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Investigation of Interaction Between Flurbiprofen and Fatty Acid Crystals

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Abstract

Objectives: Interaction between flurbiprofen and saturated fatty acids keeping in view their crystalline nature has been investigated.

Materials and Methods: Samples were prepared by co-evaporation method. FTIR, DSC, XRD, Microscopy (Optical microscopy, Scanning electron microscopy, Atomic force microscopy) techniques were employed for study of interaction between drug and fatty acid.

Results: FT-IR showed significant changes in the spectral pattern of coevaporates. Possibility of polymorphic changes and eutectic formation was completely ruled out. A better probe into interaction was obtained by microscopic techniques especially atomic force microscopy.

Conclusions: XRD was unable to detect any interaction between drug and fatty acid. FT-IR study detected chemical interaction between flurbiprofen and fatty acids. Similar observations were made by DSC study. This study has clearly demonstrated interaction between flurbiprofen and fatty acid is physicochemical in nature.

Key words:

AFM, DSC, FTIR, SEM, XRD, Interaction, Flurbiprofen, Saturated Fatty Acid.

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Introduction

Flurbiprofen is an arylpropionic nonsteroidal anti-inflammatory drug (NSAIDs) endowed with good analgesic, antipyretic, and anti-inflammatory properties. However, its very low water solubility and hydrophobic nature represent a rate-limiting step in its absorption from solid oral dosage bioavailability. Flurbiprofen is a BCS class II drug due to its good permeation properties through biological membrane but low aqueous solubility [1].

In case of topical drug delivery of NSAIDs the most commonly used permeation enhancers are fatty acids. The fatty acids enhance transport of drug molecules across the skin by a variety of mechanisms such as partitioning into the lipid bilayers and disrupting their ordered domains, improving drug partitioning into the stratum corneum and forming lipophilic complexes with drugs [2,3].

Literature is replete with the reports on permeation enhancement of NSAIDs by fatty acids. Several mechanisms also have been proposed but the study concerning the interaction between NSAIDs and fatty acids can be counted on finger tips. The interaction study between flurbiprofen and fatty acids involving the surface phenomenon have totally escape the attention of researchers.

The crystal habit of an unprocessed drug, for example, is known to be affected by many factors, including the kinetics of crystal growth, level of impurities, crystallization process parameters and the solvent and/or antisolvent used in the crystallization. Thus, changing the crystallization conditions of an API may affect the growth rates of different crystal faces, which may lead to a change in the crystal habit in relation to the dominance of the slowest growing face [4]. Crystal habits plays a very important role in determining the fate of the drug such as dissolution, bioavailability and stability of the dosage forms.

In our previous work on interaction between acetaminophen and fatty acids using modern techniques it was observed that the interaction was physical in nature [5-9]. The objective of the present study was to investigate the possibility of interaction between flurbiprofen and different fatty acids. If there is an interaction between drug and fatty acid, elucidation of the mechanism of interaction, specifically involvement of crystal surface was another objective.

Material and Method

Flurbiprofen was obtained from Sun Pharmaceuticals Ltd., Vadodara, India. Lauric acid, myristic acid, stearic acid and palmitic acid were purchased from Merck,

Schuchardt, Germany. All other materials used were of A.R. Grade.

Preparation of Coevaporated products (Crystals / Coevaporates)

Flurbiprofen and each fatty acid (1g) were dissolved separately in 10 ml. of methanol. Flurbiprofen solution was mixed with individual fatty acid to prepare 1:1 binary mixture and the solvent was allowed to evaporate at room temperature. The 1:1 ratio was selected to maximize the likelihood of observing any interaction [5-8].

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectra were obtained using a IR Prestige-21 (Shimadzu, Japan) equipped with IR solution version 1.21 (Shimadzu, Japan) in the range 400-4000 cm^{-1} with a resolution of 4 cm^{-1} (20 scans). Dry potassium bromide (50mg) was gently grounded in an agate mortar followed by the addition and mixing of (1-2mg) crystals of Flurbiprofen, fatty acids and their binary mixture.

Differential Scanning Calorimetry (DSC)

All measurements were carried out on an Indium calibrated DSC Q 10 V9.4 Build 287 (TA Instruments, USA) equipped with a refrigerated cooling system (RCS). Data acquisition and analysis was carried out using the Universal Analysis 2000 program (TA Instruments, USA). 2-4 mg. of crystal of Flurbiprofen, fatty acid and their coevaporates was weighed into pinholed aluminium pans (TA Instruments, USA) and heated under dry nitrogen (50ml/min) in the scanning range between 0 and 200°C at a rate of 10°C/min. An empty pan was used as reference. Experiments were carried out in duplicate.

X-Ray Diffraction Study (XRD)

X-ray diffraction of crystal of Flurbiprofen, fatty acid and their coevaporated products was carried out on a Rigaku rotating anode diffractometer RUH3R (Tokyo, Japan). Measurements were performed at 40kV voltage, 15mA current, at a scanning speed of 2˚/min, step size 0.02 and scanning range from 5-60˚ 2θ.

Microscopy

Optical microscopy was performed using Leica microscope FW4000 attached with digital camera. Samples were mounted on a glass slide before they were seen under the microscope. For scanning electron microscopy crystals of Flurbiprofen, fatty acid and their binary mixture were mounted on scanning electron microscope stubs with double-sided carbon tape and observed under Jeol JSM 5600 Scanning Electron Microscope (Jeol, Japan). Atomic force microscopy was done for crystal of Flurbiprofen, fatty acid and their binary mixture on a Scanning Probe Microscope (Digital Instruments: Nanoscope IV, USA) in contact mode using triangular cantilever made up of silicon nitride, having force constants of 0.38N/m. Scan sizes were taken from 50 to 1 μm.

Result and Discussion

X-Ray Diffraction

In absence of interaction in a mixture of crystalline solids, each component will retain its characteristic diffraction pattern, independent of the other components in the mixture [10]. The diffractogram of coevaporates of flurbiprofen and fatty acid (Figure. 1) retained almost all the characteristic peaks of drug and fatty acid at their respective positions with identical d-values but with a slight reduction in intensity. Since no significant change in the diffraction pattern of admixtures was noticed, the possibility of interaction does not seem to occur.

FT-IR Spectrum of Fatty Acids

The FT-IR spectrum of flurbiprofen and fatty acids (Figure. 2) showed its characteristic bands as reported in literature [11-14]. In the FT-IR spectrum of the coevaporated products of flurbiprofen and fatty acid number of bands due to wagging of methylene of fatty acid, were reduced. The additional bands of carboxylic acid dimer were absent. In the spectrum of coevaporated products of flurbiprofen- lauric acid mixture the –OH out-of-plane deformation was absent whereas, it was merged with the band at 925 cm^{-1} in the coevaporated products of flurbiprofen-myristic acid and palmitic acid. The C=O stretching band was present as a blunt band at 1705 cm^{-1} (flurbiprofen- lauric acid), 1701 cm^{-1} (flurbiprofen-myristic acid/ stearic acid), as a bifurcated band centered at 1693 cm^{-1}. The sharpness and intensity of -OH out-of-plane deformation band was considerably reduced.

Shifting, absence and merging of some of the additional bands of carbonyl group of fatty acid and –OH out-of-plane deformation band of flurbiprofen indicate interaction between flurbiprofen and fatty acids. Since, flurbiprofen and fatty acid molecules both involve hydrogen bonding, the possibility of existence of intramolecular as well as intermolecular hydrogen bonding exists [5]. Also, there are chances of mutual disruption of dimer structure of fatty acid and flurbiprofen molecules. This is further evidenced by the change in shape from broad peak to narrow one, in the region 2400-3250 cm^{-1}.

Differential Scanning Calorimetry

The DSC curve of flurbiprofen was typical of a crystalline anhydrous substance (Figure. 3). As in case of the thermogram of flurbiprofen, the DSC curve of flurbiprofen showed a single endothermic event representing the transition of flurbiprofen from solid-to-liquid state (Tm= 115°C).

Mura et al. demonstrated the importance of sample mechanical treatment in the likelihood of observing possible solid-solid interactions, especially when using co-grinding, kneading or co-evaporation for their ability to emphasize drug-excipient solid-solid interactions not visible or not clearly evident in sample blends [15]. With this objective, coevaporated mixtures of flurbiprofen with lauric acid, myristic acid, palmitic acid, and stearic acid were subjected to thermal analysis (Figure. 3). The thermal features of flurbiprofen and fatty acid per se (prepared in similar manner as coevaporated products) were similar to that observed in untreated drug and fatty acid samples. Similar to DSC curves of admixtures, in thermograms of coevaporated products of flurbiprofen with fatty acids also, depression in melting point of flurbiprofen (20-24°C) as well as fatty acids (2-5°C) towards lower temperature range was observed. There was significant enthalpy loss in all coevaporated products (minimum 9.57% and maximum 21.85%).

El-Shattawy et al. [16] have proposed that the melting peak area and heat of melting (ΔH) of the drug in mixture are decreased when an interaction occurs. The melting peak and heat of melting remains unchanged if the drug-excipient mixture is compatible. The potential

for incompatibility is proportional to the decrease in the heat of melting.

The changes in the thermal parameters in terms of lowering of Tm and change in enthalpy suggest interaction between flurbiprofen and fatty acid in coevaporated products also. The thermal parameters of coevaporated products of flurbiprofen are listed in Table 1.

Since eutectic formation has been reported [17] in some cases of flurbiprofen, in order to examine such possibility in our study, physical admixtures of flurbiprofen: palmitic acid (representative of different fatty acid) in varying ratios (1:9, 2:8, 3:7, and 7:3) were also analyzed (Fig. 4). In the resulting thermograms although the peak of flurbiprofen was shifted to lower temperature (7-10 °C), in all the cases the peak of palmitic acid was retained at its position with a negligible shift of 1-2°C lower temperature. In ratios flurbiprofen: palmitic acid (1:9 and 2:8) though only a single peak was observed at 61°C, probability of any eutectic formation seems remote on account of extremely low concentration of flurbiprofen in binary mixture.

Microscopy

Significant changes in the morphology of fatty acid crystals after crystallization with methanol were observed in scanning electron microscopic (Figure. 5). In SEM Flurbiprofen untreated can be observed as spherical aggregate (Figure. 5a), whereas coevaporated flurbiprofen (Figure. 5b) appear to be platy crystals. There was almost no difference in the FT-IR spectrum and DSC profile (melting point) of untreated flurbiprofen and crystallized flurbiprofen. Hence the possibility of polymorphic transition during crystallization seems remote (data not shown).

The crystal habit of drug was more prominent in the coevaporated product of drug and lauric acid, which showed the formation of agglomerate (Figure. 5c). The platy and tabular crystals of drug were seen entangled with lauric acid crystals show the entrapment of acicular and platy crystals of drug in fatty acid (myristic acid, palmitic acid, and stearic acid) matrix (Figure. 5, d-f). The SEM of coevaporated products of drug with myristic acid or palmitic acid showed the formation of agglomerate. Platy crystals of drug were entrapped within the fatty acid matrix. SEM of coevaporated product of drug with stearic acid showed the drug crystals form helical agglomerate with stearic acid crystals (Figure. 5f).

Investigation of interaction by Atomic Force Microscopy

Hydrogen bonding interactions and their effect along with other adsorption forces play important roles for the adsorption of macromolecules onto various solid surfaces. Many methods have been reported to study excipient adsorption on solid surface, such as solid state NMR, FTIR, UV, Raman, electron spin resonance (ESR), microcalorimetry and surface plasmon resonance (SPR). However, all these methods can only detect the change in the adsorbed excipient molecules themselves. No direct information could be obtained on how the solid surface is involved during adsorption on molecular level [5]. At present, the most efficient technique available for probing the interactions in systems is the atomic force microscopy, which was originally developed as an imaging tool [18], since then it has been applied to a number of pharmaceutical related problems. This is mainly due to its ability in characterizing the surface morphology of materials at very high resolution. It can provide insight into the interaction between polymer and crystal surface on molecular level [19].

Impurities may be additives or substances other than pure drug that are introduced for specific purposes, such as to modify the crystal habit or control the crystal size [19].
It has long been recognized that the presence of trace amounts of impurities can have substantial effects on the kinetics of crystal nucleation, growth morphology, and dissolution [20, 21]. During growth, impurities can adsorb onto the crystal surfaces, changing the relative surface free energies of the faces and blocking the active growth sites. Some impurities can suppress growth entirely, while some may enhance growth. The other may produce a selective effect, acting to varying degrees on each crystallographic surface and consequently modifying the crystal habit. Cabrera and Vermilyea [22] proposed that impurities can be adsorbed onto terraces or steps of growing crystals and become almost immobile. Impurities which are adsorbed onto a terrace can not be passed by a straight growing step, because impurities act as local pinning points. Therefore these impurities serve as a "fence" to growing steps. Consequently, growing steps must bend in order to squeeze through the impurity "fence" and, as a result the velocity of the steps is reduced compared to that of the straight steps growing in the absence of impurities.

In present study the selection of crystals suitable for AFM study was crucial factor. At the microscopic level, contamination and the roughness of the tips also affect the force measurements. In present study fatty acid serves as an impurity for flurbiprofen molecules vice versa. Figure. 6 shows the representative AFM image of flurbiprofen crystals. In the AFM image of flurbiprofen, interesting symmetrical patterns with regular, sharp edges, are visible. Flurbiprofen molecules are known to form intermolecular hydrogen bonds [22]. The addition of fatty acid produces significant changes on the surface of flurbiprofen crystal probably by disruption of hydrogen bonding of drug, as is evident from AFM images of coevaporated products of flurbiprofen with fatty acids.
Amongst all the coevaporated products of flurbiprofen, in coevaporated products of flurbiprofen and palmitic acid (Figure. 6d) the pattern of the surface of drug crystal appeared disturbed to some extent by presence of palmitic acid but still showing some arrangement on the surface of the crystals. In AFM image (Figure. 6b) of coevaporated products of flurbiprofen and lauric acid, the surface of the drug crystal was altogether changed. Figure. 6c represents the AFM image of coevaporated products of flurbiprofen and myristic acid. The changes in crystal surface of drug are evident in the form of increased roughness. AFM image of coevaporated products of flurbiprofen with stearic acid is presented in Figure. 6e, which shows the adhesion of fatty acid crystals on to the surface of drug. The difference in height between the highest and lowest points on the surface was as much as several micrometers. The outcome of AFM study is also supported by the thermal study in which the possibility of interaction was indicated between flurbiprofen and fatty acids.

Conclusion

XRD was unable to detect any interaction between drug and fatty acid. FT-IR study detected chemical interaction between flurbiprofen and fatty acids. Similar

observations were made by DSC study. Eutectic formation is a physical phenomenon. To explore the possibility of physical interaction using DSC, eutectic concept was also tested but no changes were detected. SEM and AFM clearly demonstrated how two surfaces (drug and fatty acid) are involved. Significant changes in the crystal habit of drug were observed with different fatty acids. This study has clearly demonstrated interaction between flurbiprofen and fatty acid is physicochemical in nature. AFM also revealed that although all the fatty acid have similar chemical structure except difference in number of carbon atoms their adhesion pattern on flurbiprofen crystals differ significantly. So depending up- on the requirement specific fatty acid can be selected for desired effect. Studies are in progress to see whether this interaction improves the absorption and bioavailability of drug *in vivo*.

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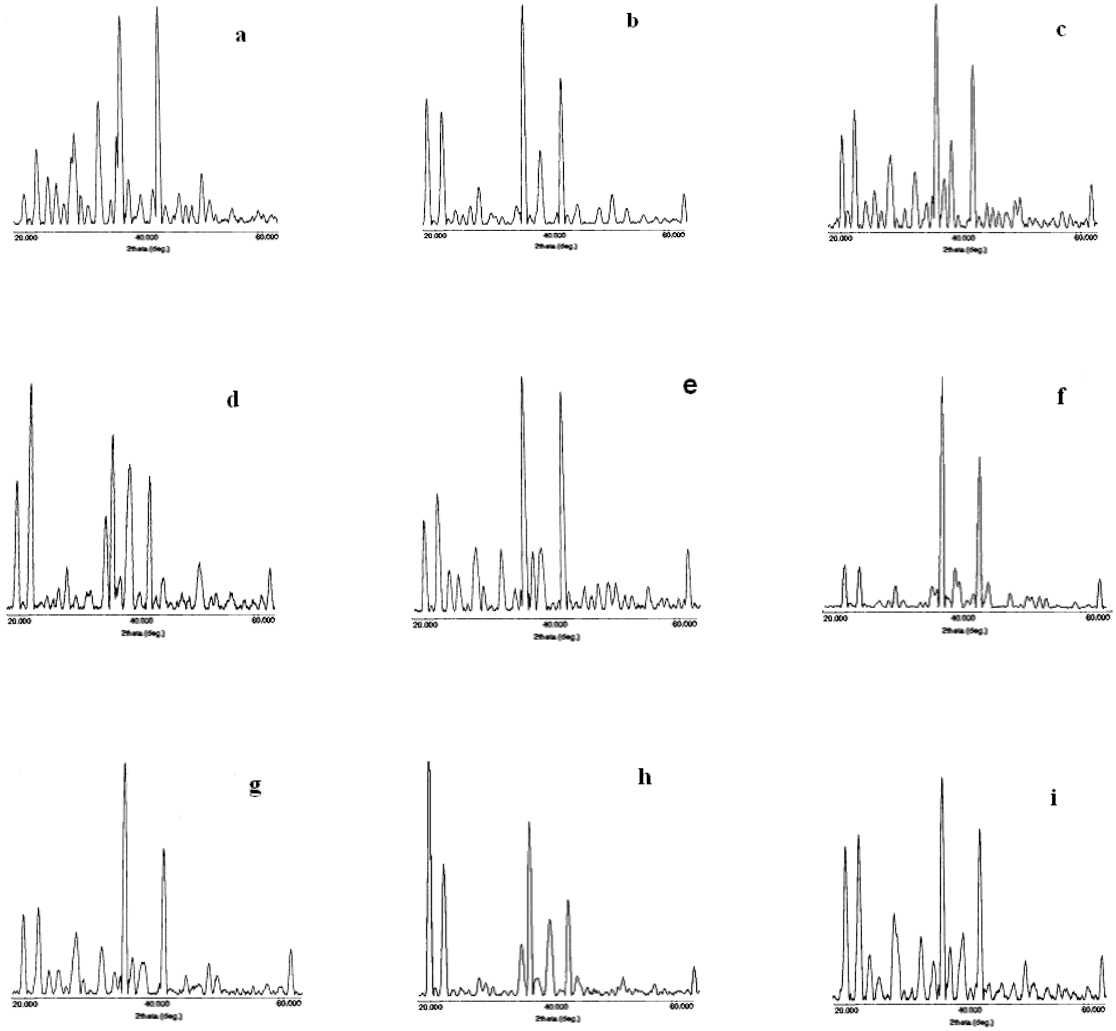
Conflict of Interests:

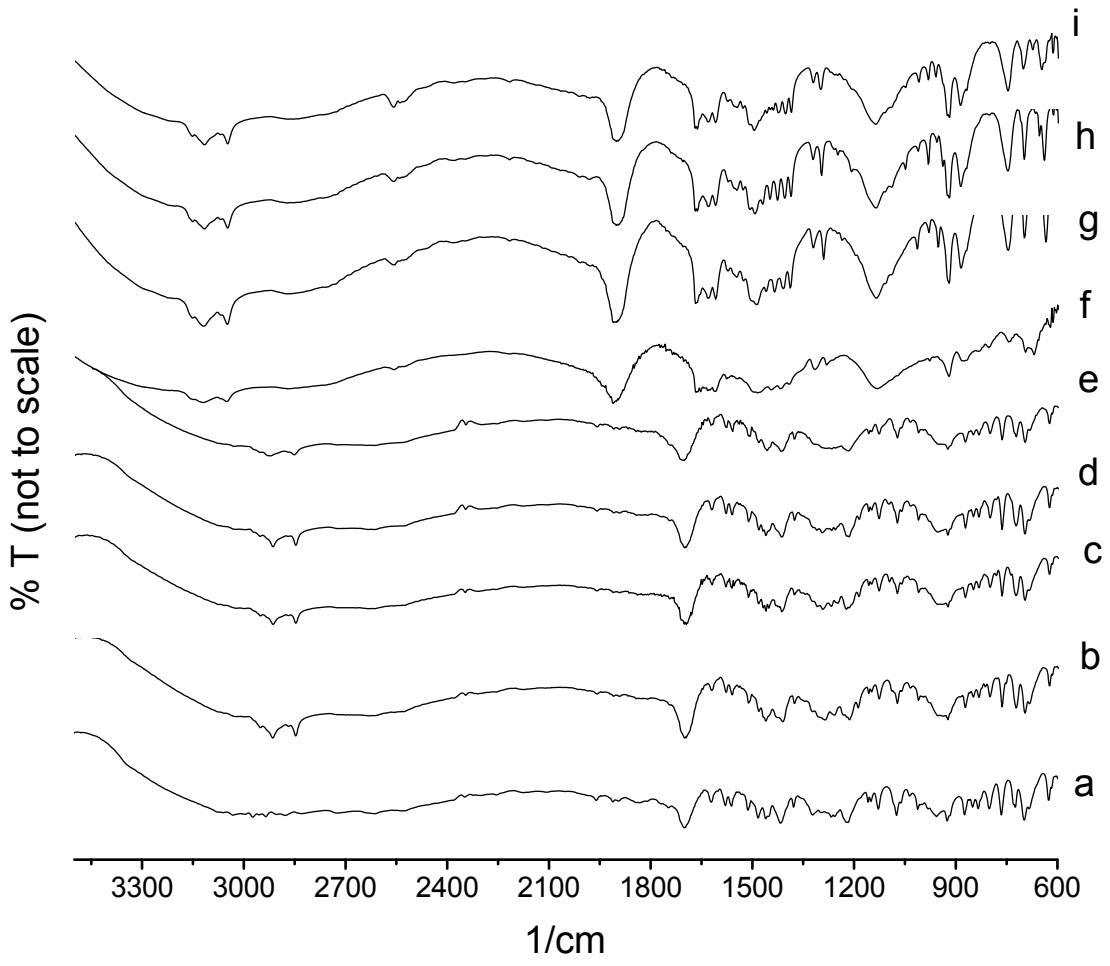
Authors have no Conflict of Interest

Figure 1 XRD Pattern Of Flurbiprofen Crystal



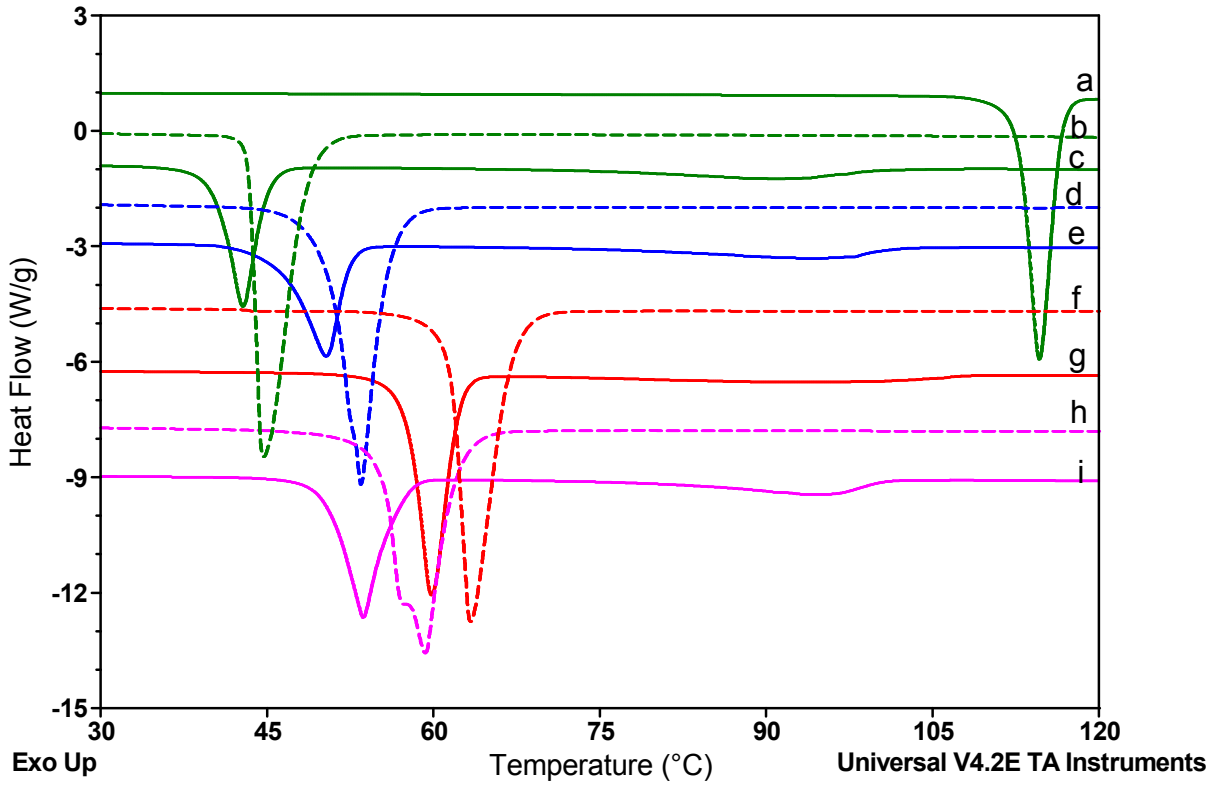
Flurbiprofen (a), lauric acid (b), Flurbi-lauric acid (c), myristic acid (d), flurbiprofen- myristic acid (e), palmitic acid (f), flurbiprofen- palmitic acid (g), stearic acid (h) and flurbiprofen- stearic acid (i).

Figure 2 FT-IR Spectrum Of Flurbiprofen Crystal



Flurbiprofen (a), lauric acid (b), Flurbi-lauric acid (c), myristic acid (d), flurbiprofen- myristic acid (e), palmitic acid (f), flurbiprofen- palmitic acid (g), stearic acid (h) and flurbiprofen- stearic acid (i).

Figure 3 DSC Thermograms Of Flurbiprofen Crystal



Flurbiprofen (a), lauric acid (b), Flurbi-lauric acid (c), myristic acid (d), flurbiprofen- myristic acid (e), palmitic acid (f), flurbiprofen- palmitic acid (g), stearic acid (h) and flurbiprofen- stearic acid (i).

Figure 4 DSC Thermograms Of Physical Admixtures Of Flurbiprofen With Palmitic Acid In Varying Ratios

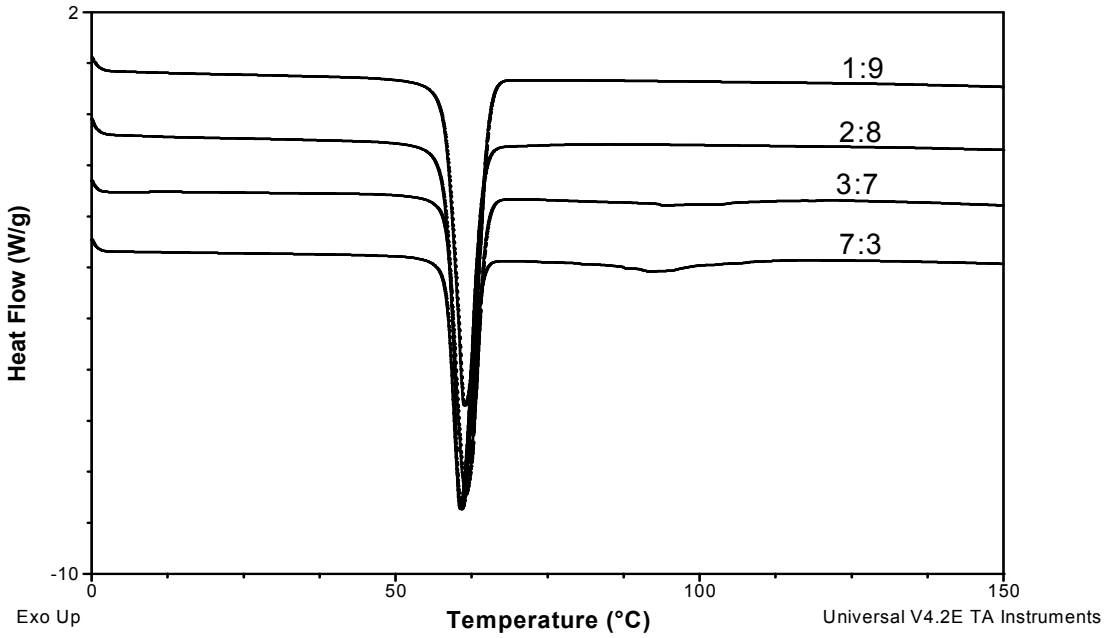
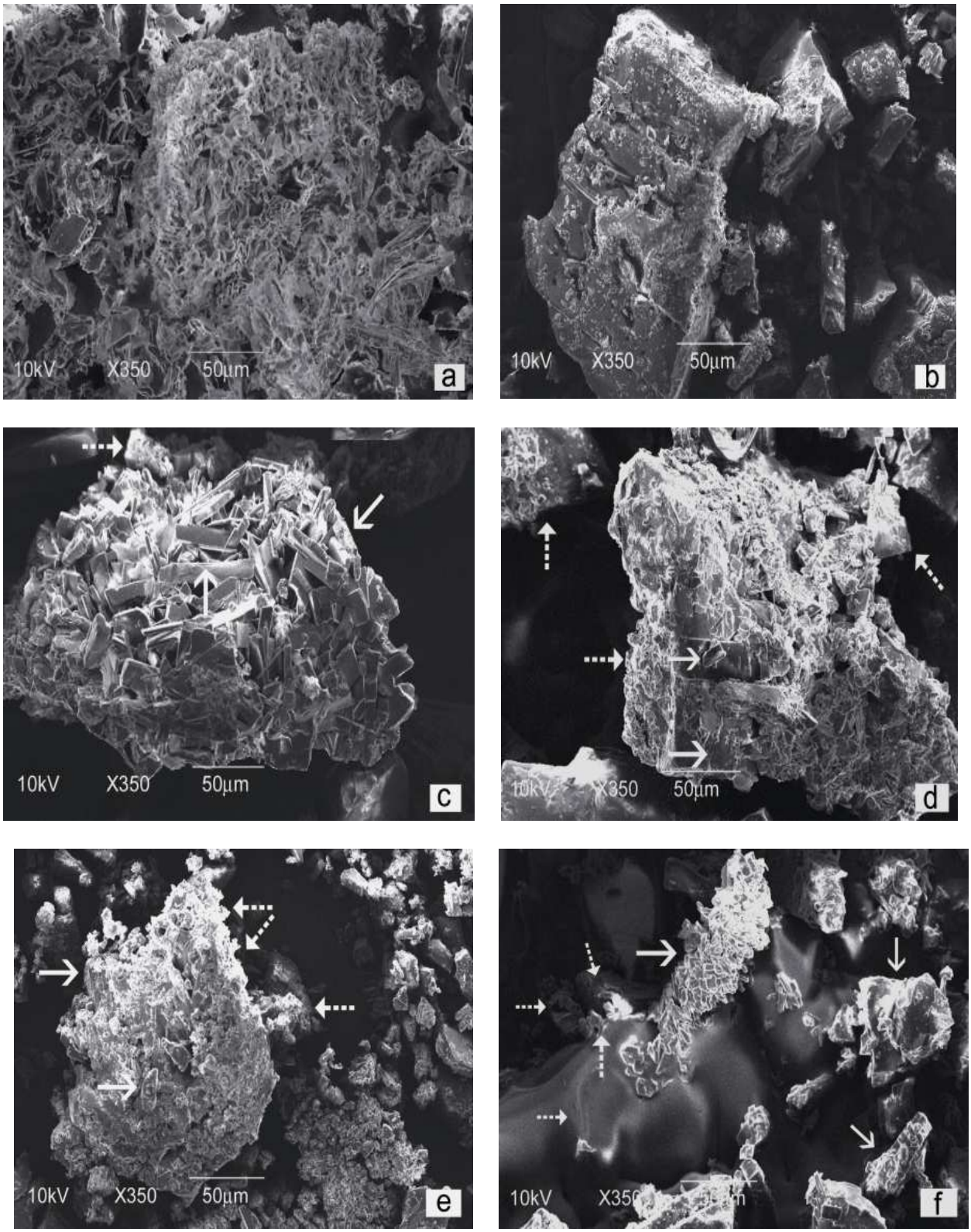
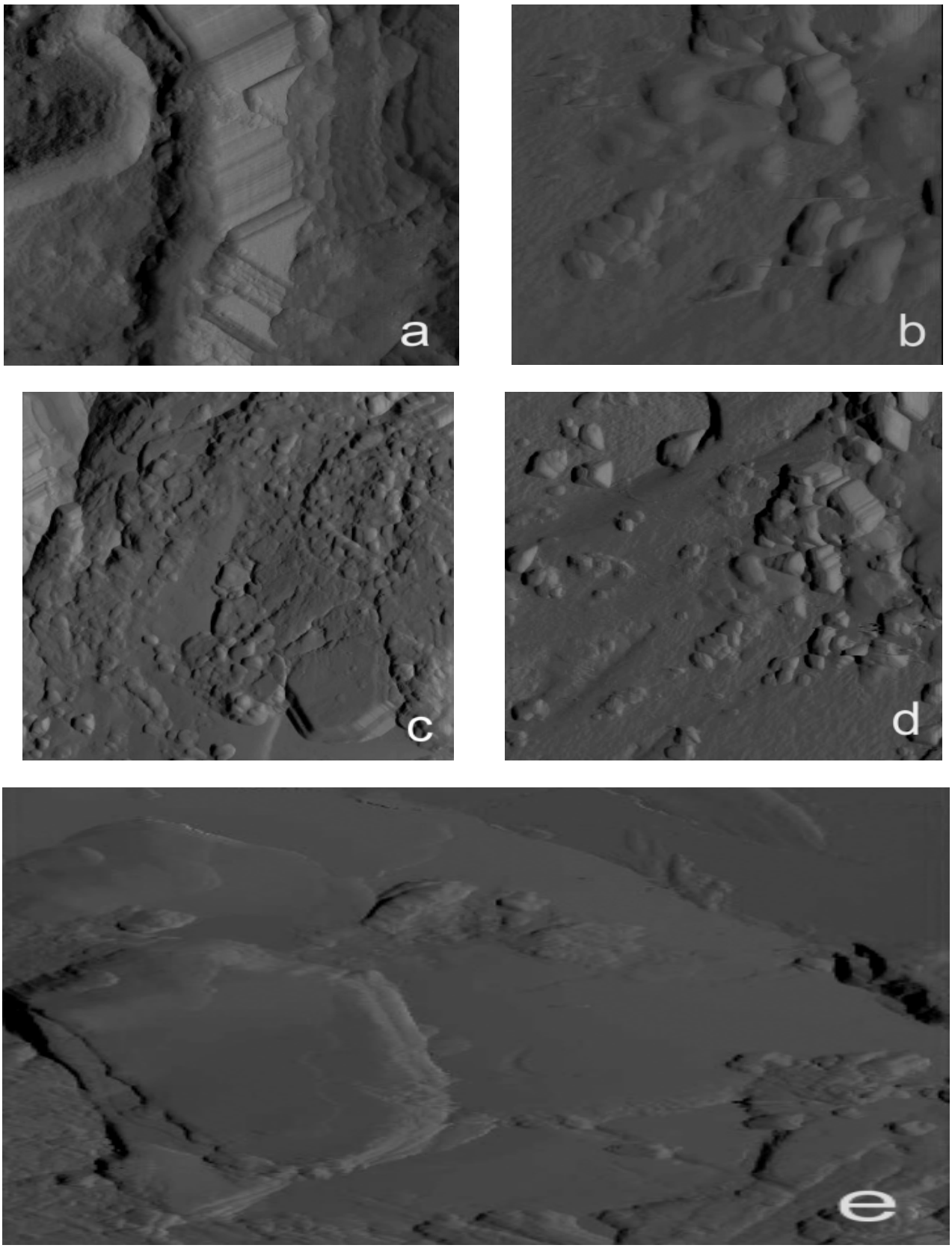


Figure 5 SEM Photomicrographs



(a) Untreated flurbiprofen, (b) coevaporated flurbiprofen and its coevaporated products with: (c) lauric acid, (d) myristic acid, (e) palmitic acid, and (f) stearic acid.
Solid arrow represents Flurbiprofen, Broken arrow represents Fatty acid.

Figure 6 AFM Image Of Coevaporated Flurbiprofen 5µm x 5µm



Flurbiprofen (a), and its coevaporated products with: lauric acid 5µm x 5µm (b), myristic acid 15µm x 15µm (c), palmitic acid 10µm x 10µm (d), and stearic acid 25µm x 25µm (e).

Table 1. Thermal parameters of coevaporated products of flurbiprofen with fatty acid

Coevaporated products of drug with	1st Transition			2nd Transition			ΔH_{cal} (J/g)	ΔH_{obs} (J/g)	ΔH %	ΔH
	T_o°C	T_m°C	ΔH(J/g)	T_o°C	T_m°C	ΔH(J/g)				
Flurbiprofen Coevaporated (Crimped)	113	115	106	-	-	-	-	-	-	-
Lauric acid	43	45	168	243	275	215	-	-	-	-
Myristic acid	50	53	182	-	-	-	-	-	-	-
Palmitic acid	61	63	199	-	-	-	-	-	-	-
Stearic acid	55	59	184	-	-	-	-	-	-	-
Flurbiprofen- Lauric	40	43	75	76	91	32	137	107	22	Loss
Flurbiprofen-Myristic	46	50	80	78	94	35	144	115	20	Loss
Flurbiprofen-Palmitic	57	60	116	72	95	22	152	138	10	Loss
Flurbiprofen- Stearic	50	54	92	81	95	32	145	124	14	Loss