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Research Article

Formulation and evaluation of floating *in situ* gel of Nizatidine

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ABSTRACT

The present investigation concerns with the development and optimization of an floating *in-situ* gelling formulation of Nizatidine. It is H2-receptor antagonist absorbed from the upper gastrointestinal tract and it is preferentially localized in parietal cells of gastric mucosa. The polymer used in the study is Sodium alginate which forms a gel when it comes in contact with simulated gastric fluid. The principle of gelling involves supply of complexed calcium ions in form of calcium carbonate that are released in the acidic environment of the stomach. The formulations are designed by using 3^2 full factorial design. Sodium alginate sols of various concentrations were prepared by dissolving variable amount of sodium alginate in deionized water. In sodium alginate solution variable amounts of calcium carbonate were added so as to obtain nine different formulations. The amount of Nizatidine is kept con- stant for all nine formulations. The F5 formulation showed optimum drug release. The gels were evaluated with respect to *In Vitro Drug Release* Appearance of gel, Viscosity of In Situ Gelling Solution, in vitro Floating Study, Content uniformity and pH Measurement. Stability study was done according to ICH guidelines. This study reports that the aqueous solutions of nizatidine containing sodium alginate forms in situ gel in acidic environment. The results of a 3^2 full factorial design revealed that the concentration of sodium alginate and concentration of calcium carbonate significantly affected on the dependent variables like viscosity, floating lag time, drug release The *in-vivo study* shows significant anti-ulcer effect of alginate based in situ gel of nizatidine.

Keywords: Nizatidine; sodium Alginate; *In-situ* gel; hydroxyl propyl methyl cellulose

INTRODUCTION

The basic goal of therapy is to achieve a steady state blood level that is therapeutically effective and non toxic for an extended period of time. The design of proper dosage regimens is an important element in accomplishing this goal.

Sustained release, sustained action, controlled release, extended action, timed release, depot and repository dosage forms are terms used to identify drug therapy systems that are designed to achieve a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose.

Sustained release systems include any drug delivery system that achieves slow release of drug over an extended period of time The design of oral sustained release delivery systems is subject to several inter related variables of considerable importance such as the type of delivery system, the disease being treated, the patient, the length of therapy and the properties of the drug. Various approaches have been made to prolong the retention time of dosage form in the stomach. Retention of drug delivery system with prolonged overall gastrointestinal transit time and slow but complete release in the stomach improves bioavailability of drugs that have site specific absorption from stomach.

The gastro-retentive formulation can be retained in the stomach to aid in improving oral prolonged delivery of the drugs that have an absorption window in particular area of gastrointestinal tract. Hence, such system helps in continuously releasing the drug while reaching the absorption window, ensuring maximum bioavailability. These considerations have led to the development of oral sustained release (SR) dosage forms possessing retention capabilities. There are different approaches such as bioadhesive system, swelling and expanding system, floating system and delayed gastric emptying system have continuously releasing the drug while reaching the absorption window ensuring maximum bioavailability (Brahmankar D et al., 2009; Chein Y.W).

In Situ Gel Forming Systems

In situ gel forming polymeric formulations are drug delivery systems that are in sol or suspension form before administration in the body, but once administered, undergo gelation *in situ*, to form a gel. *In situ* gel forming systems have been widely investigated as vehicles for sustained drug delivery. This interest has been sparked by the advantages shown by *in situ* form-

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ing polymeric delivery systems such as ease of administration and reduced frequency of administration, improved patient compliance and comfort (Mishra B. et al., 2008).

Principle of *in situ* gel formation

Formulation of gastroretentive insitugel system involves the use of gelling agent which can form a stable sol/suspension system to contain the dispersed drug and other excipients. The gelling of this sol/suspension system is to be achieved in gastric environment, triggered by ionic complexation due to change in pH.

The formulation adopted is a sodium alginate solution containing calcium carbonate (as a source of Ca^{2+}) and sodium citrate, which complexes the free Ca^{2+} ions and releases them only in the acidic environment of the stomach.

Sodium alginate acts as a gelling agent. The free Ca^{2+} ions gets entrapped in polymeric chains of sodium alginate thereby causing cross linking of polymer chains to form matrix structure. This gelation involves the formation of double helical junction zones followed by reaggregation of the double helical segments to form a three-dimensional network by complexation with cations and hydrogen bonding with water.

Sodium citrate + Ca. carbonate (Ca^{2+}) Ca. citrate complex $\xrightarrow[\text{environment}]{\text{acidic}} Ca^{2+} + COO^-$

In this way, the formulation remains in liquid form until it reaches the stomach, where gelation of sodium alginate is instantaneous. (Mishra B. et al., 2008).

EXPERIMENTAL

Materials

Nizatidine was purchased from : Lupine Ltd. Pune Maharashtra. Hydroxy propyl methyl cellulose from Colorcon Ltd., Goa, India, chitosan from Research-Lab, Mumbai, Sodium alginate from FDC Pharma. India.

Melting point determination

Melting point of Nizatidine was determined by taking a small amount of sample in a capillary tube closed at one end and placed in Thiele's melting point apparatus. Melting point was also determined by melting point apparatus. The melting point and range was noted.

UV Spectroscopy

Determination of λmax

The UV spectrum of nizatidine was obtained spectrophotometrically (UV-1800, Shimadzu, Japan). Accurately weighed 10 mg of the drug was dissolved in sufficient quantity of distilled water (DW) and volume made up to 10 ml. The stock solution was diluted to obtain a concentration of 100 µg/ml. 2 ml of aliquot was withdrawn and volume was made up to 10 ml using DW to obtain the concentration of 20 µg/ml. The resultant solution was scanned from 200 to 400 nm and the spectrum was recorded to obtain the value of maximum wavelength.

Preparation of Calibration Curves

Accurately weighed 10 mg of nizatidine was dissolved in 0.1N HCl and volume was made up to 100 ml in volumetric flask. The solution was further diluted with 0.1N HCl to obtain solution of 5 to 25 mcg/ml. Absorbance of each solution was measured using UV-Visible double beam spectrophotometer and 0.1N HCl as reference standard. The standard curve was generated for the entire range from 5 to 25 mcg/ml.

Experimental Design

A 3^2 full Factorial design was constructed where the amounts of Sodium alginate (X_1) & Calcium carbonate(X_2) were selected as factors.The levels of the two factors were selected on the basis of the preliminary studies carried out before implementing the experimental design. All other formulation and processing variable were kept constant throughout the study (Narkar M et al., 2010; Vaithiyalingam S et al., 2002).

Table 1: Factor combination as per the experimental design for Nizatidine

Formulations	Coded value	
	X1	X2
F1	0	1
F2	-1	-1
F3	-1	0
F4	0	0
F5	1	0
F6	1	-1
F7	1	1
F8	-1	1
F9	0	-1

Table 2: Translation of experimental conditions into physical units for Nizatidine

Coded Levels	Actual Levels	
	X_1 (%)	X_2 (%)
-1	0.5	0.375
0	1	0.5
1	1.5	0.625

X1= conc. of Sodium alginate

X2= conc. of Calcium carbonate

Method of Preparation of Floating *In Situ* Gel

All the ingredients used in the formulation were initially passed through sieve #60 before mixing. The required quantity of nizatidine and Sodium alginate were weighed according to the formulation. Sodium alginate sols of various concentrations were prepared by dissolving specified amount of sodium alginate in 100ml distilled water containing sodium citrate The appropriate amounts of calcium carbonate and nizatidine were

added. Various quantities of other ingredients used for preparation were mentioned in Table no.3.

EVALUATION OF Floating *In Situ* Gel

Physical appearance and pH

All the prepared alginate based in situ solutions of nizatidine were checked for their clarity and the time required for gel formation. The pH was measured of insitu solutions of nizatidine using a calibrated digital pH meter. All measurements of pH were made in triplicate

Measurement of Viscosity

The rheological properties of the solutions are of importance in view of their proposed oral administration. The viscosities of the prepared solutions were determined by Brook field viscometer.

***In Vitro* Floating Study**

Determination of Floating Lag Time

The floating lag time is defined as the time taken by the gel to reach the top from the bottom of the dissolution flask. The floating lag time of gel was determined by visual inspection using a dissolution test apparatus USP XXII type (Electrolab, EDT08, India, paddle method)containing 900 ml of 0.1N HCl at 37±0.5 °C. The readings were taken in triplicate. (Rajinikanth P et al., 2007; Shi-lei C et al., 2007).

Determination of Duration of floationg

The time for which the formulation floats constantly on the surface of the medium is known as the duration of floating. The duration of floating of gels was determined by using a dissolution test apparatus USP XXII (Electrolab, EDT08, India, paddle method)containing 900 ml of 0.1N HCl at 100 rpm at 37±0.5 °C. The readings were taken in triplicate. (Rajinikanth P et al., 2007).

Content uniformity

Accurately measured 10 ml of *in-situ* gel was transferred to 100 ml of volumetric flask. To this 70 ml of 0.1N HCL was added and shake for 30 min, followed by sonication for 15 min. Complete dispersion of contents were ensured visually and volume was made up to 100 ml with 0.1N HCL & filtered using whatman filter paper. From this solution, 10 ml of sample was withdrawn and diluted to 100 ml with 0.1N HCL. Contents of nizatidine was determined spectrophotmeterically at 313.5 nm using double beam UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan**)**

***In Vitro* Drug Release Study**

The drug release profiles of Nizatidine loaded *In Situ* Gel were carried out in 0.1 M HCl solution (pH 1.2, 900 ml) at 37±0.5 °C using a USP XXII dissolution test apparatus (Electrolab, EDT08, India, paddle method). The rotation speed of the paddle was adjusted to 100 rpm for homogenous dispersion of gel in dissolution medium. An aliquot of 5 ml was withdrawn periodically from test solution and replaced with 5 ml of fresh medium maintained at 37±0.5 °C and assayed spectrophotometrically at 313.5 nm (UV-1800, Shimadzu, Japan) for 12h. All drug release profiles were performed in triplicate. Percentage drug release was calculated using PCP Disso Software (Poona College of Pharmacy, Pune). In order to investigate the drug release mechanism, the release data were fitted to models representation zero order, first-order, Higuchi and Peppas–Korsemeyer equation. (Narkar M et al., 2010; Gattani S et al., 2010).

Stability study

The optimized formulation sealed in vial with rubber cap and kept in humidity chamber maintained 40 ± 2 °C / 75 ± 5 % RH for 3 months. After 30, 60, 90 days samples retrieved and analyzed for the drug content, *in vitro* drug release, pH and viscosity (Table 5).

RESULTS AND DISCUSSION

Characterization of Nizatidine

The characterization of drug was done by the melting point, UV, IR Spectroscopy

Melting point determination

The melting point of Nizatidine was found to be 133^0C as measured by melting point apparatus.The reported melting point range for Nizatidine 133-135^0C

Spectrophotometric characterization of Nizatidine

Determination of λ_{max} of Nizatidine

λ_{max} of Nizatidine in methanol was found to be 313.5 nm.

Calibration curve of Nizatidine

The UV absorption data at the wavelength 313.5 nm is shown in Table No.5.2. It was observed that Nizatidine showed good linearity (r^2 =0.9996) over the range of 5-25µg /mL Hence, calibration curves of Nizatidine were found to obey Beer-Lambert's law over this range. (Figure 1).

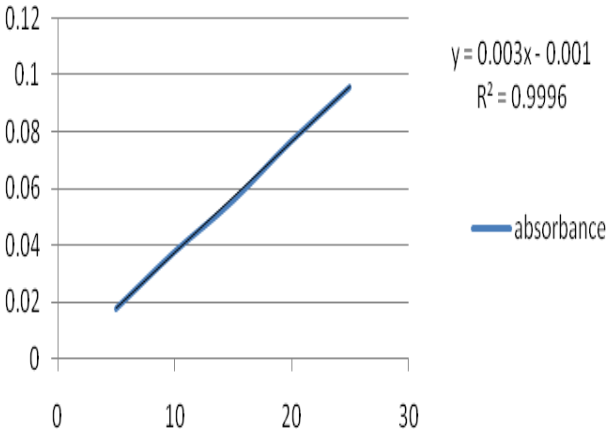

Figure 1: Standard calibration curve of Nizatidine in 0.1 N HCl

Table 3: Formulation Composition for insitu gel

Sr No.	Ingredient	Quantity in % (w/v)								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Nizatidine	3	3	3	3	3	3	3	3	3
2	Sodium alginate	0.5	0.5	0.5	1	1	1	1.5	1.5	1.5
3	Calcium Carbonate	0.375	0.5	0.625	0.375	0.5	0.625	0.375	0.5	0.625
4	Distilled water Up to	100	100	100	100	100	100	100	100	100

Each batch contain

HPMC 0.4% ,Sodium Citrate 0.25 %, Methyl Paraben 0.09%, Propyl Paraben 0.09%, D mannitol 2% and flavor 0.2 %.

Table 4: Evaluation Data for Formulations (F1-F9)

Batch	pH	Viscosity (Cps)	Floating lag time (sec)	Drug Content (%)	Drug Release(%)
F1	8.49	90	52	91.10	95.78±1.26
F2	8.65	135	38	92.52	97.58±1.83
F3	8.69	140	34	93.80	92.63±1.02
F4	8.59	150	44	95.82	97.76±1.56
F5	8.63	185	38	98.30	98.00±1.63
F6	8.54	190	30	94.22	94.55±1.09
F7	8.50	255	47	97.36	97.94±1.55
F8	8.64	265	41	98.12	94.75±1.12
F9	8.74	270	32	95.65	95.67±1.69

*Mean± S.D., (n=3)

Table 5: Result of optimized batch after three months storage

Days	% Drug release*	pH	Viscosity (Cps)	Drug Content (%)
Before Storage				
0	98.00±1.63	8.63	185	98.30
After Storage				
30	97.15±1.08	8.62	181	97.12
60	97.02±1.89	8.54	177	97.00
90	96.66±1.23	8.44	175	96.87

*All values are expressed as Mean ±SD, n = 3

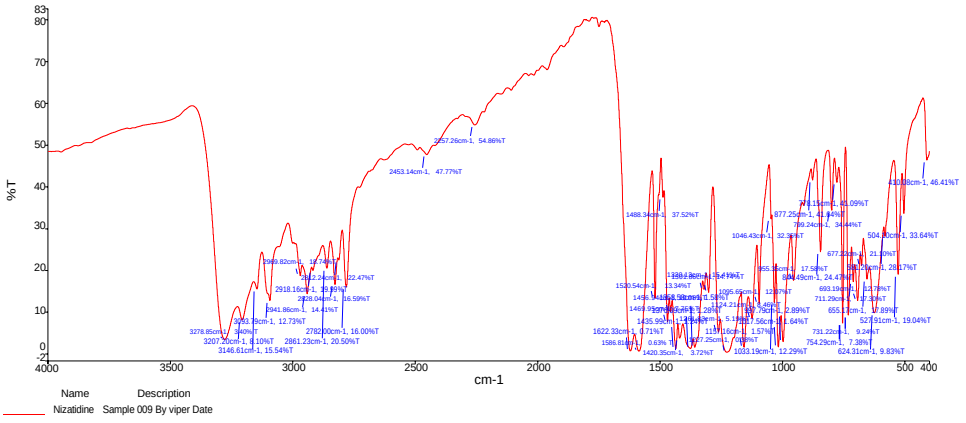

Figure 2: FTIR spectrum of Nizatidine

FTIR spectrum interpretation

The FTIR spectrum of Nizatidine is shown in Figure 2.

Viscosity

The rheological properties of the solutions are of importance in view of their proposed oral administration. The solutions showed a marked increase in viscosity with increasing concentration of sodium alginate as shown in Table 4. Increasing the calcium carbonate content in the formulation simultaneously increased the viscosity. Since the calcium carbonate is present in the formulations as insoluble dispersion, an increase in its concentration proportionally increased the number of particles dispersed, thus contributing to increased viscosity.

Physical appearance and pH

All the prepared alginate based in situ solutions of Nizatidine were checked for their clarity and the time required for gel formation. The pH was measured of in situ solutions of nizatidine using a calibrated digital pH meter at 25°C. All measurements of pH were made in triplicate and the results are given in Table 4.

***In Vitro* Floating Study**

Determination of Floating Lag Time

Floating lag times for all the formulations are depicted in Table 4. sodium alginate showed instantaneous floating when came in contact with stimulated gastric fluid. The basic mechanism behind floating was calcium carbonate is present in the formulation as insoluble dispersion and became soluble in the acidic medium. Released calcium ions and CO2 gas, caused gelation of polymer and released gas get entrapped in gel matrix, which caused the matrix system to float.

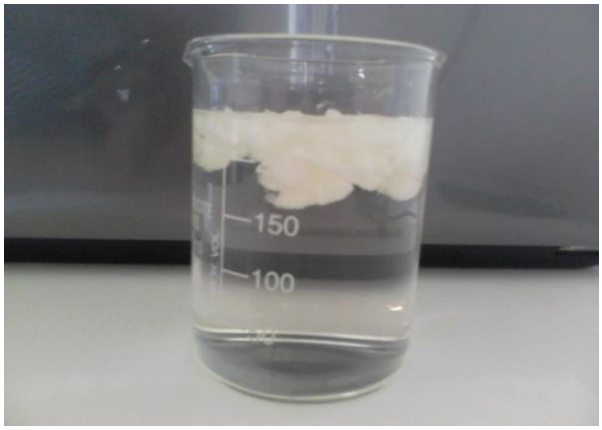

Figure 3: : In Vitro Floating Study

Determination of Duration of Floating

Duration of floating for all formulation were studied in 0.1N HCl maintained at 37.5oC at 100 RPM and are depicted in Table 4. Upon contact with acidic medium calcium carbonate effervesced, releasing carbon dioxide and calcium ions, which causes gelation and cross linking by Ca++ ions occurred to provide a gel barrier at the surface of the formulation. The released carbon dioxide got entrapped in the gel matrix producing buoyant formulation, these three-dimensional gel matrixes restrict the further diffusion of carbon dioxide and drug molecules and has resulted in extended period of floating and drug release respectively. The amount of CO2 content are responsible for the observed floating lag time and duration of floating. Similarly an increase in the polymer concentration resulted in decreased floating lag time and an increase in floating duration of the prepared systems .

Determination of drug content

The amount of nizatidine in each sample was determined by spectrophotometer

The UV absorbance of the sample was determined at a wavelength of 313.5 nm. The drug content for batches F1 to F9 are depicted in Table 4

***In Vitro* Drug Release Study**

The release of Nizatidine from floating *in-situ* gel was analyzed by plotting the % Cumulative drug release against Time (in hours).The effect of sodium alginate concentration on *in vitro* drug release from *in situ* gels is shown in Figure 4 A significant decrease in the rate and extent of drug release was observed with the increase in sodium alginate concentration in *in situ* gels. The effect of calcium carbonate concentration on *in vitro* drug release from *in situ* gels is shown in Figure 4. With increase in calcium carbonate concentration in formulations decreased percentage of drug release.

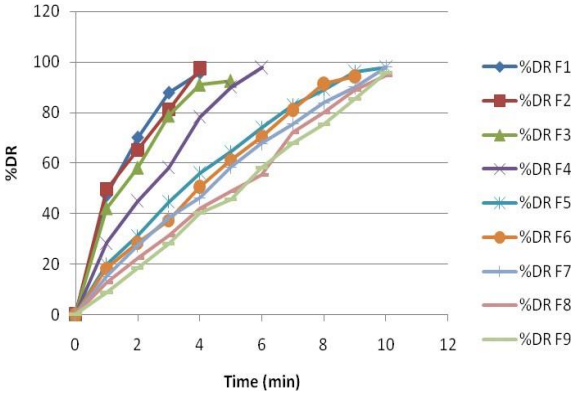

Figure 4: Percent drug release from in situ gel batches F1-F9

Stability study

The optimized formulation sealed in vial with rubber cap and kept in humidity chamber maintained 40 ± 2 °C / 75 ± 5 % RH for 3 months. After 30, 60, 90 days samples retrieved and analyzed for the drug content, *in vitro* drug release, pH and viscosity. There was no significant change in morphological condition and also in the remaining parameter during stability study.

CONCLUSION

This study reports that the aqueous solutions of nizatidine containing sodium alginate forms insitu gel in acidic environment. The results of a 3^2 full factorial design revealed that the concentration of sodium alginate and concentration of calcium carbonate significantly affected on the dependent variables like viscosity, floating lag time, drug release.

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