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Original Article

DESIGN AND DEVELOPMENT OF PULSIN-CAP CHRONOMODULATED DRUG DELIVERY OF SELECTIVE ANTI-ANGINAL DRUG: AN *IN VITRO* AND *IN VIVO* EVALUATION

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ABSTRACT

Objective: In the current work, an attempt was made to formulate the chrono pharmaceutical drug delivery of Ivabradine HCl to the colon. A time-delayed capsule was prepared by sealing the micro particles inside a gelatin capsule made up of erodible hydrogel plug.

Methods: The microparticles were formulated by counter-ion elicited aggregation methodology. A natural polymer such as chitosan was chosen as polycation and smaller molecular electrolytes like sodium citrate, sodium sulphate and sodium tripolyphosphate were chosen as poly-anions. The formulated aggregate microparticles were tested for surface morphology, size distribution, *in vitro* un-harness and drug excipient interaction. Optimized microparticles formulations were carefully chosen on the basis on dissolution studies. The whole device was enteric coated and hydrogel plug was placed in the capsule opening.

Results: The pulsatile capsule was found to be acceptable to delay the drug release in small intestinal fluid and eject out the plugin colonic fluid, thus releasing the microparticles into colonic fluid after a lag time criterion of 5 h. To mimic the pH changes along the GI tract, three dissolution media with pH 1.2, 6.8 and 7.4 were sequentially used. FT-IR study established that there was no interaction between the drug and polymer. Among all the formulations, Ivabradine HCl prepared with sodium tripolyphosphate showed prolonged release for a period of 12 h.

Conclusion: The obtained results revealed the system's capability to defer the drug release for a programmable period and prevent a sharp increase in blood pressure during the early morning hours when the risk of heart attack is the greatest.

Keywords: Counter-ion elicited aggregation, Ivabradine HCl, Microparticles, Pulsatile

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INTRODUCTION

Antianginal drugs have always been at the forefront of research because cardiovascular disorders are increasing globally. Now a day's, millions of people are suffering from cardiac diseases, and these drugs require chronic administration to get symptomatic relief. Among modified-release oral dosage form, increasing interest has currently turned to time-controlling systems for Chrono therapeutic delivery. Chrono therapeutics 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 and hypertension 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. In cardiovascular disease, capillary residence and vascular reactivity are higher in the morning and decreases latter in the day. Platelet agreeability is increased and fibrinolytic activity is decreases in the morning, leading to a state of relative hypercoagulability of the blood. Because of this, the frequencies of myocardial infarction and sudden cardiac death are higher from morning to noon [3].

A PDDS is characterized as the quick and transient release of a specific amount of drug molecule within a short period immediately after a pre-determined off-release period, that is, lag time. There are numerous circumstances where the medication should have been delivered after a lag time at the particular site [4].

Ivabradine hydrochloride (IBH) is a selective pacemaker current (I_f) ion channel inhibitor used in the treatment of angina pectoris, which is an underlying cause of heart attack when beta blockers are not responding [5]. However, the drug has low bioavailability (≈40%) due to extensive first-pass metabolism. Due to its low biological half-life of about 2 h, repetitive daily administration is needed to sustain an effective plasma level. Formulation of IBH-loaded microparticles is an attempt to avoid the frequent administration and drug release in more meticulous fashion in the colon region. Thereby increasing the bioavailability of the drug and prevent a sharp increase in blood pressure during the early morning hours, a time when the risk of heart attack is the greatest [6, 7].

In this research work, an attempt was made to formulate and evaluate the pulsatile drug delivery system of IBH to provide chronomodulated therapy for the better treatment of angina pectoris.

The pulsatile drug delivery of IBH can be taken before bedtime (10 pm) and can release drug at 3.00 am by proportioning drug concentration free cholesterol levels are more prevalent in the early morning hours [8]. The drug is taken at bedtime with a programmed start of drug release early in the morning hours. The intention of delaying the drug absorption for a lag time of 5 h is controlled by hydrogel plug.

MATERIALS AND METHODS

Ivabradine HCl was a gratis sample obtained from Cipla Ltd., India. Chitosan, Sodium sulphate, Sodium citrate, Sodium tripolyphosphate were purchased from Loba Chemicals, India. All other chemicals were of analytical grade. Prior approval by the Institutional animal's ethics committee was obtained for conducting the experiments (Ref: IAEC/IX/10/ACOP/CPCSEA, Dated 21-12-2018).

Preparation of cross-linked gelatin capsules

Hard gelatin capsules of approx. 100 number size "0" were taken. Capsule bodies and caps were separated. To generate formalin vapours, formaldehyde 25 ml of 15% (v/v) was taken into desiccators and a pinch of potassium permanganate was added to it. The wire mesh containing the empty bodies of the capsule was exposed to formaldehyde vapours. The caps were not exposed to the vapours leaving them water-soluble. The desiccators were tightly closed. The reaction was carried out for 12 h. To ensure completion

of reaction between gelatin and formaldehyde vapours, the bodies were removed and dried at 50 °C for 30 min. To facilitate removal of residual formaldehyde, the bodies were then dried at room temperature [9]. These capsule bodies were sealed with untreated caps and stored in a polythene bag.

Preparation of hydrogel plug

Plug for sealing the capsule body was prepared by compressing equal amount of HPMC K100: Lactose, Carbopol: Lactose, Na-CMC: Lactose, and Methyl Cellulose: Lactose using 7 mm punches and dies on rotary tablet press keeping varying thickness and hardness values of tablet plug [10].

Preparation of microparticles

All the microparticles formulations were fabricated by counter ion-induced aggregation method [11] and the composition was shown in table 1.1. A precisely weighed amount of chitosan was dissolved in 2% acetic acid solution; the drug was added and dissolved in it and stirred continuously until a homogeneous mixture was formed.

Formulation of 20% salt solution: Precisely weighed amount of 20g of salt (Sodium sulphate/Sodium citrate/Sodium tripolyphosphate) was dissolved in 100 ml of water by continuous stirring to get a clear solution. The prepared drug polymer mixture was added into the salt solutions with the help of a needle (24 Gauge/0.55 mm diameter) to form microparticles. The excess salt solution was removed and placed in the hot air oven at 35-40 °C for 72 h.

Designing of pulsin-cap

The Pulsin cap was formulated by filling microparticles of IBH 50 mg into formaldehyde-treated bodies by hand filling method. The capsules comprising the microparticles were then sealed with an optimized hydrogel plug. The joint of the capsule body and cap was sealed with 5% ethyl cellulose ethanolic solution [12]. The sealed capsules were completely coated by dip coating method using 5% cellulose acetate phthalate in 5:5 (v/v) mixture of acetone: ethanol plasticized with n-dibutylphthalate (0.75%), to prevent variable gastric emptying. The coating was repeated until 8 to 12% increase in weight was obtained. The percent weight gain of the capsules before and after coating was determined [13].

Table 1.1: Formulation composition of Ivabradine HCl microparticles

Cross-linking agents					
Sodium TPP		**Sodium sulphate**		**Sodium citrate**	
Composition code	**Polymer: cross-linking agent**	**Composition code**	**Polymer: cross-linking agent**	**Formulation code**	**Polymer: cross-linking agent**
F-1	1.0:0.5	F-4	1.0:0.5	F-7	1.0:0.5
F-2	1.0:0.75	F-5	1.0:0.75	F-8	1.0:0.75
F-3	1.0:1.0	F-6	1.0:1.0	F-9	1.0:1.0

Physicochemical characterization of hydrogel plug

Prepared hydrogel Plugs were evaluated for uniformity of weight, hardness, thickness and lag time [14].

Drug content uniformity

The encapsulated microparticles equivalent to 50 mg of IBH are grounded in a motor with the help of a pestle. The grounded power mixture was dissolved in 6.8 pH buffer, filtered and estimated spectrophotometrically at 286 nm [15].

***In vitro* release profile of pulsatile capsule**

Using a USP 23 dissolution testing apparatus (Apparatus 2, 100 rpm, 37 °C), drug release studies of pulsing caps were performed for 2 h in 0.1 M HCl (900 ml) because the average gastric emptying time is about 2 h. The dissolution medium was then replaced with pH 7.4 phosphate buffer (900 ml) for 3 h as this is the average small intestinal transit time. After 5 h, the dissolution medium was switched to pH 6.8 phosphate buffer (900 ml) and tested for the subsequent hours. Nine hundred millilitres of the dissolution medium were used each time. Rotation speed was maintained at 100 rpm and temperature at 37±0.5 °C. Dissolution media (5 ml) was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analysed by using UV spectroscopy at 286 nm and the cumulative percentage release was calculated over the sampling times [16].

FT-IR analysis

The FT-IR analysis for pure drugs, excipients and formulations were recorded on BRUCKER FT-Infrared Spectrophotometer using KBr pellet technique [17]. Spectrum was integrated in transmittance mode at the wave number range 380 to 4368 cm^{-1}.

Scanning electron microscopy (SEM) analysis

Scanning electron microscopy (SEM) analysis was used to determine the surface morphology (Hitachi S-3700 N). The microparticles were fixed in slabs and coated with gold/palladium using a sputter coater.

***In vivo* evaluation**

Subject selection

In this study, 12 New Zealand Male healthy rabbits with a mean age of 10±2 w and with a mean body weight of 3±0.2 kg were used. Each group consisted of six rabbits and was subjected to overnight fasting. Care was taken so that there was no stress on the animals. Each group was housed in one cage. Rabbits were randomly divided into two groups for different sampling time. During the experiment food and water were always available ad libitum. The study was conducted in a crossover design manner, with two weeks washout periods between the two experiments. The animal dose of IBH was estimated with relevance to the human dose by using the formula below. The dosage form was administered through the gastric intubation method [18].

Human dose (HD) of IBH = 5 mg.

$$\text{Animal dose} = \frac{\text{HD} \times \text{Weight of the animalman dose} \times \text{Animal weight}}{\text{Average Human weight}}$$

= 5x3/70= 0.214 mg/kg

Blood-sampling

Blood samples of 1 ml were collected from the tracheal-lobular vein of the rabbit and stored in screw-top heparinized plastic tubes. The sampling time for blood was done at 0 min (Pre-dose), 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24 and 48 h. After centrifugation at 4000 rpm for 5 min, the plasma was immediately separated by aspiration and was frozen at -20 °C until analysed by LC-MS/MS method [19].

Liquid chromatography (LC)-mass spectroscopy (MS)/mass spectroscopy method

IBH in Plasma was quantified using HPLC (Aglient series 1200) and Mass spectroscopy (Sciex API 2000). Reversed-phase C18 column (150 mm X 4.6 mm, Particle size 5 µm) was used with a mixture of ammonium acetate buffer and acetonitrile (20:80, V/V) as mobile phase.

The flow rate of the mobile phase in the column was adjusted to 1 ml/min with an Injection Volume of 20 µl. The wavelength maxima of IBH were discovered using the following approach, which had a run time of 3.0 min and a sample detection wavelength of 280 nm. UV-Vis detection at 286 nm [20, 21].

Preparation of working standard solutions

Preparation of IBH standard stock solution

IBH as a working standard equivalent to 5 mg of IBH was weighed and transferred into a 5 ml volumetric flask and then dissolved in methanol. Methanol was used to make up the volume. The conc. of the resulting solution was calculated by considering the purity of IBH. The solutions were labeled and stored in a cold store at 2-8 °C.

Table 1.2: MRM parameters for Ivabradine HCl and Nifedipine

Parameters	Ivabradine HCl	Nifedipine
Declustering Potential (DP)	70.0	70.0
Entrance Potential	10.0	10.0
Collision Energy (CE)	36.0	36.0
Collision Cell Exit Potential (CXP)	12.0	12.0

Preparation of internal standard stock solution

Nifedipine (0.00556 g) was weighed accurately and transferred in to a 5 ml volumetric flask and then dissolved in methanol. Methanol was used to make up the volume. The conc. of resulting solutions was calculated by considering the purity of Nifedipine. The solutions were labeled and stored in a cold store at 2-8 °C. The Stock solution was diluted with 60% methanol in water to get a conc. of 719.880 ng/ml.

Calibration curve standards

Preparation of stock dilutions of standard Ivabradine HCl solution

Stock solution of IBH was diluted with 60% methanol in a water solution to get a concentration ranging from 2.018-4044.260 ng/ml.

Spiking of plasma for calibration curve standards

Conc. of IBH ranging from 0.101 to 202.213 ng/ml were prepared using plasma and were labeled them as CC1 to CC8. The calibration curve standards were prepared freshly for each validation run.

Extraction procedure

Step-1: Subject samples, blank, and calibration curve standards were withdrawn from the deep freezer and allowed them to be thawed to ensure complete mixing of the contents; the thawed samples were vortexed to 0.25 ml of plasma sample in a ria vial, 25 µl of Nifedipine standard (719.880ng/ml) was added. To plasma blank, 25 µl of 60% methanol in water solution was added and vortexed the samples to ensure complete mixing of contents.

Step-2: OASIS HLB 30 mg/lcc cartridge was taken (new cartridge for each sample) onto a positive pressure processor and the following procedure was followed:

Conditioning: 1 ml methanol and followed by 1 ml of water was added.

Application: The sample was applied and allowed to dry for about 5 min. under positive pressure.

Rinsing: The cartridge was rinsed twice with 1 ml of water and was dried under positive pressure for about 5 min.

Rinsing: The cartridge was rinsed with 1 ml of 5% methanol in a water solution and was dried under positive pressure for about 5 min.

Elution: The drug was eluted into 1 ml of methanol and was dried under positive pressure for approx. 2 min. The organic layer was evaporated under a stream of nitrogen gas at 45 °C. The residue was reconstituted with 0.5 ml of mobile phase and vortexed. The samples were transferred in to auto-injector vials and loaded the vials in to autosampler. 20 µl of sample was injected in to LC-MS/MS system.

Data processing

By using the computer-based Analyst 1.4.2 version software supplied by Applied Bio-systems, Canada, the chromatograms were obtained. The conc. of the unknown samples were calculated from the equation using regression analysis of spiked plasma calibration standard with $1/x^2$ as weighting factor.

$$Y = mx + c$$

Where *Y* = Ratio of IBH peak area and IS-TD peak area (analyte area/IS-TD area);

x = Concentration of Ivabradine HCl;

m = Slope of the calibration curve; c = y axis intercept value.

Linear regression analysis equation of stock dilutions of standard IBH solution with plasma is, *Y*= 0.0118x–0.0004

Determination of pharmacokinetic parameters

Various pharmacokinetic parameters such as peak occurred (Tmax), peak plasma concentration (Cmax), elimination rate constant (Kel), time at which area under the curve (AUC), biological half-life ($t_{½}$) and mean residence time (MRT) were calculated using the non-compartmental pharmacokinetics data analysis software PK-Solutions 2.0™ (Summit Research Services, Montrose, CO, USA). The pharmacokinetic parameters of the tested formulations were statistically analysed using paired sample's t-test for normal distributed results of Ka, Ke, Cmax, MRT and AUC_0-α value. All tests were performed at 0.001 level of significance [22].

RESULTS AND DISCUSSION

The pulsin cap dosage form is a capsule which consists of a water soluble cap and a water-insoluble body. The microparticles were sealed within the capsule body using a hydrogel plug. After swallowing the pulsing cap, the water-soluble cap gets dissolved in the gastric juice and the exposed hydrogel plug will begin to swell. After ingestion at predetermined time, the swollen plug will be ejected out and the encapsulated drug formulation will then be released into the colon, where it will be dissolved and then absorbed into the bloodstream. In the current study, capsule bodies that were hardened with formaldehyde treatment for 12 h were used to prepare pulsing caps. They were sealed with unhardened cap of the capsule. The microparticles were prepared by counter ion induced gelation/aggregation method using sodium citrate (SC), sodium sulphate (SS), Sodium tripolyphosphate (STPP). Physical appearance of SC based microparticles were brown colour particles with regular shape and rough surface, whereas SS microparticles were light brown colour with flat surfaced particles STPP based particles were of white-coloured round particles. Since, chitosan has pKa value of 6.5 and bears positive charge under low pH only, associates with opposite charge anion and forms ionic complexes as aggregates [23]. The pH of the medium had a major effect on the swelling of chitosan microparticles due to the ionization of both the poly anions (SC, SS and STPP) with chitosan. The more tightly cross-linked chitosan matrix does not swell (lower water uptake) as much as the loosely cross-linked chitosan matrix. In sodium citrate and sodium sulphate cross-linking solutions swelling is very less due to poly anions tightly linked with chitosan, whereas in sodium-tripolyphosphate, the chitosan network is loose and has a high hydrodynamic free-volume to accommodate more solvent molecules, thereby inducing chitosan–STPP matrix swelling. Under microscopic examination, the formulations revealed that the microparticles were spherical and appeared as aggregates or discrete-particles.

All the prepared formulations offered good flow properties. The particle size of the microparticles ranged between 503.56-588.24µm. These microparticles were characterized for % drug content and % entrapment efficiency [24]. The results are given in table 2**.** The technique also showed good entrapment efficiency. Among the three polyanionic solutions, STPP microparticles contained maximum drug content whereas the least was observed for SS microparticles (*STPP>SC>SS*). The entrapment efficiency for the drug was more in STPP.

Formulated Hydrogel Plugs were characterized for uniformity of weight, hardness, thickness, lag time and the results were shown in table 3. The formulations fitted with the various hydrogel plugs HP1, HP2, HP3, HP4 showed 0.2%, 6.18%, 16.37% and 18.84% of drug release, respectively at the end of 5th hr. It was observed that 100 mg hydrogel plug (HPMC K100:Lactose in 1.0:1.0 ratio) having 4.5

kg/cm^2 hardness was satisfactory to retard the drug release in small intestinal fluid and to eject out the plug in colonic fluid and releasing the microparticles into colonic fluid. This suggests that the lag time could also be adjusted and influenced by the plug composition [25].

Table 2: Evaluational data of Ivabradine HCl microparticles

Composition code	Angle of-repose (θ)	Bulk-density (g/cm^3)	Tapped-density (g/cm^3)	Carr's index	Hausner-ratio	Avg. particle size (µm)	Percentage drug content	Percentage Entrapment-efficiency
F-1	28.04±0.12	0.419±0.018	0.503±0.20	14.16±0.59	1.20±0.012	561.28	48.23	96.46
F-2	28.96±0.17	0.429±0.021	0.507±0.025	14.93±0.46	1.18±0.019	579.16	39.11	97.77
F-3	29.02±0.18	0.442±0.023	0.511±0.031	14.24±0.51	1.18±0.013	588.24	32. 62	97.95
F-4	25.22±0.16	0.439±0.018	0.512±0.026	14.24±0.71	1.16±0.011	522.17	48.56	97.12
F-5	27.36±0.15	0.445±0.011	0.522±0.019	13.94±0.52	1.17±0.08	548.28	39.29	98.22
F-6	28.85±0.18	0.478±0.017	0.580±0.023	17.58±0.45	1.21±0.010	568.19	32. 41	98.21
F-7	27.46±0.15	0.452±0.019	0.543±0.023	16.75±0.53	1.20±0.012	503.56	49.04	98.08
F-8	28.12±0.12	0.469±0.021	0.571±0.022	17.86±0.46	1.19±0.013	521.37	39.34	98.35
F-9	29.30±0.18	0.478±0.023	0.580±0.018	17.58±0.49	1.21±0.09	546.61	32. 56	97.70

All values are expressed as mean±SD, (n=3)

Table 3: Physiochemical characterizations of prepared hydrogel plugs with various polymers

Hydrogel plug code	Composition (1:1)	Weight (mg)	Thickness (mm)	Hardness (kg/cm^2)	Lag time (h)
HP-1	HPMC K-100: Lactose	100	3.16	4.7	5
HP-2	Carbopol: Lactose	100	3.29	4.2	4.5
HP-3	Na-CMC: Lactose	100	3.24	3.8	4
HP-4	Methyl Cellulose: Lactose	100	3.54	3.5	3

All values are expressed as mean, (n=3)

During *in vitro* dissolution studies, it was observed that, the enteric coat of the cellulose acetate phthalate (CAP) was intact for 2 h in pH 1.2, but dissolved in intestinal pH, leaving the soluble cap of capsule, which also dissolved in pH 7.4, then the exposed polymer plug absorbed the surrounding fluid, swelled and released the drug through the swollen microparticles. After complete wetting of the plug, it formed a soft mass, which was then easily ejected out of the capsule body; releasing the microparticles into simulated colonic fluid (pH 6.8 phosphate buffer). From the *In vitro* release studies of the device, it was observed that with all formulation, there was absolutely no drug release in simulated gastric fluid (acidic pH 1.2) for 2 h and in simulated intestinal fluid (pH 7.4 phosphate buffer). Burst effect was found in colonic medium (pH 6.8 phosphate buffer) [26].

In vitro drug release profiles in colonic medium were found to have very good sustaining efficacy. Pulsin caps loaded with IBH microparticles prepared with chitosan and sodium tripolyphosphate in 1.0:0.5,1.0:0.75 and 1.0:1.0 ratios shown sustained drug release for a period of 10 h (5th h to 15th h), 11 h (5th h to 16th h) and 12 h (5th h to 17 h) respectively and are shown in fig. 1. Pulsin caps loaded with IBH microparticles prepared with chitosan and Sodium sulphate in 1.0:0.5, 1.0:0.75 and 1.0:1.0 ratios show sustained drug release for a period of 9.5 h (5th h to 14.5th h), 10.5 h (5th h to 15.5th h) and 11.5 h (5th h to 16.5 h) respectively and are shown in fig. 2.

Pulsin-caps loaded with IBH microparticles prepared with chitosan and Sodium citrate in 1:0.5,1:0.75 and 1:1 ratios shown sustained drug release for a period of 9.5 h (5th h to 14.5th h), 10.5 h (5th h to 15.5th h) and 11.5 h (5th h to 16.5 h) respectively and are shown in fig. 3.

Dissolution kinetics data pertaining to the correlation coefficient values were shown in the table 4. These values clearly indicated that the drug release followed zero order kinetics and the mechanism of drug release was governed by peppas korsmeyer model. The exponential-coefficient (n) values were found to be in between 0.7385 to 0.8882, indicating a non-fickian diffusion mechanism [27]. The SEM photograph of optimized formulation was shown in fig. 4.

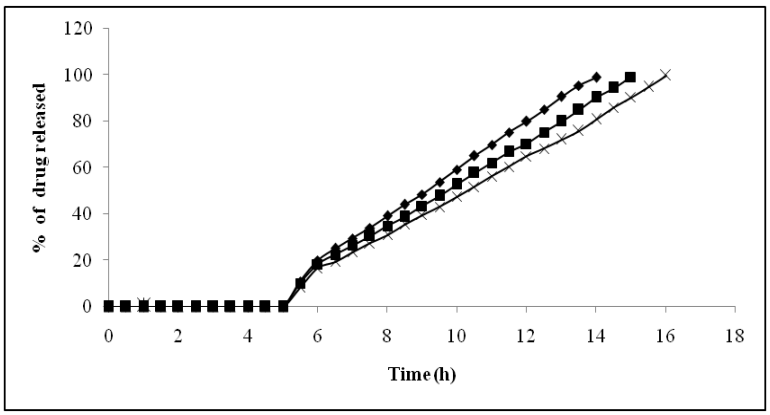

Fig. 1: Comparative *in vitro* drug release profiles of Ivabradine HCl microparticles formulated with chitosan by using Sodium TPP in different ratios (mean), n=3, (-♦-)F$_1$ Formulation prepared with 1:0.5 ratio of polymer and cross-linking agent., (-■-)F$_2$ Formulation prepared with 1:0.75 ratio of polymer and cross-linking agent, (-×-)F$_3$ Formulation prepared with 1:1 ratio of polymer and cross-linking agent

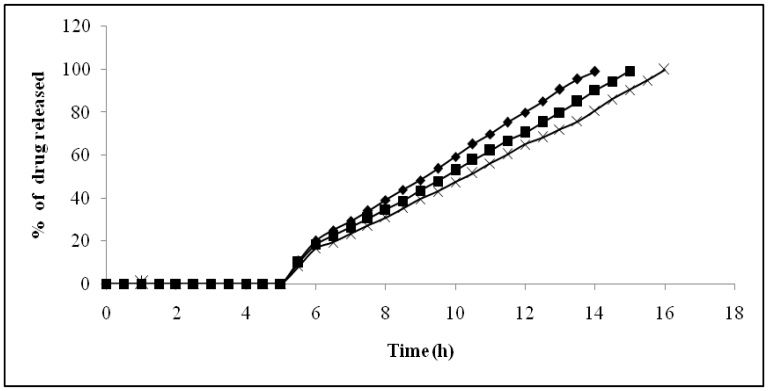

Fig. 2: Comparative *in vitro* drug release profiles of Ivabradine HCl microparticles formulated with chitosan by using sodium sulphate in different ratios (mean,n=3, (-♦-)F_4 Formulation prepared with 1:0.5 ratio of polymer and cross-linking agent. (-■-)F_5 Formulation prepared with 1:0.75 ratio of polymer and cross-linking agent, (-×-)F_6 Formulation prepared with 1:1 ratio of polymer and cross-linking agent

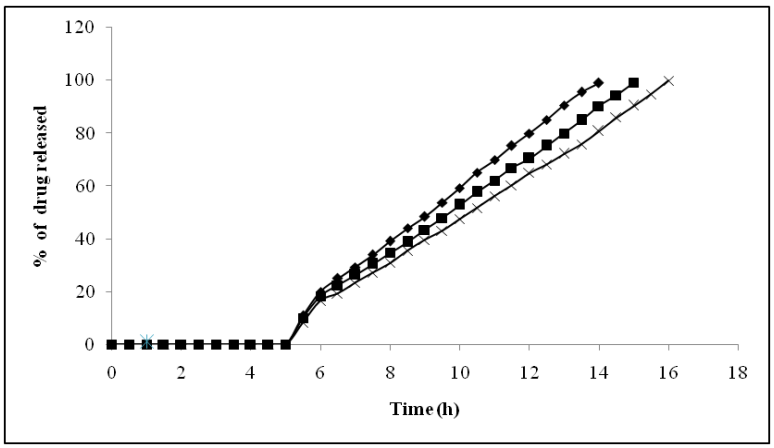

Fig. 3: Comparative *in vitro* drug release profiles of Ivabradine HCl microparticles formulated with chitosan by using Sodium citrate in different ratios (mean, n=3, (-♦-)F_7 Formulation prepared with 1:1 ratio of polymer and cross-linking agent. (-■-)F_8 Formulation prepared with 1:1.5 ratio of polymer and cross-linking agent. (-×-)F_9 Formulation prepared with 1:2 ratio of polymer and cross-linking agent

The FT-IR spectra were recorded in the region of 4000-400 cm^{-1}. The FT-IR spectrum of IBH pure drug (fig. 5) showed characteristic peaks at wave numbers were 3061.87 cm^{-1}, 1670.83 cm^{-1}, 1080.43 cm^{-1}, 1261.28 cm^{-1} and 1170.72 cm^{-1} denoting stretching-vibration of N-C stretching, C=C Ring symmetric-stretching, carbonyl-stretching C-O-C asymmetric-bending and C-O-C symmetric-bending respectively. The FT-IR spectrum of the optimized formulation was shown in fig. 6. From the figures, it was observed that similar peaks were reported in the optimized formulation. There was no shifting of characteristic peaks in drug-loaded microparticles, suggesting that there was no significant drug polymer interaction indicating the stable nature of the IBH in the optimized formulation [28].

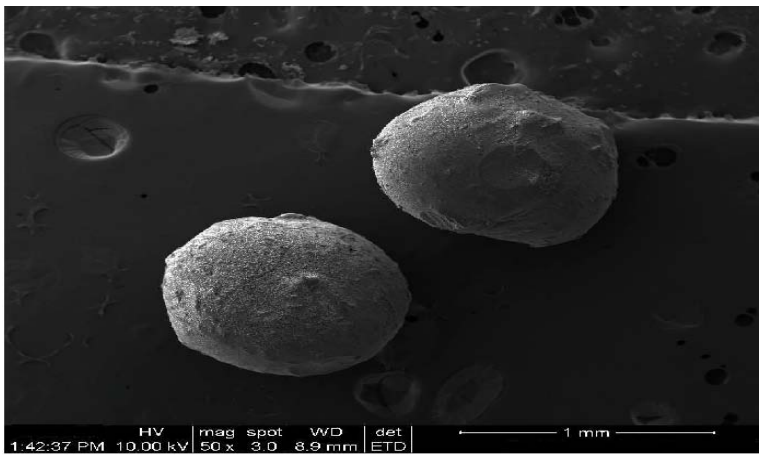

Fig. 4: Scanning electron microscope of Ivabradine HCl microparticles formulated with chitosan by using sodium TPP in 1:1 ratio

Table 4: *In vitro* release kinetics data of Ivabradine HCl microparticles

Composition code	Correlation coefficient (R^2)				Release kinetics			"n" value
	Zero-order	First-order	Higuchi	Peppas	K_0 (mg/h)	T_{50} (h)	T_{90} (h)	
F1	0.9990	0.6859	0.9271	0.9968	0.49	5.1	9.1	0.8732
F2	0.9983	0.8085	0.9352	0.9983	0.45	5.5	9.9	0.8651
F3	0.9965	0.7450	0.9355	0.9989	0.42	6	10.8	0.8594
F4	0.9907	0.8211	0.9561	0.9956	0.64	3.9	7.0	0.7385
F5	0.9895	0.8206	0.9573	0.9971	0.58	4.3	7.7	0.7508
F6	0.9925	0.8353	0.9506	0.9964	0.51	4.93	8.88	0.7643
F7	0.9926	0.7407	0.9089	0.9941	0.55	4.5	8.0	0.8882
F8	0.9968	0.7705	0.9197	0.9960	0.52	4.8	8.6	0.8804
F9	0.9990	0.7991	0.9278	0.9971	0.49	5.1	9.2	0.8750

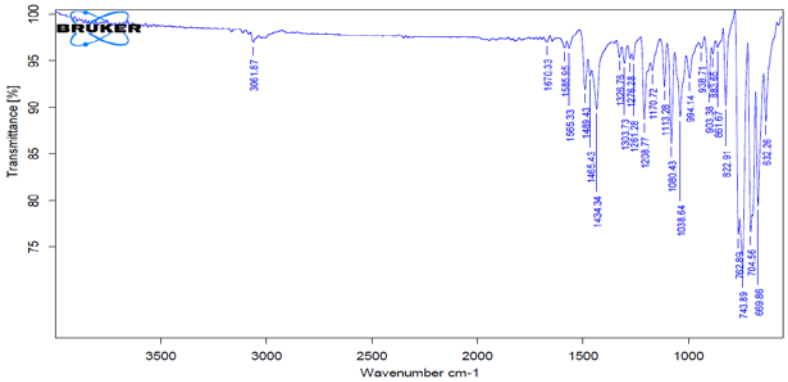

Fig. 5: FT-IR spectrum of pure Ivabradine HCl

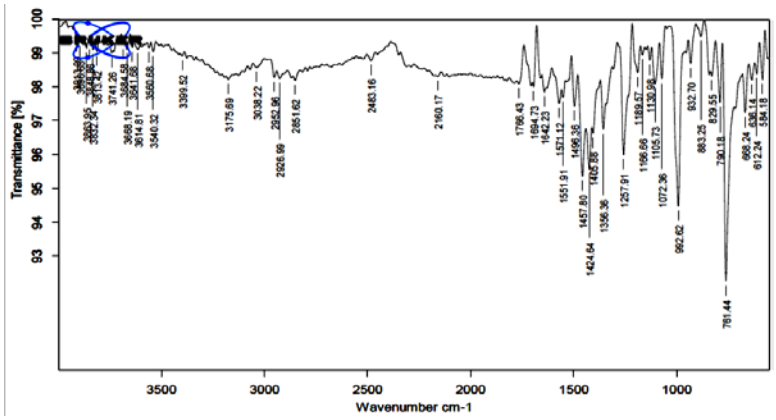

Fig. 6: FT-IR spectrum of optimized formulation

The *in vivo* experiments were conducted according to the protocol and procedure which was described earlier. Pharmacokinetic parameters such as absorption rate constant (Ka), AUC, MRT, elimination rate constant (Ke) and half-life were calculated from the plot of time versus plasma concentration and are subjected to statistical analysis. Plasma concentrations of IBH after the marketed product and pulsatile formulations at different times were determined and are shown in Tables 6 and 7 and in fig. 7 and 8.

Table 5: Analytical data Ivabradine HCl standard stock solution with plasma

S. No.	Sample name	Analyte-concentration (ng/ml)	Peak-area	Peak-area (IS)	Area ratio	Calculated-concentration (ng/ml)	Accuracy (%)
1	Aqueous-mixture	N/A	2247	1940259	0.001160	0.094	N/A
2	Plasma-Blank	0	0	0	0	N/A	N/A
3	Blank+IS-TD	0	0	1217732	0	N/A	N/A
4	CC1	0.101	1325	1102974	0.0012	0.098	97.03
5	CC2	0.202	2696	1132350	0.00238	0.197	97.52
6	CC3	0.506	7223	1258131	0.005740	0.481	95.06
7	CC4	2.022	30281	1277746	0.0237	1.995	98.66
8	CC5	10.11	150940	1281233	0.118	9.933	98.25
9	CC6	40.443	584605	1221929	0.478	40.351	99.77
10	CC7	101.107	1494406	1242395	1.2	101.455	100.34
11	CC8	202.213	2956131	1271509	2.32	196.099	96.98

Table 6: Plasma drug concentration of Ivabradine HCl of oral administration of marketed SR product

Time in h	Plasma concentration of Ivabradine HCl marketed SR product (ng/ml)
0	0
0.5	6.12±0.04
1.0	12.34±0.03
2.0	17.89±0.06
4.0	33.12±0.05
6.0	48.59±0.02
8.0	42.45±0.01
10.0	28.59±0.04
12.0	19.34±0.05
16.0	10.59±0.08
20.0	2.98±0.02
24.0	0.254±0.01
48.0	0.032±0.07

All values are expressed as mean±SD, (n=3)

Table 7: Plasma concentration of Ivabradine HCl of oral administration of optimized formulation

Time in h	Plasma concentration of Ivabradine HCl optimized formulation (ng/ml)
0	0
2	0
4	0
6	11.67 ±1.04
8	26.78±1. 06
10	42.89±1.11
12	53.78±1.23
14	46.36±1.16
16	39.27±1.03
18	34.69±1.68
20	29.86±1.56
24	24.21±1.43
28	22.01±1.26
32	16.01±1.12

All values are expressed as mean±SD, (n=3)

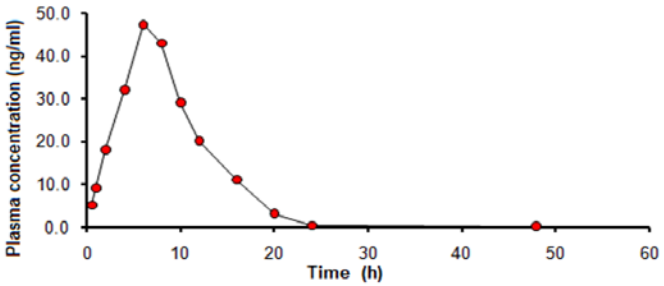

Fig. 7: Time Vs plasma drug concentration of Ivabradine HCl following oral administration of marketed SR product (mean±SD), (n=3)

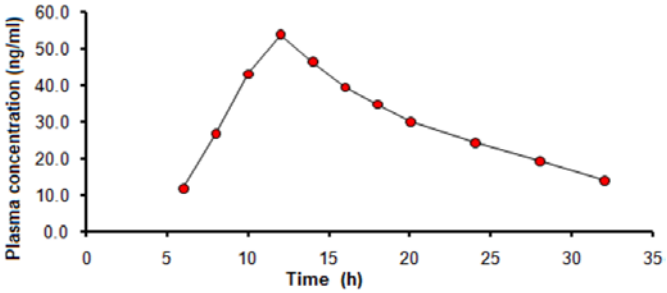

Fig. 8: Time Vs plasma drug concentration of Ivabradine HCl following oral administration of an optimized formulation

The results showed the oral administration of IBH indicated a maximum plasma concentration (C_{max}) 47.3 ± 0.26ng/ml at 1 h (t_{max}) while pulsatile formulations administration exhibited a maximum plasma concentration (C_{max}) of 53.8 ± 0.38ng/ml after an initial lag time of 5 h [29, 30]. The mean residence time (MRT) of pulsatile formulations administration (20.0 ± 0.12 h) was found to be greater

than the oral administration (14.4 ± 0.04 h) formulations [24]. The oral administration of IBH resulted in a low and quite variable AUC of 403.1±1.43ng/ml/h, while the pulsatile formulations resulted in AUC of 944.9±1.03ng/ml/h. The results were shown in table 8.

Table 8: Pharmacokinetic data of oral administration of marketed SR product and optimized formulation of Ivabradine HCl

Pharmacokinetic parameter	Marketed SR Product	Optimized formulation	Calculated value of 't'
C_{max} (ng/ml)	47.3 ± 0.26	53.8 ± 0.38	16.56***
MRT (h)	14.4 ± 0.04	20.0 ± 0.12	15.91***
$t_{1/2}$ (h)	6.19 ± 0.05	8.52 ± 0.014	5. 75***
K_{el} (h^{-1})	0.11 ± 0.002	0.08 ± 0.002	3.87***
K_a (h^{-1})	0.39± 0.06	0.25± 0.06	6.67***
$AUC_{0-\infty}$ (ng h/ml)	403.1± 1.43	944.9±1.03	56.60***
H_o: There is no change in amongst the pharmacokinetic parameters of oral ingestion of Marketed SR product and Optimized formulations of Ivabradine HCl. Table-value of't' with 10 degree-of-freedom (DF) at the 0.001 level is 4.587. Result: H_o is not accepted as the calculated't' value more than the table Value of 't' with 10 degree of freedom (DF) at 0.001 levels of significance. Therefore, it was concluded that significant difference between the pharmacokinetic data of oral administration of Marketed SR product and optimized formulations of Ivabradine HCl.			

***p<0.001 All values are expressed as mean±SD, (n=3)

CONCLUSION

It was concluded that among all the formulations, Pulsin caps loaded with IBH microparticles prepared using chitosan and sodium tripolyphosphate showed extended release for a period of 12 h. The results obtained proved the capability of the system to delay drug release for a programmable period of time and also the possibility of exploiting such delay to attain targeting to the colon. According to the chrono modulated therapy of hypertension, the lag time criterion of 5 h and sustained release for a period of 12 h was satisfied. The dosage form could be taken at bedtime which releases the contents in the early morning hours when hypertension is considered more prevalent.

LIST OF ABBREVIATIONS

IBH: Ivabradine HCl, FTIR: Fourier Transform Infrared Spectroscopy, BP: Blood pressure, IAEC: Institutional Animal Ethics Committee, CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals, HPMC: Hydroxypropyl methylcellulose, Na-CMC: Sodium carboxymethyl cellulose, USP: United States Pharmacopeia, HCl: Hydrochloric acid, UV: Ultra violet, KBr: Potassium bromide, LC-MS: Liquid chromatography–Mass spectroscopy

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AUTHORS CONTRIBUTIONS

KP and PT conceived, designed and conducted experiments. KP and PT participated in the study design and were involved in the interpretation of the results as well as in the drafting of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest regarding the publication of the paper.

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