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TABLETING COMPRESSION BEHAVIOUR OF ENZYME TRYPSIN-CHYMOTRYPSIN

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Abstract

This study is investigation of tableting compression behaviour of protein pharmaceutical in tablet formulations by the use of trypsin–chymotrypsin s as model drug. The trypsin-chymotrypsin mixture (in the activity ratio 6:1) having enzymatic activity of 2000 AU/mg.

50% Trypsin-chymotrypsin, 26 % microcrystalline cellulose , 7.5% Sodium Bicarbonate-Citric acid mixture(in ratio70:30),6.5% lactose or Starch, 5.5%Magnesium stearate were dry mixed.dry mixed blend is subjected to binding by using 4.5% Polyvinyl Pyrrolidone solution in Isopropanol.then granulated using Mesh No. 14 after dehumidification at 50 ^{0}C under vacuum. A 100-mg sample was compressed by using deep biconcave 9-mm punches and dies at the compression force of 70, 80, 90, and100 KN (Kilo Newton) at a speed of 25 mm/min. the tablet compression was performed with granules containing 6.5% lactose (without starch), in contrast, tablets were formulated by using granules contains 6.5% starch (without lactose) to investigate effect of excipients on compression behaviour of enzyme mixture. Then the tablets were coated with 12.50% w/w solution of ethyl cellulose in dichloromethane and diethyl phthalate as plasticizer. Ethyl cellulose as a coating material can prevent the influx of the dissolution medium into the tablet core, and thus decrease the premature dissolution and release of the drug from the enteric-coated tablet at acidic pH.

The hardness of tablets formulated with higher compression force significantly higher than those formulated with lower compression force. Drug dissolution profile shows significant decrease in the drug release due to denaturation from activity loss under high compression force so tablets prepared at 70 KN Compression force shows higher activity. The compression behaviour of tablet with different excipient can be study using a dissolution study. As increases pressure decreases enzymatic activity. The enzymatic activity in tablets prepared using starch at 70 KN was significantly higher than that prepared using lactose at same compression force because starch shows elastic deformation at low pressure and plastic deformation at high pressure where as lactose shows brittle behaviour.

Keywords: Protein pharmaceutical; Tableting ;Compression force; Trypsin-chymotrypsin; tablet Coating; Dissolution; Drug release; KN (Kilo Newton).

INTRODUCTION

The fact that the difficulties in the development of new tablet formulations due to increasing in complexity of drug substances. Among other new drug substances the use of proteins and peptides as pharmaceuticals is steadily increasing, specially with the fast development biotechnological process such as Isolation, purification, formulation and delivery of proteins represent significant challenges to pharmaceutical scientists, as proteins possess unique chemical and physical properties.[1,2,3] These properties pose difficult stability problems, which can be influenced by the formulation and technological factors, such as excipients, temperature, storage conditions, compression or shearing forces[4].

The simple compression of a bulk material, either powder or granulate, to a tablet is dependent on a great number of influences, mainly force transfer, particle deformation and the formation of adhesive forces. Therefore the behaviour of powder under compression is an interesting topic of wide range. Compression behaviour 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 behaviour. 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[4, 5].

Investigation of compression behaviour of protein pharmaceutical in tablet formulations done by the use of trypsin–chymotrypsin as model drug. it is anti-inflammatory agent [6]. In the present work, granules were prepared by wet granulation using isopropanol as a solvent with Polyvinyl Pyrrolidone, MCC, Starch, and second formulations starch is replaced with lactose in order to quantify possible physical interactions of excipients and drug during compression. And granules were compressed under different force i.e.70, 80, 90 KN and 100 KN. Ethyl cellulose is used as a coating material which Protect enzyme against gastric inactivation and remains intact during their transit through the stomach into the duodenum by preventing the influx of the dissolution medium into the tablet core, and thus decrease the premature dissolution and release of the drug from the enteric-coated tablet in 0.1 N HCl solutions.[3,4]Then the enzyme activity is quantified by assay. It is known to proteolytic activity decrease under pressure and which occur during tableting compression[9]. Compression of all that different formulations and their influence on enzyme activity is done to formulate an oral enzyme therapy of a trypsin–chymotrypsin.

MATERIALS AND METHODS

Materials

Trypsin-chymotrypsin was obtained from (Elder pharmaceuticals, Mumbai), microcrystalline cellulose (Avicel PH-102),sodium bicarbonate from Rankem chemicals, Mumbai,citric acid from Qualigen ltd. lactose (Lactose monohydrate) and starch (Maize starch) from Fisher scientific, Mumbai, Ethylcellulose (Cellulose Ethyl),Polyvinyl Pyrrolidone, titanium dioxide from SD Fine chemicals, Mumbai. Diethyl phthalate from Hipax fine chem ltd. Haemoglobin powder from Himedia was used. Rest of all the reagents and chemicals utilized for formulation and enzymatic assay were of analytical grades.

Methods

Granulation process

Trypsin-chymotrypsin mixture and all excipients were weighed as per formula in table No.1, then the drug and all powder excipients are shifted by using sieve No.22. Then 50% Trypsin-chemotrypsin mixture(proteolytic activity in ratio of 6:1)which is equivalent to 2000AU/mg, 26% MCC, 7.5% Sodium bicarbonate-citric acid mixture(in ratio 70:30 and 6.5% starch/lactose taken for dry mixing using multipurpose mixer (GEA Engg, Model:Ultimagral).

Then this dry mixed blend is subjected to binding by 4.5 % w/w Polyvinyl Pyrrolidone solution prepared in Isopropanol by using mechanical stirrer (Bio-Lab) and Isopropanol 700 ml/kg of blend is used as solvent[7, 8]. Then coherent mass was shifted through sieve No.14 after drying by dehumidification (Make: Tropical Model: 0330) at 50 ^{0}C under vacuum.

Tableting procedure

Setting of the Tableting Machine[10, 11]

A Tablet compression machine (Rimek Minipress, MT-II, Karnavati Engg.Ltd,) is used after proper setting of the machine and the fitting of the punches and dies.

Fitting of the punches and die

The upper and lower punches height was adjusted by fitting punches of 133.65mm length, lower punch was fitted in a such manner to work freely and when pushed through the die ,must drop back smartly under its own weight, and its tips do not protrude above the die table at the ejection point to avoid damage of base of the feed frame and punch tips, lower punch loading plugs perfectly in the turret by applying friction to the lower punch body by means of 'Nylon plug' to prevent out coming of punch, then punch guides .die bores ,die ,lock screws and turret cover were properly cleaned and fitted. Then mounting level of feed frame was correctly set, upper punch was also proper fitted after lubricating punches head using ENKLO-68 oil, Turret was firstly rotated by hand to ensure all clearances are correctly set before tableting procedure.

Tablet compression procedure [12, 13, 14]

A over load compression force regulating knob was adjusted to different compression force while tableting. A 9 mm punches and die with biconcave surface was used to compress 100 mg granule sample containing 100000 Armour unit activity at 70,80,90,100 KN force at speed of 25 mm/min.Compression forces of 70KN, 80KN, 90KN and 100 KN was optimised by using over load force regulating knob. The thickness and weight of tablets was optimised during compressing by using respective regulators. Compression force utilised for eight different formulae was as per follows

Table 1: Formulas for Core Tablet [7, 8]

S. No	Ingredients	Milligram Quantity of ingredients in each 100mg tablet				Milligram Quantity of ingredients in each 100mg tablet			
		F_1a	F_2a	F_3a	F_4a	F_5b	F_6b	F_7b	F_8b
		70 KN	80 KN	90 KN	100 KN	70 KN	80 KN	90 KN	100 KN
1.	Trypsin-chymotrypsin	50	50	50	50	50	50	50	50
2.	Polyvinyl pyrrolidone	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
3.	MCC	26	26	26	26	26	26	26	26
4.	Starch	6.5	6.5	6.5	6.5	-	-	-	-
5.	Lactose	-	-	-	-	6.5	6.5	6.5	6.5
6.	SodiumBicarbonate-Citric acid	7.5 (70:30)	7.5 (70:30)	7.5 (70:30)	7.5 (70:30)	7.5 (70:30)	7.5 (70:30)	7.5 (70:30)	7.5 (70:30)
7.	Magnesium stearate	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
8.	Isopropanol	700ml /blend	700ml /blend	700ml /blend	700ml /blend	700ml /blend	700ml /blend	700ml /blend	700ml /blend

Enteric Coating[7, 15]

Preparation of coating composition

Enteric coating composition contains 83.00%w/w Dichloromethane, 12.50% w/w Ethyl cellulose as it prevent the influx of the dissolution medium in to tablet core , 00.50% w/w Talc,03.00% w/w Diethyl phthalate and 01.00 % w/w Titanium dioxide is added as opaquant which was prepared by using mechanical stirrer and container was capped with aluminium foil. And then composition was filter through nylon cloth to remove any particles present in it.

Coating method

Enteric coating with suitable coating materials is essential requirement for core enzyme tablets to survive in stomach acid in order to be absorbed in small intestine. Enteric coating which is insoluble in acidic pH, and soluble in intestinal pH. Half liter coating composition for1kg of tablets is sprayed on tablets standard coating pan (Jaya engineers, Aurangabad) at maximum 40°C bed temperature and 8 rpm speed.

RESULTS

Evaluation of the granules [16, 17]

The prepared dried granules were then evaluated for flow properties like angle of repose, bulk density, tapped density, Hausner ratio and Carr's index.

Angle of repose

The angle of repose for the granules of each formulation was determined by the funnel method.

Density

Three densities for powdered solid were determine based on the following ratios True Density= $\rho_{t=M/Vt}$ Granular Density $\rho_{g=M/Vg}$ Bulk Density $\rho_b = M/V_b$

Bulk Density

Bulk Density is of great importance when one considers the homogeneity of low dose formulation in which there is large differences in drug and excipients densities.

Tapped Density

It is determined by placing a graduated cylinder containing a known of drug or formulation on mechanical tapping apparatus, which is operated for a fixed number of taps (1000) until the powder bed volume has reached a minimum. Using the weight of drug in cylinder and this minimum volume, the tapped density may be computed.

Hausner's ratio

Hausner ratio is calculated by

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Poured Density}}$$

Carr's index

A simple indication of the ease with which a material can be induced to flow is given by application of a compressibility index, is given by the equation.

I = [1- Tapped density / Bulk density] x 100.

All of these properties of granules were evaluated, All formulation shown angle of repose within the limit of good compressibility. Three densities values ,Hausner ratio and carr's index. All of the formulations shown values within limit as per the Table No.2

Table 2: Properties of granules

Formulation	Angle of repose*	Loose bulk density *	Tapped bulk density*	Carr's index* (%)	Hausner ratio*
F_1a	28°87□ □ 1°11□	0.556□0.031	0.646□0.034	13.85□2.53	1.16□0.03
F_2a	27°90□ □ 1°57□	0.520□0.020	0.570□0.044	8.01□4.23	1.13□0.05
F_3a	28°87□ □ 1°11□	0.532□0.027	0.650□0.038	18.15 □ 3.84	1.25□0.04
F_4a	28°43□ □ 1°87□	0.543□0.024	0.597□0.037	9.04 □ 4.52	1.09□0.02
F_5b	28°67□ □1° 07'	0.501□□0.021	0.540□0.028	7.23□3.45	1.07□0.03
F_6b	27°67□1° 60'	0.518 □0.029	0.554□0.043	6.50□3.87	1.06□0.04
F_7b	28°27'□1°43	0.530□0.032	0.584□0.026	9.25 □4.47	1.10□0.01
F_8b	28°43□□ 1° 07'	0.557□0.030	0.598□0.028	6.86□3.24	1.07□0.03

*Average of 3 determinations ± SD

Evaluation of tablets[16, 17, 18]

All the uncoated (core) and coated tablets were evaluated for following properties

Crushing strength/Hardness

The crushing strength/ Hardness of tablets were measured with a Hardness tester (Pfizer).

Uniformity of Weight: Weigh 20 tablet selected randomly and determine average weight by using electronic balance (Shimadzu, AX200).

Friability Test:

Friability test of 20 Tablets was measured with Friability tester (Electrolab, EF-1W)

Thickness

Thickness of tablets was measured by using Vernier caliper (Mitutoyo, Digimatic Caliper)

Properties of uncoated (core) tablets were as per the Table No.3 and Properties of coated tablets were as per the Table No.4

Table 3: Properties of core tablets

Formulation	Thickness±S.D.** in mm	% friability ±S.D.**	Weight variation±S.D.**	Hardness±S.D.** in Kg/cm²
F_1a	2.4 □ 0.4	0.6 □ 0.21	106 □□□□□□	3.7□0.4
F_2a	2.5 □ 0.6	0.5 □ 0.18	98.90 □□□□□□	3.2□0.6
F_3a	2.7 □ 0.8	0.6 □ 0.21	99.57 □□□□□□	3.4□ 0.2
F_4a	2.5 □ 0.6	0.7 □ 0.25	99.43 □□□□□□□	3.8 □0.5
F_5b	2.5 □ 0.4	0.6 □ 0.21	103.87 □□□□□□	3.6□0.4
F_6b	2.4 □ 0.6	0.5 □ 0.18	104.03 □□□□□□	4.0□0.6
F_7b	2.5 □ 0.4	0.6 □ 0.21	101.07 □□□□□□	3.9□ 0.2
F_8b	2.6 □ 0.6	0.7 □ 0.25	94.43 □□□□□□	4.2 □0.5

** Average of 6 determinations ± SD

Table 4: Properties of coated tablets

Formulation	Thickness ±S.D.**	Weight variation ± S.D.**	Hardness (kg/cm²) ± S.D.**
F_1a	3.4 ▯ 0.2	99.5 ▯▯▯▯▯	5.8 ▯2
F_2a	3.6 ▯ 0.4	▯▯▯▯▯▯▯▯▯▯▯▯	5.5 ▯ 1
F_3a	3.4 ▯ 0.2	▯▯▯▯▯▯▯▯▯▯▯▯	5.3 ▯ 1
F_4a	3.5 ▯0.3	▯▯▯▯▯▯▯▯▯▯▯	5.2 ▯ 3
F_5b	3.5 ▯ 0.2	▯▯▯▯▯▯▯▯▯▯▯▯▯	6.0 ▯2
F_6b	3.7 ▯ 0.4	▯▯▯▯▯▯▯▯▯▯	5.5 ▯ 1
F_7b	3.3 ▯ 0.2	▯▯▯▯▯▯▯▯▯▯▯	4.9 ▯ 1
F_8b	3.6 ▯0.4	▯▯▯▯▯▯▯▯▯▯▯	5.2 ▯ 3

** Average of 6 determinations ± SD

Disintegration study[19]

The tablet disintegration test was performed for uncoated (core) and coated tablet using the disintegration tester (Electrolab, ED2L) Disintegration media used for uncoated (core) tablet was water maintained at 37 ± 2ºC where as for coated tablet As described in USP XXI firstly using 0.1N HCl without disc for two hours and then by using phosphate buffer pH 6.8 by using disc to each tube for one hour at 37 ± 2ºC.

The time required for disintegration (DIT) of uncoated (core) tablet and coated tablet were measured by observation as per table No.5.and table No.6 respectively.

Each value reported is an average of 6 independent measurements.

Table No.5: Disintegration tests of core tablets

Disintegr-ation media	DIT ▯▯SD**							
	F_1a	**F_2a**	**F_3a**	**F_4a**	**F_5b**	**F_6b**	**F_7b**	**F_8b**
Acid media (0.1N HCl) 2 hour study	Tablet not disintegrated	Tablet not disintegrated	Tablet not disintegrated	Tablet not disintegrated	Tablet not disintegrated	Tablet not disintegrated	Tablet not disintegrated	Tablet not disintegrated
phosphate buffer, pH 6.8 for 1 hour	40.5▯2	43.2▯1	47.9 ▯ 4	50.7▯ 3	45.2 ▯ 5	47.2▯ 1	43.4 ▯ 7	59.3▯4

** Average of 6 determinations ± SD

Table 6: Disintegration tests of coated tablets

Disintegration media	Formulations ±SD**							
	F_1a	F_2a	F_3a	F_4a	F_5b	F_6b	F_7b	F_8b
Acid media (0.1N HCl) 2 hour study	Tablet not disintegrated	Tablet not disintegrated	Tablet not disintegrated	Tablet not disintegrated	Tablet not disintegrated	Tablet not disintegrated	Tablet not disintegrated	Tablet not disintegrated
phosphate buffer, pH 6.8 for 1 hour	43.7±4	47.3±3	52.5 ± 5	56.9 ± 4	48.1±4	52.4± 3	55.6 ± 5	59.3±4

** Average of 6 determinations ± SD

Assay of enteric coated tablet [20,21,25,26]

Assay principle

The proteolytic activity test which is based on the 10 minutes enzymatic hydrolysis of haemoglobin substrate at 25 ± 1^{0}C and at standard condition of time, the unhydrolyzed substrate undigested haemoglobin is precipitated with trichloroacetic acid and removed by whatman fitter paper no. 3,and the amount of un precipitated protein spilt products, which is a measure of the amount of protein present is estimated with folin ciocalteau reagent, giving a colour which detects the quantity of solubilized haemoglobin in filtrate spectrophotometrically at 660 nm using plotted standard graph of Absorbance Vs Proteolytic Activity.

Enzyme Sample Preparations

Standard preparation

Standard solution was prepared by taking 50 mg of working standard proteolytic enzyme mixture [activity approximate ≅2000 Au/mg.] in volumetric flask, dissolved slowly in 0.001 N HCL and dilute to 250 ml with 0.001 N HCL. Pipette 10ml in volumetric flask and dilute to100ml with 0.001 N HCL.

Sample preparation

five enteric coated tablets were taken after removing coating by washing them gently with water and by Triturating slowly with 0.001N HCI in a mortar and then transfer to volumetric flask and dilute to 1000ml with 0.0001 HCI. Pipette out 10 ml in volumetric flask and dilute to 100ml with 0.001 N HCL.

Assay Evaluation Procedure

After the preparation of enzymes samples. Two separate sets of standard and sample were prepared. Each set comprises 2 enzyme tests and 1 enzyme blank tubes. Marked for sample as – SP_1, SP_2, and SP_B and for standard Std_1, Std2 and Std_B. 2ml of standard solution and sample solution was taken into marked tubes. 10 ml of T.C.A was Pipette out in each of the tube in blank row marked as SP_B and Std $_B$ mixture. Closed the tube and tap gently for 30 seconds against palm of hand. Then 5 ml of the haemoglobin substrate was taken in to

the first tube of the standard set time interval of 10 minutes by using stop watch. Close the tube and tap gently against palm of hand to mix. At 30 seconds intervals, 5 ml of substrate was withdrawn in to the other tubes filling diluted upto all the blank in the last, stopwatch was reset to the zero just before the interval timer rings, and fill the T.C.A pipette in order to add it to the first tube when the alarm rings. Add 10 ml of T.C.A. at 30 seconds interval to each of the other tubes in the same order as to substrate was added and gently swirled. Filter the sample and blanks using Whatman filter No. 3 Ensure that the filtrate shall be clear from any particles. Then transfer 5 ml of digestion filtrate in tubes containing 10 ml of 0.5N NaOH. Develop colour by adding 3 ml of Folin Ciocalteau reagent beginning with blank and then sample and duplicate of each set in a definite order. After 15 minutes measure absorbance at 660 nm against respective blanks. Correct the A_{660} value of each enzyme test

Calculations

Absorbance taken at 660 nm. (A_{660nm}) value was corrected by subtracting reading of respective enzyme blanks from average of and two sample and standard test solutions.

Average Sample Reading (ASR) = $\frac{S_1+S_2}{2} - SB$

Average Standard Reading (AStR) = $\frac{St_1+St_2}{2} - StB$

Total proteolytic activity in armour unit/ tablet is calculated by formula

$$= \frac{ASR\ x\ Std.Wt\ x\ Std\ \ Activity\ x\ 10\ x\ 2\ x\ 100\ x\ 1000\ x\ 1}{AStR\ x\ 250\ x\ 100\ x\ 1\ x\ 2\ x\ 10\ x\ 5}$$

$$= \frac{ASR}{AStR}\ X\ Std.Wt\ x\ \ Std\ \ activity\ x\ 0.8$$

Where S_1, S_2 and SB are the Absorbance of sample test and sample blank solutions respectively. And St_1, St_2 and, StB are the Absorbance of standard test and standard blank solutions respectively.

The assay values were as per table No.7 and all values reported are reproducible and Represent the average of 3 determinations

Tablet 7: Assay for enzymatic activity of formulation

S.No	Formulation	Labelled activity in armour unit (AU)	Assayed activity AU/ per tablet	Assay*(%) AU ± SD*
1	F_1a		101483.3366	101.483±6.798
2	F_2a		99939.2837	99.939±2.207
3	F_3a		99814.0135	99.814±2.040
4	F_4a	**Labeled activity is 100000**	98413.0123	98.413± 7.094
5	F_5b	**AU**	78173.1758	78.173± 5.789
6	F_6b		74891.2475	74.891±4.668
7	F_7b		74313.579	74.313± 2.643
8	F_8b		71537.432	71.537± 4.342

* Average of 3 determinations ± SD

Dissolution study [21, 22]

The drug dissolution tests for the enteric coated tablets were performed according to USP by using 8 basket dissolution apparatus. (Electrolab, TDT-08L), The basket was immersed in 900 ml of dissolution medium and the

paddle rotates at 100 rpm at 37±0.5°C., first dissolution medium (0.1 N HCl) for two Hrs and second dissolution medium (phosphate buffer, pH 6.8) for one Hrs. Aliquots (4 ml) of the solution were withdrawn At after 60 min. from acid medium and 15 min. from phosphate buffer, pH 6.8 time intervals using a syringe and was suitably diluted with dissolution medium for measurement of trypsin-chymotrypsin concentration. After each withdrawal, 4 ml of fresh dissolution medium was added to maintain sink condition. The concentrations of trypsin-chymotrypsin were measured after proteolytic assay and the absorbance was measured in the ultraviolet-visible spectrophometer at 660nm.

The dissolution profile of enteric coated tablet formulations containing starch and formulations containing lactose as per table No.8.and table No.9 respectively.

Similarly marketed formulations of trypsin-chymotrypsin were taken for dissolution study as per table No.10.

All values reported were reproducible and represented the average of 6 independent dissolution tests.

Table 8: Dissolution study of formulations containing starch

Dissolution media	Time (Min)	% drug release ±SD**			
		F_1a	F_2a	F_3a	F_4a
0.1 N HCl	60	0.098±0.00	0.057±0.00	0.088±0.00	0.009±0.00
	120	0.273±0.00	0.107±0.00	0.188±0.00	0.086±0.00
PBS (pH 6.8)	135	21.52 ±0.00	16.07 ±0.00	26.59±0.00	07.62±0.00
	150	80.29 ±0.00	47.67 ±0.00	38.35±0.00	34.00±0.00
	165	87.79 ±0.00	68.03 ±0.00	40.80±0.00	35.79±0.00
	180	96.23 ±0.00	85.21 ±0.00	57.44±0.00	48.18±0.00

** Average of 6 determinations ± SD

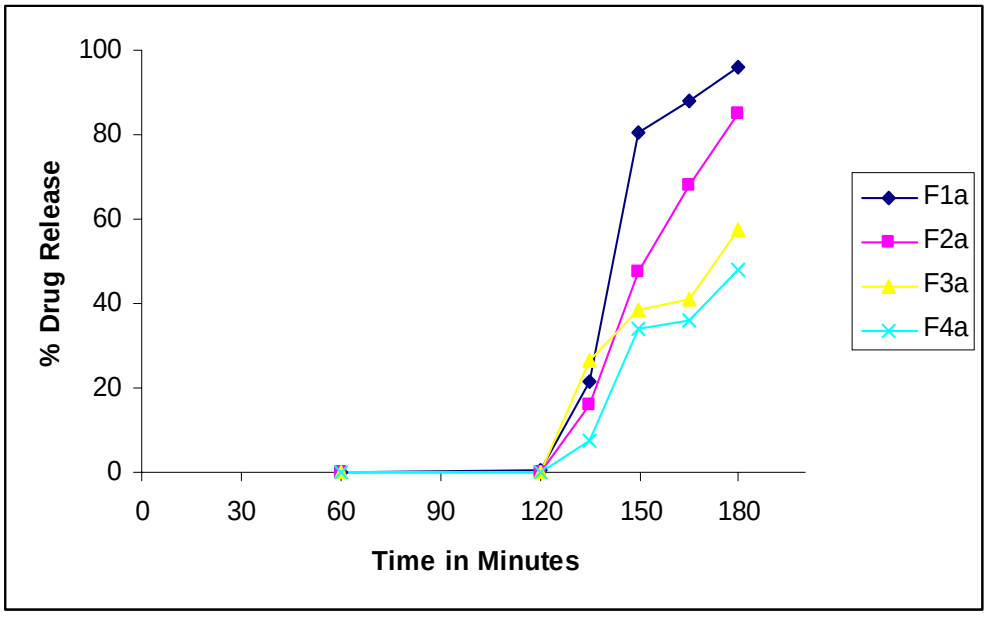

Fig. 1: Drug Release profile of Formulations containing starch

Table.9: Dissolution Study of Formulations containing lactose

Dissolution stage	Time (Min	Average % drug release ±SD**			
		F_5b	F_6b	F_7b	F_8b
Acid stage	60	0.07±0.00	0.04±0.00	0.354±0.00	0.45±0.00
	120	0.98±0.00	0.09±0.00	0.341±0.00	0.37±0.00
Buffer stage	135	12.22±0.000	17.30±0.00	15.88±0.00	11.72±0.00
	150	27.22±0.00	30.84±0.00	25.08±0.00	25.08±0.00
	165	47.79±0.00	49.15±0.00	38.57±0.00	28.73±0.00
	180	60.88±0.00	56.52±0.00	52.43±0.00	39.70±0.00

** Average of 6 determinations ± SD

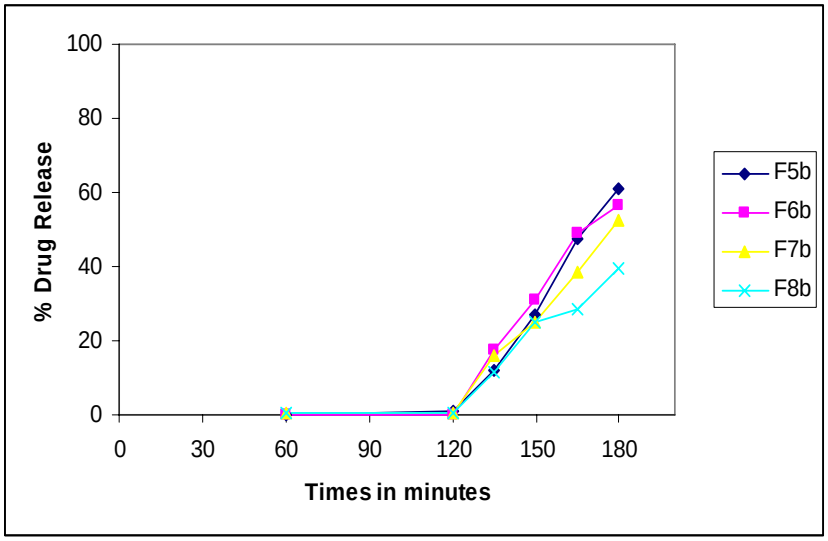

Fig. 2: Drug Release profile of formulation containing lactose

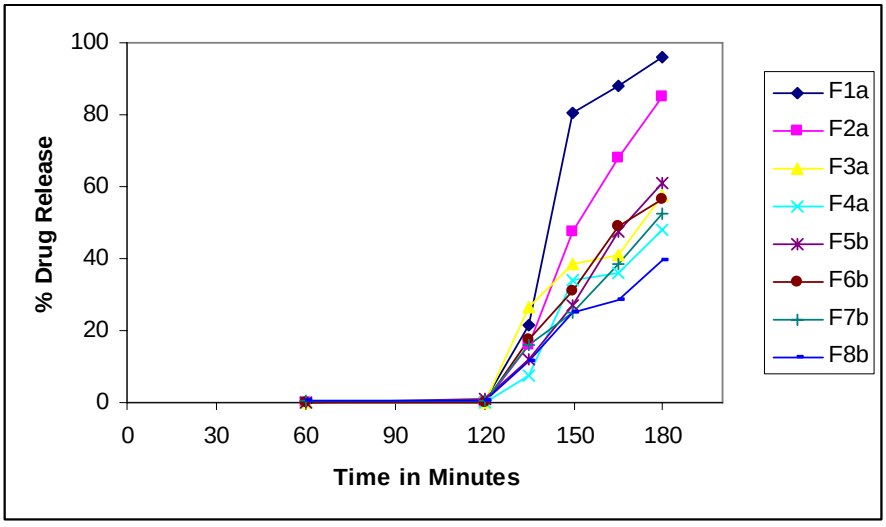

Fig. 3: Drug Release profile of all formulations

Table 10: Dissolution Studies of Marketed Formulations

Dissolution stage	Time (Min)	Average % drug release ±SD**	
		MB 1	MB 2
Acid stage	60	0.167±0.0	±0.0
	120	0.253±0.0	0.51±0.0
Buffer stage	135	24.20±0.0	27.20±0.0
	150	45.60±0.0	40.11±0.0
	165	61.14±0.0	63.63±0.0
	180	80.31±0.0	86.18±0.0

** Average of 6 determinations ± SD

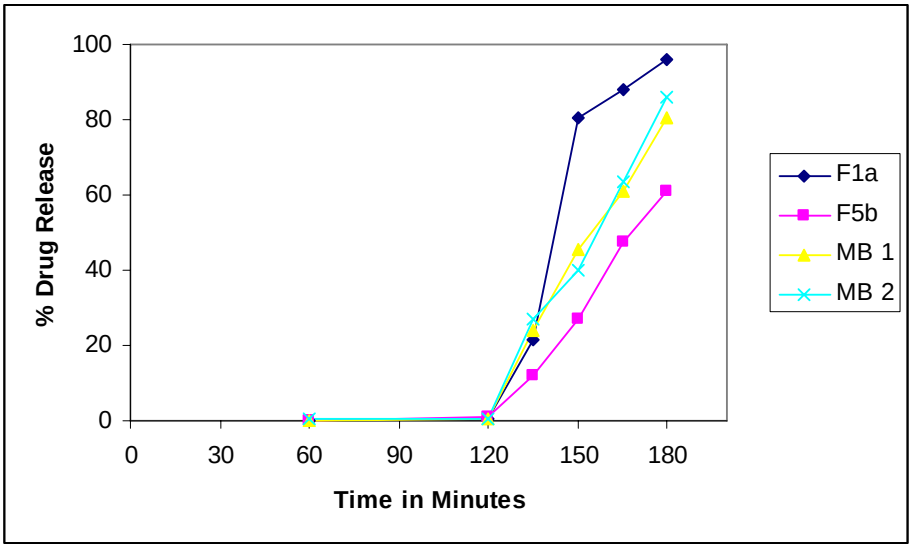

Fig. 4: Comparative Drug Release profile of F_1a and F_5b with Marketed Formulations

DISCUSSION

Effect of Excipients Addition

The different excipients were selected together for this enzyme formulation after confirmation of their compatibility with each other by FTIR study so excipients were without the risk of a negative influence on the enzyme activity. The results for Properties of granules containing starch as well as lactose were summarised in Table 2 which shows good flow properties but granules containing lactose having lower Carr's index as compared to granules containing starch because of its higher flow ability.

It is well known that the drug activity affected by excipients addition and the enzymatic activity of tablet formulation was reduced with increasing tablet hardness.[21] the results for Properties of tablets are given in Table.3 and 4 which shows tablets Formulation with lactose having higher hardness as compared to Formulation containing Starch and Table 5 and 6 shows Disintegration times for tablets containing starch were significantly shorter as compared to tablets containing Lactose because of plastic deforming property of starch. [4]

Effect of compaction force

Kuny, T., et al[4] reported Compression behaviour of the enzyme Beta-galactosidase and its mixture with

microcrystalline cellulose by applying different compaction forces. Morii M et al[23] reported the activity for alkaline protease could be reduced during the tablet compression by mechanical stress Mrvncewa, K. et al[24] reported the pressure inactivation of enzyme: Some Kinetic Aspects of Pressure Inactivation of Chymotrypsin.

This study also reports the Effect of excipients addition and activity loss during the tablet compression on dissolution followed by enzymatic activity of the trypsin-Chymotrypsin selecting compression force from 70, 80, 90, to 100 KN. Compression force was increased from 70, 80, 90, to 100 KN for F_1a, F_2a, F_3a, and F_4a formulations respectively. All these formulations were with same formula containing starch. And also compression force was increased from 70, 80, 90, to 100 KN for F_5b, F_6b, F_7b and F_8b respectively. All these formulations were with same formula containing lactose as per Table 1.

All formulations were assayed for enzymatic activity according to the above developed procedure [20,21,25,26] and % assay values for F_1a, F_2a, F_3a, F_4a were in range of 98-101 and for F_5b, F_6b, F_7b, F_8b were 71-78. It showed that the formulations containing starch having significantly higher activity as compared to formulations containing lactose, because of starch containing formulation shows elastic deformation at low pressure and plastic deformation at high pressure where as formulation containing lactose shows the brittle behaviour. Also enzymatic activity reduces with increasing compression force which is summarised in Table 7.

Tablets coated with an in non-aqueous dispersion of Ethyl cellulose showed massive swelling due to penetration of test medium into the core when acid permeability was evaluated in a 2-hour resistance test in 0.1 N hydrochloric acid. But at this point; the mechanism of drug release was primarily induced by osmotically driven release because of the influx tendency of the medium. This is consistent with the coated tablet with a membrane of ethyl cellulose. Effect of coating material observed during dissolution study 2 hr. study in 0.1 N HCl and 1 hr. study in Phosphate buffer pH 6.8.

Dissolution profile for tablet containing starch and tablet containing lactose summarised in figure 1 and Figure 2 respectively.

By comparing dissolution profiles for F_1a, F_2a, F_3a, and F_4a and F_5b, F_6b, F_7b, F_8b in figure 3 it is found that decreases in drug release with increase in compression force. Tablets compressed with 70 KN shows higher enzymatic activity than other in both formulations i.e. F_1a and F_5b shows better release profile but the formulations containing starch shows better results than formulations containing lactose. Average percentage Drug Release from optimized formulation F_1a, F_5b and Marketed Formulations MB1, MB2 after 180 minutes was 96.23, 60.88 and 80.31, 86.18 respectively. By Comparing Drug Release profile with Marketed Formulations figure 4 it is found that F_1a shows higher activity among all and F_5b shows lowest activity than Marketed Formulations.

This study reports enteric coated enzyme tablets compressed at 70 KN containing starch i.e. F_1a having higher activity than Marketed Formulations MB1, MB2 because elastic behaviour of starch at low compression force and goes under plastic deformation at higher compression force. Tablet hardness, Disintegration time and enzymatic activity loss was increased with increase in compression force.

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