Ind. J. Pharm. Edu. Res., 2026; 60(1s):s124-s135.
https://www.ijper.org

Original Article

Development and Evaluation of Levofloxacin-Based *In situ* Floating Gel for Enhanced Gastric Retention

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ABSTRACT

Background: This work focuses on the development and testing of a floating *in situ* gel system based on sodium alginate that delivers levofloxacin to treat stomach Helicobacter pylori infections. **Materials and Methods:** As the gelling and gas-forming ingredients, respectively, sodium alginate and calcium carbonate were added in different amounts to create nine distinct formulations. Drug content, pH, viscosity, *in vitro* gelation, floating behavior and *in vitro* drug release were all assessed for the produced gels. **Results:** Results demonstrated that the formulation F6, with an optimal balance of sodium alginate and calcium carbonate, exhibited desirable viscosity, prolonged floating time and sustained drug release over 24 hr. The *in vitro* gelation studies confirmed immediate gel formation and integrity maintenance in acidic conditions, ensuring effective drug retention and release in the gastric environment. The drug release kinetics followed a first-order model, indicating a controlled and predictable release profile. **Conclusion:** In conclusion, the sodium alginate-based floating *in situ* gel system presents a viable and efficient approach for the targeted delivery of levofloxacin, enhancing its therapeutic efficacy against *H. pylori* by prolonging gastric residence time and ensuring sustained drug release.

Keywords: Floating *in situ* gel, Levofloxacin, *Helicobacter pylori*, Gastro-retentive drug delivery, Sustained drug release.

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Received: 19-03-2025;
Revised: 08-05-2025;
Accepted: 28-07-2025.

INTRODUCTION

The creation of regulated and long-lasting drug delivery systems has received more attention during the last 30 years. By localizing the medicine at the site of action, these systems are designed with the intention of either reducing the frequency of dosage or increasing therapeutic effectiveness.[1] Physiological variability is a significant problem, as is gastrointestinal passage and Gastric retention time, the latter of which has a major impact on the dose form's overall transit. The oral controlled system's gastro-retentive time period is always less than 12 hr. These factors led to the development of a DDS which could consistently and for a longer duration remain in the GIT.[2]

The creation of *in situ* gel systems has drawn a lot of interest recently among the in-depth study conducted in the design of polymeric drug delivery systems.[3] These types of systems are liquid at ambient temperature, however they gel when expose to biological fluids or experience a pH shift. They can release the drug continuously while retaining comparatively stable plasma profiles.[4] Before being administered, the *in situ* gel dosage exist as a liquid; but, upon contact with the stomach contents, it converts into a gel that floats on them. Such gel transformations are caused by one or more of the following mechanisms: chemical processes (e.g., enzymatic, ionic and photo-initiated polymerization), physical changes in biomaterials (e.g., solvent diffusion and swelling) and physiological stimuli (e.g., temperature and pH).[5]

Potential benefits of *in situ* forming drug delivery systems over traditional controlled release formulations include a straightforward manufacturing process, convenience of use, less frequent administration and enhanced patient comfort and cooperation.[6,7] *H. pylori* is the causative agent of one of the most typical disease-causing bacteria. Acute chronic gastritis, peptic ulcers and gastric lymphoma are among the serious gastroduodenal illnesses associated with it. Primarily, *H. pylori* is present in the gastric mucosa, or where the mucous layer and epithelial cells converge in the stomach's upper region. To get rid of *H. pylori*, a lot of antibiotics must be taken often. Because the antibiotic does not reach the germs beneath the mucosa, it becomes unstable in the acidic pH of gastric fluid and its half-life is brief in the stomach, the infection is not completely eradicated, leaving *H. pylori* behind.[8,9]

Levofloxacin is a third-generation fluoroquinolone antibiotic with a broad spectrum. According to certain research, levofloxacin

DOI: 10.5530/ijper.20260759

exhibits exceptional *in vitro* efficacy against *H. pylori* strains that are resistant to metronidazole and clarithromycin. These positive outcomes have been verified *in vivo*, showing that the levofloxacin-based regimen cures the majority of patients with both clarithromycin and metronidazole resistance.[10] Floating *in situ* gel systems were created in an effort to extend the levofloxacin's gastric residence period, enhance the stomach's localized effects and better eradicate *H. pylori*. Being a liquid, the newly suggested sodium alginate-based levofloxacin floating *in situ* gelling systems had the benefit of being easier to administer and more patient-compliant.[11]

MATERIALS AND METHODS

Levofloxacin was purchased from Swapnroop Chemicals, Aurangabad, India. Sodium alginate was obtained from Loba Chemicals Pvt. Ltd., Mumbai, India. Propyl and methyl paraben was obtained from Loba Chemicals, Mumbai, India. Calcium carbonate was acquired from Chemdyes Corporation, Ahmedabad, India. When necessary, freshly manufactured demineralized and doubly distilled water was used.

Preparation of floating *in situ* gel

When calcium ions are present in the GI fluid as a cross-linking agent, sodium alginate forms a pH-triggered *in situ* gel. By dissolving the designated quantity of sodium alginate in 25 mL of distilled water, sodium alginate solutions of various concentrations were created. To ensure that the polymers completely swelled, the proper amount of sodium alginate was dissolved in enough distilled water and agitated for 20 min using a magnetic stirrer. A drug solution containing 300 mg of the levofloxacin in 17 mL of 0.1 N HCl was progressively incorporated into 20 mL of polymeric solution and constantly swirled for 20 min using a mechanical stirrer. Stirring continuously for 10 min, certain quantities of Calcium carbonate were produced in deionised water and added to the polymeric solution. To get the desired outcome, immediately include 0.09% w/v methylparaben and 0.09% w/v propylparaben and mix for duration of 2 min. In the end, add distilled water to get the amount up to 50 mL.[12]

Experimental design and optimization

Using Design of Experiment software, a 3^2 complete factorial design was created, with the quantities of calcium carbonate (X_2) and sodium alginate (X_1) chosen as the independent variables (Table 1). Prior to putting the experimental design into practice, preliminary research was used to determine the amounts of the two variables. The variables that were chosen as response (dependent) variables were viscosity (Y_1) and drug content (Y_2). Three levels (-1, 0 +1) were used in the study to indicate low, medium and high amounts of each component, respectively. Using ANOVA, the model's significance was assessed. Throughout the investigation, all other formulations and processing factors remained constant.

Compatibility study of drug-excipient

The presence of any incompatibility between the drug and the excipient can be evaluated using Fourier Transform Infrared (FTIR) spectroscopy. Using the KBr disk approach, a Fourier Transform Infrared Spectrophotometer (BRUKER ALPHA-II) was used to determine the infrared spectra of native levofloxacin and levofloxacin loaded *in situ* floating gel. To compensate for baseline, dried potassium bromide was used. The spectra were then scanned from 400 cm^{-1} to 4000 cm^{-1} to find the distinctive absorption bands that matched its functional groups.

Determination of drug content

Using 100 mL of water to dissolve 1 mL of the formulation of buffer of phosphate pH 1.2 and sonicating the mixture for 30 min, the drug content of an *in situ* gelling formulation containing levofloxacin was determined. Following a 0.4 µM syringe filter, the drug concentration of the resultant solution was gauged at 293 nm employing the UV-Visible Spectrophotometer (SHIMADZU UV-1900).[13]

Measurement of pH

A digital pH meter made by Welltonix was employed to determine the pH of the prepared liquid mixture. Once twice as much distilled water has stabilized the pH, recalibrate your pH meter using 0.1 N HCl and a buffer with phosphate pH 6.8. Finally, measure the pH of each batch.[14]

Measurement of viscosity

The prepared solution's viscosity was assessed using a Brookfield viscometer (Brookfield-DVE-U.S.A.). Shearing was done on samples at ambient temperature. at 100 rpm on a suitable spindle. Three measurements of the viscosity of each sample were made, with a 30-sec interval between each measurement.[15]

In vitro gelation study

Measured precisely the *in vitro* gelling abilities were evaluated by adding 10 mL of formulation to 100 mL of 0.1N HCl at 37°C in a beaker and moderate stirring to avoid breakdown of produced gel. Based on the ideas of the developed gel's rigidity, gelation time and how long the gel stays intrinsically, the *in vitro* gelling capability was classified in 3 groups. (+) Gels quickly dispersed after a few min;

(++) Gelation instant lasted for a few hours;

(+++) Gelation immediate lasted for a longer amount of time.[16]

In vitro floating study

To conduct this investigation, 10 mL of formulation was added to 500 mL of 0.1 N HCl (pH 1.2) in a beaker that was kept at 37°C with little to no stirring. Both the floating lag time (the amount of time it took for the formulation to appear on the medium's

surface) and the floating duration (the amount of time it remained floated on surface) were noted down.[17]

Swelling index

It was established what percentage of the *in situ* gel compositions' swelling index. To make an *in situ* gel, 40 mL of gastric fluid 0.1 N HCl (pH 1.2) and 5 mL of each formula were placed to a petri dish. Following the extraction of the 0.1N hydrochloric acid solution *via* the gel, any leftover and Whatman filter paper were used for blotting the solution. 10 mL of filtered water were added to the gel after the initial weight (Wo) was determined. When the water was decanted after 60 min, the ultimate weight (Wt) of the gel was determined.

The following formula determines each formulation's percentage weight gain (swelling index):

$$\% \text{ Swelling index} = (Wt - W0 / Wt) \times 100 \ldots\ldots\ldots$$

Where, W0=Initial weight of the gel. Wt=weight gain by the gel.[18]

Study *in vitro* drug release

USP type II (paddle type) dissolving test apparatus was employed to investigate the *invitro* release of levofloxacin into buoyant *in situ* gel solutions. A single-use syringe was used to transfer 5 mL of each mixture. The needle was then cleaned and any remaining formulation was extracted from the tip. With the syringe plunger steadily depressed, 5 mL was expelled into a 4.5 cm-diameter petri dish that had previously contained 10 mL of 0.1N HCl. After placing the petri dish having the formulation on the medium's surface and giving it a gentle shake, it was submerged in 900 mL of 0.1N HCl (pH 1.2) in a dissolving tank. The dissolving test device was handled at 50 rpm and 37±0.5°C for a maximum of 5 hr.[19]

RESULTS

Here, sodium alginate was utilised as release-retarding gel-forming polymer to develop nine formulations of levofloxacin floating oral *in situ* gelling system. In an effort to increase viscosity and achieve a slower rate of drug release compared to formulations containing sodium alginate alone, various kinds of viscosity-boosting polymers were introduced to sodium alginate solution. To find the ideal concentration, calcium carbonate was employed in a range of concentrations both a source of calcium ions and an agent that produces gas. The physical appearance of prepared formulation was shown in Figure 1.

Optimization

A program known as "design of experiment" was used to statistically analyze the experimental plan, the statistical examination of the linear and quadratic models using ANOVA. Using a 3^2-factorial design for floating *in situ* gel optimization, the effects of specific independent factors, namely the concentration of sodium alginate (X2) and calcium carbonate (X1), on the dependent variables, viscosity (cps) and drug content (%), were investigated (Table 2).

Effect of independent variables on viscosity

The variety of viscosity estimated was 69.91 cps to 233.6 cps. The concentration of calcium carbonate (X_1) has a significant positive effect on the viscosity (Y_1) of the floating *in situ* gel (Figure 2). The higher the concentration of calcium carbonate, the higher the viscosity, which is likely due to increased cross-linking and gel

Table 1: Independent variables with their coded levels.

Coded values Level	Independent variables	
	X1 Calcium Carbonate (mg)	X2 Sodium Alginate (mg)
-1	500	100
0	1250	650
+1	2000	1200

Figure 1: Physical Appearance of in floating *in situ* gel.

Table 2: Optimization design of levofloxacin loaded floating *in situ* gel showing variables values and responses viscosity (cps) and drug content (%).

Formulation Code	Variables (X)		Responses (Y)	
	Calcium carbonate (mg) X_1	Sodium alginate (mg) X_2	Viscosity (cps) Y_1	Drug content (%) Y_2
F1	500	100	69.91	86.81
F2	1250	100	75.92	86.85
F3	2000	100	78.05	87.68
F4	500	650	80.11	88.49
F5	1250	650	82.41	95.44
F6	2000	650	85.77	96.51
F7	500	1200	157.98	97.79
F8	1250	1200	171.1	98.72
F9	2000	1200	233.6	99.54

network density. The ANOVA analysis and equation are tabulated in Tables 3 and 4.

Effect of independent variable on drug content

The concentration of sodium alginate (X_2) has a direct effect on the drug content (Y_2) of the *in situ* gel formulation. Higher concentrations of sodium alginate generally lead to an increase in drug content (Figure 3) due to improved encapsulation efficiency and viscosity effects. The drug content was estimated from range 86.81% to 99.54%. Tables 3 and 4 represent the ANOVA analysis and equation.

Interpretation of FTIR

The FTIR spectrum of levofloxacin exhibits distinct absorption bands, each representing specific functional groups within the molecule. Notable peaks include the O-H and N-H stretching vibration at 3400 cm^{-1}, signifying hydroxyl and amine group. The C-H stretching peak at 2960 cm^{-1} also indicates aromatic and aliphatic groups. Carbonyl C=O vibrations appear at 1720 cm^{-1}, while the C=C stretching vibration at 1625 cm^{-1} suggests carbon-carbon bonds. Carbon-hydrogen bonds are indicated by the C-H stretch at 1400 cm^{-1}. These observations provide valuable insights into levofloxacin molecular composition and structure (Figure 4a). The FTIR spectra of levofloxacin-loaded *in situ* floating gel showed the characteristic peak of levofloxacin (Figure 4b).

Drug Content

Table 5 displays the percentage of drug content for each formulation that was calculated. All of the formulations' drug contents were found to be within the range of 86.81-99.54%, suggesting a consistent distribution of the drug.

Measurement of pH

Table 5 illustrates that the pH range of the formulations was determined to be 6.8-7.3.

Determination of viscosity of *in situ* gelling system

When thinking about the solutions' suggested oral administration, the rheological characteristics are very important. Easy liquid swallowing should be made easier by the formulation's ideal viscosity, which is followed by a quick sol-gel transition brought on by ionic contact. Table 5 displays the results of viscosity for formulas F1 through F9.

Study on *in vitro* gelation

Gelling investigations were conducted with 0.1N HCl (pH 1.2) and Table 5 shows the results. When any of the formulations encountered an acidic medium, they all gelled instantly and retained their integrity. When insoluble calcium carbonate reacts with an acidic media, it solubilizes and releases calcium ions and carbon dioxide, a process known as gelation. The anionic polymer in the formulation, sodium alginate, interacts with the calcium ions in the formulation to produce rapid gelation and a gel shield that limits the release of drug.

In vitro floating study

When calcium carbonate came into contact with the stomach environment, it effervesced and released calcium ions and carbon dioxide. Next, a gel-based shield was created on formulation's surface through the complexation and gelation of Ca^{2+} ions. Extended floating occurred due to the released CO_2 becoming imprisoned within the network's gel and creating a buoyant preparation. The formulation's floating qualities are mostly dependent on calcium carbonate; as this concentration increased, the floating lag time decreased, and the floating duration increased. Floating lag time and floating time were displayed in Table 5. All formulations show buoyancy (Figure 5) greater than >12 hr.

Swelling index

A polymers matrix's ability to release a drug is dependent on the system's water content. The swelling index results are shown

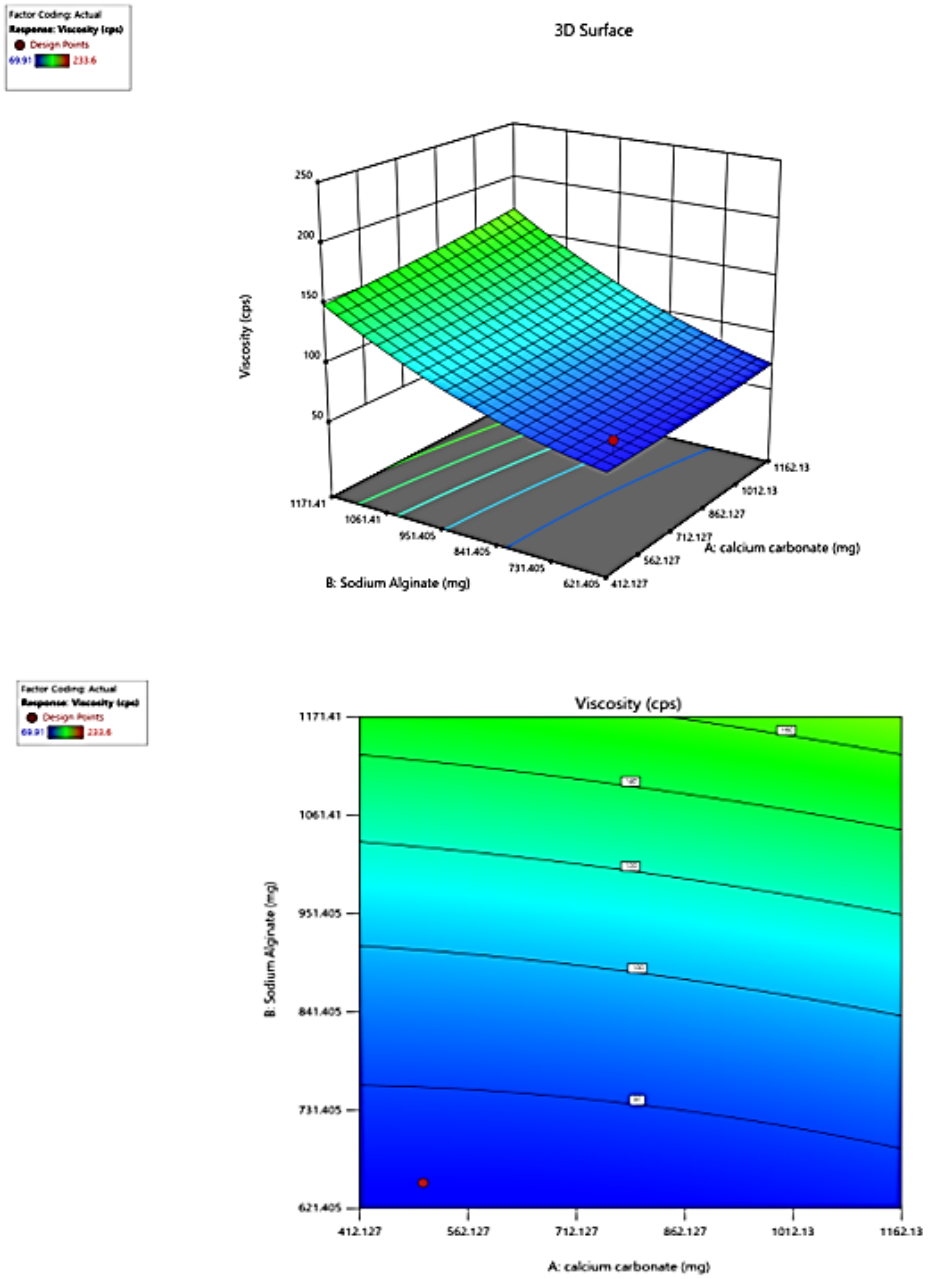

Figure 2: a) 2D Contour plots b) 3D Response surface plots for evaluating the influence of Calcium carbonate (X_1) and Sodium alginate (X_2) on viscosity (Y_1).

in Table 5; as the polymer concentration increases, so will the swelling index. Batch F1, which had a low polymer concentration, displayed a 9.3% swelling index, while Batch F9, which had a higher polymer concentration, displayed an 18.9% swelling index. Meanwhile, Batch F6, which had a medium polymer concentration, displayed an 8.2% swelling index.

In vitro drug release study

The produced formulations underwent an *in vitro* dissolving investigation in 0.1N HCl in order to examine the impact of various factors on the drug release percentage. Cumulative percent drug release (F1=89.46, F2=80.64, F3=90.21, F4=89.65, F5=90.55, F6=93.22, F7=91.11, F8=90.11 and F9=88.33%) from levofloxacin-loaded floating *in situ* gel is illustrated in Figure 6.

Kinetics of drug release

The release data of drug were fit to models of first order, zero order, Higuchi's and Korsmeyer Peppas, as well as the kinetics of the Hixson-Crowell equation to determine the release processes. Using the statistical function in MS Excel, the data were prepared for regression analysis and release kinetics graphs were created. The results are shown in Table 6. Based on the graphs (Figure

Table 3: ANOVA Analysis of variance.

Source	*F*-value	*p*-value	R^2
Response 1: Viscosity (cps)			
Model	21.81	0.0145	significant
X_1-calcium carbonate	5.51	0.1006	0.9732
X_2-Sodium Alginate	79.06	0.0030	
$X_1 X_2$	4.70	0.1186	
X_1^2	0.4977	0.5314	
X_2^2	19.31	0.0218	
Response 2: Drug Content (%)			
Model	30.30	0.0007	significant
X_1-calcium carbonate	5.21	0.0627	0.9099
X_2-Sodium Alginate	55.40	0.0003	

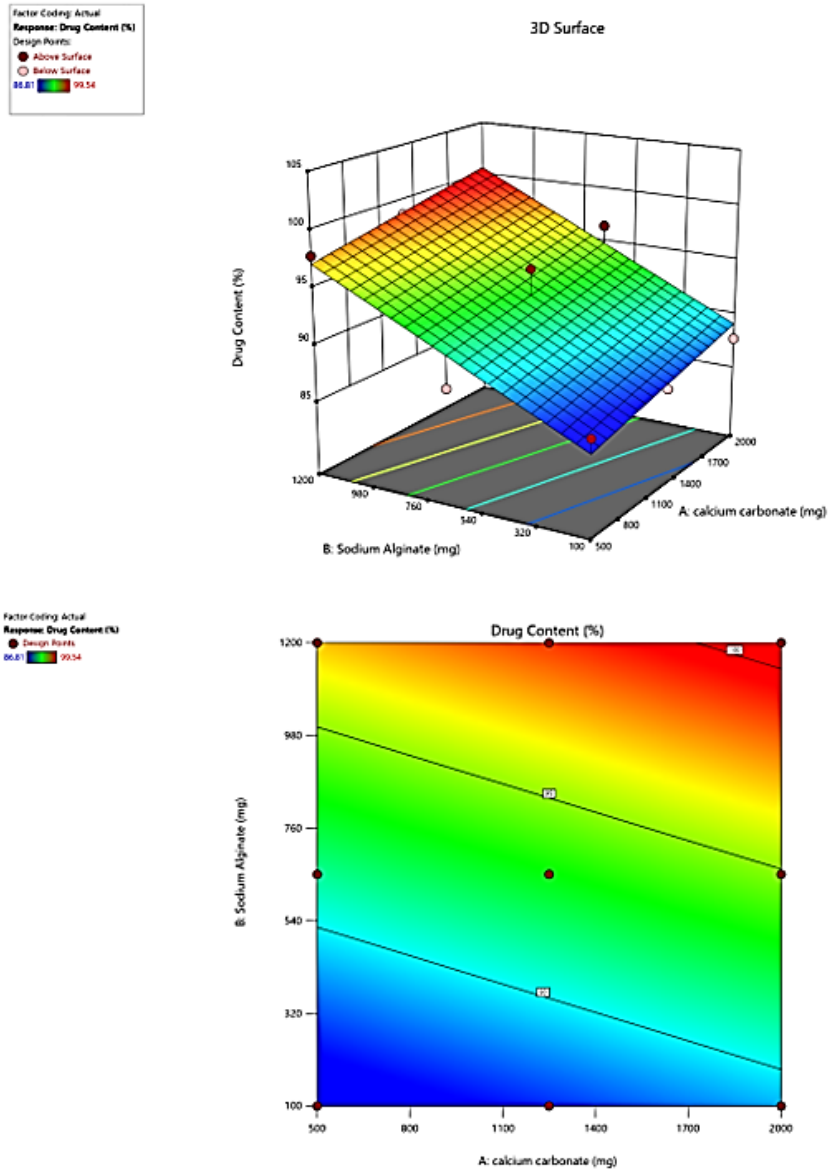

Figure 3: a) 2D Contour plots and b) 3D Response surface plots for evaluating the influence of Calcium carbonate (X_1) and Sodium alginate (X_2) on drug content(Y2).

7a-e) that displayed the maximum linearity (R^2=0.9865), it was determined that first order, best explained the *in vitro* drug release of drug code F9. The batch F9 follows the first order.

DISCUSSION

Sodium alginate and calcium carbonate significantly impact the viscosity and drug content of levofloxacin-based *in situ* floating gels. The interaction between calcium carbonate and sodium alginate can affect the gel network. For instance, calcium carbonate can cross-link with sodium alginate to form a stronger gel matrix, potentially affecting viscosity. Sodium alginate forms a gel network by interacting with calcium ions, creating a more extensive network structure as its concentration rises. This network increases the gel's resistance to flow, thus raising its viscosity. The concentration of sodium alginate determines the gel strength, while calcium carbonate contributes to the gel's buoyancy and possibly its overall structural integrity. Higher concentrations of sodium alginate can create a more gelled matrix that might entrap more drug molecules. As calcium carbonate reacts to produce carbon dioxide, the gel may expand or change in structure, which could affect how the drug is distributed within the matrix.

By comparing the FTIR spectra of levofloxacin and the final gel formulation, it confirms the presence of all components and

Table 4: Equations.

Sl. No.	Dependent Variable	Coded Equation
1	Viscosity, Y_1	$Y_1=104.73+22.44\ X_1+63.16X_2+13.80X_1\times X_2+11.17X_1^2+22.47X_2^2$
2	Drug Content, Y_2	$Y_2=95.10+2.13\ X_1+3.94X_2$

Table 5: Evaluation parameters of *in situ* gel formulation.

Batches	pH*	Viscosity (cps)	Floating lag time (sec)	Total floating time (sec)	Drug content (%)	Swelling index (%)	Gelling capacity
F1	7.3 ± 0.1	69.91	35	>12	86.81	9.3	+
F2	7.3 ± 0.1	75.92	38	>12	86.85	9.8	++
F3	7.3 ± 0.1	78.05	45	>12	87.68	11.1	++
F4	7.3 ± 0.1	80.11	57	>12	88.49	7.6	+
F5	7.3 ± 0.1	82.41	37	>12	95.44	9.5	+++
F6	7.3 ± 0.1	85.77	43	>12	96.51	8.2	+++
F7	7.3 ± 0.1	157.98	52	>12	97.79	13.2	+
F8	7.3 ± 0.1	171.1	55	>12	98.72	16.6	+++
F9	7.3 ± 0.1	233.6	60	>12	99.54	18.9	+++

*n=3, ±SD.(+) Gels quickly dispersed after a few min;(++) Gelation instant lasted for a few hours;(+++) Gelation immediate lasted for a longer amount of time.

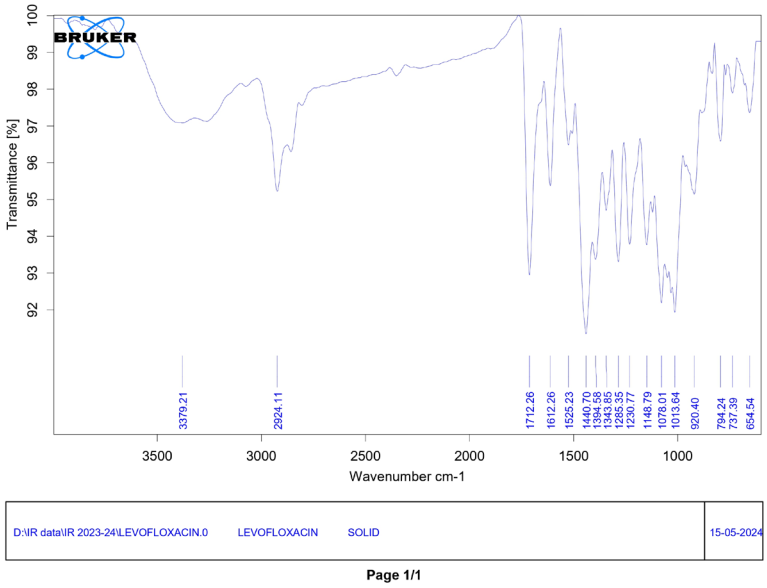

Figure 4a: FTIR of Levofloxacin.

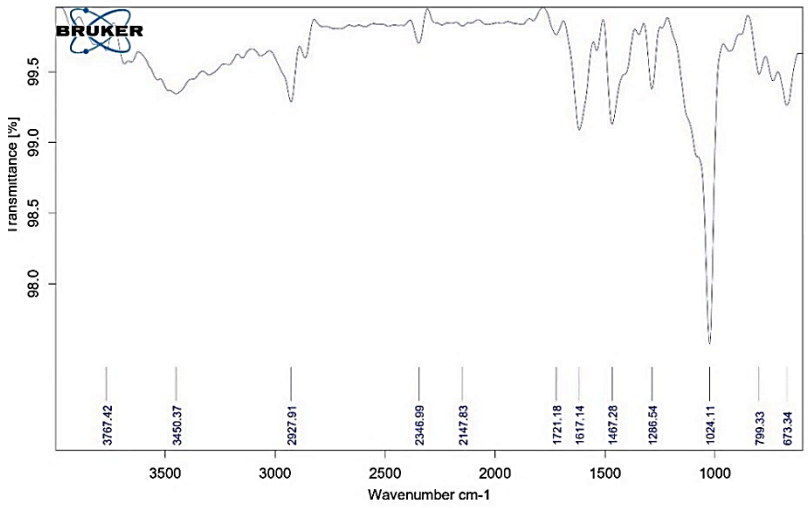

Figure 4b: FTIR of levofloxacin-loaded *in situ* floating gel.

Figure 5: Floating study of floating *in situ* gel.

Table 6: Analysis of kinetic release model formulations.

Sl. No.	Formulation Code	Regression Coefficient (R^2)				
		Zero Order	First Order	Higuchi Model	Kors peppas	Hixcon crowell
1	F1	0.9754	0.9336	0.8475	0.8907	0.9501
2	F2	0.9779	0.9507	0.8597	0.9242	0.962
3	F3	0.9098	0.9031	0.9231	0.862	0.9086
4	F4	0.9623	0.9031	0.9723	0.9341	0.9776
5	F5	0.9509	0.9752	0.9797	0.9157	0.9699
6	F6	0.9404	0.9784	0.9865	0.9072	0.9666
7	F7	0.9734	0.9867	0.9661	0.9341	0.9851
8	F8	0.9734	0.9946	0.9586	0.9569	0.9913
9	F9	0.9717	0.9865	0.9568	0.9647	0.9836

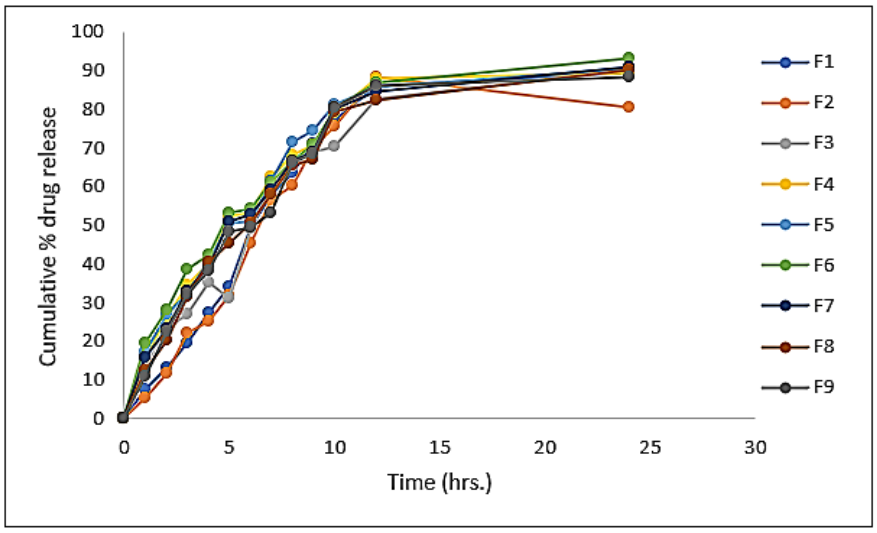

Figure 6: Graph of *in vitro* drug release.

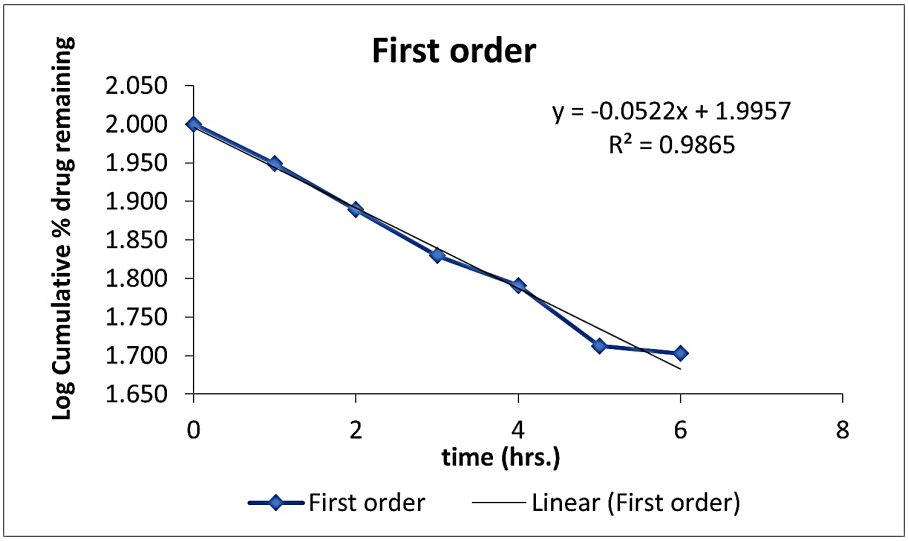

Figure 7a: First order release kinetics.

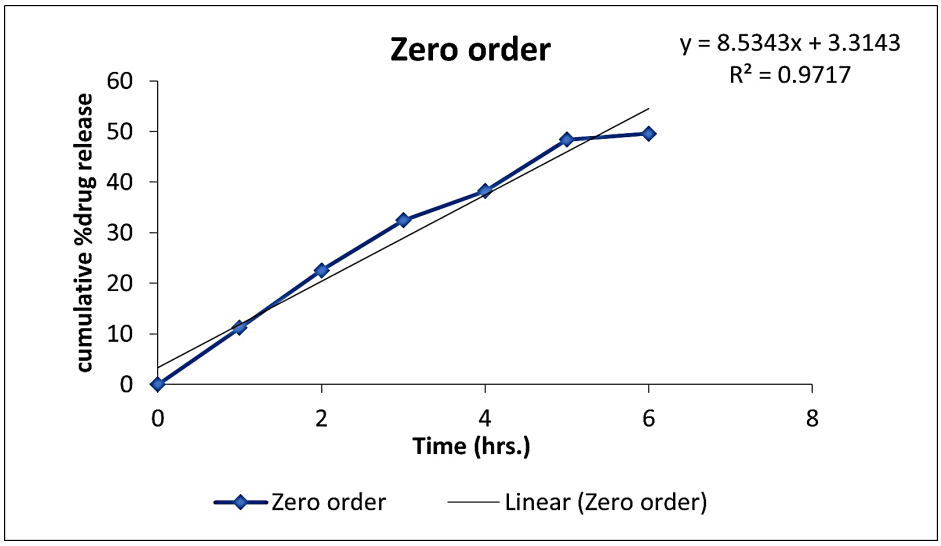

Figure 7b: Zero order release kinetics.

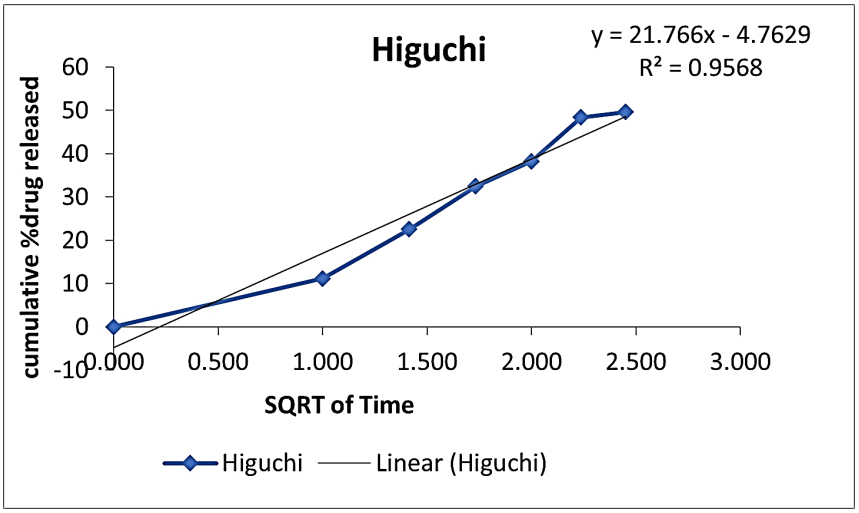

Figure 7c: Higuchi order release kinetics.

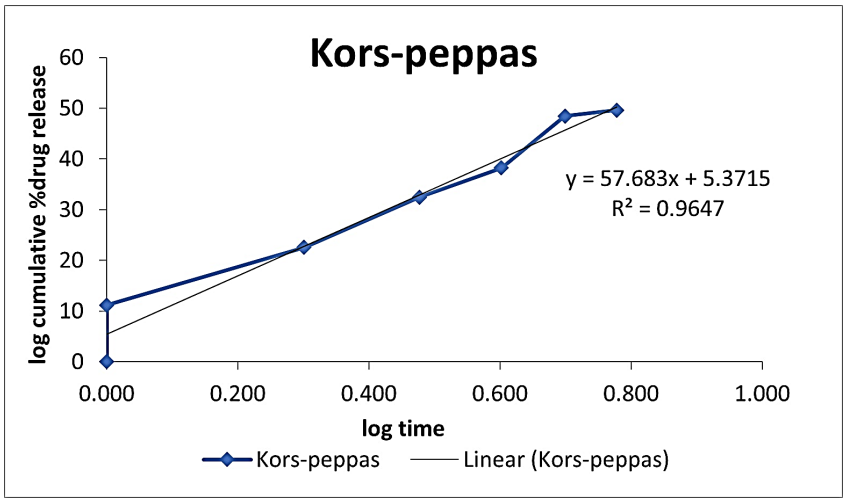

Figure 7d: Peppas model of release kinetics.

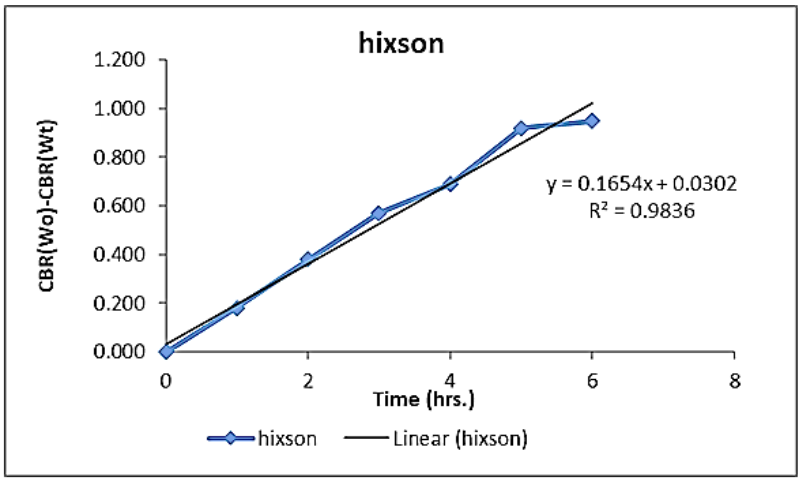

Figure 7e: Hixson release kinetics model.

ensures the stability of the drug within the gel. The formulations had basic pH because of sodium alginate and calcium carbonate. In this formulation, gelation occurs through ionotropic gelation. Sodium alginate reacts with calcium ions (Ca^{2+}) to form a gel network. When calcium carbonate is present, it reacts with gastric acid to release calcium ions, which then interact with sodium alginate to form the gel. When the gel comes into contact with the acidic environment of the stomach, calcium carbonate reacts with gastric acid (hydrochloric acid) to release Carbon Dioxide (CO_2). This gas gets trapped within the gel matrix, causing it to expand and float. A higher swelling index indicates that the gel can expand significantly upon contact with gastric fluids. This expansion is crucial for the gel to float and remain in the stomach for prolonged periods. The swelling of the gel matrix affects the drug release rate. A properly swelling gel can release levofloxacin in a controlled manner over an extended period. Drug release primarily occurs through diffusion of levofloxacin from the gel matrix into the surrounding gastric fluid. The gel matrix, which swells upon contact with gastric fluids, allows the drug to diffuse out gradually. *In situ* floating gel follows first-order kinetics, which releases the drug at a rate proportional to the remaining concentration and ensuring a controlled release.

CONCLUSION

The *in situ* floating gel was successfully formulated using appropriate gelling agent and excipients that ensure the gel's ability to remain in the stomach for prolonged periods. The process involved careful selection of polymers and other ingredients to achieve the desired floating properties and drug release profile. The characterization results confirmed that the gel exhibited desirable properties, including sufficient gelation time, floating ability and controlled release of Levofloxacin. The *in vitro* studies demonstrated that the Levofloxacin-loaded gel could effectively maintain its floating state for extended periods, which is crucial for improving the bioavailability of the drug and enhancing therapeutic outcomes. In conclusion, the Levofloxacin-loaded *in situ* floating gel presents a promising approach for oral drug delivery systems, potentially improving patient compliance and treatment efficacy.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Pharmaceutics, Shree Santkrupa College of Pharmacy, Ghogaon for providing necessary facilities to carry out this research work.

ABBREVIATIONS

FTIR: Fourier Transform Infrared; **H. pylori:** *Helicobacter pylori*; **GIT:** Gastrointestinal Tract; **ANOVA:** Analysis of Variance.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

A. A. Nale: Methodology, investigation, original draft writing. P. A. Thorat: Supervision. J. A. S. Mulla: Conceptualization, draft reviewing.

SUMMARY

The study focused on the development and analysis of an *in situ* floating gel system designed to deliver Levofloxacin, a broad-spectrum antibiotic, more effectively through oral administration. The *in situ* floating gel was formulated by incorporating Levofloxacin into a gel matrix that could transition from a liquid to a gel state upon contact with gastric fluids. Various polymers and excipients were used to achieve this transition, including gelling agents that ensure the gel's buoyancy and prolonged residence time in the stomach. The prepared gel was thoroughly characterized to assess its physical and chemical properties. Key evaluations included viscosity, gelation time, floating ability, swelling behaviour and *in vitro* drug release. The Levofloxacin-loaded *in situ* floating gel represents a promising advancement in oral drug delivery systems, offering enhanced drug bioavailability and extended therapeutic action. Further studies, particularly *in vivo* evaluations, are recommended to confirm its clinical efficacy and optimize its application in treating gastrointestinal infections.

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Cite this article: Nale AA, Thorat PA, Mulla JASG. Development and Evaluation of Levofloxacin-Based *In situ* Floating Gel for Enhanced Gastric Retention. Indian J of Pharmaceutical Education and Research. 2026;60(1s):s124-s135.