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APPLICATION OF BOX BEHNKEN EXPERIMENTAL DESIGN TO OPTIMISE PHARMACEUTICAL FLOATING TABLET FORMULATIONS OF SYNTHETIC ANTIBIOTIC OFLOXACIN

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ABSTRACT

The aim of this study was to apply Box-Behnken design for the optimization of polymer concentration in effervescent floating tablets of antibiotic drug Ofloxacin. Mean dissolution time (MDT), time required to release 50% of drug (t50%), drug release at 2 hrs (R2hrs) and dissolution efficiency in 2 hrs (DE2hrs) were taken as target responses, whereas the quantity of different polymers such as carbopol 934P (viscoelastic agent), sodium carboxymethylcellulose (Sod.CMC) (swelling agent) and tablet thickness were considered as impacting factors. A second-order polynomial equation was determined by the multiple regression analysis of the experimental data. The design space was established targeting the successful operating ranges for the mean dissolution time (MDT), time required to release 50% of drug (t50%), drug release at 2 hrs (R2hrs) and dissolution efficiency in 2 hrs (DE2hrs) as 4.5-5.0 hrs., 5.0-5.5 hrs, 25.0-30.0 % and 15.0-18.0 % respectively. The design space illustrated that the available operation range is wide at the laboratory scale and thus ensuring the product quality. From the results of study it was concluded that successful application of Box-Behnken design of experiments is helpful to select grade and concentration of polymers cost effectively to reduce cost of goods which ultimately can improve profitability of pharmaceutical production unit.

INTRODUCTION

Ofloxacin

Ofloxacin is a synthetic antibiotic of the fluoroquinolone drug class considered to be a second-generation fluoroquinolone [1][2]. Ofloxacin was first patented in 1982 (European Patent Daiichi) and received approval from the U.S. Food and Drug Administration (FDA) on December 28, 1990. Ofloxacin is sold under a wide variety of brand names as well as generic drug equivalents, for oral and intravenous administration. Ofloxacin is also available for topical use, as eye drops and ear drops (marketed as Ocuflox and Floxin Otic respectively in the United States and marketed as Optiflox, eylox respectively in Jordan and Saudi Arabia[3]).Ofloxacin is a racemic mixture, which consists of 50% levofloxacin (the biologically active component) and 50% of its "mirror image" or enantiomer dextrofloxacin[4]. Ofloxacin has been associated with adverse drug reactions, such as tendon damage (including spontaneous tendon ruptures) and peripheral neuropathy (which may be irreversible); tendon damage may manifest long after therapy had been completed, and, in severe cases, may result in lifelong disabilities[5]. It is on the WHO Model List of Essential Medicines, the most important medications needed in a basic health system[6].

Medical uses of Ofloxacin

In the U.S. ofloxacin is approved for the treatment of bacterial infections such as:

- Acute bacterial exacerbations of chronic bronchitis
- Community-acquired pneumonia
- Uncomplicated skin and skin structure infections
- Nongonococcal urethritis and cervicitis
- Mixed Infections of the urethra and cervix
- Acute pelvic inflammatory disease
- Uncomplicated cystitis
- Complicated urinary tract infections
- Prostatitis
- Acute, uncomplicated urethral and cervical gonorrhea.

Ofloxacin has not been shown to be effective in the treatment of syphilis[7]. Floxin is no longer considered a first line treatment for gonnorrhea due to bacterial resistance[8][9][10].

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Bacteriasusceptible to Ofloxacin

According to the product package insert, ofloxacin is effective against the following microorganisms[11].

- Aerobic Gram-positive microorganisms:
- Staphylococcus aureus (methicillin-susceptible strains)
- Streptococcus pneumoniae (penicillin-susceptible strains)
- Streptococcus pyogenes
- Aerobic Gram-negative microorganisms
- Citrobacter koseri (Citrobacter diversus)
- Enterobacter aerogenes
- Escherichia coli
- Haemophilus influenzae
- Klebsiella pneumoniae
- Neisseria gonorrhoeae
- Proteus mirabilis
- Pseudomonas aeruginosa
- Other microorganisms:
- Chlamydia trachomatis

Floating drug delivery systems

During the last decade, many studies have been performed conc erning the sustained releae dosage forms of drugs, which have b een aimed at the prolongation of gastric emptying time (GET). The GET has been reported to range from 2 to 6 hrs in humans in the fed state[12]. Retention of drug delivery systems in the stomach prolongs the overall gastrointestinal transit time, thereby resulting in improved bioavailability. Scintigraphic studies determining gastric emptying rates reveal ed that orally administered controlled release dosage forms are subjected to basically two complications, that is short gastric residence time and unpredictable gastric emptyingrate [13].

Depending on the mechanism of buoyancy, Two distinctly different methods viz., effervescent and noneffervescent systems have been used in the development of floating drug del ivery systems (FDDS) [14]. Effervescent drug delivery systems utilizes matrices prepared with swellable polymers such as methocel or polysaccharides and effervescent components like sodium bicarbonate and citric or tartaric acid. FDDS offers important advantages like they are less prone to g astric emptying resulting in reduced intra and inter subject variability in plasma drug levels, effective for delivery of drugs with narrow absorption windows, reduced dosing and increased patient compliance, reduced Cmax and prolonged drug levels above the minimum effective concentration and improved safety profile for drugs with side effects associated with high Cmax.

A Box-Behnken design of experiments

A Box-Behnken design is a type of response surface design that does not contain an embedded factorial or fractional factorial design. Box-Behnken designs have treatment combinations that are at the midpoints of the edges of the experimental space and require at least three continuous factors. The following figure shows a three-factor Box-Behnken design. Points on the diagram represent the experimental runs that are done:

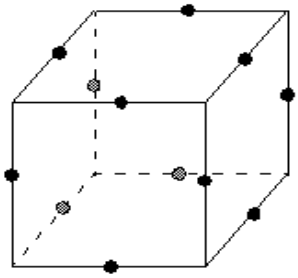

Figure 1 Points on the diagram represent the experimental runs that are done in Box-Behnken design of experiments

These designs allow efficient estimation of the first- and second-order coefficients. Because Box-Behnken designs often have fewer design points, they can be less expensive to do than central composite designs with the same number of factors. However, because they do not have an embedded factorial design, they are not suited for sequential experiments.

Box-Behnken designs can also prove useful if you know the safe operating zone for your process. Central composite designs usually have axial points outside the "cube." These points may not be in the region of interest, or may be impossible to conduct because they are beyond safe operating limits. Box-Behnken designs do not have axial points, thus, you can be sure that all design points fall within your safe operating zone. Box-Behnken designs also ensure that all factors are not set at their high levels at the same time [15].

In the present study Box-Behnken design was applied for the optimization of polymer concentration in effervescent floating tablets of ofloxacin. Mean dissolution time (MDT), time required to release 50% of drug ($t_{50\%}$), drug release at 2 hrs (R_{2hrs}) and dissolution efficiency in 2 hrs (DE_{2hrs}) were taken as target responses, whereas the quantity of different polymers such as carbopol 934P (viscoelastic agent), sodium carboxymethylcellulose (Sod.CMC) (swelling agent) were considered as independent variables.

MATERIALS AND METHODS

Ofloxacin was obtained as a gift sample from Dr. Reddy's Pharmaceuticals, Hyderabad. Carbopol 934P, sodium bicarbonate and sodium carboxymethylcellulose (Sod. CMC) were provided by Cipla Ltd., India. Lactose and magnesium stearate were supplied by Loba Chem, India.

Preparation of Floating tablets

Floating tablets were prepared by direct compression method. Ofloxacin (200 mg), required amount of polymers and other excipients were accurately weighed. Ofloxacin was well mixed with weighed quantity of polymer and then mixed with remaining ingredients i.e., sodium bicarbonate, lactose in geometric proportions. Formulations were prepared by varying drug to polymer ratio (Table 1) and keeping other ingredients such as sodium bicarbonate (15%) and lactose in required quantities to make the final weight of 400 mg/tablet. Briefly, preparation of tablets involved, passing all the ingredients except magnesium stearate through sieve #40 and mixing the blend in geometric mixing. Magnesium stearate was used for

lubrication after passing through sieve #60. The lubricated powder mixture was compressed on a sixteen station rotary tablet punching machine using 12 mm circular standard caplet shaped punches.

Central composite design (CCD) with "Box-Behnken design (BBD)

The prepared tablets were evaluated for drug release parameter with the official method described in Indian pharmacopeia, 1996 [16]. The purpose of this study was to systematically investigate the impact of several formulation variables on drug release using central composite design (CCD). The responses such as mean dissolution time (MDT), time required releasing 50% of drug ($t_{50\%}$), drug release at 2 hrs (R_{2hrs}) and dissolution efficiency in 2 hrs (DE_{2hrs}) depend on the product. The ranges of these formulation variables were chosen based on the reference listed drug labeling and literature data [17].

JMP version 11 (SAS) software" with "Minitab Software version 14

In this study, JMP version 11 (SAS) software was used to give desirability and overlay information to get optimized formulation with the possible interactions of the selected independent variables on the dependent variables. Selected factor levels for the experimental design used in the formulation of floating tablets are given in Table 1.

Table 1 Selected factor levels for the experimental design used in the formulation of floating tablets

Model factor	Actual values			Coded values		
	Low	Medium	High	Low	Medium	High
X_1: Carbopol (%)	12	14	16	-1	0	+1
X_2: Sod.CMC (%)	5	6	7	-1	0	+1
X_3: Thickness (mm)	3	3.5	4	-1	0	+1

Sod.CMC: Sodium carboxymethylcellulose

The two independent formulation variables evaluated were:
X_1: Carbopol (%); **X_2:** Sod.CMC (%)

The response variables evaluated were:
Y_1: MDT; **Y_2:** Time required for 50% of drug release ($t_{50\%}$); **Y_3:** Drug R_{2hrs}; **Y_4:** Dissolution efficacy at 2 hrs (DE_{2hrs}).

The statistical analysis of the experimental batch was performed by multiple regression analysis using Minitab Software. The coefficient of determination (r2) and adjusted coefficient of determination (adj. r2) were compared for best fitting of the model. The effect of formulation variables on the responses were statically evaluated by applying two-way analysis of variance (ANOVA) at 0.05 levels. The optimum levels of the selected variables were obtained by solving the regression equation and analyzing the desirability and overlay plot.

Establishment of the design space

Design space is defined by the ICH Q8 as ‘‘the multidimensional combination and interaction of input variables (material attributes) and process parameters that have been demonstrated to provide assurance of quality’’ [18]. The design space makes QbD a reality and the wider the design space, the more robust and flexible the process is to accommodate variations. In this case study, response surface methodology in combination with optimization was applied to establish design space. Design space was determined from the common region of successful operating ranges for fourresponses mean dissolution time (MDT), time required to release 50% of drug ($t_{50\%}$), drug release at 2 hrs (R_{2hrs}) and dissolution efficiency in 2 hrs (DE_{2hrs}). The successful operating ranges for the mean dissolution time (MDT), time required to release 50% of drug ($t_{50\%}$), drug release at 2 hrs (R_{2hrs}) and dissolution efficiency in 2 hrs (DE_{2hrs}) determined were 4.5-5.0 hrs., 5.0-5.5 hrs, 25.0-30.0 % and 15.0-18.0 % respectively.

RESULTS AND DISCUSSION

In vitro drug release studies

A study of dissolution profile for all the formulations gave an insight into the effect of polymeric fillers and tablet thickness on release profile of the formulations. From the release profiles, it was observed that the variation in type of polymer, polymer concentration and tablet thickness from F1 to F15 (Table 2) had a variable effect on drug release shown in the Table 3.

Table 2 Summary of experimental runs of the formulations in Box-Behnken design

Formulation	X1	X2	X3
F1	16	5	3.50
F2	12	6	4.00
F3	12	7	3.50
F4	14	6	3.50
F5	12	6	3.00
F6	14	5	3.00
F7	14	7	3.00
F8	14	5	4.00
F9	14	7	4.00
F10	16	7	3.50
F11	16	6	3.00
F12	12	5	3.50
F13	14	6	3.50
F14	14	6	3.50
F15	16	6	4.00

Table 3 Summary of experimental responses of the formulations in Box-Behnken design

Formulation	MDT (hrs)	$t_{50\%}$ (hrs)	R_{2hrs} (%)	DE_{2hrs} (%)
F1	4.561	4.15	31.01	18.3
F2	5.225	4.5	27.889	15.793
F3	5.05	5.8	24.55	14.358
F4	4.999	6.09	22.836	12.994
F5	4.57	4.25	30.414	17.732
F6	5.03	6.16	22.25	12.5634
F7	4.82	4.79	29.67	17.763
F8	4.96	5.05	28.069	12.5634
F9	4.88	4.93	28.88	16.648
F10	4.82	4.89	29.26	17.23
F11	4.81	4.86	29.573	17.74
F12	4.67	4.7	31.072	19.13
F13	4.678	4.6	31.823	20.209
F14	4.982	4.982	4.982	4.982
F15	4.88	4.93	28.88	16.648

Optimisation study A Box-Behnken design

Optimisation study to examine effects and interactions of significant factors on product quality attributes mainly drug release. The optimisation study typically can use one of the following experimental designs; factorial, fractional factorial, central composite, mixture design, D-optimal, or Box-Behnken design. Box Behnken design was specifically selected for mentioned reasons of requiring fewer runs than a central

composite design [15]. Summary of results of statistical analysis and optimization of the formulations using Box-Behnken design is given in Table 4. After a regression analysis for each of the responses the polynomial model established as follows:

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

where Y is the response, X_1–X_3 are the main effects of factors, X_1X_2, X_1X_3, X_2X_3 are the interaction effects of factors, X_1^2, X_2^2, X_3^2 are quadratic effects of factors, b_0 is the constant, and b_1-b_3 are the coefficients of the factors. The p values of the regression coefficients (b_1–b_3) were determined to evaluate the significance of the factors on the responses. ANOVA was also applied to determine the significance of the model.

Table 4 Summary of results of statistical analysis and optimization of the formulations using Box-Behnken design

Box-Behnken Design

Factors: 3 Replicates: 1
Base runs: 15 Total runs: 15
Base blocks: 1 Total blocks: 1

Center points: 3

Response Surface Regression: Y1, Y2, Y3, Y4 versus X1, X2, X3

Response Surface Regression: Y1 versus X1, X2, X3

The analysis was done using uncoded units.

Estimated Regression Coefficients for Y1

Term	Coef	SE Coef	T	P
Constant	-4.50037	12.8836	-0.349	0.741
X1	1.14304	0.9599	1.191	0.287
X2	0.38687	1.7747	0.218	0.836
X3	-0.01408	3.8396	-0.004	0.997
X1*X1	-0.02029	0.0289	-0.701	0.515
X2*X2	-0.02992	0.1158	-0.258	0.806
X3*X3	0.26433	0.4631	0.571	0.593
X1*X2	-0.01512	0.0556	-0.272	0.797
X1*X3	-0.14625	0.1112	-1.315	0.246
X2*X3	0.06500	0.2225	0.292	0.782

S = 0.2225 R-Sq = 49.7% R-Sq(adj) = 0.0%

Analysis of Variance for Y1

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	0.24407	0.24407	0.02712	0.55	0.796
Linear	3	0.10377	0.07386	0.02462	0.50	0.700
Square	3	0.04686	0.04686	0.01562	0.32	0.814
Interaction	3	0.09344	0.09344	0.03115	0.63	0.627
Residual Error	5	0.24750	0.24750	0.04950		
Lack-of-Fit	3	0.18225	0.18225	0.06075	1.86	0.368
Pure Error	2	0.06525	0.06525	0.03262		
Total	14	0.49157				

Response Surface Regression: Y2 versus X1, X2, X3

The analysis was done using uncoded units.

Estimated Regression Coefficients for Y2

Term	Coef	SE Coef	T	P
Constant	-11.1703	48.9532	-0.228	0.829
X1	3.6790	3.6473	1.009	0.359
X2	-3.0647	6.7431	-0.454	0.669
X3	0.0985	14.5893	0.007	0.995
X1*X1	-0.1171	0.1100	-1.064	0.336
X2*X2	0.1292	0.4399	0.294	0.781
X3*X3	-0.4830	1.7598	-0.274	0.795
X1*X2	-0.0450	0.2113	-0.213	0.840
X1*X3	-0.0450	0.4227	-0.106	0.919
X2*X3	0.6250	0.8454	0.739	0.493

S = 0.8454 R-Sq = 29.1% R-Sq(adj) = 0.0%

Analysis of Variance for Y2

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	1.46711	1.4671	0.1630	0.23	0.973
Linear	3	0.09018	1.0601	0.3534	0.49	0.702
Square	3	0.94581	0.9458	0.3153	0.44	0.734
Interaction	3	0.43112	0.4311	0.1437	0.20	0.891
Residual Error	5	3.57327	3.5733	0.7147		
Lack-of-Fit	3	2.37538	2.3754	0.7918	1.32	0.458
Pure Error	2	1.19790	1.1979	0.5989		
Total	14	5.04038				

Response Surface Regression: Y3 versus X1, X2, X3

The analysis was done using uncoded units.

Estimated Regression Coefficients for Y3

Term	Coef	SE Coef	T	P
Constant	601.583	529.716	1.136	0.308
X1	-43.608	39.467	-1.105	0.320
X2	-39.516	72.967	-0.542	0.611
X3	-91.873	157.869	-0.582	0.586
X1*X1	1.383	1.190	1.162	0.298
X2*X2	3.560	4.761	0.748	0.488
X3*X3	15.106	19.042	0.793	0.464
X1*X2	0.596	2.287	0.261	0.805
X1*X3	0.458	4.574	0.100	0.924
X2*X3	-3.305	9.148	-0.361	0.733

S = 9.148 R-Sq = 33.1% R-Sq(adj) = 0.0%

Analysis of Variance for Y3

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	206.751	206.75	22.972	0.27	0.956
Linear	3	3.288	118.37	39.457	0.47	0.715
Square	3	186.011	186.01	62.004	0.74	0.572
Interaction	3	17.452	17.45	5.817	0.07	0.974
Residual Error	5	418.398	418.40	83.680		
Lack-of-Fit	3	45.074	45.07	15.025	0.08	0.965
Pure Error	2	373.324	373.32	186.662		
Total	14	625.149				

Response Surface Regression: Y4 versus X1, X2, X3

The analysis was done using uncoded units.

Estimated Regression Coefficients for Y4

Term	Coef	SE Coef	T	P
Constant	300.800	312.130	0.964	0.379
X1	-26.506	23.256	-1.140	0.306
X2	-18.691	42.995	-0.435	0.682
X3	-26.974	93.023	-0.290	0.783
X1*X1	0.827	0.701	1.180	0.291
X2*X2	1.216	2.805	0.434	0.683
X3*X3	3.760	11.221	0.335	0.751
X1*X2	0.463	1.348	0.343	0.745
X1*X3	0.212	2.695	0.079	0.940
X2*X3	-0.558	5.390	-0.103	0.922

S = 5.390 R-Sq = 27.1% R-Sq(adj) = 0.0%

Analysis of Variance for Y4

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	53.908	53.908	5.990	0.21	0.980
Linear	3	4.685	38.992	12.997	0.45	0.730
Square	3	45.307	45.307	15.102	0.52	0.687
Interaction	3	3.916	3.916	1.305	0.04	0.986
Residual Error	5	145.270	145.270	29.054		
Lack-of-Fit	3	29.233	29.233	9.744	0.17	0.910
Pure Error	2	116.037	116.037	58.018		
Total	14	199.177				

Summary of results of statistical analysis and optimization of the formulations using Box-Behnken design is given in Table 4, shows that the responses mean dissolution time (MDT), time required to release 50% of drug ($t_{50\%}$), drug release at 2 hrs

(R_{2hrs}) and dissolution efficiency in 2 hrs (DE_{2hrs}) are not impacted significantly due to change in grade or concentration of polymers as well as tablet thickness.

No interaction effects of factors X_1, X_2 and $X_3$is observed on the responses mean dissolution time (MDT), time required to release 50% of drug ($t_{50\%}$), drug release at 2 hrs (R_{2hrs}) and dissolution efficiency in 2 hrs (DE_{2hrs})

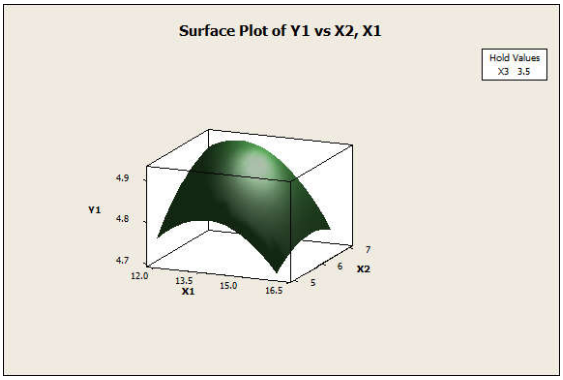

Figure 2a Surface Plot of Y1 vs X2, X1

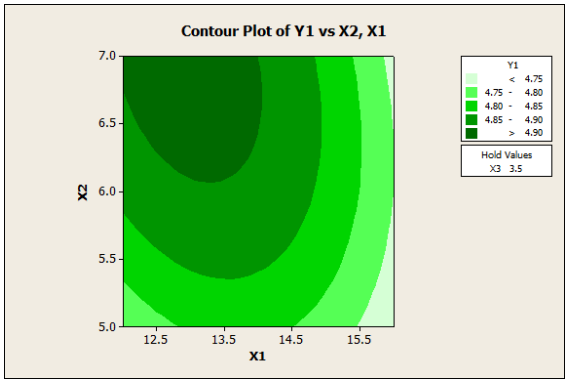

Figure 2b Contour Plot of Y1 vs X2, X1

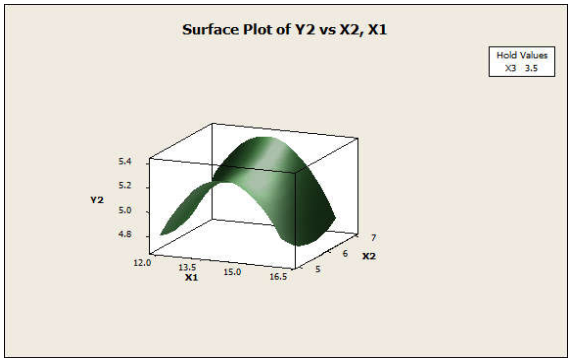

Figure 3a Surface Plot of Y2 vs X2, X1

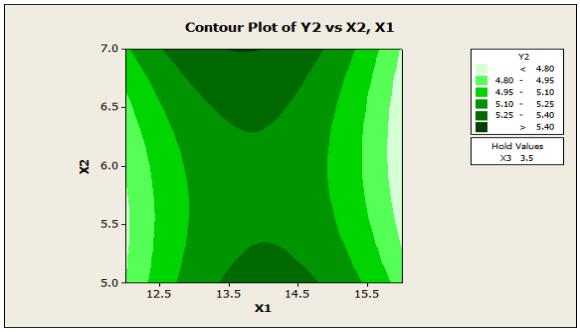

Figure 3b Contour Plot of Y2 vs X2, X1

From the regression coefficient values given in table 4 and surface and contour plots shown in Figure 2a and 2b it can be inferred that factors X_1 and X_2 have positive effect on response Y_1 while factor X_3 has negative impact on response Y_1.

From the regression coefficient values given in table 4 and surface and contour plots shown in Figure 3a and 3b it can be inferred that factors X_1 and X_3 have positive effect on response Y_2 while factor X_2 has negative impact on response Y_2. From the regression coefficient values given in table 4 and surface and contour plots shown in Figure 4a and 4b it can be inferred that factors X_1, X_2 and X_3 have negative impact on response Y_3. From the regression coefficient values given in table 4 and surface and contour plots shown in Figure 5a and 5b it can be inferred that factors X_1, X_2 and X_3 have negative impact on response Y_4.

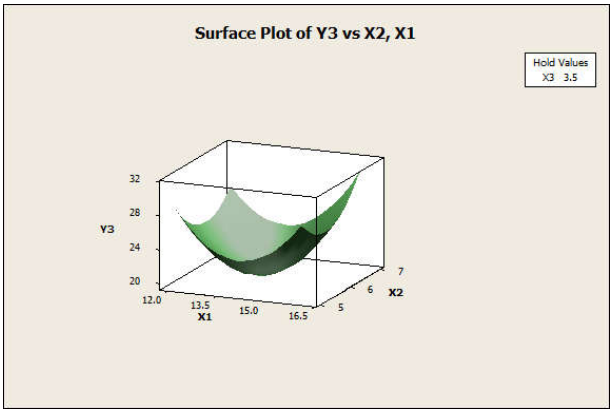

Figure 4a Surface Plot of Y3 vs X2, X1

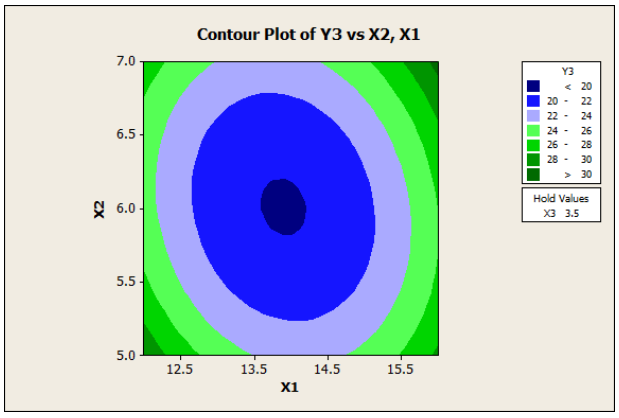

Figure 4b Contour Plot of Y3 vs X2, X1

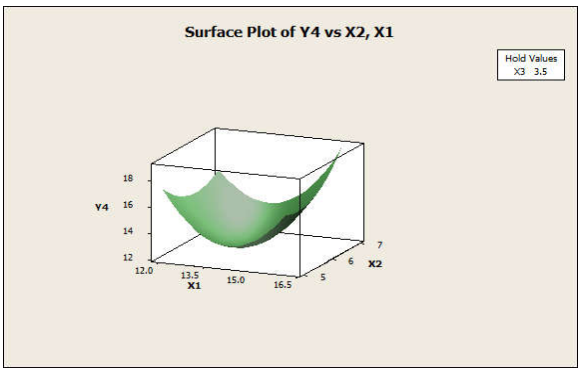

Figure 5a Surface Plot of Y4 vs X2, X1

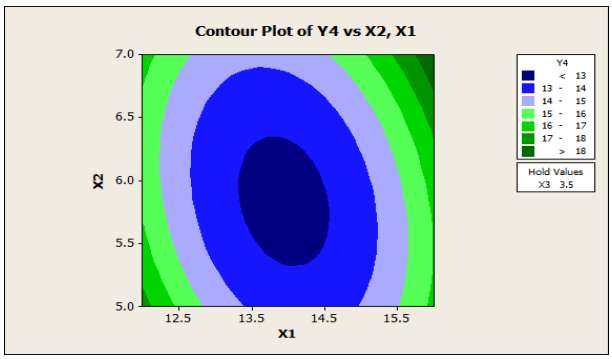

Figure 5b Contour Plot of Y4 vs X2, X1

Evaluation of the design space

The design space for floating tablets formulation of antibiotic agent Ofloxacin was established targeting the successful operating ranges for the mean dissolution time (MDT), time required to release 50% of drug ($t_{50\%}$), drug release at 2 hrs (R_{2hrs}) and dissolution efficiency in 2 hrs (DE_{2hrs}) as 4.5-5.0 hrs., 5.0-5.5 hrs, 25.0-30.0 % and 15.0-18.0 % respectively. When X_3: thickness (mm) was at 3.5mm set in experiment the proposed design space (Figure 6) comprising of the overlap region of ranges for the four responses was obtained. The design space illustrated that the available operation range is wide at the laboratory scale and thus ensuring the product quality.

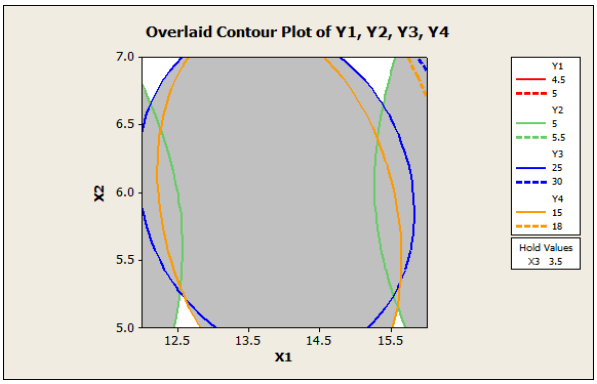

Figure 6 Overlaid Contour Plot of Y1, Y2, Y3, Y4

CONCLUSIONS

A Box-Behnken design was successfully applied for the optimisation of floating tablet formulation antibiotic agent Ofloxacin. Optimisation study results revealed that polymer grade, polymer concentration and tablet thickness do not have significant effect on drug release from the given tablet formulation. Using the design space plot obtained at the end of optimisation study decision maker can select low cost grade of polymer with minimum concentration achieve target drug release. Thus it can be concluded that successful application of Box-Behnken design of experiments is helpful to select grade and concentration of polymers cost effectively to reduce cost of goods which ultimately can improve profitability of pharmaceutical production unit.

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