British Journal of Pharmaceutical Research
9(4): 1-12, 2016, Article no.BJPR.22566
ISSN: 2231-2919, NLM ID: 101631759

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Comparision of Different Techniques Involved in the Development of Ivabradine HCL Floating Pulsatile Multiparticulate Systems for Chronotherapeutic Delivery

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Authors' contributions

This work was carried out by Author VPT. Authors TEGKM and ASSR supervised the research work. Author VPT designed the study, wrote the protocol, and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/22566
Editor(s):
(1) Rafik Karaman, Bioorganic Chemistry, College of Pharmacy, Al-Quds University, USA.
Reviewers:
(1) Parineeta Samant, MGM Medical College, Navi-Mumbai, India.
(2) Sachinkumar Vasantrao Patil, Ashokrao Mane College of Pharmacy, India.
(3) Sukesh Voruganti, Texas Tech University Health Sciences Center, USA.
(4) Syed Umer Jan, University of Balochistan, Quetta, Pakistan.
Complete Peer review History: http://sciencedomain.org/review-history/12353

Original Research Article

Received 11th October 2015
Accepted 6th November 2015
Published 20th November 2015

ABSTRACT

Aims: To develop Ivabradine HCl floating pulsatile multiparticulate (beads/ pellets) dosage forms containing calcium alginate beads/ pellets coated with pH-dependent polymer Eudragit S 100, by ionic gelation, pan coating, fluidized bed coating techniques and optimization of technique by comparing evaluation parameters with statistical data.
Study Design: Ionic Gelation, Pan Coating, Fluidized Bed Coating Techniques and comparison and optimization of suitable technique.
Place and Duration of Study: Bapatla College of Pharmacy. Bapatla, Guntur (district), Andhra Pradesh, India - 522101. June 2015 to September 2015.

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Methodology: Multiparticulates were prepared by Ionic gelation, pan coating , fluidized bed coating methods employing 1:5 drug : polymer (sodium alginate5%w/v) ratio ,5%w/v cacl2 as cross-linking agent and further coated with 6% w/w Eudragit S100 dispersed in 10% w/v of oil. These multi particulates were evaluated for % drug entrapment efficiency, Micromeritics, in vitro floating behaviour, in vitro drug release at PH 1.2 and 7.4, Kinetics and statistical analysis of these parameters.

Results: Multiparticulates prepared by Ionic gelation technique exhibited very less % drug entrapment efficiency when compared to other two techniques.

In pan coating technique lumps were formed and these particles exhibited poor flow properties besides low % of buoyancy when compared to other two techniques.

Multiparticulates prepared by fluidized bed coating technique exhibited excellent flow properties, good entrapment efficiency and floating behaviour furthermore these particles also showed lag phase and pulsatile release.

Statistical analysis revealed that there was no significant difference between pan and fluidized bed coating techniques in % drug entrapment efficiency.

In case of floating lag time significant difference was not exhibited by multiparticulates prepared by ionic gelation technique and fluidized bed coating technique as (P >.05).

Conclusion: The results confirmed that Fluidized bed coating technique was optimized for preparation of floating pulsatile multiparticulates of Ivabradine HCl.

Keywords: Ivabradine HCl; sodium alginate; eudragit s100; ionic gelation; pan coating; fluidized bed coating.

1. INTRODUCTION

Among modified release oral dosage form, increasing interest has currently turned to time controlling systems for chronotherapeutic delivery. 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 [1]. Diseases like angina pectoris, hypertension and rheumatoid arthritis relies on circadian rhythm where these diseases show peak symptoms in the early hours of the day [2]. 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.

Ivabradine HCl is I_f channel antagonist [3,4] 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 hour under fasting condition and the half-life of the drug is 2 hours. 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 early morning hours in case of heart attack patients).

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 [4-6].

Sodium alginate is a naturally occurring substance from brown sea weed and algae. It is a bio adhesive, biodegradable polysaccharide which contains varying amounts of 1, 4'-linked β-D-mannuronic acid, α-L- guluronic acid residues. It forms a bio adhesive and stable gel with divalent cations such as Ba^{2+}, Sr^{2+}, and Ca^{2+} which enabled wide spread use for sustained release of drugs. They can also be function as carriers as bifidobacteria and used for pulsatile release of drugs since alginate beads are stable in acidic media and easily degraded in alkaline media [8]. 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 [9].

Alginate beads obtained by ionotropic cross-linking of these polymers have been used to develop floating drug delivery. Various approaches like use of oils, freeze drying, and entrapment of gas or gas forming agent have

been used to induce buoyancy in cross-linked beads [8].

Eudragit polymers are series of acrylate and methacrylate polymers available in different ionic forms. Eudragit S100 is a p^H dependent polymer that gets solubilized at pH 7 and above [10].

The multiparticulate dosage forms are gaining much interest over single-unit dosage forms for pulsatile delivery. The potential advantage of multiparticulate system includes no risk of dose dumping, reduced risk of local irritation, less inter and intra subject variability and increased bioavailability [11].

The aim of the present study was to develop a floating pulsatile multiparticulate (beads, pellets) dosage forms containing calcium alginate beads/ pellets coated with pH-dependent polymer Eudragit S 100, by ionic gelation, pan coating, fluidized bed coating techniques and optimization of technique by comparing evaluation parameters with statistical data.

2. MATERIALS AND METHODS

2.1 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. Non Pareil seeds were purchased from B S Pharma. All other chemicals/ reagents used were of analytical grade.

2.2 Methods

2.2.1 Preformulation studies of drug and polymers: Drug polymer interaction (FTIR) 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. FTIR measurements of drug and drug-polymer mixtures were obtained on Shimatzu. 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^{-1} at the ambient temperature [12].

2.2.2 Preparation of drug loaded beads by ionic gelation method

Azar Danish Khan and Meenakshi Bajpai [13] formulated oil entrapped alginate beads for floating sustained release but in the current research work oil dispersion containing Eudragit S100 coated on alginate beads in order to obtain floating pulsatile behaviour.

M.S. Khan, B.K. Sridhar et al. and M.S. Shetage et al. [14,15] prepared coating solution by dissolving Eudragit S 100 in acetone for colon targeting but in the present research work coating dispersion was prepared by dispersing Eudragit S100 in oil in order to obtain floating pulsatile behaviour.

Ivabradine HCl was dispersed in sodium alginate (5% w/v) solution. The drug polymer dispersion was dropped through needle 24 gauge into 5% w/v aqueous calcium chloride solution with gentle agitation at room temperature with curing time of 2 minutes. The obtained beads with were washed distilled water and dried in hot air oven at temperature of 40℃. The time of drying was optimized by weighing the beads repeatedly, until they obtained a constant weight [13,14].

Coating of beads with Eudragit S 100:

The coating of all the beads bearing Ivabradine HCl was performed by spray coating in R and D pan coater. Coating dispersion was prepared by mixing Eudragit S 100 with oil (10%w/v) for 1 hour using a stirrer. Coating was done with a pan rotating at 20 rpm with an inlet air temperature 55℃ and outlet air temperature of 35-40℃. The dispersion was sprayed at an atomizing air pressure of 1.5 bar at a spray rate of 1.5 g/ml through 1mm nozzle diameter. The coating was continued until 6%w/w of Eudragit S100 was obtained [14,15]. The formula used for preparation of Ivabradine HCl beads batch B1 was furnished in the (Table 1).

2.2.3 Preparation of pellets by pan coating method

Pallavi M. Chaudhari and Pravin D. Chaudhari [16] used synthetic material PVPK 30 as a binder and pellets were coated with Eudragit RSPO and Eudragit L 100 solution for pulsatile release.

But in the present research work Natural polymer Sodium alginate was used as a binder and pellets were coated with Eudragit S100 oil dispersion for obtaining floating pulsatile character.

Table 1. Formula for the preparation of Ivabradine HCl beads batch B1

S. No	Ingredients	Quantities
1.	Ivabradine HCl	0.25 g
2.	Sodium alginate	1.25 g
3.	Calcium chloride	12.5 g
4.	Eudragit S100(6%w/w)	0.84 g
5.	Light liquid paraffin	8.4 ml

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

Solution of drug was prepared by dispersing drug in sodium alginate solution. This solution was sprayed onto the rotating non pareil seeds (16 # 20 i.e. 1180- 850 µm) in coating pan. Sodium alginate was used as binder. The spray rate of solution was maintained constant 1.5 g/min. The inlet air temperature was maintained at 40-50℃ and the drying time after each application was 2 min. The speed of coating pan was 20 rpm. After layering, the pellets were collected. The drug loaded pellets were dried at 45℃ for 8 hours in tray dryer to remove the moisture content. Finally these pellets were passed through sifters to remove fines. The drug loaded pellets were coated with calcium chloride solution (5%w/v) and these were allowed to dry and then over that coat, Eudragit S100 oil dispersion (10%w/v) was sprayed and the coating was continued by maintaining the same processing conditions as applied previously until 6%w/w of Eudragit S100 was obtained [16]. The composition of drug loaded beads and coating dispersion and processing conditions of batch B2 was given in (Table 2).

2.2.4 Preparation of pellets by fluidized bed coating method

H.H Gangurde et al. [17] 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 S 100 solution for obtaining pulsatile release.

Whereas in current research work drug layering solution was prepared by dispersing drug in sodium alginate solution and this was sprayed on non pariel seeds followed by Calcium chloride solution coating and finally coated with Eudragit S 100 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 non pariel seeds by fluidized bed coating technique. Drug was homogeneously dispersed in an aqueous solution of sodium alginate. The drug dispersion was then sprayed on non pareil seeds using fluidized bed coater, bottom spray with 0.5 mm nozzle at a feed rate of 0.5-2 g/min using peristaltic pump. The spraying process with the drug dispersion was continued to achieve the target drug loading level. The drug loaded pellets were finally dried at 45℃ for 15 min 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 min and then kept in hot air oven for drying purpose for 30 min at 40℃.

Application of outer p^H sensitive Eudragit S100 coating layer.

Table 2. The composition of drug loaded pellets and coating dispersion and processing conditions of batch B2

S. no.	Ingredients	Quantities	Processing condition for pan coating	
1.	Ivabradine HCl	6.25 g	Inlet air temp.	40-50℃
2.	Sodium alginate	31.25 g	Outlet air temp.	30-40℃
3.	Non pariel seeds	180.75 g	Bed temp.	40℃
4.	Calcium chloride	16.75 g	Spray rate	1.5 g/min
5.	Eudragit S100(6%w/w)	15 g	Spray nozzle diameter	1 mm
6.	Light liquid paraffin	150 ml	Spray pressure	1 Kg/cm^2
			Pan speed	20 rpm

Eudragit S100 coating solution preparation requires addition of Eudragit S100 to oil which was mixed properly with stirrer. This solution was sprayed over the drug loaded calcium alginate pellets in the fluidized bed coater until 6% w/w of Eudragit S100 was attained [17]. The composition of drug loaded beads and coating dispersion and processing conditions of batch B3 was given in (Table 3).

2.2.5 Evaluation of beads/pellets

2.2.5.1 Percentage yield

The percentage yield of beads/pellets was determined by weighing the beads/pellets after drying. The percentage yield was calculated as follows [18].

% Yield = (Practical yield of beads/pellets/Theoretical yield of beads/pellets) x 100

2.2.5.2 Drug entrapment efficiency

Accurately weighed 50 mg of drug loaded beads/pellets were added into 50 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 spectro-photometrically at 286 nm in UV- Visible spectrophotometer [18].

% Drug entrapment efficiency = Actual drug content/theoretical drug content x100

2.2.5.3 Floating behavior

Fifty milligrams of the beads/pellets were placed in 900 ml of 0.1 N hydrochloric acid. The mixture was stirred at 50 rpm in a dissolution apparatus for 7 h. After 7 h, the layer of buoyant beads/pellets 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 beads/pellets were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles [12].

% Buoyancy = [Wf / Wf + Ws)] x 100

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

2.3 Micromeritic Properties

2.3.1 Particle size analysis

2.3.1.1 Sieve analysis

Separation of the beads/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, 16, 24, 44, 60, 80, 120 were arranged in order of decreasing aperture size. Accurately weighed 1 gm. of drug loaded beads/pellets 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 beads/pellets was calculated by the following equation. The procedure was carried out three times for each product [19,20].

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

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

2.3.1.2 Angle of repose

The angle of repose for floating pulsatile beads/pellets was determined by fixed funnel method. These beads/pellets were allowed to fall freely through a funnel until apex of conical pile just touched the tip of the funnel [18].

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 beads/pellets

2.3.2 Determination of bulk density and tapped density

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

floating pulsatile beads/pellets were determined by the tapping method Accurately weighed quantity of prepared beads/pellets were transferred into a 10 ml measuring cylinder. After observing the initial volume of these beads/pellets, 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 [18].

Bulk density = mass of beads/pellets/initial volume

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

2.3.3 Percentage compressibility index /Carr's index

The percentage compressibility index was calculated according to the following formula [18].

% Compressibility Index = Tapped density – Bulk density/ Tapped density x100

2.3.4 Hausner's ratio

Hausner's ratio of beads/pellets was determined by comparing the tapped density to the bulk density using the equation [18].

Hausner's Ratio = Tapped density/ Bulk density

2.4 *In vitro* Drug Release Studies

The dissolution studies of the beads/pellets 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℃±0.5℃.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 hours as pulsatile lag time which has been adopted from Maryam maghsoodi et al. [21] who reported pulsatile lag time as 5- 6 hours. [21] followed by dissolution in phosphate buffer, p^H 7.4 till complete release of drug (30 minutes). 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 [6,22], against suitably constructed calibration curve. All measurements were conducted in triplicate, and average values were plotted.

2.4.1 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 [23].

2.5 Statistical Analysis

Results were analyzed and expressed as mean ± SD. Multipaticulates obtained by different techniques were statistically analyzed for different parameters like % Drug entrapment efficiency, % of buoyancy, particle size ,floating lag time, angle of repose and first order release rate constant by un-paired t- test using Graph Pad Prism Software-4.03 The differences were considered significant at the level of $P<.05$ [24].

3. RESULTS AND DISCUSSION

3.1 Drug Polymer Interaction (FTIR) Study

The FT-IR spectra of physical mixture, were compared with the FT-IR spectrum of pure drug (Figs. 1-2). 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.

3.2 Percentage Yield

The percentage yield for batch B1- B3 was calculated and found to be in the range of 86.24±0.38 to 90.72±0.43 as shown in (Table 4).

3.3 Percentage of Drug Entrapment Efficiency

The entrapment efficiency for batch B1- B3 was carried out and found to be in a range 10.4±1.32 to 91.3±1.08 as shown in (Table 4). Entrapment efficiency of beads prepared through ionic

gelation method found to be very less, as the drug is water soluble upon contact with aqueous curing solution (calcium chloride solution) during ionic cross-linking drug might be leached into that solution, [25] whereas in case of pan and fluidized bed coating techniques 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.

3.4 Floating Behavior

The floating test was done to investigate the floating ability of the prepared beads/pellets. All these batches remained floating for more than 6 hours but vary in the % of buoyancy and floating

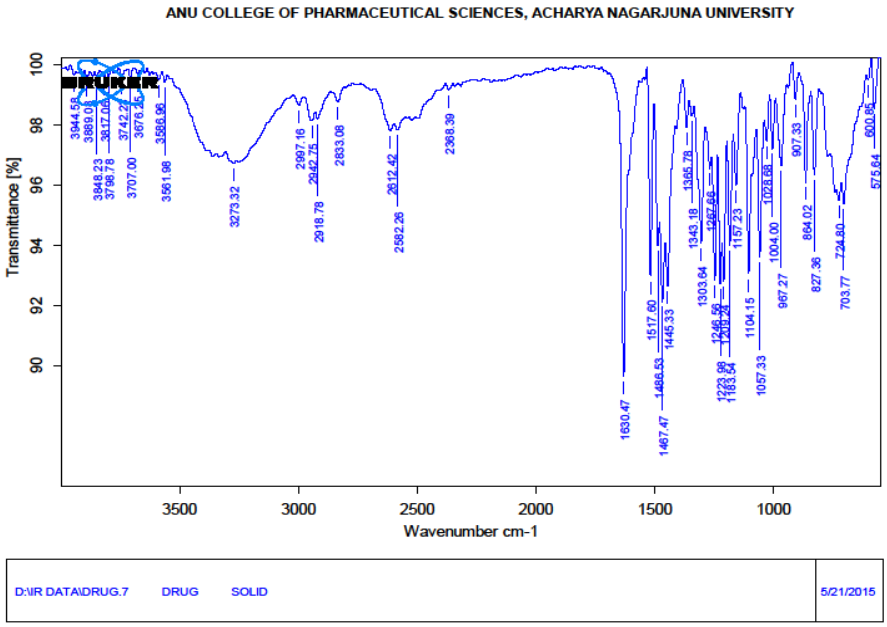

Fig. 1. FTIR spectrum of Ivabradine HCl

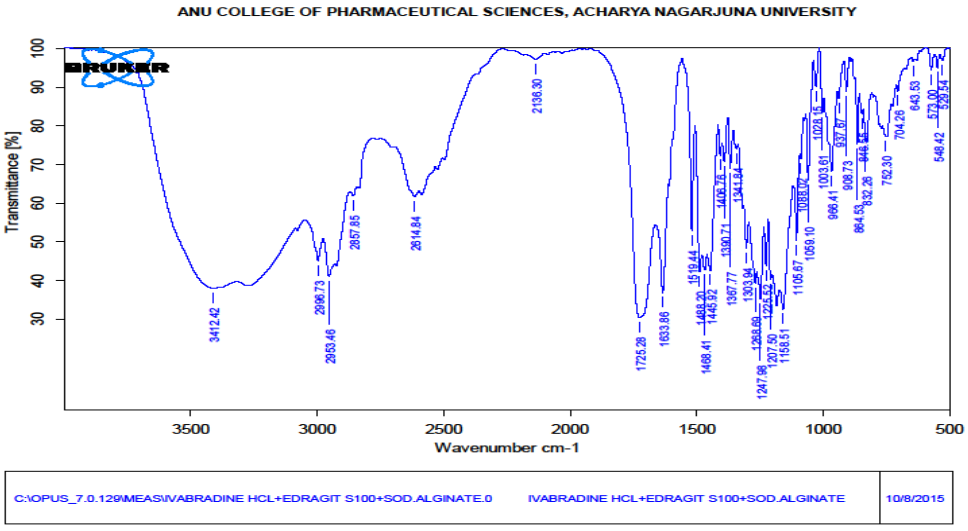

Fig. 2. FTIR spectrum of physical mixture of Ivabradine HCl, Sodium Alginate and Eudragit S100

lag time. Patel Naveen et al. [26] reported that floating lag time should be less than 10 minutes. Nitha S Hammed et al. [27] stated that particles float if buoyant force is greater than gravitational force based on density of particles i.e lighter particles float. Maryam Maghsoodi et al. [21] performed floating study for 8 hours, but in current research work floating study was carried out for 7 hours as targeted lag phase (period of no drug release) during floating is 5 hours. Floating property was attributed due to the presence of oil. Floating lag time was found to be higher in case of pellets prepared by pan coating method compared to fluidized bed coating and ionic gelation methods. % of buoyancy was less for pellets obtained by pan coating technique compared to other two techniques because lumps were formed in pan coating technique and hence mass and density was higher for these pellets which might increase gravitational pull on these particles compared to other particles and hence they sink. These values were given in (Table 4).

3.5 Micromeritic Properties

The mean particle size of floating pulsatile beads/ pellets of batch B1- B3 was found to be in the range of 1470±23 to 2530±27 as shown in (Table 5). Particle size was more in case of pan coating as lumps were formed during the process. The Angle of repose for beads prepared through ionic gelation method found to be 31.72±0.13, Compressibility index and Hausner's ratio was found to be 14.32±0.25, 1.167±0.002 respectively, which indicates good flow properties.

The Angle of repose for beads prepared through pan coating method found to be 50.28±0.21, Compressibility index and Hausner's ratio was found to be 29.78±0.11, 1.424±0.008 respectively, which indicates poor flow properties as lumps are formed during this technique. The Angle of repose for beads prepared through fluidized bed coating method found to be 24.92±0.03, Compressibility index and Hausner's

Table 3. The composition of drug loaded pellets and coating dispersion and processing conditions of batch B3

S. No	Ingredients	Quantities	Processing conditions for fluidized bed coating	
1.	Ivabradine HCl	6.25 g	Inlet air temp.	45-55℃
2.	Sodium alginate	31.25 g	Outlet air temp.	30-40℃
3.	Non pariel seeds	180.75 g	Bed temp.	40-50℃
4.	Calcium chloride	16.75 g	Spray rate	0.5-2 g/min
5.	Eudragit S100(6%w/w)	15 g	Spray nozzle diameter	0.5 mm
6.	Light liquid paraffin	150 ml	Spray pressure	1.5-2 Kg/cm^2
			Peristaltic pump rpm	1-3 rpm
			Air flow	1.2-1.5 bar

Table 4. Evaluation parameters of multiparticulates B1-B3

Batch code	% yield	% entrapment efficiency	% of buoyancy	Floating lag time (min)	Total floating time (hrs.)	Lag phase (hrs.)
B1	86.24±0.38	10.4±1.32	94.12±0.23	2	>6	--------
B2	88.59±0.54	90.7±1.14	82.5±0.81	7	>6	5
B3	90.72±0.43	91.3±0.43	93.58±0.11	4	>6	5

Values expressed as mean ± S.D, n=3

Table 5. Micromeritic properties of multiparticulates B1-B3

Batch code	Mean particle size(µm)	Angle of repose(^{o})	Bulk density(g/ml)	Tapped density(g/ml)	Carr's index (%)	Hausner's ratio
B1	1470±23	31.72±0.13	0.341±0.012	0.398±0.017	14.32±0.25	1.167±0.02
B2	2530±27	50.28±0.21	0.29±0.023	0.413±0.037	29.78±0.11	1.424±0.008
B3	1578±19	24.92±0.03	0.363±0.044	0.403±0.038	9.93±0.07	1.110±0.002

Values expressed as mean ± S.D, n=3

ratio was found to be 9.93±0.07, 1.11±0.002 respectively, which indicates excellent flow properties as per the reference standard limits of USP [28] and the values were depicted in (Table 5).

3.6 *In Vitro* Drug Release Studies

To simulate the pH variation of GI tract dissolution studies were performed first at pH 1.2 for time equivalent to floating time (5-hours) and then subsequently medium was replaced with fresh pH 7.4 phosphate buffer having maintained temperature of 37±0.20C. *In vitro* dissolution study was not performed for batch B1 because it showed very less entrapment efficiency. Pellets of batch B2 (pan coating) and B3 exhibited lag phase for 5 hours during floating in acidic medium i.e. 0.1N HCl and showed burst release within 30 minutes in Phosphate buffer pH 7.4 which was shown in (Fig. 3). Sanjay J Kshirsagar et al [29] reported that after lag phase during floating for 6 hours drug was released completely in pulsatile manner with in 30 – 45 minutes in phosphate buffer p^H 7.4. These pellets showed excellent lag in acidic p^H that may be due to insolubility of Eudragit S100 and alginate. Sanjay J Kshirsagar et al. [29] reported that at acidic p^H calcium alginate may get protonated into insoluble form having reduced swelling and moreover Eudragit S100 is p^H dependent polymer that gets soluble at p^H >7 [10]. The second phase of burst release in phosphate buffer pH 7.4, can be attributed due to solubility nature of Eudragit S100 [10] as well as rapid swelling and gel relaxation of calcium alginate gel at alkaline p^H [29].

3.7 Drug Release Kinetics

When the release data were analysed as per zero order and first order kinetic models, it was observed that the release from batch B2 and B3 followed first order kinetics as the regression values (r^2) were higher in the first order model which were shown in (Table 6) and (Fig. 4).

3.8 Statistical Analysis

Statistical results of unpaired t- test for evaluated parameters were given in (Table 7). Based on this analysis results there was no significant difference between pan coating and fluidized bed coating in % drug entrapment efficiency. In case of floating lag time parameter significant difference was not exhibited by multiparticulates obtained by ionic gelation technique and fluidized bed coating technique as *P* values are greater than .05.

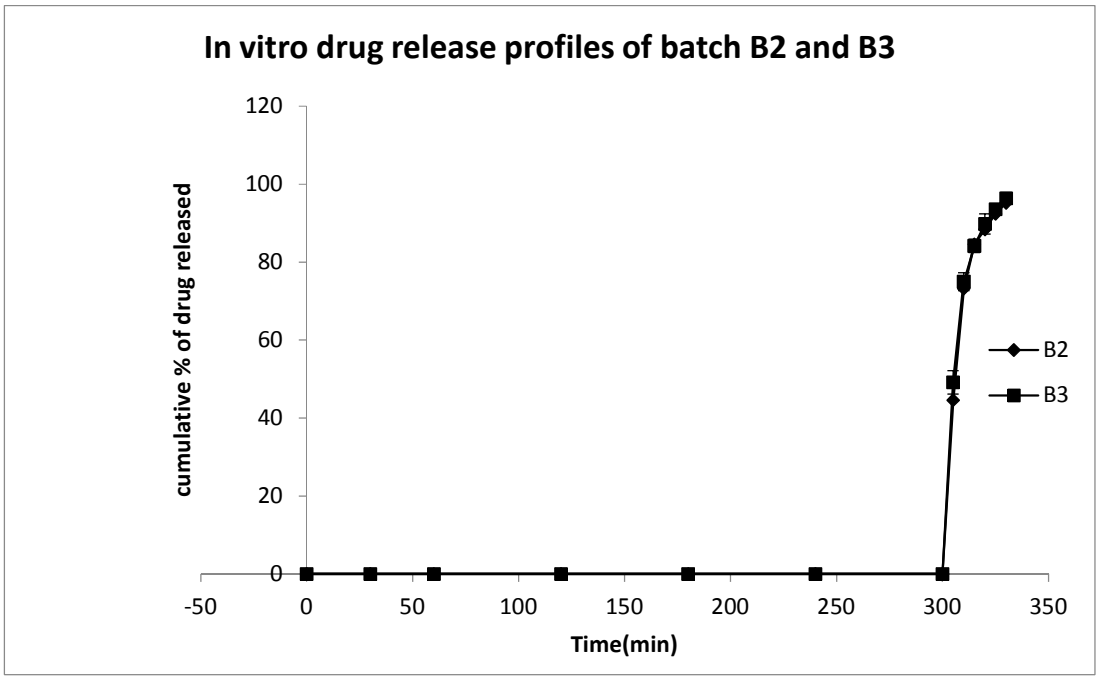

Fig. 3. *In vitro* drug release profile of floating pulsatile pellets, of Ivabradine HCl batch B2 and B3

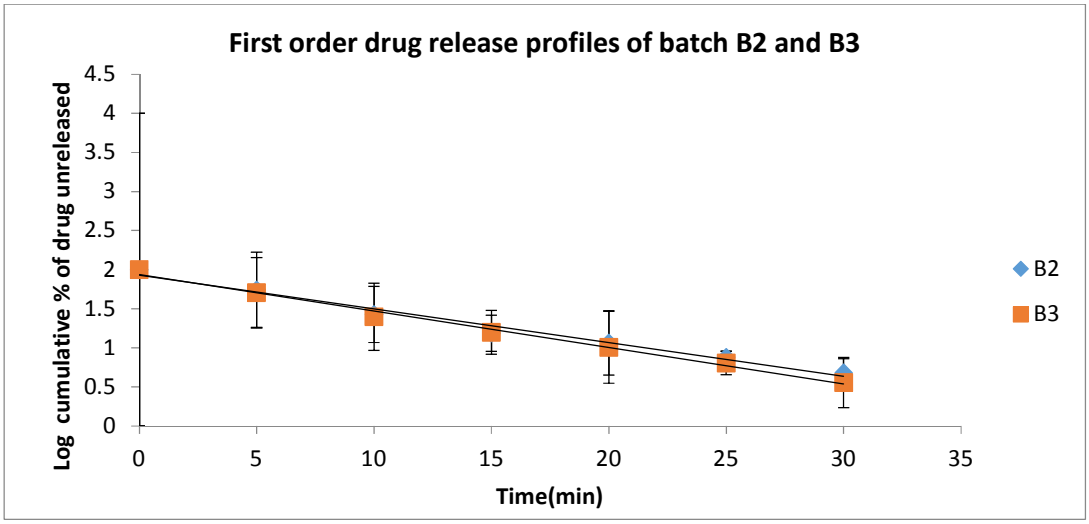

Fig. 4. First order drug release profile of floating pulsatile pellets, of Ivabradine HCl batch B2 and B3

Table 6. Drug release kinetics of B2 and B3

Batch code	Zero order		First order	
	R^2	K mg/min	R^2	K min^{-1}
B2	0.881	0.142±0.002	0.991	0.0995±0.0001
B3	0.860	0.139±0.001	0.995	0.1072±0.0001

Values expressed as mean ± S.D, n=3

Table 7. Results of unpaired t –test

Parameters	Batch code	*P* value	Degrees of freedom	t value	Significant difference
% of drug entrapment efficiency	B1:B2	*P*<0.0001	4	86.96	YES
	B2:B3	0.5507	4	0.6508	NO
	B1:B3	*P*<0.0001	4	89.96	YES
% of buoyancy	B1:B2	*P*<0.0001	4	24.18	YES
	B2:B3	*P*<0.0001	4	23.77	YES
	B1:B3	0.0214	4	3.669	YES
Particle size	B1:B2	*P*<0.0001	4	51.76	YES
	B2:B3	*P*<0.0001	4	49.94	YES
	B1:B3	0.0033	4	6.270	YES
Floating lag time	B1:B2	0.0036	4	6.124	YES
	B2:B3	0.0213	4	3.674	YES
	B1:B3	0.0705	4	2.449	NO
Angle of repose	B1:B2	*P*<0.0001	4	130.2	YES
	B2:B3	*P<0.0001*	4	207.1	YES
	B1:B3	*P*<0.0001	4	88.28	YES
First order release constant (k)	B2:B3	*P*<0.0001	4	93.08	YES

4. CONCLUSION

Ivabradine HCl floating pulsatile multiparticulate (beads, pellets) dosage forms containing calcium alginate beads/ pellets coated with pH-dependent polymer Eudragit S 100 were prepared by ionic gelation, pan coating, fluidized bed coating techniques.

Beads prepared by Ionic gelation technique, the % drug entrapment efficiency was very less when compared to other two methods, which is

an essential parameter for multiparticulate systems.

Pellets prepared by Pan Coating technique showed lag phase and pulsatile release but lumps were formed in this technique and it exhibited poor flow properties furthermore % of buoyancy was less compared to other two methods.

Pellets prepared by fluidized bed coating technique exhibited excellent flow properties, good % drug entrapment efficiency and floating behavior and also exhibited lag phase and pulsatile release.

Hence efficient floating pulsatile multiparticulate dosage form of Ivabradine HCl by using sodium alginate and Eudragit S 100 for chronotherapy of angina pectoris was optimized with fluidized bed coating technique which has proven statistically when compared with Ionic gelation technique and Pan Coating.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

Authors are thankful to Cipla Pharmaceutical Company, Mumbai for providing gift sample of Ivabradine hydrochloride, Evonik Degussa India Private Limited, Mumbai for providing gift sample of Eudragit S100 and to Bapatla College of pharmacy, Bapatla for carrying out this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shivakumar HN, Sarasija Suresh, Desai BG. Design and evaluation of pH sensitive multi- particulate systems for chronotherapeutic delivery of Diltiazem Hydrochloride. Indian Journal of Pharmaceutical Sciences. 2006;68(6):781-787.
2. Pallab R, Aliasagar S. Statistical optimization of ranitidine hydrochloride floating pulsatile delivery system for chronotherapy of nocturnal acid break through. Eur J Pharml Sci. 2009;37:363-369.
3. Chatterjee S. A short review of the i_f channel antagonist, Ivabradine. The Internet J Cardiology. 2008;6(2).
4. Richard Conti C. Ion channel therapy of ischemic heart disease: From calcium channel blockers to late sodium current inhibition. Clin. Cardiol. 2011;34:66-67.
5. Sharma Pawar. Low density multi particulate system for pulsatile release of meloxicam. Int J Pharm. 2006;313:150–158.
6. Jessy Shaji, Amol Shinde. Design and in vitro characterization of floating pulsatile microspheres of aceclofenac for rheumatoid arthritis. Int J Pharmacy and Pharm Sci. 2012;4:374-379.
7. Shivahare UD, Rathod HD, Mathur VB. Development and evaluation of floating pulsatile microspheres of metoprolol tartrate using emulsification – Solvent evaporation technique. Sch. Acad. J. Pharm. 2013;2:365-372.
8. Ramya Krishna T, Suresh B, Prabhakar Reddy V, Pavani V1, Nalini Krishna Reddy. Development of multiparticulate-floating system for pulsatile release of montelukast sodium. International Journal of Pharmacy and Biological Sciences. 2012;2(2):328-338.
9. Abaneshkumar Bansal, Vishal Pande. Development and evaluation of dual cross-linked pulsatile beads of chronotherapy of rheumatoid arthritis. J of Pharm. 2013;1-8.
10. Raymond C Rowe, Paul J Sheskey, Marian E Quinn. Hand book of pharmaceutical excipients. 6th ed. 2009; 527.
11. Bhupendra Singh, Suresh Purohit, Geetanjali Saini, Anil Bhandari, Deepak Awasthi. Colon specific chronotherapeutic drug delivery for nocturnal asthma: Eudragit S-100 coated calcium alginate gel beads-entrapped Salbutamol sulphate. International Journal of Pharmacy and Pharmaceutical Sciences. 2013;5(2):362-367.
12. Aphale Sanjivani, Shinde Swapnila, Dhat Shalaka, Bagul Uddhav, Saluja Jagdish. Development and evaluation of hollow microspheres of clarithromycin as a gastroretentive drug delivery system using eudragit polymers. International Journal of

Pharma and Bio Sciences. 2011;2(3):344-358.
13. Azhar Danish Khan, Meenakshi Bajpai. Formulation and evaluation of floating beads of verapamil hydrochloride. International Journal of PharmTech Research. 2011;3(3):1537-1546.
14. Khan MS, Sridhar BK, Srinatha A. Development and evaluation of pH-dependent micro beads for colon targeting. Indian Journal of Pharmaceutical Science. 2010;72(1):18–23.
15. Shetage MS, Sayyad FJ, Patil VG. Optimization of polymer coating level for colon targeted sustain release metoprolol succinate pellets using 32 factorial design. International Journal of Pharmaceutical Sciences and Research. 2015;6(4):1680-169.
16. Pallavi M Chaudhari, Pravin D Chaudhari. Formulation and evaluation of multiparticulate system for chron-otherapeutic delivery of salbutamol sulphate. Journal of Pharmacy & Bioallied Sciences. 2012;4(1):S71–S73.
17. Gangurde HH, Chordiya MA, Tamizharasi S, Sivakumar T. Statistical optimization of mesalamine coated pellets for possible ileo–ceacal targeting. Mahidol University Journal of Pharmaceutical Sciences. 2013; 40(2):25-44.
18. Trivedi P, Verma A, Garud N. Preparation and characterization of aceclofenac microspheres. Asian Journal of Pharmaceutics. 2008;2:110-115.
19. Meral Yuce, Kandemir Canefe. Indomethacin-loaded microspheres: preparation, characterization and in-vitro evaluation regarding ethylcellulose matrix material. Turk J. Pharm. Sci. 2008;5(3): 129-142.
20. Cvs. Subrahmanyam. Laboratory manual of physical pharmaceutics; 102.
21. Maryam Maghsoodi, Elham Hemati, Bahram Qadermazi, Zahra Yari. Hollow microspheres for gastroretentive floating-pulsatile drug delivery: Preparation and in vitro evaluation. Advanced Pharmaceutical Bulletin. 2011;1(2):55-61.
22. Prashant Singh, Senthil Kumar1 SK, Parthiban1 S, Peeyush, Tamizh Mani T. Formulation and evaluation of ivabradine hydrochloride loaded egg albumin microspheres. Journal of Pharmaceutical Research and Opinion. 2012;2:25-28.
23. Prashant Malik, Upendra Nagaich, Raj Kaur Malik, Naha Gulati. Pentoxifylline loaded floating microballons: Design, development and characterization. Journal of Pharmaceutics. 2013;1-5.
24. Shrishailgouda S Patil, et al. Formulation and characterization of TPP cross-linked chitosan microspheres loaded with lornoxicam. Journal of Biomedical and Pharmaceutical Research. 2014;3:51-58.
25. Saravana Kumar K, Jayachandra Reddy P, Chandra Sekhar KB. Formulation of neostigmine bromide-loaded muco-adhesive microspheres by emulsification-internal gelation technique and evaluation of their gastro-retentive capabilities. J. Pharm. Sci. & Res. 2011;3(11):1544-1551.
26. Patel Naveen, Patil Uma, Lanka Aparna, Patil Swaraj, Patel Krunal. Formulation and evaluation of floating tablet of metoprolol Tartrate. International Journal of Research in Pharmacy and Science. 2011;1(2):111-117.
27. Nitha S Hameed, Kuppuswami S, Arya Soman, Limson K Mathew, Saneesh V. Review article on floating drug delivery system. International Journal of Universal Pharmacy and Bio Sciences. 2014;3(3): 592-613.
28. USP 29 – NF 24. 3017.
29. Sanjay J Kshirsagar, Shrikant V Patil, Mangesh R Bhalekar. Statistical optimization of floating pulsatile drug delivery system for chronotherapy of hypertension. Int J Pharm Investig. 2011; 1(4):201-213.

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The peer review history for this paper can be accessed here:
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