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Monitoring Temperature Effect on the Polymorphic Transformation of Acitretin Using FBRM—Lasentec

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Abstract:

Polymorphs have a tendency to undergo solution-mediated transformation from one form to another during crystallization. Various in situ techniques explored for understanding the crystallization process include, Raman spectroscopy, near-infrared spectroscopy (NIR), and focused beam reflectance measurement (FBRM). Both Raman and NIR techniques are used for polymorphic identification. FBRM has gained much popularity as an ideal technique for studying and improving particle size. The crystallization process can be improved using FBRM by studying the meta-stable zone, temperature of crystallization, and primary and secondary nucleation. This FBRM technique offers a quick and time-saving approach along with insight on the particle size of the product. The same technique can be used during scale up to understand and monitor crystallization processes. This report describes a different application of FBRM, which involves monitoring the changes in chord length distribution as a result of polymorphic transformation associated with temperature during crystallization. Needle-shaped Form III of acitretin undergoes solvent-mediated transformation to cube-shaped Form II. This polymorphic transformation is monitored by monitoring the chord length distribution.

1. Introduction

Most of drug substances exist in the solid state. It has long been known that pharmaceutical solids can exist in more than one solid form, either crystalline or amorphous; i.e. they exhibit polymorphism. Each polymorphic form can exhibit different and in some cases unique physical and chemical properties including color, morphology, stability, dissolution and bioavailability, stability and other performance characteristics of drug. Change in physical properties like rate of dissolution, particle size, crystal morphology, solubility, density and hardness can cause potentially numerous problems.

The impact of polymorphism in pharmaceutical industry was first highlighted by appearance of second polymorphic form having different dissolution profile for ritonavir, which is used in the treatment of HIV.[1,2] The role of drug polymorphism with respect to the development of drug product design has been reviewed by Singhal et al.[3]

For separation and purification of an active pharmaceutical ingredients (API) crystallization is the most common and well-established technique. Development of a crystallization process for an API can be problematic. The difficulties involved in the crystallization process are failure to nucleate,[4] unwanted polymorph or contamination with unwanted polymorph,[2] polymorph with undesired properties,[5] hydrate,[6] oiling,[7] and poor purity.[8]

The various factors which influence the nucleation and crystal growth are the composition of the crystallization medium (single or binary mixture), conditions used to generate supersaturation, etc.[9−12] The most common and notable variables are the heating and cooling rates, type of anti-solvent and rate, temperature, humidity, and time of anti-solvent addition apart from the impurity level and moisture content.[13]

During the production of an API in the final step, certain unit operations such as heating, milling, and exposure to solvent may provide favorable conditions for a change in the polymorphic form or contamination by the unwanted form. Morris et al. introduced a theoretical approach for the physical transformation of an API during manufacturing process.[14]

There are two proposed mechanisms for polymorphic transformations, i.e. solid-state transformation and solution-mediated transformation.[15] Solid-state transformation occurs without passing through the liquid or the vapor phase and is more influenced by environmental factors.[16] Solvent-mediated transformation occurs in the presence of solvent and is driven by the differences in the solubilities of the two forms. Sometimes different polymorphic forms can be obtained by using the same solvent or solvent mixtures. Polymorphic transformations are influenced by environmental factors such as temperature, pressure, relative humidity, contamination from another polymorph, shear friction, crystalline defects, stirring, solvent composition, etc.

Considering the impact of polymorphism on drug performance, there is a need to monitor and understand the crystal-

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(1) Bauer, J.; Spanton, S.; Henry, R.; Quick, J.; Dziki, W.; Porter, W.; Morris, S. J. *J. Pharm. Res.* **2001**, *18*, 859.
(2) Chemburkar, S. J.; Bauer, J.; Deming, K.; Spiwek, H.; Patel, K.; Morris, S. J.; Henry, R.; Spanton, S.; Dziki, W. P.; Quick, J.; Bauer, P.; Donaubauer, J.; Narayann, B. A.; Soldani, M.; Riley, D.; Mcarland, K. *Org. Process Res. Dev.* **2000**, *4*, 413.
(3) Singhal, D.; Curatolo, W. *Adv. Drug Delivery Rev.* **2004**, *56*, 335.
(4) Parsons, A. R.; Black, S. N.; Colling, R. *Trans IchemE A* **2003**, *81* (6), 700.
(5) Wood, W. *Powder Technol.* **2001**, *121* (1), 53.
(6) Zhu, H.; Grant, D. J. W. *Int. J. Pharm.* **1996**, *135*, 151.
(7) Lafferrere, L.; Hoff, C.; Vessler, S. *Cryst. Growth Des.* **2004**, *4* (6), 1175.
(8) Black, S. N.; Cuthbert, M. W.; Roberts, R. J.; Stensland, B. *Cryst. Growth Des.* **2004**, *4* (3), 539.
(9) Threlfall, T. *Org. Process Res. Dev.* **2000**, *4*, 384.
(10) Bernstein, J. *J. Phys. D.: Appl. Phys.* **1993**, *26*, 66.
(11) Lahav, M.; Leiserowitz, L. *Chem. Eng. Sci.* **2001**, *56*, 2245.
(12) Khoshkoo, S.; Anwar, J. *J. Phys. D.: Appl. Phys.* **1993**, *26*, 90.
(13) Davey, R.; Garside. J. *Molecules to Crystallizers*; Oxford University Press: New York, 2000.
(14) Morris, K. R.; Griesser, U. J.; Eckhardt, C. J.; Stowell, J. G. *Adv. Drug Delivery Rev.* **2001**, *48*, 91.
(15) Davey, R. J.; Guy, P. D.; Ruddick, A. J. *J. Colloid Interface Sci.* **1985**, *108* (1), 189.
(16) Zhang, G. G. Z.; Law, D.; Schmitt, E. A.; Qui, Y. *Adv. Drug Delivery Rev.* **2004**, *56* (3), 371.

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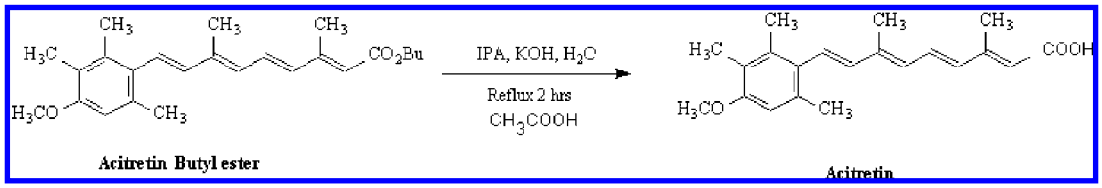
Scheme 1



lization process. The crystallization process superficially appears simple; however, its control can lead to problems in achieving the desired particle size distribution, polymorph, and morphology. These factors will have a direct impact on downstream processes such as filtration and drying. If unwanted polymorphs have resulted, then another unit operation recrystallization is followed which in turn affects the production cost due to yield loss, solvent consumption, and involvement of utility.

The techniques involved for characterization of polymorphs are differential scanning calorimetry, X-ray powder diffraction, Fourier transform infrared spectroscopy or Raman spectroscopy, hot-stage microscopy, and optical microscopy. All these are off-line techniques and are not suitable for real-time monitoring of the crystallization process.

Process analytical technology (PAT) is generating increased interest for monitoring the crystallization process online. The U.S. Food and Drug Administration (FDA) has issued the guidance for Industry, PAT-A Framework for innovative Pharmaceutical Development, Manufacturing and Quality Assurance. Currently, the techniques that are employed as PAT are FT-IR,[17,18] NIR, Raman,[19] FBRM, and PVM.[20] Solid-state transformations are less often observed due to low molecular mobility. β-glycine changes to α-glycine in air but remains unaffected in dry atmosphere.[21]

NIR and FT-IR are employed for monitoring the reaction process. Raman spectroscopy was employed for monitoring polymorphic transformation. FBRM is used for monitoring the particle size during crystallization, and PVM is used for monitoring the shape of the product during crystallization.

FBRM is used for studying the solubility of an API in a solvent and determination of the meta-stable zone and particle size distribution. Applications of FBRM in monitoring polymorphic transformations are known.[22,23]

Solution-mediated transformations are reported in the literature. Solution-mediated transformation depends on the temperature and the concentration of the solvent.[24,25] In the present work, the FBRM is used to establish the temperature of the transformation of acitretin Form III to Form II in alcoholic slurry by monitoring the change in chord length distribution.

2. Experimental Section

Three polymorphic forms of acitretin are reported, viz. Forms I, II, and III.[26] Form II is thermodynamically more stable, and Form I converts to Form II on heating above 200 °C.

Hydrolysis of 9-(2,3,6-trimethyl-phenyl)-3,7-dimethyl-nona-2,4,6,8-tetraen-1-oic acid butyl ester to acitretin using reagents such as sodium bicarbonate, sodium hydroxide, potassium carbonate, and potassium hydroxide in absolute alcohol has been reported in U.S. patent 4,105,681.

Acitretin required for the present study is prepared by hydrolysis of 9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-nona-2,4,6,8-tetraen-1-oic acid butyl ester to acitretin using potassium hydroxide in isopropyl alcohol (Scheme 1). The targeted polymorphic form is Form III.

2.1. Materials and Methods. *2.1.1. Experiments.* In a 250-mL Multimax Reactor fitted with FBRM probe, 10 g of 9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-nona-2,4,6,8-tetraen-1-oic acid butyl ester was suspended in 40 mL of isopropyl alcohol. To this was added aqueous KOH solution (3.5 g KOH in 15 mL of water). The suspension was heated to 80 °C and maintained for 4 h for complete hydrolysis. The solution was allowed to cool to 25−30 °C, and 17 mL of acetic acid was added dropwise until the pH ≈ 2−3. After complete addition of the acid the solution was warmed to 55−60 °C and maintained at the same temperature for 2 h. The separated solid was filtered and dried at 65 °C.

2.1.2. Effect of Temperature. To study the effect of temperature, the experiments were carried out as described above with a change in the acetic acid addition temperature. The addition of acetic acid was carried out at different temperatures in the range of 30−70 °C. The neutralization was studied at the following four different temperatures: 35, 45, 55, and 65 °C.

2.1.3. Determination of Solubility of Acitretin. In a 250-mL Multimax Reactor, 1.5 g of acitretin was suspended in 100 mL of isopropyl alcohol. The suspension was heated to 85 °C at a heating rate of 1 °C/min and cooled to 25 °C at a cooling rate of 1 °C/min. FBRM Lasentec probe was inserted into the reactor to determine the temperature of crystallization. After each dissolution 20 mL of isopropyl alcohol was added, and the same sequence was repeated.

2.1.4. FBRM. Focused beam reflectance measurement was performed with FBRM-Lasentec PI-14/206 manufactured by Mettler Toledo, U.S.A. The instrument is equipped with a low-energy laser light. When the light emitted by the laser hits a crystal, a sensor records and analyzes a backscattered signal. The collected datum, called the chord length, is defined as the distance of the path followed by the beam as it moves across

(17) Liotta, V.; Sabesan, V. *Org. Process Res. Dev.* **2004**, *8*, 488.
(18) Profir, V. M.; Rasmusson, A. C. *Cryst. Growth Des.* **2004**, *4*, 315.
(19) Hu, Y.; Liang, J. K.; Myerson, A. S.; Taylor, L. S. *Ind. Eng. Chem. Res.* **2005**, *44*, 1233.
(20) Chun, Y. J.; Qui, X. Y.; Mei, J. Z.; Jing, K. W.; Zhong, H. S. *Org. Process Res. Dev.* **2008**, *12*, 1223.
(21) Iitaka, Y. *Acta Crystallogr.* **1960**, *13*, 35.
(22) O'Sullivan, B.; Glennon, B. *Org. Process Res. Dev.* **2005**, *9*, 884.
(23) Zai, Q. Y.; Pui, S. C.; Reginald, B. H. T. *Org. Process Res. Dev.* **2008**, *12*, 646.
(24) Leping, D.; Huaiyu, Y.; Simon, B.; Hongyuan, W. *Org. Process Res. Dev.* **2009**, *13*, 1301.
(25) Kostas, S.; Roger, B.; Yaping, H.; Hoa., L.; Reza, F.; Stephen, W. *Org. Process Res. Dev* **2005**, *9*, 911.
(26) Luciana, M.; Grato, A. M.; Norberto, M.; Angelo, S. *J. Pharm. Sci.* **2005**, *94* (5), 1067.

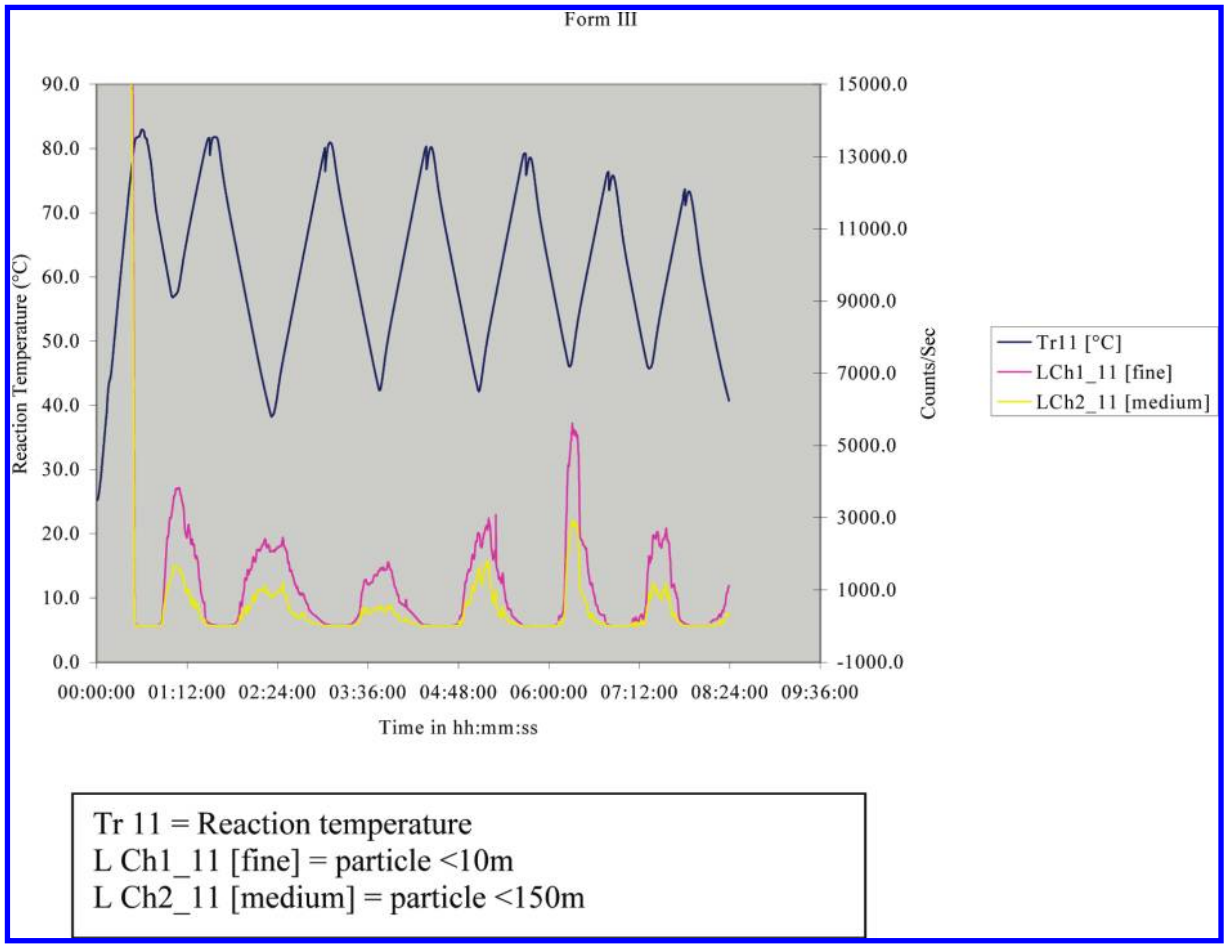


Figure 1. **Solubility determination of acitretin Form III using Multimax and FBRM.**

the crystal from one edge to the other.[27] Hundreds of thousands of chords are typically measured and counted per measurement, producing a robust chord length distribution.

The chord length distribution provides a fingerprint of the particle population.

2.1.5. X-ray Powder Diffration (XRPD). X-ray diffraction patterns of the acitretin samples were obtained by using a Panalytical X'pertPRO diffractometer. The X-ray source used copper Kα (λ = 1.5404 Å) with a tube power of 40 kV and 45 mA. The detection was performed with an accelerator detector. The scan was run from 4 to 50° 2θ, in increasing step size of 0.02° with counting time of 1 s for each step. Samples were passed through a sieve before being analyzed.

2.1.6. Optical Microscope. Microscopic images were captured with a Nikon Eclipse 80i microscope, with CF160 infinity optical system using 10× zoom, equipped with a Nikon digital camera. The software used to record and analyse the images is Q IMAGING.

3. Results and Discussion

Acitretin exists in three polymorphic forms, viz. Form I, Form II, and Form III. Forms III and II are equally stable, and Form I has an enantiotropic relationship with Form II. The higher density of Form III identifies it as the most stable Form at low temperature. Form I undergoes transformation to Form II on heating below 200 °C.[26] In the process of making acitretin, the potassium salt of acitretin crystallizes out on cooling, and it is converted to acitretin in the slurry after addition of acetic acid. When a compound exists in different polymorphic forms, isolating the desired form in lab scale and consequently scaling up to plant scale are very crucial. Solvent-mediated transformation is more likely to occur when different forms differ in their solubilities and stabilities. Hence, the possibility of polymorphic transformation needed to be explored. The study of the degree of supersaturation, the meta-stable zone, using FBRM will help to isolate the desired form.

3.1. Solubility Study. The solubility of acetritin Form III and Form II is studied in isopropyl alcohol using the Multimax Reactor coupled with a Lasentec FBRM S400Q probe. A ramp rate of 1 deg/min was employed for the heating and cooling cycles utilizing the fine range and the medium range for the dissolution point and precipitation point determinations, respectively. Figure 1 and Figure 2 represent FBRM counts indicating dissolution and precipitation of Form II and Form III in solution with heating and cooling, respectively. The data collected from this study are used to plot the solubility curve and meta-stable zone curve of both Forms III and II and is represented in Figure 3. From the curve it can be seen that the solubility of Form II in IPA is less compared to that of Form III, and hence, Form II is the more stable form. The solubility study is in accordance with the DSC results, thus confirming the monotropic relationship between the two forms.

(27) Stephanie, C. B.; Martha, A. G.; Ronald, W. R. *Cryst. Growth Des.* **2008**, *8* (9), 3316.

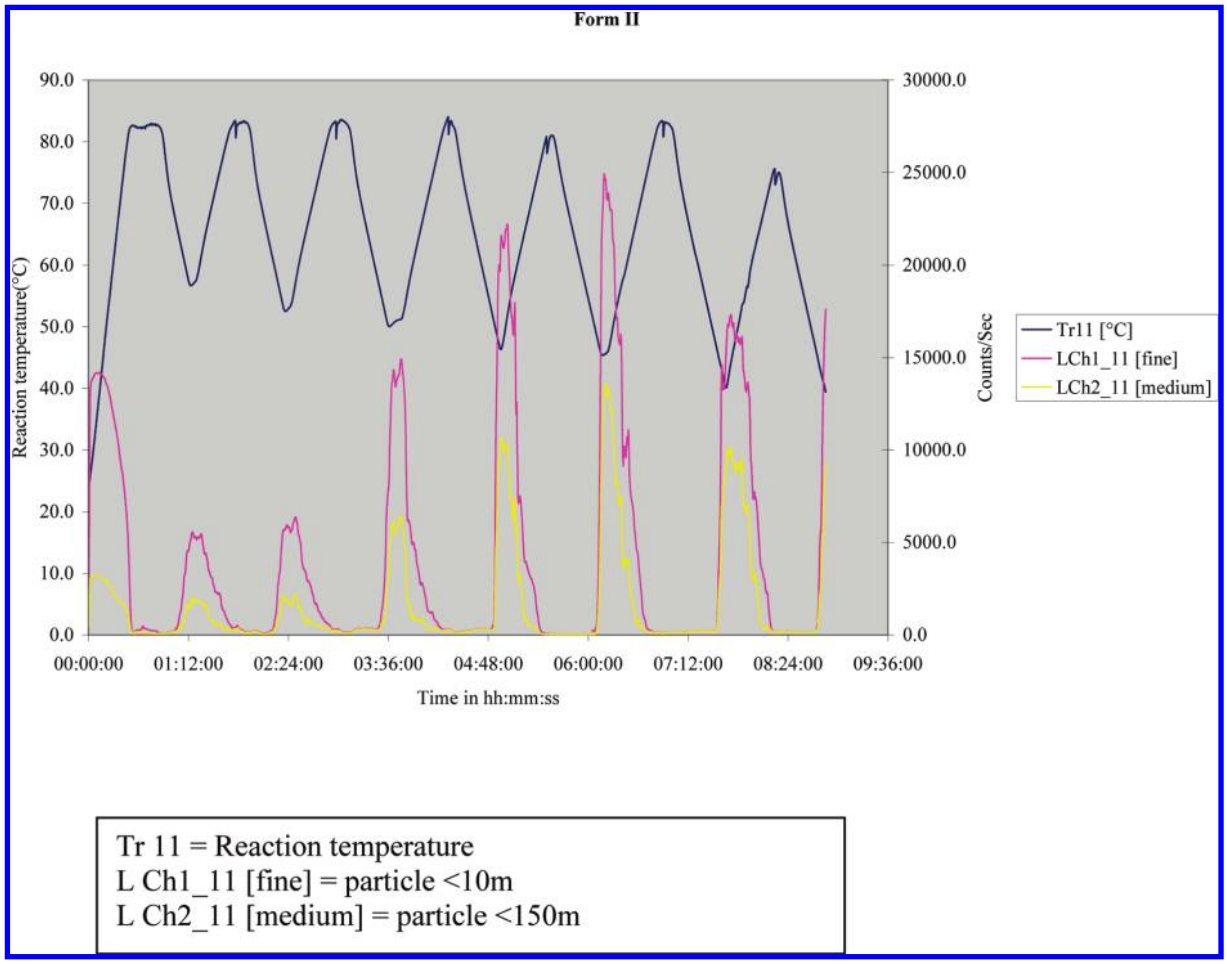


Figure 2. **Solubility determination of acitretin Form II using Multimax and FBRM.**

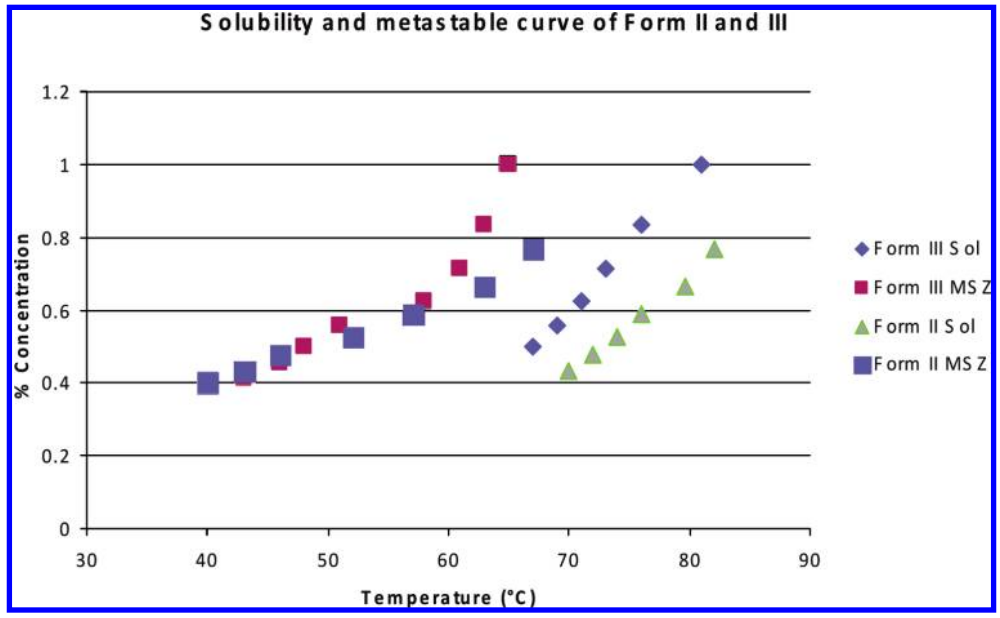


Figure 3. **Solubility and metastable curve of Form II and Form III.**

3.2. Chord Length Distribution (CLD). The unweighted CLD gives an idea about the number of small-sized particles, and weighted CLD gives an idea about the large-sized particles. In the solubility study of acitretin, the CLD showed an interesting change as a function of temperature. The CLDs of the solid crystallized above 50 °C and below 50 °C are different from each other, and this is shown in Figure 4. The solid crystallized below 50 °C shows a large number of small-sized particles in the range of 1−10 μm, and a small number of particles in the range of 30−1000 μm (Figure 4a). The solid crystallized above 50 °C shows small- and large-sized particles in the range of 20−100 μm (Figure 4b). The particles crystallized above 50 °C show more uniform CLD, although it differs in the numbers of particles.

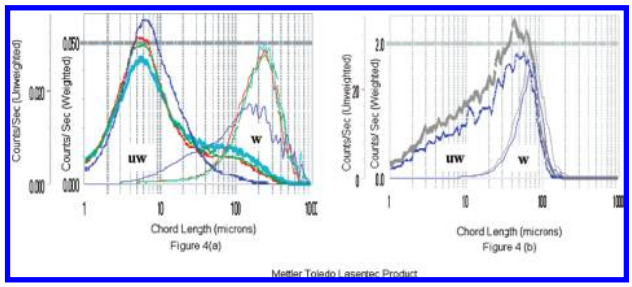


Figure 4. **Temperature effects on CLDs during the solubility curve study, (a) below 50 °C and (b) above 50 °C [unweighted (uw), weighted (w)].**

3.3. Effect of Temperature. While studying the solubility, it is very important to identify the polymorphic forms of the crystallized solid to ensure that the same form is isolated. The XRPD of the solids crystallized above 50 °C were found to match that of Form II, whereas that of the solid crystallized below 50 °C matched that of Form III (Figure 5). Two forms can be easily distinguished by the different CLDs in the unweighted distribution. To support this observation we have carried out neutralization of the potassium salt of acitretin using acetic acid at different temperatures. The potassium salt of acitretin is neutralized at 35, 45, 55, and 65 °C with the use of acetic acid and is maintained at the same temperature for 2 h. At 35 and 45 °C pure Form III was obtained, whereas neutralizing at 55 and 65 °C Form II was obtained.

The CLDs of the acitretin neutralized at different temperatures are given in Figure 6. The CLD of solids neutralized up to 50 °C is similar to the CLDs obtained for the solubility study of Form III and Form II below 50 °C. Thus, the variation in the CLDs is due to the temperature dependency of the form. To confirm this we suspended pure Form III and Form II in IPA at 25 °C and studied the CLDs, and it is observed that the results match those obtained with the experiments (Figure 7).

With FBRM, the CLD obtained will give an idea about the particle size of the crystallized material. From the CLD data, the large numbers of fine particles of Form III produced are in the range of 10 μm, whereas the large number of fine particles of Form II are produced in the range of 20−100 μm. When comparing the particle size with that determined with the Malvern particle size analyzer, the d_{90}'s of Form III and Form II particle sizes are 35 μm and 55 μm, respectively. Thus, there are no significant changes in the Malvern PSD data of the two forms, but FBRM shows significant changes in the CLD patterns of the two forms. To investigate this variation in the CLD with changes in the polymorphic forms, the microscopic images of Form III and Form II were recorded. Form II crystals are cube-shaped, and Form III crystals are needle-shaped (Figure 8). FBRM data have been used to monitor polymorphic changes through the evaluation of the shape of the CLD.[27]

Thus, the variations in the CLDs of Form II and Form III are due to the changes in the shapes. Form III is needle-shaped;

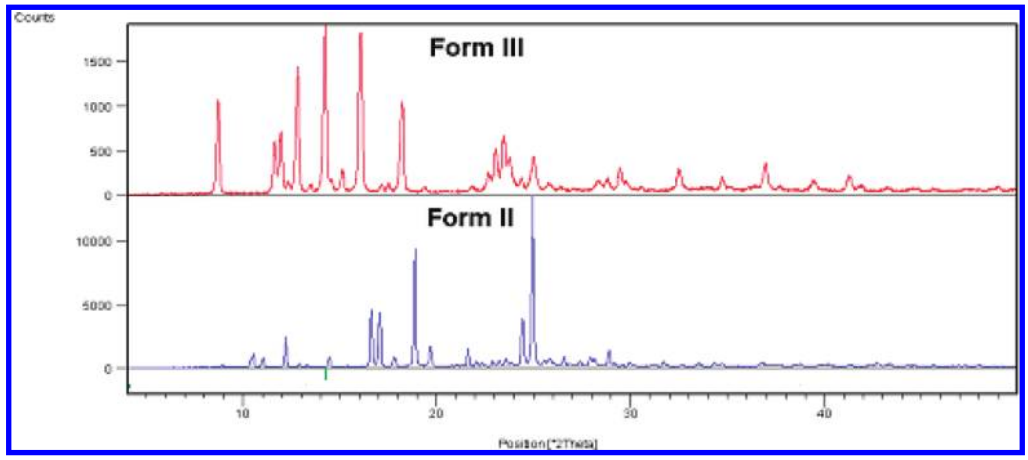


Figure 5. **X-ray powder diffraction of acitretin polymorph.**

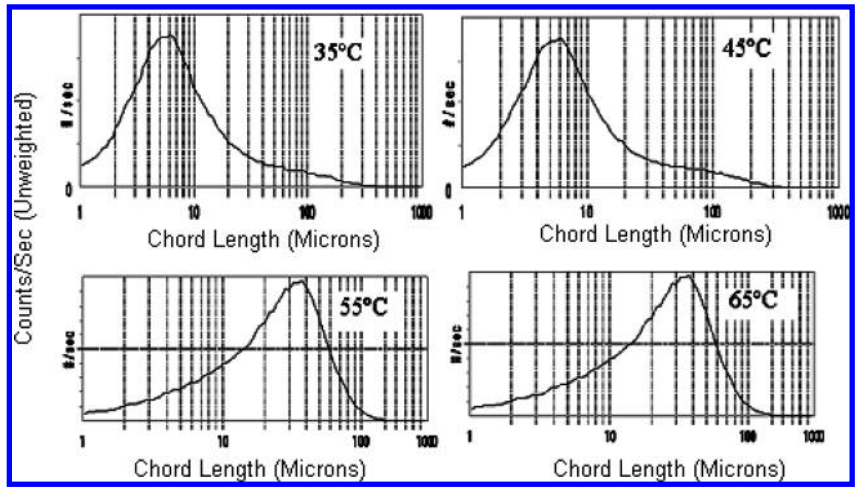


Figure 6. **Effect of temperature on CLD during hydrolysis.**

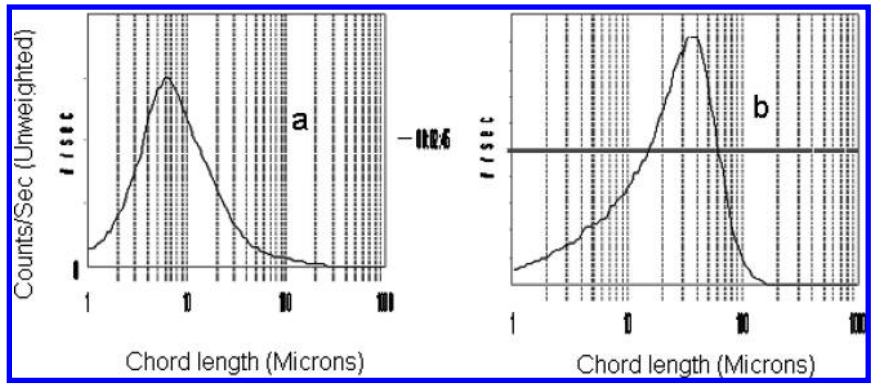


Figure 7. **CLDs of acitretin pure Form III (a) and pure Form II (b).**

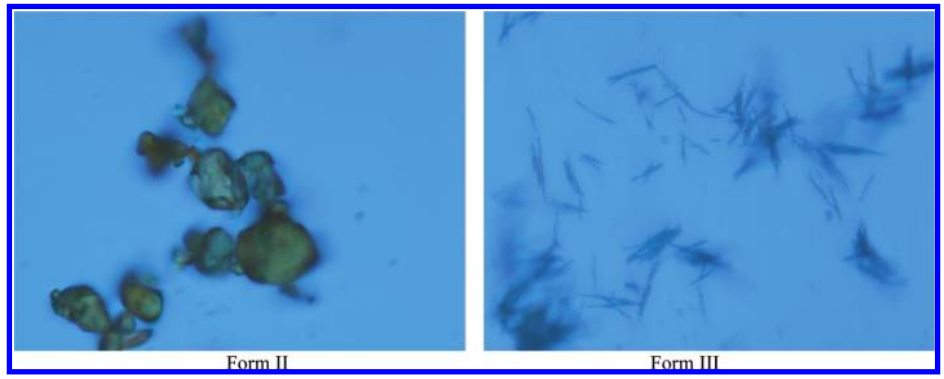


Figure 8. **Microscopic image of acitretin polymorphs, Forms II and III.**

the laser intersects along the edges of the width as well as the length of the needle. Larger numbers of chord lengths are registered with the width compared to those with the length, and hence, the CLD shows more population in the 10 μm and less with 30−1000 μm. Form II has a cubic shape, having similar length and width, and hence, the laser intersects along the edges of the cube and results in uniform CLDs in the range of 20−100 μm.

The study clearly shows that Form III has a tendency to transform to Form II in the presence of solvent. The DSC data suggest that the two forms are equally stable. Single-crystal analysis shows that Form III has *syn* disposition of the two major ring substitutions and Form II has *anti* disposition. Although DSC results showed Form III and Form II are equally stable, Form III is thermodynamically the most stable form at lower temperatures.[26] Hence, Form III undergoes transformation to Form II in solvent with increasing temperature by providing energy for the conversion of *syn* disposition to *anti* diposition. In the case of acitretin, the preference towards Form III or Form II is governed by temperature, and it can be correlated to the CLDs of the two forms. The FBRM is a very useful tool to monitor these preferences.

4. Conclusion

Form III of acitretin undergoes solvent-mediated transformation to Form II. This polymorphic transition is accompanied by changes in the crystal habit, resulting in a dramatic change in the shape of CLD measured with FBRM. Crystals of Form III have a needle shape, and those of Form II have a cube shape. The difference in the CLD is due to the different crystal shapes of the two forms. The temperature at which this polymorphic transformation takes place in acitretin is monitored by monitoring the CLD obtained by the FBRM Lasentec probe. Acitretin hydrolysis of butyl ester below 55 °C results in Form III, and above 55 °C, results in Form II.

FBRM is a well-established tool for monitoring the particle size distribution but can also be employed to reveal the polymorphic preferences which can be associated with the changes in the CLD. FBRM can be used as a PAT tool for monitoring the polymorphic transformation, provided there is a relationship between the crystal habit and the polymorph.

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