Preparation of Pharmaceutical Powders using Supercritical Fluid Technology

Pharmaceutical Applications and Physicochemical Characterisation of Powders

BY

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Abstract


The main aim of the thesis was to explore the potential of supercritical fluid (SF) techniques in the field of drug delivery. In particular, the relatively recently developed solution-enhanced dispersion by supercritical fluids (SEDS) technology has been employed in the preparation of particles/powders.

The manufacturing, stability and bioavailability of a dosage form strongly depend on the physicochemical properties of the formulation particles. For example, dry powder inhalation (DPI) for administering drugs to the respiratory tract require particles in a narrow size range (1-5 μm) to be effective. The identification of polymorphs and control of purity are also important issues since the physicochemical properties and therapeutic effects of the alternative forms of a drug may differ substantially. Solvent-based traditional crystallisation processes provide the product that may require further down-stream processing to obtain particles for advanced drug delivery applications. This can result in unwanted changes in the physicochemical properties of the particles and thus affect the performance of the dosage form. SF processing has addressed many of the challenges in particle formation research. Among several SF technologies developed for particle processing over the last decade, the SEDS process with its specially designed co-axial nozzle with mixing chamber has resulted in improved control over the particle formation process. Carbon dioxide (CO₂) was used as the SF, because it has low critical points and is non-toxic, non-flammable and relatively inexpensive.

The initial part of the thesis concerns the formation of particles of model drugs such as hydrocortisone, budesonide and flunisolide using SEDS technology and the determination of the influence of processing conditions and solvents on particle characteristics such as size, shape and crystal structure. Particles of model drugs of differing shapes in a size range suitable for inhalation delivery were prepared. In the process, two new polymorphic forms of flunisolide were identified. This was the first report of SEDS technology being shown as a polymorph-screening tool. The remainder of the thesis deals with the development of SEDS technology for precipitating therapeutic proteins such as recombinant human growth hormone (hGH) from aqueous solutions. Powders of hGH were precipitated using SEDS without significant changes in the chemical or physical stability of the protein. The addition of sucrose to hGH in the feed solution promoted precipitation and minimised the detrimental effects of the solvent and/or the process on the physical aggregation of the protein.

In conclusion, this thesis highlights the applicability of the SEDS process in drug delivery research and advances general understanding of the particle formation phenomenon. The SEDS process may also prove to be a potential alternative technology for the precipitation of stable powders of therapeutic proteins.

Keywords: Supercritical fluid, Gas Anti-Solvent, SEDS, Crystallisation, Particle design, Polymorphs, Dry powder inhalation, Solid-state behaviour, Therapeutic proteins, Precipitation, Stability, Recombinant human growth hormone (hGH)

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To my parents and family
LIST OF PAPERS

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ABBREVIATIONS

SF  Supercritical Fluid
Tc  Critical Temperature
Pc  Critical Pressure
SC-CO2 Supercritical Carbon dioxide
RESS Rapid Expansion of Supercritical Solutions
GAS Gas Anti-Solvent
SEDS Solution Enhanced Dispersion by Supercritical fluids
Nwe Weber Number
HC Hydrocortisone
hGH Recombinant Human Growth Hormone
DSC Differential Scanning Calorimetry
XRPD X-Ray Powders Diffraction
VTXRD Variable Temperature X-ray Diffraction
DPI Dry Powder Inhalation
MMAD Mass Median Aerodynamic Diameter
MLI Multistage Liquid Impinger
RP-HPLC Reversed Phase High Performance Liquid Chromatography
SEC Size Exclusion Chromatography
1 INTRODUCTION

Currently available drug delivery systems commonly contain the active pharmaceutical ingredient (API) and various functional formulation constituents (excipients). The API and excipients are invariably required to be in the powdered form at some stage in the preparation of the delivery system. The preparation, quality and performance of a dosage form strongly depend on the physicochemical properties of the powders. The physicochemical properties of interest in this thesis include chemical composition or purity, melting point, crystal structure, solubility, and particle size, shape, and surface properties. The most popular and widely used drug dosage form is the oral tablet, containing compressed API and excipient particles. The compaction behaviour of the component particles and subsequent disintegration and dissolution rate of the tablets are heavily dependent on the physicochemical and mechanical properties of the particles (1-4). However, the properties of component particles in other dosage forms, such as those for dry powder inhalation, are equally important.

1.1 Dry Powder Inhalation (DPI)

Drug delivery by inhalation for local and/or systemic effect has attracted considerable interest in recent years (5-7). Dry powder inhalers, have been developed to improve the compliance of the patients (8-10) and to avoid the usage of chlorofluorocarbons (11). Dry powder inhalers are versatile, dispensing a metered quantity of various powders in a stream of air drawn through the device by the patient’s own inspiration (12). The Spinhaler™ was the first of these inhalers; it was designed to deliver disodium cromoglycate. Subsequently, several multi-dose inhalers have been developed (e.g. Rotahaler™, Diskhaler™, Turbuhaler™ and Easyhaler™). Inhalation powders typically constitute drug particles, drug particles associated with a carrier, or drug particles bound into loose aggregates. The predominant deposition mechanism in the lungs involves inertial impaction, expressed in terms of aerodynamic diameter \( d_a = \frac{d \rho}{\rho} \). For DPI formulations, it is necessary that the particle size distribution of the active components be 1-5 \( \mu \)m to deposit the drug in the lungs (13-15). However, the cohesive force between the drug particles or the adhesion of the drug particle to a carrier particle such as lactose often results in insufficient dispersion of the drug particles at emission, thus decreasing the amount of drug delivered to the respiratory tract. The dispersion and subsequent deposition profiles of the drug particles during inhalation are governed by the physicochemical properties of both the drug and the carrier particles (13, 16-22). The deposition patterns are also regulated by the design of the inhalation device.
(23) and patient related factors such as inspiration capacity (24, 25). Therefore, it is necessary to strictly and reproducibly control the physicochemical properties of the relevant powders for the successful development of DPI systems.

1.2 Drug polymorphism

Another important issue in the development of any dosage form is control of the crystallinity and crystal properties of the constituents. Many pharmaceutical solids exhibit polymorphism, which can be defined as the ability of a substance to exist as two or more crystalline phases that have different arrangements and/or conformations of the molecules in the crystal lattice (26). The polymorphic solids have different unit cells and, thus, different physical, thermodynamic, spectral, interfacial and mechanical properties (26-28). These properties have consequences on the manufacturing processes, the physicochemical stability of the active component alone and in combination with the excipients, and the bioavailability of the drug (29-32). The unexpected appearance or disappearance of a polymorphic form can lead to serious pharmaceutical consequences, which may result in delays in product development and disruption of commercial production. For example, a thermodynamically more stable form of ritonavir (Abbott Laboratories, protease inhibitor for the treatment of HIV), with different dissolution and absorption characteristics, was discovered after the product was launched, causing production to halt completely (33). As a result, polymorphism of pharmaceutical solids has received much scrutiny in recent years, throughout the various stages of drug development, manufacturing and regulation (34, 35).

These concerns also led to the development of specific guidelines on the control of physicochemical properties which are presented and discussed by Byrne et al. in the form of decision trees/flow charts/algorithms (36). The flow chart for drug polymorphism in that review highlights a rational approach for the identification and investigation of the physical properties of polymorphs followed by discussion of the need to ensure the integrity of the drug substance, which may contain either a single morphic form or a mixture (36). The screening and identification of polymorphs has until now been limited to the tedious traditional methods of solvent crystallisation, thermal methods and more recent advanced high throughput technologies (37-40). The control of polymorphic purity/integrity has also been challenging in traditional methods of material processing.

1.3 Therapeutic protein processing

With the advent of recombinant technologies, proteins and peptide products derived from biological origins are becoming increasingly interesting as
therapeutic agents. However, the development of protein pharmaceuticals poses a significant challenge because of their limited physical and chemical stability (41-43). Protein stability is one of the main stimulants for the development of non-oral delivery systems such as respiratory, transdermal and parenteral systems (44). Moreover, advanced drug delivery systems such as DPI require drug particles to be within a specific size range. The recent advances in drug delivery research suggest several approaches for stabilising and delivering protein and peptide drugs (45-47). However, the most common practice for overcoming the instability barrier is to process the proteins into solid forms and to administer them via parenteral or inhalation routes.

In many delivery systems, including DPI, drugs are required to be in micro-particulate form to enhance dissolution and targeting purposes. The current most common methods of particle formation, size reduction, and material processing in the pharmaceutical industry include a) crystallisation from solutions, b) precipitation c) milling d) spray-drying and e) freeze-drying. In conventional crystallisation methods such as solvent crystallisations, the crystal and powder properties (notably polymorphic form, particle size, and shape) essentially depend on the conditions of crystallisation such as supersaturation, temperature, concentration, cooling rate and presence of impurities (48-50). Thus, a complex and challenging process of precisely controlling crystallisation behaviour, and thereby particle properties, is required. Furthermore, these methods lack the capacity to produce the fine particles required for current drug delivery applications. Thus, additional downstream operations such as drying, milling and sieving are often required (Fig. 1a). These operations potentially result in variations in substance characteristics such as the polymorphic nature and purity, the presence of crystal disruption and disorder, and changes in surface characteristics (51-56). These variations may have profound influence on the drug substance or product characteristics (Table 1).

Currently existing methods in the area of protein processing and formulation include freeze- and spray-drying, pulverisation and precipitation (57). Lyophilisation (freeze-drying), the most common process for preparing solid protein pharmaceuticals, requires a long, sophisticated and expensive process. This process can also generate a variety of stresses due to freezing and drying, and these can lead to pH changes and phase separation which will affect protein stability (58-60). Spray-drying process showed some promise in generating powders but higher temperature utilized to achieve thermal dehydration is a major limitation in the protein particle preparation (61).

Consequently, interest in developing new and efficient processes for controlling the physicochemical characteristics of particles is always present in the pharmaceutical industry, particularly to meet demands of novel drug
formulation and delivery. Furthermore, extending the applicability of such technologies, to aid in the screening and identification of new polymorphs and controlled crystallisation of highly pure polymorphs, is always attractive. In the development of protein therapeutics, as it is highlighted, there is a clear need for the development of alternative methods of protein processing.

**Table 1**: Solid-state and drug delivery characteristics affected by crystallisation/subsequent operations and their relationship with specific characteristics of drug substances and drug products (62)

<table>
<thead>
<tr>
<th>Solid-state properties</th>
<th>Effect on drug substance and /or drug product</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Structural</strong></td>
<td></td>
</tr>
<tr>
<td>Crystallinity (existence of amorphous and semi-crystalline forms)</td>
<td>Physical and chemical stability</td>
</tr>
<tr>
<td><strong>2. Polymorphs</strong></td>
<td></td>
</tr>
<tr>
<td>Solvates (hydrates)</td>
<td>%RH profile (hygroscopicity)</td>
</tr>
<tr>
<td>Salts</td>
<td>Solubility profile and dissolution rate</td>
</tr>
<tr>
<td>Crystal defects</td>
<td>All aspects of processing</td>
</tr>
<tr>
<td><strong>3. Dimensional</strong></td>
<td></td>
</tr>
<tr>
<td>Particle size distribution</td>
<td>Processing behavior: bulk density, agglomeration, flow/rheology, compaction</td>
</tr>
<tr>
<td>Particle morphology</td>
<td>Particle permeability (i.e particle adsorption)</td>
</tr>
<tr>
<td>Particle surface structure</td>
<td>Bioavailability (drug absorption)</td>
</tr>
<tr>
<td></td>
<td>Consistency and uniformity of the dosage form</td>
</tr>
<tr>
<td><strong>4. Chemical</strong></td>
<td></td>
</tr>
<tr>
<td>Organic and inorganic impurities, residual solvent and decomposition products</td>
<td>Toxicity</td>
</tr>
<tr>
<td>Chiral forms and chiral separation</td>
<td>Chemical, physical and enantiomeric stability</td>
</tr>
<tr>
<td>Sterility (microbial limits)</td>
<td></td>
</tr>
<tr>
<td><strong>5. Mechanical</strong></td>
<td></td>
</tr>
<tr>
<td>Brittle/ductile transitions, fracture stress, indentation hardness, stress/strain relaxation, yield pressure, Young’s modulus</td>
<td>Milling and tabletting behavior</td>
</tr>
<tr>
<td><strong>6. Electrical</strong></td>
<td></td>
</tr>
<tr>
<td>Electrostatic charge distribution</td>
<td>Agglomeration and flow properties</td>
</tr>
</tbody>
</table>

**1.4 Supercritical fluid (SF)**

In recent years, SF techniques have shown advantages in addressing many of the challenges faced in the particle formation research highlighted above.
These methods involve a single step and are environmentally benign processes (Fig. 1b).

Gases and liquids are SFs at temperatures and pressures above their critical points (Tc: critical temperature and Pc: critical pressure) (Fig 2a). An SF exists as a single phase, while retaining several advantageous properties of both liquids and gases. An SF has density values that are liquid-like, enabling appreciable solvation power, whilst the viscosity of solutes in an SF is lower than in liquids and the diffusivity of solutes is higher, which enables mass transport. Table 2 illustrates the relative density, viscosity and diffusivity values of the SF phase versus the gas and liquid phases. Additionally, an SF is highly compressible near the critical point and thus the solvation power, through density changes, can be carefully controlled by subtle changes in the temperature and pressure, a potentially interesting application for particle formation techniques. The most useful region for SF applications is 1<T/Tc< 1.1 and 1<P/Pc<2 (63).

(a) Crystallization and milling   (b) Supercritical fluid process

**Fig. 1:** a) Illustration of traditional and complex method of microparticle formation and b) the reduced complexity of SF processing (adapted from (64))

Carbon dioxide is one of the most widely used SFs because of its relatively low critical temperature and pressure (Tc=31.1 °C and Pc=73.8 bars). In particular, the low critical temperature of CO₂ makes it attractive for processing heat-sensitive materials such as protein therapeutics. In addition, carbon dioxide (CO₂) is non-toxic, non-flammable and inexpensive, and has GRAS (‘generally regarded as safe’) status.
Table 2: Density, viscosity and diffusivity of gases, liquids and SFs

<table>
<thead>
<tr>
<th>Physical state</th>
<th>Density (g/ml)</th>
<th>Viscosity (g/cm s)</th>
<th>Diffusivity (cm²/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gases</td>
<td>$10^{-3}$</td>
<td>$10^{-4}$</td>
<td>$10^{-1}$</td>
</tr>
<tr>
<td>Liquids</td>
<td>1</td>
<td>$10^{-2}$</td>
<td>$10^{-6}$</td>
</tr>
<tr>
<td>SFs</td>
<td>0.2-0.9</td>
<td>$10^{-4}$</td>
<td>$10^{-3}$</td>
</tr>
</tbody>
</table>

1.4.1 Phase behaviour

It is important to consider the phase behaviour of different SF systems, because it helps in understanding crystallisation mechanisms. This would eventually assist in proper manipulation of processing/crystallisation conditions to generate particles/products with the desired properties. An excellent review of the effects of phase behaviour on the particle formation process in SFs has been presented recently (65). Relevant systems are also discussed below.

1.4.1.1 Single component system

The phase behaviour of a pure substance is shown in Fig 2a. A pure substance can exist in three different states (solid, liquid and gas) depending on the temperature and pressure. Equilibrium between the two different phases is indicated by saturation curves; the three phases are in equilibrium at the mixture triple point. The critical point (Tc/Pc) lies at the end of the vaporisation curve; the SF phase exists above this point. The density and viscosity of the SF vary with pressure and temperature and it is exemplified in Fig. 2b.

Fig. 2: a) Phase behavior of a pure substance; b) Density and viscosity variation of CO₂ with pressure at 310 K (65)
1.4.1.2 Binary systems

1.4.1.2.1 Solid-Fluid

SFs are remarkably good solvents for high molecular weight, low vapour pressure solids. The solvent capacity of an SF can be varied over a wide range by altering pressure and/or temperature. In general, the solubility of an organic substance increases with pressure at all temperatures. The effect of temperature at constant pressure is a different situation, because of the vapour pressure changes of the solute. For example, the solubility of progesterone and methoxy progesterone acetate increases with pressure at 313 K, whilst at constant temperature the solubility decreases until 205-240 bars (crossover region) and then increases (66). The solubility of solids is increased by the addition of organic solvents to the SF (resulting in a modified SF) (67, 68). Various reviews related to the solubility of various compounds in pure and modified SF have been presented (69).

1.4.1.2.2 Liquid-Vapor/SF

Various types of liquid-vapor phase behaviors are extensively discussed in the book edited by McHugh and Krukonis (70). Figure 3a shows the simple type I phase behavior of a carbon dioxide-methanol binary vapor system (71). The binodal curve represents the boundaries of the vapor-liquid equilibrium. The solubility of CO\textsubscript{2} increases with pressure whilst the solubility of methanol remains more or less constant. The saturated liquid and vapour curves meet at the mixture critical point; above the critical point, the two fluids are completely miscible. In the miscibility region, a mixture with a composition corresponding to A is rich in ethanol and that corresponding to B is rich in CO\textsubscript{2} (Fig. 3a).

![Fig. 3](image-url)

**Fig. 3.** a) Binary vapor-liquid equilibrium of methanol-CO\textsubscript{2} (adapted from (71)); b) Ternary phase diagram for water-isopropanol-CO\textsubscript{2}. The lines in the diagram indicate tie lines (adapted from (72)).
1.4.1.3 Ternary system

The temperature and pressure should be kept constant to describe this system, as there are three degrees of freedom in a two-phase ternary system.

1.4.1.3.1 Water-solvent-vapor/SF

A more comprehensive description of various types of phase behaviour with water is also available (70). For example, a typical three-component system of CO₂-isopropanol-water determined at 60 °C and 103.4 bars is shown in figure 3b. The phase diagram shows that the SC-CO₂ is miscible in all most all proportions with isopropanol but is not soluble in water. The tie lines for a ternary system indicate that a liquid phase (the mixture of isopropanol and water in this example) is in equilibrium with a fluid phase (the mixture of SC-CO₂ and isopropanol). The point where all three fluids are miscible is referred to as the mixture critical point. A mixture in the liquid (L) region is rich in isopropanol + water and one in the vapour (V) region is rich in carbon dioxide (Fig. 3b).

1.4.1.3.2 Solute-organic solvent-vapor/SF

In this kind of system, the number of coexisting phases depends on the temperature, pressure and composition of the phases. Such systems are more relevant to SF crystallisation methods. A simple description of phase behaviour in such a ternary system has been reviewed by Palakodaty and York (65).

1.5 Particle preparation/crystallisation using SF techniques

The use of SFs to achieve precipitation was observed more than a century ago by Hanny and Hogarth (73). Following this, SF processing for particle formation has been studied for a variety of compounds (74). There have been significant efforts subsequently directed towards the processing of pharmaceuticals using SFs, using several methodologies. The principles of these technologies are presented below.

1.5.1 Rapid Expansion of Supercritical Solutions (RESS)

RESS, which is outlined in Fig. 4a, is a relatively simple, straightforward technique wherein an SF or a modified SF containing the solute is subjected to rapid expansion over the nozzle by reducing the pressure. As a result, the solvent capacity of the SF is dramatically reduced, with eventual supersaturation and particle formation (75). In such processes, the characteristics of the material are dependent on the solubility of the solute in the SF, the nozzle geometry, the temperature and pressure, and the phase path of the binary system during expansion. The RESS technique has shown some promise in the processing of a variety of compounds (76-78) and in other drug delivery applications (79, 80). However, the prospects of this
system are limited by the poor/low solubility of many pharmaceuticals in SC-CO\textsubscript{2}.

1.5.2 Gas Anti-Solvent (GAS)

The principle of this process is similar to that of conventional liquid antisolvent crystallisation. Volume expansion of the organic solvent occurs due to its high miscibility with the SF, leading to a reduction in density and a simultaneous fall in solvent capacity (81). This increases levels of supersaturation, solute nucleation and particle formation. This process, generally termed GAS recrystallisation, is suitable for crystallisation of slightly soluble/insoluble substances in an SF from organic solutions. A schematic diagram of the GAS process is shown in Fig 4b. The GAS process is typically operated as a batch process; factors influencing the particle characteristics include the solvent to SF ratio, the rate of addition of the SF, the degree of expansion of the solvent in the SF and the phase behaviour during particle formation. The GAS process has undergone major modifications in recent years, primarily as a result of the apparent increase in the temperature of the system due to the addition of compressed gas to the solvent or solution, and the requirement for additional washing or downstream processing of the product as the particles are formed in the liquid phase.

1.5.3 Supercritical Anti-Solvent (SAS)

In this process, a solution of a drug dissolved in an organic solvent is sprayed through a capillary tube into a continual stream of SC-CO\textsubscript{2} (Fig. 4c). This technique has been employed in the preparation of protein particles (82) and controlled release drug delivery systems (83).

1.5.4 Aerosol Solvent Extraction System (ASES)

This process works on the GAS principle and is a type of SAS process (84). In this process, the drug or polymer solution is sprayed into bulk SC-CO\textsubscript{2} for a period of time (see Fig. 4c). The final step involves the extraction of residual solvents by flushing with SC-CO\textsubscript{2}. ASES has been used in the preparation of microparticles of small molecular drugs (85) and proteins (86).

1.5.5 Precipitation with Compressed Antisolvent (PCA)

The PCA process, which is based on the antisolvent principle, also shares similarities with ASES (Fig. 4c). The first generation apparatus comprised a single capillary nozzle for dispersing the solution. However, later developments introduced a co-axial nozzle (87). Although the process has
shown improved mass transfer rates, the low level of control in achieving the required high degree of mixing is still a concern.

1.5.6 Precipitation from Gas-Saturated Solution (PGSS)

In this process, a molten form of the drug or polymer is dissolved in an SF under pressure and the solution is expanded over the nozzle. The SF is extracted, resulting in the formation of dry particles. The technique has been used in the co-precipitation of drugs with polymers (88). However, the high temperatures necessary to melt the compounds limit its application in the pharmaceutical industry.

![Fig. 4: Schematics of the a) RESS, b) GAS and c) SAS/ASES/PCA processes (89)](image-url)
In all the spray processes mentioned above (SAS, ASES and PCA), the droplet size depends on the nozzle diameter and the relative flow rates of solution and SF. The droplet generation is often represented by the Weber number, $N_{\text{we}}$, i.e. the ratio of inertial force to the surface tension force, which is given by eq. 1 (90).

$$N_{\text{we}} = \frac{\rho v^2 D}{\sigma}$$

(1)

where $\rho$ is the density of anti-solvent, $v$ is the relative velocity, $D$ is the jet diameter, and $\sigma$ is the interfacial tension. The higher the Weber number, the smaller the droplet size and vice versa. The interfacial tension becomes negligible under supercritical conditions where the solvent and SF are miscible. This indicates the implications of phase behaviour on the droplet formation and particle size. Thus, in the miscible region, where the droplets are extremely small, the rate of particle formation is determined by the mass transfer of the SF into the droplet, whereas particle agglomeration and aggregation phenomena are affected by the rate of solvent mass transfer into the SF from the droplet. The former mass transfer is dependent upon atomisation efficiency and the latter on dispersion and mixing characteristics between the solution droplet and the SF (65). The process path of droplet formation, nucleation and evaporation are represented in Fig. 5. Therefore, to reduce particle agglomeration and to eliminate residual solvents, enhanced mass-transfer rates are required (91). This has been achieved in the solution enhanced dispersion by supercritical fluids (SEDS) process (92, 93).

![Fig. 5: Schematic representation of I) Droplet formation; II) Nucleation and III) Solvent evaporation (adapted from (65)).](image)

1.5.7 Solution Enhanced Dispersion by Supercritical Fluids (SEDS)

The SEDS process, works on the antisolvent principle and uses a co-axial nozzle design with a mixing chamber, which facilitates perfect mixing of drug solution and SF prior to dispersion in the particle formation vessel (92, 93). In this way, high mass transfer rates are achieved with a high ratio of SF to solvent, and high velocities of the SF facilitates breakup of the solution (94, 95). This makes the SEDS process a highly controlled, reproducible and
scalable process compared to other antisolvent-based SF processes (96, 97). SEDS process, illustrated in Figure 6a, has been successfully employed in various applications including micronisation of drugs, controlling polymorph formation, chiral separations, preparation of microspheres and solid dispersions and processing of polymers and proteins (98-106). SEDS has been further developed to process water-soluble compounds, including therapeutic proteins, by introducing an organic solvent, an SF and an aqueous drug solution as separate streams into a 3-component nozzle. In a recent development of the SEDS process, a T-piece has been introduced to modify the SC-CO₂ by mixing it with a suitable solvent prior to its reaching the 2-way nozzle resulting in a homogeneous mixture (Fig. 6b). These processes (with 3-way nozzle or T-piece arrangement) have been implemented in the processing of lactose and therapeutic proteins (107-109). Though considerable advances have been made in the processing of materials using SEDS, further research and development is essential to improve the current understanding of the process. This would ultimately help in predicting and designing ‘smart particles’ effectively for drug delivery applications and in exploring new possibilities/opportunities for SF technologies in the pharmaceutical industry.
Fig. 6: Schematic diagrams of the a) Conventional and b) Modified SEDS apparatus. A: CO₂ Cylinder, B: Cooler, D: Particle formation vessel, E: Nozzle, F: Drug or Protein feed solution, G: Back pressure regulator, H: Oven, I: Solvent modifier (isopropanol), P: Pumps, C- High capacity pump for liquid CO₂, K₁- Drug/protein feed solution pump, K₂- Solvent modifier pump; T: T-piece
2 AIMS OF THE THESIS

The main aim of the thesis was to explore various applications of SF antisolvent technology, and SEDS in particular, in the preparation of drug particles for administration by pulmonary route. The general mechanisms of particle formation during the SEDS process were also of interest. The specific objectives were:

- to investigate the applicability of the SEDS technique for the formation of hydrocortisone (HC) particles and to determine the influence of SEDS processing conditions (e.g. pressure, temperature, solvents and flow rates) on the physicochemical properties of the particles such as their thermodynamic properties, morphology and in vitro aerosol deposition capabilities. (Paper I)

- to crystallise model drugs budesonide and flunisolide using the SEDS technique and to investigate the formation of a pure crystalline phase and manipulate particle size and morphology by varying the processing conditions. The applicability of VTXRD in studying solid state phase transitions was also explored. (Paper II)

- to determine the chemical purity and thermodynamic stability of different polymorphs of flunisolide (including the new forms III and IV discovered in paper II) crystallised using the SEDS technique and to investigate the suitability of budesonide and flunisolide powders produced by SEDS for administration by inhalation. (Paper III)

- to investigate the feasibility of preparing stable powders of recombinant human growth hormone (hGH) from aqueous solutions using conventional and modified SEDS processes. The study included extensive physicochemical characterisation of hGH powders precipitated using both SEDS processes and was devised to develop an alternative method of precipitating dry powders of therapeutic proteins from aqueous solutions using SF technology (Paper IV)
3 MATERIALS AND METHODS

3.1 Materials
HC (paper I) was purchased from Sigma Chemical Co., USA. Micronised budesonide and flunisolide anhydride and hemihydrate (99% purity), used in papers II and III, were gift samples from AstraZeneca, Sweden, and Alco Chemicals Ltd., Switzerland, respectively. The aqueous solution of hGH, stabilised with phosphate buffer, (paper IV) was supplied by Pfizer, Stockholm, Sweden. Methanol, acetone, acetonitrile and isopropanol of analytical grade were purchased from Merck, Germany. Copper chloride, CuCl₂·2H₂O, and tetrahydrofuran (purity >98%) were from KEBO Lab AB, Sweden. Methylparaben (purity >99%), Hydranal®-composite 2 and Hydranal®-solvent were from Sigma-Aldrich Sweden AB, Sweden. Sucrose (purity >99.5%) was obtained from Sigma Aldrich, Germany. Quenched (amorphous) sucrose was obtained by melt quenching of raw material of sucrose in liquid nitrogen. Carbon dioxide (CO₂) of high purity (99.9 %) was obtained from AGA Gas AB, Sweden. Milli-Q-water was used in all the preparations. Empty Turbuhaler® and Easyhaler® inhalers were gifts from AstraZeneca, Sweden, and Orion Pharma, Finland, respectively. All chemicals were used without further purification.

3.2 Solvent crystallisation
HC was recrystallised from various solvents for comparison with the HC particles prepared by the SEDS method (paper I). Recrystallisation involved 20 ml of almost saturated drug solutions in acetone, methanol and chloroform. 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.

3.3 General description of the SEDS processes

3.3.1 Conventional process
A schematic diagram of the conventional SEDS process, used in all papers, and a cross section of the nozzle are shown in Fig. 6a. Briefly, a suitable anti-solvent gas, in this case CO₂, is fed from the source (A) to a cooler (B) to ensure the liquefaction of the gas and to prevent cavitation. The CO₂ is then fed through a conduit from the cooler to a high-pressure pump, C (Thar Design, USA). From there, it is pumped to the high-pressure vessel (D) via a
nozzle (E). The solution containing drug is drawn from the source (F) by a conduit to the high-pressure pump, \( K_1 \) (Jasco PU-980, Japan) and is fed to the high-pressure vessel (D) via the nozzle (E) (nozzle opening is 0.20 mm). For the protein powder preparations, various proportions of isopropanol were added to the aqueous source solutions (F) to allow the extraction of solvents into SC-CO\(_2\). The supercritical CO\(_2\) leaves the high-pressure vessel and flows to the backpressure regulator, \( G \) (Jasco 880-81, Japan), which controls the pressure discharge in the system. The organic solvent is extracted into the supercritical fluid, resulting in the formation of particles in the vessel (D). A more explicit description of the equipment and operation procedure has been published elsewhere (93).

3.3.2 Modified process

This method was used only in paper IV. The minor change to the standard SEDS method involved mixing the co-solvent (isopropanol) with the SC-CO\(_2\) via a specially made T-piece prior to it entering the co-axial 2-way nozzle (which had a diameter of 0.35 mm). This created a single-phase homogeneous mixture of SC-CO\(_2\) and isopropanol. The water from the hGH solution was then extracted into the modified SC-CO\(_2\), resulting in the formation of protein particles in the vessel. A diagram of the apparatus showing the T-piece is shown in Fig. 6b.

3.4 Drug particle preparation (Corticosteroids)

The conventional SEDS technique was used in the preparation/crystallisation of particles of HC (Paper I), budesonide and flunisolide (Papers II and III). Solvents for all preparations in the study were acetone and methanol. The concentrations of HC were 9.0 mg/ml in acetone and 6.0 mg/ml in methanol (both nearly saturated solutions); the concentration of both budesonide and flunisolide was 2.5 mg/ml in both solvents. The pressure ranged from 90 to 200 bars and the temperature ranged from 40 to 90 °C (Papers I, II and III). For the HC particles, the flow rates of CO\(_2\) and drug solution were 21 ml/min and 0.1 ml/min for all combinations of pressure and temperature, except at 130 bar and 40°C, where varying flow rates were studied (paper I). For the budesonide and flunisolide crystallisations, the flow rates of CO\(_2\) and drug solution were 9 ml/min and 0.3 ml/min for all combinations of pressure and temperature. The flow rate of CO\(_2\) was varied between 9 ml/min and 25 ml/min at a pressure of 100 bars and temperature of 80°C to investigate the influence of this parameter on flunisolide particle formation. At the end of each experiment, the particles were flushed with CO\(_2\) at the respective flow rates for 15-20 minutes to remove any residual solvents. The particles are collected from the vessel and stored in a desiccator at room temperature until analysis.
3.5 Precipitation of therapeutic protein powders

The model therapeutic protein, hGH, was precipitated using both the standard and modified SEDS methods (Paper IV). Experiments were performed under various processing conditions (P: 100-200 bars and T: 40-50 °C), flow rates (for the CO₂ or isopropanol-CO₂ and protein solution) and solution compositions. At the end of each experiment, the precipitated powders were flushed with SC-CO₂ for 60 min at 25 ml/min in the conventional SEDS method and, in the modified SEDS method, for 10-12 min with the modified SF-CO₂, and again later with only SF-CO₂ for 60 min to remove residual water and the solvent. All samples were weighed and stored for 1-5 months at –20 °C before analysis.

3.6 Solid-state characterisation

3.6.1 Differential Scanning Calorimetry (DSC)

A Differential Scanning Calorimeter (DSC 220C, Seiko, Japan) was used to determine the thermal behaviour of HC, budesonide and flunisolide before and after SEDS crystallisation (Papers I and II) and flunisolide polymorphs before and after storage for different time periods (Paper III). The thermal behaviour of hGH powders was also determined (Paper IV). About 1.5-2.5 mg of drug/hGH samples were placed in aluminum pans. The pans were hermetically sealed and 2-3 holes were made on the pan cover. The hGH powders were ramped at 10 °C before the scan. The samples were scanned at a rate of 10 °C/min. Three measurements were made from each batch and reproducibility was verified using 2-3 batches.

3.6.2 X-Ray Powder Diffraction (XRPD)

Powder X-ray diffraction spectra were obtained using a Guinier–Hägg focusing powder camera (Paper I) and Siemens diffractometers D5005 (paper II) and D5000 (paper IV) operated with Cu radiation (45kV, 40 mA). Silicon powder (a = 5.431023 Å) was an internal standard in the focusing camera. The Instruments (D5000 and D5005) were operated in step scan mode in the angular range 5 - 40° 2θ.

3.6.3 Variable Temperature X-Ray Diffraction (VTXRD)

In an effort to identify the transitions indicated by DSC, the flunisolide samples in paper II were subjected to a controlled temperature programme, and X-ray powder patterns were obtained as a function of temperature. The X-Ray Diffraction (XRD) patterns were obtained by exposing the sample to CuKα radiation (45 kV 40 mA) in a wide-angle powder X-ray diffractometer (Model XDS 2000, Scintag). During the experiment, the samples were
maintained under isothermal conditions at the selected temperatures. The scan angle range was 5-40° 2θ. The range of temperature controller (Micristar, Model 828D, Hansen) was 190 °C to +300 °C. The sample was heated at a continuous rate of 10 °C/min.

3.6.4 Solubility determination
Excess drug was suspended in water in 20 ml plastic scintillating vials. These containers were kept in a thermostat maintained at 25 °C and the suspension was stirred at a constant rate. Samples were withdrawn with a syringe fitted with a 0.22 µm membrane filter after being centrifuged for 10 min at 3000 rpm. The samples of HC were taken at regular intervals over a period of 36 hrs (paper I) and samples of flunisolide were taken every 3 hrs for 24 hrs (paper II). Samples were analysed using a spectrophotometer and the average of three measurements from three vials was calculated. The solubility of three different batches was determined. The drug concentration in each sample vial was determined from the standard graph.

3.6.5 Scanning Electron Microscopy (SEM)
The size and shape of the precipitated particles (Papers I-IV) were examined using a Scanning Electron Microscope (LEO 1530, Japan) at magnifications of 5-50 K. Small amounts of the powder were loaded on to the glue tape and the excess power was blown away using a mild air stream. The particles were coated with gold-palladium (JFC – 100, Ion Sputter, Jeol, Japan) in an argon atmosphere at room temperature before examination.

3.6.6 Chemical purity
The HPLC method used was that provided by the supplier but with a different column type and an increased sampling period (paper III). The HPLC (LC-CaDL 22-14, Bischoff Chromatography, Germany) analysis was carried out to determine the chemical purity of samples freshly prepared using SEDS methodology and after storage during the stability studies. The column was a Prontosil Eurobond C18-column with packing material (particle size of 5µm) and length 4.0x125 mm (Bischoff Chromatography, Germany). The mobile phase consisted of water/acetonitrile/tetrahydrofuran in a ratio of 73:15.4:11.6. The mobile phase was run at a constant flow rate of 0.8 ml/min and the sample injection volume was 10 µl. The sampling period was 60 min, and the detector device employed was a UV-diode-array-detector operated at 254 nm. The internal standard consisted of methylparaben (0.04 mg/ml) in acetonitrile/water (1:1). The purity of the samples was estimated from the relative ratios of areas under the curve for flunisolide and internal standard, divided by the concentration of flunisolide
3.6.7 Stability studies
The thermodynamic stability of polymorphs II, III and IV and the hemihydrate of flunisolide were determined (paper III). Approximately 10-12 mg of each sample was placed on aluminium plates and maintained at 50 °C and 70±2% Relative Humidity (RH) in a desiccator. These conditions were chosen to stress the samples. The required RH was obtained from a saturated solution of copper chloride. Approximately 2 mg of the sample was withdrawn at various time intervals over one month and the thermal behaviour and chemical purity were determined using DSC and HPLC, respectively.

3.6.8 Particle size distribution
The particle size distribution of various powders of budesonide and flunisolide was determined with a Coulter Counter (Coulter Multisizer II) (paper III). An electrolyte, containing 0.9 % (w/v) NaCl and 0.01 % (w/v) Tween 80, was saturated with budesonide or flunisolide. The electrolyte was filtered through a pre-filter and a 0.22 µm membrane filter. The particles were added and samples were sonicated for 3 x 2 seconds with a Vibra Cell™ VCX600 (Sonics & Materials Inc.) to disperse aggregates. Median, arithmetic and geometric mean values were obtained from the Coulter software (Multisizer AccuComp® 1.19). Inter batch variation of the SEDS powders was checked using three batches.

3.6.9 Atomic Force Microscopy (AFM)
The samples in paper III were scanned using PicoSPM AFM (Molecular Imaging, AZ, USA) in acoustic AC (tapping) mode to investigate the surface nature of the particles. A 30 µm scanner and commercially available Si3N4 cantilevers (NCL pointprones, NanosensorsAG, Germany) were used. The particles were dispersed over an adhesive tape that was glued to a glass slide. Groups of particles were located and scanned at room temperature to provide topographic images.

3.6.10 Density measurements
The density of the budesonide and flunisolide powders was measured using a helium pycnometer (Accupyc 1330) with a 3.5 cm³ sample cup (paper III). Mean values and standard deviations were obtained using 10 measurements for every sample. These values were used for calculating theoretical aerodynamic diameter of the particles.
3.6.11 In vitro drug deposition studies

To determine the aerodynamic properties of the powders, a Multi-state Liquid Impinger (MLI), Astra Type, which is referred to as apparatus C for dry powder inhalers in the European Pharmacopoeia (EP) was used (Papers I and III). The apparatus was assembled with a low resistance filter (stage 5) and was connected to the flow system as described in the EP. The Turbuhaler (Astra-Zeneca) and Easyhaler (Orion Pharma) were used in paper I and the Turbuhaler (Astra-Zeneca) was used in paper III. The pump was programmed to pass 4 liters of air, at a speed of 51 L/min (paper I) and 60 L/min (paper III) for the Turbuhaler and 43 L/min for the Easyhaler (paper I), in order to simulate human inspiration. The resulting cutoff diameters ($\mu$m) were: stage 2 = 14.10, stage 3 = 7.38, stage 4 = 3.36 and the filter stage = 1.84 at 51 L/min for the Turbuhaler (paper I); stage 2 = 15.36, stage 3 = 8.03, stage 4 = 3.66, and the filter stage = 2.01 at 43 L/min for the Easyhaler (paper I); stage 3 = 6.8, stage 4 = 3.1 and the filter stage = 1.7 at 60 L/min for the Turbuhaler (paper III).

Methanol, solvent for the analysis, was filled into each of the four upper stages and stoppers were replaced. The apparatus was tilted to neutralise the electrostatic charges and to wet the stoppers. The end of the induction port was fitted with an appropriate mouthpiece adapter. Each sample was accurately weighed (2 mg) and loaded into the reservoir of the Turbuhaler/Easyhaler using a funnel made from aluminum foil. With the pump running and the two-way valve closed, the mouthpiece of the inhaler was inserted into the mouthpiece adapter of the MLI. The powder was discharged into the apparatus by opening the valve of the flow system.

The EP procedure was adapted for extracting the drug from different stages of the MLI. The drug concentration at each stage was determined by spectrophotometer. Each measurement was repeated three times to determine any variability in the method. Measurements were performed for powders from three different batches. The Mass Median Aerodynamic Diameter (MMAD) was determined using the IMP.XLT program (Swedish Medical Products Agency, Uppsala, Sweden). The total delivered dose was determined as the total amount of drug deposited from the throat piece to the filter stage of the MLI.

3.7 Bio-analytical characterisation (Paper IV)

3.7.1 Molecular aggregation of hGH

In order to study the effect of isopropanol on the molecular aggregation of hGH in the liquid and frozen states, various solutions of hGH in isopropanol were prepared. In some cases sucrose was also added to study its stabilising effect on isopropanol-induced hGH aggregation. Isopropanol was shaken
until completely miscible with water for injection (WFI) to form solutions of varying concentration. Sucrose, when used, was dissolved in WFI before the addition of isopropanol. A measured amount of hGH source solution (bulk) was slowly added to this solution to reach a final hGH concentration of 2% w/v. The solutions were gently rotated 5 times. All samples were analysed by SEC in order to assess the proportions of hGH monomer, dimer and polymer structures.

3.7.2 Dissolution of hGH from powders
Spectrophotometry was used to determine the content of hGH dissolved from the precipitated powders into water. Water (1-2 ml) was added to weighed amounts of protein powders. The samples were sonicated for 2 min to disperse agglomerates and then shaken gently on a shake board. After a total shaking time of 2 hours, the samples were centrifuged at 5000 rpm for 5-10 min. The supernatant was then separated and filtered through a 0.22 μm sterile filter (Millex-GV filter unit, Millipore Corp, USA). This supernatant was then analysed using SEC to determine the degree of aggregation in WFI. The concentration of soluble hGH was determined using a Lambda 2 UV/VIS spectrophotometer (Perkin-Elmer, Sweden) at 276 nm, corrected for light scattering at 320 and 340 nm. A specific absorbance of 0.79 (1 mg/ml, 1 cm) was used. The solutions obtained here were used in later analyses as described in the following sections.

3.7.3 Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)
This method was used to determine whether the undissolved solid that remained after 2h shaking and filtering contained protein, and also to investigate whether the protein was in monomer form or had been irreversibly aggregated. After separating the supernatants, which were used in SEC and spectrophotometry experiments, the remaining solids were dissolved in 0.2 ml 10% sodium dodecyl sulfate (SDS) solution. Thus, any protein present was denatured by SDS to yield negatively charged complexes of protein-SDS. The theoretical amount of hGH in the undissolved solid was calculated from the total amount of protein originally added to the feed solution minus the amount of dissolved hGH in WFI after 2 hours’ shaking (as measured in the previous section). After diluting the protein-SDS solutions to a theoretical protein concentration of 1.3 mg/ml, the disulfide bonds in the protein were reduced with β-mercaptoethanol. The silver-stained SDS-PAGE method was performed in tris-glycine buffer according to Laemmli (110).
3.7.4 Reversed Phase High Performance Liquid Chromatography (RP-HPLC)

The effect of the processing on the chemical stability and degradation of hGH (i.e. oxidation and deamidation) was studied using RP-HPLC. The aqueous hGH solutions obtained above were analysed using an HP 1090 (Hewlett Packard, Agilent technologies, Sweden) equipped with a polyvinyl alcohol-based Asahipak ODP50 column (250×4 mm, Hewlett Packard, RPC-134), equilibrated with 28% v/v 1-propanolol in 35.5 mM Tris-HCl buffer at pH 8.5 (mobile phase). The injection volume of hGH (1.3 mg/ml) was 30×1 μl and the flow-rate was 0.5 ml/min at 55 °C. UV detection was carried out at 214 nm. The amounts of each degradation product and unchanged (intact) hGH were calculated by integrating the respective areas in the chromatogram.

3.7.5 Size Exclusion Chromatography (SEC)

The molecular size distribution of hGH was determined by SEC using an HPLC 1100-3 (Hewlett Packard, Agilent technologies, Sweden). The supernatant (i.e. soluble) portion of the hGH solutions from section 3.7.2 were diluted to 0.5-1.3 mg/ml and specific volumes of these solutions were injected into a Superdex 75 HR 10×30 column (with an inlet filter of 2 μm). The mobile phase [50 mM sodium dihydrogen phosphate (NaH₂PO₄), 50 mM di-sodium hydrogen phosphate (Na₂HPO₄), 100 mM sodium chloride and 5.0% 2-propanol at pH 6.7] was pumped at a flow rate of 0.8 ml/min. Protein concentration was measured at 214 nm. The concentration of hGH was measured by comparing the area response of the sample with that of reference standards. The proportions of monomer, dimer and polymer structures were determined relative to the total integrated areas under the elution curves.

3.7.6 Residual moisture analysis

Moisture analysis was performed on a Karl Fischer Automatic Titrator (Metrohm 787 Titriso, Metrohm, Switzerland). Accurately weighed samples of hGH were transferred into the solvent vessel and titrated with the reagent. At the end point, the moisture content was automatically calculated from the titre volume and the volume of reagent consumed. Each measurement was repeated three times and the residual moisture content was measured for three batches of powders.
4 RESULTS AND DISCUSSION

4.1 Preparation of hydrocortisone particles (Paper I)

The anti-inflammatory drug HC was used as a model substance since many corticosteroid drugs are administered as inhalation powders. HC has been reported to exist in two polymorphic forms (I and II), as well as methanol and chloroform solvates; however, the definitive existence of polymorphic form II has yet to be confirmed (111).

4.1.1 SEDS crystallisation

SEDS crystallisations were performed using two solvents of different polarities at varied CO₂ densities and at different flow rates (Table 3). The solubility of HC was increased with the increased density of SC-CO₂, an observation that was confirmed by the decreased yields and the presence of HC in the CO₂ that was collected at the backpressure regulator.

4.1.2 Solid state characterisation

Solid-state characterisation of HC particles was carried out using DSC, XRPD and SEM. Dissolution studies were also performed. The initial unprocessed HC comprising irregular chunks which melted at 224±0.3 °C was identified as polymorph I (112). Conventional crystallisations from acetone and methanol also resulted in polymorph I; a solvate resulted from chloroform. Particles from different solvents showed distinctly different morphologies, from large chunks to blades of various sizes.

The crystals prepared from acetone using SEDS had a melting temperature of 222±2 °C, whereas crystals from methanol melted at 216±2 °C (Table 3). DSC results (supported by XRPD data) indicated the occurrence of polymorph I in samples of powders from acetone solutions and suggested the presence of another polymorph in samples prepared from methanol solution. Although the initial dissolution rate of the powders prepared using SEDS was higher than that of unprocessed or conventionally prepared samples, their equilibrium solubility was similar. This result, however, did not rule out possible polymorphic modification of crystals prepared from methanol, as it is quite possible that a phase transition to the stable polymorph occurred in contact with water. The higher dissolution rate of samples prepared using SEDS was possibly due to the increased surface area of the particles.
Table 3: Processing conditions and melting points for HC particles prepared using SEDS process. ND = not determined

<table>
<thead>
<tr>
<th>Pressure (in bars)</th>
<th>Temperature (°C)</th>
<th>Relative flow rates of drug solution to CO₂</th>
<th>Melting point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td>40</td>
<td>0.0048</td>
<td>219.2(±1.0)</td>
</tr>
<tr>
<td>180</td>
<td>60</td>
<td>0.0048</td>
<td>220.0(±1.0)</td>
</tr>
<tr>
<td>130</td>
<td>40</td>
<td>0.0048</td>
<td>220.0(±1.4)</td>
</tr>
<tr>
<td>130</td>
<td>40</td>
<td>0.0020</td>
<td>221.2(±1.0)</td>
</tr>
<tr>
<td>130</td>
<td>40</td>
<td>0.0300</td>
<td>222.8(N.D)</td>
</tr>
<tr>
<td>130</td>
<td>90</td>
<td>0.0048</td>
<td>221.0(±1.0)</td>
</tr>
<tr>
<td>90</td>
<td>40</td>
<td>0.0048</td>
<td>221.0(±1.0)</td>
</tr>
<tr>
<td>90</td>
<td>80</td>
<td>0.0048</td>
<td>223.0(±1.0)</td>
</tr>
<tr>
<td><strong>Samples prepared from methanol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>40</td>
<td>0.0048</td>
<td>216.0(±1.0)</td>
</tr>
<tr>
<td>180</td>
<td>60</td>
<td>0.0048</td>
<td>215.0(±1.0)</td>
</tr>
<tr>
<td>130</td>
<td>40</td>
<td>0.0048</td>
<td>215.9(±1.0)</td>
</tr>
<tr>
<td>130</td>
<td>40</td>
<td>0.0020</td>
<td>215.9(N.D)</td>
</tr>
<tr>
<td>130</td>
<td>90</td>
<td>0.0048</td>
<td>217.8(N.D)</td>
</tr>
<tr>
<td>90</td>
<td>40</td>
<td>0.0048</td>
<td>216.5(±1.0)</td>
</tr>
<tr>
<td>90</td>
<td>60</td>
<td>0.0048</td>
<td>217.1(±1.0)</td>
</tr>
</tbody>
</table>

SEM images confirmed that HC particles produced using SEDS method were either needles or flakes (Fig. 7). It is interesting that varying the processing parameters (i.e. temperature and pressure) had no effect on particle morphology when acetone was used as a solvent, but had a measurable effect when methanol was used (Fig 7). This may be because the processing parameters affected the mass transfer rates of methanol, which subsequently eventually affected the supersaturation levels, and nucleation and particle formation. In addition, the change in the morphology or crystal nature of the particles was independent of the kinetic parameters of the process (i.e. relative flow rates of solution and SC-CO₂) for powders produced from both solvents. The increased solubility of HC in CO₂ at higher pressures resulted in a decreased nuclear density, and subsequent elongated needle-shaped crystals at these pressures (113).

4.1.3 In vitro deposition studies

The Multistage Liquid Impinger (MLI) study indicated that a higher percentage of the dose was delivered and there was lower adsorption of particles to the mouthpiece and throat piece for samples prepared at 90 bars and 40°C from acetone (90/40 Ace) or methanol (90/40 MeOH) solutions compared with unprocessed particles (Table 4). This was consistent with samples crystallised at higher pressures and temperatures (such as 180 bars and 60 °C) from acetone solution (180/60 Ace) (Table 4). These results
corroborate with earlier reports wherein elongated, fibrous particles have been found to show improved deposition in the lung (114-116). This was possible because of the low inter-particulate and particle-surface interactions for SEDS particles of HC and the choice of a suitable size distribution of the particles for inhalation (117). Furthermore, SEDS processing parameters influenced the deposition of drug particles in MLI, since the delivered dose of the sample prepared at 130/40 from acetone was less than that for samples prepared under other conditions (Table 4). The Turbuhaler, which has a long flow path incorporating spiral channels which promotes the deaggregation and dispersion of long needle-shaped particles, resulted in greater uniformity and intersample consistency than seen with the Easyhaler (118).

In summary, SEDS crystallisation of HC from acetone resulted in the polymorphic form I, irrespective of the processing conditions. However, when methanol was used as a solvent, a material with a lower melting point, possibly polymorph II, was produced. The processing conditions (pressure and temperatures) influenced the morphology of the particles. Thus, the choice of solvent influenced the crystal energy of the material when it was processed using SEDS. Change in the relative flow rates of the solution and the CO₂ caused no substantial variations in the characteristics of the resultant crystals, which explains the kinetic independence of the process at a particular processing parameters. Particles prepared using SEDS methods showed better drug deposition characteristics in the lower stages of the MLI; the deposition characteristics were influenced by the processing conditions.

It is motivating from these results that the investigation of the effects of SEDS processing parameters and solvents on particle characteristics for structurally similar drugs for better understanding of particle formation. These findings also indicated that the SEDS process may be useful for the controlled crystallisation of a pure polymorph and for the identification of new polymorphs.
Fig 7: SEM pictures of SEDS processed HC samples at a) 180/60 MeOH, b) 180/40 MeOH; c) 130/40 MeOH; d) 90/60 MeOH

Table 4: The percentage deposition of unprocessed and SEDS processed HC in different stages of the impinger when Turbuhaler was used as Inhaler. Figures in brackets indicate Standard deviation of three MLI measurements

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

*Total dose is delivered dose plus amount of drug adhered to mouth piece; *Delivery dose is the amount of drug that comes into the Impinger
4.2 Crystallisation of budesonide and flunisolide (Papers II)

Budesonide, a corticosteroid with a high affinity for glucocorticoid receptors, has significant topical anti-inflammatory activity and a low propensity for systemic effects and is widely used in the treatment of asthma by pulmonary delivery (119). Anhydrous flunisolide is also a glucocorticoid given via nasal spray for the prophylaxis and treatment of allergic rhinitis, and via metered aerosol in the management of asthma. It has been reported that flunisolide exists in three different crystalline forms, designated form I, the hemihydrate and form II (120). The hemihydrate and form II are thermodynamically stable at room temperature; the hemihydrate is the more stable of the two (120). The possibility of forming pure polymorphs of flunisolide using traditional recrystallisation methods from different organic solvents is limited. Our results indicate the existence of two more polymorphs of flunisolide: forms III and IV.

4.2.1 Budesonide

4.2.1.1 SEDS crystallisation

At high pressures (e.g. 200 bars) and low temperatures (e.g. 40 °C) it was not possible to obtain crystals of budesonide from either acetone or methanol. Under these conditions, the solubility of the solute in SC-CO2 was greater than the simultaneously increased solubility of the solvent in SC-CO2, which would otherwise have increased both the rate of extraction of SF and thus the precipitation of the drug.

4.2.1.2 Solid-state characterization

The samples of budesonide crystallised from acetone and methanol solutions using SEDS technique under various conditions showed identical thermal behaviour to that of the stable form of micronised material. This implies that budesonide was crystallised in a stable crystalline form, and was less susceptible to changes in either the choice of solvent or the conditions in this range of processing parameters.

SEM examination revealed that the micronised powders of budesonide were aggregated (Fig. 8a) whilst SEDS particles prepared at 100 bars and 80 °C from acetone solutions (100/80Ace) were smooth, nearly spherical, discrete and with a size typically between 1-3 μm (Fig. 8b) and those from methanol solutions (100/80MeOH) were plate-like structures with a size distribution of 5-30 μm (Fig. 8c).
4.2.2 Flunisolide

4.2.2.1 SEDS crystallisation
As with budesonide, no flunisolide product was formed with either acetone or methanol at 200 bars. While flunisolide particles were produced at 100 bars, the product yield decreased as the temperature was lowered from 80 °C and was extremely small at 40 °C, in contrast to a complete lack of product in the case of budesonide at 40 °C. These findings and results from our earlier studies (HC and progesterone) support the hypothesis that the solubility of a drug in the mixture of SF and solvent is very important for successful crystallisation of the drug and will largely determine the nucleation phenomenon and particle characteristics.

4.2.2.2 Solid state characterization

4.2.2.2.1 Unprocessed/micronised flunisolide
The DSC profile of the micronised flunisolide (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. 9; lower panel). The exothermic peak was attributed to the recrystallisation of amorphous regions in the material, confirmed by the halo in the X-ray spectrum of the material taken at room temperature (Fig. 10; lower panel). The starting material of flunisolide comprised of irregularly shaped aggregated particles (Fig. 12a). The particles of flunisolide hemihydrate were large and cake-like (Fig. 12b).
4.2.2.2 Variable Temperature X-Ray Diffraction (VTXRD) studies

VTXRD spectra of micronised flunisolide are shown in Figure 11. The similarity of the X-ray spectrum at 30 °C to that at 130 °C indicates that the exothermic transition did not result in major phase conversions (Fig. 11). The endothermic transition in the DSC profile between 210 and 230 °C was attributed to the solid-solid transition of polymorphic form II to some other modification. This corroborates with the absence of characteristic peaks of form II in the X-ray diffraction pattern at 230 °C taken from VTXRD after isothermally holding the sample for 40 min (Fig 11). 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. 11). Similar findings were made when studied with VT-IR and FTIR coupled with thermomicroscopy, explaining the formation of an unstable intermediate form at around 230 °C which converts to form I at room temperature (120).

![DSC thermograms of flunisolide anhydrous polymorphic forms II, III and IV](image1)

![X-ray powder diffraction spectra of polymorphic modifications of flunisolide anhydrous (forms II, III and IV (reads from bottom to top)](image2)
4.2.2.2.3 *Fluniolide powders from acetone solutions*

At 100 bars, a decrease in the temperature from 80 °C to 60 °C or 40 °C resulted in the formation of a pure new polymorphic form III (Table 5). Based on DSC and XRPD profiles of the sample prepared at 100 bars and 80 °C (100/80), the material was identified as a mixture of more than two forms that contained I, III and an unidentified modification. The material crystallised 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, melting occurred at around 251.5 °C (Fig. 9; top profile). The distinct nature of the XRPD pattern of this material at room temperature combined with DSC results explained the existence of the new polymorphic form III (Fig. 10; middle panel). From the VTXRD data, the transitions observed in DSC thermograms were confirmed as true solid-solid transitions. The apparent solubility of this material was different from that of the other forms, further supporting the existence of the new polymorphic form III (Table 5). SEDS material crystallised at 100 bars and 40 °C (100/40 Ace) displayed similar thermal behaviour and a similar XRPD spectrum to that of form III, confirming its generation.

At 100/80 Ace, increasing the flow rate of CO₂ from 9 ml/min to 25 ml/min and keeping the acetone solution flow rate constant at 0.3 ml/min resulted in the formation of crystals that displayed similar thermal behaviour, morphology and XRPD patterns to those of the samples processed at 100/60, indicating the generation of polymorphic form III.

From SEM photographs, it was observed that the crystallisation of flunisolide from acetone solutions at 100/80 resulted in elongated blade-like needles (Fig. 12c), leading to partially spherical particles at 100/60 (Fig. 12d), and well faceted prisms at 100/40 (Fig. 12e). However, particles crystallised at higher flow rates were large and regularly shaped.

Fig. 11: Variable temperature XRD of flunisolide anhydrous form II at 30, 130, 230 and back at 30 °C. (Reads from bottom to top)
4.2.2.2.4  Flunisolide powders from methanol solutions

SEDS crystallisation of flunisolide was possible only between 60 °C and 80 °C at 100 bars at CO₂ and solution flow rates of 9 ml/min and 0.3 ml/min, respectively (Table 5). Thermal examination of the material precipitated at 100/80 and XRPD data suggested the generation of new polymorphic form IV (middle panel in fig. 9; top profile in fig. 10). VTXRD again proved to be a useful tool in confirming the solid-solid transition that form IV underwent before it melted. However, the apparent solubility of the material was not significantly different from that of form II (Table 5). For the powders crystallised at 60 °C, data from DSC, XRPD and VTXRD showed that the material was a mixture of forms III and IV. Interestingly, increased flow rates of CO₂ at 100/80 resulted in the formation of a mixture of forms III and IV, with a thermodynamic profile that was identical to that of the material at 100/60. The morphology of the particles (form IV and the III/IV mixture) from methanol solutions indicated elongated hexagonal plate-like structures (Fig.12f).

Table 5: Polymorphic modifications and apparent solubility in water of samples prepared using SEDS process

<table>
<thead>
<tr>
<th>Drug solution</th>
<th>Processing conditions bars/°C</th>
<th>Polymorphic modification</th>
<th>Apparent solubility (µg / ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>100/80 I+III</td>
<td>61.5 (0.5) † (1.0) ‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100/60 III ‡</td>
<td>51.5 (0.5) † (2.0) ‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100/40 III ‡</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>100/80 IV ‡</td>
<td>48.6 (0.4) † (1.0) ‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100/60 III+IV</td>
<td>47.4 (0.2) † (1.0) ‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100/40 NP</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Unprocessed material</td>
<td>___ II</td>
<td>45.9 (0.2) †</td>
<td></td>
</tr>
</tbody>
</table>

† new polymorphs. NP = no product; ND = not determined. † Standard deviation for the experiment (n=3). ‡ Standard deviation for three different batches of SEDS samples
The different crystal properties, morphology and/or size of the particles obtained in this study were possibly the result of variations in the mass transfer rates, the solubility of the drug in the SF and the mixing dynamics. The higher solubility or mass transfer rates of acetone compared to methanol in the SF would have influenced the saturation profile and subsequently the nucleation process and particle growth (113). However, the dominating factor influencing supersaturation profile and thus nucleation phenomenon appeared to be the solubility of the drug in SF. The increase in particle size with increased CO$_2$ flow rate, along with reduced nucleation density, was possibly also due to reduced residence time in the nozzle (94).

**Fig. 12:** SEM pictures of flunisolide a) Micronised (Form II), b) Flunisolide hemihydrate and samples crystallised using SEDS process c) 100/80Ace, polymorphic mixture; d) 100/60Ace, Form III; e) 100/40Ace, Form III; f) 100/80MeOH, Form IV
In summary, budesonide and anhydrous flunisolide were crystallised using SEDS process. The outcome (characteristics of the product) was dependent on the temperature and pressures employed. For both drugs, the nature of the solvent influenced the size and shape of the particles. Identification of two new polymorphs of anhydrous flunisolide (III and IV) validated this novel application of the SEDS process. Furthermore, it was possible to form pure phases of III and IV by the proper manipulations of the processing conditions. Once again, it appeared that the dominating factor controlling particle formation was the solubility of the drug in SC-CO$_2$, demonstrating the need for methods of measuring the dynamic solubility of drug substances in supercritical fluids as indicated by other researchers in the area (91, 121, 122). VTXRD was a powerful complementary tool for studying phase transitions while varying the temperature.

Though various approaches utilised in the study undoubtedly demonstrated the occurrence of two new polymorphs, it was necessary to verify any minor quantities of the chemically degraded products that might have influenced thermal behaviour and thus interpretations. Therefore, the chemical purity and thermodynamic stability of the different polymorphs of flunisolide, along with their applicability as dry powders for therapeutic use (via DPI), were the subjects of study in paper III.

4.3 Stability and aerodynamic behaviours of glucocorticoid powders (Paper III)

![Thermograms of flunisolide form IV showing transformations over 0, 1, 8, 21 and 39 days (reads from bottom to top) after storage at 50 °C and 70 % relative humidity](image)
4.3.1 Chemical purity
HPLC analysis indicated the absence of detectable quantities of impurities in the powders prepared using SEDS method, particularly in forms III and IV. The purity of the samples was ranked in the order form III and IV > form-II-hemihydrate. This excluded the influence of impurities on the thermal behaviour of the various polymorphs and thus verifies the interpretations made in paper II.

4.3.2 Stability studies
Polymorphic flunisolide forms II, III, IV and the hemihydrate were stored at 50 °C and 70% RH for more than a month. Interpretation of the thermal behaviour of the samples taken over this time period indicated that forms II, III and the hemihydrate did not undergo any transformations. However, form IV was transformed to the more stable hemihydrate form (Fig. 13). Based on the solubility data for the different polymorphs (Table 5) and the literature (120), we concluded that polymorph II and the hemihydrate are the most stable forms, whilst form I and III are less stable, and form IV is least stable under ambient conditions.

4.3.3 Particle size and surface nature
The mean particle size of powders of budesonide and flunisolide form III produced using SEDS methodology was similar or smaller than that of the micronised samples, with a narrow size distribution of 1-5 μm, suitable for inhalation delivery (Table 6). The reproducibility of the SEDS process was promising, as estimated from the standard deviations of the mean particle sizes from three batches. Atomic force microscopic examination of the particles indicated no substantial variations in the surface roughness of the particles.

4.3.4 In vitro drug deposition
The percentages of the drug dose that were delivered to the various stages of the MLI are shown in Figure 14. The mechanical agitation of the micronised samples (SEDS samples experienced such forces during handling) caused minor variation in the drug deposition pattern (Fig. 14a and 14b). The distribution of budesonide powders (produced by SEDS) in the MLI was similar to or better than that of the micronised budesonide powder (Fig. 14a and 14c). Flunisolide powders produced by SEDS, in a size range suitable for inhalation and similar to the size of the micronised particles, were preferentially deposited in the upper stages of the MLI (Fig. 14d and 14e). This implies that SEDS processing induced some aggregation in the flunisolide powders as a result of the formation of hydrophobic surfaces on the particles.
In summary, the thermodynamic stability of the different polymorphs of flunisolide was determined and was ranked as: IV<III<II=hemihydrate. SEDS is thus a promising technology for producing pure powders with particle characteristics suitable for inhalation. However, as in the case of flunisolide, some powders produced using SEDS process may be prone to aggregation as a result of the nature of the drug and the processing conditions.

Table 6: Volumetric particle size distribution of various samples of budesonide and flunisolide as measured by Coulter Counter

<table>
<thead>
<tr>
<th></th>
<th>Percent volumetric distribution of the powders&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;10</td>
</tr>
<tr>
<td><strong>Budesonide (size in µm)</strong></td>
<td></td>
</tr>
<tr>
<td>Micronised</td>
<td>4.5(0.1)</td>
</tr>
<tr>
<td>100/80Ace</td>
<td>4.2(0.0)</td>
</tr>
<tr>
<td>100/80MeOH</td>
<td>12.0(1.0)</td>
</tr>
<tr>
<td><strong>Flunisolide (size in µm)</strong></td>
<td></td>
</tr>
<tr>
<td>Micronised (Form II)</td>
<td>6.6(0.2)</td>
</tr>
<tr>
<td>100/60Ace (Form III)</td>
<td>3.7(0.0)</td>
</tr>
<tr>
<td>100/80MeOH (Form IV)</td>
<td>20.3(3.2)</td>
</tr>
</tbody>
</table>

<sup>4</sup>Calculation based on 100% volume distribution; >10 indicates that the 10% of the particles by volume are larger than the indicated size in the column; the standard deviation of the method is given in brackets, n= 3.

Fig. 14: Percentage of delivered dose of various powders of a) micronised budesonide; b) micronised budesonide, after shaking the sample; c) budesonide produced by SEDS (100/80Ace); d) flunisolide polymorph II; e) flunisolide polymorph III (SEDS)
4.4  Supercritical fluid processing of recombinant human growth hormone (Paper IV)

4.4.1  Precipitation of hGH powders

4.4.1.1  Conventional SEDS method
In this methodology, optimal processing pressure and temperature conditions were 100 bars and 40-50 °C (100/40-50) at a protein solution flow rate of 0.05-0.1 ml/min and hGH concentrations of 1 and 2% w/v. Isopropanol concentrations of 10-30% v/v were investigated and 20% v/v was essential (critical concentration) for hGH precipitation. The addition of sucrose (5 and 10% w/v) allowed precipitation without intermittent pressure rises in the system, even at low concentrations (10-20% v/v) of isopropanol. However, using this method, isopropanol was still required in the solution for precipitation and other strategies were implemented to precipitate the protein from aqueous solutions.

4.4.1.2  Modified SEDS process
In this method, isopropanol was mixed with SC-CO₂ to modify its solubility characteristics and facilitate the extraction of water. As a result, the time that the protein solution was in contact with the mixture of organic solvent and SC-CO₂ was minimised, in the hope of reducing the deleterious effects of the solvent on the protein (105, 106). Interestingly, the tendency for hGH to precipitate using this method was observed at higher pressures and lower temperatures (200/40) than with the standard method, at certain flow rates. A pressure and temperature of 100/50 and relative flow rates (CO₂/isopropanol/protein) of 25/1/0.1 were optimal for the precipitation of dry particles. Furthermore, the addition of sucrose instead of isopropanol at these conditions facilitated the precipitation of hGH from a pure aqueous solution, thus dramatically removing the adverse effects caused by solvents such as isopropanol in the modified SEDS process. Powders precipitated using this method (V1-V5) are presented in Table 7.
Table 7: Precipitation of hGH using the modified SEDS process. The flow rates of CO₂, isopropanol and hGH solutions were 25, 1 and 0.1 ml/min, respectively, in all cases.

<table>
<thead>
<tr>
<th>Sample</th>
<th>hGH (%)</th>
<th>Isopropanol (%)</th>
<th>Sucrose (%)</th>
<th>P/T (Bars/°C)</th>
<th>%Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>1</td>
<td>5</td>
<td>—</td>
<td>100/50</td>
<td>38 (6)</td>
</tr>
<tr>
<td>V2</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>100/50</td>
<td>47 (5)</td>
</tr>
<tr>
<td>V3</td>
<td>1</td>
<td>—</td>
<td>5</td>
<td>100/50</td>
<td>61 (10)</td>
</tr>
<tr>
<td>V4</td>
<td>1</td>
<td>—</td>
<td>5</td>
<td>100/40</td>
<td>24 (4)</td>
</tr>
<tr>
<td>V5</td>
<td>2</td>
<td>—</td>
<td>5</td>
<td>100/50</td>
<td>54 (2)</td>
</tr>
</tbody>
</table>

*Concentrations in the feed solution. *An approximate value calculated from the measured yield and the amount of material in the initial solution. Figures in brackets indicate standard deviations from 3 batches. P/T = pressure/temperature.

The ternary phase behaviour of water-isopropanol-CO₂ under the various processing conditions (pressure and temperature) had a profound influence on the precipitation mechanism. From the measured phase behaviour of the water-isopropanol-CO₂ system (72), at high pressures (200 bars), the mixture (water-isopropanol-CO₂) composition was in a heterogeneous vapour-liquid region in the binodal curve. Hence, supersaturation of the water-rich phase was never achieved, resulting in no or only partial precipitation of the protein. But at 100 bars and 50 °C, the critical point of the mixture exists in the CO₂-rich homogeneous phase, resulting in rapid, complete precipitation of the protein, possibly with less interaction with the solvent. On the other hand, at 40 °C, the positive slope of the tie lines indicates that the mixture at equilibrium is in a water-solvent-rich phase, resulting in poor and incomplete precipitation of the protein as a result of more interaction with the solvent.

4.4.2 Bio analytical characterisation

The results of the various bio-analytical investigations are presented in Tables 8, 9 and 10. The bulk hGH solution contained 45.3 mg/ml hGH as measured by spectrophotometry (Table 8). RP-HPLC analysis indicated that the bulk solution contained 98% native hGH, with 99.8% in the monomeric form (Tables 9 and 10). The presence of isopropanol in the hGH solutions did not cause either chemical degradation or physical aggregation at room temperature but resulted in severe aggregation when frozen.
Table 8: Amount of hGH dissolved from precipitated hGH powders. Mean values with standard deviations in brackets

<table>
<thead>
<tr>
<th>Samp les</th>
<th>Amount of hGH dissolved††(mg/ml)</th>
<th>Amount of hGH in the powder sample‡(mg/ml)</th>
<th>%Recovery</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>hGH</td>
<td>45.3</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>V1</td>
<td>0.1(0.0)</td>
<td>14.3(0.3)</td>
<td>0.9(0.0)</td>
<td>3</td>
</tr>
<tr>
<td>V2</td>
<td>2.9(0.6)</td>
<td>4.9(0.3)</td>
<td>63.2(13.2)</td>
<td>5</td>
</tr>
<tr>
<td>V3</td>
<td>3.7(0.6)</td>
<td>5.2(0.3)</td>
<td>71.2(6.8)</td>
<td>3</td>
</tr>
<tr>
<td>V4</td>
<td>1.2(0.7)</td>
<td>2.7(0.5)</td>
<td>51.8(31.8)</td>
<td>4</td>
</tr>
<tr>
<td>V5</td>
<td>1.9(0.8)</td>
<td>8.8(0.6)</td>
<td>9.7(1.8)</td>
<td>4</td>
</tr>
</tbody>
</table>

†bulk solution; ††Measured experimentally using spectrophotometry; ‡Amount of hGH in the powders is nothing but the initial amount of hGH assuming no loss of the protein during the SEDS process; n = number of batches; ND = not determined (low concentrations of hGH); %hGH dissolved or recovery = (amount dissolved/amount in the powder) x 100

Very small amounts of hGH were dissolved from precipitated powders regardless of the method, all of which contained isopropanol but not sucrose (such as sample V1, table 8). The hGH concentrations in the solutions obtained from these powders were too low to allow analysis by RP-HPLC and SEC (Tables 9 and 10). The amount of hGH dissolved from the precipitated powders increased with the addition of 5% sucrose to the feed solutions (Table 8). With a 2% hGH solution in the conventional SEDS process (i.e. a ratio of 2:5 for hGH:sucrose), 37-45% of the protein was dissolved and measured using spectrophotometry. However, with a 1% hGH solution in the modified SEDS process (ratio 1:5), 52-71% of the protein was dissolved (Table 8). The highest recovery (71%) was observed for V3. The presence of isopropanol with sample V2 and the drop in the processing temperature with sample V4 had only a minor influence on the proportion of protein recovered from the powders (Table 8). However, the percent recovery of hGH from sample V5, which contained a higher protein to sucrose ratio (2:5) than the other samples, was exceptionally low (Table 8). SDS-PAGE analysis of the insoluble solids obtained from powders precipitated using both methods with sucrose showed that 99.0% of undissolved hGH was in monomer form. It is suggested that the sucrose may limit protein aggregation during precipitation and assist dissolution by maintaining a high protein surface area and/or by diluting the protein in an amorphous hydrophilic matrix.
Table 9: Degradation of hGH, as measured by reverse-phase high performance liquid chromatography (RP-HPLC). The products of degradation are designated A, B and D. D was present in concentrations <0.2% for all samples and is not shown in the table. Mean values with standard deviations in brackets

<table>
<thead>
<tr>
<th>Samples</th>
<th>M(%)</th>
<th>A(%)</th>
<th>B(%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH†</td>
<td>98</td>
<td>0.3</td>
<td>1.7</td>
<td>1</td>
</tr>
<tr>
<td>V1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>3</td>
</tr>
<tr>
<td>V2</td>
<td>92.2(5.6)</td>
<td>2.3(0.5)</td>
<td>4.7(4.1)</td>
<td>5</td>
</tr>
<tr>
<td>V3</td>
<td>92.8(5.0)</td>
<td>2.9(2.2)</td>
<td>5.7(6.0)</td>
<td>4</td>
</tr>
<tr>
<td>V4</td>
<td>93.2(2.5)</td>
<td>1.7(0.4)</td>
<td>4.4(1.8)</td>
<td>4</td>
</tr>
<tr>
<td>V5</td>
<td>94.6(2.3)</td>
<td>1.1(0.2)</td>
<td>3.7(1.8)</td>
<td>3</td>
</tr>
</tbody>
</table>

bulk solution. M = proportion of native/intact hGH; n = number of batches; ND = not determined (low concentrations of hGH).

RP-HPLC data for powders containing sucrose (Table 9) indicated that the chemical degradation of hGH was low (i.e. 4-6%) compared with degradation of the bulk solution. Indeed, the method of precipitation, the protein:sucrose concentration ratios and the temperature did not have any considerable effect on the stability of the protein.

SEC analysis of the powders also indicated that the presence of sucrose reduced the risk of aggregation during precipitation (Table 10). This could be due to the protective nature of the sucrose against solvent/process stresses in the SEDS method (123). In powders prepared by the conventional SEDS method, the monomer content was 73-77%. The hGH:sucrose ratio and the processing temperature had no substantial influence on the monomer content in powders prepared by the conventional method. The monomer content in samples V2-V5 from the modified SEDS method was 52-91% (Table 10). The addition of sucrose to the solution of 5% v/v isopropanol (sample V2) minimised the detrimental effects of the solvent and the presence of only 5% sucrose in the solution (sample V3) further improved the monomer content (Table 10). The higher monomer content in samples V2 and V3 than in corresponding samples from the conventional process indicates an advantage associated with the modified SEDS process in that exposure of hGH to the solvents is minimised. Interestingly, decreasing the process temperature to 40 °C (sample V4) or the sucrose:protein ratio had a notable influence on the hGH aggregation behaviour (sample V5) (Table 10).
Table 10: Size exclusion chromatography (SEC) analysis of various hGH samples. Mean values with standard deviations for various batches in brackets

<table>
<thead>
<tr>
<th>Sample</th>
<th>Monomer(%)</th>
<th>Dimer(%)</th>
<th>Polymer(%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH†</td>
<td>99.8</td>
<td>0.2</td>
<td>&lt;0.1</td>
<td>1</td>
</tr>
<tr>
<td>V1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>3</td>
</tr>
<tr>
<td>V2</td>
<td>85.2(10.5)</td>
<td>9.4(6.0)</td>
<td>5.4(5.1)</td>
<td>5</td>
</tr>
<tr>
<td>V3</td>
<td>90.3(8.5)</td>
<td>7.7(5.8)</td>
<td>2.1(2.7)</td>
<td>5</td>
</tr>
<tr>
<td>V4</td>
<td>52.1(24.8)</td>
<td>21.8(10.1)</td>
<td>26.1(15.2)</td>
<td>4</td>
</tr>
<tr>
<td>V5</td>
<td>71.8(12.8)</td>
<td>14.7(5.9)</td>
<td>13.6(7.0)</td>
<td>4</td>
</tr>
</tbody>
</table>

†bulk solution. ND = not determined (amounts of hGH too low); n = number of batches.

4.4.3 Solid-state characterisation of the powders

4.4.3.1 hGH powders precipitated from solutions containing isopropanol
DSC analysis of powders precipitated from solutions (both methods) containing only isopropanol (e.g. sample V1) showed a substantial endotherm at around 80 °C and a smaller one at 214±1 °C. The initial (large endotherm) transition could be attributed to the evaporation of residual moisture and the smaller transition may have been the result of the native protein structure unfolding due to high temperature denaturation. Karl Fischer analysis confirmed a higher residual moisture content in sample V1. The precipitated powders, irrespective of method of preparation, showed diffuse peaks in the XRPD analyses, indicating that they were amorphous in nature. As exemplified by the SEM picture of sample V1 (Fig. 15a), particles of the powders, regardless of process set-up or other variables, were generally partially spherical and aggregated, with smaller particles on the surface of the larger ones.

4.4.3.2 hGH powders precipitated from solutions containing sucrose
DSC profiles of powders containing sucrose (samples V2-V5) showed an evaporation endotherm at around 30 °C followed by a sharp sucrose melting transition at around 189 °C and a final denaturation peak at 224±2 °C. Similar thermal behaviour was observed for powders precipitated from both processes and at different temperatures and protein:sucrose concentration ratios. Powders containing 5% sucrose prepared under optimum conditions had a lower moisture content than other samples (V3, V5 versus V1, V2).
XRPD patterns indicated that these powders were also amorphous, regardless of the process or formulation variables. However, an exothermic peak corresponding to the recrystallisation of the amorphous phase was not seen in the DSC profile. It may be that the recrystallisation of amorphous sucrose was delayed or prevented in the presence of the globular protein (124, 125). As seen from the SEM pictures in Figures 15a and 15b, powders containing sucrose were similar in morphology to those without sucrose, irrespective of process and formulation variables. Particles of powders precipitated under optimum conditions using the modified SEDS process (e.g. sample V3) were smooth, with a uniform narrow particle size distribution i.e. approx. 1-6 µm (Fig. 15b). The spherical appearance of the particles under SEM further supported the conclusion that the powders were amorphous in nature.

**Fig 15**: SEM pictures of the hGH powders precipitated from solutions containing a) isopropanol (V1) and b) sucrose (V3) using the modified SEDS process

In summary, optimal conditions (pressure 100 bars and temperature 40-50 ºC) for the precipitation of hGH powders using conventional and modified SEDS processes were identified. Modifying the SC-CO₂ by the addition of isopropanol prior to dispersion initiated the precipitation effectively even with low quantities of isopropanol in the hGH solution. The addition of sucrose (without isopropanol) to the aqueous solutions of the protein also facilitated precipitation. Thus, in the modified SEDS process, hGH was able to be precipitated from entirely aqueous solutions.

The optimal formulation of hGH in this study resulted in yields of about 61%. The powders were amorphous with particles in the range of 1-6 µm and containing 5% moisture. This formulation also resulted in the highest amount of hGH dissolved from the precipitated powders, with recoveries of 71%. RP-HPLC and SEC data, indicating that the powder contained 93% intact hGH with 91% in monomeric form, were comparable to the product specifications of the European Pharmacopoeia.
5 CONCLUSIONS

The studies in this thesis explored some interesting applications of a novel processing technology called Solution Enhanced Dispersion by Supercritical Fluids (SEDS) in the area of drug delivery, while also improving the general understanding of particle formation/precipitation phenomena occurring during the SEDS process.

The specific achievements of the thesis were:

- Particles of anti-inflammatory and anti-asthmatic drugs (hydrocortisone, budesonide and flunisolide), with varied physicochemical properties and in a size range suitable for inhalation delivery (1-5 µm), were prepared using SEDS process.

- SEDS was shown as a screening tool to reveal the range of polymorphs of anhydrous flunisolide. Two new polymorphs (III and IV) were identified, and were prepared with high chemical purity. The relative stability of these flunisolide polymorphs was ranked as IV<III<II=hemihydrate. Although the produced drug particles ranged from 1 to 5 µm in size, MLI studies indicated poor lung deposition.

- A stable powder form of a therapeutic protein (hGH) was formed from aqueous solution using a modified SEDS process. In the optimal formulation, the precipitated protein was stable against chemical degradation during processing and was more soluble in water than the other formulations; the dissolved hGH had a 91% monomer content. The hGH powders were amorphous, containing particles of 1-6 µm and 5% moisture.

The specific conclusions drawn from this thesis are:

- The SEDS process can be used to reproducibly prepare variously shaped drug particles in a size range suitable for inhalation delivery. However, as in the case of flunisolide, powders produced using SEDS processes may be prone to aggregation as a result of the nature of the drug and the processing conditions. Thus, alternative approaches (process and formulation oriented) are necessary for such drugs to improve drug deposition characteristics in the lungs.

- Supercritical fluid crystallisation methods may be used as screening tools for the discovery of drug polymorphs. The SEDS technology, in particular, could be employed in the controlled preparation of various polymorphs of a drug substance in highly pure form.
• The mechanisms of particle formation during the SEDS process which appear to ultimately affect the crystal structure, morphology and/or size of the precipitated particles include variations in mass transfer rates of solvents, the solubility of the drug and the mixing dynamics. However, the dominating factor controlling the generation and characteristics of the particles is the dynamic solubility of the drug in the supercritical fluid.

• The SEDS process appears to be an effective alternative means of preparing stable powders of therapeutic hormones/proteins from aqueous solution. Further exploration of the SEDS precipitation method and formulation constituents is nonetheless required to improve formulation characteristics.
6 ACKNOWLEDGEMENTS

The studies in this thesis were carried out at the Department of Pharmacy, Faculty of Pharmacy, Uppsala University. A number of persons have directly or indirectly enriched both this thesis and the time I have spent as a PhD student. I wish to express my sincere gratitude to all of them, and the following in particular.

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REFERENCES


89. R. Gaderhi, A supercritical fluids extraction process for the production of drug loaded biodegradable microparticles. Thesis from the Faculty of Pharmacy, Uppsala University (2000).


A doctoral dissertation from the Faculty of Pharmacy, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series *Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy*. (Prior to July, 1985, the series was published under the title “Abstracts of Uppsala Dissertations from the Faculty of Pharmacy”.)
## ERRATA

**Preparation of Pharmaceutical Powders using Supercritical Fluid Technology**  
*Pharmaceutical Applications and Physicochemical Characterisation of Powders*  
SITARAM P VELAGA, 2004

<table>
<thead>
<tr>
<th>Reads</th>
<th>Should read</th>
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</table>
| List of Papers  
| p1, Section 1.1  
in the lungs involves inertial impaction, expressed in terms of aerodynamic diameter  
\[ d_a = d / \rho \] | p1, Section 1.1  
in the lungs involves inertial impaction which is influenced by particle aerodynamic properties. The aerodynamic diameter \( d_a \) is the diameter of a unit density sphere that has the same settling velocity as the particle in question and is expressed as  
\[ d_a = d / \rho \] where \( d \) is geometric diameter and \( \rho \) is particle density |
| p2, Table 1  
2. Polymorphs | p2, Table 1  
Polymorphs; number the subsequent subheadings accordingly |
| p7, section 1.4.1.2.1  
...with pressure at 313 K, whilst at constant temperature... | p7, section 1.4.1.2.1  
...with pressure at 313 K, whilst with increasing temperature... |
| p7, section 1.4.1.2.2  
...A is rich in ethanol and that... | p7, section 1.4.1.2.2  
...A is rich in methanol and that... |
| p10, section 1.5.6  
The SF is extracted, resulting..... | p10, section 1.5.6  
The SF is evaporated, resulting..... |
| p14, under third bullet  
...discovered in paper II) crystallised using the SEDS technique and to... | p14, under third bullet  
...discovered in paper II that were crystallised using the SEDS technique) and to... |
| p16, heading 3.4  
... (Corticosteroids) | p16, heading 3.4  
... (Corticosteroids) |
| p17, section 3.6.2  
...operated with Cu\(\alpha\) radiation | p17, section 3.6.2  
...operated with CuK\(\alpha\) radiation |
| p18, section 3.6.4  
...were taken every 3 hrs for 24 hrs... | p18, section 3.6.4  
...were taken after 3 hrs and 24 hrs... |
| p18, section 3.6.5  
...excess powder... | p18, section 3.6.5  
...excess powder... |
| **Paper I**  
p159, section 3.1.1  
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p159, section 3.1.1  
.....modification I, instead... |
| **Paper II**  
p1566, Fig. 3 legend  
...anhydrous forms II, III and I. | **Paper II**  
p1566, Fig. 3 legend  
...anhydrous forms II, III and IV. |