FORMULATION AND OPTIMIZATION OF EPLERENONE GASTRO RETENTIVE TABLET USING DESIGN OF EXPERIMENT

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ABSTRACT

Aim

The purpose of this research is to develop and optimize a novel gastro-retentive tablet of Eplerenone which has a short half-life of 4-5 hrs, and to study the maximum drug release up to 12 hrs by designing the amount of polymer by design of experiment.

Materials

The gastro retentive tablet of Eplerenone was compressed by using the wet granulation method, with three factors, a three-level Box-Behnken design was used to optimize by incorporating Sodium bicarbonate (X1), HPMC K15M (X2), and Carbopol 934p (X3). The design suggested 15 formulations of different concentrations of X1, X2, and X3, and their effect was monitored on Y1, Y2, and Y3.

Method

Tablets were prepared by granulation and compression on a rotary compressed machine.
The gastro retentive tablet formulations were evaluated for physical characterization namely hardness, friability, weight variation, drug content uniformity, buoyancy studies, and floating lag time (Y1), in vitro drug release study at 6 hr (Y2) In vitro drug release studies at 12 hr (Y3) was performed using united states pharmacopeia XXIIV type dissolution test apparatus employing paddle stirrer at 50 rpm. The dissolution medium was 900 ml of 0.1N HCl at 37 ± 0.5° C.

Results: Accordingly, Box-Behnken design suggested an optimized formula of 73.2 mg of X1, 100 mg of X2, and 5.0 min of X3 for selected constraints of Y1, Y2, and Y3 responses. The dissolution profile of batch F16 was found to follow first-order kinetic (R2 = 0.9843) there was no difference observed in the release profile after the stability study at 40 °C/75% RH for 0,3,6 months.

KEYWORDS: Box-Behnkenexperimentaldesign,Eplerenone,gastro retentivetablet,sustainedrelease.

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INTRODUCTION

Oral sustained-release dosage forms release the drug for a longer period and aid in producing the therapeutic effect for 12 hr, for those drugs which are having low biological plasma half-life. Drugs that have narrow absorption windows in the gastrointestinal tract will have poor absorption of the drug into the systemic circulation; gastro retentive drug delivery systems have been designed. Dosage form with a prolonged mean residence time in the stomach helps in the absorption of the drugs which are less soluble or unstable in the basic pH and drugs which are absorbed from the upper gastrointestinal tract. Gastro retentive dosage systems help in the conservation of continuous therapeutic stages for prolonged periods. Floating drug delivery system has less density than gastric fluid, so they remain buoyant in gastric fluid and show sustained drug release. It was recommended that compounding narrow absorption window drugs in a unique pharmaceutical dosage form with gastro-retentive properties would enable an extended absorption phase of these drugs [1]. After oral administration, such a dosage form would be retained in the stomach and released the drug there in a controlled and prolonged manner, so that the drug would be supplied continuously to its absorption sites in the upper gastrointestinal tract which is administered orally. It is incompletely absorbed from the gastrointestinal tract and has an oral bioavailability of only 50%. A novel formulation has the advantage to prolong the gastric retention of the drug and thereby possibly improve the oral bioavailability of Eplerenone (EP). The short biological half-life of EP (4-5 hrs) also favors the development of sustained-release formulations. Drugs that are easily absorbed from the gastrointestinal tract and those with a short half-life are quickly eliminated from the systemic circulation due to which frequent dosing is required. To overcome this problem, gastro retentive drug delivery systems which provide effective plasma drug concentration for longer periods thereby reducing the dosing frequency are being formulated [2]. It also has the advantage of minimizing the fluctuations in plasma drug concentration by delivering the drug in a sustained manner. The present study aimed to analyze the effect of concentration of sodium bicarbonate, HPMC K15M, and Carbopol 934p on floating lag time, *in vitro* drug release studies at 6^{th} hr, and *in vitro* drug release studies at 12^{th} hr.

MATERIALSANDMETHODS

MATERIALS

Eplerenone was received as a gift sample from RA chem pharma., Hyderabad. Sodium bicarbonate HPMC K15, and Carbopol 934p were obtained from Himedia Laboratories Pvt., Ltd., Mumbai., Isopropyl alcohol, talc, and magnesium stearate, were purchased from S.D. Fine chemical., Mumbai. PVP K-30 was purchased from Burgoyne Burbidge & co., lactose was purchased from S.D. Fine chemicals. Mumbai Acetone was purchased from S.D. Fine chem Ltd., Mumbai. All other used ingredients and solvents were of analytical grade.

METHODS

ANALYTICAL METHOD

PreparationofcalibrationcurveforEplerenone0, 4, 8, 12, 16, 20 µg/ml solutions were prepared by

taking solution from stock IIand stock IIIandvolumewasmadeup to 10ml as shown in table 1.Theabsorbance ofrespectivesolutionsweredeterminedusingUV-visiblespectrophotometerat244nm against0.1N HClastheblank.Theexperiment was repeated six times in the same medium and a calibration curve was determinedfromthemean value as shown in fig 1.

Table1.Standardcalibrationof Eplerenone in 0.1NHCl

Concentration (µg/mL)	Absorbance
0	0
4	0.233± 0.012
8	0.439± 0.015
12	0.645± 0.020
16	0.849± 0.005
20	0.998± 0.009

*n=6 Mean ± S.D.

StandardCalibrationgraphof Eplerenoneat244nm

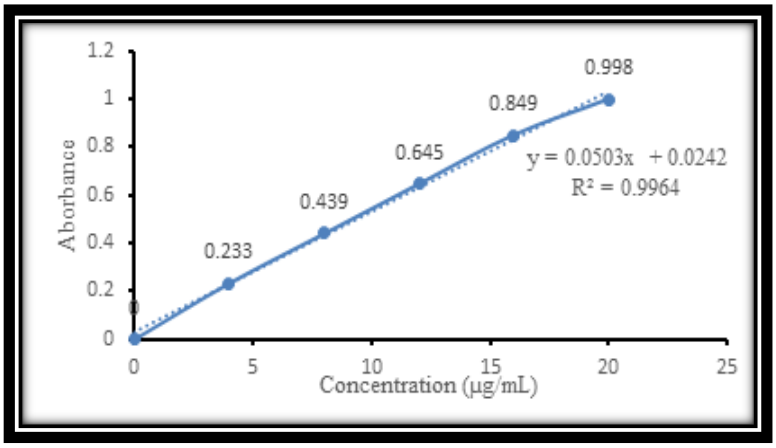


Fig 1. Standard graph of Eplerenone at 244 nm

COMPATIBILITYSTUDIES BY FTIR:

The compatibility of drugs and polymers under experimental conditions is an important prerequisite before formulation. Incompatibility between drugs and excipients can alter the stability and bioavailability of drugs, thereby, affecting their safety and/or efficacy. To confirm the purity of the test sample EP, and physical mixture. The sample was subjected to IR (FTIR Bruker) scanning over a wavelength range of 4000-400 cm-1 at a resolution of 2 cm-1 sample of 5 mg were directly placed on the probe, the spectra were recorded and compared with standard EP with reference functional group peaks as per USP/IP as shown in fig 2, 3 and table 2. [3]

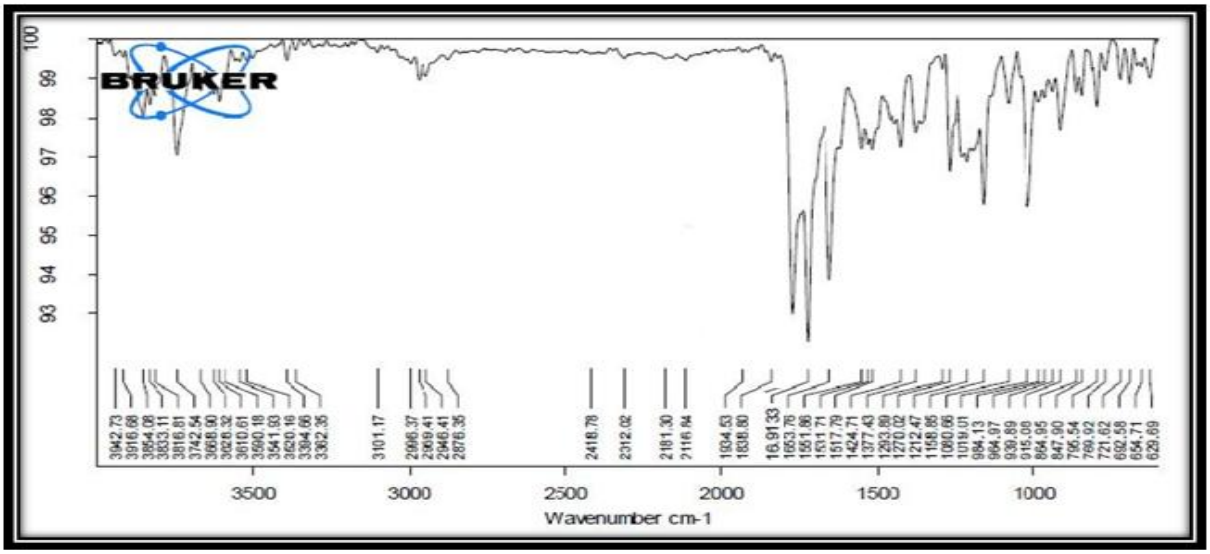


Fig 2.FTIR ofEplerenone.

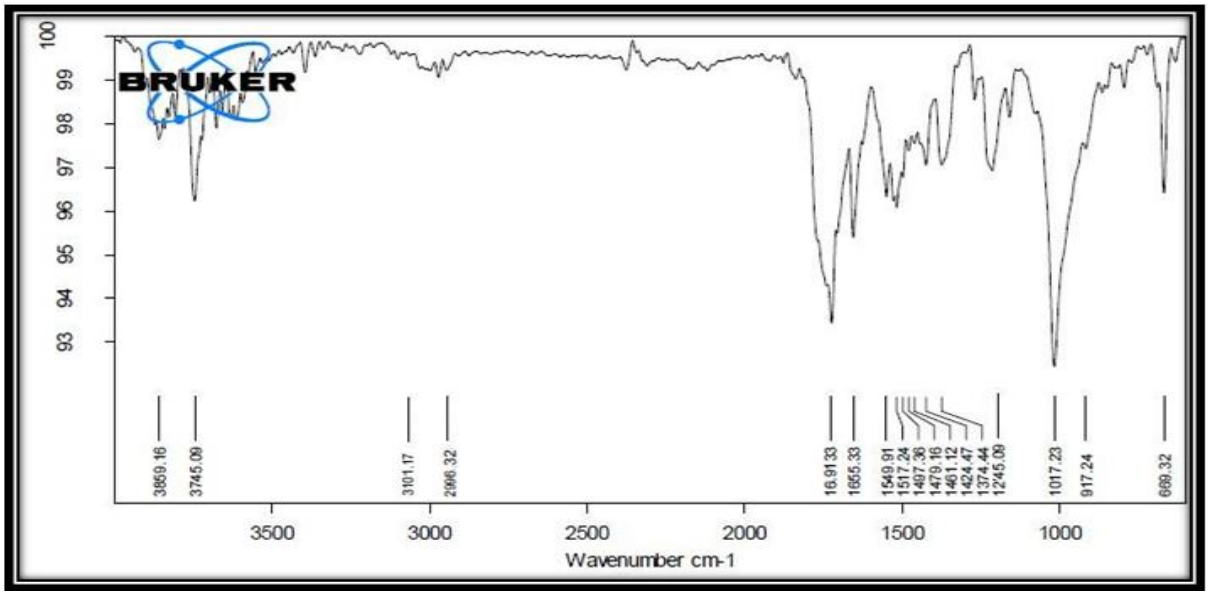


Fig 3. FTIR of Physical mixture

Table2. FTIR SPECTRA DATA OF EPAND PHYSICAL MIXTURE

Functional group	Range (cm^{-1})	Drug (Eplerenone) (cm^{-1})	Physical mixture (cm^{-1})
Aliphatic C-H stretching	2850-3000	2996.37	2996.32
Olefinic =C-H stretching	3000-3100	3010.17	3010.17
R_2 C=O (Keto)	1695	1691.33	1691.33
Olefinic C=C stretching	1642	1653.76	1655.33
C-O stretching	1245	1270.02	1245.09

BOX–BEHNKENEXPERIMENTALDESIGN

Box–Behnken experimental design (Stat-Ease, Inc. Design Expert trial version 13.0.5.0) was used to assess the effects of selected independent variables on the response to optimize the floating formulation procedure. This strategy is used to optimize the procedure using a lesser number of experimental trials by investigating quadratic response equations and by developing second-order polynomial models. The levels of factor were coded as low, medium, and high settings (−1, 0, and +1) as shown in table 3[4]. Preliminary experiments revealed the chosen independent and dependent variables along with their levels and constraints as shown in Table 3. The chosen independent variables were sodium bicarbonate (X1), HPMC K15 (X2), and Carbopol 934p (X3). The observed responses of the dependent variable were selected as Floating lag time (Y1), *In vitro* drug release study at 6 hr (Y2), *and In vitro* drug release studies at 12 hr (Y3). A Box-Behnken design was employed in this work and extended to optimize the EP floating tablet formulation for that a total of 15 experimental formulae were planned by Box-Behnken design. Through generating the polynomial equation concerning the dependent and independent variables, the procedure is to optimize the values of X1, X2, and X3, which gave the best wanted possible value of Y1, Y2, Y3 under controlled circumstances. A new formulation F16 was prepared according to the predicted levels of X1, X2, and X3. Subsequently, the observed response Y1, Y2, Y3 were matched the predicted data and the residual, as well as the residual errors (%) were then calculated. The design is suitable for the Mathematical expression of value in nonlinear quadratic response.

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

Y=representseachresponselevelb0isinterceptofallthevariablesintheequationfromb1-b33,forthesevariables,it wasfoundthat there arethreelackof fits andfour pure errors.

TABLE3.EXPERIMENTALDESIGNPARAMETERS

Factors	Levelsused(codedvalues)			Actualvalue (mg)		
IndependentVariables	Low	Medium	High	Low	Medium	High
Sodium Bicarbonate (X_1) (mg)	-1	0	1	25	50	75
HPMC K15M(X_2) mg)	-1	0	1	50	75	100
Carbopol 934p (X_3) (mg)	-1	0	1	5	10	15
Dependentvariable	Constraint			Importance		
Floating lag time (Y_1) (min)	Minimum			5		
In vitro drug release study at 6^{th}hr (Y_2) (%)	In Range (50-65)			3		
*In vitro*drug release study at 12^{th}hr (Y_3) (%)	In Range (85-100)			3		

FORMULATIONSDETAILS

FloatingtabletscontainingEplerenonewerepreparedbywetgranulationmethodusingvaryingconcentrations of sodium bicarbonate, HPMC K15M, and Carbopol 934p. The compositions of all formulations are given in table 4.

Table4.COMPOSITIONOFGASTRORETENTIVEFLOATINGTABLETSOFEPLERENONE (EP)

F Code	EP (mg)	A: $NaHCO_3$ (mg)	B: HPMC K15M (mg)	C: Carbopol 934p (mg)	Magnesium stearate(mg)	PVP-K30 (mg)	Lactose (mg)	Total(mg)
F1	70	50	100	5	3	30	42	300
F2	70	75	50	10	3	30	62	300
F3	70	50	100	15	3	30	32	300
F4	70	50	75	10	3	30	62	300
F5	70	25	75	15	3	30	82	300
F6	70	50	50	15	3	30	82	300
F7	70	50	75	10	3	30	62	300
F8	70	25	100	10	3	30	62	300
F9	70	75	75	15	3	30	32	300
F10	70	50	50	5	3	30	92	300
F11	70	25	75	5	3	30	92	300
F12	70	50	75	10	3	30	62	300
F13	70	75	100	10	3	30	12	300
F14	70	25	50	10	3	30	112	300
F15	70	75	75	5	3	30	42	300

PROCEDURE

Required quantities of all the ingredients were taken as per the Table 5, they were subjected topass through a sieve no #60. The granulation was prepared by using PVPK-30 inisopropylalcoholasagranulatingagentandthewetmasswasscreenedusingasieveno#44.Then dried at 40 ˚C in hot air oven for 24 hrs, followed by addition of lubricated(magnesiumstearate).Finally,blendwascompressedinthetabletusing16station rotatory punching machine by 10 mm punches. Further evaluation studies are done as shown in table 5.

FORMULATIONDESIGN

TABLE5.COMPOSITIONOFEXPERIMENTALDESIGN

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Std	**Run**	**A: $NaHCO_3$**	**B: HPMC K-15M**	**C: Carbopol 934p**	**Floating lag time**	**Cumulative % of drug release at 6^{th} hr**	**Cumulative % of drug release at 12^{th} hr**
		mg	mg	mg	Min	%	%
10	1	50	100	5	13	61.78	76.84
2	2	75	50	10	24	68.75	88.20
12	*3*	*50*	*100*	*15*	*18*	*40.70*	*61.67*

13	4	50	75	10	25	64.40	79.40
7	5	25	75	15	11	54.30	70.82
11	6	50	50	15	10	61.40	75.00
14	7	50	75	10	24	64.89	80.61
3	8	25	100	10	28	52.30	64.45
8	9	75	75	15	15	55.90	71.22
9	*10*	*50*	*50*	*5*	*10*	*69.65*	*99.39*
5	11	25	75	5	11	65.98	84.11
15	12	50	75	10	26	65.71	80.59
4	13	75	100	10	28	59.10	78.52
1	14	25	50	10	20	69.30	86.02
6	15	75	75	5	9	69.90	95.54

*n=3 Mean

EVALUATION

PRECOMPRESSIONPARAMETERS [5]:

BULKDENSITYANDTAPPEDDENSITY:
Bulk density(BD)andTapped density(TD)weredetermined.BDandTD was calculated using following formula:
Bulk density (ρb) = weight of the powder/Bulk volume of the powder (g/cc)
Tapped density (ρt) =weight ofthepowder/Tappedvolumeofthepowder (g/cc)

COMPRESSIBILITYINDEX:
Percentage compressibility of powder mix was determined by Carr'scompressibility index.Grading of the powders for their flow properties according to Carr's Index is calculated byfollowingformula.
Carr'sindex (%) = [(TD -BD) ×100]/TD

ANGLEOFREPOSE (Ɵ)
Thefrictionalforces inaloosepowderorgranules can bemeasured bythe angle ofrepose.
tan θ =h/r
$\theta=\tan^{-1}$ (h/r)
Where,θistheangleofrepose, h=height of pile, r =radius of thebaseofpile

HAUSNER'SRATIO
Hauser's ratio is an indirect index of ease of powder flow at certain angle. It was calculated bythefollowing formula
Hauser'sRatio=D_t/D_b
Where, D_t= Tapped densityD_b=bulk density

POSTCOMPRESSIONPARAMETERS

WEIGHTVARIATIONTEST
Twenty pills were chosen randomly from each batch, and the average weight of each tablet was calculated. The weight of each tablet was then compared to their average weight. [14].

HARDNESSTEST

The hardness of tablets affects how resistant they are to breaking during storage, transportation, and handling prior to use. The hardness of tablet was measured by Pfizer hardness tester. Kg/cm2 units were used to express the hardness.

FRIABILITY

The friability was examined using the Roche friabilator. Ten pills were precisely weighed and then placed in the rotating device that turns at a speed of 25 rpm. The pills were reweighed after 4 minutes to assess the percentage of weight loss. [15].

CONTENTUNIFORMITYTEST

Twenty tablets were broken down into a fine powder, and then portions of the powder corresponding to 70 mg of eplerenone were precisely weighed and added to a 100 ml volumetric flask. The 0.1NHCl (pH 1.2) solution was added to the volumetric flask and carefully stirred. 100 ml of 0.1NHCl was used to create the solution, which was then filtered. Add 0.1N HCl to 1 ml of the resultant solution to make it equal to 10 ml. A Shimadzu UV-visible spectrophotometer was used to measure the absorbance of the resultant solution at 244 nm.

***INVITRO*DISSOLUTIONSTUDIES**:

The *in vitro* dissolution study was performed by using a United States Pharmacopeia (USP) typeII(paddle)apparatusatarotationalspeedof50rpm.Exactly900mlof0.1NHClwasused as the dissolutionmedium and the temperaturewas maintained at 37˚C ± 0.5°C.

RESULTSANDDISCUSSION

STANDARDCALIBRATIONCURVESOF EPLERENONE

This shows the standard calibration curves for Eplerenone with slope, regression co-efficient and intercept are obtained. Theresults areshown in table 1.

COMPATIBILITYSTUDIES

CompatibilitystudiesofpuredrugEplerenonewithallexcipientswerecarriedoutpriortothepreparationoffloatingtablets.I.RspectraofpuredrugEplerenoneandcombination of Eplerenone and excipients were obtained. The results found that therein interaction betweenthedrugand excipient [16].

PRECOMPRESSIONPARAMETERS[6]:

ANGLEOFREPOSE

All formulations had angles of repose below 350, which indicates that the granules had good flow properties. The values were determined to be in the range of 23.120 to 27.040.

COMPRESSIBILITY INDEX

The range of Carr's index is 14.28 to 24.64%. Every formulation demonstrates good compressibility. Table 6 presents the outcomes.

HAUSNER'S RATIO

Hausner's ratio was found to be in the range of 1.31 to 1.02 as shown in table 6.

Table6.RESULTSOFPRE COMPRESSIONPARAMETERS

Fcode	*Bulk density(g/cc)Avg ± SD	*Tapped density(g/cc)Avg ± SD	Angle ofRepose(Ɵ)	CompressibilityIndex (%)	Hausner'sratio
F1	0.462±0.018	0.591±0.018	26.06	21.81	1.27
F2	0.469±0.026	0.561±0.014	25.42	21.39	1.19
F3	0.475±0.018	0.565±0.029	23.45	16.03	1.18
F4	0.469±0.019	0.561±0.014	27.04	16.39	1.19
F5	0.486±0.026	0.638±0.025	24.28	24.61	1.31
F6	0.478±0.028	0.586±0.025	26.06	18.43	1.23
F7	0.469±0.014	0.561±0.017	27.04	16.39	1.20
F8	0.462±0.016	0.539±0.014	23.72	14.46	1.17
F9	0.451±0.014	0.565±0.025	23.65	20.17	1.25
F10	0.487±0.012	0.637±0.014	23.72	24.64	1.30
F11	0.485±0.018	0.568±0.024	24.61	14.28	1.17
F12	0.460±0.016	0.561±0.016	25.42	21.39	1.21
F13	0.521±0.012	0.531 ± 0.014	23.12	20.16	1.02
F14	0.422±0.015	0.518 ± 0.016	23.32	18.93	1.22
F15	0.511±0.019	0.561 ±0.015	25.72	17.61	1.09

*n=3 Mean ± S.D.

POST COMPRESSION PARAMETERS [7]:

WEIGHTVARIATIONTEST:

Between 297.0 and 3.16 and 306.2 and 5.26 mg were the values of the pills. As indicated in Table 7, all of the pills passed the weight variation test since the weight variation was less than 10% of the maximum allowed by the Pharmacopoeia.

HARDNESSTEST

Thehardness of allformulations was intherangeof 5 to 5.5 kg/cm^2.

DRUGCONTENT

Thepercentageof drugcontent oftablets was found to bebetween 99.98 ±0.018 to 100.2 ±0.015 %.

FRIABILITYTEST

Thefriabilityvaluesofpreparedtabletsaregivenintable7.Thevaluesrangedfrom0.12to 0.55 %.

***INVITRO*BUOYANCYSTUDIES**

On immersion in 0.1N HCl solution pH (1.2) at 37±0.5 ^{0}C, the tablets floated, and remainedbuoyant without disintegration. Formulation F1 to F15 containing HPMCK15M showed highest floatinglag time of 28 min, and total floating time was upto12 hrs, this may be due to the amount of polymer. As the polymer concentration increased the floating lag time also increased.

Table7.POSTCOMPRESSIONPARAMETERS

FCode	Hardness(kg/cm^2)	*Weightvariation (mg)	*DrugContent (%)	Totalfloatingtime (hr)	Friability (%)
F1	5.4	300.4 ± 1.51	99.23±0.014	12	0.14
F2	5.0	300.6 ± 2.40	99.62±0.024	12	0.12
F3	5.5	301.6 ±1.92	99.17±0.036	12	0.27
F4	5.5	297.8±6.64	98.27±0.017	12	0.29
F5	5.5	303.8±3.36	98.54±0.010	12	0.30
F6	5.0	297.0 ±3.16	99.34±0.020	12	0.45
F7	5.5	300.2 ±1.64	100.2±0.015	12	0.32
F8	5.0	301.8±1.48	99.89±0.016	12	0.53
F9	5.0	301.0±1.73	99.97±0.025	12	0.46
F10	5.0	306.2 ±5.26	98.89±0.016	12	0.42
F11	5.0	305.4±6.02	99.12±0.018	12	0.47
F12	5.0	304.6 ±4.27	99.98±0.018	12	0.43
F13	5.0	301.2 ±0.44	99.28±0.015	12	0.55
F14	5.0	301.0 ±2.23	98.62±0.019	12	0.47
F15	5.0	302.6±3.43	98.81 ±0.016	12	0.54

*n=3 Mean ± S.D.

Fifteen formulations were formulated according to the Box-Behnken design,three response variables were taken floating lag time,*in vitro* drug release at 6th hour and 12th hour the selected independent variables X_1, X_2, X_3, where found to influence on three responses Y_1, Y_2, Y_3[8]. All the batches show floating lag time Y_1 in the range between 9 to 28 minutes, *in vitro* drug release at 6th hour Y_2 in the range of 42% to 69% and *in vitro* release at 12 hour Y_3 in the range of 61% to 99%. The various models fitted for each response were linear, cubic, two factor interaction and quadratic models. The result obtained were showing quadratic model was found to fits best for all three responses as indicated by greater R^2 value as shown in table 8, usingANOVA studies, the polynomial equation was generated by the software to determine the main effect and interaction factors by statistical parameters. The results of ANOVA study are shown in table 9, accordingly model F-value for responses Y_1, Y_2, $Y_3$where found to be 146.87, 127.39, 143.38 respectively, which implies that the quadratic model selected was significant for all the formulations, moreover all the models are

significant and the lack of is not significant in the selected mode[l9-12].

Table 8. MODEL SUMMARY STATISTICS

Model	R^2	Adjusted R^2	Predicted R^2	S.D.	Remark
Response (Y_1)					
Linear	0.1175	-0.1232	-0.6946	7.65	
2FI	0.1439	-0.4982	-2.7380	8.84	
Quadratic	0.9962	0.9894	0.9774	0.74	Suggested
Cubic	0.9973	0.9808		1.00	Aliased
Response (Y_2)					
Linear	0.8401	0.7965	0.6950	3.62	
2FI	0.9072	0.8375	0.6630	3.24	
Quadratic	0.9957	0.9878	0.9407	0.8851	Suggested
Cubic	0.993	0.9948			Aliased
Response (Y_3)					
Linear	0.9356	0.9181	0.8633	3.00	
2FI	0.9886	0.9801	0.9442	1.48	
Quadratic	0.9961	0.9892	0.9442	1.09	Suggested
Cubic	0.9996	0.9970		8.5774	Aliased

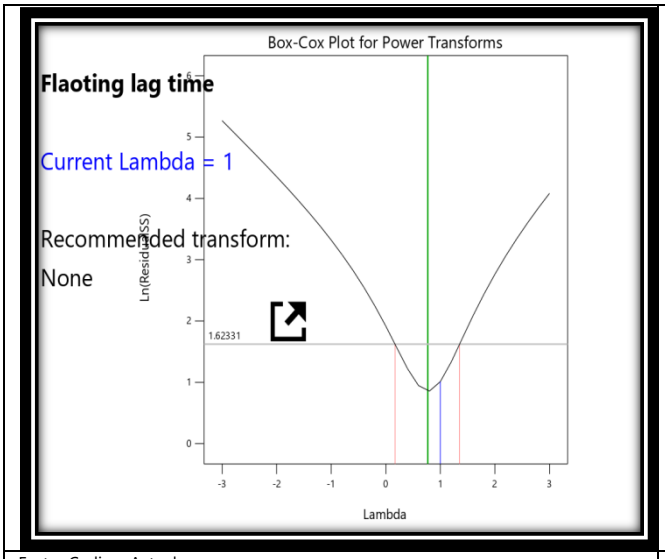
Table 9. ANOVA FOR RESPONSE SURFACE QUADRATIC MODELS

Source	Y_1		Y_2		Y_3		
	F value	P>F	F value	P>F	F value	P>F	Remark
Model	146.87	< 0.0001	127.39	< 0.0001	143.38	<0.0001	Significant
A	8.18	0.0354	19.31	0.0003	82.82	0.0003	
B	120.23	0.0001	482.71	< 0.0001	502.92	<0.0001	
C	27.50	0.0033	465.32	< 0.0001	626.30	<0.0001	
AB	7.27	0.0429	20.43	0.0027	30.42	0.0027	
AC	16.36	0.0099	2.87	0.0059	21.13	0.0059	
BC	11.36	0.0199	53.94	0.0090	17.11	0.0090	
A2	2.62	0.1663	0.5237	0.2208	1.96	0.2208	
B2	2.62	0.1663	33.52	0.0497	6.64	0.477	
C2	1112.83	<0.0001	72.31	0.0004	0.6555	0.477	
Lack of Fit	0.2500	0.8576	3.25	0.2441	5.25	0.1642	not significant

$Y1= +25.0+0.7500A +2.87B +1.38C -1.0AB +1.50AC +1.25BC -0.6250A^2 + 0.6250 B^2 -12.88 C^2$

$Y2 = +64.33 +1.37A -6.88B -6.75C +2.00AB -0.7500AC -3.25BC +0.3333A^2 -2.67B^2 -3.92C^2$

$Y3= +79.67 +3.50A -8.62B -9.63C +3.00AB -2.50AC +2.25BC +0.7917A^2 -1.46B^2 -0.4583C^2$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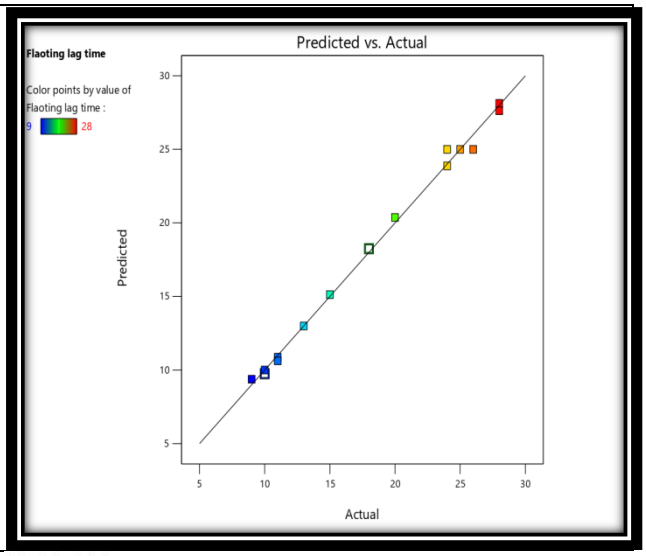
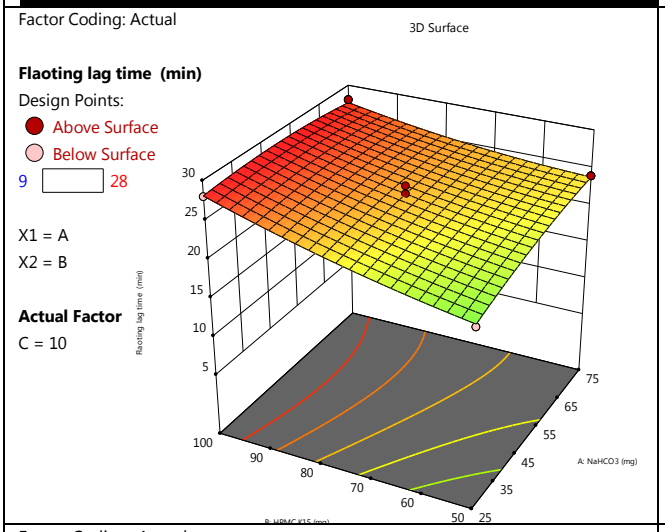
Lambda


Predicted vs. Actual
Flaoting lag time
Color points by value of
Flaoting lag time :
9
28
Predicted
Actual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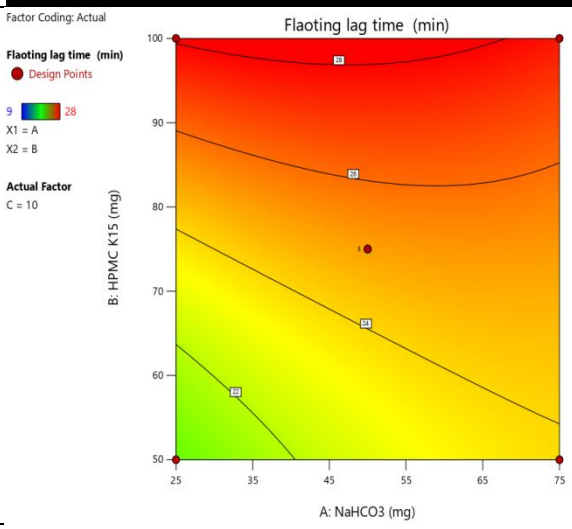
3D Surface
Flaoting lag time (min)
Design Points:
Above Surface
Below Surface
9
28
X1 = A
X2 = B
Actual Factor
C = 10
Flaoting lag time (min)
A: NaHCO3 (mg)
B: HPMC K15 (mg)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Design Points
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28
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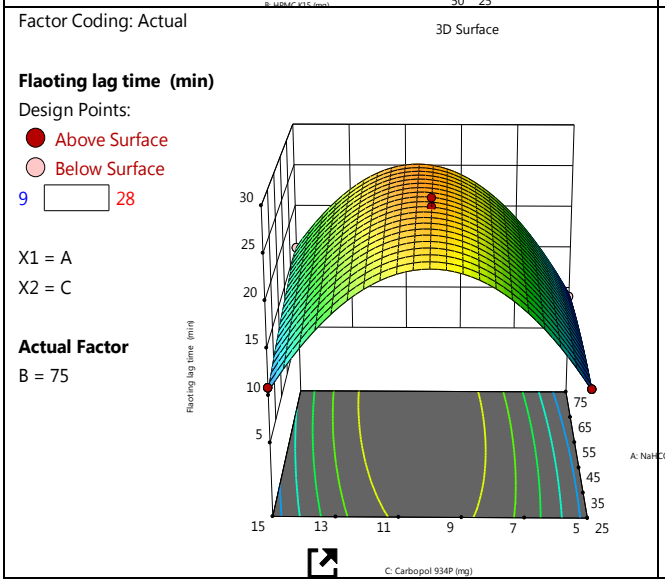
B: HPMC K15 (mg)
A: NaHCO3 (mg)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3D Surface
Flaoting lag time (min)
Design Points:
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Below Surface
9
28
X1 = A
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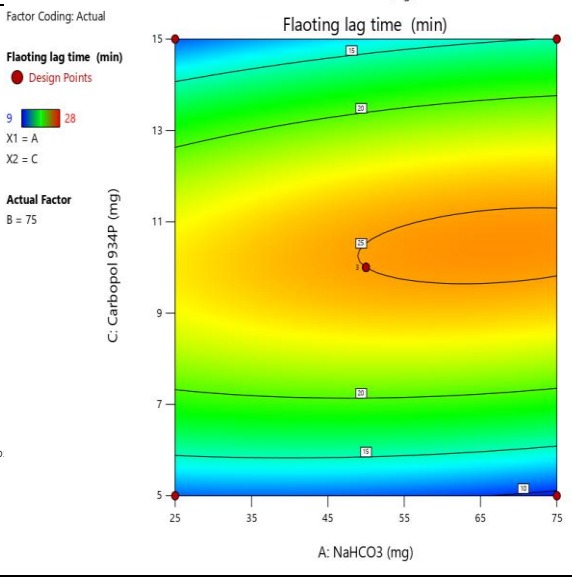
Actual Factor
B = 75
Flaoting lag time (min)
C: Carbopol 934P (mg)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Flaoting lag time (min)
Design Points
9
28
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X2 = C
Actual Factor
B = 75
Flaoting lag time (min)
C: Carbopol 934P (mg)
A: NaHCO3 (mg)

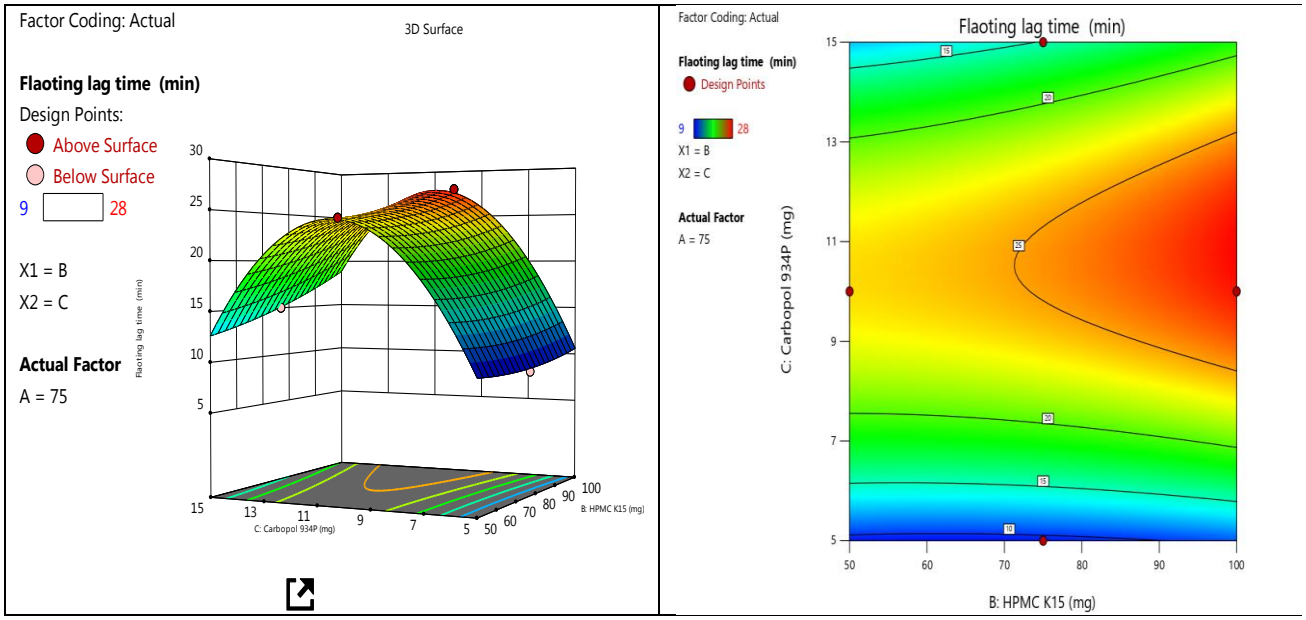
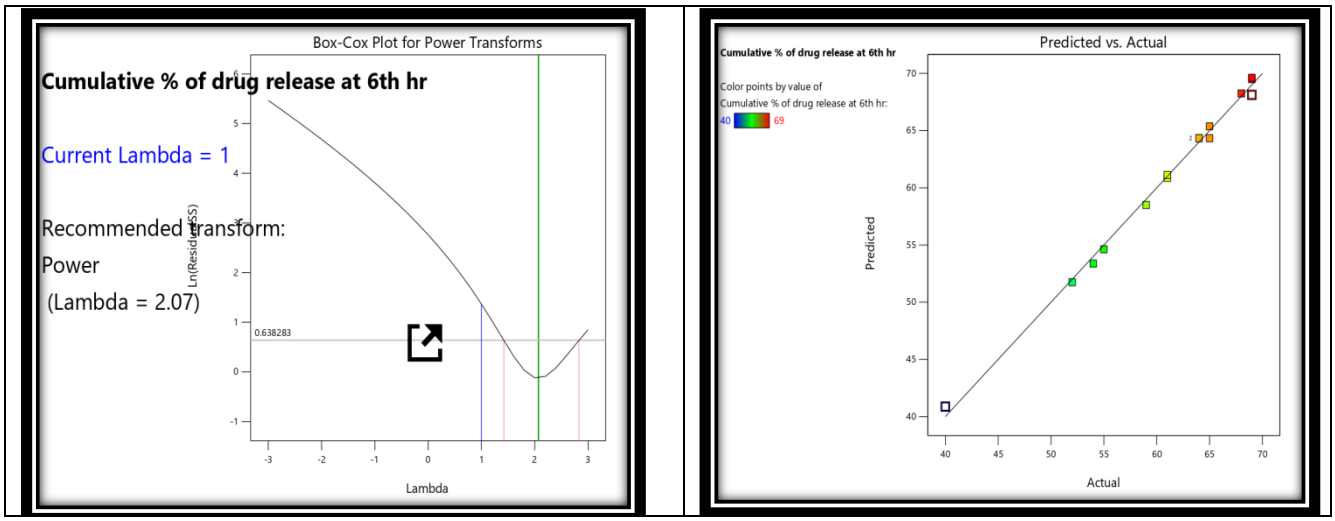


Fig 4. Fig Response surface plot three-dimensional and counter plots of Y_1

For response Y1 floating lag time A, B, C, AB, AC, BC, and C2 were found to be significant model terms the adequate precision of 30.964 indicates an adequate signal to navigate the design space. The difference between adjacent R^2 and predicted R2 was found to be less than 0.2, which shows a positive response. Surface analysis plots in three-dimensional model graphs and counterplots were constructed using the software as shown in fig 4.The effect of an independent variable on floating lag time could be quantified by the following equation. Y is equal to positive value before a factor in the equation indicates the positive response increases with the response, it was observed that the response increases as the polymer concentration increases does higher floating lag time, the negative sign indicates lesser floating time Y1 .

RESPONSE 2: CUMULATIVE % OF DRUG RELEASE AT 6th hr



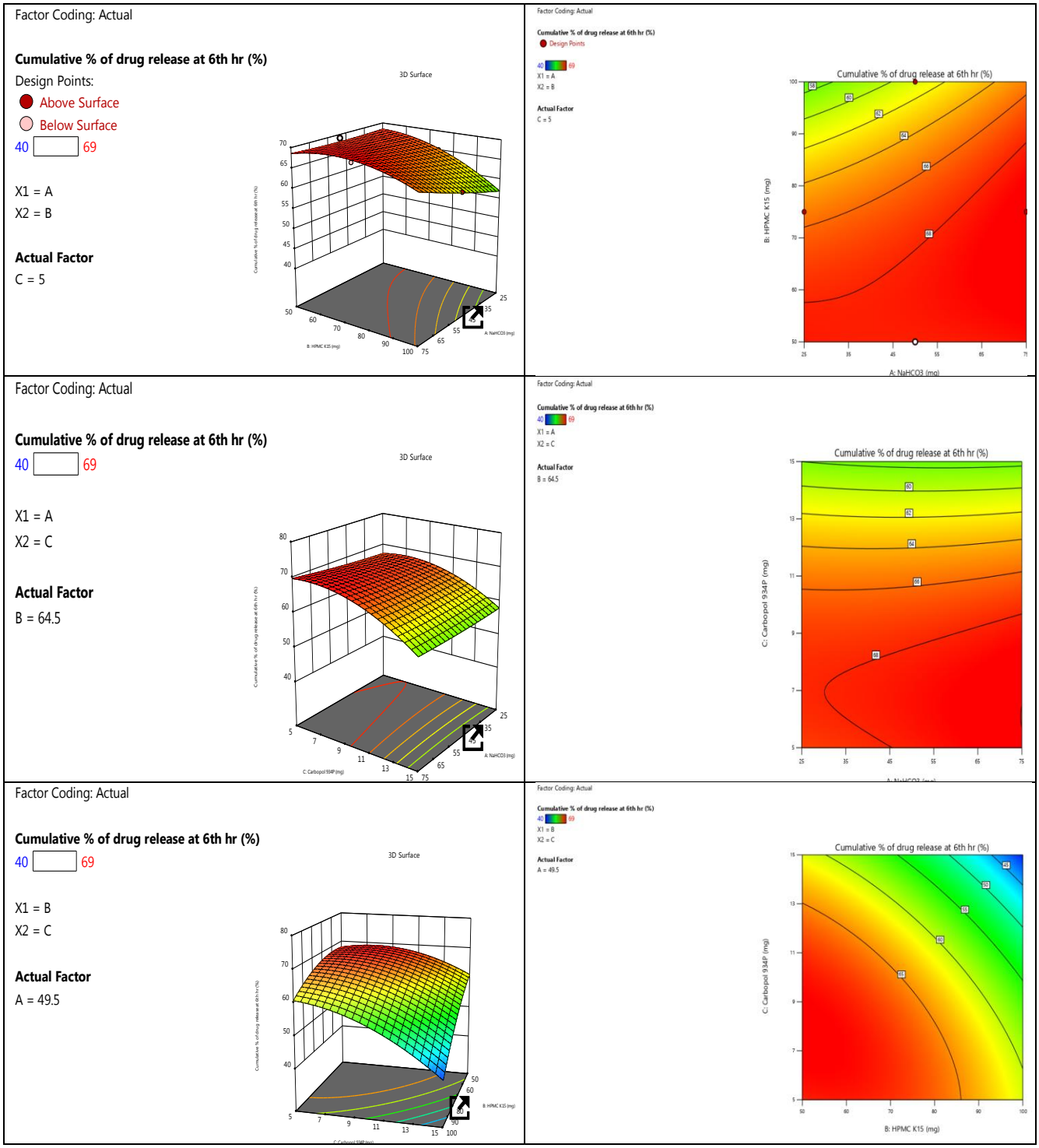


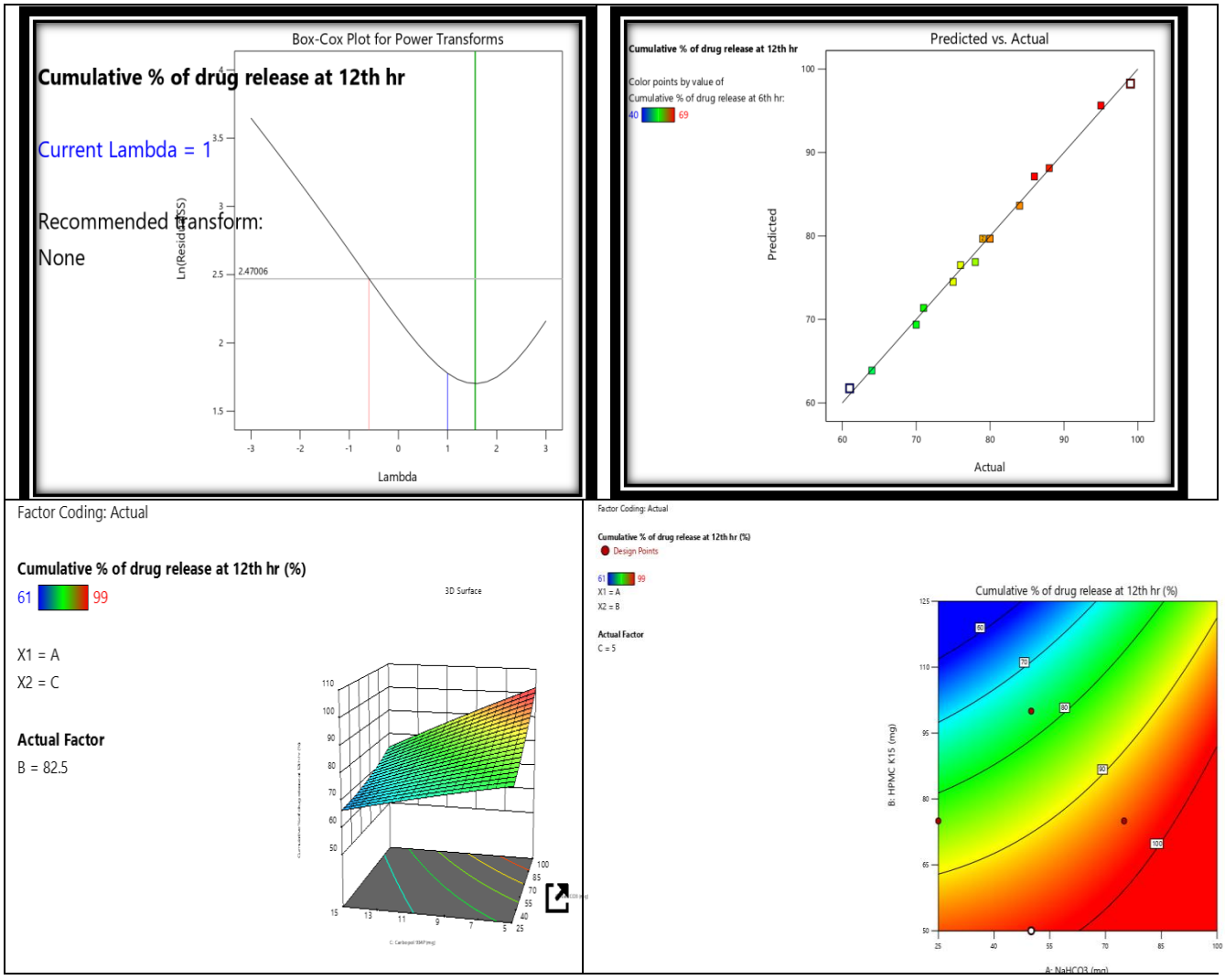
Fig5.Response surface plot three-dimensional and counter plots of Y_2

For response Y2 *in vitro* drug release at 6th hour the significant model terms obtained where A, B, C, AB, BC, B2, C2 the adequate precession of 39.78 indicates an adequate signal to navigate the design space the obtained adjusted R^2 value 0.9957 and predicted R2 value 0.9407 the difference between them where found to be less than 0.2, which shows a positive sign. Surface analysis plots in three-dimensional model graphs and counterplots were constructed using the software as shown in fig 5.

A quadratic equation was generated for the effect of *in vitro* drug release at the 6th hour is as following. A negative value indicates significance in predicting the response, and a positive value indicates significance in predicting the response.

A positive value represents an effect that positively favors optimization while a negative value indicates an inverse relationship [12] between the independent variable and dependent variable as the concentration of A2, an increases it shows a greater effect on Y2 response.

RESPONSE 3: CUMULATIVE % OF DRUG RELEASE AT 12th hr



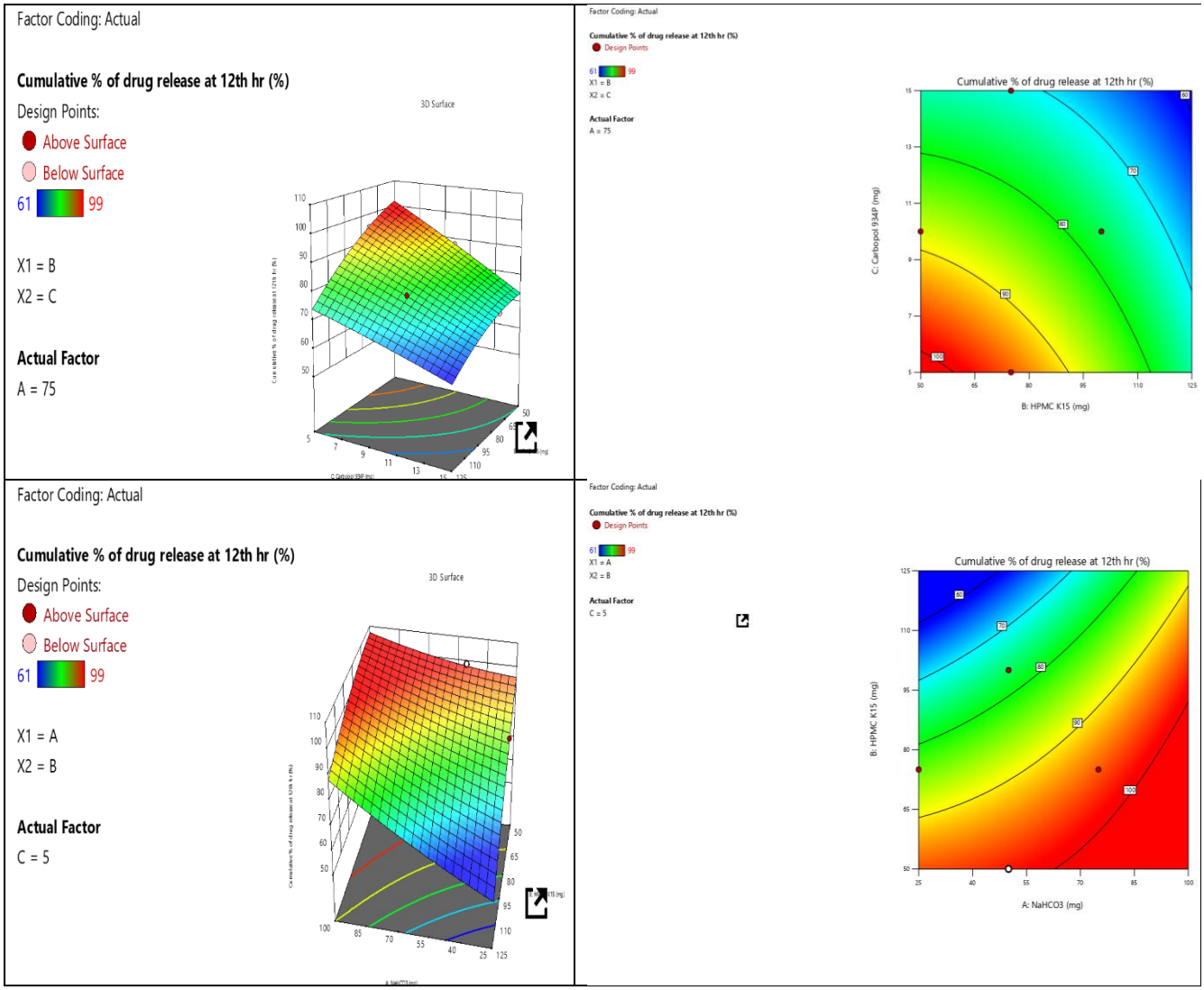


Fig6.Response surface plot three-dimensional and counter plots of Y_3

Similar to response Y3 *in vitro* drug release upto 12th hour, it was found that A, B, C, AB, AC, BC, and C2 were significant model terms, the adequate precision 41.09 indicates an adequate sign to navigate the designs space, the difference between the adjusted R^2 and predicted R^2 was found to be less than 0.2. The response Y3 was best fit to the 2FI model suggested, but in the model, the variables A, B, C, AB, AC, BC, and A2 are significant model terms other variables are included in the model to convert into a quadratic model. The higher the value indicates the positive effect on the *in vitro* drug release at the 12th hour the higher the value indicates a greater influence on *in vitro* drug release at the 12 hour as a concentration of sodium bicarbonate increases subsequently more amount of drug released from the dosage form. Surface analysis plots in three-dimensional model graphs and counterplots were constructed using the software as shown in fig 6.

To get optimize the formulation, numerical and graphical optimizations were performed using design expert software version 13.0.5.0 studies Inc., Minneapolis, MN). The various desirability were given into the software as constraints & important for three responses [9]. The optimum formulation was obtained on a set of criteria of minimum floating lag time in the range 40-69, 50-65, and 85-100 for *in vitro* drug release at the 6th hour and 12th hrs. The obtained values of X1 X2 X3 were given in desirability solutions as shown in fig 7, and the response was measured as shown in table 10. The observed values of response were compared to the predicted values and the percentage

error was calculated to validate the method.

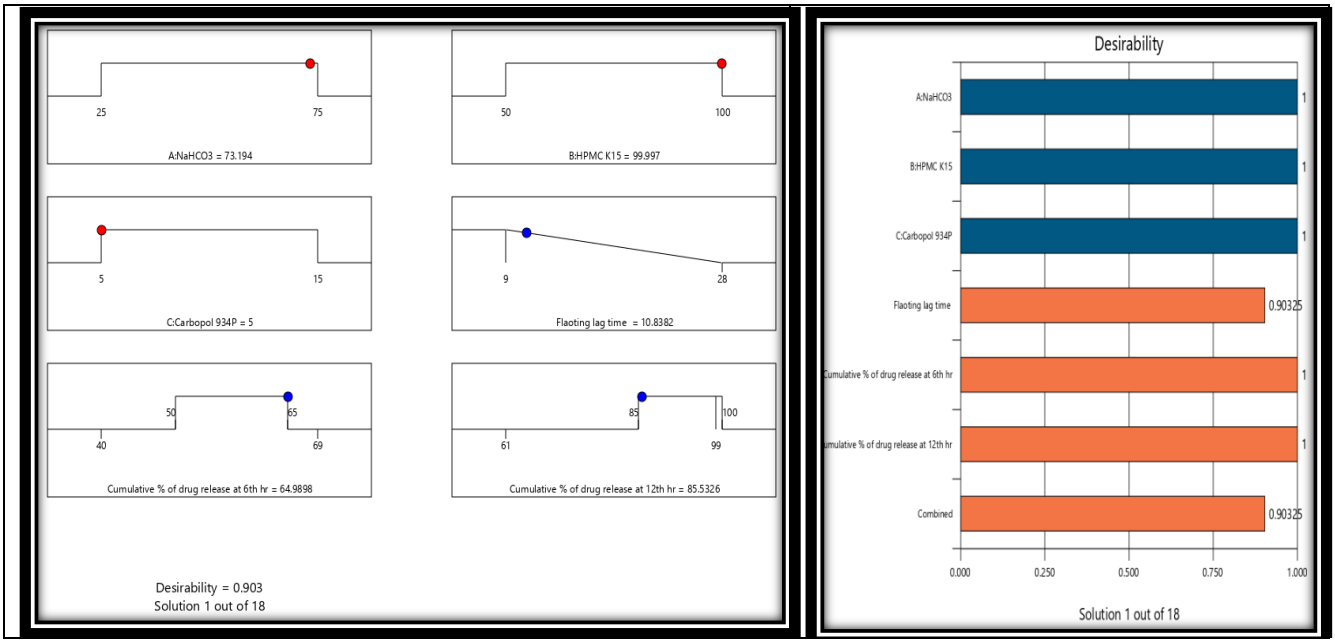
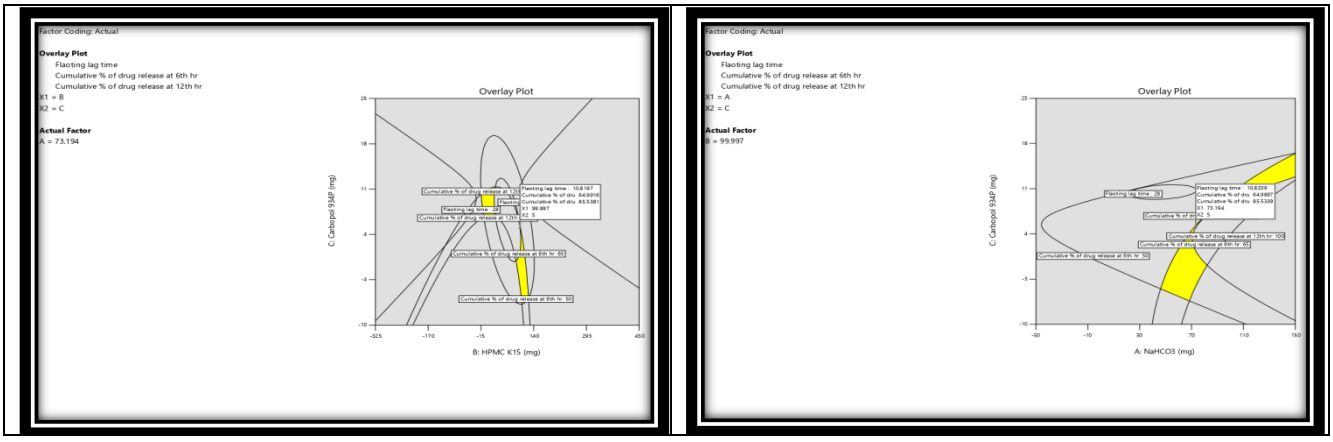


Fig7.Desirability solutions

Table 10. OPTIMIZATION OF DEPENDENT VARIABLES

Number	$NaHCO_3$	HPMC K15M	Carbopol 934p	Floating lag time	Cumulative % of drug release at 6^{th} hr	Cumulative % of drug release at 12^{th} hr	Desirability	Result
1	73.194	99.997	5.000	10.838	64.990	85.533	0.903	Selected



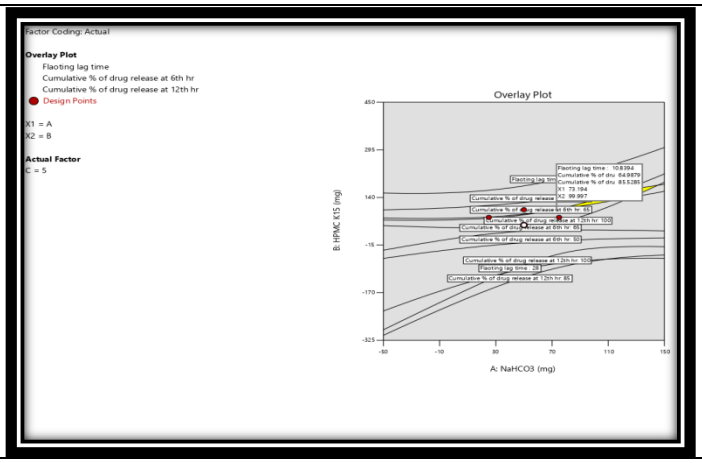


Fig8. plots for Y_1, Y_2, Y_3

Table11:*In vitro* DissolutionData forFormulation F1to F6

Time(hrs)	Cumulative% ofdrugrelease					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
0	0	0	0	0	0	0
1	10.8 ± 0.90	12.3 ± 0.50	7.23 ± 0.39	10.5 ± 0.76	8.01 ± 0.47	11.3 ± 0.68
2	24.9 ± 0.73	25.2 ± 0.44	17.36 ± 0.75	22.7 ± 0.78	13.3 ± 0.59	22.1 ± 0.86
3	36.3 ± 0.1	35.6 ± 0.69	21.49 ± 0.99	36.2 ± 0.84	24.9 ± 1.08	34.5 ± 1.09
4	48.3 ± 0.81	46.7 ± 0.85	28.62 ± 0.45	45.1 ± 0.47	35.2 ± 1.05	45.5 ± 0.59
5	56.78 ± 1.0	58.8± 0.37	36.1 ± 0.97	57.8 ± 0.78	49.8 ± 0.97	56.5 ± 0.45
6	61.78 ± 1.0	68.75 ± 0.40	40.7 ± 1.15	64.4 ± 0.69	54.3 ± 0.75	61.4 ± 0.39
8	69.81 ± 1.0	79.21 ± 0.10	52.4 ± 1.03	71.9 ± 0.53	66.4 ± 0.98	69.5 ± 0.64
12	76.84 ± 0.52	88.20 ± 0.21	61.67 ± 0.97	79.4 ± 0.22	70.82 ± 1.67	75.0 ± 0.60

*n=3 Mean ± S.D.

Table12:*Invitro*DissolutionDataforFormulationF7toF12

Time(hrs)	Cumulative% ofdrugrelease					
	F7	**F8**	**F9**	**F10**	**F11**	**F12**
0	0	0	0	0	0	0
1	11.3 ± 0.59	8.04 ± 0.39	10.4 ± 0.25	20.45 ± 0.12	17.76 ± 0.11	11.00 ± 0.11
2	20.9 ± 0.69	15.6 ± 0.34	17.8 ± 0.60	31.98 ± 0.37	26.87 ± 0.37	21.90 ± 0.67
3	30.3 ± 1.35	22.2 ± 0.79	24.3 ± 0.61	40.28 ± 0.83	36.60 ± 0.71	32.88 ±0.17
4	45.9 ± 0.84	30.35 ± 1.5	39.0 ± 0.55	52.39 ± 0.11	49.36 ± 0.37	45.00 ±0.66
5	52.5 ± 0.49	40.45 ± 0.9	49.7 ± 0.70	61.45 ± 0.73	58.50 ± 0.19	53.90 ± 0.48
6	64.89 ± 1.0	52.3 ± 0.92	55.9 ± 0.85	69.65 ± 0.65	65.98 ± 0.71	65.71 ±0.20
8	75.24 ± 1.0	59.45 ± 0.96	60.0 ± 0.32	82.23 ± 0.43	76.4 ± 0.34	72.78 ± 0.76
12	80.61 ± 0.71	64.45 ± 0.75	71.22 ± 0.61	99.39 ±0.11	84.11 ±0.90	80.59 ± 0.41

*n=3 Mean ± S.D.

Table13:*Invitro* DissolutionData forFormulation F13to F16

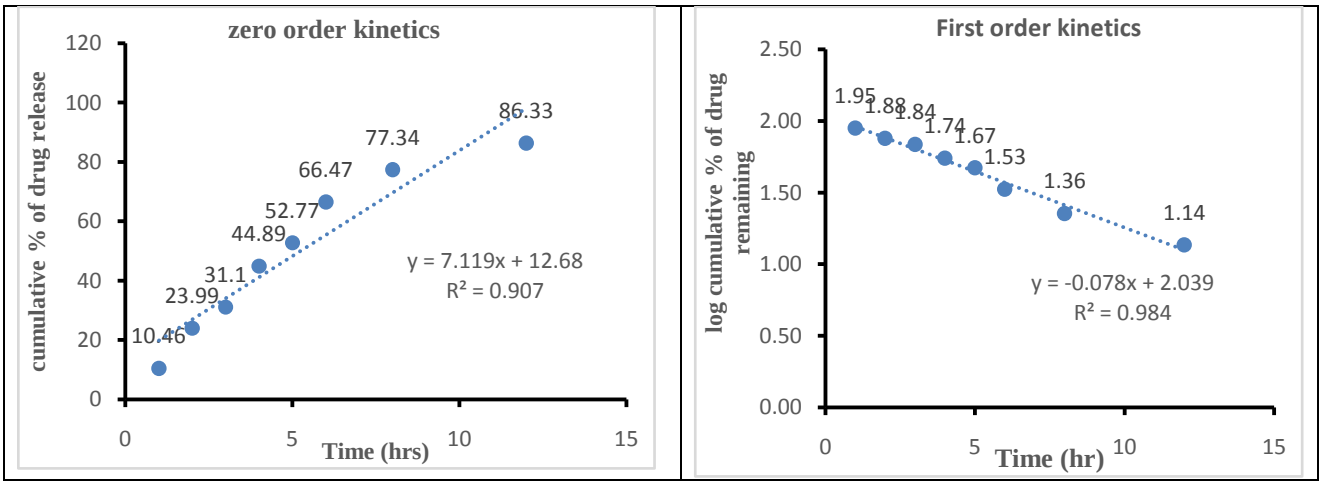
Time(hrs)	Cumulative% ofdrugrelease			
	F13	**F14**	**F15**	**F16 (from design expert)**
0	0	0	0	0
1	11.3 ± 0.57	6.04 ± 0.38	18.4 ± 0.23	10.46 ± 0.89
2	19.00 ± 0.68	15.6 ± 0.32	27.8 ± 0.64	23.99 ± 0.66
3	24.3 ± 1.31	33.2 ± 0.79	34.3 ± 0.69	31.10 ± 0.58
4	47.9 ± 0.89	49.35 ± 1.50	45.0 ± 0.51	44.89 ± 0.45
5	45.5 ± 0.41	56.45 ± 0.95	59.7 ± 0.79	52.77 ± 0.90
6	59.1 ± 1.05	69.3 ± 0.99	69.90 ± 0.84	66.47 ± 0.45
8	65.89 ± 0.45	75.5 ± 0.49	80.0 ± 0.24	77.34 ± 0.82
12	78.52±0.16	86.02±0.17	95.54 ± 0.46	86.33 ± 0.34

*n=3 Mean ± S.D.

*INVITRO*DISSOLUTIONSTUDIES

The tablets containing the highest concentration of HPMC K15 M F1, F3, F8, and F13 showed the release for 61.67 to 80.59 % at12th hr, the drug release was found to be retarded due to the presence of a high concentration of polymer in the formulation, the tablets containing batches of F2, F9, F13, F15 showed release of 71.22 to 95.54 %, respectively at the end of 12th hrs this behavior of release is due to presence of sodium bicarbonates as gas generating agent which help in the release of a drug. The tablets containing a low concentration of HPMCK15M F6, F10, and F14 showed higher drug release up to 99.39 % at the end of the 12th hrs as shown in tables 11, 12, 13.The tablets containing intermediate concentration and with the combination of the other excipients F1, F4, F5, F7, F11, and F12, showed drug release of 70.82 % to 99.39% at 12th hr. Among the various drug release patterns only a few formulations release the drug up to 12th hrs, further formulation is designed based on the results of DoE, F16 showed the release of drug 86.33 % at 12th hr.

RELEASE KINETICS GRAPHS OF F16



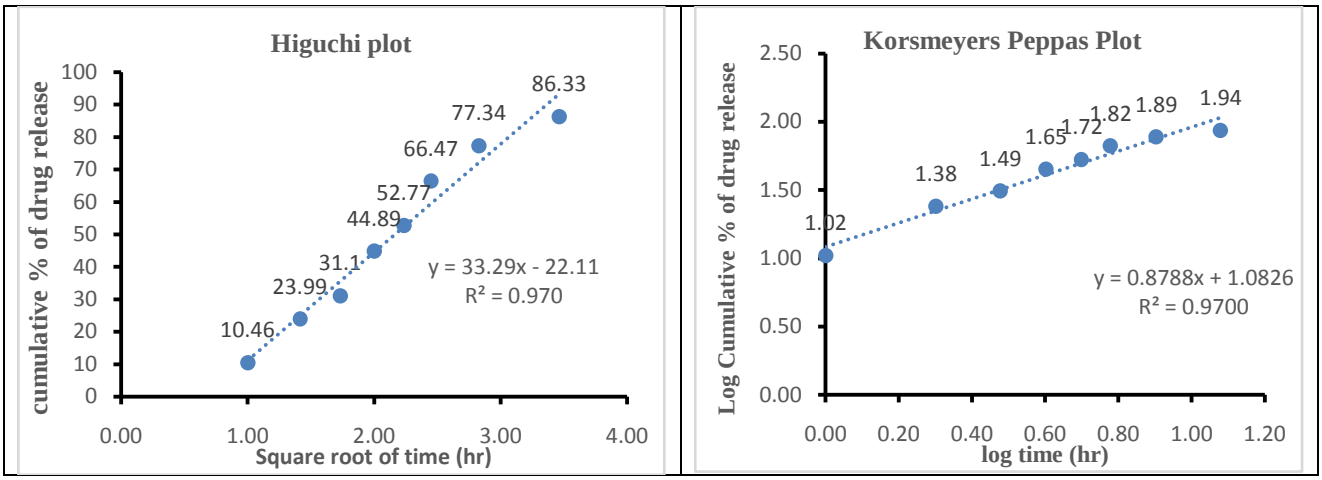


Fig 9. Release Kinetic Plots

TableNo14.RELEASEKINETICSPARAMETERS OF F16

FCode	Zero orderR^{2}values	FirstorderR^2 values	HiguchiR^{2}values	Korsmeyerpeppas	
				R^{2}Values	"n"Values
F16	0.9075	0.9843	0.9705	0.9700	0.465

To ascertain the release kinetics *in vitro* dissolution data was applied to zero order, first order, Higuchi kinetic models and Korsmeyer-Peppas equation was used to characterize the drug release mechanism. The best fit with highest regression coefficient value R^2 was predicted by first order model (R^2 = 0.9843) since the value of release exponent "n" for the proposed model was less than 0.5 as shown in table 16, the release mechanism was found to be anomalous diffusion (non-Fickian) as shown in table 14, kinetic graphs are plotted as shown in fig 9.

Table 15 CONFIRMATION LOCATIONS AND RESULTS

$NaHCO_3$ (X_1)	HPMC K15M (X_2)	Carbopol 934p (X_3)
73.194	99.997	5.00001
Floating lag time (min)	Cumulative % of drug release at 6th hr	Cumulative % of drug release at 12th hr
10.838	64.990	85.533

From the design expert software, the confirmatory locations are identified X_1 as 73.194 mg of sodium bicarbonate, X_2 as 99.99 mg of HPMCK15M, and 5 mg of Carbopol 934p as X_3. To this corresponding predicted values of Y_1-Y_3 are obtained as 10.838 min of floating lag time, 64.99 % of drug release at 6th hr and 85.53% of drug release at 12th hr as shown in table 15.

Table16COMPARATIVEVALUESOFPREDICTEDRESPONSEANDOBSERVEDRESPONSE FOR OPTIMIZE FORMULATION

Dependentvariable(Y)	Predictedresponse (%)	Observedresponse (%)	Predictederror(%)
Floating lag time (Y_1) min	10.838	11.00	+1.494
In vitro drug release at 6^{th} hr (Y_2) %	64.990	66.21	+1.877
In vitro drug release at 12^{th} hr (Y_3) %	85.583	86.33	+0.872

Table 17 COEFFICIENT TABLE FOR RESPONSES

Floating lag time (Y_1) *(P< 0.05)	*In vitro*drug release at 6^{th} hr (Y_2)*(P< 0.05)	*Invitro*drug release at 12^{th} hr (Y_3)*(P< 0.05)
P< 0.0354	P< 0.0071	P< 0.0003
Significant	Significant	Significant

STABILITY STUDIES

The optimized formulation from design expert F16 is subjected to stability studies as per ICH guidelines. Tablets packed were kept at 40 ± 2 °C and 75 ± 5 % relative humidity in a humidity chamber. Floating tablets of the final formulation were assessed for change in appearance, and *in vitro* release profile at 0 months, 3 months, and 6 months. There was not any change in morphological condition and *in vitro* drug dissolution profile during the stability studies as shown in table 18 and fig 10.

Table 18 Comparative *in vitro*drug release studies data after stability study

Time (hrs)	Cumulative % of drug release		
	0 Month	3 Month	6 months
0	0	0	0
1	10.46 ± 0.89	11.34 ± 0.76	11.65 ± 0.53
2	23.99 ± 0.66	22.45 ± 0.36	22.19 ± 0.98
3	31.10 ± 0.58	31.87 ± 0.74	31.11 ± 0.46
4	44.89 ± 0.45	42.99 ± 0.81	43.16 ± 0.27
5	52.77 ± 0.90	53.28 ± 0.84	53.12 ± 0.61
6	66.47 ± 0.45	66.73 ± 0.64	66.22 ± 0.70
8	77.34 ± 0.82	77.01 ± 0.51	77.27 ± 0.84
12	86.33 ± 0.34	86.00 ± 0.98	86.47 ± 0.53

*n=3 Mean ± S.D.

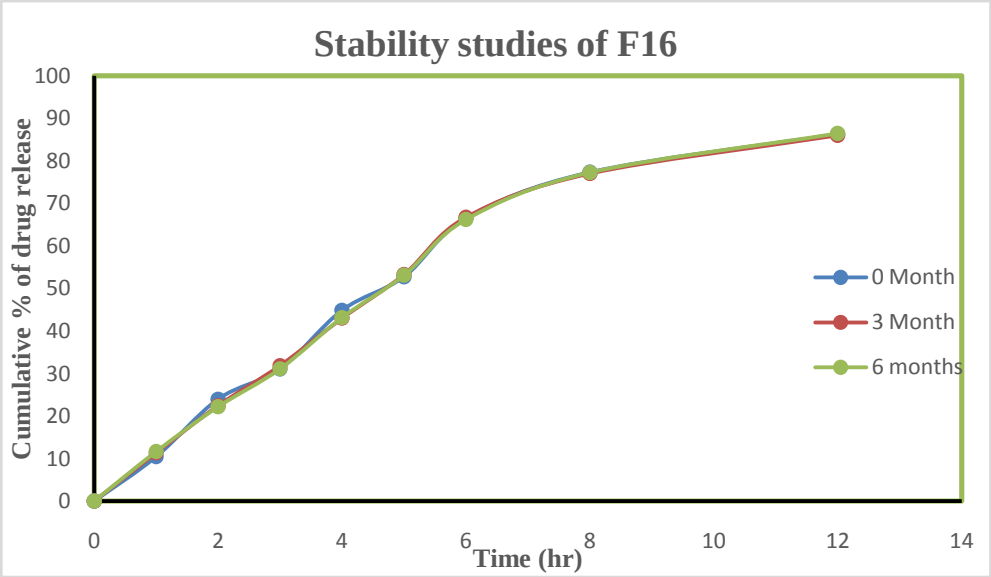


Fig 10. *In vitro* drug release graph after stability studies

DISCUSSION

Formulation F16 showed a significantly higher cumulative percentage of drug release from the floating matrix tablet at the end of 12 hours, the response observed for 15 formulations with three center points where simultaneously fitted to the 2FI model, linear model, and quadratic model, among them quadratic model is desirable with the highest R^2 value in the quadratic equation the present effect of factor influenced on the response while negative value has inverse relation between factors and response the three-dimensional response surface plot withdrawn to estimate the effect of the independent variable on response and to select the optimum formulation[20]. The cumulative percentage of drug release was found to be 86 % for the formulation and could meet the target release profile of the drug indicating the release may be enough to show sustained action of the drug release data analysis of the observed value, predicted value, and percentage error of response was found to be +1.494 for floating lag time, +1.877 for *in vitro* drug release at 6^{th} hr, +0.872 for *in vitro* drug release at 12^{th} hr, the difference between observed response and predicted response is less than 5% with significant value, as shown in table 17. Which is within the acceptable limit.

CONCLUSION

A Box-Behnken experimental design successfully helped in understanding the interaction between the three applied variables. The *in vitro* drug release varied in the presence of variables. Among the various formulation, batch F16 showed by design experts exact satisfactory results with short floating lag time, and sustained drug release up to 12 hrs. Thus, the formulated floating tablets of Eplerenone offer a superior alternative to improve patient compliance over other dosage forms[12].

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTOFINTEREST

TheAuthordeclares that thereisno conflictof interestto publishthearticlein thisjournal.

REFERENCES

1. Deshpande AA, Shah NH, Rhodes CT, Malick W. Development of a novel controlled-release system for gastric retention. Pharm Res. 1997 Jun; 14(6):815-9. doi: 10.1023/a:1012171010492. PMID: 9210203.

2. Cipolla D, Gonda I, Chan HK. Liposomal formulations for inhalation. Ther Deliv. 2013 Aug;4(8):1047-72. doi: 10.4155/tde.13.71. PMID: 23919478.

3. Hashim H, Po AL. Improving the release characteristics of water-soluble drugs from hydrophilic sustained release matrices by in situ gas generation. International journal of pharmaceutics. 1987 Mar 1; 35(3):201-9.doi.org/10.1016/0378-5173 (87)90131-1.

4. Kshirasagar NA, Thamada NA, Naik VN, Gopal MS. Design and evaluation of chitosan containing mucoadhesive buccal patch of Fluxotine HCL. Int J Sci Res. 2012;2:1-5.

5. Shaikh R, Raj Singh TR, Garland MJ, Woolfson AD, Donnelly RF. Mucoadhesive drug delivery systems. J Pharm Bioallied Sci. 2011 Jan;3(1):89-100. doi: 10.4103/0975-7406.76478. PMID: 21430958; PMCID: PMC3053525

6. Kshirasagar N, Pavani S, Adukondalu D, Pavani JK. Formulation and evaluation of sublingual strips of naratriptan. Indo Am. j. pharm. 2016 Jul 1;3(7):759-66.

7. Patel A, Modasiya M, Shah D, Patel V. Development and in vivo floating behavior of verapamil HCl intragastric floating tablets. Aaps Pharmscitech. 2009 Mar;10(1):310-5.

8. Kshirasagar N, Puchchakayala G, Balamurugan K. International journal of research in pharmaceutical sciences. Formulation and characterization of Flurbiprofen loaded micro sponge based gel for sustained drug delivery. Int. J. Res. Pharm. Sci., 2019; (10): 2765-2776
9. Porwal A, Swami G, Saraf SA. Preparation and evaluation of sustained release microballoons of propranolol. DARU: Journal of Faculty of Pharmacy, Tehran University of Medical Sciences. 2011;19(3):193.

10. Gambhire MN, Ambade KW, Kurmi SD, Kadam VJ, Jadhav KR. Development and

in vitro evaluation of an oral floating matrix tablet formulation of diltiazem hydrochloride. Aaps Pharm SciTech. 2007 Sep: E166-74.

11. Naresh Kshirasagar, Srilatha Malvey, K. Senthil Kumar, C. Vijaya, M. Venkatareddy, Formulation and evaluation of orodispersible tablets of Naratriptan using sublimation technique. Indo Am. j. pharm. 2016; (3):22-30.

12. Kshirasagar N, Deepika P, MalveyS, Adukondalu D, Pavani S,Reddy MV. Design and InvitroEvaluation of Gastro Retentive Sustained Release Tablets of KetorolacTromethamine. Journal of pharmacy science 2017; (02):1 1-5.

13. Champalal KD, Sushilkumar P. Current status of ophthalmic in-situ forming hydrogel. Int J Pharm Bio Sci. 2012; 3(3):372-88.

14. Talasaz AH, Ghahremankhani AA, Moghadam SH, Malekshahi MR, Atyabi F, Dinarvand R. In situ gel forming systems of poloxamer 407 and hydroxyl propyl cellulose or hydroxyl propyl methyl cellulose mixtures for controlled delivery of vancomycin. J. Appl. Polym. 2008 Aug 15; 109(4):2369-74.

15. Makwana SB, Patel VA, Parmar SJ. Development and characterization of in-situ gel for ophthalmic formulation containing ciprofloxacin hydrochloride. Pharma Sci. 2015 Jul 8; 6:1-6. doi: 10.1016/j.rinphs.2015.06.001. PMID: 26949596; PMCID: PMC4760229.

16. Barse R, Kokare C & Tagalpallewar A. Influence of hydroxyl propyl methyl cellulose and poloxamer composite on developed ophthalmic in situ gel: Ex vivo and in vivo characterization. . J Drug Delivery SciTechnol2016; (33):1-29.

17. P. M. A, rani n. R, k. S. Prevalence of vancomycin resistant enterococci from urinary tract infected patients. Int j pharm pharm sci. 2023 Jan. 1 15(1):1-7.

18. Elham g, sima s, javad s, a. I. Analytical method validation and bioequivalence study of erlotinib 150 mg tablets in Iranian healthy volunteers under fasting condition. Int j pharm pharm sci. 2023 15(1):27-32.

19. Abdellatif mm, ahmed sm, el-nabarawi ma, teaima m. Nano-delivery systems for enhancing oral bioavailability of drugs. Int j app pharm. 2023 Jan7; 15(1):13-9.

20. S. G, chandrakala v, srinivasan s. Development and evaluation of microsponge gel of an antifungal drug. Int j curr pharm sci. 2023 Jan.15(1):30-41.