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Research Article

Formulation and Evaluation of Multi-particulate Drug Delivery System of Pepsin Enzyme

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ABSTRACT

The aim is to develop multi-particulate drug delivery system for pepsin. Multi-particulate systems offer advantage with respect to predictable and even distribution and transportation through GI track. Multi-particulate system can be prepared by various techniques such as agitation, Powder layering, solution layering. In this study, multi-particulate systems of pepsin have been attempted using Lactose and Guar gum as principal excipients. Suitable dough of pepsin with excipients was prepared and pass through sieve # 8.The extrudets were spheronised using spheronizer. Factorial design was applied using speed (RPM) and time for spheronisation as independent variables at three levels. Formulations were evaluated for particle size, % yield, % entrapment efficiency, Disintegration time and In vitro drug release. Relationship between dependent and independent variables was established using design expert 7.0.0.

Key-words: Pepsin, Proteins & peptide delivery, multi-particulate drug delivery, extrusion spheronisation.

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Introduction:

Enzymes are macromolecular biological catalysts.[1]They are responsible for thousands of metabolic processes. Enzymes are highly selective catalysts, greatly accelerating both the rate and specificity of metabolic chemical reactions, from the digestion of food. Most enzymes are proteins. Enzymes act by converting starting molecules (substrates) into different molecules (products). [1] Amongst solid oral dosage forms, multi-particulate drug delivery systems have gained major pharmaceutical market share, due to their superior clinical performance, provision of various formulation options, and advances made in the multi-particulate technologies. In these systems, the dosage of the drug substances is divided on a plurality of subunit, typically consisting of thousands of spherical particles with diameter of 0.5-2.00mm[2]

Pepsin is an aspartic acid protease that is commonly found in the stomach of many organisms. Porcine pepsin is the most studied and is fully active at pH 1.9 but inactive above pH ~7. The aim here was to develop a multiparticulate system by incorporating Pepsin, a proteolyic enzyme, in lactose- guar gum beads. Lactose was selected as it is widely used in pharmaceutical industry and is easily available at low price and thus could result in a cost effective formulation.

Materials and Methods:

Materials:

Pepsin(1:3000), Haemoglobin powder, Guar Gum(EP), Dicalcium Phosphate (LR) ,Magnesium stearate (LR) , MCC(Pure) , Talc (LR) , Trichloro acetic acid (Pure) , PVP (Pure) from, Lactose powder (Pure) from Himedia labs Mumbai and HPMC (Methocel E-50) from Colorcon were used.

Methods:

Characterization of pepsin:

Physical properties: The enzyme powder examined for its physical properties.

Bioassay of Pepsin:

Principle:

The rate of hydrolysis of denatured hemoglobin is measured. By increasing the concentration of enzyme and keeping the other factors i.e. time, temp, pH, and substrate constant, the different tyrosine molecules of haemoglobin hydrolysis are produced. Increasing the concentration of enzyme, results in more hydrolysis of haemoglobin molecule with simultaneous increase in absorbance at 280 nm.

Procedure for standard curve:

Pepsin was estimated by modified hemoglobin hydrolysis method of USP XXVI.

Different dilutions ranging from 10-100 ug/ml of pepsin were prepared in 10 mM HCl. Form these dilutions, 1 ml of pepsin solution was added to 5.0 ml of bovine hemoglobin solution(2%) in water and incubated for 10 minutes at 37º C. The hydrolysis of hemoglobin was stopped by adding 10.0 ml of trichloroacetic acid solution (5%) and was allowed to stand for 5 minutes at 37º C. The solution was filtered through a Whatman filter paper no.42, and absorbance was measured at 280nm against water as a reference and standard curve was prepared.

IR spectroscopic study:

The powder samples of Pespin was mixed separately with Potassium Bromide with trituration. The infrared spectra were recorded and the spectral analysis was done using Fourier Transform Infra-red Spectrophotometer (Shimadzu 8400S).

Differential scanning Calorimetry:

Pepsin was assessed by carrying out thermal analysis. The samples were heated from 30°C – 300°C at the rate of 10°C/min. The inert atmosphere was maintained by purging nitrogen gas throughout the experiment at the rate of 40 ml/min. The sample (1-4mg) was carefully transferred and heated in a crimped aluminum pan for accurate results.

Preparation of Extrudets and pepsin loaded pellets

Preliminary experiments were performed to choose formulation given in table1wet mass of all the excipients was prepared and were extruded through a sieve. The granules obtained were then spheronised by lab spheronizer. [3,4] The required quantities of excipients were weighed and passed through sieve number 16. A 10%w/v solution of PVP was prepared and the required amount of pepsin powder was dissolved in it. A quantified amount of this solution was added to the powder mixture and kneading process was carried out to obtain a damp mass of appropriate consistency. This damp mass was passed through sieve number 8 and the extrudets were collected.
The extrudates were put in spheronizer with a plate size 5 mm and was allowed to rotate at speed of 1000 RPM for 5 minutes. The beads were then collected and put in the rotating pan of R&D coater which was pre-heated to 45^0 C and rotated at 14 RPM for 60 minutes to dry the beads. The dried beads were collected and passed through sieve number 16 to separate the fines.

Batch formula for Pepsin incorporated pellets[5,11]

Sr No.	Ingredients	Quantity
1.	Lactose	7 gm
2.	Pepsin	1.4 gm
3.	Talc	2.0 gm
4.	MCC	3.0 gm
5.	DCP	3.0 gm
6.	Guar Gum	8.0 gm
7.	HPMC	3.0 gm
8.	10%PVP	30 ml

Table 1: Batch formula for pepsin loaded pellets

Preparation of Extrudets and pepsin loaded pellets using 3^2 Factorial Designs

A 3^2 full factorial design was employed to study the effect of independent variables X_1 (speed) and X_2 (time) over the dependent variables like Bulk density(g/ml) ,Particle size (mm), percent yield, percent Entrapment efficiency, and *in vitro* drug release (%) Table2 and and Table3 show details of nine experiments. In this design, two factors were evaluated each at three levels (−1, 0, +1) and all possible nine experimental batches were formulated. Composition of all nine possible combinations of Pepsin loaded pellets using 3^2 full factorial design is shown in Table 3. The data was subjected to contour and 3D response surface plot using Design-expert software version 7.0.0. A multiple linear regression equation incorporating interactive and polynomial terms was used to calculate the response as follows:[6]

$Y=b0+b1X1+b2X2+b12X1X2+b11X2^1+b22X2^2$……………………………………..(1)

where Y is the dependent, that is, response variable, namely Bulk density(g/ml), Particle size (mm), yield(%), Entrapment efficiency(%),and *in vitro* drug release (%); b_0 is the arithmetic mean response of the nine runs; and b_1 and b_2 are the estimated coefficients for the factors X_1 and X_2, respectively. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction term (X_1X_2) shows how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are used to check nonlinearity. The polynomial equations can be used to draw conclusions after considering the magnitude of the coefficient and the mathematical sign it carries (i.e., positive or negative). The high values of the correlation coefficients for the dependent variables indicate a good fit.[6]

Variables	Low level (−1)	Medium level (0)	High level (+1)
X_1 = Speed (RPM)	700	1000	1300
X_2 = Time (min)	3	5	7

Table 2:Independent variables and their levels.

Batches	Lactose	Pepsin	Talc	MCC	DCP	Guar gum	HPMC	10%PVP	Speed	Time
F1	7.0	1.4	2.0	3.0	3.0	8.0	3.0	30ml	1300	7.0
F2	7.0	1.4	2.0	3.0	3.0	8.0	3.0	30ml	1300	5.0
F3	7.0	1.4	2.0	3.0	3.0	8.0	3.0	30ml	1300	3.0
F4	7.0	1.4	2.0	3.0	3.0	8.0	3.0	30ml	1000	7.0
F5	7.0	1.4	2.0	3.0	3.0	8.0	3.0	30ml	1000	5.0
F6	7.0	1.4	2.0	3.0	3.0	8.0	3.0	30ml	1000	3.0
F7	7.0	1.4	2.0	3.0	3.0	8.0	3.0	30ml	700	7.0
F8	7.0	1.4	2.0	3.0	3.0	8.0	3.0	30ml	700	5.0
F9	7.0	1.4	2.0	3.0	3.0	8.0	3.0	30ml	700	3.0

Table 3: Composition of Pepsin loaded batches at different process parameters ie different RPM and for different Time period

PHYSICOCHEMICAL CHARACTRISATION OF PEPSIN INCORPORATED BEADS:

***IR spectroscopic study*:**

The powder samples of enzyme (Pespin) were mixed separately with Potassium Bromide with trituration. The infrared spectra were recorded and the spectral analysis was done using Fourier Transform Infra-red Spectrophotometer (Shimadzu 8400S).

***Differential scanning Calorimetry*:**

Pepsin was assessed by carrying out thermal analysis. The samples were heated from 30°C – 300°C at the rate of 10°C/min. The inert atmosphere was maintained by purging nitrogen gas throughout the experiment at the rate of 40 ml/min. The samples (1-4mg) were carefully transferred and heated in a crimped aluminum pan for accurate results.

***Morphology and Particle size Analysis*:[10]**

The particle size of the prepared beads in a sample was measured using Vernier Caliper. Diameter of 10 pellets from each sample was measured; average of 10 measurements is reported.

Flow Properties of Pepsin incorporated beads:[10]

Bulk density: 13 gm of each batch was studied for bulk density and tap density in the automatic tap density measuring apparatus.

***% yield of beads*:**

Percentage yield of beads was calculated using the following formula-

$$\text{Percentage Yield} = \frac{\text{Weight of dried beads obtained}}{\text{Theoretical weight of dried beads}} \times 100$$

***% Entrapment studies*:[8,9]**

Entrapment efficiency (EE) is the amount of added drug (in percent) that is entrapped in the formulation of beads. The EE of drug from beads can be calculated in terms of ratio in the final formulation to the amount added drug. An accurately weighed quantity of 10 mg of the beads were taken and put into beaker containing 10ml of 0.1N HCl, and then beaker was kept in orbital shaker for 1 hr at 100 rpm. The resulting solution was centrifuged at 2,500 rpm for 10 minutes, and the supernatant after dilution was assayed (n= 3) for enzyme content by USP XXVI method.

Entrapment efficiency was calculated as:

$$\text{Entrapment efficiency} = \frac{\text{Actual amount of pepsin found in beads}}{\text{Theoretical amount of pepsin in beads}} \text{X 100}$$

***In-vitro dissolution studies*:[8,9]**

In vitro dissolution studies were performed for all the formulation using USP Dissolution Apparatus II (paddle type). An accurately weighed sample of beads containing equivalent amount of 10 mg of pepsin enzyme was dropped into 900 ml of 0.1N HCl maintained at a temperature of 37ºC ± 0.5ºC and stirred at a speed of 100 rpm. (USP XXVI) At different time intervals, a 5mL aliquot of the sample was withdrawn and the volume was replaced with an equivalent amount of dissolution medium kept at 37ºC. The collected samples were filtered and assayed by the method given here. Prepare 2.5% w/v Hemoglobin solution in 50 ml distilled water. Take 40 ml of this solution and to it add 10 ml 0.3M HCl. Add 2.5 ml this solution in both the sample and the blank test tubes and incubate them for 5 minutes at 37^0C. Add 0.5ml of enzyme solution(filtered dissolution sample) to Sample test tube and incubate it for 10 minutes at 37^0C. Add 5ml 5%w/v TCA solution to both the sample and blank test tubes. Add 0.5 ml of enzyme solution to the blank test tube and incubate for 5 minutes at 37^0C. Filter the solutions from all the test tubes through Whatman filter paper and measure absorbance at 280 nm.

Stability studies:

Stability studies of the formulations were carried out to know

1. Whether the chemical change or degradation of the active ingredient has occurred This may lower the therapeutic potency of active ingredient over storage period.
2. Whether gross changes in the physical form of the dosage form have occurred implying poor or substandard quality of ingredients.

Stability studies were done on optimized batches at room temperature for 30 days. Optimized formulations were sealed in aluminum packaging coated inside with polyethylene. At the end of studies, samples were analyzed for their physical appearance, drug content and drug release studies.

RESULTS AND DISCUSSION:

CHARACTERIZATION OF DIGESTIVE ENZYME PEPSIN:

Physical Characterization of Pepsin:

The pepsin powder was white or light buff in color. It was hygroscopic and amorphous with a faintly meaty but not rancid odor.

Bioassay of Pepsin:

Absorbance obtained at 280nm after reacting various concentrations of pepsin (enzyme) with Bovine Haemoglobin (substrate) is presented in figure 1. Absorbance at 280nm is indicative of tyrosine production after reaction of pepsin with Bovine Haemoglobin. It shows linear relationship with enzyme concentration.

Sr. No.	*Concentration of Pepsin (µg/ml)*	*Absorbance at 280nm*
1	0	0.0000
2	10	0.0159
3	20	0.0519
4	30	0.0889
5	40	0.1123
6	50	0.1406

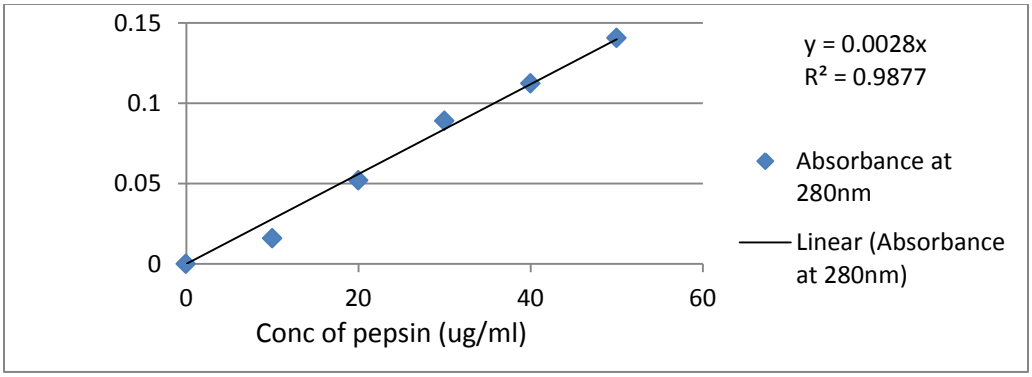


Figure: 1 Calibration curve Pepsin

Interpretation of IR spectra:

The IR spectrum of pepsin obtained on a FTIR with diffused reflectance assembly is shown in figure 2. The interpretation of IR frequencies was done and absorption bands are consistent with the structure of pepsin Data is shown in table 5.

Sr. No.	**IR Frequency (cm^{-1})**	**Assignment**
1	3490 – 3571	Free OH groups
2	3122 – 3039	H bonded OH groups
3	2891	C- H stretching
4	1656	C = O groups
5	1078	C – O groups

Table 2: IR spectral assignment of Pepsin

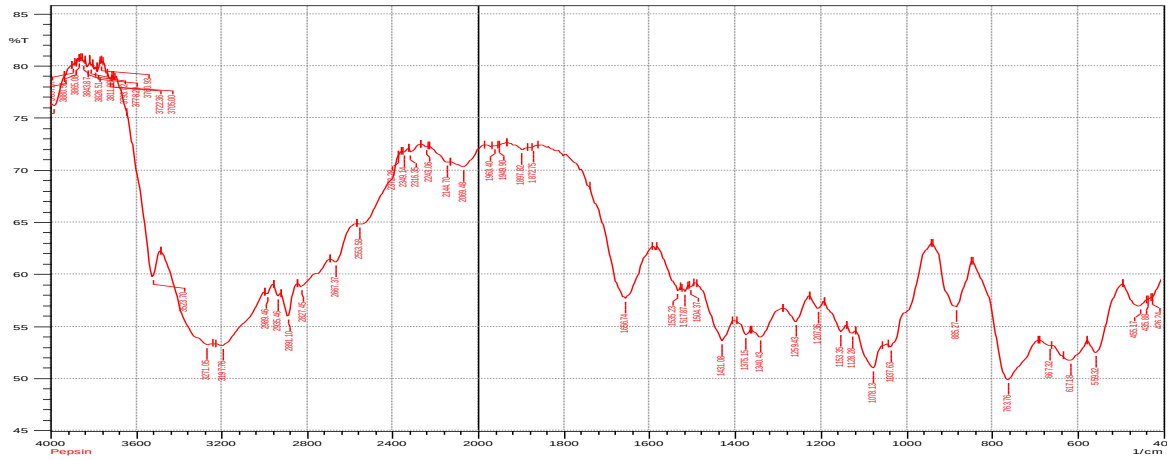


Figure 2: IR spectra of Pepsin

Differential Scanning Calorimetry:

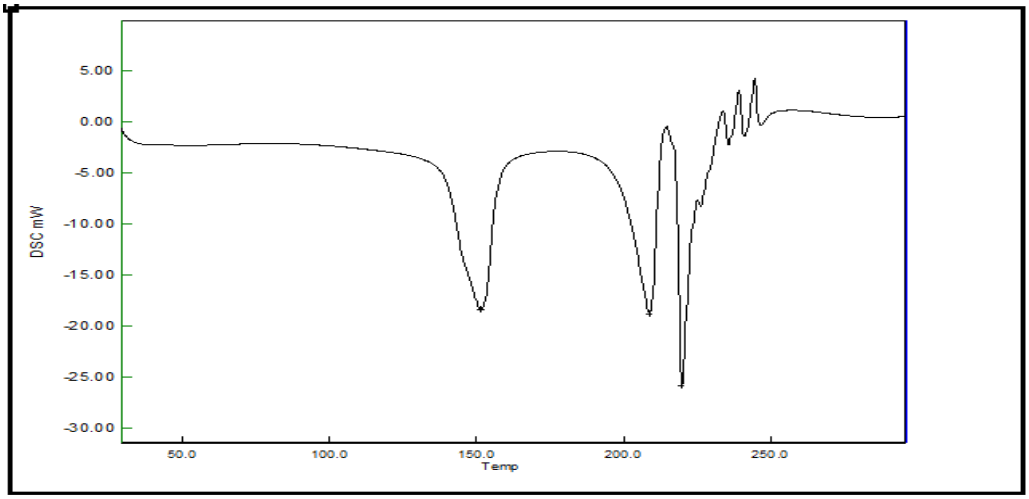


Figure 3: DSC thermogram of pure pepsin

Differential scanning calorimetry study was carried out for the pepsin. The obtained results are shown in figure 3. Pepsin exhibited endothermic peak at **151 ºC, 208ºC** and **219 ºC**. The endothermic peak at 151 ºC may be because of opening of random chains of pepsin and endothermic peak at 208 ºC may be due to the opening of β-pleated structure of pepsin and the endothermic at 219 ºC may be due to the uncoiling of the α- helix arrangement of pepsin.

Effect of factors on % yield, Avg particle size, Bulk density, Entrapment efficiency, In vitro dissolution.

FactorX 1 (Speed)	*Factor X2 (Time in min)*	*Batches*	*yield (%) (Y1)*	***particle size (mm) (Y2)*	*Bulk density (Y3)*	**Entrapment efficiency (%) (Y4)*	**Disintegration (sec) (Y5)*	**Drug release (%) (Y6)*
1300	7	F1	88.67	3.08±1.2	0.8146	88.93±0.6	424 sec	85.88±0.5
1300	5	F2	89.42	1.72±0.9	0.4549	89.54±0.6	266 sec	86.88±0.5
1300	3	F3	89.03	1.68±0.9	0.4443	89.08±1.2	260sec	86.52±0.7
1000	7	F4	90.03	2.83±1.3	0.7485	89.79±0.4	407 sec	86.06±0.7
1000	5	F5	90.41	1.76±0.4	0.4655	90.71±1.0	262 sec	87.08±1.0

1000	3	F6	89.44	1.67±1.1	0.4417	89.24±1.1	258 sec	86.43±0.7
700	7	F7	87.07	4.82±1.0	1.2748	88.03±0.3	663 sec	85.56±0.2
700	5	F8	88.09	3.88±1.0	1.0265	88.59±0.7	547 sec	85.89±0.3
700	3	F9	87.52	4.41±1.4	1.1664	88.08±0.5	609 sec	85.68±0.3

All values are expressed as Mean ±SD (*n=3, **n = 50 pellets)

Table 3:Factorial design with % yield, Avg particle size, Bulk density, Entrapment efficiency, Disintegration time and In vitro dissolution.

The results of dependent variables viz percent yield (Y1), particle size (Y2) , Bulk density (Y3) Entrapment efficiency (Y4) ,Disintegration time (Y5) and In vitro dissolution (Y6) from nine experiments are shown in Table 5 and were used to generate polynomial equation from design expert 7.0.0

Mathematical relationships generated for the studied response variables are expressed in following equations 1,2,3,4,5 and 6

Yield (Y1) = +90.19+0.91* A-0.037* B+0.023* AB-1.83* A^2-0.34* B^2…….(1)

Particle size (Y2) = +1.88-1.26* A+0.50* B+0.25* AB+1.33* A^2+0.32* B^2……(2)

Bulk density (Y3) = +0.44-0.29*A+0.13 *B + 0.065*AB+ 0.31 *A^2+0.17 *B^2……..(3)

Entrapment efficiency (Y4) = +90.29+0.57*A+0.058* B-0.025* AB-1.30* A2-0.57* B2……..(4)

Disintegrationtime(Y5)=+4.22-2.83*X1+1.00*X2+0.50*X1X2+3.17*$X1^2$+0.67* $X2^2$……..(5)

In vitro drug release (Y6) = +78.74-0.25* A+0.66* B-0.29* AB-0.35* A2-1.36* B2……..(6)

Table: 4: Significance values (probe values) of response coefficients for pepsin loaded pellets.

Coefficients	Significance values(Probe value)					
	Percent yield	Average particle size	Bulk density	Entrapment efficiency	Disintegration time	In vitro dissolution
b_0	0.0384	0.0092	0.0007	0.0930	0.0085	0.2510
b_1	0.0174	0.0023	0.0002	0.0568	0.0021	0.4809
b_2	0.8596	0.0315	0.0020	0.7770	0.0386	0.1287
b_{12}	0.9292	0.2165	0.0253	0.9205	0.2452	0.5068
b_{11}	0.0115	0.0095	0.0008	0.0284	0.0075	0.5752
b_{22}	0.3785	0.2547	0.0050	0.1797	0.2674	0.0907

In equations 1 to 6 the coefficients showed significantly high prob values and hence they were reduced in model equations 7 to 11

Yield (Y1) = +90.19+0.91* A -1.83* A^2 …….(7)

Particle size (Y2) = +1.88-1.26* A+0.50* B +1.33* A^2 ……(8)

Bulk density (Y3) = +0.44-0.29*A+0.13 *B + 0.065*AB+ 0.31 *A^2+0.17 *B^2……..(9)

Entrapment efficiency (Y4) =+90.29 -1.30* A^2 ……..(10)

Disintegration time(Y5)= +4.22-2.83*X1+1.00*B +3.17*$X1^2$……(11)

In vitro drug release (Y6) = +78.74 ……..(12)

The results of multiple regression analysis and analysis of variance test (ANOVA) are summarized in table 6

Table 5: Regression analysis data for measured responses for pepsin loaded pellets

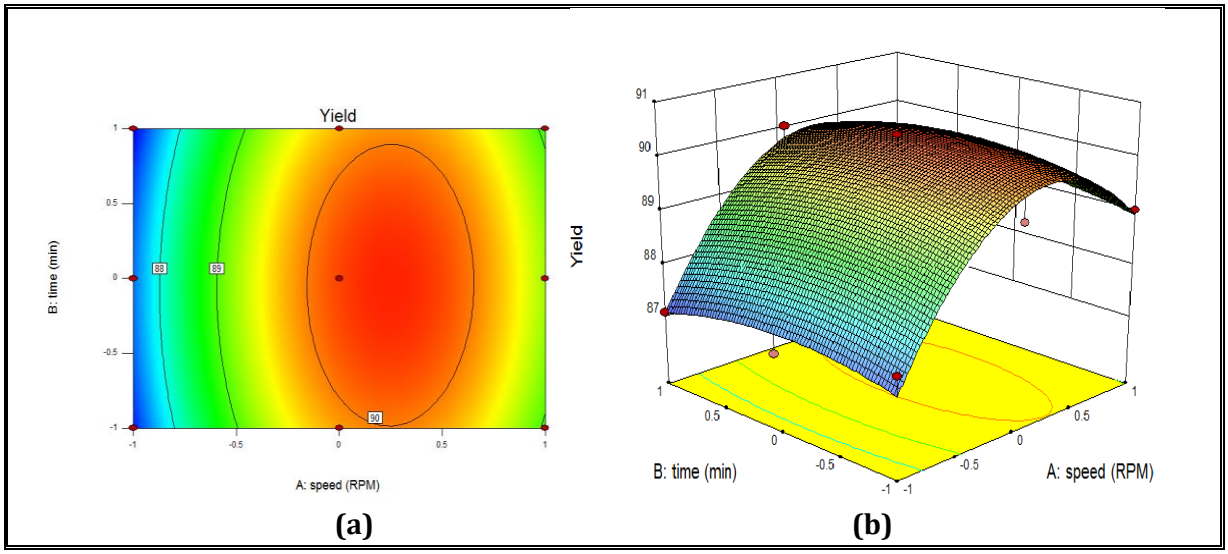
Coefficients	Percent yield		Average particle size		Bulk density		Entrapment efficiency		Disintegration time		In vitro drug release	
	FM	RM	FM	RM	FM	RM	FM	RM	FM	RM	FM	RM
b_0	+90.19	+90.19	+1.88	+1.88	0.44	0.44	+90.29	+90.29	+4.22	+4.22	+78.74	+78.74
b_1	+0.91	+0.91	-1.26	-1.26	-0.29	-0.29	+0.57	-	-2.83	-2.83	-0.25	-
b_2	-0.037	-	+0.50	+0.50	0.13	0.13	+0.058	-	+1.00	+1.00	+0.66	-
b_{12}	+0.023	-	+0.25	-	0.065	0.065	-0.025	-	+0.50	-	-0.29	-
b_{11}	-1.83	-1.83	+1.33	+1.33	0.31	0.31	-1.30	-1.30	+3.17	+3.17	-0.35	-
b_{22}	-0.34	-	+0.32	-	0.17	0.17	-0.57	-	+0.67	-	-1.36	-
R^2	0.9480	0.9480	0.9803	0.9803	0.9966	0.9966	0.9036	0.9036	0.9814	0.9814	0.8000	0.8000
Significance	0.0384	0.0384	0.0092	0.0092	0.0007	0.0007	0.0930	0.0930	0.0085	0.0085	0.2510	0.2510
F- Value	10.94	10.94	29.89	29.89	177.91	177.91	5.62	5.62	31.62	31.62	2.40	2.40

For yield, particle size , Bulk density, Disintegration time, the calculated F-value are 10.94, 29.89, 177.91, 31.62 respectively and are shown in table 9.Hence, it can be concluded that the variables selected contribute significantly in the regression of measured responses Y1 , Y2 , Y3,and Y5.

1) Effect on % yield

From equation 1, it can be seen that positive coefficient of X1 indicated increase in the yield (Y1) with increase in speed of spheronizer up to certain period. The negative coefficient of X2 indicates decrease in response Y1 ie yield with increase in time duration for spheronised the pellets from 3 to 7 min. The equation obtained was quadratic equation which shows the effect is nonlinear. The response plot and counter plots in fig 4.Indicate a relative effect of speed of spheronizer and time to allowed to spheronised on yield of pepsin loaded pellets.

Figure 4 : Different plots showing effect of independent variables on % yield of pellets.



(a) **(b)**

(a) Counter plot showing the relationship between various levels of two independent variables.(b) Response surface plot showing the influence of speed (RPM) and time (min) on the % yield of pellets.

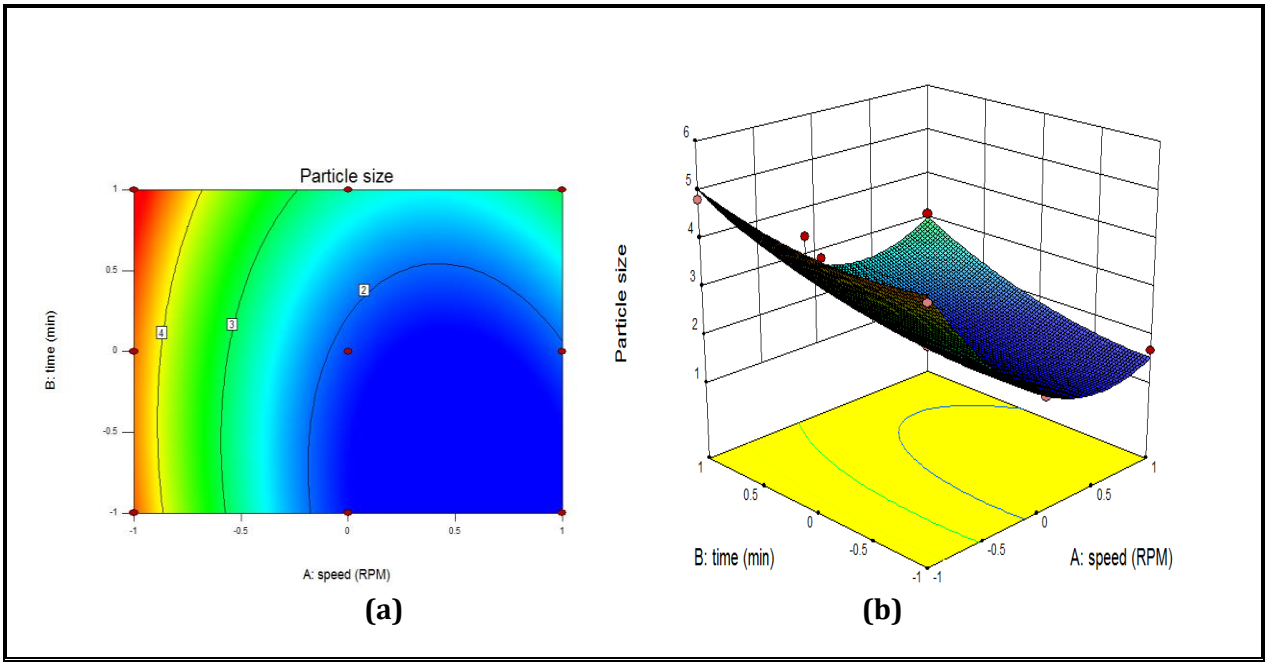
At three different levels (-1,0,1) of speed of spheronizer, as speed increase from 700 to 1300 rpm the % yield was increases and at 0 level ie 1000 rpm as time period to allowed spheronised at three different levels (-1,0,1) increases from 3 to 7 min ,yield was decreases. The % yield was increases from 87.07% to 90.41% as speed of spheronizer increases from 700 to 1000 rpm.

Yield determines the quantity of pellets per batch that were waste during the preparation process such as sieving ,drying etc

2)Effect on particle size

From equation 2, it can be seen that negative coefficient of X1 indicated decrease in the particle size (Y2) with increase in speed of spheronizer up to certain period. The positive coefficient of X2 indicates increase in response Y2 ie particle size with increase in time duration for spheronised the pellets from 3 to 7 min. The equation obtained was quadratic equation which shows the effect is nonlinear. The response plot and counter plots in fig 5.Indicate a relative effect of speed of spheronizer and time to allowed to spheronised on particle size of pepsin loaded pellets.

Figure 5 : Different plots showing effect of independent variables on particle size of pellets.



(a) **(b)**

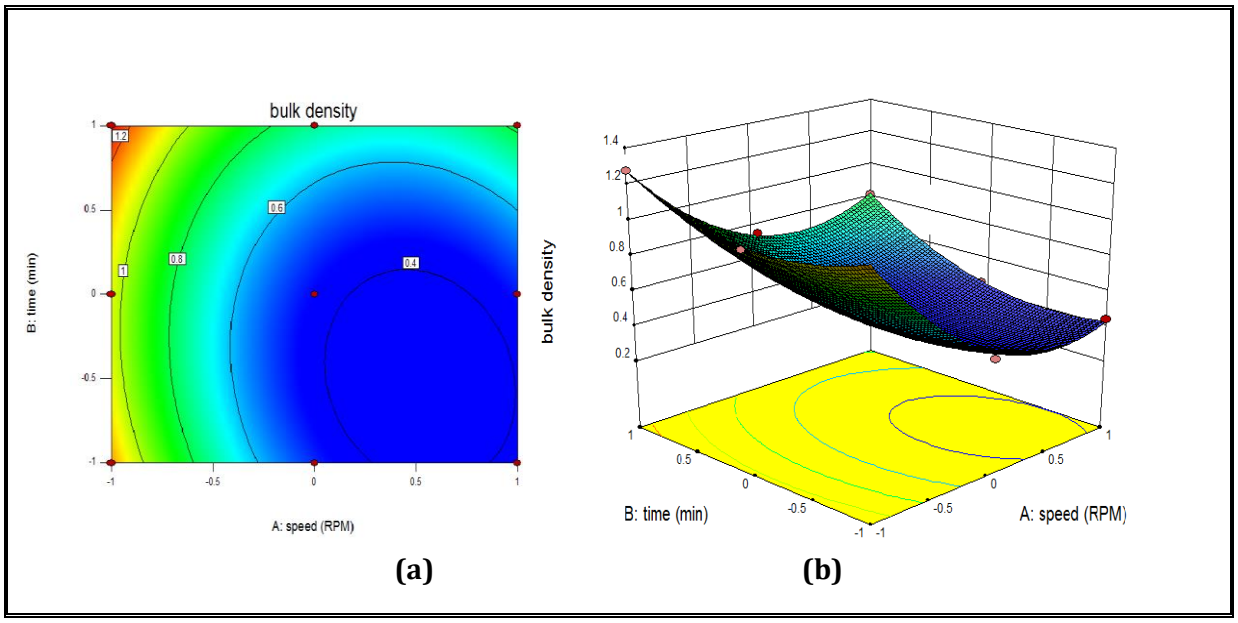
(a) Counter plot showing the relationship between various levels of two independent variables.(b) Response surface plot showing the influence of speed (RPM) and time (min) on the particle size of pellets.

Particle size determines the average size of pellet in each batch as the effect of different speed (RPM)and for different time(min) period.It was calculated with the help of vernier caliper. As the speed of spheronizer increases particle size decreases, but as time period to spheronised in the spheronizer increases beyond some time period pellets get agglomerised with each other and forms the larger size pellets.Batch F4 shows the smallest particle size at 1000RPM speed and for 3.0 min time period. Where batch F7 shows the largest particle size at slow 700 RPM and for 7.0 min time period as pellets get enough time to get agglomerised with each other.

3) Effect on Bulk density

From equation 3, it can be seen that negative coefficient of X1 indicated decrease in the bulk density (Y3) with increase in speed of spheronizer up to certain period. The positive coefficient of X2 indicates increase in response Y3 ie Bulk density with increase in time duration for spheronised the pellets from 3 to 7 min. The equation obtained was quadratic equation which shows the effect is nonlinear. The response plot and counter plots in fig 6.Indicate a relative effect of speed of spheronizer and time to allowed to spheronised on bulk density of pepsin loaded pellets.

Figure 6: Different plots showing effect of independent variables on bulk density of pellets.



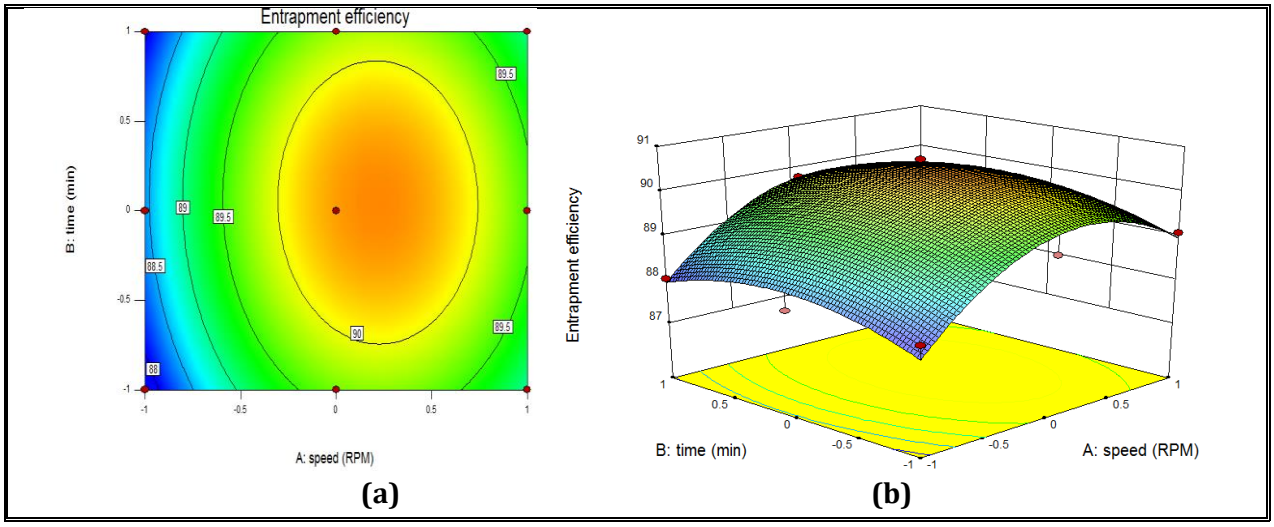
(a) Counter plot showing the relationship between various levels of two independent variables.(b) Response surface plot showing the influence of speed (RPM) and time (min) on the bulk density of pellets.

At different levels (-1,0,1) of speed of spheronizer, when speed of spheronizer changes from 700 to 1300 bulk density decreases as increase in speed causes decrease in particle size of pellets. At 0 level ie 1000 rpm speed with different time period to spheronized from 3 to 7 min bulk density increases, as more time to spheronized causes the agglomerization of pellets,as they were get more time to interact with each other.At different time period (-1,0,1) to spheronized the bulk density increases as time in spheronizer increases, they get more time to interact with each other ,forming the lager particle size of pellets.

4) Effect on entrapment efficiency

From equation 4, it can be seen that positive coefficient of X1 indicated increase in the entrapment efficiency (Y4) with increase in speed of spheronizer up to certain period. The positive coefficient of X2 indicates increase in response Y4 ie entrapment efficiency with increase in time duration for spheronised the pellets from 3 to 7 min. The equation obtained was quadratic equation which shows the effect is nonlinear. The response plot and counter plots in fig 7 .Indicate a relative effect of speed of spheronizer and time to allowed to spheronised on entrapment efficiency of pepsin loaded pellets

Figure 7: Different plots showing effect of independent variables on entrapment efficiency of pellets.

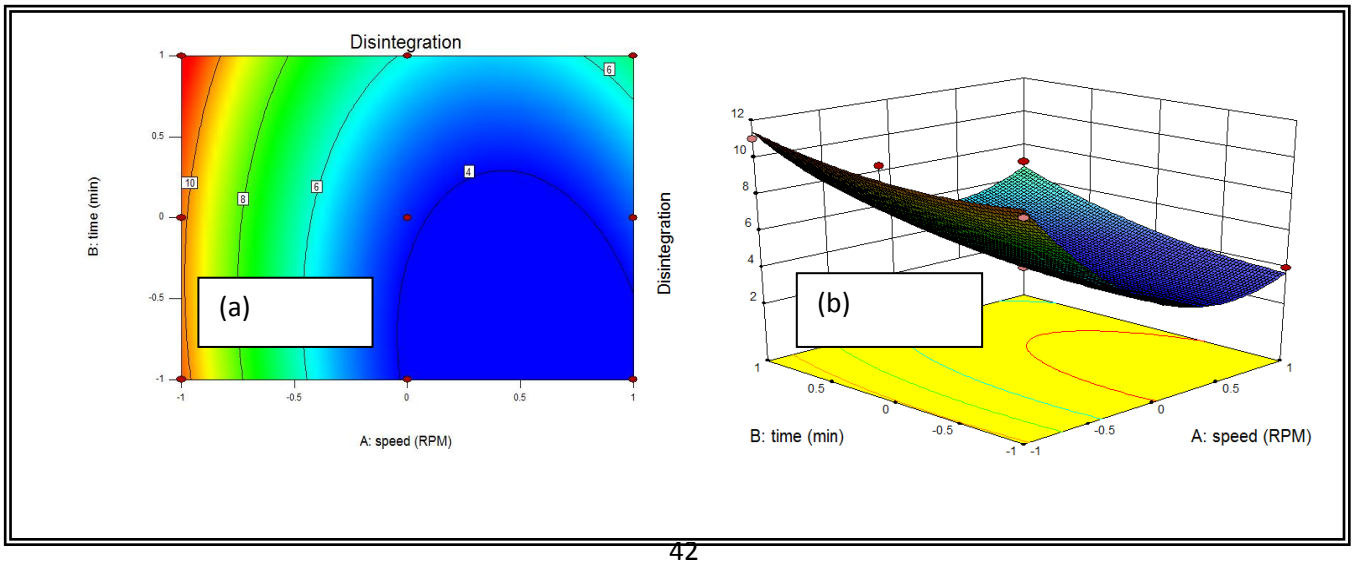


(a) Counter plot showing the relationship between various levels of two independent variables.(b) Response surface plot showing the influence of speed (RPM) and time (min) on the entrapment efficiency of pellets.

Entrapment efficiency (EE) is the amount of added drug (in percent) that is entrapped in the formulation of beads. The EE of drug from beads can be calculated in terms of ratio in the final formulation to the amount added drug.At three different levels (1-,0,1) of speed of spheronizer shows the different effect on entrapment efficiency.At 0 level ie at 1000 rpm speed shows the maximum entrapment efficiency, batch F5 which was prepared at 1000 rpm for 5 min shows the maximum entrapment efficiency 90.71% where as batch prepared at 700 rpm for 7 min shows lowest entrapment efficiency 88.03%. As the factorial was based on process variables, does not shows the much effect on entrapment efficiency.

5) Effect on disintegration time

From equation 5, it can be seen that negative coefficient of X1 indicated decrease in the yield (Y5) with increase in speed of spheronizer up to certain period. The positive coefficient of X2 indicates increase in response Y5 ie disintegration time with increase in time duration for spheronised the pellets from 3 to 7 min. The equation obtained was quadratic equation which shows the effect is nonlinear. The response plot and counter plots in fig 9.1.1 .Indicate a relative effect of speed of spheronizer and time to allowed to spheronised on disintegration time of pepsin loaded pellet



Disintegration time of beads from each batch was determined by noting the disintegration time in 0.1N HCl. (The beads were put in 5ml 0.1N HCl in a test tube and disintegration time was noted).As particle size of pellet increases, time to pellet disintegrate is also increases. At different levels (-1,0,1) of speed of spheronizer ,as the speed of spheronizer decrease the disintegration time of pellets increases ,as at low speed the larger size pellets were formed due to which it takes more time to disintegrate.Batch F6 which was prepared at (-1,1) level ie at 700 rpm speed for 7 min having particle size 4.82 mm shows the highest disintegration time 11.03min.As the speed of spheronizer increases particle size of pellets decreases which causes decrease in disintegration time as batch F3 which was prepared at 1300 rpm for 3 min shows the lowest disintegration time 4 min 20 sec.

6) Effect on In-vitro dissolution studies

Factor X1	*Factor X2*	*Batch Code* *In vitro % cumulative pepsin release in 0.1N HCl* *Time (min)*							
			0	15	30	45	60	75	90
1.00	1.00	F1	0	22.89±0.6	44.78±0.3	64.02±0.3	77.41±0.4	82.43±0.6	85.88±0.5
1.00	0.00	F2	0	21.56±0.5	45.78±0.2	64.78±0.3	78.23±0.8	82.89±0.4	86.88±0.5
1.00	-1.00	F3	0	23.09±0.5	45.88±0.8	63.45±0.5	76.08±0.2	85.19±0.3	86.52±0.7
0.00	1.00	F4	0	20.08±0.5	45.32±0.3	64.12±0.6	77.23±0.7	83.08±0.5	86.06±0.7
0.00	0.00	F5	0	24.58±1.1	49.12±1.3	65.02±1.1	79.20±1.2	84.20±0.8	87.08±1.0
0.00	-1.00	F6	0	20.54±0.3	46.06±0.5	63.55±0.5	77.09±0.5	84.11±0.6	86.43±0.7
-1.00	1.00	F7	0	21.02±0.4	46.89±0.4	63.89±0.4	78.82±0.9	81.56±0.2	85.56±0.2
-1.00	0.00	F8	0	19.48±0.2	44.08±0.4	63.11±0.4	78.11±0.6	83.22±0.2	85.89±0.3
-1.00	-1.00	F9	0	24.12±0.7	46.78±0.4	65.78±0.6	76.32±0.6	83.11±0.6	85.68±0.3

Table: 6 In-vitro cumulative pepsin release

From equation 6, it can be seen that negative coefficient of X1 indicated decrease in the yield (Y6) with increase in speed of spheronizer up to certain period. The positive coefficient of X2 indicates increase in response Y6 ie In vitro dissolution with increase in time duration for spheronised the pellets from 3 to 7 min. The equation obtained was quadratic equation which shows the effect is nonlinear. The response plot and counter plots in fig 11 .Indicate a relative effect of speed of spheronizer and time to allowed to spheronised on In vitro dissolution of pepsin loaded pellets

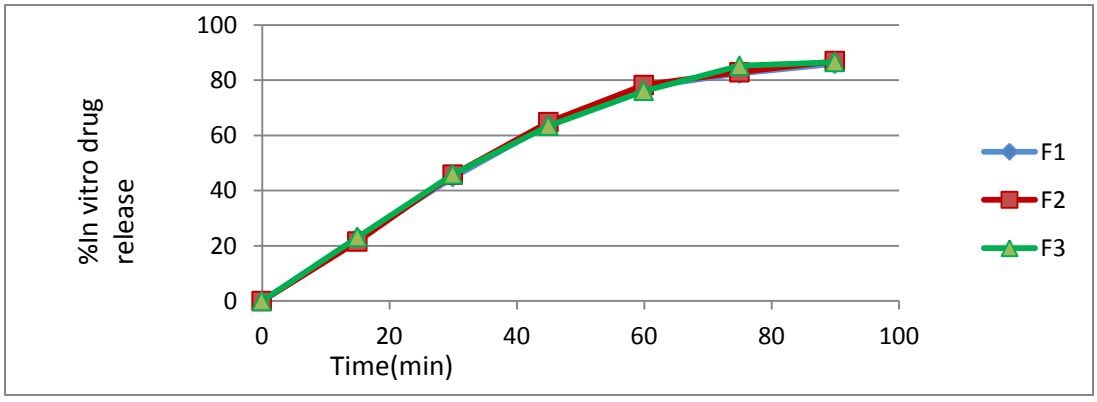


Figure 8: In-vitro cumulative pepsin release

Figure 8 shows the dissolution of three batches which were prepared at 1300 rpm for three different level (-1,0,1) of time to spheronised.Batch (F1) produced at 1300 rpm for 7 min showed 77.41 % drug release upto 60 min and

85.88% drug release upto 90 min.Batch (F2) produced at 1300 rpm for 5 min showed 78.23 % drug release upto 60 min and 86.88 % drug release upto 90 min. whereas batch(F3) produced at 1300 rpm for 3 min showed 76.08 % drug release upto 60 min and 86.52 % drug release upto 90 min.Three batches does not showed the significant change in drug release after 60 and 90 min.

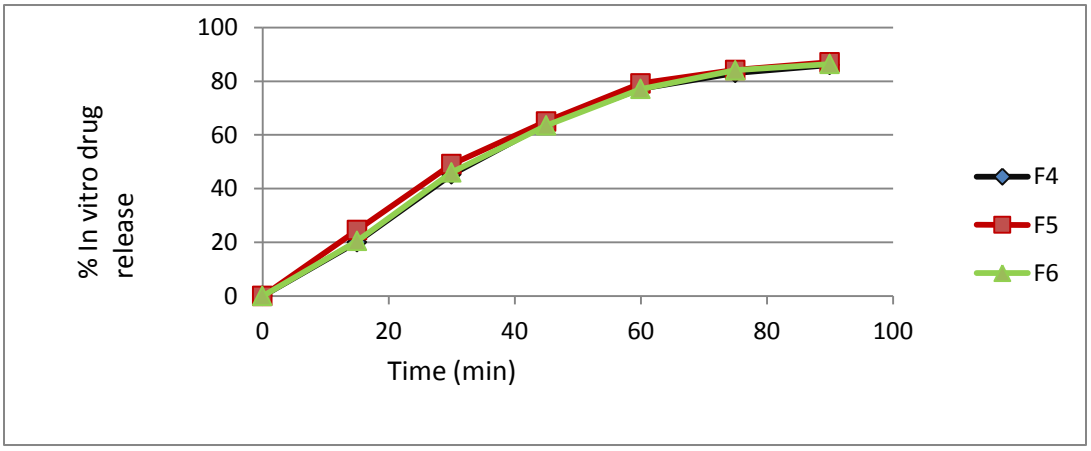


Figure 9 : In-vitro cumulative pepsin release

Figure 9 shows the dissolution of three batches which were prepared at 1000 rpm for three different level (-1,0,1) of time to spheronised.Batch (F4) produced at 1000 rpm for 7 min showed 77.23 % drug release upto 60 min and 86.06% drug release upto 90 min.Batch (F5) produced at 1000 rpm for 5 min showed 79.2 % drug release upto 60 min and 87.08 % drug release upto 90 min. whereas batch(F6) produced at 1000 rpm for 3 min showed 77.09 % drug release upto 60 min and 86.43 % drug release upto 90 min. The batch prepared at 0 level for 5 min time showed the maximum drug release after 90 min.

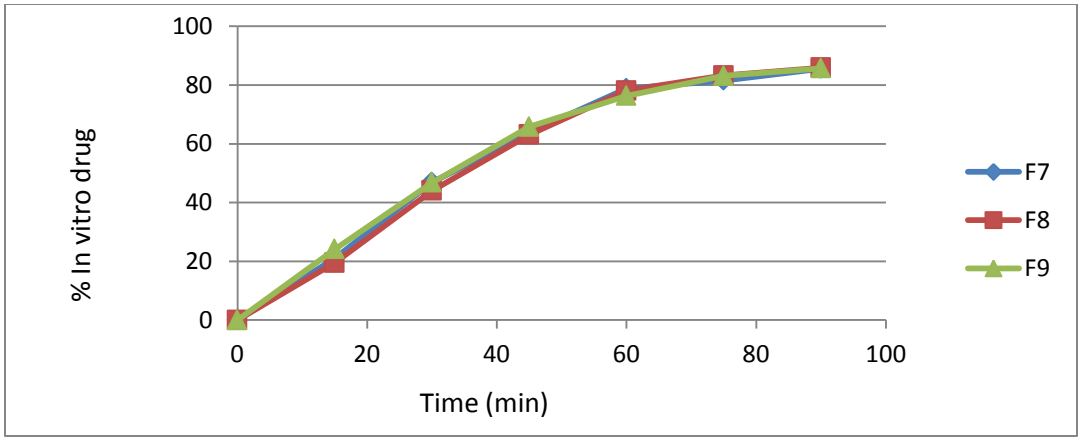
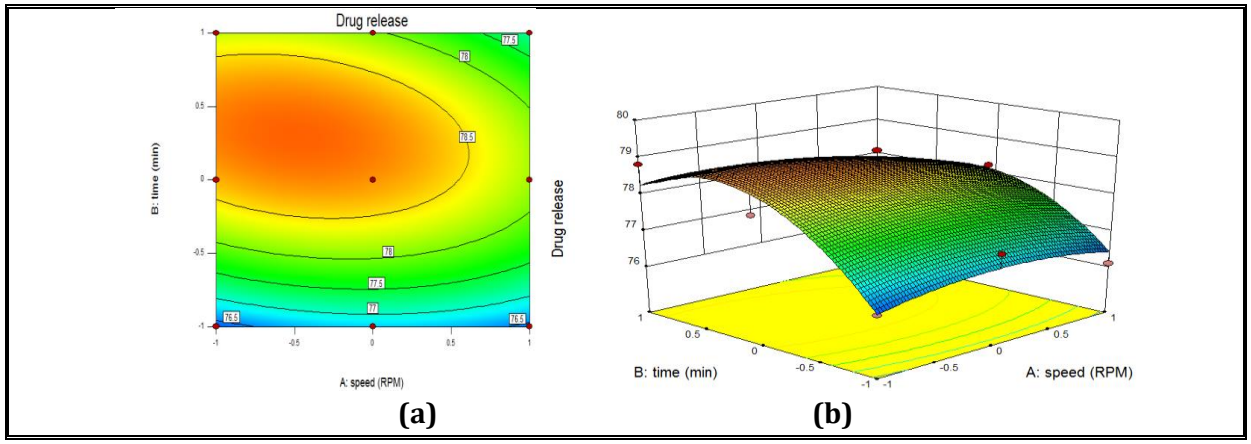


Figure 10 : In-vitro cumulative pepsin release

Figure 10 shows the dissolution of three batches which were prepared at 700 rpm for three different level (-1,0,1) of time to spheronised.Batch (F7) produced at 700 rpm for 7 min showed 78.82 % drug release upto 60 min and 85.56% drug release upto 90 min.Batch (F8) produced at 700 rpm for 5 min showed 78.11 % drug release upto 60 min and 85.89 % drug release upto 90 min. whereas batch(F9) produced at 700 rpm for 3 min showed 76.32 % drug release upto 60 min and 85.68 % drug release upto 90 min. The batch prepared at -1 level ie 700 rpm for 7 min time showed the minimum drug release after 90 min. As particle size of pellets produced at 700 rpm for 7 min was larger ie 4.82 mm showed the minimum drug release upto 90 min.

Figure 11 : Different plots showing effect of independent variables on dissolution time of pellets.



(a) Counter plot showing the relationship between various levels of two independent variables.(b) Response surface plot showing the influence of speed (RPM) and time (min) on the dissolution time of pellets.

Search for optimum formulation:

The results for the feasibility search to find the suitable region for further location of optimum formulation is presented in table 8. The criteria for selection of suitable feasible region was primarily based upon the value of percent yield, Average particle size, Entrapment efficiency, In vitro drug release from pellets up to 90 min.

Region= % yield= more than 90 %

Particle size = 1.7 to 1.8 mm

Disintegration time= less than 5 min

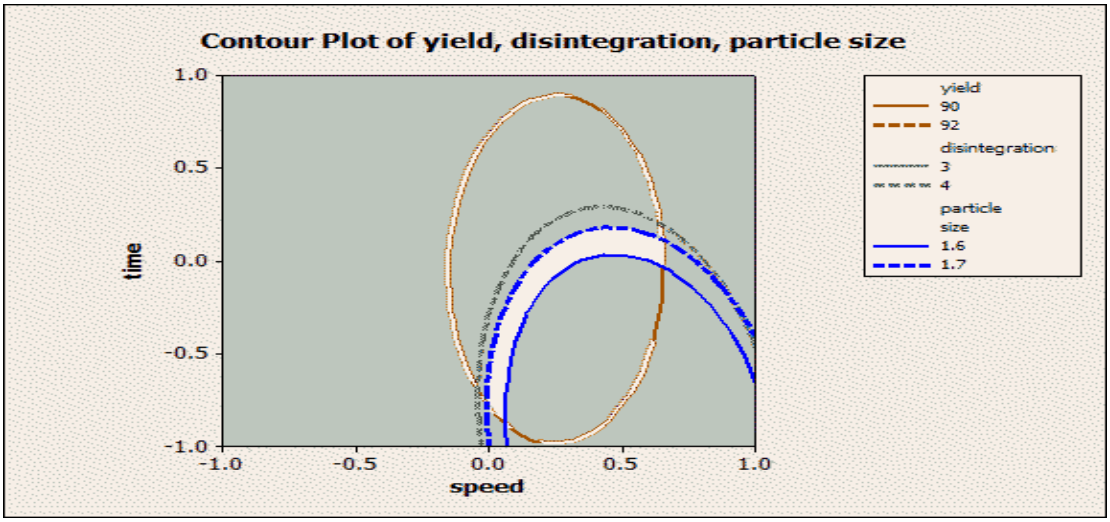


Fig 12:Counter plot showing design space for partical size,% yield,entrapment efficiency and drug release

The oral drug delivery of multi-particulates of pepsin prepared by applying the process variables of spheronizer i.e. speed (RPM) and time (min). 3^2 factorial design was used for the optimization, amongst nine formulations prepared as per the design layout, which indicates that the batch prepared at 1000 RPM speed for 5 min time period shows the suitable particle size as compared to the other batches, also shows highest entrapment efficiency and highest dissolution rate ,suitable in –vitro disintegration time and was selected as the optimized formulation.

Table 7: Composition of optimized formulation for pepsin loaded pellets

Coded form	Speed X_1 (rpm)	Time X_2 (min)	uncoded form	Speed X_1 (rpm)	Time X_2 (min)			
	0.02	0.05		980	4.75			
Batch code F5								
Batch formula(gm)	**Lactose**	**Pepsin**	**Talc**	**MCC**	**DCP**	**Guar gum**	**HPMC**	**10%PVP (ml)**
	7.0	1.4	2.0	3.0	3.0	8.0	3.0	30ml

Validation of optimum pellet process parameters for optimized formulation

For the formulation batch F5 the results of the flow properties and Percentage yield, particle size, disintegration time were carried out.Table 9. lists the predicted and experimental values of all response variables

Comparison of actual Vs experimental results of pepsin incorporated pellets.

Flow properties and Percentage yield, particle size, disintegration time of Pepsin incorporated batches shown in Table 9.

Responses	Predicted value	Experimental value	Batch code	Composition	
				X_1	X_2
Bulk density(gm/cc)	0.4413	0.4563	F5	980	4.75
Tapped density(gm/cc)	0.5614	0.4712			
Yield (%)	90.92	90.85			
Particle size (mm)	1.88	1.74			
Disintegration time(sec)	261	257			

Table: 8.Predicted and actual results for flow properties and Percentage yield, particle size, disintegration time of Pepsin incorporated batches

Comparative Study of Release Profile of The Optimized Batches With Marketed Formulation:

Time (min)	In vitro % cumulative pepsin release in 0.1N HCl	
	Marketed formulation	Optimized batch
0	0	0
15	25.23±0.8	24.58±1.1
30	38.73±1.7	49.12±1.3
45	56.89±1.9	65.02±1.1
60	69.86±1.5	79.20±1.2
75	80.36±2.0	84.20±0.8
90	90.41±0.7	87.08±1.0

Table 9: Release of Profile of the Optimized Batches With Marketed Formulation

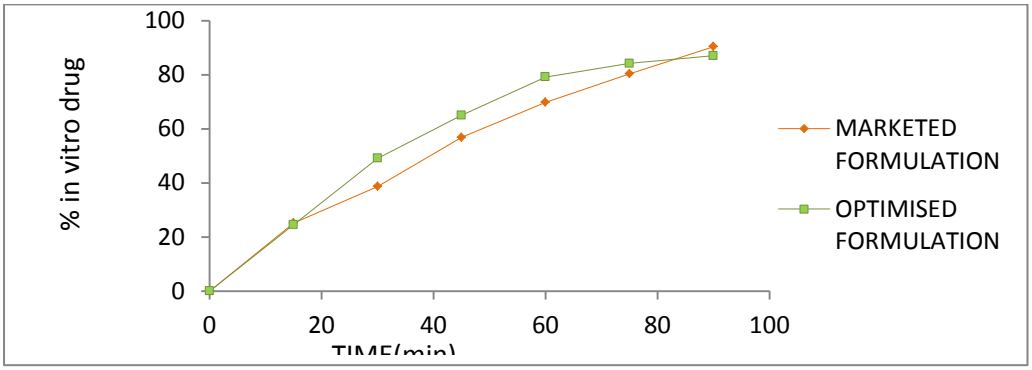


fig 13:showing Release of Profile of The Optimized Batches With Marketed Formulation:

Stability studies of optimized batch

Days	**Appearance**	**% Drug content**	**% Drug release**
Batch code F5			
Before storage			
0 day	White color	100	87.25 ±0.5
After storage			
30 days	No color change	96.89 ±0.3	86.12 ±1.1

Table 10: Stability of optimized formulation at ambient temperature and at ambient humidity

The results are given in Table 11 and the results indicated that the formulations were stable for 1 month.It was concluded that there was not significant change observed in appearance,% drug content,% drug release.The average drug content after 30 days is 96.89 ±0.3(ranging from 96.86% to 96.92%) and average drug release 86.12 ±1.1 (ranging from 85.02 % to 87.32%). The appearance of pellets remains unchanged after 1 month. Therefore prepared formulation was stable for 1 month at ambient temperature and at ambient humidity.

Stability studies of optimized batch

Optimized Batch	**F5**	
	Initial	**30 days**
Appearance	White color	No color change
%Drug content	100	96.89 ±0.3
%Drug release	87.25 ±0.5	86.12 ±1.1

Table 11: Stability of optimized formulation at ambient temperature and at ambient humidity

The results are given in Table 11 and the results indicated that the formulations were stable for 1 month. It was concluded that there was not significant change observed in appearance,% drug content,% drug release. The average drug content after 30 days is 96.89 ±0.3(ranging from 96.86% to 96.92%) and average drug release 86.12 ±1.1 (ranging from 85.02 % to 87.32%). The appearance of pellets remains unchanged after 1 month. Therefore prepared formulation was stable for 1 month at ambient temperature and at ambient humidity.

Conclusion:

The oral drug delivery of multi-particulates of pepsin prepared by applying the process variables of spheronizer i.e. speed (RPM) and time (min). 3^2 factorial design was used for the optimization, amongst nine formulations prepared as per the design layout, which indicates that the batch prepared at 1000 RPM speed for 5 min time period shows the suitable particle size as compared to the other batches, also shows highest entrapment efficiency and highest dissolution rate ,suitable in –vitro disintegration time and was selected as the optimized formulation. stability studies results showed that prepared formulation was stable enough for the period of 1 month. Therefore, the present oral multi-particulate formulation containing pepsin considered is potentially useful for the treatment of indigestion where improved patient compliance and convenience are expected and can be used as an alternative to the other dosage form.

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References

1. http://en.wikipedia.org/wiki/Digestive_enzyme.
2. Roxas M, The Role of Enzyme Supplementation in Digestive Disorders, Alternative Medicine Review 13(14), 2008; 307-314.
3. Kulkarni PA, Kulkarni AD, Gandhi JA, Shirolkar SV, Kasture PV, Pelletization Techniques as a Pharmaceutical tool in the multiparticulate drug delivery system: a review. International Journal of Drug Formulation & Research, 1(1), 2010; 89-118.
4. Koester M. and Thommes M. New Insights into the Pelletization Mechanism by Extrusion/Spheronization AAPS PharmSciTech, Vol. 11, No. 4, December 2010 (# 2010)
5. Joshi D. "Formulation and Evaluation of Multi-particulate drug delivery system for the oral delivery of Digestive Enzyme" Master of pharmacy in Pharmaceutics Submitted to Univercity of Pune. 2013-2014
6. Bala R., Khanna S., and Pawar P. Design Optimization and *In Vitro-In Vivo* Evaluation of Orally Dissolving Strips of Clobazam journal of drug delivery 2014 Sep 28
7. Valquíria Miwa Hanai Y , José M. , 'Development and Evaluation of a Floating Multiparticulate Gastroretentive System for Modified Release of AZT AAPS PharmSciTech. 2011 Jun; 12(2): 658–664.
8. Mikkilineni Bhanu P., Suryadevara V., Reddyvalam L. Sasidhar C., Talamanchi B.,and Pavuluri T.' Development and evaluation of diltiazem hydrochloride controlled-release pellets by fluid bed coating process 'J Adv Pharm Technol Res. 2013 Apr-Jun; 4(2): 101–107.
9. Chaudhari P M. and Chaudhari P. D. 'Formulation and evaluation of multiparticulate system for chronotherapeutic delivery of salbutamol sulphate 'J Pharm Bioallied Sci. 2012 Mar; 4(Suppl 1): S71–S73.
10 BELGAMWAR* V. S. and SURANA S. J. 'Design and Development of Oral Mucoadhesive Multiparticulate System Containing Atenolol' in Vitro–in Vivo Characterization Chem. Pharm. Bull. 58(9) 1168—1175 (2010)
11. Rowe RC, Sheskey PJ, Owen S, Handbook of Pharmaceutical Excipient, 5th edition, London 2006.
12. C. Lustig-Gustafsson, et al., 'The influence of water content and drug solubility on the formulation of pellets by extrusion and spheronisation,' European Journal of Pharmaceutical Sciences, 8, 1999; 147–152