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Research Article

Development and Optimization of a Novel Floating In-Situ Gel of Simvastatin for Stomach Specific Sustained Drug Delivery

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Abstract

Simvastatin has half-life of 2-3 h and required dose is 5-20 mg/day. Hence simvastatin is a suitable candidate for sustained drug delivery system. The purpose of this research work was to development and optimization of a novel floating in-situ gel of simvastatin for stomach specific sustained drug delivery which provide an increased gastric residence time and ensuing prolonged drug delivery in the gastrointestinal tract. Four different concentrations of HPMC K 4M, sodium alginate, guar gum and pectin were used. Three various concentrations of calcium carbonate and sodium citrate were taken. Sodium citrate was used to prevent gelation outside gastric environment. IR spectrum of simvastatin revealed that functional group of simvastatin present in the sample has shown their stretching in the standard range. DSC study showed that the drug is compatible with polymers and excipients. A 32 full factorial design is applied to study the effect of independent variables, i.e., concentration of HPMC K 4M (X1) and concentration of sodium alginate (X2) on dependent variables, i.e., viscosity (Y1), in vitro buoyancy time (Y2) and amount of drug release in 8 h (Y3). All the formulated in-situ gels were evaluated for their physical appearance, pH, viscosity, in-vitro floating time, in vitro gelation, drug content and in vitro drug release study. From the factorial design, R9 batch was found to be optimized batch based on the viscosity, buoyancy and %CDR at 8 h. Simvastatin was satisfactorily found to be stable for 3 months. Thus, in-situ gelling formulation is promising approach for gastroretentive sustained delivery of simvastatin.

Keywords: Floating drug delivery, in-situ gel, simvastatin, stomach specific, sustained release, 3^2factorial design

Introduction [1-3]

Oral drug delivery is a one of the simplest routes of drug delivery for systemic as well as local effect. Liquid dosage forms are more suitable to administer when contrasted with solid dosage forms but can't attain the sustained release effect because of the less residential time in the GI tract. The development of in-situ gelling systems has received significant attention over past few years as it offers best way to overcome the difficulties of immediate release and short GI residence of liquids. In-situ gelling drug delivery systems are principle, accomplished of releasing drug in sustained manner retaining comparatively constant plasma profiles. The in-situ gels are liquid at room temperature, but undergo gelation when come in contact with body fluids or change in pH, that floats on GI contents. In-situ gel formation take place because of one or combination of different stimuli like temperature modulation, pH changes and ionic cross-linking. This attains increased residence time and also sustained release. This approach is beneficial for systemic and also for local effect of drugs administered. Compared to conventional controlled release formulations, in-situ gel forming drug delivery systems possess possible advantages such as simple manufacturing process, ease of administration, reduced administration frequency and improved patient compliance. As compare to very strong gels, they can be simply applied in liquid form to the site of drug absorption, where they swell to produce a strong gel which is able to prolong the gastric residence time of the active substance. Both synthetic and natural polymers can be used in the preparation of in-situ gels. In-situ gels are administered by ocular, oral, intravenous, vaginal, intra-peritoneal and rectal route. Current advances in in-situ gels have made it possible to exploit the variations in physiological uniqueness in different parts of the GIT for improved drug absorption. Simvastatin is an antihyperlipidemic drug widely used in the treatment of hypercholesterolemia and dyslipidemia. It has short elimination half-life (2-3 h) and narrow absorption window. It is mainly absorbed from stomach. Its short biological half-life necessitates the need for its administration in two or three dosage forms of 20 to 40 mg per day. Thus, the development of sustained release dosage forms would clearly be advantageous. Simvastatin has absorption window in the upper GIT, and is tough to formulate into sustained release dosage forms, as on arrival to colon its absorption is diminished or nonexistent. Simvastatin belongs to a class of the most powerful lipid reducing drug compounds, known as the statins. In context of the above principles, a strong need was felt to develop a dosage form that delivered simvastatin into the GI tract and would increase the efficiency of the drug, providing a sustained action. In the present investigation efforts are make to increase the residence time of simvastatin at/above the absorption window by preparation of gastro-retentive floating in-situ gel.

Materials and Methods

Materials

Simvastatin was kindly gifted by Biocon Pvt. Ltd (Bangalore, India). HPMC K 4M, Guar gum and Pectin were purchased from Hi Media Pvt. Ltd (Mumbai, India). Sodium alginate and Calcium carbonate were purchased from SD Fine Chemicals Ltd (Mumbai, India). Sodium citrate was purchased from Ranbaxy Fine Chemicals Ltd (Mumbai, India). Methyl paraben and Propyl paraben were purchased from Sulab chemicals Pvt. Ltd (Ahmedabad, India). All other reagents used were of analytical grade.

Preliminary studies [4, 5]

For the optimization of polymer, four different polymers (HPMC K 4M, sodium alginate, guar gum and pectin) and four different concentrations (0.5%, 1.0%, 1.5% and 2%) were used (Table 1).

Table 1: Optimization of polymer

Batch No.	Polymer	Concentration of polymer	Viscosity of solutiona (cps)	Gelling Capacity
1	HPMC K 4M	0.5%	245.4 ± 3.38	Gel is formed
2	HPMC K 4M	1.0%	283.7 ± 2.45	Stiff gel is formed
3	HPMC K 4M	1.5%	339.2 ± 3.12	Stiff gel is formed
4	HPMC K 4M	2.0%	535.6 ± 3.19	Very stiff gel is formed
5	Sodium alginate	0.5%	172.5 ± 4.25	Gel is formed
6	Sodium alginate	1.0%	291.2 ± 3.75	Stiff gel is formed
7	Sodium alginate	1.5%	395.6 ± 2.84	Stiff gel is formed
8	Sodium alginate	2.0%	499.3 ± 2.28	Very stiff gel is formed
9	Guar gum	0.5%	203.4 ± 2.43	Gel is formed
10	Guar gum	1.0%	535.6 ± 3.19	Very stiff gel is formed
11	Guar gum	1.5%	596.2 ± 1.99	Very stiff gel is formed
12	Guar gum	2.0%	630.7 ± 2.34	Very stiff gel is formed
13	Pectin	0.5%	63.2 ± 2.52	Gel is formed
14	Pectin	1.0%	75.5 ± 3.18	Gel is not form properly
15	Pectin	1.5%	80.3 ± 2.32	Gel is not form properly
16	Pectin	2.0%	86.6 ± 2.89	Gel is not form properly

*All the formulations contain 1% w/v Calcium carbonate [a]In concentration of 1.5% w/v

Amongst the four concentrations, 0.5%, 1.0% and 1.5% were selected for further studies since in this concentration gel was formed and viscosities ranges were in acceptable limit. With 2.0% w/v concentration of HPMC K 4M, very stiff gel was formed and viscosity was also very high.

Optimization of calcium carbonate concentration [4, 5]

For the optimization of calcium carbonate concentration, three different concentrations (0.5%, 1.0% and 1.5%) of calcium carbonate were used. Calcium carbonate was used as a gas forming agent. The CaCO3 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 recognized that the formulations containing CaCO3 produce a significantly stronger gel than those containing sodium bicarbonate. Amongst the three concentrations, with 1% calcium carbonate, buoyancy time was 45 sec; total floating duration (>12 h) and viscosity (176.8 ± 2.12) were in acceptable limit. So, 1% was selected for all the formulations. Increasing the content of CaCO3 in the formulation concurrently increased the viscosity at all polymer concentrations (Table 2).

Table 2: Optimization of calcium carbonate concentration

Polymer[a]	Calcium c arbonate (%)	Buoyancy time[b] (Sec)	Viscosity[b] (cps)	Total floating duration[a] (h)
Sodium alginate	0.5%	93 ± 12	174.5 ± 2.32	> 12
Sodium alginate	1.0%	45 ± 10	176.8 ± 2.12	> 12
Sodium alginate	1.5%	67 ± 11	183.4 ± 2.85	> 12
HPMC K 4M	0.5%	115 ± 08	249.3 ± 2.95	> 12
HPMC K 4M	1.0%	55 ± 12	256.8 ± 2.45	> 12
HPMC K 4M	1.5%	68 ± 09	263.4 ± 2.32	> 12

Optimization of sodium citrate concentration [4, 5]

For the optimization of sodium citrate concentration, three different concentrations (0.10%, 0.20% and 0.30%) of sodium citrate were used. Gelation was checked in 0.1 N HCl at the same time and also after 1 day (Table 3).

Table 3: Optimization of sodium citrate concentration

Polymer[a]	Sodium citrate (% w/v)	Calcium carbonate (% w/v)	Gelation in 0.1 N HCl[b]	After 1 day
Sodium alginate	0.10	1.0	++	Gel
Sodium alginate	0.20	1.0	+++	Solution
Sodium alginate	0.30	1.0	+	Solution
HPMC K 4M	0.10	1.0	++	Gel
HPMC K 4M	0.20	1.0	+++	Solution
HPMC K 4M	0.30	1.0	+	Solution

[a]In concentration of 1.5% w/v

[b] + Gels after few minutes, dispersed rapidly

++ Gelation immediate remains for few hours

+++ Gelation immediate remains for an extended period

The formulation of in-situ gel makes contact with an acidic medium and forms gel by cross-linking with Ca+2 ions and form a 3D gel network in acidic environment. The presence of low level cations in solution was adequate 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. At low concentration (0.10%), gel was formed with 0.1 N HCl, but the formulation was converted to gel after one day 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 HPMC K 4M also.

Method of preparation of in-situ gelling solution of simvastatin [4]

An aqueous solution of matrix forming agents in deionized water was prepared. In order to get homogeneous dispersion of the drug, simvastatin was gradually added to the above solution by stirring on a magnetic stirrer. In another beaker, different concentrations of sodium alginate solutions were made by adding the alginate to purified water containing methyl paraben, propyl paraben and sodium citrate at a temperature of 60 ºC. After cooling to below 40 ºC, both solutions were mixed under stirring on magnetic stirrer. Later, calcium carbonate was added. The above formulation was sonicated for 15 min and then pH and viscosity of the solutions are determined, next, pH of solution is adjusted to 5.5-6.5. The resulting in-situ gel solution was checked for viscosity and gelling property.

3^2 Full Factorial Design [4-6]

It is required to develop an acceptable pharmaceutical formulation in shortest possible time by using minimum number of man, raw materials and hour. Conventionally pharmaceutical formulations developed by changing one variable at a time by trial and error method which is time consuming in nature and needs a lot of imaginative efforts. Furthermore, it may be challenging to develop an ideal formulation by using this classical method as the joint effects of independent variables are not considered. Thus, it is very important to understand the complexity of formulations by using conventional statistical tools like factorial design. Factorial design is an effective method of representing relative significance of a number of variables and their interactions. The number of experiments necessary for these studies are depends on number of independent variables. For each trial the response (Y) is measured.

$$Y = \beta_0 + \beta_1 X1 + \beta_2 X2 + \beta_{12} X1\ X2 + \beta_{11} X1^2 + \beta_{22} X_2^2$$

Where,

β_0 = Intercept = Constant

β_1 and β_2 = Co-efficient of X1 and X2 variable

β_{12} = Co-efficient of interaction

β_{11}, β_{22} = Co-efficient of quadratic terms = Non-linearity

X1 and X2 = Variables

$A3^2$ full factorial design is applied to study the effect of independent variables, i.e., concentration of HPMC K 4M (X1) and concentration of sodium alginate (X2) on dependent variables, i.e., viscosity (Y1), *in vitro* buoyancy time (Y2) and amount of drug release in 8 h (Y3) (Table 4 & 5). Refer (Table 6) for Composition of Simvastatin stomach specific floating in-situ gels as per factorial design.

Table 4: Factor and levels for 32 full factorial design

Variables level	Low (-1)	Medium (0)	High (+1)
Concentration of HPMC K 4M (X1)	0.5%	1.0%	1.5%
Concentration of Sodium alginate (X2)	0.5%	1.0%	1.5%

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

Batch No.	X1	X2
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
Rv1*	0	0
Rv2*	1	1

* Extra check point batches

Table 6: Composition of simvastatin stomach specific floating in-situ gels as per factorial design

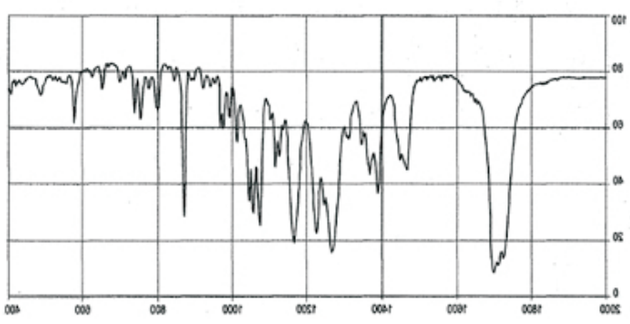
Composition	Formulation Code								
	R1	R2	R3	R4	R5	R6	R7	R8	R9
Simvastatin (mg)	50	50	50	50	50	50	50	50	50
HPMC K 4M (%)	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
Sodium alginate(%)	0.5	0.5	0.5	1	1	1	1.5	1.5	1.5
Sodium citrate (%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Calcium carbonate (%)	1	1	1	1	1	1	1	1	1
Methyl paraben & Propyl paraben (9:1) (%)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Distilled Water	up to 100 ml								

Determination of melting point [7]

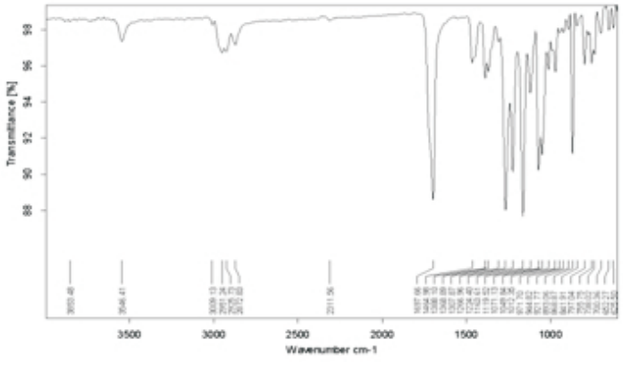
Melting point of the simvastatin was determined by taking a small amount of drug in a capillary tube closed at one end and was placed in Thiel's melting point apparatus and the temperature at which the drug melts was noted. Average of triplicate readings was noted.

Identification of simvastatin by FT-IR spectroscopy [8]

Fourier-transform infrared (FT-IR) spectra was recorded for pure drug with a Bruker Alpha FT-IR spectrophotometer using KBr zinc selanide optics of 0.01 g sample between wavelengths 400 to 4000 cm-1 and resolution is 2 cm-1 (Figure 1).



(a)

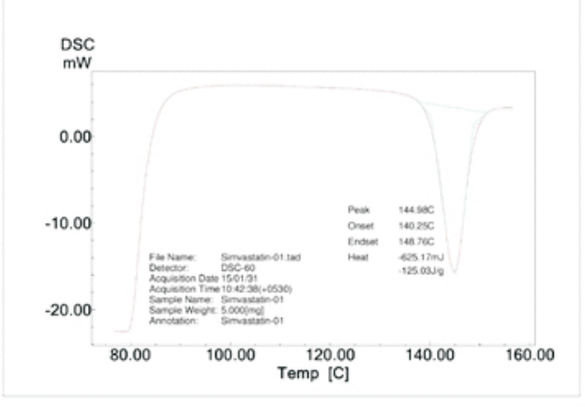


(b)

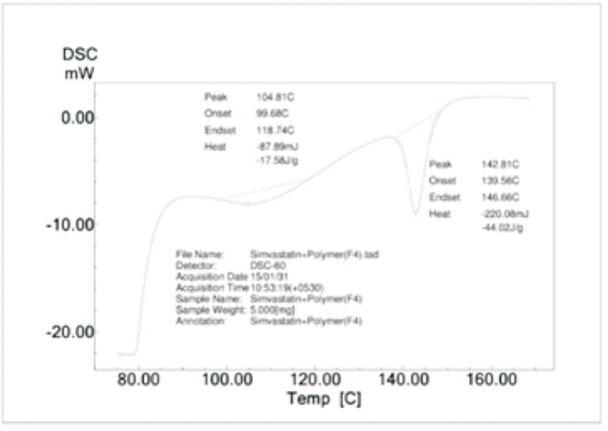
Figure 1: IR Spectrum of (a) Simvastatin (As per IP 2010) and (b) Simvastatin(Sample)

Drug-excipient compatibility study by differential scanning calorimetry (DSC) [9]

DSC scans were recorded for pure drug and the mixture of drug and polymers using DSC-Shimadzu 60 with TDA trend line software. All samples were weighed (8-10 mg) and heat at a scanning rate of 10 °C/min under dry nitrogen flow (20 ml/min) between 50 and 300°C (Figure 2).



(a)



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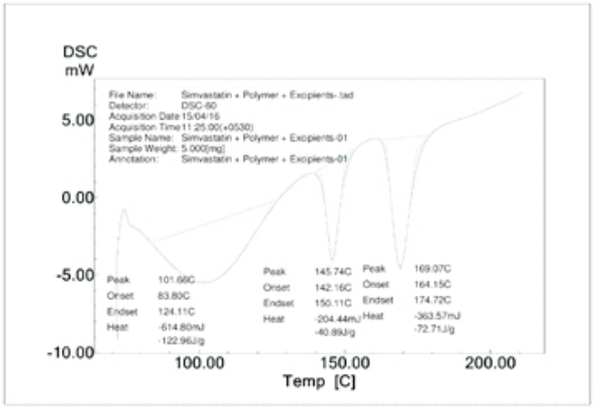
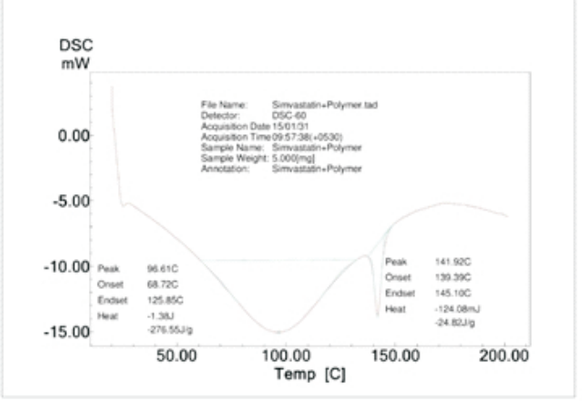


(C)



(d)

Figure 2: DSC graph of (a) Simvastatin drug, (b) Simvastatin with \polymers, (c) Simvastatin with polymers and excipients and (d) Simvastatin with polymer
(At 40 °C ± 2 °C/75% RH ± 5% RH for 15 days)

pH determination [10]

Formulations of stomach specific in-situ gels were checked for their pH (at 25 °C). A calibrated digital pH meter was used to measure the pH of formulations.

Viscosity study [11]

Viscosities of the stomach specific in-situ gel formulations were measured by using Brookfield digital viscometer with spindle 62 at 100 rpm and the temperature of formulations were maintained at 25 ± 1 °C before each measurement.

***In vitro* buoyancy time [11]**

5 ml of the stomach specific in-situ gel was placed in 100 ml of the simulated gastric fluid (0.1 N HCl, pH 1.2) at 37 ± 0.5 °C temperature. The mixture was stirred at 100 rpm with a magnetic stirrer. The time, the formulation acquired to emerge on the surface of medium (floating lag time) and the time, formulation continuously float on surface of dissolution medium (total floating time) were noticed in triplicate, and then reported the average value.

***In vitro* gelation study [6, 10]**

The in vitro gelling capacity of formulations were measured by placing 10 ml of the gelation solution in 500 ml simulated gastric fluid and maintained at 37 ± 1 °C temperature. 10 ml of formulation was added with the help of the pipette. When solution came in contact with solution of gelation, it was immediately converted into a stiff gel. The gelling capacity was evaluated based on stiffness of gel and time period for which formed gel remained as such. In vitro gelling capacity was classified in three categories depending on the time of gelation and time period for which formed gel remained.

Determination of drug content [11]

Accurately measured 10 ml stomach specific in-situ gel was taken in 250 ml volumetric flask, then made up the volume with 0.1 N HCl. Solution was shake for 30 min followed by 15 min sonication. Sonicated solution was filtered using 0.45 µ membrane filter. From that solution 10 ml of sample was withdrawn and diluted with 0.1 N HCl. The amount of simvastatin was determined using standard curve in UV spectrophotometer.

***In vitro* drug release study [12]**

The release rate of the drug from in-situ gel was determined by using USP dissolution testing apparatus II at 50 RPM. Slow speed was used to prevent breaking of gelled formulation and was maintaining with the mild agitation conditions. 500 ml of 0.1 N HCl (pH 1.2) was used as a dissolution medium, and temperature was maintained at 37 ± 0.2 °C. 10 ml of formulation was drawn up by using a disposable syringe. The syringe end was then placed into the petri dish and the syringe plunger depressed slowly to extrude 10 ml and finally petri dish was kept in dissolution vessel comprising dissolution medium without far disturbance. At every 1 hour, 10 ml sample of the dissolution medium was withdrawn and replaced with pre-warmed (37 °C) fresh medium. Absorbance of simvastatin in withdrawn samples were measured at 238 nm using UV Spectrophotometer.

Drug release kinetics study [13]

The drug release kinetic study was performed to find drug release mechanism from dissolution parameter by using various kinetic model equations. The zero-order, first-order, Hixon Crowell, KorsmeyerPeppas and Higuchi Plot models were tested (Table 7).

Contour plot and Response surface plot [5, 9]

The optimization of formulation was carried out by plotting contour plots (3-D) and surface plots (2-D) for all observed dependent variables. Here, contour plots and Response surface plots were drawn using the design expert software version 9.0 (Stat-Ease, Inc., USA). These plots are useful in study of effects of two factors on response at a time.

Table 7: Release kinetic mechanism

Release exponent 'n'	Drug transport mechanism	Rate as a function of Time
0.5	Higuchi Matrix	$t^{n-0.5}$
0.5<n<1.0	Non-Fickian Diffusion	t^{n-1}
1.0	Zero Order Release special Case-II Transport	Zero Order Release
Higher release (n>1)	Super Case-II Transport	t^{n-1}

Accelerated stability study [14]

Accelerated stability studies of the optimized formulation was carried out as per ICH guidelines. The selected formulation was stored at accelerated condition of 40ºC ± 2 ºC/75% RH ± 5% RH for 3 months and was evaluated for physical appearance, viscosity, pH, in vitro gelation, duration of floating, floating lag time, in vitro drug release profile and drug content.

Results and Discussion

Determination of melting point

The melting point of sample was found to be 137-141 °C (n=3) which indicate the purity of sample as simvastatin.

Identification of simvastatin by FT-IR spectroscopy

Refer Figure 1 for IR Spectrum of (a) Simvastatin (as per IP 2010) and (b) Simvastatin (Sample) and Table 8 for interpretation of FT-IR spectra of simvastatin.

Table 8: Interpretation of FT-IR spectra of simvastatin

Vibration	Standard wave number (cm^{-1})	Observed wave number (cm^{-1})
OH stretching	3200-2800	2951.24
C=C stretching	3010-3100	3009.13
C=O stretching	1760-1690	1697.66
C-C stretching	1100-1300	1266.96
C-O stretching	1300-1050	1224.40
C-H (Aromatic ring)	690-900	893.06

Drug-excipient compatibility study by DSC

The DSC graphs are shown in Figure 2(a-d).The thermogram of pure drug exhibits a sharp melting endotherm at 144.98 ºC (Figure 2a). DSC thermogram of simvastatin with polymers shows melting endotherm peaks at 142.81 ºC (Figure 2b). DSC thermogram of simvastatin with polymers and excipients shows melting endotherm peaks at 145.74 ºC (Figure 2c). DSC thermogram of simvastatin with polymers (At 40 °C ± 2 °C/75% RH ± 5% RH for 15 days) shows melting endotherm peak at 141.92 ºC (Figure 2d). Endothermic peak was observed in all the DSC graphs. As all the graphs do not show large derivation as compared with the DSC graph of pure drug, we can conclude that the drug and excipients are compatible with each other.

Experimental design

32 full factorial design has been applied to optimize formulation variables with basic constraint of understanding interaction of independent variables. Preliminary investigations of process

parameters shown that the factors such as concentration of HPMC K 4M (X1) and concentration of sodium alginate (X2) showed major influence on viscosity (Y1), in vitro buoyancy time (Y2) and amount of drug release in 8 h (Y3) of in-situ gel formulations. Therefore, they were employed for further studies. Both the selected dependent variables showed an extensive variation in viscosity, buoyancy time and amount of drug release for all 9 batches. The data noticeably indicated strong impact of concentration of HPMC K 4M and concentration of sodium alginate on selected responses (Y1, Y2 and Y3). The polynomial equations can be used to draw conclusions after considering magnitude of coefficients and mathematical sign, it conveys either negative or positive. Results for experimental design batches and its ANOVA were shown below.

pH determination

Results of pH measurement of formulation R1 to R9 were described in Table 9. All

the formulation has alkali pH. Minimum pH 5.8 was observed in R3 formulation and maximum pH 6.4 was observed in R6 formulation.

Table 9: Characterization of in situ gelling formulations

Batch No.	pH[a]	Viscosity[a] (cps)	*In vitro* buoyancy time[a](Sec.)	Total floating time (h)	*In vitro* gelation[b]	Drug content[a] (%)
R1	5.9 ± 0.2	124.4 ± 7.85	24±3	8	++	98.69 ± 1.36
R2	6.1 ± 0.3	184.2 ± 4.63	42±2	8	++	98.77 ± 0.98
R3	5.8 ± 0.1	273.7 ± 9.93	55±4	9	++	98.82 ± 1.43
R4	5.9 ± 0.2	183.2 ± 5.74	41±3	10	++	98.22 ± 1.19
R5	6.2 ± 0.3	261.6 ± 6.39	53±3	10	++	98.69 ± 0.81
R6	6.4 ± 0.2	353.5 ± 5.99	62±5	>12	+++	97.85 ± 1.13
R7	5.8 ± 0.2	282.7 ± 4.93	57±4	>12	+++	98.27 ± 1.21
R8	6.1 ± 0.3	374.7 ± 6.18	64±6	>12	+++	98.12 ± 1.28
R9	6.2 ± 0.3	516.3 ± 4.86	69±3	>12	+++	99.25 ± 0.52

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

[b] + Gels after few minutes, dispersed rapidly

++ Gelation immediate remains for few hours

+++ Gelation immediate remains for an extended period

Viscosity study

Results of viscosity study of formulation R1 to R9 were described in Table 9. In the selection of concentration of gelling polymer, a compromise is required between an adequately high concentration for formation of gels of satisfactory gel strength for use as a delivery vehicle, and an adequately low concentration to preserve an adequate viscosity for ease of swallowing. The solutions showed a noticeable increase in viscosity with increasing the concentration of HPMC K 4M and sodium alginate.

***In vitro* buoyancy time**

Results of in vitro buoyancy time of formulation R1 to R9 were described in Table 9. The in vitro buoyancy of the prepared formulations was performed in 0.1 N HCl (pH 1.2). The CaCO3 effervesced, releasing CO2 and calcium ions. The released CO2 is entrapped in gel network, creating buoyant formulation; then, calcium ion reacted with HPMC K 4M and produced a cross-linked 3D gel network.

***In vitro* floating time**

Results of in vitro floating time of formulation R1 to R9 were described in Table 9. The total floating time of the prepared formulations were performed in 0.1 N HCl (pH 1.2). Reason for the less floating lag time of R1 formulation might be due to escape of CO2 air bubbles from the gelling network because of low concentration of polymer. R6 to R9 formulations have total floating lag time more than 12 h. The possible reason behind it might be formation of stiff gelling system by combination of HPMC K 4M and sodium alginate after contact with HCl.

***In vitro* gelation study**

Results of in vitro gelation of formulation R1 to R9 were described in Table 9. The in-situ gel should preserve its integrity without dissolving for prolonged period to facilitate sustained release of drugs locally. R1 to R5 formulations were formed gel immediately and remained 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 hours. Formulations R6 to R9 were formed gel immediately and remained for extended period.

Determination of drug content

Results of drug content of formulation R1 to R9 were described in Table 9. The solutions showed a percentage drug content from 97.85 % to 99.25 %.

***In vitro* drug release study**

Dissolution profile of formulation R1 to R9 is shown in Figure 3. A major decrease in the rate and extent of release of the drug was observed with the increase in the concentration of polymer in in-situ gels.

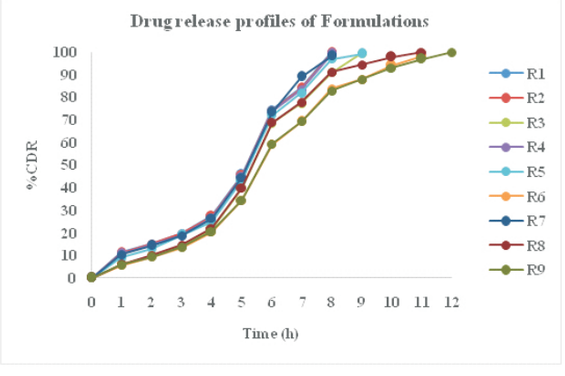


Figure 3: %Cumulative drug release of simvastatin from in-situ gelling formulations in 0.1 N HCl (pH 1.2)

Drug release kinetics study

The drug release kinetic study was performed to find out the drug release mechanism from dissolution parameter by using various kinetic model equations (Table 10).

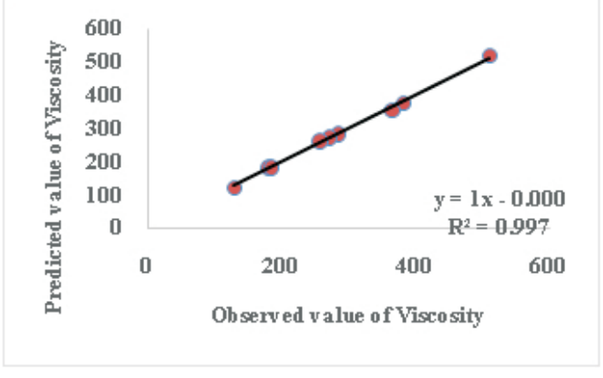
Table 10: Drug release kinetics study

	Batch	Zero Order	First Order	Hixon Crowell	Korsmeyer Peppas	Higuchi Plot
R^2 Value	R1	0.97	0.73	0.98	-0.78	0.88
	R2	0.97	0.72	0.98	-0.77	0.88
	R3	0.97	0.74	0.98	-0.68	0.88
	R4	0.97	0.72	0.98	-0.76	0.88
	R5	0.97	0.76	0.98	-0.71	0.90
	R6	0.98	0.77	0.97	-0.62	0.92
	R7	0.97	0.72	0.98	-0.76	0.88
	R8	0.97	0.78	0.96	-0.62	0.91
	R9	0.97	0.78	0.96	-0.60	0.93

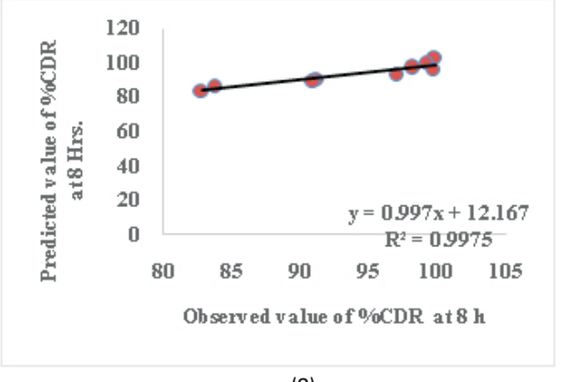
Result of kinetic model indicates that the Formulation R1-R5 & R7 follows Hixon Crowell kinetic release, Formulation R6, R8 & R9 follows Zero order release kinetic.

Statistical analysis

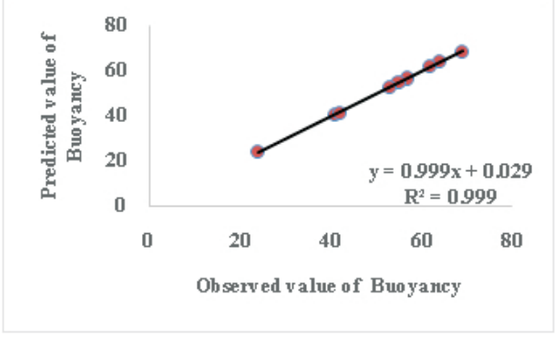
Statistical analysis was done using Microsoft Excel 2013. Linear correlation plot between observed and predicted value for viscosity, buoyancy and % CDR at 8 h are shown in Figure 4.



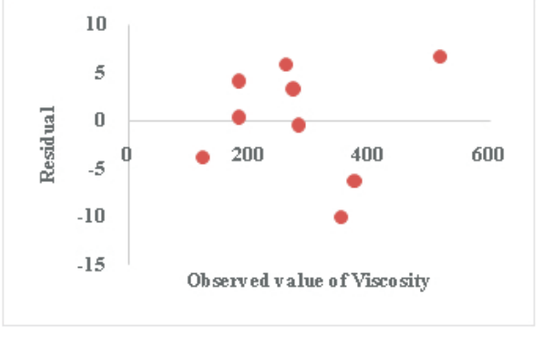
(a)



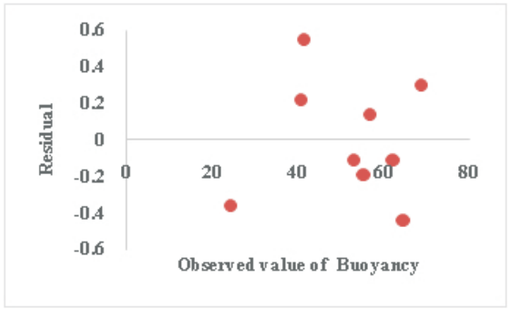
(C)



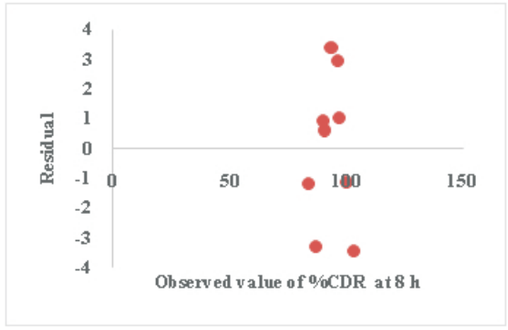
(b)



(d)



(e)



(f)

Figure 4: Linear co-relation plot of (a) viscosity (b) buoyancy and (c) %CDR at 8 h & Residual plot of (d) viscosity (e) buoyancy and (f) %CDR at 8 h

The linear correlation plots drawn between predicted and observed responses shows values of R2 are 0.9977, 0.9995 and 0.9975 for viscosity, buoyancy and % CDR at 8 h respectively, indicating excellent goodness of fit. The resultant residual plots show nearly uniform and random scatter around the mean values of response variables.

Factorial equation for viscosity

The viscosity of R1-R9 batches varied from 124.4 to 516.3 and showed correlation co-efficient 0.9977. This showed best fit to model. Positive sign in regression equation indicate the response value increases as the amount of factors increases. The P value for variable X1 and X2 were 0.0002 and 0.0001 respectively ($P<0.05$), it indicates that both variables shows prominent effect on viscosity.

Viscosity = 255.79 + 92.20 X1 + 98.57 X2 + 21.07 X1 X2 + 15.47 X12 + 26.57 X22

In the present study, coefficients b1 and b2 possessed positive sign indicating synergistic effect of variables, X1 and X2, on response Y1 (Viscosity). Both the independent variables, X1 (HPMC K 4M) and X2 (Sodium alginate) has prominent effect (b1 = 92.20 and p = 0.0002) (b2 = 98.57 and p = 0.0001) over all the three independent variables. F value is less than 0.05. The high values of the coefficient of determination indicate a good fit i.e., good agreement between the dependent and independent variables. The coefficients b1 and b2 were found to be significant at $p<0.05$.

Factorial equation for buoyancy

Observed and predicted values for buoyancy studies (Y2) for all the 9 batches are shown in Table 11. R2 value in plot of predicted v/s observed responses was 0.9995 which indicated excellent goodness of fit. The Y2 (Buoyancy) values observed for different batches showed wide variation i.e., values ranged from 24.36 to 68.69. There was not much difference between actual and predicted values.

Buoyancy = 53.11 + 10.67 X1 + 11.50 X2 - 4.75 X1X2 - 1.67 X12 - 0.17 X22

Coefficient b1 and b2 possessed positive sign, which indicated positive effect of X1 and X2 variable on response Y2 (Buoyancy). Independent variables, X1 (HPMC K 4M) (b1 = 10.67 and p = <0.0001) and X2 (Sodium alginate) (b2 = 11.5 and p = <0.0001) have almost equal effect on buoyancy. F value is less than 0.05. The coefficients b1 and b2 were found to be significant at $p<0.05$.

The buoyancy of R1-R9 batches varied from 24 to 69 and showed correlation co-efficient 0.9995. This showed best fit to model. Negative sign in regression equation indicate the response value decreases as the amount of factors increases. Positive sign in regression equation indicate the response value increases as the amount of factors increases. The P value for variable X1 and X2 was <0.0001 ($P<0.05$), it indicates that X1 and X2 have the prominent effect on the buoyancy.

Factorial equation for %CDR at 8 h

There was not much difference between actual and predicted values for all 9 batches (Table 11).

Table 11: ANOVA for dependent variables for all batches (R1-R9)

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Significance F
		For Y1 = Viscosity (F1-F9)			
Regression	1.130	5	22592.80	262.62	0.0004
Residual	75.98	1	75.98	-	-
Total	8.382	9	93128.98	-	-
		For Y2 = Buoyancy (F1-F9)			
Regression	1572.03	5	314.41	1095.35	< 0.0001
Residual	0.86	3	0.29	-	-
Total	1572.89	8	-	-	-
		For Y3 = CDR at 8 h (F1-F9)			
Regression	318.43	2	159.21	20.03	0.0022
Residual	47.70	6	7.95	-	-
Total	366.13	8	-	-	-

R2 value in plot of predicted v/s observed responses was 0.9975 which indicated excellent goodness of fit. Y3 (% CDR at 8 h) value observed for different batches showed wide variation. The response (Y3) obtained at three levels of the two independent variables (X1 and X2) were subjected to multiple regression to yield a polynomial equation. Equation clearly reflects the wide range of values for coefficients (b).

%CDR at 8 h = 93.59 - 6.63 X1 - 3.03 X2

The selected model for Y3 was linear based on adjusted R2 and press value.

Negative sign in regression equation indicate the response value decreases as the amount of factors increases. The P value for variable X1 and X2 were 0.0012 and 0.0389 respectively ($P<0.05$), it indicates that X1 has more prominent effect than X2 on the % CDR at 8 h.

Results of analysis of variance (ANOVA)

ANOVA was done using Microsoft Excel. Results of ANOVA for viscosity, buoyancy and %CDR at 8 h are shown in Table 11.

From ANOVA results, both variables show significant F value, less than 0.05. It indicates that all model terms are significant.

Contour plot and response surface plot

Contour plot and Response surface plot were drawn using Design expert software version 9.0 (Stat-Ease, Inc., USA) (Figure 5).

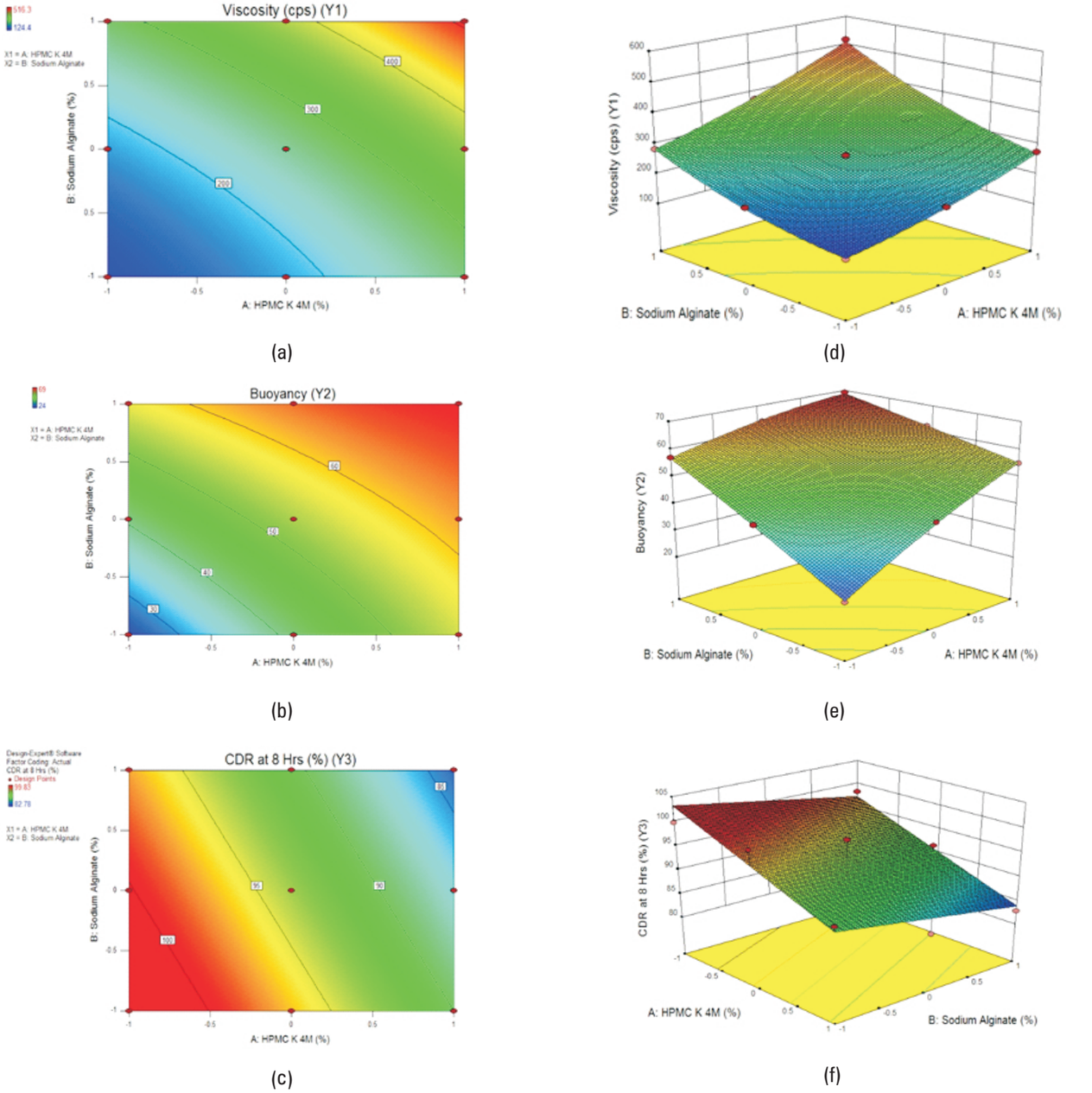


Figure 5: Contour plot of (a) viscosity Y1 (b) buoyancy Y2 and (c) %CDR at 8 h Y3; with X1 and X2 & 3D response plot of (d) viscosity Y1 (e) buoyancy Y2 and (f) %CDR at 8 h Y3; with X1 and X2

Two-dimensional contour plots and three-dimensional response surface plots are presented in Figure 5, which are useful tool to study interaction effects of the factors on responses. Figure 5(a-b) exhibited near linear relationship between factor X1 and X2 in form of almost straight lines in contour plot. Response surface plots show the relationship between these factors even more clearly but Figure 5c show perfect linear relationship.

Validation of experimental design

Polynomial equations were generated using Design expert software version 9.0 (Stat-Ease, Inc., USA) for selected responses like viscosity, buoyancy and %CDR at 8 h. The generated polynomial equations were further reduced on the basis of significant terms obtained by applying ANOVA. The 32 full factorial design was validated by preparing two extra check point formulation (RV1 & RV2). The predicted values for viscosity, buoyancy and %CDR at 8 h for both RV1 & RV2 were determined on the basis of respective polynomial equations whereas the experimental values were determined by evaluating RV1 & RV2 for the selected dependent variables (Table 12).

Table 12: Regression data for check point validation batch

Composition	Response variable	Experimental value	Predicted value
BATCH Rv1:	Viscosity	258.24	255.79
X1 = 1.0%	Buoyancy	52.19	53.11
X2 = 1.0%	%CDR at 8 h	94.97	93.59
Batch RV2:	Viscosity	514.99	509.66
X1 = 1.5%	Buoyancy	68.25	68.69
X2 = 1.5%	%CDR at 8 h	81.51	83.93

Selection of optimized formulation

Optimized formulation was selected on the basis of maximum viscosity, buoyancy and minimum %CDR at 8 h with good desirability. The desirability value of optimized formulation was found to be 0.954. The overlay plot of optimized formulation shown viscosity (420.12), buoyancy (64.99) and %CDR at 8 h (85.66). The value of X1 & X2 of optimized formulation was 1 & 0.433, respectively (Figure 6).

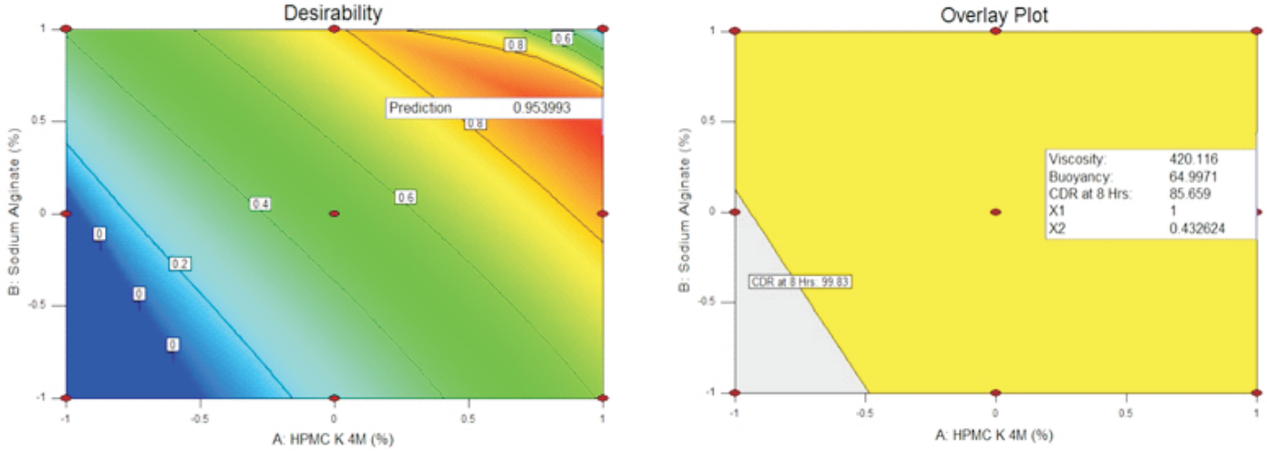


Figure 6: Desirability graph and overlay plot of optimized formulation

Optimized formulation of simvastatin floating in-situ gel is shown in Figure 7.

Figure 7: Optimized formulation of simvastatin floating in-situ gel

Accelerated stability study of the optimized formulation

R9 batch was kept for stability study under controlled environment condition (40 ± 2°C and 75 ± 5 %RH). The samples were withdrawn after 3 months and analyzed for pH, viscosity, in vitro bouncy time, total floating time, drug content and gelling capacity. The results of stability studies were described in Table 13.

Table 13: Results of stability studies

Evaluation Parameters	Time period for sampling	
	Initial	3 Month
pH	6.2	6.1
Viscosity (cps)	516.3 ± 4.86	509.5 ± 2.93
In vitro buoyancy time (Sec)	69 ± 3	72 ± 4
Total floating time (h)	>12	>12
Drug content (%)	99.25 ± 0.52	98.49 ± 0.58
In vitro gelation studies	+++	+++

It was observed that at the end of 3 months, the viscosity of the formulation was decreased from 516.3 ± 4.86 cps to 507.5 ± 2.93 cps which might be attributed to the loss of water and was insignificant to affect the rheological property of in-situ gel.

In vitro release profile of simvastatin from optimized formulation after and before stability was described in Table 14 & Figure 8.

Table 14: *In vitro* release profile of simvastatin from optimized formulation after and before stability

Time (h)	0	1	2	3	4	5	6	7	8	9	10	11	12
Initial	0	5.88	9.38	13.62	20.55	34.54	59.32	69.34	82.78	87.99	93.07	96.84	99.88
After 3 Months	0	5.98	9.54	13.52	20.45	34.39	58.14	68.47	81.91	87.07	91.95	95.97	98.96

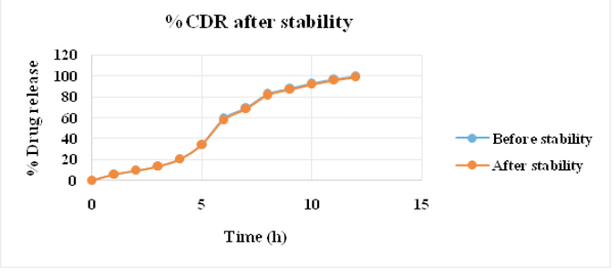


Figure 8: %CDR after stability

The graph of the release profile of drug from the optimized formulation does not show any significant change after a stability period of 3 months at 40 ± 2 °C and 75 ± 5 %RH.

Conclusion

From this study, it can be concluded that the oral administration of aqueous solutions of simvastatin containing HPMC K 4M and sodium alginate results in formation of in-situ gel at the stomach. The results of a 32 full factorial design shown that the concentration of HPMC K 4 Mand sodium alginate significantly affected the dependent variables like viscosity (Y1), in vitro buoyancy (Y2) and %CDR at 8 h (Y3). The optimized formulation R9 shown in vitro sustained drug release up to 12 h. Stability study shown that there was negligible change in pH, physical appearance, viscosity, pH, in-vitro gelation, duration of floating, floating lag time, in vitro drug release profile and drug content after 3 months. Thus, in situ gelling formulation is promising approach for gastro-retentive sustained delivery of simvastatin.

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Conflicts of interest

The authors declare that there is no competing interest regarding publication of this paper.

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