RESEARCH ARTICLE

Formulation and Evaluation of Multiple Unit Floating Beads of Antiulcer Drug

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Abstract

Aim: The present study was focused on the development of a multiple unit floating (gastroretentive) beads of lafutidine for prolongation of the gastric retention time and increase in absolute bioavailability with enhanced patient compliance in the treatment of peptic ulcer. **Materials and Methods:** Floating beads of lafutidine were prepared using ionotropic gelation method using hydrophilic polymer (hydroxypropyl methylcellulose [HPMC K4M]), gas-forming agents (calcium carbonate), gelling agent (sodium alginate), and crosslinking agent (calcium chloride). 2^3 full factorial designs were applied to optimize the developed formulation. All the formulated beads were subjected to various evaluation parameters such as micromeritics properties, percentage drug entrapment, percentage swelling index, percentage buoyancy, and *in vitro* drug release studies. Calcium chloride, HPMC K4M, and Calcium carbonate were selected as independent variables at two different levels. $t_{80\%}$ was selected as the response variable. Scanning electron microscope (SEM) study was carried out on the optimized formulation. **Results and Discussion:** The optimized formulation remains buoyant for more than 12 h. The *in vitro* drug release results indicated that increasing the concentration of HPMC K4M resulted in sustained effect with long floating duration. LF7 was selected as the optimized formulation. *In vitro* release profile of optimized formulations followed Higuchi model with non-Fickian (anomalous) diffusion. SEM studies showed their spherical shape with perforated smooth surface and cavity inside beads. **Conclusion:** Lafutidine-loaded floating beads were successfully prepared and prove to be useful for the prolonged gastric residence of the drug, better bioavailability, and patient compliance for enhanced anti-ulcer activity.

Key words: Buoyancy, Floating beads, Gastric time, Gastroretentive, Hydroxypropyl methylcellulose, Lafutidine

INTRODUCTION

Conventional oral dosage forms such as tablets, capsules provide a specific drug concentration in systemic circulation which do not release at the constant rate for prolonged period of time. Controlled release drug delivery system (CRDDS) provides drug release at a precontrolled, predictable rate either systematically or locally for intended duration of time and optimizes the therapeutic effect of a drug by controlling its release into the body with lower and less frequent dosing.[1]

Beads are distinct spherical microcapsule that works as the solid substrate on which the drug is coated or encapsulated in the core of beads. Beads can provide controlled release properties. Furthermore, the bioavailability of drugs formulated in beads can be enhanced. Floating beads fulfills the aim of development of gastroretenive drug delivery system is not only to sustain the drug release but also to prolong gastric residence of the dosage forms until all the drug is completely released at the desired period. These multiparticulate dosage forms have many advantages over single unit preparations, including uniform dispersion in the gastrointestinal tract (GIT), uniform drug absorption, less inter- and intra-individual variability, no chances of dose dumping, improve flow property, and more flexible formulation processes.[2,3]

Floating beads are suitable and beneficial for anti-ulcer drugs by improving their absolute bioavailability, therapeutics

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efficiency, increase gastric residence time (GRT), possible reduction of the dose, and improves solubility for drugs that are less soluble in a high pH environment.[4]

Antiulcer drugs especially H_2-receptor antagonist such as lafutidine are used in the treatment of peptic ulcer, duodenal ulcer, stress ulcer, gastroesophageal reflux disease, and Zollinger-Ellison syndrome. Therefore, it was thought to formulate novel drug delivery system of lafutidine in the form of floating beads to release the drug in the stomach for a prolonged period of time.[5,6]

Lafutidine is chemically 2-(furan-2-ylmethylsulfinyl) N-[4-[4-(piperidin-1-ylmethyl) pyridin-2-yl] oxybut-2-en-1-yl] acetamide as shown in Figure 1. It is used as anti-ulcerative agent as it is the new generation H_2 receptor blocker. It is reported to show potent and long-lasting antagonisms of histamine H_2 receptor-mediated effect. It is effective against the esophageal lesions induced by acid reflux through inhibition of acid secretions. The earlier studies suggest that therapy with lafutidine is effective and well tolerated in patients with acid peptic disorders. It is also useful in those patients who were previously not controlled on proton pump inhibitors (PPIs) and first-generation H_2 receptor antagonist [7,8]

Lafutidine has biological half-life (~2–3 h) with site-specific absorption in the upper part of GIT and is also stable in gastric pH. Therefore, this can be formulated in floating beads to enhance absolute bioavailability, achieve an extended gastroretentive time with potential for intragastric drug delivery and local treatment for ulcers in the upper part of GI tract. Therefore, development of controlled/sustained release formulation in the form of multiple units floating drug delivery system such as floating beads would be an ideal approach for oral delivery of lafutidine.[9]

In the present study, floating beads of suitable antiulcer drug such as lafutidine have made and optimized with the aim of increasing the GRT of the formulation thereby giving controlled release in the gastric fluid for treatment of peptic ulcer and improving the oral bioavailability of the drug.

MATERIALS AND METHODS

Pure drug sample of lafutidine with percentage purity 99.60 was supplied by Pure Chem Pharmaceutical Pvt., Ltd., Gujarat, India. Sodium alginate, calcium carbonate, and acetic acid were purchased from Sigma Laboratories, Mumbai. Hydrochloric acid, HPMC K4M and calcium chloride were purchased from Central Drug House, Delhi. All the ingredients used were of pharmaceutical grade. Solvents of analytical grade and double distilled water were used throughout the study.

Preparation of Lafutidine Loaded Floating Beads

Floating beads of lafutidine were prepared by ionotropic gelation method. Eight formulations of floating beads were prepared. Accurately weighed 40 mg of lafutidine was levigated with 10 ml of distilled water containing Tween 20. Sodium alginate (3% w/v) was accurately weighed and dissolved in distilled water using magnetic stirrer. Then, gas-forming agent, i.e. calcium carbonate and HPMC K4M was added to the solution in variable amount as shown in Table 1. The solution containing drug was dispersed in 15 ml of above sodium alginate solution. The resulting solution was dropped through a 26-G syringe needle into 50 ml of calcium chloride solution in variable amount containing 10% v/v acetic acid as shown in Table 1. The beads were formed and allowed to remain in the solution for 1 h to improve the mechanical strength. The formed beads were washed with distilled water for 3 times and dried.[10]

Bulk Density

The floating beads were accurately weighed and then filled in a 10 ml of graduated cylinder and the unsettled level of sample was known as bulk volume (cm^3) which was made in level by dropping cylinder at 2-s intervals onto a hardwood surface 3 times from a height of 1 inch to make the sample in level. The bulk density was obtained by dividing the mass of floating beads by the bulk volume in cm^3. The bulk density was calculated in g/cm^3 by the formula.

Bulk Density = Mass of bead/Bulk volume of the bead

Tapped Density

Tapped density was determined by transferring a known quantity of floating beads (2 g) into a measuring cylinder (10 ml) and was tapped mechanically on a plane hard wooden surface till a constant volume is obtained. That volume was the tapped volume (cm^3), and it includes the true volume of the powder and void space among the floating beads. Tapped density was calculated using the formula:

Tapped density = Weight of powder/Tapped volume of the powder

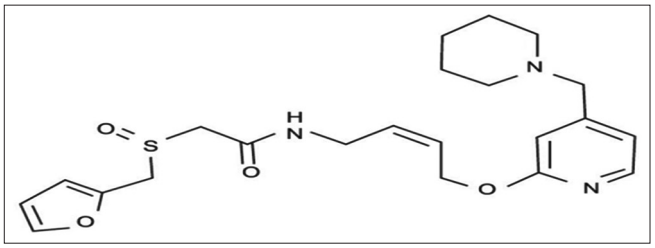

Figure 1: Structure of lafutidine

Hausner's Ratio

Hausner's ratio (packing factor) was calculated as the ratio of tapped bulk density to bulk density before tapping (poured density).

Hausner's ratio = Tapped density/Poured Density

Carr's Compressibility Index

The bulk and tapped densities were used to calculate the Carr's compressibility index.

Carr's compressibility index of each formulation was calculated as follows:

$CI = \rho_t - \rho_a / \rho_t = V_a - V_t / V_t$

Where ρ_t and ρ_a - tapped and poured bulk density; and V_t and V_a - tapped and poured bulk volume respectively.[11]

Determination of Average Size of Floating Beads

The particle size of floating beads of lafutidine was measured using calibrated micrometer attached with an electron microscope to study their shape and size. The beads were placed on a glass slide and observed by a microscope with a magnification of 45×. The slide containing beads was mounted on the stage of the microscope and diameter of at least 100 beads was measured.[12] The mean particle size was calculated, and determination was carried out in triplicate.

Determination of Drug Encapsulation Efficiency [DEE] and Drug Loading [DL]

Accurately weighed 250 mg of prepared beads from each formulation batch were taken separately and were crushed using pestle and mortar. The crushed powder of each batch was placed in 100 ml of 0.1 N HCl (pH 1.20) and kept for 24 h with occasionally shaking at 37 ± 0.5°C. After the stipulated time, the mixture was stirred at 500 rpm for 15 min on a magnetic stirrer. The polymer debris formed after the disintegration of beads was removed by filtering through Whatman filter paper (No.40). Then, the drug content in the filtrate samples was determined using a double beam ultraviolet spectrophotometer by measuring absorbance at 290 nm.[13,14] The percentage DEE of beads was calculated using following formula:

DEE (%) = [(Actual drug content in beads/Theoretical drug content in beads)×100]

DL (%) = [(Amount of drug present/Total weight of Beads)×100]

Swelling Index

The swelling properties of floating beads were determined by placing them in dissolution test apparatus in 900 ml of 0.1N HCl, pH 1.20 at 37 ± 0.5°C for 12 h. Swollen beads were removed periodically from the dissolution medium, and excess water was removed by means of a soft paper and weighed. Swelling characteristics were expressed in terms of percentage swelling index.[15]

Table 1: Composition of floating beads using 2^3 full factorial experimental design

Formulations code	Variable level in the coded form		
	X_1 (calcium chloride)	X_2 (HPMC K4M)	X_3 (calcium carbonate)
LF1	–	–	–
LF2	+	–	–
LF3	–	+	–
LF4	+	+	–
LF5	–	–	+
LF6	+	–	+
LF7	–	+	+
LF8	+	+	+
Concentration/value of independent variables			
Level	**Concentration of calcium chloride (g)**	**Concentration of HPMC K4M (g)**	**Concentration of calcium carbonate (g)**
–	7.5	0.166	1.5
+	15	0.332	3.0
Response variable			
Y ($t_{80\%}$)	Time of 80% of drug release ($t_{80\%}$)		

HPMC: Hydroxypropyl methylcellulose

$$\%\,Swelling\ Index = \frac{[(Weight\ of\ swollen\ beads - weight\ of\ dried\ beads) \times 100]}{[weight\ of\ dried\ beads]}$$

Floating Properties

In vitro buoyancy

The floating properties of beads were evaluated in a dissolution vessel (USP Type II dissolution tester) containing 900 ml of 0.1 N HCl, pH 1.20. Few beads (fifty numbers of beads) were placed in testing medium. Paddle rotation speed of dissolution tester was 50 revolutions per minute at 37 ± 0.5°C. The buoyancy of floating beads was seen by visual observation. Percentage buoyancy was calculated for each formulation batch.

% Buoyancy = [Number of floating beads/Total number of beads]×100

Floating lag time

Floating lag time was determined by weighing few mg of beads into dissolution vessel containing 900 ml of 0.1 N HCl, pH 1.20 at 37 ± 0.5°C. Time taken by the experimentally designed beads formulation to emerge on surface of dissolution medium was noted and referred as floating (or buoyancy) lag time.

Total floating duration

The time taken by the floating beads to float constantly on the surface of the simulated gastric fluid at pH 1.20, temperature 37 ± 0.5°C, paddle rotation at 50 rpm was measured using stopwatch.[16]

In Vitro Dissolution Study

In vitro dissolution study of floating beads equivalent to 40 mg lafutidine was carried out using USP basket type dissolution test apparatus. Floating beads equivalent to 40 mg of lafutidine were filled into hard gelatin capsules (HGC) of size 00. Dissolution study was carried out in 900 ml of 0.1 N HCl, pH 1.20 as dissolution medium. The dissolution medium was maintained at a temperature of 37 ± 0.5°C and rotation speed was kept at 50 rpm. At predetermined time intervals, i.e., 0, 0.5, 1, 2, 3, 4, 5, 6, 9, and 12 h 5 ml of sample was withdrawn from the dissolution apparatus and replaced with its equivalent volume of fresh dissolution medium to maintain sink condition. The withdrawn aliquots were filtered and assayed at 290 nm. The study was carried out in triplicate. Dissolution time required for 80% of the drug release ($t_{80\%}$) as response variable was calculated from the dissolutions test results.[17]

Optimization and Validation of Experimental Design

The runs or formulations, which are designed based on 2^3 full factorial designs, are evaluated for the response variables. The response values are subjected to multiple regression analysis to find out the relationship between the factors used and the response values obtained. Independent variables or factors studied were a concentration of HPMC K4M (X_1), $CaCl_2$ (X_2), and $CaCO_3$ (X_3). The response values or dependent variables subjected for this study were time of 80% of drug release ($t_{80\%}$) from floating beads. The effect of formulation variables on the response variables was statically evaluated using a commercially available software package Design Expert® 10.0.3 (Stat-Ease, USA). The optimization procedure was facilitated by the construction of a polynomial equation that describes the experimental results as a function of the effects and interactions of the factors. The polynomial equation provides sufficient data to fit in the following form:[18]

$$Y=B0+B1X1+B2X2+B3X3+B12(X1X2)+B13(X1X3)+B23(X2X3)+B123(X1X2X3)$$

Where, Y represents the experimental response, B_0 the interceptor the arithmetic mean response of 8 runs and B_1 to B_{123} represents response coefficients computed from responses of the formulations in the design. The student *t*-test was conducted to examine the probability of each coefficient being equal to zero. All tests were performed at a 95% level of significance. In the final reduced model of polynomial equation, only the significant factors and coefficients were included. The reduced polynomial equation was applied to predict the theoretical responses (predicted responses). Once the predicted response was established, the extra design checkpoint formulation after transformation was formulated and evaluated to validate the polynomial equation model.[19]

In general, the formula for transformation is as follow.[20]

$$\frac{"X - the\ average\ of\ the\ three\ levels"}{"\frac{1}{2}of\ the\ difference\ of\ the\ levels"}$$

The values after transformation of each of three variables at two levels were incorporated into the extra design checkpoint formulation designated as LF9. The study was conducted in triplicate to determine $t_{80\%}$ as response variable. Closeness between the experimental and extra design checkpoint formulation value of the responses is also presented in terms of similarity factor (f_2).

The similarity factor (f_2) is a measure of closeness or similarity of two dissolution profiles. The similarity factor (f_2) was checked for floating beads formulations (LF1–LF8) with respect to extra design checkpoint LF9 formulation. The best formulation (optimized formulation) was selected based on the highest similarity factor value (f_2) between

experimental design batch formulations (LF1–LF8) and extra design checkpoint batch (LF9). Similarity factor was calculated by the following equation:

$$f_2 = 50 \times \log \{[1+(1/n)\Sigma^{n}_{t=1}(R_t-T_t)^2]^{-0.5} * 100\}$$

Where, R_t and T_t are the cumulative percentage dissolved at each of the selected n time points of the reference and test product, respectively.

To demonstrate graphically the influence of each factor on responses and to indicate the optimum level of factors, the contour and response surface plots were generated using design-expert 10.0.3 software as shown in Figure 4. Suitable response models for the responses were selected based on the fit summaries.[21,22]

Drug Release Kinetics of Optimized Formulation

In vitro drug release data of optimized formulation (LF7) were fitted to various kinetic equations such as zero-order, first-order, and Higuchi and Peppas model to find out the mechanism of drug release from floating beads of lafutidine. All curve fitting and plotting were performed using Microsoft Excel software, and regression coefficient (r^2) values were calculated. In this by comparing the regression coefficient (r^2) values obtained, the best fit model was selected.[23-27]

Scanning Electron Microscopy (SEM) of Floating Beads

The surface characteristics and internal textures of the lafutidine loaded floating beads were studied by SEM. SEM studies were carried out before and after different time intervals of dissolution to determine the structure which is responsible for floating of the beads. Samples of floating beads for the SEM analysis were prepared by sprinkling the beads one side of double adhesive stub. The stub was then coated with gold using Jeol JFC 1100 sputter coater. SEM analysis of the floating beads was carried out. The floating beads were viewed at an accelerating voltage of 15 Kv at different magnifications. Photomicrographs were captured randomly using scanning electron microscope.[28]

Stability Studies

Stability studies were carried out as per the "ICH Q_1A stability testing guidelines." The optimized formulation LF7 was subjected to 40°C ± 2.0°C/75% RH ± 5% RH for 6 months and the samples were evaluated for floating study, *in vitro* drug release study, and percentage DEE. The sampling intervals were 0, 1, 2, 3, and 6 months.[10,29]

RESULTS AND DISCUSSION

Preparation of Lafutidine Loaded Floating Beads

Floating beads of lafutidine were prepared using ionotropic gelation methods discussed in preparation of lafutidine loaded floating beads. Eight formulations of floating beads (LF1–LF8) were prepared as per 2^3full factorial experimental design as shown in Table 1. The amount of calcium chloride (X_1), amount of HPMC K 4M (X_2), and amount of calcium carbonate (X_3) were selected as independent variables. High and low levels of each factor were coded as +1 and –1, respectively. The response parameter was chosen as a time of 80% of drug release ($t_{80\%}$).

The benefits of preparation technique include low processing time, lack of exposure of drug to high temperature due to which stability of drug increased during the processing leading to high percentage entrapment efficiency of the drug in floating beads.

Bulk Density

The result of bulk density (g/cm^3) ranged from 0.400 ± 0.0009 to 0.505 ± 0.0039 as shown in Table 2. Bulk density of different formulations of beads was found to be much less than the density of the gastric fluid (1.004 g/ml) and 0.1 N HCl, pH 1.20 (0.997 g/ml). The size of HGC was selected 00 according to bulk density determination of floating beads. The low density of beads increased the porosity and indicates good packing capacity of beads. Being less in density, the beads were expected to float immediately with less or no floating lag time. The bulk density of the hollow beads with calcium carbonate was less as compared with the beads without calcium carbonate. The higher amount of effervescent agent caused faster and higher CO_2 generation. This may be attributed to a decrease in the bulk density.

Tapped Density

The tapped density of floating beads of all formulation was found to be in the range of 0.437 ± 0.0003–0.540 ± 0.0053 g/cm^3 as tabulated in Table 2. Therefore, it was expected to be suitable for formulation of floating beads as they were having less density than 0.1 N HCl, pH 1.20. Values of tapped density also have shown good packability of beads.

Carr's Compressibility Index

Carr's compressibility index of floating beads of all eight formulations ranged from 6.10 ± 0.009 to 11.18 ± 0.003% indicating excellent compressibility of beads. Therefore, floating beads shown good packability inside the capsules with ease of filling the beads.

Hausner's Ratio

Hausner's Ratio for all eight formulations was in the range of 0.90–1.12 (<1.25) indicating good flow properties of floating beads as tabulated in Table 2.

Determination of Average Size of Floating Beads

The mean particle sizes of all formulations were ranged from 0.826 to 1.360 as shown in Table 3. Higher particle size was obtained when the proportion of HPMC K4M was increased in polymer mixture of sodium alginate: HPMC K4M. Increase in particle size diameter was also due to increase in the concentration of calcium carbonate as gas forming agent. As the amount of calcium chloride was increased, more crosslinking structure was observed that lead to a decrease in particle size.

Determination of DEE and DL

The DEE and DL of all eight formulations were found to be 39.06%–57.09% and 7.81%–14.12% as shown in Table 3. The percentage drug entrapment efficiency was more when the concentration of polymer was increased in the sodium alginate: Polymer ratio. Encapsulation efficiency was found to be increased with the increase in the concentration of gelatin solution (calcium chloride) due to crosslinking structure. DEE of some formulation was low due to high porosity ($CaCO_3$) because of leakage of the drug.

Swelling Index

Swelling index of all eight formulations was shown in Figure 2. It was found that with an increase in the concentration of HPMC K4M, swelling of beads was also increased, but the rate of drug release was found to slow down. It was due to hydration of bead when it came in contact with water due to close proximity of hydrophilic groups. The swelling of floating beads in release media ensured that beads have high GRT and do not pass through the pyloric sphincter. Swelling index is generally essential to ensure floating of beads.

Table 2: Micromeritics properties of floating beads in comparison with pure drug

Formulation code	Bulk density (g/cm³)*	Tapped density (g/cm³)*	Carr's compressibility index (%)*	Hausner's ratio
Pure drug	0.169±0.0057	0.19±0.0057	11.05±0.12	1.124
LF1	0.460±0.0002	0.500±0.0045	8.00±0.004	1.08
LF2	0.400±0.0009	0.437±0.0003	8.46±0.005	1.09
LF3	0.477±0.0023	0.508±0.0002	6.10±0.009	1.06
LF4	0.421±0.0045	0.457±0.0055	7.89±0.007	1.08
LF5	0.450±0.0001	0.506±0.0056	11.18±0.003	1.12
LF6	0.480±0.0057	0.531±0.0045	9.60±0.002	1.10
LF7	0.505±0.0039	0.540±0.0053	6.48±0.008	1.06
LF8	0.400±0.0057	0.440±0.0005	9.09±0.007	0.90

*All values are expressed as mean ± SD., n =3. SD: Standard deviation

Table 3: Comparative table of particle size, % DEE, and % DL of different formulations

Formulation code	Particle size (mm)	DEE (%)	DL (%)
LF1	0.980	47.36	11.10
LF2	0.826	54.15	13.10
LF3	1.251	51.20	12.90
LF4	0.922	57.09	14.12
LF5	1.191	39.06	7.81
LF6	0.900	48.95	10.68
LF7	1.360	46.65	10.25
LF8	1.080	52.50	10.92

DEE: Drug encapsulation efficiency, DL: Drug loading

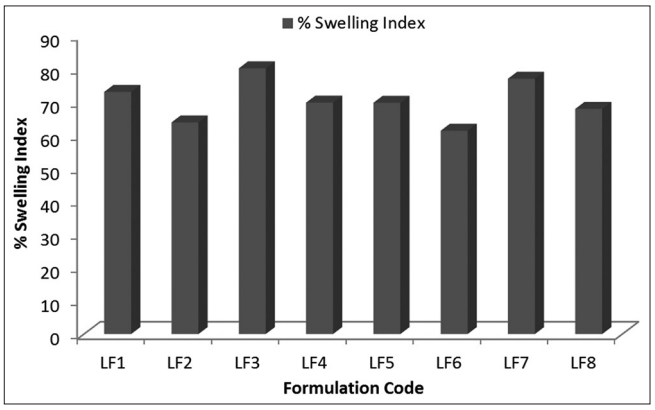

Figure 2: Comparative histogram of percentage swelling index of different formulations

Table 4: Comparative table of floating time of different formulations

Formulation code	Average floating duration (hours)	Average lag time (seconds)	% buoyancy
LF1	6.3±0.099	31±3.2	44
LF2	6.1±0.200	32±2.6	43
LF3	8.9±0.360	9±0.28	55
LF4	8.8±0.115	11±0.76	54
LF5	10.3±0.208	4±0.057	59
LF6	10.2±0.057	7±0.804	58
LF7	12.6±0.057	3±0.25	82
LF8	12±0.057	6±0.28	79

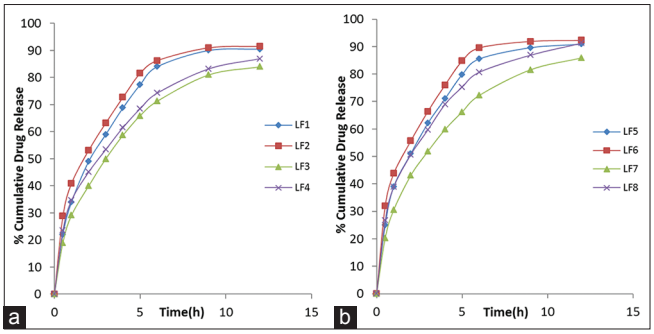

Figure 3: *In vitro* drug release profile of floating beads for (a) LF1–LF4 (b) LF5–LF8

Floating Properties

In vitro buoyancy

In vitro buoyancy study shown that incorporation of high concentration of calcium carbonate helped in floating properties when it comes in contact with aqueous fluid, produce carbon dioxide gas which reduces the density of dosage form due to the entrapment of CO_2 gas in hydrophilic matrices. Formulation containing a high concentration of hydrophilic polymer (HPMC K4M) and calcium carbonate as in case of LF7 shown high percentage buoyancy of 82% as compared to others.

Floating lag time

Floating lag time study shown that all the batches from LF1 to LF8 had taken <32 s to float or emerge on the surface of dissolution medium (0.1 N HCl and pH 1.20). It was observed that floating ability increased with increasing average particle size of beads, for example, the particle size of LF7 was bigger and had taken the lowest lag time as shown in Table 4.

Total floating duration

Total floating time for all batches LF1–LF8 was tabulated in Table 4. The reaction of calcium carbonate with the solution of acetic acid and $CaCl_2$ (dispersion medium) made the beads porous due to which it floats. On the other hand, HPMC K4M subsequently increased the floating time. Beads remained afloat for long time in the medium studied. This phenomenon might be due to the outermost hydrophilic colloid (HPMC) which on contact with an acidic medium hydrated to form an outside gel barrier that acquired and maintained a bulk density of <1 thereby being buoyant on the medium. Formulations with a high amount of HPMC led to increased floating duration.

In vitro Dissolution Study

In vitro dissolution study of lafutidine from floating beads was performed in 0.1 N HCl (pH 1.20) for 12 h using USP basket type dissolution test apparatus. Release profile shown initial burst release up to 1 h due to the surface associated drug, followed by a sustained release phase as the entrapped drug slowly diffused into the dissolution medium. There was the sustained release of drug at a constant rate. The results of study shown as the concentration of HPMC K4M was increased, it decreased drug release from floating beads and $CaCO_3$ was responsible for fast release of the drug (burst release). The *in vitro* drug release studies revealed that the formulation having less concentration of $CaCl_2$ and more concentration of HPMC K4M made the swollen beads, which ensured floating and slow diffusion of lafutidine from floating beads, for example, LF7 containing maximum concentration of HPMC K4M and low concentration of calcium chloride with fixed concentration of sodium alginate shown sustained drug release of 85.9 ± 0.021% at the end of 12 h [Figure 3]. Sodium alginate itself released in a slow manner and has main role in entrapment of drug due to which it also lead information of sustained release floating beads. The response variables ($t_{80\%}$) of different formulations were calculated from *in vitro* dissolution profiles to characterize the drug release rate from the floating beads.

Optimization and Validation of Experimental Design

The eight formulations of floating beads were prepared to study the effect of concentration of calcium chloride ($CaCl_2$), hypromellose (HPMC K4M), and calcium carbonate ($CaCO_3$) as formulation variables on time of 80% of drug

release ($t_{80\%}$). The effect of formulation variables on the response variables was statistically evaluated by applying ANOVA at 0.05 level using a commercially available software package Design Expert® 10.0.3 (Stat-Ease, USA). Mathematical polynomial cubic equations were generated for dependent variables or response parameter such as Y ($t_{80\%}$) of drug release. The mathematical models were tested for significance. The response values are subjected to multiple regression analysis to find out the relationship between the factors used and the response value obtained. Quadratic model was selected as best fit models to determine the effect of independent variables on $t_{80\%}$ as the response variable. As there were insignificant terms, the model reduction was required. After reduction, reduce cubic model was used and thus the data points were better fitted with the model and all the response models were significant with the response parameters. It was found that quadratic model is best fitted to determine the effect of independent variables on response variables. Since response model was significant, the adjusted and predicted r^2 of response model were in good agreements. Final polynomial equations of response variables in terms of coded coefficients of the formulation parameters were obtained as shown below:

$$Y(t_{80\%}) = 6.32–0.50X_1+1.30X_2–0.42X_3$$

Where X_1, X_2, and X_3 represent the coded values of the $CaCl_2$, HPMC K4M, and $CaCO_3$, respectively. Both the magnitude and sign of coefficients are important. The magnitude implies the strength whereas the sign indicates the direction of that factor variable on the corresponding response variable. The positive value of a factor in the above equations points outs the enhancement of that response and vice versa. In this study, as evidenced from Eq. response was affected positively by the percentage of HPMC K4M and negatively by the percentage of the $CaCl_2$ and $CaCO_3$ which means as the HPMC K4M increased, the release of drug was slow and sustained in manner and time taken for 80% drug release was increased.

To demonstrate graphically the influence of each factor on response and to indicate the optimum level of factors, the contour and response surface plots were generated using Design-Expert 10.03 software. Extra design checkpoint was calculated, and levels of three independent variables were found to be 13.125 g of $CaCl_2$, 0.2685 g of HPMC K4M, and 2.625 g of $CaCO_3$. Other excipients were remained same as other formulations. Then extra design checkpoint formulation (LF9) was further evaluated for *in vitro* drug release to check the similarity factor (f_2) between formulations. LF7 shown highest similarity factor (f_2) of 98% among all other formulations in the study with extra design checkpoint formulation (LF9); therefore, LF7 was selected as optimized formulation batch. The statistical insignificance of the observed values for extra design checkpoint formulation (LF9) was evaluated with the predicted value using Student's ttest. It was found to non-significant with 95% confidence interval.

Drug release Kinetics of Optimized Formulation

The dissolution profiles of best selected (optimized formulation) LF7 was fitted to different equations and kinetic models to explain the release kinetics of drug from floating beads. The kinetic treatment of the drug release data was used as an indicator for the release mechanism from matrix delivery systems. In this study, the *in vitro* drug release data were fitted to four commonly employed release kinetic models, namely zero-order, first-order, and Higuchi and Peppas models to analyze drug release mechanism from the polymeric system as shown in Figure 5a and b. The highest regression coefficient (r^2) value was obtained for Higuchi model (0.9792) followed by Korsmeyer– Peppas (0.9779), zero-order (0.8177), and first-order (0.9706) model using Microsoft Excel software. It indicates diffusion to be the predominant mechanism of drug release from floating beads. The value of diffusion exponent (n) was found to be 0.4203 that indicates Fickian diffusion-based mechanism of drug release which leads to the conclusion that prepared floating beads were spherical in shape. Zero-order, first-order, Higuchi, and Korsmeyer–Peppas plots were shown in Figure 5 (a-d).

Scanning Electron Microscopy of Floating Beads

SEM photograph revealed that floating beads had a spherical shape with the smooth perforated surface. The outer surface

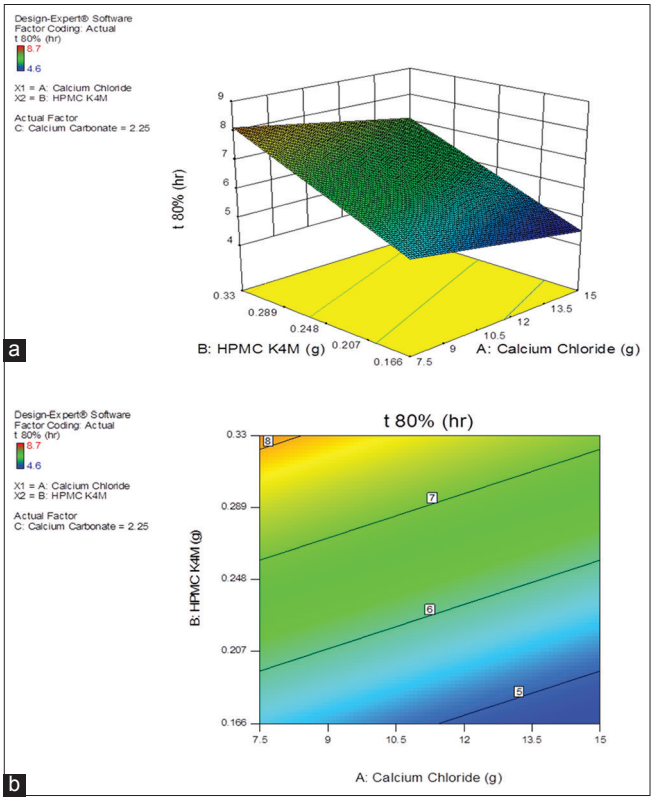

Figure 4: (a) Response surface plot of the effect of X1, X2, and X3 on time of 80% of drug release, (b) Contour plot (2 D) of the effect of X1, X2 and X3 on time of 80% of drug release

of beads was observed to be smooth, dense and less porous, whereas the internal surface was highly porous. The less porous outer surface and a highly porous internal surface supported controlled release of drug from the floating beads with good buoyancy. The formation of perforation may be due to the release of carbon dioxide. The porous nature and cavity formed would dictate the floating behavior of beads. SEM studies confirmed the presence of matrix structure, pores before and after the dissolution of the floating beads as shown in Figure 6. Differences in shape and structure of beads were seen before and after dissolution. As the time of dissolution increased, more pores and cracks were observed in the matrix structure of beads. SEM study also confirmed that these matrix beads followed non-Fickian diffusion because cracks were formed and could be seen in Figure 6b and c.

Stability Studies

The stability studies of optimized formulation batch LF7 were carried out in accordance to ICH Q_1A guidelines for 6 months to investigate the influence of humidity and temperature on floating time, *in vitro* drug release ($t_{80\%}$) and DEE. The sampling intervals were 0, 1, 2, 3, and 6 months. The results shown in Table 5 revealed that the formulation LF7 was chemically stable when stored in a closed container at 40°C ± 2.0/75% RH ±5 as the floating time, DEE, and drug release profile did not differ significantly till the end of 6 months.

Table 5: Compiled data for stability testing of optimized formulation (LF7)

Parameters studied	Time interval (months)				
	0	1	2	3	6
Floating time (h)	12.26	12.32	12.21	12.19	12.21
$t_{80\%}$ (h)	7.91	7.90	7.89	7.91	7.87
DEE (%)	46.65	46.66	46.32	46.29	46.24

DEE: Drug encapsulation efficiency

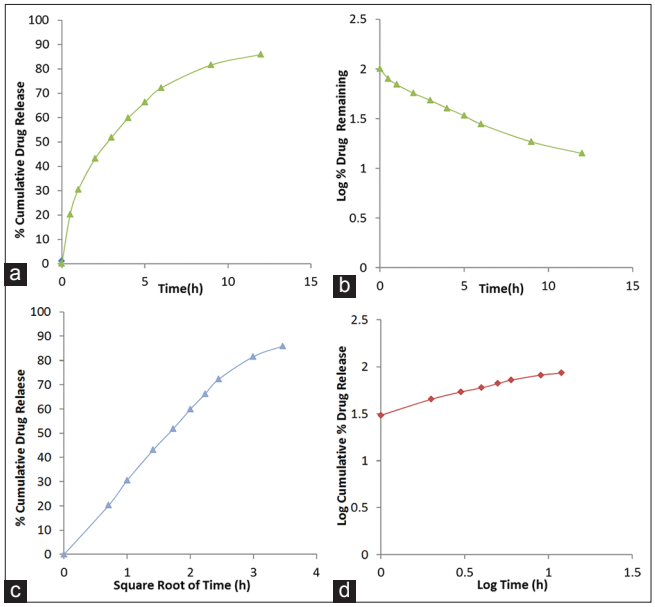

Figure 5: Drug release kinetic profile of optimized formulation LF7 (a) zero-order, (b) first-order, (c) Higuchi, (d) Korsmeyer–Peppas

CONCLUSION

Multiple unit floating beads of lafutidine with swellable hydrophilic polymer, gas-forming agent, and crosslinking agent were successfully prepared using ionotropic gelation method, by applying 2^3 factorial designs. The prepared beads had a different size and the percentage entrapment efficiency of the drug by varying the formulation variables such as polymeric concentration gas forming agent and crosslinking agent. The prepared formulations were further evaluated for micromeritic properties, particle size, percentage entrapment efficiency, percentage buoyancy, *in vitro* dissolution study, and swelling study. As the amount of polymer (HPMC K4M) increased, the drug release rate was decreased, and as the concentration of gas forming agent ($CaCO_3$) increased,

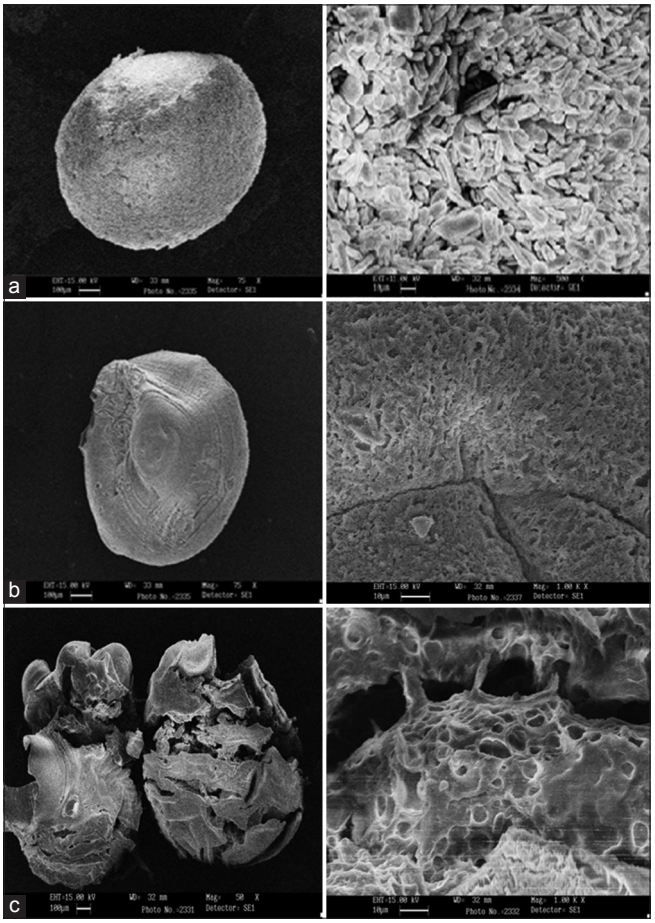

Figure 6: Scanning electron microscope photographs of the external surface and internal surface morphology (a) before dissolution (b) after 2 h of dissolution (c) after 10 h of dissolution

and the drug release rate was increased. Based on the result of dissolution study LF7 was chosen as the optimized formulation. Optimized formulation (LF7) followed Higuchi kinetics, and the release mechanism was non-Fickian diffusion ($n = 0.4956$). SEM study showed porous nature and matrix structure in the beads. The prepared floating beads were able to retain in the stomach for a prolonged period of time with the sustained release of lafutidine. Thus, floating beads of lafutidine prove to be useful for the prolonged gastric residence of the drug, sustained release, better bioavailability, enhanced patient compliance, and anti-ulcer activity.

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