Original Research Article

Pulsatile Delivery of Fexofenadine Hydrochloride Pulsincap by Box-Behnken Design

ABSTRACT

Objective: Fexofenadine hydrochloride is a selective peripheral H1-blocker, used for allergy symptoms, such as hay fever and urticaria. Allergic symptoms are aggressive during early morning hours, so a pulsatile delivery system with a lag time of 4-5 hours was formulated and optimized by Box-Behnken design.

Materials and Methods: Pulsincap system using formaldehyde-treated capsules and hydrogel plug. Box-Behnken design was applied for optimization in which three independent variables, X1= Drug: polymer ratio, X2 = Polymer: polymer ratio (Ethylcellulose: HPMC E15) and X3 = Plug weight were selected. Three dependent variables R1 = Percent release of drug after 4 hours, R2 = percent release after 10 hours and R3 = Lag time were selected.

Results: FTIR and DSC studies confirmed compatibility of drug and excipients. The empty formaldehyde-treated capsules were evaluated for physical appearance, solubility, capsule dimensions and formaldehyde content. Hydrogel plugs, powder blend and pulsincap formulations were evaluated for Physico-chemical parameters and all the parameters were within acceptable limits. Contour plots and Response surface plots indicated that as Drug: Polymer ratio (X1) and Plug weight (X3) increased, Lag time increased but % drug release decreased. As Polymer: Polymer ratio (X2) increased, the lag time was at a moderate level. Predicted vs actual responses showed the correlation of 0.786 for % release in 4hrs, 0.9744 for % release in 10hrs and 0.6281 for lag time. Optimized formulation G1 was suggested by design (with criteria 4.5-6hrs lag time, 10-20% release in 4hrs & 60-70% drug release within 10hrs). The optimised formulation was stable.

Conclusion: Pulsincap system of Fexofenadine hydrochloride can be obtained by using retarding polymers like ethyl cellulose, HPMC E15 and formaldehyde cross-linked capsules.

KEYWORDS: Fexofenadine, Polymers, Hydrogels, Ethylcellulose, Formaldehyde.

INTRODUCTION

Pulsatile drug delivery system (PDDS) is defined as the release of a certain number of molecules rapidly and transiently within a short period immediately after a predetermined off-released period. Pulsatile release is a term used for this type of release pattern [1]. PDDSs are time-controlled drug delivery systems, designed to achieve time-specific and site-specific delivery of drugs. It delivers the drug according to the circadian rhythm of the body [2]. Pulsatile release pattern is the most popular form of controlled drug delivery system because conventional systems with ~~a~~ continuous release are not ideal. Pulsatile systems are beneficial for ~~the~~ drugs ~~having~~ with chronopharmacological behaviour [3]. Pulsatile DDS can be used in many diseases and conditions where sustained-release formulations ~~don't show good efficacy~~ are not effective [4]. Many functions in our body follow biological time, i.e., their activity increases or decreases with time. The severity of diseases like asthma, myocardial infarction, angina, rheumatic disease, ulcer and hypertension is time-dependent [5]. During the early morning hours, there is a sharp rise in asthmatic attacks. In such cases, supplementing the medication at a certain time rather than maintaining a constant plasma drug level is beneficial [6]. In this case, administering the medication before bedtime, which releases the drug in a burst after the time of administration (during the morning hours), would be ideal. The same is true for preventing heart attacks within the middle of the night and therefore the morning stiffness typical of individuals affected by arthritis [7]. Fexofenadine hydrochloride belongs to a group of medicines called non-sedating H1 Antihistamines. Histamine is a substance stored in mast cells and produced by the body in response to a defence mechanism. The released histamine binds to the H1 receptors resulting in the cause of allergic symptoms [8]. Fexofenadine hydrochloride acts by blocking H1 receptors which lead to inhibition of chain reaction that result in allergic symptoms [9]. Patients who suffer from seasonal and perennial allergic rhinitis complain of disturbed sleep at night as well as troublesome symptoms in the morning on awakening. The most frequently reported symptom was sneezing with a "stuffy nose" and "red itchy eye". These symptoms were most frequent before breakfast and during the morning and least during the middle of the day and late in the afternoon. Evening administration is effective in patients exhibiting predominantly morning symptoms [10].

The main aim of the present study was to develop, design, and evaluate Fexofenadine HCl as a pulsatile release intended for chronotherapy of allergic conditions using pulsincap technology to provide maximum drug plasma concentrations at the time of its maximum need in the early morning. Formaldehyde-treated capsule bodies were used for preparing the pulsincap system. Retarding polymers like Ethylcellulose and HPMC E15 were chosen to prepare Fexofenadine HCl powder blend. HPMC K100M and HPMC K4M were chosen to prepare hydrogel plugs. The pulsincap was formulated by filling the Fexofenadine HCl powder blend into formaldehyde cross-linked capsule bodies and by placing a hydrogel plug upon it and sealing the capsule. Lag time is affected by the polymers and hydrogel plug used. Three-factor, three-level Box-Behnken design (BBD) is used for optimization.

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MATERIALS AND METHODS

Materials:

Fexofenadine hydrochloride was obtained as a gift sample from Dr. Reddy's Laboratories, Hyderabad, India. Hydroxy Propyl Methyl Cellulose (HPMC K100M & HPMC K4M), Poly Vinyl Pyrollidine (PVPK30), Ethyl Cellulose were obtained from Yarrow chemical products, Mumbai, India. Formaldehyde, sodium chloride and other chemicals of analytical grade were procured from S.D. Fine Chemicals Ltd, Mumbai, India.

Comment [AA2]: Describe each polymer composition separately in terms of molecular weight, viscosity, degree of substitution, etc.

Methods:

Drug-excipient compatibility study

Interactions by FTIR

The spectrum analysis of pure drug and physical mixture of drug with different excipients were studied by FTIR using a Shimadzu (Kyoto, Japan) facility (model-8400S). Potassium bromide (KBr) disk was placed in a suitable holder in an IR spectrophotometer and the IR spectrum was recorded from 4000 cm^{-1} to 500 cm^{-1} in a scan time of 12 minutes. The resultant spectrum was compared for any spectral changes. The spectra were observed for the presence of characteristic peaks for the respective function.

DSC Studies

The instrument was calibrated with indium standard. 3-5mg samples were weighed and placed in closed, hermetic sample pans with pinholes. Thermograms were obtained by heating the sample at a constant rate of 10°C/min from 0°C to 210.0°C. The heat of fusion, disappearance of the crystalline sharp peak of the drug and the appearance of any new peak and peak shape were noted. The thermogram of the optimized Fexofenadine HCl formulation superimposed with that of pure drug [11].

Formulation of Pulsincap drug delivery system

Crosslinking of empty capsules

Size '0' capsules were chosen for the Pulsincap system, and the solubility of these gelatin capsules was modified by crosslinking them with formaldehyde.

Comment [AA3]: The author has selected 0 size capsule which holds about 500 mg of excipients; however, the total capsule fill weight is not exceeding 200 mg. Therefore, capsule size 1 or 2 is preferred. Clarify why you chose 0 size capsule in your study?

Hard gelatin capsules of size '0' were taken with bodies separated from caps. 25 ml of 15% (v/v) formaldehyde was taken in a petri plate and placed at the bottom of the desiccator. Capsules bodies were evenly spread on the mesh as a single layer and the mesh was placed above the petri plate containing formaldehyde. The desiccators were tightly closed and empty bodies of capsules were exposed to formaldehyde vapours. The reaction time was optimized by removing capsule bodies at different time intervals from the desiccator i.e., capsule bodies were collected every 1 hour until 6 hours. They were dried at 50ºC for 30 min to ensure completion of reaction between gelatin and formaldehyde vapours. To facilitate the removal of residual formaldehyde, the capsule bodies were dried at room temperature. These capsule bodies were capped with untreated caps and stored in self-sealing covers [3].

Preparation of hydrogel plug

Two swellable hydrophilic polymers HPMC K100M and HPMC K4M were initially selected as they can control the lag time and later one hydrogel plug was optimized. Required quantity of polymers were weighed and compressed using '6mm punch' in tablet compression machine [3].

Preparation of powder blend: To produce the core physical mixture, Fexofenadine HCl was mixed with polymers (ethyl cellulose and HPMCE15) according to Table 1.

Filling of powder blend in capsule: Weighed quantity of the blend was filled into formaldehyde-treated capsule body and sealed by hydrogel plug and locked with an untreated cap [3].

Sealing of capsules: A 5% ethyl cellulose ethanolic solution was used to seal the cap with the body [3].

Table 1: Formulation table for pulsincap delivery system

Formulation	Drug (mg)	Total Polymer weight (mg)	Polymer		Plug
			Ethyl cellulose (mg)	HPMC E15 (mg)	HPMC K 4M (mg)
F1	60	90	45	45	100
F2	60	90	30	60	75
F3	60	30	15	15	100
F4	60	60	30	30	75
F5	60	90	45	45	50
F6	60	30	15	15	50
F7	60	60	20	40	100
F8	60	90	60	30	75
F9	60	60	30	30	75
F10	60	60	20	40	50
F11	60	30	10	20	75
F12	60	60	40	20	100
F13	60	60	30	30	75
F14	60	30	20	10	75
F15	60	60	40	20	50

Evaluation of empty formaldehyde-treated capsules

Physical Examination

Capsules were visually examined for any defects after 6 hours of formaldehyde cross-linking [1].

Solubility studies for formaldehyde-treated capsules

The solubility of formaldehyde cross-linked capsule bodies was checked using an orbital shaker bath. This was performed to optimize the crosslinking time. Capsules were collected at the end of every 1 hour of crosslinking and checked for their solubility in 0.1N HCl. Deformation of capsule body shape was considered as the endpoint [1,3].

Measurement of dimensions of capsule bodies

Comment [AA4]: HPMC K4M are HPMC K100M are release-controlling polymers for tableted core-based pulsatile delivery systems. HPMC K4M has a medium molecular weight with a viscosity of 4,000 cP, while HPMC K100M has a high molecular weight with a viscosity range of 75,000 to 140,000 cps. However, in case of tableted core-based pulsatile delivery systems, it is better to use one of these polymers in the form of HMPC E grades, such as E-5, E-50, etc. Clarify why you chose two HPMC K grades?

Comment [AA5]: 6 mm (Round)? Is it right?

Comment [AA6]: Clarify the tablet press type

Comment [AA7]: Both are pH-independent polymers; however, it is better to use pH-dependent and pH-independent polymers to control the release of Fexofenadine HCl in all release medias. Clarify why you used these two pH- independent polymers?

Comment [AA8]: Mention the total fill weight per capsule

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Dimensions of capsule bodies like total capsule length, capsule body diameter and capsule body length of both plain capsules and formaldehyde cross-linked capsules were measured using screw gauge to compare the differences between plain capsules and formaldehyde cross-linked capsules [1,3].

Quantitative test for free formaldehyde content

A quantitative test was employed to prove that the formaldehyde content in the cross-linked capsules is within the limits. The sample was accurately weighed (about 3 g of formaldehyde-treated capsules) and added to a mixture of H_2O_2 (25ml) and 1M sodium hydroxide (50ml) in a conical flask. Heated on the water bath until effervescence ceases (usually about 30 mins), cooled and excess of alkali was titrated with 1M hydrochloric acid, using phenolphthalein as indicator [4].

Equivalent factor: 0.03003g of HCHO ≡ 1ml of M NaOH

Evaluation of Hydrogel Plugs

Hardness and thickness

The hardness and thickness of 50mg, 75mg and 100mg HPMC K4M & 100M hydrogel plugs were measured by using Monsanto hardness tester and screw gauge respectively [3].

Swelling Index

Plugs were weighed individually (W1) and placed separately in glass beakers containing 200 ml of 0.1 N HCl, 0.001N HCl & 6.8 pH phosphate buffer and incubated at 37°C±1°C. At regular 1 hour time intervals until 6 hrs, the plugs were removed from the beakers and the excess surface liquid was removed carefully using filter paper. The swollen plugs were reweighed (W2) and the swelling index (SI) was calculated using the following formula [5].

$$\textbf{Swelling Index} = \frac{\mathbf{Weightafterswelling(Wf) - Initialweight(W0)}}{\mathbf{InitialWeight\ (W0)}} \times \mathbf{100}$$

Evaluation of powder blend

Assay

Powder blend was accurately weighed equivalent to the drug dose and dissolved in 10 ml of methanol. After required dilutions, absorbance was measured at 220 nm using UV Visible spectrophotometer [6].

Flow properties

Bulk density, tapped density, angle of repose, Hausner's ratio and the Compressibility index were determined.

Angle of repose

Angle of repose was determined by the funnel method. Accurately weighed quantity of powder blend was placed in a funnel adjusted to a height such that the tip just touched the apex of the powder blend inside. The powder blend was allowed to flow through the funnel freely and drop onto the surface. The diameter of the pile of the powder blend was measured and the angle of repose was calculated using the equation [7]: **Tan θ =h/r**

Where, θ = angle of repose, h = height of the heap (in cm) and r = radius of the base (in cm).

Bulk density (ρ_b): It is the mass of the powder divided by the bulk volume [7].

Comment [AA10]: Mention the version

Comment [AA11]: Mention the version

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Tapped density (ρ_t): It is the mass of the powder divided by the tapped volume [7].

Compressibility index: Carr's index was calculated from the following equation using the values of bulk density (ρ_b) and tapped density (ρ_t) [7].

$C = (\rho_t - \rho_b / \rho_t) \times 100$

Hausner's ratio: It is calculated by the following formula [6,7,8].

Hausner's ratio = ρ_t / ρ_b

Optimization using Box-Behnken design

A three-factor, three-level Box-Behnken design (BBD) was selected for the optimization procedure to explore quadratic response surfaces and construct second-order polynomial models using Design Expert 11 (Version 11; Stat-Ease Inc., Minneapolis, MN). Box-Behnken Design is an independent quadratic design that is used to optimize the formulations where the treatment combinations are at the midpoints of edges of the process space and the centre. Initial preliminary trials were carried out to evaluate the formulations and for the processing of pulsatile capsules. Based on this, three independent variables, X1= Drug: polymer ratio, X2 = Polymer: polymer ratio (Ethylcellulose: HPMC E15) and X3 = Plug weight were selected at three levels (low, medium, and high). The levels for these three parameters were determined from the preliminary trials. R1 = % drug release in 4hrs, R2=% drug release in 10hrs, R3=lag time (hrs) were selected as dependent factors [9]. The factors, levels, and responses are given in Table 2.

Table 2: Factors and factor levels of Box–Behnken experimental design

Independent factors	**Levels**		
	Low	**Medium**	**High**
	-1	**0**	**1**
1. X1 = Drug: polymer ratio	1:0.5	1:1	1:1.5
2. X2 = Polymer: polymer ratio (Ethyl cellulose: HPMC E15)	1:0.5	1:1	1:1.5
3. X3 = Plug weight (mg)	50	75	100
Responses (Dependent factors)			
1.R1 = % Drug release in 4 hours			
2. R2 =% Drug release in 10 hours			
3. R3= % lag time (hours)			

Evaluation of Pulsincap

Weight variation

Twenty capsules were selected randomly and weighed collectively and individually. Average weight and the % weight variation were calculated [3].

Content Uniformity

Twenty capsules were randomly selected from each batch and their contents were removed and powdered. From this sample, 60mg of powder (equivalent to drug dose) was accurately transferred to a 10ml volumetric flask. The volume was made up with methanol and sonicated for 30 mins. 1 ml of the above solution was transferred to a 10ml volumetric flask and the volume was made up to the mark with 0.1N HCl. The resulted solution was filtered through Whatman filter paper, suitably diluted and the drug content was estimated spectrophotometrically by measuring absorbance at 220nm [3].

Comment [AA15]: Add the full description (i.e. pores size, manufacturer, country, etc.)

Measurement of capsule lock length

The lock length of the capsules was measured using a screw gauge and the values were noted.

In-vitro dissolution studies

In-vitro dissolution was carried out using USP type-I (Basket) apparatus. The release of Fexofenadine HCl from the pulsincap system was studied using two different dissolution media 0.001N HCL and 6.8 pH phosphate buffer to simulate pH changes across the GI tract. The rotating basket stirrer was set at a stirring speed of 100 rpm and temperature was adjusted to 37 ± 0.5°C. Samples were withdrawn at predetermined time intervals of 0.5 hr, 1 hr, 1.5 hr, 2hr, 3hr, 4hr, 5hr, 6hr, 7hr 8 hr, 9 hr, 10 hr and replaced with 5ml of fresh dissolution medium. The withdrawn samples were assayed at 220 nm for Fexofenadine content using a UV visible spectrophotometer [3,10].

Comment [AA16]: Add a reference

Calculation of model-dependent kinetics for prepared formulations

The mechanism of drug release from the dosage form was analysed by fitting the obtained results into zero-order, first order, Higuchi and Korsmeyer-Peppas release models.

Stability studies

Pulsincap formulations were tested for their stability in amber coloured bottle containers. Optimized formulations were stored in a humidity chamber at accelerated stability conditions (40°c ± 2°c /75% ± 5% RH) as per ICH guidelines for 1month and the capsules were evaluated for drug content and in-vitro drug release every week [12].

Comment [AA17]: Glass or plastic?

RESULTS & DISCUSSION

Drug- excipient compatibility studies

Compatibility studies were carried out to study the possible interaction between Fexofenadine HCL and excipients by FTIR and DSC. FTIR spectrum of pure drug showed characteristic peaks at 3364.88cm^{-1}; 1707.03cm^{-1}; 1464 cm^{-1}; 1168.88cm^{-1} representing presence of carboxylic group, aromatic ring and tertiary alcohol respectively. The characteristic peaks of pure drug were seen in optimized formulation (Figure 1) indicating drug excipient compatibility.

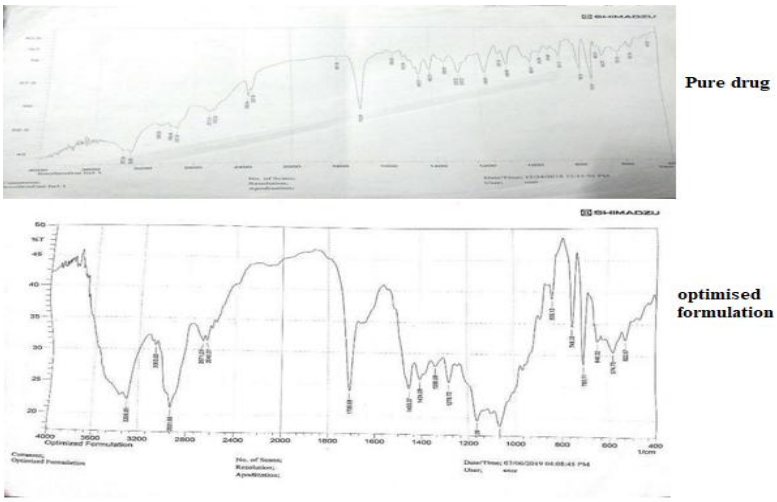

Figure 1: FTIR graph of Pure drug (Fexofenadine HCl) & Optimized formulation (Ethylcellulose: HPMC E15)

DSC thermograms are given in Figure 2. A sharp peak at 206.5°C was observed indicating the melting point of fexofenadine HCl. In the optimized formulation, three peaks were observed, one peak at 203.7°C represents drug and the other peaks of the polymer as the sample is a physical mixture of drug and polymers.

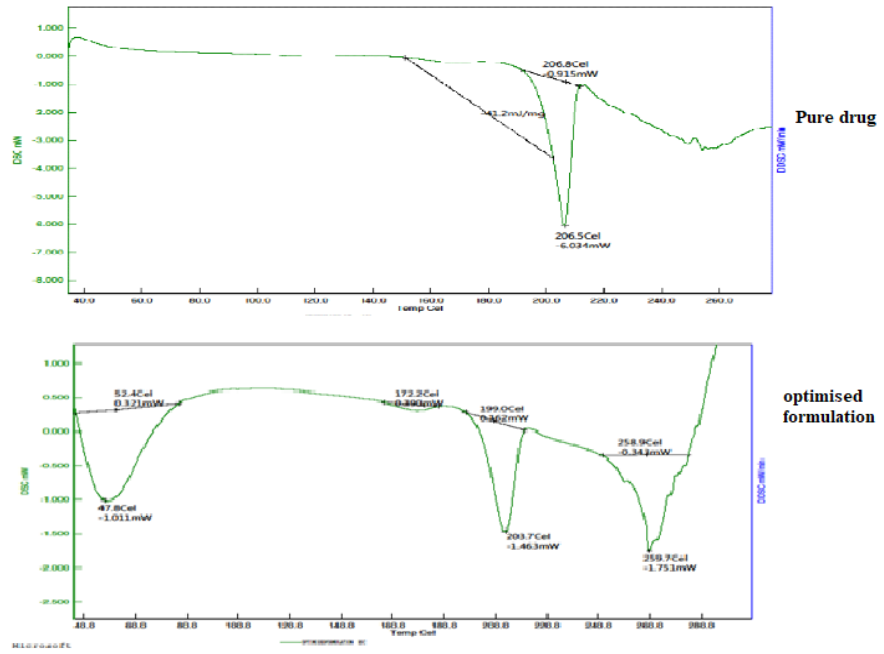

Figure 2: DSC studies of a pure drug (Fexofenadine HCl) & Optimized formulation (G1)

Comment [AA18]: Add DSC thermograms of polymers as well

Evaluation of empty formaldehyde cross-linked capsules

Physical appearance

There were no significant changes observed after exposing the capsules bodies to formaldehyde vapours for 6 hours [3].

Solubility studies for formaldehyde-treated capsules

It was found that 15% v/v formaldehyde solution and 6 hours exposure time was optimum as the capsule bodies remained intact up to 10 hours. The time at which the capsule body turns into a soft fluffy mass was noted. Deformation of capsule body shape is considered as the end point [1].

Measurement of dimensions of capsule bodies

The dimensions of the capsules (n=6) were measured and it was observed that the average capsule length before treatment was 23.73±0.14mm and after treatment, it was 22.85±0.12mm. Average capsule body diameter and average capsule body length before treatment were 6.86±0.12mm and 18.11±0.16mm and after treatment were 6.81±0.19mm & 17.98±0.18 respectively. From the dimensions obtained, it was observed that there was a slight decrease in diameter and length of capsules after formaldehyde cross-linking [1,3].

Estimation of formaldehyde content

Titration was carried out to obtain the formaldehyde content it was observed that 1.2ml of HCL was consumed during the titration. It was calculated according to the equivalent factor and obtained as:

1ml HCl ≡ 1ml NaOH ≡ 0.03003g of HCHO ≡ 30.03 mg of HCHO

Thus, the amount of formaldehyde present in the sample (10 formaldehyde-treated capsules) was found to be 36.03mg, which is within the limits (50mg/day).

Evaluation of the hydrogel plug

Hardness and thickness of hydrogel plugs of three different weights were measured and the plugs were evaluated for swelling index. The results are given in Table 3.

Table 3: Swelling Index of the hydrogel plugs (at the end of 6 hours) in three different media

Evaluation parameters	HPMC K100M			HPMC K4M		
	50 mg	**75mg**	**100mg**	**50 mg**	**75mg**	**100mg**
Hardness(kg/cm^2)	3.5±0.05	5.8±0.06	6.1±0.11	1.5±0.04	5.5±0.05	6±0.15
Thickness (mm)	1±0.13	1.5±0.01	2±0.07	1±0.21	1.5±0.06	2±0.14
Swelling Index (%) in 0.1 N HCl	330±0.18	126±0.14	120±0.11	144.4±0.14	98±0.09	120±0.13
Swelling Index (%) in 0.001N HCl	320±0.17	20±0.16	22±0.05	148.±0.09	113±0.15	114±0.18
Swelling Index (%) in 6.8pH phosphate buffer	220.6±0.19	240±0.23	310±0.16	120±0.14	140±0.19	210±0.17

Values represent n=3±SD

It was observed that the swelling index of HPMC K4M was less compared to HPMC K100M. Drug release from the higher viscosity grade, K100M was slower compared to the lower viscosity grade, K4M. As the drug used in the formulation was low soluble, using a low-grade polymer, i.e., using HPMC K4M plug was found to be effective to sustain the release of the drug [1]. Hence HPMC K4M plug was optimized. It was considered the ideal hydrogel plug and was used in the final formulation.

Evaluation of powder blend

Assay of 15 formulations was within the acceptable limits i.e., in the range of 95- 100% indicating that there was no loss of drug. The results are given in Table 4.

Flow properties [6]

Flow properties were determined and the results are given in Table 4.

Table 4: Flow properties powder blend

Formulation	Assay (%)	Bulk density (gm/ml)	Tapped density (gm/ml)	Carr's index (%)	Hausner's ratio	Angle of repose (º)
F1	97.85±0.32	0.463±0.14	0.545±0.18	12.6±0.20	1.14±0.27	16.82±0.21
F2	96.36±0.26	0.565±0.11	0.612±0.19	14.22±0.14	1.13±0.12	14.56±0.22
F3	97.90±0.34	0.595±0.14	0.622±0.14	14.4±0.22	1.17±0.2 1	15.70±0.18
F4	96.77±0.15	0.465±0.15	0.510±0.15	5.01±0.13	1.02±0.14	16.80±0.25
F5	97.0±0.18	0.526±0.13	0.643±0.12	14.9±0.17	1.15±0.20	17.60±0.23
F6	99.82±0.23	0.475±0.12	0.528±0.20	8.58±0.19	1.07±0.18	15.72±0.24
F7	98.45±0.36	0.484±0.14	0.563±0.13	6.20±0.16	1.09±0.20	13.26±0.26
F8	99.63±0.13	0.478±0.21	0.525±0.21	12.3±0.17	1.14±0.21	14.83±0.32
F9	96.56±0.35	0.445±0.20	0.480±0.19	12.1±0.15	1.12±0.14	15.80±0.29
F10	98.99±0.31	0.465±0.18	0.490±0.18	11.1±0.11	1.12±0.15	16.80±0.25
F11	99.36±0.18	0.486±0.15	0.525±0.22	14.4±0.18	1.12±0.16	17.70±0.19
F12	100.0±0.12	0.517±0.24	0.598±0.12	12.5±0.21	1.14±0.21	13.28±0.15
F13	99.63±0.21	0.536±0.14	0.570±0.14	13.2±0.10	1.12±0.37	14.32±0.15
F14	98.26±0.31	0.486±0.16	0.524±0.18	14.14±0.14	1.13±0.14	18.63±0.20
F15	97.32±0.23	0.585±0.17	0.660±0.16	7.25±0.17	1.10±0.11	18.31±0.22

Values represent n=3±SD

The results indicate that all the formulations show excellent flow properties.

Evaluation of pulsincap system

Weight variation

All the capsules passed the weight variation test as the average % weight variation was within 7.5% limits as prescribed in the pharmacopoeia.

Content Uniformity

Content uniformity was checked for all the formulations and the results obtained are within limits as prescribed in the pharmacopoeia.

Measurement of capsule lock length

The lock length of the capsules was measured using a screw gauge and the results are in co-ordination with empty capsules length.

In-vitro dissolution studies

In-vitro drug release studies were conducted for 15 formulations obtained by applying experimental design. All the formulation results are shown in Figures 3 and 4.

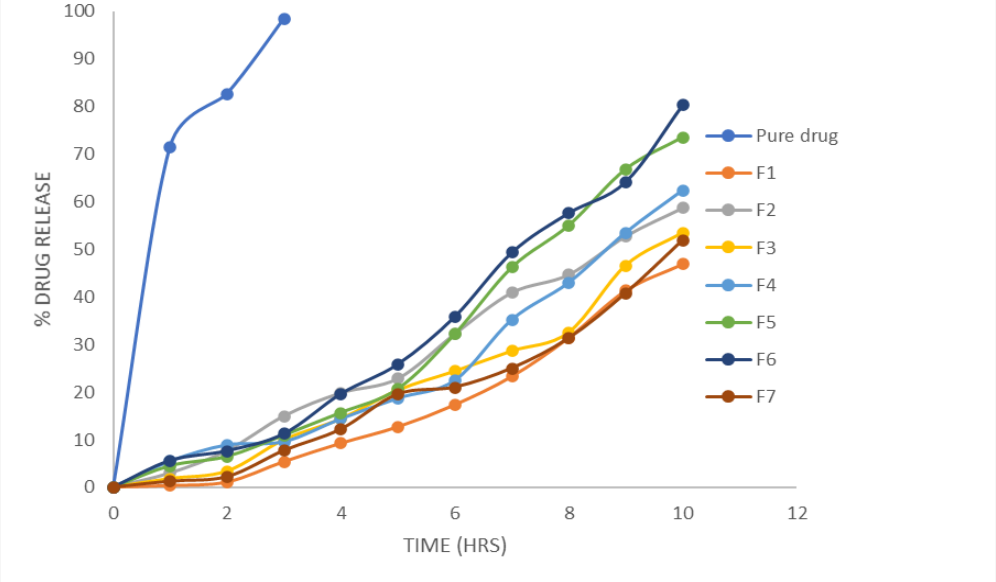

Figure 3: In-vitro drug dissolution of pulsincap in comparison with pure drug (F1- F7)

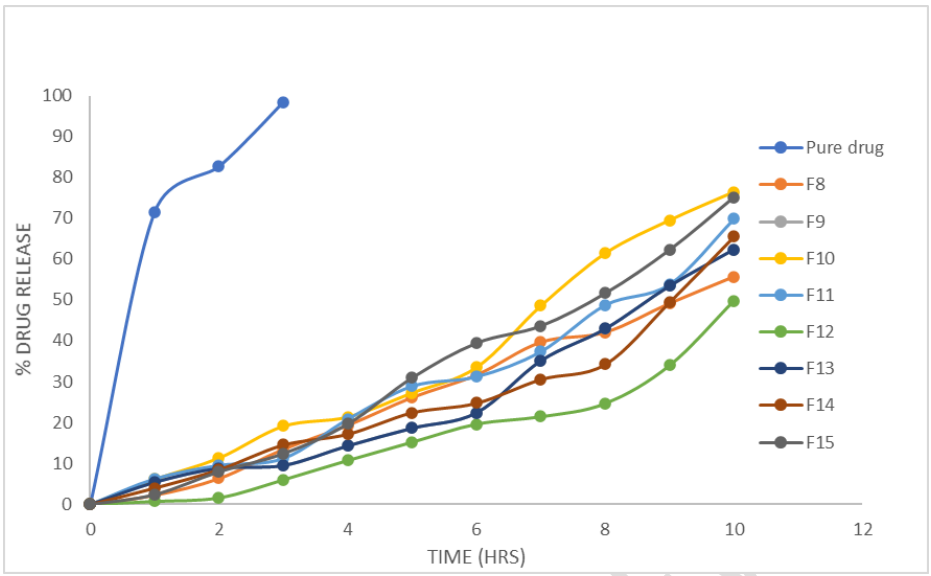

Figure 4: In-vitro drug dissolution of pulsincap in comparison with pure drug. (F7– F15)

Optimization by Box-Behnken design

Three independent variables (factors) X1 = Drug: polymer ratio, X2 = Polymer: polymer ratio (Ethyl cellulose: HPMC E15) and X3 = Plug weight were selected at three levels (low, medium, and high). Three dependent factors Y1 = Percent release in 4 hrs, Y2 = percent release in 10hrs and Y3 = Lag time were selected. It was observed that the Y1 response, i.e., percent release after 4 hours followed the reduced quadratic model whereas, Y2 and Y3 response for all formulations followed a linear model. Response parameters percentage drug release and lag time were analysed statistically by applying Analysis of variance (Table 5). ANOVA was performed to determine the effect of a factor on the responses. The results of the ANOVA were applied to identify insignificant factors. The P-value for the responses R1, R2 and R3 were found to be 0.0005, 0.0001 and 0.0017 respectively, which is less than 0.0005, indicates that model terms are significant. Lack of fit was found to be insignificant, so the model fits the data generated. R1 response (% drug release in 4 hours) followed the reduced quadratic model and the R2 and R3 response followed linear model. The Predicted R^2 is in reasonable agreement with the Adjusted R^2 for all three responses i.e., the difference was found to be less than 0.2. In this case, Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. For all three responses the adequate precision was greater than 4, indicates an adequate signal. The results are given in Table 5.

Table 5: ANOVA summary & Fit statistics for responses R1, R2 and R3

Response	Model	F- value	P-value	R^2	Adjusted R^2	Predicted R^2	Adeq Precision
R1 (% drug release after 4 hours)	Reduced Quadratic	13.48	0.0005	0.9896	0.9868	0.9785	55.786
R2(%drug release after 10hours)	Linear	349.62	<0.0001	0.7861	0.7278	0.5736	8.4809
R3(lag time)	Linear	11.44	0.0017	0.6559	0.5986	0.4191	10.4504

Response surface analysis

To study the effect of independent variables on dependent variables, 2D contour plot (Figure 5) and 3D response surface analysis (Figure 6) was done using Design of Expert 11 software. These plots provide information about the effect of two independent variables on one dependent variable at a time by keeping the third independent variable at the middle level [13].

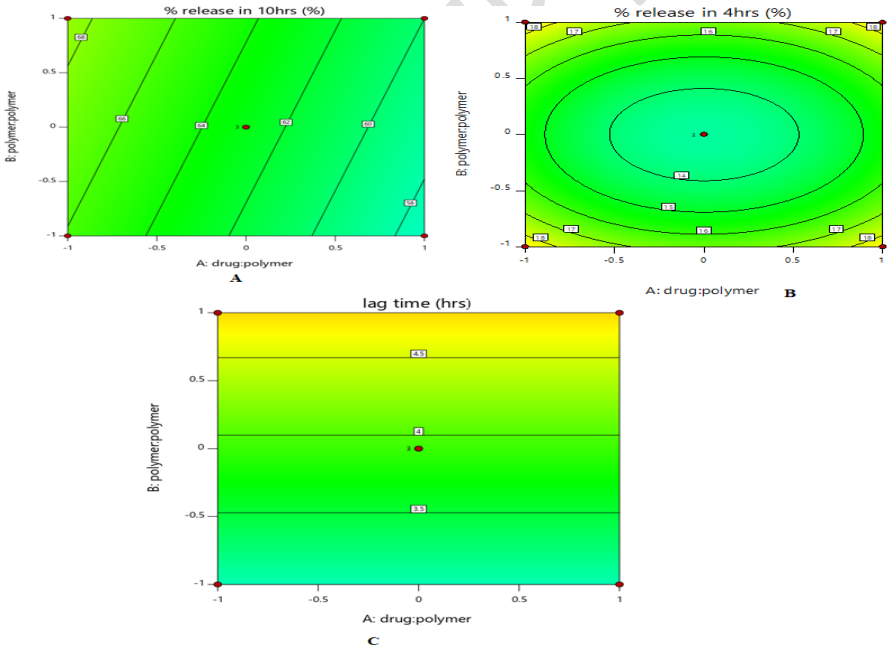

Figure 5: Contour plot for the effect of Drug: polymer ratio and polymer: polymer ratio on A) % drug release in 10 hours B) % drug release in 4 hours and C) lag time (hrs) at the center level of X3 (plug weight)

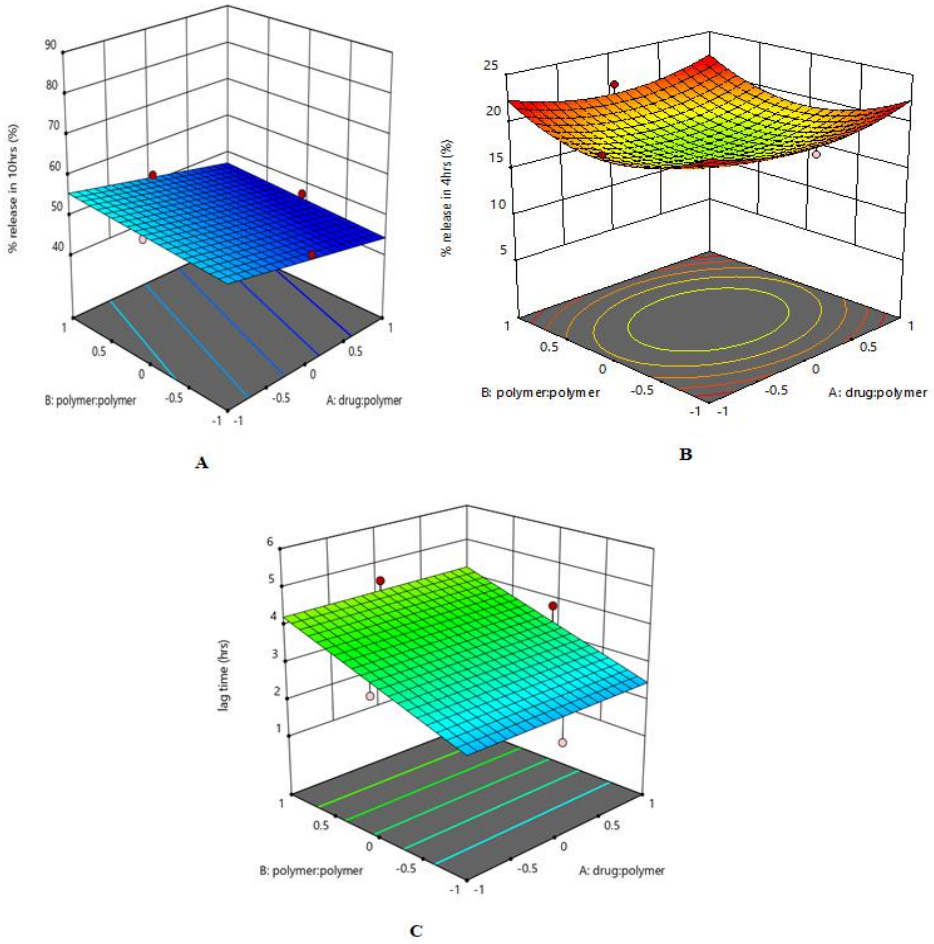

Figure 6: Response surface plot for the effect of Drug: polymer ratio and polymer: polymer ratio on A) % drug release in 10 hours B) % drug release in 4 hours and C) lag time (hrs) at a higher level of X3 (plug weight)

Effect on % drug release in 10 hours (R2)

From Figure 6A it can be observed that as the level of X1 (Drug: Polymer ratio) was increased from -1 to 1 at the centre level of X3 (Plug weight) the % drug release was found to be decreased. And with the increase in X2 (Polymer: Polymer ratio), the % drug release was found to increase. Thus, it is observed that with the increase in Drug: Polymer ratio (X1) there is a decrease in % drug release and whereas with the increase in Polymer: Polymer ratio (X2) the % drug release was also increased. With further increase in X3 (Plug weight) i.e., at a higher level of X3, the percentage drug release decreased. Contour plot at the centre level and 3D response surface plot at higher level (Figure 5A & 6A) shows the inclining trend of release rate with increase in concentrations of polymer: polymer whereas an increase in the concentration of drug: polymer showed a declining trend of release rate.

Effect on % release in 4 hours (R1)

Figure 6B shows as the level of X1 (plug weight) increased from -1 to 1 at centre level X3(Plug weight) the % drug release decreased and with the increase in X2 (drug: Polymer ratio), the % drug release decreased. With further increase in X3 (Plug weight) i.e., at a higher level of X3, the % drug release decreased. Thus, from the above plot, it is observed that with the increase in Drug: Polymer ratio (X1) there is a decrease in % drug release**.** Contour plot at the centre level of X3 (Plug wt.) (Figure 5B) and 3D response surface plot (Figure 6B) at a higher level of X3 (Plug wt.) shows the inclining trend of release rate with an increase in concentrations of the drug: polymer and polymer: polymer.

Effect on Lag time (R1)

Figure 6C shows that as the level of X1 (Drug: Polymer ratio) increased from -1 to 1 at the centre level of X3 (Plug weight) the lag time increased and as X2 (Polymer: Polymer ratio) increased from -1 to 1 at the centre level of X3 (Plug weight) the lag time was moderately affected. Thus, it is observed that with the increase in Drug: Polymer ratio (X1) there is an increase in Lag time whereas, with the increase in Polymer: Polymer ratio (X2) the lag time was at a moderate level. With further increase in X3 (Plug weight) i.e., at a higher level of X3, the lag time increased up to 4 hours. Contour plot at centre level (Figure 5C) and 3-D response surface plot at higher level of X3 (Figure 6C) shows the inclining trend of lag time with increase in concentrations of polymer: polymer and drug: polymer [13].

Validation of Design

From the Figure 7, Predicted vs. Actual responses, predicted responses of all the formulations in terms of % release in 4 hrs, % release in 10hrs and lag time are close to the actual responses. The predicted vs actual responses showed a correlation coefficient of 0.786 for % release in 4hrs, 0.9744 for % release in 10hrs and 0.6281 for lag time, it is proved that the design applied is significantly fitting the data and thus the design is validated.

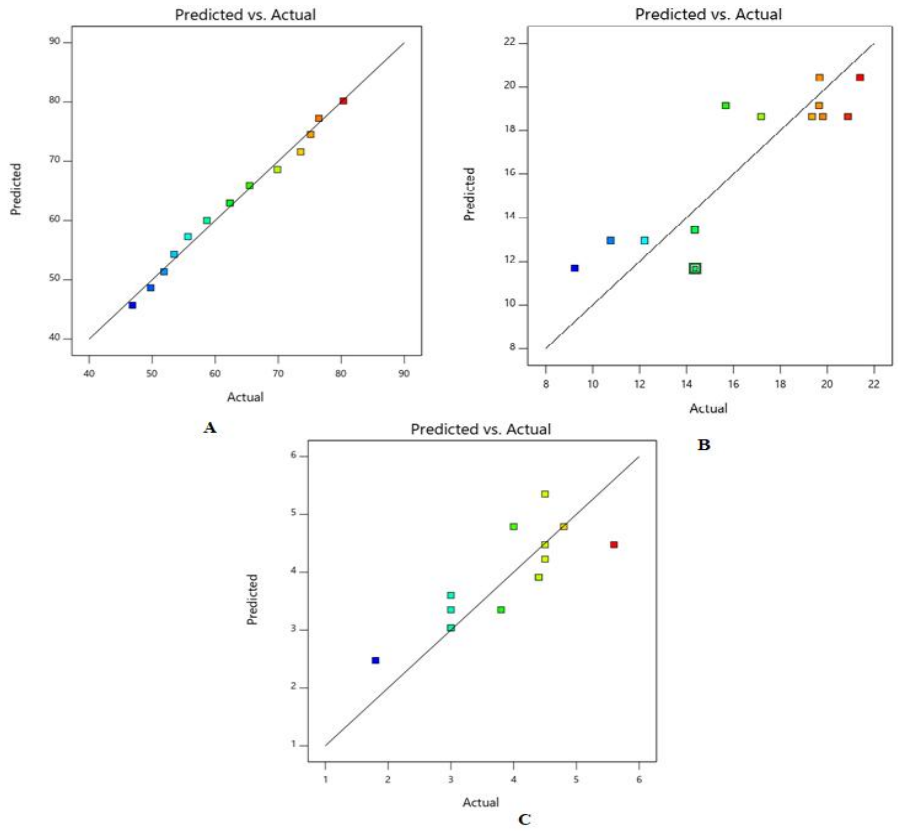

Figure 7: Predicted Vs Actual plot for responses A) % drug release in 10 hours B) % drug release in 4 hours and C) lag time (hrs)

Optimized formulation

After the application of BBD design, optimized formulations were produced which were targeted to show 10% to 20% of drug release in 4 hours, 60% to 70% of drug release in 10 hours with a lag time of 4.5 to 6hours. The optimum variables were obtained by numerical analysis based on the criterion of desirability [14]. Figure 8 shows that the suggested optimized formulation code was 0.84, 0.25 and -0.78 for X1(drug: polymer), X2 (polymer: polymer) and X3(plug weight) respectively. The value of predicted responses for R1, R2 and R3 were 17.978%, 69.79 % and 4.58h respectively. Formulation G1 was formulated and subjected to evaluation. It showed 17.16% release at the end of 4 hours, 69.45% release at the end of 10 hours and 4.58 hours lag time. The actual values were similar to predicted values with correlation coefficient of 0.9537.

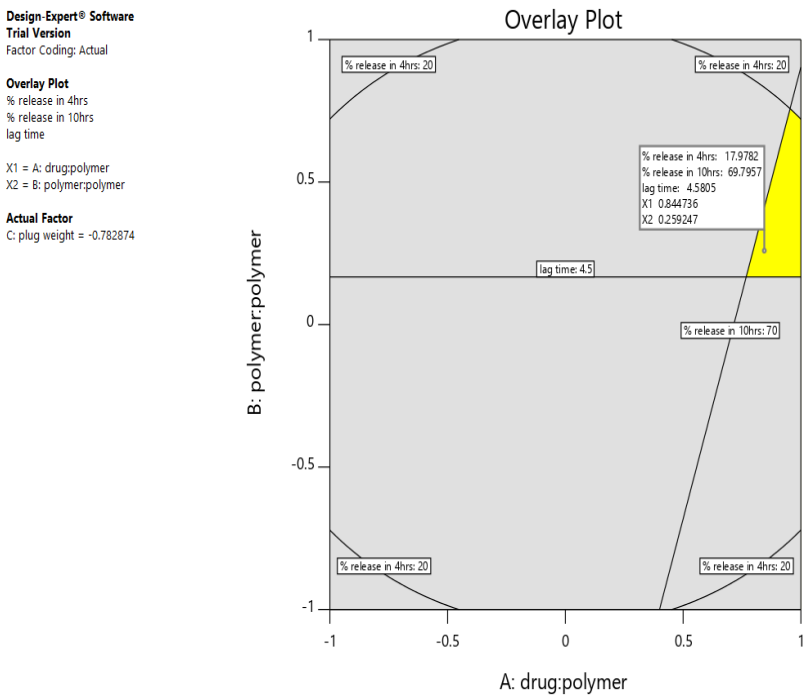

Figure 8: Overlay plot for optimization

Model dependent kinetics

Model dependent kinetics was done for all the 15 formulations obtained by design application to determine the release kinetics, release mechanism, drug transport mechanism. From the results of drug release kinetics, it was found that optimized formulation G1 follows zero-order drug release kinetics and the Korsmeyer-Peppas drug release mechanism. From the values of release component "n," it can be concluded that the formulation has a Super Case-II transport drug release mechanism.

Stability Studies

Accelerated stability studies were conducted for the optimized formulation and data revealed that there were no significant changes in the physical appearance, drug content and dissolution studies.

CONCLUSION

Fexofenadine HCl used in the treatment of allergic rhinitis has been formulated as a Pulsatile drug delivery system using the 'Pulsincap technique'. FTIR and DSC studies indicated the drug and excipients were compatible. Formaldehyde treated capsule bodies of 6 hrs formaldehyde exposure time were optimized as they were found to be intact for up to 10 hours and their formaldehyde content was found to be within official limits (50mg/day). Based on the evaluation tests results HPMC K4M was used in the formulation. Retardation of drug release was obtained by powder blend prepared using polymers HPMC E15 and Ethylcellulose which were further formulated by applying Box-Behnken design. All the evaluations of powder blend, pulsincap were within acceptable limits. Assay

of all the formulations is within the acceptable limits (95-100%). From the Box-Behnken design, it was found that the responses, i.e., % drug release after 4 hours followed reduced quadratic model, % drug release after 10 hours and lag time followed the linear model based on adequate precision, predicted R^2 and adjusted R^2. The predicted vs actual responses showed a correlation coefficient of 0.786 for % release in 4hrs, 0.9744 for % release in 10hrs and 0.6281 for lag time, this indicates the validation of the Box-Behnken design. From overlay plots and numerical optimization, optimized formulations were produced which were targeted to show 10% to 20% of drug release in 4 hours, 60% to 70% of drug release in 10 hours with a lag time of 4.5 to 6 hours. Optimized formulation G1 showed the lag time of 4.58 hours, % drug release of 17.16% at the end of 4 hours and % drug release of 69.45% at the end of 10hrs. The predicted vs actual responses showed a correlation coefficient of 0.9537. Accelerated stability studies were conducted for the optimized formulation and it was found that formulations were stable for one month.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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