OPTIMISATION OF CHRONOMODULATED DOSAGE FORM FOR ASTHMA USING FULL FACTORIAL DESIGN

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ABSTRACT

Chronotherapy is growing treatment method in various diseases. Various diseases like asthma, hypertension, and arthritis show circadian variation, that demand time scheduled drug release for effective drug action. In Asthma like diseases which follow circadian rhythms, it is necessary to modulate the drug release in synchrony with the circadian rhythm of nocturnal asthma. Different polymers like Ethyl cellulose 10, 20, 45 cps, Compritol, Sodium alginate, Sodium CMC and LH-21 were tried. The combination of Eudragit RSPO and HPMC K4M was selected for the study based on lag time and release characteristics. On the basis of the trial batches and their evaluation parameters, concentrations of the polymers were decided and the 2^3 full factorial design was applied for the formulation of tablets. Lag time prior to drug release and cumulative percentage drug release at 3hrs and 6hrs were selected as responses. Results revealed that both, the coating composition and coating level, are significant factors affecting drug release profile.

KEYWORDS- Pulsatile, Full factorial design, Press coated, Asthma

INTRODUCTION

Conventional modified and controlled release dosage forms deliver drugs at controlled rate as compared to conventional dosage forms. However, they do not provide relief from symptoms and protection from adverse events solely when necessary. Development of newer systems which provide a measured dose, at a controlled rate, at a decided time, to a targeted site is now a growing challenge.

Particular rhythms in the onset and extent of symptoms were observed in diseases such as, bronchial asthma, myocardial infarction, angina pectoris, rheumatic disease, ulcer, diabetes, attention deficit syndrome, hypercholesterolemia, and hypertension. In Asthma like diseases which follow circadian rhythms, it is necessary to modulate the drug release in synchrony with the circadian rhythm of nocturnal asthma. Salbutamol Sulphate is very highly used in chronic treatment. However, it has shortcomings like short half-life, high first pass metabolism and high tolerance rate. This makes it necessary to formulate it in Chronomodulated form which exposes the Salbutamol Sulphate when it is actually required. In the present research, we have attempted to develop a novel dosage form by using a chronopharmaceutical approach. Tablets were formulated by tablet in tablet technology. It is so programmed that a medication which is taken at 10.00 pm while sleeping will automatically give pulsed release at 4.00 am in the morning. Full factorial design was used to find the impact of various factors.

MATERIALS AND METHODS

Materials

Salbutamol Sulphate was obtained as a gift sample from Jayco chemicals, Mumbai. Eudragit RSPO was kindly gifted by Evonik Degussa pvt.ltd. HPMC K4M was kindly supplied by Colorcon Asia pvt.ltd. Micro Crystalline Cellulose, Croscarmellose Sodium, Sodium Starch Glycolate and Crosspovidone were kindly supplied by Maple biotech pvt.ltd. Magnesium Stearate was procured from J.P.fine chemicals. All chemicals used were of analytical grade.

Preparation of Core Tablets: [7, 8]

Tablet in tablet technology was used for the formulation. It is as shown in figure 1. The core tablets containing Salbutamol Sulphate were prepared by using Cross-carmellose sodium, Avicel PH 102 and Magnesium stearate. All excipients were mixed for 20 min and passed through a 40 mesh size sieve and directly compressed in to 70 mg tablets using 6 mm round flat punches on a rotary tablet machine.

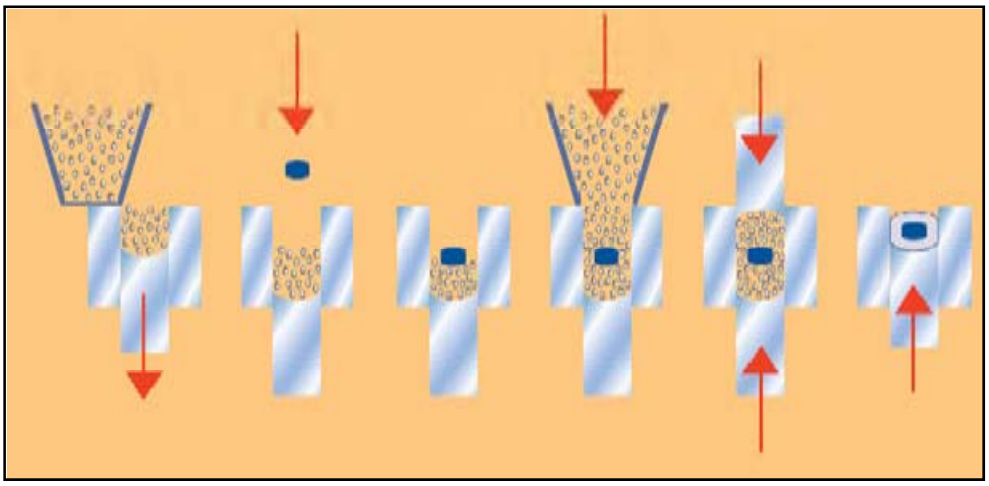

Figure 1. Method to prepare press coated pulsatile tablets[1]

Selection of Polymer and polymer concentration for optimisation: [9, 10]

Prior to selecting the suitable polymers some polymers like Ethyl cellulose 10, 20, 45 cps, Compritol, Sodium alginate, Sodium CMC and LH-21 were tried. The combination of Eudragit RSPO and HPMC K4M was selected for the study based on lag time and release characteristics.

For selecting the polymer blend ratio and to take these results as the basis for optimisation it was first necessary to use these polymers over wide concentration range. So, at first different concentrations were used to determine the concentration suitable for optimisation. Coating levels was also modified i.e. 250 mg and 300 mg.

Preparation of Press Coated Pulsatile Tablets: [6, 11, 12]

The core tablets were press coated with polymer blend. Polymer blend was composed of HPMC K4M and Eudragit RSPO in different concentrations. Half of the coating material was placed in the die cavity, the core tablet was carefully positioned in the centre of the die and cavity was filled with the other half of the coating material. Coating materials was compressed around the core tablet using 10 mm punch.

FACTORIAL DESIGN [12, 13]

On the basis of the trial batches and their evaluation parameters, concentrations of the polymers were decided and the 2^3 full factorial design was applied for the formulation of tablets. Experiments were carried out to determine the mathematical relationship between the factors acting on the system and the response of the system. The statistical evaluation of experimental outcomes was processed with Design Expert. The batches thus prepared by factorial design are evaluated and the effect of individual variable is studied by using percent drug release at 3 hrs, drug release at 6 hrs and lag time as dependent variables.

The concentrations of the variables used in the formulation of the tablets were decided on the basis of trial batches and their evaluation. The coded levels and the exact concentration of the variables used in different formulations are shown in table 1. Dependent variables that were selected are shown in table 2 with their coding. These responses were used to know the effect of independent variables.

Table 1: Coded levels and their values used in the formulation

Factor	Name	Unit	Minimum (-1)	Maximum (1)
A	HPMC K4M	mg	25	30
B	Eudragit RSPO	mg	65	70
C	Coating Level	mg	250	300

Table 2: Responses recorded with their codes

Response	Name	Units	Observations	Model
Y1	% Drug release at 3hrs	%	8	3FI
Y2	% Drug release at 6 hrs	%	8	3FI
Y3	Lag time	minutes	8	3FI

Preparation of Final Batches for Optimisation Study [12, 13, 14]

The final batches of the tablets were prepared according to the factorial design. The study was designed to study the effect of concentration of individual polymers. The remaining weight in polymer blend was adjusted by MCC. The various batches were prepared according to the concentrations as per shown in table 3.

Table 3: Formulation of optimisation (final) batches of Salbutamol Sulphate

Batch code	Eudragit RSPO (%)	HPMC K4M (%)	Weight of coating material
F_1	65	25	250 mg
F_2	70	25	250 mg
F_3	65	30	250 mg
F_4	70	30	250 mg
F_5	65	25	300 mg
F_6	70	25	300 mg
F_7	65	30	300 mg
F_8	70	30	300 mg

Evaluation of tablets [15, 16, 17]

All prepared tablets were evaluated for Thickness, Diameter, Weight Variation, Hardness, Friability, % Drug content etc. as per I.P. specifications.

***In-Vitro* drug release**

The *in vitro* drug release from coated tablets was carried out using USP paddle apparatus at 50 rpm. HCl (0.1 N) and phosphate buffer (pH 6.8) were used as the dissolution medium. Initially tablets were subjected to dissolution in 0.1 HCl for 2h and after that media were changed to phosphate buffer (pH 6.8). The samples were withdrawn at regular intervals and analyzed by UV spectrophotometer at 225 nm for the presence of the drug. Dissolution tests were performed in triplicate.

RESULTS AND DISCUSSION

Evaluation of Final batches

Table 4: Evaluation of Final batches

Batch code	Thickness (mm)	Diameter (mm)	Weight Variation	Hardness (kg/cm^2)	Friability (%)	% Drug content
F_1	2.32±0.24	10.04±0.03	319.2±0.5	5.2±0.3	0.433±0.022	99.71±0.20
F_2	2.31±0.21	10.01±0.02	320.25±0.12	5.5±0.4	0.414±0.07	99.6±0.34
F_3	2.32±0.22	10.05±0.01	322.54±0.31	5.7±0.1	0.419±0.036	99.64±0.43
F_4	2.4±0.31	9.98±0.04	321.29±0.12	5.8±0.1	0.422±0.1	99.56±0.57
F_5	2.97±0.12	10.01±0.03	371.17±0.19	5.5±0.4	0.427±0.053	99.97±0.65
F_6	2.99±0.16	10.03±0.02	369.43±0.17	5.6±0.3	0.423±0.018	99.87±0.59
F_7	3.02±0.17	10.02±0.05	370.11±0.27	5.5±0.4	0.439±0.012	99.28±0.16
F_8	3.05±0.1	10.04±0.06	370.22±0.45	5.4±0.4	0.408±0.034	99.94±0.48

n=3

Drug Content Uniformity:

The results of mean percentage drug content uniformity of all the 8 formulations are shown in table no.6.13. The results show that the % drug content uniformity is within the range of 99.28±0.16 to 99.97±0.65. It insures that each tablet contains sufficient amount of drug as per stated limits.

In-vitro drug release and lag time:

The factors that were selected as responses were carefully monitored. In-vitro drug release of formulations was totally dependent on the lag time. Formulations showed complete drug release after lag time.

Table 5: Responses of Final Batches

Batch code	% DR at 3 hrs	% DR at 6 hours	Lag time (min)
F_1	92.78	90.23	170
F_2	16.13	92.3	230
F_3	12.16	93.5	275
F_4	8.21	94.7	310
F_5	6.28	93.28	315
F_6	0	94.11	350
F_7	0	12.24	375
F_8	0	9.96	410

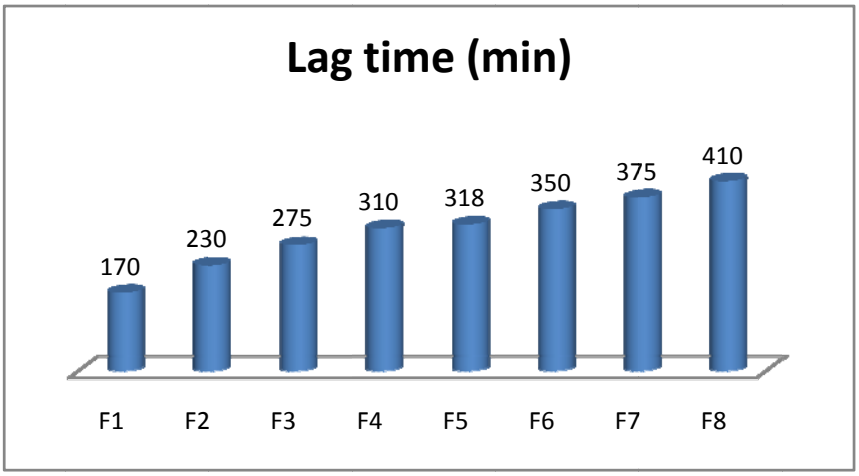

Fig. 2: Comparative Lag Time of F_1 to F_8 Formulations.

ANOVA ANALYSIS

Table 6 and 7 shows the results of the analysis of variance (ANOVA), which was used to generate mathematical models. The high values of correlation Coefficient for % Drug Release at 3 hrs indicates a good fit i.e. good agreement between the dependent and independent variable. The Model F-value of 9.52 implies the model is significant. There is only a 4.86% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case C is significant model term. High R-square values suggest that these models are significant. In this case model generated response parameters are significant. PRESS (Predicted Residual Sum of Squares) is a measure of how well the model fits each point in the design. Smaller the PRESS statistic, the better the model fits the data points. Small values for the same in this model show a good fit of the data points.

Table 6: ANOVA analysis for Drug release at 3 hrs.

Source	Sum of Squares	Mean Square	F Value	p-value Prob > F
Model	66.85728	16.71452	9.515457	0.0486
A-HPMC K4M	12.00995	12.00995	6.695555	0.0815
B-Eudragit RSPO	9.556804	9.556804	5.526519	0.1042
C-Coating Level	58.2589	58.2589	21.52295	0.0191
AB	7.051652	7.051652	5.918958	0.1421

Table 7: ANOVA analysis for various factors.

Factor	Drug release at 3 hrs	Drug release at 6 hrs	Lag time
Std. Dev.	1.3395	0.79196	8.83
Mean	2.81336	72.54	304.375
C.V. %	47.6121	1.09175	2.90392
PRESS	38.2775	40.1408	25.0

Table 8 shows the results of the analysis of variance (ANOVA), which was used to generate mathematical model. The high values of correlation coefficient for % Drug Release at 6 hrs indicate a good fit i.e. good agreement between the dependent and independent variable. The Model F-value of 2678.814 implies the model is significant.

Table 8: ANOVA analysis for Drug release at 6 hrs.

Source	Sum of Squares	Mean Square	F Value	p-value Prob > F
Model	10080.91	1680.152	2678.814	0.0148
A-HPMC K4MA	5180.829	5180.829	5071.474	0.0089
B-Eudragit RSPO	0.41405	0.41405	0.660156	0.5656
C-Coating Level	5245.762	5245.762	5175.004	0.0088
AB	1.98005	1.98005	5.156967	0.5265
AC	5649.142	5649.142	5818.148	0.0085
BC	2.7848	2.7848	4.440051	0.2821

The Model F-value of 2678.81 implies the model is significant. There is only a 1.48% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C, AC are significant model terms. High R-square values suggest that these models are significant. In this case model generated response parameters are significant. PRESS (Predicted Residual Sum of Squares) is a measure of how well the model fits each point in the design. Smaller the PRESS statistic, the better the model fits the data points. Small values for the same in this model show a good fit of the data points. ‘Adeq. Precision’ measures the signal to noise ratio. A ratio of 113.63 indicate the adequate signal.

Table 9 shows the results of the analysis of variance (ANOVA), which was used to generate mathematical model. The high values of correlation coefficient for lag time indicate a good fit i.e. good agreement between the dependent and independent variable. Model F-value of 109.22 implies the model is significant. There is only a 0.91% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant.

Table 9: ANOVA analysis for Lag time.

Source	Sum of Squares	Mean Square	F Value	p-value Prob > F
Model	42665.65	8555.125	109.224	0.0091
A-HPMC K4MA	11628.15	11628.15	148.84	0.0067
B-Eudragit RSPO	5405.125	5405.125	45.56	0.0222
C-Coating Level	27028.15	27028.15	545.96	0.0029
AB	78.125	78.125	1	0.4226
AC	528.125	528.125	6.76	0.1215

In this case A, B, C are significant model terms. High R-square values suggest that the model is significant. In this case model generated response parameters are significant. PRESS (Predicted Residual Sum of Squares) is a measure of how well the model fits each point in the design. Smaller the PRESS statistic, the better the model fits the data points. Small values for the same in this model show a good fit of the data points. 'Adeq. Precision' measures the signal to noise ratio. A ratio of 30.53 indicate the adequate signal.

Table 10: R^2 values for all the Responses

Response	R^2
% Drug Release 3 hrs (Y_1)	0.9254
% Drug Release 6 hrs (Y_2)	0.9999
Lag time (Y_3)	0.9965

RESPONSE SURFACE PLOTS

All the data obtained was used to generate 3D plots and contour plots for the responses Y_1, Y_2 and Y_3. It was observed that for Y_1 the concentration of both the polymers affect the drug release. It was as shown in figure no. 3 and 4. The 3D plot for the response Y_1 clearly reveals that the concentration of HPMC K4M and Eudragit RSPO effects at the same extent in initial release of Salbutamol sulphate. Content of quaternary ammonium groups is 4 to 7%. The ammonium groups are present as salts and make the polymers permeable but permeability is less as compared to Eudragit RLPO. This is because the Eudragit RSPO being low permeable polymer allows less amount of dissolution medium to pass. HPMC K4M performs the work of holding the tablet coat due to its gelling property. The curved lines in contour plots prove that there exists some kind of interaction between these polymers. This was not the case in response Y_2. Drug release at 6 hours was prominently found dependent on the concentration of HPMC K4M. The drug release was more retarded by HPMC K4M as compared to Eudragit RSPO. The more steep side in 3D plot as shown in figure no.5 represents the influence of HPMC K4M while the other side shows the effect of Eudragit RSPO which is less steep. Therefore, it can be concluded that HPMC K4M is better retardant. However it cannot be used alone because the tablets will neither burst nor will show initial lag time else it will show a sustained type of drug release.

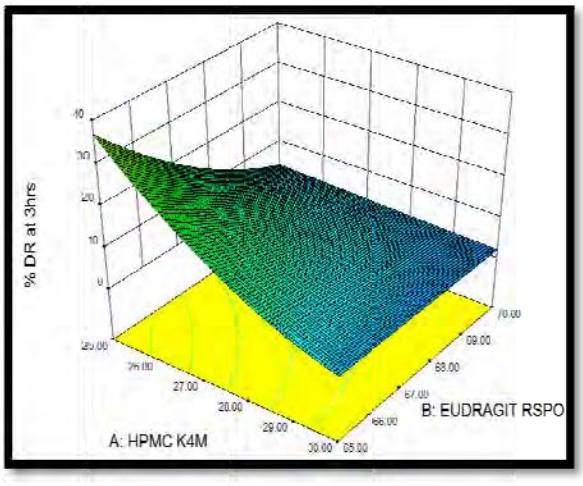

Fig. 3: 3D Response Surface Plot Showing Effect of Polymer Concentration on Drug Release at 3 Hours.

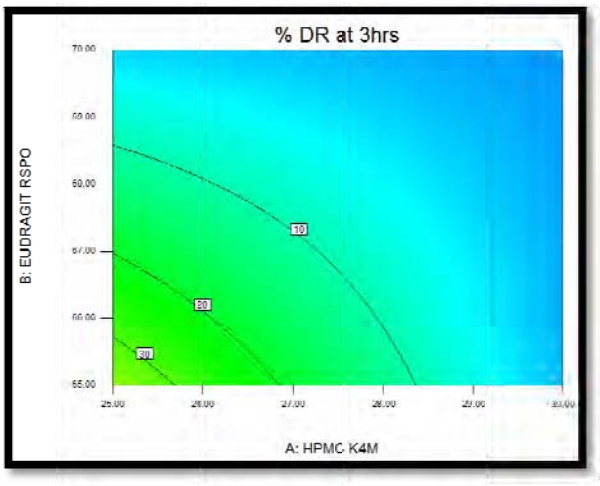

Fig. 4: Contour plot Showing Effect of Polymer Concentration on Drug Release at 3 Hours.

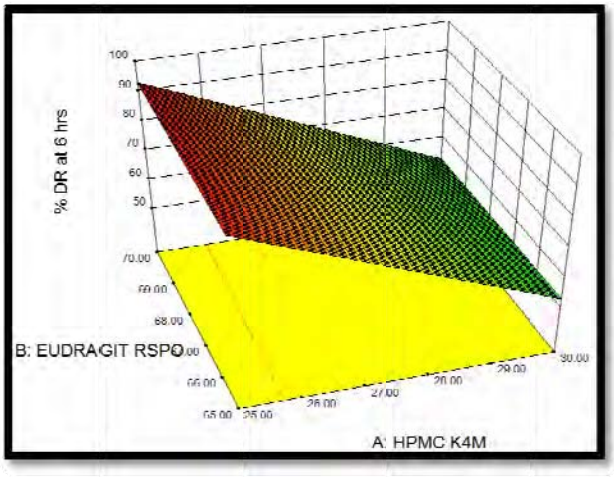

Fig. 5: 3D Response Surface Plot Showing Effect of Polymer Concentration on Drug Release at 6 Hours.

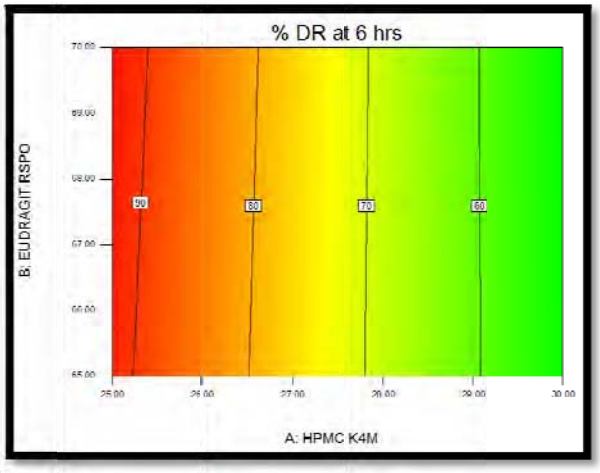

Fig. 6: Contour plot Showing Effect of Polymer Concentration on Drug Release at 6 Hours.

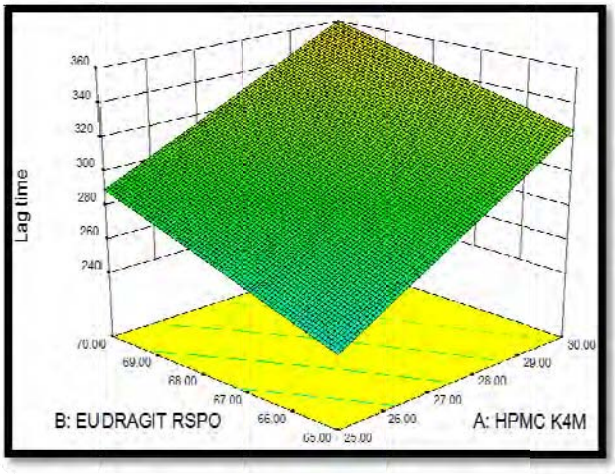

Fig. 7: 3D Response Surface Plot Showing Effect of Polymer Concentration on Lag time.

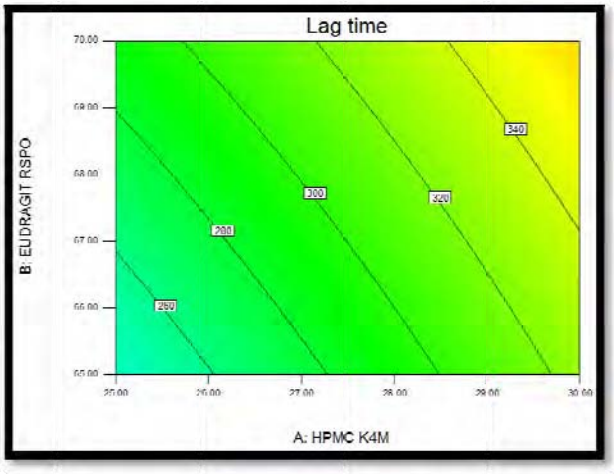

Fig. 8: Contour plot Showing Effect of Polymer Concentration on Lag time.

Comparatively straight lines were observed in contour plots indicating no interaction which was observed in initial 3 hours release. The effect on lag time was also similar as shown in figure 7 and 8. As the concentration of HPMC K4M increases it increases lag time sharply, because of its gelling strength. Eudragit RSPO also influence the lag time but less than HPMC K4M.

Equations generated were as follows:

$Y_{1=}$ 2.81336 -1.22525 X_1 -1.09298 X_2- 2.18686 X_3 + 0.937525 X_1X_2

Y_2=72.54 - 19.94X_1 + 0.2275X_2 - 20.1425X_3-0.4975X_1X_2 - 21.3575X_1X_3-0.59 X_2X_3

Y_3=304.375+38.125X_1+20.625X_2+58.125X_3-3.125X_1X_2-8.125X_1X_3

Negative sign in above equation indicate the retarding or decreasing effect and positive sign indicate enhancing or increasing effect on the respected responses.

From all the above studies including evaluation of tablets and application of statistical design it was concluded that batch F_6 could provide the best possible drug release and lag time as per the set objective of 6 hours lag time. The other batches which could also be satisfactory include F_4 and F_5 as they provide lag time of 310 and 318 minutes respectively.

CONCLUSION

The results obtained from ANOVA analysis, 3D plots and trial batches suggest that HPMC K4M plays a greater role in retarding the release of Salbutamol Sulphate. It is attributed to high viscosity of the polymer. HPMC K4M swells increasing the diffusional path length and its gelling nature holds the coat intact. Some amount of Salbutamol Sulphate diffuses out prior to lag time as it is highly soluble. Other polymer Eudragit RSPO contains quaternary ammonium groups is 4 to 7%. The ammonium groups are present as salts and make the polymers permeable but permeability is less due to very less content of ammonium groups. Eudragit RSPO

has less influence on Salbutamol Sulphate release. But, Eudragit RSPO is necessary for bursting of tablets as it is less intact in the HPMC k4M network. As time passes inner core tablets bursts due to pressure generated in it by Crosscarmellose Sodium. It was found that formulation was time dependent and formulation bursts after following predetermined lag time.

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