academicJournals

Vol. 8(7), pp. 206-210, 22 February, 2014
DOI: 10.5897/AJPP2013.3781
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Feasibility study of manufacturing coated tablet lipase from *Yarrowia lipolytica*

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Submitted 12 June, 2013; Accepted 11 February, 2014

Many studies have provided evidence to use microbial enzyme lipase such as *Yarrowia lipolytica* lipase as substitute of pancreatic lipase. However *Y. lipolytica* lipase lowest resistance at acid pH (1 to 2) limits its application in pharmaceutical industries. In order to improve *Y. lipolytica* lipase resistance to low acid pH, our study was devoted more particularly to coating lipase tablets by Eudragit L30D-55. Lipase tablets were evaluated for weight uniformity, enzyme content, friability, hardness, according to European pharmacopeia. *In vitro* release was studied using pH 1.2 acidic buffer and pH 6.8 phosphate buffer. Study revealed that the prepared tablets were able to sustain enzyme release into the intestine. *Y. lipolytica* lipase tablet was compared to Creon 150 mg (commercial drug). After 3 h incubation, coated tablets did not release lipase at pH 1.2 but was disintegrated totality at pH 6.8 same as Creon 150 mg at 17 min. After 8, 12 and 24 months storage at 25 and 37°C, enzyme tablets retain their enzymatic activity and proprieties.

Key words: Tablet, creon, Eudragit L30D-55, *in vitro* release.

INTRODUCTION

Porcine pancreatic enzymes are the current standard of treatment for pancreatic exocrine insufficiency (PEI), and when protected from exposure to gastric acid, porcine lipase will not be degraded in the stomach. Creon (pancreatin) and pancrelipase are the two primary forms of porcine pancreatic enzyme supplements (PES) available, and pancrelipase has higher enzyme content (Devi et al., 2009).

The Creon is a microgranule enteric capsule content of porcine pancreatic enzymes extract. The Creon microgranules are coated using methylhydroxypropylcellulose phthalate (enteric polymer) (Solvay, pharma). Creon helps to improve digestion of patients with exocrine pancreatic insufficiency due to cystic fibrosis, chronic pancreatitis or after deficient of the pancreas. Bovine enzymes are a potential alternative for individuals who

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refuse to consume porcine products for religious or other cultural reasons (Layer and Keller, 2003). The bovine preparations, however, contain approximately 75% less lipase activity than the porcine and human preparations, and there is some concern about transmittable pathogens (foot and mouth disease and bovine spongiform encephalopathy) from bovine preparations. A very promising approach currently being followed is the replacement of the porcine lipases by microbial lipases.

Microbial preparations of pancreatic enzymes (lipase, protease, and amylase) also exist (Zentler-Munro et al., 1992; Borowitz et al., 2006a). Certain bacteria (for example, *Burkholderia plantarii*) and fungi (for example, *Aspergillus niger*, *Rhizopus arrhizus*) produce pancreatic enzymes with substantial lipolytic activity and greater resistance to gastric acid degradation. The microbial preparations do not require colipase for activation (Layer and Keller, 2003). Of the microbial preparations some fungal lipases are remarkably stable in the acidic gastric milieu, and is rapidly inactivated by bile acids and proteases whereas bacterial lipase has remarkable stability in both gastric and duodenal milieu, particularly in moderately acidic and neutral pH. *In vitro* studies have demonstrated bacterial lipase stability against proteases and bile salts and *in vivo* canine experiments show that bacterial lipase is more potent and efficacious than porcine (PES) (Raimondo and Dimagno, 1994; Suzuki et al., 1997). However, lipase from *A. niger* (Zentler-Munro et al., 1992) and *Yarrowia lipolytica* (Aloulou et al., 2007; Turki et al., 2010a) are some examples of the most tested fungi (pH 3 to 10). The *Aspergillus* and *Yarrowia* lipases have better survival in the duodenal environment as shown *in vitro* and in rats, while the others (*R. arrhizus*) (Layer and Keller, 2003), lipase from *Candida cylindacea* appeared very sensitive to trypsin and to the detergent action of bile salts.

Nowadays in medicine and pharmaceutical domains, acrylate polymers are used to coat drugs. Acrylate polymers and their derivatives, collectively known as Eudragit polymers, were the first synthetic polymers used in pharmaceutical coatings. To form films on the surfaces of pharmaceutical materials, they are usually applied as aqueous polymeric dispersions. Among the additives that are incorporated into the film-forming aqueous polymeric dispersions, the plasticizer is the most critical component. It governs the film formation and the quality of the resulting film. Incorporation of a plasticizer is recommended for polymer coating formulations due to the high glass transition temperatures of polymers. Eudragit L 30D-55, a relatively soft polymer, can be applied in the form of latex under mild working conditions, with the addition of 10 to 20% plasticizer even at room temperature and usually good film formation can be expected (Lehmann, 1997).

Several authors (Turki et al., 2010b) have published works on lipase from *Y. lipolytica* formulated with milk and Arabic gum as good candidate for use in enzyme replacement therapy as a means of treating pancreatic exocrine insufficiency than *Candida rugosa* lipase. Our works proposed preliminary studies to formulate lipase of *Y. lipolytica* on tablet form. This enzyme is stable at pH 3 to 8 (Alloue et al., 2008). However, it is not sufficiently resistant at low pH (1) to be used as a digestive enzyme without protection. This study provides a coating technique of lipase tablets using acrylic polymers such as enteric Eudragit in order to investigate *in vitro* release compared to Creon 150 mg.

MATERIALS AND METHODS

Lipase from *Y. lipolytica* LgX6481 was produced in 2000 L bioreactor (LSL Biolafitte, Poissy, France) in the same conditions as described (Destain et al., 1997). The juice produced was centrifuged on a BTPX205 continuous centrifuge (Alfa Laval, Sweden) at 12,000 × g, at a flow rate of 500 Lh^{-1} and the supernatant was freeze dried for further studies. Eudragit L 30 D-55 (Röhm, Pharma) aqueous colloidal polymer dispersions and diethyl phthalate plasticizer were selected for the study. Silicon emulsion (Vel, Leuven Belgium), talc, lactose DCL11, aerosil 200 and polyplasdone XL were purchased from Merck (Germany). All other chemicals used for the study were of analytical grade. Creon 150 mg (commercial drugs) was purchased in a pharmacy at Gembloux (Belgium).

Tablets manufacture

For 60 g of the total powder was added 30 g of lipase, polyplasdone XL (2.5 g) , 0.2 g of aerosil 200, and finally adding 26.3 g of lactose DCL11. All ingredients was mixed in a mortar and after mixing in a turbulat (System SOHATZ, Switzerland) during 15 min, and 1 g of magnesium stearate was added. The final powder is put in an alternate machine KORCH, to seek the biconvex point (36 tablets/min).

Evaluation of sustained release tablets

In addition, weight variation, hardness, friability, disintegration time, and content uniformity of active ingredient (lipase) were determined for the quality control of Standard test. For the determination of weight variation, 20 tablets were weighed individually on an analytical balance (Sartorious, Germany), and then, mean and standard deviation were calculated. Hardness of 10 tablets were measured using a durometer COMPUTEST (Zurich, Germany). Dusted and accurately weighed 20 tablets were placed in a friabilator (Pharma Test, Germany) and rotated 4 min (100 revolutions). To remove adhering particles, the tablets were dusted again and then weighed. The test for uniformity of mass was performed on 20 tablets (European Pharmacopoeia, 2002). The deviation limit shall be 10% for tablets weighing less than 80 mg. The mean percent friability was calculated from the difference in tablet weights. The disintegration time of tablets (n=6) was determined at 37°C in water using disintegration tester (Komet, Turkey). Lipase was extracted completely from the tablets in pH 6.8 phosphate buffer and the solution was filtered. The enzymatic

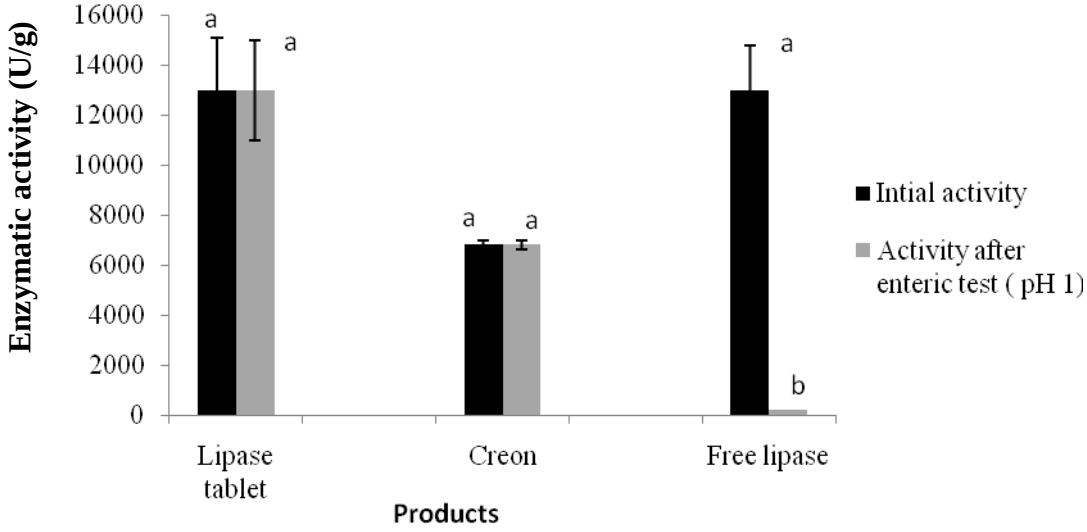

Figure 1. Enzymatic activity of products (free and lipase tablet and Creon) after Gastro-resistant test. For each product, histograms with the same letter are not significantly different, P ≥ 0.05.

activity of lipase was measured by tritimetric method using olive oil emulsion as substrate. After hydrolyse reaction, fatty acid was titrated by NaOH (0.05 N) as described by Destain et al. (1997). Activities are expressed in international units, where 1 U of lipase is the amount of enzyme able to catalyze the release of 1 µmol of fatty acid per minute at pH 7 and at 37°C.

Enteric coating of compressed tablets

In 800 g of water, 1 g of tween 80, 2 g of silicone emulsion and 25 g of talc were dispersed using ultraturax (IKA, Werk) to which was added 333 g of Eudragit L30D-55 and 20 g of diethyl phthalate. After 2 h of stirring, the homogeneous dispersions were obtained. The coating was performed by the fluidized bed drying method (Glatt, Germany) with the inlet temperature and outlet air (45 and 30°C), in an amount of 6 g/min dispersion. After coating, tablets were used for further experiments. Coating was continued till the required mass gain was achieved. *In vitro* dissolution studies were carried out using the basket method. A surplus dissolution test station (Hanson Research Corporation Chatsworth, California, and USA) was used for all dissolution studies. The volume of medium was 500 ml at 37 ± 0.5°C and a stirring rate of 100 rpm was employed. Tablet containing 30 mg of lipase and Creon containing 30 mg of lipase were used for dissolution study. To determine lipase release of the uncoated or coated tablet, a dissolution test (n = 6) was performed using two media with pH 1.2 (HCl, 0.1 M), pH 6.8 (phosphate buffer, 0.5 M), respectively at 2 h for the first and 60 min for disintegration test. For stability studies, the lipase tablets were stored at 25 and 37°C over a period of 12 and 24 months. Samples were evaluated after interval to 1 month, for different parameters such as physical appearance, hardness, mass variation, lipase activity content and dissolution.

Statistical analysis

The data obtained were subjected to analysis of variance(Statistica, 99 Edition) and mean differences determined by Duncan's multiple range test ($P < 0.05$).

RESULTS

Quality control tests

The quantitative evaluation and assessment of a tablet's chemical, physical and bioavailability properties are important in the design of tablets and to monitor product quality. These properties are important since chemical breakdown or interactions between tablet components may alter the physical tablet properties, and greatly affect the bioavailability of the tablet system. The quality control values determined for the test and standard reference are summarized in Table 1.

In vitro release study between Creon and lipase

For comparison study, lipase tablet and Creon capsule were submitted for *in vitro* release test such as enteric test at pH 1 and disintegration test at pH 6.8. We specified that Creon is composed of three pancreatic enzymes such as lipase, amylase and protease but only lipase enzymatic activity is concerned in this work. Lipase activity in Creon 150 mg is 10,000 U which represents 6800 U/g enzymatic activity. Results from Table 2 show that *Y. lipolytica* lipase freeze dried powder can be compressed in the presence of pharmaceutical excipients. After coating, lipase tablets were resistant to gastro-resistant pH (1) at 180 min (3 h). At pH 6.8, tablets were disintegrated at 17 min same to Creon 150 mg and Dry matter is ranged from 95 to 98%. Figure 1 shows clearly that enzyme tablet retains its enzymatic activity after the gastro resistant test at pH (1).

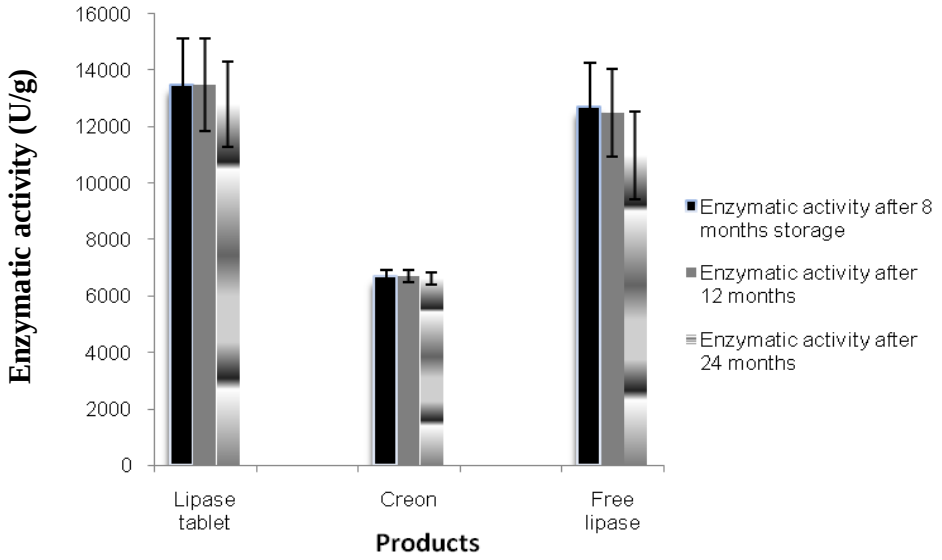

Figure 2. Enzymatic activity of products after 8 to 24 months storage at 25 and 37°C.
For each product, there is no significant difference between enzymatic activities during the storage, $P \geq 0.05$.

Table 1. Quality control of lipase tablet.

Quality control	Lipase tablet initial	Lipase tablet after 12 and 24 months at 25 and 37°C	Reference value (Euro Ph, 2002)
Color	White	No change	No change
Hadness (N)	10±3	No change	No change
Uniformity of mass (mg)	60.5±0.08	61.5±0.05	<10%
Friability	0	No change	< 1%
Disintegration time (min)	6±1	No change	<15

Stability studies

The stability study results of *Y. lipolytica* lipase tablets are shown in Table 1. There were no significant changes in their physical appearance, average mass, hardness and lipase activity content. The release profile did not show any significant changes either. Figure 2 shows that after 8, 12 and 24 months storage at 25 and 37°C lipase tablets retain their enzymatic activities.

DISCUSSION

Control quality of tablets study shows that there are various standards that have been set regarding the quality of pharmaceutical tablets. These include weight, hardness, disintegration and dissolution characters. The remaining specifications assure that tablets do not vary from one production lot to another (Liberman et al., 1990). All quality control parameters were within the specified limits (for example, weight variation < 10%, friability < 1% and disintegration time < 15 min).

In vitro release study of product show that *Y. lipolytica* lipase coated tablet can be resistant at pH 1.2 after 3 h and disintegration time after 17 min, similar to creon. This result could be explained by the fact that excipients and film coating are protected tablets against enteric environment and from moisture. Figure 1 shows that *Y. lipolytica* lipase coated tablet and Creon preserve their enzymatic activity at acid pH. It explains that the enteric coating plasticizer of diethyl phthalate and Eudragit $L_{30}D\text{-}_{55}$ were completely insoluble at pH 1.2 and soluble at pH 6.8 while free lipase lost its total activity (significantly different $P < 0.05$), as previously described by Alloue et al. (2008).

Our results confirm those of Turki et al. (2010b) who have demonstrated that the same free lipase was very sensitive to extreme gastric acidity, and lipase formulated with milk and arabic gum keeps 50% of activity at pH 7 and are resistant to intestinal protease. Tables 1 and 2, and Figure 2 show that all products retain their enzymatic activity at 25 and 37°C. Hence, it can be concluded that

Table 2. *In vitro* release of lipase tablet and Creon capsule before and after storage.

Product	Gastro-resistant test pH 1 (min)	Disintegration test pH 6.8 (min)	Gastro-resistant test pH 1 (min) after 12 and 24 months storage	Disintegration test pH 6.8 (min) after 12 and 24 months	Dry matter after 24 months storage at 25 and 37°C
Lipase tablet from *Y. lipolytica* 30 mg	180±2	17±2	120±1	17±1	98.70±0.07
Creon 150 mg	180±2	16±1	120±1	16±1	99.80±0.01
Free lipase	0	-	0	-	95.35±0.05
Reference values	120	≤60	120	≤60	99.99±0.0

the manufactured tablets were stable and retained their pharmaceutical proper-ties during a period of 24 months. It may suggest that the lipolytic activity is not destroyed in the presence of pharmaceutical additives. These results are in accordance with Wilson et al. (2013) which found a good stability after drug storage at room and 40°C temperatures.

Conclusion

Our study shows that tablet lipase from *Y. lipolytica* keep the quasi totality of activity in presence of excipients, and present similar characteristics of pharmaceutical drug (Creon 150 mg). In order to enable the use of this lipase in tablet or other galienic forms, it is very important to perform this study.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGMENT

The authors gratefully acknowledge the personnel of the laboratory of Biopharmaceutics and galenic Pharmaceutical of University of Brussels for assistance and technical supports, and the government of Cote d'Ivoire for financial support.

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