

# Gamascintigraphic *in Vivo-in Vitro* Evaluation of Floating Matrix Tablet

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## ABSTRACT

**Background of the work:** Gastroretentive drug delivery system can retain the dosage form in the gastric region for several hours. Prolong gastric retention helps in improving bioavailability and improves solubility of drugs that are less soluble in a high pH environment. For designing of GRDDS Atenolol was selected as a model drug. It has been used for the treatment of hypertension. Atenolol has short elimination half-life, stable at pH 1.2 and as the pH increase, the drugs becomes unstable and thus low oral bioavailability. In order to improve its therapeutic effectiveness Atenolol is designed in the form of floating tablet formulation. **Purpose:** The aim of the present work was to develop hydrodynamically balanced system for atenolol,  $\beta$ -blocker as a single unit floating tablet. Formulation includes the use of rate controlling polymer such as Locust Bean gum (LBG) in combination of HPMC K4M and gas generating agent Sodium bicarbonate. **Summary and conclusion:** Tablet was prepared by direct compression method and evaluated for physico-mechanical properties. The statistical method was utilized to optimize the effect of independent variables namely amount of HPMC K4M, LBG and three dependent responses such as Cumulative drug release, Floating lag time, Floating time. Graphical and mathematical analysis of the results allowed the identification and quantification of the formulation variables influencing the selected responses. To study the gastrointestinal transit of the optimized Gastroretentive formulation, the *in vivo* Gamascintigraphy was carried out in six healthy rabbits, after radio labeling the formulation with <sup>99m</sup>Tc. The transit profiles demonstrated that the dosage form was retained in the stomach for more than 5 hrs. The study signifies the potential of the developed system for stomach targeted delivery of Atenolol with improved bioavailability.

**Keywords:** Floating tablet, factorial design, Gamascintigraphy, antihypertensive model drug, HPMC, Locust bean gum.

## INTRODUCTION

Novel dosage forms have been designed to maintain relatively constant plasma drug concentration by reducing fluctuations between peak and trough drug concentration. The aim is to keep this fluctuation within the range of plasma concentration known to provide therapeutic efficacy.

In the last few decades, gastro retentive drug delivery systems have been developed extensively. The gastric floating drug delivery system offers many of benefits for the better delivery of drug which has a narrow absorption window in the upper gastrointestinal tract and exhibits poor solubility in the intes-

tinal tract. For the delivery of drug which are unstable in the intestinal environment. It also enhances the action of drug that acts locally in the stomach to prolong the gastric retention time for optimal absorption.<sup>1</sup>

In the present study Atenolol is used as model drug. Atenolol is  $\beta$ -blocker; it has been used in hypertensive and in patients with ischemic heart disease. It is incompletely absorbed from the GI tract of human with an absorption ranging from 28% to 47%, and a bioavailability of 36% of the whole dose.<sup>2</sup> Atenolol is a weak base with good solubility in acidic pH but in alkaline pH solubility significantly reduced. Atenolol is commercially available as a

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conventional tablet. Administration of atenolol conventional tablets in 100 mg/day doses may cause fluctuations in the plasma concentration resulting in side effects or reduction in the drug concentration at receptor site.<sup>3</sup> It is reported that in case of oral administration of atenolol, it can induce side effects such as diarrhea, nausea, mesenteric arterial thrombosis, ischemic colitis and dry mouth.<sup>4</sup> The short half-life of Atenolol i.e. 6 hours & narrow absorption window make Atenolol suitable candidate for formulation of floating drug delivery system.

In the present study the efforts have been focused on the development of floating drug delivery system which will help to retain the dosage form in the stomach and to increase gastric residence time, resulting in prolonged drug delivery in stomach using gel-forming polymers.

Atenolol floating tablets was developed for the treatment of hypertension with single daily dose.

The Floating polymers used in the formulations were HPMC K4M and Locust bean gum (LBG) along with sodium bicarbonate as a low density polymer, gelling agent and gas generating agent. The optimum formula obtained that combined excellent floating behavior, with controlled drug release characteristics and shown good physical stability after storage at 40 °C/75% RH for 3 months. The final formulation was further investigated in six healthy rabbits to determine the gastric retention period by Gamascintigraphy technique. The objective of the present investigation was to design and evaluate gastro retentive drug delivery system with special reference to floating effervescent Atenolol Tablet.

## MATERIAL AND METHODS

Atenolol was received as a gift sample from Dr. Reddy's laboratory, Hyderabad; India. HPMC K4 M (Signet chemical corporation Pune, India), Locust Bean Gum (HI media, India), Sodium bicarbonate, Magnesium

stearate, Talc (Thomas Baker, Mumbai, India), Croscopolvidone (Research lab fine chem. Mumbai, India) were used as excipients and obtained from the indicated sources. All other ingredients and reagents were of analytical grade and were used as received.

## METHODS

### Preparation of Atenolol floating tablets

100 mg dose of Atenolol was geometrically mixed with the required quantities of polymer blend such as hydroxypropyl methyl cellulose (HPMC K4M) as a low density hydrophilic swellable polymer & Locust bean gum (LBG) as a gelling agent and sodium bicarbonate is used as gas generating agent by geometric mixing. The powder blend was then lubricated with magnesium stearate (1%) and compressed on 12 station compression machine (CIPS Machinery) using single 10 mm flat punch. The tablet weight was not constant because that would require addition of diluent as filler; addition of this diluent may show the variation in the release profile. Thus, we do not alter the amount of diluent in the formulation to nullify any effect due to change in the proportion of diluents.

### Optimization by 3<sup>2</sup> factorial designs

A 3<sup>2</sup> randomized factorial design was selected for the optimization of Atenolol floating tablet. The design was applied to study the effect of HPMC K4M and LBG on physicochemical characteristic of the tablet. The amount of Locust bean gum (X<sub>1</sub>) and concentration of HPMC K4 M (X<sub>2</sub>) were selected as independent variables, in this study. These two factors were evaluated, each at three levels. The actual units of higher, middle and lower levels of factor X<sub>1</sub> and X<sub>2</sub> were 50 mg, 75 mg, and 100 mg. The coding was +1, 0 and -1, respectively for higher, middle and lower levels of each factor. The dependent responses are % cumulative release (Y1), floating lag time (Y2) and Floating time (Y3). The experimental design with corresponding formulations is outlined in Table 1.

**Table 1: Trial batches of Atenolol floating tablets**

Ingredients	T1	T2	T3	T4	T5	T6
Atenolol	100	100	100	100	100	100
Locust bean gum	100	100	100	50	150	100
HPMC K4M	50	100	150	100	100	25
Croscopolvidone	150	150	150	150	150	150
Sodium bicarbonate	40	40	40	40	40	40
Magnesium stearate	4	4	4	4	4	4
Talc	4	4	4	4	4	4

\*All quantities are in mg

## Physicochemical characterization of floating tablet

### Drug content and physical evaluation

The tablets were assayed for drug content using 0.1N HCl as the solvent and the samples were analyzed spectrophotometrically (JASCO, V-630) at 224 nm. The tablets were also evaluated for hardness, friability, and weight variation.

### The floating lag time and the total floating time

The floating lag time and the total floating time were determined by observation of the floating behaviours in the release test using a USP type II paddle apparatus (Electro lab, India). The dissolution medium were artificial gastric fluid (pH 1.2, simulate gastric pH in fasted condition) or hydrochloride acid solution (pH 3.0, and 5.0, simulate gastric pH in fed condition).<sup>5</sup> The time interval between the introduction of the tablet into the release medium and its buoyancy to the surface was taken as floating lag time and the total floating time was observed visually.<sup>6</sup>

### Density measurements

The apparent densities of the tablets were calculated, from their volumes and masses ( $n=6$ ). The volumes of the cylindrical tablets were calculated from their heights  $h$  and radii  $r$  (both determined with a micrometer gauge) using mathematical equation for cylinder ( $v=\pi \times r^2 \times h$ ) the density of 0.1 N HCl at 37° C was determined with a pycnometer ( $n = 3$ ).<sup>7</sup>

### Swelling ability (water uptake study)

The swelling behaviour of the tablets was determined, in triplicate, according to the method described by Dorozynski et al.<sup>8</sup> Briefly, a tablet was weighed ( $W_1$ ) and placed in a glass beaker, containing 200 mL of 0.1 N HCl, maintained in a water bath at 37° ± 0.5°C. At regular time intervals, the tablet were removed and the excess surface liquid was carefully removed by a filter paper. The swollen tablet was then reweighed ( $W_2$ ). The swelling index (SI) was calculated using the formula;

$$SI = (W_2 - W_1) / W_1$$

Where,  $W_2$ - Weight of swelled tablet,  $W_1$ - Initial weight of tablet.

### In Vitro Drug Release Study

The in vitro drug release was performed using USP 24 type-II paddle apparatus using 900 mL of 0.1N HCl at paddle rotation of 100 rpm at 37°C ± 0.5°C. The samples were withdrawn at specific time intervals 1 hrs. 2hr up to 8 hr. and replaced with the fresh medium. The samples were filtered through a 0.45-µm membrane filter, suitably diluted, and analysed at 224 nm using dou-

ble-beam UV/ visible spectrophotometer. Cumulative percentage drug release was calculated using the PCP Disso v 3 software (Poona College of Pharmacy, Pune, India).

### Kinetic modeling of drug release profiles

The dissolution profiles of all formulae in 0.1 N HCl were fitted to zero-order, first-order, Higuchi<sup>9</sup> and Korsmeyer-Pappas kinetic models.<sup>10</sup> The model with the highest correlation coefficient was considered to be the best fitting one.

### In vivo Gamascintigraphy study

#### Preparation and administration of the radio labeled tablet

Gamma scintigraphic technique was used for investigating the *In vivo* gastric retention of the optimized tablet formulation (batch F 2). Tablet formulation was radio labeled with <sup>99m</sup>Tc. It was uniformly mixed with the final tablet blend and compressed using KBr hydraulic press at suitable pressure. Radiolabel efficiency was evaluated using thin layer chromatography.<sup>11</sup> Instant thin layer chromatographic silica gel plates were used as a stationary phase and 100 % acetone was used as mobile phase. Percent radio labeling was calculated as follows.

$$\% \text{ Radio labelling} = \frac{\text{Radioactivity retained in the lower half of the strip}}{\text{Total count present with the strip}} \times 100$$

The radiolabel tablet was administered orally via feeding tube to each rabbit without anesthesia. For the study the permission was obtained from Institutional Ethical Review Board. Six 1-year-old male albino rabbits were used to monitor the in vivo transit behavior of the floating tablet. These rabbits were divided into 2 groups (group I and group II). None of them had symptoms or a past history of gastrointestinal (GI) disease. In order to standardize the conditions of GI motility, the animals were fasted for 12 hours prior to the commencement of each experiment.

### In vivo Gamascintigraphy

The location of the formulation in the stomach was monitored by keeping the subjects in front of a gamma camera a Specific stomach site (anterior) were imaged by gamma camera after definite time intervals and activity counts were recorded to calculate the counts per minute (cpm). All counts were corrected for background and isotope decay. The gamma images were recorded using an online computer system. In between the gamma scanning, the animals were freed and allowed to move and carry out normal activities but were not allowed to take any food or water until the formulation had emptied the stomach completely.<sup>12</sup>

## RESULT AND DISCUSSION

### Preliminary trials

In the preliminary trials all the tablet (Batch T1 to T6) were physically evaluated. All the formulated tablets showed percentage friability less than 1%, indicating good mechanical resistance. Thickness of tablet was in the range of 6 to 8 mm and diameter was  $10.0 \pm 0.05$  mm. hardness of the tablet was in the range of 5-6 kg/cm<sup>2</sup>. The assayed content of the drug in various formulations varied between 97.85 to 101.5%. All the tablet formulae showed acceptable physicochemical properties and complied with the pharmacopoeial specifications, drug content and friability.

In the preliminary trials the effect of HPMC K 4 M, LBG alone and in combination along with sodium bicarbonate as gas generating agent was studied. Other grades of the HPMC were not considered in the formulation due to their high viscosities which may ultimately affect the release pattern. The formulation containing HPMC K4 M alone shown that at low concentration the tablet could not float more than 5hrs and not able to maintain physical integrity, also shown initial bursting effect, this might be due to low swelling index and low polymeric viscosity thus, the floating tablet of Atenolol was not prepared using HPMC K4 M alone. Therefore to improve its physical integrity locust bean gum was added in the formulation as a gelling agent due to its gelling property it could impart viscoelasticity to the tablet. The use of viscosity gum solutions strongly increases with increasing concentration of gum. This behaviour is attributable to the interaction or entanglement, increasing the effective macromolecule dimensions and molecular weight. As a result of rheology of hydrated product, the swollen particles coalesce. This result in a continuous viscoelastic matrix that fills the interstices, maintaining the integrity of tablet and retarding further penetration of the dissolution medium ultimately the limitation of initial bursting effect was overcome by controlling the release pattern.

In the preliminary trials, 5% of sodium bicarbonate was added as a gas generating agent in all formulations, which resulted in floating lag time in the range of 1 min to 4 min. Therefore the concentration of sodium bicarbonate was varied from 10% to 20% in order to reduce the floating lag time. It was found that as the amount of sodium bicarbonate increases, the floating lag time decreases. Thus, sodium bicarbonate 15% was essential to achieve optimum *invitro* buoyancy, floating lag time less than 40 seconds and floating duration was more than 15 hrs. Further increase in concentration of

sodium bicarbonate does not show any significant effect on floating behaviour. Moreover, the increased in the amount of sodium bicarbonate caused a large amount of effervescent, which in turned resulted in pore formation, which leads to rapid hydration of the polymer matrix and thereby to rapid drug release. Therefore, level of sodium bicarbonate was kept constant at 15 % and was not considered further in the optimization as dependent variable. In the drug release profile, the drug release was in accordance with the USP specifications. According to USP test -4 the amount dissolved at 4, 8, 12 and 24 h should be 10% to 25%, 35 to 60%, 55 to 80% and more than 80% respectively. The formulation containing low concentration of HPMC K 4 M shown release pattern more than 90%. Whereas batch T2 to T5 shown greater retardation of the high concentration of polymer.

### Factorial design

Design Expert 8.0 software was used for studying effect of independent variables on responses. Experimental design layout developed for 9 possible combinations of Atenolol floating tablet formulations is shown in Table 2. Various models such as Linear, 2FI, Quadratic and Cubic, were fitted to the data and the model which fit well was suggested by software and was tested for analysis of variance (ANOVA). Regression polynomials were calculated for the individual dependent variables and then contour plots and 3D surface graphs were obtained for each individual dependent variable. Mathematical models were generated for each dependent variable or response (R) and expressed as Eqs. (1) –(3). The main effects (X1 and X2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X1, X2) show how the response changes when two factors are simultaneously Changed. The polynomial terms ( $X_1^2$  and  $X_2^2$ ) are included to investigate nonlinearity.

### Characterization of Atenolol floating tablet

All the tablet formulae showed acceptable physicochemical properties and complied with the pharmacopoeial specifications, drug content and friability. Data shown in Table 3.

### Swelling Index

The hydration ability of the formula is important because it influences: (i) tablet buoyancy, (ii) floating lag time, floating time and (iii) drug release kinetics. It could be concluded that the test medium uptake of the prepared matrices depends on the type of polymer. The formula F9 showed maximum swelling indices throughout the study period. Many authors state



**Table 2: Optimization batches by 3<sup>2</sup> factorial designs**

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Atenolol	100	100	100	100	100	100	100	100	100
Locust bean gum	50	50	50	75	75	75	100	100	100
HPMC K4M	50	75	100	50	75	100	50	75	100
Crospovidone	150	150	150	150	150	150	150	150	150
Sodium bicarbonate	75	75	75	75	75	75	75	75	75
Magnesium stearate	4	4	4	4	4	4	4	4	4
Talc	4	4	4	4	4	4	4	4	4

**Table 3: Characterization of optimized batches of Atenolol floating tablets**

Formulation Code	Floating lag time (Sec)	Floating duration (hr)	Density g/cm <sup>3</sup>	Swelling index (%)	% Cum.Drug Release (8 hr)
F1	22±5	3±2	0.6	79.9±2.8	94.99±1.2
F2	27±3	12±1	0.89	120.89±3.2	69.34±2.7
F3	38±4	18±2	0.85	110.8±2.7	63.03±1.6
F4	35±7	4±1	0.98	74.11±1.7	99.21±1.9
F5	52±8	15±2	0.89	112.31±1.5	74.03±2.1
F6	58±3	21±3	0.82	139.35±2.5	67.33±2.3
F7	60±5	7±2	0.91	103.6±2.9	94.16±2.6
F8	80±7	20±3	0.87	138.82±1.4	72.1±1.8
F9	120±6	27±2	0.85	145.79±1.9	62.04±3.1

that, greater the swelling index of the polymer, slower is the release of the drug.<sup>13-15</sup> Hence the decrease in the drug release rate may be explained due to an extensive swelling property of HPMC and LBG. So formulations containing optimum amount of swelling index was considered which in turns affected drug release rate i.e. 69%, 27 sec. FLT and tablet could float more than 12 hours as shown in **Table 3**. Swelling and erosion mechanisms of HPMC matrices have been reported by Viridena et al.<sup>16</sup> It was assumed that swelling behaviour of these hydrophilic tablets starts with water diffusion into the glassy HPMC material where the water plasticizes the polymer and reduces its glass transition temperature, T<sub>g</sub>. When T<sub>g</sub> has decreased to ambient temperature, a transformation from a glassy state to a rubbery state occurs. As the water continues to enter the tablet, a highly concentrated polymer solution is formed, denoted as a gel

layer. The solvent continues to penetrate the tablet, and the gel layer and the dimensions of the swollen tablet increase, a process normally referred to as the swelling process.

#### Density measurement

All the formulated tablet shown densities in the range of 0.6 g/cm<sup>3</sup> to 0.98 g/cm<sup>3</sup>. This may be due to gas generated in the 0.1 N HCL is trapped and protected within polymeric gel, formed by hydration of polymer, thus decreasing the density of tablet below 1 g/cm<sup>3</sup> and tablet becomes buoyant.

#### Data analysis

All responses were fitted to linear, interaction or quadratic models using Design expert software. Quadratic model was selected for percent cumulative release, floating time and factorial interaction suggested for floating lag time.

**Table 4: ANOVA of Response 1 ( $Y_1$ ) Floating lag time**

Source	Sum of Square	Df	Mean square	F value	P value Prob> F	Significant
Model	7105.6	3	2368.56	34.00	0.0009	
$X_1$ - LBG	4988.1	1	4988.17	71.60	0.0004	
$X_2$ - HPMC K4M	1633.5	1	1633.50	23.45	0.0047	
$X_1 X_2$	484.00	1	484.00	6.95	0.0462	
Residual	348.33	5	69.67			
Corr. Total	7454.0	8				

### Effect of formulation variables on floating lag time and floating time

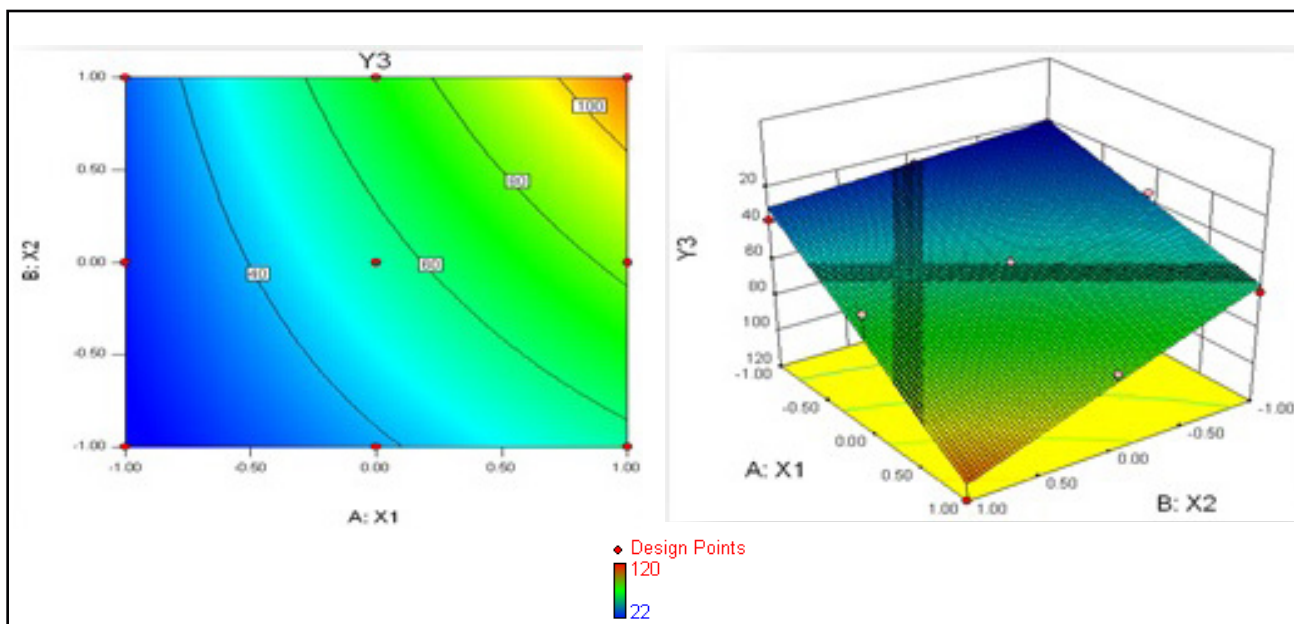
For the given response two factorial interaction models is significant, data shown in table no 4. The Model F-value of 34.00 implies the model is significant. There is only a 0.09% chance that a “Model F-Value” this large could occur due to noise. P value less than 0.0500 indicate model terms are significant. In this case A, B, AB are significant model terms.

From the Figure 1, it was observed that there was a significant effect of polymer HPMC K4M & LBG on the given response i.e. floating lag time.

**Equation (Eq.1) for response  $Y_1$  (Floating lag time) is as follows**

$$Y_1 = +54.66 + 28.83 X_1 + 16.50 X_2 + 11.00 X_1 X_2 \dots \dots \dots (1)$$

Where,  $Y_1$ –Floating lag time,  $X_1$  – Locust bean gum,  $X_2$ –HPMCK4M



**Figure 1: (a) Two dimensional contour plot; (b) three dimensional (3D) response surface plots for  $Y_3$  (Floating Lag time in sec.)**

The results of the equation indicate that there is direct effect of  $X_1$  (the concentration of LBG) & effect of  $X_2$  (the concentration of HPMC K4M) on floating lag time. Moreover, the concentration of HPMC K4M & LBG had a positive effect on floating time. Formulation F1 containing the lowest concentration HPMC K4M & LBG showed the lowest floating lag time i.e. 27 sec, Formulation containing highest concentration of HPMC K4M (100 mg) and LBG (100 mg), showed

highest floating lag time i.e.120 sec. These results may be due to the low viscosity and low bulk densities of HPMC K4M and LBG maintain the density less than gastric content and decreases the floating lag time.

### Effect of formulation variables on Floating time

For the given response Quadratic model is significant (Table 5). The Model F-value of 379.06 implies the model is significant. There is only a 0.02% chance that

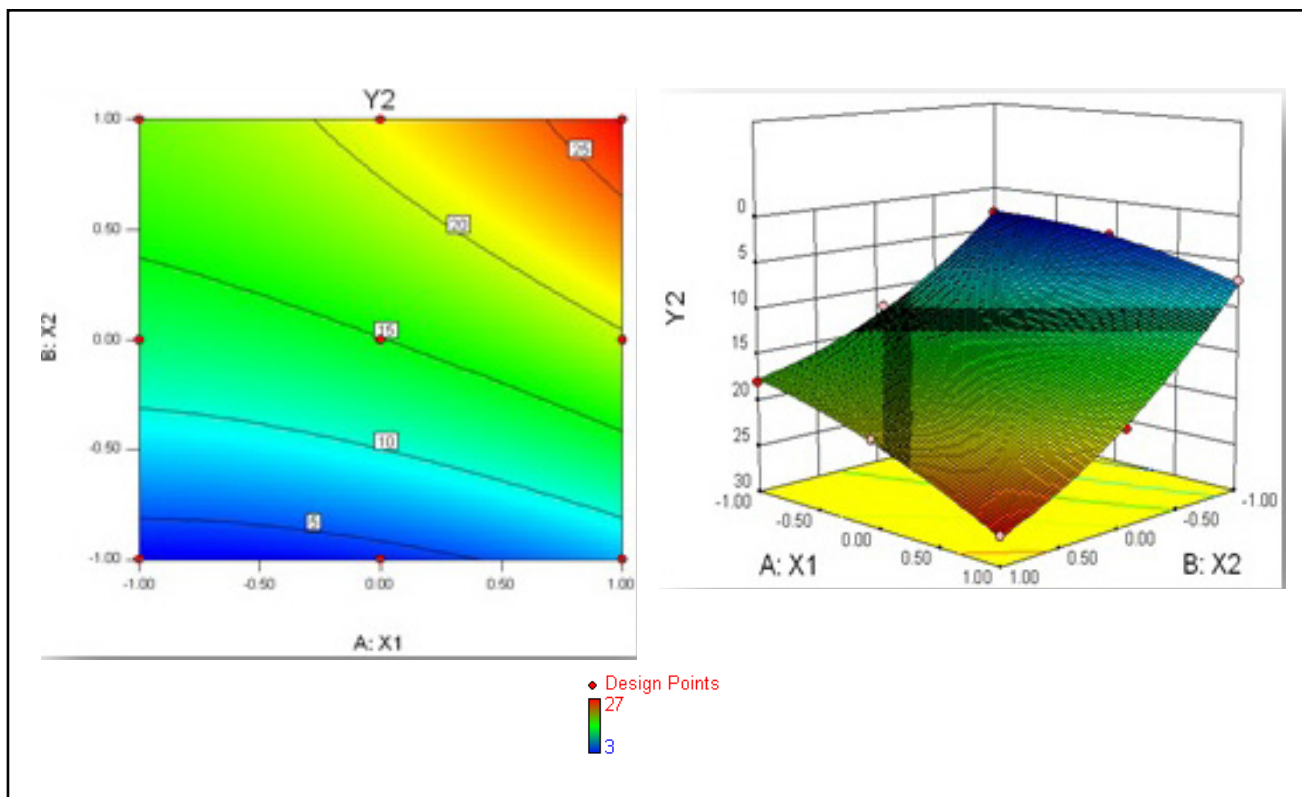
a “Model F-Value” this large could occur due to noise. The interaction terms  $X_1X_2$  show that their effect and interaction were statically significant of which P values are less than 0.0500 indicate model terms are significant. In this case A, B, AB,  $B^2$  are significant model terms.

From the Figure 2, it was observed that there is significant effect of polymer HPMC K4M & LBG on the given response i.e. floating time. As HPMC and LBG hydrophilic swelling polymer and gelling agent respectively. Upon contact with gastric fluid, HPMC takes up

water and swell, which retarded the drug release. The increased in the volume greater than the increased in mass during swelling, the density decreases and the systems, starts to float. The investigated gastric floating systems employed  $\text{NaHCO}_3$  as a gas-forming agent dispersed in a hydro gel matrix (HPMC K 4M and LBG). This suggests that the gel layers, formed by the investigated polymers, enabled efficient entrapment of the generated gas bubbles. The possible increase in tablet porosity made it float on the test medium (0.1 N HCl) for this extended period of time.

**Table 5: ANOVA of Response 1 ( $Y_2$ ) Floating time.**

Source	Sum of Square	Df	Mean square	F value	P value Prob> F	Significant
Model	544.03	5	108.81	379.06	0.0002	
$X_1$ - LBG	73.50	1	73.50	256.06	0.0005	
$X_2$ - HPMC K4M	450.67	1	450.67	1570.0	<0.0001	
$X_1 X_2$	6.25	1	6.25	21.77	0.0186	
$X_1^2$	2.72	1	2.72	9.48	0.0542	
$X_2^2$	10.89	1	10.89	37.94	0.0086	
Residual	0.86	3	0.29			
Corr total	544.89	8				



**Figure 2: (a) Two dimensional contour plot; (b) three dimensional (3D) response surface plots for  $Y_2$  (Floating time in hr.)**

These matrices are fabricated so that upon arrival in the stomach, carbon dioxide gas is liberated by the acidity of the gastric contents and is entrapped in the jellified hydrocolloid. A decrease in specific gravity causes the dosage form to float on the chime.<sup>17</sup>

**Equation (Eq.2) for response  $Y_2$  (Floating time) is as follows**

$$Y_2 = +14.88 + 3.50 X_1 + 8.66 X_2 + 1.25 X_1 X_2 + 1.16 X_1^2 - 2.33 X_2^2 \dots \dots \dots (2)$$

Where,  $Y_2$  – Floating time,  $X_1$  – Locust bean gum,  $X_2$  – HPMCK4M

**Effect of formulation variables**

For the given response Quadratic model is significant (Table 6). The Model F-value of 190.74 implies the model is significant. There is only a 0.06% chance that a “Model F-Value” this large could occur due to noise. The interaction terms  $X_1 X_2$  show that their effect and interaction were statically significant of which P values are less than 0.0500 indicate model terms are significant. In this case A, B, AB are significant model terms.

**Equation (Eq.3) for response  $Y_3$  (% Cumulative release in 8hr) is as follows**

$$Y_3 = +74.654 + 0.156 X_1 - 15.993 X_2 - 0.0400 X_1 X_2 - 4.2466 X_1^2 + 8.303 X_2^2 \dots \dots \dots (3)$$

Where,  $Y_3$  – % Cumulative release,  $X_1$  – Locust bean gum,  $X_2$  – HPMC K4M

The results of the equation indicate that the effect of  $X_2$  (the concentration of HPMC K4M) was significant than the effect of  $X_1$  (the concentration of LBG). Moreover, the concentration of HPMC K4M had a negative effect on percent Cumulative release. Formulation F3 containing highest concentration of HPMC K4M (100 mg) and lowest concentration of LBG (50 mg), showed lowest % Cumulative release in 8hr. i.e. **63.03 %**.

From the Figure 3, it observed that HPMC K4M having the significant effect on the response i.e. % cumulative release. The differences in the release may be due to the amount of gel layer formed on the surface of the tablets. HPMC K4M at higher concentrations results in a greater amount of gel being formed. This gel increases diffusion length so that drug release was decreased.

Table 6: ANOVA of Response 1 ( $Y_1$ ) – % Cumulative release						
Source	Sum of Square	Df	Mean square	F value	P value	Prob> F Significant
Model	1708.83	5	341.77	190.74	0.0006	
$X_1$ - LBG	0.15	1	0.15	0.082	0.7930	
$X_2$ -HPMC K4M	1534.72	1	1534.72	856.51	<0.0001	
$X_1 X_2$	6.4	1	6.4	3.572	0.9561	
$X_1^2$	36.07	1	36.07	20.13	0.0207	
$X_2^2$	137.89	1	137.89	76.95	0.0031	
Residual	5.38	3	1.79			
Corr. Total	1714.21	8				

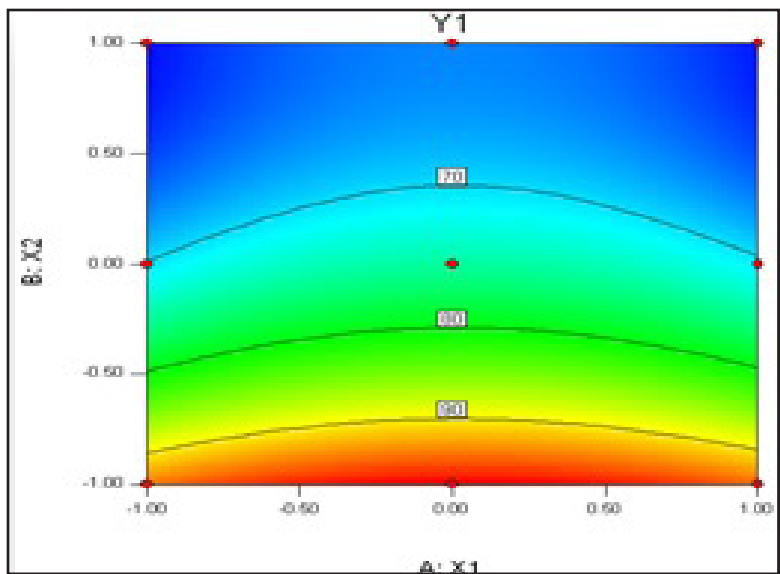


Figure 3: (a) Two dimensional contour plot; (b) three dimensional (3D) response surface plots for  $Y_3$  (% Cumulative release)



### Kinetic model

The value of coefficient of regression for different models of formulation F1 to F9 is given in Table No. 7. The coefficient of regression value for the optimized batch F2 was found to be highest i.e. **0.995** for Zero order model and hence the release mechanism was found to follow zero order kinetic release model.

### Stability Studies

Formulation showing optimum result was selected for stability studies. According to ICH guidelines,<sup>18</sup> selected formulation (F2) was stored at 40°C temperature and

75% relative humidity (RH) for a period of 3 months. Formulation was evaluated at periodical intervals of one month for % cumulative release, Floating lag time and Floating time. The drug content obtained after periodic intervals was found in the range of 98.21% to 98.05% and hence did not show any major differences. Floating lag time was also not affected by the stability conditions as shown in the Table 8. Floating lag time was observed in the range of 27 sec to 30 sec Floating time was observed at >12 hr no major differences were observed. Evaluation parameters do not show any major difference and all are in accepted limits.

**Table 7: Kinetic models of Atenolol tablet for optimization batches**

Sr. no.	Formulation	Zero Order	First Order	Higuchi Model	Hixon-Crowel	Korsmeyer-Peppas		
						R <sup>2</sup>	K	N
1	F1	0.495	0.864	0.784	0.742	0.955	1.864	0.121
2	F2	0.995	0.964	0.908	0.985	0.989	1.041	0.884
3	F3	0.902	0.965	0.980	0.950	0.963	1.394	0.417
4	F4	0.473	0.923	0.772	0.764	0.861	1.903	0.119
5	F5	0.914	0.945	0.952	0.946	0.930	1.292	0.622
6	F6	0.919	0.938	0.953	0.937	0.952	1.236	0.668
7	F7	0.966	0.939	0.959	0.941	0.945	1.419	0.572
8	F8	0.961	0.935	0.922	0.953	0.963	1.251	0.623
9	F9	0.976	0.970	0.951	0.978	0.974	1.111	0.718

**Table 8: Evaluation parameter of stability batch F2**

Days	Drug (%)	Floating Behavior	
		FLT (Sec)	Floating duration (hrs)
<b>Before Storage</b>			
0 day	98.21±2.5	27 ± 5	>12 hrs
<b>After Storage</b>			
7 days	98.17±1.5	27± 9	>12 hrs
15 days	98.15±1.5	28± 7	>12 hrs
30 days	98.10±2.0	29 ± 6	>12 hrs
60 days	98.10±2.5	30 ± 5	>12 hrs
90 days	98.05±1.5	30 ± 6	>12 hrs

**Gamascintigraphy study**

Gamma scintigraphic is a technique whereby the transit of a dosage form through its intended site of delivery can be non-invasively imaged *in vivo* via the judicious introduction of an appropriate short lived gamma emitting radioisotope. The technique of Gamascintigraphy has become the most popular method to investigate the gastrointestinal performance of the product.<sup>11</sup> Tc 99m is inexpensive and readily available through the use of portable generators.<sup>19</sup> Tc – 99 m possesses most of the characteristics of an ideal radionuclide and hence found widespread applications in nuclear and in pharmaceutical formulation development.

In the present study *in vivo* gamma scintigraphic study was done to observe the *in vivo* floating behavior of optimized floating tablet. Radio labeled formulation was evaluated for its radiolabelling efficiency it was found more than 90 %. Therefore, 99mTc acts as the marker for locating the transit of the floating atenolol tablet.<sup>11</sup> It was observed in the gamma images that optimized formulation (batch F2) was retained in the stomach more than 6 hours. A measurable number of counts of 99mTc-tagged atenolol formulation (F2) for the 6-hour Study period shown good gastro-retention (Shown in Figure 4).

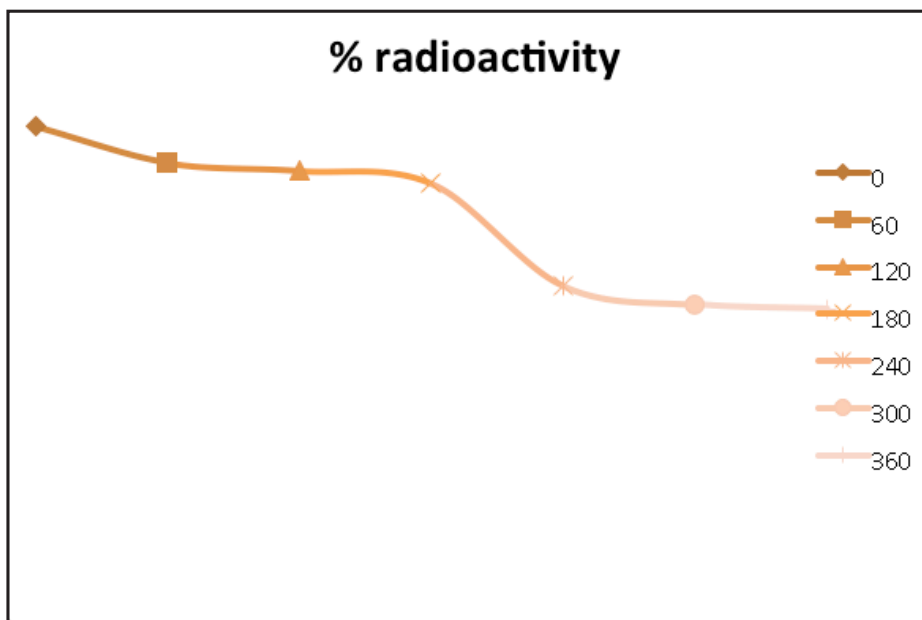


Figure 4: Representative Gastric emptying curve for the radio labeled formulation F2.

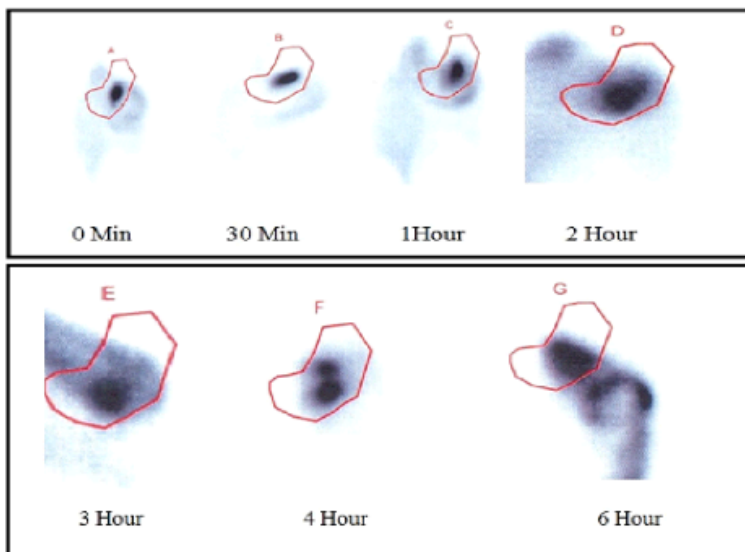


Figure 5: Representative gamma scintigraphic images of Floating matrix tablet in Rabbits

A representative rabbit gamma scintigraphic image has been shown at 0, 2 and 5 h in fig. 5. Gamma scintigraphic studies revealed the location of the capsule in 6 healthy rabbits. Posterior whole body images at various time intervals (15 minutes, 30 minutes, and 1, 2, 3, 4, and 5 hours) showed the retention of the tablet in the stomach for more than 5 hours, as shown in vitro studies.

## CONCLUSION

Floating matrix tablet of atenolol containing combination of HPMC K4 M, LBG and sodium bicarbonate shown desired characteristics of floating formulation. The optimized formulation F2 follows the zero order kinetics model mechanism. *In vitro* buoyancy studies indicate that the formulation is suitable for gastro retention. This data was supported by performing Gamascintigraphy study on Rabbits; results indicate the prolongation of gastric residence time. This retention in the stomach can improve the absorption of Atenolol.

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## CONFLICTS OF INTEREST

Authors have no conflicts of interest.

## ABBREVIATION

HPMC (Hydroxypropyl methyl cellulose), LBG (Locust bean gum), FLT (Floating lag time, FT (Floating time), Tg (glass transition temperature), ANOVA (Analysis of variance), RH (Relative humidity)

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