Hygeia.J.D.Med. July 2017 – December 2017 ISSN 2229 3590
Hygeia :: journal for drugs and medicines
July 2017
Open Access www.hygeiajournal.com
Research article section: Pharmaceutics
A Half Yearly Scientific, International, Open Access Journal for Drugs and Medicines
DOI:10.15254/H.J.D.Med.9.2017.161 Research Article

OPTIMIZED TRI-LAYERED PULSATILE TABLET OF SALBUTAMOL FOR CHRONODELIVERY IN ASTHMA

A. J. CHACKO*, JYOTI HARINDRAN
Dept. of Pharmaceutical Sciences, RIMSR, MG University, Rubber board PO. Kottayam. 686009

Key words: Chrono therapeutics, pulsatile, salbutamol sulphate, Trilayered tablet, Box-Behnken design, optimization.

Correspondence
A. J. Chacko M.Pharm.
Dept. of Pharmaceutical Sciences, RIMSR, MG University, Rubber board PO. Kottayam. 686009

Received: 11 January 2017,
Revised: 26 February 2017
Accepted: 11 March 2017,
Available online:15 July 2017

ABSTRACT

***Plan**: The study was aimed to develop a tri-layered pulsatile tablet of salbutamol sulphate for Chrono therapeutic delivery in bronchial asthma. Incidence of asthma is greatest in the early morning hours referred as ''morning dip''.*

***Preface**: A formulation which could deliver the drug in the right concentration just prior to the attack could effectively control the condition and staining drug release thereafter will further enable the dosage as once a day unit.*

***Methodology**: This formulation consists of three layers with the first layer for sustain release and the second for the burst release after a predetermined lag time and the third layer determines the lag time. A novel two stage coating was employed to achieve the burst followed by sustained release. The bottom and the sides of the tablets were coated with an insoluble polymer, ethyl cellulose which acted as a cage for the dosage form all sides except the top. The caged unit was finally dip coated with pH sensitive polymer eudragit L.*

***Outcome**: The formulation was optimized by Box-Behnken design and the composition suggested by the design expert successfully achieved the lag time of 5 hrs and cumulative drug release of 32% and 95% respectively at 6th and 24th hours*

INTRODUCTION

Chronobiology is the study of biological rhythms and their mechanisms. The term ''circadian'' was coined by Franz Halberg from the Latin circa, meaning about, and dies, meaning day. Diseases, such as hypertension, asthma, peptic ulcer, arthritis, etc, follow the body's circadian rhythm. They are naturally synchronized by the internal body clocks and are controlled by the sleep wake cycle. Each bodily system exhibits a peak time of functionality that is in accordance with these rhythmical cycles. Similarly, disease states too exhibit a peak time of activity within a circadian rhythm. Therefore the timing of drug administration in has significant impact on treatment success [1-4].

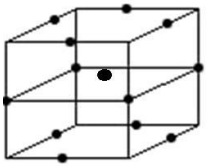
Corresponding author email: ajchacko01@yahoo.co.in
Mob: 0091 9447473432

Hygeia journal for drugs and medicines, 2229 3590
Rid: F-1516 -2017

"Morning dip" a popular term in asthma for a measurable decrease in expiratory air flow rates that commonly occurs between 2am and 4am, resulting in loss of sleep due to coughing and breathlessness. Normal lung function undergoes changes corresponding to the circadian changes with lowest in the early morning hours [4- 7].

Chronotherapy for asthma is aimed to get maximal effect from bronchodilator medications during early morning hours. It considers a person's biological rhythm in determining the timing and sometimes the amount of medication to optimize a drug's desired effect and minimize the undesired. Medications when formulated as chronotherapies results in better medication results and fewer adverse effects with better compliance [4 - 7].
Salbutamol sulphate is a short acting beta-2 adrenergic receptor agonist used for the relief of bronchospasm and chronic obstructive pulmonary disease. The plasma half life of 2.7 hours makes the drug a good candidate for the chronotherapeutic approach[8].

The Box – Behnken design an independent quadratic design was used to optimize the formulation. Here the treatment combinations are at the midpoints of edges of the process space and at the center.These designs are rotatable (or near rotatable) and require 3 levels of each factor[9, 10].



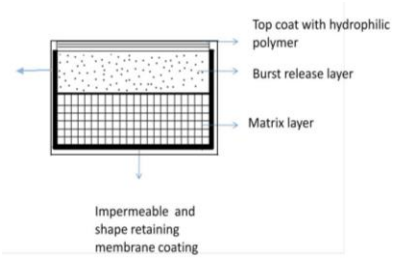


Fig.1.1: Illustration of Box- Behnken design. Fig. 1.2: Diagrammatic representation of tri-layered tablet

Box Behnken design investigated the effects of three factors such as Eudragit coating levels and amount of HPMC which determines the lag time, Guar gum the matrix forming material for sustaining drug release. The variables selected were lag time, cumulative percentage drug release at 6^{th} and 24^{th} hours. This method allows the determination of influence of the independent variables on response variables with a minimum number of experiments.

2. MATERIALS AND METHODS

2.1 Materials.

Salbutamol sulphate was used as the model drug (gifted by SANZ Pharmaceuticals, Kerala) Potassium dihydrogen phthalate and Ethyl cellulose (central drug house, New Delhi). Ethanol (Pampa Sugar Mills, Thiruvalla). All the other chemicals and solvents were of reagent grade.

2.2. Preparation of tri-layered pulsatile release tablet

The core tablet consisted of three layers; the first was the matrix layer contained 4 mg salbutamol, the second for burst release with 2 mg drug and the top layer of HPMC to control the lag time. The layered tablet was prepared[11,12] by direct compression in three stages using the formula in table 2.2. The composition for the burst release layer was arrived after performing various trials, and was kept constant throughout the study. The formulation for the matrix layer was first filled in the die cavity and compressed. The press was then reversed to fill the second layer and then compressed. The process was repeated again to fill top layer. The final compression was with maximum pressure to get a compact three layer tablet weighing 175 mg.

The excipients for all the three layers were mixed separately by geometrical dilution and passed through sieve no: 20. The tablet was compressed by 7mm concave punch in multi-station rotary tablet press.

2.3 Coating of the tri-layered core tablet.

2.3.1 Coating with ethyl cellulose.

The bottom and side portion of the tablets were coated with 1% ethyl cellulose in chloroform by dip coating. The topmost polymer layer was left uncoated. The tablets were first air dried followed by oven drying at 40°C for 1 hour to remove the residual solvent.

2.3.2 Enteric coating[13] (X_3factor as per the optimization design)

The tablets were enteric coated with Eudragit L solution in ethanol by dip coating. The tablets were first air dried followed by oven drying at 40°C for 1 hour to remove the residual solvent. The volatile coating solutions were kept in ice bath to reduce evaporation of the solvent and to maintain the concentration throughout.

2.4. Optimization design[9, 10].

The design opted for optimization was Box-Behnken with 3 factors and 3 responses. The influential factors with its range and the desired response are given in table 2.1

Table 2.1: shows the list of factors and responses with their levels and constraints.

Factors	*Levels used*		
	-1	0	+1
X_1= Amount of guar gum (mg)	50	60	70
X_2= Amount of HPMC (mg)	40	50	60
X_3= Eudragit coating (no: of dips)	2	3	4
Responses	*Constraints*		
Y_1= Lag time	4 hours		
Y_2= Cum.drug release at 6^{th} hour	30-40%		
Y_3= Cum.drug release at 24^{th} hour	85-90%		

Based on the design seventeen runs were suggested by the design expert and is shown in the following table. 2.2

Table 2.2: The formula for salbutamol sulphate tri-layered tablet.

F.Code	Ingredients (mg)							
	Polymer layer		Burst release layer Blend *	Salbutamol	Matrix layer			No of dips. Eutragit L. **X_3** factor/ level
	HPMC **X_2** factor/level	Lactose			Guar gum **X_1** factor/ level	Mg. Stearate	Lactose	
F1	50/ 0	10	35	4	60/ 0	1.5	14.5	2/ 0
F2	40/ -1	20	35	4	60/ 0	1.5	14.5	1/ -1
F3	40/ -1	20	35	4	60/ 0	1.5	14.5	3/ 1
F4	60/ 1	0	35	4	60/ 0	1.5	14.5	3/ 1
F5	60/ 1	0	35	4	60/ 0	1.5	14.5	1/ -1
F6	60/ 1	0	35	4	70/ 1	1.5	4.5	2/ 0
F7	40/ -1	20	35	4	50/ -1	1.5	24.5	2/ 0
F8	50/ 0	10	35	4	60/ 0	1.5	14.5	2/ 0
F9	50/ 0	10	35	4	70/ 1	1.5	4.5	1/ -1
F10	60/ 1	0	35	4	50/ -1	1.5	24.5	2/ 0
F11	50/ 0	10	35	4	60/ 0	1.5	14.5	2/ 0
F12	50/ 0	10	35	4	50/ -1	1.5	24.5	1/ -1
F13	50/ 0	10	35	4	50/ -1	1.5	24.5	3/ 1
F14	50/ 0	10	35	4	60/ 0	1.5	14.5	2/ 0
F15	40/ -1	20	35	4	70/ 1	1.5	4.5	2/ 0
F16	50/ 0	10	35	4	70/ 1	1.5	4.5	3/ 1
F17	50/ 0	10	35	4	60/ 0	1.5	14.5	2/ 0

*Blend of 2mg salbutamol, 2mg sodium starch glycolate, 1mg magnesium stearate and 30mg lactose.

2.5.1 Tablet evaluation

The primary evaluations like thickness, hardness, weight variation, drug content uniformity and dissolutionof the formulated tablets were carried out as per the official procedures.

2.5.2 In-vitro dissolution testing.

The in-vitro dissolution testing of the coated tablets were performed in USP apparatus II at 100 rpm. The dissolution testing was first performed for 2 hrs in 900 ml 0.1 N HCl containing 1% SLS and thereafter continued for 24 hrs in 900 ml phosphate buffer pH 6.8 containing 1% SLS.Aliquots were withdrawn at predetermined time intervals and analyzed spectrophotometrically at 225nm[14].

2.6 Optimization.

Based on the dissolution data and outer coat rupture time, 51 optimal solutions were suggested by the design expert software which could achieve the desired lag time, cumulative drug release at 6^{th} and 24^{th} hour. From 51 solutions, one was selected for the further study. The new batch of tri-layered tablet was prepared with the suggested and evaluated to confirm the result.

2.7 Stability studies.

The optimized batch of the tablets was monitored up to 6 months at accelerated conditions of temperature and relative humidity (40±2°C/ 75%±5% RH) to check its stability. Samples were withdrawn at predetermined intervals and determined the drug release [15].

3. RESULT AND DISCUSSION

The tri-layered tablets were prepared, coated and evaluated for following parameters.

3.1. Evaluation of tri-layered tablet.

Table 3.1: shows the results of thickness, diameter, average weight of one tablet and weight variation

Formulation code	**Thickness* (mm)	*Hardness* kg/cm^2	**Average weight* (mg)	*Weight variation as per USP*
F1	5.24±0.16	Above 4	201.26±0.56	Pass
F2	5.22±0.58	Above 4	185±0.17	Pass
F3	5.25±0.02	Above 4	203±0.24	Pass
F4	5.25±0.06	Above 4	184±0.56	Pass
F5	5.24±0.54	Above 4	187±0.22	Pass
F6	5.23±0.19	Above 4	201±0.16	Pass
F7	5.24±0.57	Above 4	185±0.34	Pass

F8	5.25±0.61	Above 4	204±0.85	Pass
F9	5.22±0.28	Above 4	197±0.36	Pass
F10	5.23±0.46	Above 4	199±0.33	Pass
F11	5.23±0.29	Above 4	195±0.78	Pass
F12	5.21±0.31	Above 4	192±0.86	Pass
F13	5.24±0.40	Above 4	194±0.21	Pass
F14	5.26±0.38	Above 4	195±0.64	Pass
F15	5.23±0.14	Above 4	197±0.51	Pass
F16	5.25±0.35	Above 4	195±0.44	Pass
F17	5.26±0.74	Above 4	193±0.28	Pass

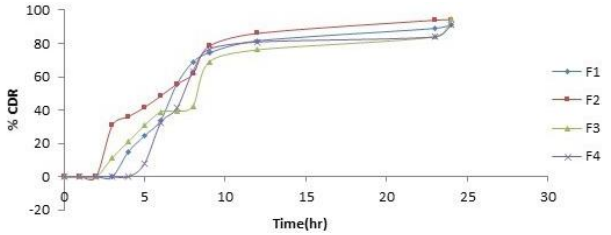


Figure 3.1: In-vitro dissolution profile of F1- F4

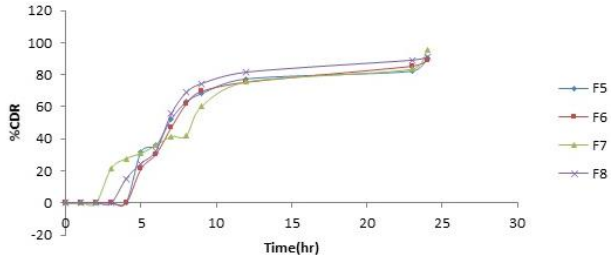


Figure 3.2: In - vitro dissolution profile of F5- F8

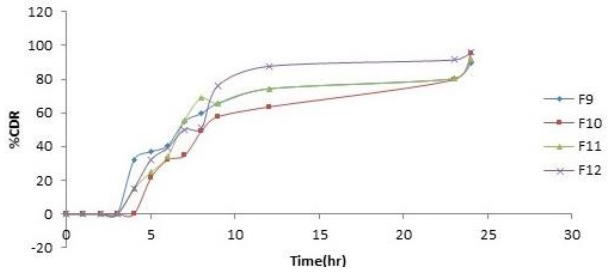


Figure 3.3: In-vitro dissolution profile of F9- F12

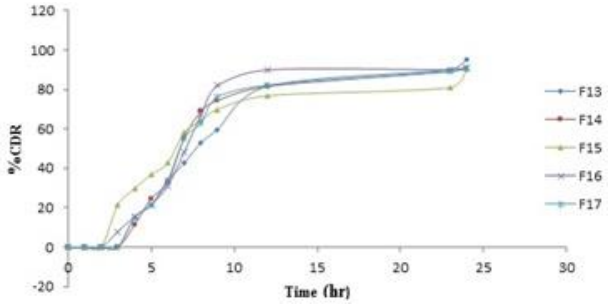


Figure 3.4: In-vitro dissolution profile of F13- F17

3.2 Optimization by Box-Behnken design.

A three factor, three level design was used. The design consists of a replicated centre point and a set of point lying at the midpoint of each edge of the multidimensional cube that defines the region of interest. The non linear computer generated quadratic model is given as

$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + E$

Where, Y = the measured response associated with each factor level combination

b_0 = intercept

b_1 to b_{33} are regression coefficients computed from the observed experimental values of Y

X_1, X_2 and X_3 = coded levels of independent variables

E = error term

Table 3.2: shows the seventeen formulations by Box-Behnken design with factors and responses.

Formulation code	Factors			Responses		
	Amount of guar gum(mg) X1	Amount of HPMC (mg) X2	Eudragit coating (No: of dips) X3	Lagtime (hrs) Y1	Cum. drug release at 6th hour (%) Y2	Cum. drug release at 24th hour (%) Y3
F1	60	50	2	4	33.96	95.42
F2	60	40	1	3	38.9	94.39
F3	60	40	3	3	32.28	91.26
F4	60	60	3	5	34	91.26
F5	60	60	1	5	48.5	94.38
F6	70	60	2	5	40.6	89.56
F7	50	40	2	3	39.3	95.68
F8	60	50	2	4	33.03	90.69
F9	70	50	1	4	35.9	90.11
F10	50	60	1	5	32.05	95.16
F11	60	50	2	3	42.8	90.59
F12	50	50	1	4	36.00	95.3
F13	50	50	3	4	32.69	90.83
F14	60	50	2	4	31.1	91.28
F15	70	40	2	3	34.11	91.82
F16	70	50	3	3	30.5	88.63
F17	60	50	2	4	34	91.27

Table 3.3: ANOVA summery of different responses.

Response model	Model		Lack of fit		PRESS	R squared
	F- value	P-value	F- value	P-value		
Lag time Y1	6.46	0.0112*	0.42	0.751#	2.37	0.8449
Cum. drug release at 6th hour.Y2	30.65	<0.0001*	5.58	0.0651#	191.53	0.7113
Cum. drug release at 24th hour.Y3	14.12	0.0010*	6.17	0.0555#	25.28	0.8240

Significance level- 0.05 or 5%, *significant, #nonsignificant

The quadratic model was selected as the suitable statistical model for formulation optimization since the values of r^2 are quite high for the three responses with corresponding smallest PRESS value. Smaller the PRESS statistics better is the model fits the data points.The polynomial equations form excellent fits to the experimental data and are highly statistically valid. Mathematical relationship in the form of polynomial equation for the measured responses was obtained with the statistical software. The equation was as follows:

$$Y1 = +3.80 - 0.13\,X_1 + 1.00\,X_2 - 0.13X_3 - 4.206E\text{-}016\,X_1X_2 - 0.25\,X_1X_3 + 8.832E\text{-}018X_2X_3 - 0.025\,X_1^2 + 0.22X_2^2 - 0.025\,X_3^2$$

$$Y2 = +33.57 + 0.46\,X_1 - 4.43\,X_2 - 3.51\,X_3 - 2.09\,X_1X_2 - 1.04\,X_1X_3 + 1.50\,X_2X_3 - 0.44X_1^2 + 2.21\,X_2^2 + 3.12\,X_3^2$$

$$Y3 = +91.17 - 2.69X_1 - 1.19X_2 + 0.33\,X_3 - 0.46\,X_1X_2 + 0.50\,X_1X_3 + 0.29\,X_2X_3 + 0.85X_1^2 + 0.40\,X_2^2 + 0.96\,X_3^2$$

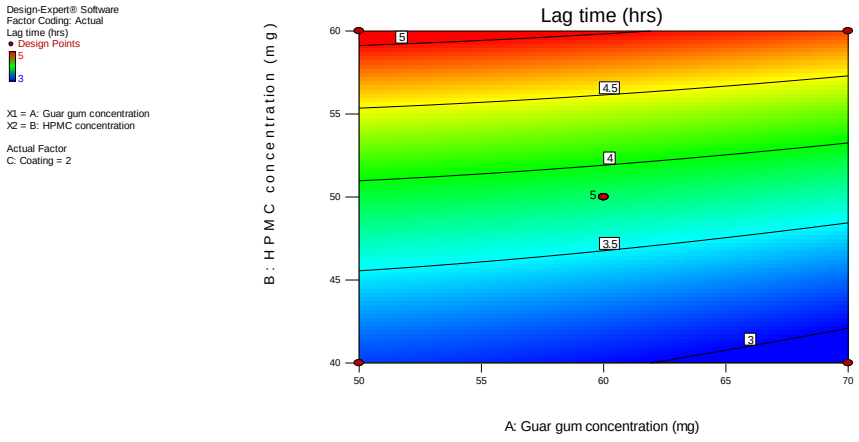


Fig 3.5: Contour plot for the effect of guar gum concentration and HPMC concentration on lag time.

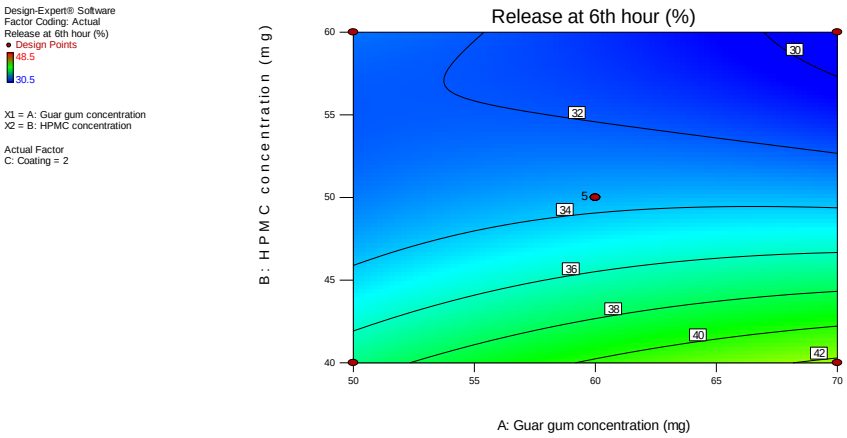


Fig 3.6: Contour plot for the effect of guar gum concentration and HPMC concentration on cumulative percentage release at 6th hour.

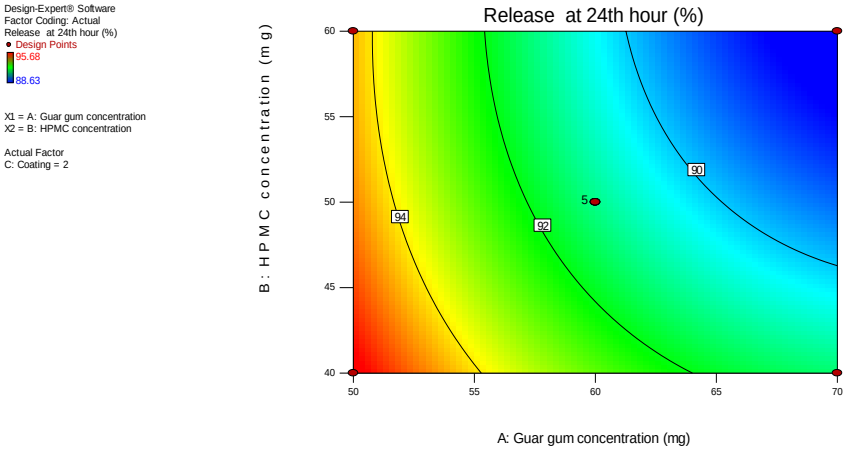


Fig 3.7: Contour plot for the effect of guar gum concentration and HPMC concentration on cumulative percentage release at 24th hour.

After generating the polynomial equation relating to the dependent and independent variables, the formulation was optimized for the responses.The optimum variables were obtained by the numerical analysis based on the criterion of desirability. A new batch of tablets with the predicted levels of formulation factors was prepared to confirm the validity of the optimization procedure.

3.3 Formulation of the optimal batch

Based on the statistical evaluations the software gave 51 solutions for the optimization from which the following was selected for further study. The formula for the optimum batch is given below.

Table 3.4: Formula for optimum batch.

Ingredients	*Amount of Guar gum*	*Amount of HPMC*	*Coating level*
Quantity (mg)	50mg	60mg	3

Table 3.5: shows the working formula for the optimized tri-layered tablet.

Ingredients (mg)							No of dips. Eutragit L. **X3** factor/ level
Polymer layer		Burst release layer Blend *	Matrix layer				
HPMC **X2** factor/ level	Lactose		Salbutamol	Guar gum **X1** factor/ level	Mag Stearate	Lactose	
60	Nil	35	4	50	1.5	24.5	3

*Blend of 2mg salbutamol, 2mg sodium starch glycolate, 1mg magnesium stearate and 30mg lactose.

3.4 In-vitro dissolution testing of the optimized batch

The in-vitro dissolution testing of the optimum formula was performed and the results were given in the table below [14].

Table. 3.6: In- vitro dissolution testing of optimized batch.

Cumulative % drug release * ± standard deviation at the following time interval (Hours)									
2 hr	3 hr	4 hr	5 hr	6 hr	7hr	8 hr	9 hr	12 hr	24 hr
0	0	0	21.38 ±0.02	2.05±1.21	34.62 ±0.52	49.08 ±0.35	57.77 ±0.69	63.52 ±0.10	95.16 ±0.63

The optimized tablet was also evaluated for thickness, hardness, weight variation and drug content uniformity as per the official procedures and was found to be within the acceptable limit. The lag time was found to be close to 5 hours followed by a burst release of above 30% drug. Thereafter the release was sustained up to 24 hrs.

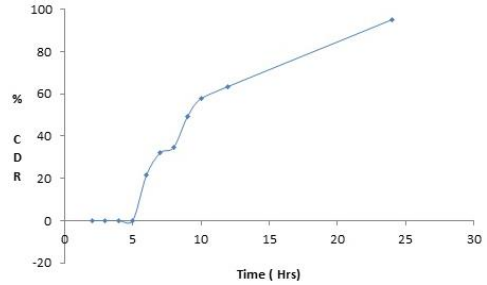


Figure 3.8: In-vitro dissolution of optimum batch

3.5 Mechanism of drug release from matrix layer.

A drug release mechanism of the optimized formula was determined by fitting the drug release data to various the kinetic models[14].

The dosage form was designed with 2mg (33.33%) of salbutamol for the burst release and 4mg (66.67%) for sustained release. The release mechanism was determined with respect to the matrix part responsible for the sustained release. The time count for the release started from the time at which the lag time determining layer of HPMC broke off from the dosage form.ie, the time at which the first dose appeared in the dissolution medium. The initial dose of 2mg drug meant for burst release was subtracted from each value of cumulative drug released.

Table.3.7: Kinetic release data of salbutamol sulphate from optimized batch.

Time (hrs)	cumulative amount of dg released (mg)	Cumulative amount of dg less 2mg in burst release layer (mg)	% cumulative release from the matrix layer containing 4mg dg	Time considered for kinetic studies (hrs)
0	0	0	0	-
1	0	0	0	-
2	0	0	0	-
3	0	0	0	-
4	0	0	0	-
5	0	0	0	-
6	1.283	0	0	-
7	1.923	0	0	0
8	2.077	0.077	1.93	1
9	2.945	0.945	23.63	2
10	3.466	1.466	36.65	3
12	3.811	1.811	45.28	5
24	5.710	3.710	92.74	17

The correlation coefficient (R^2) values for various release models: zero-order, firstorder, Higuchi model and Korsmeyer Peppas model were determined and represented in table 3.8.

Table.3.8: Kinetic study of Salbutamol sulphate release

Formulation	Zero order	First order	Higuchi	Peppas	
	R^2	R^2	R^2	R^2	n
Optimized batch	0.911	0.482	0.952	0.769	1.5

The R^2 values suggested that the drug release from the matrix layer predominately followed Zero order. This could be achieved as the dosage form was coated from all sides except the top portion from which the drug diffuses out slowly. Higuchi's R^2 value suggested that the drug release is by diffusion pattern.

Release exponent, n, was found >1, for the batch indicating a non fickian release, suggesting a super case II diffusion mechanism.

3.6 Stability studies.

The stability of the optimized batch was monitored up to 180 days at accelerated stability conditions of temperature and relative humidity. The dissolution profile showed no great differences in the drug release. The lag time of drug release was also found to be same as that of the first day. This proved that the tablet retained the expected stability requirement with regard to the dissolution profile.

CONCLUSION

The optimized tri-layered tablet designed for chronotherapeutic drug delivery was capable of retaining the drug within during the lag time of 4-5 hours followed by a burst release of about 30% drug. The guar gum containing matrix layer sustained the drug release for 24 hours. The release kinetics suggested that the drug release is by diffusion and followed a non fickian release.

This once a day tablet if consumed at bed time could effectively control the "morning dip" and manage the asthmatic symptom for the whole day. Tri-layered tablet was found to be stable even after 180 days of accelerated storage conditions.

The Box-Behnken optimization design evaluated 3 influential factors at 3 levels with 17 runs. The ANOVA summery suggested that the model selected was significant and the lack of fit is insignificant. The 3 responses chosen were the most appropriate for a chronotherapeutic drug delivery.

Disclosure

We the authors declared that there is no conflict of interest in this work.

Acknowledgement

We acknowledge the financial support of Kerala State Council for Science Technology and Environment (KSCSTE) under the student project scheme 2013-14

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