Compression Behavior of the Enzyme β-Galactosidase

Thesis

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To Adrian

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Symbols and Abbreviations

A	constant of the Heckel equation indicating the intercept
Ac	activity of the enzyme
A_{420}	Absorption at 420 nm
B	constant of the Heckel equation indicating the extent of particle rearrangement
b_1, b_2	intercept
C	proportionality constant of the modified Heckel equation
D	diameter
E_w	initial weight
K	constant of the Heckel equation indicating the slope
K_m	Michaelis constant
l	thickness
l_{eff}	effective thickness
l_{theor}	theoretical thickness
m	mass
m_1, m_2	slope
m_{eff}	effective mass
m_{theor}	theoretical mass
n	number
P	deformation hardness
p_b	bonding probability
p_{cb}	critical bonding probability
p_{cs}	critical occupation probability
p_s	occupation probability
r	correlation coefficient
R^2	squared correlation coefficient
S	surface
$s_{\bar{x}}$	standard error of average value
V	volume
V_t	true volume
V_{tot}	apparent volume
w	weight

χ_p	pressure susceptibility
ε	porosity
ε_c	critical porosity
γ	compressibility
φ	confidence interval
ρ_a	apparent density
ρ_c	critical density
ρ_n	normalized relative density (apparent density / poured density)
ρ_p	relative poured density
ρ_r	relative density
ρ_t	relative tapped density
ρ_{tr}	true density
ρ_0	starting density
σ	compaction pressure
σ_c	compression stress
σ_y	yield pressure

DCA	dry coating agent
DCP	dicalcium phosphate dihydrate
HPMC	hydroxyl propyl methylcellulose
MCC	microcrystalline cellulose
ONP	*o*-nitrophenol
ONPG	*o*-nitrophenyl-β-*D*-galactosides
SEM	scanning electron microscopy

1 Summary

This thesis is based on the investigation of the compression behavior of a solid model enzyme. It was the scope of this work to characterize the behavior of the enzyme powder under pressure to gain on the one hand information about the behavior of powder during the compression process and on the other hand to get more knowledge about the behavior of enzyme powder in pharmaceutical formulations. An important aspect was the influence of excipients because their deformation character may change the properties of pharmaceutical formulations. For that reason the physical influences of a plastic and a brittle model excipient, respectively on the enzyme powder in binary mixtures was investigated. Critical mixture ratios should be defined where the behavior of the binary mixtures shows sudden changes. If critical mixture ratios are known, they can be avoided in the development of dosage forms to get robust formulations. Since the direct compaction of powders may be difficult, the compression behavior of enzyme granulates and enzyme coated pellets was investigated as well. For that reason powders and pellets from different raw materials were layered with an enzyme binding agent solution. The enzyme activity of the various granulates, pellets and compacts was detected and the preparations were judged based on this property.

The investigated model enzyme was a solid β-galactosidase preparation from *Aspergillus oryzae*, which was chosen for its stability, the molecular weight of 105 kDa, which is an average value compared to other enzymes and the reliable and relatively simple enzyme activity assay.

Compacts were produced on a material testing machine and the activity was detected spectophotometrically. The compression properties of the various formulations were characterized by using Heckel equation and modified Heckel equation. Granulates and pellets as well as their compacts were further characterized by scanning electron microscopy pictures.

The extent of activity loss in the compacted brittle enzyme powder could not be decreased by the addition of a plastic or a brittle excipient. With the diversity of the particles even a higher number of shearing forces was built in the compacts during compression. The shearing forces seemed to have negative influences on the activity of the enzyme. In the binary powder mixtures of the enzyme powder with the plastic excipient there was found a sudden change in the behavior of the system at a mixture ratio of 20% of enzyme powder. For the brittle-brittle binary mixtures of the enzyme powder with an excipient, differences in the behavior were difficult to detect because the two powders showed a very similar behavior. Tendencies towards a critical concentration at a ratio of 60% (V/V) of enzyme powder could not be proved, although a comparison with a second brittle enzyme powder preparation in mixtures with a brittle excipient showed similar tendencies. It was found that a plastic compression character and regularity in shape and size of the compressed particles was important to protect the enzyme activity under pressure. These properties could be reached with the production of granulates and the coating of pellets by enzyme layering, whereas

especially the compacted enzyme coated pellets showed no significant activity loss under pressure due to the very regular shape and size distribution and the fact that the pellets did not break and only slightly changed their shape to reduce the spaces between the individual pellets.

A lot of new aspects in the field of particle compression have been discussed in this work. It was found that the shape and the size of the various particles may have big influences on friction and shearing forces. Shearing forces can cause a reduction of enzyme activity during the compression of an enzyme powder. The compression character of the particles showed influences on the extent of activity loss under pressure, whereas plastic properties are favorable to protect the enzyme.

As a further step it would be important to test the transferability of the results on other enzyme products and to take into consideration more practical aspects like the production on a rotary press, the investigation of economic points of view or simply the attainment of a required dosage to define an optimal formulation for an oral application of a pharmaceutical enzyme powder.

2 Introduction

Today there are a lot of different, partly very special dosage forms on the market. In this wide range of possibilities of drug dispensation, tablets are still the most common used dosage form. The importance of this quite old dosage form is evident looking at the development of tablets since the invention of a manual tablet press in 1843. Besides the standard tablets there exists also a great number of special tablet modifications, for example effervescent, film, matrix and chewing tablets that are used to influence the drug absorption. The popularity of tablets can be well explained. First of all, the application of oral dosage forms is of great importance because it is easy and safe. In addition tablets show metering accuracy, robustness and stability and their production is economic.

Looking at the big number of tablets on the market – about 48% of all drugs sold per year are tablets – it seems that the development and production process of a tablet is well known and easy. But in fact it is a matter of a very complex process. The simple compression of a bulk material, either powder or granulate, to a robust tablet is dependent on a great number of influences, mainly force transfer, particle deformation and the formation of adhesive forces. Therefore the behavior of powder under compression is an interesting topic of wide range. Although a lot of work has already been done on this topic (Leuenberger et al., 1981; Leuenberger, 1982; Jetzer et al., 1983; Kuentz and Leuenberger, 2000), it is still not known enough on it. Compression behavior and thus tablet properties depend on the different powders used. As tablet excipients as well as drugs have very different properties, it is quite difficult to make general statements about their compression behavior. For pharmaceutical application there are very complex tablet ingredient mixtures and it is still impossible to preview the properties of the end-product tablet only by knowing the exact composition of the powder mixture. Achieving the possibility of such predictions would be economic and time saving. For this reason the characterization of model excipients and drugs as well as several mixtures of them is an interesting and important research field.

The process of powder compression can be mathematically described. Celik (1992) and Hiestand (1997) give an overview of this complex process in their work. A further approach to describe the behavior of powder and powder mixtures under compression is the application of percolation theory, which has a long tradition at the Institute of Pharmaceutical Technology in Basel. Different examples illustrate the successful application of percolation theory in the field of pharmaceutical powder technology. Percolation theory provides key tools for a more rational design of pharmaceutical dosage forms and for the development of robust formulations. Percolation theory defines a percolation threshold, or in other words a critical concentration of a component in a binary or more complex powder mixture. It is evident that the critical concentration of a component in a tablet formulation is the source of lack of robustness of a formulation. Therefore it is important for a robust formulation that the relevant concentration of a component in a tablet formulation is not too close to the critical concentration and thus robustness of the formulation is guaranteed also with slight changes in the proportions of components during scale-up and large scale production of

tablet formulations. The investigation of compression processes, behavior of powder and powder mixtures under compaction and the application of percolation theory leads to a definition of robust formulations which can be used as standard formulations to enable a more rational design of pharmaceutical dosage forms.

The fact that the complexity of drug substances is increasing, can also lead to difficulties in the development of new tablet formulations. Among other new drug substances the use of proteins and peptides as pharmaceuticals is steadily increasing, especially with the fast development of biotechnological processes. Isolation, purification, formulation and delivery of proteins represent significant challenges to pharmaceutical scientists, as proteins possess unique chemical and physical properties. These properties pose difficult stability problems, which can be influenced by the formulation and technological factors, for example excipients, temperature, storage conditions, compression or shearing forces. Manning et al. (1989) give an overview of the stability of protein pharmaceuticals.
Investigation of protein pharmaceutical behavior in tablet formulations can be done by the use of enzymes as model proteins. The enzyme activity can be quantified. It is known to decrease under pressure and can thus be used as indicator of changes, which occur during compression.

The scope of this thesis is the investigation of enzyme powder under compression to gain on the one hand more knowledge about powder compression and tablet formulation in general and on the other hand to get information on behavior of enzyme powder in pharmaceutical formulations. An important aspect is the influence of excipients, because the deformation character of an excipient can of course determine the tablet properties. In addition possible chemical and physical interaction between excipients and the investigated model enzyme powder β-galactosidase is an important part of this work. It is possible to classify common tablet excipients into plastic and brittle substances. One could imagine that the physical interaction in powder mixtures of a plastic excipient and the enzyme powder is different to that of a powder mixture with a brittle excipient. The classification of all powders and mixtures of them is done in this work with the application of Heckel equation and modified Heckel equation. A protecting effect of a plastic material on the sensitive enzyme drug is expected. Besides the investigation of the influence of plastic and brittle excipient powders, the aim is also to find out if there is a mixture range in which the enzyme powder is protected by the excipient in binary mixtures. Percolation theory is applied to define the critical enzyme concentration in these binary mixtures and thus find a robust formulation that can be used as standard formulation. A small digression is dedicated to the comparison with a second enzyme powder preparation, which is shortly before its launch to market as a pharmaceutical product.
In the practice of powder compression and production of tablets there can rarely be found direct powder compaction. Thus it is a further part of investigation to produce granulates and coat pellets to find on the one hand the influences of the granulation process and the pellet coating on the enzyme activity and on the other hand to see if there are differences in the enzyme behavior under compression within these enzyme preparations. A possible protecting effect of the compacted

pellets with their smooth surface and the regularity of the particle size on the enzyme activity is investigated. For the production of granulates and the pellet coating there is also made the differentiation between plastic and brittle excipients.

A comparison of all that different formulation methods and their influence on enzyme activity is done to make a proposal for an optimum robust formulation for an oral application of a pharmaceutical enzyme powder.

3 Theoretical Section

3.1 Compression process

The compression of particles and granules as a function of the applied pressure can be described by two different properties of the particulate matter: I the compressibility, i.e. the consolidation behavior of powder materials and II the compactibility, i.e. the measure of tableting performance.
The compression process represents the concentration of free solid particles (powder) or agglomerates (granulate) under pressure into a mostly porous solid body of defined shape and sufficient mechanical strength.
Basic statements about the compression process are in general valid for powders and granulates as well. The bulk material granulate is more coarse than a powder. It is a product of agglomerated powder and has several advantages regarding the compression of tablets. The production of a granulate from a powder results in an improvement of flowability, reduction of the specific surface and a better dissolution of the drug and is often leading to a better compactibility. Nevertheless the definition of powder and granulate is often arbitrary.

Powder in a die, ready for compression is in a way a special case of a solid dispersion in gaseous state. The difference to an aerosol is founded in the fact that the particles are not isolated but keep contact in the whole bulk material. That kind of contact is not only a consequence of gravity and the limitation by the die wall, but especially of the force of attraction between the particles.

The tensile strengths, the shear strengths and the elastic moduli of compacted substances influence the process of powder compression. The parameters mentioned were reduced to dimensionless parameters by Hiestand and Smith (1984) to quantify and evaluate tableting performance. The bonding index is the ratio of the tensile strength to the dynamic indentation hardness and is interpreted as indicating the relative survival during decompression of the areas of true contact that were built at maximum compression. The strain index indicates the relative strain energy developed during the elastic recovery following the deformation. The brittle fracture index is an indicator of the brittleness of the compact. These indices can be used for comparison of the compression properties of powders.

In the following chapters the stages of uniaxial compression are described as well as the bonding forces and the deformation process that result during compression. A further important aspect is the density distribution in tablets and the porosity and relative density of compacts that are also described mathematically by pressure porosity equations. Furthermore special aspects of compression processes, i.e. pellet and enzyme compression are mentioned.

3.1.1 Uniaxial compression

The compaction of tablets is in the practice of the pharmaceutical industries an uniaxial compression process. The free particles that are filled in a die get condensed by applying force with a lower and an upper punch. The aim of this condensation is the formation of a compressed core with a definite shape, which has the new properties of a solid body (Sucker et al., 1991a).
The process that runs through in die during compression was explained by Train (1956). He investigated the relationship between the applied pressure and the relative volume of the powder accumulation in the die. Train found that there was no linear behavior in that relationship but changes in slope of the curve of applied pressure versus relative volume. According to Train the compression process can therefore be classified into four stages.

Stage I: A decrease in the relative volume is caused by interparticulate slipping of the powder, which leads to a closer packing. With the overcoming of the friction forces particles slide to energetically convenient positions. The process is limited by reaching the densest packing because the particles become immobile relative to one another.

Stage II: With the immobility of the particles a formation of temporary struts, columns and vaults results. These structures protect small voids and support generally the imposed load.
In case of cohesive powders, this low state of consolidation may be sufficient to create a loose compact as known from the filling of hard gelatine capsules.

Stage III: A higher compact strength causes a destruction of the structure of Stage II. The consequence is a deformation of the particles, either by crushing or by plastic flowing.
This behavior results because there are point and line contacts between the rough surfaces of the particles. The applied stresses are transmitted from particle to particle through these contacts. As the surface area in contact is small compared to the entire surface area of the bulk, high stresses are imposed locally causing the material to fail. With the failure of the structure, new surfaces are built and the stress distribution is more homogeneous and new bonds are built.

Stage IV: When the structure formed is strong enough to support the imposed load, any further reduction in volume of the compact involves the normal compressibility of the solid material. Any further decrease in the voids of a still porous compact can only be achieved either by exceeding the crushing strength of the structure or by plastic deformation, or both.

An elastic re-extension is resulting, when the force is taken off the system after the compression. The degree of re-extension is depending on the character of the substance.
The four stages of the compression process are shown in Fig. 3.1.

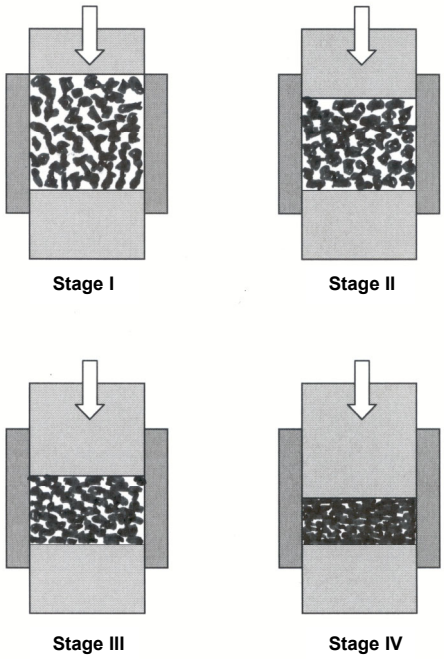

Fig. 3.1: *Schematic illustration of stages I to IV of the compression process.*

According to that description the transition between powder and tablet takes place in the third stage of compression. Yet it is important to mention, that this classification into four stages is very schematic and it must be considered that the different stages do not occur homogeneously in the compressed core. Different zones in the compressed core can be in different stages at the same time due to the inhomogeneous pressure distribution and in addition, due to the different substance properties, if the compressed core is built of a powder mixture. Nevertheless, the four stages postulated by Train are still considered as standard description of the uniaxial compression process.

3.1.2 Bonding in tablets

The mechanical strength of a compact is a consequence of the augmentation of the adhesive power resulting from the particle arrangement during the compression process. It is not only the

number of contacts between particles that increases during the compression process but also the deformation, which contributes to the strengthening of the adhesion.
There are three types of interparticle adhesion mechanisms that are of significance in tablet formation (Nyström et al., 1993):

Solid bridges

Mechanical interlocking

Intermolecular forces

Solid bridges result from recrystallisation or melting and solidification. However, these two phenomena can only appear in very special cases. For example a partly melting on so called hot spots caused by plastic flowing (Bowden and Tabor, 1958) or dissolution in adsorbed water. For that reason the solid bridges do only play a small role concerning the bonding in tablets.

Mechanical interlocking is dependent on the shape and the surface of the particles and their deformation during compression. It is possible that with very irregular surfaces interlocking between particles is built but the relevance for bonding in tablets is small.

Finally, the intermolecular forces are considered most important for mechanical strength in tablets. Intermolecular forces is a collective term for all bonding forces that act between surfaces separated by some distance. The term summarizes van der Waals forces, electrostatic forces and hydrogen bonding (Israelachvili, 1985). The term Van der Waals forces again include three different forces between atoms and molecules, i.e. dipole-dipole interaction (Keesom interaction), dipole-induced dipole interaction (Debye interaction) and the dispersion forces (London interaction).
The London interaction is an electrostatic force that affects non-polar molecules and it contributes the main part to the overall cohesivity in tablets with an amount of 75 to 100% (Wray, 1992) if the tablet consists of a high amount of an active substance, as active substances are usually rather hydrophobic. In case of a tablet with an extremely potent drug substance with e.g. 0.5 mg drug substance in a formulation with e.g. 100 mg lactose as a hydrophilic diluent representing more or less 80% (m/m), all types of van der Waal forces are contributing to the cohesive strength of the tablet.

3.1.3 Deformation process

In Stage III of the compression process described above, the concentration of the particles causes a deformation. The type of deformation can be classified and is substance-dependent.

The loading of a solid results in a first phase in an elastic deformation. The change in the shape of the solid is reversible after relief and the solid regains his natural formation. Exceeding the linear, elastic range by applying more compression pressure ends in an irreversible deformation. The transition between reversible and irreversible deformation is called yield point. The irreversible deformation can be either a plastic deformation or a destructive deformation, i.e. a brittle fracture.

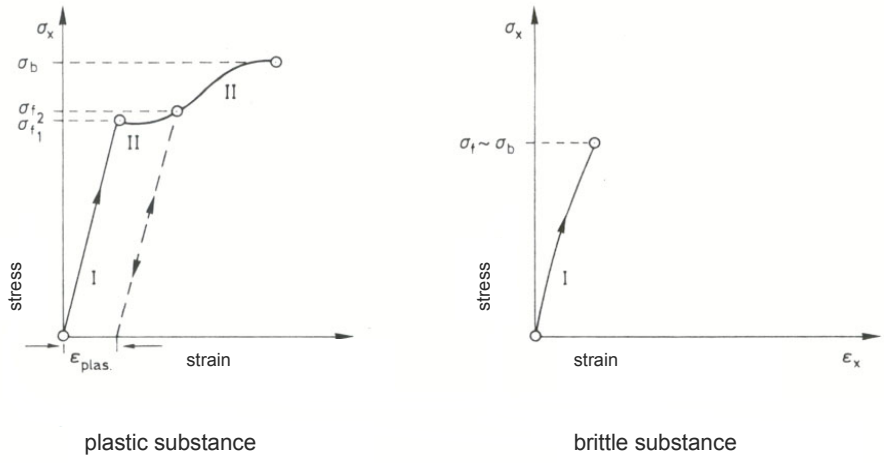

Fig. 3.2: *Stress-strain diagram (Sucker et al., 1991b).*

The stress-strain diagram (Fig. 3.2) shows the behavior of a substance under pressure. Plastic materials demonstrate a plastic flowing after the yield point, which finally ends in a fracture of the deformed particle. Brittle materials at the contrary do not have a plastic range, the elastic deformation is directly followed by a brittle fracture.

Fig. 3.3 gives a schematic overview of the three deformation behaviors.

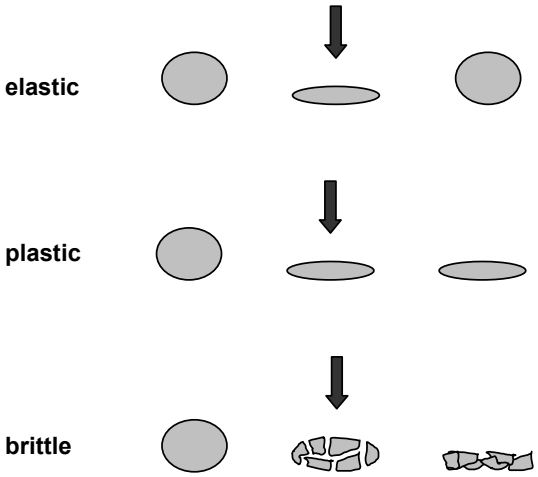

Fig. 3.3: *Schematic illustration of the typical deformation behavior before, during and after application of a compressional strength.*

3.1.4 Density distribution in tablets

Normally, a tablet is considered as a homogenous body. Yet, an exact examination of the compact exhibits heterogeneity in the density distribution (Train, 1956). Various density patterns can be observed depending on different compression conditions, for example the use of lubricant during compression, the shape of the tablet or the type of tablet press used. Stresses are transmitted through the material along force chains that make up a network of particle contacts and involve only fractions of all particles (Mueth et al., 1998). Nevertheless, in general it can be stated, that - talking about plane tablets and the use of an excenter press - there are regions of high densities in the center of the compact and on the upper edges. The lower edges of the tablet show comparatively low densities (Fig. 3.4). This result is also confirmed by Aydin et al. (1996).

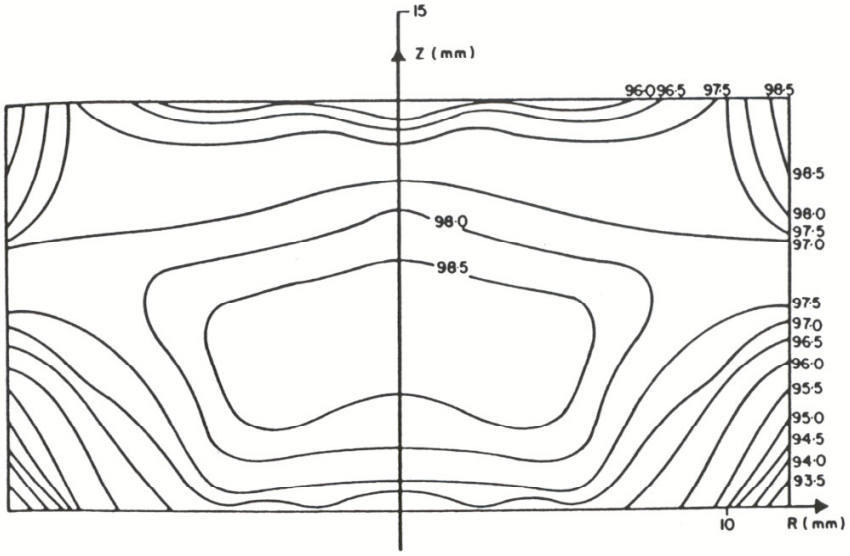

Fig. 3.4: *Density distribution in a tablet, cross section (Kandeil et al., 1977).*

3.1.5 Relative density and porosity of compacts

Although a tablet is a heterogeneous body concerning the density distribution, the relative density and thus porosity of a compact is considered as an averaged property.

Usually, the volume of the entire tablet V_{tot} (i.e. the apparent volume) is calculated from the measured height and the area of the compact. The determination of these dimensions is normally done when the compression process is finished and the tablet is ejected. This kind of measurement is called *zero-pressure* or *out-of-die* determination. The so-called apparent density, ρ_a is determined by division of the tablet weight m by the apparent volume, V_{tot}. The apparent density includes pores contrary to the true density ρ_{tr} where voids are excluded. The relative density ρ_r is obtained by dividing the apparent density by the true density:

$$\rho_r = \frac{\rho_a}{\rho_{tr}} = \frac{V_t}{V_{tot}} \tag{3.1}$$

The parameter V_t characterizes the true volume of the solid particles and therefore Eq. (3.1) shows that the relative density is essentially a solid fraction. This volume fraction, which is occupied by the solid, is linked to the volume fraction of the voids, i.e. the porosity ε.

$$\varepsilon = \frac{V_{tot} - V_t}{V_{tot}} = 1 - \frac{V_t}{V_{tot}} = 1 - \rho_r \tag{3.2}$$

The porosity is often specified in percent.

3.1.6 Heckel equation

To describe the compression characteristics of powders, the most frequently used equation was postulated by Heckel (1961a,b) and was originally developed for metallic powders. The same equation was independently postulated earlier by Shapiro and Kolthoff (1947). Heckel equation describes the relationship between the porosity of a compact and the applied pressure and is based on the assumption that the densification of the bulk powder in-die follows the first order kinetics:

$$\ln \frac{1}{1 - \rho_r} = K\sigma + A \tag{3.3}$$

where ρ_r is the relative density of the compact at pressure σ. The constants A and K are determined analytically from the intercept and slope, respectively, of the extrapolated linear region of a plot of $\ln(1/(1-\rho_r))$ versus σ. The intercept A is related to a starting density ρ_0 and an arbitrary constant B that provides a measure of volume reduction by particle rearrangements:

$$A = \ln\left(\frac{1}{1 - \rho_0}\right) + B \tag{3.4}$$

At lower pressures, there can always be shown a curved region, resulting from particle movement and rearrangement processes before interparticle bonding becomes appreciable (Fig. 3.5).

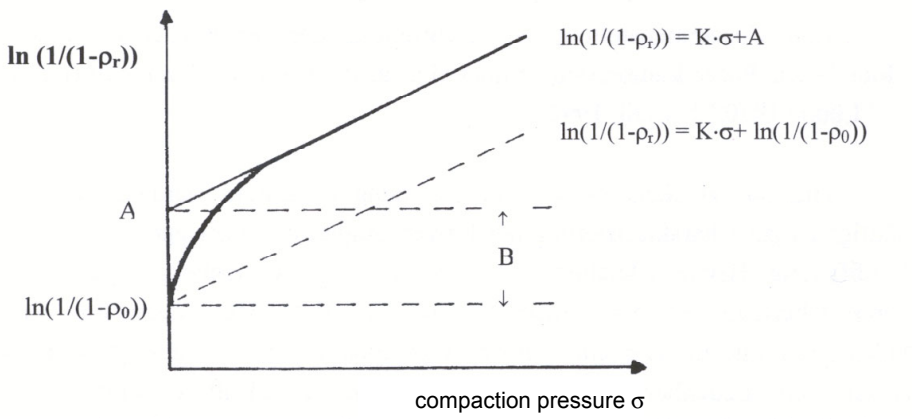

Fig. 3.5: *Schematic illustration of a Heckel plot (Heckel, 1961b).*

The linear region of the Heckel plot, i.e. shown at higher pressures, indicates the plastic deformation. Thus, *K* appears to be a material constant. Soft, ductile powders have higher *K* values than hard, brittle powders. This descriptive property of the constant *K* for deformation behavior of the material leads to the following correlation between *K* and the yield strength (Heckel, 1961a,b):

$$K \cong \frac{1}{3\sigma_y} \tag{3.5}$$

where σ_y is the yield pressure. Although Heckel (1961a) only applied pressures between 69 and 690 MPa, he postulated that extrapolation of the values to even higher pressures are justified, because linearity exists over nearly 80% of the pressure range. Contrary to that statement, it has often been seen that at high pressures the curvature of the Heckel plot arises quite exponentially. This fact is very visible with plotted data from *in-die* measurement. *In-die* or *at-pressure* measurements are often made to get more data in a faster way, but results can be influenced by an elastic deformation under pressure, which tends to lower the porosity of the powder bed. The other method used, so called *out-of-die* or *zero-pressure* measurement therefore describes the powder behavior more accurately. The reason of the curvature arise at high pressures is often considered to be a strain hardening (Gabaude et al., 1999). Sun and Grant (2001) explain the curve behavior in the last region with an elastic deformation of the powder, as already mentioned. This elastic deformation can even lead to a negative porosity and therefore to a value for relative density higher than 1. In addition, there is a mathematical reason for the curvature arise, which is valid for both *in-die* and *out-of-die* measurements (Sun and Grant, 2001). A small difference in relative density (ρ_r) values do not cause a significant difference in the expression ln(1/(1-ρ_r)), except at very high relative density values. Thus the proportional difference becomes much higher when ρ_r is high. The value of the relative density is always influenced by measurement errors, i.e. determination of true

density, tablet weight, and tablet volume. In addition it has to be kept in mind that the punch and die are elastic and compressed as well during the compaction of the powder. Therefore, data points at relative density more than 0.95 should be used with caution, because they can cause deviations from linearity.

Although Heckel plots are mostly used to characterize single materials, they can also be used to describe the behavior of powder mixtures. Ilkka and Paronen (1993) investigated some different binary mixtures. They found that all the mixtures behaved like intermediate materials between the bulk mixture components. Yet, no exact linear relationship in behavior between the mixtures and bulk components was seen. In most of the cases, one mixture component seemed to have more effect on the densification of the powder mixtures than the other.

Physical interactions in binary powder mixtures can also be detected by the following equation (Eq. (3.6)) developed by Leuenberger (1980) for the parameter, which describes the compressibility γ:

$$\gamma_{mixture} = x \cdot \gamma_A + (1-x) \cdot \gamma_B \tag{3.6}$$

with x corresponding to the percentage of the component of the binary mixture for drugs and excipients, which have similar true densities. In the same publication the strength of the compact of the binary powder mixture described with the deformation hardness P_{max} (at zero porosity) was estimated as follows:

$$P_{\max_{mixtureAB}} = P_{\max_A}{}^{x} \cdot P_{\max_B}{}^{1-x} \tag{3.7}$$

with $P_{max\ A}$, $P_{max\ B}$ describing the compactibility of the powder A, B based on the following general equations for both powders:

$$P_A = P_{\max_A}(1 - e^{-\gamma_A \rho_r \sigma_c}) \tag{3.8}$$

$$P_B = P_{\max_B}(1 - e^{-\gamma_B \rho_r \sigma_c}) \tag{3.9}$$

$$P_{A,B} = P_{\max_A}{}^{x} \cdot P_{\max_B}{}^{1-x}(1 - e^{-(x\cdot\gamma_A + (1-x)\cdot\gamma_B)\rho_r \sigma_c}) \tag{3.10}$$

with ρ_r indicating the relative density and σ_c indicating the compression stress.

3.1.7 Modified Heckel equation

For the description of the powder behavior under very low compression pressures, Kuentz and Leuenberger (1999) postulated a modified Heckel equation, which allows the description of the transition between the state of a powder and the state of a tablet. It considers that the pressure susceptibility (χ_p), which is defined as the decrease of porosity (ε) under pressure, can only be defined below a critical porosity (ε_c) or above the corresponding critical relative density (ρ_c), because a rigid structure exists there. Taking this into account, the following function was defined:

$$\chi_p \propto \frac{C}{\rho_r - \rho_c} \tag{3.11}$$

where χ_p is the pressure susceptibility, ρ_r is the relative density, ρ_c is the critical relative density and C is a constant. The new density versus pressure (σ) relationship can be described in the modified Heckel equation:

$$\sigma = \frac{1}{C}\left[\rho_c - \rho_r - (1-\rho_c)\ln\left(\frac{1-\rho_r}{1-\rho_c}\right)\right] \tag{3.12}$$

According to the constant K in the Heckel equation, the constant C in the modified Heckel equation is indicating plastic powder behavior with high values and brittle powder behavior with low values.

3.1.8 Special cases

3.1.8.1 Pellet compression

The compression of pellets is a special case of powder compression. In the recent years a lot of work has been done on that special research field, obviously because pellet compaction has some advantages in the preparation of modern dosage forms. Oral controlled release multiple dosage forms are becoming more and more important due to their improved bioavailability and safety of drug release. After disintegration of the tablets in the stomach, pellets with a particle size below 2 mm behave like liquids and have a short transit time through the stomach. The spreading of the multiparticulates across large sections of the intestine results in less variations in drug release. Pellets as multiple unit dosage forms are often filled into hard gelatine capsules. Less frequently they are compressed into tablets. Advantages of tablets compared to capsules comprise cost effectiveness and dividability (Wagner et al. 1999). In addition, the content uniformity of solid low-dosage forms is a major challenge in the pharmaceutical industry. With the compaction of drug-coated pellets, content uniformity can be reached quite easily (Martinez et al, 2001).

Pellets are a special form of granules, characterized by a very regular, round shape, a low porosity and a smooth surface (Leuenberger and Martin, 2002). Synonyms for pellets are spheres, cores and beads.
In general, powder compression principles are valid for pellet compression. The special character of pellets (round shape, low porosity and smooth surface) though, is responsible for some special compression behavior.

Some authors studied the compression behavior of pellets and it is possible to make statements about deformation behavior of pellets and the influence of various parameters like original pellet material, porosity, size, coating, fillers and cushioning substances.
Johansson et al. (1995) found that generally, discrete pellets can clearly be distinguished within a compact although the separation distances between the pellets are very low. The size and appearance of the pellets in the compact are similar to the original characteristic of the pellets. Thus, pellets tend to keep their integrity when compacted and do not fragment into smaller units. Therefore, the dominating mechanism of compression seems to be deformation in combination with a densification. A deformation of a pellet during compression is probably caused by repositioning of the primary particles, which constitute the pellet (Johansson et al., 1995). An increased tensile strength with augmentation of compaction pressure and initial pellet porosity was explained by Nicklasson et al. (1999a) with an increased degree of deformation during compaction. The occurrence of extensive deformation of the pellets facilitates the development of intergranular bonding forces.

The porosity of a tablet is generally independent on the porosity of the pellets before compaction. But increasing the pellet porosity increases the compressibility and compactibility of the pellets, i.e. the degree of deformation and densification of individual pellets can increase with pellet porosity (Nicklasson et al., 1999a). According to Johansson et al. (1995), Nicklasson et al. (1999a) and Martinez et al. (2001) the compression behavior of pellets is always rather plastic than brittle, fragmentation does not occur. Even with typical brittle pellet building substances like sugar (Martinez et al., 2001) or a 4 to 1 mixture of dicalcium phosphate dihydrate and microcrystalline cellulose (Nicklasson et al., 1999b). Nevertheless, Nicklasson et al. (1999a) found a difference in behavior of pellets derived from microcrystalline cellulose to the behavior of pellets derived from a 4 to 1 mixture of dicalcium phosphate dihydrate and microcrystalline cellulose. The degree of compression and compaction was higher for the former pellets than for the latter. The cause for that behavior is probably the difference in the hardness or deformability between the primary particles of the two substances. It is therefore easier for ductile particles to change their relative positions than it is for particles of a hard, less deformable material.
Johansson et al. (1998) studied the effect of pellet size on the compaction behavior. They found that the degree of pellet densification during compression was controlled only by the pressure applied while the degree of deformation was controlled by both, the applied pressure and the size of the pellets. The reason is probably that during uniaxial compression of an assembly of particles,

the force applied to the powder is transmitted through the powder bed at points of interparticulate contact. Increasing the size of the particles will reduce the number of force transmission points. Thus, the contact force at each interparticulate contact point will increase, which leads to increased pellet deformation.

The comparison between the compaction behavior of pellets and the compaction behavior of more irregular granules with higher porosity by Johansson and Alderborn (2001) showed that more irregular granules are more compressible. Obviously, the degree of granule deformation occurring during compression depends on the combined effects of the intragranular porosity and the granule shape. A more irregular shape increases the bed voidage, which allows an increased degree of deformation to which the granules can undergo during compression.

A further interesting point of research is the coating of pellets. On the one hand there are known drug pellets coated with a polymer film for protection or to influence the drug release. On the other hand there are cores layered with an active drug substance. For both kinds of pellets it is important to know if there is an influence of the coating on the behavior of the pellets under compression. Maganti and Celik (1994) found that coated pellets have better plastic properties than uncoated pellets of the same nature. An increase in the amount of coating applied caused a reduction in the yield pressure of the pellets. Tunon et al. (2003) also investigated the compression behavior of coated pellets and showed that the coated pellets behaved like the uncoated pellets. The coating did not significantly influence the compression behavior of the pellets. After deaggregation of the tablets the retrieved pellets were similar to the original pellets and there was no tendency for the polymer film to become convoluted. Therefore the film continued to coat the deformed pellets even after compaction, i.e. the coating is able to adapt to the densification and deformation of the pellets. Martinez et al. (2001) confirmed that statement. Pellet coating with a ductile polymer did not cause any changes in the compression characteristics of the uncoated pellets, moreover, the small value for the yield stress of the uncoated pellets, indicating plastic behavior could even be decreased by coating the pellets with the ductile polymer.

Conclusively it can be stated that pellets under pressure show on the one hand deformation, i.e. a change in shape and on the other hand a densification, i.e. a decrease in volume. Compressibility and compactibility can be influenced by the porosity of the pellets and the material of the primary particles. Nevertheless, fragmentation of pellets under compaction could not be found. The coating of pellets does not significantly influence the compression/compaction behavior.

3.1.8.2 Enzyme compression

Proteins and polypeptides are getting more and more important in the field of new drugs. As a consequence it is necessary to gain information on the stability of these substances, mainly

regarding the application of pressure. Enzymes as a special group of proteins have an activity that can be measured and quantified. Investigation of pressure-induced effects on the activity of enzymes dissolved in water showed that denaturation occurred, but was mainly reversible when the stress was released (Teng and Groves, 1988). That fact suggested a possible influence of compaction pressure on enzymes in dry state and is therefore of interest in different research groups for the last few years.

Teng and Groves (1988), Zarrintan et al. (1990), Wurster and Ternik (1994), Schulz et al. (1997) and Nürnberg and Scheler (2000) found that different enzymes investigated showed activity loss under compression. It was suspicious that between a compaction pressure of 100 and 250 MPa a sudden activity loss of 10 to 50% depending on the enzyme investigated was found. Consistently it was found that the apparent density of the compacts correlated with the enzyme activity. Thus, the sudden activity loss can be explained by a disappearance of voids in the compact and a consequent mechanical damage of the protein molecules. Volume reduction forces the molecule to change its shape, structural changes are necessary and cause a denaturation of the protein. Obviously this denaturation in dry state of the enzyme is irreversible. It is stated that the irreversible inactivation may involve the formation of incorrect enzyme conformations upon association or the loss of the ability of subunits to associate due to substantial structural changes probably because in solid state compression there is created a heterogeneous pressure distribution (Wurster and Ternik, 1994). The creation of new bonding within the enzyme molecule during compression is a further possible explanation (Schulz et al., 1997). These bonds could hinder the recreation of the native structure of the enzyme during dissolution.

A further explanation for the induced activity loss is a possible heating during compression. Enzymes are known to be heat-labile. Although several authors mentioned that possibility of thermal inactivation there is no evidence on that statement. Teng and Groves (1988) and Zarrintan et al. (1990) could show that the tableting speed had no effect on the activity loss, though it could be expected, that heat development in the compact would be dependant on the compression speed. Schulz et al. (1997) compared the activity loss after compression at 14°C and room temperature and did not find significant differences. Nürnberg and Hamperl (1986) compared the influence of compaction pressure, temperature and shearing forces and found that the compaction pressure is mainly responsible for the activity loss of compacted enzymes. Selective heat development in the compact during compression is of course possible and denaturation by hot spots is imaginable, since Weichert and Schönert (1976) found very high temperatures at the fractured surface of particles, e.g. the maximum temperature in the fracture zone in glass was 2927°C.

The compression characteristics of enzyme powders can of course be determined. Nürnberg and Scheler (2000) found a plastic behavior of their enzyme powders investigated. Heinämäki (1991) at the contrary could show that the enzyme he investigated showed a brittle compression behavior. Therefore there is no general statement possible concerning the compression character of an

enzyme powder. The compression behavior of these powders is probably dependent on the origin and production of the powder product. The enzyme powders used for compression, i.e. solid enzymes, are rarely pure enzymes. Mostly they are produced by lyophilisation, which requires an addition of excipients.

The influence of tableting excipients on the enzyme activity loss was also investigated. Graf et al. (1980) showed that microcrystalline cellulose caused a higher activity loss than dicalcium phosphate dihydrate. Unfortunately it is not stated if the enzyme powder used can be characterized with plastic or brittle compression behavior. Heinämäki (1991) stated that microcrystalline cellulose could have a negative influence on the enzyme activity because of its high compressibility. The big range of volume reduction causes friction during the compression process, which can be responsible for heat transformation and the creation of shearing forces. Yet, Heinämäki (1991) did not measure the activity loss to confirm his statement.

Summarizing the actual standard of knowledge it could be proved that enzyme activity decreases under pressure, obviously depending on the apparent density of the tablets produced. A lot of possible explanations for that behavior exist, but yet no real proof of processes happening within the compact is given. A further investigation of influences of tableting excipients seems to be helpful to get more information for the description of the compression behavior of enzymes.

3.2 Percolation theory

Percolation theory is used to explain certain regularities of critical phenomena in disordered systems. Disordered systems can be found anywhere in nature and thus, percolation theory has a broad field of application. It can be used for example to explain the fire propagation in forests as well as the gelation during egg boiling.
The description of a system is based on its classification into an (infinite) number of subunits, which are in geometrical order. In addition, each subunit needs to have the possibility to assume two different properties of condition. With an increasing probability of the one or the other condition, the physical properties of the whole system do change. In the area of a certain, so-called critical probability sudden changes occur. Percolation theory is a tool to confirm and pre calculate these critical probabilities as well as the behavior of the physical properties of a system.

Flory (1941) and Stockmayer (1943) were the first to use such concepts to describe polymers and gelation. The terminology of percolation is based on investigations on the flow of fluids through porous material by Broadbent and Hammersley (1957). The application of percolation theory in the field of pharmaceutical technology was introduced by Leuenberger in the late eighties (Leuenberger et al., 1987).

The basic concepts of percolation theory are presented in the following, more detailed introductions are given by Stauffer and Aharony (1995) and Sahimi (1994).

3.2.1 Principles of percolation theory

Percolation theory can be applied on systems, which can be characterized as a lattice. A lattice consists of an infinite number of lattice sites arranged in a certain geometrical structure. One-, two- and three-dimensional lattices can be described (Fig. 3.6).

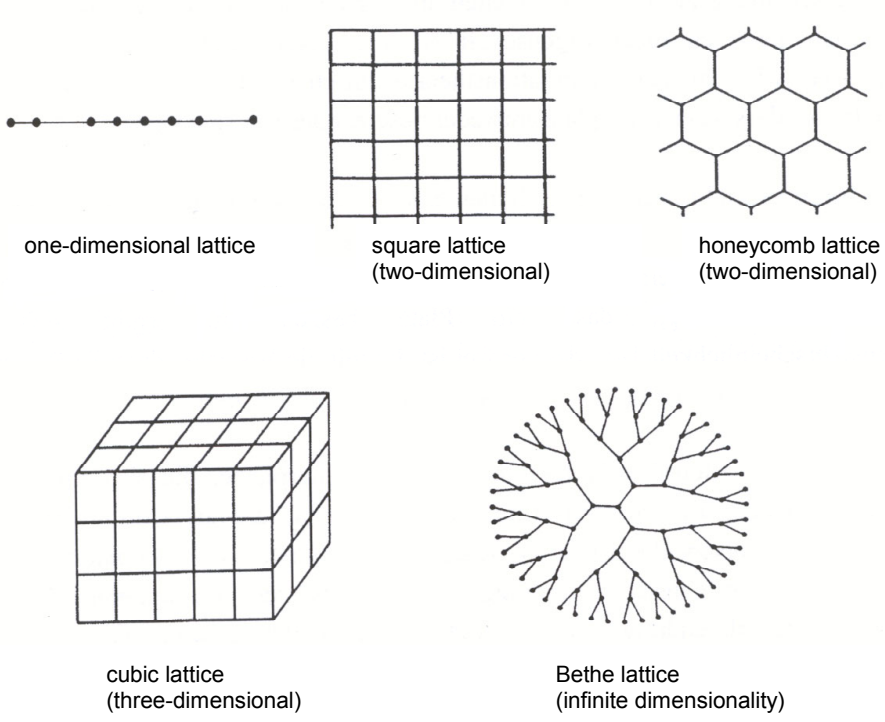

Fig. 3.6: *Examples for different lattice types (Stauffer and Aharony, 1995, Sahimi, 1994).*

In the group of two-dimensional lattices there are for example the square lattice, the honeycomb lattice or the triangular lattice. In the three-dimensional space there are described the diamond lattice, the simple cubic lattice, the body centered and the face centered cubic lattice. In addition the Bethe lattice can be mentioned as a special case because its dimensionality is infinite.

The sites of the lattice can either be occupied with the probability p_s or remain unoccupied with the probability $(1-p_s)$ That randomly occupation is independent on the occupation condition of the neighboring sites. In the case of p_s=1, every site is occupied and no empty spaces are remaining. Looking at one occupied site, a differentiation between direct and indirect neighbors is possible (Fig. 3.7). Direct or so-called nearest neighbors are sites, which share one face with the neighbor. Indirect or so-called next nearest neighbors though, touch on the edges.

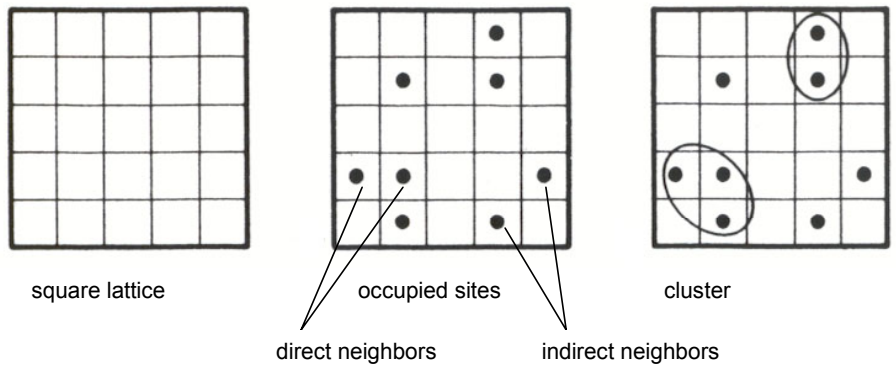

Fig. 3.7: *Illustration of some principles of percolation theory (Stauffer and Aharony, 1995).*

An isolated group of directly neighboring occupied sites is termed cluster (Fig. 3.7). The number and properties of these clusters is the main topic of percolation theory.

The type of percolation described so far is called site-percolation. The other known percolation type is the bond-percolation. With this kind of percolation every site of the lattice is occupied, i.e. the occupation probability p_s is one. Bonding between these directly neighboring occupied sites is built with the bonding probability p_b or bonding does not exist with the probability $(1-p_b)$. In the case of bond-percolation a group of occupied sites connected with bonds is called a cluster.

The combination of these two types of percolation is termed site-bond-percolation. In the case of this site-bond-percolation the sites are occupied with the occupation probability p_s and the building of bonds between two directly neighboring occupied sites results with the bonding probability p_b. A cluster is therefore a group of directly neighboring occupied sites connected with bonds.

If the occupation of the sites is not randomized but in dependence on the occupation of the neighbors, the percolation is correlated. The neighboring sites of occupied sites are either preferably occupied or avoided.

A directed system consists of a lattice in which the bonding has a special direction. This type of percolation is therefore called directed percolation.

In the special case of lack of lattice type definition where the sites are distributed randomly, the percolation type is called continuum percolation.

Fig. 3.8 gives an overview of the different types of percolation described above.

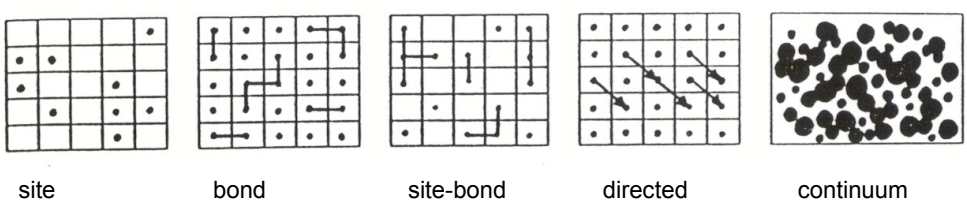

Fig. 3.8: *Schematic overview of different types of percolation.*

3.2.2 Percolation threshold

As already mentioned, percolation theory describes the number and properties of clusters. Depending on the occupation probability and the bonding probability, respectively clusters of different sizes and numbers can appear in a lattice. With a small occupation probability p_s and bonding probability p_b, respectively a lot of small clusters exist. These clusters are limited in their size. With an increasing probability p_s and p_b, respectively the size of the clusters also increases. At a certain probability p_{cs} and p_{cb}, respectively there exists for the first time a cluster which penetrates the lattice in the whole dimension describing an “infinite” cluster (see the two-dimensional case of a square lattice in Fig. 3.9).

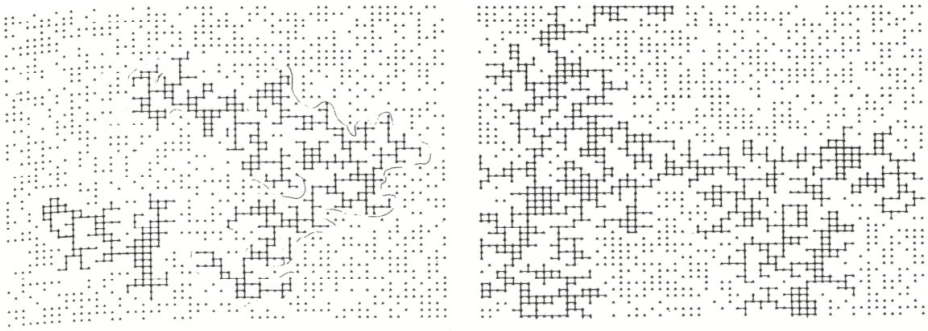

Fig. 3.9: *Square lattice with an occupation probability p_s below the percolation threshold with two finite clusters (left side) and an occupation probability p_s above the percolation threshold with an infinite cluster percolating the system (right side). (Stauffer and Aharony, 1995).*

This critical concentration is called percolation threshold. The cluster at the percolation threshold is called infinite cluster and if the probability p_s and p_b, respectively further increase, the infinite cluster also increases while the number and size of the finite clusters decrease.

The percolation threshold is dependent on the one hand on the type of percolation (site or bond) and on the other hand on the type of lattice. Generally, the percolation thresholds of three-dimensional lattices are always lower than percolation thresholds of two-dimensional lattices and the percolation thresholds for bond-percolation are lower than the one for site-percolation. The reason is obvious since in both cases, i.e. three-dimensional lattice and bond-percolation, respectively, there are more direct neighbors to build an infinite cluster.

3.2.3 Application of percolation theory in powder technology

The application of percolation theory in powder technology enables a new perspective on various problems. Leuenberger (1999) summarizes the progress in that field.

In the field of powder compression of binary mixtures the following model of percolation theory is applied.
In a binary mixture consisting of substance A and B, the lattice is formed by particles of type A or type B. As long as substance A builds a cluster in the lattice, i.e. many particles of substance A are direct neighbors it can be stated that substance A dominates the behavior of the system. With an increasing amount of substance B, clusters consisting of substance A get destroyed. More and more sites in the lattice get occupied by substance B. The system is penetrated by clusters of substance A as well as clusters of substance B. Thus, a certain point can be defined where the system domination of substance A is replaced by a system domination of substance B, i.e. a phase inversion takes place. This critical concentration is therefore called percolation threshold. The geometrical packaging is a function of the particle size, the particle size distribution and the shape of the particles. Different tablet properties of binary powder compressions have been investigated, e.g. intrinsic dissolution rate (Holman and Leuenberger, 1990), disintegration time (Leuenberger et al., 1987), Brinell hardness (Leuenberger et al., 1989; Holman and Leuenberger, 1990), tap density (Holman and Leuenberger, 1990). Percolation thresholds have been proved for each of these tablet properties with a sudden change of the tablet behavior.
For the development of formulations of solid dosage forms it is therefore necessary to avoid these critical mixture ratios to guarantee sufficient robustness of the formulation.

3.3 β-Galactosidase

β-Galactosidase, also called lactase (EC 3.2.1.23) is widely distributed in nature. This is not surprising being aware of the fact that oligo- or polysaccharides containing D-galactose joined through a β-glycosidic bond occur in most if not all organisms (Wallenfells and Weil, 1972). Thus, it is obvious that the corresponding glycosidases are as widely distributed as their substrates. This universal occurrence coupled with the simple enzymatic assay and the availability of numerous substrates made β-galactosidase one of the most widely studied glycosidases. Moreover β-galactosidase from *Escherichia coli* is of great interest regarding structure analysis due to its very big molecule weight (518 kDa). Molecule size and thus the behavior of the enzyme molecule is strongly varying from one organism source to the other, though. The great number of organisms containing β-galactosidase makes it sometimes difficult to find detailed information on a β-galactosidase from a special source.

The β-galactosidase used in this work occurred from *Aspergillus oryzae.* It was chosen as a model enzyme because of its molecular weight of about 105 kDa (Tanaka et al., 1975), which corresponds to an average molecular weight for enzymes. Moreover, the enzyme activity test is simple to handle and reliable. β-Galactosidase from *Aspergillus oryzae* is very stable and as a further important aspect the used enzyme preparation is economic, which was of importance because of the relatively big amounts used for tablet preparation.

The following chapters give an overview of the character and properties of β-galactosidase in general as well as β-galactosidase from *Aspergillus oryzae*.

3.3.1 Source and application

β-Galactosidase can be found in several plants, animals, bacteria, yeast and moulds. In plants, high enzymatic activities are found in seeds and leaves where the enzyme is probably related to the catabolism of galactolipids, such as β-D-galactosyl diacylglycerol, which are universally distributed in plants and algae (Wallenfels and Weil, 1972). Microorganisms do also produce β-galactosidase, which is used for technological use as it is economic and the availability is good. Normally the enzyme remains in the endproduct and therefore it is important that the enzyme sources are compatible with nutrition regulations if the technological process is used for nutritional products. Commonly used microorganisms for that purpose are yeasts like *Saccharomyces lactis, Saccharomyces fragilis* and *Candida pseudotropicalis*, moulds like *Aspergillus niger*, *Aspergillus oryzae* and *Mucor meihei* and bacteria like *Bacillus stearothermophilus*, *Streptococcus lactis* and *Lactobacillus bulgaricus* (Stellmach, 1988). The widespread occurrence of β-galactosidase in mammalian organs is probably related to the multiple physiological functions of the enzyme. The

role for intestinal β-galactosidase for the hydrolysis and consequently for the absorption of dietary lactose is well known (Wallenfels and Weil, 1972). About 70% of the adult world population is not able to digest lactose because of lack of the enzyme β-galactosidase in the small intestine. Absorption of milk and milk products causes symptoms like diarrhoea, convulsions and flatulence (Stellmach, 1988). Pharmaceutical application of β-galactosidase for the decomposition of lactose into glucose and galactose to avoid these symptoms is therefore necessary.

3.3.2 Structure

Structure analysis of the enzyme β-galactosidase was mostly done from the source of *Escherichia coli*. The molecule from this source was found to be a tetramer with a molecular weight of 518 kDa, its shape can be described as an oblate ellipsoid. The length and height of the molecule were calculated to be 150 and 50Å, respectively (Wallenfels and Weil, 1972). Data on the properties of β-galactosidase from other origins are very rare. Nevertheless, the small number of information shows that there are big differences between the various origins. Data from *Aeromonas formicans* and *Aerobacter cloacae* appear to have similar properties to that of *E. coli*, while the enzyme from *Staphyolcoccus aureus* has a molecular weight of 50 kDa and consists of one polypeptide chain. The molecular weights of the enzymes from *Bacillus megaterium* and *Sporobolomyces* singularis were found to be in the range of 140-150 kDa (Wallenfels and Weil, 1972).
The molecular weight of β-galactosidase from *Aspergillus oryzae* was found to be 105 kDa (Tanaka et al., 1975). This was confirmed by Akasaki et al. (1976), who also asserted that β-galactosidase from *Aspergillus oryzae* has not subunit structure.

Thus, the size of the enzyme from *Aspergillus oryzae* corresponds more or less the size of one monomer from *E. coli*. Based on that fact and the lack of subunit structure, it is supposed that β-galactosidase from *Aspergillus oryzae* has similar structure to that of the monomer of the enzyme from *E. coli*.

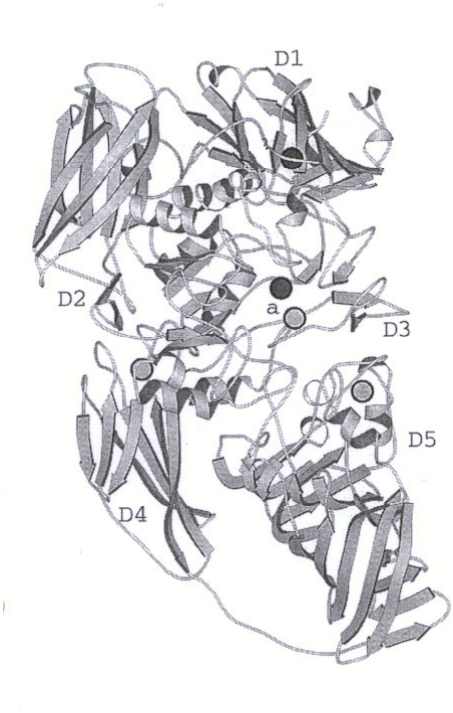

Fig. 3.10: *Structure of a monomer of β-galactosidase from E. coli.* ***D1-D5*** *indicate the five domains,* ***a*** *indicates the active site (Juers et al., 2000).*

The structure of β-galactosidase from *E. coli* was investigated by Juers et al. (2000). The monomer (116 kDa) is formed of five structural domains (Fig. 3.10). The active site is located at the C-terminal end of the central core of domain 3 and includes also portions of loops from domain 1, domain 2 and domain 5 of the monomer. The presence of magnesium ions in the active site suggests the enzyme being a metal enzyme.

The amino acid composition of β-galactosidase from *Aspergillus oryzae* was analyzed by Tanaka et al. (1975) and is shown in Tab. 3.1.

Tab. 3.1: *Amino acid composition of β-galactosidase from Aspergillus oryzae (Tanaka et al., 1975).*

Amino acid	Amino acid ($M/10^5$ g Protein)	Weight %
Alanine	58.1	5.2
Arginine	23.3	4.1
Aspartic acid	89.2	11.9
Cysteine	2.1	0.3
Glutamic acid	70.0	10.3
Glycine	82.2	6.2
Histidine	12.8	2.0
Isoleucine	29.8	3.9
Leucine	69.7	9.1
Lysine	34.5	5.0
Methionine	7.3	1.1
Phenylalanine	39.9	6.6
Proline	60.6	7.0
Serine	60.9	6.4
Threonine	53.9	6.4
Tryptophan	10.5	2.1
Tyrosine	42.6	7.7
Valine	41.3	4.8

3.3.3 Reaction

Investigations on the kinetics of the β-galactosidase catalysis proposed a Michaelis-Menten kinetic with a three-step mechanism:

$$E + S \xleftrightarrow{K_S} ES \xrightarrow{k_2} ES' + P_1 \xrightarrow{k_3} E + P_1 + P_2 \qquad (3.13)$$

It includes rapid binding of the substrate *S* to yield the Michaelis complex *ES*, formation of the intermediary complex *ES´* with simultaneous elimination of the aglyconic leaving group P_1 and hydrolysis of the *ES´* complex to yield free galactose P_2 and the Enzyme *E* (Wallenfels and Weil, 1972).

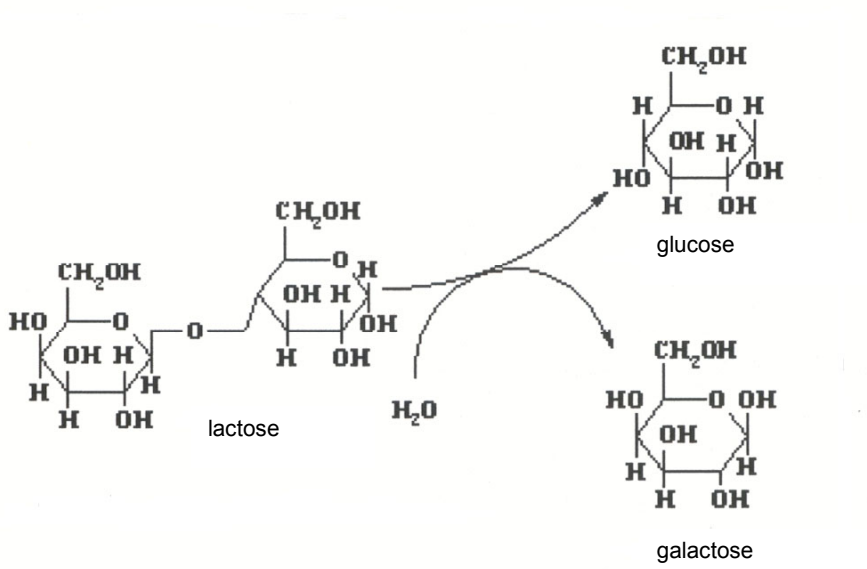

Fig. 3.11: *Reaction mechanism of β-galactosidase.*

The reaction mechanism (Fig. 3.11) catalyzed by the enzyme is a β-galactosidic cleavage of di- and oligosaccharides, respectively from the galactose side. Glycoside cleavage results between the anomeric C-atom of the galactose part and the ether-oxygen atom. The galactose part of the galactose-enzyme complex is hydrolysed releasing the enzyme (Stellmach, 1988).

3.3.4 Properties and stability

According to the investigations of Tanaka et al. (1975) the pH optimum of β-galactosidase from *Aspergillus oryzae* is at 4.5. During storage the enzyme is stable in a pH range of 4.0 to 9.0, i.e. the activity does not decrease. Influence of temperature on the enzyme activity and stability is also obvious. For the incubation temperature maximum activity is observed at 46°C. At pH 4.5 the enzyme is stable at 40°C for 10 minutes.

The hydrolysis of the substrate can be inhibited by Cu^{2+} and Ag^{+} at a concentration of 10^{-2} M and Hg^{+} at a concentration of 10^{-4} M. No influence of other metal ions is known. A complete destruction of the enzyme activity is shown by N-bromosuccinimide and sodium laurylsulfate. EDTA, mercaptoethanol, hydrogen peroxide, sodium thiosulfate, cysteine and ascorbic acid, though have no effect on the enzyme activity. Maltose, galactose and lactose at concentrations of 10^{-1} M can slightly inhibit the hydrolysis of the substrate while other sugars do not show any effect at the same concentration (Tanaka et al., 1975).

For the investigation of the enzyme activity different substrates can be employed beside the natural substrate lactose. Some requirements are essential according to Wallenfels and Weil (1972). The substrate needs to have a D-pyranoside ring, substitution at C-2, C-3, C-4 and C-6 by groups that are bigger than the hydroxyl group prevents binding of the galactoside to the active site. β-galactosidic linkage is specific. In addition, glycosidic oxygen of β-D-galactosides is essential, i.e. sulfur replacement clearly decreased the enzymatic hydrolysis.

Often used substrates for the hydrolysis of β-galactosidase are *o*-nitrophenyl-β-D-galactosides (ONPG). Akasaki et al. (1976) found that the activity measured with ONPG was almost five times higher than the activity measured with lactose in the same concentration. The Michaelis constant (K_m) value for ONPG is in the range of 10^{-4} M (Wallenfels and Weil, 1972 and Tanaka et al., 1975).

4 Materials and Methods

4.1 Substance characterization

For the characterization of the enzyme powder as well as the various excipients basic data acquisition was done with standardized methods for powder characterization.

True density was measured with a Beckman® Air Comparison Pycnometer Model 930 (Beckman Instruments, Fullerton, USA), determination of particle size was done with a laser diffractometer Malvern® Mastersizer X (MSX 6142, Malvern Instruments, Worcestershire, UK) and bulk and tapped densities were characterized according to European Pharmacopoeia 3 (1997).

4.1.1 β-Galactosidase

The enzyme preparation used for all experiments is an enzyme powder from *Aspergillus oryzae* (Fluka Chemie GmbH, Buchs, Switzerland). This yellow powder is not a pure enzyme because solid enzyme preparations are normally gained by lyophilisation. Thus, buffer salts and other excipients can be found in the powder preparation depending on the production process. The exact amount of protein in the preparation is declared to be 40%, accompanied by 60% of dextrine.
Data for true density, (relative) poured and (relative) tapped density, respectively and for the mean particle size of the β-galactosidase preparation from *Aspergillus oryzae* are shown in Tab. 4.1.

Tab. 4.1: *Characterization of β-galactosidase powder.*

Substance	Densities					Mean particle size (μm)
	True (g/ml)	Poured (g/ml)	ρ_p (rel)	Tapped (g/ml)	ρ_t (rel)	
β-Galactosidase powder	1.42	0.714	0.503	0.851	0.599	60.0

4.1.2 Excipients

The following excipients were used for powder compression as well as for granulation:

Microcrystalline cellulose (MCC)
(Avicel® PH 102, FMC, Philadelphia, USA)

Dicalcium phosphate dihydrate (DCP)
(Emcompress®, Edward Mendell, New York, USA)

The used pellet cores were the following:

Microcrystalline cellulose pellets
(Cellets®, Pharmatrans Sanaq AG, Basel, Switzerland)

Sugar pellets
(Suglets®, NP Pharm S. A. S., Bazainville, France)
The sugar pellets contain not more than 92% of sucrose, the remainder consists of maize starch.

The values for true density, (relative) poured and (relative) tapped density, respectively and for the mean particle size of the excipients mentioned above are shown in Tab. 4.2.

Tab. 4.2: *Characterization of excipients.*

Substances	Densities					Mean
	True (g/ml)	Poured (g/ml)	ρ_p (rel)	Tapped (g/ml)	ρ_t (rel)	particle size (μm)
Microcrystalline cellulose	1.55	0.325	0.210	0.403	0.260	162.8
Dicalcium phosphate	2.38	0.926	0.389	1.042	0.438	187.3
Microcryst. cellul. pellets	1.37	0.831	0.607			476.9
Sugar pellets	1.56	0.839	0.537			456.1

In addition, a buffer solution was used for all enzyme powder solutions. The citrate phosphate buffer of pH 4.5 consisted of 89.0 g disodium hydrogen phosphate dihydrate (Merck AG, Dietikon, Switzerland) and 62.0 g citric acid (Hänseler AG, Herisau, Switzerland) ad 5 l of double distilled water.

4.2 Influences on β-galactosidase activity

Enzymes are known to be sensitive on different influences. Thus, it was important to test if temperature and the number of excipients used for several studies could influence the activity of the enzyme powder β-galactosidase.

4.2.1 Temperature

The influence of different temperatures on the dry enzyme powder was investigated. The dry powder was exposed to room temperature, 30, 40, 50, 60, 70 and 80°C for 10 min, i.e. the duration of a compaction process. After this exposition the powder portions were dissolved in buffer solution at room temperature and the enzyme activity was detected and compared to a reference (see chapter 4.7.1).

In a second test, the influence of different temperatures on the dissolved enzyme powder was investigated. Solutions of enzyme powder in citrate phosphate buffer (0.015 mg/ml) were prepared and exposed to 30, 40, 50, 60, 70 and 80°C for 30 min. After cooling to room temperature, the enzyme activity of the solutions was detected and compared to a reference (see chapter 4.7.1).

4.2.2 Excipients

The influence of various excipients, which have contact with the enzyme powder during powder compression, granulation and pellet coating, respectively was investigated. Thus, solutions with 10% β-galactosidase powder and 90% of microcrystalline cellulose, dicalcium phosphate dihydrate, sucrose and maize starch, respectively in citrate phosphate buffer were prepared and the enzyme activity was detected and compared to a reference (see chapter 4.7.1). In addition, a solution of hydroxy propyl methylcellulose, also called HPMC (Pharmacoat® 603, Novartis Pharma AG, Basel, Switzerland) and β-galactosidase powder, 5% and 10% respectively was prepared and the enzyme activity was detected and compared to a reference as well (see chapter 4.7.1). Hydroxy propyl methylcellulose, which was used for the pellet coating, was also dissolved in buffer solution without

the addition of enzyme powder to detect possible influences of the polymer on the absorption in the enzyme assay.

4.3 Powder mixtures

Powder mixtures of β-galactosidase powder and microcrystalline cellulose as well as β-galactosidase powder and dicalcium phosphate dihydrate were prepared for compression. These binary mixtures with the following amounts of β-galactosidase: 1, 10, 20, 30, 40, 50, 60, 70, 80, 90% (w/w), were mixed in a blender (Turbula® T2C, W. Bachofen AG, Basel, Switzerland) for 5 minutes.

4.4 Granulation

The granulates were produced with an Aeromatic Strea fluid bed spray granulator (Aeromatic AG, Bubendorf, Switzerland). The acrylic glass granulation cylinder was limited on the bottom by a perforated base with a sieve insert and on the top by four filter bags. The filter bags were blown out alternatively every 15 seconds with compressed air. Addition of the binding agent solution resulted with a peristaltic pump (Petro Gas Ausrüstungen, Berlin, Germany) over a top spray binary nozzle positioned 28 cm above the perforated base.

The binding agent solution consisted of the binding agent, which was HPMC in a concentration of 5% in double distilled water according to some pre trials. In addition, there was β-galactosidase powder in a concentration of 10% dissolved in this solution. To ensure the stability of the enzyme powder, the solution needed to have a pH value of 4.5, which required the presence of the appropriate amount of buffer salts.

It was the aim of the granulation to layer powder particles with β-galactosidase. The powder particles were microcrystalline cellulose as a model substance for plastic behavior and dicalcium phosphate dihydrate as a model substance for brittle behavior, respectively. Layering of powder particles results cogently in an agglomeration of the small particles to granules. Nevertheless, the agglomeration was not the important part of these trials. The layering of the powder particles was necessary to be able to compare on the one hand the behavior of the mixtures of β-galactosidase powder and powder excipient and on the other hand the behavior of layered, regular shaped pellets to the irregular shaped powder excipients layered with β-galactosidase.

For that reason the amount of layering substance was calculated in the same way as for pellet layering (see chapter 4.5). The amount of binding agent solution sprayed on the powder particles

was calculated for a theoretical l-value = 2. The l-value indicates the thickness of the film coating (see chapter 4.5).

According to the different values for true density and particle sizes the amount of binding agent solution sprayed on 100 g of powder was different for the same l-value. 100 g of microcrystalline cellulose was sprayed with an amount of 271.2 g binding agent solution, whereas 100 g of dicalcium phosphate dihydrate was sprayed with an amount of 156.7 g binding agent solution.

The process parameters for both powder excipients were the same (Tab. 4.3).

Tab. 4.3: *Process parameters for granulation.*

Spray pressure (bar)	Spray rate (g/min)	Nozzle (mm)	Drying temperature (°C)	Outlet temperature (°C)
1	2.3	1.1	32	25

Granulate characterization methods were the same as described in chapter 4.1 for powders. In addition, scanning electron microscopy pictures were taken to characterize the granulates.

4.5 Pellet coating

Microcrystalline cellulose pellets with a plastic behavior of the primary particles as well as sugar pellets with a brittle behavior of the primary particles were coated with β-galactosidase binding agent solution (see chapter 4.4).

The amount of film-forming agent was calculated according to Eq. (4.1):

$$\frac{S \cdot I}{m} = \text{layering amount of DCA} \qquad (4.1)$$

where *DCA* is the dry coating agent in g per 100 g cores and per cent, respectively, *S* is the surface of the core, i.e. pellet in mm^2, *m* is the mass of one pellet in mg and *I* is the thickness of the film coating in mg *DCA* per cm^2 surface. The amount of binding agent solution sprayed on the pellets was calculated for a theoretical l-value = 5. The surface of the pellets was calculated using Eq. (4.2).

$$S = \pi \cdot D^2 \qquad (4.2)$$

where *D* is the diameter of the pellet, i.e. the mean particle size in mm.

Microcrystalline cellulose pellets were coated in a Mini-Glatt fluid bed spray granulator with Wurster insert (Glatt® GmbH, Binzen, Germany). The process parameters are shown in Tab. 4.4.

Tab. 4.4: *Process parameters for the coating of microcrystalline cellulose pellets.*

Spray pressure (bar)	Spray rate (g/min)	Nozzle (mm)	Drying temperature (°C)	Bulk temperature (°C)
2	2.6	0.5	50	34

Due to the small nozzle it was necessary to dilute the binding agent solution with buffer solution in a 1:1 ratio. 100 g of microcrystalline cellulose pellets were sprayed with an amount of 431.0 g diluted binding agent solution. The calculated value of 565.5 g binding agent solution (l-value = 5) could not be attained because the pellets started to glue up and fluidizing was no longer possible.

The coating of sugar pellets was not possible in the Mini-Glatt fluid bed spray granulator because the various cores were too heavy due to their true density. For that reason the sugar pellets were coated in an Aeromatic Strea fluid bed spray granulator (Aeromatic AG, Bubendorf, Switzerland), which was also used for granulation (see chapter 4.4). The process parameters are shown in Tab. 4.5.

Tab. 4.5: *Process parameters for the coating of sugar pellets.*

Spray pressure (bar)	Spray rate (g/min)	Nozzle (mm)	Drying temperature (°C)	Outlet temperature (°C)
1	2.6	1.1	52	32

100 g of sugar pellets were sprayed with an amount of 204.3 g binding agent solution.

In both cases the effective l-value was calculated (Eq. (4.3)).

$$\frac{\Delta m_{eff} \cdot l_{theor}}{\Delta m_{theor}} = l_{eff} \qquad (4.3)$$

where Δm_{theor} is the sum of all non-volatile substances per 100 g pellets in g, Δm_{eff} is the mass growth after coating of 100 g pellets in g, l_{theor} is the theoretically calculated l-value and l_{eff} is the effective l-value.

Characterization methods for the coated pellets were the same as described in chapter 4.1. In addition, scanning electron microscopy pictures were taken to characterize the pellets and the coating films.

4.6 Tablet compaction

Tablets of bulk β-galactosidase powder, bulk excipients, powder mixtures, granulates and pellets were compressed on the Zwick® 1478 Universal Testing Instrument (Zwick GmbH, Ulm, Germany). An 8 mm-diameter punch was used to form round flat tablets of portions of 100±0.5 mg substance.

Compaction forces of 2, 5, 7, 10, 15, 20, 40 and 60 kN (corresponding 40, 100, 140, 199, 298, 398, 796 and 1194 MPa) were applied except for microcrystalline cellulose pellets where formation of a tablet was not possible below a compaction force of 5kN. The compaction force of 2kN was hence replaced by 6kN (120 MPa).
The compression and decompression speed was 0.5 cm/min with a dwell time of 5 s at maximum pressure, the ejection speed was 5 cm/min.

Fig. 4.1 shows typical compression profiles for the applied compaction forces.

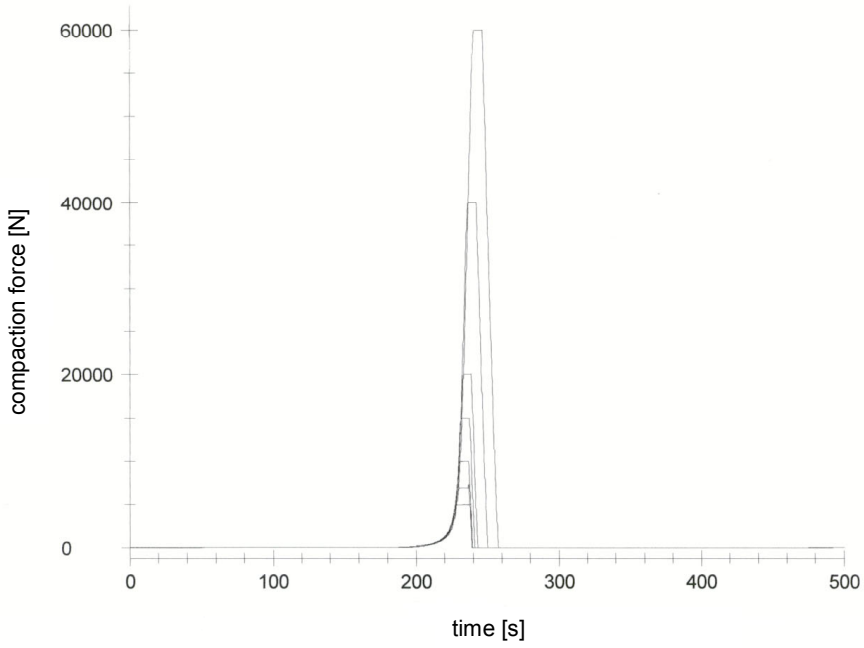

Fig. 4.1: *Typical compression profile for compaction forces of 5, 7, 10, 15, 20, 40 and 60 kN.*

For each system and compaction force five tablets were compressed, because the standard error of the average value does not significantly differ between a number of five samples and a higher number of samples. The standard error of the average value $s_{\bar{x}}$ is defined as shown in Eq. (4.4) (Lorenz, 1996).

$$s_{\bar{x}} = \frac{s}{\sqrt{n}} \tag{4.4}$$

where s is the standard error of the samples and n is the number of the samples.

A plot of the standard error of the average value against the sample number shows that the curve flattens after a sample number of five (Fig. 4.2).

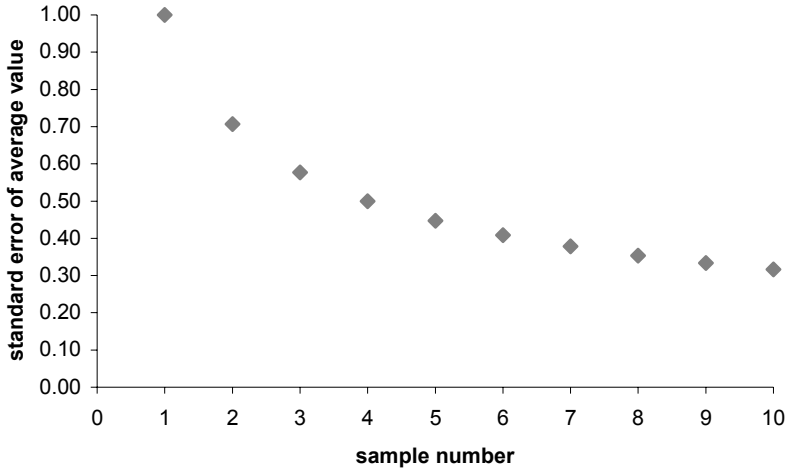

Fig. 4.2: *Plot of the standard error of average value against the sample number.*

From the compression process only out of die data were generated.

Tablet thickness was measured out-of-die after compaction with a thickness gauge (Digitcal, Tesa S.A., Renens, Switzerland) and after a storage time of 24h at 45% relative humidity in a desiccator containing calcium sulfate (Drierite®, Fluka Chemie GmbH, Buchs, Switzerland).

In addition, scanning electron microscopy pictures were taken from the compacts from pellets to characterize the behavior of the pellets under pressure.

4.7 Analytical assays

For the characterization of β-galactosidase powder, investigation of influences of temperature and excipients, characterization of the granulates and coated pellets and the studies on compression behavior of the enzyme the enzymatic activity was detected. In addition, instead of a determination of content of β-galactosidase in the granulates and pellets a determination of the amount of protein was accomplished. β-Galactosidase powder of course was also characterized by determination of the protein content.

4.7.1 Enzyme assay

The enzyme activity was detected with the synthetic substrate o-nitrophenyl-β-D-galactopyranosid, called ONPG (Fluka Chemie GmbH, Buchs, Switzerland). ONPG is decomposed into o-nitrophenol (ONP) and galactose by β-galactosidase. ONP is colorless in acidic medium but yellow colored in alkaline medium. Thus, the concentration of ONP can be detected spectrophotometrically at a wavelength of 420 nm.

The enzyme activity test was accomplished after extract chemie, modified by Amano (Stellmach, 1988), adapted to the use on a microplate reader spectrophotometer (Versamax-Tunable-Microplate-reader B02553, Molecular Devices Sunnyvale, USA). Hydrolysis of the synthetic substrate ONPG was used as a measure of the enzyme activity. The reaction mixture contained 175 µl of ONPG solution (5.7mM in citrate phosphate buffer, pH 4.5) and 25 µl of enzyme in buffer for the verum wells (n = 3) and 25 µl of buffer solution for the control wells (n = 3), respectively. After incubation for 10 min at 30°C, the reaction was stopped by adding 50 µl of 1M Na_2CO_3 (Fluka Chemie GmbH, Buchs, Switzerland). Then the absorbance of the yellow colored product o-nitrophenol (ONP) was measured at 420 nm. Absorbance value of the control was subtracted from the absorbance value of the enzyme solution and the activity *Ac* (U/mg) was calculated as shown in Eq. (4.5).

$$Ac = \frac{A_{420} \cdot 0.25}{4.45 \cdot 10 \cdot E_w} \tag{4.5}$$

where A_{420} is the absorbance at 420 nm, 0.25 is the total volume of the reagents in ml, 4.45 is the absorbance of 1 µmol ONP per ml at 420 nm, 10 is the reaction time in min and E_w is the initial weight of the enzyme in mg/0.025 ml of solution used. One β-galactosidase unit corresponds the amount of enzyme, which produces 1 µmol of o-nitrophenol per minute under the presented experimental conditions.

For the preparation of the enzyme solution enzyme powder as well as tablets, granulates and pellets were dissolved in buffer solution on the magnetic stirrer. The solution was then filtered and diluted as necessary to obtain a concentration of approximately 0.01 mg/ml. For the reference solution bulk β-galactosidase powder was dissolved and diluted in buffer solution to obtain the same concentration as for the enzyme solution. The absolute activity values measured in U/mg were converted into relative activity values assuming the absolute value of the reference being 100%.

The use of 96-well microplates (Plaque type B, non-sterile, Semadeni AG, Ostermundigen, Switzerland) allowed the measurement of 10 tablets, i.e. 2 batches or 10 other systems and five

references and their controls at the same time. The relative activity values referred to the references on the same plate and thus with the very same assay conditions.

4.7.2 Protein assay

The protein determination in β-galactosidase powder, granulates and pellets was accomplished after Lowry et al. (1951), modified by Peterson (1977) as it was proposed by Tanaka et al. (1975) and Akasaki et al. (1976) with bovine serum albumin (Sigma-Aldrich Laborchemikalien GmbH, Buchs, Switzerland) as a standard.

Lowry protein assay is based on a complexation of copper with protein under alkaline conditions. Addition of the Folin phenol reagent leads to a protein binding. The bound reagent is slowly reduced and changes color from yellow to blue.

For the determination of the protein content five standard solutions with a protein concentration of 5, 10, 20, 30 and 40 µg protein/ml were prepared to get a standard curve for the calculation of the protein content of the samples. Sample solutions were prepared with a protein content of approximately 20 µg/ml and a blank solution was also prepared for control. Standards, blanks and samples were prepared in triplicate and treated equally. To exclude interaction of substances in the protein solution the protein needed to be precipitated in a first step. To 1 ml of protein solution in a microtube (1.5 ml, Treff AG, Degersheim, Switzerland) 100 µl of 0.3% sodium deoxycholate solution (Sigma-Aldrich Laborchemikalien GmbH, Buchs, Switzerland) were added, mixed and incubated at room temperature for 10 min. Then 100 µl of 72% trichloroacetic acid were added and mixed again. After centrifugation for 30 min at 3000x g (Centrifuge 5415C, Vaudaux-Eppendorf AG, Schönenbuch, Switzerland), the tubes were placed upside-down in a rack for a few minutes. Remaining fluid on the rim of the tubes was removed. The pellets in the tubes were dissolved in 400 µl double distilled water and 400 µl of reagent A (mixture of equal parts of copper-tartrate-carbonate solution (0.1% copper sulfate pentahydrate (Fluka Chemie GmbH, Buchs, Switzerland), 0.2% tartaric acid solution (dipotassium salt, Sigma-Aldrich Laborchemikalien GmbH, Buchs, Switzerland), 10% sodium carbonate (Fluka Chemie GmbH, Buchs, Switzerland)), 0.8 N sodium hydroxide (Fluka Chemie GmbH, Buchs, Switzerland), 10% sodium dodecylsulfate (Sigma-Aldrich Laborchemikalien GmbH, Buchs, Switzerland) and double distilled water) were added. After incubation at room temperature for 10 min 200 µl of reagent B (solution of 1 volume Folin-Ciocalteu phenol reagent (Sigma-Aldrich Laborchemikalien GmbH, Buchs, Switzerland) and 5 volumes of double distilled water) were added and immediate vortexing was necessary. After incubation at 40°C for 30 min the protein solutions were filled in a 96-well microplate (n = 3) and 5 µl of ethanol were added to each well to prevent bubble formation. The absorbance of the blue solutions was detected at 750 nm.

4.8 Data interpretation

The mathematical and graphical data interpretation was done with Microsoft Excel (Office 2000, Microsoft Corporation, Redmond, USA) and a statistics program (SYSTAT Version 7.0 for Windows®, SYSTAT Inc., Evanston, IL, USA).

4.8.1 Non-linear regression

The percolation threshold of the various systems was determined by dividing the data in the graph, i.e. the curve into two straight lines. Using non-linear regression, the data were fitted to Eq. (4.6).

$$y = A(m_1 \cdot x + b_1) + B(m_2 \cdot x + b_2) \tag{4.6}$$

where x and y are properties of the system, m (respectively m_1, m_2) is the slope, b (respectively b_1, b_2) is the intercept and A and B are constants. The data points around the critical value, i.e. the percolation threshold shown as a bend in the curve, were attributed alternatively to the first section or to the second section. The final attribution was made considering the correlation coefficient R^2 for the overall fit.

4.8.2 Correlation

Several curves with different properties have been correlated using Eq. (4.7).

$$r(x,y) = \frac{(1/n)\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sigma_x \cdot \sigma_y} \tag{4.7}$$

where r is the correlation coefficient, n is the number of data, x_i and y_i are the various data points of the two correlated properties, $\bar{x}$ and $\bar{y}$ are the average values of the properties and σ_x and σ_y are the standard deviations of the properties.

5 Results and Discussion

5.1 Influences on β-galactosidase activity

The influence of temperature and different excipients on the enzyme activity was tested. These investigations led to necessary information concerning the stability of the enzyme powder. This knowledge is important for the characterization of the enzyme powder and thus for the further treatment of the enzyme powder preparation in the different studies.

5.1.1 Temperature

The measurement of the influence of the exposure of dry enzyme powder to different temperatures for the time of a compaction process showed a clear activity increase up to an exposure temperature of 80°C (Fig. 5.1).

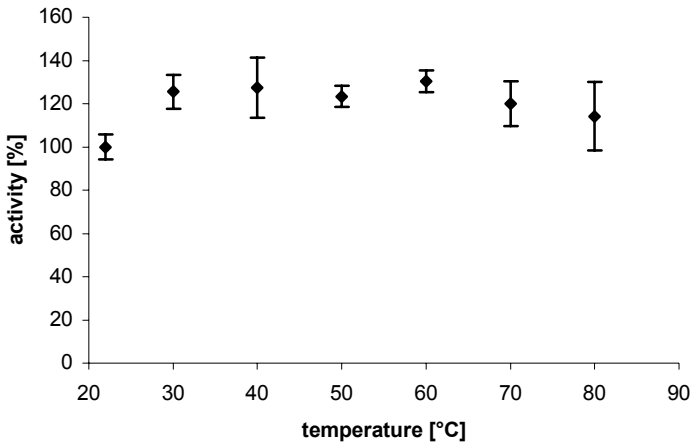

Fig. 5.1: *Temperature stability test on dry enzyme powder (n = 3). Error bars: ± 1 standard deviation.*

The activity increase between 30° and 70°C is statistically significant (φ = 95%) compared to the reference at room temperature. The activity increase at 80°C though does not significantly differ

from the reference at room temperature. None of the probes investigated showed an activity decrease.

The activity increase between room temperature and 80°C is not astonishing since optimum stability temperature of the enzyme β-galactosidase from moulds is expected at 45-50°C. Moreover, the optimum efficacy of β-galactosidases from moulds lies between 50° and 60°C (Stellmach, 1988).
For the use of dry enzyme powder for tablet compaction, problems are not expected to arise from temperature development in die. Temperature arise over 80°C during compression is not probable (Travers and Merriman, 1970; Wurster and Creekmore, 1986). Hence, possible activity loss during compression may not derive from a warming in die.

Investigation of temperature stability on enzyme solutions showed a different behavior from the temperature stability test on dry enzyme powder (Fig. 5.2).

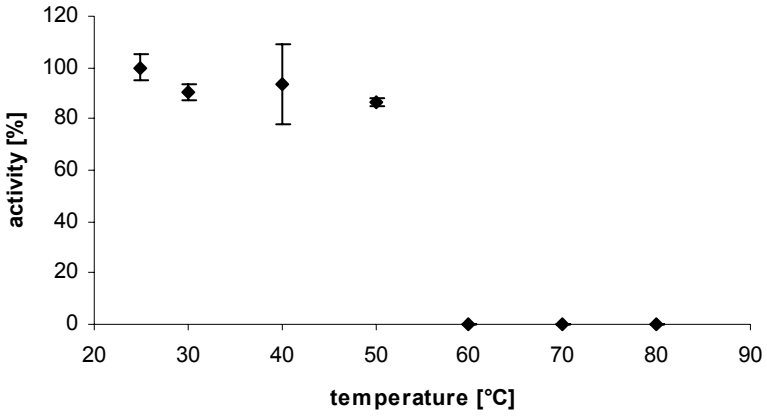

Fig. 5.2: *Temperature stability test on enzyme solutions (n = 3). Error bars: ± 1 standard deviation.*

The solutions stressed at different temperatures showed activity decrease compared to the reference at room temperature. Between 60° and 80°C there was no activity detection possible. The solutions at 30° and 40°C did not significantly (φ = 95%) differ from the reference at room temperature. At 50°C, though a significant activity decrease of more than 13% was detected compared to the reference. The statistical significance must be kept with caution, though, because the standard deviations of these two data points varied.

These investigations showed that dissolved enzyme powder was less stable than dry enzyme powder. This effect is obvious especially at stress temperatures higher than 40°C. Although in general enzymes are known to be more stable in dry state than in solutions (Hageman, 1992), it must also be kept in mind, that the exposure time in these two experiments was not the same. The exposure time for the dry enzyme powder was 10 min compared to 30 min for the enzyme solutions. Moreover the enzyme solutions had to cool to room temperature before the enzyme activity was detected. This cooling time was dependent on the stress temperature and lasted 2 to 3 hours. A possible negative effect of this cooling time on the enzyme activity is imaginable. It has to be mentioned that the lack of activity at stress temperatures higher than 50°C is at variance with literature data (Uhlig, 1998). Nevertheless, for further use of enzyme solutions, for example as binding agent solution for granulation and pellet coating, exposure temperatures higher than 50°C should be avoided.

5.1.2 Excipients

The behavior of the activity of β-galactosidase in the presence of various excipients was investigated and compared to the activity of bulk β-galactosidase powder as a reference (Fig. 5.3).

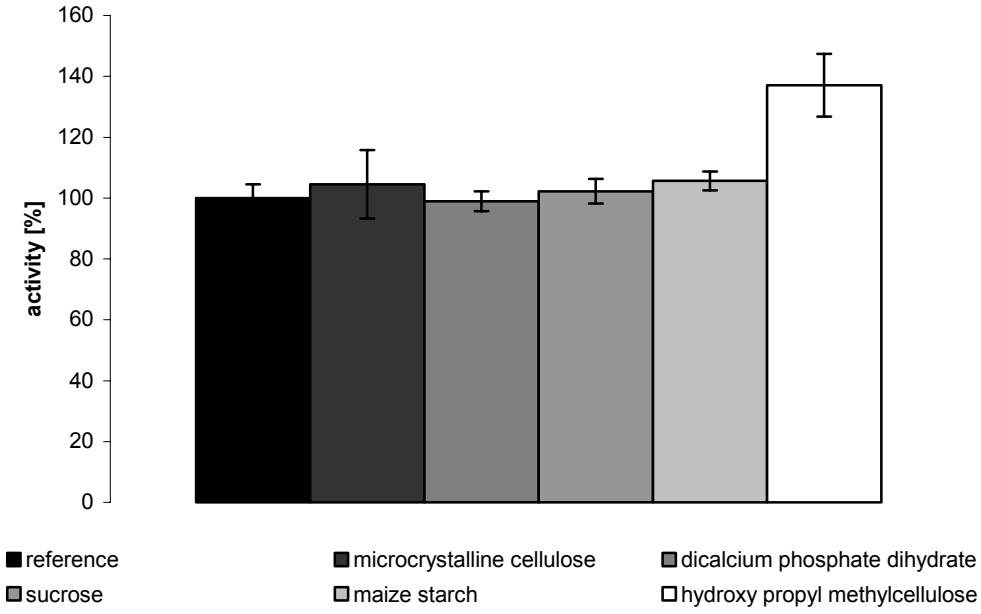

Fig. 5.3: *Activity of β-galactosidase in the presence of excipients (n = 3). Error bars: ± 1 standard deviation.*

The difference between the reference and the solutions of β-galactosidase powder and excipient, i.e. microcrystalline cellulose, dicalcium phosphate dihydrate, sucrose and maize starch was statistically not significant. Hence, influence of these excipients on the activity of β-galactosidase is not evident.

Hydroxy propyl methylcellulose at the contrary shows a significant influence on the activity of β-galactosidase powder. An activity increase of more than 37% was measured. A hydroxy propyl methylcellulose solution in the same concentration but without addition of enzyme powder had an absorption value of zero. Thus, β-galactosidase activity is directly influenced by the polymer excipient hydroxy propyl methylcellulose. The polymer is supposed to have a stabilizing effect on the enzyme. It protects the dissolved enzyme from denaturation and increases therefore the stability of the enzyme in solution. Stabilizing effects of polymers, antioxidants and other substances on enzymes are described by Halbeisen (1993) and Pikal-Cleland and Carpenter (2001).

The different excipients investigated can be used together with the enzyme preparation without the risk of a negative influence on the enzyme activity. The stabilizing effect of the polymer is even expected to have positive influences on the stability of the binding agent solutions for granulation and pellet coating.

5.2 Granulates and Pellets

The granulation and pellet coating processes have been accomplished to see if the film building behavior of the enzyme powder preparation is influenced by the raw material that was coated. For that reason, the treatment of the substances, the process parameters and the composition of the binding agent solution were kept as constant as possible to be able to make any comparisons between the four products produced. It was the aim to compare four different raw materials. For that reason the raw materials chosen had to fulfill other claims than normal raw materials for granulation. First of all, brittle raw material was compared to plastic raw material and these two material characteristics have been used in formation of regular formed particles with a smooth surface, i.e. pellets and irregular formed particles with a rough surface (Tab. 5.1).

Tab. 5.1: *Raw materials used for enzyme coating.*

	brittle	plastic
regular formed, smooth surface	**sugar pellets**	**microcrystalline cellulose pellets**
irregular formed, rough surface	**dicalcium phosphate dihydrate**	**microcrystalline cellulose**

These four raw materials have been coated with a β-galactosidase binding agent solution. It is necessary to mention that not the quality of the granulate, but the coating of the particles was decisive.

The necessary amount of β-galactosidase binding agent solution to spray on the raw material was calculated according to a theoretical l-value of two for the powders and five for the pellets. Therefore the duration of the spraying process was variable, i.e. dependent on the necessary amount of binding agent solution and the spraying rate, which was higher for the pellets than for the powders. Tab. 5.2 gives an overview of these different conditions. General process parameters are described in chapters 4.4 and 4.5.

Tab. 5.2: *Overview of the process time for particle coating.*

Preparation	Spray rate (g/min)	Amount of binding agent solution (g)	Process time (min)
β-Galactosidase-microcrystalline cellulose granulate	2.3	271.2	117.9
β-Galactosidase-dicalcium phosphate dihydrate granulate	2.3	156.7	68.1
β-Galactosidase-microcrystalline cellulose pellets	2.6	431.0	165.8
β-Galactosidase-sugar pellets	2.6	204.3	78.6

The yield of the end product was 88.6% for the β-galactosidase-microcrystalline cellulose granulate, 84.5% for the β-galactosidase-dicalcium phosphate dihydrate granulate, 87.4% for the β-

galactosidase-microcrystalline cellulose pellets and 95.1% for the β-galactosidase-sugar pellets, respectively.

Detailed information about the character of the various preparations and the influence of the coating process on the activity of the end products is given in the following chapters.

5.2.1 Characterization of granulates and pellets

An overview of the characterization of the granulates and pellets as well as the raw materials is given in Tab. 5.3.

Tab. 5.3: *Characterization of granulate and pellet preparations and raw materials.*

Substance/Preparation	Densities			Mean particle size (μm)	Median particle size (μm)
	True (g/ml)	Poured (g/ml)	ρ_p (rel)		
Microcrystalline cellulose	1.55	0.325	0.210	162.8	142.0
Dicalcium phosphate dihydrate	2.38	0.926	0.389	187.3	182.1
Microcrystalline cellulose pellets	1.37	0.831	0.607	476.9	469.3
Sugar pellets	1.56	0.839	0.537	456.1	451.0
β-Galactosidase-MCC granulate	1.47	0.307	0.208	400.1	369.3
β-Galactosidase-DCP granulate	2.02	0.895	0.442	237.7	227.7
β-Galactosidase-MCC pellets	1.41	0.836	0.594	574.1	544.5
β-Galactosidase-sugar pellets	1.45	0.858	0.590	515.1	501.4

The difference between the mean particle size and the median particle size of the pellets and granulates shows that the coating caused a broader particle size distribution, but the differences between the mean and the median values are still relatively small, which means that for all substances the layering is regularly distributed on the preparations.

The scanning electron microscopy pictures show the differences between the two granulate types. β-Galactosidase-microcrystalline cellulose granulate is formed very irregular (Fig. 5.1). On the one hand there can be found small particles similar to the raw material that was coated by the binding agent and on the other hand there are also some bigger agglomerates, showing real granulate forming. Moreover it can be seen that the surface of these granulate particles is very rough (Fig. 5.2).

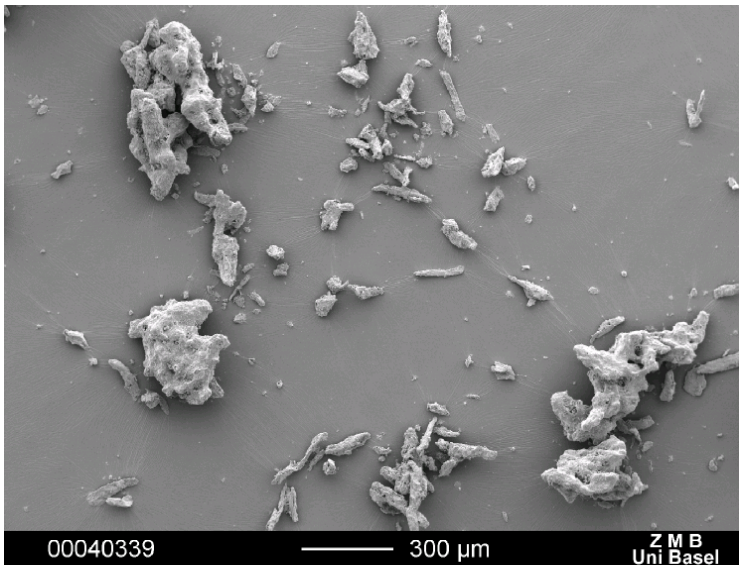

Fig. 5.1: *SEM picture of β-galactosidase-MCC granulate (50x).*

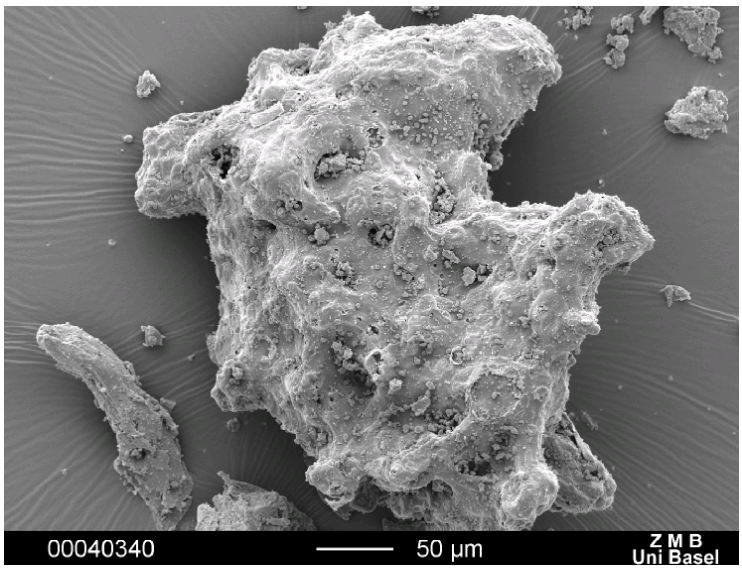

Fig. 5.2: *SEM picture of β-galactosidase-MCC granulate (250x).*

β-Galactosidase-dicalcium phosphate dihydrate granulate at the contrary is formed very regular (Fig. 5.3) and the surface of the granulate particles is very smooth (Fig. 5.4).

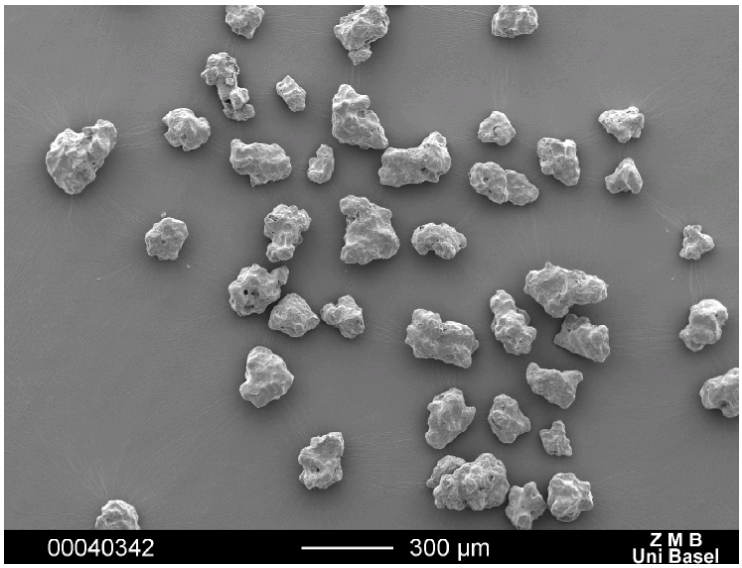

Fig. 5.3: *SEM picture of β-galactosidase-DCP granulate (50x).*

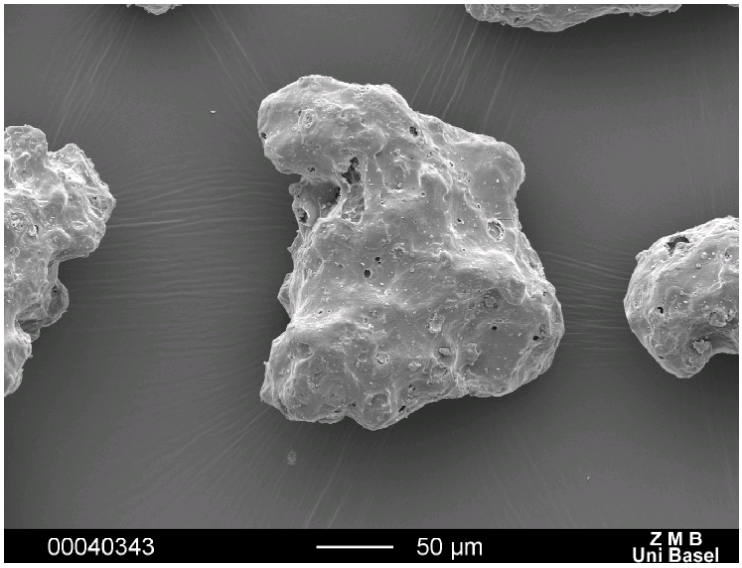

Fig. 5.4: *SEM picture of β-galactosidase-DCP granulate (250x).*

The scanning electron microscopy pictures of the raw and coated pellets give interesting information as well. The regular surface of the uncoated microcrystalline cellulose pellet (Fig. 5.5) got very smooth with the coating (Fig. 5.6).

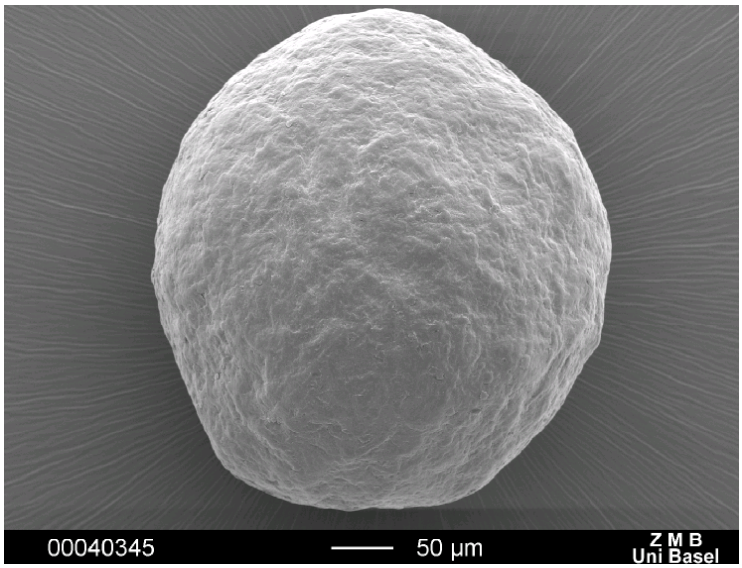

Fig. 5.5: *SEM picture of microcrystalline cellulose pellet (200x).*

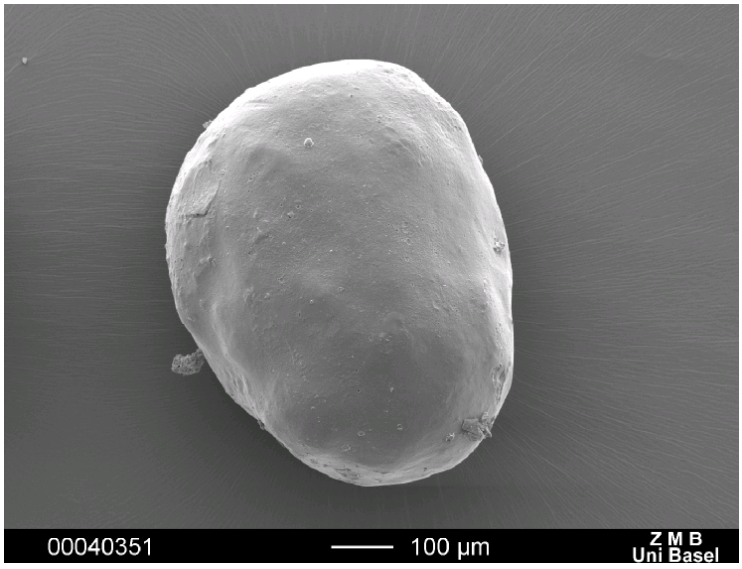

Fig. 5.6: *SEM picture of β-galactosidase-MCC pellet (100x).*

The surface of the uncoated sugar pellet (Fig. 5.7) was similar to the surface of the uncoated microcrystalline cellulose pellet and the coating lead to a rougher, more irregular surface (Fig. 5.8).

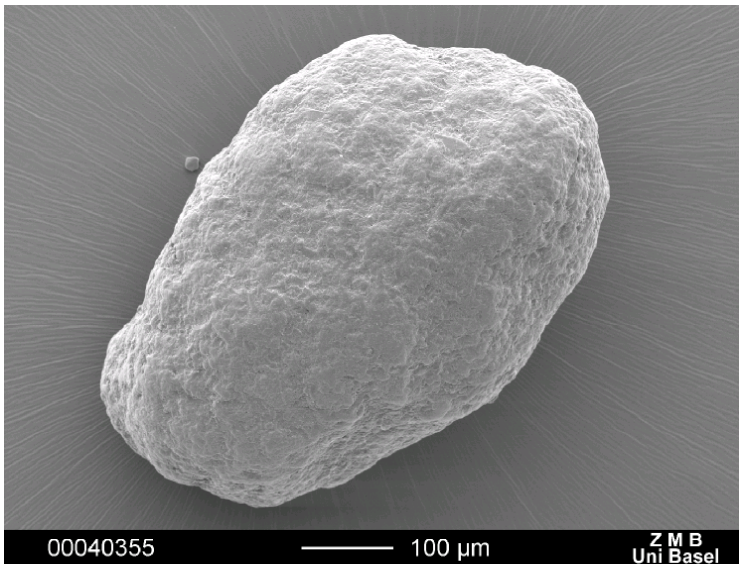

Fig. 5.7: *SEM picture of sugar pellet (150x).*

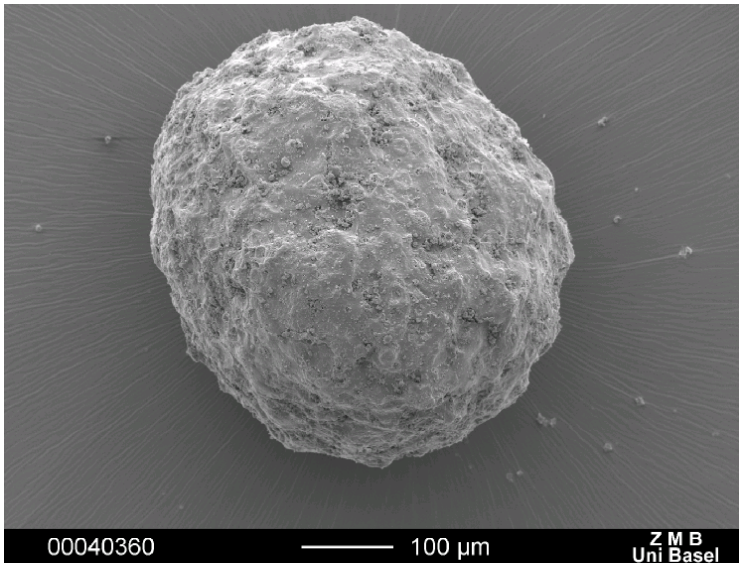

Fig. 5.8: *SEM picture of β-galactosidase-sugar pellet (150x).*

The difference in the state of these coating films (Fig. 5.9, Fig. 5.10, Fig. 5.11, Fig. 5.12) is supposed to be caused by the porosity and water absorption properties of the raw materials. Microcrystalline cellulose powder is known to absorb water during granulation processes, microcrystalline cellulose pellets are very compact and have a very small porosity. Sugar pellets at the contrary are more porous and can absorb water. The difference in the state of these two pellet types can also be seen in the cross-section pictures of the coated pellets (Fig. 5.13, Fig. 5.14). Dicalcium phosphate dihydrate is also a very compact substance, which does not absorb water

during granulation processes. Probably the fast elimination of the water in the binding agent solution by absorption during the spraying process prevents the formation of a smooth and regular coating film.

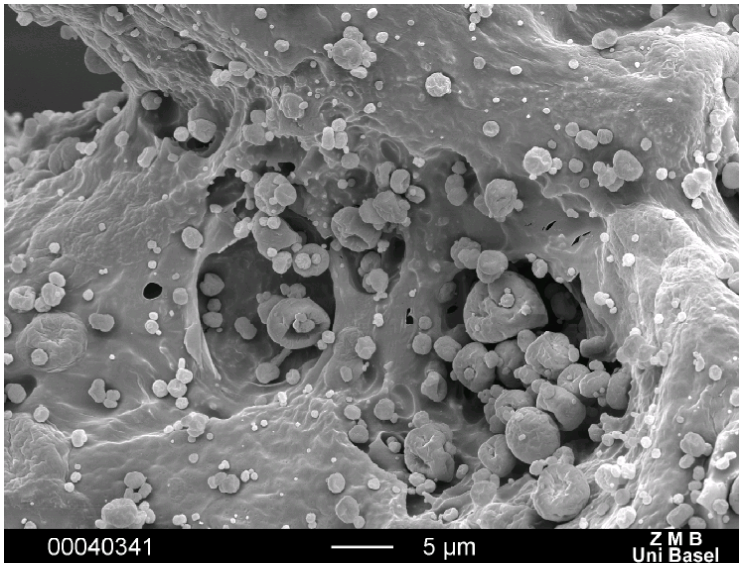

Fig. 5.9: *SEM picture of the surface of β-galactosidase-MCC granulate (2000x).*

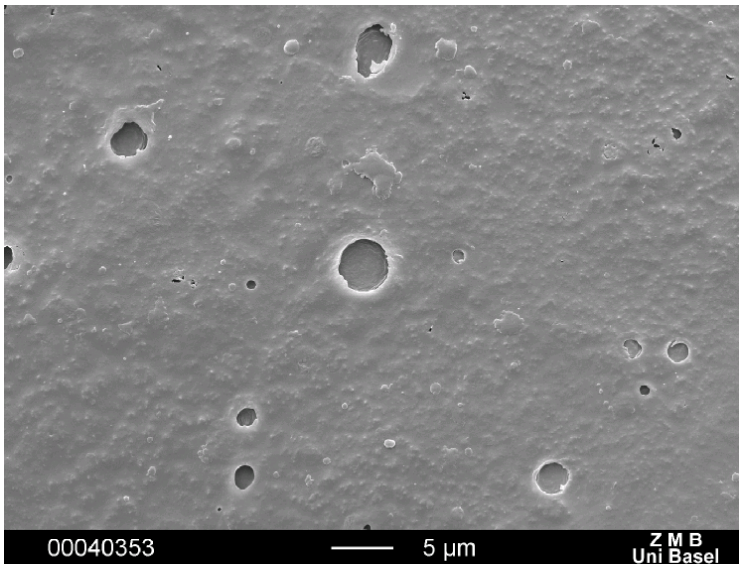

Fig. 5.10: *SEM picture of the surface of β-galactosidase-MCC pellet (2000x).*

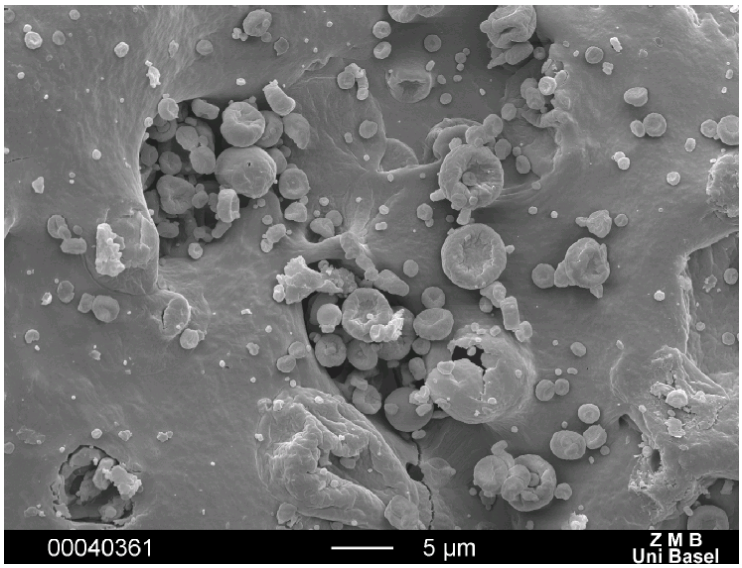

Fig. 5.11: *SEM picture of the surface of β-galactosidase-sugar pellet (2000x).*

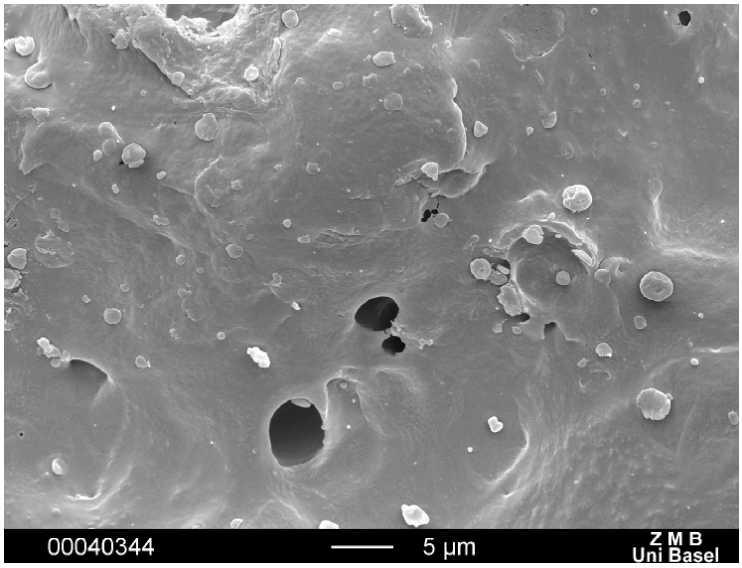

Fig. 5.12: *SEM picture of the surface of β-galactosidase-DCP granulate (2000x).*

The effective l-values were 1.3 for the β-galactosidase-microcrystalline cellulose granulate, 0.6 for the β-galactosidase-dicalcium phosphate dihydrate granulate, 3.0 for the β-galactosidase-microcrystalline cellulose pellets and 4.2 for the β-galactosidase-sugar pellets, respectively.

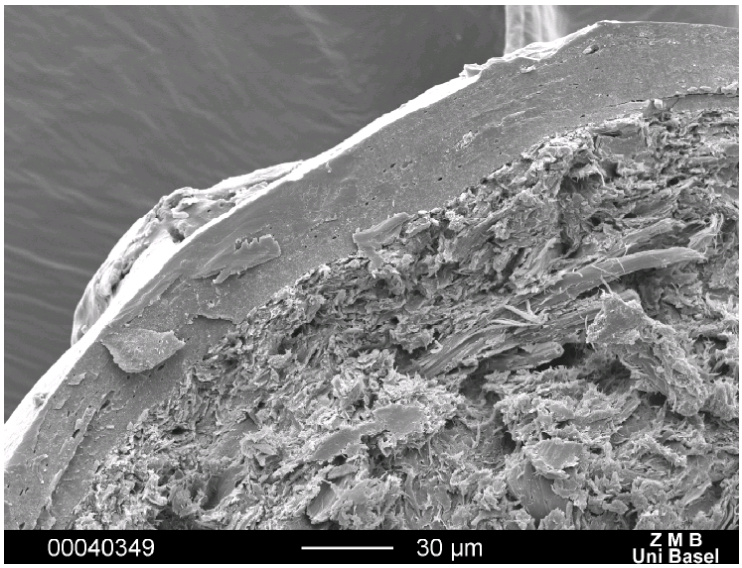

Fig. 5.13: *SEM picture of the cross-section of β-galactosidase-MCC pellet (500x).*

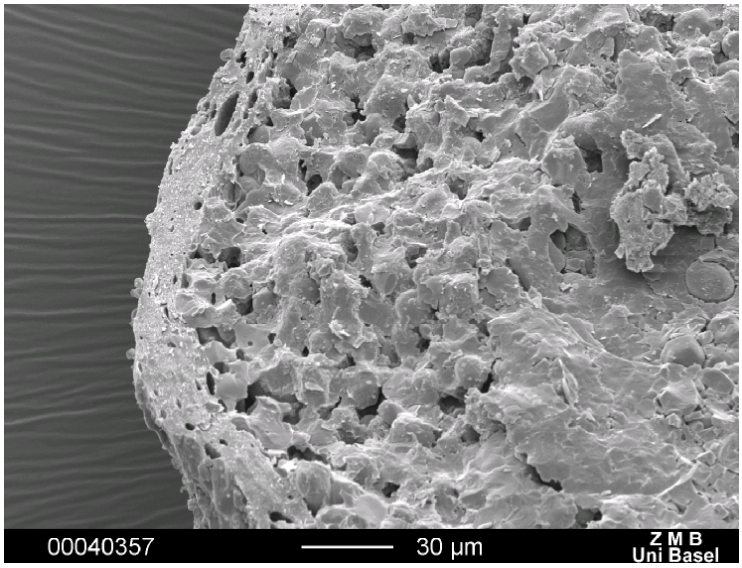

Fig. 5.14: *SEM picture of the cross-section of β-galactosidase-sugar pellet (500x).*

The growth of the particle size was 50.3 µm for the β-galactosidase-dicalcium phosphate dihydrate granulate and 237.8 µm for the β-galactosidase-microcrystalline cellulose granulate, respectively. This result shows that in the second granulate relatively big agglomerates have been formed. The growth of particle size of the pellets can be verified with the cross-section SEM-pictures (Fig. 5.13, Fig. 5.14) and was 97.2 µm for the β-galactosidase-microcrystalline cellulose pellets and 59.0 µm for the β-galactosidase-sugar pellets, respectively.

5.2.2 Influence of granulation and pellet coating on enzyme activity

Determination of the enzyme content of the layered preparations was accomplished with a measurement of the total protein content. The total protein content of the β-galactosidase powder preparation was 14.6% measured with the Lowry protein assay. Based on that result, the β-galactosidase powder content of the preparations was calculated to be 8.9% for the β-galactosidase-microcrystalline cellulose granulate, 2.3% for the β-galactosidase-dicalcium phosphate dihydrate granulate, 6.3% for the β-galactosidase-microcrystalline cellulose pellets and 10.9% for the β-galactosidase-sugar pellets. The content of total protein in the β-galactosidase powder preparation was very low compared to the value of 40% declared by the supplier. It is supposed that this difference occurred due to the reference used in the Lowry protein assay. Lowry et al. (1951) reported that the amount of color can vary with different proteins. This variation is a reflection of the contribution of specific amino acids, mainly of tyrosine and tryptophan (Sapan et al., 1999). As it was impossible to get pure β-galactosidase from the same source like the used β–galactosidase preparation to have an exact reference, bovine serum albumin was used, which is a generally used reference for standard protein tests. Nevertheless the actual yield of β-galactosidase powder in the preparations could be calculated and was found to be 43.0% for the β-galactosidase-microcrystalline cellulose granulate, 15.7% for the β-galactosidase-dicalcium phosphate dihydrate granulate, 37.1% for the β-galactosidase-microcrystalline cellulose pellets and 61.4% for the β-galactosidase-sugar pellets, respectively.

The activity of the preparations was also detected. Tab. 5.4 gives an overview of the activity increase after coating.

Tab. 5.4: *Overview of the activity increase after coating.*

Preparation	Activity increase after coating (%)
β-Galactosidase-MCC granulate	252.5 (+/- 23.7)
β-Galactosidase-DCP granulate	548.6 (+/- 22.2)
β-Galactosidase-MCC pellets	265.9 (+/- 16.8)
β-Galactosidase-sugar pellets	127.8 (+/- 9.0)

The activity increase for all preparations is very high. Probably due to two factors being involved in the granulation and pellet coating process: temperature arise and presence of binding agent. Temperature increase up to 50°C and the presence of hydroxy propyl methylcellulose do have positive influence on the behavior of the activity of the enzyme β-galactosidase. The extent of activity increase of the four preparations is very different, but it may not be correlated to the

process time (Tab. 5.2). It is supposed that the extent of activity increase is dependent on the quality of the coating film. The preparations with rough surfaces, i.e. β-galactosidase-microcrystalline cellulose granulate (Fig. 5.9) and β-galactosidase-sugar pellets (Fig. 5.11) show an activity increase of 252.5% and 127.8%, respectively. While the preparations with smooth surfaces, i.e. β-galactosidase-microcrystalline cellulose pellets (Fig. 5.10) and β-galactosidase-dicalcium phosphate dihydrate granulate (Fig. 5.12) show an activity increase of 265.9% and 548.6%, respectively. Taking into account that the binding agent solution sprayed on the β-galactosidase-microcrystalline cellulose pellets was diluted (1:1), the extent of activity increase can be associated with the quality of the coating film. Probably the viscosity of the binding agent solution is responsible for the degree of protection of the enzyme. It is conceivable that the polymer film protects the enzyme molecules from the hydrolytic covalent reactions observed in aqueous solutions and leads to an increased conformational rigidity (Volkin and Middaugh, 1992).

5.3 Compression behavior of powder and powder mixtures

The characterization of the enzyme powder preparation and mixtures of the preparation with the plastic excipient microcrystalline cellulose and the brittle excipient dicalcium phosphate dihydrate, respectively was done by the use of Heckel and modified Heckel equation. The yield strength of the mixtures was also analyzed. The activity loss of the compacted powder was investigated and correlated with the corresponding tablet porosities.

The interpretation of the Heckel plots showed some difficulties because it is necessary to define the linear range of a curve to get exact data about the compression properties of a substance. It is known that plastic substances show a small bend indicating a particle rearrangement followed by a linear segment, which can be followed by a flattened segment at very high compaction pressures. Brittle substances at the contrary show a distinct bend at the beginning of the compression followed by a linear range with a smaller slope than for plastic particles. For that reason after characterization the behavior of the substances in the Heckel plot the calculation of the *K* value was only done within a chosen compression range. The linear range of plastic materials was chosen to be between 40 and 199 MPa, whereas the linear range of brittle materials was defined between 100 and 398 MPa. With this differentiation a very exact description of the compression behavior was done but the problem was that there resulted kind of subjective characterization based on the forming of the curves in the Heckel plot. To prevent misinterpretation *K* values were also calculated from the whole date range and although the squared correlation coefficient was worse in this cases the general tendencies could always be confirmed. For the interpretation of the mixtures *K* values for both data ranges were calculated and the range with the higher squared correlation coefficient was chosen for the interpretation.

5.3.1 β-Galactosidase powder

The slope of a Heckel plot characterizes the compression behavior of a powder. The behavior of β-galactosidase powder was characterized in comparison with the excipients microcrystalline cellulose and dicalcium phosphate dihydrate, which show typical behavior for plastic and brittle properties, respectively. A Heckel plot of these three bulk materials is shown in Fig. 5.15.

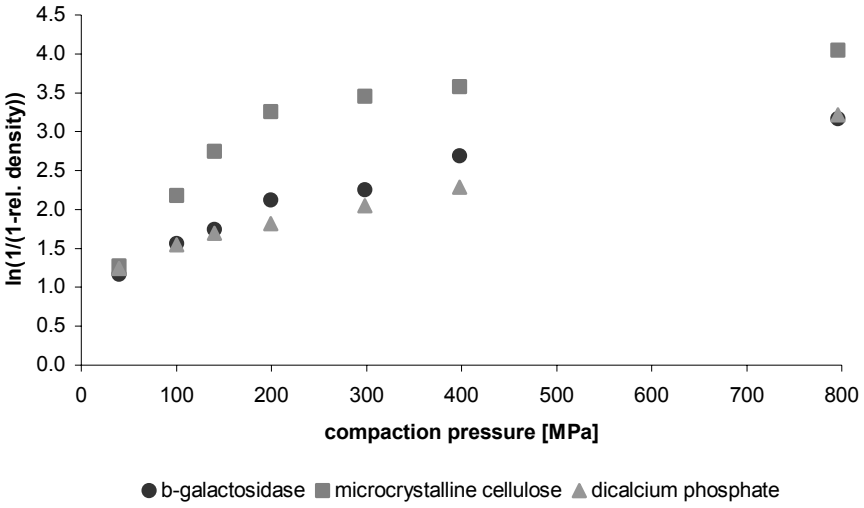

Fig. 5.15: *Heckel plots of bulk materials, comparison of β-galactosidase, microcrystalline cellulose and dicalcium phosphate dihydrate.*

Fig. 5.15 shows that the two bulk substances β-galactosidase and dicalcium phosphate dihydrate behave very similar. Contrary to that the behavior of microcrystalline cellulose is obviously different. As dicalcium phosphate dihydrate is known to have brittle properties, β-galactosidase powder can be classified as brittle substance. Calculation of the *K* values indicating the slope of the curves also confirms that statement. *K* values of β-galactosidase and dicalcium phosphate dihydrate are 0.00373 MPa^{-1} (R^2 = 0.961) and 0.00241 MPa^{-1} (R^2 = 0.997), respectively, whereas the *K* value of microcrystalline cellulose is 0.01288 MPa^{-1} (R^2 = 0.983). The use of out-of-die values has the disadvantage that much less data points are obtained. Nevertheless the similarity of the *K* values for β-galactosidase and dicalcium phosphate dihydrate is obvious and the *K* value for microcrystalline cellulose confirms the fact that plastic substances have higher *K* values than brittle substances.

A plot of the compaction pressure versus the relative density (Fig. 5.16, Fig. 5.17) and the interpretation with the modified Heckel equation (Eq. (3.12)) confirm these results.

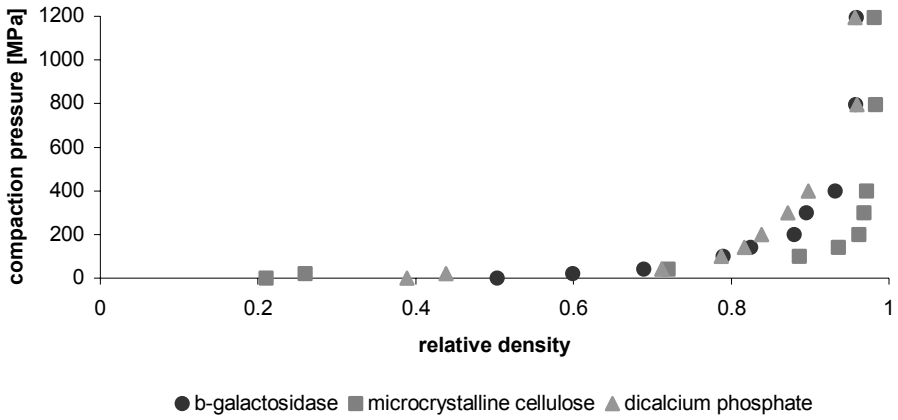

Fig. 5.16: *Compaction pressure σ as a function of the relative density. Comparison of β-galactosidase, microcrystalline cellulose and dicalcium phosphate dihydrate.*

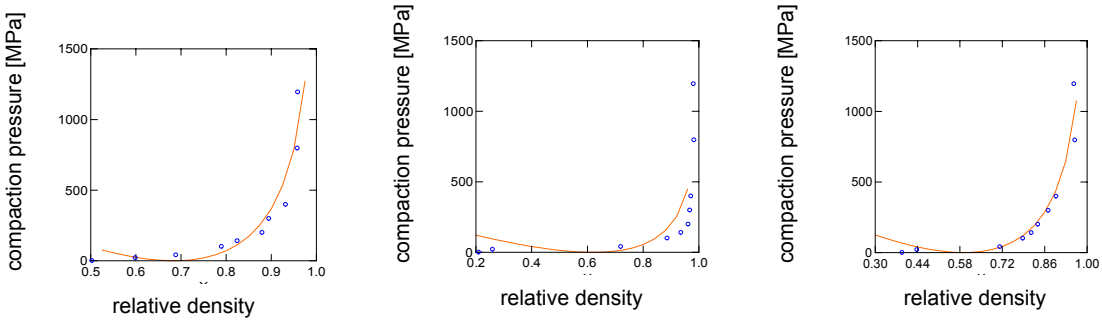

Fig. 5.17: *Compaction pressure σ as a function of the relative density with β-galactosidase, microcrystalline cellulose and dicalcium phosphate dihydrate (from left to right). The solid line represents the model according to Eq. (3.12).*

C values for β-galactosidase and dicalcium phosphate dihydrate are with values of 0.00040 MPa^{-1} (R^2 = 0.897) and 0.00058 MPa^{-1} (R^2 = 0.924) quite low and lie near together. The *C* value for microcrystalline cellulose is with 0.00114 MPa^{-1} (R^2 = 0.701) much higher, indicating a plastic powder behavior. The lower value for the squared correlation coefficient of microcrystalline cellulose can be explained with the low number of data points in the region of lower compaction pressures, which have more influence on the plastic powder behavior. Generally the interpretation with the modified Heckel plot was difficult because this model describes mainly the transition

between the state of a powder and the state of a tablet. For that reason the lack of data points at very low compaction pressures complicated the interpretation.

A further characterization of the behavior of the enzyme powder under compression was done by investigation of the activity loss (Fig. 5.18).

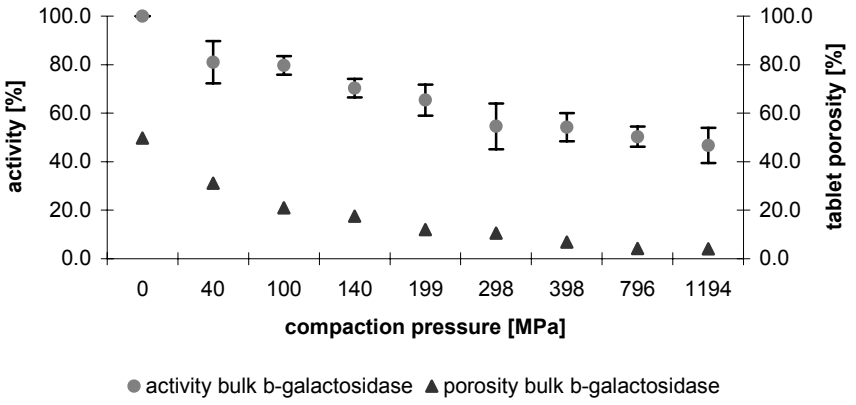

Fig. 5.18: *Comparison of activity loss and tablet porosity of bulk β-galactosidase tablets (n = 5). Error bars: ± 1 standard deviation.*

The activity decreased steadily up to a compaction pressure of 199 MPa, at higher compaction pressures, the curve flattened and the degree of the activity loss decreased. A comparison with the porosity of the tablets showed that the curve also flattened slightly after the pressure of 199 MPa. This statement was proved with the partition of each curve into two parts by non-linear regression. For both curves the partition between 199 and 298 MPa showed highest squared correlation coefficients R^2 with 0.962 for the activity curve and 0.937 for the porosity curve. The slopes in the first sections were –0.157 for the activity curve and –0.176 for the porosity curve, respectively whereas the slopes in the second sections were –0.009 for the activity curve and –0.006 for the porosity curve, respectively.

The correlation coefficient *r* (Eq. (4.7)) of these two curves showed a value of 0.965 proofing a positive correlation. This correlation can be explained with the reduction of interparticle space, which is big in the stage of particle movement and rearrangement and diminishes in the stage of particle deformation and therefore by a destruction of the native state of the enzyme under compression. This destruction is probably linear in the stage of particle movement and rearrangement. In the stage of deformation, i.e. the region of the flattening slope, shearing forces will probably decrease as a consequence of the reduced particle movement. Teng and Groves (1988) found a similar behavior for urease and proposed that at a certain compression stadium the

amount of denatured protein is already high and can protect the native protein due to a higher resistance to shear. Although a higher resistance to shearing forces from denatured proteins compared to native proteins is not proved.

5.3.2 Mixture with plastic excipient

A comparison of the bulk materials, β-galactosidase and microcrystalline cellulose, with a mixture of 50% (w/w) of each of the two substances showed that there is no linear behavior but there can be seen a slight dominance of β-galactosidase powder in the Heckel plot (Fig. 5.19).

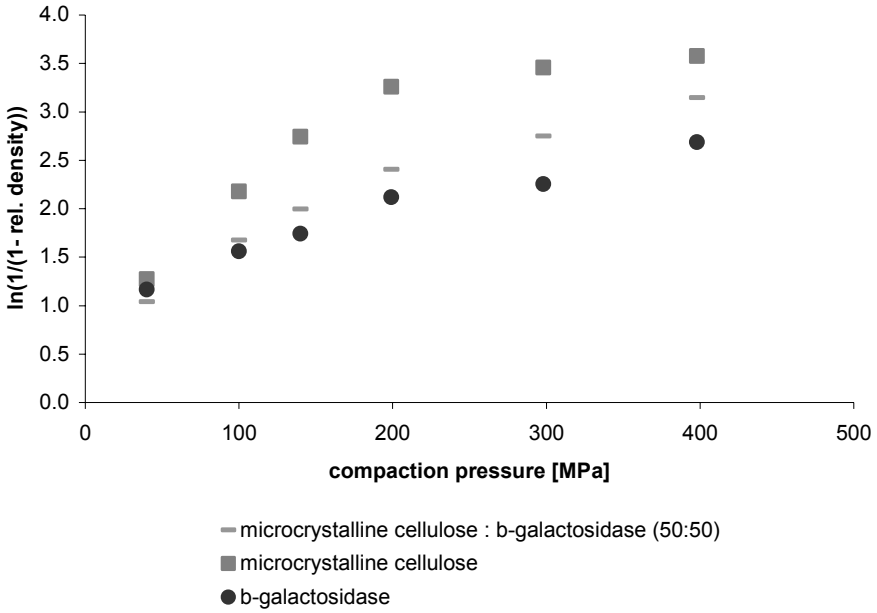

Fig. 5.19: *Heckel plots of bulk β-galactosidase and microcrystalline cellulose and their mixture (50:50).*

The calculation of the *C* value (Eq. (3.12)) of 0.00068 MPa^{-1} (R^2 = 0.847) of this 50% mixture confirmed that statement. Interpretation of the *K* value, though was difficult because the result was depending on the linear region chosen for the calculation (Eq. (3.3)). Calculation of the *K* value in the range of 100-398 MPa for brittle substances led to a value of 0.00489 MPa^{-1} (R^2 = 0.972) confirming the interpretation resulting from considering the Heckel plot, i.e. the behavior of the mixture is dominated by the bulk β-galactosidase powder. Calculation of the *K* value in the range of 40-199 MPa for plastic substances, though resulted in a value of 0.00866 MPa^{-1} (R^2 = 0.988), obviously with higher squared correlation coefficient. To prevent misinterpretation, the *K* values of

the three substances, β-galactosidase powder, microcrystalline cellulose and their 50% mixture have been recalculated over the whole compression range (without relative densities higher than 0.95). Resulting in the following *K* values: 0.00465 MPa^{-1} (R^2 = 0.947) for β-galactosidase, 0.02004 MPa^{-1} (R^2 = 0.944) for microcrystalline cellulose and 0.00690 MPa^{-1} (R^2 = 0.944) for the mixture.

These results demonstrate the difficulties and also the weakness of the use of Heckel equation for the characterization of substances. The *K* value for the slope can strongly vary with the linear range defined for the calculation.

The yield strength of all binary mixtures was calculated from the *K* values with the highest squared correlation coefficient (Eq. (3.5)) and plotted in Fig. 5.20.

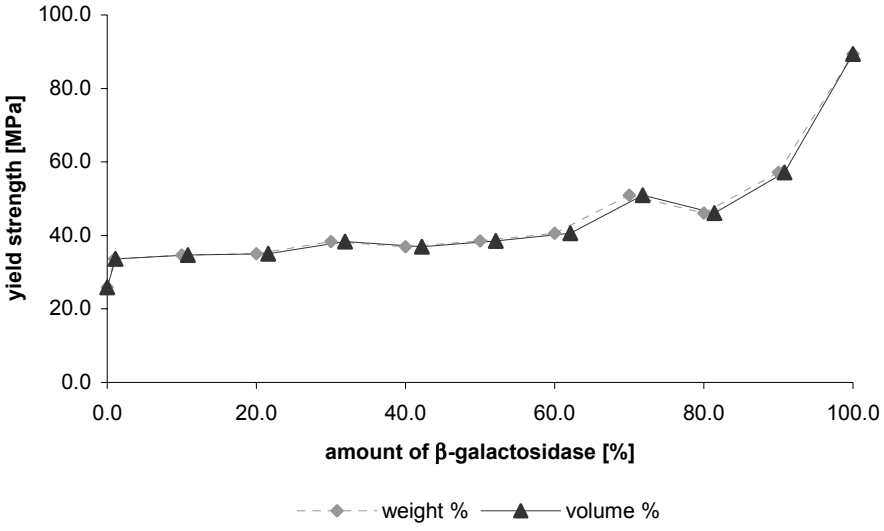

Fig. 5.20: *Yield strength of the mixtures of bulk β-galactosidase and microcrystalline cellulose.*

Looking at the yield strength behavior of plastic-brittle mixtures it can be seen that the system shows a change in behavior in the region of an amount of enzyme powder of 70%. Below that concentration of enzyme powder, the tendency of the compressed material to undergo deformation is dominated by the plastic material. This result indicates that microcrystalline cellulose forms an “infinite” cluster for concentrations above 30% (w/w). Thus according to Fig. 5.20 there is a percolation threshold between 70-80% (w/w) of β-galactosidase.

5.3.3 Mixture with brittle excipient

The comparison of two brittle bulk materials, namely β-galactosidase powder and dicalcium phosphate dihydrate with their 50% (w/w) mixture showed a negative deviation of the mixture from the bulk substances (Fig. 5.21).

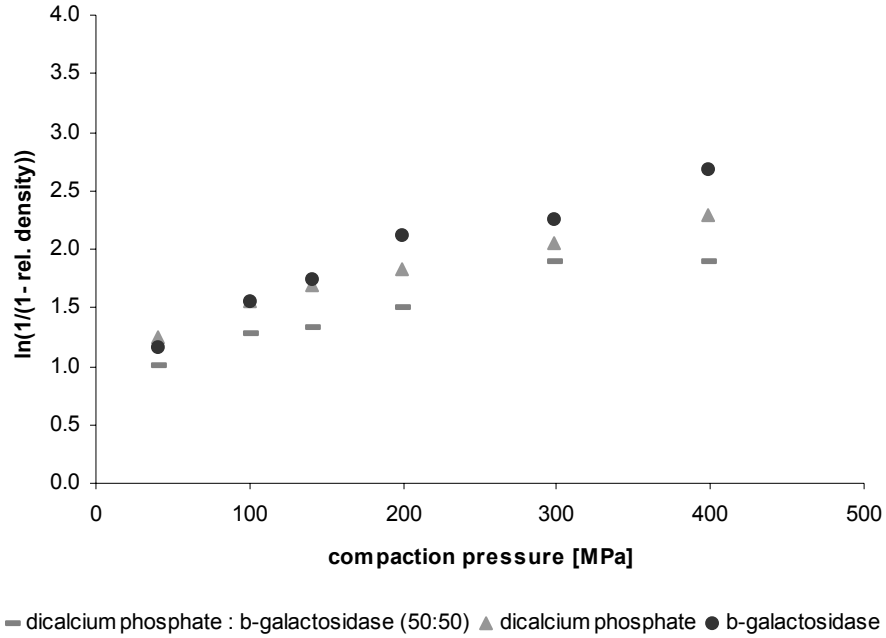

Fig. 5.21: *Heckel plots of bulk β-galactosidase and dicalcium phosphate dihydrate and their mixture (50:50).*

Calculation of the *K* value (Eq. (3.3)) and the *C* value (Eq. (3.12)) of the 50% mixture, whereas the *K* value was 0.00257 MPa^{-1} (R^2 = 0.918) and the *C* value was 0.00024 MPa^{-1} (R^2 = 0.872), showed that the slope of the mixture was almost the same as the slope of the bulk substance dicalcium phosphate dihydrate. Densification properties of different brittle particles seem to be very similar and have no big influences on each other.

Calculation of the yield pressure of all binary mixtures from the *K* values (Eq. (3.5)) confirm that statement (Fig. 5.22).

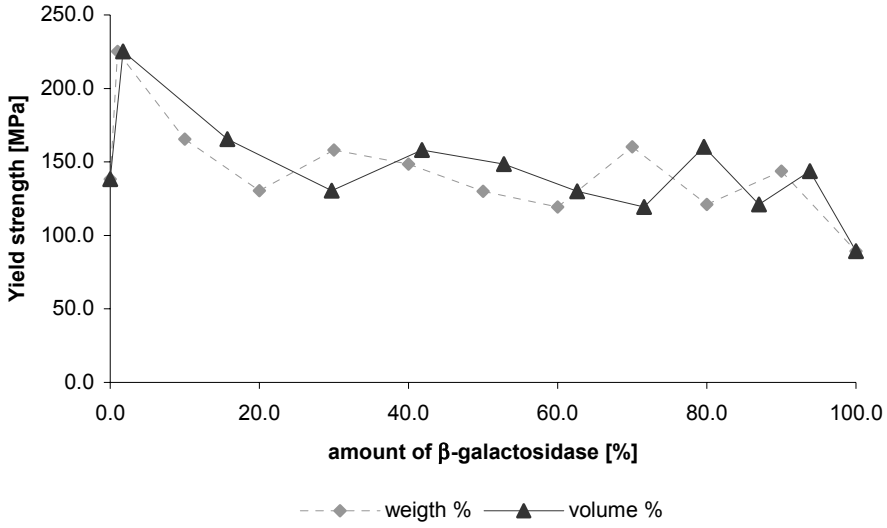

Fig. 5.22: *Yield strength of the mixtures of bulk β-galactosidase and dicalcium phosphate dihydrate.*

Although there are some irregularities in this curve the general tendency for the mixtures is the same as for the bulk substances and therefore it can be said that mixtures of brittle substances do not change their deformation behavior compared to the bulk substances of the mixtures. Due to the fact that both substances have similar yield strength it is not surprising that it is difficult to detect a percolation threshold.

5.4 Compression behavior of granulates

The produced granulates were not fractionated before compression. The reason was to have an irregular product as a contrast to the very regular product of coated pellets.

The compression behavior was characterized by investigation of the Heckel plots. In addition, the behavior of the enzyme activity under pressure was detected and correlated with the porosity of the compacts.

5.4.1 Granulate from plastic excipient

The comparison of the behavior of the β-galactosidase-microcrystalline cellulose granulate and the microcrystalline cellulose powder in the Heckel plot (Fig. 5.23) shows that the produced granulate had the same plastic properties like its raw material microcrystalline cellulose.

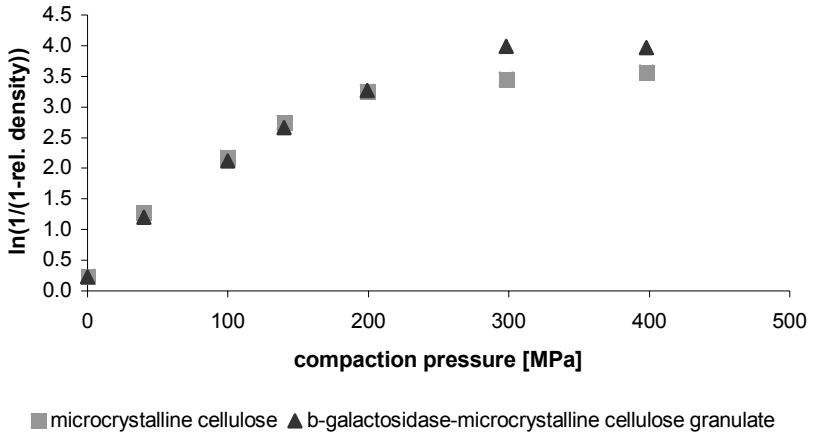

Fig. 5.23: *Heckel plots of microcrystalline cellulose and β-galactosidase-microcrystalline cellulose granulate.*

The slope indicated a plastic behavior with a *K* value of 0.01288 MPa^{-1} (R^2 = 0.983) for microcrystalline cellulose powder and 0.01318 MPa^{-1} (R^2 = 0.989) for β-galactosidase-microcrystalline cellulose granulate.

The behavior of the activity as well as the porosity of the granulate compacts are shown in Fig. 5.24.

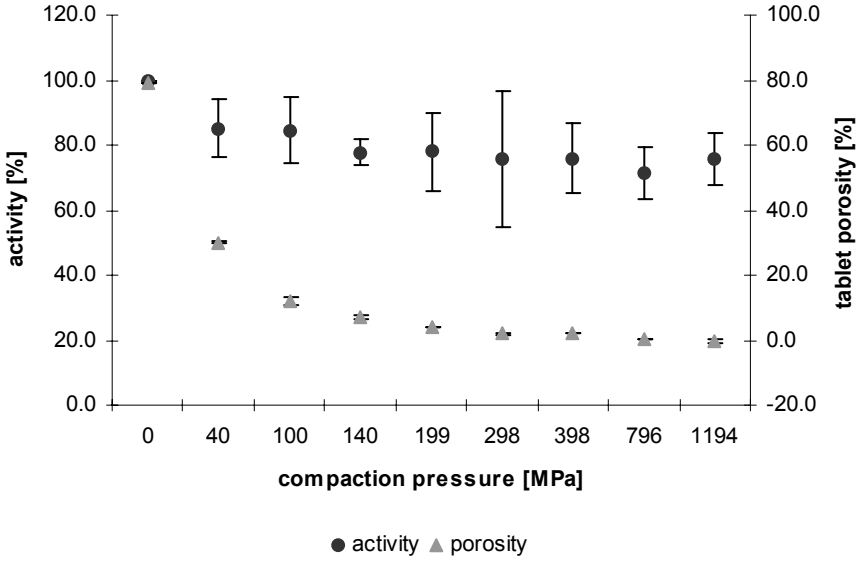

Fig. 5.24: *Comparison of activity loss and tablet porosity of β-galactosidase-microcrystalline cellulose granulate (n = 10). Error bars: ± 1 standard deviation.*

The activity at maximum compaction pressure was 75.9% at a porosity of –0.4%. The negative porosity can be explained with a total compression of the compact to zero porosity, the deviation towards a negative value is probably caused by inaccuracy of the measurement. The relative density of the compacts and thus the porosity was calculated from the weight and the thickness of the compacts, variability from the balance and the measurement with the thickness gauge are possible. The high standard deviations are probably caused by the irregularity of the compacted granules. The activity decrease of the compacted granulate was smaller than the maximum activity decrease from compacted β-galactosidase powder, which was 46.7% at a porosity of 4.1%. The correlation coefficient *r* (Eq. (4.7)) of the correlation of the activity and the porosity curve of the granulate was 0.959. Obviously the forming of the curves is still correlated and thus the activity loss is dependent on the tablet porosity. The fact that the activity loss of the compacted enzyme powder was higher with a higher final porosity suggests further influences on the activity under compression. It is conceivable that the deformation properties of the particles as well as their size and shape additionally influence the activity loss.

5.4.2 Granulate from brittle excipient

The interpretation of the Heckel plots (Fig. 5.25) of the β-galactosidase-dicalcium phosphate dihydrate granulate and the raw material dicalcium phosphate dihydrate showed that the deformation properties of the granulate were more plastic than its brittle raw material. The *K* value

was 0.00241 MPa^{-1} (R^2 = 0.997) for dicalcium phosphate dihydrate powder and 0.00785 MPa^{-1} (R^2 = 0.993) for β-galactosidase-dicalcium phosphate dihydrate granulate. This behavior was expected, since granulates are known to have better compression properties than powders, especially when the powders show brittle properties.

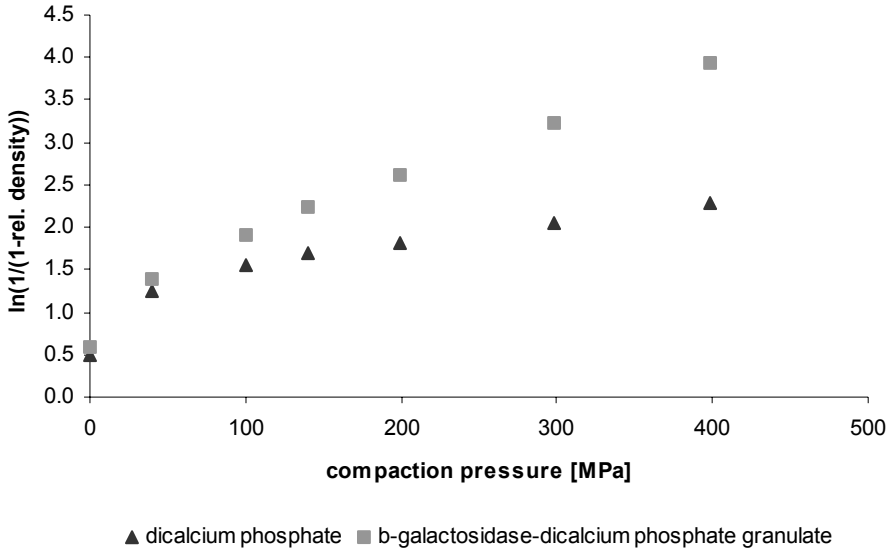

Fig. 5.25: *Heckel plots of dicalcium phosphate dihydrate and β-galactosidase-dicalcium phosphate dihydrate granulate.*

The measured activity at maximum compaction pressure (1194 MPa) was 61.2% at a porosity of –2.1%. At the very high compaction pressure of 1194 MPa there was again reached zero porosity. The behavior of the activity and the porosity of the compacted granulate over the whole compression range is shown in Fig. 5.26.

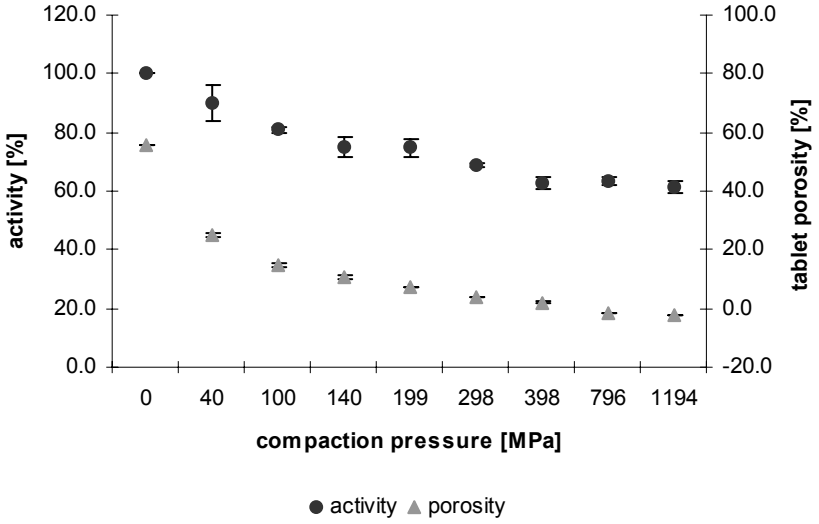

Fig. 5.26: *Comparison of activity loss and tablet porosity of β-galactosidase-dicalcium phosphate dihydrate granulate (n = 10). Error bars: ± 1 standard deviation.*

Positive correlation was proved again with the correlation of the activity and the porosity curves with a correlation coefficient *r* of 0.948. The activity loss seemed to depend again on the tablet porosity. The activity at maximum pressure (61.2%) was lower than in the compacted β-galactosidase-microcrystalline cellulose granulate (75.9%), but higher than in the compacted enzyme powder (46.7%). These results are consistent with the degree of deformation properties defined with Heckel equation, where the *K* value was 0.00785 MPa^{-1} (R^2 = 0.993) for β-galactosidase-dicalcium phosphate dihydrate granulate, 0.01318 MPa^{-1} (R^2 = 0.989) for β-galactosidase-microcrystalline cellulose granulate and 0.00373 MPa^{-1} (R^2 = 0.961) for β-galactosidase powder, respectively. The activity at maximum pressure of these three preparations was plotted against the *K* values (Fig. 5.27) and the correlation coefficient R^2 of the regression line of these three data points was 0.995, indicating dependence between the compression behavior and the activity loss under pressure.

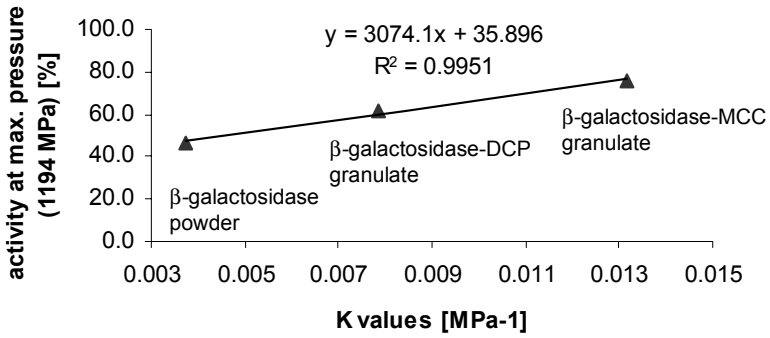

Fig. 5.27: *Comparison of the activity at maximum pressure (1194 MPa) and the K values of the investigated β-galactosidase granulates and the β-galactosidase powder.*

A comparison of the behavior of the activity under pressure of the enzyme powder and the two different enzyme granulate preparations is given in Fig. 5.28.

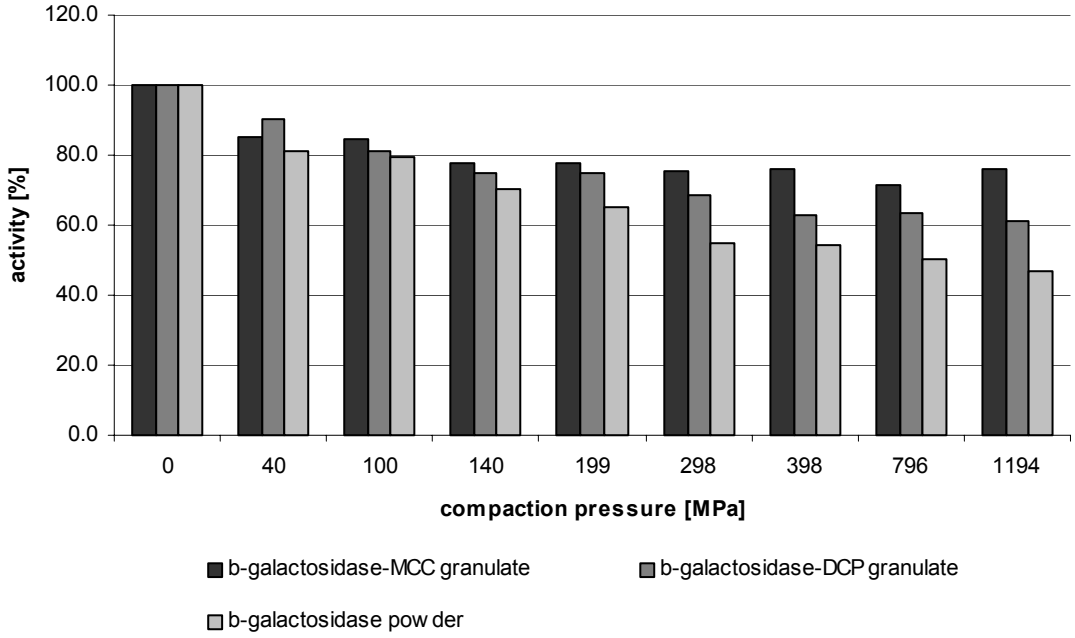

Fig. 5.28: *Comparison of the activity loss in the compacted enzyme granulates and enzyme powder.*

It is obvious that especially at higher compaction pressures the activity loss in the compacted enzyme powder was higher than in the compacted β-galactosidase-dicalcium phosphate dihydrate granulate. The lowest activity loss was found in the compacted β-galactosidase-microcrystalline cellulose granulate.

5.5 Compression behavior of pellets

The compression behavior of the uncoated raw pellets and the enzyme-coated pellets was investigated and compared. This characterization was done with Heckel equation and the interpretation of scanning electron microscopy pictures. The activity loss of the compacted enzyme pellets was also measured and correlated with the porosity of the compacts.

5.5.1 Pellets from plastic excipient

The Heckel plots of the raw microcrystalline cellulose pellets and the coated β-galactosidase-microcrystalline cellulose pellets are shown in Fig. 5.29.

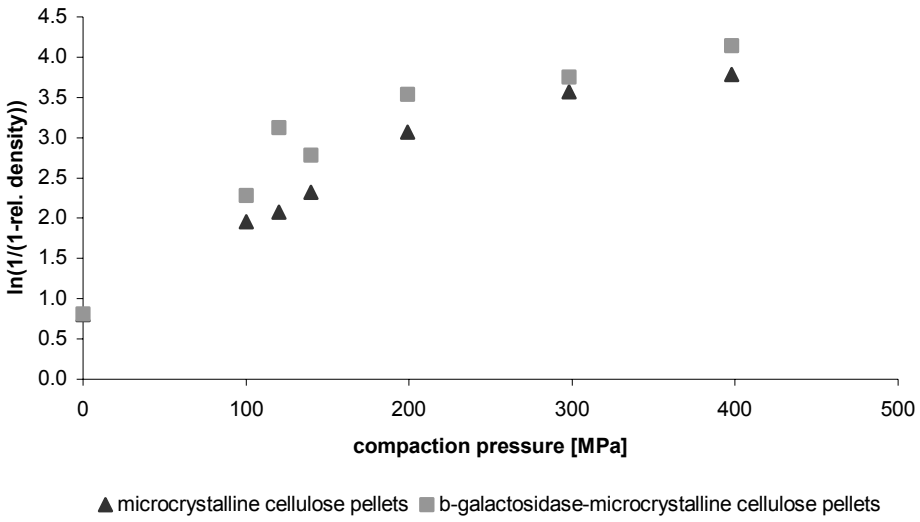

Fig. 5.29: *Heckel plots of raw microcrystalline cellulose pellets and coated β-galactosidase-microcrystalline cellulose pellets.*

The interpretation of these Heckel plots and the calculation of the *K* values was difficult, because there were no data points below a compaction pressure of 100 MPa besides the data for relative poured density at zero pressure. Generation of out-of-die data requires the production of stable compacts, which was only possible in this case at compaction pressures higher than 100 MPa. Due to the lack of data points at lower compaction pressures, there is no information about the particle rearrangement behavior, i.e. the presence of a bend in the curve. Such a behavior is typical for pellets as the spherical pellets are arranged immediately, i.e. without the application of a pressure > 0. Moreover, at a compaction pressure of 199 MPa the curves flattened, because there was no further densification possible. The zero porosity was already reached at that compaction pressure. For the calculation of the *K* value the compression range between 0 and 199 MPa was used with a resulting *K* value of 0.01128 MPa^{-1} (R^2 = 0.996) for microcrystalline cellulose pellets and 0.01507 MPa^{-1} (R^2 = 0.928) for β-galactosidase-microcrystalline cellulose pellets.

These results have been confirmed looking at the arrangement and deformation of the pellets in the compacts with a scanning electron microscope (Fig. 5.30, Fig. 5.31, Fig. 5.32, Fig. 5.33, Fig. 5.34).

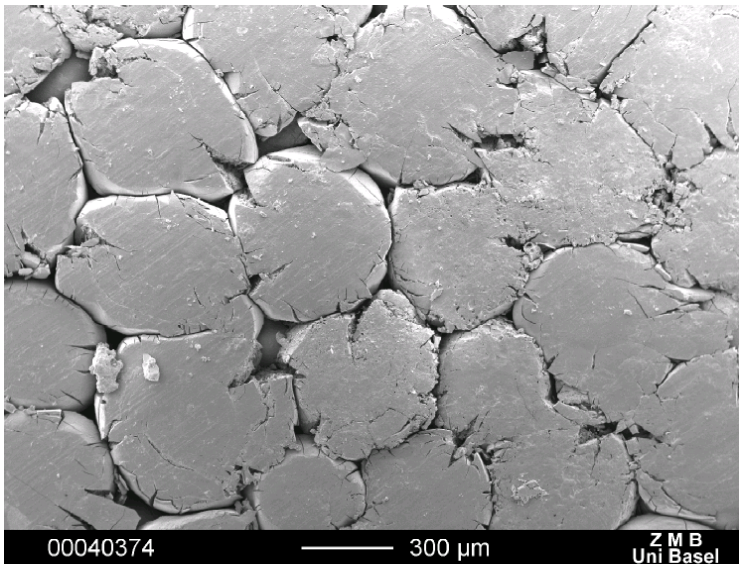

Fig. 5.30: *SEM picture of the surface of a compact of β-galactosidase-microcrystalline cellulose pellets at a compaction pressure of 100 MPa (50x).*

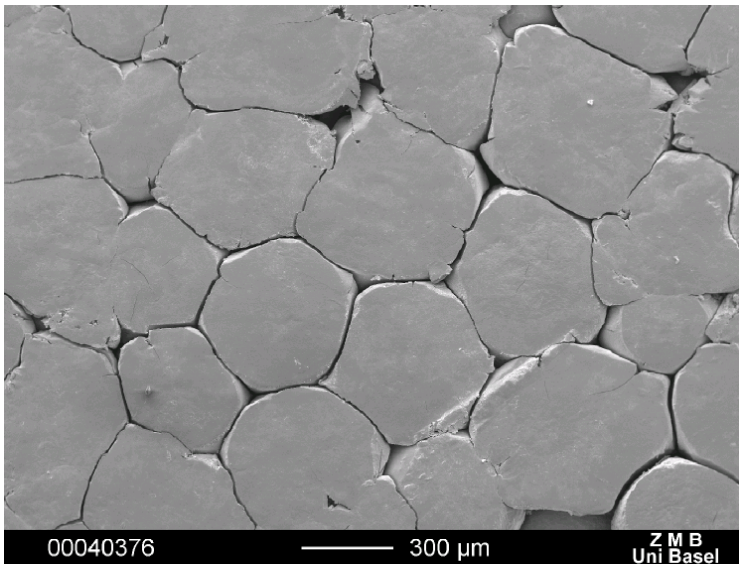

Fig. 5.31: *SEM picture of the surface of a compact of β-galactosidase-microcrystalline cellulose pellets at a compaction pressure of 199 MPa (50x).*

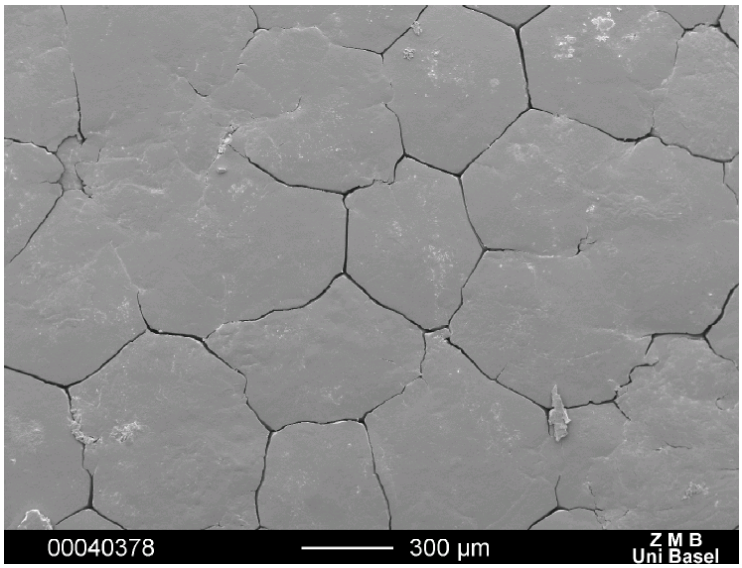

Fig. 5.32: *SEM picture of the surface of a compact of β-galactosidase-microcrystalline cellulose pellets at a compaction pressure of 1194 MPa (50x).*

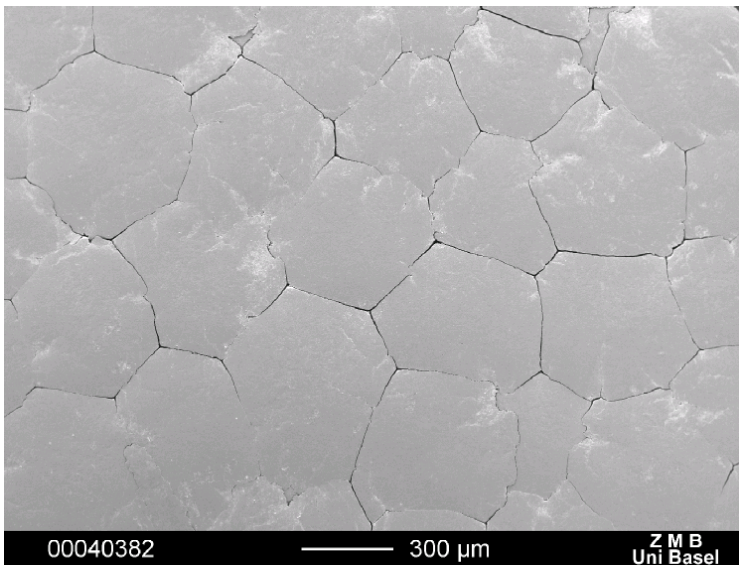

Fig. 5.33: *SEM picture of the surface of a compact of microcrystalline cellulose pellets at a compaction pressure of 1194 MPa (50x).*

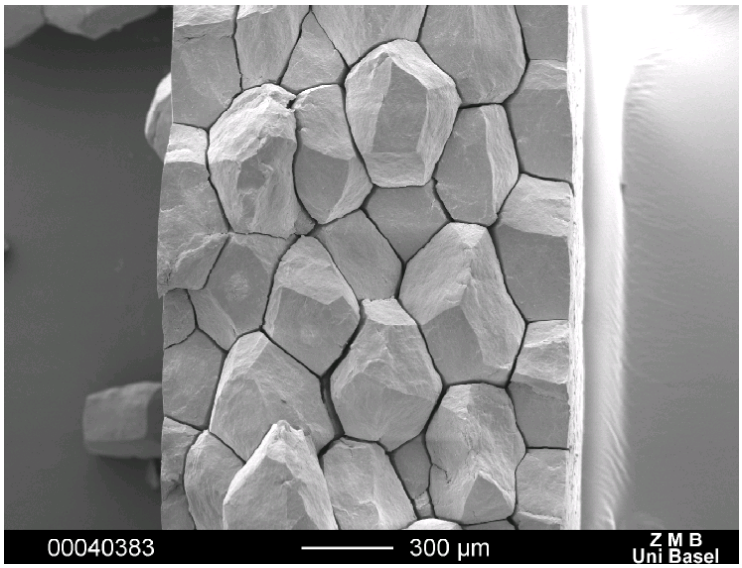

Fig. 5.34: *SEM picture of the cross-section of a compact of microcrystalline cellulose pellets at a compaction pressure of 1194 MPa (50x).*

The comparison of the behavior of the coated enzyme pellets at compaction pressures of 100, 199 and 1194 MPa on the surface of the compacts showed that there was only low porosity detectable at 100 MPa, which was fast decreasing and totally disappeared at the highest compaction pressure applied. The behavior of the compacted raw pellets did not differ (Fig. 5.33, Fig. 5.34). The cross-section of the compact illustrates the loss of porosity with a deformation of the pellets. It is also obvious that the pellets only changed their shape but did not break. Probably, there are tendencies that the coating promoted the plastic deformation capacity of the pellets, which could not be seen in the SEM pictures, but the slightly higher *K* value and the higher stability observed on the compacts from the coated pellets led to that supposition.

The investigation of the activity loss under pressure and the correlation with the porosity of the compacted β-galactosidase-microcrystalline cellulose pellets resulted in the data plotted in Fig. 5.35.

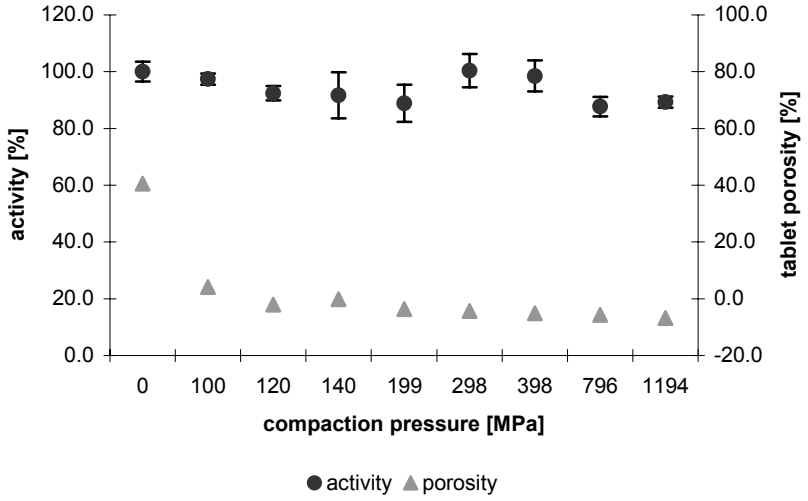

Fig. 5.35: *Comparison of activity loss and tablet porosity of β-galactosidase-microcrystalline cellulose pellets (n = 5). Error bars: ± 1 standard deviation.*

Although zero porosity was reached very fast, the forming of the activity curve was distinctly different to the activity curves of the other substances investigated. The activity loss at maximum compaction pressure was 89.3% at a porosity of –6.8%. It is supposed that the activity is stable over the whole compression range, because there was no constant activity decrease detectable. The unsteadiness of the standard deviations of the various data points, which is normal for enzyme assays, makes the interpretation of statistical significance difficult.

The calculation of the correlation between the activity and the porosity curve (Eq. (4.7)) led to a correlation coefficient *r* of 0.493. This value reflects the bad correlation between these two curves. The compression of a mixture of β-galactosidase-microcrystalline cellulose pellets with microcrystalline cellulose pellets in a mixture ratio of 50% each under the same assay conditions showed an activity of 94.5% at maximum compaction pressure and a porosity of –6.9%. The correlation coefficient *r* was 0.402. These results were consistent with the compacted β-galactosidase-microcrystalline cellulose pellets. Obviously there was no difference in the compression of bulk β-galactosidase-microcrystalline cellulose pellets and the compression of a 50% mixture of the same bulk enzyme pellets with the raw microcrystalline cellulose pellets. The absence of a significant activity loss during compression of pellets from a plastic raw material can probably be explained on the one hand again by the plastic properties seen in the Heckel plot, because obviously there is a positive effect of plastic compression properties on the behavior of the activity under pressure. On the other hand there must be an additional effect explaining the total absence of activity loss. It is supposed that the special deformation character of the pellets protects the enzyme. The fact that the pellets do not break and the various pellets keep their individuality although they fit their form to reach maximum packaging (Fig. 5.32) causes very low shearing

forces. The round shaped, regular particles get not pushed into each other. The size and the shape of the individual particles are the same due to the regularity of the pellets, thus they cannot influence each other under pressure.

5.5.2 Pellets from brittle excipient

The Heckel plots of the sugar pellets and the β-galactosidase-sugar pellets are shown in Fig. 5.36.

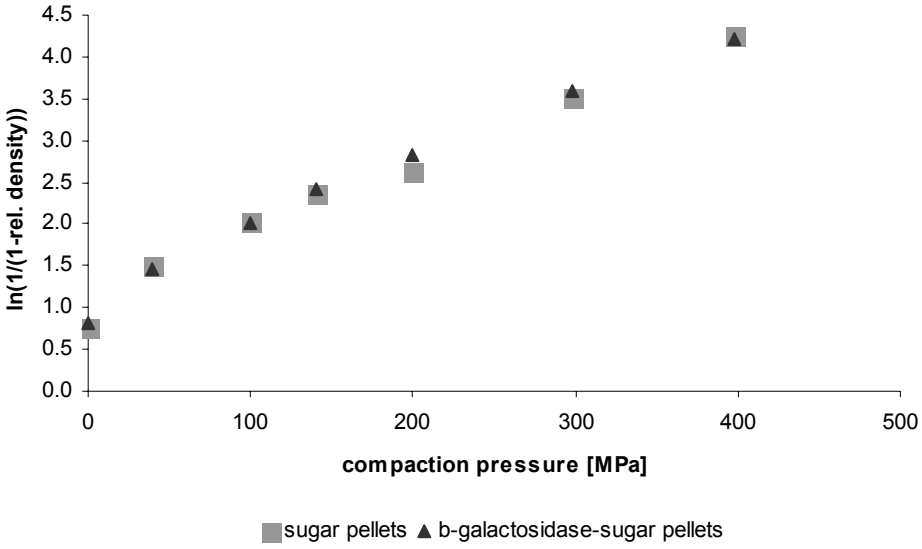

Fig. 5.36: *Heckel plots of raw sugar pellets and coated β-galactosidase-sugar pellets.*

The compression behavior of both pellets was the same in the Heckel plot, the lack of a distinct bend indicated plastic properties. The *K* value calculated between compaction pressures of 0-199 MPa was 0.00968 MPa^{-1} (R^2 = 0.944) for the sugar pellets and 0.01008 MPa^{-1} (R^2 = 0.981) for the β-galactosidase-sugar pellets. The enzyme coating did not seem to have any influences on the compression behavior of the sugar pellets.

The behavior of the single pellets in the compacts was investigated by interpretation of the scanning electron microscopy pictures (Fig. 5.37, Fig. 5.38, Fig. 5.39, Fig. 5.40, Fig. 5.41, Fig. 5.42, Fig. 5.43).

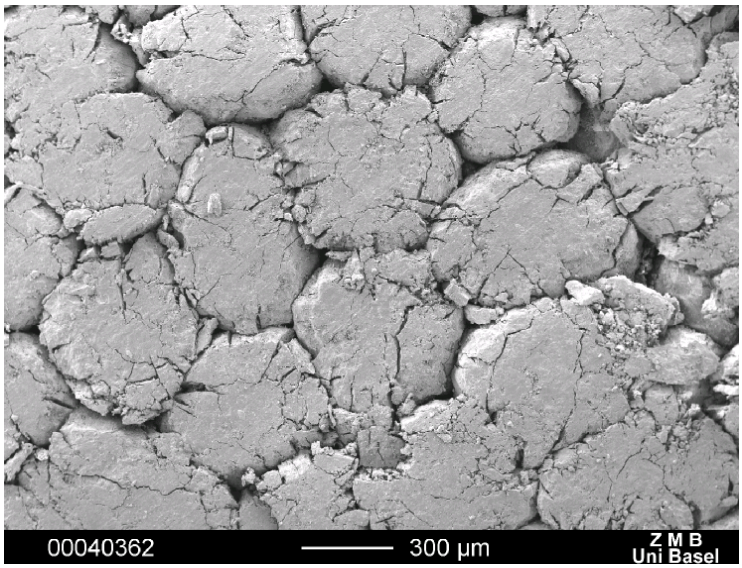

Fig. 5.37: *SEM picture of the surface of a compact of β-galactosidase-sugar pellets at a compaction pressure of 40 MPa (50x).*

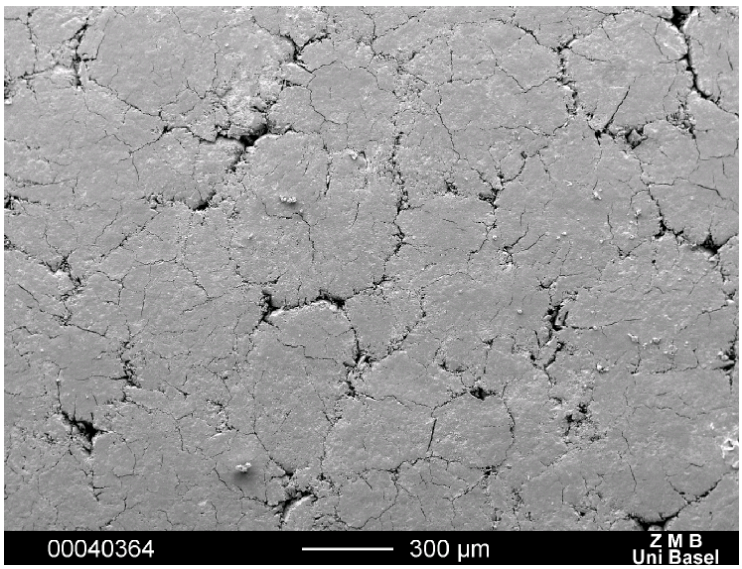

Fig. 5.38: *SEM picture of the surface of a compact of β-galactosidase-sugar pellets at a compaction pressure of 199 MPa (50x).*

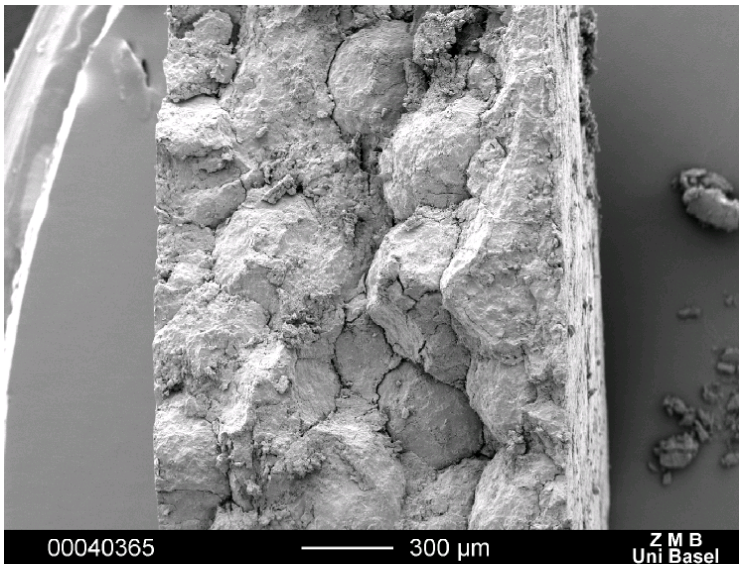

Fig. 5.39: *SEM picture of the cross-section of a compact of β-galactosidase-sugar pellets at a compaction pressure of 199 MPa (50x).*

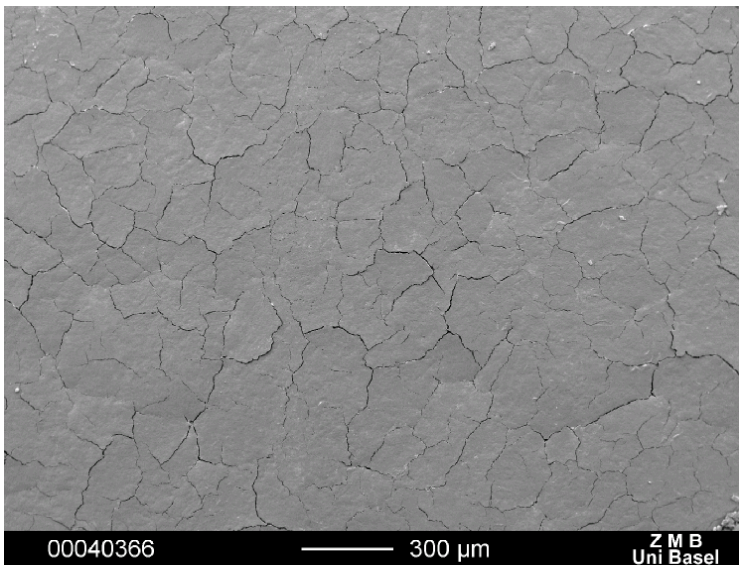

Fig. 5.40: *SEM picture of the surface of a compact of β-galactosidase-sugar pellets at a compaction pressure of 1194 MPa (50x).*

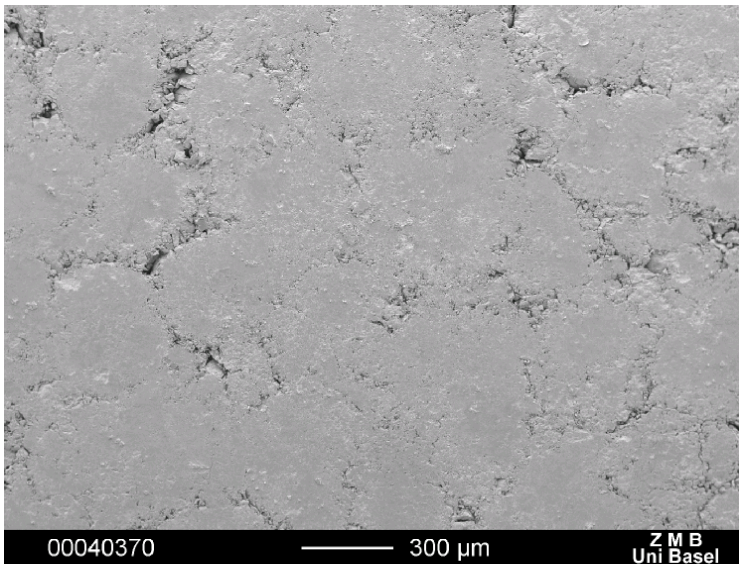

Fig. 5.41: *SEM picture of the surface of a compact of sugar pellets at a compaction pressure of 199 MPa (50x).*

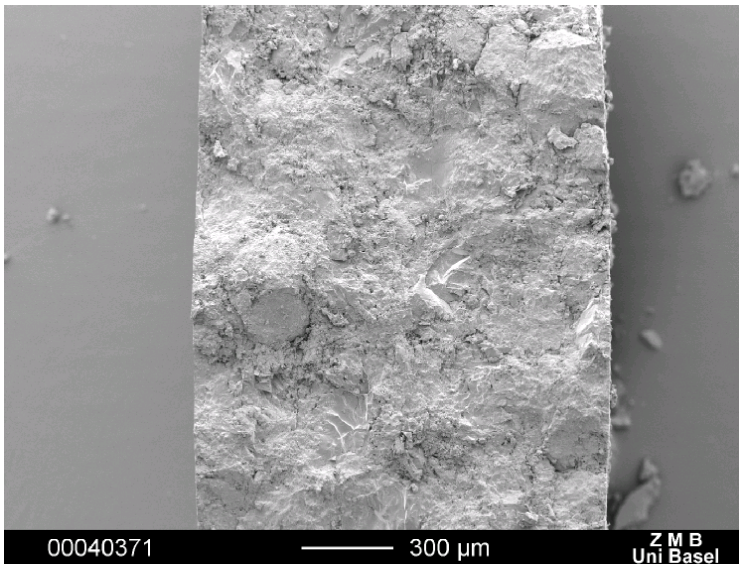

Fig. 5.42: *SEM picture of the cross-section of a compact of sugar pellets at a compaction pressure of 199 MPa (50x).*

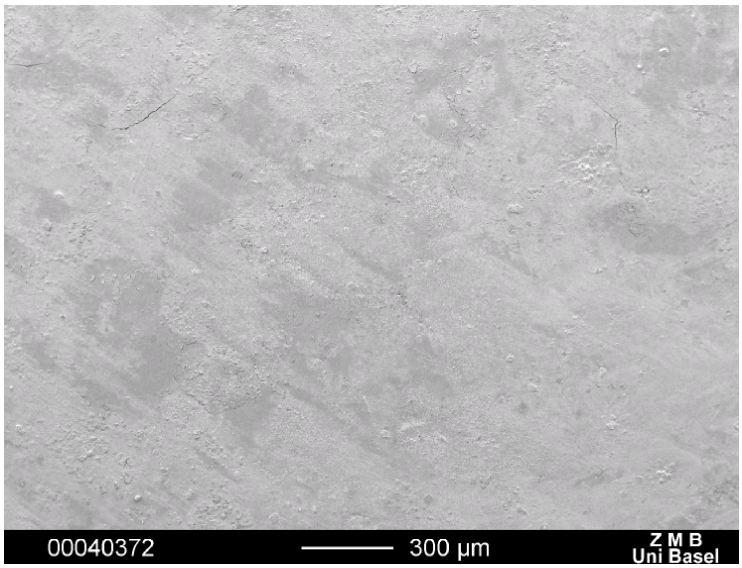

Fig. 5.43: *SEM picture of the surface of a compact of sugar pellets at a compaction pressure of 1194 MPa (50x).*

The kind of deformation of the sugar pellets under compression was different to the kind of deformation of the microcrystalline cellulose pellets, although the *K* value of the Heckel equation was very similar for all types of pellets investigated. Obviously the sugar pellets built cracks at the surface under pressure. Although the pellets did not break and the single pellets could be still distinguished in the cross-section (Fig. 5.39) the demarcation between the particles slightly disappeared. It is conceivable that with the fracture at the surface of the pellets the surface got rough and some powder particles of different size and shape were built. These particles could be pushed into each other. The surface of the coated pellets was more stable since the degree of cracking was smaller than within the uncoated pellets. Therefore the pushing of the fractured particles at the pellet surface into each other was bigger (Fig. 5.41, Fig. 5.42) and resulted in a compact, amorphous mass at the highest compaction pressure of 1194 MPa (Fig. 5.43).

The extent of the activity loss and the development of the porosity in the pellet compacts was again compared (Fig. 5.44).

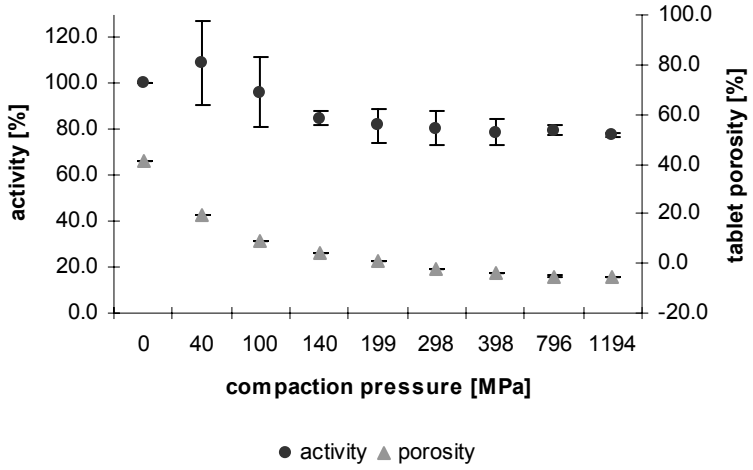

Fig. 5.44: *Comparison of activity loss and tablet porosity of β-galactosidase-sugar pellets (n = 10). Error bars: ± 1 standard deviation.*

The activity measured at maximum compaction pressure was 77.6% with a porosity of -5.25%. The correlation coefficient *r* of the two curves was 0.821. The correlation of the activity and the porosity curve of the compacted β-galactosidase sugar pellets was worse than the correlation of these two curves from the powder and granulate products, but better than the correlation of the two curves from the compacted β-galactosidase-microcrystalline cellulose pellets. Obviously the building of powder particles at the surface of the various pellets caused by the cracking leads to an enhanced number of shearing forces. Due to that reason the activity loss is higher than within the compacted β-galactosidase-microcrystalline cellulose pellets. But the fact that the pellets did not break and only slightly changed their shape although the demarcation between the single pellets disappeared seemed to cause smaller shearing forces than the one built during the compression of powders and granulates.

A comparison of the behavior of the activity under pressure of the enzyme powder and the two different enzyme pellet preparations is given in Fig. 5.45.

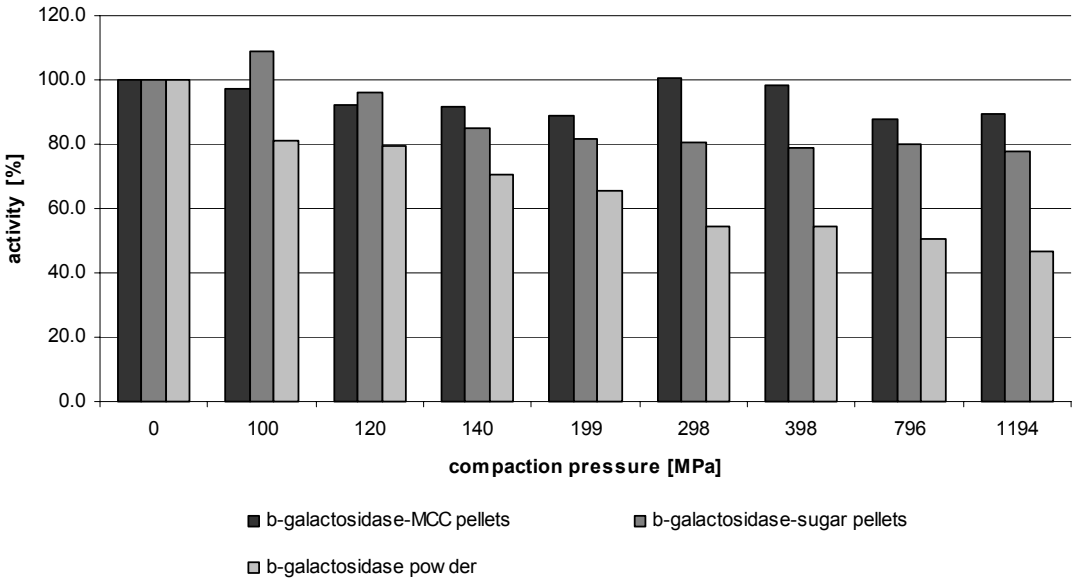

Fig. 5.45: *Comparison of the activity loss in the compacted enzyme pellets and enzyme powder.*

It is obvious that the compacted enzyme powder showed a steady activity loss, while the compacted β-galactosidase-microcrystalline cellulose pellets did not show significant activity loss. The compacted β-galactosidase-sugar pellets showed no significant activity decrease between 0 and 120 MPa. At 140 MPa though there was a significant change in the behavior of the activity probably because the built powder particles at the surface of the pellets started to get pushed into each other. Between the compaction pressures of 199 and 1194 MPa the activity loss was not significant, the shearing forces probably remained low because the single pellets did not break and only slightly changed their shape.

The behavior of all investigated β-galactosidase preparations concerning the activity loss under pressure is summarized in Fig. 5.46.

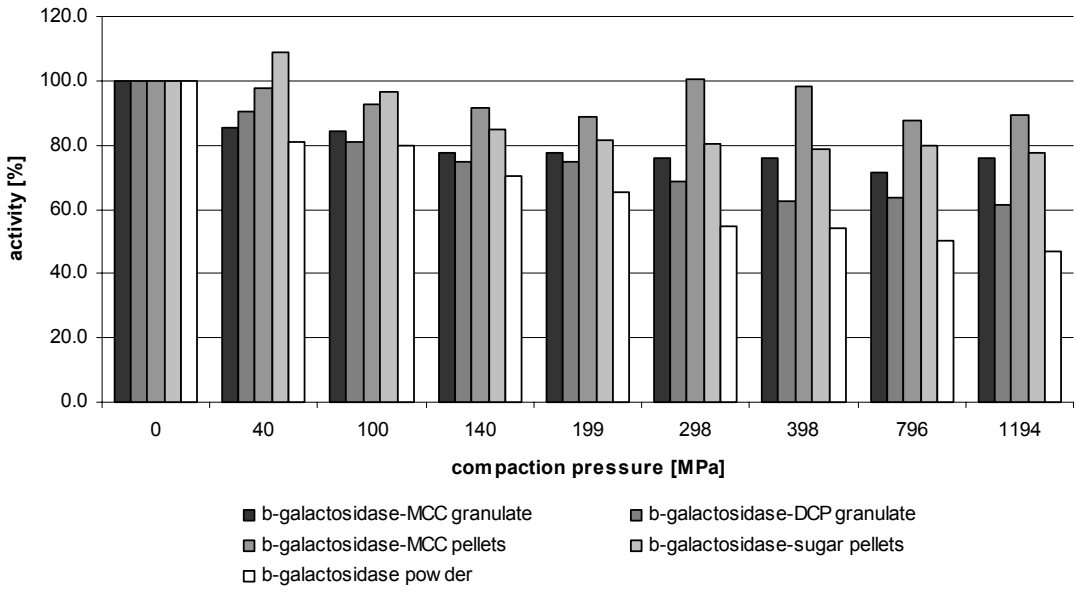

Fig. 5.46: *Summary of the behavior of the activity under pressure of the investigated β-galactosidase preparations.*

A comparison of the activity loss at maximum pressure and the *K* values of the investigated β-galactosidase preparations is given in Tab. 5.5.

Tab. 5.5: *Comparison of the activity at maximum pressure and the K values of the investigated β-galactosidase preparations.*

Preparation	*K* value (MPa^{-1})	Activity at max. pressure (%)
Bulk β-galactosidase powder	0.00373	46.7
β-Galactosidase-microcrystalline cellulose granulate	0.01318	75.9
β-Galactosidase-dicalcium phosphate dihydrate granulate	0.00785	61.2
β-Galactosidase-microcrystalline cellulose pellets	0.01507	89.3
β-Galactosidase-sugar pellets	0.01008	77.6

The investigation of the behavior of the activity under pressure of β-galactosidase powder, β-galactosidase-microcrystalline cellulose granulate, β-galactosidase-dicalcium phosphate granulate, β-galactosidase-microcrystalline cellulose pellets and β-galactosidase-sugar pellets showed that the extent of activity loss was dependent on the compression properties characterized with the Heckel equation. Preparations with a brittle behavior demonstrated a higher activity loss. Besides, there was also found a further influence on the activity loss under pressure. Obviously the degree of activity loss is also influenced by the shape and the size of the compacted particles and pellets. The compression of regular shaped particles with a constant particle size showed no activity loss, above all if the individuality of the single particles, namely pellets gets preserved in spite of a slight particle deformation.

5.6 Investigation of the critical mixture concentration

The investigation of different β-galactosidase preparations showed positive influences of plastic properties on the activity of the compacted enzyme preparations. Since the β-galactosidase powder preparation demonstrated a brittle compression character, the coating of pellets or the production of granulates seemed to have the clear advantage of higher plasticity compared to the enzyme powder. Nevertheless it must be kept in mind that the preparation of granulates and the coating of pellets is an additional production step, which is time consuming and therefore also

expensive. Moreover during the production process there are various influences on the enzyme, which must be evaluated to exclude negative consequences.

For that reason it seemed also important to investigate the influence of powder excipients mixed with the enzyme powder in various amounts to get information about formulations for direct compression. Characterization of the compression behavior of the β-galactosidase powder preparations and binary mixtures of it with a plastic and a brittle excipient, respectively (chapter 5.3) showed different behavior for the various mixtures. The aim was to detect critical densities for every mixture where the behavior of the tablet density showed sudden changes. With that approach a critical mixture concentration should be found for both excipients and that information was supposed to help to define robust formulations for the compression of β-galactosidase powder with different excipients.

5.6.1 Mixture with plastic excipient

To characterize the behavior of β-galactosidase powder in binary powder mixtures, 10 mixtures with microcrystalline cellulose, a typical plastic excipient, were investigated.

5.6.1.1 Critical densities

The enzyme activity was analyzed as a function of the apparent density of the powder compact. The apparent density of a powder compact is a result of the applied maximum compaction pressure. As 100% of the enzyme activity was measured for the material before compression, it was necessary to find a suitable representation of the enzyme activity of the compact. For this reason the apparent density of the compact was normalized with the poured density of the material before compression. Thus for the normalized relative density ρ_n a value of 1 was obtained at an enzyme activity of 100% and therefore for the higher values of ρ_n the respective values of enzyme activity.

The 11 curves obtained were approximated with two linear sections using Eq. (4.6) and each section was linearized by a regression line. The intersection of the two regression lines was determined as critical normalized density. This method for the identification of sudden changes in the behavior of a system was introduced and evaluated by Leu (1993). The relationship between activity and density of the compacts and the approximation with two linear sections for the mixtures investigated is shown in Fig. 5.47 and Fig. 5.48.

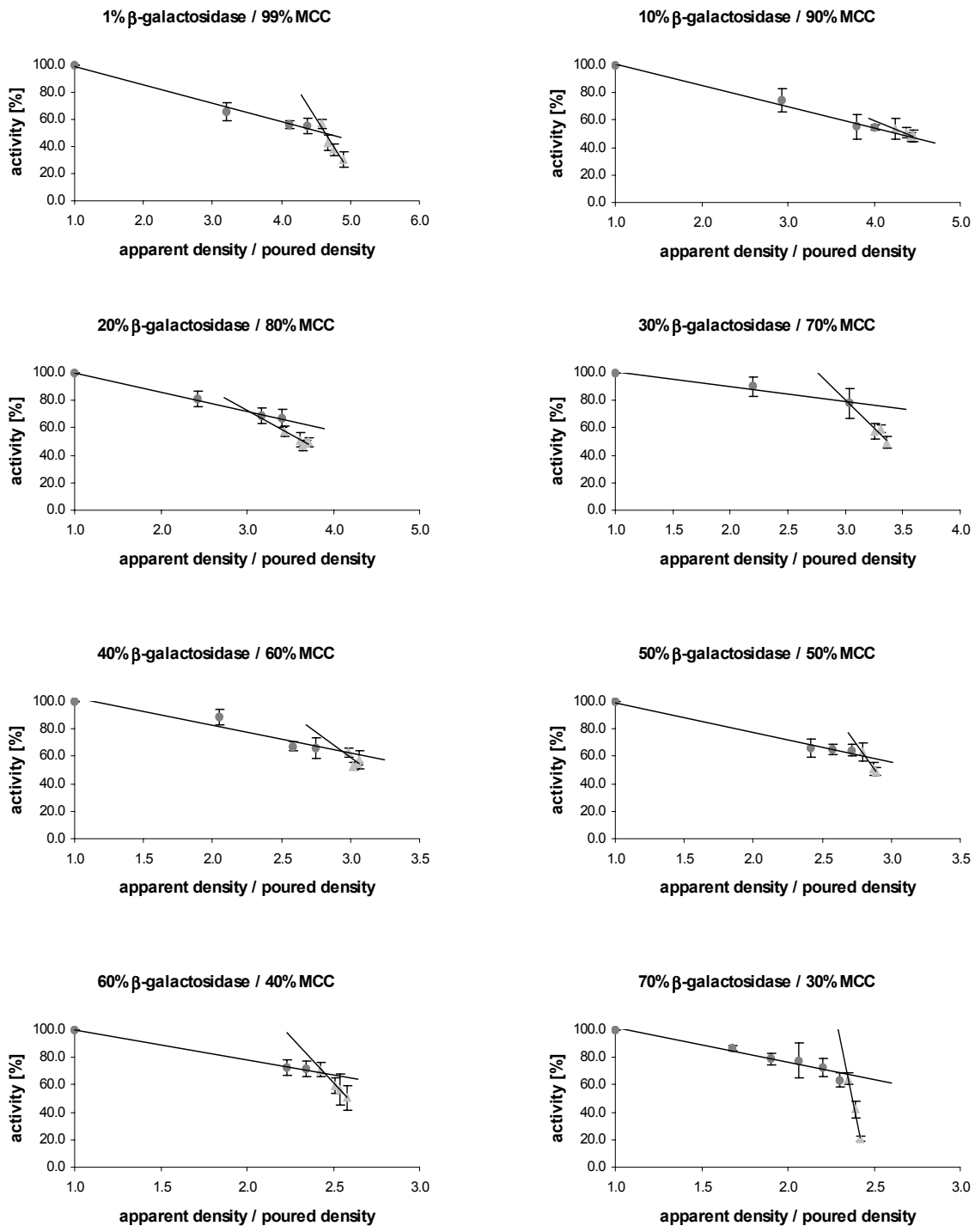

Fig. 5.47: *Relationship between activity and density for binary β-galactosidase microcrystalline cellulose powder mixtures (amounts of 1-70% β-galactosidase). N = 5, error bars: ± 1 standard deviation.*

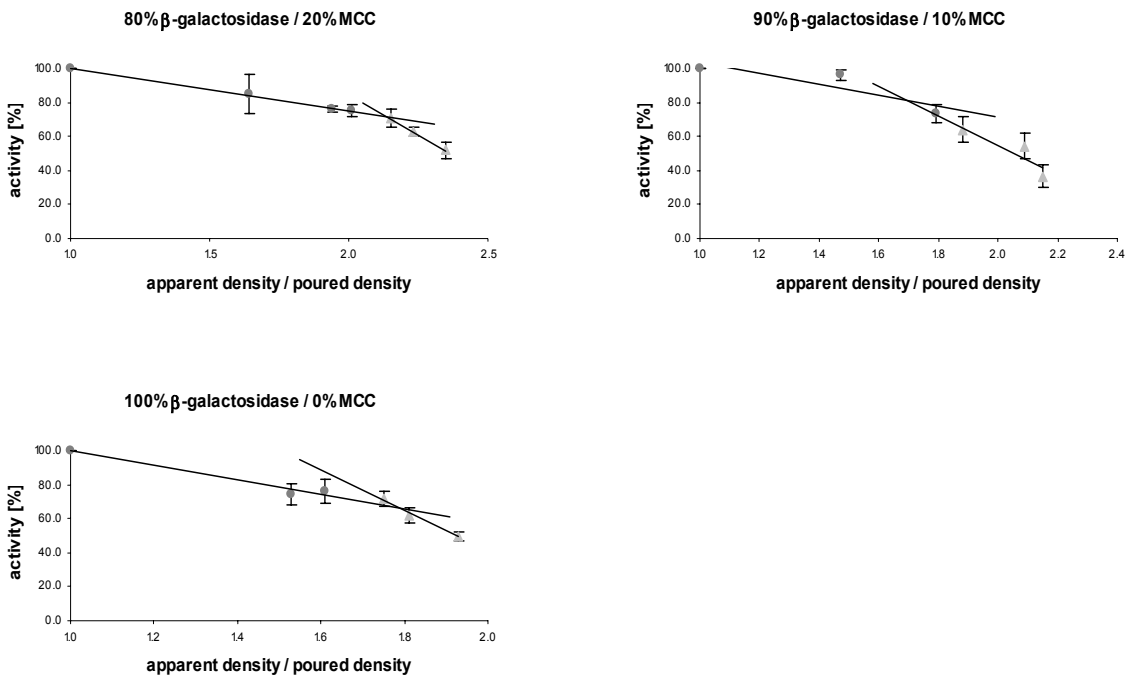

Fig. 5.48: *Relationship between activity and density for binary β-galactosidase microcrystalline cellulose powder mixtures (amounts of 80-100% β-galactosidase). N = 5, error bars: ± 1 standard deviation.*

An overview of the exact squared correlation coefficients R^2 of the non-linear regression as well as the two linear sections is given in Tab. 5.6.

Tab. 5.6: *Squared correlation coefficients of the binary β-galactosidase microcrystalline cellulose mixtures.*

Amount of β-galactosidase (%)	Squared correlation coefficient R^2		
	Non-linear regression	Section 1	Section 2
1	0.987	0.988	0.927
10	0.991	0.986	0.927
20	0.995	0.998	0.836
30	0.985	0.973	0.584
40	0.944	0.909	0.363
50	0.992	0.985	0.995
60	0.999	0.998	0.997
70	0.989	0.944	0.995
80	0.999	0.997	0.999
90	0.941	0.765	0.790
100	0.988	0.969	0.983

Each curve approximated by two straight lines showed a clear bend. At higher compaction pressures, the degree of activity loss was more important. Several plots showed that the activity loss at very high compaction pressures depended only on the final apparent density of the compact (out-of-die). The partition of the curves resulted mainly between the compaction pressure of 199 and 298 MPa, respectively. The mean squared correlation coefficient R^2 of the regression lines of section 1 and section 2 was 0.956 (±0.0687) and 0.854 (±0.2068), respectively. The low value of the correlation coefficient of section 2, as well as the high standard deviation is a consequence of the quite constant values for the normalized density at high compaction pressures.

Although there was a bend visible for every mixture ratio, the application of the evaluation method, namely the detection of the intersection of the two regression lines, showed some weaknesses. The problem was on the one hand the low number of data points and on the other hand the lack of data at low densities. Since it was necessary to get stable compacts for the out-of-die characterization and the detection of the enzyme activity it was not possible to generate data between compaction pressures of 0 and 40 MPa, because in this range stable compacts could not be produced. It is conceivable that in the region of the transition between the state of the powder and the state of the compact there is also a sudden change in the behavior of the enzyme activity, which was not detectable with the present experimental conditions. The second problem was the irregularities of the data caused by the enzyme itself. Differences in the denaturation of the molecules or small variations in the handling during the experiment can have big influences on the enzyme activity. Nevertheless with the detection of the intersection of the regression lines of the two sections the description of the deviation from linearity in the relationship between activity and density of the investigated compacts was possible.

5.6.1.2 Percolation threshold

The detected critical normalized densities of the various binary β-galactosidase microcrystalline cellulose powder mixtures have been plotted as a function of the amount of β-galactosidase (Fig. 5.49).

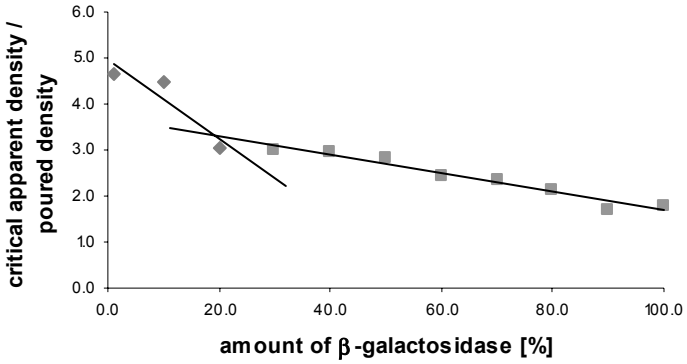

Fig. 5.49: *Critical normalized densities of each binary mixture of β-galactosidase with microcrystalline cellulose.*

The critical normalized densities as a function of the amount of β-galactosidase were again approximated by two straight lines. Thus the plot was divided into two sections with a squared correlation coefficient R^2 of 0.842 for section 1 and 0.959 for section 2, respectively. The squared correlation coefficient R^2 of the non-linear regression was 0.966. The intersection indicates the critical concentration of β-galactosidase for binary mixtures with microcrystalline cellulose. A value of 18.9% (w/w) was determined, corresponding 20.3% (V/V), i.e. roughly 20% (V/V). This critical β-galactosidase concentration can be interpreted as the percolation threshold of this binary mixture system. It defines the point where the system dominance of microcrystalline cellulose is replaced by the dominance of the β-galactosidase powder preparation. It is evident that for amounts of β-galactosidase >20% (V/V) a linear decrease of the critical normalized density is obtained with a slope being smaller than in the range <20% (V/V). The loss of activity of β-galactosidase was more important for enzyme concentrations below 20% (V/V), i.e. below the percolation threshold. Thus the question arises why the loss of enzyme activity is diminished above the percolation threshold. This behavior is probably due to the fact that β-galactosidase powder is brittle and microcrystalline cellulose powder is plastic. The brittle powder is percolating and builds a lattice in which the plastic powder is hindered to flow freely into the void space in between the brittle material. This seems to be favorable and reduces the loss of enzyme activity. In addition it is also conceivable that the rigidity of the lattice and the filling of the pore spaces with microcrystalline cellulose particles prevent the fracturing of the brittle particles and reduces therefore the shearing forces in the compact.

Below the percolation threshold the enzyme powder is completely embedded in the microcrystalline cellulose powder. Interestingly, the loss of enzyme activity is more important in case of isolated isles of enzyme in a continuum of microcrystalline cellulose. Tablet density increases because the rigid lattice of the enzyme powder is destroyed and the tablet can be compacted to higher

densities, i.e. the tablet porosity is reduced and thus activity loss is increased. In addition the shearing forces are higher with a dominance of microcrystalline cellulose because the particle movement is bigger than in the lattice dominated by the brittle substance. Thus the plastic powder microcrystalline cellulose does not work as a protecting substance.

The dependence of the activity loss on the tablet density and on the tablet porosity, respectively, of the different mixture ratios could also be proved comparing the different porosity curves. The porosity curve of each mixture was correlated separately with the porosity curve of bulk β-galactosidase (Eq. (4.7)). Correlation values are shown in Fig. 5.50.

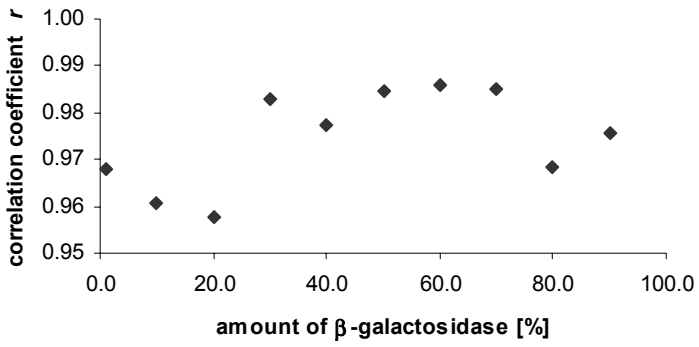

Fig. 5.50: *Correlation of porosity curves of each mixture with the porosity curve of bulk β-galactosidase.*

Correlation coefficients *r* of the porosity curves were very high, i.e. no value was lower than 0.95. But it is obvious that the correlation coefficients *r* of the mixtures with an amount of β-galactosidase powder smaller than 20% are lower than the other correlation coefficients. This means that there is a change in the porosity behavior of the compacts prepared with a mixture ratio below the percolation threshold. A comparison of the porosity curve with the corresponding activity curve of each mixture (Eq. (4.7)) showed also good correlation coefficients between 0.69 and 0.97 (Fig. 5.51).

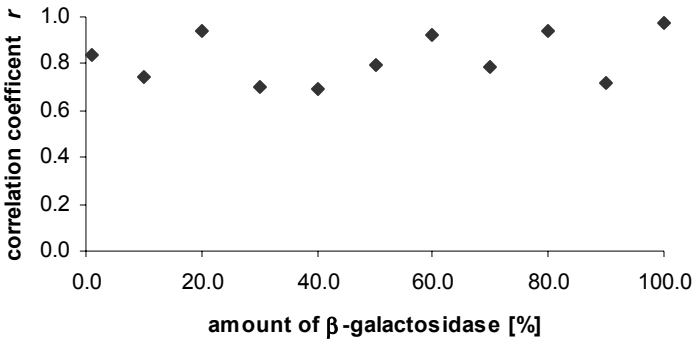

Fig. 5.51: *Correlation of porosity and activity of each mixture.*

Although the values of the correlation coefficients *r* are spread over a wide range, there may not be found a systematic trend. The wider distribution can be explained by the quite high standard deviation values of the enzyme activity. So it can be stated that for each mixture the porosity correlated with the corresponding activity values. Since the porosity is dependent on the amount of β-galactosidase in the binary mixture, it could be shown that porosity is an important factor for the activity loss in the compacted enzyme powder.

5.6.2 Mixture with brittle excipient

To characterize the behavior of β-galactosidase powder in binary powder mixtures, 10 mixtures with dicalcium phosphate dihydrate, a typical brittle excipient, were investigated.

5.6.2.1 Critical densities

The enzyme activity was analyzed again as a function of the apparent density of the powder compact, normalized as described in chapter 5.6.1.1. The 11 curves obtained were approximated with two linear sections using Eq. (4.6) and each section was linearized by a regression line. The intersection of the two regression lines was determined as critical normalized density. The relationship between activity and density of the compacts and the approximation with two linear sections for the mixtures investigated is shown in Fig. 5.52 and Fig. 5.53.

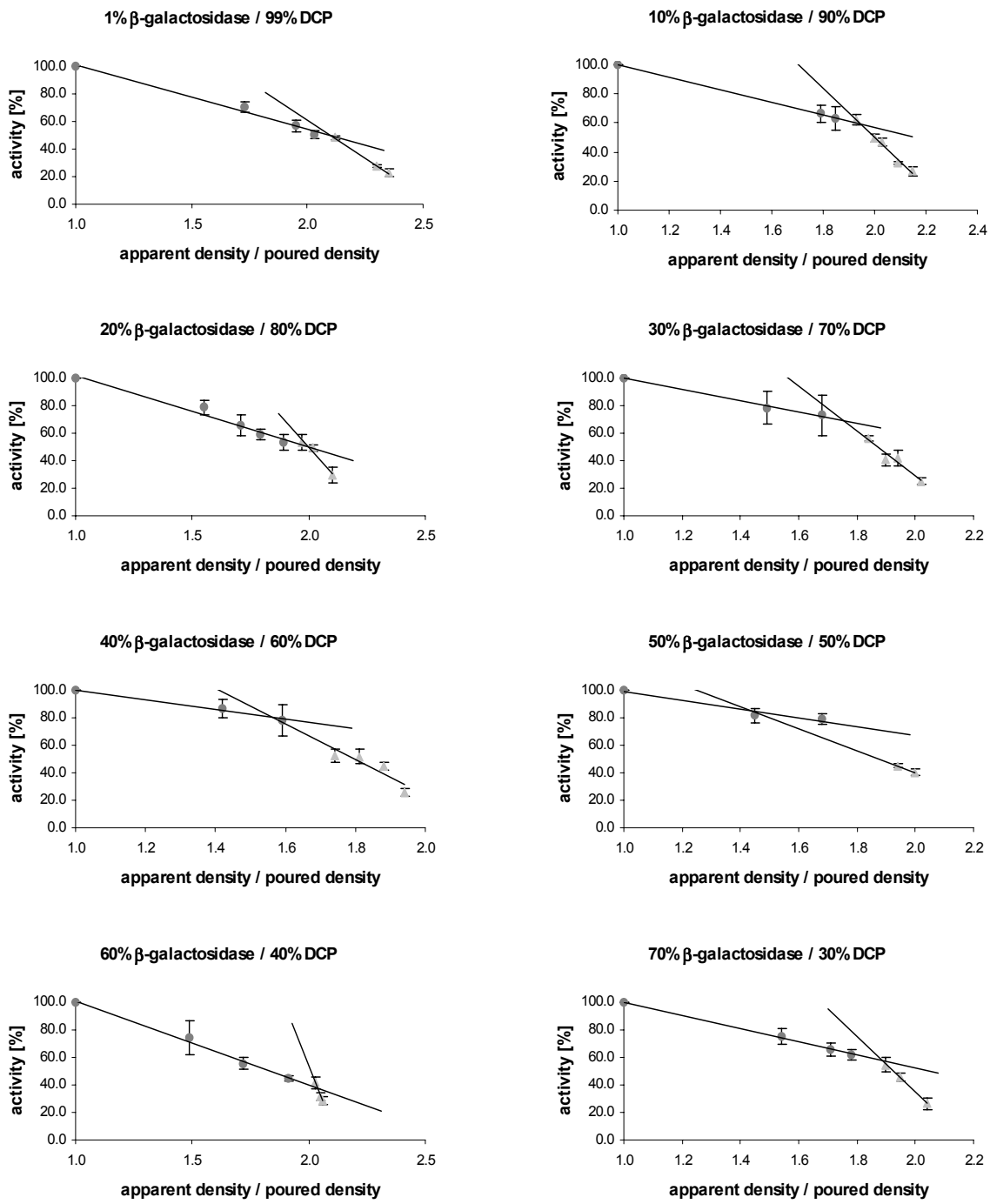

Fig. 5.52: *Relationship between activity and density for binary β-galactosidase dicalcium phosphate dihydrate powder mixtures (amounts of 1-70% β-galactosidase). N = 5, error bars: ± 1 standard deviation.*

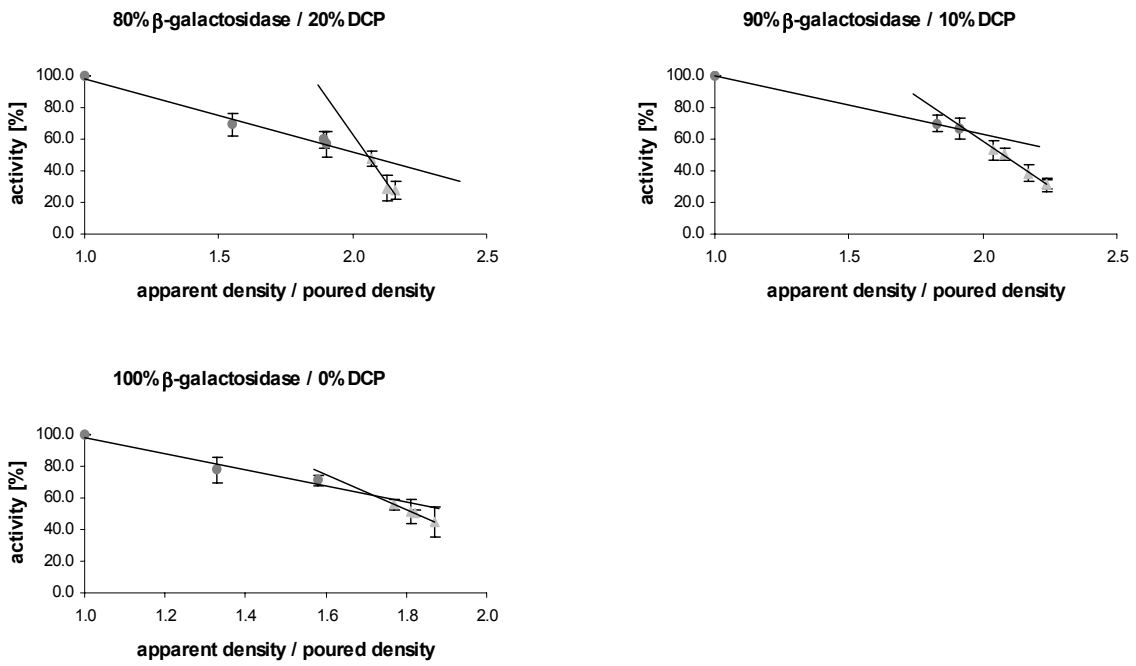

Fig. 5.53: *Relationship between activity and density for binary β-galactosidase dicalcium phosphate dihydrate powder mixtures (amounts of 80-100% β-galactosidase). N = 5, error bars: ± 1 standard deviation.*

An overview of the exact squared correlation coefficients R^2 of the non-linear regression as well as the two linear sections is given in Tab. 5.7.

Tab. 5.7: *Squared correlation coefficients of the binary β-galactosidase microcrystalline cellulose mixtures.*

Amount of β-galactosidase (%)	Squared correlation coefficient R^2		
	Non-linear regression	Section 1	Section 2
1	0.995	0.985	0.999
10	0.996	0.999	0.982
20	0.981	0.970	0.946
30	0.990	0.993	0.927
40	0.974	0.983	0.783
50	-	0.950	1.000
60	0.996	0.992	0.987
70	0.999	0.998	0.998
80	0.989	0.980	0.928
90	0.999	0.999	0.992
100	0.990	0.949	0.999

In the curves of these brittle-brittle mixtures there could also be seen clear bends. Again the degree of activity loss was more important at higher compaction pressures. The mean squared correlation coefficient R^2 of the regression lines of section 1 and section 2 was 0.982 (±0.0182) and 0.958 (±0.0648), respectively.

Although a partition into two sections was possible for every curve, some weaknesses of the method have to be faced. Due to the small number of data points, some irregularities can influence the evaluation. Irregularities can probably be caused by some unsteadiness of the degree of denaturation in the enzyme molecules. Namely the detection of the intersection of the regression lines in the curves with an amount of β-galactosidase of 40 and 50%, respectively was difficult. In the latter curve section 2 is only built of two data points, because irregularities in this region made an inclusion of more data points impossible. The values for the critical normalized densities of these two mixtures must therefore be interpreted with caution.

5.6.2.2 Percolation threshold

The detected critical normalized densities of the various binary β-galactosidase dicalcium phosphate dihydrate powder mixtures have been plotted as a function of the amount of β-galactosidase (Fig. 5.54).

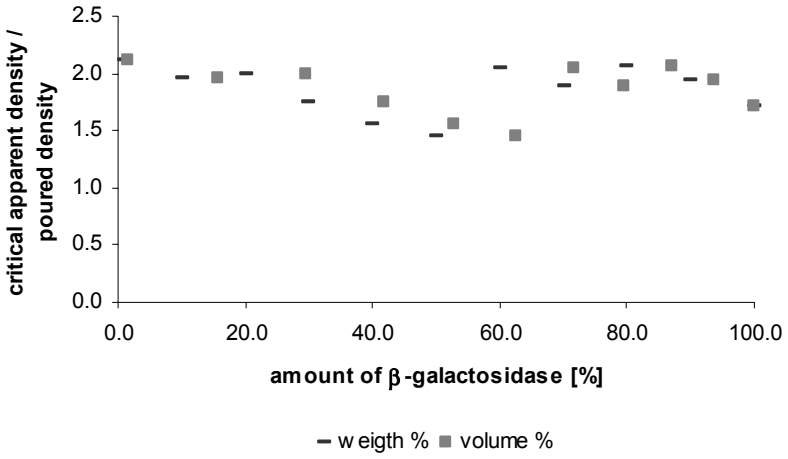

Fig. 5.54: *Critical normalized densities of each binary mixture of β-galactosidase with dicalcium phosphate dihydrate.*

The critical normalized densities as a function of the amount of β-galactosidase could not be divided into two sections. In spite of some irregularities in this curve there could not be detected a clear change in the behavior of the mixtures concerning the critical densities. The deviation mainly at 50% (m/m) corresponding 62.6% (V/V) may not be interpreted due to the inaccurate detection of the critical density in that region (chapter 5.6.2.1). Nevertheless the striking irregularities in the binary brittle mixtures with amounts of β-galactosidase of 40 and 50% found in the plot of enzyme activity against the normalized apparent density can also be a hint to some changes occurring in these mixtures. Verification of this supposition can only be done with further studies.

The definition of a percolation threshold for the mixtures with the two brittle substances β-galactosidase and dicalcium phosphate dihydrate was not possible. Probably the properties of these two substances are too similar for what reason the detection of differences in the behavior of mixtures is very difficult or even impossible.

5.7 Optimum formulation

After the investigation of various formulations for the production of a solid enzyme powder preparation, a comparison of their compression behavior and the influence on the enzyme activity was done to make a proposal for an optimum formulation.

The effective amount of β-galactosidase powder in tablets produced out of the two different kinds of granulates, the two different kinds of coated pellets and the bulk β-galactosidase preparation was compared. The absolute amount of units in the different tablets and the maximum activity loss between zero (0 MPa) and maximum (1194 MPa) compaction pressure applied were also compared. Tab. 5.8 summarizes the comparisons of these properties. The investigated powder mixtures were not taken into account as optimum formulations because there could not be found any improvement of the compression properties concerning the behavior of the enzyme activity compared to the bulk β-galactosidase powder preparation.

Tab. 5.8: Comparison of enzyme activity in tablets from different β-galactosidase preparations (1 tablet = 100±0.5 mg).

Compacted substance	Effective amount of β-galactosidase powder / tablet (mg)	Activity of uncompacted substance (units/tablet)	Activity loss between 0-1194 MPa (%)
Bulk β-galactosidase powder	100.0	434.4	59.7
β-Galactosidase-microcrystalline cellulose granulate	8.9	97.6	27.2
β-Galactosidase-dicalcium phosphate dihydrate granulate	2.3	54.6	40.1
β-Galactosidase-microcrystalline cellulose pellets	6.3	73.2	9.0
β-Galactosidase-sugar pellets	10.9	60.6	17.7

The total amount of units per tablet varied very much. On the one hand the amount of enzyme powder sprayed on the powders and pellets was different, on the other hand the increase in enzyme activity during the spraying process varied with the different substances produced. The highest amount of units per tablet was of course found in the β-galactosidase powder tablet. But there was also the highest degree of activity loss measured with the compacted powder. Although the activity of the uncompacted β-galactosidase-microcrystalline cellulose pellets was not very high it is supposed to be the best formulation because the activity loss was low, what simplifies the validation of the formulation. That suggestion is based on the concrete results of this work with its special experimental conditions.

For a more general assessment there must be taken into account some practical aspects, too. If the enzyme preparation would be produced for some pharmaceutical use, it has to be checked if the necessary dosage can be reached within the compaction of coated pellets in a dosage unit with a pleasant size. Furthermore the costs of an additional process like pellet coating must be calculated and judged. The need or the occupation of an additional production facility as well as further factors like energy consumption, raw materials (pellet cores), time and manpower used can make such processes very expensive. These considerations indicate the preference of the direct compression of the β-galactosidase powder preparation. But it has to be kept in mind that the compression in this work was done with a material testing machine in which every tablet was compacted individually. The compression of brittle powders in a rotary press is known to be difficult

and even the compression of pellets could cause some problems. For that reason the optimum formulation from a practical point of view could also be the compression of the β-galactosidase-microcrystalline cellulose granulate. That plastic granulate showed the highest enzyme activity in the uncompacted state beside the bulk enzyme powder and due to the plastic properties the activity loss was also smaller than within the bulk β-galactosidase powder. The economic aspects are the same as discussed for the pellet preparation, though. Hence it can be said that, although a lot of clarification can be done by research work, there is still needed some practice oriented development and there can be very different points of view defining an optimum formulation.

5.8 Validity for other enzyme powders

This work is limited on the investigation of one model enzyme. Of course the question arises if the results do also have validity for other enzyme preparations. Solid enzyme products are normally lyophilized powders and thus mixtures of pure enzyme, excipient and buffer salts. As a consequence the behavior of the enzyme powder under compression is dependent on the special composition. With the definition of the special compression properties of an enzyme powder preparation the product can be characterized. For similar compression properties there are expected similar effects on the enzyme activity, also in combination with compression excipients. The following chapter is a first step into the direction of testing the applicability of the results on another enzyme preparation. Of course further investigation will be necessary to get based evidence on that problem.

5.8.1 Comparison with a second enzyme powder preparation

In collaboration with a small pharmaceutical company (Meristem Therapeutics, Clermont-Ferrand, France), an enzyme powder preparation, which is going to be launched to market was investigated. Bulk enzyme powder was compressed as well as several mixtures of it with an excipient. The enzyme product was again a lyophilized powder with a composition of 40% enzyme, 10% buffer salts and 50% lactose. To exclude influences of further excipients, lactose was used to produce mixtures with the enzyme preparation in amounts of 1, 10, 20, 30, 40, 50, 60, 70, 80 and 90% of the enzyme powder preparation. The compaction conditions were the same as applied for the β-galactosidase enzyme powder (chapter 4.6), i.e. the production of tablets with a weight of 100 mg with an 8 mm-diameter punch. The compaction pressures had to be adapted and compacts were produced at 10, 20, 40, 100, 140, 199, 298 and 398 MPa (corresponding 0.5, 1, 2, 5, 7, 10, 15 and 20 kN). The enzyme activity of the produced compacts was measured by the company itself and only compacts compressed at 10, 100, 199 and 398 MPa of the following mixture ratios: 1, 10, 40, 50, 60, 70, 80, 90 and 100% have been investigated. The enzyme powder preparation and the

excipient lactose were characterized as described in chapter 4.1 and their properties are summarized in Tab. 5.9.

Tab. 5.9: *Characterization of the substances.*

Substances	Densities			Mean particle size (μm)
	True (g/ml)	Poured (g/ml)	ρ_p (rel)	
Enzyme powder	1.60	0.043	0.027	209.1
Lactose	1.55	0.490	0.317	97.9

The compression behavior was characterized with Heckel equation (Eq. (3.3)) and the Heckel plots of the bulk enzyme powder preparation, lactose and a 50% mixture of these two substances is shown in Fig. 5.55.

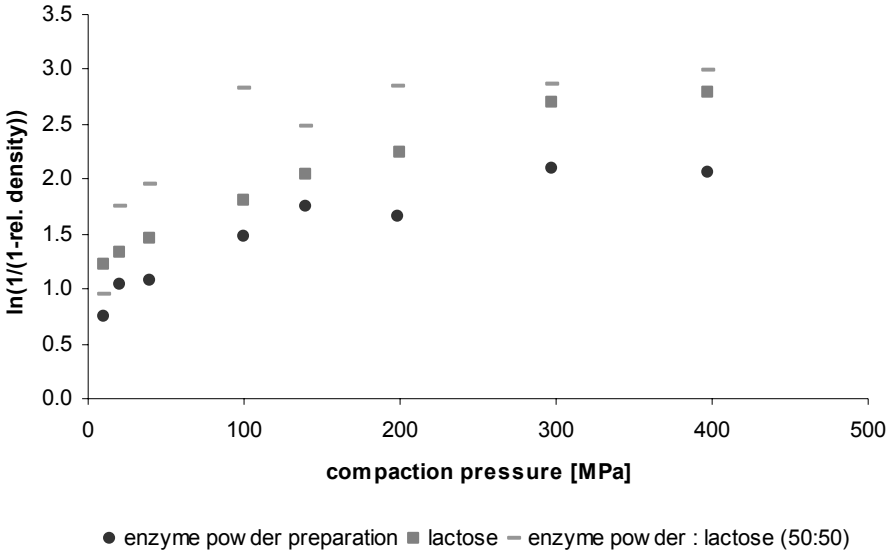

Fig. 5.55: *Heckel plots of bulk enzyme powder preparation and lactose and their mixture (50:50).*

The two bulk substances showed similar behavior in the Heckel plot and the *K* values described brittle behavior with 0.00386 MPa^{-1} (R^2 = 0.843) for the enzyme powder preparation and 0.00440

MPa^{-1} (R^2 = 0.956) for lactose. The *K* value of the 50% mixture of the bulk substance showed positive deviation with 0.00663 MPa^{-1} (R^2 = 0.610). It is to mention that the enzyme powder preparation was very fluffy and the irregularities in the curve could be caused by the special nature of the particles.

The behavior of the yield strength (Eq. (3.5)) of the mixture is shown in Fig. 5.56.

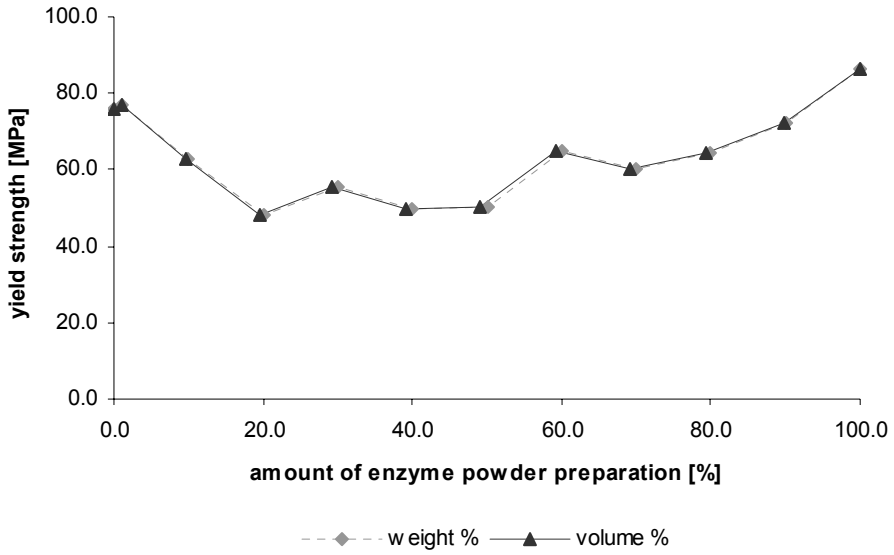

Fig. 5.56: *Yield strength of the mixtures of bulk enzyme powder preparation and lactose.*

Although the bulk substances show brittle behavior the mixtures show tendencies towards plastic properties, especially in mixtures with amounts of enzyme powder between 20 and 50%. This behavior is contrary to the behavior of the mixtures from the other brittle substances investigated, namely β-galactosidase enzyme powder and dicalcium phosphate dihydrate, which showed brittle behavior over the whole mixture range investigated. This deviation towards a plastic behavior can probably be explained by the special fluffy nature of the enzyme powder particles.

The activity detected in compacts compressed at maximum compaction pressure (398 MPa) was 89.5% at a porosity of 12.8% for the bulk enzyme powder preparation. The enzyme activity of the compacts of the different mixtures was analyzed as a function of the apparent density of the powder compact, normalized as described in chapter 5.6.1.1. The curves were approximated with two linear sections using Eq. (4.6) and each section was linearized by a regression line. The intersection of the two regression lines was determined as critical normalized density.

The problem in this special case was the very low number of data points and the big irregularities in the curves. It is not possible to make basic statements of a partition of five data points into two sections because in any case one section will consist only of two data points. Moreover, if the irregularities of the curves have been too big, a linearization of two sections was impossible. The critical density was then determined as the data point where no more big changes in density of the compacts occurred. This procedure could not be evaluated and the interpretation of the whole number of data is in this case questionable. Nevertheless the defined critical densities were plotted against the amount of enzyme powder preparation (Fig. 5.57) to judge the tendencies of the system to build critical concentrations over the mixture range.

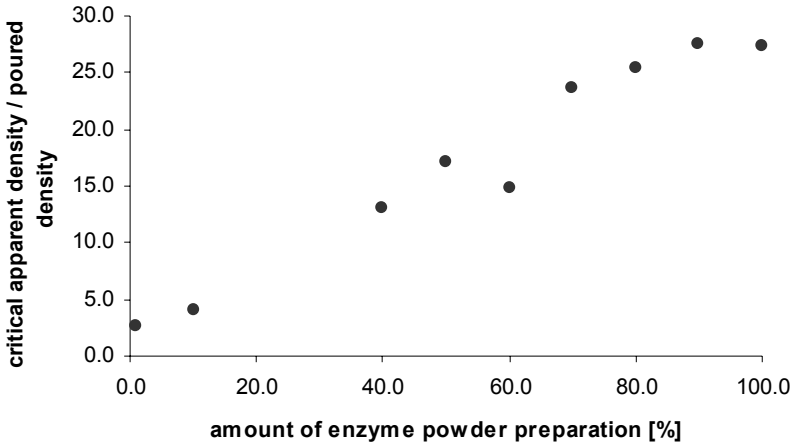

Fig. 5.57: *Critical densities of each binary mixture of enzyme powder preparation with lactose.*

The curve showed a deviation at a mixture range of 60%, but it is not sure if this deviation can be associated with an outlier or a critical concentration, i.e. a true change in the behavior of the system. In addition there cannot be given any statement on the behavior of amounts of enzyme powder preparation between 10 and 40% because there was no information on the enzyme activity in these compacts. Nevertheless the value of 60% for a critical concentration in mixtures with two brittle substances would be consistent with the results found in chapter 5.6.2.2 but in both cases the results can not really be judged due to the lack of data points and problems with the evaluation. Further investigations in that area would be interesting.

The comparison of the enzyme powder preparation from a pharmaceutical company to the model enzyme investigated in this work did not lead to a reliable result. The small amount of data does not allow any conclusive assessment of the comparison.

6 Conclusion

The investigated model enzyme β-galactosidase was influenced by different factors. The activity of the enzyme was positively influenced by temperatures up to 80°C in the solid state and up to 50°C dissolved in buffer solution. The presence of the binding agent hydroxy propyl methylcellulose in the solution had a stabilizing effect on the enzyme and led to an increased activity compared to the activity of enzyme powder dissolved in aqueous buffer solution. The increased viscosity in solutions with hydroxy propyl methylcellulose has probably a positive effect on the conformational rigidity of the enzyme molecule and may protect it from hydrolytic covalent reactions. This effect had also a big influence on the layering of powder, i.e. granulation and the coating of pellets. The products showed an increased activity, which was due to the temperature development during the spraying process and the presence of binding agent in the spraying solution. The quality of the film on the granulates and pellets was dependent on the porosity of the raw material. Fast elimination of water by absorption of the raw material caused rough surfaces, which showed lower activity increase than the smooth surfaces.

The enzyme activity was negatively influenced by application of compaction pressure, whereas the extent of the activity loss was dependent on the compression character of the compacted substances. The characterization of the β-galactosidase powder preparation after Heckel (1961a, b) indicated brittle powder behavior, which was proved in comparison with the plastic model excipient microcrystalline cellulose and the brittle model excipient dicalcium phosphate dihydrate. Binary mixtures of the enzyme powder preparation with the plastic and the brittle excipient, respectively showed deviations from linearity. The yield strength of the various mixtures showed a dominance of the plastic excipient in the plastic-brittle mixtures below a concentration of 70% of brittle enzyme powder. The yield strength of the brittle-brittle mixtures showed no changes in deformation behavior over the whole mixture ranges. Thus similarity in the general behavior of two substances can make the definition of variations between the two substances difficult.

The compression behavior of powders was partly influenced by the layering with enzyme powder solution and the consequently forming of agglomerates. The produced granulate from dicalcium phosphate dihydrate showed a more plastic behavior compared to its brittle raw material. The plastic behavior of the raw material microcrystalline cellulose at the contrary, was not further improved in the β-galactosidase-microcrystalline cellulose granulate. The extent of activity loss in the enzyme powder and the two granulates under pressure seemed to be dependent on the compression character of the preparation, whereas the activity loss in the brittle powder was the highest and the activity loss in the β-galactosidase-microcrystalline cellulose granulate, which was more plastic than the β-galactosidase-dicalcium phosphate dihydrate granulate was the lowest.

Pellets from plastic raw material microcrystalline cellulose and from brittle raw material sugar showed both plastic behavior, which was not influenced by the coating with β-galactosidase

powder solution. Although the compression character was the same for both enzyme pellet preparations there was a difference in the extent of activity loss detectable. The pellets from the raw material microcrystalline cellulose did not show significant activity loss over the whole compression range, whereas the β-galactosidase-sugar pellets showed a slight activity loss compared to the activity loss in the enzyme granulates and the enzyme powder. The assessment of the behavior of the pellets in the compact with scanning electron microscopy pictures led to the conclusion that not only the compression character of the product is decisive for the extent of activity loss but also the shape and the size of the various particles. The fact that the β-galactosidase-microcrystalline cellulose pellets did not break and only slightly changed their shape eliminating the spaces in the compact led to very low shearing forces. The β-galactosidase-sugar pellets also did not break but showed cracks in the surfaces of the various pellets. For that reason there were built small powder particles causing a higher number of shearing forces. The individual pellets only slightly changed their shape as well but the surfaces fused due to the cracking and therefore the activity loss was slightly higher for the β-galactosidase-sugar pellets than for the β-galactosidase-microcrystalline cellulose pellets. Nevertheless the regular shape and size of the pellets seemed to have positive influence on the extent of activity loss under pressure due to the reduced number of shearing forces.

Binary powder mixtures of the β-galactosidase preparation with the plastic excipient microcrystalline cellulose did not generally improve the behavior of the enzyme activity under pressure. It is supposed that over a wide mixture range the brittle enzyme powder dominates the system and builds a rigid lattice in which the plastic excipient is allowed to flow freely. The plastic deformation is in that case limited on the particles of the excipient. The plastically deformed particles can therefore fill up the free spaces in the lattice and prevent the brittle particles from fracturing, what reduces the shearing forces in the compact. With higher amounts of plastic excipient the lattice of the enzyme powder gets destroyed and microcrystalline cellulose dominates the system. Probably the dominant particles do not protect the brittle behavior with their plastic deformation but they do possibly crush the particles of the enzyme powder and cause therefore high shearing forces on the enzyme. The percolation threshold, i.e. the change in the system dominance was found to be at a mixture ratio of 20% of β-galactosidase enzyme powder. This mixture range should therefore be avoided to get robust formulations because at that mixture range a sudden change in the behavior of the system occurs. Moreover amounts of microcrystalline cellulose higher than 80% do have negative influences on the shearing forces in the compact and therefore on the activity of the enzyme powder. The expected protecting effect of the plastic excipient on the brittle β-galactosidase enzyme powder was therefore not found.

Binary powder mixtures of the β-galactosidase preparation with the brittle excipient dicalcium phosphate dihydrate had no effect on the activity under compression compared to the activity loss in bulk β-galactosidase powder. The compression behavior was brittle over the whole mixture ranges. A system dominance of the one or the other substance could not be detected, probably the two substances behaved too similar to find differences. A deviation from the general behavior of

the mixtures could be found at a mixture ratio of 50% (m/m), corresponding 62.6% (V/V), but it could not be proved if this deviation was caused by an outlier or if it was a true percolation threshold. Further investigation on brittle-brittle powder mixtures in that mixture range would therefore be interesting, although a protecting effect on the enzyme activity in this range is not expected.

A comparison with a second enzyme powder preparation, which had also a brittle compression behavior in mixtures with a brittle excipient could not help to find a clear characterization of the compression behavior of brittle-brittle mixtures. Again there was a deviation found at a mixture range of 60% of enzyme powder preparation, but due to a very low number of data, it was again impossible to associate the deviation to an outlier or to a true percolation threshold.

The problem in the characterization of powder mixtures is the fact that there can only be described a general behavior. This behavior can be judged and interpreted. But it is difficult to really prove these interpretations because the compression behavior is always influenced by the character, the size and the shape of the particles. Moreover binary powder mixtures are influenced by the character, the size and the shape of the two different powders and by the interaction between them. The fact that the behavior of the activity under compression could not be improved with powder mixtures led to the conclusion that the interaction between the two kinds of particles is important. Particles do always interact under compression, they change their shape by deformation or do break, which causes shearing forces, friction or even wedging. A further aspect is also the fact that particles that show a brittle behavior and get cut into small pieces can change their compression behavior if they reached a low size and may start flowing and deform plastically. It is evident that if there are two different kinds of particles the possibilities of interaction and variation are broad. Even if the difference in the size and shape of the particles is optimal because densest packaging can be reached by filling up the free spaces between bigger particles with some smaller particles, there are a lot of interactions due to the diversity of the particles. This is probably the reason why mixtures of the brittle β-galactosidase enzyme powder with a plastic excipient did not decrease the extent of activity loss under compression although it could be proved that plastic properties do generally improve the behavior of the enzyme activity. The fact that best results could be found in compacts from coated pellets supports that statement. The regular shaped and sized pellets with their smooth surface kept their individuality and did not break. For that reason there was a minimum number of interactions, friction and shearing forces. Therefore it is proposed to use particles with the most similar character, size and shape for the compression of enzyme powders.

The more similar and regular shaped the compressed particles are, the more problems can arise in the production of stable compacts. In the case that both kinds of particles are similar, though but have brittle properties, not only the activity loss would be high due to the brittle compression character but the production of compacts would also lead to problems because brittle substances can badly be compacted in direct compression. For that reason the production of a plastic granulate by layering the raw powder excipient with an enzyme powder solution is proposed. The

compression of a granulate is less problematic and the plastic properties would have a good influence on the behavior of the enzyme activity under pressure. The particles of the granulate have the same character and a similar shape and size, thus there are only interactions between particles with similar properties. The similarity of the particles can even be improved if the granulate is fragmented and only a special fraction is used for compression. The absence of further different kinds of particles from excipients will reduce interaction and the number of negative influences like shearing forces and friction remains low.

The optimum formulation suggested is the use of enzyme coated plastic pellets or a plastic enzyme layered granulate. To prove the applicability of these formulations for practical industrial use, the compression of these enzyme formulations should be transformed on a rotary press to investigate the influences of the higher compression speed and the different forces acting on the compacted material. Also the economic aspect would of course have an influence on the definition of an optimum formulation. The production of granulates and the coating of pellets are additional production steps which can be expensive. The costs of an additional production facility, the energy consumption, the need of raw material like pellet cores, the time and manpower used for production can be expensive. Furthermore the validation and the registration of an additional production step for the creation of an oral solid dosage form can be difficult and time consuming and therefore be also expensive. It is also necessary to check if the required dosage can be reached if the enzyme is only sprayed on a carrier. If this would not be the case for a special enzyme in a pharmaceutical application other formulation could be taken into account. Since the compression of enzyme powder is not proposed from the results in this work, the production of enzyme pellets from a plastic excipient with a low porosity would be a possibility. The amount of enzyme could be increased because in that case the whole pellet would consist of enzyme powder and not only the coating of the pellet.

These considerations demonstrate the limits of this research work. The investigated topic is dominated by basic research and can only partly be directly transferred to the development of dosage forms. Basic research is done to understand different phenomena and behavior. The development of dosage forms, though is still empirical and based on experiences. Basic research work can help to clarify general problems and develop models for the characterization of phenomena. Nevertheless the influences and variations in pharmaceutical technology are very broad and a single research work like this thesis is only a step in this direction but empirical development can still not be replaced. Practical, economical and legal aspects are also often the loser in basic research work.

A lot of new aspects in the field of particle compression have been discussed in this work. It was found that the shape and the size of the various particles may have big influences on friction and shearing forces. Shearing forces can cause a reduction of enzyme activity during the compression of an enzyme powder. The compression character of the particles showed influences on the extent of activity loss under pressure, whereas plastic properties are favorable to protect the enzyme.

This work is far from treating the topic of compression of enzyme powder in an exhaustive manner. First aspects have been discussed on the behavior of a model enzyme powder. It would be interesting to make comparisons with other enzyme types. It is possible that other enzyme molecules behave different under pressure or are differently influenced by temperature, excipients and other factors. It would be interesting to investigate an enzyme powder preparation with a plastic compression behavior and furthermore the behavior of binary mixtures with a plastic and a brittle excipient. Lyophilisation of a liquid enzyme preparation with different excipients to obtain brittle and plastic solid enzyme preparations from the same enzyme molecules would allow a very exact comparison of the influences of the compression character on the behavior of the enzyme activity. An important step towards the practical aspects would be the transformation of the compression on a rotary press or a compaction simulator (e.g. MCC Presster^{TM}, Metropolitan Computing Corporation, New Jersey, USA) and the production and compaction of enzyme pellets to reach higher dosage of enzyme preparation per tablet and also to profit from the positive aspects of the compression of pellets detected in this work.

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8 Appendix

1% β-galactosidase / 99% MCC			10% β-galactosidase / 90% MCC		
comp. force [kN]	**rel. density**	**activity [%]**	**comp. force [kN]**	**rel. density**	**activity [%]**
2.00	0.634	71.2	2.00	0.624	80.0
2.00	0.638	54.0	2.00	0.629	81.4
2.00	0.652	65.9	1.99	0.636	79.3
1.99	0.658	70.0	1.99	0.643	60.1
1.99	0.656	67.7	1.99	0.646	72.9
5.00	0.824	58.9	4.99	0.818	40.7
5.00	0.831	53.4	4.99	0.811	55.9
4.99	0.835	54.0	5.00	0.830	62.4
4.99	0.831	56.1	4.99	0.833	55.5
5.00	0.831	59.6	4.99	0.834	62.6
7.00	0.886	52.3	7.00	0.865	57.0
7.00	0.879	50.3	6.99	0.868	54.3
6.99	0.884	58.1	7.00	0.874	53.2
6.99	0.882	64.2	6.99	0.872	55.8
6.99	0.885	51.1	6.99	0.874	52.9
9.99	0.925	52.6	9.99	0.920	63.4
9.99	0.926	57.1	9.99	0.914	58.3
10.00	0.927	56.4	9.99	0.916	44.8
10.00	0.924	61.5	9.99	0.925	50.5
10.00	0.929	55.7	9.99	0.929	51.0
15.00	0.943	43.0	14.99	0.952	45.0
15.00	0.940	50.3	14.99	0.953	53.4
15.00	0.957	45.5	14.99	0.950	51.7
15.00	0.946	42.9	14.99	0.947	55.8
15.00	0.944	34.3	14.99	0.940	49.7
19.99	0.958	32.4	20.00	0.958	48.8
19.99	0.960	43.1	19.99	0.961	45.0
19.99	0.967	30.6	19.99	0.959	51.7
19.99	0.950	28.4	20.00	0.969	44.1
19.99	0.956	30.9	19.99	0.954	49.9
40.00	0.961	32.3	40.00	0.966	44.7
39.99	0.960	42.8	40.00	0.965	52.6
40.00	0.964	37.0	39.99	0.971	43.5
39.99	0.962	40.9	40.00	0.962	51.9
40.00	0.958	36.1	40.00	0.966	50.0
60.00	0.996	22.2	59.99	0.971	41.2
60.00	0.986	26.3	60.00	0.972	41.2
60.00	0.986	32.9	60.00	0.967	39.1
60.00	0.983	37.2	59.99	0.905	41.7
60.00	1.003	33.4	60.00	0.971	43.4

20% β-galactosidase / 80% MCC			30% β-galactosidase / 70% MCC		
comp. force [kN]	**rel. density**	**activity [%]**	**comp. force [kN]**	**rel. density**	**activity [%]**
2.00	0.610	72.3	2.00	0.619	97.4
2.00	0.621	80.0	2.00	0.626	96.7
2.00	0.649	86.8	2.00	0.639	86.1
2.00	0.640	83.3	2.00	0.642	89.1
1.99	0.653	83.0	2.00	0.646	81.0
4.99	0.821	63.4	5.00	0.813	71.0
5.00	0.829	62.4	5.00	0.821	71.8
5.00	0.842	73.7	4.99	0.823	68.6
5.00	0.829	71.0	4.99	0.826	75.9
4.99	0.838	74.3	4.99	0.820	70.5
7.00	0.892	65.4	6.99	0.872	62.7
7.00	0.893	73.3	6.99	0.872	69.1
7.00	0.889	56.4	7.00	0.865	85.1
7.00	0.896	67.2	6.99	0.878	87.0
6.99	0.890	73.0	6.99	0.872	85.4
10.00	0.895	58.4	10.00	0.906	57.5
10.00	0.903	56.3	10.00	0.918	59.8
10.00	0.899	63.6	10.00	0.889	47.1
10.00	0.902	57.8	10.00	0.892	58.3
10.00	0.914	53.5	10.00	0.899	55.8
15.00	0.942	50.2	15.00	0.931	51.2
15.00	0.953	53.5	15.00	0.937	51.1
15.00	0.955	43.9	15.00	0.932	59.8
15.00	0.949	57.5	15.00	0.945	61.2
15.00	0.944	51.1	15.00	0.944	63.2
20.00	0.951	45.8	20.00	0.956	56.3
19.99	0.952	46.4	20.00	0.943	56.1
20.00	0.959	46.6	20.00	0.951	62.7
20.00	0.961	43.2	20.00	0.967	60.3
19.99	0.964	53.3	20.00	0.956	60.7
39.99	0.967	50.5	40.00	0.976	61.3
40.00	0.964	49.0	40.00	0.968	60.8
40.00	0.963	47.2	40.00	0.960	66.2
40.00	0.964	50.3	40.00	0.966	65.8
40.00	0.976	46.2	40.00	0.998	60.4
60.00	0.973	46.5	60.00	0.964	44.6
60.00	0.970	45.6	60.00	0.968	48.8
60.00	0.972	54.2	60.00	0.964	47.2
60.00	0.972	51.0	60.00	0.964	56.0
60.00	0.973	50.5	60.00	0.975	48.7

40% β-galactosidase / 60% MCC			50% β-galactosidase / 50% MCC		
comp. force [kN]	**rel. density**	**activity [%]**	**comp. force [kN]**	**rel. density**	**activity [%]**
2.00	0.638	85.5	2.00	0.635	69.6
2.00	0.647	96.3	2.00	0.643	68.7
2.00	0.656	81.9	2.00	0.647	67.5
1.99	0.654	90.6	2.00	0.651	74.4
1.99	0.662	89.5	2.00	0.657	72.0
4.99	0.803	72.3	5.00	0.807	62.7
4.99	0.822	63.4	5.00	0.812	65.2
5.00	0.831	66.1	4.99	0.815	59.5
4.99	0.826	66.2	5.00	0.813	76.9
4.99	0.821	67.6	4.99	0.815	64.6
7.00	0.871	58.2	6.99	0.859	70.4
6.99	0.876	59.9	6.99	0.863	64.3
6.99	0.875	66.3	6.99	0.863	67.1
6.99	0.876	69.1	6.99	0.869	62.6
6.99	0.874	76.7	6.99	0.867	61.0
9.99	0.931	29.9	9.99	0.907	64.4
9.99	0.911	56.3	9.99	0.905	68.7
9.99	0.913	64.3	9.99	0.909	68.5
9.99	0.904	41.6	9.99	0.910	61.9
9.99	0.916	63.2	10.00	0.918	59.5
15.00	0.939	61.6	15.00	0.937	58.2
15.00	0.952	59.0	14.99	0.941	58.0
15.00	0.945	63.0	15.00	0.935	59.2
15.00	0.942	67.3	15.00	0.937	66.5
15.00	0.955	62.8	15.00	0.931	73.3
20.00	0.966	11.8	20.00	0.962	38.5
20.00	0.969	13.9	20.00	0.953	44.8
20.00	0.948	11.8	20.00	0.955	48.7
20.00	0.953	50.5	20.00	0.961	46.2
20.00	0.961	54.4	19.99	0.955	43.4
39.99	0.976	54.8	40.00	0.980	43.1
39.99	0.967	54.5	40.00	0.969	52.3
39.99	0.973	53.2	40.00	0.956	50.4
39.99	0.970	54.8	40.00	0.955	54.7
40.00	0.963	55.6	40.00	0.965	53.8
60.00	0.976	55.2	60.00	0.965	52.0
60.00	0.974	57.3	60.00	0.969	46.9
60.00	0.973	47.5	60.00	0.966	46.7
60.00	0.975	61.8	60.00	0.985	47.0
60.00	0.971	64.2	60.00	0.978	52.9

60% β-galactosidase / 40% MCC			70% β-galactosidase / 30% MCC		
comp. force [kN]	**rel. density**	**activity [%]**	**comp. force [kN]**	**rel. density**	**activity [%]**
2.00	0.697	97.8	1.99	0.668	89.2
2.00	0.702	86.8	1.99	0.674	83.6
1.99	0.684	80.5	1.99	0.673	87.5
1.99	0.693	99.2	1.99	0.680	87.5
1.99	0.702	102.3	1.99	0.678	86.4
4.99	0.865	75.2	5.03	0.779	83.0
4.99	0.838	71.8	5.02	0.770	73.5
4.99	0.837	70.2	5.01	0.761	80.3
4.99	0.827	65.2	5.01	0.752	76.2
4.99	0.829	81.2	5.01	0.756	82.3
6.99	0.885	73.0	7.05	0.820	62.6
6.99	0.878	73.9	7.05	0.835	65.3
6.99	0.885	74.5	7.01	0.816	87.6
6.99	0.879	61.7	7.02	0.834	82.8
6.99	0.881	74.9	7.10	0.831	89.9
10.01	0.911	71.2	11.50	0.887	76.2
10.12	0.914	72.6	10.01	0.888	75.7
10.02	0.912	77.9	10.01	0.884	79.4
10.02	0.916	69.4	10.00	0.883	65.4
10.01	0.923	64.4	10.12	0.872	65.0
15.02	0.964	59.4	15.00	0.932	59.3
15.02	0.953	52.8	15.00	0.925	60.9
15.01	0.939	54.0	15.00	0.921	62.6
15.00	0.933	63.5	15.00	0.928	62.3
15.01	0.934	66.7	15.03	0.921	72.7
20.01	0.945	51.4	20.01	0.955	68.3
20.02	0.946	40.0	20.00	0.936	59.2
20.01	0.955	65.9	20.30	0.928	66.8
20.02	0.977	57.2	20.00	0.960	61.5
20.02	0.965	67.7	20.11	0.947	67.5
40.01	0.963	65.6	40.00	0.961	31.9
40.04	0.969	45.2	40.00	0.955	45.2
40.03	0.988	48.7	40.00	0.964	41.1
40.00	0.957	44.3	40.00	0.965	48.0
41.57	0.979	48.8	40.00	0.959	44.8
60.00	0.988	47.7	60.05	0.991	20.4
60.00	0.998	53.1	60.06	0.963	19.7
60.00	0.984	50.3	60.36	0.996	22.7
60.00	0.994	49.0	60.04	0.970	21.3
59.99	0.988	56.1	60.01	0.954	20.9

80% β-galactosidase / 20% MCC			90% β-galactosidase / 10% MCC		
comp. force [kN]	**rel.density**	**activity[%]**	**comp. force [kN]**	**rel. density**	**activity [%]**
1.99	0.696	78.4	2.00	0.635	94.4
1.99	0.686	70.5	2.00	0.628	95.9
1.99	0.686	89.3	2.00	0.640	100.9
1.99	0.683	100.6	1.99	0.639	70.9
1.99	0.688	84.7	2.00	0.654	94.4
5.03	0.823	75.0	5.00	0.782	76.5
5.00	0.808	75.6	5.02	0.779	76.5
5.01	0.812	77.0	5.05	0.781	64.2
5.03	0.817	78.1	5.02	0.776	74.3
5.00	0.809	73.3	5.01	0.774	76.1
7.01	0.848	72.7	7.00	0.819	65.2
7.01	0.846	71.2	7.01	0.812	73.5
7.00	0.836	78.2	7.03	0.824	64.4
7.02	0.843	78.6	7.04	0.829	52.7
7.00	0.838	75.2	7.00	0.819	63.7
10.01	0.894	76.7	10.02	0.873	44.9
10.03	0.907	65.4	10.02	0.873	46.8
10.03	0.911	72.4	10.03	0.825	46.0
10.01	0.903	72.8	10.01	0.833	48.3
10.02	0.897	64.4	10.04	0.845	46.5
15.01	0.932	64.5	15.01	0.903	50.8
15.01	0.934	60.6	15.03	0.903	51.5
15.01	0.933	66.6	15.03	0.916	53.6
15.03	0.943	59.2	15.04	0.912	60.7
15.02	0.936	62.6	15.01	0.912	39.9
20.00	0.992	57.4	20.01	0.919	55.4
20.00	0.984	51.5	20.01	0.931	60.3
20.00	0.990	45.3	20.04	0.936	70.6
20.00	0.974	50.8	20.02	0.922	55.2
20.00	0.991	54.1	20.05	0.935	54.0
40.02	0.975	68.1	40.02	0.941	51.5
40.00	0.963	45.1	40.00	0.939	42.6
40.01	0.964	44.5	40.03	0.939	50.4
40.02	0.965	51.6	40.04	0.949	51.4
40.03	0.959	51.5	40.03	0.941	49.9
60.01	0.962	49.2	60.02	0.920	39.0
60.03	0.970	43.8	60.07	0.935	31.0
60.02	0.974	45.5	60.07	0.940	27.6
60.03	0.979	49.9	60.02	0.939	43.1
60.04	0.970	53.0	60.01	0.950	42.1

100% β-galactosidase / 0% MCC			1% β-galactosidase / 99% DCP		
comp. force [kN]	**rel.density**	**activity[%]**	**comp. force [kN]**	**rel. density**	**activity [%]**
2.00	0.732	94.2	2.00	0.690	71.9
2.00	0.741	82.6	2.00	0.694	66.4
1.99	0.746	92.9	2.00	0.696	76.0
1.99	0.768	84.7	2.00	0.698	66.6
2.00	0.773	90.4	2.00	0.696	71.9
5.01	0.749	68.0	5.00	0.769	68.8
5.01	0.749	73.6	5.00	0.762	56.1
5.02	0.776	69.7	5.00	0.769	77.4
5.15	0.786	77.8	5.00	0.760	74.3
5.50	0.779	83.0	5.00	0.767	83.1
7.05	0.812	68.7	7.00	0.778	60.2
7.03	0.812	71.4	7.00	0.793	58.2
8.20	0.828	74.2	7.00	0.782	56.5
7.05	0.772	86.0	7.00	0.779	59.7
7.03	0.831	79.5	7.00	0.786	49.2
10.03	0.869	77.4	10.00	0.801	64.1
10.03	0.887	71.0	10.00	0.800	56.8
10.07	0.875	75.1	10.00	0.815	59.8
10.06	0.871	66.9	10.00	0.810	60.1
10.01	0.894	68.4	10.00	0.781	66.7
15.02	0.904	62.8	15.00	0.803	46.7
15.00	0.908	60.0	15.00	0.823	50.6
15.03	0.921	64.5	15.00	0.809	49.0
15.00	0.922	66.6	15.00	0.809	50.0
15.00	0.909	55.9	15.00	0.832	55.0
20.00	0.900	48.1	20.00	0.820	46.7
20.02	0.913	52.2	20.00	0.832	49.6
20.02	0.914	55.3	20.00	0.868	45.1
20.01	0.917	57.5	20.00	0.868	49.0
20.02	0.933	58.8	20.00	0.873	47.3
40.05	0.927	49.8	40.00	0.916	29.2
40.01	0.927	48.4	40.00	0.923	27.4
40.01	0.930	47.1	40.00	0.909	26.3
40.01	0.946	52.3	40.00	0.931	27.0
40.02	0.947	50.7	40.00	0.943	27.1
60.03	0.966	47.2	60.00	0.937	22.5
60.44	0.958	53.9	60.00	0.943	21.1
60.22	0.975	47.3	60.00	0.946	19.6
60.05	0.986	50.4	60.00	0.961	24.6
60.84	0.973	50.5	60.00	0.939	26.2

10% β-galactosidase / 90% DCP			20% β-galactosidase / 80% DCP		
comp. force [kN]	**rel. density**	**activity [%]**	**comp. force [kN]**	**rel. density**	**activity [%]**
2.00	0.691	60.7	2.00	0.671	70.9
2.00	0.690	53.2	2.00	0.672	83.8
2.00	0.694	61.8	2.00	0.674	78.7
2.00	0.676	71.8	2.00	0.670	83.0
2.00	0.685	76.3	2.00	0.676	77.2
5.00	0.763	71.5	5.00	0.742	64.7
5.00	0.766	57.8	5.00	0.748	75.1
5.00	0.769	66.8	5.00	0.751	57.9
5.00	0.764	64.7	5.00	0.741	72.0
5.00	0.765	72.4	5.00	0.733	58.7
7.00	0.775	50.4	7.00	0.778	55.1
7.00	0.785	63.2	6.99	0.765	58.8
7.00	0.792	62.8	7.00	0.776	54.7
7.00	0.800	70.0	6.99	0.782	63.3
7.00	0.800	69.6	6.99	0.789	63.3
10.00	0.817	60.5	10.00	0.810	44.4
10.00	0.820	67.2	10.00	0.828	57.2
10.00	0.824	59.8	10.00	0.820	58.0
10.00	0.818	59.1	10.00	0.820	55.0
10.00	0.835	65.7	10.00	0.823	51.6
15.00	0.859	48.7	15.00	0.862	61.1
15.00	0.865	45.9	15.00	0.850	56.4
15.00	0.854	50.7	14.99	0.855	50.2
15.00	0.851	50.4	15.00	0.864	47.7
15.00	0.838	53.0	15.00	0.860	51.8
20.00	0.874	51.4	19.99	0.871	48.8
20.00	0.866	50.2	20.00	0.885	53.3
20.00	0.847	49.6	20.00	0.882	49.1
20.00	0.884	48.4	20.00	0.878	51.3
20.00	0.871	45.2	20.00	0.867	47.5
40.00	0.911	32.0	40.00	0.900	20.6
40.00	0.898	33.3	39.99	0.912	28.3
40.00	0.885	33.1	40.00	0.912	29.8
40.00	0.887	30.8	39.99	0.918	32.7
40.00	0.889	32.4	40.00	0.922	36.0
60.00	0.901	25.5	60.00	0.895	22.5
60.00	0.924	29.8	60.00	0.916	26.3
60.00	0.932	25.0	60.00	0.898	26.8
60.00	0.914	23.4	60.00	0.889	25.0
60.00	0.912	30.7	60.00	0.920	23.6

30% β-galactosidase / 70% DCP			40% β-galactosidase / 60% DCP		
comp. force [kN]	**rel. density**	**activity [%]**	**comp. force [kN]**	**rel. density**	**activity [%]**
2.00	0.655	75.8	2.00	0.632	92.1
2.00	0.673	59.7	2.00	0.645	83.1
2.00	0.650	81.6	2.00	0.648	95.5
2.00	0.667	82.0	2.00	0.657	78.7
2.00	0.653	92.5	2.00	0.648	84.8
5.00	0.761	71.0	5.00	0.714	74.4
5.00	0.735	81.5	5.00	0.720	78.3
5.00	0.735	89.6	5.00	0.716	89.1
5.00	0.734	71.7	5.00	0.726	60.8
5.00	0.767	50.4	5.00	0.734	87.4
7.00	0.764	47.8	7.00	0.714	53.1
7.00	0.770	51.2	7.00	0.740	63.3
7.00	0.770	46.6	7.00	0.751	40.7
7.00	0.768	54.8	7.00	0.751	62.3
7.00	0.770	61.8	7.00	0.763	52.9
10.00	0.818	58.7	10.00	0.790	56.1
10.00	0.809	55.5	10.00	0.788	49.5
10.00	0.817	57.6	10.00	0.795	56.2
10.00	0.817	55.0	10.00	0.794	55.8
10.00	0.815	55.2	10.00	0.795	45.7
15.00	0.844	39.1	14.99	0.811	54.5
15.00	0.838	44.0	14.99	0.829	45.0
15.00	0.843	45.4	15.00	0.828	47.6
15.00	0.838	35.5	14.99	0.824	57.2
15.00	0.849	38.8	15.00	0.832	54.1
20.00	0.854	44.4	20.00	0.856	46.9
20.00	0.854	30.3	20.00	0.852	48.0
20.00	0.866	42.6	20.00	0.858	42.3
20.00	0.863	40.8	20.00	0.855	42.5
20.00	0.861	43.7	20.00	0.861	47.9
40.00	0.889	25.6	40.00	0.889	25.6
40.00	0.897	28.1	40.00	0.885	29.4
40.00	0.905	22.6	40.00	0.884	26.4
40.00	0.889	24.1	40.00	0.878	23.1
40.00	0.899	25.9	40.00	0.885	23.2
59.99	0.920	25.8	60.00	0.895	26.8
60.00	0.883	21.1	60.00	0.893	27.0
60.00	0.884	16.0	60.00	0.903	27.1
60.00	0.879	13.7	60.00	0.897	29.5
60.00	0.879	19.1	60.00	0.897	34.0

50 % β-galactosidase / 50% DCP			60 % β-galactosidase / 40% DCP		
comp. force [kN]	**rel. density**	**activity [%]**	**comp. force [kN]**	**rel. density**	**activity [%]**
2.00	0.546	81.7	1.99	0.632	55.1
2.00	0.654	89.8	1.99	0.649	80.4
2.00	0.663	78.2	1.99	0.643	84.5
2.00	0.642	81.7	1.99	0.645	83.1
2.00	0.664	76.0	1.99	0.600	67.3
5.00	0.713	87.7	5.00	0.646	76.4
5.00	0.727	67.2	5.00	0.685	71.2
5.00	0.724	91.0	5.00	0.702	70.3
5.00	0.731	100.0	5.00	0.666	72.7
5.00	0.725	91.1	5.00	0.694	79.7
7.00	0.726	77.7	7.00	0.737	57.1
7.00	0.733	75.8	7.00	0.724	48.6
7.00	0.732	74.8	7.00	0.735	59.0
7.00	0.741	82.5	7.00	0.742	53.7
7.00	0.743	84.0	7.00	0.736	58.8
10.00	0.766	89.0	9.99	0.790	53.9
10.00	0.772	87.2	10.00	0.789	53.9
10.00	0.778	85.5	10.00	0.788	54.4
10.00	0.790	76.9	10.00	0.792	48.9
10.00	0.791	92.7	10.00	0.791	64.0
15.00	0.857	39.9	15.00	0.800	44.3
15.00	0.858	28.0	15.00	0.813	44.0
15.00	0.828	51.4	15.00	0.829	42.1
15.00	0.844	35.5	14.99	0.805	46.1
15.00	0.858	54.4	14.99	0.824	46.4
20.00	0.846	47.2	20.00	0.854	33.7
20.00	0.855	43.9	20.00	0.876	42.6
19.99	0.848	44.5	20.00	0.834	44.0
20.00	0.855	47.0	19.99	0.881	42.3
20.00	0.852	43.4	19.99	0.879	40.7
40.00	0.867	37.6	40.00	0.888	24.6
40.00	0.878	38.8	40.00	0.883	27.1
40.00	0.879	40.2	39.99	0.881	32.9
40.00	0.876	43.7	40.00	0.878	30.3
40.00	0.887	41.8	39.99	0.864	28.5
60.00	0.890	37.8	60.00	0.879	34.5
60.00	0.900	36.9	60.00	0.874	27.2
60.00	0.885	42.2	60.00	0.876	30.9
60.00	0.905	42.7	60.00	0.881	31.6
60.00	0.890	37.3	60.00	0.865	33.6

70% β-galactosidase / 30% DCP			80% β-galactosidase / 20% DCP		
comp. force [kN]	**rel. density**	**activity [%]**	**comp. force [kN]**	**rel. density**	**activity [%]**
2.00	0.638	73.8	2.00	0.611	70.7
2.00	0.634	80.0	2.00	0.642	67.7
2.00	0.646	81.8	2.00	0.636	57.7
2.00	0.655	67.5	1.99	0.656	74.5
2.00	0.655	73.9	2.00	0.647	75.6
5.00	0.707	60.2	5.00	0.710	72.4
5.00	0.717	69.0	4.99	0.688	75.1
5.00	0.721	72.1	5.00	0.712	72.2
5.00	0.712	63.6	5.00	0.718	72.8
5.00	0.713	64.3	4.99	0.717	77.1
7.00	0.735	56.3	6.99	0.773	51.2
7.00	0.737	63.6	6.99	0.777	60.5
7.00	0.748	61.2	6.99	0.784	65.4
7.00	0.746	65.5	6.99	0.792	61.7
7.00	0.752	64.6	6.99	0.783	60.1
10.00	0.799	54.6	10.00	0.777	61.9
10.00	0.795	45.8	9.99	0.776	42.6
10.00	0.789	57.1	9.99	0.776	62.1
9.99	0.804	57.6	9.99	0.771	59.3
10.00	0.791	58.1	9.99	0.814	57.7
15.00	0.812	43.6	15.00	0.845	44.1
15.00	0.811	44.6	15.00	0.846	53.0
15.00	0.818	48.7	15.00	0.852	41.1
15.00	0.817	42.4	14.99	0.859	51.2
15.00	0.818	48.4	14.99	0.876	49.1
19.99	0.818	31.3	20.00	0.812	29.2
20.00	0.837	42.1	20.00	0.844	42.2
19.99	0.859	43.3	20.00	0.808	37.6
19.99	0.847	47.1	20.00	0.803	41.9
19.99	0.860	49.1	20.00	0.818	43.5
40.00	0.852	25.7	40.00	0.878	18.9
40.00	0.848	32.4	40.00	0.894	29.1
40.00	0.860	20.3	40.00	0.894	29.7
40.00	0.851	26.8	40.00	0.894	33.4
40.00	0.844	28.1	40.00	0.898	26.7
60.00	0.879	24.8	60.00	0.859	18.9
60.00	0.886	28.6	60.00	0.895	24.7
60.00	0.885	28.5	60.00	0.875	27.8
60.00	0.889	30.3	60.00	0.877	32.4
60.00	0.897	29.5	60.00	0.886	40.8

90% β-galactosidase / 10% DCP			100% β-galactosidase / 0% DCP		
comp. force [kN]	**rel. density**	**activity [%]**	**comp. force [kN]**	**rel. density**	**activity [%]**
2.00	0.647	66.6	1.99	0.660	80.1
2.00	0.652	67.4	1.99	0.650	80.7
1.99	0.654	60.7	1.99	0.678	62.9
1.99	0.637	73.2	1.99	0.666	83.2
1.99	0.637	68.5	1.99	0.684	81.6
4.99	0.743	63.2	4.99	0.651	68.3
4.99	0.729	65.7	4.99	0.768	73.5
4.99	0.733	71.8	5.00	0.853	68.3
4.99	0.730	76.3	5.00	0.854	70.8
5.00	0.725	71.3	5.00	0.850	75.3
6.99	0.763	55.7	7.00	0.890	40.1
6.99	0.769	64.3	7.00	0.892	53.0
6.99	0.760	68.2	6.99	0.888	57.8
6.99	0.759	71.5	6.99	0.883	58.7
6.99	0.766	72.8	6.99	0.886	53.7
9.99	0.809	60.0	9.99	0.851	51.7
10.00	0.813	57.3	9.99	0.852	51.3
9.99	0.810	53.6	9.99	0.863	53.1
9.99	0.818	46.0	9.99	0.870	56.8
9.99	0.811	48.3	9.99	0.854	51.8
14.99	0.822	49.8	14.99	0.885	52.8
14.99	0.826	54.0	14.99	0.882	55.8
14.99	0.836	50.3	14.99	0.885	59.6
14.99	0.832	54.6	14.99	0.892	59.2
14.99	0.831	45.1	14.99	0.899	52.3
19.99	0.856	33.3	20.00	0.909	52.4
19.99	0.869	46.7	19.99	0.915	51.7
19.99	0.865	40.7	20.00	0.917	54.0
19.99	0.870	40.8	19.99	0.910	50.1
19.99	0.876	36.0	20.00	0.915	50.9
40.00	0.889	24.6	39.99	0.902	39.9
39.99	0.899	29.9	39.99	0.914	50.9
39.99	0.894	31.5	40.00	0.918	52.8
39.99	0.893	37.1	40.00	0.912	51.4
40.00	0.898	32.5	39.99	0.918	62.4
59.99	0.889	27.8	59.99	0.932	58.2
59.99	0.888	34.0	60.00	0.939	51.9
59.99	0.898	33.8	60.00	0.939	38.0
60.00	0.902	32.4	60.00	0.939	37.6
59.99	0.902	28.0	60.00	0.944	38.7

β-galactosidase-MCC granulate (series I)			β-galactosidase-MCC granulate (series II)		
comp. force [kN]	**rel. density**	**activity [%]**	**comp. force [kN]**	**rel. density**	**activity [%]**
2.00	0.695	61.9	2.00	0.701	92.8
1.99	0.700	78.1	2.00	0.700	84.3
1.99	0.699	81.8	1.99	0.701	93.3
1.99	0.696	79.6	1.99	0.704	91.6
1.99	0.698	75.5	1.99	0.706	95.8
5.00	0.870	86.0	4.99	0.897	91.6
5.01	0.874	73.4	4.99	0.885	85.6
5.00	0.875	66.4	4.99	0.888	96.8
4.99	0.870	78.2	4.99	0.891	94.2
4.99	0.870	82.2	4.99	0.885	90.3
6.99	0.928	71.9	6.99	0.935	60.0
6.99	0.928	79.4	6.99	0.943	86.0
6.99	0.930	75.1	6.99	0.929	84.1
6.99	0.926	70.5	6.99	0.936	85.2
6.99	0.921	77.2	6.99	0.934	87.4
9.99	0.959	72.4	10.00	0.958	85.9
9.99	0.966	69.0	10.00	0.968	95.5
9.99	0.965	65.7	10.00	0.962	80.7
9.99	0.955	68.3	9.99	0.957	78.6
9.99	0.966	71.6	10.00	0.966	90.6
14.99	0.982	65.1	15.00	0.981	86.2
14.99	0.991	55.0	15.00	0.995	87.8
14.99	0.981	64.1	15.00	0.970	95.6
14.99	0.984	64.7	15.00	0.975	93.6
14.99	0.986	56.7	15.00	0.968	88.4
19.99	0.972	62.3	19.99	0.978	88.7
19.99	0.987	64.5	19.99	0.981	86.1
19.99	0.984	70.3	20.00	0.980	84.6
19.99	0.973	72.2	19.99	0.980	80.9
19.99	0.991	73.1	19.99	0.985	76.5
40.00	1.004	69.7	40.00	1.002	77.9
40.00	1.001	57.7	40.00	0.999	73.6
39.99	0.999	66.8	40.00	0.995	70.2
40.00	0.991	69.4	40.00	0.998	78.8
39.99	0.997	63.9	40.00	0.992	85.1
59.99	1.007	68.7	60.00	0.999	99.6
59.99	1.020	72.6	60.00	0.999	82.6
60.00	1.009	70.1	59.99	1.001	74.0
59.99	0.998	73.4	59.99	1.000	83.6
60.00	0.997	66.4	59.99	0.999	67.9

β-galactosidase-DCP granulate (series I)			β-galactosidase-DCP granulate (series II)		
comp. force [kN]	**rel. density**	**activity [%]**	**comp. force [kN]**	**rel. density**	**activity [%]**
2.00	0.762	80.4	2.00	0.751	97.7
2.00	0.757	89.6	2.00	0.749	98.0
1.99	0.747	90.2	2.00	0.740	93.7
2.00	0.755	88.7	2.00	0.740	97.4
2.00	0.747	80.5	1.99	0.742	86.3
5.00	0.864	83.9	5.00	0.849	79.0
5.00	0.859	84.5	5.00	0.837	72.3
4.99	0.855	83.5	5.00	0.853	89.3
5.00	0.854	68.0	5.00	0.848	81.9
5.00	0.857	81.7	5.00	0.854	84.9
6.99	0.903	74.0	7.00	0.897	59.1
6.99	0.898	79.5	7.00	0.889	75.7
6.99	0.900	70.8	6.99	0.892	75.9
7.00	0.894	73.9	6.99	0.888	74.9
6.99	0.889	88.4	6.99	0.885	78.5
9.99	0.925	64.2	10.00	0.928	78.0
10.00	0.929	72.5	10.00	0.924	77.0
10.00	0.934	68.7	9.99	0.934	75.3
10.00	0.928	77.6	9.99	0.916	76.1
9.99	0.924	80.4	9.99	0.925	79.2
14.99	0.960	69.1	15.00	0.949	72.9
14.99	0.968	66.9	15.00	0.974	65.0
14.99	0.960	71.8	14.99	0.965	59.5
14.99	0.964	63.1	15.00	0.952	75.9
14.99	0.960	70.3	15.00	0.956	73.0
19.99	0.999	64.0	19.99	0.977	70.9
20.00	0.978	65.1	19.99	0.971	56.2
20.00	0.974	61.0	19.99	0.976	60.4
19.99	0.984	64.9	19.99	0.992	54.9
19.99	0.974	66.1	19.99	0.978	63.1
39.99	1.009	71.6	39.99	1.016	56.0
40.00	1.020	59.7	40.00	1.025	60.8
39.99	1.023	62.3	39.99	1.012	64.9
39.99	1.020	59.5	40.00	1.010	65.0
39.99	1.020	68.9	39.99	1.013	65.0
60.00	1.020	60.8	60.00	1.030	59.8
60.00	1.026	61.8	60.00	1.016	61.9
59.99	1.012	63.6	60.00	1.018	57.6
60.00	1.019	64.3	59.99	1.021	57.7
60.00	1.027	63.0	60.00	1.014	61.6

100% β-galactosidase-MCC pellets			50% β-galactosidase-MCC pellets / 50% MCC pellets		
comp. force [kN]	rel. density	activity [%]	comp. force [kN]	rel. density	activity [%]
5.00	0.965	97.3	5.00	0.968	85.8
5.00	0.949	98.6	5.00	0.968	91.5
4.99	0.953	98.7	5.00	0.977	96.2
4.99	0.962	98.3	4.99	0.982	88.0
4.99	0.969	94.0	4.99	0.979	88.2
5.99	1.021	94.6	5.99	1.003	95.5
5.99	1.022	91.4	6.00	0.999	104.9
5.99	1.031	91.2	5.99	0.993	80.9
5.99	1.006	89.2	6.00	0.996	89.3
5.99	1.025	95.4	5.99	0.999	85.4
6.99	0.997	91.6	7.00	1.003	111.6
6.99	1.002	81.2	6.99	1.009	70.5
6.99	1.006	90.9	6.99	1.008	104.7
6.99	1.003	104.0	6.99	1.007	102.5
6.99	1.004	90.8	6.99	1.001	100.7
9.99	1.035	95.1	9.99	1.030	104.4
9.99	1.036	89.6	10.00	1.033	97.7
9.99	1.029	89.9	9.99	1.039	105.3
9.99	1.044	92.0	9.99	1.029	108.8
9.99	1.040	77.8	9.99	1.030	98.6
14.99	1.042	101.1	14.99	1.040	84.4
14.99	1.049	98.1	15.00	1.040	87.1
14.99	1.043	110.2	14.99	1.041	92.4
14.99	1.039	95.5	15.00	1.045	98.6
14.99	1.040	97.0	14.99	1.044	104.6
19.99	1.050	89.9	19.99	1.045	92.7
19.99	1.054	99.7	19.99	1.046	99.0
20.00	1.056	96.6	19.99	1.043	94.1
19.99	1.046	103.6	19.99	1.043	87.9
19.99	1.047	102.5	20.00	1.040	83.1
39.99	1.056	91.7	39.99	1.060	97.3
39.99	1.055	90.7	40.00	1.055	94.3
39.99	1.052	86.3	39.99	1.050	95.7
39.99	1.060	86.5	40.00	1.052	84.1
39.99	1.061	83.4	40.00	1.052	86.9
59.99	1.067	90.3	59.99	1.070	96.4
59.99	1.077	88.9	59.99	1.075	88.3
59.99	1.067	86.0	59.99	1.066	86.6
59.99	1.064	90.9	59.99	1.067	103.2
59.99	1.066	90.4	59.99	1.067	98.0

β-galactosidase-sugar pellets (series I)			β-galactosidase-sugar pellets (series II)		
comp. force [kN]	**rel. density**	**activity [%]**	**comp. force [kN]**	**rel. density**	**activity [%]**
2.00	0.808	99.8	2.00	0.812	116.9
1.99	0.807	93.4	2.00	0.807	114.7
1.99	0.812	97.8	2.00	0.807	125.7
1.99	0.811	92.2	1.99	0.807	121.4
1.99	0.800	95.1	1.99	0.804	129.3
5.00	0.912	66.0	4.99	0.909	124.2
5.00	0.915	90.1	4.99	0.909	120.3
5.00	0.907	87.4	4.99	0.900	113.3
4.99	0.917	92.0	4.99	0.919	85.7
4.99	0.912	91.4	4.99	0.920	92.4
7.00	0.951	89.6	6.99	0.960	81.7
6.99	0.962	80.1	6.99	0.960	78.5
6.99	0.962	87.2	6.99	0.963	80.8
6.99	0.964	87.0	6.99	0.959	83.2
6.99	0.962	91.9	6.99	0.960	88.8
9.99	0.992	93.8	9.99	0.991	72.7
9.99	0.984	90.0	10.00	0.979	69.5
9.99	0.987	87.9	9.99	0.996	79.5
9.99	1.000	89.1	9.99	0.997	73.1
9.99	1.001	73.5	9.99	0.989	87.6
14.99	1.026	77.5	14.99	1.030	83.3
14.99	1.034	74.7	14.99	1.024	87.4
14.99	1.022	76.8	14.99	1.026	85.1
15.00	1.024	75.1	14.99	1.027	87.9
14.99	1.021	71.5	14.99	1.024	85.3
19.99	1.043	76.4	20.00	1.036	80.9
19.99	1.033	74.1	20.00	1.034	77.8
19.99	1.043	81.7	19.99	1.039	86.8
19.99	1.033	62.9	19.99	1.040	84.5
19.99	1.047	77.7	20.00	1.041	84.1
40.00	1.057	75.0	39.99	1.059	78.3
40.00	1.047	67.5	39.99	1.055	81.6
40.00	1.059	80.7	40.00	1.059	80.9
40.00	1.046	85.7	40.00	1.055	80.5
40.00	1.051	82.8	39.99	1.057	84.0
60.00	1.067	80.9	60.00	1.045	82.6
60.00	1.059	81.1	59.99	1.061	76.2
60.00	1.051	70.6	60.00	1.050	82.5
60.00	1.041	74.7	59.99	1.053	73.1
60.00	1.050	77.7	59.99	1.053	75.9

Curriculum vitae

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Education

2001-2004	PhD student at the Institute of Pharmaceutical Technology, University of Basel under the supervision of Prof. Dr. H. Leuenberger assistant in practical university courses of semi-solid dosage forms webmaster of the homepage of the Institute of Pharmaceutical Technology
Nov. 2000	swiss federal diploma in pharmacy
March-July 2000	diploma thesis in "non destructive dissolution testing with near infrared spectroscopy" at F. Hoffmann-La Roche Ltd., Basel
1997-1998	practical year at Barfüsser Apotheke, Basel
1995-2000	studies in pharmacy at the University of Basel
1991-1994	high school at Gymnasium Muttenz

Publications

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.

Kuny, T., Schatz, C., Ulmschneider, M., Marrer, S., Leuenberger, H., 2003. Non-destructive Dissolution Testing Correlation. Dissolution Technologies 10 (1), 22-28.