Determination of Independent Variables for the Losartan Mucoadhesive Buccal Tablets using Central Composite Design

B. Prakash Rao[1*], Beny Baby[2], Y. Vishnuvardhan Reddy[1], S. Rajarajan[1], K. Deepthi[1], K. Ramesh[1]

1.Department of Pharmaceutical Technology, Karnataka College of Pharmacy, Bangalore, India.

ABSTRACT

The Losartan Potassium buccal tablets have been developed as controlled drug delivery systems to improve the efficacy of Losartan Potassium through mucosal absorption, reduce dose frequency, toxicity, to improve bioavailability and patient compliance. Many processing variables can persuade the characteristics of the Losartan Potassium buccal tablets. The aim of the study was to study the effect of some selected process variables in the concentration of drug/locust bean gum, hydroxy propyl methyl cellulose K4M on the hardness, mucoadhesive strength and *in-vitro* release at different hours by applying central composite design, accounting the above independent variables. The central composite design with an R^2 value of 0.9695, 0.9928, 0.5839, and 0.7556 has selected as a suitable model for further analysis. The model F value of 44.52, 277.61, 7.02, 15.46 with probability P>F<0.0500 implies that this model is significant. The hardness, mucoadhesive strength and *in vitro* studies are prejudiced by mainly basic formulation factors which consist of drug/polymer ratio. Findings established the role of statistical design in predicting the values of independent variables for preparation of Losartan Potassium buccal tablets having pre determined hardness, mucoadhesive strength and *in vitro* studies.

Keywords: Mucoadhesive, central composite design, hydroxypropyl methyl cellulose K4M, locust bean gum, Losartan Potassium.

INTRODUCTION

In recent years, the transmucosal route like oral cavity, ocular, nasal, rectal, vaginal offers many advantages than the per oral route. These include an averting of both hepatic and intra-alimentary canal metabolism within GI tract. The oral mucosal cavity for drug administration has received much more consideration because of its unique advantages over other oral transmucosal routes. The buccal route is apposite for sustained and controlled release administration of drug because of its less permeability and moreover, buccal mucosa has expanse of smooth and relatively immobile mucosa. The sublingual route is suitable for rapid onset of action because of its high permeability of the mucosa and rich blood supply [1]. Buccal drug delivery facilitates safe and easy removal of dosage form in case of toxicity [2]. Various buccal adhesive dosage forms like discs, microspheres [3] and bilayered tablets [4] had been prepared and reported by several research groups [5]. Mucoadhesive polymers are crucial in the development of buccal drug delivery system. This polymer provides intimate contact between the dosage form and the absorbing tissue and augment the retention time [6]. Increasing the retention time of the dosage form is essential in the development of this system and it has been reported that increase in retention time with an increase in the mucoadhesion of the system [7]. In the literature, buccoadhesive drug delivery system for drugs like carvedilol [8], nicotine [9], triamcinolone acetonide [10], atenolol [11] are reported.

Losartan Potassium is an angiotensin II receptor antagonist used in treatment of several diseases of the cardiovascular system, especially hypertension. The half life of Losartan Potassium is 2-3 h. Losartan Potassium is completely absorbed after oral administration, but bioavailability is low (33%) because of its first pass metabolism [12]. The short half life and severe first pass metabolism of Losartan Potassium make it suitable for administration via buccal delivery system that provides controlled drug delivery by passing first pass effect. Losartan Potassium suppresses the possessions of angiotensin II and its receptors, ensuing in blocking of renin-angiotensin system. The renin-angiotensin system plays a foremost role in the control of blood pressure, and in particular it's felt to play crucial role in hypertension.

The effect of locust bean gum (A) and hydroxy propyl methyl cellulose (HPMC K4M) (B) were selected as independent variables. Hardness, mucoadhesive strength, drug release after 1 h and drug release at 8 h were selected as response variables. Computer-aided optimization technique using central composite design (CCD) Design-Expert software (Stat-Ease Inc, Minneapolis, USA) was engaged to investigate the effect of two independent variables (factors) on responses and optimization.

The aim of investigation is to yearning a mathematical model suitable for establishing a quantitative relationship among variables and predicting the quantitative values of selecting independent variables to prepare Losartan Potassium loaded locust bean gum/HPMC K4M buccal tablets having predetermined hardness, mucoadhesive strength and *in-vitro* studies.

MATERIALS AND METHODS

Materials:

Losartan Potassium was kindly gifted from Dr. Reddys Ltd (Hyderabad, India). Locust bean gum, HPMC K4M was purchased from Yarrow Chemicals Private Ltd (Bangalore, India).Ethyl cellulose was received from Karnataka Fine Chem (Bangalore, India) and other chemicals used for this study are of analytical grade.

Experimental design:

Central composite design (CCD) was selected for the development of the formulation. CCD has three groups of design points, two-level factorial, axial points (sometimes called "star" points) and centre points. The certain factor levels are summarized in (Table 1) and the centre points were recurring 4-6 times to assess the pure experimental uncertainty at the factor level. 13 runs were performed; fitting of the model was computed statistically using commercially available software Design-Expert software (Stat-Ease Inc, Minneapolis, USA). The response parameters were statistically analyzed by applying one way ANOVA at 0.05 levels and then pure error is identified.

Table 1: Central Composite Design Of Losartan Potassium Loaded Locust Bean Gum / Hydroxy Propyl Methyl Cellulose K4m Buccal Tablets

Variables	Levels				
A- Locust bean gum	-Alpha	-1	0	+1	+Alpha
	9.3	18	39	60	68.70
B- HPMC K4M	9.64	20	45	70	80.36

Preparation of Losartan Potassium buccoadhesive tablets:

Mucoadhesive buccal tablets containing Losartan Potassium were prepared by direct compression technique. The ingredients of core layer of different combinations (Table 2) were accurately weighed and mixed in a glass mortar and pestle for 15 min to obtain uniform mixture. The core layer of the above blend was compressed at minimum compaction force in 8 mm punches of single stroke tableting machine. The upper punch was raised without disturbing the core tablet and impermeable backing layer, ethyl cellulose of 30 mg was weighed and added on core layer tablet and again compressed at a compaction force of 5-7 kg/cm^{2} [13].

Table 2: Experimental Design Matrix of Hardness, Mucoadhesive Strength and *In-vitro* Release

Standard	Run	A	B	Hardness g/cm^2	Mucoadhesive Strength g/cm^2	% CDR at 1 h	% CDR at 8 h
8	1	18.00	20.00	3.1	20.0	35.27	97.93
4	2	60.00	20.00	5.0	30.0	25.96	72.37
9	3	18.00	70.00	4.0	21.0	34.39	85.37
13	4	60.00	70.00	8.0	29.0	18.09	68.32
6	5	9.30	45.00	3.2	18.0	28.11	91.14
10	6	68.70	45.00	6.0	31.0	26.54	78.03
11	7	39.00	9.64	3.0	25.4	30.32	97.59
3	8	39.00	80.36	6.5	27.1	23.06	70.19
5	9	39.00	45.00	6.0	26.5	26.07	77.09
7	10	39.00	45.00	6.0	26.2	25.00	77.00
2	11	39.00	45.00	6.0	26.0	25.50	76.24
1	12	39.00	45.00	6.0	26.4	25.09	76.29
12	13	39.00	45.00	6.0	26.3	25.09	78.00

Each formula contains 100 mg Losartan Potassium, 15 mg D.Mannitol, 5 mg Magnesium stearate, 3 mg talc, 2 mg aerosil, 30 mg ethyl cellulose, A-Locust bean gum(mg),B- Hydroxy propyl methyl cellulose-K4M (mg).

Hardness:

The hardness of the tablet was measured using the hardness tester (Pfizer AE-10085/India) [14]. The tablet is compressed between a holding anvil and a piston connected to a direct force reading gauge. The dial indicator remains at the reading where the tablet breaks and is returned to zero by depressing a reset button. It is repeated thrice and average is considered.

Mucoadhesive strength:

The bio adhesiveness of buccal mucosa was carried out by using a modified physical balance (Rider Precision PB-2/India). The apparatus consist of a right pan which was replaced with a lighter pan and left pan replaced with a glass slide which is poised by means of Teflon rings and copper wire. The balancing of apparatus achieved in such a way that by placing 5 g of removable weights on right pan and similarly equivalent amount of clay on left pan further the glass container was adjusted to a height of 6.6cm.

To find out bio adhesive strength buccal tablet wedged to the glass slide with the help of adhesive and balancing of apparatus is done by means of knob at the base of equilibrium. Further the weight of 5 g is removed from the right pan which lowers the glass slide to glass container with tablet above the membrane by a weight of 5 g. The whole setup is kept uninterrupted for 5 min after that the increments of 0.1 g weight added to the right pan until the tablet removed from

the membrane surface. The bio adhesive strength was measured by the surfeit weight on the right pan [15].

In Vitro Drug Dissolution:

The *in vitro* drug release studies were carried out using USP XXIII, type-II dissolution test apparatus (Electro lab, EDT-08Lx) [16]. 900 ml of phosphate buffer (pH 6.8) was used as the dissolution medium at the speed of 50 rpm and temperature condition of $37 \pm 1\ ^{o}C$. The impermeable layer of the tablet was attached to a glass slide with instant adhesive. The slide was put in the bottom of the dissolution vessel, so that the tablet remained on the upper side of the slide. The sample of 5 ml was withdrawn at intervals of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 h and the same volume of phosphate buffer (pH 6.8) was replaced in the dissolution apparatus followed by dilution with phosphate buffer (pH 6.8).The amount of Losartan Potassium released from the buccal tablets were analyzed spectrophotometrically at 234 nm. The mechanism of drug release from the Losartan Potassium buccal tablets was determined by finding the finest fit of the release data to Higuchi and Korsmeyer-Peppas plots. The release rate constants *k* and *n* of each model were calculated by linear regression analysis.

RESULTS AND DISCUSSION

The hardness of prepared mucoadhesive buccal tablets was from 3.1 to 8.0 kg/cm^2 and the data's were shown in (Table 2). Effect of variables on hardness was found to be increased due to increasing weight of the tablet. Also the hardness of the tablet was increased as the concentration of locust bean gum (A) and HPMC K4M (B) were increased in each formulation and it is explained.

In terms of coded factors:

Hardness R1 = + 6.00 +1.23*A + 1.11* B + 0.52*A*B − 0.61*A^2 − 0.54*B^2

In terms of actual factors:

Hardness R1= -0.37907 + 0.12202*A + 0.082649*B + 1.00000E - 003*A*B - 1.38889E - 003*A^2 − 8.60000E004*B^2

The quadratic model is selected for this response with F-value 44.52 and p value is less than 0.0001 indicates the model is significant. Values of "prob>F" less than 0.0500 indicates model terms, in this study A, B, AB, A^2, B^2 are significant model terms. The "Pred R-Squared" of 0.7832 is in reasonable agreement with the "Adj R-Squared" of 0.9477. "Adeq Precision" measures the signal. This model is used to pilot the design space. Both the factors A-locust bean gum and factor B-HPMC K4M increase the hardness of the buccal tablets. The effect of A and B

can be further elucidated with the help of response surface plot (figure. 1). At low level of factor B and as factor A increases the hardness was increasing in the beginning and again reduces, in the middle around 39mg of locust bean gum. Hardness was odserved maximum in the similar way at factor A low level and as factor B is increasing the hardness was observed in curve manner. At higher level of A and B same hardness was observed.
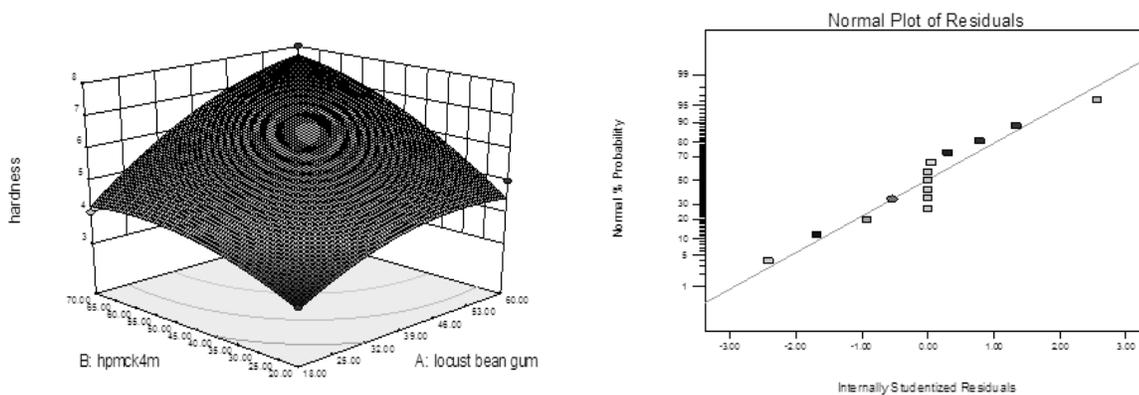

Figure. 1: 3D response plot of locust bean gum/HPMC K4M on hardness and Normal plot of residuals

The mucoadhesive strength of prepared mucoadhesive buccal tablet was studied using porcine buccal mucosa and the mucoadhesive parameters are represented in (Table 2) Bioadhesion is generally implicit to define the ability of a biological or synthetic material "stick" to a buccal membrane, resulting in adhesion of material to the tissue for a protracted period of time. The mucoadhesive strength is affected by molecular weight of polymer, contact time with membrane and degree of swelling of the polymer. Water uptake process produces polymer swelling and improves the consolidation step that increases the mobility of molecules and facilitates that interpenetration with the biological tissue layer. So, the polymer swelling is a property related to the bioadhesion of the system and it can be explained.

In terms of coded factors:

Mucoadhesive strength R2 = + 26.20 + 4.55*A + 0.30* B − 0.50*A*B − 0.97*A^2

In terms of actual factors:

Mucoadhesive strength R2 = +12.20151 + 0.43092*A + 0.049164*B − 9.52381E - 004* A*B − 2.19856E - 003*A^2

The quadratic model is selected for this response with F-value 277.61 and P-value less than 0.0001 indicates model is significant. Values of Prob > F, less than 0.0500 indicate model terms, in this study A, AB, A^2 are significant model terms. The "Pred R-Squared" of 0.9681 is in reasonable agreement with the "Adj R-Squared" of 0.9893. "Adeq Precision" measures the signal. This model

is used to navigate the design space. The effect of A and B can be further elucidated with the help of response surface plot (figure. 2). At low level and high level of factor B the bioadhesive strength was increased rapidly as locust bean gum increases and at low level and high level of factor A the bioadhesive strength shows no increase at all levels of B. Locust bean gum shows higher bioadhesive strength, when compare to the HPMC K4M due to high molecular weight, polymer chain flexibility for chain interpenetration and diffusion with mucin.
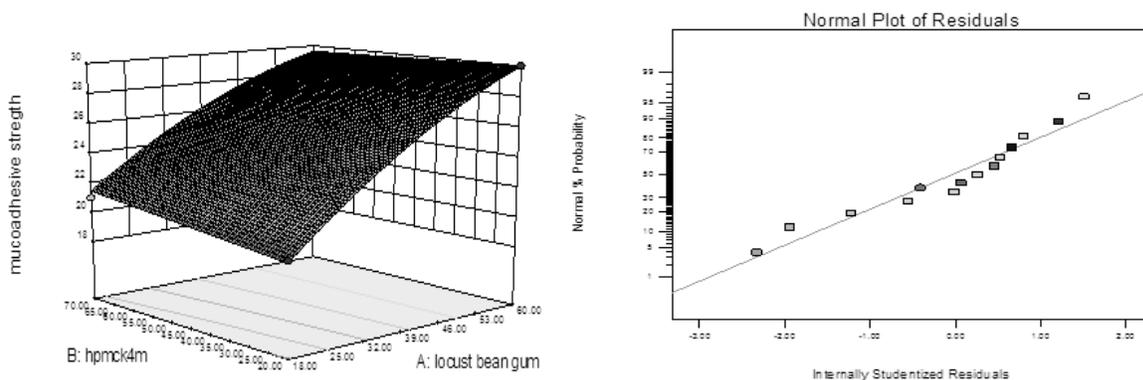

Figure. 2: 3D response plot of locustbean gum/HPMC K4M on mucoadhesive strength and Normal plot of residuals

The *in vitro* release of Losartan Potassium was performed in phosphate buffer (pH 6.8). The amount of Losartan Potassium released from all formulations ranges from 23.06% to 35.26 % in 1 h represented in (Table 2). Decreased rate of drug release was observed with increased concentration of polymers. Effect of both polymers can be explained by mathematical equation.

In terms of coded factors:

Percentage drug release at 1h R3 = + 26.87 - 3.48 *A - 2.38 *B

In terms of actual factors:

Percentage drug release at 1h R3 = + 37.60871 − 0.16566*A - 0.095086*B

The linear model is selected for this response with Model F-value 7.02 and p value is 0.0125 indicate the model is significant. Values of Prob > F, less than 0.0500 indicate model terms, in this study A is significant model terms. "Pred R-Squared" of 0.1246 is not as close to the "Adj R-Squared" of 0.5007, "Adeq Precision" measures the signal. This model can be used to pilot the design space. Both the factors A-locust bean gum and B-HPMC K4M decreases drug release from the tablet. Factor A locust bean gum shows the higher controlling effect on the release of drug than the HPMC K4M due to formation of higher viscous solution. The effect of A and B factors can be explained by using of response surface plot (figure. 3). At low level of factor A and B the drug

was found to be increased but at higher level of factor A shows lower value of drug release at all level of B indicates factor A has negative effect on drug release.
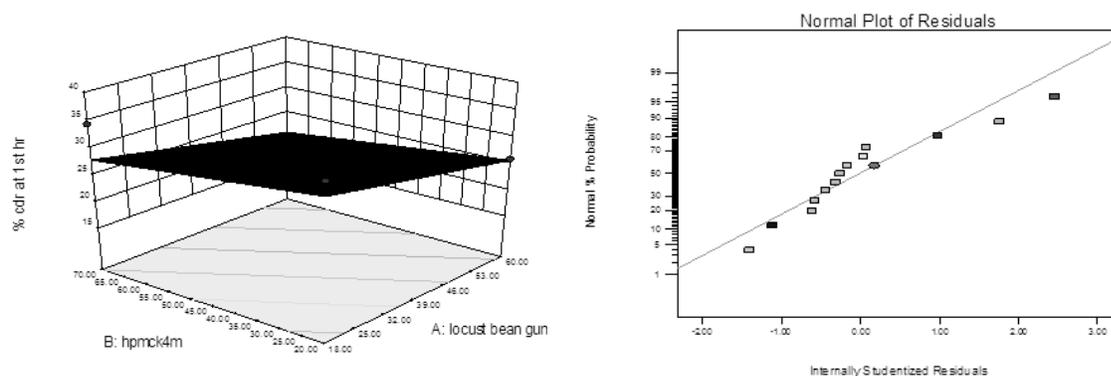

Figure. 3: 3D response plot of locust bean gum /HPMC K4M on drug release at 1 h and Normal plot of residuals

The total amount of Losartan Potassium released from all formulations in 8 h was found to be 68.33% to 97.93% and it is represented in (Table 2). Decreased rate of drug release was observed with increased concentration of polymers. Effect of both polymers can be explained by mathematical equation.

In terms of coded factors:

Percentage drug release at 8 h R4 = + 80.43 - 7.64 *A - 6.92 * B

 In terms of actual factors:

Percentage drug release at 8 h R4 = +107.07918 – 0.36399*A - 0.27680*B

The linear model is selected for this response with Model F-value 15.46 and p value is 0.0009 indicate the model is significant. Values of Prob > F, less than 0.0500 indicate model terms, so that in this study A and B are significant model terms. The "Pred R-Squared" of 0.5188 is in reasonable agreement with the "Adj R-Squared" of 0.7067. "Adeq Precision" measures the signal. This model is used to navigate the design space. Both the factors A-locust bean gum and B-HPMC K4M decreases drug release from the tablet. The factor A has shown more negative effect which indicates that drug release decrease as factor increases. The locust bean gum is a natural gum it will swells an aqueous medium to form a gel like matrix that controls release by acting as a barrier to drug dissolution and diffusion. Locust bean gum shows the higher controlling effect on the release of drug than the HPMC K4M due to formation of higher viscous solution. The effect of A and B can be further elucileted with help of response surface plot (figure. 4). At low level of factor A and B the drug release at 8 h was found to be increased, but at higher level of factor A shows lower value of drug release at all levels of B.

The result of ANOVA demostrate that the model was significant for all dependent variables (Table 3 and Table 4). Regression analysis was carried out to determine the regression coefficients. All the independent variables (Factors) were found to be significant for all R1, R2, R3, R4 response variables. The linear model were found significant for R3, R4. So, above result indicate that both the factors play an important role in the formulation of buccal tablet containing Losartan Potassium.

For the optimized formulation, hardness, bioadhesive strength, the drug release at 1 h and the drug release at 8 h were kept at maximize. The composition of optimized formula is Losartan Potassium (100 mg), locust bean gum (41.23 mg) and HPMC K4M (32.87 mg). The optimized formulation was prepared according to predicted model and evaluated for responses. The predicted values of hardness, mucoadhesive strength, drug release at 1 h and drug release at 8 h are 5.434 kg/cm^2, 26.558 gm/cm^2, 27.653%, 82.972% and actual values for the same responses are found to be 5.543 kg/cm^2, 25.956 gm/cm^2, 27.198%, and 83.124% respectively.

Table 3: Anova for Response Surface Models

Source	DF	Sum square	Mean square	F value	Probability
Hardness					
A	1	12.15	12.15	99.69	<0.0001
B	1	9.79	9.79	80.31	<0.0001 significant
AB	1	1.10	1.10	9.04	0.0197
A^2	1	2.61	2.61	21.41	0.0024
B^2	1	2.01	2.01	16.49	0.0048
Mucoadhesive strength					
A	1	165.48	165.48	1056.94	<0.00010
B	1	0.72	0.72	4.61	0.0640 significant
AB	1	1.00	1.00	6.39	0.0354
A^2	1	6.65	6.65	42.49	0.0002
% CDR at 1 h					
A	1	96.82	96.82	9.57	0.0114
B	1	45.21	45.21	4.47	0.0607 significant
% CDR at 8h					
A	1	467.42	467.42	16.99	0.0021
B	1	383.08	383.08	13.93	0.0039 significant

Table 4: Summary of Anova Grades in Analysing Lack of Fit and Pure Error

Source	Sum square	DF	Mean square	F value	Probability
Hardness					
Model	27.14	5	5.43	44.52	<0.0001
Residual	0.85	7	0.12	-	-
Total	27.99	12	-	-	-
Lack of fit	0.85	3	0.28	-	-
Pure error	0.000	4	0.000	-	-

| **Mucoadhesive strength**					
Model	173.86	4	43.46	277.61	<0.0001
Residual	1.25	8	0.16	-	-
Total	175.11	4	-	-	-
Lack of fit	1.10	4	0.28	7.46	0.0386
Pure error	0. 15	12	0.037	-	-
% CDR at 1 h					
Model	142.02	2	71.01	7.02	0.0125
Residual	101.19	10	10.12	-	-
Total	243.22	12	-	-	-
Lack of fit	100.29	6	16.72	74.10	0.0005
Pure error	0.90	4	0.23	-	-
% CDR at 8 h					
Model	850.50	2	425.25	15.46	0.0009
Residual	275.07	10	27.51	-	-
Total	1125.58	12	-	-	-
Lack of fit	273.01	6	45.50	88.31	0.0003
Pure error	2.06	4	0.52	-	-
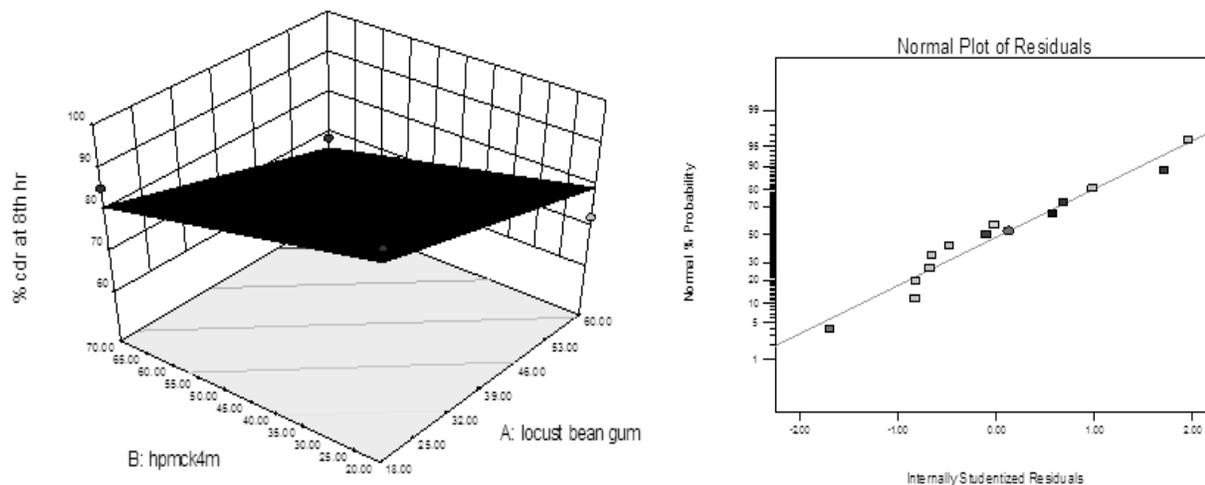
Figure. 4: 3D response plot of locust bean gum/HPMC K4M on drug release at 8 h and Normal plot of residuals

CONCLUSION

Central composite design revealed that the locustbean gum and Hydroxypropyl methyl cellulose K4M have significant effect on hardness, mucoadhesive strength and *in-vitro* release.The observed independent variables were found to be very close to predicted values of optimized formulation which demonstrates the feasibility of the optimization procedure in successful development of mucoadhesive buccal tablet containing Losartan Potassium with enhanced bioavailability and prolonged therapeutic effect for the better management of hypertension. Pertaining to these studies, this has to confirm in the in vivo settings as a separate investigation in the future.

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