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RESEARCH ARTICLE

Design, development and optimization of gastro-retentive floating in-situ gels loaded with carvedilol microspheres using response surface methodology

Latha Kukati[1*], Aisha Rahman[1], Aymen Fatima[1], Vyshak R Menon[1], Lakshmi SVVNSM[2]

1. G. Pulla Reddy College of Pharmacy, Osmania University, Hyderabad, Telangana, India
2. Department of pharmacognosy, Vishnu Institute of Pharmaceutical Education and Research, Narsapur, Medak, Telangana, India

Aim: The goal of this study was to formulate and optimize gastro-retentive floating in-situ gel comprising optimized microspheres by Box-Behnken design.

Methods: Gellan gum, k-carrageenan were used as gelling polymers and calcium carbonate as complexing and gas generating agent. For optimization X1 (concentration of k-carrageenan), X2 (concentration of calcium carbonate) and X3 (concentration of tri sodium citrate) were considered as factors and Y1 (viscosity), Y2 (floating lag time), Y3 (drug release at 8 hrs) as responses. 17 formulations obtained from the design were prepared and evaluated for various parameters.

Results and discussion: The optimized floating in-situ gel formulation obtained from the design was milky white liquid with viscosity of 355.13 cP, showed buoyancy lag time of 104 seconds and remained buoyant for >24 hrs. 27.49 % drug was released from the in-situ gel at 8 hrs indicating controlled drug release. From the stability studies which were conducted for 4 weeks, it was determined that the optimized floating in-situ gel formulation was stable.

Conclusion: This study highlights the potential utilization of microspheres in the gastro-retentive floating in-situ gel.

Keywords: gastro-retentive, polymers, *in-situ* gel, viscosity

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Introduction

In-situ is a Latin term that means "in position". An *in-situ* gel Drug Delivery System (DDS) refers to a liquid formulation that solidifies or partially solidifies upon administration, forming a depot. *In-situ* activated gel-forming systems are those that subjected to physiological conditions cause them to transition to a gel phase. Gelation occurs due to crosslinking of polymeric chains via chemical or physical crosslinking [1].

Floating gastro-retentive *in-situ* gel is a low viscosity polymer solution that, when comes in contact with stomach fluids, changes polymeric structure resulting in the formation of strong low density viscous gel [2].

Carvedilol, which exhibits non-selective β-adrenergic antagonist properties and α-1 blocking activity, is utilized in the treatment of hypertension mild to severe chronic heart failure, and ventricular dysfunction following myocardial infarction in patients who are clinically stable. It has short biological half-life of 2-6 hrs.

As carvedilol is a Biopharmaceutics Classification System (BCS) Class-II drug (low solubility and high permeability) there is a need to enhance the solubility. It exhibits pH-dependent solubility i.e., more soluble at acidic pH and insoluble at alkaline pH and has narrow absorption window (main site of absorption is upper part of Gastrointestinal tract (GIT) [3-5]. So, carvedilol is a suitable candidate for gastro-retentive drug delivery system, improving solubility, enhancing absorption and prolonging gastric residence time.

Oral administration of liquid dosage forms is suitable for measured volumes. Hence, a gastro-retentive floating *in-situ* gel was planned as such a system when administered as solution, forms a gel at gastric pH and float on gastric fluids for an extended duration, ensuring the drug remains in dissolved form at the primary absorption site.

In this study, Box-Behnken design was utilized to optimize gastro-retentive floating *in-situ* gel incorporated with optimized carvedilol microspheres (OMS) obtained from a prior investigation. The optimized microspheres contained carvedilol solid dispersion with enhanced solubility.

Materials and Methods

Materials

Carvedilol was procured from Micro Labs Ltd, Bengaluru, India. Gellan gum, k-Carrageenan, pectin, sodium saccharin, methylparaben, propylparaben were purchased from Yarrow Chem Products, Mumbai, India. Calcium carbonate, sodium bicarbonate was obtained from SD Fine Chem Ltd., Mumbai, India. All chemicals and reagents used were of analytical standard.

* Correspondence to: Latha Kukati
E-mail: lathakukatil@gmail.com

Methods

Drug-Excipient Compatibility Studies

Fourier Transform Infra-Red (FTIR) Spectroscopy

FTIR spectroscopy was utilized to examine the spectra of carvedilol and excipients used in the formulation. The sample was mixed with potassium bromide (KBr) in ratio of 1:100 to form KBr disks and placed in a suitable holder in an IR spectrophotometer (Shimadzu 8400S) and the spectra between 400 cm^{-1} to 4000 cm^{-1} were recorded [6].

Method for the Preparation of Floating In-situ Gel

Gellan gum solutions were prepared by adding the gum to 30 mL of distilled water consisting sodium citrate and heating to 90°C.

⬇

An appropriate amount of k-carrageenan was dispersed in the above solution.

⬇

After cooling to 40°C required amount of calcium carbonate was added and stirred until it dissolved completely.

⬇

Appropriate quantity of sodium saccharin, preservatives methylparaben and propylparaben were added and volume was made to 50 mL with distilled water.

⬇

The optimized microspheres were reconstituted in the gelling system [7, 8].

Optimization of floating in-situ gel loaded with carvedilol microspheres using Box-Behnken design

Trials were done to obtain the concentration range of k-carrageenan, calcium carbonate and tri sodium citrate.

Box-Behnken design (BBD) was used to study the effect of different factors- concentration of k-carrageenan, concentration of calcium carbonate and concentration of tri sodium citrate considered as independent variables, studied at levels (-1, 0 and +1). Viscosity, *in-vitro* drug release and buoyancy lag time were chosen as the responses (dependent variables).

In a BBD, the number of experimental runs

$$N = 2k (k - 1) + Co,$$

Where k is no. of factors and Co is the no. of central points.

The above equation states that there are N = 2 3(3 - 1) + 5 = 17 runs because there are three factors, three levels, and five centre points [9, 10].

The factor levels were determined according to preliminary studies and available literature [11]. The levels of the process parameters are given in Table 1.

Table 2 illustrates the formulae utilized for each experimental run. In these formulae, three factors were altered at three distinct levels, while the quantities of gellan gum, sodium saccharin, methylparaben, and propylparaben were kept constant in all the runs.

From the BBD run formulae, all the formulations were prepared and evaluated for viscosity, drug release studies for the period of 8 hrs, and floating lag time. All the results were evaluated to get the optimized formulation of floating *in-situ* gels loaded with carvedilol microspheres.

Evaluation of floating in-situ gel

Floating *in-situ* gel appearance was assessed by visual inspection [8, 12].

pH Measurement

The pH of all formulations was determined using a calibrated digital pH analyser (Analab Scientific Instruments Pvt. Ltd, Gujarat, India) at 25 ± 0.5°C. All measurements were made in triplicate [13].

Viscosity Determination

Viscosity of the solutions was determined using Brookfield viscometer LV DVII Pro. Measurements were performed using spindle number 64, sheared at 100 rpm, and the temperature was maintained at 25°C ± 1°C [11, 14, 16].

In-vitro Gelation Capacity

5 mL of the formulation solution was placed in 500 mL

Table 1. Factors and factor levels of Box-Behnken design

Independent variables	Level		
	-1	0	+1
X_1=Concentration of k-Carrageenan (%)	0.25	0.33	0.5
X_2=Concentration of $CaCO_3$ (%)	0.5	0.75	1.0
X_3=Concentration of Tri sodium citrate (%)	0.2	0.4	0.6
Dependent variables			
Y_1= Viscosity (cP)			
Y_2= Floating lag time (secs)			
Y_3= Percent drug release			

Table 2. Formulation of floating in-situ gel by Box-Behnken design

Ingredients (mg)	IG1	IG2	IG3	IG4	IG5	IG6	IG7	IG8	IG9	IG10	IG11	IG12	IG13	IG14	IG15	IG16	IG17
Gellan gum	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250
k-Carrageenan	165	165	165	250	165	165	165	165	250	125	125	250	165	250	165	125	125
$CaCO_3$	250	375	500	500	250	375	500	375	375	375	250	250	375	375	375	500	375
Tri sodium citrate	300	200	100	200	100	200	300	200	100	300	200	200	200	300	200	200	100
Sodium saccharin	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Methylparaben	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Propylparaben	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Distilled water (mL)	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50

Note: Optimized microspheres equivalent to 20 mg carvedilol were reconstituted in 5 mL of the in-situ gel formulation prior to the study.

0.1 N HCl, pH 1.2 at 37°C ± 1°C temperature. The solution is transformed into a gel as it comes in contact with the gelation medium. An ordinal scale (Table 3) ranging between + and + + + was used to assess *in-vitro* gelation characteristics [14-15].

In-vitro Buoyancy Study

5 mL of the prepared *in-situ* gelling solution was placed in a petri dish and the optimized microspheres were reconstituted. The petri dish was placed without disturbing the dissolution medium. The total floating time/duration of floating as well as floating lag time were documented [17].

Density Determination

Gel of known volume (5 mL) was placed in a petri dish with 0.1 N HCl, pH 1.2. Using a calibrated digital scale, the weight of this gel was determined, and the formulation densities were calculated [17].

In-vitro Dissolution of floating in-situ gel

United States Pharmacopeia (USP) dissolution apparatus Type -II (Paddle method, Electrolab TDT-08L USP Disso Tester, Mumbai, India) was utilised to conduct dissolution studies. Dissolution medium comprising 900 mL of 0.1 N HCl, maintained at 37°C, was employed. Stirring was set at 50 rpm. At specific time points, 5 mL samples were extracted and substituted with fresh dissolution medium up to 8 hours. After passing through Whatman filter paper, the samples were diluted and subjected to a double beam UV Visible Spectrophotometer (Lab India Analytical 3200) analysis at 242.6 nm [18].

Kinetic Mathematical Modelling of Drug Release Profile

To understand the kinetics and mechanism of release of drug, *in-vitro* data was fitted into zero- order, first- order, Higuchi and Korsmeyer-Peppas models [19-20].

Statistical analysis and optimisation

Statistical optimisation was effectuated using Design-Expert® software (version 11, Stat Ease, Inc., Minneapolis, MN).

Stability studies

Stability studies were performed as per ICH guidelines. Optimized formulation was stored for 4 weeks in a glass container and its stability was monitored at 40°C ± 2°C and humidity 75 % RH. Samples were assessed for 4 weeks for physical appearance, pH, gelling capacity, floating lag time, viscosity, density and *in-vitro* drug release [21-23].

Table 3. Grading of in-vitro gelation

Grade	Description
+	Gelation after few minutes and rapidly dispersed
++	Immediate gelation and remains for less than 24 hrs
+++	Immediate gelation and remains for more than 24 hrs

Results and Discussion

Fourier Transform Infra-Red (FTIR) Spectroscopy

The FTIR spectra of carvedilol, individual excipients and combination of carvedilol with excipients are represented in Figure 1 and the interpretation of spectra is given in Table 4.

The FTIR spectrum of drug shows characteristics peaks in close agreement with the standard reference as per IP, indicating carvedilol with high purity. The FTIR spectrum of carvedilol revealed typical peaks at 3345.27 cm^{-1} of N-H and O-H stretching (secondary amine) vibrations combined together, at 2921.96 cm^{-1} of aliphatic C-H stretching, at 1607 cm^{-1} of N-H bending. Aromatic C=C stretching at 1502.44 cm^{-1}, at 1097.42 cm^{-1} of C-O stretching (aryl alkyl ether and alkyl ether).

Both the drug and excipient peaks were identified and interpreted in the FTIR spectra, which show no evidence of drug-excipient interaction. The spectra demonstrated that the drug and polymers did not interact chemically in any way. Thus, carvedilol is not functionally altered and is compatible with the excipients.

Evaluation of Floating In-situ Gels

Result for the evaluation parameters is given in Table 5. Following ingestion, the polymeric solution undergoes a rapid transition from sol-to-gel via ionic gelation. Initially, a double helical junction zone forms during the gelation process, then double helical segments are aggregated to create a 3D network via Ca^{+2} ion complexation and H-bonding.

The floating *in-situ* gels formed from the formulations had good floating properties. The floating lag time varied with variables of the formulation. IG16 formulation had the least buoyancy lag time, while IG5 formulation had the highest lag time. All formulations remained buoyant for more than 24 hrs. Formulations comprising calcium carbonate as an effervescent agent maintain buoyancy due to carbon dioxide generation in the presence of acidic dissolution medium. The CO_2 released is trapped in the gel network, resulting in a floating formulation, and then the Ca^{+2} ions react with gellan gum, resulting in a 3D gel network and swollen structure. CO_2 diffusion is increased leading to extended periods of floating.

To float on the stomach contents, the dosage form density should ideally be less than or equal to density of the stomach contents. The density of floating *in-situ* gels was in the range of 0.621-0.845 g/cm^3.

In-vitro dissolution of floating in-situ gels

In-vitro dissolution profile of floating *in-situ* gels loaded with optimized microspheres (OMS) is depicted in Figure 2 (***i, ii***). It was observed that within 30 mins release was high as gelation was at starting. The release decreased as gelation progressed. Later, as the gel network started to loosen and dissolve high release was observed. Formula-

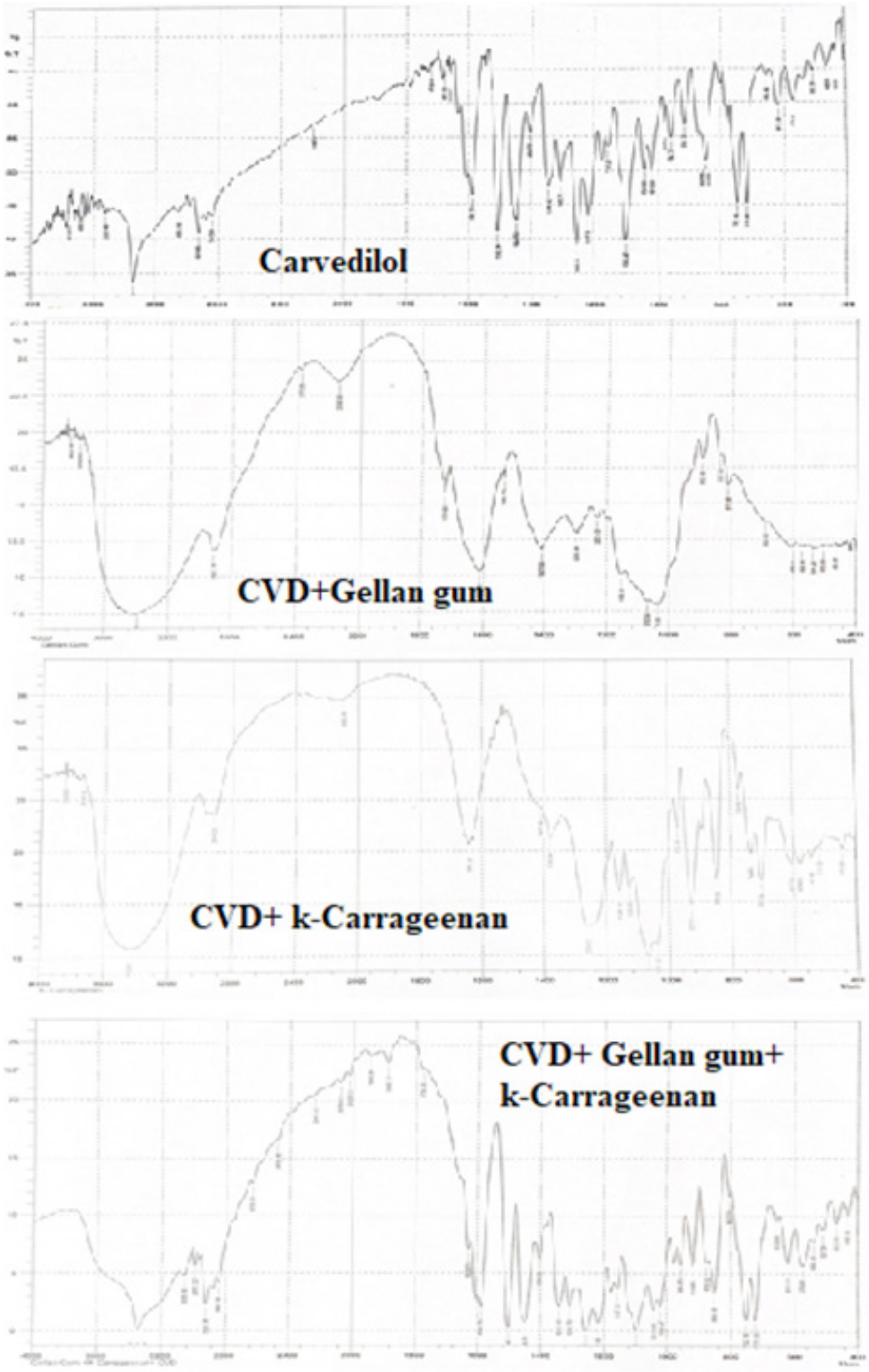


Fig. 1. Ventilatory asynchronies evaluated

Table 4. Interpretation of FTIR spectra of carvedilol, and combination of carvedilol and excipients

Functional group	Observed frequencies (cm-1)	
	Carvedilol	Carvedilol + Gellan gum + k- Carrageenan
N-H and O-H Stretching	3345.27	3345.27
C-H Stretching (Aromatic)	3056.96	3058.89
C-H Stretching (Aliphatic)	2921.96	2921.96
N-H Bending	1607.31	1607.31
C=C Stretching (Aromatic)	1502.44	1502.44
C-N Stretching	1255.57	1255.57
C-O Stretching (Aryl alkyl and alkyl ether)	1097.42	1097.42
C=CH2 Bending	617.18	617.18

Table 5. Evaluation of floating in-situ gels loaded with microspheres

Formulation	pH	Viscosity (cP)	Gelation capacity	Floating lag time (Secs)	Density (g/cm³)
IG1	8.34 ± 0.04	210 ± 0.1	++	83 ± 0.1	0.776 ± 0.02
IG2	8.83 ± 0.01	361.1 ± 0.01	+++	130 ± 0.07	0.845 ± 0.01
IG3	7.32 ± 0.01	868.2 ± 0.2	+++	75 ± 0.01	0.809 ± 0.01
IG4	8.26 ± 0.02	889.0 ± 0.03	+++	57 ± 0.2	0.777 ± 0.03
IG5	6.5 ± 0.01	450.5 ± 0.1	++	180 ± 0.03	0.843 ± 0.02
IG6	8.83 ± 0.01	361.1 ± 0.09	+++	130 ± 0.07	0.845 ± 0.02
IG7	8.24 ± 0.03	653.8 ± 0.3	+++	21 ± 0.3	0.784 ± 0.01
IG8	8.83 ± 0.01	361.1 ± 0.1	+++	130 ± 0.07	0.845 ± 0.02
IG9	8.54 ± 0.01	614.0 ± 0.04	+++	20 ± 0.03	0.678 ± 0.01
IG10	8.1 ± 0.07	194.4 ± 0.2	++	70 ± 0.01	0.652 ± 0.04
IG11	8.62 ± 0.01	111.6 ± 0.4	++	100 ± 0.2	0.783 ± 0.01
IG12	7.20 ± 0.01	607.1 ± 0.05	++	25 ± 0.01	0.712 ± 0.02
IG13	8.83 ± 0.01	361.1 ± 0.1	+++	130 ± 0.07	0.845 ± 0.03
IG14	6.8 ± 0.01	340.7 ± 0.8	+++	43 ± 0.1	0.863 ± 0.02
IG15	8.83 ± 0.01	361.1 ± 0.2	+++	130 ± 0.07	0.845 ± 0.02
IG16	8.42 ± 0.02	369.5 ± 0.1	++	17 ± 0.4	0.621 ± 0.01
IG17	6.81 ± 0.01	362.3 ± 0.1	++	113 ± 0.09	0.775 ± 0.03

Note: All the values are expressed as mean±SD, n=3. ++: Immediate gelation and remains for less than 24 hrs, +++: Immediate gelation and remains for more than 24 hrs.

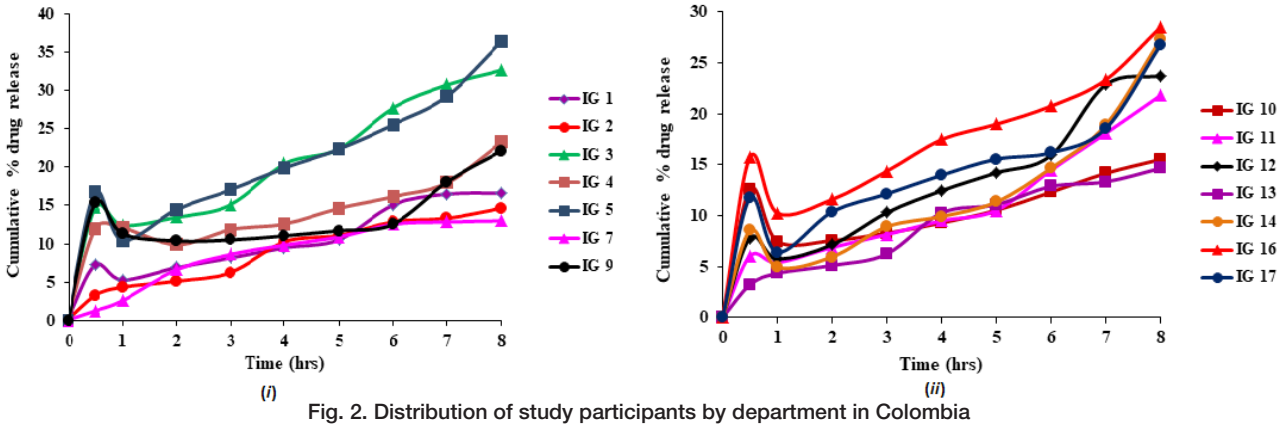


Fig. 2. Distribution of study participants by department in Colombia

tions IG2, IG7 and IG13 exhibited reduced drug release within 30 minutes, potentially due to rapid gelation. In contrast formulations IG3, IG4, IG5, IG9, IG10. IG16 and IG17 demonstrated high drug release at 30 minutes, followed by sustained release after one hour.

Statistical Analysis and Optimisation

Seventeen runs were prepared as generated by Box-Behnken design using Design-Expert® software. Using the Design-Expert® software, all the responses observed for seventeen runs were simultaneously fitted to linear, quadratic and interaction models. The R^2, Adjusted R^2, Predicted R^2, % CV values, and significant P values ($p<0.05$) were compared.

Fit Summary of responses

Table 6 shows fit summary of responses. For response 1 i.e., viscosity, fit summary suggested quadratic and linear model. For response 2 i.e., buoyancy lag time, fit summary suggested quadratic model. For response 3 i.e., percentage

Table 6. Fit summary of responses

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	Std. Dev.	R^2	
Response 1: Viscosity							
Linear	0.0003		0.6993	0.4804	119.68	0.7557	Suggested
2FI	0.9829		0.6152	-0.6122	135.37	0.7595	
Quadratic	0.0419		0.8179	-0.5748	93.13	0.9203	Suggested
Cubic			1.0000		0.0000	1.0000	Aliased
Response 2: Floating lag time							
Linear	0.0657		0.2791	-0.0449	42.23	0.4243	
2FI	0.3132		0.3330	-0.1447	40.62	0.5831	
Quadratic	0.0050		0.8321	-0.2598	20.38	0.9266	Suggested
Cubic			1.0000		0.0000		Aliased
Response 3: % Drug release							
Linear	0.1467		0.1738	-0.1989	6.56	0.3287	
2FI	0.4580		0.1619	-0.6171	6.61	0.4762	
Quadratic	0.0324		0.6330	-1.5696	4.38	0.8394	Suggested
Cubic			1.0000		0.0000	1.0000	Aliased

drug release, fit summary suggested quadratic model. Adeq Precision ratios of the responses indicated an adequate signal and hence the models were utilized to navigate the design space.

ANOVA Summary

ANOVA summary of response parameters for Box-Behnken design for floating *in-situ* gels loaded with microspheres is specified in Figure 3. ANOVA was used to assess the significance ($p<0.05$) of residual error and the ratio of mean square variation.

The P-value for viscosity, floating lag time and % drug release were 0.0043, 0.0033 and 0.0389 respectively, which is <0.05 indicating the significance of model terms.

Response 1: The model is apparently significant as the Model F-value is 8.98. There is a 0.43% chance that an F-value this large could be due to noise. A, B, C, B^2 are significant model terms in this case.

Response 2: As the F-value is 9.81, the model is apparently significant. There is a 0.33% chance an F-value this large could be due to noise. A, B, C, AB, A^2, B^2 are significant model terms in this case.

Response 3: As the F-value is 4.07, the model is apparently significant. There is a 3.89% chance that an F-value this large could be due to noise. C, AC, B^2 are significant model terms.

Diagnostic analysis of formulation characteristics

Figure 4 (i, ii, iii) shows the residual normal probability plots and the plots of the residuals vs the predicted response for the three response parameters.

The model's adequacy can be confirmed further by utilising the diagnostic plots provided by the

Design-Expert® software. The maximum number of colour points was observed on the normal probability line, proving the normality of the residuals and suggesting that the response data had provided relevant analysis. The externally studentized residuals vs predicted values showed that colour dots indicating the percentage of error were located within ranges close to zero, indicating the lack of constant error. The residual vs run plot was plotted to look for significant variables that might have affected the experiment's response parameters.

Response Surface Analysis

To assess influence of independent variables (factors) on dependent variables, 2D contour plot and 3D response surface analysis was performed. 2D contour and 3D response surface plots were plotted using Design-Expert® software.

A. Effect on Viscosity

2D contour and 3D response surface plots for the effect of X_1 and X_3 on viscosity are displayed in Figure 5.

ANOVA for Quadratic model

Response 1: Viscosity

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7.013E+05	9	77925.00	8.98	0.0043	significant
A-Conc. of k-Carrageenan	2.496E+05	1	2.496E+05	28.78	0.0010	
B-Conc. of Calcium carbonate	2.276E+05	1	2.276E+05	26.24	0.0014	
C-Conc. of Tri sodium citrate	1.021E+05	1	1.021E+05	11.77	0.0110	
AB	269.91	1	269.91	0.0311	0.8650	
AC	2487.65	1	2487.65	0.2868	0.6088	
BC	170.30	1	170.30	0.0196	0.8925	
A^2	25779.90	1	25779.90	2.97	0.1283	
B^2	95353.63	1	95353.63	10.99	0.0128	
C^2	4878.11	1	4878.11	0.5625	0.4777	
Residual	60709.67	7	8672.81			
Lack of Fit	60709.67	3	20236.56			
Pure Error	0.0000	4	0.0000			
Cor Total	7.620E+05	16				

ANOVA for Quadratic model

Response 2: Floating lag time

Source	Sum of Squares	df	Mean Square	F value	p value	
Model	36669.73	9	4074.41	9.81	0.0033	significant
A-Conc. of k-Carrageenan	3003.13	1	3003.13	7.23	0.0311	
B-Conc. of Calcium carbonate	3488.30	1	3488.30	8.40	0.0230	
C-Conc. of Tri sodium citrate	2333.06	1	2333.06	5.62	0.0496	
AB	4334.94	1	4334.94	10.44	0.0144	
AC	1884.06	1	1884.06	4.54	0.0797	
BC	462.25	1	462.25	1.11	0.3264	
A^2	8809.32	1	8809.32	21.22	0.0025	
B^2	2816.32	1	2816.32	6.78	0.0351	
C^2	835.00	1	835.00	2.06	0.1944	
Residual	2906.50	7	415.21			
Lack of Fit	2906.50	3	968.83			
Pure Error	0.0000	4	0.0000			
Cor Total	39576.24	16				

ANOVA for Quadratic model

****Response 3: % Drug release ****

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	700.61	9	77.83	4.07	0.0389	significant
A-Conc. of k-Carrageenan	1.88	1	1.88	0.0983	0.7630	
B-Conc. of Calcium carbonate	0.1973	1	0.1973	0.0169	0.8348	
C-Conc. of Tri sodium citrate	170.88	1	170.88	8.93	0.0203	
AB	5.12	1	5.12	0.2674	0.6210	
AC	117.96	1	117.96	5.16	0.0421	
BC	0.0066	1	0.0066	0.0003	0.9857	
A^2	56.70	1	56.70	2.96	0.1289	
B^2	137.97	1	137.97	7.21	0.0313	
C^2	78.56	1	78.56	4.10	0.0824	
Residual	134.01	7	19.14			
Lack of Fit	134.01	3	44.67			
Pure Error	0.0000	4	0.0000			
Cor Total	834.63	16				

Fig. 3. ANOVA summary for responses

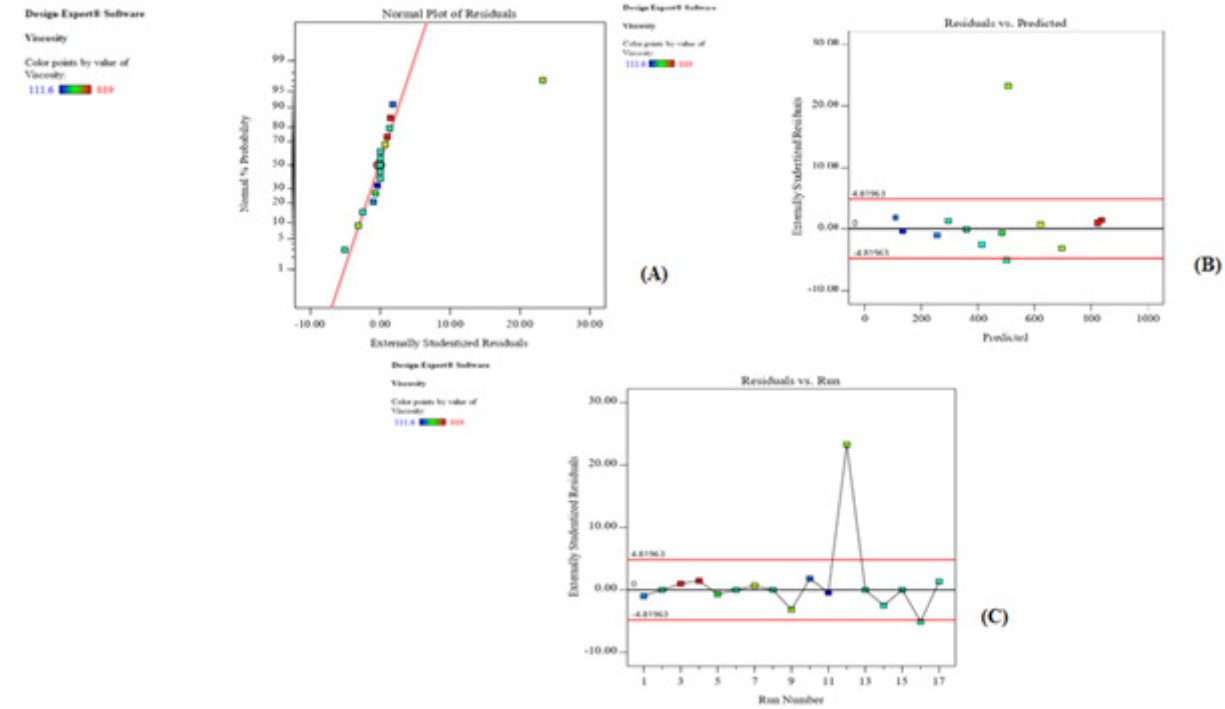
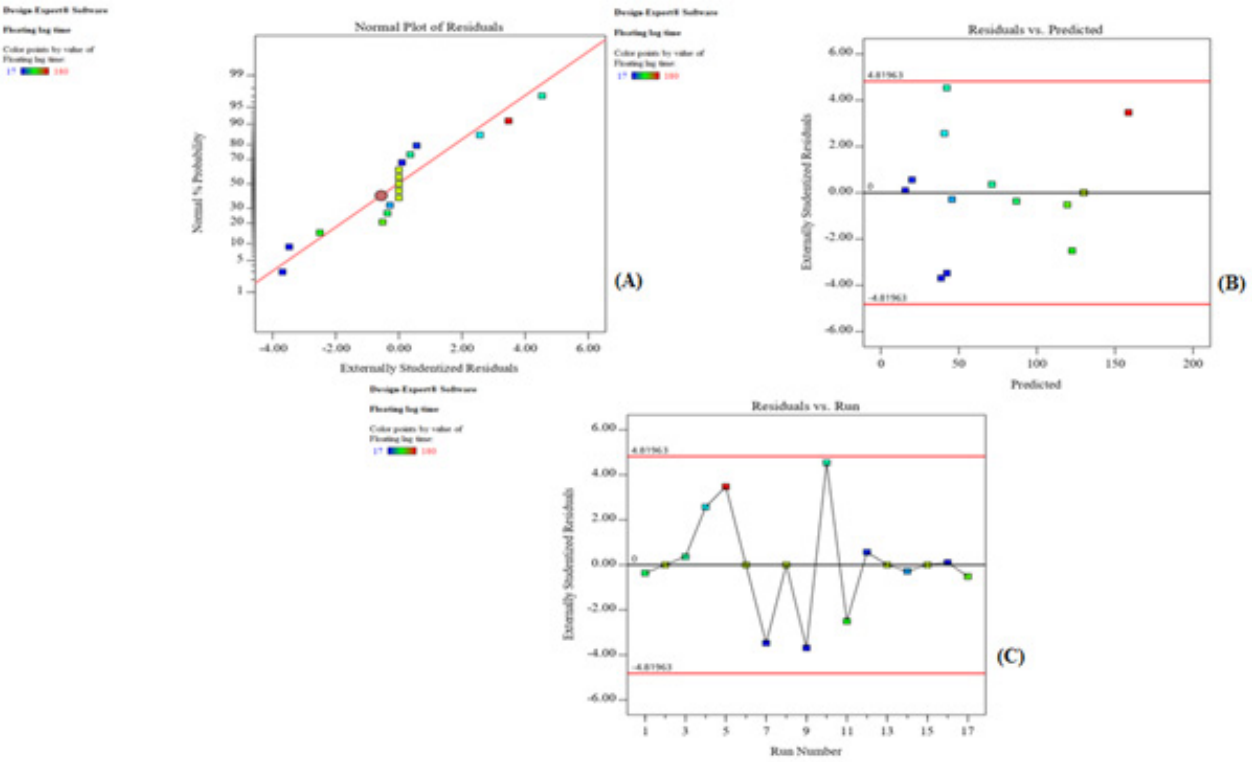


Fig. 4 (i). Viscosity. A. Residuals normal probability plot; B. Plot of residuals vs predicted response; C. Studentized residuals vs run number plot.



Fig, 4 (ii). Buoyancy lag time. A. Residuals normal probability plot; B. Plot of residuals vs predicted response; C. Studentized residuals vs run number plot.

As the level of X_1 (conc. of k-carrageenan) was increased from 0.25 % to 0.5 % at center level of X_2 (conc. of calcium carbonate) the viscosity increased from 200 to 600 cP and with the increase in X_3 (conc. of tri sodium citrate), the viscosity decreased up to 200 cP as seen through the contour plot. At higher level of X_2, the viscosity decreased from 1000 to 500 cP as X_3 was increased.

B. Effect on Buoyancy Lag Time

2D contour and 3D response surface plots for the influence of X_1 and X_2 on floating lag time are displayed in Figure 6.

As the X_1 level was increased from 0.25 % to 0.33 % at center level X_3 the buoyancy lag time increased from 40 to 140 secs and with further increase in X_1 to 0.5% the lag

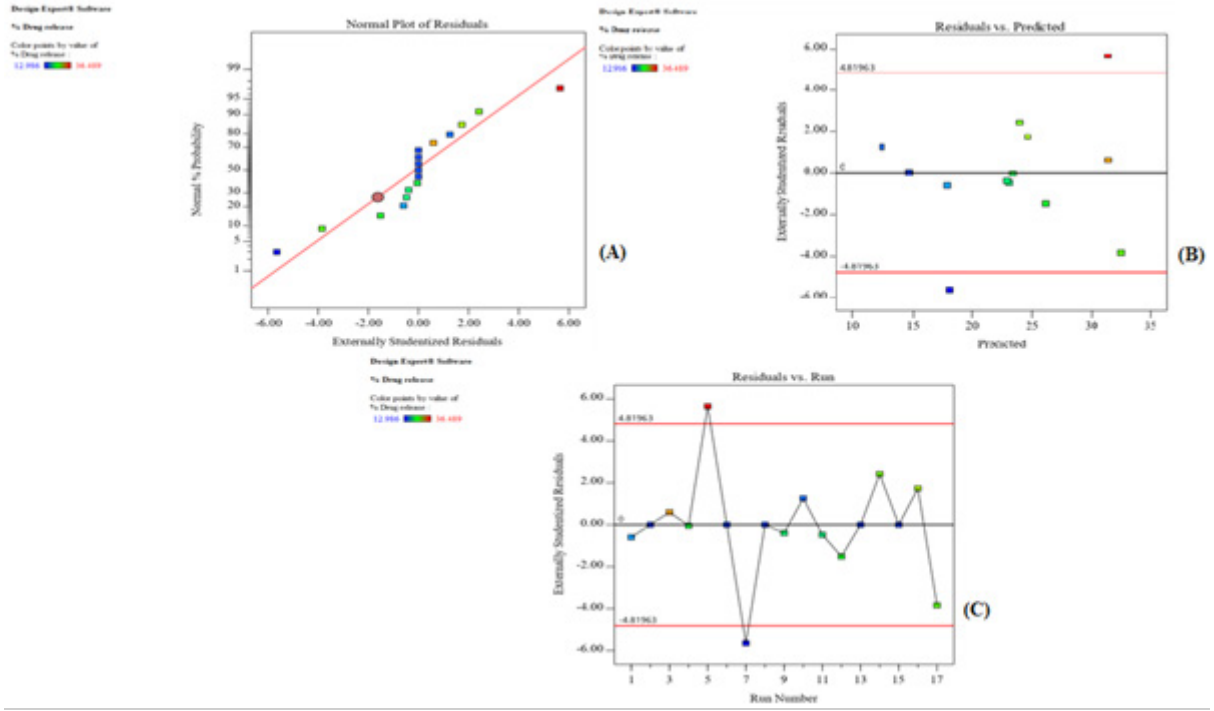


Fig. 4 (iii). % Drug release. A. Residuals normal probability plot; B. Plot of residuals vs predicted response; C. Studentized residuals vs run number plot.

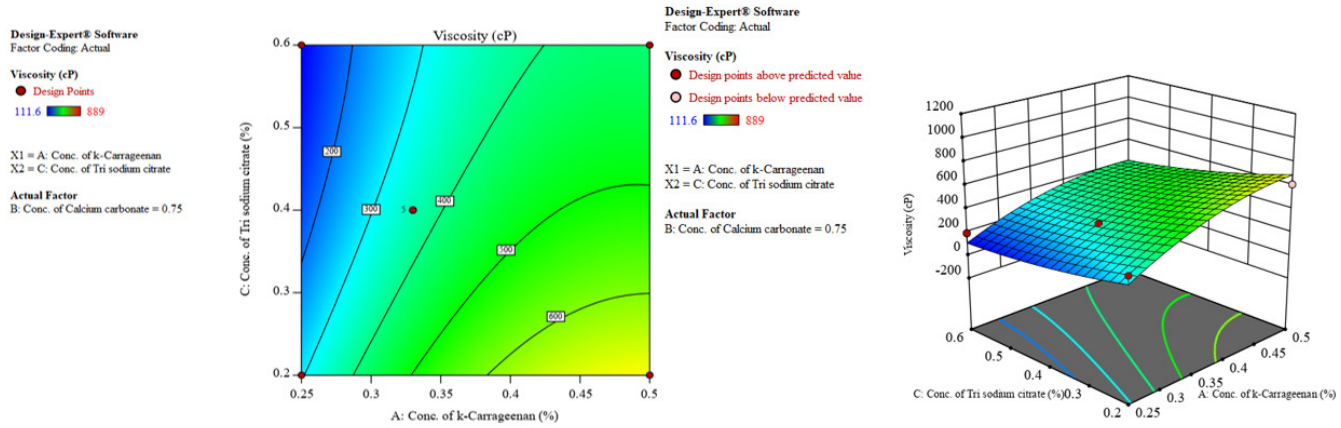


Fig. 5. 2D Contour and 3D response surface plots showing influence of X_1 and X_3 on viscosity at the Centre level of X_2

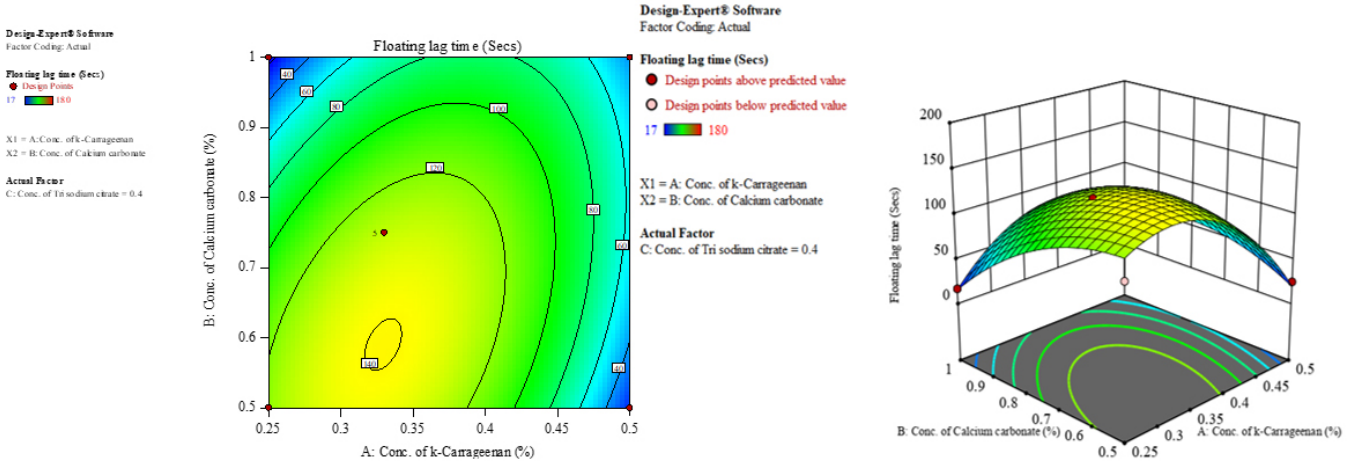


Fig. 6. 2D Contour and 3D response surface plots showing influence of X_1 and X_2 on floating lag time at the Centre level of X_3

time decreased to 40 secs. As X_2 level was increased, the floating lag time decreased from 140 to 40 secs. At the higher level of X_3, as X_2 was increased, the buoyancy lag time decreased to 20 secs.

C. Effect on % Drug Release

2D contour plots and 3D response surface plots for the effect of X_1 and X_2 on drug release are displayed in Figure 7.

As the level of X_1 (conc. of k-carrageenan) was increased at center level of X_3 (conc. tri sodium citrate), drug release decreased from 22% to 16% and with further increase in X_1 to 0.5%, the drug release increased to 24% and with the increase in X_2 (conc. of calcium carbonate), the % drug release decreased. At higher level of X_3, as the level of X_1 and X_2 was increased, there was increase in % drug release.

Validation of Box-Behnken Design

The optimum variables were determined by the numerical analysis depending on the desirability criterion. Predicted and actual responses of all the formulations are noted in the form of graph (Figure 8 (i and ii)).

To check and verify the reliability of the mathematical models built with Box-Behnken design, formulation recommended by the software was developed.

Optimized Formulation

The optimized formulation was obtained by applying constraints on independent variables. Constraint for the viscosity was in the range of 120-400 cP, floating lag time was in the range of 25-120 secs and the % drug release was targeted for 25-35% at 8 hrs. The Design-Expert® software suggested 67 solutions for optimization of floating *in-situ* gels formulation, but solution 1 was considered as its desirability was equal to 1.0.

Figure 9 shows the suggested optimized formulation code as X1=0.264%, X2=0.809% and X3=0.232%. The value of predicted responses of R1, R2 and R3 were 362.69cP, 109.769 secs and 28.59% respectively.

The overlay plot (Figure 9) gives the points not satisfying the specifications as greyed out, leaving an operating window or sweet spot highlighted in yellow colour.

Optimization was done by implementing both graphical optimization and numerical optimization techniques.

Evaluation of optimized floating in-situ gel formulation (OIG)

The evaluation results of the optimized floating *in-situ* gel are given in Table 7.

In-vitro Dissolution of Optimized Floating In-situ Gel Formulation

In-vitro dissolution of the OIG formulation was performed

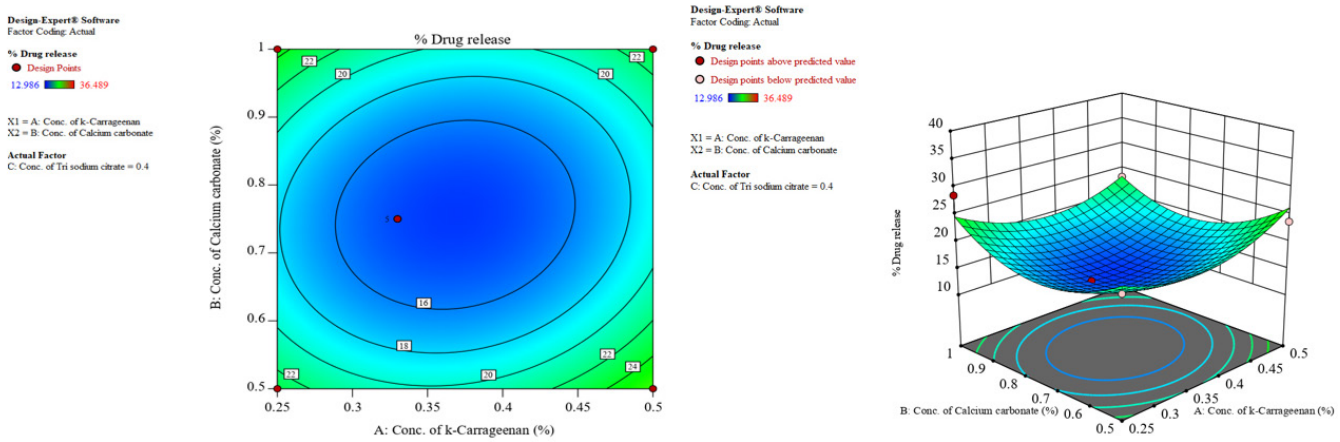


Fig. 7. 2D Contour and 3D response surface plots showing influence of X_1 and X_2 on drug release at the Centre level of X_3

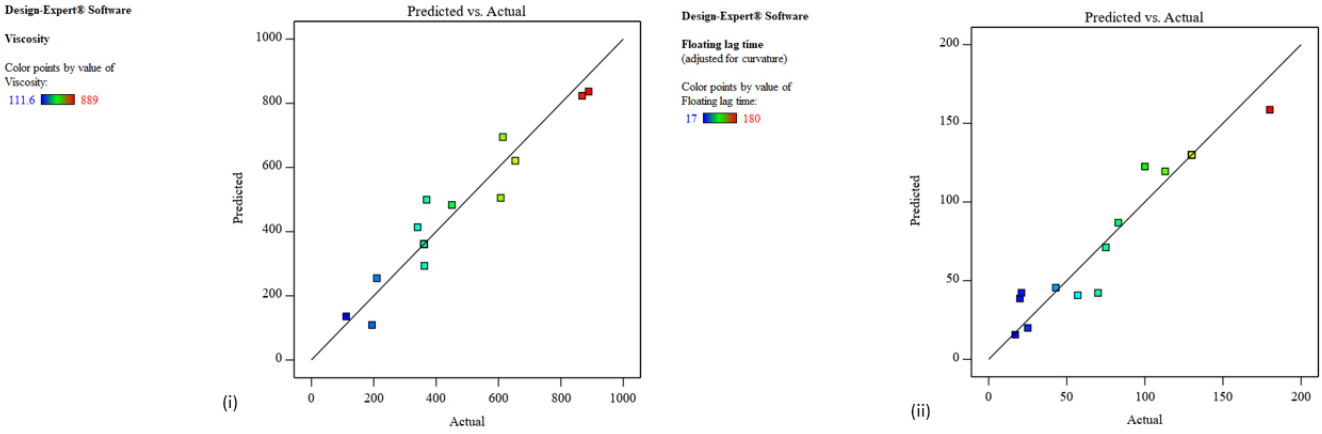


Fig. 8. Predicted vs actual responses for (i) viscosity, (ii) buoyancy lag time

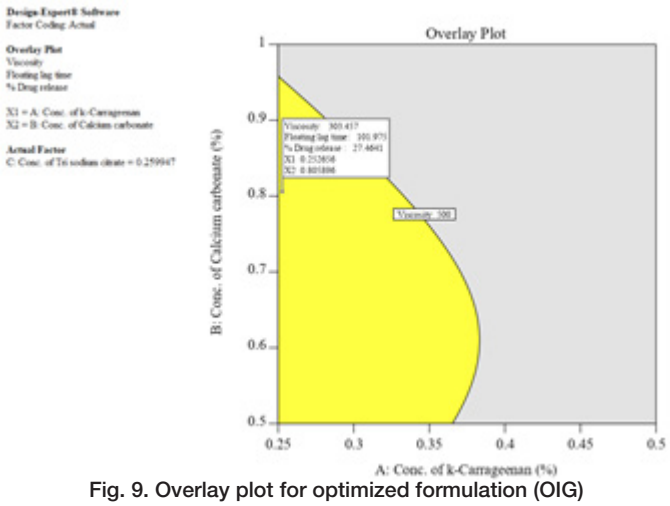


Fig. 9. Overlay plot for optimized formulation (OIG)

Table 7. Evaluation results of OIG formulation

Physical appearance	pH	Viscosity (cP)	In-vitro gelation capacity	Floating lag time (Secs)	Density (g/cm3)
Milky white solution	8.4±0.1	355.13±0.1	+++	104±0.2	0.843±0.03

Note: All the values are expressed as mean±SD, n=3. +++: Immediate gelation and remains for >24 hrs.

for 8 hrs and samples were analysed using UV spectroscopy. The dissolution profile is shown in Figure 10.

Model Dependent Kinetics

From the drug release kinetics results given in Table 8, it can be ascertained that OIG formulation follows first order release kinetics and Korsmeyer-Peppas drug release mechanism.

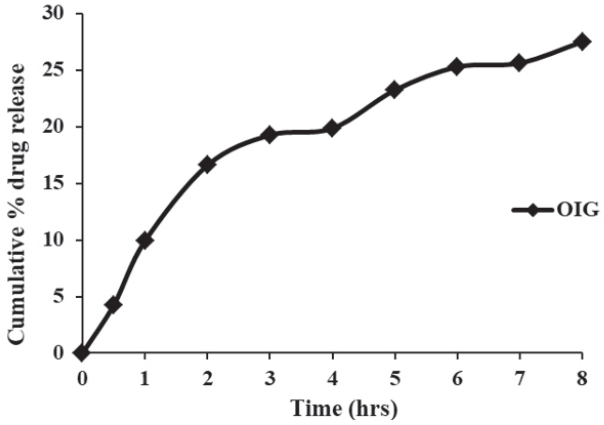


Fig. 10. In-vitro dissolution profile of OIG formulation

From the value of 'n' it can be presumed that, the mechanism of drug release from optimized floating *in-situ* gel loaded with microspheres (OIG) was Fickian diffusion.

Stability studies

To conduct stability studies, samples were assessed after one, two, three and four weeks for pH, gelling capacity, floating lag time, viscosity and density (Table 9). The *in-vitro* release profile of the OIG formulation before and after the stability studies is presented Figure 11. The stability studies revealed that there were no appreciable changes observed in the OIG formulation.

Conclusion

The optimized microspheres (OMS) obtained from the previous study were incorporated into floating *in-situ* gel which was optimized using Box-Behnken design. 17 formulations obtained from the design were prepared and assessed for pH, physical appearance, viscosity, density, gelation capacity, *in-vitro* drug release and floating behaviour. The optimized formulation (OIG) obtained from the design was milky white liquid with viscosity of 355.13 cP, showed buoyancy lag time of 104 secs and remained buoy-

Table 8. Model dependent kinetics of optimized floating in-situ gel loaded with microspheres (OIG)

Formulation	R2				n	Drug transport mechanism
	Zero order	First order	Higuchi plot	Korsmeyer-Peppas plot		
OIG	0.8742	0.8983	0.9584	0.934	0.4201	Fickian diffusion

Table 9. Stability studies of OIG formulation

Evaluation parameters	Initial	Week 1	Week 2	Week 3	Week 4
Physical appearance	Milky white solution	Milky white solution	Milky white solution	Milky white solution	Milky white solution
pH	8.4±0.1	8.4±0.2	8.5±0.1	8.7±0.01	8.2±0.1
Viscosity (cP)	355.13±0.1	356.23±0.01	354.74±0.1	355.45±0.2	357.76±0.1
Gelling capacity	+++	+++	+++	+++	+++
Buoyancy lag time (Secs)	104±0.2	105±0.1	103±0.2	104±0.1	106±0.4
Density (g/cm3)	0.843±0.03	0.845±0.11	0.841±0.1	0.843±0.1	0.843±0.05

Note: All the values are expressed as mean±SD, n=3

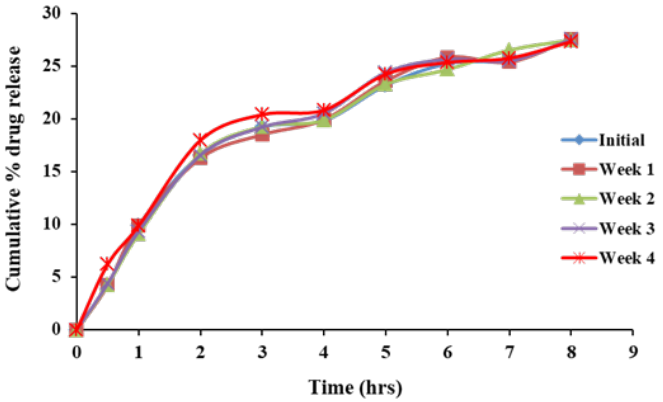


Fig. 11. Dissolution profile of OIG formulation during stability studies

ant for >24 hrs. 27.49 % drug was released from the *in-situ* gel at 8 hrs indicating controlled drug release. From the stability studies which were conducted for 4 weeks, it was determined that the OIG formulation was stable.

In conclusion, this study indicates the potential utilization of microspheres in floating *in-situ* gel for controlling the release and ease of administration as the dosage form is liquid thereby improving the patient compliance.

Discussion

Kappa carrageenan (κ-carrageenan) with a 3-linked, 4-sulfated galactose and a 4-linked 3,6 anhydro galactose. It has a double-helix conformation. The linear helical portions can associate and form a three-dimensional gel in the presence of appropriate cations. This gel is freeze-thaw stable is also able to interact with various food proteins through electrostatic interactions and increase their aggregation and stability.

κ -carrageenans are hydrophilic polymers which their solubility depend on the content of ester sulphate and the presence of potential associated cations. Higher levels of ester sulphate mean lower solubility temperature. Presence of cations such as sodium, potassium, calcium, and magnesium promote cation-dependent aggregation between carrageenan helices [24].

The *Fourier* Transform Infra-Red (FTIR) spectroscopy analysis of carvedilol, excipients, and their combinations provided critical insights into the chemical compatibility of the drug and excipients. Specifically, peaks at 3345.27 cm^{-1} (N-H and O-H stretching), 2921.96 cm^{-1} (aliphatic C-H stretching), 1607 cm^{-1} (N-H bending), 1502.44 cm^{-1} (aromatic C=C stretching), and 1097.42 cm^{-1} (C-O stretching) were observed. The spectra showed no evidence of drug-excipient interactions, suggesting that carvedilol is chemically stable and compatible with the excipients.

The study evaluated for pH, viscosity, gelation capacity, floating lag time and density for various formulations of floating *in-situ* gels and found that all formulations exhibited good floating properties, with buoyancy maintained for more than 24 hours. Notably, IG16 had the least buoyancy lag time, whereas IG5 had the highest. The rapid sol-to-gel transition facilitated by ionic gelation and the formation of a 3D network via Ca^{2}– ion complexation and H-bonding were critical in maintaining buoyancy. Calcium carbonate served as an effective effervescent agent, generating CO_2 that was trapped within the gel network, thus enhancing floating duration.

The gelation of gellan gum is strongly influenced by the chemical nature and quantity of cations present in the solution. Divalent cations promote the gelation much more strongly than monovalent cations. In monovalent cations, the gelation is mainly a result of the screening of the electrostatic repulsion between the ionized carboxylate groups on the gellan gum chains. In the case of divalent cations, the gelation and aggregation of gellan occurs via a chemical bonding between divalent cations and two carboxylate groups belonging to glucuronic acid molecules in the gellan chains, in addition to the screening effect [25]. The density of the formulations ranged from 0.621 to 0.845 g/cm^3, making them suitable for floating on stomach contents.

The *in-vitro* drug release profiles of the floating *in-situ* gels loaded with microspheres (IG1-IG17) were analysed using UV spectroscopy. The cumulative percentage of drug release was measured at various time points, ranging from 0.5 to 8 hours. In the initial phase (up to 2 hours), formulations IG5, IG7, and IG9 exhibited a rapid drug release, with IG5 showing the highest release of 16.72% at 0.5 hours and 10.43% at 1 hour. This initial burst release can be attributed to the surface-associated drug that rapidly dissolves once the gel comes in contact with the dissolution medium.

As the dissolution process progressed, a more sustained and controlled release pattern was observed. For instance, IG3 showed a cumulative release of 32.69% over 8 hours, indicating a prolonged release suitable for sustained delivery applications. Formulations such as IG12 and IG16 demonstrated similar extended-release profiles, with IG12 reaching 23.74% and IG16 achieving 28.51% cumulative release at the 8-hour mark. Highest percentage among the prepared formulations, possibility of extending the release of drug up to 24 hrs in a slow controlled manner. The variations in drug release profiles among different formulations can be attributed to the composition and concentration of the polymers and cross-linking agents used. For example, formulations with higher concentrations of gellan gum and calcium carbonate exhibited more extended-release profiles due to the stronger gel network formed, which retards the drug diffusion rate.

The mechanism of gel formation of carrageenan which is a thermo-reversible gel depends on temperature and gel-inducing agents. Structure of carrageenan at high temperature (above 80 °C) is as random coil which is due to the electrostatic repulsions between neighbouring chains. Upon temperature reducing, the conformation of chains changes to helical structure. Further cooling and presence of cations lead to intermolecular interactions between the

carrageenan chains which cause aggregation of the helical dimers and formation of a stable three-dimensional network.

The significant prolongation of drug residence caused by k-carrageenan might be attributed to both the slowdown in gel erosion and the bioadhesive property of carrageenan which was suggested by a previous report on the interaction between carrageenan and mucin *in vitro*[26].

Statistical Analysis and Optimization

The statistical analysis conducted using Design-Expert® software confirmed the significance of the models used for predicting the viscosity, buoyancy lag time, and drug release. The quadratic model was particularly effective for these responses, as indicated by the high R^2 values and significant p-values ($p<0.05$). The models provided a reliable framework for optimizing the formulation parameters to achieve desired gel properties.

Response Surface Analysis

The response surface analysis demonstrated the effects of various formulation variables on the gel properties. For instance, increasing the concentration of k-carrageenan (X1) led to an increase in viscosity the balance of water uptake, polymer mass loss during the hydration and the molecular weight of the polymer determined the viscosity of the resulting gel, whereas higher levels of tri-sodium citrate (X3) reduced viscosity. Similarly, the buoyancy lag time was influenced by the concentrations of calcium carbonate (X2) and k-carrageenan, with higher concentrations generally reducing lag time. The drug release rate was also modulated by these variables, with a higher concentration of calcium carbonate reducing drug release, likely due to the formation of a more robust gel matrix.

Validation and Optimization of Formulation

The optimized formulation was determined using numerical and graphical optimization techniques, with constraints applied to ensure the desired viscosity, buoyancy lag time, and drug release profiles. The optimized formulation exhibited a viscosity of 355.13 cP, a buoyancy lag time of 109.769 seconds, and a drug release of 28.59% at 8 hours, which were within the targeted ranges.

Stability Studies

Stability studies conducted over four weeks showed no significant changes in the pH, gelling capacity, buoyancy lag time, viscosity, or density of the optimized floating in-situ gel. The *in-vitro* release profiles before and after stability studies remained consistent, confirming the formulation's stability and reliability for extended use.

Authors' contribution

LK - Idea, design and laboratory work, guide and supervisor, Conceptualization, Formal Analysis, Data curation, Writing – original draft, Writing – review & editing

AR – Investigation, Methodology, Laboratory work, Formal Analysis, Data curation, Writing – original draft, Writing – review & editing

AF - Data curation, Formal Analysis, Writing – review & editing

VRM - Data curation, Formal Analysis, Writing – review & editing

LSVVNSM – Resources, Formal Analysis, Writing – review & editing

Conflict of interest

None to declare.

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