ORIGINAL ARTICLE

Development Optimization and *In-vivo* Evaluation of Swellable Gastroretentive Tablet by 3^2 Factorial Design

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Abstract

Aim: The present invention preferably relates to formulation and characterization of a swellable gastroretentive tablet comprising pregabalin to enhance its bioavailability and retention time in the stomach. **Materials and Methods:** Tablets were prepared by wet granulation method using hydroxypropyl methylcellulose (HPMC) K4M and Psyllium husk as swelling agents and all other excipients were used of pharmaceutical grade. **Results and Discussions:** The tablet showed mucoadhesion time, force and strength 7.5-12 h, 24-28 g and 2.35-3.62 N, respectively. Swelling index and drug release were found to be 216% and 99.2%, respectively. *In-vivo* imaging study and pharmacokinetic study showed retention of tablet in gastrointestinal tract of rabbit for 24 h and release up to 24 h. The results for stability study were also same as they were at the initial stages of the 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. **Conclusion:** It can be concluded that using polymers such as HPMC K4M and Psyllium husk the objective of this study are meet. The probability value indicates model terms are significant. The probability value, i.e., *P* value found was also <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.

Key words: Bioavailability, desirable, *in-vivo*, probability, swelling

INTRODUCTION

Gastroretentive drug delivery system is the system which is retained in the stomach for a long period and improves the bioavailability of drugs that are preferentially absorbed from upper gastrointestinal tract (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 this study is to develop swellable gastroretentive tablet of pregabalin. For this purpose, a 3^2 full factorial design was applied systematically. Pregabalin is available as an immediate release formulation in capsules and is administered to patient's 2- or 3-time daily (BID or TID). Many patients receiving pregabalin or other drugs, which are, administered 2 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 pregabalin, however, presents numerous challenges. Conventional extended release (ER) compositions are problematic for dosing because pregabalin is not absorbed uniformly in the GIT. Clinical studies indicate that pregabalin is absorbed in the small intestine and the

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Received: 02-01-2016
Revised: 19-03-2016
Accepted: 28-03-2016

ascending colon in humans, but is poorly absorbed beyond the hepatic flexure. This suggests that the mean absorption window for pregabalin is, on average, about 6 h or less and any drug release from a conventional ER dosage form beyond 6 h would thus be wasted because the dosage form has traveled beyond the hepatic flexure.[9] Thus, swellable gastroretentive tablet was formulated of pregabalin 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}). Pregabalin,(S)-3-(aminomethyl)-5-ethylhexanoic acid, is an antiepileptic, used for the treatment of neuropathic pain from post-therapeutic neuralgia and diabetic neuropathy, pregabalin is an anticonvulsant, analgesic and anxiolytic drug.[10,11]

MATERIALS AND METHODS

Materials

Pregabalin and Psyllium husk (Pharmaceutical grade) were obtained as a gift sample from Alkem Laboratories Mumbai (India). Hydroxypropyl methylcellulose (HPMC) K4M, Crospovidone, 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.

Methods

Experimental design

The 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 (X_1) and the amount Psyllium Husk (X_2) were selected as independent variables. Two-factor (X_1, X_2), 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 gastroretentive tablets according to factorial design (3^2) layout is shown in Table 1.

Response surface methodology (RSM) is also widely employed to optimize formulations with the suitable experimental design because it permits a deeper understanding of a process or product and has important applications in establishing the robustness of that product. Full factorial designs that 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 RSM (Design Expert Software Version 9, Stat-Ease, Inc.).

Formulation of swellable gastroretentive drug delivery system (SGRDDS)

The tablets 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 the formulation of tablets of pregabalin; Psyllium husk, HPMC K4M and other additives except magnesium stearate were sifted through mesh (#40). Polythene bag was used for mixing and rotated octagonal to mix content well for 15 min to get uniform mixture.

Prepared granulating fluid by dissolving PVP K30 in isopropyl alcohol and damp mass was prepared. This damp mass was shifted though mess no.12 to prepare granules. Granules were dried at 45°C for 4 h in hot air oven. These dried granules were sized through sieve no. 18 and lubricated by adding 1% magnesium stearate. This blend was then

Table 1: Factorial design formulations of pregabalin tablets prepared by wet granulation method

Ingredients (mg)	Batches								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Pregabalin	150	150	150	150	150	150	150	150	150
Psyllium husk	133.33	200	133.33	133.33	200	200	66.66	66.66	66.66
HPMC K4M	260	260	173.33	86.33	173.33	86.33	173.33	86.33	260
Crospovidone	60	60	60	60	60	60	60	60	60
Polyvinylpyrrolidone	80	80	80	80	80	80	80	80	80
Magnesium stearate	10	10	10	10	10	10	10	10	10
Microcrystalline cellulose	120	120	120	120	120	120	120	120	120
Total	813.33	880	726.66	639.66	793.33	706.33	659.99	572.99	746.66

HPMC: Hydroxypropyl methylcellulose

compressed using 8 stations Karnavati tablet compression machine with semi concave punches of diameter 12 mm.

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°C for 10 min. Then, one vial with a section of tissue was connected to the balance and another vial was fixed on height adjustable pan. To a lower vial a tablet was placed with the help of cello tape. The height of the lower vial was adjusted so that a tablet could adhere to the mucosal tissue on the upper vial. A constant force was applied on the upper vial for 20 s 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 until the two vials just separated from each other. The total weight (g) 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]

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

Ex-vivo mucoadhesive time

The *ex-vivo* mucoadhesion time studies were performed after application of tablets 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 tablet disintegration test apparatus. A side of each tablet was wetted with dissolution medium and was attached to the mucosa by applying a light force with a fingertip for 20 s. The beaker was filled with 900 ml of simulated gastric fluid and kept at 37°C; after 2 min the slide was placed in a beaker and the apparatus was started. Care was taken that while up and down motion of the arm tablet should remain in medium. Behavior and mucoadhesive time of tablet were monitored until complete detachment occurred.[14]

Swelling index

Swelling study of the tablets was carried out 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 tablets 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 tablets was given by the following formula.[15,16]

$$\text{Swelling index } (\%) = \frac{\text{Weight of swollen tablet} - \text{Initial weight of tablet}}{\text{Initial weight of tablet}} \times 100$$

In-vitro dissolution studies

Dissolution profiles of pregabalin from tablets 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 0.1 N HCl, at 37 ± 0.5°C and 50 rpm. Samples (5 ml) were withdrawn with replacement at predetermined time interval of 1, 2, 4, 6, 8, 12, 16, 24 h 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 using UV Spectrophotometer (Shimadzu UV1601).[17]

Drug release kinetics

To investigate the drug release mechanism from tablets, the drug release data was analyzed with following mathematical models and interaction of diffusion release mechanism.

Zero order kinetics:

$$Q = Q_0 - K_0 t \qquad \text{Eq. No. (1)}$$

First order kinetics:

$$Q = Q_0 \left(1 - e^{-K t}_{1}\right) \qquad \text{Eq. No. (2)}$$

Higuchi square root model:

$$Q_t = K_H t^{1/2} \qquad \text{Eq. No. (3)}$$

Hixson-Crowell cube root model:

$$\sqrt[3]{Q_0} - \sqrt[3]{Q_t} = K_{HC} t \qquad \text{Eq. No. (4)}$$

Korsmeyer–Peppas model:

$$Q_t/Q_\infty = K_k t^n \qquad \text{Eq. No. (5)}$$

Where, Q_t = Amount of drug release d at time t,

Q_o = Initial amount of drug.

Moreover, K_o, 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 were 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 R C Patel Institute of Pharmaceutical Education and Research, Shirpur having (Protocol approval No. 651/02/C/CPCSEA/2013-2014) 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 the use of laboratory animals were followed. To evaluate the *in-vivo* residence time, the tablets from the optimized formulation were selected. Four adult male New Zealand white strain rabbits of 3 months age and weighing approximately 2.5-3.0 kg were used for this study. The rabbits were fasted overnight before the start of the study. The tablets excluding drug and containing 18% $BaSO_4$ were manufactured by method as described in the preparation using 12 mm tooling. The tablet was administered through plastic tubing followed by flushing of 25-30 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 h.[22,23]

In-vivo bioavailability studies

An *in-vivo* bioavailability studies protocol was approved by the IAEC of R C Patel Institute of Pharmaceutical Education and Research, Shirpur having (Protocol approval No. 651/02/C/CPCSEA/2013-2014). Male rabbits with weight of 2.5-3 kg were selected. Total four rabbits were divided into two groups. Each group had two rabbits. The animals were housed individually under environment conditions (25°C, 12 h light and dark cycle). The rabbits were fasted overnight and allowed free accesses to water only. Best formulation was administered orally by placing the tablet in a hollow plastic tubing and flushing of 25-30 ml water. Blood samples of 1 ml were withdrawn at specific time interval for tablet. Blood samples were collected from the marginal vein of the rabbit.[24] COLUMN – 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 F3 was selected for *in-vivo* study on the basis of its *in-vitro* results. It was concluded that Pregabalin was released from the F3 tablets 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 tablets 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 ± 2°C/60% RH ± 5% for 12 months.
Accelerated testing: 40 ± 2°C/75% RH ± 5% for 6 months.

Stability studies were carried out at 40°C/75% RH for the selected formulation for 6 months.

The tablets of optimized batch were packed in air tight plastic container. They were then stored at 40°C/75% RH, for 6 months and evaluated for their physical characteristics, mucoadhesive strength, mucoadhesive time, Swelling index, and drug release at a specific interval of time per ICH guidelines. The result obtained from this evaluation test was 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 was found. Overall desirability value is an indicator of the 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 DISCUSSIONS

Tablets were evaluated for physical characters as per IP and the results are shown in shown in Table 2.

Mucoadhesive strength, force of adhesion, and mucoadhesion time

Psyllium husk and HPMC K4M have been reported to possess good mucoadhesive properties. When these polymers come in contact with water forms mucilage and swells, thus are responsible for mucoadhesion by simple bonding with mucus components. Mucoadhesion strength and mucoadhesion force were found good enough (24-28 g and 2.35-3.62 N). Mucoadhesion time was found in the range of 7.5-12 h. Based on the results of mucoadhesion study it can be predicted that gastric retention has been achieved. The results are shown in Table 3.

Swelling index

The swelling of the polymers is studied by their ability to imbibe water and swell enormously. In this study polymers used in the formulation HPMC K4M, Psyllium husk has 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 h. The results are shown in Table 3.

In-vitro dissolution study

The 3^2 full factorial design was constructed to study the effect of the amount of HPMC K4M (X_1) and Psyllium husk (X_2) on drug release from pregabalin tablets. The dependent variables chosen were drug release and swelling index. In our studies, release of pregabalin 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 pregabalin from the tablets 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 F3 showed the best 99.2% drug release, which also indicates that the bioavailability of the pregabalin formulation has also been increased as it is retained in the gastric region for a long period and does not pass the hepatic flexure.

Formulations F1, F2 and F9 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 F2 showed very less drug release, due increased concentration of Psyllium husk. The Formulations F4, F6, and F8 showed increase in drug release from 94.3% to 96.7% due to decrease in concentration of HPMC K4M and Psyllium husk. The formulations F3, F5, and F7 showed drug release from 94.1% to 99.2%, in which the polymer concentration was found optimum in batch F3 which showed drug release up to 99.2%. The batch F5 showed less drug release due to increased concentration of Psyllium husk. The drug release for all the statistically designed batches is shown in Table 4 and Figure 1.

Table 2: Tablet evaluation parameters of sustained release factorial batches

Batches	Weight variation	Hardness kg/cm²	Friability % W/W
F1	16.175±0.02	4.8±0.02	0.44±0.02
F2	17.700±0.01	4.2±0.01	0.56±0.01
F3	13.915±0.02	4.7±0.01	0.47±0.02
F4	12.131±0.02	4.6±0.03	0.48±0.03
F5	15.390±0.01	4.3±0.04	0.58±0.04
F6	13.710±0.04	4.2±0.01	0.59±0.01
F7	12.800±0.03	4.7±0.01	0.42±0.01
F8	11.159±0.01	4.5±0.03	0.44±0.03
F9	15.133±0.02	4.6±0.02	0.40±0.01

Analysis of variance (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

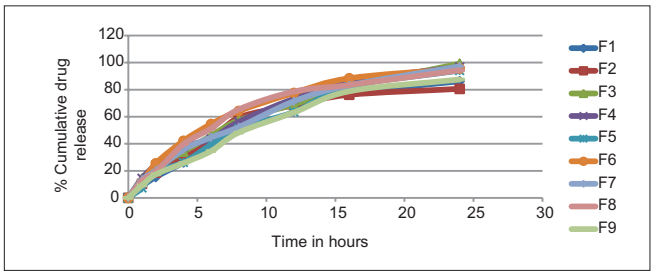

Figure 1: Percent cumulative drug release of all batches F1 to F9

Table 3: Mucoadhesive strength, force, mucoadhesive time and swelling for all the statistically designed batches

Batch code	Mucoadhesive strength (g)	Mucoadhesive force (N)	Mucoadhesive time	% Swelling
F1	28	2.74	More than 10 h	234
F2	33	3.62	More than 12 h	240
F3	26	2.55	More than 9.5 h	216
F4	24.5	2.40	More than 8 h	208
F5	27	2.64	More than 10 h	223
F6	26	2.55	More than 9.5 h	219
F7	25.5	2.50	More than 9 h	204
F8	24	2.35	More than 7.5 h	191
F9	26.5	2.59	More than 9.2 h	226

Table 4: Percent drug release for all the statistical batches

Time (h)	% drug release for all factorial batches								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	8.7	10.4	14.35	14.5	7.4	11.4	13.4	12.8	9.3
2	15.5	18.7	22.72	24.3	19.3	25.6	20.2	19.4	17.4
4	26.2	29.7	34.15	37.7	26.2	42.2	35.7	39.7	25.7
6	38.6	42.0	46.02	43.5	39.4	54.6	44.7	51.3	34.6
8	57.4	59.3	57.3	56.2	51.3	64.2	52.3	65.4	48.3
12	69.2	68.4	69.16	73.3	63.5	77.6	71.4	78.6	63.2
16	77.7	76.2	82.09	86.4	82.4	88.4	83.2	83.4	78.8
24	86.2	80.6	99.21	96.7	94.1	95.6	97.2	94.3	87.5

findings. ANOVA for the dependent variables drug release and swelling index was done. The coefficients of X_1 and X_2 were found to be significant at $P < 0.05$, hence confirmed that both the variables have a significant effect on the selected responses. Overall both the variables caused a 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 three-dimensional (3D) 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 Figures 2-5. Contour plots (Figures 2 and 4) 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. 3D surface plots (Figures 3 and 5) 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 "$P > F$" less than 0.0500 indicate model terms are significant.

The "Predicted R^2" of 0.9537 for drug release and 0.9821 for swelling index is in reasonable agreement with the "Adjusted R^2" 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 probability value, i.e., *P* value found was also <0.0500. This model can be used to develop the design. The values are shown in Tables 5-8.

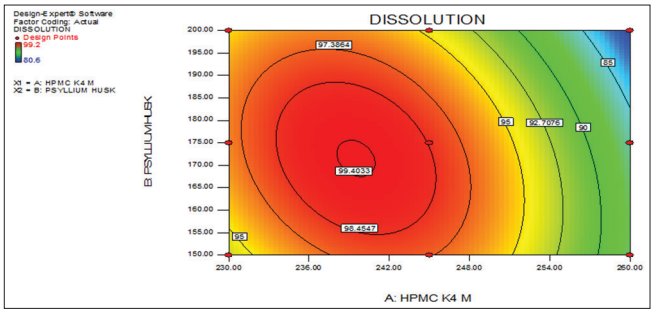

Figure 2: Counter plot of drug release

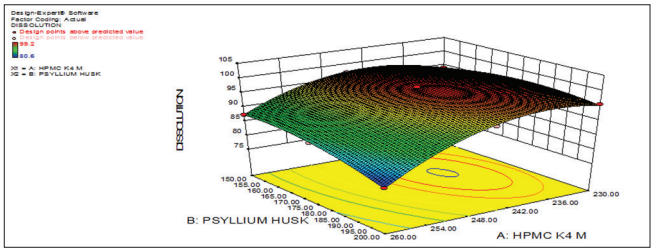

Figure 3: Three-dimensional graph of drug release

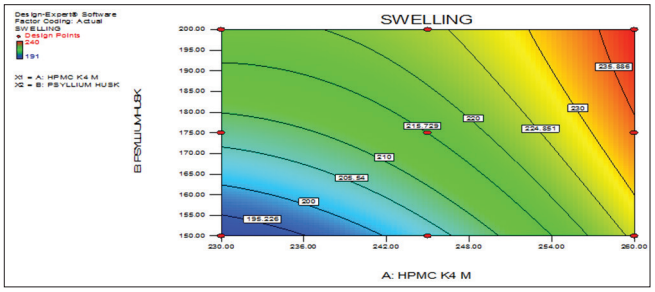

Figure 4: Counter plot of swelling index

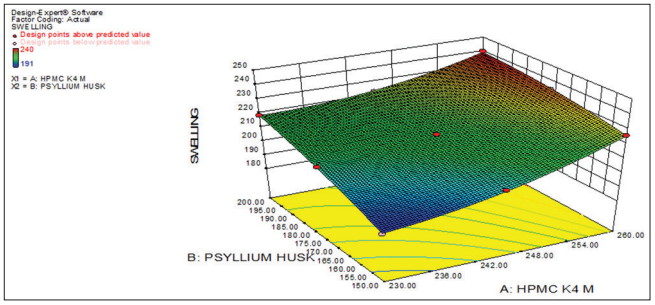

Figure 5: Three-dimensional graph of swelling index

Table 5: ANOVA table for dissolution (partial sum of squares - Type III)

Source	Sum of squares	Df	Mean square	*F* value	*P* value *P*>*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	-	-	-	-

HPMC: Hydroxypropyl methylcellulose, ANOVA: Analysis of variance

Table 6: Standard deviation, mean and R^2 for dissolution

Standard deviation	0.75	R^2	0.9943
Mean	92.30	Adjusted R^2	0.9849
C.V. %	0.82	Predicted R^2	0.9537
PRESS	13.96	Adequate precision	29.120

CV: Coefficient of variation

Response: Dissolution and swelling index

Calculation for effect of formulation variables on drug release and swelling index

Final equation for drug release for 24 h in terms of coded factors:

Dissolution = +98.40 − 5.27 * A − 1.42 * B − 2.10 * A * B − 6.80 * A^2 − 2.35 * B^2

Final equation for drug release for 24 h in terms of actual factors:

Dissolution = −1975.00000 + 15.43778 * HPMC K4M + 2.63133 * Psyllium husk −5.60000E-003 * HPMC K4M *Psyllium husk − 0.030222 * HPMC $K4M^2$ − 3.76000E-003 * $Psyllium\ husk^2$

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 * A^2 − 2.17 * B^2

Final equation for swelling index in terms of actual factors:

Swelling = +837.87037 − 9.07037 * HPMC K4M + 3.90667 * Psyllium husk − 9.33333E-003 * HPMC K4M * Psyllium husk + 0.023704 * HPMC $K4M^2$ − 3.46667E-003 * $Psyllium\ husk^2$

From the equation for dissolution the information conveyed was as follows:

1. R^2 was high indicating the adequate fitting of the quadratic model
2. As HPMC K4M and Psyllium husk (negative coefficient) showed negative sign it also indicated that drug delivery system gained more control over the release of pregabalin 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 (positive coefficient) showed positive 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 X_1 and X_2 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 tablets, 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

Table 7: ANOVA table for swelling index (partial sum of squares - Type III)

Source	Sum of source	Df	Mean squares	*F* value	*P* value *P*>*F*	Observation
Model	1856.11	5	371.22	400.92	0.0002	Significant
A-HPMC K4M	1120.67	1	1120.67	1210.32	<0.0001	Significant
B-Psyllium hush	620.17	1	620.17	669.78	0.0001	Significant
AB	49.00	1	49.00	52.92	0.0054	Significant
A2	56.89	1	56.89	61.44	0.0043	Significant
B2	9.39	1	9.39	10.14	0.0499	Significant
Residual	2.78	3	0.93			
Core total	1858.89	8				

HPMC: Hydroxypropyl methylcellulose, ANOVA: Analysis of variance

Table 8: Standard deviation, mean and R^2 for swelling

Standard deviation	0.96	R^2	0.9985
Mean	217.89	Adjusted R^2	0.9960
C.V. %	0.44	Predicted R^2	0.9821
PRESS	33.34	Adequate precision	60.670

CV: Coefficient of variation

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 F3 was found best among other formulations and n value was found $n = 0.6182$ hence it can be postulated that formulation F3 followed non-Fickian or anomalous release. The results are shown in Table 9 and Figure 6.

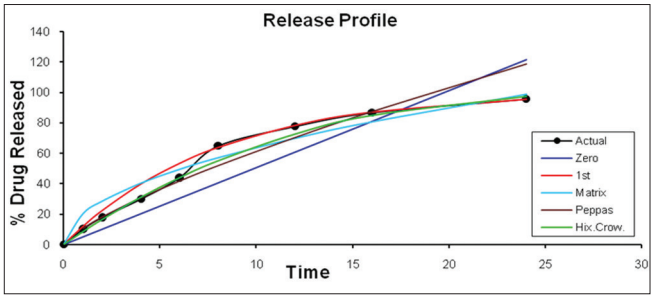

Figure 6: Drug release (average) with model fitting

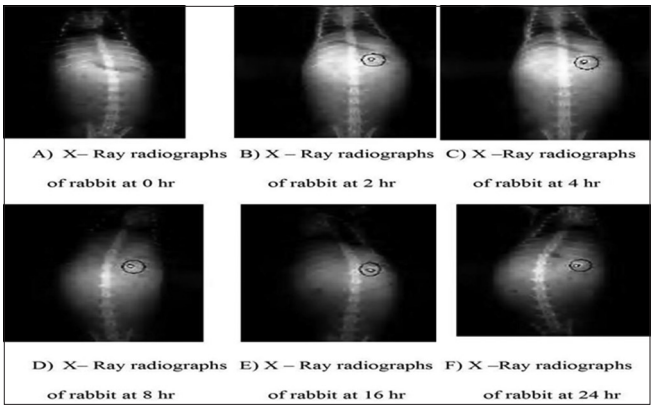

Figure 7: *In-vivo* radio imaging study in rabbits

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 sulfate as X-ray opaque substance. Replacing drug with barium sulfate made initial formulation trials, while the remaining ingredients stay in the quantities mentioned above. The quantity of incorporated barium sulfate was detectable in X-ray photographs. Photomicrographs were taken immediately after 0, 2, 4, 8, 16 and 24 h and are shown in Figure 7. The presence of tablet 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 tablets of pregabalin retained more than 24 h in GIT of rabbit. Photomicrographs were taken immediately after administration of the tablets and revealed the nature and position of the tablet up to 24 h, which also indicates that the bioavailability of the pregabalin formulation has also been increased as it is retained in the gastric region for a long period and does not pass the hepatic flexure.

In-vivo bioavailability studies

The mean pharmacokinetic parameters of the tested tablet of F3 batch and conventional tablet are shown in Table 10. The difference of bioavailability between the F3 tablet and reference tablet was a very significant. Pregabalin concentration of swellable gastroretentive tablet of pregabalin

Table 9: 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
F1	0.8974	5.06	0.9962	–0.126	0.9732	20.11	0.9889	–0.02	0.9876	10.83	0.7535
F2	0.8357	4.95	0.9777	–0.106	0.9763	19.98	0.9478	–0.02	0.9823	13.45	0.6682
F3	0.9067	4.97	0.9248	–0.155	0.9937	19.77	0.9899	–0.03	0.9983	14.77	0.6182
F4	0.8796	5.60	-	-	0.9925	22.41	0.9628	–0.04	0.9951	17.54	0.6016
F5	0.9395	5.22	-	-	0.9781	20.51	0.9417	–0.04	0.9867	10.50	0.7718
F6	0.8180	5.81	-	-	0.9853	23.54	0.9839	–0.05	0.9763	16.54	0.6516
F7	0.9040	5.49	-	-	0.9917	21.86	0.9478	–0.04	0.9968	15.77	0.6333
F8	0.8285	5.68	-	-	0.9822	22.96	0.9709	–0.04	0.9813	16.05	0.6547
F9	0.9332	4.94	0.9796	0.131	0.9718	19.44	0.9971	–0.02	0.9950	11.33	0.7152

was detected until the end of 24 h post-administration, while the plasma concentration of conventional tablet of pregabalin was detected until only the end of 12 h. This indicated prolonged release and its subsequent *in-vivo* absorption of F3 tablet. In the case of conventional pregabalin tablet, there was a quick absorption and a sharp elimination phase, while in the case of swellable gastroretentive tablet (F3); the absorption phase was slow and prolonged.

The absorption of pregabalin from conventional tablet was rapid; the mean T_{max} was 4 h, while in the swellable gastroretentive tablet of pregabalin (F3), the mean T_{max} was 8.0 h. This showed that the swellable gastroretentive tablet of pregabalin was effective in delaying the peak plasma concentration, thus indicating prolonged plasma concentration of pregabalin from the swellable gastroretentive tablet *in-vivo*. Thus, the overall absorption of pregabalin from the swellable gastroretentive tablet was more than its conventional form which shows enhancement of bioavailability of swellable gastroretentive formulation with respect to conventional tablet at the same dose. Based on these results, it can be concluded that the greater bioavailability obtained from the SGRDDS and is due to its prolonged gastric residence time. The mean peak plasma concentration (C_{max}) of swellable gastroretentive tablet was 285 ng/ml in 8 h and that of conventional tablet was 297 ng ml in 4 h. The mean biological half-life ($t_{½}$) of pregabalin from swellable gastroretentive tablet and conventional tablet was 11.05 h and 4.35 h, 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 tablets; there is continuous introduction of pregabalin into the blood stream. Therefore, the swellable gastroretentive tablet shows to have a longer plasma half-life, i.e., the drug stays in the plasma for a longer time than the conventional tablet.

Table 10: Summary of mean pharmacokinetic parameters for pregabalin from swellable gastroretentive tablet and reference (conventional tablet) in healthy rabbits

Pharmacokinetic parameters	Swellable gastroretentive tablet	Conventional tablet
C_{max} (ng/ml)	285	297
T_{max} (h)	8	4
$AUC_{0-\infty}$ (ng.h/ml)	3721	1603
Slope	–6.149	–0.351
$t_{½}$ (hr)	11.05	4.35

AUC: Area under the curve

Stability study of optimized batch

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

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

Time period of 6-month, at intervals of every 1 month, the tablets 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 tablet properties. Hence, it can be concluded that the formulated swellable gastroretentive tablets 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 F3 was having the highest desirability value among the designed formulations. Hence, it was said to be an optimum formulation. Overall

Table 11: Results for stability studies

Number of months	Hardness (kg/cm²)	Friability (%)	(%) Swelling	Mucoad-hesive strength (mg)	Mucoadhesive time (h)	*In-vitro* drug release study (%)
1	4.7±0.01	0.47±0.03	215	27	More than 9.5 h	99.23
2	4.7±0.03	0.47±0.01	216	26	More than 9.5 h	99.20
3	4.6±0.02	0.48±0.02	216	26	More than 9.4 h	99.27
4	4.7±0.03	0.46±0.01	215	27	More than 9.3 h	99.30
5	4.6±0.0	0.48±0.01	217	25	More than 9.3 h	99.23
6	4.7±0.01	0.48±0.02	217	26	More than 9.4 h	99.41

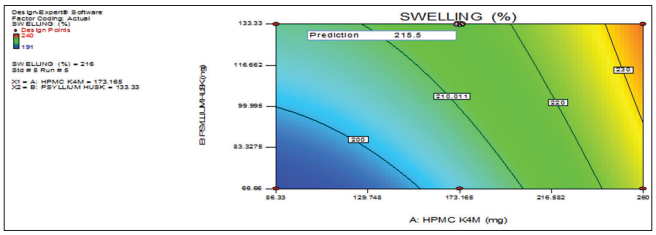

Figure 8: Prediction of swelling

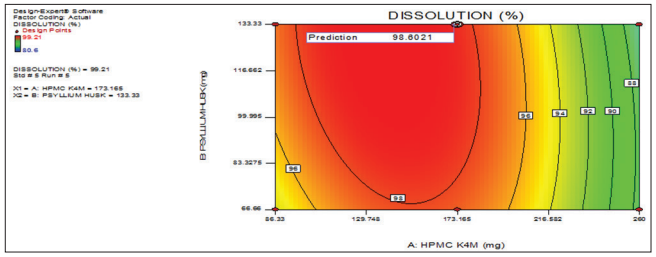

Figure 9: Prediction of dissolution

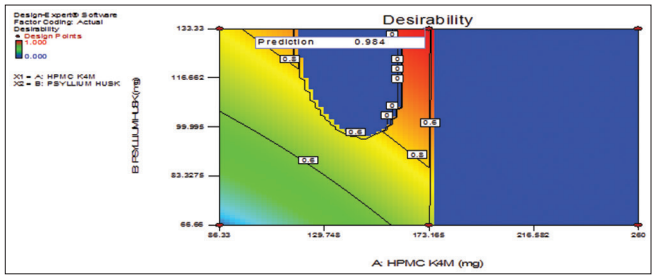

Figure 10: Prediction of desirability

desirability value is an indicator of the 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 Figures 8-10 for swelling, dissolution and desirability it is clear that the results of swelling and dissolution which were obtained from formulation F3 having HPMC K4M and Psyllium husk in the concentration range of 86.33-173.33 and 66.66-133.33 mg per tablet 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 are also validated.

CONCLUSION

The developed swellable gastroretentive tablet dosage form showed good *in-vivo* gastric retention capacity for approximately 24 h, the dissolution profile of the optimized batch (F3) also showed release up to 24 h which also indicates that the bioavailability of the pregabalin formulation has also been increased as it is retained in the gastric region for a long period 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 tablet using polymers such as HPMC K4M and Psyllium husk meets the objectives of the study.

ACKNOWLEDGMENT

I thanks to my guide Dr. S R Lahoti Sir, My Parents Shri Gyandev Narayan Shep and Sau Shiminta Gyandev Shep, My brother Sachya, Satish and Sattkar and wife Manisha.

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Source of Support: Nil. **Conflict of Interest:** None declared.