Research Article

Formulation Optimization and *In-Vivo* evaluation of Floating Gastroretentive Microsphere of Sitagliptin by 3^2 Factorial Design.

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ABSTRACT

The present invention preferably relates to formulation and characterization of gastro-retentive floating microsphere comprising Sitagliptin to enhance its bioavailability and retention time in the stomach. Microspheres were prepared by Ionotropic gelation method by using HPMC K4M and Psyllium husk as swelling agents and all other Excipients were used of Pharmaceutical grade. The Microsphere showed Mucoadhesion time, force and strength 7.5 to 12 hrs, 24-28 gm and 2.35 – 3.62 N respectively. Swelling index and drug release was found to be 216% and 99.2 % respectively. *In-vivo* imaging study and Pharmacokinetic study showed retention of Microsphere in GIT of rabbit for 24 hrs and release up to 24hrs. The results for stability study were also same as they were at the initial stages of evaluation. The method was also found optimum and valid when it was optimized using design expert software. The data obtained was best fit into Korsmeyer-Peppas model indicating non-Fickian or anomalous release and as compared to other models as R^2 value was found to be 0.9983. It can be concluded that by using polymers such as HPMC k4M and Psyllium husk the objective of the present study are meet. The Probability value indicates model terms are significant. The probablity value i.e. P-value found was also less than 0.0500. The desirability value was found 0.984 which is equal to 1. From desirability it can also be concluded that results actually obtained matches with the software prediction and hence the formulation is also validated.

Keywords: *In-vivo*, Bioavailability, Probability, Swelling, Desirable.

INTRODUCTION

Gastroretentive drug delivery system is the system which is retained in the stomach for a longer period of time and improves the bioavailability of drugs that are preferentially absorbed from upper GIT[1]. Reported methods for the design of gastroretentive systems include mucoadhesion[2,3]. Floatation, sedimentation[4-6]. Swelling and expanding drug delivery system[7,8].

The objective of present study is to develop swellable gastroretentive Microsphere of Sitagliptin. For this purpose 3^2 full factorial design was applied systematically. Sitagliptin is available as an immediate release (IR) formulation in Microsphere and is administered to patient's two- or three-times daily (BID or TID). Many patients receiving sitagliptin or other drugs, which are, administered two or more times daily would likely benefit from once daily dosing. The convenience of once daily dosing generally improves patient compliance, especially for elderly patients and for patients taking multiple medications. Once per day dosing may also lessen or prevent potentially undesirable dose-related effects by reducing peak blood levels (C MAX) and may also increase drug efficacy by increasing minimum plasma concentrations (C MIN).

Once daily dosing of sitagliptin, however presents numerous challenges. Conventional extended release (ER) compositions are problematic for dosing because sitagliptin is not absorbed uniformly in the gastrointestinal (GI) tract. Clinical studies indicate that sitagliptin is absorbed in the small intestine and the ascending colon in humans, but is poorly absorbed beyond the hepatic flexure. This suggests that the mean absorption window for sitagliptin is, on average, about six hours or less and any drug release from a conventional ER dosage form beyond six hours would thus be wasted because the dosage form has traveled beyond the hepatic flexure[9].

Thus swellable gastroretentive microsphere was formulated of sitagliptin to lessen or prevent potentially undesirable dose-related effects by reducing peak blood levels (C MAX) and also to increase drug efficacy by increasing minimum plasma concentrations (C MIN). Sitagliptin is an antidiabetic drug which is used to control blood glucose level in the body.

MATERIALS AND METHODS

Materials

Sitagliptin and Psyllium husk (Pharmaceutical grade) were obtained as a gift sample from Alkem Laboratories Mumbai (India).HPMC K4M, PVP k30 and Microcrystalline cellulose were obtained as a gift samples from Ajanta Pharma Aurangabad (India). All other solvents and reagents used were of analytical grade.

Experimental Design

A 3^2 full factorial design was selected because, an experiment may be designed to focus attention on a single independent variable or factor. An alternative approach is to study the influence of one independent

variable in conjunction with variations in one or more additional independent variables. We can study not only the effects of the two independent variables separately but also how they combine to influence the dependent variable. The amount of HPMC K4M (X1) and the amount Psyllium Husk (X2) were selected as independent variables. Two-factor (X1, X2), three-level (-1, 0, +1) design can be developed. Two-factor were evaluated each at three-level and experimental trials were performed for all nine possible combinations. *In-vitro* drug release and swelling index were selected as dependent variables. The actual formulation design of swellable gastro retentive Microspheres according to factorial design (3^2) layout is shown in **Table 1**.

Response Surface Methodology (RSM) is also widely employed to optimize formulations with suitable experimental design because it permits a deeper under-standing of a process or product and has important applications in establishing the robustness of that product. Full factorial designs, which have been widely used in response surface modeling and optimization[12]. RSM was used to establish the relative importance of two or more factors and also to indicate whether or not interaction occurs between the factors and thereby affects the magnitude of the response. The data was interpreted using response surface methodology (Design Expert Software Version 9, Stat-Ease, Inc.).

Formulation FDDS

The Microspheres were prepared by wet granulation technique using psyllium husk and HPMC K4M as swelling agent and PVP K30 as a binding agent. The various excipients used were listed in Table 1. Psyllium husk was triturated for size reduction. For formulation of Microspheres of sitagliptin; Psyllium husk, HPMC K4M and other additives except magnesium stearate. The floating microspheres containing Sitagliptin were prepared by ionotropic gelation technique. Sodium alginate alone or in combination with xanthan gum and the gas forming agent sodium carbonate was dispersed in the purified water to form a homogeneous polymer mixture. The drug, Sitagliptin was added to the polymer dispersion and mixed thoroughly on a magnetic stirrer to form a homogeneous dispersion. The gelation medium was prepared by dissolving calcium chloride in 2% glacial acetic acid. The homogenous alginate solution was extruded using 21G syringe needle into the gelation medium. The distance between the edge of the needle and surface of gelation medium was about 10cms.

The gel microspheres formed were left in the solution with gentle stirring for 30 min at room temperature to improve mechanic strength. After that, microsphere was collected and washed with distilled water twice, dried at room temperature for 24 hr and stored in desiccators. Polythene bag was used for mixing and rotated octagonal to mix content well for 15 minutes to get uniform mixture.

Measurement of Mucoadhesive Strength and Force of Adhesion

The pieces of goat stomach mucosa were used. At time of testing a section of tissue was secured keeping the mucosal side out, on the upper glass vial using a rubber band and aluminum cap. The vial with the fundus tissue was stored at 37^{O}C for 10 min. Then one vial with section of tissue was connected to the balance and another vial was fixed on height adjustable pan. To a lower vial a Microsphere was placed with the help of cello tape.

The height of the lower vial was adjusted so that a Microsphere could adhere to the mucosal tissue on the upper vial. A constant force was applied on the upper vial for 20 seconds after which it was removed and the upper vial was then connected to the balance. Then, the weight on right side pan was slowly added in an increment of 0.5 g till the two vials just separated from each other. The total weight (gm) required to detach two vials was taken as a measure of mucoadhesive strength. From this mucoadhesive strength, the force of adhesion was calculated using the following formula[13].

$$Force\ of\ Adhesion\ (N) = \frac{Mucoadhesive\ strength}{100} \times 9.81$$

***Ex-vivo* Mucoadhesive Time**

The *ex-vivo* mucoadhesion time studies were performed after application of Microspheres on freshly cut goat stomach mucosa.

The mucosa was fixed on a glass slide using double sided adhesive and one side of glass slide was fixed to thread whose another end was fixed with the arm of Microsphere disintegration test apparatus.

A side of each Microsphere was wetted with dissolution medium and was attached to the mucosa by applying a light force with a fingertip for 20 seconds. The beaker was filled with 900 ml of simulated gastric fluid and kept at 37˚C; after 2 minutes the slide was placed in a beaker and the apparatus was started. Care was taken that while up and down motion of the arm Microsphere should remain in medium. Behavior and mucoadhesive time of Microsphere were monitored until complete detachment occurred[14].

Swelling Index

Swelling study of the Microspheres was carried out by using USP dissolution apparatus type-II (LABINDIA, Disso 2000) at 37 ± 0.5°C and paddle speed was kept 50 rpm. 900 ml of distilled water is used as Dissolution medium.

The Microspheres were placed in the medium under rotation and withdrawn from the medium after selected time interval, excess water was removed by blotting and weighed. The swelling index of the Microspheres was given by following formula[15,16].

$$Swelling\ Index\ (\%) = \frac{Weight\ of\ Swollen\ Microsphere - Initial\ Weight\ of\ Microsphere}{Initial\ Weight\ of\ Microsphere} \times 100$$

In-vitro Dissolution Studies

Dissolution profiles of Sitagliptin from Microspheres were determined in triplicate at 37 ± 0.5°C using the USP dissolution apparatus type II (LABINDIA, Disso 2000). The dissolution test was performed using 900 ml of 0.1N HCl, at 37 ± 0.5°C and 50 rpm. Samples (5ml) were withdrawn with replacement at predetermined time interval of 1, 2, 4, 6, 8, 12, 16, 24 hours and filtered through a 0.45 µm pre-filter. The filtered samples were then diluted with dissolution medium and the absorbance measured at 210 nm by using U.V Spectrophotometer (Shimadzu UV1601)[17].

Drug Release Kinetics

In order to investigate the drug release mechanism from Microspheres, the drug release data was analyzed with following mathematical models and interaction of diffusion release mechanism.

Zero Order Kinetics

$Q = Q_o - K_o t$ Eq. No 1

First Order Kinetics

$Q = Q_o (1 - e^{-K_1 t})$ Eq. No 2

Higuchi Square Root Model

$Qt = K_H t^{½}$ Eq. No 3

Hixson-Crowell Cube Root Model

$\sqrt[3]{Qo} - \sqrt[3]{Qt} = K_{HC}t$ Eq. No 4

Korsmeyer-Peppas Model

$Qt/Q_\infty = K_k t^n$ Eq. No 5

Where, Qt =amount of drug release d at time t.

Qo = initial amount of drug.

And Ko, K_1, KH, K_{HC} and K_K are the coefficients of equations. The most appropriate model was selected on the basis of regression values (r^2) and diffusion release exponent (n).

Drug release kinetics and best fit model for all the selected batches was found out with the help of *PCP DISSO Version 2.08* software and Microsoft Excel[18-21].

In vivo radio Imaging Study in Rabbits

The protocol for *in-vivo* study was approved by the Institutional Animal Ethical Committee (IAEC) of Institute of Biomedical and Industrial Research, Jaipur having [Protocol approval **No.IBIR/IAEC/2016-18**] and is in accordance with guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. All institutional and national guidelines for the care and use of laboratory animals were followed. In order to evaluate the *in-vivo* residence time, the Microspheres from the optimized formulation were selected. Four adult male New Zealand white strain rabbits of three months age and weighing approximately 2.5 to 3.0 kg were used for this study. The rabbits were fasted overnight before the start of the study.

The Microspheres excluding drug and containing 18 % BaSO4 were manufactured by method as described in the preparation using 12 mm tooling.

The Microsphere was administered through plastic tubing followed by flushing of 25 to 30 ml of water. During the entire study, the rabbits had free access to water only. Photomicrographs (Wipro GeDx300 with horizontal x-ray system, Wipro GE medical system, Pune-04, India) were taken at 0, 2, 4, 8, 16 and 24 hrs[22,23].

In vivo Bioavailability Studies

An *in vivo* bioavailability studies protocol was approved by the Institutional Animal Ethical Committee (IAEC)] Biomedical and Industrial Research, Jaipur having [Protocol approval **No. IBIR/IAEC/2016-18.** Male rabbits with weight of 2.5 to 3 kg were selected. Total 4 rabbits were divided into 2 groups. Each group had 2 rabbits. The animals were housed individually under environment conditions (25°, 12 hr light and dark cycle).

The rabbits were fasted overnight and allowed free accesses to water only. Best formulation was administered orally by placing the Microsphere in a hollow plastic tubing and flushing of 25 to 30 ml water. Blood samples of 1 ml were withdrawn at specific time interval for Microsphere. Blood samples were collected from the marginal vein of the rabbit[24]. COLOUMN – C 18 hypersil stainless steel column as stationary phase, Mobile Phase – A degassed mixture of 90:10 Water and methanol solution was used as mobile phase with flow rate of 1 ml/min. The batch F-3 was selected for in vivo study on the basis of its *in vitro* results. It was concluded that Sitagliptin was released from the F-3 Microspheres in a best manner and subsequently got absorbed in vivo. Plasma samples of 0.5 ml were separated and 0.5 ml of 5N sodium hydroxide and 3N sodium chloride was added. The samples were mixed thoroughly for 1 min.

Then 8 ml of diethyl ether was added and stirred for 1 min and the samples were centrifuged at 3000 rpm for 10 min. The organic layer was separated and evaporated to dryness at 60°C. The dry residue was rediscovered using 1 ml of mobile phase and injected into the HPLC system. The samples are detected at wavelength of 210 nm.

Stability Study

The Microspheres of optimized batch were kept for accelerated stability studies according to international conference on Harmonization (ICH) guidelines. ICH specifies the length of the study and storage conditions.

Long-Term Testing

25˚C ± 2˚C / 60% RH ± 5% for 12 months.

Accelerated Testing

40˚C ± 2˚C / 75% RH ± 5% for 6 months.

Stability studies were carried out at 40˚C / 75% RH for the selected formulation for six months.

The Microspheres of optimized batch were packed in air tight plastic container. They were then stored at 40˚C / 75% RH, for six month and evaluated for their physical characteristics, mucoadhesive strength, mucoadhesive time, Swelling index, and drug release at specific interval of time per ICH guidelines. The result obtained from this evaluation test were comparing with zero month formulation[25,26].

Validation of Results of Dissolution and Swelling Based on Desirability

Validation of Results of Dissolution and Swelling Based on Desirability were performed on the basis of results obtained for dissolution and swelling using Design Expert Software Version 9, Stat-Ease, Inc., and prediction of results were found. Overall desirability value is an indicator of optimum formulation as it is calculated from the individual values which in turn and the same are calculated based on the desirable target response of independent variables[27].

RESULTS AND DISCUSSION

Swelling Index

The swelling of the polymers is studied by their ability to imbibe water and swell enormously. In the present study polymers used in the formulation HPMC K4M, Psyllium husk have been reported to show good swelling properties. These polymers in combination showed good swelling properties ranging from 191% to 240 %. This increase in swelling was possible only due to imbibitions and mucilage formation of polymers when it comes in contact with biological and or aqueous medium and due to which swelling took place. Based on the results of swelling index it can be predicted that gastric retention can be achieved preferably more than 24 hrs.

In-Vitro Dissolution Study

A 3^2 full factorial design was constructed to study the effect of the amount of HPMC K4M (X1) and Psyllium husk (X2) on drug release from sitagliptin Microspheres.

The dependent variables chosen were drug release and swelling index**.** In our studies release of sitagliptin was found to be a function of the polymer concentration. It was observed that the variation in concentration of polymer from factorial batches F1 to F9 have variability on release rate of drug.

The influence of HPMC K4M and Psyllium husk ratio on the release of sitagliptin from the Microspheres in 0.1 N HCl (pH 1.2) at 37 ± 0.5 °C is shown in Figure 1. It is clear that increase in concentration of HPMC K4M and Psyllium husk in formulae decreased the release rate. The formulation F-3 showed the best 99.2 % drug release, which also indicates that the bioavailability of the sitagliptin formulation has also been increased as it is retained in the gastric region for a longer period of time and does not pass the hepatic flexure.

Formulations F-1, F-2 and F-9 containing higher concentrations of HPMC K4M showed less drug release from 80.6% to 87.5 % as compared with other formulation batches, out of which batch F-2 showed very less drug release, due increased concentration of Psyllium husk. The Formulations F-4, F-6 and F-8 showed increase in drug release from 94.3% to 96.7% due to decrease in concentration of HPMC K4M and Psyllium husk. The Formulations F-3, F-5 and F-7 showed drug release from 94.1% to 99.2%, in which the polymer concentration was found optimum in batch F-3 which showed drug release up to 99.2%. The batch F-5 showed less drug release due to increased concentration of Psyllium husk. The drug release for all the statistically designed batches is shown in Table 2.

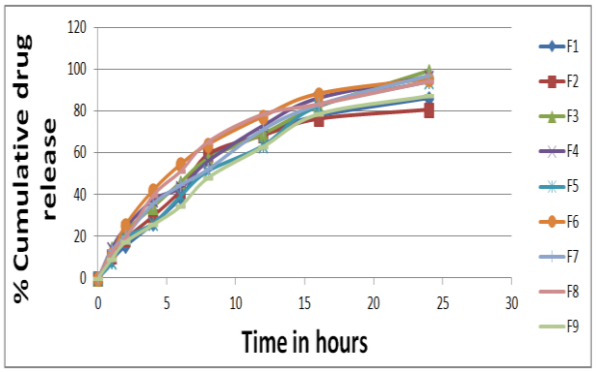

Figure 1: Percent Cumulative Drug Release of all Batches F-1 to F-9

Anova Analysis for Drug Release and Swelling Index

Evaluation and interpretation of research findings are important and the p-value serves a valuable purpose in these findings. ANOVA for the dependent variables drug release and swelling index was done. The coefficients of X1 and X2 were found to be significant at $p<0.05$, hence confirmed that both the variables have significant effect on the selected responses. Overall both the variables caused significant change in the responses. ANOVA and response surface analysis were done using Design Expert Software Version 9, Stat-Ease, Inc.

Response Surface Analysis

The quadratic model obtained from the regression analysis was used to build a 3-D graphs in which the responses were represented by curvature surface as a function of independent variables. The relationship between the response and independent variables can be directly visualized from the response surface plots presented in Figure. 2 Contour plots (Figure. 2) are two dimensional representations of the responses for the selected factors and shows that as the concentration changes of both the variables the release pattern and swelling index also varies i:e as the concentration goes on increasing the dissolution is decreased and swelling index increases and vice versa. Three dimensional (3-D) surface plots (Figure.2) for the obtained responses were drawn

based on the model polynomial functions to assess the change of the response surface. These plots explain the relationship between the dependent and independent variables i:e the effects of two factors on the response at one time. The response surface analysis for drug release and swelling index was studied which showed significant results. The Model F-value of 105.34 and 400.92 for drug release and swelling index implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant.

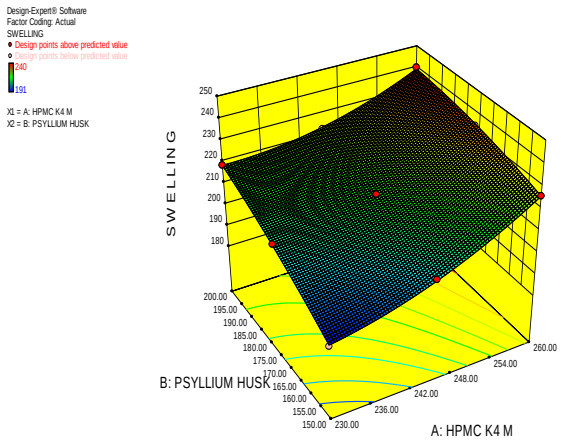

Figure 2: 3D Graph of Swelling Index

The "Pred R-Squared" of 0.9537 for drug release and 0.9821 for swelling index is in reasonable agreement with the "Adj R-Squared" of 0.9849 for drug release and 0.9960 for swelling index. The ratio of 29.120 for drug release and 60.670 for swelling index indicates an adequate signal. The probablity value i:e P-value found was also less than 0.0500. This model can be used to develop the design.

Response : DISSOLUTION AND SWELLING INDEX

ANOVA for Response Surface Quadratic Model drug release

Calculation for effect of formulation variables on Drug release and Swelling Index

Final Equation for Drug release for 24 hrs in Terms of Coded Factors

DISSOLUTION = +98.40 - 5.27* A-1.42 * B - 2.10* A * B - 6.80* A2 - 2.35 * B2

Final Equation for Drug release for 24 hrs in Terms of Actual Factors

DISSOLUTION = -1975.00000 +15.43778 * HPMC K4 M +2.63133 * PSYLLIUM HUSK -5.60000E-003 * HPMC K4 M *PSYLLIUM HUSK -0.030222 * HPMC K4 M2-3.76000E-003 * PSYLLIUM HUSK2

Final Equation for Swelling index in Terms of Coded Factors

SWELLING = +215.78 +13.67 * A +10.17 * B -3.50 * A * B +5.33 * A2 -2.17 * B2

Final Equation for Swelling index in Terms of Actual Factors

SWELLING = +837.87037 -9.07037 * HPMC K4 M +3.90667 * PSYLLIUM HUSK -9.33333E-003

* HPMC K4 M * PSYLLIUM HUSK+0.023704 * HPMC K4 M2 -3.46667E-003 * PSYLLIUM HUSK2

From the equation for dissolution the information conveyed was:

1) R^2 was high indicating the adequate fitting of the Quadratic Model.

2) As HPMC K4M and Psyllium husk (-ve coefficient) showed –ve sign it also indicated that drug delivery system gained more control over the release of sitagliptin from prepared dosage form.

From the equation for swelling the information conveyed was:

1) R^2 was high indicating the adequate fitting of the Quadratic Model.

2) As HPMC K4M and Psyllium husk (+ve coefficient) showed +ve sign it also indicated that drug delivery system gained more swelling and due to which more retention of dosage form was possible due to increase in size if dosage form.

Graphical presentation of data shows relationship between response and independent variables. The information given by graph was similar to that of mathematical equations obtained from statistical analysis. The response surface plots showed that various combinations of independent variables X1 and X2 satisfy specific requirement (i.e., drug release with swelling index while taking into consideration of various factors involved in dosage form).

Drug Release Kinetic Study

Kinetic study of drug release is often useful in obtaining one or two physically meaningful Parameters which are employed for comparative purposes and relating the release parameter. Moreover a kinetic parameter can be used to study the influence of formulation factors on the drug release for statistical optimization. The drug release kinetics was studied by plotting the data obtained from the *in-vitro* drug release in various kinetic models. To establish the mechanism involved in drug release from the Microspheres, data of percentage drug release versus log time were plotted according to Korsmeyer–Peppas equation as drug release exponent 'n' indicates the mechanism of drug release calculated through the slope of the straight line was found to be n= 0.6182. If the exponent n= 0.45, then the drug release follows the Fickian diffusion and if 0.45< n <0.85 then it is said to be non-Fickian or anomalous release. The mechanism of release for the above formulations was determined by finding the R^2 value for each kinetic model viz. zero-order,

first-order, Higuchi, and Korsmeyer–Peppas corresponding to the release data of each formulation. From most of the formulations the R^2 value of Korsmeyer–Peppas model is very near to one than the R^2 values of other kinetic models. Thus, it can be said that the drug release follows Korsmeyer–Peppas model mechanism, out of which the R^2 = 0.9983 of formulation F-3 was found best amongst other formulations and n value was found **n** = 0.6182 hence it can be postulated that formulation F-3 followed non-Fickian or anomalous release. The results are shown in Table 4.

Table 1: Factorial Design Formulations of Sitagliptin Microspheres Prepared By Ionotropic Gelation Method

S. No.	Ingredients (mg)	Batches								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Sitagliptin	150	150	150	150	150	150	150	150	150
2	Psyllium Husk	133.33	200	133.33	133.33	200	200	66.66	66.66	66.66
3	HPMC K4M	260	260	173.33	86.33	173.33	86.33	173.33	86.33	260
4	Chitosan	60	60	60	60	60	60	60	60	60
5	Polyvinylpyrolidone	80	80	80	80	80	80	80	80	80

Table 2: Percent Drug Release for all the Statistical Batches

S. No.	Time (Hrs)	% Drug Release For All Factorial Batches								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	1	8.7	10.4	14.35	14.5	7.4	11.4	13.4	12.8	9.3
2	2	15.5	18.7	22.72	24.3	19.3	25.6	20.2	19.4	17.4
3	4	26.2	29.7	34.15	37.7	26.2	42.2	35.7	39.7	25.7
4	6	38.6	42.0	46.02	43.5	39.4	54.6	44.7	51.3	34.6
5	8	57.4	59.3	57.3	56.2	51.3	64.2	52.3	65.4	48.3
6	12	69.2	68.4	69.16	73.3	63.5	77.6	71.4	78.6	63.2
7	16	77.7	76.2	82.09	86.4	82.4	88.4	83.2	83.4	78.8
8	24	86.2	80.6	99.21	96.7	94.1	95.6	97.2	94.3	87.5

Table 3: Analysis of variance table for dissolution [Partial sum of squares - Type III]

Source	Sum of Squares	Df	Mean square	F-Value	p-value Prob > F	Observation
Model	299.63	5	59.93	105.34	0.0014	SIGNIFICANT
A-HPMC K4M	166.43	1	166.43	292.55	0.0004	SIGNIFICANT
B-PSYLLIUM HUSK	12.04	1	12.04	21.17	0.0193	SIGNIFICANT
AB	17.64	1	17.64	31.01	0.0114	SIGNIFICANT
A2	92.48	1	92.48	162.56	0.0010	SIGNIFICANT
B2	11.05		11.05	19.42	0.0217	SIGNIFICANT
Residual	1.71	3	0.57	--------	--------	
Core Total	301.34	8	---------	-------	---------	

Table 4: Model Fitting (Kinetic Study) of all Factorial Batches

Batches	Zero Order		First order		Higuchi (Matrix)		Hix. Crow		Korsmeyer-Peppas		
	R^2	K	R^2	K	R^2	K	R^2	K	R^2	K	N
F-1	0.8974	5.06	0.9962	-0.126	0.9732	20.11	0.9889	-0.02	0.9876	10.83	0.7535
F-2	0.8357	4.95	0.9777	-0.106	0.9763	19.98	0.9478	-0.02	0.9823	13.45	0.6682
F-3	0.9067	4.97	0.9248	-0.155	0.9937	19.77	0.9899	-0.03	**0.9983**	**14.77**	**0.6182**
F-4	0.8796	5.60	--------	-------	0.9925	22.41	0.9628	-0.04	0.9951	17.54	0.6016
F-5	0.9395	5.22	--------	-------	0.9781	20.51	0.9417	-0.04	0.9867	10.50	0.7718
F-6	0.8180	5.81	--------	-------	0.9853	23.54	0.9839	-0.05	0.9763	16.54	0.6516
F-7	0.9040	5.49	--------	-------	0.9917	21.86	0.9478	-0.04	0.9968	15.77	0.6333
F-8	0.8285	5.68	--------	-------	0.9822	22.96	0.9709	-0.04	0.9813	16.05	0.6547
F-9	0.9332	4.94	0.9796	0.131	0.9718	19.44	0.9971	-0.02	0.9950	11.33	0.7152

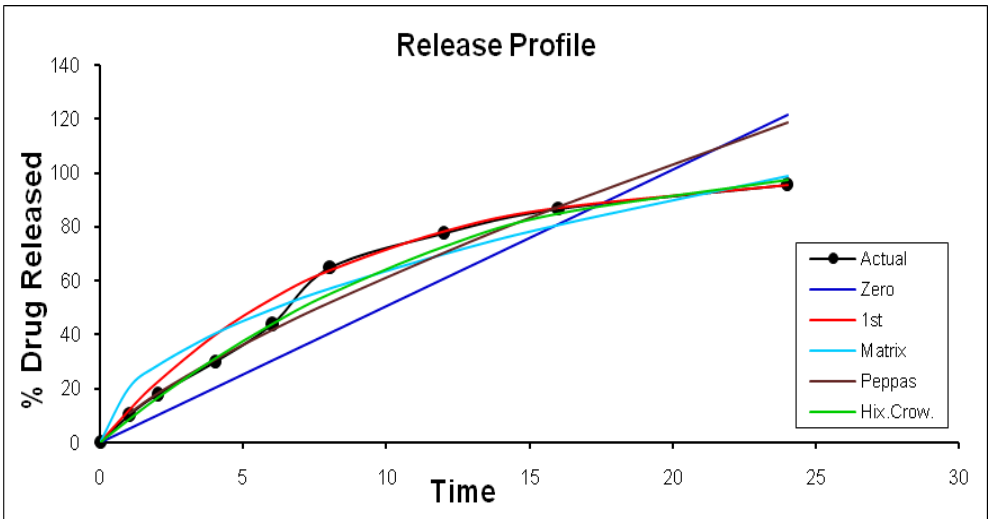

Figure 3: Drug Release (Average) with Model Fitting

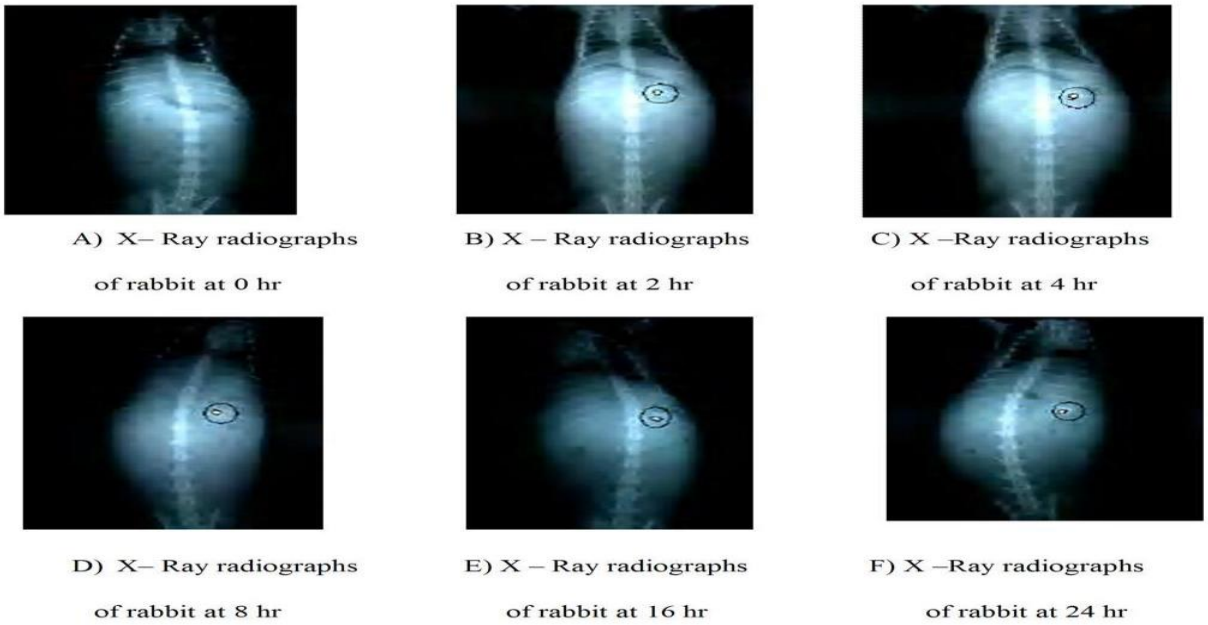

Figure 4: I*n vivo* Radio Imaging Study in Rabbits

***In-vivo* Radio Imaging Study in Rabbits**

Formulations F3 have shown good *in vitro* swelling ability, Dissolution profile and *ex-vivo* retention time in this study. Hence it was selected for *in vivo* x-ray imaging study to establish the product performance (gastric residence time) in rabbits. The optimized formulation was further modified to incorporate barium sulphate as X-ray opaque substance. Replacing drug with barium sulphate made initial formulation trials, while the remaining ingredients stay in the quantities mentioned above. The quantity of incorporated barium sulphate was detectable in X-ray photographs. Photomicrographs were taken immediately after 0, 2, 4, 8, 16 and 24 hrs and are shown in Figure. 4. The presence of Microsphere in the upper small intestine can be clearly noticed and it remains in the stomach not being subjected to disintegration in rabbits. *In vivo* x-ray imaging study clearly indicated that the prepared swelling Microspheres of Sitagliptin retained more than 24 hrs in GIT of rabbit. Photomicrographs was taken immediately after administration of the Microspheres and revealed the nature and position of the Microsphere up to 24 hrs, which also indicates that the bioavailability of the sitagliptin formulation has also been increased as it is retained in the gastric region for a longer period of time and does not pass the hepatic flexure.

***In vivo* Bioavailability Studies**

The mean pharmacokinetic parameters of the tested Microsphere of F-3 batch and conventional Microsphere. The difference of bioavailability between the F-3 Microsphere and reference Microsphere was very significant. Sitagliptin concentration of swellable gastroretentive Microsphere of Sitagliptin was detected till the end of 24 h post-administration, while the plasma concentration of conventional Microsphere of Sitagliptin was detected till only the end of 12 h. This indicated prolonged release and its subsequent in vivo absorption of F-3 Microsphere. In case of conventional sitagliptin

Microsphere, there was a quick absorption and a sharp elimination phase, while in case of swellable gastroretentive Microsphere (F-3), the absorption phase was slow and prolonged.

The absorption of Sitagliptin from conventional Microsphere was rapid; the mean T max was 4 hr, while in the swellable gastroretentive Microsphere of sitagliptin (F-3), the mean T max was 8.0 hr. This showed that the swellable gastroretentive Microsphere of sitagliptin was effective in delaying the peak plasma concentration, thus indicating prolonged plasma concentration of sitagliptin from the swellable gastroretentive Microsphere *in vivo*. Thus, the overall absorption of sitagliptin from the swellable gastroretentive Microsphere was more than its conventional form which shows enhancement of bioavailability of swellable gastroretentive formulation with respect to conventional Microsphere at the same dose. Based on these results, it can be concluded that the greater bioavailability obtained from the swellable gastroretentive drug delivery system and is due to its prolonged gastric residence time. The mean peak plasma concentration (Cmax) of swellable gastroretentive Microsphere was 285 ng/ml in 8hr and that of conventional Microsphere was 297 ng/ml in 4 hr. The mean biological half-life ($t_{1/2}$) of Sitagliptin from swellable gastroretentive Floating Microsphere and conventional Microsphere was 11.05 hr and 4.35 hr, respectively, that is higher than that of conventional dosage form. This indicates that the declining phase of the plasma concentration– time curve involves an input function in addition to the elimination function, i.e., it is not a true elimination phase. The plasma half life values are the same for the same drug substance, regardless of the dosage form. The difference observed here is due to prolonged absorption of the swellable gastroretentive Microspheres; there is continuous introduction of Sitagliptin into the blood stream.

Therefore, the swellable gastroretentive Microsphere shows to have a longer plasma half-life, i.e., the drug stays in the plasma for a longer time than the conventional Microsphere.

Stability Study of Optimized Batch

Stability study was done to see the effect of temperature and humidity on Microspheres. Storage conditions:

1. Accelerated temperature 40˚C ± 2˚C
2. Accelerated temperature at 75% RH ± 5%.

Time period of six months, at intervals of every one month, the Microspheres were visually examined for any physical changes, changes in Hardness, Friability, drug content, swelling index, Mucoadhesion Strength, Mucoadhesion Time, and *in vitro* drug release study. The results indicate no significant change in the Microsphere properties. Hence it can be concluded that the formulated swellable gastroretentive Microspheres are stable under appropriate storage conditions. The results for stability studies are shown in Table 11.

Validation of Results of Dissolution and Swelling Based on Desirability

Individual desirability values were calculated for the respective response Variables. Formulation F-3 was having highest desirability value amongst the designed formulations. Hence it was said to be an optimum formulation.

Overall desirability value is an indicator of optimum formulation as it is calculated from the individual values which in turn and the same are calculated based on the desirable target response.

From graph no. 8,9,10 for Swelling, dissolution and desirability it is clear that the results of Swelling and Dissolution which were obtained from formulation F-3 having HPMC K4M and Psyllium husk in the concentration range of 86.33 to 173.33 and 66.66 to 133.33 mg per Microsphere also showed similar results.(Design expert software version 9, Stat-Ease, Inc). The desirability found was 0.984 which is equal to 1 and hence it can also be concluded that results actually obtained matches with the software prediction and hence the formulation is also validated.

CONCLUSION

The developed gastroretentive Floating Microsphere dosage form showed good *In-vivo* gastric retention capacity for approximately 24 hrs, the dissolution profile of the optimized batch (F-3) also showed release upto 24 hrs which also indicates that the bioavailability of the sitagliptin formulation has also been increased as it is retained in the gastric region for a longer period of time as the formulation does not pass the hepatic flexure.

The formulation remains stable, along with good swelling and mucoadhesive characteristics and drug release after accelerated stability studies and thus it can be concluded that formulation, development and evaluation of swellable gastroretentive Microsphere using polymers like HPMC K4M and Psyllium husk meets the objectives of the study.

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