CHAPTER - 2
LITERATURE SURVEY AND METHODOLOGIES ADOPTED

2.1 Literature survey

Paracetamol with molecular formula \( \text{C}_8\text{H}_9\text{NO}_2 \) also known as acetaminophen is an important pharmaceutical compound that exists in three different polymorphic forms [1-3]: one of those is a thermodynamically stable monoclinic form I at ambient condition, a bilayer structure metastable orthorhombic form II and form III, that was characterized by a combination of high-throughput crystallization experiments and refinements of calculated structures from polymorph prediction [4, 5]. The relative thermodynamic stability of the polymorphs falls in the order of \( I > II >> III \), and the interconversion of the forms follows the reverse order [6].

Haisa et al. first elucidated the crystal structure of form II, which crystallizes in a centrosymmetric orthorhombic system with space group \( \text{Pbca} \) with 8 molecules in a unit cell having dimensions \( a = 7.393 \text{ Å} \), \( b = 17.164 \text{ Å} \) and \( c = 11.805 \text{ Å} \) [2]. I. D. H. Ostwald et al., have shown that the application of high-pressure can be used as a reproducible way to obtain this metastable polymorph under ambient conditions [7]. The commercially marketed thermodynamically most stable polymorph form I reported by Haisa et al. [8] crystallizes in monoclinic centrosymmetric crystal structure with space group \( \text{P2}_1/\text{n} \) with 4 molecules in a unit cell having dimensions \( a = 7.104 \text{ Å} \), \( b = 9.401 \text{ Å} \), \( c = 12.932 \text{ Å} \) and \( \beta = 97.42^\circ \). It is the polymorph used in pharmaceutical formulations, despite its poor densification properties that require a wet granulation process for tableting [9-13].

The physical structures of the two different polymorphic forms mono and ortho paracetamol have been described by several research groups [2, 8-17]. They have reported that in contrast to the stable polymorph I, the metastable polymorph II can be used for direct compression into tablets and was also reported to dissolve faster in water. The thermodynamic relationship of the mono and ortho polymorphs studied by G. L. Perlovich et al and Elena V. Boldyreva et al. [18, 19] revealed that polymorphic modifications of form I and form II are monotropically related. Di Martino et al. [6] have observed polymorphic transition II to I at nearly 156 °C with a subsequent melting of the monoclinic paracetamol polymorph I at about 169 °C, when they carried out an X-ray
diffraction experiment vs. temperature using Guinier-Lenne camera. However, despite these X-ray diffraction data, they have interpreted their DSC data (an endothermic peak at 157 °C if the heating rate was equal to 10 °C/min, and at about 169 °C, if the heating rate was equal to 0.1 °C/min) as an evidence of the melting of the orthorhombic form II.

The existence of third polymorph (form III) was first proposed by Burger in 1982 [21] and later by Di Martino et al. in 1997 [6]. A search for third polymorph by slow evaporation of an aqueous solution of commercial paracetamol produced a coupled dimer oxidation product was reported by W. Clegg et al. [22]. Different groups [6, 12, 19] have tried to isolate form III and however, its crystal structure and physical properties have never been achieved and is still elusive. Until this period, the researchers were able to only observe the form III during fusion experiments under strict geometric constraints [4, 7, 23-26] and unable to isolate them. The first PXRD pattern was recorded by M. L. Peterson et al. [27] but its profile quality proved insufficient to allow successful indexing. By in-situ thermal treatment, Marc-Antonie et al. [23] failed to index the form III reflection patterns whereas by capillary method they have isolated form III from form I and have identified its crystal system as orthorhombic. But neither its internal structure nor external morphology has been revealed completely yet. Although some work on stabilizing form III performed on the isolated polymorphs by S. Gaisford et al. and Burley et al. [24, 28] result in tentative thermal stability as it underwent solid-solid transition to form II before melting. Recently, H. M. A. Ehmann and O. Werzer reported the stabilization of form III via solid surface by standard spin coating method followed by rapid heating and resolved its crystal structure as orthorhombic [29].

Huttenburg [30] suggested that rational design of powder particles can modify the monoclinic paracetamol compression behaviour. More recently, York reviewed some possibilities offered by crystal engineering and particle design to improve compaction process [31]. A few studies have been reported, relating to the obtaining of paracetamol crystal aggregates by recrystallization or agglomeration methods, with subsequent improvement of compression behaviour [32-37]. Several recent studies have used alternative solvents in attempts to modify the crystal properties [38]. Crystallization of monoclinic paracetamol has been studied employing few selected solvents [39, 40]. W. Omar et al. [41] described that the crystal morphology highly depends on the method
of crystallization, nature of solvents, solutes and ions, temperature, pressure, cooling rate etc. B. Y. Shekunov et al. [42] stated that morphological variations of monoclinic paracetamol concerned a possible temperature dependence of the growth kinetics. R. A. Granberg et al. showed that the shape of the crystal depends on the solvent composition [43] and the influence of solvents reviewed by different authors [44-48] stated that supersaturation are prominent for variation in morphology of the grown monoclinic paracetamol crystals. J. Y. Y. Heng et al. [49] reported the bulk growth of paracetamol single crystal grown macroscopically in methanol at 20 °C by slow solvent evaporation with crystal dimensions larger than 1 cm.

Attempts to obtain and isolate the orthorhombic form from ethanolic solutions using an evaporative crystallization under ambient conditions using the method mentioned by Haisa et al. have been less successful [2]. The only successful method for the crystallization of the pure orthorhombic form reported by Di Martino et al. is based on the crystallization from the melt of the monoclinic form in a non-oxidizing atmosphere [6]. Nichols et al. reported a cooling crystallization method, which uses seeds of the orthorhombic form prepared from the fusing of the monoclinic form, to crystallize orthorhombic acetaminophen from supersaturated solution of an industrial methylated spirit. The orthorhombic form obtained via this method undergoes solution mediated phase transformation to monoclinic form within few hours of formation at 0 °C [14]. Mikhailenko et al. proposed a complex cooling crystallization method to produce large orthorhombic crystals, which includes boiling, filtration and incubation followed by slow cooling [17]. Crystals of form II have inclusions of water and induces a solvent-mediated polymorphic transformation during storage. Recently a new approach was proposed for the crystallization of metastable orthorhombic polymorph of paracetamol using specific surface topographies and surface chemistry. Cooling crystallization was performed on the surfaces of novel colloidal templates using a step rapid cooling method. The results show that in the case of crystallization on surfaces of colloidal templates, the orthorhombic form is nucleated by faster cooling rates and further stabilized by the surface topography of the templates, which is postulated to substantially delay solvent mediated polymorphic transformation to the monoclinic form [50].
Klug and Weissburg recognized that the presence of small amounts of impurities has substantial effects on the kinetics of crystal nucleation, growth morphology and dissolution [51, 52]. Mullin states that the changes in equilibrium solubility or the solution structure, or by physical and chemical adsorption of impurity on homogeneous and heterogeneous nuclei modifies the crystal habit [53]. Effects of soluble additives studied by several researchers on the nucleation kinetics and crystallization of paracetamol [54-59] have shown that the additives significantly modify the habit of the nucleated paracetamol and alter the crystal properties. According to York, changes in crystal habit could play important role in the processability of pharmaceutical raw material as well as the efficacy and performance of the final dosage form [60].

Di Profio et al. proposed a controlled evaporation approach on membrane for selective crystallization of the orthorhombic form. Polymeric membranes have been used as a selective medium for solvent evaporation by altering the rate of achievement of supersaturation, which provides consequent switching between thermodynamically and kinetically controlled nucleation and hence polymorph selectivity [61]. Capes and Cameron reported the crystallization of orthorhombic acetaminophen around the edges of evaporating aqueous droplets, due to increased supersaturation at the meniscus [62]. Thomas et al. reported the crystallization of orthorhombic form using multi-component crystallization principle. This method involves the addition of various secondary components having carboxylic acid groups in solution in addition to acetaminophen in different solvents, which do not produce multi-component molecular complexes, but provide the conditions suitable for the crystallization of the orthorhombic form [63].

Heterogeneous nucleation based methods have been applied for crystallization of selective polymorphs [64, 65]. Different polymer heteronuclei, creating specific interactions between polymer surface functional end group and acetaminophen in aqueous solutions, were used to crystallize orthorhombic form [66-68]. K. Kachrimanis and S. Malamataris reported that the addition of polymers in the crystallizing solution altered the physico-chemical properties and improves the tableting ability [69]. Grace A. Ilevbare et al. reported that the use of polymers as crystallization inhibitors may significantly impact the
extent and duration of supersaturation and bioavailability of the given compound. The effectiveness of polymers as crystal growth inhibitors starts either in nucleation or crystal growth and results in the desired polymorphs [70].

Chadwick et al. reported a method for controlling the polymorphism of the acetaminophen using crystalline nucleants [71]. Meidong Lang et al. examined the effect of solids of polymers in influencing heterogeneous nucleation of polymorphs of acetaminophen and other drug molecules. This work demonstrated that it is possible to use heterogeneous nucleation on solids with different surface energies to promote formation of different polymorphs of acetaminophen [66]. Christopher P. Price et al. reported the methodology to control the phenomenon of crystal polymorphism through the use of diverse libraries of polymers as heteronuclei. They identified the selective production of a given form from a single solvent and temperature condition by simply varying the nature of the polymer substrate [67]. V. L. Mejias et al. have studied the mechanism by which phase selection occurs during the crystallization of acetaminophen using polymers as heteronuclei, paving the way for the improvement of methods for polymorph selection and discovering based on heterogeneous nucleation parameters [68].

S. Sudo et al and J. D. Dunitz [72, 73] have proposed that seeding a solution with a crystal of the product as a well-established method to induce crystallization and to encourage the formation of desired polymorphs. Seeding with a desired polymorphic form is a promising technique for controlling polymorphic form during industrial crystallization [74]. M. D. Ward et al. [75] showed that the seed crystals possess analogous target crystal structure and induces the assembly of molecules into supramolecular motifs affording the desired polymorph. Tamura et al. [76] have investigated the influence of seeding on the crystallization of single enantiomers from racemic mixtures. They were able to form the preferred metastable δ- polymorph of a racemic sulfonate by adding previously formed single crystals of the metastable δ- polymorph to a supersaturated solution. The seeds not only promoted the desired form, but also inhibited the undesired nucleation. N. Doki et al. [77] reported that the seeding had a large effect both on the polymorphism and the size distribution of the product crystals. This study does not intend to deal with the mechanism of phase transformation nor molecular aspects in polymorphic crystallization of glycine. Ziller and Ruprecht [78] studied the seeded
growth of paracetamol in the presence of polymers in a crystallization unit in which the solution phase was filtered and passed at an elevated temperature through a detector thus permitting continuous monitoring of concentration. N. Al-Zoubi et al. crystallized the orthorhombic paracetamol from ethanolic solution by seeding at lower cooling temperatures below 0 °C [79]. But the method has faced difficulty to separate the metastable orthorhombic form from stable monoclinic form I as it undergoes solution mediated transformation upon contact with solvent.

The use of gels as growth media has been reported for a wide range of compounds, including both inorganic and organic compounds and also proteins by Robert et al, Bernard et al, Meazza et al and Aparicio et al. [80-83]. Recently the use of external field has been widely applied for designing the materials with modified properties [68, 84]. The applications of ultrasound for a polymorphic system at the right level of supersaturation can assist in isolating the ground-state polymorph (the most thermodynamically favoured and least soluble) or one near the ground state was stated by V. Lopez-Mejias et al [68]. There are reports on ultrasonic irradiation [85-87] in the crystallization process showing changes in habit, size distribution of crystals, promotes or prevents agglomeration, improves product handling, and modifies the polymorphism. Moreover, several researchers [88-91] reported that sonication initiates primary nucleation, decreases metastable zone width, shortens induction time, and increases nucleation rate. Ultrasonic irradiation has been used for enhancing the rate of crystallization of metals, inorganic salts by A. Renita et al [92] and co-crystals by N. N. Sirota [93]. Salting-out crystallization is rapidly completed with the aid of ultrasound [94]. This method has been commonly used during the nucleation phase of the crystallization process, allowing the nucleation to take place at lower supersaturation levels.

Sonocrystallization has also been applied to increase the recovered material from solution. The study carried out by R. K. Bund and A. B. Pandit [95] in lactose, 90 % of lactose was recovered from solution just in two minutes of sonication time. Without sonication, only 55-60 % was recovered after three days. The mechanism for these effects mainly depends on three factors which can be responsible for the contribution of ultrasound to crystallization: first, nucleation is enhanced by the shear forces that are produced when the bubbles produced by cavitation collapse was described by R. K. Bund;
second, M. D. Luque de Castro et al and R. Chow [88, 95, 96] stated that the rate of crystallization is enhanced by the fragmentation of seed crystals; third H. Li, J. Wang et al [94] reported that a better uniformity in crystal size distribution can be obtained under sonication. There have been some studies which indicate the formation of different polymorphs, crystal habit and crystal size distribution when systems are irradiated with ultrasound. Gracin et al. obtained the metastable form of p-amino benzoic acid under sonicated conditions [97]. Louhi-Kultanen et al. [98] studied the influence of the sonocrystallization on glycine at different temperature ranges. Ultrasound yields α-polymorph almost exclusively at lower temperature and decreases the amount of α form at higher temperatures.

Magnetic field has been recognized affecting nucleation and crystal growth rate, and polymorphism by G. Dhanaraj et al [99] and the early works by the influence of external magnetic field were observed by E. Iizuka in the poly-amino acids [100]. The major impact of the field was orientation of crystals with respect to the field direction. E. Iizuka and G. Sazaki et al. performed the experimental studies on protein crystallization and have reported that the presence of magnetic field reduced the number of crystal nuclei in solutions with highly ordered arrangement [100, 101], formation of aragonite crystal at ambient condition using magnetic field at which there is almost no aragonite formed without magnetization was reported by M. Ataka et al [102]. S.S. Stevan Wang et al, N.I. Wakayama et al and A. McPherson [103-105] stated that the gravity induced convection-suppressed environment can be achieved both in space and by magnetic-field gradients, crystal orientation occurs only in the magnetic field, and magnetically oriented crystals have been shown to have improved crystal quality when compared with those under normal-gravity conditions outside the magnetic field.

### 2.2 Materials and Experimental methodologies adopted

Acetaminophen commercially known as paracetamol, purity (assay 98.0-101.0 %) and polymers such as agar, gelatin, polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), hydroxypropylmethylcellulose (HPMC), nylon 6/6, polypropylene, polyvinylchloride, alginic acid and polymethylmethacrylate were purchased from the Sigma Aldrich (product name: A5000, CAS number: 103-90-2). Sodium metasilicate was purchased from Central Drug
House. Solvents such as ethanol, methanol, isopropyl alcohol (IPA), acetone, cyclohexanone (CH-one), tetrahydrofuran (THF), ethyl acetate, acetonitrile, acetic acid, (1, 4) - dioxane purchased from Merck and laboratory double distilled water were utilized for the present investigation.

2.2.1 Determination of solubility

Solubility of paracetamol was determined by gravimetric method. Saturated solution of paracetamol in water was prepared in an air tight round bottomed flask (RBF) fitted by PTFE stirrer shaft with ground sleeved stirring gland attachment and it was maintained in the constant temperature at 32 °C. The solution was stirred for about 6 h with continuous stirring. On reaching saturation, the solution was allowed to settle down for a few minutes. Necessary care was taken to avoid evaporation of the solvent. 1 mL clear solution of solvent comprising two different sets was taken out by means of a warmed pipette into a 5 mL beaker of solutions and allowed to evaporate. After evaporation of the solvent, weighed quantity of the sample was analyzed by averaging the data obtained for the above two samples. The above procedure was repeated for different temperature ranges and also in different solvents at ambient condition.

2.2.2 Determination of metastable zone width

The metastable zone width is defined as the region between the saturation curve and the labile zone where nucleation occurs spontaneously. The metastable zone width of paracetamol polymorphs was determined by the polythermal method. This method involves the cooling of a saturated solution at a constant rate until the nucleation occurs [53].

2.2.3 Determination of relative supersaturation

Relative supersaturation is defined as the ratio of the solute concentration in the supersaturated solution to the equilibrium concentration at a given temperature. When the solution saturated at 318 K was swift cooled from 353 K to 278 K during the investigation relative supersaturation is calculated using the formula,
\[ \sigma = \ln S = \ln(x/x_0) \]

where ‘\( \sigma \)’ is the relative supersaturation, ‘\( S \)’ is the supersaturation, ‘\( x \)’ is the actual concentration of the solute in mole fraction and ‘\( x_0 \)’ is the equilibrium concentration of the solute in mole fraction.

2.2.4 Determination of induction period

Induction period was determined by the elapsed time between the achievement of supersaturation and for the formation and detection of new crystalline particles.

2.2.5 Determination of specific gravity

Specific gravity of sodium metasilicate solution was determined by the ratio of the density of the substance to the density of water at a specified temperature.

2.2.6 Swift cooling crystallization process

The polymorphic nucleation behaviour of paracetamol has been investigated by performing laboratory scale novel swift cooling crystallization process with various process parameters such as solute concentration, temperature, agitation and using polymer as template in pure aqueous solution.

2.2.6.1 At various solute concentrations

Paracetamol and laboratory double distilled (DD) water were used for solution preparation in the crystallization experiment. About 100 mL of saturated solutions of paracetamol at 305 K was prepared in an airtight round bottom flask (RBF) with ground sleeve stirrer glands attachment for effective stirring. This setup was kept inside a digitally controlled constant temperature bath (CTB) with the inbuilt cryostat facility having temperature controlling accuracy of 0.01 °C. The temperature of the solution was raised to 353 K and maintained at the same temperature for about 3 h with continuous stirring using glass stirrer shaft and blades at a speed of 28 rpm constantly through a digitally controlled stepper motor drive. Then the solution was filtered with Duran pipeline filter having porosity in the range of 10-15 micron with the help of peristaltic pump. The filtrate was collected into another similar RBF with similar stirring assembly and maintained for about 1 h at 353 K with continuous stirring and it was swiftly
transferred to another similar CTB, which was maintained at 278 K. The solution was carefully monitored through the full visibility window of the CTB under bright light illumination and the events were recorded with time. After 22 min, the transparent solution becomes milky white indicating the initiation of nucleation. Within 3 minutes, precipitation of small crystalline particles was found at the bottom of the flask. A small portion of the solution containing these nucleated crystalline particles was carefully taken with a nucleation cell maintained at the same temperature and examined under the Olympus Stereozoom microscope SZX16 attached with Jenoptic ProgRes CT3 digital camera. The polymorphic form of nucleated crystallites was identified based on their morphology. Further growth progression of the crystallites was monitored and recorded at different time frames. The induction period, the time elapsed between the creation of supersaturation and for the formation of new detectable solid phase, was also determined for different polymorphs. The experiment was repeated with varying the initial saturation of the mother solution at different temperatures in the range from 305 K to 331.5 K. From the data obtained, the types of nucleation were identified and their corresponding induction period was deduced at different initial saturation levels of the mother solution.

2.2.6.2 At various temperatures

The similar experimental procedure was followed with fixed concentration 2.9 g/100 mL, corresponding to the saturation temperature 318 K and stirred continuously for 3 h in CTB at 353 K with constant stirring speed 100 rpm. Then the solution was swiftly transferred to another similar CTB which was kept nearby and maintained at a temperature 274 K. The experiment was repeated in steps of every 1 K in the temperature range 274-313 K and the nucleated crystals in each case were photographed.

2.2.6.3 At different agitation rates

By following the same experimental procedure as discussed above, saturated pure aqueous solutions of paracetamol at fixed concentration 2.9 g/100 mL conditions was swiftly cooled from 353 K to below ambient temperature of 278 K at different agitation rates in the range 20-120 rpm in steps of 10 rpm. Polymorph characterization was immediately carried out in order to analyze the formation of nucleated crystalline products.
2.2.6.4 Templating effect of polymers

The polymers such as nylon 6/6 \((C_{12}H_{22}N_2O_2)_n\), polypropylene \((C_3H_6)_n\), polyvinylchloride \((C_2H_3Cl)_n\), alginic acid \((C_6H_8O_6)_n\) and polymethylmethacrylate \((C_5O_2H_8)_n\), were used as received and therefore were in several different physical forms that include pellets and powder form. Laboratory double-distilled water was used for solution preparation. Paracetamol with concentration \((1.8 \text{ g}/100 \text{ mL solubility at } 32 ^\circ \text{C})\) was dissolved in 100 mL of laboratory double-distilled water in an airtight ampule and boiled at 100 °C for about 30 min with continuous stirring. Six screw-capped glass vials of each volume 5 mL were selected and 0.05 g of the five selected polymers such as nylon 6/6, polypropylene, polyvinylchloride, alginic acid and polymethylmethacrylate were taken in each volume of 5 mL of five of the glass vials. All the six vials were precooled at 1 °C for about 15 min in the digitally controlled CTB with inbuilt cryostat facility, which have a temperature controlling accuracy of ± 0.01 °C. About 1 mL of the aqueous paracetamol solution which was at boiled condition at 100 °C was pipetted out and transferred immediately into each of the six glass vials which were kept at 1 °C. The solutions in the vials were carefully monitored through the full-visibility window of the bath under bright-light illumination for nucleation, and the events were recorded over time. The solution was clear and transparent initially for a short period of 15 min, known as the induction period, i.e., the time interval between the attainment of supersaturation and nucleation, after which it yielded tiny crystalline particles. The nucleated particles were identified as one of the polymorphs of paracetamol based on their morphology. Growth of different polymorphs nucleated in pure and polymer-added solution was examined, and the images are captured at different time frames by in-situ optical microscopy. Polymorph characterization was instantly carried out to scrutinize the formation of nucleated crystalline products. The experiment was repeated by varying the initial saturation of the mother solution corresponding to the saturation temperatures in the range from 32 to 52 °C. From the data obtained, the types of nucleation were identified, and their corresponding induction period was determined at different initial saturated levels of the mother solution.
2.2.7 Specially designed seeding technique

2.2.7.1 Preparation of seeds

The seeds of paracetamol were prepared from the melt by adopting a method similar to the Bridgeman technique at optimized conditions under different pulling/cooling rates from specially designed seed preparation unit fabricated in our laboratory. About 8 g of commercial paracetamol powder of form I was loaded in an ampoule and it was tightly closed to avoid contamination with atmosphere. The loaded sample was carefully lowered and held stationary towards the hotter region inside the furnace at a temperature above 10 °C corresponding to the melting point of the sample 170 °C. The sample was allowed to melt for about 15 min and after melting the ampoule was gradually lifted towards the colder region of the furnace. The samples were prepared by repeating the experiment with different pulling speeds, such as 1, 3, 15, 30, 45, 60, 120, 150 and 200 rpms. The distance travelled by the ampoule per minute for each rpm speed was measured. The variation in the pulling rate of the ampoule as well as the variation in the cooling rate of the furnace with respect to different rpms for crystallization after melting was also measured.

From the obtained crystallization of form I under various cooling rates, the seeds were collected by gently scraping the top surface of the crystallized melt by micro spatula. The prepared seeds under different cooling rates with respect to the rate of variation in pulling were named as seed A (1.2 cm/min), seed B (2 cm/min), seed C (28 cm/min), seed D (36 cm/min), seed E (40 cm/min), seed F (57 cm/min), seed G (92 cm/min), seed H (120 cm/min) and seed I (150 cm/min) respectively. The size of the seed crystals was determined under optical microscope and their polymorphic forms were ascertained by powder X-ray diffraction analysis.

2.2.8 In-situ nucleation study

2.2.8.1 Using soluble polymers

Paracetamol and laboratory double distilled water was used for the preparation of experimental solution. The polymers used were agar, gelatin, polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP) and hydroxypropylmethylcellulose (HPMC). 250 mL pure
aqueous solution of paracetamol was prepared for room temperature saturation. The prepared solution was filtered with Whatmann no.1 filter sheet. The filtered solution was distributed in 10 similar vessels each containing a uniform quantity of 25 mL aqueous solution. 10 combinations of polymeric additive concentration in the range from 0.01-0.1 g in steps of 0.01 g were added to the saturated paracetamol solution in 10 vessels and stirring was made to the above mixture for about 15 min. The same experimental procedure was followed for each of the selected polymer additives and the nucleation study has been carried out for all the concentrations of additive added solutions under optical microscope.

2.2.8.2 Using different solvents

Commercially available paracetamol and the solvents such as polar protic [laboratory double distilled water, ethanol, methanol, isopropyl alcohol (IPA)], polar aprotic [acetone, cyclohexanone (CH-one), tetrahydrofuran (THF), ethyl acetate, acetonitrile], and non-polar solvent (1, 4 - dioxane) were used for preparing the solution. 100 mL of saturated solution of paracetamol at room temperature (305 K) was prepared with each of the above selected ten solvents separately in accordance with the solubility, filtered with Whatmann no.1 filter sheet. The apparent pH of these prepared saturated solutions was measured using Cyber pH-14L. About 10 μL of the solution was taken out using micropipette and carefully transferred to the nucleation cell maintained at the same temperature. The cell containing the solution was covered with a coverslip to avoid rapid evaporation and in-situ optical microscopic observation was carried out to observe the nucleation of the paracetamol microcrystals.

2.2.9 Slow evaporation

2.2.9.1 Growth of crystals in pure aqueous solution with sudden cooling

Paracetamol, laboratory double distilled water and 250 mL air tight round bottom flask equipped with ground sleeve stirrer glands attachment was used in the crystallization experiment. Aqueous solution of about 100 mL capacity was prepared by dissolving 1.8 g of paracetamol at different elevated temperatures in the range 40-100 °C in steps of 2.5 °C in CTB with continuous stirring for 1 h. After heating, 1 mL solution was pipetted out using micropipette from each of the prepared aqueous solution,
transferred immediately to 5 mL capacity beaker and left undisturbed to freely evaporate at ambient condition. Five repeats were carried out for each heating regime. Solution cooled naturally at room temperature 32 °C results in an initial supersaturation of $\sigma = 0.35-1.99$. Upon complete evaporation of the solution, the crystals were harvested and single crystal X-ray diffraction analysis was carried out for the grown mono and ortho paracetamol polymorphs.

2.2.9.2 Growth of crystals with and without seeding

Solution to be crystallized were prepared with the selected solvents for room temperature saturation according to the solubility by dissolving appropriate amount of paracetamol in 500 mL of polar protic water, ethanol and aprotic cyclohexanone separately. Solubility of paracetamol at room temperature in water is 1.87 g/100 mL, in ethanol 18 g/100 mL and in cyclohexanone 11.2 g/100 mL. The saturated solution was filtered with Whatmann no.1 filter sheet and the filtered solution of each solvent was equally distributed in ten crystallizing vessels totally 30 solutions for crystallization. Immediately after transferring the solution in the crystallizing vessels, seeds of weighing approximately 14 mg with average size of about 250 µm were selected. Among the thirty crystallizing vessels, three crystallizing vessels containing the selected solvents were left without seeding and the remaining vessels were seeded separately with the prepared seeds A-H in each of the selected solvents. Then the vessels were covered with perforated polyethylene sheets and kept for evaporation in the controlled slow evaporation chamber. The nucleation time was monitored carefully. Due to the evaporation of the solvent, the solution gets supersaturated and the crystal nucleation was observed. The nucleated small crystals were allowed for further growth for two weeks until they attained a desired size and finally the grown crystals were harvested.

2.2.9.3 Growth of crystals in different solvents

100 mL of saturated solution of paracetamol at room temperature 305 K was prepared with each of the above selected ten solvents such as water, ethanol, methanol, isopropyl alcohol, acetone, cyclohexanone, tetrahydrofuran, ethyl acetate, acetonitrile, and 1, 4-dioxane separately in accordance with the solubility, filtered with Whatmann no.1 filter sheet. The prepared saturated solution with different solvents were transferred
to the crystallizing vessels, closed with perforated polyethylene sheets and kept in a slow evaporation chamber in a dust free atmosphere. Solutions were continuously monitored for the nucleation and the nucleation time was noted. Depending on the evaporation of the solvent, the solution gets supersaturated and the nucleation occurred in the solution. The nucleated crystals were allowed to grow for a period of 4-45 days to achieve the reasonable size and then the crystals were harvested. The harvested crystals obtained with perfect morphology were used as the seed crystal for our bulk growth process.

2.2.10 Bulk growth of paracetamol single crystals by slow cooling method

The bulk paracetamol single crystal was grown by adopting the seed rotation technique using slow cooling method. The experimental apparatus consists of a crystallizing vessel mounted on the platform. The vessel is hermetically sealed and equipped with a smooth supporting glass rod for suspending the seed crystal. The supporting rod is connected with a stepper motor drive for rotating the seed crystal in the solution. The entire apparatus is placed in a constant temperature bath. Saturated paracetamol solution of volume 700 mL was prepared in ethanol at 318 K and filtered twice with Whatmann no.1 filter sheet and kept inside the digitally controlled full-visibility constant temperature bath (CTB) which was maintained at the saturation temperature. The CTB is capable of controlling the temperature with an accuracy of ± 0.01°C. A seed crystal of good quality with perfect morphology of weighed mass 10 mg was selected from the crystals grown by the slow evaporation method and the seed was suspended by means of a nylon thread on a smooth support and kept for seasoning at 318 K for 24 h inside the crystallizing vessel in CTB. After seed stabilization, the saturated solution was transferred to the crystallizing vessel and the vessel was kept completely closed. The crystallization setup provided with DC motor enables the seed to rotate with a constant speed at 28 rpm. Then the temperature of the CTB was reduced at a rate of 0.1 °C for every four hours according to the supersaturation \( \sigma = 0.001 \) of the solution. After the successive temperature reductions, the solution becomes supersaturated at \( \sigma = 0.02 \) and the crystal started to grow. Temperature reduction was continued until the temperature of the solution reached the ambient.
2.2.11 Growth of crystals in gel

Paracetamol, sodium metasilicate, ethanol and acetic acid were used in the experiment. Silica gel prepared from sodium metasilicate aqueous solution was used as the crystallizing growth medium and test tubes were used as crystallizing vessels. Sodium metasilicate stock solution (SMS) of specific gravity 1.06 g/cm$^3$ (A) was prepared at ambient temperature by dissolving sodium metasilicate in laboratory double distilled water, filtered and stored in an amber bottle in order to avoid the absorption of carbon dioxide from outer atmosphere. The water-ethanol solvent mixture with mixing ratio 1:1 was used as the solvent to prepare saturated paracetamol solution at ambient temperature. The experimental solution (B) was prepared by mixing equal volumes of the following two stock solutions (i) water-acetic acid (0.072 mole fraction) solution and (ii) water-ethanol (1:1) and paracetamol (0.03 mole fraction) solution. These chemical combinations were optimized by conducting several trial experiments. The prepared experimental solution was prefiltered by vacuum method followed by online pressure filtration with micro filter of 15-40 µm pore size using peristaltic pump. Different volume of SMS stock solution (A) was mixed with the experimental solution (B) to attain the desired pH of the resultant experimental solution (C) which in turn leads to the variation in the height of the gel column. The pH value of the final experimental solution (C) was measured using EUTECH pH TUTOR instrument.

In order to achieve the maximum possible uniformity of the final experimental solution (C) throughout the gel column, it was mixed effectively with magnetic stirrer before being transferred into the experimental test tube. Then the test tubes were sealed with perforated rubber corks for controlled evaporation of the solvent molecules from the top of the gel column during the growth process. Five test tubes with similar combination of solutions were prepared for each pH in order to minimize the errors during the experimental runs. The entire experimental setup was kept undisturbed in the controlled evaporation chamber to prevent atmospheric contamination of the exposed surface of the gel column. The gelation time varies from 12-24 h at room temperature and this variation of the gelation time with respect to pH of the solution was noted down. The gel column was continuously monitored for nucleation and the nucleation time of the polymorphs was measured precisely. Similar systematic procedures were followed and the pH of the
gel solution was varied in the range from 4.25-6.5 in steps of 0.05 by altering the volume of the stock solution (A) in the mixture (B) and best condition for the growth was optimized. After the completion of the growth, crystals were harvested carefully by separating them from the gel using double distilled water. The harvested crystals were air dried and photographed. The grown paracetamol single crystals were characterized by PXRD, FTIR and DSC analysis.

2.2.12 Sonocrystallization

Paracetamol and laboratory double distilled water was used for solution preparation. A desired quantity of paracetamol corresponding to saturation concentrations such as 2.4, 2.6, 2.9, 3.5, 4.3, 5.2, 7.1 and 9 g/100 mL for temperatures in steps of every 5 °C in the range 35-70 °C were chosen. Saturated solution of pure aqueous paracetamol solution was prepared in an airtight round bottom flask, stirred for about 6 h in CTB; filtered with Whatmann no.1 filter sheet. The filtered solution was maintained at their same experimental temperature for 1 h and immediately transferred into another similar CTB and kept maintained for 15 min at 32 °C. Then the experimental solutions were distributed into the measuring cell each containing the solution of 5 mL volume totally in 11 vials. One of the measuring cells was kept outside without sonication and the remaining 10 cells were subjected to ultrasonication. The experimental setup used for ultrasonication consists of ultrasonic transducer bonded at the bottom of the measuring cell, ultrasonic generator connected with the measuring cell to excite the quartz crystal fixed at the bottom of the cell at its resonant frequency to generate ultrasonic waves in the experimental solution filled in the measuring cell. The frequency of the ultrasonic waves can be varied from 1-10 MHz in steps of 1 MHz. The measuring cell containing the solution was placed inside the heavy base socket and clamped with the knurled cap. The ultrasonic waves were delivered to the medium of the cell in the above said frequencies and the duration of radiation was employed for 5 min. Solution without sonication and the ultrasound assisted solution was monitored for nucleation and the induction period was noted. The type of nucleated polymorph both in solution without and with sonication was observed under in-situ optical microscope for all the selected concentration at different supersaturation levels with variable frequency range 1-10 MHz and the events were recorded.
2.2.13 Crystallization in the presence of magnetic field

Paracetamol and laboratory double distilled water was used for solution preparation. A desired quantity of paracetamol corresponding to its saturation concentration such as 2.4, 2.6, 2.9, 3.5, 4.3, 5.2, 7.1 and 9 g/100 mL for temperatures in steps of every 5 °C in the range 35-70 °C were chosen. Saturated solution of pure aqueous paracetamol solution was prepared in an airtight round bottom flask, stirred for about 6 h in CTB; filtered with Whatmann no.1 filter sheet. The filtered solution was maintained at their same experimental temperature for 1 h and immediately transferred into another similar CTB and kept maintained for 15 min at 30 °C. The prepared experimental solutions of volume 5 mL were distributed equally into 9 glass vials each of them having 25 mL capacity. One of the vials was kept outside the magnetic field as a reference and the other 8 vials were subjected to magnetic field. The experimental setup consists of a power supply for electromagnet with variable voltage in the range 10-17 V and gauss meter with Hall probe for measuring the magnetic flux density induced. The voltage varied from 10-17 V induces the magnetic flux density in the range 160-510 Gauss between the pole pieces of the electromagnet. In operation, glass vials containing supersaturated solution were placed in the field generated in the gap between the poles of electromagnets and the exposure was given for 1 h. Solutions with and without magnetic field were monitored for nucleation and their corresponding induction period were noted. The type of nucleated polymorph both in solution with and without magnetic field was photographed using NIKON COOLPIX digital camera for all the selected concentration at different levels of supersaturation in the temperature range 35-70 °C under the variable magnetic flux density 160-510 gauss and the events were recorded with different time intervals.

2.2.14 Polymorph characterization

2.2.14.1 Powder X-ray diffraction (PXRD)

The internal structure of the nucleated paracetamol polymorphs during the in-situ observation was confirmed by subjecting them to Bruker D8 Advance X-ray diffractometer with Bragg-Brentano geometry. Powder X-ray diffraction is a rapid analytical technique primarily used as a ‘finger print’ for identification of a crystalline material. The harvested crystalline paracetamol samples were loaded immediately after
the harvestment from the mother solution to the cavity of the sample holder. The diffraction patterns were recorded at room temperature from 10 to 60° 2θ with a step size 0.05° 2θ using Cu Kα source of λ = 1.5406 Å, tube voltage 40 kV and tube current 40 mA.

2.2.14.2 Single crystal X-ray diffraction (SXRD)

In addition to the optical microscopic observation of the nucleated polymorphs, their internal structure was also confirmed by subjecting them to single crystal X-ray diffraction analysis. This study has been carried out by using a Bruker Kappa Apex II diffractometer with Mo Kα source of λ = 0.7170 Å. The structure solution and refinement were performed using SHELXTL-97. All the hydrogen atoms were refined with isotropic displacement factors and the non-hydrogen atoms were defined with anisotropic displacement factors.

2.2.14.3 Fourier Transform Infrared spectroscopy (FTIR)

The frequencies of the mode of vibrations attributed to the paracetamol molecules for the nucleated polymorphs were identified by FTIR analysis. FTIR spectrum was recorded in the range of 4000 to 400 cm⁻¹ using Bruker Tensor 27 spectrometer by KBr pellet technique.

2.2.14.4 Differential Scanning Calorimetry (DSC)

Thermal stability of the nucleated polymorphs was analyzed using TA Instruments DSC Q20 V24. The thermogram was recorded by heating the samples loaded in sealed aluminum pans covering a temperature range 40 to 190 °C at the heating rate of 1 °C/min in the presence of nitrogen atmosphere.
References


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