ORIGINAL ARTICLE

Formulation, Evaluation, and Optimization of Budesonide Pulsincap Drug Delivery System for Chronotherapy of Asthma

P Pratyusha Ande, R Nagaraju

Department of Pharmaceutics, Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam (Women's University) Tirupati – 517 502, Andhra Pradesh, India

Abstract

Aim: The present study was aimed to design and optimize colon-targeted drug delivery system of budesonide for the treatment of asthma. **Materials and Methods**: The system consists of formaldehyde-treated insoluble hard gelatin capsule body filled with budesonide-sustained release granules prepared with different polymers of $HPMCK_4M$, Eudragit L_{100}, and ethyl cellulose 20_{cps}. A response surface methodology based on central composite design was employed to investigate the influence of the amount of $HPMCK_{15}M$ (hydrogel plug) and KCl (osmogen) on the lag time and percent drug release. **Results and Discussion**: The optimized formulation (P_9) containing budesonide-sustained release granules of 120 mg, hydrogel plug of 100 mg, and osmogen of 80 mg, and formulation was prepared according to software determined levels. Both hydrogel plug and osmogen had a significant influence on the lag time and percent drug release. *In vivo* X-ray imaging study confirmed that the coated capsules dissolved at the targeted colon region. **Conclusion**: The present pulsincap formulation was effective in providing colon-targeted drug release after predetermined lag time.

Key words: Chronotherapy, modified pulsincap, pulsatile drug delivery system

INTRODUCTION

In the field of modified release, there has been a growing interest in time-specific oral delivery, which generally refers to pre-programmed release of drugs following administration to achieve improved therapeutic efficacy. These systems constitute a relatively new class of devices, with the recent advances in chronopharmacology.[1] Pulsatile drug delivery system is intended to deliver a rapid, transient, and quantified medication release after a predetermined off-release period (lag time).[2] Pulsatile system is beneficial for drugs where night time dosing is required such as antiasthmatic and antiarrhythmic drug where the disease severity is time dependent.[3] By developing pulsatile colon-targeted drug delivery system which is a site-specific drug delivery system plasma peak is achieved at an optimal time, number of doses can be reduced, and first-pass metabolism can be avoided.[4]

Pulsincap device consists of a non-disintegrating half capsule body sealed at the open end with a hydrogel plug, which is covered by a water-soluble cap. The whole unit is coated with an enteric polymer to avoid the problem of variable gastric emptying. When this capsule comes in contact with the dissolution fluid, it swells; and after a lag time, the plug pushes itself outside the capsule and rapidly releases the drug.[5]

Budesonide is an anti-inflammatory corticosteroid that exhibits potent glucocorticoid activity and weak mineralocorticoid activity. It has wide inhibitory activities against multiple cell types and mediators involved in allergic and non-allergic-mediated inflammation.[6]

Response surface methodology (RSM) is a collection of statistical and mathematical techniques, useful for developing, improving, and optimizing process. The advantage of such

Address for correspondence:
P Pratyusha Ande, Department of Pharmaceutics, Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam (Women's University) Tirupati – 517 502, Andhra Pradesh, India. Phone: +91-9441214158.
E-mail: Pharmapratyu@gmail.com

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methodology in providing a rationale for simultaneous of several variables with minimum experimentation and time, thus proving to be far more efficient and cost-effective than conventional methods of product development.[7]

The current study illustrates the development of colon-targeted pulsincap drug delivery system of budesonide for the treatment of asthma to modulate pulsatile release. A central composite design (CCD) was employed to investigate the influence of amount of $HPMCK_{15}M$ (hydrogel plug) and KCl (osmogen) on the lag time.

MATERIALS AND METHODS

Materials

Budesonide was a gift sample from Aurobindo lab, Hyderabad. $HPMCK_4M$, Eudragit L_{100}, ethyl cellulose 20_{CPS}, PvpK30, talc, Mg stearate, and lactose obtained from Bright Scientifics, Hyderabad, were used as components for the preparation of sustained release granules. Sodium alginate, guar gum, and $HPMCK_{15}M$ obtained from Bright Scientifics, Hyderabad, were used as components of hydrogel plug. All other ingredients and reagents were used of analytical grade.

Methods

Drug-excipient interactions

The physicochemical compatibilities of the drug and the used excipients were tested by Fourier-transform infrared (FTIR) studies and differential scanning calorimetry (DSC) studies.

Preparation of budesonide pulsincap delivery system

The development of pulsincap delivery system was carried out in two steps. First, sustained release granules are formulated using various polymers by wet granulation method. The formulations will then be compared and optimized based on their dissolution profile. Second, formulation of formaldehyde-treated gelatin capsules plugged with the optimized sustained release formulation and various hydrogel plugs.

Preparation of budesonide-sustained release granules

Budesonide granules were prepared by wet granulation method. The composition of different formulations used in the study is shown in the table. The $HPMCK_4M$, Eudragit L_{100}, and ethyl cellulose 20_{CPS} were sieved (no.60) separately and mixed with budesonide. The powders were blended and granulated with $PVPK_{30}$, isopropyl alcohol was used as granulating agents. The wet mass was passed through a mesh and granules were dried at 50°C for 1 h. The composition of each formulation is listed in Table 1.

Preparation of formaldehyde-treated budesonide pulsincap delivery system

The formulation of hydrogel plug was prepared by compressing different amounts of $HPMCK_{15}M$ and lactose monohydrate using tablet punching machine keeping variation in thickness and hardness values of tablet plug. This plug was then fitted into the body of hard gelatin capsule (containing granules equivalent to 9 mg of budesonide) which was cross-linked by exposing the capsule bodies to formaldehyde vapors in desiccator for 12 h. The treated body and cap of the capsules were sealed with a small amount of 5% ethyl cellulose ethanolic solution. The sealed capsules were completely coated with enteric coating (5% cellulose acetate phthalate) at 4% coating level to reduce variability in gastric emptying time.[8] The composition of each formulation is listed in Table 2.

Evaluation of budesonide-sustained release granules

Budesonide-sustained release granules were evaluated by various flow properties such as true density, bulk density, angle of repose, Hausner's ratio, and Carr's index. The granules were specifically evaluated for drug content and dissolution studies. All the above methods were performed according to the pharmacopoeial procedures, and results were expressed as mean ± standard deviation.[9]

In vitro dissolution study of budesonide-sustained release granules

Dissolution studies were carried out using USP II dissolution test apparatus (Basket) method. Capsules were placed in a

Table 1: Composition of budesonide-sustained release granules

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Budesonide	9	9	9	9	9	9	9	9	9
$HPMCK_4M$	25	50	75	-	-	-	-	-	-
Ethyl cellulose20_{CPS}	-	-	-	25	50	75	-	-	-
Eudragit L_{100}	-	-	-	-	-	-	25	50	75
$PvpK_{30}$	20	20	20	20	20	20	20	20	20
Talc	3	3	3	3	3	3	3	3	3
Mg stearate	3	3	3	3	3	3	3	3	3
Lactose	60	35	10	60	35	10	60	35	10
Total wt. (mg)	120	120	120	120	120	120	120	120	120

basket so that the capsule should be immersed completely in dissolution media but do not float. To simulate the pH changes along the gastrointestinal (GI) tract, three dissolution media with pH 1.2, 7.4, and 6.8 were sequentially used referred to as sequential pH change method. When performing experiments, the pH 1.2 medium was first used for 2 h (since the average gastric emptying time is 2 h), then removed and the fresh pH 7.4 phosphate buffer saline was added. After 3 h (average small intestinal transit time is 3 h), the medium was removed and fresh pH 6.8 dissolution medium was added for subsequent hours. 900 ml of the dissolution medium was used at each time. Rotation speed was 50 rpm and temperature was maintained at 37 ± 0.5°C. 5 ml of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analyzed at 245 nm by ultraviolet absorption spectroscopy.[9]

Evaluation of modified budesonide pulsincap formulations

Various physical tests include identification attributes as visual defect, dimensions, solubility studies of treated capsules, and chemical test were carried out simultaneously for formaldehyde-treated and untreated capsules. Variations in dimensions between formaldehyde, treated and untreated capsules were studied. The length and diameter of the capsules were measured before and after formaldehyde treatment, using dial caliper.[10]

Optimization of variables using CCD

CCD was used for optimization procedure. In this design, two factors were evaluated, each at three levels, and experimental trials were performed for all nine possible combinations by employing Design-Expert software® (Version 11.0 Stat-Ease Inc., Minneapolis, MN) listed in Table 3.

Stability studies

A short-term stability study was carried out by storing the optimized formulation at 40°C/75% RH for a period of 3 months. At monthly intervals, formulation was observed for any physical changes during the period of storage. At the end of 3rd month, formulation was analyzed for drug content and drug release profile.[11]

In vivo release study

The *in vivo* X-ray studies of dosage form were performed on healthy adult male rabbits (weighed: 3.2–3.5 Kg, mean 3.3 ± 0.12 Kg, aged: 8–10 months) using optima 646 X-ray generating unit. The studies were carried under the supervision of an expert radiologist. The protocol was approved by IAEC no. 769/2011/CPCSEA. Radiography was performed at as per the protocol requirements, and the optimized formulation was prepared for *in vivo* studies using barium sulfate as radiopaque material. The opaque should replace the active ingredient in the dosage form. The images were recorded at the tie intervals of 4, 8, 16, and 28 h using an online computer system, stored on magnetic disk, and analyzed to determine the distribution of activity in the stomach and intestine and colonic region.[12]

Pharmacokinetic studies

Four healthy adult male rabbits (weighed: 3.2–3.5 Kg, mean 3.3 ± 0.12 Kg, aged: 8–10 months) were enrolled in the study. The protocol was approved by IAEC no. 769/2011/CPCSEA. Rabbits were fasted for 12 h with free access to water by *ad libitum* before the study started. A single-dose, two cross-over design study was conducted on rabbits. There was a washout period of 1 week between the two doses. In the first stage, four rabbits received a dose of test formulation, whereas after 1 week, the second stage rabbits received the same dose of Budefex 3 mg (reference product) to complete the cross-over design. The dose was given by oral route. Blood sampling rabbits were placed in rabbit restraining box apparatus. Inserting a small needle (23 gauge) butterfly attached to a syringe in the marginal ear vein. Serial venous blood samples were collected (0.5 ml) in Vacutainer tubes according to the time schedule 0.0, 1.0, 2.0, 4.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0, 16.0, 20.0, 24.0, and 28 h after rabbits received the dose. Blood samples were centrifuged at 3000 rpm for 5 min, and serum was transferred into clean plastic tubes.

Table 2: Composition of modified budesonide pulsincap formulations

Ingredients	P1	P2	P3	P4	P5	P6	P7	P8	P9
Budesonide granules	120	120	120	120	120	120	120	120	120
$HPMCK_{15}M$	60	60	60	80	80	80	100	100	100
KCl	40	60	80	40	60	80	40	60	80
Lactose	130	110	90	110	90	70	90	70	50
Total mg	350	350	350	350	350	350	350	350	350

Table 3: Experimental design - factors and responses

Factors (independent variables)	Levels used			Responses (dependent variables)
	−1	0	+1	
X1=Amount of $HPMCK_{15}M$ (hydrogel plug) mg	60	80	100	Y1=Lag time minutes
X2=Amount of KCl (osmogen) mg	40	60	80	Y2= % drug release in 16 h

The plasma pharmacokinetic parameters were estimated. It included the observed maximum plasma concentration C_{max}, the time to reach C_{max}, T_{max}, and the area under the plasma concentration-time curve from 0 h to last measurable concentration (area under the curve [AUC_{0-t}]) and 0 h to infinity ($AUC_{0-\infty}$). The C_{max} and T_{max} were directly determined by the serum concentration versus time curves. The AUC from 0 h to t (AUC_{0-t}) was calculated by the linear trapezoidal rule. The AUC from 0 h to infinity ($AUC_{0-\infty}$) was estimated by summing the area from AUC_{0-t} to $AUC_{0-\infty}$.[13-15]

RESULTS AND DISCUSSION

Drug-excipient compatibility study by FTIR

Drug and excipient compatibility was confirmed by comparing spectra of FTIR analysis of pure drug and the optimized formulation. It was found to be identical, indicating that characteristic peaks were not altered with the excipients used in the formulations.

DSC

DSC was performed for pure drug and the optimized formulation. There were no changes in the glass transition temperature of budesonide when DSC was performed with other excipients.

Evaluation of budesonide-sustained release granules

The flow properties were carried out for sustained release granules. The results fall within the official range for good flow. Therefore, the blends have good flow property. The drug content for sustained release granules is shown in Figure 1.

In vitro dissolution study of budesonide-sustained release granules

The formulations F1-F9 were evaluated for *in vitro* drug release studies. F1 formulation showed drug release up to 6 h which is not suitable for pulsincap technology. Remaining all formulations showed drug release up to 10 h. However, F6 formulation showed drug release up to 14 h which is suitable for pulsincap drug delivery for the chronotherapeutic approach of asthma; hence, the formulation was selected for the modified pulsincap system which is plugged with the swellable polymer and osmogen fitted into the formaldehyde-treated capsule bodies. Drug release profiles of F1-F9 formulations are shown in Figure 2.

Physical evaluation of hydrogel plug

The hydrogel plugs prepared with the polymers of $HPMCK_4M$, Methocel A_4CP, and $HPMCK_{15}M$ were evaluated by thickness, hardness, and lag time which is shown in Table 4. It was found that hydrogel plug prepared with $HPMCK_{15}M$ showed 7 h of lag time which is selected for pulsincap drug delivery system.

Physical evaluation of formaldehyde-treated empty capsules

The formaldehyde-treated empty capsules (cap and body) were evaluated for diameter, length, weight, and solubility. The results are shown in Table 5. When the capsules were subjected to solubility studies conclude that 12 h formaldehyde treatment is sufficient to sustain the release for 28 h and found the capsule maintained the physical stability during the dissolution process.

Design of experiment

CCD was employed to evaluate the effects of independent variables on the responses and for optimization of the formulations. In this study, independent variables were the amount of HPCK15M (X1) and KCl (X2). Dependent variables were the lag time (Y1) and percentage drug release in 16 h (Y2). Data were fitted by Design-Expert software (version 11.0, stat-Ease, Inc., Minneapolis, MN) and three-dimensional (3D) responses were also provided. According

Table 4: Physical evaluation of hydrogel plug

Hydrogel	Thickness in mm	% Swelling index after 4 h	Lag time (h)
$HPMCK_4M$	2.13±0.36	135.8	5
Methocel A_4CP	2.46±0.32	123.6	4
$HPMCK_{15}M$	2.31±0.42	156.5	7

Table 5: Physical characteristics of formaldehyde-treated empty capsules

Parameters	Capsule		Body	
	Before treatment	After treatment	Before treatment	After treatment
Diameter (mm)	8.08 ± 0.56	7.89 ± 0.36	7.23 ± 0.33	6.94 ± 0.62
Length (mm)	21.05 ± 1.22	20.16 ± 0.89	18.24 ± 1.02	17.86 ± 0.83
Weight (mg)	65.6 ± 0.35	67.28 ± 0.34	58.6 ± 0.56	60.32 ± 0.18

Table 6: Lag time and dissolution studies as per experimental design

Std	Run	Factor 1	Factor 2	Response 1	Response 2
		A: HPMCK 15	B: KCl	Lag time	% drug release in 16 h
		mg	mg	Min	h
5	1	51.7157	60	180	83.9
2	2	100	40	310	73.1
6	3	108.284	60	340	66.1
4	4	100	80	400	56.7
3	5	60	80	240	70.6
7	6	80	31.7157	220	90.5
1	7	60	40	140	97.5
13	8	80	60	250	86.1
8	9	80	88.2843	290	78.5
11	10	80	60	250	86.1
10	11	80	60	250	86.1
9	12	80	60	250	86.1
12	13	80	60	250	86.1

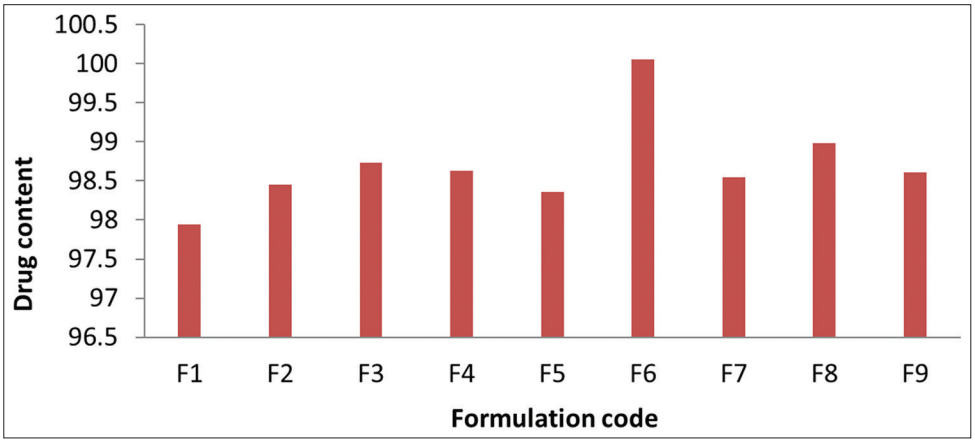

Figure 1: Drug content of formulations F1-F9

to the software, 13 runs were required to develop appropriate models. Statistical significance differences of the variables and responses were measured by ANOVA test ($P < 0.05$). Response data determined as per CCD are presented in Table 6. Drug release profiles of the experimental runs are shown in Figure 3.

The significance of the model was estimated by applying ANOVA at 5% significance level for the responses of lag time, and percent drug release is shown in Tables 7 and 8.

The predicted R^2 of 0.8587 is in reasonable agreement with the adjusted R^2 of 0.9186, i.e., the difference is <0.2. This model is significant and can be used to navigate the design space.

Table 7: Fit statistics for response 1: Lag time

SD	18.90	R^2	0.9322
Mean	259.23	Adjusted R^2	0.9186
C.V. %	7.29	Predicted R^2	0.8587
		Adeq precision	23.2736

SD: Standard deviation

Table 8: Fit statistics for response 2: Percent drug release in 16 h

SD	4.84	R^2	0.8899
Mean	80.57	Adjusted R^2	0.8112
C.V. %	6.00	Predicted R^2	0.7869
		Adeq precision	10.3637

SD: Standard deviation

The predicted R^2 of 0.78690 is as close to the adjusted R^2 of 0.8112, i.e., the difference is <0.2. This model is significant and can be used to navigate the design space.

Response surface analysis

The quadratic models generated by regression analysis were used to construct 3D response surface plots in which response parameters Y were represented by a curvature surface as a function of factors X. Figures 4 and 5 show the effect of two factors on the lag time and % drug release.

Figures 4 and 5 show a linear synergistic relationship between the two independent variables (factors) on response Y1 (lag time). This increase in lag time might be due to decreased permeability and increased hydrophobicity of CAP coating at 4% coating level as well as increased concentration of swellable polymer and osmogen. This receives confirmation from the R^2 values listed in Tables 7 and 8.

Stability studies

A short-term stability study was carried out for a period of 3 months. There were no physical changes observed after completion of 3 months. The % drug release, drug content, and lag time were found to be 97.9, 99.0, and 7 h, respectively [Table 9].

Table 9: Stability studies of optimized budesonide pulsincap formulation

Parameters	Initial	1 month	2 months	3 months	Limits as per specification
40°C/75% RH % release at 28th h	98.9	98.6	98.2	97.9	Not<85%
40°C/75% RH assay value	100.1	99.41	99.2	99.0	Not<90% not more than 110%
Lag time h	7	7	7	7	No change in the lag time

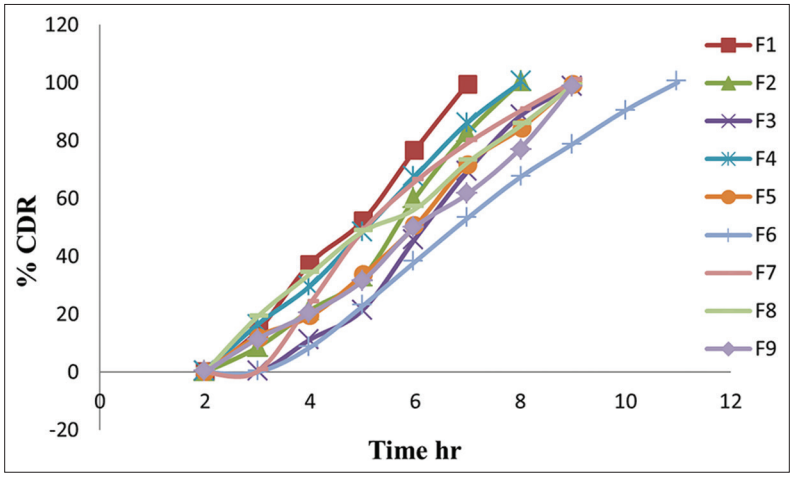

Figure 2: Drug release profile of budesonide-sustained release granules

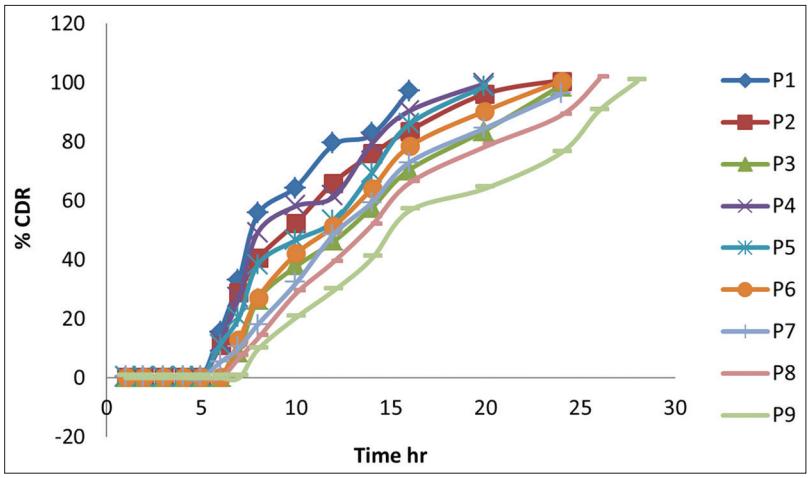

Figure 3: Dissolution profile of the experimental runs

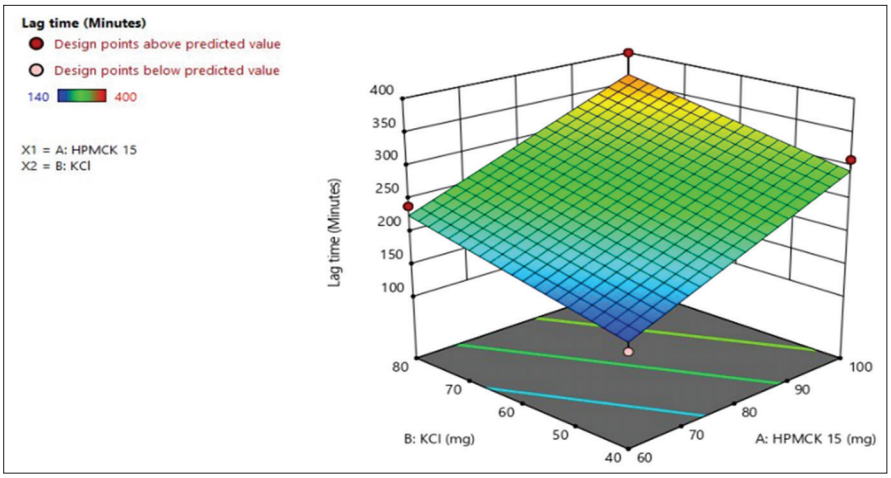

Figure 4: Response surface plot showing the influence of polymer (X1) and osmogen (X2) on response Y1 (lag time, min)

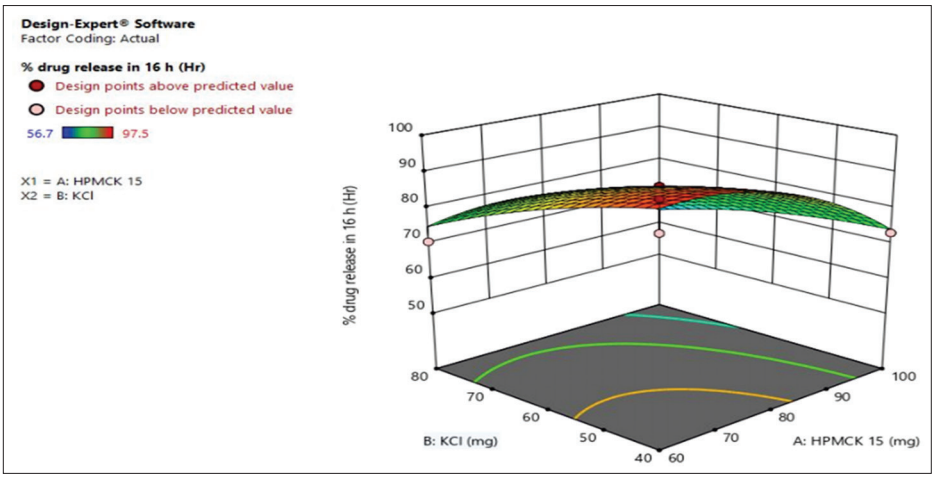

Figure 5: Response surface plot showing the influence of polymer (X1) and osmogen (X2) on response Y2 (% drug release in 16 h)

In vivo release study

In vivo X-ray imaging was employed in rabbits to trace the movement and behavior of the capsule in GI tract. The results of X-ray imaging study are shown in Figure 6a-d. It presents the capsule remained intact in the stomach by cellulose acetate coating of formaldehyde-treated capsules at 4% coating level. It was also found that no significant difference was observed in the integrity of the capsule stomach and small intestine. Reduction in size of capsule indicated that the capsules were broken down and released the drug in colon at 28th h. The results are in accordance with the fact that the optimized formulation could be targeted specifically to the colon, without any premature drug release in the stomach and small intestine.[12]

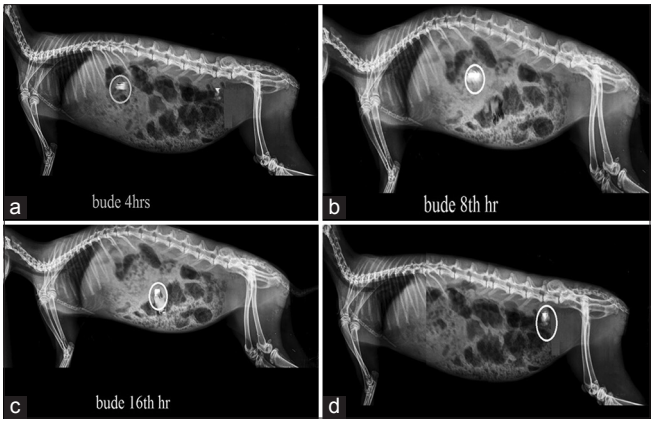

Figure 6a-d: X-ray images of the gastrointestinal tract of a rabbit, showing the movement of capsule from the stomach to the colon

Pharmacokinetic evaluation

The pharmacokinetic parameters such as C_{max}, T_{max}, and the area under the plasma concentration-time curve from 0 h to last measurable concentration AUC_{0-t} and 0 h to infinity $AUC_{0-\infty}$ were calculated for optimized formulation and the marketed formulation. The results showed that the optimized novel formulation met the chronotherapeutic approach for the better treatment of asthma. Pk parameter plots of budesonide optimized formulation and marketed formulation are shown in Figure 7a-d. The values are presented in Table 10.

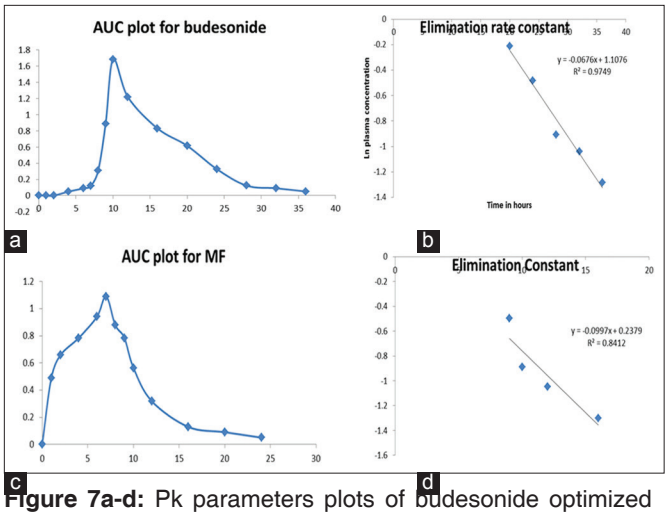

Figure 7a-d: Pk parameters plots of budesonide optimized formulation and marketed formulation

Table 10: Pharmacokinetic parameters of optimized formulation and marketed formulation

Parameters	Optimized formulation	Marketed formulation
C max (ng/ml)	1.684	1.089
Tmax	10 h	7 h
AUC ($_{0-t}$) (ng.mg/ml)	15.795	7.4
Ke	0.067	0.099
T 1/2	10.34328	7
AUC ($_{t-inf}$) (ng.mg/ml	0.776119	0
AUC ($_{0-inf}$) (ng.mg/ml	16.57112	7.4

AUC: Area under the curve

This study revealed that the modified pulsincap drug delivery system of budesonide formulated using HPMCK$_{15}$M of 100 mg and KCl of 80 mg was effective in providing colon-targeted drug release for the effective treatment of asthma. It was found to be better than marketed formulation.

CONCLUSION

The present study demonstrates that budesonide pulsincap drug delivery system could be successfully delivered to colon region which met the chronotherapeutic approach for the better treatment of asthma. Regarding the optimization, CCD can be successfully used for achieving desired responses, lag time, and drug release profile, after preprogrammed off period. From the RSM, it is easy to understand the change of responses with independent variables and for locating the desired area of interest. *In vivo* X-ray study has shown the prepared optimized formulation release the drug in the colon region met the chronotherapeutic approach. The optimized formulation when compared to the marketed formulation has shown better pharmacokinetics parameters for the effective treatment of asthma.

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