In situ monitoring of competitive coformer exchange reaction by ^{1}H MAS Solid-state NMR

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ABSTRACT: In a competitive coformer exchange reaction, a recent topic of interest in pharmaceutical research, the coformer in a pharmaceutical cocrystal is exchanged with another coformer which is expected to form a cocrystal that is more stable. There will be a competition between coformers to form the most stable product through formation of hydrogen bonds. This will cause destabilization of the pharmaceutical products during processing or storage. Therefore it is important to develop a mechanistic understanding of this transformation by

monitoring each and every step of the reaction, employing a technique such as ^{1}H nuclear magnetic resonance (NMR) . In this study, an in situ monitoring of a coformer exchange reaction is carried out by ^{1}H magic angle spinning (MAS) solid-state NMR (SSNMR) at a spinning frequency of 60 KHz. The changes in caffeine maleic acid cocrystals on addition of glutaric acid, and caffeine glutaric cocrystal on addition of maleic acid were monitored. In all the reactions, it has been observed that caffeine glutaric acid Form I is formed. When glutaric acid was added to 2:1 caffeine maleic acid, the formation of metastable 1:1 caffeine glutaric acid Form I was observed, at the start of the experiment, indicating that the centrifugal pressure is enough for the formation. The difference in the end product of the reactions with similar reaction pathway of 1:1 and 2:1 reactant stoichiometry indicate that a complete replacement of maleic acid has occurred only in the 1:1 stoichiometry of the reactants. The polymorphic transition of caffeine glutaric acid Form II to Form I at higher temperature was crucial reason which triggers the exchange of glutaric acid with maleic acid in the reaction of caffeine glutaric acid and maleic acid. This is a first report proposing, the new reaction pathways in competitive coformer exchange reactions enabled understanding the the remarkable role of stoichiometry, polymorphism, temperature and centrifugal pressure .

Introduction

The application of cocrystallization in the field of pharmaceutics is an emerging area, and cocrystallization can be used to tailor the physicochemical properties of an active pharmaceutical ingredient (API) which can reduce the cost and time associated with the drug discovery and development of innovative formulations[1]. Cocrystallization involves the interaction of API with coformers which modifies the crystal lattice through a non-covalent interaction, especially intermolecular hydrogen bonding. Coformers are neutral molecules with hydrogen bond donor

and acceptor functional groups, generally dicarboxylic acids, and are usually selected by trial and error.[2] There must be complimentary hydrogen bonding functional groups in API, and coformers for cocrystallization, which can result in the formation of cocrystals, eutectics, polymorphs, and solid solutions. Among these cocrystallization products, cocrystals are easier to characterize, since certain fingerprint regions can be identified for the cocrystal which are distinct from that of the parent components. [3] [4] [5] [6] The stability, solubility, melting point, hygroscopicity, bioavailability, and dissolution rate of an API can be effectively tuned using this method.[7] [8] The goal of every cocrystallization reaction is the formation of a stable product and hence stability studies are very important in pharmaceutics. Cocrystallization ensures the stability of an API or an excipient by an appropriate selection of coformers. The selection of a coformer and API for cocrystallization is done in such a way that the best hydrogen bond donor will interact with the best hydrogen bond acceptor to form a stable product. Competitive coformer exchange reaction is a very important phenomenon in the area of pharmaceutical cocrystals, where the least stable coformer will be replaced by the more stable one from the cocrystal. Fisher *et al*. studied competitive cocrystal reactions in ball mill using anthranilic acid and carbamazepine as model drugs and salicylic acid, threobromine and theophylline as coformers. The study showed that competitive cocrystal reactions are driven by intermolecular recognition and controlled by kinetic aspects. [9] Mukaida *et al.* studied coformer exchange reaction of caffeine cocrystals where stability order is connected with calculated hydrogen bond energy. [10] The effect of maleic acid, succinic acid and glutaric acid on carbamazepine cocrystal formation were investigated by Moribe *et al*. and found that structure, stability and mechanism has a role in these types of reactions.[11] For capturing each and every steps of the coformer exchange reaction, efficient methods are necessary. Techniques like differential scanning calorimetry (DSC),

thermogravimetric analysis (TGA), powder x-ray diffraction (PXRD), single crystal x-ray diffraction (SCXRD), IR, hot stage microscopy (HSM) and nuclear magnetic resonance (NMR) can be considered to monitor competitive coformer exchange reaction. The different cocrystals will have different crystal structure and hence exchange reaction could be easily followed through XRD. [12] [13] However, this reaction involves making and breaking of hydrogen bonds which cannot be monitored by DSC, TGA, HSM and XRD since the determination of exact position of hydrogen is challenging, using these methods. [14] Solid state NMR can give valuable information on the structural changes associated with hydrogen bonding. [15–19] ^{1}H MAS NMR spectra can provide a fingerprint region for every compound, and based on the variation in ^{1}H chemical shift one can follow the competitive coformer exchange reaction very effectively.

In several studies of pharmaceuticals, caffeine ($C_8H_{10}N_4O_2$), a central nervous system stimulant, has been used as a model compound. [20] It exists in three forms, two anhydrous forms and one hydrate form. Caffeine exhibits instability with respect to humidity. However, studies show that stability issues can be avoided by cocrystallization, without the formation of a crystalline non stoichiometric hydrate. In 2005, Trask *et al.* showed that polymorphic and stoichiometric diversity were also achieved by cocrystallization of caffeine with formic acid, acetic acid, and trifluoroacetic acid. [21] In another study, Trask *et al.* showed that the same cocrystals can be prepared using different techniques, in different stoichiometry, as well as in different polymorphic forms which highlights the importance of stoichiometry and polymorphism in cocrystallization. [22] Different stoichiometries of caffeine and maleic acid resulted in the formation of the stable 1:1 caffeine maleic acid, as well as 2:1 metastable caffeine maleic acid cocrystals.[23] Cocrystallization of caffeine with anthrallinic acid resulted in the

polymorphic and solvate forms of cocrystals.[24] Based on these studies, the natural question that occurs is about the role of polymorphism as well as stoichiometry in competitive coformer exchange reaction. As per literature, the thermodynamic stability of cocrystals will depend on the temperature and composition.[25] [26] The polymorphic transitions can be either thermodynamically reversible or irreversible with temperature. Hence temperature also has to be considered. Thus, if a study can be designed, which is able to address the effect of various parameters like stability, stoichiometry, polymorphism and temperature together in a single competitive coformer exchange reaction, it will provide maximum information at a minimum time.-

There are several studies reported in the literature which compare the stability order of caffeine cocrystals. Theoretical calculation of Hydrogen bond energies showed that caffeine oxalic acid is the most stable and 1:1 caffeine maleic acid, the least. [10] Recently, there were a few reports on competitive coformer exchange reaction, to determine the most stable cocrystal. [10] [21] [22] [27] Alsirawan *et al.*, studied competitive coformer exchange reaction of caffeine cocrystals using slurry method and dry grinding method and carried out the characterization by XRD, and also a theoretical calculation of lattice energy, and change in Gibbs energy. There is an agreement as per the reports in the literature that the most stable cocrystal is caffeine oxalic acid. [10] [28] However, the least stable cocrystal, predicted from the theoretical calculation is different from what was experimentally determined from the slurry and grinding method. Based on Alsirawan *et al.*, 1:1 caffeine glutaric acid Form I is the least stable. However, DFT studies showed that 2:1 caffeine maleic acid is the least stable. Therefore, there is a need to carry out further studies since caffeine maleic acid has two stoichiometric forms and caffeine glutaric acid has two polymorphic forms: Form I and Form II. Vangala *et al.* has reported that the caffeine glutaric acid

polymorphic system is enantiotropic with conversion temperature of 79°C and both the forms were found at this temperature.

Establishing the relationship between the structure, stability, polymorphism, stoichiometry and temperature can pave a new pathway in understanding competitive coformer exchange reaction. Based on the results of various stability studies of caffeine cocrystals reported in the literature, it can be concluded that caffeine oxalic acid is the most stable cocrystal of caffeine, and caffeine glutaric acid Form I, the least stable. It has also been reported in the literature that the comparative stability of caffeine glutaric acid Form II, 2:1 caffeine maleic acid (CAF_2MAE_1), and 1:1 caffeine maleic acid (CAF_1MAE_1) varies depending upon the method of study. [10 21 27 28] Therefore, a coformer exchange reaction, which can be monitored with a suitable technique can result in a better understanding of these reactions. Recently, Mandala *et al.* has successfully monitored the cocrystal formation by ^{13}C CPMAS solid-state NMR[29], in which spontaneous formation of a cocrystal of caffeine with malonic acid, was studied. The cocrystal formation was confirmed by comparing the spectra of the product with that of a caffeine malonic acid cocrystal, and demonstrate that it is possible to carry out an in situ monitoring of competitive coformer exchange reaction using solid state NMR. In this study, it is shown that ^{1}H MAS solid state NMR can be used as a powerful method for in situ monitoring of a cocrystal exchange reaction. The advantage of using ^{1}H solid-state NMR is that this is highly sensitive, and therefore requires much less time, compared to ^{13}C CPMAS. Caffeine maleic acid (CAF_2MAE_1) and caffeine glutaric acid (CAF_1GLU_1) are selected as model compounds. The exchange reaction of CAF_2MAE_1 with glutaric acid (GLU) was carried out and monitored in situ. To understand the role of stoichiometry in competitive coformer exchange reaction, reactions were carried out with

CAF_2MAE_1 and GLU in two ratios (1:1 and 2:1). A reverse reaction, of CAF_1GLU_1 (Form II) with MAE was also carried out.

Materials and Methods

All chemicals were purchased from Sigma Aldrich, UK. The CAF_2MAE_1 and CAF_1GLU_1 Form II were synthesized using method as reported by our group[27]. CAF_1MAE_1 was prepared in NCL by grinding caffeine and maleic acid in 1:1 ratio for 1 hour and crystallized by the addition of methanol.

Solid State NMR

The reactions were monitored in situ by recording the ^1H MAS SSNMR in a Bruker AVIII 700 MHz spectrometer. All the measurements were done in a 1.3 mm rotor at a spinning frequency of 60 KHz. To monitor the progress of the reactions, the individual components need to be identified, and the spectra of individual components were recorded, assigned and a distinct characteristic peak for each component could be identified and is discussed in the next section. The recycle delay for caffeine (CAF), maleic acid (MAE), glutaric acid (GLU), 2:1 caffeine maleic acid (CAF_2MAE_1), 1:1 caffeine maleic acid (CAF_1MAE_1) and 1:1 caffeine glutaric acid (CAF_1GLU_1) were 100s, 1000s, 500s, 75s, 250s and 100s respectively. When the reaction is monitored using SSNMR experiment, the largest recycle delay among the components was used as the recycle delay. The ^1H MAS NMR spectra of maleic acid, glutaric acid, caffeine maleic acid and caffeine glutaric acid at different temperatures were also recorded and discussed.

Procedure followed for the caffeine maleic acid (CAFMAE) -glutaric acid (GLU) reaction

Caffeine maleic acid (CAF_2MAE_1) and glutaric acid (GLU) were gently mixed in an Eppendorf in the ratio 1:1 (1(CAF_2MAE_1) +1(GLU)) and packed immediately in a Bruker 1.3 mm zirconia rotor. Initially, the reaction products were monitored at regular intervals by recording the ^1H

SSNMR at ambient temperature up to four hours till no further changes were observed. After that, the temperature was increased by 10 K from 303 K to 343 K and final temperature was set at 350 K. At every temperature after attaining stability, the reaction products were monitored at regular intervals by recording the ^{1}H SSNMR up to one hour in order to confirm that there were no further changes observed at that temperature. Then the temperature was decreased from 350 K to 303 K to the ambient temperature following the same procedure. After attaining the ambient temperature in the spectrometer, the rotor packed with the sample was removed and kept overnight at the ambient temperature and a ^{1}H MAS NMR spectrum was recorded after that. The experiment was then carried out with the same reactants, with a ratio 2:1 (2(CAF_2MAE_1) +1(GLU)) following the same procedure. The two experiments were also repeated after grinding the reactants in order to understand the effect of grinding in addition to the temperature and the centrifugal pressure. In this case also, the ^{1}H MAS NMR spectra were recorded at different temperatures starting from ambient temperature to 350 K.

Procedure followed for the caffeine glutaric acid (CAFGLU)-maleic acid (MAE) reaction

Caffeine glutaric acid (CAF_1GLU_1) and maleic acid (MAE) were gently mixed in an Eppendorf in the ratio 1:1 (1(CAF_1GLU_1) +1(MAE)) and packed immediately in a 1.3 mm rotor. The reaction products were monitored at regular intervals by recording the ^{1}H MAS NMR with time and temperature, following the same procedure as described above for the CAFMAE and GLU system.

RESULTS AND DISCUSSIONS

Identification of the characteristic peaks of the individual components

The ^{1}H MAS NMR spectra of CAF (1a), MAE (1b), GLU (1c), CAF_2MAE_1 (1d), CAF_1MAE_1 (1e) and CAF_1GLU_1 Form II (1f) spectra were recorded and is shown in Figure 1. The spectrum of caffeine (1a) contains three methyl groups and one methylene group, and the peaks at 7.8 ppm, and 3.4 ppm corresponds to the methylene hydrogen and the methyl groups respectively. In the spectrum of glutaric acid (Figure 1b), two peaks are seen, one at 12.6 ppm which is assigned to the acidic group and the other at 2.2 ppm, corresponding to the $-CH_2$ group. In maleic acid, both intramolecular and intermolecular hydrogen bonds are present, indicated by peaks at 15.7 ppm and 13 ppm, and a peak at 6.9 ppm corresponding to its methylene hydrogens in the spectrum (Figure 1c). The very small peak around 2 ppm may be due to an impurity. In the 2:1 caffeine maleic acid (CAF_2MAE_1), only the intermolecular hydrogen bond is present which is seen at 14.5 ppm in the spectrum (Figure 1d). The methylene peaks of caffeine and maleic acid in caffeine maleic acid are seen at 7.2 ppm and the peaks at 3.9 ppm and 3 ppm are methyl groups of caffeine (Figure 1d). In CAF_1MAE_1, the peaks corresponding to the intramolecular and intermolecular hydrogen bonds are seen at 18.8 ppm and 14.2 ppm respectively (Figure 1e). The methylene peaks are at 8.1 and 6.9 ppm respectively and the methyl peaks are seen at 3.2 ppm (Figure 1e). In 1:1 CAF_1GLU_1 Form II, two acidic peaks are seen at 13.5 ppm and 9.8 ppm, the methylene peaks are at 8.09 ppm and 7.1 ppm, and the methyl peaks are at 3.1 ppm respectively (Figure 1e). Based on these results, a distinct characteristic peak for each of the compounds could be identified as 7.8 ppm for CAF, 12.6 ppm for GLU, 15.7 ppm for MAE, and 14.5 ppm for CAF_2MAE_1, 18.8 ppm for CAF_1MAE_1 and 13.5 ppm for CAF_1GLU_1 Form II respectively. Due to the metastable nature of CAF_1GLU_1 Form I, its preparation was not successful and the characteristic peak of caffeine glutaric acid Form I (10.9 ppm) is identified from the variable temperature study of caffeine glutaric acid Form II (figure 2e).

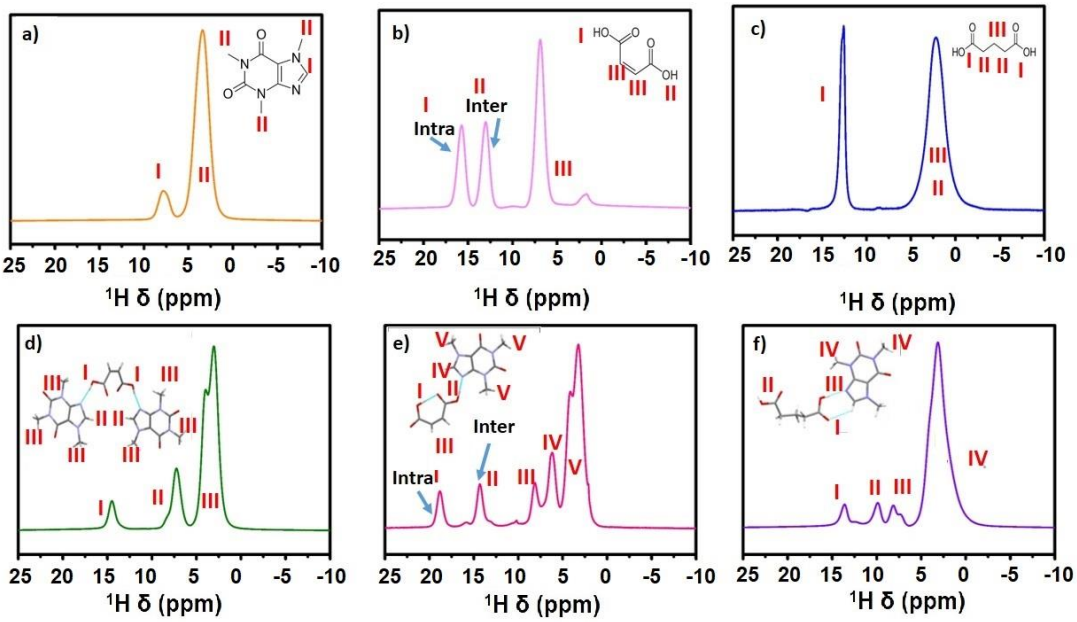

Figure 1: ^{1}H MAS NMR spectra of a) CAF b) MAE c) GLU d) CAF_2MAE_1 e) CAF_1MAE_1 f) CAF_1GLU_1 Form II.

Variable temperature study of individual components

Since the coformer exchange reactions are carried out at higher temperatures, the ^{1}H MAS spectra from 298 K to 350 K were recorded for the individual components CAF_2MAE_1, CAF_1MAE_1, CAF_1GLU_1 Form II, MAE and GLU and the results are shown in Figure 2No changes were observed in the spectra of CAF_2MAE_1, CAF_1MAE_1, MAE and GLU at higher temperatures. Remarkable changes are observed in the case of CAF_1GLU_1 Form II (Figure 2e). In ^{1}H MAS spectrum of CAF_1GLU_1 Form II, two acidic peaks were seen at 13.5 ppm and 9.8 ppm at 298 K. Up to 323 K no changes were obtained and after this a shift of peak from 9.8 ppm to 10.9 ppm was observed (333 K) and the methylene peaks which were observed at 8.09 ppm and 7.1 ppm (298 K) converged into a single peak around 7.5 ppm. These observations indicated that the polymorphic transition of CAF_1GLU_1 Form II to Form I is taking place at this

temperature. The polymorphic transformation of CAF_1GLU_1 Form II into Form I, similar to that observed by us has already been reported. [30] Based on this result, the peak at 10.9 ppm is assigned as the characteristic peak of CAF_1GLU_1 Form I.

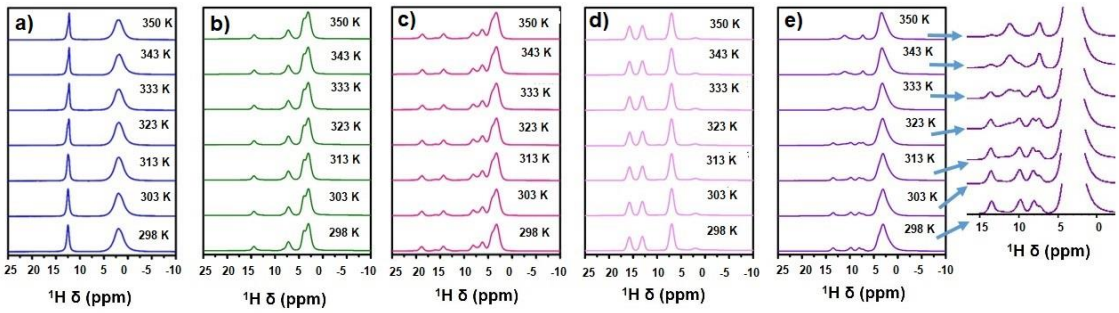

Figure 2: ^1H MAS NMR spectra of a) GLU b) CAF_2MAE_1 c) CAF_1MAE_1 d) MAE e) CAF_1GLU_1 Form II from 298 K to 350 K.

Coformer exchange reaction in caffeine maleic acid cocrystals

Two types of reactions were carried out in caffeine maleic acid cocrystals. In the first type, the reactants are gently mixed in an Eppendorf and immediately packed in the 1.3 mm rotor, and the reaction is monitored with time and temperature. In the second type, the reactants are grounded, and immediately packed in 1.3 mm rotor and the reaction monitored with temperature. Our observations are discussed below.

a) Reactants gently mixed in the ratio 1:1 (1(CAF_2MAE_1) +1(GLU))

The ^1H MAS NMR spectra recorded during the coformer exchange reaction of gently mixed caffeine maleic acid (CAF_2MAE_1) and glutaric acid (GLU) in the ratio 1:1 (1(CAF_2MAE_1) +1(GLU)) is shown in Figure 3a. Initially, the three characteristic acidic peaks at 14.5 ppm, 12.6 ppm and 10.9 ppm were observed. After two hours, a peak at 18.9 ppm appeared. No further changes were observed, up to 4 hours except for the slight increase in intensity of the peak at 10.9 ppm and peaks in alkyl (0 to 5 ppm) and alkene (6 to 8 ppm) regions. The temperature was

then increased from the ambient temperature to 303 K and the ^{1}H MAS NMR spectra were recorded after every 10 minutes, up to one hour till no further changes were observed. The same procedure was followed for every temperature step, up to 350 K. Then the temperature was brought down from 350 K to 298 K following the same procedure. The characteristic acidic peaks at 14.5 ppm, 18.9 ppm and 12.6 ppm disappeared at 333 K and were absent till cooled down to the ambient temperature. The characteristic peaks at 13.5 ppm and 13 ppm appeared immediately after cooling and the peak at 15.7 ppm appeared when the spectrum was recorded after keeping the sample out of the spectrometer for a night, and the peak at 14.5 ppm, which was present at the start of the experiment, completely disappeared. These results can be understood as follows. At the start of the measurements, the individual components CAF_2MAE_1, and GLU are present, indicated by their characteristic peaks at 14.5 ppm and 12.6 ppm. An additional peak at 10.9 ppm, which is assigned to CAF_1GLU_1 Form I is also observed. The formation of CAF_1GLU_1 Form I is the most important product in this exchange reaction. The presence of this peak, unexpected at the initial phase implies that centrifugal pressure and high rotational kinetic energy due to spinning at 60 KHz is sufficient for the formation of CAF_1GLU_1 Form I. After two hours, a peak appeared at 18.9 ppm which is a characteristic peak of CAF_1MAE_1 and remains unchanged up to 4 hours indicating that stoichiometric conversion of CAF_2MAE_1 to CAF_1MAE_1 is taking place, releasing one CAF. CAF_1MAE_1 and CAF_1GLU_1 Form I coexists at this stage. From 333 K, all the acidic peaks disappeared. After cooling, the CAF_2MAE_1 peak at 14.5 ppm which was observed at the beginning of the experiment, completely disappeared. The other CAF_1MAE_1 (18.9 ppm) peak was also absent after cooling and after keeping the sample out overnight, suggesting a complete replacement of maleic acid from caffeine maleic acid (CAF_2MAE_1) has occurred. There is an increase in the sharpness of

peaks when reaction happens and these sharp peaks were not present in the ^{1}H MAS spectra of the individual components at higher temperature. The increase in the sharpness of the peaks observed in the alkyl and alkene region could be due to the combined effect of centrifugal pressure and higher temperature which results in the increase in mobility of these groups.[3132] Immediately after cooling, we can see both forms of CAF_1GLU_1 (13.5 ppm and 10.9 ppm) peaks. As per the reported literature, the transition temperature of CAF_1GLU_1 Form I to CAF_1GLU_1 Form II is 66 ℃ and at 79℃ Form I and Form II can remain in equal amount. Depending upon the environment in which the Form I is present, it can remain either unchanged or can be converted into Form II.[30] Here the formation of CAF_1GLU_1 Form I and its presence throughout the reaction is only due to centrifugal pressure at high spinning, which stabilizes the CAF_1GLU_1 Form I and hence both forms coexists at the end of the reaction. The expected maleic acid peak, was observed only in the spectrum that was recorded after keeping the sample out overnight. This result was quite surprising and this is attributed to the isomerization of maleic acid to fumaric acid at high temperatures, as reported earlier by other workers.[333435] In addition to temperature, centrifugal pressure also has a role in isomerisation since Kawamura *et al.* have also reported that increased pressure induced by fast MAS have generated isomerization of retinal from all-trans to 13-cis state in the membrane protein Bacteriorhodopsin. [36] To confirm the above observation, the spectrum of fumaric acid at 298 K was recorded, and one of the acidic peaks of fumaric acid is at 13 ppm (Figure 3b), and it was observed that even when the reactants were cooled to the ambient temperature, this peak remained. The maleic acid peak at 15.9 ppm, appeared in the spectrum recorded after keeping the rotor overnight at the ambient temperature, suggesting that the fumaric acid has converted back to maleic acid, as expected. Since the reaction was carried out only up to 350 K, and the

sublimation temperature of MAE is 368K, there is no possibility of the loss of MAE due to sublimation, which is also consistent from variable temperature study of MAE (Figure 2e).

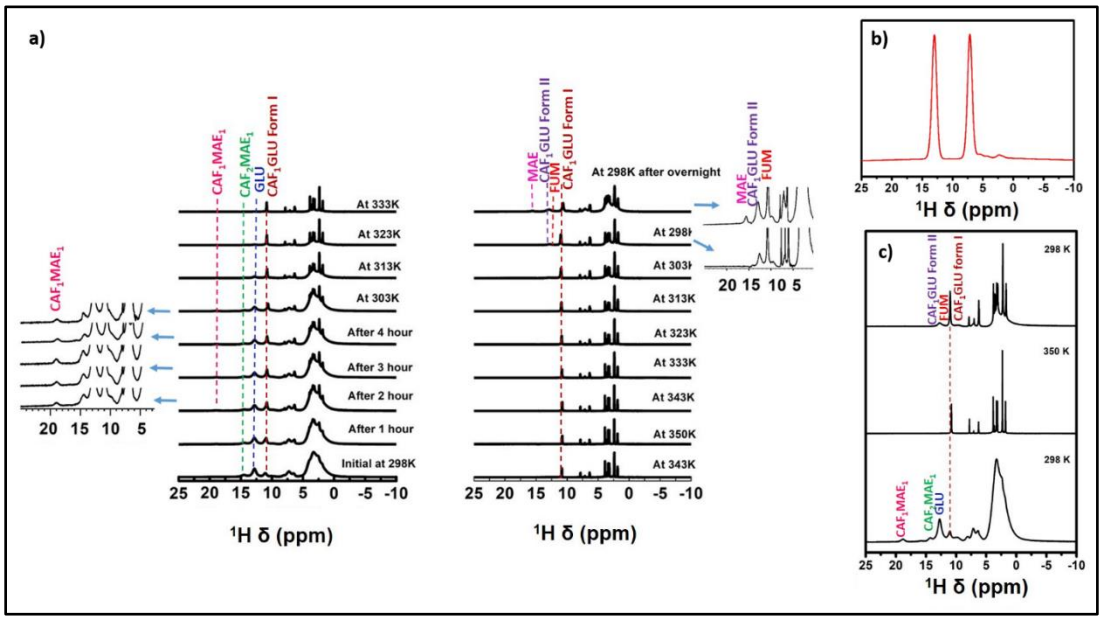

Figure 3: ^{1}H MAS NMR spectra of a) gently mixed 1(CAF_2MAE_1) +1(GLU) from 298 K to 350 K and back b) Fumaric acid at 298 K and c) grounded 1(CAF_2MAE_1) +1(GLU) at 298 K, heated to 350 K and then cooled to 298 K

b) Reactants grounded in the ratio 1:1 (1(CAF_2MAE_1) +1(GLU))

The ^{1}H MAS NMR spectra recorded during the coformer exchange reaction of CAF_2MAE_1 with GLU, which was grounded in the ratio 1:1, is shown in figure 3c. The characteristic acid peaks of CAF_1 MAE_1 (18.8 ppm), CAF_2MAE_1 (14.5 ppm), GLU (12.6 ppm) and CAF_1GLU_1 Form I (10.9 ppm) are observed in the initial spectrum. The remarkable difference between the grounded and the gently mixed samples was that the peak (18.8 ppm) which was observed after 4 hours for the sample that was gently mixed, was seen at the starting itself. Therefore, this reaction was monitored only with changing temperature and not with time. The temperature was

increased from the ambient temperature to 303 K and the ^{1}H MAS NMR spectrum was recorded after 10 minutes when the temperature stabilizes. The same procedure was followed at every temperature step up to 350 K. The temperature was brought down from 350 K to 298 K following the same procedure, and after cooling down to 298 K, in the spectrum that was recorded, the peaks corresponding to CAF_2MAE_1 (14.5 ppm) and CAF_1MAE_1 (18.8 ppm) had completely disappeared and only peaks corresponding to CAF_1GLU_1 (13.5 ppm and 10.9 ppm) were observed, indicating that a complete replacement was taking place. In this case also the fumaric aicd peak was observed at 13 ppm after cooling which is attributed to the isomerization of maleic aicd to fumaric acid at higher temperature, indicating that the reaction pathways are similar in the gently mixed and the grounded system..

The reaction pathways of gently mixed and grounded components are given in the Figure 4.

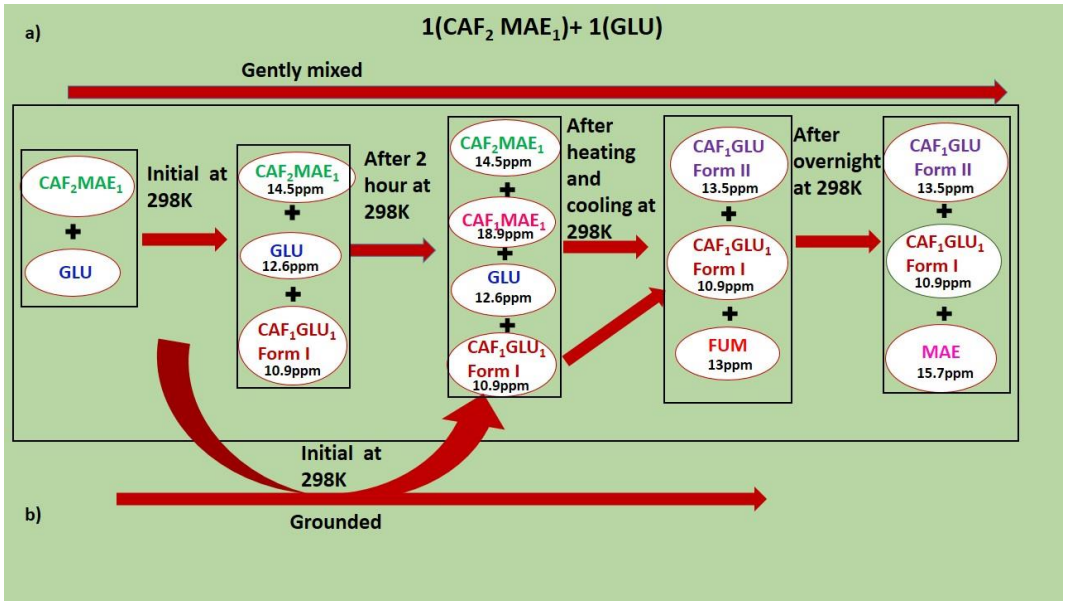

Figure 4: Reaction pathway of 1(CAF_2MAE_1)+1(GLU) coformer exchange reaction

<u>Coformer replacement reaction in caffeine glutaric acid cocrystal</u>

The results of the ^{1}H MAS NMR spectra recorded during the coformer exchange reaction of gently mixed caffeine glutaric acid (FII, 1:1) and maleic acid, in the ratio 1:1 (1(CAF_1GLU_1)+1(MAE)) is shown Figure 5. The peaks at 15.7 ppm, and 13.5 ppm are clearly the characteristic peaks of MAE and CAFGLU Form II. No changes were observed in the spectra at ambient temperature up to 1 hour, and up to 323 K. However, from 333 K, the characteristic peaks of CAF_1MAE_1 (18.9 ppm) and CAF_1GLU_1 Form I (10.9 ppm) started to appear, and were present up to 343 K and then disappeared at 350 K. After cooling to 298 K, the final spectra show the presence of both forms of CAF_1GLU_1 (13.5 ppm and 10.9ppm), MAE (15.7 ppm), and CAF_1MAE_1 (18.9 ppm). These unexpected results can be rationalized as follows. As per the reported literature, CAF_1GLU_1 exists in two forms: metastable Form I which is stable at higher temperature and Form II stable at low temperature and CAF_1GLU_1 Form II is more stable than CAF_2MAE_1. Therefore, maleic acid cannot replace glutaric acid from the stable CAF_1GLU_1 Form II. However, at 323 K, when some of the CAF_1GLU_1 Form II gets converted to Form I, the reaction with MAE occurs. Vangala *et al.* reported that at 79℃ both forms can coexists in equal amount.[30] Thus, the polymorphic transformation of Form II to Form I trigger the reaction at 333 K, which could be happening, since in addition to the temperature of 333 K, the centrifugal pressure due to spinning of the sample is also playing a significant role in facilitating the polymorphic transformation which is taking place at a much lower temperature than that is reported in the literature. The variable temperature ^{1}H MAS SSNMR study of caffeine glutaric acid also showed that the Form I appears at 333 K (Figure 2e). The coformer exchange reaction pathway in caffeine glutaric acid cocrystal is given in the Figure 6.

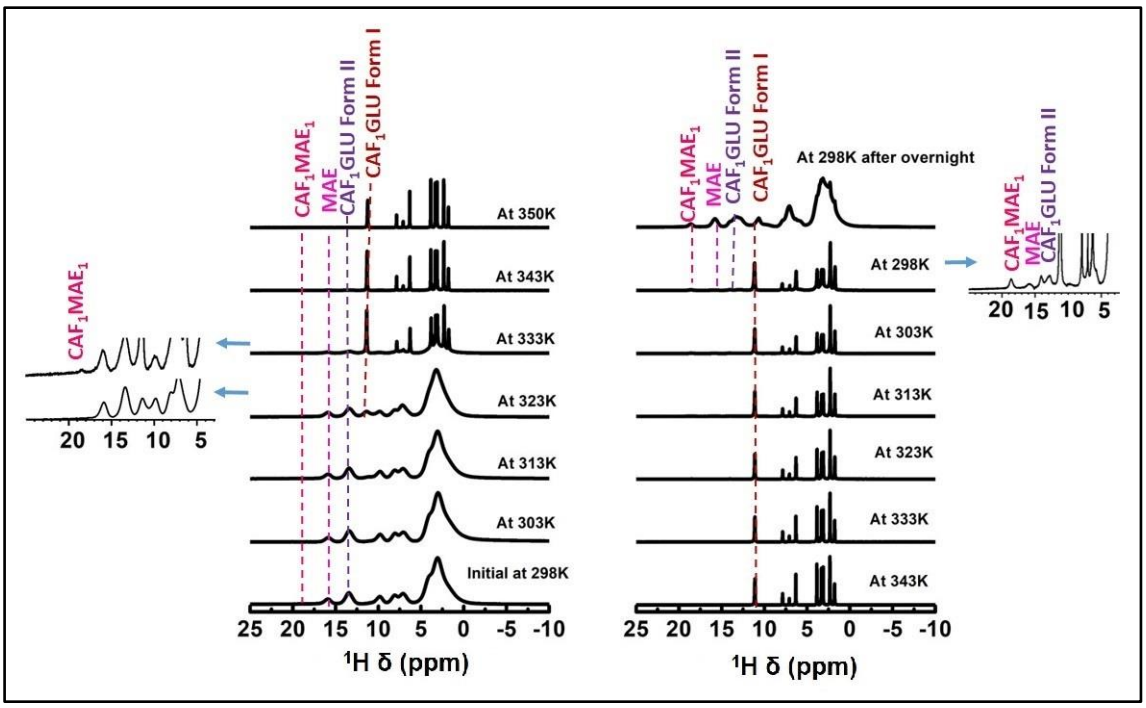

Figure 5: ^{1}H MAS NMR spectra of gently mixed a) 1(CAF_1GLU_1) +1(MAE) at various temperatures from 298 K to 350 K and back.

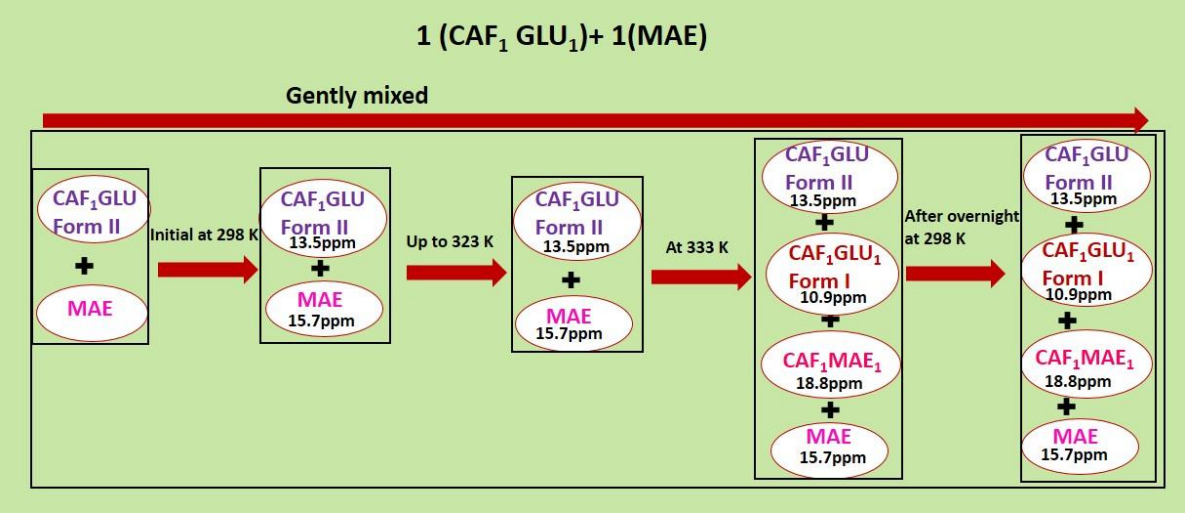

Figure 6: Reaction pathway of 1(CAF_1GLU_1)+1(MAE) coformer exchange reaction

Coformer exchange reaction in caffeine maleic acid cocrystal at a different stoichiometry

These experiments were carried out to understand the effect of stoichiometry of reactants on the coformer exchanges reactions.

a) Reactants gently mixed in the ratio 2:1 (2(CAF_2MAE_1) +1(GLU))

The ^1H MAS NMR spectra recorded during the coformer exchange reaction of gently mixed CAF_2MAE_1 and GLU in the ratio 2:1 is shown in Figure 7a. The reaction was carried out using the same procedure as that was done for the 1:1 ratio. Initially, three characteristic acidic peaks at CAF_2MAE_1 (14.5 ppm), GLU (12.6 ppm) and CAF_1GLU_1 (10.9 ppm) were observed. After two hours, a peak at 18.9 ppm appeared which can be attributed to the CAF_1MAE_1. The temperature was then increased from the ambient temperature to 303 K and ^1H MAS NMR spectra were recorded after every 10 minutes, up to one hour till no further changes were observed. The same procedure was followed for every temperature step, up to 350 K. Then the temperature was brought down from 350 K to 298 K following the same procedure. After cooling, the peak at 14.5 ppm and 13.5 ppm appeared, corresponding to CAF_2MAE_1 and CAF_1GLU_1 Form II and no other characteristic peaks were observed immediately after reaching 298 K. When the ^1H spectrum was recorded after keeping the sample overnight, the characteristic peaks of MAE at 15.7 ppm and that of CAF_1MAE_1 at 18.8 ppm appeared along with that of CAF_2MAE_1 at 14.5 ppm, CAF_1GLU_1 Form I at 10.9 ppm and CAF_1GLU_1 Form II at 13.5 ppm. Thus, since the peak corresponding to CAF_2MAE_1 has reappeared, it can be concluded that a complete replacement of maleic acid with glutaric acid has not taken place. If a complete replacement reaction has happened, the expected products would be MAE, CAF_1MAE_1 and CAF_1GLU_1. However, it is observed that CAF_2MAE_1 coexists with CAF_1MAE_1 and CAF_1GLU_1 which clearly implies that stoichiometry of the reactants plays a crucial role in competitive coformer exchange reaction.

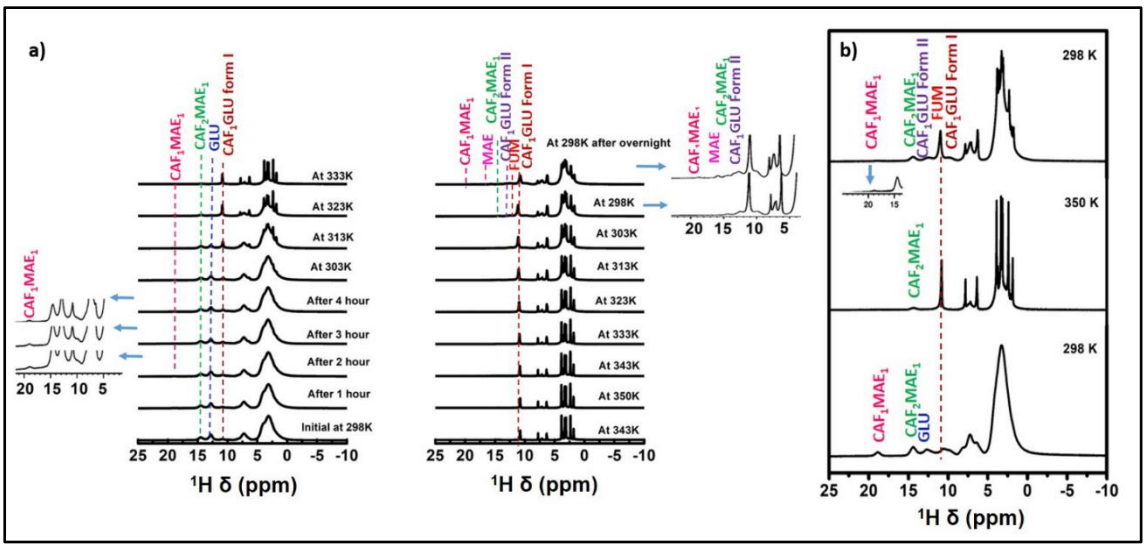

Figure 7: ^{1}H MAS NMR spectra of gently mixed a) 2(CAF_2MAE_1) +1(GLU) at various temperatures from 298 K to 350 K and back and b) ground 2(CAF_2MAE_1) +1(GLU)

a) Reactants grounded in the ratio 2:1 (2(CAF_2MAE_1) +1(GLU))

The ^{1}H MAS NMR spectra recorded during the coformer exchange reaction of grounded 2(CAF_2MAE_1) +1(GLU) is shown in Figure 7b. Initially the characteristic acid peaks observed are of CAF_1MAE_1 at 18.8 ppm, CAF_2MAE_1 at 14.5 ppm, GLU at 12.6 ppm and CAF_1GLU_1 Form I at 10.9 ppm. In this experiment also, the reaction was monitored only with the temperature and not with time since the peak at 18.8 ppm which was observed after 4 hours in the gently mixed case was seen at the starting itself. The temperature was then increased from the ambient temperature to 303 K and the ^{1}H MAS NMR spectrum was recorded after 10 minutes when the temperature stabilizes. The same procedure was followed at every temperature step up to 350 K. The temperature was brought down from 350 K to 298 K following the same procedure, after cooling, in the final spectra, the characteristic peaks of CAF_2MAE_1 at 14.5 ppm

and CAF_1MAE_1 at18.8 ppm, CAF_1GLU_1 at 13.5 ppm and 10.9 ppm peaks were observed. In this case also, the results indicate that complete replacement of MAE with GLU is not taking place.

The reaction pathway of both gently mixed and grounded components are given in the Figure 8.

Our study shows that polymorphism, and stoichiometry plays significant role in competitive coformer exchange reaction which is influenced by other reaction conditions such as temperature, pressue etc, and ^{1}H MAS NMR captured the reaction pathway of competitve coformer exchange reaction very effectively.

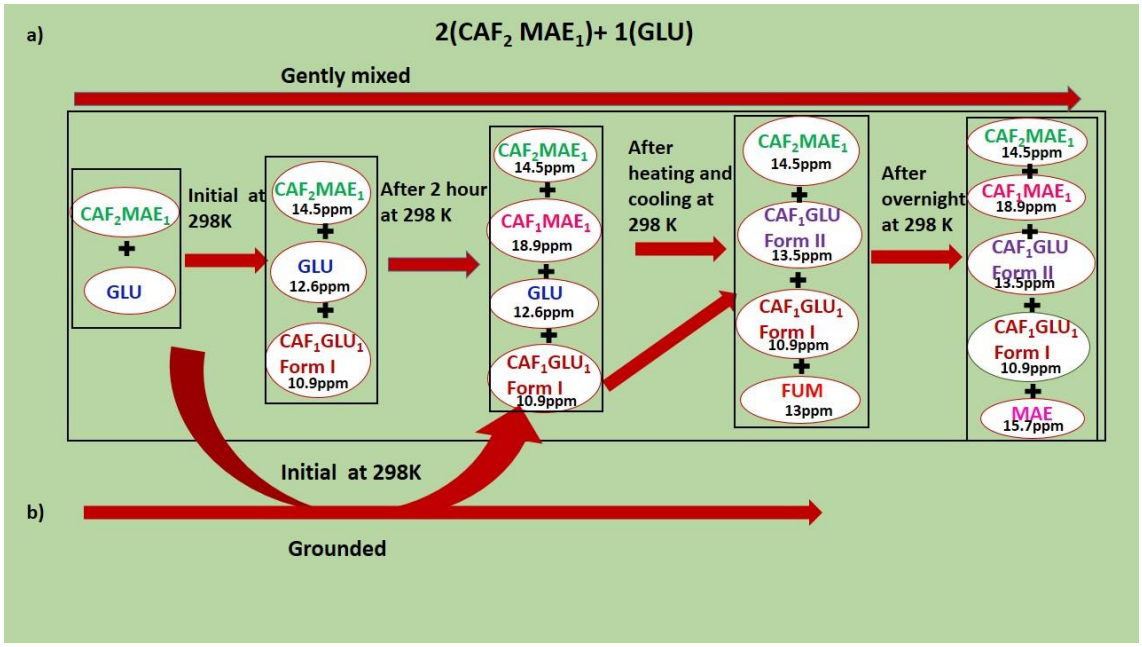

Figure 8: Reaction pathway of $2(CAF_2MAE_1)+1(GLU)$ coformer exchange reaction

Conclusions

The competitive coformer exchange reactions were in situ monitored using ^{1}H MAS SSNMR, capturing the reaction pathways very effectively. This study shows that a complete replacement of maleic acid from CAF_2MAE_1 occurred, resulting in the formation of CAF_1GLU_1, when the reactants were in the ratio 1:1 ($1(CAF_2MAE_1)$ +1(GLU)). In all the reactions, an important

intermediate product is CAF_1GLU_1 Form I, which was observed at the starting itself even though it is metastable as per the reported literature. This observation indicates that the centrifugal pressure due to high frequency sample spinning imparts stability to Form I, enabling it to be present throughout the reaction. Absence of maleic acid at the end of the reaction was surprising, but the presence of fumaric acid suggest that isomerisation of maleic acid to fumaric was taking place at higher temperatures. The difference in the end products of the reactions at reactant stoichiometry of 1:1 and 2:1 points out that a complete replacement of maleic acid has occured only in the 1:1 stoichiometry of the reactants. The reverse reaction (CAF_1GLU_1 + MAE), resulted in the formation of CAF_1MAE_1, despite CAF_1GLU_1 considered to be more stable, and no reaction expected, is attributed to the polymorphic transition of CAF_1GLU_1 Form II to Form I triggering the exchange reaction at 333 K. Thus new reaction pathways in competitive coformer exchange reactions were revealed, and the remarkable role of stoichiometry, polymorphism, temperature and centrifugal pressure could be distinguished. These pathways will provide significant support to the pharmaceutical formulators in selection of coformers and product excipient.

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ABBREVIATIONS

API

CAF

MAE

GLU

CAFMAE

CAFGLU

MAS

NMR

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