DEVELOPMENT AND EVALUATION OF NOVEL IN-SITU GEL FOR THE TREATMENT OF GASTRIC ULCER DISEASE USING NATURAL POLYMER

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Abstract:

Objective: The aim of the present study was to formulate and evaluate novel gastroretentive *in-situ* gel of Roxatidine acetate for the treatment of gastric ulcer disease.

Methods: A gastroretentive *in-situ* gel of Roxatidine acetate was formulated by natural polymer using different concentrations of chitosan and *vigna mungo* gum as gelling agent. The compatibility study was done by DSC and FTIR. The prepared formulations of *in-situ* gel were evaluated with various parameter like gelling capacity, viscosity, pH, drug content, gel strength, floating ability, gel density, water uptake, *in-vitro* drug release and stability studies.

Results: Selected S4 and G4 gastroretentive *in-situ* gel of Roxatidine acetate formulations were found to be best on the basis of results of evaluation parameters. The S4 & G4 drug release rate was retained up to 12 hours. The rises in concentrations of gelling agent decrease the rate of drug release. It is indicated that prepared formulation exhibited controlled release of drug at prolonged time. The release rate of drug from the formulation surface was found to be best fit Korsmeyer-Peppas model model for S4 & G4 (R^2=0.9956, n value=0.3699; R^2=0.9984, n value=0.3767;) and were implies that the optimised formulations patterns of release preceded by Fickian diffusion. The stability of Roxatidine acetate optimised formulations S4 & G4 showed no changes in physical appearance, drug content, dissolution profile and HPLC chromatograms. The evaluation parameter and results revealed that Roxatidine acetate gastroretentive in-situ gel formulation was found to be stable at 40°C/75 percent RH for three months.

Conclusion: The development of a once daily dosage, from *in situ* gel prepared S4 & G4 formulation can able to provide prolonged drug release, improve the bioavailability of the drug, and increase patient compliance. The result of evaluation revealed that S4 & G4 formulation can improve therapeutic action for the treatment of gastric ulcer disease.

Keywords: *In-situ* gel, pH induced ion gelation, calcium carbonate, HPMC K100M, natural polymer of *vigna mungo* gastro retentive, Roxatidine acetate**,** Sodium citrate.

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1. INTRODUCTION

Most of the drugs given via oral route are subjected to absorption throughout the gastrointestinal tract, with major absorption from stomach and intestine.

Prolonged gastric retention of a drug is required in the following conditions:

1. Drug is best absorbed from stomach e.g. aspirin, phenylbutazone etc.
2. Gastric fluids facilitate and improve the disintegration and dissolution of the drug.
3. Dissolution and absorption of drug is promoted by the food e.g. griseofulvin.
4. Slow dissolving drugs.
5. Drug show local effect within stomach.

In order to fulfill all these conditions, various approaches of the controlled drug delivery have been developed. One of these types of the approaches, which ensure that a particular drug or dosage form remains within stomach for longer duration of time, is Gastro retentive drug delivery system (Hirtz et al., 2021).

Gastro retentive system ensures that whole drug delivery system remains within the gastric region for longer duration of time. This improves gastric retention time for such drug in comparison to conventional dosage form and further minimum effective concentration of drug remains maintained in systemic circulation for longer duration. This also improves the solubility of drugs which are less soluble at alkaline pH of intestine and wastage of drug during the absorption process is reduced remarkably (Alexander et al., 2021).

1.1 The objective of the present study was to formulate and evaluate the in situ gel floating drug delivery system of Roxatidine acetate for the treatment of gastric ulcer disease using calcium carbonate, HPMC K100M and natural polymer of *vigna mungo*.. (Basavarajet al., 2018).

- Identification of a minimal cut off size above which floating dosage form retained in the stomach for prolonged periods of time. This would permit a more specific control to be achieved in gastro retentivity.
- Design of an array of floating in situ gel having a narrow GRT for use according to the clinical need.ex. Dosage and disease state.
- Design of floating in situ gel having low density, desired swell ability, minimized use of polymers. This may be achieved by compounding polymeric matrices with various biodegradation properties.

Such systems also provide higher concentrations of drug released around gastric mucosa to efficiently treat the gastric diseases like ulcer, gastritis, oesophagitis etc (Ponchel et al., 2021).
Comparisons between conventional and gastro retentive drug delivery system are shown in table.1

Table: 1. Comparisons between conventional and gastro retentive drug delivery system

Parameter	Gastro retentive drug delivery system	Conventional drug delivery system
Risk of toxicity	Lower	Higher
Patient compliance	High compliance level	Less compliance level
Dose dumping	High risk	No risk
Drugs	Advantageous for drugs: • Having rapid gastrointestinal absorption • Degrade in colon • Showing local action in the stomach	Dis-advantageous for drugs : • Having low gastrointestinal absorption • Degrade in colon •Showing relatively less effect in the stomach

1.2 Floating drug delivery system

One of the best preferred routes among the patients is the oral administrations as compared to other mode of administration. However, oral administration has only limited use for important drugs, from various pharmacological categories, that have poor oral bioavailability due to incomplete absorption and/or degradation in the gastrointestinal (GI) tract. Some of these drugs are characterized by a narrow absorption window (NAW) at the upper part of the gastrointestinal tract. This is because of proximal part of the small intestine exhibits extended absorption properties (including larger gaps between the tight junctions, and dense active transporters).Despite the extensive absorption properties of the duodenum and jejunum, the extent of absorption at these sites is limited because the passage through this region is rapid. Enhancing the gastric residence time (GRT) of a NAW the drug may significantly improve the net extent of its absorption

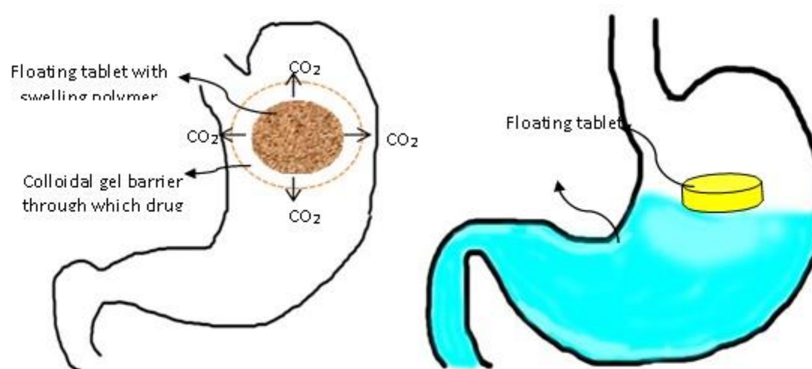

Figure: 1. (a) Drug release mechanism and (b) Floating drug delivery systems

Gastric emptying of dosage forms is an extremely variable process and ability to prolong and control emptying time is a valuable asset for dosage forms, which reside in the stomach for a longer period of time than conventional dosage forms. Several difficulties are faced in designing controlled release systems for better absorption and enhanced bioavailability. One of such difficulties is the inability to confine the dosage form in the desired area of the gastrointestinal tract (Groning et al., 2019). Drug absorption from the gastrointestinal tract is a complex procedure and is subject to many variables. It is widely acknowledged that the extent of gastrointestinal tract drug absorption is related to contact time with the small intestinal mucosa. Thus small intestinal transit time is an important parameter for drugs that are incompletely absorbed.

2. MATERIALS AND METHODS

2.1Materials

Selected drug Roxatidine acetate as gift sample was received from Cipla Limited, Mumbai and others reagents were used analytical grade.

2.2 Compatibility study

The compatibility study of drug and excipient was studied by using FTIR and Differential Scanning Calorimetry (DSC) method details of procedure discussed below (Shukla et al., 2019).

2.2.1 Differential scanning calorimetry studies

Thermal analysis was performed using Mettler Toledo DSC-912 system with a differential scanning calorimeter equipped with a computerized data station. The sample of pure drug, physical mixture of drug and polymer and mixture of polymers was weighed and heated at a scanning rate of 10°C/min between 40 and 200$^{\circ C}$ and 40 ml/min of nitrogen flow. The differential scanning calorimetry analysis gives an idea about the interaction of various materials at different temperature.

2.2.2 FTIR Spectroscopy

The dry sample of Roxatidine acetate was mixed with FTIR grade KBr in the ratio of 1:100. This mixture was compressed in form of a pellet by applying 10 tons of pressure in hydraulic press. The pellets were scanned over wave number range of 4000 to 400 cm-^{1}. FTIR instrument and spectral analysis was done.

2.3 Preparation of floating *in situ* gelling polymeric solution

Chitosan and *vigna mungo* at different concentrations (1 to 3.5% w/v) was prepared in half volume of deionised water containing calcium chloride (0.05 to 0.2% w/v) and sodium citrate (0.5% w/v).This solution was heated to 60°C with stirring. After cooling below 40°C; add another one third quantity of deionised water containing HPMC K100M (0.75 to 1.5 % w/v) with continuous stirring. Add drug (220 mg/10 ml), sodium bicarbonate (0.5 to 1.5% w/v) and remaining excipients to above mixture and make up volume with water. The resulting *in-situ* gelling solution containing Roxatidine acetate was finally stored in amber colour narrow mouth bottles until further use. All batches were prepared in triplicate (Dave et al., 2018).

2.3.1 Physical appearance of the *in-situ* solution

The sample of Roxatidine acetate was studied for organoleptic characters such as color, odour and appearance.Various formulations were prepared using chitosan and *vigna mungo* 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).

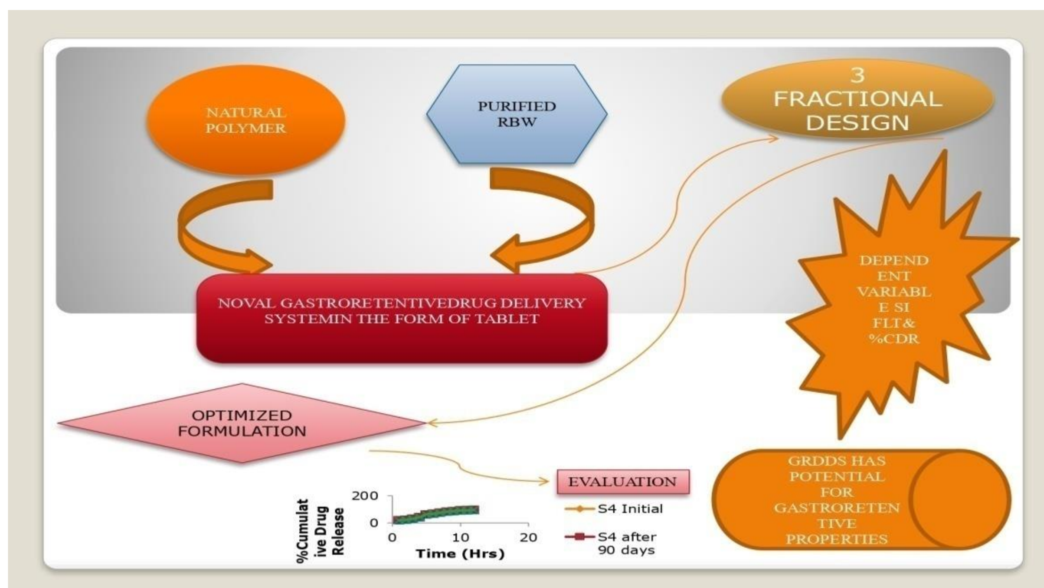

Figure: 2. Schematic representation for preparation of gastro retentive in situ gel of Roxatidine acetate.

Table:2. Formulation of chitosan (S 1 to S6) and *vigna mungo* l (G 1 to G6) batches

Ingredients(%W/V)	S1	S2	S3	S4	S5	S6	G1	G2	G3	G4	G5	G6
(RXD) (mg/10ml)	220	220	220	220	220	220	220	220	220	220	220	220
Chitosan/*vigna* Mungo	1.0	1.5	2.0	2.5	3.0	3.5	1.0	1.5	2.0	2.5	3.0	3.5
Calcium chloride	0.15	0.15	0.15	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	0.5	0.5
HPMC K 100M	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Sodium bicarbonate	1	1	1	1	1	1	1	1	1	1	1	1
Sodium Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Sodium saccharine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Water q.s	100	100	100	100	100	100	100	100	100	100	100	100

2.3.2 Batches for selection of gel forming polymer and its concentration range.

The batches were prepared by using different concentration of chitosan and *vigna mungo,* as mentioned in Table-2 respectively.

2.3.3 Selection of working range of critical excipient concentration

The selection of working range of excipients were evaluated using chitosan (1 to 3.5% w/v) calcium chloride, HPMC K100 M and Sodium bicarbonate as described in Table-2, These selected excipients were used for the development of *in-situ* gel formulations. The best critical concentration of excipients was selected on the basis of physical parameters of *in-situ* gel formulation and on the basis of its effect on drug release pattern.

2.3.4 Evaluation of preliminary batches

Various formulations were prepared using chitosan and *vigna mungo* 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).

2.3.5 *In-vitro* gelling capacity

To evaluate the formulations for their *in-vitro* gelling capacity by visual method, solutions of *in-situ* gelling formulations were prepared. The *in-vitro* gelling capacity of prepared formulations was measured by placing 5 ml of the gelation solution in 500 ml 0.1N HCl (pH 1.2) maintained at 37± 1ºC temperature. The formulation was transferred at the surface of fluid using pipette by placing it at surface of fluid and slowly released from the pipette. The *in-situ* gel solution when comes in contact with gastric fluid gelation process start. It was immediately converted into stiff gel like structure. The gelling capacity of solution was evaluated on the basis of stiffness of formed gel and time period for which they formed gel remains as such. The *in-vitro* gelling capacity was graded in three categories on the basis of

gelation time and time period for which formed gel remains (Debnath et al., 2011).
(+) Gels after few minutes, dispersed rapidly
(++) Gelation immediate remains for less than 12 h
(+++) Gelation immediate remains for more than 12 h.

2.3.6 Determination of viscosity
The viscosity of *in-situ* gelling formulations was determined at 25°C with Anton Paar Rheometer with probe using 1 ml aliquot of the sample. Measurements on each value were performed in triplicate at a fixed shear rate of 50 (1/sec) using Rheoplus software (Shukla et al., 2019).

2.3.7 pH measurement
pH of preliminary batches was measured using a digital pH meter (Shukla et al., 2019).

2.3.8 Determination of drug content
Accurately 10ml of *in-situ* gelling formulation from different batches (equivalent to 220 mg of Roxatidine acetate) were measured and transferred to 50 ml of volumetric flask. Then after 20-30ml of 0.1N HCl were added. Above solution was subjected to magnetic stirring for 30 min followed by 30 min sonication. Followed by volume was adjusted to 50 ml and filtered using Whatman Filter Paper (No 41). From this solution, 1 ml of sample was withdrawn and determines drug content.

2.3.9 Measurement of gel strength
Prepared *in-situ* gel formulation of gel strength was determined by using Anton Paar Rheometer. The prepared *in-situ* gel formulation was taken in gastric fluid (o.1N HCL) at 37°C. The *in-situ* gel was determined using probe load measurement method. Probe of reometer was inserted in prepared *in-situ* gel solution that measured the change in load of a probe in terms of gel strength. The stress strain plots were used to demonstrate values of gel strength using Rheoplus software. Stress point showing a sudden decrease of stress after increasing strain values taken as gel strength.

***2.3.10 In vitro* floating ability**
Floating study of *in-situ* gelling solution was carried out in 500 ml of 0.1N HCl (pH 1.2) in a beaker. Accurately measured 10 ml of solution was added to HCl with mild agitation. Time required for the gels to float on the surface after adding the solution (floating lag time) was measured.

2.3.11 Gel Density
The densities of the formulations were measured by calculating the mass and volume of the gels formed for each of the formulation. The polymeric solution converted to form stiff gel when it comes in contact in acidic buffer. Form the gels, the mass of gel was recorded using weighing balance; gel transferred to measuring cylinder and the volume was noted.

2.3.12 Water Uptake Test

Water uptake test was measured by using 20 ml of 0.1N HCl, 10 ml of *in-situ* solution and was transferred for sol to gel transition. The excess buffer was removed through sieving. Later the stiff formed gel was weighed using the electronic weighing balance. The initial weight was noted. Further, the gel obtained was put in 10 ml distilled water for 1 h and weighed again. The continuous process of replacing the water after every hour along with weighing of the gel was continued till 6 h. The final weight of the swollen gel was reported.

2.3.13 *In-vitro* drug release
The drug release study from the *in-situ* gel was done using USP Type II Dissolution Apparatus. 10 ml of *in-situ* solution was taken equivalent to 220 mg of the dose of Roxatidine acetate. The 10 ml solution was put in the dissolution basket using measuring cylinder containing 900ml of 1.2pH buffer (simulated gastric fluid). The temperature was set at 37°C and rpm at 50. The drug release was studied up to 12h. 5 ml sample was withdrawn at every hour and the content was determined by measuring absorption at 223nm using UV spectrophotometer.

2.3.14 Release kinetic study
To analyze the mechanism of drug release from the *in-situ* gel, data obtained from the drug release studies from optimized batch was subjected to different kinetic model treatments (Zero order, First order, Matrix, Hixson-Crowell and Korsemeyer-Peppas.). The correlation coefficient (r^2) was used as an indicator of the best fitting for each of the models considered.

2.3.15 Stability studies
In any rational design and evaluation of dosage forms for drugs, the stability of the active component must be major criteria in determining their acceptance or rejection. Stability studies were carried out as per ICH Q1A (R2) guidelines. The optimized Roxatidine acetate *in-situ* gelling

formulations were subjected to stability studies (Mishra et al., 2007).

2.3.15.1 Stability protocol

a) Types of packaging material

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

b) Storage condition.

The formulations were subjected to stability as per ICH guidelines.

Table:3. Stability storage conditions

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

2.3.15.2 Stability study by HPLC

The optimized formulations were subjected to HPLC study and observation for any changes in retention time was noted.

2.3.15.3 Determination of drug content by HPLC

Accurately, 10 ml of *in-situ* gelling optimized formulations (equivalent to 220mg of Roxatidine acetate) were taken and transferred to 100 ml of volumetric flask. To this 50-70ml of methanol was added. Above solution was subjected to magnetic stirring for 30 min followed by 30 min sonication. Volume was adjusted to 100 ml. and filtered using Whatman Filter Paper. From this solution, 1 ml of sample was withdrawn and diluted to 50 ml methanol. Triplicate dilutions of each concentration of drug were prepared. These diluted drug solution was injected into the HPLC system. Detection of Roxatidine acetate was performed with the UV detector set at 223nm

3. RESULT AND DISCUSSION

The compatibility study of drug and excipient was studied by using FTIR and Differential Scanning Calorimetry (DSC) method details of procedure discussed below

3.1 Compatibility test for drug in formulation blend using DSC studies

The DSC thermo gram of pure drug and formulation blend is shown in the Figure 3. Figure depicts the DSC thermo gram of Roxatidine acetate (A) Chitosan polymer (B), *vigna mungo* (C), optimized formulation (S4 & G4) blend (D) & (E). Differential Scanning Calorimetry studies indicated a sharp endothermic peak at 131.6°C corresponding to melting of pure Roxatidine acetate. The peaks broadening of drug in the samples B and C indicated the pure form of gelling agents. From observation of DSC spectra of samples D and E the relative intensities were changed due to dilution. It indicated that drug didn't form a complex with the excipients and endothermic peaks remained unchanged in position. The interpretation of DSC spectra represented that the excipients and drug do not interact with each other in the formulation (Shukla et al., 2020).

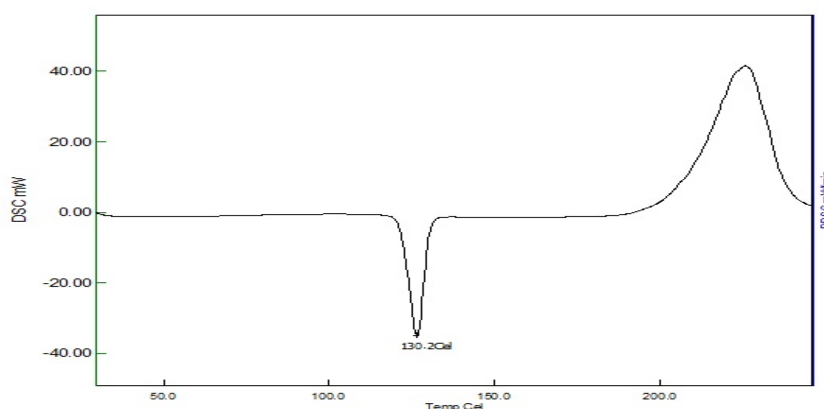

Figure: 3. DSC Thermogram of Roxatidine acetate

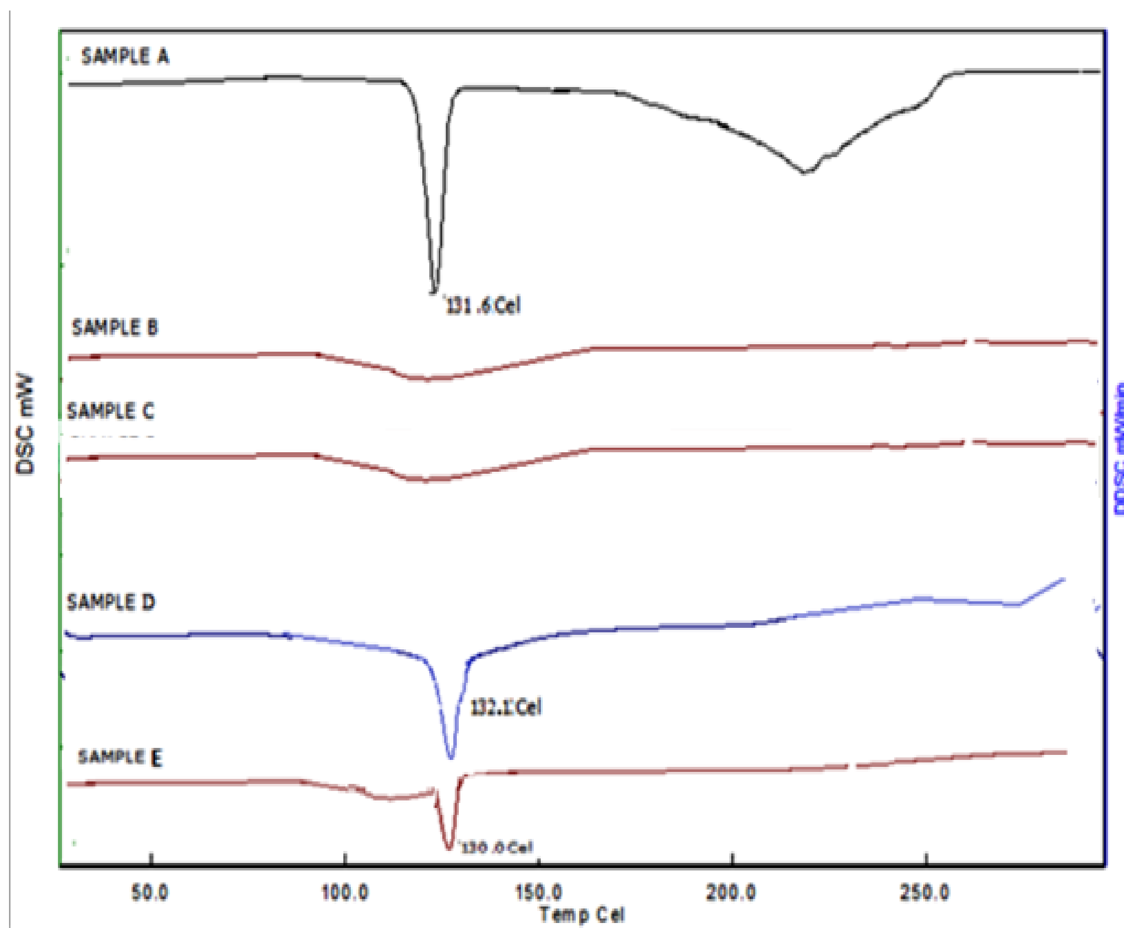

Figure: 4. DSC Thermogram of (a) Roxatidine acetate, (b) Chitosan(c) *vigna mungo* (d) & (e) Optimised formulation (S4 & G4) blend.

3.2-*In-vitro* gelling capacity

In the preliminary trial batches Roxatidine acetate *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 12h, (+++) Gelation immediate remains for more than 12 h The observations in the formulations were noted as shown in Table -4. From Table 4.batches having desired results with respect to *in-vitro* gelling capacity were taken into account and further studies were carried out.

3.3-Determination of viscosity

All preliminary batches were evaluated for viscosity. Viscosity for chitosan and *vigna mungo* containing formulation varies from 253.8 cps to 371.0cps and 353.2cps to 370.0cps respectively (Yadav et al., 2020).

3.4-pH measurement

The pH of all the formulations was found to be in the range of 6.3 to 6.8 respectively.

3.5-Determination of drug content

All preliminary batches were evaluated for drug content (Table 9). Drug content for chitosan and *vigna mungo* containing formulation varies from range 98.06 to 101.3% and 97.06 to 100.3 % respectively. From the results of tables 4 indicated that *vigna mungo* does not show appreciable drug content. It was decided that these batches would be critically evaluated for further parameters and noted.

3.6-Measuremen of gel strength

The *in-situ* gel strength of all batches was determined by Antoh Pear Rheometer. Selected all batches from preliminary studies were subjected to gel strength determination (dyne/cm^2). All gel strength measurements were performed in triplicate with good reproducibility as shown in Table-4. All the gels showed good gelling strength.

Table: 4. Result of evaluation parameter of in situ gel formation

Batches	In vitro gelling capacity	Viscosity (cps)	pH	Drug content (%)Mean ±SD,(n=3)	Gel strength(dyne/ cm^2)	Buoyancy Test	Gel Density (gm/cm3)	Water Uptake (%)
S 1	- - -	253.8±0.752	6.5	101.3±0.5315	30.50±0.5477	> 15 hr	0.661± 0.11	22.141
S 2	+ + -	260.7±0.816	6.5	98.53±0.3321	31.33±0.5164	> 15 hr	0.683± 0.18	25.30
S 3	+ + +	282.8±2.137	6.5	98.11±0.3441	34.50±0.5477	> 15 hr	0.698± 0.06	27.92
S 4	+ + +	311.5±2.345	6.5	99.38±0.3321	39.33±0.8165	> 15 hr	0.738± 0.04	36.561
S 5	+ + +	331.3±1.871	6.5	99.48±0.2139	33.33±0.5164	> 15 hr	0.771± 0.20	37.712
S 6	+ + +	371.0±0.894	6.5	98.06±0.2139	37.17±0.7528	> 15 hr	0.8671± 0.13	43.378
G 1	- - -	253.2±1.772	6.5	100.3±0.5201	29.83±1.472	> 15 hr	0.610± 0.17	20.410
G 2	+ - -	257.2±4.665	6.5	98.53±0.3668	30.33±1.211	> 15 hr	0.633± 0.06	23.163
G 3	+ + -	279.0±2.683	6.5	98.12±0.3366	33.00±1.095	> 15 hr	0.648± 0.04	25.273
G 4	+ + +	308.8±2.041	6.5	99.39±0.3364	33.00±2.098	> 15 hr	0.678± 0.03	34.424
G 5	+ + +	324.8±9.390	6.5	99.48±0.2160	33.00±0.8944	> 15 hr	0.721± 0.05	36.291
G 6	+ + +	370.0±2.757	6.5	97.06±0.2020	36.33±1.033	> 15 hr	0.817± 0.11	40.201

3.7. *In vitro* floating ability

The floating ability of all the selected formulation was found to be more than 15h. All the *in-situ* gel formulations exhibited good floating ability. The results of floating evaluation indicated that calcium carbonate and gelling excipient concentration was suitable to float and gelling formation in 900ml of simulated gastric fluid at 37 ± 0.5^{0}C.

3.8. Gel density

The gel strength of selected all batches were found to be under limit as per official method.

3.9. Water uptake test

The water uptake test of selected all batches were found to be under limit as per official method.

3.10. *In-vitro* drug release study

The Optimization Batches S4 & G4 drug release rate was retained up to 12 hours. The floating lag time of optimised formulations was 18 sec and the floating time was over 12hours. The rise in concentrations of complexing agent (calcium chloride) and thickening agent (HPMC K100 M) indicated that prolonged drug release Raises the gas forming agent concentration and that the drug release from formulations has decreased continuously. It was observed that the best fit model for optimised formulations of S4 & G4 were fit for Korsmeyer-Peppas model (R^2=0.9956, n value=0.3699; R^2=0.9984, n value=0.3767) and was implies that the optimised formulations patterns of release were preceded by Fickian diffusion (Shukla et al., 2022).

3.11. Release kinetic study

The optimized formulations were subjected to various mathematical models to understand the drug release pattern. The study was carried out using PCP-Disso-v3software.The value of the coefficient of regression suggested the best fit kinetic model. The best fitted kinetic model was found to be korsmeyer- peppas model. In the present study diffusion value of exponent is 0.3767 for *vigna mungo*& 0.3699 for Chitosan therefore release of the formulations is mainly by Fickian transport. It indicates that the drug release is controlled by diffusion process.

Table: 5. Various model fittings for optimized formulation of *vigna mungo* and chitosan

Model	S4		G4	
	R^2	K	R^2	K
Zero order	0.9308	10.0736	0.923	11.0756
1st order	0.9829	-0.3430	0.9726	-0.3530
Matrix	0.9870	19.5021	0.9860	19.5614
Korsmeyer Peppas	0.9984	21.9898	0.9959	22.0128
Hixson Crowell	0.9396	-0.607	**0.9897**	**-0.506**

Table: 6. Model and its parameters for optimized formulation of *vigna mungo* and chitosan

Best fit Model Korsemeyer-Peppas model	**S4**	**G4**
Parameters for Korsmeyer-Peppas equation	*n*= 0.3767 – fickian transport k = **27.9898**	n= 0.3699 – fickian transport **k = 28.0112**

Table: 7. Interpretation of diffusion release mechanisms from polymeric films of *vigna mungo* and chitosan

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	*t-0.5*
0.5 < n < 1.0	Anomalous transport	*tn-1*
1.0	Case-II transport	*Zero order release*
Higher than 1.0	Super Case-II transport	*tn-1*

3.12. Stability studies

The optimized batch formulations S4 & G4 were subjected to stability study as per ICH guidelines at the following condition of 40°C/75 % RH for 3 months. These formulations were evaluated by using following parameters such as physical appearance, pH, drug content and area under curve study etc.

3.12.1 Physical appearance

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

3.12.2 pH measurement

The pH of the optimized formulations were found to be unchanged before (pH 6.9) and after (pH 6.9) stability studies

3.12.3 *In-vitro* drug release study

Drug release profile of optimised formulation (*vigna mungo* and chitosan) after stability study is shown in Figure-6-7.

Table: 8. Drug release profile of optimized formulation of S4 and G4 before and after 90 days

Time (Hrs)	S4		G4	
	Initial	after 90 days	Initial	after 90 days
1	20.01	20.20	19.91	20.10
2	23.80	24.00	22.80	23.00
3	30.69	31.10	31.69	32.00
4	42.79	43.10	41.59	42.10
5	60.89	61.20	62.00	62.80
6	67.77	68.00	66.74	67.20
7	75.77	76.10	75.16	76.00
8	81.91	82.10	82.31	83.20
9	85.56	86.04	86.66	87.10
10	92.16	93.00	91.16	92.10
11	93.62	94.00	92.62	93.10
12	95.62	96.10	94.55	95.20

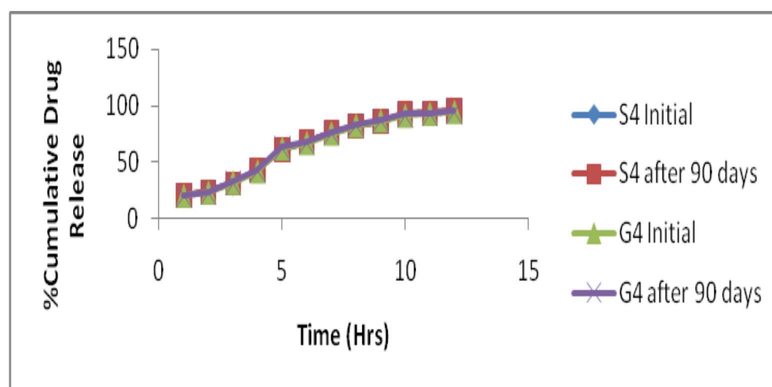

Figure: 5. Drug release profile study of optimized formulation of *vigna mungo*

It can be observed that optimised formulation S4 showed 95.62% release before stability study (0 day) and showed 96.10% release at 12 h after stability studies (90 days.). While the formulation G4showed 91.16% release before stability study (0 day) and showed 92.10% release at 12 h after stability studies (90 days.). It was concluded that there was significant similarity (G4 value more than 50) in release profiles of both the formulations at initial and after 90 days. So the formulations were found to be stable. (Basavaraj et al., 2018).

3.12.4 Stability study by HPLC

The optimised formulations S4 & G4 were subjected to HPLC study and observed for any changes in retention time. The chromatograms are shown below.

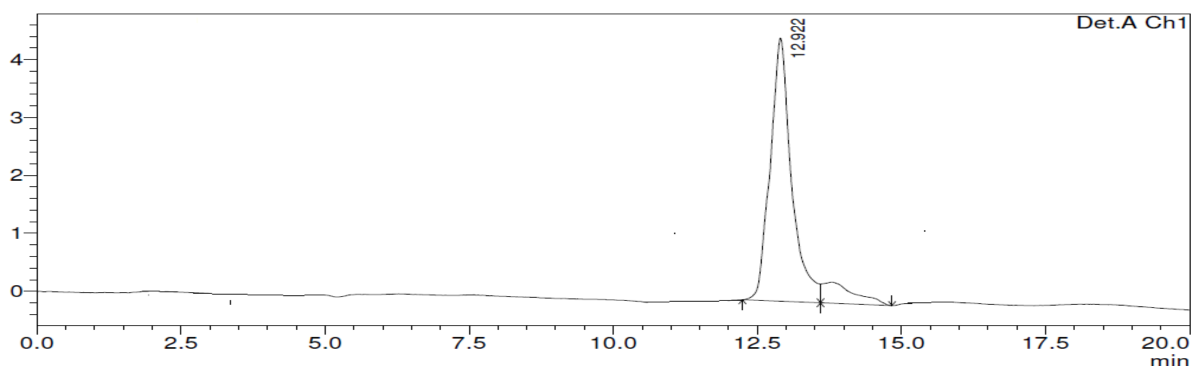

Figure: 6. Chromatogram of optimised formulation S4 of Chitosan.

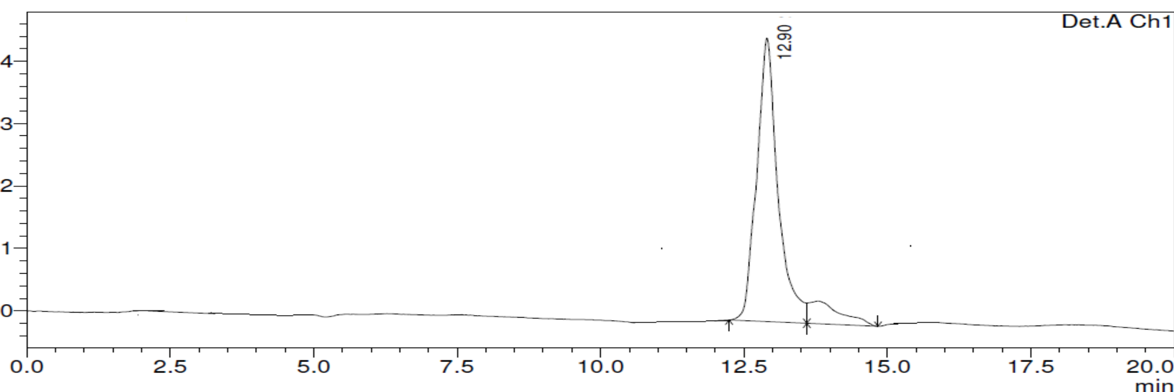

Figure: 7. Chromatogram of optimised formulation G4 of *vigna mungo*

From the above chromatograms it can be concluded that there is no significant change (0.02 min) in the retention time for optimised batch formulations. So the formulation was found to be stable after 3 months.

3.12.5 Determination of drug content by HPLC

Drug content was determined for optimised formulations of S4 & G4 after 90 days. The drug content was determined on the basis of HPLC study.

Table: 9. Drug content for optimised formulation S4 and G4 before and after stability study

Formulations	Days	AUC	Tailing factor	Drug content (%)
S4	0	324878	1.765	99.43%
	30	324047	1.769	99.32%
	60	324062	1.767	99.21%
	90	324064	1.759	99.29%
G4	0	324876	1.761	99.01%
	30	324040	1.669	99.46%
	60	324052	1.669	99.82%
	90	324061	1.748	99.34%

The *in-situ* gelling formulations were prepared using two separate Chitosan polymers and *vigna mungo*, as gelling polymers. Changes in amounts of Chitosan (1-3.5 per cent w/v), *vigna mungo* (1-3.5 per cent w/v), and calcium chloride (0.05-0.2 per cent w/v) was used in different formulations. Based on their effects on release trends and contingent reactions, sodium bicarbonate (1% w/v) and HPMC K100 M (1.25% w/v) levels were set. The stability of Roxatidine acetate optimised formulations S4 & G4 as a solution showed no changes in appearance, drug content, dissolution profile and HPLC chromatograms observed that Roxatidine acetate *in-situ* floatable gelling method was stable at 40ºC/75 percent RH for three months.

Finally, the current research has succeeded in designing, planning and testing oral continuous release of floating Roxatidine acetate gelling device with stomach-specific drug delivery, preserving the release of drugs and achieving gastric preservation for the required duration. This study reports that oral administration of aqueous solutions of Roxatidine acetate containing chitosan and *vigna mungo* results in formation of *in-situ* gel at the stomach site. chitosan and *vigna mungo* were selected as best suitable polymer on the basis of evaluation parameters. Optimized batches were also evaluated for viscosity and gel strength determination Formulation F6 (2.5% w/v chitosan and 2.5% w/v *vigna mungo*) was found to be the optimized formulation. The release of Roxatidine acetate retarded significantly with an increase in drug concentration. The prepared formulation can provide a site-specific delivery of Roxatidine acetate for 10 to 12 h with zero-order release kinetics. The stability study data reveals that all formulations were stable and there is no interaction among ingredients. Finally it can be conclude that chitosan and *vigna mungo* are the best suitable *in-situ* gelling polymers for GRDDS.

Future studies are needed to investigate the bioavailability of Roxatidine acetate in blood to confirm the effect of formulation on C_{max} and T_{max} of the drug for the reducing dose and dose frequency to minimize side effects after administration of Roxatidine acetate floating gastroretentive dosage form.

4. CONCLUSION

In this study, it was empirically proven that an increase in selected *vigna mungo* gum concentration led to a decrease in drug release, aligning with their respective properties. Moreover, the G4 and S4 GRDDS *in-situ* gel formulations were identified as the optimized formulations. It was based on their superior drug release profile over a period of 12 hours. The analysis of the conducted evaluations yielded statistically significant results. In summary, the formulated gastric floating *in-situ* gel exhibited sustained release characteristics for duration of 12 hours, potentially augmenting the drug's bioavailability and improving patient adherence through its innovative development.

5. ACKNOWLEDGEMENT

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6. CONFLICT OF INTEREST

There were no instances of conflicts of interest associated with the dissemination of this research endeavor.

ABBREVIATIONS

Gastroretentive drug delivery (GRDDS), Fourier transform infrared (FTIR), High Performance Liquid Chromatography (HPLC), Differential Scanning Calorimetry (DSC).

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