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Research Paper

Increasing the Dissolution Rate of Itraconazole Processed by Gas Antisolvent Techniques using Polyethylene Glycol as a Carrier

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Purpose. The purpose of this study was to increase the dissolution rate of the poorly water soluble, antifungal drug Itraconazole.

Methods. Itraconazole was successfully micronized using both the gas antisolvent (GAS) and aerosol solvent extraction systems (ASES) using Acetone as the solvent. The affects of operating conditions such as temperature, pressure and solvent choice on variables such as morphology, particle size and dissolution were investigated. The influence of temperature in the range 25 to 40°C and pressure between 90 and 190 bar were investigated.

Results. Solvent choice was found to have the largest affect on particle production, with acetone found to be the optimal solvent choice when compared with dimethyl formamide (DMF), tetrahydrofuran (THF) and dichloromethane (DCM). Itraconazole particles with an average particle size of 6.9 μm were formed at the optimal ASES processing conditions of 40°C and 190 bar. More significantly, in the first 100 minutes of dissolution 71.1% of the dense gas processed itraconazole was dissolved compared with 52.5% of Sporonox (the commercially available formulation) and 14.6% of the unprocessed material. Additional studies demonstrated that the formation of an itraconazole/PEG composite resulted in a 6-fold increase in dissolution rate in the first 100 min, to 89.8%, when compared to the unprocessed material.

Conclusions. Using ASES, microparticles of itraconazole were produced with an increased dissolution rate compared with raw material and commercially available product.

KEY WORDS: dissolution rate; gas antisolvent; itraconazole; polyethylene glycol; supercritical fluids.

INTRODUCTION

Many pharmaceutical compounds exhibit low bioavailability due to poor solubility in aqueous media. The Biopharmaceutical Classification System categorises drugs into four categories based on their solubility and permeability. Class II drugs are classified as having low solubility and high permeability (1). The oral bioavailability of these drugs is more likely to be dissolution dependent with the *in vitro* dissolution rate being similar to the *in vivo* dissolution rate unless the dosage amount is very high (2). Due to its low solubility in water of less than 1 μg/ml, and low bioavailability of around 50%, itraconazole is classified as a class II pharmaceutical compound (2).

Itraconazole is a synthetic triazole antifungal drug used in the treatment of mycotic infections caused by organisms that are untreatable by topical therapy. Despite the excellent *in vitro* anti-fungal effect of itraconazole it has been described as "practically impossible to apply to the body" (3). The relatively low potency of itraconazole necessitates the need for large dosages of 200–400 mg (4). Itraconazole is marketed as Sporanox in two oral dosage forms; as a capsule and as an oral emulsion formulation. The observed absolute oral bioavailability of the capsules is 30% whereas the emulsion formulation has a bioavailability of approximately 55% (5). The relative low solubility and thus oral bioavailability of itraconazole presents a great opportunity for the drug to be improved.

The formation of drug–polymer composites can have a significant impact in the pharmaceutical industry for dry formulations. Polymer blends have been used in drug delivery to protect the drug from its surroundings, to manipulate the drug properties, to increase the drug stability, to increase the solubility of the drug, to target the drug to a specific part of the body and in controlled drug release formulations (6–8). Polymers form composites with drugs by coprecipitation, encapsulation and complex formation mechanisms. The choice of polymer for use in drug–polymer composites is largely dependent upon approval of the polymer as safe by the appropriate regulatory body (the Food and Drug Administration (FDA) in the USA and the Therapeutic Goods Association (TGA) in Australia). The polymer will be non-toxic, most often biodegradable and have properties suitable to the desired application such as the ability to increase solubility or wettability of the pharmaceutical.

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Polyethylene Glycol (PEG) is a polymer synthesised to the desired molecular weight by the polymerisation of ethylene oxide. PEGs of molecular weight above 3000 are generally flaked solids and are available in molecular weights up to 35000 Daltons. Due to their outstanding toxicological safety, PEGs are used in the cosmetic and food industries and are approved for use in the pharmaceutical industry up to a daily maximum dose of 10 mg/kg body weight. In the pharmaceutical industry PEG has been used as an excipient and as an active ingredient. PEG has been used as an ophthalmic demulcent, laxative and in the preservation, protection and restoration of cells, tissue or organ function (9). More commonly PEG is used as an excipient; in ointment and suppository bases and in tablets. In tablets PEG may act as a binder, lubricant, be used for tablet coating or as a carrier, solubilizer and to manipulate the dissolution properties of an Active Pharmaceutical Ingredient (API) (9).

The Mechanism by which PEG increases the dissolution rate of an API has been the subject of many studies (10–13). Mechanisms that have been identified include improved aqueous solubility due to a reduction in the incorporated API particle size in the formation of solid dispersions, transformation of the drug from a crystalline to amorphous form, formation of complexes, reduction in aggregation and agglomeration, the formation of solid solutions, improved wetting properties of the drug and solubilisation of the drug by the carrier at the diffusion layer (13). Verheyen *et al.* suggests that it is widely accepted that often more than one of the suggested mechanisms is responsible for the increase in dissolution rate (13).

Polyethylene Glycol has been proven to increase the dissolution rate of some pharmaceutical compounds. Clofibrate, a pharmaceutical used to lower cholesterol, had an increased dissolution rate when processed as a solid dispersion with PEG. The dissolution rate of clofibrate increased as the molecular weight of the PEG was increased with the best results obtained with PEG 20000 (14,15). Wantanabe *et al.* prepared granules of nifedipine and PEG by direct tablet compression. Nifedipine incorporated with PEG 6000 and PEG 20000 was found to rapidly dissolve with complete dissolution, in pH 1.2 medium, being attained at 60 min (16).

Micronization is another technique that can be used to increase the bioavailability of pharmaceuticals. Conventional techniques for the manufacture of pharmaceuticals can be placed into three categories; mechanical methods, physical methods and physico-chemical methods. Mechanical methods can include crushing, grinding, granulation and spray formation. Grinding can result in particles as small as several microns, however this process can often result in a product that has a broad particle size distribution. Granulation can result in a loss of microscopic homogeneity and the additives used may have a negative impact on disintegration and dissolution performance (17). Wet granulation can produce spherical-like, cylindrical-shaped, free-flowing multiparticulate systems with a constant composition and mean particle size of 0.1 to 2 mm. However wet granulation is an expensive, complex, multi-stage process and is not suitable for use with heat sensitive pharmaceuticals as they may be destroyed or degraded in the final drying stage (18). Spray drying, one of the most common conventional mechanical methods for producing pharmaceuticals, can be used for particle production and microencapsulation. Traditionally, spray drying typically resulted in spherical or regularly shaped particles 50 to 500 μm. However, advances in the technique have led to smaller micron and sometimes nano sized particles being produced. Problems can occur due to production issues such as over-heating brought about by wall accumulation and loss of volatiles in the drying process resulting in low yield (19). Furthermore, in order to remove the solvent from the drug after atomization, traditional spray drying techniques generally require extremely high temperatures, approximately 150°C, which can result in degradation of the active pharmaceutical ingredient (20). Physico-chemical methods such as crystallization produce particles larger than 1 μm in size. Additionally, crystallization is limited in pharmaceutical production by its inability to ensure solvent-free particles. Conventional techniques for micronization and coprecipitation of pharmaceuticals are often not ideal for use with many pharmaceuticals since the properties of the drug can be altered by thermal and mechanical stress. Moreover, conventional techniques often result in high residual solvent content, requiring further purification, and material with a broad and/or irregular particle size distribution (21). The use of dense gas technology for the precipitation of pharmaceuticals has been the topic of a number of recent studies. The technology has been used for the micronization of pharmaceuticals with low or no residual solvent, a narrow particle size distribution and, in the case of oral drugs, with increased dissolution rate profiles (21–30).

A dense gas is a fluid above or near its critical point and includes both supercritical and subcritical conditions. The main advantages of utilising dense gas technology in pharmaceutical processing are the reduction of solvent use, the subsequent reduction in residual solvent levels in the final product and the ability to create particles with a small particle size distribution. The unique tuneable solvation strength and transport property characteristics of dense gases are responsible for their successful application to pharmaceutical production. Several dense gas techniques have been developed for drug formulation, crystallisation and micronization. These include gas antisolvent techniques, depressurization of an expanded liquid organic solvent (DELOS), carbon dioxide assisted nebulization with a bubble dryer (CAN-BD), supercritical-assisted atomization (SAA), rapid expansion of supercritical solutions (RESS) and particles from gas-saturated solutions (PGSS) (21). In this study two gas antisolvent techniques, namely the gas antisolvent (GAS) process and aerosol solvent extraction system (ASES), were used to process itraconazole.

The ability of dense gases such as CO_2 to dissolve and expand or extract organic solvents, thus lowering their solvation power, allows for the precipitation of solids from organic solutions. Dense gases have been operated predominantly in two modes as anti-solvent. The first method, which is a semi-batch process known as the GAS process, involves the gradual addition of a dense gas to expand a solution until supersaturation occurs and the solute precipitates. Once the solute has been precipitated, additional dense gas is added to remove the residual solvent and produce a dry precipitate upon depressurization. The second technique can be conducted continuously, is known in various references as the aerosol solvent extraction system (ASES), supercritical antisolvent (SAS), solution-enhanced dispersion by supercritical

fluids (SEDS) and precipitation with a compressed antisolvent (PCA), and involves spraying a dispersion of an organic solution, containing the compound to be precipitated, via a nozzle, into a flowing or static dense gas.

Both the ASES and GAS processes have been used to re-engineer a number of pharmaceutical compounds. In gas antisolvent processes various process parameters can have a significant impact on the physiochemical properties of the precipitate. The choice of solvent can change the morphology, yield and particles size. The precipitation of copper indomethacin (Cu-Indo) from *N*-methyl-2-pyrrolidone (NMP) by the ASES process, at temperatures and pressures above 25°C and 6.9 MPa, resulted in the formation of two distinct particle morphologies being produced; namely large spheres with diameters of 20 μm and irregular particles of 5 μm. However when Cu-Indo was precipitated from DMSO the resultant particles were irregular platelet shaped (31). Reverchon and Della Porta used the SAS process to precipitate the antifungal drug griseofulvin (32). Griseofulvin precipitation from NMP proved unsuccessful with almost all the griseofulvin being extracted from the system with the solvent. However, long needles (>1 mm) were formed from both dichloromethane (DCM) and dimethyl sulfoxide (DMSO) (32). The difference in morphology caused by solvent choice can be attributed to the solvent–solute interaction. Further to this, a change in solvent can result in changes in vapour pressure, solute solubility, viscosity, phase behaviour and the mass transfer properties of the system.

Griseofulvin has also been precipitated using a modified GAS process involving a stirring device. Acetone was used as the solvent and the system was pressurised up to 100 bar at 1.3 bar/min. Two morphologies were obtained at low stirring rate (33 rpm) generating long needles ranging in length from 12–20 μm and 100–250 μm in width. As the stirring rate increased to 500 rpm, bipyramidal shape particles were produced with length 360–550 μm and width of 171–180 μm. The increase in stirring rate lead to both a switch in morphology from needles to bipyramid (33). Warwick *et al.* reported that the expansion rate of the solution in the GAS process was found to have a significant effect on the morphology of the particles produced. At operating conditions of 25°C and 5.8 MPa a slow expansion rate lead to the production of uniform rhombic shaped particles between 20 and 50 μm. Conversely when a rapid expansion rate was employed the Cu-Indo precipitate was a non-uniform mixture of rhombic and bipyramidal crystals as large as 100 μm (31). For drugs delivered orally the change in morphology of particles is significant as these changes can result in the dissolution rate of the final product being modified. However, in the case of some APIs, such as itraconazole it has been found that micronization alone is not enough to significantly increase the dissolution rate (4). Thus more recently dense gas processes have been used to co-precipitate pharmaceutical compounds with suitable polymers.

Processing itraconazole by dense gas techniques has been previously investigated by other authors. Nektar Therapeutics has applied for a patent for a series of formulations comprising itraconazole and processed by dense gas processes, namely GAS and SEDS (4). Formulations containing itraconazole and polyvinylpyrrolidone (PVP) or hydroxypropylmethylcellulose (HPMC) were produced. Production of itraconazole co-formulated with PVP, using tetrahyrofuran (THF) as the solvent, resulted in a dispersed particulate powder which was non-cohesive and easy-flowing. When blended with lactose the itraconazole:PVP formulation had a dissolution profile comparable to that of the commercially available product. Co-formulations of HPMC and itraconazole with a 1:1 drug ratio and using dichloromethane:methanol (1:1) as the solvent, resulted in a finely dispersed easy flowing powder. Similarly to the co-formulation formed with PVP this powder had a dissolution profile comparable to that of the commercially available product (4). Lee *et al.* produced solid dispersions of itraconazole and the hydrophilic polymer HPMC 2910 using the ASES process. The resulting solid dispersion had a particle size ranging from 100–500 nm and contained itraconazole in its amorphous state. The product formed had a dissolution profile similar to that of Sporonox (the commercial formulation) capsules (34,35). The SAS process was used to produce solid state inclusion complex powders of itraconazole and 2-hydroxyproply-β-cyclodextrin (HP-β-CD). The particle size of the inclusion complexes was found to be between 0.1–0.5 μm—a significant decrease from the untreated itraconazole which had a particles size distribution of 50–100 μm (36).

Itraconazole has previously been processed using supercritical carbon dioxide as the solute by a process similar to PGSS (37). Hassan *et al.* established this new method whereby a desired amount of sample was loaded into an extraction chamber and heated to 130°C. Supercritical fluid was then used to pressurise the cell to 300 atm and then constantly flowed through the system at a rate of 0.2–2 ml/min. After a desired period of 10 to 60 minutes the pressure was suddenly reduced to atmospheric. The product was then cooled in dry ice but there was no secondary solvent removal step (37). In this study by Hassan *et al.* an itraconazole formulation including PEG (M_W 20,000), HPMC, sodium starch glycolate and glycerol was developed. The dissolution rate of itraconazole was found to increase by approximately 10% as the molecular weight of PEG was increased from 3,350 to 20,000 (37). The supercritical carbon dioxide was then added to the formulation creating a highly porous powder when solidified. The dissolution rate was increased by increasing the operating pressure as a result of enhancing the dissolution of the CO_2 in the molten itraconazole formulation. In turn, the increased dissolution of the CO_2 resulted in an increase in surface area and porosity upon depressurisation (37). Complete dissolution, in gastric fluid, of itraconazole processed at 135°C and 300 atm, was achieved in 1 h. Comparatively when the same dissolution media was used 90% of the Sporonox dissolved in an hour (37).

The effect of using pressurised carbon dioxide as a temporary plasticizer and foaming agent on the hot stage extrusion process for the production of solid dispersions of itraconazole with polyvinylpyrrolidone-co-vinyl acetate 64 (PVP-VA 64) was investigated by Verreck *et al.*. (38) Amorphous dispersions were obtained. However, due to agglomeration of the particles, the initial dissolution rate in simulated gastric fluid, of those processed with CO_2 was slower. A maximum of 60% of the itraconazole was dissolved in 60 min (38).

Commercially available oral dosage forms of itraconazole have a relatively low bioavailability. Furthermore the dependence and thus variance of the bioavailability of

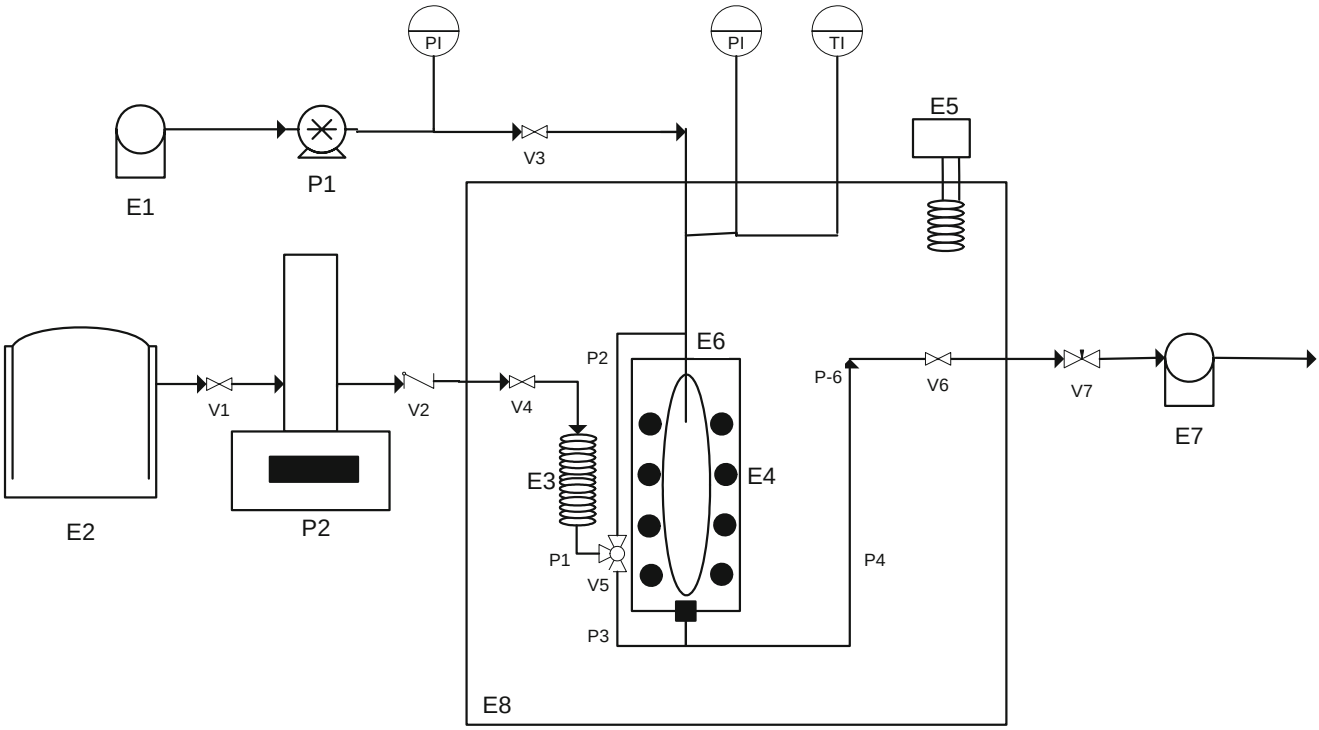

Fig. 1. Multipurpose ASES/GAS apparatus (*E1*, *E2* solution and gas reservoirs, *E3* heating coil, *E4* Jerguson, *E5* heater, *E6* nozzle, *E7* solvent trap, *E8* water bath, *P* pumps, *V* valves, *S* streams).

itraconazole caused by the consumption of food, or lack there of, can be problematic (4). These limitations of bioavailability coupled with the issues surrounding current complicated methods of production provide an opportunity to develop improved formulations of itraconazole. Some research using dense gases to process itraconazole has already taken place with significant and obvious improvements being made. However there remains the need to eliminate the use of class 2 solvents, the use of which should be limited in pharmaceutical production and to keep operating conditions, such as temperature, low to limit the chance of depleting the drug efficacy and to maintain the financial viability of processing. In this study, dense gas anti-solvent techniques, namely the GAS and ASES processes, were used to produce itraconazole and an itraconazole/PEG composite. Processing conditions were optimised and the use of various solvents was compared. The objective of the study was to improve the dissolution rate and thus *in vivo* bioavailability of itraconazole.

MATERIALS AND METHODS

Materials

Itraconazole was obtained from Australian Pharmaceutical Industries. Polyethylene glycol (PEG), with an average M_W of 20,000, was purchased from Fluka Chemie. HPLC grade dimethyl formamide (DMF) and dichloromethane (DCM) were purchased from Univar, tetrahydrofuran (THF) from Unichrom and Acetone from AnalR. Carbon Dioxide was acquired from Linde Gas Pty. Ltd. The sodium chloride (NaCl) and hydrochloric Acid (HCl) used for making the simulated gastric fluid (SGF) used in dissolution testing were both manufactured by Univar.

Melting Point Depression

Prior to processing itraconazole and PEG using dense gas processes it is important to establish what effect, if any, the elevated temperature and pressure will have on the compounds. Initial testing of the compounds is of particular significance for polymers, which have been shown to have a depressed glass transition temperature in the presence of dense gases (39).

The melting point depression was studied by filling a glass capillary tube with approximately 20 mg of the drug or polymer compound. The glass tube was then placed inside a Jerguson sight gauge (series no. 32) and then in a temperature controlled water bath set at 40°C. Carbon dioxide was

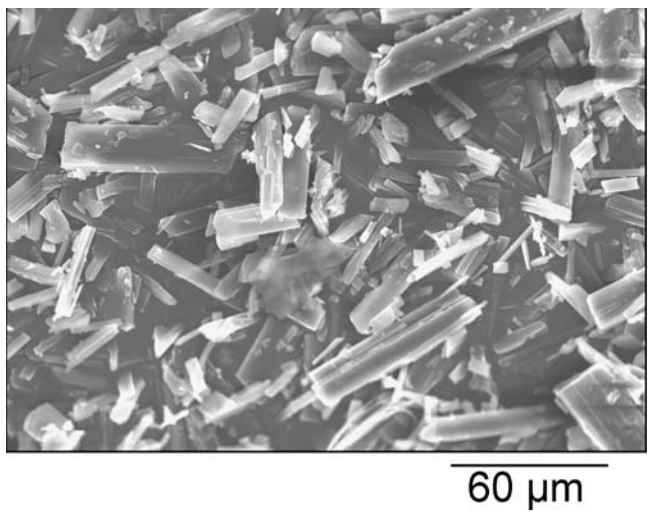

Fig. 2. SEM image of Unprocessed Itraconazole.

pumped into the system at a constant rate and the pressure slowly increased in 5 bar increments, allowing at least 20 min between each pressure interval. The respective compounds were observed for any visible signs of melting. For both itraconazole and PEG (M_W 20,000) there were no signs of melting up to a pressure of 195 bar.

Processes for Particle Production—Dense Gas and Grinding

A schematic diagram of the multipurpose ASES/GAS apparatus used for the production of itraconazole is shown in Fig. 1.

In the ASES experiments the solutes were dissolved in the solvent and stored in a solution reservoir (E1). The precipitation chamber (E4: Jerguson sight gauge series no.32) was maintained at constant temperature by submerging it in a water bath (E8). The water bath was heated by a recirculation heater (E5: Ratek TH5-2KW) with a thermocouple (TI: Martin) used to monitor the temperature. A syringe pump (P2: ISCO 260D) was used to pump the anti-solvent (CO_2) into the top of the precipitation chamber until the required pressure was reached. The pressure was monitored by a pressure indicator built into the HPLC pump (P1: Agilent 1100 series) and a Druck pressure indicator (PI: PTX7517-1).

The solution was pumped by a HPLC pump at 0.1 ml/min through a nozzle (E6: stainless steel tubing with inner diameter 100 μm) thus dispersing the solution as fine droplets, into the precipitation chamber (E4). A frit with 0.5 mm porosity was assembled at the bottom of the Jerguson to collect the precipitate.

In all the ASES experiments a minimum ratio of 1:100 was maintained between the solvent and antisolvent flow rates. The solute concentration was varied depending on the solubility of the drug in the solvent being used. For itraconazole processed with DCM and DMF a 2 wt% solution was used. When THF and acetone were employed as the solvents 1 and 0.25 wt% solute concentrations, respectively were used.

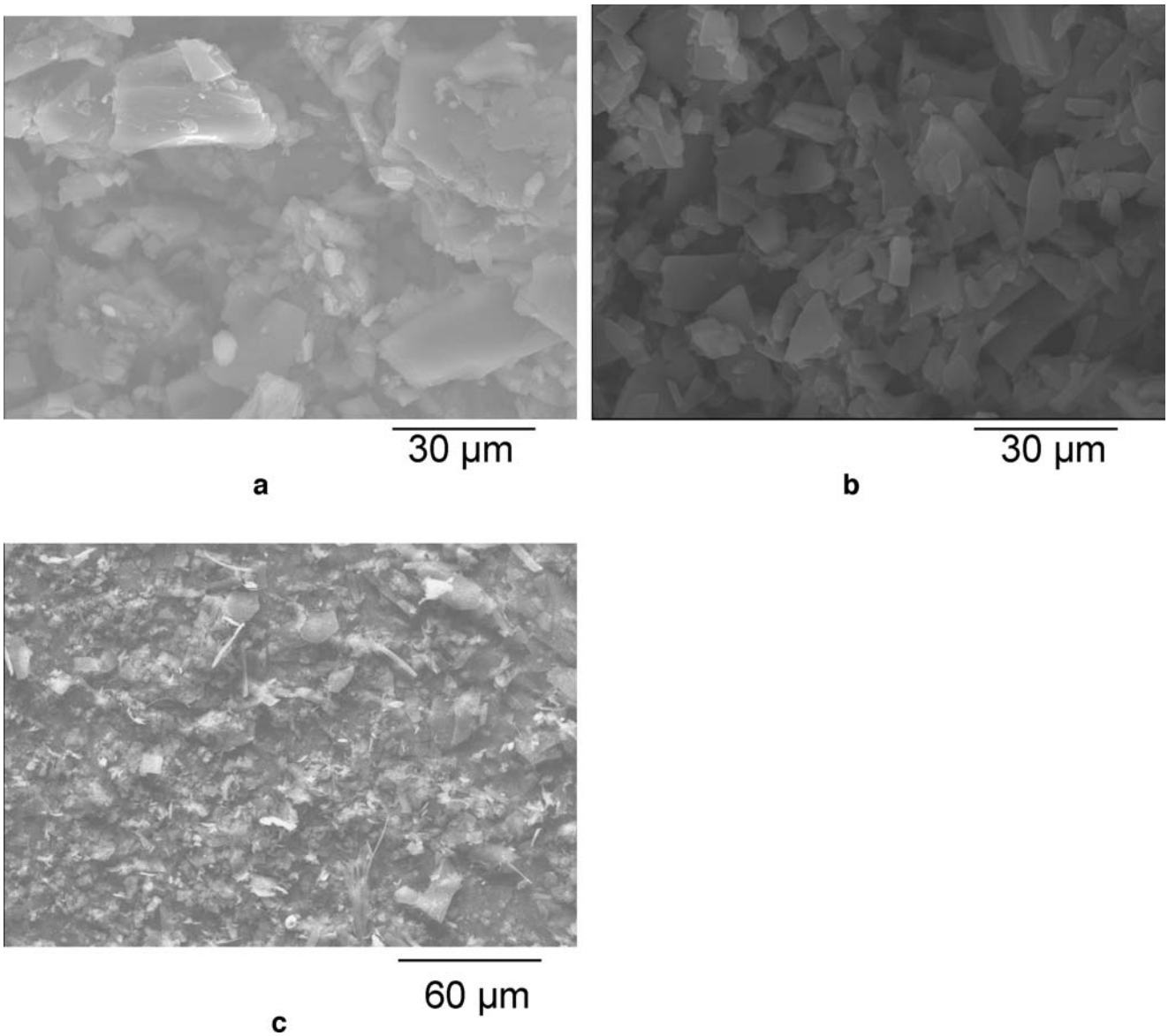

Fig. 3. SEM image of Itraconazole Processed by the GAS Process: **a** using acetone at 40°C, **b** using acetone at 25°C, **c** using DMF at 40°C.

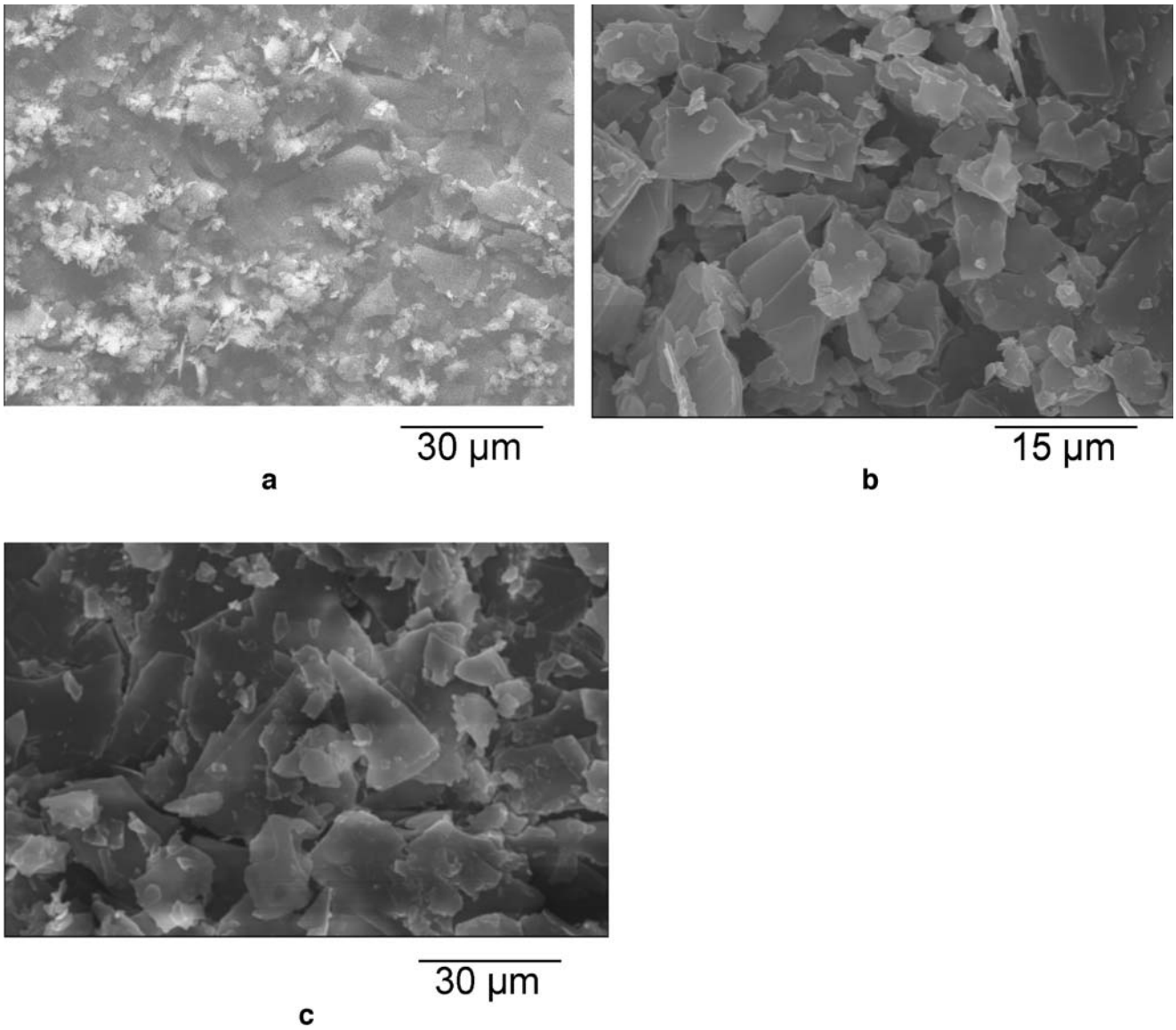

Fig. 4. SEM image of Itraconazole Processed by the ASES Process using: **a** acetone at 40°C/190 Bar, **b** acetone at 25°C/140 bar, **c** DMF at 40°C/190 bar.

In the GAS experiments the nozzle was removed from the system. The solution containing drug/excipient (12 ml) was injected into the precipitation chamber. After thermal equilibrium was reached carbon dioxide was gradually fed into the precipitation chamber (3 barmin^{-}) through the bottom of the chamber, passing through the frit which facilitated mixing of the solution and dense gas. The other components of apparatus remained the same.

In both the ASES and GAS experiments an average of 650 ml of anti-solvent was used to wash the precipitate in order to remove residual solvent when using DMF, DCM or THF. However when acetone was used as the solvent only 150 ml of carbon dioxide was required for washing.

For comparative purposes a ground mixture of itraconazole: PEG (50:50) was prepared using a Rocklabs bench top ring mill. The sample was loaded into the mill and then the sample was processed for 6 min.

Particle Morphology

Sample particle size and morphology were analysed using a Scanning Electron Microscope (SEM: Hatachi S4500II). A small amount of the itraconazole and itraconazole: PEG composites were mounted onto a metal stub using double-sided carbon-conductive tape. The samples were then vacuum coated with 1–2 nm chromium using the Xenosput (Emitech K575X).

Drug Loading

The drug loading of a co-precipitate sample was determined by UV Spectrophotometric techniques using a Cary UV 50 Spectrophotometer at 262 nm. A solvent namely, acetone, that dissolved both PEG and itraconazole was selected. A known amount of sample was dissolved in solvent

Table I. Dissolution Data for Itraconazole

Process	Sample Processing Conditions	K_W (min^{-1})	Dissolved in 100 min (%)	Yield
Unprocessed Powder	–	–	14.6	–
Sporanox	–	–	52.5	–
Itraconzole Dissolution Data				
GAS	DMF 40°C	0.004	45.5	33.5
	DMF 25°C	–	23.2	31.0
	DMF 10°C	–	22.2	20.9
	DCM 40°C	–	26.7	29.5
	DCM 25°C	–	14.3	20.3
	DCM 10°C	–	13.5	15.6
	Acetone 40°C	0.010	64.5	73.4
	Acetone 25°C	0.007	53.1	61.2
ASES	DMF 40°C/190 Bar	0.006	44.2	40.2
	DMF 40°C/90 Bar	–	24.1	10.1
	DMF 25°C/140 Bar	0.004	33.7	30.1
	DCM 40°C/190 Bar	0.003	25.8	31.3
	DCM 40°C/90 Bar	–	6.1	6.8
	DCM 25°C/140 Bar	–	21.1	19.3
	THF 40°C/190 Bar	0.004	42.1	60.3
	THF 40°C/90 Bar	–	2.3	27.1
	THF 25°C/140 Bar	–	23.4	36.4
	Acetone 40°C/190 Bar	0.012	71.1	89.7
	Acetone 25°C/140 Bar	0.010	63.0	83.4
Itraconzole/PEG Dissolution Data				
Physical Mixture 50:50	–	–	29.2	–
ASES (All Processed using Acetone at 40°C/190 Bar)	Itra/PEG=30:70	0.016	89.8	79.3
	Itra/PEG=50:50	0.014	72.7	82.8
	Itra/PEG=70:30	0.011	64.8	84.7
	Itra/PEG=90:10	0.010	66.3	86.3

and the concentration was obtained for each system prior to the analysis. The effect of PEG concentration on itraconazole absorption was negligible.

Thermal and Crystallinity Analysis

Differential Scanning Calorimetry (DSC: 2010 TA Instruments) analysis was used to examine the polymorphic forms of both unprocessed and processed itraconazole and to detect the presence of residual organic solvent in the sample. In this analysis 10 mg of the sample to be analysed was placed in an aluminium pan and along with an empty reference pan and the heat flow was measured between 0–400°C with a 10°C min^{-1} temperature gradient in an inert atmosphere of nitrogen. The crystallinity of the powders was determined using X-ray diffraction (XRD) analysis. Measurements were made on a Siemans D-5000 diffractometer using CuKα radiation (λ=1.54056 Å). Samples were placed in an aluminium sample holder and were scanned from 4 to 60° at a scanning rate of 2 min^{-1}.

Particle Size Analysis

The particle size of the drug and drug/polymer composites were analysed using the Malvern Mastersizer 2000, with a Hydro 2000 cell. All data collected was then analysed using version 5.2.2 of the Malvern software. The analysis was carried out by dispersing approximately 10 mg of the powder in the dispersant (ethanol) and using approximately 30 s of sonication to create a suspension. The suspension was introduced into the Malvern which already contained ethanol.

Dissolution

The dissolution rate of itraconazole was measured using the Vankel 7000 Dissolution Equipment and Cary UV 50 Spectrophotometer. The dissolution testing was conducted in sink conditions using the USP paddle method at 37°C in USP 26 Simulated Gastric Fluid (SGF). The composition of the pepsin free SGF was 2 g NaCl, 7 ml HCl and 1 l of deionized water (1). The dissolution vessels contained 900 ml of SGF and absorbances were measured at a wavelength of 262 nm. Experimental data was the average of at least three replicates. To achieve suspension of the powder in the SGF all dissolution experiments were carried out by suspending the dry powders in 25 ml of the SGF before adding them to the dissolution vessels. In order to carry out the dissolution procedure on the Sporonox formulation the Sporanox powder was removed from the capsule and dissolution testing proceeded by first forming a suspension as per the other samples. Intrinsic disc dissolution was conducted using the same dissolution conditions and equipment as all other dissolution testing. The apparatus and method is similar to that described in Chan *et al.* and consists of stainless steel powder holders containing compressed sample (40). The powder samples were pressed directly into the holders by a hydraulic press (Carver Hydraulic Unit model number 3912) at a compression rate of 2 metric ton for 60 s to form a compact disc 1 cm in diameter. The same compression rate was used for all samples as it has been previously demonstrated that increases in the compaction pressure may result in a decreased dissolution rate (40,41).

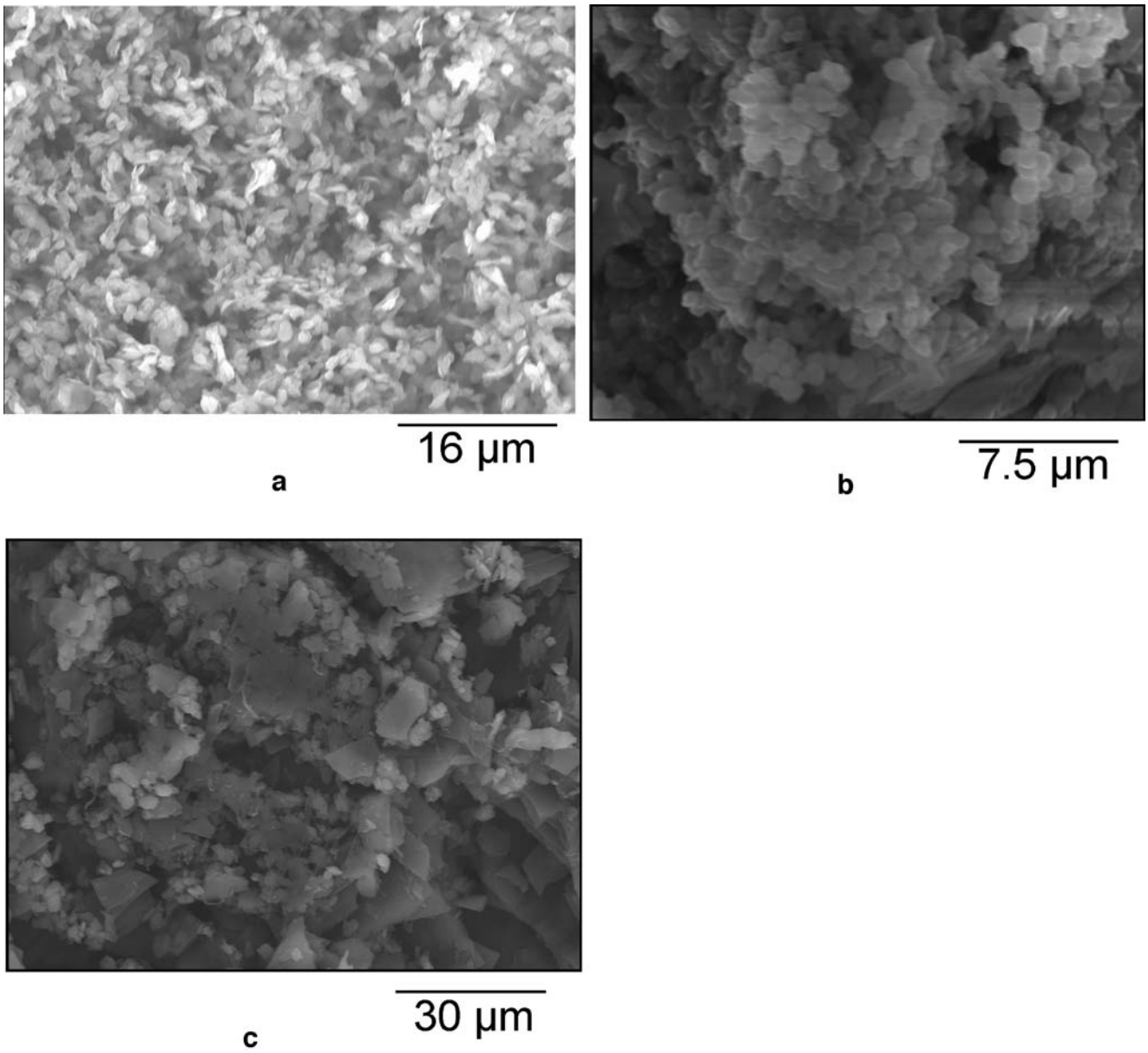

Fig. 5. SEM image of **a** PEG precipitated by the ASES process and **b**, **c** Itraconazole/PEG composite produced using the ASES process.

RESULTS AND DISCUSSION

Itraconazole Precipitation Using Dense Gas Processes

Unprocessed itraconazole, Fig. 2, is a white crystalline powder made up of irregular shaped rectangular prisms with a broad particle range of 2–70 μm with a volume average particle size of 13.8 μm. Itraconazole particles produced by the GAS process at various conditions were generally irregular flat flakes. The effects of precipitating itraconazole from different organic solvents and of operating temperature on the characteristics of the final itraconazole powder were examined. As illustrated in Fig. 3 the particle morphology was not significantly altered by the change in solvent type or operating temperature.

Similarly to GAS precipitated itraconazole, the choice of solvent and operating conditions such as temperature and pressure did not have a significant effect on the particle morphology of material processed by the ASES process (Fig. 4). However, the mean particle size, particle size distribution and yield were affected by the choice of solvent and by the operating temperature.

In all cases, regardless of process or solvents used, an increase in temperature resulted in an increase in the yield of the precipitate (Table I). In the GAS process, with acetone as the solvent, there was a 16% increase in the yield from 61.2% at 25°C to 73.4% at 40°C. For the ASES process 83.4 and 89.7% yields were achieved at process conditions of 25°C/140 bar and 40°C/190 bar respectively representing a 7% increase in the yield. Similarly, Velaga *et al.* reported a decrease in product yield for flunisolide and budesonide produced using the SEDS process as temperature was decreased from 80 to 40°C. The decreased yield at lower temperatures could be attributed to the increased density and corresponding decreased diffusivity resulting in a greater amount of drug being solubilized hence preventing the formation of particles (42).

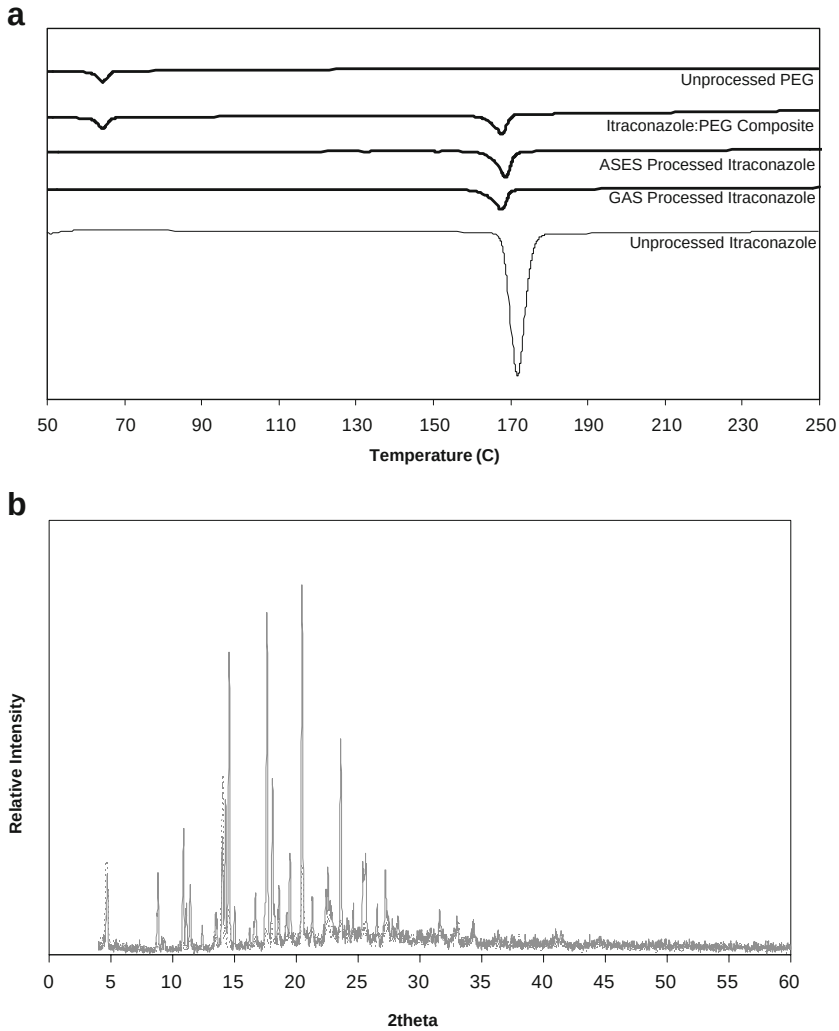

Fig. 6. **a** DSC Thermogram for Itraconazole. **b** XRD analysis of Itraconazole: (*solid line*) unprocessed and (broken line) dense gas processed.

An increase in pressure also resulted in an increase in yield regardless of solvent. Using the ASES technique and DMF at an operating temperature of 40°C an increase in pressure from 90 to 190 bar resulted in a 75% increase in the yield from 10.1 to 40.2%. Furthermore, the solvent used was also found to have a large effect on the yield of the precipitate. As reported in Table I when itraconazole was processed using the GAS technique, at an operating temperature of 40°C, the yields of 73.4, 33.5 and 29.5% were achieved using acetone, DMF and DCM respectively. Using the ASES technique at operating conditions of 40°C and 190 bar yields of 89.7, 60.3, 40.2 and 31.3 were achieved using acetone, THF, DMF and DCM respectively. The decrease in yield in the presence of certain solvents could be because of three reasons namely, the co-solvent effect, failure in the solvent removal process and low supersaturation. Since the solubility of itraconazole in carbon dioxide is limited, the presence of certain solvents may have increased the solubility and the mixture may be in a homogeneous region for the ternary phase diagram of itraconazole-CO_2-solvent. Thus, as the solubility of itraconazole increased in particular solvents the yield decreased, this is referred to as the cosolvent effect (32). A failure in the solvent removal process, whereby the conditions do not allow for adequate solubility of the liquid solvent in the dense gas, can lead to accumulation of a liquid phase at the bottom of the reaction vessel. In turn, this solvent accumulation will result in any particles that have been formed being redissolved (43). Solvent accumulation was visually observed in some experiments using DCM, DMF and THF which are all at least 130 times more viscous than acetone. Also, as has been previously reported by Subra *et al.*, in the ASES process conditions exist in which the supersaturation is not high enough to induce crystal nucleation. These conditions could include low initial concentration, high solubility in the solvent/antisolvent mixture and a large metastable zone and result in negligible powder production as crystallisation does not occur (43).

Coprecipitation of Itraconazole and Polyethylene Glycol

The precipitation of itraconazole from acetone using the ASES process at operating conditions of 40°C and 190 bar proved to be optimal for itraconazole powder production based on the decrease in particle size, dissolution profiles and high yield of 89.7%. As such it was decided that, pending successful precipitation of PEG using the determined operat-

Table II. Summary of Particle Size Analysis for Itraconazole and Itraconazole:PEG Formulation

Process	Sample Processing Conditions	*d*(0.1)	*d*(0.5)	*d*(0.9)
Itraconazole				
Unprocessed	–	5.6	13.7	31.0
GAS	DCM at 40°C	7.1	15.2	29.5
	DMF at 40°C	6.0	11.4	20.4
	Acetone at 40°C	5.4	9.7	16.6
ASES	DCM at 40°C/190 Bar	7.2	11.9	23.5
	DMF at 40°C/190 Bar	7.2	11.7	18.4
	THF at 40°C/190 Bar	4.7	9.9	16.6
	Acetone at 25°C/140 Bar	5.3	8.7	13.0
	Acetone at 40°C/190 Bar	4.1	6.9	11.5
Itraconazole-PEG Formulation				
ASES (All Processed using Acetone at 40°C/190 Bar)	PEG/Itraconazole 10:90	9.5	20.7	40.8
	PEG/Itraconazole 30:70	7.4	18.2	36.6
	PEG/Itraconazole 50:50	5.9	15.4	35.4
	PEG/Itraconazole 70:30	6.0	13.6	27.9
	PEG/Itraconazole 90:10	5.8	12.9	25.1

ing parameters, these conditions should be used for the co-precipitation of itraconazole and PEG with the aim to produce a drug formulation with an increased dissolution rate. Precipitation of PEG from acetone by the ASES process at the optimal conditions for itraconazole processing resulted in non-discrete, slightly agglomerated, uniform semi-spherical particles of 3 μm in size (Fig. 5a). Average yields of 75% were able to be achieved and so the conditions established for itraconazole production were deemed suitable for the co-precipitation experiments.

Scanning Electron Microscope (SEM) images of the drug/polymer composite (Fig. 5b,c) show that there are two unique morphologies present in the composite product. Firstly, there were the rectangular prism shaped particles similar to that formed by the dense gas precipitation of itraconazole. Secondly, there were semi-spherical shaped particles of PEG. The SEM image suggests that the co-precipitation of the drug and polymer resulted in the two compounds being precipitated separately, rather than as an encapsulated matrix product, with the polymer microspheres coating the drug particles. The independent precipitation of the drug and polymer is not uncommon in dense gas processes where coprecipitation largely depends on the similarity of the compounds involved (29,44).

The composition of itraconazole in the mixture of PEG/Itraconazole, to be referred to as the drug loading, was determined using UV analysis and compared with the theoretical drug loading. In all cases the drug loading was

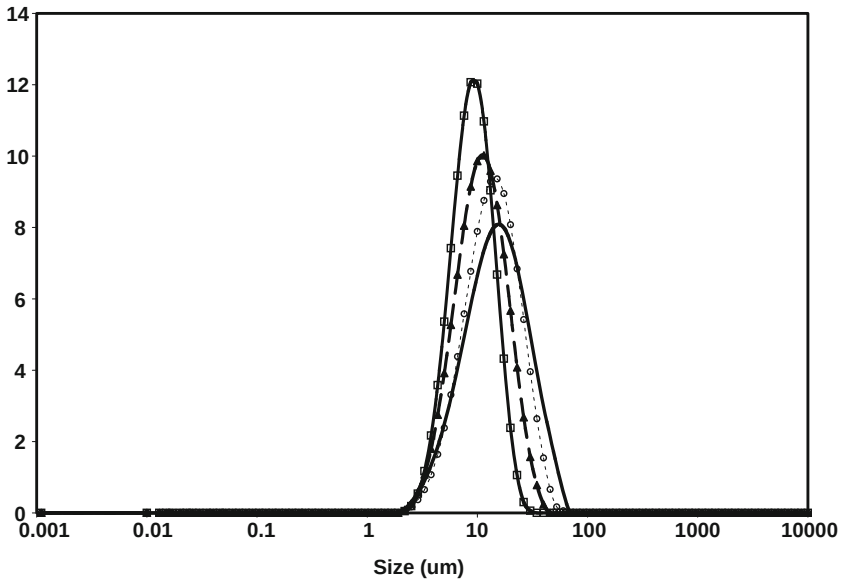

Fig. 7. Effect of solvent used on the particle size distribution of Itraconazole processed using the GAS Technique at 40°C: *square* itraconazole processed using acetone as the solvent; *triangle* itraconazole processed using DMF as the solvent; *X* itraconazole processed using THF as the solvent; (*circle*) itraconazole processed using DCM as the solvent; *line* raw itraconazole.

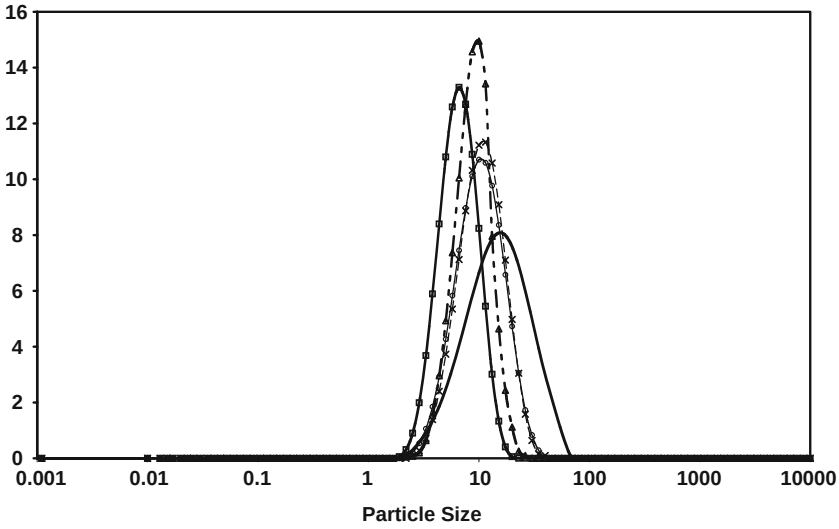

Fig. 8. Effect of solvent used on the particle size distribution of itraconazole processed using the ASES Technique at 40°C and 190 Bar: *square* itraconazole processed using acetone as the solvent; *triangle* itraconazole processed using DMF as the solvent; *X* itraconazole processed using THF as the solvent; *circle* itraconazole processed using DCM as the solvent; *line* raw itraconazole.

within 2% of the theoretical value, which, it could be said is within the limits of experimental error and accuracy. Furthermore, the fact that the composition of the mixture has not changed demonstrates that both components were either not soluble or had negligible solubility in the CO_2-solvent system.

Drug Stability and Crystallinity

A major concern when re-processing pharmaceutical compounds is that the properties of the drug will not be jeopardised. Changes in drug properties can affect the *in vitro* efficacy of a pharmaceutical compound. The DSC thermogram in Fig. 6a shows that the melting point of itraconazole was unaffected by processing using dense gas techniques. The DSC also further confirms the notion that the two compounds have been precipitated independently with the polymer and the drug having separate and discrete melting peaks. Furthermore, the absence of any secondary peak indicates that any residual solvent in the dense gas processed material is negligible. The absence of residual solvent is a good result given that the FDA guidelines for residual solvents in pharmaceutical products state that residual solvent should be minimised. In particular the permitted daily exposure (PDE) to acetone is 50 mg (45). However, as is apparent from

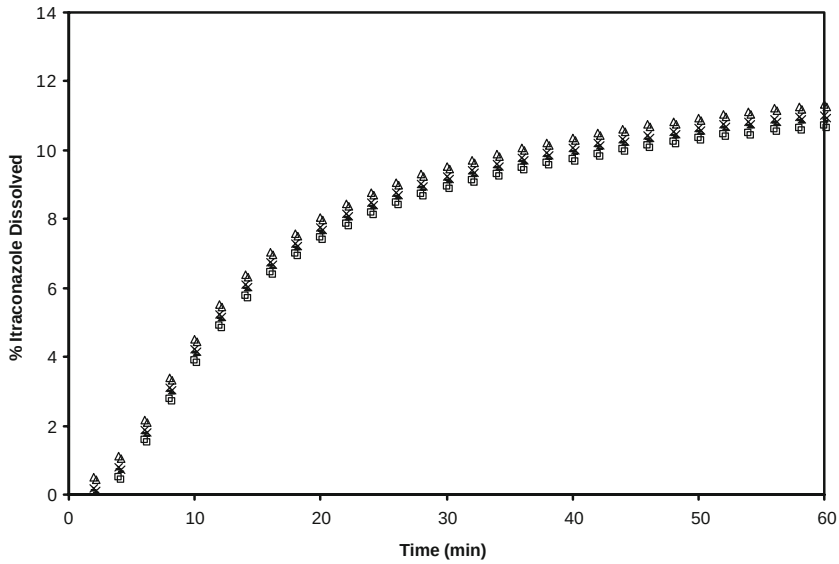

Fig. 9. Intrinsic Dissolution Profile for Itraconazole Processed at 40°C: (□) using the GAS technique and acetone as the solvent; (Δ) using the ASES technique and acetone as the solvent;(X) using the GAS technique and DMF as the solvent;(−) unprocessed.

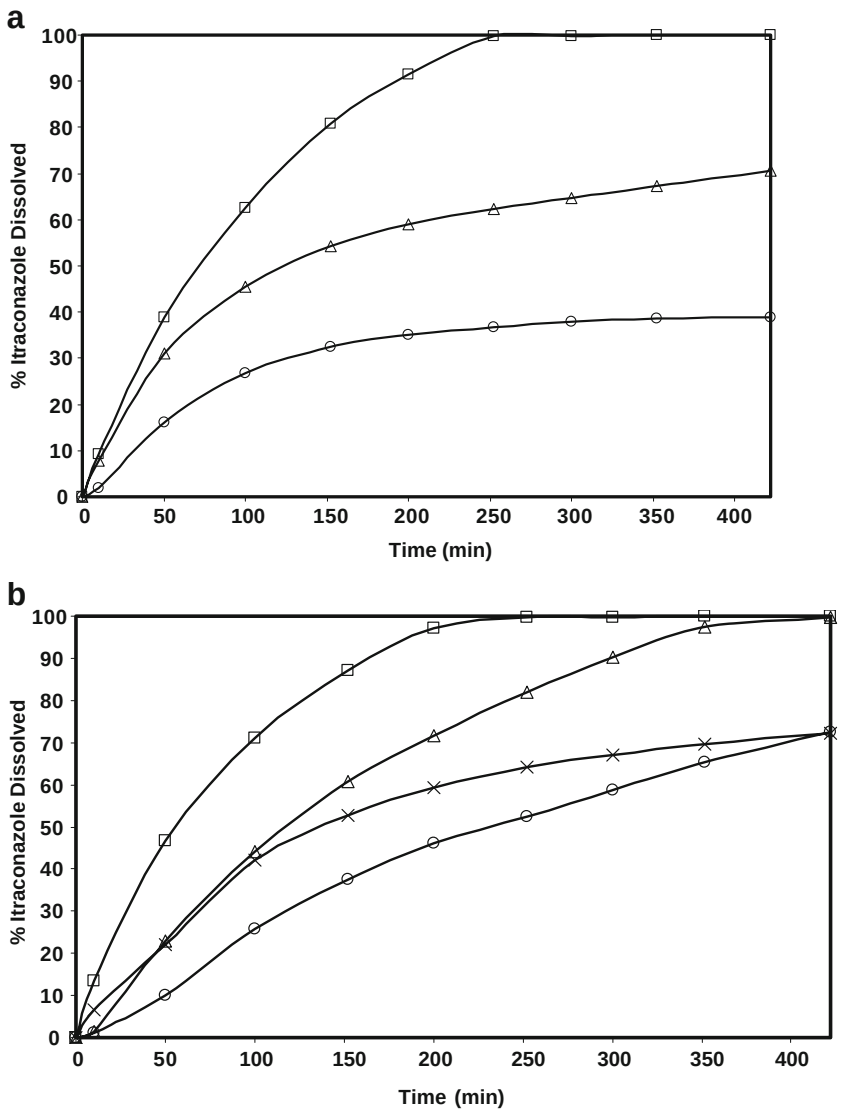

Fig. 10. **a** Effect of solvent used on the dissolution profile for itraconazole processed using the GAS technique at 40°C: *square* itraconazole using acetone as the solvent; *triangle* itraconazole using DMF as the solvent; *circle* using DCM as the solvent. **b** Effect of solvent used on the dissolution profile for itraconazole processed using the ASES technique at 40°C and 190 bar: *square* itraconazole processed using acetone as the solvent; *triangle* itraconazole processed using DMF as the solvent; *X* itraconazole processed using THF as the solvent; *circle* itraconazole processed using DCM as the solvent.

the thermogram, the enthalpy (ΔH) has been varied when itraconazole has been processed with dense gases. Any variance in enthalpy can be indicative of changes in the degree of crystallinity of the drug. The XRD analysis, shown in Fig. 6b, confirmed that the processed itraconazole exhibited a loss in the intensity of the crystallinity peaks present in the unprocessed material. The decreased intensity implies that there is some shift towards the processed drug being amorphous. Loss of particle crystallinity is not uncommon in dense gas processing and has been attributed to the rapid nucleation rates caused by extremely high levels of supersaturation. Rapid nucleation of the particles can prevent the molecules from organising themselves into the crystalline form (31,46–49). The significance of this result is that the amorphous or crystalline nature of a drug has been shown to affect its therapeutic activity with the amorphous form of drugs often more readily absorbed than the crystalline form thus decreasing the time for the drug to achieve its therapeutic action (1).

Particle Size Distribution

The average particle size, measured by Malvern Particle Size Detector, of pure unprocessed itraconazole was 13.8 μm. Processing by dense gas CO_2 resulted in a decrease in the particle size of pure itraconazole (Table II). The particle size of itraconazole produced by gas antisolvent processes was significantly affected by the choice of solvent, the operating

temperature, pressure and choice of process. The particle size distribution for itraconazole particles produced by the GAS and ASES processes are shown in Figs. 7 and 8, respectively. Regardless of the process used itraconazole processed with acetone had the smallest average particle size whereas that processed with DCM had the largest average particle size. Itraconazole precipitated by the ASES process was found to have a smaller particle size than that processed, with the same solvent, by the GAS process. At a temperature of 40°C using the GAS process itraconazole had an average particle size of 9.7 μm. Whereas, when the ASES process was used the mean particle size was 6.9 μm and over 90% of the particles were less than 11.5 μm. It should be noted that while the use of acetone proved the most efficient, regardless of the solvent used dense gas processed drug always had a smaller particle size than the raw material. These results are significant as the micronization of pharmaceutical compounds has been shown to have a direct relationship to an increase in dissolution rate.

Further to the choice of solvent other operating conditions such as temperature and pressure have previously been found to have a significant effect on the particle size (31,50). As can be seen in Fig. 7 regardless of the dense gas process or solvent used an increase in temperature and pressure resulted in a decrease in average particle size. The decreased particle size can be attributed to the higher carbon dioxide density which increases the deforming pressure forces necessary to break up the liquid droplet into smaller droplets hence creating smaller particles. The hydrodynamic theory surrounding the spray process during the ASES process is of particular importance when investigating particle size and in understanding the reasons for achieving certain results. For the ASES process the hydrodynamic theory for the spray process, relating particle size to antisolvent density, has been explained in terms of the Weber number (51).

The Weber number is a dimensionless number used for the analyses of fluid flows where the surface tension influences the flow (52). In terms of atomisation and spray processes the Weber number is the ratio of the deforming external pressure forces and the reforming surface tension forces experienced by a liquid droplet encountering flowing air (53). The Weber number can be defined by Eq. 1 where ρ_A is the antisolvent density, V is the relative velocity, d is the initial droplet diameter and σ is the interfacial surface tension.

$$\mathrm{We} = \frac{\rho_A V^2 d}{\sigma} \quad (1)$$

Introducing polymer into the system can reduce the particle size of the drug as it limits the crystal growth of the drug. However, in this study given the drug and polymer precipitated as individual compounds it is difficult to determine whether the growth of the drug particle has been minimised. It was found that increasing the amount of polymer in the system resulted in a decrease in particle size decreased. The reduction in particle size is due to the shift in the spray hydrodynamics as the surface tension increased with increased polymer ratios. As shown in Table II, in real terms this resulted in a 37% decrease in composite particle size from 20.7 μm when 10% PEG was used compared with a 12.9 μm when 90% PEG was precipitated. If the Weber number were to remain constant, regardless of the amount of polymer in the system, then it would be expected that an increase in polymer content would result in an increase in particle size. The experimental results, repeated in triplicate, indicate that the Weber number was not constant throughout the experiments.

Effect of Dense Gas Processing on Drug Dissolution Rate

The dissolution of a drug refers to the process by which a solid phase, for example a powder, goes into an aqueous solution phase. Often, dissolution is the rate limiting step in the absorption of drugs with low solubility as it is the slowest step in the various stages of drug release (1). The dissolution rate of unprocessed itraconazole, dense gas processed itraconazole and itraconazole-PEG composite, a milled mixture of itraconazole-PEG and commercially available Sporanox capsules were measured in simulated gastric fluid (SGF).

The dissolution of a solid into a liquid is determined by physical parameters such as the surface area of the solid, the thickness of the solid/liquid interface known as the diffusion

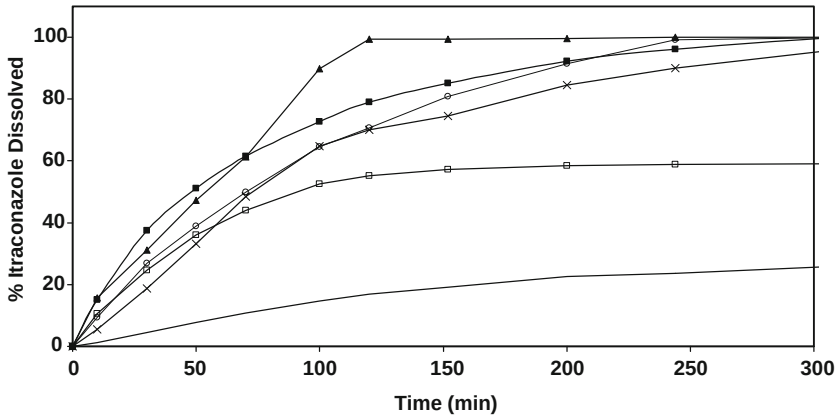

Fig. 11. Dissolution profile of itraconazole processed by the ASES technique at 40°C/190 bar: *triangle* 30:70 Itraconazole/PEG composite; *square* 50:50 Itraconazole/PEG composite; *circle* itraconazole produced by the ASES process; *X* 70:30 Itraconazole/PEG composite; *square* Sporonox; *line* unprocessed itraconazole.

layer and the solubility of the solid in the liquid. The factors affecting dissolution rate are expressed in quantitative terms by the Noyes–Whitney equation (Eq. 2) where dM/dt is the mass of solute released per unit time, D is the diffusion coefficient of the solute in solution, s is the surface area, h is the thickness of the diffusion layer, C_s is the solubility of the compound in the dissolution medium and C is the concentration of the solute in the bulk solution at time (t).

$$\frac{dM}{dt} = \frac{Ds}{h}(C_s - C) \tag{2}$$

When the concentration of the solute is considerably less than the saturation concentration ($C_s>>>C$) the system is said to be operating under sink conditions. Under sink conditions Equation 2 can be further refined as represented as Eq. 3 where K_W is the dissolution rate constant (1). The dissolution rate constant, K_W, is calculated by finding the inverse of the time taken for 63.2% of the drug to dissolve and can be used to compare the dissolution rates of the various samples, these are reported in Table I.

$$\frac{dM}{dt} = K_w s(C_s) \tag{3}$$

When surface area is constant, by means of creating a compressed tablet and performing intrinsic disc dissolution, there was no significant difference in the dissolution rate of the itraconazole powders (Fig. 9). Conversely when powder dissolution was performed there were significant differences in the dissolution rate between each of the samples. These results confirm that size reduction was a dominant factor in enhancing the dissolution rate of itraconazole.

The effect of particle size on the dissolution rate of itraconazole is further demonstrated in Fig. 10 which contains the dissolution profiles for itraconazole processed by the GAS process at 40°C and the ASES process at 40°C and 190 bar using various solvents. In both cases the dissolution rate proved to have a direct relationship with particle size as the smaller particles allowed for a greater surface area to be achieved. Itraconazole processed using the ASES process with a temperature of 40°C, pressure of 190 Bar and using acetone as the solvent, had the quickest dissolution. At 100 minutes only 14.6% of the unprocessed powder and 52.5% of the Sporonox had dissolved compared with 71.1% of the ASES processed material. Given the fact that for class 2 drugs the *in vitro* dissolution rate is considered similar to the *in vivo* dissolution rate, these results present an opportunity to increase the efficacy of itraconazole drug delivery.

Production of an itraconazole:PEG composite resulted in further increases in the itraconazole dissolution profile with 89.8% dissolved in 100 minutes. As evident in Fig. 11 the co-precipitate with a drug to polymer ratio of 30:70 had a substantially higher dissolution rate than both other ratios of drug/polymer and it could be seen that as the amount of polymer increased so did the dissolution rate of the itraconazole. Meure *et al.* similarly found that increasing the amount of polymer in a drug/polymer formulation manufactured using dense gases resulted in an increase in drug dissolution (54). Due to the fact that the incorporation of PEG did not result in further decreases in the particle size it is more likely that the mechanism for increasing dissolution of itraconazole was a reduction in aggregation and agglomeration and improved wettability of the drug.

The increase in dissolution rate represents an outstanding fivefold increase in the first 100 min of dissolution between the unprocessed itraconazole and the dense gas drug processed drug. Furthermore the drug/polymer composite resulted in a sixfold increase in the dissolution rate indicating a substantial increase to the bioavailability of the drug. Perhaps more importantly the itraconazole/PEG composite showed an improved dissolution when compared with the commercially available product. These results demonstrate the great opportunity that dense gas processing presents to the pharmaceutical industry.

CONCLUSIONS

Dense gas technology has been shown to be suitable for the re-engineering of pharmaceutical compounds. Specifically, both the GAS and ASES process can be used to produce the anti-fungal drug itraconazole powder and an itraconazole/PEG composite in a one-step process. The qualities of the final powder are dependent on operating parameters such as temperature, pressure and choice of solvent. The optimum conditions for itraconazole, within the range investigated, were determined to be 40°C and 190 bar using acetone as the solvent in the ASES process. At these conditions there was a significant decrease in particle size and an increase in dissolution rate from 14.6% for the unprocessed powder to 71.1% for the ASES processed material in the first 100 minutes. The crystallinity of the dense gas process material was slightly amorphous which, coupled with the decreased particle size, increased the dissolution of the drug. Furthermore the itraconazole/PEG composite resulted in a sixfold increase in Dissolution rate in the first 100 min when compared with the unprocessed material. Differential Scanning Calorimetry indicated that there was negligible residual solvent in the final dense gas processed product. As itraconazole is a class 2 drug the increased *in vitro* dissolution rate can be thought of as similar to the *in vivo* dissolution. Thus this study presents an opportunity to develop itraconazole with an increased bioavailability.

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