Characterization of the Crystallinity of Drugs: B02669, a Case Study

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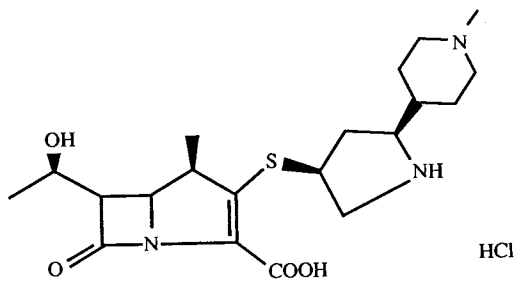
Introduction

Many reports have been written concerning the use of various techniques to assess crystallinity. In some studies, model systems were examined after preparation through varying degrees of mechanical stress (e.g. grinding). Other reports examined several lots of material which by various processing techniques represented different degrees of crystallinity. Techniques utilized to evaluate this property include powder X-ray diffraction[6-15], differential scanning calorimetry (DSC)[12], optical microscopy, infrared spectroscopy[11,12], and solution calorimetry[10,13-15,17]. Properties which were monitored as a function of crystallinity include stability[3-5,18,19], dissolution[15,16], tabletting, and hygroscopicity[16]. These methods vary in the stress (mechanical and thermal) that is applied to the sample, the amount of time and sample that is required, the sensitivity of the technique to small changes, and the necessity of internal or external standards to quantitate crystallinity.

The purpose of the studies described in this report is to use a model compound and compare various techniques to assess extent of crystallinity. In this study, crystalline and amorphous samples were mixed together with minimal mechanical stress to prepare materials of varying percent crystallinity. The model compound employed was BO2669 a beta-lactam antibiotic. It is well documented that as a class, the solid state form of these materials plays an important role in stability. The 2 moles of the HCl salt (shown below) form a crystal hydrate with 5 moles of water.



Like most compounds in this class, the major mode of degradation is by hydrolysis resulting in opening of the beta lactam ring. Moisture content and crystallinity will both contribute to its thermal stability in the solid state.

Materials and Methods

Laboratory samples of crystalline BO2669 were used as received. Crystalline material was lyophilized from water to form what is designated as the 100% amorphous compound. Samples with varying amounts of crystalline and amorphous materials were mixed by weight and ground with a mortar and pestle. The effects of grinding were minimal as determined by solution calorimetry comparing heats of solution of ground and unground materials. These samples were then analyzed by a variety of techniques. Samples were stored in a freezer following preparation to avoid degradation. Isothermal calorimetry studies were conducted following at least one week of freezer storage. Samples where amorphous content was low were prepared individually to avoid content uniformity issues.

Solution calorimetry studies were conducted at 35°C using the Setaram C-80 calorimeter. Heats of solution were determined in 0.02 M Na_2HPO_4 pH 7.0 buffer. Studies which analyzed the solutions following the heat of solution experiment confirmed no sample degradation under these conditions. The accuracy of the calorimetric measurement was checked by the determination of the heat of solution of KCl in water.

Powder X-ray diffraction studies were conducted at room temperature using a Sieman's model D5000 diffractometer. No internal standard was utilized. TGA measurements for moisture content were determined with a Perkin Elmer DSC/TGA model 7 instrument by weight loss over the temperature range of 40 to 100°C. Moisture levels represent equilibrium values at ambient conditions (approximately 40% rh). Isothermal calorimetry stability studies were conducted at 60°C using a Thermometric Bioactivity Monitor. The thermal activity was monitored for at least 24 hours at intervals of 10 minutes.

To assess solid state stability, samples representing a range of percent crystallinity were placed in screw topped vials and inserted into an oven at 60°C. At various times ranging from one to 3 weeks, the samples were assayed by HPLC to monitor stability of the compound. The HPLC method used a Beckman C18 column 4.6 × 250 mm and a mobile phase of 7% acetonitrile and 93% 0.01M Na_2HPO_4 pH 7.0 with UV monitoring at 290nm. Samples were injected in duplicate.

Results

Thermal Stability

The thermal stability of the mixtures at 60°C are shown in Figure 1 which is a graph of the percentage of the intact compound as a function of time for the prepared mixtures. The results expressed as percent intact after one week are shown in Table I. It is important to note that the decompo-

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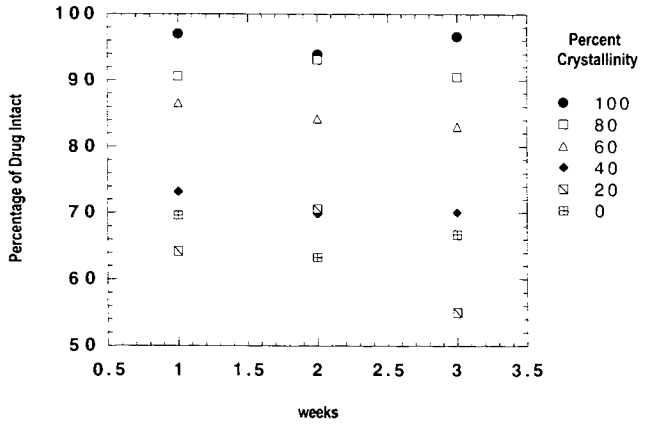

Figure 1 Thermal Stability of BO2669 at 60C Stress. Percent Intact as a Function of Time.

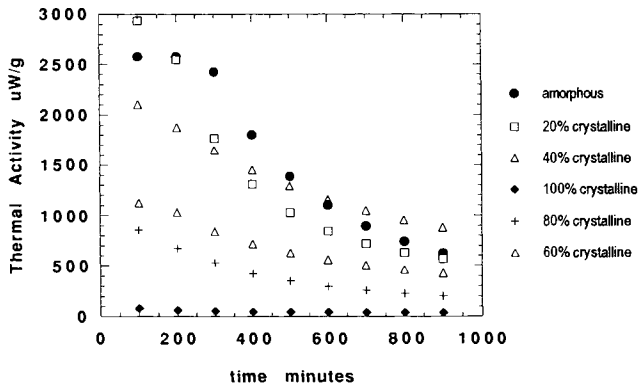

Figure 2 Summary of Isothermal Calorimetry Studies BO2669 at 60C. Thermal Activity as a Function of Time for Each of the Mixtures.

sition rate is not first order for any of these mixtures and the kinetics of decomposition at 60°C were too complex for further study.

An additional method to assess thermal stability of the samples is to monitor thermal activity (Figure 2). The thermograms from the samples all had the same general appearance consisting of two phases, a rapid phase producing a maximum in thermal activity followed by a slower phase as the thermal activity returns to the baseline. After four days in the calorimeter, the samples were significantly degraded. The thermal activity is presented as uW/g after 5 hours in Table I. This time interval was to minimize the effects of insertion of the cells into the calorimeter which influenced data for the first hour and to collect data concerning the initial reaction rate to minimize effects from second order degradation processes.

Physical and Chemical Properties

The powder X-ray diffraction pattern of the various mixtures are shown in Figure 3. The intensity of the strongest peak for each of the amorphous/crystalline mixtures is shown in Table I. There is a decrease in the intensity of this peak as the amorphous nature of the mixture increases.

The moisture content of the mixtures are listed in Table I. Other studies have shown that there is an increase in moisture content as crystallinity decreases. In general, as the percent amorphous increases, the mixture's equilibrium moisture content increases. The change with this material is not as pronounced as reported in other studies.[16,18] The percentage change of moisture content with increasing relative humidity did not show an enhancement of hygroscopicity for the more amorphous samples. Beyond 76% humidity, all the samples liquefied and were discolored. There is no evidence of further crystallization upon exposure to higher humidities.

Discussion

In both of these measures of thermal stability, the greater the percentage of amorphous material in the mixture the more exothermic the thermal activity and the larger the percentage of compound lost after one week at 60°C. The glass transition of the amorphous material occurs at 35°C and thermal stability profiles at 60°C are complicated by this physical change which promotes additional instability. Crystalline material has a glass transition temperature of approximately 62°C but this value is accompanied by sample decomposition at 75°C. Note that thermal activity measurements can be accomplished in a few hours with minimal sample preparation whereas the thermal stability studies by HPLC take at least one week and depend upon the availability of a stability indicating chromatographic method.

Table I. Summary of Physical and Chemical Data BO2669 100 to 0 Percent Crystalline

mixture crystallinity %	ΔHsol[a] j/g	moisture[b] at 50% rh %	x-ray peak counts	thermal[c] activity uW/g	intact drug[d] 60C 1wk %
100	15.3	9.7	1313	52	97
80	11.2	10.6	1203	528	91
60	6.6	12.0	494	840	87
40	−2.5	13.0	317	1650	73
20	−4.6	13.3	218	1766	64
0	−12.4	13.1	61	2423	67

Notation: a. Average error 5% with final concentration of 1 mM. When running the standard KCl in water, the error of this instrument is less than 1%. The homogeniety of the sample mixtures is not known. b. average error is 0.3%, 9% moisture is bound to form the hydrate any additional moisture is unbound. c. Thermal activities listed are obtained after 5 hours with an average of three ten minute time points. Error ± 2%. d. Assay results are from an average of duplicate time points. Error ± 2%.

Heats of solution, listed in Table I, correlate with crystallinity for these samples. As the amorphous content of the solid increased, the heat of solution in aqueous buffer became more exothermic. DSC, another thermal technique, could not be utilized for these samples because the samples decomposed prior to melting.

A linear relationship is found to exist between the properties of heat of solution and thermal activity as a function of degree of crystallinity as illustrated in Figures 4 and 5. In addition, there is a general correlation between thermal stability as determined by HPLC and percent crystallinity. If one compares thermal stability with moisture content (Figure 6), the data suggest that there is an initial linear trend up to 12% water after which, the presence of additional water appears to accelerate the rate of decomposition. It is important to note that these studies were conducted at 60°C where the relative humidity inside the calorimeter cell or in a vial may be greater than ambient due to desorption of moisture from the solid. The sample may therefore be stressed by temperature as well as humidity. This temperature is in excess of the glass transition of the amorphous material and may contribute to this non-linearity. Thermal activity, as determined by isothermal calorimetry, is clearly not first order for all of these systems.

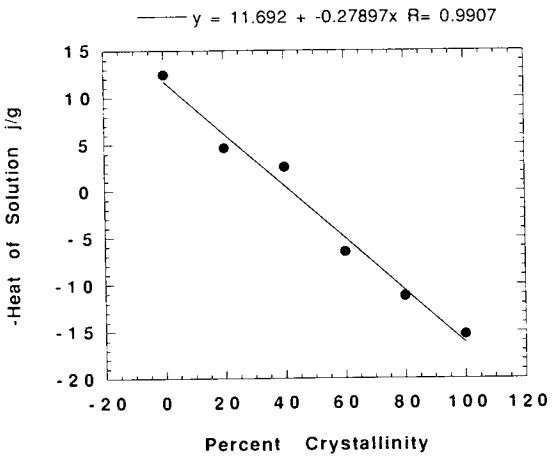

Figure 4 Heat of Solution BO2669 in 0.02M Na_2HPO_4 at 35C as a Function of Percent Crystallinity.

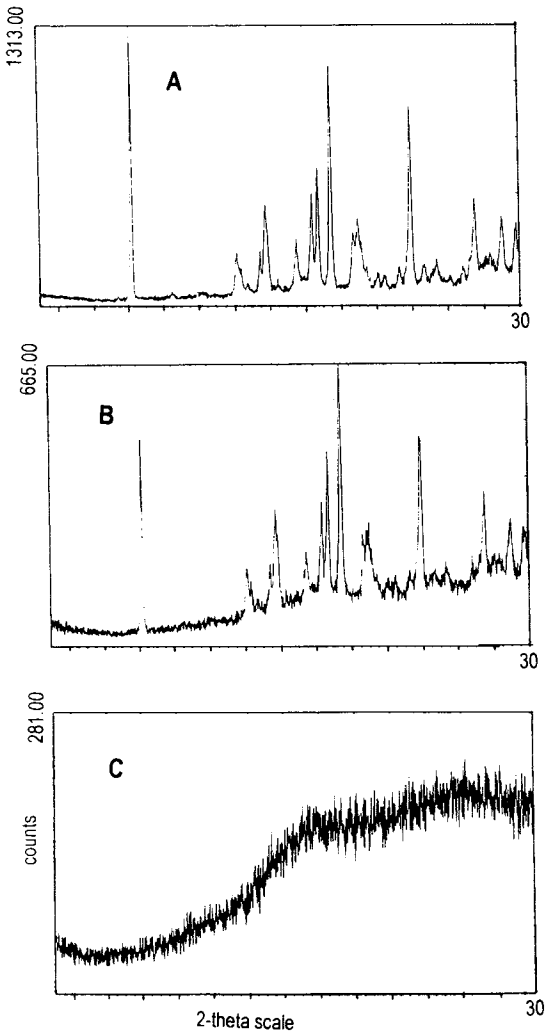

Figure 3 Powder X-ray Diffraction Patterns for the BO2669 Mixtures: A. 100% Crystalline, B. 60% Crystalline, C. 0% Crystalline.

This paper proposes various means to assess the crystallinity of a model compound. The results show that as crystallinity decreases, stability decreases. The heat of solution measurement is a relatively quick means to assess crystallinity and in this model system proves to be helpful as a means to screen various batches to assure batch to batch reproducibility and stability. Isothermal calorimetry could also be used in this system to assess crystallinity due to the greater thermal activity of the more amorphous samples. A 20% decrease in crystallinity causes a 10-fold increase in thermal activity. The work presented here does demonstrate the greater equilibrium moisture content of the more amorphous material. It appears that once the equilibrium moisture content was obtained, relative changes in weight under higher humidity conditions compared with that of the crystalline material.

Further reduction of the amorphous content in primarily crystalline materials, using the mixing and sampling method for preparation of the samples, may be hindered because of the need to grind for longer times and hence increase mechanical stress to assure a homogeneous sample. Content uniformity will certainly impact on the ability to probe

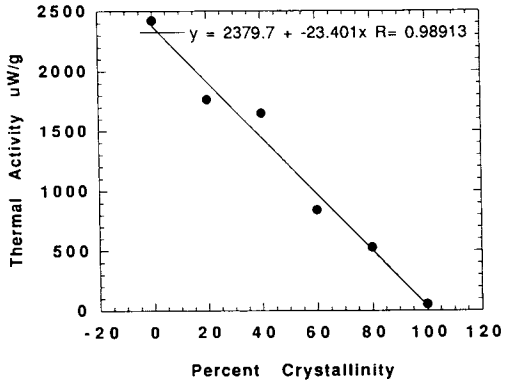

Figure 5 Thermal Activity of Mixtures of BO2669 After 5 hours at 60C as a Function of Percent Crystallinity.

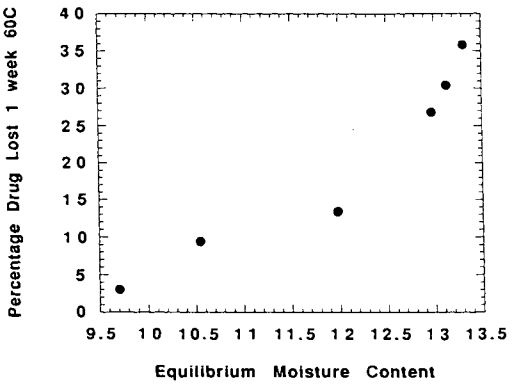

Figure 6 Thermal Stability as Determined by Percentage of Drug Lost After 1 Week at 60C as a Function of Equilibrium Moisture Content at 40% rh.

smaller increments of amorphous in a crystalline sample. An alternative procedure will have to be used.

A beta-lactam antibiotic, BO2669, was used as a model to assess crystallinity by various techniques. Heats of solution, isothermal calorimetry, X-ray powder diffraction and thermal stability all showed correlations with crystallinity of prepared mixtures of amorphous and crystalline materials. The most quantitative correlations with degree of crystallinity were obtained by measurements of thermal activity and heats of solution determined by isothermal calorimetry and solution calorimetry, respectively.

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