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Stabilization of Metastable Flufenamic Acid by Inclusion of Mefenamic Acid: Solid Solution or Epilayer?

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ABSTRACT: The physical stability of metastable form I of flufenamic acid (FFA) increased by using mefenamic acid (MFA) as an inclusion compound. We studied the extent of this effect and explained the mechanism by investigating the effect of the presence of MFA on nucleation and crystal growth of the mixed crystals and the effects it has on the surface morphology. We conclude that the polymorphic transformation of FFA was inhibited in the presence of MFA both by lowering the difference in free energy of the MFA/FFA I and MFA/FFA III solid solution crystals, and also by forming an epilayer, thus affecting the kinetics of the polymorphic transformation. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:4013–4022, 2010

Keywords: flufenamic acid; mefenamic acid; inclusion compound; solid-state polymorphic transformation; solid solution; epilayer

INTRODUCTION

Polymorphism exists when a compound can crystallize with different crystal structures.[1] The differences in structure necessarily lead to differences in free energy between polymorphs. There is only one thermodynamically stable polymorph; others are called metastable under given temperature and pressure except at the exact equilibrium conditions. Crystallization often does but not always produce the stable phase at a given set of experimental conditions. The classical view on this phenomenon was stated by Ostwald namely that a system does not seek for the most stable form but rather the form that causes the least amount of change in free energy.[2] A more modern theory suggests that the nuclei of different polymorphs compete during the crystallization process, where the form is decided depending on the rate of nucleation and/or crystal growth.[3] Regardless of the mechanism, metastable crystal formation is caused by kinetic rather than thermodynamic factors. The concept of thermodynamic metastability indicates that there is a driving force toward a more stable phase, that is, there is a tendency for a polymorphic transformation.

Being in a higher state of energy, metastable forms may have advantages over the stable form in terms of their solubility, manufacturability, and/or hygroscopicity.[4,5] When solubility and/or bioavailability are limited, formulating a metastable crystalline phase may improve a drug's performance, amongst other approaches such as amorphization or using nanocrystals. As a general rule, the less stable the state of the material, the higher its solubility and dissolution rate, but at the same time this inherently introduces stability issues. Besides chemical stability, on the physical level, a lower stability invariably creates a driving force for (re-)crystallization, which diminishes the positive effect of the chosen form over time and thereby lowers the product shelf-life. It depends on the rate, or kinetics, of the reaction whether this becomes a prohibitive problem. From a product formulation (or "form selection") point of view, there is therefore always a trade-off between solubility and stability. As a mediator between the inherent properties of different forms, stabilizing agents can sometimes be employed, which may offer a potential to get the best of two worlds and optimize product performance.

In order to understand the working mechanism of a stabilizing agent, it is necessary to first understand the mechanism of polymorphic transformations. Paul and Curtin[6] proposed a four-step mechanism for solid-state chemical reactions: (1) "loosening of the molecules at the reaction site; (2) molecular change; (3) solid-solution formation; and (4) separation of product." A logical extension of this concept was proposed

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by Byrn, Pfeiffer, and Stowell (see Ref.[1], p. 259) for solid-state physical transformations: (1) "molecular loosening in the initial phase, (2) formation of an intermediate solid solution, (3) nucleation of the new solid phase, and (4) the growth of the new phase." Similarly, a solution-mediated transformation occurs via the dissolution of the metastable form followed by the nucleation and crystal growth of the stable form from that solution. The difference between the solid-state and the solution-mediated polymorphic transformation is hypothesized to be the first two steps: "molecular loosening and formation of an intermediate solid solution" in the solid-state polymorphic transformation and dissolution of the metastable form in the solution-mediated polymorphic transformation. For the solid-state process it may be more appropriate to think of the solid solution in step 2 as a "disordered phase" perhaps containing molecular arrangements of both the starting phase and the final phase.

It is well known that structurally related compounds affect solution-mediated polymorphic transformations and crystallization by inhibiting the nucleation of the stable form or by providing seeds for the stable form.[4,7–12] However, there has been little work regarding the stabilization of metastable phases or organic molecular crystals by using additives during solid-state transformation.

An interesting yet little pursued avenue is the stabilization of the metastable phase by creating solid solutions by incorporation of a structurally related compound.[13] It was argued that the incorporation of additives can change the relative stability between the metastable phase and the stable phase thus reducing the driving force for a transformation to the stable form. When different components form mixed crystals, they can either be stoichiometric (co-crystals or salts) or nonstoichiometric. In the latter case, there are many different types of structural arrangement but when the distribution is random we refer to these crystals as solid solutions. Analogs to solutions, when the mixing of the components in the solid solution is ideal, they can mix at any ratio. However, nonideal mixing, typically caused by the misfit of a foreign molecule in the crystal lattice, will lead to a certain limit of mixing, or "solubility," at a given temperature and pressure. Therefore, just like mixtures of liquids, phase separation will occur above the mixing limit, which leads to conglomeration.[14]

One specific type of heterophase system, when two compounds crystallize in a same vessel, is an epilayer formation of one compound to the other. It is well known that impurity adsorption on the surface of a substrate crystal changes the surface property by altering the surface energy of the substrate crystal. For example, impurity adsorption on the surface can introduce different lateral interactions and thus strains on the surface of a substrate crystal. In most cases, an adsorbed impurity causes point defects and changes the crystal structure locally. However, it is also possible to restructure the whole surface with a different preferred orientation.[15]

It was shown that an *Fe* epilayer induced the phase transformation of *Co*-crystal from *h.c.p.* phase to *b.c.c.* structure.[16] *Co*-crystals were grown sandwiched between *Fe* layers. The *Fe* layer, when it served as a substrate, induced the epitaxial growth of *Co*-crystals with *b.c.c.* structure. In addition, when the *Fe* epilayer was formed on *Co*-crystals with *h.c.p.* structure, the *Fe* epilayer induced the recrystallization of *Co*-crystals with *b.c.c.* structure.

It is hypothesized that impurity adsorption, epilayer formation, might not affect the bulk property of the substrate, in our case, relative phase stability; however, it might be possible to alter the energy barrier of the transformation of a substrate having considered that the nucleation of new phase during solid-state transformation starts on the surface of the substrate crystal.

Flufenamic acid (FFA) is known to have at least eight polymorphs.[17] When seeded, both FFA forms I and III can be grown at room temperature. FFA I and FFA III are enantiotropically related. FFA III is stable below 42°C, whereas FFA I is the stable form from 42°C all the way up to the melt. The reversible solid-state transformation between these two forms shows a large hysteresis effect. At room temperature FFA I crystals show a slow transformation to FFA III. FFA III crystals transform to FFA I at around 115°C.[17] FFA III crystals are yellow and typically grow as rod-shaped crystals. FFA I is white and grows to tablet-shaped crystals. Albeit slow at room temperature, the phase transition from FFA I to FFA III can be observed easily by the color change from white to yellow.

In our studies, we used mefenamic acid (MFA) as a structurally related impurity compound. Figure 1 shows how it is related to FFA. Both compounds are within the same class of fenamic acid derivatives and as such have the same pharmaceutical application, as a nonsteroidal antiinflammatory drug. Therefore, at least in principle, mixed crystals of these two compounds could be used as a real drug product instead of just serving as an academic exercise.

We grew crystals of both pure forms I and III of FFA as well as mixed crystals of these forms with MFA as an inclusion compound at different mixture ratios to investigate the effect it would have on the solid-state phase transition of the metastable FFA I to the stable FFA III at room temperature. Further, we studied the effect of MFA on the crystal growth of these crystals, particularly the surface morphology. Bulk properties of the mixed FFA/MFA crystals were investigated by PXRD, DSC, and solubility measurements.

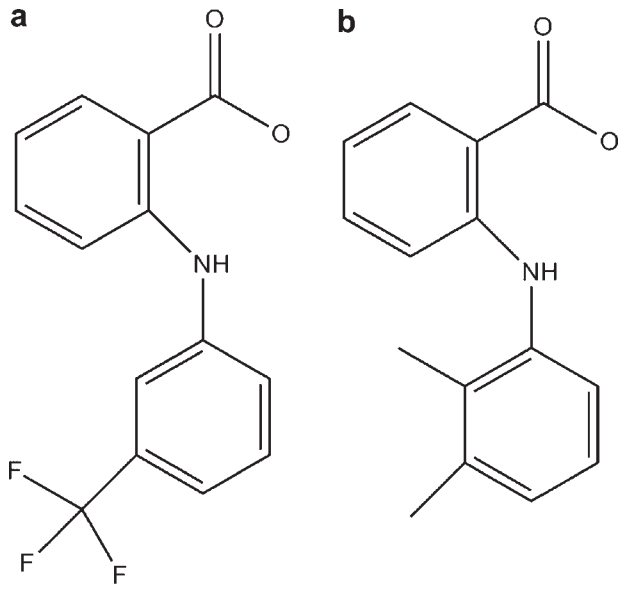

Figure 1. Chemical structures of (a) flufenamic acid ($C_{14}H_{10}F_3NO_2$: 281.24 g/mol) and (b) mefenamic acid ($C_{15}H_{15}NO_2$: 241.29 g/mol) showing the similarity between flufenamic acid (FFA) and mefenamic acid (MFA).

MATERIALS AND METHODS

Materials

FFA and MFA (Fig. 1) were purchased from Sigma–Aldrich (St. Louis, MO). Water with trifluoroacetic acid 0.1% (v/v) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). Toluene and HPLC-grade acetonitrile were obtained from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). Water was double-distilled and filtered with Milli-Q® ultrapure water purification system (Billerica, MA).

Crystallization of FFA I and III Crystals in the Presence of MFA

Pure FFA I and III crystals were crystallized, respectively, by dissolving 2 g of pure FFA I or FFA III in 10 mL toluene in a scintillation vial in an oven at 65°C. The solutions were then transferred to Petri dishes, cooled, and then the toluene was allowed to evaporate slowly with Petri dish covers on at room temperature. After the growth the crystals were rinsed with toluene. Crystals of FFA III can be easily grown since it is the stable form at room temperature. However, FFA I is the metastable form at room temperature. Therefore, it was necessary to grow FFA I crystals starting with FFA I powder. We believe that there is seeding effect on the formation of FFA I crystals although we completely dissolved FFA I powder at 65°C. Mixed crystals of FFA/MFA were grown in a similar way of growing pure FFA crystals. However, a known amount of MFA was mixed with FFA powders in a vial to reach mixtures of MFA/FFA in the range 0.25–9.01% by weight.

The crystallization rate was varied in order to investigate its effect on the uptake of MFA in FFA I and FFA III: a fast rate of crystallization for mixed crystals of FFA I/MFA was achieved by cooling the solution from 65 to 15°C with a rate of 10°C/min, and (2) a slow crystallization for mixed crystals of FFA III/MFA was achieved by using the solution-mediated polymorphic transformation of mixed crystals of FFA I/MFA to mixed crystals of FFA III/MFA. The mixed crystals of FFA I/MFA were grown by dissolving 2 g of FFA I with the known amounts of MFA in 10 mL toluene in a scintillation vial in an oven at 65°C. The vial was removed from the oven and then cooled at room temperature in order to grow the mixed crystals of FFA I/MFA. The vial was kept at room temperature until the mixed crystals of FFA I/MFA transformed to the mixed crystals of FFA III/MFA. Since FFA I is the metastable form at room temperature, the solution-mediated polymorphic transformation from the mixed crystals of FFA I/MFA to FFA III/MFA in toluene occurs. In this case, it took several weeks to months to obtain FFA III crystals containing MFA.

Images of FFA Crystals

Pictures of FFA crystals were obtained using a digital camera, Nikon Coolpix 995 (Nikon, Inc., Melville, NY). ISO speed ranged from 285 to 400, exposure time from 0.25 to 1 s, focal length from 11 to 22 mm. All pictures were taken without flash.

High-Performance Liquid Chromatography (HPLC) of FFA and MFA

The amounts of FFA and MFA were determined by HPLC, Agilent 1100 series (Agilent Technologies, Inc., Santa Clara, CA) with a diode array detector. HP Chemstation (Agilent Technologies, Inc.) was used for data analysis. A zorbax reverse phase-C_8, 4.6 mm × 250 mm analytical column (Agilent Technologies, Inc.) was used at 30°C. The mobile phase consisted of A (water with trifluoroacetic acid 0.1%, v/v) and B (acetonitrile) (60:40, v/v). The flow rate was 2 mL/min. Samples were analyzed at UV $\lambda = 280$ nm.

Powder X-Ray Diffraction (PXRD)

PXRD analysis was performed on FFA powders obtained by crushing FFA crystals containing MFA using a Siemens D5000 X-ray diffractometer (Bruker AXS, Inc., Madison, WI) equipped with Cu K_α radiation. Samples were analyzed over the range 6–40° (2θ) at the rate of 1°/min with step size of 0.01°. For reference purposes, a powder pattern for FFA I was calculated using Mercury 1.4 from the single crystal structure data obtained from the CSD crystal structure, refcode FPMCA11 (The Cambridge Crystallographic Data Centre, Cambridge, UK).

Differential Scanning Calorimetry (DSC)

Thermal analysis was conducted using a Q2000 DSC (TA Instruments, New Castle, DE). The temperature scale and heat flow were calibrated by measuring the onset temperature and the enthalpic response of an Indium standard. The aluminum pans were hermetically sealed and the samples were heated from 0 to 150°C and subsequently cooled from 150 to 0°C at a rate of 5, 10, or 20°C/min. Data were acquired and analyzed using Universal Analysis 2000 software v. 4.1D (TA Instruments).

Scanning Electron Microscopy (SEM)

The surface morphology of the (1 0 0) face of pure FFA I and FFA I crystals grown with various amounts of MFA were analyzed using a JEOL JSM-840 (Jeol USA, Inc., Peabody, MA). Samples were prepared for analysis by placing a crystal on carbon double-sided tape fixed to an aluminum mount. Samples were sputter coated using a Hummer I Sputter Coater (Technics, Alexandria, VA) at ~10 mA and ~100 mTorr (Ar) with Au/Pd for 3 min. Under high vacuum mode, an Everhart Thornley detector was used with a beam voltage of 0.5 kV. Data were analyzed using digital image scan generator ver. 2.10.

RESULTS AND DISCUSSION

Solid-State Polymorphic Transformation of Pure FFA

Figure 2 shows a typical solid-state polymorphic transformation of FFA I to FFA III followed over the course of time. In this particular case two independent nuclei were formed on the surface (on the top and right side). The growth of the stable phase can be seen to gradually traverse through the crystal by the advance of the yellow front. As opposed to what was reported for 4-methyl-2-nitroacetanilide,[13] the shape of the crystal did not change during the transformation, which McCrone classified as the formation of a pseudomorph.[18] The area in transformation is indicated by the black lines. The rate of the polymorphic transformation was reported to decrease with time, which is consistent with our present result. More than half the crystal had transformed after 48 h, where it took at least another 150 h to complete the transformation. When MFA (0.25–9.01, w/w, %) was used as an additive during crystallization, the solid-state polymorphic transformation did not occur within 2 years.

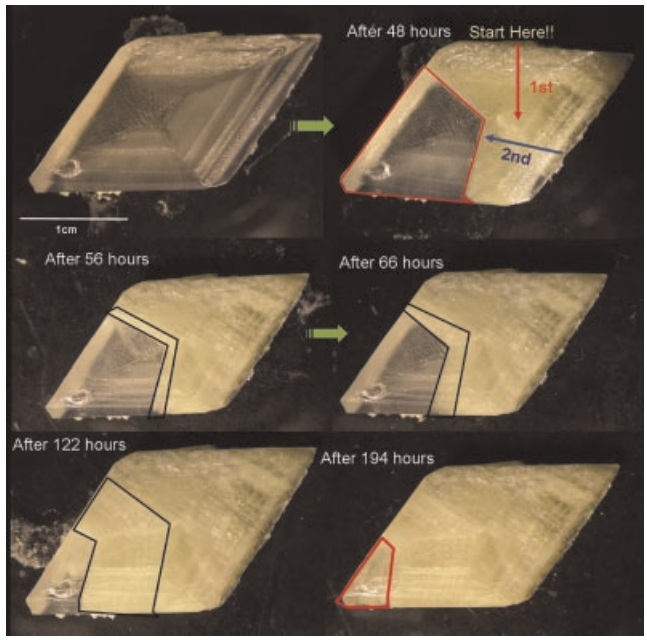

Figure 2. The solid-state polymorphic transformation of FFA I to FFA III followed over time. The transformation is observed as a change in color from white to yellow. At 48 h the area outlined in red is still in the original FFA I, whereas the rest had transformed to FFA III. The difference between subsequent observations is indicated with black lines. After a total of 194 h, the transformation is almost fully complete. These observations suggest that the actual transformation happens at a straight growth front that traverses through the crystal.

Effect of MFA on Crystallization of FFA

Inhibition of Nucleation

According to classical nucleation theory (Eq. 1), the rate of nucleation increases as interfacial tension decreases and as supersaturation and/or temperature increases. In this respect, additives can decrease the rate of nucleation by increasing the critical supersaturation for nucleation, at which induction time approaches 0, and/or by increasing the interfacial tension, and vice versa

$$J = A\exp\left(\frac{-\Delta G_{\text{crit}}}{kT}\right) = A\exp\left[-\frac{16\pi\gamma^3\upsilon^2}{3k^3T^3(\ln S)^2}\right] \quad (1)$$

where A is some arbitrary factor, γ the interfacial tension between the nucleus and the supersaturated solution, υ the molecular volume, k the Boltzmann constant, T the temperature, and S the supersaturation. In Eq. 1, it is assumed that the shape of the critical nucleus is spherical.[19]

In our system, MFA significantly increased the induction time from several hours to almost a week. Related studies conducted in our laboratories showed that FFA increases the solubility of MFA (unpublished results: FFA solubility without MFA: 64.589 ± 1.58 mg and FFA solubility with MFA: 71.747 ± 1.78 mg at 25°C in toluene). The total amounts of MFA used in our study ranged from 0.25% to 9.01%. Even at 0.25%, we clearly observed the increase in induction time. Therefore, it is unlikely that the delay

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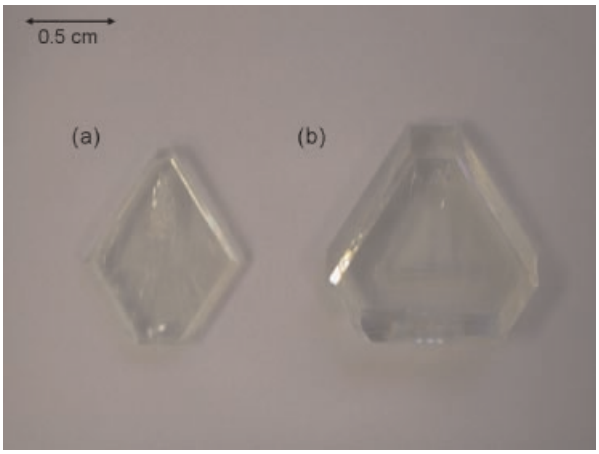

Figure 3. FFA I crystals grown (a) with (0.25, w/w, %) and (b) without MFA showing that the FFA crystal grown with MFA is thinner than the FFA crystal grown without MFA.

of the induction time is solely due to increasing the solubility of FFA, in other words, decreasing the supersaturation, in the presence of MFA. Several types of additives which affect the rate of nucleation can exist: (1) an additive which interacts with the host molecules can attach to a prenuclear aggregates and thus, prevent the aggregates from growing to a critical size for nucleation, (2) an additive which is incorporated into the prenuclear aggregates accelerates dissolution by creating defects in the aggregates, and (3) an additive which inhibits the growth by attaching to the fast growing surface of prenuclei of the host crystal. A related study conducted previously in our laboratories showed evidence of the strong interaction between FFA and MFA during the nucleation processes.[20] In the previous study, the metastable form of MFA was grown in the presence of FFA. It was suggested that the specific interaction between FFA and the stable form of MFA inhibited the nucleation of the stable form of MFA and, thus, allowed the formation of the metastable form of MFA. Therefore, it can also be assumed that the delay of the nucleation of FFA in the presence of MFA is due to the specific interaction between FFA and MFA during nucleation processes.

Morphology

Morphology is an indicator of the growth rate of each face of a crystal. Depending on the growth rate of each face of a crystal, final morphology of the crystal is determined. For example, a relatively large surface implies a slow growth rate perpendicular to the large surface while a small surface implies the fast growth rate perpendicular to the small surface. Therefore, comparison of morphology of FFA crystals with and without the additive, MFA, was made to investigate the effect of MFA on the growth of FFA crystals. In 1958, Cabrera and Vermilyea[21] classified additives to mobile and immobile impurities. Mobility of impurities is important when molecular recognition at the interface is taken into consideration, because sometimes one impurity can show different reactivity on the various faces of a crystal. However, it should be pointed out that this difference can be attributed to the various growth mechanisms at different faces of a crystal.

Crystals grown in the presence of MFA were thinner than crystals of pure FFA as shown in Figures 3 and 4. As the amounts of MFA added during crystallization processes increased, crystals became thinner. This kind of morphology change can imply that MFA inhibits the growth of a large top face of FFA I crystals. There are several types of additives which can induce morphology changes. Additives that share a similar functional group and the same binding mechanism and which are stereospecifically similar to the host molecules such as paracetamol in the presence of metacetamol or *p*-acetoxybenzoic acid can be well adsorbed on the surface of the crystals and will generally block the addition of host molecules.[22] Therefore, the amount of uptake in the host crystals will be high when compared to that of other types of additives. The effect of these additives on the

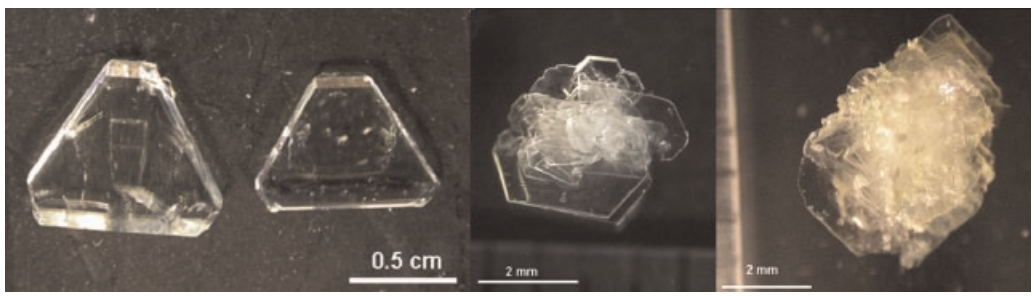

Figure 4. Pictures of FFA I crystal grown from the solution containing FFA and (a) MFA/FFA 0.25 (w/w, %) (b) 0.5 (w/w, %), (c) 2.5 (w/w, %), and (d) 5 (w/w, %) in an initial solution for crystallization showing that the FFA crystals become thinner as the concentration of MFA increases in the initial solution.

morphology change of crystals will not be significant, and these additives will also have many chances to be incorporated into the crystal lattice forming a solid solution. A second group of additives has similar functional groups to attach to the host molecules but also has sterically different functional groups so that additives block the subsequent attachment of host molecules.[22] For this group, the amount of uptake of additives in a crystal will be low since they can only exist on the surface of the crystals, but crystal morphology will be significantly changed. A third group of additives has opposite charges to the molecules on the surface of the crystals. Interestingly, these additives apparently do not form strong bonds with the moiety of the opposite sign but can form nonionic interactions so that they affect the crystal habit. An example of this type is ionic surfactants which interact with the surface carboxyls of an adipic acid crystal.[23] A fourth type is those which reduce the edge free energy of steps so that the two-dimensional nucleation rate on the perfect surface of the crystals and the rate of step advance increases.[21,24] MFA seems to belong to the second type additives. The effect of MFA on the surface morphology of FFA I crystals was studied.

Surface Morphology of FFA Crystal Grown With MFA

The related study conducted in our laboratories showed the epitaxial growth of MFA on FFA crystals.[25] The previous study, which investigated epitaxial growth upon sublimation, showed strong lattice matching as well as similarities of hydrogen bonding patterns and conformations between FFA and MFA. Therefore, it was expected that MFA would prefer to attach on the FFA (1 0 0) face which contains fluoride atoms and/or carboxylic groups. However, based on the fact that FFA crystals became smaller when grown with the high concentration of MFA, it was assumed that MFA can also be incorporated into the crystals or in the sites other than the (1 0 0) face. Figure 5 shows the (1 0 0) face of FFA crystal grown in the presence/absence of MFA in toluene. The surfaces were generally clean and etching pits are small and sharp-edged when FFA crystals were grown without MFA (Fig. 5d). On the contrary, a vertical crack (Fig. 5a) was observed when FFA crystals were grown in the presence of MFA. The large crack indicates that the presence of MFA induces the strain in FFA crystals. Layers near the crack were formed on the surface. Figure 5b shows the enlarged picture of the ends of layers. Edges of the end of layers are rounded with a different slope on each side. In addition, contrary to the small sharp edges of the pure FFA crystal (Fig. 5d), the etching pits became generally curved and rounded indicating that MFA was incorporated into FFA crystals (Fig. 5c). On the basis of Figure 5, MFA seems not only to be involved in an epilayer type attachment (Fig. 5a and b) on the (1 0 0) face of the FFA crystals but also to be incorporated into the crystal (Fig. 5c). The incorporations behavior was further studied using PXRD, DSC, and HPLC.

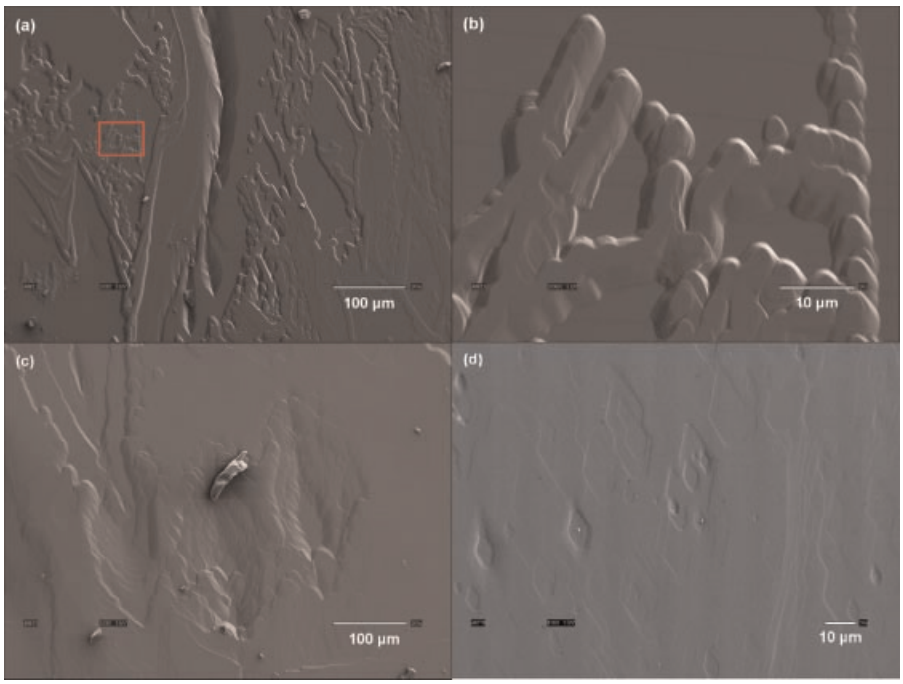

Figure 5. SEM pictures of (a) the (1 0 0) face of FFA I grown with MFA (0.5, w/w, %), (b) the enlarged picture of a boxed area in (a) showing the ends of layer, (c) etching pits on the (1 0 0) face of FFA I containing MFA (0.5, w/w, %), and (d) etching pits of pure FFA I.

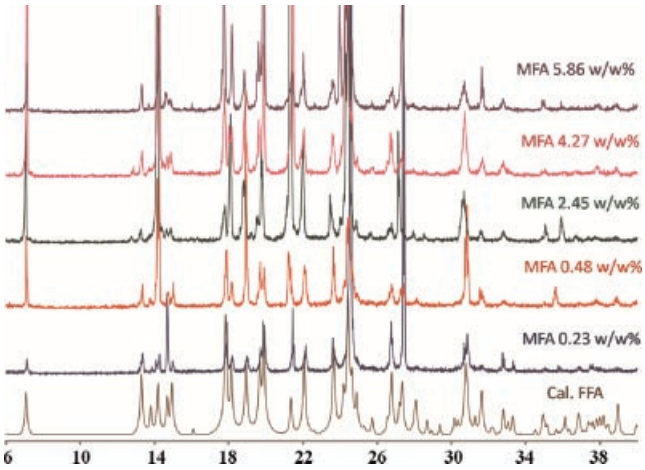

Figure 6. PXRD patterns of calculated FFA I and FFA I crystals containing (1) 0.23, (2) 0.48, (3) 2.45, (4) 4.27, and (5) 5.86 (w/w, %) of MFA.

Solid Solution Formation

Solid Solution Formation Analyzed by PXRD

Lattice parameter changes of FFA due to the incorporation or attachment of MFA were studied using PXRD. The maximum concentration of MFA which can be taken by FFA was 5.86%. No significant structural changes were observed resulting from the incorporation of MFA in FFA except the relative intensity changes and the peak broadening (Fig. 6). This result suggests the formation of a solid solution.

Solid Solution Formation Analyzed by DSC

PXRD analysis and SEM study of etching pits on the (1 0 0) face of FFA crystals demonstrated the solid solution formation between FFA and MFA. The definition of an "ideal" solid solution is the homogeneous mixture of two solids but not a co-crystal (in the case of co-crystal, we call it a co-crystal rather than an ideal solid solution). What is the meaning of "homogeneous" in the solid state? In the case of the powder mixing, it is almost impossible to obtain a homogeneous mixture of two components at molecular level. So, if we sample the powder mixtures, the composition of each sample will be at least slightly different. Such differences in composition are due to the properties of each components, for example, size, shape, and/or density. Especially, taking into consideration that almost all properties of crystals are anisotropic, it is reasonable to assume that additives or impurities can have a tendency to attach to specific sites rather than to random sites.[26,27] This tendency reflects on the morphology changes of FFA in the presence of MFA. Although they are not distributed homogeneously throughout the crystal, the DSC thermogram indicated the solid solution formation by an indefinite start of the melting endotherm, which is typical for solid solution,[19] as well as the melting point depression (Fig. 7 and Tab. 1). Therefore, this system can be referred as an "anisotropic solid solution." In addition to the formation of the solid solution, it was also noted that the transition behavior of FFA III containing MFA appears to be quite different from that of pure FFA transition (Fig. 7). FFA I is the stable form at high temperature. Therefore, FFA I shows only melting at 133.7°C during DSC measurement. However, FFA III is the metastable form at high temperature. Therefore, FFA III shows three events, melting, recrystallization, and remelting when it is pure. Figure 7a shows the DSC thermograms of FFA I containing various amounts (0, 0.24, 2.45, and 4.27, w/w, %) of MFA. As the concentration of MFA increased in FFA I crystals, the melting point as well as enthalpy of fusion decreased (Tab. 1). Figure 7b, c, and d shows the DSC thermograms of pure FFA III versus FFA III containing 0.24% (w/w) of MFA with different heating rates. In this particular case, we varied the heating rate since it was difficult to separate the two events, melting of FFA III and recrystallization of FFA I. Transition of pure FFA III occurs via the melting of FFA III followed by the recrystallization and the remelting of FFA I. At the heating rates of 5 and 10°C/min, we can clearly see the melting point depression. In addition, the melting endotherm of pure FFA I, which was formed after recrystallization of pure FFA III, is similar to that of FFA I, which was formed after recrystallization of FFA III containing MFA. However, the DSC thermograms obtained at all rates show that the melting endotherm and recrystallization exotherm of FFA III containing MFA are much smaller than those of pure FFA III. One explanation is that the energy required to melt and/or recrystallize is smaller for FFA III containing MFA than for pure FFA III.

A necessary requirement for a formation of a solid solution is the similarity between the molecules including the sizes and shapes of two molecules, and a necessary condition for the formation of continuous series of solid solution is the identical space group, the same number of molecules in the unit cell and similar packing in crystal lattice.[28] Otherwise, they will not form a solid solution or they will form a solid solution with discontinuity in solubility. As shown in Figure 1 and Table 2 structures of FFA and MFA are very similar in size and shape; however, the space groups of FFA and MFA are different. Therefore, the discontinuity in solubility between FFA and MFA was expected. The solubility of MFA in two polymorphs of FFA was measured by HPLC.

Solubility of MFA in FFA

Figure 8 shows that the amounts of MFA in FFA I crystals are similar to those of MFA in the solution up

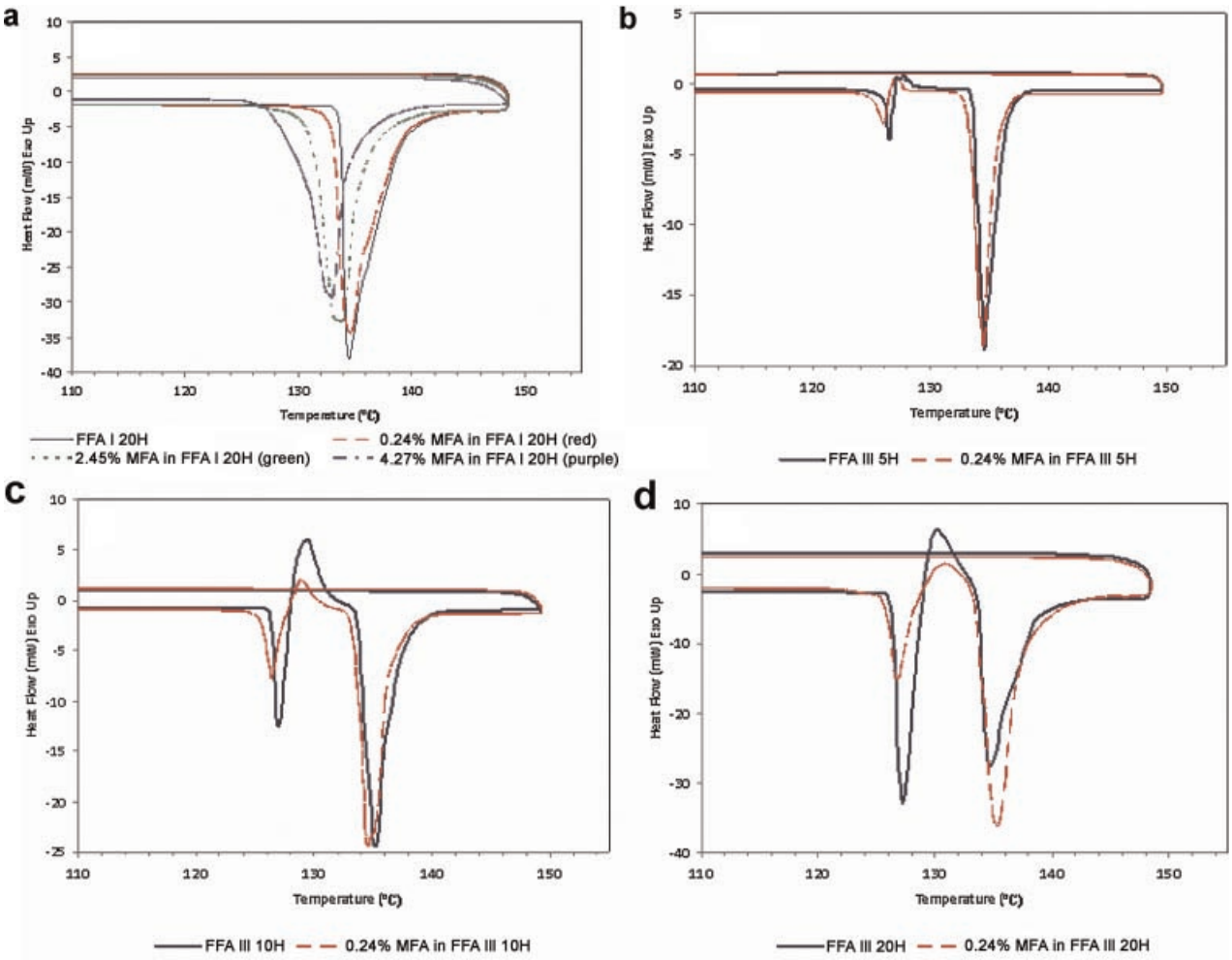

Figure 7. DSC thermograms of (a) pure FFA I and FFA I containing 0.25, 2.45, and 4.27 (w/w, %) of MFA and DSC thermograms of pure FFA III versus FFA III containing 0.24 (w/w,%) of MFA at heating rates of (b) 5°C/min., (c) 10°C/min., and (d) 20°C/min.

to a certain level and then they level off. Empty diamonds in Figure 8 indicate the concentration range that the amounts of incorporation are smaller than the concentration in the solution. The decrease in the extent of incorporation is likely to be due to the solubility limit of MFA in FFA I crystals. In the case of MFA/FFA III mixed crystals, the ratio of MFA to FFA III crystals is lower when compared to that of MFA to FFA I crystals (Fig. 8). The amounts of MFA in MFA/FFA III mixed crystals are only about 20% of MFA in a solution. Another interesting point of this incorporation data is that the amounts of MFA in FFA I crystals were higher when crystals were grown at a slow rate than when crystals were grown at a high rate (cooling from 60 to 15°C at a rate of 10°C/min). However, FFA III showed the opposite phenomenon, namely, that the amounts of MFA in FFA III crystals were higher when crystals were grown at a high rate than when

Table 1. Melting Points and ΔH_f^m of FFA I With/Without MFA at a Heating Rate of 20°C

Ratio of MFA/FFA I (w/w, %)	MP (°C)	ΔH_f^m (kJ/mol)
	133.7	28.3
0.24	133.0	27.4
2.45	131.3	26.7
4.27	130.3	25.3

Table 2. Crystallographic Data of FFA I, FFA III, and MFA I

Parameter	FFA I	FFA III	MFA I[29]
Space group	$P2_1/c$	$C2/c$	$P\bar{1}$
Crystal system	Monoclinic	Monoclinic	Triclinic
a (Å)	12.4329 (6)	39.589 (8)	14.556
b (Å)	7.7790 (2)	5.0568 (10)	6.811
c (Å)	12.7096 (6)	11.981 (2)	7.657
α (°)	90	90	119.57
β (°)	94.764 (3)	92.09 (2)	103.93
γ (°)	90	90	91.30
V (Å^3)	1224.97	2396.9 (8)	631.766
Z	4	8	2

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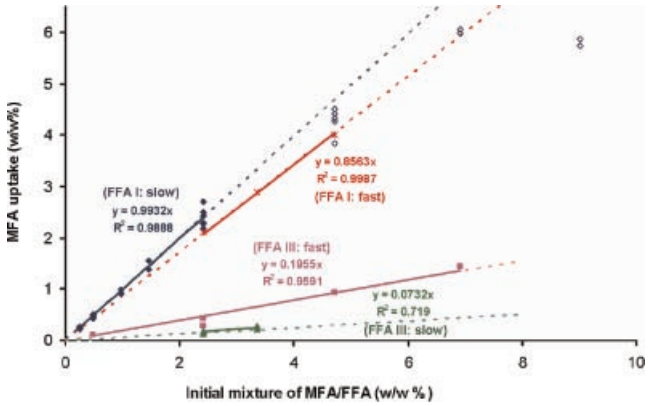

Figure 8. Uptake of MFA in FFA I and III crystals grown at different crystallization rates.

crystals were grown at a slow rate. These two contradictory results suggest that the attachment of MFA on FFA III follows a "transport-reaction controlled" mechanism, and that of MFA on FFA I follows a "surface-reaction controlled" mechanism. In other words, molecular recognition at the interface between FFA and MFA is required for MFA to be incorporated or attached to FFA I but not to FFA III. Therefore, it also suggests that MFA has a lower solubility in FFA III than in FFA I in an equilibrium state indicating more similarity of MFA to FFA I than to FFA III. Detailed studies on the structural analysis between MFA and FFA polymorphs were published previously.[25]

When two organic molecules are mixed forming a solid solution, a "solute" molecule replaces a "solution" crystal normally by substitution. This substitution causes the changes in the free energy of the solution crystal by (1) causing "strains" and thus, changing the lattice energy, (2) increasing the entropy of mixing, (3) changing the lattice vibrational spectrum, and (4) possibly changing the conformation of the solute molecules.[28]

When the molecules with similar size, shape, and/or density are mixed in a solid solution, the free energy of the solid solution does not rise or lower significantly as compared to a pure solid. However, when the molecules with different size, shape, and/or density are mixed in a solid solution, the substitution by the molecules in the lattice of a pure solid causes "strains" in the lattice and thus changes the free energy of the solid solution much more than the substitution by the molecules with a similar size, shape, and/or density does. In our case where both FFA I solid solution and FFA III solid solution have the same level of MFA, the substitution of MFA in FFA III solid solution will rise more than that in FFA I since MFA has a similar size, shape, and density to FFA I as compared to FFA III. Therefore, the difference in the free energy, which is the driving force for polymorphic transformation, will reduce and thus, delay the solid-state polymorphic transformation. However, it should be pointed out that it only applies to the level of substitution of MFA studied in this work since changes in the free energy of a solid solution crystal depends on the magnitude of the four parameters mentioned above.

This study showed that FFA containing MFA did not undergo solid-state polymorphic transformation within 2 years. Byrn, Pfeiffer, and Stowell[1] proposed the "four-step solid to solid physical transition" model: "molecular loosening in the initial phase, formation of an intermediate solid solution, nucleation of the new solid phase, and the growth of the new phase." In this respect, it can be expected that MFA can affect the each step of solid-to-solid physical transition: (1) molecular loosening in the initial phase can be inhibited by the formation of epilayer by MFA, (2) the formation of an intermediate solid solution (disordered phase) can be inhibited: during solid-to-solid physical transition process, the formation of an intermediate solid solution (disordered) between two phases is necessary. There are several scenarios possible during the formation of an intermediate solid solution in case of "solid solution." Depending on the energy state of the solid solution and the concentration of the solute in the solid solution, a new pure phase can grow in the crystalline medium, or solid solution phase can grow into a new solid solution phase. Or, intermediate cases are also possible and probably most common.[14] As a result, a time required to form an intermediate solid solution of solid solution is likely to take longer than the time to form an intermediated solid solution of the pure metastable form, especially in our case where MFA has higher affinity to FFA I than to FFA III, and (3) the nucleation of a new solid phase and the growth of the new phase can be inhibited: defect sites or the crystal surface are the most probable site for nucleation although the lattice restructuring can occur at any point of the crystalline medium. As shown and discussed previously, an epilayer of MFA can inhibit the nucleation by blocking the possible sites, surface, for restructuring. In addition, as discussed above, the substitution of MFA in FFA III solid solution will rise more than that in FFA I since MFA has a similar size, shape, and density to FFA I as compared to FFA III. Therefore, the difference in the free energy, which is the driving force for polymorphic transformation, will decrease and thus, delay the solid-state polymorphic transformation.

CONCLUSION

This study reports the stabilization of the solid-state polymorphic transformation of FFA using a structu-

rally related compound, MFA, with very small amounts as low as 0.25%. Effects of MFA on crystallization of FFA and the solid-state polymorphic transformation of FFA were studied. Morphology, surface morphology, the formation of the solid solution, the solubility limit of the solid solution was investigated and studied in conjunction with the previous studies: the epitaxial relationship between FFA and MFA during sublimation[25] the effect of FFA on the solubility of MFA (unpublished results), and the polymorph selection of MFA in the presence of FFA.[20] It can be concluded that the polymorphic transformation of FFA was inhibited in the presence of MFA not only by reducing the difference in the free energy between FFA I and FFA III solid solutions but also by forming an epilayer, and thus inhibiting each step of "four-step solid to solid physical transition" proposed by Byrn, Pfeiffer, and Stowell. We believe that this work provides another insight into the stabilization of solid-state polymorphic transformation by a structurally related additive.

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REFERENCES

1. Byrn SR, Pfeiffer RR, Stowell JG. 1999. Solid-state chemistry of drugs. 2nd edition. West Lafayette: SSCI, Inc. p 259.
2. Ostwald WZ. 1897. Studien über die Bildung und Umwandlung fester Körper. 1. Abhandlung: Berstattigung und Begrundlung. Z Phys Chem 22:289–330.
3. Weissbuch I, Lahav M, Leiserowitz L. 2003. Toward stereochemical control, monitoring, and understanding of crystal nucleation. Cryst Growth Des 3:125–150.
4. Bauer J, Spanton S, Henry R, Quick J, Dziki W, Porter W, Morris J. 2001. Ratinovir: An extraordinary example of conformational polymorphism. Pharm Res 18:859–866.
5. Aguiar AJ, Zelmer JE. 1969. Dissolution behavior of polymorphs of chloramphenicol palmitate and mefenamic acid. J Pharm Sci 58:983–987.
6. Paul IC, Curtin DY. 1973. Thermally induced organic reactions in the solid state. Acc Chem Res 6:217–225.
7. Mukuta T, Lee AY, Kawakami T, Myerson AS. 2005. Influence of impurities on the solution-mediate phase transformation of an active pharmaceutical ingredient. Cryst Growth Des 5: 1429–1436.
8. Gu C-H, Chatterjee K, Young V, Jr., Grant DJW. 2002. Stabilization of a metastable polymorph of sulfamerazine by structurally related additives. J Cryst Growth 235:471–481.
9. Ferrari ES, Davey RJ. 2001. Solution-mediated transformation of α to β L-glutamic acid: Rate enhancement due to secondary nucleation. Cryst Growth Des 4:1061–1068.
10. Blagden N, Davey RJ, Rowe R, Roberts R. 1998. Disappearing polymorphs and the role of reaction byproducts: The case of sulphathiazole. Int J Pharm 172:169–177.
11. Davey RJ, Blagden N, Potts GD, Docherty R. 1997. Polymorphism in molecular crystals: Stabilization of a metastable form by conformational mimicry. J Am Chem Soc 119:1767–1772.
12. Davey RJ, Maginn SJ, Andrews SJ, Buckley AM, Cottier D, Dempsey P, Rout JE, Stanley DR, Taylor A. 1993. Stabilization of a metastable crystalline phase by twinning. Nature 366:248–250.
13. He X, Stowell JG, Morris KR, Pfeiffer RR, Li H, Patrick Stahly G, Byrn SR. 2001. Stabilization of a metastable polymorph of 4-methyl-2-nitroacetanilide by isomorphic additives. Cryst Growth Des 1:305–312.
14. Kitaigorodsky AI. 1984. Mixed crystals. Berlin Heidelberg, New York: Springer-Verlag. pp 26–40.
15. Godqik G, Pfnur H, Lyuksyutov IF. 2001. Impurity-induced changes of overlayer symmetry and of phase transitions. Europhys Lett 56:67–73.
16. Dekoster J, Jedryka E, Meny C, Langouche G. 1993. Epilayer-induced structural transition to b.c.c. Co during epitaxial growth of Co/Fe superlattices. Europhys Lett 22:433–438.
17. Burger A, Ramberger R. 1980. Thermodynamische beziehungen zwischen polymorphen modifikationen: Flufenaminsaure und mefenaminsaure. Mikrochim Acta 73:17–28.
18. McCrone WC. 1965. Polymorphism. In: Fox D, Labes MM, Weissberger A, editors. Physics and chemistry of the organic solid state. New York: John Wiley & Sons. pp 725–767.
19. Mullin JW. 2001. Crystallization. 4th edition. Oxford, Burlington: Elsevier Butterworth-Heinemann. pp 154–155, 181–215.
20. Lee EH, Byrn SR, Carvajal MT. 2006. Additive-induced metastable single crystal of mefenamic acid. Pharm Res 23:2375–2380.
21. Cabrera N, Vermilyea DA. 1958. The growth of crystals from solution. In: Doremus RH, Roberts BW, Turnbull D, editors. Growth and perfection of crystals. New York: John Wiley & Sons, Inc. pp 393–410.
22. Hendriksen BA, Grant DJW. 1995. The effect of structurally related substances on the nucleation kinetics of paracetamol (acetaminophen). J Cryst Growth 156:252–260.
23. Michaels AS, Tausch FW, Jr. 1961. Modification of growth rate and habit of adipic acid crystals with surfactants. J Phys Chem 65:1730–1737.
24. Sears GW. 1958. The effect of poisons on crystal growth. In: Doremus RH, Roberts BW, Turnbull D, editors. Growth and perfection of crystals. New York: John Wiley & Sons, Inc. pp 441–445.
25. Lee EH, Boerrigter SXM, Byrn SR. 2010. Epitaxy of a structurally related compound on the (100) faces of flufenamic acid form I and III single crystals. Cryst Growth Des 10:518–527.
26. Chikazumi S. 1950. Ferromagnetic properties and superlattice formation of iron nickel alloys (I). J Phys Soc Jpn 5:327–333.
27. Iwata T. 1995. A statistical theory of the anisotropic distribution of atom pairs in binary alloys. J Jpn I Met 19:95–99.
28. Kitaigorodsky AI. 1973. Conditions for formation of solid solution. In: Loebl EM, editor. Molecular crystals and molecules. New York, London: Academic Press, Inc. pp 94–105.
29. McConnell JF, Company FZ. 1976. N-(2,3-Xylyl) antranilic acid, $C_{15}H_{15}NO_2$ mefenamic acid. Cryst Struct Comm 5:861–864.