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4.1 Materials:
Selected drugs

Mefenamic acid : Micro labs, Bangalore.
Ketoprofen : Micro labs, Bangalore.
Piroxicam : Ipca laboratory, Mumbai.

Solvents:

Isopropyl Alcohol : Ranbaxy laboratories
Tetra hydro furan (THF) : Ranbaxy laboratories
Isopropyl acetate : Ranbaxy laboratories
Chloroform : Ranbaxy laboratories

All other chemicals and reagent used were of analytical grade.

4.2 Instruments and equipments:

UV-Vis Spectrophotometer: UV-1800 Shimadzu, Japan.
FT-IR Spectrophotometer: 8400S Shimadzu, Japan.
Differential Scanning Calorimeter: DuPont 9900, USA Thermal analyzer with 910 DSC module
X-ray powder diffractometer: A JDX - 8 P Jeol Japan
Scanning electron microscope: Joel- LV-5600, USA,
Submicron particle analyzer-Zetasizer Nano ZS: Malvern Instruments, UK
USP XXIV Dissolution test apparatus: Electrolab. Mumbai
pH Meter: Elico Pvt. Ltd., India

Computer analysis
1. Microsoft excel was used to obtain graphs and figures
2. Multi-dimension minimization program was used to analyze X-ray diffraction pattern
4.3 **Reagents and Buffers**:\textsuperscript{154,156}

**Hydrochloric Acid, 0.07M:** Solution of 0.07M HCl was prepared by diluting 5.95ml of hydrochloric acid to 1000ml with distilled water.

**Potassium Dihydrogen Phosphate, 0.2M:** Dissolved 27.218 g of potassium dihydrogen phosphate in water and diluted with water to 1000 ml.

**Sodium Hydroxide, 0.2M:** Dissolved sodium hydroxide in water to produce a 40% w/v solution and allowed to stand. Precaution was taken to avoid absorption of carbon dioxide, clear supernatant solution was siphoned, diluted to suitable volume to contain 8.0 g of sodium hydroxide in 1000 ml. Solution was standardized as mentioned in Indian pharmacopoeia (Vol. I, 1996).

**Phosphate buffer of pH 7.4:** 50ml of 0.2M potassium dihydrogen phosphate was placed in 200 ml volumetric flask, 39.1 ml of 0.2M sodium hydroxide was added, water was added to volume.

4.4 **Analytical methods**

**Mefenamic acid:** Method described in USP XXII was followed \textsuperscript{(156)}

**UV Scanning Range:** 200-400nm.

**Stock solution:**

Stock solution (1000 µg/ml) was prepared by dissolving 100 mg of mefenamic acid in 100 ml of pH 7.4 phosphate buffer. Stock solution (2.5 ml) was further diluted to 25 ml (100mcg/ml).

This solution was scanned between 200-400 nm. It was found that the sample showed a \( \lambda_{max} \) of 286 nm (Figure 13) and was used for further studies.
MATERIALS AND METHODS

Figure 13 UV Spectra of Mefenamic acid in Phosphate buffer, pH 7.4

Standard plot of mefenamic acid:
From the above stock solution, aliquots of 0.5, 1.0, 1.5, 2.0 and 2.5 ml were transferred to 10 ml volumetric flasks and diluted with pH 7.4 phosphate buffer (Table 3). Absorption of solutions were measured at 286 nm.
The calibration curve of Mefenamic acid in pH 7.4 phosphate buffer is shown in Figure 14.

Table 3 Calibration curve data of mefenamic acid in pH 7.4 phosphate buffer

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Concentration in μg/ml</th>
<th>Absorbance Mean ± SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0.173± 0.001</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0.323± 0.002</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>0.487± 0.003</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>0.652± 0.001</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>0.83± 0.002</td>
</tr>
</tbody>
</table>

*SD Standard deviation: n=3


**MATERIALS AND METHODS**

*Figure 14* Calibration curve of mefenamic acid in pH 7.4 phosphate buffer

**Ketoprofen:** Method described in USP XXII was followed (157).

**UV Scanning Range:** 200-400nm.

**Stock solution:**

Stock solution (1000μg/ml) was prepared by dissolving 100 mg of pure ketoprofen in 100 ml of pH 7.4 phosphate buffer.

Solution showed a λ\text{max} of 260 nm (Figure 15) and was used for further studies.

*Figure 15* UV Spectra of Ketoprofen in Phosphate buffer, pH 7.4
**MATERIALS AND METHODS**

**Standard Plot of Ketoprofen:**

From the above stock solution, aliquots of 4, 6, 8, 10 and 12 ml were transferred to 100 ml volumetric flasks and diluted with pH 7.4 phosphate buffer (Table 4). The absorption of solutions was measured at 260 nm.

The calibration curve of Ketoprofen acid in pH 7.4 phosphate buffer is shown in Figure 16.

**Table 4 Calibration curve data of Ketoprofen in pH 7.4 phosphate buffer**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Concentration in μg/ml</th>
<th>Absorbance Mean ± SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>0.223±0.25</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>0.352±0.14</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>0.465±0.27</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>0.608±0.11</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>0.731±0.23</td>
</tr>
</tbody>
</table>

*mean ± SD, n = 3

![Figure 16 Calibration curve of Ketoprofen in 7.4 phosphate buffer](image-url)
MATERIALS AND METHODS

Piroxicam:

*Piroxicam:* Method described in USP XXII was followed (158)

*UV Scanning Range:* 200-400nm.

*Stock solution:*

Stock solution (1000μg/ml) was prepared by dissolving 100 mg of pure piroxicam in 100 ml of pH 7.4 phosphate buffer. Stock solution (10ml) was further diluted to 100 ml with pH 7.4 phosphate buffer. This solution is suitably diluted yield 100mcg/ml solution.

Solution showed a \( \lambda_{\text{max}} \) of 334 nm (Figure 17) and was used for further studies.

![Figure 17 UV Spectra of Piroxicam in pH 7.4 phosphate buffer (100μg/ml)](image-url)
Standard Plot of Piroxicam:

From the above stock solution, aliquots of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml were transferred to 10 ml volumetric flasks and diluted with pH 7.4 phosphate buffer Table 5. The absorption of solutions was measured at 334 nm.

The calibration curve of Piroxicam in pH 7.4 phosphate buffer is shown in Figure 18.

Table 5 Calibration curve data of piroxicam in pH 7.4 phosphate buffer

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Concentration in μg/ml</th>
<th>Absorbance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0.114±0.15</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>0.234±0.26</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>0.345±0.13</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>0.465±0.14</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>0.582±0.22</td>
</tr>
<tr>
<td>6</td>
<td>300</td>
<td>0.723±0.10</td>
</tr>
</tbody>
</table>

*mean ± SD, n = 3

![Figure 18 Calibration curve of Piroxicam in 7.4 phosphate buffer](image-url)
4.5 **Preparation of crystals by different crystallization techniques:**

**Spherical crystallizations:**

There are several methods described for Spherical crystallization. One of the methods involves three solvent systems. Liquid I is solvent (S) for the drug and other being the non-solvent (NS) and the third liquid used is bridging solvent. The drug solution is added to an emulsion of non-solvent and bridging solvent under continuous stirring.

The drug precipitates and agglomerates as spherical crystals.

All three drugs selected for the study were crystallized to obtain spherical crystals using equipment shown in Figure 19.

![Figure 19 Equipment used for spherical crystallization](image)

A – Thermostat B – Glass vessel C – Paddle D – Motor

Apparatus consisted of flat-bottomed glass vessel (B) with a glass lid, a variable speed motor (D) and a propeller agitator with four blades(C). Each experiment was repeated at least twice to ensure reproducibility. The vessel was placed in a thermostat (A) to maintain the desired temperature.

**Optimization of spherical crystallization techniques:**

To optimize the spherical crystallization by several parameters were considered; among these are effect of amount of bridging liquid, effect of the rotation speed of the stirring, effect of the temperature and effect of mode of addition of bridging liquid
4.5.1 Mefenamic acid:

4.5.1.1 Preparation of spherical crystals of Mefenamic acid (SA):

Mefenamic acid (3.5 gm) was dissolved in 20 ml tetra hydro furan (THF) heated at 45°C. The drug solution was poured quickly in to 70 ml of water maintained at 20°C under continuous stirring. When fine crystals of Mefenamic acid began to form, 10 ml of isopropyl acetate was added drop wise under continuous stirring for 45 min at 500 (±5) rpm. Spherical crystals formed were separated from the solution by filtration. Spherical crystals were dried at 40°C for 12 hours and kept in a desiccator at room temperature until further experiment.

4.5.1.2 Preparation of spray dried crystals of Mefenamic acid (SD):

Mefenamic acid (3.5g) was dissolved in 20 ml of tetra hydro furan (THF) heated at 45°C until a clear solution was obtained. The drug solution was poured in to solvent mixture consists of water (70 ml) and isopropyl acetate (10ml) maintained at room temperature. The resulted solution was spray dried using Mini Spray Dryer LSD -48; (Jay instrument & systems Pvt. Ltd. Mumbai) at a Feed rate of 12%, vacuum in the system maintained at -65 MM WC, Atomization pressure rate of 1 kg/cm², Aspirator level was 35%, inlet temperature of 110 ±2°C and outlet temperature of 45 ±5°C. The crystals were separated from cyclone separator, collected and kept in a desiccator at room temperature until further experiment.

4.5.1.3 Preparation of freeze dried crystals of Mefenamic acid (FD):

Mefenamic acid (3.5g) was dissolved in 20 ml of tetra hydro furan (THF) heated at 45°C until a clear solution was obtained. The drug solution was poured into solvent mixture consists of water (70 ml) and isopropyl acetate (10ml) maintained at room temperature. The resulted solution was transferred to 100 ml glass bottle and kept in a
ultra low freezer at -40°C at a rate of 0.5°C/min, for 24 hr. The frozen drug solution was placed in a lyophilizer for 72 hr using a Freeze Dryer (ILSHIN Lab. Co. Ltd. Korea) with a condenser temperature of -40°C and a pressure of 7×10^{-2} mbar followed by a secondary drying at 25°C for 24 hr. The resulted crystals were kept in a desiccator at room temperature until further experiment.

4.5.1.4 Preparation of super cooling crystals of Mefenamic acid (SC): Super cooling crystals were prepared by melting the drug (5 gm) at 235 ±5°C using heating mantle. The molten drug was brought to room temperature and then transferred to deep freezer for 1 h. The resulted crystals were kept in a desiccator at room temperature until further use.

4.5.1.5 Recrystallization of Mefenamic acid (RS): Mefenamic acid (3.5 gm) was dissolved in 20 ml tetra hydro furan (THF) heated at 45°C and isopropyl acetate (10ml) was added. The drug solution was poured quickly in to 70 ml of water maintained at 20°C with occasional stirring. The crystals of Mefenamic acid were collected by filtration and were dried at 45°C for 12 hours and were kept in a desiccator at room temperature until further use.

4.5.2 Ketoprofen:

3.5.2.1 Preparation of spherical agglomerates of ketoprofen (SA): Ketoprofen (4 gm) was dissolved in 25 ml of isopropyl alcohol (IPA) heated at 45°C until a clear solution was obtained. The drug solution was poured quickly in to 60 ml of water maintained at 20°C, under continuous stirring at 500 rpm with a paddle. When fine crystals of ketoprofen began to precipitate (5-10 min), 10 ml of chloroform (bridging liquid) was added. After 10 min of stirring, 5 ml of chloroform was added again drop wish. The temperature was reduced to 5°C, after about 1 hour stirring;
spherical agglomerates were formed and were separated from the solution by filtration. Spherical agglomerates were dried at 45°C for 12 hours. The resulted crystals were kept in a desiccator at room temperature until further use.

**3.5.2.2 Preparation of spray dried crystals of Ketoprofen (SD):**

Ketoprofen (4 g) was dissolved in 25 ml of isopropyl alcohol (IPA) heated at 45°C until a clear solution was obtained. The drug solution was poured into chloroform (15ml) maintained at room temperature. The resulted solution was spray dried using Mini Spray Dryer LSD -48; (Jay instrument & systems Pvt. Ltd. Mumbai) at a Feed rate of 12%, vacuum in the system maintained at -65 MM WC, Atomization pressure rate of 1 kg/cm², Aspirator level was 35%, inlet temperature of 85 ±2°C and outlet temperature of 45 ±5°C. The crystals were separated from cyclone separator, collected and kept in a desiccator at room temperature until further experiment.

In spray drying crystallization techniques, water is not used as the melting point of the ketoprofen is lower than the boiling point of water.

**3.5.2.3 Preparation of freeze dried crystals of Ketoprofen (FD):**

Ketoprofen (4 g) was dissolved in 25 ml of isopropyl alcohol (IPA) heated at 45°C until a clear solution was obtained. The drug solution was poured into solvent mixture consists of water (60 ml) and chloroform (15ml) maintained at room temperature. The resulted solution was transferred to 100 ml glass bottle and kept in ultra low freezer at -40 °C at a rate of 0.5°C/min for 24 hr. The frozen drug solution was placed in a lyophilizer for 72 hr using a Freeze Dryer (ILSHIN Lab. Co. Ltd. Korea) with a condenser temperature of -40°C and a pressure of 7×10⁻² mbar followed by a secondary drying at 25°C for 24 hr. The resulted crystals were kept in a desiccator at room temperature until further use.
3.5.2.4 Preparation of Super cooling crystals of Ketoprofen (SC):

Super cooling crystals were prepared by melting the drug (5 gm) at 95 ±5°C using heating mantle. The molten drug was brought to at room temperature and then transferred to deep freezer for 1 h. The resulted crystals were kept in a desiccator at room temperature until further use.

3.5.2.5 Preparation of Recrystallization sample of Ketoprofen (RS):

Ketoprofen (4 g) was dissolved in 25 ml of isopropyl alcohol (IPA) heated at 45°C and chloroform (15 ml) was added. The drug solution was poured quickly in to water (60ml) maintained at 20°C with occasional stirring. The crystals of Ketoprofen were collected by filtration and were dried at 45°C for 12 hours and were kept in a desiccator at room temperature until further use.

4.5.3 Piroxicam:

3.5.3.1 Preparation of spherical agglomerates of piroxicam (SA):

Piroxicam (2.5 g) was dissolved in 25 ml of N, N-dimethylformamide (DMF) maintained at 45°C until a clear solution was obtained. The drug solution was quickly poured in to 68 ml of water maintained at 20°C, under continuous stirring at 600 rpm with a Paddle. When fine crystals of Piroxicam began to precipitate (2-5 min), 5 ml of chloroform was added. After 2 min of stirring, 2 ml of chloroform was added again. After 30 min of stirring spherical agglomerates were formed and were separated from the solution by filtration. Spherical agglomerates were dried at 45°C for 12 Hr and were kept in a desiccator at room temperature until further use.
3.5.3.2 Preparation of spray dried crystals of Piroxicam (SD):

Piroxicam (2.5g) was dissolved in 25 ml of is N, N-dimethylformamide (DMF) heated at 45°C until a clear solution was obtained. The drug solution was poured into 68 ml solvent mixture consists of water and chloroform (7ml) maintained at room temperature. The resulted solution was spray dried using Mini Spray Dryer LSD -48; (Jay instrument & systems Pvt. Ltd. Mumbai) at a Feed rate of 12%, vacuum in the system maintained at -65 MM WC, Atomization pressure rate of 1 kg/cm², Aspirator level was 35%, inlet temperature of 165 ±2°C and outlet temperature of 45 ±5°C. The crystals were separated from cyclone separator, collected and kept in a desiccator at room temperature until further experiment.

Preparation of freeze dried crystals of Piroxicam (FD):

Piroxicam (2.5g) was dissolved in 25 ml of is N, N-dimethylformamide (DMF) heated at 45°C until a clear solution was obtained. The drug solution was poured into solvent mixture consists of water (68 ml) ml and chloroform (7ml) maintained at room temperature. The resulted solution was transferred to 100 ml glass bottle and kept in ultra low freezer at -40°C at a rate of 0.5°C/min for 24 hr. The frozen drug solution was placed in a lyophilizer for 72 hr using a Freeze Dryer (ILSHIN Lab. Co. Ltd. Korea) with a condenser temperature of -40°C and a pressure of 7×10⁻² mbar followed by a secondary drying at 25°C for 24 hr. The resulted crystals and were kept in a desiccator at room temperature until further use.

3.5.3.3 Preparation of Super cooling crystals of Piroxicam (SD):

Super cooling crystals were prepared by melting the drug (5 gm) at 205 ±5°C using heating mantle. The molten drug was brought to at room temperature and then
transferred to deep freezer for 1 h. The resulted crystals were kept in a desiccator at room temperature until further use.

3.5.3.4 Preparation of recrystallization sample of piroxicam (RS):

Piroxicam (2.5 g) was dissolved in 25 ml of N, N-dimethylformamide (DMF) heated at 45°C and chloroform (7ml) was added. The drug solution was poured quickly in to water (68 ml) maintained at 20°C with occasional stirring. The crystals of piroxicam were collected by filtration and were dried at 45°C for 12 hours. The resulted crystals were kept in a desiccator at room temperature until further use.

4.6 Characterization of prepared different crystals:

4.6.1 Differential Scanning Calorimetry (DSC):

Thermograms were obtained using a DSC DuPont 9900, with thermal analyzer. Accurately weighed samples were in an aluminum crucible Calorimetric measurements were made with empty cell (High purity alumina discs) as the reference. The instrument was calibrated using high purity indium metal as standard. The system was purged with nitrogen gas at a flow rate of 100 ml/min to maintain an inert atmosphere. Heating was done at the rate of 10°C / min\(^{159}\).

The heat of fusion of drug in prepared crystals was calculated from the peak area of the melting endotherm. The heat of fusion of pure crystalline drug was determined in a separate experiment. The ratio of these fusion energies was used to calculate the percent crystallinity of drug in the prepared crystals using the following equation\(^{160}\):

\[
\text{% Crystallinity} = \frac{\text{Melting enthalpy of the prepared crystals}}{\text{Melting enthalpy of the commercial sample}} \times 100
\]
4.6.2 **Fourier Transform Infrared spectroscopy:**

The FTIR spectral measurements were taken at ambient temperature using a Shimadzu, Model 8400S (Japan). About 2 mg of the commercial sample and prepared crystals were selected separately. Drug samples were dispersed in KBr powder and the pellets were made by applying 5000-6000 kg/cm.\(^2\) pressure. FT-IR spectra were obtained by powder diffuse reflectance on FT-IR spectrophotometer\(^{161}\).
4.6.3 X-ray powder diffraction:

X-Ray powder diffraction patterns were used to detect possible polymorphic transition during the crystallization process. X-Ray powder diffraction patterns were recorded using Rigaku Miniflex II X-ray Diffractometer with Ni filtered radiation of wavelength 1.5406 Å (Cu Target). Samples were scanned in the 2θ range of 0-50°. The scanning speed used for the recording was 3°/min with step size of 0.02°. Diffraction pattern was analyzed using multi dimension minimization program. The program helps to calculate 2θ values and cell parameters a, b, c, α, β and γ which fits the observed reflections to less than 5% of the mean value.162

Sample Preparation: About 1 g of the sample was ground to fine powder in a mortar, Appropriate quantity of sample was taken in the sample holder. The sample was pressed by using glass slides. The sample holder was wiped to avoid any spillage on the instrument.

Procedure: Blank scanning was performed using the empty sample holder. Samples was scanned, correction for blank was made. The pattern of diffractogram was compared with standard pattern.

4.6.4 Scanning electron microscopy:

The Scanning electron microscopic photographs were obtained to identify and confirm crystals nature and morphological characteristics of the crystals. Scanning electron microscopic studies were carried out using SEM Model Joel- LV-5600, USA.
**4.6.5 Determination of residual solvents present in prepared crystals by gas chromatography:**

GC studies were carried out on Shimadzu model 2014 (Shimadzu Technologies, Japan) coupled with a split/split less injector, operated in a split-mode and FID. The computer with GC solutions software has been used to control the gas chromatograph. Rtx-5 capillary column (cross bond 5%di-phenyl/95%di-methyl-polysiloxane) with a length of 30 meters coil and an internal diameter of 0.25 mm was used for all samples studied.

**Procedure:** solution of reference sample (1ml) was injected into headspace of GC and recorded the chromatogram.

**Solvent residue was calculate using following formula:**

Content of residual solvents (ppm):

\[ \frac{\text{Area ratio of methanol to 1, 4 Dioxane in sample} \times \text{Wt (g) of Methanol in stock standard} \times 1 \times 5 \times 10^6}{\text{Area ratio of methanol to 1, 4 Dioxane in std.} \times 100 \times 100 \times \text{Wt (g) of sample}} \]

**4.6.6 Melting point:**

Melting point of drug was determined by capillary method. A few crystals of the drug were placed in a thin walled capillary tube and closed at one end. The capillary, which contains the sample, and a thermometer are then suspended so they can be heated slowly and evenly. The temperature range over which the sample is observed to melt is taken as the melting point.
4.7 Evaluation of crystals for their micromeritic properties, solubility and dissolution:

4.7.1 Percentage yield:
The percentage yield of each prepared crystals by different crystallization technique was determined according to the total recoverable final weight of crystals and the initial weight of drugs used for crystallization.

4.7.2 Drug content:
Spherical crystals (50mg) were triturated and dissolved in 250ml of phosphate buffer pH7.4. The solution was filtered. After suitable dilution with phosphate buffer pH7.4, solution was analyzed spectrophotometrically (Shimadzu). Drug contents were calculated from calibration curves.

4.7.3 Water content:
Water content was determined by Karl-fisher titration. About anhydrous methanol (20 ml) was transferred to the titration vessel and neutralize it with the reagent potentiometrically to consume any moisture that may be present. The weighed amount of the substance was quickly transferred to the titration vessel, stirred for 1 minute and again titrates with Karl Fischer reagent to the end point potentiometrically. The water content in sample was calculated.

\[
\text{Water} \ (\% \ w/w) = \frac{V \times F \times 0.1}{W}
\]

Where,

\(V\) = volume in mL of Karl Fischer reagent consumed

\(F\) = Water equivalence factor of the reagent (mg/mL)

\(W\) = weight of sample taken in g
4.7.4 Micromeritic properties:

**Particle size determination:**

Particle size of commercial drug samples, recrystallized samples, spray dried and super cooled crystals samples were determined by microscopic method and particle size of spherical agglomerates was determined by sieving method. Freeze dried crystals size was determined by a submicron particle analyzer-Zetasizer Nano ZS (Malvern Instruments, UK). Particle size distribution of crystals is reported by intensity. The measurement was performed at 250°C. DTS software (version 4.0) was used to collect the data that were analyzed using “multinarrow modes”. Sample preparation and procedure is presented below:

**Sample preparation:**

About 10 ml of Miglyol 812 (used as Dispersant) was taken in a test tube. About 0.05g of sample to be tested was added to the test tube. Sonicated using a sonicator with an operating frequency of 20 – 40 KHz, for complete dispersion of particles, with gentle swirling for 3 minutes.

**Procedure:**

1. Instrument was switched on and initialized
2. Sample handling units were cleaned with isopropyl alcohol alcohol for two times, rinsed with n-hexane for 2- 3 times and finally rinsed by Miglyol 812.
3. Miglyol 812 was poured into sample handling unit till it reached the level.
4. Background measurement was performed.
5. Sample was introduced into sample handling unit till the obscuration was between 10 to 30%.
6. Sample was circulated for about three minutes to achieve uniform distribution of sample.

7. Sample measurement was performed three times and means value was reported.

8. The weighted residual obtained from the measurement should be less than 1.0%.

**Bulk density and Tapped density:**

Bulk density was determined by pouring the samples into a dried measuring cylinder without tapping. Samples were carefully leveled without compacting and the initial volume to the nearest graduated unit was recorded and untapped density in g/ml was calculated.

\[
\text{Bulk density Untapped (g/ml)} = \frac{W}{V}
\]

Where,

- \( W \) = weight of the sample in g
- \( V \) = Volume occupied by the sample in ml.

Tapped density was determined by placing a graduated cylinder containing a known mass of powder on a mechanical tapper apparatus (Electro lab-tap density tester-USP). Samples were tapped until no further reduction in volume of the sample was observed. The tapped density was calculated by following equation:

\[
\text{Tapped Bulk density (g/ml)} = \frac{W}{V_f}
\]

Where,

- \( W \) = Weight of the sample (g).
- \( V_f \) = Volume occupied by the sample (ml)
Carr’s Index:

Flowability of commercial sample and prepared crystals was quantified using Carr’s Index (CI). The CI was determined from their apparent bulk density and the tapped densities. The test was carried out in triplicate.

\[
\% \text{ Compressibility} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100
\]

Hausner’s Ratio:

Hausner’s ratio is an indication of the flowability of powder and the ratio greater than 1.25 is considered to be an indication of poor flowability. Hausner’s ratio was determined by the following equation. The test was done in triplicate.

\[
\text{Hausner’s ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}
\]

Angle of repose (\(\theta\)):

Fixed funnel method was employed. A funnel that was secured with its tip at a given height above the graph paper that was placed on a flat horizontal surface, Powder or agglomerates was carefully poured through the funnel until the apex of the conical pile just touches the tip of the funnel. The radius and height of the pile were then determined. The angle of repose (\(\theta\)) for the samples were calculated:

\[
\tan(\theta) = \frac{\text{height}}{\text{radius}}.
\]

4.7.5 Mechanical Properties:

Tensile strength:

Tensile strength of different prepared crystals was determined by compressing 500 mg of crystals using hydraulic press at different loads (1000-5000 kg) for 1 min. The compacts were stored in desiccator for overnight to allow elastic recovery. The thickness and diameter were measured for each compact. The hardness of each
compact was then measured using Pfizer hardness tester. The tensile strength (σ) of the compact (kg/cm²) was calculated using following equation.

\[ \sigma = \frac{2F}{\pi Dt} \]

Where, F, D and t are hardness (kg), compact diameter (cm) and thickness (cm), respectively.

**Mechanical strength (Friability (%)):**

Two grams (Wo) of spherical crystals was placed in a friabilator, and this was subjected to the impact test at 50 rpm for two minutes. After passing this through a sieve having a mesh size 125μm, the weight (W) of the material which did not pass through the sieve was determined, and friability (X) was calculated using equation

\[ X = \frac{Wo - W}{Wo} \times 100 \]

**Crushing strengths:**

Crushing strengths of spherical crystals were determined by using mercury load cell method. It was carried out using 10ml glass hypodermic syringe. Tip of syringe and top end of the plunger were removed. The barrel was used as hallow support and guide tube with close fitting to the plunger. A window was cut at the lower end of the barrel to facilitate placement of the agglomerate on the base of platen. The plunger acted as movable platen. It was set directly on the agglomerates, positioned on the lower platen. Mercury is added to the plunger at a predetermined rate from burette from a fixed height. The total weight of mercury plus weight of plunger required to break the agglomerates was the measure of crushing strength.
**Heckel plot:**

Deformation of crystals under influence of stress was evaluated by the Heckel equation (1) Crystals were lubricated with magnesium stearate and were compressed (average mass 500 mg ± 2%), at different pressures, up to constant density of compacts using the 13 mm flat faced punch and die set on a hydraulic press (Spectra lab, India). The ranges of different pressures (1000-5000 kg cm–2) were applied to get constant density. The pallets were stored in airtight moisture-proof containers for 24 hours to enable elastic recovery and hardening. The compressibility behavior was studied using the Heckel equation:

\[
\ln \left[ \frac{1}{1-D} \right] = kP + A
\]

Where, D is relative density and k and A are constants.

The slope of the straight line portion, k, is the reciprocal of the mean yield pressure, \(P_y\), of the material. From the intercept A, the relative density, \(D_A\), can be calculated using the following equation:

\[
D_A = 1 - e^{-A}
\]

Relative density of the powder at the point when the applied pressure equals zero, \(D_0\), is used to describe the initial rearrangement phase of densification as a result of die filling.

Relative density, \(D_B\), describes the phase of rearrangement at low pressures and is the difference between \(D_A\) and \(D_0\).

\[
D_B = D_A - D_0
\]

Each sample was done in triplicate.
4.7.6 Solubility studies:
The solubility of different crystals in water and pH 7.4 phosphate buffer was determined. Excess quantities of prepared crystals were taken into screw-capped 50 ml glass vials filled with water and pH 7.4 phosphate buffer. The vials were shaken for 24 hours on mechanical shaker. The solution was filtered through whatmann filter paper No.1 and the drug concentration was determined spectrophotometrically.

4.7.7 In-vitro Dissolution Studies:
USP dissolution apparatus XXIV-Type II (Electro Lab, Mumbai) was employed in the studies.

4.7.7.1 Mefenamic acid:
The dissolution of commercial sample of mefenamic acid and prepared Mefenamic acid crystals was determined by using USP dissolution apparatus XXIV-Type II (Electro Lab, Mumbai). Dissolution medium (900ml) consisted of pH 7.4 Phosphate buffer maintained at 37 ± 0.2°C with a paddle rotation speed at 100 rpm. 10 ml of dissolution medium was withdrawn at every 10 min interval for 1 h and replaced by equal amount of fresh medium and then filtered through a membrane filter (0.45µm) maintained at 37 ± 0.2°C. The amount of dissolved drug was determined using UV spectrophotometric method (UV 1800 A Shimadzu, Japan) at 286 nm. Each sample was done in triplicate.

4.7.7.2 Ketoprofen:
The dissolution of ketoprofen commercial sample and prepared Ketoprofen crystals was determined by using USP dissolution apparatus XXIV-Type II (Electro Lab, Mumbai). Dissolution medium (900ml) consisted of pH 7.4 Phosphate buffer maintained at 37 ± 0.2°C with a paddle rotation speed at 100 rpm. 10 ml of dissolution
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Medium was withdrawn at every 10 min interval for 1 h and replaced by equal amount of fresh medium and then filtered through a membrane filter (0.45µm) maintained at 37 ± 0.2°C. The amount of dissolved drug was determined using UV spectrophotometric method (UV 1800 A Shimadzu, Japan) at 260 nm.

4.7.7.3 Piroxicam:
The dissolution of piroxicam pure commercial sample and prepared Piroxicam crystals was determined by using USP dissolution apparatus XXIV-Type II (Electro Lab, Mumbai). Dissolution medium (900ml) consisted of pH 7.4 Phosphate buffer maintained at 37 ± 0.2°C with a paddle rotation speed at 100 rpm. 10 ml of dissolution medium was withdrawn at every 10 min interval for 1 h and replaced by equal amount of fresh medium and then filtered through a membrane filter (0.45µm) maintained at 37 ± 0.2°C. The amount of dissolved drug was determined using UV spectrophotometric method (UV 1800 A Shimadzu, Japan) at 334 nm.

4.7.8 Model fittings of dissolution of different prepared crystals samples:
The dissolution data obtained were fitted into various mathematical models (PCP-Disso-v2.08). The parameters, n the time exponent, k the release rate constant and R the regression coefficient were determined to know the release mechanism. The various models studied were

- First order
- Zero order
- Matrix model
- Hixson- Crowell model
- Peppas model
4.7.9 Determination the physical stability:

Different crystals prepared were subjected to stability testing. Prepared crystals were filled in glass vials, closed with gray butyl rubber closures and sealed with an aluminium caps. The vials containing optimized crystals were kept in stability chamber (Thermo lab, humidity chamber), maintained at 40 ± 2°C and 75 ± 5 % RH for six month. Samples were withdrawn first 1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd} & 6\textsuperscript{th} month and studied for Description, characterization by FT-IR and XRD, drug content and \textit{in vitro} drug release and compared with freshly prepared crystals.

4.8 Preparation of tablets containing different prepared crystals:

4.8.1 Mefenamic acid:

Mefenamic acid conventional tablets were prepared by direct compression. Commercial sample and prepared crystals were mixed with Avicel and aerosol used as filler (Table 6) for 10 min in a cubic mixer. The mixture was mixed with sodium starch glycolate as disintegrant agent or povidone as dispersing agent for 10 min and lactose as diluents was added to the mixture. Blend was compressed on a 10 mm punch and equipped with strain gauge (10-400 kg). Sufficient compression load between 80-100 kg was applied in order to produce tablets hardness of 5-6 kg using Rimek, mini press-1 tablet machine. The punched tablets were subjected to dissolution study and compared with marketed product (Meflup) of mefenamic acid. Each tablet contained 250 mg of Mefenamic acid.
Table 6 Composition of Mefenamic acid (Commercial sample and prepared crystals) tablets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Tablet (1 &amp; 2) (mg)</th>
<th>F-2 (1 &amp; 2) (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefenamic acid</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>Avicel-101</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Sodium starch glycolate</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Povidone*</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Aerosol</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Lactose</td>
<td>80</td>
<td>80</td>
</tr>
</tbody>
</table>

Total weight of the tablets = 500 mg

1’Sodium starch glycolate, 2-Povidone

4.8.2 Ketoprofen:

Ketoprofen conventional tablets were prepared by direct compression. Commercial sample and prepared crystals were mixed with Avicel and aerosol used as filler (Table 7) for 10 min in a cubic mixer. The mixture was mixed with sodium starch glycolate as disintegrant agent or povidone as dispersing agent for 10 min and lactose as diluents was added to the mixture. Blend was compressed on a 10 mm punch and equipped with strain gauge (10-400 kg). Sufficient compression load between 80-100 kg was applied in order to produce tablets hardness of 5-6 kg using Rimek, mini press-1 tablet machine. The punched tablets were subjected to dissolution study and compared with marketed product (rhofenid (AHPL) of Ketoprofen. Each tablet contained 25 mg of ketoprofen.
Table 7 Composition of Ketoprofen (Commercial sample and prepared crystals)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Tablet (1 &amp; 2) (mg)</th>
<th>Tablet (1 &amp; 2) (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoprofen</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Avicel-101</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Sodium starch glycolate</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Povidone</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Aerosol</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Lactose</td>
<td>90</td>
<td>90</td>
</tr>
</tbody>
</table>

Total weight of the tablets = 250 mg

1'Sodium starch glycolate, 2-Povidone

4.8.3 Piroxicam:

Piroxicam conventional tablets were prepared by direct compression. Commercial sample and prepared crystals were mixed with Avicel and aerosol used as filler (Table 8) for 10 min in a cubic mixer. The mixture was mixed with sodium starch glycolate as disintegrant agent or povidone as dispersing agent for 10 min and lactose as diluents was added to the mixture. Blend was compressed on a 10 mm punch and equipped with strain gauge (10-400 kg). Sufficient compression load between 80-100 kg was applied in order to produce tablets hardness of 5-6 kg using Rimek, mini press-1 tablet machine. The punched tablets were subjected to dissolution study and compared with marketed product (Ugesic (Meyer) of Piroxicam. Each tablet contained 20mg of Piroxicam.
Table 8 Composition of Piroxicam (Commercial sample and prepared crystals) tablets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Tablet (1 &amp; 2) (mg)</th>
<th>Tablet (1 &amp; 2) (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piroxicam</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Avicel-101</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Sodium starch glycolate</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Povidone</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Aerosol</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Lactose</td>
<td>70</td>
<td>70</td>
</tr>
</tbody>
</table>

Total weight of the tablets = 200 mg

1'Sodium starch glycolate, 2-Povidone

4.8.4 Evaluation of tablets:

4.8.4.1 Hardness:

The hardness of tablets was measured using iNWEKA iHT100 (ERWEKA Gmbh) hardness tester.

4.8.4.2 Weight variation:

Twenty tablets were randomly picked up and weighed individually. The average weight was calculated. The individual weights were compared with the average weight. The tablets pass the test if, not more than two tablets fall outside the percentage limits of ± 5%.

\[
\text{% Deviation} = \frac{\text{Diff. between average weight & individual tablet weight}}{\text{average tablet weight}} \times 100
\]

4.8.4.3 Friability test:

Six tablets of each formulation were tested for their friability using EF-2 Friabilator (USP) (Serve well instrument Pvt Ltd.).
4.8.4.4 **Drug content:**

**Mefenamic acid:**
Tablets (20) were powdered, powder equivalent to 5000 mg of Mefenamic acid was mixed with 10 ml of water, and solution was allowed to stand for 10 minutes, with occasional shaking. 75 ml of methanol was added and shaken well, and sufficient amount of methanol was added to produce 100 ml and filtered. 5 ml of filtrate was mixed with equal volume of methanol and pH 7.4 phosphate buffer to produce 100 ml. The drug concentration was determined using UV Spectrophotometer (UV 1601 A Shimadzu, Japan) at 286 nm. The results are mean of three experiments.

**Ketoprofen:**
Tablets (20) were powdered, powder equivalent to 250 mg of Ketoprofen was transferred to 100ml volumetric flask diluted with methanol & phosphate buffer to produce 100 ml & filtered. 5 ml of filtrate was further diluted to produce 100 ml. The drug concentration was determined using UV Spectrophotometer (UV 1601 A Shimadzu, Japan) at 260 nm. The results are mean of three experiments.

**Piroxicam:**
Tablets (20) were powdered, powder equivalent to 200 mg of Piroxicam was transferred to 100ml volumetric flask diluted with methanol & phosphate buffer to produce 100 ml & filtered. 5 ml of filtrate was further diluted to produce 100 ml. The drug concentration was determined using UV Spectrophotometer (UV 1601 A Shimadzu, Japan) at 260 nm. The results are mean of three experiments.
4.8.4.5  Dissolution studies of tablets containing crystals:

4.8.4.5.1  Mefenamic acid:
The dissolution of Mefenamic acid tablets was determined by using USP dissolution apparatus XXIV-Type II (Electro Lab, Mumbai). The dissolution medium was 900 ml of pH7.4 phosphate buffer maintained at 37 ± 0.2°C with a paddle rotation speed at 100 rpm. 10 ml of dissolution medium was withdrawn at every 10 min interval for 1 h and replaced by equal amount of fresh medium and then filtered through a membrane filter (0.45µm) maintained at 37 ± 0.2°C. The amount of dissolved Mefenamic acid was determined using UV Spectrophotometer method (UV 1800 A Shimadzu, Japan) at 286 nm. The results are mean of three experiments.

4.8.4.5.2  Ketoprofen:
The dissolution of Ketoprofen tablets was determined by using USP dissolution apparatus XXIV-Type II (Electro Lab, Mumbai). The dissolution medium was 900 ml of pH7.4 phosphate buffer maintained at 37 ± 0.2°C with a paddle rotation speed at 100 rpm. 10 ml of dissolution medium was withdrawn at every 10 min interval for 1 h and replaced by equal amount of fresh medium and then filtered through a membrane filter (0.45µm) maintained at 37 ± 0.2°C. The amount of dissolved Ketoprofen was determined using UV Spectrophotometer method (UV 1800 A Shimadzu, Japan) at 260 nm. The results are mean of three experiments.

4.8.4.5.3  Piroxicam:
The dissolution of Piroxicam tablets was determined by using USP dissolution apparatus XXIV-Type II (Electro Lab, Mumbai). The dissolution medium was 900 ml of pH7.4 phosphate buffer maintained at 37 ± 0.2°C with a paddle rotation speed at 100 rpm. 10 ml of dissolution medium was withdrawn at every 10 min interval for 1 h
and replaced by equal amount of fresh medium and then filtered through a membrane filter (0.45µm) maintained at 37 ± 0.2°C. The amount of dissolved Piroxicam was determined using UV Spectrophotometer method (UV 1800 A Shimadzu, Japan) at 334 nm. The results are mean of three experiments.
4.8.4.6 Dissolution data analysis:

The dissolution profiles of the prepared formulations were compared using differential \((f_1)\) and similarity factor \((f_2)\).

Differential factor \(f_1\) was calculated by the percentage difference between the two curves at each time point and was a measurement of the relative error between the two curves.

\[
f_1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \times 100
\]

Where, Differential factor, \(n\) is the sampling number, \(R_t\) and \(T_t\) are the percent dissolved of the reference and test products at each time point \(t\). The acceptable value for \(f_1\) is 0-15.

The similarity factor is a logarithmic reciprocal square-root transformation of the sum of squared error and is a measurement of the similarity in the percentage of dissolution between two curves using the following equation;

\[
f_2 = 50 \log \left\{ \left[ 1 + \left( \frac{1}{n} \right) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \right\} \times 100
\]

Where, \(f_2\) similarity factor, \(n\) is the sampling number, \(R_t\) and \(T_t\) are the percent dissolved of the reference and test products at each time point \(t\). Two dissolution profiles are considered similar when the \(f_2\) value is greater than or equal to 50.

If both test and reference products show >85% dissolution in 15 minutes, profiles are considered similar and \(f_2\) calculation is unnecessary. Otherwise, calculate \(f_2\). If \(f_2 > 50\), the profiles are considered similar.