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Ultrasound-assisted surface engineering of pharmaceutical powders

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ACADEMIC DISSERTATION

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Abstract

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Effective processing of powdered particles can facilitate powder handling and result in better drug product performance, which is of great importance in the pharmaceutical industry where the majority of active pharmaceutical ingredients (APIs) are delivered as solid dosage forms. The purpose of this work was to develop a new ultrasound-assisted method for particle surface modification and thin-coating of pharmaceutical powders. The ultrasound was used to produce an aqueous mist with or without a coating agent.

By using the proposed technique, it was possible to decrease the interparticular interactions and improve rheological properties of poorly-flowing water-soluble powders by aqueous smoothing of the rough surfaces of irregular particles. In turn, hydrophilic polymer thin-coating of a hydrophobic substance diminished the triboelectrostatic charge transfer and improved the flowability of highly cohesive powder. To determine the coating efficiency of the technique, the bioactive molecule β-galactosidase was layered onto the surface of powdered lactose particles. Enzyme-treated materials were analysed by assaying the quantity of the reaction product generated during enzymatic cleavage of the milk sugar. A near-linear increase in the thickness of the drug layer was obtained during progressive treatment. Using the enzyme coating procedure, it was confirmed that the ultrasound-assisted technique is suitable for processing labile protein materials. In addition, this pre-treatment of milk sugar could be used to improve utilization of lactose-containing formulations for populations suffering from severe lactose intolerance. Furthermore, the applicability of the thin-coating technique for improving homogeneity of low-dose solid dosage forms was shown. The carrier particles coated with API gave rise to uniform distribution of the drug within the powder. The mixture remained homogeneous during further tabletting, whereas the reference physical powder mixture was subject to segregation.

In conclusion, ultrasound-assisted surface engineering of pharmaceutical powders can be effective technology for improving formulation and performance of solid dosage forms such as dry powder inhalers (DPI) and direct compression products.

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Natalja Genina

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List of original publications

This thesis is based on the following publications, which are referred to in the text by their respective roman numerals (I-IV).

I **Genina N.**, Räikkönen H., Heinämäki J., Antikainen O., Siiriä S., Veski P., Yliruusi J., 2009. Effective modification of particle surface properties using ultrasonic water mist. AAPS PharmSciTech, 10 (1), 282-288. doi:10.1208/s12249-009-9208-3

II **Genina N.**, Räikkönen H., Ehlers H., Heinämäki J., Veski P., Yliruusi J., 2010. Thin-coating as an alternative approach to improve flow properties of ibuprofen powder. Int. J. Pharm. 387 (1-2), 65-70. doi:10.1016/j.ijpharm.2009.12.005

III **Genina N.**, Räikkönen H., Heinämäki J., Veski P., Yliruusi J., 2010. Nano-coating of β-galactosidase onto the surface of lactose by using an ultrasound-assisted technique. AAPS PharmSciTech. 11(2), 959-965. doi: 10.1208/s12249-010-9462-4

IV **Genina N.**, Räikkönen H., Antikainen O., Heinämäki J., Yliruusi J., 2010. Ultrasound-assisted powder coating technique to improve content uniformity of low-dose solid dosage forms. AAPS PharmSciTech (in press). doi: 10.1208/s12249-010-9514-9

Abbreviations

AFM	Atomic force microscopy
API	Active pharmaceutical ingredient
a_w	Water activity
BET	Brunauer-Emmett-Teller sorption model
DC	Digital camera
DPI	Dry powder inhaler
DSC	Differential scanning calorimetry
DVS	Dynamic vapour sorption
HPMC	Hydroxypropyl methylcellulose
IFM	Inverted fluorescence microscopy
IR	Infrared
LMH	α-lactose monohydrate
M	Mesh
MCC	Microcrystalline cellulose
m/V	mass per volume
OM	Optical microscopy
Ph. Eur.	European Pharmacopoeia
PIT	Process induced transformation
R^2	Correlation coefficient (quantitative measure of the goodness of fit)
R_a	Arithmetic average roughness
RH	Relative humidity
R_q	Root mean square roughness
RSP	Riboflavin sodium phosphate
SD	Standard deviation
SEM	Scanning electron microscopy
SFT	Spatial filtering technique
SM	Stereomicroscopy
Srel	Relative standard deviation
SSA	Specific surface area
UV	Ultraviolet
UV-Vis	Ultraviolet-Visible
XRPD	X-ray powder diffraction

1 Introduction

Powder technology is an important research area of pharmaceutical manufacturing, since solid dosage forms are the most common preparations for drug administration. Nowadays in the pharmaceutical industry, quality by design and economic pressures demand optimized production from the outset of a project to prevent delays in new drug approvals by regulatory agencies (Freeman, 2010). Powder characterization can give rise to better understanding of critical parameters during the earliest stages of product development. Effective processing of powdered substances can lead to enhancement in powder performance and thus help to prepare a clinically effective formulation, develop robust technologies and manufacture a high quality product.

It is evident that powder properties, such as particle size, shape, surface and density as well as flowability and compressibility can be critical for successful production of solid dosage forms. By improving powder morphological and rheological characteristics at the beginning of the drug development process, it is possible to avoid powder handling problems and to obtain a product with desirable properties in a cost-effective way. Effective treatment of particle surfaces may be one option for affecting powder behaviour during industrial processes. Preparation of particles with a modified exterior can prevent a tendency for them to interact. It may result in better flow and aerosolization properties that are crucial in the manufacture of newer delivery technologies such as dry powder inhalers (DPIs) (Podczeck, 1998; Schiavone et al., 2004; Edge et al., 2008). In addition, optimized flowability of powders is important in the preparation of tablets and capsules of uniform mass and content (Prescott and Hossfeld, 1994). Functional coating and homogeneous distribution of the active pharmaceutical ingredient (API) in the production of low-dose solid dosage forms can be another set of issues that makes surface engineering of powdered carrier particles valuable (Grawe et al., 2001; Chan and Chew, 2003). In addition, efficient processing of particle surfaces could avoid the time, energy and cost consuming granulation step and allow the direct compression of a physical powder blend.

The main goal of this work was to increase insight into the effects of particle surface engineering on the performance and quality of powders during pharmaceutical processing. Various visual and analytical methods were used to study changes in the technical properties of powdered materials and the relationship between these changes and those in the particle surface morphology. Carrier particle surface coating with APIs was used to increase the utilization of lactose in some pharmaceutical formulations and to improve content uniformity of low-dose solid dosage forms. The above-mentioned modifications of particle surface properties were done using an ultrasound-assisted technique developed during this work. The literature review is focused on the theoretical characterization of powder properties and methods used to improve powder behaviour during drug development.

2 Literature review

2.1 Pharmaceutical powders and granules

To date the majority of medicines are available as solid dosage forms such as powders and their derived products (e.g. granules, pellets, tablets and capsules). However, powdered materials possess some unfavourable properties that must be overcome during drug development and design to maintain satisfactory performance of powders in dosage forms. Therefore, an understanding of particle properties is fundamentally important to enable their effective modification and further improvement of powder bulk behaviour.

A powder is a multiple system consisting of solid and gaseous phases. Powders have characteristics related to both phases. Powdered materials can deform permanently, acting as solids, and can be compressed, behaving as gases. In addition, they can flow and possess rheological properties typical for liquids. Pharmaceutical powders are electrical insulators. They are good at generating, holding and transferring particle surface charge. Powders are considered to be composed of a large number of fine particles with a maximum dimension of less than 1000 µm (Staniforth, 2002). Powders consisting of particles of less than 100 µm are considered to be very interactive with each other and with the walls of handling devices, which causes problems during processing of these materials, especially in conditions of very low humidity. On the other hand, powders of particle size greater than 250 µm are less electrostatic and rather free flowing (Antikainen, 2003). Granular material is the product of size enlargement processes with particle dimensions greater than 1000 µm (Staniforth, 2002).

2.1.1 Particle size and shape

The particle morphology of powders plays a critical role in the performance of APIs and excipients during the manufacturing of dosage forms. Obtaining detailed information about particle size and shape of raw material is important if the final product contains solid particulates (Heng and Chan, 1997). Different methods such as microscopy in connection with image analysis, sieving, light scattering, sedimentation, electrical sensing and photon correlation spectroscopy have been used to measure the size of particles (Heng and Chan, 1997). Among them, the first three have been the most widely-used techniques. However, all methods have their advantages and special limitations.

Characterization of spherically-shaped particles with a narrow size distribution is easy to perform by microscopic methods along with image analysis. However, the shape of particles is usually not regular, and being elongated, acicular, angular or a mix, thereof makes determination of particle size difficult. Therefore, equivalent spherical diameters of different particle properties, such as volume, surface area and projected area, have been used to describe the particle size of powdered materials (Laitinen, 2003). The common treatment of image data provides quantitative descriptions of particle shape such as aspect or elongation ratio (length/breadth), bulkiness factor (area/(length + breadth)) and shape or

form factor (4π area/(perimeter)^2) (Heng and Chan, 1997). Aspect ratio shows the deviation from a spherical shape towards an elongated form. The bulkiness factor provides an indication of solidity. The shape factor gives a measure of sphericity. The ideal sphere has form factor and aspect ratio values of unity. In spite of these valuable data, particle image analysis is a relatively slow and tedious process, and flaws and statistical errors in particle size determination can happen due to a preferred orientation of the particles, insufficient dispersion of powder samples and an inadequate number of powdered units. A novel approach based on analysis of digital images of powder surfaces was introduced for particle size analysis of pharmaceutical materials (Laitinen et al., 2002). The method is fast, non-destructive and requires less than a half gram of the sample. However, the resolution of this technique is limited and the accuracy of the applied models depends on the quality of the results obtained from the reference methods. Preparation of powder surface samples may be a source of error as well (Laitinen et al., 2004).

Sieving is an appropriate method if the amount of material is not limited. However, it is a time-consuming method, in which inaccuracies may occur due to incorrect sieve load and sieving time, aggregation of highly cohesive powder and a tendency of particles to pass through the mesh on their smaller cross-sectional area (Heng and Chan, 1997).

Nowadays the popularity of the light scattering method is related to its quick performance, which gives reproducible results for particle size distribution. The difficulties of this technique can be associated with finding a suitable solvent for dispersion of the powder in the case of particle size determination in liquids. Agglomeration of particles and a large sample size can be problematic for measurement of particle dimensions using particles in an air-based method.

2.1.2 Particle surface

A surface is a boundary that separates an object from another object, space or substance (Seitavuopio, 2006). One important surface characteristic is its free energy. The origin of surface energy is the net inward force on the surface molecules applied by molecules in the bulk (Buckton, 2002). Unlike liquids, the surface free energy of solids has a non-uniform structure. On contact, interactions between particles depend on the energy level of their surfaces. A change in the solid-state form or surface polarity of a substance modifies its surface energy. Measurement of surface free energy for solids is complicated due to heterogeneity of the forces acting from the bulk on each molecule. Several methods based on the contact angle and vapour sorption analysis have been used to assess surface energy of powdered substances (Table 1). Determination of the contact angle requires powders to be compacted before measurements to produce a flat surface. The compaction process may change the superficial properties of particles. Therefore, methods based on vapour sorption analysis are more favourable to evaluate surface energy of powders.

Table 1 *Methods used to determine surface energy of powders.*

Method	Phenomenon	Measured characteristics	Reference
Contact angle	Wetting	The contact angle between a solid surface and a drop of liquid placed on it	Nikolakakis et al., 2002; Butt et al., 2006; Baki et al., 2010
Gravimetric analysis	Vapour sorption to the powder surface	The mass change of the powder due to vapour sorption	Buckton, 1997
Calorimetric analysis (Isothermal microcalorimetry)	Vapour sorption to the powder surface	The heat associated with sorption of vapour to the powder	Buckton, 1999
Inverse gas chromatography	Vapour sorption to the powder surface	The retention volume of carrier gas required to elute the different adsorbate vapours from the powder being investigated	Schiavone et al., 2004; Steckel et al., 2004; Kumon et al., 2006; Steele, 2009

Surfaces of particles are characterized as having a certain degree of roughness. This parameter depends on the material properties and processing history of the substance. Particle surface roughness has an influence on the bulk characteristics of powders. Particles with an uneven surface are generally more interactive and may have a greater tendency to interlock than particles with smooth surfaces. This phenomenon may have an influence on powder flowability (Holgado et al., 1995). In addition, surface roughness may change wettability of the powder particles. This fact plays a significant role during coating procedures and may affect the adhesion between the coating agent and the solid (Muster and Prestidge, 2002).

Optical microscopy (OM), scanning electron microscopy (SEM), laser profilometry and atomic force microscopy (AFM) are the most widely-used analytical techniques for studying the nature of surfaces of pharmaceutical materials (Seitavuopio, 2006). OM is a simple and fast tool to visualize particle morphology. However, due to the limited resolution and two-dimensional view of the specimen in focus it cannot provide accurate data about surface heterogeneity. SEM and AFM imaging can be used to investigate surface morphology at a nanometre scale. There is a large number of existing transformation approaches that can be applied to image analysis to obtain quantitative information about particle surface irregularities.

Height parameters, wavelength parameters (mean spacing, S_m), shape parameters (skewness, S_{sk}), statistical functions and fractal dimension (D) are the commonly-used roughness parameters to get numeric values for particle surface topography (Fini et al., 1996; Provder and Kunz, 1996; Li and Park, 1998; Passerini et al., 2002a; Seitavuopio, 2006). The most important roughness height descriptors are the arithmetic average roughness:

$$R_a = \frac{1}{n}\sum_{i=1}^{n}\left|y_i - \bar{y}\right| \quad \text{(Eq. 1)}$$

and the root mean square roughness:

$$R_q = \frac{1}{n}\sqrt{\sum_{i=1}^{n}\left(y_i - \bar{y}\right)^2} \quad \text{(Eq. 2)}$$

where n is the number of measurements, y_i is the i-th measurement of surface height and $\bar{y}$ is the mean value of height over all measurements (Seitavuopio, 2006). R_a and R_q are different representations of the same concept, and for many surfaces can even be used interchangeably. It is worth to mention that these parameters are differently sensible for different shapes of roughness. This fact, as well as historical reasons, explains their simultaneous usage.

Specific surface area (SSA) is a property of powder which represents the total surface area of particles per unit mass, per net volume of particles or per apparent volume of powder (Shinohara, 1997). Gas adsorption and air permeability are the common methods used to measure SSA of solid particles. The first is based on the BET theory (Brunauer et al., 1938) and has the advantage of determining the SSA of fine particulates, taking into account pores smaller than 50 nm (Heng and Chan, 1997). The air permeability technique measures the resistance of a porous powder bed to gas flow. The method is robust and quick; however, it cannot accurately determine the deep surface texture of particles. During powder processing, a decrease in the SSA may be used as an indicator of particle agglomeration and/or loss of fines (Shur et al., 2008). In addition, the measured SSA values of powders of the same particle size allow comparison of the roughness of particle surfaces (Iida et al., 2004a).

2.1.3 Interparticulate interactions and effect of relative humidity

The attractive forces acting in bulk powder are governed by the nature of the particles, predominantly the properties of their surfaces and the interfaces between them, and the interparticulate distances between interacting units (Führer, 1996). Interparticulate interactions occurring between the surfaces of the same materials are called cohesion, whereas interactions between unlike surfaces are called adhesion. These forces are of molecular and electrostatic origins. The atoms of some molecules with limited charges can be involved in molecular interactions that act over short distances. These interactions are non-specific van der Waals attractions that can be divided into ion-dipole, dipole-dipole and van der Waals-London interactions (Führer, 1996). Van der Waals forces of attraction are the main forces acting between powdered particles (Visser, 1989). The magnitude of the forces involved depends on the morphological properties of the particles and the distances between them. Generally, interactions increase with decreases in particle size, the roughness of particle surfaces and the separation distances. However, the surface topography plays a critical role for interparticular interactions and can give the opposite effect (Figure 1). For instance, mechanical interlocking of pharmaceutical particles due to a rough surface structure can lead to intensification of interparticulate interactions (Figure

1e,f). During pharmaceutical process operations such as mixing, sieving and grinding, friction between the particles causes the formation of local charges, especially on the tops of crystal edges and where there are crystal defects. This initiates triboelectrostatic charge transfer that gives rise to particle attraction. The properties of the interacting materials determine the magnitude of charge and hence the relative position of these materials in the triboelectric series.

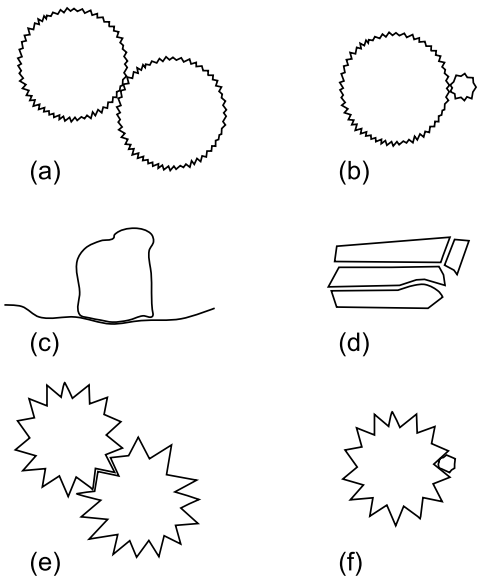

Figure 1 *Effect of surface morphology and contact geometry on interparticular interactions; (a) and (b): small scale surface roughness preventing particle close contact; (c) and (d): smooth surface structure increasing particle interactions; (e) and (f): large scale surface roughness promoting particle approach due to mechanical interlocking (modified from Visser, 1989).*

Relative humidity (RH) has a strong influence on the interparticulate interactions within a powder bed. The impact of RH on powder cohesion and adhesion strongly depends on the polarity of particle surfaces. In conditions of low RH, a tightly bound water sorption layer may be formed on the powder particle surface (Figure 2). At points of close contact between particles, water layers may act as a binder and merge particles together through hydrogen bonds. In a humid atmosphere, water may condense on the powder surface, forming the capillary bridges, and join particles together due to the surface tension force of liquid (Führer, 1996; Iida et al., 2004a; Young and Price, 2004). During powder handling, relative humidity has to be routinely adjusted and controlled, since a water layer is able to distribute the transferred charge homogeneously over the surface, reducing electrostatic interactions and equilibrating the powder. In addition, at high humidity transferred charge can leave particles due to an exchange of water molecules between particle surfaces and the atmosphere, stabilizing powders and making them easy to deal with (Führer, 1996; Kawashima et al., 1998a).

Residual moisture has a significant effect on stability (Mahlin et al., 2006), flow (Führer, 1996; Faqih et al., 2007), compactibility (York, 1981) and adhesion properties

(Podczeck et al., 1997; Bérard et al., 2002; Price et al., 2002; Young and Price, 2004) of pharmaceutical materials.

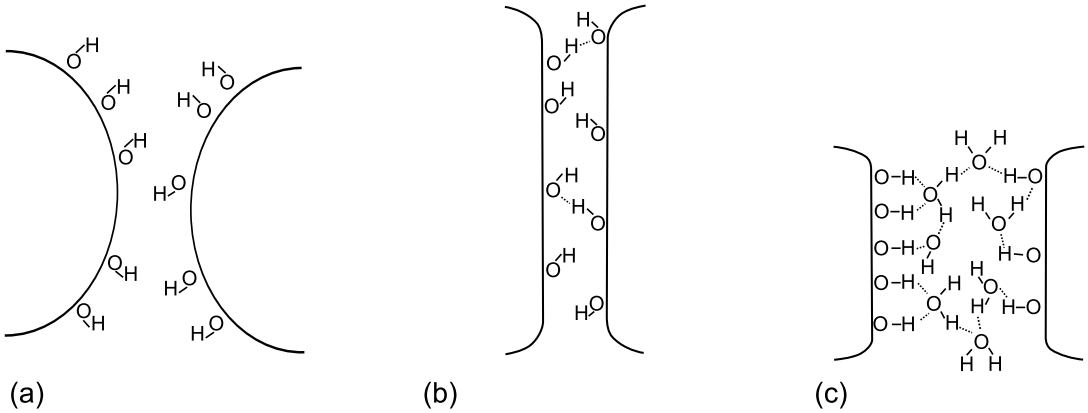

Figure 2 *The effect of relative humidity (RH) on the bonding mechanisms of powdered materials: (a) tightly bond water sorption monolayer at low RH, (b) after particle contact in a dry atmosphere, (c) mobile water layer at high RH (Führer, 1996).*

2.1.4 Flow and aerosolization properties of powders

Modification and control of powder flow characteristics is of extreme importance in the case of DPIs, where the active substance is often micronized and, therefore, very cohesive (Thalberg et al., 2004). Maintaining good flowability of powders is a prerequisite for the successful production of tablets and capsules. Insufficient flow properties of powdered mixes may cause variations in the weights of tablets and capsules and, as a consequence, fluctuation in the API dose.

Particle characteristics such as density, size, shape, surface roughness and surface energy determine the interparticulate interactions and, therefore, affect the flow properties of powders. Spherically-shaped particles with a smooth surface and narrow particle size distribution flow better than particles with irregular morphology and a broad size distribution. However, free-flowing particles segregate easily, causing problems in dose uniformity of the final product. Flowability of powders depends strongly on the particle size (Liu et al., 2008). Powders of small particle size are generally cohesive and poorly flowing. An increase in the particle size brings about an improvement in the flow properties of powders. Therefore, granulation of powders is usually performed to increase the bulk density of dusty materials and decrease the segregation tendency of powder mixtures with different sizes, shapes and densities of particles (Alderborn, 2002). Triboelectrostatic charges on particle surfaces make the powder flow properties worse and, consequently, cause problems in the uniformity of dosage units. Storage in conditions of sufficient air humidity may partly discharge the material (Führer, 1996). The effect of humidity on the stabilization of cohesive powders is most significant in the case of polar particle surfaces (Kawashima et al., 1998a).

Several approaches have been used to measure flowability of powders (Räsänen et al., 2003; Lindberg et al., 2004; Thalberg et al., 2004; Rios, 2006; Jiang et al., 2009; Seppälä et al., 2010). The flow properties of powdered material may be determined directly (European Pharmacopoeia (Ph. Eur.), 2005) using a flow cup, which is often vibrated, or

indirectly by examination of the angle of repose (Geldart et al., 2006), avalanching behaviour (Bhattachar et al., 2004) or yield stress (Staniforth, 2002). Good-flowing powders give a small angle of repose and have minimal shear stress at zero normal stress. Determination of the compressibility index or Carr`s index (the ratio of the difference between the tapped and poured densities to the tapped density) has been a convenient method to give an estimation of rheological properties of powders: the higher the compressibility index, the poorer the flowability (Carr, 1965).

2.1.5 Consolidation and compression behaviour of powders

Compression of powders is defined as a volume reduction mechanism of powdered material subjected to compressive stress. Pharmaceutical powders differ from each other in their consolidation behaviours during tabletting. Particles can deform plastically, elastically or can fragment (Figure 3). The majority of pharmaceutical powders seem to be strain-rate sensitive and deform viscoelastically, and the degree of deformation depends on the applied stress and the time of loading (Müller, 1996).

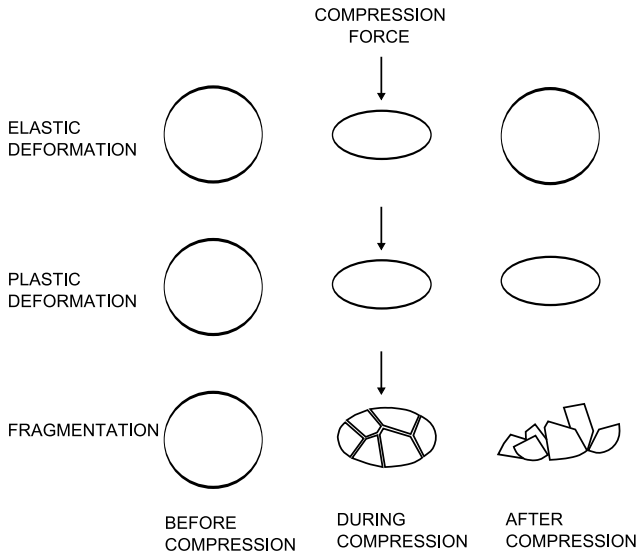

Figure 3 *Powder deformation during compression (Ragnarsson,1996).*

Before compression, particle size, shape and surface properties affect the bulk density of a powder. In the beginning of the tabletting process, spherically-shaped particles rearrange easily and produce fewer defects in the compacts than irregular species. Several mechanisms are simultaneously involved in the formation of bonds between particles in the consolidated powder. The most important of these are distance attraction forces (van der Waals forces, hydrogen bonds, electrostatic forces), solid bridges (sintering, melting, crystallization), mechanical interlocking (shape- and roughness-related), non-freely mobile binder bridges (adsorption layers) and bonding due to mobile liquids (capillary forces) (Nyström and Karehill, 1996). Relative humidity (RH) has a strong impact on the last two bonding mechanisms (York, 1981).

The majority of drug powders have poor compression and consolidation properties. Therefore, before tabletting a careful selection of components for the formulation is very important. If the main ingredient deforms plastically, the minor components should be brittle (Wells, 2002) since lubricant sensitivity of the former leads to insufficient bonding (Bolhuis and Hölzer, 1996).

2.1.6 Dissolution and solubility of powders

Controlled dissolution of API is of great interest in the pharmaceutical sciences. Dissolution is defined as the transport of molecules or ions from a solid state into a solution, whereas solubility is the amount of a substance that can be transferred into a solution until equilibrium is reached between the solution and the undissolved substance at given conditions. Diffusion and convection are the primary processes responsible for producing a dissolved drug in solution during dissolution testing (Chen and Flanagan, 2009). Diffusion predominates at short distances, where atoms, molecules and small particles (<0.1 µm) are transported irreversibly from a region of high concentration to lower concentration. Mass transfer occurs by random molecular motion and is driven by an increase in entropy. Over larger distances, convection (heat mixing) is an important transport mechanism for material transfer.

Temperature, pressure, the nature of the solvent, crystal characteristics, pH, and the presence of additives are the main factors affecting the balance of intermolecular forces between solvent and solute and, consequently, solubility (Heng and Chan, 1996). In addition to the above mentioned factors, particle size, shape and surface roughness of a drug determine the dissolution rate of the API powder (Ashford, 2002). Improving the dissolution rate is extremely important in the case of poorly water-soluble drugs.

Particle size is a critical characteristic that affects dissolution rate and, consequently, therapeutic effect of a poorly-soluble API (Shaw and Carless, 1974). Generally, smaller particles have higher intrinsic solubility than larger ones (Heng and Chan, 1997). The existence of a higher interfacial free energy on smaller particles is responsible for formation of a thermodynamically unstable system that causes greater dissolution of them. Particles of the same size but with different surface morphologies may exhibit different dissolution times as well. Particles with a rough surface have larger specific surface areas and, consequently, greater dissolution rates.

Co-processing of hydrophobic API with hydrophilic carrier or wetting agent has been used to avoid a decrease in the effective surface area (agglomeration) during particle size reduction. In addition, wetting of hydrophobic surfaces enhances penetration of the solvent and, consequently, improves dissolution of solids (Passerini et al., 2002b; Rasenack and Müller, 2002; Cavallari et al., 2005a). Additive-related defects produced in the crystal structure during crystallization make particles thermodynamically unstable, leading to faster dissolution (Chiou et al., 1976). Pharmaceutical co-crystallization provides a new method for enhancing the dissolution rate of poorly-soluble drugs (Remenar et al., 2003). On the other hand, by inclusion of hydrophobic excipients in the formulation, it is possible to control the release of freely-water soluble API from lipid microparticles (Passerini et al., 2003).

Granulation of powdered particles causes a marked increase in the particle size of the mixture, which can affect the dissolution characteristics of the API. A reduction in available surface area can inhibit the dissolution rate and affect bioavailability of the drug. However, the use of hydrophilic excipients in preparation of granules can help to avoid this problem and even facilitate solubilization of API (Walker et al., 2007).

2.1.7 Solid-state properties

Solid materials may exist as crystalline or amorphous substances. The crystalline particles have a specific three dimensional structure, the so-called crystal lattice, in which molecules and atoms are arranged in a regular manner. Amorphous substances have no regular long-range order in their molecules and no definite structure. The majority of pharmaceutical materials are crystalline and exhibit polymorphism - the ability of a solid to exist in more than one crystalline form (Grant, 1999). Solvent molecules can be incorporated into the crystal lattice. These forms are called solvates or pseudopolymorphs. If the solvent is water, the solvate form is termed hydrate. The different polymorphs differ in many physical characteristics, such as packing, thermodynamic, spectroscopic, kinetic, surface and mechanical properties (Kahela et al., 1983; Byrn et al., 1988; Nichols and Frampton, 1998; Grant, 1999; Sun and Grant, 2001). The differences in solubility and dissolution rate between polymorphic forms of the same substance are the most important for pharmaceutical applications. The use of metastable polymorphs in comparison with the stable forms results in higher dissolution rate (Guillory, 1999). Hydrated crystals tend to exhibit slower dissolution rate in water than their anhydrous forms (Aaltonen et al., 2006). The aqueous solubility of non-aqueous solvates is often higher than that of the non-solvated counterparts if solvates formed from water-miscible solvents (Grant and Higuchi, 1990; Heng and Chan, 1997). Amorphous substances are more soluble than the corresponding crystalline solids. Any change in the crystalline form may affect the therapeutic efficacy of a pharmaceutical product. For that reason, process induced transformation (PIT) has been widely studied over recent years. It has been reported that polymorphic transformations take place easily during various unit operations such as granulation (Aaltonen et al., 2007a), drying (Römer et al., 2008) and tabletting (Kogermann et al., 2007), among others. In addition, surface amorphization can happen during milling of lactose powder for DPIs (Young and Price, 2004), whereas the stability of amorphous regions depends on the surrounding atmosphere (Steckel et al., 2006). Therefore, PIT of pharmaceutical compounds must be well controlled to exclude undesirable conversion during manufacturing and storage of the product.

The appropriate tools for solid state analysis are X-ray powder diffraction (XRPD), spectroscopic techniques (Raman, terahertz pulsed, infrared (IR) and near-infrared spectroscopy), thermal analysis (differential scanning calorimetry (DSC), thermogravimetric analysis), microscopic methods (hot stage microscopy, OM, SEM) and dynamic vapour sorption (DVS). Vibrational spectroscopy techniques are fast, non-destructive and non-invasive tools that allow real-time process monitoring, whereas thermal analysis, DVS and XRPD demand sample preparation. However, spectroscopic methods require complex data analysis in order to extract and systematically group the

useful information (Lakio, 2010). All these techniques provide complementary information about the solid-state properties of the examined material (Römer, 2008). Using a combination of different methods makes it possible to obtain a more complete picture of the molecular structure of the substance and its transformations than use of a single technique (Giron et al., 2002; Aaltonen et al., 2007b).

2.2 Particle surface engineering

2.2.1 Particle surface smoothing

Modification of particle surfaces may be one possibility to improve powder behaviour during industrial processing. Preparation of particles with smooth surfaces may decrease the attraction forces between them, reducing the tendency to interlock. This results in better flow and packing properties, which is of great importance in the production of tablets and capsules of uniform mass and content. By modifying particle surface roughness, it is possible to adjust powder demixing and aerosolization properties that are crucial in the preparation of ordered mixture for DPIs (Kawashima et al., 1998b; Podczeck, 1998; Schiavone et al., 2004).

Many approaches have been used in the field of particle surface engineering (Davies et al., 1998), especially in the processing of α-lactose monohydrate (LMH) carrier surfaces for DPIs. Controlled crystallization of lactose from different solvents has been carried out to produce regular crystals with smooth surfaces (Zeng et al., 2000a,b). Iida et al. used a number of methods for effective surface treatment of excipient such as dissolving surface asperities in aqueous ethanol solution (Iida et al., 2003a) and applying shear stress in a high-speed powder mixer (Iida et al., 2004b). A smoothing procedure, based on wetting in a water-ethanol mixture with or without a ternary component (magnesium stearate) and drying of the lactose powder in a high-shear mixer was successfully carried out (Young et al., 2002; Ferrari et al., 2004). In another study, decantation with absolute ethanol presaturated with lactose was used to remove fines from carrier lactose powder (Islam et al., 2004). In addition, high-temperature experiments with alcoholic solution were conducted to control surface dissolution of lactose particulates (El-Sabawi et al., 2006a,b). One of the proposed mechanisms responsible for changes in particle morphology is related to the ‘Ostwald ripening’ phenomenon. The process is driven by solubility differences between smaller and bigger particles (Ferrari et al., 2004). Fine particulates attached to the surfaces of bigger particles dissolve and recrystallize in the valleys of the larger ones, giving rise to a smooth particle surface.

Promising attempts have been made to improve flow and/or aerosolization properties of API through modification of surface characteristics. A surface treatment approach, based on interactions at the solid-liquid interface in a drug suspension was successfully applied to APIs with poor flowability (Hammouda et al., 1999; Ramadan et al., 2006). Molecules of solvent and impurities can interact with growing faces of crystals during crystallization leading to a change in morphology of ripening units (Rodríguez-Hornedo et

al., 2007) (Figure 4). The recrystallization of sulphadiazine, methyldopa (Hammouda et al., 1999), ibuprofen (Rasenack and Müller, 2002), erythromycin A dihydrate (Mirza et al., 2008) and salbutamol sulphate (Larhrib et al., 2003) from preselected solvents in the absence or presence of additives has been carried out to change the habit and surface topography of the drug crystals.

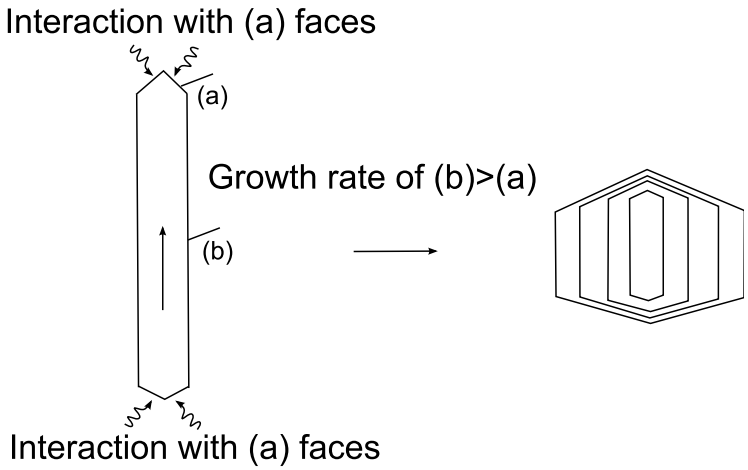

Figure 4 *A schematic representation of changes in crystal morphology via specific interactions of an additive/solvent with crystal faces (modified from Rodríguez-Hornedo et al., 2007).*

In addition, several other approaches have been used to produce particles with desired properties, e.g. supercritical fluid technology (Schiavone et al., 2004) and the aerosol flow reactor method (Eerikäinen et al., 2003). The former has been used to produce API particles with increased surface smoothness and decreased surface energy and, as result, with improved drug aerosolization. The latter has been developed to yield spherical nanometer-sized drug particles with smooth surfaces and a unimodal size distribution.

2.2.2 Coating of powders

Coating of powder particles can be performed to change the nature of the powder surface and improve the dispersion, dissolution and flow rate of pharmaceutical bulk materials (Fernández-Arévalo et al., 1990; Kawashima et al., 1998a; Kawashima et al., 1998c; Chan and Chew, 2003). In addition, the coating of the particulate system can have a positive effect on taste masking, environmental protection and controlled release properties. However, the coating of particles is challenging due to their small size, irregular shape and high adhesion and cohesion, which lead to the agglomeration of individual particulates.

Several approaches have been used for coating powder particles with liquid. Recently, particle thin-coating in a top-sprayed fluidized bed system has been performed to improve the flow properties of ibuprofen powder (Ehlers et al., 2009). A Würster fluidized bed has been used to cover lactose particles with a solution containing lactose and HPMC (Iida et al., 2005). However, both techniques are complex systems that require comprehensive

knowledge of the properties of materials and the influence of process variables (Dewettinck et al., 1999; Jono et al., 2000; Tang et al., 2008).

Another way to coat a substance is to use a spray-drying technique. This method has been used to modify particle properties and enhance their manufacturing performance (Chan et al., 1997; Elversson and Millqvist-Fureby, 2006). The resulting spray-dried product is generally a free-flowing powder, but the operating equipment is very bulky and energy consuming. In addition, the material obtained would most likely be amorphous, which may lead to instability of the formulation due to recrystallization and particle fusion (Young and Price, 2004).

Coating of solid particles can be performed by means of induced coacervation of the coating agent in a stirred suspension. The resulting microencapsulated particles can be separated as a powder by filtering the suspension, washing out non-encapsulated particles with a suitable solvent and drying the wetted material. The polymeric coating serves as a barrier to physicochemical degradation and can act as a release modifier for surface-treated micro-sized drug products (Albertini et al., 2005; Sam et al., 2008). In addition, encapsulation of drug material is a common procedure in the preparation of nanoparticles. Emulsification/solvent diffusion, emulsification/solvent evaporation, nanoprecipitation and salting-out methods are widely-used techniques for nanoencapsulation of APIs (Hirsjärvi, 2008). In addition, further surface modification or functional coating of prepared nanoparticles can be performed to enhance stability and controlled release properties of APIs (Olivier et al., 1995; Nobs et al., 2003; Trimaille et al., 2003). Nanoparticle dispersions can be dried to a powder by means of freeze-drying and vacuum heating. However, the challenges in nanoencapsulation of a drug are related to low yield due to poor drug encapsulation efficiency, difficulties with scaling-up and removal of non-adsorbed surface modifiers.

The drawbacks of the above-mentioned liquid coatings, such as air pollution and long processing times, were overcome using an environmentally-friendly and safe dry powder coating technique (Pfeffer et al., 2001; Luo et al., 2008). The disadvantage of this method is the heat applied or generated during mechanical interactions that can cause the degradation of temperature-sensitive materials.

Physical dry blending of DPI carriers with small amounts of impurities is another approach to improving particle surface characteristics. Covering of lactose particles with the antistatic compounds sucrose tristearate (J-1803F) (Iida et al., 2003b) and magnesium stearate (Iida et al., 2004c) has been performed to modify the superficial morphology of the particles. A novel mechanofusion approach in the presence of hydrophobic additives has been used to alter the shape and surface energy of lactose (Kumon et al., 2006). In addition, hydrophobic coating of polar surfaces may diminish the effect of relative humidity on powder stability and formulation performance (Iida et al., 2004a). Pre-blending of lactose carriers with a fine lactose fraction can be used to enhance performance of DPIs (Zeng et al., 1998). Generation of fines on the surface of lactose excipient during milling could give the same result (Steckel et al., 2006). The effect was proposed to be due to occupation of the most active binding sites by fine lactose on the carrier surface, leaving passive sites for API adhesion. This mechanism of reduced adhesion between drug and carrier surface was claimed to be responsible for an increase in API aerosolization. In other studies, the effect of fine excipient was suggested to be related

to redistribution of API between the surfaces of the carrier and fine particles to form agglomerates consisting of fines and API particles that could improve the separation of API from carrier lactose (Lucas et al., 1998; Islam et al., 2004).

2.3 Dry powder preparation of proteins

The manufacturing of complex macromolecular compounds (e.g. proteins) presents more difficulties due to their labile structure. Proteins suffer from both physical and chemical instability and are therefore very sensitive to processing conditions (Manning et al., 1989). Spray-drying (Mumenthaler et al., 1994; Lucas et al., 1998; Maa et al., 1998), spray freeze-drying (Maa et al., 1999) and lyophilization (Carpenter et al., 1997) are the most common methods for the dry powder preparation of protein pharmaceuticals. However, these techniques have some drawbacks, such as thermal stress, shear forces, low yield and long processing times that can affect the stability of polypeptides (Broadhead et al., 1994; Ståhl et al., 2002). The spray-coating of recombinant human deoxyribonuclease onto microcarriers by means of top and bottom fluid bed techniques was successfully performed to evaluate the stability and integrity of the protein layer (Maa et al., 1996; Maa and Hsu, 1997). The resulting preparation was compared with the spray-dried product. Both techniques caused the partial loss of protein activity during the drying stage, although spray-drying was less damaging. However, some sources (Maa et al., 2004) mention that spray coating of vaccine antigens onto the carrier microparticles did not change the activity of the proteins.

2.4 Ultrasound and its implementation

2.4.1 Principles of ultrasound

Ultrasound is a sound-like oscillation of pressure travelling through the air which is above the human auditory limit. More widely, it can be defined as a mechanical wave propagating through any form of matter (gas, liquid, solid, etc) at a frequency higher than 20 kHz. High-power ultrasonic probes and sonotrodes consisting of electromechanical transducers are used in ultrasonic apparatuses for generation of energy in the form of sound waves (Ruecroft et al., 2005). Ultrasound can be applied at different frequencies and levels of intensity (Levina and Rubinstein, 2000). At low intensity, ultrasound is used to non-destructively study structural properties of materials. At high intensity, ultrasound is applied to alter physicochemical characteristics of matter irreversibly. Various effects derived from ultrasound-induced particle motion are responsible for changes made in the structural and chemical properties of materials. Most of these effects are related to heat, stirring, chemical activity, mechanical stress and cleansing (Levina and Rubinstein, 2000).

2.4.2 Implementation of ultrasound in pharmaceutical technological processes

From the pharmaceutical point of view, sonochemistry and sonocrystallization are the most common processing applications of acoustic waves. Converted to heat, ultrasonic energy can accelerate or modify chemical reactions such as oxidation, polymerization, depolymerization and others (Ruecroft et al., 2005). In sonocrystallization, ultrasound can be applied to the medium to initiate primary nucleation and, consequently, to avoid conventional seeding. This effect is related to high energy transient cavitation and microstreaming (Ruecroft, 2007). Acoustic energy-based techniques have been used for controlled crystallization of solid materials to produce crystals with desired physicochemical properties using the technology designed by Prosonix (Kaerger and Price, 2004; Ruecroft et al., 2005; Ruecroft, 2007). Nucleation of the low-saturated solution, initiated by a short ultrasound pulse, gives rise to the growth of large crystals, whereas continuous sonication of the medium yields small crystals (Ruecroft, 2007). By selection and optimization of ultrasound parameters, it is also possible to achieve crystallization of the stable polymorphic form. In addition, combined particles consisting of two or more different APIs or API and excipient have been prepared by an ultrasound-assisted technique that raises new possibilities for particle engineering (Ruecroft, 2007; Pitchayajittipong et al., 2009).

Besides sonocrystallization, ultrasound has been used to improve fluidization of nanoparticle agglomerates (Zhu et al., 2004). Additionally, acoustic wave-based techniques have been used for dry granulation during compact formation (Fini et al., 2002; Cavallari et al., 2005b; Passerini et al., 2006), ultrasound-assisted powder compression (Levina and Rubinstein, 2000; Levina and Rubinstein, 2002), homogenization of nanoparticle preparations (Hirsjärvi, 2008), dispersion of suspended particles and cleaning. Spray-congealing technology using an ultrasound atomizer has been used to obtain surface-modified microparticles (Rodriguez et al., 1999; Albertini et al., 2005; Cavallari et al., 2005a). Recently, ultrasound-assisted extrusion and cutting has been applied to the processing of viscous amorphous materials with a low glass transition temperature (Hoppu et al., 2009a,b).

Atomization of aqueous solutions by means of an ultrasonic nebulizer has been widely used in pulmonary drug delivery to produce a cold mist where the size of droplets is less than 8 µm (Omron-healthcare, 2010). The small size range is vital to guarantee the deposition of API into the airways. The size distribution of droplets in atomized solutions is also extremely important in coating operations for pharmaceutical powders. Smaller droplets reduce the risk of agglomerate formation and result in a powder formulation with better performance (Maa et al., 2004). However, the ultrasound-induced increase in the solution temperature may have an effect on the stability of heat-sensitive pharmaceutical molecules (Taylor, 2002). In the case of coating with a protein solution, foaming during atomization might provoke loss of the native protein structure and aggregation of the macromolecules, with a further loss of activity (Branchu et al., 1999). Nebulized droplets possess a large air-liquid interface that might also affect the stability of bioactive compounds (Niven et al., 1996).

Degradation of pharmaceutical residues by means of ultrasound waves has been studied to determine whether it might have a promising future in the area of wastewater treatment. Sonolysis of ibuprofen (Méndez-Arriaga et al., 2008), and levodopa and paracetamol (Quesada-Peñate et al., 2009) has been successfully performed for remediation of aqueous solutions.

2.4.3 Application of ultrasound beyond the pharmaceutical field

Beyond pharmaceuticals, ultrasound has been widely used in many different areas such as diagnostic sonography, cleaning, biological cell disintegration, plastic welding, foam extrusion and under-water ranging (Ruecroft et al., 2005). Additionally, acoustic energy has been applied to a variety of activities in the food processing industry. Mixing, homogenization, tenderization of meat and degassing are related to the mechanical effects of ultrasound, whereas bactericidal action, alteration of enzyme activity, sterilization of equipment and others are related to chemical and biological effects (Hoppu, 2008).

2.5 Content uniformity of low-dose solid dosage forms

The uniform distribution of highly potent API during preformulation and the preservation of content uniformity during further processing are essential for obtaining the correct effect in the final product. Poor content uniformity of the formulation can lead to a fluctuation in the dose. Single dose ineffectiveness can be a consequence of insufficient API, while an overdose can cause toxic side effects. Both situations pose health risks to patients.

2.5.1 Direct compression versus granulation

It is well known that direct compression is a cost effective method for tablet manufacturing because it requires fewer unit operations. In addition, it avoids stability problems during formulation due to the absence of moisture and heat during the process (Jivrai et al., 2000). However, direct compression has some disadvantages such as segregation of the physical mixture, as well as cohesion of drug substances during mixing and tabletting (Prescott and Hossfeld, 1994; Bolhuis and Chowhan, 1996). These limitations are of most concern if a low-dose drug with a narrow therapeutic index and high toxicity is in use. Because of that, an intermediate wet or dry granulation of the mixture is usually performed to increase the bulk density of the powder and distribute the API uniformly (Thiel and Nguyen, 1982; Thiel et al., 1986; Michoel et al., 1988; Wan et al., 1992; Greaves et al., 1995; de Haan and Thys, 1997; Parikh, 1997; am Ende et al., 2007). In wet granulation, the drug compound can be dissolved or suspended in the granulation liquid, or it can be a part of the powder mixture intended for granulation. Nevertheless, granulation is a complicated processing stage that involves several steps and

adds extra costs to tablet manufacture. In addition, it causes a noticeable increase in the particle size of the mixture, which can affect various characteristics of the API, with the dissolution rate being the most important one.

2.5.2 Improvement of content uniformity of low-dose solid dosage forms

Besides granulation processes, several other approaches have been used to improve content uniformity of low-dose solid dosage forms. The sandwich method and geometric mixing of API and carrier lactose particles have been widely applied for preparation of low-dose ordered mixtures intended for lung delivery (Hersey, 1975; Wu et al., 2000; Schiavone et al., 2004; Steckel et al., 2006). In a United States patent (de Haan and Deurloo, 1995), ordered mixing of micronized steroidal compounds and excipients was performed to design low-dose solid dosage forms. However, in this case, only carefully preselected diluents with superior binding properties and very low demixing potentials could be successfully used to obtain a sufficiently homogeneous mixture. In another case (Lerner, 1971), a very potent medical compound was deposited onto the surface of the excipient by dissolving the drug in an organic solvent such as chloroform, mixing the solution obtained with carrier particles, and drying the mixture. A structured system was successfully obtained, but the use of unsafe volatile liquids is strongly criticized nowadays. Spray-drying of an alcoholic suspension containing dissolved API and dispersed adjuvant particles was carried out to produce a preformulation with the drug substance adhering to the surface of the carrier particles (Grawe et al., 2001). This admixture can be further dry blended with directly compressible excipients to produce solid dosage forms of a uniform content, avoiding demixing and sizing effects. By using a fluid bed technique, it was possible to coat particles of an ordered mixture with polymer multilayers (Thiel and Nguyen, 1984; Thiel and Sberna, 1986). This method provided a free-flowing product with good content uniformity and low demixing potential. In addition, the fluidized bed technique has been used to spray API solution onto sugar spheres in order to produce a homogeneous drug layer without any significant change in the particle size distribution of carrier particles (Martinez et al., 2001).

3 Aims of the study

The primary goal of this thesis was to develop a new ultrasound-assisted technique for controlled thin-coating and modification of particle surfaces of pharmaceutical powders.

The specific objectives of this study were:

1. to minimize surface free energy and improve flowability of poorly-flowing powders by aqueous smoothing of particle surfaces (**I**)

2. to optimize flowability of a cohesive API powder using polymer thin-coating (**II**)

3. to increase the utilization of lactose-containing pharmaceutical formulations for a population with adverse reactions to milk sugar by nano-coating powdered lactose particles with the labile enzyme β-galactosidase (**III**)

4. to develop a method for precise determination of coating efficiency of the presented technique (**III, IV**)

5. to demonstrate the superiority of the API-coated powder approach over conventional physical powder blending methods in terms of content uniformity in low-dose tablets (**IV**)

4 Experimental

4.1 Materials

Thiamine hydrochloride (USP, Hawkins, Minneapolis, MN, USA) (**I**), β-galactosidase (Lactase 14-DS, Nagoya, Japan) (**III**) and riboflavin sodium phosphate (RSP) (Ph. Eur., Fluka Analytical, Sigma-Aldrich, France) (**IV**) were used as water-soluble model drugs. Ibuprofen (IBUPROFEN 50, Boots Pharmaceuticals, U.K.) (**II**) was used as a poorly-flowing, practically water-insoluble model drug. Two grades of LMH (coarser grade Pharmatose® 80M (**I, III**) and finer grade Pharmatose® 200M (**I**), Division of Campina BV, the Netherlands), hydroxypropyl methylcellulose (HPMC) (Methocel E5 Premium LV EP, Dow Chemical Company, USA) (**II**) and microcrystalline cellulose (MCC) (Avicel® PH-200, FMC, Ireland) (**IV**) were utilized as model excipients. The standardized reagents for the ultraviolet (UV) method (R-Biopharm, Darmstadt, Germany) (**III**) were used for a quantitative determination of coating efficiency. Purified water (**I-IV**) was used as a solvent.

4.2 Methods

4.2.1 Particle coating and surface modification (I-IV)

The method for particle coating (**II-IV**) and surface modification (**I-IV**) of powders has been developed at the Division of Pharmaceutical Technology, University of Helsinki. A schematic diagram of the technique is presented in Figure 5.

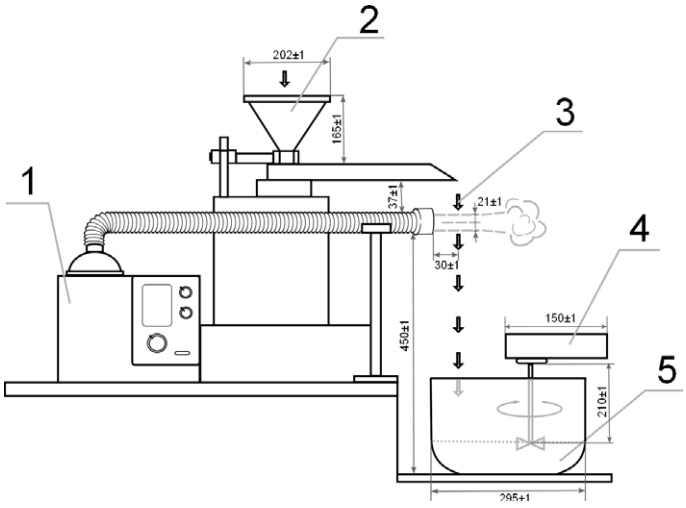

Figure 5 *Schematic diagram of the developed technique: (1) ultrasound nebulizer, (2) vibrating feeder, (3) solid powder, (4) stirrer (**I-III**) and (5) collector (the dimensions of the system are given in millimetres).*

In study **I**, the powder particles of lactose and thiamine as solid substances were supplied by a vibratory feeder (Fritsch Laborette 24, Germany) and exposed to the instantaneous effect of a stream of pure water mist generated from an ultrasound nebulizer (Ultrasonic Nebuliser NE-U17, Ultra Air, Omron, the Netherlands). The droplet size of the water mist was approximately 1 to 8 µm (Omron-healthcare, 2010). In studies **II-IV**, a mist of coating solution was applied to the surface of carrier particles. Due to the open setup of the technique, some loss of coating agent occurred during the entire coating procedure. In studies **I-III**, powders were occasionally mixed by a stirrer (IKA®-WERKE, RW 11 basic, Staufen, Germany) to prevent the formation of liquid bridges between individual particles. No stirring of the surface-covered cellulose was performed in study **IV**. In total, 5 (**I**) and 30 (**II-IV**) treatment cycles were done with the same solid material. Representative samples of 5 g (**III, IV**) and 20 g (**II**) were withdrawn every 5th cycle. After the last treatment round, slightly wetted powders were left to equilibrate in ambient conditions. In study **IV**, the experimental procedures were performed in a dim room to minimize the destructive effect of light on the riboflavin salt. All process parameters (**I-IV**) are summarized in Table 2.

Table 2 *Applied process parameters.*

	Study I	**Study II**	**Study III**	**Study IV**
Batch size (g)	200-250	120	33	350
Number of cycles	5	30	30	30
Time between cycles (min)	10	10	7	2
Feeding rate of powder (g/min)	7.3-42.3	2.2	2.3	9.7
Air flow (L/min)	17	17	17	8.5
Flow rate of mist (g/min)	3.0	3.0	2.8	1.1
Coating agent	-	HPMC	β-galactosidase	RSP
Amount of coating agent in solution (%, m/V)	-	0.15	1	4
Carrier particles	LMH, Thiamine·HCl	Ibuprofen	LMH	MCC
Rotation rate of stirrer (rpm)	240	240	240	-
Sample size (g)	-	20	5	5
Yield (%)	98.1-99.5	95.2	90.0	-
Equilibration time before packing (h)	1	1	1	24

4.2.2 Particle size and morphology (I-IV)

4.2.2.1 Spatial filtering technique (SFT) (I, II)

The measurements of particle size and size distribution were performed by the SFT (Petrak, 2002). The samples were fed into the SFT apparatus (Parsum® IPP 70; Gesellschaft für Partikel-, Strömungs und Umweltmesstechnik GmbH, Chemnitz, Germany) through the orifice (diameter 4 mm) using a funnel and dispersed by pressurized air. The chord length of each particle that passed through the laser light beam was transformed to particle size for subsequent data analysis (n = 3).

4.2.2.2 Laser diffractometry (III)

A Malvern laser diffractometer (Malvern 2600c, Malvern, England) was used to measure the particle size distribution of powders in an isopropyl alcohol suspension (n = 3).

4.2.2.3 Sieve analysis (IV)

An automatic sieve shaker (Fritsch analysette, Germany) was used to measure the particle size distributions of the powders using the following sieves: 45, 71, 90, 125, 180, 250, 355 and 500 µm. The sample size was 5 g. A stacked set of the sieves was shaken for 5 min with an amplitude setting value of 6 (n = 3).

4.2.2.4 Scanning electron microscopy (SEM) (I-IV)

The morphological properties of the powders were investigated using SEM (Zeiss DSM 962, Carl Zeiss, Oberkochen, Germany). Before scanning, the samples were coated with platinum using a vacuum evaporator. SEM images were acquired at an acceleration voltage of 10 kV.

4.2.2.5 Atomic force microscopy (AFM) (I, II)

The detailed surface texture of the particles was studied by AFM (Autoprobe CP, Thermomicroscopes, USA) over 5 µm x 5 µm (**I, II**) and 10 µm x 10 µm (**I**) areas. AFM mapping was performed in noncontact (**I**) and contact (**II**) modes. AFM imaging was carried out in ambient conditions using a large area scanner (100 µm lateral scan size). The average roughness parameter (R_a) (AFM User's guide, 1998) for each sample was calculated (n = 5-6).

4.2.2.6 Inverted fluorescence microscopy (IFM) (IV)

In study **IV**, RSP is autofluorescent (Guo et al., 1999) and therefore, IFM (Olympus IX71/IX51, Olympus Optical Co., LTD, Japan) was used to determine the homogeneity of the API surface coating of the carrier particles and to visualize the distribution of API within the binary mixture.

4.2.2.7 Stereomicroscopy (SM) and digital imaging (IV)

SM (Leica M26, Germany) in connection with a digital camera (DC) was used to acquire colour images of prepared powders and tablets at a magnification of 0.63x. The powder samples were photographed using a separate DC (Casio EX-F1, Japan).

4.2.3 Moisture content (I-IV)

An IR moisture analyser (Sartorius MA 100, Sartorius AG, Germany) was used to determine the moisture content of the powders based on loss of weight at 105°C (**I, III, IV**) (n = 3). The measurements of the water activity of the samples (n = 3) were made using an AquaLab water activity meter (AquaLab 3 TE, Decagon Devices, Inc., Washington, DC, USA) (**II-IV**).

4.2.4 Particle packing and flow properties (I, II)

The flow characteristics of powders were measured using a new method for a direct determination of flow rate of very cohesive powders on a small scale (Seppälä et al., 2010) (**I, II**) (Figure 6). The system, which implements the method introduced here, induces up and down movements of the sample holder that are sufficient to discharge poorly flowable powders through the orifice onto the analytical scale. Using the data collected from the scale and other input parameters (e.g. dimensions of the sample holder, elapsed time), a computer calculates the flow rate (mg/s and µl/s) of powder particles (n = 3-5). In study **II**, the samples were equilibrated for 72 hours at different levels of controlled RH (low (26 ± 4%), intermediate (50 ± 2%) and high (70 ± 3%)) to evaluate the effect of RH on the rheological properties of powders.

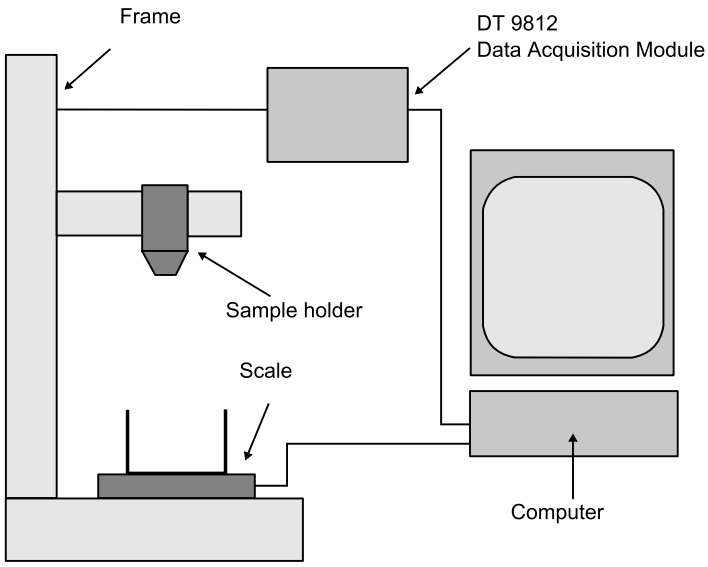

Figure 6 *Block diagram of the powder flow measuring arrangement (Seppälä et al., 2010).*

In study **I**, the angle of repose was measured according to the modified method presented by Zeng et al. (2000a). Powder particles were poured into a copper tube (2.8 cm x 3.15 cm), which had been placed vertically over a flat metal base with a diameter of 3.0 cm. After the powder filled the tube completely, it was slowly raised vertically, leaving a cone of powder. The cones were photographed by a digital camera (Olympus Stylus 820 *I μ* 820, Hamburg, Germany) using super macro mode. Analysis of pictures was done by Matlab software (version 7). The angle of repose was calculated from the tangent (cone height/cone base radius) (n = 3).

To evaluate packing properties of powders, the bulk density was determined from the mass of analysed material occupying 150 ml volume (**I**). The flow rate as a practical property, describing flowability, was determined as the time for a fixed amount of powder (50.0 g) to flow through a glass tunnel with a standard orifice diameter according to the method described in Ph. Eur. 5 (n = 3) (**I**).

4.2.5 Solid-state properties (I-III)

4.2.5.1 X-ray powder diffraction (XRPD) (I, II)

Treated and untreated samples were analysed with a theta-theta X-ray diffractometer (D8 Advance, Bruker AXS GmbH, Karlsruhe, Germany). Measurements were performed in symmetrical reflection mode with Cu Kα radiation (λ=1.54 Å) using a Göbel mirror.

4.2.5.2 Differential scanning calorimetry (DSC) (I, III)

Calorimetric analysis was carried out in a differential scanning calorimeter (Mettler Toledo DSC 823e, Greifensee, Switzerland). The sample sizes were 4.5±0.5 mg (**I**) and 9±1 mg (**I, III**) in 40-µl aluminium pans with two pinholes. The heating rate was 10°C/min, and the cooling rate approximately 70°C/min.

4.2.5.3 Raman spectroscopy (III)

The Raman spectra were collected using a Raman spectrometer (PhAT System^{TM}, RamanRXN1^{TM}, Kaiser Optical System Inc., Ann Arbor, MI, USA) equipped with a fibre-optically coupled PhAT probe head. All Raman spectra were acquired using HoloGram software (HoloPro^{TM}, Kaiser Optical System Inc., Ann Arbor, MI, USA).

4.2.6 Determination of enzyme activity, coating efficiency and stability of enzyme preparations (III)

To measure the enzyme activity in solution, a standardized spectrophotometry method (R-Biopharm, 2010) was used. The standard curve was obtained after incubation of 200 mg of LMH and 200 mg of lactase powder in 100 mL of purified water. To determine the effect of ultrasound-assisted nebulization on β-galactosidase activity, a 1% (m/V) protein solution was prepared, part of the enzyme solution was used as prepared, and another part was atomized and the mist containing β-galactosidase was collected as a fluid. Both solutions were used to prepare intermediate solutions containing 0.005-0.2 g/L of enzyme and 1.9 g/L of lactose. These solutions were incubated for precisely 24 h at 22±1°C and assayed.

To measure the change in the amount of surface-attached β-galactosidase during the coating procedure, 200 mg of coated powder from each sample was dissolved in 100 mL of purified water. The solutions were incubated and analysed. The stability of the β-galactosidase preparation was tested after two weeks and after one month of storage.

To evaluate the potential enzymatic degradation of lactose during the treatment procedure, 200 mg of the final freshly-coated powder was dissolved in 100 mL of purified water and assayed immediately for D-galactose without incubation. The same procedure was conducted with raw LMH, and the results were compared.

4.2.7 Properties of API-coated MCC powder in comparison with physical mixtures of MCC and API (IV)

4.2.7.1 Riboflavin content of the coated powder and physical mixture

In order to determine the coating efficiency of the described procedure, the withdrawn representative samples were analysed for RSP content. Two hundred fifty milligrams of the treated powder from each sample (n = 3) was suspended in 100 mL of water. The absorbance of the filtered solution was measured at 444 nm using a UV-Vis spectrophotometer (Pharmacia Ultrospec III, Pegasus Scientific Inc., Rockville, MD, USA). To generate the standard curve, 200 mg of RSP and 2300 mg of MCC were used to prepare 0.2 mg/mL stock solution of RSP in water.

A physical mixture of RSP and MCC was prepared to compare formulations. To calculate the exact amount of API required for getting a binary mix with the same RSP content as in the coated powder, the latter (250 mg of powder in 100 mL of water, n = 11) was assayed at 444 nm. The total mass of the physical mixture was 310 g. The powder components were placed in the glass jar and the RSP placed in the middle of the MCC powder. The powders were mixed for 30 min with a rotation speed of 46 rpm in a Turbula blender (Willy A. Bachofen AG Maschinenfabrik, Basel, Switzerland). The physical mixture was assessed for content uniformity of RSP (250 mg of powder in 100 mL of water, n = 11) by taking samples from different locations in the blender. The standard deviation (SD) and the relative standard deviation (*Srel*) were calculated.

4.2.7.2 Tabletting and tablet properties

Lubricant-free tablets were compressed from both the coated powder and the physical mixture by using an instrumented single punch tabletting machine (Korsch EK-0, Erweka Apparatebau, Berlin, Germany) equipped with flat-faced 9-mm punches. The compression force used was 2.3 kN for both formulations. The batch size was 950 tablets with a speed of 37 tablets per minute. The device was set up to produce tablets with a target weight of 250 mg and a target crushing strength of 90 N. The same settings were used for the coated powder as for the physically mixed reference powder. The tablets were collected sequentially one by one into the plastic tubes: 100 tablets per tube.

To analyse the homogeneity of mass and content of low-dose forms and observe the differences in these parameters with tabletting time, the first 10 tablets in the series from each sequential tube were weighed individually and assayed at 444 nm using a UV-Vis spectrophotometer (Pharmacia Ultrospec III, Pegasus Scientific Inc., Rockville, MD, USA).

Surface coating of the MCC particles with the API could have an impact on the mechanical properties of tablets prepared from this powder. Therefore, the crushing strength of tablets was measured by using a crushing strength device (Schleuniger-2E, Dr. K. Schleuniger & Co, Germany) (n = 100).

5 Results and discussion

5.1 Moisture content (I-IV)

In studies **I-III**, the treatment by successive steps of ultrasound-assisted spraying of water (**I**), polymer (**II**) or protein (**III**) mist and a brief equilibration of the powder bed did not give any increase in the water content of modified samples. The values for water activity were in good agreement with those for the processing environment. Obviously, the open setup of the ultrasound technique facilitates the evaporation of excessive liquid during processing and, hence, makes an additional drying step unnecessary. It in turn prevents the attrition and breakage of the treated powder, minimizing coating defects (**II, III**). In study **IV**, the water content of the processed powders increased slightly during the coating procedure. However, keeping the treated and untreated materials in ambient conditions for one day equilibrated the moisture content of the powders.

5.2 Morphological properties (I-IV)

5.2.1 Particle size distribution (I-IV)

Permanent binding of particles to each other (as a result of cohesion and adhesion forces, capillary pressure, solid bridges, etc) can take place during the water mist treatment and coating procedure (Maa and Hsu, 1997). This leads to a broader particle size distribution. Agglomerated particles behave as granules and lose their native powder characteristics. Therefore, prevention of particle agglomeration was highly desirable. In all studies (**I-IV**), particle surfaces of powders were modified successfully without granule formation, whereas particle size characteristics remained practically unchanged. A slight increase in the particle size of the processed powders was sometimes observed. This was most likely due to dissolution of fines and their subsequent recrystallization on the surface of the larger species (**I, III**) as well as due to a thin API layer on the surface of the carrier particles (**III, IV**). Besides, loss of dust could take place as a result of the open setup of the technique. However, in study **II**, the values of size descriptors of the surface-treated powders slightly decreased in comparison with the raw material. Obviously, a thin HPMC coat on the surface of individual particles provided a barrier that reduced the cohesiveness of the ibuprofen powder.

5.2.2 Particle surface properties (I-IV)

In study **I**, the surface of treated lactose particles became smoother, whereas the shape of the particles remained almost unchanged (Figure 7). The aqueous mist, applied directly to the powder brought about dissolution of superficial prominences and fine particulates.

Dissolved material was most probably deposited in the pits of bigger particles due to the 'Ostwald ripening' phenomenon (Ferrari et al., 2004), making particle surfaces smoother. For flake-like particles of thiamine, it was difficult to draw clear conclusions based on visual observations of the changes in the surface topography (Figure 7).

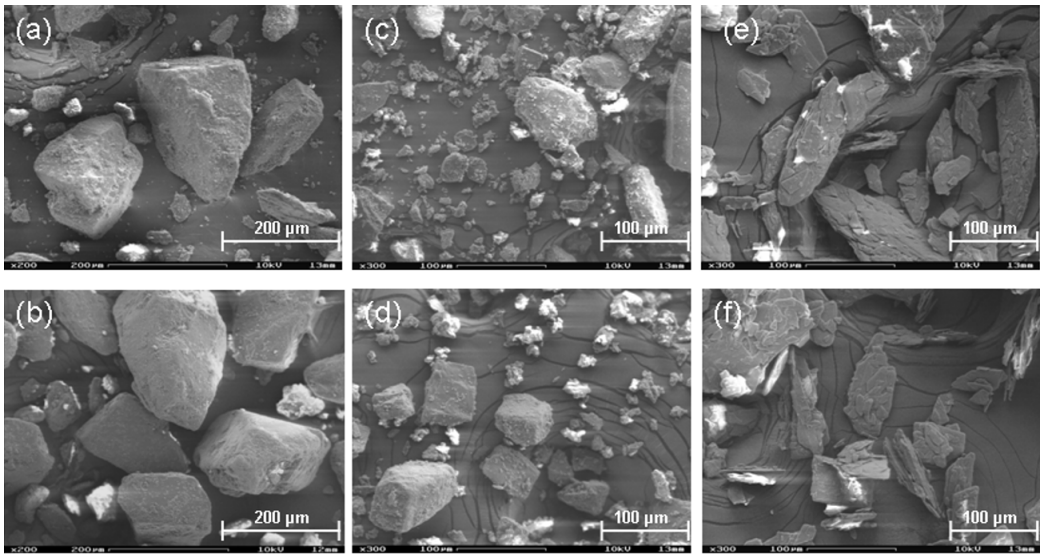

Figure 7 *Scanning electron micrographs of untreated (a) and treated (b) coarser grade lactose monohydrate (LMH); untreated (c) and treated (d) finer grade LMH; untreated (e) and treated (f) thiamine hydrochloride powders.*

Representative AFM images obtained over 5 µm x 5 µm areas (Figure 8) showed a decrease in surface roughness of powder particles after water mist processing. The directly measured R_a parameter confirmed this observation: the decreased values of R_a over 5 µm x 5 µm areas were obtained for all aqueous-mist treated samples.

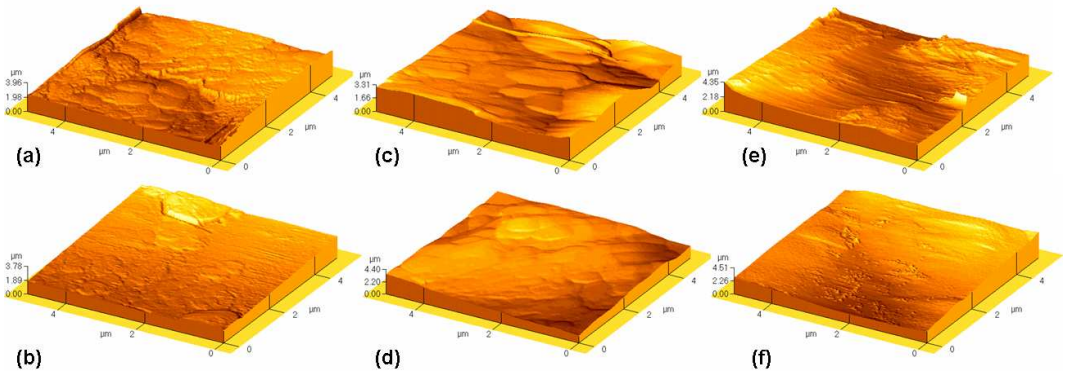

Figure 8 *Representative AFM height images showing particle surface roughness of untreated (a) and treated (b) coarser grade lactose monohydrate (LMH); untreated (c) and treated (d) finer grade LMH; untreated (e) and treated (f) thiamine hydrochloride powders.*

Particle thin-coating gave rise to a relatively smooth polymer layer on the surface of rough ibuprofen species (**II**). During progressive enzyme coating of LMH, the surface of modified lactose particles became smoother as well (**III**). After the last coating cycles, the

surfaces of the carrier particles appeared to be visually smooth (Figure 9). However, using straight visual examinations, it was not possible to identify individual coating layers. Therefore, in study **IV**, the carrier particles were coated with autofluorescent API to observe the coating film on the particle surfaces (Figure 10). In addition, it made it possible to compare distribution of the active substance within the RSP-coated powder with the homogeneity of the reference physical mixture. API coating produced a uniform drug layer on the surface of the carrier particles. The formulation used for direct compression appeared to be a random mixture, where API agglomerates of spherical particles were still observable. The cohesive nature of RSP, which is related to its small particle size, prevented the homogeneous dispersion of API particles within the physical system.

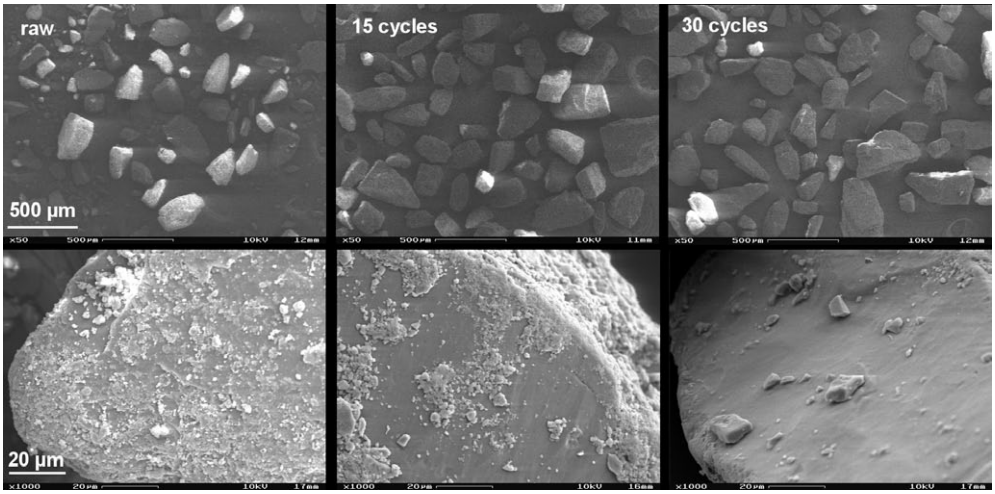

Figure 9 *Scanning electron micrographs of untreated and β-galactosidase-coated lactose monohydrate (LMH) powder after 15 and 30 cycles of treatment.*

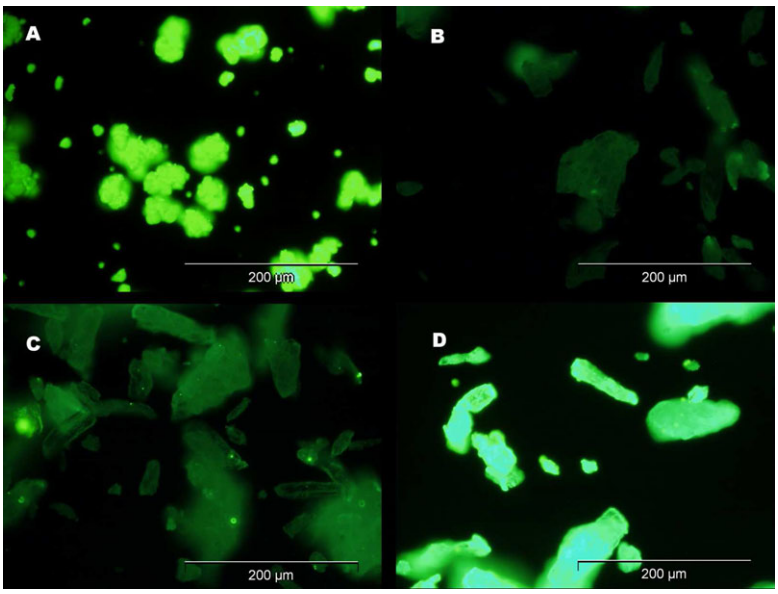

Figure 10 *The inverted fluorescence microscope images of raw riboflavin sodium phosphate (A), microcrystalline cellulose (B), the physical mixture of powders (C) and the surface-coated powder (D).*

5.3 Powder rheological properties (I, II)

5.3.1 Flow and packing properties (I, II)

An aqueous mist treatment at the solid-liquid-air interface was expected to decrease particle surface roughness and cause changes in the particle size range. The smoothing procedure was supposed to reduce the internal friction between particles and improve the flow rate of the powder. Results from the new flowability measurement tool (cf. 4.2.4) revealed that flow rates of surface-treated coarser LMH and thiamine powders increased approximately two fold, whereas the flowability of finer lactose tended to improve slightly less (Table 3). A similar increase in the flow rate of coarser LMH was obtained from the flowability test done according to Ph. Eur. (18.0 ± 1.7 g/s for raw material and 28.3 ± 0.32 g/s for surface-modified substance). The impossibility of applying the last method for poorly-flowing powders highlights the usefulness of the new flowability measurement tool.

Table 3 *Flowability, angle of repose and bulk density values for water mist treated and untreated materials (data are presented as mean ± SD, n = 3).*

Sample	Flowability		Angle of repose (Degrees)	Bulk density
	mg/s	µl/s		g/cm^3
LMH coarser grade, untreated	86.5±7.6	146±11	49.6±0.66	0.766±0.009
LMH coarser grade, treated	174±10*	278±21*	46.5±0.79*	0.787±0.005*
LMH finer grade, untreated	9.30±1.7	23.8±3.7	56.5±2.1	0.505±0.007
LMH finer grade, treated	11.4±1.7	29.3±5.5	54.8±1.9	0.501±0.008
Thiamine·HCl, untreated	6.39±0.61	24.1±2.1	64.9±0.11	0.326±0.004
Thiamine·HCl, treated	12.1±0.66*	41.5±2.3*	62.6±1.8	0.361±0.004*

* $p<0.01$, significant difference compared to untreated substance by Student's unpaired *t*-test

The smaller values of angle of repose for processed powders underlined the fact that treated substances exhibited superior flowability in comparison with the original materials. But the angle of repose was less sensitive to the changes in flow properties of modified powders than the values obtained using the new flowability measurement technique. Only coarser lactose showed statistically significant reductions in the angle values. This could be due to the fact that the angle of repose is determined by the least stable particles (Zeng et al., 2000a), whereas the results from the new tool depend primarily on the bulk properties of the powder bed. Also, more dense packing was observed for processed coarser LMH and thiamine samples. Their poured densities increased significantly.

Improvement in the flow properties of the coarser lactose was probably due to smoothing of surfaces and a decrease in the level of fines with a very slight increment in particle size. The changes in mechanical characteristics of thiamine powder were assumed

to be mainly due to a narrowing of the particle size distribution, whereas the tendency for better flow properties of finer lactose was related to surface and size modifications.

In study **II**, HPMC thin-coating improved the flow characteristics of ibuprofen powder (Figure 11). The higher number of cycles gave a more uniform coating of particle surfaces, as can be seen from the gradual growth in the flow rate. It increased greatly after twenty-five cycles of treatment, whereas further processing did not give any significant improvement. Obviously, the raw surface of ibuprofen particles has already been completely hidden by polymer thin-coat after twenty-five cycles of treatment. The effect of the coating layer on the flow properties of the powder could be explained by the increase in the hydrophilicity of particle surfaces, which reduced electrostatic interactions and thus decreased the cohesive forces between hydrophobic ibuprofen particles. The influence of hydrophilic species on the flow properties of hydrophobic substances was previously reported by other authors (Kawashima et al., 1998a).

5.3.2 The effect of relative humidity on flow properties (II)

It is a well-known fact that moisture has a significant influence on the flow properties of cohesive powders (Forsyth et al., 2002; Faqih et al., 2007). In study **II**, it was found that the flow rate decreased as the water content of the surface-treated powders increased (Figure 11). It is obvious that at high relative humidity the water vapour condensed onto particle surfaces, creating a water sorption layer (Führer, 1996; Nyström and Karehill, 1996). A further increase in relative humidity caused separation of the water phase, which gave rise to capillary forces (Iida et al., 2004a). These liquid interactions held particles together, preventing their flow. The effect of humidity on the flow properties of the modified powder became more noticeable when the uniformity of the HPMC layer increased. The thin-polymer coating made the surface of the ibuprofen particles more hydrophilic, as mentioned above. The hydrophilic surface had more affinity for water molecules and as a consequence became more subject to pronounced capillary effects. As well, the smoother surfaces of HPMC-coated particles could have enhanced hydrostatic forces (Podczeck et al., 1997). At low water activity levels, having less adsorbed water probably acted as a lubricant, facilitating powder flow (Kaerger et al., 2004). Changes in water activity did not have a significant effect on the flowing characteristics of the raw material.

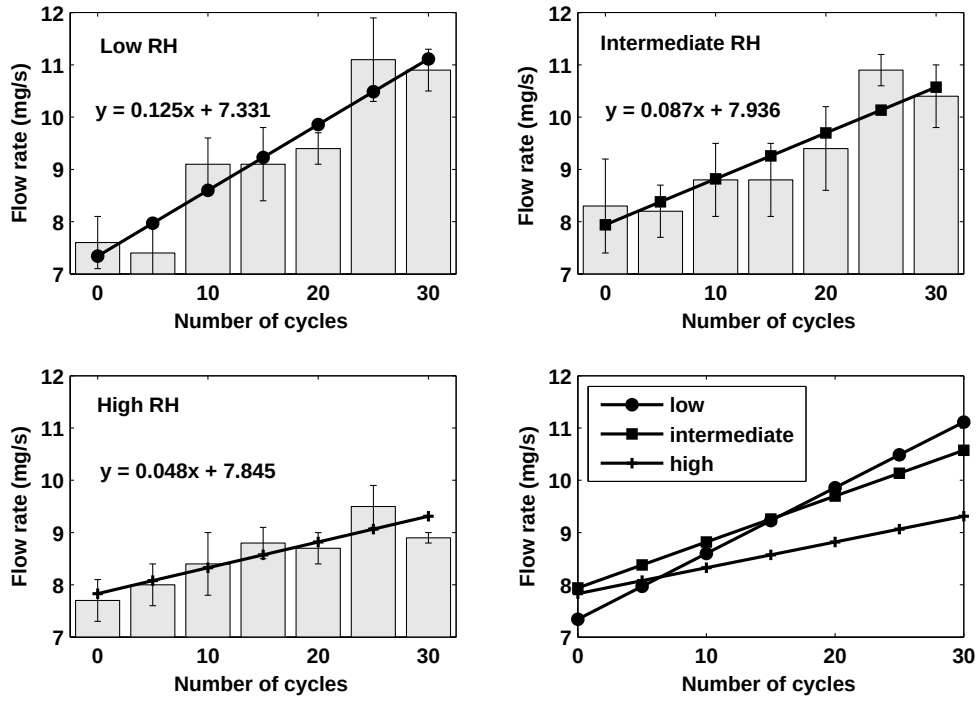

Figure 11 *The influences of the uniformity of the HPMC coating layer and relative humidity on the flow properties of ibuprofen powders (n = 5).*

5.4 API-coating of carrier powder (III, IV)

5.4.1 Protein mist coating (III)

In study **II**, thin-coating of ibuprofen particles with HPMC was performed to reduce the cohesiveness of the highly charged material and improve the flow properties of the powder. However, the increase in the thickness of the coating layer was measured with an indirect method, and quantitative analysis of the particle coat was not performed. Thus, the coating efficiency of our technique needed to be determined. There were several reasons for selecting β-galactosidase as a model substance. Firstly, the stability of the nebulized product needed to be checked, and β-galactosidase is a fragile molecule that can lose its activity very easily. Secondly, a precise method exists for the determination of the substrate/product concentration for the β-galactosidase reaction, and consequently, the amount of enzyme. The method is well standardized since the quantitative estimation of lactose in diary products is a common procedure due to the large number of people with lactose intolerance (Onwulata et al., 1989; Montalto et al., 2005; O'Connell and Walsh, 2006). Thirdly, it can provide proof that the surface-modified powder remained dry if no reaction product appeared, as an enzymatic reaction requires water. The amount of D-

galactose produced in the maximally treated powder in the freshly prepared solution was below the detection limit of the UV-Vis spectrophotometer .

5.4.2 Effect of nebulization on β-galactosidase activity (III)

It is well known that proteins are stress-sensitive bioactive molecules that can undergo irreversible conformational changes with subsequent activity loss (Yoshioka et al., 1993; Alavi et al., 2002; Kuny and Leuenberger, 2003). In our study the ultrasound nebulization did not affect the catalytic activity of lactase (Figure 12). The amount of enzyme needed to cleave a predetermined amount of lactose sugar was the same for the atomized protein as for the commercial protein.

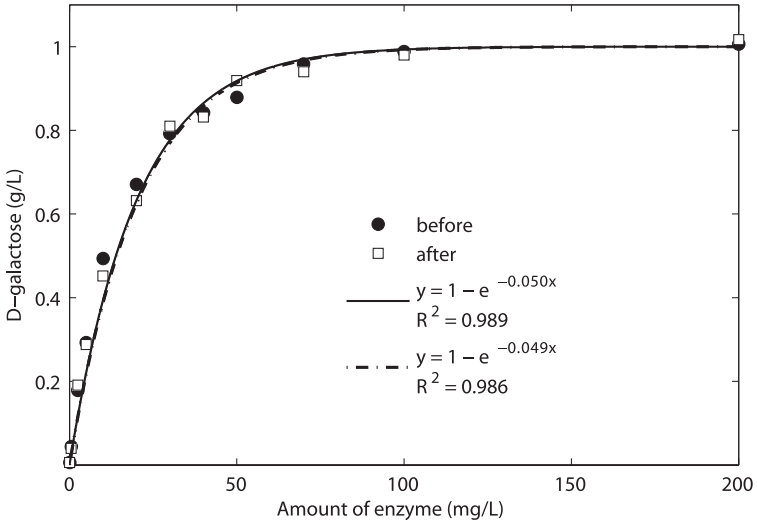

Figure 12 *Retention of β-galactosidase activity before and after nebulization of the enzyme solution. The incubation time of substrate/enzyme solutions is 24 h.*

5.4.3 Determination of API loading (III, IV)

In study **III**, the quantity of β-galactosidase on the lactose particle surface was determined via its reaction product, D-galactose, by UV-Vis spectrophotometry. The enzyme loading increased gradually with the treatment cycles, suggesting a constant increase in the thickness of the coating layer (1st run) (Figure 13). The stability of the enzyme preparation was examined after two weeks and after one month of storage. β-galactosidase tended to remain unchanged in its ability to cleave disaccharide bonds. Furthermore, the repeatability of our technique was tested by conducting an analogous coating experiment (2nd run). The results from the second run did not differ from those of the first. The technique therefore appears to give rise to an enzyme coat in a reproducible way.

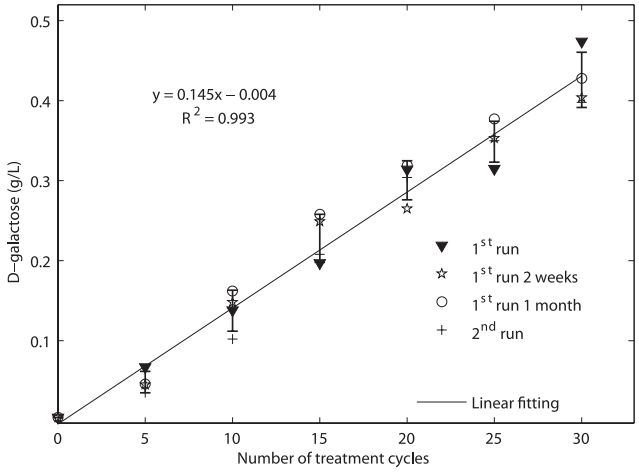

Figure 13 *Coating efficiency, expressed as amount of D-galactose (g/L) produced by a progressively increasing enzyme coat as the number of coating cycles increased (1st run). Stability of the β-galactosidase formulation prepared in the first coating experiment after 2 weeks and after 1 month of storage (1st run) and repeatability of the technique by conducting an analogous coating experiment (2nd run). Error bars: mean ± standard deviation (n = 4).*

The results shown in Figure 12 and Figure 13 allow us to calculate the precise amount of surface-deposited enzyme material using a fitted exponential function. There were on average 0.5 and 5.7 mg of β-galactosidase coat per 1 g of lactose powder after 5 and 30 cycles, respectively. This means that every coating cycle increased the amount of surface-attached enzyme by 0.16±0.03 mg/g of treated LMH.

From the ratio (α) between the weight of the enzyme coat and the weight of lactose powder, assuming that lactose particles were spheres, the thickness of the coating layer can be calculated according to Eq. 3:

$$r_2 = r_1 \times \sqrt[3]{\frac{\alpha \times \rho_1 + \rho_2}{\rho_2}} \qquad \text{(Eq. 3)}$$

where r_1 and r_2 are the radii of uncoated and coated lactose particles, and ρ_1 and ρ_2 are the true densities of pure lactose and enzyme powder, respectively. From the literature, ρ_1 is 1.545 g/cm^3 (Rowe et al., 2009), and ρ_2 is 1.42 g/cm^3 (Kuny and Leuenberger, 2003). The volume median diameter of raw lactose particles was measured to be 226 µm.

The calculated thickness of the β-galactosidase layer was up to 234 nm, increasing nearly linearly with every treatment cycle (Figure 14). The uniform increase in the quantity of the surface-attached protein is important for the homogeneous distribution of the API in the powder.

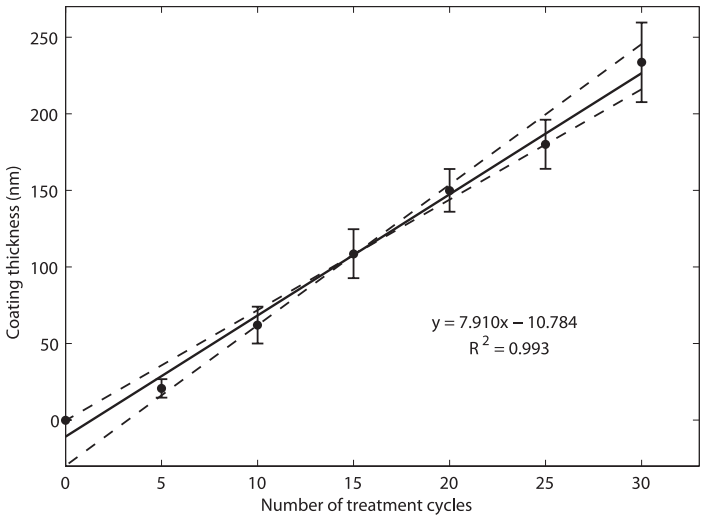

Figure 14 *Calculated thickness of the coating layer. Error bars: mean ± standard deviation, n = 4. The results were fitted within the approximation of error (broken line).*

In study **IV**, RSP coating produced an ultra-thin yellow layer on the surface of the polymer carriers. The colour's intensity increased as the coating proceeded (Figure 15). A quantitative analysis of the surface-treated powders, taken at predetermined intervals during the coating procedure, also revealed a near linear increase in the thickness of the drug layer. This means that the amount of the surface-attached active substance can be controlled easily during the coating process. The results revealed that the coating was homogeneous, as nearly every single particle was treated with a mist of RSP solution, preventing the formation of 'dead zones' in the processed powder.

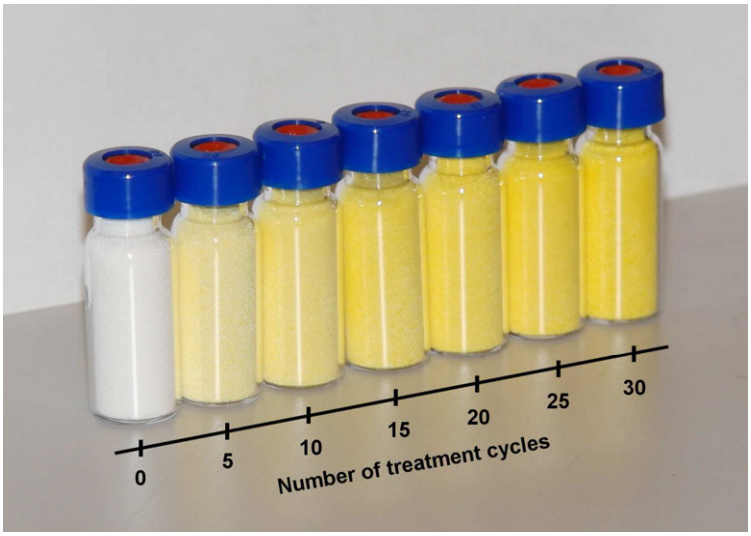

Figure 15 *The increase in the colour intensity of riboflavin sodium phosphate-treated powders as the number of coating cycles increased.*

5.4.4 Content variation of API-coated and physically mixed powders (IV)

API-coating of powdered particles offers some advantages relative to the widely applied mechanical mixing; with the latter method, problems are experienced with demixing due to cohesion of particular components, segregation of powder and formation of "dead spots" inside the blender.

In study **IV**, the average amount of riboflavin was 1.48±0.04 mg per 250 mg of coated powder (*Srel* = 2.8%). This value was used to calculate the amount of riboflavin salt that needed to be added to MCC to get a physical mixture with the same quantity of the API for both formulations. However, we obtained a physical mixture with a lower amount of riboflavin: 1.31±0.08 mg per 250 mg of powder (*Srel* = 5.7%). It appeared that some API stuck to the walls and to the cork of the bottle. This observation, and a higher SD in riboflavin content within the physical mixture in comparison with the coated powder, could indicate that the physical mixture was not completely homogeneous and that areas of high and low drug content were formed.

5.4.5 Weight and content variation of tablets (IV)

Since tablets are the final product and the most convenient solid dosage forms, compacts from API-coated powder and the physical mixture of both components were prepared to evaluate the weight and content uniformity. Tablets from both formulations were successfully compressed without addition of lubricant. API coating of MCC powder did not change the mechanical properties of tablets significantly in comparison with tablets prepared from the physical mixture.

The average mass of the tablets made from the coated powder was slightly higher, indicating denser packing of the powder during tablet compression: 252.4±0.9 mg (*Srel* = 0.4%) for the coated powder and 247.0±1.3 mg (*Srel* = 0.5%) for the physical mixture. The denser arrangement of the solid material could be related to the changes in the surface properties and, consequently, in the bulk density of API-coated MCC particles. The SD and *Srel* in weight variation of compacts from the coated powder were smaller and consequently, the uniformity of weight was higher.

The content variation was clearly less for the surface-treated material, where the drug coating gave rise to a uniform distribution of API within the powder (Figure 16). *Srel* (n = 100) was almost 4 times higher for the directly compressed compacts of the physical mixture (6.5%) than for the tablets prepared from the RSP-coated formulation (1.8%), indicating a smaller long-time scale variation in the latter preparation. Additionally, the short-time scale variation within the first 10 compacts from each subsequent series was better for tablets prepared from the surface-coated powder than for tablets prepared from the physical mix. Obviously, segregation of the physical mixture occurred during tabletting, since a strong vibration of the hopper can provoke separation of the binary formulation. This caused an increase in the content variation of the reference tablets. Visual observation of the tablet surfaces confirmed the above finding (Figure 17).

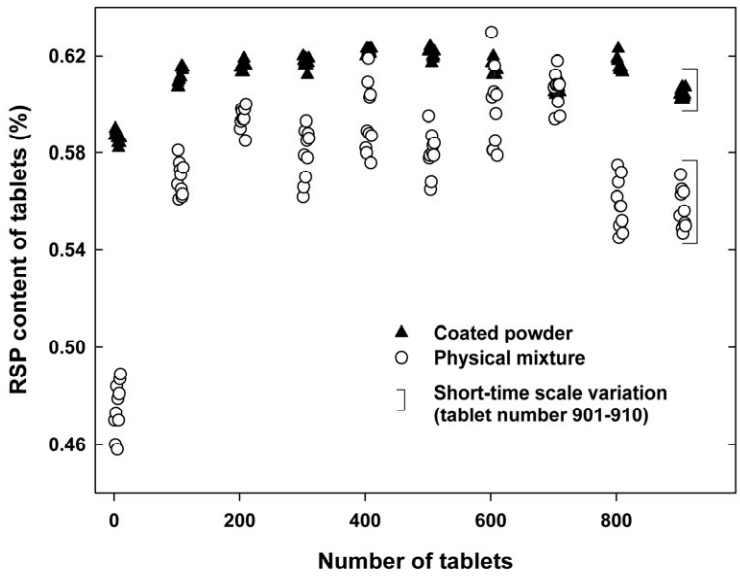

Figure 16 *The content variation of tablets prepared from the coated powder and the physical mixture of powders as a function of the number of tablets compressed (n = 100).*

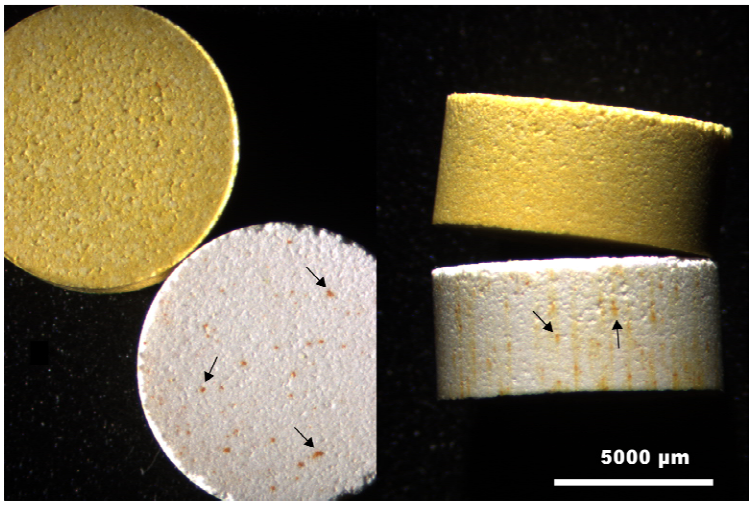

Figure 17 *Stereomicrographs of tablets prepared from the surface-coated powder (yellow tablets) and physical mixture of powders (white tablets with yellow-orange spots). Arrows point to poorly mixed riboflavin sodium phosphate particles (small yellow-orange spots).*

5.5 Solid-state properties (I-III)

XRPD, DSC and Raman spectra analyses were performed in order to determine whether any solid state modifications had occurred on the particle surface of powders. No crystal lattice changes nor the halo patterns were observed after water mist treatment and thin-coating of powder particles.

6 Summary and conclusions

A new ultrasound-assisted technique for modification of particle surfaces of pharmaceutical powders was developed. The proposed method is promising as an effective and repeatable tool for improvement of the physico-technical properties of powders. This technique can be used to dissolve and smooth particle surfaces of water-soluble excipients and drug substances as well as to thin-coat cohesive APIs, giving rise to powders with desired characteristics. Improvement of rheological properties, measured by the novel flowability equipment, may have practical application for the effective processing of powders for DPIs as well as for direct compression of tablets. Furthermore, the introduced method is insensitive to batch size and environmental conditions. This technique can be used in an uninterrupted manner to improve the flow properties of heat, moisture and shear/stress sensitive powders. In addition, it can be applied to freshly milled, highly charged materials to make further unit operations such as granulation, weighing and packing easier and more cost effective.

Uniform deposition of the enzyme β-galactosidase onto lactose particles was carried out by the technique presented in this thesis. During coating, overwetting of the powder mass and activation of the enzyme were avoided, both of which are difficult to achieve using more complex methods such as the fluid bed technique. Determination of the coating efficiency of the surface-treated powder was precisely done using a simple approach. The thickness of the protein layer increased progressively. Such pre-treatment of lactose may be useful for formulating pharmaceutical dosages for patients with lactose intolerance. However, further studies are required to demonstrate the pharmaceutical processability of the functionally coated lactose.

By using the ultrasound-assisted technique, it was possible to make a homogeneous powder formulation of a low-dose API that remained uniform during the entire tabletting process. The API coating produced an even drug layer on the surface of the carrier particles. Tablets prepared from the coated powder showed significantly improved content uniformity in comparison with the tablets compressed from the physical mixture of the powders. The increase in content uniformity of low-dose tablets improves quality of the final dosage forms and consequently, ensures the safe delivery of a potent active substance to patients. However, the duration of treatment by the ultrasound-assisted technique has to be considered when the modification/coating procedure is done several tens of times. Furthermore, the method is not fully optimized to date, and the loss of mist to the environment has to be taken into account.

References

Aaltonen J., Heinänen P., Peltonen L., Kortejärvi H., Tanninen V.P., Christiansen L., Hirvonen J., Yliruusi J., Rantanen J., 2006. In situ measurement of solvent-mediated phase transformation during dissolution testing. J. Pharm. Sci. 95(12), 2730-2737.

Aaltonen J., Kogermann K., Strachan C., Rantanen J., 2007a. In-line monitoring of solid-state transitions during fluidisation. Chem. Eng. Sci. 62(1-2), 408-415.

Aaltonen J., Strachan C., Pöllänen K., Yliruusi J., Rantanen J., 2007b. Hyphenated spectroscopy as a polymorph screening tool. J. Pharm. Biomed. Anal. 44(2), 477-483.

AFM User's guide to autoprobe CP and M5. Park Scientific instruments. Part III: Software Reference 48-101-1101, Rev. A. For PSI ProScan Software Version 1.5, 1998.

Alavi A.K., Squillante III E., Mehta K.A., 2002. Formulation of enterosoluble microparticles for an acid labile protein. J. Pharm. Pharm. Sci. 5(3), 234-244.

Albertini B., Passerini N., Rodriguez L., 2005. Evaluation of ultrasonic atomization as a new approach to prepare ionically cross-linked chitosan microparticles. J. Pharm. Pharmacol. 57(7), 821-829.

Alderborn G., 2002. Tablets and compaction. In: Aulton, M.E. (Ed.), Pharmaceutics: The Science of Dosage Form Design. 2nd edition, Churchill Livingstone Elsevier, 397-440.

am Ende M.T., Moses S.K., Carella A.J., Gadkari R.A., Graul T.W., Otano A.L., Timpano R.J., 2007. Improving the content uniformity of a low-dose tablet formulation through roller compaction optimization. Pharm. Dev. Technol. 12(4), 391-404.

Antikainen, 2003. New methods to evaluate applicability of powders and granules for tablet compression. Ph. D. Thesis, University of Helsinki, Finland.

Ashford M., 2002. Bioavailability–physicochemical and dosage form factors. In: Aulton, M.E. (Ed.), Pharmaceutics: The Science of Dosage Form Design. 2nd edition, Churchill Livingstone Elsevier, 234-252.

Baki G., Bajdik J., Djuric D., Knop K., Kleinebudde P., Pintye-Hódi K., 2010. Role of surface free energy and spreading coefficient in the formulation of active-agent layered pellets. Eur. J. Pharm. Biopharm. 74(2), 324-331.

Bérard V., Lesniewska E., Andrès C., Pertuy D., Laroche C., Pourcelot Y., 2002. Dry powder inhaler: influence of humidity on topology and adhesion studied by AFM. Int. J. Pharm. 232(1-2), 213-224.

Bhattachar S.N., Hedden D.B., Olsofsky A.M., Qu X., Hsieh W.-Y., Canter K.G., 2004. Evaluation of the vibratory feeder method for assessment of powder flow properties. Int. J. Pharm. 269(2), 385-392.

Bolhuis G.K., Chowhan Z.T., 1996. Materials for direct compression. In: Alderborn G., Nysröm C. (Eds.), Pharmaceutical Powder Compaction Technology, Marcel Dekker Inc., New York, 420-500.

Bolhuis G.K., Hölzer, A.W., 1996. Lubricant sensitivity. In: Alderborn G., Nysröm C. (Eds.), Pharmaceutical Powder Compaction Technology, Marcel Dekker, Inc., New York, 517-560.

Branchu S., Forbes R.T., York P., Petrén S., Nyqvist H., Camber O., 1999. Hydroxypropyl-β-cyclodextrin inhibits spray-drying-induced inactivation of β-galactosidase. J. Pharm. Sci. 88(9), 905-911.

Broadhead J., Rouan S.K.E., Hau I., Rhodes C.T., 1994. The effect of process and formulation variables on the properties of spray-dried β-galactosidase. J. Pharm. Pharmacol. 46(6), 458-467.

Brunauer S., Emmett P.H., Teller E., 1938. Adsorption of gases in multimolecular layers. J. Am. Chem. Soc. 60 (2), 309-319.

Buckton G., 1997. Characterization of small changes in the physical properties of powders of significance for dry powder inhaler formulations. Adv. Drug Deliver. Rev. 26(1), 17-27.

Buckton G., 1999. Isothermal microcalorimetry and inverse phase gas chromatography to study small changes in powder surface properties. Int. J. Pharm. 193(1), 13-19.

Buckton G., 2002. Solid-state properties. In: Aulton, M.E. (Ed.), Pharmaceutics: The Science of Dosage Form Design. 2nd edition, Churchill Livingstone Elsevier, 141-151.

Butt H.J., Graf K., Kappl M., editors. Physics and chemistry of interfaces. 2nd edition, WILEY-VCH Verlag GmbH&Co. KGaA, Weinheim, 2006.

Byrn S.R., Sutton P.A., Tobias B., Frye J., Main P., 1988. Crystal structure, solid-state NMR spectra, and oxygen reactivity of five crystal forms of prednisolone tert-butylacetate. J. Am. Chem. Soc. 110(5), 1609-1614.

Carpenter J.F., Pikal M.J., Chang B.S., Randolph T.W., 1997. Rational design of stable lyophilized protein formulations: some practical advice. Pharm. Res. 14(8), 969-975.

Carr R.L., 1965. Evaluating flow properties of solids. Chem. Eng. 18, 163-168.

Cavallari C., Rodriguez L., Albertini B., Passerini N., Rosetti F., Fini A., 2005a. Thermal and fractal analysis of diclofenac/Gelucire 50/13 microparticles obtained by ultrasound-assisted atomization. J. Pharm. Sci. 94 (5), 1124-1134.

Cavallari C., Albertini B., Rodriguez L., Rabasco A.M., Fini A., 2005b. Release of indomethacin from ultrasound dry granules containing lactose-based excipients. J. Control. Release 102 (1), 39-47.

Chan H.-K., Clark A., Gonda I., Mumenthaler M., Hsu C., 1997. Spray dried powders and powder blends of recombinant human deoxyribonuclease (rhDNase) for aerosol delivery. Pharm. Res. 14(4), 431-437.

Chan H.-K., Chew N.Y.K., 2003. Novel alternative methods for the delivery of drugs for the treatment of asthma. Adv. Drug Deliver. Rev. 55(7), 793-805.

Chen Y., Flanagan D., 2009. Theory of diffusion and pharmaceutical applications. In: Qiu Y., Chen Y., Zhang G.G.Z. (Eds.), Developing Solid Oral Dosage Forms: Pharmaceutical Theory and Practice, Academic Press Elsevier, USA, 147-162.

Chiou W.L., Chen S.J., Athanikar N., 1976. Enhancement of dissolution rates of poorly water-soluble drugs by crystallization in aqueous surfactant solutions I: sulfathiazole prednisone and chloramphenicol. J. Pharm. Sci. 65(11), 1702-1704.

Davies R., Schurr G.A., Meenan P., Nelson R.D., Bergna H.E., Brevett C.A.S., Goldbaum R.H., 1998. Engineered particle surfaces. Adv. Mater. 10(15), 1264-1270.

de Haan P., Deurloo M., inventors. Low steroid dose dry pharmaceutical preparation. United States patent US 5382434. 1995 Jan 17.

de Haan P., Thys C.P., inventors. Compressed dry-granulation desogestrel tablets. United States patent US 6063403. 1997 Dec 18.

Dewettinck K., Messens W., Deroo L., Huyghebaert A., 1999. Agglomeration tendency during top-spray fluidized bed coating with gelatin and starch hydrolysate. Lebensm. Wiss. Technol. 32(2), 102-106.

Edge S., Mueller S., Price R., Shur J., 2008. Factors affecting, defining the quality and functionality of excipient used in the manufacture of dry powder inhaler products. Drug Dev. Ind. Pharm. 34(9), 966-973.

Eerikäinen H., Watanabe W., Kauppinen E.I., Ahonen P.P., 2003. Aerosol flow reactor method for synthesis of drug nanoparticles. Eur. J. Pharm. Biopharm. 55(3), 357-360.

Ehlers H., Räikkönen H., Antikainen O., Heinämäki J., Yliruusi J., 2009. Improving flow properties of ibuprofen by fluidized bed particle thin-coating. Int. J. Pharm. 368(1-2), 165-170.

El-Sabawi D., Price R., Edge S., Young P.M., 2006a. Novel temperature controlled surface dissolution of excipient particles for carrier based dry powder inhaler formulations. Drug Dev. Ind. Pharm. 32(2), 243-251.

El-Sabawi D., Price R., Edge S., Young P.M., 2006b. Continued investigation into the influence of loaded dose on the performance of dry powder inhalers: surface smoothing effects. Drug Dev. Ind. Pharm. 32(10), 1135-1138.

Elversson J., Millqvist-Fureby A., 2006. *In situ* coating–An approach for particle modification and encapsulation of proteins during spray-drying. Int. J. Pharm. 323(1-2), 52-63.

European Pharmacopoeia. 5th edition, Council of Europe, Strasbourg, 2005.

Faqih A.M.N., Mehrotra A., Hammond S.V., Muzzio F.J., 2007. Effect of moisture and magnesium stearate concentration on flow properties of cohesive granular materials. Int. J. Pharm. 336(2), 338-345.

Fernández-Arévalo M., Vela M.T., Rabasco A.M., 1990. Rheological study of lactose coated with acrylic resins. Drug Dev. Ind. Pharm. 16(2), 295-313.

Ferrari F., Cocconi D., Bettini R., Giordano F., Santi P., Tobyn M., Price R., Young P., Caramella C., Colombo P., 2004. The surface roughness of lactose particles can be modulated by wet-smoothing using a high-shear mixer. AAPS PharmSciTech. 5(4), e60.

Fini A., Fazio G., Fernández-Hervás M.J, Holgado M.A., Rabasco, A.M., 1996. Fractal analysis of sodium cholate particles. J. Pharm. Sci. 85(9), 971-975.

Fini A., Rodriguez L., Cavallari C., Albertini, B., Passerini N., 2002. Ultrasound-compacted and spray-congealed indomethacin/polyethyleneglycol systems. Int. J. Pharm. 247(1-2), 11-22.

Forsyth A.J., Hutton S., Rhodes M.J., 2002. Effect of cohesive interparticle force on the flow characteristics of granular material. Powder Technol. 126 (2), 150-154.

Freeman T., 2010. The importance of powder characterization. Pharm. Technol. Eur. 22(6).

Führer C., 1996. Interparticulate attraction mechanisms. In: Alderborn G., Nysröm C. (Eds.), Pharmaceutical Powder Compaction Technology, Marcel Dekker, Inc., New York, 1-15.

Geldart D., Abdullan E.C., Hassanpour A., Nwoke L.C., Wouters I., 2006. Characterization of powder flowability using measurement of angle of repose. China Part. 4(3-4), 104-107.

Giron D., Goldbronn C., Mutz M., Pfeffer S., Piechon P., Schwab P., 2002. Solid state characterization of pharmaceutical hydrates. J. Therm. Anal. Calorim. 68(2), 453-465.

Grant D.J.W., Higuchi T., 1990. Solubility behaviour of organic compounds. In: Saunders W.H. (Ed.), Techniques of Chemistry, Vol. 21, John Wiley & Sons, New York, 12-88.

Grant D.J.W., 1999. Theory and origin of polymorphism. In: Brittain H. (Ed.), Polymorphism in Pharmaceutical Solids, Marcel Dekker, Inc., New York, 1-33.

Grawe D., Hoesel P., Moellmann P., Timpe C., Dittgen M., Matthey K., inventors. Homogeneous preformulations containing high concentrations of steroids, for producing low-dose solid and semi-solid pharmaceutical preparations. United States patent US 6290931B1. 2001 Sep 18.

Greaves F.C., Beasley M.W., Suddith A.W., Swarbrick J., 1995. Novel approaches to the preparation of low-dose solid dosage forms. Pharm. Technol. 188, 60-64.

Guillory J.K., 1999. Generation of polymorphs, hydrates, solvates, and amorphous solids. In: Brittain H. (Ed.), Polymorphism in Pharmaceutical Solids, Marcel Dekker, Inc., New York, 183-226.

Guo H.X., Heinämäki J., Yliruusi J., 1999. Characterization of particle deformation during compression measured by confocal laser scanning microscopy. Int. J. Pharm. 186(2), 99-108.

Hammouda Y.E., El-Khordagui L.K., Darwish I.A., El-Kamel A.H., 1999. Manipulation of powder characteristics by interactions at the solid-liquid interface: 1-sulphadiazine. Eur. J. Pharm. Sci. 8 (4), 283-290.

Heng P.W.S., Chan L.W., 1997. Drug substance and excipient characterization. In: Parikh D.M. (Ed.), Handbook of Pharmaceutical Granulation Technology, Marcel Dekker, Inc., New York, 25-57.

Hersey J.A., 1975. Ordered mixing: a new concept in powder mixing practice. Powder Technol. 11(1), 41-44.

Hirsjärvi S., 2008. Preparation and characterization of poly(lactic acid) nanoparticles for pharmaceutical use. Ph. D. Thesis, University of Helsinki, Finland.

Holgado M.A., Fernández-Hervás M.J., Fernández-Arévalo M., Rabasco A.M., 1995. Use of fractal dimensions in the study of excipients: application to the characterization of modified lactoses. Int. J. Pharm. 121(2), 187-193.

Hoppu P., 2008. Characterization and processing of amorphous binary mixtures with low glass transition temperature. Ph. D. Thesis, University of Helsinki, Finland.

Hoppu P., Grönroos A., Schantz S., Juppo, A.M., 2009a. New processing technique for viscous amorphous materials and characterization of their stickiness and deformability. Eur. J. Pharm. Biopharm. 72(1), 183-188.

Hoppu P., Virpioja J., Schantz S., Juppo, A.M., 2009b. Characterization of ultrasound extrudated and cut citric acid/paracetamol blends. J. Pharm. Sci. 98(6), 2140-2148.

Iida K., Hayakawa Y., Okamoto H., Danjo K., Leuenberger H., 2003a. Preparation of dry powder inhalation by surface treatment of lactose carrier particles. Chem. Pharm. Bull. 51(1), 1-5.

Iida K., Hayakawa Y., Okamoto H., Danjo K., Luenberger H., 2003b. Effect of surface covering of lactose carrier particles on dry powder inhalation properties of salbutamol sulfate. Chem. Pharm. Bull. 51(12), 1455-1457.

Iida K., Hayakawa Y., Okamoto H., Danjo K., Luenberger H., 2004a. Influence of storage humidity on the *in vitro* inhalation properties of salbutamol sulfate dry powder with surface covered lactose carrier. Chem. Pharm. Bull. 52(4), 444-446.

Iida K., Inagaki Y., Todo, H., Okamoto H., Danjo K., Luenberger H., 2004b. Effect of surface processing of lactose carrier particles on dry powder inhalation properties of salbutamol sulfate. Chem. Pharm. Bull. 52(8), 938-942.

Iida K., Hayakawa Y., Okamoto H., Danjo K., Luenberger H., 2004c. Effect of surface layering time of lactose carrier particles on dry powder inhalation properties of salbutamol sulphate. Chem. Pharm. Bull. 52(3), 350-353.

Iida K., Todo H., Okamoto H., Danjo K., Leuenberger H., 2005. Preparation of dry powder inhalation with lactose carrier particles surface-coated using a Wurster fluidized bed. Chem. Pharm. Bull. 53(4), 431-434.

Islam N., Stewart P., Larson I., Hartley P., 2004. Lactose surface modification by decantation: are drug-fine lactose ratios the key to better dispersion of salmeterol xinafoate from lactose-interactive mixtures? Pharm. Res. 21(3), 492-499.

Jiang Y., Matsusaka S., Masuda H., Qian Y., 2009. Development of measurement system for powder flowability based on vibrating capillary method. Powder Technol. 188(3), 242-247.

Jivraj M., Martini L.G., Thomson C.M., 2000. An overview of the different excipients useful for the direct compression of tablets. Pharm. Sci. Technol. Today. 3(2), 58-63.

Jono K., Ichikawa H., Miyamoto M., Fukumori Y., 2000. A review of particulate design for pharmaceutical powders and their production by spouted bed coating. Powder Technol. 113(3), 268-277.

Kaerger J.S., Edge S., Price R., 2004. Influence of particle size and shape on flowability and compactibility of binary mixtures of paracetamol and microcrystalline cellulose. Eur. J. Pharm. Sci. 22(2-3), 173-179.

Kaerger J.S., Price R., 2004. Processing of spherical crystalline particles via a novel solution atomization and crystallization by sonication (SAXS) technique. Pharm. Res. 21(2), 372-381.

Kahela P., Aaltonen R., Lewing E., Anttila M., Kristofferson E., 1983. Pharmacokinetics and dissolution of two crystalline forms of carbamazepine. Int. J. Pharm. 14(1), 103-112.

Kawashima Y., Serigano T., Hino T., Yamamoto H., Takeuchi H., 1998a. Design of inhalation dry powder of pranlukast hydrate to improve dispersibility by the surface modification with light anhydrous silicic acid (AEROSIL 200). Int. J. Pharm. 173(1-2), 243-251.

Kawashima Y., Serigano T., Hino T., Yamamoto H., Takeuchi H., 1998b. Effect of surface morphology of carrier lactose on dry powder inhalation property of pranlukast hydrate. Int. J. Pharm. 172(1-2), 179-188.

Kawashima Y., Serigano T., Hino T., Yamamoto H., Takeuchi H., 1998c. A new powder design method to improve inhalation efficiency of pranlukast hydrate dry powder aerosols by surface modification with hydroxypropylmethylcellulose phthalate nanospheres. Pharm. Res.15(11), 1748-1752.

Kogermann K., Zeitler J.A., Rantanen J., Rades T., Taday P.F., Pepper M., Heinämäki J., Strachan C.J., 2007. Investigating dehydration from compacts using terahertz pulsed, Raman and near-infrared spectroscopy. Appl. Spectrosc. 61(12), 1265-1274.

Kumon M., Suzuki M., Kusai A., Yonemochi E., Terada K., 2006. Novel approach to DPI carrier lactose with mechanofusion process with additives and evaluation by IGC. Chem. Pharm. Bull. 54(11), 1508-1514.

Kuny T., Leuenberger H., 2003. Compression behaviour of the enzyme β-galactosidase and its mixture with microcrystalline cellulose. Int. J. Pharm. 260(1), 137-147.

Laitinen N., Antikainen O., Yliruusi J., 2002. Does a powder surface contain all necessary information for particle size distribution analysis? Eur. J. Pharm. Sci. 17(4-5), 217-227.

Laitinen N., 2003. Opening new perspectives for visual characterization of pharmaceutical solids. Ph. D. Thesis, University of Helsinki, Finland.

Laitinen N., Antikainen O., Rantanen J., Yliruusi J., 2004. New perspectives for visual characterization of pharmaceutical solids. J. Pharm. Sci. 93(1), 165-176.

Lakio S., 2010. Towards real-time understanding of processes in pharmaceutical powder technology. Ph. D. Thesis, University of Helsinki, Finland.

Larhrib H., Martin G.P., Marriott C., Prime D., 2003. The influence of carrier and drug morphology on drug delivery from dry powder formulations. Int. J. Pharm. 257(1-2), 283-296.

Lerner L.J., inventor. Modified sequential oral contraceptive. United States patent US 3568828. 1971 Mar 9.

Levina M., Rubinstein M.H., 2000. The effect of ultrasonic vibration on the compaction characteristics of paracetamol. J. Pharm. Sci. 89(6), 705-723.

Levina M., Rubinstein M. H., 2002. The effect of ultrasonic vibration on the compaction characteristics of ibuprofen. Drug Dev. Ind. Pharm. 28(5), 495-514.

Li T., and Park K., 1998. Fractal analysis of pharmaceutical particles by atomic force microscopy. Pharm. Res. 15(8), 1222-1232.

Lindberg N.-O., Pålsson M., Pihl A.-C., Freeman R., Freeman T., Zetzener H., Enstad G., 2004. Flowability measurements of pharmaceutical powder mixtures with poor flow using five different techniques. Drug Dev. Ind. Pharm. 30(7), 785-791.

Liu L.X., Marziano I., Bentham A.C., Litster J.D., White E.T., Howes T., 2008. Effect of particle properties on the flowability of ibuprofen powders. Int. J. Pharm. 362(1-2), 109-117.

Lucas P., Anderson K., Staniforth J.N., 1998. Protein deposition from dry powder inhalers: fine particle multiplets as performance modifiers. Pharm. Res. 15(4), 562-569.

Luo Y., Zhu J., Ma Y., Zhang H., 2008. Dry coating, a novel coating technology for solid pharmaceutical dosage forms. Int. J. Pharm. 358(1-2), 16-22.

Maa Y.-F, Nguyen P.-A., Hsu C.C., 1996. Spray-coating of rhDNase on lactose: effect of system design, operational parameters and protein formulation. Int. J. Pharm. 144(1), 47-59.

Maa Y.-F., Hsu C.C., 1997. Feasibility of protein spray coating using a fluid-bed Würster processor. Biotechnol. Bioeng. 53(6), 560-566.

Maa Y.-F., Nguyen P.-A.T., Hsu S.W., 1998. Spray-drying of air-liquid interface sensitive recombinant human growth hormone. J. Pharm. Sci. 87(2), 152-159.

Maa Y.-F., Nguyen P.-A., Sweeney T., Shire S.J., Hsu C.C., 1999. Protein inhalation powders: spray drying vs spray freeze drying. Pharm. Res. 16(2), 249-254.

Maa Y.-F., Ameri M., Rigney R., Payne L.G., Chen D., 2004. Spray-coating for biopharmaceutical powder formulations: beyond the conventional scale and its application. Pharm. Res. 21(3), 515-523.

Mahlin D., Berggren J., Gelius U., Engström S., Alderborn G., 2006. The influence of PVP incorporation on moisture-induced surface crystallization of amorphous spray-dried lactose particles. Int. J. Pharm. 321(1-2), 78-85.

Manning M.C., Patel K., Borchardt R.T., 1989. Stability of protein pharmaceuticals. Pharm. Res. 6(11), 903-918.

Martinez L., Tchoreloff P., Leclerc B., Couarraze G., 2001. Active layering and direct compression of sugar spheres: content homogeneity in low-dosage tablets. Pharm. Technol. Eur. 13(10), 1-5.

Méndez-Arriaga F., Torres-Palma R.A., Pétrier C., Esplugas S., Gimenez J., Pulgarin C., 2008. Ultrasonic treatment of water contaminated with ibuprofen. Water Res. 42(16), 4243-4248.

Michoel A., Verlinden W., Rombaut P., Kinget R., De Smet P., 1988. Carrier granulation: a new procedure for the production of low-dosage forms. Pharm. Technol. 12(6), 66-84.

Mirza S., Miroshnyk I., Heinämäki J., Rantanen J., Antikainen O., Vuorela P., Yliruusi J., 2008. Hydroxypropyl methylcellulose-controlled crystallization of erytromycin A dihydrate crystals with modified morphology. Cryst. Growth Des. 8(10), 3526-3531.

Montalto M., Nucera G., Santoro L., Curigliano V., Vastola M., Covino M., Manna R., Gasbarrini A., Gasbarrini G., 2005. Effect of exogenous β-galactosidase in patients with lactose malabsorption and intolerance: a crossover double-blind placebo-controlled study. Eur. J. Clin. Nutr. 59(4), 489-493.

Mumenthaler M., Hsu C.C., Pearlman R., 1994. Feasibility study on spray-drying protein pharmaceuticals: recombinant human growth hormone and tissue-type plasminogen activator. Pharm. Res. 11(1), 12-20.

Muster T.H., Prestidge C.A., 2002. Face specific surface properties of pharmaceutical crystals. J. Pharm. Sci. 91(6), 1432-1444.

Müller C., 1996. Viscoelastic models. In: Alderborn G., Nysröm C. (Eds.), Pharmaceutical Powder Compaction Technology, Marcel Dekker, Inc., New York, 99-132.

Nichols G., Frampton C.S., 1998. Physicochemical characterization of the orthorhombic polymorph of paracetamol crystallized from solution. J. Pharm. Sci. 87(6), 684-693.

Nikolakakis I., Newton J.M., Malamataris S., 2002. Solid state 'adsorption' of fine antibiotic powders onto sorbitol: effects of particle size, state of sorbed water and surface free energy characteristics. Eur. J. Pharm. Sci. 17(4-5), 229-238.

Niven R.W., Prestrelski S.J., Treuheit M.J., Ip A.Y., Arakawa T., 1996. Protein nebulization II. Stabilization of G-CSF to air-jet nebulization and the role of protectants. Int. J. Pharm. 127(2), 191-201.

Nobs L., Buchegger F., Gurny R., Allémann E., 2003. Surface modification of poly(lactic acid) nanoparticles by covalent attachment of thiol groups by means of three methods. Int. J. Pharm. 250(2), 327-337.

Nyström C., Karehill P.-G., 1996. The importance of intermolecular bonding forces and the concept of bonding surface area. In: Alderborn G., Nysröm C. (Eds.), Pharmaceutical Powder Compaction Technology, Marcel Dekker, Inc., New York, 17-53.

O'Connell S., Walsh G., 2006. Physicochemical characteristics of commercial lactases relevant to their application in the alleviation of lactose intolerance. Appl. Biochem. Biotech. 134(2), 179-191.

Olivier J.-C., Vauthier C., Taverna M., Ferrier D., Couvreur P., 1995. Preparation and characterization of biodegradable poly(isobutylcyano acrylate) nanoparticles with the surface modified by the adsorption of proteins. Colloid. Surface B. 4(6), 349-356.

Omron-healthcare. Instruction manuals, UltraAir nebuliser NE U-17, 2010. http://www.omron-healthcare.com/export/sites/default/_global/RespiratoryTherapy/InstructionManualsNebuliser/IM-_NE-U17-E_2.0-EN.pdf (Accessed June 13th, 2010).

Onwulata C.I., Rao D.R., Vankineni P., 1989. Relative efficiency of yogurt, sweet acidophilus milk, hydrolyzed-lactose milk, and a commercial lactase tablet in alleviating lactose maldigestion. Am. J. Clin. Nutr. 49, 1233-1237.

Parikh D.M., 1997. Introduction. In: Parikh D.M.(Ed.), Handbook of Pharmaceutical Granulation Technology, Marcel Dekker, Inc., New York, 1-5.ˇ

Passerini N., Albertini B., Gonzáles-Rodríguez M., Cavallari C., Rodriguez L., 2002a. Preparation and characterization of ibuprofen-poloxamer 188 granules obtained by melt granulation. Eur. J. Pharm. Sci. 15 (1), 71-78.

Passerini N., Perissutti B., Moneghini M., Voinovich D., Albertini B., Cavallari C., Rodriguez, L., 2002b. Characterization of carbamazepine-Gelucire 50/13 microparticles prepared by a spray-congealing process using ultrasounds. J. Pharm. Sci. 91(3), 699-707.

Passerini N., Perissutti B., Albertini B., Voinovich D., Moneghini M., Rodriguez L., 2003. Controlled release of verapamil hydrochloride from waxy microparticles prepared by spray-congealing. J. Control. Release. 88(2), 263-275.

Passerini N., Albertini B., Perissutti B., Rodriguez L., 2006. Evaluation of melt granulation and ultrasonic spray congealing as techniques to enhance the dissolution of praziquantel. Int. J. Pharm. 318 (1-2), 92-102.

Petrak D., 2002. Simultaneous measurement of particle size and particle velocity by the spatial filtering technique. Part. Part. Syst. Charact. 19(6), 391-400.

Pfeffer R., Dave R.N., Wei D., Ramlakhan M., 2001. Synthesis of engineered particulates with tailored properties using dry particle coating. Powder Technol. 117(1-2), 40-67.

Pitchayajittipong C., Shur C., Price R., 2009. Engineering of crystalline combination inhalation of a long-acting beta(2)-agonist and a corticosteroid. Pharm. Res. 26(12), 2657-2666.

Podczeck F., Newton J.M., James M.B., 1997. Variations in the adhesion force between a drug and carrier particles as a result of changes in the relative humidity of the air. Int. J. Pharm. 149(2), 151-160.

Podczeck F., 1998. The relationship between physical properties of lactose monohydrate and the aerodynamic behaviour of adhered drug particles. Int. J. Pharm. 160(1), 119-130.

Prescott J.K., Hossfeld R.J., 1994. Maintaining product uniformity and uninterrupted flow to direct compression tablet presses. Pharm. Technol. 18(6), 99-114.

Price R., Young P.M., Edge S., Staniforth J.N., 2002. The influence of relative humidity on particulate interactions carrier-based dry powder inhaler formulations. Int. J. Pharm. 246(1-2), 47-59.

Provder T., Kunz B., 1996. Application of profilometry and fractal analysis to the characterization of coating surface roughness. Prog. Org. Coat. 27(1-4), 219-226.

Quesada-Peñate I., Julcour-Lebigue C., Jáuregui-Haza U.-J., Wilhelm A.-M., Delmas H., 2009. Sonolysis of levodopa and paracetamol in aqueous solutions. Ultrason. Sonochem. 16(5), 610-616.

R-Biopharm. Lactose/D-galactose, 2010. http://www.r-biopharm.com (Accessed February 5th, 2010).

Ragnarsson G., 1996. Force-displacement and network measurements. In: Alderborn G., Nysröm C. (Eds.), Pharmaceutical Powder Compaction Technology, Marcel Dekker, Inc., New York, 77-97.

Ramadan A., El-Massik M., El-Khordagui L., Daabis N., Hammouda Y., 2006. Surface treatment: a potential approach for enhancement of solid-state photostability. Int. J. Pharm. 307(2),141-149.

Rasenack N., Müller B.W., 2002. Ibuprofen crystals with optimized properties. Int. J. Pharm. 245(1-2), 9-24.

Remenar J.F., Morissette S.L., Peterson M.L., Moulton B., MacPhee J.M., Guzmán H.R., Almarsson Ö., 2003. Crystal engineering of novel cocrystals of a triazole drug with 1,4-dicarboxylic acids. J. Am. Chem. Soc. 125(28), 8456-8457.

Rios M., 2006. Developments in powder flow testing. Pharm. Technol. Eur. 30, 38-49.

Rodriguez L., Passerini N., Cavallari C., Cini M., Sancin P., Fini A., 1999. Description and preliminary evaluation of a new ultrasonic atomizer for spray-congealing processes. Int. J. Pharm. 183(2), 133-143.

Rodríguez-Hornedo N., Kelly R.C., Sinclair B.D., Miller J.M., 2007. Crystallization: general principals and significance on product development. In: Swarbrick J. (Ed.). Encyclopedia of Pharmaceutical Technology, Informa Healthcare USA, Inc., New York, 834-854.

Rowe R.C., Sheskey P.J., Quinn M.E., editors. Handbook of Pharmaceutical Excipients. 6th edition, Pharmaceutical Press, London, 2009.

Ruecroft G., Hipkiss D., Ly T., Maxted N., Cains P.W., 2005. Sonocrystallization: the use of ultrasound for improved industrial crystallization. Org. Process Res. Dev. 9(6), 923-932.

Ruecroft G., 2007. Sonocrystallisation to the rescue. Innovations in Pharmaceutical Technology. 22, 74-76.

Räsänen E., Antikainen O., Yliruusi, J., 2003. A new method to predict flowability using a microscale fluid bed. AAPS PharmSciTech. 4(4), E53.

Römer M., 2008. Investigating physical properties of solid dosage forms during pharmaceutical processing. Process analytical applications of vibrational spectroscopy. Ph. D. Thesis, University of Helsinki, Finland.

Römer M., Heinämäki J., Miroshnyk I., Kivikero N., Sandler N., Rantanen J., Yliruusi J., 2008. Phase transformation of erythromycin A dehydrate during fluid bed drying. J. Pharm. Sci. 97(9), 4020-4029.

Sam M.T., Gayathri D.S., Prasanth V.V., Vinod B., 2008. NSAIDs as microspheres. Internet J. Pharmacol. 6(1). http://www.ispub.com (Accessed June 17th, 2010).

Schiavone H., Palakodaty S., Clark A., York P., Tzannis S.T., 2004. Evaluation of SCF-engineered particle-based lactose blends in passive dry powder inhalers. Int. J. Pharm. 281(1-2), 55-66.

Seitavuopio P., 2006. The roughness and imaging characterization of different pharmaceutical surfaces. Ph.D. Thesis, University of Helsinki, Finland.

Seppälä K., Heinämäki J., Hatara J., Seppälä L., Yliruusi J., 2010. Development of a new method to get a reliable powder flow characteristics using only 1 to 2 g of powder. AAPS PharmSciTech. 11(1), 402-408.

Shaw T.R.D., Carless J.E., 1974. The effect of particle size on the absorption of digoxin. Eur. J. Clin. Pharmacol. 7(4), 269-273.

Shinohara K., 1997. Fundamental and rheological properties of powders. In: Fayed M.E., Otten L. (Eds.), Handbook of Powder Science & Technology, Chapman & Hall, New York, 96-145.

Shur J., Harris H., Jones M.D., Kaerger J.S., Price R, 2008. The role of fines in the modification of the fluidization and dispersion mechanism within dry powder inhaler formulations. Pharm. Res. 25(7), 1631-1640.

Staniforth J., 2002. Powder flow. In: Aulton M.E. (Ed.), Pharmaceutics: The Science of Dosage Form Design, 2nd edition, Churchill Livingstone Elsevier, 197-210.

Steckel H., Markefka P., teWierik H., Kammelar R., 2004. Functionality testing of inhalation grade lactose. Eur. J. Pharm. Biopharm. 57(3), 495-505.

Steckel H., Markefka P., teWierik H., Kammelar R., 2006. Effect of milling and sieving on functionality of dry powder inhalation products. Int. J. Pharm. 309(1-2), 51-59.

Steele G., 2009. Preformulation as an aid to product design in early drug development. In: Gibson M. (Ed.), Pharmaceutical Preformulation and Formulation, 2nd edition, Informa Healthcare USA, Inc., New York, 188-246.

Ståhl K., Claesson M., Lilliehorn P., Lindén H., Bäckström K., 2002. The effect of process variables on the degradation and physical properties of spray dried insulin intended for inhalation. Int. J. Pharm. 233(1-2), 227-237.

Sun C., Grant D.J.W., 2001. Influence of crystal structure in the tableting properties of sulfamerazine polymorphs. Pharm. Res. 18(3), 274-280.

Tang E.S.K., Wang L., Liew C.V., Chan L.W., Heng P.W.S., 2008. Drying efficiency and particle movement in coating–Impact on particle agglomeration and yield. Int. J. Pharm. 350(1-2), 172-180.

Taylor K., 2002. Pulmonary drug delivery. In: Aulton, M.E. (Ed.), Pharmaceutics: The Science of Dosage Form Design. 2nd edition, Churchill Livingstone Elsevier, 473-488.

Thalberg K., Lindholm D., Axelsson A., 2004. Comparison of different flowability test for powders for inhalation. Powder Technol. 146(3), 206-213.

Thiel W.J., Nguyen L.T., 1982. Fluidized bed granulation of an ordered powder mixtures. J. Pharm. Pharmacol. 34(11), 692-699.

Thiel W.J., Nguyen L.T., 1984. Fluidized bed film coating of an ordered powder mixture to produce microencapsulated order units. J. Pharm. Pharmacol. 36(3), 145-152.

Thiel W.J., Nguyen L.T., Sberna F.J., 1986. Content uniformity of microdose tablets (dosage 1 microgram-10 mg) produced by fluid bed granulation of interactive mixtures. J. Pharm. Pharmacol. 38(5), 335-343.

Thiel W.J., Sberna F.J., 1986. Fluidized bed film coating of an interactive powder mixture to produce microencapsulated 2-5 microns particles. J. Pharm. Pharmacol. 38(3), 166-171.

Trimaille T., Pichot C., Delair T., 2003. Surface functionalization of poly(D,L-lactic acid) nanoparticles with poly(ethylenimine) and plasmid DNA by the layer-by-layer approach. Colloid. Surface A. 221(1-3), 39-48.

Visser J., 1989. Van der Waals and other cohesive forces affecting powder fluidization. Powder Technol. 58(1), 1-10.

Walker G.M., Bell S.E.J., Andrews G., Jones D., 2007. Co-melt fluidised bed granulation of pharmaceutical powders: improvements in drug bioavailability. Chem. Eng. Sci. 62(1-2), 451-462.

Wan L.S.C., Heng P.W.S., Muhuri G., 1992. Incorporation and distribution of a low dose drug in granules. Int. J. Pharm. 88(1-3), 159-163.

Wells J., 2002. Pharmaceutical preformulation: the physicochemical properties of drug substances. In: Aulton, M.E. (Ed.), Pharmaceutics: The Science of Dosage Form Design. 2nd edition, Churchill Livingstone Elsevier, 113-138.

Wu L.-S., Pang J., Chen J.-G., Hussain M.A., 2000. Dry blending process scale-up for a very low dose drug candidate. AAPS PharmSciTech. 1(3), article TN2.

York P., 1981. Analysis of moisture sorption hysteresis in hard gelatine capsules, maize starch, and maize starch: drug powder mixtures. J. Pharm. Pharmacol. 33(5), 269-273.

Yoshioka S., Aso Y., Izutsu K., Terao T., 1993. Aggregates formed during storage of β-galactosidase in solution and in the freeze-dried state. Pharm. Res. 10(5), 687-691.

Young P.M., Cocconi D., Colombo P., Bettini R., Price R., Steele D.F., Tobyn M.J., 2002. Characterization of a surface modified dry powder inhalation carrier prepared by "particle smoothing". J. Pharm. Pharmacol. 54(10), 1339-1344.

Young P.M., Price R., 2004. The influence of humidity on the aerosolisation of micronised and SEDS produced salbutamol sulphate. Eur. J. Pharm. Sci. 22(4), 235-240.

Zeng X.M., Martin G.P., Tee S.K., Marriott C., 1998. The role of fine particle lactose on the dispersion and deaggregation of salbutamol sulphate in an air stream in vitro. Int. J. Pharm. 176(1), 99-110.

Zeng X.M., Martin G.P., Marriott C., Pritchard J., 2000a. Crystallization of lactose from Carbopol gels. Pharm. Res. 17(7), 879-886.

Zeng X.M., Martin G.P., Marriott C., Pritchard J., 2000b. The influence of crystallization conditions on the morphology of lactose intended for use as a carrier for dry powder aerosols. J. Pharm. Pharmacol. 52(6), 633-643.

Zhu C., Liu G., Yu Q., Pfeffer R., Daves R.N., Nam C.H., 2004. Sound assisted fluidization of nanoparticle agglomerates. Powder Technol. 141(1-2), 119-123.