International Journal of Drug Delivery 5 (2013) 43-55
http://www.arjournals.org/index.php/ijdd/index

Original Research Article

ISSN: 0975-0215

Formulation and characterization of novel floating *in-situ* gelling system for controlled delivery of ramipril

Vora Vipul[1], Biswajit Basu*[1]

*Corresponding author:

Biswajit Basu

[1]Department of Pharmaceutics, Atmiya Institute of Pharmacy, Yogidham Gurukul, Kalawad Road, Rajkot-360005, Gujarat State, India..

Abstract

The present study mainly focuses on the novel floating *in-situ* gelling system for controlled delivery of ramipril. Ramipril has half-life of 2-4 hours and required dose is 10 mg day. Hence ramipril is a suitable candidate for sustained drug delivery system. A gastro retentive drug delivery system of ramipril was formulated to increase the resident time in stomach and to modulate the release behavior of the ramipril. Different formulations of ramipril were prepared by using different concentration of gelling polymer such as sodium alginate, gellan gum and calcium carbonate. Sodium citrate was used to prevent gelation outside the gastric environment. The formulation was studied for FT-IR study and DSC study to interpret the interaction between drug and polymer used. Formulation containing 0.50 % of sodium alginate, 0.50 % of gellan gum and 1.0 % of calcium carbonate showed the best gelling ability. For optimization of in-situ gelling system 3^2 full factorial design was employed to study the effect of independent variables, concentration of gellan gum (X_1) and concentration of sodium alginate (X_2) and dependent variables like viscosity, *in vitro* bouncy time, % drug release at 4 hr (Y_3), % drug release at 6 hr (Y_4) and % drug release at 8 hr (Y_5). F8 batch was selected as optimized batch based on buoyancy time (71 sec), viscosity 356.9cp, drug content 99.06 % and CPR 99.80 % at 12 hrs. The controlled release of ramipril from *in-situ* gelling system was observed and good fit to the Zero order and Korsmeyer Peppasmodel whichshows fickian diffusion (n=0.351) mechanism. Stability revealed that there was no noticeable change in characterizations. Thus, *in-situ* gel formulation is promising approach for gastroretentive controlled delivery of Ramipril.

Keywords: *In-situ* gel, Floating drug delivery system, Ramipril, Sodium alginate, Gellan gum.

.

Introduction

Ramipril is angiotensin converting enzyme (ACE) inhibitor antihypertensive class of medication [1]. ACE is a peptidyldipeptidase that catalyzes the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II[2]. In hypertensive patients with normal renal function treated with ramipril alone for up to 56 weeks. The extent of absorption is at least 50-60% and is not significantly influenced by the presence of food in the GI tract[3]. Ramipril is a whitecrystalline or microcrystalline powder with 2-10 mg/day dose, it is readily absorbed from the stomach, but undergoes extensive first-pass hepatic metabolism, with half-life of 2to 4 hours[4-6]. Protein binding of ramipril is about 73-90 %[5]. Due to short half-live it requires frequent dosing which lead to fluctuation in blood levels and decrease patient compliance. These attributes make ramipril agood candidate for controlled release dosage form.

In-situ gel-forming preparations are “stimuli-responsive” polymeric drug-delivery systems that are conveniently delivered orally as a liquid, followed by a transition into a gel upon contact with the gastric uids in the stomach[7]. *In-situ* gelling formulations provide a novel idea of delivering drugs to patient as a liquid dosage form. It present control or sustain release of drug for the desire duration.

Materials and Methods

Materials

Ramipril was obtained as gift sample from Ipca Laboratories Pvt. Ltd., Mumbai, India, Gellan Gum (Sisco Research Laboratories Pvt. Ltd., Mumbai, India), Sodium Alginate (SD fine Chem. Limited., Mumbai), Carrageenan (Himedia Laboratories Pvt. Ltd., Mumbai, India), Low Methoxy Pectin and High Methoxy Pectin (Krishna Pectines Pvt. Ltd., Sirsoli, India). All other reagents and chemicals used were of analytical grade.

Methods

Preliminary studies

Four various concentrations of gellan gum were used, Among the four concentrations, 0.25%, 0.5% and 0.75% were selected for further studies because in this concentration gel was formed and

viscosities ranges were in acceptable limit. With 1% w/v concentration of gellan gum, very stiff gel was formed as well as viscosity was very high (Table 1)

Table 1: Preliminary Studies

Batch No.	Polymer	Concentration of polymer	Viscosity of solution[a] (cps)	Gelling capacity
T1	Gellan gum	0.25 %	163.4 ± 3.23	Gel is formed
T2	Gellan gum	0.50 %	241.6 ± 2.98	Stiff gel is formed
T3	Gellan gum	0.75 %	303.6 ± 3.21	Stiff gel is formed
T4	Gellan gum	1.00 %	542.6 ± 4.32	Very Stiff gel is formed
T5	Sodium alginate	0.25 %	136.5 ± 3.24	Gel is formed
T6	Sodium alginate	0.50 %	169.3 ± 3.78	Gel is formed
T7	Sodium alginate	1.00 %	287.6 ± 4.24	Stiff gel is formed
T8	Sodium alginate	1.50 %	393.6 ± 2.94	Stiff gel is formed
T9	Low methoxy pectin	1.00 %	75.6 ± 2.17	Gel is not formed
T10	Low methoxy pectin	4.00 %	92.3 ± 3.02	Gel is formed
T11	High methoxy pectin	1.00 %	72.5 ± 2.42	Gel is not formed
T12	High methoxy pectin	4.00 %	79.8 ± 3.41	Gel is not formed
T13	Carrageenan	1.00 %	68.3 ± 2.97	Gel is not formed
T14	Carrageenan	4 .00 %	83.4 ± 3.14	Gel is not formed
*All the formulations contain 1% w/v calcium carbonate				

Selection and optimization of calcium carbonate

Calcium carbonate was used as gas forming agent. The calcium carbonate present in the formulation as insoluble dispersion is dissolved and releases carbon dioxide on reaction with acid, and the *in-situ* releases calcium ions resulting in formation of gel with floating characteristics. It is established that formulations containing calcium carbonate produce a significantly stronger gel than those containing sodium bicarbonate. This is due to the internal ionotropic gelation effect of calcium on gellan [13,14].

Three various concentrations of calcium carbonate were taken. Among these three concentrations, with 1% calcium carbonate, buoyancy time was 42 sec; total floating duration and viscosity (174.6±1.98) were in acceptable limit. So, 1% was selected for all the formulations. Increasing the calcium carbonate content in the formulation simultaneously increased the viscosity at all polymer concentrations studied (Table 2).

Table 2: Optimization of calcium carbonate

Polymer[a]	Calcium carbonate (%)	Buoyancy time[b] (Sec)	Viscosity[b] (cp)	Total floating duration[a] (h)
Sodium alginate	0.5 %	90 ± 10	171.4 ± 2.48	> 12
Sodium alginate	1.0 %	42 ± 06	174.6 ± 1.98	>12
Sodium alginate	1.5 %	63 ± 12	179.3 ± 2.75	>12
Gellan gum	0.5 %	113 ± 11	246.2 ± 3.75	>12
Gellan gum	1.0 %	52 ± 08	252.5 ± 2.38	>12
Gellan gum	1.5 %	65 ± 07	261.31 ± 2.26	>12

[a]In concentration of 0.50 % w/v

[b]All the values are in mean ± SD (n=3)

Optimization of concentration of sodium citrate

The *in-situ* gelling formulation makes contact with an acidic medium and forms gel by cross linking with Ca++ ions and form a three dimensional gel network in acidic environment. Low level of cations present in the solution was sufficient to hold the molecular chains together so low level of sodium citrate is required to prevent gelation of *in-situ* gelling formulation before it comes contact with acidic medium[11]. At low concentration (0.15%), gel was formed with 0.1 N HCl, but after one day the formulation was converted to gel during storage. At medium concentration (0.20%), gelation was very good with 0.1 N HCl and the formulation was also stable (solution form) during storage. So, 0.20% was selected for final formulations. Same concentration was selected for gellan gum also (Table 3).

Table 3: Optimization of concentration of sodium citrate

Polymers[a]	Sodium citrate (% w/v)	Calcium carbonate (% w/v)	Gelation in 0.1 N HCl[b]	After 1 day
Sodium alginate	0.15	1.0	++	Gel
Sodium alginate	0.20	1.0	+++	Solution
Sodium alginate	0.25	1.0	+	Solution
Gellan gum	0.15	1.0	+++	Gel
Gellan gum	0.20	1.0	+++	Solution
Gellan gum	0.25	1.0	+	Solution

[a] In concentration of 0.50 % w/v

[b]Gels after few minutes, dissolves rapidly;

++ Gelation immediate remains for few hours;

+++ Gelation immediate remains for extended period;

++++ Gels after few minutes, remains for extended period.

Preparation of floating *in-situ* gelling solution

Polymer solution of different concentration was prepared in deionized water containing sodium citrate using magnetic stirrer. Low level of cations present in the solution is sufficient to hold the molecular chains together and inhibit hydration. A polymeric solution was heated at 60ºC to uniform dispersion of polymer with stirring on magnetic stirrer. After cooling below 40ºC, Drug is dissolve separately in deionized water, various concentrations of gas forming agent were added which is and dispersed well with continuous stirring. Finally drug and gas forming agent containing solution were added to the polymeric solution. The resulting in situ gel solution was finally stored in amber color narrow mouth bottles until further use.

3^2 Full Factorial Designs

A 3^2 full factorial design was applied to examine the combined effect of two formulation variables, each at 3 levels and the possible 9 combinations of ramipril in situ gel were prepared (Table 2). The Concentration of gellan gum (X_1) and the Concentration of sodium alginate (X_2) were taken as independent variables. The viscosity, Buoyancy time (sec), cumulative percentage drug release at 4, 6 & 8 hrs were taken as dependent variables.[17] (Table 4, 5 & 6)

Table 4: Factor and levels for 3^2 factorial design

Variables level	Low (-1)	Medium (0)	High (+1)
Concentration of gellan gum (X_1)	0.25 %	0.50 %	0.75 %
Concentration of sodium alginate (X_2)	0.00 %	0.25 %	0.50 %

Table 5: Coded value of factor in different batches of *in-situ* gelling formulations

Batch No.	X_1 (A)	X_2(B)
R1	-1	-1
R2	0	-1
R3	1	-1
R4	-1	0
R5	0	0
R6	1	0
R7	-1	1
R8	0	1
R9	1	1

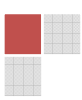
Table 6: Formulation table of various *in-situ* gelling formulations

Batch No.	Ramipril (mg/ml)	X_1(%)	X_2(%)	Calcium carbonate	Sodium Citrate
R1	2	0.25	0.00	1%	0.2 %
R2	2	0.50	0.00	1%	0.2 %
R3	2	0.75	0.00	1%	0.2 %
R4	2	0.25	0.25	1%	0.2 %
R5	2	0.50	0.25	1%	0.2 %
R6	2	0.75	0.25	1%	0.2 %
R7	2	0.25	0.50	1%	0.2 %
R8	2	0.50	0.50	1%	0.2 %
R9	2	0.75	0.50	1%	0.2 %

Measurement of melting point of ramipril

Melting point was determined by taking small amount of ramipril in a capillary tube closed at one end. The capillary tube was placed in an electrically operated digital melting point apparatus and the temperature at which the drug melts was recorded. This was performed thrice and average value was noted.

FT-IR spectroscopy



The pure drug and drug with excipients were scanned separately. Potassium bromide was mixed with drug and/or polymer in 9:1 ratio. Mixture of drug and/or polymer was compressed in palate using KBR press palate and the spectra were taken in FTIR spectrophotometer (Thermo Fisher Sci. Inc. USA Nicolat iS10).

Differential scanning calorimetry (DSC) Study

DSC analysis of pure drug and optimized formulation was performed with Shimadzu DSC 60 thermal analyser at the heating flow rates of 10 C per min between 50 and 300 C under static air using aluminium pans.

Measurement of pH

The pH of the prepared formulations was measured by digital pH meter (Systronics Ltd., Ahmedabad, India).

Measurement of viscosity of *in-situ* gelling solution

Viscosity of the samples was determined using a Brookfield digital viscometer (Model no: LVDV-III ULTRA Programmable Rheometer) with spindle S62. The sample temperature was controlled at 25±1ºC before the each measurement [8].

Measurement of *in vitro* buoyancy of *in-situ* gelling solution

The *in-vitro* floating study was determined using USP dissolution apparatus II having 500 ml of 0.1 N HCl solution (pH 1.2). The medium temperature was maintained at 37±2 C. 10 ml prepared *in-situ* gel formulations was drawn up using disposable syringe and placed into the Petridish (4.5 cm internal diameter) and finally Petridis containing formulation was kept in the dissolution vessel containing medium without much disturbance. The time the formulation takes to emerge on the medium surface (floating lag time) was noted [9,10].

Measurement of *in-vitro* duration of floating of *in-situ* gelling solution

The *in vitro* floating study was determined using USP dissolution apparatus II having 500 ml of 0.1 N HCl solution (pH 1.2). The medium temperature was maintained at 37±2 C. 10 ml prepared *in-situ* gel formulations was drawn up using disposable syringe and placed into the Petridis (4.5 cm internal diameter) and finally Petridis containing formulation was kept in the dissolution vessel containing medium without much disturbance. The time the formulation constantly float on the dissolution medium surface (duration of floating) was noted [9,10].

Measurement of *in vitro*gelation study

The gelation study was carried out as described by Zhidong et al., with slight modification. The gelation cells were fabricating locally using Teflon. The cells were cylindrical reservoirs capable of holding 3 ml of the gelation solution (0.1 N HCl of pH 1.2). 500 µl transparent plastic cup was located at the bottom of cell within the cells to hold the gel sample in place after its formation. Then, 500 µl of the preparation will be carefully placed into the cavity of the cup using micropipette, and 2 ml of the gelation solution (0.1 N HCl of pH 1.2) was added slowly in reservoir. Gelation will be observed by visual examination.

The in vitro gelling capacity was graded in four categories on the basis of gelation time and time period for which the formed gel remains [8].

+ Gels after few minutes, dissolves rapidly
++ Gelation immediate remains for few hours
+++ Gelation immediate remains for extended period.
++++ Gels after few minutes, remains for extended period.

Measurement of drug content in formulation

The drug content of the formulation will be determine by dissolving 5 ml of *in-situ* gelling formulation in 40ml of methanol containing 50 ml volumetric flask followed by sonication for 30 min. Than volume is makeup to the mark. The resulting solution will be filter and the drug content of solution will be measured at maximum absorbance at 222 nm using UV-Visible Spectrophotometer.

Measurement of *in vitro* drug release

The release of ramipril from the *in-situ* gel preparations was determined as described by Zatz and Woodford (1987) with some modification using USP dissolution test apparatus (USP XXIV) with a paddle stirrer at 50 rpm. This speed was slow enough to avoid the breaking of gelled formulation and will be maintaining with the mild agitation conditions believed to exist *in vivo*. The dissolution medium used will 500 ml of 0.1 N HCl (pH 1.2), and temperature were maintained at 37 C. 10 ml formulation was drawn up using disposable syringe. The syringe end was then place into the Petridis (4.5 mm internal diameter) and the syringe plunger depressed slowly to extrude 10 ml and finally Petridis containing formulation will keep in the dissolution vessel containing dissolution medium without much disturbance. At each time interval, a precisely measured sample of the dissolution medium will removes and replace with prewarmed (37 C) fresh dissolution medium. Absorbance of ramipril in withdrawn samples was measured using UV Visible Spectrophotometer (Shimadzu Corporation, Tokyo, Japan UV-1700 Pharma Spec) [11].

Stability study

Prepared in situ gel formulation of ramipril was stored in a amber colored glass containers (well stoppered) for three months and the stability of the in situ gel suspension formulation of Ramipril was monitored up to 2 months at Controlled temperature (40 ± 2 C) and controlled humidity (75 ± 2 % RH) conditions. Periodically (initial, 1and 2 months) samples were removed and evaluated for pH, viscosity, drug content and in vitro release[8,12].

Results & Discussion

Melting Point of Ramipril

Melting point of Ramipril was determined by capillary tube method and it was found to be 109±1.98 (n = 3). This value is similar as that of the literature citation 109 ºC.

FT-IR spectroscopy

FT-IR studies were carried out for pure drug alone and final optimized formulation. FTIR spectrum of pure Ramipril was shown in the Figure 1. Similarly FTIR spectra of optimized formulation were shown in Figures 1 and 2. Characteristic peaks were not affected and prominently observed in FTIR spectra given in Figures 1 and 2. This indicates that there is no interaction between Ramipril and polymers and the drug was compatible with the formulation components.

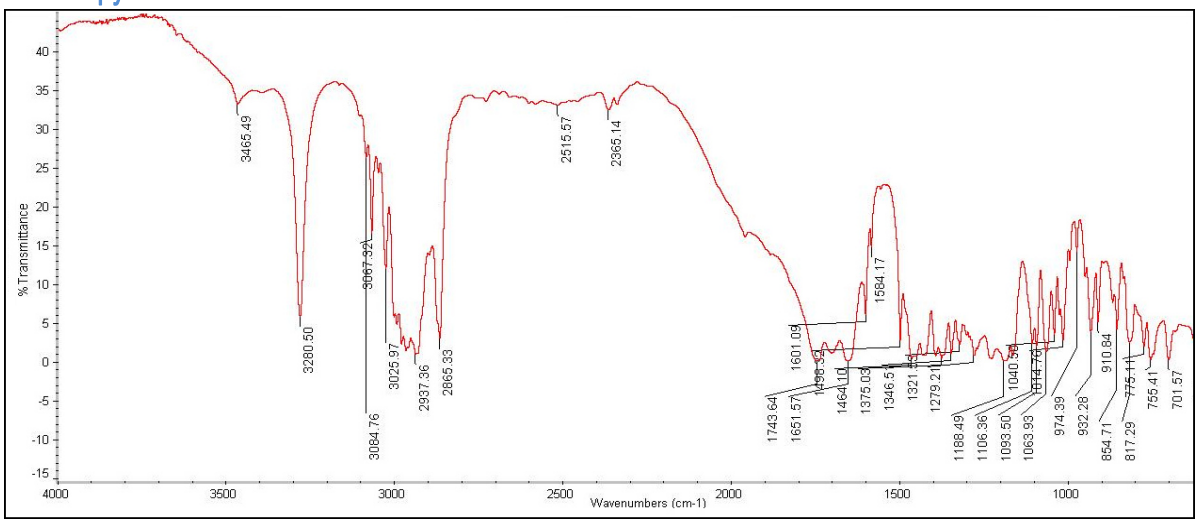


Figure 1: IR Spectrum of Ramipril

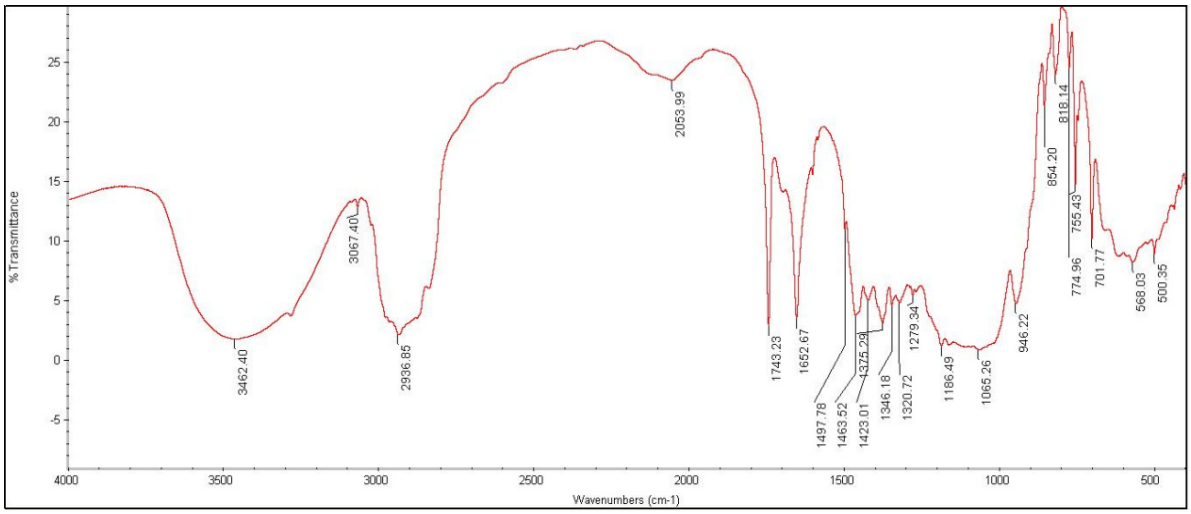


Figure 2: IR Spectrum of optimized formulation

Differential Scanning Calorimetry (DSC) Study

Thermograph of Ramipril is shown in Figure 3. Melting transition of Ramipril was observed from 109.44ºC (Onset) to 115.47ºC (Endset). Sharpmelting transition of Ramipril was observed at 112.06ºC. In the optimized formulation R8 (Figure 4) drug and excipients melting endotherm was observed from 108.95ºC (Onset) to 114.98ºC (Endset). Sharp melting transition of Ramipril in R8 formulation was observed at 112.64ºC.

In case of optimized formulation drug peak is shifted to slightly lower temperature and decreases the intensity of peak which may be due to baseline shifting. There was no much difference in the melting point of the drug in all the thermographs.

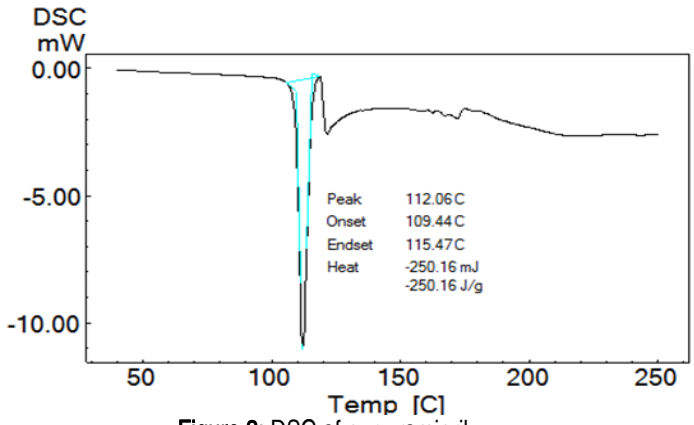


Figure 3: DSC of pure ramipril

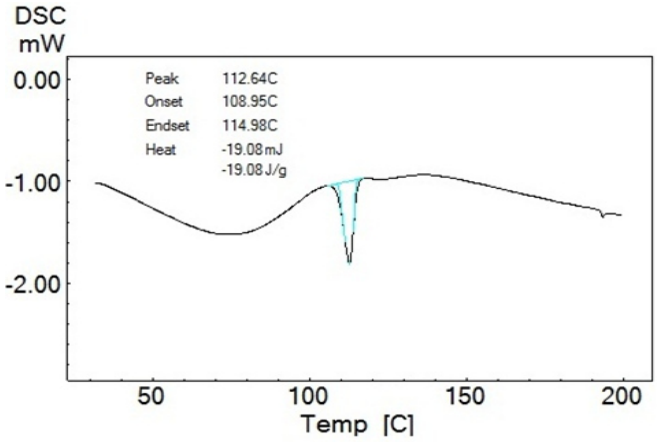


Figure 4: DSC of optimized formulation-R8

Experimental design

3^2 full factorial design has been applied to optimize the formulation variables with basic requirement of understanding interaction of independent variables. Preliminary investigations of the process parameters revealed that factors like concentration of gellan gum (X1) and concentration of sodium alginate (X2) showed significant influence on viscosity (Y1), in vitro buoyancy time (Y2), amount of drug release in 4 hrs (Q4; Y3) and amount of drug release in 6 hrs (Q6; Y4) and amount of drug release in 8 hrs (Q8; Y5) of in situ gel formulations. Hence, they were utilized for further systematic studies. For all 9 batches, both the selected dependent variables (X1 and X2) showed a wide variation in viscosity, amount of drug dissolve and buoyancy time. The data clearly indicated strong influence of A and B on selected responses (Y1, Y2, Y3, Y4 and Y5). The polynomial equations can be used to draw conclusions after considering magnitude of coefficients and mathematical sign it conveys either positive or negative. Results for experimental design batches and its ANOVA were shown in table 7, 8, 9 and figure 5 – 9.

pH of *in-situ* gelling solutions

Results of pH measurement of formulation R1 to R9 were described in Table 7. All the formulation has pH around neutral or slightly alkali. Maximum pH 7.4 was observed in R3 formulation and minimum pH 6.8 was observed in R8 formulations.

Table 7: Characterizations of *in situ* gelling formulations

Batch No.	pH[a]	Viscosity[a] (CP)	*In vitro* buoyancy time[a] (Sec)	Total floating time (h)	Drug content[a] (%)	*In vitro* gelation studies[b]
R1	7.2±0.4	118.4±9.2	38±4	>4	97.500± 0.83	++
R2	7.1±0.3	195.6±8.5	79±5	>8	99.688± 0.94	++
R3	7.4±0.3	261.6±10.6	66±3	> 12	98.542±1.48	++
R4	6.9±0.4	195.5±7.5	45±3	> 10	96.667± 1.18	++
R5	7.0±0.2	258.3±5.8	56±2	> 12	98.333± 0.79	++
R6	7.3±0.3	345.4±8.8	83±6	> 12	98.750± 0.63	+++
R7	6.8±0.4	274.5±7.4	58±5	> 12	97.604±1.48	+++
R8	7.2±0.2	356.9±6.3	71±3	> 12	99.06± 0.63	+++
R9	7.0±0.4	496.5±12.3	103±4	> 12	97.292± 1.91	++++

a All the values are in mean ± SD (n=3)
b+ Gels after few minutes, dissolves rapidly,
++ Gelation immediate, remains for few hours,
+++ Gelation immediate, remains for extended period,
++++ Gels after few minutes, remains for extended period.

Table 8: Summary of experimental design

Batch code	Factor 1 Gellan Gum	Factor 2 Sodium Alginate	Response 1 Viscosity (cps)	Response 2 Buoyancy (Sec)	Response 3 CPR at 4 hr	Response 4 CPR at 6 hr	Response 5 CPR at 8 hr
R1	-1	-1	118.4	38	95.24	99.86	100.05
R2	-1	0	195.6	79	85.13	99.77	99.89
R3	-1	1	261.6	66	72.5	86.28	99.53
R4	0	-1	195.5	45	83.13	94.19	99.48
R5	0	0	258.3	56	78.03	74.10	98.20
R6	0	1	345.4	83	67.67	83.98	93.96
R7	1	-1	274.5	58	69.41	81.25	91.05
R8	1	0	356.9	71	59.61	72.29	85.09
R9	1	1	496.5	103	55.89	67.94	81.11

Table 9: Polynomial coefficient of all five responses

Coefficient		β_0	β_1	β_2	β_{11}	β_{22}	β_{12}
Y1	FM	259.08	92.05	87.52	19.70	15.22	14.42
	RM	-	-	-	-	-	-
Y2	FM	63.307	8.17	18.5	-	-	-
	RM	63.307	-	18.5	-	-	-
Y3	FM	77.54	-11.32	-8.62	-3.96	-0.93	2.30
	RM	77.27	-11.32	-8.62	-4.31	-	2.30
Y4	FM	85.08	-10.73	-6.18	-	-	-
	RM	-	-	-	-	-	-
Y5	FM	97.93	-7.036	-2.666	-4.78	-0.550	-2.355
	RM	97.77	-7.036	-2.663	-4.990	-	-2.355

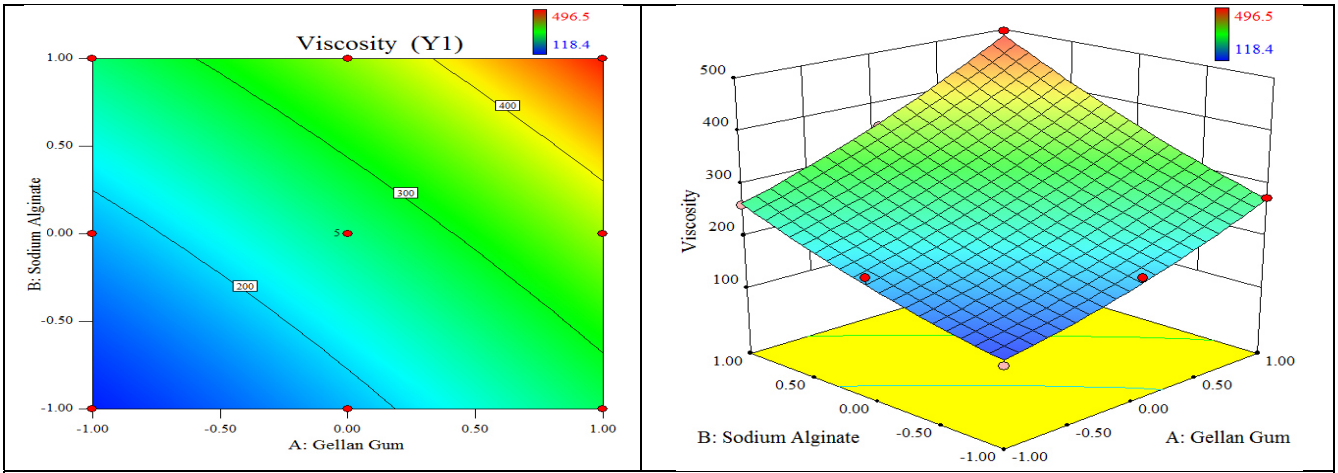


Figure 5: Counter plot and 3D response plot of viscosity (Y1) with X1 and X2

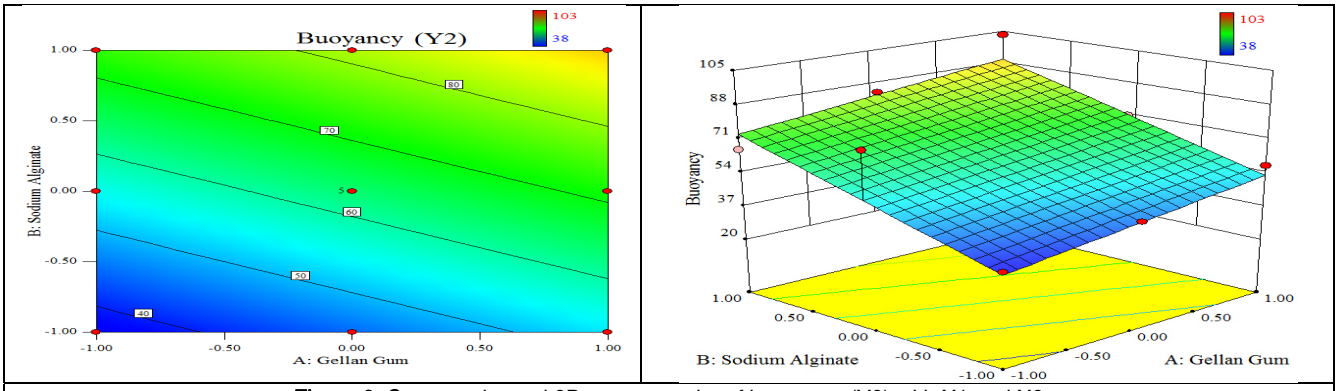


Figure 6: Counter plot and 3D response plot of buoyancy (Y2) with X1 and X2

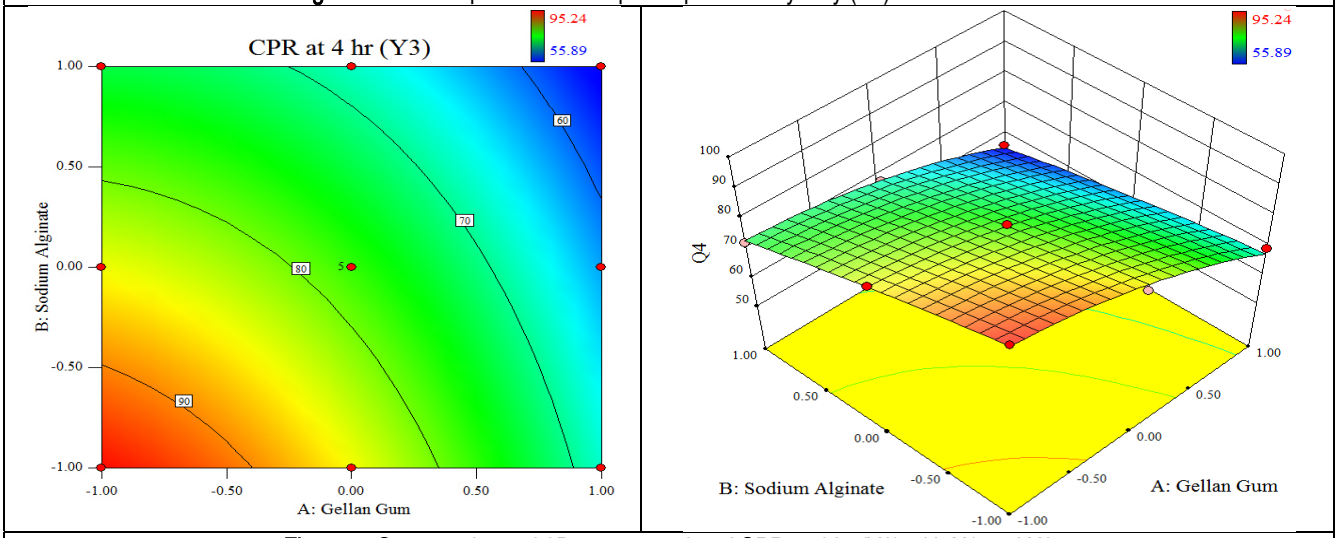


Figure 7: Counter plot and 3D response plot of CPR at 4 hr (Y3) with X1 and X2

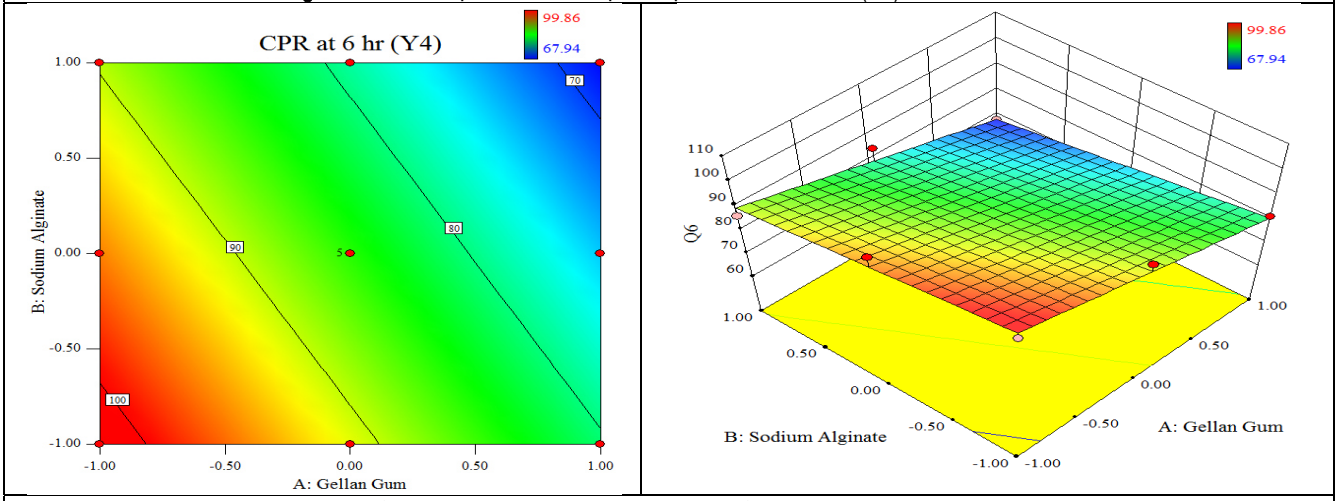


Figure 8: Counter plot and 3D response plot of CPR at 6 hr (Y4) with X1 and X2

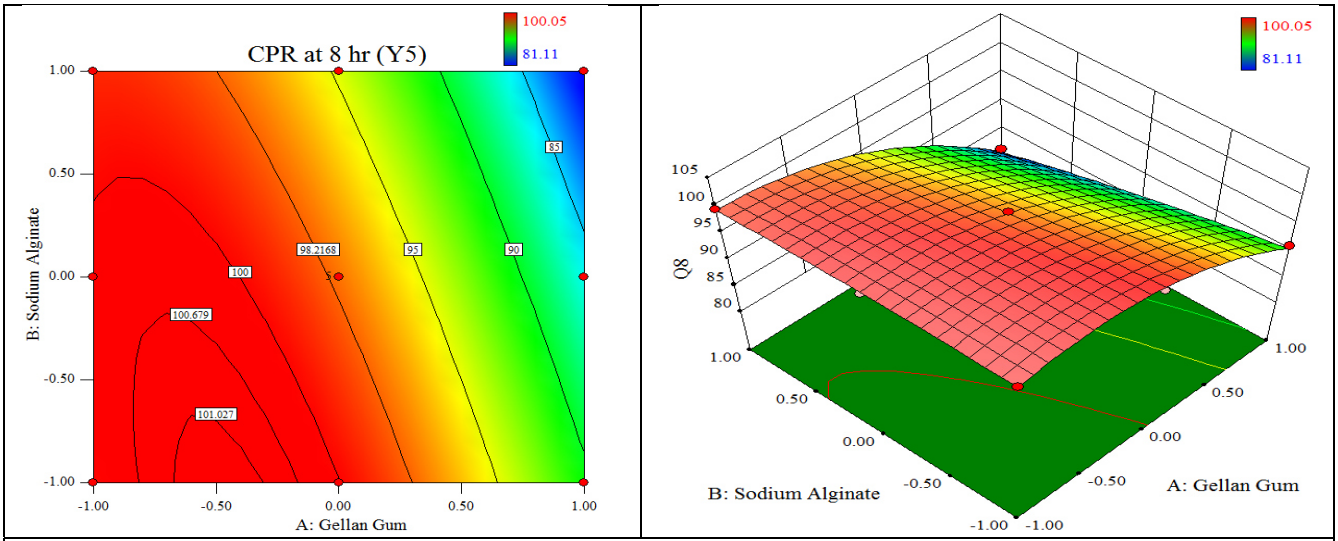


Figure 9: Counter plot and 3D response plot of CPR at 8 hr (Y5) with X1 and X2

Viscosity of *in-situ* gelling solutions

The rheological properties of the solutions are of importance in view of their proposed oral administration. In the selection of the concentration of gelling polymer a compromise is sought between a sufficiently high concentration for the formation of gels of satisfactory gel strength for use as a delivery vehicle, and a sufficiently low concentration to maintain an acceptable viscosity for ease of swallowing[15].

Results of viscosity formulation R1 to R9 were described in Table 7. The solutions showed a marked increase in viscosity with increasing concentration of gellan and sodium alginate.

In-vitro buoyancy of *in-situ* gelling solution

The buoyancy of the prepared formulations was performed in 0.1 N HCl (pH 1.2). Results of in vitro buoyancy time of formulation R1 to R9 were described in Table 7. Formulations containing calcium carbonate demonstrated excellent floating ability, while formulations not containing this agent settled at the bottom of the medium. The calcium carbonate effervesced, releasing carbon dioxide and calcium ions. The released carbon dioxide is entrapped in the gel network, producing buoyant formulation; then, calcium ion reacted with gellan and produced a cross linked three dimensional gel network.

In-vitro duration of floating of *in-situ* gelling solution

The total floating time of the prepared formulations were performed in 0.1 N HC (pH 1.2). Results of *in vitro* total floating time of formulation R1 to R9 were described in Table 7. Reason for the less floating lag time of R1 formulation might be due to escape of CO_2 air bubbles from the gelling network because of low concentration of polymer. R5 to R9 formulations have total floating lag time more than 12 hr. The possible reason behind that, combination of sodium alginate and gellan gum form stiff gelling system after contact with HCl.

In-vitro gelation study

Results of in vitro gelation are graded on arbitory scale from formulation R1 to R9 were described in table 7.The *in-situ* formed gel should preserve its integrity without dissolving or eroding for prolonged period to facilitate sustained release of drugs locally. R1 to R5 formulations are forms gel immediately and remain for few hours. Low concentration of polymer is responsible for weak cross linked three dimensional network of gel, might be that is the reason for the degradation of gel after few hour. Formulations R6, R7 and R8 are forms gel immediately and remain for extended period. Formulation R9 form gel after few minutes and remains for extended period. Because of high concentration of gellan gum and sodium alginate it forms high stiff gel.

Drug content

Results of drug content of formulation R1 to R9 were described in Table 7. The solutions showed a percentage drug content from 96.66% to 99.06 %.

In-vitro drug release

Dissolution profile of formulation of R1 to R9 is shown in Figure 10. The effect of polymer concentration on *in vitro* drug release from *in-situ* gels is shown in Figure 5. A significant decrease in the rate and extent of drug release was observed with the increase in polymer concentration in *in situ gels* and is attributed to increase in the density of the polymer matrix and also an increase in the diffusional path length which the drug molecules have to traverse[16]. The release of drug from these gels was characterized by an initial phase of high release (burst effect). However, as gelation proceeds, the remaining drug was released at a slower

rate followed by a second phase of moderate release. This bi-phasic pattern of release is a characteristic feature of matrix diffusion kinetics. The initial burst effect was considerably reduced with increase in polymer concentration[9].

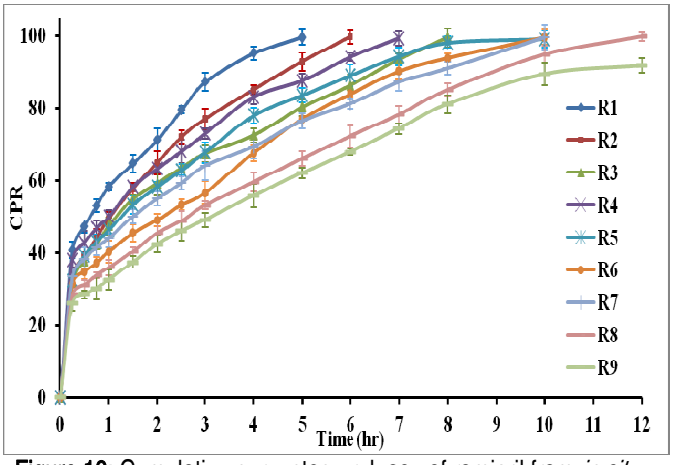


Figure 10: Cumulative percentage release of ramipril from *in situ* gelling formulations in 0.1 N HCl (pH 1.2)

Multiple regression analysis[8]

The factorial design was carried out using the software DESIGN EXPERT® version 8.0.7.1 trial (Stat-Ease Inc., Minneapolis, USA). Response surface graphs were used to determine the factor of interaction between the considered variables. Values of $p < 0.05$ indicate model terms are significant.

The concentration of gellan gum (X_1) and amount of sodium alginate (X_2) were chosen as independent variables. The statistical model comprising incorporated interactive and polynomial terms was utilized to evaluate the response. The resulted equations for all five dependent variables Y_1 (viscosity), Y2 (buoyancy), Y^3 (CPR at 4 hr), Y_4 (CPR at 6 hr), and Y_5 (CPR at 8 hr) in terms of coded factors are presented in Table 8.

For quadratic models

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1 X_1 + \beta_{22} X_2 X_2 + \beta_{12} X_1 X_2 \quad \textit{(eq. 1)}$$

For linear models

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1 X_1 \quad \textit{(eq. 2)}$$

Kinetics of drug release [17,18]

The data of average values were processed as per Zero order, First order, Higuchi, Hixon Crowell and Korsmeyer peppa's models are represented in the Figures 11 to 15 respectively. The release data of ramipril from all the formulations were given in Figure 5. Data of the *in vitro* release were fit into different equations and kinetic models to explain the release kinetics of ramipril from the *in-situ* gelling solutions.

On the basis of the R^2 value R3 to R5 formulations were followed the first order release kinetics and all the other formulations (R1, R2, R6, R7, R8 and R9) were followed the zero order release kinetics. The better fit (highest R^2 values) was observed incase of Higuchi's model than Hixon Crowel model in all the formulations. Hence mechanism of drug release from the *in situ* gelling formulations R1 to R9 are followed diffusion controlled. Application of Higuchi's equation ($M = K t^{1/2}$) provides information about the release mechanism, namely diffusion rate limited. Application of Hixon Crowell cube root law, the equation ($M_0^{1/3} - M^{1/3}$) = kt, provides information about the release mechanism, namely dissolution rate limited. Korsmeyer Peppas model indicates that release mechanism is not well known or more than one type of release phenomena could be involved. The 'n' value could be used to characterize different release mechanisms. According to Korsmeyer Peppas model, a value of slope <0.5 indicates fickian diffusion. So, it indicates that release mechanism from the formulation R8 follows fickian diffusion.

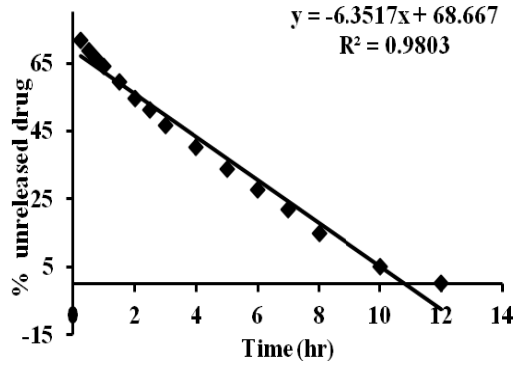


Figure 11: Zero order

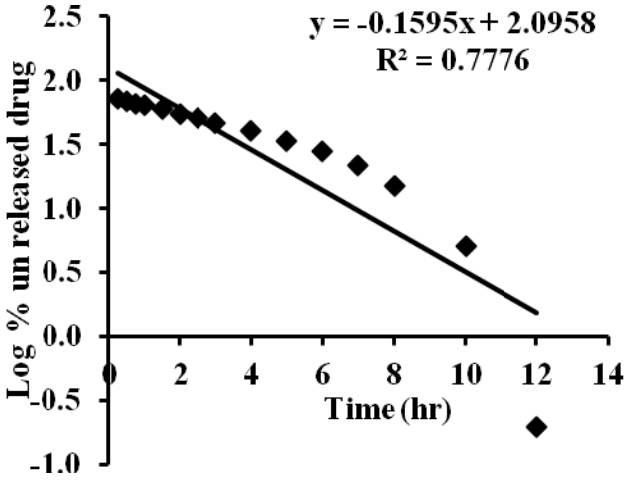


Figure 12: First Order

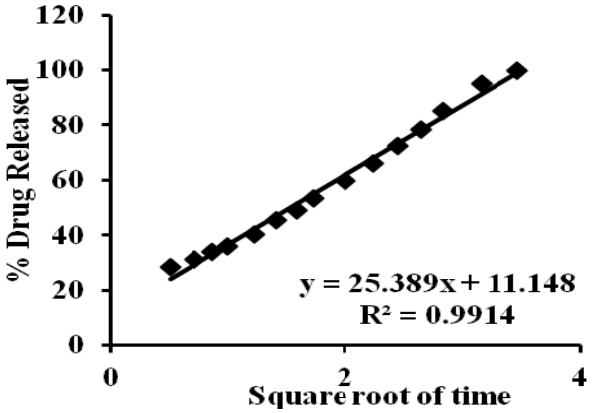


Figure 13: Higuchi square root of time

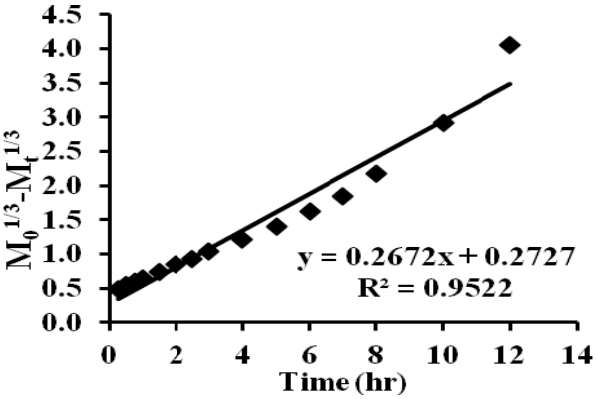


Figure 14: Hixon Crowell

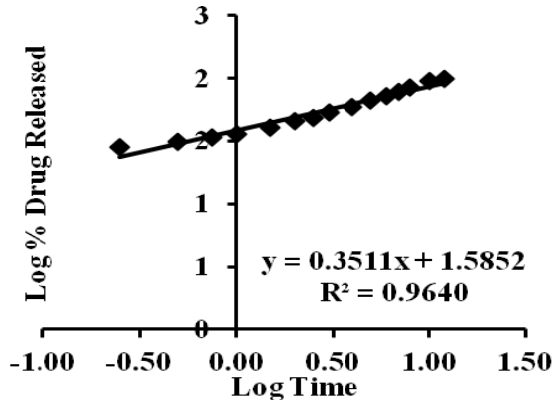


Figure 15: Korsmeyer Peppas model

Stability study of optimized batch

From the above result R8 batch was found to be an optimized batch and the same was kept for stability study under controlled environment condition (40± 2 C and 75 ± 5 %RH). The samples were withdrawn at interval of one and two month and analyzed for pH, viscosity, gelling capacity, bouncy time, total floating lag time, drug content. The results were described in Table 10. It was observed that at the end of two months the viscosity of the formulation was decreased from 365.9 ± 9.3 cp to 359.2 ± 7.3 cpwhich might be attributed to the loss of water and was insignificant to affect the rheological property of *in-situ* gel. The *in vitro* drug release profiles were evaluated by similarity factor (f2) for optimized batch before and after two months. Similarity factor of (f2) 87.06 indicate similarity of both the profiles[7,19]. *In vitro* release profile of ramipril from optimized formulation after and before stability was described in table 11. Hence it can be concluded that there isn't any significant change in the *in-situ* gel at the end of stability study of two months.

Table 10: Results of stability studies

Evaluation Parameters	Time period for sampling		
	Initial	1 month	2 month
pH	7.2 ± 0.20	7.12 ± 0.09	7.10 ± 0.12
Viscosity (cp)	356.9 ± 9.3	358.8 ± 5.6	359.2 ± 7.3
In vitro gelling cpacity	+++	+++	+++
In vitro buoyancy (sec)	71 ± 3	75 ± 5	79 ±4
Total floating time (hr)	>12	>12	>12
Drug content (%)	99.06 ± 0.63	98.32 ± 0.67	97.56 ± 0.84

Table 11: *In vitro* release profile of ramipril from optimized formulation after and before stability

Time	Initial	After 1 Month	After 2 Month
0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.25	28.28 ± 2.15	29.05 ± 2.35	26.71 ± 1.01
0.50	31.19 ± 1.51	30.22 ± 1.32	29.54 ± 1.45
0.75	33.91 ± 0.76	32.94 ± 1.98	32.17 ± 1.35
1.00	35.99 ± 2.15	34.59 ± 1.45	33.26 ± 2.15
1.50	40.40 ± 1.26	39.45 ± 2.01	38.25 ± 1.68
2.00	45.50 ± 3.12	44.03 ± 1.12	42.52 ± 2.05
2.50	48.89 ± 2.76	47.21 ± 2.46	46.15 ± 1.49
3.00	53.19 ± 1.05	50.20 ± 1.93	48.70 ± 2.13
4.00	59.61 ± 2.47	57.18 ± 3.02	55.06 ± 1.54
5.00	66.09 ± 1.90	64.23 ± 2.45	63.14 ± 2.16
6.00	72.29 ± 3.15	69.36 ± 2.04	68.41 ± 3.15
7.00	78.23 ± 2.48	76.96 ± 1.65	75.39 ± 1.09
8.00	85.09 ± 1.87	86.07 ± 1.84	80.99 ± 2.94
10.00	94.87 ± 2.54	92.39 ± 2.01	90.64 ± 1.86
12.00	99.80 ± 1.21	98.10 ± 1.79	96.93 ± 2.73

* All the values are in mean ± SD (n=3)

Conclusions

This study reports that oral administration of aqueous solutions of Ramipril containing gellan gum and sodium alginate results in formation of *in situ* gel at the stomach. From compatibility studies it was found that there was no interaction between the drug and polymer. The results of a 3^2 full factorial design revealed that the concentration of gellan gum sodium, alginate and concentration of calcium chloride significantly affected on the dependent variables like viscosity (Y_1), *in vitro* buoyancy (Y_2), CPR at 4hr (Y_3), CPR at 6hr (Y_4) and CPR at 8hr (Y_5).The optimized formulation R8 show *in vitro* sustains drug release up to 12 hr. From the release kinetic it was concluded that R8 formulation indicate zero order release as a best fit model. Stability revealed that there was no noticeable change in pH, viscosity, gelling capacity, buoyancy, total floating time, drug content and *in vitro* drug release profile. Thus, *in situ* gelling formulation is promising approach for gastroretentive controlled delivery of ramipril.

Acknowledgements

We are thankful to Ipca Laboratories Pvt. Ltd., Mumbai for providing us the drug sample for our project work. We are also thankful to Krishna Pectines Pvt. Ltd., Sirsoli for providing us the polymers.

References

[1]. Brunton LL, Lazo JS, Parker KL. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th ed. McGraw Hill, New York:2006; 805.

[2]. Rang HP, Dale MM, Ritter JM, Moore PK, Pharmacology, 5th ed. Churchill Livingstone,New York: 2003; 296.

[3]. Shukla J. Formulation and evaluation of oral self microemulsifying drug delivery system of ramipril. M. Pharm Thesis. Veer Narmad South Gujarat University, Surat, July 2010.

[4]. British Pharmacopeia.The stationary office of the medicine and healthcare products regulatory agency, Majesty's Stationary Office Ltd., Great Britain, 2004; Volume-II: 1680-1682.

[5]. Drug bank, "Ramipril" November 2011,www.drugbank.ca/drugs/DB00178.

[6]. Wikipedia, "Ramipril", November 2011, www.en.wikipedia.org/wiki/Ramipril. Kotreka U, Adeyeye M. Gastroretentive Floating Drug Delivery Systems: A Critical Review. Crit. Rev. Ther. Drug. Carrier. Syst. 2011;28(1): 47–99.

[7]. Patel RP, Dadhani B, Ladani R, Baria AH, Patel J. Formulation, evaluation and optimization of stomach specific in situ gel of clarithromycin and metronidazole benzoate. International Journal of Drug Delivery. 2010;2:141-153.

[8]. Paruvathanahalli R, Balasubramaniam J, Mishra B. Development and evaluation of a novel floating in situ gelling system of amoxicillin for eradication of Helicobacter pylori. International Journal of Pharmaceutics. 2007; 335(1-2): 114-122.

[9]. Paruvathanahalli R and Mishra B.Floating in situ gelling system of acetohydroxamic acid for clearance of h. pylori. Drug Delivery and Industrial Pharmacy. 2008; 34: 577-587.

[10]. Paruvathanahalli R, Mishra B. Floating in-situ gelling system for stomach site specific delivery of clarithromycin to eradicate H. pylori. Journal of Controlled Release. 2008; 125(1): 33-41.

[11]. Chavda J, Patel K, Modasiya K. Floating In Situ Gel based on Alginate as Carrier for Stomach-Specific Drug Delivery of famotidine. International Journal Pharmaceutical Science and Nanotechnology2010; 3(3): 1092-1104.

[12]. Choi BY, Park HJ, Hwang SJ and Park JB. Biologicalintractions between divalent cation and polysaccharides. International Journal of Pharmaceutics. 2002; 23(9): 81–91.

[13]. Kedzierewicz F, Lombry C, Rios R, Hoffman M and Maincent P. Effect of the formulation on the in vitro release of propranolol from gellanbeads. International Journal of Pharmaceutics. 1999; 178: 129–136.

[14]. Miyazaki S, Takahashi A, Itoh K, Ishitani M, Dairaku M, Togashi M, Mikami R and Attwood D. Preparation and evaluation of gel formulations for oral sustained delivery to dysphagic patients. Drug Deve. Ind. Pharmacy. 2009; 35(7): 780-787.

[15]. Rajalakshmi R, Sireesha A, Subhash KV, Pavani PV, Naidu KL. Development and Evaluation of a Novel Floating Insitu Gelling System of Levofloxacin Hemihydrate.International Journal of Innovative Pharmacy and Research. 2011; 2(1): 102-108.

[16]. Dash S, Murthy Pn, Nath Land Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Poloniae Pharmaceutica and Drug Research. 2010; 67(3): 217-223.

[17]. Biswajit B. Release kinetics studies of pimozide from buccal mucoadhesive patches. Journal of Pharmacy Research. 2011; 4(6): 1806-8.

[18]. U.S. Department of Health and Human Services Food and Drug Administration, CDER, Januray 2012,

[19]. www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070246.pdf. April 2012.