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Original Article

DEVELOPMENT AND EVALUATION OF ORAL CONTROLLED RELEASE MATRIX TABLETS OF LAMIVUDINE: OPTIMIZATION AND *IN VITRO-IN VIVO* STUDIES

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ABSTRACT

Objectives: To develop and evaluate a controlled release matrix tablets containing lamivudine (LAM).

Methods: A central composite design (CCD) of the experiment were employed with the amount of hydrophilic polymer (HPMC K100M) (X_1) and amount of hydrophobic polymer cellulose acetate phthalate (X_2) as independent variables. Four response variables were considered in the formulation, which includes the % drug release at 1hr (Y_1), % drug release at 8hr (Y_2), diffusion coefficient (Y_3) and $T_{50\%}$ (Y_4). The design was quantitatively evaluated by the quadratic model.

Results: Statistical analysis revealed that factor X_1 was found to be highly significant for responses Y_2 and Y_4, whereas factor X_2 for response Y_1. The quadratic factor of X_1 and X_2 is found to be highly significant in response Y_3. A numerical optimization technique for desirability function was used to optimize the response variables with different target and the observed responses were highly agreed with experimental values. The response Y_1-Y_4 and the optimized formulation was arrived by restricting to $17\% < Y_1 > 18\%$; $72.0\% < Y_2 > 75\%$; $0.55 < Y_3 > 0.65$; $4.2 < Y_4 > 4.52$h. The results showed a good relationship between the experimented and predicted values. The dissolution profiles of the optimal formulation before and after stability studies were evaluated by using a similarity factor (f_2) and were found to be similar. *In vivo* studies indicate that the formula generated by CCD showed a controlled release profile.

Conclusion: The results of *in vivo* studies revealed that the optimized formulation exhibited a controlled release of lamivudine.

Keywords: Hydrophilic and hydrophobic polymer, Central composite design, Quadratic model, *In vitro-in vivo* correlation, Response surface methodology.

INTRODUCTION

Lamivudine (3-TC), 2-deoxy-3-thiacytidine (LAM), is a potent nucleoside analog reverse transcriptase inhibitor with very low cellular cytotoxicity. Moreover, LAM is active against zidovudine-resistant human immunodeficiency virus (HIV) [1, 2]. 3-TC has approximately 80% oral bioavailability in human with the usual dosage of 150mg twice daily in combination with other antiretroviral agents [3]. Conventional oral formulations of LAM are administered multiple times a day because of its moderate half-life (5-7 hrs) [4]. Treatment of HIV using conventional formulations of LAM is found to have many drawbacks, such as drug accumulation due to frequent dosing, plasma concentration fluctuation, poor patient compliance, and high cost [5].

Oral controlled drug delivery system represents one of the frontier areas of drug delivery system in order to fulfill the need for a long-term treatment with anti-HIV agents [6]. Among the different controlled drug delivery (CDD) systems, matrix based controlled release tablet formulations are the most popularly preferred for its convenience to formulate a cost effective manufacturing technology in commercial scale. Development of oral controlled release matrix tablets containing water-soluble drug has always been a challenging because of dose dumping due to improper formulation resulting in plasma fluctuation and accumulation of toxic concentration of drug [7]. Over many years, numerous studies have been reported in the literature on the application of hydrophilic polymers in the development of oral controlled release matrix systems for various drugs [8,9,10]. Among the hydrophilic polymers, cellulose derivatives such as carboxymethyl cellulose (CMC) [11], sodium carboxymethyl cellulose [12], hydroxyproyl cellulose (HPC) [13], and hydroxypropyl methyl cellulose (HPMC) [14-16] have been extensively studied as a matrix forming polymer in the controlled release tablet formulations. These polymers are most preferred because of its cost effectiveness, broad regulatory acceptance, non-toxic and easy of compression [17]. However, the use of hydrophilic matrix alone in controlling drug release for water soluble drugs is restricted due to the rapid diffusion of the dissolved drug through the hydrophilic gel network. For such drugs, it becomes essential to include hydrophobic polymers in the matrix system [18]. Hence an attempt is made in this research work to formulate controlled release (CR) matrix tablets of LAM using Hydroxypropyl methyl cellulose (HPMC) K100M as hydrophilic polymer with cellulose acetate phthalate (CAP) as a hydrophobic polymer. Instead of normal and trial method, a standard statistical tool design of experiments is employed to study the effect of formulation variables on the release properties. The *in vivo* behavior of the optimized formulation was further evaluated by using the rabbit as an animal model.

MATERIALS AND METHODS

Materials

Lamivudine was received as a gift sample from M/s Strides Arcolab Ltd., Bangalore, India. Hydroxypropyl methyl cellulose (METHOCEL™) K100M procured from Colorcon Asia Pvt. Ltd., Goa, India and Cellulose acetate phthalate procured from G. M. Chemie Pvt. Ltd., Mumbai, India. Other materials, including Magnesium stearate (Loba Chemie Pvt. Ltd., Mumbai, India), Polyvinylpyrrolidone (PVP) K30 (Sigma-Aldrich Co. LLC., Bangalore, India), Aerosil (S D Fine-Chem Ltd, Mumbai, India), Talc (**Nice Chemicals (P) Ltd.,** Kochi, India), and Lactose (Sigma-Aldrich Co. LLC., Bangalore, India) was purchased from a commercial source. All other chemicals used in the study were of analytical grade.

Drug excipients compatibility study

Sample of pure drug, physical mixture of excipients with drug and polymers in a 1:1 ratio was placed in an accelerated stability

condition of 40 ± 2^{0}C and 75 ± 5% RH for a period of 3 months. At the end of 3 months, samples were evaluated for drug excipient compatibility by using Fourier-transform infrared (FT-IR) spectrometer (8400s, Shimadzu Corporation, Japan) and differential scanning colorimeter (DSC) (Pyris-1, Perkin-Elmer, USA).

FT-IR spectrometer

The FT-IR analysis was performed on the drug sample and drug-excipients to examine the interactions using the spectra. 3-5mg of the composite sample was added to approximately 100mg of KBr. The mixture was then ground to a fine powder using a mortar and pestle, and transparent discs were formed using a pellet press. The discs were then placed in the FTIR spectroscopy analyzer, and the spectra were collected at the range of 4000-500 cm^{-1}.

Differential scanning colorimeter

DSC curves were obtained using aluminum pans containing about 1 mg of samples, under dynamic nitrogen atmosphere (50 mL min^{-1}) and heating rate of 10^{0}C min^{-1} in the temperature range from 25 to 450^{0}C. The DSC cell was calibrated with indium (mp 156.6^{0}C) and lead (mp 327.5^{0}C).

Experimental design

Central composite design (CCD) is an experimental design technique, by which the factor involved and its relative importance can be assessed was adopted for optimization of controlled release tablets of LAM [19,20,21]. According to the model, it contains four full factorial design points, four axial points and three center points. The selected factor levels are summarized in Table 1. The center points were repeated 3 times to estimate the pure experimental uncertainty at the factor levels. The two independent formulation variables evaluated include:

Independent variables

X_1 = Amount of HPMC K100 (75mg to 150mg)

X_2 = Amount of Cellulose Acetate Phthalate (75mg to 100mg)

Dependent variables (Responses)

Y_1 = Percentage drug release at 1hr

Y_2 = Percentage drug release at 8 hr

Y_3 = Diffusion Coefficient (n)

Y_4 = Time required for 50% of the drug release in hr ($T_{50\%}$)

Preparation of CR matrix tablets

The formulations were prepared by wet granulation technique at random following CCD; table 2 shows the experimental design. All the ingredients were passed through an 80 mesh screen. The required quantities of HPMC K100M, CAP, PVP K30, aerosil and lactose were mixed in a suitable stainless steel vessel in a tumbler mixer (Rimek, Karnavati Engineering Pvt. Ltd. Ahmedabad, India) at 100 rpm for 30 min. LAM (200mg) was added to the above mixer in a geometric ratio and mixed at 30 rpm for 30 min. Isopropyl alcohol was used as a granulating agent. The granules were dried at room temperature for 1 hr and passed through 20 mesh screen. Talc was added to the above granules and finally lubricated with magnesium stearate. The granules were compressed by using a 10 station rotary tablet compression machine (Rimek, Karnavati Engineering Pvt. Ltd., Ahmedabad, India) fitted with 12 mm biconcave punches. The compression was controlled to produce 13±0.5 kg/cm^2 tablet crushing strength.

Characterization of granules

Prior to compression, the granules were evaluated for their characteristic parameters [22]. Angle of repose was determined by funnel method; Bulk density (BD) was determined by using a measuring cylinder and tapped density (TD) was determined by Tap Density Tester (ETD-1020, Electrolab, India). Carr's index (CI) was calculated using the following equation (1),

$$CI = (TD - BD) \times 100 / TD \quad \text{..... (1)}$$

Characterization of tablets

The properties of the compressed matrix tablets, such as hardness, friability and weight variation were determined as per United States Pharmacopoeia-27 and National Formulary-22 specifications [23]. The content of 06 randomly selected CR matrix tablets from each batch was determined by using UV double beam spectrophotometer (UV-1601, Shimadzu Co., Japan). Friability was determined using friability testing apparatus (Electrolab, India). Weight variation of tablets was determined as per official procedure for randomly selected 20 tablets by using an electronic balance (Denver APX-100, Arvada, Colorado).

***In vitro* dissolution studies**

The drug release profile of the formulated tablets was studied using USP dissolution apparatus II (TDT-06T, Electrolab, India) at 37^{0}C ± 1^{0}C using 900 ml of pH 1.2 buffer for the first 2 hr, followed by pH 7.4 buffer till the end of dissolution studies. The paddle rotation speed was set to 100rpm. Aliquot samples were withdrawn at every 1 hr and after suitable dilutions the samples were analyzed spectrophotometrically 264 nm. The volume of the sample withdrawn each time was replaced with the same volume of the respective buffer solutions. The studies were carried out in triplicate and mean values plotted versus time with standard error of mean, indicating the reproducibility of the results. The release data were fitted to various mathematical models for describing the release mechanism from tablets; such as Korsmeyer-Peppas model [24], Zero-order model [25], and Higuchi release model [26]. All curve fitting, simulation and plotting were carried out by using commercially available softwares (SigmaPlot® version 9, Systat Software, Inc.; and GraphPad PRISM® version 3.02, GraphPad Software, Inc.).

Statistical analysis

The effect of formulation variables on the response variables was statistically evaluated by applying one-way ANOVA at 0.05 level using a commercially available software package Design-Expert® version 6.05 (Stat-Ease, Inc.). The design was evaluated by the quadratic model, which bears the form of an equation (2).

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_1^2 + b_4 X_2^2 + b_5 X_1 X_2 \quad \text{..... (2)}$$

Where Y is the response variable, b_0 the constant and b_1, b_2, b_3 ... b_5 is the regression coefficient. X_1 and X_2 stand for the main effect; X_1, X_2 are the interaction terms and show how the response changes when two factors are simultaneously changed. X_1^2, X_2^2 are quadratic terms of the independent variables to evaluate the nonlinearity.

Stability studies

Stability studies were conducted on the optimized formulation. The optimized formulation was packed in a screw capped amber colored glass container. The containers were exposed to 40°C ± 2°C/ 75% ± 5% RH as per ICH guidelines for 6 months. Sampling was done at predetermined time intervals and evaluated for various physico-chemical parameters viz., appearance, drug content and hardness. *In vitro* drug release studies were also performed at the end of stability studies. To confirm the similarity of drug release profiles before and after stability studies, a model-independent statistical tool for comparison of dissolution profile *"similarity factor"* (f_2) was used with the equation (3) [27].

$$f_2 = 50 \cdot \log\{[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2]^{-0.5} * 100\} \quad \text{..... (3)}$$

***In vivo* Pharmacokinetic studies**

The i*n vivo* pharmacokinetics studies was carried out using six male New Zealand white rabbits, weighing 2.5-3.2kg after obtaining approval from the institutional animal ethical committee. Animals were housed in a 12-hr light-dark, constant temperature environment prior to the study. All rabbits were fasted for one day before the experiment and water was supplied *ad libitum*. The optimal CR tablet containing 100mg of LAM was orally administered with small amount of water. At pre-determined time intervals, 1 ml

of blood was collected from a marginal ear vein into heparinized plastic tubes. Blood samples collected were centrifuged at 2000rpm for 10 min and stored at -20°C till further use. The concentration of the drug was determined by a standard HPLC method with minor modifications [28]. The pharmacokinetic parameters were computed by using plasma concentration time profile data utilizing a commercially available software Kinetica@ 2000 Version 3 (Inna Phase Corp., USA).

RESULTS

Drug excipient compatibility studies

In order to confirm the drug excipient compatibility, samples were analyzed by FT-IR spectroscopy. The FT-IR spectra of LAM and its physical mixtures are presented in fig. 1. The characteristic absorption peak of LAM was found to be 1643 cm^{-1} (C=O stretching), 3073 cm^{-1} (C-H stretching, aromatic), 2957 & 2829 cm^{-1} (C-H stretching, aliphatic), 3324 & 3263 cm^{-1} (N-H stretching), 1612 cm^{-1} (N-H bending), 1493 cm^{-1} (C=N stretching), and 3549 cm^{-1} (O-H stretching). These characteristic peaks were also present in the FT-IR spectra of physical mixtures, but with reduced intensity which may be due to the presence of other excipients.

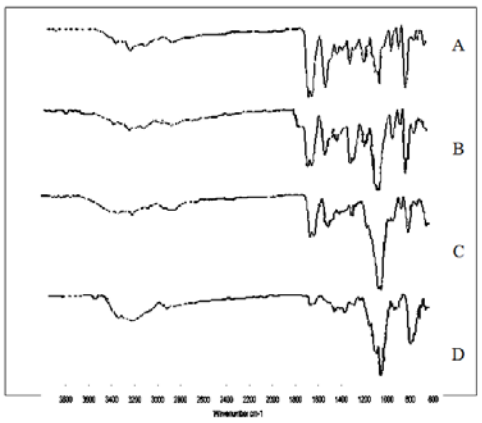

Fig. 1: FT-IR spectra of LAM (A) physical mixture of LAM with HPMC K100M (B), physical mixture of LAM with CAP (C) physical mixture of LAM with lactose (D)

The DSC thermogram of LAM shows a sharp endothermic peak at 178.71°C, where as physical mixtures of drug with excipients and polymers exhibited an endothermic peaks ranging from 170.01 to 178.47°C (fig. 2) which is corresponding to the melting point of the drug, thus indicating no interaction between the drug-excipients and drug-polymers used for this study.

Micromeritic properties

The micromeritic properties were evaluated for all the batches of the granules. The angle of repose values ranged between 18.53 ± 0.80 to 21.54 ± 0.24. The results indicate good flow properties. The cars index measures the propensity of a powder to consolidate when undergoing vibration, shipping and handling. The result ranges from 5.62 ± 1.25 to 11.11 ± 2.15 %, which indicate good flow properties.

Evaluation of prepared tablets

The tablets of different batches showed a uniform thickness (4.93 ± 0.03 to 5.16 ± 0.06 mm) and hardness (12.50 ± 0.23 to 13.39 ± 0.15 kg/cm^2). The assayed content of drug in various formulations varied between 99.15 ± 1.34 to 104.25 ± 2.56 %. The average percentage weight deviations for 20 tablets were found to be less than 5% and friability was found to be less than 1%. Thus, all the physical parameters were found to be within the permissible limits of USP.

Release profile

Fig. 3,4,5 illustrates the release profiles of four factorial points, four axial points and three central points. It is evident from formulations K1 to K4 that as the amount of polymer in the tablet increases, the drug release decreases which may be due to strong polymeric gel network. From fig. 3, it can be inferred that the release of all three centre points overlaps each other, indicating that the error due to the experimental procedure was found to be less in generating a meaning full fitting for the dependent variables.

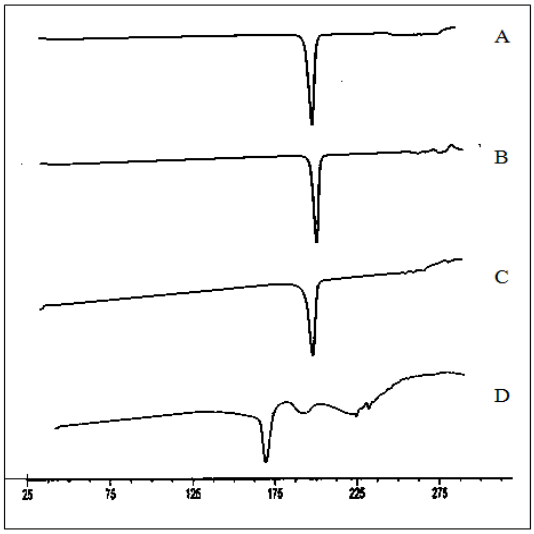

Fig. 2: DSC thermogram of LAM (A) physical mixture of LAM with HPMC K100M (B), physical mixture of LAM with CAP (C) physical mixture of LAM with lactose (D)

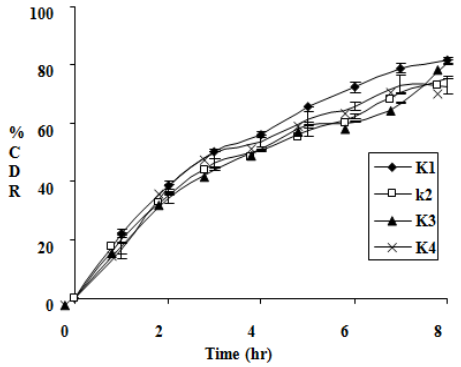

Fig. 3: The release profiles for formulations prepared from four factorial points; () K1, () K2, () K3, (x) K4

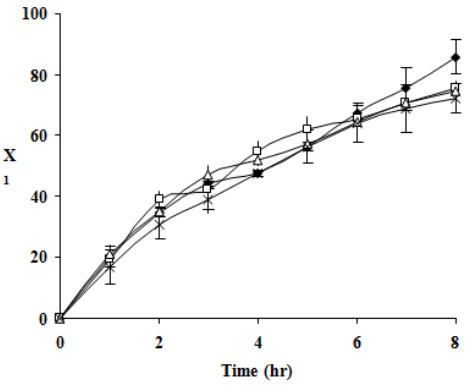

Fig. 4: The release profiles for formulations prepared from four axial points; () K5, () K6, () K7, (x) K8

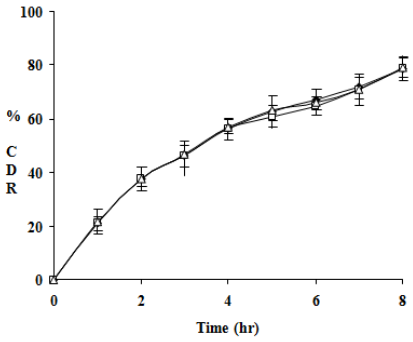

Fig. 5: The release profiles for formulations prepared from three centre points; () K5, () K6, () K7
The results of the $T_{50\%}$ values are summarized in table 1. Formulations K1, K3 and K5 showed a low $T_{50\%}$ values due to rapid release of LAM from the delivery system

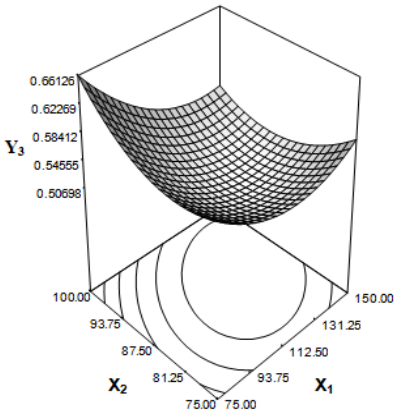

Fig. 6: Response surface plot showing the effect on amount of HPMC (X_1) and amount of CAP (X_2) on the response diffusion co-efficient (Y_3)

Table 1: Factor combinations as per CCD

Factor	Factor level				
	-1.41	**-1**	**0**	**1**	**1.41**
X_1: Amount of HPMC K100	59.46	75	112.5	150	165.54
X_2: Amount of CAP	69.82	75	87.50	100	105.18

The diffusion exponent values thus obtained were ranged between 0.51 and 0.65; this indicates anomalous (non-fickian) diffusion (Table 1). These formulations also yielded a quality adjustment with Higuchi release model (Table 2).

Table 2: Coded levels as per CCD with observed responses

Formulation code	X_1	X_2	Y_1 (%)	Y_2 (%)	Y_3 (n)	Y_4 (h)
K1	-1	-1	22.12	81.70	0.57	3.66
K2	1	-1	20.48	75.38	0.57	4.21
K3	-1	1	17.73	80.81	0.69	3.61
K4	1	1	17.10	72.53	0.55	4.52
K5	-1.41	0	20.09	85.65	0.69	3.28
K6	1.41	0	19.37	75.59	0.56	4.35
K7	0	-1.41	21.31	74.66	0.56	4.09
K8	0	1.41	16.79	72.16	0.65	4.28
K9	0	0	21.21	78.80	0.56	4.09
K10	0	0	21.49	78.51	0.55	4.10
K11	0	0	21.55	79.32	0.55	4.07

Effect of formulation variables

The results of curve fitting analysis for various formulations were given in table 3.

Table 3: Results of curve fitting analysis

Formulation code	Korsmeyer-Peppas K_{KP} (h^{-n})	R^2	Zero- order K_0 (% h^{-1})	R^2	Higuchi K_H (% $h^{-1/2}$)	R^2
F1	24.89 ± 0.91	0.9975	12.13 ± 0.72	0.8806	29.19 ± 0.54	0.9879
F2	22.74 ± 1.25	0.9941	10.77 ± 0.68	0.8645	25.97 ± 0.49	0.9873
F3	23.28 ± 1.18	0.9945	10.52 ± 0.71	0.8388	25.42 ± 0.39	0.9913
F4	24.9 ± 1.35	0.9937	11.24 ± 0.75	0.8412	27.14 ± 0.43	0.9907
F5	25.62 ± 1.26	0.9946	11.23 ± 0.79	0.8210	27.19 ± 0.37	0.9931
F6	20.84 ± 1.25	0.9940	11.05 ± 0.57	0.9127	26.49 ± 0.73	0.9748
F7	23.27 ± 1.00	0.9963	10.92 ± 0.69	0.8625	26.34 ± 0.43	0.9903
F8	15.43 ± 0.90	0.9968	11.92 ± 0.25	0.9876	28.12 ± 1.57	0.9164
F9	27.19 ± 0.71	0.9983	11.37 ± 0.83	0.7970	27.57 ± 0.18	0.9982
F10	26.42 ± 0.99	0.9966	11.08 ± 0.83	0.7935	26.88 ± 0.26	0.9964
F11	26.5 ± 1.03	0.9964	11.34 ± 0.82	0.8087	27.47 ± 0.28	0.9959

The regression coefficients for each term in the regression model are summarized in table 4.

Table 4: Regression coefficients for the response variables

$Y_1 = 21.42 - 0.41X_1 - 1.77X_2 - 0.85X_1^2 - 1.19X_2^2$
$Y_2 = 78.88 - 3.60 X_1 - 0.91 X_2 1.02 X_1^2 - 2.59 X_2^2$
$Y_3 = 0.51 - 0.03 X_1 + 0.01 X_2 + 0.04 X_1^2 + 0.04 X_2^2 - 0.03X_1X_2$
$Y_4 = 4.09 + 0.37 X_1 + 0.07 X_2 - 0.14 X_1^2 + 0.05 X_2^2 + 0.09 X_1X_2$

The model parameters are affecting the response variables described in table 5. In case of Y_1, factor X_1, X_2, X_1^2 and X_2^2 were found to be significant and their effect was found to negative i. e. as the amount of HPMC and CAP increases the drug release from the matrix tablets decreases. Similar effect was also observed in case of response Y_2.

Table 5: Summary of ANOVA table for dependent variables from CCD

Source	d. f.	Sum square	Mean square	F value	Probability
% drug release at 1h (%) R^2 = 0.9862					
X_1	1	1.36	1.36	13.41	0.0146
X_2	1	25.10	25.10	248.10	< 0.0001
X_1^2	1	4.08	4.08	40.32	0.0014
X_2^2	1	7.99	7.99	79.02	0.0003
MT release at 8 hr (%) R^2 = 0.9937					
X_1	1	103.91	103.91	160.21	< 0.0001
X_2	1	6.61	6.61	492.81	< 0.0001
X_1^2	1	5.88	5.88	31.35	0.0025
X_2^2	1	37.82	37.82	27.86	0.0032
Releae exponent (n) R^2 = 0.9599					
X_1	1	0.0143	99.84	0.0143	0.0008
X_2	1	0.0069	7.32	0.0069	0.0039
X_1^2	1	0.0055	109.12	0.0055	0.0063
X_2^2	1	0.0024	102.18	0.0024	0.0306
X_1X_2	1	0.0047	29.38	0.0047	0.0087
$T_{50\%}$ (hr) R^2 = 0.9993					
X_1	1	1.10	6656.42	1.10	< 0.0001
X_2	1	0.03	210.48	0.03	< 0.0001
X_1^2	1	0.10	627.65	0.10	< 0.0001
X_2^2	1	0.01	82.23	0.01	0.0003
X_1X_2	1	0.03	195.18	0.03	< 0.0001

In case of Y_3, all the studied variables, its quadratic effect and interaction effect were found to be significant. As the amount of HPMC increases the diffusion coefficient value decreases. A similar but opposite effect was observed in case of increasing the amount of CAP. The interaction effect between X_1 and X_2 are shown in the response surface plot (fig. 6).

If X_1 is kept at the highest level and X_2 was increased from -1 level to +1 level, the effect on diffusion coefficient was found to be minimal. And If X_1 was at lower level, the same diffusion coefficient value increases from 0.57 to 0.69.

In case of Y_4, all the studied variables, their quadratic effect and the interaction term were found to be significant. High level of factor X_1 shows a high value of $T_{50\%}$ at all the levels of X_2 thus indicating that increasing the amount of HPMC in the matrix tablets, increases the strength of gel viscosity which in turn decreases the water diffusion into the core layer and thereby decreases the release rate and in turn increases the $T_{50\%}$ (fig. 7).

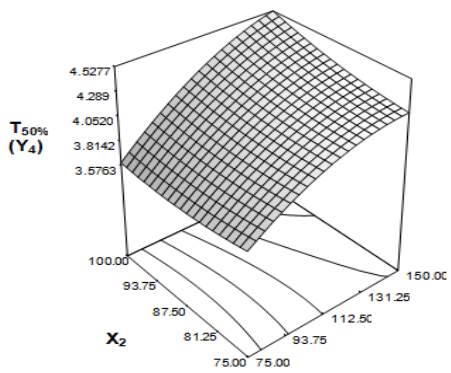

Fig. 7: Response surface plot showing the effect of amount of HPMC (X_1) and amount of CAP (X_2) on the response $T_{50\%}$ (Y_4)

Optimization

The process was optimized for the response Y_1-Y_4 and the optimized formulation was arrived by restricting to 17% < Y_1 > 18%; 72.0% < Y_2 > 75%; 0.55 < Y_3 > 0.65; 4.2 < Y_4 > 4.52h. The optimal levels of factor X_1 and X_2 were 145mg, and 98.96mg with a maximum desirability value of 1. Even though, to challenge the reliability of the response surface model, new optimized formulation was prepared according to the predicted model and evaluated for the responses. The results are showed in the table 6.

Table 6: Comparison between the Experimented (E) and Predicted (P) values for the most probable optimal formulation

Dependent variables	Optimized formulation	
	E	P
Y_1(%)	18.34 ± 2.61	17.82
Y_2 (%)	74.78 ± 4.45	72.91
Y_3 (n)	0.55 ± 0.08	0.54
Y_4 (hr)	4 ± 0.15	4.5

Stability studies

The drug content (204.22 ± 1.29mg) and hardness (11.16 ± 0.16 kg/cm^2) of optimized formulation before and after 6 months of stability studies were subjected to statistical analysis using the paired *t*-test and based on the p-value (drug content; 0.1132 and hardness; 0.1917) it was concluded that no significant difference were observed before and after stability studies (fig. 8). The release profiles appear to be almost super impossible and the calculated f_2 value was 89.18.

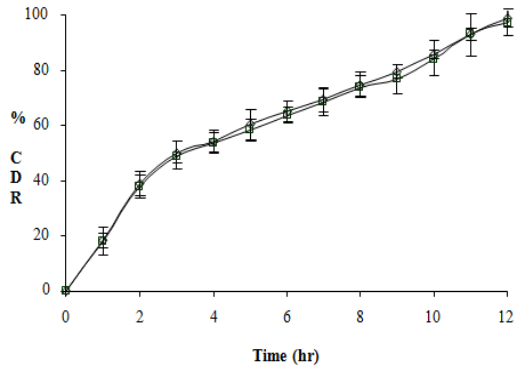

Fig. 8: Comparison of release profile of optimized dosage form of LAM before (BSS) and after (ASS) stability studies; () BSS () ASS

***In vivo* studies**

For *in vivo* studies in rabbit, the optimal formula obtained was reduced to half the quantity and compressed by using 8 mm.

The mean plasma concentration of LAM (100mg) following oral administration of optimized CR tablets is shown in fig. 9.

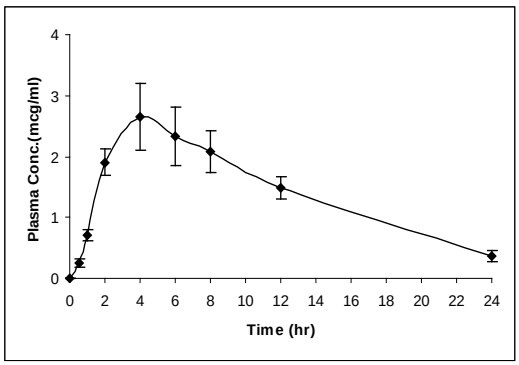

Fig. 9: Mean plasma concentration time profile of optimized CR LAM matrix tablet in rabbits (n = 6)

The average time required for maximum plasma concentration (2.65 ± 1.84µg/ml) is 4hr.

The average half life of optimized CR tablets was found to be 6.31± 0.50 hr with average mean residence time (MRT) of 11.12 ± 0.52hr (Table 7).

Table 7: Pharmacokinetics parameters of LAM after oral administration of optimized formulation to rabbits (n = 6)

Parameters	Optimized formulation
C_{max} (µ/ml)	2.65 ± 1.84
T_{max} (hr)	4 ± 0.0
AUC_{0-24} (µ. hr/ml)	33.89 ± 5.35
AUC_{tot} (µ. hr /ml)	37.32 ± 6.53
$AUMC_{0-24}$ (µ. hr^2/ml)	301.86 ± 56.42
$AUMC_{tot}$ (µ. hr^2/ml)	415.12 ± 68.81
$t_{1/2}$ (hr)	6.31 ± 0.50
MRT (hr)	11.12 ± 0.52
K_e (hr^{-1})	0.1098 ± 0.02

Level A *in vitro-in vivo* correlation was performed by using percent LAM dissolved versus the percent LAM absorbed data at the same point (fig. 10).

DISCUSSION

The drug excipient compatibility was confirmed by FT-IR and DSC thermogram, both the studies were indicating no interaction between the drug-excipients and drug-polymers used for this study. The angle of repose and cars index data results shows good flow properties of the granules. All the physical parameters of the prepared tablets were found to be within the permissible limits of USP.

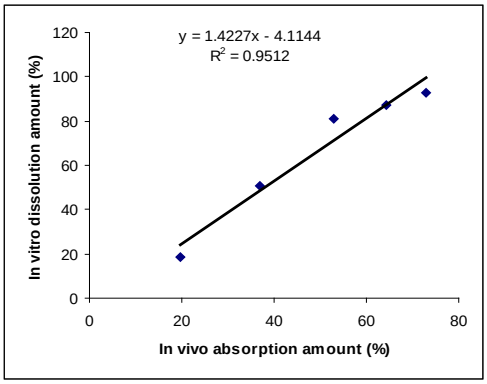

Fig. 10: Relationships between the percent LAM released and absorbed for optimized CR matrix tablet in rabbit

The release profile of all the formulations showed a linear pattern of LAM release at least in their initial phase, which indicates the appropriate choice of selected range of formulation variables. The decrease in drug release may be attributed due to the increased strength of HPMC gel layer; the drug diffusion was controlled by the penetration of liquid through the gel layer. CAP in spite of having more solubility at alkaline pH with minimum swelling, the release of drug was further hindered. Such behavior may be due to the thick gel layer of HPMC prevented the dissolution of CAP in alkaline medium. This indicated that CAP was not present in sufficient proportion to influence drug release pattern from the matrix because the solubility of the CAP was masked by the gel strength of HPMC [29].

This type of behavior is attributed due to low HPMC concentration in the delivery system makes the tablet matrix weaker leading to very fast release of drug. Such formulations with low $T_{50\%}$ values relatively have high percentage LAM release at 8hr. In order to understand the complex mechanism of drug release from the tablet, the *in vitro* release data was fitted to Korsmeyer-Peppas release model and interpretation of diffusion exponent values (n) enlightens in understanding the release mechanism from the dosage form.

The probable explanation for this behavior may be due to the increased polymer load in the delivery system and the system takes a complete control on the release of LAM due to polymer chain relaxation and disentanglement leading to erosion. Since presence of only HPMC in the matrix would not give the desired release profile of low initial drug release followed by increased release rate, hence CAP was included in the matrix. It was expected that presence of CAP would confer pH modulated release characteristics with very low drug release in acidic environment of the upper GI tract followed by higher release rate in the alkaline pH on account of formation of a porous matrix due to dissolution of CAP and erosion of gel matrix of HPMC [30].

Multiple response optimization approach was considered more useful and suitable for optimizing the release properties from controlled release matrix tablets. To optimize four responses with different targets, a multi-criteria decision approach, like numerical optimization technique by the desirability function was used to generate the optimum settings for the formulation [31,32]. A good relationship was found between the experimented and predicted values, which confirm the practicability and validity of the model.

The *in vitro* drug release profiles of the optimal CR matrix tablets before and after stability studies were presented with no significant differences, which found to be stable. The results of *in vivo* studies indicate that the formula generated by CCD exhibited a controlled

release profile of LAM. Also level A, *in vitro-in vivo* correlation studies resulted with the same point. Hence the optimized formulation of LAM matrix tablet provides controlled release.

CONCLUSION

A central composite design was performed to study the effect of formulation variables on release properties by the application of computer optimization technique. Amount of HPMC K100M along with its interaction with amount of CAP was found to be significantly affected the studied response variables indicating that an appropriate balance between the studied independent variables is imperative to get a controlled release of LAM. The mechanism of drug release from the optimized formulation was confirmed as non-fickian (anomalous) transport.

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CONFLICT OF INTEREST

Declared None

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