Development And Evaluation of In-Situ Floating Gel in Treatment of Gastroesophageal Reflux Disease (Gerd)

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Abstract

Gastroretentive drug delivery systems can deliver narrow-window drugs to the upper stomach at a controlled rate. The raft forming process is better than floating structures, liquid dosing mediums, and in situ gelation for sustained drug release in the upper GI tract. According to statistics, lafutidine is exactly put in the parietal cells of the stomach mucosa, where the upper gastro-intestinal tract absorbs it. Lafutidine capsules had 65–70% bioavailability compared to intravenous infusion. Stability and absorption are acceptable between 1 and 4. The colon and lower gastrointestinal tract degenerate. In this study, the formulations were changed into gel for simulated gastric fluid (pH 1.2) and floated in the gastrointestinal environment, releasing medication for 12 hours. The finished device was tested. UV was linear from 5–30 g/ml with a regression value of 0.999. IR and DSC demonstrated negligible interaction between chemical, polymers, and excipients. Gelling polymers and calcium chloride were complexed with two xyloglucan polymers, gellan gum, and in situ gelling formulations. Xyloglucan was the best polymer for rheological tests, medication content, mucoadhesive strength, and gel strength. Lafutidine's solution formulae X10 and G10 did not improve in appearance, drug content, or dissolving profile after three months at 40°C and 75% RH. Wistar rats tested the in situ optimized Lafutidine gelling (X10, G10) formula. The ulcer index, stomach acid volume (ml), acidity (mEq / l / g), and pH were significantly affected by a single-way ANOVA solution and Tucky's multiples comparison test. The current work successfully developed, tested, and produced an oral continuous release floating Lafutidine gelling device with stomach-specific drug delivery, maintaining drug release and gastric preservation for the required time.

Key Words: In-Situ Floating Gel, Gastroesophageal Reflux Disease (GERD), Lafutidine

DOI Number: 10.14704/nq.2021.19.11.NQ21238 **NeuroQuantology 2022; 19(11):356-374**

Introduction

Low-density floating systems can float over stomach contents for a long time without effecting gastric emptying. The mechanism gently releases medication while floating on gastric contents.[1] Floating system increases stomach retention and plasma medication concentration management. The buoyancy retention concept requires minimal stomach content and floating force (F) to keep the dose form reliably buoyant on the meal. Gelling agents provide a stable sol/suspension system to hold dispersed medication and excipients in gastro retentive in situ gel systems. Ionic complexation caused by pH shift gels this sol/suspension system in the stomach [2]. Gastric contents involuntarily enter the esophagus in gastroesophageal reflux. Most healthy people experience gastroesophageal reflux numerous times a day without symptoms or injury. Reflux of stomach contents into the esophagus causes frequent or severe symptoms that might harm the esophagus, pharynx, or respiratory tract.[3] Lafutidine is a unique second-generation histamine H2-receptor antagonist. It is absorbed in the small intestine, circulates to gastric cells, and immediately binds to gastric cell histamine H2 receptors to block stomach acid release. Lafutidine treats stomach ulcers, duodenal ulcers, and acute gastritis mucosal lesions. Lafutidine binds to H2 receptors 2-80 times better than famotidine, ranitidine, and cimetidine [4].

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Lafutidine's short biological half-life and limited absorption require regular doses. Thus, gastroretentive drug delivery of lafutidine in oral in situ gel increases drug residence time and release time, reducing dosing frequency and increasing bioavailability.[4]

Materials And Methods

Materials

Lafutidine was obtained from Pure Chem Pvt. Ltd., Gujrat, India. All other chemicals obtained from local suppliers.

Methods

Characterization of Lafutidine [5-8]

1. Organoleptic properties

The sample of Lafutidine was studied for organoleptic characters such as color, odor and appearance.

2. Melting point

The melting point of lafutidine was found using melting point apparatus. For the purpose of determining the m.p., the drug was injected into a glass capillary with a flame-sealed aperture. The capillary containing the drug was submerged in liquid paraffin inside the magnetic stirring m.p. apparatus.

3. FTIR Spectroscopy

Lafutidine dry sample and IR grade KBr were mixed at a ratio of 1:100. This mixture was compressed into a pellet by applying 10 tonnes of pressure with a hydraulic press. A wave number range of 4000 to 400 cm-1 was used to scan the pellets. An FTIR instrument was used to conduct the spectral study.

4. Differential scanning calorimetry studies

The thermal examination was carried out using the Mettler Toledo DSC-823'e system, which includes a differential scanning calorimeter and a computerized data station. A nitrogen flow was applied while weighed samples of pure medicines, physical mixes of drugs and polymers, and mixtures of polymers were heated at a rate of 10°C/min between 40 and 200°C. Differential scanning calorimetry research sheds light on how various materials interact with one another at various temperatures.

5. Physical compatibility test

A preformulation study employing potential formulation polymers was done to determine the interaction/compatibility between the drug and the polymer. In a 1:1 ratio, lafutidine and xyloglucan were equally distributed. The combination was stored in glass vials that were kept at room temperature. Utilizing the aforementioned differential scanning calorimetry and infrared spectroscopy, samples were examined for compatibility after 15 days.

6. Determination of solubility

In solutions of 0.1 N HCl, pH 4.6 acetate buffer, and pH 6.8 phosphate buffer, the solubility of lafutidine was measured. Extra Lafutidine was added to 25 ml of each medium after all the media had been prepared, and the mixture was then placed in a 50 ml conical flask and agitated on a mechanical shaker for 48 hours. 1 cc of each sample was extracted after 48 hours of shaking and placed through Whatman filter paper No 41. Calculations of solubility were done after measuring absorbance in the 200–400 nm range using a UV–visible spectrophotometer.

7. UV Spectroscopy (Determination of λ_{max})

Lafutidine stock solution (100 g/ml) was made in 0.1 HCL. To achieve a concentration of 100 g/ml Lafutidine in 0.1 N HCl and water, respectively, this solution was properly diluted with 0.1 N HCl separately. The UV visible spectrophotometer captured the UV spectrum in the 200–400 nm range.

8. Calibration curve of Lafutidine in 0.1 N HCl.

Following careful weighing, lafutidine (10 mg) was added to a volumetric flask with a 100 ml capacity. It was next dissolved in 25 ml of 0.1 N HCl and then diluted with the same solution to a final volume of 100 ml. To attain a concentration of between 5 and 30 g/ml, the aforementioned solution was further diluted. The absorbance of the resulting solutions was measured at 290 nm using a UV Visible Spectrophotometer. HCl (0.1N) was used as the standard for the blank. After creating calibration plots, the linearity was determined. Three runs of the calibration curve were made.

Formulation and development [9-12]

1. Calculation of theoretical drug release profile and fixation of dose

Theoretical sustained release drug profile was

calculated on the basis of pharmacokinetic parameters of drug.

2. Selection of gelling polymers

Literature survey revealed the use of aqueous solution of 1 to 3.5% w/v Xyloglucan and gellan gum used for preparation of in situ floating cation induced gels.

3. Selection of complexing agent

Literature survey revealed the use of 0.05 to 0.2% w/v calcium chloride as a cation (Ca++) source to induced instant gelation.

4. Method for preparation of in situ gelling solutions

In a half volume of deionized water that also contained calcium chloride (0.1 to 0.25% w/v) and sodium citrate (0.5% w/v), xyloglucan and gellan gum were made at various concentrations (2 to 4% w/v). With stirring, this solution was heated to 60 °C. Add another third of the deionized water containing HPMC K750PH (1 to 1.75% w/v) once it has cooled below 40°C while stirring continuously. To the aforementioned combination, add the medicine (220 mg/10 ml), sodium bicarbonate (0.5 to 1.5% w/v), and the remaining excipients. Fill the remaining volume with water. The final step was to store the Lafutidine-containing in situ gelling solution in amber-colored narrow mouth bottles until usage. Triplicates of each batch were created.

Preparation of preliminary trial batches for selection of gel forming polymer and its working concentration range.

Various trial batches were prepared by using different concentration of Xyloglucan and gellan gum, alone and in combination (G: X) as mentioned in Table below, as per procedure mentioned above.

Table 1: Formulation of Xyloglucan trial batches.

Ingredients (%W/V)	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10
(LFD) (mg/10ml)	200	200	200	200	200	200	200	200	200	200
Xyloglucan	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Calcium chloride	0.5	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Sodium citrate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
HPMC K 750PH	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Sodium bicarbonate	1	1	1	1	1	1	1	1	1	1
CMTG	-	0.1	0.3	0.5	-	-	-	-	-	-
NH-TG	-	-	-	-	0.1	0.3	0.5	-	-	-
Thio-TG	-	-	-	-	-	-	-	0.1	0.3	0.5
Sodium Propyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sodium saccharine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Water	Q.S.									

Table 2: Formulation of gellan gum trial batches.

Ingredients (%W/V)	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
(LFD) (mg/10ml)	200	200	200	200	200	200	200	200	200	200
Gellan Gum	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Calcium chloride	0.5	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Sodium citrate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
HPMC K 750PH	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Sodium bicarbonate	1	1	1	1	1	1	1	1	1	1
CMTG	-	0.1	0.3	0.5	-	-	-	-	-	-
NH-TG	-	-	-	-	0.1	0.3	0.5	-	-	-
Thio-TG	-	-	-	-	-	-	-	0.1	0.3	0.5
Sodium Propyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sodium saccharine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Water	Q.S.									

Evaluation of preliminary batches for selection of working concentration range of gelling polymers

Various formulations were prepared using Xyloglucan and gellan gum as described above and were used to select working concentration range of gelling polymers on the basis of in vitro gelling capacity and pourability (relative viscosity).

In vitro gelling capacity

Solutions of in situ gelling formulations were created in order to assess the formulations' ability to gel in vitro using a visual technique. By adding 5 ml of the gelation solution to 500 ml of 0.1N HCl (pH 1.2) kept at a constant temperature of 37°C, the in-vitro gelling capacity of the produced formulations was determined. Using a pipette, the formulation was delivered to the fluid's surface by being placed there and then being gradually released. The solution was instantly transformed into a rigid gel-like structure as soon as it came into contact with the gelation solution. The stiffness of the produced gel and the length of time it lasts as such were used to assess the solution's ability to gel. Based on the length of the gelation process and the amount of time the created gel lasts, the in-vitro gelling capability was divided into three groups.

(+) Gels after few minutes, dispersed rapidly
(++) Gelation immediate remains for less than 12 h
(+++) Gelation immediate remains for more than 12 h

Determination of viscosity

The viscosity of in situ gelling formulations was determined at 25°C with Brookfield Viscometer.

Evaluation of preliminary batches for selection of gelling polymer

All selected preliminary batches were subjected to determination of pH, drug content, gel strength, mucoadhesive strength, and rheological study to select gelling polymer.

pH measurement

pH of preliminary batches was measured using a digital pH meter.

Determination of drug content

The exact amount of 10 ml of in-situ gelling formulation (equal to 220 mg of lafutidine) from several batches was measured and transferred to a 50 ml volumetric flask. 20–30 ml of 0.1 N HCl were then added. The above solution underwent 30 minutes of magnetic stirring and 30 minutes of sonication. Whatman Filter Paper (No. 41) was used to filter the mixture after volume was increased to 50 ml. 1 ml of the sample was taken out of this solution and diluted to 50 ml with 0.1N HCl. Using a double beam UV-Visible spectrophotometer, the contents of lafutidine were measured spectrophotometrically at 290 nm.

Measurement of gel strength

Using a Brookfield Viscometer, the gel strength of in-situ gelling compositions was assessed. Gels were made in simulated stomach fluid (0.1 N HCL) at 37°C, and the load change of a probe pushed slowly through the gel was quantified using a straightforward approach. Rheoplus software was used to display gel strength results using stress-strain graphs.

Stress point displaying a sharp drop in stress following an increase in strain values, which are interpreted as gel strength.

In vitro mucoadhesive force study

Using a texture analyzer (CT3, Brookfield, USA) with a 50 N load cell and a mucoadhesive holder, mucoadhesion testing of the in situ gelling formulations was performed. For testing different formulations' mucoadhesive strengths, rat stomach mucosa was used as the model membrane. Before inserting the tissue (about 20 X 20 mm) into the holder stage of the mucoadhesive probe, it was equilibrated for 15 min at 37.0 0.5oC. At a pace of 0.5 mm/s, the homogenous gel-coated probe was lowered until it made contact with the membrane. A 1N contact force was maintained for 60 seconds before the probe was removed at 0.5 mm/s to a 15 mm distance. The maximum detachment force, or Fmax, needed to remove the probe from the tissue may be determined using the texture analyzer.

Evaluation of rheological behavior (shear thinning property)

To investigate the shear thinning behavior of polymers, a rheological investigation of in situ floating solutions was conducted. Shear thinning characteristic reflects viscosity reduction as continuous shear rate increase. Rheological analysis was carried out graphically utilizing Brookfield Viscometer's measurements of viscosity (mPa) and shear rate (1/sec).

Evaluation and characterization of floating in situ gel

In vitro dissolution studies

Table 3: Details of dissolution test

1.	Apparatus	USP Type II
2.	Speed	50 rpm
3.	Volume of medium	900 ml
4.	Stirrer	Paddle type
5.	Aliquot taken at each time interval	05 ml
6.	Time intervals	12 h
7.	Medium used	0.1 N HCl (pH 1.2)
8.	Temperature	37 ± 0.5°C
9.	Spectrophotometric analysis	UV-Visible analysis at 290nm

The graphs of cumulative release (%) versus time (h) were plotted.

In vitro floating ability

The in situ gelling solution's flotation investigation was conducted in 500 ml of 0.1N HCl (pH 1.2) in a beaker. The 10 ml of solution was added to the HCl with a light stir. The lag time (floating lag time) between introducing the solution and the gels floating on the surface was measured.

Stability Studies

The stability of the active ingredient must be a significant criterion in any logical design and evaluation of dosage forms for medications in order to determine their approval or rejection. Stability studies were conducted in accordance with ICH Q1A (R2) recommendations. Stability tests were performed on the improved formulation of lafutidine in situ gelling.

Stability protocol

Types of packaging material

The formulations were packed in PET (Polyethylene terephthalate) bottles.

Storage condition

The formulations were subjected to stability as per ICH guidelines at the following conditions (Table 9). Samples were kept in stability chamber.

Table 4: Stability storage conditions.

Description	Storage conditions
Accelerated testing	40°C ±2 °C / 75% RH ±5 % RH

In vivo study [13-16]

Male wistar rats (200-250 g each) were utilized for in vivo experiment study. All the animal studies were conducted in accordance with the protocol approval by the Institutional Animal Ethics Committee (IAEC).

Procedure

In vivo pharmacodynamic investigations were conducted on rats using the pylorus ligation procedure to induce ulcers. Additionally, it was examined to see if the gel had developed in the rat gastric juice. Four groups of six creatures each were created from the animals. The second, third, and fourth groups received immediate treatment with placebo in situ gel, a plain Lafutidine solution (equal to 22 mg/kg p.o.), and an improved in situ gelling formulation (equivalent to 22 mg/kg p.o., respectively), while the first group served as the control. The rats were given a 24-hour fast before being given ether anesthesia, during which time a tiny incision was made under the xiphoid process to expose part of the abdomen. The stomach's pylorus region was raised and bound. Care was taken during this procedure to prevent traction on the pylorus or harm to its blood supply. The stomach was stitched up with broken sutures.

Group-1 Control

After 8 hours of pylorus ligation the animals were sacrificed and the stomach of each animal was removed, cut along the greater curvature and subjected to analysis of parameters given below.

Group-2

Xyloglucan-based placebo in situ gel is given orally to the animals in the immediate treatment group 20 minutes before they are slaughtered (at 8 hours). The stomachs are then removed, sliced along the greater curvature, and the gel's presence or absence is checked before the stomachs are submitted to the study of the parameters listed below.

Group-3

In this group, plain Lafutidine drug solution (equivalent to 22 mg/kg p.o) in water was administered orally 30 mins before starting the experiment in rats and after 8 hours of surgery animal is sacrificed and subjected to analysis of parameters given below.

Group-4

In this group, optimized in situ gelling Lafutidine formulation (equivalent to 22 mg/kg p.o) was administered orally 30 mins before starting the experiment in rats and after 8 hours of surgery animal was sacrificed and subjected to analysis of parameters given below.

Parameters Studied

Ulcer index

The incised stomachs were first washed with running tap water and placed on a watch glass and subjected to measurement of ulcer index. The ulcer index was determined using the formula (5)

Ulcer index = 10/X Formula 1

Where,

X = Total mucosal area/Total ulcerated area.

Volume of gastric secretion

The gastric juice was centrifuged at 1000 rpm for 10 min and volume of decanted juice was measured by using measuring cylinder.

Determination of total acidity of the gastric secretion

The volume of the gastric juice was increased to 10 ml by diluting an aliquot of 1 ml with distill water in a 50 ml conical flask. It received 2 drops of phenolphthalein indicator. After more 0.01 N NaOH titration, a stable shade of pale pink emerged. It was noticed how much alkali was consumed. The following formula was used to translate the total acidity into mEq/l/g.

$$\text{Total acidity } \left({}^{\text{mEq}}/_{100\text{g}}\right) = \frac{\text{volume of NaOH Consumed} \times \text{N} \times 100}{0.1}$$ Formula 2

Where,

N = Normality of NaOH (0.01 N)

pH of the gastric secretion

pH was measured using a digital pH meter.

Statistical analysis

All the data were expressed as the mean ± S.E.M. data were subjected to One-Way analysis of variance (ANOVA) followed by Tucky's multiple analysis test, where ***P < 0.001, **P < 0.01and *P < 0.05 was considered as statistically significant.

Results And Discussion

Preformulation Studies

Table 5: Melting point & Organoleptic properties of Lafutidine.

Drug	Melting Point range		Organoleptic properties	
	Literature	Practical	Colour	State
Lafutidine	98-101°C	98-100°C	white to off–white	crystalline powder

Fourier transform infrared spectroscopic (FTIR) studies

The FTIR spectrum of Lafutidine is shown in Figure below and interpretation of FTIR spectra is given in Table. FTIR spectrum of Lafutidine showed all the peaks corresponding to the functional groups present in the structure of Lafutidine.

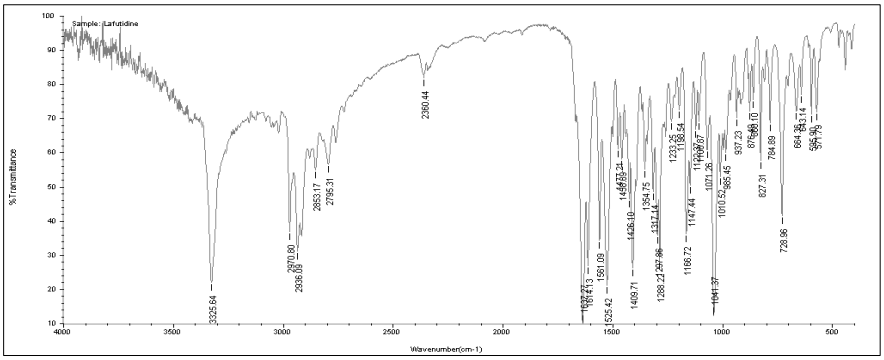

Figure 1: FTIR spectrum of Lafutidine.

The major peaks for pure Lafutidine were seen as below, 3325.64 for–NH stretching, 2360.44 for–CH (alkyl) stretching, 1632.24 for-C=O stretching, 937.23 for SO stretching which confirms the important functional group Lafutidine.

Differential Scanning Calorimetric (DSC) studies

Differential Scanning Calorimetry studies indicated a sharp endothermic peak at 99.21°C corresponding to melting of pure Lafutidine is as shown in Figure.

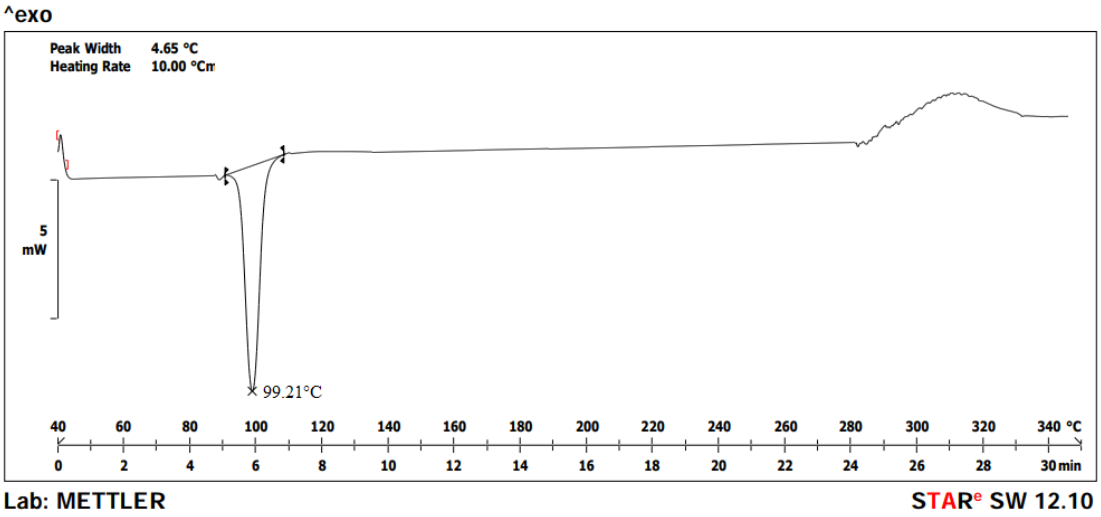

Figure 2. DSC thermogram of Lafutidine

Physical compatibility test

Physical compatibility test using Infrared Spectroscopy

For physical compatibility test FTIR of drug and excipients were mixed and kept strictly for 30 days. The spectrum was scanned over a frequency range 4000-400 cm-1. FTIR spectra of drug-excipient mixtures retained the characteristic functional peaks of the drug as shown in Figures below. Thus, the polymer and the drug show no interaction.

The major peaks for pure Lafutidine were seen as below, 2360.44 for–CH (alkyl) stretching, 1632.24 for-C=O stretching, 937.23 for SO stretching which confirms the important functional group Lafutidine.

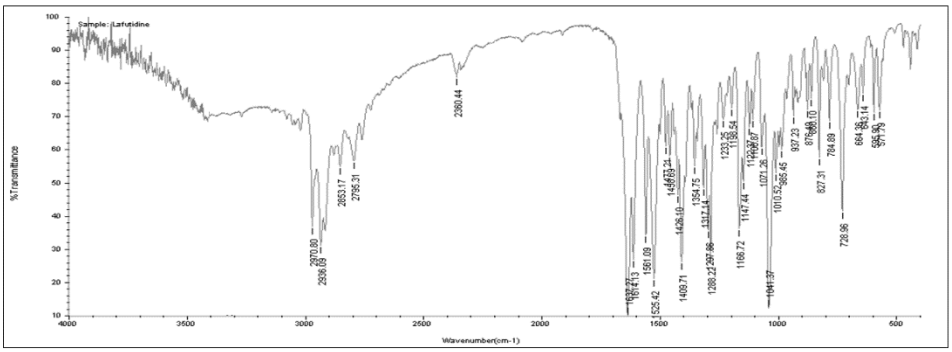

Figure 3: IR spectrum of Lafutidine + gellan gum + HPMC K100 M physical mixture.

The three main peaks for pure Lafutidine were seen as follows: 2360.44 for-CH (alkyl) stretching, 1632.24 for-C=O stretching, and 937.23 for SO stretching, confirming Lafutidine as an essential functional group.

There is no interaction between the drug and excipients, according to the FTIR spectra of pure Lafutidine and physical mixtures, which demonstrate the vibration of functional groups found in the drug's structure.

Physical compatibility test using Differential Scanning Calorimetric (DSC) studies

The Figure displays the DSC thermograms of both the pure medication and its physical mixes with different polymers. Comparative Scanning Studies using a calorimeter showed a pronounced endothermic peak at 99.21°C, which corresponded to the melting point of pure lafutidine. Dilution of the drug in physical mixes of the drug with both polymers led to the observed widening of the peaks as well as changes in relative intensities. It might be said that there is no interaction between the medicine and the polymers. The location of the endothermic peaks prevented the medicine from forming a compound with the excipients.

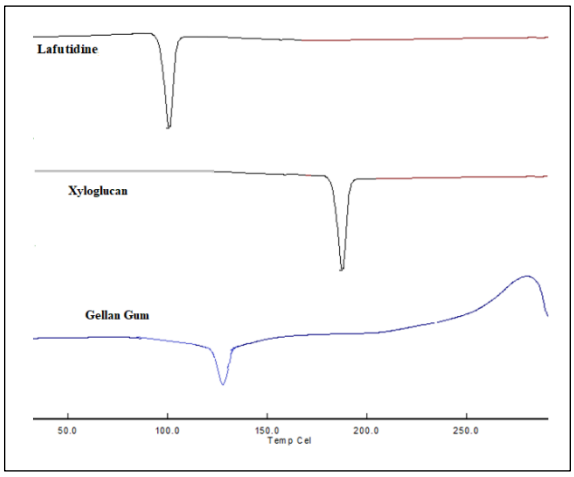

Figure 4: DSC thermogram of Lafutidine, Xyloglucan and gellan gum

From the above observation from FTIR and DSC study, it was concluded that the polymer and drug did not interact with each other and are compatible.

Determination of solubility of Lafutidine

The solubility of Lafutidine as observed in 0.1 N HCl (pH 1.2) and buffers of pH values 4.6 (acetate buffer) and 6.8 (phosphate buffer) are presented in Table. Lafutidine exhibited a pH dependent solubility in these aqueous buffers. Higher solubility of Lafutidine was observed at acidic pH values, while the solubility dropped rapidly as the pH increased (Figure).

Table 6: Solubility analysis of Lafutidine.

Solvent	Solubility (mg/ml)
pH 1.2 (0.1N HCl)	52.22
pH 4.5	40.12
pH 6.8	11.44

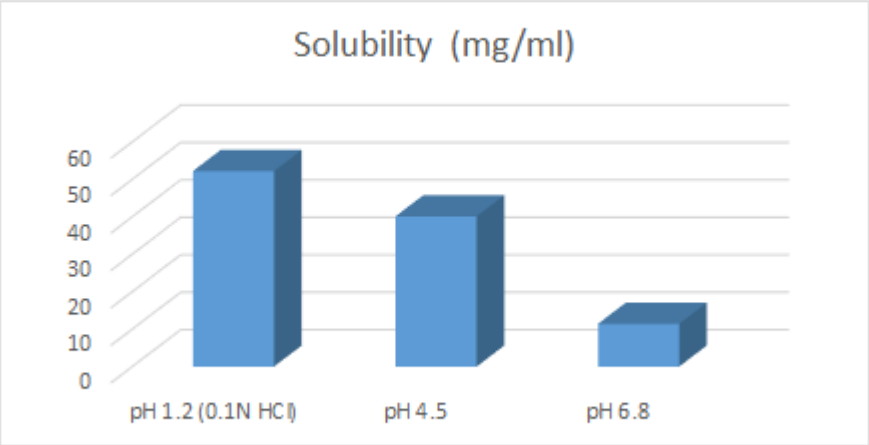

Figure 5: Solubility analysis of Lafutidine

UV-VIS Spectrophotometric method for Lafutidine

Determination of λ_{max}

Wavelength of maximum absorbance (λmax) of Lafutidine was found to be 290 nm in 0.1 N HCl.

Calibration curve for Lafutidine

The calibration curve for Lafutidine in 0.1 N HCl is shown in Figure 25. The graph of absorbance vs. concentration for Lafutidine was found to be linear in the concentration range of 5-30 µg/ml at 290 nm. The r2 of the calibration curve was found to be 0.9996.

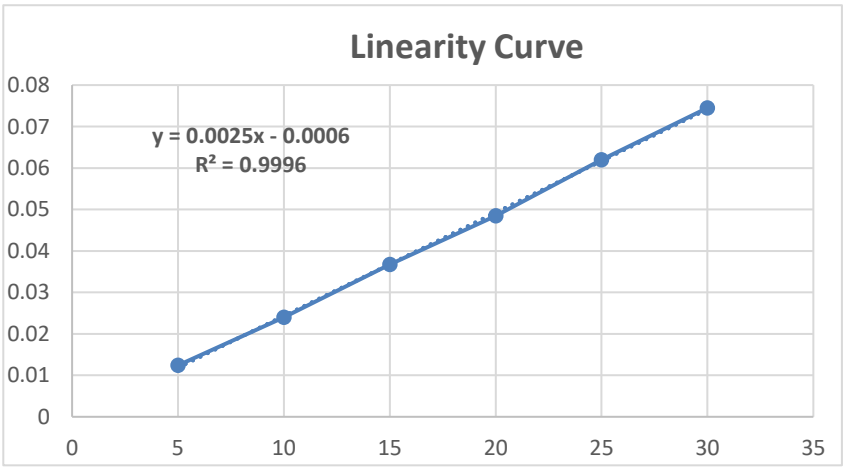

Figure 6: Calibration cure of Lafutidine in 0.1 N HCl.

Preliminary studies

Evaluation of preliminary trial batches for selection of gel forming polymer and its working concentration range.

In vitro gelling capacity

In the preliminary trial batches Lafutidine in situ gelling formulations were prepared using various gelling polymers. The in vitro gelling capacity was graded in three categories on the basis of gelation

time and time period for which formed gel remains.
(+) Gels after few minutes, dispersed rapidly
(++) Gelation immediate remains for less than 12 h
(+++) Gelation immediate remains for more than 12 h

The observations in the formulations were noted as shown in below Table.

Table 7: In vitro gelling capacity of formulations containing Xyloglucan.

Batches	Xyloglucan (% w/v)	In vitro gelling capacity
X 1	1.5	+ - -
X 2	2.0	+ + +
X 3	2.5	+ + +
X 4	3.0	+ + +
X 5	3.5	+ + +
X6	4	+ + +

Table 8: In vitro gelling capacity of formulations containing gellan gum.

Batches	Gellan gum (%w/v)	In vitro gelling capacity
G 1	1	- - -
G 2	1.5	+ +-
G 3	2	+ + -
G 4	2.5	+ + +
G 5	3	+ + +
G 6	3.5	+ + +

From Table batches having desired results with respect to in vitro gelling capacity were taken into account and further studies were carried out.

Determination of viscosity

All preliminary batches were evaluated for viscosity.

Table 9: Viscosity of formulations containing Xyloglucan mean ± SD (n=3).

Batches	Viscosity (cps)	Pourability
X 1	274.52±1.25	Pourable
X2	312.45±2.12	Pourable
X 3	421.52±1.47	Pourable
X 4	328.65±3.26	Pourable
X 5	336.22±4.51	Pourable
X 6	407.12±1.02	Pourable
X 7	398.56±4.55	Pourable
X 8	345.32±3.23	Pourable
X9	457.55±4.55	Pourable
X 10	401.22±2.55	Pourable

Table 10: Viscosity of formulations containing gellan gum, mean ± SD (n=3).

Batches	Viscosity (cps)	Pourability
G 1	278.45±2.15	Easy to pour
G 2	214.56±4.12	Easy to pour
G 3	302.35±4.66	Pourable
G 4	402.33±5.14	Pourable
G5	415.44±2.66	Pourable
G 6	328.47±3.14	Pourable

G 7	451.55±2.78	Pourable
G8	314.77±6.23	Pourable
G 9	304.78±9.10	Pourable
G 10	406.33±7.44	Pourable

Selection of working concentration range of gelling polymers

All Xyloglucan and Gellan gum containing formulations were showing gel forming properties in the range of 1.5 to 3.5% w/v along with batch (G:X) containing both polymers. Formulations containing 1.5% w/v Xyloglucan and 2% w/v gellan gum were rejected since formed gel does not have duration of floating for more than 12 h. Formulations containing 3.5% w/v Xyloglucan and 3 to 3.5% w/v gellan gum were rejected since they were found to be difficult to pour. Working concentration for Xyloglucan and gellan gum was found to be 2 to 3% w/v and 2.5% w/v respectively for further evaluations. All selected formulations (including batch G: X) were subjected to evaluation of mucoadhesive strength and gel strength

Evaluation of preliminary batches for selection of gelling polymer

pH measurement

The pH of all the formulations was found to be in the range of 6.3 to 7.2 respectively.

Table 11: pH of formulations containing natural polymers. (Xyloglucan)

X1	X2	X3	X4	X5	X6	X7	X8	X9	X10
6.8	6.8	6.9	7.1	7.2	7.2	6.3	6.4	6.5	6.6

Table 12: pH of formulations containing natural polymers. (gellan gum)

G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
6.3	6.3	6.4	6.7	6.8	6.3	6.6	6.7	6.7	6.8

Determination of drug content

All preliminary batches were evaluated for drug content. Drug content for xyloglucan and gellan gum containing formulation varies from range 98.54 to 101.05% and 97.52 to 102.01% respectively. From the tables it can be seen that gellan gum does not show appreciable drug content. It was decided that these batches would be critically evaluated for further parameters and then only rejected.

Table 13: Drug content of formulations containing Xyloglucan.

Batches	Drug content (%) Mean ±SD,(n=3)
X 1	98.54±0.56
X 2	99.12±0.87
X 3	99.23±0.45
X 4	99.21±0.48
X 5	100.21±0.65
X 6	101.05±0.36
X7	99.58±0.54
X 8	100.52±0.44
X 9	100.14±0.12
X 10	99.56±0.58

Table 14: Drug content of formulations containing gellan gum.

Batches	Drug content (%) Mean ±SD,(n=3)
G 1	97.52±1.12
G 2	98.56±098
G 3	99.21±1.25

G 4	98.51±2.04
G 5	99.62±1.51
G 6	102.01±1.44
G 7	101.05±2.45
G 8	99.58±1.70
G 9	98.47±1.14
G10	97.66±2.12

Determination of gel strength

All of the batches that were chosen from the early investigations were evaluated for gel strength. A straightforward technique that monitored the change in load of a probe moved slowly through the gel was used to investigate gel behavior under applied stress. All of the chosen batches' rheograms (stress vs strain graphs) are displayed. Values of the tension just before it collapsed were taken into account when calculating the gel strength (dyne/cm2). As demonstrated in the table, each measurement of gel strength was made in triplicate with high levels of repeatability. All of the gels demonstrated strong gelling.

Table 15: Gel strength of preliminary batches, mean ± SD (n=3). [Xyloglucan]

Formulation	Gel strength (dyne/cm2)
X 1	33.02±0.21
X 2	41.02±0.41
X 3	38.51±0.52
X 4	36.22±0.36
X 5	34.12±0.14
X 6	33.02±0.55
X7	31.20±0.47
X 8	33.20±0.22
X 9	37.46±0.56
X 10	40.12±0.12

Table 16: Gel strength of preliminary batches, mean ± SD (n=3). [Gellan Gum]

Formulation	Gel strength (dyne/cm2)
G 1	27.45±0.78
G 2	29.54±0.25
G 3	28.41±0.23
G 4	33.20±0.74
G 5	34.25±0.41
G 6	38.55±0.95
G 7	39.12±0.78
G 8	41.20±0.66
G 9	40.15±0.14
G10	39.65±0.55

Mucoadhesive strength

All of the batches that were chosen from the early trials had their mucoadhesive strength measured. By measuring the force (dyne/cm2) necessary to separate gel from the mucosal surface, Texture Analyzer was used to evaluate the in vitro bioadhesive property of all formulations on rat stomach mucosa. As can be seen in the table, each measurement of mucoadhesive strength was carried out in triplicate with high repeatability.

Table 17: Mucoadhesive strength of preliminary batches, mean ± SD (n=3).[Xyloglucan]

Formulation	Mucoadhesive strength (dyne/cm2)
X 1	901.2±0.56
X 2	1024.1±0.14
X 3	978.2±0.78
X 4	856.3±1.02
X 5	1254.2±0.56
X 6	1150.2±0.78
X7	965.2±0.56
X 8	863.2±0.14
X 9	914.2±0.59
X 10	978.1±0.78

Table 18: Mucoadhesive strength of preliminary batches, mean ± SD (n=3).[Gellan Gum]

Formulation	Mucoadhesive strength (dyne/cm2)
G 1	903.2±0.36
G 2	891.2±0.58
G 3	1021.5±0.69
G 4	1124.7±0.47
G 5	941.6±0.59
G 6	896.3±0.47
G 7	988.6±0.59
G 8	902.8±0.48
G 9	1126±0.89
G10	1267.5±0.74

Evaluation of rheological behavior

Rheological investigations were conducted on every batch that was chosen from the preliminary studies. Each measurement was carried out three times with high levels of repeatability. All of the chosen formulation batches showed shear thinning behavior. The formulations' viscosities, especially those that contain xyloglucan, are suitably low and have improved shear thinning properties. In comparison to xyloglucan alone as a gel forming polymer, formulations containing gellan gum alone exhibit poor shear thinning properties.

Selection of gelling polymer

In comparison to formulations containing xyloglucan alone, all of the selected batches of gellan gum had higher drug contents (97.52 to 102.01%), higher gel strengths (27.45-41.20 dyne/cm2), and higher mucoadhesive strengths (891.2 to 1267.5 dyne/cm2).

The most effective gel forming polymer for in situ gelling formulation was chosen to be xyloglucan, which has a working concentration range of 2 to 3% w/v.

Effect of complex forming agent (calcium chloride) on release pattern and selection of its working concentration range

Since its working concentration range of 2 to 3% w/v was chosen as the best suitable gel forming polymer for in situ gelling formulation from preliminary batches, Xyloglucan 2.5% w/v was chosen for further evaluations. Its working concentration range was chosen using the impact of calcium chloride concentration variations on drug release patterns. Calcium chloride was produced in six batches (CC-1 to CC-6) at varying concentrations. Based on the analysis of the drug release profiles of the manufactured batches, it was determined that a rise in calcium chloride concentration causes the medication's sustained release impact to increase. This might be explained by the formulation's inclusion of calcium chloride, which releases calcium ions into the acidic medium and causes xyloglucan to gel. Only batches CC-3 to CC-6, which include 0.1 to 0.2% w/v of calcium chloride, were found to have cumulative percent release for more than 10 hours, indicating improved gel

stiffness. The working calcium chloride concentration range was chosen between 0.1 and 0.2% w/v based on the relative gel stiffness needed for prolonged drug release. Each measurement was carried out three times with high levels of repeatability.

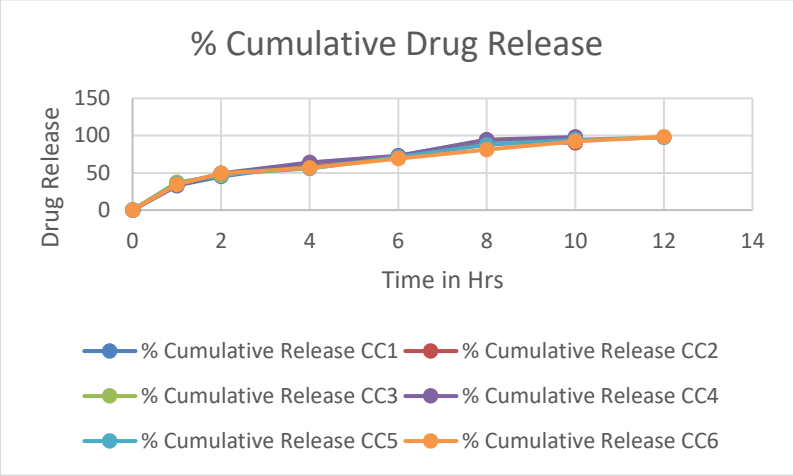

Figure 7: Effect of calcium chloride on drug release profile.

Table 19: Effect of calcium chloride on drug release profile, mean ± SD (n=3).

Formulation	CC1	CC2	CC3	CC4	CC5	CC6
CC	92.15	93.65	94.55	98.15	97.58	98.26
Time (h)	8	10	10	10	12	12

In vitro drug release study

Dissolution data of in situ gelling formulations (X1-X10 and G1-G10) are reported in below. Dissolution study for each formulation was carried out in triplicate, in acid and buffer stage.

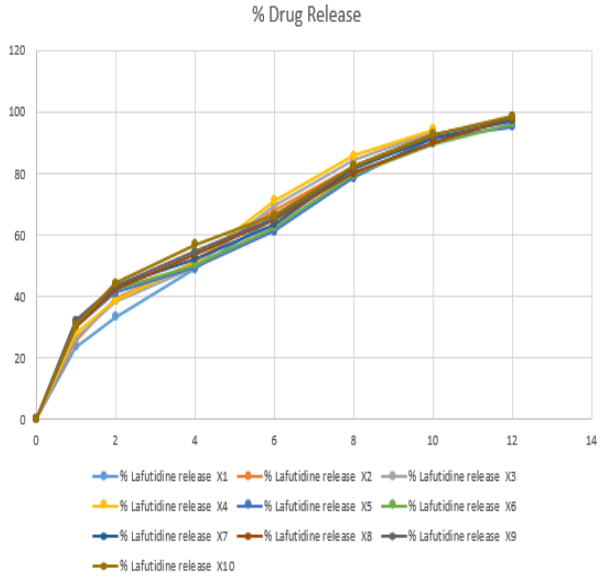

Figure 8: Cumulative drug release and % Lafutidine release- Batch- X1-X10

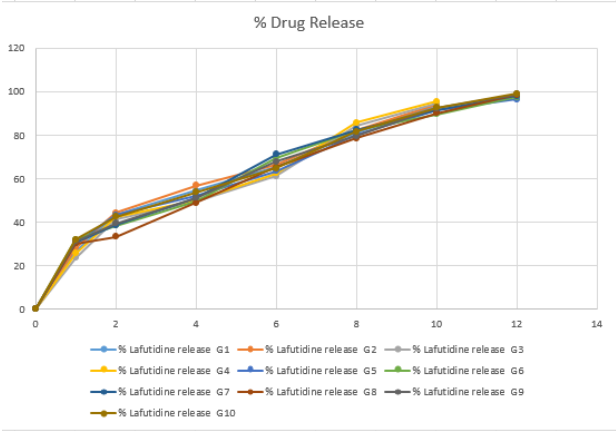

Figure 9: Cumulative drug release and % Lafutidine release- Batch- G1-G10

Think about the sustained release formulations X1-X10 and G1-G10, which all demonstrated sustained release of lafutidine over a period of 10–12 hours.
In comparison to formulations X5-X10 and G5-G10, formulations (XI, X2, X3, X4) including HPMC K750PH and G1-G4 led to an earlier release.
Lafutidine release from all of the formulations was modeled using various release kinetic theories. For all of the formulations X1 to X10, the diffusional exponent 'n' value showed that the release mechanism was non-fickian drug transport and that the Korsemeyer Peppas Model was used.

Stability studies

The formulation X10 and G10 was subjected to stability study as per ICH guidelines at the following condition of 40°C/75 % RH for 3 months. These formulations were evaluated for following parameters after stability study.

Appearance

Formulations X10 and G10 kept for stability studies were examined for its appearance. The colour and consistency of formulation was found to be unchanged after stability studies.

pH measurement

The pH of the optimized formulation X10 and G10 was found to be unchanged before (pH 7.2) and after (pH 7.1) stability studies and before (pH 7.1) and after (pH 6.9) respectively.

In-vitro dissolution study

Dissolution profile of optimised formulation (X10 and G10) after stability study is shown in Figure below.

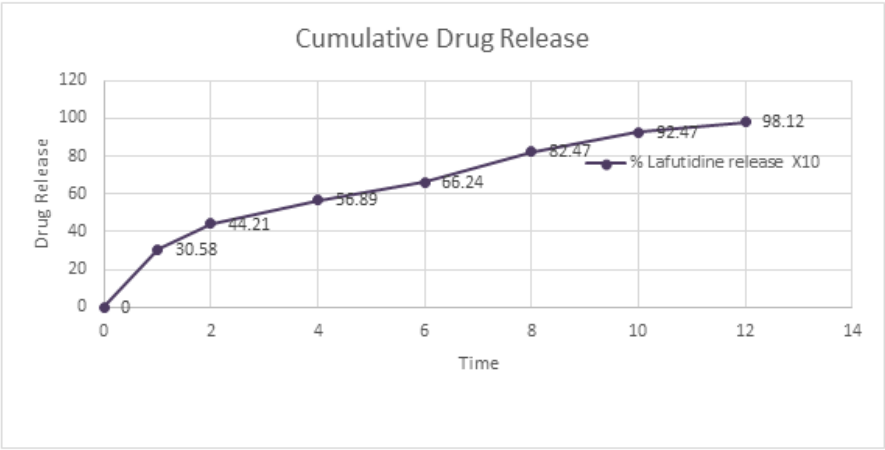

Figure 10: Dissolution profile study of optimized formulation (X10) before and after 90 days.

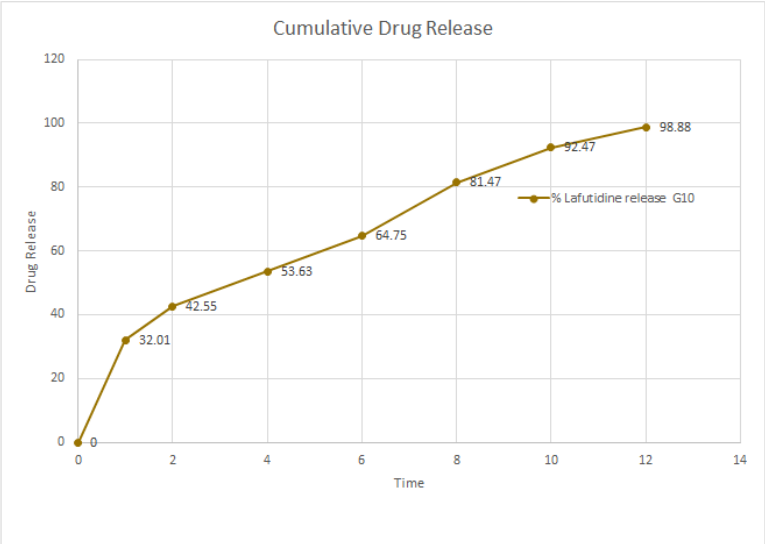

Figure 11: Dissolution profile study of optimized formulation (G10) before and after 90 days.

The optimized formulation X10 showed 98.56% release prior to the stability study (0 day) and showed 98.12% release at 12 hours following the stability studies (90 days), while the optimized formulation G10 showed 99.02% release prior to the stability study (0 day) and showed 98.88 release at 12 hours following the stability studies (90 days). It was determined that the release characteristics of the two formulations varied significantly. It was determined that the formulations were stable.

Determination of drug content by HPLC

Drug content was determined for optimized formulations of X10 and G10 after 90 days. The drug content was determined on the basis of HPLC study.

Table 20: Drug content for optimized formulation X10 and G10 before and after stability study.

Formulations	Days	Drug content (%)
X10	0 30 60 90	99.56% 99.48% 99.20% 99.11%
G10	0 30 60 90	97.66% 97.54% 97.46% 97.11%

From Tables given above, it was concluded that there was no significant change in drug content of formulation after 90 days.

In vivo pharmacodynamic study

The current in vivo studies showed that, when compared to the plain Lafutidine solution and the control group, the reduction of ulcer index and the volume of gastric acid secretion from Lafutidine in situ gelling formulation (optimized formulations X10 and G10) was significantly different (P 0.01). While the group treated with the Lafutidine in situ gelling formulation significantly decreased both pH and total acidity as compared to control (P 0.001) and plain Lafutidine drug solution (P 0.001), it was found that the plain Lafutidine solution did not significantly decrease total acidity and pH when compared with the control group (P > 0.05). The ulcer index for the control group was determined to be 2.204 0.75. In the case of the immediate treatment group, the gel that was seen in the rat's stomach juice that was collected was created, and the ulcers were also detected by the ulcer index of 2.98 0.26. The ulcer index was only 1.15 0.25 in the case of lafutidine in situ gel after 8 hours of administration. After 8 hours of administration, the ulcer index for the simple medication was

discovered to be 2.47 0.78. The ulcer index for the control group was determined to be 2.204 0.75. In the case of the immediate treatment group, the gel that was seen in the rat's stomach juice that was collected was created, and the ulcers were also detected by the ulcer index of 2.98 0.26. The ulcer index for lafutidine in situ gel was only 1.15 0.25 after 8 hours of treatment for XA0 and 1.25 0.89 for XA1. After 8 hours of administration, the ulcer index for the simple medication was discovered to be 2.47 0.78.

Table 21: Effect of Lafutidine in situ floating gel on ulcer induced by pylorus ligation in rat.

Group	Ulcer index	Volume of gastric acid secretion (ml)	Total acidity (mEq/l/100 g)	pH
Control	2.204± 0.75	6.154± 2.45	68.45±1.56	1.12 ±0.78
Lafutidine Gel (IT)	2.98 ± 0.26	7.1 ±1.15	65.23 ±0.59	1.17 ±0.98
X10	1.15 ± 0.25	5.12 ±0.96	57.45 ±2.05	1.24±0.58
G10	1.25± 0.89	4.99±1.02	56.84±1.05	1.19±0.56
Lafutidine	2.47 ± 0.78	3.65± 0.45	29.56 ±2.05	1.78± 0.25

n = 6 animals in each group; Values are mean ± SEM; *P <0.05,**P < 0.01. ***P < 0.001when compared with control; P value was calculated using one way ANOVA followed by Tuckey's multiple comparison test.

The possible reason for this observation may be the drug concentration in the body that was maintained for longer duration in case of Lafutidine in situ gel as compared with that of plain Lafutidine solution.

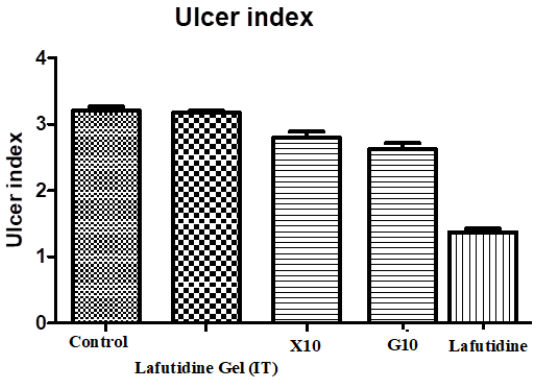

Figure 12: Effect of Lafutidine in situ floating gel on ulcer index.

α (P<0.01) compared to Control (One way ANOVA followed by Tucky's test; β (P<0.001) compared to Control (One way ANOVA followed by Tucky's test) β (P<0.001) compared to α (One way ANOVA followed by Tucky's test)

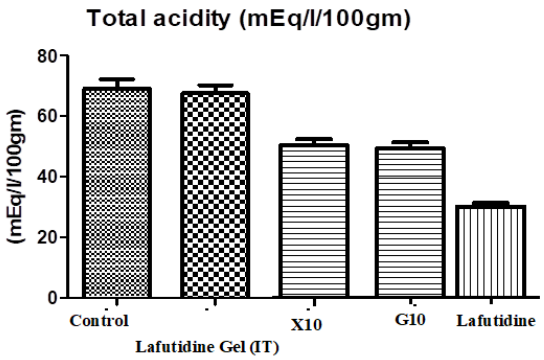

Figure 13: Effect of Lafutidine in situ floating gel on volume of gastric acid secretion

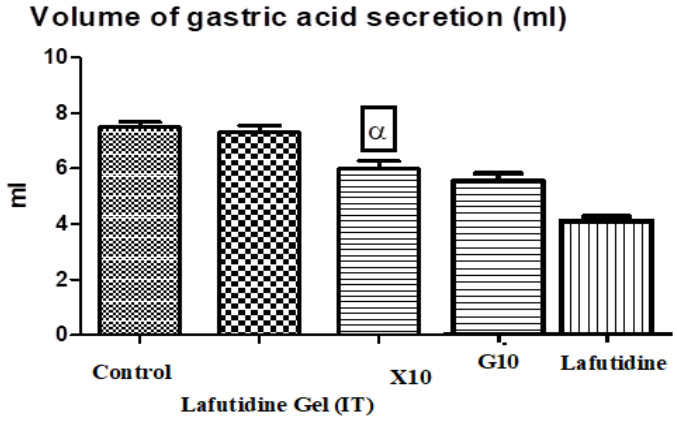

Figure 14: Effect of Lafutidine in situ floating gel on total acidity.

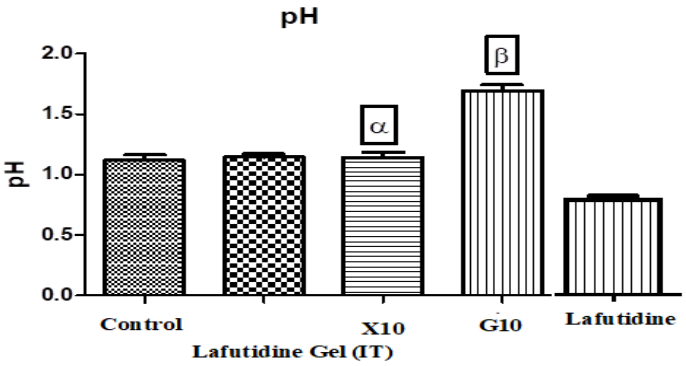

Figure 15: Effect of Lafutidine in situ floating gel on pH.

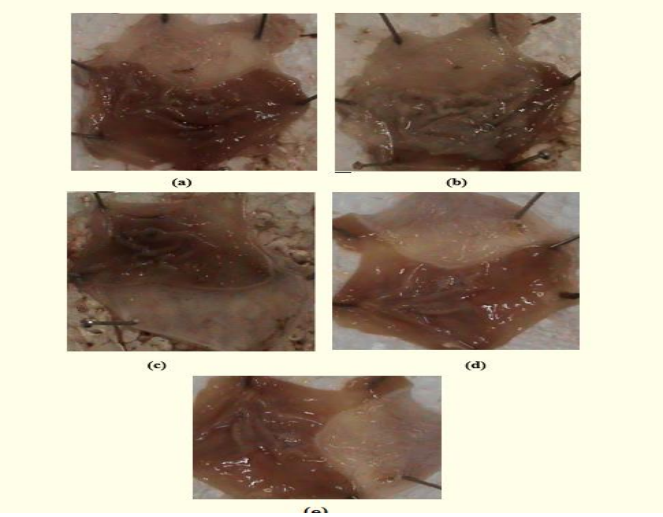

Figure 16: Animal groups : (a) control, (b) control plus immediate treatment of in situ gelling formulation, (c) plain Lafutidine solution, Lafutidine in situ gelling optimized formulations X10 (d) and G10 (e)

Conclusion

Using gastroretentive drug delivery devices, medications with a narrow window of absorption can be delivered at a controlled rate to the upper stomach region. The raft formation process has a considerable advantage over methods of gastric preservation based on floating structures, the use of pharmaceuticals as a liquid dosing medium, and in situ gelation of the solution for a prolonged drug

release in the upper GI tract. Lafutidine is precisely placed in the parietal cells of the stomach mucosa, where the upper gastro-intestinal tract consumes it, according to the data. The bioavailability of Lafutidine in pills is 65–70% compared to intravenous infusion. Its stability and absorption are acceptable between 1 and 4. The colon and lower gastrointestinal tract's environment progressively deteriorates. The formulations used in the current investigation were transformed into gel for simulated gastric fluid (pH 1.2), where they immediately floated in the gastrointestinal environment and demonstrated sustained drug release for 12 hours. Various parameters of the finished device were examined. Lafutidine concentrations in formulations are estimated using UV and HPLC Spectrophotometric analytical methods. With a regression value of 0.999, the HPLC methodology was demonstrated to be linear in the concentration range of 05 to 30 g/ml and was therefore registered as a stability-suggesting method. UV was also shown to be linear in the concentration range of 5–30 g/ml, with a regression value of 0.999. IR studies and differential calorimetry scanning (DSC) showed that the chemical, polymers, and excipients displayed little interaction. Two distinct xyloglucan polymers, gellan gum, and the in situ gelling formulations were developed as complex agents for gelling polymers and calcium chloride.

Based on examination criteria including rheological testing, medicine content, mucoadhesive strength, and gel strength, xyloglucan was shown to be the most suited polymer. Over the course of three months at 40°C and 75% RH, lafutidine's stability in its optimized formulas X10 and G10 as a solution didn't improve in terms of appearance, drug content, or dissolving profile.

For the in vivo testing of the in situ optimized Lafutidine gelling (X10, G10) formula, Wistar rats were employed. In comparison to a single-way ANOVA solution and Tucky's multiples comparison test, the ulcer index, stomach acid volume (ml), overall acidity (mEq / l / g), and pH were all considerably impacted. Not to mention, the current study was effective in developing, testing, and producing an oral continuous release floating Lafutidine gelling device with stomach-specific drug delivery, continuing the release of medicines and achieving gastric preservation for the required period of time.

Conflict Of Interest

None declared by authors.

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eISSN 1303-5150