Effect of Different Organic Solvents on Crystal Habit of Mefenamic Acid

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ABSTRACT

Mefenamic acid (MA) crystals which have different types of habits were prepared by antisolvent technique using different organic solvents; ethanol, acetone, and isopropanol. The physicochemical characteristics of the crystals were studied using various techniques: scanning electron microscope, X-ray powder diffractometry, FTIR spectrometry, differential scanning calorimetry, dissolution behavior, and wettability. All crystals were found to be isomorphic. Dissolution studies were performed. Crystals prepared using acetone as a solvent showed the highest dissolution rate among other solvents used in this study. The improvement in the dissolution is mainly due to the changes in the outer appearance of the crystals and surface modification. The choice of optimal solvent would influence the crystal habit and dissolution of MA.

Keywords: Crystal habit; Habit modification; Mefenamic acid; Anti-solvent method.

INTRODUCTION

More than 40% of newly discovered drugs have little or no water solubility. This would present serious challenge to the successful development and commercialization of new drugs in the pharmaceutical industry [(1)]. Therefore, developing novel methods to increase bioavailability of drugs that inherently have poor aqueous solubility is of great challenge to solid dosage form formulations.

One of the methods used to increase the dissolution rate is the formation of high specific surface area by micronization technique. In micronization two strategies are utilized; mechanical milling and precipitation from solution [(2)].

Chaumeil [(3)] describes the improvement of dissolution rate and in bioavailability by micronization of sparingly water-soluble drugs using jar milling and fluid energy mill.

Evaporative precipitation into aqueous solution (EPAS) and anti-solvent precipitation are among the most recent methods to obtain micronization of drug particles [(4-12)]. Evaporative precipitation into aqueous solution (EPAS) is a process used to form micron to submicron-sized particles of poorly water soluble drugs. It involves dissolving the drug in water immiscible organic solvent which is then sprayed through an atomizer into aqueous solution containing hydrophilic stabilizer to produce aqueous dispersion.

Intense atomization leads to rapid evaporation of the small organic droplets in aqueous solution. The rapid evaporation of small organic droplets produces large supersaturation and rapid nucleation of the drug. Hydrophilic stabilizers in water surrounding the shrinking organic droplets diffuse to the surface of the growing particles to inhibit particle growth.

Stabilization of drug particles with water soluble stabilizers in the aqueous suspension facilitates

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dissolution rate of the final powder after drying.

The second technique in micronization, which is the anti-solvent technique, is widely used process to prepare inorganic and organic particles. A number of recent studies have utilized anti-solvent precipitation technique to produce nanoparticles of poorly water- soluble drugs. In this process the poorly soluble drug with or without surfactant is dissolved in water miscible organic solvent, including methanol, ethanol, etc. The organic solution is then mixed with an anti-solvent usually an aqueous solution containing surfactant either by sonication or just pouring upon mixing. The supersaturated solution leads to nucleation and growth of drug particles which can be stabilized by surfactants [11, 13, 14]. Both previous particle engineering technologies have advantages and disadvantages; in EPAS, the high temperature favors particle growth which can lead to larger drug particles than in anti-solvent process, and high shear is necessary in EPAS to atomize the organic solution to increase heat and mass transfer while it is optional in anti-solvent technique. In contrast to EPAS technique nanoparticles of poorly water soluble drugs can be produced with anti-solvent precipitation at low temperature and in this technique the recovery of the particles needs more attention than in EPAS.

The existence of active ingredients in various crystal forms affect the bioavailability and therapeutic efficacy of a drug [15]. A physical form having improved dissolution rate and solubility is useful for improving the bioavailability of drugs [16]. Optimization of crystal properties through modification of surface or habit without formation of polymorphic form is preferred in drug development [17].

In pharmaceutical manufacturing, crystal habit is an important variable. Different crystal forms of a particular drug possess different planes and differ in their specific surface and their free surface energy. They exhibit different physicochemical properties such as dissolution rate, powder flow and compressibility which are of pharmaceutical interest and can differ for different habits of the same drug [18].

The overall shape of a growing crystal is determined by the relative rate of growth of its various faces. Growth rate of a surface will be controlled by a combination of structurally related factors such as intermolecular bonds and dislocations, and by external factors such as supersaturation, temperature, solvent and impurities [19]. Among those external factors, solvent strongly affects the habit of crystalline materials; however, the role played by solvent interactions in enhancing or inhibiting crystal growth is still not completely understood. It was noted that crystals obtained from different solvents exhibited different crystal habits. Crystals of the required physicochemical properties may be obtained by selecting solvents of different solubility parameters and dielectric constants [17]. Different crystal forms of the analgesic drug Ibuprofen were found to have different physicochemical properties by changing only the solvent of crystallization [18, 20]. The role-played by solvent-solvent interactions in enhancing or inhibiting crystal growth is still not completely resolved. It has been shown that favorable interactions between solute and solvent on specific faces leads to a reduction in the interfacial tension that leads to a transition from smooth to rough interface and a concomitant faster surface growth [21,22].

The objectives of this work were to enhance wettability of poorly soluble drugs, and to study the effect of solvents on the crystal habit of mefenamic acid. Mefenamic acid (MA) was chosen as a model drug since it is poorly soluble in water, requires a high dose and has poor compressibility [23]. The appearance of hydrophobic groups on the outer side of crystals may be responsible for polymorphism, cohesivisity and the poor flow properties of the drug [24].

MATERIALS AND METHODS

Materials

Mefenamic acid (MA) was supplied by JPM (Naour, Jordan), absolute ethanol, acetone, isopropyl alcohol, potassium dihydrogen orthophosphate, sodium hydroxide, were all supplied by Aldrich and were of pharmaceutical grade.

Methods

Different crystal samples of MA drug were prepared

in the presence of different solvents aiming to achieve better physicochemical properties to the drug. Crystallization was carried out using the anti-solvent technique. One gram of MA was dissolved in 50 ml of the relevant organic solvent (ethanol, acetone or isopropyl alcohol), and the resultant clear solution was placed in an atomizer under controlled temperature (25°C). The organic solution was then sprayed out through a nozzle 3 cm above the surface onto 50ml of distilled water heated up to 90°C which is a temperature above the boiling point of the different organic solvents being used in the study. The atomized solvent quickly evaporates, precipitating MA in the aqueous phase. The solutions were left overnight at room temperature, and the crystals were collected and stored in a Pyrex desiccators containing fresh silica gel. The crystals were filled into clean vials, closed, labeled and covered with parafilm and placed in the Pyrex desiccators at room temperature to be used in the study.

Scanning Electron Microscopy (SEM)

Electron micrographs of crystals were obtained using scanning electron microscope (Camera SU 30, Semprobe, France) operating at 12 KV. The specimens were mounted on a metal stub with double–sided adhesive tape and coated under vacuum with gold in an argon atmosphere prior to observation (Polaron E 6100 vacuum coater, UK).

X-Ray powder diffraction:

Powder diffraction patterns of all samples were determined using a PW 3040 diffractometer (Xpert MPD, Phillips, Netherlands) with cobalt radiation. The sample tubes were filled completely with MA as a starting material and also the other crystallized samples and were measured at a generator tension of 40kv and a generator current of 40 mA.

Fourier Transform Infrared (FTIR)

The FTIR spectroscopy, Nicolet Avatar 5.1 ESP 360 Spectrometer, connected to Omnic software running under Microsoft Windows was used. KBr blank was used, and each sample was diluted by mixing 0.1 g of the sample with 0.9 g of KBr, and then placed for analysis. Samples were analyzed using diffuse reflectance cells.

Thermal Analysis (Differential Scanning Calorimetry)

Differential scanning calorimetric (DSC) measurements were performed using Quick cooling differential scanning calorimeter (DSC-50 Q Shimadzu, Japan) equipped with a Shimadzu TA-50 WSI instrument controller and a Shimadzu professional computer.

Samples were weighed in aluminum pans that were sealed with a crimper. The thermal behavior was studied under a dry nitrogen purge (20ml/min) at a heating rate of 15ºC/min.

Contact angle (θ):

Compacts of sample powder were prepared at a 44 KN compression force using Roell & Korthaus RKM 50 with a highly polished stainless steel punch and die of ½ inch diameter (approximately 20,000 pound/ inch pressure). Each disc was placed on the shadomaster instrument (r11M, No.S3896, baTY& Co.Ltd., UK) then a droplet of purified water (5µl) was placed onto the surface of the compact using a micropipette; Transferpette®, from a height of 1 cm perpendicular to the disc. The contact angle was read using a magnification of 20X. Each sample was tested three times.

Dissolution studies:

100 mg of MA starting material and other crystallized samples were filled in a transparent hard gelatin capsule of size 5, placed in 500ml phosphate buffer (pH 7.4) using Ereweka dissolution apparatus at a paddle speed of 100rpm with temperature maintained at 37°±1ºC under sink conditions. Aliquots of 5 ml were withdrawn at appropriate intervals, filtered using 0.45µm millipore filter and an equal volume of phosphate buffer was replaced. MA was assayed spectrophotometrically at λ = 333 nm. The dissolution experiment was done in triplicate.

RESULTS AND DISCUSSION

The contact angle is used as an indicator for the wettability of mefenamic acid crystals

prepared by antisolvent. The contact angles for mefenamic acid prepared with different solvents are listed in table (1). The limits of contact angle are 0° for complete wetting and 180° for no wettability [25]. The contact angle of MA prepared in the presence of acetone in table (1) had the highest wettability compared with untreated MA powder, and MA crystallized with other organic solvents.

Table (1): Average contact angle for different solutions

Type of Solvent	Average contact angle* (θ)
MA crystals (untreated)	121 ± 1.1
MA crystals (with acetone)	84 ± 1.2
MA crystals (with ethanol)	96.5 ± 0.7
MA crystals (with isopropanol)	99 ± 1.2
n=3*	

DSC profiles of the untreated and the recrystallized samples using different solvents showed a single endothermic peak at *circa* 234ºC corresponding to the melting point of the drug (Figure 1). The DSC profiles of MA crystals crystallized using different solvents showed that all crystals were structurally similar and isomorphic. Polymorphic modification is ruled out. This would suggest that any improvement of the physical characteristics of MA is achieved by changes in outer appearance of the crystals, and in surface modifications.

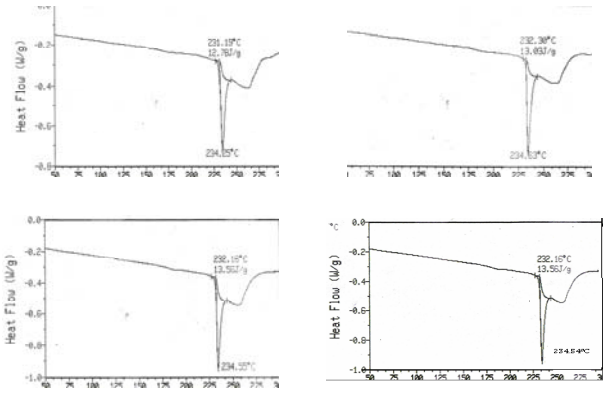

Fig (1): DSC profiles of MA crystals

The IR absorption bands in the FTIR spectrum of all crystals of mefenamic acid were similar (Figure 2). These unassociated changes at the molecular level shows that there are no differences between the internal structure and conformations of these samples. This agrees with the conclusion of Adhiyaman and Sanat about crystal modification [15].

Mefenamic acid has two polymorphic forms, the stable polylmorph I and the metastable polymorph II. The FTIR absorption spectra of form I and II, shows characteristic difference in the detailed shape and intensities of some of the major absorption bands that can be used to identify each polymorph specifically in the region of wave number between 3350 cm^{-1} and 3300 cm^{-1}, the NH stretching frequency occur at (3310-3250) cm^{-1} for form I and at 3347 cm^{-1} for form II [23]. All FTIR absorption profiles of mefenamic acid for crystalline samples are consistent with those of polymorph I (Figure 2).

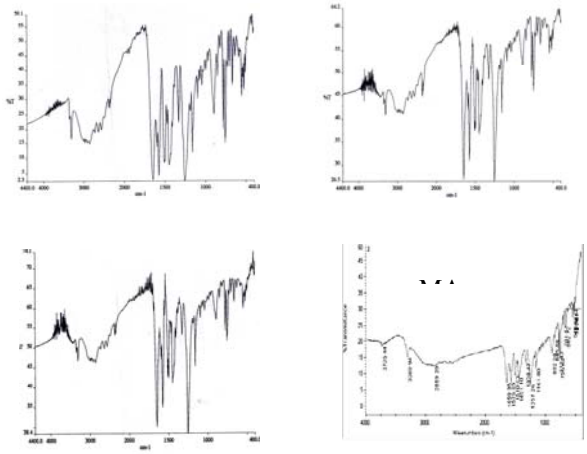

Fig (2): FTIR profiles of MA crystals.

X-ray powder diffraction spectra for all MA crystals are shown in (Figure 3) in addition to MA powder. X-ray powder diffraction is very useful method in determining whether pair of crystals of a particular drug is polymorphs. In general, when the peak position pattern for two forms of crystals is identical, the particles have the same internal structure. If the pattern is different then the crystals have different internal structures and are polymorphs [26, 27]. All samples in this work exhibited spectra with similar

positions of peaks (2θ) values. Crystallization solvents did not alter the polymorphic nature of MA and hence polymorphs are also ruled out here.

However, the relative intensities of their peaks were different. This occurred as the crystals exhibited preferred orientations in their packing within the sample holder as they have markedly different crystal habits, and arrangement of particles will affect their repacking under pressure. Therefore, the relative abundance of the planes exposed to the X-ray source would have been altered, producing the variations in the relative intensities of the peaks.

Garekani et al.[19] reported that paracetamol crystals obtained from different solvents exhibited similar X-ray diffraction patterns but different intensities. This was attributed to differences in crystal size [26]. The same trend was also found with phenytoin crystals, the intensity of the peaks of the treated crystals in the X-ray diffracton was higher than that of untreated one. This was explained with higher crystal perfection in the sample holder [21].

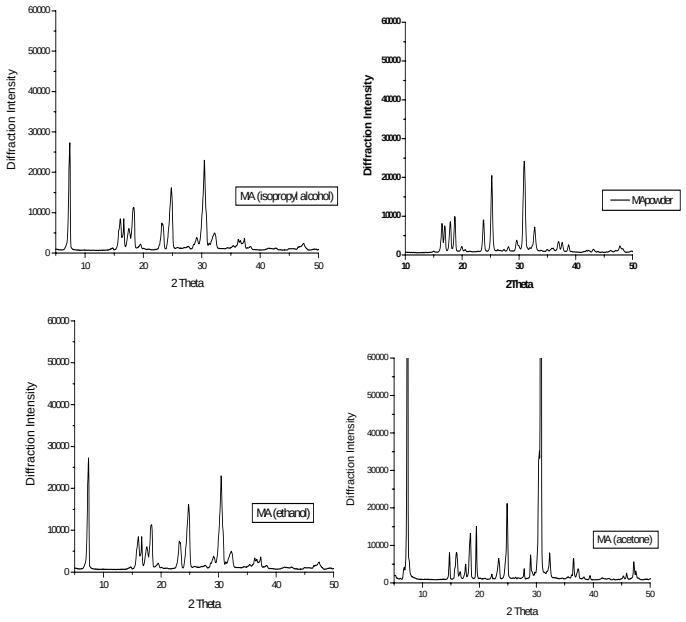

Fig (3): X-ray powder diffraction pattern of MA crystallized from a- isopropyl alcohol b- acetone c- ethanol and d- MA powder

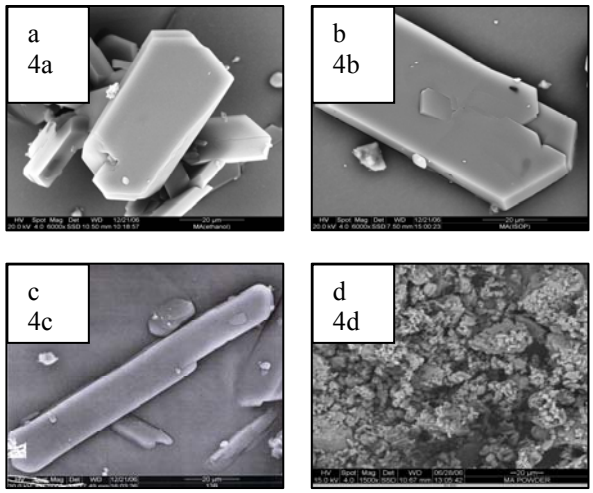

Fig (4): SEM of MA crystals; 4a. MA + Ethanol, 4b. MA + isopropanol, 4c. MA + Acetone, 4d. Untreated MA.

(Figure 4) shows the scanning electron micrographs of untreated and crystallized MA from different solvents (ethanol, acetone and isopropanol) at similar conditions. It is clear from the figure that the untreated MA is having small irregular needle shaped crystals whereas the samples crystallized from ethanol and isopropanol had a plate-like crystal habit, and those from acetone were needle-like. This habit modification is caused by surface-solvent interactions, which affect the growth rate of polar faces differently. Depending on the nature of the solvent, difference is mainly caused by the hydrogen bond interactions. Solvents influence the crystal growth from dissolved drug molecules through various mechanisms. Solvent properties such as polarity, molecular weight, and interaction with dissolved drug are factors that influence the direction in which crystals grow on nuclei. The formation of different habits of MA is attributed to interactions of MA and these crystallization solvents. This would agree with the finding of Garekani et al. 2001 [26].

It is suggested that polar solvents were preferentially adsorbed by polar faces and non-polar solvents by non-polar faces. Both alcohol and acetone as crystallization media interact through hydrogen bonds with MA hydroxyl groups. As the interaction of acetone will be

stronger than alcohol due to its relatively high polarizability, and has better ability to form Schiff-base reaction (π =0.71 for acetone and 0.54 for alcohol), the growth of crystals from that side is more inhibited and crystal growth is continued from other sides. The results showed that the size of the produced crystals is regular and differs from the size of the untreated MA.

Table (2): Parameters of the solvents used

Solvent	Dielectric constant (€)	Dipole moment (μ)	Boiling point (° C)	Structure
Ethanol	24.3	1.69	78	C_2H_5
Iso-propanol	18.3	1.66	82.3	$CH_2CHOHCH_2$
Acetone	20.7	2.91	56.3	CH_3COOCH_3

Dissolution profiles of mefanamic acid recrystalizad from ethanol, isopropyl alcohol and acetone are shown in (fig 5) there is a marked enhancement in the dissolution characteristics of mefanamic acid recrystallized from acetone and alcohols compared with untreated one. The dissolution rate was found to be in the order of acetone> ethanol> isopropyl alcohol> untreated powder.

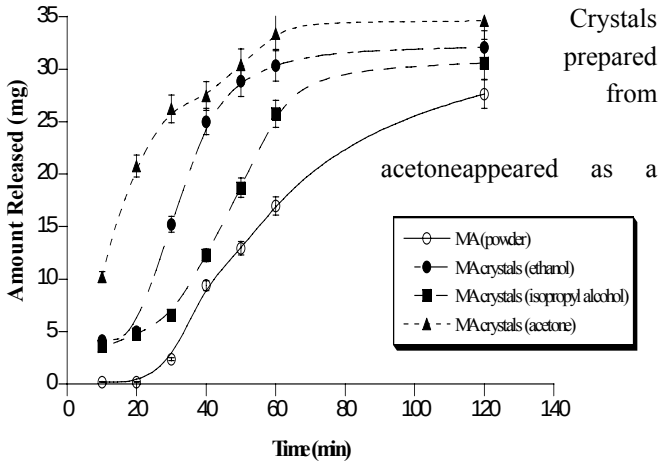

Fig (5): Dissolution profile of MA crystals

Crystals prepared from acetoneappeared as a needle -like shape. Consequently, expected an increase in the specific surface area exposed to dissolution medium compared with the plate- like crystals prepared from alcohols which exhibit a lesser specific surface area exposed to dissolution medium.

In addition to that the interaction between acetone and mefanamic acid crystals resulting in a product with a higher solubility and wettibility (Schiff_base reaction) this was confirmed with a contact angle data. (Table 1)

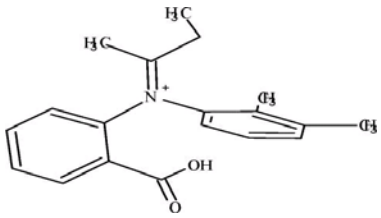

Fig (6): Product of reaction between MA and acetone (Schiff-base reaction).

CONCLUSION

The crystallization medium and method of crystallization have major effect on MA crystal habit. Crystallization of MA in acetone resulted in needle shape crystals, while crystallizing in ethanol and isopropanol resulted in plate shaped crystals.

The crystals showed significant changes in dissolution rate. This suggests that solvent used in preparation of crystals has an important role in the bioavailability of a drug as dissolution data is a good primaty indicator for in-vivo performance.

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تأثير المذيبات على الشكل الخارجي لبلورت حامض المفيناميك

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ملخـص

تم تحضير بلورات ذات شكل خارجي مختلف لحامض المفيناميك اعتماداً على تقنية anti solvent وباستخدام مذيبات مختلفة. تمت دراسة هذه البلورات باستخدام أجهزه مختلفة. أتضح بأن جميع بلورات حامض المفيناميك لها نفس التركيب البلوري وعند قياس سرعة انفكاك هذه البلورات مع الوقت وجد بأنها مختلفة. كما وجد بأن هذه البلورات لها شكل خارجي مختلف عن بعضها البعض ولهذا السبب تم تفسير نتائج اختلاف الذائبية في الماء. عند مقارنة المذيبات مع بعضها تبين أن البلورات المحضرة باستخدام الأسيتون أعطت أعلى انفكاك. أوضحت الدراسة أن اختيار المذيب المناسب عند تحضير البلورات له تأثير رئيسي على انفكاك الدواء ومن ثم على توافره الحيوي في الجسم.

الكلمـات الدالـة: الشكل الخارجي للبلورة، حامض المفيناميك، Anti-solvent method.

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