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Research Article

FORMULATION DEVELOPMENT AND STATISTICAL OPTIMIZATION OF IVABRADINE HYDROCHLORIDE FLOATING PULSATILE PELLETS BY FLUIDIZED BED COATING TECHNIQUE

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ABSTRACT

Objective: The objective of the current work was to develop Ivabradine hydrochloride (HCl) floating pulsatile pellets containing drug loaded calcium alginate pellets coated with pH-dependent polymer Eudragit S100 oil dispersion.

Methods: Fluidized bed coating technique was used to develop pellets. A 2^2 factorial design was employed to study the effect of independent variables (inlet air temperature, spray rate), on dependent variables (% entrapment efficiency, % friability, and average particle size). Optimization was done by fitting experimental data to the software program (Minitab). Obtained pellets were subjected to different evaluation parameters which are critical in the development of the dosage form. An *in vitro* lag phase study was carried out for all batches in simulated gastric fluid (0.1N HCl) for 5 hrs and *in vitro* drug release study was carried out for optimized batch (B4) of two different sizes (10/12#, 12/16#) in simulated intestinal fluid (pH 7.4 phosphate buffer).

Results: The optimized batch (B4) showed satisfactory % entrapment efficiency of 92.66±1.52; % friability of 0.57±0.03; and average particle size of 1424±16 (µm). All batches maintained lag phase for 5 hrs in 0.1N HCl. An optimized batch of two different sizes exhibited a burst release within 30 minutes in simulated intestinal fluid with no significant difference in release rate constant (*p>0.05) and followed first order kinetics.

Conclusion: Thus, ivabradine HCl floating pulsatile pellets was successfully developed for treating angina pectoris which is an underlying cause of heart attack by fluidized bed coating technique employing factorial design.

Keywords: Ivabradine hydrochloride, Sodium alginate, Eudragit S100, Pellets, Fluidized bed coating, Optimization, Central composite design

INTRODUCTION

In the present era, multiparticulate dosage forms are gaining interest over single-unit dosage forms for pulsatile delivery. The potential advantage of multiparticulate system includes no risk of dose dumping, reduced the risk of local irritation, less inter- and intra-subject variability and increased bioavailability [1]. Pelletization is one of the most promising techniques for the multiparticulate drug delivery system. Pellets are agglomerates of fine powders or granules of bulk drugs and excipients. They consist of small, free-flowing, spherical or semi-spherical solid units, typically from about 0.5-1.5 mm and are intended usually for oral administration [2]. Pellets are typically coated for the purpose of producing modified release dosage forms, and the coating is preferably done by fluid bed technology as it promotes uniform coating leading to an efficient and predictable drug release.

Among modified release oral dosage forms, chronotherapeutic dosage forms are gaining popularity as they release the drug when the symptoms of diseases are at peak level. Chronotherapeutics refers to a clinical practice of synchronizing drug delivery in a manner consistent with the body's circadian rhythm including disease states to produce maximum health benefit and minimum harm [3]. Diseases such as angina pectoris, hypertension, and rheumatoid arthritis rely on circadian rhythm where these diseases show peak symptoms in the early hours of the day [4]. To treat these types of diseases, a rationale therapeutic system is required that would synchronize the drug delivery with the circadian variation in periods of increased risk.

In this context, floating pulsatile drug delivery systems has been utilized for chronotherapeutic drug administration, where lag phase (period of no drug release) is maintained during floating in acidic medium followed by burst release in intestinal fluid [5-7].

Ivabradine hydrochloride (HCl) is I_f channel antagonistused in the treatment of angina pectoris which is an underlying cause of Heart attack when beta blockers are not responding [8,9]. Ivabradine HCl is rapidly and almost completely absorbed after oral administration with a peak plasma level reached in about 1 hr under a fasting condition, and the half-life of the drug is 2 hrs. The absolute bioavailability is around 40%, due to the first-pass effect in the gut and liver.

Due to these factors, the currently available marketed ivabradine HCl formulations could not able to release the drug when the symptoms of diseases were at peak level in early morning hours in case of heart attack patients.

This rationale formulation improves patient compliance by releasing the drug at specific time, specific site, and specific amount when the symptoms of heart attack are at peak level in the early hours even though this dosage form is taken at bedtime by maintaining lag phase during floating in stomach followed by burst release in intestinal pH, which is the principle of floating pulsatile drug delivery system.

A novelty of the study involves in the improvement of entrapment efficiency of water-soluble drug by solution layering technique using fluidized bed coater compared to ionic gelation method and also to obtain lag phase for water-soluble drug in acidic medium.

Sodium alginate is a naturally occurring substance from brown seaweed and algae. It forms a bioadhesive and stable gel with divalent cations such as Ba^{2+}, Sr^{2+}, and Ca^{2+} which enabled widespread use for sustained release of drugs. They can also be function as carriers as bifidobacteria and used for the pulsatile release of drugs since alginate beads are stable in acidic media and easily degraded in alkaline media [10]. This polymer swell at higher p^H and release the drug by

diffusion or degradation mechanism in sustained or burst manner after predetermined lag time [11].

Alginate beads obtained by ionotropic crosslinking of these polymers have been used to develop floating drug delivery. Various approaches such as the use of oils freeze drying and entrapment of gas or gas-forming agent have been used to induce buoyancy in cross-linked beads.

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 [12].

The objective of this research work was to formulate ivabradine HCl floating pulsatile pellets using **s**odium alginate and Eudragit S100 by fluidized bed coating technique and optimization of pellets by response surface methodology. Initially**,** preliminary trials were done with 1:1, 1:3, and 1:5 drug:polymer (sodium alginate) ratio for obtaining lag phase in acidic medium (0.1N HCl). With 1:5 drugs:polymer ratio lag phase was obtained. Therefore**,** this ratio was selected for developing floating pellets using 2**%** w/w, 4**%** w/w, and 6% w/w of Eudragit S100 oil dispersion. With 6 % w/w of Eudragit S100 93% of pellets remained floating for more than 6 hrs. Hence**,** 1:5 drug:polymer (sodium alginate) ratio and Eudragit S100 6%w/w were selected for the factorial design. During optimization**,** the effect of two independent variables, i.e. inlet air temperature (X_1) and spray rate (X_2) on responses such as % entrapment efficiency, % friability**,** and average particle size (µm) 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. Sodium alginate was purchased from Molychem Mumbai. Nonpareil seeds were purchased from B S Pharma. All other chemicals/reagents used were of analytical grade.

Methods

Preformulation studies of drug and polymers

Drug-polymer interaction (Fourier transform-infrared [FT-IR]) study

Ivabradine HCl, Sodium alginate Eudragit S100 were subjected to drug-excipient compatibility study. The drug and polymers were mixed physically in 1:1 ratio and the mixtures were placed in sealed vials for 3 months at room temperature. FT-IR measurements of drug and drug-polymer mixtures were obtained on Shimatzu. FT-IR 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 [13].

Preparation of pellets by fluidized bed coating method

Gangurde *et al*. prepared drug layering solution by dispersing drug in hypromellose E_5 solution and this was sprayed on celpheres followed by croscarmellose sodium solution coating and finally coated with Eudragit S100 solution for obtaining pulsatile release [14].

Whereas in current research work, drug layering solution was prepared by dispersing drug in sodium alginate solution and this was sprayed on nonpariel seeds followed by calcium chloride solution coating and finally coated with Eudragit S100 oil dispersion for obtaining floating pulsatile release.

Preparation of drug pellets by solution layering technology and further coating with calcium chloride and Eudragit S100

Drug loaded pellets were prepared by spraying drug solution over nonpariel seeds by fluidized bed coating technique. Drug was homogeneously dispersed in an aqueous solution of sodium alginate. The drug dispersion was then sprayed on nonpareil seeds using fluidized bed coater, bottom spray with 1 mm nozzle at a feed rate of 4 ml/minute using the peristaltic pump. The spraying process with the drug dispersion was continued to achieve the target drug loading level. The drug loaded pellets were dried in the fluidized chamber for 10 minutes and were used for further coating with calcium chloride solution (5%w/v) which was prepared by mixing calcium chloride in water. After layering, the pellets were gently fluidized for 10 minutes.

Application of outer pH-sensitive Eudragit S100 coating layer

Eudragit S100 coating dispersion preparation requires the addition of Eudragit S100 (6%w/w) to oil (10% w/v) which was mixed properly with a stirrer. This solution was sprayed over the drug loaded calcium alginate pellets in the fluidized bed coater [14]. The composition of drug loaded pellets and coating dispersion and processing conditions of batch B was given in Table 1.

Experimental design

The design of experiments (DOE) was used to provide an efficient means to optimize the fluidized bed coating technique with the minimum number of experiment runs and to find out which process variables have the maximum influence on the prepared pellets. The number of experimental runs required for the study depends on a number of variables. DOE is an effective and efficient approach for exploring the variability in responses, and establishment of a relationship between process variables and the responses studied [15]. In the present study, 2^2 (two factors 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 pellets'. The experimental design was generated within the domain of levels using the Minitab. Different batches of ivabradine HCl pellets were prepared based on the 2^2 face-centered central composite design. In all cases, triplicate was done, and the results were averaged. The variables were inlet air temperature (°C) (X_1), spray rate (ml/min) (X_2). The design matrix including investigated variables along with their levels and responses are shown in Table 2.

Optimization of data analysis and validation of models

Analysis of variance (ANOVA) is inextricably linked to experimental design [16]. ANOVA was used to analyze the significance of the model and each response parameters and 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 [17]:

$$Y_i=b_0+b_1X_1+b_2X_2+b_1\ b_2\ X_1\ X_2.$$

Table 1: The composition of drug loaded pellets and coating dispersion and processing conditions of batch B

Serial number	Ingredients	Quantities	Processing conditions for fluidized bed coating	
1	Ivabradine HCl	6.25 g	Inlet air temperature	40°C
2	Sodium alginate	31.25 g	Outlet air temperature	40°C
3	Nonpariel seeds	180.75 g	Bed temperature	50°C
4	Calcium chloride	16.75 g	Spray rate	4 ml/minutes
5	Eudragit S100 (6%w/w)	15 g	Spray nozzle diameter	1 mm
6	Light liquid paraffin	150 ml	Spray pressure/atomizing air pressure	1.2 bar

Weight of batch 250 g. Divided into 1250 capsules. HCl: Hydrochloride

Table 2: Design matrix and measured responses

Run order	Batch number	Variables levels in coded form		Response 1% entrapment efficiency[a] (Y_1)	Response 2% friability[a] (Y_2)	Response 3 average particle size[a] (Y_3)
		X_1	X_2			
1	B1	−1	−1	84.1±0.9	0.57±0.01	1510±15
2	B2	1	−1	88.2±0.8	0.51±0.01	1276±30
3	B3	−1	1	85.66±1.6	0.63±0.02	1536±33
4	B4	1	1	92.66±1.52	0.57±0.03	1424±16

Translation of coded levels in actual units

	Low (−1)	High (+1)
Inlet air temperature (°C) (X_1)	40	50
Spray rate (ml/minute) (X_2)	2	4

[a]Mean±SD; sample size (n)=3. SD: Standard deviation

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

After fitting the response data in the run design as in Table 2, 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), and predicted multiple correlation coefficient (R^2) which authenticated the 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 the industry to optimize multiple-response problems [18]. Desirability function approaching 1 is desired to determine the optimal setting [19]. Finally, product optimization was carried out by numerical optimization technique using the desirability function approach.

Evaluation of pellets

Percentage yield

The percentage yield of different batches was determined by weighing the floating pellets that were recovered after preparation. The percentage yield of different batch B1-B4 was calculated as follows [20].

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

Drug entrapment efficiency

Accurately weighed 50 mg of drug loaded pellets were added into 50 ml of phosphate buffer, pH 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 a UV-visible spectrophotometer.

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

Floating behavior

1 g of the pellets was placed in 900 ml of 0.1N HCl. The mixture was stirred at 50 rpm in a dissolution apparatus for 7 hrs. After 7 hrs, the layer of buoyant pellets was pipetted and separated by filtration. Pellets in the sinking layer were separated by filtration. Pellets of both types were dried in a desiccator until constant weight was obtained. Both the fractions of pellets were weighed, and buoyancy was determined by the weight ratio of floating pellets to the sum of floating and sinking pellets.

% Buoyancy=$[W_f/W_f+W_s]\times100$

Where, W_f and W_s are the weights of the floating and settled pellets, respectively. All the determinations were made in triplicate.

Micromeritic properties

Particle size analysis

Sieve analysis

Separation of the pellets 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, 12, 16, 22, 44, 60, and 120 were arranged in order of decreasing aperture size. Accurately weighed the amount of drug loaded pellets (10 g) from each batch were placed on the uppermost sieve The sieves were shaken for a period of 10 minutes the amount retained on different sieves' were weighed and mean particle size of the pellets was calculated by the following equation. The procedure was carried out three times for each product [21,22].

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

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

Friability

Friability of the pellets was determined by subjecting 10 g of the pellets of the #12/16 mesh fraction with 200 glass beads to abrasion in an automated USP friabilator (Electrolab EF-2, India) for 4 minutes at 25 rotations/minutes. The abraded samples were sieved using #16 mesh for 2 minutes. The pellets retained on the sieve were weighed and % friability was calculated from the difference in the weight of pellets before and after friability.

% Friability=(Initial weight−Final weight/Initial weight)×100

Angle of repose

The angle of repose for floating pulsatile pellets of 10 g was determined by fixed funnel method. These pellets were allowed to fall freely through a funnel until the apex of conical pile just touched the tip of the funnel [20].

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 pellets

Determination of bulk density and tapped density

It is the ratio between a given mass of floating pulsatile pellets and its volume after tapping. The bulk density and tapped density of floating

pulsatile pellets were determined by the tapping method accurately weighed quantities (1 g) of prepared pellets were transferred into a 10 ml measuring cylinder. After observing the initial volume of these pellets, the tapping was continued manually on a hard surface until the constant volume was noted. The bulk density and tapped density were calculated according to the following formula.

Bulk density=mass of pellets/initial volume

Tapped density=mass of pellets/volume of pellets after tapping

Percentage compressibility index/Carr's index
The percentage compressibility index was calculated according to the following formula [20]

% Compressibility index=Tapped density−bulk density/tapped density×100

Hausner's ratio
Hausner's ratio of pellets was determined by comparing the tapped density to the bulk density using the equation.

Hausner's Ratio=Tapped density/Bulk density

***In vitro* drug release studies (lag phase and drug release studies)**
The dissolution studies of the pellets equivalent to 5 mg of ivabradine HCl were performed using USP Type II dissolution test apparatus. The 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 lag phase study was carried out in 0.1 N HCl (pH 1.2) for a time period equivalent to floating time, i.e. 5 hrs as the pulsatile lag time which has been adopted from Maryam Maghsoodi *et al*., who reported pulsatile lag time as 5-6 hrs [23]. For all the batches by analyzing the samples at 5th hr in 0.1N HCl spectrophotometrically at 286 nm against suitably constructed calibration curve. Drug release study was carried out for optimized batch of two different sizes (10/12 # mesh and 12/16 # mesh) in phosphate buffer pH 7.4 till the complete release of the drug (30 minutes) [24]. 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 against suitably constructed a calibration curve. All measurements were conducted for 3 times, 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 released versus time) and first order (log cumulative percentage of drug unreleased versus time). Regression (r^2) and K values were calculated from the linear curves obtained by regression analysis [25].

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 [26].

RESULTS AND DISCUSSION

Drug-polymer interaction study

FT-IR spectroscopy analysis

The FT-IR spectra of physical mixture were compared with the FT-IR spectrum of pure drug (Figs. 1 and 2). The FT-IR 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) FT-IR spectra of ivabradine HCl, Sodium alginate, and Eudragit S100 mixture also showed identical peaks which indicated that there was no interaction between drug and polymers.

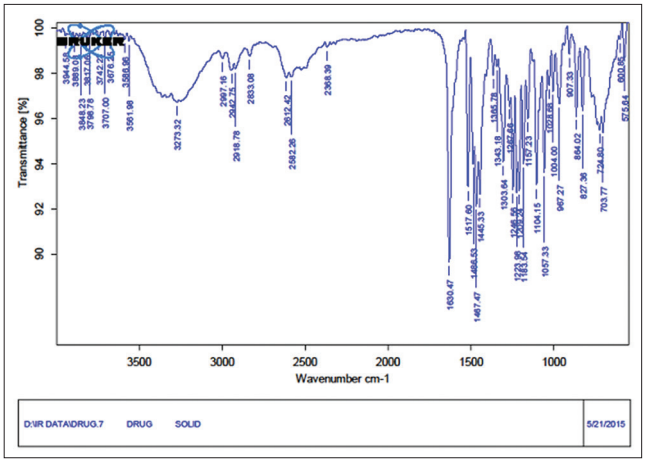

Fig. 1: Fourier transform-infrared spectrum of ivabradine hydrochloride

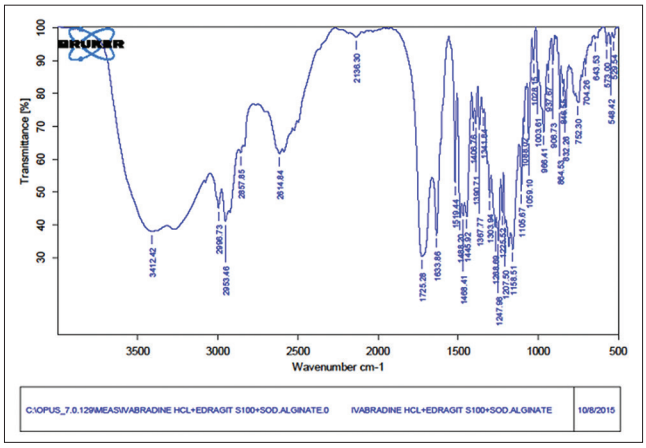

Fig. 2: Fourier transform-infrared spectrum of physical mixture of ivabradine hydrochloride, sodium alginate and Eudragit S100

Preparation of pellets

One of the important features of this process was to improve entrapment efficiency of water-soluble drug. In ionic gelation method, water-soluble drug leaches out on contact with aqueous curing solution (calcium chloride) during ionic crosslinking but in fluidized bed coating technique drug is not in contact with water even though cross-linking calcium chloride aqueous solution is coated on drug dispersed alginate layer because simultaneous drying of water occurs during coating process.

Triplicates of four batches of "ivabradine HCl floating pulsatile pellets" were prepared by fluidized bed coating technique using 2^2 face-centered central composite design, in which the important variables were inlet air temperature (X_1) and spray rate (X_2) and % entrapment efficiency (Y_1), % of friability (Y_2), and average particle size (Y_3) from "ivabradine HCl floating pulsatile pellets" were taken as response parameters.

Data analysis and model validation

Fitting of data to the model

Two factors with two levels in the coded values were shown in Table 3. All the response variables were observed experimentally for triplicates of 4 runs as proposed by the 2^2 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, and predicted R^2 were shown in Table 3 for each response along with their ANOVA results. Values of probability *p<0.05 indicate significant model terms.

After elimination of non-significant (*p>0.05) coefficients in Table 3, following correlations for response variables were obtained in terms of coded factors.

% Entrapment efficiency: $Y_1=87.6417+2.7917X_1+1.4917X_2$

% Friability: $Y_2=0.57-0.03X_1+0.03X_2$

% Average particle size: $Y_3=1436.50-86.5X_1+43.50\ X_2+30.50\ X_1\ X_2$

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

The entrapment efficiency (%) for triplicates of 4 batches was found to be in the range of 84.1±0.9 to 92.66±1.52% as evidenced from Table 3. The ANOVA result indicated that the main factors X_1 (inlet air temperature) and X_2 (spray rate) their interaction terms $X_1\ X_2$ caused variation on the encapsulation efficiency. The model shows that both X_1 (inlet air temperature), X_2 (spray rate), and their interaction term $X_1\ X_2$ had positive effects on entrapment efficiency. The % entrapment efficiency was found to be increasing with increasing (X_1) inlet air temperature (In run 3 and run 4 mean difference is 7.06; *p<0.05). The mean difference for run 2 and run 4 is 4.46 (p<0.05) which indicates that with increasing spray rate (X_2) % entrapment efficiency is increasing.

The data of % friability for all batches ranging from 0.51±0.01% to 0.63±0.02% were observed from Table 3. The result indicated that the main factors X_1 (inlet air temperature) and X_2 (spray rate) caused variation on % friability. The model shows that X_1 (inlet air temperature) had a negative effect, and X_2 (spray rate) had a positive effect on % friability. The % friability was found to be decreasing with increasing (X_1) inlet air temperature (In run 3 and run 4 mean difference is 0.06 (*p<0.05). The mean difference for run 2 and run 4 is 0.05 (*p<0.05) which indicates that with increasing spray rate (X_2) % friability is increasing.

Average particle size was found to be in the range of 1276±30 to 1536±33 as indicated in Table 3. The ANOVA result indicated that the main factors X_1 (inlet air temperature) and X_2 (spray rate) their interaction terms $X_1\ X_2$, and caused variation on the average particle size. The model shows that X1 (inlet air temperature) had a negative effect and X_2 (spray rate) had positive effects on the average particle size. The average particle size was found to be decreasing with increasing (X_1) inlet air temperature (In run 3 and run 4 mean difference is 112 (*p<0.05). The mean difference for run 2 and run 4 is 148 (*p<0.05) which indicates that with increasing spray rate (X_2) average particle size was found to be increasing. In this model, their interaction term $X_1\ X_2$ showed positive coefficients possibly because of the dominance of X_2.

Counter and three-dimensional response surface plot analysis

Design expert software generated the counter and three-dimensional response plots which visualized the effects of the process parameters on the response variables (% entrapment efficiency, % friability, and average particle size).

% entrapment efficiency (Y_1) was increased with increasing levels of inlet air temperature (X_1) and spray rate (X_2) as depicted in the 2D-Iso response curves and response plots in Fig. 3a1 and b1). % Friability (Y_2) decreased with increasing levels of inlet air temperature (X_1) and increased with increasing levels of spray rate (X_2) and as reported in Fig. 3a2 and b2. Similarly, the effect of chosen variables on average particle size was indicated in Fig. 3a3 and b3. As the levels of inlet air temperature (X_3) decreases and spray rate (X_2) level increases, the average particle size was found to be increasing.

Optimization

A numerical optimization technique using the desirability function approach was employed to generate the optimum settings for the batch. Suitable levels of constraints (Target) were chosen to achieve desired characteristics (responses) of the batch. It was found to satisfy the requisites of an optimum batch when the desirable ranges of responses were restricted to % entrapment efficiency at 95%, % friability at 0.6%, and average particle size at 1400 µm. On analyzing various response variables and comprehensive evaluation of feasibility

Table 3: ANOVA results for predicting % entrapment efficiency (Y_1, %), % of friability (Y_2, %), and average particle size (Y_3, µm)

Source	b-coefficient	Sum of squares	df	Mean square	F value	*p value, prob>F
For Y_1 (%)						
Model	87.6417	126.823	3	42.27	37.90	0.000
X_1	2.7917	93.521	1	93.521	58.97	0.000
X_2	1.4917	26.701	1	26.701	16.84	0.003
$X_1\ X_2$	0.7417	6.601	1	6.601	4.16	0.076
Residual	-	12.687	8	12.687	-	-
Pure error	-	12.687	8	12.687	-	-
Total	-	139.509	11	-	-	-
Other statistics: R^2=0.9091, adjusted R^2=0.8750, predicted R^2=0.7954						
For Y_2 (%)						
Model	0.57	0.0216	3	0.072	28.8	0.000
X_1	-0.03	0.0108	1	0.0108	28.8	0.001
X_2	0.03	0.0108	1	0.0108	28.8	0.001
$X_1\ X_2$	-	0.000	1	0.000	-	1
Residual	-	0.003	8	0.003	-	-
Pure error	-	0.003	8	0.003	-	-
Total	-	0.0246	11	-	-	-
Other statistics: R^2=0.8780, adjusted R^2=0.8323, predicted R^2=0.7256						
For Y_3 (µm)						
Model	1436.50	123657	3	41219	91.09	0.000
X_1	−86.5	89787	1	89787	145.40	0.000
X_2	43.5	22707	1	22707	36.77	0.000
$X_1\ X_2$	30.5	11163	1	11163	18.08	0.940
Residual		4940	8	4940	-	-
Pure error		4940	8	4940	-	-
Total		128597	11	128597	-	-
Other statistics: R^2=0.9616, adjusted R^2=0.9472, predicted R^2=0.9136						

*p<0.05 is considered as significant, df: Degrees of freedom, ANOVA: Analysis of variance

of exhaustive grid search, the following combination of variables was suggested by the software with desirability function of 0.81811 as reported in Table 4, inlet air temperature=50 (°C); spray rate=4 (ml/minute); the desirability function value (0.81811) is closer to 1. The variables of batch B4 coincides with software suggested variables and responses are compared. The optimized batch of pellets (B4) showed % entrapment efficiency of 92.66±1.52, % friability 0.57±0.03, and average particle size 1424±16 with no error value. It was suggested that the generated models were well suited to optimize the ivabradine HCl floating pulsatile pellets.

Evaluation of pellets

Percentage yield

The percentage yield of pellets of various batches varied from 90.08±0.17 to 90.58±0.11, which was shown in Table 5. The optimized batch exhibited 90.58±0.11% yield which was shown in Table 5.

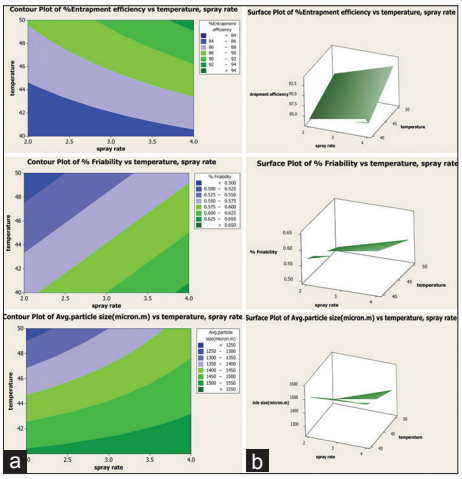

Fig. 3: (a) Contour and (b) response surface plots showing the effects of factors on % entrapment efficiency, % friability, and average particle size

Drug entrapment efficiency

The entrapment efficiency of all batches was carried out and found to be in a range of 84.1±0.9 to 92.66±1.52% as shown in Table 2. For optimized batch (B4), entrapment efficiency was observed to be 92.66±1.52% as shown in Table 4.

Floating behavior

All these batches remained floating for more than 6 hrs with the insignificant difference in % of buoyancy and in floating lag time which ranges from 93.06±0.14% to 93.58±0.11% and 239±2 to 242±1 seconds, respectively, which were indicated in Table 5. Optimized batch (B4) also remained floating for more than 6 hrs, and it showed to 93.58±0.11% of buoyancy and 240±1 seconds floating lag time as indicated in Table 5.

Micromeritics

The mean particle size of batches ranges from 1276±30 to 1536±33 µm as given in Table 2. Mean particle size for optimized batch (B4) was found to be 1424±16 µm which was reported in Table 4. % friability of all batches was found to be <1% which was reported in Table 2. The bulk density and tapped density of batch B1-B4 ranges from 0.338±0.01 to 0.365±0.02 and 0.385±0.03 to 0.412±0.02 g/cc, respectively. The bulk density and tapped density for optimized batch (B4) was found to be 0.364±0.01 and 0.404±0.03 g/cc, respectively which were reported in Table 6. The values of Carr's index, Hausner's ratio, the angle of repose which was reported in Table 6 indicated good flow properties as per USP limits. For optimized batch, free flow properties were categorized as an excellent flow which was reported in Table 6.

In vitro release studies (lag phase and drug release studies)

The lag phase study revealed that all these batches maintained lag phase for 5 hrs in 0.1N HCl as indicated in Table 5. The optimized batch of two different sizes (10/12 #, 12/16 #) also showed lag phase in 0.1N HCl for 5 hrs and with no significant difference in burst release of drug in phosphate buffer pH 7.4 as depicted in Fig. 4.

Drug release kinetics

When the release data were analyzed as per zero order and first order kinetic models, it was observed that the release from optimized batch

Table 4: The criterion for numerical optimization

Parameters	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
X_1: Inlet air temperature (°C)	Is in range	−1	1	1	1	1
X_2: Spray rate (ml/minute)	Is in range	−1	1	1	1	1
% Entrapment efficiency (Y_1)	Target=95	90	99	1	1	1
% Friability (Y_2)	Target=0.6	0.5	0.7	1	1	1
Average particle size (Y_3)	Target=1400	1300	1500	1	1	1

Solutions						Desirability
Process variables			Response variables			
Code	X_1: Inlet air temperature (°C)	X_2: Spray rate (ml/minute)	Experimental values[a]		Predicted values	% Error[b]	
B5 (optimized)	50	4	% EE	91.93±1.16	92.66	0	0.81811
			% Friability	0.57±0.03	0.57	0	
			Average particle size	1424±16	1424	0	

[a]Mean±SD; n=3. [b]Percentage of error (%)=[(actual value−predicted value)/predicted value)]×100. SD: Standard deviation

Table 5: Evaluation parameters of pellets

Batch code	% yield[a]	Floating lag time[a] (seconds)	% of Buoyancy[a]	Total floating time (hrs)	Pulsatile lag phase (hrs)
B1	90.08±0.17	239±2	93.12±0.11	>6	5
B2	90.28±0.54	241±3	93.19±0.12	>6	5
B3	90.34±0.83	242±1	93.06±0.14	>6	5
B4 (optimized)	90.58±0.11	240±1	93.58±0.11	>6	5

[a]Mean±SD; n=3. SD: Standard deviation

Table 6: Micromeritics

Batch code	Micromeritics				
	Angle of repose (°)[a]	Bulk density (g/cc)[a]	Tapped density (g/cc)[a]	Carr's index (%)[a]	Hausner's ratio[a]
B1	31.97±0.25	0.36±0.05	0.408±0.02	11.8±0.12	1.133±0.001
B2	32.43±0.11	0.338±0.01	0.385±0.03	12.2±0.11	1.139±0.002
B3	31.76±0.34	0.365±0.02	0.412±0.02	11.4±0.13	1.129±0.002
B4	29.12±0.17	0.364±0.01	0.404±0.03	9.9±0.15	1.109±0.001

[a]Mean±SD; n=3. SD: Standard deviation

Table 7: Drug release kinetics of optimized Batch B4 (10/12#, 12/16#)

Batch code	Zero order		First order	
	r^2	K mg/minute	r^2	K/minute
B5 (10/12#)	0.851	0.140	0.993	0.127
B5 (12/16#)	0.850	0.140	0.995	0.129

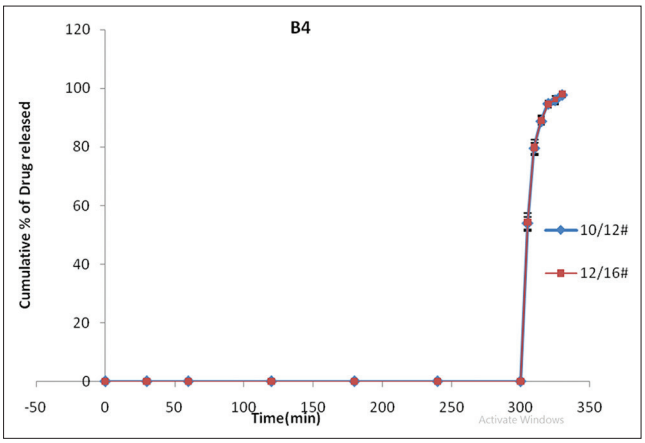

Fig. 4: *In vitro* drug release profile of different sizes of optimized batch B4 (n=3)

followed first order kinetics as the regression values (r^2) were higher in the first order model which were shown in Table 7.

CONCLUSION

Currently in the market, sustained release and immediate release dosage forms of ivabradine HCl are available, but there are no floating pulsatile dosage forms of ivabradine HCl. So, the present research carried out and this research work disclosed that ivabradine HCl floating pulsatile pellets were successfully formulated by fluidized bed coating technique. The effects of two independent variables (inlet air temperature and spray rate) on three responses were studied and optimized systematically using response surface methodology. The work revealed that independent variables had a significant effect on the measured responses. The optimized batch (B4) showed the % entrapment efficiency of 92.66±1.52; % friability of 0.57±0.03; and average particle size of 1424±16. *In vitro* drug release studies revealed that lag phase was maintained during floating in acidic medium, i.e. 0.1N HCl for 5 hrs which are a targeted time followed by burst release within 30 minutes in phosphate buffer pH 7.4 (intestinal pH). These pellets have the flexibility in filling any desired dosage amount in specific size capsule. Micromeritic study revealed that these pellets exhibited free flow properties which are essential in attaining uniformity of dosage amounts during capsule filling. FT-IR 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, the applicability of response surface methodology to optimize the variables in the preparation of ivabradine HCl floating pulsatile pellets is apt enough.

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