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A novel method for the production of crystalline micronised particles

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ABSTRACT

The aim of this study was to develop a method for converting an amorphous drug to a crystalline form to enhance its stability and inhalation performance. Spray-dried amorphous salbutamol sulphate powder was conditioned with supercritical carbon dioxide ($scCO_2$) modified with menthol. The effect of menthol concentration, pressure, temperature and time on the characteristics of the resulting salbutamol sulphate powder was investigated. Pure $scCO_2$ had no effect on the physical properties of amorphous salbutamol sulphate; however, $scCO_2$ modified with menthol at 150 bar and 50 °C was efficient in converting amorphous drug to crystalline form after 12 h of conditioning. The average particle size of powders decreased slightly after the conditioning process because of reducing agglomeration between particles by increasing surface roughness. Emitted dose measured by the fine particle fraction ($FPF_{emitted}$) of amorphous salbutamol sulphate was enhanced from 32% to 43% after conditioning with $scCO_2$ + menthol and its water uptake was significantly decreased. This study demonstrates the potential of $scCO_2$ + menthol for converting amorphous forms of powders to crystalline, while preserving the particle size.

1. Introduction

More than 75% of pharmaceutical formulations use drugs in powder form (Roberts and Debenedetti, 2000). The crystallinity and particle size are key factors in drug stability and bioavailability. Methods such as spray drying, grinding, jet milling, and advanced liquid–liquid antisolvents are currently used for drug micronisation. Their broad application is limited due to use of high temperature operation (Tong and Chow, 2006), powders with broad particle size range, the use of organic solvents and their residues (Subra-Paternault et al., 2007), and the appearance of amorphous fractions (Brodka-Pfeiffer et al., 2003).

Salbutamol sulphate is a β_2-sympathomimetic for the treatment of asthma, which is broadly used in inhalation and oral formulations (Corrigan et al., 2006a; Columbano et al., 2002; Brodka-Pfeiffer et al., 2003). It would be desirable to enhance the aerosol performance and the bioavailability of each drug to minimise the dose and decrease the side effects of each drug. The micronised-crystalline salbutamol sulphate is produced by crystallisation followed by grinding and milling (Fages et al., 2004). Dry powder of crystalline salbutamol sulphate was also recently produced by a liquid–liquid antisolvent using high gravity packed bed followed by spray drying (Chiou et al., 2007; Hu et al., 2008) and a sonocrystallisation technique (Dhumal et al., 2009). The aerosol performance of salbutamol sulphate was significantly enhanced, but large amounts of organic solvent were used in these processes. The amorphous form of micronsied salbutamol sulphate produced by spray drying of an aqueous solution exhibited poor aerosol performance (Chawla et al., 1994).

The Gibbs free energy of amorphous solids is higher than crystalline forms, thus there is always a tendency for the glassy materials to recrystallise into the more stable crystalline form (Hancock and Zografi, 1994). The amorphous drug transforms into the thermodynamically stable crystalline state at ambient conditions when the glass-transition temperature (T_g) is below the room temperature. The rate of crystallisation can be explained by the William–Landel–Ferry equation (Williams et al., 1955) (WLF equation) where the rate of the amorphous to crystalline transition (r) is defined as the ratio of the time for crystallisation (θ_{cr}) at any temperature (T) to the time for crystallisation (θ_g) at the T_g which can be related by the following equation:

$$\log_{10} r = \log_{10}\left(\frac{\theta_{cr}}{\theta_g}\right) = \frac{-17.44(T - T_g)}{51.6 + (T - T_g)} \quad (1)$$

Thus by increasing the ΔT (difference between process temperature (T) and T_g) the rate of crystallisation is promoted.

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Adsorption of water and other solvents into an amorphous powder may result in decreasing the glass transition, reducing the energy to convert amorphous powder to crystalline form and plasticization (Ward and Schultz, 1995; Buckton et al., 1995; Columbano et al., 2002; Raula et al., 2008; Hancock and Zografi, 1994; Roos, 1995). Amorphous powders are not thermally stable, agglomerate easily and convert to crystalline forms with a larger particle size and consequently, have short shelf-life (Chan and Chew, 2003).

Crystalline, non-aggregated micronised particles with a narrow particle size distribution are most desirable for use in commercial dry powder inhalers (DPI). The major challenges in micronisation processes are the production of a narrow particle size, preclusion of amorphous phase formation, and reduction of surface electrostatic charge that increase powder aggregation and cohesiveness, leading to handling problems and potentially decreasing the emitted doses from the inhalation devices.

Several approaches were attempted to convert amorphous drugs into crystalline form. Amorphous drugs were converted to crystalline by exposure of the powder to elevated humidity (Buckton et al., 1995; Columbano et al., 2002), however, the aerosol performance was decreased due to powder aggregation (Chawla et al., 1994; Corrigan et al., 2004, 2006b; Raula et al., 2008). Amorphous and spherical particles of salbutamol sulphate with an average size suitable for the inhalation delivery were produced by the spray-dried technique without affecting its chemical integrity (Chawla et al., 1994). Co-spray drying of salbutamol sulphate with excipients such as polyethylene glycol (PEG) (Corrigan et al., 2004), lactose and chitosan (Corrigan et al., 2006a) and other drug such as ipratropium bromide (Corrigan et al., 2006b) was examined to convert the amorphous powder of salbutamol sulphate to the crystalline form, but none of these additives were efficient.

Supercritical fluids, which are fluids at above critical temperature and pressure, have been used for the production of crystalline particles of drugs using various techniques, such as rapid expansion of supercritical solutions (RESS), particles from gas-saturated solutions, and gas antisolvent (Tong and Chow, 2006; Palakodaty and York, 1999; Shekunov and York, 2000; Shariati and Peters, 2003; Gallagher et al., 1989; U.S.P. 28, 2005). The RESS process is designed for a drug with a high degree of solubility in a supercritical fluid. The particles from gas-saturated solutions are designed for drugs and carriers that exhibit a melting-point depression upon exposure to a supercritical fluid while the gas antisolvents are designed for the micronisation of a drug with a low solubility in a supercritical fluid (Gallagher et al., 1989). Many companies currently assess the feasibility of using SCF process in large scale (Tong and Chow, 2006). A gas antisolvent technique was used for the crystallisation of salbutamol sulphate (Najafabadi et al., 2005). The crystalline flake-like particle of salbutamol sulphate with an average particle size of 7 μm was formed from methanol solution using CO_2 as an antisolvent. However, the powder was not suitable for inhalation drug delivery formulation as the particle size was above 5 μm which is not desirable for inhalation (Prime et al., 1997; Malcolmson and Embleton, 1998; Patton et al., 2004; Sellers et al., 2001; Stanton et al., 2002; Thi et al., 2008).

It was reported that amorphous fluticasone fumarate could be converted to crystalline form by conditioning spray-dried powder with menthol vapour at 50 °C (Brown et al., 2003). Menthol is currently obtained from extracts of various mint oils or prepared synthetically. It is stable in room temperature and store as solid crystalline. Presently, menthol is used in many pharmaceutical formulations for its fragrance and flavors and in foods, beverages, cigarettes, tooth pastes and food flavors for the refreshment taste (Hamasaki et al., 1998). Menthol is accepted by US Food and Drug Administration (FDA) for oral use (Patel et al., 2007). The solubility of menthol in high pressure CO_2 is considerably high (e.g. 0.68×10^{-3} mole fraction at 75 bar and 40 °C), therefore, it can be used as a harmless modifier to enhance the solubility of pharmaceutical compound in many dense gas processes (Sovova et al., 2007). Menthol was used as a cosolvent in micronisation of griseofulvin using the RESS process (Thakur and Gupta, 2005). Menthol increased the solubility of griseofulvin in $scCO_2$ and decreased the degree of aggregation between particles. The preliminary results acquired in this study demonstrate that the addition of menthol to CO_2 did not increase the solubility of salbutamol sulphate in CO_2 at the conditions examined significantly; it was not, therefore, practical to apply RESS process for the crystallisation and micronisation of salbutamol sulphate.

The primary objective of this study was to produce a stable salbutamol sulphate powder suitable for aerosol drug delivery. In this study we assess to use $scCO_2$ + menthol for converting amorphous powder to crystalline form. Rapid sorption of CO_2 + menthol on the surface of powder may decrease the drug transition temperature and allow the amorphous drug to convert to crystalline form at moderate temperature at a predetermined time. The effects of process variables on the powder crystallinity and particle size were determined.

2. Materials and methods

2.1. Materials

Salbutamol sulphate (99.9% purity), *n*-hexane (95% purity), food grade carbon dioxide with 99.99% purity and menthol (99.9% purity) were supplied by Inter-Chemical Ltd., China, Unichrom, British Oxygen Company (BOC) and Sigma–Aldrich, respectively. Milli-Q water was used to dissolve salbutamol sulphate for the spray drying processing and analysis.

2.2. Preparation of amorphous salbutamol sulphate

Salbutamol sulphate was dissolved in water and spray dried to generate amorphous salbutamol sulphate using a Buchi 290 mini spray drier (Buchi Laboratory-Techniques, Switzerland). In each run a 10 wt% aqueous solution of salbutamol sulphate was sprayed at 2.4 mL/min via a two-fluid nozzle with an internal diameter of 2.5 mm. The nozzle's air aspiration rate and the pressure were kept at 38 mL/min and 4.14 bar, respectively. The drying air inlet set at 150 °C. The drying air temperature at the outlet was 103 °C. Finally, dried powder was collected from the spray drier collection pot, kept in a sealed container, and stored in a desiccator at room temperature for analysis and further processing.

2.3. Powder conditioning by $scCO_2$ + menthol

The schematic diagram of apparatus used for conditioning amorphous powders by $scCO_2$ + menthol is shown in Fig. 1. In each run, 2.0 g amorphous salbutamol sulphate powder was loaded into a custom-made stirred high-pressure vessel with a total volume of 45 mL. The vessel was stirred using a magnetic stir bar. The temperature of the water bath and stirrer speed was adjusted and controlled using a hot plate (Daihan Scientific Co. Ltd., MSH-30D Wise Stir). A high pressure pump (Thar Technologies, USA, P50 Series,) was used for the delivery of CO_2 at high pressure to the system.

The required amount of menthol was loaded into the menthol container as shown in Fig. 1. The amount of menthol and salbutamol sulphate was loaded in the system to keep the weight ratio at a desired level. The concentration of menthol in CO_2 was calculated at the determined pressure, temperature and volume of the vessel. At all conditions examined, the amount of menthol dissolved in CO_2 was below the solubility (saturation concentration) of menthol in

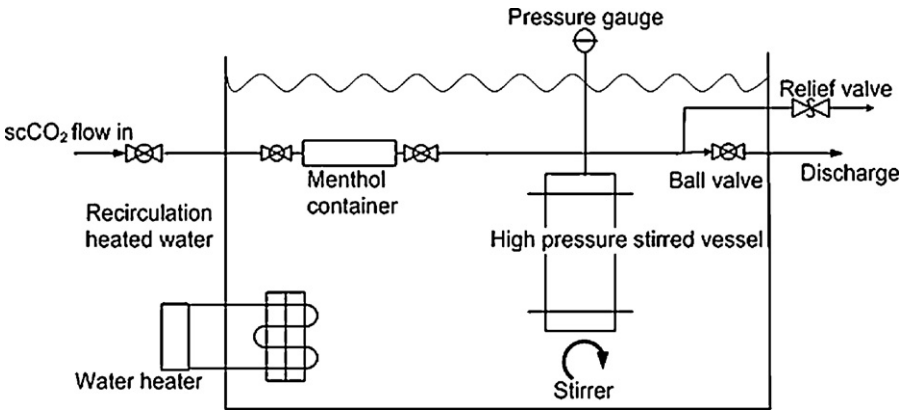

Fig. 1. Schematic diagram designed for the conditioning of salbutamol sulphate with $scCO_2$ + menthol.

CO_2. Therefore, total amount of menthol added to the system was dissolved in CO_2 at the set temperature and pressure.

In each experiment carbon dioxide was first mixed with menthol prior to pressurizing the high-pressure vessel and then fed at a rate of 10 mL/min into the vessel loaded with salbutamol sulphate until the desired pressure was achieved. The system was then isolated and kept at these operating conditions for a period of time. After conditioning, the stirrer was stopped, and the system was purged of menthol by passing pure CO_2 at the operating pressure and temperature for 1.5 h at constant flow rate of 10 mL/min. Finally, the system was depressurized; the powder was collected, placed in a closed sealed container and kept at room temperature in a desiccator for characterisation. At each condition the experiment was repeated at least three times.

The experiments were conducted to assess the effect of variables and determine the optimum conditions for crystallinity of salbutamol sulphate while maintaining the particle size. An experimental design was used with four factors and two levels (pressure, temperature, conditioning time; and weight ratio of menthol to salbutamol sulphate).

2.4. Solid-state characterisation

2.4.1. Particle morphology

A scanning electron microscope (SEM) (Phillips 505, Holland) was used to determine particle morphology. The equipment was operated at 15 kV and spot size 20. Samples were gold coated (~20 nm thickness) using a modified Edwards E306A coater.

2.4.2. Particle size distribution (PSD)

The particle size distribution was measured using Mastersizer 2000 laser diffractometer (Malvern, Worcs, UK) as a dry powder. 10–30 mg of powder was loaded into the dry powder disperser and the particle size distribution was determined at a maximum pressure of 4 bar. The measurements were carried out in triplicate. The size distributions were expressed by the cumulative volume diameter at 10%, 50% and 90%.

2.4.3. Particle crystallinity

Powder crystallinity was determined by X-ray diffraction (XRD, D5000, Siemens, Germany) operated at room temperature using Cu Kα radiation at 30 mA and 40 kV, with an angular increment of 0.05°/s count time of 2 s. A Universal Analysis 2000 modulated differential scanning calorimetry (DSC, TA Instruments, USA) was also used to analyse the crystallinity and melting points of the powders. The samples (3–7 mg) were loaded into closed aluminum pans and subjected to heat at a rate of 10 °C/min to a maximum temperature of 250 °C under a nitrogen purge. Fourier transform infrared (FTIR) spectroscopy (Varian Schimitar 2000, with a PIKE ATR-an attenuated total reflectance accessory) was also used to assess the crystallinity of the powder with 4 cm^{-1} resolution and 32 scans.

Prior to testing the degree of crystallinity of salbutamol sulphate with DSC, a calibration data was acquired for synthetic samples produced by physical mixture of crystalline and amorphous salbutamol sulphate. Amorphous fraction was prepared by spray drying and the crystalline one by conditioning raw salbutamol sulphate with water vapour at room temperature for 48 h. Turbular System (Schatz Mixer) was used for 15 min to prepare a 2 g mixture of salbutamol sulphate with various compositions from 0 to 100 wt% crystalline. The mixtures were analysed by DSC analyser to measure the heat of crystallisation (J/s) that corresponds to amorphous contents of the samples.

2.4.4. Quantify of surface roughness

Surface fractal dimensions (D_S) of the spray dried and conditioned salbutamol sulphate particles were obtained to quantify the surface roughness of particles. The determination of D_S by light scattering utilizes the Rayleigh–Gans–Debye (RGD) scattering theory. There are two conditions to be met in order to utilize the RGD theory to predict D_S accurately (Tang et al., 2003).

(a) $|m-1| \ll 1$, where m is the complex refractive index of the particle relative to that of the surrounding medium. This condition implies that the incident beam is reflected negligibly at the particle-medium interface so that the direction of incident light is the same everywhere in the scattering medium.
(b) $kR|m-1| \ll 1$, where R is the radius of particle and k is a function of incident light's wavelength. This condition implies that the amplitude of incident beam does not change significantly after it meets the particle.

This theory neglects the multiple scattering effect because it assumes that the radiation illuminating each particle in the aggregate and is totally unperturbed by the presence of other particles in the aggregate. Nevertheless, it has been shown theoretically and experimentally that although multiple scattering does change the magnitude of the scattering intensity, it does not change the evaluated fractal dimension of the aggregates (Chen et al., 1988).

The surface fractal dimension was measured using a Mastersizer 2000 laser diffractometer (Malvern, Worcs, UK). During the experiment, a laser light hits the particles suspended in liquid and it is scattered. The intensity of the scattered light, which is measured by a number of detectors positioned at different angles, can be used to generate D_S. The value of surface fractal dimension varies from 2 for a perfectly smooth surface to 3 for very rough surface particles.

Table 1
The effect of processing conditions on the particle size and/or crystallinity of salbutamol sulphate powder.

Time (hours)	Pressure (bar)	Temperature (°C)	Weight ratio menthol to salbutamol sulphate	$D_{0.5}$ (μm)	Crystallinity
Preliminary experimental data					
24	1	50	1:1	3.06(±0.03)	No
15	150	50	–	3.05(±0.001)	No
5	150	50	1:1	3.21(±0.04)	Partially crystalline
5	150	50	1:4	3.74(±0.31)	No
8	150	45	1:3	3.71(±0.01)	No
10	150	35	1:4	3.17(±0.01)	No
12	150	30	1:2	2.88(±0.04)	No
12	150	50	1:1	2.56(±0.02)	Crystalline
Experimental design					
5	100	40	1:1	nm	No
5	100	50	1:1	nm	No
5	150	40	1:1	nm	No
5	150	50	1:1	nm	No
8	100	40	1:1	nm	No
8	100	50	1:1	nm	No
8	150	40	1:1	nm	No
8	150	50	1:1	nm	Partially crystalline

nm—not measured.

Scattering momentum (q) is used to relate the scattering angle (θ), the laser wavelength (λ), and the refractive index of suspending liquid (η):

$$q = \frac{4\eta\pi}{\lambda} \sin\left(\frac{\theta}{2}\right) \quad (2)$$

In the case of surface fractal, when $q >$ (1/particle diameter):

$$I(q) \propto q^{-6+D_S} \quad (3)$$

where $I(q)$ is the intensity of scattered light.

Based on Eq. (3), when $I(q)$ versus (q) is plotted on logarithmic scale, D_S can be obtained from the slope.

2.4.5. Aerosol performance

The method of aerosol performance analysis is based on the standard technique described in British Pharmacopeia (British Pharmacopeia, *Appendix XII*, *Aerodynamic assessment of fine particles-fine particle dose and particle size distribution*, *Apparatus C*. 2001). The aerosol performance of salbutamol sulphate powders were assessed using an Aeroliser® (Novartis Pharmaceuticals, Australia) coupled through an USP stainless steel throat to a multi-stage liquid impinger (MSLI) (Copley, UK), operating at 100 L/min. The powder (20.0 ± 0.5 mg) was filled into hydroxypropyl methylcellulose capsule (size 3, Capsugel®, USA) and three capsules were used in each experiment. Each experiment was performed in duplicate. Particles deposited at different locations in MSLI were assayed by UV spectrophotometry (U-2000, Hitachi, Japan) at 276 nm. A calibration curve of salbutamol sulphate in water was prepared prior to conducting the aerosol performance test at the concentration range of 0.4–100 μg/mL. The data were used to determine the capsule and device retention (the mass fraction of drug particles remaining in capsule and device relative to the total mass recover), the impaction loss (the mass fraction of drug particles remaining in throat and stage 1 relative to the total mass recover), the emitted dose ($FPF_{emitted}$, the mass fraction of drug particles collected from stages 3, 4 and filter relative to the emitted dose) and the loaded fine particle fraction (FPF_{loaded}, the mass fraction of drug particles collected from stages 3, 4 and filter relative to the total mass recover). Total mass recover is the total of emitted dose and the capsule and device retention, while emitted dose is the drug mass collected from the throat, stages 1–4 and filter of the MSLI.

2.4.6. Dynamic vapour sorption (DVS)

The physical stability of dried powders of salbutamol sulphate was assessed using a dynamic vapour sorption (DVS, DVS-1000, Surface Measurement System, Cheadle, UK). The sample was allowed to dry, then loaded into a glass sample pan and finally exposed to a series of regulated relative humidities (RH). The sample on the pan was equilibrated at one RH until the change in mass over 5 min was less than 0.0002% before progressing to the next RH. Two cycles of 0–90% RH and back to 0% in step of 10% RH were used, with mass and humidity recorded every 20 s. Isothermal condition was produced through the software provided (DVS Analysis Suite Version 3.6), and the mass change was calculated relative to the dry mass.

2.4.7. Gas chromatography (GC) analysis

A Hewlett-Packard 5890A with flame ionization detector (FID) was used for the GC analysis. The column used for the GC analysis was from J & W DB5 series with 15 m × 0.32 mm i.d. The GC column, injector and detector temperature were set at 150 °C. The flow rate of the nitrogen carrier gas was 20 mL/min. The injection volume was 2 μL for standard solution of menthol (0.001%, w/v) and conditioned salbutamol sulphate solution which were dissolved in *n*-hexane. The method used to determine menthol was a single point external standard (G.C. Education, 1998). In this method, a standard solution with known amount of menthol (0.001%, w/v) was injected in GC column and the peak area was recorded. Then a response factor (R_f) was calculated using Eq. (4):

$$R_f = \frac{\text{peak area}}{\text{amount of known menthol (g)}} \quad (4)$$

A series of unknown concentration of menthol in conditioned salbutamol sulphate were injected in GC column and the peak areas were recorded. The amount of menthol in the conditioned salbutamol sulphate can be calculated using Eq. (5):

$$\text{Amount of menthol} = \frac{\text{peak area}}{R_f} \quad (5)$$

The retention time for menthol was at 3.234 ± 0.007 min with peak area of 141.89 ± 47.54 μV s for sample solution. Standard solution of menthol with concentration 0.001% (w/v) gave peak area of 2647 μV s, thus the detection of limit by GC method would be much less than 0.001% (w/v) or 2×10^{-8} g in 2 μL solvent.

3. Results and discussion

Our preliminary data showed that pure $scCO_2$ at 150 bar and 50 °C had no plasticization effect on salbutamol sulphate and was not efficient in converting amorphous salbutamol sulphate to crystalline for even after 15 h exposure. Conditioning amorphous salbutamol sulphate with menthol was also not efficient to generate crystalline powder even after 24 h conditioning at 50 °C in atmospheric pressure. In contrast, it was reported that amorphous fluticasone fumarate was converted to crystalline form by conditioning spray-dried powder with menthol vapour at 50 °C in atmospheric pressure (Brown et al., 2003). We assessed the feasibility of using $scCO_2$ supplemented with menthol for the conditioning of amorphous salbutamol sulphate. The amount of menthol was below the saturation concentration in CO_2 at all conditions examined (Sovova et al., 2007). The effects of menthol, pressure, temperature and time on the particle size and crystallinity of salbutamol sulphate were investigated.

As shown in Table 1, the time, pressure, temperature and weight ratio of menthol:salbutamol sulphate had a significant effect on converting amorphous drug to crystalline. Partially crystalline salbutamol sulphate was created, when conditioning spray-dried powder with $scCO_2$ for 5 and 8 h at 50 °C, 150 bar with 1:1 weight ratio of menthol to salbutamol sulphate while maintaining the particle size. A high degree of crystallinity was achieved when the powder was conditioned for 12 h. The results of DSC and XRD analysis demonstrate that salbutamol sulphate was maintained as an amorphous powder, when conditioned with CO_2 + menthol at lower menthol:drug weight ratios such as 1:2, 1:3 and 1:4 as well as temperatures below 50 °C and conditioning period less than 12 h.

Our results showed that stirring and dispersion of the powder during the conditioning play a critical role in particle size. Large crystals of salbutamol sulphate were formed after 12 h of conditioning without stirring with a 1:1 weight ratio menthol:salbutamol sulphate using CO_2 at 50 °C and 150 bar. The formation of non-uniform large crystals (Fig. 2a) may be resulted from the integration of particles in the vicinity of each other during the crystallisation process. Stirring is, therefore, critical during the conditioning process to minimise particle integration.

Spray drying followed by conditioning with $scCO_2$ + menthol had a significant impact on the salbutamol sulphate particle morphology. As shown in Fig. 2b, untreated salbutamol sulphate had irregular particles greater than 20 μm that were not suitable for dry powder inhaler (DPI) formulation. Amorphous, uniform spherical particles of salbutamol sulphate with average particle size of 3.1 μm were formed by spray drying of an aqueous solution. The surface of particles had some pitting, dimple and shrunken area (Fig. 2c), similar to the one observed in previous studies (Chawla et al., 1994; Columbano et al., 2002, 2003; Corrigan et al., 2004). After conditioning the amorphous salbutamol sulphate with $scCO_2$ + menthol the particles were spherical with nano-scale needle-shape crystals on the surface (Fig. 2d); which may occur because of decreasing the T_g of salbutamol sulphate upon addition of $scCO_2$ and menthol into the solid phase, leading to increasing the rate of crystallisation. According to the WLF equation (Williams et al., 1955) by decreasing the T_g of any compound the rate of crystallisation increases. The greater the temperature difference (ΔT) in Eq. (1), the greater the rate of crystallisation. As reported the rate of crystallisation increases dramatically when ΔT is above 30 °C (Langrish, 2008).

3.1. Particle size distribution

The particle size distributions were measured for various samples of salbutamol sulphate. As shown in Table 2, the average particle size of the powder processed by spray drying and conditioning with pure CO_2 were 3.1 μm. The particle size of powder conditioned with $scCO_2$ + menthol was decreased to 2.6 μm. The presence of nanosized crystals on the surface of particles may decrease the degree of agglomeration between particles, therefore, enhancing the dispersion and slightly decreasing

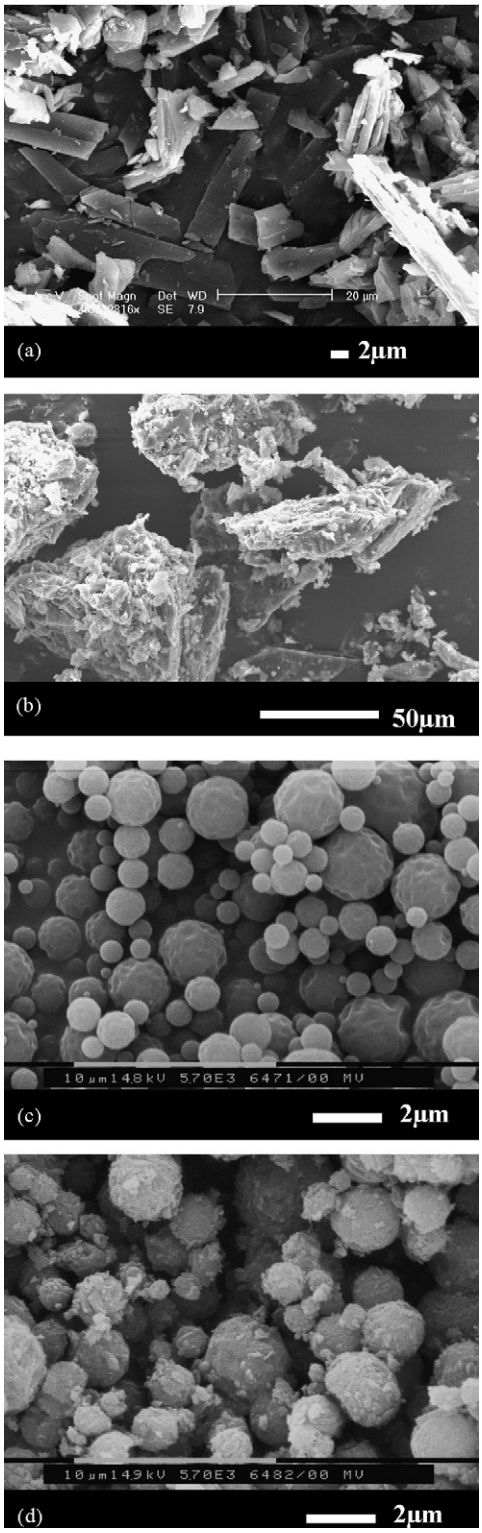

Fig. 2. SEM images of salbutamol sulphate: (a) after conditioning with $scCO_2$ + menthol at 50 °C and 150 bar for 12 h without stirring (b) untreated powder, (c) spray drying, (d) after conditioning with $scCO_2$ + menthol at 50 °C and 150 bar for 12 h with stirring.

Table 2
The effect of processing conditions on the particle size distributions of salbutamol sulphate.

Technique	Particle size distribution (μm)		
	$D_{0.1}$	$D_{0.5}$	$D_{0.9}$
Spray dried	1.10(±0.004)	3.05(±0.02)	6.20(±0.03)
Conditioned with menthol at 50 °C, 1 bar, 24 h	1.11(±0.02)	3.06(±0.03)	6.20(±0.04)
Conditioned with pure $scCO_2$ at 150 bar, 50 °C, 12 h	0.88(±0.01)	3.05(±0.001)	6.59(±0.02)
Conditioned with $scCO_2$ + menthol at 150 bar, 50 °C, 12 h	1.06(±0.01)	2.56(±0.02)	5.18(±0.12)

the particle size measured for dry powder by light scattering technique.

3.2. Crystallinity

The crystallinity of the powder was examined using techniques such as XRD, FTIR spectroscopy and DSC. The XRD profile of the salbutamol sulphate conditioned with $scCO_2$ + menthol was compared with the untreated and the spray-dried samples. The sharp peaks at different angles on the XRD profile of menthol (Fig. 3a) and untreated salbutamol sulphates (Fig. 3b) demonstrated that both were crystalline powders. As can be seen in Fig. 3e, there were no sharp peaks on the XRD profile of the spray-dried salbutamol sulphate indicating that the powder was in the amorphous form (Chawla et al., 1994; Columbano et al., 2002). The XRD profiles of conditioned salbutamol sulphate (Fig. 3c) were similar to untreated salbutamol sulphate with sharp peaks at the same angles. The absence of peaks corresponding to menthol in the conditioned sample corroborated that the residue was negligible in the final product. These results demonstrate that the crystal structure of the drug conditioned by the $scCO_2$ + menthol has not changed. The difference in the relative intensity of peaks may be due to the crystal orientation in the sample (Hu et al., 2008); the reduction of the intensity revealed that the particle size was reduced after conditioning with $scCO_2$ + menthol (Yang et al., 2008). The residence time for the conditioning had a significant impact on the crystallisation of amorphous powder. The sample that was conditioned for shorter time was only partially crystalline (Fig. 3d).

FTIR spectroscopic analysis was used for assessing the crystallinity of the powders. It was found that amorphous powder had broader spectrums compared with crystalline powder. Previously it was reported that slightly broader peak appeared in the FTIR profile of spray-dried salbutamol sulphate/ipratropium bromine when it was in the amorphous form, compared with the physical mixture of both materials in crystalline form (Corrigan et al., 2006b). The broad peaks in Fig. 4e at various wavenumbers for spray-dried salbutamol sulphate sample confirm that the powder was in the amorphous form. The difference in broadness of absorbance peaks may result from the differences in the amount of the powder that was in contact with the ATR crystal because of variation in applied pressure or hardness of materials. The broadness of the peaks for

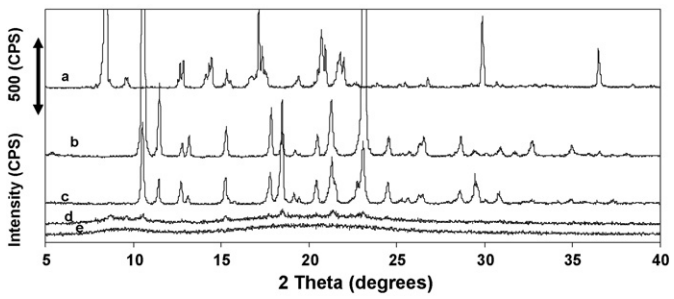

Fig. 3. XRD patterns of (a) menthol, (b) untreated salbutamol sulphate, (c) treated with $scCO_2$ + menthol at 50 °C and 150 bar for 12 h and (d) 5 h. (e) Spray-dried salbutamol sulphate.

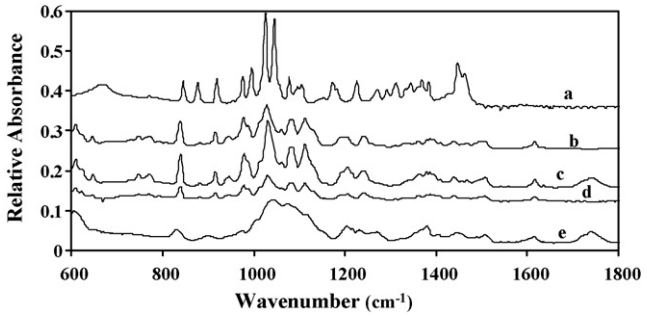

Fig. 4. FTIR spectra of (a) menthol, (b) untreated salbutamol sulphate, (c) treated with $scCO_2$ + menthol at 50 °C and 150 bar for 12 h and (d) 5 h. (e) Spray-dried salbutamol sulphate.

the spray-dried salbutamol sulphate powder that was treated with $scCO_2$ + menthol for 5 h was slightly decreased (Fig. 4d), indicating that the powder was partially crystalline. This is in agreement with the results acquired from XRD analysis. However, the FTIR spectrum of the sample conditioned with $scCO_2$ + menthol for 12 h (Fig. 4c) was close to untreated salbutamol sulphate with a high degree of crystallinity. The results demonstrate that the chemical integrity of the conditioned salbutamol sulphate was maintained. The large peak at 1450 cm^{-1} as reported previously for menthol was not found on the FTIR spectrum of the conditioned sample, confirming that the residue of the menthol in the processed sample was negligible (Narishetty and Panchagnula, 2005). The absorbance peaks were observed for the OH bond (Corrigan et al., 2006b) and carbonyl bond (Celebi et al., 1996) within a range of wavenumbers for salbutamol sulphate (Fig. 4b–e).

The results from DSC analysis presented in Fig. 5 confirmed the data acquired from XRD and FTIR spectroscopy. The absence of an endothermic peak at 45 °C for menthol sublimation in DSC profile of salbutamol sulphate conditioned with $scCO_2$ + menthol demonstrated that menthol was not detected by the methods used. The DSC curves for untreated salbutamol sulphate (Fig. 5a) and the one

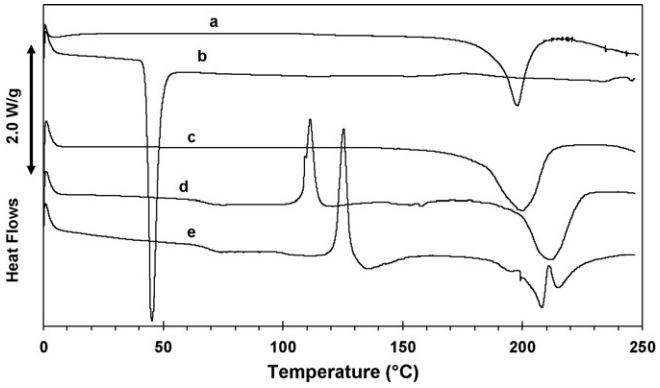

Fig. 5. DSC curves of salbutamol sulphate: (a) untreated, (b) menthol, (c) treated with $scCO_2$ + menthol at 50 °C and 150 bar for 12 h (d) Spray-dried salbutamol sulphate and (e) 5 h.

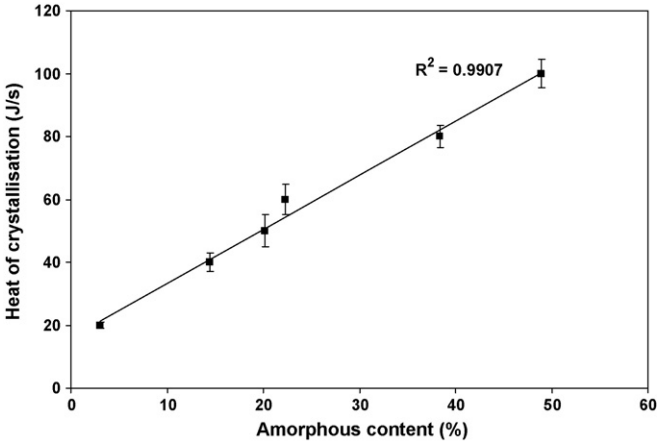

Fig. 6. Heat of crystallization obtained by DSC analyser.

produced from conditioning with $scCO_2$ + menthol (Fig. 5c) had a single endothermic peak at 200 °C, which is its melting point as reported in the literature (Ward and Schultz, 1995). The presence of a peak between 106 and 111 °C (exothermic heat) that corresponds to the heat of crystallisation in the spray-dried sample and similarly shown in the one conditioned with pure $scCO_2$ confirm that these powders were amorphous. The second endothermic peak at 208 °C in Fig. 5d and e corresponds to the melting of the crystalline form.

3.3. Measuring menthol residue

GC analysis with a single point external standard method was used to quantify menthol in the sample conditioned with $scCO_2$ + menthol. The residue of menthol was between $0.00107 \pm 0.00036\%$ and $0.00536 \pm 0.00180\%$ for all samples examined; these values are much lower than the toxic level reported by the Royal Pharmaceutical Society of Great Britain (minimum lethal dose of menthol in human body is 2 g (R.P.S.G.B., 2006)). The results of GC analysis corroborated the data acquired by XRD, FTIR and DSC; menthol concentration was negligible in salbutamol sulphate after conditioning. Menthol is accepted by US Food and Drug Administration (FDA) for oral use (Patel et al., 2007).

3.4. Quantify amorphous contents

DSC analyser was used to measure the amorphous fraction quantitatively. No heat of crystallisation was detected for synthetic samples produced with less than 10 wt% amorphous fraction. It has been reported that quantification limit of amorphous sucrose was 10% by using the DSC analyser (Saleki-Gerhardt et al., 1994). Heat of crystallisation of 2.29 J/s was detected for a mixture that contained 20 wt% amorphous salbutamol sulphate as shown in Fig. 6. No heat of crystallisation was observed for untreated salbutamol sulphate and the one conditioned with $scCO_2$ + menthol for 12 h as shown in Fig. 5a and c, respectively; this results confirmed that the degree of crystallinity of conditioned samples was above 90%, which can be adequate to promote the stability of an inhalation formulation.

3.5. Scattering exponent (S_E)

The scattering patterns of the spray-dried salbutamol sulphate particles and conditioned salbutamol sulphate are shown in Fig. 7a and b. The dimension obtained from slope of the scattering curve cannot be called surface fractal dimension because the second condition ($kR|m-1| \ll 1$) of the Rayleigh–Gans–Debye theory was not met due to the size of the particles. Instead, we called this parameter

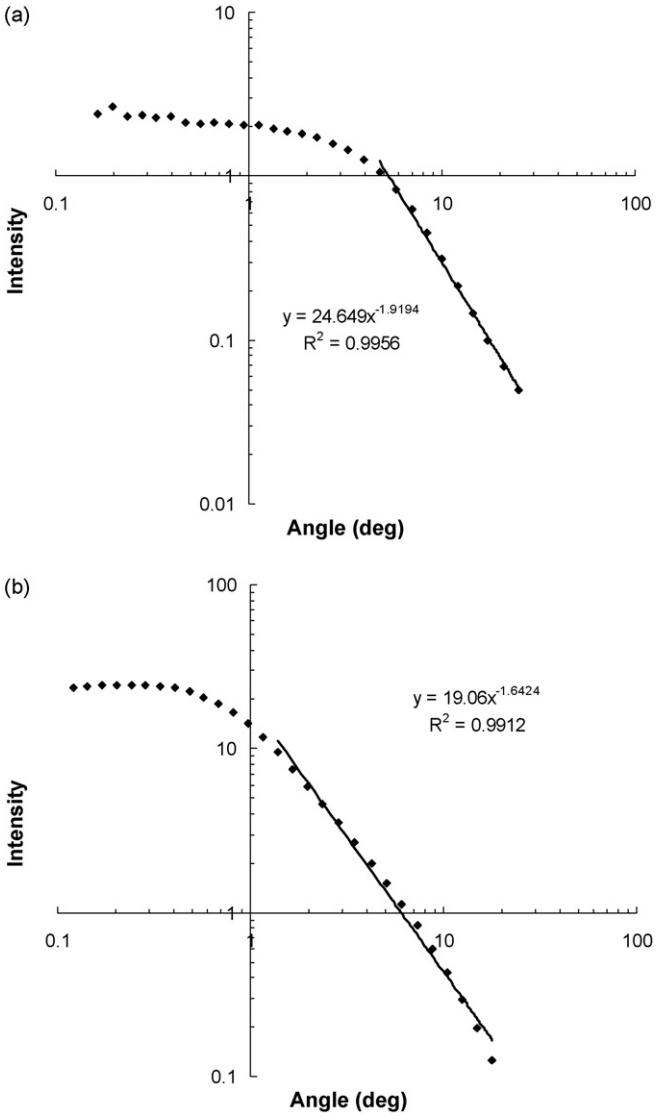

Fig. 7. Scattering curves obtained from light scattering of salbutamol sulphate produced from (a) spray drying ($S_E = 4.08$) and (b) $scCO_2$ + menthol ($S_E = 4.36$).

scattering exponent. The values of scattering exponent, S_E, obtained for salbutamol sulphate particles is higher than 3 because the second condition was not satisfied. However, the S_E still corresponds to the expected trend i.e. the value is higher for rougher particles (as shown by the scanning electron micrographs). The S_E for smoother spray-dried salbutamol sulphate is 4.08 ± 0.01 and for rougher conditioned salbutamol sulphate is 4.36 ± 0.01. Although the absolute value of surface fractal dimension might not be correct, the light scattering technique can nevertheless be used as a tool to compare the roughness of particle surface in this work since the particles have similar size.

3.6. Particle stability

The highly crystalline products from conditioning salbutamol sulphate with $scCO_2$ + menthol were much less hygroscopic (0.5% water uptake at 90% RH) (Fig. 8a) than the amorphous salbutamol sulphate produced by spray drying (10.13% water uptake at 60% RH) (Fig. 8b). The lower moisture uptake and reproducibility in the repeated cycle of the DVS run showed the stability of the product to moisture exposure and a low level of amorphous content (if any) present. The first cycle of amorphous salbutamol sulphate showed

Table 3
The effect of $scCO_2$ + menthol on the aerodynamic properties of salbutamol sulphate using Aeroliser® at 100 L/min with.

Technique	FPF_{loaded} (%)	$FPF_{emitted}$ (%)	Capsule and device retention	Impaction loss
Spray dried	31.2(±6.5)	32.5(±6.8)	3.84(±0.6)	52.4(±12.2)
Conditioned with $scCO_2$ + menthol	40.3(±3.4)	42.7(±4.0)	5.36(±0.9)	31.7(±6.3)

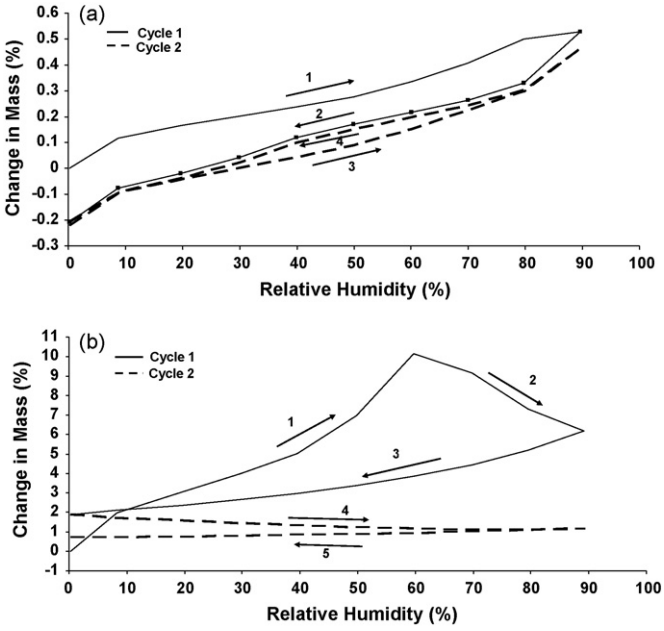

Fig. 8. DVS isotherms of salbutamol sulphate produced by (a) conditioning with $scCO_2$ + menthol and (b) spray drying.

a continuous uptake of moisture until 60% RH, at which moisture content of the powder started to expel the absorbed water as it recrystallised and consequently reduced its dispersibility (Chan, 2003). Previously, it was reported that amorphous salbutamol sulphate is recrystallised at a RH of around 75% (Columbano et al., 2002).

3.7. Multi-stage liquid impinger (MSLI)

The results of the aerosol performance analysis show that the crystalline salbutamol sulphate produced by $scCO_2$ + menthol had a lower impaction loss and higher FPF (loaded and emitted) in comparison with spray-dried powder (Table 3). The powder dispersion behaviors of these two powders were different; the presence of crystalline salbutamol sulphate was significantly higher in the rest of the stages and the filter but lower in the throat (Fig. 9). It was found that the agglomerated powders were broken into individual particles by the shear force from the air and/or collision between the agglomerates or between the agglomerate and the inhaler device during dispersion (Coates et al., 2005), thus enhancing the $FPF_{emitted}$ of the powder from 32.5% (spray-dried product) to 43.0% (conditioning with $scCO_2$ + menthol). The rough surface effectively prevents close packing between agglomerates (if any), thus increasing the inter-agglomerate distance and reducing the van der Waals attractive force (Chiou et al., 2007; Chew et al., 2005; Dhumal et al., 2009). The irregular surface is also contributes to a small aerodynamic size, less cohesiveness that increases flowability and the aerosol performance of the powders emitted from the capsule (Chiou et al., 2007; Chew et al., 2005). In addition, particles that were dispersed with particle size less than 5 μm (Fig. 9), had a greater fraction of crystalline salbutamol sulphate (38%) compared with the spray-dried products (31%).

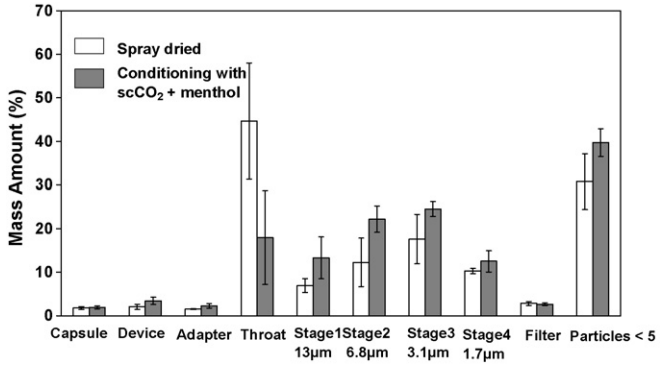

Fig. 9. Comparison between the powder dispersion behaviors of amorphous salbutamol sulphate that was spray-dried and crystalline salbutamol sulphate produced after conditioning with $scCO_2$ + menthol.

4. Conclusions

This study demonstrates the potential of $scCO_2$ + menthol for converting amorphous forms of powders to crystalline, while decreasing slightly the particle size. This new approach has a great potential in powder processing for various formulations where crystalline structures are critical. The process is capable of removing residues of amorphous fractions, which are produced in a product by various techniques, to enhance their thermodynamic stability and to produce a long shelf-life drug formulation. Menthol used in the process was efficiently removed from the product, and it could be potentially recycled in a large scale production. Conditioning by $scCO_2$ + menthol is an organic solvent-free process that operates at a moderate temperature and is attractive for processing fragile and heat-sensitive compounds. The FPF of salbutamol sulphate conditioned by $scCO_2$ + menthol was increased, hence decreasing the dosage required for the treatment of asthma, reducing the side effects and promoting patient compliances. The benign technique developed has a potential for promoting the degree of crystallinity for the production of inhalable powders at moderate temperatures without using any organic solvents.

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