Adhesive mixtures for inhalation

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Adhesive mixtures for inhalation

The cohesion between formulation variables,
inhalation variables and dispersion performance

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Cover: Coherent anti-Stokes Raman scattering (CARS) microscopy images of a lactose carrier particle with adhering budesonide drug particles (front) and a lactose carrier particle as we like to see it after dispersion: free of drug particles (back). Imaging was performed by Andrew L. Fussell from the Optical Sciences Group of the University of Twente.

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Chapter 1

Introduction

Adhesive mixtures for inhalation

Scientists often find themselves in the situation where answering a question or solving a challenge raises a new one. A striking example can be found in the formulation of therapeutic powders for the local treatment of respiratory diseases like asthma or chronic bronchitis and emphysema (chronic obstructive pulmonary disease, COPD). Being one of the wonders of evolution, the respiratory tract is shaped in such a way that under normal breathing conditions the vast majority of dust and other particles is either 'filtered out' of the inhaled airstream by the oropharynx, or, if they happen to pass this barrier, exhaled before deposition onto the mucosal lining fluid. As a result, the targeted delivery of therapeutic powders with the inhaled airstream involves the challenge of bypassing an effective natural defence mechanism. The solution to this challenge lies in the presentation of drug particles to the inhaled airstream with such an aerodynamic size range that the forces causing transport and deposition of the particles are optimally balanced. Typically, only a very narrow aerodynamic particle size range of roughly 1-5 μm may lead to satisfactory deposition profiles, where oropharyngeal drug deposition by inertial impaction is minimised, yet deposition of drug particles by sedimentation in the lower airways occurs quickly enough to prevent their exhalation [1, 2].

For such fine drug particles other forces are balanced in a particularly unfavourable way: adhesion or cohesion forces dominate the force of gravity, which renders the particles very sticky and gives them undesired bulk properties like poor flowability [3] (Fig. 1A). This poses a challenge with respect to reproducible dose metering, especially considering the fact that low doses of only 6-500 μg are common for the treatment of mentioned diseases. The most widely applied solution to this challenge is to blend the fine drug with a lactose excipient that consists of relatively coarse 'carrier' particles, roughly in the size range of 40-150 μm. The drug particles, as a result of their stickiness, will adhere to the surfaces of the carrier particles during blending to form so-called 'adhesive units' (Fig. 1B). Because of this characteristic phenomenon, these blends are preferably referred to as 'adhesive mixtures' (rather than 'binary mixtures', 'ordered mixtures' or 'interactive mixtures') [4]. The force of gravity acting on adhesive units dominates the adhesion and cohesion forces between them, and the powder will have strongly improved flow properties as a result. The lactose excipient thus acts as a diluent (formulations mostly contain between 95 to 99.9% lactose) and as an enhancer of bulk flowability, which solves the challenge of reproducible dose metering when homogeneous mixtures are produced.

Adhesive units do not fall within the respirable size range of 1-5 μm. If drug particles do not detach from the carrier surface during inhalation they will therefore inevitably deposit in the mouth or throat region by inertial impaction. Here they do not exert any therapeutic activity, but may cause adverse reactions. Dispersion of the drug from adhesive mixtures into the inhaled airstream thus has to include the detachment of

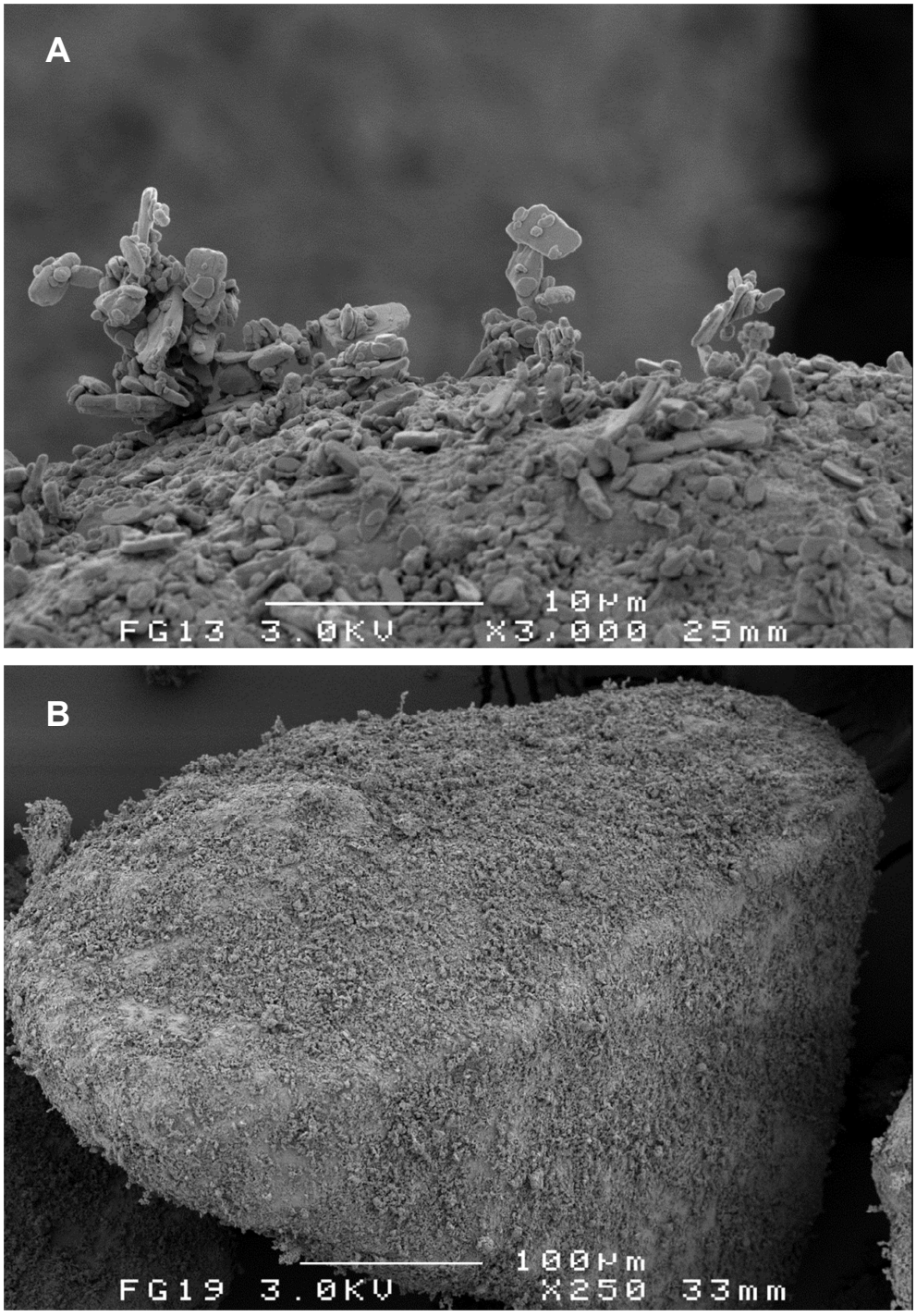

Figure 1: scanning electron microscopy (SEM) images of adhesive mixtures for inhalation. A: sticky salmeterol xinafoate particles on the surface of a lactose carrier particle. **B:** an adhesive unit of salmeterol xinafoate particles adhered to a coarse lactose carrier particle (250-315 µm sieve fraction).

drug particles from the carrier surface and the breaking up of any drug agglomerates > 5 μm. Dry powder inhalers serve the purpose of reproducible dose metering and enhancing the efficiency with which kinetic energy from the inhaled airstream is converted into forces causing dispersion of the powder formulation. However, despite their use, it is currently a great challenge to develop and produce carrier-based dry powder inhalation products that efficiently and reproducibly disperse the drug with the pressure drop generated by the patient across the inhaler as the only driving force. In addition, there is a quest for measurable particle or powder properties that can be used as predictors of dispersion performance, as this could greatly improve the efficiency and efficacy of the development and production processes themselves. Obtaining a fundamental understanding of how different formulation and dispersion variables affect the dispersion performance of adhesive mixtures will be an important step towards solving these challenges, and it is the purpose of this thesis to contribute to such an understanding.

Dispersion of adhesive mixtures

The dispersion of adhesive mixtures during inhalation may be regarded as a three-step process of consecutively fluidisation of the powder in the airstream, detachment of primary and agglomerated drug particles from the carrier surface and the break-up of detached agglomerates into primary particles or small 'multiplets'. It is common practice to express the *in vitro* dispersion performance of dry powder inhalation products as the fraction of the drug mass that is aerosolised as particles (including agglomerates) with an aerodynamic diameter below a certain cut-off value, mostly 5 μm. This fraction is referred to as the 'fine particle fraction' (FPF). Alternatively, the drug fraction that is detached from the carrier surface during inhalation can be used as a measure of dispersion performance (Fig. 2). Referring to the three-step process, it may be clear that this detached fraction is not necessarily representative of the fine particle fraction, as released agglomerates from the carrier surface may be too large to fall within the size range < 5 μm.

The degree of dispersion obtained during inhalation depends on the balance between adhesive and cohesive interaction forces in the powder on the one hand and the separation forces generated by the kinetic energy of the inhaled airstream on the other. These forces are therefore briefly discussed in the following sections.

Interaction forces

Adhesion or cohesion forces in adhesive mixtures include forces such as Van der Waals forces, capillary forces and electrostatic forces and they are all well described and

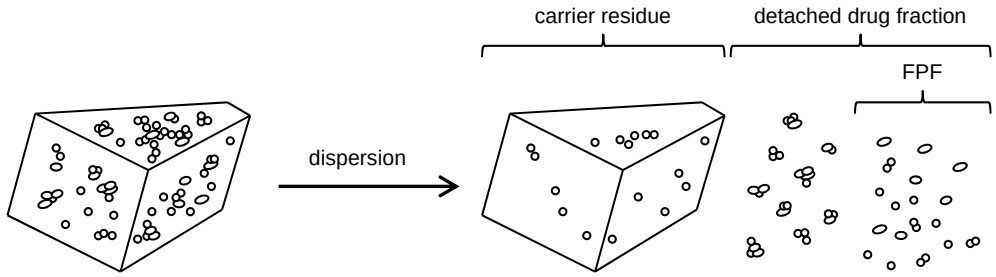

Figure 2: different measures of dispersion performance. FPF = fine particle fraction. A difference between the FPF and the detached drug fraction is caused by detachment of particles (including agglomerates) from the carrier surface that do not fall within the defined size range for the FPF. In this thesis, a classifier-based test inhaler will be used in which the carrier particles are retained during a dispersion experiment. This allows the simple determination of the carrier residue (CR), from which the detached drug fraction can be calculated (i.e. 100-CR).

understood as far as their origin is concerned. Although the relevance of the different types of interaction forces in adhesive mixtures is strongly dependent on factors such as relative humidity and the particles' chemical nature, it is generally accepted that adhesion of fine drug particles to lactose carriers or their cohesion to other drug particles is caused primarily by Van der Waals forces [5]. Basically, the magnitude of the Van der Waals force between two particles depends on their contact area, separation distance and material specific properties. A drug-carrier interaction is best represented by that of a sphere to a plane and the Van der Waals force for such an interaction may be described by [6]:

$$F_{ad} = Ad/12h^2 \qquad 1.1$$

in which F_{ad} is the adhesion force (N), A is the (material specific) Hamaker constant (usually around 10^{-19} J), d is the diameter of the drug particle (m) and h the separation distance between the particles (m). Alternatively, Johnson, Kendall and Roberts developed a model (the JKR model) from which it follows that [7]:

$$F_{ad} = 1.5\pi r W_{ad} = 1.5\pi r(\gamma_d + \gamma_c - \gamma_{dc}) \qquad 1.2$$

In which r is the drug particle radius (m), W_{ad} the work of adhesion (J/m^2) and γ_d, γ_c and γ_{dc} are the surface free energies (J/m^2) of the drug particle, carrier particle and drug-carrier interface, respectively. Other models exist, such as those by Hertz [8] and Derjaguin, Muller and Toporov (DMT, [9]), which differ in their assumption regarding the influence of Van der Waals interaction forces on particle deformation. Of the

different models, the JKR model is often considered most applicable to the situation of drug-carrier adhesion in adhesive mixtures for inhalation.

From the above it follows that particle properties such as size and surface energy are directly related to the strength of an adhesive interaction. However, the above equations are valid only for perfectly smooth, rigid (Eq. 1.1) or elastically deformable (Eq. 1.2) particles. In reality, lactose carrier surfaces exhibit various degrees of surface roughness and may contain plastically deformable impurities such as protein residues from the production process. Drug particles have a certain surface roughness too and throughout adhesive mixtures they will have different shapes, sizes, orientations, degrees of plastic deformation and numbers of contact points with the carrier surface. Such factors influence the separation distance between contacting particles and may decrease or increase their contact surface area by several orders of magnitude, and hence, cause a significant spread in the adhesion force throughout the mixture. Techniques to satisfactorily quantify or model properties like surface roughness are currently unavailable, and therefore, exact determination of the interaction forces between particles in adhesive mixtures is very difficult despite the understanding of their origin.

In addition to the physicochemical properties of the drug and carrier, interaction forces will be strongly dependent on formulation variables such as mixing time and drug content. During the mixing process, frictional and inertial mixing forces will affect the distribution of the drug particles over the carrier surface. These forces may furthermore change the orientation of the drug particles, cause their plastic deformation by compression onto the carrier surface, change their degree of agglomeration or change the drug and carrier particle surfaces by attrition. The drug content in adhesive mixtures will determine the number of drug-drug contacts relative to the number of drug-carrier contacts and thus, the ratio of cohesive to adhesive interactions. It determines the propensity for agglomeration and may, therefore, to great extent determine the particle size distribution of the drug as present in the mixture (i.e. including agglomerates).

Dispersion forces

Separation or dispersion forces generated during inhalation may include aerodynamic drag, lift and shear forces, friction forces from sliding of the carrier particles against other particles or the inhaler wall and inertial forces from collisions, rotations and vibrations. Of these forces, friction forces are generally highest, followed by inertial forces and aerodynamic forces. Friction forces may result in a transfer of drug particles from one carrier particle to another or to the inhaler wall rather than their entrainment by the airstream. The relevance of these forces to the dispersion performance of dry powder products is therefore questionable. Aerodynamic drag forces are described by the following Equations:

$$F_{drag} = C\pi\rho d^2 v^2/8 \quad 1.3$$

$$F_{drag} = 3\pi\eta v d \quad 1.4$$

in which F_{drag} is drag force (N), C is the drag coefficient, ρ is the density of the air (kg/m^3), d is the diameter of the drug particle (m), η the viscosity of the air (m^2/s) and v the velocity difference between the air and the particle. Equation 1.3 is applicable at turbulent air flow, whereas Equation 1.4 applies to the non-turbulent Stokes regime. The relevance of aerodynamic forces to dispersion may be questioned too, however. These forces depend on the velocity difference between the air and the carrier, which may be significant only at the onset of fluidisation depending on the degree of turbulence of the air. Drug particles may also reside in a stationary boundary layer around the carrier. Furthermore, it was suggested that drug particles may find shelter from drag, lift, shear and friction forces in clefts and depressions or against steep faces on the carrier surface [10]. Such forces may therefore not be effective in combination with rough, granulated carriers. This applies to a lesser extent to inertial forces, which may be described by the simple formula:

$$F_{in} = ma = \rho\pi d^3 a/6 \text{ (for spheres)} \quad 1.5$$

in which F_{in} is the inertial separation force (N), m the mass of the drug particle (kg), a the deceleration of the carrier surface upon impaction (m/s^2), ρ the density of the drug particle, and d the diameter of the drug particle. The deceleration of the carrier partly depends on its velocity (dv/dt) and should thus depend on the velocity of the entraining airstream. Based on Equations 1.3-1.5 a positive relationship between drug particle size or air flow velocity (e.g. flow rate, inhaler resistance) and the magnitude of dispersion forces should thus be expected. Of course, the different types of forces can only be effective as dispersion forces when they act on the drug particles in the right direction.

The predominant type of dispersion force will depend on several factors. First, as already mentioned, the carrier surface properties may be of influence. Second, the geometry of the inhaler will be important. For example, in air classifier based inhalers the kinetic energy of the airstream is used to generate many carrier-inhaler collisions. As a result, inertial forces with such devices will contribute to dispersion to a greater extent than with devices in which very few carrier-inhaler collisions are generated (e.g. most capsule inhalers). Third, the inertia (mass or size and velocity) of the carrier in the inhaler may determine its trajectory and thus the number of collisions with the inhaler wall. For example, it was found that an increase in carrier size resulted in more carrier-inhaler collisions in the Cyclohaler [11]. The flow rate or air flow velocity in the inhaler could, therefore, have a similar effect.

As for interaction forces, an understanding of the different types of dispersion forces does not lead to them being well defined, since they too are dependent on many factors that are difficult to quantify. These forces will also exhibit a broad distribution throughout the mixture as a result of heterogeneity of the drug and carrier particle properties on which they depend. Furthermore, formulation variables will greatly affect dispersion forces as they determine, for example, the (agglomerated) particle size distribution of the drug and its distribution over the carrier surface.

Past research focuses, current understanding and future opportunities

The current understanding of the relationships between particle properties, formulation variables and the dispersion performance of adhesive mixtures is very limited, despite several decades of intensive research [12]. Although this fact may at first seem discouraging, there are some clearly identifiable causes which offer an opportunity for a more effective future research direction.

Because of the basic notion that interaction forces are directly related to particle properties such as size, surface free energy, surface roughness and shape, there has been a strong focus on the relationships between such properties of the individual mixture components and dispersion performance [13, 14]. However, these properties are difficult to quantify and can rarely be changed in isolation from one another. Furthermore, a direct relationship between such properties and dispersion performance is very unlikely due to the influence of the formulation process on interaction and dispersion forces. For example, it may be expected that not the primary particle size distribution of the drug is relevant, but that of the drug as present on the carrier surface (i.e. including drug agglomerates and drug-fine lactose composite agglomerates). Furthermore, the carrier surface properties may be of negligible influence at high drug contents causing the formation of drug multi-layers, and the shapes of the drug particles from the starting material may be subject to plastic deformation under the influence of mixing forces. There is no question that the physicochemical properties of the drug and carrier particles in the starting materials influence the mixture properties, but the role of the formulation process cannot be neglected. The dispersion performance of adhesive mixtures is a direct result of the mixture properties and the inhalation conditions, and therefore, a 'top-down approach' (Fig. 3), in which attention is first focussed on the relationship between dispersion performance and mixture properties, and at a later stage on the relationship between mixture properties and physicochemical particle properties may be more beneficial. In such an approach attention would be focussed on identifying and defining relevant mixture properties, and on developing analysis methods to quantify them.

Interactions between formulation variables and inhalation variables are often poorly understood, which may lead to suboptimal study designs and conflicting results

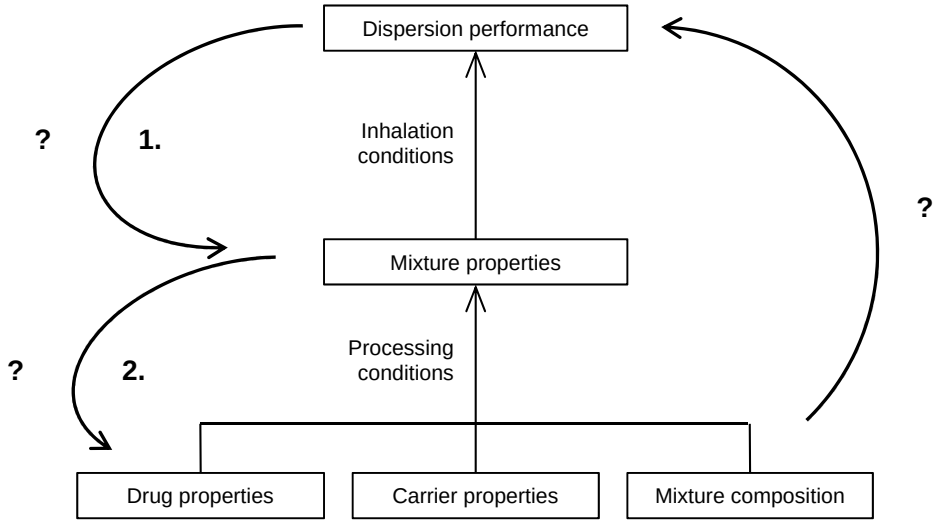

Figure 3: schematic presentation of the factors that lead to a certain dispersion performance of adhesive mixtures for inhalation. The arrow on the right represents the conventional 'bottom-up approach', which is concerned with the study of a direct relationship between properties of the starting materials and dispersion performance. The arrows on the left represent the 'top-down approach' chosen in this thesis, which focusses first on the relationship between dispersion performance and the mixture properties (1) and in a following stage on that between the mixture properties and the properties of the starting materials (2).

[14]. A striking example can be found in the research area concerning the role of fine lactose particles (i.e. 'fines') added to adhesive mixtures. To test one of the hypotheses concerning the working mechanism of fines, the order in which the drug and fine lactose were mixed with the carrier lactose has been changed in several studies. Some studies did find an influence of mixing order [15, 16], whereas others did not [17, 18]. Zeng et al. showed that such conflicting results may be caused by differences in the inhalation flow rate during dispersion [15] and Jones et al. showed that the mixing time and the drug content can cause conflicting results of mixing order experiments as well [16]. Interactions between formulation and inhalation variables thus greatly affect the outcome of dispersion performance tests. Clearly, a better understanding and anticipation of such interactions is required to effectively study the influence of individual factors or variables on the dispersion performance of adhesive mixtures.

Although the FPF is without a doubt the therapeutically most relevant measure of dispersion performance, it may not be the most applicable measure to use for obtaining a mechanistic understanding of the influence of formulation variables on dispersion performance. The FPF includes drug detachment from the carrier surface as well as the subsequent dispersion of detached agglomerates. The FPF is furthermore

dependent on any drug losses to the inhaler parts and inlet of the measuring instrument. Hence, the origin of differences in the FPF cannot be attributed to a specific step of the dispersion process, which increases the uncertainty regarding the underlying working mechanisms of formulation variables. In addition, if cascade impaction analyses are performed, measuring the FPF is very time consuming. Contrary to the fine particle fraction, the detached drug fraction includes only one step of the dispersion process; that is, drug detachment from the carrier surface. It is a direct measure of the drug fraction for which dispersion forces or, rather, drug-carrier separation forces dominate drug-carrier adhesion forces. Changes in the detached drug fraction can thus be more directly related to changes in the adhesion and/or separation force distributions throughout the mixture, as was explained by De Boer et al. in their 'force distribution concept' (Fig. 4) [19]. Drug detachment experiments may be performed by air jet sieving [20], centrifugation [21] or dispersion testing of adhesive mixtures with classifier based inhalers [19]. Drug detachment can be measured directly by analysing the non-detached drug fraction on the surfaces of carrier particles collected after a dispersion experiment (i.e. 'carrier residue', Fig. 2). Such experiments are generally less time consuming then cascade impaction analyses and, therefore, allow more data to be collected within the same timeframe.

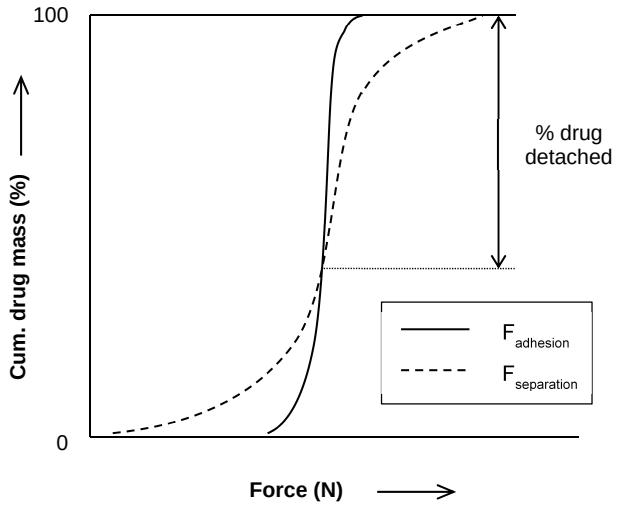

Figure 4: hypothetical example of the 'force distribution concept' [19]. Throughout adhesive mixtures the adhesion force between drug and carrier particles ($F_{adhesion}$) is 'distributed' due to variation in drug particle size, local carrier surface composition, surface roughness, particle orientation, etc. The separation force exerted on the drug particles ($F_{separation}$) during dispersion is also 'distributed' throughout the mixture, mostly because of a variation in drug particle size or mass. The drug fraction for which the separation force exceeds the adhesion force in the exact opposite direction will detach from the carrier surface during dispersion. Determination of the detached drug fraction based on the adhesion and separation force distributions in this way assumes that both types of forces are completely correlated (i.e. the drug particle that experiences the highest separation force also experiences the highest adhesion force).

Clear and useful definitions of terms used in discussing formulation studies have not always been formulated. An example is the term 'active sites'. It mostly refers to sites on the surface of lactose carrier particles where drug particles can bind relatively strongly [22-24], such as ductile contaminations that result in a large contact area under the influence of press-on forces during the mixing process, or large carrier surface irregularities that result in multiple contact points. Although this view on what constitutes 'active sites' is generally agreed upon, it may not be the most useful: the strength of a drug-carrier interaction alone does not determine the dispersion likelihood of the drug particle concerned; the balance between dispersion and interaction forces does. Therefore, a direct relationship between the activity of carrier surface sites in terms of binding strength and dispersion performance does not necessarily exist, yet conclusions about changes in the activity of carrier surface sites (e.g. due to the 'saturation of active sites' [25]) are often drawn directly based on changes in dispersion performance. This way, changes in dispersion performance may be attributed to surface phenomena that determine the strength of drug-carrier interactions, whereas in reality they result from a change in the dispersion force generated.

The aim and outline of this thesis

Worldwide an estimated 235 million people suffer from asthma (2013 WHO estimate) and another 64 million from COPD (2004 estimate, [26]). In Europe, around 6 million dry powder inhalation products were sold between 2002 and 2008, comprising roughly 40% of all inhalation devices [27]. In addition, currently marketed dry powder inhalation products generate FPFs that are roughly between only 10(!)-50% of the label claim [28]. Several powder inhalation products have recently been marketed of which the carrier-based formulations contain excipients such as magnesium stearate to obtain a satisfactory dispersion performance (e.g. Foster Nexthaler, Seebri Breezhaler), even though no evidence can be found in literature about their safety on inhalation in the long term. Clearly, the efficient production of safer, more efficient and effective dry powder inhalation products is a goal worthwhile of striving towards. Understanding the dispersion performance of adhesive mixtures is pivotal in this respect. Therefore, the aim of this thesis is to improve the understanding of the relationships between formulation variables, inhalation variables and the dispersion performance of adhesive mixtures for inhalation. Drug detachment from lactose carriers during dispersion will be the primary measure of dispersion performance studied throughout this thesis for the reasons mentioned in the previous section. Chapter 2 describes in a theoretical way the interacting role of inhalation flow rate towards the effects of formulation variables on dispersion performance. The knowledge from this theoretical chapter is applied for a rational design of experiments in following chapters. Chapters 3-6 present studies on the

effects of three of the most relevant formulation variables (the drug content, the mixing time and the fine lactose content) on drug detachment. An important aspect of these studies is the accounting for interactions with multiple inhalation and formulation variables, such as the inhalation flow rate, the type of drug or the carrier size fraction. Furthermore, as part of the mentioned 'top-down approach', an attempt will be made to identify and define the mixture properties most relevant to drug detachment and to characterise them with existing or new techniques. For example, chapter 7 presents a new definition of the activity of carrier surface sites, and the use of coherent anti-Stokes Raman scattering (CARS) microscopy in combination with scanning electron microscopy (SEM) for the chemical-selective imaging of adhesive mixtures is evaluated in chapter 8. The final chapter is a general discussion about the work presented in this thesis. It highlights some practical implications, shortcomings and future perspectives.

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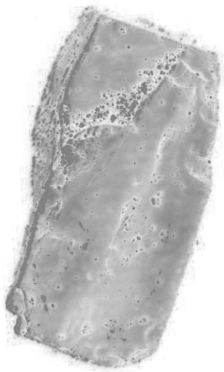
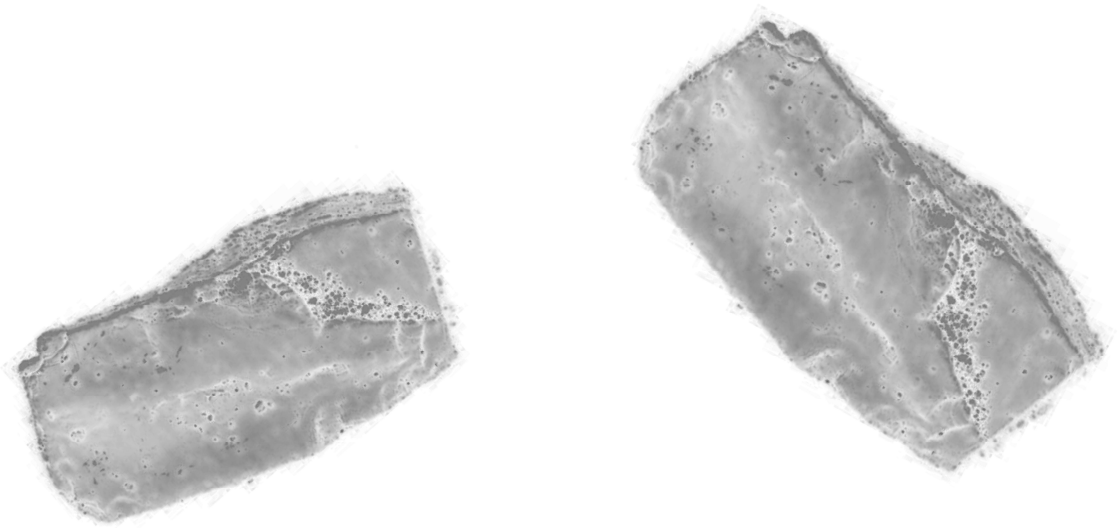


Chapter 2

The dispersion behaviour of dry powder inhalation formulations cannot be assessed at a single inhalation flow rate

Floris Grasmeijer, Anne H. de Boer



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Abstract

The dispersibility or dispersion behaviour of inhalation powders with a particular dry powder inhaler can be expressed by their dispersion performance as a function of inhalation flow rate. For carrier-free formulations the dispersibility was shown to directly reflect the agglomerate tensile strength distribution. For adhesive mixtures the dispersibility reflects the 'energy ratio distribution', which is introduced in this chapter. Formulation variables are likely to affect the tensile strength or energy ratio distribution in such a way that their effect on dispersion performance differs in magnitude or direction between inhalation flow rates. The effects of formulation variables on powder dispersibility can, therefore, only be properly assessed by dispersion performance testing over a range of inhalation flow rates.

Introduction

In mechanistic studies focussing on the formulation of powders for inhalation it is common practice to assess and compare the dispersion performances of powders at a single inhalation flow rate. Most often a flow rate of 60 L/min is chosen (e.g. [1-3]), likely because this particular flow rate has long been considered 'optimal' for inhalers having a medium resistance to air flow [4]. In this chapter it is explained why such an approach may severely hamper the fundamental understanding of dry powder inhalation formulations, and therefore, cannot be considered rational.

First, it is important to distinguish a powder's 'dispersion performance' from its 'dispersibility' or 'dispersion behaviour'. The dispersion performance of powders for inhalation at a specific flow rate results from the balance between interparticulate interaction forces and opposing dispersion forces. Increasing the flow rate during dispersion results in a higher air flow velocity through the inhaler, and thus, in a higher kinetic energy of the airstream. This means that higher dispersion forces may be generated relative to the interaction forces in the powder and that a greater dispersion performance can be expected. The dispersion performance of an inhalation powder as a function of inhalation flow rate is referred to as the powder's dispersibility or dispersion behaviour in this chapter.

Carrier free formulations: the agglomerate tensile strength distribution

For carrier-free or 'cohesive' powders dispersion concerns the break-up of agglomerates. The tensile strength of these agglomerates is, therefore, considered to be the descriptor of interaction forces most relevant to dispersion performance [5]. Cohesive powders are inherently heterogeneous with a tensile strength distribution throughout the powder bed as a result. During dispersion, only the agglomerates with a tensile strength below the dispersion force generated will break up. Therefore, if it is assumed that at a certain flow rate all agglomerates experience more or less the same dispersion force, then the dispersion performance of a powder at any flow rate directly relates to its tensile strength distribution. Indeed, differences in the width of the tensile strength distribution between cohesive powders of milled lactose and Lactohale 300 could explain why the order of dispersion performance of these powders inverted with increasing flow rate [5]. Hence, the dispersibility of cohesive powders cannot be properly assessed and compared by measuring dispersion performance at only one flow rate, because it neglects the distributed nature of a primary factor determining dispersion performance (i.e. the tensile strength of the agglomerates).

Adhesive mixtures: the energy ratio distribution

A certain analogy exists between the dispersion of cohesive powders and that of adhesive mixtures. The dispersion of adhesive mixtures primarily concerns the detachment of drug particles (including drug and drug/fine-lactose agglomerates) from a lactose carrier surface. Although this process was previously described by de Boer et al. in terms of opposing adhesion and separation forces (i.e. the so-called 'force distribution concept' [6], as already explained in more detail in chapter 1, Fig. 4), a slightly different description may be preferable for the following reasons: Two assumptions underlying the force distribution concept are that the magnitude of a drug-carrier interaction force does not depend on the mode and rate of drug detachment from the carrier surface and that the drug particles experiencing the highest adhesion forces also experience the highest separation forces (i.e. both forces are completely correlated). However, for a more rapid separation the force of detachment can be higher, whereas for a slower or more prolonged separation it may be lower. This is the result of a difference in the degree of viscoelastic deformation of the contact surfaces that allows their separation by 'peeling' [7] and of the accumulation of vibrational energy in the fine particles [8]. Furthermore, although the adhesion and separation force may to a certain extent be correlated because of a shared proportionality to drug particle (agglomerate) size [6], the assumption of complete correlation may greatly underestimate the importance of other factors determining either one or both types of forces, such as the geometry of the carrier surface.

The disadvantages associated to the force distribution concept can be circumvented by describing drug detachment in terms of energy instead of force, and by regarding the distribution in the ratio of the 'opposing' types of energy rather than the distribution in both types of energy separately. The strength of a drug-carrier interaction may be expressed in terms of the minimum amount of energy needed to break it. This 'binding energy' (E_b) is independent of the mode and rate of particle separation for similar end-situations (e.g. equal drug particle displacement and no difference in the degree of plastic deformation) [7]. The energy equal to the maximum binding energy that may potentially be overcome for a certain drug particle in the mixture and a specific dispersion process is referred to as the 'potential separation energy' ($E_{s,pot}$). Thus, detachment of a drug particle from the carrier surface occurs during dispersion if its energy ratio $E_{s,pot}/E_b \geq 1$, regardless of the exact underlying adhesion and drug detachment mechanisms. Because of variability of the parameters that determine the magnitude of $E_{s,pot}$ and E_b for individual drug particles throughout the mixture (e.g. drug and carrier particle surface roughness, local carrier surface composition, number of contact points, drug particle shape, size and orientation), these types of energy will exhibit a distribution, and consequently, so will the energy ratio $E_{s,pot}/E_b$. The relationship between the energy ratio and the drug fraction that is detached from the

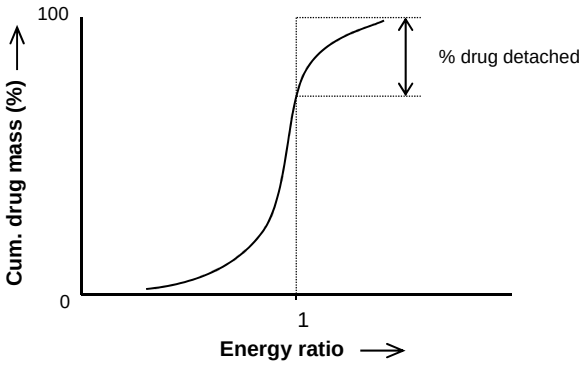

Figure 1: hypothetical drug mass distribution as a function of the energy ratio (i.e. energy ratio distribution). The detached drug fraction equals the cumulative drug mass fraction for which the ratio of potential separation energy ($E_{s,pot}$) to binding energy (E_b) ≥ 1. An increase in flow rate results in a shift of the energy ratio distribution to higher values and thus leads to an increase in the detached drug fraction.

carrier surface is further clarified in Figure 1, where a hypothetical drug mass distribution as a function of the energy ratio (i.e. energy ratio distribution) is presented for an adhesive mixture subjected to a particular dispersion process. A higher flow rate is represented by a shift of the energy ratio distribution curve to higher values for the energy ratio as it increases $E_{s,pot}$. Hence, the dispersion performance (detachment efficacy) at any flow rate directly relates to the energy ratio distribution. The dispersion performances of two adhesive mixtures from a specific inhaler are the same at any single flow rate if their energy ratio distributions are exactly the same. If two formulations have a different energy ratio distribution as a result of a change in a relevant variable in the formulation process, then the difference in dispersion performance between these formulations theoretically cannot be the same over the range of flow rates that correlates with 0-100% dispersion (detachment) efficacy. This is elucidated in Figure 2 for a number of different imaginary energy ratio distributions, including an extreme (D). Therefore, quantitative interactions between the variable that caused the difference in the energy ratio distributions and inhalation flow rate will **always** occur if the maximum range of dispersion efficacies is covered, and possibly qualitative interactions too (i.e. Fig. 2C). It should be noted that for the same reason interactions between formulation variables will always depend on inhalation flow rate as well, giving rise to multi-order interactions. It follows that, analogues to cohesive powders, the dispersion behaviour of adhesive mixtures and the effects of formulation variables thereon neither can be properly assessed by dispersion performance testing at only one flow rate.

Contrary to the tensile strength of agglomerates, there is currently no model available that quantitatively describes the energy ratio. A quantitative determination of

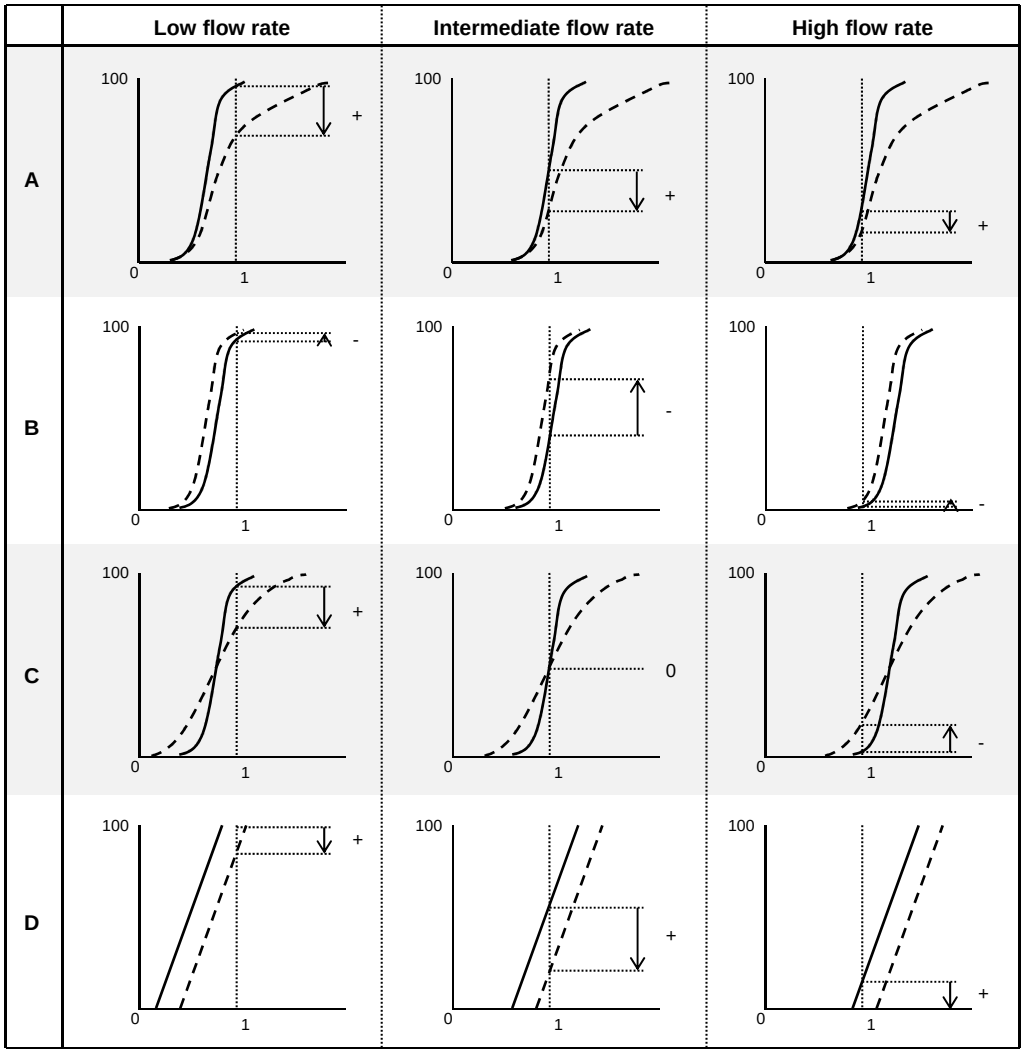

Figure 2: different effects on the detached drug fraction. Solid lines represent hypothetical energy ratio distributions in the starting situation, dashed lines the hypothetical energy ratio distributions after changing a certain variable in the formulation process. Y-axes of the individual figures represent the cumulative drug mass (%), X-axes the energy ratio. Situations differ in the inhalation flow rate, the way in which the energy ratio distribution is changed (examples A-D) and the shape of the energy ratio distribution in the starting situation (examples A-C versus example D). For every situation a different flow rate may result in a different sign (+ or -) or magnitude (length of arrow) of the effect on the detached drug fraction, representing qualitative and quantitative interactions, respectively. Even in the extreme case of a linear energy ratio distribution that is shifted without changing its shape, the effect cannot be the same in magnitude over the entire range of flow rates (situation D).

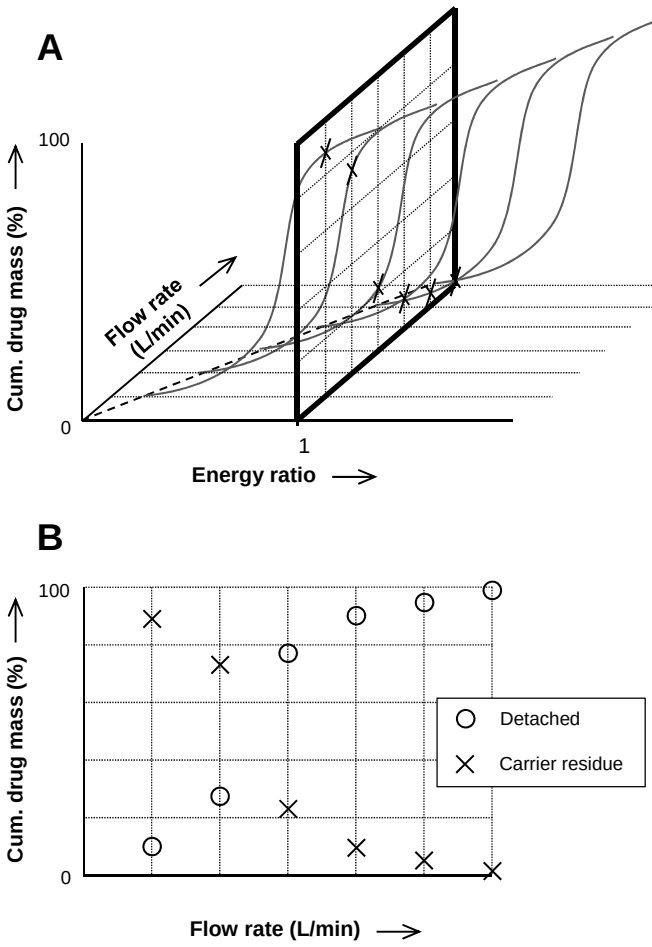

Figure 3: the relationship between the theoretical energy ratio distribution and the dispersion behaviour of adhesive mixtures in practice. A: hypothetical energy ratio distribution at different flow rates (z-axis). The plane parallel to the y and z-axis at x = 1 shows the measurable dispersibility profile of the formulation, represented by 'carrier residue' (i.e. the undetached drug fraction) as a function of flow rate in Figure B. **B:** powder dispersibility profiles resulting from the energy ratio distribution in Figure A. The detached drug fraction equals 100-carrier residue. Although the energy ratio distribution cannot be directly measured, the dispersibility profiles can be considered 'apparent' energy ratio distributions, which are similar in shape to the 'real' energy ratio distribution in Figure A if a linear scaling exists between flow rate and energy ratio (i.e. the dashed line in the plane formed by the x- and z-axis which marks the start of every energy ratio distribution in Figure A), and if the shape of the 'real' energy ratio distribution does not change with flow rate.

the energy ratio distribution will therefore not be possible. As a result, energy ratio distributions only provide a theoretical argument against the common practice in carrier-based formulation studies of dispersion performance testing at a single inhalation flow rate. This argument is supported, however, by previously reported interactions between flow rate and formulation variables such as the mixing order of drug and lactose fines in ternary formulations [9] and mixing time [10]. Furthermore, the theoretical argument can be easily tested by measuring the effects of formulation variables on the detached drug fraction over a range of inhalation flow rates, as will be done in following chapters of this thesis. It should be noted that dispersibility profiles (i.e. drug detachment as a function of inhalation flow rate) measured this way can be considered 'apparent energy ratio distributions' (see Figure 3).

Conclusions

The effects of formulation variables on the dispersibility or dispersion behaviour of dry powder inhalation formulations cannot be properly assessed by dispersion performance testing at a single inhalation flow rate. This neglects the distributed nature of the factors that directly relate to dispersion performance and, as a result, provides a limited mechanistic insight. The utility of inhalation formulation studies may therefore benefit from an approach in which dispersion performance is by default tested over a range of inhalation flow rates instead.

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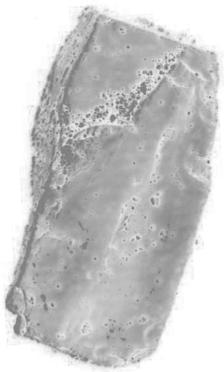

Chapter 3

Drug content effects on the dispersion performance of adhesive mixtures for inhalation

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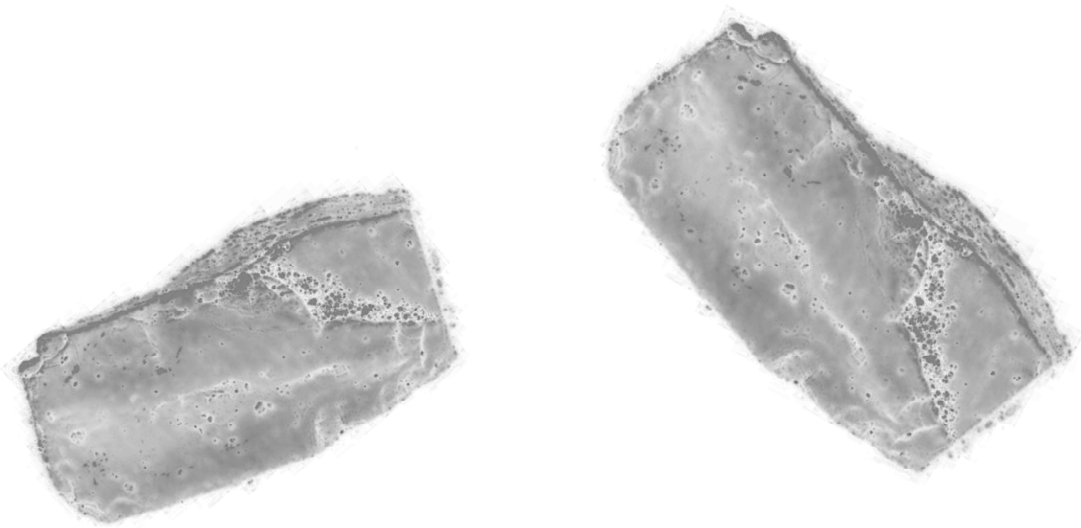

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Abstract

The drug content in adhesive mixtures for inhalation is known to influence their dispersion performance, but the direction and magnitude of this influence depends on other variables. In the past decades several mechanisms have been postulated to explain this finding and a number of possible interacting variables have been identified. Still, the role of drug content in the formulation of adhesive mixtures for inhalation, which includes its significance as an interacting variable to other parameters, is poorly understood. Therefore, the results from a series of drug detachment experiments are presented in which the effect of drug content and its dependence on inhalation flow rate, the mixing time and the type of drug is studied. Furthermore, it is investigated whether the effect depends on the range within which the drug content is changed. Quantitative and qualitative multi-order interactions are observed between these variables, which may be explained by a shifting balance between three different mechanisms. The results therefore demonstrate that accounting for (multi-order) interactions between variables has to be part of quality by design activities and the rational design of future experiments.

Introduction

The therapeutic doses of the drugs used in the local treatment of asthma and COPD differ substantially. For example, the long-acting beta agonist formoterol and the anticholinergic tiotropiumbromide are usually administered in doses from 6 to 18 μg, whereas for the corticosteroid fluticasone doses from a single inhalation up to 500 μg are given. The large variation in doses is reflected in the drug contents that are present in commercially available inhalation powder formulations. For adhesive mixtures drug contents typically range from 0.1 to 4% (Tables 1 and 2).

Table 1: drug contents in commercially available carrier based dry powder formulations containing a single drug.

Preparation	Drug	Carrier/dose	Drug/dose	Drug content
Eklira Genuair 400	ACL	12600 μg	400 μg	**3.08%**
Beclometason 100 Cyclocaps	BDP	25000 μg	100 μg	**0.40%**
Beclometason 200 Cyclocaps	BDP	25000 μg	200 μg	**0.79%**
Beclometason 400 Cyclocaps	BDP	25000 μg	400 μg	**1.57%**
Budesonide Easyhaler 200	BUD	7903 μg	200 μg	**2.47%**
Budesonide Easyhaler 400	BUD	14507 μg	400 μg	**2.68%**
Budesonide Novolizer 200	BUD	10700 μg	200 μg	**1.83%**
Budesonide Novolizer 400	BUD	10500 μg	400 μg	**3.67%**
Flixotide Diskus 100	FP	12500 μg	100 μg	**0.79%**
Flixotide Diskus 250	FP	12500 μg	250 μg	**1.96%**
Flixotide Diskus 500	FP	12500 μg	500 μg	**3.85%**
Formoterol Novolizer 6	FFD	5744 μg	6 μg	**0.10%**
Formoterol Novolizer 12	FFD	11488 μg	12 μg	**0.10%**
Formoterol Easyhaler 12	FFD	8000 μg	12 μg	**0.15%**
Seebri Breezhaler	GLY	23600 μg	63 μg	**0.27%**
Onbrez Breezhaler 150	IM	24800 μg	194 μg	**0.78%**
Onbrez Breezhaler 300	IM	25000 μg	388 μg	**1.53%**
Salbutamol Cyclocaps 200	SS	25000 μg	200 μg	**0.79%**
Salbutamol Cyclocaps 400	SS	25000 μg	400 μg	**1.57%**
Salbutamol Novolizer 100	SS	11420 μg	120 μg	**1.04%**
Ventolin Diskus 200	SS	40000 μg	200 μg	**0.50%**
Serevent Diskus	SX	12500 μg	50 μg	**0.40%**
Spiriva Handihaler 18 μg	TBM	5500 μg	22.5 μg	**0.41%**

ACL = aclidinium bromide; BDP = beclometasone dipropionate; BUD = budesonide; FP = fluticasone propionate; FFD = formoterol fumarate dihydrate; GLY = glycopyrronium bromide; IM = indacaterol maleate; SS = salbutamol sulphate; SX = salmeterol xinafoate; TBM = tiotropium bromide.

Table 2: drug contents in commercially available carrier based combination preparations.

Preparation	Drug 1	Drug 2	Carrier/dose	Drug 1 /dose	Drug 2 /dose	Total content
Foster NEXThaler	BDP	FFD	9894 µg	100 µg	6 µg	**1.06%**
Seretide Diskus 50/100	FP	SX	12500 µg	100 µg	50 µg	**1.19%**
Seretide Diskus 50/250	FP	SX	12500 µg	250 µg	50 µg	**2.34%**
Seretide Diskus 50/500	FP	SX	12500 µg	500 µg	50 µg	**4.21%**

BDP = beclometasone dipropionate; FP = fluticasone propionate; FFD = formoterol fumarate dihydrate; SX = salmeterol xinafoate.

In contrast to the wide range of drug contents in marketed inhalation products, it is almost common practice to use a fixed drug to carrier ratio of 1:67.5 (1.46%) in studies on the relationship between certain variables and carrier based formulation performance. This ratio is most likely adopted from earlier carrier based formulations for the Rotahaler®, Diskhaler® and Cyclohaler [1]. Therefore, the choice for this particular drug to carrier ratio seems to be based on historical reasons, rather than it having a sound scientific foundation.

A scientific foundation for the choice of a certain drug to carrier ratio may be found in several studies which have already shown that drug content affects blend homogeneity and drug detachment from the carrier. Adhesive mixtures for inhalation can be considered 'total mixes', in which a dynamic process of ordering and randomisation determines the outcome of the blending process [2]. With increasing drug content the equilibrium is displaced towards randomisation [2-4], which results in less homogeneous mixtures that are more prone to segregation during handling as well [5]. On the upside, this adverse effect may come along with better drug detachment from the carrier particles when separation forces are generated, as was reported for a model interactive system containing smooth spherical carrier particles [6]. For inhalation formulations that contain lactose carriers the reported effects of drug content on dispersion performance are not consistent, however. Beneficial effects of an increasing drug content from 0.25 to 2.8% on the fine particle fraction (FPF) from two commercially available inhaler devices were reported by Steckel et al [7], whereas other evaluation studies described unfavourable effects as well [8, 9].

In explaining the effect of the drug content on the dispersion performance of adhesive mixtures for inhalation several mechanisms have been postulated. Firstly, so-called active sites on the lactose carrier surface may become saturated with increasing drug content [10, 11]. This results in a decreasing mass percent of drug adhering strongly to the carrier surface, which improves drug particle detachment. Secondly, layer formation or agglomeration of drug particles with increasing drug content can cause detachment of large agglomerates rather than single drug particles if failure of adhesive bonds between the inner drug layer and the carrier surface occurs during inhalation [6,

12-14]. This too may improve drug detachment, because it increases the magnitude of the lift and inertial separation forces more so than that of the drug-carrier interaction forces. Thirdly, a higher drug content increases the effectiveness of inertial and frictional mixing forces as drug particles fill up clefts and depressions in the carrier surface [14, 15]. By being transferred through the powder bed, this enables such mixing forces to act effectively as press-on forces on drug particles that would have found shelter from them in carrier surface irregularities at a much lower drug content. It causes the particle interaction forces to increase by reducing their separation distance and increasing their contact surface area, and therefore, it causes drug detachment to be negatively affected [16]. And lastly, it was hypothesised that with increasing drug content detached particles may be more likely to collide with neighbouring drug particles to cause enhanced drug detachment; a mechanism which is referred to as the 'collision effect' [6]. Collision effects may marginally contribute to improved drug detachment with increasing drug content, but particularly for irregular lactose surfaces their relevance is questionable. Therefore, they are not further discussed in this chapter.

Since not all of the aforementioned mechanisms result in improved drug detachment, the net effect of a change in drug content depends on which mechanism is dominant under the given circumstances. Therefore, rather the balance of a number of different mechanisms determines the effect of drug content than either of these mechanisms alone, and any variable by which this balance is affected will be an interacting variable. Some variables that are suspected to interact with the effect of drug content are the range within which the drug content is changed [8, 9]; the dispersion principle used and its efficacy (e.g. flow rate) [14, 15]; the mixing conditions applied; and the characteristics of the used carrier product [15]. In addition, agglomerate formation may be drug dependent, and therefore, so may be the effect of drug content.

A better understanding of the influence of drug content on dispersion performance may lead to a more rational approach in future studies regarding the choice for this variable. Furthermore, it would help to understand the functionality and performance of carrier products and marketed formulations and to explain the results from powder dispersion performance tests that are presented in literature. The objective of this study is therefore to deepen the understanding of the effect of drug content on drug particle detachment from lactose carriers during inhalation. To this end, a series of drug detachment experiments is performed with formulations containing a range of drug contents. A special focus is put on the role of flow rate, the type of drug, the range within which the drug content is changed and mixing time as interacting variables. Scanning electron microscopy (SEM) and laser diffraction analysis are used in an attempt to qualify and quantify the occurrence of the different mechanisms behind the effect of drug content.

Materials and methods

Starting materials

Alpha lactose monohydrate (Pharmatose 80M) was kindly donated by DFE Pharma (Goch, Germany). The micronised drugs used for this study are salbutamol sulphate (supplied by DFE Pharma), salmeterol xinafoate and fluticasone propionate (granted by Novartis, Germany) and budesonide (purchased from Fagron, The Netherlands). All drugs were passed through a 90 μm sieve to break up larger agglomerates prior to mixing with the carrier.

Carrier classification

A lactose carrier with a size fraction of 250-315 μm was obtained from Pharmatose 80M by 20 minutes of vibratory sieving at an amplitude of 1.5 mm (Retsch AS 200 control, Germany) followed by 15 minutes of air jet sieving (Alpine AS200, Augsburg, Germany). Such a coarse size fraction of the crystalline carrier material stresses the effect of press-on forces during the mixing process. It is therefore expected to aid in identifying and determining the relative significance of this mechanism behind the effect of drug content on drug detachment. Sieving was performed under uncontrolled environmental conditions (with relative humidity values ranging from 25-65%). After sieving, the lactose was left to rest for at least 2 days before further processing to allow electrostatic charges to dissipate.

Blend preparation

Drug-carrier blends were prepared in batches of 25 g at ambient conditions. To prevent electrostatic charge effects as much as possible, a stainless steel mixing vessel with a volume of 160 cm^3 was used (the filling degree was approximately 20%). During filling of the vessel the drug was 'sandwiched' in between two equal parts of the lactose carrier after which the powder was gently pre-blended with a spatula for approximately 20 orbits. Blends were then further mixed in a Turbula blender (WA Bachhofen, Basel, Switzerland) at 90 rpm for a total of 2, 10 or 60 minutes. Every two minutes any powder adhering to the mixing vessel wall was scraped loose and returned into the blend. For salmeterol and fluticasone, blends containing 0.1, 0.2, 0.4, 1, 2 and 4% of drug by weight were prepared, whereas for salbutamol and budesonide only drug contents of 0.4% and 4% were used. These drug contents are representative for the typical range found in commercial preparations (Tables 1 and 2). However, it is important to realise that the nature of particle interactions in adhesive mixtures for inhalation is ultimately determined not so much by the drug content as by the drug particle density on the carrier surface. This can be expressed as the drug mass per unit carrier surface area (i.e. carrier surface

payload) or the degree of carrier coverage relative to a single monolayer. This means that a comparison between mixtures based on drug content is only valid if they contain a lactose carrier with the same specific surface area (and a comparable surface roughness and surface 'activity'). As will become clear from the calculated drug contents for monolayer carrier coverage presented in Table 3, drug contents in this study are chosen such that carrier coverage values range from 6% (for the 0.1% fluticasone mixture) to 328% (for the 4% budesonide mixture), representing sub-monolayer to multilayer carrier coverage. It should be noted that these values for the carrier coverage are based on the assumption of a uniform drug distribution over the carrier surface, which is unlikely to represent the situation in practice. Therefore, they should merely be regarded as a rough indication for the average drug particle density on the carrier surface.

Content uniformity testing

Content uniformity of the blends was tested by randomly taking 20 samples of 25 ± 1 mg. Samples were analysed as explained in the section 'sample analysis'. Content uniformity was considered acceptable at relative standard deviations (RSDs) < 3%.

Helium pycnometry

The density of the drugs was measured by helium pycnometry (model MVP-1, Quantachrome, USA). Sample sizes ranged between 5.75 g and 12.35 g. Results are the mean of 4 measurements.

Laser diffraction analysis

The particle size distributions (PSDs) of the drugs were determined by laser diffraction technique with the HELOS BF diffractometer (100 mm lens, calculations based on the Fraunhofer theory) after dispersion of the powders with a RODOS disperser at 3 bar (Sympatec, Clausthal-Zellerfeld, Germany). Increasing the pressure drop to 5 bar did not have any influence on the PSDs measured, confirming that the primary PSDs were already obtained at the lower pressure.

The PSDs of salmeterol and fluticasone particles (including agglomerates) as present on the carrier surface in mixtures containing 0.1% to 1% of drug were measured by wet laser diffraction. To prepare saturated aqueous drug solutions, suspensions of fluticasone and salmeterol containing 0.03% polysorbate 80 (Tween 80) were prepared by ultrasonication for at least 30 minutes with a Helma Transsonic 700/H ultrasonic bath (Elma Hans Schmidbauer, Singen, Germany) and subsequent continuous stirring. After cooling down to room temperature, 35-40 mL of these suspensions was passed through a 0.2 μm cellulose acetate filter. To the saturated solutions 20 mg (for 1% mixtures) to 200 mg (for 0.1% mixtures) of the blends was added to dissolve the lactose carrier and suspend the drug. For the sample sizes used, optical concentrations ranged

between 6 and 14%. It was checked that dissolved lactose did not influence the results. Measurements were performed in the CUVETTE SC-40 module (50 mL cuvette) with the HELOS BF diffractometer with a 100 mm lens and the FREE computation mode (Sympatec, Clausthal-Zellerfeld, Germany). A stirring speed of approximately 500 rpm in the cuvette was used continuously to prevent sedimentation of the suspended drug particles. After 12 minutes all lactose particles were fully dissolved for all samples and, immediately afterwards, the PSD of the suspended drug particles was measured for 10 seconds. The primary PSDs of fluticasone and salmeterol were measured in a similar way after a pre-suspension step comprising sonication of approximately 0.5 mg of the drugs in 2 mL of the saturated solution for 11 minutes using a 70 W, 42 kHz ultrasonic cleaner (Electris UC449UP, France). The amount of pre-suspension that was added to the CUVETTE was titrated to an optical concentration of around 10%. It was checked that the optical concentration and characteristic PSD data of the primary particles thus measured remained constant for at least 12 minutes. Because wet suspension methods as described here are sensitive to bias especially from (de-)agglomeration effects, the reliability of the data from these measurements will be discussed further on.

Scanning electron microscopy

Scanning electron micrographs of the drugs and blends were taken with a JSM-6301F (Jeol, Japan) at an acceleration voltage of 3kV and probe current 7. Samples were fixed to an aluminium specimen mount by means of double sided adhesive carbon tape. For the pure drugs any excess sample was blown from the tape with pressurised air and excess particles from the blends were gently tapped (instead of blown) from the specimen mount to avoid drug particle detachment from the carrier crystals. The drugs were sputter coated with 10 nm of a gold-palladium alloy, whereas for the mixture samples a coating thickness of 20 nm was found to be necessary to prevent charging effects.

Drug detachment experiments

An air classifier based test inhaler was used for the drug detachment experiments [17]. The design of the classifier used is such that > 95% of the carrier particles is retained during the dispersion measurement. The residual amount of drug present on the surface of the retained carrier particles after a dispersion experiment was analysed and normalised to 100% retention (referred to as carrier residue, CR). The percentage of drug detached was calculated as 100-CR. Note that drug detachment in this study refers to the relative amount of drug detached. Therefore, if drug detachment is concluded to be deteriorated with increasing drug content, the absolute amount of drug detached may have increased nonetheless. A dose weight of 25 ± 1 mg was used for the dispersion tests and all results presented are the mean of 5 measurements. All measurements were

performed at ambient conditions and with a fixed suction time of 3 seconds at 10 to 80 L/min (corresponding to pressure drops of up to 20.1 kPa). Such high pressure drops are not representative for the pressure drops to be attained by patients across dry powder inhalers [18]. However, the purpose of this study was not to simply conduct functionality experiments under conditions relevant to the practice of dry powder inhalation. Rather, the aim is to obtain a mechanistic insight into the effect of drug content, and it will become clear from the results presented in this chapter that this requires detachment to almost complete removal of all drug particles from the carrier surface. Finally it is to be noted that changes in drug detachment that are measured in this way do not always correlate to fine particle fractions, since large drug agglomerates may be detached too. However, because the first step to obtaining finely dispersed particles is drug detachment, understanding the mechanisms behind drug particle liberation from the carrier is highly relevant.

Sample analysis

Collected samples of the salmeterol, fluticasone, budesonide and salbutamol blends and carrier residues were spectrophotometrically analysed at wavelengths of 228, 228, 243 and 225 nm, respectively (Unicam UV-500, ThermoSpectronic, Cambridge, UK). Salbutamol was dissolved in demineralised water and all other drugs in ethanol. Samples were allowed to dissolve for at least one hour. Prior to UV-analysis the samples in ethanol were centrifuged for 5 minutes at 3000 rpm to obtain solutions free from suspended lactose (Hettich Rotanta D-7200, Hettich AG, Switzerland). Absorption values were corrected for any absorption caused by the dissolution of lactose when applicable.

Statistical analysis

A heteroscedastic 2-tailed Student's t-test was performed using Microsoft Excel 2010 to test the significance of the difference in drug detachment at 20 and 50 L/min between formulations containing 0.4% and 4% of drug.

Results

Content uniformity

The highest RSD in drug content measured was 2.04%, and therefore all blends prepared were sufficiently homogeneous and considered suitable for the dispersion experiments.

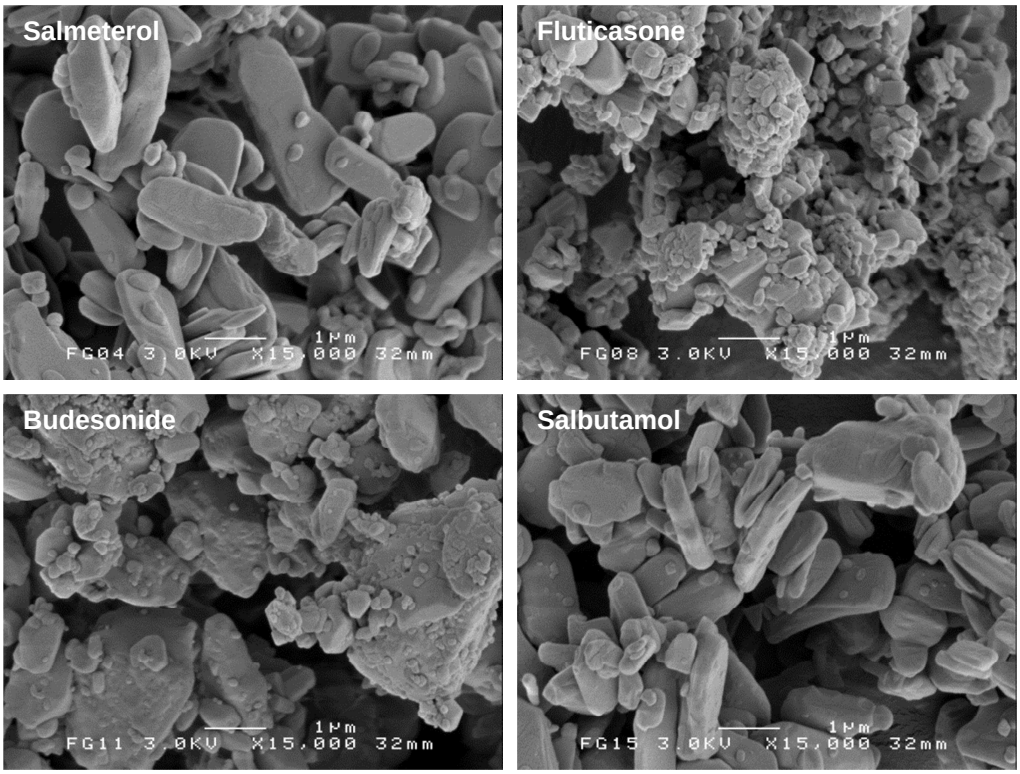


Figure 1: representative SEM images of the four micronised drugs.

SEM imaging

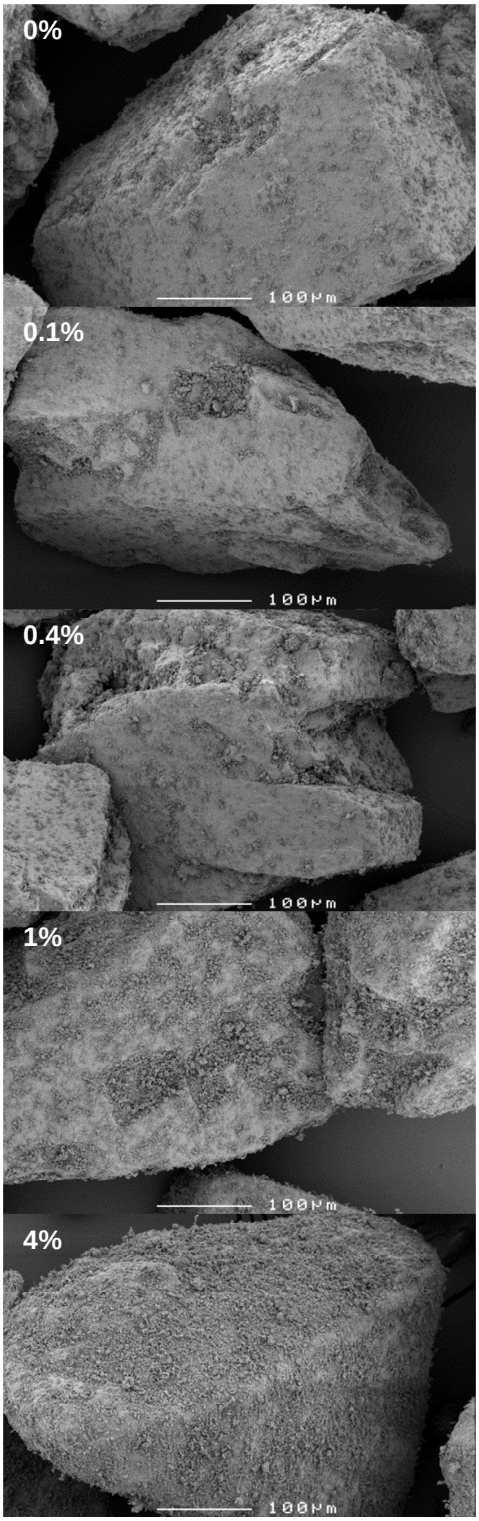
SEM images presented in Figure 1 show that both bronchodilator drugs salmeterol and salbutamol consist of plate like particles, whereas the corticosteroids budesonide and fluticasone are more irregularly shaped. In addition, the corticosteroids seem to contain more submicron particles, although this is not reflected in the X_{10} values obtained from RODOS dispersion (Table 3). This suggests that these fines are firmly attached to the coarser particles or comprise only a negligible volume fraction.

Table 3: PSDs (n = 2) and densities (n = 4) of the drugs.

Drug	X_{10}	X_{50}	X_{90}	V% ≤ 5 µm	ϱ (g/cm³ (SD))	Monolayer (%)*	$m_{particle}$ (10^{-12} g)**
Salmeterol	0.66	1.41	3.04	99.56	1.23 (0.004)	1.24	1.81
Fluticasone	0.72	1.80	3.99	95.66	1.36 (0.007)	1.75	4.15
Salbutamol	0.63	1.24	2.60	100	1.30 (0.012)	1.16	1.30
Budesonide	0.65	1.36	2.90	99.96	1.25 (0.008)	1.22	1.81

* The drug content for theoretical monolayer coverage is calculated as explained previously [15].

** Drug particle mass is calculated based on the X_{50} and density of the drugs.



The lactose carrier contains a notable amount of adhering lactose fines after being subjected to the same mixing conditions as the blends, despite the preceding classification procedure (Fig. 2, 0%). It is difficult to distinguish between these fines and the drug particles, particularly at a relatively low magnification of 250 x (Fig. 2, 0.1-4%). This limits the possibility to accurately monitor the spatial distribution of the drug particles over the carrier surface with SEM. At salmeterol contents of 1% and higher, carrier surface irregularities become filled up and covering of the carrier surface by drug particles becomes more apparent. For the other drugs the observations from SEM were similar.

In Figure 3, representative carrier surface close-ups of 4% mixtures are shown at the same magnification. No notable differences in the degree of agglomeration between the different drugs can be observed.

Pycnometry and laser diffraction analysis

True density values and characteristic values for the primary PSD of the drugs used in this study are presented in Table 3. PSDs of salmeterol and fluticasone in the adhesive mixtures as released from the carrier surface during the suspension experiments are shown in Figure 4. With increasing drug content the median diameter of the drug agglomerates on the surface increases. At drug contents > 1% continuous hollow drug

Figure 2: representative SEM images of the carrier after the mixing process (0%) and of salmeterol blends. The percentage indicates the salmeterol content of the mixture.

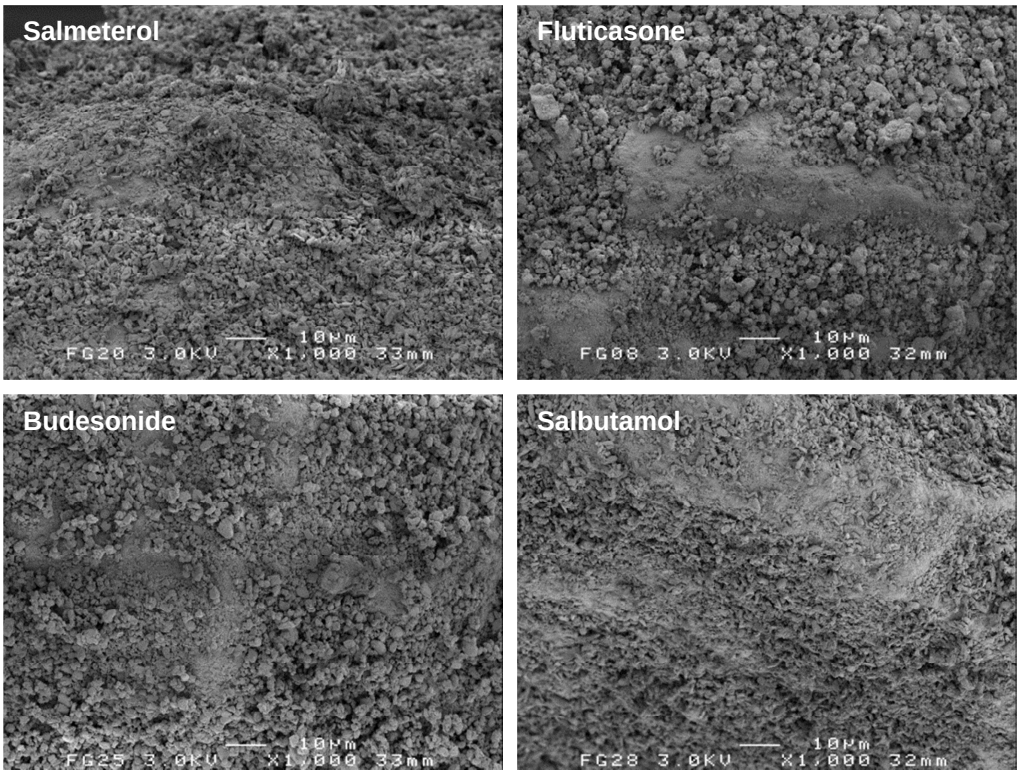


Figure 3: representative SEM images of the 4% mixtures.

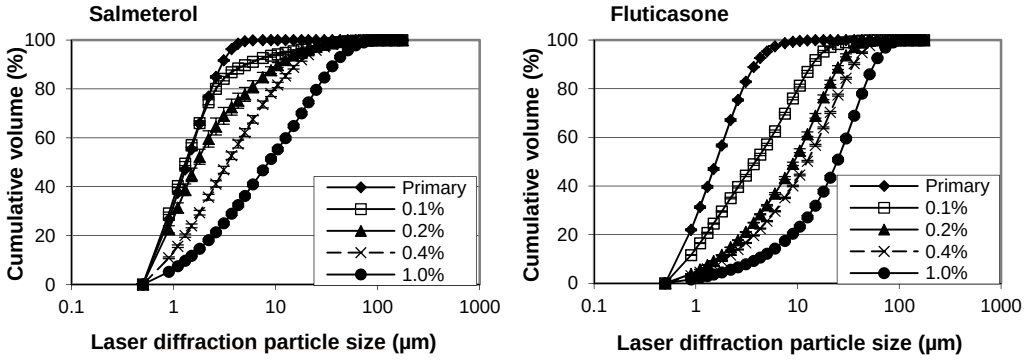


Figure 4: agglomeration behaviour within the mixture of salmeterol and fluticasone with increasing drug content. Data are obtained by laser diffraction in saturated aqueous drug solutions after complete dissolution of the lactose carrier. Y-error bars represent minimum and maximum values measured (n = 2).

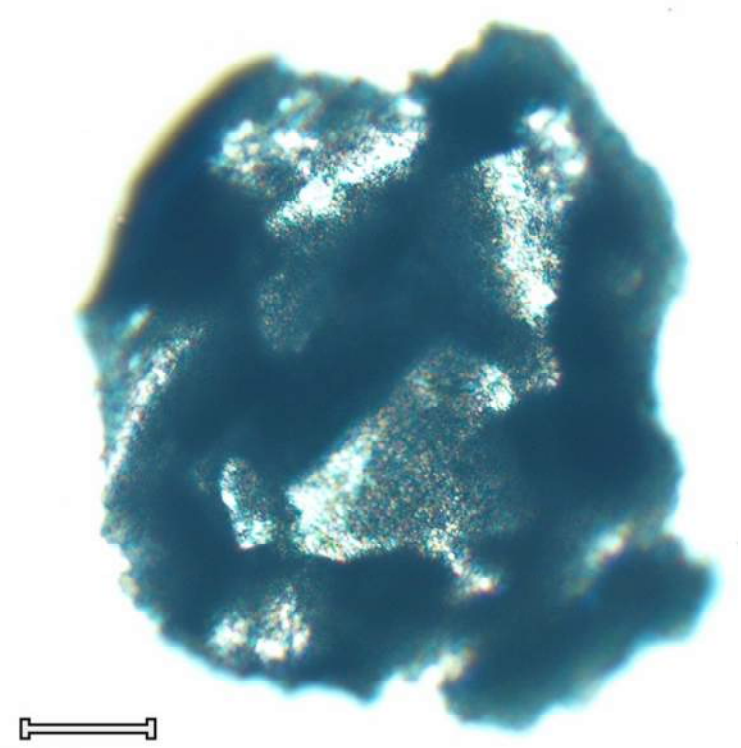


Figure 5: salmeterol network in suspension after dissolution of the lactose carrier from a 4% blend. The scale bar represents 60 μm.

particle networks remained in suspension after dissolution of the lactose carrier, as shown for 4% salmeterol in Figure 5. The X_{50} values of these hollow networks did not deviate much from those of the carrier crystals. Obviously, such values do not provide information on the size of detachable drug agglomerates but only on the size of the carrier particles, and therefore, these data are not shown. In many cases the shapes of the carrier particles from which the networks were detached remained recognisable after complete dissolution of the lactose.

Drug detachment experiments

Drug detachment as function of flow rate for the four drugs tested shows a less pronounced sigmoidal relationship at a drug content of 0.4% than for the 4% blends (Fig. 6). At flow rates below 30 to 35 L/min the relative amount of drug detached decreases when the drug content is increased from 0.4 to 4%, whereas at higher flow rates the effect is opposite. The difference in drug detachment at 20 and 50 L/min between formulations containing 0.4% and 4% of drug is statistically significant ($p < 0.001$) for all drugs used.

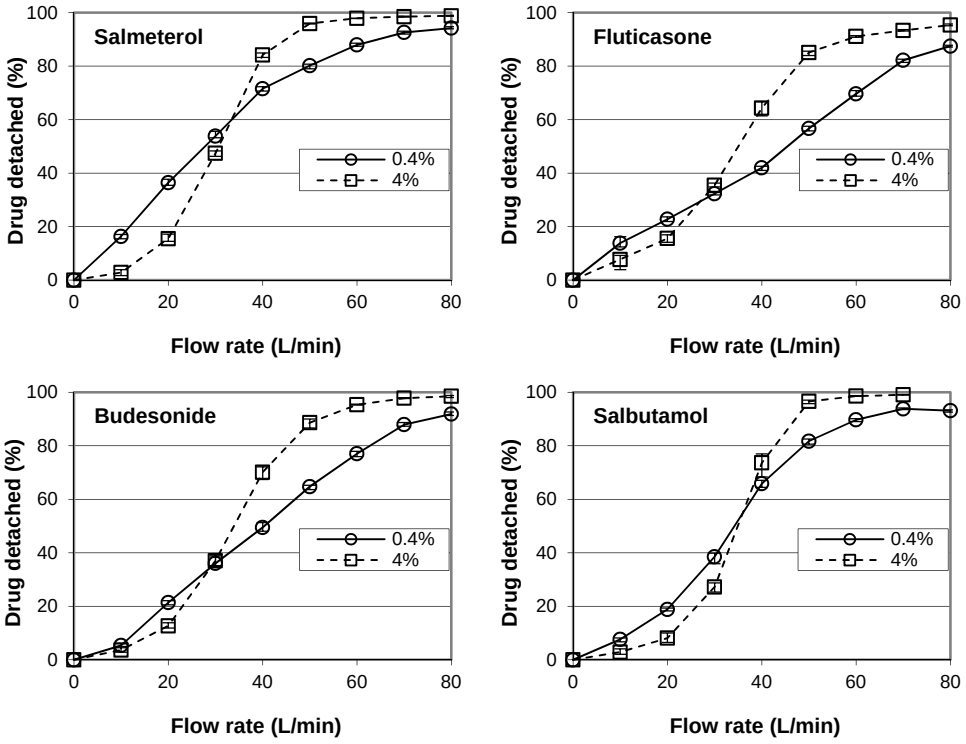


Figure 6: effect of drug content on drug detachment and its dependence on flow rate and drug type. The difference between the 0.4% and 4% curves represents the effect of drug content on drug detachment at the different flow rates. The mixtures were prepared by 10 minutes of mixing. The y-error bars represent maximum and minimum values measured (n = 5).

For salmeterol and fluticasone the relationship between drug content and drug detachment at different flow rates has been examined further by preparing blends with drug contents of 0.1%, 0.2%, 1% and 2% as well. For salmeterol, an increase in drug content from 0.1% to 0.2% leads to a higher drug detachment for all flow rates up to 60 L/min (Fig. 7). At flow rates from 10 to 30 L/min a maximum drug detachment is reached between drug contents of 0.2 and 1%. Drug detachment at higher contents tends to decrease for these flow rates before reaching a constant value at the highest drug contents. At 50 and 60 L/min, drug detachment continues to increase and approaches the 100% value at a drug content of 4%. Different trends are observed for fluticasone (Fig. 7). At all flow rates drug detachment decreases initially when the drug content is increased, starting at 0.1%. After reaching a minimum value at drug contents of 0.2% (at low flow rates) and 0.4% (at high flow rates), an increase in detachment is obtained when the drug content is further increased to 0.4% (low flow rates) and 1 to 2% (high flow rates), respectively. Further increasing the drug content results in a

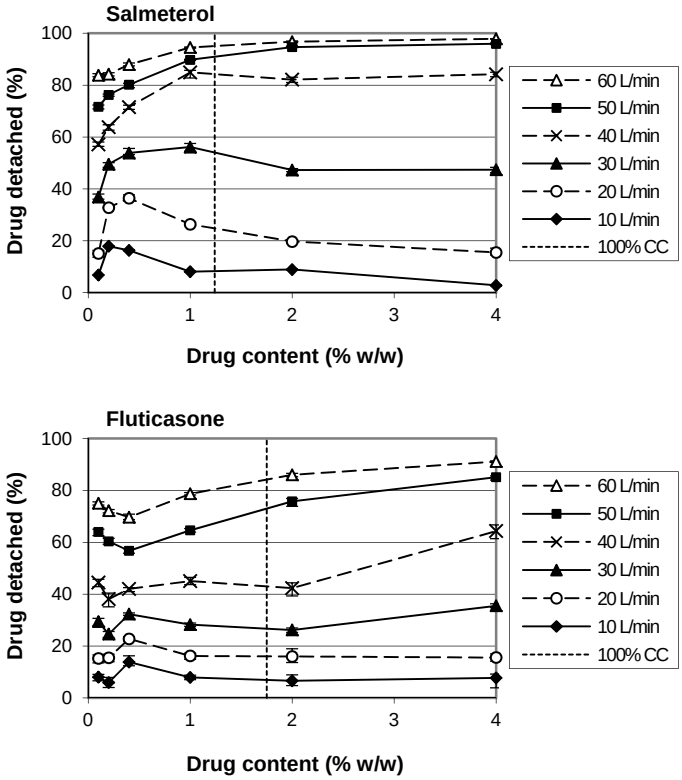


Figure 7: effect of drug content on drug detachment and its dependence on flow rate and drug type. In addition to Figure 6, the dependence on the drug content range is shown. The mixtures were prepared by 10 minutes of mixing. '100% CC' refers to the drug concentration corresponding to a theoretical monolayer carrier coverage. The y-error bars represent maximum and minimum values measured (n = 5).

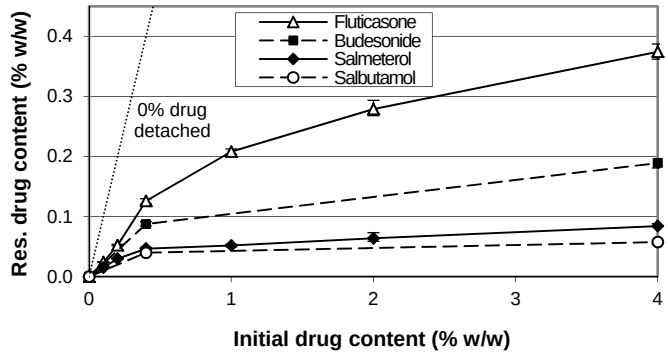


Figure 8: residual drug content after a drug detachment experiment at 60 L/min as function of initial drug content. Data are a different representation of the data presented in Figures 6 and 7. The y-error bars represent minimum and maximum values measured (n = 5).

decrease in detachment again at low flow rates, whereas detachment continues to increase marginally at 50 and 60 L/min towards a maximum value obtained at 4% drug content. It is difficult to draw unequivocal conclusions from these differences in fluticasone detachment behaviour between drug content ranges at the different flow rates, because the changes are small and stay within a narrow range of at most 8% of the initial drug content. From our experience with these drug detachment experiments we know that environmental conditions (especially relative air humidity) can very well account for differences of up to 5%, and therefore, some of these changes might not be physically relevant in relation to the changes in drug content, even if statistical significance could be proven.

A different representation of the drug detachment data at 60 L/min from Figures 6+7 is given in Figure 8. With increasing drug content the slope of the relationships decreases, which means that the fraction of drug that is not detached from the carrier decreases. This may be indicative for the saturation of active sites, as explained previously for similar relationships [11]. Different plateau values for the

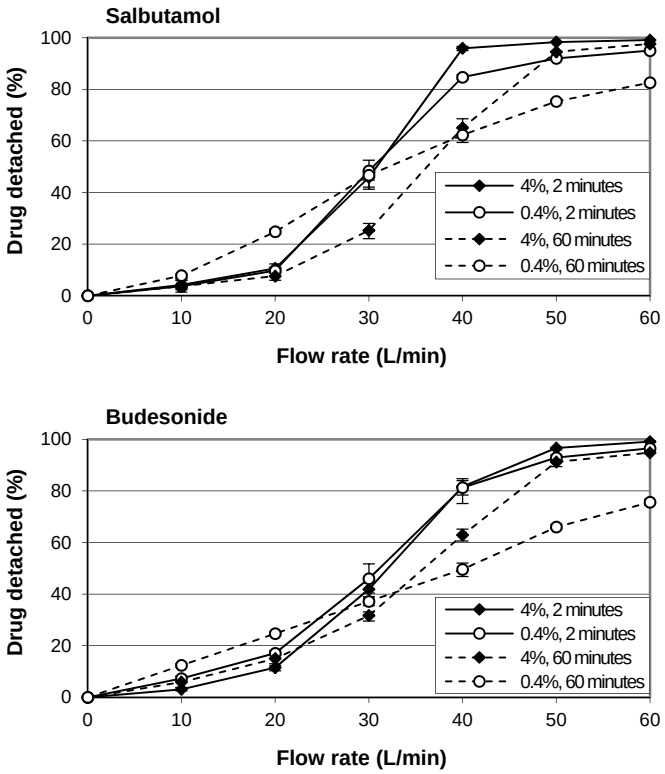


Figure 9: the effect of drug content on drug detachment and its dependence on mixing time. Data are shown for salbutamol and budesonide. The difference between the 0.4% and 4% curves for equal mixing times represents the effect of drug content on drug detachment at the different flow rates. The y-error bars represent maximum and minimum values measured (n = 5).

different drugs are reached upon extrapolation in the order of fluticasone > budesonide > salmeterol > salbutamol.

Figure 9 illustrates the role of mixing time as an interacting variable for the effect of drug content on drug detachment. After a relatively short mixing time of 2 minutes the absolute difference in drug detachment between a drug content of 0.4 and 4% is at most 11% for salbutamol and 5% for budesonide. These differences increase to 22% and 25%, respectively, after 60 minutes of mixing. At a drug content of 0.4% longer mixing results in higher drug detachment at 10 and 20 L/min for both drugs, whereas at higher flow rates drug detachment decreases. With a drug content of 4%, increasing the mixing time from 2-60 minutes results in a negative effect on drug detachment at intermediate flow rates of 30 and 40 L/min, whereas at flow rates ≤ 20 and ≥ 50 L/min the effect is negligible for both drugs.

Discussion

About the occurrence of the different mechanisms

Drug agglomeration. It is reasonable to expect that more drug agglomerates (with or without lactose fines) are formed, and that their size as they are detached from the carrier during dispersion increases when the drug content in the mixture is increased. Such an expectation is supported by the data presented in Figures 2-4. This results in a change of the ratio of separation to binding forces in such a way that drug detachment is enhanced, especially for inhaler devices relying primarily on inertial separation forces such as the device used in this study [17]. An absolute quantification of this effect based on the data from this study is not possible, however. During the wet laser diffraction measurements (Fig. 4) a slow decrease of the characteristic PSD descriptors (X_{10}, X_{50} and X_{90}) was observed after the 12 minute period in which the lactose carrier dissolved. This is likely the result of partial deagglomeration of the drug particles in the liquid and it may be expected that this process occurs during dissolution of the lactose carrier as well. The deagglomeration process may further be influenced by dissolution of fine lactose particles that have co-agglomerated with the drug particles. The size distribution of the agglomerates could thus depend on their strength in suspension, which, in turn, is likely to depend on the degree of co-agglomeration of the drug with lactose fines. The degree of deagglomeration in suspension may vary for different drugs, which limits the direct comparison between fluticasone and salmeterol in this respect. Nevertheless, the measurements proved to be highly repeatable, as can be concluded from the y-error bars in Figure 4. In addition, the observed trend is the same for both drugs investigated. Therefore, although the data may not be correct in absolute sense, they certainly are indicative of drug particle enlargement with increasing drug content. Furthermore, the

formation of drug layers and agglomerates is confirmed with SEM, especially at drug contents > 1% (Fig. 2+3).

Drug compression onto the carrier surface. An increased effectiveness of press-on forces is difficult to measure, but SEM images can be used to quantify at least the drug content above which the propensity for this effect will increase. The complete filling of irregularities in the carrier surface is approached within the range of drug contents between 0.4 and 1% (Fig. 2). From this content upward the efficacy of the mixing forces as press-on forces is likely to increase, which, as explained in the introduction, is expected to contribute in a negative way to the effect of drug content on drug detachment during inhalation.

Saturation of active sites. Based on the results presented in Figure 8 and the data presented in previous studies [10, 11], the occurrence of the saturation of active sites with an increasing drug content seems very plausible. However, at low drug contents (i.e. below theoretical monolayer carrier coverage) this effect is expected to be strongly dependent on the degree of preferential occupation of active sites: if no preferential occupation of active sites occurs during mixing, then saturation of active sites is not possible within this drug content range. Dispersion experiments do not provide the ability to distinguish between the saturation of active sites, agglomeration effects and press-on effects. Therefore, to definitively prove the occurrence of the saturation of active sites it is necessary to measure differences in the relative amount of drug attached to active sites (for example the non-detached drug fraction after dispersion at 60 L/min) between the different drug contents in the absence of agglomeration and press-on effects. Because in this study at least agglomeration already occurs from the lowest drug content used upward (as discussed), this is not possible. Strong (indirect) indications for the occurrence of the saturation of active sites as a result of changes in drug content have never been presented along with definitive proof of the absence of other mechanisms either. Therefore, although the saturation of active sites is likely to occur, it cannot be proven beyond reasonable doubt with the current data presented and the relevance of this mechanism to the overall effect of drug content in this study remains uncertain.

To further illustrate this problem, several hypothetical relationships between the residual drug content on the carrier after a drug detachment experiment at 60 L/min and the initial drug content in the blend are displayed in Figure 10. For the purpose of this example we define active sites as sites where drug particles can only be detached at flow rates > 60 L/min through the inhaler used in this study. Furthermore, we assume that 100% monolayer coverage of the carrier corresponds to a drug content of 1.5% and that the maximum binding capacity of active sites for the primary drug particles corresponds to a drug content of 0.2%. In situation 1 the preferential occupation of active sites is

complete, no agglomeration or press-on effects occur and multilayer coverage of the carrier by the drug does not result in the formation of new active 'apparent carrier surface sites' by underlying drug layers. Under such conditions the saturation of active sites is characterised by a relationship that follows the '0% drug detached' line (for which y = x) until a plateau value is reached that is equal to the binding capacity of active sites. The saturation of active sites results in a positive effect on drug detachment at drug contents > 0.2%. In situation 2 completely non-preferential occupation of active sites occurs (i.e. a random distribution of drug particles over all carrier sites is obtained), which causes active sites to be saturated only when the complete carrier surface is covered with a drug monolayer. In this situation the saturation of active sites results in a positive effect on drug detachment only at initial drug contents > 1.5% (i.e. the concentration at which the complete surface is covered). In situation 3 completely preferential occupation of active sites in combination with an increasing effectiveness of press-on forces with increasing drug content causes an increasing drug mass to be attached to 'pseudo-active sites' [19], thereby gradually increasing the total binding capacity of active sites. In the case of multilayer coverage of the carrier, underlying drug layers may also form new active sites to increase their total binding capacity. This results in a higher drug concentration after detachment at drug contents > 0.2% compared to relationship 1. Relationship 4 represents the situation in which drug agglomeration dominates the overall effect of drug content: a higher agglomeration potential of the drugs with increasing drug content causes the ratio of separation to binding force to increase due to agglomeration for an increasing fraction of the drug mass. As a result, the

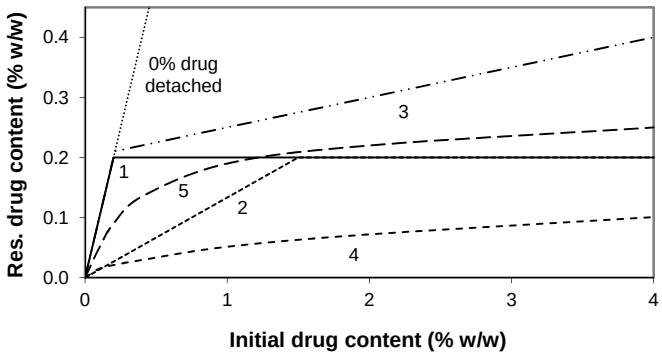


Figure 10: relationships between residual and initial drug content for different hypothetical scenarios. Residual drug content = drug content after a drug detachment experiment at 60 L/min. Drug contents that equal the maximum binding capacity of active sites and monolayer coverage are assumed to be 0.2% and 1.5%, respectively. The relationships represent: **1)** completely preferential occupation of active sites, absence of effects from agglomeration, press-on forces and layer formation; **2)** same as situation 1 with completely non-preferential occupation of active sites; **3)** same as situation 1 with effects from press-on forces and layer formation; **4)** same as situation 1 with a high degree of agglomeration; **5)** intermediate situation with an unknown balance of mechanisms.

saturation content of active sites (for the primary drug particles) may never be reached after detachment and saturation of active sites does not contribute to improved drug detachment with increasing drug content. Relationship 5 displays an intermediate situation between situations 3 and 4. The lower slope with increasing drug content is representative for a larger fraction of the drug mass being detached. In the absence of the exact binding capacity of active sites as a reference (which is the situation in practice), situation 5 cannot be distinguished from situation 4 when regarded individually. This means that the increased drug detachment may be completely the result of either agglomeration effects, or the saturation of active sites (with a gradually decreasing degree of preferential occupation of active sites with increasing drug content) or a combination of both. Therefore, only in the absence of agglomeration effects such a relationship, which is representative of those experimentally determined for the different drugs (Fig. 8), would be proof of the occurrence of the saturation of active sites.

About the variables that interact with the effect of drug content

The flow rate. The results presented in Figure 6 show that flow rate interacts with the effect of drug content in a quantitative and qualitative way. This is in agreement with the expectations based on energy ratio distributions discussed in chapter 2 of this thesis. The interaction could be explained by the flow rate causing a shift in the balance of the different mechanisms behind the effect of drug content on drug detachment. At low flow rates (< 30-35 L/min) the negative effect from press-on effects has to dominate any positive contribution from agglomeration effects or the saturation of active sites. At flow rates above 30-35 L/min a dominance of agglomeration effects and the saturation of active sites over press-on effects gives an opposite result. In the following section it will be discussed that this finding is strongly dependent on the chosen drug content range of 0.4 to 4%.

Because flow rate does not influence the properties of the powder mixtures, the shift in the balance of the different mechanisms concerns a change in their significance to drug detachment. For example, a decrease in the drug fraction that is only detached at flow rates > 60 L/min due to the saturation of active sites does not necessarily increase the fraction that is detached at flow rates < 30 L/min. It may also just increase the fraction that is exclusively detached at flow rates between 30 and 60 L/min. In that case, the saturation of active sites would be significant to drug detachment only at flow rates > 30 L/min.

The drug content range. As explained, the occurrence of press-on effects and the saturation of active sites may be strongly dependent on the range over which the drug content is changed. It therefore determines their balance and thus the overall effect of drug content on drug detachment. Strictly speaking, the drug content range is not an interacting variable for the effect of drug content, but a specific property of the variable

itself. Therefore, this interaction is an example of a conditional effect [20]. Its occurrence is confirmed by the results presented in Figure 7, which shows both quantitative and qualitative interactions. The difference between salmeterol and fluticasone in this respect shows that also a drug specific influence exists.

In the discussion about the occurrence of the three mechanisms it was reasoned that agglomeration is likely to occur starting at the lowest contents used, and that press-on effects become relevant from drug contents of 0.4% upwards. The concentration dependent salmeterol detachment at flow rates up to and including 30 L/min presented in Figure 7 can therefore be explained with an initial dominance of agglomeration effects (causing the initial increase in drug detachment between 0.1 and 0.2-0.4%) followed by a dominance of press-on effects with increasing drug content (causing the decrease in drug detachment). Apparently, at higher flow rates separation energies have become so high that the increase in binding energy by press-on effects has become insignificant. As discussed, the saturation of active sites may contribute to the overall effect too, but their relevance is uncertain.

The trends observed for fluticasone detachment within the same drug content range are not the same (Fig. 7). Considering the data presented in Figure 4, agglomerate formation also occurs for fluticasone. Apparently, the effect from agglomeration is balanced by the effect of press-on forces and the saturation of active sites in a different way as for salmeterol. A more effective redistribution of fluticasone towards active sites, a different interaction with the lactose fines that are still present on the carrier surface after sieving or a higher effectiveness of equally high press-on forces due to drug specific characteristics (which will be discussed further on) may explain this difference.

The mixing time. The data presented in Figure 9 show that mixing time interacts with the effect of drug content on drug detachment in a quantitative way. The small difference in drug detachment between 0.4% and 4% after 2 minutes of mixing for both drugs indicates that mixing is the driving force for the three mechanisms discussed. Within such a short mixing time insufficient 'chance effects' (i.e. drug redistribution towards active sites, drug de- or re-agglomeration and compression of the drug particles onto the carrier surface) seem to have occurred to significantly influence drug detachment. Alternatively, if these chance effects would have been sufficient to induce significant agglomeration and press-on effects and the saturation of active sites between 0.4 and 4% after 2 minutes of mixing, they would have to counteract each other over the range of flow rates that is applied. This is not very likely. Therefore, the mechanisms seem to become more significant during prolonged mixing but to a different extent, which magnifies any overall effect that results from their balance. It should be realised that the data from Figures 6+7 (obtained with 10 minutes of mixing) are therefore also to a great extent the result of the chosen mixing time, at least with respect to the magnitude of the observed effects.

An increase in drug content from 0.4-4% causes a notable interaction with the effect of mixing time on drug detachment too. At higher concentrations, higher flow rates are required to achieve the same relative amount of drug detached when mixing is prolonged. This negative effect is likely the result of a dominance of press-on effects. For the lower drug content, the effect of prolonged mixing more explicitly depends on the flow rate. The negative effect at the higher flow rates can be explained by a dominance of press-on effects and the redistribution of drug particles towards active sites. The positive effect at low flow rates seems contradictory to the redistribution of drug particles towards active sites, but it can be explained by agglomeration effects and the migration of drug particles towards sites of low binding activity nonetheless. The mixing forces cause partial agglomeration of the drug particles with each other and with lactose fines. These resulting larger particles are most easily detached due to a larger ratio of separation to binding force. Hence, they are likely first detached at low flow rates. Drug particles also find shelter from collisional and frictional mixing forces in carrier surface irregularities without necessarily being attached with a high binding force. Because the drug particles do not find shelter from inertial separation forces at these sites during inhalation, migration of particles towards these sites may result in a higher ratio of separation to binding force too and can thus be regarded as the redistribution of drug particles towards sites with a low activity. This process can very well occur concurrently with the redistribution of drug particles towards highly active sites. Which mechanism is most relevant for the effect of mixing time on drug detachment for mixtures that contain low drug contents will be the subject of a future study.

The type of drug. Previously presented values for the cohesion-adhesion balance (CAB) of salbutamol, salmeterol, fluticasone and budesonide in combination with alpha lactose monohydrate are 0.63, 2.39, 0.22 and 0.82 respectively [21, 22]. Although it has been described that CAB-values can be highly batch dependent [23], it is expected that the choice for the different drugs brings about a difference in adhesion and cohesion characteristics. This is supported by Figure 8, in which a higher plateau value indicates that the average binding force for that drug to lactose is higher, or that the average separation force is lower due to a lower tendency to form agglomerates (or both). In this respect the low CAB-value (0.22) of fluticasone could be indicative of a high intrinsic adhesion to lactose, whereas salmeterol (2.39) would be more prone to form agglomerates which are more easily detached [24]. However, the literature CAB-values for budesonide and salbutamol are not in agreement with the trends observed in Figure 8, and neither are the observations with SEM regarding the degree of agglomeration in Figure 3 (visually there seems to be hardly a difference in the degree of agglomeration between the different drugs). The different plateau values for the drugs could also be the result of a difference in their average particle mass, which is the result of the PSD of the drug and its density. In a classifier based test inhaler the ratio of separation to binding

force is lower for lighter particles and they will therefore be less likely to detach during inhalation. This would result in a higher plateau value in Figure 8. However, the drug with the highest calculated average particle mass (i.e. fluticasone, Table 3) also has the highest plateau value, and therefore, differences in the average particle mass neither explain the different plateau levels. Other drug specific characteristics that are of influence on the adhesive and cohesive interactions, such as the observed differences in particle shape and roughness, but also surface energy, moisture adsorption, deformability (e.g. Young's modulus, Poisson ratio) and electrostatic properties are thus more likely to be the cause of the observed differences.

Regardless of the underlying mechanism, the difference in cohesion and adhesion characteristics between the drugs is expected to cause a different balance of mechanisms with a change in drug content. For example, the saturation of active sites may be more pronounced for strongly adhesive drugs, especially when the drug content is increased beyond monolayer coverage of the carrier surface. For strongly cohesive drugs, however, agglomeration effects may be more pronounced. The data presented in Figures 6 and 9 show that the drug dependence of the effect of a change in drug content is expressed not so much in the direction as in the magnitude of the effect. However, from the data presented in Figure 7 it follows that an independence of the direction of the effect from the drug properties can be a result of the chosen drug content range. For example, a change in drug content from 0.1-0.4% results in opposite effects on drug detachment between salmeterol and fluticasone at flow rates of 40 L/min and higher. Therefore, the type of drug causes both qualitative and quantitative interactions with the effect of drug content, depending on the drug content range.

Other variables. The results from this study may be a consequence of the choice for other variables as well. For example, the dispersion principle of the test inhaler used relies mostly on inertial separation forces. This likely enhances the contribution of agglomeration effects to the overall balance of effects. Furthermore, the coarse crystalline carrier fraction used may cause higher press-on forces than a finer or granulated carrier fraction. The negative contribution of an increased effectiveness of these forces to the overall balance of mechanisms in this study may therefore have been more dominant than will be the case for other carrier materials. The opposite may be true for the low shear mixing process and small batch size that was used. Finally, the drug contents at which certain effects have been observed are likely biased by the presence of lactose fines on the carrier surface after the sieving procedure. This may especially be true at low drug contents, where the lactose fines comprise a significant part of the total fine particle mass.

About practical implications

Based on the results from this study it is conceivable that the differences in drug content between commercially available formulations result in a different dispersion behaviour. This is especially relevant for drugs from the same manufacturer that are available in different dose strengths. Furthermore, a drug to lactose ratio of 1:67.5 (1.46%) is not used in any of the formulations presented in Tables 1 and 2, which should stimulate researchers to use other than historical reasons for the choice of a certain drug to lactose ratio. Examples could be a certain desired carrier coverage (monolayer or multilayer), the desire to make use of the sheltering capacity of carrier surface irregularities or the approximate maximum binding capacity of active sites for the carrier-drug combination that is being used. In addition, it follows from the discussion that the drug content can greatly affect the balance of different mechanisms. This has to be taken into account when investigating the influence of other variables on the dispersion performance of adhesive mixtures.

In explaining the effect of added lactose fines on the dispersion performance of adhesive mixtures, the same mechanisms as for the effect of drug content may be expected. This means that also quantitative and qualitative interactions of flow rate with the effect of added fines can occur. Such interactions have been demonstrated previously for lactose fines with a similar size distribution to the drug particles [20]. The effects of added lactose fines on dispersion performance are further studied in chapter 5.

Conclusions

The effects of drug content on drug particle detachment from lactose carriers can be explained with a balance between agglomeration effects, press-on effects and the saturation of active sites. The flow rate, the type of drug, mixing time and the drug content range are shown to cause quantitative and qualitative interactions, likely by shifting the balance between the mechanisms in play. Our findings explain conflicting results that were previously reported in literature regarding the effect of drug content on dispersion performance. They furthermore irrefutably show that the choice for variables other than the ones under investigation should not be based on historical reasons, but rather on how they may interact with the primary variable(s) of interest. A higher drug content may lower the significance of a mechanism such as the saturation of active sites and can therefore shift the balance of effects of other variables towards agglomeration or press-on effects, as is shown for the effect of mixing time in this study.

Acknowledgements

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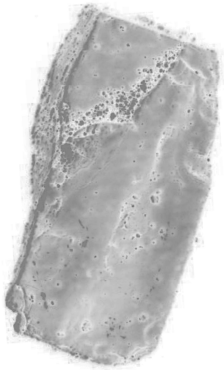

Chapter 4

Mixing time effects on the dispersion performance of adhesive mixtures for inhalation

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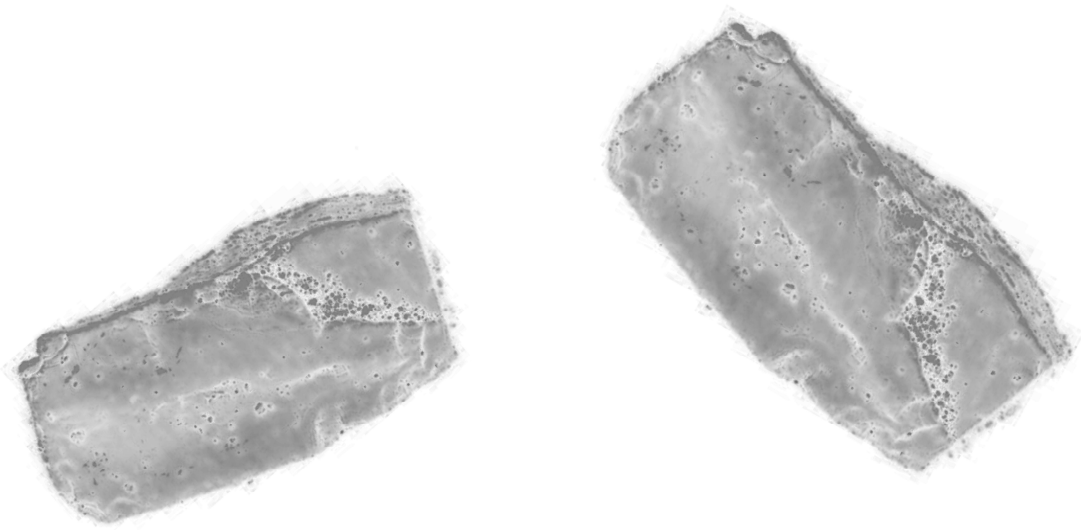

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Abstract

This chapter deals with the effects of mixing time on the homogeneity and dispersion performance of adhesive mixtures for inhalation. Interactions between these effects and the carrier size fraction, the type of drug and the inhalation flow rate were studied. Furthermore, it was examined whether or not changes in the dispersion performance as a result of prolonged mixing can be explained with a balance of three processes that occur during mixing, knowing drug redistribution over the lactose carrier; (de-) agglomeration of the drug (and fine lactose) particles; and compression of the drug particles onto the carrier surface. For this purpose, mixtures containing salmeterol xinafoate or fluticasone propionate were mixed for different periods of time with a fine or coarse crystalline lactose carrier in a Turbula mixer. Drug detachment experiments were performed using a classifier based inhaler at different flow rates. Scanning electron microscopy and laser diffraction techniques were used to measure drug distribution and agglomeration, whereas changes in the apparent solubility were measured as a means to monitor the degree of mechanical stress imparted on the drug particles. No clear trend between mixing time and content uniformity was observed. Quantitative and qualitative interactions between the effect of mixing time on drug detachment and the type of drug, the carrier size fraction and the flow rate were measured, which could be explained with the three processes mentioned. Generally, prolonged mixing caused drug detachment to decrease, with the strongest decline occurring in the first 120 minutes of mixing. For the most cohesive drug (salmeterol) and the coarse carrier, agglomerate formation seemed to dominate the overall effect of mixing time at a low inhalation flow rate, causing drug detachment to increase with prolonged mixing. The optimal mixing time will thus depend on the formulation purpose and the choice for other, interacting variables.

Introduction

Lacey was one of the first to describe the mixing process as the creation of disorder by allowing chance to determine the positions of the particles. With increased mixing time these chance effects accumulate, eventually resulting in a more or less stable equilibrium of maximum disorder [1]. The theoretical 'random' mixing process addressed by Lacey excludes particle interaction phenomena and is therefore fundamentally different from the 'ordered', or rather, 'total' mixing process that best describes the formation of adhesive mixtures for inhalation [2-4]. In both instances the role of mixing time is essentially the same, however, in that it allows chance effects to accumulate and therefore determines the extent to which certain processes within the mixture take place.

On blending of adhesive mixtures for inhalation several such processes can be distinguished. For example, it has been shown that drug agglomerates which are present in the starting material will be broken up [5, 6] and evidence suggests that at the same time new, less coherent agglomerates can be formed [7]. Furthermore, the inhomogeneous nature of lactose carrier surfaces allows for redistribution of drug particles to occur between surface sites with a different binding activity or sites with a different capacity to offer sheltering to drug particles from the redistribution forces during further mixing [2, 4, 8, 9]. In addition, repeated press-on forces and possibly triboelectrification effects may gradually increase interaction forces between drug and carrier particles [10-12]. Lactose fines might also be generated by attrition of the carrier, but this process seems to be restricted to high shear blending operations [13, 14].

The three processes that are expected to principally affect the dispersion performance of adhesive mixtures and that are therefore subject of this study can be summarised as agglomeration, distribution and press-on processes, respectively. They each may alter the dispersion performance of the mixture in a different direction and with a different magnitude. Therefore, it is to be expected that the overall effect of mixing time on the dispersion behaviour of any carrier-based inhalation formulation is primarily related to the obtained balance between these three principal processes. This also implies that any variable that alters this balance may interact with the effect of mixing time.

The carrier size fraction, the drug content and the flow rate were previously identified as variables that can interact with the effect of mixing time on drug detachment during inhalation [15, 16]. These variables either directly affect the different processes during mixing by influencing the potential and propensity for drug redistribution and (de-)agglomeration or the magnitude and efficacy of press-on forces, or they affect the significance of their occurrence in relation to drug detachment by altering the dispersion efficacy. For example, the agglomeration behaviour of drugs in adhesive mixtures was shown to be proportional to the carrier size fraction, or more specifically, the interparticulate pore size of the carrier powder bed [7, 17]. Furthermore,

the significance of a change in drug particle mass (agglomerate size) in relation to drug detachment from lactose carriers during inhalation was found to decrease with increasing dispersion efficacy (e.g. with higher flow rates through the inhaler) [18]. Therefore, for a given drug-carrier combination agglomerate formation is most likely to be a dominant process determining the effect of mixing time on drug detachment when coarse carrier particles are used and dispersion tests are performed at a low flow rate. Another variable that may change the balance of the different processes during mixing and could therefore interact with the effect of mixing time is the type of drug: the balance of intrinsic cohesive to adhesive interaction energy in combination with lactose can be different between drugs, and therefore, so may be their propensity towards agglomeration [19].

Mixing is unquestionably the most important unit operation in the formulation of adhesive mixtures for inhalation and mixing time is an easily controllable process parameter. Despite this, the effect of mixing time on formulation performance and its relation to all the other variables to be considered is still not fully understood for carrier-based inhalation formulations. As a start to straightening out this discrepancy this thesis chapter deals with the effect of mixing time on drug detachment for adhesive mixtures with a relatively low drug content. In particular interactions between mixing time and the type of drug, the carrier size fraction and the flow rate are investigated. Starting point of this study is the idea that the balance of mainly drug (de-)agglomeration, redistribution over and compression onto the carrier surface determines the overall effect of mixing time on drug detachment. Therefore, different characterisation techniques have been used in an attempt to measure or monitor the occurrence of these three 'principal processes' and, by that, to study their relative contribution to the overall effect.

Materials and methods

Starting materials

Alpha lactose monohydrate (Pharmatose 80M, DMV-Fonterra Excipients, Goch, Germany) was used to prepare the different carrier size fractions. Micronised salmeterol xinafoate and fluticasone propionate were granted by Novartis (Germany). These drugs were chosen for their difference in cohesion-adhesion balance in combination with lactose, which was previously reported to be 2.39 and 0.22, respectively [20]. The drugs were passed through a 90 μm sieve to break up larger agglomerates and triboelectric charge resulting from the screening process was allowed to decay for at least 2 days afterwards.

X-ray diffraction

The change in characteristics of drug particles when subjected to mechanical stress during mixing may depend on their initial solid state. For example, disordering or amorphisation of initially crystalline particles results in a higher apparent solubility [21, 22]. Therefore, the solid state of the drugs as used was measured by X-ray diffraction with a D2 PHASER equipped with a 1 mm divergence slit and a LYNXEYE™ detector (Bruker AXS B.V., Delft, The Netherlands). Approximately 15 mg of the micronised material was evenly spread on a zero background Si sample holder without the use of an adhesive. The sample holder was rotated at 60 rpm during measurement and it was made sure that the powder was still in place afterwards. An air-scatter screen of 1 mm was used to prevent radiation from the X-ray source to directly reach the detector. The scans were performed from 5 to 40 °2θ with a step size of 0.01 °2θ and a 1 s step duration. CuKα radiation with a wavelength of 1.5406 Å was generated at 30 kV and 10 mA. The measurements were performed on at least two different specimens from the same powder sample to ascertain the reproducibility of the obtained diffraction pattern.

Carrier classification

A coarse (250-315 μm) and a fine (63-90 μm) carrier fraction were obtained from Pharmatose 80M by 20 minutes of vibratory sieving at an amplitude of 1.5 mm (Retsch AS 200 control, Germany). To further remove lactose fines from the surface of the carrier particles, the carrier fractions were then air jet sieved for 10 minutes (e200LS, Hosokawa Alpine AG, Augsburg, Germany); the coarse fraction on a 250 μm sieve at an underpressure of 2000 Pa and the fine fraction on a 63μm sieve at 3000 Pa.

Blend preparation

Blends were prepared at ambient conditions. The coarse carrier fraction was blended with 0.4% of drug and the fine carrier with 1.48% to obtain a similar carrier surface payload (in mg/m^2, calculated based on the ratio of the arithmetic mean fraction diameters). The drug was sandwiched in between two equal parts of the carrier material in a stainless steel mixing vessel with a volume of 160 cm^3 and pre-mixed with a spatula for approximately 20 orbits. The blends were subsequently mixed with a Turbula blender operated at 90 rpm (WA Bachofen, Basel, Switzerland) for 0.5 to 780 minutes. Data for different mixing times are obtained from the same batch, starting with a batch size of 25 g which decreased to approximately 15 g for the final blending step due to the extraction of samples. For both carrier fractions placebo blends (containing only the carrier material) were prepared in the same way.

Content uniformity testing

Content uniformity of the blends was tested by taking 10 samples of 25 ± 1 mg from random positions. Blend homogeneity was considered acceptable at relative standard deviations (RSDs) of the content < 3%. The mean value of the 10 samples was taken as the drug content in the mixture.

Segregation sensitivity testing

To confirm a possible explanation for the content uniformity data, the segregation sensitivity of the salmeterol mixtures containing a coarse carrier and being mixed for 2 and 420 minutes was tested. To this end, 1 g samples were subjected to the described vibratory sieving procedure during 1 minute on a 150 μm test sieve. The salmeterol content of the sieving residue was determined from 5 samples of 25 ± 1 mg and expressed relative to the salmeterol content of the original blends.

Scanning electron microscopy (SEM)

The (re-)distribution and agglomeration behaviour of the drug on the carrier with mixing time was studied with SEM. Images were obtained with a JSM-6301F (Jeol, Japan) at an acceleration voltage of 3kV and probe current 7. Samples were fixed on an aluminium specimen mount by means of double sided adhesive carbon tape. For the pure drugs excess sample was blown from the tape with pressurised air. Any excess particles from the carrier material and blends were gently tapped from the specimen mount to avoid detachment of lactose fines or drug from the carrier crystals, respectively. The drugs were sputter coated with 10 nm of a gold-palladium alloy, whereas for the carrier and mixture samples a coating thickness of 20 nm was found to be necessary for preventing charging effects.

Laser diffraction analysis

All laser diffraction experiments have been performed with the HELOS BF diffractometer (Sympatec, Clausthal-Zellerfeld, Germany) equipped with an R3 lens (measuring range 0.5-175 μm) or an R5 lens (measuring range 0.5-875 μm, for the coarse carrier fraction). The FREE calculation mode was used, which is based on the Fraunhofer theory.

Dry dispersion. The particle size distributions (PSDs) of the drugs were measured after dispersion of the powders with a RODOS disperser at 3 bar (Sympatec, Clausthal-Zellerfeld, Germany). The PSDs did not change when the pressure drop for dispersion was increased to 5 bar, which indicates that the primary PSDs of the drugs were measured. Results are the mean of 2 measurements, which was deemed a sufficient number of replicates with the added control measurements at a dispersion pressure of 5

bar and considering the small deviations that were observed between individual measurements.

The PSDs of both lactose carrier fractions were measured in the same way at 3 and 5 bar. Approximately 2 g of the sieved and placebo blended material was fed to the RODOS disperser through a funnel. Results are the mean of 3 measurements.

Wet dispersion. To further quantify the qualitative information from SEM, the agglomeration behaviour of the drugs on the carrier with prolonged mixing was measured with laser diffraction too. The agglomerate size of the hydrophobic drugs as present in the screened starting material and in the blends was measured in aqueous suspensions using the CUVETTE SC-40 module (50 mL cuvette, Sympatec, Clausthal-Zellerfeld, Germany). A sample of the blend was added to saturated aqueous solutions of the drugs containing approximately 0.03% of polysorbate 80 (Tween 80). The particle size distributions of suspended drug agglomerates were measured for 10 s after precisely 12 minutes (coarse carrier) or 2 minutes (fine carrier); the duration in which the lactose completely dissolved for all samples. Dissolution of the lactose carrier was apparent from a disappearing peak corresponding to the size of the carrier material and further confirmed by optical microscopy of the suspensions (see further). A stirring speed of approximately 500 rpm was used throughout the entire procedure to prevent sedimentation of the suspended drug particles. It was made sure that the hydrophobic drugs did not adhere to the wall of the cuvette during measurement. Sample sizes were chosen such that an optical concentration of around 10% was obtained. It was checked that dissolved lactose did not influence the laser diffraction results. The primary PSDs of the drugs were measured after wet dispersion in a similar way after a pre-suspension step comprising sonication of approximately 0.5 mg of the drugs in 2 mL of the saturated solution for 11 minutes using a 70 W, 42 kHz ultrasonic cleaner (Electris UC449UP, France). The amount of pre-suspension that was added to the CUVETTE was titrated to an optical concentration of around 10%. It was checked that the optical concentration and characteristic PSD data of the primary particles thus measured remained constant for minimally 12 minutes. The chosen conditions and procedures are the result of their careful evaluation concerning reliability and reproducibility during many exploratory measurements. Results are the mean of at least 3 measurements.

Optical microscopy

Aqueous suspensions of the adhesive mixtures were inspected by optical microscopy to confirm the dissolution of the lactose carrier (BX50F, Olympus Optical Co., Ltd., Japan). Several drops of the suspensions were placed on a glass microscope slide without using a cover slip.

Solubility testing

The apparent solubility of salmeterol was measured as a means to quantify the degree of mechanical stress imparted on the drug particles during the mixing process. An aqueous suspension of salmeterol was prepared by suspending the micronised starting material in demineralised water containing approximately 0.03% polysorbate 80 (Tween 80). After sonication for at least 30 minutes in a Helma Transsonic 700/H ultrasonic bath (Elma Hans Schmidbauer, Singen, Germany) the suspension was stored in the dark for 1 week without stirring before further use. Thereafter, the suspension was passed through a 0.2 μm cellulose acetate filter to obtain a saturated solution. The apparent solubility strongly depends on the suspended drug concentration [21, 22]. Therefore, to 10 mL of the saturated solution, 0.7 mg of the salmeterol starting material was added or a sample of the different salmeterol mixtures that resulted in an equivalent added salmeterol mass (calculated based on the measured content). The resulting suspensions were regularly vortexed during 1 hour. Exploratory measurements showed that, for the longest mixing times, maximum dissolution is attained within 1 hour and that the concentration consecutively decreases towards the equilibrium saturation concentration in the course of several days to weeks. The procedure was continued by passing the suspensions through a 0.2 μm cellulose acetate filter. The samples were further analysed as discussed in the section 'spectrophotometric analysis'. Results are the mean of 2 measurements.

Drug detachment experiments

Drug detachment was measured by analysing the residual amount of drug present on the carrier surface after a dispersion experiment with a classifier based test inhaler [23]. The carrier crystals could be collected for analysis after a drug detachment experiment, since they were retained in the classifier of the inhaler. The residual amount of drug normalised to 100% of the carrier is referred to as 'carrier residue' (CR). The percentage of drug detached is calculated as 100-CR. Doses of 25 ± 1 mg were used for the drug detachment experiments, which were performed at flow rates of 20 and 60 L/min for a fixed duration of 3 seconds. Drug detachment experiments for the same flow rate and drug-carrier combination (at different mixing times) were performed on the same day to minimise environmental effects. Results are the mean of 5 measurements.

Spectrophotometric analysis

Samples from the content uniformity analyses and drug detachment experiments were analysed for salmeterol and fluticasone content by spectrophotometric analysis at a wavelength of 228 nm (Unicam UV-500, ThermoSpectronic, Cambridge, UK). Calibration curves for the concentration of both drugs were constructed with a coefficient of determination of 0.9998. All samples were dissolved in ethanol and subsequently centrifuged for 5 minutes at 3000 rpm (Hettich Rotanta D-7200, Hettich

AG, Switzerland) to clear the drug solutions from suspended lactose particles prior to measurement. If necessary, samples were diluted for the drug concentrations to fall within the range covered by the calibration curves.

The absorbance of the samples from the solubility tests was measured spectrofotometrically at a wavelength of 280 nm. The measurement at this wavelength avoids the necessity of dilution of the supersaturated salmeterol solutions in order for the absorption values to fall within the linear measuring range of the spectrophotometer used. Water containing 0.03% of polysorbate 80 and the dissolved pure lactose carrier (if applicable) was used as a blank. Because only the relative difference in the apparent solubility of salmeterol between the different samples is of interest, an exact quantification of the salmeterol concentration is not necessary and no calibration curve was constructed.

Results and discussion

Solid state of the drugs

The X-ray diffraction patterns of the drugs are presented in Figure 1. Judging from the sharp peaks and the lack of a 'halo' both drugs are crystalline.

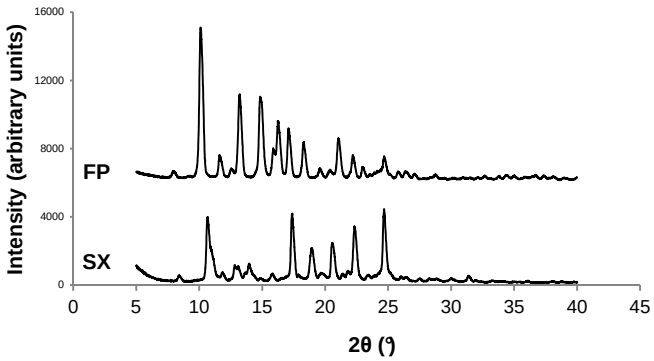


Figure 1: X-ray diffraction data for salmeterol xinafoate (SX) and fluticasone propionate (FP).

Particle size distributions of the drugs and carrier materials

Because the volume median diameters of the drugs are well within the desirable size range for inhalation of 1-3 μm (see Table 1), the drugs are considered suitable for use in these experiments.

Table 1. Characteristic PSD data (average (SD)) of the drugs (n = 2) and carrier material (n = 3).

	X50 (μm)	V% < 10 μm
Salmeterol (dry)	1.41 (0.01)	99.99 (0.01)
Salmeterol (wet)	1.43 (0.01)	100
Fluticasone (dry)	1.80 (0.06)	99.85 (0.03)
Fluticasone (wet)	1.59 (0.02)	99.53 (0.04)
Fine carrier	72.04 (0.28)	1.65 (0.06)
Fine placebo*	74.75 (0.60)	1.15 (0.51)
Coarse carrier	289.97 (4.29)	0.34 (0.41)
Coarse placebo*	288.69 (0.87)	0.15 (0.01)

* The fine and coarse carrier as obtained after the sieving procedure have been placebo blended for 600 and 420 minutes, respectively.

The carrier material as obtained after the classification procedure still contained a measurable amount of fines, which is presented in Table 1 as the volume fraction < 10 μm. Placebo mixing of both carrier fractions unexpectedly resulted in a trend of decreasing volume fraction < 10 μm. The loss of fines with mixing was confirmed by visual observation of the carrier particles with SEM (Figure 2). The reduction of the amount of fines may be explained by their adhesion to the inner walls of the mixing vessel, which resulted in a faint white haze being visible after placebo mixing.

The data from RODOS dispersion suggest that the fine carrier fraction contains a higher volume fraction of lactose fines than the coarse carrier fraction. However, because the specific surface area of the fine carrier fraction is approximately 3.7 times that of the coarse carrier (calculated based on the ratio of the arithmetic mean fraction diameters), their fines content per unit carrier surface area may well be comparable. This statement is supported by Figure 2, in which both carrier fractions are shown at a different magnification so as to display the carrier particles at the approximate same size. There is no notable difference in surface coverage by fines between the fine and coarse carrier fractions. Differences in the size distribution of the lactose fines may exist between the carrier fractions, however.

The appearance of lactose fines in commercial carrier products is inevitable as they cannot be effectively removed. They will influence the balance of the principal processes (i.e. drug (de-) agglomeration, redistribution and compression) during mixing of the blends to certain extent. Based on the placebo experiments it may be expected that in our study drug detachment will not be affected by mixing time through the generation of new lactose fines. Furthermore, any difference in the effect of mixing time on drug detachment between both carrier fractions is not likely to be the result of a difference in the covering of the carrier by lactose fines. However, it is not clear to what

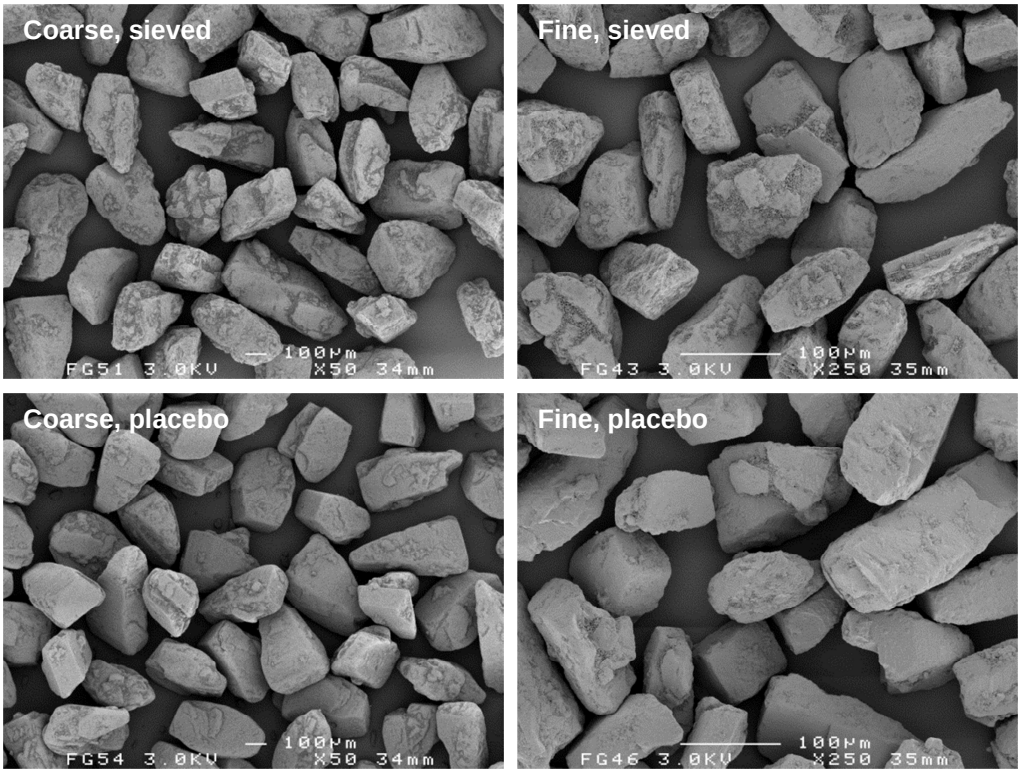


Figure 2: representative SEM images of the sieved and placebo blended carrier material. Mixing times for the placebo blends displayed are 420 and 600 minutes for the coarse and fine fraction, respectively.

extent this may be determined by other factors too, such as differences in the scale of carrier surface discontinuities between the carrier fractions.

Content uniformity

A trend of decreasing drug content with prolonged mixing was observed for both drugs mixed with the coarse carrier (Table 2, 0.4% of drug). This is the result of drug adhesion to the inside of the mixing vessel, which was observed as the formation of a white haze that became more opaque as mixing was continued. For the fine carrier, drug losses occurred mostly at the early onset of mixing after which the content stayed relatively constant (Table 2, 1.48% of drug). The difference in absolute drug loss to the mixing vessel wall between the carrier fractions is small (approximately 15 and 18.5 mg for the coarse and fine carrier fraction, respectively), which suggests that the drug loss is largely determined by saturation of the mixing vessel's inner walls, which is independent of the carrier type. The losses are of the same order of magnitude for both drugs.

Table 2. Content uniformity test results for blends containing 0.4% drug on a coarse lactose carrier or 1.48% drug on a fine lactose carrier (n = 10).

Mixing time (min)	0.4% salmeterol		0.4% fluticasone		1.48% salmeterol		1.48% fluticasone	
	Content*	RSD (%)	Content*	RSD (%)	Content*	RSD (%)	Content*	RSD (%)
0.5	95.3	0.91	97.4	1	95.5	0.96	96.5	1.23
2	94.9	0.89	97.1	0.8	96.5	0.75	95	1.47
10	93.5	0.61	94	0.82	96.7	0.37	95.7	0.53
30	90.3	0.56	90.7	0.56	96.3	0.86	94.5	0.68
60	87.3	0.92	89.9	0.51	96.4	0.49	94.4	0.88
120	76.5	0.82	86.1	0.73	96.2	0.72	93.9	0.88
420	84.9	2.54	84.2	1.16	94	0.89	92.6	0.34
600	-	-	-	-	93.4	0.82	94.7	0.41
780	-	-	-	-	-	-	94.6	0.49

* Content = % of drug weighed.

For all blends the RSD of the content is < 3% and they are therefore considered homogeneous (Table 2). No meaningful change in RSD with increased mixing time is observed, only the RSD of the 0.4% salmeterol mixture after 420 minutes of mixing is notably higher. This higher RSD accompanies an increase in segregation sensitivity. Between 2 and 420 minutes of mixing the drug loss resulting from 1 minute of vibratory sieving increases from 15.6% to 52.4%.

Agglomeration effects

Scanning electron microscopy. The relatively lower degree of homogeneity and increased segregation sensitivity for the 420 min 0.4% salmeterol mixture are likely the result of a high degree of agglomeration of the salmeterol (and possibly fine lactose) particles onto or in between the coarse carrier particles. For the large agglomerates of approximately 25 μm that are visible in Figure 3 (C1 and C2) gravitational forces start to dominate adhesion forces. This shifts the balance between randomisation and 'ordering' or adhesion in favour of randomisation [4]. The reproducibility of the formation of large agglomerates was confirmed by SEM for at least three different batches of 0.4% salmeterol prepared. A less pronounced agglomeration of salmeterol with prolonged mixing is observed on the fine carrier (Fig. 4). The agglomerates are less numerous and they are smaller than those on the coarse carrier (no larger agglomerates than about 10 μm could be found in the specimen). This observation suggests that agglomeration may occur in the carrier surface irregularities, which are larger for the coarse carrier. These data are in line with the conclusion from previous studies that the size of drug

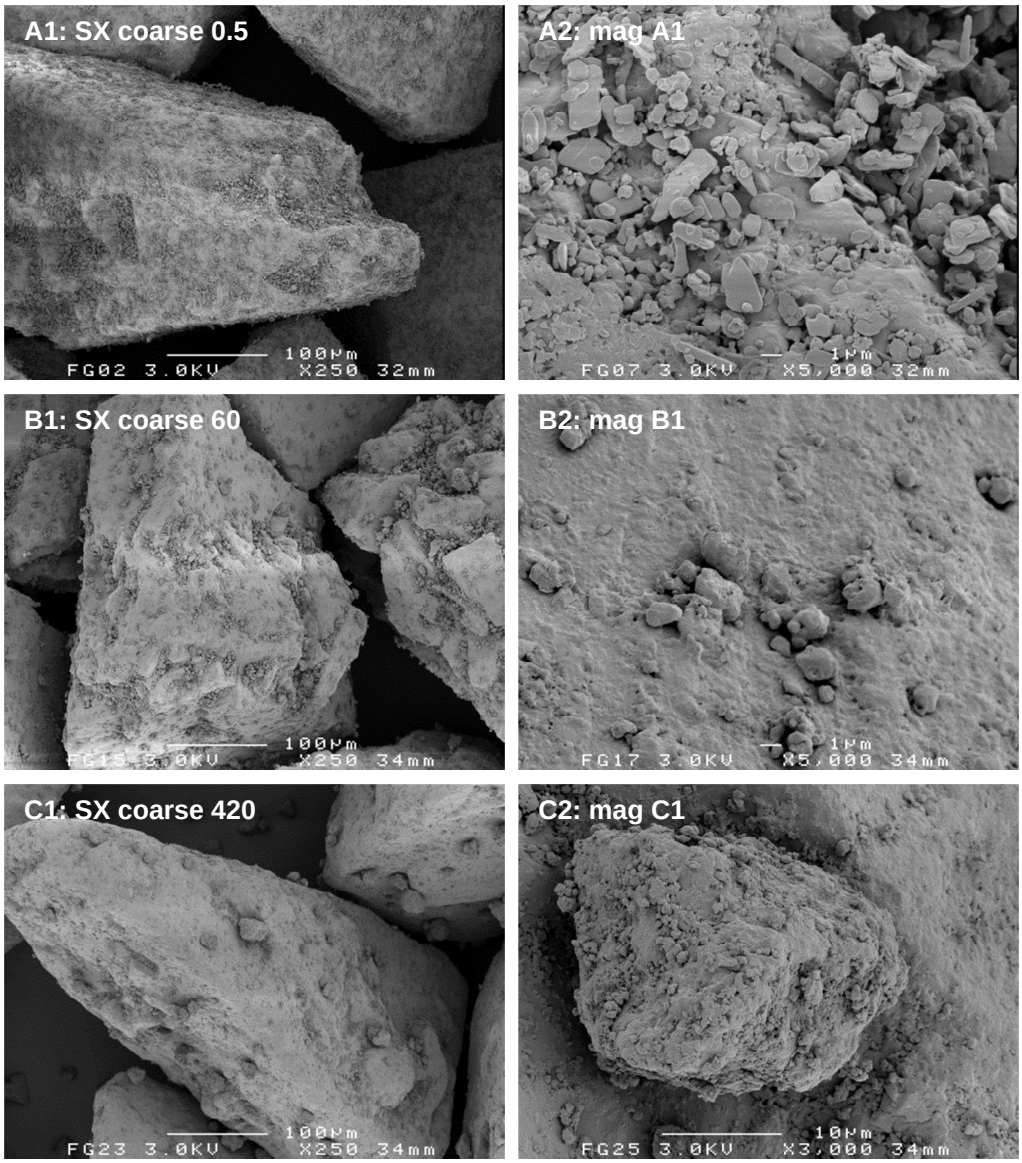
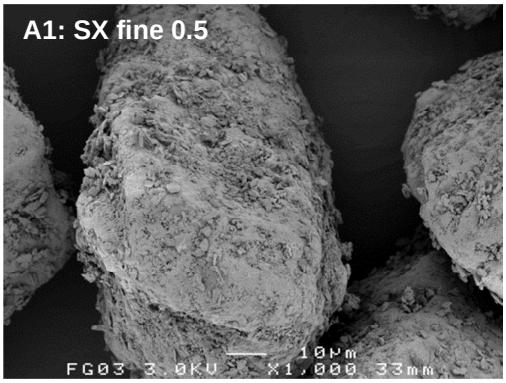


Figure 3: representative SEM images of the salmeterol blend containing a coarse carrier at different mixing times. Mixing times are given in minutes. Magnifications on the right hand side are taken from the images on the left hand side.

agglomerates after mixing increases with increasing carrier particle size [7, 17]. However, the conclusions from those studies are based on mixtures containing equal drug contents. This results in a higher carrier surface payload (mg/m^2) for larger carrier size fractions, which may also cause an increase in agglomerate size (as discussed in chapter 3 of this thesis). In our study a difference in carrier surface payload cannot have caused the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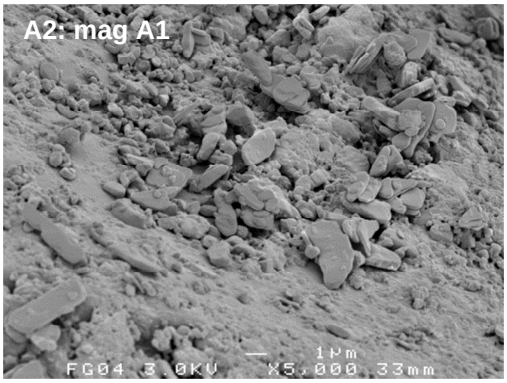
10µm
FG03 3.0KV X1,000 33mm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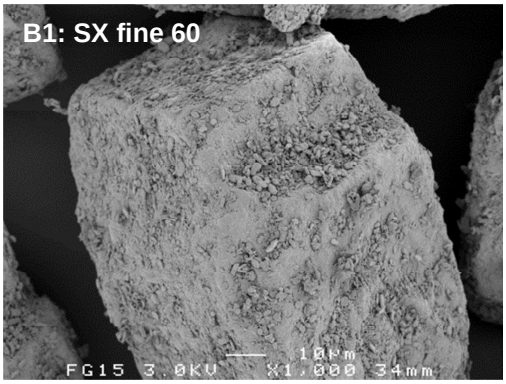
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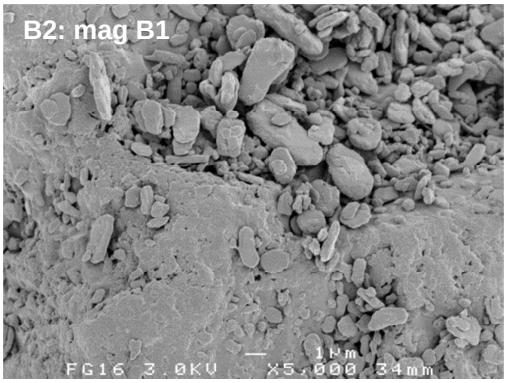
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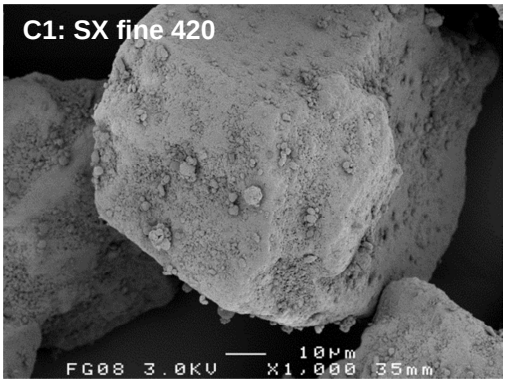
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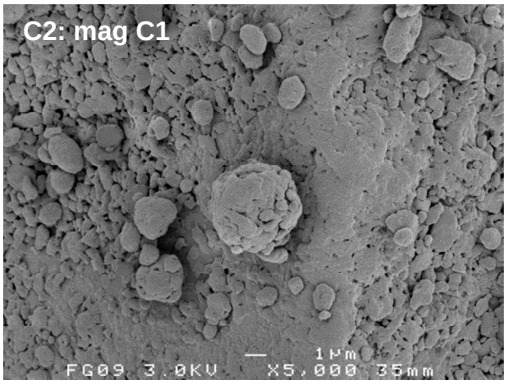
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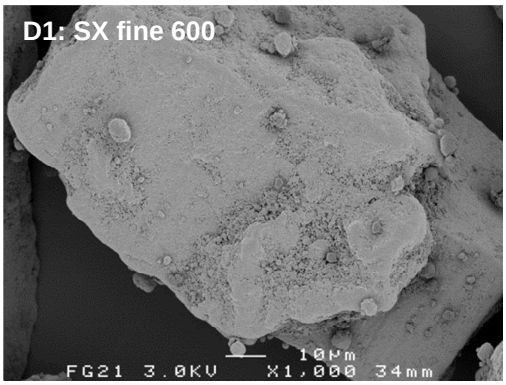
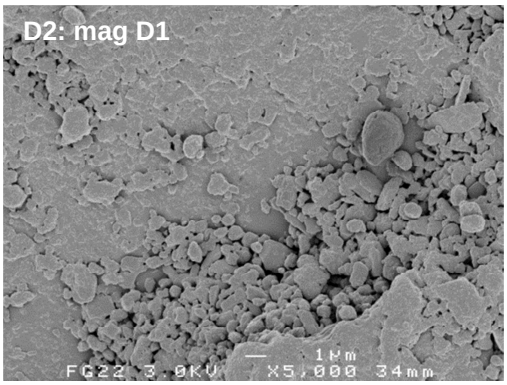
FG09 3.0KV X5,000 35mm


D1: SX fine 600
10µm
FG21 3.0KV X1,000 34mm


D2: mag D1
1µm
FG22 3.0KV X5,000 34mm

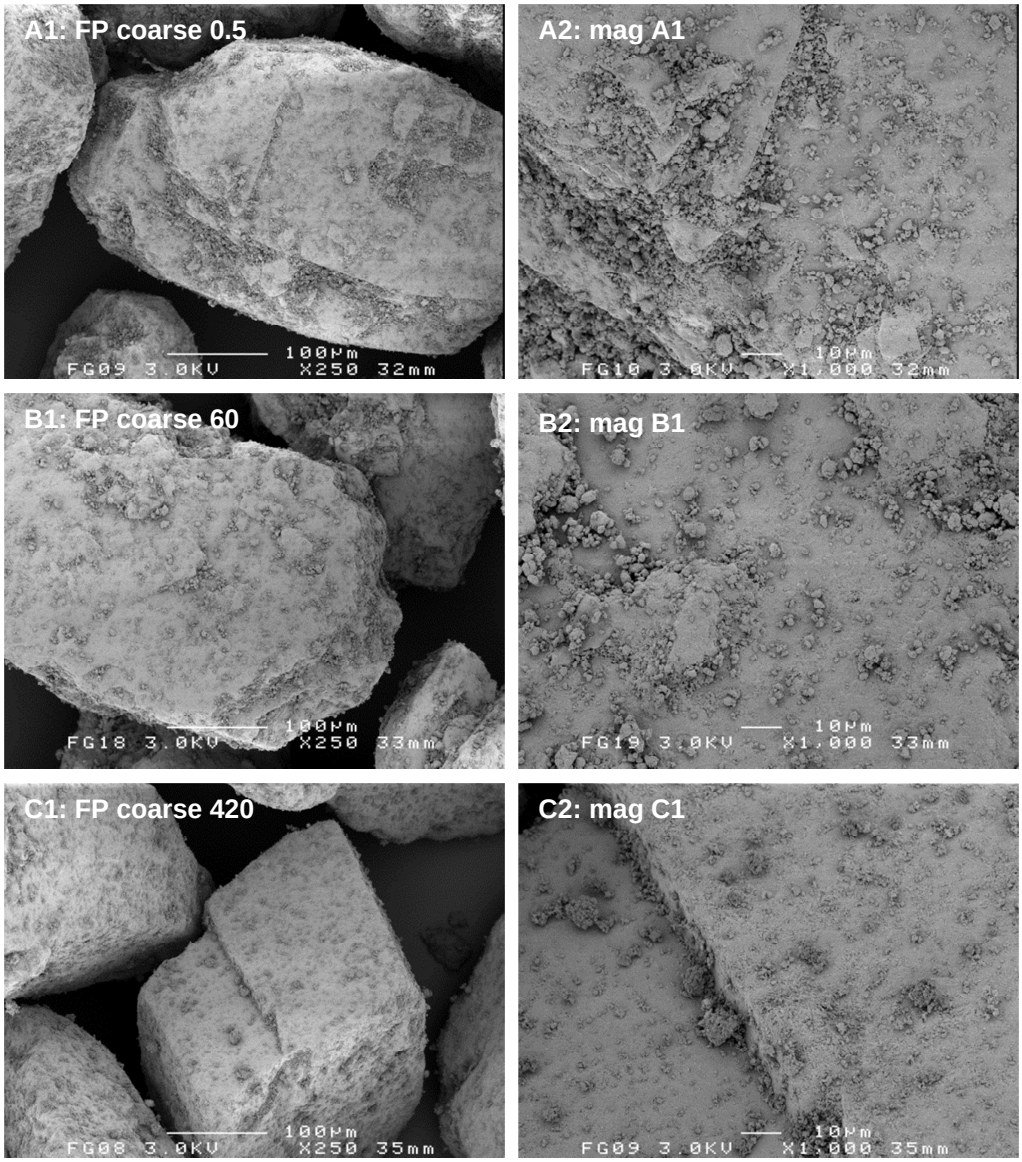
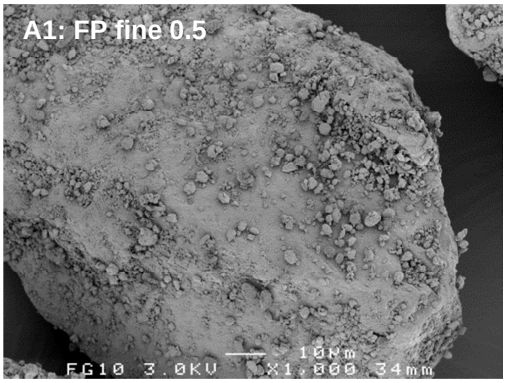


Figure 5: representative SEM images of the fluticasone blend containing a coarse carrier at different mixing times. Mixing times are given in minutes. Magnifications on the right hand side are taken from the images on the left hand side.

← **Figure 4: representative SEM images of the salmeterol blend containing a fine carrier at different mixing times.** Mixing times are given in minutes. Magnifications on the right hand side are taken from the images on the left hand side.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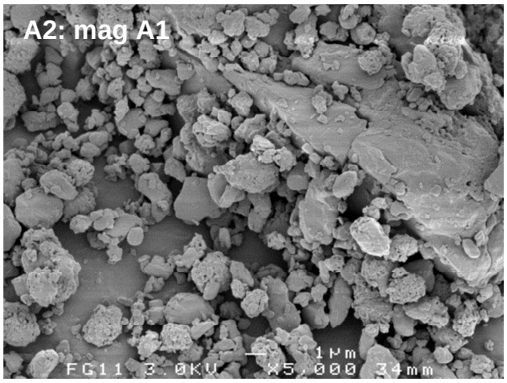
10µm
FG10 3.0KV X1,000 34mm


A2: mag A1
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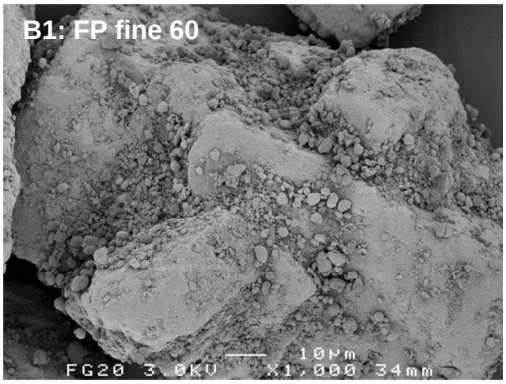
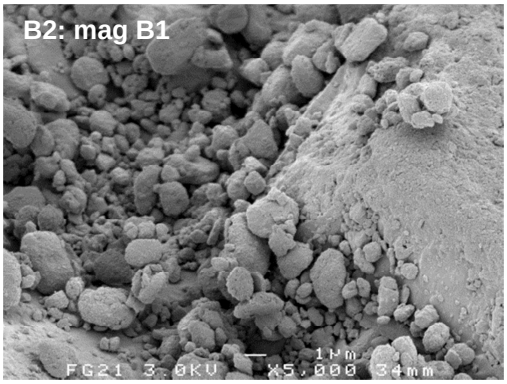
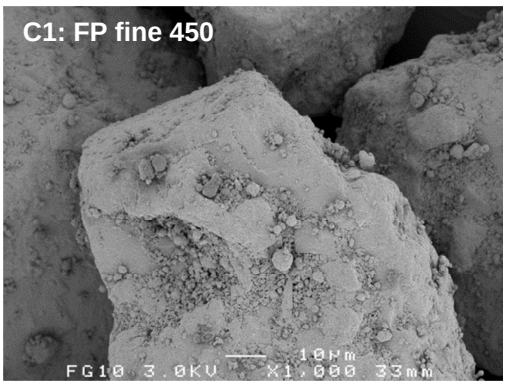
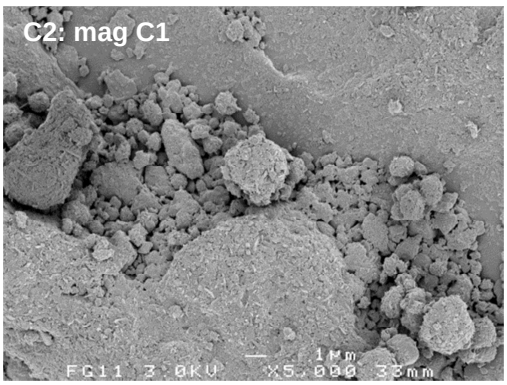
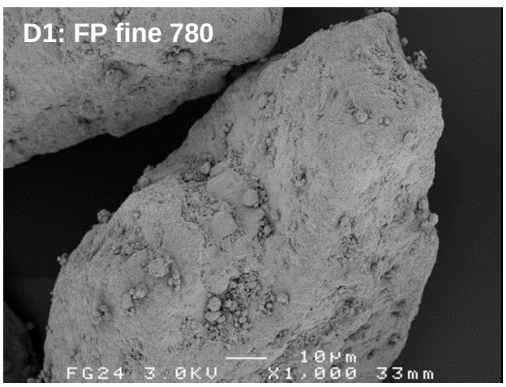
FG11 3.0KV X5,000 34mm

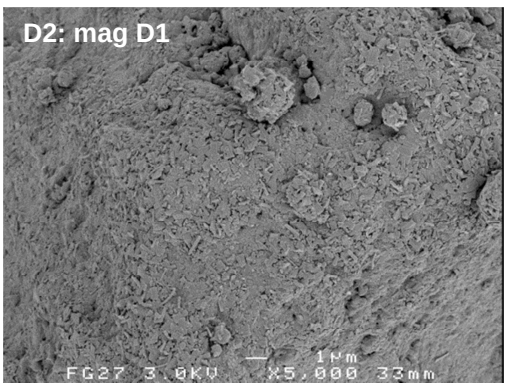

B1: FP fine 60
10µm
FG20 3.0KV X1,000 34mm


B2: mag B1
1µm
FG21 3.0KV X5,000 34mm


C1: FP fine 450
10µm
FG10 3.0KV X1,000 33mm


C2: mag C1
1µm
FG11 3.0KV X5,000 33mm


D1: FP fine 780
10µm
FG24 3.0KV X1,000 33mm


D2: mag D1
1µm
FG27 3.0KV X5,000 33mm

difference in agglomeration behaviour between both carrier size fractions, as it was kept constant.

The agglomeration behaviour of fluticasone is markedly different from that of salmeterol when mixed with the coarse carrier depending on the mixing time. Some fluticasone agglomerates could be found in the specimen after 420 minutes of mixing (Fig. 5, C1 and C2), but they are smaller (maximally about 10 μm) and less numerous than is the case for salmeterol (Fig. 3, C1 and C2). When mixed with the fine carrier, the difference in agglomeration behaviour between fluticasone (Fig. 6) and salmeterol (Fig. 4) is not as pronounced.

The difference in the propensity towards agglomeration between both drugs, which becomes especially notable after prolonged mixing with the coarse carrier, can be explained by a difference in their balance of intrinsic cohesive to adhesive interaction energy in combination with lactose. Values for the cohesion-adhesion balance (CAB) of salmeterol and fluticasone with lactose have previously been reported to be 2.39 and 0.22, respectively [20]. This means that the cohesiveness of salmeterol is 2.39 times its adhesiveness to lactose, whereas for fluticasone the adhesiveness to lactose is 4.55 times its cohesiveness. Although these results have been obtained with different batches of material (which may influence CAB values significantly [24]), they are in agreement with the greater agglomeration tendency of salmeterol than that of fluticasone observed in this study.

It should be noted that evaluations based on SEM images can lead to biased or incorrect conclusions. Disadvantages inherently associated with SEM imaging include the difficulty of representative sampling from the mixture, representative imaging of a specimen and the possible altering of the sample during its preparation. SEM images presented in this chapter are therefore images that have been obtained with utmost care to ascertain their representativeness. Their selection has been made after studying multiple samples and batches and imaging different spots of the same specimen. In addition, care was taken for the gentle handling of the powder during sample preparation. Nevertheless, one should keep in mind that conclusions from SEM imaging are based on a very limited number of observations, which is why laser diffraction was used in this study as an additional characterisation technique.

Laser diffraction analysis

The wet suspension laser diffraction method provides data on the agglomeration behaviour of salmeterol that are in agreement with the observations from SEM (Fig. 7). The X_{50} of 93 μm at t = 0 min represents the agglomerate size of the screened starting

← **Figure 6: representative SEM images of the fluticasone blend containing a fine carrier at different mixing times.** Mixing times are given in minutes. Magnifications on the right hand side are taken from the images on the left hand side.

material. These agglomerates are quickly dispersed during blending with the coarse carrier until a minimum X_{50} of only 2.71 μm is reached after 10 minutes, which approaches the primary particle size of the drug (Table 1). Continued mixing then results in a gradual increase of the X_{50} to a value of 25.4 μm after 420 minutes. As discussed, agglomerates of the same order of magnitude have been observed with SEM (Fig. 3, C1 and C2). For the fine carrier fraction, a minimum in X_{50} is reached after 120 minutes, which remains very much the same (around 5 μm) during continued mixing. Compared to the X_{50}-value of the primary salmeterol particles (Table 1) this confirms the occurrence of only minor agglomeration, as was concluded from SEM images too.

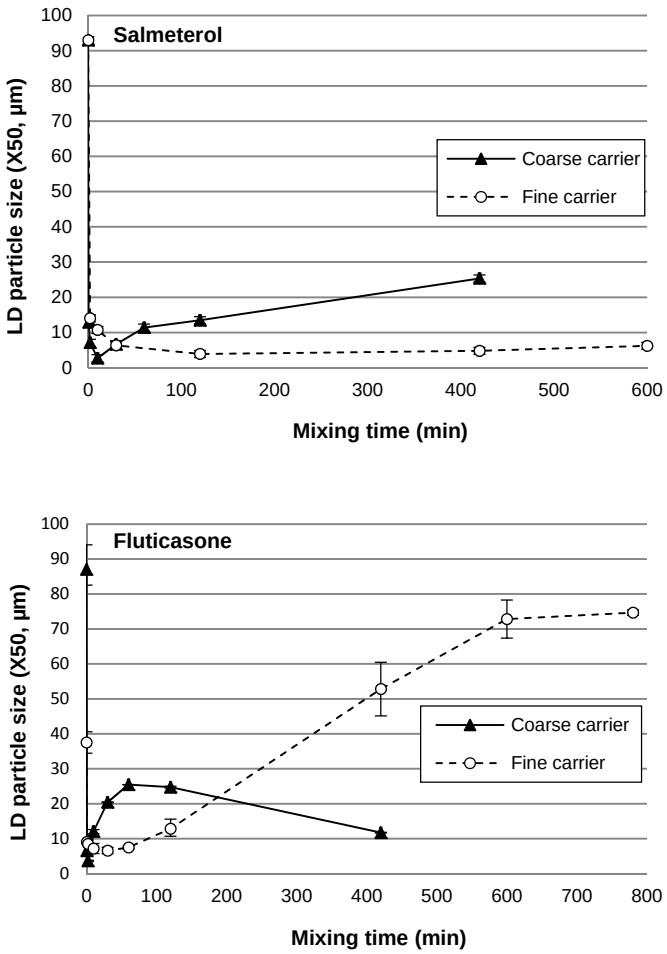


Figure 7: X_{50} **from laser diffraction analysis of suspended drug particles after dissolution of the carrier material.** Y-error bars represent minimum and maximum values measured (n = 2).

Although these data for salmeterol may be in agreement with the images from SEM, they are likely to be biased by dissolution effects and subsequent deagglomeration in suspension. It was noticed that with prolonged mixing a larger sample of the blends was required to attain the desired optical concentration of around 10% (up to a 6-fold difference between the shortest and longest mixing times for both carrier fractions). This observation can neither be explained by the slightly lower drug content with prolonged mixing (Table 2), nor by differences in the particle size distribution of the agglomerates (especially not for the fine carrier). It is much more likely the result of an increasing apparent solubility of salmeterol with increased mixing time that causes improved dissolution of the drug and thus the formation of a supersaturated solution (Fig. 8). Therefore, the conclusion has to be drawn that the salmeterol data in Figure 7 may to certain extent be biased by dissolution effects, especially for long mixing times. This may result in enhanced dispersion of agglomerates, and thus underestimation of the agglomerate size.

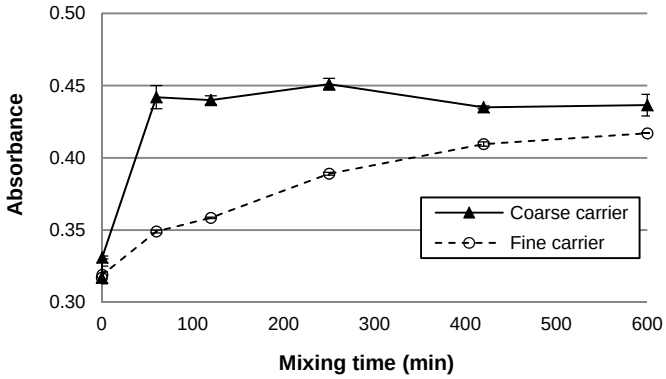


Figure 8: change in apparent solubility of salmeterol during mixing with the coarse and fine lactose carrier. The apparent solubility is represented by the absorbance of the solution at 280 nm.

For fluticasone, laser diffraction data (Fig. 7) are not in agreement with the SEM images (Figs. 5+6). With the coarse carrier, a maximum agglomerate size in the mixture of 25 μm is measured after 60 minutes of mixing, but agglomerates of this size could not be observed in the mixture by SEM (Fig. 5, B1 and B2). For the fine carrier the difference is even more pronounced with a maximum of 75 μm after 780 minutes of mixing determined by laser diffraction measurements, whereas only small agglomerates of maximally 4-10 μm could be found with SEM (Fig. 6, D1 + D2). The value of 75 μm coincides remarkably well with the size of the carrier particles. Light microscopic imaging of the suspensions revealed that this discrepancy is the result of the formation of

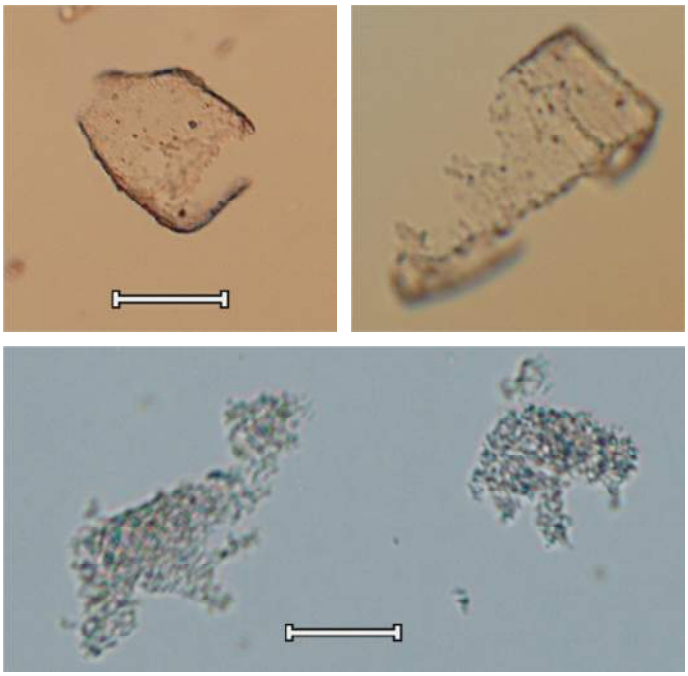


Figure 9: light microscopic images of suspended fluticasone films after dissolution of the lactose carrier. Top: 1.48% fluticasone mixed with the fine carrier for 600 minutes, the scale bar represents 60 μm; bottom: 0.4% fluticasone mixed with the coarse carrier for 420 minutes, the scale bar represents 15 μm.

insoluble, thin films that consist of the drug material and have sufficient structural integrity to remain intact in suspension after complete dissolution of the carrier. For the fine carrier these films have shapes similar to those of the carrier particles (Fig. 9, top), whereas for the coarse carrier the films cover a relatively smaller part of the particle surface (Fig. 9, bottom). Possibly the larger surface irregularities prevent the formation of a continuous film over the complete surface of the coarse carrier. Spontaneous re-agglomeration of drug particles after suspension is a well-known possible source of bias with wet laser diffraction measurements too. However, such a process is not likely to have contributed to the discrepancy between results from laser diffraction and SEM for fluticasone: no increase in the X_{10}, X_{50} or X_{90} values was observed for any of the mixture samples during or after dissolution of the carrier material or for the suspended primary particles.

The formation of a film or coating on the carrier surface by both drugs can be observed with SEM too, especially on the fine carrier (Figs. 4 and 6, D2). For salmeterol, film formation on the fine carrier could also be confirmed by suspension of the blend

mixed for 600 minutes in a supersaturated drug solution. Then, similar films as shown in Figure 9 (top) for fluticasone were observed by light microscopic imaging several minutes after submersion of the particles (images not shown). This confirms that dissolution and dispersion prior to the laser diffraction measurements (Fig. 7) must have occurred for salmeterol. The apparent solubility of fluticasone in the medium used in this study is too low to be reliably measured by spectroscopy, even after prolonged mixing. This explains why dissolution effects did not occur for fluticasone and the films remained intact in the suspension medium during the laser diffraction measurements.

Dissolving the carrier material to allow the measurement of drug agglomerates in suspension is a commonly applied technique [5, 6, 17]. However, such data are a measure of the strength of agglomerates as well as their size, because de-agglomeration can occur during the time required for dissolution of the carrier. This will be more pronounced for weaker agglomerates. In addition, fines of the same material as the carrier will be dissolved, which may cause the dispersion of composite agglomerates. Our results furthermore show that changes in the apparent solubility of the drugs and film formation may introduce additional sources of bias. With the mixing conditions applied in this study this is especially true for mixing durations longer than 1 hour. Therefore, the data from such wet laser diffraction methods have to be interpreted with good knowledge of all the processes involved and cannot be used unconditionally as a measure for the size of detachable drug agglomerates. To aid in their correct interpretation, supporting data from other characterisation techniques should preferably be provided.

Press-on effects

The increase in apparent solubility of salmeterol (Fig. 8) can be explained by the mechanical stress that is imparted on the drug particles during mixing. This causes disordering or amorphisation of the initially crystalline particle surface [25, 26]. Mixing with the coarse carrier results in a faster increase in apparent solubility than does mixing with the fine carrier. Apparently, the higher mass of the coarse carrier particles results in increased mechanical stress on the drug particles during collisions and this leads to faster amorphisation. Within 60 minutes of mixing a plateau is reached in the apparent solubility which is roughly 1.5 times the supposed equilibrium solubility of the starting material. This means that a maximum degree of disordering of the salmeterol particles is reached within this time or that the suspension concentration chosen in these experiments is not optimal for distinguishing further disordering [26].

The fact that the drug particles are subjected to significant mechanical stress during mixing does not only become clear from the increase in apparent solubility of salmeterol. SEM imaging of the blends reveals a change in morphology of the individual drug particles upon prolonged mixing too. This is most clearly illustrated in Figure 10, where the starting material is compared with agglomerates found in the mixture with a fine carrier after 420 (salmeterol) or 780 minutes (fluticasone) of mixing. Similar

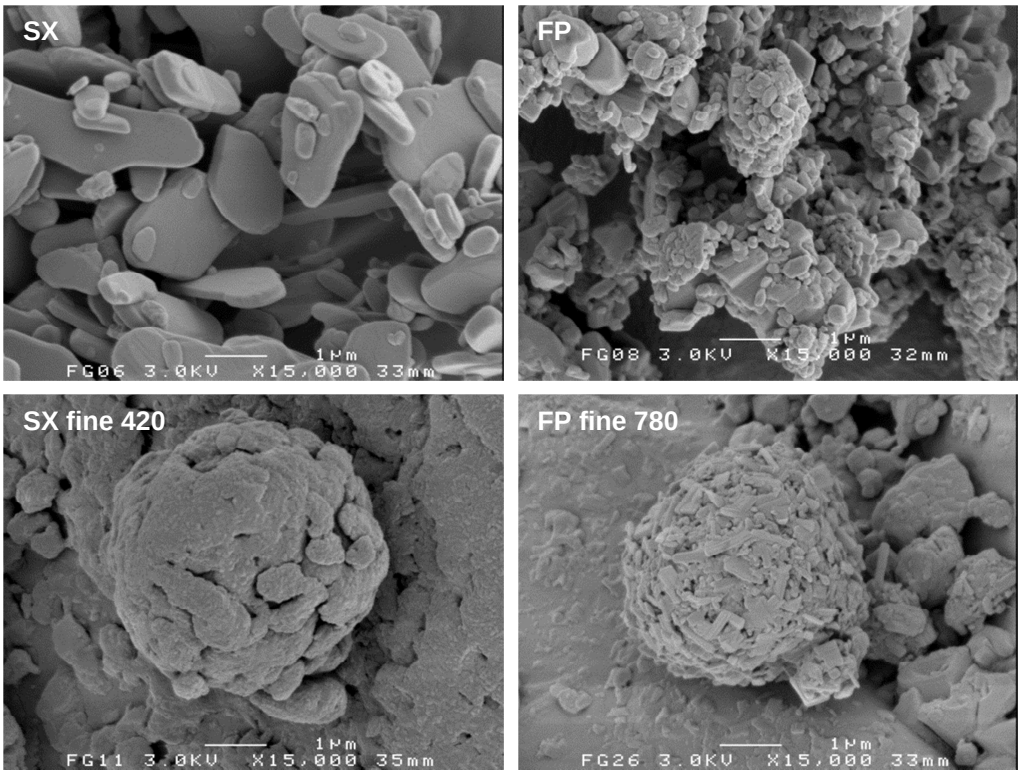


Figure 10: representative SEM images showing morphological changes to the drug particles occurring during mixing. Top: the salmeterol (SX) and fluticasone (FP) starting materials; bottom: drug agglomerates on the fine carrier after a certain mixing time (in minutes).

agglomerates and similarly shaped primary particles were not observed in the placebo blends. Therefore, the agglomerates shown in Figure 10 are likely to consist primarily of the drug component. The original plate-like salmeterol particles are extensively plastically deformed, whereas fluticasone appears to be fragmented into small needle shaped particles. In the mixture with the coarse carrier, plate-like salmeterol particles have mostly been deformed to spherical particles already after 60 minutes of mixing (Fig. 3, B2). When mixed with the fine carrier, the original plate-like shape of the salmeterol particles is still recognisable after 60 minutes of mixing (Fig. 4, B2). Only after 420 minutes of mixing their original shape is completely lost (Fig. 4, C2). Therefore, the conclusion from the apparent solubility data that greater mechanical stress is caused by the coarser carrier is in agreement with the SEM images. Apparently, the larger carrier surface irregularities of the coarser carrier particles do not offer sufficient protection to the drug particles from mechanical stress to balance the higher frictional and inertial forces that result from a higher mass of this carrier.

The introduction of a higher degree of solid-state disorder with prolonged mixing may affect the chemical stability of the formulation. In addition, recrystallisation at the drug-carrier interface during storage may severely affect the powder's dispersion behaviour in a negative way. The inertial and frictional mixing forces that cause an increase in apparent solubility may partly act as press-on forces, which cause increased drug-carrier interactions and thus negatively influence drug detachment during inhalation. Based on the faster increase in apparent solubility (Fig. 8) a greater negative contribution from press-on forces to the overall effect of mixing time on drug detachment may be expected for the coarse carrier than for the fine carrier.

Drug (re-)distribution

Drug particles are primarily located in carrier surface irregularities after 0.5 minutes of mixing (Figs. 3-6, A1 and A2). The presented data indicate that the drug particles are subsequently redistributed over the complete carrier surface during prolonged mixing, where they may become immobilised by compression on the carrier surface to form continuous, coherent films. In addition, certainly for salmeterol, a substantial fraction of the drug forms large agglomerates (whether preceded by drug redistribution into carrier surface irregularities or not). Agglomeration and compression (as a result of mechanical stress that concurrently causes the film formation and increased solubility) of the drug particles are especially pronounced after mixing for at least 60 minutes. Therefore, after such long mixing times, the redistribution of drug particles between carrier surface sites with a different intrinsic binding activity is likely to be of minor importance than press-on and agglomeration effects to the overall effect of mixing time on drug detachment. With shorter mixing distribution effects may be more significant.

Drug detachment

The data presented in Figure 11 confirm that the effect of mixing time on drug detachment is dependent on the type of drug, the carrier size fraction and the flow rate. The effect of mixing time is most pronounced within the first 120 minutes of mixing.

For salmeterol mixed with the coarse carrier, drug detachment at a flow rate of 20 L/min increases from 23.9% after 2 minutes of mixing to 43.4% after 420 minutes of mixing. These values are in line with the change in the amount of drug that is contained in easily detachable agglomerates, as was measured with the segregation sensitivity test (i.e. 15.6% to 52.4%, respectively). Therefore, the positive effect may be the result of a dominance of agglomerate formation. However, the maximum increase in drug detachment is already attained within the first 30 minutes of mixing, whereas agglomeration seemed to occur mostly from 10 to 420 minutes of mixing (based on the laser diffraction data in Fig. 7) or from 60-420 minutes of mixing (based on the SEM images in Fig. 3). Therefore, other effects may have contributed to the positive effect as

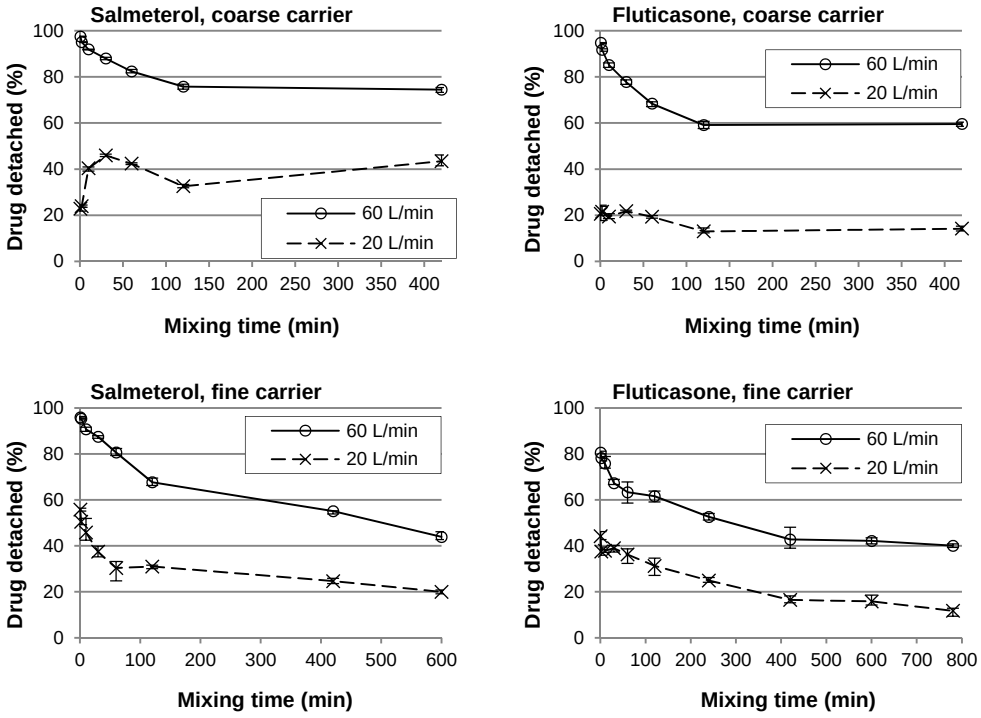


Figure 11: drug detachment at 20 and 60 L/min as function of mixing time. Y-error bars represent minimum and maximum values measured (n = 5).

well. The observed change in the shape of the salmeterol particles by SEM could be one of those additional effects (as shown in Fig. 3, B2). At 60 L/min, the effect of mixing time is dominated by press-on effects and possibly some migration of drug particles towards active binding sites (distribution effects). In the first 10 minutes of mixing deagglomeration may also contribute to a lower drug detachment (i.e. a lower ratio of inertial separation to binding force due to a lower drug agglomerate mass). As a result, drug detachment decreases from nearly 100% after 0.5 minutes of mixing to 75% after 120 minutes of mixing and an opposite effect from that at 20 L/min is thus obtained. In other words, not the whole drug particle mass is affected by mixing time in the same way. The salmeterol fraction that is contained in easily detachable agglomerates increases (Fig. 11: coarse, 20 L/min) concurrently with the fraction that is contained in a strongly bound film on the carrier surface (Fig. 11: coarse, 60 L/min). Which effect is measured depends on the dispersion efficacy (i.e. flow rate) during the drug detachment experiments. The fact that, at 60 L/min, a plateau in drug detachment is reached suggests that during mixing a dynamic equilibrium is established between drug that is present in the strongly bound film on the carrier surface and drug that is present as more readily detachable drug particles (including agglomerates).

In contrast to the coarse carrier, prolonged mixing with the fine carrier results in a negative effect on drug detachment at 20 L/min. Because the agglomeration of salmeterol is less pronounced with the finer carrier, the positive contribution from this effect is not dominant over any negative contribution from increased compression of the drug onto the carrier surface and redistribution of drug towards active binding sites. The greater dominance of press-on and possibly distribution effects is also apparent at 60 L/min, because no plateau value is reached and the minimum amount of drug detached is lower than with the coarse carrier (42% versus 75%, respectively). Based on the SEM images and apparent solubility data it was concluded that mechanical stress and thus press-on forces on the drug particles are lower for the fine carrier. This effect, which should result in improved drug detachment, is apparently offset by the decrease in agglomeration from the coarse to the fine carrier.

For fluticasone, drug detachment at 20 L/min stays relatively constant on continued mixing with the coarse carrier. This suggests that effects from agglomeration are balanced by press-on effects and distribution effects and are thus less dominant than is the case for salmeterol. This is in agreement with the lower degree of agglomeration that was observed for fluticasone. Also the lower plateau value in drug detachment that is reached at 60 L/min indicates that the dynamic equilibrium between drug being present as detachable agglomerates and as strongly adhering films is shifted towards the latter when changing salmeterol for fluticasone. The effect of mixing time is comparable for both drugs when mixed with the fine carrier. This too is in agreement with the less pronounced difference in their agglomeration behaviour (which for salmeterol is likely restricted by the smaller surface irregularities).

Practical implications

The results show that the mixing time should be carefully considered for the formulation of carrier-based inhalation powders. The optimal mixing time may vary depending on the formulation purpose and the choice for other, interacting variables. A balance between satisfactory homogeneity and mechanical stability on the one hand and dispersibility on the other may be achieved at relatively short mixing times. However, the effect of mixing time is also most pronounced at the start of mixing, and therefore, a sufficiently robust process may require longer mixing instead. For research purposes it may be desirable to stress press-on effects or agglomeration effects, which can be achieved by prolonged mixing, especially when using a coarse carrier fraction.

The need for a careful consideration of the applied mixing time is further stressed by the fact that the relevance of mixing time is not restricted to the homogeneity and dispersion performance of the powder formulation. The mechanical stress from (prolonged) mixing may influence the solid state of the drug and, with that, its dissolution behaviour (absorption) after inhalation and subsequent deposition in the lungs and the physical and chemical stability of the mixture during storage.

The long mixing times that have been applied in this study may not be representative for the mixing times applied in industrial dry powder inhalation formulation. However, many of the effects that are shown in is this chapter may occur at a higher rate when larger batch sizes and higher shear mixing principles are used, as is common for industrial processes. For example, it was shown that 1 hour of mixing with the low shear Turbula blender used in this study (operated at 90 rpm) results in a similar dispersion performance as 5 minutes of mixing with a lab-scale high shear blender (i.e. Picomix operated at 1000 rpm) [27].

Future perspectives

The overall effect of mixing time on drug detachment is likely to depend on more variables than the ones addressed in this chapter. Examples are the dispersion principle, the drug content, the carrier surface roughness, the mixing principle and the mixing intensity. Furthermore, the change in drug detachment is only an indication of the change in the maximum fine particle fraction that can be obtained, depending on the degree of agglomerate dispersion following detachment. Therefore, much is still to be learned from future investigations that address these factors in combination with the right techniques to measure or monitor the relevant powder properties.

Conclusions

Quantitative and qualitative interactions occur between the effect of mixing time on drug detachment and the type of drug, the carrier size fraction and the flow rate used. This can be satisfactorily explained with a balance of three processes which take place during mixing: i.e., drug (de-)agglomeration, compression onto and (re-)distribution over the carrier surface. A combination of SEM, laser diffraction techniques and the measurement of the apparent solubility of the drug can be used to qualitatively analyse these processes, but an exact quantification requires further improvement of these or the development of other techniques.

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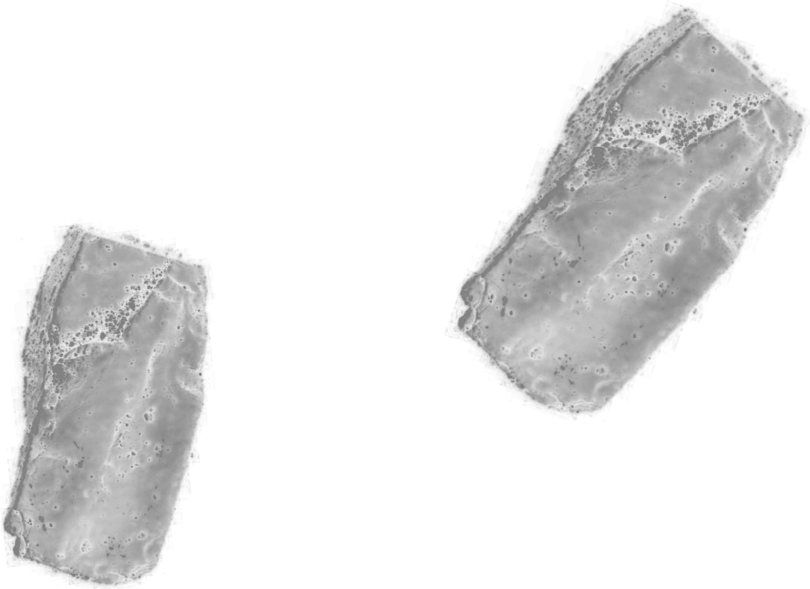

Chapter 5

New mechanisms to explain the effects of added lactose fines on the dispersion performance of adhesive mixtures for inhalation

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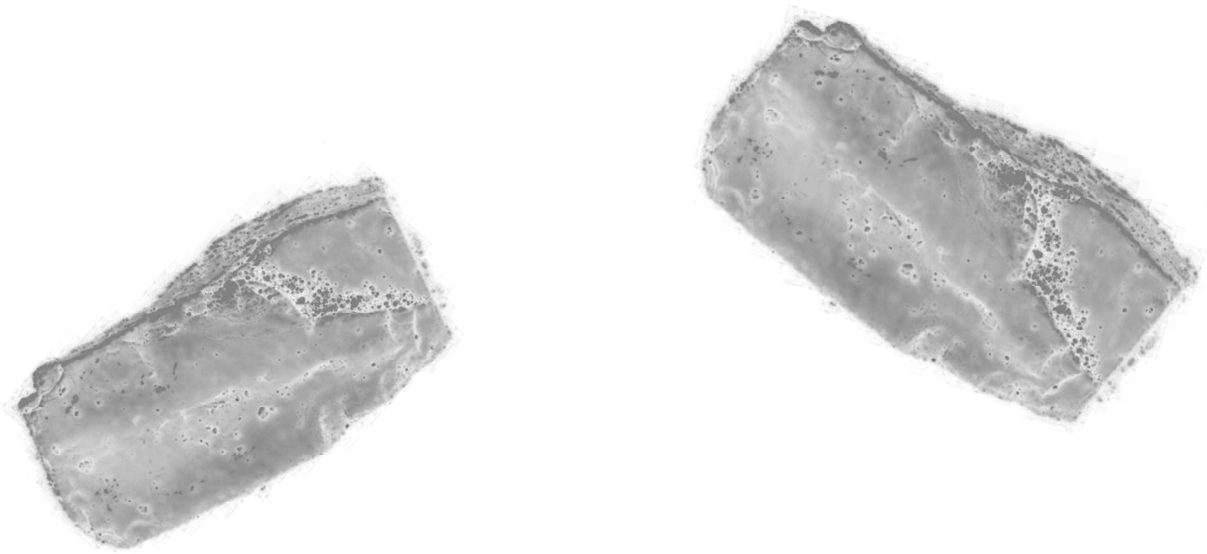

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Abstract

Fine excipient particles or 'fines' have been shown to improve the dispersion performance of carrier-based formulations for dry powder inhalation. Mechanistic formulation studies have focussed mainly on explaining this positive effect. Previous studies have shown that higher drug contents may cause a decrease in dispersion performance, and there is no reason why this should not be true for fines with a similar shape, size and cohesiveness as drug particles. Therefore, the effects on drug detachment of 'fine lactose fines' (FLF, X_{50} = 1.95 μm) with a similar size and shape as micronised budesonide were studied and compared to those of 'coarse lactose fines' (CLF, X_{50} = 3.94 μm). Furthermore, interactions with the inhalation flow rate, the drug content and the mixing order were taken into account. The observed effects of FLF are comparable to drug content effects in that the detached drug fraction was decreased at low drug content and low flow rates but increased at higher flow rates. At high drug content the effects of added FLF were negligible. In contrast, CLF resulted in higher detached drug fractions at all flow rates and drug contents. The results from this study suggest that the effects of fines may be explained by two new mechanisms in addition to those previously proposed. Firstly, fines below a certain size may increase the effectiveness of press-on forces or cause the formation of strongly coherent fine particle networks on the carrier surface containing the drug particles. Secondly, when coarse enough, fines may prevent the formation of, or disrupt such fine particle networks, possibly through a lowering of their tensile strength. It is recommended that future mechanistic studies are based on the recognition that added fines may have any effect on dispersion performance, which is determined by the formulation and dispersion conditions.

Introduction

The dispersion performance of adhesive mixtures for inhalation may be modified by the addition of a third, fine particulate component or 'fines' [1]. The resulting powders are often referred to as tertiary blends. Few techniques in the preparation of adhesive mixtures for inhalation have been given as much attention in scientific literature as the addition of fines, and an excellent overview of the majority of this literature is given by Jones and Price [2]. Fines generally improve the dispersion performance of adhesive mixtures, and different hypotheses have been proposed to explain this finding (Table 1).

Table 1: hypotheses concerning the working mechanism of added lactose fines.

Hypothesis	Explanation	Reference
Active sites hypothesis	Fines occupy so-called 'active sites' on the carrier surface, leaving only weaker binding sites available for the drug particles to bind to.	[3]
Agglomeration hypothesis	Fines form agglomerates, multiplets or multi-layers with drug particles, which are supposedly more easily detached from the carrier surface.	[3, 4]
Buffer hypothesis	Fines coarser than the drug particles may act as a buffer between colliding carrier particles and protect drug particles from press-on forces during mixing.	[5]
Fluidisation hypothesis	Fines increase the tensile strength of the bulk powder, which increases the minimum energy required for fluidisation and thus the energy available for dispersion.	[6]

Obtaining solid experimental support for the proposed hypotheses has proven to be a true challenge. As a result, the exact working mechanisms of added fines are largely unknown to date. This is not only the result of a limited number of techniques available to measure relevant powder properties. Interactions between formulation and dispersion variables also greatly add to the challenge [7]. This was, for example, shown in a study by Jones et al., in which the effect of lactose fines on dispersion performance was studied in relation to the drug content, mixing time and mixing order of the drug and fines [8]. The influence of any of these variables was apparently dependent on the levels of the other variables taken into account. Thus, interactions may explain why contradictory results have been obtained between studies on the working mechanisms of fines that were performed under different conditions, and hence, why the plethora of available data has not led to a fundamental understanding of powder performance [2].

The addition of lactose fines to adhesive mixtures in essence does not differ from an increase in drug content, since both result in a higher total amount of fines. Especially when the lactose fines have roughly the same size distribution, shape and

cohesiveness as the drug particles, an effect of added fines on dispersion performance similar to that of drug content may be expected, despite a difference in chemical composition [9]. This means that also the same mechanisms might play a role.

In chapter 3 of this thesis it was shown that the effects of drug content on dispersion performance may be explained by multiple mechanisms and that interactions likely result from a shift in their balance. Two of the mechanisms used to explain drug content effects have also been proposed as working mechanisms for the effect of fines in the agglomeration and active sites hypothesis (Table 1). However, under certain conditions, increasing the drug content resulted in a lower dispersion performance. Such an unfavourable effect cannot be explained by agglomeration or the saturation of active sites alone, since these mechanisms would lead to a better dispersion performance [3, 4]. It was proposed that the unfavourable effect of drug content on dispersion performance was caused by an increased effectiveness of press-on forces during mixing, since drug particles filled up carrier surface irregularities at higher contents and, thus, exhibited greater susceptibility to compressive mixing forces. Furthermore, the formation of strongly coherent drug particle networks on the carrier surface was observed, from which the drug may be difficult to detach. The inhalation flow rate interacts with drug content in both quantitative and qualitative ways and is, therefore, likely to cause a shift in the balance between the mechanisms in play.

Studies described in this chapter address the hypotheses that, analogous to the effect of drug content, the effect of added lactose fines on the dispersion performance of adhesive mixtures can be explained by a balance between multiple mechanisms and that these mechanisms may include an increase of the effectiveness of press-on forces or the formation of coherent networks. For this purpose, the effects of added lactose fines with a median particle size close to that of the drug (i.e. < 2 μm) on the dispersion performance of adhesive mixtures with different budesonide contents were studied over a range of flow rates. Furthermore, interactions with the size distribution of the fines and mixing order of the drug and fines were studied. These variables are expected to change especially the contribution of the 'buffer mechanism' and the 'active sites mechanism', respectively, to the overall effect of added fines.

Materials and methods

Starting materials

Alpha-lactose monohydrate of different grades was obtained from DFE Pharma (Goch, Germany). Pharmatose 80M was used to obtain a coarse size fraction of carrier particles, whereas Respitose ML006 was micronised to obtain lactose with a size distribution roughly comparable to that of the drug (i.e. fine lactose fines, FLF). Lactose fines coarser

than the drug (i.e. coarse lactose fines, CLF) were obtained by micronisation of a Lactohale product (Borculo Domo Ingradients, Borculo, The Netherlands). The FLF and CLF had been stored for at least one year under environmental conditions before use. During this period they have been exposed multiple times to air with a relative humidity varying closely around 50%, and therefore, any amorphous surfaces formed during the milling process had likely recrystallised over time. Micronised budesonide (Fagron, The Netherlands) was the drug used in this study. To break up larger agglomerates, the drug and lactose fines were passed through a 90 μm test sieve at least several days prior to preparation of the mixtures.

Carrier classification

Pharmatose 80M was sieved for 20 minutes with a vibratory sieve (Retsch AS 200 control, Germany) to obtain a carrier size fraction of 250-315 μm. The vibratory sieving procedure was then followed by 15 minutes of air jet sieving (Alpine A200, Augsburg, Germany) to remove as many intrinsic lactose fines from the carrier material as possible. The carrier classification procedure is the same as that used in the study on drug content effects presented in chapter 3, which allows the data from both studies to be compared.

Laser diffraction analysis

Particle size distributions of the mixture components were measured with the HELOS BF laser diffractometer after dispersion with a RODOS powder disperser at 3 bar (Sympatec, Clausthal-Zellerfeld, Germany). For the drug and lactose fines a 100 mm lens with a measuring range of 0.5/0.9-175 μm was used, whereas the carrier material was measured with a 500 mm lens (4.5-875 μm measuring range). Data are based on the Fraunhofer theory. Increasing the dispersion pressure to 5 bar did not result in a change of the measured particle size distributions, which indicates that the size distributions of the approximate primary particles were obtained at 3 bar. Results are the mean of three measurements.

Blend preparation

Blends were prepared at ambient conditions in batches of 25 g. Different amounts of the drug and lactose fines were 'sandwiched' simultaneously between two equal parts of the carrier material in a 160 cc stainless steel mixing vessel and gently pre-mixed with a spatula for several orbits. Mixing was then continued with a Turbula blender (WA Bachhofen, Basel, Switzerland) operated at 90 rpm for 5 or 10 minutes. Mixing order experiments involved blending of the carrier material with either budesonide or lactose fines for 5 minutes. Subsequently, the other fine particulate component (lactose fines or budesonide, respectively) was added and mixing was continued for another 5 minutes.

Content uniformity testing

Blends were tested for content uniformity by taking 20 samples of 25 mg from randomly chosen positions in the powder bed. The content of the samples was determined as described under 'sample analysis'. Blends were considered homogeneous and suitable for further testing at relative standard deviations (RSDs) of the content < 3%.

Scanning electron microscopy (SEM)

Scanning electron micrographs were obtained with a JSM-6301F (Jeol, Japan) at an acceleration voltage of 2 or 3 kV. Samples were mounted on an aluminium sample holder by means of conducting double sided adhesive tape. Loose particles were tapped off very gently to leave the drug-fines adherence to the remaining carrier particles on the sample holder unchanged. The specimens were then sputter coated with 20 nm of a gold/palladium alloy (120B, Balzers AG, Liechtenstein).

Drug detachment experiments

Drug detachment experiments were performed as described in chapters 3 and 4 of this thesis. In summary, inhalation experiments were performed with a classifier based test inhaler [10] at fixed inhalation flow rates from 10 to 60 L/min. The resistance of the test inhaler is 0.056 $kPa^{0.5}$ min L^{-1}, and therefore, the inhalation flow rates correspond to pressure drops of 0.2-11.4 kPa. For each measurement an accurately measured amount of 25 mg of an adhesive mixture was loaded by hand into the classifier of the test inhaler. Carrier particles were collected from the inhaler's classifier after the experiment to measure the residual (non-detached) drug content (carrier residue, CR) and CR-values were corrected for minor carrier passage fractions towards 100% retention. Drug detachment was calculated as 100-CR. Results are the mean of five measurements.

Sample analysis

Samples were analysed by spectrophotometry to determine their drug content (Unicam UV-500, ThermoSpectronic, Cambridge, UK). Budesonide mixtures were suspended in ethanol and after 1 hour suspended lactose was removed by centrifugation at 3000 rpm for 5 minutes (Hettich Rotanta D-7200, Hettich AG, Switzerland). The resulting clear solution was then analysed at a wavelength of 243 nm.

Table 2. Particle size distributions of the mixture components (average (SD); n = 3).

Component	X_{10} (µm)	X_{50} (µm)	X_{90} (µm)
Budesonide	0.70 (0.00)	1.58 (0.02)	3.10 (0.03)
FLF	0.86 (0.01)	1.95 (0.00)	3.48 (0.01)
CLF	1.22 (0.02)	3.94 (0.07)	9.15 (0.24)
Carrier	241.6 (0.1)	344.8 (0.2)	475.8 (1.1)

Table 3: formulations studied and their total carrier surface coverage (CC) by fine components.

Formulation	CC (%)*
0.4% Bud	28
4% Bud	283
0.4% Bud + 4% FLF	217
0.4% Bud + 4% CLF	124
4% Bud + 4% FLF	471
4% Bud + 4% CLF	378

* The carrier surface coverage is calculated as explained previously [11]. For these calculations, the X_{50}-values of the fine components from Table 2 were used and densities of budesonide and alpha-lactose monohydrate were considered to be 1.25 (chapter 3, Table 3) and 1.53 g/cm^3, respectively. The calculated values are based only on added fine components (drug and lactose fines) and do not take into account the presence of lactose fines intrinsic to the carrier material after the sieving procedure.

Results

Laser diffraction analysis

The particle size distributions of the mixture components from laser diffraction analysis are presented in Table 2. The particle size distribution of the FLF is comparable to that of the drug, with the X_{50} being only 0.37 μm higher. The CLF are markedly coarser, with an X_{50} more than two times higher than those of the FLF and the drug. No particles < 10 μm were measured in the sieved carrier material after RODOS dispersion. Based on the X_{50}-values of the fine components the total carrier surface coverages of the different formulations were calculated, which are presented in Table 3.

Content uniformity

RSDs of the drug contents in the mixtures ranged from 0.6-2.2%. Therefore, all mixtures were considered homogeneous and suitable for the drug detachment experiments.

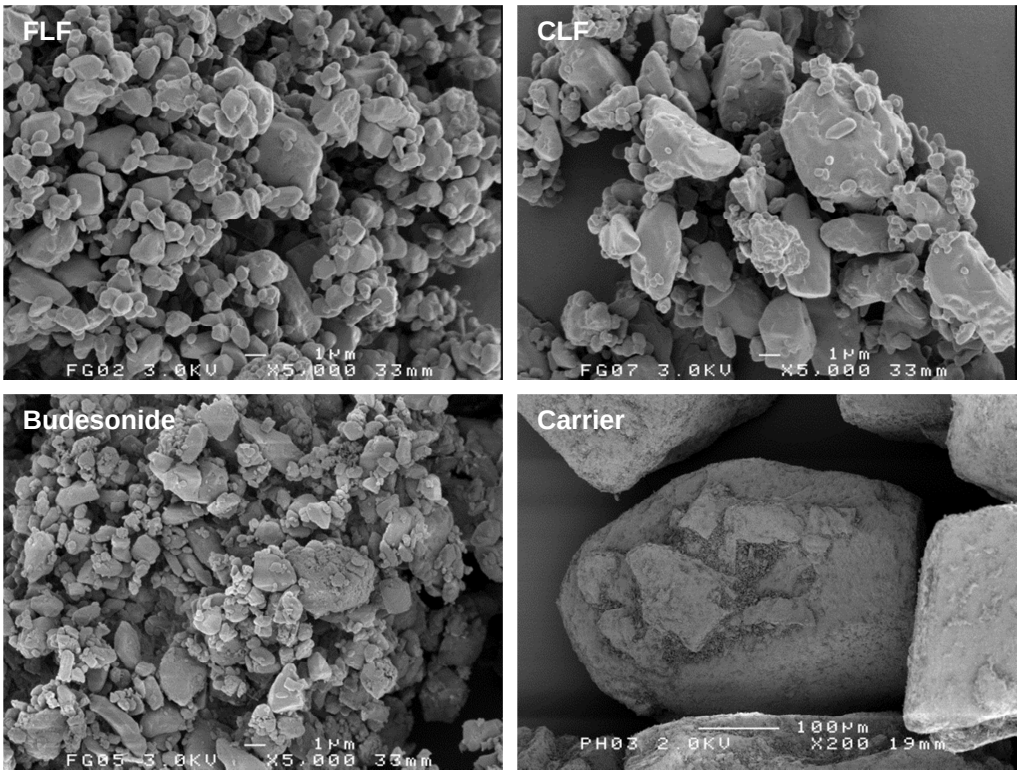

Figure 1: representative SEM images of the pure mixture components.

SEM imaging

Representative scanning electron micrographs of the pure mixture components are presented in Figure 1. Fines are visible in carrier surface irregularities of the sieved carrier particles, despite the fact that they were not measured with the laser diffraction technique. Apparently neither the double sieving procedure nor RODOS dispersion does effectively remove all of the fines from the carrier surface. The images confirm that the FLF have a size distribution similar to budesonide and that CLF are markedly coarser.
The shapes of the budesonide particles roughly resemble those of the lactose fines particles.

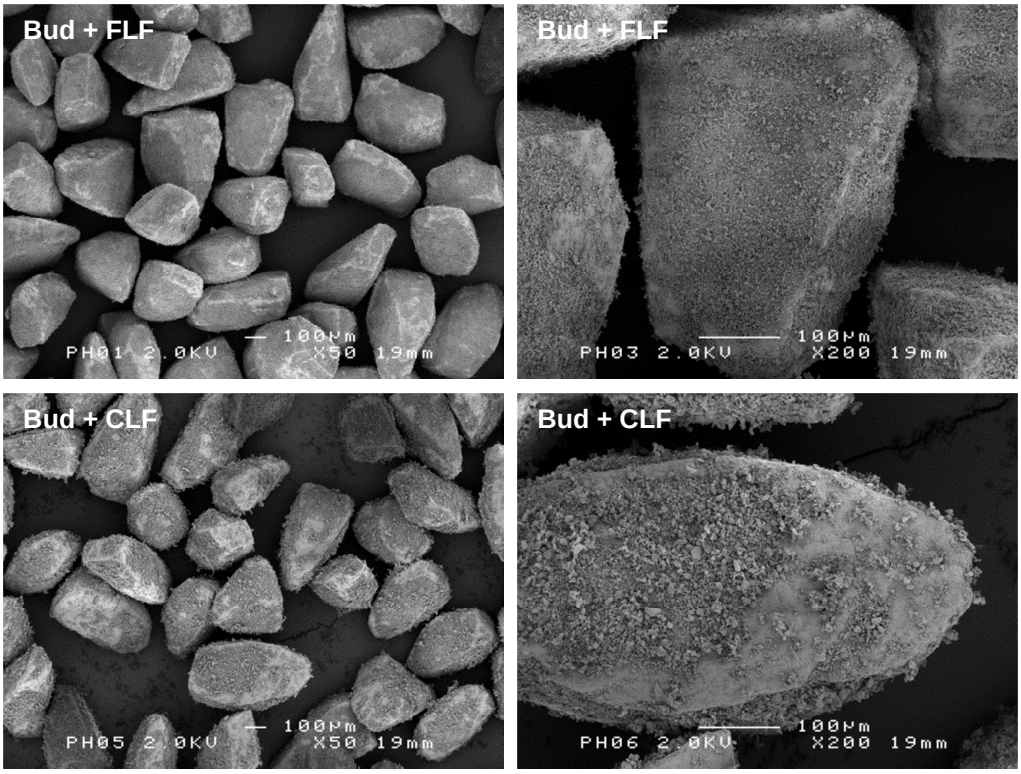

Figure 2: representative SEM images of mixtures. Top: mixtures containing 4% budesonide and 4% fine lactose fines. **Bottom:** mixtures containing 4% budesonide and 4% coarse lactose fines. Images on the right hand side are a magnification of images on the left hand side.

SEM images of mixtures containing 4% budesonide and 4% of FLF or CLF (mixed simultaneously for 10 minutes) are presented in Figure 2. A more dense and continuous layer around the coarse carrier particles appears to be formed by FLF and budesonide than by CLF and budesonide. The typical difference in structure of the fine components between FLF and CLF has been observed with SEM for batches of 0.4 and 4% budesonide mixtures as well as for mixtures containing other drugs (salbutamol sulphate and salmeterol xinafoate; unpublished data). The images shown in Figure 2 are therefore likely to be obtained from representative samples and they are representative for different drugs as well.

Drug detachment experiments

Figure 3 shows that the effect of 4% added lactose fines on the detached drug fraction depends on the size distribution of the fines, the budesonide content and the flow rate.

The effect of 4% added FLF. The addition of 4% FLF to a 0.4% budesonide mixture results in a lower detached drug fraction at flow rates of 10-30 L/min (Fig. 3A). At 20 L/min this effect is most pronounced with a decrease in the detached drug fraction from 24.7% to 6%. At flow rates between 40 and 60 L/min the detached drug fraction increases compared to 0.4% budesonide alone, with a highest absolute increase of 18% occurring at 50 L/min. Hence, flow rate interacts in both qualitative and quantitative ways with the effect of added FLF on drug detachment at a low drug content. This is similar to the effects on the detached drug fraction of an increase in budesonide content from 0.4% to 4%, although then the negative effect at low flow rates is less pronounced (Fig. 3A). For 4% budesonide mixtures, the effect of adding 4% FLF is relatively small. At this drug content the detached drug fraction decreases at most 8.4% (absolute difference; Fig. 3B, 30 L/min).

The effect of 4% added CLF. For mixtures containing 0.4% (Fig. 3A) or 4% of budesonide (Fig. 3B), the addition of 4% CLF results in higher detached drug fractions over the range of flow rates applied. Thus, in contrast to FLF, there is no qualitative interaction between flow rate and the effect of CLF. Flow rate does interact in a quantitative way with the effect of CLF, however. The absolute increase in the detached drug fraction is highest at 30 L/min with 32% and 40.5% for budesonide contents of 0.4% and 4%, respectively. At flow rates of 10, 50 and 60 L/min the effect of CLF on the detached drug fraction is negligible for 4% budesonide mixtures.

The influence of the fines content. The amount of added lactose fines may influence the effect on the detached drug fraction, depending on the size distribution of the added fines. At 30 L/min, the detached drug fraction of 4% budesonide mixtures is positively and nearly linearly related to the CLF content (0-6%), whereas it is almost independent of the FLF content (Fig. 4). Some exploratory experiments revealed that the inhalation flow rate and the drug content may also be of influence. For example, at 20 L/min the detached drug fractions of 0.4% budesonide mixtures containing 0%, 0.4% or 4% fines were 24.7%, 14.4% and 6%, respectively (FLF), or 24.7%, 32.1% and 42.9%, respectively (CLF). Hence, detached drug fractions with 0.4% added fines were intermediate to those with 0% and 4% added fines at 20 L/min. However, at a flow rate of 50 L/min the effect on the detached drug fraction of 0.4% of fines was negligible (< 4.2%) for both FLF and CLF, whereas the addition of 4% fines increases the detached drug fraction by 18% (see Fig. 3A).

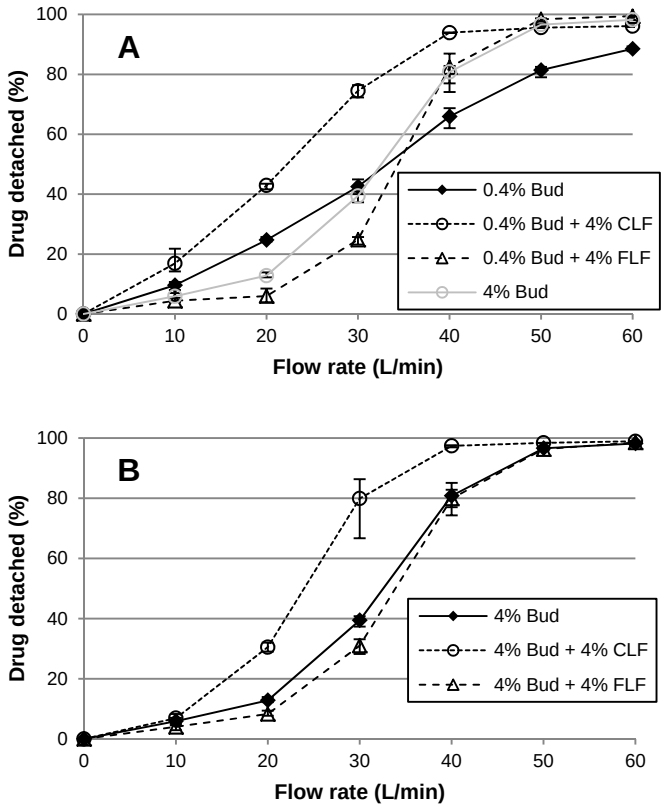

Figure 3: the effects of added lactose fines on drug detachment over a range of flow rates. A) effects of 4% fine lactose fines (FLF) or 4% coarse lactose fines (CLF) on drug detachment of 0.4% budesonide mixtures and **B)** for 4% budesonide mixtures. Error bars represent maximum and minimum values measured (n = 5).

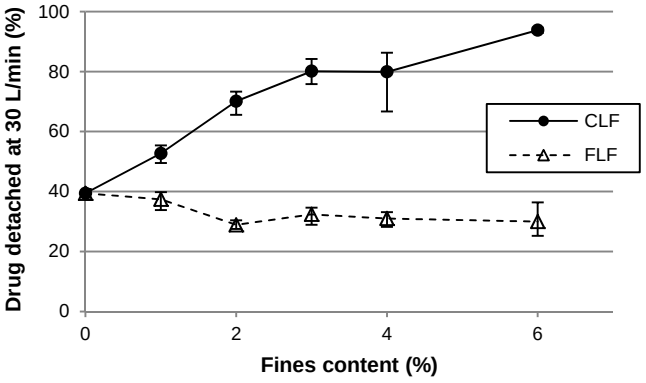

Figure 4: drug detachment of 4% budesonide mixtures at 30 L/min as a function of added fines content. FLF = fine lactose fines; CLF = coarse lactose fines. Error bars represent maximum and minimum values measured (n = 5).

The influence of mixing order on the effect of 4% added fines. The mixing order of the fine components interacts with the effect of 4% added fines on drug detachment from the lactose carrier. The magnitude of this interaction depends on the applied flow rate, the size distribution of the fines and the drug content (Fig. 5). In all scenarios where mixing order causes a pronounced difference in the effect of added fines on drug detachment, the largest effect on the detached drug fraction compared to the situation without added fines is obtained by mixing of the fines first. In contrast, the smallest effect on the detached drug fraction is obtained by mixing the drug first in all scenarios. At 50 and 60 L/min, the addition of 4% FLF or CLF to and subsequent 5 minutes of mixing with a 0.4% budesonide only mixture (mixed for 5 minutes) does not result in a change in the detached drug fraction (Figs. 5A and 5B, "0.4% Bud; 5 min" and "Bud first"). The absolute difference in the detached drug fraction between the fines first and budesonide first mixtures is pronounced especially at flow rates of 30 and 40 L/min for 0.4% budesonide with 4% FLF (17.3% and 25.5%, respectively, Fig. 5A) and 20 and 30 L/min for 0.4% budesonide with 4% CLF (18.2% and 28.1%, respectively, Fig. 5B). The mixing order of 4% budesonide and 4% CLF affects the detached drug fraction at a flow rate of 30 L/min (29.8% difference in the detached drug fraction, Fig. 5C), whereas at all other flow rates applied the influence of mixing order is negligible. Mixing of fines and budesonide simultaneously for 5 or 10 minutes results in detached drug fractions intermediate to those obtained by mixing the fines or budesonide first at 30 and 40 L/min (0.4% budesonide + 4% FLF, Fig. 5A) or at 20 and 30 L/min (0.4% budesonide + 4% CLF, Fig. 5B). At higher flow rates the simultaneous mixing of budesonide and fines results in detached drug fractions equal to those obtained by mixing the fines first for these two formulations. For the formulation containing 4% budesonide + 4% CLF (Fig. 5C, 30 L/min), mixing both components simultaneously for 10 minutes results in a detached drug fraction intermediate to those obtained by mixing the fines or the drug first. Mixing both fine components simultaneously for only 5 minutes results in a detached drug fraction equal to that obtained by mixing the fines first.

Discussion

About the presence of intrinsic lactose fines

The presence of intrinsic lactose fines on the carrier surface after the sieving procedure (Fig. 1) has likely affected the balance of effects resulting from the addition of FLF or CLF. For example, intrinsic fines may occupy part of the active sites on the carrier surface or cause a 'buffering effect' during mixing of the drug with the lactose carrier. This would decrease the potential for the 'saturation of active sites' or a buffering effect by FLF or CLF. Therefore, it would be desirable to use more effective methods for the

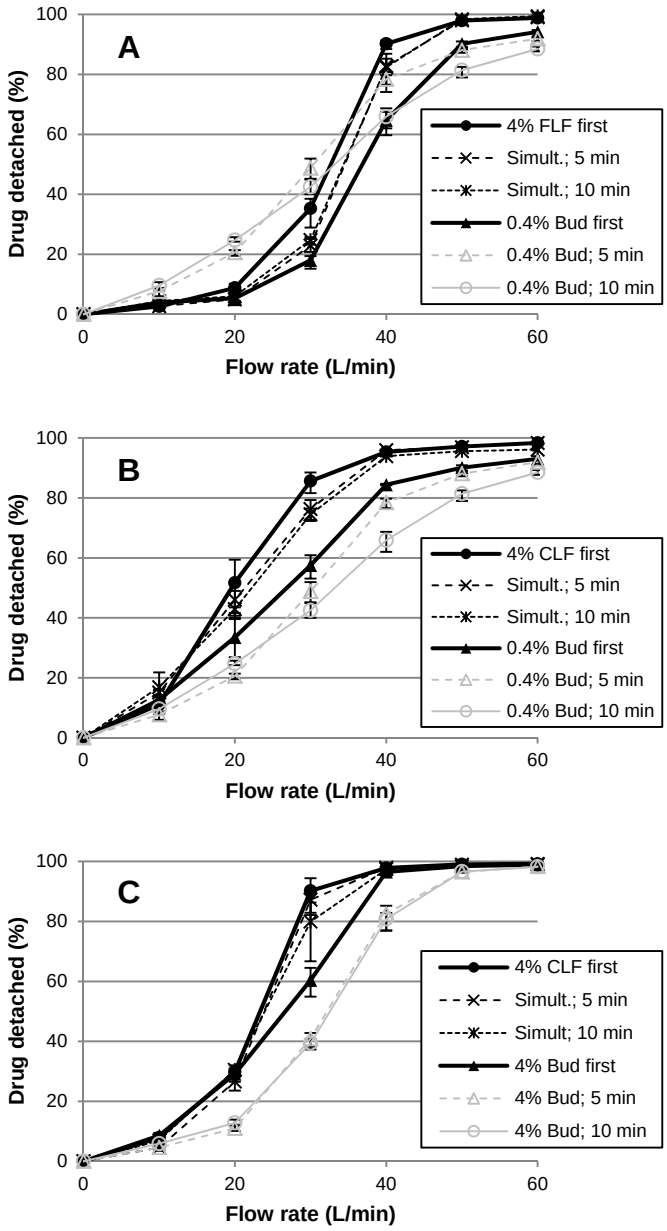

Figure 5: the influence of mixing order on the effects of added fines on drug detachment. A) mixtures containing 0.4% budesonide and 4% fine lactose fines (FLF); **B)** mixtures containing 0.4% budesonide and 4% coarse lactose fines (CLF); **C)** mixtures containing 4% budesonide and 4% CLF. Drug detachment versus flow rate profiles of mixtures containing only 0.4% or 4% of budesonide (mixed for 5 or 10 minutes) are shown as a reference. Fine components were mixed separately by mixing one component with the carrier for 5 minutes, after which the second component was added and mixing was continued for another 5 minutes. Simult. = drug and fines mixed simultaneously with the carrier material for 5 or 10 minutes. Error bars represent maximum and minimum values measured (n = 5).

removal of intrinsic lactose fines. Other workers have used techniques that included submersion of the carrier material in ethanol based solutions [5, 12]. Although such techniques may result in lower intrinsic fines contents, they also change carrier surface properties like surface roughness and the degree of contamination with protein residues. This too will change to unknown extent the potential for certain effects when adding lactose fines, and therefore, such techniques are not ideal either. The carrier classification method used in this study may better represent the practical situation, since commercial carrier products will always contain a certain amount of intrinsic lactose fines too. Furthermore, the same classification method was used in chapter 3 for the study on drug content effects, which allows a better comparison of these effects with those obtained by the addition of lactose fines in the current study.

About the effects of FLF

The effects of FLF on the detached drug fraction are similar to drug content effects. With a drug content of 0.4%, flow rate interacts in the same qualitative way with the effects on drug detachment of 4% added FLF as with those of 3.6% added budesonide (i.e. for a total drug content of 4%, Fig. 3A). Similar effects of an increase in drug content from 0.4-4% are shown in chapter 3 (Fig. 6), even though these drugs differed in their adhesiveness and cohesiveness, particle shape and density. This suggests that these component specific properties are of minor importance to the effect of fines content on dispersion performance. It may therefore be expected that the effects of FLF obtained with budesonide in this study are representative for other drugs as well, which we have already been able to confirm for salbutamol sulphate (unpublished data) and salmeterol xinafoate (see chapter 6). The 0.4% difference in added fine particle content between FLF and budesonide is unlikely to have affected the presented finding, because minor drug content effects were observed at drug content ranges > 2% in chapter 3. This too agrees well with the minor effects of FLF on drug detachment of 4% budesonide mixtures (Figs. 3B and 4).

The similarity between the effects of FLF and drug content on the detached drug fraction suggests that the same working mechanisms play a role. In chapter 3 it was shown that drug content effects may be explained by a balance between agglomeration effects, press-on effects and the saturation of active sites. Agglomeration effects and the saturation of active sites are addressed in the 'agglomeration' and 'active sites' hypothesis, respectively (Table 1). The press-on effect refers to an increase in interaction forces between the mixture constituents due to inertial and frictional forces exerted on the mixture during the blending process. A stronger effect of such press-on forces may be expected with increasing fines contents. This stronger effect is caused by fine particles filling up carrier surface irregularities, allowing the forces caused by mixing to be transferred through the powder bed and to act effectively on fine particles that would have been sheltered in the irregularities from these forces at lower fines contents. The

qualitative interaction of flow rate with the effect of drug content on the detached drug fraction of 0.4% mixtures was previously explained by a change in the relevance to drug detachment of the different mechanisms that occur during mixing. At flow rates roughly < 40 L/min press-on effects are most relevant or dominant, which shifts to a dominance of agglomeration effects and the saturation of active sites at higher flow rates. This may also apply to the interaction of flow rate with the effect of FLF. With an initial drug content of 4%, active sites are already saturated, press-on forces are likely already maximally efficacious and the addition of FLF does not lead to pronounced agglomerate formation (Fig. 2). This may explain the minor effect of FLF on drug detachment when added to 4% budesonide mixtures.

About the influence of the size of the added fines

The difference in effect on drug detachment between FLF and CLF is in agreement with the 'buffer hypothesis' as explained in Table 1. According to this hypothesis, CLF should have a more beneficial effect on the detached drug fraction than FLF, because their larger size should allow them to better protect the drug particles from press-on forces by acting as a buffer between colliding carrier particles or between their carrier particle and the wall of the mixing vessel. This would result in weaker drug-carrier interactions and thus lead to a higher chance of drug particle detachment during inhalation.

Several other explanations for the difference in effect on drug detachment between FLF and CLF may be given, however, which causes the occurrence and significance of a 'buffer effect' with CLF to remain unclear. Extensive, coherent networks of fine particles are formed around the coarse carrier particles at total drug and FLF contents ≥ 4% (Fig. 2, top and chapter 3, Fig. 5). The formation of such particle networks is often considered as part of the 'agglomeration hypothesis' (Table 1). However, in contrast to single, detachable agglomerates, the detachment of particles from such networks requires 'cracking' or failure of some of the cohesive and adhesive bonds between the fine particles. Drug detachment thus likely depends on the tensile strength of the particle network. The tensile strength of agglomerates can be described as in Eq. 1 [13]:

$$\sigma = 15.6\phi^4 W/d \qquad \text{Eq. 1.}$$

in which σ is the tensile strength of the agglomerate, ϕ the packing fraction (volume of particles per volume of agglomerate), W the work of adhesion or cohesion and d the particle diameter. From Eq. 1 it follows that the use of fines with a larger diameter may decrease the tensile strength of any fine particle network formed, which should benefit drug detachment. Such an effect may be expected to depend on the fines content relative to the drug content, as observed for CLF (Fig.4). This idea is supported by the work of Adi et al., who studied the effect of lactose fines with different size fractions on the

dispersion performance of carrier-based and carrier-free mixtures with salmeterol xinafoate [14]. Fines with a median diameter of 7.9 μm led to a better dispersion performance of the mixtures than fines with a median diameter of 3.0 μm. For carrier-based formulations, the influence of the fines content on their effect on dispersion performance was studied. The magnitude of the effect of the coarser fines was proportional to their content over a range from 5 to 20%. These workers also observed differences in the structure of agglomerates formed and stated that an influence on the tensile strength was the most likely cause of the difference in dispersion performance between both fines size fractions. In addition to a direct particle size effect on the tensile strength of the fine powder bed, there may also be some indirect effects. Coarser fines most probably rise above carrier surface irregularities and have a higher mass and inertia. This would make them more susceptible to redistribution by frictional and inertial forces during mixing, which may prevent the formation of particle networks with a high packing fraction. Furthermore, the addition of an equal weight fraction of a coarser material represents fewer particles, which reduces the carrier coverage (Table 3) and the number of contact points that can be formed per unit agglomerate volume and thus the potential for the formation of vast and coherent particle networks. These factors may also explain the observed difference between FLF and CLF in the structure of the fine particle layer they form with the drug on the carrier surface (Fig. 2). The above hypothesis may be referred to as the 'tensile strength hypothesis', not to be confused with the 'fluidisation hypothesis' (Table 1), which refers to the tensile strength of the powder bulk. In light of the above, agglomeration (i.e. the formation of single particle-like clusters from primary particles) will be distinguished from network formation throughout the rest of this chapter. Lastly, particle detachment from the lactose carrier is supposedly for the larger part caused by inertial separation forces, especially in the classifier based test inhaler used in this study [10, 15]. Inertial separation forces are proportional to d^3, whereas most types of interaction forces are only proportional to d^1. Hence, coarser particles will detach more easily from the carrier surface during inhalation, as was shown by Dickhof and co-workers for different budesonide size fractions [16]. It may therefore be expected that CLF detach more easily from the carrier surface than FLF. This likely increases drug detachment too as drug particles may to some extent be co-agglomerated with the fines or liberated from simultaneously 'cracking' particle networks. The given hypotheses to explain the difference in effect between FLF and CLF do not rely heavily on the properties of the drug component. A similar difference between the effects of CLF and FLF may therefore be expected for other drugs with different adhesion and cohesion characteristics, as has already been observed for salmeterol xinafoate (0.4% drug content only, see chapter 6) and salbutamol sulphate (unpublished data).

About the influence of the mixing order

The finding that the influence of the mixing order of drug and fines on the detached drug fraction depends on the flow rate, on the particle size of the fines and on the drug concentration (Fig. 5) is in agreement with results from previously presented studies. For example, Zeng et al. observed that mixing of milled lactose (5.0 μm) with the carrier material first, followed by addition of the drug resulted in a higher fine particle fraction at 60 L/min through a Rotahaler than mixing of the drug first. However, at a different flow rate (90 L/min) or when fines with a different volume median diameter (15.9 μm) were used, the influence of mixing order on the fine particle fraction was insignificant [17]. In another study Jones et al. showed that mixing order did affect the fine particle fraction of salbutamol mixtures at 60 L/min through a Rotahaler, but only at drug contents of 0.5 and 1.5% and not at drug contents of 2.5, 3.5 or 4.5% (with a carrier size fraction of 63-90 μm)[8]. These workers, furthermore, showed that the influence of mixing order was dependent on mixing time too. Because the formulation and dispersion conditions chosen in the above studies are different from those in the current study, the observed interactions with the influence of mixing order are not restricted to a specific set of formulation and dispersion conditions.

Mixing order experiments have mostly been performed to test the active sites hypothesis (Table 1) [2]. Theoretically, if fines indeed act on dispersion performance through the occupation of active sites, then mixing the fines first would maximally exploit this mechanism and should result in a more beneficial effect on dispersion performance than any other mixing order. As a result, the presence of a mixing order effect has always been interpreted as support of the active sites hypothesis, whereas the absence of such an effect has always been interpreted as a falsification of the active sites hypothesis. The occurrence of interactions with the effect of mixing order shows that the interpretation of mixing order experiments is not that straightforward, however. Several assumptions underlying the active sites hypothesis and the classical interpretation of mixing order experiments are, therefore, extensively discussed in the following sections before interpreting the results presented in Figure 5.

Firstly, once fine excipient particles bind to active sites, the new surface they form for drug particles to settle on should be less 'active' than the original carrier surface. If this is not the case, then binding of the fines to active sites will not result in a higher chance for drug particles to bind to sites with a lower activity. This point was addressed by Louey and Stewart, who used atomic force microscopy to measure the adhesion force between a silica sphere and the carrier surface [4]. They measured higher adhesion energies after the addition of a ternary component and reasoned that drug particles would be more likely to bind to the ternary component than to carrier sites with a low activity. As a result, it was concluded that the saturation of active sites was unlikely to be an important mechanism for the effect of added fines on dispersion performance.

Secondly, once bound to active sites, the redistribution or replacement of particles should be limited. If a highly dynamic situation exists during mixing in which fine particles are continuously redistributed to and from active sites, similar equilibrium situations may be reached independent of the mixing order of the fine components, even after mixing for a relatively short duration as in this study. The fact that drug detachment was influenced by mixing order suggests that at least a highly dynamic situation did not exist during mixing in our experiments. Especially noteworthy in this respect may be the small difference in drug detachment at 50 and 60 L/min caused by the addition of FLF or CLF to a mixture containing 0.4% budesonide mixed for 5 minutes (Figs. 5A and 5B, "0.4% Bud; 5 min" and "0.4% Bud first"). At these high flow rates it may be expected that the non-detached drug particles are attached to the most active sites. After mixing 0.4% budesonide with the carrier material for 5 minutes, continued mixing for another 5 minutes with added FLF or CLF does not change the detached drug fraction, whereas mixing the fines first does result in a 4.6-7.7% higher detached drug fraction. Because drug detachment of the 'budesonide first' mixture would be expected to be closer to the 'fines first' mixture in the case of a highly dynamic situation, at least in between the 'fines first' and the '5 minutes budesonide alone' mixture, this may be indicative of a limited dynamic situation. Furthermore, because continued mixing of budesonide alone for a total of 10 minutes ("0.4% Bud; 10 min") does result in a 6.7% and 3.7% lower detached drug fraction at 50 and 60 L/min, respectively, compared to only 5 minutes of mixing, the addition of fines may prevent the further distribution of drug particles towards active sites or prevent the firm compression of drug particles onto the carrier surface (i.e. onto sites previously referred to as 'pseudo-active sites' [18]).

Thirdly, in order for the active sites hypothesis to be relevant, active sites should have the potential to significantly influence dispersion performance. This means that: 1) if no fines are added to the mixture, a significant drug mass relative to the total drug mass should be bound to active sites; and 2) drug particles which are displaced from active sites by fines have to contribute to the dispersion performance characteristic measured (i.e. fine particle fraction or drug detachment). Regarding the first point it can be stated that with a fixed binding capacity of active sites a potentially higher drug fraction may be bound to active sites at lower drug contents. Hence, the potential contribution of the active sites mechanism to the effect of added fines on dispersion performance will be larger at lower drug contents. This may explain the influence of drug content as found by Jones et al. [8], mentioned previously, and the observation in the present study that mixing order influences the detached drug fraction at 40-60 L/min for the 0.4% budesonide mixtures (Figs. 5A and B), whereas it does not for the 4% mixture (Fig. 5C). It should be noted, however, that the binding capacity of active sites, and thus the potential for the active sites mechanism to play a significant role, depends on the definition of active sites that is used. To further explain the second point, it may be clarifying to think of the drug mass being distributed as function of the flow rate at

which detachment from the carrier surface will occur. The choice of flow rate during dispersion experiments determines whether a certain shift within the mass distribution caused by the addition of fines can be measured. For example, drug particles in a binary mixture that detach at flow rates ≥ 60 L/min, but after the addition of lactose fines detach already at 40 L/min, do not contribute to a higher mass fraction (i.e. an improved dispersion performance) if drug detachment is only measured at flow rates ≤ 30 L/min. Thus, attributing a positive effect of added lactose fines on dispersion performance measured at 30 L/min to the saturation of active sites implicitly defines active sites as those sites on the carrier surface from which drug is not detached by dispersion at 30 L/min. This point also highlights the importance of the definition of active sites. In Figure 5C, the difference in the detached drug fraction at 30 L/min between the 'fines first' and 'drug first' mixing orders is the result of a shift in the drug mass distribution from the subfraction that is detached at 30-40 L/min to the subfraction detached at 20-30 L/min. Therefore, only if the definition of active sites includes that part of the carrier surface from which drug particles are not detached at 30-40 L/min this mixing order effect may be considered evidence in favour of the active sites mechanism. However, such a definition would be very broad.

Finally, the saturation of active sites should be the only mechanism of which the occurrence or significance is affected by mixing order. If this is not the case, then an observed mixing order effect does not form conclusive evidence in favour of the active sites hypothesis and the likelihood of this mechanism playing a role has to be inferred from the formulation and dispersion conditions used. Mixing order effects may reasonably be expected for at least the 'buffer hypothesis', the 'tensile strength hypothesis' and the 'agglomeration hypothesis' too. For example, the compression of drug particles onto the carrier surface and the formation of drug particle networks with a high tensile strength in the absence of fines (i.e. mixing the drug first) may not be fully reversible after the addition of fines. Furthermore, it may prevent the drug particles from being available for agglomeration with the added lactose fines.

It may be concluded based on the above that the mixing order data presented in Figure 5 do not provide evidence against or in favour of just one working mechanism for the effect of added fines. At best one may conclude that multiple mechanisms are likely to be relevant, which all seem to depend on the mixing order of the fine components to some extent. At low drug contents and high dispersion efficacies (i.e. flow rates), an influence of mixing order is most likely to be an indication that the active sites hypothesis could be true, but its significance relative to other mechanisms remains questionable.

About practical implications

The results from this study show that the addition of lactose fines to adhesive mixtures for inhalation is an important technique that can serve multiple purposes. Traditionally,

fines are added to carrier-based inhalation formulations solely to improve their dispersion performance. However, Figures 3 and 5 show that lactose fines may also be used to influence the flow rate dependence of the formulation's dispersion performance. This flow rate dependence of the dispersion performance should ideally be chosen such, that it compensates for the shift in drug particle deposition towards larger airways with increasing flow rate, as shown *in vivo* by Usmani et al. [19]. It then aids in obtaining a flow rate independent lung deposition or therapy. Of course, this assumes the use of a dispersion principle that effectively converts the higher kinetic energy of the air resulting from higher flow rates into higher dispersion forces. Added lactose fines, especially FLF, may furthermore be used to lower the effect on dispersion behaviour of drug content. The difference in dispersion behaviour between formulations containing 0.4 and 4% of drug is smaller with FLF added to the formulation with the lower drug content (Fig. 3A). This may be especially useful for formulations of the same brand and drug that are available in different dose strengths.

About future perspectives

The carrier size fraction and the dispersion principle of the inhaler used may be important factors determining the balance between the different mechanisms in play, and therefore, there is a demand for studies focussing specifically on the interaction between these variables and the effects of added lactose fines on formulation dispersion performance. For example, in chapter 4 it was shown that the use of a finer carrier likely reduces the relevance of the press-on hypothesis and may also greatly affect the potential for agglomeration of the fine components on the carrier surface. Furthermore, the test inhaler used in this study relies predominantly on inertial (collisional, vibrational and rotational) forces for the separation of drug particles from the carrier surface. This may cause agglomeration effects to be more dominant, and the difference between FLF and CLF to be more pronounced, than would be the case for dispersion principles relying on aerodynamic (drag, lift and shear type) forces. However, because favourable as well as unfavourable effects of drug content on the fine particle fraction from different devices (dispersion principles) were observed previously (as discussed in the introduction of chapter 3), and because a great similarity between the effects of drug content and FLF was observed in this study, it is anticipated that the general findings from this study will apply to a range of different dispersion principles.

The fact that added lactose fines may either improve or deteriorate the dispersion performance of carrier-based inhalation powders means that a much broader perspective is needed than has previously been presented to obtain a mechanistic understanding of this formulation technique. Future investigations should be based on the idea that added fines may have any effect, which is determined by the formulation and dispersion conditions. This means that mechanistic studies in which just one fixed set of conditions is used can be considered obsolete from this moment onwards. Further

unravelling of the significance of different possible mechanisms may also rely heavily on the development of new or improved techniques to measure relevant powder properties. Especially relevant properties may be the agglomeration behaviour of the fine components on the carrier surface, the tensile strength of fine particle networks and the spatial distribution of the different fine components over the carrier surface. In addition, the previously expressed desire for sensitive methods to measure mixture flowability remains [8].The influence of added fines on this mixture property was not considered in the current study, because its relevance to dispersion performance was minimised by directly weighing samples into the dispersion principle (i.e. the air classifier) of the test inhaler. However, for other devices (e.g. capsule based inhalers) the fluidisation hypothesis (Table 1) could well be relevant. Furthermore, fines may influence press-on effects during mixing through an influence on powder flowability.

Conclusions

Added lactose fines are likely to exert their effect on the dispersion performance of carrier-based inhalation formulations through a combination of different mechanisms. The balance between the different mechanisms can be shifted by formulation and dispersion variables, such as the inhalation flow rate, the size distribution of the added fines, the drug content and the mixing order. Two new mechanisms may play a role in addition to those proposed previously. Firstly, it was shown that added lactose fines may lower dispersion performance, likely by increasing the effectiveness of press-on forces and the formation of coherent fine particle networks on the carrier surface. Secondly, a remarkable difference in effect on drug detachment between fine (X_{50} = 1.95 μm) and coarse lactose fines (X_{50} = 3.94 μm) may be explained by the coarse lactose fines weakening or preventing the formation of such coherent particle networks. It is of importance to the further mechanistic understanding of the effect of fines to better define 'active sites', to acknowledge that multiple mechanisms may play a role simultaneously and to study the interactions with other formulation and dispersion variables.

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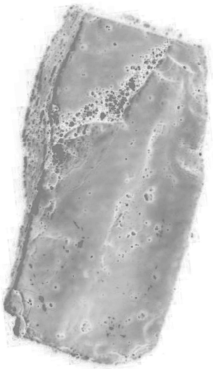
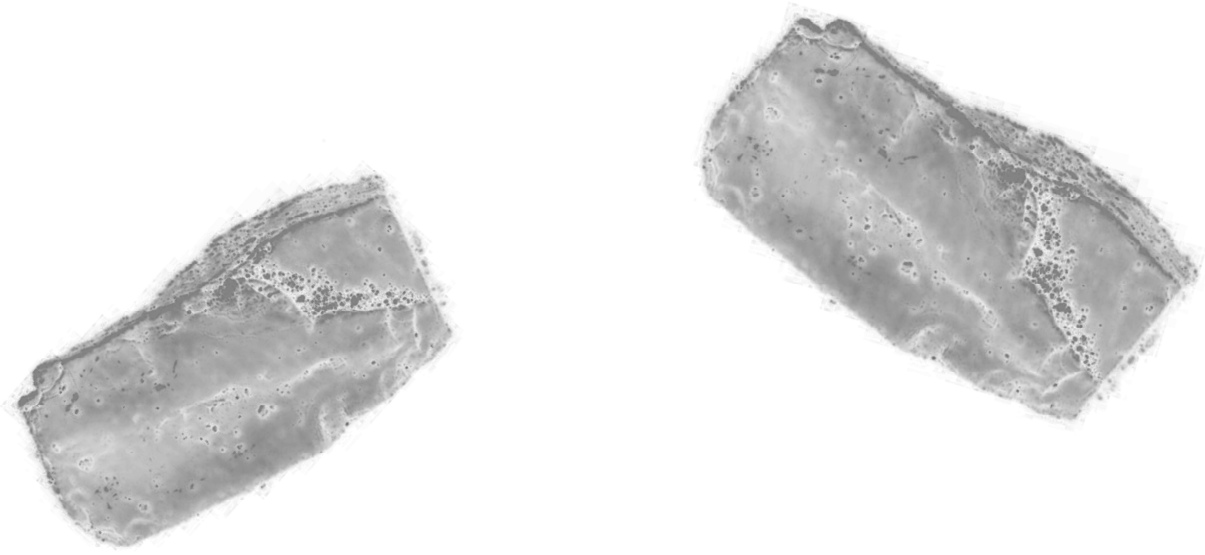


Chapter 6

A preliminary study on press-on effects in adhesive mixtures for inhalation

The study presented in this chapter is part of a collaboration with Peter Stewart and Shyamal Das from the Faculty of Pharmacy and Pharmaceutical Sciences at Monash University (Melbourne, Australia).



Abstract

Press-on effects are thought to play an important role in the effects of drug content and added fine lactose particles on the dispersion behaviour of adhesive mixtures for inhalation. The apparent solubility of salmeterol xinafoate (SX) in adhesive mixtures is an indication of the mechanical stress experienced by the drug particles during the mixing process. It is used as an indirect measure of press-on effects in this preliminary study to test the hypotheses regarding the occurrence of these effects following a change in the variables mentioned. An increase in salmeterol content and the addition 'fine lactose fines' (FLF, X_{50} = 1.95 μm) resulted in a lower apparent solubility of SX after prolonged mixing, whereas 'coarse lactose fines' (CLF, X_{50} = 3.94 μm) did not. These findings do not support the previously proposed hypotheses that higher fine particle contents increase the susceptibility of drug particles to press-on forces, and that CLF may act as a buffer between colliding particles to reduce the susceptibility of drug particles to press-on forces. However, a direct relationship between press-on effects and apparent solubility does not necessarily exist, especially when the carrier surface is covered by a multi-particulate layer. Therefore, the hypotheses cannot be conclusively rejected. It is shown that the apparent solubility of salmeterol xinafoate is a useful measure for the characterisation of carrier-based dry powder inhalation formulations. For a deeper understanding of press-on effects other powder properties, such as bulk flowability or the tensile strength of fine particle networks on the carrier surface, should be considered as well.

Introduction

The strength of adhesive or cohesive bonds depends on the compression applied on the contacting materials during or after bond formation. When compression causes plastic deformation of either one or both of the contacting materials, it increases their contact area and reduces their separation distance, with higher Van der Waals interaction forces as a result. Previously, Lam and Newton [1] found a linear increase in geometric median adhesion force with applied compression force for pharmaceutical powders (i.e. polyethyleneglycol, lactose, starch and calcium carbonate) on a surface of stainless steel. As can be expected, they found that this effect was more pronounced for materials with a lower yield stress. The powders from this study were sieved into size fractions of 45-56 or 32-45 μm, but similar findings regarding the relationship between compressive force and adhesion force have been reported for micronised powders of, for example, lactose, salmeterol xinafoate and budesonide [2-4].

During blending of drug and lactose carrier particles into adhesive mixtures for inhalation inertial and frictional forces are generated that act on the constituent particles. These mixing forces are necessary to obtain homogeneous blends, but they may also negatively affect the mixture's dispersion performance. Drug particles may be compressed onto the lactose carrier surface by a vector component of the mixing forces that is directed towards the carrier surface. The mixing forces are then mostly referred to as 'press-on forces'. Similar to the centrifugal forces applied in previous studies [1-3], these press-on forces likely increase the average drug-carrier binding energy, which lowers the chance of drug particle detachment from the carrier surface during inhalation. Some workers have hypothesised that press-on forces play an important role in the effects of numerous formulation variables, such as drug content, mixing time, carrier size fraction and the size and content of added fine lactose particles [5-8], as has also been extensively discussed in chapters 3-5 of this thesis. Basically, these variables are suggested to partly affect dispersion performance through an influence on the susceptibility of drug particles to press-on forces, the magnitude of these forces or the number of 'compression events' during the mixing process. In the remainder of this chapter such effects will be referred to as 'press-on effects'.

Currently, no method exists to directly measure the press-on forces to which drug particles are subjected during mixing. This makes it difficult to test the hypotheses regarding the occurrence of press-on effects. However, chapter 4 introduced a method in which the apparent solubility of salmeterol xinafoate was used as an indirect measure of the mechanical stress imparted on the drug particles during mixing. This method was based on the work by Mosharraf and Nyström [9, 10]. As can be expected, the degree of mechanical stress and thus the apparent solubility was positively related to mixing time and carrier size fraction, which relate to the number of compression events and the magnitude of the press-on forces, respectively. Because the forces that cause mechanical

stress are also the forces that result in compression, it was reasoned that the apparent solubility could also be used as an indirect measure of press-on effects.

The method introduced in chapter 4 is again used in this chapter to study the occurrence of press-on effects with a change in drug content, as was hypothesised in chapter 3. In addition, it is used to test the 'buffer hypothesis', which was discussed in chapter 5 as one of the explanations for a difference in effect on drug detachment between fine and coarse added lactose fines. It is hypothesised that the significance of press-on effects depends on the baseline degree of compression in the mixture; e.g. if drug particles are hardly compressed against the carrier surface after short mixing, there is no buffering potential for significant reduction of compression. Mixtures were therefore prepared at different mixing times.

Materials and methods

Similar materials en methods were used as described in previous chapters. Therefore, they are only very briefly summarised in the following section with references to the chapters giving a more detailed description.

Lactose carrier particles with a size fraction of 250-315 μm were obtained from Pharmatose 80M (DMV-Fonterra excipients, Goch, Germany) by vibratory and air-jet sieving (Ch. 4). Micronised salmeterol xinafoate (SX) was used with a volume median diameter of 1.41 μm, as determined by dry laser diffraction analysis using a RODOS disperser (Ch. 3). Fine lactose fines (FLF) and coarse lactose fines (CLF) used in this study are from the same batch as those used in chapter 5. The volume median diameters of FLF and CLF, also determined by dry dispersion laser diffraction analysis, are 1.95 and 3.94 μm, respectively. SEM images of FLF and CLF are presented in chapter 5, Figure 1.

The lactose carrier was mixed with 0.4 or 4% SX, or 0.4% SX and 3.6% FLF or CLF. The drug and fine lactose were mixed with the carrier simultaneously. Mixtures were prepared in batches of 25 g using a Turbula blender at 90 rpm for 2, 10 or 60 minutes (WA Bachofen, Basel, Switzerland). A stainless steel mixing vessel was used with a volume of 160 mL. Each mixture occupied roughly 20% of this volume.

The mixtures were tested for content uniformity (Ch. 4), apparent solubility of SX (n =3; Ch. 4) and drug detachment during inhalation (Ch. 4). Furthermore, mixtures were studied by scanning electron microscopy (SEM, Ch. 4). Blends were considered homogeneous and suitable for the experiments with a relative standard deviation (RSD) of the drug content < 3%. To minimise the influence of environmental conditions on differences in apparent solubility of SX between mixtures, single measurements (n = 1) of the apparent solubility were performed on all mixtures simultaneously. Drug detachment experiments were performed at 20, 40 and 60 L/min through a classifier based test inhaler. Samples were analysed spectrofotometrically (Ch. 4).

Homoscedastic 2-tailed Student's t-tests were performed to determine the statistical significance of differences in apparent solubility. The F-test was used to test for

the equality of standard deviations between compared data sets. The correlation between apparent solubility and detached drug fraction was determined by simple linear regression. Calculations were performed using Microsoft Excel 2010.

Results

Content uniformity

The RSDs in the salmeterol content of 10 samples was between 0.7-2% for all mixtures prepared. The mixtures were therefore considered sufficiently homogeneous.

SEM

Figure 1 is an SEM image of micronised salmeterol xinafoate particles in the starting material. The primary particles exhibit a distinct plate-like shape. SEM images of the mixtures that were mixed for 60 minutes are presented in Figure 2. Salmeterol particles in the 0.4% mixture are plastically deformed and agglomerated after 60 minutes of mixing (Fig. 2A2). This is in agreement with SEM observations of 0.4% SX mixtures after prolonged mixing presented in chapter 4. More large agglomerates were observed on the carrier surface in the 4% SX mixture than in the mixture containing 0.4% SX + 3.6% FLF (Figs. 2B1 and 2C1). Denser and more continuous fine particle networks are formed around the lactose carrier particles in the mixture containing FLF than in the mixture containing CLF (Figs. 2C1 and 2D1). This is in agreement with previous SEM observations on mixtures containing 0.4% budesonide and 4% FLF or CLF, as presented in chapter 5. At the higher magnification of 5000 times, plate-like particles,

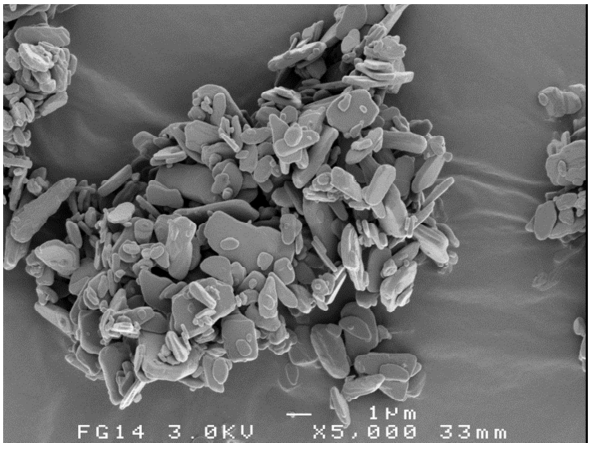
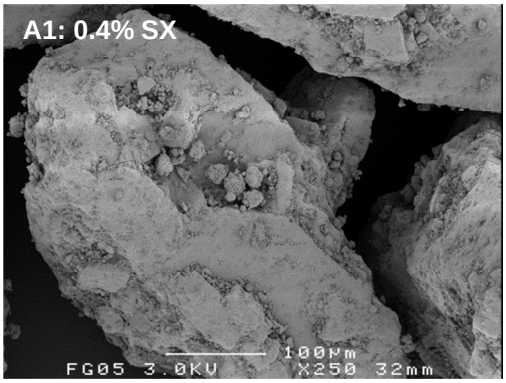
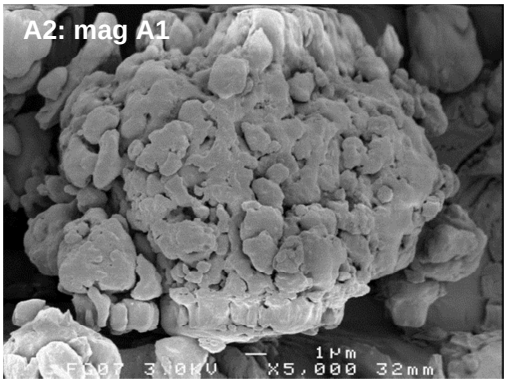
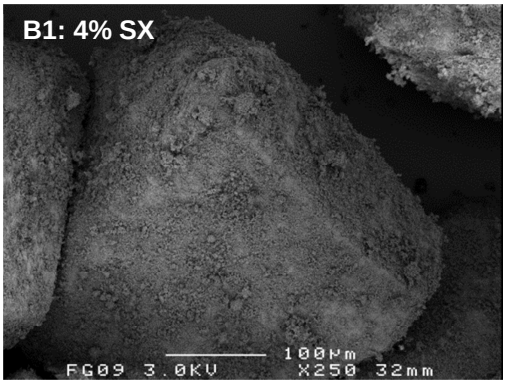
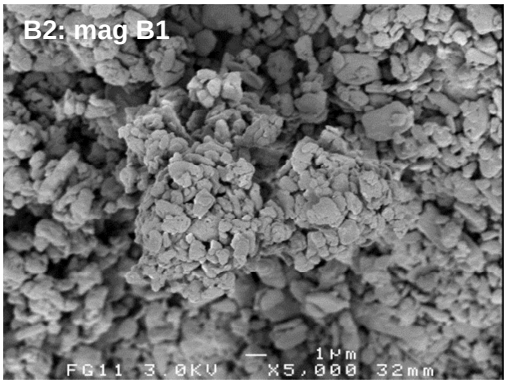


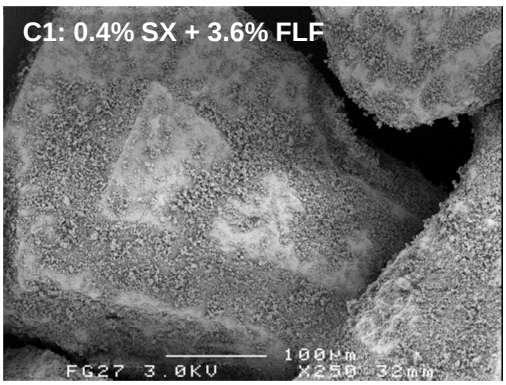
Figure 1: representative SEM image of micronised salmeterol xinafoate.

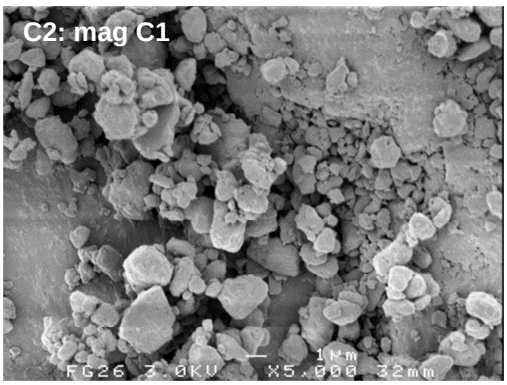

A1: 0.4% SX
100µm
FG05 3.0KV X250 32mm

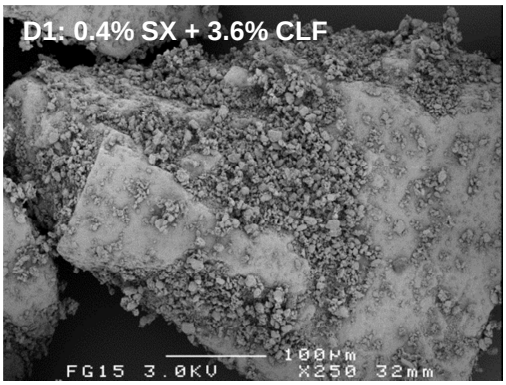

A2: mag A1
1µm
FG07 3.0KV X5,000 32mm

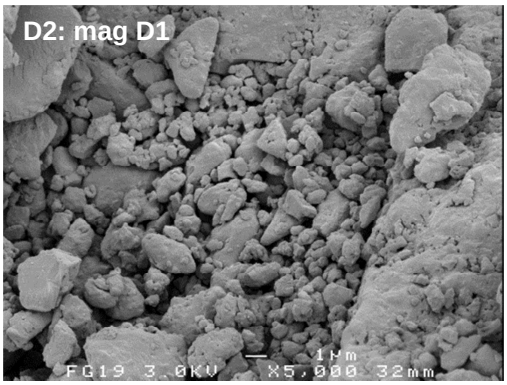

B1: 4% SX
100µm
FG09 3.0KV X250 32mm


B2: mag B1
1µm
FG11 3.0KV X5,000 32mm


C1: 0.4% SX + 3.6% FLF
100µm
FG27 3.0KV X250 32mm


C2: mag C1
1µm
FG26 3.0KV X5,000 32mm


D1: 0.4% SX + 3.6% CLF
100µm
FG15 3.0KV X250 32mm


D2: mag D1
1µm
FG19 3.0KV X5,000 32mm

supposedly SX, can still be distinguished in the 4% mixture after 60 minutes of mixing, whereas this is not possible for the other mixtures.

Drug detachment

An overview of the detached drug fractions at 20, 40 and 60 L/min for the different mixtures is presented in Figure 3. Overall, the observed effects on the detached drug fraction of mixing time, drug content, FLF and CLF, as well as any interactions with inhalation flow rate, are very similar to the effects of these variables previously presented in chapters 3-5 for salmeterol or other drugs. New in this chapter is the study of possible interactions between mixing time and the effects of FLF and CLF on drug detachment.

At a flow rate of 20 L/min, the highest detached drug fraction was obtained with the mixture containing CLF, followed by the mixtures containing 0.4% SX, 4% SX and FLF respectively. This order of dispersion performance does not change with increased mixing time from 2-60 minutes, although the absolute difference in the detached drug fraction between the mixtures changes considerably. For example, after 2 minutes of mixing, CLF result in a 29.4% absolute increase in the detached drug fraction of 0.4% SX, whereas after 60 minutes of mixing this increase is only 4.6%. The lower beneficial effect of CLF after a longer mixing time is mostly the result of an increase in the detached drug fraction of 0.4% SX alone, which was attributed to the agglomeration and deformation of a fraction of the salmeterol particles in chapter 4. At 40 L/min, both the order of dispersion performance and the absolute difference in drug detachment between the formulations changes from 2-60 minutes of mixing. At this flow rate, the detached drug fraction decreases from 2-60 minutes of mixing for all formulations, but this decrease is strongest for the mixture containing FLF (36%) followed by 0.4% SX (24.4%), CLF (24.3%) and 4% SX (17%). At 60 L/min, mixing time only affects the difference in the detached drug fraction between the formulations, but not their order of dispersion performance (which is 4% SX > FLF > CLF > 0.4% SX). After 2 minutes of mixing differences in the detached drug fraction at 60 L/min between the formulations containing 4% SX, CLF or FLF are statistically significant ($p < 0.05$), but not relevant.

Apparent solubility

The results of the apparent solubility tests are presented in Figure 4. In the 0.4% SX mixture, the apparent solubility of SX increases with mixing time, which results in a significant increase of the measured absorbance from 0.35 (reference) to 0.39 after 2 minutes of mixing ($p < 0.05$) up to 0.51 after 60 minutes of mixing ($p < 0.001$). These observations are in agreement with the apparent solubility data presented in chapter 4,

← Figure 2: representative SEM images of the different mixtures.

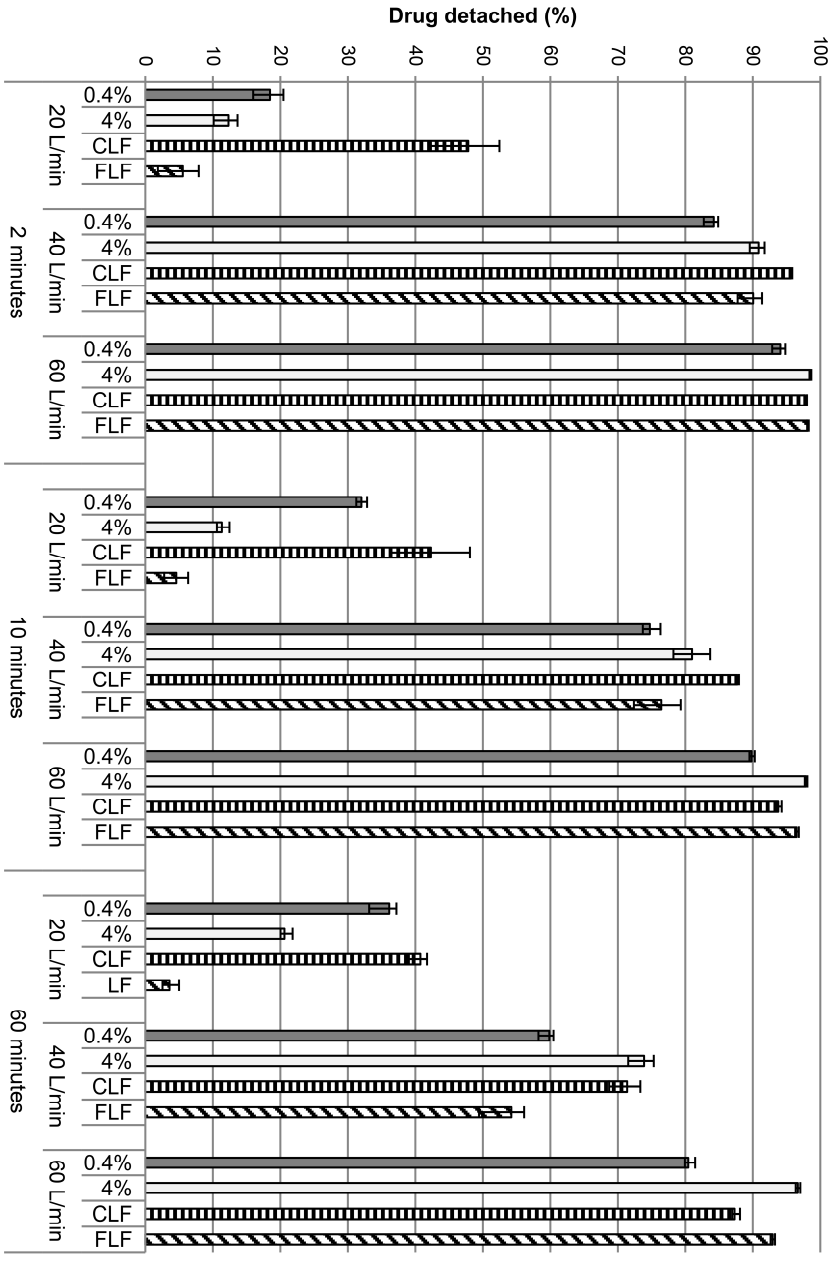


Figure 3: detached drug fractions of the different formulations at different flow rates and mixing times. Error bars represent maximum and minimum values measured (n = 5).

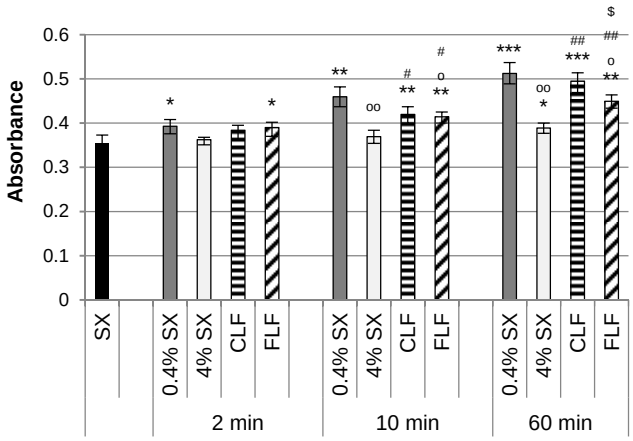


Figure 4: apparent solubility of SX in the mixtures, represented by the absorbance at 280 nm. SX = reference (micronised salmeterol xinafoate starting material). *, o, # and $ denote a significant difference relative to SX, 0.4% SX, 4% SX and CLF, respectively. One, two or three symbols represent p-values < 0.05, < 0.01 and < 0.001, respectively. Error bars represent maximum and minimum values measured (n = 3).

although the absolute absorbance values measured differ between both studies. This may result from differences in the environmental conditions (temperature), batch variations in the saturated aqueous salmeterol solution (e.g. polysorbate 80 concentration) or batch variations in the mixture. After 2 minutes of mixing, neither an increase in salmeterol content from 0.4 to 4% nor the addition of FLF or CLF to 0.4% SX results in a significant change in the apparent solubility of SX. After 10 minutes of mixing, the increase in drug content from 0.4 to 4% and the addition of FLF to 0.4% SX result in a significantly lower absorbance of 0.09 ($p < 0.01$) and 0.05 ($p < 0.05$), respectively. This is the same after 60 minutes of mixing, although absolute differences are then larger (0.12 and 0.06, respectively). The addition of CLF to 0.4% SX does not result in a significantly lower apparent solubility of SX in the mixture for any of the mixing times applied. The increase in drug content causes a larger decrease in apparent solubility than the addition of either FLF or CLF after 10 ($p < 0.05$) and 60 ($p < 0.01$) minutes of mixing. Furthermore, after 60 minutes of mixing, a significant difference exists ($p < 0.05$) between the effects of FLF and CLF on the apparent solubility of SX in the mixture (i.e. no effect of CLF, and a reduction by FLF).

It was observed during the apparent solubility measurements that the suspensions obtained with 4% SX mixtures were clearer and contained larger agglomerates than those obtained with the other formulations. To check whether this had affected the dissolution rate of SX and could thus explain the lower absorbance values in Figure 4, a control measurement was performed in which the four formulations mixed for 60 minutes were dispersed by short sonication (2 x 10 seconds with a 10

second pause in between) at the start of the dissolution hour. Visually similarly opaque suspensions were obtained this way. The resulting absorbance values were 0.556, 0.409, 0.530 and 0.505 for 0.4% SX, 4% SX, CLF and FLF, respectively. Thus, although all values were higher than those presented in Figure 4 due to the sonication process, the same trend was observed and agglomeration was unlikely to be the cause of the lower apparent solubility of SX in the 4% mixture.

Discussion

The apparent solubility data presented in Figure 4 do not support the press-on hypothesis and buffer hypothesis for the effects on drug detachment of drug content, FLF and CLF. According to these hypotheses, higher drug or lactose fines contents result in a higher susceptibility of drug particles to press-on forces [5, 6]. Furthermore, CLF could act as a buffer between colliding carrier particles or between carrier particles and the wall of the mixing vessel, thereby reducing compression of the drug particles onto the carrier surface [8]. Assuming that the degree of compression of the salmeterol particles is related to the degree of mechanical stress and, ultimately, the apparent solubility of SX in the mixture, then the apparent solubility of SX in the mixtures containing 4% SX or FLF should be higher than that in the mixture containing 0.4% SX. Furthermore, the apparent solubility of the mixture containing CLF should be lowest. However, none of this is true in practice; on the contrary, CLF does not result in a significantly lower apparent solubility, whereas FLF and a higher drug content do. This suggests that the hypothesised press-on effects of these variables do not occur, that they are dominated by other effects or that compression of drug particles is not related to their apparent solubility as assumed.

To determine which of the above suggestions is most plausible, it is necessary to discuss the process of drug compression in more detail, starting with the mixture containing 0.4% of salmeterol. From SEM observations and drug suspension tests it was inferred in chapter 4 that salmeterol particles are redistributed during mixing from carrier surface irregularities (where they are not susceptible to press-on forces) towards planes and protrusions (where they are immobilised by press-on forces). When compressed between lactose carrier particles or a carrier particle and the wall of the mixing vessel, salmeterol is the most likely material to plastically deform, because it has a much lower yield strength than lactose (i.e. 19.7 [11] vs 150.1 MPa [12], respectively). This causes defects in the crystal structure of the salmeterol particles and is likely responsible for the measured increase in apparent solubility with mixing time. A fraction of the mechanically stressed and deformed particles (roughly 50% of the total drug mass) was not immobilized on the carrier surface, but formed large agglomerates that were likely responsible for the beneficial effect on drug detachment at a low flow rate of 20 L/min.

When the drug content is increased from 0.4 to 4%, the drug particles form a continuous network over the entire carrier surface that is multi-layered (see Fig. 2B and chapter 3, Fig. 5). Although the filling up of carrier surface irregularities may indeed result in a higher susceptibility to press-on forces for a larger fraction of the drug particles, the magnitude of the press-on forces acting on individual drug particles may not be similar to the situation with a salmeterol content of 0.4%. For example, it is known that higher fine particle contents lower powder flowability (e.g. [13, 14]). As a result, carrier particles may collide at lower velocity when mixtures contain a higher drug or added lactose fines content, which lowers the press-on force. In addition, when press-on forces act on particle multi-layers, the mechanical stress imparted on individual drug particles may be reduced because the compressive pressure is distributed over multiple particles. Furthermore, part of the energy involved is likely absorbed by the fine powder bed on the carrier surface due to particle rearrangement and consolidation. This could cause a reduction in drug detachment efficacy during inhalation due to an increased coherence or tensile strength of the fine particle network. Hence, a paradoxical situation is imaginable, where the detrimental effect of compression to drug detachment increases even though the mechanical stress on individual drug particles is reduced. In such cases, the apparent solubility obviously is not a good indication of the occurrence of press-on effects. As mentioned, lactose is a harder material than salmeterol xinafoate. Furthermore, lactose fines may cause a lower tensile strength of the fine particle bed on the carrier surface due to differences in adhesion or cohesion properties. This may cause the energy from 'compression events' to be more directly or efficiently transferred to the individual salmeterol particles contained in multi-layers with lactose fines. In addition, the lactose fines have a more rounded shape than the salmeterol particles (chapter 5, Fig. 1 and Fig. 1 of this chapter), which may cause them to act in a way similar to ball bearings, thereby affecting powder bulk flowability in a less disadvantageous way than the plate-like SX particles. This may explain the higher apparent solubility of SX in the mixtures containing FLF and CLF than that in the 4% SX mixture.

If high mechanical stresses are most strongly experienced by drug particles that are directly compressed to the carrier surface instead of those arranged in multi-layer networks, then it could be that especially those drug particles that are bound to the carrier surface with the highest energy, and therefore are the most difficult to detach, are responsible for an increase in apparent solubility. This is further explored in Figure 5, in which the drug detachment data obtained by dispersion at 60 L/min (see Fig. 3) are displayed as a function of absorbance (see Fig. 4). Simple linear regression results in a negative relationship with a coefficient of determination (R^2) of 0.8896. Hence, approximately 89% of the variation in drug detachment at 60 L/min correlates to a variation in apparent solubility or mechanical stress. Even though such a fairly strong correlation does not necessarily result from a causal relationship between the factors, it does provide an indication that drug detachment at such a high flow rate is

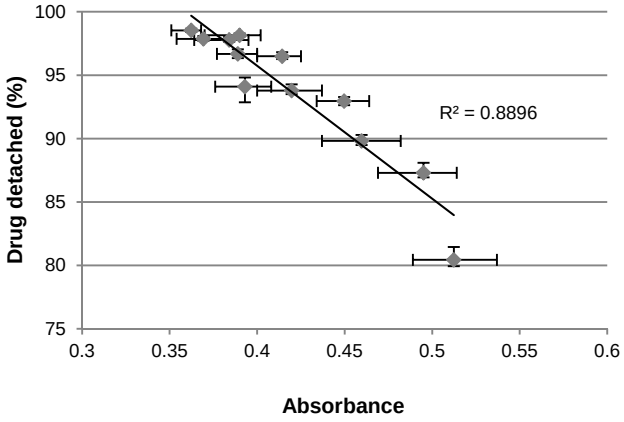


Figure 5: relationship from simple linear regression between the detached drug fraction at 60 L/min and absorbance. All formulations are included.

predominantly determined by press-on effects (i.e. only the most strongly bound or compressed particles do not detach). At flow rates of 20 and 40 L/min the correlation between drug detachment and absorbance is much lower, with R^2 values of 0.1584 and 0.5315, respectively. Hence, at these flow rates effects other than drug particle compression to the carrier surface dominate. This could include agglomeration effects, but also the compression of multi-layered particle networks, as discussed in the previous paragraph. The correlation between drug detachment and apparent solubility may depend on the type of dispersion principle used. For dispersion principles relying predominantly on aerodynamic (drag, lift and shear type) forces for the separation of drug particles from the carrier surface, drug detachment at high flow rates may to a large extent also be determined by sheltering of drug particles from these separation forces in carrier surface irregularities. This could result in a lower influence of the binding energy or press-on effects on drug detachment, and therefore, a lower correlation between drug detachment and apparent solubility.

From the above discussion it follows that a further understanding of press-on effects may result from the use of methods to assess powder flowability and the tensile strength of networks formed by the fine components. However, many different methods exist to measure powder flow properties, and the relationship between powder flowability and the magnitude or occurrence of press-on effects may not be straightforward. Furthermore, although an interesting technique has recently been demonstrated by Das et al. [15] for the determination of powder strength distributions, such a technique does not provide information about the consolidation and compaction behaviour of fine powders under mechanical loads representative of those experienced

by the fine particle networks on carrier particles during mixing. Therefore, the road towards a further understanding of press-on effects will be a very challenging one.

Conclusions

The apparent solubility of salmeterol in adhesive mixtures for inhalation can provide a greater fundamental understanding of the mechanisms behind the effects of formulation variables. The results from this study are not in agreement with the previously hypothesised increased susceptibility of drug particle to press-on forces with an increase in drug or FLF content. They neither support the 'buffer hypothesis' that was previously used to explain the favourable effects of CLF on drug detachment. However, a direct relationship between 'press-on effects' and apparent solubility does not necessarily exist, especially when the carrier surface is covered by multi-layers of fine particles. Therefore, a better understanding of press-on effects may result from studies that also consider other factors, such as powder bulk flowability and the tensile strength of fine particle networks on the carrier surface.

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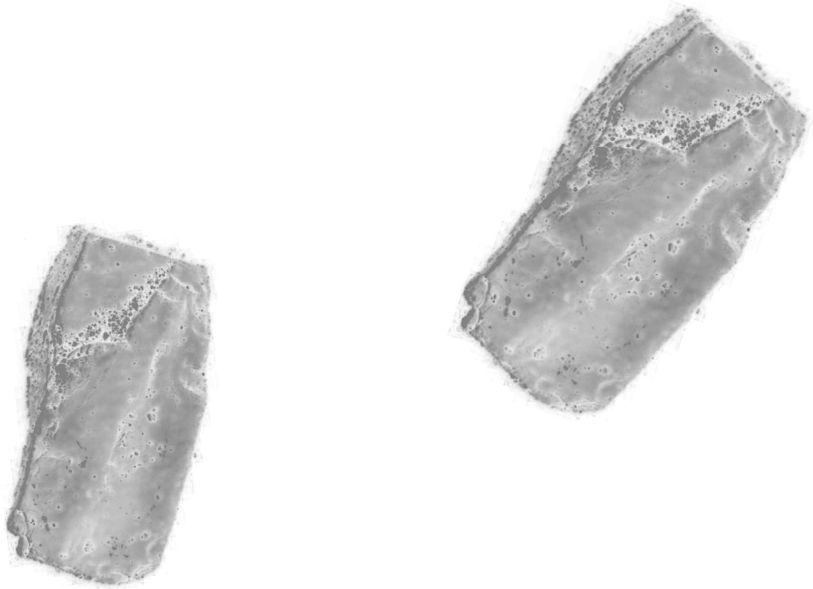
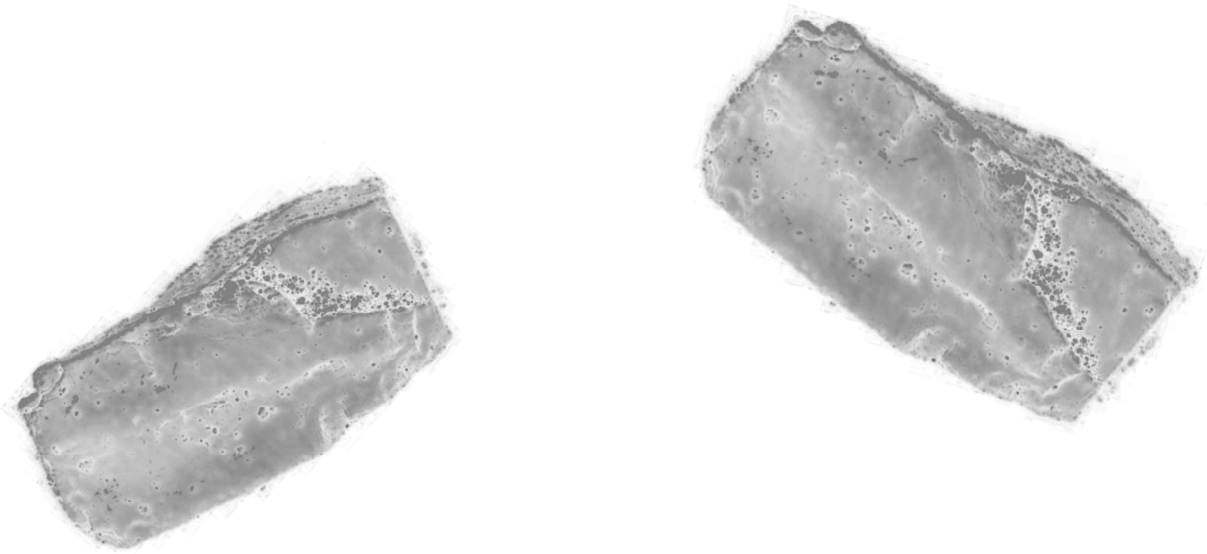


Chapter 7

A proposed definition of the 'activity' of surface sites on lactose carriers for dry powder inhalation

Floris Grasmeijer, Henderik W. Frijlink, Anne H. de Boer



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Abstract

A new definition of the activity of surface sites on lactose carriers for dry powder inhalation is proposed which relates to drug detachment during dispersion. The new definition is expected to improve the understanding of 'carrier surface site activity', which stimulates the unambiguous communication about this subject and may aid in the rational design and interpretation of future formulation studies. In contrast to the currently prevailing view on carrier surface site activity, it follows from the newly proposed definition that carrier surface site activity depends on more variables than just the physicochemical properties of the carrier surface. Because the term 'active sites' is ambiguous, it is recommended to use the term 'highly active sites' instead to denote carrier surface sites with a relatively high activity.

Introduction

The notion that some sites on the surfaces of carrier particles in adhesive mixtures are more 'active' than others was already introduced by Hersey in 1975 [2]. The term 'active sites' has been widely used in literature concerning adhesive mixtures for inhalation ever since, and it has also been extensively used in the discussions in chapters 3-5. However, a specific definition of 'active sites', or 'carrier surface site activity' in general, has never been formulated. This may lead to ambiguous communication about the subject and misconceptions regarding the significance of active sites to powder dispersion performance. It also hinders the rational design of formulation studies aiming to investigate mechanisms such as the 'saturation of active sites' and it could lead to the incorrect use of this mechanism as an explanation for certain effects of formulation variables on dispersion performance. A definition of carrier surface site activity is therefore proposed in this chapter.

The currently prevailing view on carrier surface site activity and its disadvantages

Carrier surface site activity is mostly related to the energy or force with which carrier surface sites bind drug particles, and sites that bind drug particles with a high energy relative to other sites are generally considered to be 'active sites' [2-4]. Such sites may for example be macroscopic surface irregularities where drug particles can form multiple contact points with the carrier surface, or amorphous regions and contaminations that lead to increased capillary interactions or larger contact areas due to plastic deformation upon compression. In addition, the term 'pseudo-active sites' was introduced for carrier surface sites of which a high binding energy is mostly the result of a high susceptibility of the drug particles to press-on forces during mixing [5]. Active sites are generally considered to be occupied preferentially over sites with lower activity by drug particles during the mixing process [2]. Although it is not clearly defined what constitutes a discrete 'carrier surface site', the view of carrier surface site activity in terms of binding energy implicitly defines a carrier surface site as any location on the carrier surface that can host or bind a drug particle. There are several disadvantages associated to the currently prevailing view on carrier surface site activity, two of which are highlighted in the following paragraphs.

Firstly, the term 'active sites' to denote carrier surface sites with a relatively high activity is deceptive, because it implies that 'inactive sites' also exist. However, when a drug particle is in contact with the carrier surface, at least London-Van der Waals interaction will occur regardless of the carrier surface site. Therefore, if the activity of carrier surface sites is related to their interaction force or binding energy towards a contacting drug particle, then all sites are active to some extent, whereas none are truly inactive. Only if the occurrence of drug particle detachment from carrier surface sites during dispersion is considered, a discrete classification of carrier surface sites as either

'active' or 'inactive' could be consistent (e.g. 'inactive' sites would denote sites from which drug detachment occurs, whereas 'active sites' would denote sites from which it does not, under defined dispersion conditions).

Secondly, and more importantly, a definition of site activity solely in terms of binding energy is flawed if one wishes it to be directly related to drug detachment from the lactose carrier during dispersion. It was explained chapter 2 that drug detachment is not so much directly related to the binding energy as it is to the ratio of separation to binding energy (i.e. energy ratio) . Hence, drug particles that are attached to the carrier surface with a relatively high binding energy may still be detached, whereas drug particles bound with a relatively low energy may not, if the separation energy for these drug particles results in energy ratios ≥ 1 (detachment) or < 1 (no detachment), respectively. This could be especially relevant for dispersion principles primarily relying on aerodynamic drag, lift and shear type separation forces in combination with carrier particles having a high surface rugosity. Drug particles may find shelter from these separation forces in depressions in the carrier surface [6], which could result in an energy ratio < 1, even if the binding energy at such sites would be relatively low. For inertial (collisional, vibrational, rotational) separation forces such a dependence on carrier surface geometry should theoretically be less pronounced. A dependence of the presence of sites that are apparently 'active' on the type of dispersion principle is, therefore, to be expected. This expectation is corroborated by the data presented in Figure 1. A disperser of the venturi type (RODOS, Sympatec), which relies primarily on aerodynamic separation forces, is not able to detach more than 70-75% of drug with increasing dispersion pressure above 200 kPa from coarse lactose carriers with relatively large

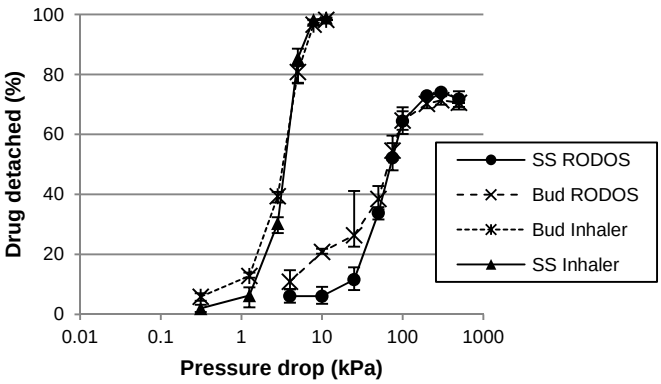

Figure 1: detached drug fraction with increasing pressure drops for two different dispersion principles. Mixtures contain 4% budesonide (Bud) or salbutamol sulphate (SS) on a lactose carrier with a 250-315 μm size fraction. Inhaler = classifier based test inhaler; device and procedures as described previously [1]. RODOS = RODOS dry powder disperser (Sympatec, Clausthal-Zellerfeld, Germany); carrier was collected after dispersion and the non-detached drug fraction analysed following the same procedure as for the inhaler measurements (n = 10).

surface irregularities. According to the current perception of site activity this could lead to the conclusion that 25-30% of the drug is bound to active sites. However, by dispersing the same mixtures with a classifier based test inhaler that relies predominantly on inertial separation forces, a drug detachment efficacy of 70-75% is already obtained between 2.7 and 5 kPa (equivalent to 30 and 40 L/min, respectively) and almost 100% detachment efficacy is obtained by further increasing the pressure drop (to only 10 kPa). Hence, sites that are apparently 'active' with one dispersion principle may not be so with another.

A proposed definition of carrier surface site activity

Based on the preceding considerations, it is proposed to define carrier surface site activity as the ability of carrier surface sites to retain drug particles during dispersion. Drug particles are all types of particles on the carrier surface that contain drug and that are detached as such from the carrier surface (i.e. primary drug particles as single entities and drug agglomerates or drug/lactose-fines composite agglomerates as clustered particles). Different approaches may be used to determine 'the ability of carrier surface sites to retain drug particles during dispersion', of which two examples are further discussed in the following paragraphs. An important difference between the possible approaches is the resulting definition of the term 'active site'. Therefore, it is proposed to use the term 'highly active site' to refer to a carrier surface site with a high activity relative to other sites in a less ambiguous way.

The ability of a carrier surface site to retain a single drug particle during inhalation may be expressed as the inverse of the energy ratio of that specific drug particle at that particular binding site at a defined dispersion effort (i.e. flow rate or pressure drop with a particular type of inhaler). With this approach carrier surface site activity is expressed on a continuous (non-discrete) scale. For non-spherical drug particles, the binding energy and thus the energy ratio may depend on their orientation. Therefore, even for a well-defined drug particle in terms of size and shape the activity of a specific carrier site is likely to be distributed based on the chance of different orientations of the drug particle. Because the binding energy, and thus the inverse of the energy ratio, cannot be equal to zero, all sites on the carrier surface should be considered 'active'. Highly active sites should thus be referred to as such, and may be defined by a minimum value for the activity (e.g. > 1, or no detachment) at a defined high dispersion effort. However, because the energy ratio cannot be measured, the activity of carrier surface sites cannot exactly be determined going by this approach. Despite this, highly active sites may be identified by imaging non-detached drug particles on the carrier surface after dispersion at the defined high dispersion effort. One should keep in mind then, that non-detached fine lactose particles may cause 'false positives' if they cannot be distinguished from drug particles. In addition, the absence of drug particles from carrier

surface sites may cause 'false negatives' if no drug particles were bound to those sites after the mixing process prior to dispersion. Furthermore, with this approach, the activity of lactose carrier surface sites does not take into account the potential storage capacity of active sites. In the case of multi-layer formation, sites will be formed by drug particles adhering to the lactose carrier, thus changing the surface properties. These 'apparent carrier surface sites' have their own activity, which is not assigned to the underlying real carrier surface site, even though the separation energy component of this activity may to a large extent result from the real carrier surface geometry.

A more pragmatic approach could be to express the ability of carrier surface sites to retain drug particles in terms of their residual carrier surface payload (volume drug/unit surface area) after dispersion. In this case, 'active sites' are all sites with an activity > 0 (m^3 drug/m^2 carrier surface area), whereas sites with an activity of 0 can be considered 'inactive'. Contrary to the previous approach, this approach accounts for differences in the storage capacity between active sites. Obviously, the occurrence and storage capacity of 'active sites' depends on the flow rate or pressure drop applied. 'Highly active sites' may be defined by an activity > 0 at a well-defined high dispersion effort. With this approach, quantification of the activity of a discrete carrier surface site will be very difficult still. Furthermore, the activity of all 'inactive sites' at a certain dispersion effort is equal (i.e. 0), even though differences in their ability to retain drug particles at lower dispersion effort will exist. As for the previous approach, highly active sites may be identified by imaging non-detached drug particles on the carrier surface after dispersion at the well-defined effort, but one should be wary of false positives and negatives.

Implications of the newly proposed definition

Regardless of the approach chosen to define and quantify the ability of carrier surface sites to retain drug particles during dispersion, the given definition implies that site activity, and also the occurrence and storage capacity of 'highly active sites', will depend on other variables than just the physicochemical characteristics of the carrier surface, including:

- the type of dispersion principle that is operated (Fig. 1);
- the mixing process (including the type of mixer, mixing speed, mixing time (see chapter 4), batch size);
- conditions determining capillary and electrostatic interactions;
- the drug properties; and
- the drug content (see chapter 3).

Hence, 'carrier surface site activity' should not be considered a carrier specific property:

it changes with the formulation and dispersion conditions. Because of this, the terms 'site activity' and 'highly active site' should always be put into context.

Highly active sites according to the proposed definition are not necessarily preferentially occupied by drug particles during mixing. During mixing, drug particles are separated from and redistributed over the carrier surface by the mixing forces. Redistribution will most likely be favoured towards sites on the carrier surface where the ratio of redistribution to binding energy is lowest. This may be strong binding sites, but also sites where drug particles can find shelter from the mixing forces, such as carrier surface irregularities. If the redistribution forces during mixing are dependent on the geometry of the carrier surface in a different way than on the separation forces during dispersion, then occupation of sites with a relatively low activity may be favoured during mixing. In other words, sites with a low activity from a dispersion perspective could be highly active from a mixing perspective. Such considerations can have important implications. For example, they question the validity of the hypothesis that a higher degree of 'saturation of (highly) active sites' by added lactose fines may be obtained when these fines are mixed with the carrier material before the drug instead of after [7].

The 'saturation of (highly) active sites' is often clearly distinguished from 'agglomeration' as a mechanism of action of formulation variables, such as the drug or fine lactose content (see chapter 3, and [7]). However, agglomeration may result in a lowering of carrier surface site activity according to the proposed definition, because the drug particle size is generally positively related to detachment from the carrier surface. Agglomeration thus saturates the drug (particle size) retaining ability of carrier surface sites, and therefore can be considered a form of (highly) active site saturation.

Conclusions

It is proposed to define the activity of carrier surface sites as their drug retaining ability during dispersion. In contrast to a definition of site activity in terms of binding energy or binding force, this new definition directly relates site activity to drug detachment from the lactose carrier during dispersion, and hence, increases its relevance. The term 'active site' may have a different meaning, depending on the approach chosen to define the drug retaining ability of carrier surface sites. Therefore, it is recommended to refer to carrier surface sites with a relatively high activity as 'highly active sites'. According to the proposed definition, the activity of carrier surface sites is the result of physicochemical properties of the carrier surface, as well as relevant formulation and dispersion conditions. A rational choice of such conditions is therefore of utmost importance in studies concerning carrier surface site activity.

Acknowledgements

We sincerely thank Maarten van den Noort for collecting the experimental data presented in Figure 1.

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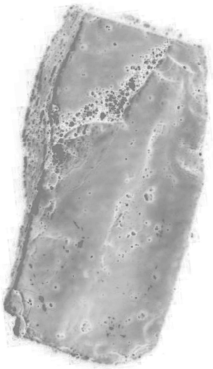
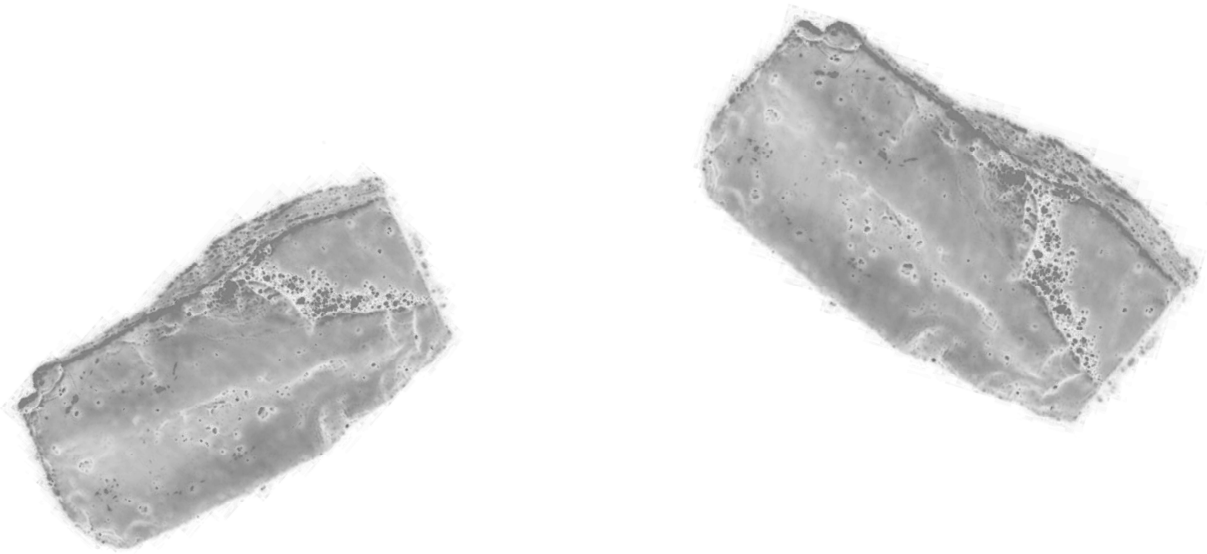


Chapter 8

Chemical-selective imaging of adhesive mixtures for inhalation using coherent anti-Stokes Raman scattering (CARS) microscopy



Based on: Andrew L. Fussell[1], Floris Grasmeijer[2], Henderik W. Frijlink[2], Anne H. de Boer[2], Herman L. Offerhaus[1], CARS microscopy as a tool for studying the distribution of micronised drugs in adhesive mixtures for inhalation, J. Raman Spectr, 2014. 45(7): 495-500

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Abstract

The spatial distribution and degree of agglomeration of drug particles throughout adhesive mixtures for inhalation are thought to be relevant to the dispersion behaviour of such mixtures. The physical state of these properties is very difficult to assess with conventional high resolution imaging techniques, because the small drug particles often cannot be sufficiently distinguished from other fine mixture components. Chemical-selective imaging techniques may be very helpful in this regard. Although several such techniques have been described, chemical-selective imaging is not yet routinely used in the study of adhesive mixtures. Reasons may be found in insufficient contrast formation for the specific chemicals used in adhesive mixtures for inhalation, or insufficient accessibility of the techniques. Additional techniques are therefore desirable. This chapter presents a preliminary study on the suitability of coherent anti-Stokes Raman scattering (CARS) microscopy for the chemical-selective imaging of adhesive mixtures. Its use in combination with image analysis software to determine the particle size distribution of budesonide in adhesive mixtures is explored. Furthermore, its combination with high resolution scanning electron microscopy (SEM) is presented. The results show that CARS microscopy is a promising technique for the characterisation of adhesive mixtures. It provides a way for their rapid chemical-selective imaging, even when the drug (e.g. budesonide) does not differ from lactose with respect to the chemical elements of which it is composed. However, the attainable field of view with the CARS setup from this study is very limited and not all drug particles appear to be detected with the settings used. Therefore, a further optimisation and validation of the techniques is required.

Introduction

Imaging of adhesive mixtures for inhalation is an important tool in unravelling the relationships between formulation variables, mixture properties and mixture dispersion behaviour. Scanning electron microscopy (SEM) is routinely used for visual observation and the characterisation of adhesive mixtures. Its high resolution makes it suitable to image the spatial distribution over and clustering of fine particles on the carrier surface, or micron and even nano scale surface topographical features of the individual mixture components. However, with the secondary electron signal that is normally used with SEM, chemical-selective imaging of adhesive mixtures is not possible. Identification of different components therefore has to be based on differences in morphological features, such as particle size and shape.

Identification of the different mixture components based only on morphological features may not always be possible. This is, for example, the case when different mixture components have a similar size and shape distribution, as is often true for the drug component and intrinsic or added fine lactose particles. Furthermore, it was shown in chapter 4 for micronised fluticasone propionate and salmeterol xinafoate particles that they lose their original shape during blending with the carrier material. As a result, their chemical identity cannot be established based on a morphological comparison of the particles before and after mixing. Identification of the different mixture components may allow to determine the composition of agglomerates on the carrier surface, the spatial distribution of the drug and fine lactose over the carrier surface, or the identification of 'highly active' carrier surface sites after dispersion, as explained in chapter 7. Furthermore, selective imaging of the drug component allows the use of image analysis software to determine, for example, its particle size distribution as present in the mixture. Such properties are expected to be relevant to powder dispersion behaviour, and therefore, chemical-selective imaging techniques may potentially be a valuable tool in inhalation formulation studies.

Several techniques exist which may be applied for the chemical-selective imaging of adhesive mixtures, some of which have been reviewed by Shur and Price [1]. Examples include backscatter electron imaging, energy-dispersive X-ray spectroscopy (EDX or EDS) and cathodoluminescence. Backscatter electron, X-ray and cathodoluminescence signals are all produced in addition to the secondary electron signal during SEM. Chemical-selective and topographical images of the same sample may therefore be easily obtained and combined with these techniques. However, contrast formation in backscatter electron and EDX imaging relies on differences in weighted average atomic number (z) or elemental composition of the sample material, respectively. Because the drugs used in the treatment of asthma and COPD and lactose are all organic compounds, only few drugs may be distinguishable from lactose based on these characteristics (e.g. those that contain elements heavier than oxygen, such as fluticasone

propionate and salbutamol sulphate, see Table 1) [1, 2]. Salmeterol xinafoate on lactose carriers has been selectively imaged using the cathodoluminescence signal from SEM [2]. For this, a "state-of-the-art detector" was necessary to capture the emitted light signal of very low intensity.

Table 1: chemical formulas of lactose and several drugs commonly used in the treatment of asthma and COPD.

Compound	Chemical formula
Alpha-lactose monohydrate	$C_{12}H_{22}O_{11}\cdot H_2O$
Budesonide	$C_{25}H_{34}O_6$
Salbutamol sulphate	$(C_{13}H_{21}NO_3)_2\cdot SO_4$
Salmeterol xinafoate	$C_{25}H_{37}NO_4\cdot C_{11}H_8O_3$
Fluticasone propionate	$C_{25}H_{31}F_3O_5S$

Raman techniques use a different principle based on the inelastic scattering of monochromatic light [3, 4]. Basically, incident light excites molecules from the ground state to a 'virtual energy state', which is characterised by an oscillating polarisation in the molecules. This virtual energy state is an intermediate state from which the molecule can pass into an excited vibrational state under the emission of a photon with a lower energy or longer wavelength than the incident photon (i.e. the incident light is 'red shifted').

This process is referred to as 'Stokes scattering' (Fig. 1) and it is the basis for spontaneous Raman techniques. The vibrational state is characterised, for example, by the stretching and bending of chemical bonds, and its energy level equals the energy difference between the incident and emitted photon. The transition from a ground state to a certain vibrational state via an intermediate virtual state is independent of the wavelength of the incident light. Therefore, a 'Raman spectrum', which is chemical specific, shows the intensity of the scattered light as a function of the energy difference between incident and emitted photon (and thus the energy level of the vibrational state). In addition to the ground state also a vibrational state of molecules can be excited to a virtual energy state by incident light, after which the molecule can pass into the ground state under the emission of light with a higher energy or shorter wavelength (i.e. the incident light is 'blue shifted'). This nonspontaneous process is referred to as 'anti-Stokes scattering' (Fig. 1) and is the basis for coherent anti-Stokes Raman scattering (CARS). In CARS three photons are used to cause and stimulate Stokes scattering, followed by anti-Stokes scattering (Fig. 1). CARS probes the same molecular vibrational frequencies as spontaneous Raman techniques [4]. It differs from spontaneous Raman mapping techniques by an approximate 100 times faster imaging speed [5]. This is because spontaneous Raman techniques collect information over a wide spectral range while with CARS information is collected from only a single Raman shift. Furthermore, because CARS produces an anti-Stokes (blue) shifted signal it is free from single photon

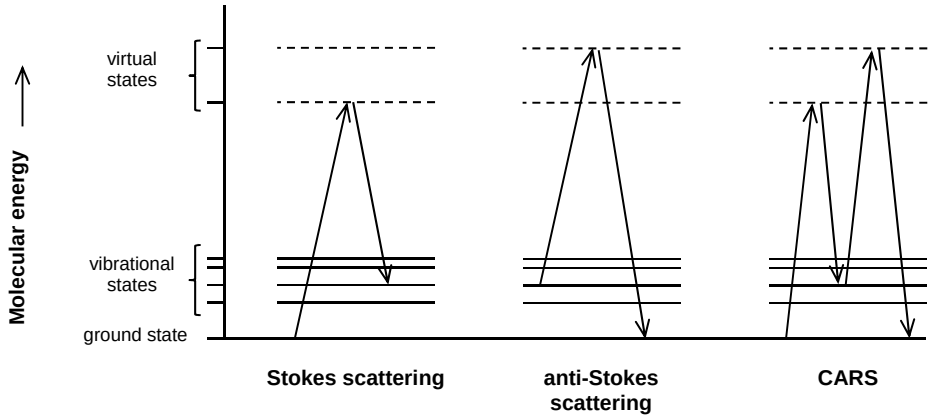

Figure 1: energy diagrams representing Stokes, anti-Stokes and coherent anti-Stokes Raman scattering (CARS).

fluorescence, which hampers spontaneous Raman measurements.

Microscopes that employ the CARS technique have been widely applied in biological and medical imaging [6, 7]. So far, the application of CARS microscopy to pharmaceutical systems has been scarce. Kang et al. [8-10] imaged in situ release of paclitaxel from polymeric films in a static medium using CARS microscopy. In the first application focusing on orally administered drugs and dosage forms, Windbergs et al. [11] and Jurna et al. [12] used CARS microscopy to image the distribution of theophylline in lipid dosage forms and monitored the release of theophylline during dissolution in a flow through cell setup. They were able to image both drug release and conversion from theophylline anhydrate to theophylline monohydrate in real time.

The resolution of CARS microscopy is restricted to the diffraction limit of light, which is considerably lower than that of electron imaging techniques such as SEM. However, because CARS microscopy is a non-destructive imaging technique, SEM may be performed of a sample after CARS microscopy and the obtained images can be subsequently compared. Combining the imaging modalities of light microscopy and electron microscopy is well-established in the area of biomedical sciences, with fluorescence microscopy commonly being combined with SEM. Comparative light and electron microscopy (CLEM) allows a cell to be imaged from the micron to nanometre scale while maintaining its spatial orientation [13]. Additionally, spontaneous Raman microscopy [14] and spectroscopy [15] have been combined with SEM. CARS microscopy has also been combined with electron microscopy [16, 17], but to the best of our knowledge has not yet been demonstrated in a comparative manner with SEM and never before been applied in studies on drug distribution in adhesive mixtures.

In this chapter we explore the suitability of CARS for the chemical-selective imaging of adhesive mixtures for inhalation. In addition, the use of image analysis software to determine the particle size distribution of drug particles in the mixture based on CARS images is demonstrated. Furthermore, we present an imaging system that combines CARS microscopy with SEM, allowing direct comparison of images recorded by both modalities.

Materials and methods

Adhesive mixtures

Adhesive mixtures previously prepared for the studies presented in chapters 3 and 4 were used for the imaging study presented in this chapter. Mixtures of 0.4% budesonide and a coarse lactose carrier (250-315 μm) mixed for 10 minutes or 1.48% salmeterol xinafoate and a fine lactose carrier (63-90 μm) mixed for 0.5 or 600 minutes were used. Further details about the preparation and characterisation of these mixtures can be found in the materials and methods sections of chapters 3 and 4, respectively.

Hyperspectral and z-stacked CARS methods

CARS microscope setup. The CARS microscopy system is described in detail elsewhere [18]. Briefly, A Nd:YVO4 picosecond pulsed laser (Coherent, USA) operating at a fundamental wavelength of 1064 nm was frequency doubled to pump an optical parametric oscillator (OPO) (APE, Germany) which produced two dependently tunable laser beams. The fundamental laser beam was combined with one of the beams from the OPO and directed into an inverted microscope (Olympus IX71, Japan), where they were focused using either a 60X/1.2 NA objective (distribution / particle size measurements with budesonide mixtures) or a 40X/0.9 NA objective (CLEM imaging with salmeterol mixtures). The backscattered CARS signal was collected by the focusing objective and detected with a photomultiplier tube (Hamamatsu, Japan). The CARS microscope system using the 60X objective had an axial spatial ('depth') resolution of about 1 μm and a lateral spatial ('in focal plane') resolution of about 0.4 μm.

Hyperspectral imaging. Hyperspectral CARS imaging provides a rapid method to extract the CARS intensity profile (CARS spectra) over a range of wavenumbers allowing the identification of peaks suitable for recording chemically specific CARS images. The method for conducting hyperspectral imaging was published previously [18]. Hyperspectral scans were recorded over the wavenumber range of 2800-3100 cm^{-1} using a step size of about 4 cm^{-1} and took about 4 minutes to collect.

Z-stacked imaging. CARS microscopy is inherently confocal with a signal generated only within the focus of the objective. This feature provides the ability to record z-depth or axially stacked images to gain a greater understanding of the drug distribution on the rough surface of the lactose carrier particles. Z-stacked imaging was conducted by stepping the microscope objective in the z-direction in increments of 1 μm. Each z-stacked image (512x512 pixels) consists of about 20 slices and took about 1 minute to record.

Particle size analysis

Laser diffraction. The particle size distribution of the primary budesonide particles was measured with a HELOS BF diffractometer using a RODOS dry disperser at 3 bar (Sympatec, Clausthal-Zellerfeld, Germany). The diffractometer was equipped with a 100 mm lens and calculations were performed based on the Fraunhofer approximation theory. Increasing the dispersion pressure to 5 bar did not affect the particle size distribution of the drug, indicating that indeed the particle size distribution of the primary particles was measured at 3 bar. To compare the RODOS data with the results from the analysis using Image J (see further) laser diffraction particle sizes were recalculated to projected surface areas (SA) according to the equation $SA = ¼ * \pi * d_{drug}^2$.

Image J. Particle size analysis was performed using the particle analyser command available in the Image J software (http://rsbweb.nih.gov/ij/) according to the following steps. The image file was firstly opened by Image J and the scale bar from the image used to set the scale in image J. The image was then converted to 8 bit type and converted to binary using the inbuilt automated routine based on the IsoData algorithm [19].With this routine, pixels with an intensity of 84 or lower (on a scale of 0-255) are discarded from the image. The scale bar was then removed from the image before setting the particle analyser command to analyse for particles between 0-infinity μm^2.

CLEM method

Samples were mounted on a glass microscope slide using double-sided adhesive tape and suspended in the air above a 40X/0.9NA objective. Carrier particles of interest were identified using the transmission signal. Z-stacked CARS images (512x512 pixels) were recorded for both the lactose carrier particle (2888 cm^{-1}) and the drug (budesonide, 3046 cm^{-1}, salmeterol, 3050 cm^{-1}) loaded onto the surface. After CARS imaging, the glass microscope slide was removed from the CARS microscope and mounted on double-sided carbon tape and placed on the SEM sample holder. The samples were sputter coated with 20 nm of a gold/palladium alloy. SEM images were then obtained at an acceleration voltage of 3 kV (JSM-6301F, Jeol, Japan).

Results and discussion

CARS spectra

Hyperspectral CARS images of the pure chemical compounds were recorded to identify the key vibrational bands that would allow selective imaging of the adhesive mixtures. Hyperspectral scans covered the wavenumber range from 2800-3100 cm^{-1}, corresponding to the C-H stretch region. Figure 2 shows the CARS spectra extracted from the hyperspectral data for alpha-lactose monohydrate, budesonide and salmeterol. The frequencies chosen for single wavelength imaging were 2888 cm^{-1} (lactose), 3046 cm^{-1} (budesonide), and 3050 cm^{-1} (salmeterol).

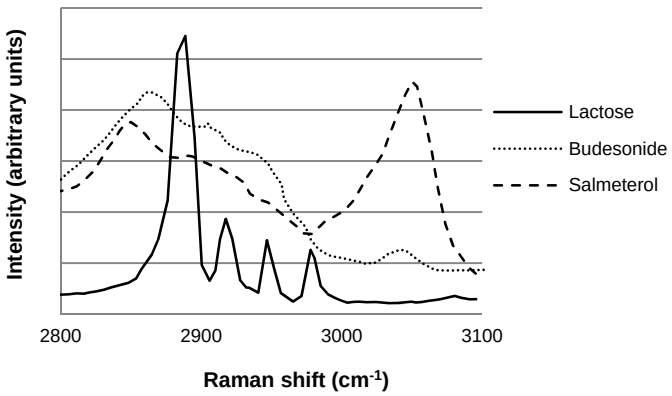

Figure 2: CARS spectra extracted from hyperspectral data covering the C-H stretching range from 2800-3100 cm^{-1} for lactose, budesonide and salmeterol.

Drug distribution

Changing the wavelength of one of the lasers allowed different components in the same sample to be subsequently imaged. Figure 3A shows a z-stacked CARS image (512x512 pixels) collected at 3046 cm^{-1}, which is selective for budesonide. Figure 3B shows a z-stacked image (512x512 pixels) of the same area as Figure 3A recorded at 2888 cm^{-1}, which is selective for lactose. Figure 3C shows the overlaid z-stacked images from Figures 3A and B, while Figure 3D shows the corresponding transmission light image.

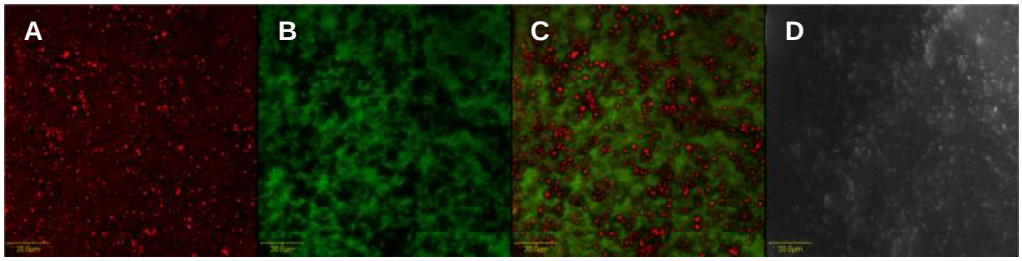

Figure 3: projected z-stack CARS images. A: budesonide (3046 cm^{-1}), **B:** lactose (2888 cm^{-1}), **C:** overlay image showing distribution of budesonide on the surface of a lactose carrier particle, and **D:** transmission light image.

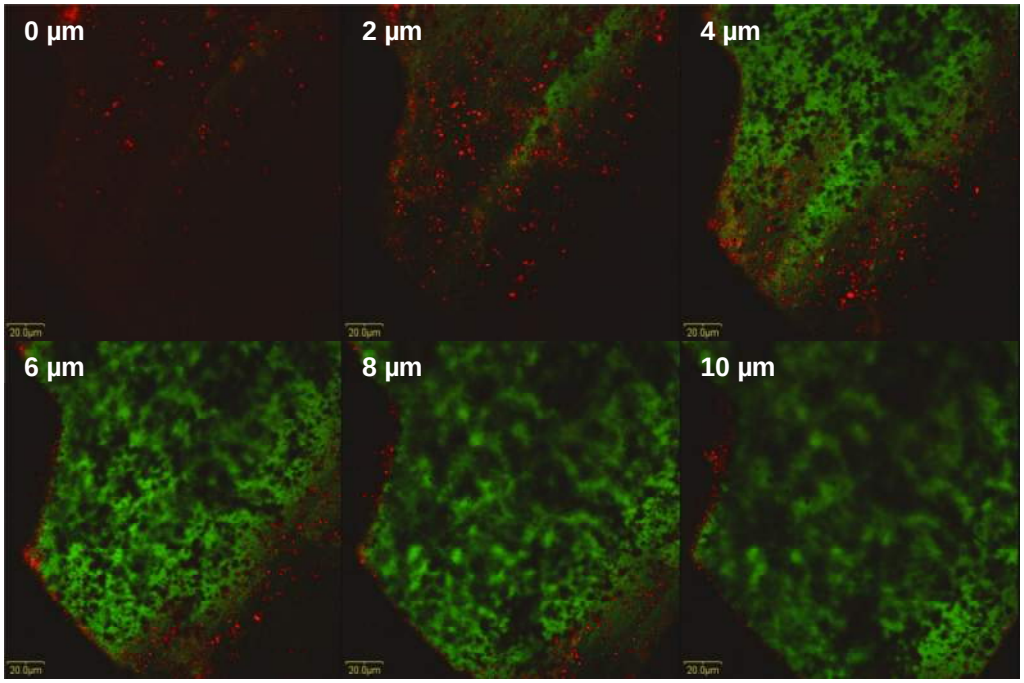

Figure 4: frames from a z-stacked image of a lactose carrier particle (green) loaded with budesonide (red).

From Figure 3C we can see that the budesonide (red) is distributed in clusters covering the surface of the lactose particles. Comparing the CARS image in Figure 3C with the transmission image in Figure 3D highlights the strength of the CARS, as the transmission image suggests little more than a rough carrier surface and does not reveal the spatial distribution of the drug. Furthermore, it should be noted that budesonide does not contain any other elements than lactose (carbon, hydrogen and oxygen, see

Table 1), which excludes a technique such as EDX for the chemical-selective imaging of a mixture of both compounds.

Figure 4 shows frames from a z-stacked image (512x512 pixels) of a budesonide (red) loaded lactose carrier particle (green) starting from 0 μm depth and stepping every 2 μm until a depth of 10 μm. Budesonide particles at different z-depths, for example due to large carrier surface irregularities, can be imaged this way. The confocal nature of CARS microscopy may also offer the possibility to image changes in the depth of the drug in a multi-layer of drug and added lactose fines when, for example, studying the effect of the order in which the different fine components are added to the blend. Of course, for such applications one has to take into account any effects of differences in the z-depth of the carrier surface itself when interpreting the images.

The field of view in Figures 3 and 4 is restricted to only part of a carrier particle in the size range of 250-315 μm. As a result, multiple images are needed in order to sufficiently corroborate certain findings regarding drug distribution. With the 40X objective a larger field of view was attainable, but this was still not sufficient to completely capture a coarse carrier particle. With a 20X objective, the signal strength of the drug component was too low to be detected. It should be noted that by using a different objective the resolution of the microscope system will also change.

Particle size analysis

The Image J particle analysis command calculates the particle area by setting a threshold intensity below which pixels are removed and the remaining pixels are counted to give an area in μm^2. Figure 5A shows the original CARS image (reproduced from Figure 3A) prior to particle size analysis. Figure 5B shows the background removed image with only the drug particles (black) remaining. This image contains roughly 6% of the number of pixels in Figure 5A. Figure 5C shows Figure 5A and B overlaid. From Figure 5C it can be seen that there is a good correlation with most of the particles in the CARS image represented in the background removed image. In Figure 5D particle size distributions determined using Image J particle analysis (grey) and laser diffraction (red) are compared. The particle size distribution obtained with Image J analysis reports 68% of the particles to have a median projection area of $0.4\mu m^2$, while laser diffraction analysis results in a particle size distribution with 94% of the primary particles within the same size class. The larger particle sizes measured with Image J analysis can be attributed to agglomeration of the primary particles during mixing. The sizes of composite agglomerates containing drug and lactose particles will likely be underestimated using the combination of CARS microscopy and Image J. Furthermore, the process of obtaining a binary image from the CARS image was not further validated, which would be required for future application of the technique in practice.

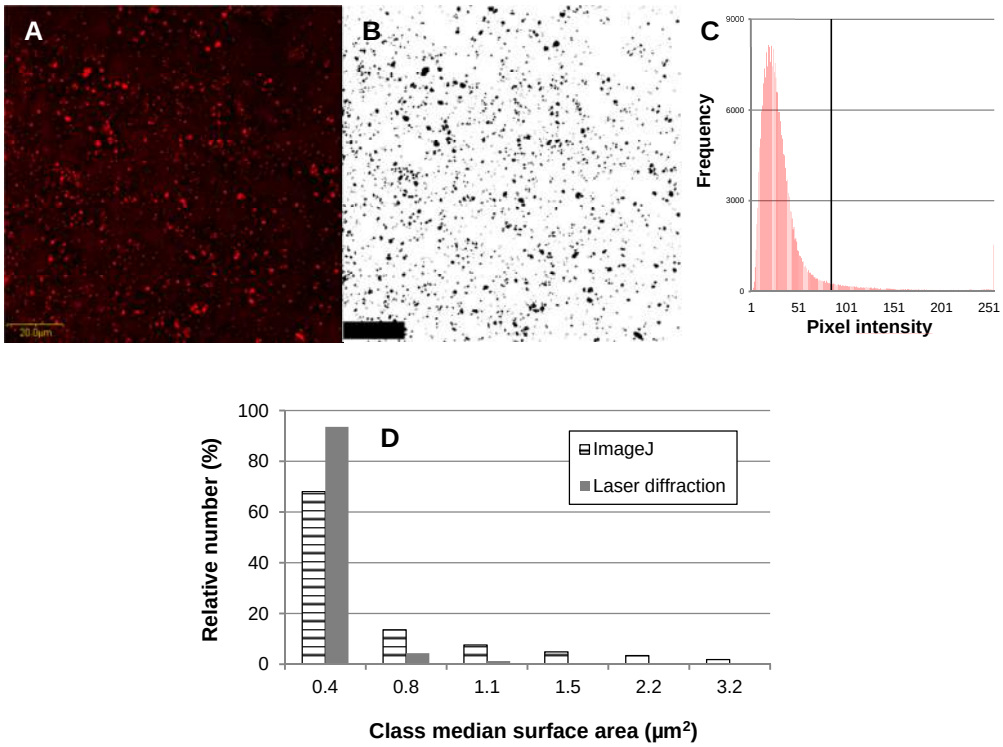

Figure 5: particle size distributions obtained by laser diffraction and ImageJ. A: original CARS image of a budesonide coated lactose carrier particle. **B:** binary representation of the CARS image containing the particles (scale bar represents 20 µm). **C:** Pixel intensity distribution in Fig. A. The vertical line at an intensity of 84 marks the cut-off intensity below which pixels are discarded for the binary representation in Fig. B. **D:** particle size distributions from ImageJ and laser diffraction analysis.

Comparative CARS and SEM imaging

Combining SEM with CARS microscopy provides the advantages of the high resolution from SEM and the chemical selectivity from CARS. With the combined techniques it is therefore possible to image the spatial distribution of the drug on the carrier surface and to relate this to morphological characteristics of the drug and carrier particles. This is especially useful when the drug particles do not deviate in size or shape distribution from added or intrinsic lactose fines (as observed in chapter 5), or when the mixing process causes the drug particles to change in such a way that they cannot be identified by comparison with the starting material (as observed in chapter 4). Figure 6 shows the result of comparative CARS and SEM imaging of the salmeterol blends prepared by 0.5 and 600 minutes of mixing. After only 0.5 minutes of mixing, the characteristic plate-like shape of the salmeterol particles as observed in chapters 4 and 6 is still intact, allowing

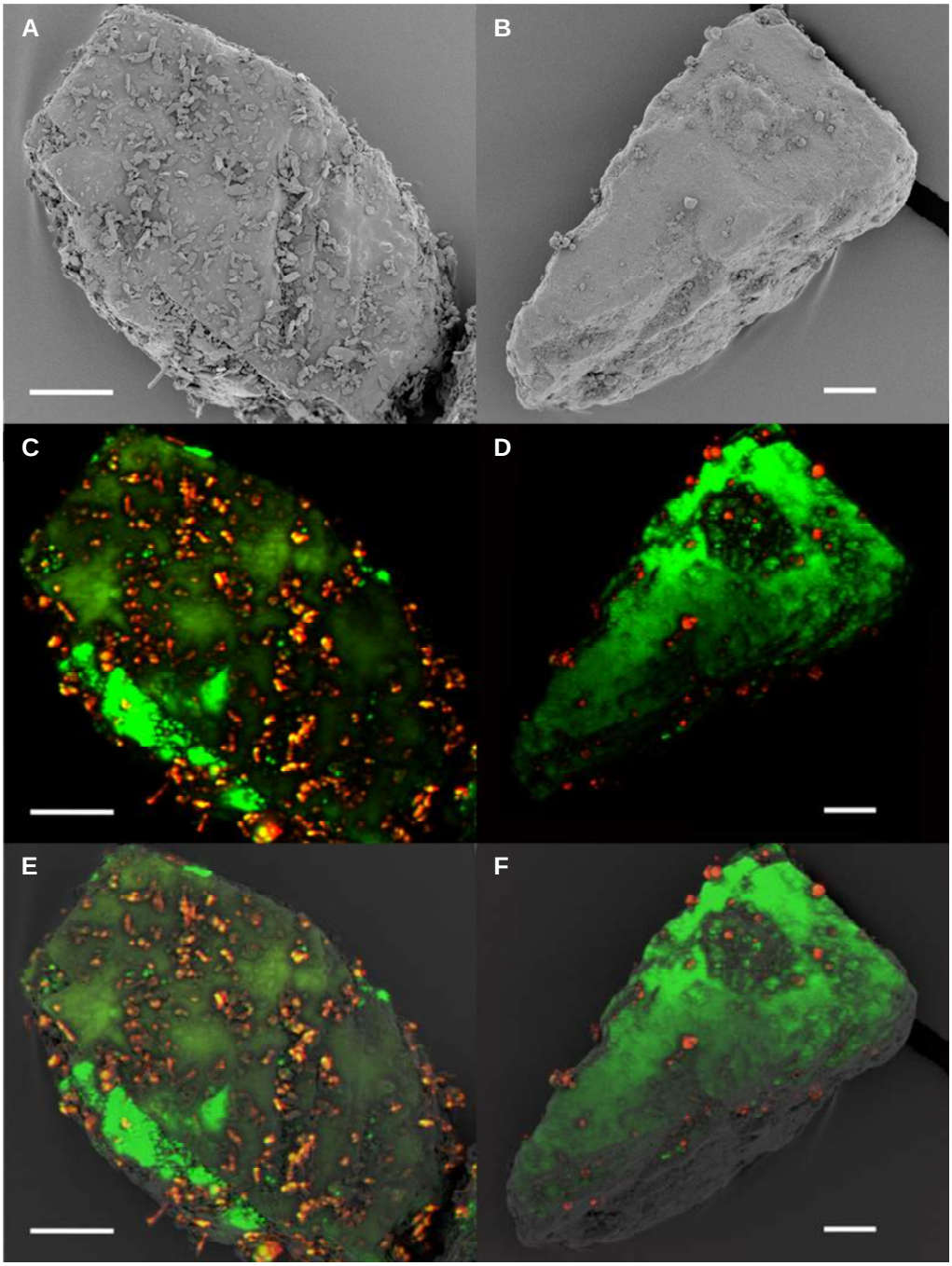

Figure 6: SEM (A+B), CARS (C+D) and CLEM images (E+F) of salmeterol mixtures after 0.5 (left) and 600 minutes of mixing (right). Scale bars represent 20 μm.

the identification of the drug particles on the carrier surface by only high resolution SEM imaging. The distribution of drug particles appears to be similar between both techniques. Interestingly, upon close examination not all particles that may be identified as drug particles based on their plate-like shape by SEM imaging are identified as such by CARS. These particles could consist of lactose, but it is possible that due to a high carrier surface roughness combined with a large z-stack step size (1 μm) CARS imaging missed some of the drug particles observed using SEM. In case of the latter, CARS imaging can possibly be further optimised by reducing the image scanning speed and by using a smaller step size for z-stacked imaging, thereby improving the correlation with SEM imaging. CARS confirms that the plastically deformed and agglomerated particles on the carrier surface after 600 minutes of mixing consist of salmeterol. In chapter 4 it was also described that salmeterol forms a film on the carrier surface in addition to the spherical agglomerates after such long mixing times. However, this film formation is not observed with CARS, which may be due to the film thickness being below the resolution limit for CARS microscopy.

Conclusions

CARS microscopy provides a way for the rapid chemical-selective imaging of adhesive mixtures. Its suitability does not depend on the presence of chemical elements that distinguish the drug from the lactose carrier material, and for that reason it provides a valuable addition to other chemical-selective imaging techniques. When combined with image analysis software it can aid in obtaining quantitative data about the particle size distribution of the drug as present in the mixture. Furthermore, CLEM by combining CARS and SEM is a promising tool for the qualitative study of adhesive mixtures for inhalation. However, a further optimisation and validation of these techniques is required. For example, the attainable field of view with the CARS setup in this study is too small for completely capturing adhesive units that contain a coarse (250-315 μm) lactose carrier particle. Furthermore, not all drug particles appear to be detected with a 1 μm z-stack step size, nor is a salmeterol film that is suppposedly formed on the carrier surface after prolonged mixing.

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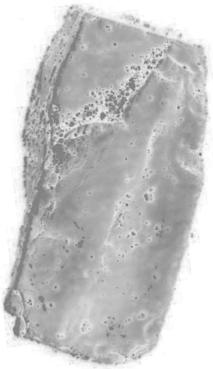
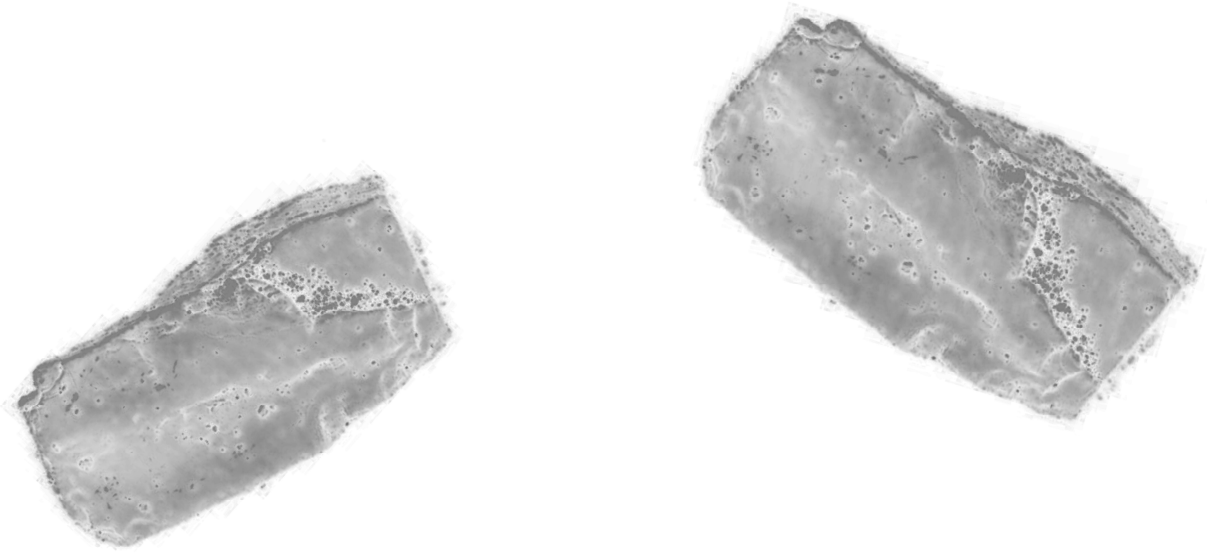


Chapter 9

General discussion



Introduction

In the previous chapters a variety of studies and discussions have been presented, all with the aim to improve the understanding of the relationships between formulation and inhalation variables and the dispersion behaviour of adhesive mixtures for inhalation. Of course, a better understanding of adhesive mixtures is merely a means to an end in which patients are treated more effectively and safely by dry powder inhalation therapy in general. While fewer and fewer pages separate us from the back cover of this thesis, several questions become more and more in need of being addressed: Do the studies on the effects of drug content, mixing time, added fines or new analysis techniques and the discussions on 'carrier surface site activity' or 'dispersion performance' versus 'dispersion behaviour' indeed lead to an improved overall understanding of adhesive mixtures? And if so, how exactly does that result in a safer and more effective dry powder inhalation therapy for patients?

These and other questions are addressed in the following general discussion. It summarises the most important results and condenses them into a schematic model of the interplay between variables. In addition, some implications for future research studies and dry powder inhalation therapy in general will be addressed. And finally, a last conclusions section will mark the end of this thesis.

The interplay between variables

In chapters 3-6 multi-order interactions between formulation and inhalation variables were shown to be omnipresent. Interactions of inhalation flow rate with formulation variables were predicted based on energy ratio distributions in chapter 2. In all formulation studies presented in successive chapters experimental support for their existence was indeed obtained. Anticipation of this specific interaction has resulted in a more complete assessment of the effects on drug detachment of the formulation variables studied, and therefore, has been crucial in unravelling their mechanisms of action. This is in direct contrast to the common practice of dispersion performance testing at a single inhalation flow rate in mechanistic inhalation formulation studies, e.g. [1-3]. A better conception of the mechanisms by which certain formulation variables affect drug detachment could furthermore be obtained by studying their interactions with other formulation variables. In chapter 6, for example, the interaction of drug content, added lactose fines and the size fraction of added lactose fines with mixing time was rationally used to study the occurrence and significance of press-on effects.

The data presented in chapters 3-6 have consistently suggested that formulation variables exert their influence on dispersion performance by affecting processes that occur within the mixture during blending. Multiple powder properties relevant to

dispersion performance (referred to as 'principal factors') are thereby changed, each with its own effect on dispersion performance (referred to as 'principal effects'). The net effect of a formulation variable thus depends on the 'sum' of its principal effects. Three principal factors have been identified and studied in this thesis, and they can be summarised as:

- the size distribution of the drug particles (including drug/drug and drug/fine-lactose agglomerates) as they are detached from the carrier surface;
- the degree of compression of the drug particles onto the carrier surface and each other; and
- the 'activity distribution' of carrier surface sites (see further) occupied by drug particles.

The principal effects that result from a change in one of the three principal factors listed will from here on be referred to as particle size effects, press-on effects and activity effects, respectively. The first two principal factors are quite self-explanatory, but the activity distribution of occupied carrier surface sites is more complex. It is a result of the distribution in activity of carrier surface sites that can potentially host drug particles, as extensively discussed in chapter 7, and the actual spatial distribution of the drug over these carrier surface sites. The activity of carrier surface sites was defined in chapter 7 as the ability of carrier surface sites to retain drug particles during dispersion. This ability is affected by the size distribution and degree of compression onto the carrier surface of the drug particles. Therefore, particle size and press-on effects are actually specific types of activity effects. What makes activity effects especially complex is the fact that carrier surface site activity also depends on inhalation variables such as the dispersion principle, although they do not physically alter the mixture. Hence, a paradoxical situation is possible in which the third 'mixture property' is altered without any physical changes to the mixture. Obviously, this presents challenges with respect to the qualification or quantification of this principal factor. Other examples of activity effects besides a change of dispersion principle (and excluding particle size and press-on effects) are changes in the carrier surface morphology [4-7]; coating of the carrier surface with 'force control agents' (e.g. magnesium stearate) [7, 8]; altering the drug distribution over the carrier surface by changing the mixing time (chapter 4); altering the type of drug; or the saturation of 'highly active' carrier surface sites with an increasing drug or lactose fines content in the mixture (chapters 3 and 5) and [3, 5, 9].

The principal factors and their effects are not only key to understanding the influence of formulation variables on mixture dispersion performance, but also to understanding the interactions that occur between formulation and inhalation variables. Each interaction essentially results in a different sum of principal effects. In chapters 3-6 two distinctly different types of interactions have been observed:

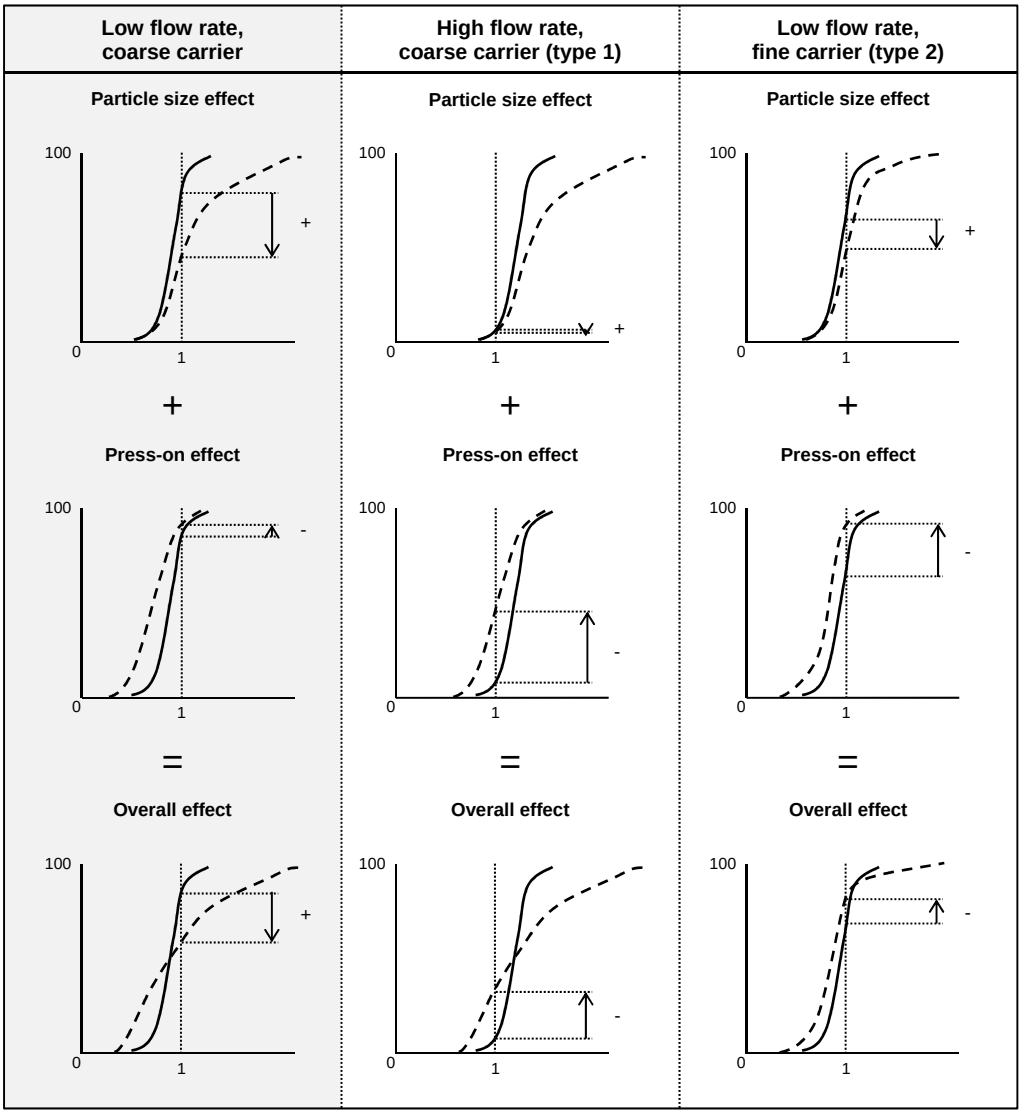


Figure 1: the two types of interactions explained by means of energy ratio distributions. Y-axes of the individual figures represent the cumulative drug mass (%), X-axes the energy ratio. The **left column** loosely represents the effect of mixing time on salmeterol xinafoate detachment from a coarse carrier at a low flow rate (see chapter 4, Fig. 11). The overall effect (bottom figure) is the sum of a particle size effect (top figure) and a press-on effect (middle figure). The **middle column** shows an interaction of the first type. Flow rate alters the relevance to drug detachment of the changes in the particle size distribution and the degree of compression of the drug. This is represented by a shift of the energy ratio distributions to higher values. The **right column** shows an interaction of the second type. A finer carrier causes mixing time to change the particle size distribution and degree of compression of the drug in a different way, resulting in a different change in the energy ratio distributions. As a result, different particle size and press-on effects amount to a different overall effect. The magnitudes and signs of the effects on drug detachment are represented by the length of the arrows and '+' or '-' signs, respectively.

1. those in which one variable affects the relevance to drug detachment of equal changes in the principal factors that are caused by another variable, and
2. those in which variables affect each other in how they alter the principal factors.

The first type of interaction occurs between inhalation variables (which do not physically alter the mixture properties) and formulation variables (which do). The second type occurs mainly between formulation variables, but can also occur between inhalation and formulation variables (e.g. because of the mentioned potential 'activity effect' of the dispersion principle). Both types of interactions can be illustrated by means of energy ratio distributions, as depicted in Figure 1. The examples in this figure do not consider activity effects: the overall effect shown in the bottom graphs is the sum of only the particle size and press-on effect presented in the top and centre graphs, respectively. They are loosely based on the effect of mixing time on salmeterol detachment from lactose carriers and its interaction with inhalation flow rate and carrier size fraction as presented in chapter 4 (Figure 11). The above conception of the interplay between variables is schematically presented in Figure 2.

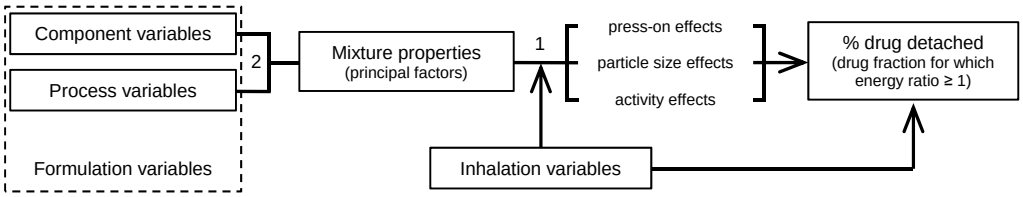


Figure 2: schematic presentation of the interplay between variables in the formulation and dispersion of adhesive mixtures for inhalation. Component variable: any variable related to the physicochemical properties and relative amounts of mixture components (e.g. carrier surface roughness, carrier size fraction, type of drug, drug content); **process variable:** any variable related to the mixing and mixture handling processes (e.g. mixing time, mixing intensity, mixing principle, batch size, powder filling or dosing principle); **inhalation variable:** any variable related to the dispersion process (e.g. type of dispersion principle, inhalation flow rate, loading of the dispersion principle or dose weight, residence time of the powder in the dispersion principle); **energy ratio:** the ratio of separation to binding energy (E_s/E_b) for a drug particle that is adhered to the surface of a carrier particle (chapter 2); the principal factors and effects are defined in the text. The numbers **1** and **2** refer to the two types of interactions identified and defined in the text and further explained in Figure 1.

The recognition that interactions between formulation and inhalation variables play an important role in the dispersion of adhesive mixtures is not new, although it has only quite recently emerged with an extensive review by de Boer, Chan and Price [10]. A focus on interactions in inhalation formulation studies is not new either, although it is

demonstrated on very rare occasions only, most notably in a study on the mechanisms of added lactose fines [3]. However, the notion that multi-order interactions between formulation and inhalation variables are omnipresent and that they may be explained by changes in the relevance or physical state of only a few mixture properties at once has never been presented before. This notion provides a framework that allows individual studies to be put in their proper perspective and may form a guide to the rational design of future experiments.

Major challenges and future research directions

The ability to accurately measure the principal factors of adhesive mixtures is a prerequisite for unravelling their relationship to dispersion performance under different inhalation conditions (e.g. inhalation flow rate, type of dispersion principle) on the one hand and to the properties of the starting materials and formulation processes on the other. Several existing techniques were explored and new ones were introduced in this thesis for the measurement of the three principal factors (or aspects thereof). However, they are either inherently biased (e.g. the wet laser diffraction technique applied in chapters 3 and 4 for measuring the particle size distribution of the drug in the mixture), do not provide a direct indication of the factor of interest (e.g. the apparent solubility as a measure of compression in chapters 4 and 6) or need further fine-tuning regarding the conditions under which they are used (e.g. the imaging technique evaluated in chapter 8). Therefore, a most urgent and major challenge lies in the development of new or the optimised use of existing techniques for the characterisation of principal factors of adhesive mixtures. It should be noted in this respect that more principal factors may be relevant than just the three mentioned in the previous section. For example, they do not include effects of the fluidisation behaviour of the mixture on dispersion performance, which was hypothesised to be one of the possible mechanisms of effect of added lactose fines [11]. Therefore, attention should also be focussed on the identification and characterisation of other principal factors than the ones studied in this thesis.

The principal factors are intermediary between the properties of the starting materials and the dispersion behaviour of adhesive mixtures. Therefore, as already mentioned in the previous paragraph, an understanding of the relationship between properties of the starting materials and dispersion performance can only result from unravelling the relationships between the properties of the starting materials and the principal factors on the one hand, and the principal factors and dispersion performance on the other hand. Considering the fact that large changes in some of the physicochemical properties of drug particles as a result of the mixing process were observed in chapter 4, studying properties of the starting materials that determine their change in response to the mechanical stresses generated during mixing would be highly relevant. An example may be their hardness or yield stress. Such a property could also

relate to the cohesion-adhesion balance (CAB) [12] for drugs and lactose, which for salmeterol and fluticasone was found to be in agreement with their agglomeration behaviour during mixing (chapter 4). Furthermore, the observed changes in the physicochemical properties of the starting materials during mixing diminish the relevance of their initial characteristics such as the size and shape distribution or surface roughness. The relationship between the principal factors and dispersion performance is affected by inhalation variables such as inhalation flow rate and the type of dispersion principle. In this thesis much attention has been focussed on the influence of flow rate, but only for one type of dispersion principle. Therefore, studies on how the type of dispersion principle affects the specific results obtained in this thesis would be of interest.

Drug detachment from lactose carriers was chosen as the measure of dispersion performance in this thesis. As pointed out in previous chapters, the detached drug fraction may not always correlate to the drug fraction that has the correct size distribution for inhalation beyond the oropharynx. Furthermore, it is known that the dispersion performance of adhesive mixtures may change over time depending on storage conditions [13], an aspect that has not been taken into account in the current study. Therefore, the framework as discussed in the previous section can be refined by studies focussing on agglomerate dispersion after detachment from lactose carriers as well as the physicochemical stability of adhesive mixtures.

Last, but most certainly not least, accurate definitions can form a useful aid in identifying research directions and preventing ambiguous scientific communication. They are therefore of major importance to the advancement of dry powder inhalation therapy. In this thesis, definitions of new terms (e.g. energy ratio and energy ratio distribution) as well as new definitions of existing terms (e.g. carrier surface site activity) were proposed, and vivid discussions should be stimulated to come up with more useful definitions of these or other terms in the future.

Practical implications

The safe and efficient treatment of patients by dry powder inhalation therapy requires the production of dry powder inhalation products that effectively and efficiently deliver the drug to the airways and that are of constant quality. Patient health risk is further minimised by use of the least amount of excipients. The better understanding of the relationships between formulation and inhalation variables resulting from the work in this thesis allows for a more efficient and efficacious implementation of a quality by design approach to dry powder inhalation product development. Specifications of so-called 'critical quality attributes' (CQAs) of the starting materials and 'critical process parameters' (CPCs) can be better defined if their relationships to dispersion performance are known [14]. This, in turn, can help manufacturers of the starting materials to develop and produce products that better meet the demands of the pharmaceutical companies. In

addition, it should directly benefit therapy efficacy and patient safety because of a resulting more constant product quality. If the advanced understanding of adhesive mixtures is also applied to the development of more efficiently dispersing formulations based purely on an advanced, more rational choice of process variables and variables of the lactose and drug components, patient safety would further benefit as lower doses are then required (leading to fewer adverse reactions) and the use of dispersion performance enhancers such as magnesium stearate may become obsolete.

A constant therapy, i.e. a constant lung deposition of the drug, does not only require a constant product quality. For example, inter- and intra-patient variability in inhalation flow profile will result in variability in lung deposition pattern of the drug. Drug deposition will shift to larger airways with increasing inhalation flow rate [15]. Such an effect can be compensated for by a flow rate dependence of the fine particle fraction or particle size distribution within the fine particle fraction. For this, an increase in inhalation flow rate should result in a higher fine particle fraction or a finer particle size distribution. We have seen in this thesis that formulation variables such as drug content, the addition of lactose fines and mixing time can greatly affect the flow rate dependence of dispersion performance. An improved understanding of the interplay between variables can thus be used for the development of inhalation products that deliver a flow rate independent therapy.

A more constant product quality that results from an effective quality by design approach will also lower production costs by reduced batch rejection. This would either be an attractive incentive for the pharmaceutical industry, or it may benefit society if it results in a lower cost of dry powder inhalation therapy. Lower costs may also result from a rise of the market in generic products, because an improved understanding of adhesive mixtures would better allow the development and subsequent approval of 'equivalent' dry powder inhalation products. One of the aspects of equivalence would be a similar flow rate dependence of the product's dispersion performance, as elaborated on in the previous paragraph.

Conclusions

The work presented in this thesis has led to an improved conception of the interplay between variables in the formulation and dispersion of adhesive mixtures for inhalation as summarised schematically in Figure 2. Multi-order interactions between these variables are omnipresent, and they can be explained by changes in the relevance or physical state of only a few mixture properties. This notion provides a framework for future research efforts, which can accelerate the further understanding of adhesive mixtures. A major and most urgent challenge lies in the development or optimisation of analysis techniques for the characterisation of mixture properties relevant to dispersion performance. It is anticipated that the improved understanding of adhesive mixtures resulting from the

current and future work will stimulate the development of generic products and the effective implementation of quality by design approaches to product development. It may furthermore lead to the development of more efficiently dispersing formulations, without the need for potentially harmful excipients. This benefits patient safety and could reduce the cost of dry powder inhalation therapy.

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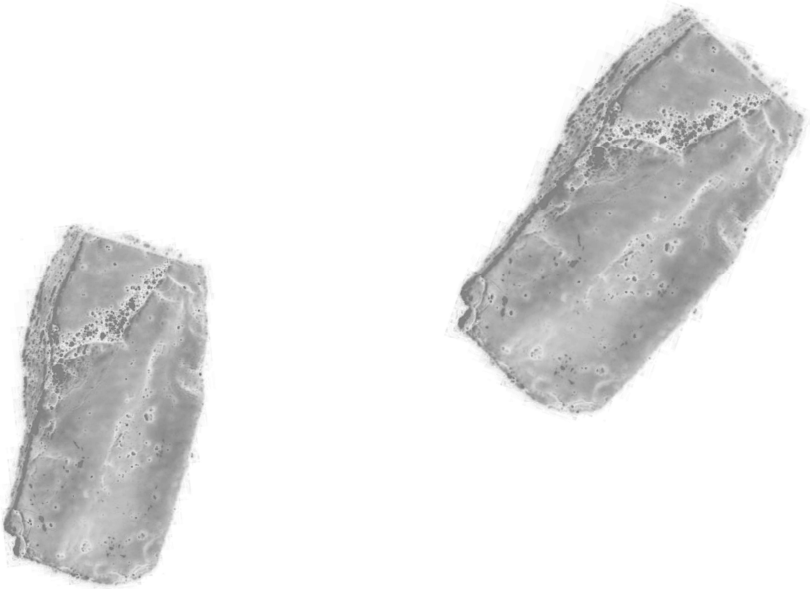
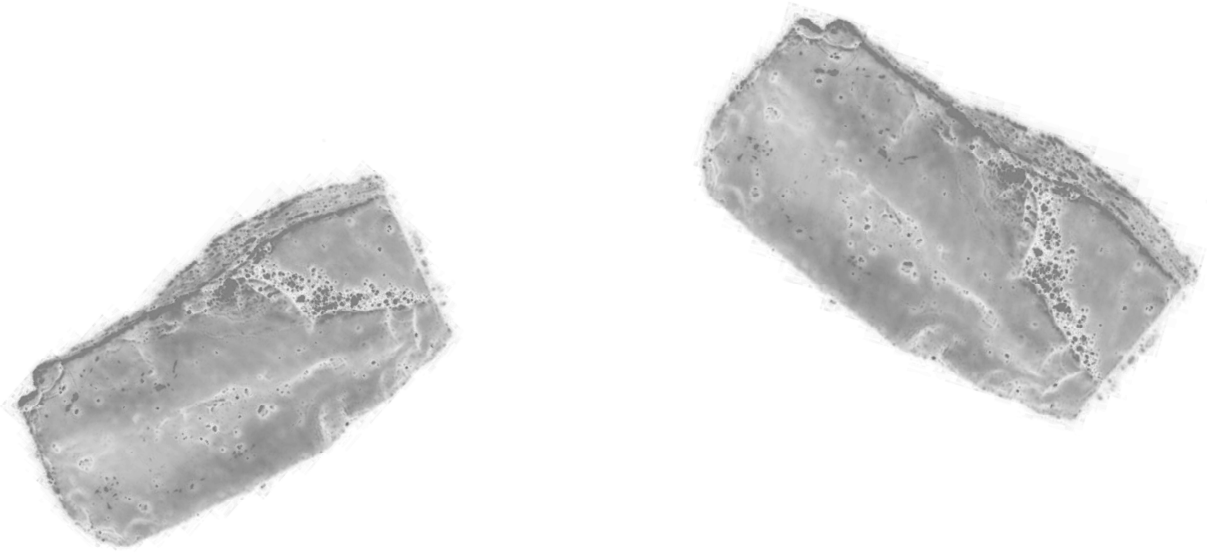


Appendix A

Summary



Chapter 1: general introduction. Adhesive mixtures provide a means for accurate dosing of drugs in dry powder inhalation therapy, but they pose challenges with respect to efficient dispersion during inhalation. A better understanding of the relationships between formulation and inhalation variables and mixture dispersion performance is necessary to be able to improve the therapeutic safety and efficacy of adhesive mixtures, and it is the aim of this thesis to contribute to such an understanding. To fulfil this aim, a study approach is chosen with a special focus on identifying interactions between formulation and inhalation variables, and on identifying and measuring adhesive mixture properties relevant to dispersion performance.

Chapter 2: the interacting role of inhalation flow rate. The first interaction studied in this thesis in a theoretical way is that between inhalation flow rate and formulation variables. It is common practice in formulation studies for dry powder inhalation to test the dispersion performance of formulations at only one inhalation flow rate. However, interactions may be expected between inhalation flow rate and the effects of formulation variables on dispersion performance, which may in the extreme lead to contradictory conclusions drawn at different flow rates. This has previously been shown for carrier-free formulations and this could be explained by their dispersion performance being directly related to the agglomerate tensile strength distribution. An analogy is drawn between carrier-free formulations and adhesive mixtures. The ratio of potential separation to binding energy, or 'energy ratio', and the distribution of its value for the population of drug particles in the mixture is introduced to describe drug detachment from lactose carriers during inhalation. The dispersion performance of adhesive mixtures (i.e. drug detachment) at any flow rate directly relates to their energy ratio distributions. Based on hypothetical examples of energy ratio distributions it is shown that the effect of a variable on drug detachment cannot be the same in magnitude over the range of inhalation flow rates that corresponds to 0-100% detachment efficacy. It may even change in direction. Therefore, dispersion performance should be more commonly tested over a wide range of flow rates to increase the utility of mechanistic formulation studies. Based on these considerations, the interaction with inhalation flow rate is anticipated in all experimental studies presented in following chapters.

Chapter 3: drug content effects on dispersion performance. Although several studies in the literature result in conflicting conclusions about the effects of drug content on the dispersion performance of adhesive mixtures, it is well recognised that drug content is a relevant variable in this respect. Therefore, it is surprising to observe that the choice of drug content in formulation studies is often based on historical considerations and is the same in many studies (i.e. 1.46%). It is hypothesised in chapter 3 that the conflicting results can be explained by a balance of different mechanisms, which shifts under the influence of interacting variables. This hypothesis is tested by studying the effect of drug

content on drug detachment from lactose carriers and its dependence on inhalation flow rate, the mixing time, the type of drug and the drug content range. Scanning electron microscopy (SEM) and wet laser diffraction technique have been applied to study the occurrence of three mechanisms: agglomeration of the drug particles, a change in the susceptibility of drug particles to press-on forces and the saturation of strongly bonding carrier sites, or so-called 'active sites'. It was found that multi-order interactions exist between the variables tested. Qualitative and quantitative interactions were observed with flow rate. This means that both favourable and unfavourable effects on the detached drug fraction of different magnitudes were obtained with the same change in drug content. The interactions could indeed be explained with a shifting balance of the three mechanisms mentioned. However, the exact relevance of each of these mechanisms could not be determined with the powder characterisation techniques applied. Because of the interactions between flow rate and the other variables considered, the study provides support for the conclusions drawn in chapter 2. Furthermore, it provides a basis for a more rational choice of drug content in future formulation studies.

Chapter 4: mixing time effects on dispersion performance. A similar approach to that in chapter 3 has been applied to the study of mixing time effects on the dispersion performance of adhesive mixtures in chapter 4. The dependence of these effects on inhalation flow rate, carrier size fraction and the type of drug was studied. In addition to the application of SEM and wet laser diffraction technique, the apparent solubility of salmeterol xinafoate after mixing was determined and used as a measure of the degree of mechanical stress exerted on the drug particles. The agglomeration behaviour of the drug component during mixing was strongly affected by the type of drug and the carrier size fraction. Agglomeration upon prolonged mixing was most pronounced for the most cohesive drug tested (i.e. salmeterol xinafoate) when mixed with a coarse carrier size fraction. The agglomeration behaviour of this drug/carrier combination could explain a favourable effect of prolonged mixing on the detached drug fraction at an inhalation flow rate of 20 L/min. At a flow rate of 60 L/min, with a fine carrier size fraction or fluticasone propionate as the drug, prolonged mixing resulted in an unfavourable effect on drug detachment. This suggests that under these conditions the overall effect of mixing time on drug detachment is dominated by the break-up of drug agglomerates from the starting material, compression of drug particles onto the carrier surface or their redistribution towards carrier surface sites with a higher activity. Drug particles were found to be extensively plastically deformed (salmeterol) or fragmented (fluticasone) during prolonged mixing, and they formed a coherent particulate layer or 'film' over the carrier surface. In addition, the apparent solubility of salmeterol increased upon mixing. This all is an indication of considerable mechanical stress on the drug particles, and therefore, of the relevance of press-on effects during the mixing process. As for chapter

3, the interactions between flow rate and the other variables considered provide support for the conclusions of chapter 2. Furthermore, the results again show that seemingly conflicting results for the effect of a formulation variable on dispersion performance can be explained by a shifting balance of different mechanisms.

Chapter 5: effects of added lactose fines on dispersion performance. A hypothesised analogy between drug content effects and the effects of added lactose fines on dispersion performance is studied in chapter 5. If true, the effect on drug detachment of added lactose fines with a similar size and shape distribution as the drug could interact in a qualitative way with flow rate, even though mechanisms resulting in an unfavourable effect of added lactose fines on dispersion performance have never been proposed. The effects on drug detachment of added lactose fines with different size fractions and their dependence on budesonide content, fines content, inhalation flow rate and mixing order were studied. Mixtures were characterised by SEM. The effects on drug detachment of fine lactose fines (FLF, X_{50} = 1.95 μm) are very similar to drug content effects: there is a similar qualitative interaction with flow rate at a low drug content of 0.4%. Coarse lactose fines (CLF, X_{50} = 3.94 μm) only result in a favourable effect on drug detachment at all flow rates compared to the drug alone. The difference in effect between FLF and CLF could support a previously proposed 'buffer hypothesis'. However, FLF and CLF also result in a different structure of the fine particle network on the carrier surface, as observed with SEM. This suggests that lactose fines, when they are fine enough, may contribute to the formation of strongly coherent particle networks, whereas they may prevent the formation or cause the disruption of such networks when they are larger. These possible mechanisms behind the effects of added lactose fines were never considered before. Interactions with the order in which the drug and lactose fines were mixed with the carrier material were observed, but they could not be considered evidence in favour of or against specific mechanisms. An issue in discussing the results is the lack of a proper definition of 'active sites', which is extensively addressed in chapter 7. Similarly to chapters 3 and 4, the results from this chapter support the conclusions about the interacting role of inhalation flow rate in chapter 2. In addition, it is again shown that a formulation variable may result in any possible effect on dispersion performance, depending on the choice of other, interacting variables. The improved understanding of the effects of added lactose fines may lead to advanced applications of this formulation technique, including optimisation of the flow rate sensitivity of dispersion performance.

Chapter 6: press-on effects in adhesive mixtures. Hypotheses regarding the occurrence of press-on effects as a result of changes in drug content, added lactose fines content or the size distribution of added lactose fines are tested in a preliminary study presented in chapter 6. The apparent solubility of salmeterol xinafoate after mixing is measured and

used as an indication of the amount of mechanical stress and the degree of drug particle compression. An increase in drug content from 0.4% to 4% results in a significant decrease of the apparent solubility of salmeterol. A significant but lower decrease in apparent solubility results from the addition of 3.6% fine lactose fines (FLF, X_{50} = 1.95 µm) to 0.4% salmeterol, whereas the addition of 3.6% coarse lactose fines (CLF, X_{50} = 3.94 µm) does not significantly lower the apparent solubility. The effects on drug detachment were similar to those reported in chapters 3 and 5. The findings indicate that a higher drug content and FLF reduce the mechanical stress on drug particles during mixing, whereas CLF do not. This conflicts with the hypothesis of an increased susceptibility to press-on forces and the buffer hypothesis, respectively. Furthermore, the apparent solubility of salmeterol after mixing does not correlate to the detached drug fraction at inhalation flow rates of 20 and 40 L/min. Therefore, other effects, such as agglomeration, may dominate at these flow rates. In addition, especially for multi-layered fine particle networks a paradoxical situation is imaginable, where the detrimental effect on drug detachment of press-on effects increases even though the mechanical stress on individual drug particles is reduced. At a flow rate of 60 L/min there is a strong correlation (R^2 = 0.8896) between apparent solubility and the detached drug fraction, which may indicate that drug detachment at this flow rate is predominantly determined by the degree of compression of drug particles onto the carrier surface. For a further understanding of press-on effects a focus on other powder properties, such as flowability or the tensile strength of fine particle networks on the carrier surface, could be helpful.

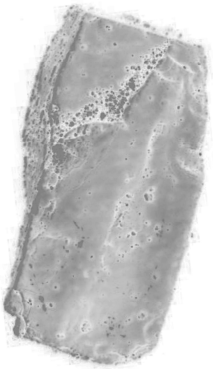
Chapter 7: a proposed definition for 'carrier surface site activity'. 'Active sites' have been discussed especially in chapters 3 and 5, but this was complicated by the lack of a proper definition of this term. To stimulate the ambiguous communication about this topic in scientific discussions and to improve the interpretation of results from formulation studies a definition of the phenomenon 'carrier surface site activity' is proposed in chapter 7. The activity of carrier surface sites is proposed to be defined as their 'ability to retain drug particles during dispersion'. This definition relates carrier surface site activity directly to drug detachment during dispersion and is, therefore, more practical than a definition in terms of binding energy or binding force towards drug particles. Two possible approaches for the definition and determination of the ability of carrier surface sites to retain drug particles during dispersion are discussed. They differ, amongst other things, in the resulting definition of 'active site', and therefore, it is proposed to refer to carrier surface sites with a high activity as 'highly active sites'. Carrier surface site activity, according to the newly proposed definition, depends not only on physicochemical properties of the carrier surface, but also on other variables such as the dispersion principle, the type of drug or the mixing process. The new definition offers a different perspective on the idea that (highly) active sites are preferentially occupied by drug

particles during mixing or that a mechanism such as the 'saturation of active sites' is markedly different from agglomeration of drug particles on the carrier surface.

Chapter 8: coherent anti-Stokes Raman scattering (CARS). The use of Coherent anti-Stokes Raman scattering (CARS) microscopy for the chemical-selective imaging of adhesive mixtures is evaluated in chapter 8. Chemical-selective imaging of adhesive mixtures can be applied to measure the particle size distribution of the drug on the carrier surface, the spatial distribution of the drug over the carrier surface, or to identify highly active sites on lactose carrier particles after a dispersion experiment. CARS microscopy can be used for the rapid chemical-selective imaging of adhesive mixtures, as is shown for mixtures containing budesonide or salmeterol xinafoate. It can be easily combined with image analysis software such as ImageJ, or with high resolution imaging techniques such as scanning electron microscopy (SEM). Validation of the technique is required to ensure that all drug particles are detected within the field of view. The technique would furthermore be optimised by enlarging the attainable field of view.

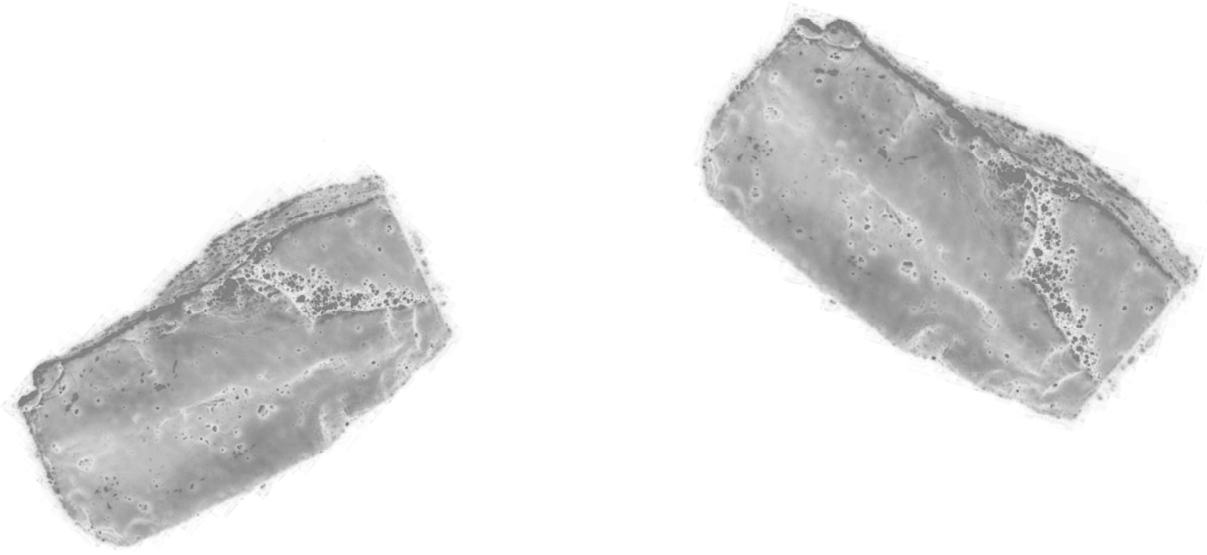
Chapter 9: general discussion. The effects of formulation variables on mixture dispersion performance can be explained by changes in three mixture properties or 'principal factors': the size distribution of the drug particles (including drug/drug and drug/fine-lactose agglomerates) as they are detached from the carrier surface; the degree of compression of the drug particles onto the carrier surface and onto each other; and the 'activity distribution' of occupied carrier surface sites. Changes in drug detachment through changes in these three principal factors are referred to as 'principal effects': i.e. particle size, press-on and activity effects, respectively. Two types of interactions between formulation and inhalation variables have been observed and they can be explained by changes in either the physical state or the relevance to drug detachment of the principal factors. A major and most urgent challenge lies in the development of techniques for the characterisation of the principal factors. The availability of such techniques is a prerequisite for unravelling the relationships between the principal factors and dispersion performance on the one hand, and the principal factors and the properties of the starting materials on the other. Regarding the latter, changes to the physicochemical properties of the starting materials under the influence of mixing forces have been observed, which diminishes the relevance of initial properties such as size and shape distribution and surface roughness. Properties that determine their change during mixing, such as hardness or yield stress, could therefore be more relevant. The improved conception of the interplay between variables in the formulation and dispersion of adhesive mixtures resulting from this thesis may stimulate the effective implementation of quality by design approaches to dry powder inhalation product development. In addition, it may facilitate the development of generic or more efficiently dispersing

products. This results in improved patient safety and potentially lower costs of dry powder inhalation therapy.



Appendix B

Samenvatting



Hoofdstuk 1: algemene inleiding. Adhesieve mengsels zijn poederformuleringen waarmee laaggedoseerde farmaca voor inhalatie nauwkeurig kunnen worden gedoseerd. Deze mengsels bestaan uit relatief grote hulpstofdeeltjes (lactose 'dragerdeeltjes') met aangehechte fijne farmacondeeltjes. Een nadeel van adhesieve mengsels is dat de drager- en farmacondeeltjes moeilijk van elkaar te scheiden of te dispergeren zijn tijdens inhalatie, wat een voorwaarde is voor een effectieve en patiëntveilige inhalatietherapie. Een beter begrip van de manier waarop variabelen uit het formulerings- en dispergeerproces het uiteindelijke dispergeergedrag van adhesieve mengsels bepalen is noodzakelijk om het dispergeergedrag van adhesieve mengsels te optimaliseren. Het is de doelstelling van dit proefschrift om hieraan bij te dragen. Om deze doelstelling te halen zal het onderzoek dat in dit proefschrift wordt gepresenteerd zich sterk richten op factoren die in voorgaande formuleringsstudies vaak onderbelicht zijn gebleven, zoals de identificatie van interacties tussen formulerings- en dispergeer- of inhalatievariabelen en de identificatie en analyse van mengseleigenschappen die bepalend zijn voor het dispergeergedrag van adhesieve mengsels.

Hoofdstuk 2: interacties met inhalatiedebiet. Begonnen wordt met een theoretische studie van de interactie tussen het inhalatiedebiet en formuleringsvariabelen. Over het algemeen wordt in formuleringsstudies de dispergeerbaarheid van een bepaalde poederformulering vanuit een bepaalde inhalator bij slechts één inhalatiedebiet gemeten. Echter, voor dragervrije poederformuleringen is aangetoond dat het inhalatiedebiet van invloed is op het effect dat een formuleringsvariabele heeft op de mengseldispergeerbaarheid. Dit is te verklaren met behulp van de verdeling in treksterkte van agglomeraten in de formulering. In extreme gevallen kan een verschil in inhalatiedebiet zelfs tot tegenstrijdige conclusies leiden voor wat betreft het effect van een bepaalde formuleringsvariabele op de dispergeerbaarheid van een poederformulering. In dit hoofdstuk wordt aannemelijk gemaakt dat hetzelfde valt te verwachten voor adhesieve mengsels. Naar analogie van de agglomeraattreksterkte voor dragervrije formuleringen wordt de 'energieratio' voor adhesieve mengsels geïntroduceerd. De energieratio is de verhouding tussen de potentiële scheidingsenergie en de bindingsenergie behorende bij een specifiek farmacondeeltje dat gebonden is aan een dragerdeeltje. De verdeling in energieratio in een adhesief mengsel is direct gerelateerd aan het dispergeergedrag van dat mengsel. Met behulp van hypothetische energieratioverdelingen wordt aangetoond dat een verschil in dispergeergedrag tussen twee adhesieve mengsels nooit volledig hetzelfde kan zijn over een bereik van inhalatiedebieten dat overeenkomt met een dispersie-effectiviteit van 0-100%. Het verschil kan zelfs tegengesteld zijn. Het inzicht dat voortvloeit uit mechanistische formuleringsstudies zou daarom aanzienlijk kunnen worden vergroot door standaard de dispergeerbaarheid van adhesieve mengsels over een groot bereik van verschillende inhalatiedebieten te meten. Op basis van deze bevinding is de dispergeer-

baarheid van adhesieve mengsels altijd bij meerder inhalatiedebieten gemeten in de experimentele studies gepresenteerd in dit proefschrift.

Hoofdstuk 3: de effecten van farmacongehalte op mengseldispergeerbaarheid. Het is algemeen bekend dat het farmacongehalte in adhesieve mengsels van invloed is op hun dispergeerbaarheid. Desalniettemin wordt in formuleringsstudies de invloed van het farmacongehalte vaak buiten beschouwing gelaten door te kiezen voor een standaard gehalte van 1,46%. Waarschijnlijk wordt deze gewoonte ingegeven door onduidelijkheid omtrent het exacte effect van farmacongehalte op mengseldispergeerbaarheid, welke wordt veroorzaakt door tegenstrijdige conclusies in de wetenschappelijke literatuur. Deze tegenstrijdige conclusies kunnen worden verklaard door interacties van farmacongehalte met andere formulerings- en inhalatievariabelen. Tegenstrijdige effecten van het farmacongehalte zouden bijvoorbeeld op kunnen treden wanneer een balans van meerdere, tegengestelde mechanismen verschuift onder invloed van interacterende variabelen. Deze hypothese is getoetst door het effect van farmacongehalte op de tijdens inhalatie van carrierdeeltjes losgemaakte farmaconfractie te meten, en daarbij tevens de invloed van het inhalatiedebiet, de mengtijd, het type farmacon en het gehaltebereik op dit effect te bestuderen. Met behulp van rasterelektronenmicroscopie (SEM) en een natte laserdiffractietechniek is het optreden van drie verschillende mechanismen of processen in het mengsel bestudeerd, te weten: agglomeratie van farmacondeeltjes, het veranderen van de vatbaarheid voor compressie op het drageroppervlak van de farmacondeeltjes en de verzadiging van sterke bindingsplaatsen op het drageroppervlak (zogenaamde 'active sites'). Hogere-orde interacties treden op tussen de variabelen die zijn onderzocht. Kwantitatieve en kwalitatieve interacties treden op tussen farmacongehalte en inhalatiedebiet. Dit betekent dat een bepaalde verandering in farmacongehalte soms leidt tot zowel een verhoging als een verlaging van de losgemaakte farmaconfractie, afhankelijk van het inhalatiedebiet. Deze interacties kunnen inderdaad worden verklaard met een verschuiving in de balans van de drie genoemde mechanismen. De exacte bijdrage van elk van deze mechanismen kan met behulp van de gebruikte analysetechnieken echter niet goed worden bepaald. De interacties met het inhalatiedebiet vormen een ondersteuning voor de conclusies getrokken in hoofdstuk 2. Bovendien vormen de bevinding betreffende de effecten van farmacongehalte op mengseldispergeerbaarheid een basis voor een betere keuze van deze variabele in toekomstige formuleringsstudies.

Hoofdstuk 4: de effecten van mengtijd op mengseldispergeerbaarheid. De effecten van mengtijd op de dispergeerbaarheid van adhesieve mengsels zijn bestudeerd in hoofdstuk 4. Hiervoor is een vergelijkbare aanpak toegepast als in hoofdstuk 3 voor de bestudering van effecten van het farmacongehalte. De invloed van het inhalatiedebiet, de dragergrootte en het type farmacon is onderzocht. Naast SEM en de natte laserdiffractie-

techniek is tevens de schijnbare oplosbaarheid van salmeterol xinafoaat na mengen bepaald. Deze schijnbare oplosbaarheid is gebruikt als een maat voor de mechanische belasting die op de farmacondeeltjes is uitgeoefend tijdens het mengproces. Zowel SEM als laserdiffractiemetingen tonen aan dat het agglomeratiegedrag van het farmacon tijdens mengen sterk afhankelijk is van het type farmacon en de dragergrootte. De vorming van agglomeraten is het meest uitgesproken voor het meest cohesieve farmacon (salmeterol xinafoaat) in combinatie met de meest grove dragerfractie die is bestudeerd. De agglomeraatvorming kan verklaren waarom langer mengen met deze farmacon-dragercombinatie resulteert in een grotere farmaconfractie dat van de drager loskomt bij een inhalatiedebiet van 20 L/min. Echter, bij een inhalatiedebiet van 60 L/min, met een fijne dragerfractie of met fluticasonpropionaat als farmacon resulteert langer mengen in een kleinere farmaconfractie die van de drager loskomt tijdens inhalatie. Dit is een indicatie dat onder de laatstgenoemde condities het effect van mengtijd op mengseldispergeerbaarheid niet wordt bepaald door agglomeraatvorming, maar bijvoorbeeld door het opbreken van agglomeraten aanwezig in het uitgangsmateriaal, het aandrukken van farmacondeeltjes op het drageroppervlak of de verplaatsing van farmacondeeltjes naar plekken op het drageroppervlak met een hogere 'activiteit'. Tijdens het mengproces treedt plastische deformatie (salmeterol) of fragmentatie (fluticason) van de farmacondeeltjes op. Bovendien vormen de farmacondeeltjes een sterk coherente laag of 'film' verspreid over het drageroppervlak en neemt de schijnbare oplosbaarheid van salmeterol toe naarmate langer wordt gemengd. Dit duidt er allemaal op dat de farmacondeeltjes onderhevig zijn aan mechanische belasting tijdens mengen, wat de waarschijnlijkheid van 'compressie-effecten' tijdens mengen vergroot. Net als in hoofdstuk 3 vormen de interacties met het inhalatiedebiet een verdere ondersteuning van de conclusies getrokken in hoofdstuk 2. Daarnaast tonen de resultaten weer aan dat ogenschijnlijk tegenstrijdige effecten kunnen worden verklaard door een balans van meerdere mechanismen welke verschuift onder invloed van interacterende variabelen.

Hoofdstuk 5: de effecten van toegevoegde fijne lactosedeeltjes op mengseldispergeerbaarheid. Een mogelijke overeenkomst tussen de effecten van farmacongehalte (hoofdstuk 3) en de effecten van toegevoegde fijne lactosedeeltjes op mengseldispergeerbaarheid wordt onderzocht in hoofdstuk 5. Indien deze overeenkomst bestaat zouden fijne lactosedeeltjes met een vergelijkbare vorm- en grootteverdeling als het farmacon kunnen resulteren in een tegengesteld effect op mengseldispergeerbaarheid afhankelijk van het inhalatiedebiet. Dit zou nieuw licht werpen op het werkingsmechanisme van fijne lactosedeeltjes, omdat vrijwel uitsluitend mengseldispergeerbaarheidverhogende effecten van toegevoegde fijne lactosedeeltjes zijn beschreven in de wetenschappelijke literatuur. De verandering in de van de drager losgemaakte fractie budesonide als gevolg van het toevoegen van fijne lactosedeeltjes is daarom onderzocht. Hierbij is rekening gehouden met een invloed van het farmacongehalte, het gehalte aan fijne lactosedeeltjes, de

grootteverdeling van de fijne lactosedeeltjes, het inhalatiedebiet en de mengvolgorde van farmacon en fijne lactose met de drager. Naast het meten van de dispergeerbaarheid zijn de adhesieve mengsels ook onderzocht met behulp van rasterelektronenmicroscopie (SEM). De effecten op dispergeerbaarheid van 'fijne' fijne lactosedeeltjes (X_{50} = 1.95 μm) zijn inderdaad vergelijkbaar met de effecten van farmacongehalte. Er is een vergelijkbare kwalitatieve interactie met het inhalatiedebiet bij een laag farmacongehalte van 0,4 gewichtsprocent. Toevoeging van 'grovere' fijne lactosedeeltjes (X_{50} = 3.94 μm) resulteert uitsluitend in een verhoging van de dispergeerbaarheid van de budesonidemengsels. Het verschil in effect tussen de fijnere en grovere fijne lactosedeeltjes kan een in het verleden aangedragen 'bufferhypothese' ondersteunen. Daarentegen is met SEM een opmerkelijk verschil in de fijne-deeltjesstructuur op het oppervlak van de dragerdeeltjes waarneembaar tussen beide typen fijne lactosedeeltjes. Deze structuur is compacter en meer aaneengesloten voor de fijnere lactosedeeltjes. Dit duidt erop dat fijne lactosedeeltjes enerzijds kunnen bijdragen aan de vorming van een sterk coherent en moeilijk dispergeerbaar netwerk op de dragerdeeltjes wanneer ze fijn genoeg zijn, en anderzijds zulke netwerken kunnen opbreken of hun vorming voorkomen wanneer de deeltjes grover zijn. Deze werkingsmechanismen zijn nog niet eerder beschreven en onderzocht. Er is een invloed van de volgorde waarin het farmacon en de fijne lactose met de dragerlactose worden gemengd op het effect van fijne lactose op mengseldispergeerbaarheid, maar met de in deze studie toegepaste analysetechnieken leidt dit niet tot een groter inzicht in de exacte balans van verschillende mechanismen. Het ontbreken van een goede definitie voor 'de activiteit' van het oppervlak van de dragerdeeltjes heeft de interpretatie van de data en het voeren van de discussie hierover sterk bemoeilijkt. Een definitie voor deze term wordt daarom voorgesteld in hoofdstuk 7. In aansluiting op hoofdstukken 3 en 4 vormt dit hoofdstuk verdere ondersteuning betreffende de interacterende invloed van het inhalatiedebiet. Ook is weer aangetoond dat een bepaalde verandering in een formuleringsvariabele elk mogelijk effect op mengseldispergeerbaarheid kan hebben, afhankelijk van de keuze voor andere variabelen. Het inzicht in de effecten van fijne lactosedeeltjes als gevolg van de gepresenteerde studie zou kunnen leiden tot verschillende innovatieve toepassingen van deze formuleringstechniek. Hiertoe behoort bijvoorbeeld het optimaliseren van een inhalatiedebiet afhankelijke mengseldispergeerbaarheid voor het bereiken van een inhalatiedebiet onafhankelijke inhalatietherapie.

Hoofdstuk 6: compressie-effecten in adhesieve mengsels. 'Compressie-effecten' zijn veranderingen in de mate waarin farmacondeeltjes worden aangedrukt op het oppervlak van dragerdeeltjes tijdens het mengproces. Het optreden van compressie-effecten als gevolg van veranderingen in farmacongehalte, het gehalte toegevoegde fijne lactose-deeltjes of de grootteverdeling van toegevoegde fijne lactosedeeltjes wordt onderzocht in hoofdstuk 6. Hiervoor wordt gebruik gemaakt van de 'schijnbare oplosbaarheids-

methode' zoals gepresenteerd in hoofdstuk 4. De schijnbare oplosbaarheid van salmeterol xinafoaat is een maat voor de mechanische belasting van farmacondeeltjes tijdens het mengproces en daarom mogelijk indirect voor de mate van compressie van de farmacondeeltjes op het drageroppervlak. Een verhoging in het salmeterolgehalte van 0,4 tot 4% resulteert in een significante daling van de schijnbare oplosbaarheid van salmeterol na mengen. Hoewel in mindere mate, resulteert ook het toevoegen van 3,6% 'fijne' fijne lactosedeeltjes (X_{50} = 1.95 μm) aan 0,4% salmeterol in een significante daling van de schijnbare oplosbaarheid. Echter, het toevoegen van 'grovere' fijne lactosedeeltjes (X_{50} = 3.94 μm) resulteert niet in een significante daling van de schijnbare oplosbaarheid van salmeterol na mengen. De effecten van de onderzochte formuleringsvariabelen op mengseldispergeerbaarheid komen overeen met de resultaten gepresenteerd in hoofdstukken 3 en 5. De resultaten geven aan dat een verhoging van het farmacongehalte of het toevoegen van 'fijne' fijne lactosedeeltjes resulteert in een verlaging van de mechanische belasting van farmacondeeltjes tijdens het mengen, terwijl dit niet het geval is voor grovere fijne lactosedeeltjes. Deze bevindingen zijn in tegenspraak met de veronderstelde verhoogde vatbaarheid van het farmacon voor compressie bij verhoging van het farmacongehalte en ook met de 'bufferhypothese' ter verklaring van het verschil in effect op dispergeerbaarheid tussen de fijne en grovere toegevoegde fijne lactosedeeltjes. De schijnbare oplosbaarheid vertoont geen correlatie met de van de drager gescheiden farmaconfractie bij een inhalatiedebiet van 20 of 40 L/min. Andere effecten dan compressie-effecten, zoals agglomeratie van farmacondeeltjes, zijn daarom waarschijnlijk dominant bij deze debieten. Daarnaast is het denkbaar dat een verlaging van dispergeerbaarheid optreedt als gevolg van compressie-effecten terwijl de mechanische stress op individuele deeltjes afneemt, wanneer de fijne deeltjes op de dragerdeeltjes meerdere lagen vormen. Bij een inhalatiedebiet van 60 L/min is er een sterke correlatie (R^2 = 0.8896) tussen de van de drager gescheiden salmeterolfractie en de schijnbare oplosbaarheid van salmeterol. Dit kan betekenen dat de mengseldispergeerbaarheid bij dit hoge debiet voornamelijk door compressie-effecten wordt bepaald. Een beter begrip van compressie-effecten zou voort kunnen vloeien uit bestudering van effecten van formuleringsvariabelen op de stromingseigenschappen van adhesieve mengsels. Daarnaast is waarschijnlijk ook de treksterkte van de netwerken gevormd door farmacon- en fijne lactosedeeltjes een relevante, doch moeilijk te meten factor.

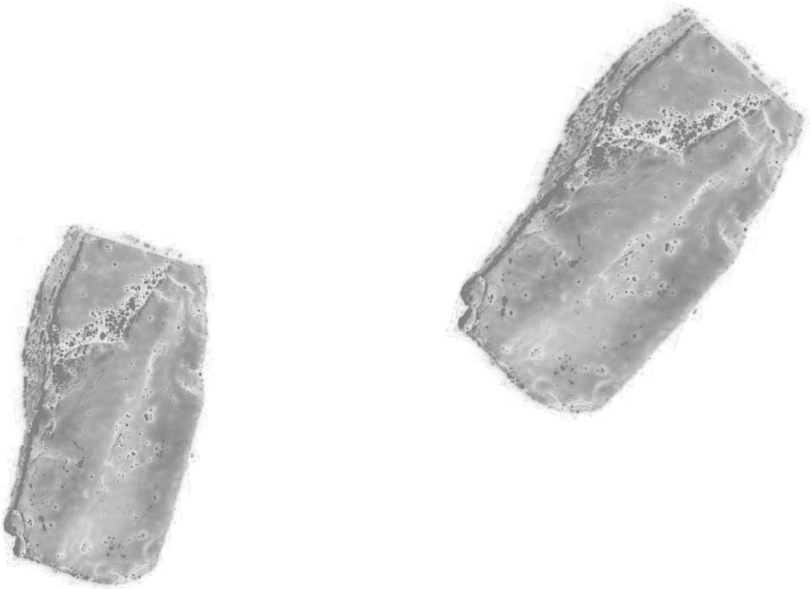
Hoofdstuk 7: een definitievoorstel voor de 'activiteit' van bindingsplaatsen op het drageroppervlak. Actieve bindingsplaatsen (eng: 'active sites') zijn uitgebreid bediscussieerd in hoofdstukken 3 en 5, maar dit werd bemoeilijkt door het ontbreken van een definitie voor deze term. Daarom is in hoofdstuk 7 een definitie voor de 'activiteit' van bindingsplaatsen op het drageroppervlak voorgesteld, welke eenduidige wetenschappelijke communicatie over dit onderwerp dient te stimuleren en tevens kan helpen bij de correcte interpretatie van resultaten uit formuleringsstudies. Het is voorgesteld om

de 'activiteit' van bindingsplaatsen op het drageroppervlak te definiëren als hun vermogen om farmacondeeltjes vast te houden tijdens het dispergeerproces. Deze definitie relateert de activiteit van bindingsplaatsen direct aan de scheiding van farmacondeeltjes van de dragerdeeltjes en dus aan mengseldispergeerbaarheid. Het is daarom een nuttiger definitie dan een definitie waarin slechts de bindingskracht of -energie tussen drager en farmacon wordt beschouwd. Er worden twee verschillende benaderingen toegelicht voor het definiëren en bepalen van het vermogen van een bindingsplaats om farmacondeeltjes vast te houden tijdens het dispergeerproces. Een belangrijk verschil tussen de benaderingen is de resulterende definitie van een 'actieve bindingsplaats'. Ter bevordering van eenduidigheid is het daarom aan te raden om bindingsplaatsen met een relatief hoge activiteit 'zeer actieve bindingsplaatsen' te noemen in plaats van het gebruikelijke 'actieve bindingsplaatsen'. Volgens de voorgestelde definitie is de activiteit van bindingsplaatsen op het drageroppervlak niet alleen maar afhankelijk van de fysisch-chemische dragereigenschappen. Ook andere variabelen, zoals het dispergeerprincipe, de farmaconeigenschappen en het mengproces spelen een bepalende rol. De voorgestelde definitie biedt een andere kijk op het idee dat actieve bindingsplaatsen tijdens het mengproces als eerste worden bezet door farmacondeeltjes. Bovendien gaat het in tegen de heersende opvatting dat een mechanisme als de 'verzadiging van actieve bindingsplaatsen' wezenlijk verschilt van agglomeratie van het farmacon.

Hoofdstuk 8: coherent anti-Stokes Raman scattering (CARS). CARS-microscopie is een chemisch selectieve beeldvormingstechniek. Het zou mogelijk gebruikt kunnen worden om de deeltjesgrootteverdeling van het farmacon op de dragerdeeltjes te bepalen of om de verdeling van farmacondeeltjes over het drageroppervlak of de locatie van '(zeer) actieve bindingsplaatsen' in kaart te brengen. De geschiktheid van CARS-microscopie voor de karakterisering van adhesieve mengsels met budesonide of salmeterol is onderzocht in hoofdstuk 8. Met CARS-microscopie kunnen de afzonderlijke (chemische) componenten in adhesieve mengsels snel in beeld worden gebracht. Bovendien kan het eenvoudig worden gecombineerd met beeldanalysesoftware zoals ImageJ, of met beeldvormingstechnieken met een hoge resolutie zoals rasterelektronenmicroscopie (SEM). Verdere validatie van CARS is noodzakelijk om er zeker van te zijn dat alle farmacondeeltjes in het monster worden gedetecteerd. Bovendien zou een groter gezichtsveld dan mogelijk was in deze studie de betrouwbaarheid van de techniek vergroten.

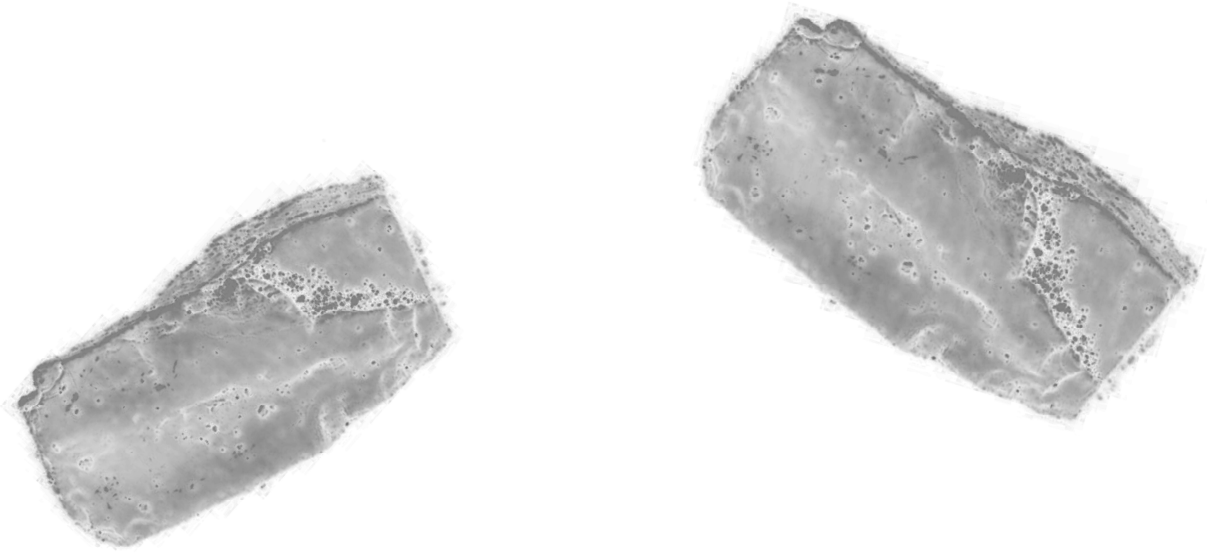
Hoofdstuk 9: algemene discussie. De onderzoeken uitgevoerd in het kader van dit proefschrift hebben tot verschillende nieuwe inzichten geleid met betrekking tot de samenhang van formuleringsvariabelen, inhalatievariabelen en mengseldispergeer-baarheid. De effecten van formuleringsvariabelen op mengseldispergeerbaarheid kunnen

worden verklaard door een verandering in drie verschillende mengseleigenschappen. Deze 'hoofdeigenschappen' zijn: de grootteverdeling van farmacondeeltjes (inclusief farmacon/farmacon en farmacon/fijne-lactose agglomeraten) zoals ze worden gescheiden van het drageroppervlak; de mate waarin farmacondeeltjes op het drager-oppervlak en op elkaar zijn aangedrukt; en de activiteitsverdeling van bezette bindings-plaatsen op het drageroppervlak. Veranderingen in de van de drager gescheiden farmaconfractie door een verandering in één van deze hoofdeigenschappen zijn respectievelijk de 'hoofdeffecten': deeltjesgrootte-effecten, compressie-effecten en activiteitseffecten. Twee typen interacties kunnen zich voordoen tussen formulerings- en inhalatievariabelen. Deze interacties zijn het gevolg van een verandering in ofwel de fysische staat, ofwel de relevantie met betrekking tot mengseldispergeerbaarheid van de hoofdeigenschappen van adhesieve mengsels. De meest urgente uitdaging voor een verder begrip van adhesieve mengsels is de ontwikkeling van technieken voor de karakterisering van de hoofdeigenschappen. Zonder deze technieken is het niet mogelijk om de samenhang tussen formuleringsvariabelen, inhalatievariabelen en mengseldis-pergeerbaarheid volledig te ontrafelen, omdat de hoofdeigenschappen van adhesieve mengsels daarin een verbindende rol spelen. Zowel de relatie tussen deze hoofdeigenschappen en mengseldispergeerbaarheid, alsook de relatie tussen deze hoofdeigenschappen en de fysisch-chemische eigenschappen van de uitgangsmaterialen dient te worden onderzocht. Hierbij is het van belang om rekening te houden met het feit dat deze relaties respectievelijk afhankelijk zullen zijn van de inhalatievariabelen en de meng- of procesvariabelen. Veranderingen tijdens het mengproces in fysisch-chemische eigenschappen van de uitgangsmaterialen, zoals deeltjesgrootte- en -vormverdeling en oppervlakteruwheid, maken deze eigenschappen waarschijnlijk minder relevant met betrekking tot mengseldispergeerbaarheid. Daarentegen zouden eigen-schappen welke bepalend zijn voor het optreden van deze veranderingen tijdens het mengproces, zoals hardheid en zwichtspanning, juist wel relevant kunnen zijn en daarom een interessant onderwerp voor verder onderzoek vormen. Het betere inzicht in de samenhang tussen formuleringsvariabelen, inhalatievariabelen en mengseldispergeer-baarheid kan de implementatie van een 'quality-by-designaanpak' voor de productie van poederinhalatieproducten verbeteren. Bovendien kan het een stimulans vormen voor de ontwikkeling van generieke en efficiënter dispergerende producten. Dit zou op termijn de kosten van poederinhalatietherapie drukken en de veiligheid en het welzijn van patiënten verhogen.



Appendix C

Biography and list of publications



Floris Grasmeijer finished high school at the Vincent van Gogh gymnasium in Assen in 2003. Because of an interest in the natural sciences Floris decided to study Pharmacy at the University of Groningen later that year. In 2009 he successfully finalised this study after having written a master's thesis on the characterisation of high dose aerosols from dry powder inhalers. The research for this thesis was performed at the inhalation division of the Department of Pharmaceutical Technology and Biopharmacy of the University of Groningen under the supervision of Anne Haaije de Boer and Paul Hagedoorn. Late 2009 Floris started a PhD study on the subject of adhesive mixtures for inhalation at the same department, which was finalised in 2014. Findings from this study were presented at international conferences and meetings and published in multiple peer-reviewed journals (see further). Currently, Floris holds a post-doctoral position at the Department of Pharmaceutical Technology and Biopharmacy of the University of Groningen, where he is involved in several inhalation technological research projects.

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Grasmeijer, F., Hagedoorn, P., Frijlink, H.W., de Boer, A.H., Adhesive mixtures for inhalation: variables determining interparticulate forces and their interactions, Pharmacy day University of Groningen, 2012, Groningen (received award for best presentation)

Grasmeijer, F., Hagedoorn, P., Frijlink, H.W., de Boer, A.H., Adhesive mixtures for inhalation: the coherence of formulation and inhalation variables, Pharmacy day University of Groningen, 2013, Groningen (received award for best presentation)

Grasmeijer, F., Hagedoorn, P., Frijlink, H.W., de Boer, A.H., Adhesive mixtures for inhalation: interactions and mechanisms determing dispersion behaviour, Meeting of the Belgian-Dutch Biopharmaceutical Society, 2013, Ghent

Grasmeijer, F., Hagedoorn, P., Frijlink, H.W., de Boer, A.H., Adhesive mixtures for inhalation: the 'activity' of surface sites on lactose carrier particles, Pharmacy day University of Groningen, 2014, Groningen (received award for best presentation)

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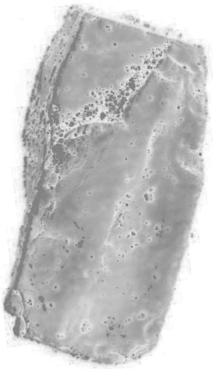
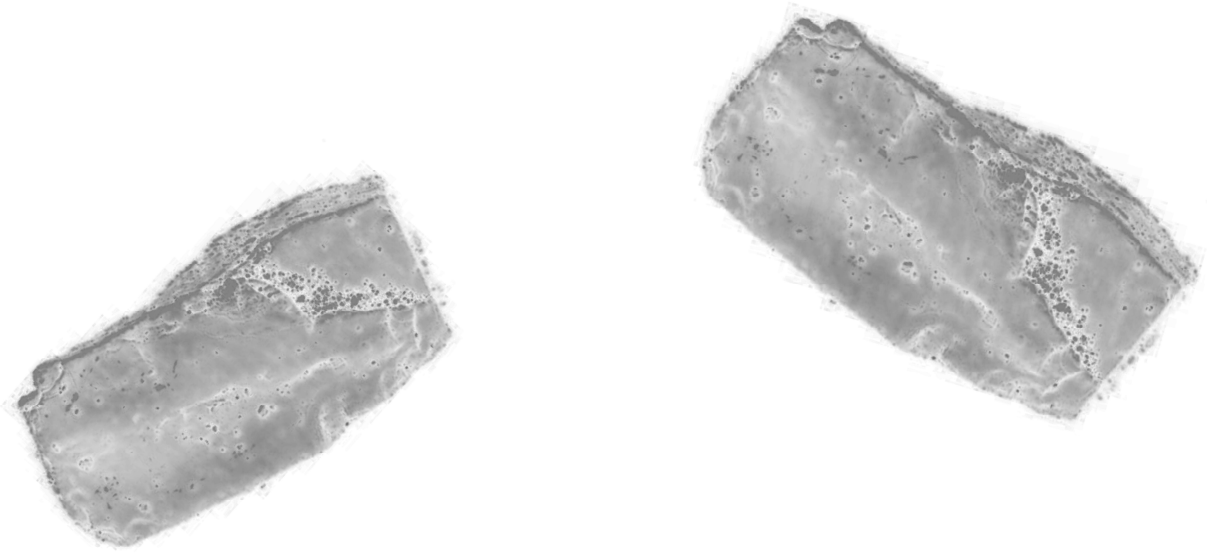


Appendix D

Dankwoord



Donderdagavond 7 mei 2014.
Temperatuur: 17°C.
Relatieve luchtvochtigheid: 49%.
Zwaarbewolkt met af en toe een miezertje.

Misschien nu op het eerste gezicht niet relevant, maar ja, je weet het maar nooit. Mochten de verwachtingen van dit dankwoord niet helemaal worden ingelost, dan zou dat immers zomaar aan de omgevingsfactoren op het moment van schrijven kunnen liggen. Er zijn er namelijk nogal een aantal die gedurende de afgelopen jaren een vinger voor me hebben uitgestoken. Of twee. Of die er bewust of onbewust voor zorgden dat mijn motivatie en 'weerbaarheid' op peil bleven. En ofschoon in het merendeel van de gevallen een eervolle vermelding in dit hoofdstuk daar niet de drijfveer voor zal zijn geweest, ligt teleurstelling toch al snel op de loer wanneer deze achterwege blijft. En wat te denken van die enkelen, die dit proefschrift of de reis ernaartoe zo bijzonder hebben verrijkt, dat elke in woorden uitgedrukte waardering sowieso tekort zal schieten? Enfin, het is me een gewaagde bezigheid, dat schrijven van een dankwoord. Geen cursus die je daar tijdens het promotietraject op voorbereidt.

Mijn oprechte dank gaat uit naar de volgende personen voor hun bijdrage aan dit proefschrift:

Mijn promotor prof. dr. **Henderik Willem Frijlink**. Beste Erik, dank dat je mij de kans en het vertrouwen hebt gegeven om op het gebied van de inhalatietechnologie te promoveren. Een veld dat mijn interesse wekte als student farmacie tijdens het door jou op informele manier en met veel enthousiasme gegeven vak 'innovatieve geneesmiddelvormen'. Dat jouw stuiterend enthousiasme de afgelopen jaren ook andere rimpelingen heeft veroorzaakt (vaak foutief aangemerkt als 'aardgasbevingen'), realiseren zich denk ik maar weinigen. Ik houd het met alle liefde voor je stil.

Mijn copromotor dr. **Anne Haaije de Boer**. Beste Anne, het koken van een lekker soepje vergt een goede basis. Bij de bereiding van mijn soepje wist ik mij ervan verzekerd dat de basis door een chef-kok was bereid. Daarnaast vormde jij in al onze gesprekken en discussies het klankbord dat nodig is om muziek te kunnen maken. Wanneer ik aanklopte, vergrootte je een voor jou reeds bestaand probleem van tijdgebrek rustig met een uurtje. Zelden kwam ik met dezelfde ideeën weer naar buiten als waarmee ik naar binnen ging. En dan had ik er voor mijn gevoel toch echt al goed over nagedacht. En dan dat oog voor detail in geschreven teksten: dit proefschrift is een beetje van mezelf, en een beetje van jou.

Mijn begeleider **Paul Hagedoorn**. Beste Paul, "In de handen van de grootste kneus is zelfs het beste gereedschap waardeloos" zou zomaar jouw lijfspreuk kunnen zijn, getuige de grondige uitleg van procedures en technische apparatuur die ik van jou heb mogen ontvangen. Je hebt je zelfs weleens min of meer verontschuldigd voor het ongelooflijk gedetailleerde niveau waarop je dat doet (je houdt naar eigen zeggen erg van mieren, of zoiets). De kwaliteit van de data en interpretaties, en de mogelijkheden die we uit de

beschikbare apparatuur hebben gehaald zijn dan ook in niet geringe mate jouw verdienste. Daarnaast ben je ook nog eens een zwaargewicht in de subtiele (en minder subtiele) humor. Werken met jou is een aaneenschakeling van mooie momenten.

Mijn vriend en collega-inhalatiepromovendus **Marcel 'alleskunner' Hoppentocht**. Beste Marcel, dank voor… tsja, alles eigenlijk.

The Optical Sciences Group Twente, in particular dr. **Andrew L. Fussell** and dr. ir. **Herman L. Offerhaus**. If indeed a picture is worth a thousand words, then this brief acknowledgement does not make up for all the CARS-images that enrich this thesis. Thank you for your hospitality and fruitful collaboration.

Voormalig analist op het inhalatielab **Maarten van den Noort**. Beste Maarten, ondanks dat je al vrij snel na het begin van mijn promotieonderzoek vertrok, drijft dit proefschrift deels op 'jouw data'. En daarvan weten we één ding heel zeker: ze zijn betrouwbaar!

Prof. ir. **Hans Wesselingh**. Beste Hans, jouw werk met betrekking tot het modelleren van het dispersieproces in de gebruikte testinhaler is (nog?) niet wereldkundig gemaakt, maar heeft mij enorm geholpen dit proces beter te begrijpen. Ik hoop dat je daar het bewijs van in dit proefschrift terugvindt.

The assessment committee formed by prof. dr. **Wim E. Hennink**, prof. dr. **Francesco Picchioni** and prof. dr. **Peter Stewart**. Thank you for taking the time.

Mijn broer en collega-promovendus **Niels Grasmeijer**. Beste Niels, dank dat je zo vaak mijn proefkonijn wilde zijn. Jouw commentaren op mijn teksten hebben ervoor gezorgd dat mijn gedachtenkronkels ook voor anderen enigszins begrijpelijk werden. Daarnaast bleek jouw scherpe en frisse blik niet zelden de sleutel tot het oplossen van praktische ~~problemen~~ uitdagingen op het lab.

En jij ook bedankt, voor je interesse, je helpende hand, je gezelschap of luisterend oor. Ik hoop dat ik je die gunst net zo onzelfbaatzuchtig zal verlenen mochten de rollen een keertje omgedraaid zijn.

Floris