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Research paper

Influence of tableting on the enzymatic activity of different α-amylases using various excipients

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Abstract

The purpose of the study was to show the influence of compression pressure on the enzymatic activity of different types of α-amylases and to analyze the loss of activity of α-amylase in mixtures with different excipients. Following that, the properties of excipients used for tableting enzymes were evaluated. Tablets were produced on an instrumented single punch tableting machine. The pure amylases were tableted with increasing graded compaction pressures. Mixtures were tableted to different maximum relative densities, $\rho_{rel,max}$. The remaining enzymatic activity of the α-amylase in the tablets was determined by the starch iodine reaction. The results show a difference between different types of α-amylase depending on their origin and additives. Enzymatic inactivation occurs for the pure materials at all pressures used. It is initiated during and continues after compaction. It can be inhibited by freezing the tablets. Another possibility is to tablet the enzyme in a mixture with excipients, which prevent inactivation by softly embedding the enzyme. One example which even stabilizes α-amylase at high volume reduction is κ-carrageenan. In conclusion, enzymatic inactivation can be markedly reduced when excipients are used for tableting, which require little compaction pressure and are able to release the mechanical stress in the form of expansion.

Keywords: Tableting; α-Amylase; Pressure; Carrageenan; Microcrystalline cellulose; Dicalcium phosphate dihydrate

1. Introduction

Enzymes have been used extensively in therapy during the last century [1]. Tablets are a suitable dosage form for application of these materials because the enzymes are formulated in the dry state and no decomposition in solution can occur. However, one major factor influencing the stability of enzymes in tablets is compaction pressure [2–12]. Losses of activity of up to 50% have been reported for different enzymes due to compaction pressure [12]. Overcoming this problem will be a major challenge in the future, since enzymes are proteins like many new drugs, which are now evaluated for therapeutic use [13].

A major enzyme used for replacement of pancreatic enzymes is α-amylase [9,14]. This enzyme is necessary in the digestive tract. For this drug no resorption in the intestine is necessary like for other proteins, which are used for systemic therapy. As part of pancreatine, this enzyme has been found to exhibit volume reduction during compaction, resulting in loss of activity [9,14], whereas thermal and tribomechanical factors are of little importance [9]. α-Amylases from sources other than pancreatine can also be used for replacement [8,15,16]. These different α-amylases show different structural properties [8,15,16].

Thus, one objective of this study was to show the influence of compression pressure on the enzymatic activity of different types of α-amylases and to determine the factors that result in loss of activity on compaction.

Usually, a tablet consists of a mixture of excipients and the pharmaceutical ingredient. The excipient that influences the activity of the α-amylase the most is the filling material [14]. Well-known filling excipients can be used, e.g. microcrystalline cellulose, dicalcium phosphate dihydrate or lactose. In most cases, the filling material is expected to show a lot of plasticity because this is advantageous for the cohesion of the tablet and thus for the compaction process; especially when poorly binding active substances are tableted [17]. However, in case of enzymes which lose their activity due to volume reduction it would be of advantage to have a filling material which is able to produce a mechanically stable tablet without a lot of pressure. Additionally, the filler should release the compaction stress after volume reduction by showing a high elastic recovery

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[18,19]. Miyamoto [3,20] used a special microcrystalline cellulose which needs little pressure, but it still shows plasticity [21]. Carrageenans have shown a tableting behavior from which a soft embedding of enzymes or other ingredients can be expected [18,19]. They can be tableted very easily under low pressures and at the same time show a lot of elastic recovery. Due to this fact, they can be expected to store less stress in the tablet and to embed the active ingredient, here the α-amylase, softly.

Thus, the second objective of the study was to analyze the loss of activity of α-amylase in mixture with different excipients. One important aim is to find out whether carrageenan was superior compared to other excipients for tableting this pharmaceutically active protein.

2. Materials

The enzymes used were α-Amylase EC, a pure α-amylase (*Aspergillus oryzae*, Lot # WE 30446, 14.000 FIP Units, Extrakt Chemie, Stadthagen, Germany), α-Amylase S, an α-Amylase on the basic of wheaten flour and starch which contains cellulase (*Bacillus subtilis*, Lot # S9811527, 75.600 FIP Units; Biopract GmbH, Berlin, Germany) and Amylopract, an α-amylase in mixture with β-glucanase and protease (*Bacillus amyloliquifaciens*, Lot # M/T, 5.400 FIP Units; Biopract GmbH, Berlin, Germany). The excipients used for the mixtures were dicalcium phosphate dihydrate (Emcompress®, Lot # R 19 K, Mendell, Patterson, NY, USA), microcrystalline cellulose (Avicel ® PH 101, Lot # 14204, FMC Corporation, Princeton, NJ, USA) and κ-carrageenan (Gelcarin® GP-911 NF, Lot # ZC502, FMC Corporation, Princeton, NJ, USA).

3. Methods

3.1. Mixtures

Binary mixtures of α-Amylase EC with the three excipients dicalcium phosphate dihydrate, microcrystalline cellulose and κ-carrageenan were produced in a cubic mixer (Erweka GmbH, Heusenstamm, Germany). Mixing time was set to 15 min. The percentage of α-amylase was held constant at 25% (w/w).

3.2. Tableting

Tableting was performed on an instrumented single punch tableting machine (EK0/DMS, No. 1.0083.92, Korsch GmbH, Berlin, Germany) with 11 mm diameter flat faced punches (Ritter GmbH, Hamburg, Germany).

3.2.1. Enzymes

For all experiments, 100 mg of the pure amylases were tableted. Three tablets of each type of amylase were produced at a compaction pressure of 340 ± 10 MPa. For increasing graded compaction pressures between 50 and 380 MPa single tablets of Amylase S and Amylase EC were produced. Force, time and displacement were recorded for each compaction cycle. All tablets were stored dry at ambient conditions except for amylase EC, for which some tablets were frozen at −25°C.

3.2.2. Mixtures

Equal true volumes of the mixtures were tableted to four different maximum relative densities of the tablets, $\rho_{rel,max}$ ranging from 0.71 to 0.90 (±0.001).

The definition of $\rho_{rel,max}$ is as follows:

$$\rho_{rel,max} = \frac{\rho_{max}}{\rho_{true}}$$

with $\rho_{rel,max}$ = maximum relative density, ρ_{max} = density at minimum height of the tablet under load and ρ_{true} = true density. The true densities of all substances were determined by helium pycnometry (Accupyc 1330, Micromeritics, Norcross, GA, USA). The true densities of the mixtures were calculated from the true densities of the pure materials.

Displacement of the punch faces was measured using an inductive transducer (W 20 TK, Spectris GmbH, Langen, Germany) and was corrected for elastic deformation of the punches. Elastic deformation was measured by punch-to-punch deformation. Forces were measured by calibrated strain gauges. The depth of filling was held constant at 13 mm. The production rate was ten tablets per min. The die was externally lubricated with a solution of 2% (w/w) stearic acid in ethanol. The amount of material necessary for each tablet with a given $\rho_{rel,max}$ was calculated. The powder was manually filled into the die and one compaction cycle was performed. Three single tablets were produced at each condition. Data acquisition was performed by a DMC-plus system (Spectris GmbH, Langen, Germany) and data were stored by BEAM-Software (AMS-Flöha, Germany). Force, time and displacement were recorded for each compaction cycle.

3.3. Tablet height

The height of all tablets was determined manually with a micrometer screw (Mitotuyo Corp. Tokyo, Japan) directly after production. Elastic recovery according to Armstrong and Haines-Nutt [22] was calculated. Tablet height was not studied later because the height at this time refers to the measured enzymatic activity of the frozen tablets.

3.4. Analysis of enzymatic activity

The enzymatic activity of the α-amylases was determined by an assay method measuring the starch digestive power using the starch iodine reaction, here by the Willstätter hypoiodite method. The method used is described in Ref. [23]. The method was optimized for the amylase used and in modification the reaction temperature was set to 30°C. The percentage of enzymatic activity was calculated always for

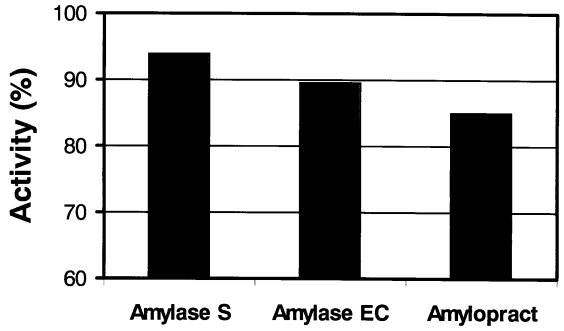

Fig. 1. Influence of the type of α-amylase on the remaining enzymatic activity of tablets ($n = 3$, mean and SD).

three tablets. Two determinations were performed for each tablet. The reproducibility of the method was determined to be 3.26% for the pure enzyme.

4. Results and discussion

4.1. Enzymes

4.1.1. Influence of the type of α-amylase

Fig. 1 shows the loss of enzymatic activity of three different types of α-amylase at a compaction pressure of 340 MPa. All the α-amylases show a partial inactivation. The resulting tablets have a height of less than 0.6 mm. This means that a calculated $\rho_{rel,max} > 1$ results and the enzymes will be totally deformed. Amylase S, a *Bacillus subtilis* α-amylase, shows the lowest reduction of enzymatic activity after tableting. It is an amylase, which is unique among other amylases in that it contains no sulfhydryl groups or disulfide linkages; furthermore, it contains zinc and calcium. Sulfhydryl groups or disulfide linkages are known to increase pressure stability [12]. This industrial amylase is standardized by wheaten flour and starch and contains additionally some cellulase. Most probably the wheaten flour and starch may prevent a further loss of activity. The highest inactivation is exhibited by Amylopract, an industrial *Bacillus amyloliquifaciens* α-amylase, which contains other enzymes like glucanases and proteases. This industrial α-amylase which does not contain any wheaten flour and starch is more easily inactivated during compaction. One reason for the inactivation compared to *Bacillus subtilis* α-amylase S may be that *Bacillus amyloliquifaciens* α-amylase contains no calcium. Calcium stabilizes the enzymatically active conformation of the α-amylase [12,15], and the absence of calcium is the major difference in *Bacillus subtilis* α-amylase [12,15]. The other reason may be that there is no additive like wheaten flour and starch present, which can prevent inactivation. The stability of amylase EC, an *Aspergillus oryzae* α-amylase, is intermediate to the other two amylases. This α-amylase contains both sulfhydryl and disulfide groups [15], but it contains no additives and no other enzymes. It is used in pharmaceutical production. According to the structure it should be more resistant to pressure than *Bacillus subtilis* α-amylase (Amylase S). Since the results show the opposite, it seems likely that by addition of an additive or excipient stability during tableting could be improved.

Summarizing, stability during compaction seems to be enhanced by increase of sulfhydryl or disulfide groups and by the increase of stabilizing calcium or zinc ions. Furthermore, the presence or absence of excipients is important. The influence of different excipients on inactivation during tableting will be discussed later.

4.1.2. Influence of compaction pressure

Amylase S and Amylase EC were both compacted at graded compaction pressures up to 380 MPa. For both α-amylases a loss of activity is obvious (Fig. 2). It is about the same at all compaction pressures used. There is no dependency on compaction pressure seen when a certain pressure has been exceeded. The pressure was always higher than 50 MPa because at lower pressures no stable tablets resulted. Following that, it can be assumed that at all pressures the final inactivation is reached. This means a remaining activity of 95.7 ± 1.5% for Amylase S and of 90.3 ± 1.0% for Amylase EC. The result is in accordance with results in literature [9,12,14].

4.1.3. Influence of storage conditions

Since Amylase EC is the commonly used α-amylase in pharmaceutical industry, the influence of storage conditions was studied for this enzyme. Fig. 3 shows the loss of enzymatic activity for tablets which were directly frozen after tableting compared to those which were stored at ambient conditions for at least 7 days (30–40% relative humidity). For the frozen tablets a continuous increase of inactivation due to compaction was observed, whereas the loss of activity was higher for those stored at ambient conditions. Compaction seems to be an event, which only starts the inactivation of enzymatic activity, and this continues over some days. For the frozen tablets the inactivation is stopped by freezing. Similar results were obtained by Hachino and

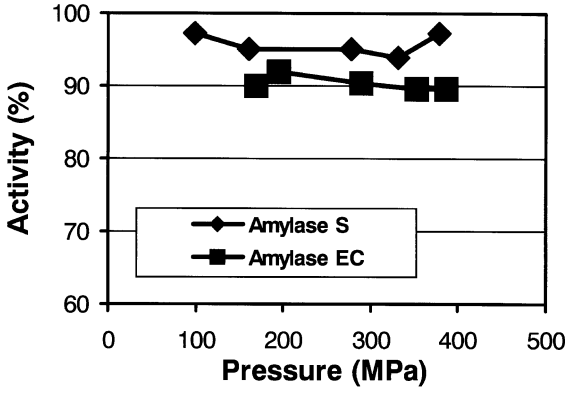

Fig. 2. Influence of compaction pressure on the remaining enzymatic activity of tablets made of different α-amylases.

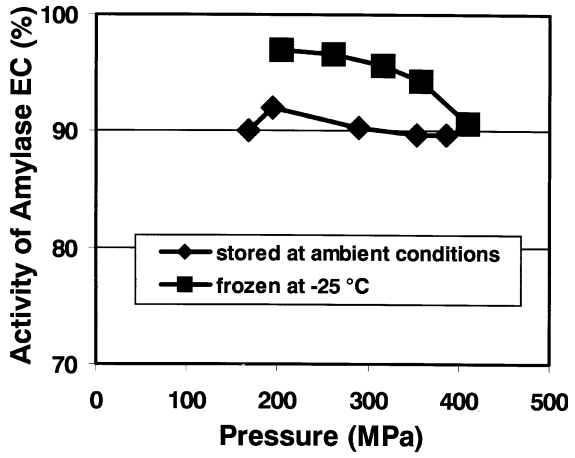

Fig. 3. Influence of storage conditions on the remaining enzymatic activity of tablets made of α-Amylase EC.

Furukawa [24] for thrombin tablets. For the frozen tablets a dependency on compaction pressure can be seen. The higher the compaction pressure the higher is the loss of activity during compaction. Thus, storage conditions are of high importance for the stability. The most important influence is the combination of moisture [12,25] and temperature [26]. If the tablets are frozen, moisture cannot interact with the enzyme because the mobility of the water molecules is reduced.

4.2. Mixtures

To freeze all tablets containing α-amylase directly after tableting would be a very cost-intensive factor for the pharmaceutical industry. Thus, it is of importance to find excipients which can inhibit inactivation of α-amylase during tableting. Then, no special storage is necessary.

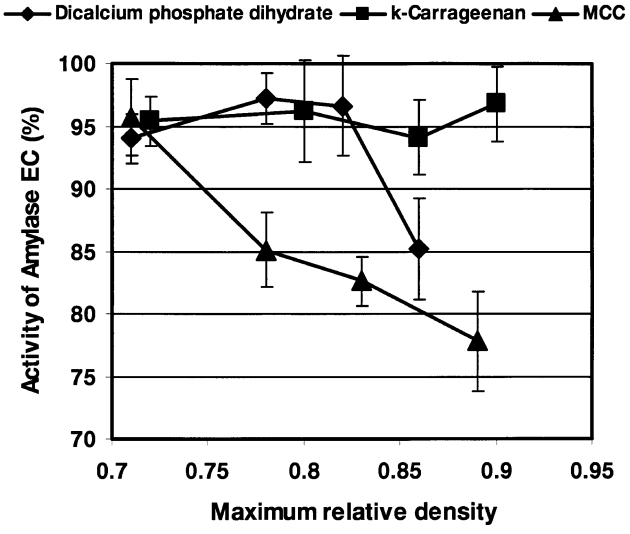

Fig. 4. Influence of the type of excipient on the remaining enzymatic activity of tablets made of mixtures with α-Amylase EC ($n = 3$, mean and SD).

4.2.1. Influence of mixing

Firstly, the α-amylase should not be inactivated during the mixing process. Therefore, samples of the binary mixtures were analyzed for their enzymatic activity after mixing. Mixtures with dicalcium phosphate dihydrate showed a value of 101.3%, those with microcrystalline cellulose a value of 97.9% and mixtures with κ-carrageenan a value of 101.1% activity. Thus, mixing did not influence the stability.

4.2.2. Influence of pressure and volume reduction

Fig. 4 shows the influence of $\rho_{rel,max}$ on the stability of Amylase EC in mixture with the three different excipients. The corresponding pressures and the elastic recovery of the tablets are given in Table 1. Dicalcium phosphate dihydrate is a brittle material, which requires a lot of pressure for compaction. Microcrystalline cellulose is one of the most widely used pharmaceutical excipients. It is plastically deforming. The tablet height is the lowest in Table 1. Compared to the other excipients the tablets with κ-carrageenan require the lowest pressure for compaction and the tablet height is the highest afterwards.

Looking at the remaining enzymatic activity, for microcrystalline cellulose, inactivation is continuously increasing with increasing $\rho_{rel,max}$. This means that due to the plastic deformation the enzyme was inactivated even when the pressures were lower than in mixture with dicalcium phosphate. For both dicalcium phosphate dihydrate and the κ-carrageenan, the loss of activity is less than for the pure enzyme up to a $\rho_{rel,max}$ of 0.83 (see Section 4.1.1). The influence of moisture to destabilize the α-amylase in combination with microcrystalline cellulose can be excluded since carrageenan contains more moisture than microcrystalline cellulose [19]. Thus, inactivation is inhibited by the excipient. Obviously, the plastic deformation with microcrystalline cellulose has a greater influence on the activity than the

Table 1
Pressure and tablet height of the differently produced tablets ($n = 3$, mean and SD)

Excipients	$\rho_{rel,max}$	Pressure (MPa)	Elastic recovery (%)
Dicalcium phosphate dihydrate	0.71	79.7 (2.3)	5.24 (0.41)
	0.78	166.7 (3.6)	3.59 (0.55)
	0.82	277.4 (8.9)	5.32 (0.55)
	0.86	410.0 (4.9)	6.87 (0.22)
κ-Carrageenan	0.72	54.5 (4.4)	12.48 (0.61)
	0.80	100.1 (1.5)	10.79 (0.13)
	0.86	184.9 (0.4)	10.69 (0.24)
	0.90	315.4 (2.0)	12.70 (0.06)
Microcrystalline cellulose	0.71	100.6 (0.4)	5.28 (0.16)
	0.78	167.1 (2.2)	4.77 (0.06)
	0.83	258.0 (2.4)	7.14 (0.85)
	0.89	335.8 (7.6)	9.27 (0.31)

particle fracture of the brittle dicalcium phosphate dihydrate. However, when the carrageenan and dicalcium phosphate were more compressed, dicalcium phosphate dihydrate showed a further loss of activity of more than 5%, whereas inactivation in mixture with the κ-carrageenan remained the same as at the other $\rho_{rel,max}$. At this $\rho_{rel,max}$ the combination of the low pressure and the high elastic recovery (tablet height) of the κ-carrageenan enables the enzyme to be 'softly tableted': the pressure necessary compared to both the other excipients is low and the tablet height the highest. The use of carrageenan prevents inactivation and the α-amylase is embedded very softly inside the tablet.

Graf and Sakr [14] related the inactivation of the α-amylase to the transformation of mechanical energy into heat and concluded that heat was absorbed by some materials better than the others. The conclusion would be to find materials, which can absorb heat preferentially. On the other hand, Teng and Groves [12] showed that the loss of activity is due to volume reduction. The results of this study show that it is both the volume reduction and the release of mechanical energy in form of elastic expansion, which are responsible for the stability of the enzyme. Only if the excipients are able to release the mechanical stress necessary for tableting, inactivation can be prevented.

5. Conclusions

The results show that there is a difference between different types of α-amylase depending on their origin and their additives. The inactivation occurs for the pure materials at all pressures used. It is initiated during and continues after compaction. It can be inhibited by freezing the tablets directly after production. Another possibility is to tablet the enzyme in mixture with excipients, which are able to prevent inactivation by softly embedding the enzyme, since inactivation was markedly reduced on using excipients, which were able to release the mechanical stress necessary for tableting. An example of such an excipient shown in this study was κ-carrageenan which stabilizes α-amylase even at high volume reduction.

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