Supercritical Fluids Crystallization of Budesonide and Flunisolide

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Received March 24, 2002; accepted July 1, 2002

Purpose: Budesonide and flunisolide anhydrate were crystallized using the solution enhanced dispersion by supercritical fluids (SEDS) technique. The aim was to investigate the possibility of preparing different pure polymorphs.

Methods: 0.25% w/v solutions of each drug were prepared from acetone and methanol. Operating conditions were 40–80°C and 80–200 bars. The flow rate of drug solution was 0.3 mL/min and that of CO_2 was 9–25 mL/min. Sample characterizations included differential scanning calorimetry, X-ray powder diffraction, variable temperature X-ray diffraction, scanning electron microscopy, and solubility studies.

Results: The particle morphology of budesonide was dependent on the nature of the solvent. SEDS processing of flunisolide with acetone at 100 bars resulted in the formation of polymorphic mixtures at 80°C and a new polymorph III at 60 C and 40°C. With methanol at 100 bars another new polymorph IV was formed with different particle morphology at 80°C and a polymorphic mixture at 60°C.

Conclusion: Using the SEDS, microparticles of crystalline budesonide were prepared and new polymorphs of flunisolide were produced. Particle characteristics were controlled by the temperature, pressure and relative flow rates of drug solution and CO_2.

KEY WORDS: budesonide; flunisolide; supercritical fluids; microparticles; variable temperature X-ray diffraction; and polymorphism.

INTRODUCTION

Many organic and inorganic substances of pharmaceutical interest crystallize in one or more crystalline forms. These forms of a crystalline solid include polymorphs and psuedopolymorphs. Polymorphism occurs quite often among organic compounds (e.g., steroids) and many polymorphic drugs have been identified and presented in the review by Borka (1). Different polymorphs show differences in physical properties, such as density, melting point, solubility, dissolution rate due to notable differences in crystal packing, molecular conformations, and lattice energies (2). The variations in these properties of different crystal forms potentially affect the processing of drug substances into a formulation and its bioavailability (3). Apparently, the crystallization methods or processes determine the final product chemical purity and its physical properties.

Conventional crystallization processes (e.g., solvent crystallization) may suffer from lack of control over the material characteristics and often may need subsequent processing, such as drying and milling. These pharmaceutical processes have the potential to form activated solids and to cause a phase transition of the active ingredient that can eventually affect the quality of the formulation (4–6). In view of this, it is crucial to design and optimize a robust process for precisely controlling material characteristics in terms of polymorphic form and its purity, size, and shape of particles through the manipulation of kinetic and thermodynamic factors of the process.

During the years, it has been shown that crystallizations using supercritical fluids offer some advantages like single-step particle formation and environmentally benign over conventional crystallization techniques (7–8). Supercritical fluids (SFs) processes that have been developed use SFs as a solvent (Rapid Expansion of Supercritical Solutions, RESS) or anti-solvent (Gas AntiSolvent, GAS) in the particle formation. GAS was used to crystallize pure forms of sulfathiazole (form I and II) (9). Solution enhanced dispersion by supercritical fluids (SEDS), a modified form of GAS processes, is a one-step process that uses a coaxial nozzle with a mixing chamber that facilitates control over the particle formation phenomenon and the direct formation of dry and fine particles, because of the increased mass transfer rates. In fact, this technique was successfully used to crystallize the new polymorphs of salmeterol (10) and sulfathiazole (11)

Budesonide, is a potent glucocorticoid with a high topical anti-inflammatory activity and low systemic effects, widely used in the treatment of asthma by pulmonary delivery. Flunisolide anhydrous is also a glucocorticoid used as a nasal spray for the prophylactic and treatment of allergic rhinitis, and as a metered aerosol in the management of asthma. It has been reported that flunisolide exists in three different crystalline forms namely form I, hemihydrate and form II. The hemihydrate and form II are thermodynamically stable at room temperature of which hemihydrate is the most stable form (12). In this study, crystallization of flunisolide anhydrous using different organic solvents resulted in the formation of polymorphic mixtures. The aim of the present study was to crystallize the model drugs budesonide and flunisolide anhydrous using SEDS technique and manipulating the formation of pure crystalline phases by varying the processing conditions. The particles prepared from SEDS were characterized using differential scanning calorimetry (DSC), scanning electron microscopy (SEM), X-ray powder diffraction (XRPD) and variable temperature X-ray diffraction (VTXRD). In addition, apparent solubility of selected samples was determined. From the results, the mechanisms of particle formation during the crystallization using the SEDS technique were discussed.

MATERIALS AND METHODS

Materials

Micronized budesonide and flunisolide anhydrous (99% purity by the USP HPLC assay procedure) were gift samples from AstraZeneca, Sweden and Alco Chemicals Ltd., Switzerland, respectively. Methanol and acetone of analytical grade were purchased from Merck, Germany. Carbon dioxide (CO_2) of high purity (99.9%) was obtained from AGA Gas

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AB, Sweden. All chemicals were used without further purification.

Crystallization Using SEDS

A schematic diagram and explicit description of the SEDS apparatus used in this study was presented (13–14). The experimental procedure was as follows: crystallizations were carried out for budesonide and flunisolide anhydrous. Acetone and methanol solutions of drugs with a concentration of 2.5 mg/mL were fed through the nozzle into the high pressure vessel. During the crystallization, the pressure was varied between 100 bars and 200 bars and the temperature ranged from 40°C to 80°C. Several experiments were performed with different relative flow rates of CO_2 and drug solution. The flow rates of CO_2 and drug solution were 9 mL/min and 0.3 mL/min at all combinations of pressure and temperature. The influence of flow rate of CO_2 on flunisolide particle formation phenomenon was determined by varying the flow rate of CO_2 between 9 mL/min and 25 mL/min at a pressure of 100 bars and temperature 80°C. At the end of each experiment, the crystals were flushed with CO_2 at respective flow rates for 15 min to remove any residual solvent.

Solid-State Characterization of Drug Particles

Differential Scanning Calorimetry

Drug samples (1.5–2.5 mg) were placed in aluminum pans. The pans were closed and holes were made on pan covers. The samples were scanned at a rate of 10 C/min in a differential scanning calorimeter (DSC) (220C; Seiko, Japan) to determine the thermal behavior of different samples before and after the SEDS crystallization.

X-Ray Powder Diffraction

XRPD spectra were obtained using conventional diffractometer (model D5005, Siemens, Germany) operated with Cu_α radiation (45kV, 40 mA). The instrument was operated in a step scan mode in the angular range 5–40° 2θ. Some samples were investigated by a Guinier–Hägg focusing powder camera with silicon powder (a = 5.431023 Å) as an internal standard and the $CrK\alpha_1$ as the radiation source.

Variable Temperature X-Ray Diffraction

In an effort to identify the transitions indicated by DSC, the samples were subjected to a controlled temperature program, and X-ray powder patterns were obtained as a function of temperature. The VTXRD patterns were obtained by exposing the sample to CuK_α radiation (45 kV 40 mA) in a wide-angle powder X-ray diffractometer (Model XDS 2000, Scintag, USA). During the experiment, the samples were maintained under isothermal conditions at the selected temperatures. The scan angle range was 5–40° 2θ. The temperature controller (Micristar, Model 828D, Digital Systems Inc., USA) could be used over the temperature range of –190°C to +300°C. The sample was subjected to a continuous temperature program with a heating rate of 10°C/min.

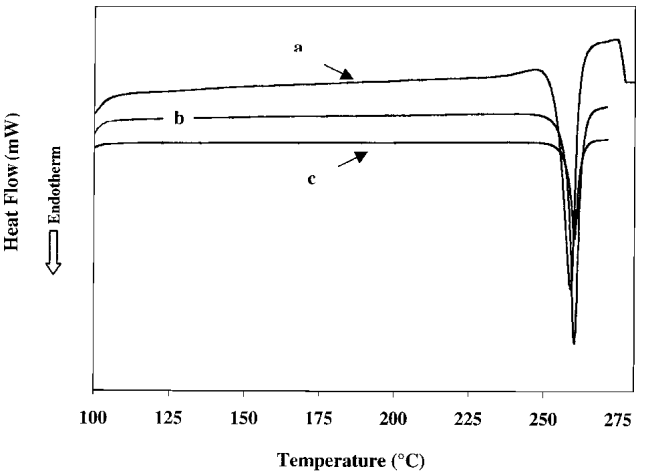


Fig. 1. Differential scanning calorimetry thermograms of micronised (unprocessed) (a) and solution enhanced dispersion by supercritical fluids technique crystallized budesonide at 100/80Acetone (b) and 100/80 Methanol (c).

Scanning Electron Microscopy

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

Solubility Determination of Flunisolide Particles

The absorbances of the solutions was measured using a UV spectrophotometer (U1100, Hitachi Ltd., Tokyo, Japan) at 246 nm. Every measurement was conducted in triplicate. A standard curve was drawn by plotting the absorbance as a function of concentration.

The solubility values of different crystalline forms of flunisolide were determined by suspending excessive quantities of the drug (2 mg) in 5 mL of milliQ water in 20 mL plastic scintillating vials. These containers were kept in a thermostat maintained at 25°C and were stirred at the rate of 300 rpm for 3 h. Samples were withdrawn with a syringe fitted with a 0.22 μm membrane filter. These samples were analyzed

Table I. Various Solution Enhanced Dispersion by Supercritical Fluid Processing Conditions Employed during the Crystallization of Budesonide at CO_2 Flow Rate of 9 mL/min and Drug Solution Flow Rate of 0.3 mL/min

Pressure (Bars)	Temperature (°C)		
	40	60	80
Crystallization from acetone solution			
100	NP	P	P
200	NP	NP	NP
Crystallization from methanol solution			
100	NP	P	P
200	NP	NP	NP

[P] stands for the product.
[NP] stands for no product.

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. The apparent solubility values were confirmed by determining the solubility after 24 h.

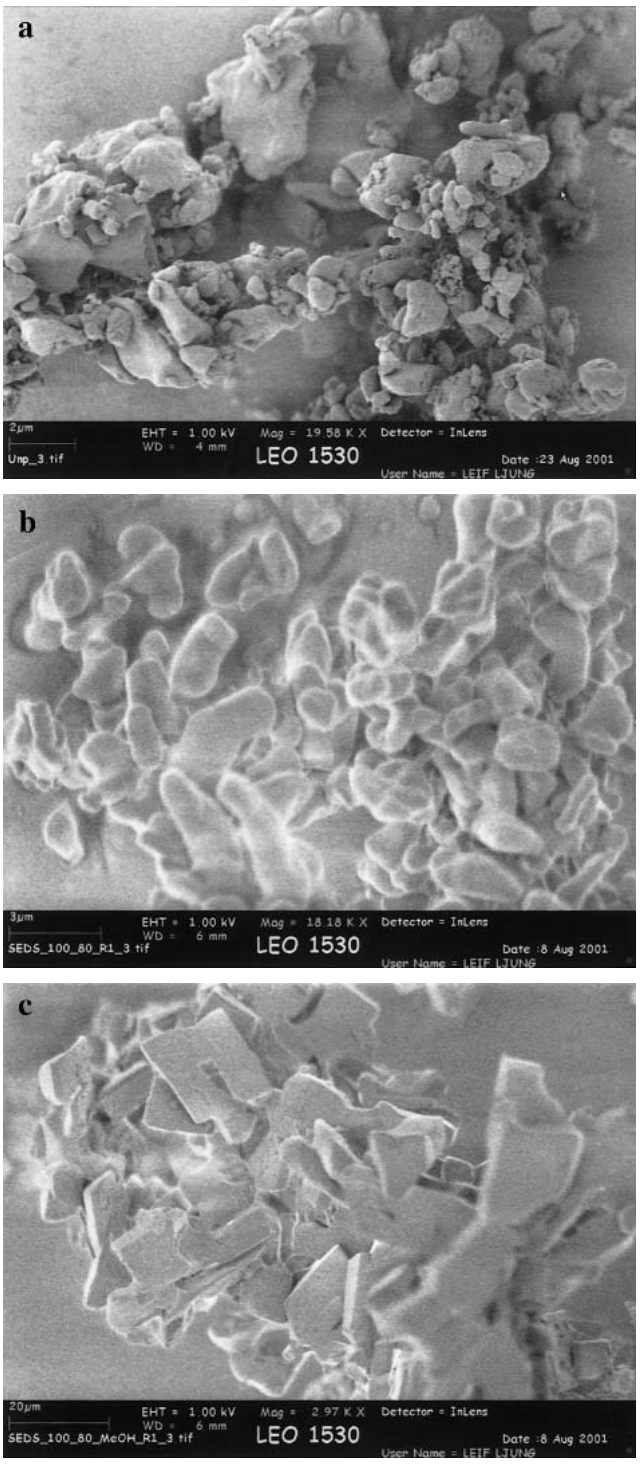


Fig. 2. Scanning electron microscopy pictures of (a) unprocessed micronized budesonide and solution enhanced dispersion by supercritical fluids technique processed of budesonide particles at (b) 100/80 Ace c) 100/80MeoH.

RESULTS AND DISCUSSIONS

Budesonide

The DSC thermogram of the micronized budesonide (unprocessed) showed a single endothermic melting transition at 258.7°C, which was typical for the crystalline material Fig. 1). The Table I shows various SEDS processing conditions employed during the crystallization of budesonide at CO_2 flow rate of 9 mL/min and 0.3 mL/min for the drug solution. At high pressure, 200 bars and low temperature, 40°C, no product was formed with either acetone or methanol. This was probably due to the increased solubility of budesonide in SF-CO_2 at these conditions that resulted in complete extraction of both the solvent and the drug. This was confirmed by the presence of budesonide in the sample collected at the back pressure regulator at these conditions. Obviously, this mechanism dominated over the simultaneously increased solubility of solvent in SF-CO_2, which would have caused the faster extraction and hence the increased precipitation of the drug.

The SEDS crystallized samples from acetone and methanol solutions at various conditions showed identical thermal behavior as the micronised material (Fig 1). This implies that the samples were crystalline, and any change in the crystal structure was less susceptible to either nature of the solvent or the conditions in this range of processing conditions. Similar results were obtained when budesonide was micronized by means of the Aerosol Solvent Extraction System (ASES) process (15) and the SEDS process (16).

SEM photographs of micronised and SEDS crystallized samples are shown in Fig. 2. From the SEM pictures, it is clear that the micronized material was comprised of irregular spherical particles constituting aggregates (Fig. 2a). This is the typical morphology of micronization of drug using high-energy operations. SEDS crystals formed at 100 bars and 80°C (100/80) from acetone were smooth, nearly spherical and discrete whilst those from methanol showed plate like structures (Fig. 2b and 2c). It is also seen from SEM pictures that the size of the particles from acetone was typically

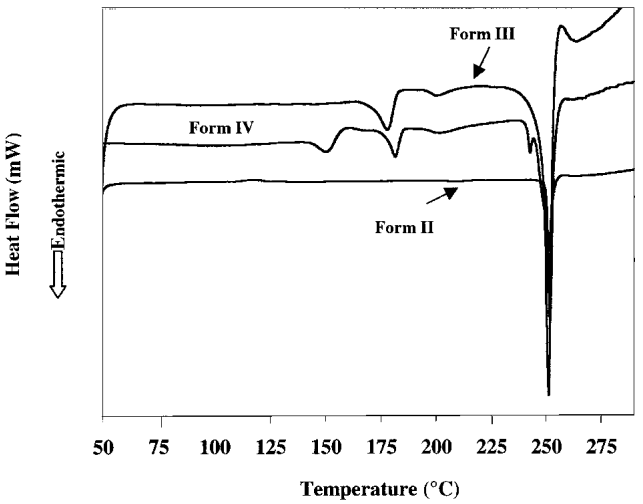


Fig. 3. Differential scanning calorimetry thermograms of flunisolide anhydrous polymorphic forms II, III, and I.

between 1–3 μm and from methanol, it was 5–30 μm. The higher solubility of acetone compared with methanol in SF could have influenced the saturation profile with a consequent affect on the nucleation and growth, producing particles with different size distributions and morphologies (13,17). Apparently, the slower extraction rate, using methanol would favor the formation of large regular crystals. The differences in size distribution and particle shape between acetone and methanol samples could be partially due to the bond formation possibilities and capabilities between the solvents and the drug affecting the extraction process. The uniformity in particle size and morphology demonstrated the ability of the SEDS process to control the particle formation (14).

Flunisolide Anhydrous

Solid-State Characterization of Unprocessed Micronized Material

The DSC of the micronized flunisolide anhydrous (unprocessed) showed a small exothermic peak at 117 ± 1.4°C and a slight endothermic transition at around 210°C to 230°C prior to the melting endotherm at 250 ± 0.9°C Fig. 3). The exothermic peak was attributed to the recrystallization of amorphous regions in the material, confirmed by the halo in X-ray spectrum of the material taken at room temperature Fig. 4). The similarity in the X ray spectrum at 30°C and 130°C in VTXRD proves that the exothermic transition did not result in major phase conversions (Fig. 5). The presence of an exothermic peak in the DSC profile was not observed or reported in the earlier investigation (12). Contradiction in the result was possibly due to the formation of activated phases as a result of the micronization process that our sample had experienced. The endothermic transition at 210°C–230°C was attributed to the solid-solid transition of polymorphic form II to other modification that could be adjudged by the absence of characteristic peaks of form II in the X-ray difraction (XRD) pattern at 230°C taken from VTXRD after isothermally holding the sample for 40 min (Fig 5). There was no change in X- ray pattern until 210°C, where the intensities of characteristic peaks started to decrease, indicating that no phase change had occurred until 210°C. However, the phase change was completed at 230°C. The X-ray pattern, obtained when the sample was cooled to room temperature, was different from the one at 230°C and was identified as characteristic of polymorphic form I (Fig. 5). Similar findings were obtained with Fourier transform infrared spectroscopy coupled with thermomicroscopy, explaining the formation of an unstable intermediate form at 230°C that converts to the form I when cooling the sample to room temperature (12). The SEM pictures showed partially spherical particles, constituting large agglomerates with variations in the size of the particles (Fig. 7a).

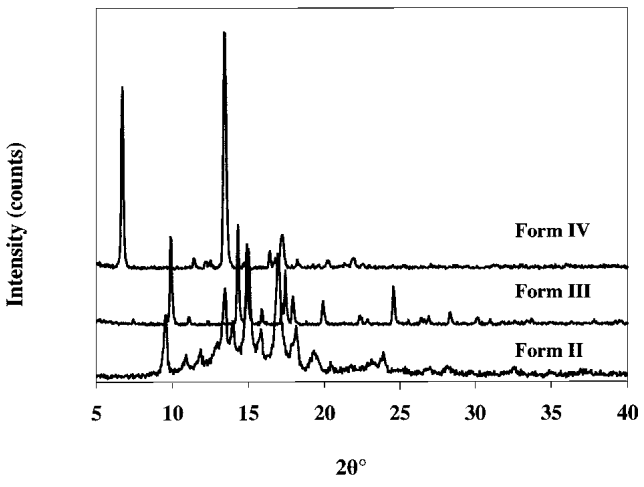
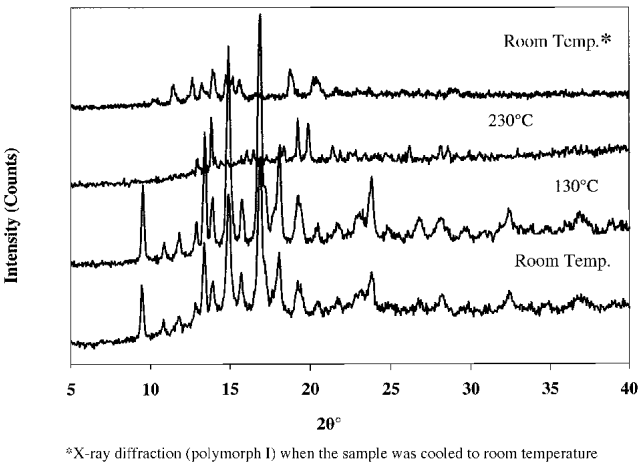


Fig. 4. X-ray powder diffraction spectra of polymorphic forms II, III, and IV of flunisolide anhydrous.



*X-ray diffraction (polymorph I) when the sample was cooled to room temperature

Fig. 5. Variable temperature X-ray diffraction (XRD) of flunisolide anhydrous form II. XRD patterns were obtained at indicated in the figure.

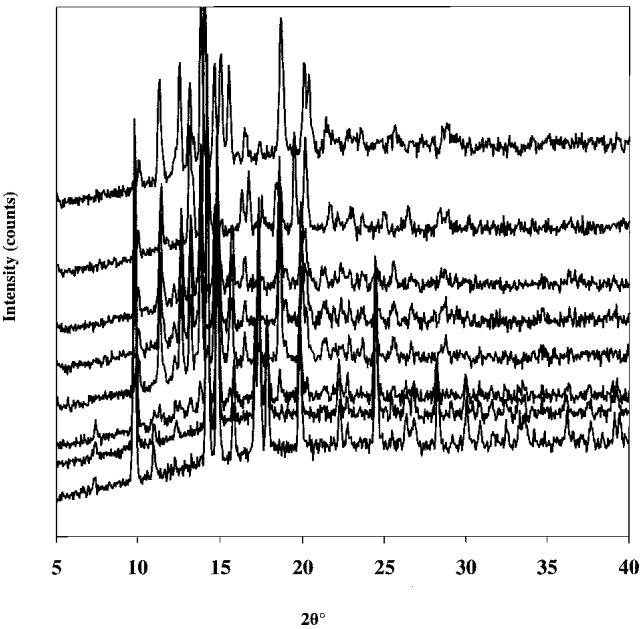


Fig. 6. Variable temperature X-ray diffraction (XRD) of polymorph III (solution enhanced dispersion by supercritical fluids technique sample 100/60 Ace) of flunisolide anhydrous. The XRD patterns shown were obtained at room temperature, 130°C, 155°C, 170°C, 190°C, 210°C, 230°C, and room temperature on cooling (reads from bottom to top).

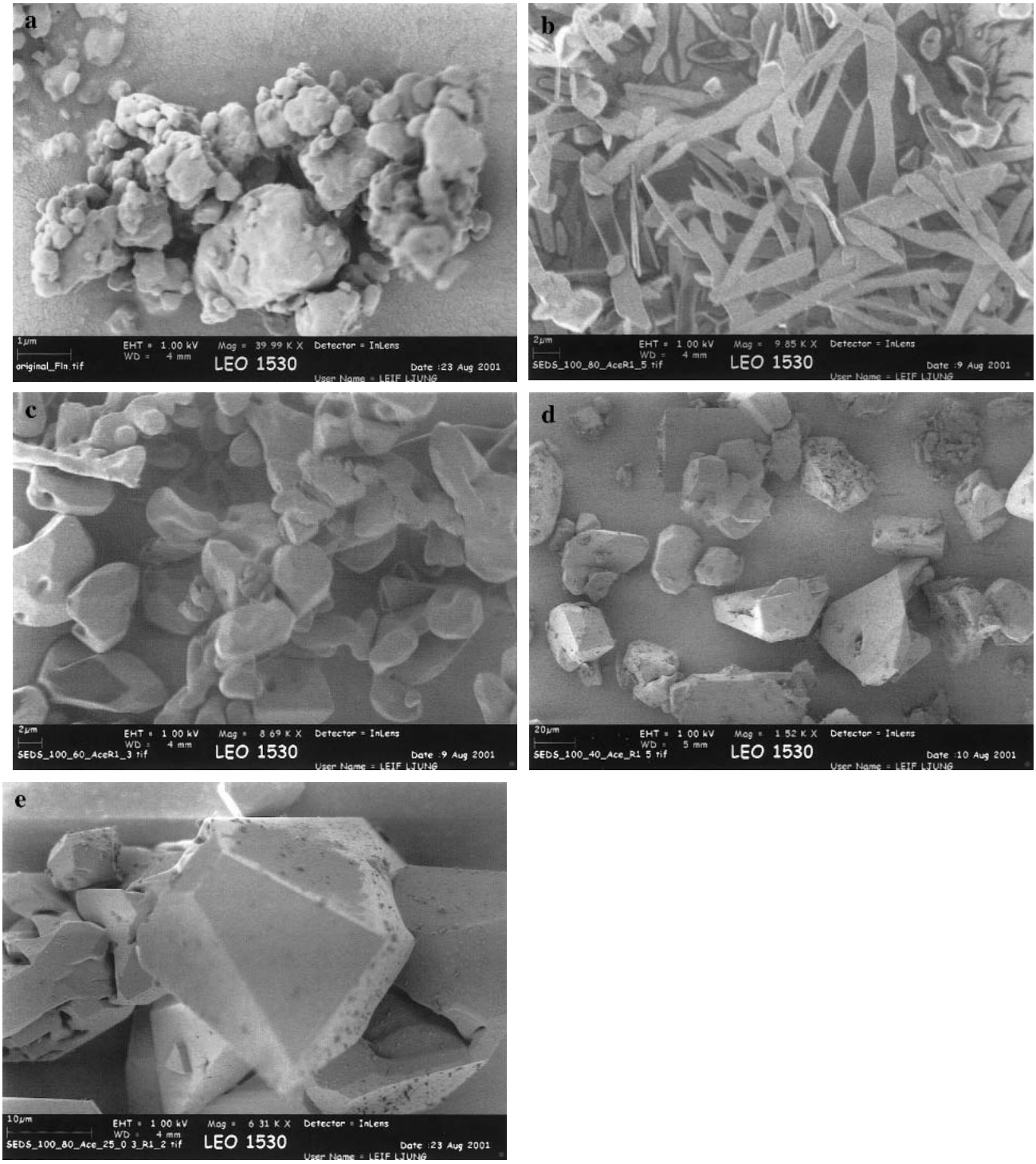


Fig. 7. Scanning electron microscopy pictures of flunisolide anhydrous (a) unprocessed, micronized (Form II) and solution enhanced dispersion by supercritical fluids technique crystallized samples at (b) 100/80 Ace, polymorphic mixture (c) 100/60 Ace, Form III (d) 100/40 Ace, Form III (e) 100/80 Ace at CO_2 flow rate of 25 mL/min.

SEDS Crystallization

Table II shows various SEDS processing conditions employed during the SF crystallization of flunisolide at different CO_2 and drug solution flow rates. It is apparent from the Table II that at 200 bars no product was formed either acetone or methanol. Particles were produced at 100 bars. However, the product yield decreased as the temperature was lowered from 80°C and was extremely low at 40°C. Thus, most of the drug is solubilized in SF at higher pressures and low temperatures disabling the formation of particles. This effect was also observed for budesonide. In fact, in our earlier studies (unreported results), crystallization of progesterone using SEDS technique was not possible at all the processing condi-

Table II. Various Solution Enhanced Dispersion by Supercritical Fluid Processing Conditions Employed during the Crystallization of Flunisolide Anhydrous at Different CO_2 and Drug Solution Flow Rates

Pressure (Bars)	Temperature (°C)		
	40	60	80
Crystallization from acetone solution			
100	P	P	P, P[a], P[b]
200	NP	NP	NP
Crystallization from methanol solution			
100	NP	P	P, P[a], P[b]
200	NP	NP	NP

[P] stands for the product.
[NP] stands for no product.
[a,b] indicate products at 18 and 25 mL/min of CO_2, respectively.

ions. This was also possibly because of the complete solubility of the drug in SF, further supporting the above explanation.

Solid-State Characterization of SEDS Crystallized Flunisolide

Samples from Acetone Solutions

The SEDS crystallization of flunisolide anhydrous was accomplished between 40°C and 80°C at 100 bars as presented in Table II. It is interesting to note from the Table III that the decrease in the temperature from 80°C to 60°C or 40°C resulted in the formation of a pure new polymorphic form III. DSC thermogram of the material formed at 100 bars and 80°C (100/80 Ace) showed an endothermic transition at 198.2°C before it melted at 251°C (Fig. 3). Based on the XRD examination, the material was identified as a mixture of more than two forms that contained I, III, and unidentified modifications. As reported earlier, even small fractions of form I could have significant affect on the thermal behavior of the sample that explains the similarity between the thermal behavior of the present sample and polymorph I (12). The crystals were mostly elongated flat rods (Fig. 7b). The higher apparent solubility of this polymorphic mixture could be due to its morphology and the presence of different polymorphic phases (Table III). In fact, a higher apparent solubility of polymorphic form I was reported earlier (12)

The material crystallized at 100 bars and 60°C (100/60 Ace) showed two endothermic transitions, the major one at around 176°C followed by the minor one at 200.4°C. Finally, the melting occurred at around 251.5°C (Fig. 3). The distinct nature of the XRPD pattern of the material at room temperature combined with DSC results explain the existence of the new polymorphic form III (Figs. 3 and 4). As seen in the VTXRD (Fig. 6), some peaks started to disappear at 170°C and some other new peaks were appearing, indicating the solid-solid transformation and the pattern at 190°C completely resembles the XRD profile of the polymorphic form I. On further heating to 230°C, the XRD pattern again changes to the one similar to an intermediate unstable modification and the pattern obtained when cooled the sample to room was identical to the one for form I. Crystals exhibited partially spherical morphology (Fig. 7c). The apparent solubility of this material was different from the other forms, supporting the existence of the new polymorphic form III (Table III).

SEDS prepared material at 100 bars and 40°C (100/40 Ace) displayed similar thermal behavior and XRPD spectrum to that of form III confirming the generation of form III. However, the particles were discrete, prismatic and larger (Fig.7d). This is possibly due to the nucleation density is limited owing to lower supersaturation levels achieved in the droplet due to the higher solubility of the solute in SF at lower temperatures.

From the SEM photographs, it was seen that the crystallization of flunisolide anhydrous at 100 bar and 80°C (100/80 Ace), at low CO_2 density, formed elongated blade like needles, while at 100 bar and 60°C (100/60 Ace), partially spherical particles were produced. At 100 bars and 40°C (100/40 Ace) well-faceted prisms were obtained (Fig. 7 b, c, and d).

Shekunov et al. have explained the particle formation mechanisms involved in the SEDS technique. They claimed that the extraction rate, the solubility function of the drug and the mixing dynamics of the fluids control the particle formation in the process (18). Our findings support their conclusions. Moreover, we found that the dominating factor that controls particle formation was the solubility function of the drug in SF.

Table III. Different Solution Enhanced Dispersion by Supercritical Fluid (SEDS) Processed Samples and Their Corresponding Polymorphic Forms and Their Apparent Solubility in Water

Drug solution	Processing conditions press. (Bars)/Temp. (°C)	Polymorphic modification	Apparent solubility (μg/mL)
Acetone	100/80	I + III	61.5 (0.5)† (1.0)[c]
	100/60	III[a]	51.5 (0.5)† (2.0)[c]
	100/40	III	ND
Methanol	100/80	IV[a]	48.6 (0.4)† (1.0)[c]
	100/60	III + IV	47.4 (0.2)† (1.0)[c]
	100/40	NP	ND
Unprocessed material	—	II	45.9 (0.2)[b]

[a] new polymorphs.
[b] SD for the experiment (n = 3).
[c] SD for three different batches of the SEDS samples processed at respective processing conditions.
[NP] indicates no product.
[ND] indicates not determined.

At 100 bar and 80°C, increasing the flow rate of CO_2 from 9 mL/min to 18 mL/min, keeping the acetone solution flow rate constant at 0.3 mL/min, resulted in the formation of crystals that displayed similar thermal behavior, morphology, and XRPD pattern that are similar to the samples processed at 100 bar/60°C, indicating the generation of polymorphic form III. A further increase in flow rate of solution to 25 mL/min produced a material with similar modification (form III); nevertheless, these particles were larger with a regular shape (Fig. 7e). The increase in particle size with increase in CO_2 flow rate is caused by the increased extraction capacity of the drug which results in lower supersaturation levels and lower nucleation density resulting in fewer but larger particles. Furthermore, this effect could be because of reduced residence time in the nozzle and increased variability of solvent concentration in the flow (17–18). Similar results were obtained with nicotinic acid during crystallization using SF-CO_2 (19)

Samples from Methanol Solutions

The SEDS crystallization of flunisolide anhydrous was possible only between 60°C and 80°C at 100 bars at the CO_2 and solution flow rates of 9 mL/min and 0.3 mL/min respectively (Table II). Thermal examination of the material, precipitated at 100 bars and 80°C (100/80 MeOH) revealed endotherms at 150°C, 182°C, 200°C 230°C with eventual melting of the final form at 255°C (Fig. 3). The XRPD spectrum of the material was distinctly different from other modifications indicating the generation of polymorphic form IV (Fig. 5). With VTXRD set up (Fig. 8), powder patterns were obtained while a sample was subjected to a controlled temperature program in an effort to identify the transitions that were seen in the DSC pattern. The endothermic transitions at 150°C, 182°C and 210°C were attributed to the transition of form IV to series of unidentified phases, as revealed by the disappearance of characteristic peaks of form IV. At 230°C, the XRD pattern appeared to be similar to the unstable intermediate modification identified in case of form II. On cooling the sample to room temperature, the characteristic peaks of form I were identified. The particles showed hexagonal-platelike morphology (Fig. 9). The unique XRD pattern of the material, endothermic heat effects in DSC and its different morphology confirm the formation of the new polymorphic form IV. However, the apparent solubility of the material was not significantly different from the modification II (Table III). This result gives an indication that the stability of the new form IV is equal to that of thermodynamically stable anhydrous form (Form II) at room temperature. For the sample formed at 100 bars and 60°C (100/60 MeOH), the DSC trace showed slight initial transition at 148°C leading to the steep endothermic transition at 182°C followed by the final melting at 253°C. It was apparent from the ambient temperature XRD and VTXRD profiles that the material was a mixture of forms III and IV. SEM examination confirmed the elongated hexagonal-platelike morphology of the crystals that was typical for the modification IV. Nevertheless, the apparent solubility of the sample was equal to the polymorphic modification IV.

The morphology, thermal behavior, XRPD pattern, and apparent solubility of the material crystallized at higher CO_2 flow rate (i.e., 18mL/min and 25mL/min) at 100/80 were found to be identical to the one produced at 100/60 which demonstrated the formation of the material with mixture of phases. This outcome with methanol share similarity with the results from acetone solution in a sense that the increase in CO_2 flow rate at 100/80 resulted the formation of material with similar characteristics as the material formed at 100/60.

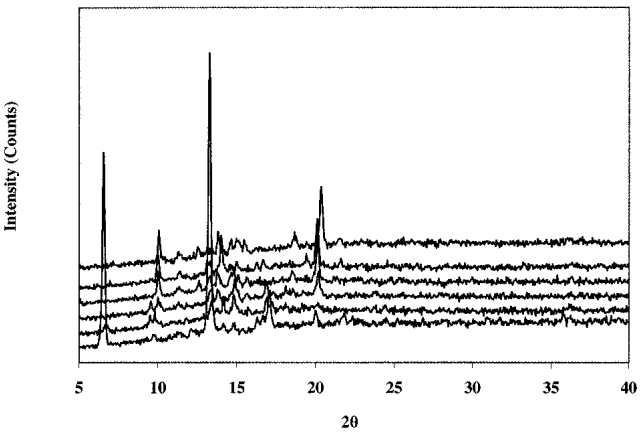


Fig. 8. Variable temperature X-ray diffraction of polymorph IV solution enhanced dispersion by supercritical fluids technique sample 100/80 MeOH) of flunisolide anhydrous. The XRD patterns shown were obtained at room temperature, 160°C, 182°C, 210°C, 230°C, and room temperature on cooling (reads from bottom to top).

CONCLUSIONS

Budesonide and flunisolide anhydrous can be crystallized using the SEDS process. The outcome depends on the temperature and pressures employed. For both drugs, the nature of the solvent influences the size and shape of the particles. VTXRD was proved powerful complementary tool to study phase transitions during temperature variation. The crystal structure of budesonide was impervious to either the processing conditions or the nature of solvent, whereas different modifications of flunisolide were produced by proper choice of processing conditions. SEDS processing of flunisolide using acetone as solvent at 100 bars and 80°C resulted in the formation of material consisting of a mixture of different

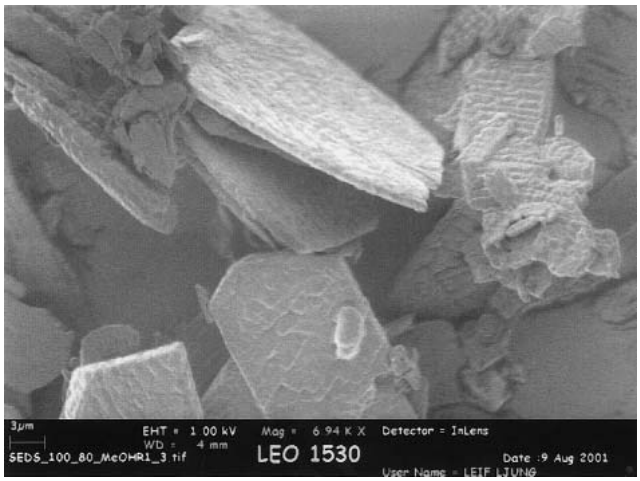


Fig. 9. Scanning electron microscopy picture of solution enhanced dispersion by supercritical fluids technique crystallized flunisolide anhydrous at 100/80 MeOH (Form IV).

polymorphic modifications. As the temperature was lowered a pure new form III was obtained. SEDS processing of flunisolide at 100 bars and 80°C using methanol as the solvent, resulted in the formation of pure new form IV. At 100/60 with methanol, a mixture of III and IV was obtained. By varying the flow rate of CO_2, the formation of the polymorph and the size of the particles could be controlled. The extraction rate of the solvent into SF and mixing dynamics between the solution and SF are important. However, we conclude from our results that the dominating factor controlling particle formation is the solubility function of the drug in SF. Thus, the crystallization of drugs from supercritical fluids using the SEDS process is a promising alternative to conventional methods in the generation of pure polymorphic phases. The later part of the project deals with further characterization of these particles and their pharmaceutical implications.

ACKNOWLEDGMENTS

We thank Professor Raj Suryanarayanan and his research group at the Department of Pharmaceutics, University of Minneapolis, MN, U.S.A. for their assistance in XRPD and VTXRD studies and for valuable discussions. We also thank AstraZeneca, Sweden, and Sicor S.p.A., Italy, for providing gift samples.

REFERENCES

1. L. Borka. Review on the crystal polymorphism of substances in The European Pharmacopoeia. *Pharm. Acta Helv.* **66**:16–22 (1991).
2. D. J. W. Grant. Theory and origin of polymorphism. In H. G. Brittain (ed.), *Polymorphism in Pharmaceutical Solids*, Marcel Dekker, New York, 1999, pp. 1–33.
3. J. K. Haleblian and W. McCrone. Pharmaceutical applications of polymorphism. *J. Pharm. Sci.* **58**:911–929 (1969).
4. A. Saleki-Gerhardt, C. Ahlneck, and G. Zografi. Assessment of disorder in crystalline solids. *Int. J. Pharm.* **101**:237–247 (1994).
5. R. K. Cavatur and S. Raj. Characterization of phase transitions during freeze-drying by in situ X-ray powder diffractometry. *Pharm. Dev. Technol.* **3**:579–586 (1998).
6. H. G. Brittain and E. F. Fiese. Effects of pharmaceutical processing on drug polymorphs and solvates. In H. G. Brittain (ed.), *Polymorphism inPharmaceutical Solids*, Marcel Dekker, New York,1999, pp. 331–361.
7. J. W. Tom and P. G. Debenedetti. Particle formation with supercritical fluids - a review. *J. Aerosol Sci.* **22**:555–584 (1991).
8. S. Mawson and K. P. Johnston. Precipitation with a compressed fluid antisolvent: advanced concepts. *Polym. Mater. Sci. Eng.* **74**: 180–181 (1999).
9. M. Kitamura, Y. Yoshinaga, and H. Masuoka. Crystallization of polymorphous sulfathiazole by gas-antisolvent method. Proceedings of the 14th Int. Symp. on Ind. Cryst., Cambridge, United Kingdom 1999, pp. 1596–1603.
10. S. Beach, D. Latham, C. Sidgwick, M. Hanna, and P. York. Control of the physical form of salmeterol xinofoate. *Org. Process Res. Dev.* **3**:370–376 (1999).
11. A. Kordikowski, T. Shekunov, and P. York. Polymorph control of sulfathiazole in supercritical CO_2. *Pharm. Res.* **18**:682–688 (2001).
12. M. Bartolomei. Solid-state studies on the hemihydrate and the anhydrous forms of flunisolide. *J. Pharm. Biomed. Anal.* **24**:81–93 (2000).
13. S. P. Velaga, R. Ghaderi, and J. Carlfors. Preparation and characterisation of hydrocortisone particles using a supercritical fluids extraction process. *Int. J. Pharm.* **231**:155–166 (2002).
14. M. Hanna and P. York. U.S. patent 5851453; Eur.patent 0706421B1, 0767702B1 (1998).
15. H. Steckel, J. Thies, and B. W. Muller. Micronizing of steroids for pulmonary delivery by supercritical carbon dioxide. *Int. J. Pharm.* **152**:99–110 (1997).
16. M. Bisrat and S. Moshashaee. WO 98/52544 (1998).
17. B. Y. Shekunov, M. Hanna, and P. York. Crystallization process in turbulent supercritical flows. *J. Cryst. Growth.* **198**:1345–1351 (1999).
18. B. Y. Shekunov, J. Baldyga, and P. York. Particle formation by mixing with supercritical antisolvent at high Reynolds numbers. *Chem. Eng. Sci.* **56**:2421–2433 (2001).
19. M. Rehman, B. Y. Shekunov, P. York, and P. Colthorpe. Solubility and precipitation of nicotinic acid in supercritical carbon dioxide. *J. Pharm. Sci.* **90**:1570–1582 (2001).