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Effect of Compression Pressure on the Activity of Lipase

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Abstract

Enzymes have been used extensively in therapy during the last century. 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 is tablet compaction pressure. Overcoming this problem will be a major challenge in the future. It was the scope of this work to characterize the behavior of the lipase powder and immobilized lipase under pressure to gain 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. The free enzyme could not survive the smallest pressure used, where as the CLEA could withstand the compression.

Keywords: Lipase, *Burkholderia cepacia,* Cross Linked Enzyme Aggregate (CLEA), Glutaraldehyde, Kappa carrageenan

Introduction

Enzymes are very efficient catalysts for biochemical reactions. They speed up the reactions by providing an alternative reaction pathway to lower activation energy. The enzymes are usually highly selective, and are usually globular[1]. The intra- and intermolecular bonds that hold proteins in their secondary and tertiary structures are disrupted by changes in temperature, pH, pressure and many other factors. Above the optimum temperature and pH, the enzyme structure begins to break down (denature) since at higher temperatures intra- and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy, enzymes have been used extensively in therapy during the last century[2]. Tablets are a suitable dosage form for the applications of these materials because the enzymes are formulated in the dry state and no decomposition in solution can occur[3]. However, one major factor influencing the stability of enzymes is tablet compaction pressure. Losses of activity of up to 50% have been reported for different enzymes due to compaction pressure. Overcoming this problem will be a major challenge in the future[2].

There are three major classes of enzymes as classified according to their function as, metabolic enzymes, food enzymes, and digestive enzymes. To make the enzyme stable in harsh environment it can be immobilized by fixing them to a solid surface, by covalent bonding to a solid support, by adsorption onto an insoluble substance, entrapment within a gel, and cross linking. Lipase is an enzyme necessary for the absorption and digestion of nutrients in the intestines.

This digestive enzyme is responsible for breaking down lipids (fats), in particular triglycerides, which are fatty substances in the body that come from fat in the diet. Once broken down into smaller components, triglycerides are more easily absorbed in the intestines. Lipase is primarily produced in the pancreas but is also produced in the mouth and stomach. Most people produce sufficient amounts of pancreatic lipase, but pancreatitis and such digestive disorders has to be treated with enzyme replacement therapy. The effect of pressure on the activity of the lipases has not been studied in detail. It was the scope of this work to characterize the behavior of the lipase powder and immobilized lipase under pressure to gain 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.

Materials and Methods

Enzyme lipase PS from *Burkholderia cepacia* (from Amano, Nagoya, Japan), BSA (bovine serum albumin, Sigma, St.Louis, USA), Sodium Alginate (Sigma, St.Louis,USA), Tris buffer (pH 8.5), phosphate buffer (pH 7.2) ammonium sulphate (enzyme grade), calcium chloride 5M, Folin – phenol ciocalteau reagent, gluteraldehyde, etc are from

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standard chemical companies. Purified cassava starch was obtained from fresh tubers. Phosphate buffer pH 7.0, 0.1M and Tris HCl buffer 0.01M, pH 8.5 prepared by standard methods.

Lipase Assay
Lipase assay was done using Olive oil as substrate in Tris-HCl buffer, pH 8.5, at 30 ±2 °C. To 10 ml buffer and 10 ml olive oil emulsion, 0.5 ml enzyme was added and under shaking for 20 minutes. 20 ml acetone was added to stop the reaction. It was then titrated against 0.05M NaOH using phenolphthalein as indicator. From the titre value enzyme activity was calculated. One unit (U) of lipase activity is defined as the amount of enzyme, which liberated 1µmol of free fatty acid per minute under assay conditions.

Protein estimation
Protein estimation was done by Lowry's method; (9) using BSA as the standard and the blue colour develop was read at 660 nm in a spectrophotometer (Shimadzu UV2100, Japan).

Compression equipment.
The compression experiments were conducted in a precision compressing machine (Carver, model no: 3851, USA). Pellets were made by applying different vertical compression force.

Extraction procedure
Weighed 10gm of lipase PS enzyme was dissolved in 100 ml of phosphate buffer 0.1M, pH 7.0. It was centrifuged at 1957 x g for 20 min and the supernatant was collected and the lipase activity was measured.

Ammonium sulphate precipitation method
The previously purified enzyme supernatant was precipitated with 80% saturated ammonium sulphate solution at 4 °C under stirring for 12 hr centrifuged at 1957 x g for 5 min. and the pellet was taken and measured the activity of the enzyme and stored in refrigerator.

Cross linking of lipase (CLEA)
Fifty mg of lipase precipitate was cross-linked by using of 2% glutaraldehyde solution in phosphate buffer for 1hour at 25°C and the enzyme got cross linked and formed aggregates. After cross linking, it was washed 2-3 times with phosphate buffer, pH 7.0, 0.1M, to remove the excess glutaraldehyde and stored in the same buffer, and measured the activity of the enzyme. Aggregated enzymes were immobilized by entrapment in three different hydrogels such as chitosan, gelatin, and sodium alginate by ionotropic gelation.

Entrapment of lipase aggregates in hydrogel
Weighed 50 gm of enzyme aggregate (CLEA) and mixed it with 3% sodium alginate solution. Poured sodium alginate solution mix in 3ml of 5M calcium chloride solution in Petri dish kept at 4°C for 1 hour. The gel was washed with water and the immobilized slab was cut into small bits and freeze dried and then measured the activity of the enzyme.

Similarly gelatin entrapment was carried out by using 10 % gelatin in water heated to dissolve and cooled to room temperature and added 2ml of the hardening solution which consisted of 20% formaldehyde, 50% ethanol, 30% water. The gel was frozen at -20°C for 4 hours. Kappa carrageenan, 3% was taken in water and the cross linking agent was 3% KCl.

Preparation of compressed tablets
Weighed 80% of dried purified starch and 20% of immobilized enzyme (total weight 0.4 to 0.5g) and mixed well to get a fine uniform powder. The mix was filled in the die (11mm dia.) of the compressing apparatus and compressed it at different pressure of 0.5, 1.0, 1.5 and 2.0 tonn and a pellet was made. The experiment was repeated three times.

The enzyme release studies
Release rate were determined in dissolving the pellets in 15ml of phosphate buffer (pH 7.4) in a conical flask under shaking condition at 100rpm for 1 hour at ambient temperature of 28 ±3 °C and the activity was measured.

Results and discussion:
The influence of the type of lipase at different compaction pressures were studied. This is a EC 3.1.1.3 Triacylglycerol lipase. The lipase enzyme as such as well as the cross linked aggregates (CLEA) was used for the study and it was found that both preparations behaved differently. The free enzyme could not survive the smallest pressure used, where as the CLEA could withstand the compression and the data is shown in Fig-1. The mixture ratio of 20% of enzyme powder and the filler used was cassava starch in both the cases. It was found that a plastic compression character and regularity in shape and size of the

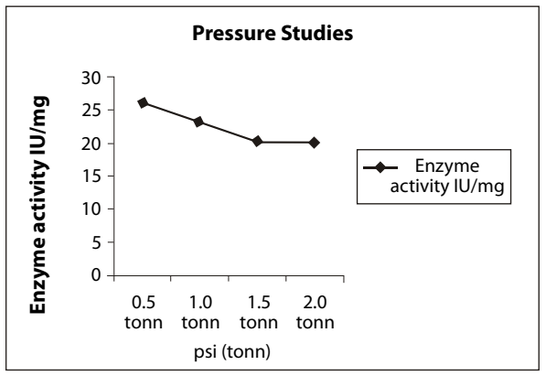
Table-1 Characteristics of tablet on compression

S.No	Compression pressure on tablets (tonn)	Weight g	Height mm	Diameter mm	Density
1	0.5	0.456	4.0	11.09	1.17
2	1.0	0.409	3.61	11.08	1.18
3	1.5	0.535	4.64	11.08	1.19
4	2.0	0.509	4.92	11.10	1.21

Table: 2 Effect of compression on the lipase activity

S.No	Compression pressure on tablet. (tonn)	Enzyme activityIU/mg
1	0.5	25.94
2	1.0	22.95
3	1.5	19.96
4	2.0	19.96

Fig: 1 Effect of compression on the enzyme activity



compressed particles was important to protect the enzyme activity under pressure.

The investigated model enzyme was a solid lipase preparation from *Burkholderia cepacia*, which was chosen for its stability, the molecular weight of 28kDa, and relatively simple enzyme activity assay. Compacts were produced on a material testing machine and the activity was detected. The shearing forces seemed to have negative influence on the activity of the enzyme.

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. 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

Conclusions

The free lipase enzyme could not survive the smallest pressure used, where as the CLEA could withstand the compression. Immobilization of the enzyme makes it robust and the resulting modified enzyme is stable. This preparation can be used for pharmaceutical as well as animal feed. 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.

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