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Comparison of solid dispersions produced by supercritical antisolvent and spray-freezing technologies

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ABSTRACT

Oxeglitazar is a new orally administered poorly water soluble active substance used in the treatment of type II diabetes. The objective of this work was to improve its dissolution kinetics using supercritical antisolvent (SAS) and spray-freezing (SF) techniques. Oxeglitazar was formulated with various excipients, including: Poloxamer 188 and 407, polyethylene glycol (PEG) 8000 and polyvinilpyrrolidone (PVP) K17 in a 1:1 weight ratio. In the SAS technology, pharmaceutical ingredients were dissolved in an appropriate solvent, and the feed solution was dispersed through a capillary nozzle in supercritical CO_2 (SC CO_2). Dichloromethane (DCM), chloroform ($CHCl_3$), and a binary co-solvent system of chloroform–ethanol (EtOH/$CHCl_3$ 50:50, v/v%) were tested. In the SF process, tert-butanol (*t*BuOH) was used as solvent. The feed solution was injected into liquid nitrogen through a capillary nozzle located above the surface of the boiling nitrogen. Frozen particles were collected and freeze-dried for 30 h. Formulations were compared in terms of particle morphology, particle size, flow properties, crystallinity, polymorphic purity, residual solvent content, precipitation yield, drug content, specific surface area and dissolution kinetics. SAS and SF processed formulations exhibited enhanced dissolution rates. Within 5 min, the amount of dissolved drug varied from 31.6 to 64.3% for SAS and from 77.9 to 96.9% for freeze-dried formulations while only 30.5% was dissolved from raw drug. Apart from oxeglitazar/PVP K17, SAS prepared solid dispersions were characterized by high crystallinity and acicular shape. Freeze-dried formulations consisted of porous spherical particles with high amorphous content (94.2–100%).

1. Introduction

The bioavailability of an orally administered active pharmaceutical ingredient (API) depends on its solubility and dissolution kinetics in aqueous media over the pH range of 1.0–7.5 and the permeability across the gastrointestinal tract (FDA, 2002). In fact, only a few percent of biologically active compounds possess adequate solubility and permeability. In the other cases special formulation techniques are involved or other routes of administration are recommended. Active substances with high permeability and low aqueous solubility are classified by the Biopharmaceutics Classification System (BCS) as Class II APIs (Amidon et al., 1995). Since the absorption of these drugs is dissolution rate-limited their bioavailability can only be increased by enhancing their dissolution rate. Several methods are currently used to overcome difficulties associated with hydrophobic drugs, the most importants are: inclusion complexation with cyclodextrin (CD) derivates (Tavornvipas et al., 2002; Perrut et al., 2002a,b; Charoenchaitrakool et al., 2002; Nakate et al., 2003), and the formation of solid dispersions with water-soluble polymers (Rouchotas et al., 2000; Jung et al., 1999). The main drawback of cyclodextrin-based drug delivery is the 1:1 stoichiometric ratio that can result in low drug-carrier weight ratio. Occasionally, high molecular weight APIs require even lower drug-carrier ratio (Nakate et al., 2003). Although, the drug content of a solid dispersion may vary over wide ranges, the optimal concentration of excipient exceeds rarely 50%. Conventional methods i.e. spray-drying, solvent evaporation and hot melt method often result in low yield, high residual solvent content or thermal degradation of the active substance. Another important limitation of solid dispersions is the inherent stability problems. The amorphous state was considered for long time as unsuitable for pharmaceutical application due to its metastable nature (Debenedetti and Roberts, 2002). Amorphous formulations of drug substrates having low glass transition temperature may crystallize during storage. However, the

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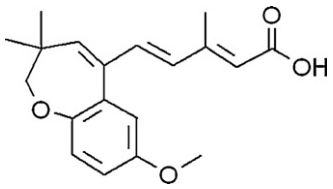

Fig. 1. The molecular structure of oxeglitazar.

use of polymers with a high glass transition temperature for the formulation of solid dispersions is often sufficient to prevent crystallization. Several technologies have been developed to meet more stringent regulatory and environmental demands. These methods use compressed gases, supercritical fluids (SCF) or liquefied gases as solvent, antisolvent or cryogenic medium (Rogers et al., 2001; Jung and Perrut, 2001; Charbit et al., 2004; Majerik et al., 2004; Badens et al., 2005).

A SCF can be defined as a substance existing as a single fluid phase above its critical temperature (T_c) and critical pressure (P_c). Physical properties of SCFs including density, viscosity, diffusivity, surface tension and solvent strength vary between gas-like and liquid-like values depending on the temperature and pressure conditions. In addition, carbon dioxide, the most commonly used fluid is chemically quite inert, non-toxic, non-flammable and abundant. Owing to its mild critical temperature (31.06 °C) and critical pressure (73.8 bar), CO_2 is suitable to treat heat-sensitive APIs like peptides, steroids and DNA with relatively low energy costs. Particle formation is one of the most researched areas of SCF application. Unlike conventional processes, SCF techniques may reduce particle size and residual solvent content in one step and allow formulation chemists to control polymorphic purity and particle morphology (Beach et al., 1999; Fargeot et al., 2003; Badens et al., 2004). These methods use SCFs either as solvent: Rapid Expansion from Supercritical Solution (RESS) (Türk et al., 2002; Perrut et al., 2005); or antisolvent: Gas Antisolvent (GAS) (Krukonis et al., 1994; Moneghini et al., 2001; Corrigan and Crean, 2002; Sethia and Squillante, 2004), supercritical antisolvent (SAS) (Perrut et al., 2005; Falk et al., 1997; Taki et al., 2001; Majerik et al., 2007a,b), Aerosol Solvent Extraction System (ASES) (Bitz and Doelker, 1996; Ruchatz et al., 1997), Solution Enhanced Dispersion by Supercritical Fluids (SEDS) (Hanna and York, 1995; York et al., 2001); and/or dispersing fluid: SEDS, Particles from Gas-Saturated Solution (PGSS) (Kerc et al., 1999; Juppo et al., 2003).

Solid dispersions can also be obtained by the ultra-rapid freezing of a solution containing the pharmaceutical ingredients (Rogers et al., 2001; Majerik et al., 2004). In the first step the feed solution is dispersed through an injection device (capillary, rotary, pneumatic or ultrasonic nozzle) in a cryogenic medium (N_2(l), Ar(l), O_2(l), hydrofluoroalkanes or organic solvents). In the second step frozen particles are freeze-dried to remove the organic solvent (Briggs and Maxvell, 1973; Gombotz et al., 1990; Williams et al., 2002; Rogers et al., 2002a,b, 2003; Yu et al., 2002; Hu et al., 2003, 2004; Vaughn et al., 2005). The mean particle size can be controlled by choosing an appropriate injection device and changing its location relative to the refrigerant. Owing to the liquid–liquid collision, dispersion beneath the surface of refrigerant may considerably reduce particle size. Williams et al. (2002) have prepared sub-micron particles of carbamazepine/Poloxamer 407/PVP K17 solid dispersion by injecting the feed solution in liquid nitrogen through a submerged insulating nozzle (spray-freezing into Liquid, SFL).

Our research aimed to improve the bioavailability of oxeglitazar (Fig. 1), an orally administered Class II API with an aqueous solubility of 240 mg/L (at pH 7.4; 37 °C). Two particle formation methods were compared: SAS with SC CO_2 antisolvent and SF with liquid N_2 cryogenic medium ($T_b = -195.8$ °C). The active substance was embedded in various polymer matrices: PEG 8000, PVP K17, Poloxamer 188 and 407. Solid dispersions were characterized by powder X-ray diffraction (XRD), scanning electron microscopy (SEM), optical microscopy, gas chromatography (GC), UV/vis spectroscopy, BET surface area measurement and dissolution tests.

2. Materials

Oxeglitazar was obtained from Merck Santé, Lyon, France; Carbon dioxide (99.7%) was supplied by Air Liquide, France; ethanol (99.8%) and chloroform (99%) were purchased from Carlo Erba, Italy; dichloromethane (99.95%) and dimethylsulfoxide (99.5%) were purchased from SDS, France; tert-butanol (99.5%) was purchased from Reanal, Hungary; potassium phosphate monobasic and sodium phosphate dibasic dodecahydrate were obtained from Acros Organics, Belgium; Poloxamer 188 (Lutrol F68), Poloxamer 407 (Lutrol F127), PEG 8000 (Pluriol E 8005) and PVP K17 (Kollidon 17 PF) were received from BASF, Germany.

3. Methods

3.1. Supercritical antisolvent precipitation

The schematic diagram of the SAS apparatus is shown in Fig. 2. About 2 g of the pharmaceutical ingredients in a 1:1 weight ratio were dissolved in DCM, $CHCl_3$ or $CHCl_3$/EtOH. Feed solution was dispersed through a capillary nozzle (125 μm ID) in a co-current SC CO_2 stream. Feed solution was delivered by a reciprocating HPLC pump (Gilson 307, France) at a flow rate of 3 ml/min, CO_2 was compressed to 80 bar by a water-cooled membrane pump (Dosapro Milton Roy, France) at a flow rate of 10 g/min. Compressed CO_2 and solution were both heated to 35 °C before entering the precipitation vessel (Top Industrie S.A., France). Particles formed by the antisolvent effect were retained by a 0.1 μm metal frit filter and washed with pure SC CO_2 for 30 min to remove residual solvents. A cold trap was installed between the heated expansion valve and the flow meter to condense the organic solvents. Pressure and CO_2 flow rate were manually controlled.

3.2. Spray-freezing

Since SF experiments include a freeze-drying step, *t*BuOH was chosen owing to its high vapor pressure and melting point. The feed solution was injected into liquid nitrogen through a capillary nozzle (125 μm ID) located 50 mm above the surface of the

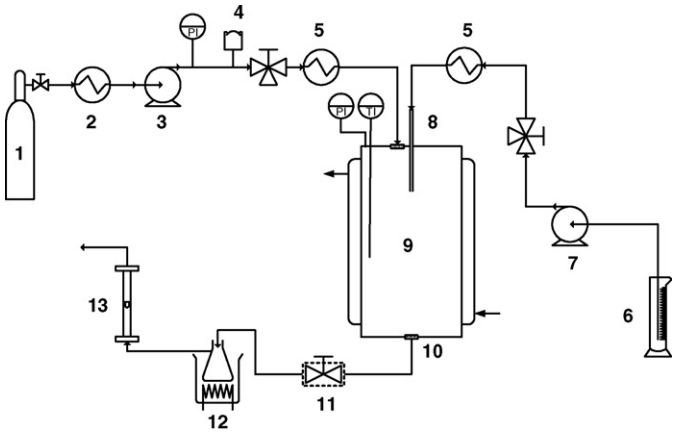

Fig. 2. Schematic diagram of SAS apparatus: (1) CO_2 source, (2) cooler, (3) CO_2 metering pump, (4) bursting disc, (5) heat exchanger, (6) solution source, (7) solution metering pump, (8) capillary nozzle, (9) precipitation vessel, (10) frit filter, (11) expansion valve, (12) cold trap, and (13) gas flow meter.

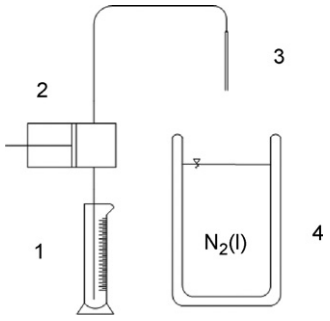

Fig. 3. Schematic diagram of SF apparatus: (1) solution source, (2) metering pump, (3) capillary nozzle, and (4) liquid nitrogen.

cryogenic medium (Fig. 3). Though, smaller particle size can be achieved by using submerged nozzle, low temperature cryogenic media may block the submerged capillary nozzle and result in processing difficulties. Solution was delivered by an HPLC pump at 8 ml/min. Frozen particles were filtered, incubated at 10 °C for 15 min to remove N_2 and freeze-dried in a jacketed vacuum vessel. The shelf temperature was controlled according to the following program: primary drying at 10 °C for 10 h, secondary drying at 15, 20, 25, 30 and 35 °C for 20 h (4 h at each temperature). Vacuum was maintained by a two-stage rotary vacuum pump (Edwards, UK) having an ultimate vacuum of ~0.01 mbar. The ice condenser was submerged in liquid N_2.

3.3. Powder X-ray diffraction (XRD)

XRD patterns of SAS formulations were obtained using a Philips Analytical X-ray diffractometer MPD3710 (Philips Analytical Inc., The Netherlands). Samples were ground in agate mortar prior to analysis. Ground powders were placed in the cavity of an aluminum sample holder and flattened with a glass slide. Samples were scanned over the range of 4.0–47.0° 2θ with a step size of 0.020° 2θ and a count time of 2 s per step using Co Kα source with a wavelength of 1.78896 Å. SF powders were analyzed on Philips Analytical X-ray diffractometer B.V. PW3710 (Philips Analytical Inc., The Netherlands) over the range of 4.0–40.0° 2θ with a step size of 0.020° 2θ and a count time of 1 s per step using Cu source (λ = 1.54056 Å).

3.4. Dissolution studies

Dissolution tests were carried out in pH 7.4 phosphate buffer medium (6.4 g $Na_2HPO_4{\cdot}12H_2O$; 0.6 g KH_2PO_4 and 5.85 g NaCl dissolved in 1000 ml distilled water). About 100 mg sample (equivalent to ~50 mg oxeglitazar) was added to 1000 ml dissolution medium. Bath temperature and paddle speed were set at 37 ± 0.5 °C and 75 rpm. Aliquots of 10 ml were taken through a filtering rod at 5, 10, 15, 30, 45, 60 and 120 min, diluted to 50 ml and analyzed on Spectronic AquaMate 9423 AQA 2000E spectrophotometer (Thermo Spectronic, UK) at λ = 292.0 nm. Error bars represent the standard error of the mean.

3.5. UV/vis spectroscopy

The drug content of formulations was determined using Spectronic AquaMate 9423 AQA 2000E spectrophotometer (Thermo Spectronic, UK). 100 mg sample was dissolved in 50 ml ethanol, 300 μl of the stock solution was diluted to 50 ml with ethanol. Drug content was calculated from the absorbance measured at 292.0 nm.

3.6. Gas chromatography

Residual solvent analysis was carried out on a HP 8590 gas chromatograph (Hewlet Packard, Germany) equipped with flame ionization (FID) detector. About 100 mg sample was dissolved in 2 ml DMSO. Sample solutions (2.0 μl) were introduced by direct injection on a Chrompack Fused Silica column (25 m × 0.53 mm) with Poraplot Q coating (Chrompack International, The Netherlands). Samples were analyzed using Ar carrier gas at a constant oven temperature of 210 °C while the injector and detector temperatures were maintained at 260 °C. The method of external standardization was used to calculate the residual solvent content.

3.7. Scanning electron microscopy (SEM), optical microscopy

SEM micrographs were taken using Philips XL30 ESEM (Philips Analytical Inc., The Netherlands) Environmental Scanning Electron Microscope. Samples were coated by gold before examination (cathode dispersion). Particle size and morphology of SAS prepared formulations were investigated using a Motic B2 optical microscope (Motic Paris, France).

3.8. Specific surface area

Micromeritics ASAP 2000 (Micromeritics, USA) apparatus was used to determine BET surface areas. A known amount of powder was loaded into the sample cell and degassed at ambient temperature for at least 2 h (p < 5 Hgμm) prior to analysis. Specific surface area was calculated using the model of Brunauer, Emmett, and Teller (Brunauer et al., 1938).

4. Results and discussion

4.1. Previous studies

XRD and differential scanning calorimetry (DSC) studies revealed that oxeglitazar has two polymorphic forms. The higher melting form (A) is the thermodynamically stable one at all temperature. Due to a periodic hydrogen bond chain, the crystal growth of both polymorphs is preferred in one crystallographic direction, leading to acicular crystal. This habit is undesirable because of its poor flow properties. There are several methods that aim to modify the crystal shape: combination of two or more forms, crystal twinning, crystallization under controlled conditions (i.e.: temperature) or in presence of additives and trace impurities. However, these techniques failed to affect the crystal habit of oxeglitazar (Fargeot et al., 2003).

4.2. Particle morphology

Fig. 4a shows an optical micrograph of the raw API crystals (as received) which exhibit an acicular habit. The same habit has also been obtained in presence of some excipients. Indeed, acicular crystals of SAS prepared formulations containing PEG 8000, Poloxamer 188 and 407 gave a thick cottony layer on vessel wall. Optical micrographs showed needle-like drug crystals with a length up to 5 mm (Fig. 4b–d). In most cases, crystals were partly covered by spherical polymer coating (~50 μm) suggesting that the nucleation of the active substance was much faster than the precipitation of the excipient. A bit larger crystals were obtained with the Poloxamer 407 from DCM but polymer coating was not observed (Fig. 4d). Experiments with PVP K17 led to irregular particles with high apparent density and good flowability (Fig. 4e). The morphology of oxeglitazar/PVP K17 particles was very different from those observed for the other polymers.

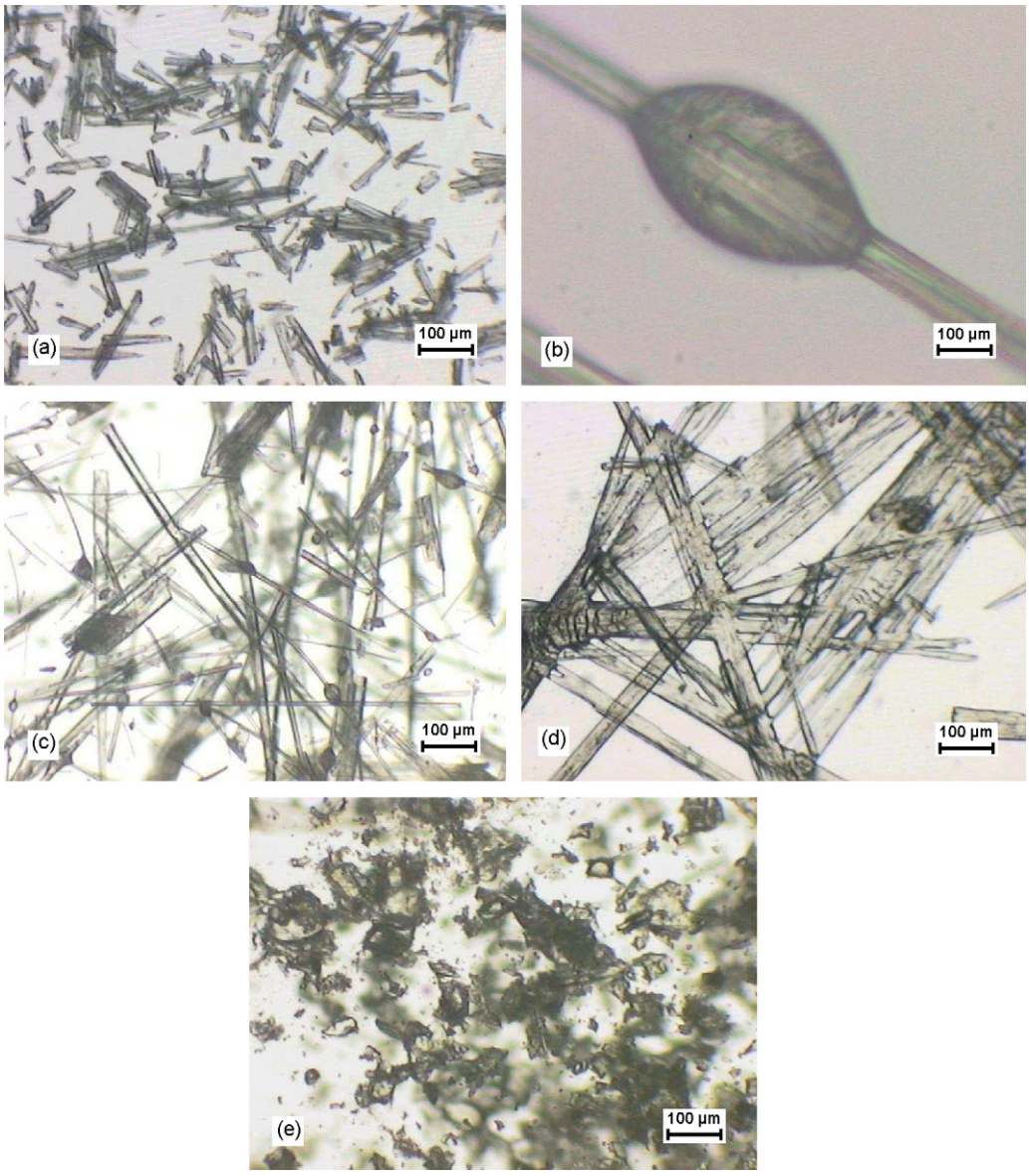

Fig. 4. Optical micrographs of the raw API and of SAS formulations: (a) raw API, (b) oxeglitazar/Poloxamer 188 (EtOH/$CHCl_3$), (c) oxeglitazar/PEG 8000 (EtOH/$CHCl_3$), (d) oxeglitazar/Poloxamer 407 (DCM), and (e) oxeglitazar/PVP K17 (EtOH/$CHCl_3$).

SEM micrographs of the raw API crystals (as received) showed very narrow needle-like crystals that ranged in length from 100 μm to 1 mm (Fig. 5a). The freeze-dried formulations were composed of porous hollow particles in the size range of 50 μm to 2 mm and the microstructure of these particles varied. SF processed pure oxeglitazar contained aggregated acicular particles between 1 and 10 μm in length (Fig. 5b). Drug-carrier particles consisted of porous lattice and aggregates of microparticles (Fig. 5c–f). The highest porosity was observed in PVP K17 matrix (Fig. 5f) while PEG 8000 had the smallest primary particle size (Fig. 5e). In all cases, free-flowing spherical particles were obtained that were easy to disintegrate and micronize. Solid dispersions were free from acicular drug crystals. Distinction could not be made between individual ingredients suggesting that oxeglitazar was homogenously dispersed throughout the polymer matrix.

4.3. Precipitation yield and drug content

Precipitation yield was defined as the percentage of recovered pharmaceutical ingredients with respect to the amount delivered with the liquid solution. The yields for SAS process ranged from 56 to 91% (Fig. 6). The highest yield was obtained from $CHCl_3$ between 86 and 91%, the average yields from DCM and EtOH/$CHCl_3$ were 70 and 67%. Compared to other supercritical antisolvent methods these values are rather promising (York et al., 2001; Juppo et al., 2003; Jung et al., 2003). Material loss can be partly decreased by improved filtration of the outlet stream and more careful collection of the particles retained in the precipitation vessel. The effect of these factors may be reduced by scale-up. Jung et al. (2003) have increased the particle recovery yield of SAS prepared inulin from 61 to 97% by multiplying the capacity by 100. What cannot be eliminated is the material loss rising from the fact, that pharmaceutical ingredients are not completely insoluble in SC CO_2-solvent systems. Hence these latter dissolve and wash out a part of these ingredients. The difference in average yields and the presence of the pharmaceutical ingredients in the cold trap at the end of the precipitation process confirmed this theory.

In SF process, neither freezing in liquid nitrogen, nor lyophilization is a potential source of loss. Material loss of freeze-dried formulations was owing to laboratory scale protocol. Harvesting of

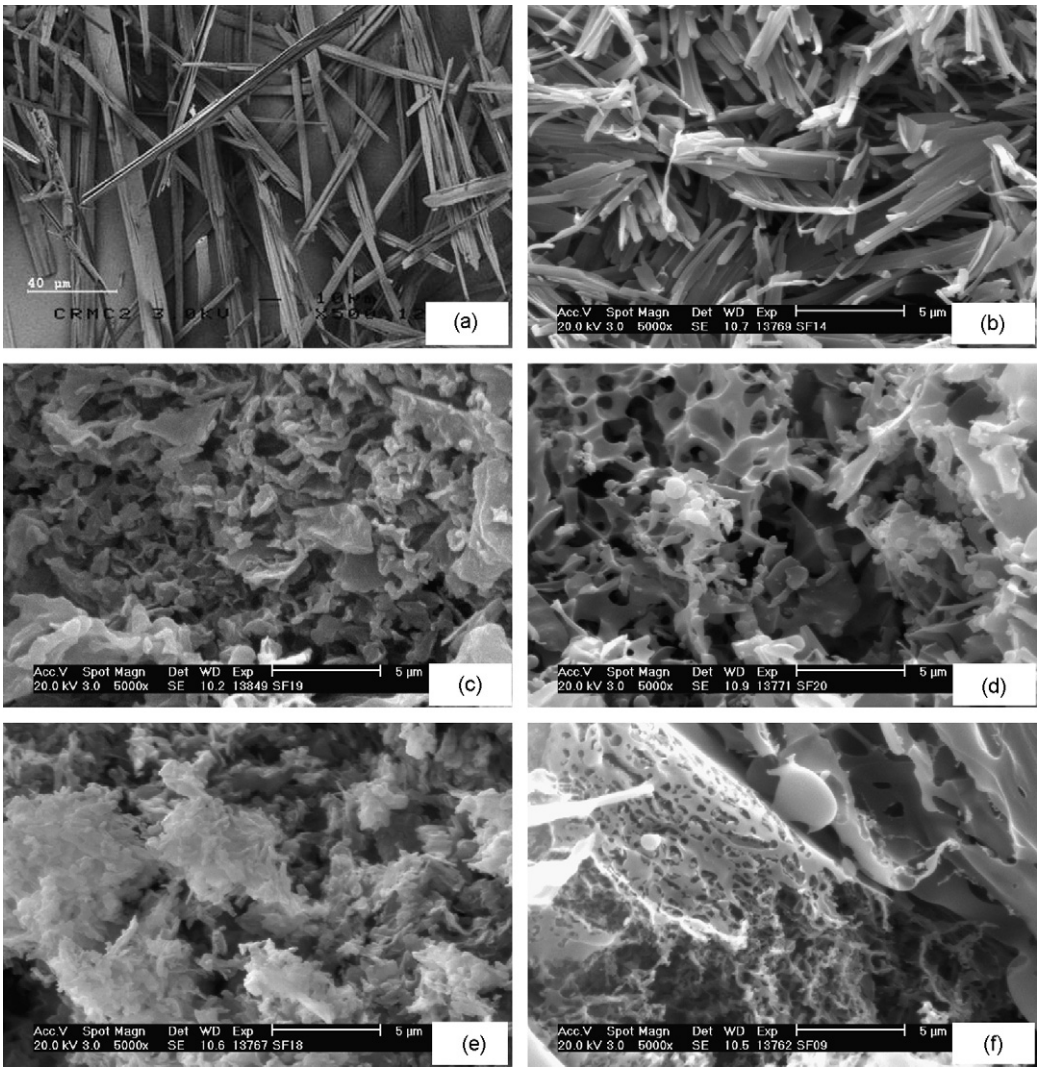

Fig. 5. SEM micrographs of the raw API and of SF formulations: (a) raw API, (b) freeze-dried pure API, (c) oxeglitazar/Poloxamer 188, (d) oxeglitazar/Poloxamer 407, (e) oxeglitazar/PEG 8000, and (f) oxeglitazar/PVP K17.

frozen particles and fast vacuumation during freeze-drying have decreased process yield but still it was very high, nearly 100% (Fig. 6).

Total drug content of SAS formulations ranged from 41.8 to 57.9% suggesting that the SC CO_2-solvent system does not always dissolve the two ingredients to the same extent (Fig. 7). In SF powders drug content showed smaller deviation, it ranged from 47.5 to 50.0%.

4.4. Crystallinity and polymorphic purity

XRD measurements were performed to determine the polymorphic purity and the degree of crystallinity of embedded oxeglitazar.

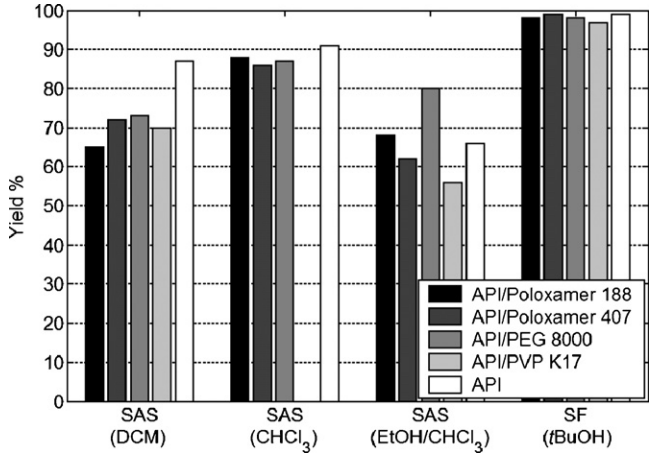

Fig. 6. Precipitation yield.

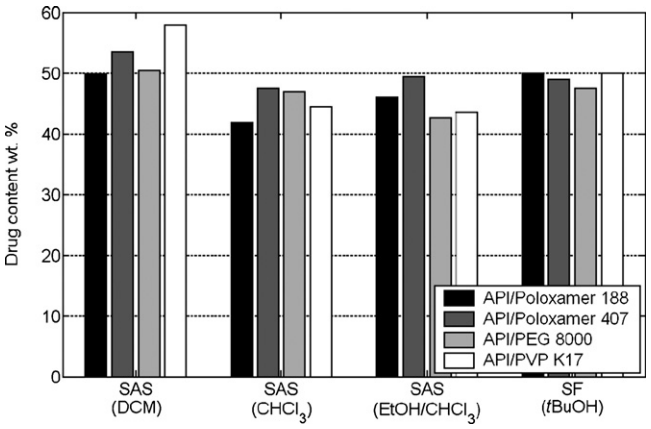

Fig. 7. Total drug content.

Table 1
Crystallinity (%).

Method	Solvent	Without excipient	Poloxamer 188	Poloxamer 407	PEG 8000	PVP K17
SAS	DCM	100	72	61	72	18
SAS	$CHCl_3$	100	77	99	51	4
SAS	EtOH/$CHCl_3$	92	100	100	58	27
SF	*t*BuOH	6	6	4	4	0

Table 2
Polymorphic purity.

Method	Solvent	Without excipient	Poloxamer 188	Poloxamer 407	PEG 8000	PVP K17
SAS	DCM	A (B)	A	A	A (B)	A and B
SAS	$CHCl_3$	A	A and B	A and B	A (B)	A and B
SAS	EtOH/$CHCl_3$	A	A (B)	A (B)	A (B)	A
SF	*t*BuOH	A and B	A	A	A	ND

A: Form A; A (B): Form A containing a few Form B; A and B: nearly equivalent proportions of the two polymorphs; ND: not detectable.

Table 3
Residual solvent level in ppm.

Method	Solvent	Limit	Poloxamer 188	Poloxamer 407	PEG 8000	PVP K17
SAS	DCM	7500	765	804	233	13,500
SAS	$CHCl_3$	750	341	132	826	28,600
SAS	EtOH/$CHCl_3$	750	52	89	441	3,240
SF	*t*BuOH	–	420	680	1100	4,600

DSC was found unsuitable because melted polymers have dissolved the embedded drug crystals before they could have melted. Therefore crystallinity was calculated on the basis of peak area in XRD patterns, according to the following equation:

$$\text{Crystallinity} = \frac{\text{Peak area (sample)}}{\text{Peak area (raw drug)}} \times \frac{100}{\text{Drug content}} \quad (1)$$

XRD measurements have confirmed our visual observations on drug crystallinity. SAS processed pure oxeglitazar was highly crystalline regardless of solvent (Table 1). SAS formulations with crystalline polymers (PEG 8000, Poloxamer 188 and 407) contained high fraction of crystalline drug, typically between 51 and 100% (Fig. 8). Coprecipitation with PVP K17 led to quasi amorphous solid dispersions with a crystallinity between 4 and 27%. While drug crystallinity was more influenced by applied polymer, polymorphic composition was likely to depend equally on solvent and excipient choice. Polymorphic compositions of different formulations are listed in Table 2. The stable form was always dominant notwithstanding that in most cases the metastable form was also detected.

SF formulations exhibited particularly low crystallinity. The highest value was measured in SF prepared pure oxeglitazar (6.2%), the lowest one in PVP K17 matrix. This latter can be considered as solid solution since crystalline drug was not detectable at all. These results are consistent with previous studies on particle formation using ultra-rapid freezing (Williams et al., 2002; Rogers et al., 2002a,b; Hu et al., 2004). Freeze-dried formulations were characterized by high polymorphic purity. Lower melting form was only detectable in the control formulation (SF prepared pure oxeglitazar). It is also evident that PVP has a certain ability to inhibit crystallization of active substances regardless of the method of preparation (Sethia and Squillante, 2004). Different XRD patterns are shown on Fig. 9.

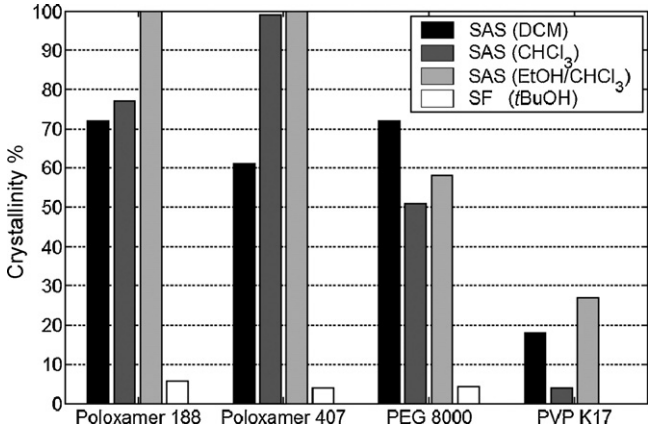

Fig. 8. Crystallinity.

XRD analysis was also used to perform stability studies. Freeze-dried formulations were stocked for 3 months at ambient temperature in sealed glass containers. XRD patterns showed no significant change in crystallinity and polymorphic composition after 3 months.

4.5. Residual solvent

The residual solvent content was determined by gas chromatography analysis. Among the organic solvents used is SAS process $CHCl_3$ and DCM are Class 2 solvents with permitted daily exposure (PDE) of 0.6 and 6 mg, EtOH belongs to the third and less toxic solvent class with a PDE of 50 mg (FDA, 1997). The concentration of Class 2 solvents in pharmaceutical products is limited because of their inherent toxicity. Some of these solvents – like $CHCl_3$ – are animal carcinogens without adequate evidences of carcinogenicity in humans. Although current requirements can be satisfied using conventional technologies, there is a clear tendency in pharmaceutical industry to replace Class 2 solvents or limit their application i.e.: to avoid them in the final stages of manufacturing. Two options are available when setting limits of Class 2 solvents: Option 1 may be applied if the daily dose is not known or fixed. This option assumes a high dose (10 g/day) that is rarely exceeded. Option 2 takes into account the daily dose or the maximum administered daily mass of a drug product, if the drug is not regularly administered. The maximum allowed daily dose for oxeglitazar is 400 mg, which makes 800 mg together with the inactive pharmaceutical ingredient. According to the Eq. (2), limits of $CHCl_3$ and DCM are 750 and 7500 ppm, under Option 2. Solvents in Class 3 are less toxic in short-term studies and negative in genotoxicity studies. Their

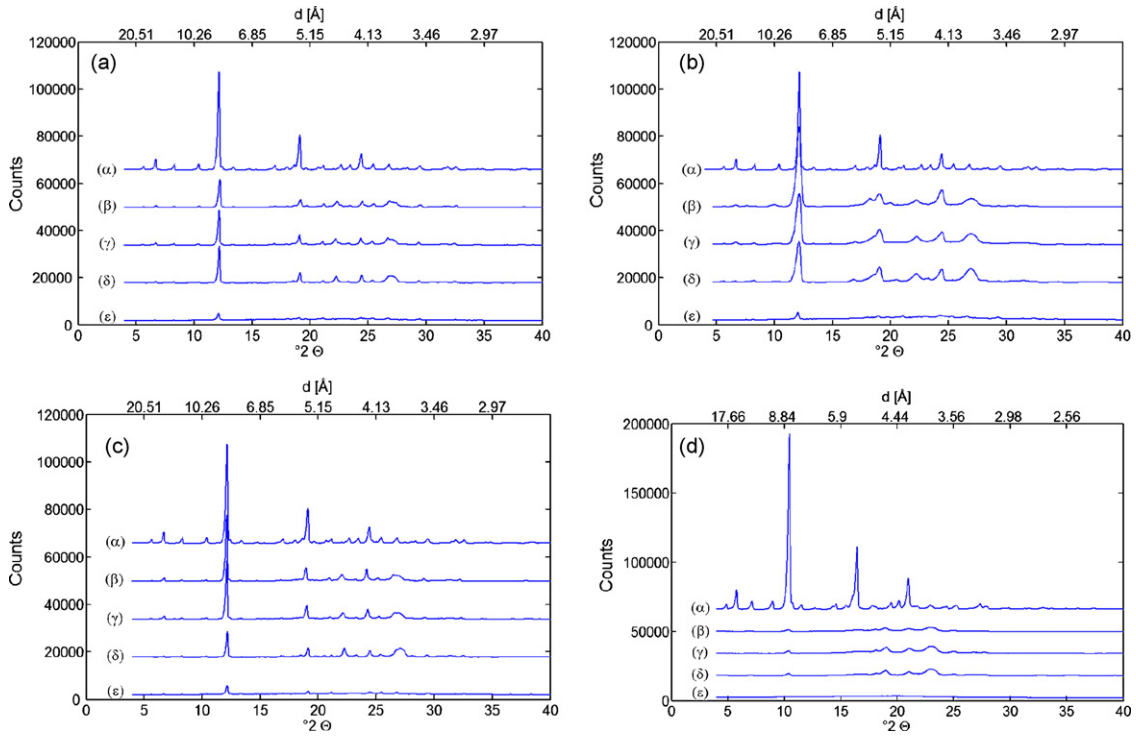

Fig. 9. XRD patterns: (a) SAS formulations from DCM solution; (b) SAS formulations from $CHCl_3$ solution; (c) SAS formulations from EtOH/$CHCl_3$ solution; (d) SF formulations from *t*BuOH solution. Greek letters refer to: (α) raw drug (Form A), (β) oxeglitazar/Poloxamer 188, (γ) oxeglitazar/Poloxamer 407, (δ) oxeglitazar/PEG 8000, and (ε) oxeglitazar/PVP K17.

presence in pharmaceutical products is acceptable without justification below 5000 ppm (under Option 1). The residual amount of EtOH was not determined in this work:

$$\text{Conc}\,(\text{ppm}) = \frac{1000 \times \text{PDE}\,(\text{mg/day})}{\text{Dose}\,(\text{g/day})} \quad (2)$$

Results of gas chromatography studies are shown in Table 3. With some exception all formulations meet ICH requirements. In the case of PVP K17 and PEG 8000 (precipitated from $CHCl_3$ solution), longer solvent stripping is recommended. PVP K17 formulations were characterized by high density and low crystallinity which makes solvent stripping more difficult. In addition, the precipitation processes of PVP formulations were frequently perturbed because the polymer has partly blocked the outlet filter.

Since *t*BuOH is not classified and no recommendation is available in ICH guidance, the concentration limit was assessed based on available toxicity data. Teagarden and Baker (2002) have evaluated several organic/water co-solvent systems for their use in freeze-drying process. Authors have pointed out that *t*BuOH/water system is particularly advantageous since *t*BuOH has high vapor pressure and high melting point, readily sublimes during primary drying and increases the sublimation rate of water, too. In addition, its acute toxicity is similar to those of Class 3 solvents. Residual *t*BuOH concentrations in freeze-dried pharmaceutical products ranged from 100 to 180,000 ppm in previous studies (Wittaya-Areekus and Nail, 1998; Nuijen et al., 2000; Teagarden et al., 1998). The amount of residual *t*BuOH was found to depend on solvent composition, excipient crystallinity, freezing-rate, cake thickness and the temperature of secondary drying. Although ultra-rapid freezing-rate and amorphous matrix are expected to result in high residual solvent level, it was not the case in this work. *t*BuOH concentration in SF prepared powders ranged from 420 to 4600 ppm after 30 h of lyophilization. These are below the limit applied to Class 3 solvents (5000 ppm, Option 1). Moreover, corresponding values in Poloxamer matrices are in the range of recommended limits for Class 2 solvents.

4.6. Specific surface area

Specific surface areas of raw drug, SAS and freeze-dried formulations are listed in Table 4. Three representative SAS powders were chosen that cover the whole range of particle morphology. Oxeglitazar/Poloxamer 188 and oxeglitazar/Poloxamer 407 powders consisted of acicular drug crystals with and without spherical polymer coating. Oxeglitazar/PVP K17 was a semi-crystalline solid dispersion with dense irregular particles. Raw drug prepared by cooling crystallization and SCF processed solid dispersions exhibited low specific surface area (<1 m^2/g). In contrast, SF formulations were characterized by enhanced BET surface area ranging from 6.1 to 39.1 m^2/g. PVP K17 showed the highest surface area (39.1 m^2/g), which was not surprising seeing SEM micrographs. The other drug-carrier powders exhibited lower surface area compared to SF prepared oxeglitazar (18.9 m^2/g), still they were at least 6 times higher than raw drug.

4.7. Dissolution kinetic study

Dissolution tests were performed in simulated intestinal fluid (pH 7.4). Dissolution profiles of the various formulations are shown

Table 4
Specific surface area.

Method	Excipient	Solvent	Surface area (m^2/g)
Raw drug	–	–	<1
SAS	Poloxamer 188	EtOH/$CHCl_3$	<1
SAS	Poloxamer 407	DCM	<1
SAS	PVP K17	EtOH/$CHCl_3$	<1
SF	–	*t*BuOH	18.9
SF	Poloxamer 188	*t*BuOH	6.1
SF	Poloxamer 407	*t*BuOH	7.5
SF	PEG 8000	*t*BuOH	15.0
SF	PVP K17	*t*BuOH	39.1

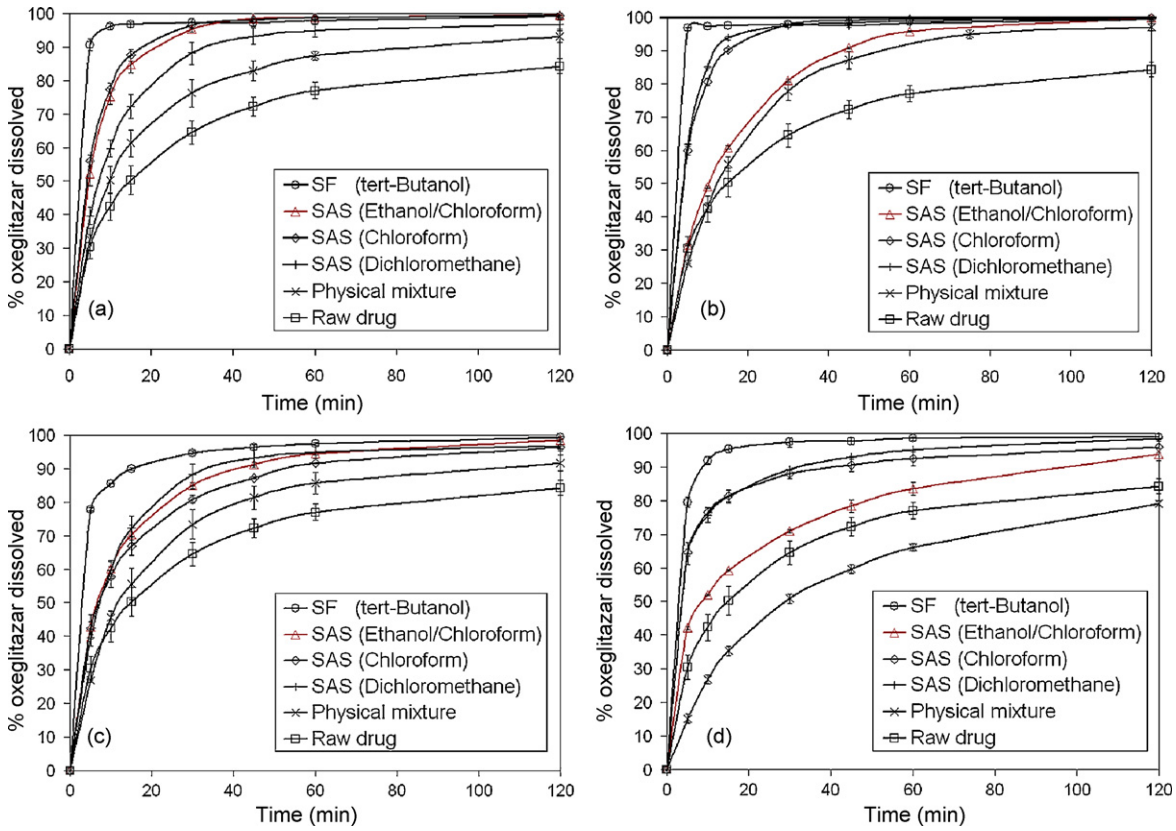

Fig. 10. Dissolution profiles of SAS and SF prepared powders: (a) oxeglitazar/Poloxamer 188, (b) oxeglitazar/Poloxamer 407, (c) oxeglitazar/PEG 8000, and (d) oxeglitazar/PVP K17.

in Fig. 10. Approximately 30% of raw drug were dissolved within 5 min and 85% after 2 h. SAS and SF processed formulations exhibited higher dissolution rate compared to raw drug and physical mixtures. The percentage of dissolved oxeglitazar at 5 min varied from 31.6 to 64.3% for SAS and from 77.9 to 96.9% for freeze-dried formulations. Dissolution profiles of SAS prepared PEG 8000 formulations showed up to 1.4-fold higher concentrations than raw drug at 5 min. Corresponding values for Poloxamer and PVP K17 formulations varied between 1.0 and 2.1. Dissolution curves of Poloxamer 407 and PVP K17 formulations showed high initial slope but dissolution rate of PVP K17 formulation dropped considerably in the first 10 min. At 30 min, dissolved oxeglitazar from PVP K17 matrix did not exceed 90% while corresponding values of Poloxamer 407 formulations achieved 97%.

Freeze-dried powders showed very high dissolution rates. The amount of dissolved oxeglitazar from SF formulations was 2.6–3.2 times greater than raw drug within 5 min. The highest concentration was measured from Poloxamer 407 matrix (96.9% within 5 min), followed by Poloxamer 188 (90.7%). Although, PVP K17 was better in terms of specific surface area and crystallinity it led to lower rate of dissolution (79.6%) compared to Poloxamers. This can be attributed to the lower dissolution rate of PVP K17 carrier which was proved to be a factor more important than specific surface area and crystallinity. Enhanced dissolution rate of freeze-dried formulations was consistent with those in earlier studies (Williams et al., 2002; Rogers et al., 2002a,b; Hu et al., 2004).

5. Discussion

SAS and SF are two solution-based particle formation methods with different mechanisms. SAS is based on the high supersaturation induced by the antisolvent effect of the supercritical fluid simultaneous with the evaporation of the organic solvent. Under the working conditions, solvents and SC CO_2 form a single phase and solution jet is characterized by intensive mass transfer. In the SF process, solution is dispersed in air and forms droplets stabilized by superficial tension forces. The atomized droplets freeze immediately upon the impact of the jet in liquefied gas. As liquid nitrogen is boiling throughout the process, injected solution keeps on evaporating nitrogen. This may induce intense boiling as nitrogen has relatively low heat of evaporation (5.594 J/mol) and forms an insulating "vapor barrier" of gaseous nitrogen around droplets. Briggs and Maxvell (1973) used dichlorodifluoromethane ($T_b = -30\,^\circ C$) as cryogenic medium to prevent the formation of extensive vapor barrier. However industrial application of greenhouse gases like Freon is avoided and is unnecessary as temperature gradient in liquid nitrogen was found high enough to ensure fast freezing.

The particle morphology of most SAS processed formulations was similar to raw material obtained by cooling crystallization. However, unexpectedly large particle size was observed in comparison with other SAS and ASES prepared pharmaceutical products (Bitz and Doelker, 1996; Carretier et al., 2003). Increased particle size and equilibrium shape are obtained when crystallization occurs in low supersaturation conditions. Although, both processes are known to be kinetically controlled, this characteristic is more dominant in ultra-rapid freezing. The bulk powder properties of freeze-dried powders were independent of applied polymer; all formulations were composed of highly porous free-flowing spherical particles. The active substance and the excipient were not visually distinguishable in SEM micrographs suggesting that oxeglitazar was homogenously dispersed throughout the amorphous polymer matrices. This hypothesis was confirmed by XRD measurements. The amorphous state was dominant in all freeze-dried formulations and in SAS prepared oxeglitazar/PVP

K17 powders. PVP K17 inhibited the crystallization of oxeglitazar leading to a solid solution in SF and non-acicular amorphous particles in SAS process. Although, particle size of freeze-dried formulations was much larger compared to SAS, they were easy to disintegrate owing to their porous structure.

Polymorphic purity of SAS processed powders was heavily influenced by solvent and excipient choice. Though higher melting form was dominant in all cases the presence of metastable polymorph is a crucial factor supposed to induce phase transition in the pharmaceutical product and shorten shelf life. The effect of solvent was not studied for SF process but excipient showed favorable effect on polymorphic composition. Lower melting form was not detectable in any freeze-dried drug-carrier systems contrary to control formulation. Such a high polymorphic purity was unexpected as non-equilibrium conditions often result in metastable forms as it was the case for SF processed pure oxeglitazar. Additionally, XRD measurements performed 3 months after the preparation showed no significant evolution in drug crystallinity and polymorphic purity for SF formulations suggesting that excipient matrix can stabilize the incorporated amorphous drug phase.

Residual solvent content was of the same order of magnitude in powders prepared by the two techniques. Residual DCM contents were consistent with published works on SCF processed pharmaceutical products. Bitz and Doelker (1996) compared spray-drying, solvent evaporation and ASES processes. Residual DCM concentrations in L-poly(lactic acid) (L-PLA) and L-PLA/tetracosactide powders after 4 h of drying at a CO_2 flow rate of 6 kg/h were 5283 and 758.3 ppm, respectively. Ruchatz et al. (1997) studied the effect of spraying rate and CO_2 flow rate on residual solvent content, particle size, yield and morphology of ASES prepared L-PLA particles. Authors achieved low residual concentrations of DCM (71.5–449.9 ppm) after 5 h of solvent stripping by varying the CO_2 flow rate in the range of 2–11 kg/h. Experiments, carried out at constant spraying rate and drying time revealed that residual solvent level and CO_2 flow rate were inversely proportional. Thus, residual solvent level can be reduced by increasing the CO_2 flow rate or extending the solvent stripping. Residual $CHCl_3$ content was not previously reported in the literature of SCF based particle engineering. Residual *t*BuOH content in SF powders was lower than in most conventional freeze-dried pharmaceutical products (Wittaya-Areekus and Nail, 1998; Nuijen et al., 2000; Teagarden et al., 1998). Corresponding values of *t*BuOH in SF or SFL processes are not available. Hu et al. (2003) prepared solid dispersions by SFL technology from acetonitrile and tetrahydrofuran/water co-solvent solutions. Acetonitrile and tetrahydrofuran impurities were not detectable in SFL micronized carbamazepin/Poloxamer 407/PVP K15 powders after 15 and 72 h of lyophilization. PVP K17 has retained the higher amounts of solvent in both technologies involved.

BET surface areas of raw drug and SCF processed powders did not exceed 1 m^2/g. In contrast, SF formulations were characterized by enhanced surface area. Measured values were more than 39-fold higher than raw drug. Owing to the ultra-rapid freezing all drug-carrier systems consisted of highly porous particle. Interestingly, apart from oxeglitazar/PVP K17 system, drug-carrier particles exhibited lower surface area than SF prepared pure oxeglitazar. Corresponding value for oxeglitazar/PVP K17 was considerably higher in accordance with previous studies (Williams et al., 2002; Rogers et al., 2002a,b; Hu et al., 2004).

SAS and SF processed formulations exhibited improved dissolution properties that allow more of the drug to be absorbed. Within 5 min, the amount of dissolved oxeglitazar varied from 31.6 to 64.3% for SAS and from 77.9 to 96.9% for freeze-dried formulations. The higher dissolution rate of these latter can be attributed to their glassy state, high specific surface area and homogenous distribution of oxeglitazar in the polymer matrix. However, even though SF prepared PVP K17 formulation exhibited considerably higher specific surface area and higher amorphous content, the best dissolution kinetic was obtained with Poloxamer 407 (96.9% within 5 min).

6. Conclusion

This paper compares a SCF and a cryogenic particle formation method. SAS and SF technologies were evaluated for their potential use in preparation of immediate release solid oral dosage forms. Owing to the different mechanisms of particle formation, powders exhibited very different particle morphologies, crystallinities and dissolution rates. SF technology was proved to be a versatile method to prepare fast-dissolving solid dispersions. SAS prepared formulations showed higher crystallinities and lower dissolution rates but SAS is more favorable in terms of time consumption. As the above results indicated requirements on residual solvent, polymorphic purity and stability can also be satisfied.

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