PREPARATION AND CHARACTERIZATION OF POLYMORPHS OF CHLORZOXAZONE

Talluri chandrashekar 1,*, Bairy padma1, Kantham Srinivas2, Kusuma praveen kumar 3

1 Department of Pharmaceutics and Pharmaceutical Chemistry Sree Nagarjuna College of Pharmacy, Kadiipikonda, Near Kazipet, Hanumakonda, Warangal, Andhra Pradesh, India – 506 003.
2 Department of Pharmaceutical Chemistry, SRR College of Pharmaceutical Sciences, Valbhapur, Elkahurthy, Karimangar, Andhra Pradesh, India – 506 476.
3 Department of Pharmaceutical Chemistry, Vaagdevi Institute of Pharmaceutical Sciences, Bollikunta, Warangal, Andhra Pradesh, India -506002.

ABSTRACT

Chlorzoxazone, 5-chlorobenzo[d]oxazol-2(3H)-one is a centrally active muscle relaxant which is insoluble in water. By considering this property we have developed four different polymorphs (Form I, Form II, Form III and Form IV) of Chlorzoxazone in different solvents. However, the stable crystal form of the parent compound may exhibit inadequate solubility or dissolution rate resulting in poor oral absorption, particularly for water-insoluble compounds. In this case, alternative polymorphs of different forms may be investigated. The prepared polymorphs were characterized by using Optical Microscopy, DSC, XRD and IR spectroscopic methods. This study proved that the Form I, Form II shown higher solubility than Form III, Form IV and pure Chlorzoxazone.

Keywords: Chlorzoxazone, polymorphs, optical microscopy, DSC, XRD, IR.

INTRODUCTION

Over 85% of active pharmaceutical ingredients (APIs) have been reported to possess more than one polymorphic form in the solid state. Different polymorphs of an APIs have different physical and chemical properties, leading to changes in its solubility, stability, dissolution and bioavailability and, finally, in changes in the efficacy of drugs (Shan- Yang Lin et al., 2007). Therefore, it is important to evaluate each with regard to polymorphism and select most stable polymorph. The most stable polymorphs should be used in the marketed formulation to prevent the polymorphic transition during manufacturing, delivery or storage. The polymorphs selected during the drug development process should be thermodynamically stable and remain stable during the manufacturing process (Karpinski et al., 2006).

Chlorzoxazone, chemically 5-chlorobenzo[d]oxazol-2(3H)-one and is a centrally active muscle relaxant for the treatment of painful muscles spasm associated with musculoskeletal disorders, such as fibrositis, bursitis, myositis, spondylitis, sprains, and muscle strains (Harry G.Brittain et al., 1986). Basically Chlorzoxazone is...
insoluble in water. In this study we summarize the results of our studies performed on the different polymorphs of the Chlorzoxazone based on the solubility and dissolution profile of the different forms obtained from different solvents. This study presents the spectral characterization of each of these different forms, by using, Optical Microscopy, FT-IR, DSC and X-ray data.

Materials and Methods
Preparation of crystal forms from different solvents:
Preparation of Chlorzoxazone Form I from chloroform
The drug (0.5 g) was dissolved in chloroform (50 ml) to check its solubility. To this solution, another weighed amount of Chlorzoxazone (2.5 g) was added and refluxed with chloroform (250 ml), for minutes. The solution was filtered through whatmann filter paper and the filtrate was kept at room temperature to afford well-defined crystals of Chlorzoxazone. The crystal Form I, obtained were collected by filtration, dried under vacuum for 24 hours and stored in well-closed container.

Preparation of Chlorzoxazone Form II from ethanol
The drug (0.5 g) was dissolved in ethanol (50 ml) at its boiling point to check its solubility. To this solution, another weighed amount (2.5 g) of Chlorzoxazone was added and refluxed with ethanol (200 ml) for 90 minutes. The solution was filtered through whatmann filter paper and concentrated by recovery of the solvent to one third of its original volume and kept at room temperature to afford well-defined crystals of Chlorzoxazone. The crystal Form II, obtained were collected by filtration, dried under vacuum for 24 hours and stored in well container.

Preparation of Chlorzoxazone Form III from acetone
The drug (0.5 g) was dissolved in acetone (50 ml) at its boiling point to check its solubility. To this solution, another weighed amount (2.5 g) of Chlorzoxazone was added and refluxed with acetone (220 ml) for 2.5 hours. The solution was filtered through whatmann filter paper and the filtrate was kept at room temperature to afford well-defined crystals of Chlorzoxazone. The crystal Form III, obtained were collected by filtration, dried under vacuum at room temperature for 48 hours and stored in well-closed container.

Preparation of Chlorzoxazone Form IV from ethyl acetate
The drug (0.5 g) was dissolved in ethyl acetate (45 ml) at its boiling point to check its solubility. To this solution, another weighed amount (2.5 g) of Chlorzoxazone was added and refluxed with ethyl acetate (250 ml) for 2.5 hours. The solution was filtered through whatmann filter paper and the filtrate was concentrated by recovery of the solvent to one third of its original volume and kept at room temperature to afford well defined crystal of Chlorzoxazone. The crystal Form IV, obtained were collected by filtration, dried under vacuum at room temperature for 48 hours and stored in well-closed container.

Characterization of crystals:
Optical microscopy
All the crystals so prepared were viewed under optical microscope for their physical characterization. The samples were prepared by placing a small amount of respective crystal powder (previously passed through No.100 sieve) on the slide, dispersed in a drop of mineral oil (liquid paraffin) and covered with cover slip. The slides were visualized by means of binocular polarizing microscope under 10X/0.25 Ph1 & 40X/0.45 Ph 2. When Polarized transmitted light was used to illuminate the sample, the background of the image appeared dark and the sample appeared bright. Samples were observed at a magnification of 100X also. Photomicrographs were taken by using Kodak film roll (Byrn SR et al., 1999).

Differential Scanning Calorimetry (DSC)
The instrument was calibrated using Indium as standard. The sample (2-10mg) was weighed accurately in aluminum pan and sealed hermetically using a crimpers. thermo grams were obtained by heating the encapsulated samples at a constant heating rate of 5 °C/min with chart speed of 5 mm/min under an atmosphere of nitrogen. The exact peak temperatures, melting point and heat of fusion were determined. The temperature range for the scan was 30 °C to 450 °C for all the samples (Brittain H et al., 1999).

IR spectroscopy
The crystal samples (2-2.5 mg) were triturated with dried potassium bromide (100 mg) using agate mortar and pestle. These quantities were usually sufficient to give a disc of 13 mm diameter and a spectrum of suitable intensity. The mixture after grinding into a fine powder was spread uniformly in a suitable die and compressed into a pellet form at a pressure of about 10 kg/cm² for three minutes by using hydraulic press. The resultant pellet was mounted in a suitable holder in the FT-IR spectrophotometer and full range spectra of all crystals were recorded form 4000 cm⁻¹ to 400 cm⁻¹ (Berge SM et al., 1977).

Powder X-ray diffraction spectroscopy
All crystal samples were ground and screened through 100 sieve. The x-ray diffraction pattern was recorded using phillips analytical automatic powder diffractometer at 30 mA, 40 KV. The samples were scanned at 25 °C at diffraction angle of 20 over the range of 5° to 40° (Gould PL et al., 1986).
Formulation studies:
Selected polymorphs previously passed through 100 mesh were mixed with sufficient quantity of microcrystalline cellulose, magnesium stearate and talc by geometrical dilution. The powder mixture was compressed in an electrically driven Tablet punching machine (Rimek, Mumbai) using 8 mm punch to obtain tablets. The weight of the tablets was maintained at 200±10 mg. The composition of the tablets was as given below (ingredients per tablet):
- Chlorzoxazone crystals: 50 mg
- Microcrystalline cellulose: 140 mg
- Magnesium stearate: 5 mg
- Talc: 5 mg

Hardness test
Measurement of hardness was carried out using pfizer hardness tester as per Indian Pharmacopeia by gradual application of increasing force on the tablet held along its circumference at two diametrically opposite points in the jaw of the tester. The value of load at the point of fracture was determined in Kg/cm$^2$. The hardness value of 3 randomly picked tablets was determined and the average hardness value was calculated (Table 1).

Table-1: Hardness, Disintegration time and Dissolution of the tablets of selected crystal forms of Chlorzoxazone

<table>
<thead>
<tr>
<th>Crystal Form</th>
<th>Hardness (Kg/cm$^2$)</th>
<th>Disintegration time (Sec)</th>
<th>Dissolution</th>
<th>Percentage Drug release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Time (min)</td>
<td>Form I</td>
</tr>
<tr>
<td>Form I</td>
<td>4.12</td>
<td>98</td>
<td>10</td>
<td>62.1</td>
</tr>
<tr>
<td>Form II</td>
<td>4.18</td>
<td>118</td>
<td>20</td>
<td>70.2</td>
</tr>
<tr>
<td>Form III</td>
<td>4.40</td>
<td>129</td>
<td>30</td>
<td>78.3</td>
</tr>
<tr>
<td>Form IV</td>
<td>4.63</td>
<td>145</td>
<td>40</td>
<td>86.4</td>
</tr>
<tr>
<td>Pure</td>
<td>4.82</td>
<td>170</td>
<td>50</td>
<td>91.8</td>
</tr>
</tbody>
</table>

Disintegration test
It was carried out by using thermionic disintegration test apparatus as per Indian Pharmacopeia. To test for disintegration time, one tablet was placed in each tube and disintegration testing was carried out in distilled water at 37 ± 2°C. The time when all the 6 tablets disintegrated and all the particles passed through 10 mesh (wire mesh) was recorded as the ‘Disintegration Time’ of the tablets (Table 1).

Dissolution studies
The in vitro dissolution of tablets made for certain of the crystal forms was performed using USP dissolution apparatus. 0.1 N HCl (900 ml) was used as the dissolution medium and paddles rotating at 60 r.p.m as stirring devices. The dissolution was carried out for 60 min. Samples (5 ml) were withdrawn at 10, 20, 30, 40, 50 and 60 minutes intervals. The same volume of medium was replaced immediately to maintain the sink condition. The absorbances of resulting solutions (after filtering through whatman filter paper) were taken at 284 nm after suitable dilutions of the solutions using the same medium (Table 1).
**Figure 1**: Optical Microscopy images of different crystal forms of Chlorzoxazone

<table>
<thead>
<tr>
<th>Form I</th>
<th>Form II</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Form III</td>
<td>Form IV</td>
</tr>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Figure 2**: DSC spectra of different crystal forms of Chlorzoxazone

<table>
<thead>
<tr>
<th>Form I</th>
<th>Form II</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>Form III</td>
<td>Form IV</td>
</tr>
<tr>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Figure 3: XRD spectra of different crystal forms of Chlorzoxazone

<table>
<thead>
<tr>
<th>Form I</th>
<th>Form II</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="XRD Spectrum Form I" /></td>
<td><img src="image2.png" alt="XRD Spectrum Form II" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Form III</th>
<th>Form IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3.png" alt="XRD Spectrum Form III" /></td>
<td><img src="image4.png" alt="XRD Spectrum Form IV" /></td>
</tr>
</tbody>
</table>

Figure 4: IR spectra of different crystal forms of Chlorzoxazone

<table>
<thead>
<tr>
<th>Form I</th>
<th>Form II</th>
<th>Form III</th>
<th>Form IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image5.png" alt="IR Spectrum Form I" /></td>
<td><img src="image6.png" alt="IR Spectrum Form II" /></td>
<td><img src="image7.png" alt="IR Spectrum Form III" /></td>
<td><img src="image8.png" alt="IR Spectrum Form IV" /></td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION
The title compounds were characterized by their physical, analytical and spectral data and the title compounds were obtained in good yields and purity. Chlorzoxazone was obtained as a free base from the supplier and it was used as such for the preparation of different crystal forms. The free base showed appreciable solubility only in chloroform, ethanol, acetone, and ethylacetate to be used as solvents for crystallization.

Optical Microscopy images (Figure 1) shown that the prepared crystals of Form I and Form II were same in their shapes and sizes, whereas Form III and Form IV are different in their shapes and sizes. Melting points of the crystals were obtained same except for Form III and Form IV. From DSC spectra, (Figure 2) it was observed that there was a slight change in the melting point between Form I and Form II in onset of peak (179.48 °C and 182.72 °C, respectively) and heat of fusion (98.42 °C and 102.16 °C, respectively). But there was a significant difference in onset of peak (190.09 °C, 121.88 °C and heat of fusion (190.15 °C and 97.08 °C) for Form III and Form IV respectively.

From the above observation it was concluded that Form III and Form IV were significantly existed in different crystal form. But Form I and Form II structures are all most same.

The XRD pattern of Form I (Chloroform) and Form II (Ethanol) was shown that the intensity of scattering angle (2θ of 20 angle) was same and the main scattering peaks of each form are clustered between 20 to 30° on 20 angle. But these solvate morphs could not be differentiated from an inspection of the pattern. This was evidenced that the two crystal structures were similar.

Therefore it was concluded that Form I and Form II were existed in same crystal forms.

The XRD pattern of Form III (Acetone) and Form IV (Ethylacetate) was shown the existence of two different structure of crystal. This can be easily differentiated from an inspection of the pattern. The main scattering peaks of Form III was between 25-32° on 20 angle and for Form IV is between 27-34° on 20 angle. The most intense peak of Form III was at 140° on 20 angle but Form IV appearance of two most intense peak was noted between 13 and 140° on 20 angle.

Therefore it was concluded that Form III and Form IV were existed in different crystal forms was shown in Figure 3.

IR spectra of the pure drug (Chlorzoxazone) and prepared crystal (Form I Form II, Form III and Form IV) were recorded using potassium bromide disc method. The observations confirmed that the drug (Chlorzoxazone) present at all prepared crystal forms and also this study confirms, both the drug and solvents are compatible and are not interacted each other. Comparison of IR spectra of all four forms is shown in Figure 4.

The dissolution study of the crystals afforded very interesting results (Table 1). Form I and Form II offered fastest dissolution. Form III dissolved a bit slowly in comparison to Form I and Form II. The Form IV gave constant rate of dissolution at the expiry of 80min. and it was slow comparatively in their rate of dissolution compared to Form I and Form II.

Acknowledgments
The authors are thankful to the Director and Principal, Sree Nagarjuna College of Pharmacy, Kadipikonda, JNTU for providing laboratory facilities and financial support.

REFERENCES
Gould PL. Salt selection for basic drugs. Int J Pharm. 1986; 33; 201-17