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Design and development of sodium alginate/ carboxymethyl cellulose *in situ* gelling system for gastroretentive delivery of lisinopril

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ABSTRACT: Lisinopril is a potent ACE (angiotensin-converting enzyme) inhibitor used to treat hypertension and congestive heart failure. It exhibits 25% of low bioavailability. Hence, in the current study, the major objective was to increase the gastric transient time of lisinopril and develop *in situ* gel formulation for better absorption and modulating release behavior of lisinopril. Different formulations of lisinopril were prepared by using gelling polymers such as Carboxymethyl cellulose (CMC), pectin, and calcium carbonate. Sodium citrate was used to prevent gelation outside the gastric environment. The formulation was studied using Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC) to interpret the interaction between drugsand polymers. For optimization of *in situ* gelling system 3^2 full factorial design was employed to study the effect of independent variables, the concentration of CMC (X_1) and concentration of sodium alginate (X_2), on the dependent variables viscosity, and % drug content. Formulation (F5) containing 1.25% of sodium alginate, and 0.75% of CMC showed good gelling ability. The composition F5 was optimized on the basis of viscosity (5.03 Pa.s), drug content (94.06±1.0%), and cumulative drug release (95±0.73%) at 12 h. Floating *in situ* gelling system improved bioavailability and gastric transit time of lisinopril. A stability study indicated the absence of any noticeable change in the formulation. Thus, *in situ* gel formulation is a promising approach for gastro-retentive sustained delivery of lisinopril. These results ensure that the developed system is an alternative to conventional drug delivery systems and can enhance patient compliance.

KEYWORDS: *In situ* Gel; Sodium Alginate; Carboxymethyl cellulose; Gastro-retentive drug delivery; Lisinopril.

1. INTRODUCTION

Nowadays hypertension is a very communal disease found all over the world. Hypertension is a serious condition that can harm your heart, brain, kidneys, and other organs. Hypertension affects an estimated 1.28 billion individuals aged 30 to 79 worldwide [1]. The force produced by blood circulation against the walls of the body's primary vessels, the arteries, is known as blood pressure. If your systolic blood pressure is greater than 140 mmHg and your diastolic blood pressure is greater than 90 mmHg, you have hypertension [2]. Many orally taken medications have a low rate of absorption and poor bioavailability. Some medicament shows less absorption. To compensate for this impact, a big dose of medication is given [3]. To create an effective controlled release system, a variety of formulation approaches have been used, including super porous hydrogel, bio/mucoadhesive, raft forming, magnetic, ion-exchange, and low- and high-density systems [4].

The oral drug delivery system shows a systemic effect upon drugs absorbed through the gastrointestinal tract (GIT). As a result, numerous ways to keep the medication in the stomach after delivery are available. Oral delivery includes floating, swelling, expanding systems, and delayed gastrointestinal emptying systems. The oral drug delivery system is most frequently employed because it is relatively easy for children and the elderly to ingest the medication. Many orally administered drugs showed a poor rate of absorption while elimination at a faster rate. To achieve desired therapeutic action and maximum absorption window a large dose of a drug can be given. Furthermore, poorly absorbed drugs have a wide range of bioavailability. These issues could be solved by altering the way drugs are delivered [5].

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The gastroretentive drug delivery system (GRDDS) is advantageous for medications with low bioavailability, therapeutic efficacy, and dosage. Drug absorption depends upon gastric residence time, absorption site, dosage form, etc. [6-7]. Some drugs are easily absorbed in the stomach and quickly eliminate from the body because of their shorter half-lives. Hence, frequent dosing is important for therapeutic activity [8]. As a result, medications with a gastrointestinal absorption window have limited bioavailability. GRDDS refers to a method of allowing medicine to enter the stomach gradually [9].In a floating system, the drug should float on the gastric fluid surface hence it increases the gastric residence. It is a low-solidity methodology in which a drug that has a lower density floats on the gastric surface in the stomach, discharging the drug for more time. The delivery system is extended after the drug has been eliminated from the stomach [10].

The "*in situ* gel" system is a new drug delivery system, where the on-site swelling system helps to release drugs in a sustainable and controlled manner, improving patient compliance, and comfort with its own 'sol-to-gel' behavior. Temperature, pH change, solvent exchange, and UV radiation all influence the transition from 'sol-to-gel'. The above-mentioned 'sol-to-gel' properties are widely used in the preparation of biocompatible compounds, and there are many advantages in '*in situ* gel' systems, which include ease of application of the dose, reduced frequency of administration, and even protection against change in environmental conditions. Various polymers are made to form a gel at the site and used in the oral, eye, transcutaneous, buccal, peritoneal, injection, injection, rectum, and vagina [11-12].

Lisinopril is a potent ACE (angiotensin-converting enzyme) inhibitor [13]. In hypertensive patients with diabetes mellitus, lisinopril is used to treat hypertension and congestive heart failure, and to prevent the progression of renal impairment [14]. It belongs to BCS class III and has a 25% bioavailability and a half-life of 12 h. Lisinopril is absorbed uniformly from the gastrointestinal tract but has a lower solubility in the intestinal fluid. Lisinopril's bioavailability is improved, and adverse effects are reduced, due to the sustained delivery [15-16]. The retention period of the delivery system in the stomach must be increased to make the drug available in solution form when it reaches the place of maximum absorption. This will increase the oral bioavailability of therapeutics. The developed floating gastroretentive tablets improved retention time and sustained drug release in the stomach, which increased the local availability of the drug [17]. The oral liquid *in situ* gel has advantages over oral tablets. As a result, they are distinguished by ease of administration, extended residence time, and sustained drug release at the administration site, as well as a reduction in administration frequency and an improvement in patient compliance [18]. Design of experiments (DOE) is a systematic and organized strategy for developing pharmaceutical dosage forms. Several applied statistics tools are used in the design of experiments to systematically categorise and quantify the cause-and-effect relationships between inputs and output variables in the phenomenon or process being studied [19]. To examine the effects of several variables and their interactions on response quantity, several factors varied during each experimental run. Full factorial design (FFD) relates to a factorial experiment whose design includes all possible combinations of the selected variables and levels [20].

In the present study, 3^2full factorial design was employed where 2 factors, CMC concentration (X_1) and sodium alginate concentration (X_2) were evaluated at 3 levels, *viz*. low (-1), medium (0), and high (+1) using experimental trials performed at all 9 possible combinations with minimum failure of batches

The objective of this study was to use sodium alginate and carboxymethyl cellulose (CMC) to make a controlled-release gastro-retentive *in situ* gel for lisinopril to extend the gastric transient time. The developed formulation, *in situ* gel, will form a gel when it comes into contact with an acidic environment of the stomach and will stay for a long time, ensuring higher medication bioavailability.

2. RESULTS AND DISCUSSION

Sodium alginate is a natural hydrophilic anionic unbranched binary copolymer comprising (1,4)-linked -D-mannuronic acid and -L-glucuronic acid residues. It has a better ion binding characteristic for multivalent cations, which is the basis for their gelling characteristics. Sodium alginate acts as a gelling agent [21]. CMC is a well-known mucoadhesive, ionizable, hydrophilic, non-toxic, biocompatible, and biodegradable polymer. Also, it has no known side effects on human health. The carboxylate group in the CMC is responsible for gelation, bioadhesion, etc., Hence, it is used as a viscosity-enhancing and rheological control agent [22]. Lisinopril shows low bioavailability and has a low solubility in the intestinal fluid which makes it an ideal candidate for the oral *in situ* gel for its sustained delivery [15].

2.1 Preparation and optimization of *in situ* gelling solution

The formulation was optimized by using 3^2 full factorial design. The formulation was optimized using a statistical approach to study the effect of all the factors and their interaction on responses. The design is useful to investigate the quadratic effects. Hence, the design was employed for the development of a

statistically optimized formulation with minimum experimental runs. The detailed optimization of formulation and effect of an independent variable on responses has been studied using Design expert® and evaluated using ANOVA[23].

Table 1. Compositions of formulations F1 to F9 made using 3^2 full factorial design and the results of their characterization

Formulation code	Factor 1 CMC (g)	Factor 2 Sod. Alginate (g)	Response 1 Viscosity (Pa.s)	Response 2 Floating lag time (s)	Response 3 Floating time (h)	Response 4 CDR (%)
F1	1.25	1.50	3.92±0.120	40±0	>12	87.51±1.10
F2	1.00	1.00	5.44±0.460	43±1	>12	91.40±0.86
F3	0.75	1.00	3.11±0.250	42±0	>12	84.22±0.91
F4	0.75	1.50	8.57±1.120	39±1	>12	82.60±0.98
F5	0.75	1.25	5.03±0.041	40±0	>12	95.00±0.73
F6	1.25	1.00	3.30±0.052	41±0	>12	90.08±0.89
F7	1.00	1.50	7.74±1.020	45±1	>12	80.00±1.03
F8	1.25	1.25	5.34±0.890	50±1	>12	91.66±0.76
F9	1.00	1.25	7.40±0.710	48±0	>12	89.00±0.87

(n= 3, mean±SD)

On the basis of characterization outcomes of the designed formulations, formulation F5 was optimized. The optimized formulation contained 0.75% CMC and 1.25% sodium alginate. It showed a floating lag time of 40 s, a floating time of more than 12 h, a viscosity of 5.03 pa.s., and drug release of 36.20% at 4 h and 95.00% at 12 h. In design expert full factorial design was applied to the formulation of two independent factors namely; polymer concentration of CMC (A) and concentration of sodium alginate (B) at three different levels were used (-1, 0, +1) (Table 5). For various responses, fit analysis, ANOVA, and surface response 3D plots (Figure 1) were studied in depth. The impacts of each factor on the associated response are expressed by the equations below, where CMC concentration is denoted by A, and sodium alginate concentration is denoted by B.

$$Y1 = 5.03 - 0.9929X_1 + 1.24X_2 - 1.22X_1X_2 + 0.2331X_1^2 + 0.3431X_2^2 \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$Y2 = 95.00 + 1.91X_1 - 2.58X_2 - 0.1600X_1X_2 - 2.86X_1^2 - 5.18X_2^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$Y3 = 40.00 + 0.3536X_1 - 0.1464X_2 + 0.5000X_1X_2 + 3.00X_1^2 + 0.5000X_2^2 \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

The linear model was best suited for all of the responses, with p-values less than 0.05 ANOVA results were significant. The modified and expected R^2 values were relatively similar. Adequate precision compares the range of predicted values at design points to the average prediction error. Adequate precision, i.e., the signal-to-noise ratio should be larger than 4, which is desirable. If the signal-to-noise ratio is greater than 4, it indicates that the model can be used to navigate the design space. The optimized formulation contained 0.75 % CMC and 1.25 % sodium alginate to get a floating lag time of 40 s, a floating time of more than 12 h, the viscosity was found at 5.03 pa.s., drug release at 4 h 36.20±0.46 %, and 12 h 95.00±0.73 % respectively.

There was a directly proportional relationship between viscosity and amount of sodium alginate whereas it was inversely proportional for the CMC. Hence, the optimum amount of these polymers was needed to achieve the desired viscosity ranges. The response plot of CDR, Fig. 1(B), showed that as the concentration of sodium alginate increased, the % CDR decreased.

However, at increased CMC concentration, the drug release rate was increased. The surface response plot of buoyancy time against a concentration of sodium alginate indicated a direct relationship between the concentration of sodium alginate and the buoyancy time. Besides, CMC also presented similar results [24].

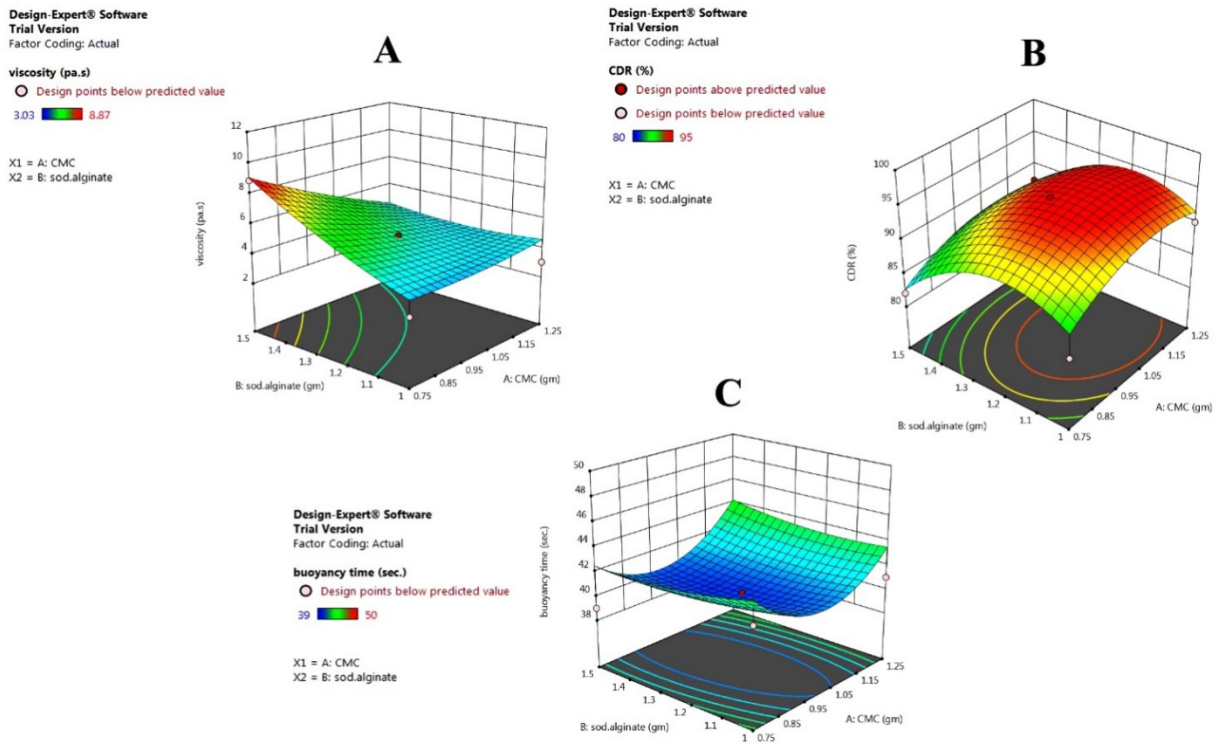

Figure 1. 3D Surface response plots: (A) viscosity, (B) %CDR, and (C) buoyancy obtained using design-expert software

2.2 *In vitro* gelling capacity

Gelling capability at pH 1.2 was measured at a point when all prepared solutions transformed to floating gel as soon as they came into contact with 0.1N HCl. The sodium alginate anionic polymer in the formulation reacts with the calcium ions to instantly gel it, forming a gel barrier that controls the release of the medication. The gelation time and the time required for the produced gel to float on the medium are classified into three types, for example, +, ++, and +++. All the formulations showed instant gelation upon contact with an acidic medium [29]. The observations are mentioned in Table 2.

Table 2. Characterization of *in situ* gel formulations

Formulation Code	pH	*In vitro* gelling capacity	Drug content (%)
F1	7.2±0.01	++	86.92±1.2
F2	7.2±0.04	++	92.00±1.1
F3	7.2±0.01	++	83.01±1.3
F4	7.2±0.05	+++	82.50±1.1
F5	7.2±0.01	+++	94.06±1.0
F6	7.2±0.04	+++	91.00±1.2
F7	7.2±0.03	++	81.75±1.4
F8	7.2±0.01	+++	90.00±1.3
F9	7.2±0.04	++	89.85±1.5

(n= 3, mean±SD)

+ = After few minutes, the gels get dispersed immediately
++ = Rapid gelation lasts only a few hours
+++ = The immediate gelation remains for prolonged time

2.3 Drug polymer compatibility studies

The results of the pre-formulation study indicated that all the major peaks of the drug and polymer were found in the FTIR analysis. The melting point of the drug and polymer was studied in DSC thermographs. The results indicated the drug and polymer compatibility.

FTIR spectrum for lisinopril showed functional peaks for lisinopril were observed for groups C=O Stretching at 1654.14 cm^{-1}, C=O at 1389 cm^{-1}, C-H Stretching at 2961 cm^{-1}, N-H at 3556.97 cm^{-1}, C=C at 1571.27 cm^{-1}, C-H Bending at 748.29 cm^{-1} and for the formulation. There was no significant variation in the FTIR peaks and for the formulation, similar peaks and bonds were identified it indicating that the drug and excipients indicated an absence of chemical interaction i.e., no effect of the excipients on the pure drug [30].

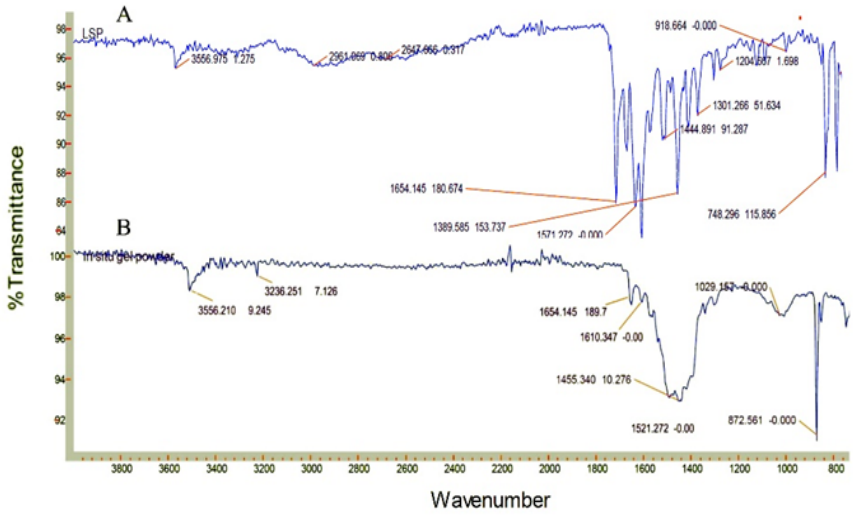

Figure 2. FTIR spectra: (A) pure drug Lisinopril and (B) Optimized formulation (F5)

The thermogram of pure lisinopril, Fig. 2 (A), shows a sharp endothermic peak at 180.56 °C. The DSC thermogram of the drug with formulation excipients, Fig. 2 (B), recorded a melting peak at 164.03 °C. There was a shift in the lisinopril characteristic peak to the left as well as a reduction in peak intensity. The shift in endothermic peak to low temperature indicates the transition of crystalline lisinopril to its amorphous form suggesting complete dissolution of the drug in the polymer matrix

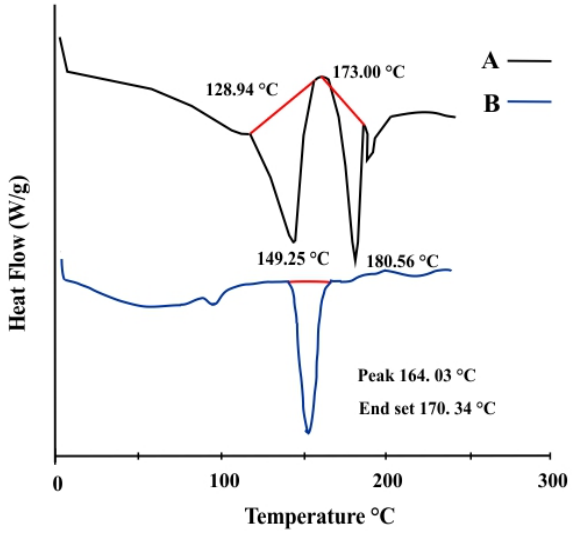

Figure 3. DSC thermographs: (A) pure drug lisinopril and (B) optimized formulation (F5)

2.4 Rheological properties of the formulation

In the development of *in situ* gels, the rheological characteristics are crucial. In selecting the concentration of gelling polymer, a ratio between sufficiently high concentrations of polymers is essential for the production of gels. The ideal viscosity of the formulation makes it simple to administer and swallow as a liquid and produces gel strength that is suitable for use as a delivery vehicle. The results of viscosity are presented in Table 1. The viscosity diagram and thixotropic analysis of the optimized formulation (F5) are shown in Figures 4 and 5 respectively. With higher concentrations of CMC and sodium alginate, the viscosity

of the solutions increased. The thixotropic analysis showed some non-Newtonian pseudoplastic time-dependent change in viscosity. It was concluded that fluids attained an even viscosity with respect to shear rate [34].

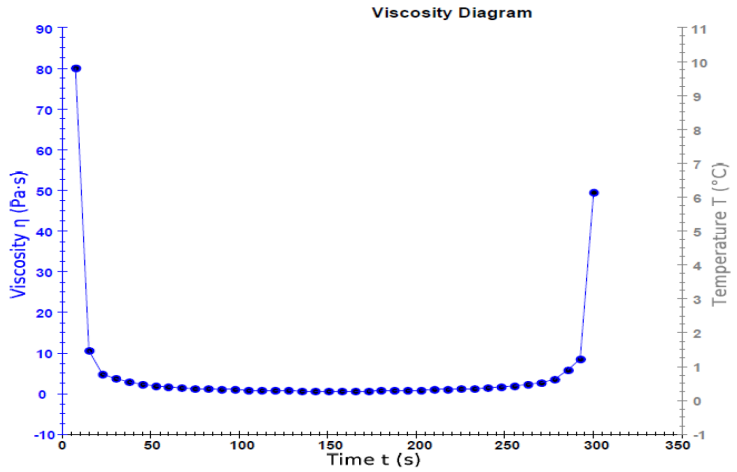

Figure 4. Viscosity versus time graph of optimized batch (F5) of *in situ* gel formulation

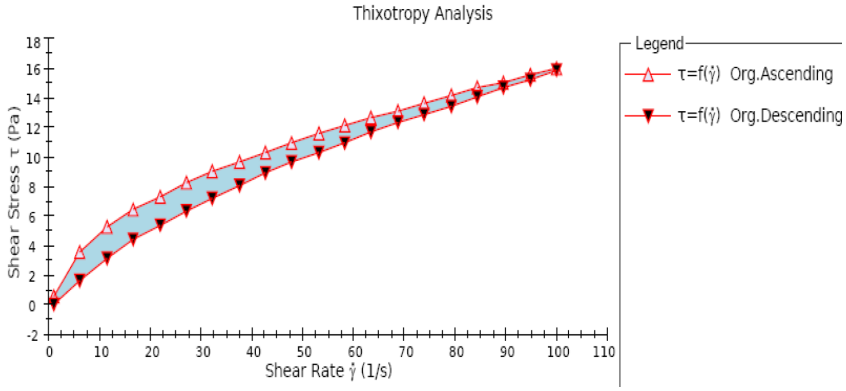

Figure 5. Thixotropic analysis of optimized composition (F5)

2.5 Buoyancy studies

The minimum floating lag time is required in the formulation. Floating lag times of all formulations' ranged from 38 to 58 s. Formulation F5, which contains 1.50% sodium alginate and 1.0% calcium carbonate, takes less time to float on the surface of the medium from the bottom. Increase in floating lag time because of less concentration of calcium carbonate [37].

2.6 *In vitro* release study

The dissolution profile of all formulations showed in Table 1. Increases in polymer content *in situ* gels resulted in a considerable reduction in drug release rate and extent, which can be related to an increase in the density of the polymer matrix as well as an enhancement in dispersion path length. The discharge of the drug from the gel was considered the initiation of a high-release phase. The initial releasing impact was considerably diminished as the polymer concentration was increased. All of these drug release and floating lag time studies revealed that calcium carbonate affects the gelling properties of sodium alginate. The *in situ* formed gel preserved its integrity without dissolving or eroding for a prolonged period to facilitate the sustained release of drugs. The results assure that the given floating *in situ* gelling system will increase the gastric residence time of lisinopril in the stomach, which can be attributed to enhancing the bioavailability of lisinopril. The floating study showed that all formulations remain afloat for more than 12 h. and there is no significant difference in %CDR observed between the batches after the further drug release. Hence, floating times and %CDR for all the batches are mentioned as > 12 as per the previously reported literature [39].

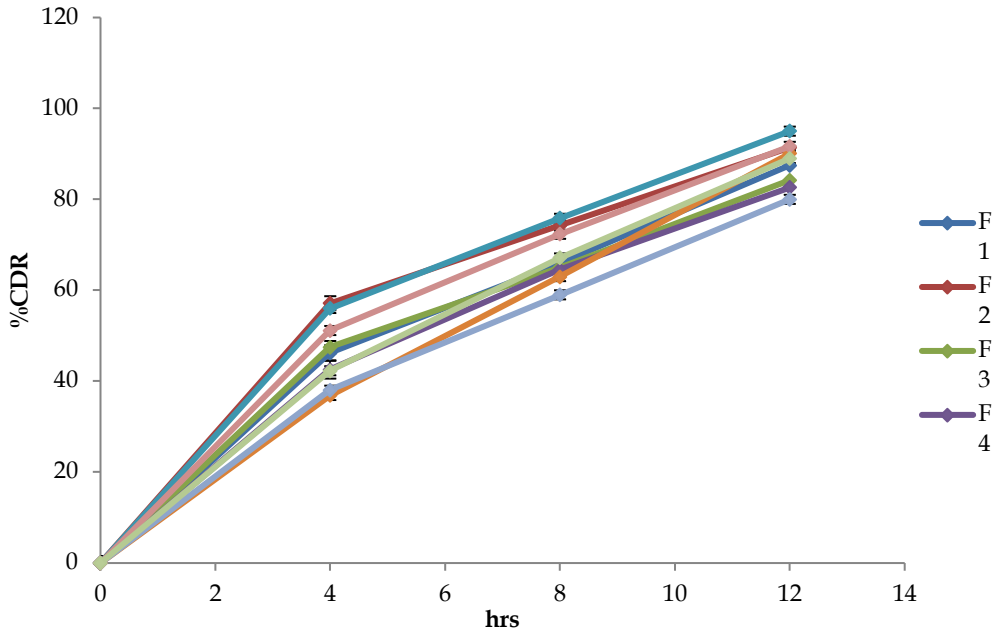

Figure 6.% Cumulative drug release of F1 to F9 batches at 4h, 8h, and 12h time intervals

2.7 Drug Release Kinetics

To evaluate the mechanism of drug release, the *in vitro* release profile was fitted to multiple kinetic models such as zero order, first order, Higuchi, and Korsmeyer-Peppas. The regression coefficient (R^2) values were used to assess the goodness of fit. Table 3 shows the outcome of the optimized batch R^2 values mentioned in Table 3. The release mechanism was determined by fitting the data into the Korsmeyer-Peppas equation Mt/Ma = Ktn, where 'Mt/Ma' is the proportion of the drug released at time t,' 'K' is the kinetic constant, and 'n' is the release exponent that specified the process for releasing the drug. From the obtained data it was observed that the Korsmeyer-Peppas model was the best-fit kinetic release model [41].

Table 3. Model fitting for optimized *in situ* gel formulation (F5)

Parameter	Zero-order	First-order	Higuchi	Korsmeyer-Peppas
Regression coefficient (R^2)	0.9203	0.9928	0.9956	0.9957

2.8 Stability study

The prepared formulations were stored in a vial with a rubber closure for three months. After 1, 2, or 3 months, samples were collected and examined for viscosity, drug content, pH, floating lag time, and floating time. The stability study of the optimized F5 batch is shown in Table 4.

Table 4. Stability study of optimized formulation (F5) up to 3 months for the following parameters

Parameter	0 Month	1 Month	2 Months	3 Months
Viscosity (pa.s)	5.03±0.041	5.11±0.13	5.10±0.21	5.10±0.35
Drug content (%)	94.06±1.0	94.00±1.1	93.89±1.3	93.59±1.2
Floating lag time (s)	40±0	41±1	41±0	41±0
Floating time (h)	>12	>12	>12	>12
pH	7.2±0.01	7.2±0.04	7.1±0.04	7.1±0.02

(n= 3, mean±SD)

3. CONCLUSION

The present investigation deals with the formulation, characterization, and optimization of the floating *in situ* gelling systems of lisinopril. Oral administration of a lisinopril suspension consisting of sodium alginate and CMC results in the creation of *in situ* gel in the stomach. There was no chemical interaction between the drug and polymers. Development of *in situ* gel was conducted using 3^2 full factorial design to investigate the effects of independent variables on dependent variables. The formulation (F5) containing 0.75% CMC and 1.25% sodium alginate sustained *in vitro* release for up to 12 h, which could improve drug bioavailability. Developed *in situ* gelling formulation (F5) of lisinopril could float in the stomach and release the drug slowly and maintain its integrity without eroding or dissolving, allowing sustained drug release. The optimized formulation is easy to administer as well as showed desired gastrointestinal retention properties enabling greater efficacy. These results ensure that the developed *in situ* gel system could be the best alternative to the conventional drug delivery of lisinopril to enhance patient compliance.

4. MATERIALS AND METHODS

4.1 Materials

The drug lisinopril was obtained from Aarti pharma ltd. Mumbai. Sodium alginate, CMC, Sodium citrate, and calcium carbonate were supplied by Molychem Lab, Mumbai, and all other reagents used were from Fine chemicals, Mumbai, India.

4.2 Preparation and optimization of *in situ* gelling solution

CMC and sodium alginate with various concentrations of 0.25%, 0.5%, 1.0%, and 1.5% (% w/v) were primed by combining the sodium alginate with distilled water consisting of 0.25% (w/v) sodium citrate and 1.0% (w/v) calcium carbonate and heating to 60°C while stirring. After cooling to below 40°C, lisinopril (50 mg) was combined with 10 mL of 0.1 N hydrochloride acid (HCl) solution (pH 1.2) and added to the resultant solution. The formation of suspension occurs after the addition of the solution. 0.1N sodium hydroxide was used to neutralize the suspension. In ultrapure water, a 1% (w/v) solution of lisinopril was produced. The viscosity of the solution was measured in an *in situ* gel suspension containing lisinopril. The concentrations of calcium carbonate and sodium citrate were held constant at 1.0% and 0.25%, respectively, in the final product. CMC concentrations ranged from 0.75% to 1.25% (w/v), based on the batch (Table 6). The concentrations of sodium alginate (X_2) and CMC (X_1) varied from 0.75% and 1.25% (w/v) in factorial design batches [25-26].

The polymer effect on variables was studied using a 3^2 Full Factorial Design (FFD) for lisinopril *in situ* gel formulation. CMC concentration (X_1) and sodium alginate concentration (X_2) were used as independent variables. The viscosity and drug release were taken as dependent variables. In the present study, the main objective is to increase stability and control viscosity. Hence, in a Design Expert® version 11.0 (Stat-Ease Inc., Minneapolis, USA) software, the full factorial design was selected for a study of viscosity and stability at 2 factors at 3 levels that gave 9 possible combinations of polymers. A 2-factor at 3 levels design was used for polynomial models [27-28].

Table 5. Coded values for the formulations at three levels (low, medium, and, high)

Variables level	Low (-1)	Medium (0)	High (+1)
Concentration of CMC (X_1)	0.75%	1.0%	1.25%
Concentration of sodium alginate (X_2)	1.0%	1.25%	1.50%

Table 6. Compositions of all formulations (F1 to F9) set by software using 3^2 full factorial design

Formulation code	Lisinopril (mg)	CMC (g)	Sodium alginate (g)	Sodium citrate (%)	Calcium carbonate (%)
F1	50	1.25	1.50	0.2	1.0
F2	50	1.00	1.00	0.2	1.0
F3	50	0.75	1.00	0.2	1.0
F4	50	0.75	1.50	0.2	1.0
F5	50	0.75	1.25	0.2	1.0
F6	50	1.25	1.00	0.2	1.0
F7	50	1.00	1.50	0.2	1.0
F8	50	1.25	1.25	0.2	1.0
F9	50	1.00	1.25	0.2	1.0

4.3 Characterization of *in situ* gel

4.3.1 *In vitro* gelling capacity

In a beaker, 500 mL of 0.1N HCl (pH 1.2) was used to determine the *in vitro* gelling capability of the *in situ* gelling solution. From the prepared solution, a 10 mL sample was properly measured and added to HCl with gentle agitation to prevent the gel from splitting. The gelation property was measured qualitatively and expressed in strokes in Figure 7 [29-30]

\+ = After a few minutes, the gels get dispersed immediately
++ = Rapid gelation lasts only a few hours
+++ = The immediate gelation remains for prolonged time

Figure 7. Physical appearance of formation of *in situ* gel in 0.1 N HCl

4.3.2 Drug polymer compatibility studies

The drug lisinopril and polymer compatibility were investigated using FTIR and DSC on both the drug and the polymer (sodium alginate, CMC) separately and in a physical mixture. FTIR is another parameter to identify the purity of the drug and formulation. The FTIR spectrum shows the peaks analogous to the chemical environment of the drug. The FTIR spectra of lisinopril were verified in the range 4000 – 400 cm^{-1} (Agilent Technologies Cary 630 FTIR) [31-32].

The thermal performance of pure lisinopril and the optimized batch was investigated using the DSC apparatus in the study. 2 mg of each sample was placed in aluminum crucibles that were heated at 10°C/min at a temperature range of 25 °C to 500 °C. Changes in drug crystallinity and homogenous dispersion can be detected by DSC (SDT Q600 V20.9 Build 20, TA instrumentation, USA) [33].

4.3.3 Rheological properties of the formulations

By using a Brookfield digital viscometer (*R/S plus* Rheometer Engineering Laboratories Inc.) viscosity of the solution was measured. At room temperature (25°C), the *in situ* gel sample was sheared at a rate of 100 rpm using a spindle [34-35].

4.3.4 Determination of the drug content

The formulation's drug concentration was evaluated by dissolving 5 mL of the *in situ* gel formulation in 80 mL of 0.1N HCl, pH 1.2, and stirring for 1 h on the magnetic stirrer. The solution was diluted with 0.1N HCl, pH 1.2, after 1 h. The resultant solution will be filtered, and the drug content will be detected using a UV-visible spectrophotometer at maximum absorbance of 206 nm (UV-1900 UV-vis spectrophotometer, Shimadzu, Japan) [36-37].

4.3.5 Buoyancy studies

The floating time was evaluated by checking the time required for the *in situ* gel to be afloat on the surface of the gastric fluid at pH 1.2 (floating lag time). The duration of time the preparation stayed afloat on the medium's surface (duration of floating) was recorded [38].

4.3.6 *In vitro* drug release

To determine the drug release of solution a USP dissolution test apparatus type II (Electrolab, Dissolution Tester) with a paddle stirrer at 50 rpm was used. To avoid disrupting the gel composition, this speed was kept low. The temperature was maintained at 37°C and the dissolution medium used was 500 mL of 0.1 N HCl (pH 1.2), 10 mL formulation was removed and put in the dissolution vessel. Using a UV-visible spectrophotometer (UV-1900, Shimadzu, Japan), the absorbance of the lisinopril withdrawal sample was measured at 206 nm. In order to maintain the sink condition, a fresh dissolution medium was added immediately after the test sample was removed. The drug release studies were carried out for a period of 12 h [39-40].

4.3.7 Stability study

Prepared solution of *in situ* gel preparation kept in amber color glass bottles and kept for 3 months in a stability chamber (Remi, Programmable Environmental Test Chamber). The stability of *in situ* gel of lisinopril was monitored for 3 months and the stability study was mentioned in Table 4. At regular time intervals of 30, 60, and 90 days sample was removed and characterized for physical and chemical testing to ensure formulation safety and efficacy. The stability study was performed as per ICH guideline Q1 [42-43].

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