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Article

# Influence of solvent choice and operating conditions on Chlorzoxazone crystal shape and size

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Solubility of pharmaceutical drugs in organic solvents is one of the important parameters to understand the equilibrium concentration of solute-solvent, which helps optimize and design crystallization conditions to obtain the desired product crystals. In the present study, Chlorzoxazone (CHZ) is used as a model pharmaceutical compound to investigate the equilibrium solubility, the influence of solvent and the operating conditions on the shape, and the size distribution. The solubility of CHZ is determined in organic solvents like Isopropanol, Ethanol, and 2-Ethoxyethylacetate, Ethylacetate and Ethyllactate using shake flask method from -5ºC to 60ºC. The solubility of CHZ in these solvents shows an increasing trend as the temperature increases in the following order: ethyllactate + water (0.5+0.5) < ethylacetate < isopropanol < ethanol < 2-ethoxyethylacetate < ethyllactate + water (0.75+0.25). The solvents, isopropanol, ethanol, and ethyl lactate, produce needle-shaped crystals, while 2-ethoxyethylacetate and ethyl acetate tend to produce plate shaped crystals. CHZ crystals obtained from 2-ethoxyethylacetate tend to have plate shaped crystals with a lower aspect ratio and are selected for batch cooling crystallization experiments performed at different cooling rates, and agitation. It is found that the agitation at 300 rpm and the cooling rate 0.2ºC/min produce more uniform crystal size distribution.

**Keywords**: Chlorzoxazone. Solubility. Cooling crystallization. Solvent choice. Crystal shape. Size distribution.

## INTRODUCTION

Crystallization is used for the separation and the purification of products in fine chemicals, pharmaceuticals, biopharmaceutical, and food industries (Chen *et al.*, 2011). The challenge in pharmaceutical crystallization of an active pharmaceutical ingredient (API) is to achieve the desired shape and size of crystals with high purity. In addition, pharmaceutical industries are also focusing on the flow ability of the API during the tableting process, its stability for long storage and transportation. The solubility of the drug in organic solvents plays an important role in the crystallization process. The solubility of the drug depends on the polarity of the solvent, and its molecular interaction which plays a major role in altering the size and the shape of drug crystals (Gracin, Rasmuson, 2002).

The particle size and the shape have a greater impact on the bioavailability of the drug (Mallick, 2004). The thermodynamic stability, flow ability, and transformation behavior differ with each solvent, solvent composition, and temperature (Kitamura, Nakamura, 2002). The formation of the crystal lattice of the molecular solids is based on the choice of solvent and additives in the solution (Vedantam, Ranade, 2013). The solubility data accurately predict the equilibrium concentration of an API in a solvent and the mode of crystallization operations (Widenski, Abbas, Romagnoli, 2010). The control of crystal shape, size, and form is vital in the batch crystallization process. The

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micrometric properties of crystals have a direct impact on the downstream operations such as filtration, drying, and milling as well as the physicochemical properties of the solid such as dissolution rate and bioavailability (Zhang *et al.*, 2017).

Literature shows that solubility studies are available for various pharmaceutical solutes (API) such as Ibuprofen (Aragon, Rosas, Martinez, 2010), Diphenoxylate (Qing *et al.*, 2015), 2-Methylnapthalane (Zhang *et al.*, 2015), Phenacetin (Croker *et al.*, 2015), Piroxicam (Hansen, Qu, 2015), Aceclofenae and Fenofibrate (Patil, Patil, Navale, 2016), Vanillin (Noubigh, Oueslati, 2014) and some other drugs in various organic solvents. Chlorzoxazone (CHZ) is a centrally acting agent for the painful musculoskeletal conditions with sedative properties and is a poorly water-soluble compound of solubility 0.2–0.3mg/ml (Raval *et al.*, 2015). CHZ is a Class II drug under the biopharmaceutical classification system (BCS), which has a limited dissolution rate and poor compressible property (Raval *et al.*, 2015). The drugs with low solubility have extended dissolution rates with lesser convenience to reach the targets in patients. There is a greater impact while administering the drug through the oral route. To the best of our knowledge, the solubility for CHZ has not been reported in the open-source. Because of the biological importance and low solubility, Chlorzoxazone (Figure 1) is selected as a model compound for this study. The experimental solubility is measured for various solvents using the shake flask method at different temperatures. The analysis of CHZ crystals in terms of shape and size produced from various solvents by cooling crystallization is reported. Further, an experimental batch cooling crystallization is conducted for CHZ using 2-Ethoxyethylacetate at different cooling and stirring rates to understand the effect of these operating parameters on crystal habit.

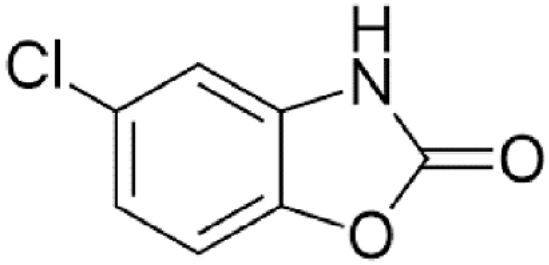


**FIGURE 1 -** Chemical structure of Chlorzoxazone.

## MATERIAL AND METHODS

All chemicals purchased are of analytical grade and are used without further purification. The solvents [ Ethanol (Changshu Hong sheng Fine Chemicals, 99.9% purity), Isopropanol (Merck Life science,99% purity), Ethyl acetate (Sisco research laboratories,98% purity), 2-Ethoxyethylacetate (Sigma-Aldrich,98% purity), Ethyl lactate (Tokyo Chemical Industry,98.0% purity)] are purchased and their characteristics are presented in Table I. Distilled water is used in all the experiments. Chlorzoxazone ($C_7H_4ClNO_2$), Molecular weight-169.58, CAS-ID 95-25-0, Melting Point-191.5ºC, Aqueous solubility 0.2-0.3 mg/ml) is obtained as a gift sample from the manufacturer.

**TABLE I - List of solvents and their properties**

| Solvent | Boiling point (ºC) | Density (g/cm$^3$) | Solvent type | Saturation Temperature (ºC) |
|---|---|---|---|---|
| Ethanol | 78.37 | 0.789 | Polar, protic | 50 |
| Isopropanol | 82.5 | 0.786 | Polar, protic | 60 |
| Ethyl acetate | 77.1 | 0.902 | Mid polar, aprotic | 50 |
| 2-Ethoxyethylacetate | 156 | 0.973 | Mid polar, aprotic | 60 |
| Ethyl lactate | 158 | 1.03 | Mid polar, protic | 60 |

**TABLE I - List of solvents and their properties**

| Solvent | Boiling point (ºC) | Density ($g/cm^3$) | Solvent type | Saturation Temperature (ºC) |
|---|---|---|---|---|
| Water | 100 | 0.997 | Polar, protic | 60 |

## Solubility measurements

The solubility of CHZ in various solvents is determined using the shake flask method (Baka, Comer, Takács-Novák, 2008). The solvents chosen for the study are: Ethanol, Isopropanol, Ethyl acetate, 2-Ethoxyethylacetate, Ethyl lactate + water (0.75+0.25), and Ethyl lactate + water (0.5+0.5). The solubility measurement of CHZ in the selected solvents is carried out within the temperature range from -5°C to 60°C. The upper saturation temperature for the solubility measurement is fixed, approximately 10°C below the boiling point of the selected solvents. The outer jacketed glass vessel is connected with a circulating water bath and is placed on the magnetic stirrer as shown in (Figure 2). A 2 ml of solvent is taken in a test tube and allowed to equilibrate at a set temperature in a water bath. An excess amount of the solid drug CHZ is added to the solvent. A Teflon-coated magnetic bead is added to each tube and the solution is agitated at 300 rpm for 6 hrs. Agitation is stopped after 6 hrs and the solution is allowed to stand for 18 hours at the same set temperature for settling. Then, clear supernatant is pipetted out from the sample and it is added to the diluting solvent for measuring the absorbance. The drug concentration in the samples is determined using UV-spectroscopy at 283 nm (Stewart, Janicki, 1987). The solubility measurement procedure is repeated for all the chosen solvents of the study.

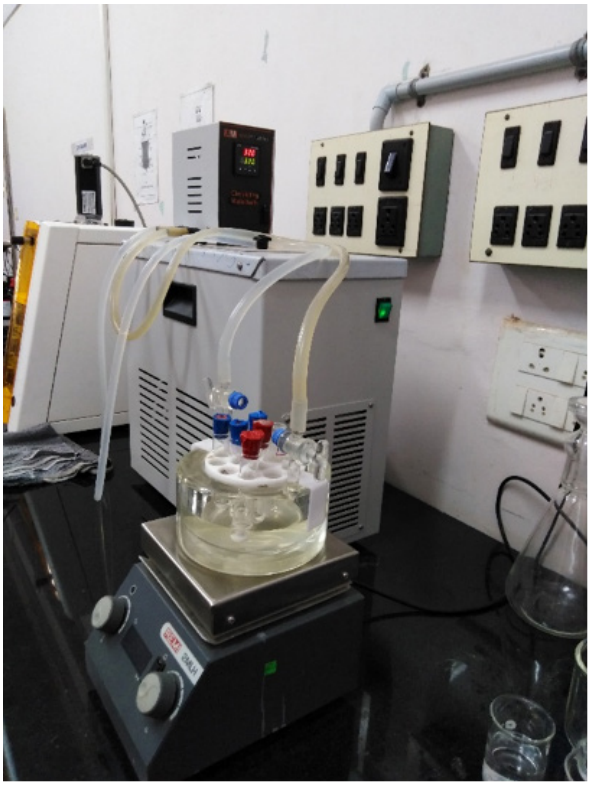

**FIGURE 2 -** Experimental set up for solubility measurement.

### Cooling crystallization

To understand the effect of solvents on the crystal shape, the batch cooling crystallization experiments are performed. The solvent (5 ml) is taken in the outer jacketed glass reactor of capacity 15 ml connected with a circulating water bath. The reactor is placed on the magnetic stirrer with a Teflon-coated magnetic bead. The saturation temperature is chosen to be 10–20ºC below the boiling point of the solvents. Then, stirring rate is set at 300 rpm to mix the contents in the reactor. The saturated solution is prepared by dissolving the known amount of drug to the exact volume of the solvent in the reactor. The solution is heated 5ºC above the saturation temperature to dissolve the solute completely. The saturated solution is maintained at the same temperature for half an hour to obtain a clear solution. Then, the solution is gradually cooled to 5ºC, at a specified cooling rate of 0.5ºC/min. Nucleation is observed in the supersaturated solution. After nucleation, crystals are produced continuously in the solution. The crystals are separated from the mother solution using vacuum filtration method. The crystals are dried in a vacuum oven and used for further analysis. The cooling crystallization experiments are repeated for all the selected solvents of the study.

### Effect of operating conditions in batch crystallization

The experimental batch cooling crystallization of CHZ is conducted using 2-ethoxyethylacetate at different operating parameters such as cooling rate and agitation. The experiments are carried out in a double jacketed glass vessel connected with programmable circulating water bath. The saturation temperature (60°C) is maintained initially to dissolve the desired amount of CHZ in the selected solvent. Based on the solubility data, 0.652 g of the CHZ drug is dissolved in 5 ml of 2-ethoxyethylacetate, and the temperature is raised 5°C above the saturation temperature until a clear solution is obtained. The solution is stabilized for half an hour at that temperature before cooling. The solution is cooled to 5°C at two different cooling rates of 0.2°C/min and 0.4°C/min and three different agitation speeds of 300, 400, and 500 rpm. The solution is filtered through a vacuum filter and crystals are dried before analysis.

### Image Analysis

The images of crystals are investigated using optical microscope (Olympus Model - CX21i). The microscopic lens connected with Magvision image analysis software is used for taking images and to measure the length of crystals. The images are taken for each of the crystals formed. The mean size of the crystals was determined using OriginPro software for determining the statistical analysis. The measurement of crystal size is taken by counting 100 crystals from different images taken at different positions of the sample. Similarly, image analysis is performed for the crystals obtained from each solvent.

### Fourier Transform Infrared Spectroscopy (FTIR)

The molecular-level interactions are analyzed using Perkin Elmer/spectrum 2 Fourier Transform Infrared Spectroscopy (FTIR) in dry air at room temperature. The equipment set up is a horizontal type model, attenuated total reflectance mode on diamond crystals, and the samples are scanned in the wavelength range 4000–450 $cm^{-1}$. The CHZ crystals obtained from the selected solvents are analyzed at 32 scans at a resolution of 4 $cm^{-1}$ and a scan speed of 0.2 cm/s.

### Powder X-ray Diffraction (PXRD)

The recrystallized solids from each solvent and raw CHZ are analyzed using Riagku Ultima IV X-ray unit, Japan. The unit is rigged with source of energy emitting Cu Kα radiation (λ=1.5406 Å) scanning at a speed of 1°/min. The tube voltage is 40 kV and tube current is 30mA. All the samples are scanned from 10-90° with a step size of 0.05°. The characteristics spectrum and the resulting intensity of the peak are obtained. The analysis is performed to examine the polymorph transformation or solvate formation that could have occurred during recrystallization. The samples of crystals are smoothly deposited and pressed on a zero back ground plate prior to analysis.

## RESULTS AND DISCUSSION

Drug solubility is an important property in solution crystallization operations to obtain the desired purity, crystal habit, flow ability, and for improving the dissolution rate. For crystallization studies, the individual solute-solvent system solubility data are important (Wang *et al.*, 2010). The equilibrium concentration of CHZ in various solvents at different temperatures was determined as shown in Table II. The solubility data for CHZ in isopropanol, ethyl acetate, 2-ethoxyethylacetate, ethanol, and ethyl lactate are determined as a function of temperature from -5ºC to 60ºC. The solubility data are obtained in an increasing order: Ethyl lactate + Water (0.5+0.5) < Isopropanol < 2-Ethoxyethylacetate < Ethyl lactate + Water (0.75+0.25). The solubility increases with increasing temperature till saturation temperature (60°C) as shown in Figure 3. Similarly, Ethyl acetate < Ethanol solubility hierarchy is shown in Figure 4 with increasing temperature till saturation temperature (50°C). The saturation temperature is selected based on the boiling pointof selected solvents. If solubility experiments are performed beyond the saturation temperature, there exists a possibility of solvent evaporation resulting in deviation in concentration from the equilibrium concentration.

The solubility of CHZ in ethyl lactate and water as a mixed solvent in defined composition is also chosen for the present study. Pure ethyl lactate dissolves about 428 mg/ml of the drug and the crystals become more solid at a lower temperature in cooling crystallization operations. The solubility of crystallizing solute should be in the range of 5–20 mg/ml at room temperature to promote easy separation of crystals from the solvent (Rohani, Horne, Murthy, 2005). The concentration of the API should be in a specified limit at respective temperatures; else, crystals become solid during crystallization leading to the formation of immovable mass which is difficult to filter. The solubility limit of any drug in a given solvent is 5–20 mg/ml at lower temperatures and 50–200 mg/ml at a higher temperature. Because of this factor, it is proposed to decrease the solubility of the drug using a mixed solvent (ethyl lactate and water). The solubility of the drug in ethyl lactate + water (0.75+0.25) is 171.65 mg/ml at 60ºC. The solubility of CHZ in ethyl lactate + water (0.5+0.5) is found to be 74.25 mg/ml at 60ºC. It clearly shows that water is attracted more towards ethyl lactate and hindered the solvent bonding potential with the CHZ, and thereby the solubility of the drug is reduced as the proportion of water increases in the solvent mixture. Since, CHZ is a water-insoluble compound, increasing the volume fraction of water from 0.25 to 0.5 with ethyl lactate lowers the solubility of the drug.

**TABLE II -** Experimental solubility data of CHZ in select solvents at different temperatures

| Temperature ºC | Equilibrium Concentration of CHZ (mg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | Ethanol | Ethyl acetate | Isopropanol | 2-Ethoxyethyl acetate | Ethyl lactate + water (0.75+0.25) | Ethyl lactate + water (0.5+0.5) |
| -5 | 24.57 | 28.98 | 24.06 | 37.87 | 34.58 | 7.57 |
| 5 | 33.15 | 34.41 | 33.32 | 57.21 | 46.61 | 12.79 |
| 10 | 37.86 | 40.39 | 40.55 | 67.90 | 57.64 | 15.31 |
| 20 | 54.02 | 54.77 | 52.33 | 82.87 | 72.26 | 19.94 |
| 30 | 74.04 | 65.14 | 70.56 | 93.40 | 92.32 | 32.57 |
| 40 | 84.65 | 72.12 | 91.04 | 102.82 | 115.11 | 40.22 |
| 50 | 113.26 | 85.49 | 100.47 | 118.78 | 142.51 | 54.78 |
| 60 | x | x | 125.37 | 123.86 | 171.65 | 74.72 |

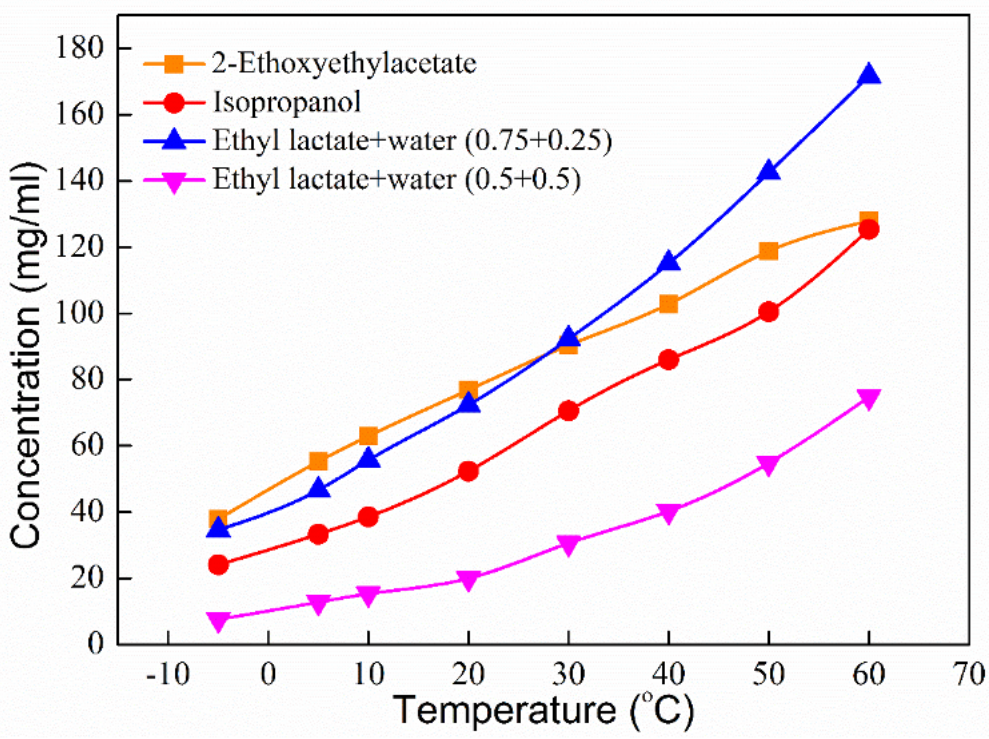


**FIGURE 3 -** Solubility of CHZ in select solvents at -5 ˚C to 60˚C.

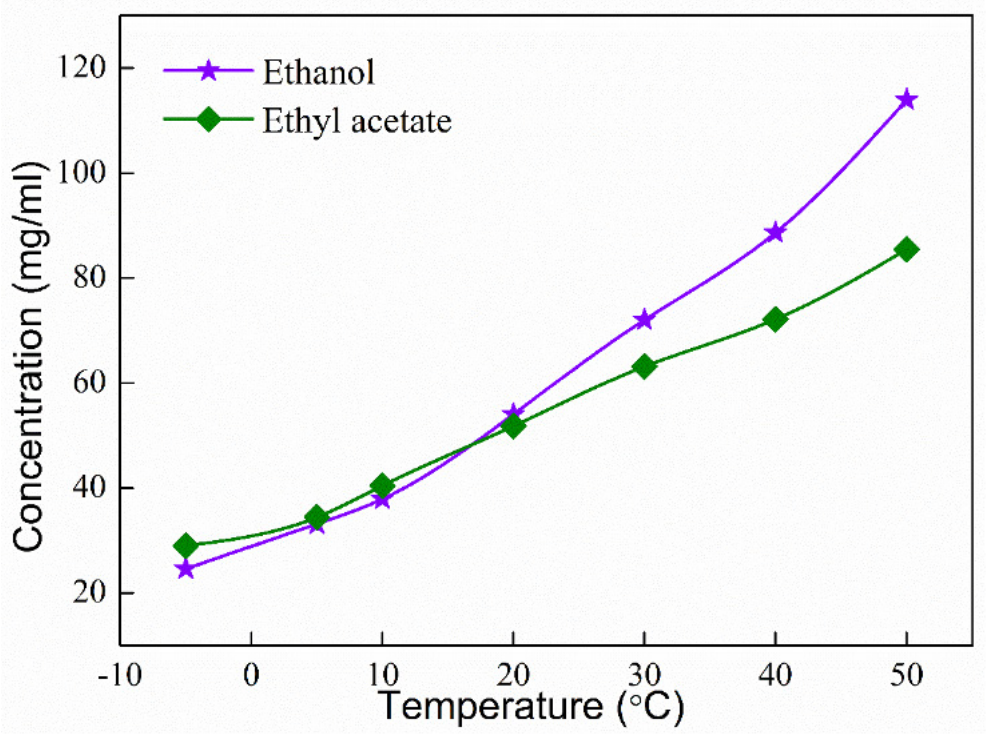


**FIGURE 4 -** Solubility of CHZ in select solvents at -5 ˚C to 50˚C.

**Influence of solvent characteristics on crystal habit**

After cooling crystallization experiments, crystals are separated from the mother solution using vacuum filtration. The crystals are allowed to dry in a vacuum oven for 24 hours. Image analysis of the dried crystals is performed to examine the shape and the size distribution of crystals. The image analysis techniques are used to determine the crystal size distribution and for descriptors like roundness, elongation, and aspect ratio. The image analysis is carried out for the crystals obtained from each solvent. The ethyl lactate–water mixed solvent system affects the shape and the size of the crystals. The solvent composition (0.5+0.5) of ethyl lactate + water shows a tendency towards the long thin needle-shaped crystals as shown in (Figure 5A) with a size of 319.07±36.49 μm. The composition of ethyl lactate + water (0.75+0.25) produces short thin needle-shaped crystals with a size of 130.83±31.25 μm, as shown in (Figure 5B). It is observed that small changes in the solvent composition are sufficient to alter the shape, the size, and the stability.

The alcoholic solvents like isopropanol and ethanol have hydroxyl groups interacting with N-H and C=O group of CHZ compounds leading to the formation of hydrogen bonding. CHZ compound contains the geometrical position N-H and C=O, projecting outwards in its structural arrangement, and so it freely interacts with polar solvents. Due to this alcoholic solvent interaction, the CHZ compound tends to extend the length of crystals resulting in a very long thin needle shape for isopropanol shown in Figure 5C. Similarly, the crystals from ethanol are of needle shape, but slightly different from the crystals obtained from isopropanol. The CHZ crystals obtained from ethanol are shorter in length with fair aspect ratio than that of isopropanol as shown in Figure 5D. The observed difference in shapes also influences the size of crystals estimated to be 254.06±41.15 μm and 147.13±32.15 μm for isopropanol and ethanol, respectively as presented in Table III. Theoretically, the drug compound acts as a Lewis acid (electron pair acceptor) to establish hydrogen bonds with proton-acceptor functional groups of the solvents (oxygen in O=C< and nitrogen in N-H). Similarly, the observed results also have shown such interactions of alcoholic solvents with the CHZ compound. Ethyl acetate and 2-ethoxyethylacetate are a little different from other solvents because of their mid polarity, and aprotic group. Ethyl acetate is a midpolar solvent which interacts with N-H &C=O and nonpolar C-CL bonds of the CHZ compound. 2-Ethoxyethylacetate is a midpolar, aprotic solvent that interacts with C-CL and C=O of the CHZ compound. The partial polar interaction allows the crystals to grow in one direction through hydrogen bonding. The presence of aprotic group in the solvent broaden the crystals surface resulting in plate shaped crystals. Due to geometric restrictions, CHZ crystals produced from 2-ethoxyethylacetate and ethyl acetate solvents tend to grow as an elongated plate shaped crystals as shown in (Figures 5E and 5F) respectively. The influence of solvent can also be seen in the size distribution of crystals which is estimated to be 48.80±14.15 μm for 2-ethyoxyethylacetate and 96.45±24.85 μm for ethylacetate as shown in Table IV. The size of crystals obtained from ethylacetate is larger than that from 2-ethyoxyethylacetate because of its mid polar interaction which elongates the growth of crystals. But, in terms of aspect ratio, CHZ crystals produced from 2-ethyoxyethylacetate are better than those from ethyl acetate because of strong nonpolar interactions.

The CHZ compound interactions with polar protic solvents like isopropanol, ethanol, and ethyl lactate result in elongated needle crystals, whereas mid polar and nonpolar aprotic solvents like ethyl acetate and 2-ethyoxyethylacetate result in plate shaped crystals (The solvent properties are listed in Table I). The aprotic solvents tend to produce broad crystals, while protic solvents tend to produce thin crystals, which coincide with present predictions (Croker *et al*., 2015). The selection of solvents is important to obtain the desired morphology of the crystals. Similar to CHZ, a class II drug ibuprofen has been reported to produce crystals with a high aspect ratio from nonpolar solvents like hexane and a low aspect ratio from polar solvents like ethanol and methanol (Acquah *et al*., 2009). In the present study, the crystals obtained from 2-ethyoxyethylacetate would be more desirable because of the plate shaped with a low aspect ratio.

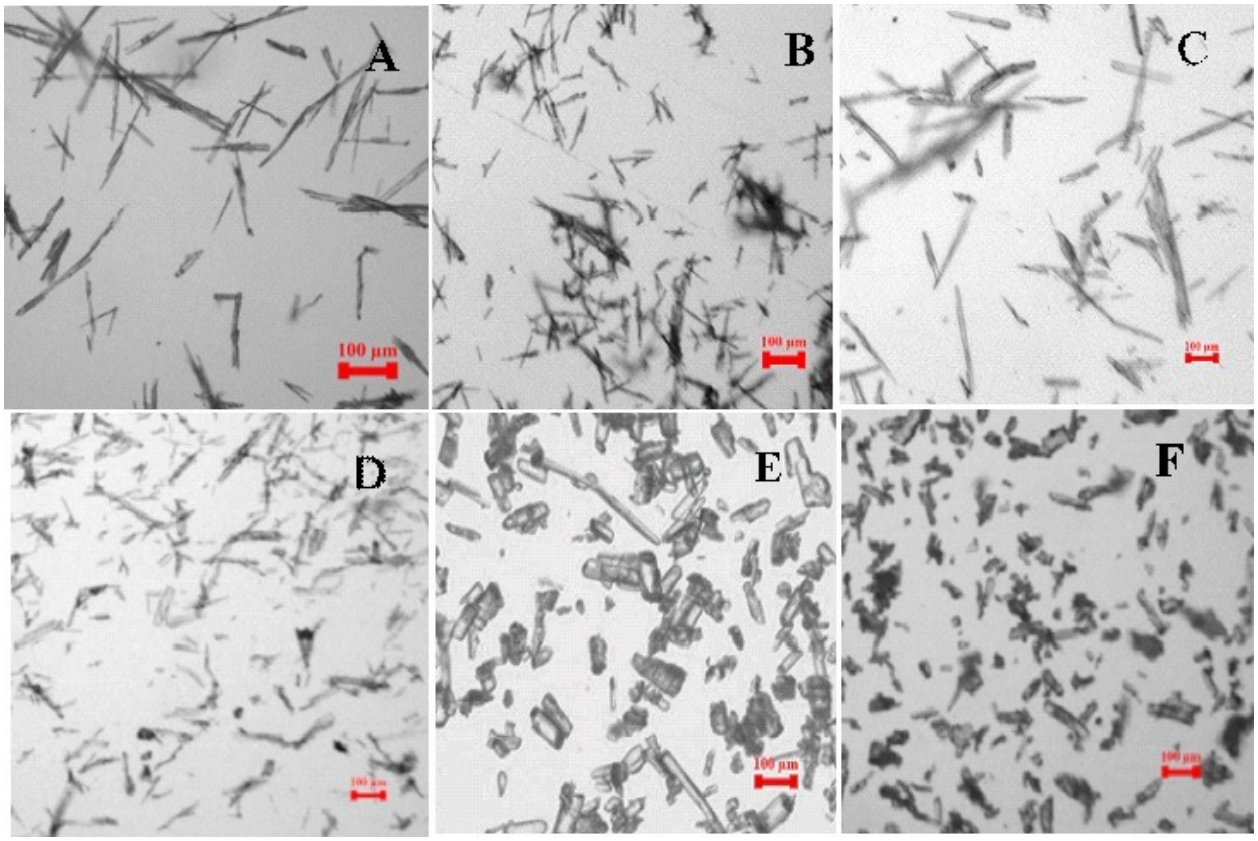


**FIGURE 5 -** Optical microscopic images (4x resolution) of CHZ crystals obtained from A) Ethyl lactate + water (0.5+0.5) B) Ethyl lactate + water (0.75+0.25) C) Isopropanol D) Ethanol E) 2-Ethoxyethyl acetate F) Ethyl acetate.

**TABLE III - Shape and size of CHZ crystals observed in select solvents**

| S.No | Solvents | Shape | Description | Mean size of crystal (µm) |
|---|---|---|---|---|
| 1. | Ethanol | Needle | Needles with fair aspect ratio | 147.13±32.15 |
| 2. | Ethyl acetate | Plate shaped | Thin elongated plate shaped | 96.45±24.85 |
| 3. | Isopropanol | Needle | Very thin brittle long needle | 254.06±41.15 |
| 4. | 2-Ethoxyethylacetate | Plate shaped | Small thick plate shaped | 48.80±14.15 |
| 5. | Ethyl lactate + water (0.75+0.25) | Needle | Short thin needle | 130.83±31.25 |
| 6. | Ethyl lactate + water (0.5+0.5) | Needle | Long thin needle | 319.07±36.49 |

**TABLE IV - Operating conditions for batch cooling crystallization and effect on CHZ crystals**

| Solvent / Concentration | Cooling rate °C/ min | Agitation rpm | Shape |
|---|---|---|---|
| 2-Ethoxyethylacetate / Concentration 0.625 g/5ml | 0.2 | 300 | Plate shaped crystals |
| | | 400 | Plate shaped crystals |
| | | 500 | Plate shaped crystals along with aggregate formation |
| | 0.4 | 300 | Plate shaped crystals |
| | | 400 | Plate shaped crystals |
| | | 500 | Plate shaped crystals along with aggregate formation |

## Fourier transformation of Infrared spectroscopy (FTIR)

The FTIR results show the presence of the functional groups and their stretching vibrations in the CHZ crystals obtained from selected solvents as shown in the Figure 6. In these spectrograms, the high-intensity stretching vibrations are observed in the region 1762-450 $cm^{-1}$ for all the solvents. The FTIR spectrum of CHZ crystals obtained from ethanol has also exhibited similar broad and intense absorption bands in the range of 550–1750 $cm^{-1}$ (Yurdakul, Yurdakul, 2014). The intense absorption bands are due to the intramolecular interaction of N-H and C-N bending, and C=O stretching vibrations of the oxazole and carbonyl groups of CHZ. The FTIR spectrum showed no shift change when compared with the raw form of crystals (Figure 6A), indicating that the selected solvents do not alter the position of functional groups. But, the intensity of the peaks pattern varies due to the shape of CHZ crystals.

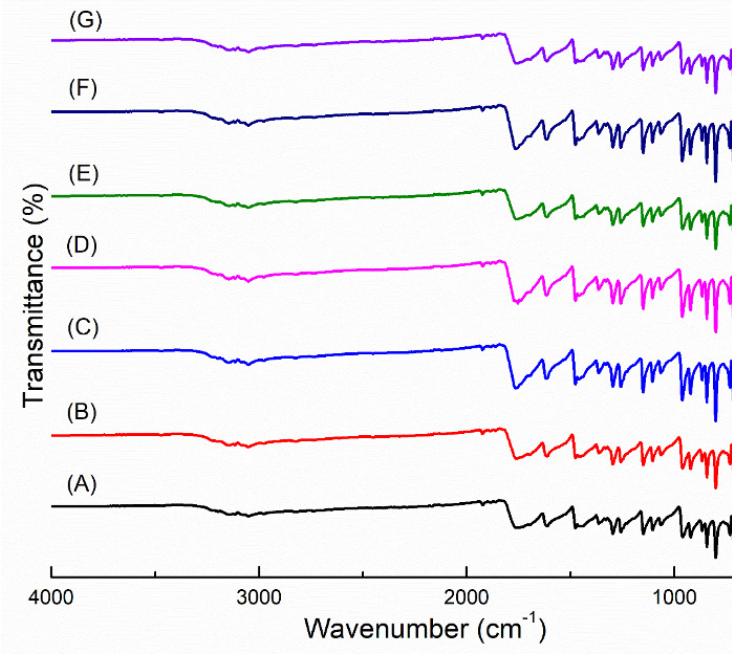


**FIGURE 6 -** FTIR spectra for recrystallized CHZ in the solvent (A) raw CHZ (B) 2-ethoxyethylacetate (C) isoproponal (D) ethanol (E) ethyl lactate + water (0.75+0.25) (F) ethyl lactate + water (0.5+0.5) (G) ethylacetate.

## PXRD analysis

Figure 7 shows the typical PXRD results of raw CHZ and recrystallized CHZ in the solvents used in the present study. The PXRD pattern confirms that the CHZ crystals recrystallized from the selected solvents show similar pattern as that of the raw drug. It means that there is no evidence for solvate formation or polymorphic transition during crystallization process. The peak patterns confirm that geometrical position of the CHZ crystal lattice is found to be similar for all the selected solvents. However, the intensity of the peak pattern showed the variation due to the difference in the alignment of crystal surfaces to the incident beam.

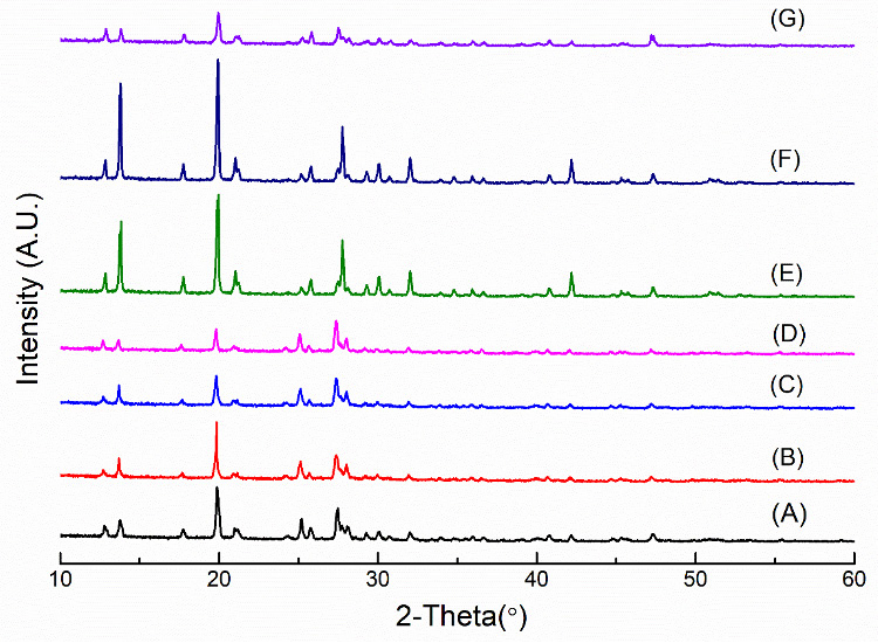


**FIGURE 7 -** PXRD patterns of recrystallized CHZ in the solvent (A) raw CHZ (B) ethyl lactate + water (0.5+0.5) (C) ethyl lactate + water (0.75+0.25) (D) ethyl acetate (E) ethanol (F) isoproponal (G) 2-ethoxyethylacetate.

## Batch crystallization of CHZ using 2-Ethoxyethylacetate at different operating conditions

The best choice of the solvent for batch cooling crystallization of CHZ is based on the shape of crystals. The plate shaped crystals obtained from 2-ethoxyethylacetate is likely to show better flow-ability during formulation. The crystallization process conditions like temperature range, cooling rate, agitation, seeded conditions, and additives could be altered to control the shape and the size distribution of crystals (Brittain, 2016). Hence, batch cooling crystallization experiments are performed using 2-ethoxyethylacetate to determine the effect of agitation and the cooling rate on the shape of crystals. The saturated solution is prepared and the solution is cooled down to 5ºC for all combinations of operating conditions (Table IV) considered in the present study. Crystals from each experiment are analyzed for their shape, and are presented in Table IV.

### *Effect of agitation on the shape of CHZ crystals*

The range of agitation is selected based on the dimensions of the crystallizer used in the present experiment. The speed of 300, 400 and 500 rpm is selected for the experiments to determine the effect of crystal collisions under suspensions. The effects of agitation speed of 300 and 500 rpm with respective cooling rates

of 0.2 and 0.4 ºC/min on crystal shape are shown at 10X resolution (Figure 8A-8D). At 300 rpm with the cooling rate 0.2ºC/min, the crystals are found to be plate-like crystals as shown in Figure 8A, whereas at 500 rpm with 0.2ºC/min cooling rate, there is a formation of aggregates among the crystals as shown in Figure 8B. Similarly, with 0.4ºC/min cooling rate, the crystals are found to be plate-like crystals at 300 rpm and crystal aggregates are formed at 500 rpm as shown in Figures 8C and 8D. At 300 rpm, the suspension velocity of the solution is optimal and thus crystals get more space for their growth instead of crystal collisions. The effect of agitation is pronounced at 500 rpm inducing the formation of aggregates, as clearly observed in Figures 8B and 8D. The collision of crystal due to agitation results in the formation of broken crystals (Kim, *et al.*, 2011). At a speed of 500 rpm, the smaller crystal fragments are produced due to collision of crystals with the crystallizer internals or from the breakage of larger crystals. The fragmented crystals tend to adhere to the energetically more stable surface of the larger crystals leading to aggregation. It shows that the effect of agitation at a higher rate induces aggregates and reduces the growth of crystals. Thus, the agitation for both cooling rates is found to have the same effect and control over the size of crystals. The plate shaped crystals of CHZ obtained at 300 rpm would be more desirable for better tableting ability.

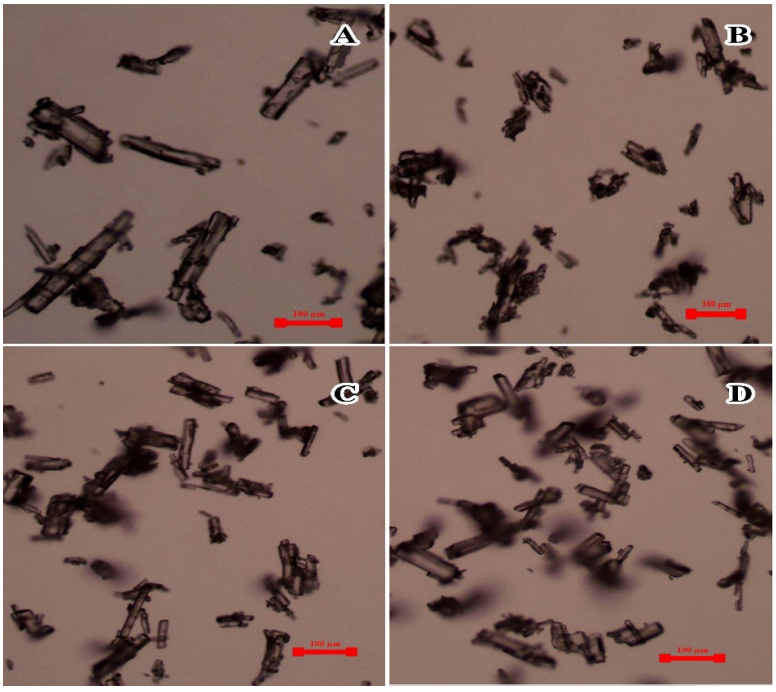


**FIGURE 8 -** Optical microscopic images (10x Resolution) of CHZ crystals from batch crystallization experiments for cooling rate and agitation A) 0.2 ºC/ min with 300 rpm B) 0.2 ºC/ min with 500 rpm C) 0.4 ºC/ min with 300 rpm D) 0.4 ºC/ min with 500 rpm.

*Effect of cooling rate on the size distribution of CHZ crystal*

The cooling rates of 0.2 and 0.4 °C/min are chosen and the experiments are carried out with agitation speeds 300, 400 and 500 rpm to examine the CHZ crystal obtained from 2-ethoxyethylacetate. The FTIR band spectrum of crystals from 2-ethoxyethylacetate has shown no shift change when compared with the pure form of crystals (Figure 6), indicating that the operating conditions do not change the morphology of CHZ. The mean size of crystals obtained from different operating conditions is presented in Table V. The crystals obtained

for a cooling rate of 0.2°C/min have a mean size of 115.56±32.35 µm at 300 rpm and 52.43±13.62 µm at 500 rpm. The cooling rate of 0.4°C/min produced mean size of 99.26±27.64 µm at 300 rpm and 56.87±17.65 µm at 500 rpm. The mean size of CHZ crystal obtained from 2-ethoxyethylacetate is found to be 48.80±14.15 µm (shown in Table III) during the cooling crystallization experiment operated at 300 rpm with 0.5°C/min cooling rate. The size of CHZ crystals produced at lower cooling rates is found to be bigger than the crystals produced at higher cooling rates at a speed of 300 rpm. Thus, the results indicate the cooling rate for 0.2°C/min at 300 rpm and 500 rpm produces larger crystals and 0.4°C/min at 300 and 500 rpm produces smaller crystals. This is due to the fact that the slower cooling rate (0.2 °C/min) tends to extend the level of supersaturation for a longer time and so the formation of nuclei is controlled resulting in a growth of crystals to a larger size. In contrast, at a faster cooling rate (0.4°C/min) the supersaturation is reduced suddenly which leads to uncontrolled nucleation and the subsequent growth resulting in smaller sized crystals. The cooling rate is found to have control over the crystal size distribution as shown in Figure 9. The CHZ crystals produced at a lower cooling rate of 0.2°C/min with 500 rpm and 300 rpm are found to be uniformly distributed and also closer to its mean size as shown in Figure 9C and 9D, but CHZ crystals formed at a higher cooling rate of 0.4°C/min with 500 rpm and 300 rpm are randomly distributed as shown in Figure 9A and 9B. The lower cooling rate controls the spontaneous nucleation and leads more uniform size distribution (Fevotte, Klein, 1996).

**TABLE V - E**ffect on the size of CHZ crystals at different operating conditions

| Cooling Rate (ºC/ min) | Agitation (rpm) | Mean size of crystal (µm) |
|---|---|---|
| 0.2 | 300 | 115.26±32.35 |
| | 500 | 52.43±13.62 |
| 0.4 | 300 | 99.62±27.64 |
| | 500 | 56.87±17.65 |

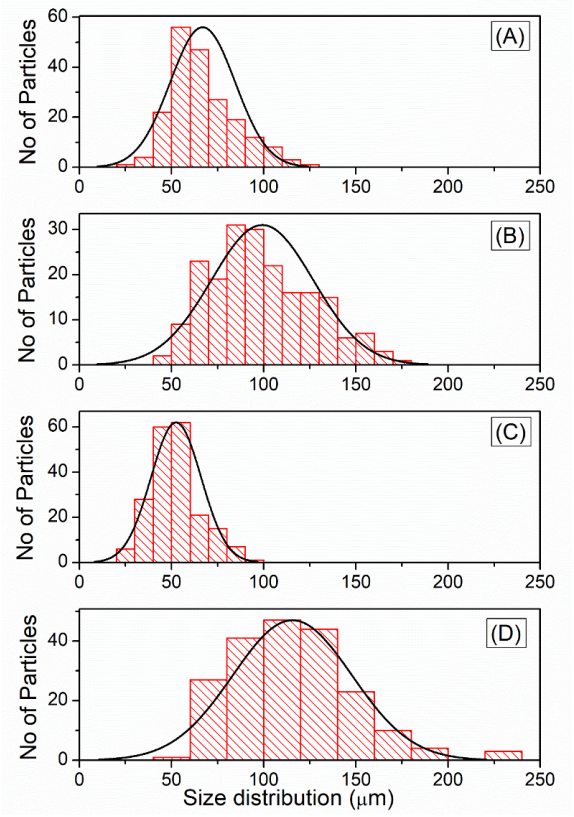


**FIGURE 9 -** Size distribution comparison of CHZ crystals from experiments operated at (A) 0.4ºC/min with 500 rpm (B) 0.4ºC/min with 300 rpm (C) 0.2ºC/min with 500 rpm (D) 0.2ºC/min with 300 rpm.

## CONCLUSION

The solubility curve helps to predict the equilibrium concentration of the solute-solvent system and aids in deciding the type of crystallization and the operating conditions. The solubility of CHZ in the selected solvents has been determined using the shake flask method. The polarity of the selected solvents and their interaction with CHZ influence the shape and the size of crystals. Plate shaped CHZ crystals with a lower aspect ratio is obtained from 2-ethoxyethylacetate which is found to be more desirable for improving flow-ability and compressibility. Hence, the batch cooling crystallization experiments are performed for CHZ in 2-ethoxyethylacetate at different cooling rates and agitation speeds to understand their effects on crystal shape and size. The agitation speed at 300 rpm leads to the growth of plate shaped crystals with a low aspect ratio. The lower cooling rate of 0.2ºC/min produces larger size crystals with uniform size distribution.

## DECLARATION

The authors report that they have no conflicts of interest.

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