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FORMULATION, OPTIMIZATION AND EVALUATION OF GASTRO RETENTIVE NOVEL FLOATING IN-SITU GELLING SYSTEM OF PHYLLANTHUS NIRURI

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ABSTRACT

The oral liquid dosage forms are more prone to low bioavailability because of their quick transit from the stomach. Producing sustained release formulation of an oral liquid dosage form could be successfully augmented through a strategy of liquid in-situ gelling system. There are many advantages of in situ forming polymeric delivery systems viz. ease of administration and reduced frequency of administration, improved patient compliance and comfort. In-situ forming polymeric formulation is in sol form before administration in the body, but once administered undergoes in-situ gelation. The objective of this study was to develop a novel in-situ gel system of *Phyllanthus niruri* for treatment of peptic ulcer. The delivery system consists of varying concentrations of sodium alginate and calcium carbonate. The system was subjected to various in vitro study. In vitro drug release studies were conducted in 0.1 N HCl. The finalised formulation (F7) contained sodium alginate (0.5 % w/v), calcium carbonate (1.5 % w/v) which showed drug release of 90.7 %. The floating lag time was found to be less than 1 min and system was found to be floating throughout the drug release time of 12 h. The gelation occurred immediately after addition in acidic medium. The optimized cream showed maximum drug release and floating gel was found to give good results.

KEYWORDS: *Phyllanthus niruri*, Corilagin, Floating in situ gel, Gastroprotective, Optimization.

INTRODUCTION

In situ gel forming drug delivery systems are in principle capable of releasing drug molecule in a sustained manner affording relatively constant plasma profiles. These hydrogels are liquid at room temperature but undergo gelation when in contact with body fluids or change in pH. These have a characteristic property of temperature dependent, pH dependent and cation induced gelation. Compared to conventional controlled release formulations, in situ forming drug delivery systems possess potential advantages like simple manufacturing processes and ease of administration.[1,2]

Gastric ulcers are common pathologies that affect a significant number of people around the world. The increased incidence of gastric ulcers is associated with aggressive factors against the gastric mucosa such as ethanol exposure, stress, smoking, nutritional deficiencies and frequent ingestion of non-steroidal anti-inflammatory drugs. Although many conventional drugs are available to treat ulcers, most of these drugs have adverse reactions when used over long term. Moreover, short residence time of drug leads to incomplete eradication of gastric ulcer as there is insufficient concentration of the drug in the gastric mucous layer or epithelial cell surface. The instability of the drug in the low pH of gastric fluid can also be a reason for it. Therefore, it is necessary to design drug delivery systems that not only alleviate the shortcomings of conventional delivery system but also deliver the drug into the epithelial cells.[3-5]

Different therapeutic strategies have been studied for complete eradication of the gastric ulcer. One way to improve the efficacy in eradicating the gastric ulcer is to deliver the drug locally in the stomach. Better stability and longer residence time (gastro retentive system) will allow more of the drug to contact the gastric mucus layer. Many approaches have been reported in the literature for the formulation of gastro retentive systems. Bioadhesive systems may result in irritation of the mucous layer due to high localized concentration of the drug. In addition, single-unit systems such as tablets or capsules may exhibit the all-or none emptying phenomenon leading to variability in bioavailability. Floating in-situ gel (FIG) formulations present a novel and interesting approach to obtain gastro retentive sustained release of drugs and FIG has been developed for several drugs. This system would have the advantage of ease of administration, as being a liquid and also be more patient compliant.[6-9]

Natural products have gained powerful attention due to its effective role in chemotherapeutic agents and chronic disease prevention, including gastric ulcer. Phyllanthus niruri L (Euphorbiaceae family) is an Indian herbal medicine. The active phytoconstituents such as flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins and saponins have been identified from various parts of P. niruri. Extracts of this herb have been proven to have therapeutic effects in many clinical studies. Recently, P. niruri extract showed gastroprotective activities by mechanism of regulating pH, increase mucous production and anti-oxidant property. Tannins are the primary constituents found in leaves of P. niruri , in particular, corilagin is having excellent anti-ulcer effect along with other beneficial activities. Present research has been undertaken with the aim to formulate the floating in situ gel containing methanolic extract of Phyllanthus niruri Linn. plant for the treatment of gastric ulcer.[10-12]

The formulation was optimized for sodium alginate content and calcium carbonate content to obtain in situ floating gel with optimum performance parameters such as floating lag time and drug release using a 3^2 full factorial design.

MATERIALS AND METHODS
The plant parts (leaf, stem and root) of *P.niruri* were collected and the authenticity of the plant was confirmed by the Botanical Survey of India, Koregaon Park (Pune-411001), India. Methanolic extract of Phyllanthus niruri was purchased from Shamantak Enterprises, Pune. Sodium alginate, HPMC K15M, Sodium citrate and calcium carbonate were purchased from Loba Chemie, Bombay, India. All materials and chemicals used were of either pharmaceutical or analytical grade.

Preparation of emulsion
The accurate quantity of drug and Tween 80 were taken in mortar and water was added slowly drop by drop with continuous stirring until the homogenous emulsion was formed.

Preparation of in situ floating solution
The solutions containing various concentrations of sodium alginate (0.5-2 % w/v) were prepared in deionised water containing 0.25% w/v of sodium citrate. This solution was heated up to 70°C with stirring and allowed to cool below 40°C. After cooling, various concentrations of calcium carbonate, HPMC K 15M and drug emulsion were added in respective batches with continuous stirring to form homogeneous dispersion.

Development and formulation of floating in situ gel
Selection of experimental design
The formula was optimized by employing full factorial design. Design-Expert (Version 12; Stat-Ease Inc., Minneapolis, Minnesota, USA) was used for mathematical modeling and assessment of the responses. A 3^2 factorial design was chosen to optimize a formula which will provide floating lag time and drug release as shown in Table 1.

Table 1: Factors and levels for 3^2 factorial design of floating in situ gel formulation.

Variables		Levels		
A	Sodium alginate	-1	0	+1
		0.5	1.25	2.0
B	Calcium carbonate	0.5	1.0	1.5
Responses		**Goals**	**Acceptance range**	
X_1	Floating lag time	Maximum	<63	
X_2	Drug release	Minimum	<93.5	

3^2 full factorial design was chosen as the optimization design since it allows the comparison of all the levels and factors to obtain significant results. The responses such as floating lag time, drug release were chosen for optimization which depended mainly on quantity of polymer and the crosslinking agent. Hence these were selected as factors. The three levels give good idea about interaction between factors if any.

Evaluation parameters
1. Physical appearance and pH
All the prepared sodium alginate based in situ solutions of Phyllanthus niruri (Corilagin) were checked for their clarity and the pH of the solutions. After administered of the prepared solutions in 0.1 N HCl, pH 1.2, the time required for gel formation and consistency of gel formed was checked visually. The pH was also measured in each of the solution of sodium alginate based in situ solutions of using a calibrated digital pH meter at 25°C.

2. Viscosity of in situ gelling solutions
The viscosity of formulations was determined by a Brookfield viscometer (DV-III Brookfield, USA). The spindle (S06) was rotated at 50 rpm.

3. Floating behavior
The buoyancy lag time and buoyancy duration of the formulations were determined in simulated gastric fluid (0.1 N HCl, pH 1.2). The time in minutes taken by the formulation to emerge on the dissolution medium surface (buoyancy lag time) and buoyancy duration was noted.

4. In-vitro gelling capacity
To evaluate the formulations for their in-vitro gelling capacity by visual method, solutions of in situ gel forming drug delivery system were prepared. The in-vitro gelling capacity of prepared formulations was measured by placing five ml of the gelation solution (0.1N HCl, pH 1.2) in a 15 ml borosilicate glass test tube

and maintained at 37±1ºC temperature. One ml of formulation solution was added with the help of pipette. The formulation was transferred in such a way that places the pipette at surface of fluid in test tube and formulation was slowly released from the pipette. As the solution comes in contact with gelation solution, 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 the 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 the formed gel remains.

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

5. Drug content
Ten mL of the solution was added to 900 mL of simulated gastric fluid (0.1N HCL) and stirred for 1 h on a magnetic stirrer. The solution was filtered, suitably diluted with simulated gastric fluid and the drug concentration was determined by using a UV visible spectrophotometer (UV-1601 Shimadzu, Japan) at 268 nm against a suitable blank solution.

6. In vitro release
The release of Phyllanthus niruri (Corilagin) from the formulations was determined using a USP/24 dissolution test apparatus (Tab Machines, India) with a paddle stirrer at 50 rpm. The dissolution medium used was 900 mL of simulated gastric fluid (0.1 N HCl, pH 1.2) and temperature was maintained at 37 ± 0.2 °C. Ten mL of the formulation were kept in the dissolution vessel and simulated gastric fluid was carefully added to the vessel avoiding any disturbance of the Petri dish. At each time interval, a precisely measured sample of the dissolution medium was pipetted out and replenished with fresh medium .Phyllanthus niruri (corilagin) concentration in the aliquot was determined spectrophotometrically. Each study was conducted in triplicate.

7. Stability study
The selected formulations were packed in amber-colored bottles, which were tightly plugged with cotton and capped. They were then stored at 40ºC/75% RH for 1 month and evaluated for their physical appearance, drug excipients compatibility by FTIR.

RESULT AND DISSCUSSION
The two main pre-requisites of the floating in situ gelling systems include free flowing liquid easy for swallowing and instant gelation in acidic environment. The mechanism of gelation involves the formation of double helical junction zones followed by aggregation of double helical segments to form a three-dimensional network by complexing with cations and hydrogen bonding with water. The solution to gel transformation of sodium alginate occurs in the presence of either monovalent or divalent cations in contacts with the gastric fluids. The calcium carbonate present in the formulation as insoluble dispersion dissolves on reaction with acid and releases carbon dioxide. The in situ released calcium ions results in formation of gel. The released carbon dioxide is entrapped in the gel network of the formulation and the gel rises to the surface of the dissolution medium (in-vitro) or the stomach (in-vivo).

The gelling capacity and floating study were carried out in 100 ml of 0.1 N HCl. The batches F1-F9 prepared for the selection of polymer concentration. Batches F1-F9 contains various concentrations of sodium alginate. It was observed that as the concentrations of polymer increases, viscous nature of the formulation increases and they become more difficult to pour. The F3, F6, F9 batches showed gelation immediately that remained for 12hr. The F3, F6 and F9 batches were rejected because they were more viscous in nature as compared to batches F1, F2, F4, F5, F7 and F8. All batches showed drug content in the range of 93.74-98.17 %. F7and F8 batches were selected for in vitro dissolution study because of their high drug content and good pourability. F7 batch showed 90.32% of drug release after 8 h drug release as shown in Table 2.
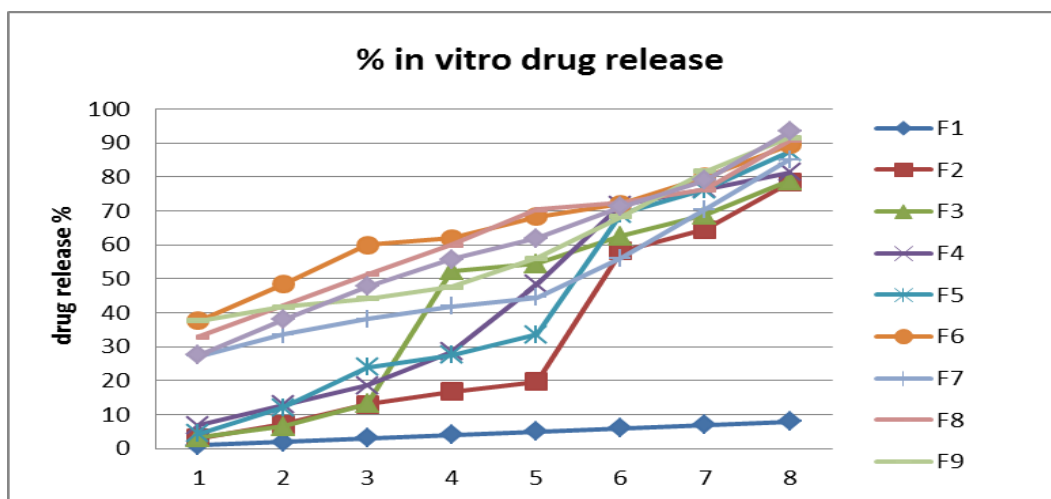

Figure 1: % In vitro drug release of optimization batches.

Table 2: Evaluation parameter of formulation.

| Formulation code	Sodium alginate X1	Calcium Carbonate X2	Floating lag time (sec)	Drug Release (%)	Total floating time (h)	Viscosity (Cps)	pH
F1	0.5	0.5	24	78.00	08	201	7.8
F2	1.25	0.5	45	79.00	08	224	7.9
F3	2.0	0.5	62	81.18	12	264	8.4
F4	0.5	1.0	23	87.00	08	205	8.2
F5	1.25	1.0	46	89.00	08	230	8.3
F6	2.0	1.0	63	85.10	08	275	8.3
F7	0.5	1.5	25	90.32	08	212	8.6
F8	1.25	1.5	44	91.29	12	244	8.4
F9	2.0	1.5	60	93.50	12	283	8.7

Batch Optimization

3^2 full factorial design was chosen as the optimization design since it allows the comparison of all the levels and factors to obtain significant results. All are independent variables, i.e. concentration of sodium alginate and concentration of calcium carbonate are seen to affect the dependant variables, i.e. floating lag time and Drug release . The Equations with coded values was obtained to be:

Floating lag time= $+45.44+18.83A-0.333B+0.7500AB -2.17A^2-0.6667B^2$…........ (1)

Drug Release = $+87.42+0.7467A+6.15B+0.0050AB-0.5833 A^2-1.49 B^2$ …….... (2)

From equation 1 and Figure 2, it was found that floating lag time increases with increasing concentration of sodium alginate. It was observed that as the concentrations of polymer increases, viscous nature of the formulation increases and they become more difficult to pour. From equation 2 and Figure 3, it can be deduced that the concentration of calcium carbonate have positive influence on in vitro drug release. The floating lag time values were in direct relationship with increase in amount of sodium alginate. It was found that as the concentration of calcium carbonate increases, in vitro drug release increases.
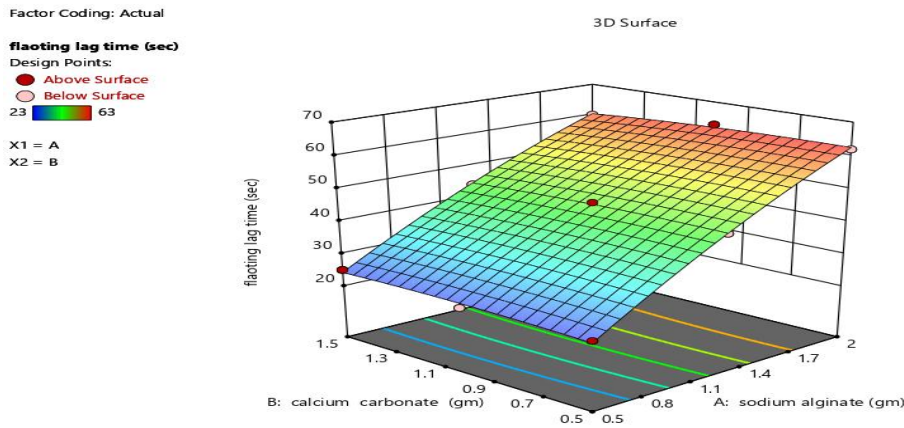
Figure 2: Response surface showing effect of sodium alginate, calcium carbonate on floating lag time.
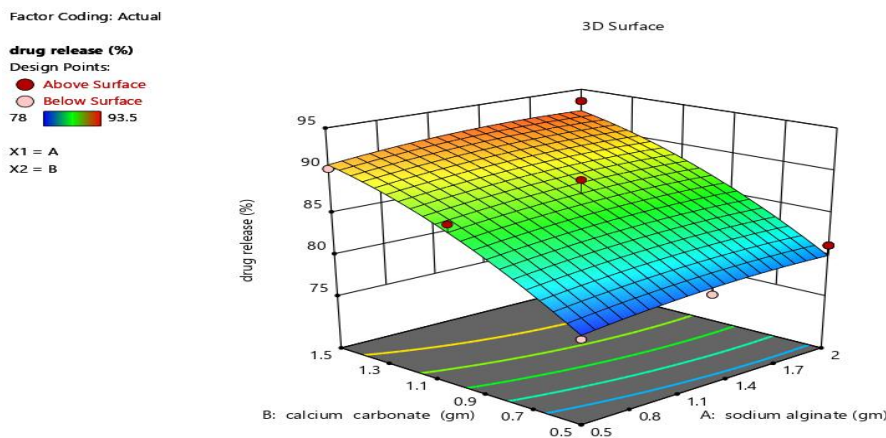
Figure 3: Response surface showing effect of sodium alginate, calcium carbonate on drug release.

Table 3: Summary of statistical parameters for the optimization model.

Parameters	Floating lag time	Drug release
R^2	0.9977	0.9430
P value	0.0004	0.0439
F value	264.31	9.93

Table 4: The optimum formulation obtained after numerical optimization.

Ingredients	Composition
Phyllanthus niruri extract	625 mg
Sodium alginate	0.5 gm
Sodium citrate	0.25 gm
HPMC K15	0.5 gm
Calcium carbonate	1.5
Tween 80	4 ml
Water	Q.s 50

The comparison of observed versus predicted values and the small % difference indicates the prognostic ability of developed model and can be used to navigate the deign space.

Table 5: Predicted vs. observed values.

Parameter	observed values	Predicated values	% Error
Floating lag time(sec)	0.9940	0.9739	0.2
Drug release (%)	0.9977	0.9940	2.39

CONCLUSION
Batch F7 formulation or in situ floating gel was found to be best and satisfactory compared to all other formulations. It had light brown appearance, easy to pour. The prepared floating in situ gelling system has feasibility of forming gel in stomach and sustaining the drug release from the over the period of 12 h. Its ease of administration with reduced frequency of administration results in better patient acceptance. The pH of the formulation was found to be more than 7. The prepared floating in situ gelling system has feasibility of forming gel in stomach and sustaining the drug release from the over the period of 12 h. The viscosity of floating in situ gel was found to be 212 cps at 50 rpm which indicated that the prepared floating in situ gel was easily pourable. Hence from all the results, it can be concluded that formulation and optimization of floating in situ gel was successfully done.

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