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FORMULATION AND OPTIMIZATION OF DELAYED RELEASE TABLET CONTAINING DOXYCYCLINE HYCLATE

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ABSTRACT

Doxycycline hyclate act by broad spectrum antibacterial agent belonging to chemical class of tetracycline derivative. In this research paper various enteric coating polymers are used in formulation to attained delayed release of drug. Tablet formulation was prepared by using various excipients and delayed release polymers and their evaluation test are performed. Nine formulation trial batches were prepared by using Minitab 18 3^2 full factorial design. In that three formulation level (High, Medium and Low) of delayed release polymer as a factor and dissolution and drug content as an response were taken. From all nine batches batch F3 were selected as an optimized batch because of %Cumulative drug release and drug content were found satisfactory. Batch F3 gives results of % CDR (95.16%) and % drug content (101.25 %) having the concentration of 21.63 mg and 64.88 mg of HPMC K100M and Ethyl cellulose respectively and hence batch F3 was selected as an optimized batch.

INTRODUCTION

Each tablet made up of one or more active substances and usually obtained by compressing uniform volumes of particles. The particle containing one or more active substances with or without excipients such as diluents, binders, disintegrating agents, glidants, lubricants, substances capable of modifying the behavior of the preparation in the digestive tract, coloring matter authorized by the competent authority and flavoring substances.[1] To achieve the better therapeutic effect drugs needs to be reach at right place at right time. These kind of result obtained for certain drugs by simple solution or by conventional dosage form but for other drugs some modifications for release the drug in the body.[2] Modified release are the dosage form which release the drug at chosen location in at right time to achieve the

therapeutic effect which never achieved by the conventional dosage form. Modified release dosage forms are categorized into two types, one is delayed-release dosage form which does not immediately release the drug after administration and another one is extended-release dosage form. ER formulations can be referred to as dosage forms that allow at least a two fold reduction in the dosing frequency compared to a conventional dosage form.[3] In delay-release dosage form the drug is purposefully delayed before it reaches to intestine because of several reasons such as degradation of drug in Gastric pH, to minimize irritation to stomach.[4] Delayed release dosage form are formulated by using various enteric coated polymers or delayed release polymers either by incorporated into formulation or by enteric coating. In this research paper various enteric coating polymers are used in formulation to attained delayed release of drug.

MATERIALS AND METHODS

Doxycycline Hyclate provided by Athena drug delivery solution Pvt. Ltd., HPMC K100M, Ethyl cellulose, HPMC phthalate, Microcrystalline Cellulose, Magnesium stearate, PVP K 30M, Aerosil.

EXPERIMENTAL WORK

Identification of drug

Absorption maxima determination:

The solution of Doxycycline hyclate in the concentration of 10mg/ml was prepared using water. It was scanned over the wavelength range of 200-400 nm using double beam U.V. spectrophotometer with water as blank.

Melting point determination

Melting point was determined by open capillary method.

Preparation of calibration curve of standard plot Doxycycline hyclate

Doxycycline hyclate was accurately weighted (10mg) and dissolved in 10 ml of phosphate buffer 6.8 and 1.2 to produce a primary stock solution of 1mg/ml (1000ug/ml). The 1ml of primary stock solution was suitably diluted with 100ml of distilled water to produce working stock solution in concentration of 10ug/ml. From the above solution withdraw 1ml, 2ml, 3ml, 4ml and 5ml solution in volumetric flask with continues dilution to make final volume of 10 ml for each withdrawing WSS solution, so as to final concentration achieves was 1ug/ml to 5

ug/ml respectively. Absorbance of the solution was recorded at 268 nm using double beam UV spectrophotometer with water as a blank.

Formulation of delay release tablets for trial batches

The tablets were formulated by using direct compression technique. The compositions of tablet formulation were given in table no.1. All ingredients were mixed in geometrical order as mentioned in formula tablet by passing through sieve #24 except only magnesium stearate passed through sieve #60 sieve. After trituration mixture was blended and then directly compressed using capsule shaped punches.

Table 1: Formulation Table of All Preliminary Batches.

Batch No. / Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
API	50	50	50	50	50	50	50	50	50
HPMC K100 M	21.62	21.62	21.62	43.25	43.25	43.25	64.88	64.88	64.88
Ethyl cellulose	21.62	43.25	64.88	21.62	43.25	64.88	21.62	43.25	64.88
HPMC Phthalate	10	10	10	10	10	10	10	10	10
MCC	34.26	72.63	51	72.63	51	29.57	51	29.37	7.74
Aerosil	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mg Stearate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PVP K 30	12	12	12	12	12	12	12	12	12

Evaluation of preliminary (trial) batches

Thickness

Thickness and diameter were measured using vernier caliper.

Hardness

The hardness of tablet of each formulation was measured by monsanto hardness tester. The hardness was measured in terms of kg/cm^2.

Weight variation

Randomly 20 tablets were selected after compression and average weight was determined. None of the tablets deviated from the average weight by more than $\pm$ 7.5%. The weight values were expressed in milligrams. This is an important parameter in process quality control test to be checked frequently. Corrections were made during the compression of tablets. Any variation in weight of tablets (for any reason) leads to either under medication in overdose. So every tablet in each batch should have a uniform weight.

Table 2: Weight variation specifications.

Sr.no	Average weight of tablets (mg)	Maximum % difference allowed
1	80 mg or less	10
2	80 mg to 250 mg	7.5
3	More than 250 mg	5

Friability test

Friability is the measure of tablet strength. Roche type friabilator was used for testing the friability using the following procedure. Twenty tablets were weighed accurately and placed in the tumbling apparatus that revolves at 25 rpm dropping the tablets through a distance of six inches with each revolution. After 4 min the tablets were weighed and the percentage loss in tablet weight was determined.

$$\text{Friability \%} = \frac{\text{Initial weight of tablets} - \text{Final wt of tablets}}{\text{Initial weight of tablets}}$$

Content uniformity

The content uniformity test is used to ensure that every tablet contains the amount of drug substance intended with little variation among tablets within a batch due to awareness of physiological availability. The content uniformity test has been involved in the monograph of all coated and uncoated tablets and all capsules intended for oral administration where the range of size of the dosage form available include 50 mg or smaller size.

In vitro dissolution studies

The release rate of doxycycline hyclate delay release tablet was determined using USP dissolution testing apparatus type II (paddle type). 900 ml of the dissolution medium (6.8 pH phosphate buffer) was taken in covered vessel and the temperature was maintained at 37° $\pm$ 0.5°. The speed of paddle was set at 100 rpm. Sampling was done at regular intervals. For each sample 10 ml of the dissolution medium was withdrawn and same amount was replaced. The sample was filtered and diluted with 6.8 phosphate buffer and then analyzed in UV spectrophotometer. The absorbance was measured at 268 nm and % drug release was calculated.

Drug content

10 tablets were weighed and powdered. Powder equivalent to50mg of doxycycline hyclate was weighed and dissolved in 6.8 pH phosphate buffer. Different concentrations of drug were prepared and analyzed U.V. Visible Spectroscopy.

Optimization of formulation with QbD paradigram

Selection of optimized batch from above trial batches

For the optimization of formulation by qbd principle it is very important to take preliminary batches and select optimized batch from normal evaluation test of formulation so as to it is easy to finalize the ratio of polymers which may give better results than other ratio. Using this final ratio in factorial design paradigm we can obtain perfect design of experiment and to make control strategy for obtaining formulation which is more effective as possible. Formulations were prepared by changing ratio of polymers to optimize formulation. The hydroxyl propyl methyl cellulose k100M and ethyl cellulose concentration was maintained from 21.62 to 64.88mg (ranges are selected from 9 trial batches).

3^2 Factorial design

A factorial design is used to evaluate two or more factors simultaneously. The treatments are the combinations of levels of the factors. The advantages of factorial design over one factor at a time experiment are that they are more efficient and they allow interactions to be detected. Intervention studies with 2 or more categorical explanatory variables leading to a numerical outcome variable are called as "Factorial design" Based on preliminary trails a 2 factor 3 levels full factorial design (3^2) by using minitab 17 was employed to design delay release doxycycline hyclate tablet. This design was suitable for exploring quadratic response surfaces and analysis of variance study.

Table 3: Actual Values of Factorial Design.

Batches	Independent Variable		Actual values	
	X_1	X_2	X_1 (mg)	X_2 (mg)
F1	1	1	21.62	21.62
F2	1	2	21.62	43.25
F3	1	3	21.62	64.88
F4	2	1	43.25	21.62
F5	2	2	43.25	43.25
F6	2	3	43.25	64.88
F7	3	1	64.88	21.62
F8	3	2	64.88	43.25
F9	3	3	64.88	64.88

Table 4: Levels of Variables in 3^2 Factorial Design.

Sr. No	Variable levels	Low	Medium	High
1	HPMC K100M	21.62mg	43.25mg	64.88mg
2	Ethyl cellulose	21.62mg	43.25mg	64.88mg

Each factor was evaluated at three levels and experimental trials were performed. All the nine formulations were subjected to evaluation for physical, mechanical and performance properties. For the purpose of factorial design % cumulative drug release and content drug were considered as dependent variables.

RESULT AND DISCUSSION

Identification of drug

Determination of UV Absorption Maxima

The absorption maxima of Doxycycline hyclate (10ug/ml) in water was found to be at 268 nm.

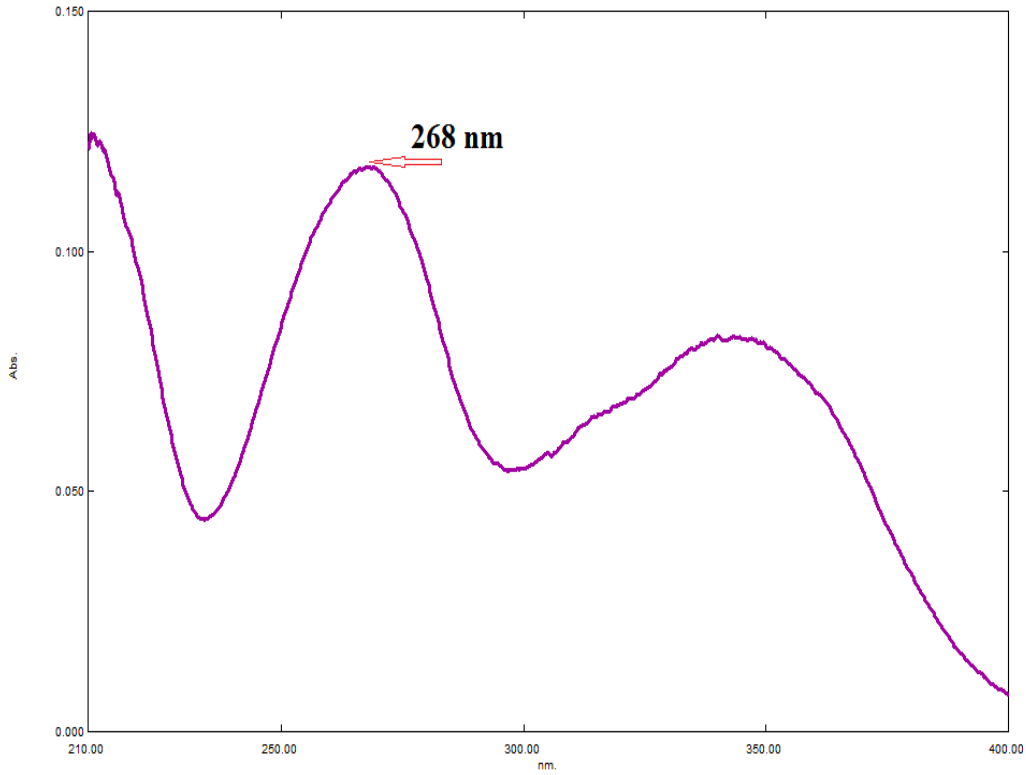

Figure 1: Determination of UV Absorption Maxima (λ_{max}).

Melting Point

The melting point of Doxycycline hyclate was found to be 207.33^{0}C.

Table 5: Melting Point of Doxycycline Hyclate.

Sr.No	Melting point (^{0}C)	Average (^{0}C)
1.	206	207.33
2.	207	
3.	209	

Preparation of calibration curve of standard plot Doxycycline hyclate

Doxycycline hyclate was accurately weighted (10mg) and dissolved in 10 ml phosphate buffer pH 6.8 & pH 1.2 to produce a primary stock solution of 1mg/ml (1000ug/ml). The 1ml of primary stock solution was suitably diluted with 100ml of distilled water to produce working stock solution in concentration of 10ug/ml. From the above WSS withdraw 1ml, 2ml, 3ml, 4ml and 5ml solution in volumetric flask with continues dilution to make final volume of 10ml for each withdrawing WSS so as to final concentration achieves was 1ug /ml to 5ug/ml respectively. Absorbance of solution was recorded at 268 nm using double beam spectrophotometer with water as blank. The plot of absorbance versus concentration was plotted.

Table 1: Calibration Curve of Doxycycline Hyclate in Phosphate Buffer 6.8.

Sr. No.	Concentration	Absorbance at 268 nm
1.	10	0
2.	20	0.291
3.	30	0.451
4.	40	0.758
5.	50	1.003

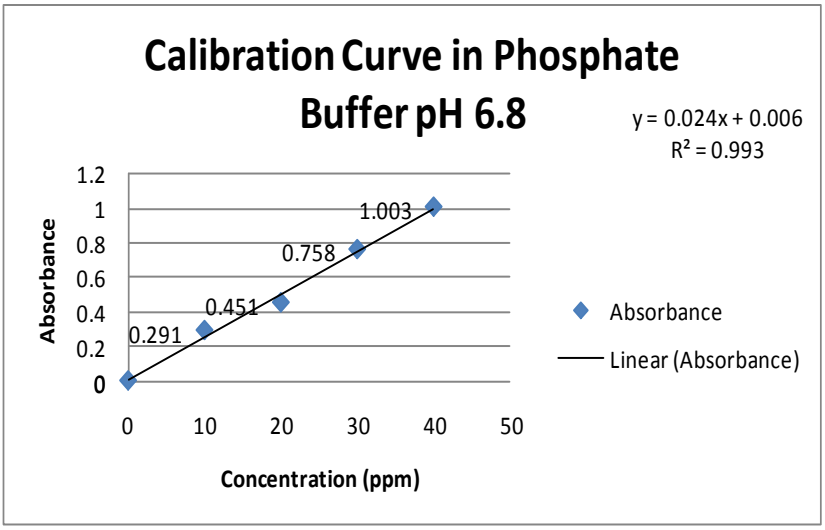

Figure 2: Calibration Curve in Phosphate Buffer PH 6.8.

Table 7: Calibration Curve of Doxycycline Hyclate in Phosphate Buffer 1.2.

Sr. No.	Concentration	Absorbance at 268 nm
1.	10	0.128
2.	20	0.261
3.	30	0.531
4.	40	0.69
5.	50	0.884

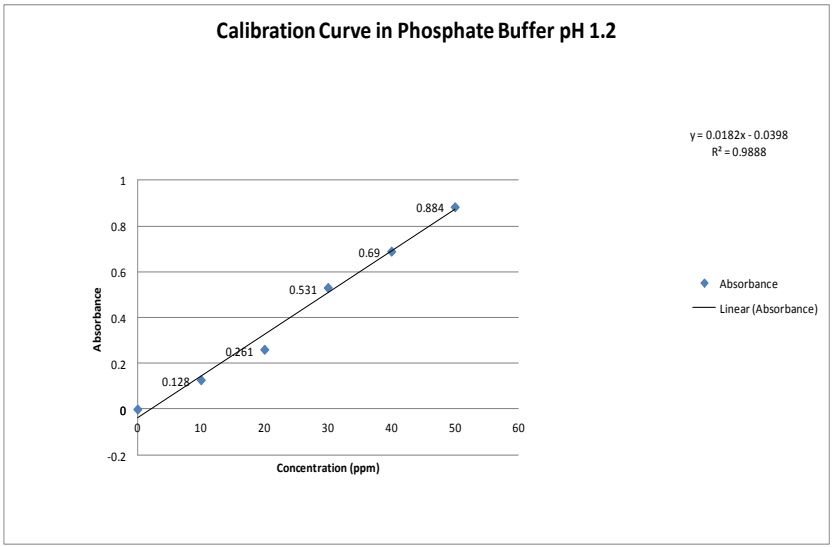

Figure 3: Calibration Curve in Phosphate Buffer PH 1.2.

Table 2: Physicochemical Parameter Study of all Preliminary Trial Batches.

Batches	Bulk Density	Tapped Density	Compressibility Index	Hoasner's Ratio	Porosity	Angle of Repose
F1	2.25	3	21	1.33	25	45
F2	2.5	2.8	12.5	1.12	75	45
F3	0.26	0.32	19.1	1.23	69	36.69
F4	0.26	0.34	24	1.3	97	41.34
F5	0.26	0.33	20	1.26	68.8	38.65
F6	0.26	0.34	22.82	1.3	88.04	37.23
F7	0.24	0.30	21.21	1.25	77.77	36.5
F8	0.20	0.30	33.25	1.5	58.41	40.69
F9	0.23	0.30	21.42	1.3	68.36	40.69

From the powder characteristics i.e. angle of repose, compressibility index and hausner's ratio it was concluded that the powder possesses Very poor, good and Cohesive flow characteristics.

Table 3: Post Evaluation Test of all Preliminary Trial Batches.

Batches	Weight variation	Friability test	Hardness test	Thickness	% Drug Content
F1	211.5	0.14	3.4	4.33	102.22
F2	212.5	0.23	4.5	4.32	96.42
F3	210.9	0.23	4.1	4.27	101.25
F4	210.9	0.43	4.2	4.35	104.15
F5	209.8	0.19	3.2	4.29	100.28
F6	211.8	0.47	3.4	4.32	97.39
F7	209.8	0.33	3.6	4.28	99.32
F8	210	0.19	3.5	4.30	100.2
F9	209.1	0.14	4.2	4.33	98.35

Table 4: In vitro dissolution study by using phosphate buffer 6.8.

Time (Hrs)	% Cumulative Drug Release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	29.39	47.47	36.22	24.49	20.49	26.99	13.82	12.54	15.60
2	34.20	55.42	41.69	33.92	31.65	35.16	17.68	17.25	21.12
3	45.32	56.24	44.86	47.60	36.66	41.45	22.95	24.44	32.70
4	47.84	64.85	48.70	49.43	41.07	43.82	37.64	38.74	28.45
5	52.63	75.49	58.74	51.55	49.84	46.88	43.62	43.95	33.68
6	61.02	73.32	66.73	64.87	55.73	55.11	56.94	53.50	44.80
7	70.90	75.64	69.79	72.01	60.17	59.68	65.12	58.67	50.82
8	81.59	77.91	77.77	74.93	62.40	66.48	68.90	68.20	59.81
9	88.82	80.95	90.71	78.48	66.80	73.28	71.17	74.82	68.04
10	99.71	87.79	95.16	86.21	79.19	84.58	74.14	82.17	71.84

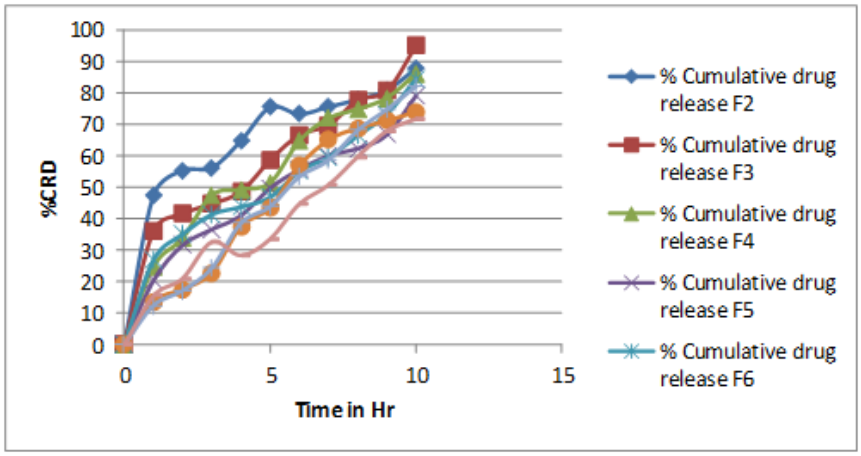

Figure 4 In Vitro Dissolution Study Graph of All preliminary trial batches.

ANOVA

General Factorial Regression: Drug Content versus HPMC... l Cellulose.

Factor Information

Table 11: Factor Information.

Factor	Level	Values
HPMC K100M	3	21.62,43.25, 64.88
Ethyl Cellulose	3	21.62,43.25, 64.88

Analysis of Variance

Analysis of variance (ANOVA) (Table 23) indicated that the assumed regression model was significant and valid for each considered response. Main effects are having significant impact hence reduced model was taken into consideration for ANOVA analysis. Comparison of regression analysis with respect to full model and reduced model is demonstrated. Among

main effects the main compression force is having a significant impact on all response variables. Difference between adjust ss and adjust Ms value.

Table 12: Analysis of Variance.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	659.96	82.49	*	*
Linear	4	523.63	130.91	*	*
HPMC K 100M	2	501.76	250.88	*	*
Ethyl Cellulose	2	21.87	10.94	*	*
2-Way Interactions	4	136.32	34.08	*	*
HPMC K 100M*Ethyl Cellulose	4	136.32	34.08	*	*
Error	0	*	*		
Total	8	659.96			

Table 13: One –Way ANOVA of % CDR, Drug Content Method.

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Means

Factor	N	Mean	StDev	95% CI
%CDR	9	99.968	2.415	(95.272, 104.664)
Drug Content	9	84.53	9.08	(79.84, 89.23)

Pooled StDev = 6.64563

Multiple Response Prediction

Variable	Setting
F3 batch	
HPMC	21.62
Ethyl	64.88

Response	Fit	SE Fit	95% CI	95% PI
% CDR	95.16	*	(*,*)	(*,*)
Drug Content	101.25	*	(*,*)	(*,*)

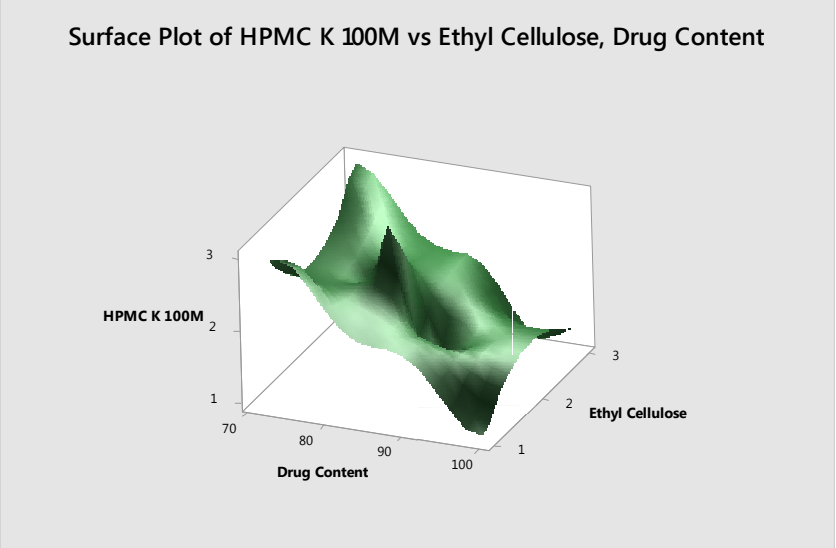

Figure 2: Surface Plot of HPMC K 100M Vs Ethyl Cellulose, Drug Content

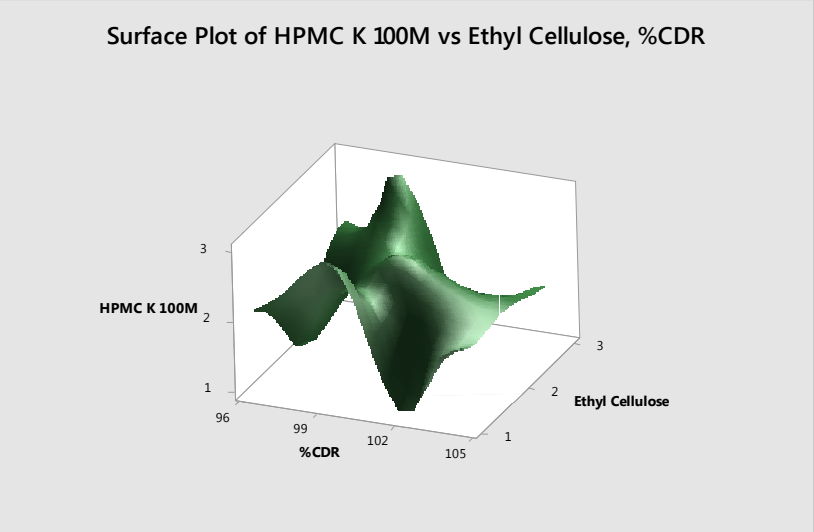

Figure 6: Surface Plot of HPMC K 100M Vs Ethyl Cellulose, % CDR.

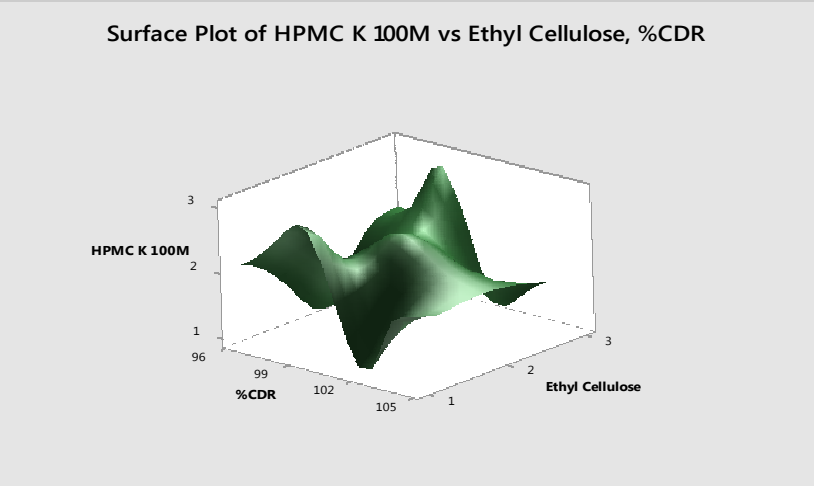

Figure 7: Surface Plot of HPMC K 100M Vs Ethyl Cellulose, % CDR.

Counter plot and Response Surface Plot

The two dimensional plot obtained by contour plots are superimposed for simultaneous optimization of the independent variables (figure 5.06). The desired values for the HPMC K100M Vs Ethyl cellulose in %CDR were set to obtain the predicted values from the set coded values. From the predicted values obtained by overlay of contour plots of both the responses, the actual values were calculated and experimental trials were performed for ensuring the proper validation of the process.

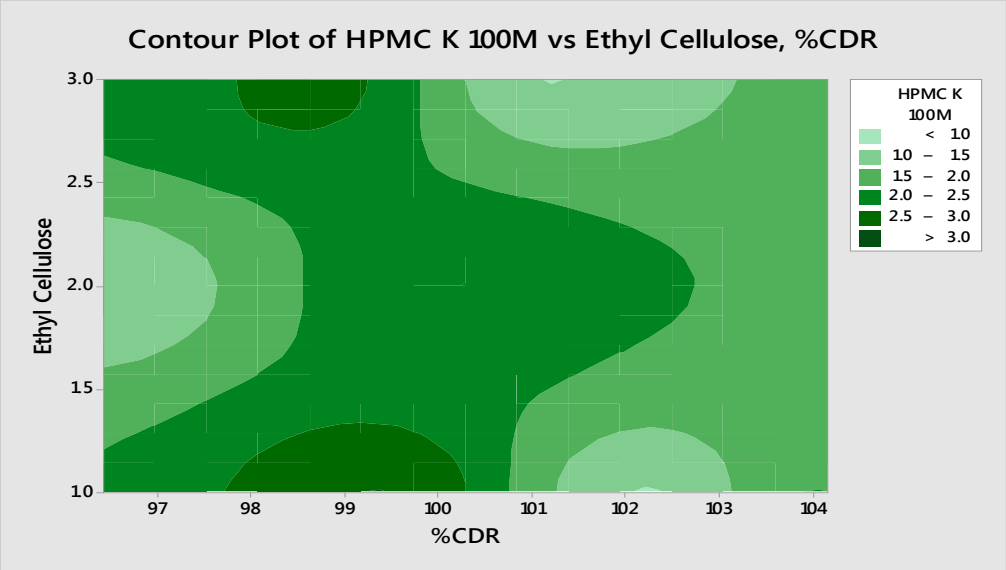

Figure 8: Contour Plot of HPMC K 100M Vs Ethyl Cellulose, % CDR.

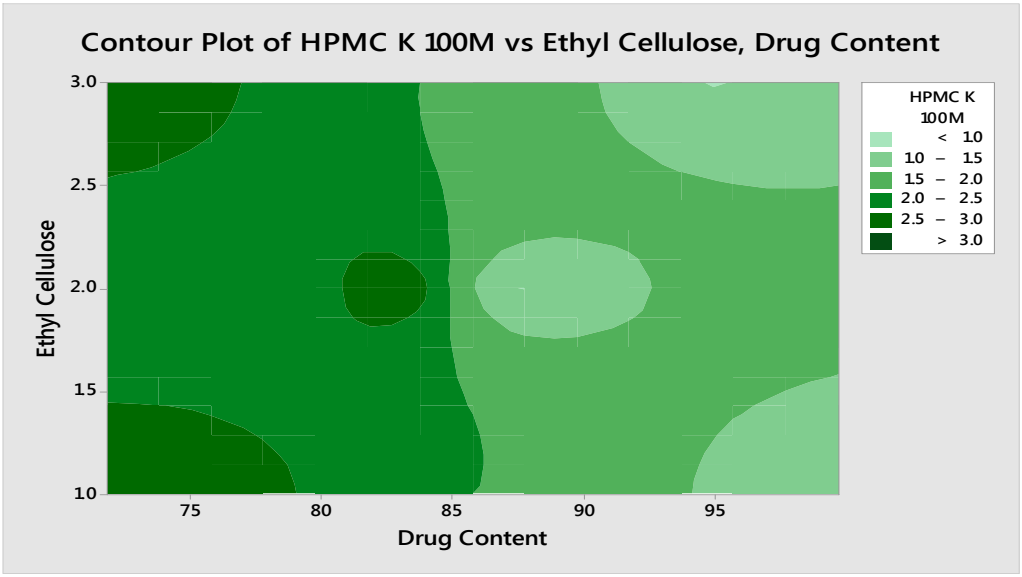

Figure 9: Contour Plot of HPMC K 100M Vs Ethyl Cellulose, Drug Content.

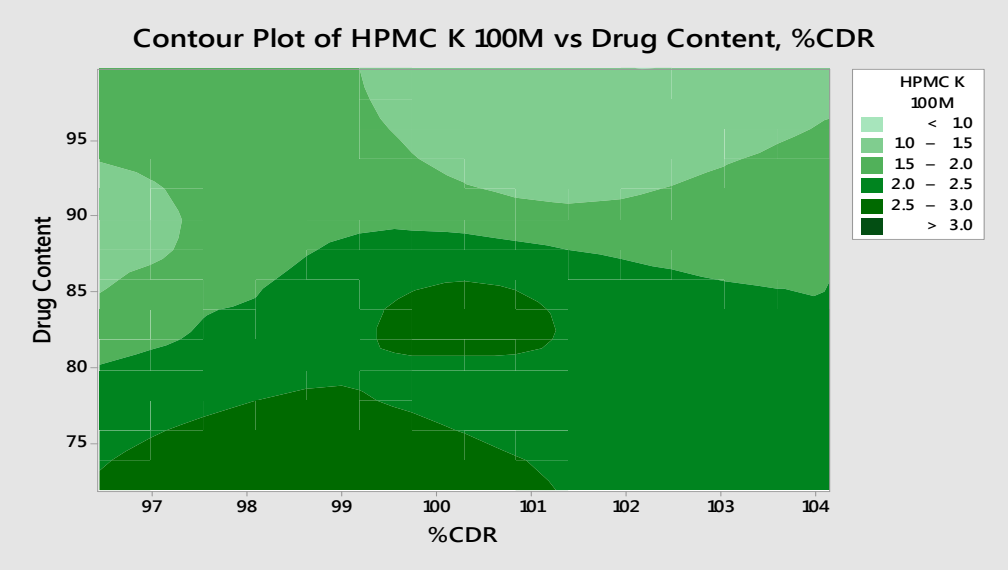

Figure 10: Contour Plot of HPMC K 100M Vs Drug Content, % CDR.

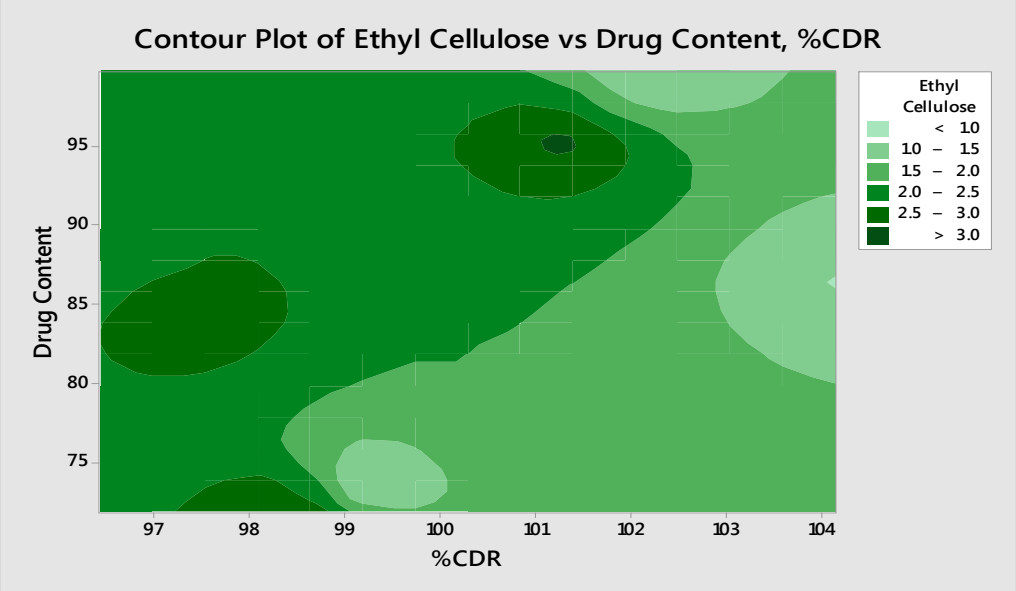

Figure 11: Contour Plot of Ethyl Cellulose Vs Drug Content, %CDR

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Table 14: Tukey Pairwise Comparisons.

Factor	N	Mean	Grouping	
%CDR	9	99.968	A	
Drug Content	9	84.53		B

Means that do not share a letter are significantly different.

Tukey Simultaneous 95% CIs

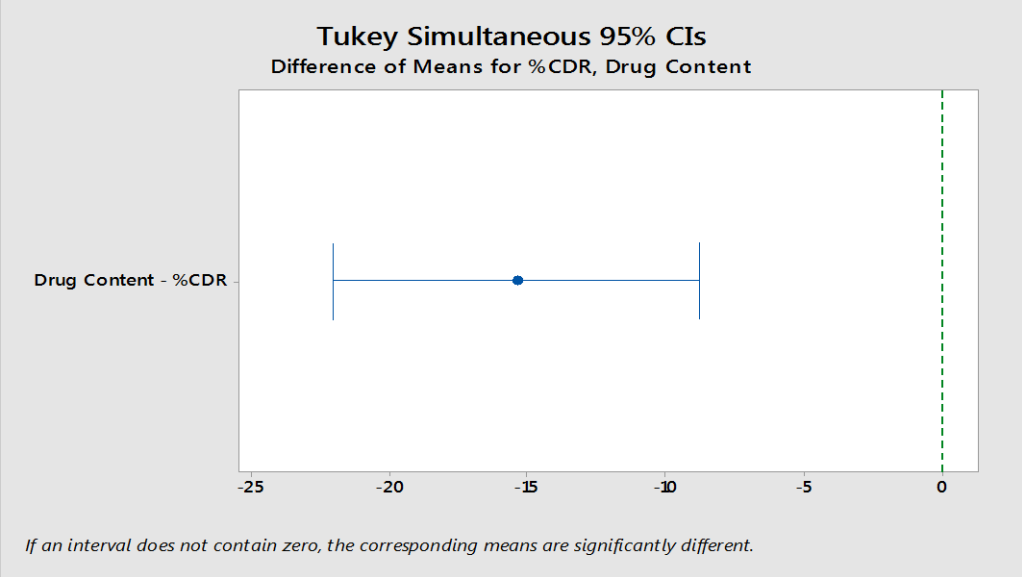

Figure 12: Tukey Simultaneous 95% Cls Difference of Means For %CDR, Drug Content.

Fisher Pairwise Comparison

Grouping Information Using the Fisher LSD Method and 95% Confidence

Table 15: Fisher Pairwise Comparison.

Factor	N	Mean	Grouping	
%CDR	9	99.968	A	
Drug Content	9	84.53		B

Means that do not share a letter are significantly different.

Fisher Individual 95% CIs

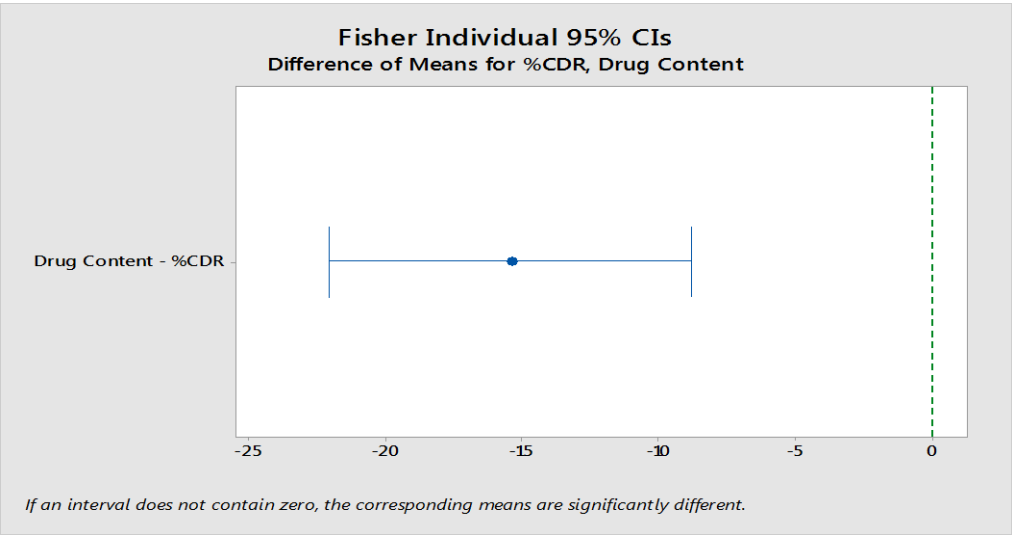

Figure 13: Fisher Individual 95% ClsDifference of Means For %CDR, Drug Content.

Interval Plot of %CDR, Drug Content

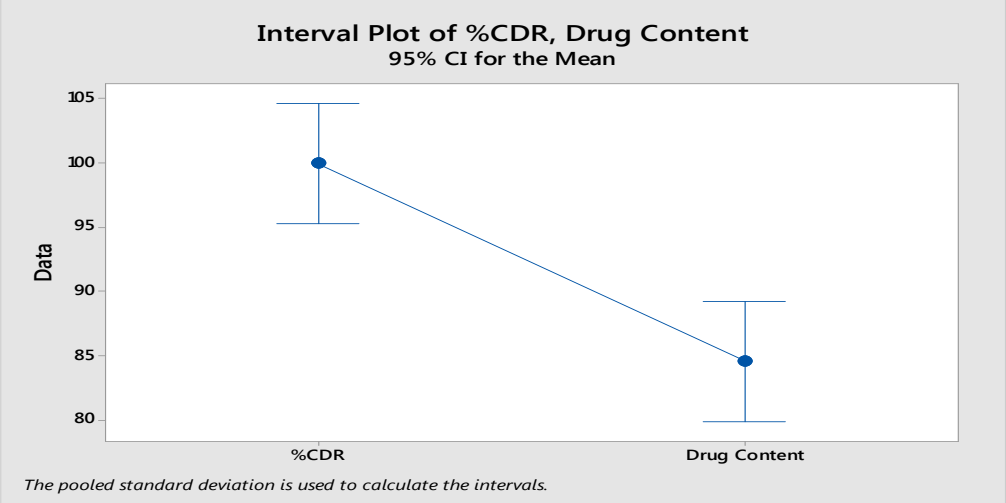

Figure 14: Interval Plot of % CDR, Drug Content 95% Cls For the Mean.

Boxplot of %CDR, Drug Content

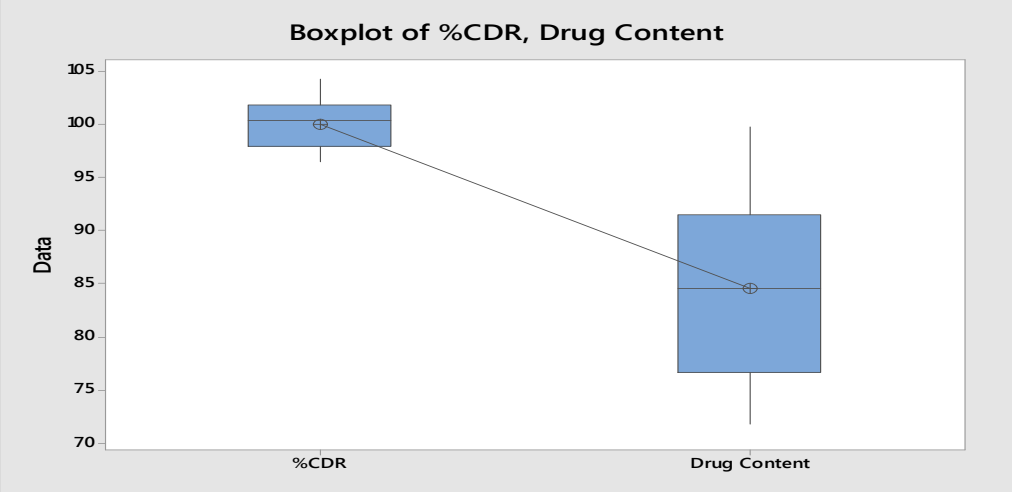

Figure 15: Box plot of % CDR, Drug Content.

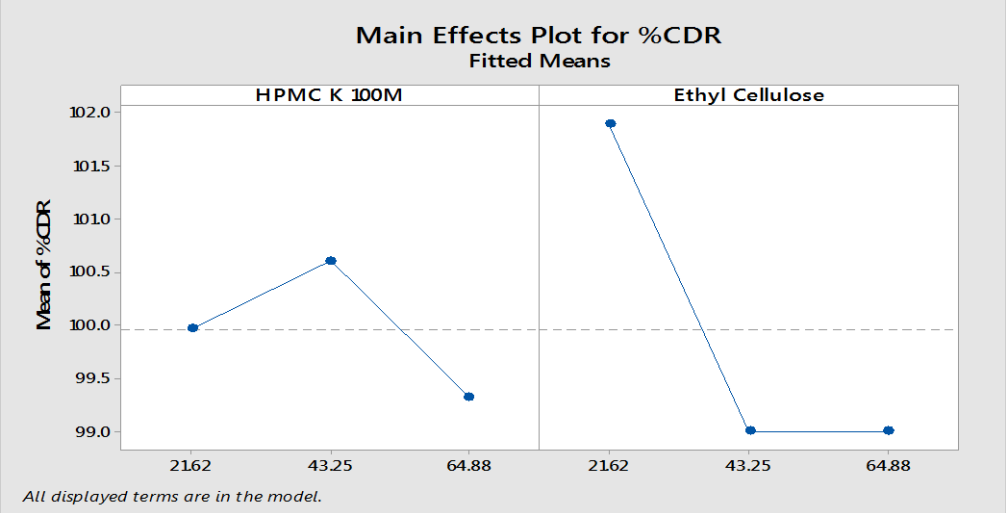

Figure 3: Main Effects Plot For %CDR Fitted Means.

Residual Plots for %CDR, Drug Content

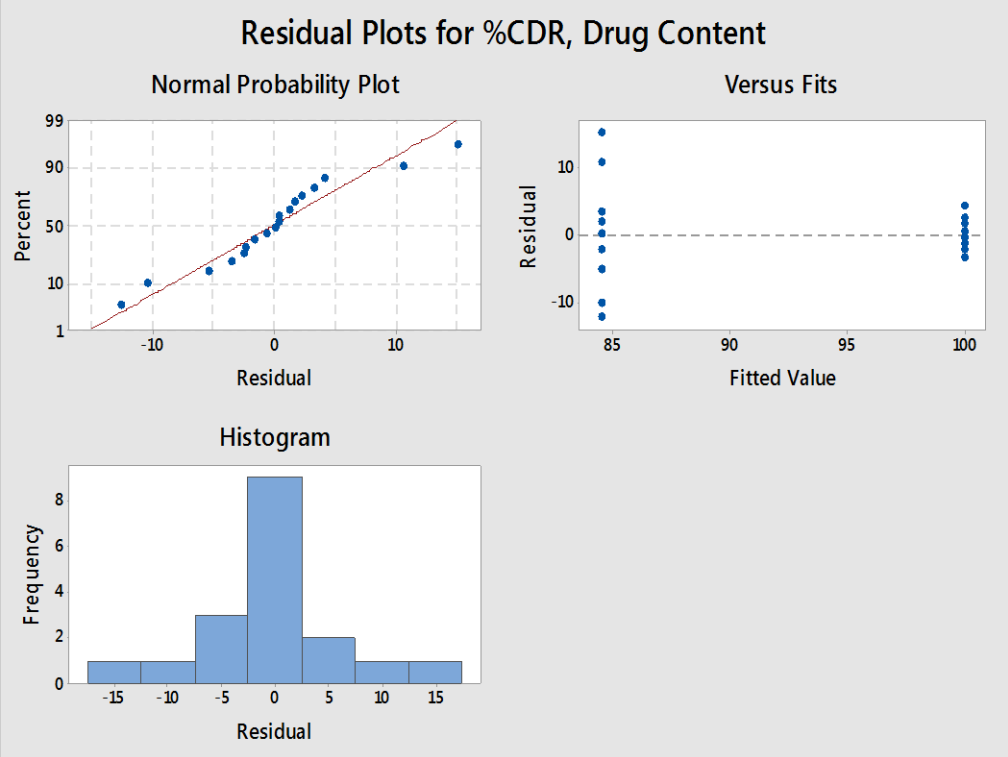

Figure 17: Residual Plots For % CDR, Drug Content.

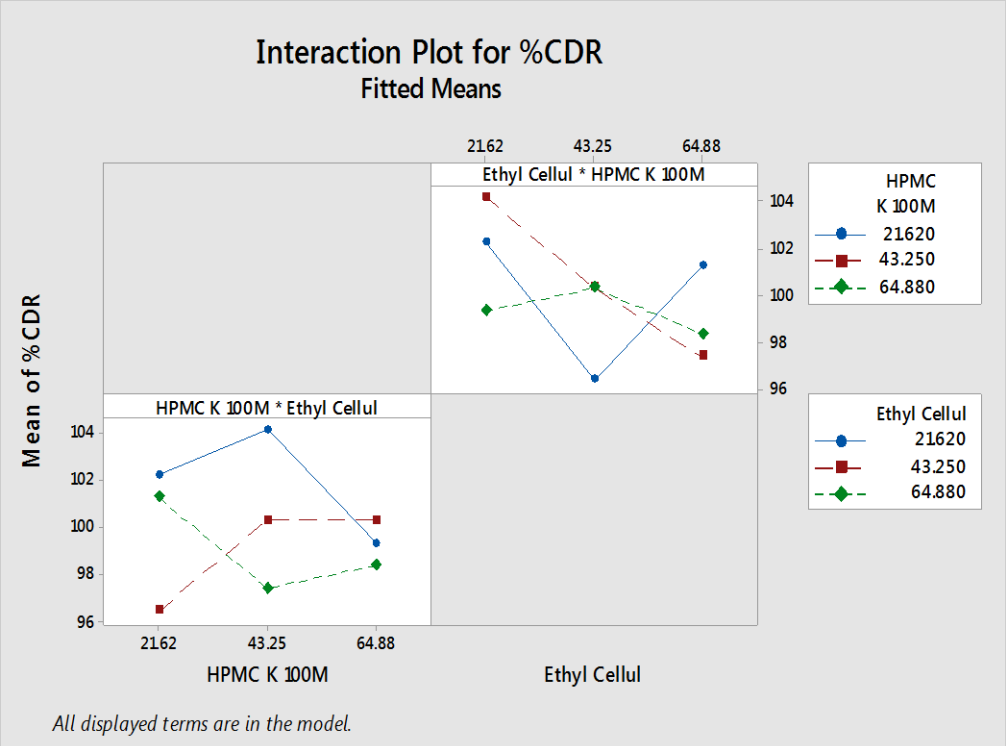

Figure 18: Interaction Plot For %CDR Fitted Means.

Selection of Optimized Batch by Dissolution study

Table 5: Dissolution Study of optimized batch (F3).

Time (Hr)	% Cumulative drug release
0	0
1	36.22
2	41.69
3	44.86
4	48.70
5	*58.74*
6	66.73
7	69.79
8	77.77
9	80.71
10	95.16
% Drug Content	101.25

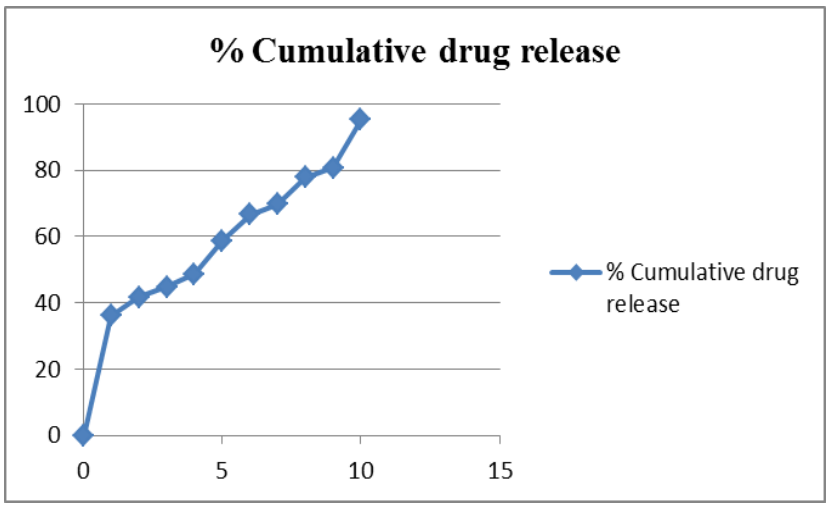

Figure 19: % CDR of Finally Optimized Delay Release Doxycycline Hyclate Tablet Batch.

IR spectrum of Drug with different Excipients

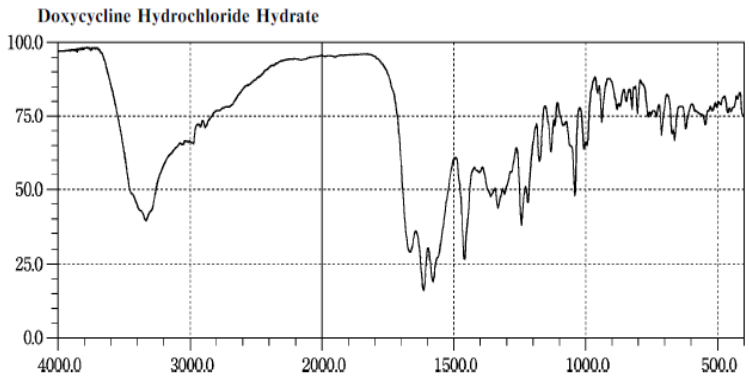

Figure 20: FTIR of doxycycline hyclate.

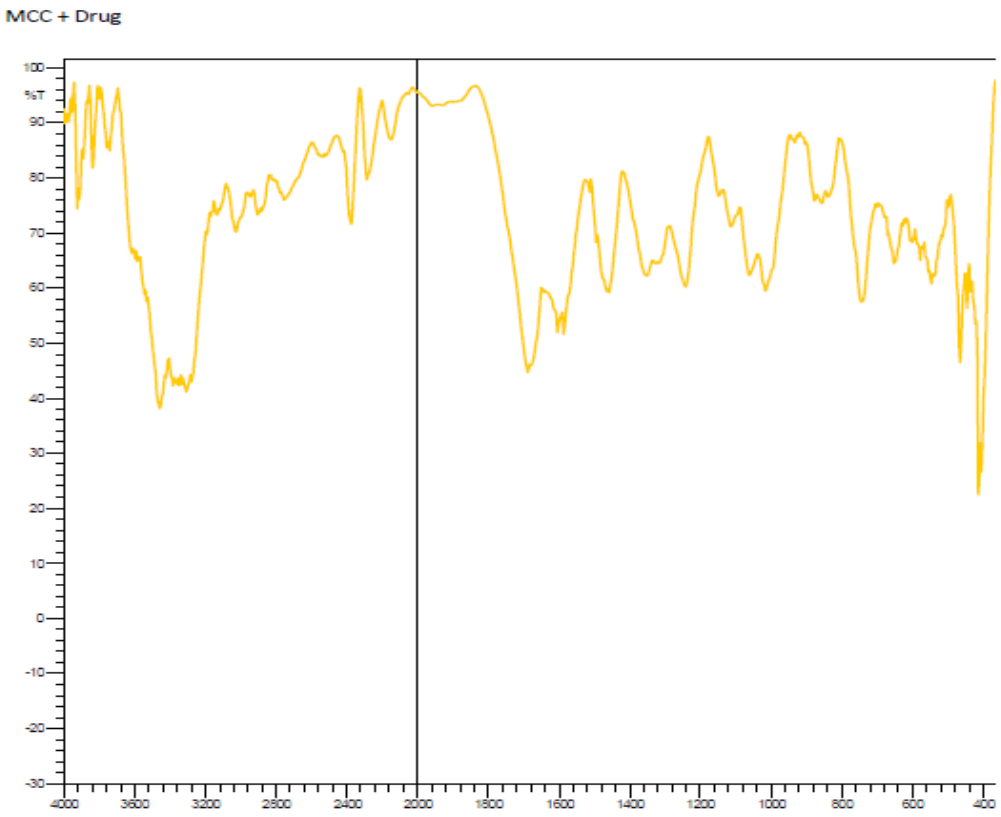

Figure 21: FTIR graph of MCC + Drug.

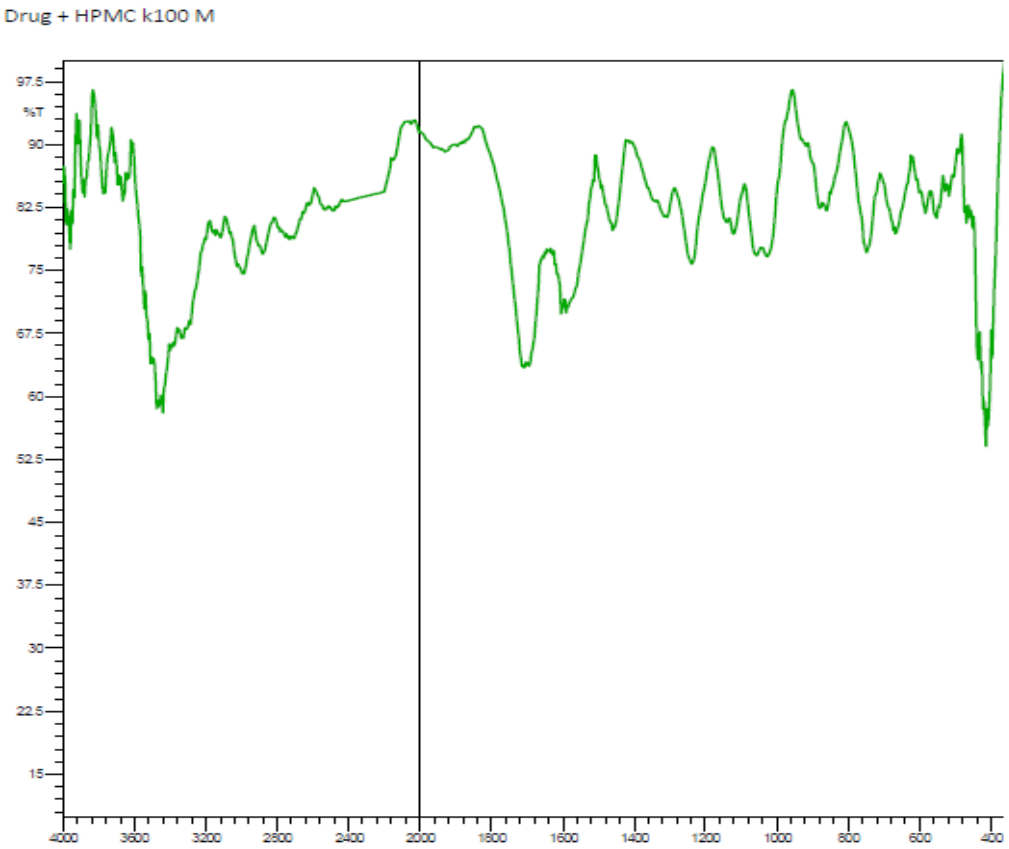

Figure 22: FTIR Graph of HPMC K 100 + Drug.

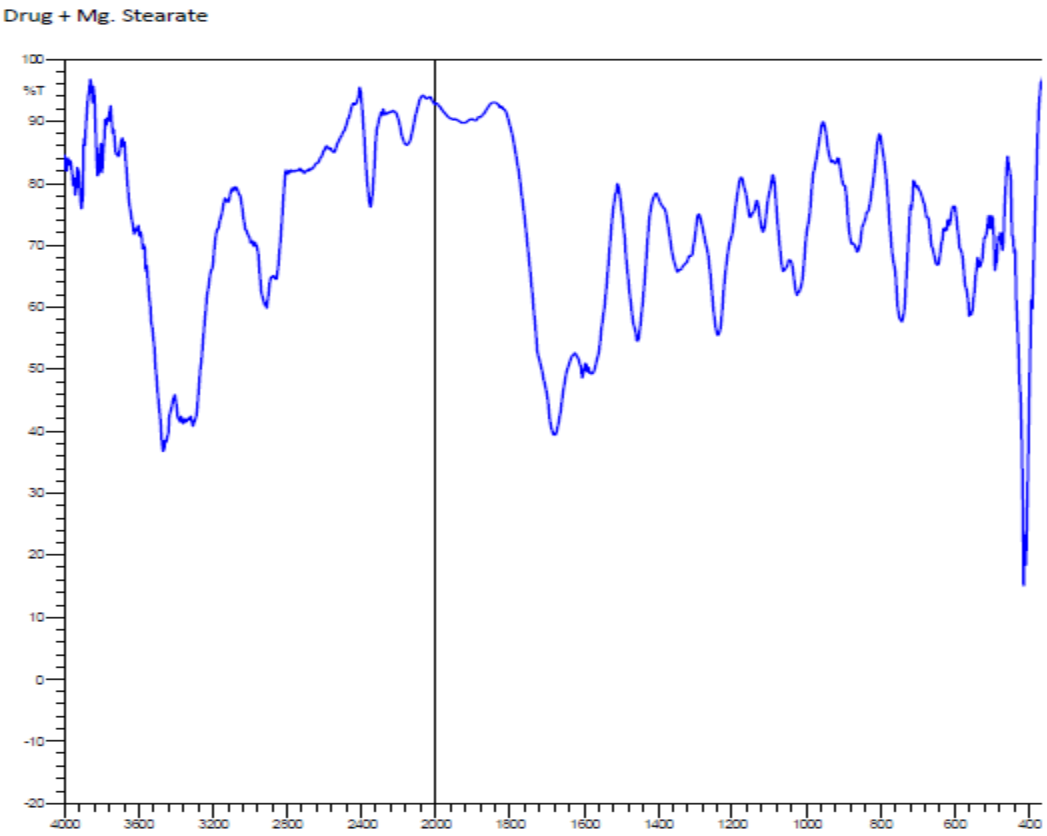

Figure 23: FTIR Graph of Magnesium Stearate.

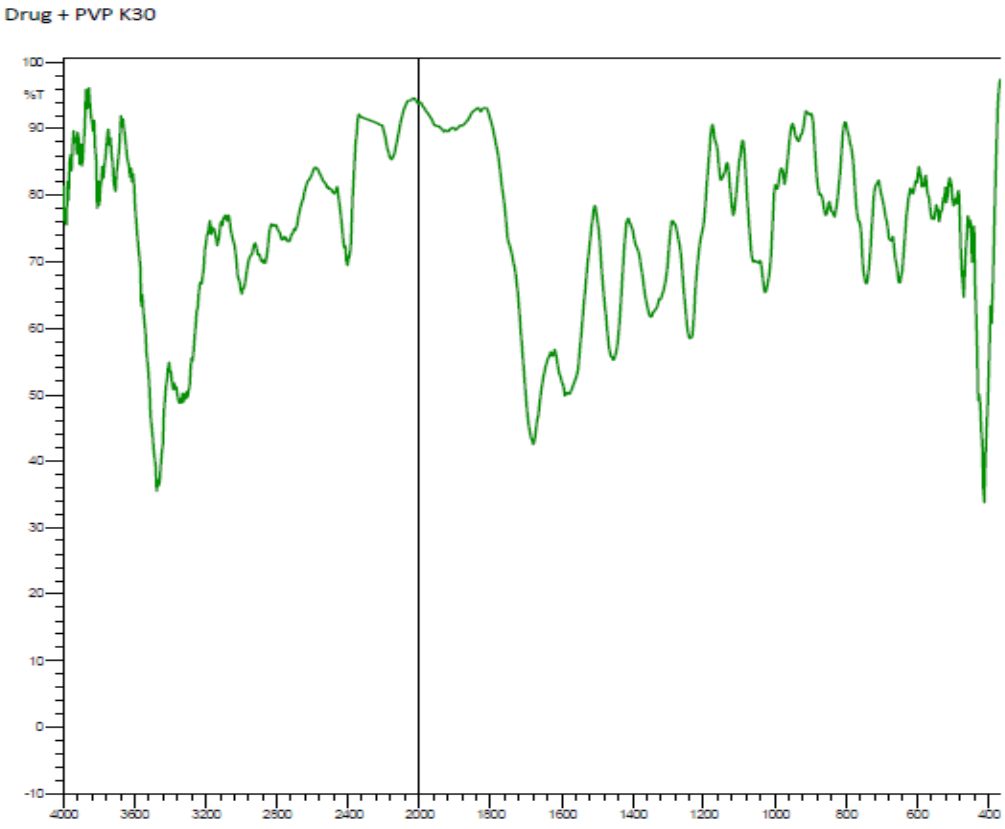

Figure 24: FTIR Graph of PVP K30 + Drug.

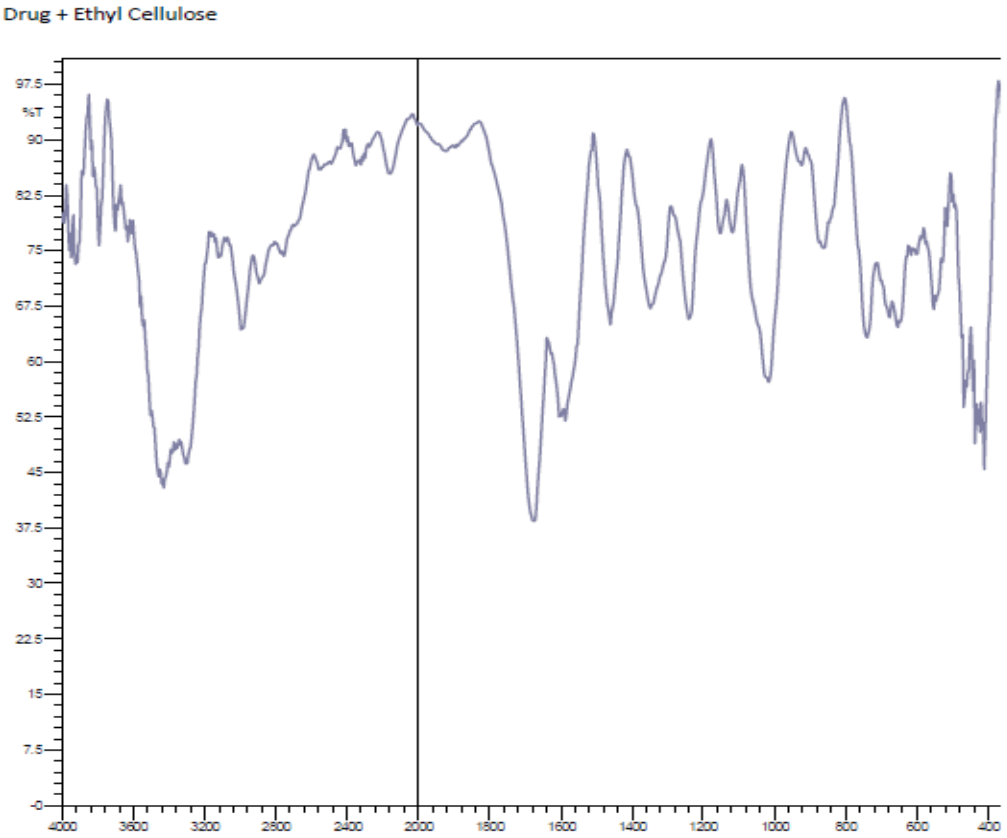

Figure 25: FTIR Graph of Ethyl Cellulose + Drug.

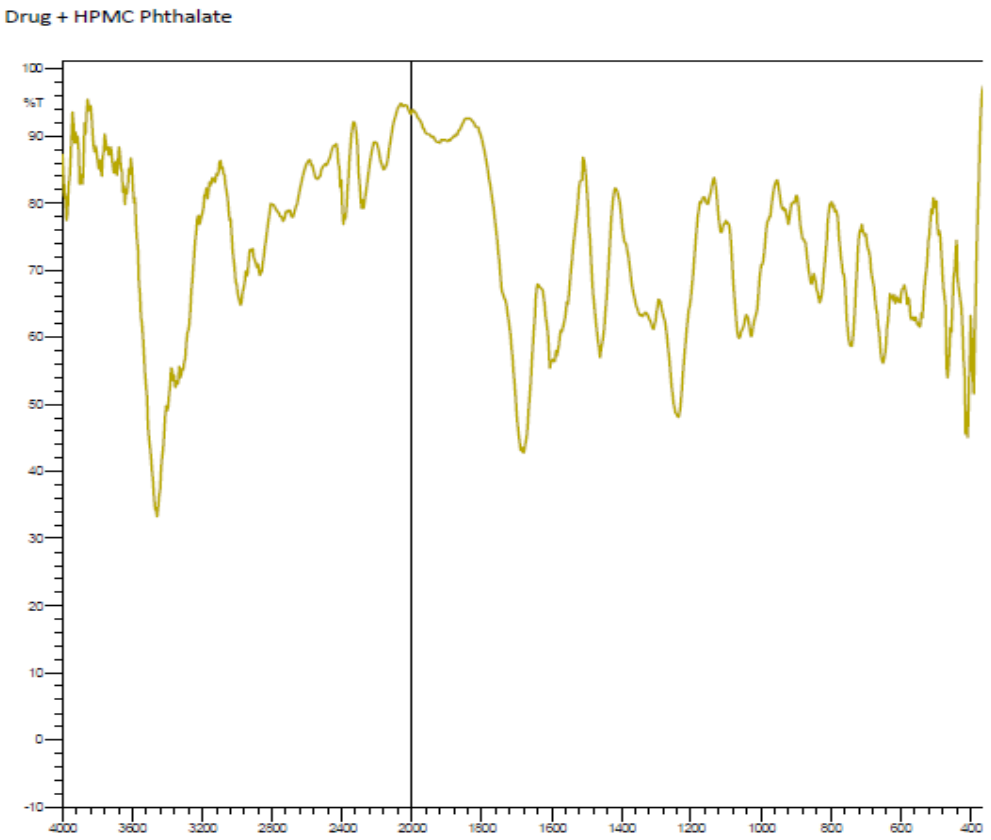

Figure 26: FTIR Graph of HPMC Phthalate + Drug.

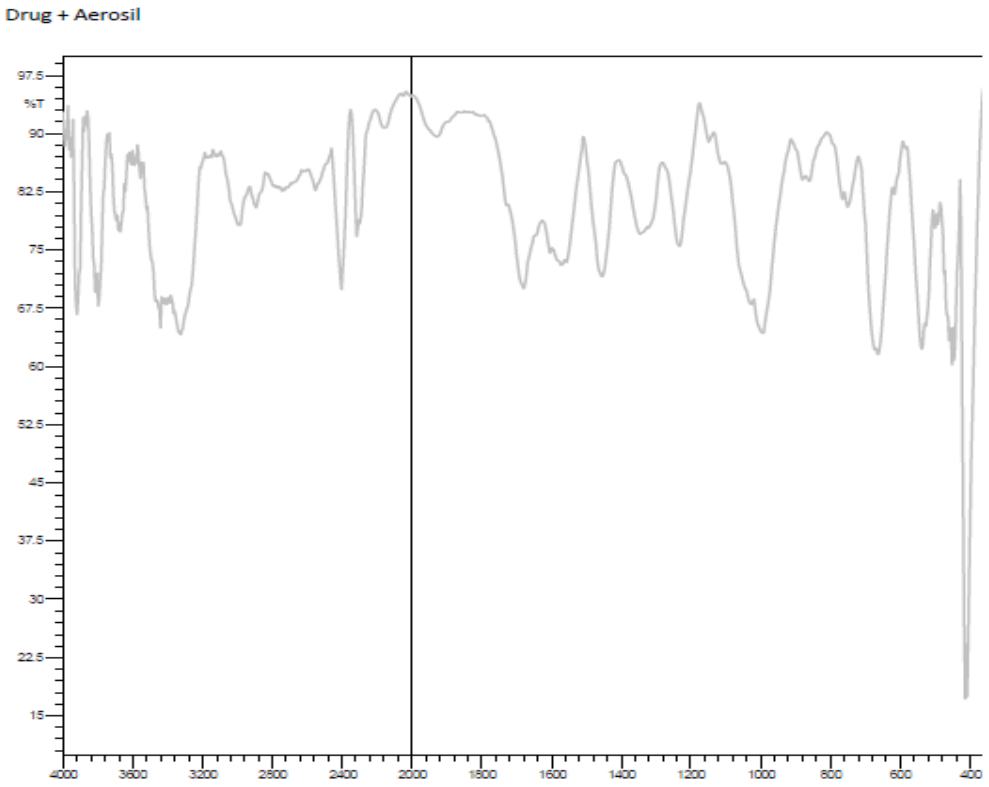

Figure 27: FTIR Graph of Aerosil + Drug.

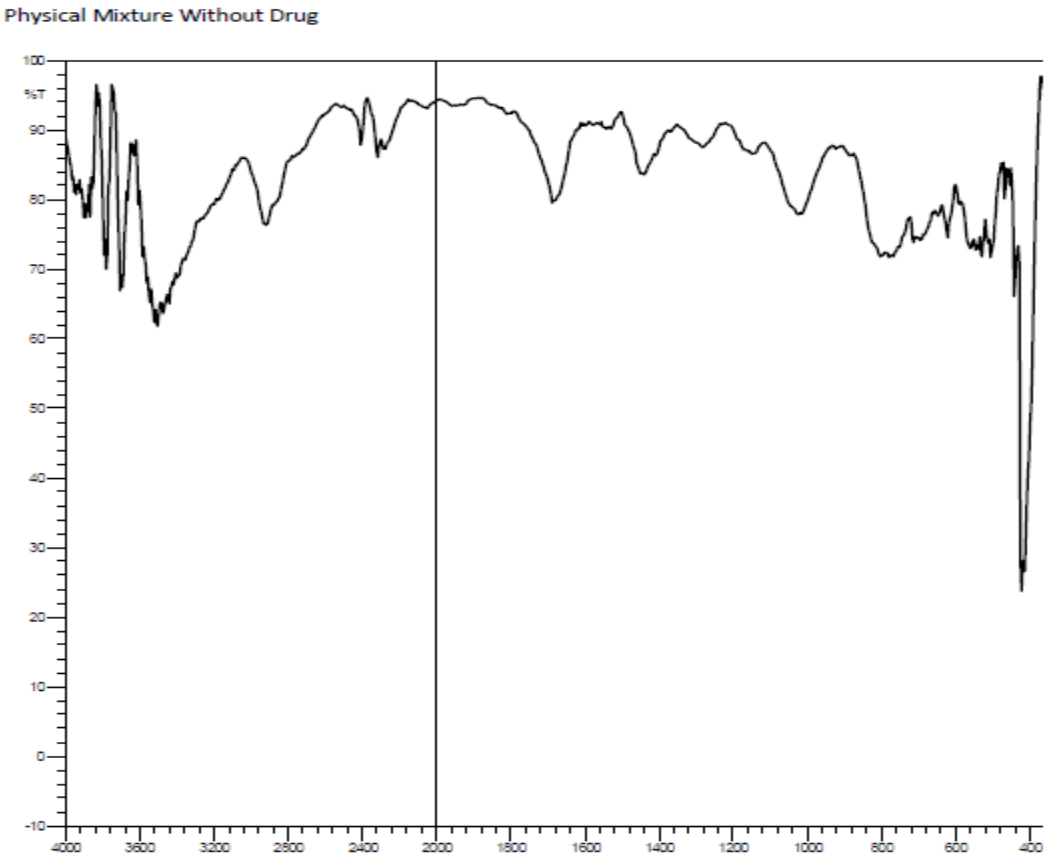

Figure 28: FTIR Graph of Physical mixture Without Drug.

SUMMARY

Doxycycline hyclate act by broad spectrum antibacterial agent belonging to chemical class of tetracycline derivative and use in treatment of various bacterial infection like UTI, respiratory track infection, malaria, acne vulgaris etc. The Preformulation studies of drug and excipients like identification test which include melting point, preparation of calibration curve by using pH 1.2 and pH 6.8 and measurement of λ max were performed. Tablet formulation were prepared by using various excipients and delayed release polymers and their evaluation test are performed. Nine formulation trial batches were prepared by using Minitab 18 3^2 full factorial design. In that three formulation level (High, Medium and Low) of delayed release polymer as a factor and dissolution and drug content as an response were taken. From all nine batches batch F3 were selected as an optimized batch because of %Cumulative drug release and drug content were found satisfactory.

CONCLUSION

In the present study Doxycycline hyclate delayed-release tablet were prepared using HPMC K100M and Ethyl cellulose as delayed release polymer. From the result obtained from nine trail batches it is concluded that prepared formulation shows optimum in-vitro dissolution at the end of 10 hours and % drug content. Formulation trial batches were prepared by using minitab 18 3^2 full factorial design. In that ratio of ethyl cellulose and HPMC K100M as delayed release polymer were selected as low, medium and high concentration and total nine formulation batches were obtained. From all nine batches (F1 to F9), batch F3 gives satisfactory results of % CDR(95.16%) and % drug content (101.25%) having the concentration of 21.63 mg and 64.88 mg of HPMC K100M and Ethyl cellulose respectively and hence batch F3 was selected as an optimized batch.

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