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Crystallization Process Development for a Stable Polymorph of Treprostinil Diethanolamine (UT-15C) by Seeding

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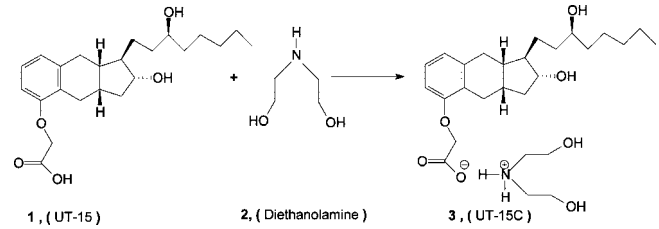
Abstract:

Process development of treprostinil diethanolamine salt (UT-15C) involved the development of crystallization and slurry protocols to address the polymorph and morphology control issues. Two forms of UT-15C were evaluated by differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD) and thermogravimetric analysis (TGA). Two crystallization solvent systems were developed to produce the thermodynamically stable form in high quality and yield. One solvent system gave dense particles while the other gave lighter and fly-away particles. Slurrying the lighter particles in heptane converted them to denser particles. The protocol was executed successfully on large-scale cGMP batches.

Introduction

Polymorphism[1] is defined as the ability of a substance or compound to crystallize into different, yet chemically identical, crystalline forms. In the pharmaceutical industry, the significance of polymorphism was realized recently through some relatively high-profile cases.[2] In particular, the unexpected appearance in early 1998 of a more thermodynamically stable form (Form II) of ritonavir[2] (Norvir, Abbott Laboratories, protease inhibitor for the treatment of HIV), with different dissolution properties compared to those of the earlier commercial Form I. Form II is <50% as soluble as Form I, resulting in the observed poor dissolution behavior and eventual withdrawal of the capsule from the market. This incident had serious implications for the marketed product and the patients receiving the drug.[2a,b] The project was suspended until a modified procedure was found. Renitidin, sertraline, and frentizole are some important examples of pharmaceuticals that exhibit polymorphism.[3] These incidents have led to an increased awareness of the importance of early-stage polymorph identification and characterization. It is evident from the number of publications and patents being granted that polymorphism is a topic of high importance for the pharmaceutical industry. To cite a few: a publication on a polymorph study of the L-arginine salt of ragalitazar describes evaluation of its 12 polymorphs[4] and a paper about sertraline[3] describes eighteen polymorphic forms assessed via high-throughput crystallization. There were over 3600 crystallizations conducted during the course of this study.[5] United States patent U.S. 5,700,820[6] discloses six polymorphs of troglitazone; U.S. 5,248,699[7] discloses five polymorphic forms of sertraline hydrochloride (Zoloft); European patent EP 490648[8] describes four polymorphic forms of frentizole; and EP 022527[9] also deals with the subject of polymorphism in drugs.

Scheme 1



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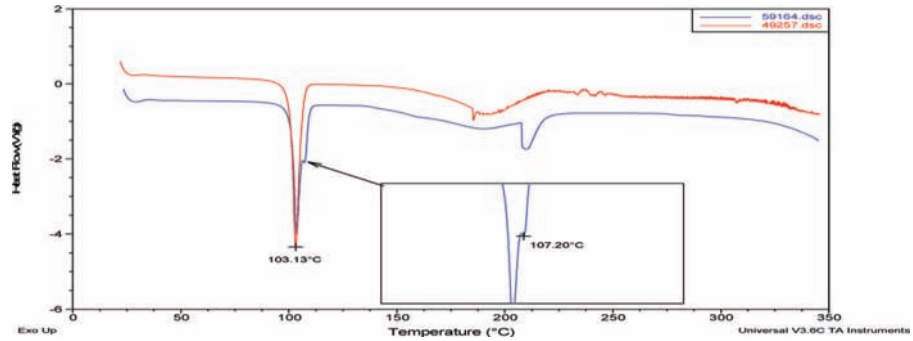

Figure 1. **DSC overlay of treprostinil diethanolamine (top to bottom) and sample after storage.**

Treprostinil (**1**, **UT-15**) (Scheme 1) belongs to a class of stable analogues of PGI_2 called benzindene prostacyclins.[10] **UT-15** (**1**) is effective in the treatment of pulmonary arterial hypertension (PAH), a debilitating and often fatal lung disease, and has been approved by the FDA for treatment of PAH.[11] **UT-15** is delivered subcutaneously or intravenously via a microinfusion device, has a relatively short biological half-life and is not degraded upon passage through the lungs.

The goal of this project was to indentify an oral prostacyclin analogue for the treatment of PAH that was bioavailable, soluble in water, and easy to deliver. Various salts of **UT-15** (**1**) were screened, and the treprostinil diethanolamine salt (**UT-15C**, **3**) showed promising physical characteristics for formulation as an oral drug.

Polymorphism. Two polymorphic forms of **UT-15C** (**3**), Form A and Form B, have been identified to date. Preparation of early developmental batches of **UT-15C** produced Form A. However, upon storage, some of Form A partially converted to Form B to form a mixture of Forms A and B (based on melting point and confirmed by differential scanning calorimetry (DSC) and XRPD data; Figures 1 and 2). On the basis of these observations, it was hypothesized that Form B was thermodynamically more stable and Form A was a metastable form, but kinetically crystallized more readily.

This observation was also further supported by solubility and heat of solution results. According to the "Oswald rule of stages",[12] often in crystallization processes a metastable form crystallizes from the solution initially and transforms to a more stable form at a rate specific to the compound, depending upon the relative solubility of the two phases in the solvent system. This phenomenon is widely observed with many active pharmaceutical ingredients (APIs) in the pharmaceutical industry. The melting temperatures of Form A (T_m^A) and Form B (T_m^B) were about 103 and 107 °C, respectively, and the measured heat of fusion for Forms A and B were 109.0 J/g (53.955 kJ/mol) and 109.2 J/g (54.054 kJ/mol), respectively.

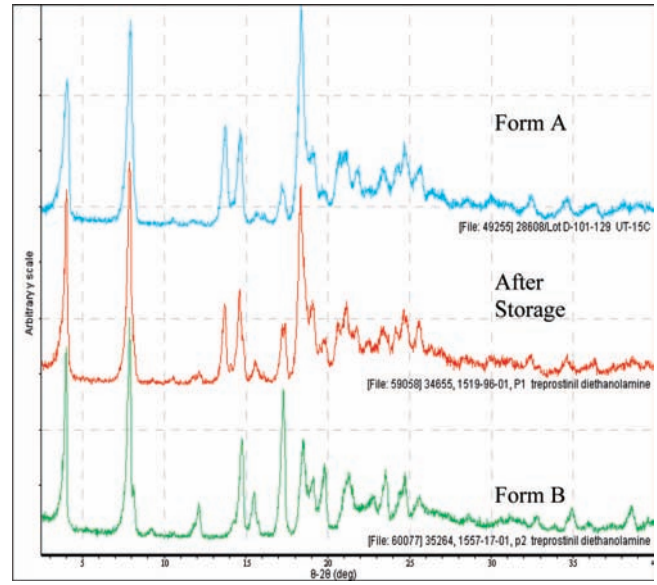

Figure 2. **X-ray powder diffraction (XRPD) pattern comparison of treprostinil diethanolamine salt (UT-15C) Form A, Form A after storage, and Form B.**

The synthesis of **UT-15C** (**3**), faced a number of challenges during the early development of the final crystallization step. The first problem to overcome was the tendency of the compound to oil-out (formation of gummy-mass) by finding the right solvent ratio. The second obstacle was designing a crystallization process that produced the desired form (Form B) consistently.

In light of the above-mentioned issues, it was important to develop a more controlled crystallization process to achieve only one form and desired morphology from a formulation standpoint. This paper describes the problems faced during the crystallization development and provides the findings and solutions that successfully resulted in a robust crystallization process for **UT-15C**, producing the desired form with desired particle properties (Figure 3 shows the overlay of XRPD pattern of Form A and Form B). The peaks at 13.7° 2θ and 17.2° 2θ were the characteristic values for Forms A and B, respectively, in the XRPD analysis.

Form A is a crystalline material that melts at 103−104 °C. Form B is a crystalline form that melts at a higher temperature, 106−108 °C, and was observed to form under a variety of conditions (Figure 4 shows the DSC and thermogravimetric analysis (TGA) of Form A and Form B). Evaluation of the

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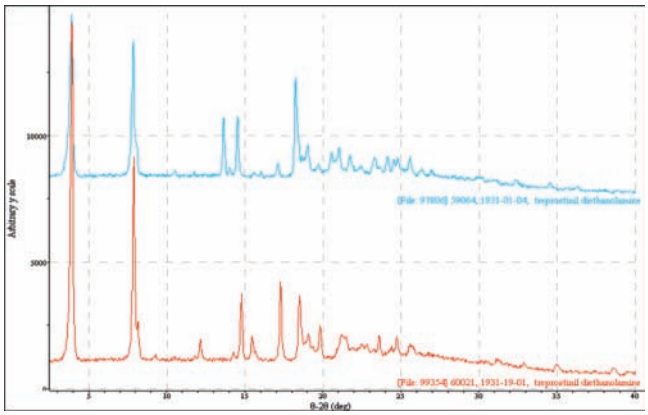

Figure 3. **Overlay of XRPD pattern of Form A (top) and Form B (bottom).**

relative thermodynamic relationships of Form A and Form B indicated that Form B was the more thermodynamically stable form. The energy difference between the two forms was found to be about 0.2 J/g (0.1 kJ/mol). The crystal structures of the two forms of **UT-15C** appear to be very similar, and the small differences in the large lattice parameters account for the similar stabilities of **UT-15C** Forms A and B. The experimental XRPD patterns of Forms A and B were analyzed to provide unit cell parameters for each form.

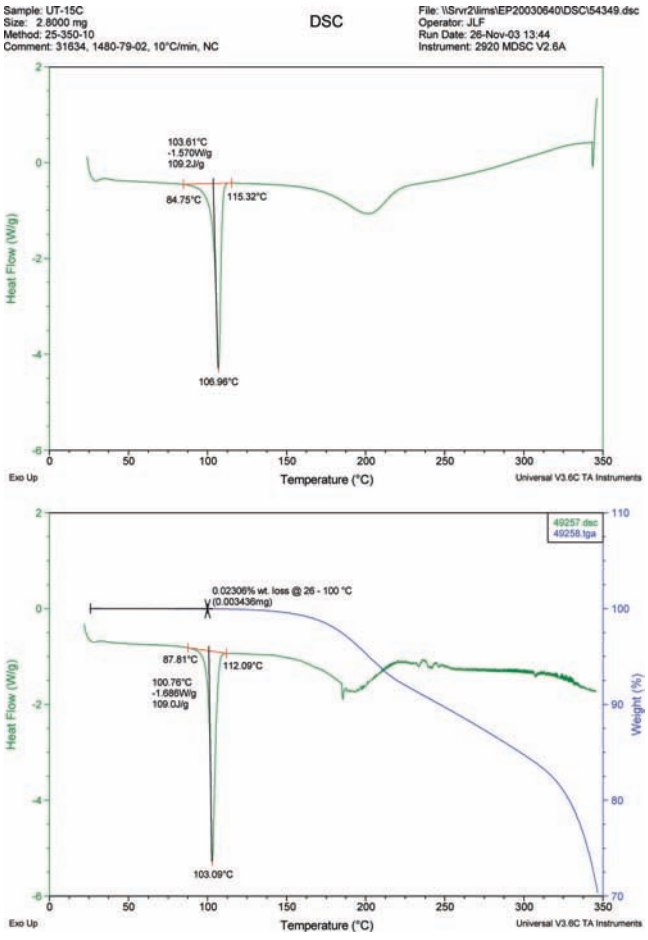

Figure 4. **DSC of Form B (top) and DSC and TGA of Form A (bottom).**

The experimental XRPD patterns of Form A and Form B were indexed using the SSCI indexing software (version 1.8.4) and DICVOL. The indexing method searches for crystal unit cells initially containing one molecule per asymmetric unit and then proceeds by increasing the number of molecules per asymmetric unit until viable solutions are found. The indexing begins with the highest orthorhombic symmetry and then proceeds to lower symmetries through to monoclinic and triclinic. Orthorhombic solutions for each form were independently found that describe all of the measured peaks in each experimental XRPD pattern within a 2% error in precision. The space group and unit cell dimensions for each form can initially be described as:

Form A: $P2_12_12_1$, $a = 45.736$ Å, $b = 12.737$ Å, $c = 4.704$ Å, volume $= 2740$ Å^3

Form B: $P2_12_12_1$, $a = 45.212$ Å, $b = 12.482$ Å, $c = 4.811$ Å, volume $= 2715$ Å^3

The unit cell parameters were refined and electron density models were evaluated using MAUD. Based on the possible indexed unit cells the measured XRPD patterns were fit to find solutions which provide the best description of the measured data. These unit cell results present the smallest and most precise determination of unit cell volumes and improve upon the initial precision to within 0.5% resolution limit.

Form A: $P2_12_12_1$, $a = 45.3676$ Å, $b = 12.6856$ Å, $c = 4.6893$ Å, volume $= 2699$ Å^3

Form B: $P2_12_12_1$, $a = 45.1804$ Å, $b = 12.4707$ Å, $c = 4.8283$ Å, volume $= 2720$ Å^3

The initial indexing results indicate that Form B has a smaller volume. Upon refinement, unit cell results show the inverse is true. However, in each case, the volume differences fall within the precision error or resolution limit of the calculation method. This indicates that the unit cell volumes are actually nearly identical from an XRPD perspective.

For structures which appear to be so similar (Forms A and B), the differences in the large lattice parameters determine stability. The largest lattice parameter corresponds to the weakest bond direction and, therefore, the most likely to fail (Donnay−Harker).[13] This indicates that Form B is the more stable form, but only by a fractional amount. The modified Donnay−Harker[13] theory predicts the same morphology for both Forms A and B, and we observed that Forms A and B were similar (needlelike) as predicted (Figure 5). Both forms readily dissolve in water with solubilities greater than 500 mg/mL (pH 6.95).

Form A has hydrogen bonds linking cations together along the shortest crystallographic *c*-axis and the anions together along the medium crystallographic *b*-axis (Figure 6). Although these hydrogen-bond networks give an indication as to the origin of differences between the two forms, it must be pointed out that the unit cell values for Forms A and B suggest that the bonding networks should be reversed (the unit cell values have higher precision than the placement of hydrogen bonds). In Form B (Figure 7), the *c*-axis is longer, indicating a weaker bond direction, and in Form A, the *b*-axis is shorter, indicating a stronger bond direction.

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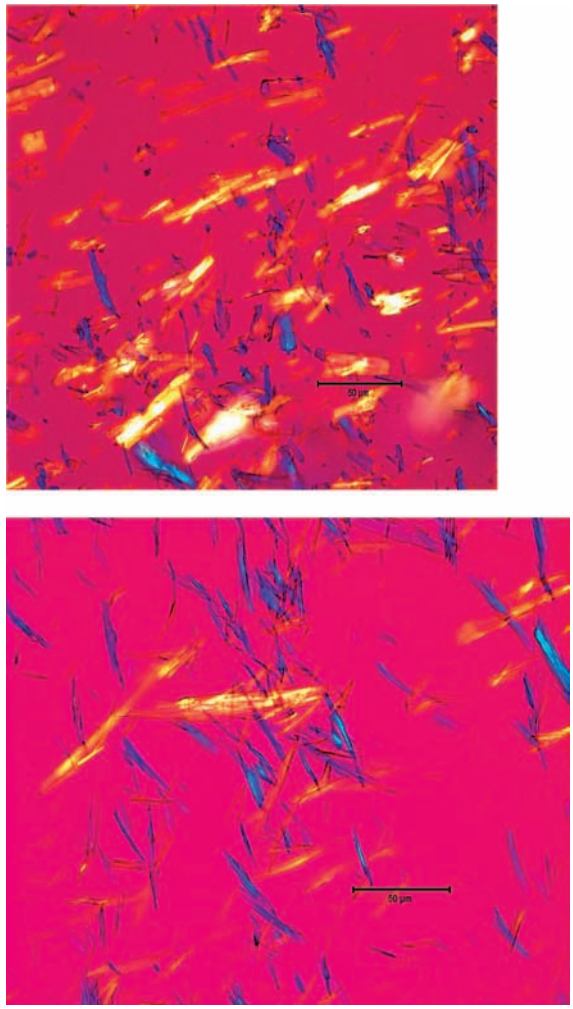

Figure 5. **Optical microscope images of crystals Form A (top) and Form B (bottom).**

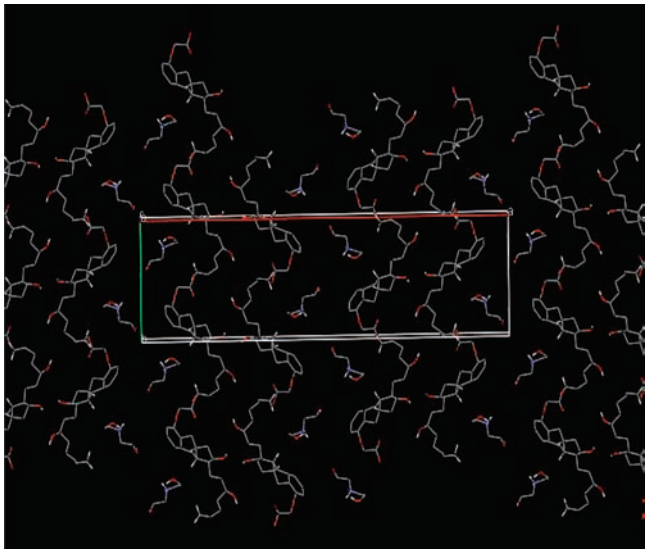

Figure 6. **Packing diagram of UT-15C Form A viewed down the *c*-axis.**

Results and Discussion

Various methods for obtaining polymorph B were considered, and a large number of experiments were conducted using several solvents with emphasis on slurry and crystallization experiments. Table 1 shows the solubility data of **UT-15C** (**3**)

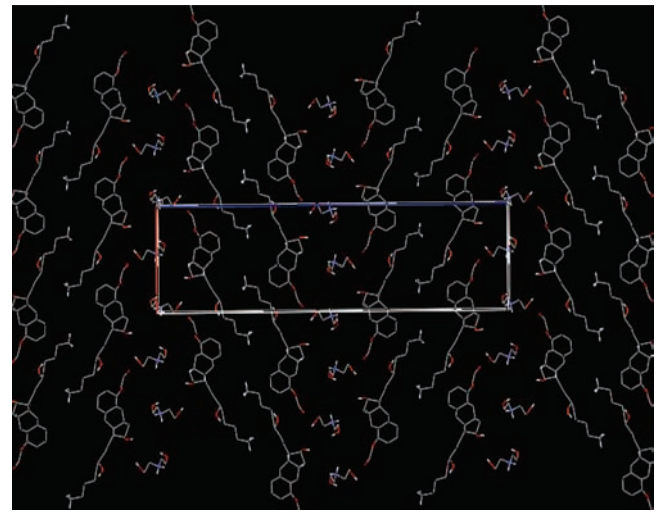

Figure 7. **Packing diagram of UT-15C Form B viewed down the *b*-axis.**

in various solvents, Form A and Form B did not have noticeable differences in solubility. On the basis of these solubility data, slurry, and crystallization experiments were conducted to obtain Form B exclusively.

Table 1. **Solubility of treprostinil diethanolamine (UT-15C) at 25 °C**

solvent	solubility (mg/mL)
acetone	2
ethanol/acetone (1:5)	9
ethanol/acetone (1:6)	6
ethanol/acetone (1:7)	5
ethanol/acetone (1:8)	3
ethanol (EtOH)	110
ethyl acetate (EtOAc)	1
ethanol/ethyl acetate (1:5)	3
ethanol/ethyl acetate (1:6)	2
ethanol/ethyl acetate (1:7)	1
ethanol/ethyl acetate (1:10)	<1
1,4-dioxane	<3
2-propanol (IPA)	9
methyl *tert*-butyl ether (MTBE)	<3
ethanol/MTBE (1:7)	<2
tetrahydrofuran (THF)	3
toluene	<2
water	>500
IPA/MTBE (1:1)	13
IPA/MTBE (1:2)	5
IPA/MTBE (1:3)	2
IPA/MTBE (1:5)	1

Several slurry preparations of **UT-15C** in various solvent/antisolvent ratios and solvent volumes were performed. Initially, the conversion from Form A to Form B occurred within 23−26 h at lower solvent volumes of isopropyl alcohol (4 mL IPA/g) at both 1:1 and 1:2 ratios of isopropyl alcohol (IPA) and methyl *tert*-butyl ether (MTBE). No conversion was observed using higher solvent volumes 8 mL/g slurry and 12 mL/g slurry utilizing the 1:1 and 1:2 IPA/MTBE solvent system (Table 2). The two forms were evaluated for their relative thermodynamic stability by slurry interconversion experiments conducted in a mixture of IPA and MTBE at various temperature conditions for several hours.

Form A was completely converted to Form B as confirmed by XRPD (Figure 8), and DSC (Figure 9). Initial studies

Table 2. **Slurry preparation attempts of treprostinil diethanolamine Form B using isopropyl alcohol/methyl *tert*-butyl ether (IPA/MTBE) at 25 °C**

solvent ratio (v/v)	solid/ solvent ratio (w/v)	slurry time (h)	XRPD result
–	–	0	A + B
1:1	1:4	5.25	A + B
		7.25	A + B
		23.25	B
1:1	1:8	1	A + B
		7	A + B
		24	A + B
1:2	1:4	18.5	A + B
		26	B
1:2	1:8	1	A + B
		2.5	A + B
		6	A + B
		23	A + B
1:2	1:12	1	A + B
		23	A + B
1:3	1:12	1	A + B
		5	A + B
		24	A + B
1:5	1:12	1	A + B
		5	A + B
		24	A + B

produced Form B by slurry experiments using a mixture of IPA and MTBE, but the process was not reproducible on large scale. From these trials, it was concluded that some of the conditions were not appropriate for obtaining only Form B and could be ruled out (i.e., fast cooling and crashing the compound out at low temperature always gave the less stable Form A).

Concurrent to the slurry experiments, several crystallization systems were examined to determine if they would exclusively provide Form B on a consistent basis. As Form B was thermodynamically more stable than Form A, it was important to isolate Form B and therefore, it was necessary to ensure that crystallization occurred slowly and in a controlled manner. Seeding with Form B prior to start of crystallization was helpful to obtain the desired form. Crystallization using a mixture of IPA/MTBE at various ratios was studied but less-stable Form A was obtained.

Development of a new crystallization protocol involved investigations of various solvent systems such as ethanol/acetone and ethanol/ethyl acetate. Both ethanol/acetone and ethanol/ethyl acetate solvent systems provided promising results. Various experiments were conducted using these two solvent systems, and various parameters were investigated to identify a process using either the ethanol/ethyl acetate or ethanol/acetone solvent systems that would consistently produce Form B. The variables studied included: (i) solvent ratio, (ii) seeding with Form B, and (iii) cooling rate during crystallization. Using a 1:7 ratio of ethanol/acetone and seeding with 1% Form B at 40 °C provided Form B with high quality and yield (>90%) as confirmed by XRPD and melting point data. Using various ratios of ethanol/acetone such as 1:5 provided predominantly Form A, and 1:6 provided predominantly Form B; however, yields were slightly lower (85−90%) than using a ratio of 1:7. When a ratio of 1:8 ethanol/acetone was used, a mixture of Forms A and B was obtained as confirmed by melting point. When crystallization was performed without any seeds of Form B, a mixture of

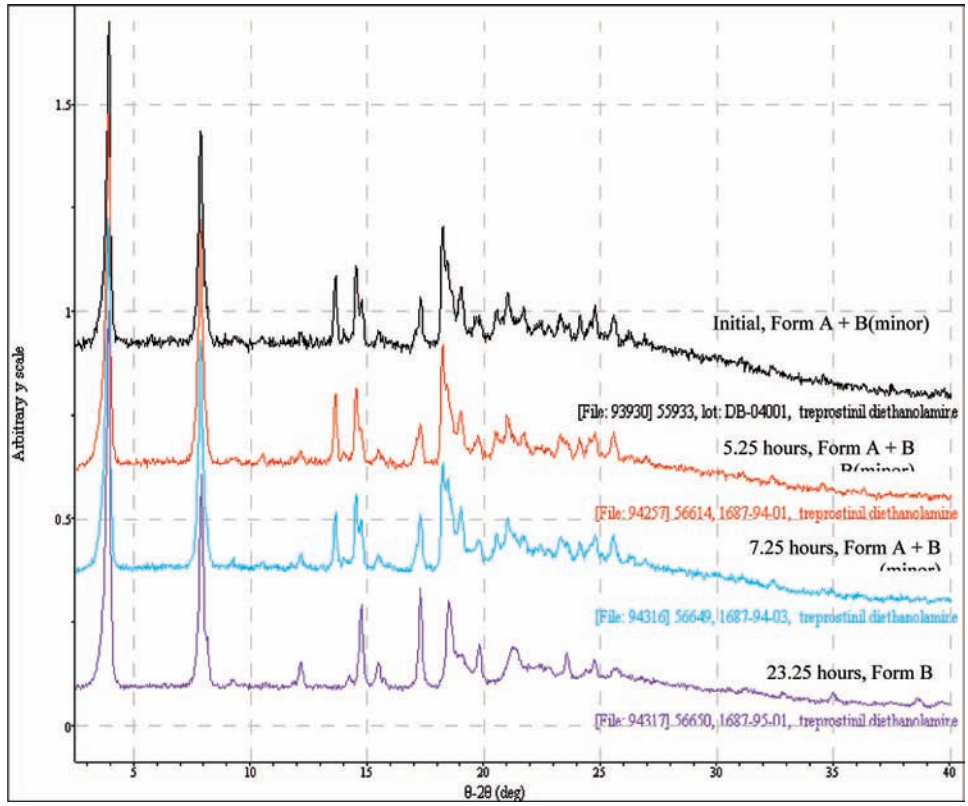

Figure 8. **XRPD Patterns of UT-15C samples from 1:1 IPA/MTBE, 4 mL/g slurry (top to bottom: initial, 5.25 h, 7.25 h, and 23.25 h).**

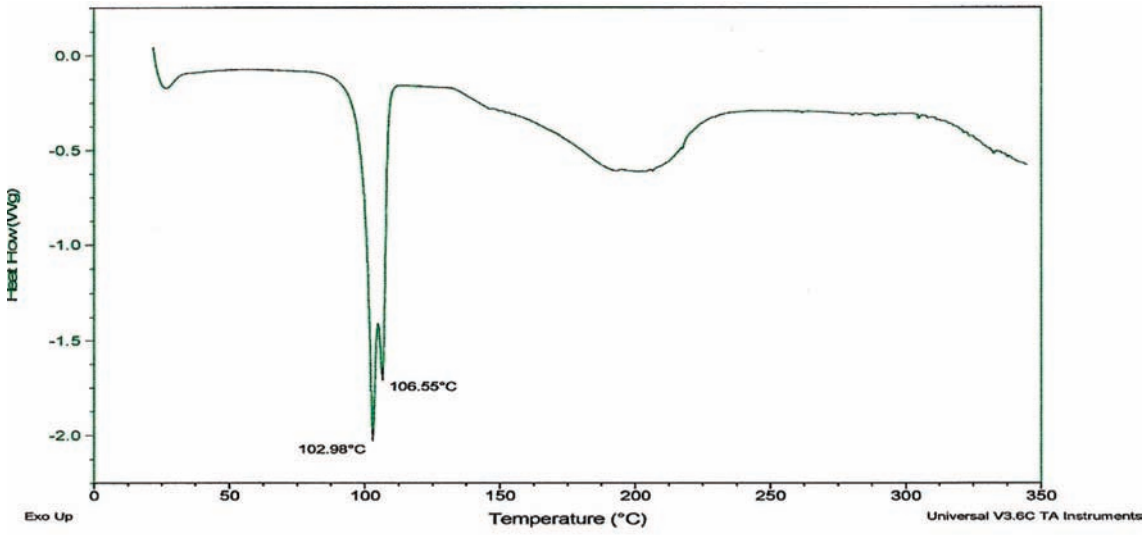

Figure 9. **DSC thermogram of treprostinil diethanolamine (UT-15C; mixture of Forms A and B).**

Table 3. **Summary of various crystallization conditions and the resulting form(s) as determined by XRPD**

entry	solvents	ratio v/v	Form A or B	seed of Form B (%)
1	EtOH/acetone	1:5	A major	1
2	EtOH/acetone	1:6	B major	1
3	EtOH/acetone	1:7	B	1
4	EtOH/acetone	1:7	A major	no seed
5	EtOH/acetone	1:8	A + B	1
6	EtOH/EtOAc	1:5	A + B	1
7	EtOH/EtOAc	1:6	A + B	1
8	EtOH/EtOAc	1:7	B	1
9	EtOH/EtOAc	1:7	A major	no seed
10	EtOH/EtOAc	1:10	A major	1

Forms A and B was obtained with greater percentage of Form A (Table 3).

From these experiments, it was obvious that two conditions were required to produce Form B: (i) seeding with Form B to induce nucleation and (ii) maintaining a 1:7 ratio of ethanol/acetone while controlling temperature. Nucleation was induced, at a 1:7 ratio of ethanol/acetone, by seeding to the clear solution with 1% Form B at 40 °C. At these conditions, most of the seeds remain undissolved to induce nucleation. Once nucleation started, cooling was stopped for ~1 h to allow the crystallization to progress at low supersaturation. Hence, the crystallization occurred under controlled conditions. As the crystallization proceeded, more crystals started to grow, and at this stage the solution was kept at 35 °C for 14 h and then cooled slowly to ambient at a rate of 3 °C/h. This crystallization process provided Form B consistently. In later experiments, once nucleation was induced by adding 1% seed of Form B at 40 °C to a clear solution of **UT-15C**, cooling was stopped for ~1 h to allow the crystallization to progress at low supersaturation. At this stage the mixture was cooled to ambient conditions at a cooling rate of 1 °C /h, and this crystallization process provided Form B consistently.

Various ratios of ethanol/ethyl acetate such as 1:5 or 1:6 provided a mixture of Forms A and B, and yields were slightly lower (85−90%) than using a ratio of 1:7 (Table 3). A ratio of ethanol/ethyl acetate, 1:7, produced Form B as the major form as confirmed by XRPD and yields were in the range of 90−95%. Crystallization of **UT-15C** using a 1:7 ratio of either ethanol:acetone or ethanol/ethyl acetate combined with 1% polymorph B seeding at 45−50 °C provided Form B. However, one noteworthy point is the crystallization from ethanol/acetone provided a denser material as compared to a light, fly-away material obtained from crystallization using ethanol/ethyl acetate. Table 3 summarizes the results of various conditions used to obtain Form B.

Finally, ethanol/acetone was selected as the solvent system for final recrystallization of **UT-15C** because of the acceptable physical properties of the final material. As scale-up of this crystallization process continued, the yields and form purity improved, but the particle obtained was rod-shaped (Figure 10) which was not ideal for formulation development.

A dry-milling operation was not desirable for this compound because **UT-15C** (**3**) is a highly potent prostaglandin mimic,

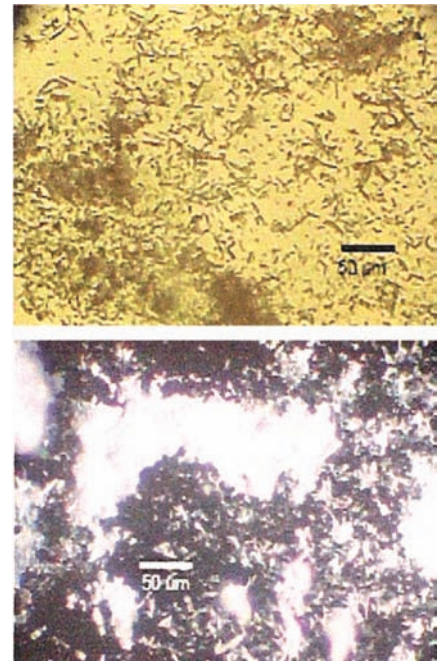

Figure 10. **Particle shape image before heptane slurry.**

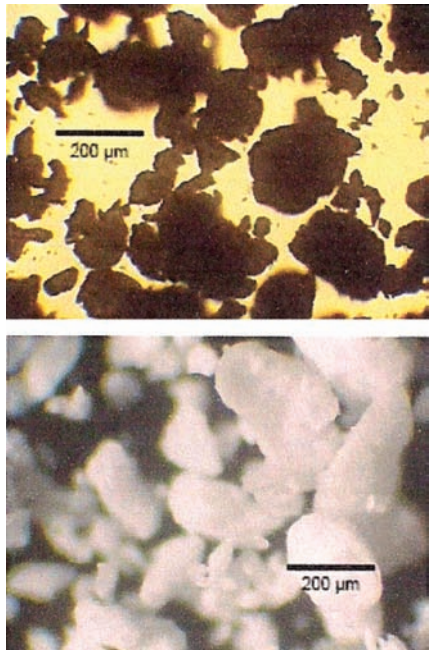

Figure 11. **Particle shape image after heptane slurry.**

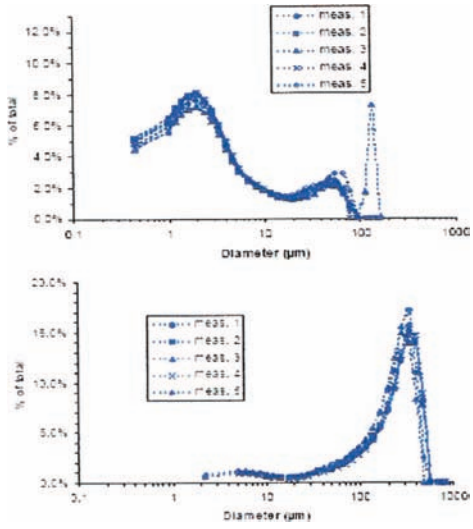

Figure 12. **Particle size distribution before heptane slurry (top) and after heptane slurry (bottom).**

and any acute exposure would be highly undesirable. The subsequent drug product formulation incorporated a wet granulation process, and as a result, a milling step was not required to control particle size after synthesis of the API.

UT-15C obtained after the synthesis/crystallization from ethanol/acetone was subjected to a heptane slurry at 75−78 °C to obtain the desired particle habit. On the basis of small-scale experiments this process was scaled up to kilogram scale, and the nature of material obtained from the heptane slurry was granular and denser (Figure 11) as compared to the rod-shaped material before the heptane slurry (Figure 10).

Microscopically, before heptane slurry, **UT-15C** appeared to be composed of fine, rodlike particles and large aggregates of particles; after heptane slurry it appeared to be composed of large agglomerates with a low level of fines (Figure 12). The microscopic appearances were generally consistent with the particle sizing data (Figure 12). This observation provided a hypothesis that the particle characteristics change after initial crystallization (1:7 ratio of ethanol/acetone) and subsequent slurry (Figure 11). The particle characteristics obtained after heptane slurry were more suited for formulation development.

In conclusion, two crystalline forms of **UT-15C** were studied. There was only 0.1 kJ/mol in enthalpy difference between the two crystalline forms. A solubility difference was observed between forms, but this solubility difference is not expected to influence bioavailability from a solid-dosage form. Form B was the more stable form, and both ethanol/acetone and ethanol/ethyl acetate as crystallization solvents consistently provided Form B of **UT-15C**. The use of ethanol/acetone for crystallization provided a denser material as compared to crystallization from ethanol/ethyl acetate. Finally, ethanol/acetone was selected as a solvent system for the final crystallization of **UT-15C** because of the acceptable physical properties of the final material. Performing a heptane slurry operation on crystallized **UT-15C** gave the desired granular and compact particle shape as compared to the rodlike material before the heptane slurry.

3. Experimental Section

X-ray powder diffraction (XRPD) analyses were performed by SSCI Incorporation using Shimadzu XRD-6000 X-ray powder diffractometer. Thermogravimetric analyses (TGA) and differential scanning calorimetry (DSC) were performed using TA Instruments 2950 thermogravimetric analyzer and TA Instruments 2950 differential scanning calorimeter, respectively. XRPD, TGA, and DSC analyses were performed by SSCI Incorporation. Particle size analysis and microscopy of treprostinil diethanolamine (**UT-15C**) were performed by Cirrus Pharmaceutical Inc. Melting temperatures were determined using a capillary tube melting point apparatus and are uncorrected. The following experimental procedure was used for large-scale production of Form B of **UT-15C**.

3.1. Experimental Procedure using Ethanol/Acetone Solvent System. To a suspension of **UT-15** (1200 g) in ethanol (1000 mL) was added a solution of diethanolamine (352 g) in ethanol (2200 mL). The mixture was heated to a clear solution at 50 °C. The warm solution was filtered to remove any suspended, insoluble materials, and the clear solution was transferred to 50-L jacketed reactor. It was heated to 65 °C, and acetone (34 L) was slowly added in portions while maintaining the temperature of the reaction solution between 45−55 °C. At this temperature, the clear solution was stirred for 0.5 h. The temperature of the solution was decreased to 40 ± 2 °C over a period of 2 h. At this temperature, the reaction was seeded with Form B (12 g), and the solution was stirred for 2 h at 40 ± 2 °C. The temperature of the reaction was decreased to 35 °C over 2 h and then stirred at 35 °C overnight. The following day, the reaction temperature was decreased to 22 °C over a period of 4 h. The reaction mixture was stirred at this temperature overnight. The third day, the reaction mixture was cooled to 10 °C over a period of 1 h in order to maximize recovery of **UT-15C**. At 10 °C,

UT-15C was isolated by filtration using an Aurora filter. The cake was washed with acetone (18 L), and the **UT-15C** was dried in the Aurora filter for 2 h under house vacuum. It was then transferred into trays for air-drying overnight. At this stage, the melting point of the free-flowing **UT-15C** (106 °C) indicated Form B was predominantly present (≥98.5%). This was also confirmed by XRPD data. The weight of the **UT-15C** was 1414 g (94%). This product was slurried in heptane (12 L) at 50−55 °C for 14−16 h to change its crystal morphology. After the heptane slurry and drying, the melting point of this batch increased to 106−108 °C.

3.2. Experimental Procedure using Ethanol/Ethyl Acetate Solvent System. A 50-L cylindrical reactor equipped with a heating/cooling system, a mechanical stirrer, a condenser, and a thermocouple was charged with a solution of **UT-15** (1250 g, 3.20 mol) in ethyl acetate (40 L), and a solution of diethanolamine in ethanol (337 g of diethanolamine was dissolved in 5.1 L of ethanol, 3.20 mol). While stirring, the reaction mixture was heated to 60−75 °C for 0.5−1.0 h to obtain a clear solution. The clear solution was cooled to 50 ± 5 °C. At this temperature, some seeds of Form B of **UT-15C** (∼12 g) were added to the clear solution. The suspension of polymorph B was stirred at this temperature for 1 h. The suspension was cooled to 20 ± 2 °C overnight (over a period of 16−24 h). The resulting **UT-15C** was collected by filtration using an Aurora filter equipped with a filter cloth, and the collected solid was washed with ethyl acetate (2 × 8 L). The **UT-15C** was transferred to a HDPE/glass container for air-drying in a hood, followed by drying in a vacuum oven at 50 ± 5 °C under high vacuum for 1−2 h. The weight of the **UT-15C** was 1 507 g (95%), mp 104−105 °C. XRPD data indicated Form B was predominantly present (≥98.5%).

3.3. Experimental Procedure for Heptane Slurry. A 50-L cylindrical reactor equipped with a heating/cooling system, a mechanical stirrer, a condenser, and a thermocouple was charged with a slurry of **UT-15C** (3071 g, obtained from the EtOH/EtOAc solvent system, mp 104−105 °C) in heptane (36 L). The suspension was heated to 70−75 °C for 16 h. The suspension was cooled to 22 ± 2 °C over a period of 1−2 h. **UT-15C** was collected by filtration using an Aurora filter. The cake was washed with heptane (15−30 L) and the material was dried in the Aurora filter for 1 h. **UT-15C** was transferred to trays for air-drying overnight in a hood, followed by vacuum drying at 50 °C under high vacuum for 1 h to obtain 3.0 kg of **UT-15C** (99%), mp 105.0−106.5 °C

3.4. Experimental Procedure for Preparation of Form B Seed. To a suspension of **UT-15C**(8.5 g, mixture of Forms A and B) in MTBE (40 mL) was slowly added IPA in four portions (4 × 40 mL) over a period of 2 h while stirring. The slurry was stirred at ambient temperature (∼22−25 °C), and the conversion of Form A to Form B was observed by recording the XRPD after regular intervals (an aliquot was taken from the slurry, filtered, and dried for XRPD). After stirring for 24 h, the slurry was filtered in vacuo, dried at room temperature to obtain Form B (8 g, mp 106−108 °C, confirmed by XRPD)

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