**Summary, conclusions and some future perspectives**

There is a growing interest in the use of dry powder inhalers (dpi's) for inhalation therapy. Dpi's can deliver much higher doses than metered dose inhalers and they do so in much shorter times than is necessary for the administration of the same dose with a nebuliser. Because the drug is in the dry state, stability problems are widely excluded and operation of a dpi requires no hand-lung coordination, nor the application of external energy sources such as pressurised air and electricity. However, most dpi's do generate rather ineffective friction, shear, drag or lift forces for powder de-agglomeration and fine particle generation during inhalation. As a result, requirements on carrier properties in adhesive mixtures for inhalation in relation to the drug-to carrier interaction are rather extreme. This is the reason why most studies in the field of dry powder inhalation focus on understanding and controlling the factors that influence this interaction. For instance, the use of so-called 'force control agents' (during the mixing process) has been proposed and several particle engineering technologies have been developed to produce particles with decreased adhesiveness. Also different carrier surface modification methods have been presented to reduce carrier surface impurities and discontinuities, of which particularly the latter are considered unfavourable. In this respect, it has frequently been described that only fine carriers with smooth surfaces yield acceptable fine drug particle fractions. However, most smart particle technologies produce expensive, instable and/or highly voluminous powders, whereas the choice for fine carriers conflicts with the demands on good flow properties, which are mandatory for good dose reproducibility from multi-dose reservoir systems.

Usually, fine particle fractions from inhalation systems are measured with cascade impactors. Impactors classify the aerosol from an inhaler into mass fractions (of the dose) as a function of the aerodynamic diameter. The data are processed into cumulative mass distribution curves from which the mass fractions of the dose in the therapeutic size range can be assessed. It is generally believed that the pharmacopoeial cascade impactor procedures are well validated and that impactor data are predictive for lung deposition, but several recent studies have shown that this is not true. Cascade impactors are inaccurate devices. Moreover, they are laborious in use. They provide insufficient information when studying the mechanisms of particle interaction in inhalation powders and drug detachment from these powders during inhalation. They can only be operated under stationary flow conditions which excludes the simulation of realistic inspiratory flow manoeuvres. Besides, the size distribution (in the aerosol) can not be studied as function for the inhalation time. Hence, they are inappropriate for fundamental studies as well as for formulation and device development.

In this thesis, some alternatives have been investigated and evaluated in order to address previously mentioned shortcomings and problems in dry powder aerosol generation and characterisation.

Specifically:

1. Laser diffraction technique has been made applicable as an alternative for cascade impactor analysis.
2. Device and formulation development have been directed to establish an appropriate balance between the adhesive forces in the powder mixture and the separation forces during inhalation (rather than to focus on achieving a favourable drug-to-carrier interaction alone).
3. Inertial separation (instead of drag, lift, shear and friction) forces have been utilised for fine particle generation during inhalation, so as to benefit (rather than to suffer) from large carrier surface discontinuities.
4. The action of the inertial separation forces during inhalation has been sustained.

The aspects 3 and 4 have been realised by development and application of air classifier technology (ACT) and they have made the exploration of relatively coarse and narrow lactose fractions as carrier in adhesive mixtures possible.

As a starting point for all investigations, the relevant aspects for inhalation therapy are reviewed in **chapter 1** of this thesis. The preferable aerodynamic size distribution has been assessed in relation to the site of deposition (receptor or absorption area) and the flow manoeuvre through the inhaler. Assessment has been made on the basis of fluid and particle dynamics and deposition modelling in the respiratory tract. Different types of particle-particle interactions (and their orders of magnitude) in powder formulations for inhalation are discussed, as well as some other relevant parameters in adhesive mixtures preparation, such as carrier payload and mixing time. Special attention is given to the effect of carrier surface and bulk properties on the drug-to-carrier interaction in these mixtures. Finally, the complex system of variables and interactions that influence the performance of a dpi is explained. Not only proper choices regarding process (mixing) conditions and physical properties of the starting materials have to be made, also the influence of inhaler design, air flow resistance and patient parameters have to be considered (e.g. instruction, clinical picture, age and gender). In many respects, an optimum has to be found, as between the cohesive and adhesive forces in the mixture, the interparticulate forces in the mixture and the separation forces during inhalation, and the generated fine particle fraction and its deposition in the respiratory tract. A scheme of balancing steps is therefore included.

*With respect to laser diffraction technique* (chapters 2 and 3), it is shown that this technique can be a powerful and valuable tool in mechanistic studies, inhaler (and formulation) design and development and quality control of all types of aerosol generation systems.

In **chapter 2**, theoretical considerations and practical pros and cons of the laser diffraction and cascade impactor techniques are discussed in a comparative way. It is explained that laser diffraction analysis produces a volumetric size distribution of the aerosol for which (in dpi development) the primary particle size distribution of the drug (from RODOS dispersion) is the reference. The objective is to get as close as possible to this primary particle size distribution, at lowest possible inspiratory effort. For nebulisers, producing droplet-like (spherical) aerosol particles from aqueous drug solutions (with unit density), the laser diffraction size distribution equals by definition the aerodynamic size distribution. The only problem to solve is to make a proper choice between the two diffraction theories, Mie and Fraunhofer. The Mie theory is theoretically more correct, but the Fraunhofer theory is more practical, as it does not require knowledge of the complex refraction index whereas considerably less smoothing is involved in the deconvolution of the diffraction integral. Laser diffraction technique is fast, highly reproducible and much more sensitive than cascade impactor analysis. Single doses can be analysed with great accuracy and laser diffraction technique produces much more size classes in the range of interesting drug particles sizes between 1 and 5 μm. The possibility of time sliced measurement enables one to follow the size distribution in the aerosol as function of the inhalation time, which yields valuable information in drug detachment (from carrier crystals) and aqueous droplet generation studies, particularly when borderline effects are likely to occur. Such effects may be expected during short term aerosol generation (e.g. in provocation tests) or when the inspiratory flow conditions are instable. Time sliced measurements yield information that can not be obtained with a cascade impactor.

One of the practical limitations of standard laser diffraction technique in aerosol characterisation is that the apparatus do not have the possibility to generate or inhale the aerosol with a controlled flow manoeuvre. Therefore, in **chapter 3** the design, development and evaluation of a special inhaler adapter is described. The adapter has a modular construction with a pre-separator for the removal of large carrier particles from the aerosol cloud and a fine particle collector for chemical analysis of the generated fine particle fraction. Different applications for nebulisers and dry powder inhalers with this (prototype) adapter are presented and discussed, including data from a dry powder inhaler development for colistin sulfate treatment in CF therapy. This dpi and formulation development has been completed with laser diffraction technique only. Only in its final phase, validation with cascade impactor analysis has been performed. One of the more recent applications (not described in this thesis) is exploring the principle of Rayleigh break-up for medical aerosol generation. Droplet size analysis in the aerosol as function of the pressure profile of the spraychip (membrane), droplet coalescence and evaporation as function of chip geometry and the effect of mouthpiece design on the aerosol properties would not have been possible with cascade impactors or aerodynamic particle sizers.

Many future perspectives of laser diffraction technique for aerosol characterisation exist. For example, the particle growth (of hygroscopic drug formulations) in moist air could be studied as function of the exposure time and droplet evaporation as function of the travelling time (and ambient conditions) could be investigated. For future investigations, a breath simulator may be developed. In contrast with cascade impactor analysis, the particle sizing with laser diffraction technique is independent of the flow rate through the inhaler, which makes it possible to vary this flow rate without having to ‘store’ the aerosol in a spacer where sedimentation and electrostatic particle attraction may occur.

*The air classifier technology* (chapters 4 and 5 for the basic design and performance and chapters 9 and 10 for their application in the Novolizer® multi-dose dry powder inhaler) and *adhesive mixture studies* (chapters 6 to 8) have a strong interaction with each other.

In **chapter 4**, the basic design and working principle of air classifiers for inhalation are discussed. Particles entrained by the inspiratory air stream from the dose system are subjected to two different forces (in addition to the force of gravity) upon entering the classifier: the drag force of the air (propelling the particles) and a centrifugal force. It is shown that a classifier can be designed in such a way that only the small particles (drug particles released from the carrier crystals) leave the classifier and large (carrier) particles remain in circulation for as long as the inspiratory air flow through the classifier is maintained. This has many advantages. During circulation, carrier particles collide with each other and with the walls of the classifier. Collision and centrifugal forces separate the drug from the carrier particles and drug particle separation continues for the whole duration of the inhalation. This, in contrast with most marketed inhalers which use the available energy for separation rather ineffectively as the powder rapidly passes the de-agglomeration principle of the inhaler. Another advantage of carrier retention is that the patient is not burdened with the inhalation of a large amount of excipient. Finally, the retention is useful when studying the drug detachment (rate) during inhalation. Carrier residue analyses are much more accurate and reproducible than cascade impactor analyses. In chapter 4, also a Force Distribution Concept (FDC) is presented. This concept includes a visualisation of the size distributions of the adhesive forces in the mixture and the separation forces during inhalation. The shapes of the distribution curves can be assessed from the primary particle size distribution of the drug. Their positions relative to each other are indicated by their intersection which has a relationship with the carrier residue after inhalation. Changes in the size distributions, which

are the result of changing the mixing or inhalation conditions, change the intersection between both curves and thereby the carrier residue. Therefore, mapping of the size distribution curves can be obtained from measuring the carrier residue at different flow rates. This allows drawing conclusions regarding drug particle size distribution on the carrier surface, the effect of press-on forces during mixing, the variation in bonding places on the carrier surface, etc. Although FDC has been developed primarily to improve air classifier technology, it finds more widespread application in adhesive mixture optimisation (to be published)

In **chapter 5** it is shown that air classifiers are rather insensitive to carrier surface properties and carrier size distribution, compared with most marketed inhalers. Carrier fractions with different mean diameters from the same batch of crystalline alpha lactose monohydrate have been prepared and characterised, and this has been repeated for different batches. It was found that the surface roughness and impurity increase with mean fraction diameter, which is quite obvious for industrial crystals, as the number of lattice disturbances and the size of the irregularities both increase with the crystal diameter. Residues of the mother liquor accumulate in these irregularities when the crystals are removed from the crystallisation tank. After drying of the crystals, small quantities of salts en soluble proteins and peptides remain on the carrier surface. The amount of water of adhesion may be relatively high in the impurities and decomposition reactions with the lactose (e.g. Maillard reaction) may occur. Such high impurity sites and surface cavities are often considered as so-called ‘active sites’. These are strong bonding sites where the adhesive forces between drug and carrier particles are highest and also where drag, lift and friction types of removal forces during inhalation can not get hold effectively of adhering drug particles. However, no clear correlations have been found between the fraction of drug detached and the surface impurities or irregularities when air classifier technology is used as de-agglomeration principle. One of the explanations is that drug particles inside carrier surface irregularities can not find shelter from inertial removal forces. As a result, carrier surface irregularities are not necessarily active sites in combination with ACT.

With a basic classifier with carrier retention (described in chapter 4), the rate and mode of drug particle detachment during inhalation have been studied. Good knowledge of the detachment rate was necessary to optimise the classifier concept for the Novolizer® multi-dose dry powder inhaler, and the mode of detachment has been studied to explain the dramatic changes in detachment rate during the first second of inhalation.

In **chapter 6** it is shown that the drug detachment rate from adhesive mixtures in a classifier follows first order reaction kinetics reasonably well within the first half second of inhalation. This is independent of the carrier payload (0.4 or 4% drug) and the carrier size fraction (45-63 or 150-200 µm). It suggests that the drug detachment rate is initially proportional with the drug concentration on the carrier surface, but the proportionality constant (k) appears to depend on the flow rate and on the carrier size fraction. After approximately 0.5 s, when the detachment rate slows down, other parameters become involved. From comparison of scanning electron micrographs of the mixtures before and after different inhalation times the impression was obtained that initially the largest particles (and drug agglomerates) are detached, which seems logical as the magnitude of the inertial separation forces (during inhalation) increases with the drug particle diameter.

The mode of drug particle detachment for the same mixtures is discussed in **chapter 7**. With laser diffraction technique (time sliced measurements), using the previously described adapter, it could be confirmed that the largest drug particles and agglomerates are detached

first. It could also be shown that the size of released particles at lower flow rates (e.g. 10 l/min) from mixtures with 4% drug (high carrier coverage) is considerably larger than that of the primary drug particles. This seems to confirm literature in which it is postulated that drug particles tend to agglomerate on the carrier surface during mixing with the carrier particles. It was found that the size of the induced agglomerates increases with the carrier particle diameter. This is in agreement with the supposition that large carrier particles, with good flow properties, exert much higher inertial and frictional kneading and press-on forces during mixing than small particles. The same forces may also increase the adhesive forces between drug and carrier particles, which explains why the detachment rate constant (in chapter 6) was found to decrease with increasing mean carrier diameter. When the flow rate is increased to values > 10 l/min, the released drug agglomerates are further disintegrated before they are discharged from the classifier. Already at 30 l/min the size distribution of particles in the aerosol from the classifier approaches that of the primary drug particles.

The press-on forces during mixing are not only responsible for the induction of (relatively soft) drug agglomerates on the carrier surface. They also gradually break-up small but relatively strong agglomerates in the starting material, unless the carrier payload is so low (e.g. 0.4%) that most of the drug particles are wiped together in carrier surface discontinuities where they find shelter from these press-on forces. Most of the natural agglomerates in the starting material, after being detached from the carrier crystals, are too strong to be disintegrated completely in the classifier. As a result of the difference in strength and size between naturally present and induced agglomerates, the size of particles released from mixtures with low carrier payloads may differ from that of particles from mixtures with high payloads. At low flow rates (10 l/min), detached particles may be smallest for low payload mixtures, as the natural agglomerates are generally smaller than newly formed ones, particularly those induced on large carrier crystals. But at higher flow rates detached particles may be largest for the low payload mixtures, because natural agglomerates are broken up during the mixing process and newly formed ones in the classifier after detachment.

The role of carrier surface discontinuities has been further investigated in **chapter 8** in relation to the carrier bulk properties. In this chapter also the drug binding capacity of strong bonding (‘active’) sites has been studied more in detail. There is strong evidence that a fundamental difference exists between these active sites and carrier discontinuities, which (in literature) are often considered as the same. It could be shown that drug particles tend to assemble in carrier surface discontinuities which have to be filled first before saturation of the active sites can be completed. This seems to indicate that the active sites are primarily within the surface discontinuities and that powder densification in these cavities by the press-on forces during mixing is necessary to make good contact between the drug particles and the active sites. This also explains the effect of mixing time, which depends on the drug content in the mixture. At low payloads, there is a relatively great effect of mixing time on drug detachment during inhalation. During mixing, relocation of drug particles from weak to strong bonding sites occurs, but the relocation process proceeds relatively slowly. Therefore, increasing the mixing time at lower payloads decreases the fraction of drug detached during inhalation over a long range of mixing times. At higher payloads, when a multi-particulate drug layer around the carrier particle exists, there is little room left for drug particle relocation over the carrier surface. On the contrary, densification of the drug particles on the carrier surface and in its cavities occurs, which increases the number of drug particles making contact with strong binding places. However, densification is more or less completed within the first 10 minutes of mixing. Thus, elongation of the mixing time for high payload mixtures has little effect on the fraction of drug detached.

Because drug particles in carrier surface cavities may find shelter from the press-on forces during mixing at low carrier payloads (which keeps the adhesive forces in the mixture low), a certain carrier surface rugosity may be beneficial to air classifier technology. This emphasizes the need for good characterisation methods for the surface discontinuities, particularly regarding their size and drug storage volume, the number of strong bonding places inside the pores, the pore depth in relation to the average drug particle diameter, etc. Scanning electron micrographs indicate that the depth of the discontinuities and the fraction of the carrier surface over which they exist, increase proportionally with the carrier diameter (for carrier fractions from the same batch of lactose). This may have wide-ranging consequences, particularly if the depth of the discontinuities decreases to values smaller than the diameter of the adhering drug particles. This not only increases the effectiveness of press-on forces during mixing, but also that of drag, lift and friction type of separation forces.

Future perspectives include the preparation of carrier crystals with controlled carrier rugosity. In combination with air classifier technology, proper mixing conditions and a carrier payload (with drug) adjusted to the storage volume of the carrier discontinuities, 80% drug detachment at a relatively low flow rate of 30 l/min is possible without having to use specially particle engineered powders. Future research may focus on measurement of the press-on forces during the mixing process as function of the carrier bulk properties. Also the role of naturally adhering lactose fines on the carrier surface is still far from understood.

**Chapter 9** describes the development of the air classifier family for the marketed Novolizer. This family includes in addition to the basic classifier which is described in chapter 4, a classifier that is optimised for spherical pellets and a classifier with a controlled residence time for the powder. On the basis of the drug detachment rate study in chapter 6 and the requirement that the total dose is released within 2 l of inhaled air, it can be concluded that a circulation time of 0.5 to 1.5 s for the powder in the classifier is optimal. After 0.5 s the drug detachment rate from carrier crystals slows down, but the particles released are smaller than those detached in the first 0.5 s and therefore they may be meaningful for the fine particle fraction. At 60 l/min, 1.5 s corresponds with an inhaled volume of 1.5 l. Longer inhalation times at this flow rate are not desired, because some air (approximately 0.5 l) is necessary for transport of the drug particles into the deep lung. In chapter 9 it is explained how the residence time in a classifier can be measured and controlled and what influence the carrier size distribution and the flow rate have on the powder residence time in the classifier. It is also explained how the fine particle losses (by adhesion) in the classifiers can be minimised for spherical pellets by creating an internal air barrier and how a sheath of clean air around the aerosol can be used to control the inhaler resistance to air flow and to minimise aerosol deposition in the mouth.

The performance of the standard Novolizer® classifier and the classifier with controlled residence time is evaluated in **chapter 10**. The fine particle fraction can be controlled to match target values quite precisely up to nearly 60% of the nominal dose for a wide range of doses (50-400 µg), thereby making use of the knowledge of the properties of adhesive mixtures (from chapters 5-8). Even with coarse standard lactose products (not meant for inhalation) relatively high fine particle fractions over 40% of the nominal dose can be achieved. Whereas other marketed devices (e.g. the GSK Diskus) produce strongly decreasing fine particle fractions (to less than 10%) with increasing median carrier diameters or a decreasing percent of fine lactose particles in the mixture, classifier technology is relatively unaffected by carrier size and the presence of lactose fines in the mixture. In fact, the presence of lactose fines (< 15 µm) should rather be avoided in adhesive mixtures for a classifier based inhaler, as they slow down the carrier particle velocity in the classifier (by tribocharge). They

also reduce the mixture homogeneity and dose reproducibility. The fine particle fraction (< 5 μm) from the Novolizer® increases with increasing flow rate through the classifier, which compensates for a shift in drug deposition to higher airways when the particle velocity in the respiratory tract is increased.

The Novolizer® has been marketed in several European countries with generic formulations for budesonide, salbutamol and formoterol, and registration has been obtained on the basis of equivalence with the AstraZeneca Turbuhaler. Future expectations include that formulations for new chemical entities are developed for the Novolizer®, which may result in much higher fine particle fractions than obtained with the currently marketed generic drugs.

Finally **chapter 11** describes the design and performance of an inhaler with two classifiers in a parallel arrangement for the de-agglomeration of high drug doses. The inhaler consists of three plate like parts and a blister for the drug formulation. The simple construction and assembling of parts make the inhaler very cheap and therefore appropriate for single use. The disposable multiple classifier inhaler (named Twincer™) has been developed particularly for the administration of high dose antibiotics in CF-therapy. In vitro deposition results show that powder quantities of 25 mg can be dispersed into nearly primary particles at a pressure drop across the inhaler of only 1 kPa in one single inhalation manoeuvre. Such a low pressure drop can be attained easily by small children or patients with extremely poor lung functions. Its excellent dispersion performance at low pressure drops for high doses and its good moisture protection of the drug formulations in aluminium blisters make the Twincer™ also appropriate for the administration of moisture sensitive (sugar glass) formulations which contain high percentages of excipient(s). Such formulations can be used for instance to prepare vaccines as a dry powder. The disposable design furthermore eliminates the risk of bacterial resistance development (e.g. in CF therapy) and inhaler contamination. Substitution of vaccine injection by dry powder inhalation also reduces the risks of patient infection (polluted syringes or water) to zero. Not to mention that replacement of time consuming antibiotic nebulisation with dry powder administration in one or two single inhalation(s) will increase the patient's compliance dramatically.