mg blend mixture containing Theophylline and Oxalic in a 2:1 mole ratio and about 400 mgs of 3.0 mm Steel beads. The resulting solid phase from these experiments was characterized by XRPD and the data are shown in Fig. 6.

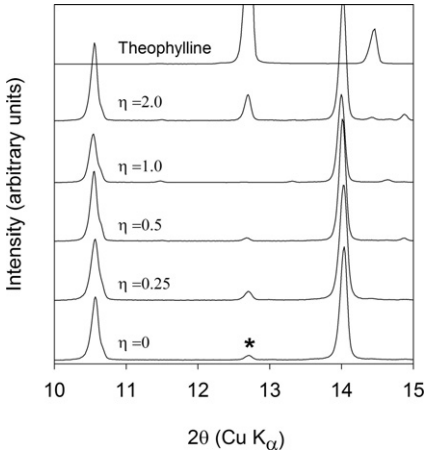

Fig. 6. XRPD data from RAM of Theophylline-Oxalic acid system as a function of increasing amount of water. "*": unreacted Theophylline.

The data show that for η values from 0 to 1.0 nearly complete conversion to cocrystal was observed (based on visual examination of the XRD pattern for the absence of the peak at 12.7° 2θ associated with unreacted theophylline, marked with a "*" in Fig. 6). For η value of 2.0, unreacted theophylline was clearly observed. This observation is likely related to the relative solubilities of the cocrystal formers in water. The solubility of Theophylline in water is around 8.3 mg/mL while that of Oxalic acid is around 143 mg/mL. As the ratio of water to solids is increased, oxalic acid is increasingly solubilized in the solvent and selective solubilization competes with cocrystal formation during RAM. Thus at η value of 2 the system shows unreacted theophylline. It should be noted that even at $\eta = 2.0$ significant conversion to the cocrystal was obtained. For future experiments, $\eta = 0.5$ was selected as the least amount of solvent that provided the greatest conversion to the cocrystal for the given experimental set up.

3.4. Process parameters that affect scale up of theophylline-oxalic acid system: design of experiments approach

Based on prior knowledge, the mixing intensity (acceleration) and mixing time are known to be the important process parameters that affect the extent of conversion in mechanochemical reactions that are performed by RAM. These parameters were studied using a DOE approach. Two DOEs were conducted to optimize the acceleration and mixing time. The design of DOE 1 and 2 are presented in Tables 1 and 2 respectively. The results from DOE 1 and 2 are shown in Fig. 7. DOE 1 and 2 were designed based on a continuous change in factors investigated. The outcome of the DOE experiments was analyzed using the JMP software by correlating the acceleration and mixing time used in the RAM with the percentage of unreacted Theophylline. The percentage of unreacted Theophylline was calculated using the quantitative XRPD method developed (c.f. XRPD Methods section). DOE 1 investigated acceleration in the range of 50 to 80 g and a mixing time in the range of 15 to 120 min. The data indicated that lower accelerations and longer mixing times resulted in higher conversion to the cocrystal. Based on this data, DOE 2 was designed to extend acceleration to a lower range of 30 to 60 g and mixing time to a higher range of 2 to 6 h. DOE 2 data indicated that an acceleration of 60 g with a mixing time of 2 h was optimal for the production of the cocrystal. DOE 2 data also shows that there is no tangible effect of mixing time (2 to 6 h range) on the level of conversion to the cocrystal. For an acceleration of 60 g and a mixing time of 2 h, only 0.4% of unreacted Theophylline was observed in the sample.

Apart from ranges for parameters investigated, the other main differences between DOE 1 and 2 was in the amount of solids used and the nature of the beads used. DOE 1 was carried out with about 400 mgs of total solids while DOE 2 was carried out with 1.2 g of solids. Samples examined from DOE 1 showed that there was metal shedding from the stainless steel beads used. Therefore for DOE 2 it was decided to use Zirconia beads which have been shown to be highly wear resistant. In both the DOEs the η value was maintained at 0.5.

3.5. Scale up of theophylline-oxalic acid system and kinetics of cocrystal formation

Using the optimized mixing time and acceleration of 2 h and 60 g, a study was conducted to understand the effect of solids loading on conversion to the cocrystal. The total solids content in the reaction mixture was increased from 1.2 g (used in DOE 2) to 24 g. The XRPD data is shown in Fig. 8. The level of unreacted Theophylline in samples increased with increasing solids loading. At a scale of 24 g, about 2.1% unreacted Theophylline was detected. While this level of unreacted coformer is still within an acceptable range, it was unclear whether this trend would continue if the solids loading was increased.

One potential method to increase conversion to the cocrystal is to increase bead loading. It was speculated that an increase in bead loading would lead to increased particle contact and regeneration of new surfaces which in turn could promote conversion to cocrystal. A study at a 5 g scale was done to investigate the effect

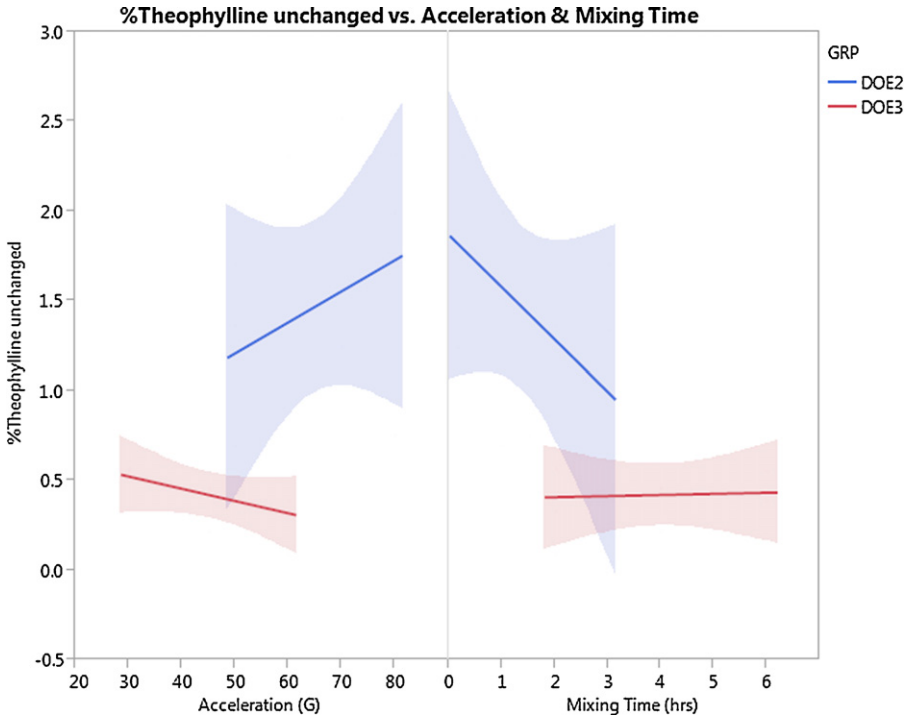

Fig. 7. DOE data trends for correlation of acceleration and mixing time with the percentage of unreacted theophylline.

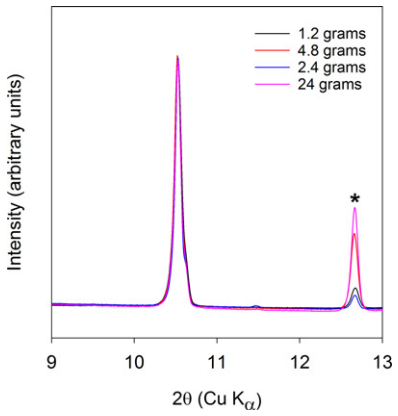

Fig. 8. XRPD data of cocrystal samples produced with increasing amount of solids content in the reaction mixture. Increasing amount of unreacted theophylline ("*") was observed as solids loading was increased.

of bead loading at 1×, 5×, 7×, and 15× amounts. The sample temperature was significantly increased in RAM when 7× and 15× beads were used. Moreover at a 15× bead loading highly defective cocrystals were obtained. There was no major difference between the 1× and 5× bead loadings in terms of conversion to the cocrystal. For the scale up attempt at 80 g, as a conservative approach it was decided to increase the bead loading to 5× (400 g of 3 mm Zirconia beads) to improve the conversion to the cocrystal. In addition it was also decided to monitor the kinetics of conversion to the cocrystal at this scale. The XRPD, DSC, SEM, and PSD data of the cocrystal obtained from the 80 gram scale up run are shown in Fig. 9.

The XRPD of the cocrystal samples obtained after 6 and 10 h of mixing time compares well with the powder pattern calculated from the published single crystal structure. The XRPD of the 6-h sample shows unreacted Theophylline (labeled 'UT' in Fig. 9) at about a 1.8% level. The XRPD of the 10-h sample shows almost complete conversion to the cocrystal based on the Theophylline peak at 12.7° 2θ. However, in the 10-h sample several new reflections (labeled '*' in Fig. 9) were observed. Based on comparison with published data (Seton et al., 2010) it is likely that these new reflections correspond to a newly discovered polymorphic Form IV of Theophylline. Thus the 10-h sample may also contain some unreacted Theophylline, the amount of which cannot be quantified due to lack of an appropriate standard. The DSC thermogram of the cocrystal shows an endotherm with a peak temperature of 233.2 °C (labeled T_{m1}) that corresponds to the melting of the cocrystal. Two further endotherms with peak temperatures at 273 and 277.6 °C (labeled T_{m2} and T_{m3}) correspond to the melting of two polymorphic varieties of anhydrous Theophylline. It is interesting to note that similar thermal behavior (two melting endotherms) was noted for the monohydrate or the dehydrate forms of Theophylline (Zhang and Rasmuson, 2012). The SEM micrograph of the cocrystal show

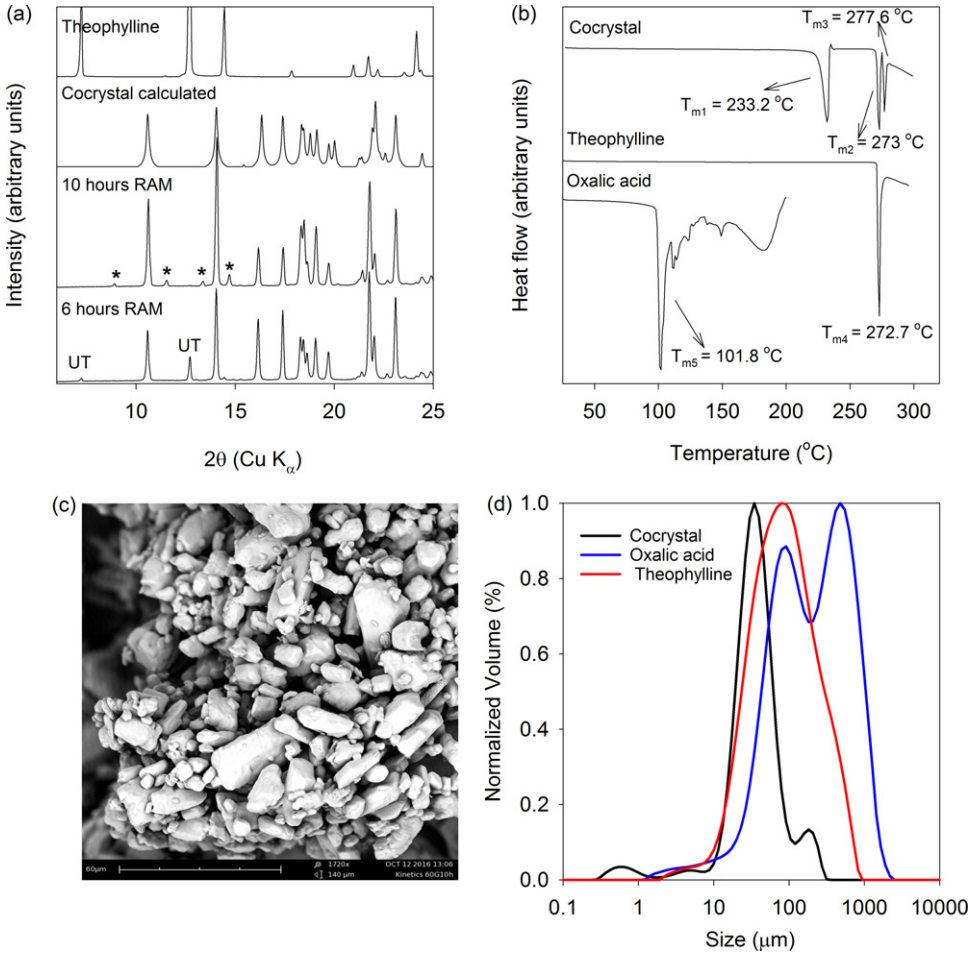

Fig. 9. (a) XRPD data of cocrystals from the 80 gram scale up run (6 h and 10 h time point) in comparison to a calculated pattern and pure theophylline. (b) DSC thermograms of the cocrystal from the 80 g scale up run (10 h time point), Theophylline and Oxalic acid. T_{m1}: melting point of cocrystal, T_{m2} and T_{m3}: melting point of Theophylline polymorphs, T_{m4}: melting point of as-received Theophylline and T_{m5}: melting point of oxalic acid. (c) SEM micrograph of the cocrystal from the 80 gram scale up run (10 h time point). (d) Particle size distribution of cocrystal (10 h time point), oxalic acid, and theophylline measured using laser light diffraction.

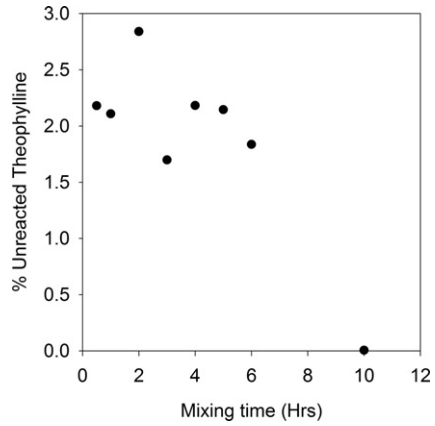

Fig. 10. Percentage of unreacted theophylline as a function of mixing time for the 80 gram scale up run using RAM.

irregular plate-like morphology with a majority of the particles less than 80 μm. PSD measurement conducted on the cocrystal further confirmed that the mean particle size based on volume was around 50 μm. It should be noted that the mean particle size based on volume of the Oxalic acid and Theophylline starting material was around 310 and 123 μm respectively. Thus in addition to accelerating the kinetics of cocrystal formation, the use of beads in the RAM also helps to reduce the particle size to a level that is readily amenable for further formulation development.

For the 80-gram scale up lot, the kinetics of conversion to the cocrystal formation was monitored at periodic intervals by stopping the RAM and retrieving about a gram of material for analysis by quantitative XRPD. The data for unreacted Theophylline as a function of mixing time is shown in Fig. 10. It is interesting to note that within the first half hour of mixing at 60 g the conversion to the cocrystal reaches 97–98%. From half hour to 6 h there is very little change in the conversion levels. At the 10-h mixing time almost complete conversion to the cocrystal was observed. However as discussed previously, the caveat here is that new reflections are seem in the XRPD pattern of the 10-h sample which are most likely from a polymorphic variety of anhydrous Theophylline. Thus, it is possible that the conversion to the cocrystal remains similar at around 98.2% for the 6 and 10 h mixing time samples. Based on the kinetic data, acceleration at 60 g for a mixing time of 2 h seems sufficient to convert the starting solids to the cocrystal even at the 80-gram scale.

4. Discussion

In this work we have demonstrated that Resonant Acoustic mixing can be used for high throughput screening and scale up of cocrystals. RAM employs low frequency high intensity acoustic waves as the mixing medium. The advantages of this type of mixing are: (i) uniform distribution of mixing energy through the sample, (ii) no contact between mixing parts and the sample, and (iii) minimal temperature excursion during the mixing process. Furthermore, if kinetics of mechanochemical reactions has to be accelerated then beads can be used to simulate the milling process. These advantages coupled with the fact that small scale instruments are inexpensive, makes the RAM methodology very attractive for screening and making cocrystals.

In contrast to RAM, the other mechanochemical technique that has been shown to be effective in scaling up cocrystals is Twin Screw Extrusion (TSE) (Daurio et al., 2014; Dhumal et al., 2010). While TSE can be used for scale up of cocrystals, it is not a technique that can be adapted for screening of cocrystals as the material requirements are high. However, TSE provides higher shear and access to a greater temperature range than the RAM. For cocrystal systems in which conversion is facilitated by eutectic formation or higher shear forces, TSE may become more attractive than RAM. TSE is also a continuous process which may provide added benefits

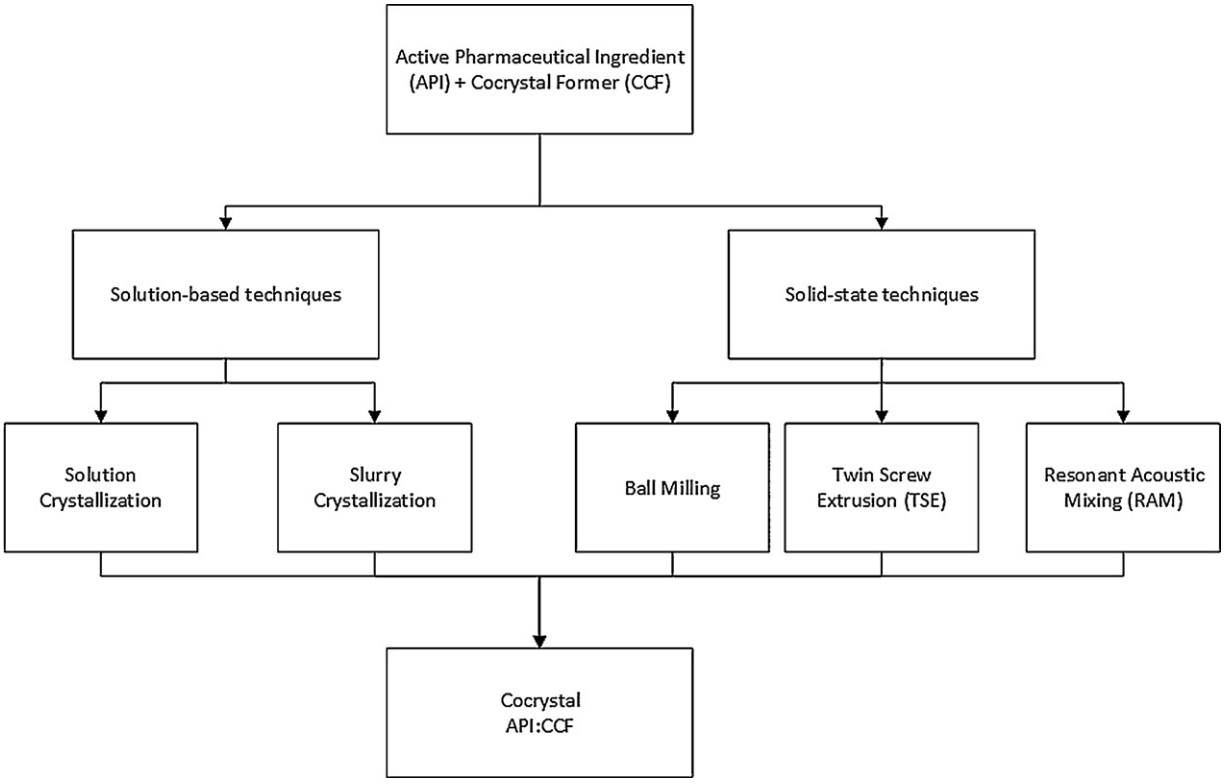

Fig. 11. Solvent-based and solid-state techniques for making cocrystals.

during manufacturing. Thus it seems that RAM could be become the technique of choice for early development of cocrystals (hundreds of grams to 1 kilogram scale) while TSE is well placed for large scale manufacture (multi-kilogram scale).

The currently available techniques for making cocrystals are summarized in Figs. 4–11.

5. Conclusions

The use of 96 well plate methodology to screen for cocrystals was adapted for resonant acoustic mixing. High success rate in terms of cocrystal hits was demonstrated for three APIs: Caffeine, Theophylline, and Carbamazepine. The application of RAM in the production of both anhydrated and hydrated forms of Theophylline-Citric acid cocrystals was demonstrated. Furthermore the production of Theophylline-Oxalic acid cocrystal was demonstrated at a 80-gram scale. Acceleration and mixing time were identified as the key parameters to be optimized for successful production of cocrystals. The use of beads along with liquid assisted-acoustic mixing was introduced in this work as a means to accelerate kinetics of cocrystal formation. RAM is thus established as a scalable and environmentally friendly mechanochemical technique for the production of cocrystals.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2017.02.027.

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