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Formulation Development and Statistical Optimization of Ivabradine Hydrochloride Floating Pulsatile Microspheres Using Response Surface Methodology

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Abstract

Aim: The objective of this work is to develop floating pulsatile microspheres of ivabradine Hydrochloride and statistical optimization using software based response surface methodology. **Materials and Methods:** The microspheres were prepared by nonaqueous solvent evaporation method in which three process variables were of utmost importance such as stirring speed, stirring time, and polymer concentration. The desired responses were % entrapment efficiency, % of buoyancy, and %DE at 20 min of microspheres. Optimization was done by fitting experimental data to the software program (Minitab). Obtained microspheres were subjected to different evaluation parameters which are essential in the development of the dosage form. **Results and Discussion:** The optimized batch of formulation showed satisfactory drug entrapment efficiency of 88.56 ± 1.12, % of buoyancy of 91.42 ± 1.09, and %DE at 20 min of 64.4 ± 0.36. Scanning electron microscopy analysis revealed that particles were spherical with smooth surface. Particles were free flowing and its average particle size 794 ± 1.43 µm. The developed optimized batch of microspheres maintained lag phase during floating in acidic medium (simulated gastric fluid) for 5 h followed by pulsatile release of ivabradine HCl within 30 min in phosphate buffer P_H 7.4 (Simulated intestinal fluid). Fourier transform infrared spectroscopy and differential scanning calorimetry studies revealed that there was no interaction between ivabradine HCl and Eudragit S100. **Conclusion:** Ivabradine HCl floating pulsatile microspheres were successfully made using response surface methodology.

Key words: Central composite design, Eudragit S100, floating pulsatile microspheres, ivabradine, nonaqueous solvent evaporation, optimization

INTRODUCTION

Diseases, such as angina pectoris, hypertension, and rheumatoid arthritis, rely on circadian rhythm where these diseases show critical conditions in the early hours of the day.[1] These diseases require rationale therapy where the drug is released from the dosage forms at a specific time when the symptoms are worsen particularly in the early hours.

Ivabradine Hydrochloride is I_f channel antagonist[2,3] used in the treatment of angina pectoris which is an underlying cause of heart attack when beta blockers are not responding. Ivabradine HCl is rapidly and almost completely absorbed after oral administration with a peak plasma level reached in about 1 h under fasting condition, and the half-life of the drug is 2 h. The absolute bioavailability is around 40%, due to first-pass effect in the gut and liver. Due to these factors, the available marketed ivabradine HCl formulations could not able to release drug when the symptoms of diseases were at peak level in the early morning hours in the case of heart attack patients.

To satisfy this condition, floating pulsatile concept was applied to formulate ivabradine HCl microspheres, which is a challenging task to increase gastric residence of dosage form having lag phase during floating in acidic medium followed

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by burst release in intestinal fluid. The combination of floating pulsatile principles of delivery system would have the benefit that a drug can be released in the upper gastrointestinal (GI) tract after a lag phase.[4,5] In addition, multiple unit dosage forms has many relative advantages over single unit dosage forms such as predictable GI transit time, maximum drug absorption with reduced inter- and intra-subject variability due to difference in gastric emptying rate, thus giving greater product safety.[6]

Eudragit polymers are series of acrylate and methacrylate polymers available in different ionic forms. Eudragit S100 is a pH dependent polymer that gets solubilized at pH 7 and above.[7]

The objective of this research work was to formulate ivabradine HCl floating pulsatile microspheres using Eudragit S100 by nonaqueous solvent evaporation technique and optimization of microspheres by response surface methodology. Initially, preliminary trials were done with 1:1, 1:3, 1:5 drugs:polymer ratio for obtaining lag phase during floating. With 1:5 drug:polymer ratio lag phase was obtained. Hence, this ratio was selected for the factorial design. During optimization, the effect of three independent variables, i.e. stirring speed (X_1), stirring time (X_2), and polymer concentration (%w/v) (X_3) on responses, such as % entrapment efficiency, % of buoyancy, and %DE at 20 min, were studied. This study encompasses the development of a new dosage form which was analyzed by various characterization tests.

MATERIALS AND METHODS

Materials

Ivabradine HCl was a generous gift sample obtained from Cipla Pharmaceutical Company, Mumbai. Eudragit S100 was obtained as a gift sample from Evonik Degussa India Private Limited, Mumbai. All other chemicals/reagents used were of analytical grade.

Methods

Drug polymer interaction studies

Fourier transform infrared spectroscopy (FTIR) study

Ivabradine HCl and Eudragit S100 were subjected to drug-excipient compatibility study. The drug and polymer were mixed physically in 1:1 ratio and the mixtures were placed in sealed vials for 3 months at room temperature. FTIR measurements of drug and drug-polymer mixtures were obtained on Shimadzu. FTIR samples were prepared by mixing with KBr and placing in the sample holder. The spectra were scanned over the wave number range of 4000-400/cm at the ambient temperature.[8]

Differential scanning calorimetry (DSC) analysis

DSC was carried out on pure drug ivabradine HCl, ivabradine HCl, and Eudragit S100 mixture. The accurate amount of samples was weighed into aluminum pans and sealed. All samples were run at a heating rate of 10°C/min over a temperature range of 30-300°C in atmosphere of nitrogen.[5]

Preparation of microspheres

Each batch of microspheres was prepared loading fixed quantity of pure ivabradine HCl (50 mg). Microspheres containing ivabradine HCl as core material was prepared by nonaqueous solvent evaporation technique. Accurately weighed amount of Eudragit S100 was dissolved in 5 ml/2.5 ml, of ethanol to form homogeneous polymer solution. Core material, i.e., ivabradine HCl was added to polymer solution and mixed thoroughly (1:5 drug:polymer ratio). The resulting drug-polymer solutions were poured gradually at room temperature into 50 ml of heavy liquid paraffin using disposable syringe of 24 G needle and the preparation using a mechanical stirrer. The microspheres were collected by decantation, and the product was washed repeatedly with petroleum ether until free from liquid paraffin and dried at room temperature for 1 h and stored in desiccators for further use.[9]

Experimental design

The design of experiments (DOE) was used to provide an efficient means to optimize the solvent evaporation method with the minimum number of experiment runs and to find out which process variables have the highest impact on the prepared microspheres. The number of experimental runs required for the study depends on number of variables. DOE is an effective and efficient approach for exploring the variability in responses and establishment of relationship between process variables and the responses studied.[10] In this study, 2^3 (three-factor and two-level) face centered central composite design was adopted to analyze the interaction of each level of factors on the desired responses and for optimization of ivabradine HCl floating pulsatile microspheres.' The experimental design was generated within the domain of levels using the Minitab. Different batches of ivabradine HCl microspheres were prepared based on the 2^3 face centered central composite design. The variables were stirring (X_1), stirring time (X_2), and polymer concentration (%w/v) (X_3). The design matrix including investigated variables along with their levels and responses are shown in Table 1.

Optimization of data analysis and validation of models

Analysis of variance (ANOVA) is inextricably linked to experimental design.[11] ANOVA was used to estimate the significance of the model and each response parameters and

Table 1: Design matrix and measured responses

Run order	Batch no	Variables levels in coded form			Response 1% entrapment efficiency[a] (Y_1)	Response 2% Buoyancy[a] (Y_2)	Response 3% DE at 20 min[a] (Y_3)
		X_1	X_2	X_3			
1	F1	−1	−1	−1	92.23±0.75	92.3±0.8	63.18±0.8
2	F2	1	−1	−1	84.64±0.8	94.2±0.6	69.05±0.1
3	F3	−1	1	−1	86.37±1.56	93.6±1	68.3±0.2
4	F4	−1	−1	1	92.97±1.4	84.7±2	48.1±0.6
5	F5	1	1	−1	82.47±1.1	94.26±0.6	69.55±0.5
6	F6	−1	1	−1	92.2±1.4	88.1±0.9	62.78±0.55
7	F7	1	−1	1	92.06±0.5	87.3±1	61.6±1
8	F8	1	1	1	92.5±0.9	90.8±0.5	62.9±0.8

Translation of coded levels in actual units

Variables level	Low (−1)	High (+1)
Stirring speed (rpm) (X_1)	700	1000
Stirring time (min) (X_2)	60	105
Polymer concentration (%w/v) (X_3)	5	10

[a]Mean±SD; *n*=3

also to establish the statistical validation of the polynomial equations.

The response (Y_i) in each trial was measured by carrying out a multiple factorial regression analysis using the generalized quadratic model.[12]

$$Y_i = b_0+b_1X_1+b_2X_2+b_3X_3+b_1b_2X_1X_2+b_1b_3X_1X_3 +b_2b_3X_2X_3+b_1b_2b_3X_1X_2X_3.$$

Where Y_i is the measured response; b_0 is the arithmetic mean response of the eight runs, and b_i is the estimated coefficient for the factor X_i. The main effect (X_1, X_2, and X_3) represents the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2, X_1X_3, and X_2X_3) show how the response changes when two factors are changed simultaneously. The interaction term ($X_1X_2X_3$) shows how the response changes when three factors are changed simultaneously.

After fitting the response data in the run design as in Table 1, the experimental results were analyzed by ANOVA technique. It displayed b-coefficients, F-values, and P-values of model terms. Other statistical parameters: The multiple correlation coefficient (R^2), adjusted multiple correlation coefficient (R^2), predicted multiple correlation coefficient (R^2) which authenticated he suitability of models. The models with significant terms were the best fit polynomials which explained the effects of different model terms on the responses. The desirability function approach is one of the most widely used methods in industry to optimize multiple response problems.[13] Desirability function approaching 1 is desired to determine the optimal setting.[14] Finally, product optimization was carried out by numerical optimization technique using the desirability function approach.

Evaluation of microspheres

Percentage yield

The percentage yield of different formulations was determined by weighing the floating microspheres after drying. The percentage yield of different formulation F_1-F_9 was calculated as follows.[15]

% Yield = (Total weight of microspheres/total weight of drug and polymer)×100

Drug entrapment efficiency

Accurately weighed 10 mg of drug loaded microspheres was added into 10 ml of phosphate buffer, P_H 7.4 in a volumetric flask and kept as such for overnight later sonicate it until the drug leaches out. The drug concentrations were determined spectrophotometrically at 286 nm in UV-visible spectrophotometer.[15]

% Drug entrapment efficiency=Actual drug content/theoretical drug content×100

Micromeritic properties

Particle size analysis

Sieve analysis

Separation of the microspheres into various size fractions was carried out using a mechanical sieve shaker. A series of standard stainless steel sieves (Erweka, DIN 4188) of number 10, 16, 24, 44, 60, 80, 120 were arranged in order of decreasing aperture size. Accurately weighed amount of drug loaded microspheres from each batch were placed on the uppermost sieve The sieves were shaken for a period of 10 min. the amount retained on different sieves were weighed and mean particle size of the. Microspheres were calculated

by the following equation. The procedure was carried out three times for each product.[16,17]

$d_{avg} = \Sigma nd/\Sigma n$

Where d_{avg} = Mean size of particles
n = Frequency of particle in a particle size range
d = Average particle diameter of a particular sieve number
nd = Weight size.

Angle of repose

The angle of repose for floating pulsatile microspheres was determined by fixed funnel method. These microspheres were allowed to fall freely through a funnel until apex of conical pile just touched the tip of the funnel.[15]

The angle of repose θ was determined according to the following formula:

$\theta = \tan^{-1} h/r$

Where,
h = Height of pile
r = Radius of the pile formed by the floating pulsatile microspheres.

Determination of bulk density and tapped density

It is the ratio between a given mass of floating pulsatile microspheres and its volume after tapping. The bulk density and tapped density of floating pulsatile microspheres were determined by the tapping method accurately weighed quantity of prepared microspheres were transferred into a 10 ml measuring cylinder. After observing the initial volume of these microspheres, the tapping was continued on a hard surface until constant volume was noted. The bulk density and tapped density were calculated according to the following formula.[15]

Bulk density = Mass of microspheres/initial volume

Tapped density = Mass of microspheres/volume of microspheres after tapping

Percentage compressibility index/Carr's index

The percentage compressibility index was calculated according to the following formula[15]

% Compressibility index = Tapped density−Bulk density/Tapped density×100

Hausner's ratio

Hausner's ratio of microspheres was determined by comparing the tapped density to the bulk density using the Equation[15].

Hausner's ratio = Tapped density/bulk density.

Floating behavior

About 50 mg of the microspheres was placed in 900 ml of 0.1 N HCL. The mixture was stirred at 50 rpm in a dissolution apparatus for 7 h. After 7 h, the layer of buoyant microspheres was pipetted and separated by filtration. Particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in a desiccator until constant weight was obtained. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles.[8]

% Buoyancy = [Wf/Wf+Ws)]×100

Where Wf and Ws are the weights of the floating and settled microspheres, respectively. All the determinations were made in triplicate.

In vitro release studies

The dissolution studies of the microspheres equivalent to 5 mg of ivabradine HCl were performed using USP Type II dissolution test apparatus. Volume of the dissolution medium was 900 ml with a stirring speed of 50 rpm, and the temperature was maintained at 37°C ± 0.5°C. These conditions were kept constant for all dissolution studies. The drug release study was carried out in 0.1 N HCl (P_H 1.2) for a time period equivalent to floating time, i.e. 5 h as pulsatile lag time which has been adopted from Maghsoodi *et al*., who reported pulsatile lag time as 5-6 h[18] followed by dissolution in phosphate buffer P_H 7.4 till complete release of drug (30 min). Periodically 5 ml of samples were withdrawn and replaced with equal amount of fresh dissolution media immediately after sampling, filtered through Whatman filter paper and the concentration of ivabradine HCl was measured spectrophotometrically at 286 nm[5,19] against suitably constructed calibration curve. All measurements were conducted in triplicate, and average values were plotted.

Drug release kinetics

Data obtained from *in vitro* release study was fitted into kinetic equations. The kinetic models used were zero order (amount of drug dissolved versus time), first order (log cumulative percentage of drug undissolved versus time). Regression (r^2) and K values were calculated from the linear curves obtained by regression analysis.[20]

Percent dissolution efficiency (%DE)

$$\%DE = \left[\int_{t1}^{t2} y\,dt / y100 \times (t_2 - t_1)\right] \times 100$$

Where y is the percentage of dissolved product.

DE is the area under the dissolution curve between time points t_1 and t_2 expressed as a percentage of the curve at maximum dissolution, y100, over the same time period. The integral of the numerator, i.e., the area under the curve is calculated by a

model independent method, the trapezoidal one.[21,22] The area under the curve is the sum of all the trapeziums defined by:

$$AUC = \sum_{i=1}^{i=n} \frac{(t_i - t_{i-1})(y_i + y_{i-1})}{2}$$

Where t_i is the i^{th} time point, y_i is the percentage of dissolved product at time t_i.

Scanning electron microscopy (SEM)

The shape and surface morphology of ivabradine HCl microspheres were investigated using SEM. The samples for SEM study were prepared by lightly sprinkling the formulation on a double adhesive tape stuck to an aluminum stub. The stubs were coated with gold to a thickness of about 300 Å under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a SEM. Images were taken at an acceleration voltage of 30 kV and magnifies of 200 µm.[8]

Statistical analysis

All estimated data were expressed as mean ± standard deviation. Each measurement was done in triplicate and significance was tested by unpaired *t*-test wherever necessary.[23]

RESULTS AND DISCUSSION

Drug polymer interaction study

FTIR analysis

The FTIR spectra of physical mixture were compared with the FTIR spectrum of pure drug [Figures 1 and 2]. The FTIR spectra of pure ivabradine HCl showed sharp peak at 1246.56, 1057.33 (O-CH_3 stretching), 1630.47 (C=O stretching), 2918.78 (symmetric CH stretching), 1445.33 (CH def), 1517 (C=C stretching), 1057.33 (C-N stretching of tertiary aliphatic amine) FTIR spectra of ivabradine HCl and Eudragit S100 mixture also showed identical peaks which indicated that there was no interaction between drug and polymer.

DSC

DSC thermograms [Figures 3 and 4] revealed that melting point of ivabradine HCl starts at 189.39°C (peak at 193.02°C) in ivabradine HCl and Eudragit S100 mixture and in the case of pure drug melting point starts at 192.56°C (peak at 195.58°C). The result confirmed that there was no interaction between drug and polymer.

Preparation of microspheres

One of the important features of this process was the use of ethanol as a dispersed phase and heavy liquid paraffin ethanol as dispersed phase and heavy liquid paraffin as an immiscible continuous phase to enable the formation of oil_1 – in – oil_2

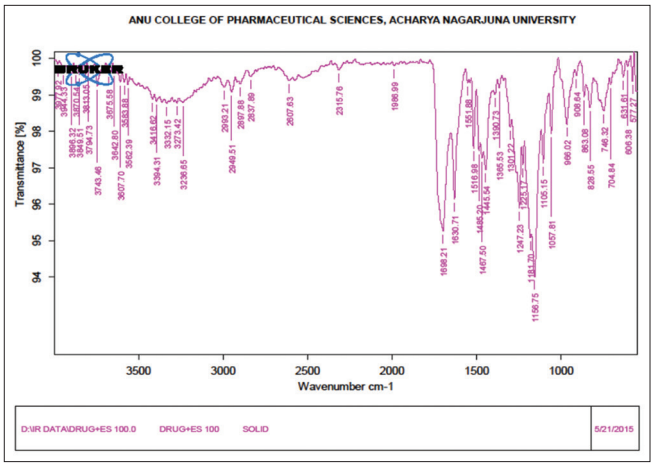

Figure 1: Fourier transform infrared spectroscopy spectrum of physical mixture of ivabradine Hydrochloride and Eudragit S100

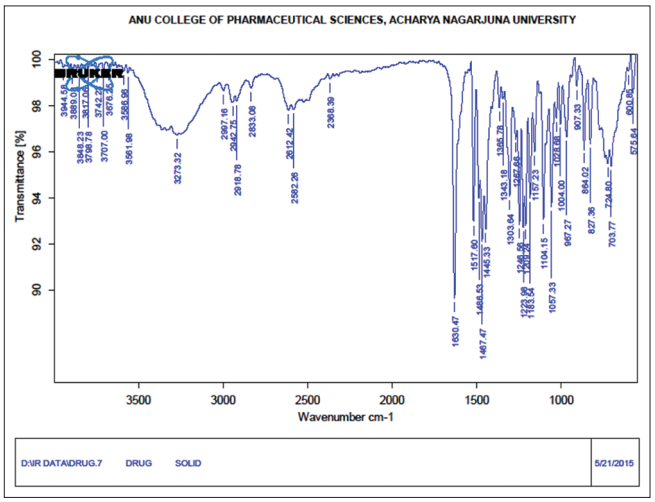

Figure 2: Fourier transform infrared spectroscopy spectrum of ivabradine Hydrochloride

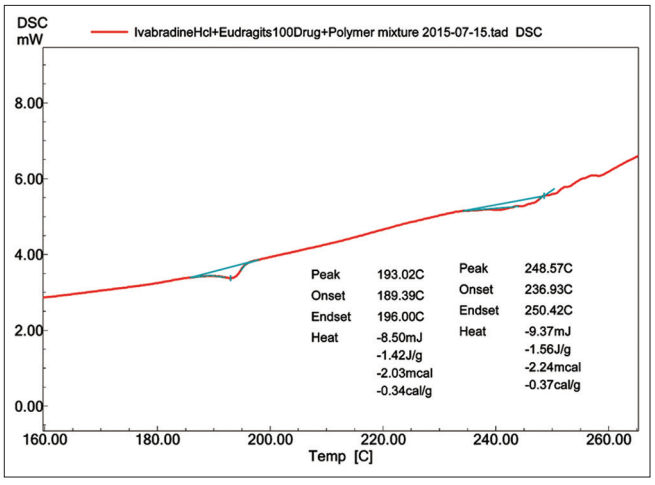

Figure 3: Differential scanning calorimetry thermogram of ivabradine Hydrochloride and Eudragit S100

emulsion. To dissolve both ivabradine HCl and Eudragit S100 ethanol was found effective and its dielectric constant was 24.3. As solvents with dielectric constants between 10 and 40 show poor miscibility with the nonpolar heavy liquid paraffin, so a heavy liquid paraffin was selected as a bulk phase in which both ivabradine HCl and Eudragit S100 were scantily soluble.

Eight formulations of "Ivabradine HCl floating pulsatile microspheres" were prepared by nonaqueous (O_1/O_2) solvent evaporation technique using 2^3 face-centered central composite design, in which the important variables were stirring (X_1), stirring time (X_2) and polymer concentration (%w/v) (X_3) and % entrapment efficiency (Y_1), % of buoyancy (Y_2), %DE at 20 min (Y_3) from "ivabradine HCl floating pulsatile microspheres" were taken as response parameters.

Data analysis and model validation

Fitting of data to the model

Three factors with two levels in the coded values were shown in Table 1. All the response variables were observed experimentally for 8 runs as proposed by the 23 face-centered central composite design and were fitted to run design chart. Models for various responses were obtained using Minitab software. The values of R^2, adjusted R^2, predicted R^2 were shown in Table 2 for each response along with their ANOVA results. $P < 0.05$ indicates significant model terms.

After elimination of non-significant ($P > 0.05$) coefficients Table 2, following correlations for response variables were obtained in terms of coded factors.

$$\% \text{ Entrapment efficiency: } Y_1 = +84.429 - 1.512X_1 - 1.046X_2 + 3.003X_3 + 0.613X_1X_2 + 1.361X_1X_3 + 0.964X_2X_3$$

$$\% \text{ of buoyancy: } Y_2 = +90.653 + 1.037X_1 + 1.162X_2 - 2.853X_3$$

$$\%\text{DE at 20 min: } Y_3 = 63.141 + 2.551X_1 + 2.742X_2 - 4.379X_3 - 2.208X_1X_2 + 0.771X_1X3 + 1.337X_2X_3 - 1.053X_1X_2X_3$$

The above model equations carry factors along with coefficients (positive/negative) which quantify response values. A positive sign of coefficient indicates synergistic effects, while negative sign represents an antagonistic effect (10).

The entrapment efficiency (%) for 8 batches were found to be in the range of 82.47 ± 1.1 to 92.5 ± 0.9% as evidenced from Table 1 ANOVA result indicated that the main factors X_1 (stirring speed), X_2 (stirring time), X_3 (polymer concentration), their interaction terms X_1X_2, X_1X_3, and X_2X_3 caused variation on the encapsulation efficiency. The model shows that both X_1 (stirring speed), X_2 (stirring time) had negative effects, and X_3 (polymer concentration) had positive effect on encapsulation efficiency. The % entrapment efficiency was found to be decreasing with increasing (X_1) stirring speed (In run 1 and run 2, mean difference is 7.59 ($P < 0.05$). The mean difference for run 1 and run 3 is 5.86 ($P < 0.05$) which indicates that with increasing stirring time (X_1) % entrapment efficiency is decreasing. The mean difference for run 2 and run 7 is 7.42 ($P < 0.05$) which indicates that with increasing polymer concentration (X_3) % entrapment efficiency is increasing. In this model, their interaction terms X_1X_3 and X_2X_3 showed positive coefficients, even though negative coefficients were shown by X_1 and X_2; possibly because of dominance of X_3.

The data of % of buoyancy for all batches ranging from 84.7 ± 2 to 94.26 ± 0.6% were observed from Table 1. The result indicated that the main factors X_1 (stirring speed), X_2 (stirring time), X_3 (polymer concentration) caused variation on % of buoyancy. The model shows that both X_1 (stirring speed), X_2 (stirring time) had positive effects, and X_3 (polymer concentration) had negative effect on % of buoyancy. The % of buoyancy was found to be increasing with increasing (X_1) stirring speed (In run 1 and run 2, mean difference is 1.9 ($P < 0.05$). The mean difference for run 7 and run 8 is 3.5 ($P < 0.05$) which indicates that with increasing stirring time (X_1) % of buoyancy is increasing. The mean difference for run 1 and run 4 is 7.6 ($P < 0.05$) which indicates that with increasing polymer concentration(X_3) % of buoyancy is decreasing.

%DE at 20 min was found to be in the range of 48.1 ± 0.6 to 69.55 ± 0.5 as indicated in Table 1. ANOVA result indicated that the main factors X_1 (stirring speed), X_2 (stirring time), X_3 (polymer concentration), their interaction terms X_1X_2, X_1X_3, X_2X_3 and caused variation on the encapsulation efficiency. The model shows that both X_1 (stirring speed), X_2 (stirring time) had positive effects, and X_3 (polymer concentration) had negative effect on %DE at 20 min. The %DE at 20 min was found to be increasing with increasing (X_1) stirring speed (In run 1 and run 2, mean difference is 5.87 ($P < 0.05$). The mean difference for run 1 and run 3 is 5.12 ($P < 0.05$) which indicates that with increasing stirring time (X_1) %DE at 20 min was found to be increasing. The mean difference for run 2 and run 7 is 7.45 ($P < 0.05$) which indicates that with increasing polymer concentration (X_3) %DE at 20 min was found to be decreasing. In this model, their interaction terms X_1X_3 and X_2X_3 showed positive coefficients while the interaction terms X_1X_2 and $X_1X_2X_3$ showed negative coefficients.

Counter and three-dimensional (3D) response surface plot analysis

Design expert software generated the counter and 3D response plots high visualized the effects of the process parameters on the response variables (% entrapment efficiency, % of buoyancy, % DE at 20 min).

% entrapment efficiency (Y_1) was decreased with increasing levels of stirring speed (X_1) and stirring time (X_2) as depicted

Table 2: ANOVA results for predicting entrapment efficiency (Y_1, %), % of buoyancy (Y_2, %), % DE at 20 min (Y_3, %)

Source	*b*-coefficient	Sum of squares	d.f	Mean square	*F*-value	*P* value, *P*>*F*
For Y_1 (%)						
Model	84.429	375.592	7	53.656	81.58	0.000
X_1	−1.512	54.904	1	54.904	45.16	0.000
X_2	−1.046	26.205	1	26.205	21.59	0.000
X_3	3.003	216.360	1	216.360	177.98	0.000
X_1X_2	0.613	9.004	1	9.004	7.41	0.015
X_1X_3	1.361	44.445	1	44.445	36.56	0.000
X_2X_3	0.964	22.311	1	22.311	18.35	0.001
$X_1X_2X_3$	−0.311	2.319	1	2.319	1.91	0.186
Residual		19.415	16	1.22		
Pure error		19.415	16	1.22		
Total		395.043	23			
Other statistics: R^2=0.9508, adjusted R^2=0.9292, predicted R^2=0.8892						
Source	***b*-coefficient**	**Sum of squares**	**d.f**	**Mean square**	***F*-value**	***P* value, *P*>*F***
For Y_2 (%)						
Model	90.653	266.332	7	38.05	93.74	0.000
X_1	1.037	25.792	1	25.792	28.61	0.000
X_2	1.162	32.387	1	32.387	35.92	0.000
X_3	−2.853	195.396	1	195.396	216.71	0.000
X_1X_2	−0.222	1.179	1	1.179	1.31	0.270
X_1X_3	0.213	1.092	1	1.092	1.21	0.287
X_2X_3	0.638	9.779	1	9.779	10.85	0.005
$X_1X_2X_3$	0.172	0.707	1	0.707	0.78	0.389
Residual		14.427	16	0.902		
Pure error		14.427	16	0.902		
Total		280.761	23			
Other statistics: R^2=0.9486, adjusted R^2=0.9261, predicted R^2=0.8844						
Source	***b*-coefficient**	**Sum of squares**	**d.f**	**Mean square**	***F*-value**	***P* value, *P*>*F***
For Y_3 (%)						
Model	63.141	997.62	7	142.5	594.22	0.000
X_1	2.551	156.16	1	156.16	349.37	0.000
X_2	2.742	180.40	1	180.40	403.60	0.000
X_3	−4.379	460.25	1	460.25	1029.69	0.000
X_1X_2	−2.208	117.04	1	117.04	261.85	0.000
X_1X_3	0.771	14.26	1	14.26	31.90	0.000
X_2X_3	1.337	42.88	1	42.88	95.93	0.000
$X_1X_2X_3$	−1.053	26.63	1	26.63	59.57	0.000
Residual		7.15	16			
Pure error		7.15	16			
Total		1004.78	23			
Other statistics: R^2=0.9929, adjusted R^2=0.9898, predicted R^2=0.984, *P*<0.05 is considered as significant, d.f: Degrees of freedom						

in the two-dimensional -Iso response curves and response plots in Figure 5a1 and b1. % of buoyancy (Y_2) increased with increasing levels of stirring speed (X_1) and stirring time (X_2) as reported in Figure 5a2 and b2. Similarly, the effect of chosen variables on %DE at 20 min was indicated in Figure 5a3 and b3. As the levels of stirring speed (X_1) and

stirring time (X_2) increases %DE at 20 min was found to be increasing.

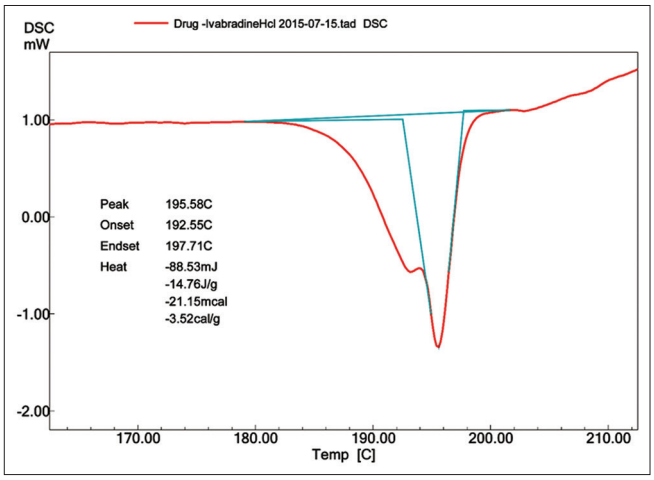

Figure 4: Differential scanning calorimetry thermogram of ivabradine Hydrochloride

Optimization

A numerical optimization technique using the desirability function approach was employed to generate the optimum settings for the formulation. Suitable levels of constraints (Target) were chosen to achieve desired characteristics (responses) of the formulation. It was found to satisfy the requisites of an optimum formulation when the desirable ranges of responses were restricted to % entrapment efficiency at 95%, % of buoyancy at 95%, and %DE at 85%. On analyzing various response variables and comprehensive evaluation of feasibility of exhaustive grid search, the following combination of variables was suggested by the software with desirability function of 0.854 as reported in Table 3, stirring speed =700 (rpm); stirring time =75.9 min; polymer concentration =5. The desirability function value (0.854) is closer to 1. Ivabradine HCl microspheres were prepared using the optimal "variables" settings and evaluated for the responses. The optimized batch of microspheres (F_9) showed % entrapment efficiency of 88.56 ± 1.12, % of

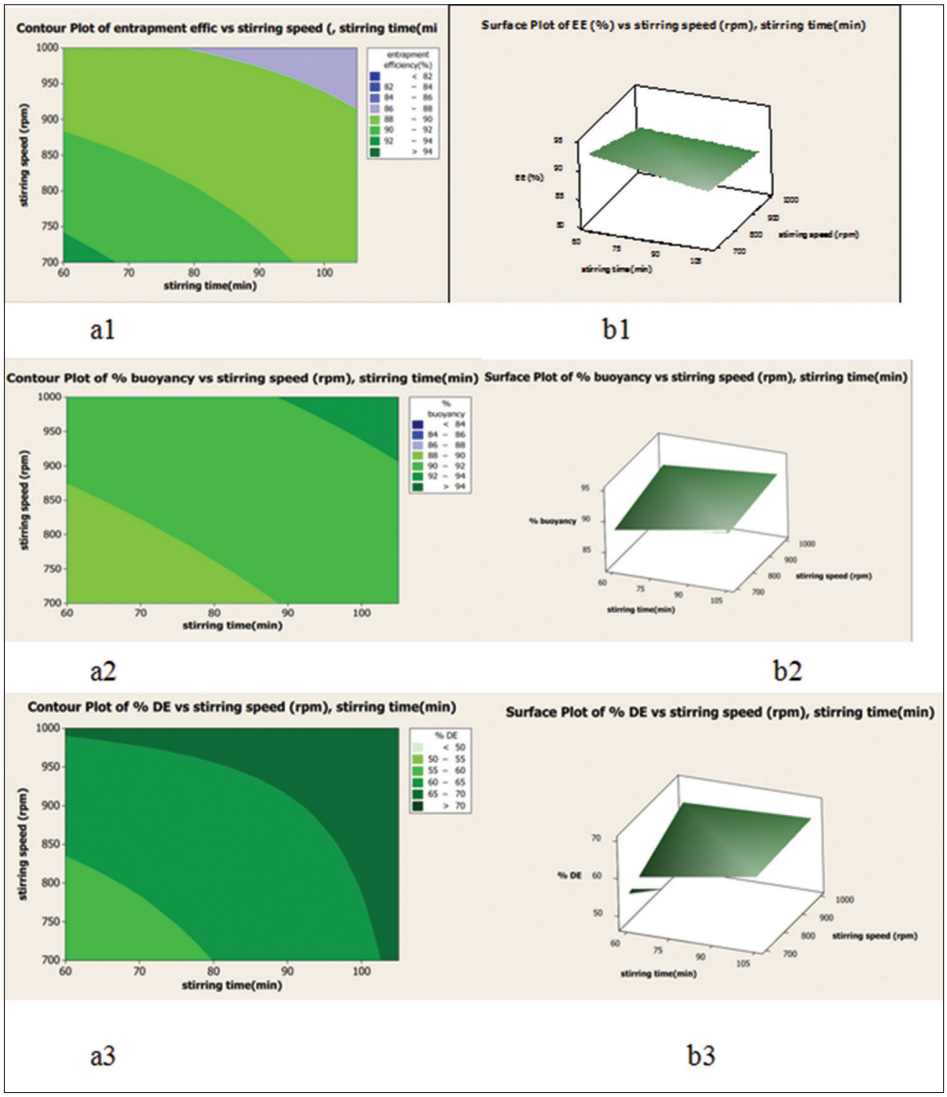

Figure 5: (a - a1-a3) Count hour and (b - b1-b3) response surface plots showing the effects of factors on % entrapment efficiency, % of buoyancy, % DE at 20 min

buoyancy 91.42 ± 1.44, %DE of 64.4 ± 0.91 with small error value (<1.77%). It was suggested that the generated models were well suited to optimize ivabradine HCl floating pulsatile microspheres.

Evaluation of microspheres

Percentage yield

The percentage yield of microspheres of various formulations varied from 73.12 ± 1.14 to 88.23 ± 1.65 which were shown in Table 4. The optimized formulation exhibited 82.12 ± 1.43 % yield which was shown in Table 4.

Drug entrapment efficiency

The entrapment efficiency of formulation F_1-F_9 was carried out and found to be in a range of 84.64 ± 0.8 to 92.97 ± 1.4 as shown in Table 1. For optimized formulation entrapment efficiency was observed to be 88.56 ± 1.12 as shown in Table 3.

Micromeritics

The mean particle size of formulation F_1-F_8 ranges from 456 ± 1.07 to 1250 ± 1.39. Mean particle size for optimized formulation (F_9) was found to be 794 ± 1.43 which were reported in Table 4. The bulk density and tapped density of formulation F_1-F_8 ranges from 0.293 ± 0.016 to 0.427 ± 0.01 and 0.347 ± 0.03 to 0.481 ± 0.02 gm/cc, respectively. The bulk density and tapped density for optimized formulation (F_9) was found to be 0.343 ± 0.02 and 0.398 ± 0.03 gm/cc, respectively, which were reported in Table 4. The values of Carr's index, Hausner's ratio, angle of repose which were reported in Table 4 indicated good to fair flow properties as per USP

Table 3: The criterion for numerical optimization

Parameters	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
X_1: Stirring speed (rpm)	Is in range	−1	1	1	1	1
X_2: Stirring time (minutes)	Is in range	−1	1	1	1	1
X_3: Polymer concentration (% w/v)	Is in range	−1	1	1	1	1
% Entrapment efficiency (Y_1)	Target=93	85	99	1	1	1
% Of buoyancy (Y_2)	Target=93	85	99	1	1	1
% DE at 20 minutes (Y_3)	Target=65	60	70	1	1	1

Solutions				Desirability				
Process variables				Response variables				
Code	X_1: Stirring speed, rpm	X_2: Stirring time, min	X_3: Polymer concentration, %w/v	Experimental values[a]		Predicted values	% Error[b]	Desirability
F9 (optimized)	700	75.9	5	% EE	88.56±1.12	90.16	−1.77	0.854
				% of buoyancy	91.42±1.09	92.76	−1.44	
				% DE	64.4±0.36	64.99	−0.91	

[a]Mean±SD; n=3, [b]Percentage of error (%)

Table 4: Evaluation parameters of microspheres

Formulation code	% yield[a]	Micromeritics					
		Mean particle size (μm)[a]	Angle of repose (°)[a]	Bulk density (gm/cc)[a]	Tapped density (gm/cc)[a]	Carr's index (%)[a]	Hausner's ratio[a]
F1	84.98±1.17	873±1.32	32.27±0.21	0.369±0.02	0.425±0.04	13.2±0.19	1.15±0.018
F2	74.28±1.54	505±1.65	35.12±0.12	0.296±0.03	0.349±0.04	15.2±0.17	1.18±0.014
F3	78.34±1.83	635±1.46	34.71±0.34	0.306±0.02	0.359±0.05	14.8±0.11	1.17±0.012
F4	88.23±1.65	1250±1.39	31.12±0.14	0.427±0.01	0.481±0.02	11.2±0.09	1.127±0.034
F5	73.12±1.14	456±1.07	35.76±0.23	0.293±0.016	0.347±0.03	15.6±0.06	1.18±0.019
F6	85.92±1.13	1056±1.18	31.8±0.31	0.412±0.032	0.468±0.043	11.9±0.05	1.136±0.04
F7	87.19±1.23	1123±1.98	31.75±0.26	0.421±0.05	0.475±0.06	11.37±0.02	1.138±0.017
F8	85.12±0.9	976±1.56	32.01±0.42	0.381±0.03	0.435±0.05	12.4±0.03	1.142±0.02
F9 (optimized)	82.12±1.43	794±1.43	33.78±0.37	0.343±0.02	0.398±0.03	13.8±0.04	1.16±0.01

[a]Mean±SD; *n*=3

limits. For optimized formulation, free flow properties were categorized as good flow which was reported in Table 4.

Floating behavior

All these formulations remained floating for more than 6 h but vary in % of buoyancy which ranges from 84.7 ± 2 to 94.26 ± 0.6 which were indicated in Table 1. Optimized formulation (F_9) also remained floating for more than 6 h and it showed 91.42 ± 1.09 % of buoyancy as indicated in Table 3. The floating properties of microspheres may be attributed to their low density.

In vitro drug release

To simulate the pH variation of GI tract dissolution studies were performed first at pH 1.2 (acidic medium) for time equivalent to floating time (5 h) and then subsequently medium was replaced with fresh phosphate buffer pH 7.4 (Intestinal p^H) having maintained temperature of 37°C ± 0.2°C. All these formulations maintained lag phase during floating in acidic medium and exhibited burst release in pH 7.4 phosphate buffer but vary in % of drug release as depicted in Figure 6. The optimized formulation also showed lag phase in acidic medium and burst release in phosphate buffer pH 7.4 as displayed in Figure 7.

Drug release kinetics

When the release data were analyzed as per zero and first order kinetic models, it was observed that the release from all the formulations followed first order kinetics. The regression values (r^2) were higher in the first order model which were shown in Table 5.

Percent dissolution efficiency (%DE)

The %DE at 20 min of formulations F_1-F_8 ranges from 48.1 ± 0.6 to 69.55 ± 0.5 as reported in Table 1. The %DE at 20 min for optimized formulation (F_9) was found to be 64.4 ± 0.36 which were reported in Table 3.

SEM

Photomicrograph of SEM revealed that the microspheres were spherical in shape with smooth surface as shown in Figure 8 which revealed the absence of any drug crystal on the surface and conformed uniform distribution of drug in the polymer matrix.

Table 5: Drug release kinetics of formulations F1-F9

Formulation code	Zero order		First order	
	r^2	K mg/min	r^2	K min^{-1}
F1	0.882	0.145	0.998	0.124
F2	0.907	0.226	0.994	0.158
F3	0.873	0.1752	0.998	0.144
F4	0.964	0.148	0.975	0.092
F5	0.903	0.226	0.995	0.159
F6	0.8815	0.145	0.996	0.116
F7	0.885	0.144	0.995	0.108
F8	0.883	0.145	0.998	0.119
F9 (optimized)	0.905	0.179	0.993	0.139

CONCLUSION

This work disclosed that ivabradine HCl floating pulsatile microspheres were successfully formulated by nonaqueous solvent evaporation technique. The effects of three

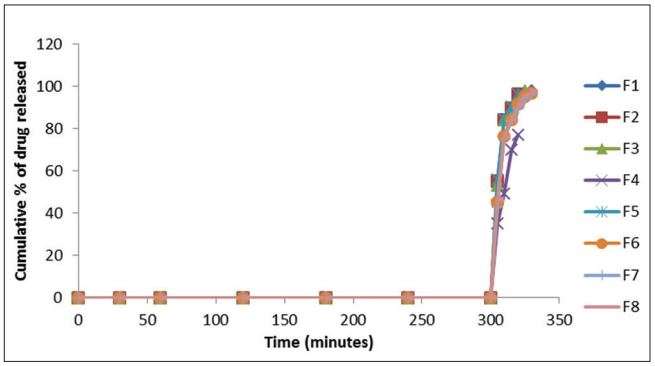

Figure 6: *In vitro* drug release profile of ivabradine Hydrochloride floating pulsatile microspheres of formulations F_1-F_8

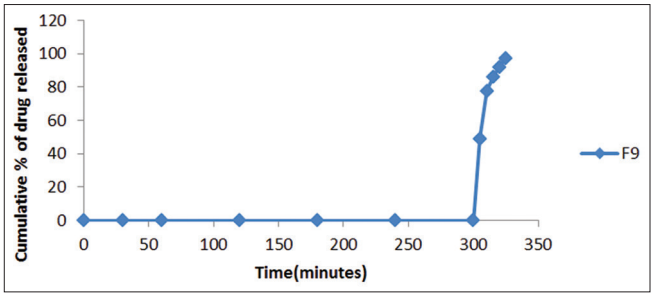

Figure 7: *In vitro* drug release profile of optimized formulation (F_9) of ivabradine Hydrochloride floating pulsatile microspheres

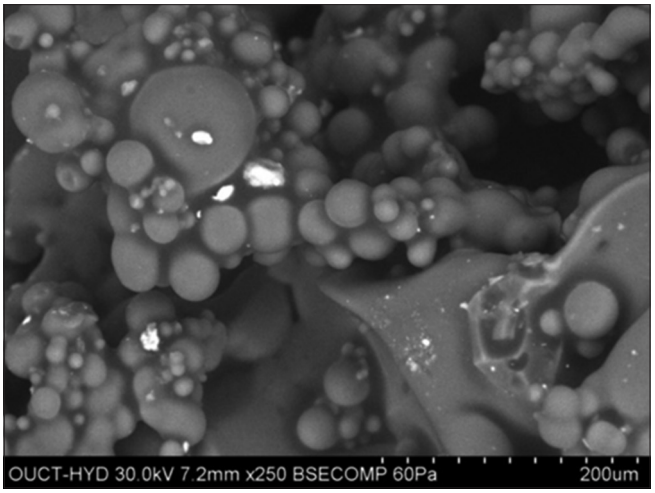

Figure 8: Scanning electron microscopy of optimized formulation (F_9)

independent variables (Stirring speed, stirring time, and polymer concentration) on three responses were studied and optimized systematically using response surface methodology. The work disclosed that all three independent variables had significant effect on the measured responses. The optimized formulation (F_9) showed the % entrapment efficiency of 88.56 ± 1.12, % of buoyancy of 91.42 ± 1.09, and %DE at 20 min of 64.4 ± 0.36. *In vitro* drug release studies revealed that lag phase was maintained during floating in acidic medium, i.e., 0.1N HCl for 5 h which is a targeted time followed by burst release within 30 min in phosphate buffer pH 7.4 (intestinal pH). These microspheres have the flexibility in filling any desired dosage amount in specific size capsule. Micromeritic study revealed that these microspheres exhibited free flow properties which are essential in attaining uniformity of dosage amounts during capsule filling. SEM analysis conformed that these microspheres were spherical with smooth surface. FTIR and DSC studies reported that there was no interaction between drug and polymer. The optimized formulation can be used as an alternative to the single unit marketed formulation which releases the drug in right time, right place and right amount when the symptoms of heart attack are at peak level in the early hours. Therefore, applicability of response surface methodology to optimize the variables in the preparation of ivabradine HCl floating pulsatile microspheres is highly advantageous.

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