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RESEARCH ARTICLE

Formulation and Evaluation of Bilayer Tablets Containing L-Arginine

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ABSTRACT

L-Arginine is a semi-essential amino acid involved in numerous areas of human physiology, including production of nitric oxide (NO) a key messenger molecule. The purpose of the present work was to develop an optimized bilayer tablet for cardiac patient using L-Arginine as a drug candidate by optimization technique. In preliminary study, L-Arginine bilayer tablets were prepared by wet compression method. Preliminary study for immediate release and sustain release was done using different excipients like HPMC K15M, HPMC K100M, ethyl cellulose, Polyvinyl pyrrolidone, light magnesium oxide, Microcrystalline cellulose, sodium starch glycolate. Among them HPMC K100M, light magnesium oxide, SSG showed influence on drug release. A Box Behnken experimental design was employed in formulating bilayer tablets. HPMCK100M (X_1), Light Magnesium Oxide (X_2) and SSG (X_3) were selected as independent variables. Two dependent variables % CDR at 2 hrs (Y_1) and at 8 hrs (Y_2) were considered. The main effect and interaction terms were quantitatively evaluated using mathematical model. Bilayer tablets were evaluated for thickness, hardness, friability, drug content and *in vitro* dissolution studies. The drug release of L-Arginine obeyed the Korsmeyer-Peppas kinetic model which depicted fickian diffusion. Stability study was carried out at 25ºC / 60 %RH and 40ºC/ 75 %RH for 1 month and checked for the drug content and % CDR at 2 hrs and 8 hrs. Result of stability study indicated that optimized batch gives satisfactory result.

KEYWORDS

Bilayer tablet, L-Arginine, SSG, Poly vinyl pyrrolidone, HPMC K100M, Light Magnesium Oxide

INTRODUCTION

Modified release dosage form is a general term used to describe the dosage forms having drug release features based on time, course and/or location and which are designed to accomplish therapeutic or convenience objectives not offered by conventional or immediate release forms. There are several terms which are used interchangeably with respect to modified release dosage forms, viz., controlled release, sustained release, prolonged release, extended release and other such dosage forms.

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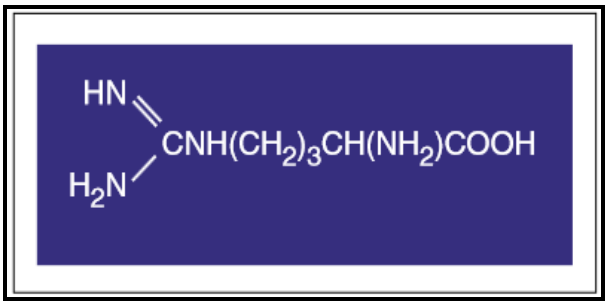
Controlled release system differs from other systems which simply prolong the drug release and hence the plasma drug levels for an extended period of time. Controlled release systems are those which can provide some control, whether this is of a temporal or spatial nature, or both, of the drug release in the body. An ideal controlled release system aims at delivering the drug at a predetermined rate, locally or systemically, for a specified period of time.[1,2] The design of controlled release dosage forms holds many advantages over conventional dosage forms like reduction in frequency of drug administration, improved patient compliance, reduction in drug level fluctuation in blood, reduction in total drug

usage when compared with conventional therapy.[3-4] Bilayer tableting technology has been specially developed to provide two different release rates or biphasic release of a drug from a single dosage form. For these types of drugs, extended release formulations generally lead to a delayed appearance of effective plasma levels and they cannot provide a prompt disposition of the dose immediately after administration.[5] To fulfil the specific therapeutic needs of the different diseases, new drug delivery devices are required for a more accurate time-programmed administration of the active ingredients. The optimization of pharmaceutical formulations with regard to one or more attributes has always been a subject of importance and attention for pharmaceutical scientists in formulation research.[6]

L-Arginine is an amino acid that has numerous functions in the body. It helps the body get rid of ammonia (a waste product), it is used to make compounds in the body such as creative, L-glutamate and L-praline, and can be converted to glucose and glycogen if needed. L-Arginine is used to make nitric oxide, a compound in the body that relaxes blood vessels. Preliminary studies have found that L-Arginine may help with conditions that improve when blood vessels are relaxed (called vasodilatation), such as atherosclerosis, erectile dysfunction.



Arginine's effects on cardiovascular function are due to arginine-induced endothelial NO production. Endothelial nitric oxide synthase (eNOS) catalyses this reaction, which produces NO and ornithine. Nitric oxide diffuses into the underlying smooth muscle and stimulates guanylyl cyclase, producing guanosine-3',5'-cyclic monophosphate (cGMP), which in turn causes muscle relaxation and vasodilation. Arginine supplementation has been shown to increase flow-mediated brachial artery dilation in normal individuals as well as those with hyperlipidemia and hypertension. Nitric oxide is also responsible for creating an environment in the endothelium that is anti-atherogenic. Adequate NO production inhibits its processes at the core of the atherosclerotic lesion, including platelet aggregation, monocyte adhesion and migration, smooth muscle proliferation, and vasoconstriction. Thus, the aim of the present study is to formulate and evaluate bilayer tablets containing L-arginine.

MATERIAL AND METHODS

L-Arginine was obtained from Desang Corporation. HPMC K100M & HPMC K15M was purchased by Colorcon Ltd. Ethyl cellulose & Microcrystalline cellulose were purchased by Accent micocell India. PVP K-30 were purchased by ISP technology ltd. Sodium starch glycolate were purchased by DMV International.

Drug-Excipient Compatibility Study

Before initiating formulation development, compatibility of L-Arginine with different excipients were tested using the techniques of FT-IR (Shimadzu, Model 8400S, Japan).

Manufacturing Procedure (Wet Granulation Method)

Preparation of Immediate Release Layer

Immediate release layer of L-Arginine was prepared by wet granulation technique. L-Arginine passed through sieve no. 20#. Sodium Starch Glycolate, HPMC K100M, Micro crystalline cellulose passed through sieve no. 40# & mixed in polybag. PVP K-30 was dissolved in 60 ml isopropyl alcohol and used for preparing the granules. The granules were dried in tray dryers at 60°C (LOD 1.5 to 2.5 % w/w). The erythrosine lake colour was passed through the sieve no 20#. All the above powders were mixed in geometric proportion in a polybag for 15 minutes. Talc and Magnesium stearate were passed through sieve no. 40#. Sifting was performed and lubricated material was mixed in the polybag for 2 minutes. The final weight of the IR layer was fixed to 600 mg.

Preparation of Sustained Release layer

Granules of sustained release layer were formulated by uniformly mixing required quantity of L-Arginine with measured quantities of polymer and diluent as specified in formulation table 5. Wet dump mass was passed through sieve no 20# and the granules were dried in tray dryer 60°C (LOD 1.5 to2.5 % w/w). Talc and magnesium stearate were added and mixed thoroughly before compression of granules. The final weight of the S.R. layer was fixed to 800 mg.

Compression of Bilayer Tablets

Bilayer tablets were compressed using 19.7 × 9.0 mm standard concave capsule shape punch on 27 station bilayer compression machines. (Cadmach Machinery Co. Pvt. Ltd., India). Various parameters like hardness and thickness maintain within limit 10.2-10.7 kg/cm^2, 7.0-7.8 mm respectively and Tablet weight was maintained at 1400 mg.

Experimental Design[7,8,9]

A Box-Behnken design was employed in the present study. In this design 3 factors were evaluated, each at 3 levels, and experimental trials were performed for all 15 possible combinations with 3 replicate center points. The concentration of Hydroxy Propyl Methyl Cellulose K100M (X_1), Light Magnesium Oxide (X_2) and Sodium Starch Glycolate (X_3) were chosen as independent variables in box-behnken design, while % CDR at 2 hrs (Y_1) and % CDR at 8 hrs (Y_2) was taken as dependent variable.

A three-factor, three-level BBD was used to explore quadratic response surfaces and construct second order polynomial models using Design Expert 8 (Version 8.0.7.1; Stat-Ease Inc. Minneapolis, MN). Since there are three factors, three levels, and three center points, the number of runs according to the above equation is N = 2 × 3(3 – 1) + 3 = 15 runs. The 15 experiments included the use of three center runs, which were necessary to avoid singularity and to verify any change in the estimation procedure. This design is suitable for exploration of quadratic response surfaces and for construction of second-order polynomial models, thus helping to optimize the process by using a smaller number of experimental runs (15 runs). This is more feasible than normal three-level three-factor (3^3) full factorial design (27 runs).

The model is of the following form:

$$\mathbf{Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X1^2 + b_{22}X2^2 + b_{33}X3^2 + E}$$

Where, Y is the selected response, b_0–b_3 are the regression coefficients, X_1, X_2, and X_3 are the factors studied, and E is an error term.

Table 1: Selection of independent variables and dependent variables

Independent variables			**Dependent variable**	
X_1	**X_2**	**X_3**	**Y_1**	**Y_2**
Concentration of Hydroxy Propyl Methyl Cellulose (mg)	Concentration of Light Magnesium Oxide (mg)	Concentration of Sodium Starch Glycolate (mg)	%CDR at 2 hrs.	%CDR at 8 hrs.

Table 2: Selection of levels for independent variables and coding of variable

Levels	**Coded value**	**Independent Variables**		
		X_1 (mg)	**X_2 (mg)**	**X_3 (mg)**
Low	-1	190	20	10
Intermediate	0	200	25	12
High	1	210	30	14

Table 3: Formula of different batches according to Box-Behnken Design

Batch No.	Variables Levels in Coded Form			Transformed Form		
	X_1	X_2	X_3	Conc. of HPMC K100	Conc. of Light Magnesium Oxide	Conc. of Sodium Starch Glycolate
F1	-1	-1	0	190	20	12
F2	+1	-1	0	210	20	12
F3	-1	+1	0	190	30	12
F4	+1	+1	0	210	30	12
F5	-1	0	-1	190	25	10
F6	+1	0	-1	210	25	10
F7	-1	0	+1	190	25	14
F8	+1	0	+1	210	25	14
F9	0	-1	-1	200	20	10
F10	0	+1	-1	200	20	10
F11	0	-1	+1	200	30	14
F12	0	+1	+1	200	30	14
F13	0	0	0	200	25	12
F14	0	0	0	200	25	12
F15	0	0	0	200	25	12

Table 4: Composition of Factorial Design Batches of IR Layer

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
L-Arginine	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500
HPMC K100M	0	0	0	0	0	0	0	10	12	22	40	40.4	46.4	41.4	41.4
Micro Crystaline Cellulose	27.5	32.5	42.5	37.5	39.5	44.5	40.5	30.5	37.5	27.5	10.1	10.1	6.1	6.1	6.1
Sodium Starch Glycolate	12	12	12	12	10	10	14	14	10	10	14	14	12	12	12
Poly Vinyl Pyrodine K30	45	40	30	35	35	30	30	30	25	25	20.4	20	20	25	20
Colour Erythrocine	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Talc	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10
Magnesium Stearate	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
I.P.A.	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
Total(mg/tab)	600														

Table 5: Composition of Factorial Design Batches of SR Layer

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
L-Arginine	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500
HPMC K100M	190	210	190	210	190	210	190	210	200	200	200	200	200	200	200
Light Magnesium Oxide	20	20	30	30	25	25	25	25	20	20	30	30	25	25	25
Poly Vinyl Pyrodine K30	65	45	55	35	60	40	60	40	55	55	45	45	50	50	50
Magnesium Stearate	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Talc	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
IPA	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S.	Q.S	Q.S	Q.S	Q.S
Total (mg/tab)	800														

After application of Box-Behnken design and with the help of produced polynomial terms, amount of formulation variable were optimized.

Evaluation of Bilayer Tablets[11]

Prepared powder blends were evaluated for the following parameters.

Pre-compression Parameters

All pre-compression parameter including bulk density, tapped density, compressibility index, Hausner's ratio and angle of repose were evaluated.

Post Compression Parameters

Prepared bi-layer tablets were evaluated for the following parameters.

a. Thickness

Thickness of the each 3 tablet was measured by using Venire callipers from the centre of top and bottom part of the tablet.

b. Uniformity of Weight

20 units were selected and weighed individually at random or, for single dose preparations in individual containers, the contents of 20 units, and calculated the average weight.

Not more than two of the individual weights deviate from the average weight by more than the percentage and none deviates by more than twice that percentage.

c. Hardness

The hardness of tablet (n=5) was an indication of its strength. Measuring the force required to break the tablet across tests it. Hardness was measured using the Monsanto hardness tester. Measure the pressure required to break diametrically placed matrix tablet, by a coiled spring.

d. Friability

Friability is the loss of weight of tablet in the container due to removal of fine particles from the surface. Friability test is carried out to access the ability of the tablet to withstand abrasion in packaging, handling and transport. Twenty tablets from each batch were selected randomly and weighed. These tablets were subjected to friability testing using for 100 revolutions. Tablets were removed, de-dusted and weighed again. Following formula was used to calculate the friability.

$$\textbf{Friability} = [(W_1-W_2)100]/W_1$$

Where,

W_1= Weight of tablet before test, W_2 = Weight of tablet after test.

e. In-vitro Dissolution Study

Dissolution studies were carried out using a USP dissolution apparatus type II with 900ml dissolution mediums at 37 °C ± 0.5 and 50 rpm in phosphate buffer (pH 7.2). At fixed time intervals, 10ml aliquots were withdrawn, filtered, suitably diluted and then assayed for L-Arginine content by measuring the absorbance at 217 nm. Fresh media (10 ml), which was pre-warmed at 37 °C, was replaced into the dissolution medium after each sampling to maintain its constant volume throughout the test. Dissolution studies were performed in three replicates (n = 3), and calculated mean values of cumulative drug release were used while plotting the release curves.

Checkpoint Analysis

A checkpoint analysis was performed to confirm the role of the derived polynomial equation and contour plots in predicting the responses. Values of independent variables were taken at three points, one from each contour plot, and the theoretical values of % CDR were calculated by substituting the values in the polynomial equation. Bi-layer tablets were prepared experimentally at three checkpoints and evaluated for the responses.

Optimization of Formulation

From overlay plot of responses, optimized formulation was selected as check point to validate RSM. The bilayer tablets were formulated using the chosen optimal composition and evaluated for various parameter and % drug release at 2 hrs and 8 hrs. The observed and predicted responses were critically compared.

Curve Fitting Analysis[12]

In order to describe the kinetics of drug release from a controlled release formulation, various mathematical equations have been proposed, namely, zero-order rate, first-order equation, Higuchi model, and Hixson Crowell cube root law. To authenticate the release model, dissolution data can further be analyzed by Korsmeyer Peppas equation. The selection criteria for the best model were based on goodness of fit, akaike information criteria (AIC), and residual sum of squares (SSQ).

Stability Study[13,14]

The purpose of stability study is to provide evidence on the quality of a drug substance or drug product which varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. Formulations were selected for stability on the basis of the *in vitro* drug release profile. The formulations were subjected to accelerated stability studies as per ICH (The International Conference of Harmonization) guidelines. Stability studies on the optimized formulation was carried out to determine the effect of presence of formulation additives on the stability of the drug and also to determine the physical stability of the formulation under accelerated storage conditions. Optimized bilayer formulations were packed in strips of thick aluminium foil laminated with polyvinyl chloride at elevated temperature and humidity conditions of 25ºC ± 2ºC / 60 ± 5% RH and 40 ± 2ºC/ 75 ± 5 % RH for time period of 1 month in stability chamber. Samples were withdrawn at the end of every week till six month and evaluated for % drug content and % CDR at the end of 2 hrs and 8 hrs.

RESULTS AND DISCUSSION

Drug-Excipient Compatibility Study

Drug and Excipients compatibility study was performed by using FT-IR spectrophotometer. Here, the peak of L-Arginine was correlated with drug in presence of other excipients. In all the FT-IR spectra, identical peak of L-Arginine was not varied than of its original peak. So, it would be concluded that, the drug is compatible with all the excipients used in the formulation.

Evaluation of Bi-layer Tablets

The granules for all fifteen batches prepared by wet granulation method were evaluated for various pre-compression parameters.

Table 6: Pre compression Evaluation Parameters of Factorial Batches for IR Layer

Batch Code	Bulk density (gm/ml)	Tapped density (gm/ml)	Carr'sindex (%)	Hausner's ratio	Angle of repose (θ)
F1	0.51±0.02	0.58±0.05	13.72±0.04	1.13±0.05	26.44±0.02
F2	0.52±0.02	0.59±0.05	13.46±0.05	1.14±0.01	27.46±0.05
F3	0.52±0.05	0.58±0.03	11.53±0.03	1.12±0.01	26.05±0.03
F4	0.54±0.06	0.61±0.01	12.96±0.04	1.13±0.05	27.52±0.05
F5	0.52±0.01	0.59±0.05	13.46±0.01	1.14±0.03	28.27±0.06
F6	0.53±0.02	0.60±0.02	13.21±0.05	1.13±0.01	26.65±0.05
F7	0.52±0.05	0.59±0.05	13.46±0.04	1.13±0.03	25.12±0.05
F8	0.51±0.03	0.58±0.07	13.72±0.02	1.14±0.07	27.11±0.03
F9	0.54±0.05	0.59±0.05	9.26±0.05	1.09±0.05	24.96±0.01
F10	0.55±0.02	0.62±0.05	12.72±0.04	1.12±0.05	26.44±0.02
F11	0.53±0.02	0.60±0.05	13.20±0.05	1.13±0.01	24.46±0.05
F12	0.54±0.05	0.61±0.03	12.96±0.03	1.13±0.01	22.05±0.03
F13	0.50±0.06	0.57±0.01	14.00±0.04	1.14±0.05	21.42±0.05
F14	0.52±0.01	0.58±0.05	11.54±0.01	1.11±0.03	26.87±0.06
F15	0.53±0.02	0.61±0.02	15.09±0.05	1.15±0.01	24.45±0.05

(All values are expressed as mean ± standard deviation, n=3).

The granules for all factorial batches were evaluated for bulk density which ranged from 0.50 to 0.55 gm/ml, tapped density which ranged from 0.57 to 0.62 gm/ml, Carr's index ranged from 9.25 to 15.09 %, Hausner's ratio ranged from 1.09 to 1.15 and angle of repose ranged from 21.42 to 28.27°. All these results indicated that, the granules possess excellent flowability and compressibility properties.

Table 7: Pre compression Evaluation Parameters of Factorial Batches for SR Layer

Batch Code	Bulk density (gm/ml)	Tapped Density (gm/ml)	Carr's index (%)	Hausner's ratio	Angle of repose (°)
F1	0.56±0.030	0.64±0.0152	14.28±0.355	1.14±0.05	28.43±0.5
F2	0.630±0.03	0.723±0.045	14.76±3.258	1.15±0.05	29.30±0.8
F3	0.566±0.041	0.653±0.030	15.37±3.66	1.15±0.02	26.99±0.3
F4	0.453±0.015	0.516±0.034	13.90±2.61	1.13±0.5	28.41±0.7
F5	0.540±0.04	0.617±0.032	14.25±3.16	1.14±04	27.64±0.5
F6	0.550±0.04	0.633±0.032	15.09±3.66	1.15±0.5	28.41±0.2
F7	0.566±0.041	0.653±0.030	15.37±2.89	1.15±0.1	26.99±0.4
F8	0.543±0.003	0.620±0.04	14.18±3.16	1.14±0.6	27.49±0.6
F9	0.57±0.04	0.651±0.056	14.21±0.039	1.14±0.64	28.15±0.4
F10	0.558±0.6	0.631±0.48	13.08±0.087	1.13±0.03	28.45±0.6
F11	0.569±1.15	0.646±0.098	13.53±0.34	1.13±0.07	27.42±0.2
F12	0.549±1.6	0.627±0.95	14.20±0.48	1.14±0.07	26.87±0.3
F13	0.559±0.18	0.643±1.58	15.02±0.49	1.15±0.59	25.97±0.8
F14	0.521±0.092	0.591±0.23	13.43±0.07	1.13±0.09	28.97±0.1
F15	0.573±0.04	0.659±0.54	15.00±0.068	1.15±0.54	26.15±0.7

(All values are expressed as mean ± standard deviation, n=3)

The granules for all SR factorial batches were evaluated for bulk density which ranged from 0.453 to 0.630 gm/ml, tapped density which ranged from 0.510 to 0.723 gm/ml, Carr's index ranged from 13.08 to 15.37 %, Hausner's ratio ranged from 1.13 to 1.15 and angle of repose ranged from 25.97 to 29.30°. All these results indicated that, the granules possess excellent flowability and compressibility properties.

Table 8: Post compression Evaluation Parameters of Factorial Design Batches

Batch Code	Thickness (mm) [n=3]	Weight Variation Test (±5%) [n=20]	Friability test (<1%) [n=5]	Hardness (kg/cm^2) [n=3]	Drug Content (%) [n=3]
F1	7.6 ±0.03	Pass	0.23 ±0.05	10.5±0.02	93.47±0.35
F2	7.8 ±0.5	Pass	0.3 ±0.01	10.5 ± 0.8	97.25±0.13
F3	7.4 ±0.01	Pass	0.5 ±0.01	10.2 ±0.4	98.80±0.55
F4	7.2 ±0.1	Pass	0.76 ±0.5	9.7±0.01	98.71±0.90
F5	7.6 ±0.03	Pass	0.43±0.06	10.3±0.01	96.93±0.83
F6	7.5 ±0.01	Pass	0.71 ±0.1	10.2±0.02	97.95±0.66
F7	7.6±0.08	Pass	0.62 ±0.11	10.7 ±0.1	93.47±0.35
F8	7.5 ±0.04	Pass	0.75 ±0.08	10.5±0.02	98.42±0.15
F9	7.4 ±0.02	Pass	0.23 ±0.05	10.9±0.05	98.94±0.93
F10	7.3 ±0.1	Pass	0.26 ±0.04	10.2±0.05	93.94±0.82
F11	7.8±0.3	Pass	0.39±0.84	10.5±0.01	98.75±0.87
F12	7.2±0.9	Pass	0.48±0.47	10.4±0.3	96.28±0.13
F13	7.4±0.4	Pass	0.26±0.9	10.2±0.45	99.6±0.54
F14	7.4±0.6	Pass	0.64±0.3	10.7±0.79	96.27±0.69
F15	7.8±0.2	Pass	0.31±0.9	10.2±0.3	97.87±0.12

Tablets of all 15 factorial batches (F1 to F15) passed weight variation test as the % weight variation was within the pharmacopoeia limits of ±5%. Thickness of all tablets was in the range between 7.2 mm to 7.8 mm. Hardness of tablets was in range between 9.7 to 10.9 kg/cm^2. Friability was in range between 0.23 to 0.75 %. Thus, all the physical parameters of the manually compressed tablets were quite within control. Friability values were less than 1 % in all cases shows good mechanical strength at the time of handling and transports.

***In-Vitro* Drug Release Study**

Table 9: *In-vitro* Drug Release of Factorial Batches

Time (hrs)	% Cumulative Drug Release														
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**	**F14**	**F15**
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	45.8± 3.45	37.87± 3.12	39.45± 4.15	43.98± 4.56	49.3± 3.28	42.58± 3.32	50.48± 4.19	41.98± 3.47	38.97± 3.36	38.97± 3.21	41.89± 4.32	38.97± 3.67	34.79± 4.72	33.76± 4.82	35.18± 3.84
2	54.42± 4.32	49.87± 3.78	45.87± 4.78	51.19± 5.18	59.22± 3.75	51.65± 4.76	58.98± 3.92	52.28± 4.53	46.39± 4.95	50.15± 4.65	47.98± 4.89	48.76± 5.63	39.15± 3.67	38.07± 5.29	40.87± 5.89
4	75.28± 4.65	68.79± 4.89	70.48± 5.63	73.21± 3.67	71.97± 5.29	76.87± 5.89	72.45± 4.79	75.15± 3.95	71.89± 3.82	69.81± 5.13	72.68± 4.65	67.97± 6.21	66.97± 5.89	67.86± 5.73	68.79± 5.24
8	83.29± 6.35	81.76± 4.78	84.97± 4.91	81.59± 4.98	86.97± 3.81	81.47± 5.62	84.79± 5.26	83.48± 4.72	82.97± 5.14	82.49± 5.68	82.98± 3.69	83.68 ±4.56	82.99± 4.63	85.79± 4.62	82.98± 4.84
12	97.58± 5.12	98.48± 5.41	98.78± 4.74	93.81± 5.25	96.32± 5.29	98.24± 3.76	97.32± 4.67	98.89± 5.98	97.58± 5.12	98.48± 5.41	98.78± 4.74	93.81± 5.25	96.32± 5.29	98.24± 3.76	97.32± 4.67

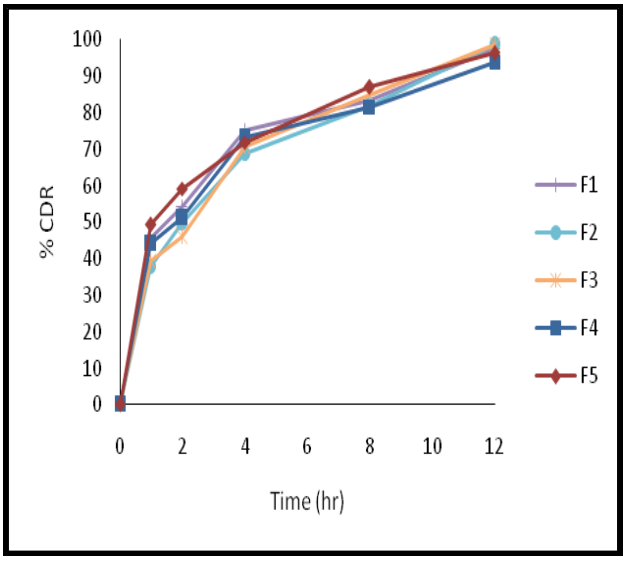


Figure 1: *In-vitro* Drug Release of Factorial Batches F1 to F5

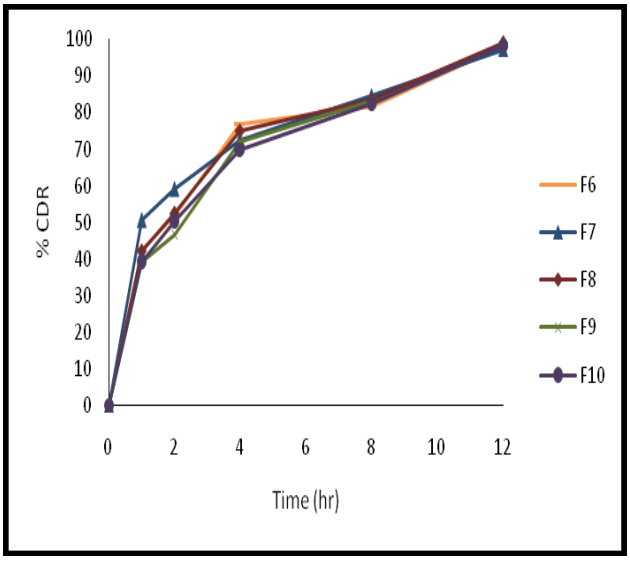


Figure 2: *In-vitro* Drug Release of Factorial Batches F6 to F10

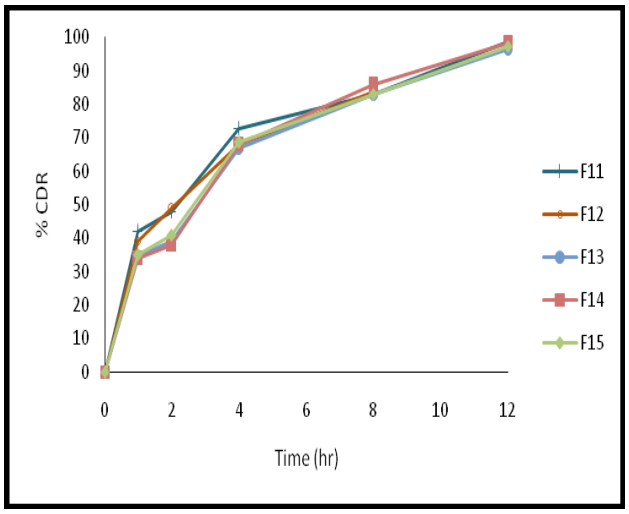


Figure 3: *In-vitro* Drug Release of Factorial Batches F11 to F15

From the dissolution data obtained from factorial batches, it was observed that in formulations containing same quantity of HPMC K100M as quantity of Light Magnesium Oxide decreased the % CDR at the end of 2 hrs and so as 8 hrs decreased that was due to hydrophobic nature of Light Magnesium Oxide which will impart hydrophobicity to the drug molecule so reduced bursting effect at the initial stage of release profile.

It was also observed that in I.R. formulations containing HPMC K100M decreased the % CDR at the end of 2 hrs as per the acceptance criteria this is due to drug in I.R. layer also bind and released in control manner. % CDR from all factorial batches was shown in table. It was concluded that batch F13 containing 200 mg of HPMC K100M, 25mg of Light Magnesium Oxide and 12mg of Sodium starch glycolate released maximum amount of drug at the end of 8 hrs that is 82.99 % as per the acceptance criteria (at the end of 2 hrs %CDR was 39.15%). So batch F13 was optimized.

Statistical analysis

A Box-behnken Design with 3 independent variable at 3 different levels is used to study the effect on dependent variable. All 15 batches of bilayer tablets within the experimental design were evaluated for % CDR at 2 hrs and % CDR at 8 hrs.

Linear, 2FI, Quadratic & Cubic Models were studied to data for dependent response simultaneously using design expert software. Quadratic model was suggested to obtain P-value, Adjusted R^2 and Predicted R^2 values.

Table 10: Selection of Model

Model Type	Y_1			Y_2			
	P-value	**Adjusted R^2**	**Pred. R^2**	**P-value**	**Adjusted R^2**	**Pred. R^2**	**Remark**
Linear	0.9192	-0.21864	-0.54874	0.4779	-0.0248	-0.5841	
2FI	0.9329	-0.59175	-1.73403	0.1146	0.3025	-0.5326	
Quadratic	0.0059	0.751354	-0.32262	0.042	0.7555	-0.001	Suggested
Cubic	0.1182	0.950001		0.4514	0.7984		

The % CDR (dependent variable) obtained at various levels of the 3 independent variables (X_1, X_2, and X_3) was subjected to multiple regression analysis to yield a second-order polynomial equation (full model) as shown in table 11 and 12. The value of the correlation coefficient (r^2) of response Y_1 and Y_2 was found to be 0.9112 and 0.9127 respectively, indicating good fit. The results clearly indicate that the % CDR value is strongly affected by the variables selected for the study. This is also reflected by the wide range of values for coefficients of the terms of equations.

The main effects of X_1, X_2, and X_3 represent the average result of changing 1 variable at a time from its low level to its high level. The interaction terms (X_1X_2, X_1X_3, X_2X_3, X_1^2, X_2^2, and X_3^2) showed the % CDR changes when 2 variables are simultaneously changed.

Coefficient may be positive or negative for synergistic or antagonistic effect respectively. From the model, it is evident that both synergistic and antagonistic effects on % CDR at 2hr and 8hr are observed.

Table 11: ANOVA for response surface quadratic model for Y_1

Source	**SS**	**Df**	**MS**	**F Value**	**p-value prob > F**	**R^2**
Model	508.7868	9	56.53187	5.700548	0.0349	0.9112
X_1 –HPMC K100	22.78125	1	22.78125	2.297211	0.1900	
X_2- LMO	0.904513	1	0.904513	0.091209	0.7748	
X_3- SSG	0.043513	1	0.043513	0.004388	0.9498	
X_1X_2	24.35423	1	24.35423	2.455826	0.1779	
X_1X_3	0.189225	1	0.189225	0.019081	0.8955	
X_2X_3	2.2201	1	2.2201	0.22387	0.6560	
X_1^2	305.3122	1	305.3122	30.787	0.0026	
X_2^2	13.06166	1	13.06166	1.317109	0.3030	
$X3^2$	184.8643	1	184.8643	18.64131	0.0076	
Residual	49.58459	5	9.916918			
Lack of fit	45.59633	3	15.19878	7.621745	0.1182	
Pure Error	3.988267	2	1.994133			
Cor Total	558.3714	14				
Equation:						
Full Model						
$Y= 39.36-1.69X_1-0.34X_2+0.074X_3+2.47X_1X_2+0.22\ X_1\ X_3-0.74\ X_2X_3 +9.09X_1^2+1.88\ X_2+7.08\ X_3^2$						
Reduced Model						
$Y= 39.36+9.09X_1^2+7.08X_3^2$						

Table 12: ANOVA for response surface quadratic model for Y_2

Source	SS	Df	MS	F Value	p-value prob > F	R^2
Model	31.1	9	3.46	5.81	0.0336	0.9127
X_1–HPMC K100M	3.81	1	3.81	6.4	0.0525	
X_2- LMO	0.71	1	0.71	1.19	0.3251	
X_3- SSG	2.12	1	2.12	3.57	0.1176	
X_1X_2	0.13	1	0.13	0.22	0.6561	
X_1X_3	0.37	1	0.37	0.62	0.4684	
X_2X_3	13.36	1	13.36	22.45	0.0052	
X_1^2	0.64	1	0.64	1.07	0.3473	
X_2^2	4.85	1	4.85	8.15	0.9316	
X_3^2	10.25	1	10.25	17.22	0.0089	
Residual	2.98	5	0.6			
Lack of fit	1.99	3	0.66	1.35	0.4514	
Pure Error	0.98	2	0.49			
Cor Total	34.08	14				
Equation:						
Full Model						
$Y= 82.35+0.69\ X_1-0.30\ X_2+0.52X_3+0.18\ X_1\ X_2+0.30\ X_1X_3-1.83X_2X_3+0.42\ X_1^2+0.036\ X_2^2+1.67\ X_3^2$						
Reduced Model						

Contour Plots for % CDR at 2 hr (Y_1)

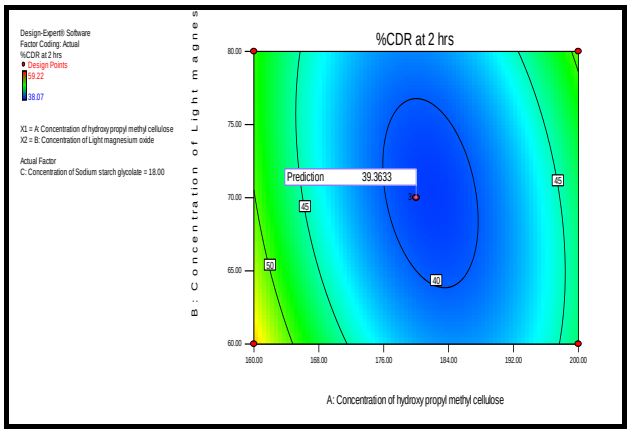


Figure 4: Two-Dimensional Contour Curve of HPMC K100M (X_1) and Light Magnesium Oxide (X_2) showing effect on % CDR at 2 hrs (Y_1)

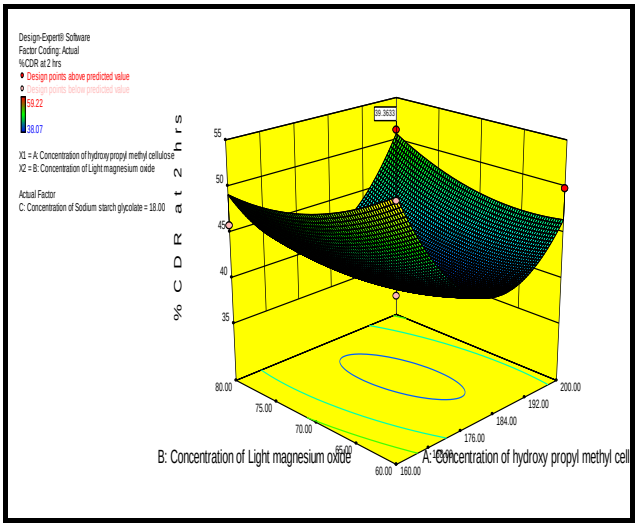


Figure 5: 3-D graph showing effect of HPMC K100M (X_1) and Light Magnesium Oxide (X_2) showing effect on % CDR at 2 hrs (Y_1)

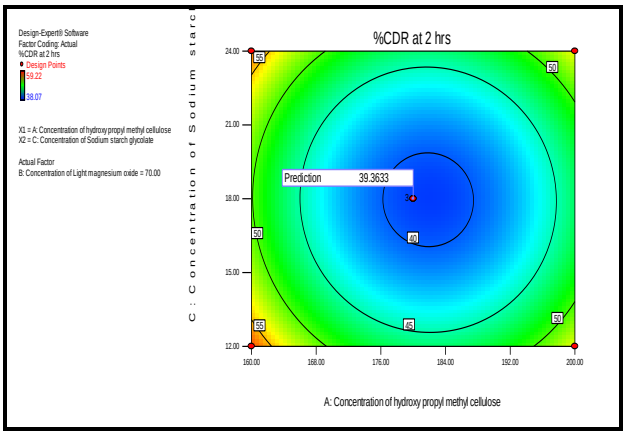


Figure 6: Two-Dimensional Contour Curve of HPMC K100M (X_1) and Sodium Starch Glycolate (X_3) showing effect on % CDR at 2 hrs (Y_1)

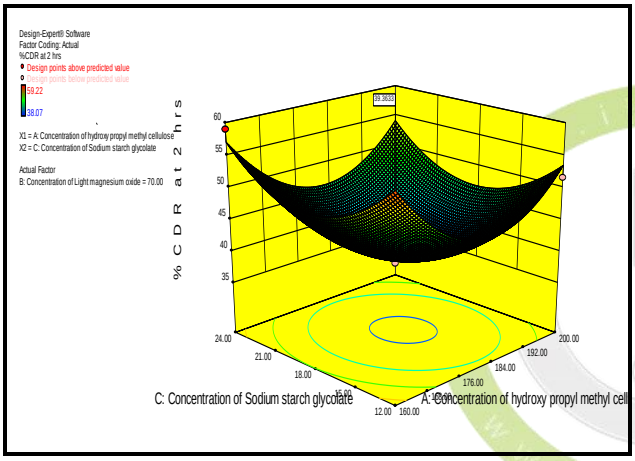


Figure 7: 3-D graph showing effect of HPMC K100M (X_1) and Sodium Starch Glycolate (X_3) showing effect on % CDR at 2 hrs (Y_1)

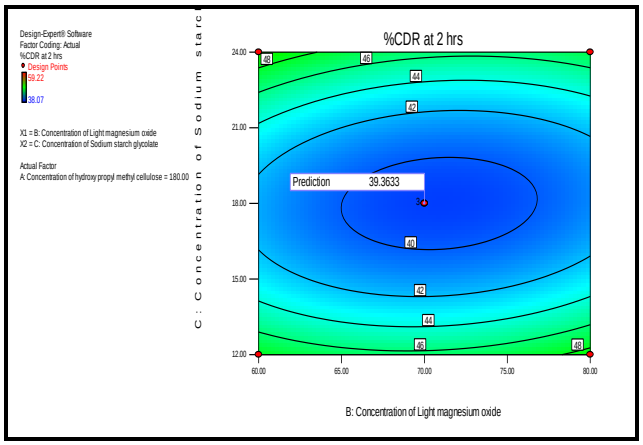


Figure 8:Two-Dimensional Contour Curve of Light Magnesium Oxide (X_2) and Sodium Starch Glycolate (X_3) showing effect on % CDR at 2 hrs (Y_1)

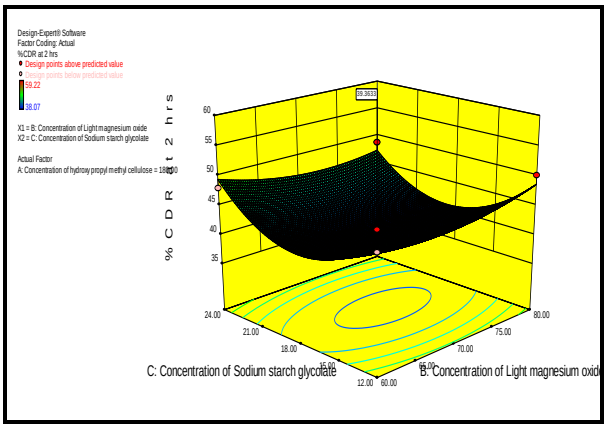


Figure 9: 3-D graph showing effect of Light Magnesium Oxide (X_2) and Sodium Starch Glycolate (X_3) showing effect on % CDR at 2 hrs (Y_1)

Contour Plots for % CDR at 8 hr (Y_2)

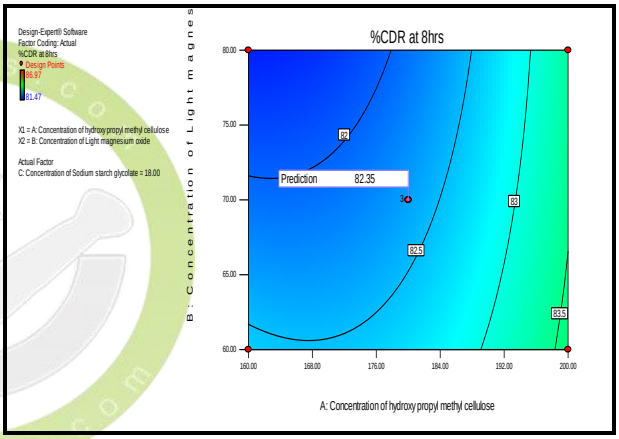


Figure 10: Two-Dimensional Contour Curve of HPMC K100M (X_1) and Light Magnesium Oxide (X_2) showing effect on % CDR at 8 hrs (Y_2)

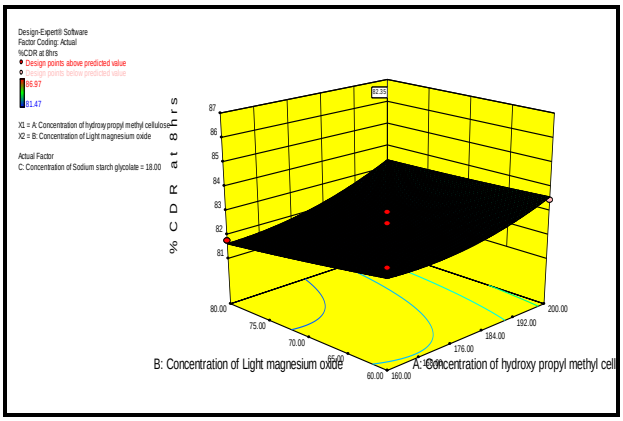


Figure 11: 3-D graph showing effect of HPMC K100M (X_1) and Light Magnesium Oxide (X_2) showing effect on % CDR at 8 hrs (Y_2)

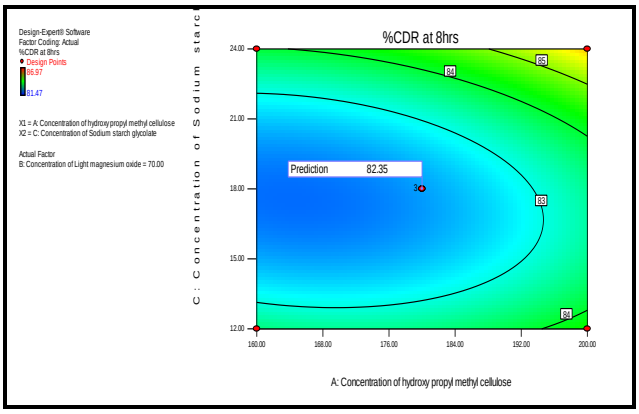


Figure 12: Two-Dimensional Contour HPMC K100M (X_1) and Sodium Starch Glycolate showing (X_3) effect on % CDR at 8 hrs (Y_2)

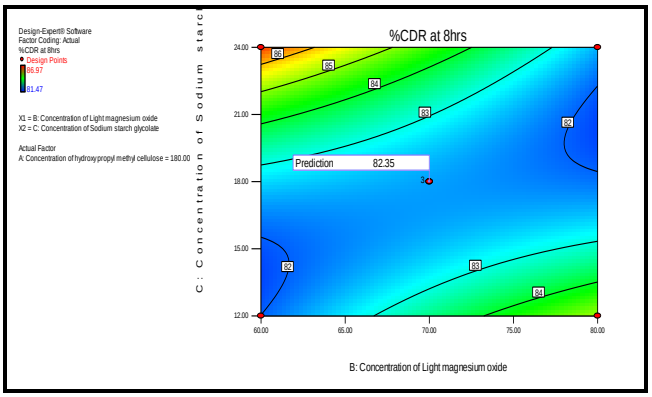


Figure 14: Two-Dimensional Contour Curve of Light Magnesium Oxide (X_2) and Sodium Starch Glycolate (X_3) showing effect on % CDR at 8 hrs (Y_2)

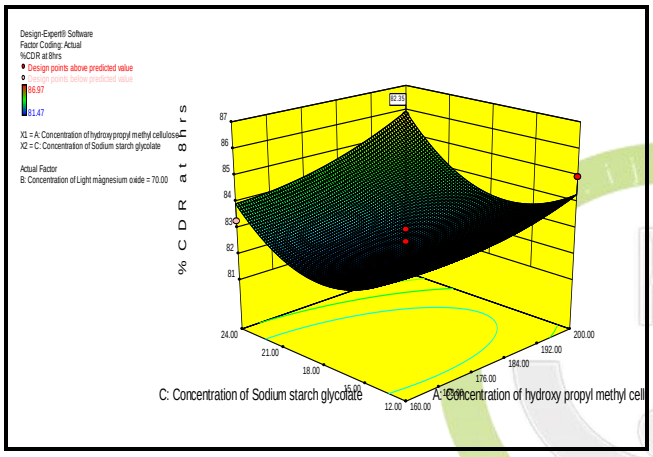


Figure 13: 3-D graph showing effect of HPMC K100M (X_1) and Sodium Starch Glycolate (X_3) showing effect on % CDR at 8 hrs (Y_2)

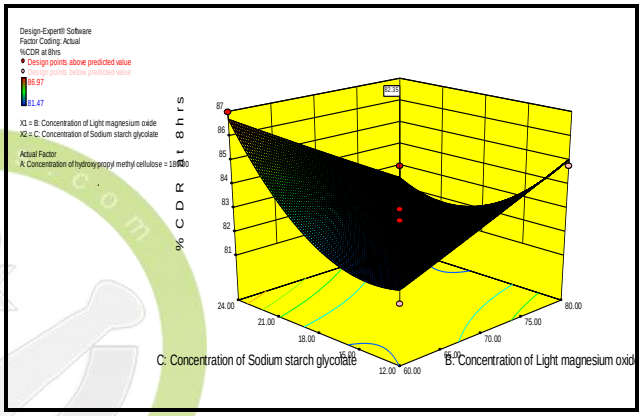


Figure 15: 3-D graph showing effect of Light Magnesium Oxide (X_2) and Sodium Starch Glycolate (X_3) showing effect on % CDR at 8 hrs (Y_2)

Check Point Analysis

Table 13: Checkpoint Batches with Predicted and Measured Value of % CDR

Batch Code	X_1	X_2	X_3	Y_1		Y_2	
				Measured	Predicted	Measured	Predicted
F16	-0.5	0	1	39.78	39.25	83.305	82.16
F17	0	1	-0.5	41.97	40.19	82.27	81.97
F18	1	-0.5	0	40.52	39.24	83.79	82.57

To validate the evolved mathematical models, three check point batch (F16, F17 and F18) were prepared and evaluated for % CDR at 2 hrs Y_1 and % CDR at 8 hrs Y_2. The observed and predicted values were shown in Table 13. Results indicate that the good correlation was found between observed and predicted values. Thus, it can concluded that the result analysis indicated good correlation between the experimental values and the predicated once, thereby suggested that the model was satisfactory and accurate.

Optimization of the Formulation

The optimum formulation was selected by "trading off" response variable % CDR at 2 hrs and 8 hrs allowing the maximizing criteria of release of < 40% & >80% respectively. Upon comprehensive evaluation, the formulation with 200 mg of Hydroxy Propyl Methyl Cellulose K100M, 25 mg of Light Magnesium Oxide, 12 mg of Sodium Starch Glycolate fulfilled the optimal criteria of % cumulative drug release at 2 hrs and 8 hrs. The optimization was performed by superimposing the contour plots of the response Y_1 and Y_2 and locating the region of optimal surface common to both the plots as shown in figure 16.

The overlay plot of the responses generates an optimized area, as per the desired criteria. It can be concluded that by adopting a systemic formulation approach, one can reach to an optimum point in the shortest time with minimum efforts. So, batch F13 was selected as an optimized formulation.

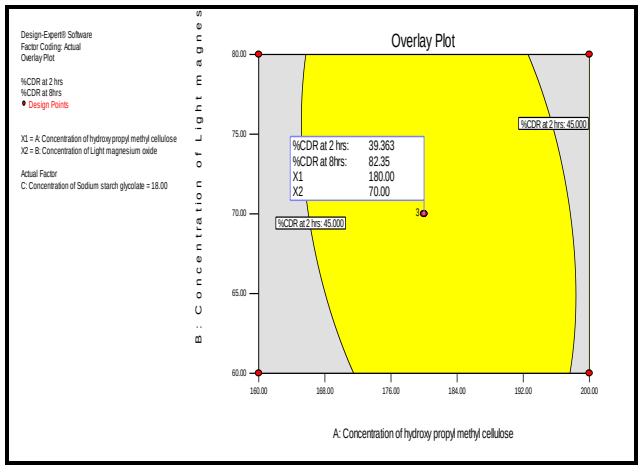


Figure 16: Overlay Plot of Response Variables

Table 14: Evaluation of Optimized Batch F13

Pre-compression Evaluation	**IR Layer**	**SR Layer**
Bulk density(gm/ml)	0.535±0.038	0.559±0.18
Tapped density(gm/ml)	0.615±0.087	0.643±1.58
Carr's Index (%)	14.95±0.15	15.02±0.49
Hausner's ratio	1.15±0.8	1.15±0.59
Angle of repose(°)	28.98±0.4	25.97±0.8
Post Compression Evaluation		
Weight variation	Pass	
Hardness (kg/cm^2)	10.2 ± 0.01	
Thickness (mm)	7.4 ± 0.4	
Friability (%)	0.26 ± 0.01	
% Drug content	99.6 ± 0.02	
% Cumulative Drug Release at 2 hrs	39.15%	
% Cumulative Drug Release at 8 hrs	82.99%	

(All values are expressed as mean ± standard deviation, n=3)

Application of Pharmacokinetic Study

The best fit model was selected on the basis of R^2 value. To confirm the diffusion mechanism, the data were fit into Korsmeyer-Pappas's equation. The formulations F13 showed good linearity (R^2: 0.9890), with slope (n) values ranging from 0.45, indicating that diffusion is the dominant mechanism of drug release with these formulations. This n value, however, appears to indicate a coupling of diffusion and erosion mechanisms so called fickian diffusion. The relative complexity of this formulation and its components may indicate that the drug release is controlled by more than one process. From the above analysis by using different model the Korsemeyer model was good fit with linearity value 0.9890.

Table 15: Model Fitting for Optimized Batch F13

Model	R^2	R	K	SSR	AIC
Zero Order Kinetic	0.6667	0.9288	9.723	2163.5371	48.0770
First Order Kinetic	0.9745	0.9891	0.276	165.2693	32.6455
Higuchi	0.9828	0.9925	29.627	111.7157	30.2957
Korsmeyer-Peppas	0.9890	0.9945	0.440(n=0.45)	71.6657	29.6321
Hixon-Crowell	0.9522	0.9846	0.076	310.4045	36.4273

R^2 = Regression Coefficient, r = Correlation Coefficient
K= Release Rate Constant, SSR= Sum of Squared Residuals,
AIC=Akaike Information Criterion

Stability Study

Optimized bilayer formulations were packed in strips of 0.04 mm thick aluminium foil laminated with polyvinyl chloride and placed for stability study at 40˚C/75% RH and 25 ± 2ºC / 60% ± 5% RH for 1 month in stability chamber. Sample was collected after 1 month and evaluated for dissolution in phosphate buffer pH 6.8, USP- II paddle apparatus at 50 rpm. Result was applied to stability study to show the effect of storage on *in-vitro* drug release of formulation. The results of accelerated stability studies were shown in Table 16.

Table 16: Stability Study of Optimized Formulation at 25ºc ± 2ºC / 60 ± 5% RH and 40 ± 2ºC/ 75 ± 5 % RH

Test	Initial	After1 Month	
		40± 2ºC/ 75± 5%RH	**25 ± 2ºC/ 60 ± 5% RH**
Appearance	Pink and white colour bilayer tablet and oblong shape plain on both side.	No change in appearance	No change in appearance
Hardness (kg/cm^2)	10.2±0.45	10.2±0.68	10.2±0.58
Thickness (mm)	7.4±0.4	7.4±0.3	7.4±0.4
Drug Content (%)	99.92±0.13	99.87±0.24	99.56±0.65
% CDR at 2 hrs	39.54±2.18	39.45±2.18	39.73±1.89
% CDR at 8 hrs	82.99±4.63	82.79±3.63	82.48±4.25

All values are expressed as mean ± standard deviation, n=3

CONCLUSION

The formulations of L-Arginine bilayer tablets showed good results in case of physicochemical parameters and prepared by using super disintegrant sodium starch glycolate for the fast release layer and HPMC K100M, Light magnesium oxide for the sustaining layer tablet which release drug up to 12 hrs. Pre-compression and post-compression parameters were found to be within the satisfactory limits and hence suitable to formulate bilayer tablets. Batch F13 has been selected as optimize formulation among all the other formulation. Batch F13 provides better drug release profile. It can be concluded that by considering the concentration of super disintegrant as a SSG for immediate release layer and the concentration of binder as a HPMC K100M and diluent light magnesium oxide for sustained release layer, bilayer tablets were successfully developed.

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