Article

Preparation and Evaluation of an Elusive Polymorph of Mefenamic Acid by High Pressure Techniques

Nasir Abbas [1,*], Iain Oswald [2] and Colin R. Pulham [3]

[1] University College of Pharmacy, University of the Punjab, Lahore, Pakistan
[2] Strathcltyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow, G4 0RE, UK; iain.oswald@strath.ac.uk
[3] School of Chemistry, University of Edinburgh, David Brewster Road, Edinburgh EH9 3FJ, UK; c.r.pulham@ed.ac.uk
* Correspondence: nasirabbas77@gmail.com

Abstract: High pressure crystallization technique has been successfully used to prepare an elusive form II of a non-steroidal anti-inflammatory drug, Mefenamic acid. Single crystal of form II was grown at 0.3 GPa from an 4:1 methanol/ ethanol mixture as a solvent using Diamond Anvil Cell. Comparison of crystal structures show that the efficient packing of MA molecules in Form II results from the structural flexibility of MA acid molecules. Compression studies performed on the single crystal of form I resulted in 14 % decrease of unit cell volume up to 2.5 GPa. No phase transition was observed up to this pressure. A reconstructive phase transition is required to induce conformational changes in the structure, which is confirmed by crystallization at high pressure results.

Keywords: mefenamic acid; diamond anvil cell; high-pressure; polymorphism; high-pressure crystallization; non-steroidal anti-inflammatory drugs

1. Introduction

The phenomenon polymorphism is solid state is the ability of a substance to crystallize with more than one crystal forms; called as polymorphs. The polymorphism of organic compounds is of fundamental interest to experimental and theoretical chemists, and is also of crucial importance to industry for a wide range of materials that includes pharmaceuticals, pigments, explosives (energetic materials), food products and proteins [1, 2]. In pharmaceuticals, polymorphism of the active pharmaceutical ingredient (API) is of great importance. Difference in the three dimensional arrangements and/or conformations of molecules within the unit cell of polymorphs can have profound effects on the physical and chemical properties of the API such as melting point, sublimation temperature, heat capacity, solubility, dissolution and bio-availability [2]. Many processing properties are also dependent on the polymorphic behaviour of the active pharmaceutical agents (API) [1]. Polymorphic inter-conversion during manufacturing process (such as compression, milling and drying) and storage of drug compounds is also of crucial importance, where changes in conditions e.g. temperature, relative humidity and pressure may results in the formation of new forms. Patent rights are also become an issue, as polymorphs of a same compound can be patented separately. The cases of Ritonavir (an HIV drug by Abbot Laboratories) and ranitidine hydrochloride (Zantac, the largest selling anti-ulcer drug from GlaxoSmithKline), provide examples of the importance of polymorphism in processing and patent litigation, where emergence of new polymorphs results in huge financial loss to the companies [3, 4]. For all the above reasons

Peer-reviewed version available at Pharmaceutics 2017, 9, , 16; doi:10.3390/pharmaceutics9020016

pharmaceutical companies deploy a great effort and resources for the identification of all the possible polymorphs in the early stage of development.

In recent years considerable effort has been put into developing high-throughput, automated approaches to polymorph screening where key crystallization variables such as solvent and antisolvent selection, evaporation and cooling rate followed by the characterization by spectrosopic, diffraction and thermodynamical techniques are employed [5]. The main aim is to develop methods that are capable of producing and identifying large numbers of samples in a relatively short period of time. Even after these exhaustive tests, a new polymorph may remain undetected for many years, or a sample of a new polymorph may be obtained once but never again, the phenomenon of so-called "disappearing polymorphs" [6, 7]. Polymorphism remains one of the major challenges for both the industry and academic sectors. The development of complementary methods with better control on the crystallization conditions, and more importantly the understanding of factors that result in different packing arrangements of organic molecule in the solid state would therefore be highly desirable [8].

There is a need to explore alternative crystallisation conditions *e.g.* use of pressure as additional variable in conjunction with conventional strategies. Polymorph screening is normally performed at ambient conditions of pressure. Pressure can change the relative thermodynamic stability of polymorphs and also can have effect on the conformation of molecules as they pack within a crystal structure. It can act as an additional variable for the identification of new polymorphs. High-pressure has already been extensively applied in polymorphic studies of many simple organic compounds such as amines, ketones, carboxylic acids, alcohol and amino acids [9-15], where direct compression has successfully been used to generate new polymorphs. However sometime only compression is not enough for these compounds where kinetic barrier of molecular rearrangement is high and cannot be achieved by simple compression. To overcome this problem a technique has been developed which involves recrystallization of compound from solutions under elevated pressure (i.e. pressure induced crystallization). This has successfully been used to generate new polymorphs of simple compounds e.g. new polymorphs of parabanic acid [8], urea [16], acetamide and paracetamol [8, 17].

Mefenamic acid (2-[(2, 3-dimethyphenyl)-amino] benzoic acid), is a well known fenamate derivative showing potent analgesic effect [18] and is marketed as Ponstel®. It belongs to the group of medicines called non-steroidal anti-inflammatory drugs (NSAIDs), which are among the most frequently used medicinal drugs. MA is known to exist as three polymorphic forms, denoted as forms I, II and III. These polymorphs show different solubility and stability behaviour [19, 20]. Form I is the most stable form under ambient conditions, but form II is the stable form above 160^{o}C, form III is the least stable form at ambient conditions and converts back to form I immediately [21]. The crystal structures of form I, II and II was reported in 1976, 2008 and 2013, respectively [22]. NMR spectroscopy and theoretical studies have shown that form I has some conformational similarities with related fenamate compounds *e.g.* flufenamic acid, niflumic acid and maclofenamic acid [23, 24]. MA molecule shows structural flexibility that may results in more conformational polymorphs using high pressure conditions. Compression behaviour of the two polymorphs has been studied in connection with the mechanical properties of the finished tablets, but very little attention has been paid to possible polymorphic transitions of the active ingredient under mechanical treatment [25]. All polymorph screening of MA has been performed at ambient pressure [26]. For all the above

Peer-reviewed version available at Pharmaceutics 2017, 9, , 16; doi:10.3390/pharmaceutics9020016

reasons the aims of the current investigation were therefore to explore whether high-pressure methods could be used to obtain the elusive form II, or other new forms.

2. Materials and Methods

Mefenamic acid was obtained from the Sigma Aldrich, UK. Single crystals were obtained by slow cooling of a hot saturated solution (*ca.* 1M) in THF. A small, colourless, rectangular shaped crystal was taken directly from the crystallised sample and was confirmed as being form I by indexing the diffraction data from single crystal X-ray diffraction. Crystals from the same solution were used in all of the single crystal compression experiments. Analytical grade solvents were used for the recrystallisation experiments.

High-pressure experiments were carried out using Merrill-Bassett diamond-anvil cell (DAC) (half-opening angle 40°) [27], equipped with 600 μm culet diamonds and a pre-indented tungsten gasket of thickness 250 μm and a 300 μm diameter hole. For high-pressure crystallisation experiments, diamond-anvil cells using tungsten carbide backing disc [28] were used.

2.1. Compression Studies

For compression studies a single crystal of suitable size was loaded into DAC along with a small piece of ruby in order to allow the determination of the pressure by laser fluorescence method [29] using a 632.8 nm excitation line from a He-Ne laser. The fluorescence was detected by a Jobin-Yvon LabRam 300. A methanol-ethanol (4:1) mixture was used as the pressure-transmitting medium [30].

2.2. Crystallization at High Pressure

For high pressure crystallization, a *ca.* 1M solution of mefenamic acid was prepared in ethanol. The solution was then loaded along with a few crystallites of form I at 293(2) K into a DAC. The pressure was increased to a minimum in order to form a sealed system and the cell was then gently heated to dissolve all of the solid material. Pressure was then applied by tightening the screws of the DAC to induce precipitation. Crystallisation was achieved at *ca*. 0.6 GPa. The pressure was then reduced to 0.3 GPa in order to facilitate growth of a single crystal. The temperature was then cycled near 353 K in order to dissolve all but one of the crystallites. On slow cooling to 298 K a single crystal grew.

2.3. Single Crystal X-ray Diffraction

Diffraction data were collected on a Bruker APEX II CCD diffractometer at 293(2) K using Mo-Kα radiation (λ = 0.71073). Data indexing was performed using *CELL_NOW* [31]. Data processing was performed according to the procedure described by Dawson *et al*. (2004) [32]. Integration of data sets and global cell refinement were carried out using the program *SAINT* [33], in which 'dynamic masks' were used in order to prevent integration of areas of the detector shaded by the body of the DAC. An analytical correction for the absorption by the DAC component was then applied using *SHADE* [34] and an absorption correction for the crystal was applied using *SADABS* [35]. Known coordinates were taken from the literature [22]. Refinement was then performed using *CRYSTALS* [36]. All non-hydrogen atoms were refined isotropically and hydrogen atoms were placed in

Peer-reviewed version available at Pharmaceutics 2017, 9, , 16; doi:10.3390/pharmaceutics9020016

calculated positions. A completeness of only *ca.* 40 % was obtained for this dataset and this led to a rather high *R*-factor of 9.5 %. Nevertheless, this was sufficient to identify the main structural feature of form II and make a comparison between the polymorphs. Full structural refinements details and Crystallographic data in CIF format is attached as supplementary information.

3. Results

3.1. Direct compression of a single crystal of form I

The first data-set was collected at ambient pressure. Indexing of the reflections confirmed the known triclinic form I of MA. Diffraction data were then collected in incremental steps of pressure up to 2.5 GPa. Lattice parameters at pressures up to 2.5 GPa are shown in Table 1. On raising the pressure to 3.0 GPa the crystal disintegrated.

Table 1: Lattice parameters for form I with increasing pressure up to 2.50 GPa.

Pressure	**Ambient**	**0.18 GPa**	**0.49 GPa**	**0.90 GPa**	**1.67 GPa**	**2.50 GPa**
Crystal System	Triclinic	Triclinic	Triclinic	Triclinic	Triclinic	Triclinic
Space group	*P*-1	*P*-1	*P*-1	*P*-1	*P*-1	*P*-1
***a* (Å)**	6.7582(14)	6.775(2)	6.7120(13)	6.6432(3)	6.5642(13)	6.5153(13)
***b* (Å)**	7.3391(15)	7.288(2)	7.2369(14)	7.1516(6)	7.1406(14)	7.0188(14)
***c* (Å)**	14.3127(29)	14.303(15)	14.0762(28)	13.7098(8)	13.4047(27)	13.1342(26)
***α* (°)**	76.69(3)	76.72(5)	77.02(3)	77.724(6)	77.888(3)	78.38(26)
***β* (°)**	79.83(3)	79.11(5)	79.43(3)	78.962(4)	77.426(3)	77.11(3)
***γ* (°)**	65.70(3)	65.55(2)	65.72(3)	65.606(6)	63.846(3)	62.82(3)
V (Å^3)	626.94(31)	622.1(7)	604.1(31)	575.69(6)	545.78(2)	535.0(17)
Z	2	2	2	2	2	2
T (K)	298	298	298	298	298	298

All data-sets up to 2.50 GPa were processed according to the procedure described above. As a result of the limitations caused by shading from the steel body of the diamond-anvil cell, high-pressure data-sets are frequently incomplete compared with data-sets recorded at ambient pressure. These factors combined to make structural refinement particularly challenging and in this case resulted in poor structural refinement with high *R*-factors. Therefore, only lattice parameters are reported here. Figure 2 shows the variation of unit cell volume with increasing pressure. There was a *ca.* 14 % decrease in the unit cell volume over the studied pressure range. Within the limits of experimental errors, the volume decreases smoothly over this pressure range, indicating that there is no phase transition associated with direct compression of the single crystal.

Peer-reviewed version available at Pharmaceutics 2017, 9, , 16; doi:10.3390/pharmaceutics9020016

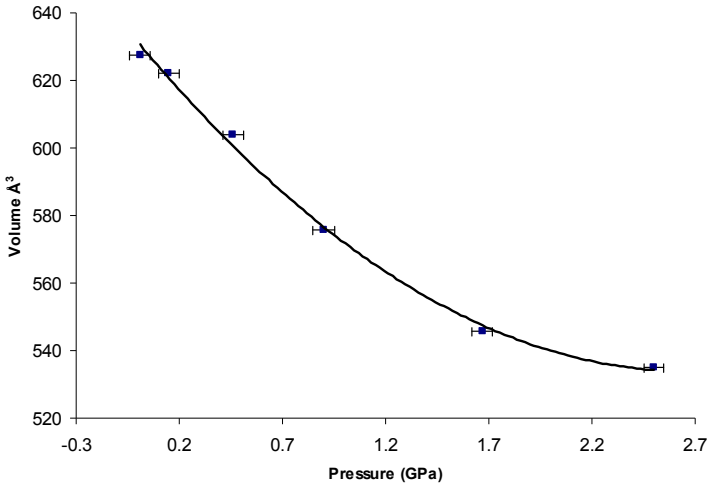

Figure 1: variation of unit cell volume of form I with pressure.

The observation that at 3.0 GPa the crystal disintegrated to give a polycrystalline powder, suggests reconstructive phase transition at this pressure. These observations prompted a study by recrystallisation from solution at high pressure.

3.1.2. Crystallization from solution at high pressure

Crystal structure obtained from the single crystal grown at 0.3 GPa gave a triclinic unit cell with lattice parameters shown in Table 1. Crystallographic data obtained from the high pressure crystallization experiments is compared with already published forms I, II and III (Table 1). All three polymorphs (I–III) crystallize in the triclinic space group (P-1) with a molecule in the asymmetric unit; such an observation is relatively rare, wherein all the polymorphic forms consist of Z′ = 1.

Table 2: Crystallographic information of various polymorphs of MA compared with high pressure structure.

Parameter	MA II (high-pressure crystallisation)[a]	MA II (Lee, Byrn and Carvjal)[b]	MA III	MA I (CSD:XYANAC)[c]
Chemical formula	$C_{15}H_{15}NO_2$	$C_{15}H_{15}NO_2$	$C_{15}H_{15}NO_2$	$C_{15}H_{15}NO_2$
Formula weight	241.29	241.29	241.29	241.29
Crystal system	*P*-1	*P*-1	*P*-1	*P*-1
Space group	Triclinic	Triclinic	Triclinic	Triclinic
a (Å)	7.7900(15)	7.6969	7.723(2)	14.556
b (Å)	9.1890(18)	9.1234	7.9340(10)	6.811
c (Å)	9.4120(19)	9.4535	11.2320(10)	7.657
α (°)	106.751(10)	107.113	83.590(10)	119.57
β (°)	92.287(12)	91.791	80.940(10)	103.93
γ (°)	101.377(11)	101.481	67.510(10)	91.30
V (Å^3)	629.1(2)	618.89	626.96)	631.766
Z	2	2	2	2
T (K)	298(2)	150	298(2)	298
$R(F_o)$	0.095	0.052	0.042	0.045
$Rw(F^2_o)$	0.095	0.134	0.109	

Programme used	CRYSTALS	SHELXTL	MULTAN

[a] this study [b] E. H. Lee *et al.* [24] [c] McConnell *et al.*[22]

3.1.3. Description of crystal structure

The molecular structure of MA with numbering scheme used in this work is shown in Figure 1. The conformation of the molecule in the crystal structure can be described by three torsional angles (see Figure 1): θ_1 (O1-C7-C6-C1) *i.e.* twisting of the carboxylic group with respect to C6-C7 axis; θ_2 (C1-N1-C8-C13) *i.e.* twisting of the second phenyl group with respect to the N1-C8 axis; and θ_3 (C6-C1-N1-C8) *i.e.* twisting of bridging group relative to the C1-N2 axis. These torsional angles are shown by arrows in Figure 1 and are calculated by using the program *MERCURY* [37].

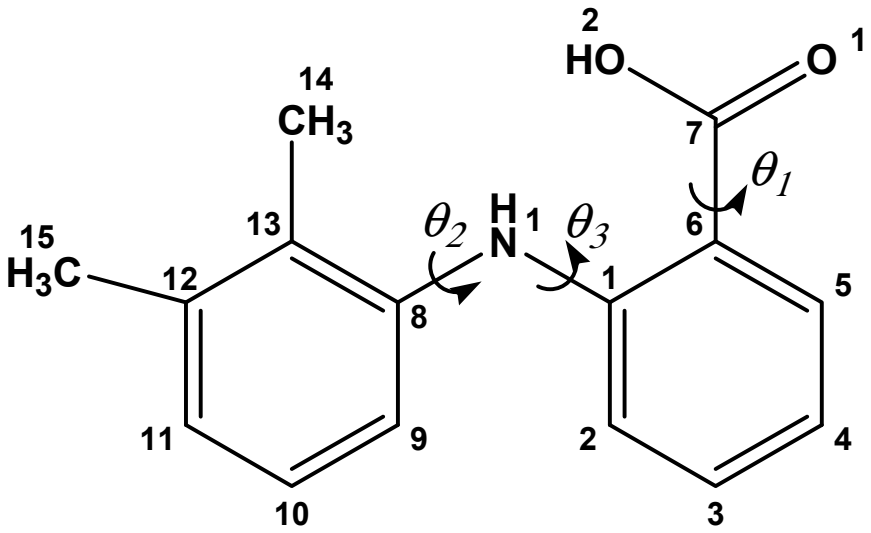

Figure 2: Mefenamic acid molecule, with numbering scheme used in this work. Definition of torsional angles: θ_1 is the angle involving O1-C7-C6-C1, θ_2 is the angle involving C1-N1-C8-C13 and θ_3 is the angle involving C6-C1-N1-C8.

The crystal packing diagram of form I, II and II (high pressure form) are compared in Figure 3. Although the polymorphs exhibit significant variation in the unit cell dimensions, the MA molecules pack as dimer units through an intermolecular hydrogen bond involving the carboxylic acid group (O2-H…O1) with an O2…O1. The length of this hydrogen bond is comparable in all the three forms with the value of ~1.68 Å. The phenyl ring bearing the carboxyl group is coplanar with the carboxyl- and bridging amino-groups. This coplanar conformation is stabilised by the resonance interaction and internal hydrogen bond between the amino- and carboxyl-groups [38]. The distance between the atoms (N1…O1) involved in this interaction in form I, II and III 1.88, 2.01 and 2.09 Å, respectively.

The description of the hydrogen-bond pattern according to the graph-set notation at the first level graph set is N_1 = S(6)R_2^2 (8) (The graph-set notations are assigned by the method described by Bernstein *et al.* [39], and is obtained from *PLUTO* [40] and *MERCURY* [37]). Where S and R motifs represents internal and external hydrogen bonds, respectively.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 March 2017 doi:10.20944/preprints201703.0050.v1

Peer-reviewed version available at Pharmaceutics 2017, 9, , 16; doi:10.3390/pharmaceutics9020016

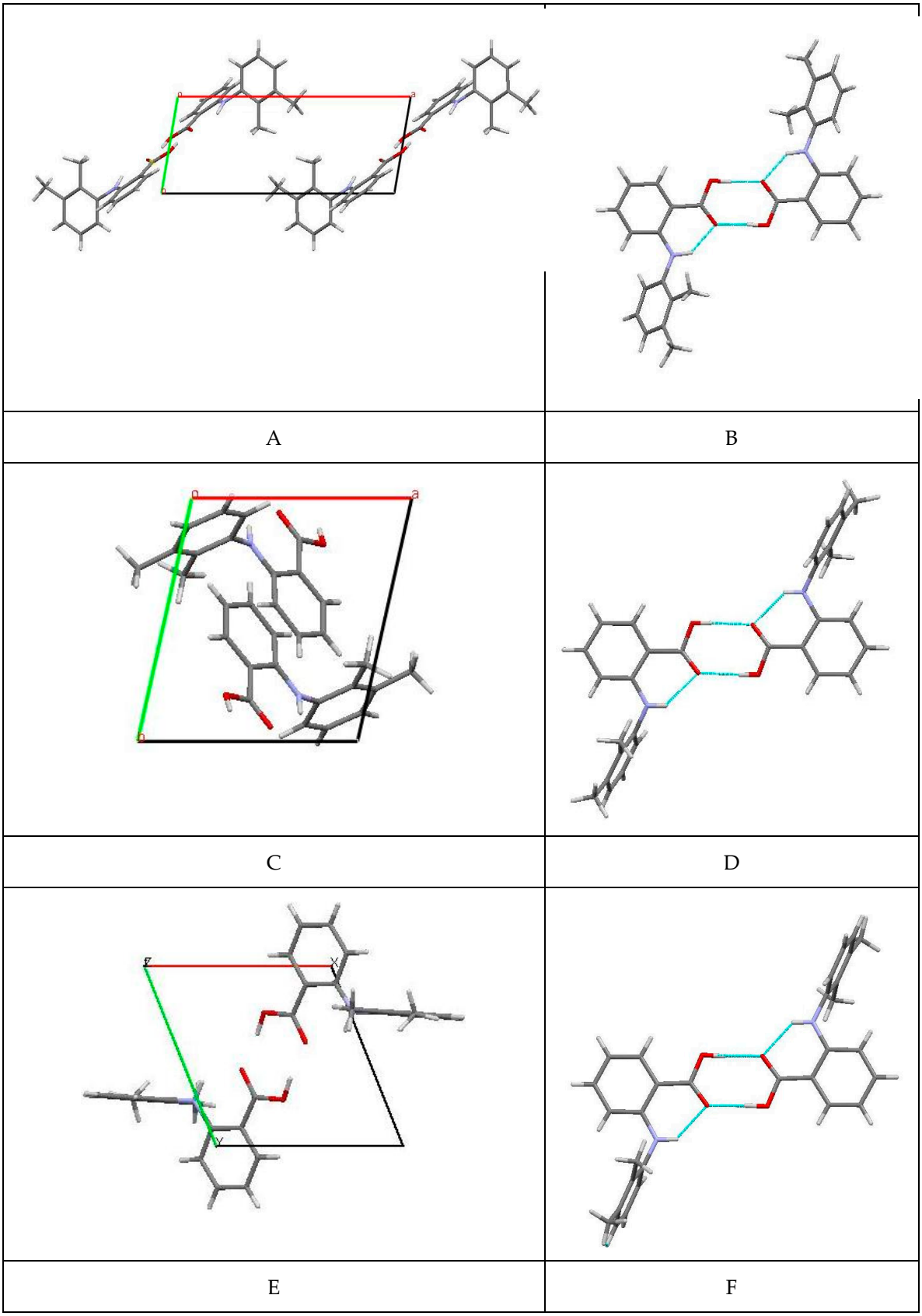

Figure 3: (A) Crystal packing diagram of form I viewed down the *c*-axis (B) Dimer unit of MA molecule in form I (C) Crystal packing diagram of form II (D) dimer unit of MA molecule in form II (E) Crystal packing diagram of form III viewed down the *c*-axis (F) Dimer unit in form III.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 March 2017 doi:10.20944/preprints201703.0050.v1

Peer-reviewed version available at Pharmaceutics 2017, 9, , 16; doi:10.3390/pharmaceutics9020016

The comparison of space-filling diagrams (Figure 4) of forms I, II and III indicates more efficient packing of MA molecules in form II. The difference in the crystal packing arises from the torsional angle θ_2 (Table 3), where the values has changed significantly from -119.99° to -85.19°, whilst the deviation in the other two torsional angles, (θ_1 and θ_3) are very small. Due to changes in θ_2, the carboxylic acid group and the amino group are no longer coplanar and as a result the length of the intramolecular hydrogen bond (N1...O1) increases from 1.82 Å to 2.01 Å.

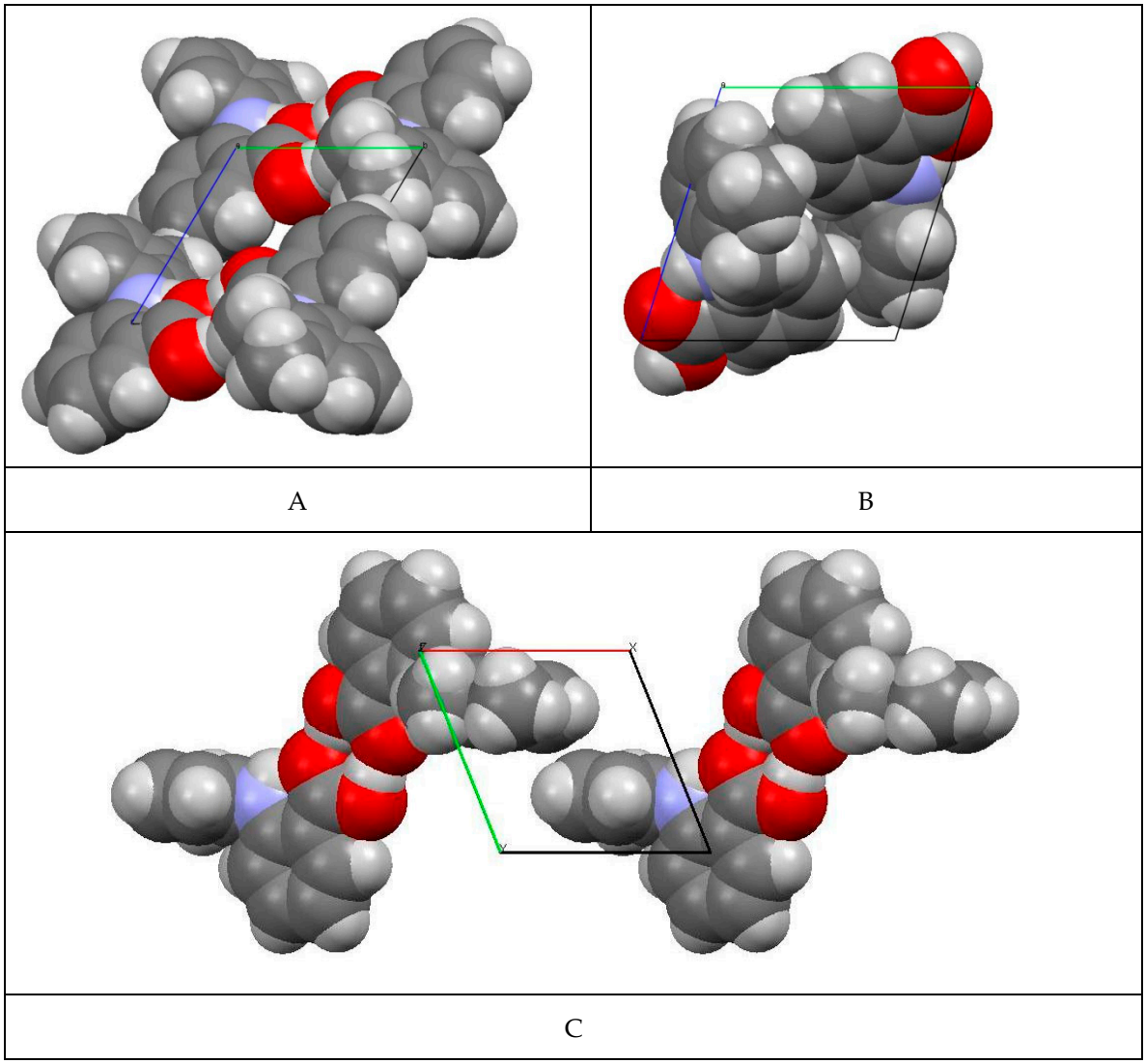

Figure 4: Space-filling diagrams for (A) forms I (B) form II (C) Form III.

Table 3: Comparison of torsional angles between forms I and II of MA.

Torsional angle	MA I [22]	MA II [this study]	MA III [21]
θ_1	-1.71	-5.30	-5.20
θ_2	-119.99	-90.07	-80.82
θ_3	-179.34	-171.56	-178.31

3.1.4. Decompression studies

On progressive decompression from 0.3 GPa at 293 K to ambient pressure, no colour change or destruction of the crystal was observed. This tiny crystal was then removed from gasket, placed on

Peer-reviewed version available at Pharmaceutics 2017, 9, , 16; doi:10.3390/pharmaceutics9020016

the goniometer and was successfully indexed as form II. However, owing to poor quality and the damage done during unloading from the gasket it was not possible to obtain sufficiently good data for full structure refinements. The crystal was also used as a seed in order to attempt the growth of single crystals of form II at ambient pressure, but this resulted in the production of form I instead. This suggests that presence of form I seeds in solution may dominate the crystallisation process.
In order to demonstrate reproducibility, repeated crystallisation at high pressure always resulted in form II. These results are of great significance as they demonstrate that form II can be prepared reproducibly at high pressure and recovered successfully to ambient pressure. Furthermore, unlike in the study by Lee *et al*., [24] no additives were required. This may suggest that the form II is the more thermodynamically stable form at high pressure, mirroring the behaviour of paracetamol [41].

4. Discussion

Mefenamic acid (MA) is an example of polymorphic system where the stable form (form I) can be obtained with relative ease, and its crystal structure is known from beginning (1976).
However, metastable elusive forms (form II and III) are very difficult to obtain. Our attention was drawn to this compound by the observation that form II is slightly denser than form I and has better packing of molecules within the unit cell. Hence our aim was to explore effect of pressure on the polymorphic transformation of MA and to explore weather form II could be prepared reproducibly either high pressure techniques.

There are two known methods to obtain the metastable form II; (i) heating of form I above the transition temperature (ii) cooling of a supersaturated solution in *N*,*N*-Dimethylformamide or chloroform. The thermal method resulted in the formation of a polycrystalline mass, and solution methods resulted in the conversion to form I on cooling. A recently reported method [18] involves crystallization of MA in the presence of structurally similar additive, flufenamic acid (FFA). They have described that MA molecules in form I have similar conformation as compared to FFA form I crystal structure. When MA crystallized in the presence of FFA, FFA blocked the nucleation process of MA form I and resulted in production of form II. This allowed crystal structure of form II to be determined for first time. This procedure is tedious, involves a number of controlled and lengthy processes (takes more than 20 days) and often results in failure. This is undesirable from both a scientific plus regulatory point of view, especially as crystal structures of as many polymorphic forms as possible, are required quickly and at an early stage of development.

Our initial attempts to obtain form II by recrystallisation from solution, using variety of solvent and at ambient pressure conditions were always resulted in the stable form I (see supplementary material). Different level of supersaturation was also applied but the results were same as stable form I was produces every time. Optical observation of recrystallisation process showed that on crystallisation the solution turned yellowish green first before white precipitates came out, which is indication of crystallization of form II. This may be explained by the fact that initially nucleated metastable polymorph (form II) dissolved rapidly into the surrounding phase and again nucleated as the stable form I. All these observations suggested that MA is an example of concomitant polymorphism where two or more crystal forms that nucleate and grow simultaneously under identical conditions but ends with the conversion of metastable form to most stable form. Occasionally, in case of concomitant polymorphs metastable form is not detected during the conventional screening process as it remains in contact with solution and may convert to the more

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 March 2017 doi:10.20944/preprints201703.0050.v1

Peer-reviewed version available at Pharmaceutics 2017, 9, , 16; doi:10.3390/pharmaceutics9020016

stable form via a solution-mediated transformation process by means of dissolution and recrystallisation [42, 43]. There was a need to explore alternative crystallisation conditions *e.g.* use of pressure as additional variable in conjunction with conventional strategies.

Compression behaviour of MA has been studied in connection with mechanical and dissolution properties [44], but very little attention has been paid to polymorphic transition under the conditions of high pressure. Previous studies have shown that the difference between polymorphs of MA comes from the torsional flexibility (figure 2) of molecules where rotation of one phenyl ring to other (torsional angle $\theta 2$), at the polymorphic transition from form I to form II. Direct compression of either single crystal or powder sample is one way to exploit this torsional flexibility. However our result on the single crystal compression studies shows that no phase transition has been observed by compressing single crystal of form I up to 1.62 GPa. Pressure was then raised to 3.8 GPa. At this pressure crystal disintegrated, suggesting a destructive phase transition. This is confirmed by the recrystallization from solution at high pressure, where form II was generated at 0.3 GPa pressure. Further, a nice single crystal of form II was produce by this procedure, which was previously very difficult to achieve.

High pressure encourages the efficient packing of molecules to normally give a denser structure. In the case of MA, conformation of MA molecule changed from form I to form II with the applied pressure in order to achieve better packing and the torsional angle θ_2 has been changed from -119.9° to -85.1° respectively. This conformational change results in weakening of internal hydrogen bond as length of this contact increases from 1.88 to 2.01 Å. This is depicted by IR spectra of two forms (figure 5) where NH stretching frequency has changed from 3311cm^{-1} to 3500 cm^{-1} as a result of transformation from form I to II.

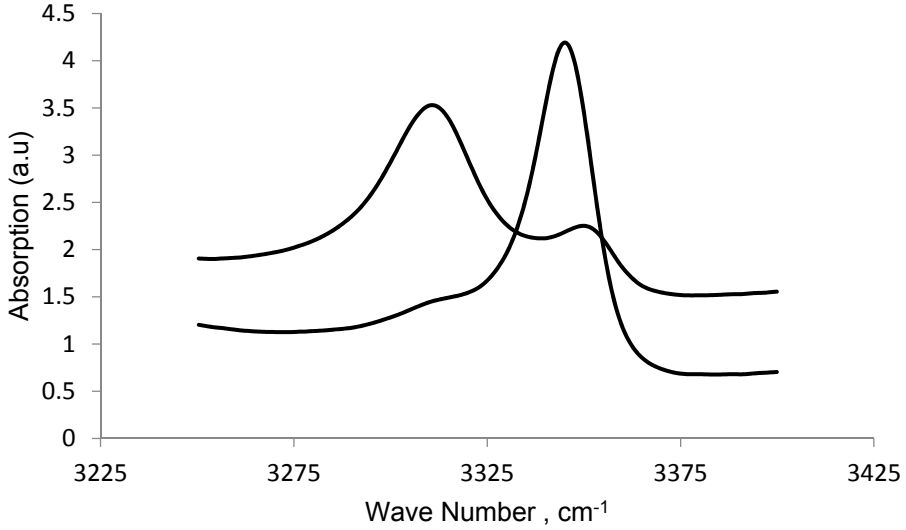

Figure 5: Comparison of IR absorption spectra (3250-3400 cm^{-1}) of MA recorded over short periods of milling.

5. Conclusions

Single crystal of form II was obtained by high pressure crystallization technique at 0.3 GPa. The example of mefenamic acid has demonstrated that pressure can be used to induce polymorphism in

organic compounds where conformational flexibility in the structure may results in the production of new and elusive polymorphs. Direct compression of solid substance may encounter by a kinetic barrier which can be overcome by this process.

Supplementary Materials: The following are available online at www.mdpi.com/link, crystallographic information files for the structures

Acknowledgments: Authors would like to acknowledge University of Edinburgh and EastChem, UK for providing funding for this project.

Author Contributions: "We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors". The author, Nasir Abbas has done the lab work and prepared manuscript under the supervision of Colin Pulham. Iain Oswald helped to design the studies and help in structure solution and refinements. Iain Ostwald has also critical reviewed the manuscript and provided a valuable input in the refinement of the manuscript.

Conflicts of Interest: "The authors declare no conflict of interest."

References

1. Giron, D., Contribution of thermal methods and related techniques to the rational development of pharmaceuticals—part 1. *Pharm. Sci. Technol. Today.* **1998,** *1* (5), 191-199, http://dx.doi.org/10.1016/S1461-5347(98)00046-7
2. Bernstein, J., *Polymorphism in Molecular Crystals*. Oxford University Press: 2007; Vol. 14, DOI:10.1093/acprof:oso/9780199236565.001.0001
3. Chemburkar, S. R.; Bauer, J.; Deming, K.; Spiwek, H.; Patel, K.; Morris, J.; Henry, R.; Spanton, S.; Dziki, W.; Porter, W., Dealing with the impact of ritonavir polymorphs on the late stages of bulk drug process development. *Org. Process Res. Dev.* **2000,** *4* (5), 413-417, DOI:10.1021/op000023y
4. Raw, A. S.; Furness, M. S.; Gill, D. S.; Adams, R. C.; Holcombe, F. O.; Lawrence, X. Y., Regulatory considerations of pharmaceutical solid polymorphism in Abbreviated New Drug Applications (ANDAs). *Adv. Drug Deliv. Rev.* **2004,** *56* (3), 397-414. http://dx.doi.org/10.1016/j.addr.2003.10.011
5. Ismail, S. Z.; Anderton, C. L.; Copley, R. C.; Price, L. S.; Price, S. L., Evaluating a crystal energy landscape in the context of industrial polymorph screening. *Cryst. Growth Des.* **2013,** *13* (6), 2396-2406. DOI:10.1021/cg400090r
6. Dunitz, J. D.; Bernstein, J., Disappearing polymorphs. *Acc. Chem. Res.* **1995,** *28* (4), DOI:193.10.1021/ar00052a005
7. Bernstein, J.; Henck, J.-O., Disappearing and reappearing polymorphs—an anathema to crystal engineering? *Cryst. Eng.* **1998,** *1* (2), 119-128. http://dx.doi.org/10.1016/S0025-5408(98)00086-5
8. Fabbiani, F. P.; Allan, D. R.; Marshall, W. G.; Parsons, S.; Pulham, C. R.; Smith, R. I., High-pressure recrystallisation—a route to new polymorphs and solvates of acetamide and parabanic acid. *J. Cryst. Growth* **2005,** *275* (1), 185-192. http://dx.doi.org/10.1016/j.jcrysgro.2004.10.083
9. Hemley, R. J.; Dera, P., Molecular crystals. *Rev. Mineral. Geochem.* **2000,** *41* (1), 335-419. DOI:10.2138/rmg.2000.41.12
10. Boldyreva, E. V., High-pressure diffraction studies of molecular organic solids. A personal view. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **2008,** *64* (1), 218-231. http://dx.doi.org/10.1107/S0108767307065786
11. Boldyreva, E. V., High-pressure studies of the hydrogen bond networks in molecular crystals. *J. Appl. Crystallogr.* **2004,** *700* (1), 151-155. http://dx.doi.org/10.1016/j.molstruc.2004.03.026

Peer-reviewed version available at Pharmaceutics 2017, 9, , 16; doi:10.3390/pharmaceutics9020016

12. Boldyreva, E. V., High-pressure studies of the anisotropy of structural distortion of molecular crystals. *J. Appl. Crystallogr.* **2003,** *647* (1), 159-179. http://dx.doi.org/10.1016/S0022-2860(02)00520-3
13. Katrusiak, A., High-Pressure X-ray Diffraction Studies on Organic Crystals. *Cryst. Res. Technol.* **1991,** *26* (5), 523-531. DOI:10.1002/crat.2170260502
14. Oswald, I. D.; Allan, D. R.; Motherwell, W. S.; Parsons, S., Structures of the monofluoro-and monochlorophenols at low temperature and high pressure. *Acta Crystallogr., Sect. B: Struct. Sci* **2005,** *61* (1), 69-79. https://doi.org/10.1107/S0108768104030617
15. Moggach, S. A.; Parsons, S.; Wood, P. A., High-pressure polymorphism in amino acids. *Crystallogr. Rev.* **2008,** *14* (2), 143-184. http://dx.doi.org/10.1080/08893110802037945
16. Olejniczak, A.; Ostrowska, K.; Katrusiak, A., H-bond breaking in high-pressure urea. *J. Phys. Chem. C* **2009,** *113* (35), 15761-15767. DOI:10.1021/jp904942c
17. Fabbiani, F. P.; Allan, D. R.; Dawson, A.; David, W. I.; McGregor, P. A.; Oswald, I. D.; Parsons, S.; Pulham, C. R., Pressure-induced formation of a solvate of paracetamol. *Chem. Commun.* **2003,** (24), 3004-3005. DOI:10.1039/B310394C
18. Flower, R. J., Drugs which inhibit prostaglandin biosynthesis. *Pharmacol. Rev.* **1974,** *26* (1), 33-67. http://pharmrev.aspetjournals.org/content/26/1/33
19. Aguiar, A. J.; Zelmer, J. E., Dissolution behavior of polymorphs of chloramphenicol palmitate and mefenamic acid. *J. Pharm. Sci.* **1969,** *58* (8), 983-987. DOI:10.1002/jps.2600580817
20. Romero, S.; Escalera, B.; Bustamante, P., Solubility behavior of polymorphs I and II of mefenamic acid in solvent mixtures. *Int. J. Pharm.* **1999,** *178* (2), 193-202. http://dx.doi.org/10.1016/S0378-5173(98)00375-5
21. SeethaLekshmi, S.; Guru Row, T. N., Conformational polymorphism in a non-steroidal anti-inflammatory drug, mefenamic acid. *Cryst. Growth Des.* **2012,** *12* (8), 4283-4289. DOI:10.1021/cg300812v
22. McConnell, J., FZ Company. N-(2, 3-Xylyl) anthranilic acid, C15H15NO2. Mefenamic acid. *Cryst. Struct. Commun* **1976,** *5*, 861-864
23. Munro, S. L.; Craik, D. J., NMR conformational studies of fenamate non-steroidal anti-inflammatory drugs. *Magnetic resonance in chemistry* **1994,** *32* (6), 335-342. DOI:10.1002/mrc.1260320605
24. Lee, I. S.; Lee, A. Y.; Myerson, A. S., Concomitant polymorphism in confined environment. *Pharm. Res.* **2008,** *25* (4), 960-968. DOI:10.1007/s11095-007-9424-z
25. Iwasaki, T.; Takahara, M.; Sonoda, R.; Watano, S., Dry grinding of mefenamic acid particles for enhancement of its water dissolution rate. *Part. Part. Syst. Char.* **2007,** *24* (3), 236-241. DOI:10.1002/ppsc.200601091
26. Alvarez, A. J.; Singh, A.; Myerson, A. S., Polymorph screening: comparing a semi-automated approach with a high throughput method. *Cryst. Growth Des.* **2009,** *9* (9), 4181-4188. DOI: 10.1021/cg200546g
27. Merrill, L.; Bassett, W. A., Miniature diamond anvil pressure cell for single crystal x-ray diffraction studies. *Rev. Sci. Instrum.* **1974,** *45* (2), 290-294. http://dx.doi.org/10.1063/1.1686607
28. Moggach, S. A.; Allan, D. R.; Parsons, S.; Warren, J. E., Incorporation of a new design of backing seat and anvil in a Merrill–Bassett diamond anvil cell. *J. Appl. Crystallogr.* **2008,** *41* (2), 249-251. https://doi.org/10.1107/S0021889808000514
29. Piermarini, G. J.; Block, S.; Barnett, J.; Forman, R., Calibration of the pressure dependence of the R 1 ruby fluorescence line to 195 kbar. *J. Appl. Phys.* **1975,** *46* (6), 2774-2780. http://dx.doi.org/10.1063/1.321957

30. Piermarini, G.; Block, S.; Barnett, J., Hydrostatic limits in liquids and solids to 100 kbar. *J. Appl. Phys.* **1973,** *44* (12), 5377-5382. http://dx.doi.org/10.1063/1.1662159
31. Sheldrick, G. M. *CELL NOW, program for unit cell determination*, 2003. http://shelx.uni-ac.gwdg.de/SHELX/
32. Dawson, A.; Allan, D. R.; Parsons, S.; Ruf, M., Use of a CCD diffractometer in crystal structure determinations at high pressure. *J. Appl. Crystallogr.* **2004,** *37* (3), 410-416
33. Bruker, A.; SAINT, A. *Inc., Madison, WI, 2004 Search PubMed;(b) GM Sheldrick*, 2008
34. Parsons, S.; SHADE, S. *The University of Edinburgh*, 2004
35. Sheldrick, G.; SADABS, B.-S. A. D. A. *Other Correction*, 2006
36. Betteridge, P. W.; Carruthers, J. R.; Cooper, R. I.; Prout, K.; Watkin, D. J., CRYSTALS version 12: software for guided crystal structure analysis. *J. Appl. Crystallogr.* **2003,** *36* (6), 1487-1487. https://doi.org/10.1107/S0021889803021800
37. Macrae, C. F.; Edgington, P. R.; McCabe, P.; Pidcock, E.; Shields, G. P.; Taylor, R.; Towler, M.; Streek, J. v. d., Mercury: visualization and analysis of crystal structures. *J. Appl. Crystallogr.* **2006,** *39* (3), 453-457. https://doi.org/10.1107/S002188980600731X
38. Dhanaraj, V.; Vijayan, M., Structural studies of analgesics and their interactions. XII. Structure and interactions of anti-inflammatory fenamates. A concerted crystallographic and theoretical conformational study. *Acta Crystallogr., Sect. B: Struct. Sci* **1988,** *44* (4), 406-412. https://doi.org/10.1107/S0108768188001107
39. Bernstein, J.; Davis, R. E.; Shimoni, L.; Chang, N. L., Patterns in hydrogen bonding: functionality and graph set analysis in crystals. *Angew. Chem. Int. Ed. (English), Angew. Chem. (German)* **1995,** *34* (15), 1555-1573. DOI:10.1002/anie.199515551
40. Motherwell, W. S.; Shields, G. P.; Allen, F. H., Visualization and characterization of non-covalent networks in molecular crystals: automated assignment of graph-set descriptors for asymmetric molecules. *Acta Crystallogr., Sect. B: Struct. Sci* **1999,** *55* (6), 1044-1056. https://doi.org/10.1107/S0108768199006497
41. Fabbiani, F. P.; Allan, D. R.; David, W. I.; Moggach, S. A.; Parsons, S.; Pulham, C. R., High-pressure recrystallisationa route to new polymorphs and solvatesBased on the presentation given at CrystEngComm Discussion: New Trends in Crystal Engineering, 810 September 2004, Nottingham, UK. *CrystEngComm* **2004**,
42. Mukuta, T.; Lee, A. Y.; Kawakami, T.; Myerson, A. S., Influence of impurities on the solution-mediated phase transformation of an active pharmaceutical ingredient. *Cryst. Growth Des.* **2005,** *5* (4), 1429-1436. DOI:10.1021/cg049646j
43. Bernstein, J.; Davey, R. J.; Henck, J. O., Concomitant polymorphs. *Angew. Chem. Int. Ed. (English), Angew. Chem. (German)* **1999,** *38* (23), 3440-3461. DOI:10.1002/(SICI)1521-3773(19991203)38:23<3440::AID-ANIE3440>3.0.CO;2-#
44. Iwasaki, T.; Takahara, M.; Sonoda, R.; Watano, S., Dry grinding of mefenamic acid particles for enhancement of its water dissolution rate. *Part. Part. Syst. Char.* **2007,** *24* (3), 236-241. DOI:10.1002/ppsc.200601091