PÄIVI HARJUNEN

Modification by spray drying of the physicochemical properties of lactose particles used as carriers in a dry powder inhaler

Doctoral dissertation

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ABSTRACT

Drug delivery by inhalation has traditionally been used for topical action to treat pulmonary diseases but systemic absorption has also been investigated over the past few years. Three main delivery systems have been devised, namely metered dose inhaler (MDI), nebuliser and dry powder inhaler (DPI). Dry powder formulations for inhalation consist of micronized drug alone or of drug blended with some kind of inert coarse carrier particles. A carrier is typically used in DPI to improve the flowability of drugs particles into the inhalation device during the filling process, to increase dispersing property of the cohesive drug particles during emission and to dilute the drug on lower dosing.

Spray drying is extensively used in the pharmaceutical industry to produce solid particles. The spray drying technique transforms liquid feed into dry powder in one step. Typically, a polymorphic change from a crystalline form to an amorphous form occurs during spray drying.

The objective of this study was to evaluate how the physicochemical properties of the carrier particles, such as their amorphous content, particle surface roughness, bulk density and particle size, the drug-carrier ratio and the storage (40°C and 75% RH) of the formulation affect in vitro deposition of the drug from multiple dose reservoir based DPI (Taifun®). The physicochemical properties of lactose were modified by spray drying. In the present study mannitol, glucose, α-lactose monohydrate (110M, 325M), spray-dried lactose, crystallized spray-dried lactose, Flowlac-100® with and without crystalline micronized lactose and crystalline lactose were used as the carrier, and salbutamol sulphate and budesonide were used as model drugs. The physicochemical properties of the drugs and carriers were studied by various techniques, including a scanning electron microscope (SEM), a laser light diffraction, a differential scanning calorimetry (DSC), an isothermal microcalorimetry (IMC) and solution calorimetry.

The physicochemical properties of carrier particles strongly affected in vitro deposition of budesonide and salbutamol sulphate from the multiple dose reservoir based DPI, and the physical stability of the inhalation powder. Prominent surface roughness, small particle size and high amorphous content of the carrier decrease the respirable fraction (RF) of the drugs. Further, the emitted budesonide dose increased as a function of bulk density of formulation. The effect of the chemical composition of carrier was evident on the RF% values of budesonide; the highest RF% values of budesonide were achieved when mannitol was used as the carrier. In the case of salbutamol sulphate, carrier has no substantial effect on RF% value. The drug-carrier ratio affected the RF% values of budesonide and salbutamol sulphate before and after storage (40°C and 75% RH) period. Typically the RF% values increased with an increase in the drug-carrier ratio. The effect of the storage on the RF% values of budesonide was dependent on the carrier and the drug-carrier ratio. The RF% value of salbutamol sulphate decreased after storage irrespective of the chemical composition of the carrier and irrespective of the drug-carrier ratio.

This study shows that the amorphous content of the spray-dried lactose can vary from 0% to 100%, depending on ethanol concentration of the feed solution. When the solubility of lactose in the feed solution was decreased by increasing the ratio of ethanol to water, the amorphous content in the spray-dried lactose decreased. The enthalpy of solution (∆H_{so}) and the enthalpy accompanied with an addition of a lactose sample in a saturated aqueous solution (∆H_{sol}) were determined by solution calorimetry. A linear correlation was observed between both ∆H_{so} and ∆H_{sol} and amorphous content of the lactose samples.

In conclusion, the present results suggest that highly crystalline carrier particles, which have a smooth surface and optimal size are required to formulate physically stable inhalation powders for multiple dose reservoir based DPI. The present results suggest that the amorphous content of spray-dried lactose can be controlled by selecting the appropriate ethanol concentration in the feed solution. Further, the present study demonstrates that solution calorimetry may be a rapid and simple method for determining the amorphous content also in samples that are not completely dissolved in the solvent.

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Medical Subject Headings: technology, pharmaceutical / methods; administration, inhalation; drug carriers; powders; lactose; mannitol; glucose; chemistry, physical; surface properties; budesonide; particle size; drug storage; calorimetry
To my family
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Kuopio, December 2003

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<tr>
<td>ACI</td>
<td>Andersen cascade impaction</td>
</tr>
<tr>
<td>d</td>
<td>geometric particle diameter</td>
</tr>
<tr>
<td>D_{50%}</td>
<td>average particle size</td>
</tr>
<tr>
<td>d_{ac7}</td>
<td>particle aerodynamic diameter</td>
</tr>
<tr>
<td>DPI</td>
<td>dry powder inhaler</td>
</tr>
<tr>
<td>DSC</td>
<td>differential scanning calorimetry</td>
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<tr>
<td>ESEM</td>
<td>environmental scanning electron microscopy</td>
</tr>
<tr>
<td>FPD</td>
<td>fine particle dose</td>
</tr>
<tr>
<td>FPF</td>
<td>fine particle fraction (%)</td>
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<tr>
<td>FPM</td>
<td>fine particle mass</td>
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<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>ΔH_{sat}</td>
<td>enthalpy accompanied with an addition of a lactose sample in the saturated aqueous solution</td>
</tr>
<tr>
<td>ΔH_{sol}</td>
<td>enthalpy of solution</td>
</tr>
<tr>
<td>IMC</td>
<td>isothermal microcalorimetry</td>
</tr>
<tr>
<td>MDI</td>
<td>metered dose inhaler</td>
</tr>
<tr>
<td>MMAD</td>
<td>mass median aerodynamic diameter</td>
</tr>
<tr>
<td>MSLI</td>
<td>multistage liquid impinger</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>XRPD</td>
<td>X-ray powder diffraction</td>
</tr>
<tr>
<td>ΔP</td>
<td>pressure drop across an inhaler device</td>
</tr>
<tr>
<td>Q</td>
<td>flow rate</td>
</tr>
<tr>
<td>ρ</td>
<td>tap density</td>
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<tr>
<td>R_{D}</td>
<td>specific resistance</td>
</tr>
<tr>
<td>RF</td>
<td>respirable fraction (%)</td>
</tr>
<tr>
<td>RH</td>
<td>relative humidity (%)</td>
</tr>
<tr>
<td>R.S.D.</td>
<td>relative standard deviation</td>
</tr>
<tr>
<td>S.D.</td>
<td>standard deviation</td>
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<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
</tr>
<tr>
<td>t</td>
<td>time</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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</tr>
<tr>
<td>T</td>
<td>temperature</td>
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<tr>
<td>Tg</td>
<td>glass transition temperature</td>
</tr>
<tr>
<td>TG</td>
<td>thermal gravimetry</td>
</tr>
<tr>
<td>TI</td>
<td>twin impinger</td>
</tr>
<tr>
<td>UDCA</td>
<td>ursodeoxycholic acid</td>
</tr>
<tr>
<td>v/v</td>
<td>volume/volume</td>
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<tr>
<td>w/w</td>
<td>weight/weight</td>
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LIST OF ORIGINAL PUBLICATIONS

This doctoral dissertation is based on the following publications, referred to in the text by Roman numerals I-IV:


IV Päivi Harjune, Vesa-Pekka Lehto, Mikko Koivisto, Elina Levonen, Petteri Paronen and Kristiina Järvinen: Determination of amorphous content of lactose samples by solution calorimetry. Submitted.
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1 INTRODUCTION

Interest in drug delivery via inhalation is rapidly expanding. Drug delivery by inhalation is currently intended for topical administration to treat pulmonary diseases but recently systemic absorption has also been investigated after pulmonary inhalation for the possible delivery of proteins and macromolecules (Adjei and Gupta, 1994; Byron and Patton, 1994; Patton, 1996; Smith, 1997; LiCalsi et al., 1999; Maa et al., 1999). The interest in dry powder inhalers (DPIs) has increased, since the use of chlorofluorocarbons will soon become prohibited even in medical sprays. Novel DPIs result in even better lung deposition of drugs than pressurized metered dose inhalers (MDI), and coordination of actuation and inhalation are easier with DPI (Borgström and Newman, 1993). A DPI formulation may consist of a drug alone, or of a drug blended with a carrier material (Prime et al., 1997). The aerodynamic diameter of a drug particle needs to be between 1 and 5 μm for deep lung deposition. Small drug particles generally have poor flow properties and they are notoriously difficult to disperse, due to the highly cohesive nature of small particles. Thus, a carrier is typically used in DPI to aid in the flow and dispersion properties of the drug (Podczeck, 1998; Larhrib et al., 1999; Zeng et al., 2000a).

The dispersion and subsequent deposition of drug particles in the respiratory tract from DPIs are actuated by patient’s inhalation flow rate (Prime et al., 1997). The design of the inhaler device (Vidgren et al., 1988c; Steckel and Müller, 1997a; Palander et al., 2000) and the physicochemical properties of both the drug (Vidgren et al., 1987a; Jashnani et al., 1996; Dickhoff et al., 2002) and carrier (Podczeck, 1998; Larhrib et al., 1999; Zeng et al., 2000a) particles appear to have significant influence on drug deposition into the deep parts of the lung. Particulate interactions within the formulation govern the drug dissociation from carrier particles. Any change in the physicochemical properties of carrier particles, such as particle size (Braun et al., 1996; Larhrib et al., 1999, 2003; Podczeck, 1998; Srichana et al., 1998; Steckel and Müller, 1997b; Vanderbist et al., 1999; Zeng et al., 2000b, 2001a), particle surface (Ganderton, 1992; Kawashima et al., 1998a; Larhrib et al., 1999, 2003; Zeng et al., 2000a), specific surface area (Kawashima et al., 1998a; Steckel and Müller, 1997b) particle shape (Iida et al., 2001; Larhrib et al., 1999, 2003; Zeng et al., 2000a, b), the chemical composition (Tee et al., 2000; Chan et al., 1997; Braun et al., 1996; Bosquillon et al., 2001a), crystallinity (Kawashima et al., 1998a; Zeng et al., 2001a), bulk density (Bosquillon et al., 2001a) and water content (Podczeck, 1998) may have an impact on the delivery and deposition of drug.
α-Lactose monohydrate has been most frequently employed as a carrier in DPIs. In addition, glucose (Braun et al., 1996; Steckel and Müller, 1997a), mannitol (Naini et al., 1998; Tee et al., 2000; Bosquillon et al., 2001a), trehalose (Bosquillon et al., 2001a), sorbitol (Tee et al., 2000) and sucrose (Naini et al., 1998) have been studied over the past few years because new drugs and new designs of devices have created a demand for new carriers.

The production of powders for dry DPIs is an important area where the spray drying process has been commonly utilized (Vidgren et al., 1987a, 1989; Broadhead et al., 1994; Vanbever et al., 1999; Dellamary et al., 2000; Bosquillon et al., 2001a, b). Spray drying is a useful method when the physical and biopharmaceutical properties, such as, dissolution rate, crystallinity, particle size distribution, bulk and particle density, morphology and moisture content of drugs or excipients are preferred to change. Currently, new additives have also made it possible to utilize the spray drying method to produce hollow and porous drug particles for inhalation (Ben-Jebria et al., 1999; Dellamary et al., 2000). Spray drying is a convenient method also for the production of pharmaceutical complexes (Broadhead et al., 1992).

Amorphous regions within the crystal structure of the solids can be produced by spray drying (Vidgren et al., 1987a, 1989; Ueno et al., 1998; Broadhead et al., 1992; Briggner et al., 1994; Sebhatu et al., 1994). The amorphous form will have different physical properties, and as such will interact with other phases in a different manner to that of the crystalline form (Buckton et al., 1998). This can be important in many products, not least in certain inhalation powders where micronized drugs must bind in a reversible manner to a carrier. Typically highly crystalline materials are used in drugs. The amorphous content of a compound can be determined by a variety of techniques, including isothermal microcalorimetry (IMC) (Buckton and Beezer, 1991; Angberg et al., 1992a, b; Buckton et al., 1995a), differential scanning calorimetry (DSC) (Saleki-Gerhardt et al., 1994; Sebhatu et al., 1994), thermal gravimetry (TG) (Chidavaenzi et al., 1997; Yao et al., 2003), X-ray powder diffraction (XRPD) (Saleki-Gerhardt et al., 1994; Stephenson et al., 2001) and solution calorimetry (Pikal et al., 1978; Thompson et al., 1994; Ward and Schultz, 1995; Gao and Rytting, 1997; Hogan and Buckton, 2000).
2 REVIEW OF THE LITERATURE

2.1 Inhalation therapy

Drug delivery by inhalation is primarily intended for local topical administration to treat pulmonary diseases but recently inhalation and then systemic absorption have also been investigated for the possible delivery of protein and peptides (Adjei and Gupta, 1994; Byron and Patton, 1994; Patton, 1996; Smith, 1997; LiCalsi et al., 1999; Maa et al., 1999). The pulmonary delivery route offers several advantages compared to more traditional modes of administration (e.g. the oral route): (1) Medication is directly delivered to the tracheobronchial tree allowing for rapid and predictable onset of action. (2) The first-pass metabolism and degradation within the gastrointestinal tract are avoided. (3) The large surface area of bronchioles and minimal resistance associated with the pulmonary gaseous exchange properties promote the rapid absorption of the inhaled medicament. However, the efficiency of inhalation therapy is not high; typically only about 10-25% of the inhaled dose of the drug reaches the alveolar region in in vivo studies (Braun et al., 1996). Losses of inhaled therapeutics can be attributed to a variety of factors; for example, inhaled drug particles must possess a very narrow range of aerodynamic diameters to pass through the filter of the mouth and throat (Edwards et al., 1998). Three main delivery systems have been devised, namely MDI, nebuliser and DPI.

The principal mechanisms of inhaled drug particles which contribute to lung deposition are inertial impaction, sedimentation and Brownian motion (Heyder et al., 1986). In vitro methods (impaction techniques) have been used to predict pulmonary deposition of the drug particles (Broadhead et al., 1995; Steckel and Müller, 1997a; Kawashima et al., 1998b; Zeng et al., 1999; Newman et al., 2000; Dunbar et al., 2002). They have been designed to reproduce the anatomical dimensions of an average healthy human airway. Measurement of the in vitro particle size distribution of an inhaler yields parameters such as mass median aerodynamic diameter (MMAD), fine particle fraction (FFP%), fine particle dose (FPD) and respirable fraction (RF%). The FFP% and FPD express the percentage of the drug dose and mass of drug contained in respirable particles, (smaller than about 5 μm diameter) respectively (Newman et al., 2000). RF% expresses the percentage of the respirable drug dose of drug contained in emitted dose. It is generally accepted that the aerodynamic diameter of a drug particle needs to be between 1 and 5 μm for deep lung deposition. The aerodynamic diameter of particles,
\[ d_{ac} = \sqrt{\frac{d}{\rho}} \]  

(1)

where \( d \) is the geometric diameter of particle, \( \rho \) is the powder tap density and \( \rho_l \) is 1 g/cm\(^3\).

The multistage Andersen cascade impactor (ACI) (Ph. Eur. 4\(^{th}\) Ed. 2002) is a widely used inertial method for the characterization of the aerodynamic particle size distribution. It consists of a throat, pre-separator, eight stainless steel stages and a final filter. Briefly, drug doses travel through the simulated upper airways onto metal plates resembling different stages of the respiratory tract. The cascade impactor utilizes the relationship between velocity and mass where large particles impact on upper stages whereas finer aerodynamic particles can penetrate to the lower stages of the separator. The multi-stage liquid impinger (MSLI), the glass impinger and the metal impinger are also commonly used inertial impactors (Ph. Eur. 4\(^{th}\) Ed. 2002). In comparison to ACI, these differ in their design, but they are all approved for use in testing the aerodynamic particle size distribution of aerosols by the European Pharmacopoeia. Earlier these testing devices could give varying aerodynamic size distributions for the same drug formulation because they were intended to be operated at different flow rates and drug delivery from DPI is a function of inspiratory flow rate. At present, the European Pharmacopoeia requires the aerodynamic particle size distribution of aerosols to be measured under a flow rate achievable at a pressure drop of 4 kPa across the inhaler device, which represents the inhalation effort of an average asthmatic patient. It is possible to calculate the flow rate, \( Q \), that will be achieved across an inhaler device at a pressure drop, \( \Delta P \), of 4 kPa when a specific resistance, \( R_d \), of device is known using the following equation (Zeng et al., 2001b):

\[ \Delta P^{4kPa} = R_d Q \]  

(2)

All in vitro models are simplified and the potential impactions differ from the tissue surfaces comprising the airway. Thus in vivo studies provide a more realistic evaluation of a formulation’s deposition characteristics. Animals, like rats (Todo et al., 2001; Lombry et al., 2002; Ikegami et al., 2003), rabbits (Dubus et al., 2001) and quinea pigs (Kawashima et al., 1998c; Ben-Jebria et al., 1999) are used in in vivo studies. In vivo
deposition studies in human subjects have been carried out using radioactively labelled particles (Vidgren et al., 1987b; Karhu et al., 2000; Pitcairn et al., 2000; Dunbar et al., 2002). Pulmonary drug delivery has been assessed by the two-dimensional method of gamma scintigraphy or three-dimensional radionuclide imaging methods of single photo emission computed tomography and positron emission tomography (Newman et al., 2003).

2.1.1 Dry powder inhalers (DPIs)

The dry powder inhalation system consists of a DPI (device) and a dry powder formulation. The interest in DPIs has increased, since the use of chlorofluorocarbons will be banned even in medical sprays. DPIs are propellant-free, portable, easy to operate and low-cost devices. Novel DPI can result in even better lung deposition of drugs than can be obtained from pressurized MDI, and coordination of actuation and inhalation is easier with DPI (Vidgren et al., 1988a, b; Borgström and Newman, 1993). The patient’s own inspiration disperses a metered quantity of powder in a stream of air drawn through the device.

Dry powder formulations for inhalation consist of micronized drug alone or of drug blended with some inert coarse carrier particles (Prime et al., 1997; Zeng et al., 2000a, b; Tee et al., 2000). To be effective, the DPI device must protect the powder from the ambient environment, dose a repeatable amount each time and deagglomerate the powder into particles for lung deposition. The design of the DPI device appears to have a significant influence on drug deposition to the deep parts of the lung (Vidgren et al., 1988c; Steckel and Müller, 1997a). The inhaler must generate forces that will not only entrain the powder, but also deagglomerate the powder into particles of a respirable size for inhalation (Voss and Finlay, 2002). A high air flow rate is required when a DPI is operated, in order to break up the interparticulate binding, while a low air flow is required to penetrate drug particles into the deep lung. The nature of the air flow can be laminar or turbulent as it passes through the DPI. Turbulent air flow is more effective than laminar airflow for dispersing the powder mixture (Voss and Finlay, 2002) and the smaller the diameter of the air channel, the more turbulent the air flow (Ward et al., 1992). A reduction in the internal dimensions leads to an increase in the resistance of the inhaler to airflow. When inhalers have a relatively high resistance to air flow, the flows through inhalers are smaller than those through low resistance inhalers for the same pressure drop, as indicated by Equation 2. Deaggregation forces in the device may
be caused some combination of turbulence, mechanical impaction, particle uptake or mechanical vibration (Voss and Finlay, 2002).

DPIs can be subdivided into unit- and multi-dose inhalers (Fig. 1). In the early 1970s, the first successful unit-dose DPI was Spinhaler® (Bell et al., 1971). In Spinhaler®, a single dose of the micronized drug had been packed into a gelatin capsule which was inserted into the device. Rotahaler® and Berotec® are unit-dose DPIs where the drug mixture, which include a bulk carrier to aid powder flow is prefilled into a hard gelatin capsule and loaded into the device. The primary disadvantage of the unit-dose device is the cumbersome nature of loading during an asthma attack. The development of multi-dose DPI facilitated a better acceptance of inhalers. At present, drug alone or drug-carrier mixtures are packed into blisters or disks and thus, a number of unit-doses can be placed into the device. The Diskus® device represents a further development of the Dishaler® approach, with the pre-metered doses sealed in blisters on a foil strip (Prime et al., 1997). The precision of metering dose can be determined in the factory and the pre-metered doses can be individually sealed and protected from the environment (Brindley et al., 1995). Alternative DPI designs are based on multi-dose reservoir systems. The inhaler includes a reservoir of the bulk powder formulation and dose metering unit. The single unit dose can be dispensed by different mechanisms into the dosing chamber. The Turbuhaler® and Easyhaler® devices are reservoir based powder inhalers.
Figure 1. Types of dry powder inhalers and some of the commonly used DPIs.

2.1.1.1 Formulations of DPI

A DPI formulation may consist of a drug alone or a drug blended with a carrier material (Fig. 2). The physicochemical properties of the drug and carrier particles such as particle size, shape, density, and crystallinity of the particles can influence the lung deposition of the drug.

Figure 2. Dry powder formulation may consist of conventional small carrier-free drug particles (A), small drug and larger carrier particles (B) or large porous drug particles (C).
When no carrier is used, the dry powder formulation consists of 1-5 μm solid particles in variable states of aggregation (Timsina et al., 1994; French et al., 1996). Drug particles in this particle-size range are cohesive and exhibit typically poor flow properties which cause problems in packing drug particles into the capsules and the inhalation device. However, it has been shown by designing drug particles with very low mass density, porous structure and a larger geometric diameter, that these particles can be successfully inspired into the lungs (Ben-Jebril et al., 1999). Low forces of cohesion allow large porous particles to deaggregate easier than smaller, non-porous particles. These particles, with a geometric size range of 10-30 μm but an aerodynamic diameter of 1-5 μm, have been shown to be suitable for deep lung deposition (Vanbever et al., 1999).

Some kind of carrier is commonly used in DPI to improve the flowability of drugs particles into an inhalation device during filling process, to increase the dispersing property of cohesive drug particles during emission and to permit dilution of the drug for lower dosing (Podczeck, 1998; Tee et al., 2000). The drug is thought to adhere to the carrier to form an ordered mix (Hersey, 1975). The adhesion of the drug particles to the carrier particles has to be strong enough to hinder deaggregation during packing and storage but must permit deaggregation during inhalation. α-Lactose monohydrate has been most frequently employed as a carrier in DPIs. In addition, glucose is used as a carrier in commercial DPIs (Braun et al., 1996; Steckel and Müller, 1997a) but also other carriers, like mannitol (Naini et al., 1998; Tee et al., 2000; Bosquillon et al., 2001a), trehalose (Bosquillon et al., 2001a), sorbitol (Tee et al., 2000) and sucrose (Naini et al., 1998) have been studied over the past few years.

Carrier particles are typically the main component in inhalation powders and thus, any change in the physicochemical properties of carrier particles, such as particle size (Braun et al., 1996; Larhrib et al., 1999, 2003; Podczeck, 1998; Srichana et al., 1998; Steckel and Müller, 1997b; Vanderbist et al., 1999; Zeng et al., 2000b, 2001a), particle surface roughness (Ganderton, 1992; Kawashima et al., 1998a; Larhrib et al., 1999, 2003; Zeng et al., 2000a), specific surface area (Kawashima et al., 1998a; Steckel and Müller, 1997b), particle shape (Larhrib et al., 1999, 2003; Zeng et al., 2000a, b), crystallinity (Kawashima et al., 1998a; Zeng et al., 2001a), density (Bosquillon et al., 2001a) and water content (Podczeck, 1998) may affect lung deposition of drug (Table 1). The influence of the physicochemical properties of the carrier particles on the drug deposition depends on the drug and the design of the device (Vidgren et al., 1988c; Broadhead et al., 1995; Palander et al., 2000).
Table 1. Examples of physicochemical properties of carrier affecting pulmonary deposition of a drug from DPI.

<table>
<thead>
<tr>
<th>Increase (↑) in parameter of carrier</th>
<th>Decrease (↓) or increase (↑) in pulmonary deposition of a drug</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unit-dose DPI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particle size ↑</td>
<td>↓</td>
<td>Braun et al., 1996; Larhrib et al., 1999, 2003; Srichana et al., 1998; Steckel and Müller, 1997b; Zeng et al., 2000b, 2001b, 2001c</td>
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<tr>
<td></td>
<td>↑</td>
<td>Byron et al., 1990; French et al., 1996; Vanderbist et al., 1999</td>
</tr>
<tr>
<td>Particle surface roughness ↑</td>
<td>↓</td>
<td>Ganderton, 1992; Kawashima et al., 1998a; Larhrib et al., 1999; Zeng et al., 2000a, 2001c</td>
</tr>
<tr>
<td></td>
<td>↑</td>
<td>Larhrib et al., 2003</td>
</tr>
<tr>
<td>Specific surface area ↑</td>
<td>↑</td>
<td>Kawashima et al., 1998a; Steckel and Müller, 1997b</td>
</tr>
<tr>
<td>Density ↑</td>
<td>↓</td>
<td>Bosquillon et al., 2001a</td>
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<tr>
<td>Elongation ratio ↑</td>
<td>↓</td>
<td>Larhrib et al., 1999</td>
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<td></td>
<td>↑</td>
<td>Zeng et al., 2000a, b; Larhrib et al., 2003</td>
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<tr>
<td>Crystallinity ↑</td>
<td>↑</td>
<td>Kawashima et al., 1998a; Zeng et al., 2001b</td>
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<tr>
<td><strong>Multi-dose DPI</strong></td>
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<tr>
<td>Pre-metered</td>
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<tr>
<td>Particle size ↑</td>
<td>↓</td>
<td>Steckel and Müller, 1997b; Podezeck, 1998</td>
</tr>
<tr>
<td>Particle surface roughness ↑</td>
<td>↓</td>
<td>Young et al., 2002; Podezeck, 1998</td>
</tr>
<tr>
<td>Elongation ratio ↑</td>
<td>↑</td>
<td>Podezeck, 1998</td>
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<tr>
<td>Reservoir</td>
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<tr>
<td>Particle size ↑</td>
<td>↓</td>
<td>Steckel and Müller, 1997b</td>
</tr>
<tr>
<td>Specific surface area ↑</td>
<td>↑</td>
<td>Steckel and Müller, 1997b</td>
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</tbody>
</table>

The size of carrier particles affects the pulmonary delivery of the drug because the particles of different size ranges have different dispersion and deaggregation properties. Larger carrier particles have been shown to exert stronger adhesion forces on drug particles than smaller carriers (Staniforth et al., 1982). Drug dispersion is also
dependent upon the surface roughness of the carrier particles. Increasing the surface smoothness of lactose carrier particles was shown to improve the respirable fraction of salbutamol sulphate from either a Rotahaler® or a Cyclohaler® (Zeng et al., 2000a) and this was attributed to a reduction in the adhesion forces between the drug and carrier particles with a smooth surface. However, Kawashima et al. (1998a) showed that if the carrier had a fairly large surface area with microscopically increased surface roughness, this could improve the RF% of pranlukast hydrate from a Spinhaler®. The shape of carrier particles influences powder properties such as flowability, mixing uniformity, powder dispersion and deaggregation (Wong and Pilpel, 1990; Iida et al., 2001). Lactose crystals of different shape were shown to produce different FPF and dispersibility of drugs (Kawashima et al., 1998a; Larhrib et al., 1999). Zeng et al. (2000a) found that increasing the elongation ratio of lactose crystals increased the respirable fraction of salbutamol sulphate from Rotahaler® and Cyclohaler®.

The addition of fine particles of carrier or fine particles of ternary components were found to improve drug delivery from DPI by reducing interparticulate forces between the drug and the carrier particles (Lucus et al., 1998; Zeng et al., 1998; Tee et al., 2000). The FPF value of a drug can also be increased by optimizing both the concentration of the drug particles in the formulation (Steckel and Müller, 1997b; Vanderbist et al. 1999) and the mixing sequence of the components (Zeng et al. 1999). Further, the inhalation flow rate (Hindle and Byron, 1995; Braun et al., 1996; De Boer et al., 1997; Zeng et al., 1999) and the storage conditions (Jashnani et al., 1995; Maggi et al., 1999) have been shown to influence lung deposition of the drug.

2.2 Spray drying

2.2.1 Spray drying process

The production of powders for DPIs is an important area where the spray drying process has been commonly utilized lately. Different types of particles have been produced by spray drying, such as small spherical particles with a narrow size distribution, encapsulated particles and large porous drug particles with a low mass density. Further, spray drying is useful method for the production of protein and peptide particles of a suitable size for inhalation (Broadhead et al., 1994; Vanbever et al., 1999; Stähl et al., 2002).

Spray drying is a method in which a fluid mixture is usually sprayed into hot dry air (Fig. 3). The mixture can be a solution, emulsion, suspension or dispersion. It is
atomized into millions of individual droplets in a nozzle (Fig. 3b). The solvent is vaporized immediately by the hot air (Fig. 3c). Initially, the vaporization rate of moisture is constant on the surface of the drop and the majority of the solvent is removed in this stage (Venthoey, 1997). Subsequently a solid phase forms on the surface of the drop, inhibiting the vaporization of solvent from the interior. At this time particles form and the vaporization rate becomes dependent on the diffusion rate of moisture through a crust of increasing thickness (Broadhead et al., 1992). The vaporization process rapidly removes most of the available heat so that the product is dried gently without thermal shocking. Generally the particles reach a maximum temperature which is 15-20°C below the outlet temperature of the dryer (Masters, 1985). A fluid mixture is converted into a powder in a one step process and dried product becomes entrained in the drying air from the drying chamber into the recovery section. Separation of dried product from the drying medium occurs in the cyclone and the final product is collected into a collection vessel. The resultant product properties are dependent on the operating variables, such feed solution composition (Chidavaenzi et al., 1997, 2001; Di Martino et al., 2001; Corrigan et al., 2002), inlet / outlet air temperature (Broadhead et al., 1994; Maa et al., 1997; Ueno et al., 1998), feed flow rate (Masters, 1985; Ståhl et al., 2002) and air flow (Masters, 1985; Ståhl et al., 2002). In addition, design and dimensions of spray drying equipment, atomiser design and orifice size have an effect on spray drying product.

![Diagram](image)

**Figure 3.** Diagram of a) the dry air flow, b) the feed solution flow and spray nozzle, c) nozzle and product in the spray drying (modified from Büchi training papers, 1998).
2.2.1.1 Feed solution composition

Physicochemical properties of the spray-dried products, such as particle size, shape and amorphous content, can be controlled by adjusting the feed composition. Typically water has been used as a feed solution in the spray drying, but other solvents such as chloroform/methanol (Matsuda et al., 1992) and ethanol/dichloromethane (Ueno et al., 1998) can also be used. Generally, amorphous particles are produced when a material is dissolved in the feed solution before the spray drying. For example, the spray-dried lactose (Chidavaenzi et al., 1997; Naini et al., 1998), sucrose (Naini et al., 1998), trehalose (Naini et al., 1998), salbutamol sulphate (Chawla et al., 1994) and disodium cromoglycate (Vidgren et al., 1987a) were highly amorphous (amorphous content > 80%) when distilled water was used as a feed solution. A short average droplet drying time during spray drying is often believed to offer an insufficient opportunity for nucleation and crystal growth. However, e.g. in the case of mannitol, this time appeared to be sufficient to enable crystallization, because the spray dried mannitol was 100% crystalline when distilled water was used as a feed solution (Phillips et al., 1996; Naini et al., 1998). However, the spray drying induced polymorphic transition of mannitol from its α to the β form during the spray drying (Yoshinari et al., 2002). Chidavaenzi et al. (1997) showed that by selecting the appropriate feed concentrations in water, spray dried lactose could be produced with various polymorphic proportions. At higher feed concentrations, incomplete dehydration of the suspended lactose particles occurred resulting in some lactose monohydrate in the products (Chidavaenzi et al., 1997). In addition, the concentration of lactose in a feed solution can influence the amorphous content of lactose; the amorphous content of the spray-dried lactose varied from 100% to 82% when the lactose concentration in water increased from 0.1 g/mL to 0.4 g/mL. Also, coarser particles could be produced by increasing the concentration of the particles in the feed solution (Mastor, 1985).

Additives in a feed solution can affect the physicochemical properties of the spray-dried particles. Chidavaenzi et al. (2001) showed that polyethylene glycol (PEG) 4000 caused lactose to crystallise during the spray drying process. They found that spray drying lactose with PEG 4000, even at level of 1% of the solids, resulted in crystalline α-monohydrate, α-anhydrous and crystalline β-lactoses. Their relative proportions were dependent on the concentration of PEG 4000. In contrast, Corrigan et al. (2002) reported that all spray dried lactose/PEG 4000 samples contained amorphous lactose together with varying amounts of crystalline lactose, but none of the samples contained α-lactose monohydrate after spray drying. The crystallinity of spray dried lactose/PEG
4000 composites varied depending on the percentage of PEG 4000 present. The lower crystallinity of the lactose/PEG 4000 systems in the study by Corrigan et al. (2002) may be related to the different spray drying conditions employed, such feed concentration and airflow rate. In the case of mannitol, addition of sodium phosphate to the feed solution hindered mannitol crystallization during spray drying (Costantino et al., 1998).

Di Martino et al. (2001) used 0.1 N ammonium solution as a feed solution in spray drying of acetazolamide, and a mixture of two polymorphic forms I and II were produced. The glycine powders spray dried from aqueous solution having a pH of either 1.7 or 10 had different particle morphologies and sizes, which could well have modified their suitability for incorporation into pharmaceutical formulations (Yu and Ng, 2002).

Respirable hollow and porous powders can be produced by co-spray drying with suitable excipients (Ben-Jebria et al., 1999; Dellamary et al., 2000). For example, Dellamary et al. (2000) produced both hollow and porous cromolyn sodium, salbutamol sulphate and formoterol fumarate particles with different excipients and with a relatively high levels of solid (>20%) in the spray-feed. Also hollow protein and peptide particles can be prepared by spray drying (Chan et al., 1997; Johnson, 1997; Maa et al., 1998). However, degradation during the atomization process may be a problem for some macromolecules, and different excipients are required to optimize the spray drying process (Johnson, 1997).

### 2.2.1.2 Inlet/outlet air temperature

The inlet temperature affects the dryer evaporative capacity at a constant air rate. The effect of temperature on the physicochemical properties of particles appears to be highly dependent on the material. Increased inlet air temperature increased the insulin particle size (Ståhl et al., 2002). The increased particle size may be explained by the fact that at high inlet air temperatures, a skin is formed on the outer surface of the spray droplets, but this skin is destroyed and the outer surface collapses when the inner water phase evaporates through the skin. Broadhead et al. (1994) suggested that the increase in particle size of β-galactosidase might be due to increased agglomeration at the higher inlet air temperatures. An increased temperature often causes a reduction in bulk density also, as evaporation rates are faster and products dry to a more porous or fragmented structure (Masters, 1985). Ueno et al. (1998) observed that the inlet-air temperature could affect the amorphous content of ursodeoxycholic acid (UDCA) samples. When the inlet-air temperature of spray drier was increased beyond 140°C the amorphous content of UDCA increased clearly. In contrast, increased inlet air temperature resulted
in a decreased moisture content of product (Billon et al., 2000; Ståhl et al., 2002). Increased inlet air temperature increases the outlet temperature because of the increased supply of heat energy. Thus a clear correlation was observed between the moisture content of spray dried insulin (Ståhl et al., 2002), paracetamol (Billon et al., 2000) and β-galactosidase (Broadhead et al., 1994) and the outlet air temperature. Broadhead et al. (1994) showed that the yield of β-galactosidase increased with increased inlet temperature during spray drying. Maa et al. (1997) reported that a decreased outlet temperature gave rise to more regular, spherical protein particles.

2.2.1.3 Feed flow rate

If the feed flow rate is increased, the residual moisture content in powder increases, coarser particles are produced and powder bulk density increases at constant atomizer operating conditions (Masters, 1985). Ståhl et al. (2002) indicated that the increased feed flow rate lowered also the outlet air temperature, resulting in lower drying capacity and thus a higher moisture content of the spray-dried insulin. Billon et al. (2000) observed that an increase in the feed flow rate contributed to a higher moisture content of paracetamol, especially at low inlet temperature. Broadhead et al. (1994) showed that the yield of the spray-dried β-galactosidase increased with a decreased feed rate.

2.2.1.4 Air Flow

The rate of air flow controls the residence time of the product in the drying chamber. Air flow has a bearing on the product being handled and on its properties (Masters, 1985). Increased residence time leads to a greater degree of moisture removal. An increase in the energy available for atomization, such as air flow, can reduce particle size (Masters, 1985). Ståhl et al. (2002) showed that an increased atomization nozzle flow reduced the particle size which they attributed to the fact that the higher atomization flow, the more energy is supplied for breaking up the liquid into droplets during the atomization step.

Air flow may influence the yield of spray dried product. Reduced air flow assists product recovery from the drying chamber. Ståhl et al. (2002) showed that when the nozzle flow rate was decreased, the yield of insulin increased. Decreased atomization energy produced enlarged droplets and the large dry particles were more easily captured through the centrifugal force in the cyclone.
2.3 Investigation of amorphous content

2.3.1 The amorphous state

If the degree of disorder is more extensive than the occasional molecular dislocation, it can be viewed as an amorphous region within the crystal structure (Phipps and Mackin, 2000; Yu, 2001) (Fig. 4). Amorphous regions within the crystal structure of the solids can be produced by many pharmaceutical processes, such as milling (Ward and Schultz, 1995), mixing (Konno, 1990), spray drying (Vidgren et al., 1987b, 1989; Ueno et al., 1998; Broadhead et al., 1992; Briggner et al., 1994; Sebhatu et al., 1994), tablet compaction (Ahlneck and Alderborn, 1989), and lyophilization (Pikal et al., 1978). Some drugs and excipients, such as proteins, peptides, some sugars and polymers, have a tendency to exist as amorphous solids.

![Micronization Diagram](image)

**Figure 4.** Schematic representation of a crystalline surface before and after micronization (modified from Ward and Schultz, 1995).

The formation of disorder in a solid produces regions that are thermodynamically unstable, i.e. they are in a higher energy state than the crystalline form (Saleki-Gerhardt et al., 1994; Yu, 2001). The amorphous form has different physical properties, and as such will interact with other phases in a different manner to that of the crystalline form. Amorphous solids have higher solubility, higher dissolution rates, and sometimes better compression characteristics than the corresponding crystals (Hancock and Zografi, 1997; Yu, 2001). However, the amorphous region may significantly decrease the physical and chemical stability of a compound, since any unstable system has to have a mechanism by which it can transfer to its stable state, especially on exposure to heat and humidity (Hancock and Zografi, 1997; Naini et al., 1998; Yu, 2001). The transition from the amorphous to the crystalline form will depend upon the mobility of the molecules (Hancock et al., 1995; Buckton and Darcy, 1999; Yu, 2001). The transition
of the amorphous solid depends upon the difference between the temperature (T) and the glass transition temperature (Tg). Amorphous regions can absorb a sufficient quantity of water while the free volume increases and the Tg decreases. The presence of water is of significance, because the absorption of water generally lowers the Tg until the material is able to crystallise. Figure 5 shows one possible scenario involving a moisture mediated amorphous to crystalline conversion (Ward and Schultz, 1995). The molecules with increased mobility can rearrange into a stable crystalline structure, forming aggregates and expelling water. This change can dramatically change the physicochemical properties of a material. Overall, there is an urgent need to characterise materials as to their amorphous content and to understand how this relates to the functionality of the material in the subsequent processes which it is to be used. The amorphous content of a compound can be determined by a variety of techniques, including isothermal microcalorimetry (IMC), differential scanning calorimetry (DSC), thermal gravimetry (TG), differential thermal analysis, X-ray powder diffraction (XRPD) and solution calorimetry.

Two partially amorphous particles $\rightarrow$ H$_2$O $\rightarrow$ crystalline particles

\[ \text{Tg} \downarrow, \text{Free volume} \uparrow \]

Figure 5. An amorphous material can absorb a sufficient quantity of water to increase the free volume and lower the Tg. The molecules adjust into a stable crystalline structure forming a bridge between the two particles (modified from Ward and Schultz, 1995).

2.3.2 Isothermal microcalorimetry (IMC)

IMC is now widely used in the pharmaceutical sciences. It is based on the fact that all physical and chemical processes are accompanied by a heat exchange. Isothermal heat-conduction microcalorimetry measures the heat flow (dQ/dt) from ongoing processes. The heat flow curve for the crystallization process can be divided into three distinct phases: adsorption or absorption of moisture, crystallization of amorphous regions within
the sample, and evaporation of excess moisture from the solid following structural collapse (Phipps and Mackin, 2000). Figure 6 shows microcalorimetric heat flow curves for the recrystallization of lactose samples spray dried from various ethanol:water mixtures. Several studies have shown that IMC is a suitable technique to probe small amounts of amorphous material present in powders (Briggner et al., 1994; Sebhatu et al., 1994; Buckton et al., 1995a; Buckton, 1997; Chidavaenzi et al., 1997). It has been demonstrated that IMC is capable of detecting as little as 0.5% amorphous material in a solid (Buckton et al., 1995b).

The instrument consists of a water bath which serves as a large heat sink. This heat sink is maintained at a pre-set temperature to an precision of 10⁻⁵°C. The measuring channels are housed in the heat sink, and are composed of a sample and reference cell surrounded by heat conducting thermopiles. Cells are maintained at isothermal conditions, so any process requiring or evolving heat within the cell will result in a flow of heat either into the cell from the heat sink, or from the cell to the heat sink. The miniature vapour chamber technique can be used in the quantification of amorphous material (Lehto and Laine, 1997; Angberg et al., 1992a, b; Briggner et al., 1994; Buckton and Darcy, 1999). The sample under investigation is exposed to a controlled humidity or solvent atmosphere and the vapour phase is generated within the sample chamber. Another technique that can be used in the quantification of amorphous content is the RH perfusion unit. With the RH perfusion unit technique the wetting and adsorption or absorption response is detected as the solvent vapour is generated outside the sample chamber (Phipps and Mackin, 2000).
Figure 6. Typical microcalorimetric heat flow curves for the recrystallization of lactose samples spray dried from various ethanol:water mixtures, using the miniature humidity chamber technique (54% RH, 25°C) (Harjunen et al., 2002).

2.3.3 Differential scanning calorimetry (DSC)

DSC measures the difference in energy input into a sample and a reference material as a function of temperature. The required energy is directly proportional to the energy change in the systems and therefore permits measurements of enthalpy associated with the process (Venthoye, 1997). Endothermic or exothermic changes are registered as a peaks or troughs in the DSC trace. In addition to determination of the amorphous content of a sample, DSC can be used to identify changes associated with melting, boiling, degradation, release of solvate and polymorphic transformation. DSC appears to be able to estimate the degree of disorder with an overall sensitivity of ± 5-10% (Saleki-Gerhardt et al., 1994). Figure 7 shows typical DSC traces for 100% crystalline, 15% amorphous and 100% amorphous lactose samples. Thermograms of 100% crystalline and 15% amorphous lactose have an endothermic peak at 110-150°C, this is due to dehydration of readily desorbable (i.e. it is not physically adsorbed water), and/or hydrate water (Chidavaenzi et al., 1997; Buckton et al., 1998). Thermograms of 15%
and 100% amorphous lactose have an exothermic peak at 150-170°C due to the crystallization of amorphous lactose (Sebhatu et al., 1994). An endothermic peak at 215-225°C is due to melting of α-lactose followed by melting of β-lactose (Buckton et al., 1998).

![DSC traces](image)

**Figure 7.** Typical DSC traces for 100% crystalline (A), 15% amorphous (B) and 100% amorphous (C) lactose samples. Lactose samples were prepared by spray drying. Ethanol:water ratio in a feed solution was 100:0 (A); 40:60 (B) and 0:100 (C) (Harjunen et al., 2002).

### 2.3.4 X-ray powder diffraction (XRPD)

When a solid is exposed to X-ray beams, the radiation is scattered in all directions. In some directions, the scattered beams are completely in phase and reinforce one another to form diffracted beams, a phenomenon that can be described by Bragg’s law (Zeng et al., 2001b). XRPD is a well-establish method used to analyse the crystallography of particles but it has been shown to be of limited use in detecting the existence of the very small amount of amorphous material (< 10% w/w) often present in pharmaceutical powders (Saleki-Gerhardt et al., 1994; Sehatu et al., 1994). The XRPD is often used in combination with other methods, such as thermal analysis, to characterise such powders ‘fully’ (Zeng et al., 2001b). The X-ray powder pattern of every crystalline form of a compound is unique, making this technique particularly suited to the identification of different polymorphic forms of a compound. Amorphous materials typically exhibit a broad band whereas predominantly crystalline powders will give a trace with an array of
distinct sharp peaks. As an example, the X-ray diffraction patterns of lactose samples whose amorphous content varies from 0% to 100% are shown in Figure 8.

![X-ray diffraction patterns of lactose samples](image)

**Figure 8.** X-ray diffraction patterns of lactose samples prepared by spray drying. Ethanol:water ratio in a feed solution was 100:0 (0% amorphous), 40:60 (15% amorphous), 30:70 (45% amorphous), 20:80 (77% amorphous) and 0:100 (100% amorphous) (Harjunen et al., 2002).

### 2.3.5 Solution calorimetry

Solution calorimetry is a thermal analysis technique in which the temperature change produced by a chemical or physical interaction during the mixing of two solutions or of a solid and a liquid in a constant temperature environment is monitored as a function of time (Gao and Rytting, 1997). The heat of solution of any particular solid in one of its solvents can be measured (Hogan and Buckton, 2000). The two phase system comprises of a solvent and a known amount of solute in a reaction cell /glass ampoule, which are housed together in the reaction vessel and equilibrated to the temperature at which the reaction is to take place. Following equilibrium, the reaction cell is opened or the glass ampoule is broken, thus initiating the reaction and allowing the heat of solution to be
measured. The heat is directly measured without other invasive processes except for dissolution or wetting of the materials (Gao and Rytting, 1997).

Solution calorimetry has been used to determine the amorphous content of drugs and excipients accurately (Pikal et al., 1978; Gao and Rytting, 1997; Suryanarayanan and Mitchell, 1984; Grant and York, 1986; Hendriksen, 1990; Hogan and Buckton, 2000). It has been demonstrated that solution calorimetry is capable of detecting the presence of as little as 0.5% amorphous material in a sample (Hogan and Buckton, 2000). However, the enthalpy of solution measurement may be limited by the solvent, since the solubility and dissolution rate of the compound in the chosen solvent need to be reasonably high (Gao and Rytting, 1997; Buckton and Darcy, 1999). As an example, thermograms of various amorphous (100%, 15%, 0%) spray-dried lactose samples are shown in Figure 9.

**Figure 9.** Thermograms ($\Delta H_{sol}$) of 100%, 45% and 0% amorphous spray-dried lactose samples determined by solution calorimetry (Harjunen et al., 2002).
3 AIM OF STUDY

The general objective of the present study was to evaluate the effects of physicochemical properties of lactose used as a carrier in dry powder inhalation on pulmonary deposition of a drug from multiple dose reservoir based DPI. The physicochemical properties of lactose were modified by spray drying. The specific aims of the study can be summarized as follows:

1. To investigate how the physicochemical properties of a carrier, the drug:carrier ratio and the storage of the formulation affect in vitro deposition of a drug from a reservoir based DPI.

2. To investigate the effect of composition of feed solution on the crystallinity of spray-dried lactose.

3. To develop a solution calorimetry method for the determination of the amorphous content of spray-dried lactose. The relationship between the enthalpy accompanied with the addition of a spray-dried lactose sample in a saturated aqueous solution and the amorphous content of the lactose samples was determined by solution calorimetry.
4 EXPERIMENTAL

4.1 Materials (I-IV)

α-Lactose monohydrate (Pharmatose® 325M and Pharmatose® 110M, DMV, The Netherlands), Flowlact-100® (Meggle GMBH, Germany), mannitol ($D_{90\%} 116 \mu m$), glucose anhydride ($D_{90\%} 106 \mu m$), micronised budesonide ($D_{90\%} 0.9 \mu m -1.3 \mu m$), micronized salbutamol sulphate ($D_{90\%} 1.4 \mu m$) and Taifun® DPI were kindly supplied by Leiras Oy and Focus Inhalation Oy (Turku, Finland). Absolute ethanol (AA) was obtained from Primalco (Rajamäki, Finland). Hexane (HPLC grade) and methanol (HPCL grade) were purchased from Rathburn (Scotland). Sodium dihydrogen phosphate was purchased from Riedel-de Haën (Germany) and acetonitrile (HPLC grade) was purchased from Merck (Germany). Tris(hydroxymethyl)aminomethane (Tris®) was purchased from Parr Instrument Company (Moline, USA). All other used materials were of analytical grade, and used as received.

4.2 Production of 0-100% crystalline lactose by spray drying (II-IV)

The spray-dried lactose carrier (II) was prepared from a 30% (w/w) α-lactose monohydrate (325M) suspension by spray drying with a Büchi Mini-Spray Drier 190 (Büchi Laboratorium-Technic AG, Switzerland) under the following conditions; air flow rate 15 (dial setting), atomizer air flow rate 500 Nl, heating rate 8 (dial setting), inlet temperature 120°C, outlet temperature 78°C and feed rate 5 ml/min. The nozzle diameter was 0.7 mm. A 30% (w/w) lactose suspension was prepared by dispersing α-lactose monohydrate into ethanol/water (20:80 w/w) solution. The amorphous content of the spray-dried lactose was 64% (w/w).

The crystalline micronized lactose (II) was prepared from a 15% (w/w) micronized lactose suspension by spray drying under the following conditions; air flow rate 15 (dial setting), atomizer air flow rate 800 Nl, heating rate 7 (dial setting), inlet temperature 107°C, outlet temperature 79°C and feed rate 5 ml/min. The nozzle diameter was 0.7 mm. A 15% (w/w) micronized lactose suspension was prepared by dispersing micronized lactose to absolute ethanol. The crystalline micronized lactose was 100% crystalline.

A crystalline lactose carrier (Table 2) was prepared by spray drying from the suspension containing 30 g α-lactose monohydrate (325M) and 70 g absolute ethanol. The spray drying variables were as follow; inlet temperature 105°C, outlet temperature
78°C, atomizer air flow rate 700 NL, feed rate 7 ml/min, air flow rate (dial setting) 15 and heating rate 7 (dial setting). The diameter of the nozzle was 0.7 mm. The crystalline lactose was 100% crystalline.

0-100% crystalline lactose samples were prepared by spray drying using the feed solution where the ratio of ethanol to water varied between 0:100 to 100:0 (III, IV). A 15% (w/w) lactose suspension or solution was spray dried with a Büchi Mini-Spray Drier 190 under the following conditions; air flow rate 15 (dial setting), atomizer air flow rate 700 NL, heating rate 7 (dial setting), inlet temperature 106-108°C, outlet temperature 72-80°C and feed rate 5 ml/min. The only exception was that lactose was spray dried from pure distilled water (0% ethanol) at an outlet temperature of 110°C and at inlet temperature of 160°C. The diameter of the nozzle was 0.7 mm.

Each type of lactose was packed into tightly closed plastic bottles and stored in a desiccator (with silica gel at room temperature) prior to the studies.

4.3 Characterization of the materials (I-IV)

4.3.1 Isothermal microcalorimetry (IMC) (I-IV)

The amorphous content of lactose (I-IV), glucose anhydrate (I), mannitol (II), budesonide (I, II) and salbutamol sulphate (I) samples was measured by IMC TAM 2277 (Thermometric AB, Sweden). The miniature humidity chamber technique (Angberg et al., 1992a, b) was employed to detect the thermal response for the recrystallization of amorphous samples. The extent of heat evolution was directly related to the degree of amorphicity. During the measurement, the sample was recrystallized in the moisture absorbed from the saturated salt solution, which was included in the hermetically sealed 3 ml glass ampoule as a desiccant, together with the sample. The samples stored in a silica desiccator at room temperature were accurately weighed just prior to the measurements. The samples and identical reference ampoules containing only the saturated salt solution were immediately placed in the equilibrium position of the TAM. The samples were lowered into the measuring position after 15 minutes of equilibration.

The lactose sample that was spray dried from pure water was considered to be totally amorphous (100%) because XRPD studies showed only diffuse scattering with no characteristic reflections of crystallinity in the diffractogram. The corresponding heat for the recrystallization process was taken as the reference value in the calculations of amorphous content of the lactose samples (I-IV). The reference value for the totally
amorphous salbutamol sulphate was 27.6 J/g (I). This reference value was taken from the literature (Backton et al., 1995a). Mannitol (I), glucose (I) and budesonide (I, II) were approx. 100% crystalline, as verified with XRPD measurements (Philips PW 1820, the Netherlands).

4.3.2 Differential scanning calorimetry (DSC) (II, III)

DSC analyses were made with a Perkin-Elmer DSC 7 (Perkin-Elmer, Norwalk, Connecticut, USA). The accurately weighed lactose (II, III) and the formulation containing budesonide (II) samples (3-10 mg) were measured in aluminium pans (40 µL). A single empty aluminium pan was used as a reference for all measurements. All DSC analyses were performed under an atmosphere of dry nitrogen. The heating rate was 10°C/min (the temperature range 25-233°C (II) and 2°C/min (the temperature range 30-350°C) (III). Temperature calibration was accomplished by a two-point calibration, using gallium and indium (II) or indium and tin as standards (III).

4.3.3 Solution calorimetry (IV)

The enthalpy of solution (ΔH_{sol}) and the enthalpy accompanied with an addition of a lactose sample in a saturated aqueous solution (ΔH_{sat}) were determined at room temperature using a Parr 1455 Solution Calorimeter (Parr Instrument Company, Moline Illinois, USA) equipped with a chart recorder. 0-100% crystalline lactose samples were prepared by the spray drying as described earlier (4.2). Physical mixtures were prepared by mixing 100% amorphous lactose and 100% crystalline lactose with a mortar and pestle for 10 min. The 100% amorphous lactose was prepared by spray drying of lactose from water. IMC measurements showed that an untreated commercial lactose (325M) was 100% crystalline and thus, it was used as the 100% crystalline lactose in these studies.

The solution calorimeter was calibrated using 0.50 g tris(hydroxymethyl)- aminomethane (Tris® Parr Instruments) and 100.00 g 0.1 N hydrochloride acid. The thermograms were recorded at a sensitivity of 10 mV or 0.1°C full scale and a chart speed of 3 cm/min. The samples were pre stored in a silica desiccator at room temperature, and accurately weighed (about 400 mg) just before the measurements. The ΔH_{sol} was determined in distilled water (100.00 g). The ΔH_{sat} was determined in a saturated aqueous solution (100.00 g), prepared from the corresponding lactose. A
negative value for the $\Delta H_{\text{lat}}$ and $\Delta H_{\text{vol}}$ indicated an evolution of heat (exothermic process), and a positive value indicated an absorption of heat (endothermic process).

4.3.4 Moisture content (II)

The moisture content of the lactose grades was measured by a Karl-Fischer titrator (Mettler DL 35 Karl-Fischer titrimeter, Greifensee, Switzerland).

4.3.5 Thermal gravimetry (TG) (III)

The TG measurements were made with a Perkin-Elmer configuration TGA 7, which was controlled by a TAC 7/DX controller and personal computer with Pyris Thermal Analysis System software version 3.52. Temperature calibration was accomplished by four thermomagnetic substances: alumel, nickel, nicoseal and perkalloy.

Measurements were made in platinum pans under a nitrogen flow rate of 50 ml/min. The weights of the spray-dried lactose sample were between 5 and 10 mg, and the temperature range was 30-200°C. A heating rate of 2°C/min was used in the measurements. Lactose sample was assumed to contain 100% $\alpha$-lactose monohydrate when the sample contained 5.2% w/w hydrate water. When sample contained less than 5.2% w/w hydrate water, the amount of lactose that was in the form of monohydrate in % of the total lactose was calculated as follows: (hydrate water % (w/w) in the sample / 5.2% (w/w)) x 100%.

4.3.6 Particle size (I, II, IV)

The particle size was analysed by dispersing a small amount of mannitol (I), glucose anhydrate (I) or lactose samples (I, II, IV) in 2-propanol. The particles were treated in an ultrasonic bath for 1 minute, to destroy aggregates. The measurements were carried out by laser light diffraction using a Malvern Mastersizer S (I, II) or Mastersizer 2000 Hydro 2000S (IV) (Malvern Instruments Ltd., Malvern, UK).

4.3.7 Specific surface area (III)

The specific surface areas of spray dried lactose samples was measured with a FlowSorb 2300 (Micromeritics, Norcross, Georgia, U.S.A.) by determining the quantity of gas adsorbed as a single layer of molecules on a sample. The gas was composed of
30% nitrogen and 70% helium. Samples were stored in a vacuum silica desiccator (at least 24h at room temperature) before measurements.

4.3.8 Scanning electron microscopy (SEM) (I-III)

The surface roughness and shape of particles were evaluated by a scanning electron microscope (SEM) (JSM-35 Scanning microscope, Joel Tokyo, Japan (I, III) or XL30 ESEM TMP microscope, FEI/Philips, the Czech Republic (I, II)). The samples were coated with gold under vacuum (Sputter Coater II- E 5100, Polaron Equipment, England), and all micrographs were obtained at an acceleration voltage of 15 kV.

4.4 Preparation and characterization of inhalation powders (I, II)

4.4.1 Preparation of lactose grades used as a carrier (I, II)

Commercially available α-lactose monohydrate 325M and 110M were used as the carriers (I, II). The spray-dried lactose carrier (II) was prepared from a 30% (w/w) α-lactose monohydrate (325M) suspension by spray drying as described earlier (4.2). The crystallized spray-dried lactose (II) was prepared by mixing the spray-dried lactose in ethanol at 60°C for one hour. The crystallized spray dried lactose was collected after filtration and evaporation.

In addition, a commercially available spray-dried lactose, Flowlac-100® (II), was used as a carrier, either untreated or mixed with the crystalline micronized lactose (II). The crystalline micronized lactose (II) was prepared by passing α-lactose monohydrate (325M) through a jet mill (JM-80, Fryma, Herts, UK), up to two times, and operated at an air pressure of 5 bars. After milling the micronized lactose was spray dried from absolute ethanol as described in 4.2 section. The mixing of Flowlac-100® with the crystalline micronized lactose (II) was performed as follows; 2.5 g of the crystalline micronized lactose was dispersed for 30 seconds in an ethanol:hexane medium (2:98 v/v) by ultrasonication. Flowlac-100® (50 g) was added and dispersion was mixed for 20 minutes at 200-400 rpm. The suspension was filtered through a Büchner funnel (GF 52 Ref. No. 428248, Schleicher & Schuell), and the powder was dried for 2.5 h at 40-50°C under vacuum (100 mbar).

The crystalline lactose (Harjunen et al., 2002) was prepared by spray drying from the suspension containing 30 g untreated commercial α-lactose monohydrate (325M) and 70 g absolute ethanol as described 4.2 section.
Each type of lactose carrier was packed into tightly closed plastic bottles and stored in a desiccator (with silica gel at room temperature) prior to the studies.

### 4.4.2 Formulation of inhalation powders (I, II)

The studied formulations are summarized in Table 2. Budesonide (I, II) and salbutamol sulphate (I) were used as model drugs. Different lactose grades, i.e. α-lactose monohydrate, 325M (II) and 110M (I), the spray-dried lactose (II), the crystallized spray-dried lactose (II), Flowlac-100® (II), Flowlac-100 mixed with crystalline micronized lactose (II), the crystalline lactose (Harjunen et al., 2002), mannitol (I) and glucose anhydrate (I) were used as the carrier.

**Table 2. The compositions of the studied inhalation powders (I, II).**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug (µg) / dose</th>
<th>Composition</th>
<th>Drug</th>
<th>Carrier</th>
<th>Drug:Carrier (w/w%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSS5</td>
<td>5</td>
<td>Salbutamol sulphate</td>
<td>Mannitol</td>
<td>1:296.6</td>
<td></td>
</tr>
<tr>
<td>MSS25</td>
<td>25</td>
<td>Salbutamol sulphate</td>
<td>Mannitol</td>
<td>1:58.5</td>
<td></td>
</tr>
<tr>
<td>MB10</td>
<td>10</td>
<td>Budesonide</td>
<td>Mannitol</td>
<td>1:160.3</td>
<td></td>
</tr>
<tr>
<td>MB50</td>
<td>50</td>
<td>Budesonide</td>
<td>Mannitol</td>
<td>1:31.3</td>
<td></td>
</tr>
<tr>
<td>MB100</td>
<td>100</td>
<td>Budesonide</td>
<td>Mannitol</td>
<td>1:15.1</td>
<td></td>
</tr>
<tr>
<td>GSS5</td>
<td>5</td>
<td>Salbutamol sulphate</td>
<td>Glucose</td>
<td>1:296.6</td>
<td></td>
</tr>
<tr>
<td>GSS25</td>
<td>25</td>
<td>Salbutamol sulphate</td>
<td>Glucose</td>
<td>1:58.5</td>
<td></td>
</tr>
<tr>
<td>GB10</td>
<td>10</td>
<td>Budesonide</td>
<td>Glucose</td>
<td>1:160.3</td>
<td></td>
</tr>
<tr>
<td>GB50</td>
<td>50</td>
<td>Budesonide</td>
<td>Glucose</td>
<td>1:31.3</td>
<td></td>
</tr>
<tr>
<td>GB100</td>
<td>100</td>
<td>Budesonide</td>
<td>Glucose</td>
<td>1:15.1</td>
<td></td>
</tr>
<tr>
<td>L1B100</td>
<td>100</td>
<td>Budesonide</td>
<td>Lactose 110M</td>
<td>1:15.1</td>
<td></td>
</tr>
<tr>
<td>L2B100</td>
<td>100</td>
<td>Budesonide</td>
<td>Lactose 325M</td>
<td>1:15.1</td>
<td></td>
</tr>
<tr>
<td>L3B100</td>
<td>100</td>
<td>Budesonide</td>
<td>Spray-dried lactose</td>
<td>1:15.1</td>
<td></td>
</tr>
<tr>
<td>L4B100</td>
<td>100</td>
<td>Budesonide</td>
<td>Crystallized spray-dried lactose</td>
<td>1:15.1</td>
<td></td>
</tr>
<tr>
<td>L5B100</td>
<td>100</td>
<td>Budesonide</td>
<td>Flowlac-100®</td>
<td>1:15.1</td>
<td></td>
</tr>
<tr>
<td>L6B100</td>
<td>100</td>
<td>Budesonide</td>
<td>Flowlac-100® with crystalline micronized lactose</td>
<td>1:15.1</td>
<td></td>
</tr>
<tr>
<td>L7B100*</td>
<td>100</td>
<td>Budesonide</td>
<td>Crystalline lactose</td>
<td>1:15.1</td>
<td></td>
</tr>
</tbody>
</table>

* Harjunen et al., 2002
The budesonide:carrier ratio was 1:160.3 (w/w) (10 µg dose) (I), 1:31.3 (w/w) (50 µg dose) (I) and 1: 15.1 (w/w) (100 µg dose) (I, II). The formulations were prepared in suspension as follows (Lankinen, 1999); micronized budesonide, 0.31 g (10 µg dose), 1.55 g (50 µg dose) or 3.10 g (100 µg dose) was dispersed for 5 minutes in hexane (100 ml) by ultrasonication (Ultrasonic cleaner, Laborette 17, 170 W, Fritsch GmbH, Germany) and suspension was mixed at 300-400 rpm. After that, hexane (100 ml) and carrier, 49.69 g (10 µg dose), 48.45 g (50 µg dose) or 46.90 g (100 µg dose) were added while mixing was continued for 10 minutes without ultrasound. The suspensions were filtered through a Büchner funnel (GF 52 Ref. No. 428248, Schleicher & Schuell). The budesonide formulations were dried in a rotary evaporator for 1.5 h, rotating at 10 rpm under a vacuum of about 100 mbar at 40°C.

The salbutamol sulphate:carrier ratios were 1:296.6 (w/w) (5 µg dose) (I) and 1:58.5 (w/w) (25 µg dose) (I). Micronized salbutamol sulphate, 0.17 g (5 µg dose) or 0.84 g (25 µg dose), was mixed for 30 min at 55°C (200-400 rpm) in 4% (v/v) ethanol/n-hexane solution (35 ml) and after that, n-hexane (35 ml) and carrier, 49.83 g (5 µg dose) or 49.16 g (25 µg dose), were added while mixing was continued for 10 minutes by ultrasound (30 s). Salbutamol sulphate was mixed at 55°C in order to crystallize its initial 11% amorphous content. The suspensions were filtered through a Büchner funnel (GF 52 Ref. No. 428248, Schleicher & Schuell). The salbutamol sulphate inhalation powder was dried in a rotary evaporator for 2.5 h, and the powder was rotated a few rounds at intervals of 30 minutes under a vacuum of about 100 mbar at 40-50°C.

Finally the budesonide and salbutamol sulphate formulations were manually sieved (Ø 1.0 mm) and packed into tightly closed plastic bottles. After preparation, all formulations were stored for 5-7 days in a desiccator (33% RH at room temperature) and then the formulation (0.5 g) was accurately measured into the two Taifun® inhalers and stored for one day in a test chamber at 25°C/60% RH (WK 11-180/40, Weiss Tecnik GmbH) prior to the studies. All formulations were also placed in the stability chamber at 40°C/75% RH, in a permeable polystyrene tubes (Brennan et al., 1974; Bellamy et al., 1980), for one month for later evaluation. This means that the inhalation powders were exposed to more moisture vapor in the polystyrene tubes than would be the case if they were stored inside Taifun® inhalers (Focus Inhalation Oy, data on file). Thus, the effect of moisture on the performance of the inhalation powder could be evaluated more rapidly via the use of the polystyrene tubes.
4.4.3 HPLC analysis of drugs (I, II)

Budesonide and salbutamol sulphate were analysed by high performance liquid chromatography, HPLC. In the case of budesonide, a mobile phase of methanol, acetonitrile and 0.017 M sodium dihydrogen phosphate buffer (pH 3.2) (30:30:40 v/v/v) at a flow rate of 1.0 ml/min was used (I, II). In the case of salbutamol sulphate, a mobile phase of acetonitrile and 0.025 M sodium dihydrogen phosphate buffer (pH 3.0) (4:96 v/v) at a flow rate of 1.0 ml/min was used (I). The HPLC system consisted of a Spectra System® detector, a Spectra Series® pump, a Spectra Series® autosampler, a Spectra Series® solvent degasser (Thermo Separation Products, USA). Budesonide and salbutamol sulphate were analysed by a 150 x 4.0 mm I.D. column packed with 5 μm Inertsil C-8 (GL Sciences inc.) and by a 125 x 4.0 mm guard column packed with 5 μm LiChrosphere 60 RP-Select B, respectively. The retention times of budesonide and salbutamol sulphate were about 6 and 5 min, these being monitored at wavelengths of 249 nm and 224 nm, respectively. The precision of the HPLC method was tested daily by analyzing the appropriate budesonide or salbutamol sulphate standard solution 5 times in a row and the R.S.D. of the peak area was always < 2%.

4.4.4 The uniformity of emitted drug dose (I, II)

The uniformity of the emitted drug dosage was investigated in the test chamber (25°C/60% RH). The first 25 doses were individually emitted by Taifun® into the dosage unit sampling apparatus (Ph. Eur. 4th Ed. 2002). The first 5 doses were omitted from the calculations (i.e., only doses from 6 to 25 were included). The test was performed at a flow rate of 30 L min⁻¹, at a flow time of 8 s. Each budesonide dosage unit sampling apparatus was carefully washed with 10 ml of methanol:sodium dihydrogen phosphate buffer (0.017 M, pH 3.2) mixture (50:50 v/v) (I, II). In the case of salbutamol sulphate, the dosage unit sampling apparatus was washed with 10 ml of sodium dihydrogen phosphate buffer (0.025 M, pH 3.0) (I). The concentrations of the drugs were determined by HPLC, as described above. The emitted drug doses were measured from two inhalers before and after a storage period of one month at 40°C/75% RH.
4.4.5 In vitro deposition of drugs (I, II)

The pulmonary deposition of drugs was evaluated by the Andersen Sampler (Ph. Eur. 4th Ed. 2002) (the impactor stages were not coated with a viscous liquid), using a vacuum pump and 3-way valve, operated at a flow rate of 28.3 L min⁻¹, at a flow time of 8 s. The Andersen sampler consists of a throat, a preseparator, eight stages and a final filter. The test was carried out on the same DPs described in section 4.4.4. A total of 20 doses were released from the Taifun³ DPI to a cascade impactor at intervals of one minute. The deposition of drug from each inhaler was determined twice; i.e., depositions of doses from 26 to 45 and from 46 to 65 were studied. The budesonide formulation was washed from the collection stages of the impactor, the preseparator and throat with methanol:sodium dihydrogen phosphate buffer (0.017 M, pH 3.2) mixture (50:50 v/v) (I, II). In the case of salbutamol sulphate, the collection stages of the impactor, the preseparator and throat were washed with sodium dihydrogen phosphate buffer (0.025 M, pH 3.0) (I). The drug concentrations in these samples were analysed by HPLC, as described in section 4.4.3.

A variety of parameters were employed to characterize the deposition profiles of the drugs. The recovered mass (RM) was the sum of the drug from each of the cascade impactor stages (plates + frame 0-7 and filter), metal throat, which included the DPI adapter, and from the preseparator. The fine particle mass (FPM) (particle size < 5.8 μm) was the sum of the amount of the drug recovered from stages 2 to 7 and the filter. The respirable fraction (RF%) was calculated as the ratio of FPM to RM. The mass median aerodynamic diameter (MMAD) was calculated with the cumulative drug percentages at each stage, from filter to stage 0. The MMAD value was taken as the particle size at a cumulative percentage value of 50%.

4.4.6 Bulk density of formulations (II)

The bulk densities (g/ml) of formulations were determined by measuring the volume of a known mass of formulation that has been passed through a screen into a graduated cylinder (25 ml). The bulk density was measured twice, after preparation and then after storage.
4.4.7 Statistical analysis (I-IV)

The non-parametric Kruskal-Wallis test was used to test the differences between multiple groups; significance in the differences in the means was tested using the Games-Howell’s multiple range test (I-IV). Mann-Whitney U-test was used to test the differences between means of two independent groups (I, II). The level of significance was taken as $p < 0.05$. 

5 RESULTS AND DISCUSSION

5.1 Effect of carrier on in vitro pulmonary deposition of drugs (I, II)

The multiple dose reservoir based Taifun® was used as a DPI to study the effects of the physicochemical properties of a carrier on the pulmonary deposition of a drug (I, II). The Taifun® has a high resistance to airflow while drug delivery to the lungs is relatively independent of inhalation flow (Pitcairn et al., 2000). In the majority of previous studies, single-unit-dose DPIs have been used as the device (e.g. Kawashima et al., 1998a; Tee et al., 2000; Zeng et al., 1999, 2000a, b, 2001c).

5.1.1 Physicochemical properties of carriers (I, II)

Carrier particles are typically the main component in inhalation powders and thus, any change in the physicochemical properties of carrier particles may affect lung deposition of drug (Tee et al., 2000; Clarke et al., 2001; Iida et al., 2001; Zeng et al., 2001c). In this study, mannitol (I), glucose (I), α-lactose monohydrate 325M (II) and 110M (I), spray-dried lactose (II), crystallized spray-dried lactose (II), Flowlac-100®, Flowlac-100® with crystalline micronized lactose (II) and crystalline lactose (Harjunen et al., 2002) were used as the carriers (Table 2).

The average particle size (D_{50%}) of different carriers was quite similar before storage, about 120 μm, except α-lactose monohydrate 325M (D_{50%} = 58 μm), spray-dried lactose (D_{50%} = 46 μm) and crystalline lactose (D_{50%} = 55 μm).

The particle shape of the carriers varied from irregular (α-lactose monohydrate 325M (II: Fig. 1a) and 110M (I: Fig. 1a), glucose (I: Fig. 1c), crystallized spray-dried lactose (II: Fig. 1c), crystalline lactose (Harjunen et al., 2002)) to spherical (spray-dried lactose (II: Fig. 1b), Flowlac-100® (II: Fig. 1d), and Flowlac-100® with crystalline micronized lactose (II: Fig. 1f)). The shape of mannitol was elongated (I: Fig. 1b).

The surface area of the mannitol was higher than that of glucose and α-lactose monohydrate 110M (I: Table 1).

The moisture content of α-lactose monohydrate 325M, crystallized spray-dried lactose, Flowlac-100® and Flowlac-100® with crystalline micronized lactose was between 5.2% and 5.5% (w/w) initially (II: Table 2). This amount of moisture content corresponds to hydrate water. The moisture content of spray-dried lactose was 3.6% (w/w) initially but the moisture content of spray-dried lactose increased substantially during the storage period (II: Table 2). The moisture content of Flowlac-100® without
and with crystalline micronized lactose slightly increased during the storage period (II: Table 2).

Spray-dried lactose (II), Flowlac-100° and Flowlac-100° with crystalline micronized lactose (II) contained 64%, 3% and 2% amorphous material, respectively, initially while α-lactose monohydrate 325M (II) and 110M (I), glucose (I), mannitol (I) and crystallized spray-dried lactose (II) and crystalline lactose (Harjunen et al., 2002) were 100% crystalline. All carriers were 100% crystalline after 1 month’s storage at 40°C / 75% RH due to recrystallization of amorphous portions. This result can be explained by the fact that crystallization of lactose occurs spontaneously when RH is higher than 52% (Naini et al., 1998).

Thermograms of α-lactose monohydrate 325M, spray-dried lactose, crystallized spray-dried lactose, Flowlac-100° and Flowlac-100° with crystalline micronized lactose were measured both with and without budesonide by DSC initially and after storage (II). These results proved that the DSC thermograms of carriers, both with and without budesonide, were identical indicating that the suspension method did not change the thermodynamic properties of the carriers during the manufacturing. The only exception was that the DSC thermogram of the highly amorphous (64%) spray-dried lactose sample was slightly modified due to the suspension. This is not surprising since the amorphous state is metastable with respect to the crystalline form leading to significant physical stability problems during dosage form development and subsequent manufacture.

5.1.2 Formulations containing mannitol as the carrier (I)

The ratio of the emitted salbutamol sulphate dose to the theoretical dose (5 or 25μg) varied from 90% to 96% when mannitol was used as the carrier before and after storage period (Fig. 10, A). The storage increased (5 μg) or did not change (25 μg) the emitted dose of salbutamol sulphate (Fig. 10, A).

The RF% values of salbutamol sulphate particles initially increased when the drug:carrier ratio was increased from 1:296.6 (5 μg/dose) to 1:58.5 (25 μg/dose) (Fig. 10, B). The increases in the RF% values accompanying the increases in the drug:carrier ratio may be due to the fact that the detachment of the drug from the carrier does not occur efficiently when the amount of the drug is low in the mixture, since the drug particles initially occupy the high energy adhesion sites on the carrier particles, as described by Staniforth (1996). As a result, the fraction of irreversible bonds between the drug particles and the carrier particles becomes higher when the drug concentration
is low. At higher drug doses, more drug particles may be adhering to sites with less strong binding affinities on the surface of carriers (I: Figs. 2-5). However, the effect of the drug:carrier ratio on the RF% values of the drug is not straightforward because some studies have demonstrated that the fine particle fraction of the drug can decrease when there is an increase in the ratio of drug to the carrier (Braun et al., 1996; Steckel and Müller, 1977b). In this study, the storage also strongly decreased the RF% values of salbutamol sulphate irrespective of the dose (Fig. 10. B).

A)

B)

**Figure 10.** The emitted dose (%) pattern (A) and respirable fraction (RF%) pattern (B) of salbutamol sulphate (5 and 25 μg/ dose) before and after storage (1 month at 40°C and 75% RH) when either mannitol or glucose was used as the carrier. Mean values ± S.D. are shown.
Figure 11. The emitted dose (%) pattern (A) and respirable fraction (RF%) pattern (B) of budesonide (10, 50 and 100 μg/ dose) before and after storage (1 month at 40°C and 75% RH) when either mannitol or glucose was used as the carrier. Mean values ± S.D. are shown.

In the case of budesonide, the ratio of the emitted budesonide dose to the theoretical dose (10-100 μg) varied from 84% to 94% when mannitol was used as the carrier before and after storage period (Fig. 11. A). The storage period had an effect on emitted doses so that the emitted dose decreased with a low budesonide dose (10 μg) and it increased
with a high dose (100 µg) after storage. The exception was the budesonide dose of 50 µg at which the storage period had no significant effect on emitted dose (I: Table 2).

The RF% values of budesonide increased with an increase in the drug:carrier ratio from 1:160.3 (10 µg/dose) to 1:15.1 (100 µg/dose) before and after storage (Fig. 11. B). The storage decreased the RF% values of budesonide at the dose of 10 µg. In contrast, the RF% value of budesonide at the dose of 50 µg increased after storage while the storage period had no significant effect on the RF% value of budesonide when the dose was 100 µg (I: Table 2).

When mannitol was used as the carrier, the results indicate that the drug:carrier ratio had no clear effects on the emitted doses. Instead, the RF% values of salbutamol sulphate and budesonide increased when the drug:carrier ratio was increased before and after storage.

5.1.3 Formulations containing glucose as the carrier (I)

The ratio of the emitted salbutamol sulphate dose to the theoretical dose (5 or 25µg) varied from 77% to 99% when glucose was used as the carrier before and after the storage period (Fig. 10. A). The emitted salbutamol sulphate doses decreased after storage (Fig. 10. A). When compared to mannitol at the corresponding dose, the emitted salbutamol sulphate dose was higher when glucose was used as the carrier before storage, while the emitted salbutamol sulphate dose was lower when glucose was used as the carrier after storage (Fig. 10. A).

The RF% values of salbutamol sulphate particles initially increased when the drug:carrier ratio was increased from 1:296.6 to 1:58.5 (Fig. 10. B). This trend was not obvious after the storage of the formulations as the storage strongly decreased the RF% values of salbutamol sulphate irrespective of the dose. When compared to mannitol before and after storage at the corresponding salbutamol sulphate dose, the glucose containing formulations tended to have lower RF% values (except for the formulation with the salbutamol sulphate dose of 25 µg before storage) (Fig. 10. B).

These results can be explained by the fact that glucose can absorb a significant amount of moisture at 40°C and 75% RH humidity (Callahan et al., 1982; Kibbe, 2000). Maggi et al. (1999) showed that the high moisture content of the powder strongly decreased the RF% values of disodium cromoglycate. The sorption of water vapour by the solid material increases the interparticulate forces via liquid bridging and fusion among the particles (Zeng et al., 2001b). When glucose was used as the carrier, the
agglomerates were formed in the presence of the salbutamol sulphate (data not shown), whereas no agglomerates were observed in the presence of budesonide.

In the case of budesonide, the ratio of the emitted budesonide dose to the theoretical dose (10-100 μg) varied from 96% to 106% when glucose was used as the carrier before and after the storage period (Fig. 11. A). The emitted budesonide dose of 10 μg decreased significantly after storage while the storage period had no effect of the emitted budesonide dose of 50 and 100 μg. When compared to mannitol at the corresponding budesonide dose, the emitted budesonide dose doses were higher when glucose was used as the carrier before and after storage (Fig. 11. A).

The drug:carrier ratio had no significant effect on the RF% values of budesonide before storage with glucose used as the carrier (Fig. 11. B). However, the RF% values of budesonide increased with an increase in the drug:carrier ratio after storage.

When compared to mannitol at the corresponding budesonide dose, the glucose containing formulation tended to have lower RF% values before and after storage (Fig. 11. B).

5.1.4 Formulations containing lactose as the carrier (I, II)

The effects of physicochemical properties lactose on in vitro deposition of budesonide (dose 100 μg) were investigated. Lactose grades that were studied are shown in Table 2. The selected lactose carriers have different physicochemical properties, such as particle size, shape and amorphous content, as described in section 5.1.1.

The ratio of the emitted budesonide dose to the theoretical dose (100 μg) varied from 3% to 98% when lactose was used as the carrier before and after the storage period (Fig. 12. A). When untreated α-lactose monohydrate 110M (L1B100) and 325M (L2B100) were used as the carrier, the ratio of emitted budesonide dose to the theoretical dose (100 μg) varied from 89% to 98% before and after storage period (Fig. 12. A). Initially, the ratio of emitted budesonide dose to the theoretical dose (100 μg) was only 3% when spray-dried lactose was used as a carrier (L3B100) but it increased up 65% after storage. Similarly the emitted budesonide dose was substantially lower than the theoretical dose when crystallized spray-dried lactose (L4B100) was used as a carrier before (39%) and after storage (47%) (Fig. 12. A). The emitted budesonide doses from the formulation containing crystalline lactose as a carrier (L7B100) were 86% and 87% before and after storage, respectively (Fig. 12. A). Also, the emitted budesonide dose was close to the theoretical dose before and after storage when Flowlac-100® was used as the carrier (L5B100) (Fig. 12. A). The emitted budesonide dose decreased when
Flowlac-100\textsuperscript{®} with crystalline micronized lactose was used as the carrier (L6B100) instead of Flowlac-100\textsuperscript{®} (Fig. 12 A).

Taifun\textsuperscript{®} is a reservoir based powder inhaler, and the volume of the measure cup is constant (Pitcairn et al., 1995). Thus the bulk density of formulation affects the emitted dose. The bulk densities of the formulations varied from 0.33 g/ml to 0.58 g/ml (II: Fig 3A). The emitted budesonide dose increased as a function of bulk density of formulation (II: Fig. 3B). When the bulk density of the formulation ranged between 0.48 and 0.58 g/ml, the emitted budesonide dose varied between 83 – 98%. Emitted budesonide doses were clearly lower than the theoretical dose when either spray-dried lactose or crystallized spray-dried lactose were used as the carrier. These low emitted budesonide dose results are at least partly due to the low bulk density of these formulations (0.33 g/ml – 0.39 g/ml). The low emitted dose of spray-dried lactose or crystallized spray-dried lactose containing formulation cannot be explained only by the low bulk density, also the flow properties of these formulations may be poor. Spray-dried lactose contains a great many small particles with a diameter < 10 \mu m at which van der Waals forces will predominate over gravitational forces and such particles are often highly cohesive and have poor flowability (Zeng et al., 2001b). Iida et al. (2001) have shown that the flow properties of the powder mixture can markedly affect drug emission from the device. In the present study, the emitted budesonide dose substantially increased after storage of the formulation containing spray-dried lactose (Fig. 12 A), suggesting that the flow properties of the powder increased. This might be due to reduced effects of triboelectrification on powder behaviour, as moisture sorption on the particle surface is an efficient way to dissipate any surface charge (Zeng et al., 2001b). The amorphous content of spray-dried lactose was 64% before storage. It is well-known that amorphous lactose can adsorb a significant amount of moisture at 40°C / 75% RH (Briggner et al., 1994; Stubberud and Forbes, 1998; Naini et al., 1998).
A) 

B) 

Figure 12. The emitted dose (%) pattern (A) and respirable fraction (RF%) pattern (B) of budesonide before and after storage (1 month at 40°C and 75% RH) when different lactose grades were used as the carrier. Mean values ± S.D. are shown. Symbols are shown in Table 2. * Data significantly different if compared to L1B100 at the corresponding time (P<0.05). # Data significantly different if compared to corresponding carrier before storage (P<0.05).

The effects of lactose grade on the RF% values of budesonide are shown in Figure 12. B. The formulation containing crystallized spray-dried lactose as a carrier (L4B100) showed a high RF% value (45%) before and (44%) after storage, as the storage period
had no effect on RF%. The lowest value of RF% (10%) was initially observed when Flowlac-100® was used as a carrier (L5B100). The RF% was low, probably due to fact that the surfaces of Flowlac-100® carrier particles are very rough. Staniforth (1996) produced a schematic surface energy map for a lactose crystal, showing iso-energetic adhesion sites between the drug and carrier surfaces. When drug particles occupy the highest energy binding sites of a carrier, pulmonary deposition of the drug decreases due to incomplete powder deaggregation with DPI. According to Podczeck (1998), separation of the drug from the carrier in the air stream does not occur when surface roughness of the carrier material is large. Earlier studies have demonstrated that drug particle adhesion to carrier surfaces can be decreased by altering the size distribution of the carrier (Staniforth, 1996; Podczeck, 1998; Zeng et al., 1998). For example, Zeng et al. (1998, 2001a) have reported that the FPF of the salbutamol sulphate can be increased by adding a third component to the inhalation powder formulation, such as micronized lactose or magnesium stearate. In the present study, when compared to Flowlac-100® (L5B100), RF% increased clearly when Flowlac-100® was mixed with crystalline micronized lactose (L6B100) (Fig. 12. B). This result demonstrates that adhesion of budesonide to Flowlac-100® particles decreased in the presence of micronized lactose. The RF% value of budesonide increased when α-lactose monohydrate (325M and 110M), Flowlac-100®, Flowlac-100® with crystalline micronized lactose and crystalline lactose (L7B100) were used as a carrier after storage. In the case of spray-dried lactose (L3B100), the RF% value significantly decreased during storage.

Figure 12 shows that the highest RF% values of budesonide were achieved when the crystallized spray-dried lactose (L4B100) was used as a carrier before and after storage, but emitted budesonide doses were lower than the theoretical dose. When Flowlac-100® with crystalline micronized lactose was used as a carrier (L6B100), the emitted budesonide dose was close to the theoretical dose and high RF% values were achieved, but RF% values increased after storage (Fig. 12). Crystalline lactose (L7B100) seems to be the best carrier for budesonide because the emitted budesonide dose was close to the theoretical dose before and after storage and the storage period changed the RF% values less than in the cases of other carriers. Such crystals may be particularly beneficial for multiple dose reservoir DPI, which usually requires its powders to possess good flow properties to ensure a uniform delivery of the drug.
5.1.5 The effects of storage (I, II)

The effects of storage of formulation on in vitro deposition of salbutamol sulphate (I, II) and budesonide (I, II) were evaluated after a 1 month storage period at 40°C and 75% RH humidity. Environmental conditions (temperature, humidity) may adversely affect aerosol generation and the particle size distributions from DPIs either by modifying adhesive or cohesive properties of the formulation or by inducing hygroscopic growth (Jashnani et al., 1995; Jashnani and Byron, 1996).

When glucose was used as the carrier, the emitted salbutamol sulphate dose decreased irrespective of the dose after storage (Fig. 10. A) whereas the emitted budesonide dose did not change (except at the budesonide dose of 10 μg) (Fig. 11. A). Glucose and hydrophilic salbutamol sulphate were observed to form tight agglomerates during the storage (data not shown). Budesonide and glucose did not form these kinds of agglomerates. Further, salbutamol sulphate and budesonide did not form any agglomerates with mannitol. The effects of storage can be explained by the fact that glucose can absorb a significant amount of moisture at 40°C and 75% RH humidity while the moisture sorption capacity of mannitol is less (Kibbe, 2000). It must be noted that in addition to glucose, salbutamol sulphate is also sensitive to moisture sorption (Jashnani et al., 1995). The sorption of water vapour by the solid material increases the interparticulate forces via liquid bridging and fusion between the particles (Zeng et al., 2001b).

In the cases of different lactose grades, the emitted budesonide dose clearly increased after storage of the formulation containing spray-dried lactose (Fig. 12. A). This result suggests that the flow properties of the powder had improved. This might be due to reduced effects of triboelectricity on powder behaviour, as moisture sorption on the particle surface is an efficient way to dissipate surface change (Braun et al., 1996). In addition, spray-dried lactose and budesonide formed agglomerates during storage, as indicated by the increased MMAD values after storage (II: Table 3). This in not surprising because amorphous lactose is sensitive to moisture sorption and the sorption of water vapour by the solid material increases the interparticulate forces via liquid bridging and fusion among the particles (Zeng et al., 2001b).

The storage decreased the RF% values of salbutamol sulphate irrespective of the carrier (I: Table 3, Fig. 10. B). These results indicate that the interparticulate forces via liquid bridging and fusion among the particles within the powders had increased and this resulted in incomplete detachment of the drug from the carrier after storage. Mixing and deaggregation processes depend on the competition between the adhesion and
cohesion forces existing between drug-carrier, carrier-carrier or drug-drug (Bérard et al., 2002). Both cohesion and adhesion forces of solids are related to the surface energies of the interacting particles. Solids with a high surface energy have a high tendency to adsorb other materials onto their surface and form strong bonds with adhered particles. When an adherent particle is exposed to a compressed air stream, it will be detached from the adhering surface if the drag forces of the stream can overcome the adhesional forces of the particle (Zeng et al., 2001b).

The storage decreased the RF% values of budesonide at the dose of 10 μg irrespective of carrier (I: Table 2, Fig. 11. B). These results indicate that interparticulate forces increased between the carrier and budesonide particles at the low drug concentration during storage hindering the drug detachment from the surface of the carrier. This trend was also found at the budesonide dose of 50 μg when glucose was used as a carrier while the RF% value of budesonide at the dose of 50 μg increased after storage when mannitol was used as a carrier (Fig. 11. B). This difference may be explained by differences in moisture sorption capacity of glucose and mannitol, as described earlier.

In the case of budesonide at the dose of 100 μg, the storage significantly increased the RF% value when glucose (Fig. 11. B) and α-lactose monohydrate (325M, 110M) (Fig. 12. B), Flowlac-100% (Fig. 12. B), Flowlac-100% with crystalline micronized lactose (Fig. 12. B) and crystalline lactose (Fig. 12 B) were used as a carrier. When mannitol (Fig. 11. B) was used as the carrier, the storage period had no effect on RF% of budesonide at the dose of 100 μg. One explanation for the increased RF% values of budesonide particles after storage could be that surface electrostatic properties of budesonide and the carrier may have decreased due to moisture sorption on the particle surfaces during storage (Zeng et al., 2001b). As a result, fine budesonide particles could more easily become detached from the surface of the carriers during inhalation (Zeng et al., 2001b). The RF% value clearly decreased when spray-dried lactose was used as the carrier (Fig. 12. B). This result indicates that adhesion of budesonide to spray-dried lactose particles increased during storage. It must be noted that amorphous lactose is sensitive to moisture sorption (Naini et al., 1998; Stubberud and Forbes, 1998) and binding forces acting within the powder particles increase when the formulation is exposed to a high humidity level (75% RH) during storage.

These results clearly demonstrate that one must have highly crystalline drugs and excipients if one wishes to formulate stable inhalation powders.
5.2 Physicochemical properties of spray-dried lactose (III, IV)

Spray drying is widely used in the pharmaceutical industries; e.g., in the preparation of spherical particles of drugs and excipients, granulation, microencapsulation, and complex formation (Broadhead et al., 1992). Spray drying is known to produce predominantly amorphous material due to rapid solidification (Sebhatu et al., 1994).

In this study, the effect of the ratio of ethanol to water in the feed solution on crystallinity of the spray-dried lactose was studied (III). The ratio of ethanol to water varied between 0:100 to 100:0. The ratio of ethanol to water influenced the crystallinity of the spray dried lactose (III: Table 2; IV: Table 2). When pure water was used as the feed solution, spray drying produced 100% amorphous lactose. When the ratio of ethanol to water in the feed solution was increased, the degree of disorder in the lactose was decreased in the product (III: Table 2; IV: Table 2). A crystalline lactose was achieved when lactose was spray dried from pure ethanol.

The solubility of lactose at room temperature is 1 g in 4.63 ml of water, and lactose is practically insoluble in ethanol (Kibbe, 2000). When the solubility of lactose in the feed solution was decreased by increasing the ratio of ethanol to water, the amorphous content in the spray dried lactose decreased (III: Table 2; IV: Table 2). This is probably due to fact that most of the lactose that was dissolved in the feed solution became solidified as amorphous lactose. The relationship between the fraction of dissolved material in the feed solution and the amorphous content of the spray-dried sample is, however, not straightforward. Chidavaeni et al. (1997) observed that the amorphous content of spray-dried lactose was higher than the dissolved amount of lactose in the feed solution. This might be due to a milling effect on the suspended lactose particles in the atomiser; i.e., milling resulted in the formation of an amorphous material by solid state transition, or enhanced solubility, or more likely a combination of both processes (Chidavaeni et al., 1997).

The water content of spray-dried lactose samples was determined by TG (III). The adsorbed water of samples removed at 30-90°C, and hydrate water of lactose removed at 80-150°C. Commercial α-lactose monohydrate that was not spray dried contained hydrate water (5.1%) and no adsorbed water was observed (III: Table 2). The total amount of water, which included the adsorbed and hydrate water in the spray-dried samples, varied from 3.9% to 5.3%, as determined by TG (III: Table 2). The feed solution strongly affected the ratio of adsorbed water to hydrate water, since the content of adsorbed water increased and hydrate water decreased as the crystallinity of the spray-dried lactose decreased (III: Table 2). It is well-known that the capacity of
amorphous lactose for moisture sorption is higher than that of crystalline lactose (Stubberud and Forbes, 1998; Naini et al., 1998).

The number of small and spherical lactose particles increased, when the concentration of water in the feed material was increased (IV: Fig. 2). The particle size of spray dried lactose decreased (IV: Table 1) and surface area increased (III: Table 2) as a function of the amorphous content (IV: Table 1). However, the surface area of 100% amorphous lactose was significantly smaller than the surface area of 86% amorphous lactose (III: Table 2), since the 100% amorphous lactose was fairly cohesive and aggregated to form larger units.

In this study, crystalline lactose (L7B100) was produced by spray drying from absolute ethanol. Figure 12 A shows that when the crystalline lactose was used as the carrier, the emitted budesonide dose was close to the theoretical dose both initially and after storage of formulation one month at 40°C/75% RH. In addition, when compared to commercial α-lactose monohydrate (325M or 110M), RF% value of budesonide was higher before storage and the formulation was physically more stable when the crystalline lactose was used as the carrier.

5.3 Determination of amorphous content of spray-dried lactose by solution calorimetry (IV)

The amorphous content of a materials can be determined by a variety of techniques, including XRPD (Stephenson et al., 2001), DSC (Sebahat et al., 1994), TG (Yao et al., 2003), IMC (Buckton and Beezer, 1991; Angberg et al., 1992a) and solution calorimetry (Gao and Ryting, 1997; Hogan and Buckton, 2000). In the earlier studies, where solution calorimetry has been utilized, the dissolution process in a sample has been fast and not limited by saturation (Gao and Ryting, 1997; Hogan and Buckton, 2000).

In this study, the enthalpy of solution (ΔH_{sol}) of lactose samples was determined in water at room temperature. The physical mixtures with varying contents of 100% amorphous spray-dried lactose and 100% crystalline lactose and samples of spray-dried lactose with varying amorphous content were measured. The amorphous content of the physical mixtures and the spray-dried samples varied from 0% to 100% as determined by IMC (IV: Tables 2, 3).

In the present study, the ΔH_{sol} of the 100% amorphous lactose was -53.3 ± 2.7 J/g, (n= 4), indicating an exothermic process. The untreated commercial 100% crystalline lactose revealed the value of the ΔH_{sol} to be 54.2 ± 0.9 J/g, (n= 4), indicating an
endothermic reaction. Similarly, the value of the $\Delta H_{\text{sol}}$ for the 100% crystalline spray dried lactose was $53.5 \pm 0.7$ J/g, ($n=4$). Our results are in line with an earlier report from Hogan and Buckton (2000) who reported the enthalpy of solution of the 100% amorphous lactose and the 100% crystalline lactose to be $-56.50$ J/g and $56.2$ J/g, respectively, in water. When the enthalpies of solutions of the present 25-51% amorphous lactose samples were determined, an initial exothermic response was observed, followed by an endothermic response. The overall result for the dissolution process of the 25-51% amorphous lactose samples was endothermic. A linear correlation between the $\Delta H_{\text{sol}}$ and the amorphous content of the sample was obtained ($R^2 = 0.9952$ for the spray dried samples and $R^2 = 0.9956$ for the physical mixtures) (IV: Figs. 1A, 2A). A good correlation between the $\Delta H_{\text{sol}}$ and the amorphous content of the physical mixtures of some drugs and excipients has been demonstrated also earlier (Pikal et al., 1978; Thompson et al., 1994; Gao and Ryting, 1997; Hogan and Buckton, 2000). Hogan and Buckton (2000) found a good correlation between the $\Delta H_{\text{sol}}$ and the amorphous content of lactose (the amorphous content of lactose ranged from 0 to 10%). Pikal et al. (1978) observed a correlation between the $\Delta H_{\text{sol}}$ and the extent of crystallinity of antibiotics. Gao and Ryting (1997) determined the crystallinity of sucrose and warfarin sodium by measuring the $\Delta H_{\text{sol}}$ of the samples. Thompson et al. (1994) found a linear relationship between the $\Delta H_{\text{sol}}$ and the crystallinity of an undefined drug (BO2669).

The enthalpy of solution measurement may be limited by the solvent if the solubility and dissolution rate of a compound in the chosen solvent needs to be reasonably high (Gao and Ryting, 1997; Hogan and Buckton, 2000). Solubility and dissolution rate of a sample in the chosen solvent has been quite high in all earlier studies where the amorphous content of the sample has been determined by using the values of $\Delta H_{\text{sol}}$ (Pikal et al., 1978; Thompson et al., 1994; Gao and Ryting, 1997; Hogan and Buckton, 2000). In the present study, the enthalpy accompanied with an addition of a lactose sample in a saturated aqueous solution ($\Delta H_{\text{sat}}$) was determined. The saturated aqueous solutions were prepared from the corresponding lactose samples. The $\Delta H_{\text{sat}}$ of the 100% amorphous lactose was $-68.7 \pm 3.5$ J/g ($n=8$), indicating an exothermic reaction. The value of the $\Delta H_{\text{sat}}$ was $5.45 \pm 0.9$ J/g, ($n=4$) for the 100 % crystalline untreated commercial lactose and $4.1 \pm 0.7$ J/g ($n=4$) for the 100% crystalline spray-dried lactose, indicating endothermic processes. When the $\Delta H_{\text{sat}}$ of the 0-100% amorphous lactose was determined, we observed only either a clear exothermic or a clear endothermic response.
The present results demonstrate the clear correlation between the $\Delta H_{\text{sol}}$ and the amorphous content for the spray-dried lactose ($R^2 = 0.9852$) and for the physical mixtures ($R^2 = 0.9895$) (IV: Figs. 2A, B). To our knowledge this observation has not been reported earlier.

A linear relation between the $\Delta H_{\text{sol}}$ and the $\Delta H_{\text{sat}}$ for lactose samples ($R^2 = 0.9933$ for the physical mixtures and $R^2 = 0.9846$ for the spray-dried samples) was observed (IV: Figs. 3A, B).

The present results show that when compared to IMC, the value for the amorphous content of a sample was comparable when determined by solution calorimetry (IV: Tables 2, 3). Either the values for $\Delta H_{\text{sol}}$ or for $\Delta H_{\text{sat}}$, could be utilized if the amorphous content was to be determined by solution calorimetry. This study indicates that the amorphous content of sample can be determined by solution calorimetry even though the sample is not completely dissolved in the solvent.
6 CONCLUSIONS

The present study evaluates the effects of the physicochemical properties of carrier particles, the drug:carrier ratio and storage of formulation on in vitro deposition of drugs from a novel multiple dose reservoir based DPI. In vitro deposition of the drug was found to be highly dependent on the formulation. Optimization of in vitro aerosolization properties of inhalation powders includes optimizing of the chemical composition and physicochemical properties of the carriers. The results demonstrate that spray drying is a useful method for the production of crystalline lactose particles to be used as a carrier in a DPI.

1. The physicochemical properties of carrier particles, such as their amorphous content, particle surface roughness, bulk density and particle size, strongly affect in vitro deposition of a drug from the multiple dose reservoir based DPI, and the physical stability of the inhalation powder. Highly crystalline particles, having a smooth surface and optimal size are required if one wishes to formulate inhalation powders for the multiple dose reservoir based DPI.

2. The effect of the chemical composition of carrier was evident on the RF% values of budesonide. In the present study, mannitol, glucose, α-lactose monohydrate (110M, 325M), spray-dried lactose, crystallized spray-dried lactose, Flowlac-100®, Flowlac-100® with crystalline micronized lactose and crystalline lactose were used as the carrier. The highest RF% values of budesonide were achieved when mannitol was used as the carrier. In the case of salbutamol sulphate, the carrier has no substantial effect on RF% value.

3. The drug:carrier ratio affected the RF% values of budesonide and salbutamol sulphate before and after the storage period. Typically the RF% values increased in parallel with an increase in the drug:carrier ratio.

4. The effect of the storage (40°C and 75% RH) on the RF% values of a hydrophobic budesonide was dependent on the carrier and the drug:carrier ratio. The RF% value of a hydrophilic salbutamol sulphate decreased after storage irrespective of the chemical composition of the carrier and irrespective of the drug:carrier ratio.
5. The amorphous content of spray-dried lactose can vary from 0% to 100%, depending on the solubility of lactose in the feed solution. When the concentration of lactose was kept constant in the feed solution, an increase in the ratio of ethanol to water in the feed solution decreased the amorphous content of the spray-dried lactose samples.

6. A linear correlation was observed between $\Delta H_{\text{sat}}$ and the amorphous content of the lactose samples (spray-dried samples or physical mixtures). Further, a linear relationship was observed between the $\Delta H_{\text{sat}}$ and the $\Delta H_{\text{sol}}$ of the lactose samples. The results indicate that solution calorimetry may represent a rapid and simple method for determining the amorphous content also in samples that are not completely dissolved in the solvent.
7 REFERENCES


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