

## **Influence of Moisture on the Crystal Forms of Niclosamide Obtained from Acetone and Ethyl Acetate**

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### **ABSTRACT**

The purpose of this study was to elucidate the formation of crystal hydrates of niclosamide and to delineate the effect of relative humidity on the crystal forms obtained from acetone and ethyl acetate. Recrystallization of niclosamide was performed in the presence and absence of moisture. Two hydrates and their corresponding anhydrides were isolated. The hydrates obtained by the process of recrystallization from acetone (Form I) and that obtained from ethyl acetate (Form II) were classified based on differences in their dehydration profile, crystal structure, shape, and morphology. Crystals obtained in the absence of moisture were unstable, and when exposed to the laboratory atmosphere transformed to their corresponding hydrates. Differential scanning calorimetry thermograms indicate that Form I changes to an anhydrate at temperatures below 100°C, while Form II dehydrates in a stepwise manner above 140°C. This finding was further confirmed by thermogravimetric analysis. Dehydration of Form II was accompanied by a loss of structural integrity, demonstrating that water molecules play an important role in maintaining its crystal structure. Form I, Form II, and the anhydrate of Form II showed no significant moisture sorption over the entire range of relative humidity. Although the anhydrate of Form I did not show any moisture uptake at low humidity, it converted to the monohydrate at elevated relative humidity (>95%). All forms could be interconverted depending on the solvent and humidity conditions.

**KEYWORDS:** niclosamide, isomorphic desolvates, polymorphism, hydrate formation, relative humidity, channel-type solvates

### **INTRODUCTION**

The solid-state characteristics of drugs are highly influenced by the existence of different crystal forms.<sup>1-3</sup> The occurrence

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of solvated or hydrated crystal forms, crystals in which the solvent molecules occupy regular positions in the crystal lattice, is widespread among compounds of pharmaceutical interest.<sup>4,5</sup> The behavior of pharmaceutical hydrates in response to changes in environmental conditions can have a significant impact on the development process and dosage-form performance. The obvious situation that favors the formation of hydrates is recrystallization, using water as a solvent. However, recrystallization using organic solvents can generate a metastable crystal form, which, when exposed to moisture, absorbs water and changes into a hydrate. The water molecule, because of its small size and multidirectional hydrogen bonding capability, is particularly suited to fill structural voids.<sup>6</sup> Pfeiffer et al have described such systems wherein the crystalline form is solvated while in equilibrium with a saturated solution and loses most of its solvent upon drying but retains the 3-dimensional structure of the parent solvate. Pfeiffer further refers to the crystals as "desolvated" crystals and emphasizes the need to differentiate them from a truly unsolvated unique structure like the anhydrate.<sup>7</sup> Stephenson et al introduced the term "isomorphic desolvates" as it accurately defines a desolvate that retains the structure of its parent solvate form. They cited several compounds exhibiting such behavior, some of which include cephalixin, cefaclor, erythromycin A, and spirapril hydrochloride.<sup>8</sup>

Niclosamide (5-Chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide), categorized as an anthelmintic, is widely used for the treatment of various worm infestations. The ethanolamine salt is used as a molluscicide. Niclosamide is practically insoluble in water and sparingly soluble in ethanol, chloroform, and ether.<sup>9</sup> Two monohydrates, an anhydrate, and various solvates of niclosamide have been reported in the literature.<sup>10</sup> Single-crystal studies performed on the one monohydrate of niclosamide, indicate that the water molecule is bound to the asymmetric unit of the drug and occupies a cavity bounded by van der Waals envelopes of surrounding drug molecules.<sup>11</sup> A review of the literature indicates that no studies have been published that address the mechanism of formation of these hydrates. Moreover, a detailed study characterizing the effect of moisture on the various pseudopolymorphs of niclosamide has not been reported. A study to better understand the behavior of

niclosamide in the presence of organic solvents as well as its interaction with humid air is thus warranted. This article reports an attempt to better understand the formation of crystal hydrates of niclosamide from anhydrous solvents like acetone and ethyl acetate and to delineate the effect of relative humidity (RH) on the various pseudopolymorphs of niclosamide.

## MATERIALS AND METHODS

### *Materials*

Niclosamide was obtained commercially (ICN Biomedicals Inc, Aurora, OH) and dried under vacuum at 80°C for 24 hours to remove any residual solvent present. Analytical-grade acetone and ethyl acetate were used for recrystallization. The drug was dissolved in the solvents with the aid of heat and left to crystallize at room temperature. Recrystallization was performed in 2 batches for each solvent. In one batch, the drug was dissolved in the solvent and sealed in a vial to avoid any contact with humid air. In the other batch, the vials were left open to the atmosphere and the solvent was allowed to evaporate at controlled room temperature.

### *Thermal Analysis*

Differential scanning calorimetry (DSC) thermograms were obtained using a DSC 2920 Modulated DSC (TA Instruments, New Castle, DE). The temperature axis and cell constant of the DSC cell were calibrated with indium (10 mg, 99.999% pure, melting point 156.60°C, heat of fusion 28.40 J/g). Samples (0.2-0.7 mg) were weighed in aluminum pans on an analytical balance (Sartorius Corp, Edgewood, NY). The escape of solvent was facilitated by placing a pinhole in the lid prior to sealing. The samples were equilibrated at 25°C and heated to 250°C at a heating rate of 5°C/min under continuous nitrogen flow. Thermogravimetric analysis (TGA) of the crystalline samples was performed with a Hi-Res Modulated TGA 2950 (TA Instruments). Samples (2-5 mg) were analyzed in open aluminum pans under a nitrogen flow (50 mL/min) at 25°C to 210°C with a heating rate of 5°C/min. The microbalance was calibrated using calcium carbonate.

### *Moisture Sorption Analysis*

Moisture sorption isotherms were generated at 20°C using an SGA-100 Symmetric Vapor Sorption Analyzer (VTI Corp, Hialeah, FL). The SGA-100 is equipped with an electronic microbalance (CI Electronics, Wiltshire, UK) and a dew point analyzer (Edgetech, Milford, MA) for the accurate measurement of weight and RH, respectively. The instrument was calibrated using sodium chloride and PVP

K30. The procedure involved drying the samples at varying temperatures and increasing the RH in 10% steps from 10% to 95% RH.

### *X-Ray Powder Diffraction*

X-ray powder diffraction (XRPD) data was collected using a Miniflex Tabletop XRD system (Rigaku/MSK, The Woodlands, TX) from 5° to 55° 2 $\theta$  with steps of 0.1°, and the measuring time was 1.0 second/step. All samples were ground to similar size before exposure to radiation. The powder samples were illuminated using CuK $\alpha$  radiation ( $\lambda = 1.54056\text{\AA}$ ) at 30 kV and 15 mA. A nickel filter was used to reduce the K $\beta$  contribution to the x-ray signal, and a scintillation counter was used for detection. Background reduction, K $\alpha$ 2 stripping, and data analysis were performed using the MDI Jade 5.0 software (Materials Data Inc, Liverpool, CA).

### *Optical Microscopy*

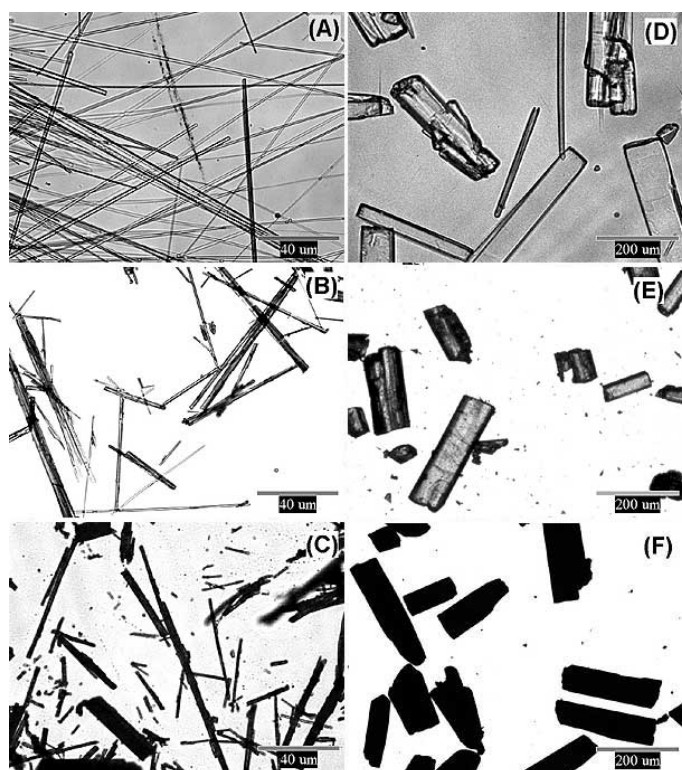
Microscopic observations of the various crystals were made using an Eclipse TE 2000-U (Nikon Instruments, Melville, NY) inverted light microscope. Photomicrographs were obtained using a monochrome digital camera equipped with a CCD image sensor. The data were analyzed using MetaMorph imaging software (Universal Imaging, Downingtown, PA). Optical micrographs of the solvates were obtained in a cavity slide while in equilibrium with the solvent, while other crystals were imaged on a glass slide.

## RESULTS

### *Recrystallization*

It was observed that crystals were readily formed from both solvents, acetone and ethyl acetate, as the saturated solutions were allowed to cool to room temperature. Photomicrographs of the various crystals are shown in Figure 1. It was noted that crystals obtained both from acetone and ethyl acetate were transparent and colorless while submerged in the solvents and became yellow when the solvent was allowed to evaporate to the atmosphere. This color change was not observed when the solvent was allowed to evaporate from a small sample in a desiccator maintained at 0% RH. However, these "desolvated" crystalline forms are very hygroscopic and rapidly develop color (colorless to yellow) when brought in contact with humid air. As seen in Figures 1A and 1B for Form I and in Figures 1D and 1E for Form II, this transformation is effected without any noticeable change in morphology of the crystals. The crystals obtained by the evaporation of the solvents in the presence of moisture were further characterized and determined to be hy-

drates. The crystalline form recovered from acetone, in the presence of moisture, is referred to as Form I and that recovered from ethyl acetate, in the presence of moisture, is referred to as Form II. Both Form I and Form II were dehydrated, and the corresponding anhydrites are referred to as Anhydrate I and Anhydrate II, respectively. Dehydration of the hydrates rendered the anhydrites opaque as seen in Figures 1C and 1F. This phenomenon of increasing opacity accompanying dehydration is well documented in the literature.<sup>12-14</sup> The recrystallization studies clearly demonstrate that both solvents generate a colorless crystal form that is stable while in equilibrium with the solvent and then, when exposed to humid air, quickly converts to a yellow hydrate by losing the solvent from the crystal lattice. This transformation is induced by the incorporation of water molecules into the lattice from the humid air.



**Figure 1.** Photomicrographs of the crystals: (A) acetone solvate in equilibrium with acetone, (B) Form I, (C) Anhydrate I, (D) ethyl acetate solvate in equilibrium with ethyl acetate, (E) Form II, and (F) Anhydrate II.

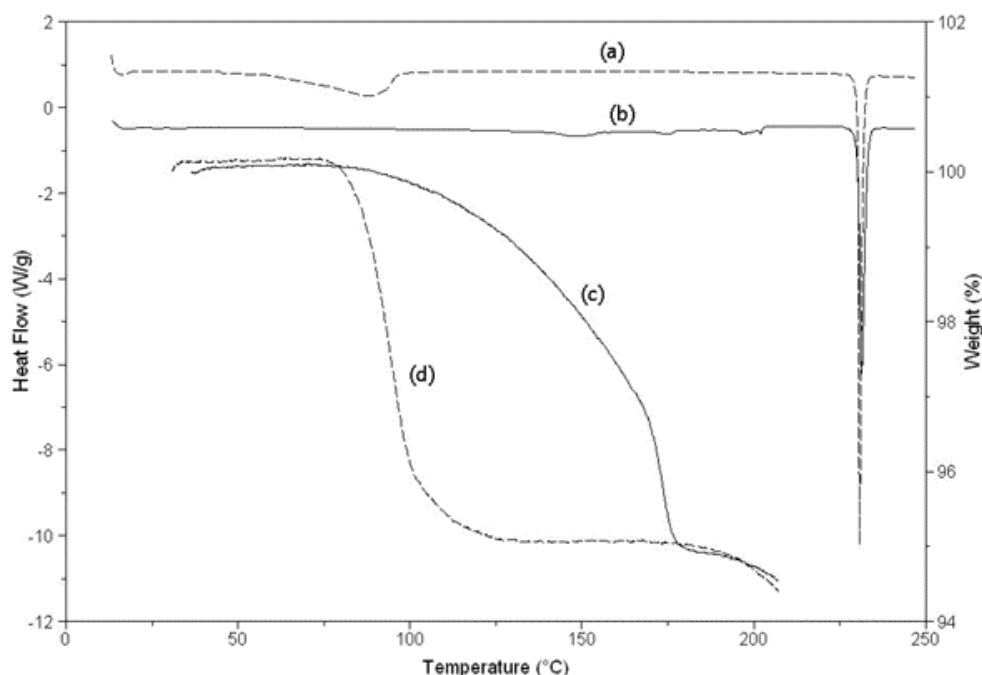
### **Thermal Analysis**

The DSC and TGA thermograms of Form I and Form II are depicted in Figure 2. Detailed characterization of other pseudopolymorphs of niclosamide has been reported.<sup>15</sup> Thus, to avoid redundancy, only the salient features pertinent to the elucidation of the mechanism of hydrate forma-

tion are discussed here. Form I exhibits 2 endothermic transitions. The first transition has an onset temperature of  $65.61^{\circ}\text{C} \pm 3.3^{\circ}\text{C}$  and an enthalpy value of  $0.11 \pm 0.02$  kJ/g ( $n = 3$ ). This transition is associated with loss of water and is confirmed by the single step dehydration seen during TGA. The second endothermic peak is a sharp peak at  $230.71^{\circ}\text{C} \pm 0.07^{\circ}\text{C}$  with an enthalpy of  $0.13 \pm 0.01$  kJ/g ( $n = 3$ ) and is due to melting. Dehydration of Form II occurs gradually over a wide temperature range, and the peak is much broader as compared with the one seen for Form I. The onset of this transition is at  $139.6^{\circ}\text{C} \pm 3^{\circ}\text{C}$  and has an enthalpy value of  $0.14 \pm 0.01$  kJ/g ( $n = 3$ ). Although the enthalpy values for dehydration in both samples are comparable, the transition for Form II occurs over a wide range of temperature; hence more heat is required during this transition as compared with Form I. This continuous dehydration behavior, with the onset at relatively low temperatures, is common for channel-type hydrates and has been explained by Morris based on the concept of sigma cooperativity.<sup>16-18</sup> The higher amount of heat absorbed during dehydration for Form II also indicates that some structural rearrangement should be occurring during dehydration. Of interest, the heat flow seems to increase at  $149.87^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$ ,  $173.35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ , and  $201.96^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$  ( $n = 3$ ). However, the first 2 peaks are broad, while the one at  $201.96^{\circ}\text{C} \pm 0.60^{\circ}\text{C}$  is a sharp spike. The TGA thermogram of Form II indicates that weight loss occurs in a stepwise manner, and complete dehydration occurs below  $190^{\circ}\text{C}$ . It was concluded that the peak at around  $201^{\circ}\text{C}$  is not due to dehydration but due to structural collapse based on the shape and location of the peaks. The final endothermic effect for Form II is seen at  $231.23^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$  with an enthalpy value of  $0.13 \pm 0.00$  kJ/g ( $n = 3$ ) and is attributable to melting. In summary, thermal analysis supports the hypothesis that the 2 hydrates have different strengths of interaction with water molecules as well as a different topology of inclusion.

### **Moisture Sorption Analysis**

Moisture sorption data are invaluable for evaluating not only the equilibrium weight gain, but also the kinetics of hydration/dehydration. The 2 hydrates and their corresponding anhydrites were exposed to various RH conditions between 10% and 95% RH. All forms were also exposed to ~100% RH for a prolonged period of time (~60 days) and were characterized periodically for any change. Form II and Anhydrate II did not show any change over the entire range of RH. Although Form I did not show any change in morphology, thermal profile, or XRPD, a light-brown coloration was observed at the surface of the crystals when exposed to high humidity. However, this change was temporary and the brown coloration disappeared as the crystals were removed from conditions of elevated humidity. This change is attrib-



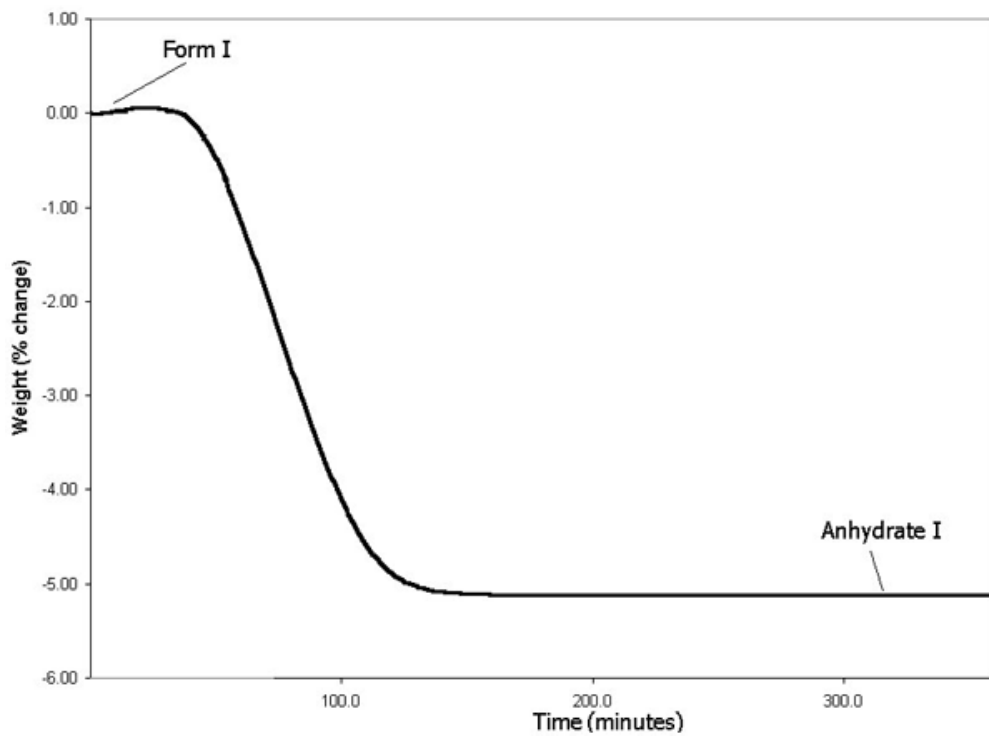
**Figure 2.** DSC thermograms, represented by curves (a) and (b), indicating the changes in heat flow with respect to temperature and TGA thermograms, represented by curves (c) and (d), indicating the percentage change in weight with respect to temperature for the 2 hydrates, Form I (-----) and Form II (—).

uted to the high surface free energy of the crystals, but the exact mechanism cannot be delineated at this time. Anhydrate I did not show any significant moisture sorption at low humidity, but when exposed to 95% RH for 2 weeks, it changed to a structure closely related to Form I. The moisture sorption profile of Anhydrate I is depicted in Figures 3 and 4. Figure 3 (initial 360 minutes of Figure 4) shows the isothermal dehydration of Form I at 60°C. It can be seen that Form I changes to Anhydrate I at 60°C and 0% RH, and the weight loss is stoichiometrically equivalent to the loss of a water molecule (~5.2%). When further exposed to 95% RH, the anhydrate weight gain is ~4.9%. XRPD of this rehydrated form indicates that its structure is closely related to the structure of Form I. This transformation is shown in Figure 4. It is generally accepted that polymorphs are only affected by changes in water vapor pressure if moisture sorption allows molecular motion, which in turn, allows reorganization into a different form via solution-mediated transformation.<sup>16</sup> Thus, it would be justified to claim that some structural differences exist between Anhydrate I and Anhydrate II based on the behavior of the 2 anhydrates at elevated RH.

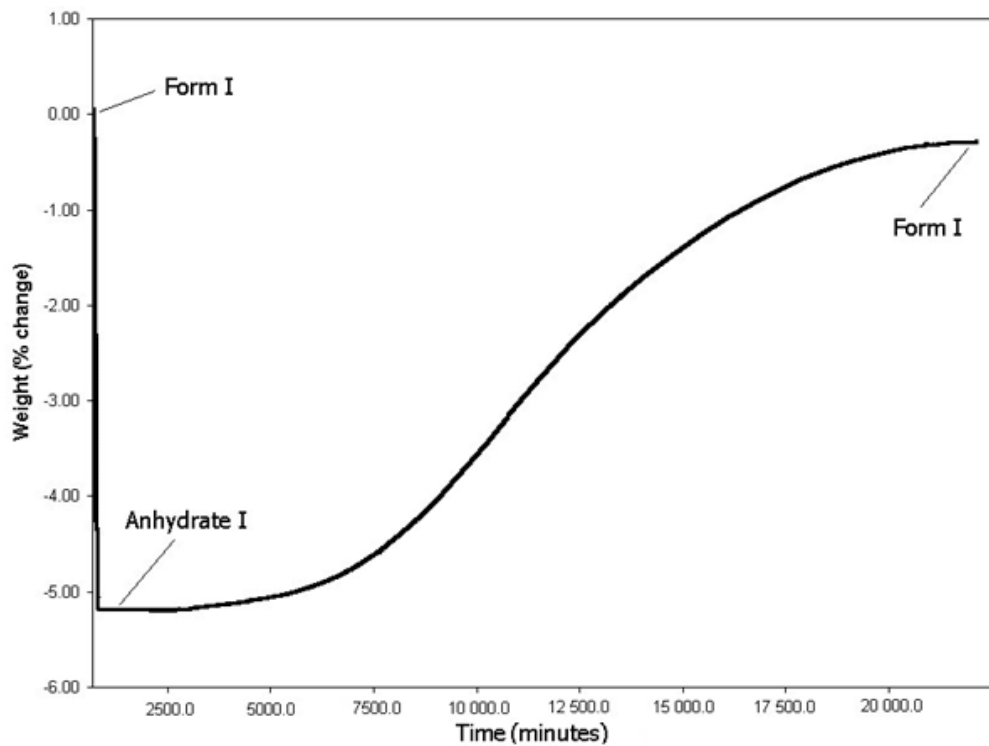
### ***X-Ray Powder Diffraction***

XRPD patterns of the pseudopolymorphs of niclosamide are shown in Figure 5. Attempts to obtain the XRPD pattern of the desolvates were unsuccessful, as the loss of solvent is rapidly accompanied by the incorporation of water molecules. The desolvates are very unstable and convert to hydrates even at very low humidity conditions. Visual inspection of the diffraction pattern clearly indicates significant differences in the structures of Form I and Form II. In a similar way, major differences are noted between the 2 hydrates and their corresponding anhydrates. The  $2\theta$  vs relative intensities of the

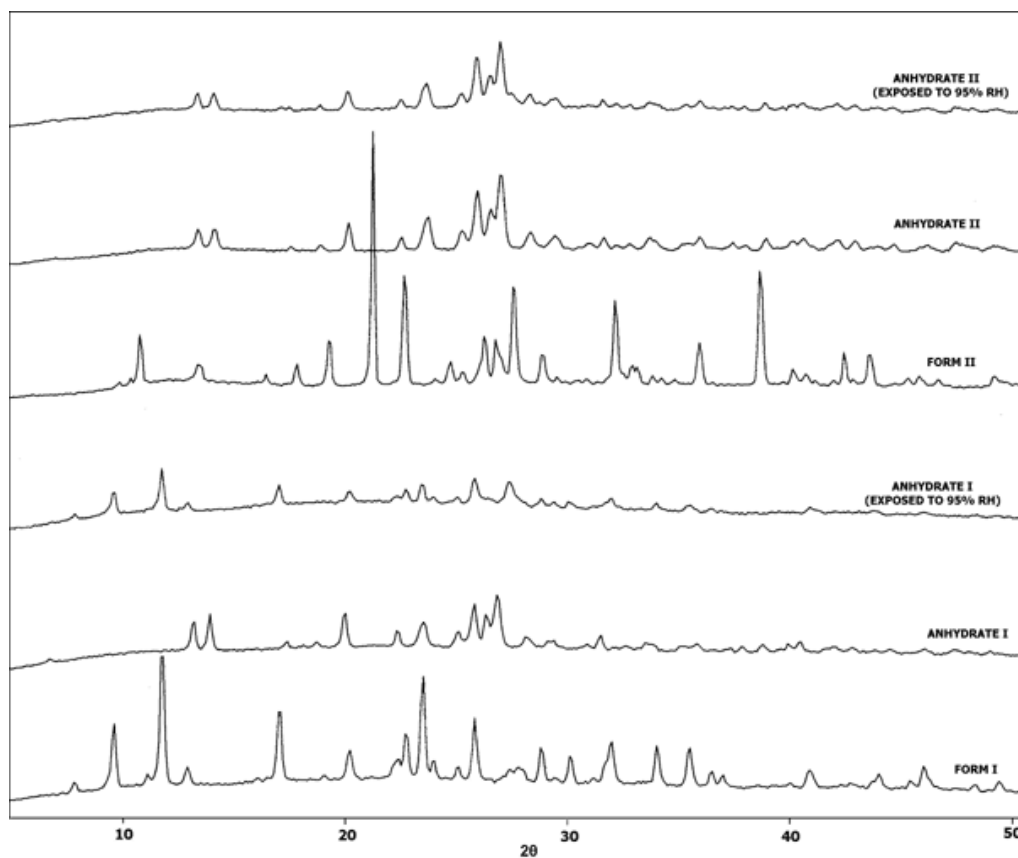
various forms are shown in Table 1. The pseudopolymorphs were compared on the basis of the *United States Pharmacopeia (USP)* general chapter on x-ray diffraction, which states that identity is established if the scattering angles of the 10 strongest reflections agree to within  $\pm 0.20$  degrees and if the relative intensities of these reflections do not vary by more than 20%.<sup>19</sup> It is observed that when Anhydrate I is exposed to elevated RH, the majority of the peaks are similar to the peaks obtained for Form I. This was further corroborated by the thermal behavior of rehydrated Anhydrate I. In the case of Form II, as seen by DSC analysis, dehydration leads to struc-



**Figure 3.** Isothermal dehydration of Form I at 60°C and 0% RH.



**Figure 4.** Moisture sorption profile of Form I initially dried at 0% RH and 60°C followed by exposure to 95% RH.

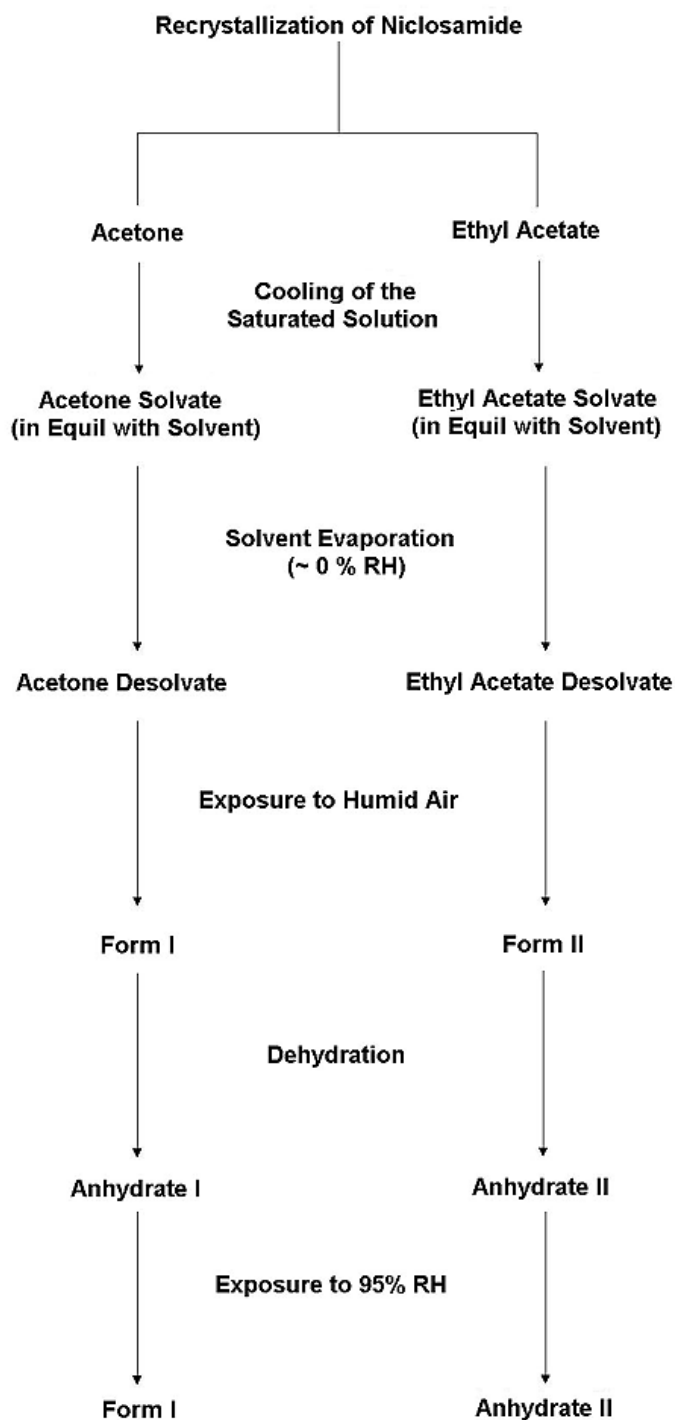


**Figure 5.** X-ray powder diffraction pattern of the pseudopolymorphs of niclosamide.

**Table 1.** X-Ray Powder Diffraction Data of Some of the Pseudopolymorphs of Niclosamide\*

Form I		Anhydrate I		Anhydrate I (Exposed to 95% RH)		Form II		Anhydrate II		Anhydrate II (Exposed to 95% RH)	
2θ	I %	2θ	I %	2θ	I %	2θ	I %	2θ	I %	2θ	I %
9.56	39.9	13.15	66.7	9.54	49.0	10.71	19.2	13.30	24.4	13.26	31.3
11.73	100.0	19.96	79.7	11.69	100.0	13.35	6.3	20.09	38.9	20.06	37.9
17.03	59.1	22.34	35.3	16.99	43.1	17.79	8.0	23.65	42.3	23.57	46.6
20.18	23.0	23.42	57.6	20.18	24.7	19.23	17.7	26.92	100.0	26.92	100.0
22.70	41.6	26.82	100.0	22.69	23.2	21.19	100.0	31.57	12.1	31.55	13.6
23.46	85.9	28.16	21.3	23.43	33.7	22.63	42.8	33.71	14.3	33.70	12.1
25.79	56.7	31.46	31.3	25.79	52.9	24.67	7.4	35.90	16.0	35.90	16.1
27.81	10.6	33.56	15.9	27.38	48.1	28.86	10.2	40.59	13.8	40.55	10.0
30.12	26.1	35.79	15.9	31.95	24.2	32.12	29.7	42.09	10.6	42.08	11.5
31.91	36.9	38.77	14.6	33.98	17.4	35.91	15.1				
34.00	39.3	40.45	22.9	35.47	13.4	38.64	44.5				
35.45	33.3					40.17	3.9				
40.86	15.5					42.44	12.2				
43.97	13.8					43.58	11.4				
46.00	22.9										

\*RH indicates relative humidity.



**Figure 6.** Schematic representation of the formation of some pseudopolymorphs of niclosamide.

tural rearrangement and the diffractogram of Anhydrate II is shown. Exposure of Anhydrate II to elevated RH does not change the crystal structure, indicated by the powder patterns of Anhydrate II before and after exposure to elevated RH, which do not show any changes. Although the powder patterns of Anhydrate I and Anhydrate II show similarities, certain differences are noted in the intensity values below

20° 2θ as shown in Table 1. These data support the hypothesis that the crystal structures of Anhydrate I and Anhydrate II are closely related, but they may not be isostructural. Moisture sorption analysis under similar conditions of RH and temperature, substantiates this conclusion. The exact differences might be explicated by the use of a higher performance diffractometer or the determination of unit cell parameters using single-crystal XRD. Unfortunately, the small size of the Anhydrate I crystals precludes single-crystal studies at this time.

## DISCUSSION

Stephenson has shown that crystalline forms that desolvate yet retain their original crystal lattice belong to a common category of crystalline organic compounds known as isomorphic desolvates.<sup>8</sup> When solvent is lost from the crystal but the lattice retains its 3-dimensional order, the resultant structure is in a high-energy state relative to the original solvate crystal. The desolvated lattice can reduce its internal energy by either resorbing the solvent or water molecules, or by relaxation processes, which increase the packing efficiency of the solid by unit cell volume reduction. In the case of niclosamide, it is hypothesized that 2 such desolvated structures are formed; namely, the desolvated form of acetone and ethyl acetate, in the absence of moisture. These forms seem to maintain the structure of the parent solvate, yet no longer possess the solvent of crystallization. This void, created by the loss of solvent molecules, acts as a driving force for the sorption of water. Thus, when exposed to humid air, these desolvated structures absorb moisture to transform into Form I or Form II, as described above. Photomicrographs shown in Figure 1 demonstrate that the morphologies of the solvated crystals and those of the hydrated crystals are very similar. When the solvent is allowed to evaporate from the solvated crystals, the transformation to the hydrate is very rapid (less than 1 minute), even at room temperature and low values of RH. Comparing the kinetics of this transformation to the transformation of Anhydrate I to Form I (~14 days), it seems very unlikely that there would be any structural rearrangement during the transformation of the desolvate to the hydrate. However, further studies analyzing the crystal structure in equilibrium with the solvent are required to confirm this.

The difference in moisture sorption profiles of the 2 anhydrides could be explained on the basis of the ease of molecular diffusion through the crystal lattice. The theory of crystal packing is well developed, and it is accepted that the lowest energy structure is the one with the highest density and least amount of void spaces in the crystal structure.<sup>20-22</sup> In the case of Form I, dehydration occurs at low temperatures and the unit cell probably does not attain maximum packing density.

This is shown by the rehydration of Anhydrate I at high RH, albeit slowly. In the case of Form II, dehydration occurs at elevated temperature with structural rearrangement to maximize packing efficiency. This leads to a comparatively stable anhydrate, which does not interact with moisture at elevated RH as evidenced by no change in structure over a period of 60 days at ~100% RH. These results also indicate the relative reactivity of the 2 anhydrates to moisture, which has implications for form selection in product development.<sup>23</sup> The importance in differentiating between the desolvate and the anhydrate is also emphasized by the moisture sorption analysis shown in this study. A schematic representation of the behavior of niclosamide, with respect to solvents and RH, is shown in Figure 6. The complexity of form interconversion demonstrated in this work emphasizes the need for well-defined studies that explore mechanistic models.

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