Study of the Transformations of Micro/Nano-crystalline Acetaminophen Polymorphs in Drug-Polymer Binary Mixtures

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Abstract. This study elucidates the physical properties of sono-crystallised micro/nano-sized acetaminophen/paracetamol (PMOL) and monitors its possible transformation from polymorphic form I (monoclinic) to form II (orthorhombic). Hydrophilic Plasdone® S630 copovidone (S630), N-vinyl-2-pyrrolidone and vinyl acetate copolymer, and methacrylate-based cationic copolymer, Eudragit® EPO (EPO), were used as polymeric carriers to prepare drug/polymer binary mixtures. Commercially available PMOL was crystallised under ultrasound sonication to produce micro/nano-sized (0.2–10 microns) crystals in monoclinic form. Homogeneous binary blends of drug-polymer mixtures at various drug concentrations were obtained via thorough mixing. The analysis conducted via the single X-ray crystallography determined the detailed structure of the crystallised PMOL in its monoclinic form. The solid state and the morphology analyses of the PMOL in the binary blends evaluated via differential scanning calorimetry (DSC), modulated temperature DSC (MTDSC), scanning electron microscopy (SEM) and hot stage microscopy (HSM) revealed the crystalline existence of the drug within the amorphous polymeric matrices. The application of temperature controlled X-ray diffraction (VTXRPD) to study the polymorphism of PMOL showed that the most stable form I (monoclinic) was altered to its less stable form II (orthorhombic) at high temperature (>112°C) in the binary blends regardless of the drug amount. Thus, VTXRD was used as a useful tool to monitor polymorphic transformations of crystalline drug (e.g. PMOL) to assess their thermal stability in terms of pharmaceutical product development and research.

KEY WORDS: monoclinic; nano-sized crystals; orthorhombic; sono-crystallisation; variable temperature XRPD.

INTRODUCTION

‘Polymorphism’ generally referred as the ability of a crystalline material to exist in two or more crystalline forms is considered a pivotal part of the crystallisation processes (1, 2). A metastable state of the polymorphs which may not necessarily be obtained easily, can accelerate physicochemical and mechanical properties of a drug substance compared to that of the marketed or naturally occurred counter stable form (1). Moreover, different polymorphic forms may result on significant changes in the solubility and dissolution rates of an active entity (1). By definition, crystalline polymorphs are those crystal lattice in which the crystalline components represent different and discrete phases from a thermodynamic viewpoint.

The current demand for micro/nano-material various applications in medical and pharmaceutical industry especially in drug delivery has triggered the research in organic crystal engineering and development (3, 4). Crystal engineering is particularly important for the development of pharmaceutical molecules in the context of improved drug delivery or bio-labelling/bio-sensing (5, 6). Micronising of molecular crystals has also become an emerging approach mainly in the development of different water insoluble drugs by enhancing the dissolution rates (7, 8). Interestingly, despite the unique physicochemical properties of the molecular crystals, majority of the reported studies have primarily focused on the solubility aspects whilst other applications such as mechanical properties have been relatively unexplored in pharmaceutical research and developments. There is an immense need to explore the potential of crystal engineering to enhance the physical/mechanical properties of drug candidates (e.g. paracetamol). It has been seen that the implementation of sono-crystallization—the use of ultrasound to facilitate crystallisation (9)—can produce micro to
nano-meter-sized crystals with improved mechanical properties. Moreover, it will be of significant interest in crystal engineering and science to monitor the stability of the polymorphic forms or the transformations of these sono-crystallised micro/nano-sized drug crystals.

Paracetamol (PMOL) exists as a white crystalline powder used as an analgesic pain reliever (10). It is sparingly soluble in water (~12.78 mg/ml), and it exhibits multi-polymorphic forms such as monoclinic, orthorhombic and a rarely occurred meta or less stable form with less stability and melting points (11, 12). These forms are also known as forms I, II and III, respectively. Thus, PMOL serves an excellent model drug candidate to study the effects of manufacturing techniques and the subsequent conversions on its crystal forms (1, 11). Also, the glass transition temperature of PMOL at 25°C makes it an interesting system (11). The crystallisation technique and process to engineer the micronised (or nano-sized) crystals of PMOL may provide a useful mean for the characterisation and evaluations of the physico-chemical properties of the pharmaceutical dosage forms.

Temperature variable X-ray powder diffraction (VTXRPD) analysis has already been used as a powerful tool to characterise the polymorphism of pharmaceutical crystalline drugs and their stability as a function of increasing temperature (13, 14). In one of our previous study, the effect of temperature on the transformation of PMOL crystal was studied by implementing VTXRD as a predictive tool during the HME processing (15). However, the effect of crystal engineering, e.g. sono-crystallisation on the polymorphic stability of the PMOL crystals, was not studied.

We report a new case to fabricate micro/nano-sized PMOL crystals and monitor its polymorphic transformation upon heating using a VTXRD method to assess the stability of the manufactured crystals alone or in combination with either hydrophilic vinylpyrrolidone-vinyl acetate polymers (S630) or methacrylate copolymers (EPO).

MATERIALS AND METHOD

Materials

The drug paracetamol (PMOL) was bought from Sigma-Aldrich (Gillingham, UK). Vinylpyrrolidone-vinyl acetate, Plasdone S630 (S630) and Eudragit EPO (EPO) were donated by ISP (Germany) and Evonik Industries (Germany), respectively, and were used as received.

Calculations of Hansen Solubility Parameters (\(\delta\))

Hansen solubility parameters (16) were determined for the assessment of the possible miscibility of the drug with two polymers and were determined by utilising the Hoftyzer and van Krevelen method (17) as shown in the equation below:

\[
\delta^2 = \delta_d^2 + \delta_p^2 + \delta_h^2
\]

(1)

where

\[
\delta_d = \frac{\sum F_{di}}{V_T}, \quad \delta_p = \sqrt{\frac{\sum F_{pi}^2}{V_T}}, \quad \delta_h = \sqrt{\frac{\sum E_{hi}}{V_T}}
\]

\(i\) = group contributions in the drug/polymer molecules, \(\delta\) = solubility parameters, \(F_{di}\) = dispersion energy, \(F_{pi}\) = molar polarization energy, \(E_{hi}\) = hydrogen bonding and \(V\) = molar volume.

Preparation of Paracetamol Crystals and Drug-Polymer Binary Mixtures

Excessive amount of PMOL (500 mg) was dissolved in minimal amount of ethanol (3 ml) and stirred with a magnetic stirrer in ambient until all PMOL got completely dissolved in the solvent making the solution completely transparent. The PMOL solution was then poured into n-hexane solution (200 ml) kept in an ultra-sonic bath at 50-kHz frequency (Fisher Chemicals, UK) until sono-crystallised PMOL particles precipitated completely. The precipitation of crystallised PMOL was then isolated by using a vacuum pump and washed with additional n-hexane and kept in oven at 50°C overnight to obtain dry PMOL crystals.

Manufactured crystallised PMOL was mixed with two hydrophilic polymers to investigate the possible drug/polymer miscibility and hence any interactions that may lead to a possible polymorphic transformation. The loadings of PMOL in the formulations were kept between 30 and 60% (w/w) as shown in Table 1. Appropriate amount of drug and polymer was mixed in a mortar and pestle prior to a thorough blending in a TF2 Turbula mixer (Switzerland) for 10 min in order to obtain a homogeneous drug/polymer binary mixture.

Microscopic Imaging

The surface properties of the bulk drug, pure polymers and mixtures of drug/polymer mixtures were evaluated by Stereo-Scan S360 SEM (Cambridge Instruments, UK) at the accelerating voltage of 20 kV. For this purpose of the study, the samples were mounted on an aluminium stub using adhesive carbon tape and were sputter coated with gold. A Leica high-magnification microscope was used to take photographs of the crystalline PMOL particles for the comparison with the commercial drug. The average particle size was determined by investigating an area having at least 200–500 particles of the sono-crystallised PMOL and measuring the diameter by using the scale bar presented in the images.

Thermal Analysis Via DSC and MTDSC

The solid state of the bulk drug, bulk polymers and mixtures of drug/polymer in different ratios was studied by using a differential scanning calorimeter (DSC) 823e manufactured by Mettler-Toledo (Greifensee, Switzerland).

| Name       | F1 | F2 | F3 | F4 | F5 | F6 |
|------------|----|----|----|----|----|----|
| Paracetamol| 30 | 50 | 60 | 30 | 50 | 60 |
| Eudragit EPO| 70 | 50 | 40 | 70 | 50 | 40 |
| Plasdone S630| 28.2 | 48.2 | 58.3 | 27.9 | 47.7 | 58.7 |

Table 1. Formulation Compositions of PMOL and Percentage Crystallinity of PMOL in Various Drug/Polymer Binary Blends
RESULTS AND DISCUSSION

Drug/Polymer Miscibility (Hansen Solubility Parameters)

The drug/polymer miscibility was estimated by correlating the energy of mixing from inter- and intra-molecular interactions of the materials used (drug and polymers) (19). For this purpose, the Hansen parameters (i) calculated based on the structural orientation of the component informs that the drug-polymers with similar δ values used in a system are likely to interact with each other as matter of being miscible. It has widely been accepted that two compounds are generally treated miscible when Δδ is less than 7 MPa\(^{1/2}\) (17, 20). The higher values of Δδ (>7 MPa\(^{1/2}\)) between a drug/polymer pair generally indicate an immiscibility.

It has been seen in the literature that the solubility parameters provides a general indication of two components which are generally a drug and a polymer to develop solid drug-polymer mixtures in which drug particles can be dispersed in the polymer matrices. The estimated δ values of PMOL, S630 and EPO are summarised in Table II, where it can be seen that the Δδ of both hydrophilic polymeric carriers and the drug in the binary systems are less than 7 MPa\(^{1/2}\). This indicates that PMOL is miscible with both S630 and eudragit grade copolymer EPO. The Δδ values observed in both drug/polymer binary systems are less than 7 MPa\(^{1/2}\). Therefore, based on the theoretical calculation, it can be claimed that both polymers are expected to be miscible with the drug used in the binary systems.

Microscopic Imaging

The surface properties of the bulk API and the drug/polymer binary mixtures studied by SEM are shown in Fig. 1a. The micrographs of crystallised PMOL exhibited octahedral shaped crystals representing monoclinic form (2). Similarly, the commercial PMOL revealed similar characteristic monoclinic crystalline structures (data not shown). The surface analysis conducted via SEM of all binary mixtures also showed the presence of monoclinic form of PMOL in nano- to micro-scale with both S630 and EPO polymers in all formulations. These findings indicated the presence of crystalline PMOL in its original monoclinic form without any transformations yet. Interestingly, the photographs captured via a High-Magnification Leica microscope of both commercial PMOL (un-micronized) and the sono-crystallised PMOL (micronized) revealed significant size differences as shown in Fig. 1b. The average particle size of the commercial PMOL crystals ranged from 50 to 250 μm (by investigation at

| Sample       | δ\(\rho\) (MPa\(^{1/2}\)) | δ\(\rho\) (MPa\(^{1/2}\)) | δ\(\rho\) (MPa\(^{1/2}\)) | δ\(\rho\) (MPa\(^{1/2}\)) | δ (MPa\(^{1/2}\)) | Δδ |
|--------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----|
| Paracetamol  | 19.67           | 7.93            | 21.20           | 13.96           | 25.39           | 28.04 |
| Plasdone S630| 15.14           | 0.45            | 15.15           | 12.18           | 19.43           | 7.25 |
| Eudragit EPO | 17.89           | 0.65            | 17.89           | 6.08            | 18.90           | 5.96 |

Table II. Hansen Solubility Parameters of PMOL and Polymers Used in the Binary Blends
Fig. 1. **a** SEM images of the micro-crystalline PMOL and the formulations; **b** microscopic photographs of commercial PMOL and sono-crysallised PMOL (micro-nano sized)

Fig. 2. **a** DSC thermal transitions of bulk PMOL and polymers.  **b** DSC thermograms of PMOL/EPO binary systems
least 200–500 particles) while the sono-crystallised PMOL (PMOL) showed a particle size ranging from 0.2 to 10 μm. This was also evident in all drug/polymer binary mixtures using two different polymers. The optimised sono-crystallisation approach produced nano-sized crystals of PMOL which may prove advantageous to enhance the physico-mechanical properties of the very poorly compactible actives such as PMOL. Similar studies have been reported elsewhere (21).

### Table III. Thermal Transition Summary of Bulk Drug, Polymers and the Drug/Polymer Binary Formulations

| Formulations | Glass transition/ enthalpy (°C/ΔH, Jg⁻¹) | Melting endotherms/ enthalpy (°C/ΔH, Jg⁻¹) |
|--------------|------------------------------------------|------------------------------------------|
| PMOL         | 24.55                                    | 169.1/137.06                             |
| S630         | 105.5                                    | N/A                                      |
| EPO          | 48.56                                    | N/A                                      |
| F1           | 45.90                                    | 143.31/20.49                             |
| F2           | 44.09                                    | 148.48/23.83                             |
| F3           | 41.67                                    | 151.47/33.81                             |
| F4           | 52.85                                    | 137.04/13.27                             |
| F5           | 105.80                                   | 131.30/7.20                              |
| F6           | 102.25                                   | 123.39/6.50                              |

![Fig. 3. HSM images of PMOL and PMOL loaded formulations](image)
Thermal Analysis

The thermal transitions of the bulk drug, polymers and the formulations were analysed by DSC. The DSC thermograms of crystallised PMOL showed a sharp transition due to its melting at 169.1°C with an enthalpy $\Delta H = 137.00$ J/g. This transition represents the polymorphic form I (monoclinic) (Fig. 2a) and complements the findings from the literature (2, 10, 11). In the case of both polymers, a modulated temperature DSC was used as the conventional DSC thermogram showed enthalpy relaxation peak that overlapped with the glass transition temperatures ($T_g$s) of the polymers. For methacrylate EPO, an endothermic thermal step change was observed at 48.38°C whilst for S630 at 105.51°C. Both endothermic thermal events correspond to the $T_g$s of the respective amorphous copolymers.

The DSC thermograms of the drug/EPO mixtures (F1-F3) (Table III) showed endothermic thermal transitions at 143–151.57°C at various drug loadings (30–50% w/w ratios) (Fig. 2b). Similarly, all formulations with S630 showed two endothermic thermal transitions as shown in Table III: one corresponding to the melting of the drug at higher end (123–137°C) and another one at lower end due to the $T_g$s of the amorphous polymer. From the results, it is quite evident that the shifted melting transitions have become broader compared to those of pure drug indicating the decrease in the crystallinity of PMOL (12). Moreover, this could also possibly be attributed to the drug-polymer interactions in the binary blends. However, the study of volume fraction of PMOL in the formulations has played a pivotal role on the melting temperature of the drug with EPO. The increase in the drug concentration in the formulations resulted in the increase in the melting endotherms (data not shown). In contrast, a different result was observed with the S630 polymer due to the nature of the polymer and possible drug/polymer interaction strength.

Fragility indicates the degree of viscosity and relaxation time change of a material at its glassy state. The fragility index ($m$) of strong glasses has typical values of $m < 100$ and weak glasses $100 < m < 200$ (11). In this study, the activation enthalpy and thus the fragility index ($m$) at the $T_g$s were estimated by using DSC as expressed in the following equation:

$$
\Delta H^{act} = \Delta H_0 e^{-m/T_g}
$$

Fig. 4. Single X-ray crystallographic image of PMOL-monoclinic form (stacking)
equations: where \( m \) = fragility index, \( E_a \) = activation energy and \( R \) = gas constant (22, 23).

\[
\ln q_0 = -\frac{E_a}{RT} \tag{2}
\]

\[
m = \frac{E_a}{(2.303 \times R \times T_{gm})} \tag{3}
\]

By using Eq. (3), it has been calculated that the crystallised PMOL has a fragility index of 83.8, which is less than that found by Qi et al. 86.7 (11). It simply indicates that the PMOL system in this study is a stronger glass compared to that of previously studied amorphous paracetamol. Similarly, the \( m \) values calculated for PMOL in PMOL/S630 (F4-F6) formulations are between 97.7 and 188.9 for 30–60% PMOL loadings which indicates that PMOL is neither same as amorphous paracetamol nor crystalline paracetamol form I. Rather PMOL in the formulations upon heating represents more fragile systems (e.g. form II) may be stuck together by weak van der Waals forces (11, 23).

Thermal analysis conducted via HSM determined the thermal transitions due to the melting of crystalline PMOL within the polymer matrices as a function of heating. Various images of both the bulk drug and drug/polymer binary mixtures taken using HSM are depicted in Fig. 3. As expected, the bulk drug exhibited no thermal changes up to its melting (168°C) complementing the results obtained from the DSC. In the DSC results, there were no thermal events that occurred until about 169.10°C when it melted. Similar to the DSC findings, the drug/polymer binary mixtures displayed minimal drug melting at the heating temperature that reaches 130–140°C and afterward presented a complete melting of the drug crystals present in the polymer matrices (Fig. 3).

**Variable X-Ray Powder Diffraction (VTXRPD) Studies**

The variable temperature effect on the alteration in the crystal structure of sono-crystallised PMOL was monitored by VTXRPD approach. The results obtained from the XRPD upon heating the samples were recorded for bulk PMOL, PMOL/S630 and PMOL/EPO binary systems.

**Fig. 5.** a XRD diffractorams of PMOL and all binary formulations. b VTXRD diffractograms of sono-crystallised PMOL micro/nano crystals. c VTXRD diffractograms of PMOL/EPO binary systems. d VTXRD diffractograms of PMOL/S630 binary systems
The single X-ray crystallographic imaging revealed that the sono-crystallised PMOL existed as monoclinic form \( \text{(21)} \) and the details of the crystal structure are depicted in Fig. 4. The standard XRPD diffractogram of the monoclinic sono-crystallised PMOL form I showed characteristic peaks at 2\( \theta \) values at 11.9–26.50 followed by a series less intense peaks at different 2\( \theta \) values at ambient temperature (Fig. 5a). Similarly, various drug/polymer binary mixtures showed the characteristic diffraction peaks of PMOL with slightly lower intensity indicating that PMOL is present in its same form. Further analysis via a VTXRPD analysis of crystallised PMOL clearly showed the characteristic peak of form I at 24.0 degree 2\( \theta \) at ambient temperature (24) which started shifting as the temperature increased. This could be a sign of the crystalline structural change of the drug (e.g. a distorted lattice becoming less stable). This alteration of the monoclinic lattice increases with temperature up to 161°C (Fig. 5b). Thereafter, a slight temperature increase directed to the polymorphic transformation of paracetamol to its less stable form orthorhombic and completed at about 164–166°C (Fig. 5b). The signature diffraction peak for monoclinic form at 24.35 2\( \theta \) shifted to a new position at 24.04 2\( \theta \) confirming the transformation of the monoclinic form to less stable form II.

Similar studies with the formulations with two polymers showed that regardless of the drug loadings, the signature peak at 24.36 2\( \theta \) for monoclinic form starts shifting as a function of the applying temperature and the transformation was completed at about 112°C when the peak was positioned at 24.03 2\( \theta \) with higher intensity corresponding to the orthorhombic structure (Fig. 5c). There was no obvious change observed upon increasing the temperature further up to 120°C. In contrast, the formulations of PMOL/S630 formulations (F4-F6) exhibited a transformation of PMOL at slightly higher temperature 120°C (Fig. 5d). This could be linked to the higher \( T_g \) (~105°C) of S630 polymer which may have resulted in the thermal stability to retain the crystal structure of the drug while heating. In the case of EPO, since the \( T_g \) was quite lower (~48°C), the transformations occurred at about 112°C. However, the polymorphic transformation occurring temperature for PMOL depended on the nature and thermal stability of the polymers used. It can be concluded that the VTXRPD is a useful tool to study the temperature effects on the polymorphic transformation (25).

![Fig. 5. continued.](image-url)
of a crystalline drug. The thermal stability of a crystalline molecule is pivotal in terms of long-term product stability in pharmaceutical product manufacturing and development.

CONCLUSIONS

The calculated solubility parameters indicated the possible drug-polymer miscibility complemented by the results exhibited by thermal analysis. The approach adopted by the sono-crystallisation proved to be an effective technique to produce nano-micro sized crystals with high thermal stability. The temperature-assisted VTXRPD was successfully applied to study the alteration in the crystal structure of the sono-crystallised PMOL from various water soluble polymer matrices as a function of increasing the temperature. In this study, it has been seen that the polymorphic change was temperature dependant (at a range of 112–120°C) while the nature of the polymers played a vital role. In conclusion, VTXRPD can effectively be exploited as a useful approach to study possible polymorphic change of various drug candidates to develop different dosage forms. This is particularly suited to enhance the dissolution rates of poorly water soluble crystalline drugs or increasing physical/mechanical properties of various crystalline actives, e.g. paracetamol.

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