ELSEVIER

International Journal of Pharmaceutics 231 (2002) 155–166

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

# Preparation and characterisation of hydrocortisone particles using a supercritical fluids extraction process

Sitaram P. Velaga *, Raouf Ghaderi, Johan Carlfors

*Department of Pharmacy, BMC, Uppsala University, P.O. Box 580, Uppsala SE 751 23, Sweden*

Received 22 May 2001; accepted 20 August 2001

**Abstract**

Crystallisation and subsequent milling of pharmaceutical powders by traditional methods often cause variations in physicochemical properties thereby influencing bioavailability of the formulation. Crystallisation of drug substances using supercritical fluids (SFs) offers some advantages over existing traditional methods in controlling particle characteristics. The novel particle formation method, solution enhanced dispersion by supercritical (SEDS) fluids was used for the preparation of hydrocortisone (HC) particles. The influence of processing conditions on the solid-state properties of the particles was studied. HC, an anti-inflammatory corticosteroid, particles were prepared from acetone and methanol solutions using the SEDS process. The solutions were dispersed with supercritical $CO_2$, acting as an anti-solvent, through a specially designed co-axial nozzle into a pressured vessel maintained at a specific constant temperature and pressure. The temperatures and pressures studied were 40–90 °C and 90–180 bar, respectively. The relative flow rates of drug solution to $CO_2$ were varied between 0.002 and 0.03. Solid-state characterisation of particles included differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD), solubility studies and scanning electron microscopy (SEM) examination. The aerodynamic properties of SEDS prepared particles were determined by a multistage liquid impinger (MLI). Particles produced from acetone solutions were crystalline needles, melting at $221 \pm 2$ °C. Their morphology was independent of processing conditions. With methanol solutions, particles were flakes or needles depending on the processing temperature and pressure. This material melted at $216 \pm 1$ °C, indicating a different crystal structure from the original material, in agreement with observed differences in the position and intensity of the XRPD peaks. The simulated lung deposition, using the MLI, for HC powder was improved after SEDS processing. It was possible to produce and control the crystallinity, morphology, and aerodynamic properties of HC particles with the SEDS technique. This method may be useful for the processing of inhalation powders. 

*Keywords:* Hydrocortisone; Supercritical fluids; Particles; Solid-state properties; Inhalation powders; Multistage liquid impinger (MLI)

## 1. Introduction

The successful formulation development of a new drug depends on physical and chemical prop-

* Corresponding author. Tel.: +46-18-471-4259; fax: +46-18-471-4223.

*E-mail address:* sitaram.velaga@galenik.uu.se (S.P. Velaga).

0378-5173/02/$ - see front matter 
PII: S0378-5173(01)00870-5

erties of excipients and active ingredients. Physicochemical properties, such as crystal features, polymorphism and the surface properties of drug substances determine the stability, reproducibility, handling and bioavailability of dosage forms. (Haleblian and McCrone, 1969). These properties of drug particles are even more precisely defined in the case of powders for inhalation, as these properties have a great influence on the deposition of particles in the lungs and the uniformity of dosing (Hickey et al., 1994; Visser and Maassluis, 1989). Thus, the manufacture of an elegant pharmaceutical formulation of a drug through the control and manipulation of their physical properties using robust processes is inevitable in formulation development. Recently, particle formation processes based on the use of supercritical fluids (SFs), have been introduced as a viable means of controlling crystal formation.

The SF state is the state attained by gases and liquids when subjected to temperatures and pressures above their critical parameters ($T_c$ and $P_c$). The SF state exists as a single phase exhibiting low viscosity, low surface tension and high diffusivity, facilitating high mass transfer rates in comparison to liquids, and which is highly compressible and can, therefore, have its density and thereby its solvation power altered by careful control of changes in the temperature and pressure. Carbon dioxide is the most widely used SF because it has low critical points ($T_c = 31.1$ °C and $P_c = 73.8$ bar). Additionally, It is non-toxic, non-flammable and cheap.

The particle formation processes involving SF that are most often referred to are based on rapid expansion of supercritical solutions (RESS) and gas anti-solvent (GAS) crystallisation. RESS is based on the principle that solvent strength can be dramatically reduced by decreasing the density of the SF, resulting in the crystallisation of dissolved solutes (Debenedetti et al., 1993; Matson et al., 1987). A range of substances, including polymers, fibres and pharmaceuticals has been processed by RESS (Charoenchaitrakool et al., 2000; Mawson et al., 1995; Tom and Debenedetti, 1991). Anti-solvent crystallisation processes, such as GAS, work on the principle that, when mixed with a solution, SF acts as an anti-solvent to the dissolved substance. Upon mixing, volume expansion of the solution occurs with a subsequent fall in the solvent strength followed by supersaturation and particle formation. Various supercritical anti-solvent techniques and materials processed have been reviewed by Reverchon (1999). Whilst, these approaches show advantages over the conventional methods for producing fine particles, they need to be further explored and optimised to provide a completely controlled environment for particle nucleation and growth. Furthermore, RESS has limited application since most drugs are insoluble in SFs.

Based on the principle of GAS, a novel technique called solution enhanced dispersion by supercritical (SEDS) fluids has been developed (York, 1995). SEDS is a one-step process which uses a coaxial nozzle design with a mixing chamber that facilitates control of the particle formation characteristics and the direct formation of dry and fine particles because of the increased mass transfer rates compared with other techniques. This technique has been used for the processing of diverse materials including low molecular weight substances, proteins and polymers (Palakodaty et al., 1998; Moshashaee et al., 2000; Ghaderi et al., 2000).

The objective of this study was to explore the applicability of the SEDS technique for the formation of hydrocortisone (HC) particles and for controlling their characteristics, through determining the influence of processing conditions (viz. solvent, pressure, temperature and the flow rates of the drug solution and $CO_2$) on solid-state properties of drug particles. HC, an anti-inflammatory drug, was used as a model substance since it belongs to the corticosteroid family from which many drugs are often used for pulmonary delivery. The solid-state properties of unprocessed, conventionally crystallised and SEDS crystallised samples were characterised using DSC and XRPD. The morphology of samples was examined by SEM. In addition, the solubilities of processed and unprocessed HC samples were determined. The applicability of SEDS processed HC particles for inhalation was studied in-vitro using a multistage liquid impinger (MLI).

## 2. Materials and methods

### 2.1. Materials

HC was purchased from Sigma Chemical Co., USA. Methanol, acetone and chloroform of analytical grade were purchased from Merck, Germany. Carbon dioxide ($CO_2$) of high purity (99.9%) was obtained from AGA Gas AB, Sweden. All chemicals were used without further purification.

### 2.2. Particle preparation by the SEDS technique

The experiments were performed in SEDS equipment. A schematic diagram of the SEDS apparatus, used in this study, is shown in Fig. 1. Briefly, a suitable anti-solvent gas, in this case $CO_2$, is fed from the source (A) to a cooler (B) to ensure the liquefaction of the gas and to prevent cavitation. The $CO_2$ is then fed through a conduit from the cooler to a high-pressure pump (C). From there, it is pumped to the high-pressure vessel (D) via a nozzle (E). The drug dissolved in a suitable organic solvent is drawn from the source (F) by a conduit to the high-pressure pump (C) and is fed to the high-pressure vessel (D) via the nozzle (E). The supercritical $CO_2$ leaves the high-pressure vessel and flows to the backpressure regulator (G), which controls the pressure discharge in the system. The organic solvent is extracted into the SF, resulting in the formation of solid microparticles in the vessel (D). The microparticles are collected from the vessel and stored in a desiccator at room temperature, until they were analysed. A more explicit description of the equipment and operation procedure has been presented elsewhere (Hanna and York, 1998).

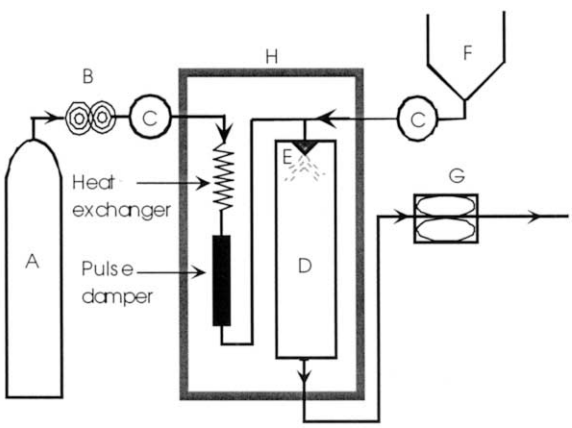


Fig. 1. Schematic representation of SEDS apparatus. (A) $CO_2$ cylinder; (B) cooler; (C) high pressure pump; (D) vessel; (E) nozzle; (F) drug solution; (G) back pressure regulator; (H) oven.

During the particle formation, the SEDS processing conditions were varied as follows, the pressure was varied between 90 and 180 bar and the temperature ranged from 40 to 90 °C. The flow rates of $CO_2$ and drug solution were 21 and 0.1 ml/min at all combinations of pressure and temperature, except at 130 bar and 40 °C, where the flow rates were studied. Thus at a pressure of 130 bar and a temperature 40 °C, the $CO_2$ flow rate was varied between 10 and 25 ml/min while the drug solutions had flow rates from 0.005 to 0.3 ml/min. The nearly saturated solutions of acetone (9.0 mg/ml) or methanol (6.0 mg/ml) with HC were used throughout the experiments. At the end of each experiment the microparticles were flushed with $CO_2$ at the flow rate as was used for the experiment for 15 min to remove any residual solvent.

### 2.3. Conventional recrystallisation of hydrocortisone

Crystallisations were carried out in acetone, methanol and chloroform on a laboratory scale. These were prepared as reference material for comparison with SEDS produced samples. Twenty millilitres of almost saturated drug solutions of acetone (9.0 mg/ml), methanol (6.0 mg/ml) and chloroform (1.6 mg/ml) were prepared. The acetone and methanol solutions were kept on a hot plate at 50 °C, while the chloroform solution was kept at room temperature for the solvent to evaporate. The crystals were collected and dried under airflow at room temperature.

### 2.4. Solid-state characterisation of drug particles

#### 2.4.1. Differential scanning calorimetry (DSC)

Drug samples (1.5–2.5 mg) were placed in alu-

minium pans and covered with lids. The samples were scanned at a rate of 10 °C/min in a differential scanning calorimeter (DSC 22 °C, Seiko, Japan) to determine the melting temperatures of the HC, before and after the SEDS processing.

#### 2.4.2. Powder X-ray diffraction (XRPD)

X-ray powder diffraction (XPRD) spectra were obtained using the Guinier–Hägg focusing powder camera with silicon powder ($a = 5.431023$ Å) as an internal standard and the Cu $K\alpha_1$ as the radiation source.

#### 2.4.3. Scanning electron microscopy (SEM)

The particle shape and topography were observed and examined by SEM (JSMT 330-Scanning Microscope, Jeol, Japan and Philips SEM525, the Netherlands). Particles of representative samples were coated with gold–palladium (metallisation; JFC-100, Ion Sputter, Jeol, Japan) in an argon atmosphere at room temperature before examination.

### 2.5. Solubility determination

A standard curve was drawn by plotting the absorbencies as a function of concentration. The absorbencies of respective solutions were measured using an ultra violet (UV) spectrophotometer (U1100, Hitachi Ltd., Tokyo, Japan) at 232 nm. Every measurement was conducted in triplicate.

For the sample solubility determination, excessive quantities of the drug (6.0 mg) were suspended in 12 ml of water in 20 ml plastic scintillating vials. These containers were kept in a thermostat maintained at 25 °C and were stirred at a constant rate. Samples were taken from the supernatant carefully at regular intervals over a period of 36 h after being centrifuged for 10 min at 3000 rpm. These samples were analysed using a spectrophotometer (U1100, Hitachi, Tokyo, Japan) and the average of three measurements from three different vials was calculated. The drug concentration in each sample vial was determined from the standard graph.

### 2.6. In vitro drug deposition

In this study, a MLI was used for the assessment of fine particle dose and particle distribution. This is referred to as Apparatus C for dry inhalation powders in the European Pharmacopoeia (EP). MLI has such a design that flow rate can be altered between 30 and 100 l/min and has the advantage of avoiding interstage losses of particles. The study was performed with Turbuhaler (Astra-Zeneca) and Easyhaler (Orion Pharma). The MLI apparatus was assembled in accordance with the EP procedure for dry powder inhalers and connected to a flow system that has been adjusted to provide 4 l (over 4.7 s at the rate of 51 l/min ($\pm$ 5%) in the case of Turbuhaler and 43 l/min ($\pm$ 5%) over 5.6 s in the case of Easyhaler) to simulate human inspiration. The resulting cut off diameters of the stages 2–4 and of the filter were 14.10, 7.38, 3.36, 1.84 μm at 51 l/min for the Turbuhaler and 15.36, 8.03, 3.66, 2.01 μm at 43 l/min for the Easyhaler. The critical and steady state flow through the system were measured at the specified rates and maintained within the limits following the procedure outlined in EP. The schematic diagram is shown in Fig. 2. The EP method was followed when dispensing 20 ml of milliQ water into each stage from stage 1 to stage 4 and subsequent experimentation. Two milligrams (which is equivalent to ten doses of 200 μg) of each sample was accurately weighed and placed in the reservoir of the devices. The dry powder inhaler (Turbuhaler or Easyhaler) was attached to the inlet port of the Impinger, which was fixed with a suitable mouthpiece adapter. Powder was discharged into the apparatus by opening the two way valve for the times specified above. Samples from each stage were collected following the EP procedure. Measurements were repeated three times for each sample following a similar procedure at each step. The absorbance of samples from each stage was measured. For each sample produced by SEDS under the specified processing conditions, two batches were measured to study the reproducibility of the particle formation process. The amount of drug deposited on each stage was determined from the standard graph.

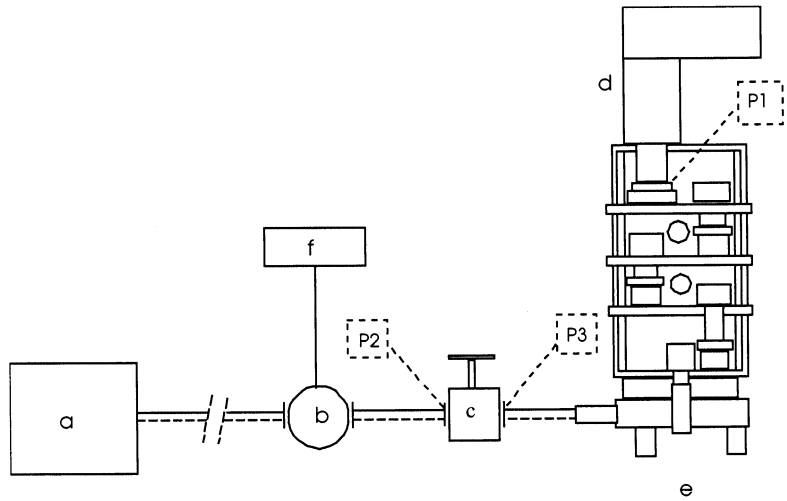


Fig. 2. Schematic illustration of experimental set-up for testing powders for inhalation. (a) Vacuum pump; (b) shut off valve; (c) regulating valve; (d) induction port (throat); (e) impinger; (f) timer, P1–P3, pressure reading points.

## 3. Results and discussion

### 3.1. Solid-state properties

#### 3.1.1. Conventional crystallisation

HC was crystallised from acetone, methanol and chloroform to obtain the reference material. Representative SEM micrographs of these samples and of unprocessed samples are shown in Fig. 3. The starting material (or unprocessed material) comprised irregular chunks that melted at $224 \pm 0.3$ °C (Fig. 3a). This material was identified as polymorph I based on the melting range and XRPD data (Florey, 1983). The crystals from the methanol were large chunks with a broad particle size distribution (Fig. 3b), whilst acetone crystallisation resulted in large prisms partially connected to each other (Fig. 3c). However, samples from these solvents had a melting temperature of $224 \pm 1$ °C, which is equivalent to the endothermic transition of the unprocessed material (Fig. 4a). This demonstrated that the crystallisation from acetone resulted in particles with polymorphic modification I, which was further, confirmed by the equivalence of the equilibrium solubility with the unprocessed sample (Fig. 5). This result was in agreement with previous studies (Kuhnert-Brandstatter and Gasser, 1971). With respect to methanol crystallisation, Kuhnert-Brandstatter and Gasser (1971) interpreted their results as the formation of a solvate, which, on heating, loses solvent at 95–105 °C and transforms into polymorphic modification I. Contrary to this, we obtained crystalline material that melted at $224 \pm 1$ °C, polymorphic modification ?, instead of the solvate. Chloroform crystallisation resulted in crystals with long blade-like structures that showed two endothermic transitions one at $109 \pm 0.7$ °C and the other at $222 \pm 0.1$ °C (Fig. 3d and Fig. 4a). This demonstrated the formation of chloroform solvate that lost solvent around 110 °C leading to an another crystalline form melted at 222 °C. The solvate formation from chloroform, whose morphology is different from methanol crystals, was in agreement with the previous study (Kuhnert-Brandstatter and Gasser, 1971). Additionally, it was determined that the equilibrium solubility of these samples was the same as for the unprocessed sample, however, the samples exhibited different dissolution rates because of differences in their morphology and particle sizes. The experimental values of the equilibrium solubility of unprocessed, conventionally crystallised and SEDS processed material under various processing conditions, shown in Fig. 5, was $0.290 \pm 0.01$ mg/ml, which is the same as

the value (0.297 mg/ml) reported in the literature for HC at 25 °C (Hagen and Flynn, 1983).

### 3.1.2. SEDS crystallisation

SEDS processing parameters that were employed during the microparticle preparation from methanol and acetone in this study, are summarised in the Table 1 along with the melting points of the various samples. Typical SEM pictures of SEDS processed material using methanol and acetone at various processing parameters are shown in Fig. 6. The SEM images show that particles produced from SEDS were either needles or flakes. It was interesting that varying the processing parameters (i.e. the temperature and pressure) had no effect on particle morphology when acetone was used as a solvent whilst particle morphology depended on temperature and pressure with methanol as a solvent (Fig. 6). In addition, the change in the relative flow rates of drug solution to $CO_2$ (0.002–0.03) had no influence on either morphology or crystallinity of particles produced from both the solvents at 130 bar and 40 °C.

When processing with methanol at 40 °C, predominantly flake-like particles were observed at 180 bar (180/40); a mixture of flakes and needles was obtained at 130 bar (130/40) and, predominantly, needles were formed at 90 bar (90/40; Fig. 6). At 60 °C, particles were needle-like at all pressures, with the size or length of the needles increased when the pressure was lowered from 180 to 90 bar as observed from a SEM examination of the samples (Fig. 6a and d). This may be due to increased solubility of HC in $CO_2$ at higher pressures resulting in a decreased nuclei density, thereby forming elongated needles. It was observed that the production yield of HC, obtained

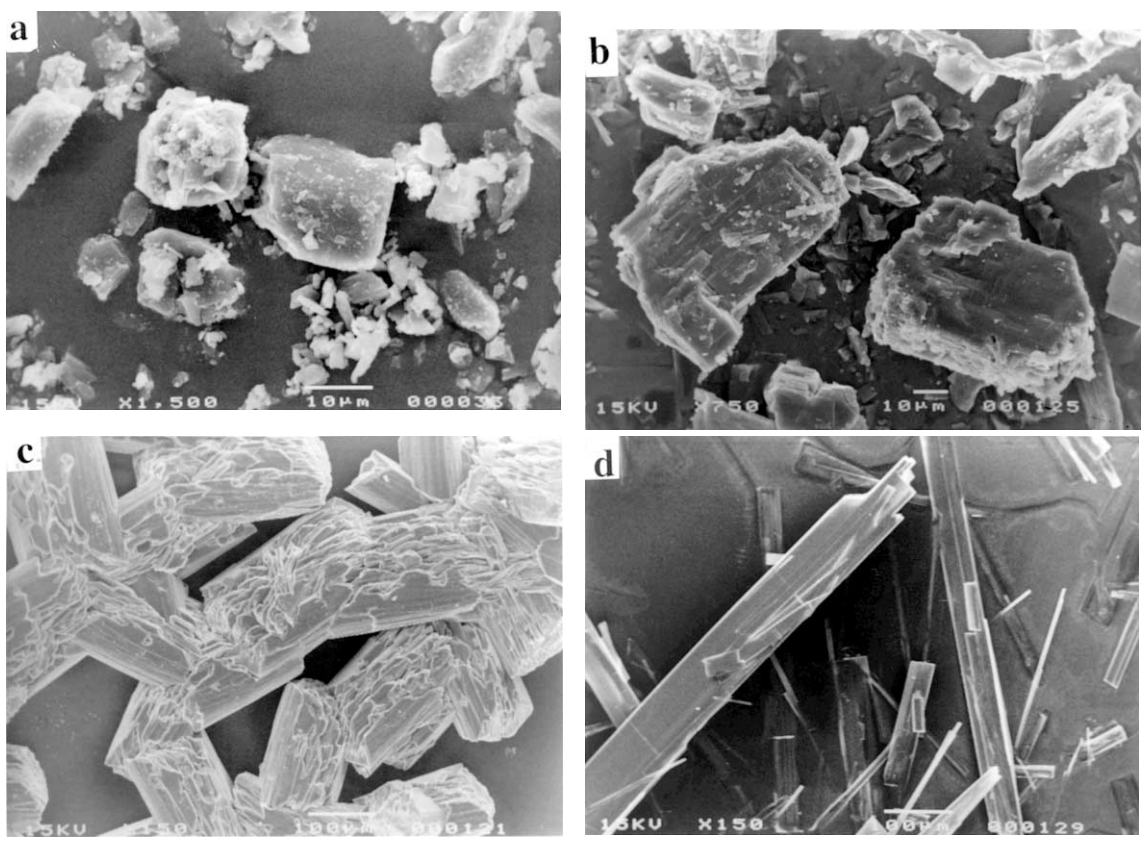


Fig. 3. SEM photographs of (a) unprocessed and conventionally recrystallised samples of HC from (b) methanol; (c) acetone and (d) chloroform.

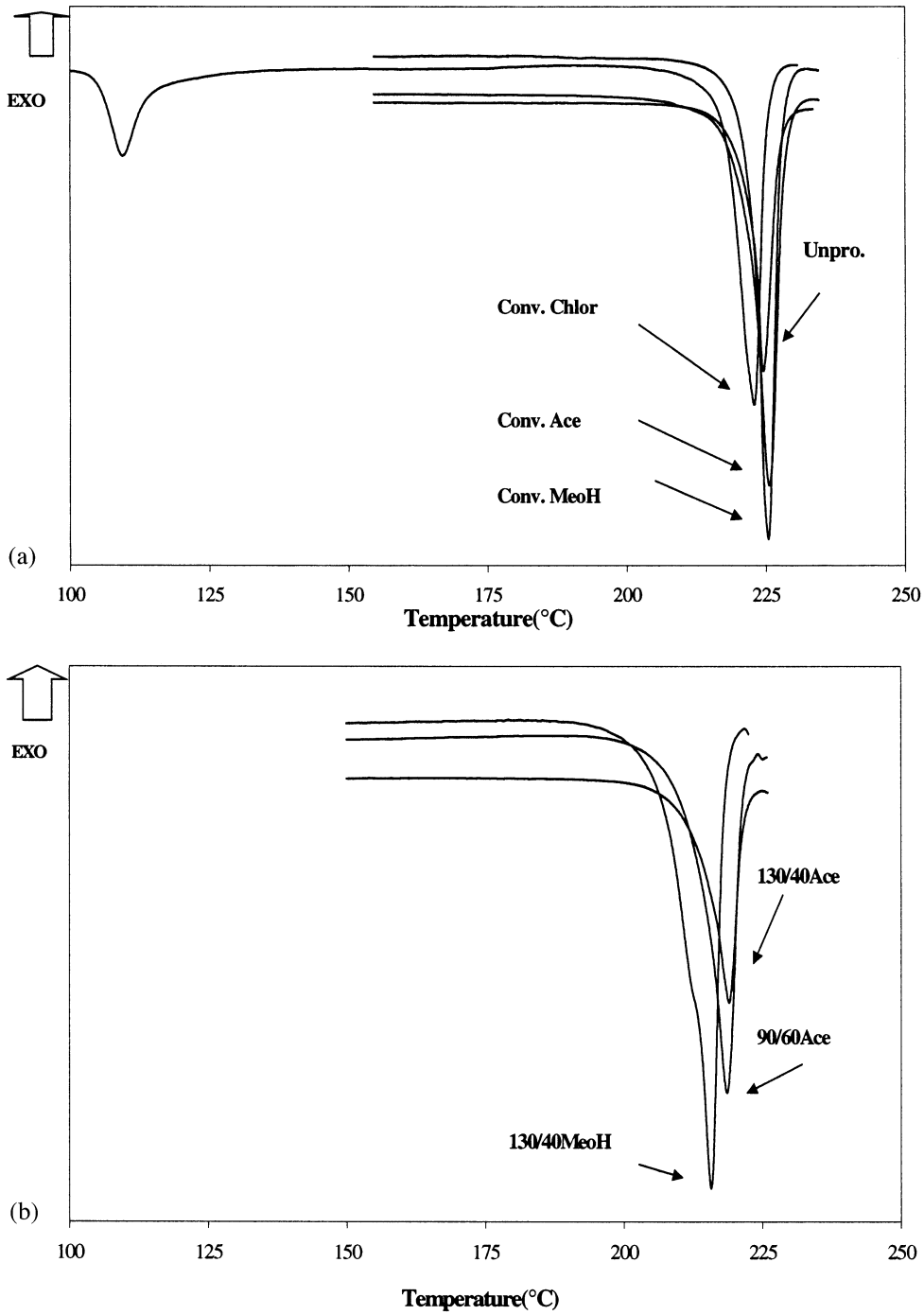


Fig. 4. (a) DSC thermograms showing melting temperatures of unprocessed, conventionally recrystallised samples by methanol, acetone and chloroform. The arrows for the respective samples are pointed to their maxima. (b) DSC thermograms showing melting temperatures of SEDS processed HC samples at various conditions. The arrows for the respective samples are pointed to their maxima.

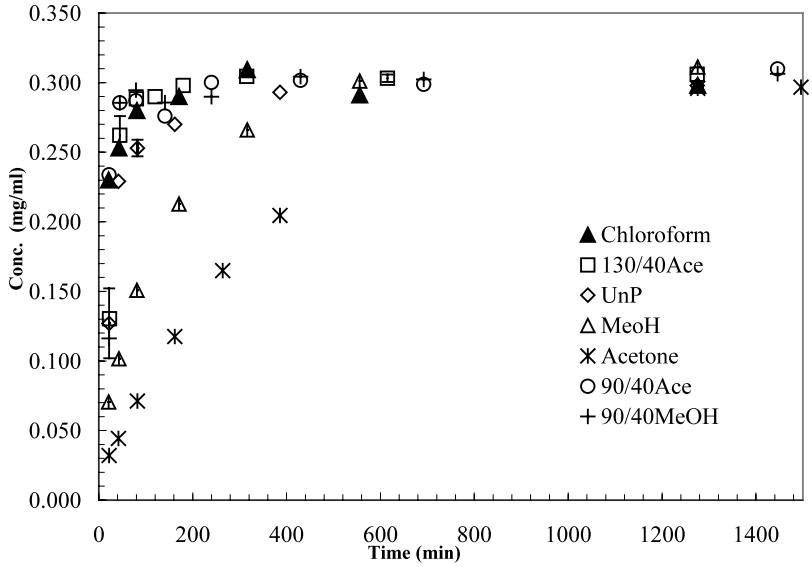


Fig. 5. Solubility profiles of unprocessed, conventionally and SEDS processed HC samples.

Table 1
Processing conditions and melting points for particles prepared by SEDS

| Pressure (in bars) | Temperature (°C) | Relative flow rates of drug solution to $CO_2$ | Melting point (°C) |
|---|---|---|---|
| *Samples prepared from acetone* | | | |
| 180 | 40 | 0.0048 | 219.2 ($\pm$1.0) |
| 180 | 60 | 0.0048 | 220.0 ($\pm$1.0) |
| 130 | 40 | 0.0048 | 220.0 ($\pm$1.4) |
| 130 | 40 | 0.0020 | 221.2 ($\pm$1.0) |
| 130 | 40 | 0.0300 | 222.8 (N.D) |
| 130 | 90 | 0.0048 | 221.0 ($\pm$1.0) |
| 90 | 40 | 0.0048 | 221.0 ($\pm$1.0) |
| 90 | 80 | 0.0048 | 223.0 ($\pm$1.0) |
| *Samples prepared from methanol* | | | |
| 180 | 40 | 0.0048 | 216.0 ($\pm$1.0) |
| 180 | 60 | 0.0048 | 215.0 ($\pm$1.0) |
| 130 | 40 | 0.0048 | 215.9 ($\pm$1.0) |
| 130 | 40 | 0.0020 | 215.9 (N.D) |
| 130 | 40 | 0.0300 | 217.8 (N.D) |
| 130 | 90 | 0.0048 | 216.5 ($\pm$1.0) |
| 90 | 40 | 0.0048 | 217.1 ($\pm$1.0) |
| 90 | 60 | 0.0048 | 216.2 ($\pm$1.0) |

Brackets show the standard deviation of the measurements of three SEDS batches produced at the same processing parameters. N.D. stands for not determined.

at higher temperatures and pressures, was decreased, reflecting an increased solubility of HC in $CO_2$. A similar observation was made by Shekunov et al. (1999) when processing paracetemol particles using the SEDS process. With acetone as a solvent for HC, the samples produced were a network of needles of the same size as shown in Fig. 6e irrespective of the processing conditions.

It can be discerned from Table 1 that samples prepared from acetone were crystalline with a melting temperature of $221 \pm 2$ °C, whereas crystals from methanol melted at $216 \pm 1$ °C. This indicated the occurrence of polymorph modification I for the samples prepared from acetone and probably the presence of another polymorph in the case of samples prepared from methanol. This finding was further supported by differences in the position and intensity of the XRPD peaks for

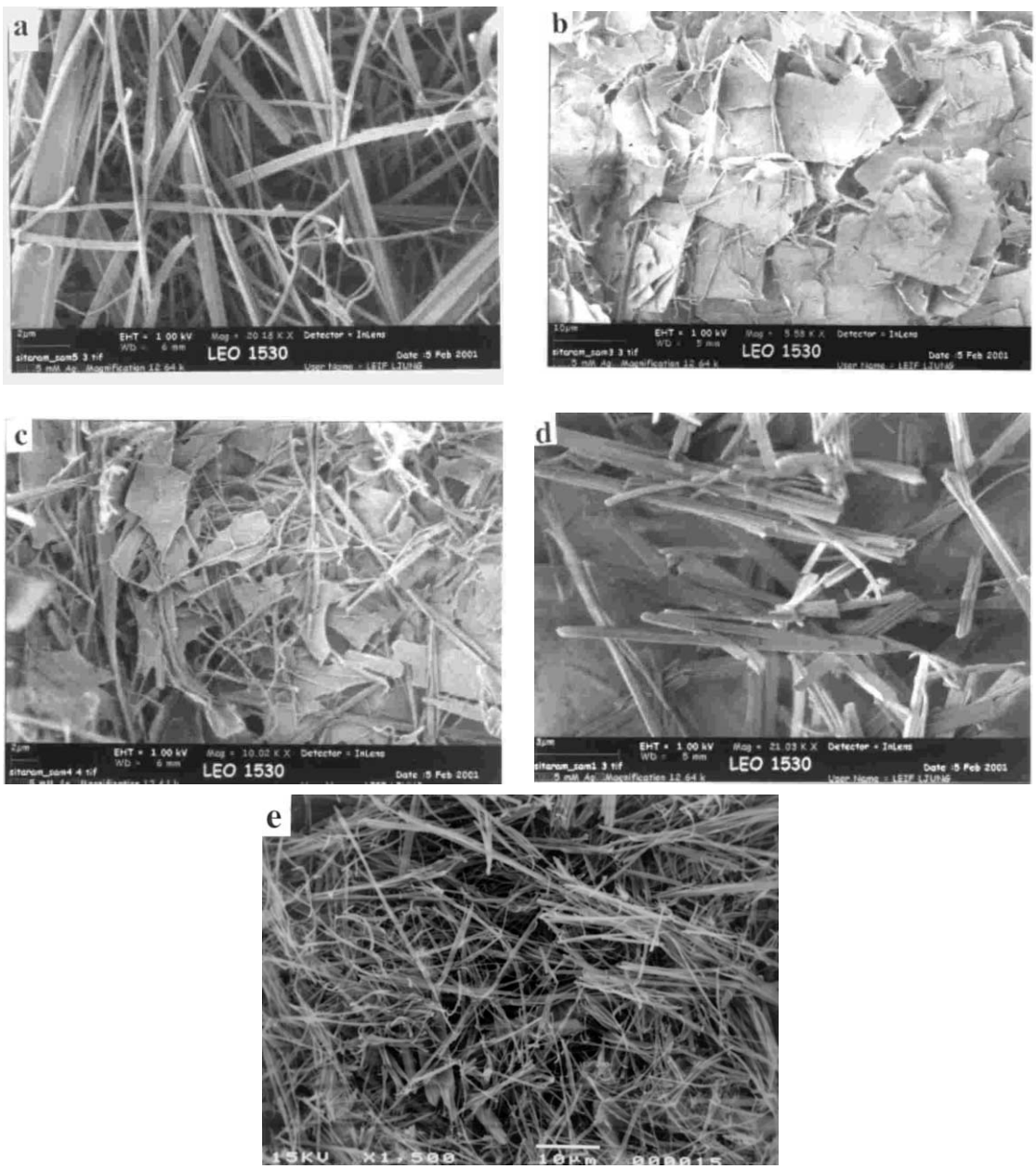


Fig. 6. SEM pictures of SEDS processing HC samples at (a) 180/60 MeOH; (b) 180/40 MeOH; (c) 130/40 MeOH; (d) 90/60 MeOH; (e) 180/40 Ace.

Table 2
XPRD data of unprocessed, SEDS processed and conventionally crystallised HC samples

| Unprocessed | | 130/40 Methanol | | 130/40 Acetone | | Crystallised from chloroform | |
|---|---|---|---|---|---|---|---|
| Intensity[a] | $d$ (Å)[b] | Intensity[a] | $d$ (Å)[b] | Intensity[a] | $d$ (Å)[b] | Intensity[a] | $d$ (Å)[b] |
| 1000 | 6.0992 | 1000 | 6.0834 | 1000 | 6.1135 | 244 | 6.0869 |
| 469 | 5.0736 | 642 | 5.0539 | 478 | 5.0658 | 145 | 5.1446 |
| 93 | 5.4755 | 136 | 4.6823 | 180 | 5.4941 | 12 | 5.0485 |
| 77 | 4.6990 | 121 | 3.0331 | 106 | 4.6971 | N.A. | N.A. |

N.A. marks those data that are not available.
[a] Relative intensity scale in arbitrary units.
[b] The interplanar spacing.

Table 3
The percentage deposition of unprocessed and SEDS processed HC in different stages of the impinger when Turbuhaler was used

| | Unprocessed HC (%) | 90/40 Ace (%) | 90/40 MeOH (%) | 180/60 Ace (%) | 130/40 Ace (%) |
|---|---|---|---|---|---|
| Mouth piece | 5.4 (2.4) | 1.9 (0.5) | 1.7 (0.3) | 1.5 (0.3) | 5.1 (6.0) |
| Throat | 33.3 (7.7) | 10.5 (1.8) | 10.8 (2.3) | 10.6 (1.2) | 6.0 (2.3) |
| Stage 1 | 5.6 (0.7) | 46.0 (3.3) | 48.0 (2.8) | 46.6 (4.5) | 23.4 (9.1) |
| Stage 2 | 3.0 (0.6) | 12.8 (0.9) | 6.2 (0.4) | 12.0 (0.5) | 8.4 (2.5) |
| Stage 3 | 3.6 (0.2) | 6.0 (0.7) | 3.5 (0.6) | 4.9 (0.4) | 4.4 (1.6) |
| Stage 4 | 1.6 (0.3) | 2.7 (0.3) | 0.8 (0.2) | 2.7 (0.3) | 1.4 (0.3) |
| Filter | 5.7 (0.7) | 4.6 (0.8) | 3.1 (0.1) | 4.0 (0.5) | 3.9 (1.9) |
| Total dose [a] | 58.1 (8.8) | 84.7 (1.2) | 74.1 (1.9) | 82.2 (5.0) | 54.5 (12.5) |
| Delivered dose[b] | 52.6 (8.3) | 82.8 (0.8) | 72.4 (1.9) | 80.7 (5.3) | 49.5 (17.3) |

The figures in brackets indicate the S.D. of three MLI measurements.
[a] Total dose is the delivered dose plus the amount of drug adhered to mouth piece.
[b] The delivered dose is the amount of drug that enters the impinger.

samples produced from methanol, as presented in Table 2. However, further examination with solid-state nuclear magnetic resonance (NMR), infra red (IR) spectroscopy and single crystal diffraction techniques is required to confirm the findings. DSC thermograms of samples processed under various conditions are shown in Fig. 4b.

It is apparent from Fig. 5 that the equilibrium solubility of SEDS processed samples was the same, although the initial dissolution rate of samples is higher than for unprocessed and conventionally processed samples obtained with acetone and methanol. A possible explanation for the equivalence of the equilibrium solubility of SEDS processed samples, despite their higher crystal energy, could be due to their transition to the stable polymorph in contact with water. The higher dissolution rate of SEDS samples was obviously caused by the increased surface area in the case of needles, as shown in the SEM pictures (Fig. 6a and e).

### 3.2. In vitro deposition of drug particles

The purpose of this study was to compare the in vitro deposition capabilities of different SEDS processed samples and unprocessed samples using Turbuhaler or Easyhaler. The delivered dose is the amount of drug that comes into the MLI and the percentage of drug deposited in different stages is calculated on the basis of the initial amount of drug charged into the reservoir of the DPI device. As shown in Table 3, it was apparent that the percentage of the dose delivered was higher for the SEDS samples than for the unprocessed samples when Turbuhaler was employed.

In addition, the percentage of drug that adhered to the mouthpiece and throat was negligible for the SEDS processed sample (Table 3). This was probably caused by lowered particle and surface interactions (Cooper, 1998). For the SEDS samples, the better deposition of the drug in stages 1–4 was attributed to decreased inter-particulate forces. These results share similarity with the work by York and Hanna (1996), who reported the better deposition of SEDS processed salmeterol as compared with conventionally crystallized and micronised material. It was apparent that the SEDS processing parameters influenced the deposition of drug particles in MLI, as the sample prepared at 130 bar and 40 °C with acetone (130/40 Ace) had a delivered dose that was less than samples prepared under other conditions (Table 3). In an effort to compare the delivered dose from Easyhaler and Turbuhaler, it was observed that variations in the drug deposition in different stages of MLI were higher for Easyhaler for the sample processed at 90 bar and 40 °C from acetone (90/40 Ace) (Table 3). This may be caused by the design of Turbuhaler with a long flow path with spiral channels promoting the deaggregation and the dispersion of long needles, resulting in high uniformity and consistency (Wetterlin, 1988). However, the delivered dose for SEDS processed powder from Easyhaler was 99%, demonstrating complete discharge of material from the reservoir, again verifying low surface adsorption (Tables 3 and 4). The reproducibility was good for the in vitro measurement method as can be seen from the reported standard deviations in the Tables 3 and 4. We also observed a good reproducibility in MLI measurements between two SEDS produced batches at a specific processing condition.

Table 4
The percentage deposition of unprocessed and SEDS processed HC in different stages of impinger when easy haler was used

| | Unprocessed HC (%) | 90/40 Acetone (%) |
|---|---|---|
| Mouth piece | 0.0 (0.0) | 0.0 (0.0) |
| Throat | 34.8 (0.7) | 5.2 (0.5) |
| Stage 1 | 10.7 (0.7) | 76.5 (7.0) |
| Stage 2 | 8.2 (0.1) | 9.3 (6.2) |
| Stage 3 | 6.4 (0.2) | 4.5 (4.5) |
| Stage 4 | 1.8 (0.1) | 0.1 (0.2) |
| Filter | 4.3 (0.0) | 3.4 (2.2) |
| Total dose[a] | 66.1 (1.2) | 98.9 (17.6) |
| Delivered dose[a] | 66.1 (1.2) | 98.9 (17.6) |

Figures in brackets indicate the S.D. of three MLI measurements.

[a] The total dose is equal to the delivered dose, since the EP method for an Easyhaler excludes the determination of amount of drug adhered to mouth piece.

## 4. Conclusion

SEDS crystallisation of HC from acetone resulted in polymorphic modification I with similar morphologies, irrespective of processing conditions. On the other hand, when methanol was used as the solvent, the morphology of crystals was influenced by the pressure and the temperature of the process. Additionally, SEDS crystallisation of HC from methanol solutions produced a material with a lower melting point, possibly polymorph II. In any case, it was evident that the choice of solvent influenced the crystal energy of material. At 130 bar and 40 °C, changes in the relative flow rates of the solution to $CO_2$ showed no substantial variations in the characteristics of crystals, explaining the kinetic independence of the process for particular parameters. The solubility of all crystallised HC material was the same irrespective of whether the preparation was by SEDS or through conventional crystallisation. It is possible to prepare crystalline microparticles with the SEDS technique because of the controlled higher supersaturation followed by fast nucleation and the controlled crystal growth compared with conventional methods. Drug particles prepared by SEDS technique appeared to be a viable alternative for the preparation of powders intended for pulmonary delivery.

## Acknowledgements

The authors gratefully acknowledge Professor Rolf Berger, Department of Inorganic Chemistry

of Uppsala University for his great help in acquiring X-ray diffraction patterns of samples. We also wish to thank Mona Carlsson, Medical Products Agency, Uppsala, Sweden and Ahmad Pirizadeh for their valuable support during the experimentation with MLI.

## References

Charoenchaitrakool, M., Dehghani, F., Foster, N.R., Chan, H.K., 2000. Micronisation by rapid expansion of supercritical solutions to enhance the dissolution rates of poorly water-soluble pharmaceuticals. Ind. Eng. Chem. Res. 39, 4794–4802.

Cooper, S.M., 1998. Preparation of pharmaceutical compositions containing polymorph of fluticasone. Glaxo Group Ltd., UK PCT Int. Appl. WO 98/17676.

Debenedetti, P.G., Tom, J.W., Kwauk, X., Yeo, S.D., 1993. Rapid expansion of supercritical solutions (RESS): fundamentals and applications. Fluid Phase Equilib. 82, 311–321.

Florey, K., 1983. Hydrocortisone. Anal. Profiles Drug Subst. 12, 277.

Ghaderi, R., Artursson, P., Carlfors, J., 2000. A new method for preparing biodegradable microparticles and entrapment of hydrocortisone in DL-PLG microparticles using supercritical fluids. Eur. J. Pharm. Sci. 10, 1–9.

Hagen, T.A., Flynn, G.L., 1983. Solubility of hydrocortisone in organic and aqueous media: evidence for regular solution behavior in apolar solvents. J. Pharm. Sci. 72, 58–64.

Haleblian, J.K., McCrone, W., 1969. Pharmaceutical applications of polymorphism. J. Pharm. Sci. 58, 911–929.

Hanna, M., York, P., 1998. US patent 5851453 Eur. patent 0706421B1, 0767702B1.

Hickey, A.J., Concessio, N.M., Van Oort, M.M., Platz, R.M., 1994. Factors influencing the dispersion of dry powders as aerosols. Pharm. Tech. 8, 58–64.

Kuhnert-Brandstatter, M., Gasser, P., 1971. Solvates and polymorphic modifications of steroid hormones II. Microchem. J. 16, 577–589.

Matson, D.W., Fulton, J.L., Petersen, R.C., Smith, R.D., 1987. Rapid expansion of supercritical fluid solutions: solute formation of powders, thin films, and fibers. Ind. Eng. Chem. Res. 26, 2298–2306.

Mawson, S., Johnston, K.P., Combes, J.R., DeSimone, J.M., 1995. Formation of poly(1,1,2,2-tetrahydroperfluorodecyl acrylate) submicron fibers and particles from supercritical carbon dioxide solutions. Macromolecules 28, 3182–3191.

Moshashaee, S., Bisrat, M., Forbes, R.T., Nyqvist, H., York, P., 2000. Supercritical fluid processing of proteins. I: lysozyme precipitation from organic solution. Eur. J. Pharm. Sci. 11, 239–245.

Palakodaty, S., York, P., Pritchard, J., 1998. Supercritical fluid processing of materials from aqueous solutions: the application of SEDS to lactose as a model substance. Pharm. Res. 15, 1835–1843.

Reverchon, E., 1999. Supercritical antisolvent precipitation of micro-and nano-particles. J. Supercrit. Fluids 15, 1–21.

Shekunov, B.Y., Hanna, M., York, P., 1999. Crystallization process in turbulent supercritical flows. J. Cryst. Growth 198–199 (Pt. 2), 1345–1351.

Tom, J.W., Debenedetti, P.G., 1991. Formation of bioerodible polymeric microspheres and microparticles by rapid expansion of supercritical solutions. Biotechnol. Prog. 7, 403–411.

Wetterlin, K., 1988. Turbuhaler: a new powder inhaler for administration of drugs to the air-ways. Pharm. Res. 5, 506–508.

Visser, J., Maassluis, N., 1989. Van der Waals and other cohesive forces affecting powder fluidisation. Powder Technol. 58, 1–10.

York, P., 1995. A novel approach for controlled particle formation using supercritical fluids. Pharm. Res. 12, S 141.

York, P., Hanna, M., 1996. Particle engineering by supercritical fluid technologies for powder inhalation drug delivery. Respir. Drug Delivery V, Program Proc., Interpharm Press, 231–239.