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Effects of Solvents on Polymorphism and Shape of Mefenamic Acid Crystals

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Abstract. Mefenamic acid [2-(2, 3-dimethylphenyl) amino benzoic acid] is an active pharmaceutical compound that exist in different polymorphic form and shape. In this work the effect of solvents on polymorphism and shape of mefenamic acid crystals were examined. The solvents used were ethanol, isopropanol, ethyl acetate, dimethyl acetamide, dimethyl formamide, and acetone. Natural cooling was employed during the crystallisation process. The crystals produced were dried and analysed using optical microscopy, differential scanning calorimetry, thermal gravimetric analysis, x-ray powder diffraction (XRPD) and fourier transform infrared spectroscopy (FTIR). The analysis confirmed that the crystals obtained using ethyl acetate, ethanol, isopropanol, and acetone are pure Form I with a needle-like flat shape. Meanwhile, the crystallisation using DMF produced polymorphic Form II in cubic shape.

1 Introduction

Solvent demonstrate an important role in the crystallisation process as it can contribute to the polymorphic selectivity of targeted pharmaceutical compound [1, 2]. This is because, at the molecular level, the solvent can selectively adsorb onto the crystals faces or interact with the solute clusters. The solvent-solute interactions, i.e. hydrogen bonding have been proven to affect the birth of different polymorphic forms [3]. A solvent molecule that has a stronger ability to donate or accept hydrogen bonding than the solute molecule may establish hydrogen bonding network with the solute molecule, resulting in selective nucleation [4]. For example, crystallisation of sulfathiazole using water produces polymorphic Form II and III. The polymorphic Forms I and IV are obtained from acetone, while n-propanol gives only Form I [5].

The types of solvent used during the crystallisation process also influence the shape of the crystals. Garti and co-worker reported that the crystallisation of cholesterol using ethanol and acetonitrile produced needles and plate-like crystal, respectively [6]. Ibuprofen crystals that crystallized from polar solvents, such as ethanol and acetone and non-polar solvents, such as diethyl ether and hexane, produced needle-like crystals. In contrast, crystallisation of ibuprofen using dichloromethane and acetonitrile produced cubic and spherical agglomerates, respectively [7]. The external structure has profound effect on orientation of particle, and thus may cause differences in packing, powder flow, compaction, syringability, stability of suspension, and dissolution characteristics [2].

Mefenamic acid (2-[(2,3-dimethylphenyl) amino] benzoic acid, $C_{15}H_{15}NO_2$) is a widely used nonsteroidal anti-inflamatory and analgesic agent for treatment of pain cause by menstrual disorders was reported to exist in three polymorphic forms: Form I, Form II and Form III [8-10]. The polymorphs are enantiotropically related and show different physicochemical properties which in turn affect the process acceptability, bioavailability and performance of the final product. The habit of mefenamic acid crystals can be either plate or needle shaped depending on the crystallisation conditions. Numerous research works on mefenamic acid crystals have resulted in the availability of different methods using various solvents for producing a particular polymorph [8, 9]. However, most of the literatures are difficult to follow as they do not state the initial solute concentration.

This paper presents the work on investigation of various types of solvents, which classified into polar

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protic (ethanol and isopropanol) and dipolar aprotic (ethyl acetate, dimethyl acetamide, dimethyl formamide, and acetone) on polymorphism and habit of mefenamic acid crystals. Apolar aprotic solvents such as hexane, cyclohexane and heptane were not considered as crystallisation solvent due to its poor solubility characteristics [11]. In order to understand and differentiate the characteristics of crystals produced, comprehensive characterizations were performed using various analytical techniques, i.e. thermal, microscopy, infrared spectroscopy and x-ray diffraction. The experimental results were compared with those of previous workers whenever possible.

2 Methodology

2.1. Material

Mefenamic acid powder with purity of 98 wt% was obtained from Baoji Tianxin Pharmaceutical Co. Ltd., China. The solvents used namely ethanol, ethyl acetate (EA), isopropanol (IPA), dimethyl acetamide (DMA), dimethyl formamide (DMF) and acetone were analytical grade with >99 wt % purity and supplied by Fisher Scientific.

2.2 Crystallisation method

Saturated solution of mefenamic acid was prepared by dissolving known amount of mefenamic acid in 50 mL of solvents studied. The amount of mefenamic acid used as tabulated in Table 1 is based on solubility data reported in our previous work [11]. The solution prepared was heated on a hot plate at 60°C except for DMF and acetone, which was at 50°C until dissolution. Then, the solution was allow to cool to room temperature through natural cooling. The crystals produced were filtered and dried in an oven at 60°C. The crystals were periodically dried and weighed until constant weight was achieved. The dried crystals were stored in a screw cap glass vials for further characterization works.

Table 1. Amount of mefenamic acid used in different solvents.

Method	Solvent	Amount of Mefenamic Acid (g)
1	Ethyl actetate	1.59
2	Ethanol	1.14
3	Isopropanol	1.23
4	Dimethyl formamide	8.60
5	Dimethyl acetamide	33.87
6	Acetone	2.70

2.3 Characterisation methods

The images of the crystals were captured using Leica microscope DM750 with a total magnification of 200x4x/0.10 and processed using Leica Application Suite Software version 3.6. The FTIR spectra of crystals from 500 to 4000 cm^{-1} were obtained using Perkin Elmer's ATR-FTIR Spectrometer (Frontier). The spectra were analyzed using OMNIC software with an average scan of 16 [12].

The melting properties analysis was performed using a Mettler Toledo DSC-1 from temperature of 25 to 300°C at a heating rate of 10 °C/min under constant purging of nitrogen [9]. About 2-10 mg of crystals was weighed into a 40 µL standards aluminum pan with lid and sealed hermetically prior to analysis. An empty aluminum pan was used as a reference in all the runs. The results were analyzed using Mettler Toledo Stare SW 9.10. The TA Instruments (Q500/50) was also used to study the changes of weight derivatives with temperature. Sample with a weight range of 4-6 mg was placed on a platinum pan and heated from 25 to 300°C at a constant heating rate of 10 °C / min.

The x-ray powder diffraction patterns were recorded using Shimadzu XRD 6000 equipped vertical x-ray goniometer and Cu Kα radiation with angle reproducibility of ±0.001° (2θ). Prior to analysis, the crystals were initially placed into an aluminum holder and gently grinded, pressed and flatten softly using spatula and glass plate. The measurement conditions used were: 40 kV of voltage; 30 mA of current; 5-50° (2θ) of scan range; 0.05° of step size and 3°/min of scan mode in a continuous mode [9].

3 Results and Discussion

3.3.1 Shape of crystals

Shape or habit of crystals of may influence the bulk density, flowability and mechanical strength of the pharmaceutical compound [13]. Fig. 1 shows the shapes of crystals produced using different solvents. As seen in Fig. 1 (a), (b), (c) and (f), mefenamic acid which crystallised using EA, ethanol, IPA, and acetone, respectively are elongated needle-like flat shaped crystals and consistent with those reported in the literature [8, 14, 15]. Fig. 1 (d) shows that the crystals crystallised from DMF are cubic-shaped. Meanwhile, the crystallisation using DMA produced a plate-like-shaped crystals (Fig. 1 (e)). A comparison, however, cannot be made for crystals obtained using DMA as it is yet to be reported in the literature.

The difference in crystal shapes produced in this work are probably due to the variation of solvents' polarity which results in different solute-solvent interactions at the crystal's facets. The crystallographic structure of mefenamic acid obtained from Lee and co-workers shows the presence of –OH, -C=O and $–C_6H_5$ which were exposed on the crystal facet (0 0 1) [16]. The –OH and -C=O have significant hydrogen bonding ability and thus, may permit strong hydrogen bonding interactions between facet (0 0 1) and solvents with high polarity. Based on the polarity index, DMF is expected to form stronger interactions with the facet (0 0 1)

followed by DMA, ethanol, propanone IPA, and EA. The strong hydrogen bonding interactions between the facet and solvent will consequently reduce the interfacial tensions and enhance the growth of the crystal facet. The relative contribution of the facet, however, decreases with the increase of solvent's polarity [15]. This phenomenon explained the reduction of crystal length produced using DMF and DMA solvents as shown in Fig. 1 (d) and (e), respectively.

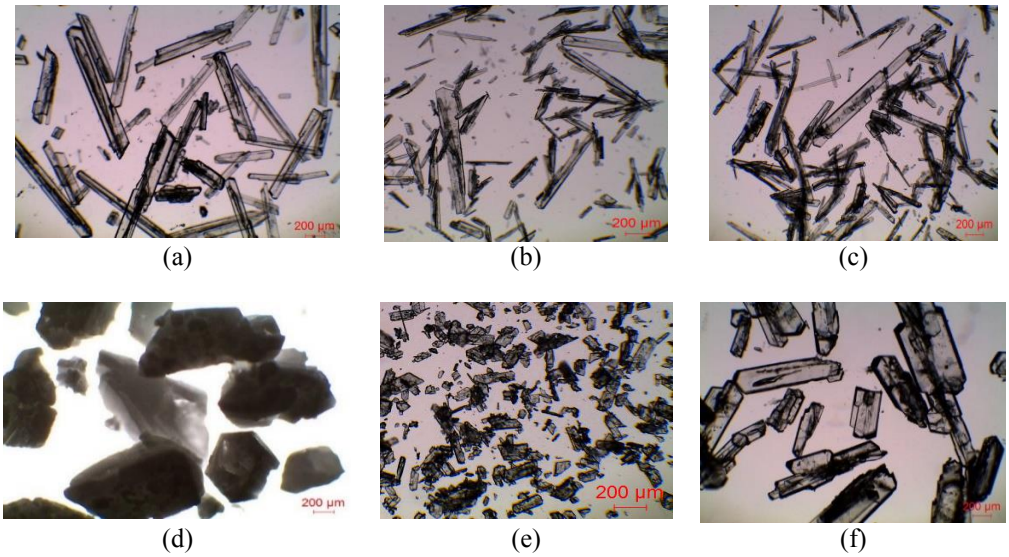

Fig. 1. Optical microscopy images of crystals obtained from crystallisation using different solvents: (a) ethyl acetate; (b) ethanol; (c) IPA; (d) DMF; (e) DMA; and (f) acetone.

3.3.2 Infrared spectroscopy

Fig. 2 shows partial infrared spectra for mefenamic acid crystals as received from the supplier and after crystallisation using different solvents. As shown by the dashed vertical line in Fig. 2, the crystals obtained after crystallised using EA, ethanol, IPA, DMA, and acetone have N-H stretching band at approximately 3313 cm^{-1}, which corresponds to Form I of mefenamic acid. The crystals obtained using DMF as solvents show N-H stretching at 3347 cm^{-1}, which correspond to Form II. The higher wavelength observed for the N-H stretching of mefenamic acid Form II suggests a weaker hydrogen interaction between carboxylic groups in comparison with mefenamic acid Form I [14]. Previous works also highlighted that the N-H stretching band that occurs between 3300 and 3350 cm^{-1} is an important spectrum that can be used to distinguish either Form I or Form II of mefenamic acid [17, 21].

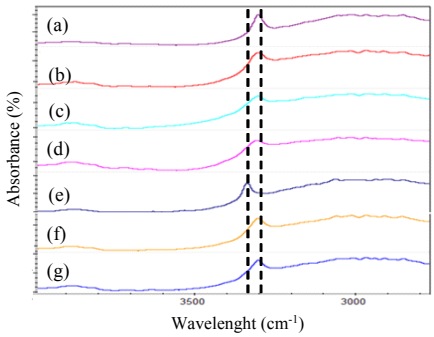

Fig. 2. Partial infrared spectra of of mefenamic acid samples: a) as received, and after crystallised using (b) ethyl acetate, (c) ethanol, (d) IPA, (e) DMF, (f) DMA, and (g) acetone.

3.3.3 Thermal behaviour

The differential scanning calorimetry (DSC) and thermal gravimetric (TG) curves of mefenamic acid after crystallisation using different solvents are shown in Fig. 3 (a) until (f). The DSC curve in Fig. 3 (a), (b), (c), (e), and (f) have two endothermic peaks. The onset temperature of the first endothermic peak of mefenamic acid shown in those figures varied from 168.82 to 190.16°C. Meanwhile, for the second endothermic peak, the values are from 226.75 to 232.08°C. These values concur with those values of mefenamic acid Form I reported in the literature [9, 12, 14, 17]. The temperature of the first and second endothermic peaks represent the transition temperature, T_T of Form I to Form II and the melting or fusion, T_f of Form II, respectively. The presence of an endothermic peak before the melting point shows that the mefenamic acid polymorphs are enantiotropic, where it can reversibly transform into one another by cooling or heating process [14].

The DSC curve of mefenamic acid crystallised using DMF as shown in Fig. 3 (d) on the other hand, only has one endothermic peak with molar enthalpy of fusion of 38.31±5.51 $kJmol^{-1}$. This value is comparable with those reported by literature, which is 38.243, 38.7 and 40.39 $kJmol^{-1}$ [12, 18]. The endothermic peak is associated with the molecule decomposition [14]. The DSC curve with one endothermic peak is consistent with mefenamic acid Form II characteristic [14]. The derivative TG curves shown in Fig. 3 (a) until (f) are presented to provide correlation between the weight loss and the calorimetric profile. The curves show a significant derivative weight change between 165 and 245°C, which

is approximately at the second endothermic peak of the DSC curve. The change in the derivative weight is believed due to the decomposition of mefenamic acid crystals during the melting process. In addition, no significant derivative weight was detected in the region of 80 to 100°C, whereas in many cases, it may cause the decomposition of the substrate material or volatilisation of residual solvents [19].

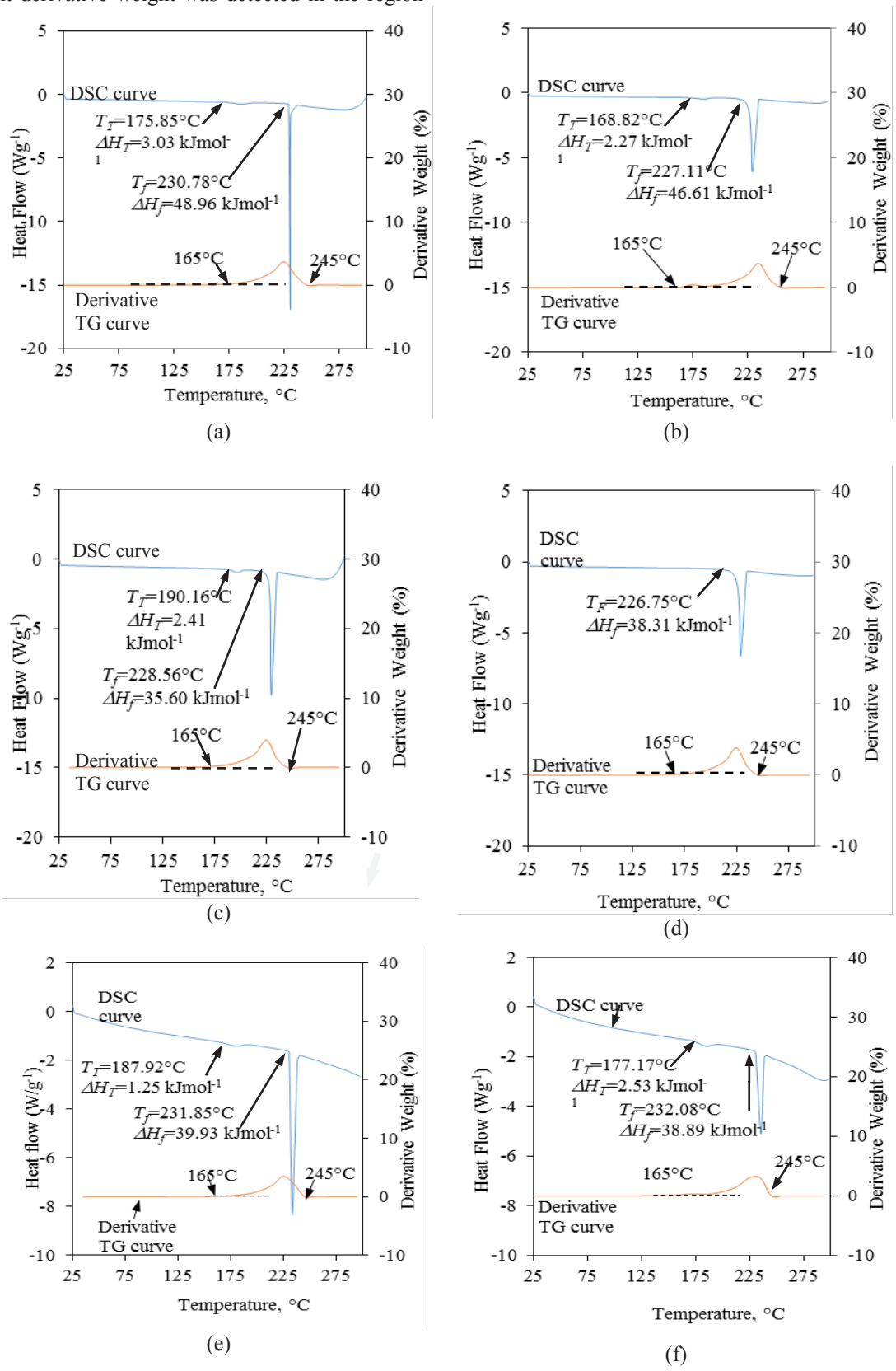

Fig. 3. DSC and derivative TGA curves of mefenamic acid crystals obtained by (a) ethyl acetate; (b) ethanol; (c) IPA; (d) DMF; (e) DMA; and (f) acetone.

3.3.4 Phase analysis

The XRPD pattern of mefenamic acid crystals obtained from EA, ethanol, IPA, DMA and acetone in comparison with some major reflections in the reference pattern of Form I reported literature [8, 20] is shown in Fig. 4 (a), (b), (c), (d) and (e), respectively. The pattern concurs with the literature. In addition, any significant peaks at 11.8°, 17.9°, 23.8° and 25.6°, as well as 9-12° (2θ) which represent Form II of mefenamic acid were not observed. This confirms that pure Form I crystals were successfully produced using ethyl acetate, ethanol, IPA, DMA and acetone.

The comparison of XRPD pattern of the crystals obtained from DMF with some major reflection of Form II reported by Kato et al. [20] and Cesur and Gokbel [8] are illustrated in Fig. 4 (f). The crystallisation of mefenamic acid using N, N-dimethyl formamide as a solvent produced an XRPD pattern that concurred very well with XRPD pattern of Form II reported by those literature [8, 20]. This finding demonstrates that crystallisation from DMF able to produced mefenamic acid crystals Form II.

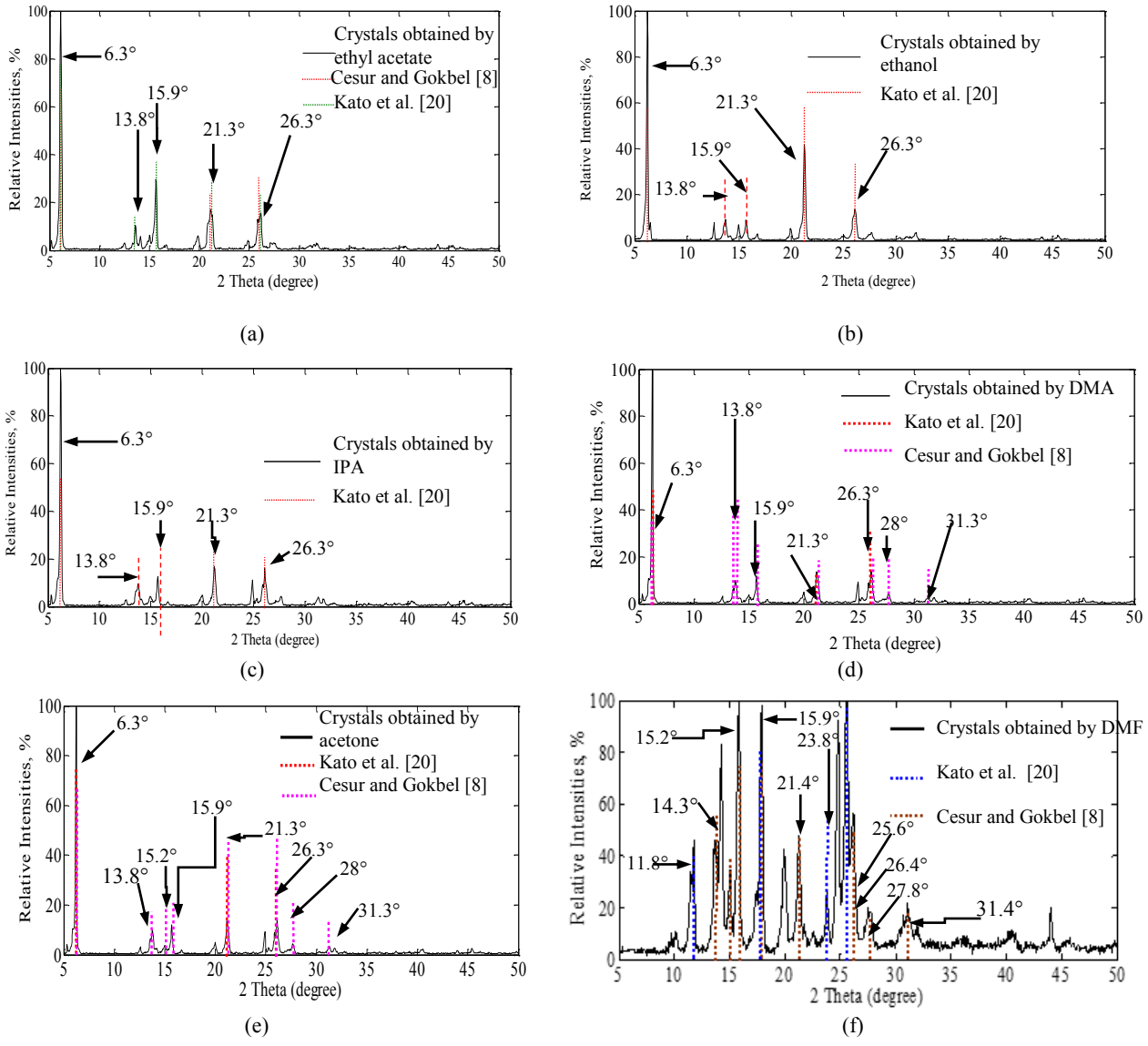

Fig. 4. XRPD pattern of crystals obtained from (a) ethyl acetate; (b) ethanol; (c) IPA, (d) DMA, (e) acetone and (f) DMF in comparison with some major reflections with the reference pattern of Form I or Form II obtained from literature.

Conclusion

The crystallisation of mefenamic acid using different solvents with natural cooling mode produced crystals with different polymorphic forms and habits. The results of the thermal analysis (DSC), FTIR spectroscopy and XRPD have shown that the crystals obtained from EA, ethanol, IPA, DMA and acetone are pure Form I, while DMF is pure Form II. No solvent entrapment in the mefenamic acid crystals were observed during thermal studies and the crystals were completely decompose during melting. The crystals produced were needle-like and cubic shaped crystals, depending on the solvents used.

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