HEINRICH HEINE
UNIVERSITÄT DÜSSELDORF

THE INFLUENCE OF THE CARRIER SURFACE CHARACTERISTICS ON DRY POWDER INHALATION

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Abbreviations and symbols

a	Surface area
AFM	Atomic force microscopy
ANOVA	Analysis of variance
B	Particle mechanical mobility
BET	Brunauer-Emmet-Teller
BSA	Bovine serum albumin
C_c	Slip correction factor
COPD	Chronical obstructive lung disease
D	Particle diameter
D_a	Aerodynamic particle diameter
D_f	Diffusion
DIN	Duetsches Institute fuer Normung
DSC	Differential scanning calorimetry
DPIs	Dry powder inhalers
F	Force
F_E	Force of interaction
F_g	Gravitational force
FPF	Fine particle fraction
g	Acceleration gravity
H	thickness
HPLC	High pressure liquid chromatography
I	Intensity
K	Boltzman constant
M	Mass
MDIs	Metered dose inhalers
MW	Molecular weight
MOC	Micro-Orifice Collector
n	number of measurements
N_2	Nitrogen
NGI	Next generation impactor
Δp	Difference in pressure
Ph.Eur	European Pharmacopeia
Q	Flow rate
r	Radius
R	Standard gas constant
Re	Reynolds number
RH	Relative humidity
SEM	Scanning electron microscopy
S	Distance
S_w	Specific surface area
SD	Standard deviation
T	Temperature
T_c	Crystallization temperature
T_g	Glass transition temperature
T_m	Melting temperature
t	Time
V	Volume
VdW	Van der Waals force

V_o	Initial velocity
V_{ts}	Terminal settling velocity
VMD	Volume median diameter
%RH	Percentage relative humidity
%w/w	Percent by weight
η	Viscosity
π	Constant ≈ 3.1416
γ	Surface tension
ρ	Particle density
ρ_o	Unit density

SUPPLY SOURCE OF MATERIALS

Absolute ethanol	VWR International, France
Acetone p.a.	Riedel de Haën, Seelze, Germany
Acetonitril	VWR International, Germany
Acetic acid	VWR International, Germany
Polyoxyethylen-20-cetylether (Brij)	Serva Elctrophoresis GmbH, Germany
Dichloromethane	KME Laborchemie Handels GmbH, Germany
Glycerol anhydride	Merk, Darmstadt, Germany
Lactose (InhaLac120)	Meggle, Germany
Isopropanol alcohol HPLC grade	Sigma-Aldrich chemie GmbH, India
Magnesium nitrate hexahydrate	Sigma-Aldrich chemie GmbH, India
Methanol	VWR International, Germany
Mannitol (Pearlitol160C)	Roquette Freres, France
Mannitol (Pearlitol25C)	Roquette Freres, France
Mannitol (Pearlitol100SD)	Roquette Freres, France
Mannitol (Pearlitol200SD)	Roquette Freres, France
Potassium sulphate	Merk, Darmstadt, Ger
Salbutamol sulphate	Selectchemie AG/Lindopharm, Germany
Sodium hydrochloride	Mallinckrodt Baker, Holland

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A THESIS ABSTRACT

THE INFLUENCE OF CARRIER PARTICLE CHARACTERSTICS ON DRY POWDER INHALATION FORMULATION

Respiratory diseases such as asthma and chronic obstructive pulmonary disease are treated with a range of drugs which may be delivered either systemically or by inhalation. Pulmonary delivery of active pharmaceutical agents via dry powder inhalers (DPIs) has been attracting much attention in recent years as an alternative to pressurized metered dose inhalers (MDIs). DPI formulations typically consist of either drug alone or drug blended with inert carriers that are FDA-approved for inhalation as lactose and mannitol in an ordered mixture. Upon aerosolization the powder formulation must deaggregate into fine drug particles in the 1-5μm range for effective pulmonary delivery. The use of carrier particles enhances drug particles flowability and increases the inhalation efficiency. The adhesion between drug and carrier particles, as well as the fine particle fraction generated from this ordered mixture, can be influenced by a variety of factors, including the surface properties of drug and carrier particles, drug to carrier ratio, carrier particle size, shape, mixing time and method, humidity and electrostatic behavior. However, according to literature, impacting dry powder inhalant performance is most successful by targeting the surface topography of the carrier particles. In this study carrier particles of lactose and mannitol were used. The carrier surface was modified by wet decantation, addition and removal of carrier fines and storage at different relative humidities to improve drug particle separation upon inhalation. The modified carriers were mixed with salbutamol sulphate in a tumbling mixer. DPI formulation performance was examined by the assessment of the respirable fraction of the drug in vitro. The results obtained using preconditioned carriers as opposed to unconditioned carriers suggest that preconditioning could be advantageous or disadvantageous in terms of the performance of dry powder inhalation.

B INTRODUCTION

1 Inhalation therapy

The origin of inhalation can be traced back to early civilization where this route of administration was relatively uncommon. Since a few decades the respiratory tract has been used for direct delivery of drugs to the lung via inhalation to treat respiratory diseases such as asthma, cystic fibrosis, obstructive lung disorders and other airways diseases. More recently, research has focused on using the lung as a conduit to deliver biomolecules such as peptides and proteins to the systemic circulation due to the large absorptive surface area of the respiratory system ($30m^2$-$100m^2$) with a thin epithelial lining (Okamoto et al., 2002; Byron et al., 1994; Zijlstra et al., 2004). In addition, epithelial surfaces of the lung express several receptors, some of which facilitate the transport of macromolecules and small hydrophilic drugs. This approach of drug delivery has the advantage of rapid drug absorption since most of the surface area resides in the alveolated regions of the deep lung which contain a rich capillary network to facilitate rapid gas exchange. Furthermore the first pass metabolism is circumvented (Hickey et al., 1992).

Inhalers are well known devices for administering pharmaceutical products to the respiratory tract by inhalation. Inhalers are widely used particularly in the treatment of the respiratory tract diseases. There are a number of types of inhalers currently available. The most widely used type is the pressurized metered dose inhaler (MDI) which uses a propellant to expel droplets containing the pharmaceutical product. Those devices are disadvantageous because of environmental reasons as they were use chlorofluorocarbons (CFCs) as propellant, and because of clinical reasons related to the inhalation characteristics of the devices (coordination between drug release and inhalation manoeuver is necessary, amount of drug impacted on the upper airways due to the high velocity of the aerosol cloud leaving the inhaler is high). CFCs are being phased out (Hindle et al., 1995), due to their implication in stratospheric ozone depletion (Houghton et al., 2004). Unfortunately, the transition to the environmentally more acceptable HFA hydrofluoroalkane propellants has been difficult, due in large part to the poor solvency of the

HFA, which limits the solubility of the drug as well as approved surfactants. The solubility can be increased by the addition of a co-solvent such as ethanol.

However, an alternative device to the MDI is the dry powder inhaler (DPI). Dry powder inhalers have many advantages in comparison to MDIs such as lack of stability problems, no need of propellant, no need of coordination, while the DPI disadvantages are flow rate dependence of the delivered dose and the respirable fraction, cost/complexity of formulation use or dose, and moisture sensitivity. The delivery of dry powder particles of pharmaceutical products to the respiratory tract presents certain problems. The inhaler should deliver the maximum possible proportion of the active particles to the lungs, including a significant proportion to the lower lung, preferably at the low inhalation capabilities to which some patients, especially asthmatics, are limited (Hickey et al., 1992). It has been found, however, that, when currently available dry powder inhaler devices are used, in many cases only about 20-30% of the active particles that leave the device on inhalation are deposited in the lower lung (Steckel et al., 1997, a). The type of dry powder inhaler used is of significant importance to the efficiency of delivery over a range of airflow conditions of the active particles to the respiratory tract.

In addition, the physical properties of the powder used affect both the efficiency and reproducibility of delivery of the active particles and the site of deposition in the respiratory tract. The size of the active particles is particularly important. For effective delivery of active particles deep into the lungs, the active particles should be small, with an equivalent aerodynamic diameter substantially in the range of 1μm to 5μm (Zanen et al., 1994; Timsina et al., 1994). Small particles are, however, thermodynamically unstable due to their high surface area to volume ratio, which provides significant excess surface free energy and encourages particles to agglomerate. In the inhaler, agglomeration of small drug particles and adherence of drug particles to the walls of the inhaler are problems, which result on the one hand in poor flowability as well as un-ability of those agglomerates to break up into the primary particles upon inhalation, on the

other hand in the un-ability to leave the inhaler remaining adhered to the interior walls of the inhaler. In an attempt to improve that situation, dry powder for use in dry powder inhalers often include coarse carrier particles mixed with fine particles of active material (Louey et al., 2004). The active particles adhere to the surfaces of the carrier particles whilst in the inhaler device, and are dispersed on inhalation into the respiratory tract to give a fine particle cloud. The carrier particles are often large particles greater than 90μm in diameter, which lead to an improvement of the flowability and an increase in the dosing properties of cohesive drug particles. Furthermore, they may act as a diluent of drug in lower dosing.

In ideal adhesive mixtures for inhalation, micronized drug particles are distributed homogeneously over the surface of much larger carrier particles. The interaction forces between drug and carrier surface are predominantly Van der Waals forces, but also electrostatic and capillary forces may play a role.

The most important factors that affect dry powder inhalation are particle size, shape, density, stability, moisture, crystallinity (polymorphism and amorphous), surface chemistry, area and texture, plasticity, and electrostatics of the components of the adhesive mixture. The use of a carrier is not free of drawbacks. Strong interparticle forces between the drug and the carriers may prevent the separation of the micronized drug particles from the surface of the coarse carrier on inhalation, thereby preventing the availability of the drug to the respiratory tract. Strong attachment of the drug to the carrier surface may occur within asperities and clefts of the carrier surface, which are highly energetic sites on which the active particles are preferably attracted to and adhere more strongly. Because of such strong interparticle forces, drug particles will be unlikely to leave the surface of the carrier particles and to be deposited in the lower respiratory tract. Surface asperities could be classified into two types macroscopic asperities which entrap drug particles and decrease drug release upon inhalation (mentioned above), and microscopic asperities which could contact drug by one point at the edges of those asperities, this decrease of the contact area between drug particle and carrier

particle reduces the interactions. Therefore, the features of the carrier particles should be such as to give sufficient adhesion force to hold the active particles to the surface of the carrier particles during manufacturing and in the delivery device before use. Nevertheless, that force of adhesion should be low enough to allow the dispersion of the active particles in the respiratory tract (Louey et al., 2003; de Boer et al., 2003, a).

Carrier particles can be chosen according to their median particle size, taking into account the fact that an increase in median particle size increases the adhesion force between drug and carrier particles (Staniforth et al., 1982). Many authors use lactose exhibiting a size between 63 and 90µm (Timsina et al., 1994; Bennett et al., 1999; Zeng et al., 2000). Furthermore, the carrier particles must be pharmacologically safe, and must have high crystallinity. Carrier particles also decrease the residual fine drug particles that adhere to inhalation devices and capsules upon inhalation of fine drug particles. With the use of carrier particles, drug particles are emitted from capsules and devices more easily, and the inhalation efficiency increases (Kawashima et al., 1998).

Lactose is the predominant carrier in dry powder inhalation (te Wierik et al., 2002). However, lactose has reducing properties and would not be the appropriate excipient for some drugs such as proteins or peptides (Zeng et al., 2000). Lactose has been recognized in several earlier studies which clarify that the efficiency of a powder formulation is highly dependent on the lactose quality and source (Steckel, 2004, a), the size distribution of the used lactose carrier (Zeng et al., 1996), and the content of fine lactose (Lucas et al., 1998), or ternary additive particles (Kassem, 1990), with respect to the used dry powder inhaler and the flow properties of that device. Differences in surface topography, surface energy and air-entrainment behavior of the used lactose crystals were suggested to be responsible for these dependencies (Price et al., 2002). Lactose mostly has small amorphous parts at the surface (Buckton et al., 1999) and it may cause the CSE (cerebrospinal encephalopathy), so there is a need for safe alternatives. Most literature and books for dry powder inhalation refer to carbohydrates such as fructose, glucose, galactose,

sucrose, trehalose, raffinose, melezitose, alditols, such as mannitol and xylitol, maltodextrins, dextrans, cyclodextrins and amino acids such as glycine, arginine, lysine, aspartic acid and glutamic acid, and peptides such as HAS (human serum albumin) and gelatin (Gonda et al., 2000).

This study uses mannitol as a carrier instead of lactose. Mannitol has no reducing property, is stable, crystalline and causes no cerebrospinal encephalopathy (CSE). In addition, it provides no toxic effects. Mannitol is a sugar-alcohol which is not absorbed in the gastro-intestinal tract, does not cross the blood brain barrier and is not metabolized to any substantial extent when injected. Mannitol is stable as a powder and resists moisture at relatively high humidities up to 95%RH.

Mannitol is introduced as an alternative carrier for lactose, some modifications of the surface area of the mannitol carrier in comparison with lactose were carried out to improve the fine particle fraction. The carrier surface modifications include wet decantation, addition of carrier fines to the treated carriers, removal of carrier fines using an air jet sieving process and storage of the carrier at different relative humidities for longer period of time. These modifications have been carried out to change the carrier surface area which subsequently affects the drug to carrier interactions and the effect of those modifications on the fine particle fraction and the delivered dose was challenged.

2 Structure of respiratory tract

The respiratory airways are often described as the pulmonary tree. They originate at the trachea that bifurcates to form the main bronchi. The bronchi divide to form smaller bronchus that lead to the individual lung lobes, three lobes on the right hand side and two lobes on the left hand side. The bronchi undergo further division to form bronchioles, alveolar ducts and terminate in alveolar sacs. The surface area will increase through these divisions of the airways descendly also the capillary blood supply will be more in the terminal alveolar sac than the main bronchi. The alveolus is the principle site of gas exchange in the airways, a function compatible with the increased surface

area that promotes extensive and efficient diffusional gas exchange between the alveolar space and the blood in the alveolar capillary. The alveolar region is the target site area for drug deposition. The various levels of the airways may be categorized functionally as being either conducting or respiratory airways. The airway epithelium comprises a variety of cell types, the luminal surfaces of the airways are lined by ciliated cells and mucus (mixture of water, inorganic salts, glycoproteins, glycosaminoglycans, lipids and other proteins and peptides), that covers the luminal surface of the epithelium. The mucus fulfils three functions. Firstly, it protects the epithelium from becoming dehydrated. Secondly, the water in the mucus is involved in the saturation of the inhaled air with water. Thirdly, the mucus is involved in airway protection. Mucus clearance from airways is enhanced by coughing which rapidly propels the mucus toward the pharynx. Failure to clear mucus hypersecretion (as may occur in chronic bronchitis) can result in airway obstruction and infection. Such situation may adversely affect the therapeutic activity of an inhaled drug due to the increased thickness of the mucus layer through which the drug must diffuse to reach its site of action and retard the penetration of the aerosolized particles through the airways as a result of mucus plugging of the airway lumen. Goblet cells and mucus glands are not present in airways distal to the bronchi, and, therefore, mucus layers do not line the peripheral airways. The human lungs, however, have efficient means of removing deposited particles over periods ranging from minutes to hours. In the upper airways, ciliated epithelia contribute to the mucociliary escalator, by which particles are swept from the airways towards the mouth. In the deep lungs, an army of alveolar macrophages is capable of phagocytosing particles soon after their deposition. An effective slow-release inhalation therapy therefore requires a means of avoiding the lung's natural clearance mechanisms until encapsulated drugs have been effectively delivered (Hickey et al., 1992).

3 Respiratory tract diseases

The term respiratory tract diseases includes many disorders affecting the lungs, such as asthma, chronic obstructive pulmonary disease (COPD),

infections like influenza, pneumonia and tuberculosis, lung cancer, and many other breathing problems. It is well known that inhalation therapy, especially for asthma and chronic obstructive pulmonary disease, is very successful even though only a small fraction of the inhaled dose (20-30%) reaches the peripheral parts of the respiratory tract.

Asthma is a clinical syndrome characterized by increased responsiveness of the tracheobronchial tree to a variety of stimuli, which may be spontaneous, allergen-related or drug-induced, and the primary pathophysiological abnormality being bronchial wall inflammation leading to airway narrowing. Asthma is characterized by recurrent breathing problems and symptoms such as breathlessness, wheezing, chest tightness and coughing. Asthma symptoms vary over time, and also differ in severity from one individual to another. When it is not effectively treated, asthma often leads to hospitalization, missed work and school, limitations on physical activity, sleepless nights and in some cases death. Asthma disease is estimated to affect about 300 million people worldwide, particularly among children. This number could be increased by a further 100 million by 2025.

Chronic obstructive pulmonary disease is characterized by primary bronchitis and emphysema causing progressive loss of lung function. COPD is associated with symptoms of chronic cough and purulent sputum and is strongly related to a history of smoking especially in elderly people.

Asthma symptoms remain poorly controlled despite the availability of inhaled corticosteroids and β_2-adrenergic agonists. One reason why asthma remains poorly controlled is the improper use of pressurized metered-dose inhalers and dry powder inhalers in most countries (Africa's press. The global Initiative for Asthma, 2004).

4 Aerosol deposition in the respiratory tract

The amount of drug reaching the lower airways and the pattern of deposition are essential for pharmacologic effects. There are many factors that may affect the particle deposition in the lung as aerosol physical properties and patient properties such as the anatomy and air velocities within the respiratory tract, the passage of air through the various anatomic regions during the

breathing. Presence of mucus may act to enhance further deposition. Deposition of aerosols increases during brochoconstruction, primarily as a result of changes in inertial impaction and turbulent flow experienced by aerosol particles after irregular airway obstruction. In the respiratory tract there are inspiratory and expiratory muscles. During inhalation the chest is expanded longitudinally by contraction of the main inspiratory muscle, the dome shape diaphragm in the lower part of the chest. This enlargement of the chest volume creates an under pressure in the lung which is the driving force for an airflow. Flow rates within the lung affect deposition. The nature of particle deposition forces and their relationship to the aerodynamic particle size have been subject to many studies and reports. The degree of polydispersity of an aerosol has been shown to influence significantly the aerosol deposition in the respiratory tract (Hickey et al., 1992).There are five basic physical mechanisms that cause the deposition of aerosol in the lung: inertial impaction, sedimentation, diffusion, interception, electrostatic precipitation (Hickey et al., 1992; Thompson et al., 1998). The smaller particles of drug (1μm-5μm) can change direction in the inhaled airstreams and deposit deep in the lung, whereas the larger carrier particles impact in the throat and are swallowed.

The main three deposition mechanisms are shown in Figure 1. Inertial impaction is caused by the tendency of particles and droplets to move in a straight direction, instead of following the gas streamlines when they change direction. It depends mainly on the mass and shape of the particle and the velocity by which it will move. It occurs mainly in the mouth and bronchi and to the particles larger than 10μm. Sedimentation occurs to small particles of 1μm-5μm under the influence of gravity in the middle and alveolar regions, while the larger particles are already impacted. The sedimentation velocity follows the Stokes-Cunningham Law. Diffusion (Brownian movement) occurs when the particle becomes sufficiently small, this means that diffusion increases with decreasing particle size. Brownian motion is only important when the particle diameter is small e.g. <1μm. Brownian diffusion will be more prevalent in regions where airflow is very low or absent, such as in alveoli. The submicrometer size particles are exhaled or deposited by random Brownian motion in the distal regions. Interception denotes the situation under

which the center of gravity of the particle is within the streamlines of the gas phase but the distal end of the particle is already touching a solid or liquid. Electrostatic precipitation occurs when a charged particle can induce a charge of opposite sign in the walls of airways and then becomes electrostatically attracted to it.

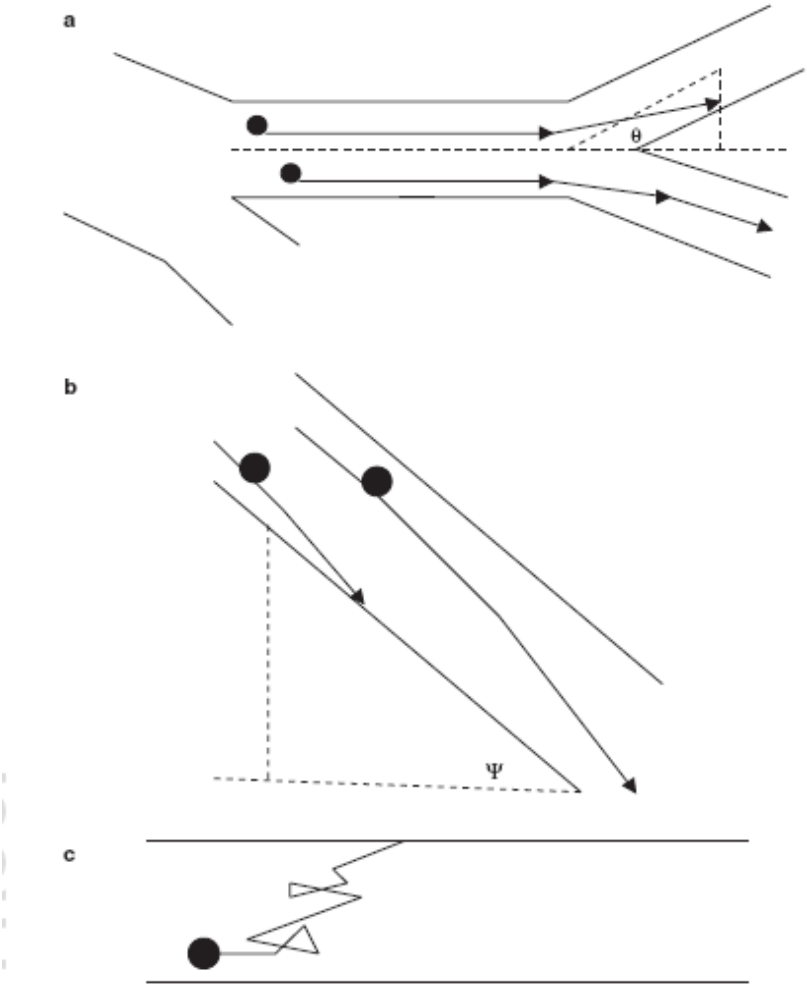


Figure 1: Mechanisms of particle deposition within the respiratory tract (**a**) inertial impaction (**b**) sedimentation and (**c**) Brownian diffusion (Taylor et al., 2004).

Therapeutic effect depends directly on the site of deposition. Optimal localization varies with drugs and their pharmacological site of action. It seems evident that the optimal site of action for bronchodilators is located in distal airways. For example in case of antibiotics, the alveoli must be targeted, while for glucocorticosteroids, the site of action remains uncertain.

4.1 Inertial impaction

The inhaled air constantly changes direction as it flows from mouth down through the branching airway system. The inhaled particles will have to follow the air stream in order to get deeper into the lungs. However particles are unable to do so when their inertia is too high: Due to high mass or high velocity or both they will deposit. Therefore, the largest particles are deposited by the mechanism of inertial impaction in the throat. Impaction depends on the particle mass (M) that moves with the initial velocity (V_o) through still air. As a result of frictional forces, the particle will stop after traveling the stopping distance (S) (Hickey et al., 1992).

$$S = B \cdot M \cdot V_{\circ} \qquad \textbf{Equation 1}$$

B is the mechanical mobility of the particle (i.e. the velocity per unit of force). Impaction of the particles with the same D_{ae} (aerodynamic diameter) will vary depending on the flow rate Q. The impaction parameter $\rho \cdot D^2 \cdot Q$ was found to predict uniquely the deposition in the human mouth and nose, where ρ is the particle density.

4.2 Sedimentation

Sedimentation in the lung occurs to small particles of 1µm-5µm under the influence of gravity in the middle and alveolar regions, while the larger particles are already impacted in the mouth and throat regions. Sedimentation is the process whereby the particles deposit through gravity, which causes the particles to fall to the surface. Sedimentation arises whenever the gravitational force acting on a particle overcomes the total force maintaining it into suspension. Inhaled particles will then fall out of the gas stream. This is an important mechanism in the small airways because the gas velocity dramatically decreases at this level. The probability of sedimentation is proportional to both residence time of the particle in the airways and particle size. The sedimentation velocity follows the Stokes-Cunningham Law. If the particle (sphere) has the diameter D and the density ρ, and moves under the

influence of the gravitational force F_g, it will have a terminal settling velocity V_{ts} in the laminar region governed by the Stokes-Cunningham Law.

$$V_{ts} = \frac{\rho \cdot D^2 \cdot g}{18\eta} = B \times F_g \qquad \textbf{Equation 2}$$

η is the viscosity of the air, when the particles become very small so that using the Stokes's picture of an objective moving in a continuous medium is no longer permissible, the so-called slip correction factor, C_c, must be applied. The particle can slip through the medium, and consequently, its velocity is greater than that predicted by the previous equation.

$$V_{ts} \text{ (with slip)} = V_{ts} \cdot C_c \qquad \textbf{Equation 3}$$

The Cunningham correction factor needs to be applied to aerosols with particles or droplets in the regimens with Re>1.
D_{ae} is the aerodynamic diameter (which is the diameter of the sphere of unit density that has the same terminal sedimentation velocity).

$$D_{ae} = D\frac{(\rho)^{0.5}}{\rho_\circ} \qquad \textbf{Equation 4}$$

D is the diameter of the sphere and ρ its density, ρ_o is the unit density, which equals 1g/cm³ or 1kg/m³.

4.3 Diffusion

Diffusion (Brownian motion) is only important when the particle diameter is small e.g. <1μm. Diffusion will be more prevalent in the regions where the airflow is very low or absent, such as in the alveoli. Nevertheless it is also present in bronchioles and at bronchial airway bifurcations. Diffusion of small particles in the respiratory tract depends mainly on the Stokes-Einstein Equation (Equation 5). This mechanism is slow. Therefore, these particles may be exhaled before they touch the respiratory epithelium. Effectiveness of Brownian motion is inversely proportional to the particle diameter.

$$D_f = \frac{k \cdot T \cdot C_c}{3Q\eta D}$$ **Equation 5**

D_f is the diffusion coefficient, k is the Boltzman constant and T is the absolute temperature and C_c is the Cunningham correction factor. Because such particles (e.g. 1µm) have negligible probability of deposition in the extra thoracic and bronchial regions, the alveolar delivery should be more selective.

In the small airways, the drug deposition might occur due to particle interception, when the particles have a fiber shape and just touch the airway surface, although they do not deviate from the streamlines (Crowder et al., 2002). Furthermore, a charged particle may deposit by producing an electric force upon contact with the respiratory walls. Although, these two mechanisms are considered to be of lower importance.

5 Types of aerosols used for pulmonary applications

5.1 Introducing remarks

The history of inhalation therapy can be traced back to 4000 years ago, when in India the leaves of the Atropa Belladonna plant were smoked as a cough suppressant. More than a millennium later, the ancient Greeks recognised the benefit of the volatile gases from the pine forests of Libya in the treatment of tuberculosis of the lungs. Inhalation of sea mists, hot vapours and aerosols to ease airway obstruction were remedies used by Hippocrates. “Asthma cigarettes“ containing stramonium from the plant Datura Stramonium were probably introduced in the times of the industrial revolution. The late 19th and the first half of the 20th centuries were marked by a variety of creative designs for nebulizers driven either by compressed air or ultrasonic vibrations. These devices were quite inconvenient for patients and were not very widely used. The development of highly effective asthma drugs in the second half of the century was coupled with a desire to reach large numbers of patients with highly portable delivery systems: these efforts culminated in the launch of the

chlorofluorocarbon (CFC) propellant driven metered dose inhalers (MDI) and dry powder inhalers (DPI) (Gonda et al., 2000).

Inhalation offers many benefits as direct deposition tool of the drug to the lung, which results in a lower required dose compared to oral delivery. Normally, the required amount of drug for inhalation is about ten percent of the orally required amount of drug to obtain similar effects. DPIs offer fast onset of the effect by using inhaled medication. Especially when using β_2-agonists (fenoterol, formoterol, salbutamol, terbutaline), the effect can be seen within 1-5 minutes after inhalation. For anticholinergics (ipratropium) and β_2-agonist salmeterol, the effect takes 5-20 minutes. DPIs offer also a decrease in the systemic side effects. In case of common dosages, systemic side effects for the inhaled corticosteroids hardly occur.

Inhalation provides an excellent delivery route for the treatment of both pulmonary and nonpulmonary conditions. Delivering medications via inhalation generally requires small doses, offers a rapid onset of drug action, and reduces systemic effects, compared with other routes of administration. Portable nebulizers, metered dose inhalers (MDIs) and dry powder inhalers (DPIs) have been competing in the marketplace for many years. In addition, each device (MDI, Nebulizers, and DPI) has taken a side to accommodate the consumer need.

5.2 Nebulizers

A nebulizer is a type of inhaler that sprays a fine, liquid mist of medication (often used by persons who cannot use a metered-dose inhaler, such as infants and young children, and persons with severe asthma). The two major types of nebulizers are the jet and ultrasonic nebulizer. The jet nebulizer works by passing air at high speed over the end of a capillary tube, liquid may be drawn up the tube from the reservoir in which it is immersed, and when the liquid reaches the end of the capillary, it is drawn into the air stream and forms droplets that disperse to become an aerosol. The ultrasonic nebulizer works by using a piezoelectric transducer to induce waves at the reservoir surface that leads to the production of droplets in the atmosphere above the reservoir. An air stream is passed through the atmosphere to transport the droplets as

an aerosol. The droplets produced from nebulizers are small enough to penetrate to the periphery of the lung (Zeng et al., 2001). These devices are more effective generators of small particle than both MDIs and DPIs. Nebulizers produce a greater proportion of the dose reaching the lower airways, although each solution droplet contains less drug than each particle generated from an MDI or dry powder generator. However, the solution flow rate and airflow rate have a significant influence on the total output of these devices.

Nebulizers are used mainly in hospitals due to simplicity of use. The nebulizer can be used to administer a variety of drugs and can be adapted for use with a mouthpiece, adult or pediatric face mask, tracheostomy collar, T piece, or ventilator circuit. For children, nebulizers seem to be preferred, but MDIs with spacers can also be used. Nebulizers are used for the generation of special aerosols such as liposome. Nebulizers present in portable form and compressor-driven nebulizers, which designed to meet patient's desires for portability.

5.3 Metered dose inhalers

The development of the first pressurized metered dose inhaler (MDI) in the mid-1950s was a major advance in the administration of drugs locally to the lung, especially for the treatment of asthmatics (Clark et al., 1993). They are formulated as micronized drug suspended in a liquefied chlorofluorocarbon CFC propellant mixture. In the past 30 years, there has been evidence that CFC damages the ozone layer and consequently the environment and CFCs were substituted by hydrofluoroalkanes (HFA). Some MDIs using hydrofluoroalkane (HFA) propellants have proven a safe, effective and have successfully placed into use. Improvements in MDI design and reformulation with propellants such as HFAs may offer significant advantages over MDIs that used CFCs, and may prolong the widespread use of pressurized drug-delivery systems for many years to come. The original particle size of the suspended powder is very important because this dictates the smallest particle size generated from the device. The powder prepared most commonly by milling the drug to the appropriate size (3μm-5μm), then suspending it in

the propellant by means of surfactants, for example, oleic acid. Because of the size of the particles, the suspension is not colloidal, and therefore, is stable for only minutes. This means it is important to shake the suspension to redisperse the particles before use. In many studies, the MDIs deliver at best only 10%-15% of the dose actuated into the respiratory airways. The balance of the dose is either lost to the inner surface of the adapter (about 10%), or is deposited through inertial impaction in the oropharynx area (80%). The deposition in the oropharynx leads to swallowing and possible systemic absorption of the therapeutic agent. To reduce this fraction that is lost to the oropharynx and swallowed, a number of tube spacers of various geometric shape and dimensions were considered. Patient co-ordination of actuation with inhalation can be a problem with MDIs particularly in young, elderly or chronically ill patients.

5.4 Dry powder inhalers

The worldwide phasing out of CFC (Chlorofluorocarbon) propellants in the last years presents difficulties to the pharmaceutical industry and shifts the researcher attention to the dry powder inhaler as a substituent delivery tool. It is notoriously difficult to use the MDI correctly, mainly because of poor coordination between actuation of the device and inspiration. Poor inhalation technique has been suggested to be related to reduce clinical effect (Gonda et al., 2000). Dry powder inhalers (DPIs) were developed as a breath-actuated and environmentally friendly alternative to the MDI. The powders are formulated by mixing cohesive micronized drug particles with larger carriers. These breath-actuated devices rely on the patient's inspiratory effort to deaggregate and subsequently inhale the drug particles. In this manner, the problems of coordinating actuation and inhalation do not exist (Hindel et al, 1995). Powder dispersion in DPI devices is accomplished either passively, by the patient's own inspiratory effort, or actively, by a component (e.g. electronic vibrator, impeller) in the DPI device, because micronized particles are difficult to deaggregate. The efficacy of passive DPI devices depends strongly on the patient’s inspiratory flow rate. This has led to the development of the more sophisticated, active DPI devices. The efficacy of inhaled medications may be

affected by the patient's age, severity of disease, and inhalation technique, as well as the specific pharmacological properties of the drug. Oropharyngeal deposition is a consistent confounding factor that continues to challenge each delivery model. Cost, convenience, and ease of use can all affect patient compliance.

DPIs are propellant free, portable, easy to prepare and low cost devices with improved stability of the formulation as a result of dry state, less need for patient coordination and less potential for formulation problems. DPIs drawbacks are, the performance depends mainly on the patient's inspiratory flow profile, resistance of the device and other design parameters, potential difficulties to obtain dose uniformity, less protection from environmental effects and patient abuse, and not available worldwide.

DPIs generally are described as breath actuated devices, because the inspiratory air stream releases the dose from the dose system and supplies the energy for the generation of fine drug particles from the powder formulation. The difficulty in deaggregation of the micronized therapeutic substance is due to the relatively high surface area to mass ratio, which leads to a high energetic surface after micronization and consequently leads to greater cohesiveness and adhesiveness compared to larger, unprocessed particles (Young et al., 2005). For low dose drugs, this high adhesivity and cohesivity can result in product development hindrance in regard to issues such as content uniformity, stability and drug metering. To solve this problem most dry powder formulators blend the micron-sized drug particles with a larger inert carrier material, such as lactose, to form an 'ordered mix'. This approach results in improvement of handling and processing properties, accurate dosing also by dilution of the drug to mass ratio and an increase in device drug emptying. The forces exerted by patient and required to successfully remove drug particles from the surfaces of the carrier will be dependent on the physico-chemical properties of the drug and the carrier, and the environmental conditions under which they are formulated, stored and used. The fine drugs must be reseparated from the coarse carrier particles during inhalation. After emission of what is called interactive powder mixture (micronized drug mixed with coarse carrier) from a capsule or inhalation device, the separation of fine drugs from the carrier particles and their delivery

to the targeted sites are important (Figure 2). However the separation efficiency of fine drug particles from the carrier surface is low and effective drug delivery to the targeted sites (alveoli) is very low. For all breath-actuated devices the fine particle fraction (FPF) is dependent on the flow rate generated by the patient. Increasing the flow rate produces a greater amount of drug delivered and increases the respirable fraction from DPIs. The dose which a patient inhales, and hence the effectiveness of the therapeutic agent, depends upon the inspiration capacity (Cegla et al., 2004).

The in vitro inhalation properties of DPIs are reported in literature to be very much related to surface properties of the carrier particles, which directly affect the drug to carrier adhesion properties and subsequently the drug particle detachment upon inhalation (Staniforth et al., 1982; Ganderton et al., 1992; Kawashima et al., 1998; Bennett et al., 1999; Zeng et al., 1999, 2000; Voss et al., 2002). In general, the morphology and roughness of carrier particles are not uniform. This is in a part due to the fact that carriers, such as lactose, are produced on a relatively large scale from natural sources. The processing and production of such organic materials will invariably lead to particles containing regions which exhibit different roughness (peaks and troughs). Furthermore, since DPI carrier based systems are based on organic crystalline materials there may also be specific crystal faces with different surface free energies present. In addition, production and processing methods may also result in the presence of surface macroscopic and/or microscopic amorphous regions. Variations in physico-chemical properties in the surface of a carrier material may lead to differences in apparent adhesion properties of drug particles. Furthermore, during the dynamic process of mixing, the number of contacts and collisions, increased by increasing the agitation of the mixer and the time of processing, for example, during the preparation of an interactive mixture, the exchange of adherent drug particles between the carriers occurs as the particles are displaced from one carrier and readhere randomly to another. If particles can interact tribioelectrically, this will allow the particle to experience contact events with other carriers thereby forming a large number of point charges on their surfaces (Louey et al., 2002). These points called active sites and described in literature to be the best sites at which the drug particle adheres to the coarse carrier (Staniforth et al., 1982; Lucas et al., 1998; Zeng

et al., 2001). Several studies are known in which the positive effect of the presence of very fine lactose in carrier-based formulations on the fine particle dose is described (e.g. Arnold et al., 1993; Srichana et al., 1998; Zeng et al., 1998, 2000a). Others reported the complex effects of lactose grade (Larhirb et al., 1999) and carrier surface properties (Kawashima et al., 1998; Podczeck et al., 1998a, 1999; Zeng et al., 2000b; de Boer et al., 2003, b) on the interaction forces between drug and carrier particles. It has been recommended to use carrier particles with a very specific surface rugosity (Ganderton et al., 1991; Podczeck et al., 1998b), to treat the carrier surface mildly in a (ball) milling process (Staniforth et al., 1995) or to co-process carrier particles with so-called force control agents or the fine additives (Begat et al., 2001; Iida et al., 2003, b; 2005). Staniforth et al., 1995 proposed mild treatment in a ball mill as a suitable means to dislodge naturally adhering fines from the surface of coarse carrier crystals and to reattach them to areas of high energy (clefts and grooves), so as to passive these active sites before drug is mixed with the carrier (preconditioning of the carrier). This so-called corrosion process (although in a different manner) has also been applied by Podczeck et al.,1998a, 1999 who concluded that its effect is only meaningful above certain threshold values for the initial surface roughness. Fine carrier fractions, or the addition of certain fractions of fine lactose particles to coarser carriers, have been shown to increase the fine particle fractions from most marketed inhalers (e.g. Steckel et al., 1997; Zeng et al., 1998, 2001; Louey et al., 2002). Additionally, fine carrier fractions used mostly to enhance powder discharge from capsule inhalers.

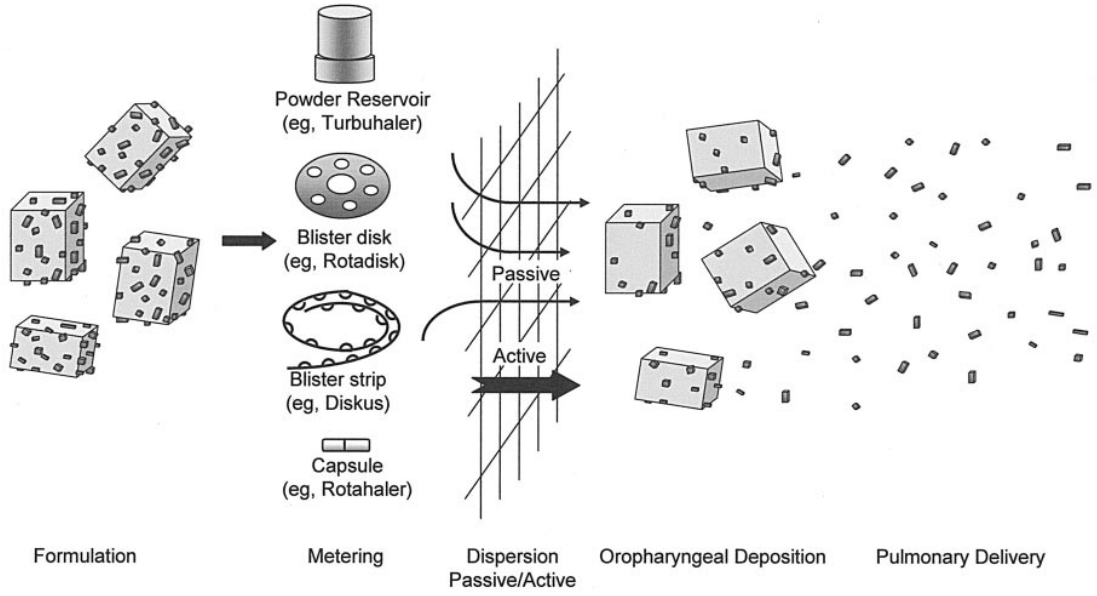


Figure 2: Principles of dry powder inhaler design (Telko et al., 2005).

Particle-particle and particle-container interactions are important in every aspect of micronized powder handling. Drug adhesion to equipment, carrier particles and inhaler surfaces occurs following two major types of contact. During powder processing, powder filling and inhaler emptying, bulk drug or drug-blend particles are most typically moved over surfaces with which they are in frictional contact. Typical shear forces acting on particles in this scenario exceed gravitational forces and are imposed by mixers, filling equipment, the impellers and air jets used as powder deaggregating mechanisms in inhalers. Contact of an aerosolized powder with an inhaler mouthpiece, or other component in the flowing aerosol stream is the other major type of powder-surface interaction in inhalers. These result in deposition and adhesion, clumps or multi-particle assembly formation, due primarily to new particles impacting on and around those that are already attached. Clarke et al. (2001) reported that for the smaller 2.1µm–3.3µm size fraction, forces of autohesion (cohesion) were greater in impacted agglomerates than forces of adhesion between the agglomerate and the surface on which it was formed, causing powder detachment as an agglomerated mass. This was not always the case for the larger 4.7µm–5.8µm size fraction where autohesive and adhesive fracture of the heap (possibly due to less efficient packing of the larger particles/aggregates), caused detachment of several small aggregates.

5.4.1 Types of dry powder inhalers

The choice of the inhaler device should be an integral part of obstructive lung disease management decision. The design of the delivery system for administering drugs to the lung can have as much, or more, impact as the choice of the inhaled drug itself. The inhaler device must deliver the therapeutic agent to the lungs with a small particle size (diameter 1µm-5µm).
There are many types of inhalers used in the DPI field. Different inhaler devices have different efficacy due to differences in lung deposition. The drug powder itself has to be prepared in the same way as the one used in MDIs, by micronization. Excipients have to be added to enhance the flowability. The drug is often present in low concentration on the coarse carrier surface. Upon inhalation, the carrier should ideally be retained in the inhaler device or deposit in the oropharyngeal region due to its large particle size. Therefore drug detachment from the carrier is thought to be crucial in determining the overall delivery efficiency of drugs from dry powder aerosols. The inhaler design influences the fine particle output which also depends strongly on the patient inspiratory performance, in which the fine particle output is more or less flow dependent. However, a higher resistance to air flow limits the range of possible flow rates. Furthermore reduced particle velocity results in a reduced mouth and throat deposition. The principle of operation of those types of generators are using the patient's breathing airflow to draw the drug from the DPI inhaler. The inspiratory flow rate and the turbulence created within the device as the air flows through it break the bonds formed between adhering particles. Therefore, the minimum air flow needed should be determined in order to estimate the likelihood that patients with obstructive lung diseases and airflow limitations will be able to discharge the dose. For the same reason, it is also valuable to determine the resistance to airflow through the device (Timsina et al., 1994). For example, the Spinhaler®, a so-called single dose inhaler delivers the active ingredient from a capsule by rotating it under the influence of the patient's breath, ejecting aerosol particles into the air stream. These particles pass through rotor blades, driving the capsule rotation, and are collected or deaggregated to ensure that smaller particles are administered to the patient (Timsina et al., 1994, de Boer et al.,

1996, b). A more recent device, the Turbuhaler® for delivery of active ingredient, uses a reservoir of drug that fills a series of conical-shaped holes with the powder, by twisting a grip at the base of the Turbuhaler; the holes are filled and scraped at the surface to eliminate excess material. Thus, the dose is governed by the volume of the holes. (In which this inhaler contains the powder as small pellets that deaggregate upon inhalation). The Rotahaler® works by twisting motion of the device cracks a gelatin capsule containing the drug, which then available for inhalation (Timsina et al., 1994). The Aerolizer® is a single dose inhaler work by pressing two levers pierces a capsule. The pierced capsule rotates during inhalation and releases the powder into stream. The multidose Diskhaler® contains individual doses stored in blisters that are arranged in a tangential pathway on a disk, mostly eight blisters per disk, and each dose is activated by sliding the tray out and in and lifting the rear end of the lid so that the needle punctures the blister. The doses are numbered, making it possible for the patient to know how many doses remain (Timsina et al., 1994, de Boer et al., 1996, a). In the Inhalator Ingelheim® devices the gelatin capsule is punctured by pressing a button which is linked to needles, rendering its contents available for inhalation. The drug is inhaled through the pierced holes of a stationary capsule contained in a narrow vertical chamber. The Cyclohaler® has similar features to the Inhalator Ingelheim® device. However, the former device has a longer mouth-piece with a plastic mesh, wider air channels and the capsule is inserted in a narrow horizontal chamber (Timsina et al., 1994). In the Taifun® DPI the inhaled air goes through a cyclone chamber made of propylene, in which the powder is lifted into the air stream. This setup causes the powder to go through multiple contacts with the chamber surface. The Clickhaler® DPI is constructed in a manner which allows the powder to flow with fewer contacts with device surface during actuation. Hard gelatin capsules might have the potential to retain more drug due to adhesion than, for example, plastic blisters. Those gelatin capsules which contain pigments appear to be especially prone to adhesion (Podczeck, 1998). Novolizer® is a multidose inhaler provides a combination of technical features that ensure that every inhalation maneuver is performed with adequate inspiratory and sufficient drug particles are delivered to the patient’s lung. Novolizer® is characterized by low airflow

resistance and provides a refillable cartridge system, and an accurate dose counter. The principal forces leading to deagglomeration within the inhaler reservoirs remain unclear, but are often divided into the following categories, shear force fluidization, turbulence and particle collision (Wang et al., 2004). The Novolizer is the inhaler of choice used in this research work, which generates inertial separation forces instead of turbulent shear or frictional forces. Inertial forces enable successful detachment of drug particles not only from smooth carrier surfaces, but also from carrier irregularities. The Novolizer works on the cyclone principle and air classifier technology. These are robust concepts (de. Boer et al., 2006, c and d) involving both centrifugal force and drug force acting on the particles swirling in a cylindrical chamber (cyclone), which coupled with collision force achieves drug particle detachment from carrier surfaces (Figure 3).

The majority of the literature points to turbulence as the principle factor without considering the detailed nature of turbulent fluid flow and its interaction with dispersed particles (Ganderton et al., 1992; Tismina et al., 1994). There are large differences in fine particle output between different types of DPIs as proved by many publications. These differences depend on the drug load on the carrier crystals, inhaler design parameters, as well as batch to batch variation in powder formulation. On the other hand these differences should be taken into account when changing the prescription of the inhaler device. For the tested inhalers it should be recommended to patients to inhale forcefully and deeply through the DPI to obtain the highest fine particle output. Normally for an optimal use of an inhaler, it is important that the patient is familiar with the optimal inhalation technique. Unfortunately, many mistakes are being made during inhalation. These mistakes can be reduced by giving the patient only one type of inhaler as much as possible for the bronchodilators as well as the corticosteroids. In this way the patient needs to be familiar with only one inhalation technique. In contrast significant head tilting reduced lung deposition of sodium cromoglycate compared to holding the head in the normal position, suggesting that the upper airways are narrowed during the extremes of head tilting, perhaps because of changes induced in the shape of the larynx, pharynx or soft palate (Newman et al., 1994).

Early devices had very low dispersion of respirable-sized particles, often around 10% (de Boer et al., 1996, b; Prime et al., 1997; Steckel et al., 1997). In general, this poor performance can be attributed to the incomplete deaggregation of smaller drug particles from larger carrier particles used as an aid to powder flow during dispersion. More modern devices use means of generating significant turbulence to aid in the deaggregation process. Initially, flow is diverted around the drug and is allowed to pass through the drug only when the optimal flow rate has been obtained (Crowder et al., 2001).

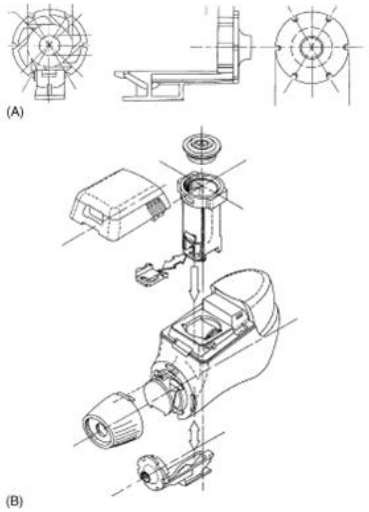


Figure 3: The Novolizer air classifier technique (de Boer et al., 2005).

5.4.2 Resistance to airflow in DP inhalers

The production of fine particles by nebulization or disintegration of agglomerates, and the delivery of fine drug particles in the inspiratory airflow, requires an energy source. MDIs and nebulizers usually use an external energy source, either the propellant or pressurized air is used as energy source to generate the fine particle cloud, which can be taken up by the inspiratory airflow for further transport into the respiratory tract. The inhalation process using dry powder inhalers principally occurs by using the patient-generated inspiratory flow as energy source for emptying of the dose system and the delivery of fine drug particles into the respiratory tract. Inspiratory flow through a breath-controlled dry powder inhaler is the result of an inspiratory pressure generated by the respiratory muscles. Therefore, resistance to

airflow is one of the design parameters for DPIs, that could be used to control the inspiratory flow profile, and is one of the parameters used to optimize particle deposition in the distal airways. There are numerous research works in this field, but they mostly focus on the *in vivo* study using volunteers, since the consistent and precise drug delivery and reliable lung deposition from a DPI depend on the device characteristics and also on the inspiratory flow rate achieved by the patient. In general, the resistance to airflow of the inhaler device restricts the inspiratory flow through the DPI that can be generated by the patient, and it is major determinant for the inspiratory flow profile. Whereas the fine particle output of the inhaler device depends mainly on the generated flow profile and the powder formulation used in the device. The inspiratory flow profile as generated by different patients is variable and depends on the patient's inhalation performance. The inhalation through DPI is in fact inhalation through an external resistance to airflow; this can be simulated by inhalation through simple orifices having the same resistances to airflows as these DPIs. The design of the DPI results in an inhaler specific resistance to airflow. This specific resistance can be calculated as the slope in the linear relationship between the square root of pressure drop (Δp) against the volumetric flow (Φ), according to the simplified equation for an orifice type of flow constriction (de Boer et al., 1996, a, b, 1997).

$$\sqrt{\Delta p} = R \cdot \Phi \qquad \textbf{Equation 6}$$

In which R is the specific airflow resistance.

The drug particle release from DPIs is both actuated and controlled by the patient's inspiratory flow. This provides the advantage of automatic coordination between drug delivery and inhalation on the one side, but makes the efficacy of DPIs strongly dependent on patient's performance on the other.

C THE AIM OF THIS WORK

It is clear that inhalation therapy using dry powder inhalers is not a simple type of treatment and just administration of some powder to the patient which will result in a clinical effect. There are too many variables or factors which are involved in the way between powder formulation and the clinical efficacy in the human body. The carrier of choice in DPIs is lactose monohydrate with few exceptions of some formulations using glucose (Steckel et al., 1997). The reducing property of lactose makes it not always the preferred carrier for some drugs as proteins and peptides due to incompatibility of lactose with the amine group in these compounds (Hickey et al., 1992). In addition, lactose monohydrate is produced from material of bovine source so that the transmissible spongiform encephalopathy discussion is still an issue for this compound (Steckel et al., 2004). On the other hand, there are many suggestions including carbohydrates. The aim of this work is to use mannitol as an alternative to lactose and in comparison with lactose. The aim was extended to establish a correlation between carrier surface characteristics and the dispersibility of drug from the interactive mixture with salbutamol sulphate. Modifications of the surface area of these carriers (lactose and mannitol) were carried out using wet decantation and air jet sieving, those both methods act to remove inherent surface fines and smoothen the carrier particle surfaces. Furthermore, the investigation of the impact of storing the carrier at different relative humidities on the surface characteristics of the carrier and the performance of the dry powder inhalate is also an aim of this study. Finally, the carrier surfaces smoothed by wet decantation were inspected microscopically and compared to the same smoothed carrier surfaces after addition of ascending amounts of fines. Inspection of the effect of elevated environmental humidity on the carrier surface characteristics were carried out. In this study, there is a series of trials to alter the carrier surface smoothness in order to impact interparticle interactions between the drug and the carrier, thereby tailoring the performance of dry powder inhalation formulations.

D MATERIALS AND METHODS

1 Carrier materials

1.1 α-Lactose monohydrate

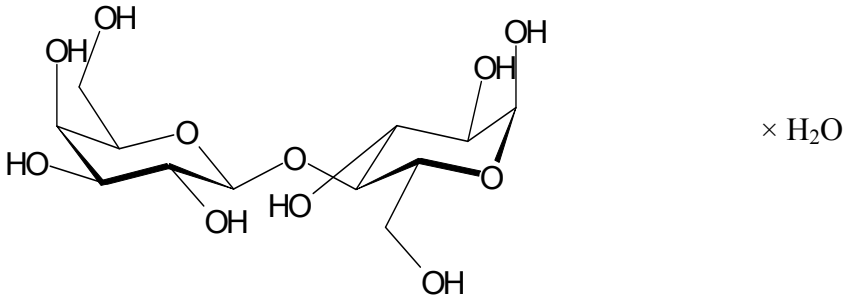


Figure 4: O-β-D-Galactopyranosyl-(1→4)-α-D-glycopyranose monohydrate, $C_{12}H_{22}O_{11}H_2O$

Lactose is a natural disaccharide consisting of galactose and glucose and is present in milk of most mammals. Lactose is extracted from cow's milk, often as a by-product of cheese manufacture. There are many grades of lactose used in pharmacy as spray dried or crystalline powders of α-lactose and β-lactose. These grades are different in physical properties and flow characteristics. α-Lactose monohydrate is obtained by crystallization from super-saturated solutions at temperatures <93.5°C, whereas β-lactose crystals are obtained at temperatures >93.5°C. Microscopically α-lactose monohydrate appears as pyramid and tomahawk shaped crystals (Figure 5). It may contain amorphous fractions that are present when it is either spray-dried from a suspension or lyophilized. This noncrystalline portion is responsible for the improved compressibility of spray-dried lactose. Lactose is soluble in 2.04 parts of water at 50°C (José et al., 2000). Lactose monohydrate used in pharmaceutical formulations as a diluent in oral capsule and tablet formulations. It may also be used in intravenous injections. In dry powder formulations it serves either as a diluent to increase dose accuracy, or as a carrier improving flow properties and decreasing agglomeration of the active ingredient. The advantages of lactose are the known toxicity profile, broad availability with low price, low hygroscopicity as well as the smoothness of the lactose crystals surfaces and the regular shape, which lead to good flowability. Lactose as a carrier in DPI used mostly as sieved, spray dried and

spray freeze-dried particles to provide certain character in the DPI formula. Lactose is used to a more limited extent in the lyophilized products and infant formulas. There are adverse reactions due to lactose intolerance occurring in persons with deficiency of the intestinal enzyme lactase. This results in lactose being undigested and may lead to clinical symptoms including abdominal cramps, diarrhoea, distension and flatulence. (Handbook of Pharm Exp. 1994).

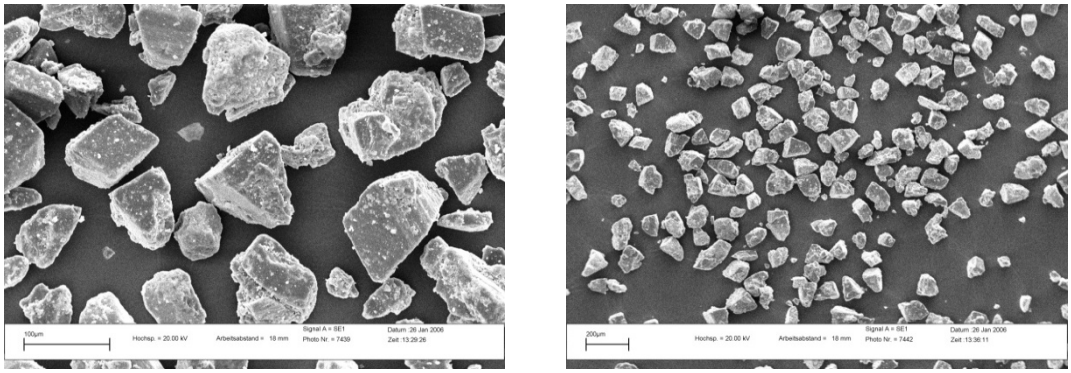


Figure 5: Scanning electron micrographs of lactose crystals.

1.2 Mannitol

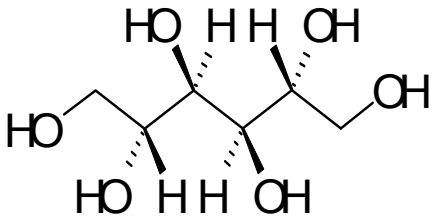


Figure 6: 1, 2, 3, 4, 5, 6 Hexanehexol, $C_6H_{14}O_6$

Mannitol is D-mannitol. It is a hexahydric alcohol related to mannose and is isomeric with sorbitol. The difference between the two polyols represents the orientation of the OH-group on the second carbon atom and the response to moisture, since sorbitol is highly hygroscopic whereas mannitol resists moisture sorption at relatively high humidity. Microscopically mannitol appears as orthorhombic crystalline needles (Figure 7).

Mannitol is a naturally occurring sugar alcohol found in animals and plants. It is present in small quantities in almost all vegetables. Mannitol occurs naturally in manna, the exudates of the manna ash *Fraxinus ornus* (Oleaceae). Mannitol is soluble in water, very slightly soluble in ethanol (96%), practically insoluble in chloroform and ether (Handbook of Pharm Exp. 1994).

Mannitol is stable substance and flows well when prepared as a spray-dried powder, because the particles are close to being spherical. As spray-dried powder, mannitol retains its crystallinity and resists moisture sorption at high relative humidities. These characteristics make it an attractive substance for pulmonary drug delivery (Barben et al., 2003). Inhalation of mannitol may be an efficient method to clear excessive secretions of the airways in patients with bronchiectasis (Daviskas et al., 1997; 1999). Only small amounts of mannitol are absorbed from the gastrointestinal tract following ingestion. When consumed orally in large amounts laxative effects may occur. Mannitol does not cross the blood brain barrier and is not metabolized to any substantial extent when injected (Anderson et al., 1997). Mannitol induces airway narrowing indirectly through changing the osmolarity of the airway surface, leading to the release of mediators, so leading to bronchial smooth muscle contraction (Koskela et al., 2000). Inhaled mannitol may not only permit a point of need bronchial provocation testing but also serves to identify potentially useful drugs that can be used acutely to prevent attacks of asthma (Brannan et al., 1998; 2000). Inhalation of dry powder mannitol increases mucociliary clearance in asthmatic and healthy subjects and may benefit patients with abnormal mucociliary clearance. Mannitol may also alter the rheological properties of the mucus (Daviskas et al., 1997). However, the use of mannitol in small quantities as a carrier in dry powder inhalation has been approved by FDA (Food and drug administration).

Mannitol is widely used in pharmaceutical formulations (Zeng et al., 2000) and food products. It is used as a diluent in tablet formulations. Since it is not hygroscopic it can thus be used with moisture sensitive active ingredients. Mannitol is commonly used as an excipient in the manufacture of chewable tablet formulations, because of its negative heat of solution, sweetness and mouth feel. In lyophilized preparations, mannitol has been used mostly with trehalose as a carrier for protein and peptides to produce a homogenous cake that improves the appearance of the lyophilized plug in a vial. Mannitol is the most commonly used excipient in freeze-dried pharmaceutical products. One of the reasons for the wide spread use of mannitol is its tendency to crystallize from frozen aqueous solutions and the high melting temperature of the mannitol/ice eutectic mixture (about -1.5°C). This property promotes efficient

freeze-drying and physically stable pharmaceutically elegant freeze-dried solids. Mannitol has been used to prevent thickening in aqueous anti-acid suspensions and has been used as a plastizer in soft gelatin capsules, as a component of sustained release tablet formulations and in food applications as a bulking agent. Therapeutically, supersaturated aqueous solutions of mannitol (20-25% w/v) are wieldy used as osmotic diuretics. Mannitol is approved from the FDA to be used in the respiratory tract (ICH guidelines, 1998), mannitol is shown to be feasible alternatives to lactose in this study, and it is expected that this sugar alcohol will eventually find their way into approved products (Steckel et al., 2004; Tee et al., 2000).

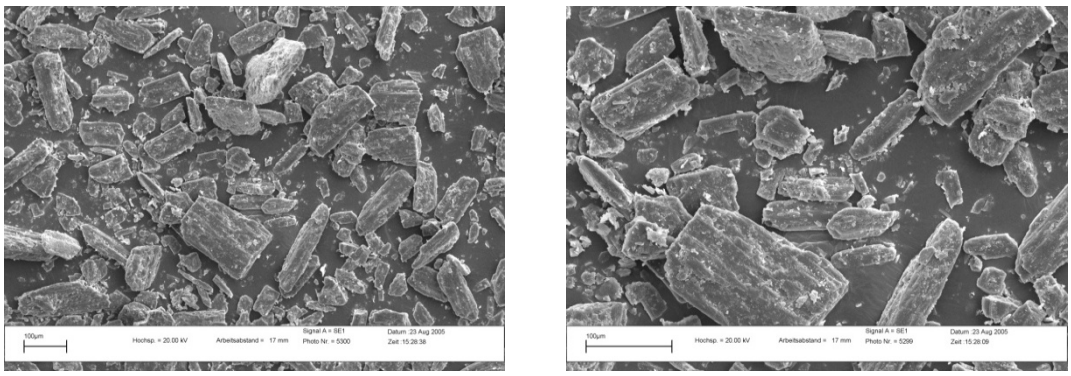


Figure 7: Scanning electron micrographs of Mannitol crystals.

1.3 Active ingredient

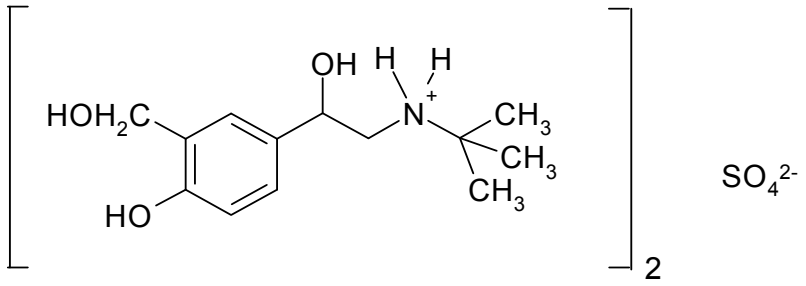


Figure 8: Bis [(1RS)-2-[(1, 1-dimethylethyl) amino]-1-[4-hydroxy-3-(hydroxymethyl) phenyl] ethanol] sulphate.

Salbutamol sulphate is a β_2-adrenergic agonist that is commonly used to treat asthma and chronic obstructive lung disease (COPD) (O'Callaghan et al., 2002), and as such is most commonly delivered respiratorily, both in dry powder and pressurized inhalers. It can be used orally and through parenteral routes. The effect of salbutamol can take place within 5 to 20 minutes of dosing. Recent research has focused on the use of β_2-adrenergic stimulations

as prophylactic drugs because of their ability to inhibit the release of spasmogens and inflammogens from human mast cells. Intravenous salbutamol can be used to relax the uterine smooth muscles to delay premature labour. Salbutamol sulphate is a salt of salbutamol base, freely soluble in water, and very slightly soluble in ethanol and ether. The basic structure of phenyl ethylamine and the substitution on the phenyl ring protects the compound from the effect of the enzyme catechol-O-methyl-tranferase (Hickey et al., 1992). Salbutamol sulphate appears as needle shaped crystals (Figure 9) probably offering a good deposition in the distal lung parts.

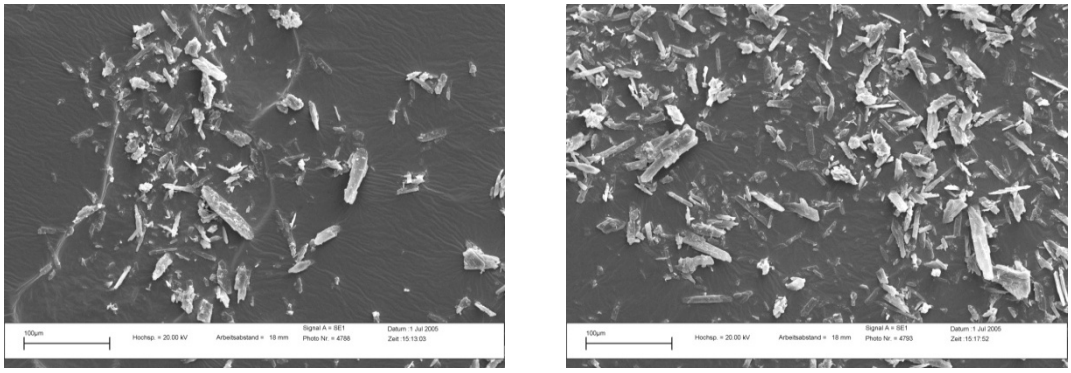


Figure 9: Scanning electron micrographs of salbutamol sulphate.

2 Characterization of the materials

2.1 Particle size

2.1.1 Preliminary considerations

Aerosols that contain particles which have the same size are called monodisperse aerosols. Monodisperse aerosols almost never exist naturally and are difficult to produce. An aerosol which contains particles of various sizes is called polydisperse. The distribution of a polydisperse aerosol typically follows a log-normal distribution. The particle size distribution affects the deposition of drug in the respiratory tract (Telko et al., 2005).

Defining the size of particles inevitably raises the problem of specifically geometrical properties such as shape and dimensions. When particles are not spherical, the number of geometrical parameters defining them is numerous and the concept of size becomes ambiguous. The selected parameter depends on the field of investigated particles. Particle size analysis methods

can be divided into different categories based on several different criteria: size range of analysis, wet or dry methods, manual or automatic methods, speed of analysis. A dry powder inhalation drug delivery system's overall efficiency is a function of the device performance as well as the drug formulation. If the device has high performance, meaning it is highly effective at deagglomerating and aerosolizing the drug into particles capable of penetrating into the deep lung, the fine particle fraction (FPF) will be largely determined by the size distribution of the drug particles in the formulation (Brown et al., 1998). Therefore, the particle engineering of the drug substance may play a significant role in improving the FPF.

2.1.2 Sieving Methods

2.1.2.1 Analytical sieving

Sieving is one of oldest methods of classifying powders by particle size distribution. Sieving will essentially sort the particles by their intermediate size dimension (i.e. breadth or width). Small weight powders have insufficient force during sieving to overcome the surface forces of cohesion and adhesion, which cause the particles to stick to each other and to the sieve. For such material other means of agitation such as air jet sieving or sonic sifting may be more appropriate. In pharmaceutical terms sieving is usually the method of choice for classification of the coarse grades of powders like the carrier particles of dry powder inhalants. One of the limitations of sieving is the appreciable amount of sample (normally at least 25g, depending on the density of powder and the diameter of the test sieve). The sieving method is essentially a two dimensional estimate of size because passage through the sieve aperture is frequently more dependent on maximum width and thickness then on length. Sieving is carried out under conditions that do not cause the test sample to gain or lose moisture, the relative humidity of the environment in which the sieving is carried out must be controlled to prevent moisture uptake or loss by the sample. Sieving is a useful technique in particle sizing, if the goal is to produce a certain size fraction. It is especially convenient for easily flowing and fairly coarse material above e.g. 100 µm with a few fines. The choice of sieving time and the amount of material sieved can have

considerable effects on the results and reproducibility of the results. Material cohesiveness may also cause errors in measurements and result in false size distributions. The amount of sample in sieving is relatively large and sieve analysis is also very time consuming. Analytical test sieves are constructed from a mesh, which is of simple weave that is assumed to give nearly square aperture, and are sealed into the base of an open cylindrical container. The basic analytical method involves stacking the sieves on top of one another in ascending degree of coarseness. The nest of sieves is subjected to a standardized period of agitation, and then the weight of material retained in each sieve is accurately determined, the test gives the weight percentage of powder in each sieve size range (USP 29/NF 24, 2006).

2.1.2.2 Air jet sieving

Another form of sieve analysis, called air jet sieving, uses individual sieves rather than a complete nest of sieves. The air jet method is an effective single-sieve system used for dry powders. The sieve is mounted in a sealed chamber, and air is drawn upwards through the sieve from a slowly rotating slotted nozzle to fluidize the sample. The exit airflow carries under-sized particles downward through the sieve to a collection canister. Starting with the finest sieve the amount of material passing is determined by weighing. The retained sample is transferred to the next larger sieve size, and the procedure is repeated until sieving has been done on all required sieves in succession. Air jet action is gentle and effective especially for fragile or low specific gravity material. Air jet sieving is often more efficient and reproducible than using mechanically vibrated sieve analysis.

2.1.3 Laser light scattering

Both the large-particle and the small-particle analyzers are based on the interaction of laser light with particles. For particles which are much larger than the wavelength of light, any interaction with particles causes light to be scattered in a forward direction with only a small change in angle in comparison to particles which are smaller than the wavelength. This phenomenon is known as Fraunhofer diffraction and produces light intensity

patterns which occur at regular angular intervals and are inversely proportional to the particle diameter producing the scatter. Therefore, as the size of the particles decreases, the scattering angle increases. Fraunhofer diffraction theory is useful for particles which are significantly larger than the wavelength of laser light. As particles approach the dimension of the wavelength of the light, some light is still scattered in the forward direction, according to Mie scatter theory, but there is also some side scatter at different wavelengths and polarizations. The use of the Mie theory requires knowledge of the refractive index of the sample material for calculation of the particle size distribution.

The laser diffraction results may be presented as 10, 50 and 90 percentile (X_{10}, X_{50} and X_{90}). The calculation method relies on the equivalent diameter of a sphere exhibiting the same diffraction pattern. This can lead to an error in calculation as the particle may strongly deviate from the spherical shape.

Powder samples can be measured as dry sample or dispersion in a non-dissolving medium to promote deagglomeration of cohesive particles. Laser diffraction has many advantages such as ease of operation and faster mode of measuring in comparison to the sieving process. On the other hand laser diffraction has also disadvantages, such as poor submicrometer performance and computing artifacts. Furthermore, the amount of sample to be measured is very large when using the dry sample method. Additionally, submicrometer particles scatter most intensely at higher angles, where not all scattered light is collected (Kelly et al., 2006).

2.1.4 Impactors

The term impactor is generally used for instruments where the particles impact on impaction plates or cups due to their inertia. Impactors are useful tools in the assessment of the aerodynamic diameter of powder particles to be used for inhalation. The impactor has the capacity to separate the inhalation powder in a number of fractions. Various studies have shown that the aerodynamic particle size distribution of aerosols generated by inhalation products correlates with the amount of drug deposited in the lungs. The aerodynamic size distribution of an aerosol cloud defines where the particles

in that cloud are deposited by impaction following inhalation. It is generally known, that the particles to be therapeutically effective should be in the range of 1µm-5µm in order to settle in the lungs. The theoretical aerodynamic diameter of the individual particle, D_{ae}, is calculated based on the following definition:

$$D_{aer} = \sqrt{\rho / \rho`} \cdot d \cdot \chi \qquad \textbf{Equation 7}$$

ρ equals the particle density, $\rho`$ is 1 g/cm^3, χ is the dynamic shape factor, defined as the ratio of the drag force on a particle to the drag force on the particle volume-equivalent sphere at the same velocity. d is the mass median particle diameter as measured by light microscopy, which is the reference technique applicable to each powder considered.

Particles having an aerodynamic diameter in excess of 5µm will generally impact on the oropharynx and be swallowed, whereas below 1µm, the possibility exists that the particles will remain entrained in the air stream and be exhaled. Anyway, particles as small as a 0.5µm sphere delivers only 0.1% of the mass that a 5µm sphere carries into the lungs. Traditionally, reduction of the 'D_{ae}' has been effected for solid drug particles by micronization, usually by jet-milling. Decrease of both, particle density and size is currently achieved by spray drying (Edwards et al., 1997; 1998) or more recently, by spray-freeze drying (Zijlstra et al., 2004). Theoretically, a smaller 'D_{ae}' can also be obtained with particles of non-spherical shapes, such as platelets, rods or fibers (Crowder et al., 2002), because the χ-value for such particles can be as high as 10. In terms of aerodynamic performance, changing the "ruggedness" of particle surface, as quantified by the surface fractal dimension, is analogous to a simultaneous reduction of the particle density and increase of the dynamic shape factor. However, for all these non-spherical shapes, deagglomeration may depend on the particle packing (Chow et al., 2007). The aerodynamic diameter is the most appropriate measure of aerosol particle size, because it relates to the particle dynamic behavior and describes the main mechanisms of aerosol deposition. Both gravitational settling and inertial impaction depend on the aerodynamic diameter.

The Next Generation impactor (NGI) is the recent impactor used nowadays for analyzing the in vitro deposition of dry powder particles (Figure 10). The NGI is a cascade impactor with 7 stages and a micro-orifice collector (MOC), Operating over the flow rate range of 30 litres/min to 100 litres/min, the 50 percent-efficiency aerodynamic cut-off diameter (D_{50} values) ranges between 0.24μm to 11.7μm, evenly spaced on a logarithmic scale. In this flow range there are always at least 5 stages with D_{50} values between 0.5μm and 6.5μm. The collection efficiency curves for each stage are sharp and minimize overlap between stages. The cut-off points of stages 1 to 7 are 8.06, 4.46, 2.82, 1.66, 0.94, 0.55 and 0.34 micron respectively at 79.3L/min. A suitable mouthpiece adapter is used to provide an airtight seal between the inhaler and the induction port. The micro-orifice collector (MOC) for most formulations will eliminate the need for a final filter as determined by validation, and most particles not captured on stage 7 of the impactor will be captured on the cup surface below the MOC. For impactors at 60 litres/min, the MOC is capable of collecting 80 percent of 0.14μm particles. Particle separation and sizing is achieved by successively increasing the velocity of the air stream as it passes through each stage by forcing it through a series of nozzles, containing progressively reducing jet size. The inhalation portion of the single breathing cycle is simulated through the use of a two-way switching valve connected to a vacuum pump. The operation of the solenoid valve and the duration of the cycle are controlled by means of a timer. One channel from the valve is connected to the impactor and the other to the vacuum pump. The NGI is applicable to all single shot inhaled delivery systems (MDIs, DPIs, aqueous inhalers) (Marple et al., 2003, a, b, c). The NGI uses a preseparator to catch any powder boluses and large non-inhalable particles. The NGI preseparator consists of a two-stage preseparator with a sharp and reproducible cut-point between 10μm and 15μm, dependent on the flow rate. The impactor itself comprises just three main parts, the bottom frame (which holds the cup tray containing the eight collection cups used to collect the samples prior to analysis. The seal body that holds the nozzles in place, the lid that contains the inter-stage passageways. In routine operation the three parts are held together using a handle clamping mechanism, each part sealed with O-rings such that they are leak-free.

Impactors are used to determine the fine particle fraction which represents the percentage of particles <5µm, which potentially can penetrate and settle on the distal airways. The fine particle fraction (FPF) is calculated as the ratio of fine particle dose to the recovered dose. The fine particle dose (FPD) is calculated as the dose of drug exhibiting an aerodynamic diameter <5µm by addition of the doses of drug on stages 3-7 plus the part of drug <5µm on stage 2 obtained by interpolation at 79.3l/min. The recovered dose (RD) is the sum of the drug collected in the throat piece and the 8 stages of the NGI. The emitted dose (ED) is the amount of drug released from the inhaler device, the amount of drug discharged from the dose system of the DPIs does not equal the amount delivered to the respiratory tract (mouthpiece release). Losses occur inside the inhaler, partly due to waste directly from the dose system even before inhalation and partly as a result of accumulation of primary drug particles in the mouthpiece region during inhalation. This situation depends mainly on the flow rate exerted by the patient through the inhaler device. Additionally, the large carrier particles in the drug formulation may sweep previously accumulated drug particles from the walls of the mouthpiece, which reduces the mouthpiece deposition.

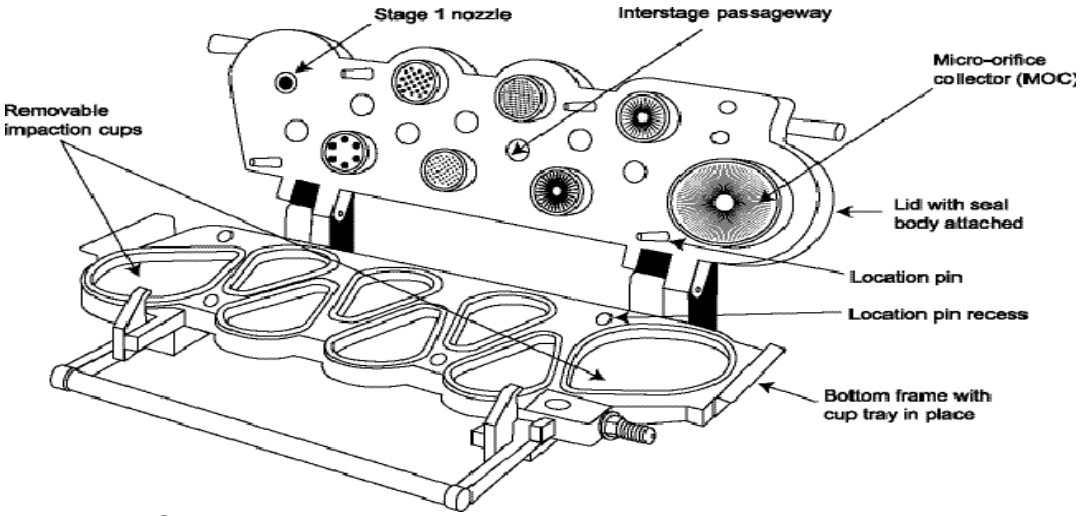


Figure 10: Next Generation Impactor (British pharmacopoeia 2007).

2.2 Particle shape and surface topography

Therapeutic efficacy of inhaled drug therapy depends on more variables and parameters than oral or systemic drug administration and is affected by the device performance, formulation characters, the variability in airway anatomy,

disease status, breathing pattern profiles, handling skills, etc. The delivery system and skills to master inhalation will affect therapeutic efficacy which depends in general on the dose available at the site of action.

The shape can profoundly effect on the aerodynamic behaviour of particles. Particle shape is largely governed by the method used to prepare the powders e.g. comminution, screening, spray drying, crystallization etc. The best non-spherical shapes are those of elongated particles. Because of the investigations on toxic effects of mineral fibers such as asbestos, it is known that the aerodynamic diameter of particles with high axial ratio is almost independent of their length and diameter. The deposition of these fibers in the peripheral lung is enhanced by interception (Crowder et al., 2001). These types of shapes can be obtained by e.g. control of the crystallization conditions. For DPIs, small variations in the homogeneity of a carrier surface may result in large differences in aerosolisation performance, the microstructure of a carrier may be altered by treatment of the carrier surface. Examples, include solvent smoothing methods (Ferrari et al., 2004; Young et al., 2002), coating with laminar molecules such as magnesium stearate (Iida et al., 2004, a) and the addition of fines to either fill high-energy active sites or form multiple agglomerates (Islam et al., 2004; Louey et al., 2002; Lucas et al, 1998). However, these approaches, generally aim to smooth out existing morphological differences or cover them up completely.

The surface area of corrugated (rough) particles is higher than of smooth particles that occupy the same volume. Thus, particle morphology is a basic factor used in DPI formulation design. By modifying or engineering the particles in order to modify the surface area, the interparticulate forces can be modulated to enhance drug deposition. Ideally, the contact area and interparticulate forces should be adjusted to a level that provides a stable formulation and easy release of drug substance from the coarse carrier upon inhalation. However, there are some studies showing that a smooth carrier surface increases the fine particle fraction and the dispersibility of micronized drug (Iida et al., 2003, a; 2004, c; Flament et al.; 2004; Zeng et al., 2000), while other studies show that corrugated carrier particles increase the fine particle fraction (Heng et al., 2000). These results appear contradictory, but both may be correct. The increased FPF with smoothed carriers was

attributed to the reduction of binding sites with multiple contacts, and removal of surface irregularities which increases the contact area of a single contact point between drug and carrier particle (Podczeck et al., 1999; Price et al., 2002). In contrast Heng suggested that the size of the carrier surface irregularities is at a scale that reduces the total contact area between the drug and the carrier particle. Thus, it reduces the adhesive interaction force and subsequently increases the FPF (optimized level of rugosity is required). The carrier particle should not only be homogeneous but also have a specific roughness beneficial to formulation stability and aerosolisation, whereas the carrier which contain rough areas can result in a higher contact area available for drug particles and various libration of drug particles during inhalation in comparison to the smoothed carriers (Young et al., 2002; 2007).

Scanning electron microscopy is particularly appropriate when a two-dimensional particle image is required. In a typical SEM, electrons are emitted from a tungsten cathode and are accelerated towards an anode; alternatively, electrons can be emitted via field emission (FE). The electron beam passes through pairs of scanning coils in the objective lens, which deflect the beam horizontally and vertically so that it scans in a raster fashion over a rectangular area of the sample surface. The energy exchange between the electron beam and the sample results in the emission of electrons and electromagnetic radiation which can be detected to produce an image. Before the scan the sample is exposed to a gold coating process, which is needed to make samples conductive.

2.3 Particle surface area

The adsorption of an inert gas such as nitrogen, helium, or krypton onto solid materials provides the most widely used method for surface area determination. The basic theory of this method is that the amount of gas adsorbed onto a particle is proportional to the surface area of that particle. The amount of the adsorbed gas can be calculated by measurement of its volume and pressure. In other words, the method involves the determination of the quantity of adsorbate (gas) required to cover all the available surface areas with a layer one molecule thick. The volume (V_m) of gas required to

achieve monolayer adsorption at equilibrium under a specific temperature and pressure can be calculated according to the Langmuir equation:

$$\frac{p}{V} = \frac{1}{bV_m} + \frac{p}{V_m}$$ **Equation 8**

V is the volume of the gas adsorbed at pressure p and b is a constant. Thus, a plot of p/V against p will yield a straight line with slope $1/V_m$.

However, it is unlikely that the adsorbed gas will be limited to a monolayer and multimolecular layers are often formed on the surface caused by condensation of gas molecules onto the adsorbed monolayer. This problem was solved by Brunauer, Emmett and Teller, who extended the Langmuir theory to account for multimolecular layer adsorption. The equation they generated is the classic BET equation.

$$\frac{p}{V(p_0 - p)} = \frac{1}{V_m C} + \frac{(C-1)p}{V_m C p_0}$$ **Equation 9**

V is the volume of gas adsorbed at pressure p (the partial pressure of the adsorbate), V_m is the volume of gas adsorbed as a monolayer, p_0 is the saturation pressure of adsorbate at the experimental temperature and C is a constant exponentially relating the heats of adsorption and condensation of the adsorbate. Therefore, using various concentrations of adsorbate, a graph of $p/V(p_0-p)$ against p/p_0 yields a straight line. The value of V_m can be calculated from the reciprocal of the sum of the slope and the intercept. Then, the total surface area of the sample powder is calculated using:

$$S_t = \frac{V_m N_0 A_{cs}}{M_w}$$ **Equation 10**

where S_t is the total surface area, N_0 is the Avogadro's number, A_{cs} is the cross-sectional area of the adsorbent molecule and M_w is the molecular weight of the adsorbent.

The specific surface area (S) of the solid then can be obtained from:

$$S=\frac{S_t}{m} \qquad \textbf{Equation 11}$$

where m is the mass of powder measured.

The BET theory is based upon many assumptions such as all adsorption sites are energetically equivalent and the heat of adsorption for the second layer and above are equal to the heat of liquefaction. Although such assumptions are not strictly true under normal conditions, this method has nevertheless proven to be an accurate representation of surface area for a wide range of materials examined (Newman, 1995). Nitrogen has been found to be an appropriate adsorbent gas for most materials which have surface areas of more than $1.0 m^2 g^{-1}$, while krypton should be used for those with smaller areas.

Nitrogen adsorption may also be used to determine the surface areas of both, the carrier and the drug particles of a DPI formulation. However, the surface area of carrier particles determined by nitrogen adsorption will consist of a portion of the surface that is not significant as far as physical interaction between the drug and carrier particles is concerned. It would be reasonable to assume that this part of the surface should be ignored in order to obtain a more accurate correlation between surface area and particle behavior.

2.4 Crystalline behavior of powder

2.4.1 Introducing remarks

The crystallinity of the powder particle may be changed by powder processing, such as milling, decantation processes as well as by storage at different relative humidities. This accidental production or removal of amorphous parts may cause changes in the powder performance, especially in the forces acting between the drug and the carrier. Amorphous parts of the powder material are thermodynamically unstable and will tend to revert to the crystalline form on storage (devitrification). The onset of the devitrification process may be so slow as to be effectively irrelevant within the shelf life of the powder. Powder particles were examined in relation to their crystallization

by using: differential scanning calorimetry, X-ray diffraction and water vapor sorption to get more understanding of the carrier and drug substance stability.

2.4.2 Differential scanning calorimetry

The differential scanning calorimetry (DSC) technique is used for measuring the energy necessary to establish a nearly zero temperature difference between a substance and an inert reference material, as the two samples are subjected to identical temperature regimes in an environment heated or cooled at a constant rate. This supply or removal of thermal energy may induce physical or chemical processes in the sample, e.g. melting, crystallization or decomposition, accompanied by a change in enthalpy, the latent heat of fusion, heat of reaction etc. Such enthalpy changes may be detected by thermal analysis and related to the processes occurring in the sample. The reference sample should have a well-defined heat capacity over the entire range of temperatures to be scanned. The basic principle underlying this technique is that, when the sample undergoes a physical transformation such as phase transitions, more (or less) heat will need to flow to the sample than to the reference to maintain both at the same temperature. DSC can also be used to observe changes like the glass transition. It has often been difficult to detect very low levels of amorphous contents using DSC (below 10% w/w), because of the small energy changes associated with the transition at these low levels. Other studies (Hey et al., 1997) showed a DSC coupled with an optical video microscopic equipment to record optical and thermal data of the inspected material simultaneously in order to enhance the reliability of the DSC results.

2.4.3 X-ray powder diffraction

X-ray powder diffraction is one of the most important characterization tools used in solid-state chemistry and materials science. In the pharmaceutical field X-ray diffraction used mostly to detect how crystalline the powder is. X-rays are electromagnetic radiation of a wavelength of about 1Å (10^{-10}m), which is about the same range as interatomic distances. They occur in the portion of the electromagnetic spectrum between gamma-rays and the

ultraviolet. X-ray diffraction has been used to determine the structure of crystalline substances. Each crystalline solid has its unique characteristic X-ray powder pattern which may be used as a "fingerprint" for its identification. Once the material has been identified, X-ray crystallography may be used to determine its structure, i.e. how the atoms pack together in the crystalline state and what the interatomic distances and angles are etc.
The path difference between two waves:

$$n \times wavelength = 2d \sin(theta)$$ **Equation 12**

Here *d* is the spacing between diffracting planes, θ is the incident angle between the incident ray and the scattering planes. n is an integer which is equal 2 in this case of two waves, this equation called Bragg's equation .

The X-ray diffraction experiment requires an X-ray source, the sample under investigation and a detector to pick up the diffracted X-rays. The X-ray radiation most commonly used is that emitted by copper, whose characteristic wavelength for the Kα radiation is =1.5418Å.

When the incident beam strikes a crystalline powder sample, diffraction occurs in every possible orientation of 2theta. The diffracted beam may be detected by using a moveable detector, which is connected to a chart recorder. In normal use, the counter is set to scan over a range of 2theta values at a constant angular velocity. Routinely, a 2theta range of 5 to 70 degrees is sufficient to cover the most useful part of the organic powder patterns.

2.4.4 Water vapour sorption

Water vapour sorption is a relatively new method used for amorphous content determination in a powder sample. The vapour sorption profile of amorphous material may be markedly different from crystalline material. Once water is incorporated into an amorphous region, it acts as plastizer increasing the molecular mobility of the solid and causing a reduction of the glass transition temperature T_g. As the T_g drops below the experimental temperature T, the amorphous material may crystallize. During crystallization the water that was

present in the amorphous regions is expelled from the solid. The availability of a machine with good temperature and humidity control makes it rather simple to detect changes in crystallinity gravimetrically. Additionally the determination of the water content depending on the relative humidity in this system at constant temperature gives information about the hygroscopicity of the compound used and clarifies behavior of this compound at different climatic environments (Young et al., 2007).

E RESULTS AND DISCUSSION

I Influence of micronization on the physical properties of salbutamol sulphate as a model drug used in dry powder inhalation

1 Introduction

Many studies have been reported regarding the effect of the physico-chemical properties of the drug on the drug-to-carrier interaction and the fine particle fraction obtained during inhalation. For example, the type of drug (Price et al., 2002; Podczeck et al., 1995; Clarke et al., 2002), the drug particle size and the drug concentration in the interactive mixture and the duration of the blending time (Kulvanich and Stewart, 1987, a and b). Several techniques have been applied to prepare drug particles in the desired aerodynamic size range. These techniques include standard techniques such as milling, and more advanced techniques such as spray drying (Maa et al., 1998; Chawla et al., 1994), spray freeze drying (Costantino et al., 2002), super critical fluid extraction (SCF) (Shekunov et al., 2006) and crystallization which are used to produce drug particles with controlled surface characteristics, compared to 'jet milling' during which this cannot be achieved.

The following part of this study focuses on the influence of the micronization process on the physicochemical properties of salbutamol sulphate which is used as a model drug interactively mixed with lactose and mannitol as carrier materials.

2 Results and discussion

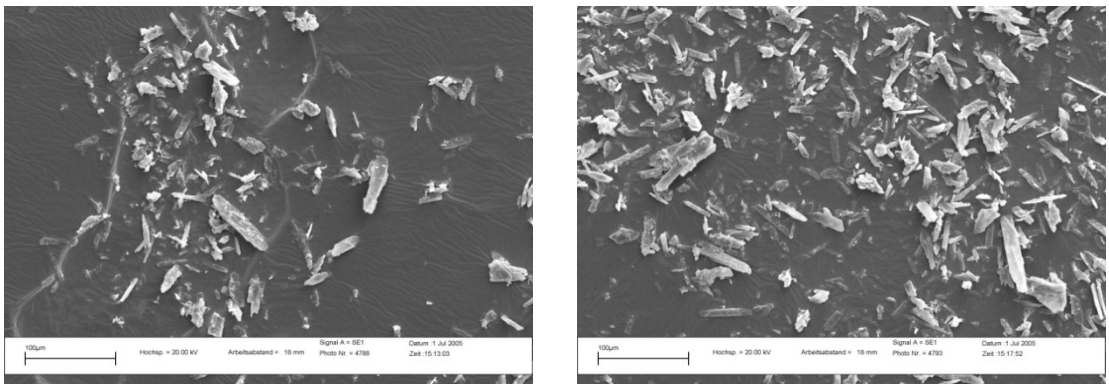
2.1 Determination of particle size distribution using laser diffraction

The size of the active ingredient is particularly important in the dry powder inhalation field to offer effective delivery of the drug particles to the distal parts of the lung, where the drug particles should be small particles with an aerodynamic diameter of 1µm-5µm. The particle size distribution of salbutamol sulphate was measured using laser diffraction, the original size

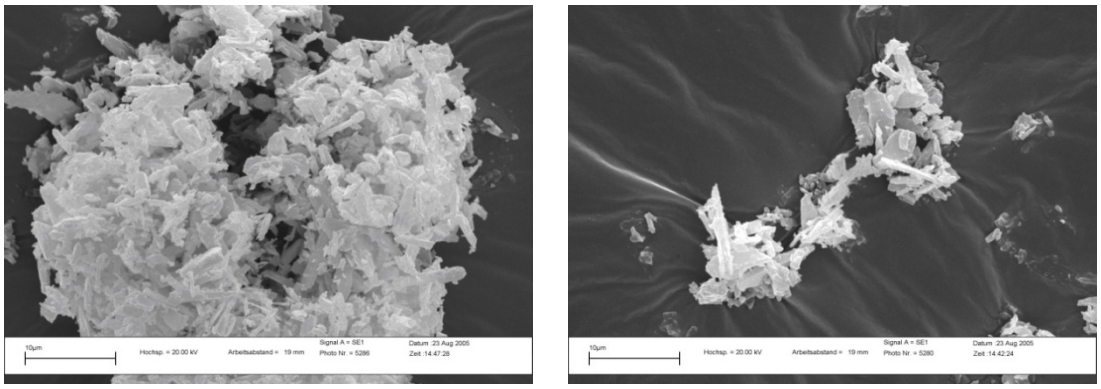
was about 8.3µm in diameter. After micronization the particle size using laser diffraction was summarized using x_{10}, x_{50}, and x_{90} values. The vast majority of salbutamol sulphate particles were smaller than 5µm (x_{50}=2.03µm±0.07µm (mean±s.d.), x_{10}=0.63µm±0.32µm, x_{90}=5.33µm±0.06µm, suggesting that the drug powder was suitable for DPI formulation. However it should be noted that laser diffraction measures the geometric diameter, whereas particle deposition is determined by the aerodynamic diameter, so a direct comparison between the proportion of particles exhibiting a geometric diameter <5µm and the FPF cannot necessarily be drawn.

2.2 Determination of particle shape and surface characteristics by scanning electron microscopy

SEM micrographs were taken by scanning fields, selected randomly, at several magnifications. As shown in Figure 11, the salbutamol sulphate particles appear as needle shaped particles before as well as after micronization by air jet milling. Micronized salbutamol sulphate tends to adhere to each other forming agglomerates. Unmicronized particles show larger particles in comparison to micronized particles, which is in agreement with the laser diffraction analysis results.



a



b

Figure 11: Scanning electron micrographs of unmicronized (**a**) and micronized (**b**) salbutamol sulphate respectively.

2.3 Determination of the extent of crystallinity

2.3.1 Introducing remarks

Micronization is a high energetic process that may induce changes in the crystallinity of materials on the particle's surface and form amorphous areas (Figure 12). The reduction in the degree of crystallinity, if this disorder is more extensive than inherent crystallographic molecular defects and dislocations, can be viewed as an amorphous region at the surface or near-surface of a particle (Begat et al., 2003; Feely et al., 2002). The presence of even small amounts (as little as 1% of total weight) of amorphous regions on the particle surface has a significant impact on the physicochemical nature and may affect especially surface related properties like interparticle interactions of the powdered material (Buckton et al., 1995). It is hypothesized that the milling process changes the orientation of molecules on the surface of the powder particles and thus alters surface energetics (Briggner et al., 1994; Ward et al., 1995).

Conditioning of salbutamol sulphate was carried out in order to remove the amorphous parts possibly present on the particle surface and revert them into crystalline parts which are stable. Conditioning was carried out by storage at 52.8% and 75% for 1 day, 7 days, 10 days and 14 days. Storage was also carried out without conditioning using silica gel to compare the material with conditioned powders.

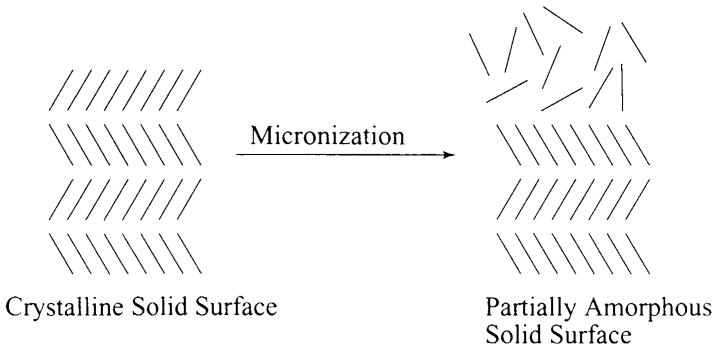


Figure 12: Modified crystal orientation after micronization (Ward et al., 1995).

The amorphous part can be transferred into crystalline by reducing the Tg of the substance below room temperature. This recrystallization may result in size increase of the micronized substance. Particle growth may generate fractions of the particles with diameters outside of the respirative range (1µm-5µm). Conditioning of salbutamol sulphate may convert the amorphous parts into crystalline solids under storage conditions, which are controlled with respect to relative humidity and temperature. These conditions aim at reducing the glass transition temperature Tg of the solid material by sorption of water and setting the surrounding temperature to values above Tg so that the molecular mobility and consequently the crystallization process is accelerated. Salbutamol sulphate stored at different relative humidities was investigated by differential scanning calorimetry, X-ray diffraction and water vapour sorption to show the effect of the conditioning process on crystallinity.

2.4 Water vapour sorption

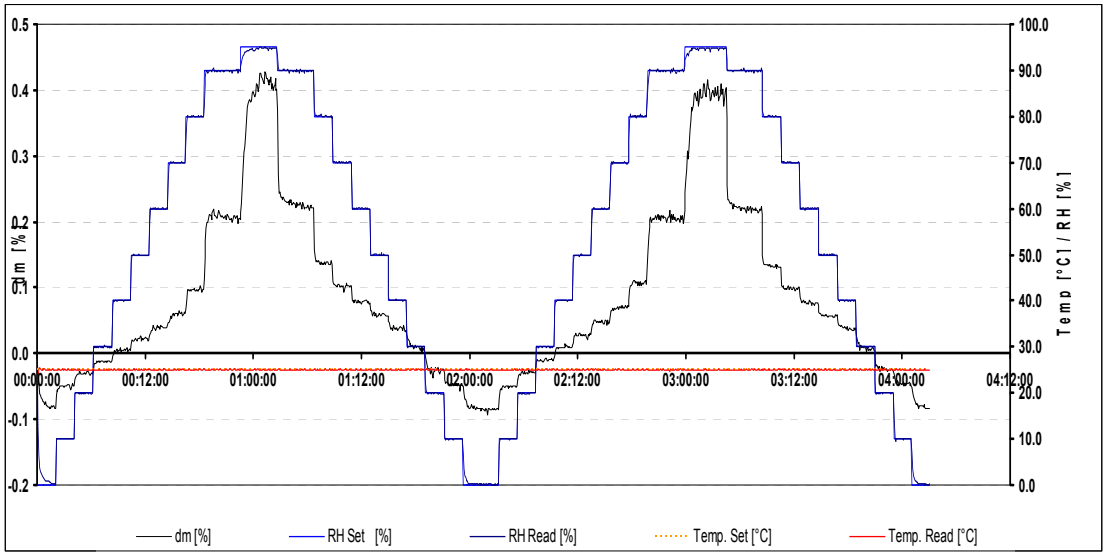
Over recent years a number of research papers have been published using isothermal microcalorimetry to quantify low levels of amorphous contents (Briggner et al., 1994; Sebhatu et al., 1994b; Buckton et al., 1995; Mackin et al., 2002; Young et al., 2007). Besides, water vapour sorption was used to determine the amount of amorphous material in salbutamol sulphate (Buckton et al., 1995) which was used as active ingredient in this study.

The water vapour sorption data of crystalline salbutamol sulphate are shown in Figure 13 (a). The higher the humidity increases, the higher is the mass gain and decreasing the relative humidity after reaching 95%RH leads to a

decrease in the mass. By spray drying amorphous salbutamol sulphate particles are obtained. The corresponding sorption data are shown in Figure 13 (b) (data documented in Ref. 56, Gorny M. et al., 2007). In contrast to crystalline salbutamol sulphate, there is a mass decrease observable at 50% RH and above due the expulsion of excess water following recrystallization of the amorphous content. The calculation of the amorphous content as stated in Gorny M. et al., 2007 shows that the micronized salbutamol sulphate contains 1.65% amorphous material. From Figure 13 (b), it is obvious that the micronized salbutamol sulphate contains a small amorphous content and this content recrystallizes at 50% relative humidity by expelling the water. This leads to a decrease of the mass of salbutamol sulphate.

In order to remove amorphous parts from the drug particles and in order to ensure that crystalline material, that is stable upon storage, will be used for the preparation of ordered mixtures with the carrier, conditioning of the drug was performed at 52.8% RH and 75% RH. Storage of micronized salbutamol sulphate at 52.8% and 75% RH for 1 day and subsequent water sorption experiments lead to the results shown in Figure 14 (a) and (b) respectively. Both samples still show amorphous parts indicating that recrystallization has not been completed yet. By using the calculating method mentioned by Gorny M. et al., 2007, it has been found that the amorphous content of salbutamol sulphate stored at 52.8% and 75% relative humidity is 0.35% and 0.29% respectively. Conditioning of micronized salbutamol sulphate at 52.8% and 75%RH for 2 weeks respectively was done at ambient temperature in order to obtain a stable milled material. Again, the samples were analyzed by water vapour sorption (Figure 15). Both samples show almost fully crystalline behaviour. It has been found that the amorphous content of salbutamol sulphate stored at 52.8% and 75% relative humidity for 2 weeks is 0.022 % and 0.018 % respectively. Additionally the first mass loss is detectable at 0%RH, this is caused by the loss of water, which has not been removed during storage over silica gel prior to analysis. Significant differences in the total amount of water sorbed are observed, with the unmicronized material having a maximum value approximately 12 fold greater than the micronized material at 95%RH. This was somewhat unexpected based on the large

differences in both particle size and surface area between these two samples. Uptake of such large quantities of water can occur as the result of hydrate formation, deliquescence or capillary condensation. To the author's knowledge, no hydrate forms of salbutamol sulphate have been reported (Ward et al., 1994).



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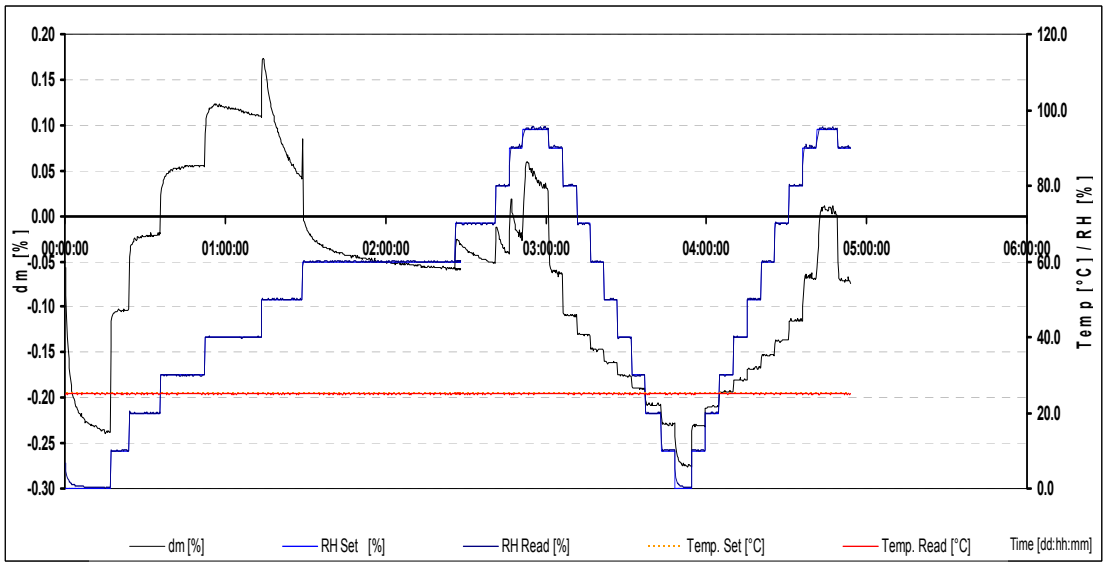
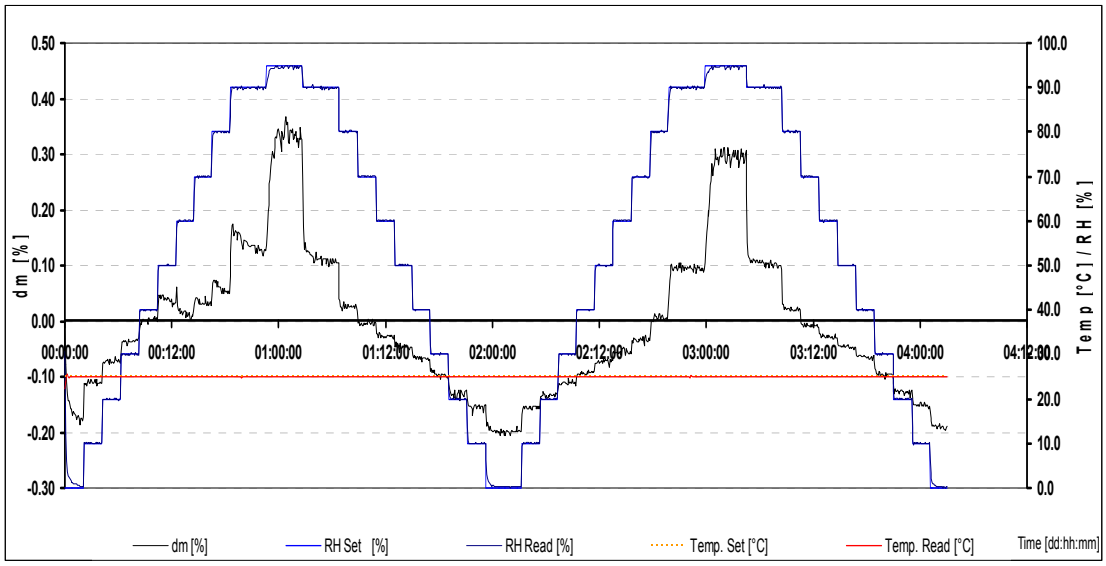


b

Figure 13: Water vapour sorption of crystalline (**a**) and amorphous (**b**) salbutamol sulphate (spray dried).



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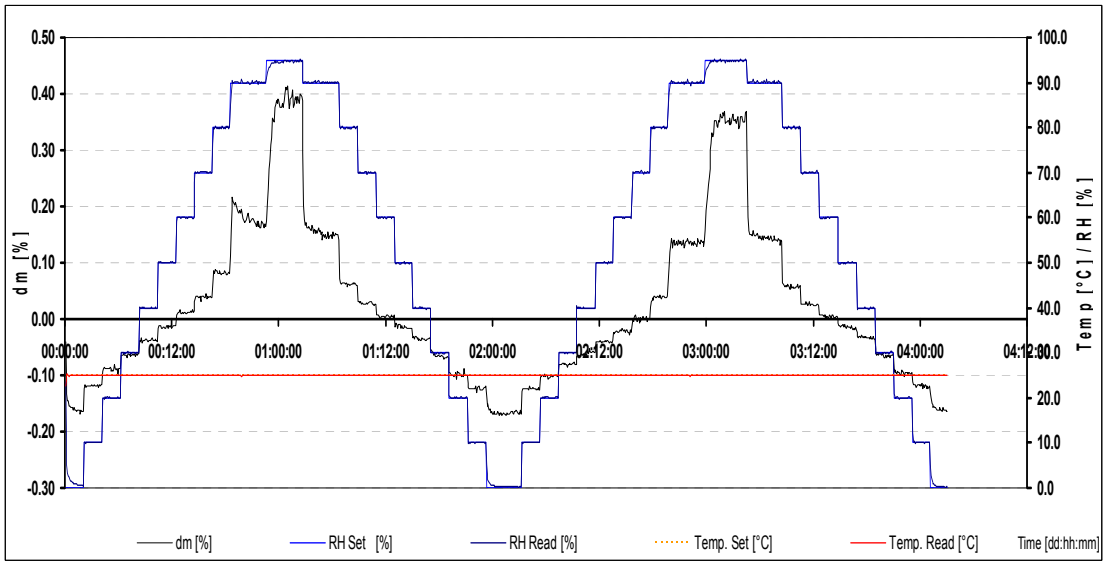

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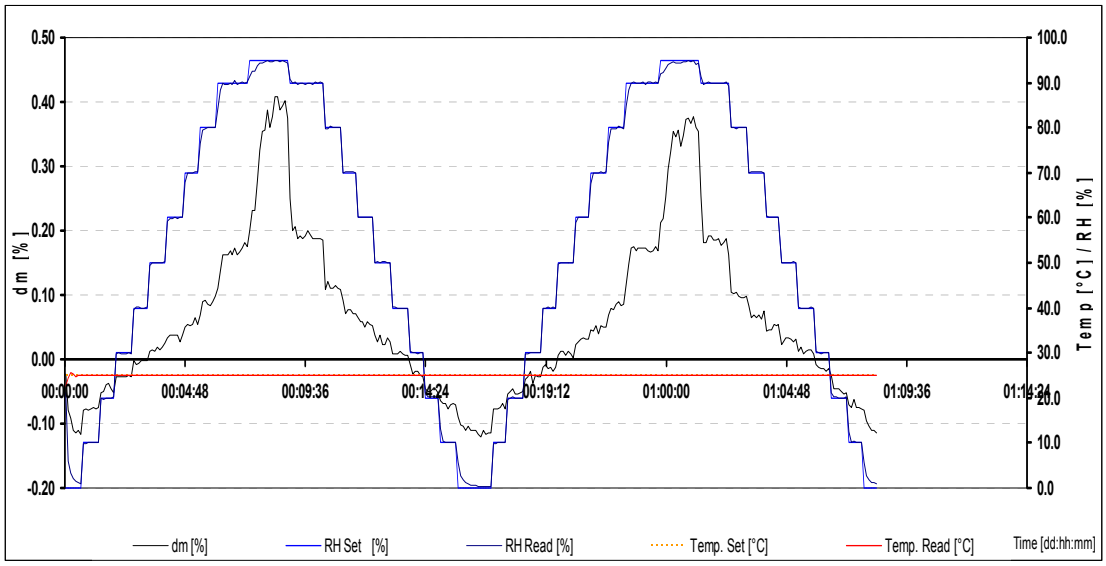
Figure 14: Water vapour sorption of milled salbutamol sulphate stored for 1 day at 52.8% relative humidity (a) and 75% relative humidity (b).



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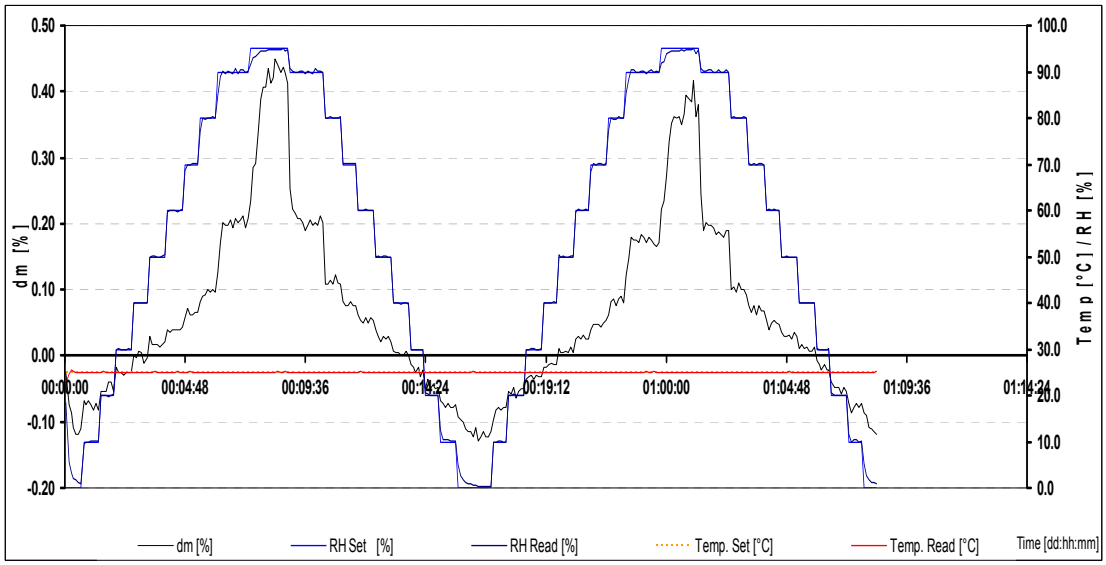


b

Figure 15: Water vapour sorption of milled salbutamol sulphate stored for 2 weeks at 52.8% relative humidity (a) and 75% relative humidity (b).

2.5 Differential scanning calorimetry

Differential scanning calorimetry as one of the standard techniques for the determination of crystallinity was performed on unmicronized salbutamol sulphate, salbutamol sulphate directly after micronization, micronized salbutamol sulphate stored for 24h at silica gel, at 50%RH and 75%RH, salbutamol sulphate stored for 7 days at 50%RH and 75%RH, salbutamol sulphate stored for 14 days at 50%RH and 75%RH respectively. The DSC

thermograms (Figure 16) show approximately similar thermal behaviour, i.e. there is no exothermic peak which indicates amorphous content. The amorphous parts that were detected by water vapour sorption of the micronized salbutamol sulphate show no glass transition. This can be related to the detection limit of this technique, which is limited to 5%-10% of amorphous content (Buckton et al., 1999).

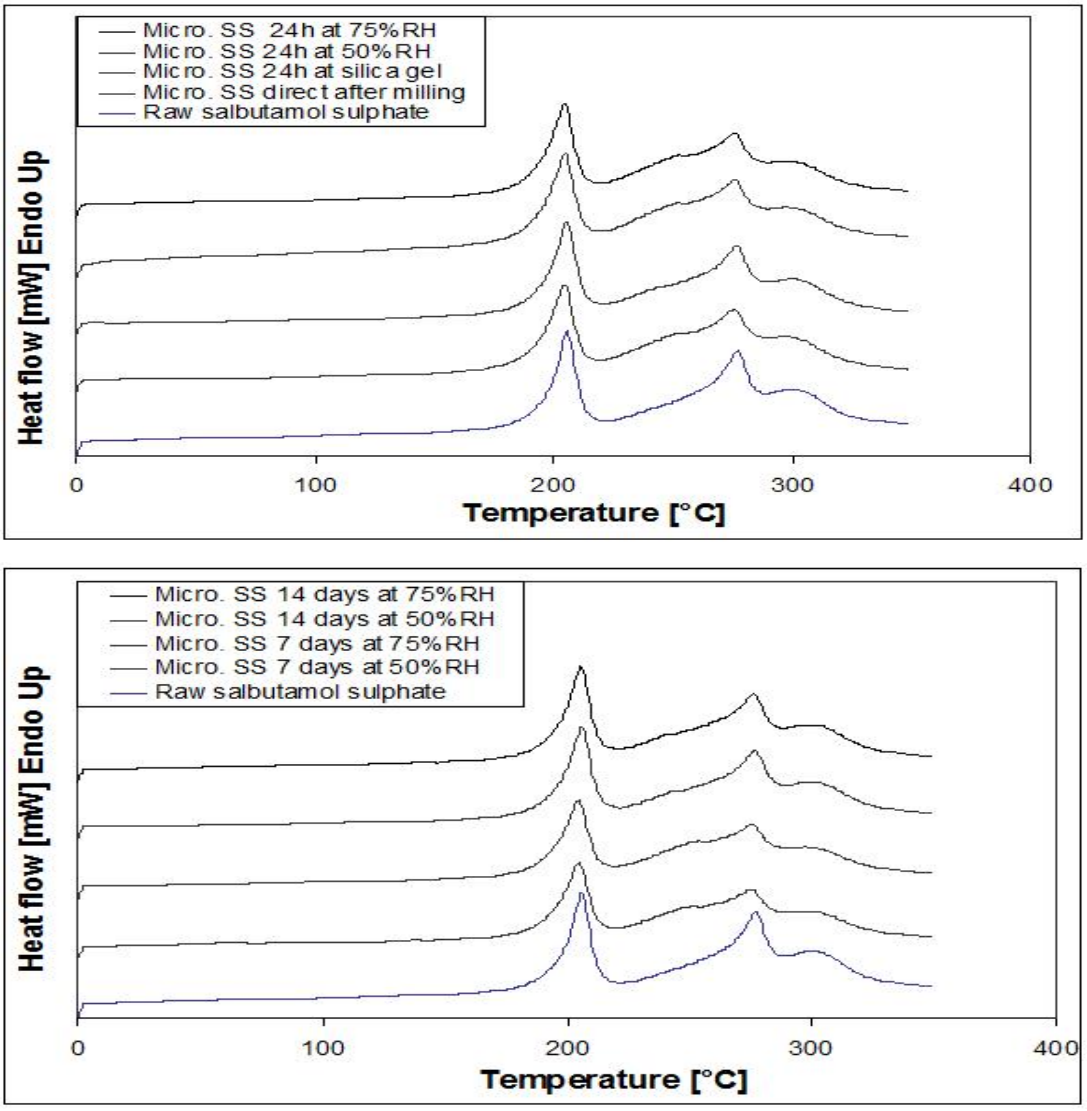


Figure 16: DSC thermograms of raw and micronized salbutamol sulphate (micro.SS) conditioned for different times at different relative humidities (n=2), the second of two measurements is shown.

The enthalpy of fusion of salbutamol sulphate (Table I) was changed directly after milling through the air jet mill and after conditioning at different relative humidities and for different periods. This enthalpy change could be related to

the presence of small amorphous part in the milled and conditioned powder, which was under the detection limits of the DSC technique.

Table I: DSC parameters of conditioned and raw salbutamol sulphate, n=2, mean ± range (the range indicates the maximal and minimal values).

SUBSTANCE	ENTHALPY [J/G]	ONSET[°C]	PEAK[°C]
Raw salbutamol sulphate at orange gel	232.93±2.75	196.16±0.07	205.84±0.07
Salbutamol sulphate direct measured after milling	274.32±10.5	191.27±0.22	204.88±0.09
Micronized salbutamol sulphate 24h at orange gel	230.60±1.05	194.17±0.18	205.85±0.08
Micronized salbutamol sulphate 24h at 50%RH	251.12±2.77	192.26±0.03	205.21±0.59
Micronized salbutamol sulphate 7 days at 50%RH	254.31±9.76	193.03±0.02	204.91±0.07
Micronized salbutamol sulphate 14 days at 50%RH	234.45±9.02	194.51±0.14	205.69±0.25
Micronized salbutamol sulphate 24h at 75%RH	245.29±5.23	192.24±0.01	204.74±0.04
Micronized salbutamol sulphate 7days at 75%RH	236.11±4.11	191.95±0.04	204.90±0.08
Micronized salbutamol sulphate 14days at 75%RH	234.77±9.81	194.59±0.07	205.59±0.01

2.6 X-ray powder diffraction

The unconditioned and conditioned salbutamol sulphate were examined using X-ray diffraction as a further standard technique. The unconditioned and conditioned samples appear to be crystalline in nature and no broad peaks due to amorphous parts are visible (Figure 17). Those results come in agreement to the results obtained from differential scanning calorimetry, which suggest the absence of amorphous parts. However, if there are amorphous parts less than 5%-10%, which are the detection limits for amorphous amount with this technique, they won't be detected.

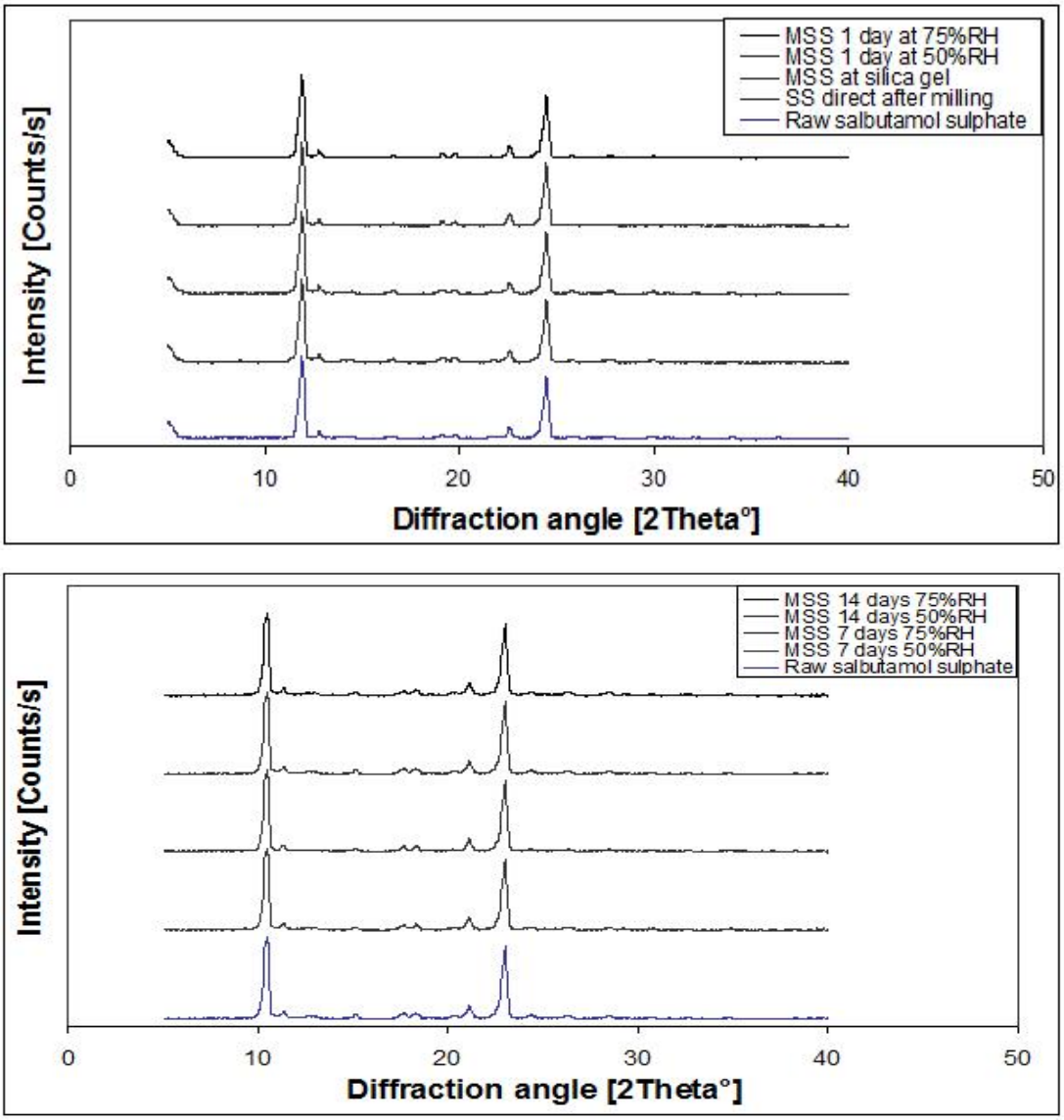


Figure 17: X-ray diffraction of conditioned raw and micronized salbutamol sulphate at 52.8% and 75%RH for 1 day, 1 week and 2 weeks, the second of two measurements is shown.

3 Conclusion

This study shows that the use of water vapour sorption is a good tool to detect small amorphous contents in micronized salbutamol sulphate powder. However, the use of differential scanning calorimetry and X-ray diffraction were not adequate to detect an amorphous content less than 10%, which comes in agreement with the results of Buckton, 1999. Furthermore, the results show that conditioning of milled salbutamol sulphate is not only dependent on relative humidity but also on storage time. So relative humidity

as well as storage time has to be carefully controlled in order to obtain a thermodynamically stable product. Additionally, the micronized salbutamol sulphate must be conditioned minimally for two weeks at 52.8%RH to get stable crystallized salbutamol sulphate.

II The influence of relative humidity on the carrier particle surface characteristics

4 Introduction

A comprehensive investigation was undertaken to evaluate the effect of humidity on the carrier surface characteristics used in dry powder inhalation formulations. Lactose (InhaLac120) and mannitol (Pearlitol160) carrier particles (112µm - 140µm) were subjected to different relative humidities for 6 weeks period of time in order to get a smoother surface. Storage of carriers at elevated relative humidities may allow water vapour to condensate in the interparticulate capillaries. Highly soluble materials such as lactose monohydrate and mannitol may undergo limited dissolution at interparticulate contact points with subsequent solidification, thus resulting in solid bridge formation between particles. Especially the carrier fines present on the coarse carrier are prone to undergo partial dissolution due to their enhanced solubility caused by their small particle size and possibly due to their amorphous content. This leads to particulate fusion between the carrier fines and the coarse particles which is expected to increase the carrier surface smoothness. The carrier powders were placed in Petri dishes at different relative humidities in desiccators for 6 weeks. Then they were stored over silica gel for another week before the preparation of interactive mixtures.

5 Results and discussion

5.1 Determination of the extent of crystallinity

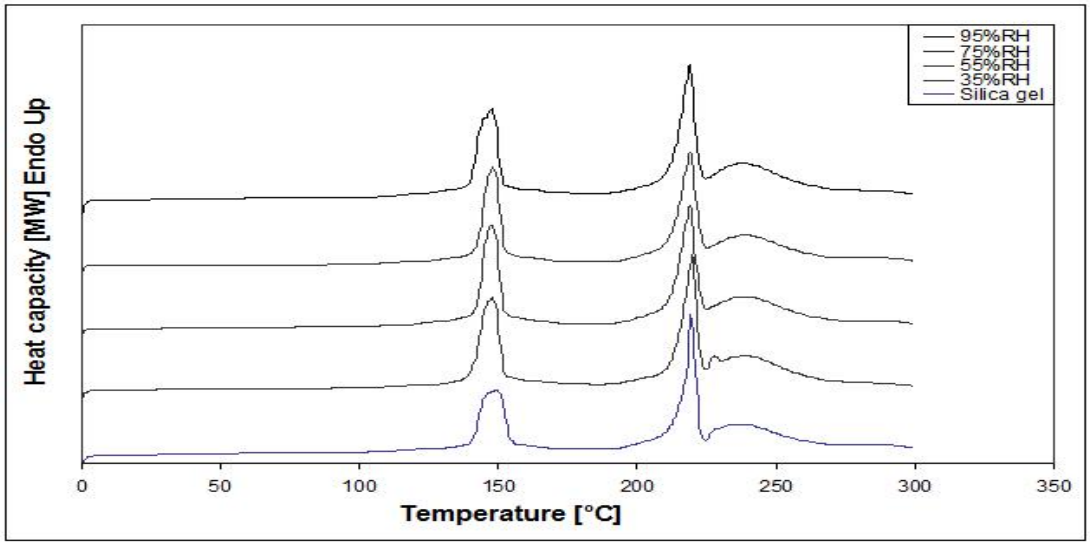
5.1.1 Differential scanning calorimetry

The extent of crystallinity was examined using a differential scanning calorimeter (DSC). The DSC was performed on lactose and mannitol in order to provide an indication of the variation of the crystalline/amorphous content and polymorphism induced by the storage at elevated relative humidities. DSC thermograms of mannitol (Figure 18, b) show a single peak at approximately 166.19°C with a total enthalpy of 293.16J/g while lactose may exhibit complex thermo-analytical transitions because of its several crystalline,

as well as amorphous forms. α-lactose monohydrate becomes anhydrous at 120°C and has a melting point of 146°C-147°C, whereas the endothermic peak at approximately 217°C is the melting endotherm of β-lactose dehydrate (Figure 18, a). Thermograms and enthalpies of fusion (Table II) of both carrier materials after storage at low (silica gel) and different elevated relative humidity reveal no differences in the crystalline behaviour irrespective of storage conditions. This indicates the stability of lactose and mannitol carrier materials even when they were stored for long time at elevated relative humidity. Additionally, the detection limits for amorphous contents with such technique have a lower cut off of 5-10%, this detection limit is due to the fact that this technique measures the entire sample (Buckton et al., 1999).

Table II: DSC parameters of the carriers after 6 weeks storage at different relative humidities (n=2), mean ± range (the range indicates the maximal and minimal values).

SAMPLE NAME	ENTHALPY [J / G]	ONSET [°C]	PEAK [°C]
Lactose stored at silica gel	153.51±1.19	141.17±4.06	147.40±1.86
Lactose stored at 35%RH	153.60±0.73	140.51±0.39	147.73±0.08
Lactose stored at 55%RH	159.80±1.69	143.43±1.04	146.61±1.16
Lactose stored at 75%RH	158.77±2.99	142.08±0.21	147.77±0.18
Lactose stored at 95%RH	164.72±0.58	137.47±2.26	147.74±0.10
Mannitol stored at silica gel	293.16±7.26	165.49±0.14	166.19±0.04
Mannitol stored at 35%RH	294.66±0.26	166.25±0.11	167.03±0.07
Mannitol stored at 55%RH	293.37±1.25	166.32±0.14	167.18±0.28
Mannitol stored at 75%RH	297.89±9.94	166.03±0.01	166.63±0.01
Mannitol stored at 95%RH	292.59±1.69	165.95±0.08	166.77±0.12



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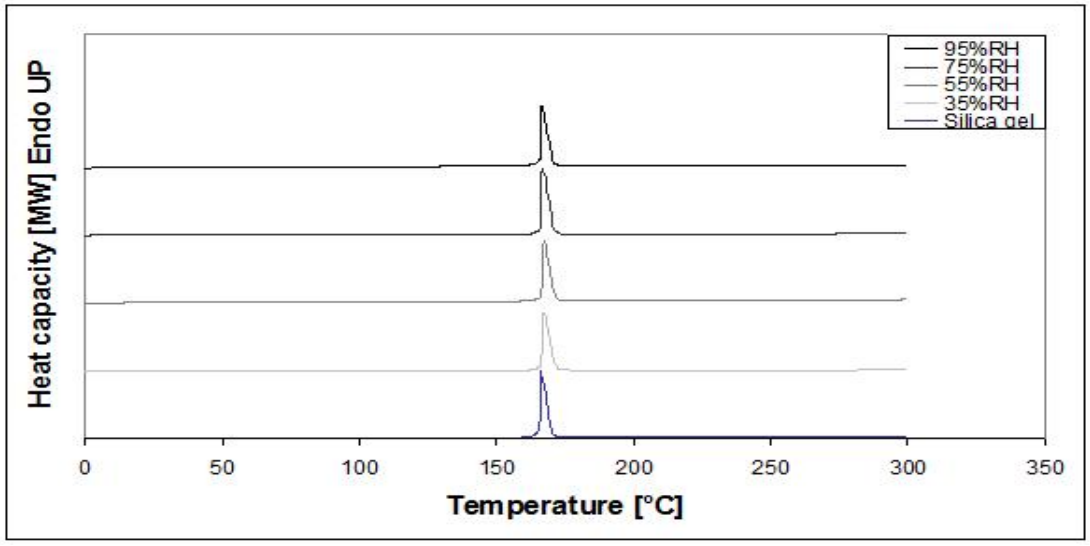

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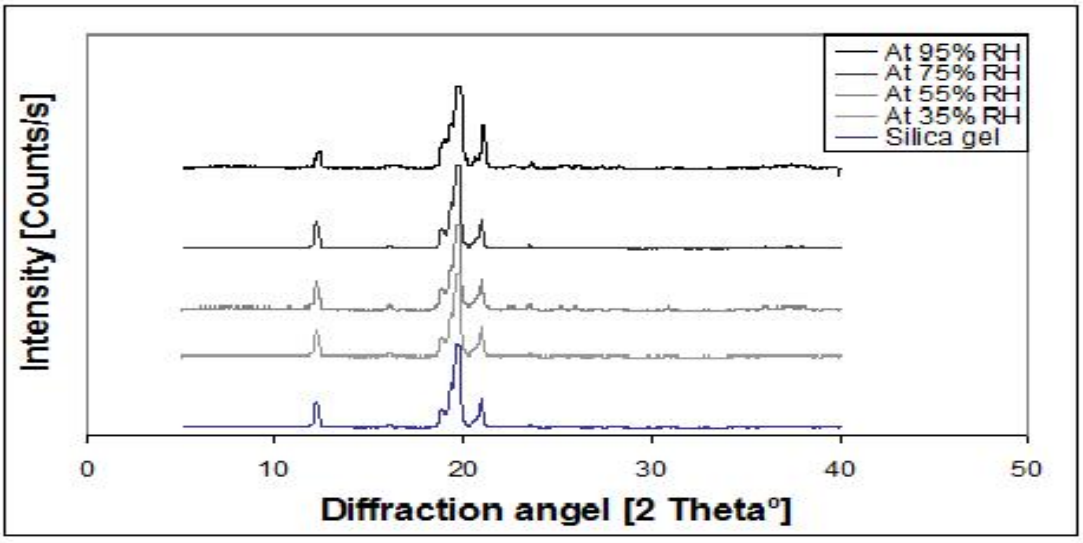
Figure 18: DSC traces of lactose (**a**) and mannitol (**b**) after storage at different relative humidities for 6 weeks, the second of two measurements is shown.

5.1.2 X-ray powder diffraction

The X-ray diffractograms of stored carriers irrespective of the storage conditions show characteristic sharp peaks as indication of crystallinity and absence of a broad, amorphous halo peak as shown in Figure 19. The diffractograms of both, lactose and mannitol carrier materials, show no humidity induced changes occurring in the crystallinity after storage at elevated relative humidity, suggesting that the presence of water vapour has no effect on the crystal lattice of these carrier particles within the range of the

investigation. If those carrier samples had contained an amorphous part before storage it would be expected to recrystallize during storage at elevated relative humidities resulting in differences of the diffractograms of samples stored under silica gel conditions and at elevated relative humidity. The results obtained by X-ray diffraction come in agreement with the results obtained from the DSC thermograms, where also no storage induced differences in the crystallinity were observed. Furthermore, DSC and X-ray diffraction techniques will measure the properties of the sample as a whole. The detection limits for amorphous content with such techniques can vary, but will generally have a lower cut off of 5–10% (Buckton et al., 1999)



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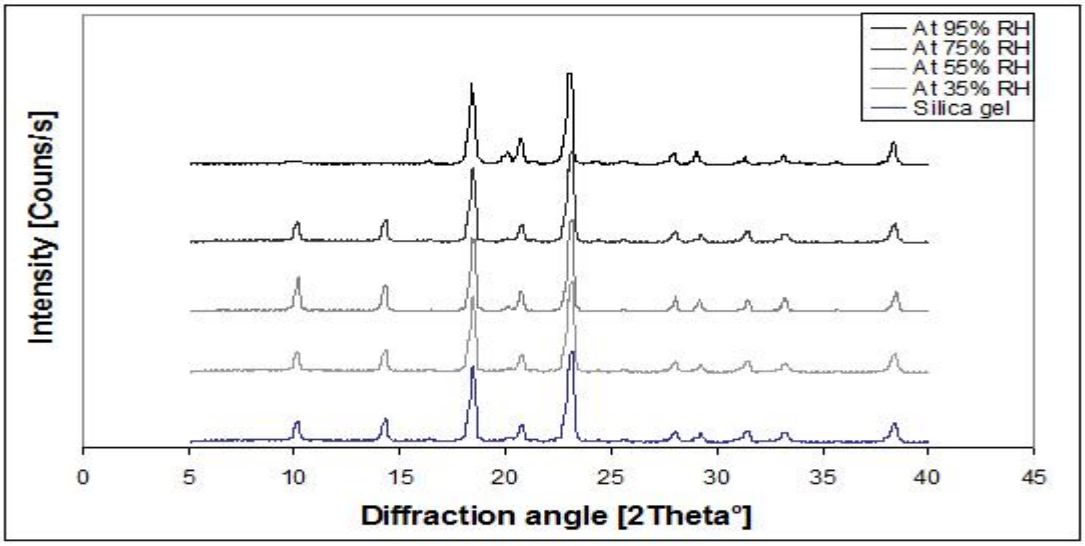
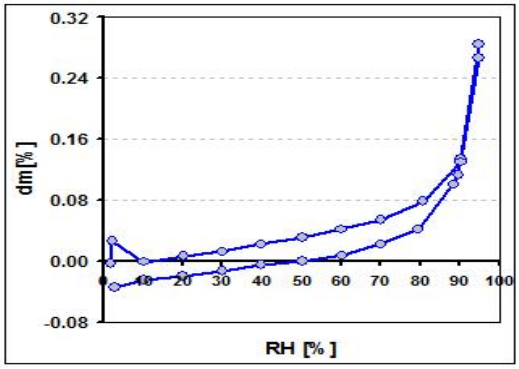


b

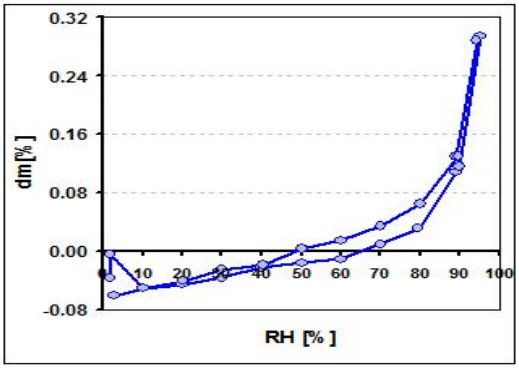
Figure 19: X-ray diffraction of lactose (**a**) and mannitol (**b**) after storage at different relative humidities for 6 weeks, the second of two measurements is shown.

5.1.3 Water vapour sorption

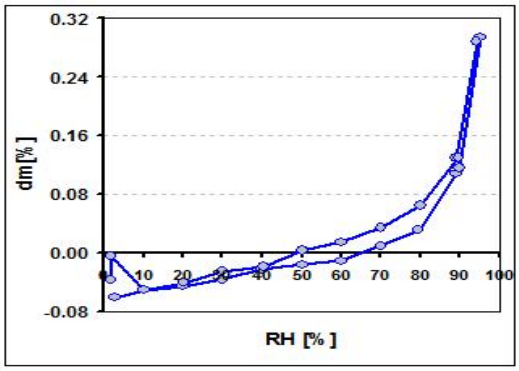
Water vapour sorption is used to investigate the relative moisture sorption of carrier samples. 6 weeks storage of lactose at 35% and 55% RH showed an unchanged water uptake with respect to the sample stored under silica gel conditions. This suggests no change can be predicted in the lactose carrier water uptake at those two percents of relative humidities with respect to the sample stored under silica gel conditions. Storage of lactose at 75% and 95% RH (Figure 20) showed a decrease of water uptake with respect to 35% and 55%RH respectively, which indicates the merging of the carrier fines with the coarse one which subsequently results in a decrease of the carrier surface area exposed to those relative humidities. This decrease in the carrier surface area results in less water sorption by lactose carrier at 75% and 95%RH. The storage of mannitol carrier at 35%, 55% and 75% RH showed no change of the water uptake with respect to the sample stored under silica gel conditions. This suggests no detectable change in the mannitol carrier water uptake at those relative humidities after 6 weeks storage. However, the storage at 95% (Figure 20) showed an increase of water uptake, which might be explained that, the dissolution of carrier fines into the coarser particles and the aimed smoothing of the mannitol carrier surface needs more quantity of water than lactose carrier fines. Additionally mannitol is not affected by the elevated relative humidity unless they reach high level of increase (95%RH). Those results come in agreement with the results obtained from DSC and X-ray diffraction before. Since the water vapour sorption indicates no change in the carrier's crystallinity and proofs the merging of carrier fines to the coarse lactose which results in smoothing of lactose particle surfaces.



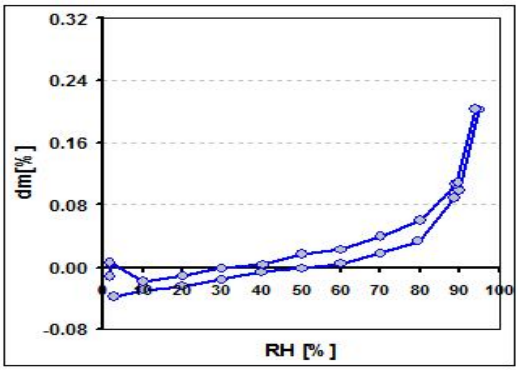
Lactose stored at silica gel



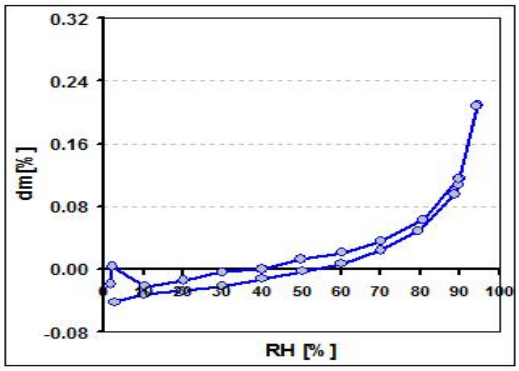
Lactose stored at 35%RH



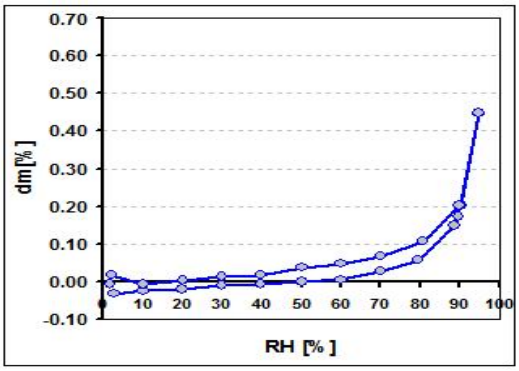
Lactose stored at 55%RH



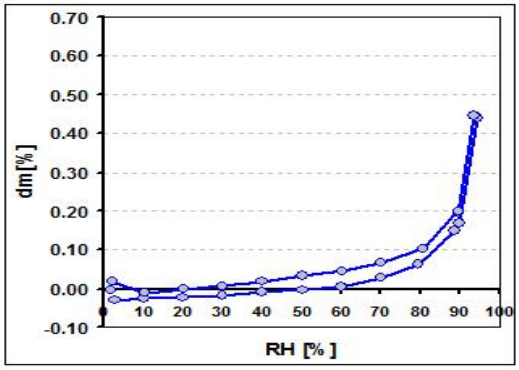
Lactose stored at 75%RH



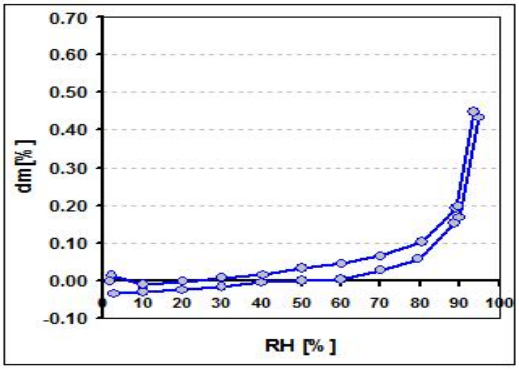
Lactose stored at 95%RH



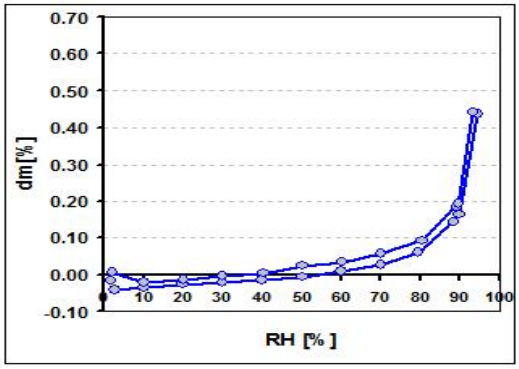
Mannitol before storage

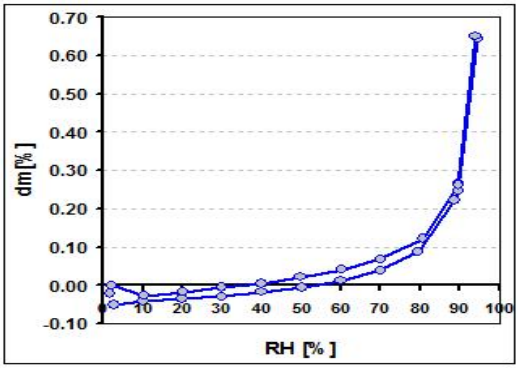


Mannitol stored at 35%RH



Mannitol stored at 55%RH





Mannitol stored at 75%RH Mannitol stored at 95%RH

Figure 20: Water vapour sorption isotherms of lactose and mannitol carrier materials after storage at different relative humidities for 6 weeks.

5.2 Determination of particle size distribution by laser diffraction

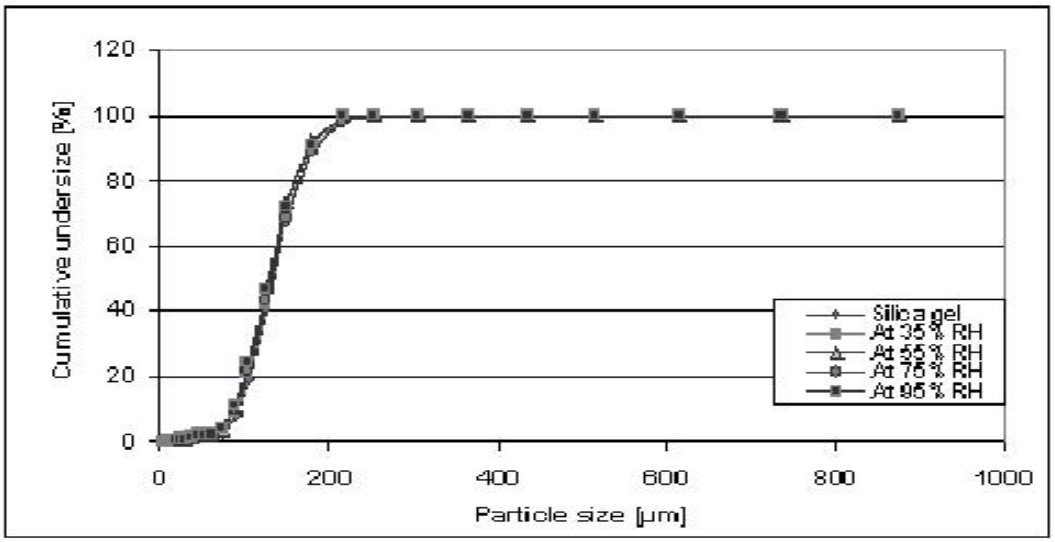
As water uptake, partial dissolution and subsequent interparticulate fusion is not limited to the carrier fines but may also happen to the coarse carrier particles, substantial particle size increase may occur (Maggi et al., 1999).

Figure 21 and Table III suggest that no change occurred after storing both carrier materials at different relative humidities for 6 weeks with respect to the carriers stored at silica gel.

Table III: Particle size distribution of lactose and mannitol after storage for 6 weeks at elevated relative humidities using laser diffraction (n=3, mean ± SD)

Substance	X_{10}[µm]	X_{50}[µm]	X_{90}[µm]
Lactose stored at silica gel	91.98±1.71	133.48±0.69	184.56±0.71
Lactose stored at 35%RH	91.95 ±0.61	132.96±0.48	183.27±1.04
Lactose stored at 55%RH	88.63±1.23	128.32±1.07	177.49±1.02
Lactose stored at 75%RH	90.14±1.01	130.62±1.03	180.63±3.75
Lactose stored at 95%RH	87.62±1.83	128.06±1.16	177.16±0.95
Mannitol stored at silica gel	23.33±1.38	132.43±2.43	240.04±5.32
Mannitol stored at 35%RH	22.47±2.17	131.35±1.65	238.09±5.12

Mannitol stored at 55%RH	20.81±0.49	130.09±0.42	239.69±2.02
Mannitol stored at 75%RH	22.14±0.55	130.34±1.67	236.77±1.91
Mannitol stored at 95%RH	23.79±2.21	131.16±3.61	246.28±5.07



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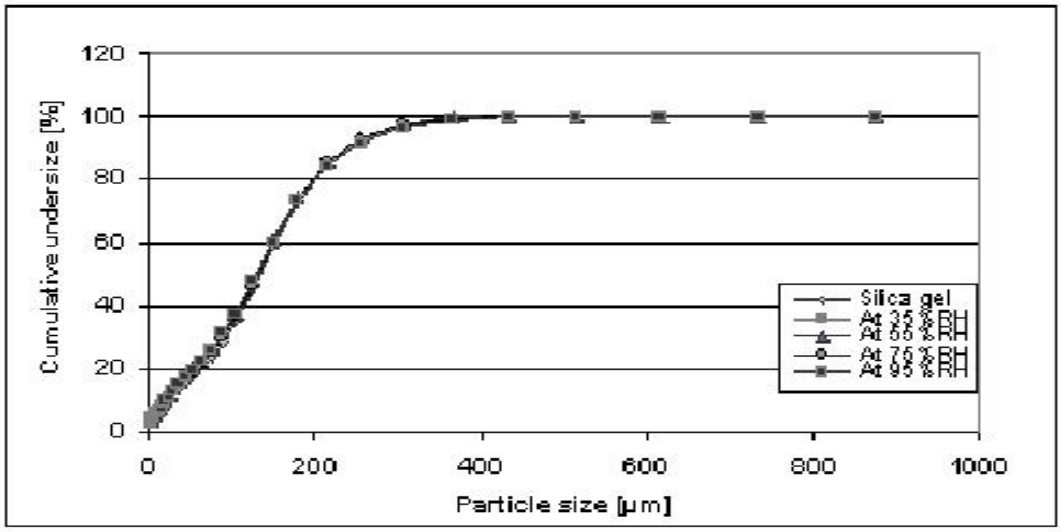

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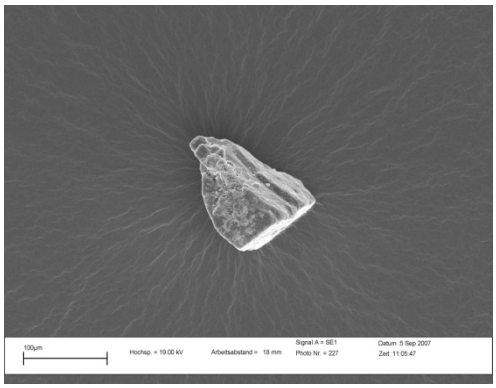
Figure 21: Particle size distribution of lactose (**a**) and mannitol (**b**) after storage for 6 weeks at different relative humidities, the second of three measurements is shown.

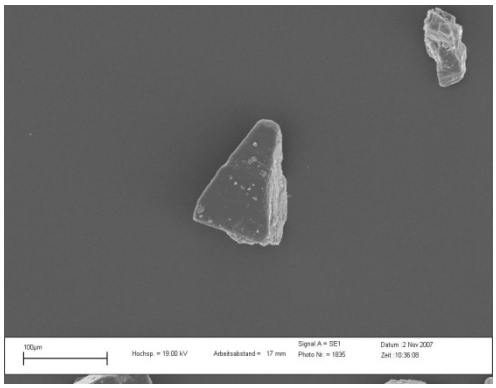
This unchanged particle size of both lactose and mannitol carrier material after 6 weeks storage time at different relative humidities (Figure 21) indicates that interparticle fusion between coarse particles does not take place at least.

5.3 Determination of particle shape and surface characteristics by scanning electron microscopy

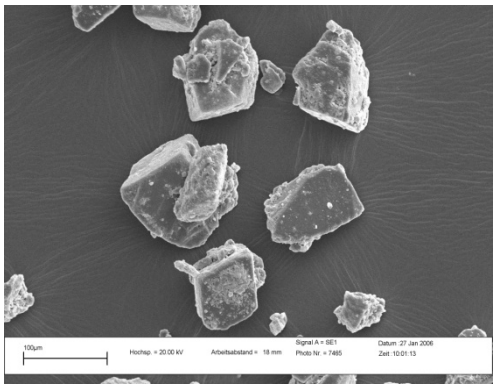
Moisture uptake and loss due to changes in relative humidity can result in local dissolution and recrystallization, leading to irreversible aggregation through solid bridge formation (Dunbar et al., 1998), which might be detected by scanning electron microscopy (SEM). Therefore, the morphology and surface characteristics of lactose and mannitol were investigated using SEM. The scanning electron micrographs show, that there is no significant difference of the surface of carriers stored under silica gel conditions and those stored at different elevated relative humidities (Figure 22). Although the interparticulate fusion of the carrier surface fines with the coarse carrier surface following a water uptake, partial dissolution and subsequent solid bridge formation, induced by storage at elevated relative humidities and subsequent decrease of the relative humidity, has been expected, obviously the extent to which this phenomenon occurs is too small to be reliably detected by SEM if there is any effect at all.



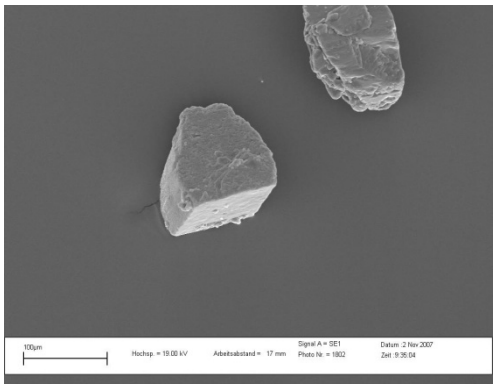
Lactose stored at silica gel



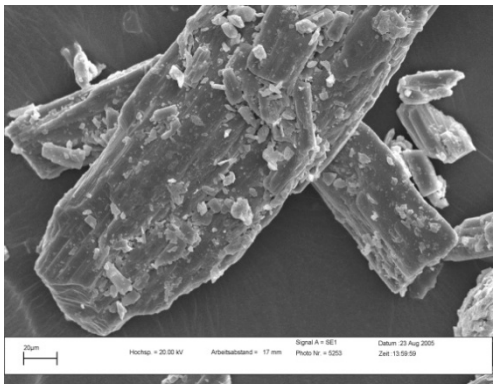
Lactose stored at 55%RH



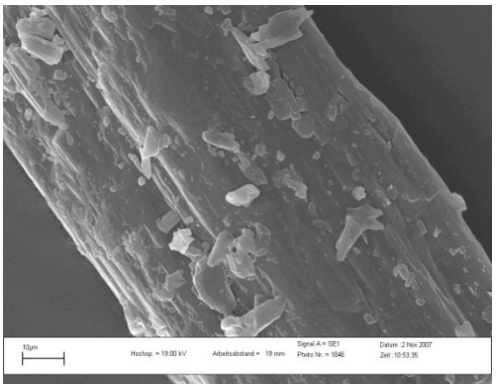
Lactose stored at 75%RH



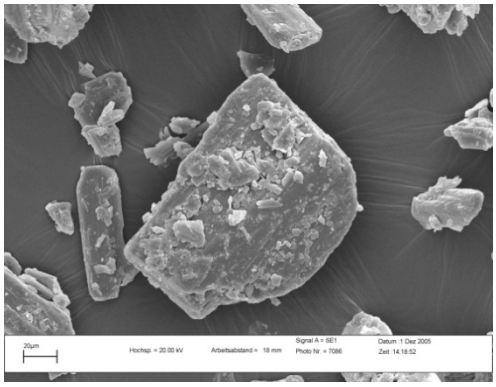
Lactose stored at 95%RH



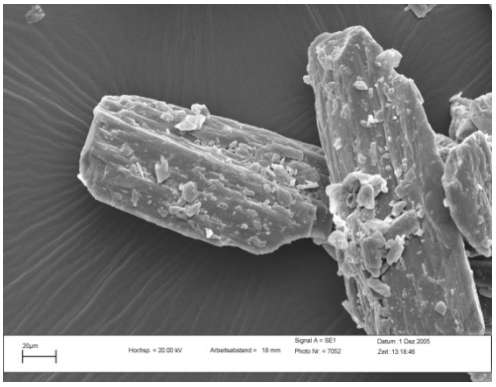
Mannitol stored at silica gel



Mannitol stored at 55%RH



Mannitol stored at 75%RH



Mannitol stored at 95%RH

Figure 22: Scanning electron micrographs of lactose and mannitol after storage for 6 weeks at different relative humidities.

5.4 Determination of surface area by BET measurement

The specific surface area of the lactose and mannitol carriers was measured by multi-point Brunauer-Emmett-Teller (BET) nitrogen adsorption. It is expected, that humidity induced merging of the carrier surface fines with the coarse carrier surface will result in a decrease of the specific surface area.

Figure 23 shows a significant decrease of the lactose carrier surface area after 6 weeks storage at 95%RH with respect to the sample stored under silica gel conditions, which is an indication for an increased smoothness of the lactose carrier material stored at this relative humidity. The surface area of mannitol shows a significant decrease at 55%RH and above after 6 weeks storage. This significant decrease of the carrier surface area of lactose and mannitol after 6 weeks indicates enhanced smoothness of the carrier surface due to merging of carrier fines with the coarse carrier. Those results come in agreement with the results obtained by the water vapour sorption technique in case of lactose carrier material, while the mannitol carrier exhibited a decrease of the specific surface area with BET technique which was not so predictable with the water vapour sorption technique.

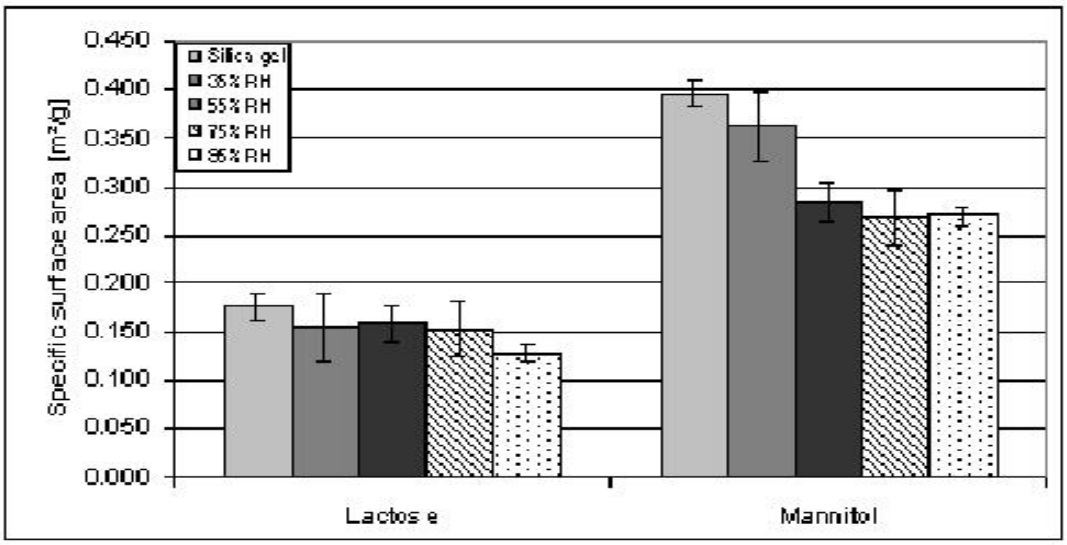


Figure 23: Specific surface area of lactose and mannitol carriers after storage for 6 weeks at different relative humidities determined by nitrogen adsorption, n=3, mean ±SD.

5.5 In vitro deposition test

The results obtained from the in vitro deposition tests are shown in Figure 24 and are represented as fine particle fraction. Figure 25 depicts the mass of the delivered dose. A plot of the fine particle fraction depending on 6 weeks storage time at different relative humidities is presented in Figure 24 . Those plots suggest that a change occurred due to storage at higher relative

humidities in comparison to the sample stored under silica gel conditions. However, the delivered dose shown in Figure 25 has not been markedly changed after storage at different relative humidities. The relationship between the increased relative humidity and the unchanged performance of both carrier materials up to 75%RH could be attributed to non-hygroscopic nature of those two carrier powders. This property of the lactose and mannitol carriers can be considered a useful character of both carriers to ensure stability of dry powder formulation against the storage at elevated humidity. On the other hand storage at 95%RH of both carrier materials shows a significant decrease in the fine particle fraction with respect to the samples stored under silica gel conditions, which indicates the serious role of this high relative humidity in reducing the in vitro deposition after 6 weeks storage time. This reduction suggests that particle smoothness is achieved by storage of lactose and mannitol carrier materials at 95%RH. This carrier surface smoothness was also predicted with the BET surface area measurement for lactose and mannitol carrier substances. Additionally the water vapour sorption has indicated the smoothness of lactose carrier surface, while mannitol carrier still takes high quantity of water at this high relative humidity (95%RH) to achieve the aimed smoothness. Storage of carrier materials at elevated relative humidities may allow water vapour to condensate in the capillaries that exist between individual particles. Furthermore, highly soluble materials such as lactose and mannitol may undergo limited dissolution at interparticulate contact points with subsequent solidification, thus resulting in solid bridge formation between particles, leading to particulate fusion. This fusion results in carrier particles with smoother surface. The enhanced smoothness provides a higher contact area with the drug particles yielding stronger adhesion forces and finally reducing the FPF.

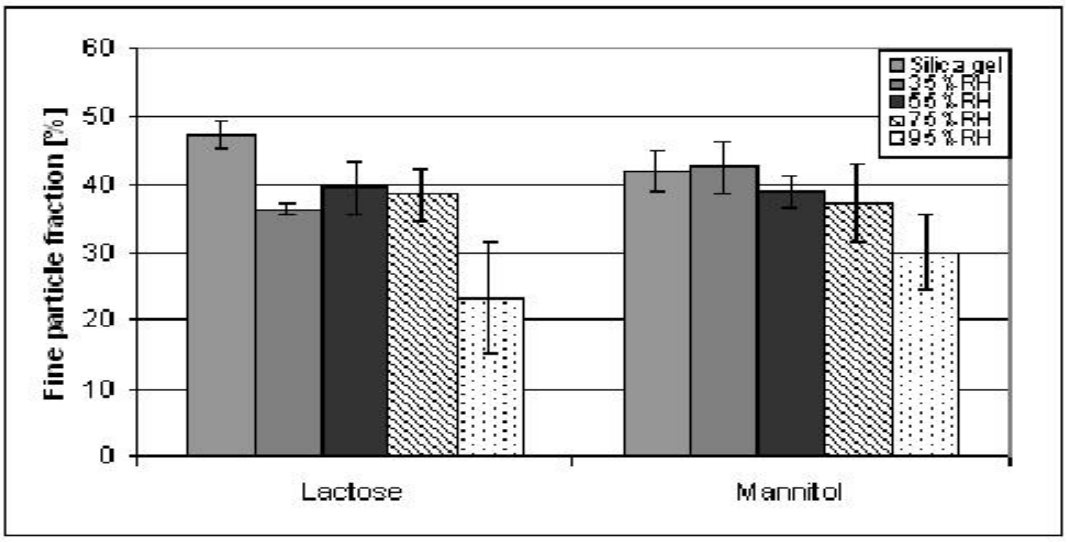


Figure 24: Fine particle fraction of mixtures of salbutamol sulphate with lactose and mannitol carriers after 6 weeks storage at different relative humidities, n=3, mean ±SD.

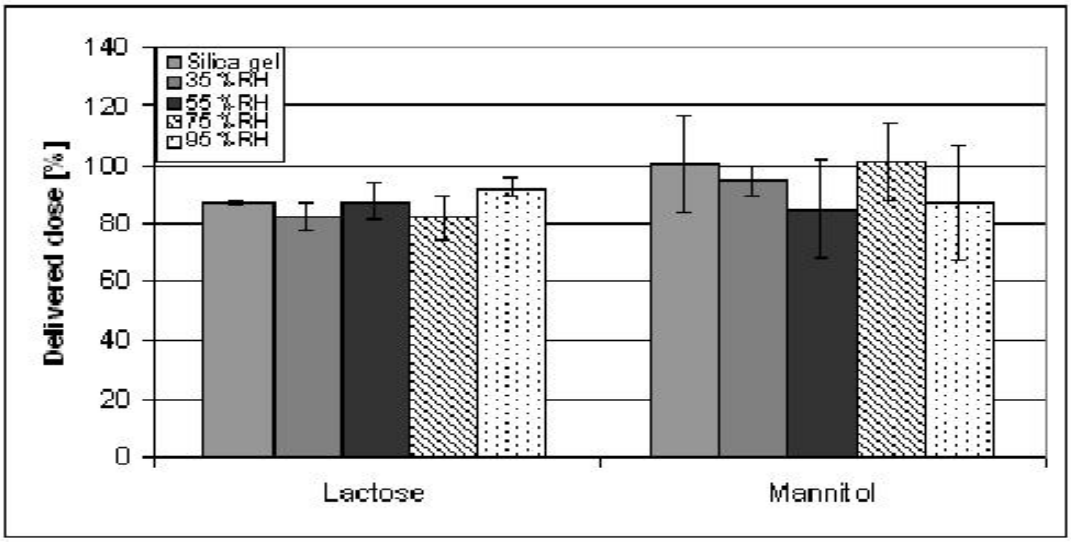


Figure 25: Effect of humidity on the delivered dose after 6 weeks storage time n=3, mean ±SD.

6 Conclusion

This study demonstrates that the storage of lactose and mannitol carrier materials at different relative humidity up to 75%RH has not improved the in vitro deposition of the carrier used in DPI based formulations. Additionally storage of those carrier materials at 95%RH reveals a significant decrease in the fine particle fraction. This might be explained by the assumption that during storage the fine particles of the carrier itself will fuse with the coarse

carrier surface leading to a smoother surface, which provides a higher contact area with the drug particles yielding stronger adhesion forces and finally reducing the FPF. Lactose and mannitol carrier materials showed a smoothed surface and no change in the crystallinity after this storage of the carrier materials before formulation with the active constituent.

III Influence of the removal of carrier surface fines by wet decantation on dry powder inhalation formulations

7 Introduction

Smooth carrier particles using solvents can be made with special preparation techniques. These techniques include the surface treatment of the carrier crystals by submersion in a mixture of ethanol and water (Zeng et al., 2001; Iida et al., 2003 a), by wetting the carrier with simultaneous continuous mixing (Young et al., 2002) and by decantation using lactose saturated absolute ethanol (Islam et al 2004). Surface smoothness was also attained by crystallization from carbopol gels (Zeng et al., 2000, c).

In this chapter, the effect of carrier fines on the drug to carrier interaction forces has been investigated by modifying the carrier particle surface using a wet decantation procedure using a new sequence of solvents. The wet decantation process consisted of successive steps of superficial washing of the carrier particles (sieve-fraction 112-140µm) using absolute ethanol. The decantation process is expected to result in detachment of the fine carrier particles from the surface and probably dissolution into the absolute ethanol. The decantation was followed by washing the carrier particles with dichloromethane, in which solubility of the carrier is negligible, in order to prevent solid bridging upon drying. The decantation process was applied for five and nine times respectively to the lactose and mannitol carrier materials.

The treated and the untreated samples were characterized by differential scanning calorimetry (DSC), X-ray powder diffraction and water vapour sorption in order to investigate whether there is any influence of the decantation process on the crystallinity of the carriers. Their particle sizes and morphologies were investigated by laser diffraction and scanning electron microscopy (SEM) respectively. The impact of the decantation process on the surface properties of the carriers was investigated by gas adsorption according Brunauer Emmet and Teller (BET). Finally, the influence of decantation on the in vitro deposition of the ordered mixtures consisting of salbutamol sulphate and the modified carriers was examined.

8 Results and discussion

8.1 Determination of the extent of crystallinity

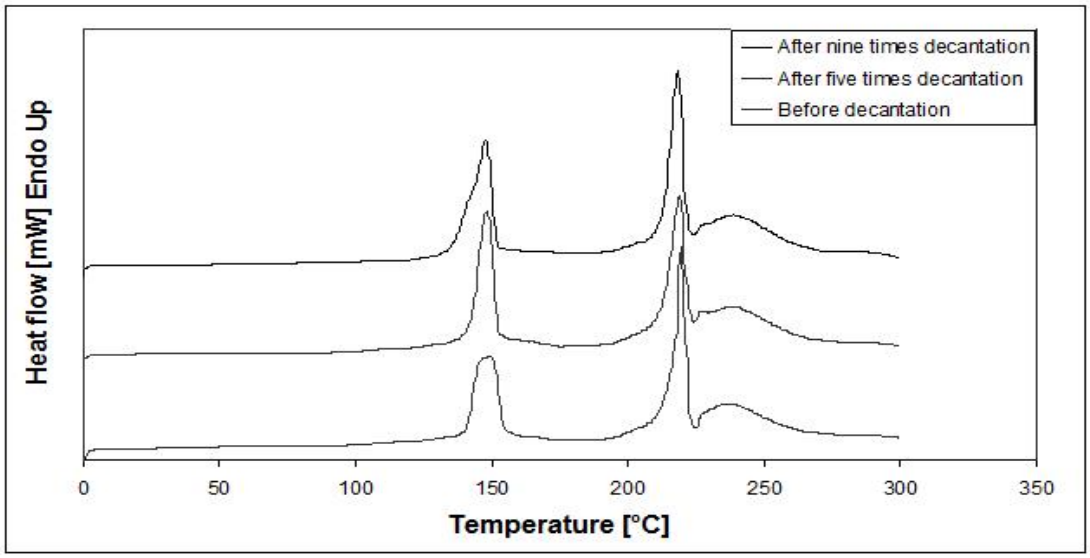
8.1.1 Differential scanning calorimetry

The extent of crystallinity was examined using a differential scanning calorimeter. It's known from literature (e.g. Buckton et al., 1999) that lactose may have a small part of amorphous forms at the carrier surface, which may recrystallize during the decantation process by the effect of the organic solvents that were used in this process.

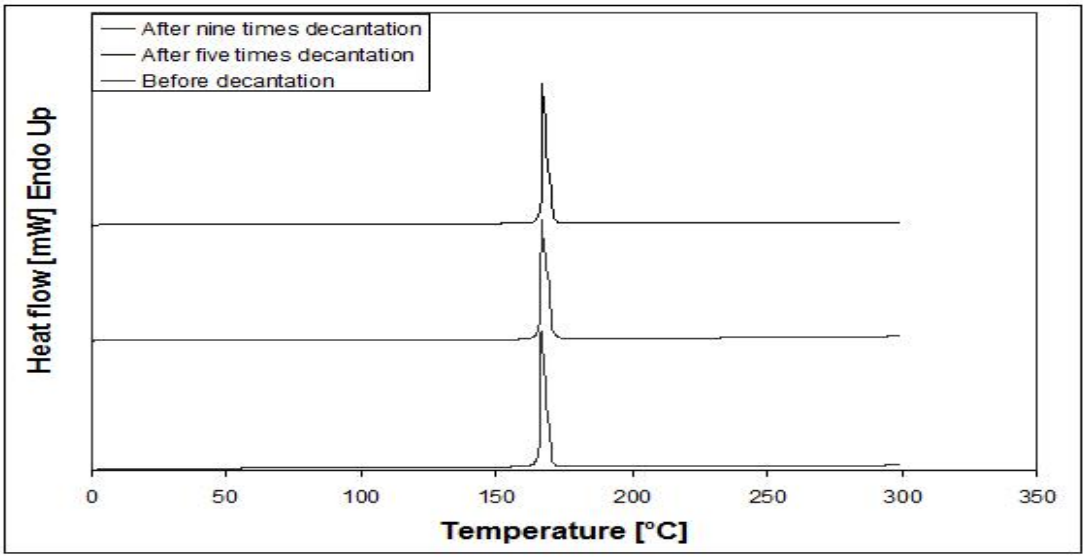
Untreated as well as treated mannitol show only one endothermic peak between 165°C and 166°C (Figure 26 (b)). The enthalpy of fusion of treated mannitol does not significantly exceed the enthalpy of fusion of untreated mannitol (Table IV). All lactose samples exhibit complex thermo-analytical transitions because of several crystalline as well as amorphous forms of lactose. As shown in Figure 26 (a), α-lactose monohydrate becomes anhydrous at 120°C and has a melting point at 146°C to 147°C whereas the endothermic peak at approximately 217°C is the melting endotherm of β-lactose dehydrate. The value for the enthalpy of dehydration ranges from 149.84J/g to 153.51J/g, which is similar to the value reported in literature. There was no evidence of any crystallization exotherm, indicating that the amorphous part of the material, if there was any before decantation, has already recrystallized before the DSC measurements. Comparing the enthalpy of fusion of the carrier materials before and after decantation reveals no change, which indicates the crystallinity of both carrier materials after wet decantation. However amorphous character in highly crystalline solids might be difficult to detect using traditional analytical techniques such as differential scanning calorimetry (DSC), where the limit of detection is 5% to 10% (Buckton et al., 1999).

Table IV: DSC parameters of lactose and mannitol carrier materials before and after decantation (n =2), mean ± range (the range indicates the maximal and minimal values).

SUBSTANCE	ENTHALPY [J / G]	ONSET [°C]	PEAK [°C]
Lactose before decantation	153.51±1.19	141.17±6.04	147.40±1.86
Lactose after five times decantation	153.37±0.18	142.12±0.05	148.21±0.10
Lactose after nine times decantation	158.51±3.10	139.58±0.38	147.74±0.08
Mannitol before decantation	293.16±7.26	165.49±0.14	166.19±0.03
Mannitol after five times decantation	293.33±1.43	166.04±0.01	166.66±0.09
Mannitol after nine times decantation	301.69±2.85	166.06±0.01	166.61±0.08



a

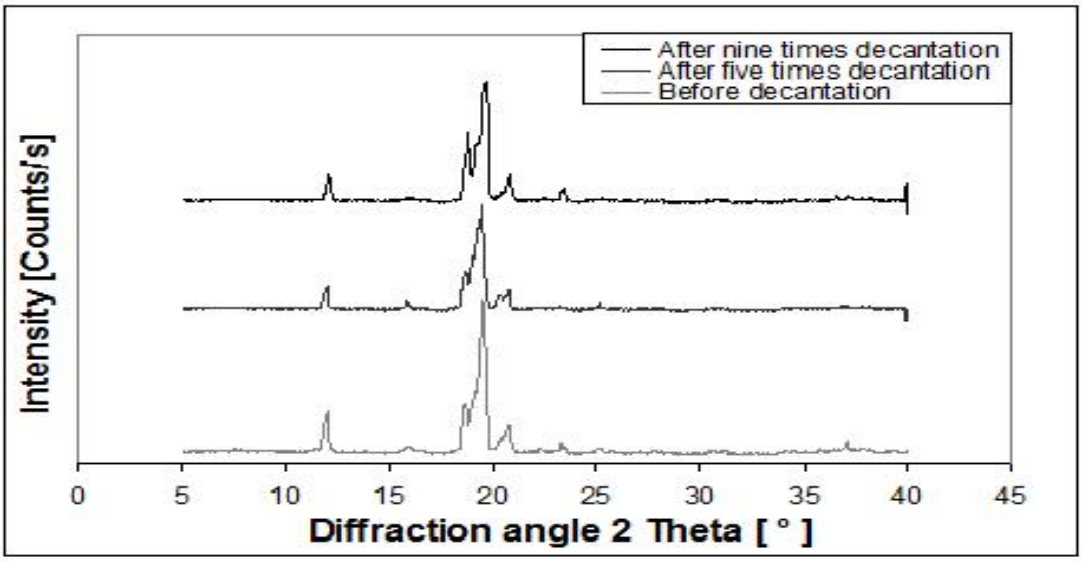


b

Figure 26: DSC traces of lactose (**a**) and mannitol (**b**) carriers before and after decantation, the second of two measurements is shown.

8.1.2 X-ray diffractometry

The X-ray diffractograms of untreated and treated lactose and mannitol carrier materials show sharp peaks as an indication of crystallinity and the absence of a broad, amorphous halo peak (Figure 27). From the diffractograms shown in Figure 27 (a) and (b), it could be concluded that lactose and mannitol carrier materials are not affected by the decantation process in terms of crystallinity. However X-ray is better at detecting small amounts of crystalline materials of an amorphous sample than it is at detecting a small amount of the amorphous form in a crystalline sample.



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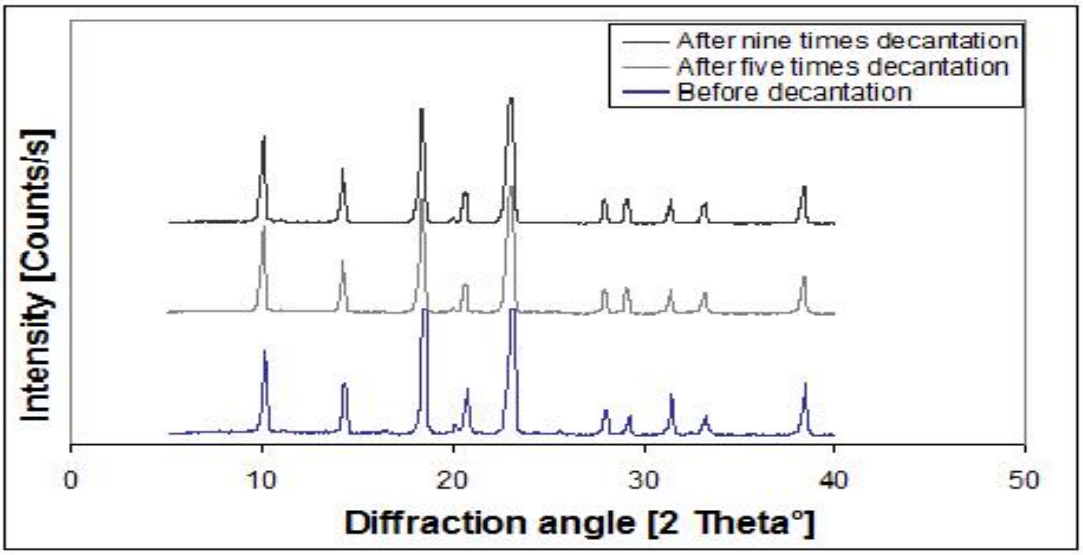


b

Figure 27: X-ray diffractograms of lactose (**a**) and mannitol (**b**) carriers before and after decantation, the second of two measurements is shown.

8.1.3 Water vapour sorption

Lactose and mannitol carrier materials are non-hygroscopic and show an increase of mass with increasing relative humidity (Figure 28a, Figure 29a). However, the maximum water uptake of untreated lactose during the second sorption cycle is somewhat lower in comparison to the first one. This may be due to a humidity induced liquid and subsequent solid bridge formation especially between the carrier surface fines and the coarse carrier surface.

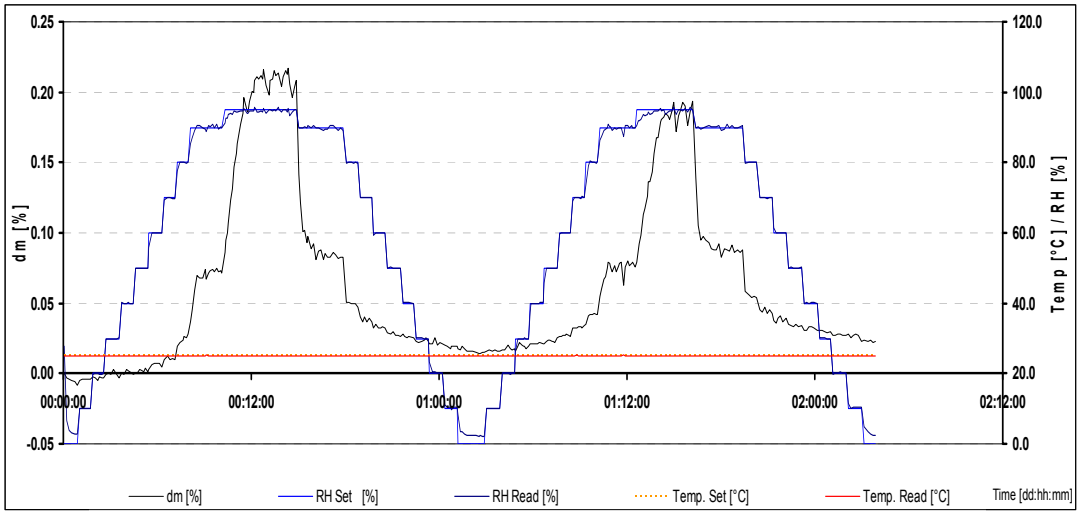
This leads to a reduction of the surface area followed by a decrease of water adsorption in the second cycle. The assumption that this effect is related especially to the merging of the carrier surface fines with the coarse carrier surface is supported by the fact that this behaviour is not observed with lactose that has been subjected to wet decantation in order to remove surface fines. The difference between the first and the second sorption cycle is less pronounced in mannitol samples, indicating an even lower hygroscopicity with respect to lactose.

Buckton et al., 1995 used water vapour sorption to assess the amorphous content of lactose by using the mass loss at 60%RH as indication of the conversion of the amorphous form to crystalline α-lactose monohydrate by the expulsion of water. Untreated as well as nine times decanted lactose used in this study show an increase of mass with increasing relative humidity followed by a decrease of mass with the decreasing relative humidity in the first sorption cycle (Figure 28). The sorption behavior of the second cycle is similar. The absence of the mass loss at 60% RH in the first cycle and the similarity of the sorption behavior in the second cycle assures that there is no amorphous part in this lactose. Similarly, mannitol shows a sorption behavior of a crystalline carrier with no amorphous content (Figure 29).

The water vapour sorption isotherms show that untreated carrier particles are taking up more water than the treated carriers (Figure 30). The lower lines are the adsorption responses and the upper lines are the desorption responses. The maximum weight gain for untreated lactose is 0.21% and for untreated mannitol 0.28%, which comes in agreement with water vapour sorption results obtained by storing both carrier materials at elevated humidities. Whereas those results indicated that mannitol carrier adsorbs more water quantity than lactose carrier.

The water vapour sorption isotherm of treated lactose shows a decrease in water uptake by the increase of the decantation frequency in comparison to the untreated lactose. This can be explained by the part of fine carrier particles which has been removed from the carrier surface by wet decantation leading to a decrease of the surface area and subsequent decrease of water

sorption. The water vapour sorption of treated mannitol shows a decrease of water sorption after five times decantation also caused by the decrease of the surface area and subsequent decrease of water sorption. However, nine times decantation does not further reduce the water sorption.



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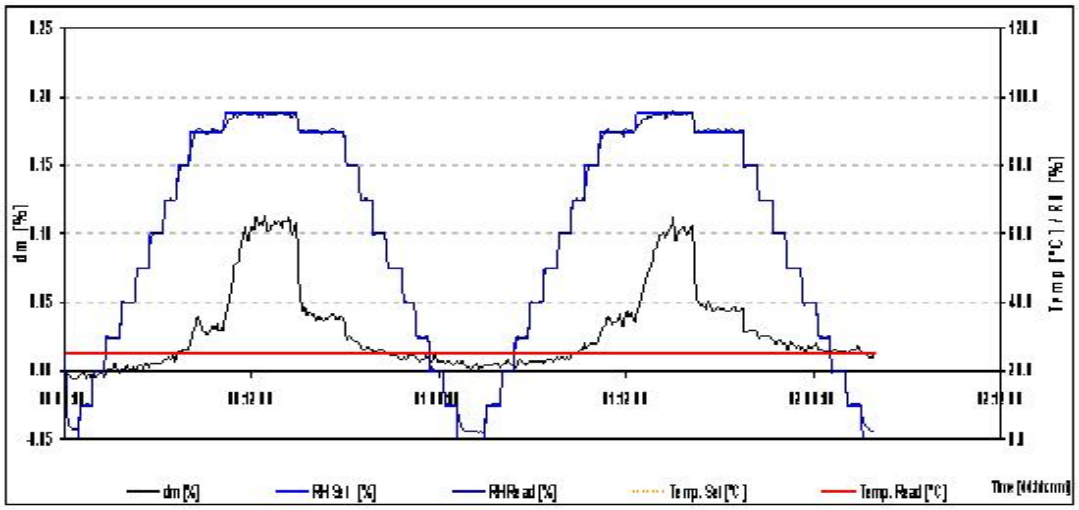
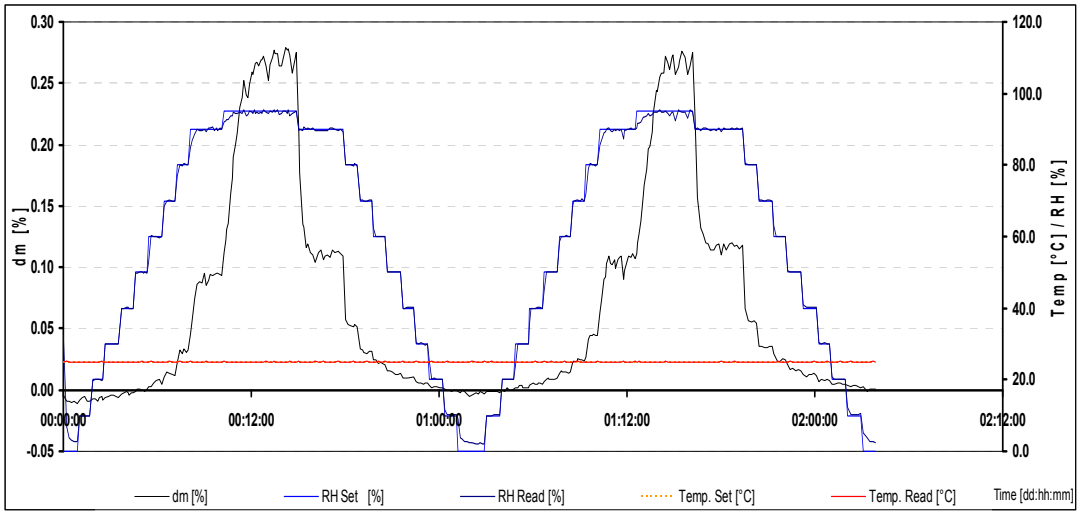


b

Figure 28: Water vapour sorption isotherms of lactose before decantation (**a**) and after nine times decantation (**b**).



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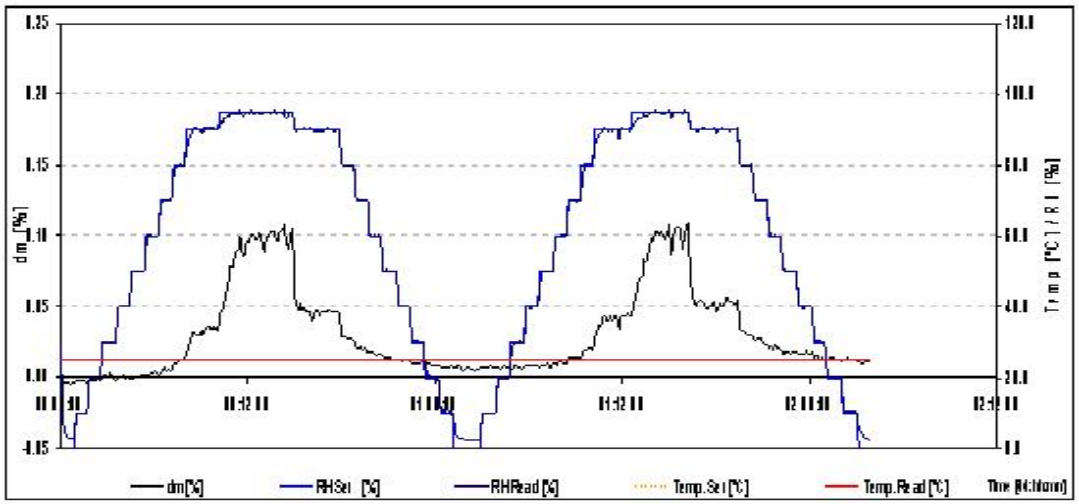

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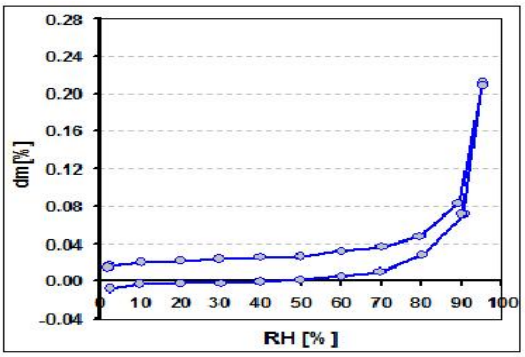
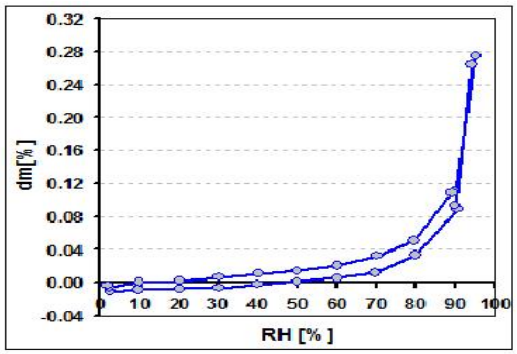
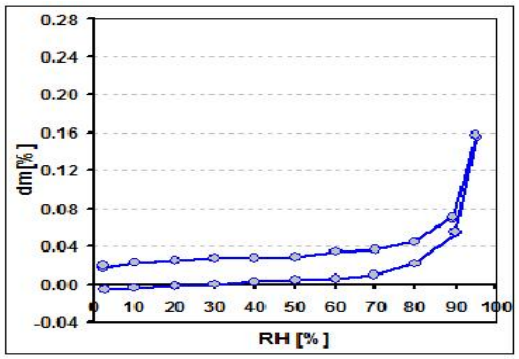
Figure 29: Water vapour sorption isotherms of mannitol before decantation (**a**) and after nine times decantation (**b**).



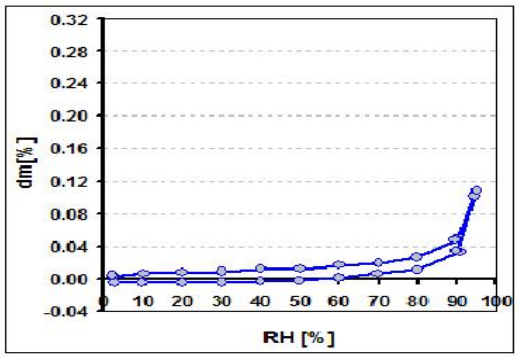
Lactose before decantation



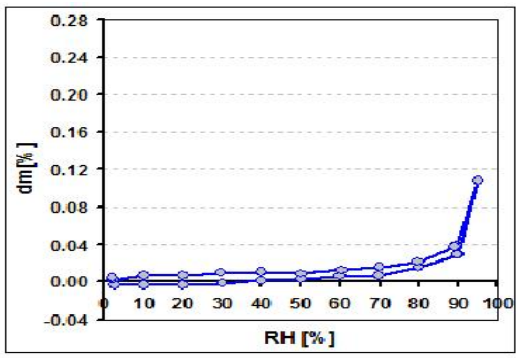
Mannitol before decantation



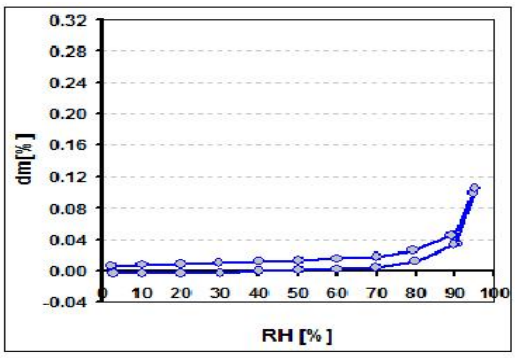
Lactose after five times decantation



Mannitol after five times decantation



Lactose after nine times decantation



Mannitol after nine times decantation

Figure 30: Water vapour sorption isotherms of lactose and mannitol before and after decantation.

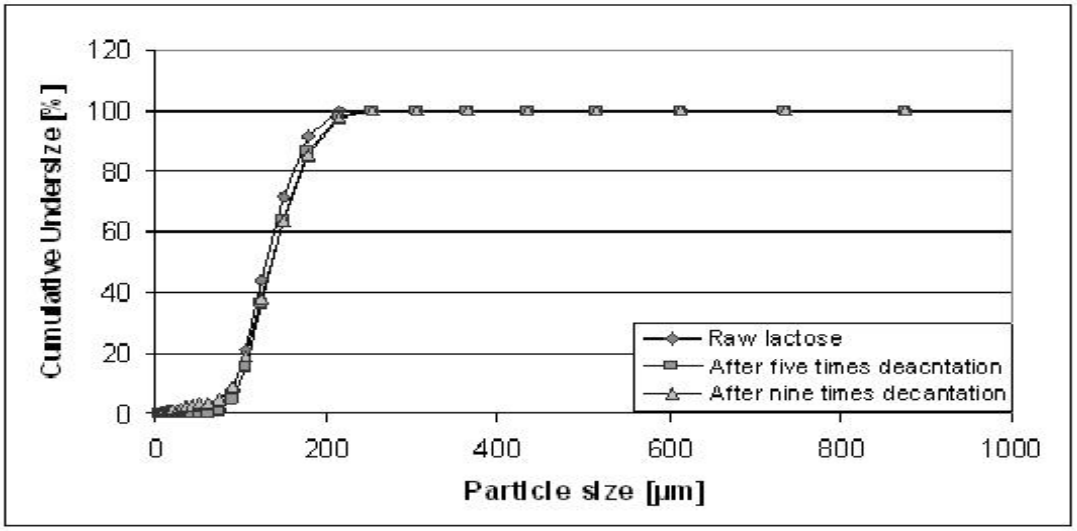
8.2 Determination of the particle size distribution by laser diffraction

To investigate the effect of the decantation process on the particle size of lactose and mannitol carriers, laser diffraction analysis was carried out. The median of the particle diameter of the carriers after the smoothing process is shown in Figure 31 and Table V. The size distributions show that no substantial change in size occurred after decantation. Mannitol decanted nine times shows a small change x_{10} which could be due to the removal of carrier surface fines. In contrast, Zeng et al. (2001) found a reduction in particle size of lactose as well as an increase in the specific surface area. They used similar procedures (rinsing with 96% ethanol), but their submersion experiments lasted considerably longer (over 48 hrs). Furthermore they also used a much higher drying temperature (70 °C for 3 h). The likely consequence of this procedure is partial dissolution and decrease of the crystal size. The other consequence of this procedure is (surface) dehydration

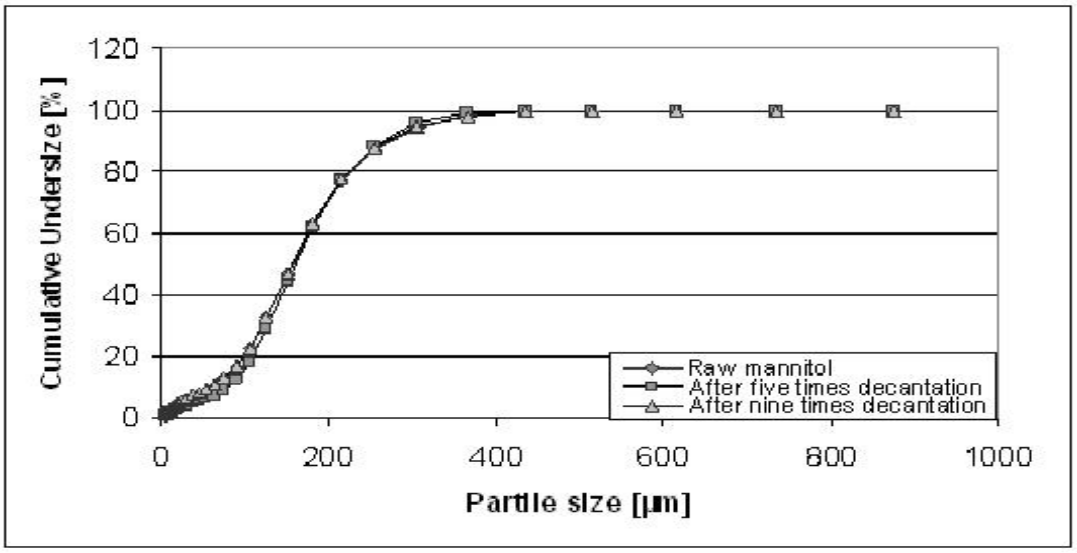
of the alpha-lactose monohydrate crystals, which may lead to changes of the sorption behaviour (Dickhoff et al., 2006). In this study the size distribution of the carrier particles is not changed markedly which indicates that the decantation process has no major influence on the particle size distribution. On the one hand there is no decrease of the particle size indicating that ethanol did not solubilize the coarse carrier particles and on the other hand no increase in particle size indicating that dichloromethane inhibited solid bridging efficiently.

Table V: Laser diffraction analysis of carrier particle size before and after decantation (n=3, mean ± SD).

Substance	X_{10}[µm]	X_{50}[µm]	X_{90} [µm]
Lactose before decantation	92.40±3.35	130.12±3.97	178.14±2.48
Lactose after five times decantation	93.24±1.70	133.95±1.76	184.26±3.23
Lactose after nine times decantation	91.42± 207	136.76±1.00	193.2±1.15
Mannitol before decantation	63.15±11.6	156.06±2.32	247.67±5.17
Mannitol after five times decantation	69.27±5.41	156.49±6.56	262.00±3.91
Mannitol after nine times decantation	56.50±4.54	155.70±5.23	269.48±1.23



a



b

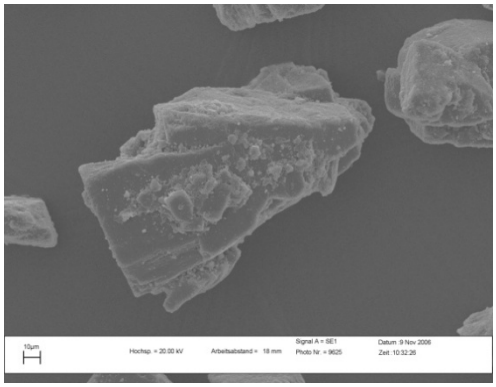
Figure 31: Laser diffraction analysis of lactose (**a**) and mannitol (**b**) before and after decantation (n=3, mean ± SD), the second of three measurements is shown.

8.3 Determination of the particle shape and the surface characteristics by scanning electron microscopy

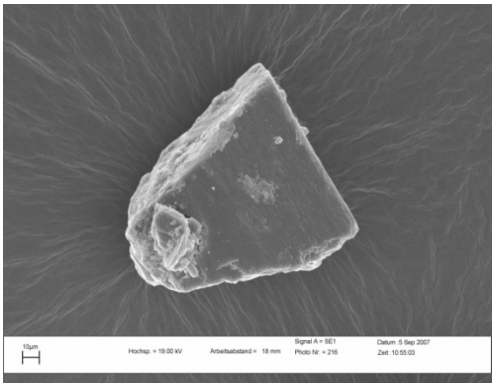
The particle morphology of lactose and mannitol carriers was examined by using a scanning electron microscope. Several photomicrographs are produced by scanning fields, selected randomly, at several magnifications. Decantation of lactose and mannitol with absolute ethanol for several times

and finally with dichloromethane did not change the shape of the carrier crystals. According Figure 32, lactose after five and nine times decantation shows a smoother surface with less fines in comparison to lactose before decantation, whilst mannitol still has fines even after nine times decantation frequency. Furthermore, some studies (Zeng et al. 2001) have shown that treatment of lactose with 95% ethanol induced small asperities and cavities into the surface causing the fine particle dose of salbutamol sulphate to decrease. Examination of the scanning electron micrographs of the decanted lactose used in this current study demonstrated little evidence of asperities and cavities resulting from the treatment of the lactose and mannitol by a wet decantation.

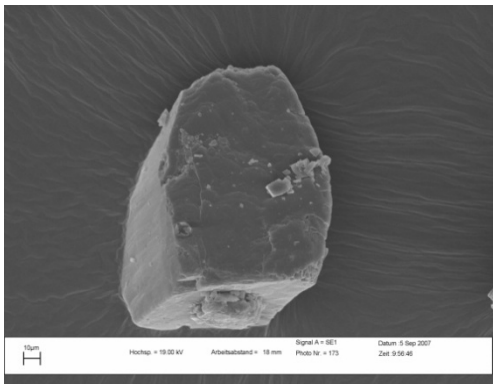
However, the possibility of investigating the whole carrier sample by using scanning electron microscopy cannot be attained. Scanning electron micrographs capture just a few particles of the whole bulk and do not provide representative information about the whole number of particles and how they will appear. This clarifies why the resultant micrographs before and after decantation may give a rough idea about the effect of decantation but it wouldn't be appropriate to indicate the definite success of the applied decantation process.



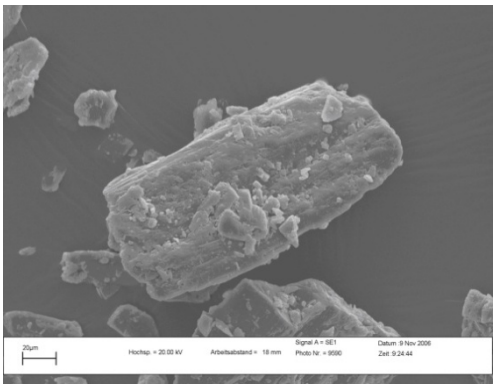
Untreated lactose



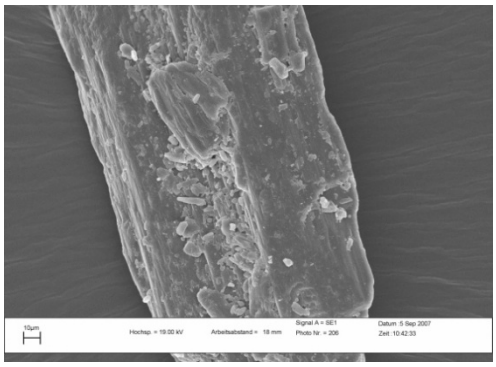
Lactose after five times decantation



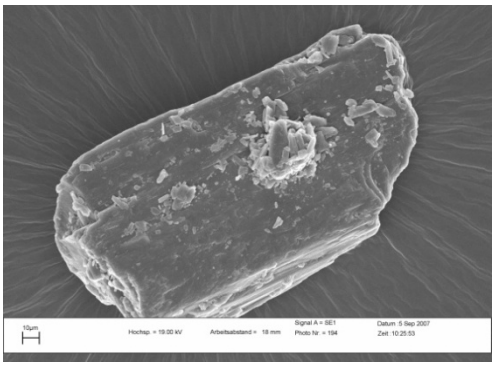
Lactose after nine times decantation



Untreated mannitol



Mannitol after five times decantation



Mannitol after nine times decantation

Figure 32: Scanning electron micrographs of lactose and mannitol before and after decantation.

8.4 Estimation of the amount of carrier surface fines present in the untreated carriers by air jet sieving

In order to roughly quantify the amount of fine carrier particles present on the surface of the coarse carrier and to estimate the effectiveness of the decantation process, the carriers were treated using air jet sieving to remove fine carrier particles. The amount of carrier fines that is removed from the carrier surface is weighed after sieving. Figure 33 shows the percent of fines that are removed from the untreated carrier surface at different compressed air pressures and sieving times. The increase of the air pressure and the elongation of the sieving time lead to an increase in the percent of surface fines detached from the coarse carrier until a certain time, where a plateau is reached. Figure 33, indicates that mannitol contains more fines at the coarse carrier surface or the fines are bound more loosely in comparison to lactose. Mannitol shows the removal of fines up to 13% whereas lactose shows about

5% at the maximum compressed air pressure applied (4000 Pa). This method of using the air jet sieving gives a rough estimate on whether the carrier contains fines at all, that possibly will be removed by decantation.

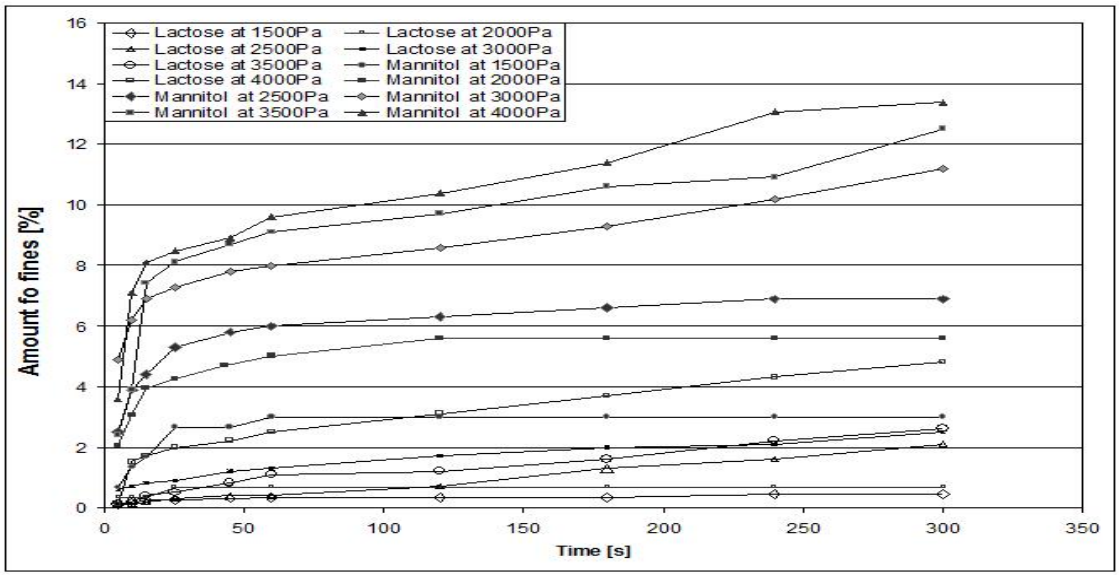
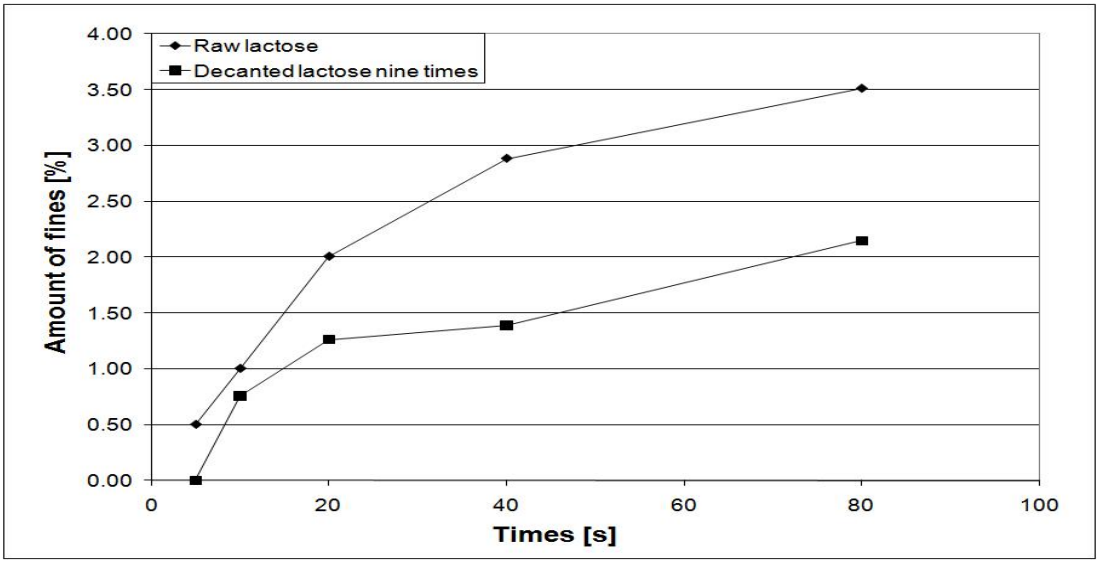


Figure 33: Percent of fine carrier particles removed from the carrier surface of untreated lactose and mannitol determined using air jet sieving at different air pressures and for different time periods, the second of three measurements is shown.

To test the effectiveness of the wet decantation process to remove fines from the coarse carrier surface, the method of fines determination by air jet sieving was applied to the untreated and nine times decanted carriers. Air jet sieving was applied at 4000Pa for 5, 10, 20, 40 and 80 seconds to both carrier materials. Figure 34 (a, b) shows that, the untreated carrier materials contain higher percents of fines than the treated ones, which indicates the success of the decantation process. The air jet sieve method has proven to be a valuable tool for roughly estimating the amount of fines that may be present at the carrier surface and for checking the effectiveness of the decantation procedure.



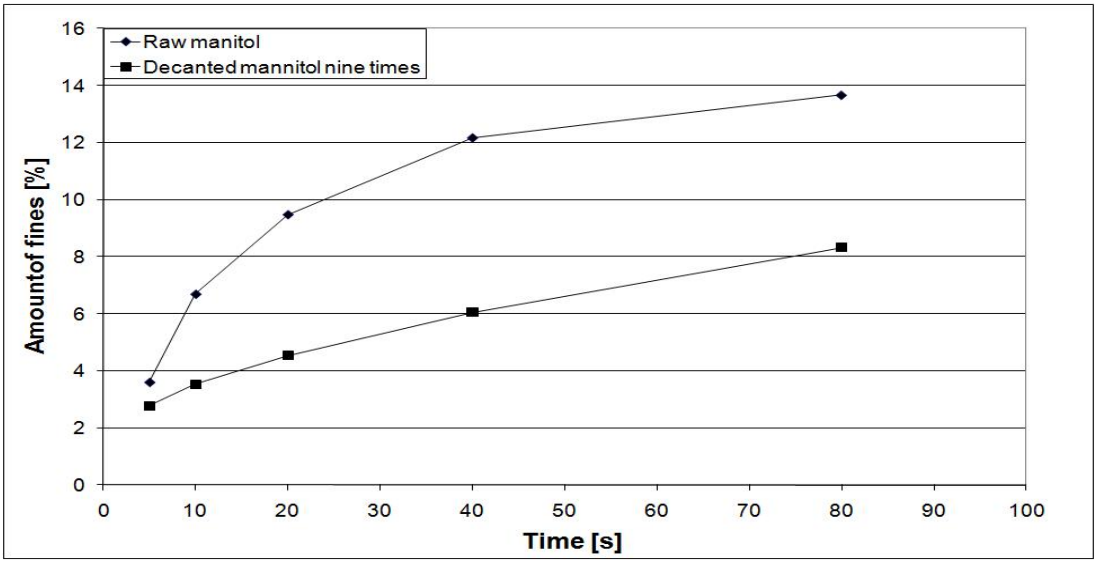


Figure 34: Percent of fine carrier particles removed from the carrier surface of lactose and mannitol by air jet sieving at 4000 Pa before and after decantation, the second of the three measurements is shown.

8.5 Determination of surface area by BET measurement

Particle size, shape and roughness are primary determinants of the surface area. Gas adsorption characterizes both internal and external surface area. Figure 35 shows the specific surface area of treated and untreated lactose, treated and untreated mannitol respectively. It is obvious that decantation decreases the specific surface area of the carriers. This is attributed to the removal of fine particles from the coarse surface of the carrier. The extent to which this occurs depends on the frequency of the decantation method applied (Figure 35). Also Young et al. (2002) reported a decreasing specific

surface area after particle smoothing as a result of wetting of lactose with 5:3 water: ethanol solution in a high-speed mixer under vacuum at 50°C.

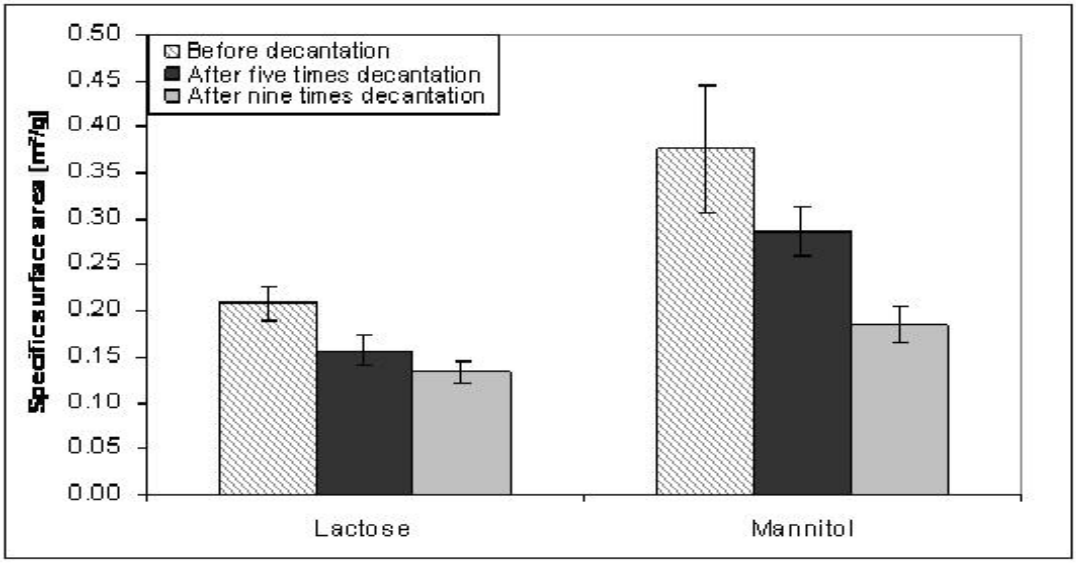


Figure 35: Specific surface area determination before and after five and nine times decantation process, (n=3, mean ± SD).

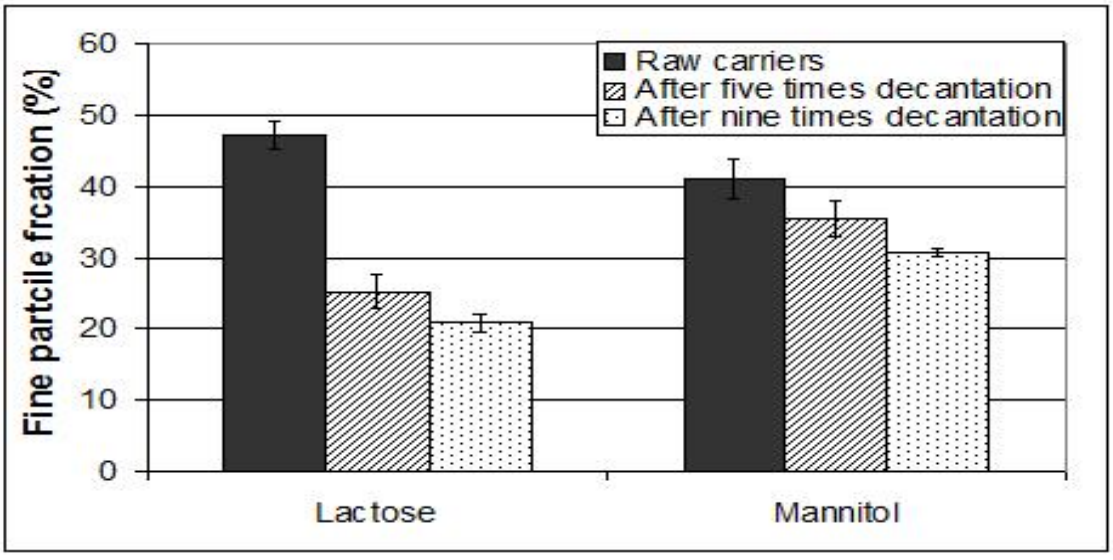
The results of the BET determination of lactose are in agreement with the results of the water vapour sorption measurements, where the decrease of surface area due to the removal of fines is accompanied with a decrease of water uptake after decantation. However, mannitol water sorption is decreased up to five times decantation only. After nine times decantation the mannitol water sorption is not significantly decreased anymore, although the specific surface area is markedly decreased. Nevertheless, it has to be kept in mind that water vapour sorption may give a rough idea about the surface area of a powder, but is not solely reliant on this parameter. This may cause the above mentioned deviation of the results.

8.6 In vitro deposition test

The FPF of salbutamol sulphate using untreated lactose and mannitol as carriers is higher than that of the modified carriers (Figure 36). The FPF is significantly reduced after five times decantation (Anova, $P < 0.05$) and it is significantly reduced after nine times decantation in comparison to the untreated carrier samples of lactose and mannitol respectively. One

explanation of these findings is the removal of fine particles from the coarse carrier surface leading to free high energetic sites on the carrier surface that can be occupied by the added micronized drug. This provides stronger adhesion between the drug and the carrier which leads to difficulties in separation of the drug particles from the carrier upon aerosolization. Additionally, the increase in surface smoothness of the carrier results in an increase of the area of contact in comparison to a rough surface built by the carrier fines and consequently in higher adhesion forces between the drug and the smoothed carrier particle. This results in poor detachment of drug particles from the carrier particles upon inhalation. The results of this study demonstrate the importance of adhered carrier fines on the coarse carrier surface. The results with lactose are in agreement with the results of Islam et al., 2004, who reported that ordered mixtures with lactose treated by decantation showed a decline of the fine particle fraction in comparison to untreated lactose. These mechanisms of action are also valid for the newly introduced mannitol carrier.

The delivered dose of lactose and mannitol carrier materials have not so much been affected by the removal of the surface fines, although this removal was expected to smoothen the carrier surfaces and reduce the drug amount delivered from the inhaler device.



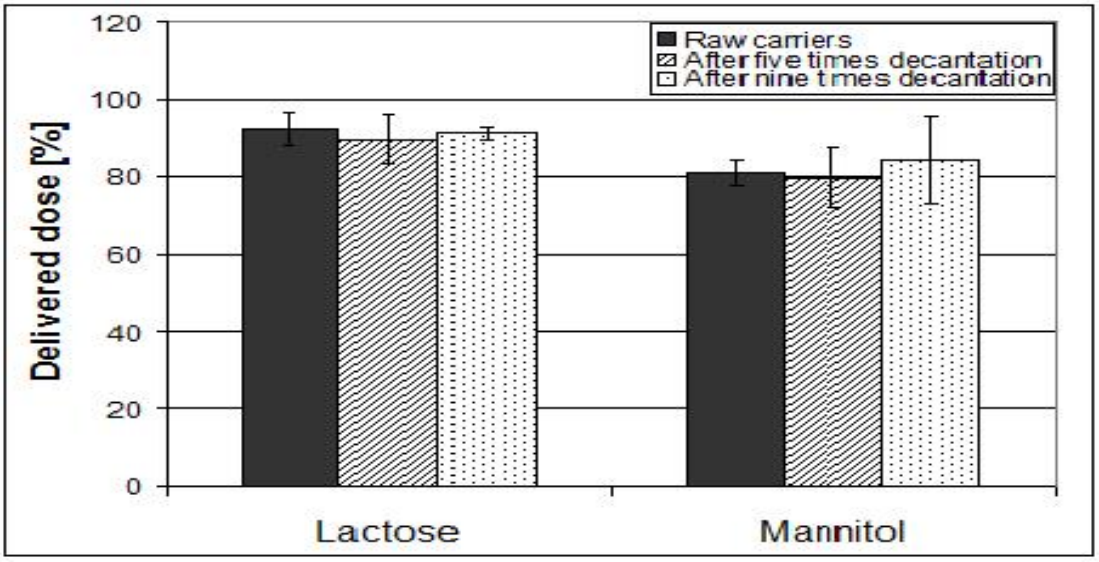


Figure 36: Fine particle fraction and delivered dose of mixtures of salbutamol sulphate with lactose and mannitol before and after five and nine times decantation (n=3, mean ± SD).

9 Conclusion

This study introduces a new decantation process with a new sequence of solvents, which is a valuable method of removing the fine carrier particles from coarse lactose and mannitol carrier surfaces without solid bridge formation and without changing particle size. Lactose and mannitol show successful removal of surface fines by decantation in comparison to as supplied starting carrier materials. The increase of the frequency of the decantation process results in a decrease of the specific surface area due to removal of fines from the particle surface. The engineering of lactose and mannitol carrier surfaces using this new decantation process as carrier fines removing and particle smoothing process resulted in a significant decrease of the fine particle fraction when compared with the as supplied starting materials. This decrease of the FPF can be explained by two possible mechanisms. The first one is that the removal of fine particles leads to an increase of free high energetic spots at the carrier surface which can be occupied with the added drug. This occupation results in higher adhesion forces between drug and carrier particle and reduced fine particle fraction. The second mechanism is that the removal of carrier fines from the carrier

surface leads to an increase in surface smoothness by decreasing the micro-roughness caused by the carrier fines, which possibly results in the formation of stronger adhesion forces due to the increase of the contact area between this smoothed carrier surface and the drug particle. Those strong adhesion forces result in difficulties of drug particle detachment from the carrier particles during the inhalation process and subsequently in a low FPF. Those results are in agreement with the results obtained after storing the lactose and mannitol carrier materials at 95%RH, which showed a decrease in the FPF due to merging of the carrier surface fines with the coarse one and increasing the smoothness of the carrier surface**.** This increased smoothness results subsequently in a higher contact area with the drug particles yielding stronger adhesion forces and finally reducing the FPF.

IV Influence of added fines to the decanted carrier surfaces used in dry powder formulations

10 Introduction

Previous investigations reported that the carrier surface directly affects the aerosolization efficiency from a DPI (Kawashima et al. 1998; Podczeck 1998; Larhrib et al. 1999; Zeng et al. 2000). Carrier fines may be present on the carrier surface from attrition (naturally adhering fines), or by the addition of certain quantities of fine or intermediate sized lactose to coarser lactose carrier fractions (Zeng et al., 1998; Podczeck, 1998a). The effect of very fine particles may play an important role in the flow and aerosol dispersion behaviour of particles in the 1 µm-10 µm range (Zeng et al., 1998).

This part of this thesis discusses the possibility of adding carrier fines to not only lactose but also to mannitol carriers which were smoothed using the wet decantation process. The effect of those added fines on the in vitro deposition will be evaluated.

The percent of naturally adhering carrier fines present on the untreated coarse carrier surface was determined using an air jet sieve (chapter III). Lactose fines removed by air jet sieving yielded about 4.5%, whilst mannitol was determined to contain 13.7% fines (see decantation study for more details, chapter III). Accordingly carrier fines were added to the decanted carriers, namely 2%, 4%, 8% to the lactose, and 4%, 8%, 16% to the mannitol carrier in order to find out whether the FPF might be reverted to the original value of the raw carriers before applying the wet decantation process.

Publications by Louey et al., 2003; Islam et al., 2004; Staniforth et al., 1996 and Zeng et al.,1998 report that maximum dispersion occurs when about 10%-15% fine particles are present in the mixture. Interactive mixtures consisting of very large proportions of fine lactose particles showed a significantly lower FPF. Zeng et al., 1999, found that formulations produced by blending the coarse carrier and the carrier fines before adding the drug (thus giving the fines the first opportunity to bind to areas of high adhesion on the carrier) gave greater performance (Figure 37, A) than formulations produced

by blending the coarse carrier and the drug first (thus giving the drug particles the first opportunity to bind to areas of high adhesion on the carrier). The magnitude of this effect ranged between a 60% and 70% increase in FPF. Another possible blending order (drug and fine excipient particles before addition of coarse carrier) tended to yield an intermediate FPF (Figure 37, B).

In this study the coarse carrier particles and the carrier fines were mixed before the addition of the drug. All mixtures showed a good content uniformity with the coefficient of variation <5%.

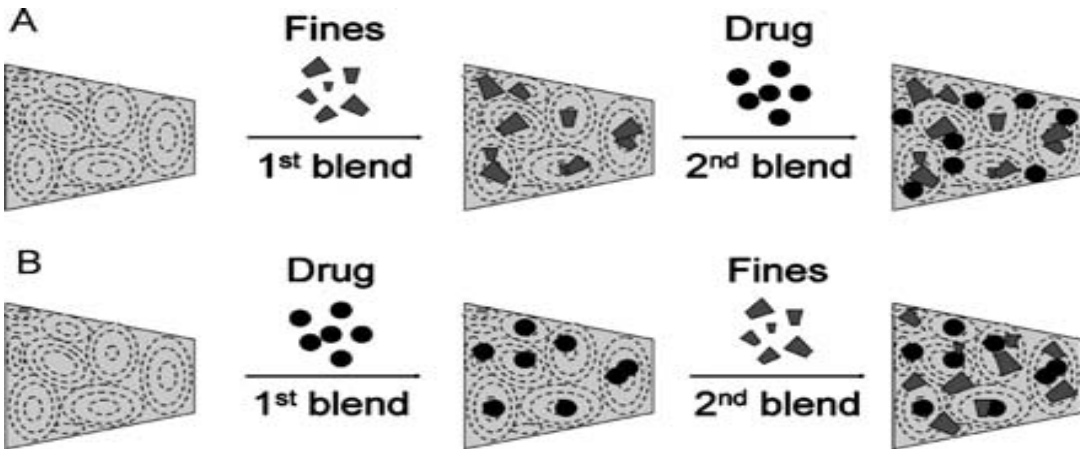


Figure 37: Effect of mixing sequence (Chow et al., 2007).

The carriers treated by nine times decantation (see previous chapter) and the treated carriers with added carrier fines (lactose plus 2%, 4%, 8% lactose fines, mannitol plus 4%, 8%, 16% mannitol fines) were characterized by differential scanning calorimetry (DSC), X-ray powder diffraction and water vapour sorption in order to find out whether a modification of the crystallinity of the carrier materials has been occurred. Particle size, morphology and specific surface area were investigated by laser diffraction, scanning electron microscopy (SEM) and Brunauer Emmet and Teller (BET) gas adsorption. Finally, the influence of the addition of carrier fines to the decanted carriers on the in vitro deposition was examined.

11 Results and discussion

11.1 Determination of the extent of crystallinity

11.1.1 Differential scanning calorimetry

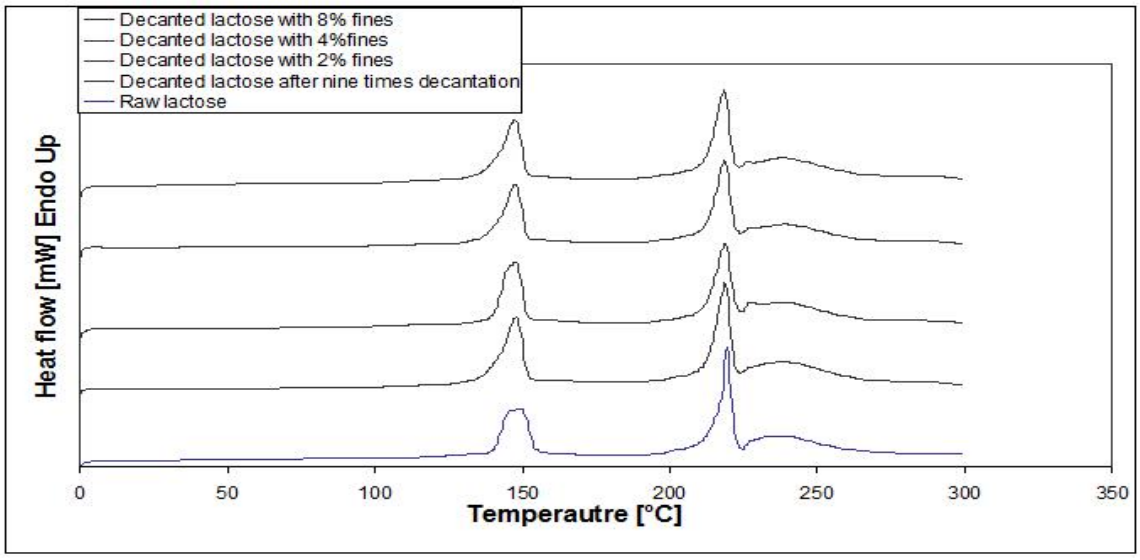
The influence of the added fines on the overall crystallinity of the formulations is depending on the amount, particle size, shape, surface and crystallinity of the added smaller-sized lactose and mannitol particles. As discussed previously, micronized lactose may be rich in amorphous regions, which are physically softer than the crystalline form. If micronized drug particles are bound to these amorphous regions, then deformation of the binding sites between drug and amorphous regions would be more likely to occur than between drug and crystalline regions, which subsequently result in a increased area of contact, enhanced interparticle interactions and lower FPF (Zeng et al., 2000).

The thermal properties of treated lactose and mannitol before and after addition of carrier fines are similar as shown in Table VI. Also the amount of fines added to treated lactose and mannitol does not result in a substantial change of the overall crystallinity of both carriers. The DSC thermograms (Figure 38) show approximately similar thermal behaviour of the carriers before and after addition of fines, which suggests that no change of the crystallinity of those carriers has been occurred after addition of carrier fines. Furthermore, The DSC technique has narrow detection limits of amorphous parts (above 5-10%).

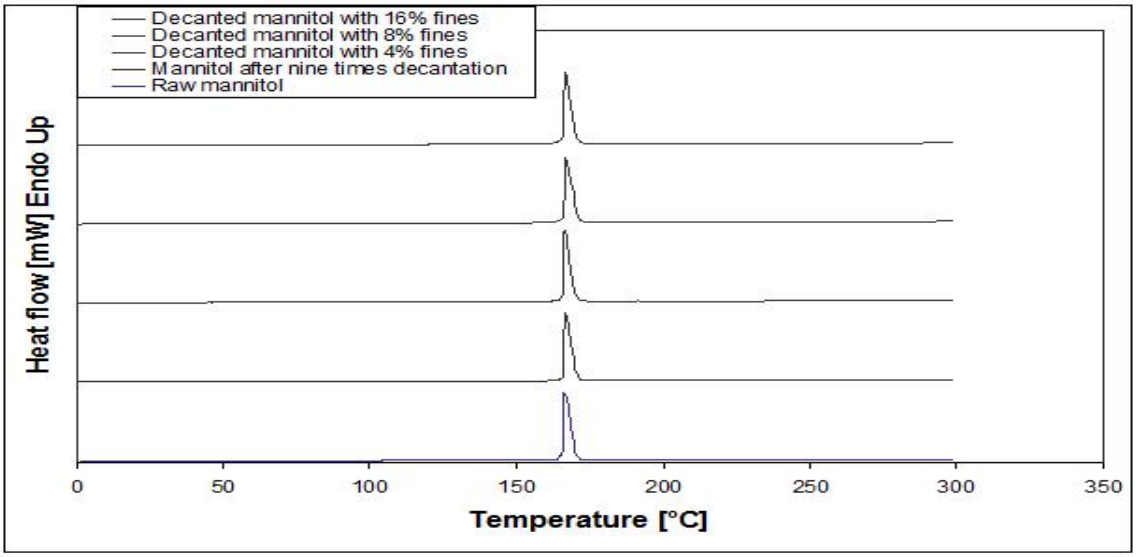
Table VI: DSC parameters of decanted lactose and mannitol before and after addition of fines (n=2), mean ± range (the range indicates the maximal and minimal values). The values of carriers before decantation are given for reasons of comparison.

SUBSTANCE	ENTHALPY [J / G]	ONSET [°C]	PEAK [°C]
Lactose before decantation	153.51±1.19	141.17±6.04	147.40±1.86

Lactose after nine times decantation	158.51±3.10	139.58±0.38	147.74±0.08
Decanted lactose with 2% fines	162.97±0.81	140.35±0.17	147.73±0.05
Decanted lactose with 4% fines	156.15±4.01	140.16±0.15	147.60±0.09
Decanted lactose with 8% fines	157.84±6.78	139.48±0.23	147.59±0.08
Mannitol before decantation	293.16±7.26	165.49±0.14	166.19±0.03
Mannitol after nine times decantation	301.69±2.85	166.06±0.01	166.61±0.08
Decanted mannitol with 4% fines	305.25±5.67	165.96±0.02	166.69±0.03
Decanted mannitol with 8% fines	307.38±2.51	165.99±0.07	166.77±0.08
Decanted mannitol with 16% fines	299.20±6.53	166.00±0.07	166.83±0.17



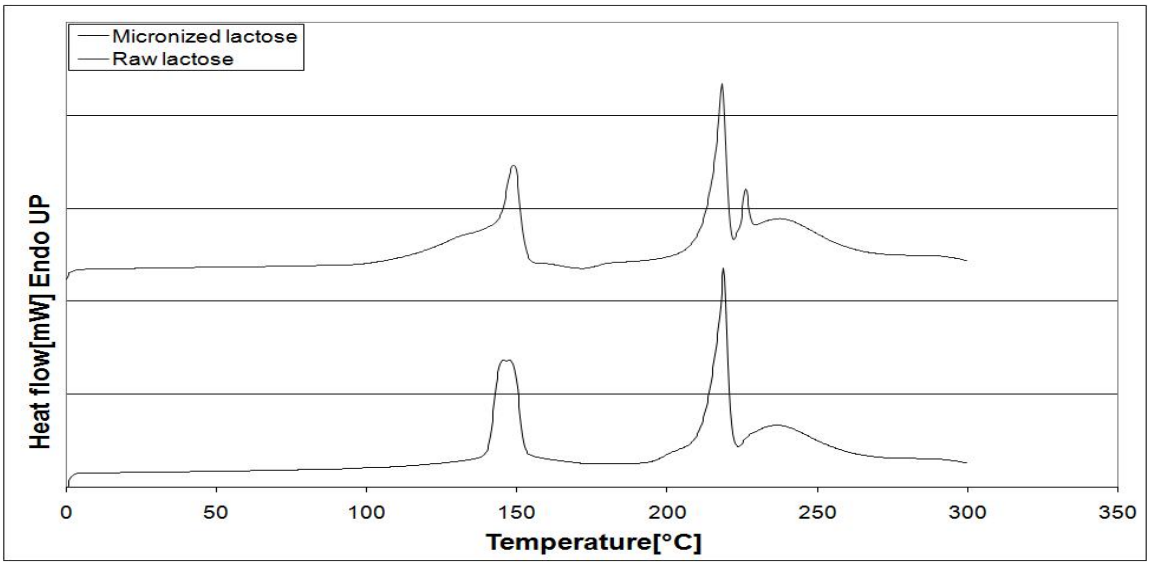
a



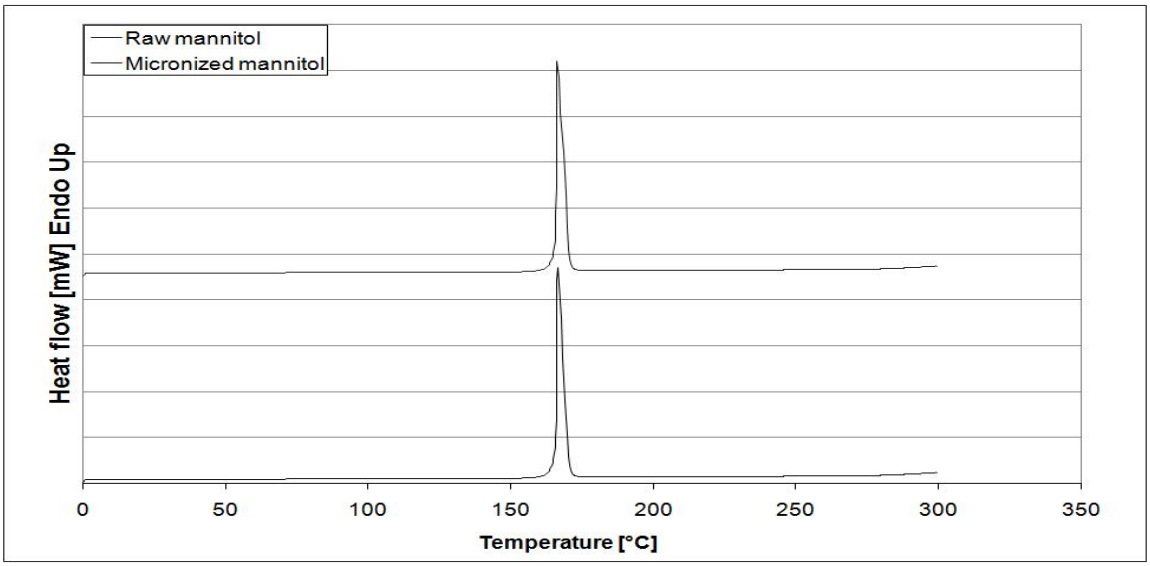
b

Figure 38: DSC traces of decanted lactose (**a**) and mannitol (**b**) before and after addition of fines, the second of two measurements is shown. The traces of carriers before decantation are given for reasons of comparison.

Moreover, the thermal behavior of lactose after micronization shows a broadening of the first melting peak and small endothermic peak appeared after the second melting peak (Figure 39, a) which could be related to artefact during measuring this sample in DSC. Additionally the mannitol DSC thermograms before and after micronization process (Figure 39, b) are approximately the same, which suggest no detectable amorphous part could be attained by using the DSC technique for those micronized carrier materials.



a



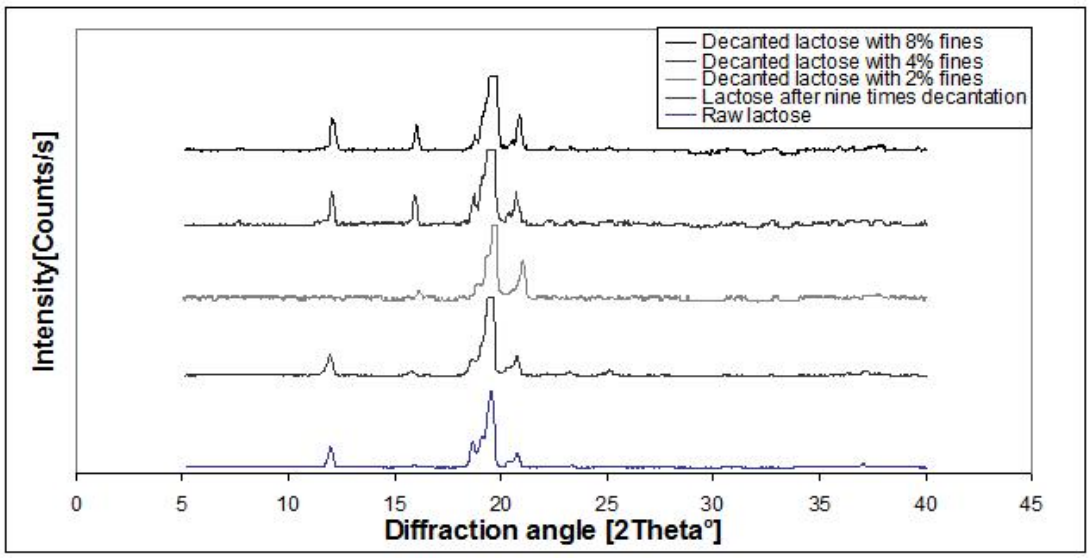
b

Figure 39: DSC traces of lactose (**a**) and mannitol (**b**) before and after micronization, the second of two measurements is shown.

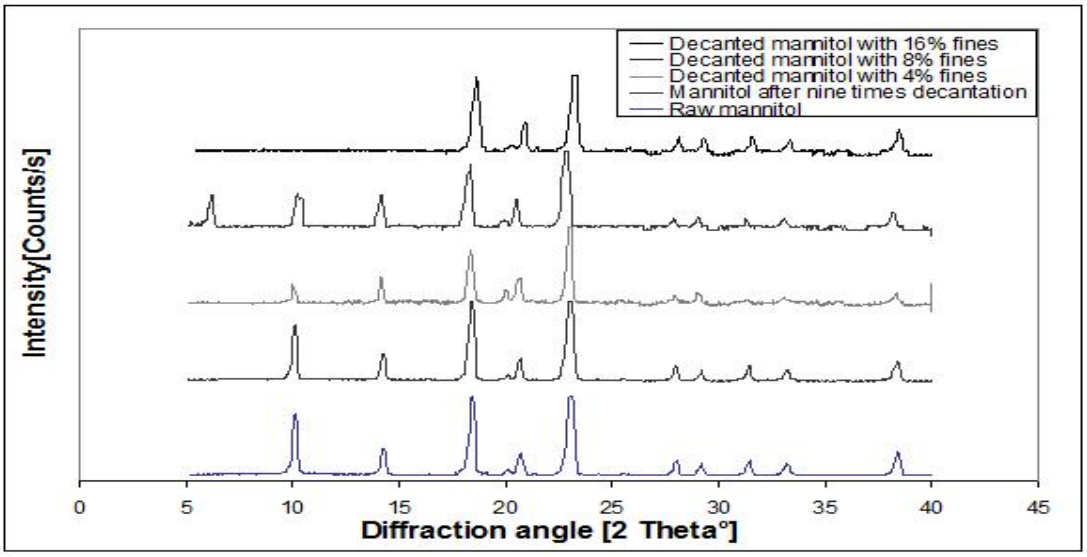
11.1.2 X-ray powder diffraction

The XRD diffractograms (Figure 40) are approximately the same for the decanted carriers and the decanted carriers after addition of fines, which suggest that no change in the crystallinity of those carriers has been occurred after addition of carrier fines. Although, the addition of micronized carriers was expected to affect the crystallinity of decanted carriers adversely. This step of

characterisation suggests the crystalline nature of lactose and mannitol carrier materials even after adding different percents of micronized carriers. However, the peaks at 10° and 15° 2theta of decanted mannitol with 16% added mannitol fines are missing, which may be related to the needle shape of mannitol crystals. During sample preparation of those needle shaped mannitol crystals in the aluminium form, orientation of the needles may have been occurred, which may explain the disappearance of certain peaks. Whereas the detection of amorphous parts with such technique is limited to values above 5%-10%.

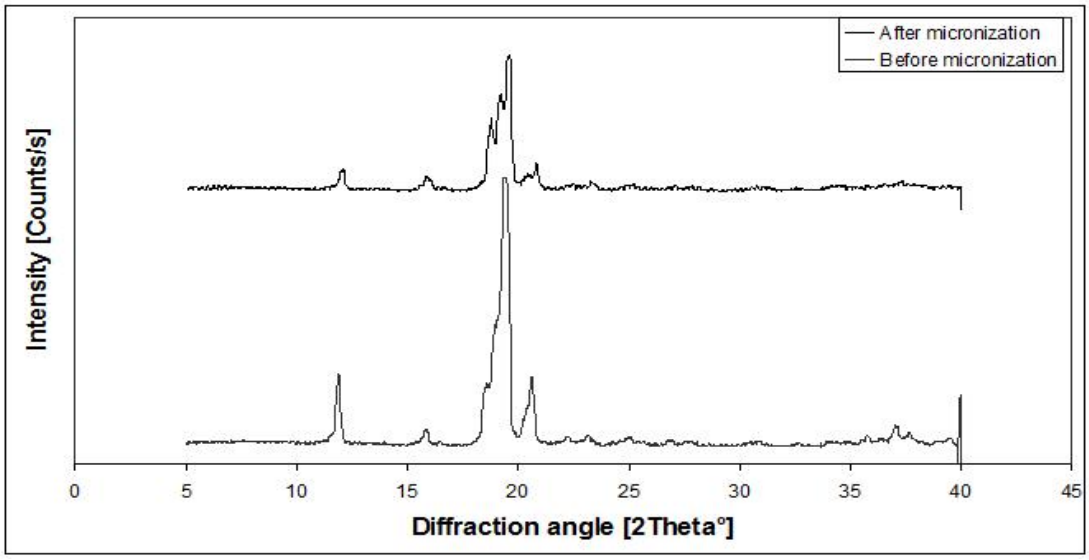


a

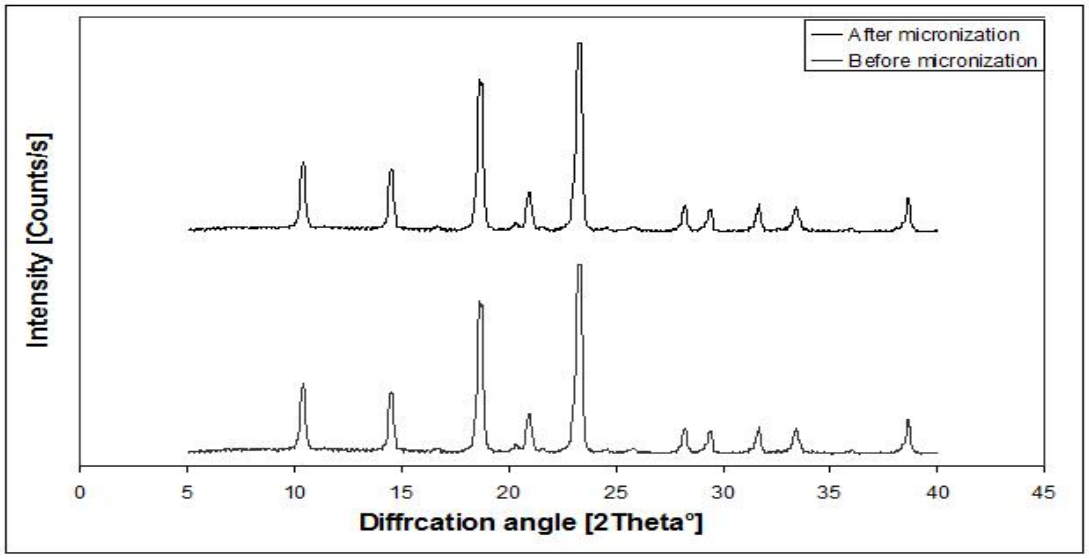


b

Figure 40: XRD diffractograms of lactose (**a**) and mannitol (**b**) before and after addition of fines, the second of two measurements is shown. The diffractograms of carriers before decantation are given for reasons of comparison.



a



b

Figure 41: XRD diffractograms of lactose (**a**) and mannitol (**b**) before and after micronization, the second of two measurements is shown.

Moreover, the XRD diffractograms (Figure 41) are approximately the same for the lactose and the mannitol carrier materials before and after micronization, which suggests that no change in the crystallinity of those carriers has been occurred after milling of those carriers.

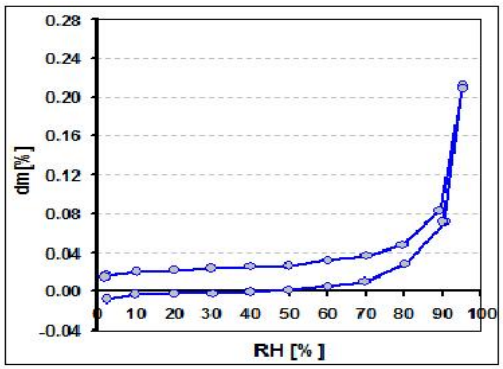
11.1.3 Water vapour sorption

Lactose and mannitol are non-hygroscopic carriers and show an increase of the mass with the increase of relative humidity (Figure 42). Nine times treated lactose used in this study shows an increase of the mass with the increase of the relative humidity followed by a decrease of the carrier mass with the decrease of the relative humidity in the first sorption cycle. The sorption behaviour of the second cycle is similar. The absence of the mass loss at 60% RH in the first cycle and the similarity of the sorption behaviour of the second cycle assure that there is no amorphous part in this lactose after nine times decantation.

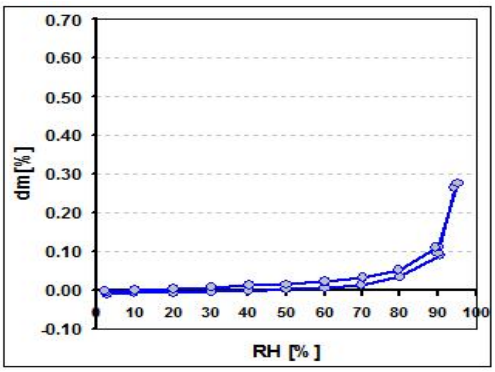
Similarly mannitol shows the sorption behaviour of a crystalline carrier with no amorphous content.

The isotherms (Figure 42) show that the carriers with added fines are taking up more water than the treated carriers without fines. The lower lines are the adsorption responses and the upper lines are the desorption responses. The maximum weight gain for lactose without fines is 0.12% and for mannitol without fines is 0.10%.

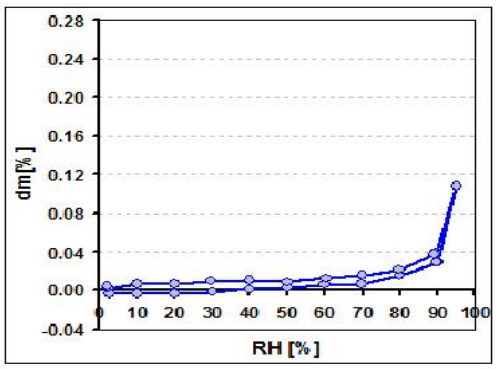
The water vapour sorption isotherms of lactose and mannitol after addition of fines show an increase in water uptake with increasing the fines amount in comparison to lactose and mannitol without fines. This can be explained by the part of fine carrier particles which has been added to the decanted carrier surface leading to an increase of the surface area and subsequent increase of water sorption. However, the extent to which the increase in water uptake happens is less pronounced in lactose than in mannitol. The reason for this phenomenon may be the fact, that lactose surface fines may merge with the coarse carrier surface during the sorption cycle at higher relative humidities resulting in almost the same smooth carrier surfaces at 95% RH independent on the amount of fines previously present. Mannitol is less susceptible to this event, which has already been shown with samples stored at different relative humidities and subjected to water vapour sorption (see chapter II).



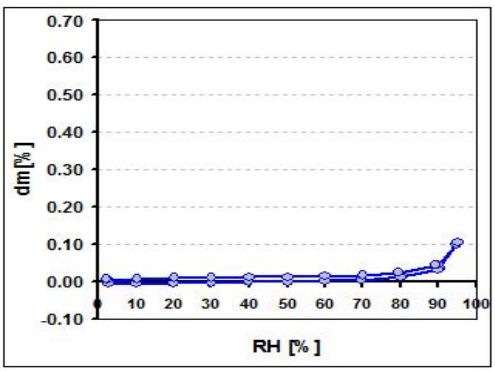
Lactose before decantation



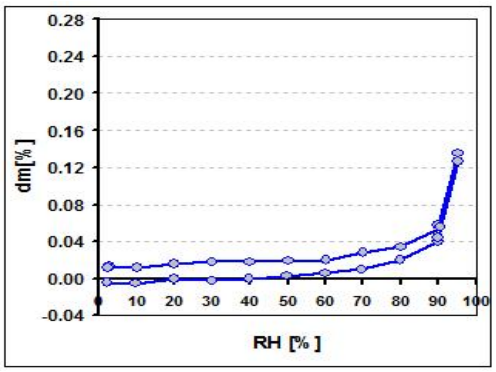
Mannitol before decantation



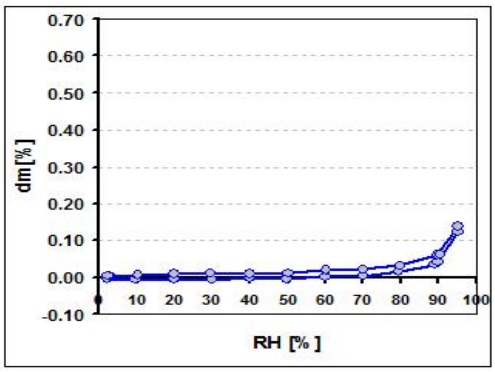
Lactose after nine times decantation



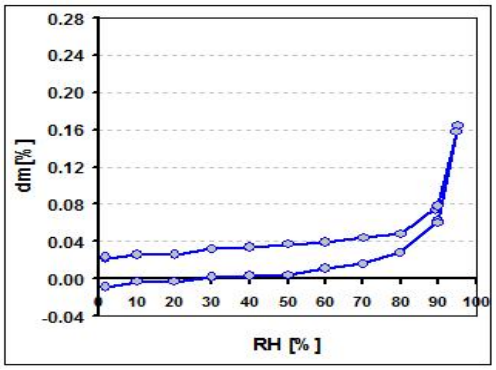
Mannitol after nine times decantation



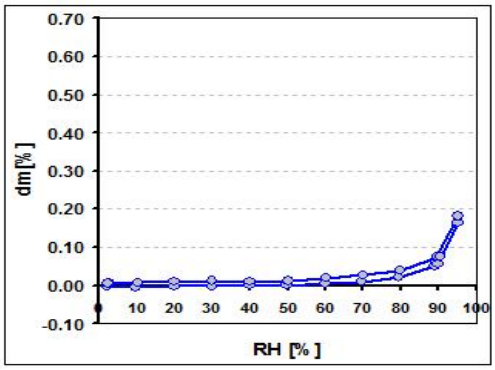
Decanted lactose with 2% fines



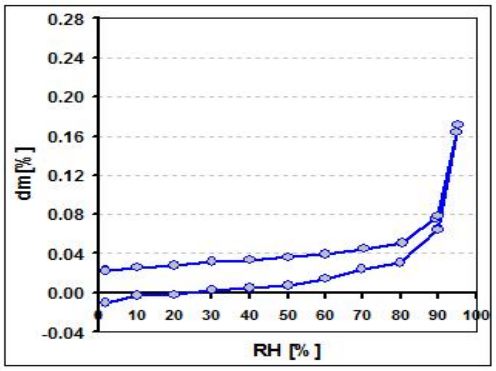
Decanted mannitol with 4% fines

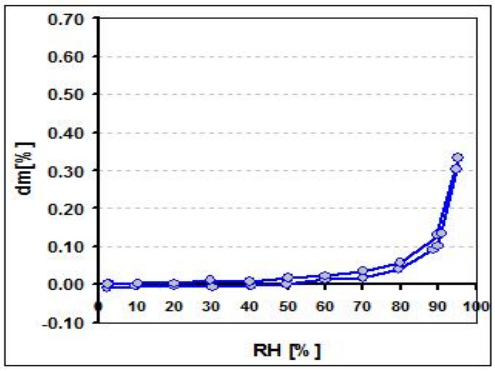


Decanted lactose with 4% fines



Decanted mannitol with 8% fines





Decanted lactose with 8% fines Decanted mannitol with 16% fines

Figure 42: Water vapour sorption isotherms of lactose and mannitol before and after addition of fines. The isotherms of carriers before decantation are given for reasons of comparison.

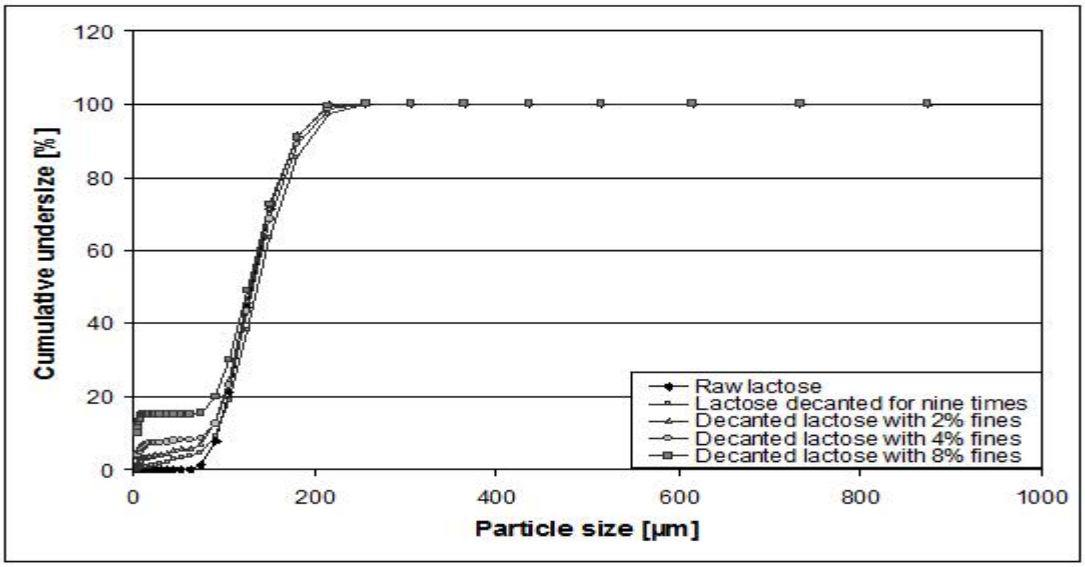
11.2 Determination of the particle size distribution by laser diffraction

The lactose and mannitol carrier fine particles themselves have the median diameter of 3.55µm-5.73µm, whereas the lactose fines have diameter of 3.55µm±0.42µm and mannitol fine have a diameter of 5.73µm±1.29µm. As shown below in Figure 43 and Table VII there are some differences of the particle size distributions before and after the addition of fines to decanted carriers. Especially striking is the decrease of the x_{10} value with increasing amount of fines, which results from the detachment of the fines from the coarse carrier surface caused by the dry dispersing system of the laser diffraction instrument. The increased x_{90} value after addition of 16% micronized mannitol might be related to the proposal of fine layer formation around the coarse carrier itself or formation of large agglomerates on the carrier surface which were difficult to remove by dispersing air. Mannitol x_{50} show a slight variation which could be also related to the formation of agglomerate at coarse carrier surface between the rest of the inherent fines at the carrier surface and the artificially added fines in a high percent. Additionally SEM shows that mannitol carrier still have fines even after subjecting them to nine times decantation sequence.

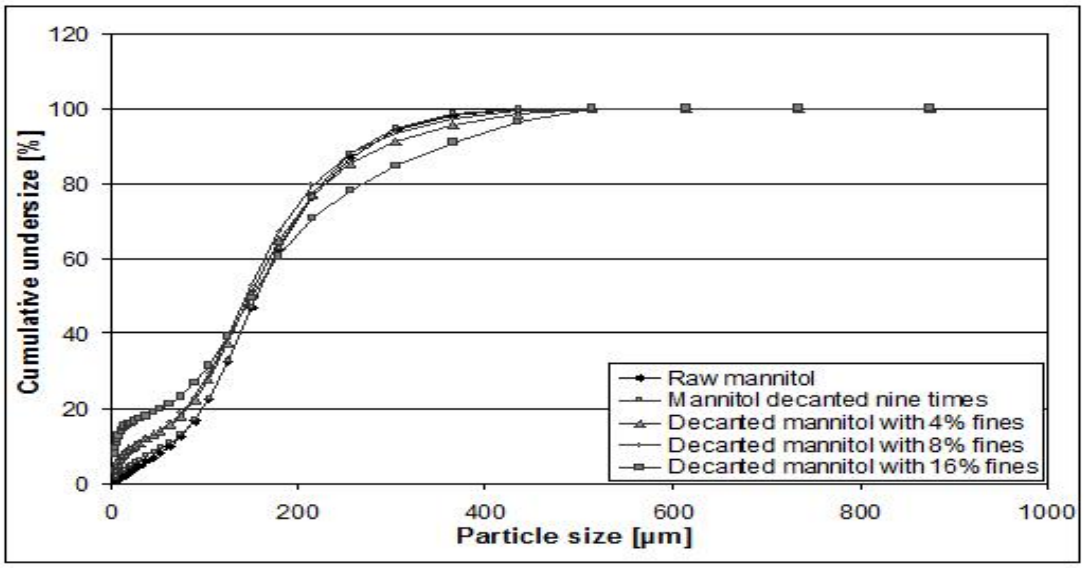
It has to be mentioned that this difference between carriers carrying surface fines and those that do not, for example carriers before wet decantation versus those after wet decantation. This difference may be due to the fact that the fines present in those carriers were not added artificially but were fines inherent to the carrier material itself and originating from its manufacturing process. Obviously the fines inherent to the carrier material adhere more strongly to the coarse carrier surface and can't be detached by the forces exerted by the dry dispersing system than fines which are added later on.

Table VII: Laser diffraction analysis of carrier particle size before and after addition of fines (n = 3, mean ± SD). The values of carriers before decantation are given for reasons of comparison.

Substance	X_{10} [µm]	X_{50} [µm]	X_{90} [µm]
Lactose before decantation	92.40±3.35	130.12±3.97	178.14±2.48
Lactose after nine times decantation	91.42±2.07	136.76±1.00	193.29±1.15
Decanted lactose with 2% fines	81.80±1.46	127.98±0.69	177.75±0.45
Decanted lactose with 4% fines	81.11±1.09	132.37±0.65	185.05±1.22
Decanted lactose with 8% fines	7.035±1.78	124.86±2.77	177.72±2.09
Mannitol before decantation	63.15±11.6	156.06±2.32	247.67±5.17
Mannitol after nine times decantation	56.50±4.54	155.70±5.23	269.48±1.23
Decanted mannitol with 4% fines	29.83±8.04	149.86±3.16	283.49±11.51
Decanted mannitol with 8% fines	18.01±0.94	145.60±2.93	278.69±1.59
Decanted mannitol with 16% fines	6.41±1.55	148.88±5.50	374.59±5.47



a



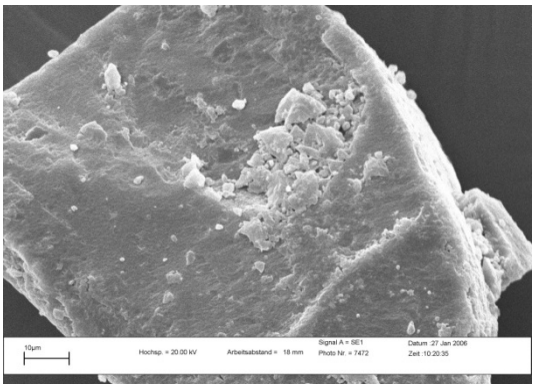
b

Figure 43: Laser diffraction analysis of lactose (**a**) and mannitol (**b**) before and after addition of fines, the second of three measurements is shown. The particle size distributions of carriers before decantation are given for reasons of comparison.

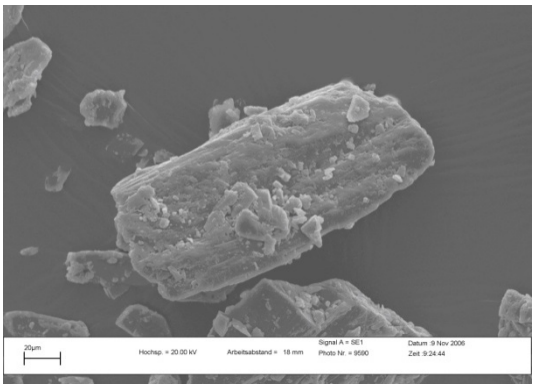
11.3 Determination of the particle shape and the surface characteristics by scanning electron microscopy

The added fines are shown to adhere to the coarse carrier (Figure 44). Mannitol crystals appear to have more fines than lactose crystals after addition of micronized carriers. The photomicrographs of the binary carriers indicate the formation of fine particle layer, whereas other areas of the carrier surface are still free of fines. Probably, the strong active sites are saturated first, and the subsequent particles selectively bind with their own species to form particle layers. This tendency of particle layer formation is increased with increased added fine concentration despite the free space that is available on the carrier surface.

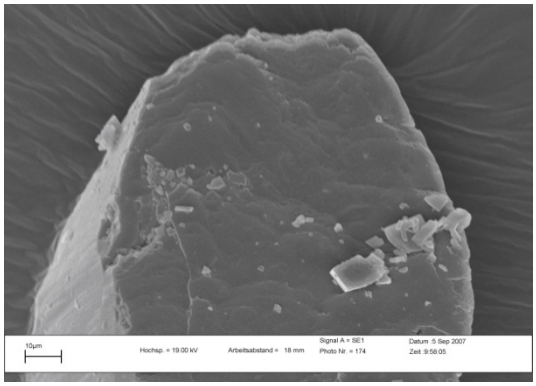
However, as already mentioned previously, scanning electron micrographs capture just a few particles of the whole bulk and do not provide representative information about the whole number of particles.

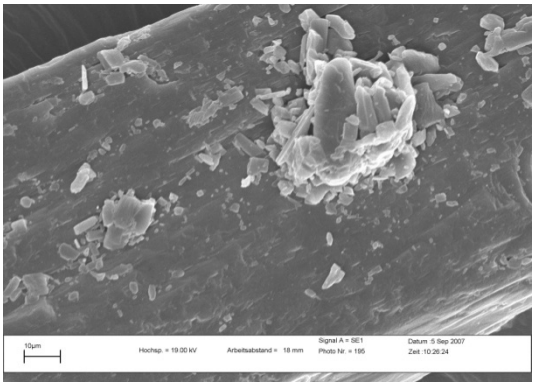


Untreated lactose

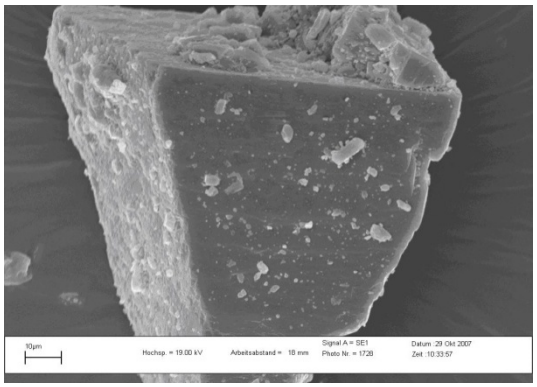


Untreated mannitol

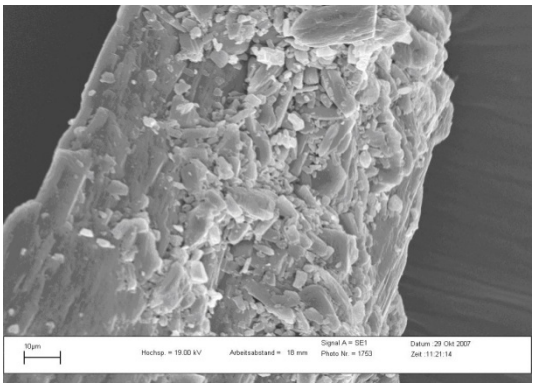




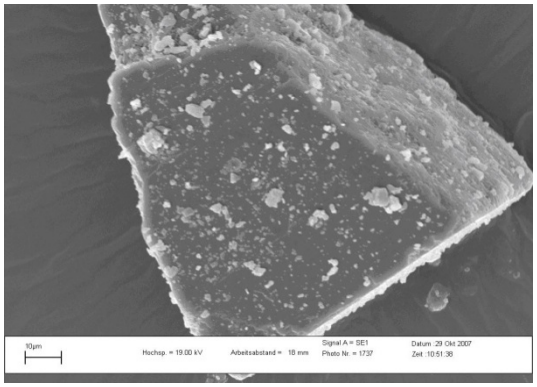
Lactose after nine times decantation



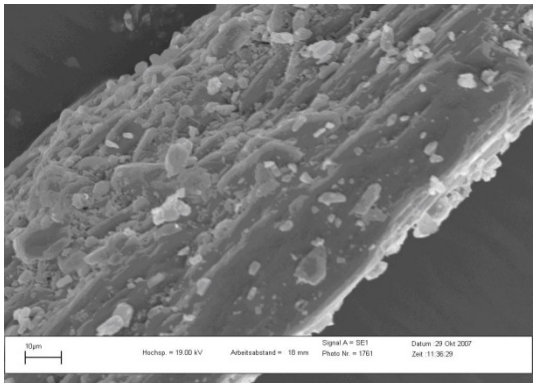
Mannitol after nine times decantation



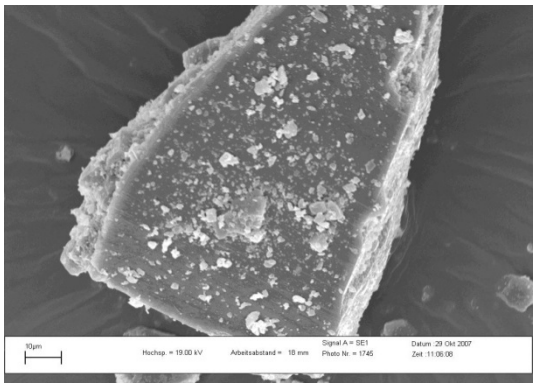
Decanted lactose with 2% fines



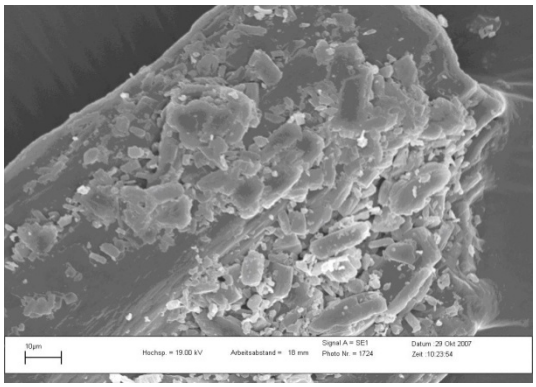
Decanted mannitol with 4% fines



Decanted lactose with 4% fines



Decanted mannitol with 8% fines



Decanted lactose with 8% fines

Decanted mannitol with 16% fines

Figure 44: Scanning electron micrographs of nine times decanted lactose and mannitol before and after addition of fines. Micrographs of carriers before decantation are given for reasons of comparison.

11.4 Determination of surface area by BET measurement

In comparison to the untreated carriers, the decanted carrier materials have a smaller surface area due to the removal of fines from these carriers as shown

in Figure 45. The added fines increase the specific surface area as expected. The results of the BET measurements of lactose and mannitol are in agreement with the results of the water vapour sorption, where the increase of surface area due to the addition of fines is accompanied by a more or less pronounced increase of water uptake.

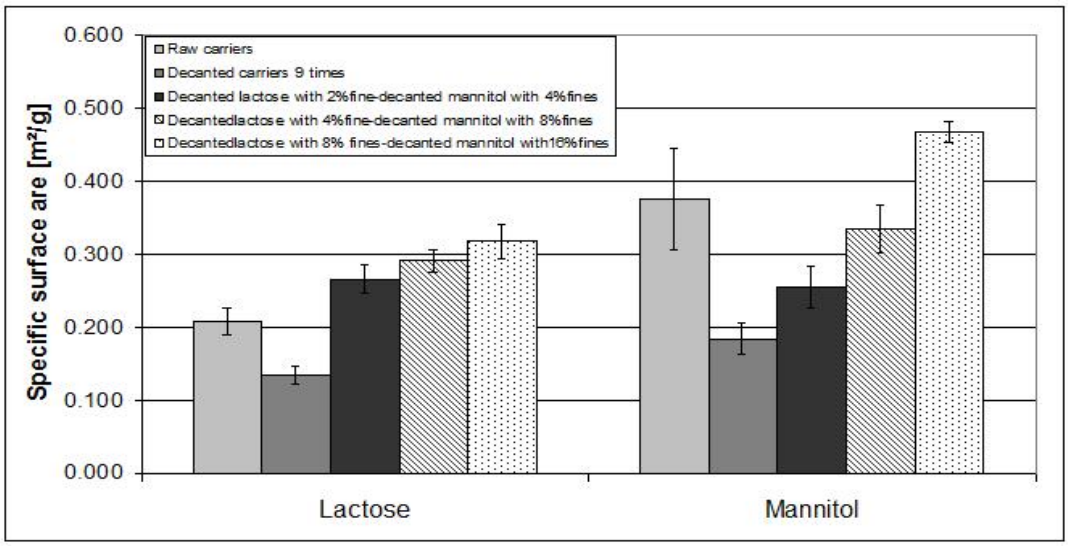


Figure 45: Specific surface area of nine times decanted lactose and mannitol before and after addition of fines (n = 3, mean ± SD). Results of carriers before decantation are given for reasons of comparison.

11.5 In vitro deposition test

The removal of carrier fines from the coarse carrier surface by decantation leads to a significant decrease in the FPF as shown in Figure 46. As already mentioned above, this may be attributed to the removal of the carrier fines from the carrier surface, which may lead to a higher number of free active sites on the carrier surface that may be occupied with drug particles, and to an enhanced smoothness of the carrier surface resulting in a higher contact area between the drug and the carrier. Both mechanisms result in higher adhesion forces between the drug and the carrier leading to a poor drug particle detachment from the carrier particles during the inhalation process.

When this smoothed carrier quality is now pre-blended with carrier fines, the active sites may be covered by the carrier fines and the smoothness of the coarse carrier surface may be reduced leading to less adhesion of the drug

particles. This reduced adhesion of the drug to the carrier surface results in the increase of the FPF with respect to the mixtures containing the fines-free carriers treated by wet decantation (Figure 46). Increasing the concentration of carrier fines further enhances the FPF. However, increasing the amount of fines is limited, since exaggerated addition of fines may result in poor flow properties of the powder formulation.

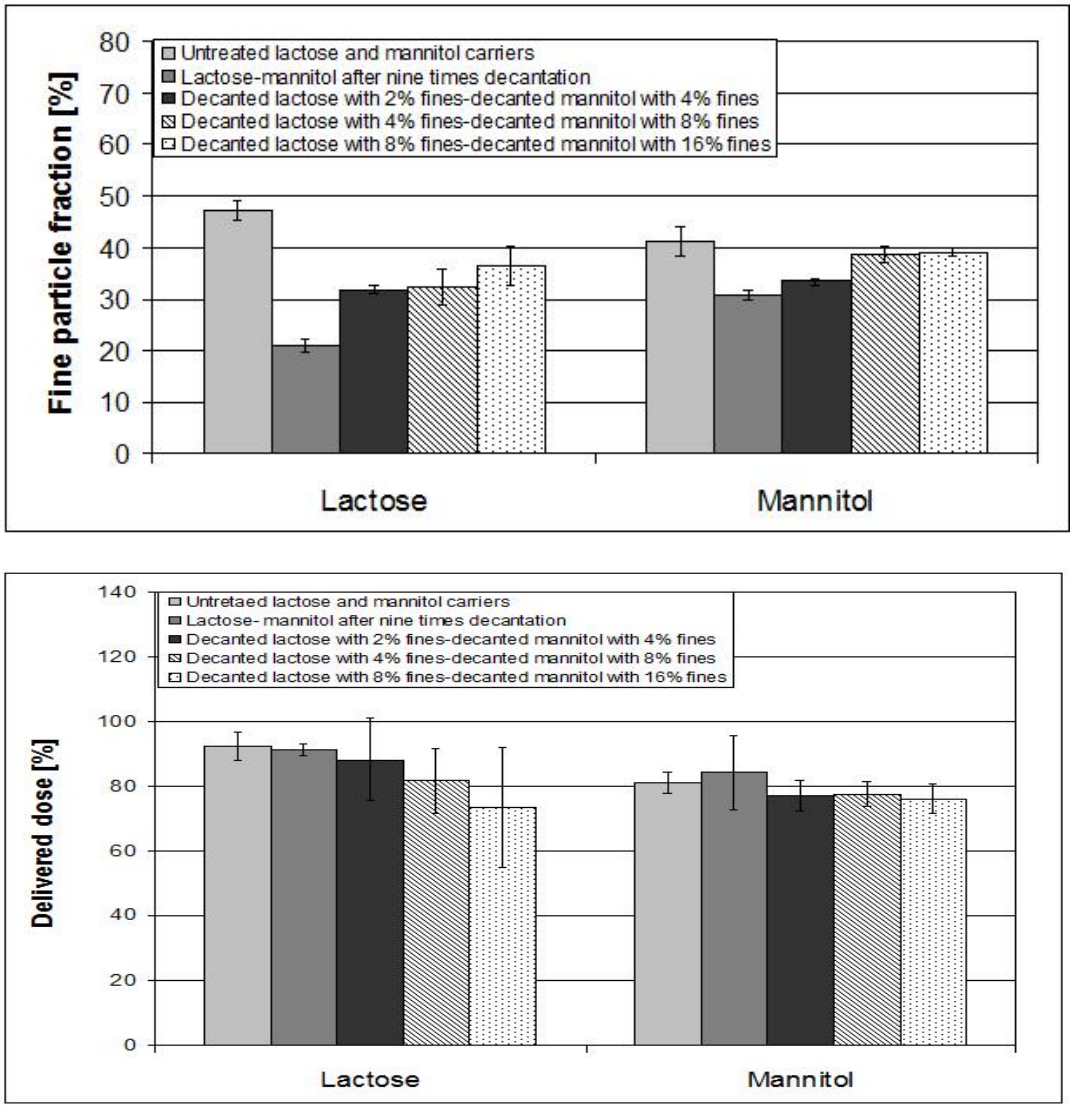


Figure 46: Fine particle fraction and delivered dose of salbutamol sulphate with lactose and mannitol decanted nine times before and after addition of fines (n = 3, mean ± SD). Results of carriers before decantation are given for reasons of comparison.

Also, Staniforth et al., 1995 and; Zeng et al., 1998 reported that the addition of carrier fines displaces drug from such sites on the coarse carriers and hence more of the drug is located at sites where the binding action might be weaker. Consequently the more weakly adhered drug particles are dislodged and detached more easily from the surface of carrier particles, and dispersed in the air stream during inhalation.

Another study that used atomic force microscopy (AFM) to measure the adhesive force between a 10μm silica sphere and various sites on the surface of a lactose carrier, however, found a log-normal distribution of forces, suggesting that, the division of a carrier surface into areas of strong and weak adhesion may be too simplistic (Louey et al., 2001). Furthermore, the authors speculated that during the blending process, drug particles were distributed between the surface of the carrier and multiplets formed by the aggregation of fines and drug particles. The experimental evidence to support this was limited to SEM of the formulations that showed the presence of both drug-coarse carrier adhesion units and fine particle multiplets. This had been described previously for other ternary powder blends by Soebagyo et al., 1985.

Lucas et al., 1998 further speculated that upon aerosolization, drug particles were more easily liberated from fine particle multiplets than from the surface of coarse carrier particles, as fine lactose was thought to have a smoother surface than coarse lactose, giving a reduced force of adhesion between drug and fines. They also suggested that certain fine particle multiplets might also be small enough to form part of the FPF without dispersion, a premise supported by the work of Srichana et al., 1998, whose work on the deposition of salbutamol sulphate and lactose found that these two components can travel together to the lower regions of the lung.

Anyhow, although the FPF increases with increasing amount of carrier fines, the FPF of the mixtures containing the original carriers, namely the ones before wet decantation, is not obtained.

12 Conclusion

Removal of carrier fines by decantation decreases the in vitro deposition of the drug in comparison to the untreated carrier. Addition of micronized carriers to decanted carriers is found to increase significantly the dispersion of the drug probably by reducing drug to carrier adhesion and allowing easy detachment of the drugs from the carrier surface. Drug to carrier adhesion is supposed to be reduced either by the occupation of active sites by carrier fines or by the reduction of the contact area between the drug and the carrier due to surface roughness enhancement. Although the addition of fines to the treated carriers could not revert the fine particle fraction to the original value of the untreated carriers. This may indicate that the added fines were not equal to the original fines which had been removed by wet decantation. Added carrier fines exhibit another size, shape, surface, energetic charge, etc. than the inherent fines.

V Influence of added fine lactose and mannitol on inhalable dry powder formulations

13 Introduction

The previous parts of the present study aimed to obtain a smoothed carrier surface using wet decantation and storage of the carrier materials at different relative humidities, which clarifies that a certain level of carrier rugosity is required to improve the in vitro deposition. To which extent the carrier rugosity should be available is the aim of this part of the thesis. As substantiated by previous studies (Lucas et al., 1998; Podczeck et al., 1999), the presence of a small amount of adhered fines on coarse lactose carrier is critical for facilitating particle deaggregation in the air turbulence generated by inhalation. Addition of carrier fines was also tried with the wet decanted lactose and mannitol carriers. That part of the study was carried out to investigate the effect of added fines on the treated carriers in vitro deposition (carriers without surface fines). The added fines were not enough to achieve the FPF before decantation, probably because they had not the same fines shape, electrostatic properties and size as the original ones. So it is of interest, what would be the effect of carrier fines if they were added to the carrier as its supplied.

The untreated sieve fraction 112µm-140µm of mannitol (Pearlitol 160C) and lactose (InhaLac120) as well as these carriers with added micronized carrier fines (2%, 4%, 8% lactose fines added to coarse lactose and 4%, 8%, 16% mannitol fines added to coarse mannitol) were characterized by differential scanning calorimetry (DSC), X-ray powder diffraction and water vapour sorption in order to inspect whether crystallinity of the carrier materials is modified by the addition of fines. Particle size and specific surface area were investigated by laser diffraction and Brunauer Emmet and Teller (BET) gas adsorption. Finally, the influence of this corrugation process on the in vitro deposition was examined.

14 Results and discussion

14.1 Determination of the extent of crystallinity

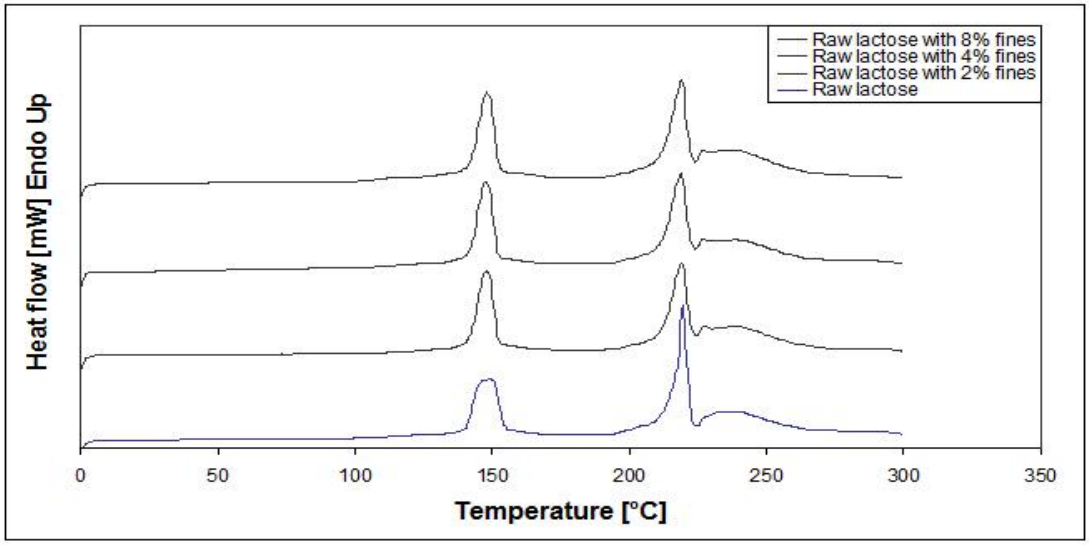
14.1.1 Differential scanning calorimetry

The untreated lactose and mannitol carrier materials alone and with added fines show negligible differences in terms of the molar heat of fusion and the onset as well as peak temperature. Figure 47 and Table VIII prove that no considerable modification of crystallinity has taken place after the addition of the carrier fines. Additionally, the detection limits for amorphous contents with such technique have a lower cut off of 5-10% .This detection limit is due to the fact that this technique measures the entire sample (Buckton et al., 1999)

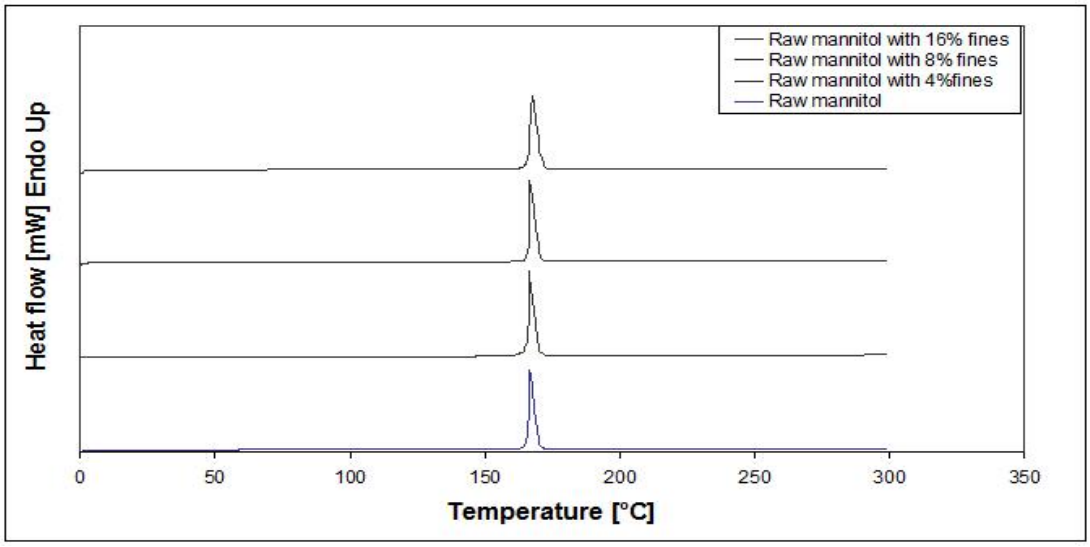
Further, addition of different amounts of carrier fines in an ascending manner has not changed the crystallinity of lactose and mannitol carriers.

Table VIII: DSC parameters of lactose and mannitol carriers before and after addition of different amounts of carrier fines (n=2), mean ± range (the range indicates the maximum and the minimum values).

SAMPLE NAME	ENTHALPY [J / G]	ONSET [°C]	PEAK [°C]
Untreated lactose	153.51±1.19	140.17±6.04	147.40±1.86
Lactose with 2% fines	158.34±2.20	142.33±0.51	148.08±0.08
Lactose with 4% fines	147.39±3.99	142.21±0.18	148.06±0.25
Lactose with 8% fines	164.67±0.88	141.96±0.05	147.89±0.25
Untreated mannitol	293.16±7.26	165.49±0.14	166.19±0.03
Mannitol with 4% fines	297.56±15.35	165.98±0.41	167.19±0.90
Mannitol with 8% fines	297.07±3.92	165.72±0.09	166.82±0.18
Mannitol with 16% fines	287.49±5.86	165.84±0.06	167.39±0.22



a

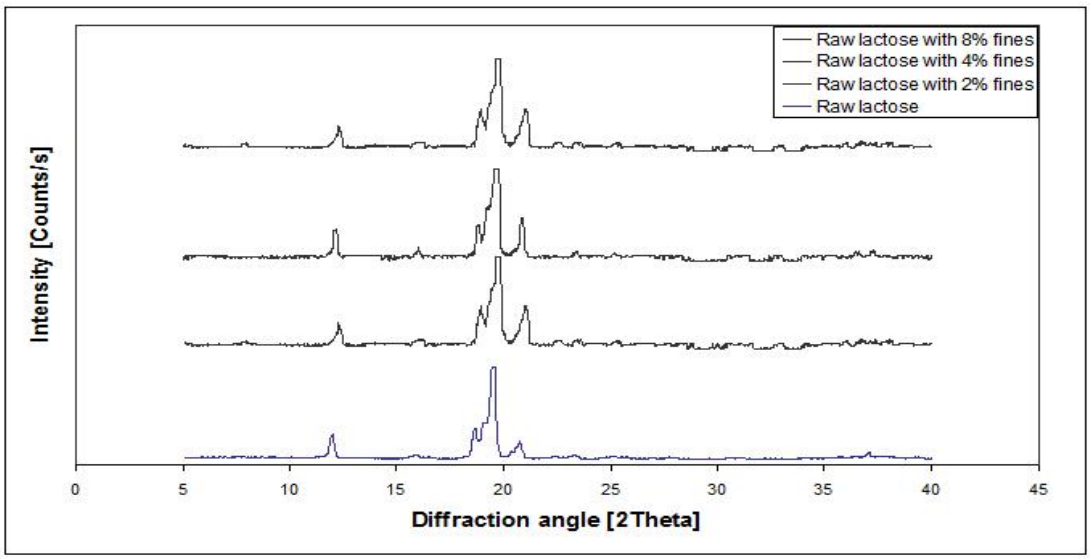


b

Figure 47: DSC traces of lactose (**a**) and mannitol (**b**) carriers before and after addition of fines, the second of two measurements is shown.

14.1.2 X-ray powder diffraction

X-ray analysis was performed on lactose and mannitol carrier materials with and without added fines in order to assess the possible relevant changes of crystallinity. Figure 48 shows that no significant differences are found comparing the X-ray spectra before and after the addition of fines. These results come in agreement with the DSC results which proof that no change of the crystallinity of the original carriers has occurred. Peaks between 5° and 9° are artefacts from the powder diffractometer.



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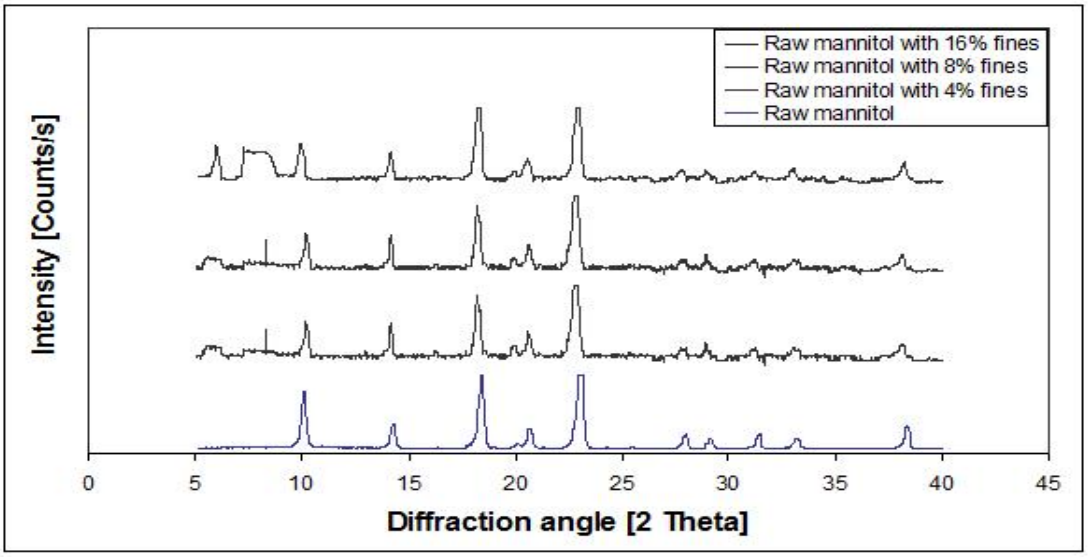


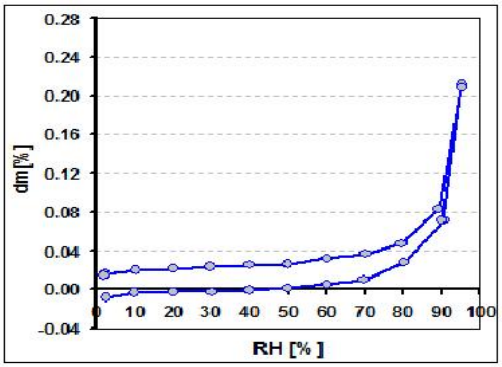
b

Figure 48: XRD diffractograms of lactose (**a**) and mannitol (**b**) before and after addition of fines, the second of two measurements is shown.

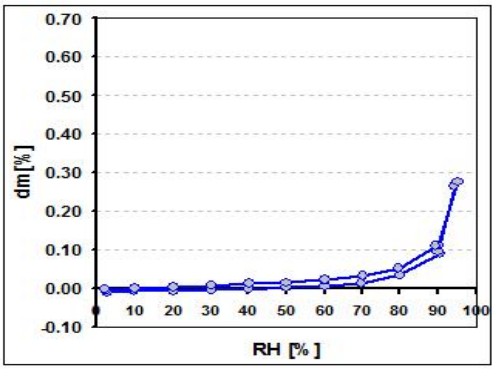
14.1.3 Water vapour sorption

Lactose and mannitol carrier materials show an increase of the mass with an increase of the relative humidity followed by a decrease of the carrier mass with a decrease of the relative humidity in the first sorption cycle. The sorption behaviour of the second cycle is similar. The isotherms (Figure 49) plots show that the carriers with added fines are taking up more water than the carriers without fines. The lower lines are the adsorption responses and the upper lines are the desorption responses. The maximum weight gain for lactose

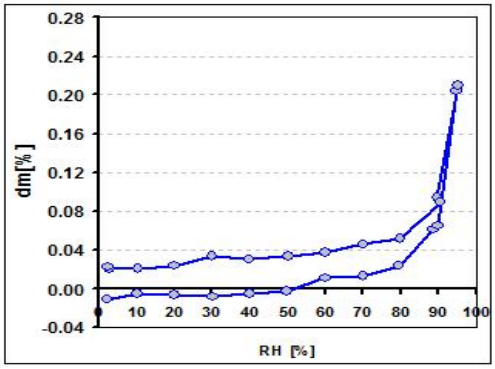
without fines is 0.20% and for mannitol without fines is 0.30%. The increase in water uptake by the increase of the amount of fines can be explained by an increase of the surface area after addition of fines. The water sorption isotherms of lactose and mannitol carrier materials show an increase in the water uptake with the subsequent increase of the added fines, which reflect no change in the crystallinity of the both carrier materials; this unchanged crystallinity comes in agreement with the DSC and X-ray diffraction results.



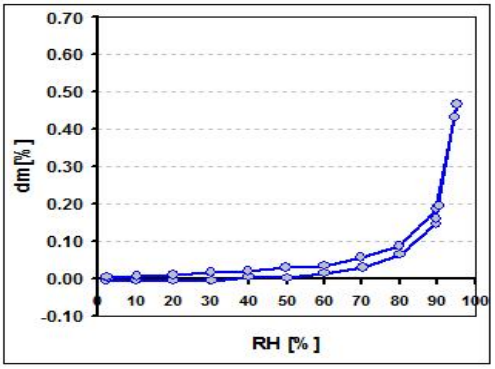
Lactose



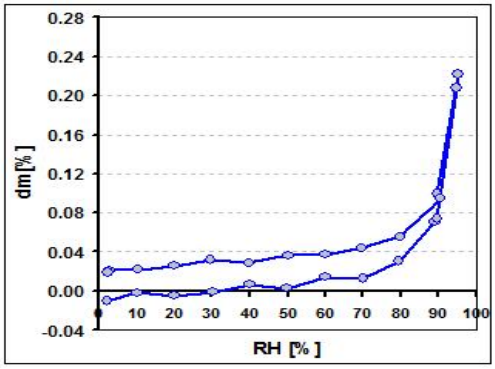
Mannitol



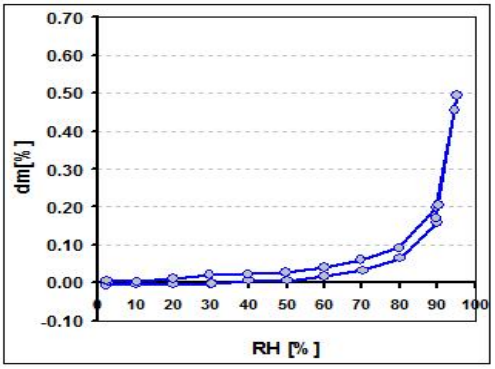
Lactose with 2% fines



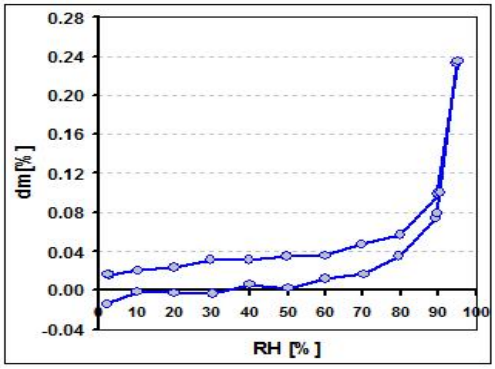
Mannitol with 4% fines

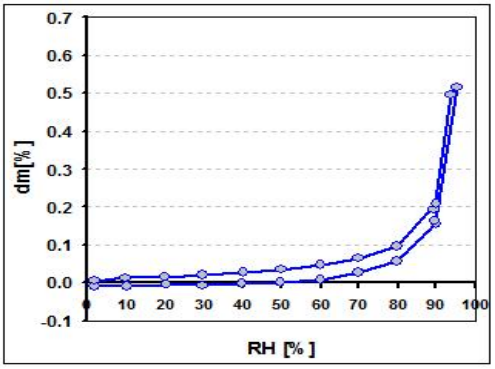


Lactose with 4% fines



Mannitol with 8% fines





Lactose with 8% fines Mannitol with 16% fines

Figure 49: Water vapour sorption isotherms of lactose and mannitol before and after addition of fines.

14.2 Determination of the particle size distribution by laser diffraction

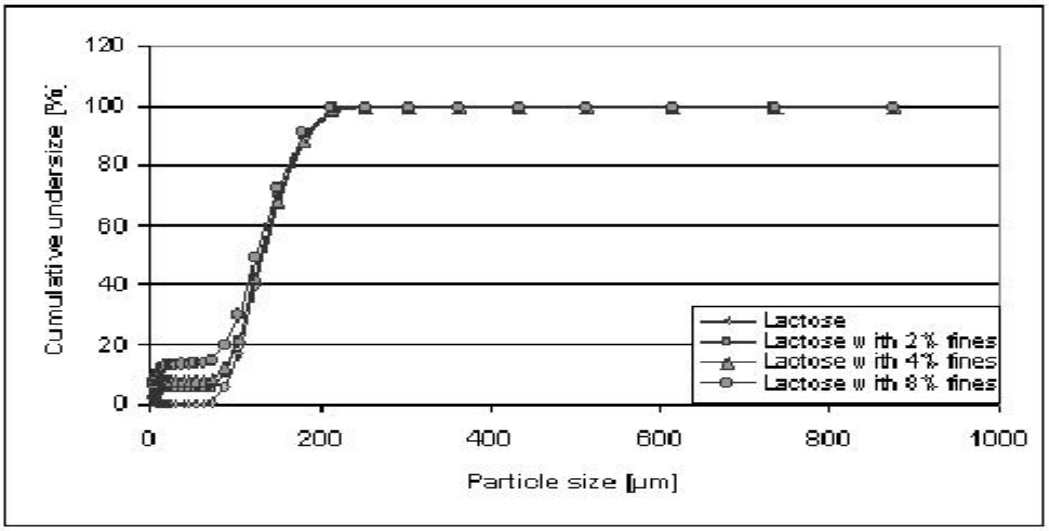
The lactose and mannitol carrier fine particles themselves have the median diameter of 3.55µm-5.73µm, whereas the lactose fines have diameter of 3.55µm±0.42µm and mannitol fine have a diameter of 5.73µm±1.29µm. Figure 50 and

Table IX show some change in the median of the particle size distribution before and after the addition of fines which could be related to the formation of agglomerates at coarse carrier surface between the inherent carrier fines and the artificially added fines in a high percent. Nevertheless, a decrease of the x_{10} percentile is observed which as already mentioned in the previous chapter, results from the detachment of the fines from the coarse carrier surface caused by the dry dispersing system of the laser diffraction instrument. The increased x_{90} value after addition of micronized mannitol in ascending manner might be related to the proposal of formation of large agglomerate on the carrier surface which were difficult to remove by dispersing air.

Table IX: Laser diffraction analysis of the carrier particle size distribution before and after addition of fines (n=3, mean ± SD).

Substance	X_{10} [µm]	X_{50} [µm]	X_{90} [µm]
Lactose	95.71±0.16	134.79±0.32	185.11±0.91

Lactose with 2% fines	85.71±2.84	130.93± 0.58	181.01±1.27
Lactose with 4% fines	80.63±1.78	132.09±0.39	184.75±0.45
Lactose with 8% fines	8.81±0.02	126.04±0.20	178.85±0.04
Mannitol	63.15±11.6	156.06±2.32	247.67±5.17
Mannitol with 4% fines	24.62±1.05	142.52±1.12	272.93±5.38
Mannitol with 8% fines	25.19±5.04	163.22±4.23	305.29±3.69
Mannitol with 16% fines	6.45±0.47	159.51±4.90	316.57±32.7



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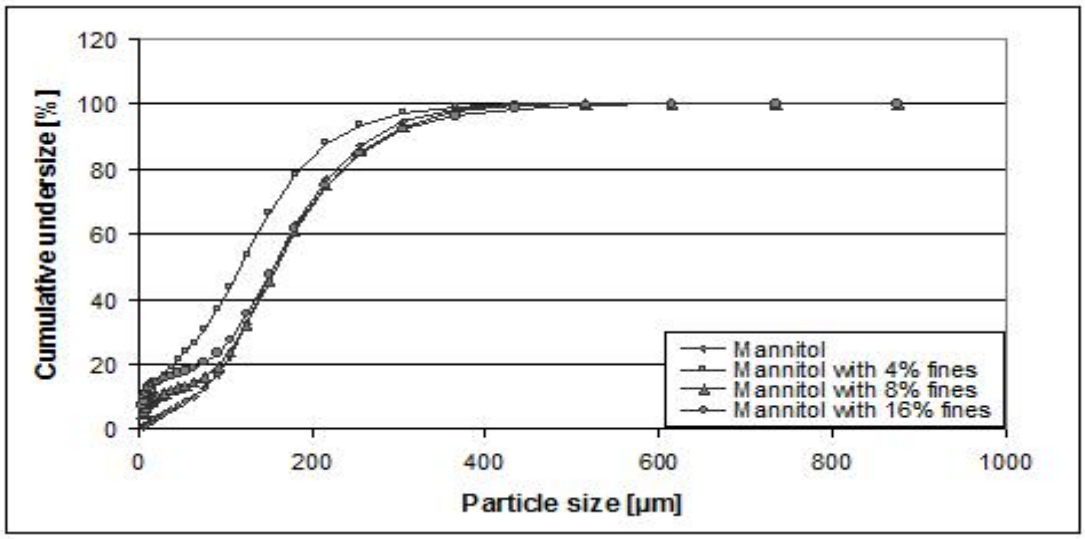


b

Figure 50: Laser diffraction analysis of lactose (**a**) and mannitol (**b**) carriers before and after addition of carrier fines, the second of three measurements is shown (n = 3, mean± SD).

14.3 Determination of the particle shape and the surface characteristics by scanning electron microscopy

The particle morphology of lactose and mannitol carriers using scanning electron microscopy was not examined, since the added carrier fines could not be distinguished from the inherent carrier fines themselves.

14.4 Determination of surface area by BET measurement

In general. Figure 51 shows an increase in the specific surface area due to the addition of fines with respect to the carriers without fines. However, this increase is not significant (ANOVA $P< 0.05$) for some carrier fines percent to each other.

Lactose as it is shows a significant lower specific surface area in comparison to all lactose samples containing fines, whereas lactose with 2% fines is not significantly different with respect to the carrier containing 4% of fines. Nevertheless, the surface area of samples containing 4% fines is significantly lower than the one of carriers containing 8% of fines. Those results are somewhat in contrast with the results obtained from the water vapour sorption

analysis, which showed a slight, but continuous increase of water uptake by increasing the amount of added fines.

Furthermore, mannitol shows no change in the specific surface area after the addition of carrier fines up to 8%, whereas the addition of 16% carrier fines shows a significant increase in the specific surface area. Comparison of the mannitol specific surface area change to the water uptake obtained from water vapour sorption analysis shows a disagreement of the results, because the water uptake of mannitol increases continuously from zero to 16% of fines.

Nevertheless, as already mentioned, it has to be kept in mind that water vapour sorption may gives a rough idea about the surface area of a powder, but is not solely reliant on this parameter. This may cause the above-mentioned deviation of the results.

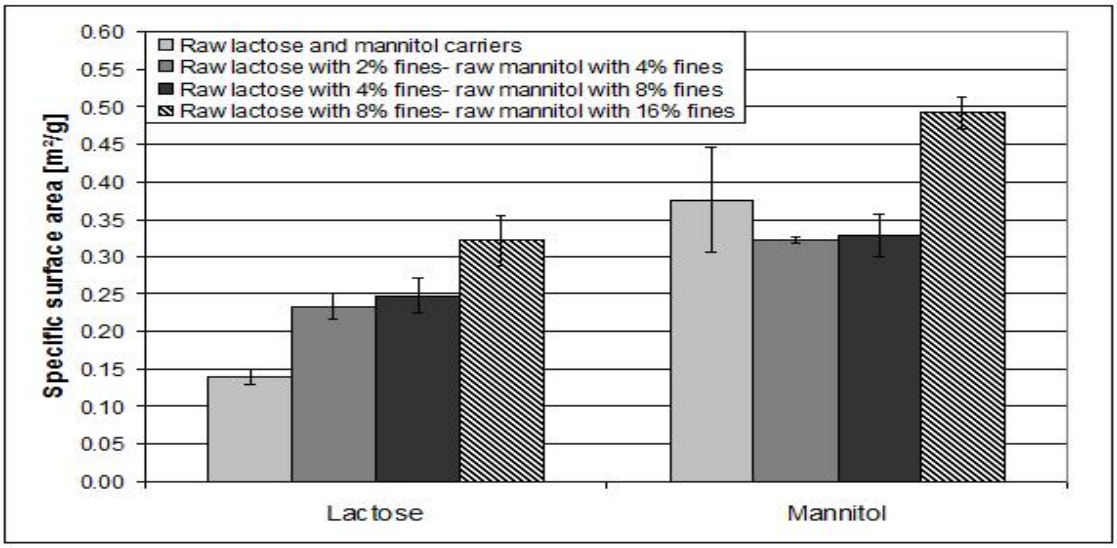


Figure 51: Specific surface area of lactose and mannitol carriers before and after addition of fines (n = 3, mean ± SD).

14.5 In vitro deposition test

The addition of additional carrier fines to the sieve fractions 112µm-140µm of lactose (InhaLac120) and mannitol (Pearlitol 160C) resulted in the highest fine particle fractions found in this study. Lactose (Figure 52) shows an increase in the FPF after adding 2% and 4% carrier fines with respect to the untreated

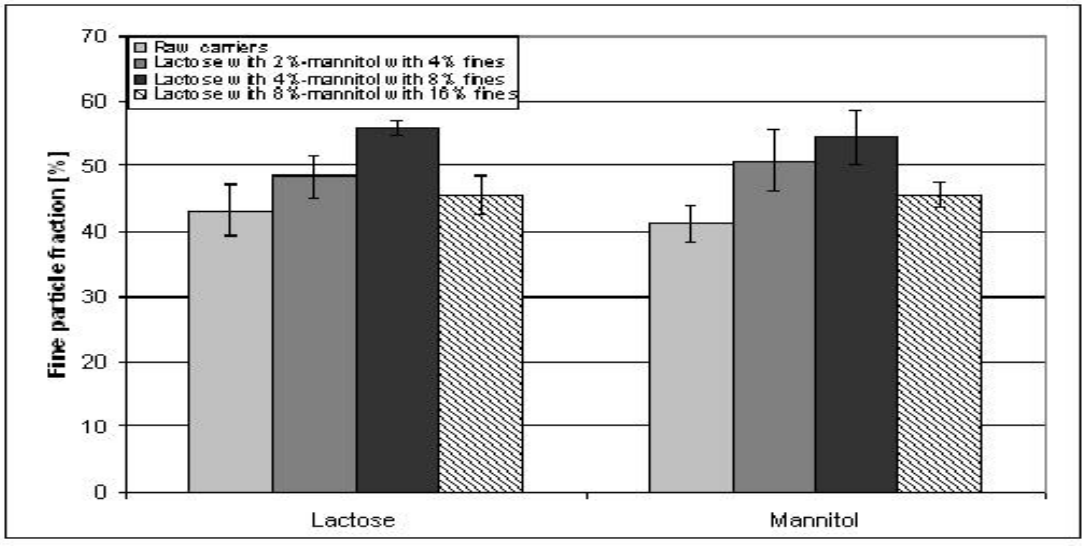
carrier. However, after adding 8% fines, the FPF is reduced with respect to samples containing 2% and 4% carrier fines. Mannitol exhibits the same manner of increasing the FPF with 4% and 8% of added fines. After adding 16% the FPF is reduced.

Despite the large body of research, there is disagreement as to the mechanism by which considerable amounts of fines improve the formulation performance. Besides the fact already discussed in the previous chapter, namely that moderate amounts of carrier fines may increase the roughness of the coarse carrier surface thereby decreasing the drug-carrier contact area and increasing the FPF, there are two main hypotheses found in literature explaining the impact of higher amounts of fines. The first hypothesis and the one also already mentioned in the previous chapter suggests that fines prevent the drug from adhering to the strongest binding sites on the carrier (the term active site may refer to variations in morphology and surface free energy), which will directly influence the thermodynamic work of adhesion (Young et al., 2005). Whilst the second hypothesis proposes that fine particles of drug and excipient form mixed agglomerates that are more easily dispersed and deaggregated during aerosolization with respect to drug-coarse carrier agglomerates (Frijlink et al., 2004).

In contrast, there are also studies reporting the formation of mixed agglomerates, which possess strong adhesion forces between drug and fines resisting the break up in the air stream. This assumption is supported by Louey et al., 2002 who indicated that interactive mixtures consisting of salbutamol sulphate and very large proportions of fine lactose particles show a decreased FPF. Podczeck et al., 1998, 1999 indicated that addition of 20%-30% of fine carrier particle fraction apparently resulted in the adhesive layer structure to be built around the coarse carrier, where the frictional removal is less likely, because the drug particles will be embedded in the macro-waviness of the adhesive layer. Lucas et al., 1998, reported that a higher FPF of spray dried bovine serum albumin occurs with increasing concentrations of fine lactose up to 5%. The evidence from these in vitro studies is supported by data from one *in vivo* study, which found that the inclusion of lactose fines in a

salbutamol sulphate carrier-based formulation increased both the urinary excretion of the drug and the post administration forced expiratory volume in one second (FEV1) (Tee et al., 2001). Increases in both of these parameters suggest increased pulmonary delivery of the drug. However, Lucas et al., 1998 also describe no further increase of the FPF at concentrations of 7.5% to 10%. It was assumed that the treated carrier with higher amounts of carrier fines is nearly saturated with the added carrier fines, and the addition of drug to such carriers could be expected to have no chance to adhere onto the carrier surface and will form strong agglomerates with those added fines and subsequently due to inertial impaction, the major part of drug particles will be lost in throat and the fine particle fraction will decrease.

Figure 52 shows a slight decrease in the delivered dose with the ascending increase of added percent of carrier fines to both lactose and mannitol carrier materials, which might be due to the reduced flowability of both carriers after comprising those carrier fines.



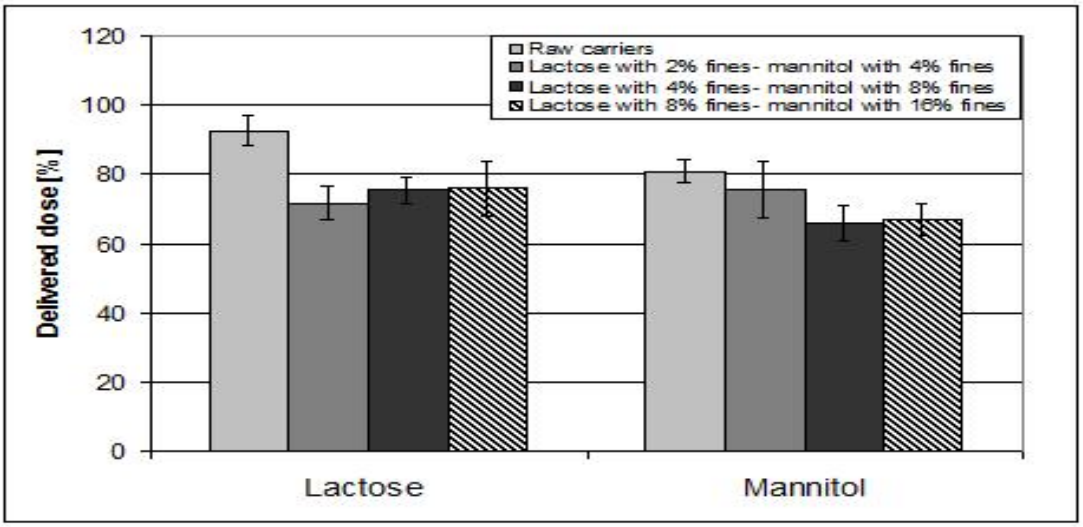


Figure 52: The fine particle fraction and delivered dose of lactose and mannitol carriers mixed interactively with salbutamol sulphate before and after addition of carrier fines (n = 3± SD).

15 Conclusion

It has been shown in this chapter that it is relevant to prepare carrier crystals with a controlled surface rugosity built by carrier surface fines. This rugosity may offer weaker adhesion forces with drug particles and subsequently enhanced in vitro deposition. Enhanced amounts of fine particles, in contrast, may also lead to a decrease of the fine particle fraction. The mechanisms by which this decrease is driven, however, is controversially discussed in literature including the hypothesis of mixed agglomerates formation, which possess strong adhesion forces between drug and fines resisting the break up in the air stream and autoadhesion layer structure formation, where the frictional drug removal is less likely, because the drug particles are embedded in the macro-waviness of the autoadhesion layer.

VI Influence of carrier surface fines removal by air jet treatment on the dry powder inhalation formulations

16 Introduction

This study aims to remove the inherent particle fines from the carrier surface using an air jet sieving technique. The impact of using the air jet treated carriers on the in vitro deposition was investigated. The results were compared with those obtained with untreated carriers. The method proposed using air-jet sieving is simple and easy to perform.

The sieve fraction 112µm-140µm of lactose (InhaLac120) and mannitol (Pearlitol 160C) was either used as it is or was placed on a 63µm air jet sieve. The air stream was blown for 15, 30, 60, 120 seconds (Total time of air jet treatment was 225 s) from down side to remove the carrier fines at 4000Pa pressure respectively in order to get rid of the loosely bound fines and to get a smoothed carrier surface.

Both parts of the carrier materials were characterised using differential scanning calorimetry (DSC), X-ray powder diffraction and water vapour sorption to test the impact of this method of fines removal on the carrier material's crystallinity. The particle size, morphology and surface area were investigated using laser diffraction, scanning electron microscopy (SEM) and gas adsorption (BET). Finally, the influence of this smoothness increasing process on the in vitro deposition was examined.

17 Results and discussion

17.1 Determination of the extent of crystallinity

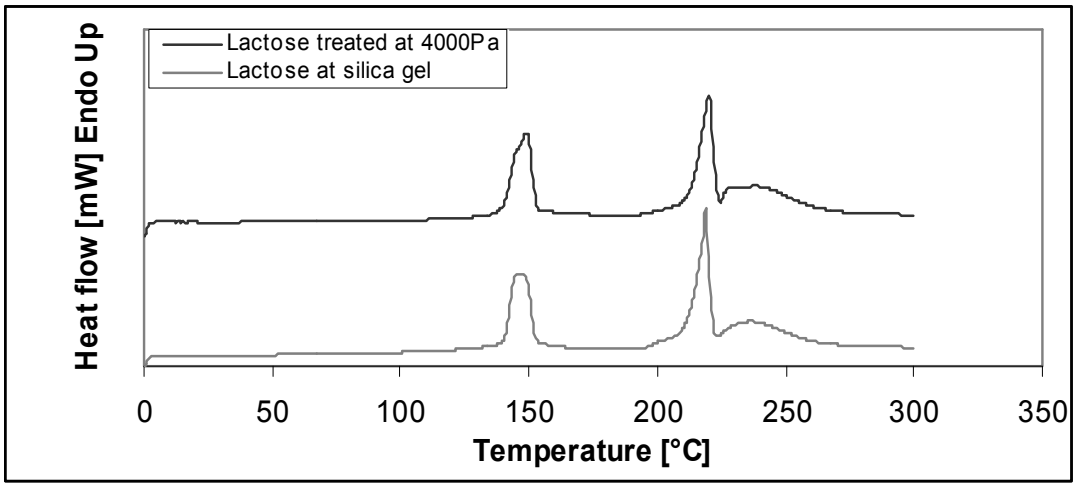
17.1.1 Differential scanning calorimetry

DSC analysis was performed on the untreated and air jet treated lactose and mannitol carrier materials in order to assess possible relevant modifications of crystallinity. Figure 53 and Table X show no significant differences comparing the samples before and after the compressed air treatment. The samples show negligible differences in terms of molar heat of fusion and onset as well

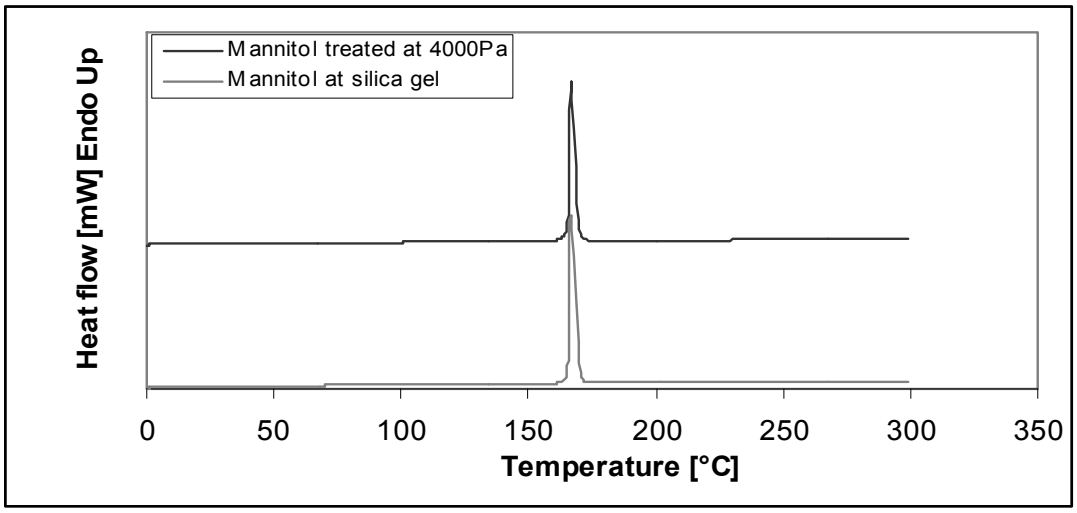
as peak temperature. This suggests that no considerable change of crystallinity has taken place after the carrier fines removal process by compressed air treatment. Additionally, the detection limits for amorphous contents with such technique have a lower cut off of 5-10%. This detection limit is due to the fact that this technique measures the entire sample (Buckton et al., 1999).

Table X: DSC parameters of lactose and mannitol carriers before and after compressed air treatment (n=2), mean ± range (the range indicates the maximum and the minimum values).

SAMPLE NAME	ENTHALPY [J/G]	ONSET [°C]	PEAK [°C]
Lactose before treatment	152.93±2.19	141.26±0.34	147.52±0.08
Lactose after air jet treatment	147.80±3.71	140.40±0.67	148.27±0.58
Mannitol before treatment	293.92±0.55	165.98±0.12	166.66±0.13
Mannitol after air jet treatment	289.25±0.17	165.65±0.07	166.58±0.07



a

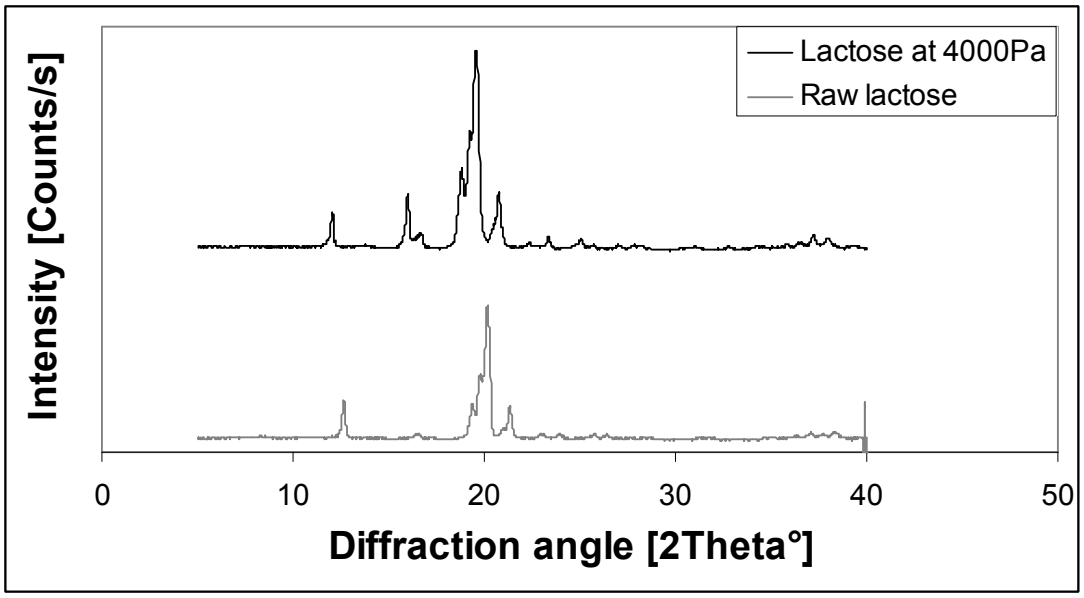


b

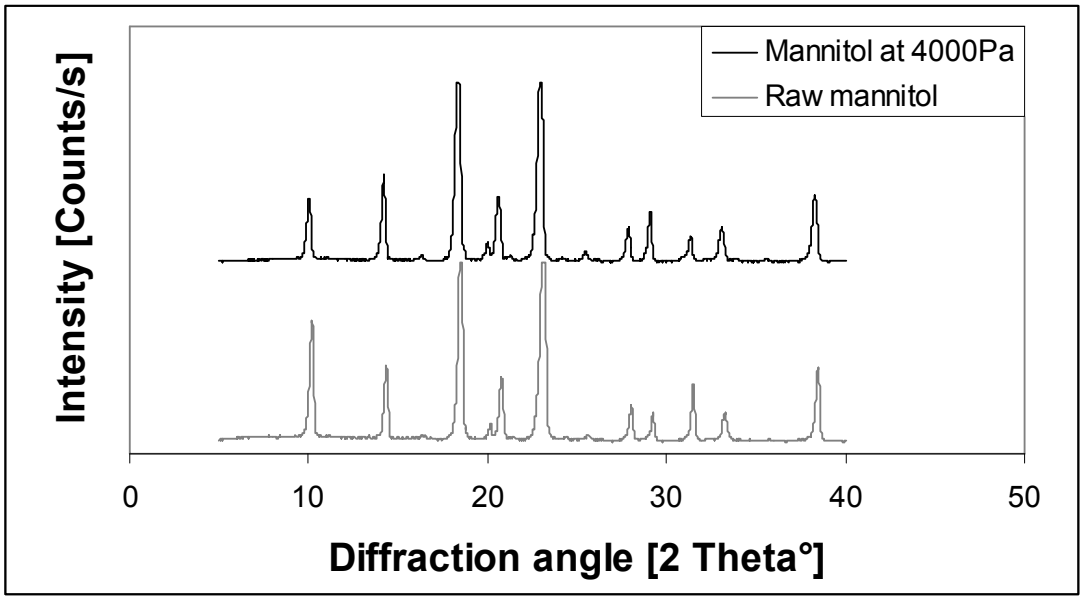
Figure 53: DSC traces of lactose (**a**) and mannitol (**b**) before and after treatment with compressed air, the second of two measurements is shown.

17.1.2 X-ray powder diffraction

In fact, the comparison between the X-ray spectra before and after the air jet treatment (Figure 54) revealed that the positions of the peaks were not changed. Although, the intensities of some peaks are changed in the treated lactose and mannitol carriers which might be due to the removal of carrier surface fines. This test indicates no changes of crystallinity after subjecting the carrier materials to compressed air treatment, although the compressed air treatment was expected to introduce amorphous parts by attrition. Those results come in agreement with the DSC studies, where the crystallinity of lactose and mannitol carriers and the absence of amorphous parts after removal of the carrier surface fines were shown**.**



a



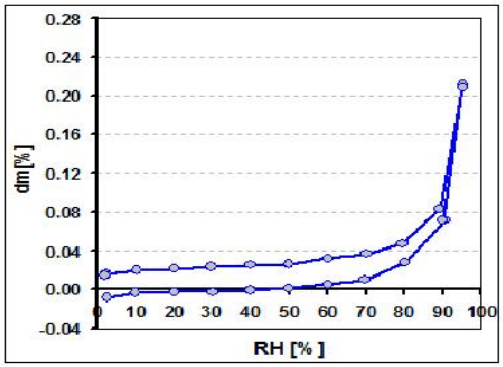
b

Figure 54: XRD diffractograms of lactose (**a**) and mannitol (**b**) before and after treatment with compressed air, the second of two measurements is shown.

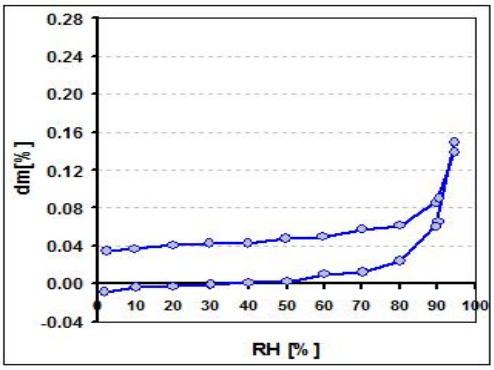
17.1.3 Water vapour sorption

Comparisons of water vapour sorption isotherms of lactose and mannitol carrier materials before and after compressed air treatment show a decrease in the final water uptake at 95%RH. Figure 55 shows that the carrier materials subjected to compressed air treatment exhibit reduced mass after one cycle of water sorption, which could be attributed to the removal of carrier surface

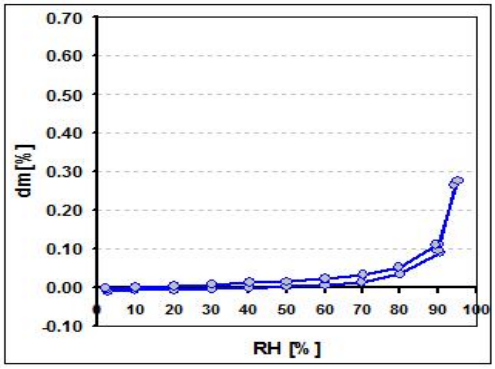
fines by air jet technique. Those results are in agreement with the wet decantation process results obtained before, where they also show a decrease of water uptake after subjecting both carrier materials to the frequent wet decantation. The water vapour sorption isotherms showed no change in the crystallinity of lactose and mannitol carrier materials after subjecting both of them to the air jet sieving treatment, whereas they only showed a decrease in water uptake due to the carrier surface fines removal.



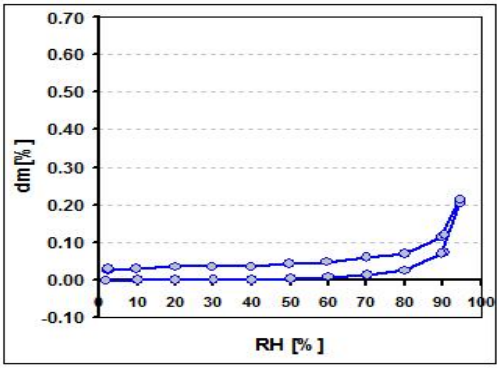
Lactose before treatment



Lactose after treatment at 4000Pa



Mannitol before treatment



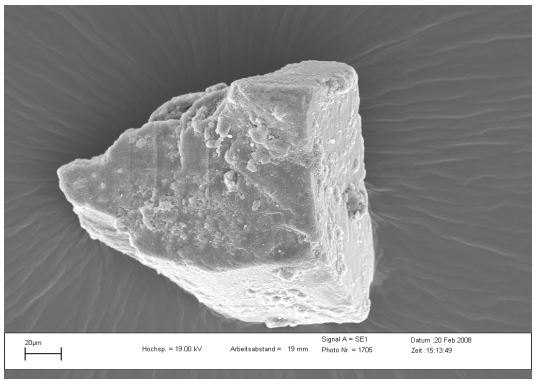
Mannitol after treatment at 4000Pa

Figure 55: Water vapour sorption isotherms of lactose and mannitol before and after treatment with compressed air.

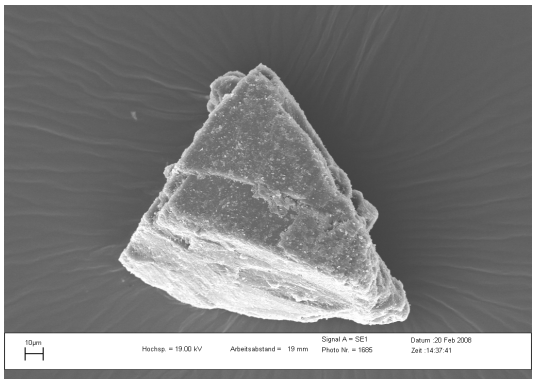
17.2 Determination of the particle shape and the surface characteristics by scanning electron microscopy

Treatment of lactose and mannitol carrier materials with compressed air seems to result in a reduction of the surface fines of both carrier particles (Figure 56). The carriers after compressed air treatment show clearly fewer fine particles adhering to the larger carrier particles in comparison to the

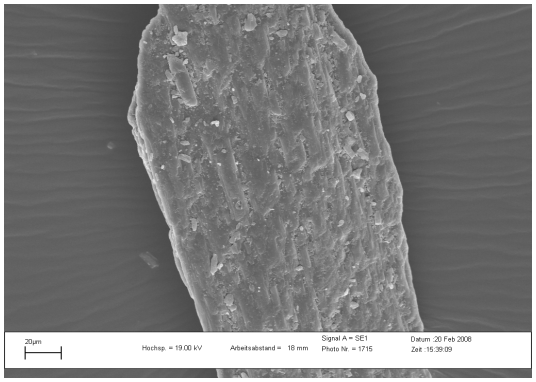
carriers before treatment. The inherent lactose and mannitol surface fines may have been raised due to wear (e.g. mixing or transport). However, according to the photographs of Figure 56, complete removal of fines was not attained by this method of treatment, which proves that the energy input was not sufficient to remove strongly adhered fine particles.



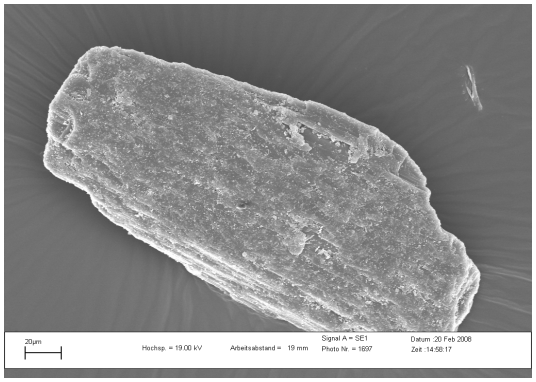
Lactose before treatment



Lactose at 4000Pa



Mannitol before treatment



Mannitol at 4000Pa

Figure 56: Scanning electron micrographs of lactose and mannitol before and after treatment with compressed air.

17.3 Determination of surface area by BET measurement

The air jet treatment of lactose and mannitol carrier particles results in the separation of the weakly adhered carrier fines from the coarse carrier particles and subsequently decreased specific surface area of the treated carrier in comparison to the same carrier before treatment (Figure 57). Those results come in agreement with those results obtained by water vapour sorption analysis, where the decrease in water uptake of the treated lactose and

mannitol carrier was due to the carrier surface fines removal which was proven by the BET results.

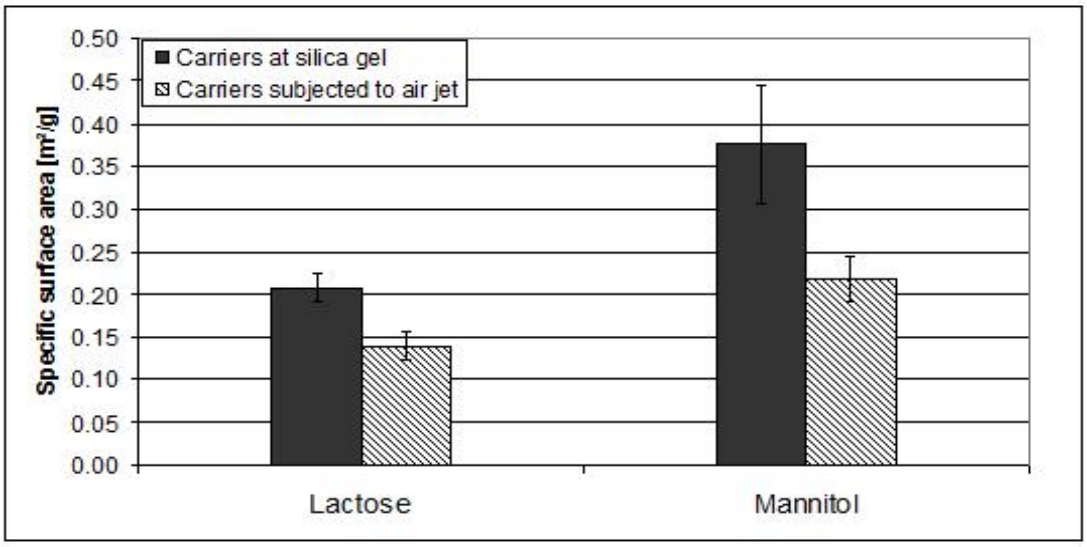


Figure 57: Specific surface area of lactose and mannitol before and after compressed air treatment (n=3, mean± SD).

17.4 In vitro deposition test

The aerodynamic behaviour of lactose and mannitol carrier materials was estimated with the Next Generation Impactor (NGI) to study the in vitro deposition profile of salbutamol sulphate carrier based formulations containing the untreated and air jet treated carriers. A summary of the data is presented in Figure 58. The use of compressed air treatment of carrier particles by air-jet sieving shows a significant decrease of the fine particle fraction compared to the untreated carriers as shown in Figure 58. Accordingly the emitted dose was also reduced from 70-80% of recovered dose emitted from the formulations containing raw untreated carriers to 50-60% of recovered dose being emitted from the formulation containing the treated carriers by air-jet sieving. Air-jet sieving or air washing might lead to triboelectric charging of the formulations, which has been shown to decrease DPI performance.

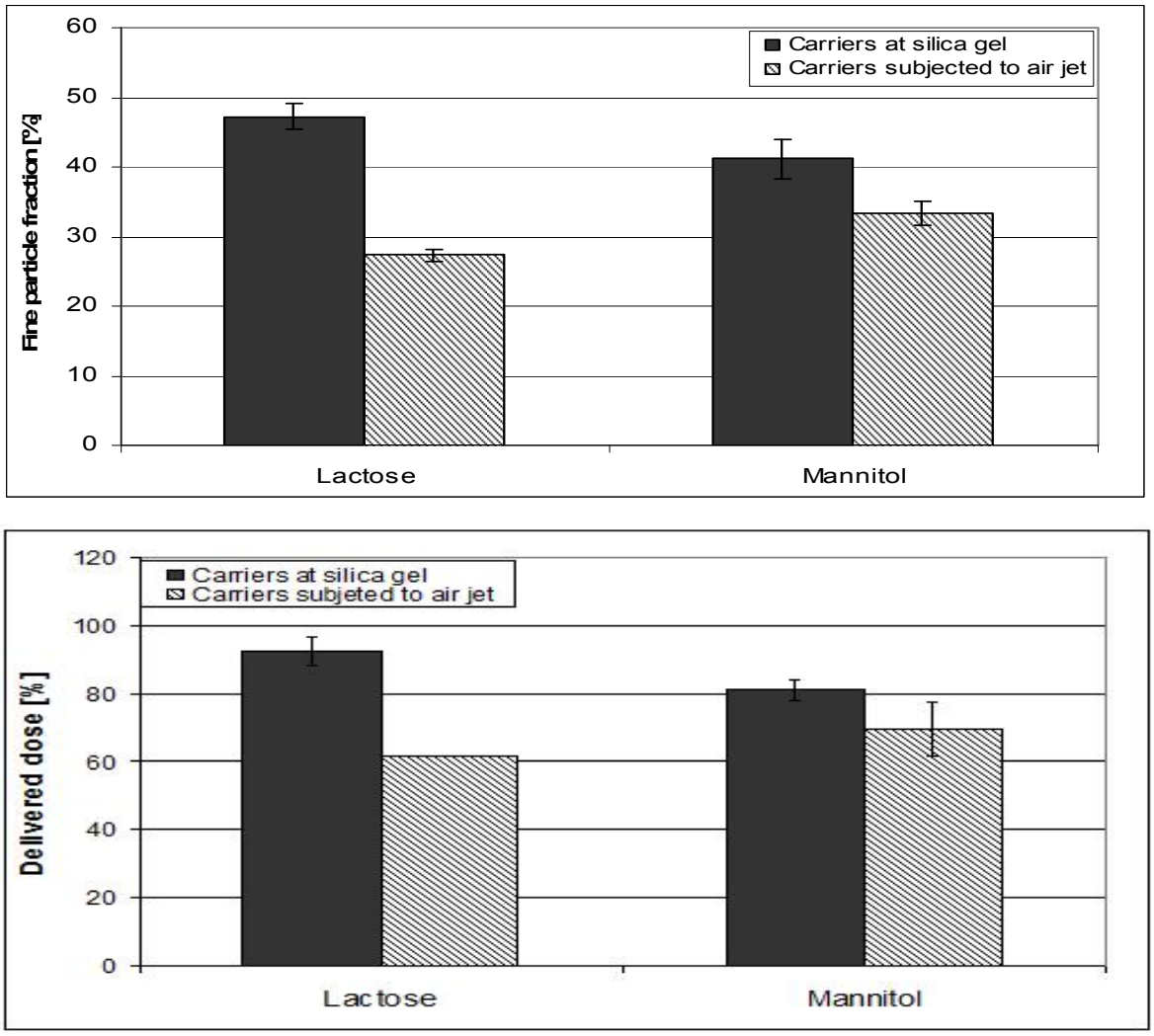


Figure 58: Fine particle fraction and delivered dose of carriers before and after compressed air treatment (n=3, mean ± SD).

18 Conclusion

This study provides an approach to remove the inherent carrier fines from the carrier surface. This fines removal leads to subsequent reduced fine particle fraction which comes in agreement with the results obtained from wet decantation studies mentioned previously. This decrease of the FPF can be explained by two possible mechanisms. The first one is that the removal of fine particles leads to an increase of free high energetic spots at the carrier surface which can be occupied with the added drug. This occupation results in higher adhesion forces between drug and carrier particle and reduced fine particle fraction. The second mechanism is that the removal of carrier fines from the carrier surface leads to an increase in surface smoothness by

decreasing the micro-roughness caused by the carrier fines, which possibly results in the formation of stronger adhesion forces due to the increase of the contact area between this smoothed carrier surface and the drug particle. Those strong adhesion forces result in difficulties of drug particle detachment from the carrier particles during the inhalation process and subsequently in a low FPF. Those results are in agreement with the results obtained after applying the wet decantation process, whereas the both mechanisms aim to remove carrier surface fines and resulted in a reduction of the FPF. The drawback of this method was the resultant highly electrostatic particles, which need a careful processing. Mannitol as a new substitutive carrier also shows a good response in removing the surface fines by the air jet technique.

F SUMMARY

Dry powder formulations consist mainly of fine drug particles mixed with inert coarse carrier particles to aid the flow and dispersion of the fine drug particles. A number of different carriers have been used although lactose has been employed most frequently, because it is the most available safe excipient. This study introduces mannitol as a new alternative to lactose in order to avoid the lactose drawbacks. Carrier particles used in this study have a 112μm-140μm size range. The fine drug particles which consist of salbutamol sulphate were obtained by micronization using air jet milling in the current study.

As micronized salbutamol sulphate might be affected by the milling process which may result in a crystal dislocation and high energetic surfaces, this study investigated the changes of the crystallinity of salbutamol sulphate particles. The drug particles were conditioned via storage at elevated relative humidities at room temperature for different time periods. The crystallinity of the conditioned drug particles was investigated using differential scanning calorimetry, X-ray diffraction and water vapour sorption. Additionally particle size and shape were also investigated using laser diffraction and scanning electron microscopy. The results indicated that the relative humidity as well as storage time has to be carefully controlled in order to obtain thermodynamically stable fine drug particles. Additionally, the micronized salbutamol sulphate must be conditioned minimally for two weeks at 52.8%RH to get stable crystallized salbutamol sulphate.

The performance of dry powder formulations might be affected by the geometric and physicochemical properties of carrier particles, which include the carrier particle size, shape, electrical charge, surface texture, crystallinity etc. In order to modify the surface properties of the raw carrier materials, different powder compositions were formulated with the aim of studying the influence of the carrier surface on the in vitro deposition of the powder formulation.

In this study lactose and mannitol carrier materials were stored at elevated relative humidities for 6 weeks and investigated with respect to the lactose and mannitol carriers which were not stored at elevated relative humidities. The results showed a decrease in the in vitro deposition due to the storage of the carrier materials at higher relative humidities up to 95%, which allowed the condensation of water vapour on the carrier surfaces and formation of solid bridges between carrier fines and the coarse carrier particles. This bridge formation results in an increasing of the carrier surface smoothness, which usually increases the adhesion forces between the carrier and drug particles as a result of the increased contact area. Subsequently, this results in a less dispersion of drug particles from the carrier materials upon inhalation.

Another approach used to modify the surface properties of raw carriers was to remove the carrier fine particles with a novel wet decantation process. The carriers were subjected to successive washing processes with absolute ethanol and lastly with dichloromethane to prevent the solid bridge formation between the carrier particles. As a result the decanted carriers showed a reduced FPF in comparison with the raw carriers themselves, which was authorized to the increased carrier surface smoothness due to a decrease in the carrier surface micro-roughness. This increased carrier surface smoothness offers a higher contact area for the drug particles and stronger adhesion to the carrier material which subsequently reduces the drug particle detachment upon aerosolization and decreases the FPF. Additionally, other mechanisms can be encountered to explain the reduced FPF for example the removal of fine particles leads to an increase of free high energetic spots at the carrier surface which can be occupied with the added drug. This occupation results in higher adhesion forces between drug and carrier particle and reduced fine particle fraction.

Carrier fines were added back to the cleaned coarse carrier (wet decanted carrier), which improves the FPF of the drug to a certain limit. Moreover, these results seem to be influenced by the amount of carrier fines added to the powder formulation. The improved FPF of salbutamol sulphate with increased concentration of added fines may be caused by the saturation of the active

sites on the carrier surface and a reduction of the adhesion forces between drug and carrier particles. Although the addition of fines to the decanted carriers could not revert the fine particle fraction to the original value of the raw carriers. This may indicate that the added fines were not equal to the original fines which had been removed by wet decantation. Added carrier fines exhibit another size, shape, surface, energetic charge, etc. than the inherent fines.

Another interesting approach was to increase the carrier roughness via addition of carrier fines to the raw lactose and mannitol carrier materials. This increased roughness may offer weaker adhesion forces with drug particles and subsequently enhanced in vitro deposition. This improvement in FPF could be attained to a certain limit of the added carrier fines and then any further increase of the amount of added carrier fines was accompanied by a decrease of the FPF. The mechanisms by which this decrease is driven, however, is controversially discussed in literature and include the hypothesis of mixed agglomerates formation, which possess strong adhesion forces between drug and fines resisting the break up in the air stream and autoadhesion layer structure formation, where the frictional drug removal is less likely, because the drug particles are embedded in the macro-waviness of the autoadhesion layer.

In order to confirm the results obtained after smoothing the carrier surface materials by wet decantation, the lactose and mannitol carriers were subjected to compressed air treatment using the air jet sieving process. The use of compressed air was shown to produce a cleaner carrier surface when compared to that before treatment. Striping of carrier fines from the coarse carrier with compressed air produced more adhesion sites for drug particles. This would be expected to increase the overall particulate interaction between the drug and coarse carrier particles, which would in turn reduce the FPF of the drug. This reduction in the FPF reflects the validity of the wet decantation process used in this study.

The results of this study clarify the role of the surface carrier fines and indicated that the increasing surface smoothness and/or roughness must be

balanced to get an improved in vitro deposition from the dry powder formulations. However, the added or removed carrier fines must not affect adversely the carrier physicochemical properties.

G EXPERIMENTAL DETAILS

1 Materials

Mannitol was kindly supplied by Roquette Freres (Lestrem, France) as Pearlitol160C, Pearlitol25C, Pearlitol100SD and Pearlitol200SD, Pearlitol160C was chosen to be used as a new carrier in comparison to lactose. Lactose was used as InhaLac120 (α-lactose monohydrate) supplied kindly by Meggle GmbH (Wasserburg, Germany). Salbutamol sulphate was supplied kindly by Lindopharm (Hilden, Germany). Absolute ethanol, methanol and acetonitril (HPLC degree) were purchased from VWR international GmbH, Darmstadt, Germany. Dichloromethane was purchased from KMF, Laborchemie Handels GmbH, Lohmar, Germany. Acetic acid was purchased from Mallinckrodt Baker B. V., Deventer, Holland.

2 Methods

2.1 Particle size distribution

2.1.1 Analytical sieving

The particle size distribution was measured with sieve analysis according to DIN 66165 (Deutsches Institut for Normung, 1987a, b). The particle size distribution parameters were determined from the distribution curves using the sieve shaker machine AS 200 control (Retsch GmbH&Co. KG, Haan, Germany). The sieves 0.045µm, 0.060µm, 0.080µm, 0.112µm, 0.125µm, 0.140µm, 0.180µm, 0.200µm and 0.315µm were used. Each sieve was weighed before the test and then assembled with the other sieves in an ascending degree of coarseness. The sample size was 30g and the nest of sieves was agitated for 20 minutes with an amplitude of 1.5mm. Then each sieve was carefully removed from the nest without loss of the material, and weighed to determine the mass of the material on each sieve. The experiment was carried out in triplicate (n = 3).

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2.1.2 Laser diffractometry

Laser diffraction analysis relies on the fact, that particles scatter light at angles in inverse proportion to their size. The particle size distributions of the micronized drug and the excipients were determined with a Sympatec HELOS laser diffraction spectrometer equipped with a RODOS dry powder dispersing system (Helos H1402/Kf-magic and dry dispenser Rodos, Sympatec GmbH, Clausthal-Zellerfeld, Germany). The powder samples were fed to the dispersing air stream using a funnel connected to the injector of the dry disperser. Depending on the pressure and speed of the passing air stream, the dispersing force could be adjusted. The carrier and active ingredient powders were dispersed by compressed air at 0.3bar - 4.0bar. The pressure at which only agglomerates are dispersed without destroying single particles was evaluated to be 2.5bar. The size distributions of the samples were obtained using the dispersion pressure at 2.5bar. All calculations were made with the Fraunhofer theory. All data given represent the average values of at least three determinations at dispersing pressure of 2.5bar. Volume median diameter and span (50% undersize and 90% undersize - 10% undersize) were calculated. The span is a measure of the distribution of the particle size, where, D90% is the diameter for which 90% of the sample is smaller, D50% is the diameter for which 50% of the sample is smaller, i.e. the median diameter. D10% is the diameter for which 10% of the sample is smaller.

2.2 Preparation of coarse carrier

The sieved fractions (112µm-140µm) of lactose (InhaLac120) and mannitol (Pearlitol 160C) were obtained by sieving the sugar particles sequentially through test sieves with an aperture width of 112µm and 140µm, using a sieve shaker (Retsch, Haan, Germany). The sieved carriers were placed over silica gel in a desiccator until further required.

2.3 Micronization of salbutamol sulphate

Salbutamol sulphate particle was milled by using an air jet mill (50AS, Hosokawa Alpine AG, Augsburg, Germany) injection pressure was set to 3bar, milling pressure to 2bar and feeding rate was adjusted to approximately

1g/min, to prevent a variable particle size distribution, a uniform feed rate was maintained, by using mechanical controller feed part. Micronized salbutamol sulphate was placed over silica gel in desiccators until further required.

2.4 Preparation of fine carriers

Micronized mannitol (MM) was prepared by passing the coarse mannitol (Pearlitol 160C) through the air jet mill (50AS, Hosokawa Alpine AG, Augsburg, Germany). The injection pressure was set to 4.5bar, the milling pressure to 4bar and the feeding rate was adjusted to approximately 1g/min. The micronized lactose (ML) was obtained after 13 passes of lactose carrier (InhaLac120) through the air jet mill, the injection pressure was set to 5bar and the milling pressure to 4bar and feeding rate was adjusted to approximately 1g/min. Micronized carriers were kept over silica gel in desiccators until further required.

2.5 Particle morphology

The particle morphology of lactose, mannitol and salbutamol sulphate was examined by using scanning electron microscopy (LEO VP 1430, LEO Electron Microscopy Ltd, Cambridge, England), operated by using an electron beam at the acceleration voltage of 19kV and a working distance of approximately 18mm. Samples (≈0.5mg) were mounted via a graphite tape to an aluminum stub. After stripping off the upper side of the adhesive, a small amount of particles was scattered on the stub and dispersed by tapping lightly on the edge of the stub with a spatula to break agglomerates or by using an air stream. The particles were then coated with ~15nm - 20nm of gold with an Agar manual sputter coater (Agar Scientific Ltd., Stansted, Essex, England), using an electrical potential of 1.5kV and the current of 20mA. Photomicrographs were taken randomly of several different areas of the powder on each stub. Representative areas were photographed with different magnification power.

2.6 Particle crystallinity

2.6.1 Determination of the extent of crystallinity by differential scanning calorimetry

Thermal stability and changes in crystallinity were investigated using a differential scanning calorimeter (DSC30, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) calibrated with indium. A small amount (≈ 3mg) of carrier or drug was crimp-sealed in an aluminium pan with pierced lid. The pan was then placed in the sample chamber and an empty matched aluminium pan was used as the reference for all measurements. The experiments were performed in the range from 0°C-300°C under nitrogen flow of 50ml/min. The scanning rate was adjusted to 10°C/min. The onset and/or peak temperatures and heat of enthalpy (ΔH) for each peak were determined from the normalized DSC thermogram. Each experiment was carried out twice. The second of the two determinations is shown in the figures of this thesis unless otherwise stated.

2.6.2 Determination of the extent of crystallinity by X-ray diffractometry

Powder X-ray diffraction patterns of samples were obtained using the Miniflex powder diffractometer (Rigaku corporation, Tokyo, Japan) with a Cu-Kα-radiation (λ = 1.5406 Å) as the source of radiation. This diffractometer was operated at the voltage of 30 kV and the current of 10mA. Each sample was placed in the cavity of an aluminium sample holder flattened with a glass slide to present a good surface texture and inserted into the sample holder. In order to measure the powder pattern, the sample holder and detector were moved in a circular path to determine the angles of scattered radiation and to reduce preferred sample orientation. All samples were measured in the 2θ angle range between 5° and 40° with the scan rate of 0.02° for 2s and a step size of 0.02°. All samples were analyzed in triplicate. The diffractometer was operated at room temperature and humidity. The second of the three samples is shown in the figures of this thesis unless otherwise stated.

2.6.3 Water vapour sorption

Plots of water, either adsorbed or desorbed, as a function of relative humidity at constant temperature, are commonly known as sorption isotherms. The sorption/desorption profiles were determined gravimetrically. The gravimetric studies were undertaken in a humidity controlled microbalance system (Projekt Messtechnik, Ulm, Germany). The construction of the water vapour sorption system SPS11 is based on a microbalance capable of measuring changes in sample mass lower than 1 part per million. The system is housed in an incubator to control temperature and surrounding humidity. The apparatus is computer controlled, allowing a pre-programming of sorption and desorption isotherms. Approximately 3gm of samples were loaded; the relative humidity was first set to 0%, and then raised in 9 steps of 10% to 90% and one step more to 95%. Subsequently the relative humidity was decreased from 95% to 90% then to 0% by the same way. This cycle was repeated once more. The equilibrium condition was set to 0.01% mass change per 60 minutes, which had to be reached before the program moved to the next humidity step. The temperature was set to 25°C. Samples were weighed in time intervals of 6 minutes, the whole measurement was run for only one time due to the time consuming of this type of experiments.

2.7 Surface area measurement

The surface area was measured by nitrogen adsorption for carrier and drug particles. Prior to surface area measurement, known masses of the samples were accurately weighed into sample tubes and out gassed for 24h at 40°C and vacuum for mannitol and at 50°C and N_2 current for lactose to remove any adsorbed gases from the surfaces of the particles. This difference of preparation temperatures between the two carriers was due to the sensitivity of each carrier and depending on preliminary experiments before. After outgassing, the sample tubes were connected to a surface area apparatus connected to a computer. The carrier specific surface area was obtained by BET nitrogen adsorption measurement from a Micromeritics Tristar 3000 (Micromeritics GmbH, Moenchengladbach, Germany). Each sample was measured in triplicate and the mean with the standard deviation was

calculated. The specific surface area was calculated according to DIN 66131 [31] (Deutsches Institut for Normung, 1973).

2.8 Determination of surface fines

The percentage of the fine particles present at the lactose and mannitol carrier surfaces (112µm-140µm), and originating from the carrier itself was calculated and expressed as percentage of carrier fines. 30g of the carrier were subjected to an air pressure of 1500Pa, 2000Pa, 2500Pa, 3000Pa, 3500Pa and 4000Pa, at 5, 10, 15, 25, 45, 60, 120, 180, 240 and 300 seconds using the 63µm sieve of the Haver test sieves (A200, Alpine AG, Augsburg, Germany). The sample was weighed after finishing the sieving process and the percentage of fine particles was calculated as follows:

Percentage of carrier fines = [(mass of the sample before sieving - mass of the sample after sieving)/mass of the sample before sieving)] × 100

Equation 13

2.9 Preparation of interactive mixtures

Blends of salbutamol sulphate with coarse lactose or coarse mannitol (112µm-140µm) were prepared in a ratio of 1:99 w/w in 8g batch in a stainless steel mixing container using a Tumbling mixer (Turbula T2C, WA Bachofen AG, Switzerland) for 90min, 42 rotations per minute . Micronized salbutamol sulphate was blended with lactose or mannitol in a sandwich method to prepare a binary mixture. Briefly, an amount of a carrier, equivalent to about half the total mass of the carrier was used to ‘sandwich’ the drug in the blend. i.e. the half part of the carrier material was put firstly in the mixing container followed by the drug followed by the other half of the carrier material. To minimize the effects of tribocharge, a stainless steel container was used, of 5.2cm diameter and 3.2cm height. The sample was then stored in vacuum desiccators over silica gel minimum at least 24 hours to allow electrostatic charge decay.

For ternary mixture preparation, the three components of the formulation were blended using the sequence of addition reported by Zeng et al., 1998, and Louey et al., 2002. The micronized carriers were sandwiched with the coarse smoothed and raw carriers and mixed at first 45 minutes, and then the micronized salbutamol sulphate was added and mixed for another 45 minutes.

2.10 Content uniformity test

The blend uniformity was determined by taking 12 samples with a special sample taker which were individually weighed. Each dose was dissolved in 50ml acetate buffer of pH3 in a graduated cylinder and the amount of salbutamol sulphate in each sample was analyzed using high performance liquid chromatography (HPLC) at the wavelength of 276nm. The formulations used throughout this study showed an acceptable degree of homogeneity with the mean drug content across all blends being within 100.0% $\pm$ 5.0% of the theoretical value and each blend exhibiting a coefficient of variation (CV) below 5% (n = 3). This suggests that the overall process of mixing, sampling and analyzing is accurate and reproducible.

2.11 In vitro deposition determined using the Next Generation Impactor (NGI)

The aerodynamic particle size distribution of salbutamol sulphate was carried out using the Next Generation Impactor (Copley Scientific, Nottingham, UK). Methodology followed that of the British Pharmacopoeia (2007, 5.8). The NGI was connected to a vacuum pump with adjustable flow rate between 60 and 100 l/min. The flow manoeuver through the inhaler was controlled with a previously adjusted corresponding pressure drop across the device with a flow controller (Copley, Nottingham, UK). The inspiration time was controlled with a time controlled solenoid valve and was set to 3 seconds. The adhesive mixtures were filled into Novolizer® cartridges (Viatris GmbH & Co. KG, Frankfurt, Germany) and dosing was performed with the built-in metering system. The aerosolization was done at 79.3 L/min.

The impactor plates were coated with a viscous solution of emulgator (Brij 58-0.25%) in glycerol anhydride (4.75%) and isopropanol HPLC degree

(95.00%). A specified volume (2ml for stages 2-7 and 4ml for stage 1 and the micro-orifice collector) of this solution was distributed on each collection plate to provide a film. The plates were left to dry under ambient room conditions for at least 2 hours prior to each analysis. The NGI plates were covered with this viscous solution to eliminate the bouncing of the powder particles off of the plates, which can give incorrect size distributions. Prior to each measurement, the temperature and relative humidity of the surrounding environment was measured using a thermo-hygrometer, in order to measure the influence of surrounding environment on the FPF produced.

The impactor was assembled and a Novolizer® was then fitted into the moulded rubber mouthpiece attached to the throat of the impinger. The TPK Copley pump (Copley Scientific, Nottingham, UK) which was connected to the outlet of the apparatus was switched on and allowed to run for 3 seconds prior to the release of the dose. The pump was then allowed to run for another 3 seconds at 79.3L/min. This process was repeated 49 times.

The impactor was dismantled and the individual plates as well as the micro orifice collector (MOC) were carefully washed with acetate buffer pH3. The inhaler mouthpiece, the throat and preseparator were washed into volumetric flasks of 100ml, and the washing solution was made up to a set volume with the same solvent (acetate buffer, pH3). The concentration of salbutamol sulphate in each of the samples was analyzed by high performance liquid chromatography (HPLC). The particles of less than 5μm were expected to be deposited in the lung after inhalation, which used to describe the inhalation properties of DPIs. The fine particle fraction (FPF) is calculated as the dose of drug exhibiting an aerodynamic diameter <5μm by addition of the doses of drug on stages 3-7 plus the part of drug <5μm on stage 2 obtained by interpolation at 79.3l/min. Experiments were run in triplicate, each time on new mixture of salbutamol sulphate with the carrier at the same proportion (3 batches of each formulation (8 g) were prepared).

2.12 HPLC analysis of salbutamol sulphate

Salbutamol sulphate was analyzed by high performance liquid chromatography (HPLC) employing a mixture of 50% acetonitril and 50% acetate buffer (2.5 gm glacial acetic acid (100%) in 1000 ml distilled water). The pH was adjusted to pH 3.0. The flow rate of 0.52 ml/min was used. The HPLC system consisted of a pump (LC 6A Shimdazu, D-Duisburg), a multiple wavelength detector (SPD-6AV, Shimdazu) operating at 276nm. The system was equipped with an auto sampler (SIL-6B, Shmidazu) and a 15cm x 4.6mm internal diameter reversed phase column packed with 5µm C-18 Nucleosil HD RP 18 MN 250/4 (Machery u. Nagel, Dueren, Germany). The retention times for salbutamol sulphate and the standards were 2.71min and 5.49min, respectively. Standard solutions were made to contain drug concentrations between 0.100µg/50ml-0.700µg/50ml. These standard solutions were employed to construct a calibration curve of peak height against drug concentration. The calibration was prepared on a daily basis, and a calibration curve with r^2>0.99 was considered acceptable. The injection volume was 10µl. Each sample was measured in duplicate. The content of salbutamol sulphate from the impactor experiment of each cup and the preseparator was calculated from the peak heights of the chromatograms. The total amount of the delivered drug (or mass balance) was also calculated, the amount of salbutamol sulphate was calculated in µg/dose and as the percentage of the total amount of drug delivered.

2.13 Statistical tests

The in vitro deposition data were examined for statistically significant differences by the ANOVA single variance test. A P-value of <0.05 was considered significant.

2.14 Carrier surface modifications

2.14.1 Preparation of carriers with reduced fines by storage at different relative humidities

This step of this research work started with storage of the sieve fraction 112µm-140µm of lactose (InhaLac120) and mannitol (Pearlitol 160C) carriers at different relative humidities for different periods of time in order to get smoother surfaces. Saturated salt solutions were used to maintain constant relative humidity levels inside small desiccators during the storage of carrier particles. The desiccators were incubated at 25°C. The following series of saturated salt solutions were used: magnesium chloride (35%), magnesium nitrate (55%), sodium chloride (75%) and potassium nitrate (95%). Relative humidity was measured within the desiccator using a thermo-hygrometer (Testo, Lenzkirch, Germany) with relative variability being <3%.The carrier powders were placed in Petri dishes over these different relative humidities for 6 weeks, then stored over silica gel before the preparation of interactive mixtures.

2.14.2 Preparation of carriers with reduced fines by wet decantation

The sieve fraction 112µm-140µm of mannitol (Pearlitol 160C) was treated with absolute ethanol to remove impurities and adhering fines from the particle surfaces and to reduce surface irregularities in order to produce carrier crystals with an increased surface smoothness. 60g of the fraction was washed with absolute ethanol to remove the fine particles. The mixture was stirred to obtain a homogenous suspension and allowed then to settle for 10 minutes at ambient conditions. The cloudy supernatant fluid was decanted and replaced by 60ml dichloromethane (CH_2Cl_2). Again the mixture was allowed to settle for 10 minutes at ambient conditions; the cloudy supernatant layer was out excluded. During the supernatant removal, special care was taken to ensure minimum disturbance of the lower part of suspension. The powder sample was left for 2 to 4 days under the fumed hood to dry, and sieved through the 112µm and the 140µm sieve. The 112µm-140µm fraction was recovered. The obtained powder was stored over silica gel before the

preparation of interactive mixtures. The same procedure was applied to lactose (InhaLac120) carrier particles.

2.14.3 Preparation of ternary mixtures

The decanted carrier particles of the sieve fraction 112µm-140µm of lactose (InhaLac120) and mannitol (Pearlitol 160C) were mixed with 2%, 4%, 8% of micronized lactose and 4%, 8%, and 16% of micronized mannitol in a tumbling mixer for 45 minutes respectively. The same carrier fines amounts were added to the untreated sieve fraction 112µm-140µm of lactose (InhaLac120) and mannitol (Pearlitol 160C). The prepared binary carriers were subsequently mixed with salbutamol sulphate for further 45 minutes in a sandwiching manner. The prepared ternary mixtures were kept over silica gel for at least 24 hours to allow for electrostatic charge decay.

2.14.4 Preparation of carriers with reduced fines by air jet treatment

The sieve fraction 112µm-140µm of lactose (InhaLac120) and mannitol (Pearlitol 160C) was divided into two portions. One portion was saved in an open glass container over silica gel in a desiccator until further needed. The other portion was placed on a 63µm air jet sieve. The air stream was blown from down side to remove the carrier fines at 4000Pa pressure for 15, 30, 60, 120 seconds respectively (i.e. the powder sample was subjected for 225 s to air jet treatment). The sample was then saved in a desiccator over silica gel for at least 24 hours before use to get rid of the electrostatic charge that may have raised during the air jet treatment.

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