POLYMORPHIC FORMS OF 3-(4-AMINO-1-OXO-1,3 DIHYDRO-ISOINDOL-2-YL)-PIPERIDINE-2,6-DIONE

This application claims the benefit of U.S. provisional application 60/499,723, filed September 4, 2003, the contents of which are incorporated by reference herein their entirety.

1. FIELD OF THE INVENTION

This invention relates to polymorphic forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione, compositions comprising the polymorphic forms, methods of making the polymorphic forms and methods of their use for the treatment of diseases and conditions including, but not limited to, inflammatory diseases, autoimmune diseases, and cancer.

2. BACKGROUND OF THE INVENTION

Many compounds can exist in different crystal forms, or polymorphs, which exhibit different physical, chemical, and spectroscopic properties. For example, certain polymorphs of a compound may be more readily soluble in particular solvents, may flow more readily, or may compress more easily than others. *See, e.g.*, P. DiMartino, *et al.*, *J. Thermal Anal.*, 48:447-458 (1997). In the case of drugs, certain solid forms may be more bioavailable than others, while others may be more stable under certain manufacturing, storage, and biological conditions. This is particularly important from a regulatory standpoint, since drugs are approved by agencies such as the U.S. Food and Drug Administration only if they meet exacting purity and characterization standards. Indeed, the regulatory approval of one polymorph of a compound, which exhibits certain solubility and physico-chemical (including spectroscopic) properties, typically does not imply the ready approval of other polymorphs of that same compound.

Polymorphic forms of a compound are known in the pharmaceutical arts to affect, for example, the solubility, stability, flowability, fractability, and compressibility of the compound, as well as the safety and efficacy of drug products comprising it. *See, e.g.*, Knapman, K. *Modern Drug Discoveries*, 2000, 53. Therefore, the discovery of new polymorphs of a drug can provide a variety of advantages.

U.S. Patent Nos. 5,635,517 and 6,281,230, both to Muller *et al.*, disclose 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione, which is useful in treating and preventing a wide range of diseases and conditions including, but not limited to,

inflammatory diseases, autoimmune diseases, and cancer. New polymorphic forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione can further the development of formulations for the treatment of these chronic illnesses, and may yield numerous formulation, manufacturing and therapeutic benefits.

3. SUMMARY OF THE INVENTION

This invention encompasses polymorphs of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. In certain aspects, the invention provides polymorphs of the compound identified herein as forms A, B, C, D, E, F, G, and H. The invention also encompasses mixtures of these forms. In further embodiments, this invention provides methods of making, isolating and characterizing the polymorphs.

This invention also provides pharmaceutical compositions and single unit dosage forms comprising a polymorph of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. The invention further provides methods for the treatment or prevention of a variety of diseases and disorders, which comprise administering to a patient in need of such treatment or prevention a therapeutically effective amount of a polymorph of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Specific aspects of the invention can be understood with reference to the attached figures:

FIGURE 1 provides a representative X-ray powder diffraction (XRPD) pattern of Form A;

FIGURE 2 provides a representative IR spectrum of Form A;

FIGURE 3 provides a representative Raman spectrum of Form A;

FIGURE 4 provides a representative thermogravimetric analysis (TGA) curve and a representative differential scanning calorimeter (DSC) thermogram of Form A;

FIGURE 5 provides a representative moisture sorption/desorption isotherm of Form A;

FIGURE 6 provides a representative XRPD pattern of Form B;

FIGURE 7 provides a representative IR spectrum of Form B;

FIGURE 8 provides a representative Raman spectrum of Form B;

FIGURE 9 provides a representative TGA curve and a representative DSC thermogram of Form B;

FIGURE 10 provides representative TG-IR results of Form B;

FIGURE 11 provides a representative moisture sorption/desorption isotherm of Form B;

FIGURE 12 provides a representative XRPD pattern of Form C;

FIGURE 13 provides a representative IR spectrum of Form C;

FIGURE 14 provides a representative Raman spectrum of Form C;

FIGURE 15 provides a representative TGA curve and a representative DSC thermogram of Form C;

FIGURE 16 provides representative TG-IR results of Form C;

FIGURE 17 provides a representative moisture sorption/desorption isotherm of Form C;

FIGURE 18 provides a representative XRPD pattern of Form D;

FIGURE 19 provides a representative IR spectrum of Form D;

FIGURE 20 provides a representative Raman spectrum of Form D;

FIGURE 21 provides a representative TGA curve and a representative DSC thermogram of Form D;

FIGURE 22 provides a representative moisture sorption/desorption isotherm of Form D;

FIGURE 23 provides a representative XRPD pattern of Form E;

FIGURE 24 provides a representative TGA curve and a representative DSC thermogram of Form E;

FIGURE 25 provides a representative moisture sorption/desorption isotherm of Form E;

FIGURE 26 provides a representative XRPD pattern for a sample of Form F;

FIGURE 27 provides a representative thermogram of Form F;

FIGURE 28 provides a representative XRPD pattern of Form G;

FIGURE 29 provides a representative DSC thermogram for a sample of Form G;

FIGURE 30 provides a representative XRPD pattern of Form H;

FIGURE 31 provides a representative TGA curve and a representative DSC thermogram of Form H;

FIGURE 32 provides a representative XRPD pattern of Form B;

FIGURE 33 provides a representative XRPD pattern of Form B;

FIGURE 34 provides a representative XRPD pattern of Form B;

FIGURE 35 provides a representative XRPD pattern of Form E;

FIGURE 36 provides a representative XRPD pattern of polymorph mixture;

FIGURE 37provides a representative TGA curve of Form B;

FIGURE 38 provides a representative TGA curve of Form B;

FIGURE 39 provides a representative TGA curve of Form B;

FIGURE 40 provides a representative TGA curve of Form E;

FIGURE 41 provides a representative TGA curve of polymorph mixture;

FIGURE 42 provides a representative DSC thermogram of Form B;

FIGURE 43 provides a representative DSC thermogram of Form B;

FIGURE 44 provides a representative DSC thermogram of Form B;

FIGURE 45 provides a representative DSC thermogram of Form E;

FIGURE 46 provides a representative DSC thermogram of polymorph mixture;

FIGURE 47 provides a UV-Vis scan of dissolution medium;

FIGURE 48 provides a UV-Vis scan of 0.04 mg/ml of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in dissolution medium;

FIGURE 49 provides a UV-Vis scan of 0.008 mg/ml of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in dissolution medium;

FIGURE 50 provides a calibration curve for 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione;

FIGURE 51 provides a solubility curve of Form A;

FIGURE 52 provides a solubility curve of Form B;

FIGURE 53 provides an intrinsic dissolution of Forms A, B and E; and

FIGURE 54 provides an intrinsic dissolution of Forms A, B and E.

5. DETAILED DESCRIPTION OF THE INVENTION

5.1 DEFINITIONS

As used herein and unless otherwise indicated, the terms "treat," "treating" and "treatment" refer to the alleviation of a disease or disorder and/or at least one of its attendant symptoms.

As used herein and unless otherwise indicated, the terms "prevent," "preventing" and "prevention" refer to the inhibition of a symptom of a disease or disorder or the disease itself.

As used herein and unless otherwise indicated, the terms "polymorph" and "polymorphic form" refer to solid crystalline forms of a compound or complex. Different polymorphs of the same compound can exhibit different physical, chemical and/or spectroscopic properties. Different physical properties include, but are not limited to stability (*e.g.*, to heat or light), compressibility and density (important in formulation and product manufacturing), and dissolution rates (which can affect bioavailability). Differences in stability can result from changes in chemical reactivity (*e.g.*, differential oxidation, such that a dosage form discolors more rapidly when comprised of one polymorph than when comprised of another polymorph) or mechanical characteristics (*e.g.*, tablets crumble on storage as a kinetically favored polymorph converts to thermodynamically more stable polymorph) or both (*e.g.*, tablets of one polymorph are more susceptible to breakdown at high humidity). Different physical properties of polymorphs can affect their processing. For example, one polymorph might be more likely to form solvates or might be more difficult to filter or wash free of impurities than another due to, for example, the shape or size distribution of particles of it.

Polymorphs of a molecule can be obtained by a number of methods known in the art. Such methods include, but are not limited to, melt recrystallization, melt cooling, solvent recrystallization, desolvation, rapid evaporation, rapid cooling, slow cooling, vapor diffusion and sublimation. Polymorphs can be detected, identified, classified and characterized using well-known techniques such as, but not limited to, differential scanning calorimetry (DSC), thermogravimetry (TGA), X-ray powder diffractometry (XRPD), single crystal X-ray diffractometry, vibrational spectroscopy, solution calorimetry, solid state nuclear magnetic resonance (NMR), infrared (IR) spectroscopy, Raman spectroscopy, hot stage optical microscopy, scanning electron microscopy (SEM), electron crystallography and quantitative analysis, particle size analysis (PSA), surface area analysis, solubility, and rate of dissolution.

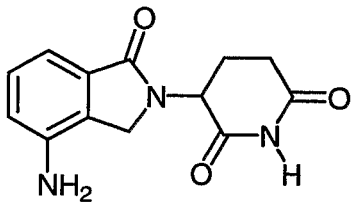
As used herein to refer to the spectra or data presented in graphical form (*e.g.*, XRPD, IR, Raman and NMR spectra), and unless otherwise indicated, the term "peak" refers to a peak or other special feature that one skilled in the art would recognize as not attributable to background noise. The term "significant peaks" refers to peaks at least the median size (*e.g.*, height) of other peaks in the spectrum or data, or at least 1.5, 2, or 2.5 times the median size of other peaks in the spectrum or data.

As used herein and unless otherwise indicated, the term "substantially pure" when used to describe a polymorph of a compound means a solid form of the compound that

comprises that polymorph and is substantially free of other polymorphs of the compound. A representative substantially pure polymorph comprises greater than about 80% by weight of one polymorphic form of the compound and less than about 20% by weight of other polymorphic forms of the compound, more preferably greater than about 90% by weight of one polymorphic form of the compound and less than about 10% by weight of the other polymorphic forms of the compound, even more preferably greater than about 95% by weight of one polymorphic form of the compound and less than about 5% by weight of the other polymorphic forms of the compound, and most preferably greater than about 97% by weight of one polymorphic forms of the compound and less than about 3% by weight of the other polymorphic forms of the compound.

5.2 POLYMORPHIC FORMS

This invention is directed to polymorphic forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione, which has the structure shown below:



This compound can be prepared according to the methods described in U.S. Patent Nos. 6,281,230 and 5,635,517, the entireties of which are incorporated herein by reference. For example, the compound can be prepared through catalytic hydrogenation of 3-(4-nitro-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. 3-(4-Nitro-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione can be obtained by allowing 2,6-dioxopiperidin-3-ammonium chloride to react with methyl 2-bromomethyl-4-nitrobenzoate in dimethylformamide in the presence of triethylamine. The methyl 2-bromomethyl-4-nitrobenzoate in turn is obtained from the corresponding methyl ester of nitro-ortho-toluic acid by conventional bromination with N-bromosuccinimide under the influence of light.

Polymorphs of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione can be obtained by techniques known in the art, including solvent recrystallization, desolvation, vapor diffusion, rapid evaporation, slow evaporation, rapid cooling and slow cooling. Polymorphs can be made by dissolving a weighed quantity of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in various solvents at elevated temperatures. The solutions of the compound can then be filtered and allowed to evaporate either in an open vial

(for fast hot evaporation) or in a vial covered with aluminum foil containing pinholes (hot slow evaporation). Polymorphs can also be obtained from slurries. Polymorphs can be crystallized from solutions or slurries using several methods. For example, a solution created at an elevated temperature (*e.g.*, 60 °C) can be filtered quickly then allowed to cool to room temperature. Once at room temperature, the sample that did not crystallize can be moved to a refrigerator then filtered. Alternatively, the solutions can be crash cooled by dissolving the solid in a solvent at an increased temperature (*e.g.*, 45-65 °C) followed by cooling in a dry ice/solvent bath.

One embodiment of the invention encompasses Form A of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form A is an unsolvated, crystalline material that can be obtained from non-aqueous solvent systems. Another embodiment of the invention encompasses Form B of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form B is a hemihydrated, crystalline material that can be obtained from various solvent systems. Another embodiment of the invention encompasses Form C of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form C is a hemisolvated crystalline material that can be obtained from solvents such as, but not limited to, acetone. Another embodiment of the invention encompasses Form D of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form D is a crystalline, solvated polymorph prepared from a mixture of acetonitrile and water. Another embodiment of the invention encompasses Form E of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form E is a dihydrated, crystalline material. Another embodiment of the invention encompasses Form F of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form F is an unsolvated, crystalline material that can be obtained from the dehydration of Form E. Another embodiment of the invention encompasses Form G of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form G is an unsolvated, crystalline material that can be obtained from slurrying forms B and E in a solvent such as, but not limited to, tetrahydrofuran (THF). Another embodiment of the invention encompasses Form H of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form H is a partially hydrated crystalline material that can be obtained by exposing Form E to 0 % relative humidity. Each of these forms is discussed in detail below.

Another embodiment of the invention encompasses a composition comprising amorphous 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione and crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of form A, B, C, D, E, F,

G or H. Specific compositions can comprise greater than about 50, 75, 90 or 95 weight percent crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione.

Another embodiment of the invention encompasses a composition comprising at least two crystalline forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione (*e.g.*, a mixture of polymorph forms B and E).

5.2.1 FORM A

The data described herein for Form A, as well as for Forms B-H, were obtained using the experimental methods described in Examples 6.3 – 6.7, provided below.

Form A can be obtained from various solvents, including, but not limited to 1-butanol, butyl acetate, ethanol, ethyl acetate, methanol, methyl ethyl ketone, and THF. Figure 1 shows a representative XRPD pattern of Form A. The pattern is characterized by peaks, preferably significant peaks, at approximately 8, 14.5, 16, 17.5, 20.5, 24, and 26 degrees 2θ. Representative IR and Raman spectra data are provided in Figures 2 and 3.

Representative thermal characteristics of Form A are shown in Figure 4. TGA data show a small weight increase up to about 150°C, indicating an unsolvated material. Weight loss above 150°C is attributed to decomposition. The DSC curve of Form A exhibits an endotherm at about 270°C.

Representative moisture sorption and desorption data are plotted in Figure 5. Form A does not exhibit a significant weight gain from 5 to 95% relative humidity. Equilibrium can be obtained at each relative humidity step. As the form dries from 95% back down to 5% relative humidity, it tends to maintain its weight such that at 5% relative humidity it has typically lost only about 0.003% by weight from start to finish. Form A is capable of remaining a crystalline solid for about 11 days when stored at about 22, 45, 58, and 84% relative humidity.

Interconversion studies show that Form A can convert to Form B in aqueous solvent systems and can convert to Form C in acetone solvent systems. Form A tends to be stable in anhydrous solvent systems. In water systems and in the presence of Form E, Form A tends to convert to Form E.

When stored for a period of about 85 days under two different temperature/relative humidity stress conditions (room temperature/0% relative humidity (RH) and 40°C/93% RH), Form A typically does not convert to a different form.

In sum, Form A is a crystalline, unsolvated solid that melts at approximately 270°C. Form A is weakly or not hygroscopic and appears to be the most thermodynamically stable anhydrous polymorph of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione discovered thus far.

5.2.2 FORM B

Form B can be obtained from many solvents, including, but not limited to, hexane, toluene, and water. Figure 6 shows a representative XRPD pattern of Form B, characterized by peaks at approximately 16, 18, 22 and 27 degrees 2θ.

Solution proton NMR confirm that Form B is a form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Representative IR and Raman spectra are shown in Figures 7 and 8, respectively. Compared to Form A, the IR spectrum for Form B has peaks at approximately 3513 and 1960 cm^{-1}.

Representative DSC and TGA data for Form B are shown in Figure 9. The DSC curve exhibits endotherms at about 146 and 268 °C. These events are identified as dehydration and melting by hot stage microscopy experiments. Form B typically loses about 3.1% volatiles up to about 175°C (per approximately 0.46 moles of water). Comparison of the IR spectrum of the volatiles with that of water indicates that they are water (*See* Figure 10). Calculations from TGA data indicate that Form B is a hemihydrate. Karl Fischer water analysis also supports this conclusion.

Representative moisture sorption and desorption data are shown in Figure 11. Form B typically does not exhibit a significant weight gain from 5% to 95% relative humidity, when equilibrium is obtained at each relative humidity step. As Form B dries from 95% back down to 5% relative humidity, it tends to maintain its weight such that at 5% relative humidity it typically has gained only about 0.022% by weight (about 0.003 mg) from start to finish. Form B does not convert to a different form upon exposure to about 84% relative humidity for about ten days.

Interconversion studies show that Form B typically converts to Form A in a THF solvent system, and typically converts to Form C in an acetone solvent system. In aqueous solvent systems such as pure water and 10% water solutions, Form B is the most stable of the polymorphic forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. However, it can convert to Form E in the presence of water. Desolvation experiments show

that upon heating at about 175°C for about five minutes, Form B typically converts to Form A.

When stored for a period of about 85 days under two different temperature/relative humidity stress conditions (room temperature/0% RH and 40°C/93% RH), Form B does not convert to a different form.

In sum, Form B is a hemihydrated, crystalline solid that melts at about 267°C. Interconversion studies show that Form B converts to Form E in aqueous solvent systems, and converts to other forms in acetone and other anhydrous systems.

5.2.3 FORM C

Form C can be obtained from evaporations, slurries and slow cools in acetone solvent systems. A representative XRPD pattern of this form is shown in Figure 12. The data are characterized by peaks at approximately 15.5 and 25 degrees 2θ.

Solution proton NMR indicates that the 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione molecule is intact. Representative IR and Raman spectra are shown in Figures 13 and 14, respectively. The IR spectrum of Form C is characterized by peaks at approximately 3466, 3373, and 3318 cm^{-1}. The Raman spectrum of Form C is characterized by peaks at about 3366, 3321, 1101, and 595 cm^{-1}.

Representative thermal characteristics for Form C are plotted in Figure 15. Form C loses about 10.02% volatiles up to about 175°C, indicating it is a solvated material. Weight loss above about 175°C is attributed to decomposition. Identification of volatiles in Form C can be accomplished with TG-IR experiments. The representative IR spectrum captured after several minutes of heating, as depicted in Figure 13, when compared with a spectral library, shows acetone to be the best match. Calculations from TGA data show that Form C is a hemisolvate (approximately 0.497 moles of acetone). The DSC curve for Form C, shown in Figure 15, exhibits endotherms at about 150 and about 269°C. The endotherm at about 150°C is attributed to solvent loss based on observations made during hot stage microscopy experiments. The endotherm at about 269°C is attributed to the melt based on hot stage experiments.

Representative moisture sorption and desorption balance data are shown in Figure 17. Form C does not exhibit a significant weight gain from 5 to 85% relative humidity, when equilibrium is obtained at each relative humidity step up to 85% relative humidity. At 95% relative humidity, Form C experiences a significant weight loss of about 6.03%. As the

sample dries from 95% back down to 5% relative humidity, the sample maintains the weight achieved at the end of the adsorption phase at each step down to 5% relative humidity. Form C is capable of converting to Form B when stored at about 84% relative humidity for approximately ten days.

Interconversion studies show that Form C typically converts to Form A in a THF solvent system and typically converts to Form E in an aqueous solvent system. In an acetone solvent system, Form C is the most stable form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Desolvation experiments performed on Form C show that upon heating at about 150°C for about five minutes, Form C will typically convert to Form A.

In sum, Form C is a crystalline, hemisolvated solid, which melts at approximately 269°C. Form C is not hygroscopic below about 85% RH, but can convert to Form B at higher relative humidities.

5.2.4 FORM D

Form D can be obtained from evaporation in acetonitrile solvent systems. A representative XRPD pattern of the form is shown in Figure 18. The pattern is characterized by peaks at approximately 27 and 28 degrees 2θ.

Solution proton NMR indicates that the 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione molecule is intact. Representative IR and Raman spectra are shown in Figures 19 and 20, respectively. The IR spectrum of Form D is characterized by peaks at approximately 3509, 2299, and 2256 cm^{-1}. The Raman spectrum of Form D is characterized by peaks at approximately 2943, 2889, 2297, 2260, 1646, and 1150 cm^{-1}.

Representative thermal characteristics for Form D are plotted in Figure 21. Form D loses about 6.75% volatiles up to about 175°C, indicating a solvated material. Weight loss above about 175°C is attributed to decomposition. TG-IR experiments indicate that the volatiles are water and acetonitrile. Calculations from TG data show that about one mole of water is present in the sample. A representative DSC curve for Form D exhibits endotherms at about 122 and about 270°C. The endotherm at about 122°C is attributed to loss of volatiles based on observations made during hot stage microscopy experiments. The endotherm at about 270°C is attributed to the melt based on hot stage experiments.

Representative moisture sorption and desorption data are plotted in Figure 22. Form D does not exhibit a significant weight gain from 5 to 95% relative humidity when equilibrium is obtained at each relative humidity step. As the form dries from 95% back

down to 5% relative humidity, it maintains its weight such that at 5% relative humidity the form has typically gained only about 0.39% by weight (about 0.012 mg) from start to finish. Form A is capable of converting to Form B when stored at about 84% relative humidity for approximately ten days.

Interconversion studies show that Form D is capable of converting to Form A in a THF solvent system, to Form E in an aqueous solvent system, and to Form C in an acetone solvent system. Desolvation experiments performed on Form D show that upon heating at about 150°C for about five minutes Form D will typically convert to Form A.

In sum, Form D is a crystalline solid, solvated with both water and acetonitrile, which melts at approximately 270°C. Form D is either weakly or not hygroscopic, but will typically convert to Form B when stressed at higher relative humidities.

5.2.5 FORM E

Form E can be obtained by slurrying 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in water and by a slow evaporation of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in a solvent system with a ratio of about 9:1 acetone:water. A representative XRPD pattern is shown in Figure 23. The data are characterized by peaks at approximately 20, 24.5 and 29 degrees 2θ.

Representative thermal characteristics of Form E are plotted in Figure 24. Form E typically loses about 10.58% volatiles up to about 125°C, indicating that it is a solvated material. A second weight loss of an additional about 1.38% was observed between about 125°C and about 175°C. Weight loss above about 175°C is attributed to decomposition. Karl Fischer and TG-IR experiments support the conclusion that the volatile weight loss in Form E is due to water. The representative DSC curve for Form E exhibits endotherms at about 99, 161 and 269°C. Based on observations made during hot stage microscopy experiments, the endotherms at about 99 and about 122°C are attributed to loss of volatiles. The endotherm at about 269°C is attributed to the melt based on hot stage experiments.

Representative moisture sorption and desorption data are plotted in Figure 25. Form E typically does not exhibit a significant weight change from 5 to 95% relative humidity when equilibrium is obtained at each relative humidity step. As the sample dried from 95% back down to 5% relative humidity, the sample continues to maintain weight such that at 5% relative humidity the sample has lost only about 0.0528% by weight from start to finish.

Interconversion studies show that Form E can convert to Form C in an acetone solvent system and to Form G in a THF solvent system. In aqueous solvent systems, Form E appears to be the most stable form. Desolvation experiments performed on Form E show that upon heating at about 125°C for about five minutes, Form E can convert to Form B. Upon heating at 175°C for about five minutes, Form B can convert to Form F.

When stored for a period of 85 days under two different temperature/relative humidity stress conditions (room temperature/0% RH and 40°C/93% RH) Form E typically does not convert to a different form. When stored for seven days at room temperature/0% RH, Form E can convert to a new form, Form H.

5.2.6 FORM F

Form F can be obtained by complete dehydration of Form E. A representative XRPD pattern of Form F, shown in Figure 26, is characterized by peaks at approximately 19, 19.5 and 25 degrees 2θ.

Representative thermal characteristics of Form A are shown in Figure 27. The representative DSC curve for Form F exhibits an endotherm at about 269°C preceded directly by two smaller endotherms indicative of a crystallized form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. The DSC thermogram does not show any thermal events prior to the melt, suggesting that it is an unsolvated material.

5.2.7 FORM G

Form G can be obtained by slurrying forms B and E in THF. A representative XRPD pattern of this form, shown in Figure 28, is characterized by a peak at approximately 23 degrees 2θ. Two other peaks unique to Form G appear at approximately 21 and 24.5 degrees 2θ.

Representative thermal characteristics of Form G are plotted in Figure 29. A representative DSC curve for Form G exhibits an endotherm at about 248°C followed by a small, broad exotherm at about 267°C. No thermal events are seen in the DSC thermogram at lower temperatures, suggesting that it is an unsolvated material.

5.2.8 FORM H

Form H can be obtained by storing Form E at room temperature and 0% RH for about 7 days. A representative XRPD pattern is shown in Figure 30. The pattern is characterized by a peak at 15 degrees 2θ, and two other peaks at 26 and 31 degrees 2θ.

Representative thermal characteristics are shown in Figure 31. Form H loses about 1.67% volatiles up to about 150°C. Weight loss above about 150°C is attributed to decomposition. Karl Fischer data shows that Form H typically contains about 1.77% water (about 0.26 moles), suggesting that the weight loss seen in the TG is due to dehydration. The DSC thermogram shows a broad endotherm between about 50°C and about 125°C, corresponding to the dehydration of Form H and a sharp endotherm at about 269°C, which is likely due to a melt.

When slurried in water with either Forms A or B, after about 14 days Form H can convert to Form E. When slurried in THF, Form H can convert to Form A. When slurried in acetone, Form H can convert to Form C.

In sum, Form H is a crystalline solid, hydrated with about 0.25 moles of water, which melts at approximately 269°C.

5.3 METHODS OF USE AND PHARMACEUTICAL COMPOSITIONS

Polymorphs of the invention exhibit physical characteristics that are beneficial for drug manufacture, storage or use. All polymorphs of the invention have utility as pharmaceutically active ingredients or intermediates thereof.

This invention encompasses methods of treating and preventing a wide variety of diseases and conditions using polymorphs of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. In each of the methods, a therapeutically or prophylactically effective amount of the compound is administered to a patient in need of such treatment or prevention. Examples of such disease and conditions include, but are not limited to, diseases associated with undesired angiogenesis, cancer (*e.g.*, solid and blood borne tumors), inflammatory diseases, autoimmune diseases, and immune diseases. Examples of cancers and pre-cancerous conditions include those described in U.S. patent nos. 6,281,230 and 5,635,517 to Muller *et al.* and in various U.S. patent applications to Zeldis, including application nos. 10/411,649, filed April 11, 2003 (Treatment of Myelodisplastic Syndrome); 10/438,213 filed May 15, 2003 (Treatment of Various Types of Cancer); 10/411,656, filed April 11, 2003 (Treatment of Myeloproliferative Diseases). Examples of other diseases and disorders that can be treated or prevented using compositions of the invention are described in U.S. patent nos. 6,235,756 and 6,114,335 to D'Amato and in other U.S. patent applications to Zeldis, including 10/693,794, filed October 23, 2003 (Treatment of Pain Syndrome) and 10/699,154,

filed October 30, 2003 (Treatment of Macular Degeneration). The entirety of each of the patents and patent applications cited herein is incorporated herein by reference.

Depending on the disease to be treated and the subject's condition, polymorphs of the invention can be administered by oral, parenteral (*e.g.*, intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implantation), inhalation spray, nasal, vaginal, rectal, sublingual, or topical routes of administration and may be formulated, alone or together, in suitable dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles appropriate for each route of administration. Because individual polymorphs have different dissolution, stability, and other properties, the optimal polymorph used in methods of treatment may depend on the route of administration. For example, forms that are readily soluble in aqueous solutions are preferably used to provide liquid dosage forms, whereas forms that exhibit great thermal stability may be preferred in the manufacture of solid dosage forms (*e.g.*, tablets and capsules).

Although the physical characteristics of polymorphs can, in some cases, affect their bioavailability, amounts of the polymorphs that are therapeutically or prophylactically effective in the treatment of various disease and conditions can be readily determined by those of ordinary skill in the pharmacy or medical arts. In certain embodiments of the invention, a polymorph is administered orally and in a single or divided daily doses in an amount of from about 0.10 to about 150 mg/day, or from about 5 to about 25 mg/day. In other embodiments, a polymorph is administered every other day in an amount of from about 0.10 to about 150 mg/day, or from about 5 to about 25 mg/day.

The invention encompasses pharmaceutical compositions and single unit dosage forms that can be used in methods of treatment and prevention, which comprise one or more polymorphs of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione and optionally one or more excipients or diluents. Specific compositions and dosage forms are disclosed in the various patents and patent applications incorporated herein by reference. In one embodiment, a single dosage form comprises a polymorph (*e.g.*, Form B) in an amount of about 5, 10, 25 or 50 mg.

6. EXAMPLES

6.1 POLYMORPH SCREEN

A polymorph screen to generate the different solid forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione was carried out as follows.

A weighed sample of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione (usually about 10 mg) was treated with aliquots of the test solvent. Solvents were either reagent or HPLC grade. The aliquots were usually about 200 μL. Between additions, the mixture was usually shaken or sonicated. When the solids dissolved, as judged by visual inspection, estimated solubilities were calculated. Solubilities were estimated from these experiments based on the total solvent used to provide a solution. Actual solubilities may have been greater than those calculated due to the use of too-large solvent aliquots or to a slow rate of dissolution.

Samples were created by generating solutions (usually about 30 mg in 20 mL) at elevated temperatures, filtering, and allowing the solution to evaporate whether in an open vial (hot fast evaporation) or in a vial covered with aluminum foil containing pinholes (hot slow evaporation).

Slurry experiments were also performed. Usually about 25 mg of solid was placed in either 3 or 5 mL of solvent. The samples were then placed on orbital shakers at either ambient temperature or 40 °C for 4-10 days.

Crystallizations were performed using various cooling methods. Solid was dissolved in a solvent at an elevated temperature (*e.g.*, about 60 °C), filtered quickly and allowed to cool to room temperature. Once at room temperature, samples that did not crystallize were moved to a refrigerator. Solids were removed by filtration or decantation and allowed to dry in the air. Crash cools were performed by dissolving solid in a solvent at an increased temperature (*e.g.*, about 45-65 °C) followed by cooling in a dry ice/acetone bath.

Hygroscopicity studies were performed by placing portions of each polymorph in an 84% relative humidity chamber for approximately one week.

Desolvation studies were carried out by heating each polymorph in a 70 °C oven for approximately one week.

Interconversion experiments were carried out by making slurries containing two forms in a saturated solvent. The slurries were agitated for approximately 7-20 days at

ambient temperature. The insoluble solids were recovered by filtration and analyzed using XRPD.

6.2 PREPARATION OF POLYMORPHIC FORMS

Eight solid forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione were prepared as described below.

Form A was obtained by crystallization from various non-aqueous solvents including 1-butanol, butyl acetate, ethanol, ethyl acetate, methanol, methyl ethyl ketone, and tetrahydrofuran. Form B was also obtained by crystallization from the solvents hexane, toluene and water. Form C was obtained from evaporations, slurries, and slow cools in acetone solvent systems. Form D was obtained from evaporations in acetonitrile solvent systems. Form E was obtained most readily by slurrying 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in water. Form F was obtained by complete desolvation of Form E. It is found to be an unsolvated, crystalline material that melts at about 269 °C. Form G was obtained by slurrying forms B and E in THF. Form H was obtained by stressing Form E at room temperature and 0% RH for 7 days.

6.2.1 SYNTHESIS OF POLYMORPHS B AND E

Form B is the desired polymorph for the active pharmaceutical ingredient (API) of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. This form has been used in the formulation of API into drug product for clinical studies. Three batches were produced as apparent mixtures of polymorphs in the non-micronized API of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione.

Development work was carried out to define a process that would generate polymorph B from this mixture of polymorphs and could be implemented for strict polymorphic controls in the validation batches and future manufacturing of API of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Characterization of polymorphic forms produced during the work was performed by XRPD, DSC, TGA and KF.

A process was also developed for the large-scale preparation of Form E. Polymorph E material was prepared in order to carry out a comparison with polymorph B drug product in capsule dissolution testing of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. 150 g of a mixture of polymorphs in 3L of water was stirred at room temperature for 48 hours. The product was collected by filtration and dried at 25 °C for 24 hours under

vacuum. XRPD, DSC, TGA, KF and HPLC analyses confirmed that the material isolated was polymorph E.

In a preliminary work, it was demonstrated that stirring a suspension of a mixture of polymorphs of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione with water at high temperature (75 °C) for an extended period of time converted this mixture of polymorphs exclusively to form B. Several specific parameters were identified including temperature, solvent volume and drying parameters (temperature and vacuum). XRPD, DSC, TGA, KF and HPLC analyses were used to characterize all of the batches. After completing the optimization work, the optimized process was scaled-up to 100-200 g on three lots of API. Drying studies were carried out at 20 °C, 30 °C and 40 °C, and 65 °C with a vacuum of 150 mm of Hg. The results are shown in Tables 1-5.

The cooling and holding periods of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione slurry were studied. The experimental laboratory data suggests that polymorph B seems to be forming first, and overtime equilibration to polymorph E at RT conditions occurs, therefore generating a mixture of polymorphs B and E. This result supports the fact that polymorph B seems to be a kinetic product, and that prolonged processing time converts the material to polymorph E resulting in a mixture of polymorphs B and E.

A laboratory procedure was developed to exclusively produce polymorph B of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. The procedure includes a stirred 10 volume water slurry at ~ 75 °C for 6-24 hours. The following preferred process parameters have been identified:

1. Hot slurry temperature of 70-75 °C.

2. Product filtration of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione at 65-75 °C.

3. Drying under vacuum at 60-70 °C is preferred for an efficient removal of unbound water in 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione wet cake.

4. The filtration step of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione may be a time sensitive operation. The use of efficient solid-liquid separation equipment is preferred.

5. Holding periods of water-wet cake of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione at KF higher than 5% may cause the kinetic equilibrations of polymorph B to mixed polymorphs of E and B.

Drying to KF <4.0 % water was achieved in ~ 3 hours (30-70 °C, 152 mm Hg). Polymorphs B and E were distinguished by the water levels as measured by KF and TGA. The reference sample of polymorph B is micronized API. In order to make accurate comparison by XRPD samples were gently grinded before submission for analysis. This increases the clarity of the identification of the polymorphic form. All samples were analyzed for XRPD, DSC, TGA, KF and HPLC.

Table 1: Preliminary Studies

Amount	Reaction conditions	Analysis	Results/conclusion
2 g	Water, rt, 48 h	XRPD, DSC, TGA, KF	Polymorph E
25 g	Water, rt, 48 h	XRPD, DSC, TGA, KF	Polymorph E
5 g	Water, 70-75 °C, 24 h then rt 24 h	XRPD, DSC, TGA, KF	Polymorph B
1 g	9:1 Acetone – water, Slow evpo.	XRPD, DSC, TGA, KF	Polymorph Mixture
1 g	175 °C 1 h in an oven	XRPD, DSC, TGA, KF	Polymorph A
0.5 g (polymorph A)	Water, rt, 24 h	XRPD, DSC, TGA, KF	Polymorph E
1 g polymorph B	Water, rt, 48 h	XRPD, DSC, TGA, KF	Polymorph E
1 g polymorph E	Water, 70-75 °C, 24 h	XRPD, DSC, TGA, KF	Polymorph B
1 g	Slurry in heptane	XRPD, DSC, TGA, KF	No change

Table 2: Optimization of Temperature, Time and Solvent Volume

Amount	Amount Water (mL)	Temp (°C)	Time (h)	Results/ conclusion
10 g	50	75	6	Mix
10 g	50	75	24	Polymorph B
10 g	100	70	6	Polymorph B
10 g	100	70	14	Polymorph B
10 g	100	70	21	Polymorph B

Amount	Amount Water (mL)	Temp (°C)	Time (h)	Results/ conclusion
10 g	100	75	6	Polymorph B
10 g	100	75	24	Polymorph B
10 g	100	75	6	Polymorph B
10 g	100	75	19	Polymorph B
10 g	100	75	14	Polymorph B
10 g	100	75	24	Polymorph B
5 g	100	75	18	Polymorph B
10 g	100	80	6	Polymorph B
10 g	100	80	20	Polymorph B
10 g	200	45	6	Polymorph B+E
10 g	200	45	24	Polymorph E
10 g	200	60	48	Polymorph B
10 g	200	75	6	Mix
10 g	200	75	24	Polymorph B
10 g	200	75	13	Polymorph B
10 g	200	75	24	Polymorph B

Optimum conditions were determined to be 10 volumes of solvent (H_2O), 70-80 °C for 6-24 hours.

Table 3: Holding Time

Amount	Reaction Conditions	Holding Time (h)	Holding Temp (°C)	Results/ Conclusion
5g	Water, 70-75 °C, 24 h	24	23-25	Polymorph B
1g Polymorph B	Water, 70-75 °C, 24 h	48	23-25	Polymorph E
2 g	Water, 40 mL	16	23-25	Polymorph E
150 g	Water, 3.0 L	24	23-25	Polymorph E
150 g	Water, 3.0 L	48	23-25	Polymorph E
10 g	Water, 100 mL, 24h, 75 °C	18	23-25	Polymorph B

Amount	Reaction Conditions	Holding Time (h)	Holding Temp (°C)	Results/ Conclusion
10 g	Water, 100 mL, 24h, 75 °C	18	40	Polymorph B
10 g	Water, 200 mL, 24h, 75 °C	14	-5	Mix
10 g	Water, 200 mL, 24h, 75 °C	14	23-25	Polymorph E
10 g	Water, 200 mL, 24h, 75 °C	14	40	Mix
10 g	Water, 100 mL, 24h, 75 °C	21	23-25	Polymorph E
10 g	Water, 100 mL, 24h, 75 °C	21	40	Mix
10 g	Water, 100 mL, 14h, 75 °C	2	23-25	Mix

Holding time gave mixed results and it was determined that the material should be filtered at 60-65 °C and the material washed with 0.5 volume of warm (50-60 °C) water.

Table 4: Scale-up Experiments

Amount	Amount Water (L)	Temp (°C)	Time (h)	Results/Conclusion
100 g	1.0	75	6	Polymorph B
100 g	1.0	75	22	Polymorph B
100 g	1.0	75	6	Polymorph B
100 g	1.0	75	24	Polymorph B
100 g	1.0	75	6	Polymorph B
100 g	1.0	75	22	Polymorph B

Table 5: Drying Studies

Amount	Drying Time (h)	Drying Temp (°C)	Vacuum (mm Hg)	KF§ (%)	Results/ Conclusion
100 g	0	-	-	3.690	Polymorph B
100 g	3	30	152	3.452	Polymorph B
100 g	8	30	152	3.599	Polymorph B
100 g	0	-	-	3.917	Polymorph B

Amount	Drying Time (h)	Drying Temp (°C)	Vacuum (mm Hg)	KF§ (%)	Results/ Conclusion
100 g	5	40	152	3.482	Polymorph B
100 g	22	40	152	3.516	Polymorph B
100 g	3	40	152	3.67	Polymorph B
100 g	22	40	152	3.55	Polymorph B

*Reaction Conditions: Water 1L, 75 °C, 22-24h; §Average of 2 runs.

Drying studies determined that the material should be dried at 35-40 °C, 125-152 mm Hg for 3 to 22 h or until the water content reaches ≤ 4 % w/w.

For a large scale preparation of polymorph E (5222-152-B), a 5-L round bottom flask was charged with 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione (150 g, 0.579 mol) and water (3000 mL, 20 vol). The mixture was mechanically stirred at room temperature (23-25 °C) for 48 h under nitrogen atmosphere.

Samples were taken after 24h and 48h before the mixture was filtered and air-dried on the filter for 1h. The material was transferred to a drying tray and dried at room temperature (23-25 °C) for 24 h. KF analysis on the dried material showed water content of 11.9 %. The material was submitted for XRPD, TGA, DSC and HPLC analysis. Analysis showed the material was pure polymorph E.

For a large scale preparation of polymorph B (5274-104), a 2L-3-necked round bottom flask was charged with 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione (polymorph mixture, 100g, 0.386 mol) and water (1000 mL, 10.0 vol). The mixture was heated to 75 °C over approximately 30 minutes with mechanical stirring under nitrogen atmosphere.

Samples were taken after 6h and 24h before the mixture was allowed to cool to 60-65 °C, filtered and the material washed with warm (50-60 °C) water (50 mL, 0.5 vol). The material was transferred to a drying tray and dried at 30 °C, 152 mm Hg for 8h. KF analysis on the dried material showed water content of 3.6 %. After grinding the material was submitted for XRPD, TGA, DSC and HPLC analysis. Analysis showed the material was pure polymorph B. The results of the analyses are shown in Figures 32-46.

6.3 X-RAY POWDER DIFFRACTION MEASUREMENTS

X-ray powder diffraction analyses were carried out on a Shimadzu XRD-6000 X-ray powder diffractometer using Cu *Kα* radiation. The instrument is equipped with a fine-focus

X-ray tube. The tube voltage and amperage were set at 40 kB and 40 mA, respectively. The divergence and scattering slits were set at 1° and the receiving slit was set at 0.15 mm. Diffracted radiation was detected by a NaI scintillation detector. A theta-two theta continuous scan at 3 °/min (0.4 sec/0.02° step) from 2.5 degrees 2θ to 40 degrees 2θ was used. A silicon standard was analyzed each day to check the instrument alignment.

X-ray powder diffraction analyses were also carried out using Cu *Kα* radiation on an Inel XRG-3000 diffractometer equipped with a curved position-sensitive detector. Data were collected in real time over a theta-two theta range of 120° at a resolution of 0.03°. The tube voltage and current were 40 kV and 30 mA, respectively. A silicon standard was analyzed each day to check for instrument alignment. Only the region between 2.5 and 40 degrees 2θ is shown in the figures.

6.4 THERMAL ANALYSIS

TG analyses were carried out on a TA Instrument TGA 2050 or 2950. The calibration standards were nickel and alumel. Approximately 5 mg of sample was placed on a pan, accurately weighed, and inserted into the TG furnace. The samples were heated in nitrogen at a rate of 10 °C/min, up to a final temperature of 300 or 350 °C.

DSC data were obtained on a TA 2920 instrument. The calibration standard was indium. Approximately 2-5 mg samples were placed into a DSC pan and the weight accurately recorded. Crimped pans with one pinhole were used for analysis and the samples were heated under nitrogen at a rate of 10 °C/min, up to a final temperature of 350 °C.

Hot-stage microscopy was carried out using a Kofler hot stage mounted on a Leica Microscope. The instrument was calibrated using USP standards.

A TA Instruments TGA 2050 interfaced with a Nicolet model 560 Fourier transform IR spectrophotometer, equipped with a globar source, XT/KBr beamsplitter, and deuterated triglycine sulfate (DTGS) detector, was utilized for TG-IR experiments. The IR spectrometer was wavelength calibrated with polystyrene on the day of use while the TG was temperature and weight calibrated biweekly, using indium for the temperature calibration. A sample of approximately 10 mg of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione was weighed into an aluminum pan and heated from 25 to 30 °C to 200 °C at a rate of 20 °C/min with a helium purge. IR spectra were obtained in series, with each spectrum representing 32 co-added scans at a resolution of 4 cm^{-1}. Spectra were collected with a 17-second repeat time. TG/IR analysis data are presented as Gram-Schmidt plots and IR spectra

linked to the time. Gram-Schmidt plots show total IR intensity vs. time; hence, the volatiles can be identified at each time point. They also show when the volatiles are detected. From the Gram-Schmidt plots, time points were selected and the IR spectra of these time points are presented in the stacked linked spectra. Each spectrum identifies volatiles evolving at that time point. Volatiles were identified from a search of the HR Nicolet TGA vapor phase spectral library. The library match results are also presented to show the identified vapor.

6.5 SPECTROSCOPY MEASUREMENTS

Raman spectra were acquired on a Nicloet model 750 Fourier transform Raman spectrometer utilizing an excitation wavelength of 1064 nm and approximately 0.5 W of Nd:YAG laser power. The spectra represent 128 to 256 co-added scans acquired at 4 cm^{-1} resolution. The samples were prepared for analysis by placing the material in a sample holder and positioning this in the spectrometer. The spectrometer was wavelength calibrated using sulfur and cyclohexane at the time of use.

The mid-IR spectra were acquired on a Nicolet model 860 Fourier transform IR spectrophotmeter equipped with a globar source XT/KBr beamsplitter and a deuterated triglycine sulfate (DTGS) detector. A Spectra-Tech, Inc. diffuse reflectance accessory was utilized for sampling. Each spectrum represents 128 co-added scans at a spectral resolution of 4 cm^{-1}. A background data set was acquired with an alignment mirror in place. A single beam sample data set was then acquired. Subsequently, a log 1/R (where R = reflectance) spectrum was acquired by rationing the two data sets against each other. The spectrophotometer was calibrated (wavelength) with polystyrene at the time of use.

6.6 MOISTURE SORPTION/DESORPTION MEASUREMENTS

Moisture sorption/desorption data were collected on a VTI SGA-100 moisture balance system. For sorption isotherms, a sorption range of 5 to 95 % relative humidity (RH) and a desorption range of 95 to 5 % RH in 10 % RH increments was used for analysis. The sample was not dried prior to analysis. Equilibrium criteria used for analysis were less than 0.0100 weight percent change in 5 minutes with a maximum equilibration time of 3 hours if the weight criterion was not met. Data were not corrected for the initial moisture content of the samples.

6.7 SOLUTION PROTON NMR MEASUREMENTS

NMR spectra not previously reported were collected at SSCI, Inc, 3065 Kent Avenue, West Lafayette, Indiana. Solution phase ^{1}H NMR spectra were acquired at ambient

temperature on a Bruker model AM spectrometer. The ^{1}H NMR spectrum represents 128 co-added transients collected with a 4 μsec pulse and a relaxation delay time of 5 seconds. The free induction decay (FID) was exponentially multiplied with a 0.1 Hz Lorentzian line broadening factor to improve the signal-to-noise ratio. The NMR spectrum was processed utilizing GRAMS software, version 5.24. Samples were dissolved in dimethyl sulfoxide-d_6.

The scope of this invention can be understood with reference to the appended claims.

6.8 INTRINSIC DISSOLUTION AND SOLUBILITY STUDIES

Intrinsic dissolution experiments were conducted on Form A (anhydrous), Form B (hemihydrate), and Form E (dihydrate) of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Equilibrium solubility experiments were conducted on Forms A and B. Aliquots were analyzed by ultraviolet-visible spectrophotometry, and the solids remaining from each experiment were analyzed by X-ray powder diffraction (XRPD).

6.8.1 EXPERIMENTAL

6.8.1.1 Dissolution

Dissolution experiments were carried out in a VanKel VK6010-8 dissolution apparatus equipped with a VK650A heater/circulator. An intrinsic dissolution apparatus (Woods apparatus) was used. Samples were compressed at 1.5 metric tons (1000 psi) for 1 min using the Woods apparatus in a hydraulic press, giving a sample surface of 0.50 cm^2. A dissolution medium consisting of 900 mL HCl buffer, pH 1.8, with 1% sodium lauryl sulfate, was used for each experiment. The medium was degassed by vacuum filtration through a 0.22-μm nylon filter disk and maintained at 37 °C. The apparatus was rotated at 50 rpm for each experiment. Aliquots were filtered immediately using 0.2-μm nylon syringe filters. In some cases, the undissolved solids were recovered and analyzed by X-ray powder diffraction (XRPD).

6.8.1.2 Solubility

Equilibrium solubility experiments were conducted in a 100-mL, three-neck, round-bottom flask immersed in a constant temperature oil bath maintained at 25 °C. A solid sample of 400-450 mg was stirred in 50 mL of dissolution medium (HCl buffer, pH 1.8, with 1% sodium lauryl sulfate) using a mechanical stir rod. Aliquots were filtered using 0.2-μm nylon syringe filters and immediately diluted 1 mL → 50 mL, then 5 mL → 25 mL with dissolution medium in Class A glassware, a final dilution factor of 250.

6.8.1.3 UV-Vis Spectrophotometry

Dissolution and solubility samples solutions were analyzed by a Beckman DU 640 single-beam spectrophotometer. A 1.000-cm quartz cuvette and an analysis wavelength of 228.40 nm were utilized. The detector was zeroed with a cuvette filled with dissolution medium.

6.8.1.4 X-Ray Powder Diffraction

XRPD analyses were carried out on a Shimadzu XRD-6000 X-ray powder diffractometer using Cu Kα radiation. The instrument is equipped with a fine focus X-ray tube. The tube power and amperage were set at 40 kV and 40 mA, respectively. The divergence and scattering slits were set at 1° and the receiving slit was set at 0.15 mm. Diffracted radiation was detected by a NaI scintillation detector. A theta-two theta continuous scan at 3 °/min (0.4 sec/0.02° step) from 2.5 to 40 °2θ was used. A silicon standard was analyzed each day to check the instrument alignment. Samples were packed in an aluminum holder with silicon insert.

6.8.2 RESULTS

The results of these solubility and intrinsic studies are summarized in Table 6. Both the solubility and dissolution experiments were conducted in a medium of HCl buffer, pH 1.8, containing 1% sodium lauryl sulfate. Form A was found to be unstable in the medium, converting to Form E. The solubilities of Forms A, B, and E were estimated to be 6.2, 5.8, and 4.7 mg/mL, respectively. The dissolution rates of Forms A, B, and E were estimated to be 0.35, 0.34, and 0.23 mg/mL, respectively.

6.8.2.1 UV-Vis Spectrophotometry Method Development

A UV-Vis scan of the dissolution medium (blanked with an empty cuvette) was done to identify any interfering peaks. A small peak at 225 nm was present as shown in Figure 47.

Solutions of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione at varying concentrations were analyzed by UV- Vis spectrophotometry. A preliminary scan of a 1.0 mg/mL solution was done, with the instrument blanked with dissolution medium. The solution was highly absorbing and noisy from 200 - 280 nm, making dilution necessary.

A 0.04 mg/mL solution of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione was then scanned from 200 - 300 nm. The plot was still noisy between 200 and 230 nm as shown in Figure 48. The sample was further diluted to 0.008 mg/mL. A wavelength scan of 200 - 350 nm for this sample showed a peak a 228.4 nm with no

interference, as shown in Figure 49. Therefore, a wavelength of 228.4 was chosen for analysis of the solubility and dissolution samples.

A six-point calibration curve was generated with standards of the following concentrations: 0.001 mg/mL, 0.002 mg/mL, 0.005 mg/mL, 0.010 mg/mL, 0.015 mg/mL, and 0.020 mg/mL (Notebook 569-90). A linearity coefficient of $R^2 = 0.9999$ was obtained as shown in Figure 50.

6.8.2.2 Solubility

A sample consisting of 449.4 mg Form A was slurried in dissolution medium. Particle size was not controlled. Aliquots were taken at 7, 15, 30, 60, 90, and 150 min. The concentration reached 6.0 mg/mL by the first time point. The highest concentration reached was 6.2 mg/mL, at 30 min. From that point the concentration decreased, reaching 4.7 mg/mL at 150 min as in Figure 51. The solids remaining at the final time point were analyzed by XRPD and found to be Form E as shown in Table 7. No peaks attributed to Form A can be seen in the pattern. Since the concentration did not plateau at 4.7 mg/mL, the solubility of Form E may be lower than that.

A sample consisting of 401.4 mg Form B was slurried in dissolution medium. Particle size was not controlled. Aliquots were taken at 7, 15, 30, 60, 90, 180, 420, and 650 min. Form B dissolved much more slowly than Form A, reaching 3.3 mg/mL in 90 min. The concentration stabilized at 5.6 - 5.7 mg/mL at the final three time points as in Figure 52. The remaining solids were shown to be Form B as in Table 7, suggesting Form B has good stability in water.

A summary of the solubilities is given in Table 6. The amounts dissolved at each time point are shown in Tables 8 and 9.

Table 6: Summary of Results

Form	Solubility	Intrinsic Dissolution #1	Intrinsic Dissolution #2	Average Intrinsic Dissolution Rate
Form A	6.2 mg/mL	0.35	0.22[a]	0.29[a]
Form B	5.8 mg/mL	0.35	0.32	0.34
Form E	4.7 mg/mL	0.21	0.25	0.23

a. The Form A dissolution experiment #2 may have converted to Form E on the surface of the disk, skewing the average rate lower.

Table 7: Experimental Details

Experiment	Final Form
Pressed Form A	A
Pressed Form B	B
Form A Solubility	E
Form B Solubility	B
Form A Dissolution	-
Form A Dissolution	A
Form B Dissolution	-
Form B Dissolution	B
Form E Dissolution	E
Form E Dissolution	-

Table 8: Form A Solubility

Time Point (min)	Concentration (mg/mL)
7	6.00
15	6.11
30	6.16
60	6.10
90	5.46
150	4.73

Table 9: Form B Solubility

Time Point (min)	Concentration (mg/mL)
7	1.63
15	2.14
30	2.33
60	2.94
90	3.34
180	5.67
420	5.76
650	5.61

6.8.2.3 Intrinsic Dissolution

Approximately 200 mg each of Forms A and B were compressed into disks in the Woods apparatus using 2 metric tons of pressure. The samples were subsequently scraped out, ground gently, and analyzed by XRPD. The study showed that compression and grinding does not cause a form change in either case. (See Table 7).

Two preliminary dissolution runs were performed. The disks fractured to some extent in both experiments, compromising the requirement of constant surface area.

The first experiment of intrinsic dissolution that strictly followed the USP chapter on intrinsic dissolution utilized approximately 150 mg each of Forms A and B. Seven aliquots, beginning at 5 min and ending at 90 min, were taken to maintain sink conditions. The experiment resulted in linear dissolution profiles, with a rate of 0.35 mg per cm^2 per minute for both forms. The Form E experiment was done later under the same conditions and added to the graph for comparison. (See Figure 53). The Form E dissolution rate was 0.21 mg per cm^2 per minute, significantly lower than the dissolution rate of Forms A and B. This is in line with expectations based on the solubility data. The crystal form of the remaining solids did not change in any case.

The second experiment utilized approximately 250 mg each of Forms A and B. The Form E experiment (135 mg) was done later and added to the graph for comparison. (See Figure 54). Nine aliquots were taken, beginning at 5 min and ending at 150 min. The dissolution rates were 0 22, 0.32, and 0.25 mg per cm^2 per minute, respectively, for Forms A, B, and E. The dissolution rate for Form A in this experiment was low, while the rates for Forms B and E were similar to those found in the first experiment. It is believed that in this case, a thin layer of the Form A sample disk may have converted to Form E upon exposure to water. This is supported by the evidence of rapid conversion of Form A to Form E in the solubility experiment. The diffraction pattern of the undissolved solids does not indicate a form change. However, the bulk of the sample disk is not exposed to water. Therefore, the true intrinsic dissolution rate of Form A is believed to be close to 0.35 mg per cm^2 per minute. An insufficient quantity of Form A was available to repeat the experiment.

A summary of the intrinsic dissolution rates is given in Table 6. The amounts dissolved at each time point are summarized in Tables 10 and 11.

Table 10: Intrinsic Dissolution Experiment #1 Results

Time Point	Form A [a]	Form B [a]	Form E [a]
5 min	5.76	10.80 [b]	2.70
10 min	7.73	6.85	4.13
20 min	11.31	10.25	6.96
30 min	15.59	14.35	9.60
45 min	21.98	20.57	12.57
60 min	27.11	25.70	15.16
90 min	34.17	34.34	20.82

a. Results are reported as Cumulative Amount Dissolved per Unit Area (mg/cm2)

b. This date point not included in graph since the value is higher than the next two data points.

Table 11: Intrinsic Dissolution Experiment #2 Results

Time Point	Form A [a]	Form B [a]	Form E [a]
5 min	4.50	5.04	3.06
10 min	5.22	6.12	4.31
20 min	7.54	7.73	11.40
30 min	11.46	12.72	11.93
45 min	15.01	17.33	14.72
60 min	18.38	21.93	18.52
90 min	24.38	31.64	26.24
120 min	30.35	41.31	33.56
150 min	35.26	49.54	40.82

a. Results are reported as Cumulative Amount Dissolved per Unit Area (mg/cm2)

6.9 ANALYSES OF MIXTURES OF POLYMORPHS

This invention encompasses mixtures of different polymorphs. For example, an X-ray diffraction analysis of one production sample yielded a pattern that contained two small peaks seen at approximately 12.6° and 25.8° 2θ in addition to those representative of Form B. In order to determine the composition of that sample, the following steps were performed:

1) Matching of the new production pattern to known forms along with common pharmaceutical excipients and contaminants;

2) Cluster analysis of the additional peaks to identify if any unknown phase is mixed with the original Form B;

3) Harmonic analysis of the additional peaks to identify if any preferred orientation may be present or if any changes in the crystal habit may have occurred; and

4) Indexing of the unit cells for both Form B and the new production sample to identify any possible crystallographic relationships.

Based on these tests, which can be adapted for the analysis of any mixture of polymorphs, it was determined that the sample contained a mixture of polymorph forms B and E.

6.10 DOSAGE FORM

Table 12 illustrates a batch formulation and single dosage formulation for a 25 mg single dose unit of a polymorphic form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione.

Table 12: Formulation for a 25 mg capsule

Material	Percent By Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Polymorphic Form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione	40.0%	25 mg	16.80 kg
Pregelatinized Corn Starch, NF	59.5%	37.2 mg	24.99 kg
Magnesium Stearate	0.5%	0.31 mg	0.21 kg
Total	100.0%	62.5 mg	42.00 kg

The pregelatinized corn starch (SPRESS B-820) and polymorphic form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione components are passed through a screen (i.e., a 710 μm screen) and then loaded into a Diffusion Mixer with a baffle insert and blended for about 15 minutes. The magnesium stearate is passed through a screen (*i.e.*, a 210 μm screen) and added to the Diffusion Mixer. The blend is then encapsulated in capsules using a Dosator type capsule filling machine.

The entire scope of this invention is not limited by the specific examples described herein, but is more readily understood with reference to the appended claims.

CLAIMS

What is claimed is:

1. Crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione having an X-ray powder diffraction pattern comprising a peak at approximately 17.5 degrees 2θ.

2. The crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 1, wherein the pattern further comprises peaks at approximately 8, 14.5, 16, 20.5, 24 and 26 degrees 2θ.

3. The crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 1 that has a differential scanning calorimetry melting temperature maximum of about 270 °C.

4. Crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione having an X-ray powder diffraction pattern comprising a peak at approximately 27 degrees 2θ.

5. The crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 4, wherein the pattern further comprises peaks at approximately 16, 18 and 22 degrees 2θ.

6. The crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 4 that has a differential scanning calorimetry melting temperature maximum of about 268 °C.

7. Crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione having an X-ray powder diffraction pattern comprising a peak at approximately 25 degrees 2θ.

8. The crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 7, wherein the pattern further comprises a peak at approximately 15.5 degrees 2θ.

9. The crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 7 that has a differential scanning calorimetry melting temperature maximum of about 269 °C.

10. Crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione having an X-ray powder diffraction pattern comprising a peak at approximately 28 degrees 2θ.

11. The crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 10, wherein the pattern further comprises a peak at approximately 27 degrees 2θ.

12. The crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 10 that has a differential scanning calorimetry melting temperature maximum of about 270 °C.

13. Crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione having an X-ray powder diffraction pattern comprising a peak at approximately 20 degrees 2θ.

14. The crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 13, wherein the pattern further comprises peaks at approximately 24.5 and 29 degrees 2θ.

15. The crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 13 that has a differential scanning calorimetry melting temperature maximum of about 269 °C.

16. Crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione having an X-ray powder diffraction pattern comprising a peak at approximately 19 degrees 2θ.

17. The crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 16, wherein the pattern further comprises peaks at approximately 19.5 and 25 degrees 2θ.

18. The crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 16 that has a differential scanning calorimetry melting temperature maximum of about 269 °C.

19. Crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione having an X-ray powder diffraction pattern comprising a peak at approximately 23 degrees 2θ.

20. The crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 19 that has a differential scanning calorimetry melting temperature maximum of about 267 °C.

21. Crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione having an X-ray powder diffraction pattern comprising a peak at approximately 15 degrees 2θ.

22. The crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 21 that has a differential scanning calorimetry melting temperature maximum of about 269 °C.

23. The crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 1, 4, 7, 10, 13, 16, 19 or 21, which is substantially pure.

24. A mixture of the crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 4, 5 or 6 and the crystalline of claim 13, 14 or 15.

25. A composition comprising amorphous 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione and crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 1, 4, 7, 10, 13, 16, 19 or 21.

26. The composition of claim 25, which comprises greater than about 50 weight percent crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione.

27. A pharmaceutical composition comprising crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of claim 1, 4, 7, 10, 13, 16, 19 or 21 and a pharmaceutically acceptable excipient.

28. The pharmaceutical composition of claim 27, wherein the composition is a single unit dosage form.