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"DEVELOPMENT AND OPTIMIZATION OF CLARITRHOMYCIN PEDIATRIC CHOCOLATE DOSAGE FORM"

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ABSTRACT
Clarithromycincn is an antibiotic used for skin and urinary tract infection. It is available in suspension and tablet dosage form but due to its bitter taste pediatric patient compliance is very poor.The objective of present study was to develop a chocolate formulation containing Clarithromycin for pediatric administration. The formulation consist of chocolate base containing cocoa butter, cocoa powder and mannitol. Clarithromycin is Biopharmaceutics classification system class II drug with low solubility and high permeability. Solubility of Clarithromycin was enhanced by using β-cyclodextrin. The prepared chocolate formulation was evaluated for appearance, blooming test, in vitro drug content, in vitro drug release, etc. Batch was optimized using 3^2 factorial design on the basis of hardness and drug dissolution. Fourier transfer infra-red and Differential scanning calorimetry study showed that the drug and excipients were compatible with each other's. No significant changes were observed in melting point and wave number. Phase solubility study showed 1:3 ratio of drug and β-cyclodextrin enhancement in solubility. The quantitative effect of dependent and independent variable at different level was determined by polynomial equation. Linearity was observed between actual and predicted values. Optimized batch showed 95% cumulative drug release in 70 min. Stability study of optimized batch was studied at 0-8°C and at 25°±5°C.It showed no changes in physical and chemical characteristic. Clarithromycin containing medicated chocolate was successfully formulated as effective pediatric dosage form with improved patient compliance.

KEYWORDS: Clarithromycin, Medicated chocolate, β-CD, 3^2 full factorial design.

INTRODUCTION
Clarithromycin is used in skin infection, urinary tact infection, etc. which is very common in pediatrics but it is very bitter in taste. Current marketed formulations are available in tablet (film coated) and suspension dosage form which has disadvantage like difficulty in swallowing and suspension need to maintain dosage consistency. This problem can be overcome by chewable tablet which leave no bitter or unpleasent taste upon disintegration. In order to increase patient acceptance and compliance chocolate formulation is prefered. Chocolate is highly sophisticated and infinitely versatile food that can be combined to create completely different taste and texture sensations.[1] Clarithromycin is a second generation semi-synthetic macrolide antibiotic derived from erythromycin A by 6-O-methylation.

MATERIALS AND METHODS
Chemical and reagents
Clarithromycin was gifted by Envee drug pvt. Limited, cocoa butter, cocoa powder, mannitol, magnesium stearate, talc were used as ingredients.

Instruments
UV spectrophotometer (Shimadzu- UV-1700 P.C. Tokyo, Japan), FTIR (Perkin Elmer, USA), DSC (Perkin Elmer instruments, Pyris-1 DSC, USA.), Brookfield viscometer (Brookfield LVDV- 11 pro, Germany), tablet dissolution tester, magnetic stirrer, microwave oven, weighing balance.

Calibration curve of Clarithromycin
Standard stock solution: 100 mg Clarithromycin was dissolved in 100 ml of 0.1N HCl to prepare stock solution. From stock solution 0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml were taken out to prepare different concentrations of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml respectively. The baseline was corrected and λ_{max} was determined by UV spectrophotometer. Same strength of Clarithromycin solution were also prepared in phosphate buffer (Ph6.8).

SOLUBILITY ENHANCEMENT BY INCLUSION COMPLEXATION METHOD
A mixture of Clarithromycin and β-cyclodextrin (β-CD)

(1:1, 1:2, 1:3 and 1:4 %w/w) was wetted with a mixture of acetone: water (1:1%v/v) and kneaded thoroughly for 30 minutes in glass mortar. The paste formed was dried under vaccum for 24 hours. Dried powder was scrapped, crushed, pulverized and passed through sieve no.100 and stored in desiccator for further studies.

PREPARATION OF FAST DISSOLVING GRANULES

Fast dissolving granules were prepared by wet granulation method.[2] Prepared batches were evaluated for bulk density, tapped density, angle of repose, Carr's index, Hausner's ratio and disintegration time. (**Table 1).**

Table 1: Preliminary batches of fast dissolving granules.

Batch	Drug (Clarithromycin) + β-CD (mg)	SSG (mg)	Syrup	Magnesium stearate (mg)	Talc (mg)
F1	375	5	q.s	30	60
F2	375	10	q.s	30	60
F3	375	15	q.s	30	60

PROCEDURE FOR MAKING MEDICATED CHOCOLATE

Chocolate base was melted in oven at 50°C till it becomes free flowing liquid. Required quantity of drug containing granules was added to it. Whole mass was stirred well with the help of magnetic stirrer to ensure uniform mixing. Mixture was poured in a polycarbonate set mould and refrigerated for 15 min till it becomes solid.[1]

Table 2: Full factorial design for optimization of medicated chocolate.

Independent Variables Levels			
X_1 = Amount of chocolate base (gm)	1.70	1.85	2.0
X_2 = Amount of SSG (mg)	10	12.50	15
Transformed values	-1	0	1
Dependent variables	Y_1 = %CDR (C)		
	Y_2 = Hardness		

Table 3: Presentation of 9 experimental runs with actual values using 3^2 Factorial Design.

BATCH No.	X1 (Amount of chocolate base)	X2 (Amount of SSG)
F1	1	1
F2	1	-1
F3	-1	-1
F4	- 1	0
F5	1	0
F6	0	0
F7	0	1
F8	0	-1
F9	-1	1

EVALUATION OF MEDICATED CHOCOLATE[1]

(1) Texture evaluation and consistency

Texture of the medicated chocolate in terms of stickiness and grittiness was evaluated by visual inspection.

(2) Hardness of chocolate

Chocolate crushing strength was measured with a Pfizer tablet hardness tester.

(3) Blooming test

a) Fat bloom

Fat bloom is caused by the recrystallization of the fats and/or a migration of a filling fat to the chocolate layer. Storage at a constant temperature was delayed the appearance of fat bloom.

b) Sugar bloom

This is a rough and irregular layer on top of the chocolate formulation. Sugar bloom is caused by condensation (when the chocolate was taken out of the refrigerator). This moisture dissolves the sugar in the chocolate. When the water evaporates afterwards, the sugar recrystallizes into rough, irregular crystals on the surface.

(4) Preservative Efficacy Test (PET) as per USP[3]

Culture of bacteria E coli (ATCC 4352), P aeruginosa (ATCC 9027), S aureus (ATCC 6538) and fungi C albicans (ATCC 10231), A Niger (ATCC 16404) were grown in Sabouraud Glucose Agar Media and Soya bean Casein Digest Agar Media for respective bacteria and fungi. The test microorganism cultures were diluted with sterile WFI to obtain 10^{-6} CFU/ml and transferred into 5 test tubes containing prepared medicated chocolate and 0.1% prepared cultures in each. Initial counts were noted. Numbers of colony of microorganism at 7^{th}, 14^{th} and 28^{th} day was recorded.

(5) Evaluation of an extent of taste masking[4]

Extent of taste masking was done by spectrophotometric determination of amount of drug dissolved.

(6) Determination of drug content in the medicated chocolate[3]

Drug content of a medicated chocolate was determined by UV Spectrometer. Medicated chocolate was taken in 25ml beaker. It was dissolved in 10ml of methanol and sonicated. Sample was centrifuged for 15min at 2500 rpm. Supernatant containing drug was filtered to remove any traces of chocolate. This sample was analysed by UV spectrophotometer against methanol as a blank.

(7) In vitro dissolution study

In vitro drug release study was carried out using USP test dissolution apparatus type- II rotating paddle at a speed of 50 rpm using 900 ml of 0.1 N HCl as dissolution

media at 37±0.5ºC.

(8) Stability Study

Optimized formulation was packed in aluminium foil and kept in wide mouth air tight container, kept in a stability chamber at specified temperature 25 ± 5°C and refrigerated at 0-8°C for one month. Chemical stability of the formulation was assessed by the estimation of %CDR in the formulation and physical stability was evaluated by monitoring any change in hardness, melting point and organoleptic property.

RESULTS AND DISCUSSION

SPECTROPHOTOMETRICS ESTIMATION OF CLARITHROMYCIN IN 0.1N HCl

Calibration curve was taken in 0.1N HCl at 253 nm. It obeyed Beer's law in the range of conc. 10-50 µg/ml. Linear regression of absorbance on concentration gave equation y= 0.0161x with a correlation coefficient of 0.983 in 0.1 N HCl.

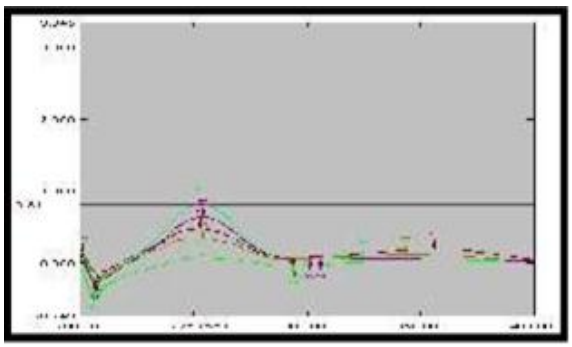

Figure 1: UV spectra of Clarithromycin in 0.1N HCl at λmax253 nm.

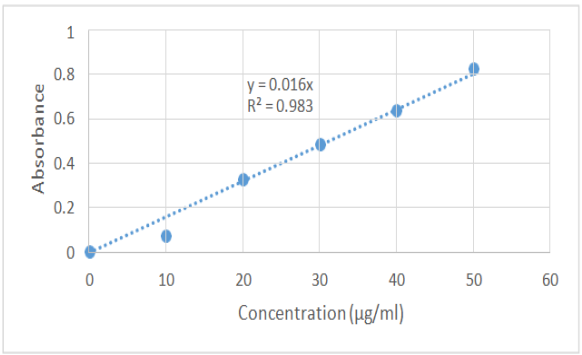

Figure 2: Calibration curve of Clarithromycin in 0.1 N HCl

SPECTROMETRIC ESTIMATION OF CLARITHROMYCIN IN PHOSPAHTE BUFFER (PH 6.8)

Calibration curve was taken in phosphate buffer (PH 6.8) at 264 nm. It obeyed Beer's law in the range of conc.10-50 µg/ml. Linear regression of absorbance on concentration gave equation y = 0.0161x with a correlation coefficient of 0.983 in phosphate buffer.

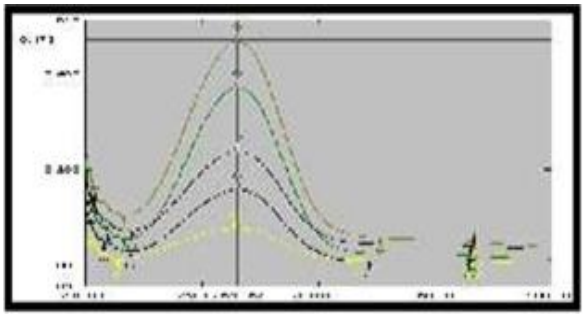

Figure 3: UV spectra of Clarithromycin in phosphate buffer (pH 6.8) at λmax 264nm.

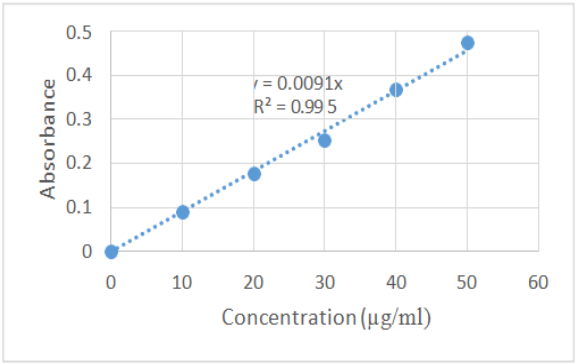

Figure: 4 Calibration cure of Clarithromycin in phosphate buffer (PH 6.8).

FTIR SPECTRA FOR CLARITHROMYCIN

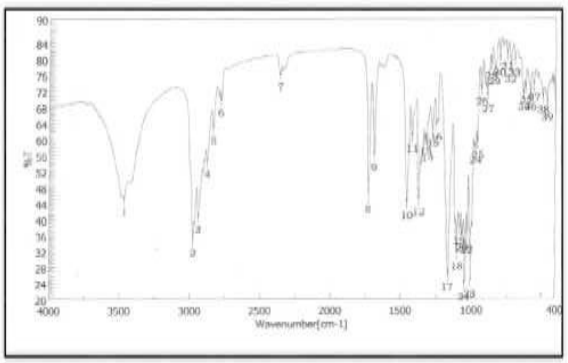

Figure: 5 FTIR study of Standard Clarithromycin.

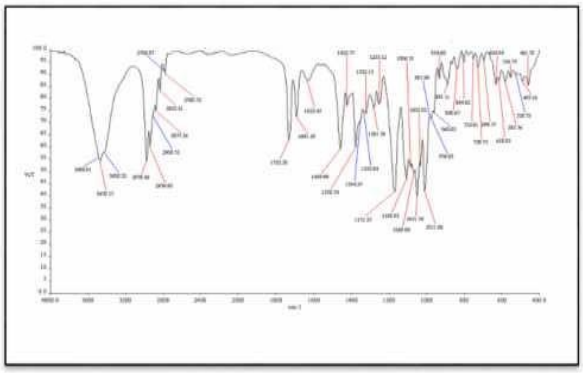

Figure: 6 FTIR study of test Clarithromycin.

Table 4: Identification of functional group of Clarithromycin.

Sr. No.	Functional group stretching	Wave number(cm^{-1})
1	-C=O stretching vibration from ketone group	1691
2	-O-C=O Stretching vibration in the lactone ring	1732
3	-O- ether functional bands	1051
4	-O- ether functional bands	1171
5	-O- ether functional bands	1107
6	Alkyl-CH3 substitution bands	2939

FTIR studies were carried out for test Clarithromycin and inclusion carriers. The results are summarized as follows and peak is given in **Table 7**. It can be considered as characteristic peak of drug. This indicated, that there was minute interaction between drug and carrier.

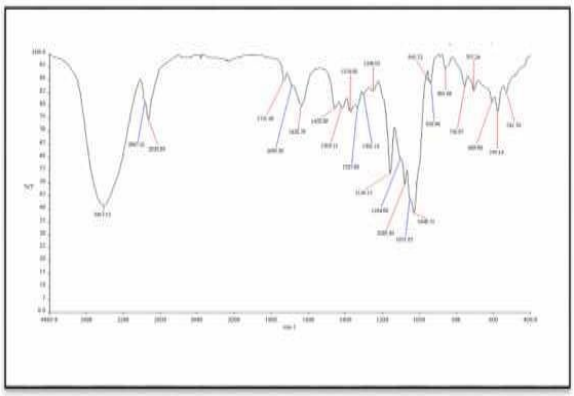

Figure: 7 FTIR study of final mixture.

The characteristic IR absorption peak of Clarithromycin for-C=O stretching was around 1691cm^{-1}, -O-C=O was seen at 1732cm^{-1}at present in test Clarithromycin and these was not shifted in the physical mixture of all excipients in the final formulation. So, it was confirmed that chemical modification of the drug had been taken place due to Complexation with β-CD. This indicated that there was significant difference between the internal structures and conformation of these samples at the molecular level due to formation of H-bond during complexation. Thus it was concluded that there was no chemical interaction between drug and excipients.

DSC SPECTRA OF CLARITHROMYCIN

Differential scanning Calorimetry study of test Clarithromycin

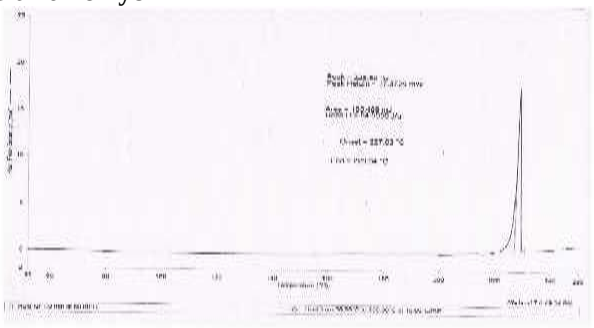

Figure 8: DSC spectra of test Clarithromycin.

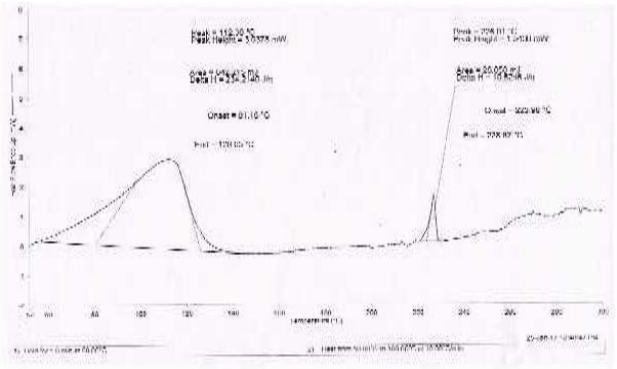

Figure 9: DSC spectra of final mixture.

DSC spectra for test Clarithromycin is shown in **Figure 8 and 9** which shows peak at 228°C which was near to ideal melting point of Clarithromycin. So from this it was concluded that there was no thermal changes observed after inclusion complexation process.

SOLUBILITY DETERMINATION OF CLARITHROMYCIN

Solubility studies were carried out according to Higuchi and Connors. The water solubility of Clarithromycin was inspected by preparation of its saturated solution. An excess amount of the drug was placed in separate 10 ml of water and the mixture was stirred for 48 hr. at 37˚C. After removing the insoluble substance by filtration, the absorbance of the filtrate was recorded at 253 nm for Clarithromycin in UV at 37°C and the concentration was obtain by the standard curve of drugs. The results proved that the water solubility of Clarithromycin was 0.085 mg/ml.

Phase solubility

Solubility of drug was obtained by using β-CD prepared in different molar ratio. The result showed that the solubility of Clarithromycin increased with the increase in the concentration of β- CD. The solubility plot of drug in various concentration of β cyclodextrin suggests that as concentration of β-cyclodextrin increased from1:1 to 1:3. 1:3 Drug: β-CD shows good enhancement of solubility of Clarithromycin and was found in distilled water. The result were shown in **Table 8**.

Table 5: Solubility value of Clarithromycin using different molar ratio of β-CD.

Ratio of β-CD	Solubility of Clarithromycin (mg/ml)
1:0	0.085
1:1	0.163
1:2	0.175
1:3	0.177
1:4	0.172

Table 6: Percentage yield of inclusion complex.

Ratio of β-CD	Theoretical yield	Practical yield	% yield
1:1	2	1.5	75.3
1:2	3	2.8	93.3
1:3	4	3.8	95.0
1:4	5	4.6	92.0

1:3 drug: β-CD shows highest percentage yield compare to other ratio of β-CD.

Table 7: Evaluation of the fast dissolving granules.

atch No.	Bulk density (gm/cm^3)	Tapped density (gm/cm^3)	Angle of repose (˚)	Carr's Index (%)	lausner's Ratio
F1	1.25	1.42	29.7	11.97	1.13
F2	0.76	0.90	26.6	15.5	1.18
F3	1.11	1.42	33.4	21.8	1.27

EVALUATION OF MEDICATED CHOCOLATE FORMULATION

Table 8: General appearance.

Sr. No.	Characteristic	Result
1	Colour	Brown
2	Odour	Pleasant

Colour of medicated chocolate is brown and its odour is pleasant which help in increasing patients compliance. Colour and odour plays an important role in increasing patients compliance. If odour of medicated chocolate is not good then it becomes difficult for pediatric patients to swallow the medicament.

Table 9: Physicochemical properties of Clarithromycin containing medicated chocolate.

Batch No.	Appearance	Shape	Thickness (mm)	Melting point(°C)
F1	Smooth	Poor	6.5	35
F2	Gritty	Poor	6.5	32
F3	Gritty	Good	7.5	28
F4	Smooth	Good	7.1	40
F5	Gritty	Good	6.5	26
F6	Smooth	Poor	6.5	32
F7	Smooth	Good	6.0	40
F8	Poor	Poor	7.5	28
F9	Smooth	Good	6.1	35

Studies concluded that batch F9 showed good appearance, good shape, proper thickness and proper melting point compared to other batches. Batch F1 shows good appearance and melting point but its thickness and shape were poor. Batch F2, F3 and F5 showed poor appearance and thickness compared to F9 batch. The various studied parameters results were shown in table.

EVALUATION OF BLOOM TEST

Table 10: Blooming test data of batches.

Sr. No.	Storage condition	Evaluated condition	Fat Blooming	Sugar Blooming
1	Refrigerated condition (2-8°C) for 24 hours	At room temperature	No blooming	No blooming
2	Room temperature (25°C) for 24 hours	At refrigerated condition	No blooming	No blooming

Storage at constant temperature delay the condensation of the chocolate and appearance of Sugar bloom. There occur formation of white layer on chocolate if storage at proper temperature is not done. In order to avoid this situation chocolate should be stored at proper temperature and under proper condition. Results showed that there was no fat blooming and sugar blooming observed in medicated chocolate.

EVALUATION OF BATCHES

Table 11: Evaluation of batches.

Batches	Friability (%)	Hardness (Kg/cm^2)	Average Weight variation (gm)	Disintegration time (sec)	Viscosity (cps)
F1	0.16±0.04	4.1	2.4±0.06	30	57269
F2	0.20±0.02	4.5	2.4±0.09	60	60152
F3	0.18±0.01	2.7	2.1±0.05	60	61295
F4	0.13±0.05	3.4	2.1±0.07	45	60289
F5	0.12±0.01	4.1	2.4±0.08	45	60152
F6	0.17±0.02	3.8	2.3±0.06	45	58569
F7	0.12±0.01	3.8	2.3±0.04	30	61296
F8	0.17±0.03	3.9	2.3±0.07	60	58569
F9	0.13±0.02	3.1	2.1±0.05	30	62245

Effect of different variables like amount of SSG, amount of chocolate base, mixing time, heating temperature over formulation were studied.F9 batch showed the optimum result for hardness and melting point.

PRESERVATIVE EFFICACY TESTING (PET)

PET was carried out to determine the efficiency of preservative to maintain stability of formulation. Data of table showed that the fungi Candida Albican and Aspergilles Niger shows inhibition in growth after 7, 14, 28 days from initial count. While bacterial count is shown in table. As per USP there should be 1 log reduction after 7 days, 3 log reduction after 14 days and no growth was observed compared to 14th days and 28 days. In case of fungi, as per USP there should be no growth/inhibition for PET. B* obeyed the similar pattern of reduction as per standard limit and complies with the result.

Table 12: Microbial count of fungi at specified time interval for PET.

Culture	Batch	Initial	7^{th} days	14^{th} days	28^{th} days
Candida albicans	B*	14×10^6	92×10^5	47×10^4	53×10^3
Aspergillus Niger	B*	8×10^6	27×10^5	38×10^4	13×10^3

Table 13: Microbial count of bacteria at specified time interval for PET.

Culture	Batch	Initial	7^{th} days	14^{th} days	28^{th} days
Escherichia Coli	B*	24×10^5	16×10^4	340	340
Pseudomonas Aeruginosa	B*	18×10^5	10×10^4	260	260
Staphylococcus aureus	B*	$21x\ 10^5$	$11x\ 10^4$	230	230

EVALUATION OF EXTEND OF TASTE MASKING

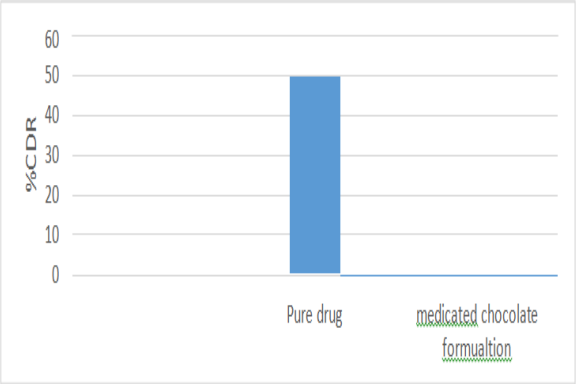

Figure: 10 Taste masking.

In present study taste masking of formulation was evaluated by spectrophotometric method. If the amount of drug dissolve less in medicated chocolate formulation compared to the pure drug then it can be said that the bitter taste of the drug had been masked. The result of the present study was shown in **Figure 10** which shows that the bitter taste of Clarithromycin was masked by chocolate formulation. The amount of drug dissolved in the original drug powder was considered as 50% while the chocolate formulation showed bitterness equal to 2.65.

DRUG CONTENT

Table 14: Evaluation Of Different Batches For Drug Content.

Batches	Drug content (%)
F1	84.04 ±0.02
F2	86.56 ±0.01
F3	91.83 ±0.01
F4	90.43 ±0.01
F5	88.63 ±0.00
F6	89.94 ±0.02
F7	90.52 ±0.01
F8	91.87 ±0.01
F9	99.43 ±0.01

Batch F9 shows the highest amount of drug content compare to other batches.

IN VITRO DISSOLUTION STUDY

Table 15: Dissolution profile of Clarithromycin.

Batches	% CDR of Clarithromycin
F1	84.62
F2	85.862
F3	87.476
F4	90.256
F5	86.051
F6	86.207
F7	87.546
F8	85.97
F9	95.614

The drug release profile of the Clarithromycin containing medicated chocolate formulations was shown in **Figure no.11** and the data shown in **Table no 17**%CDR was found to be up to 97%. Batch F9 showed highest amount % CDR compare to other batches. This showed that as the amount of SSG was increased the %CDR of drug also increased. Drug release was decreased with the increased in amount of chocolate base.

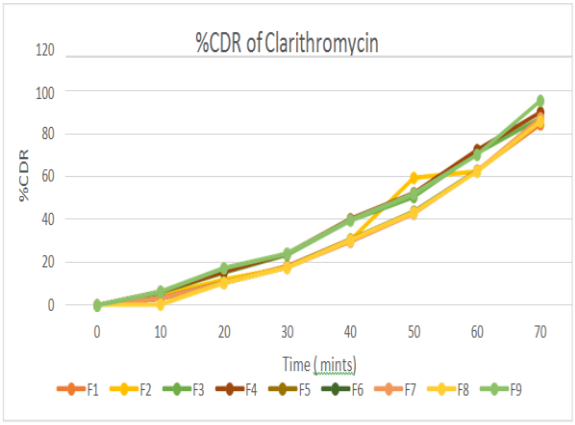

Figure 11. Dissolution profile of different batches of Clarithromycin.

OPTIMIZATION OF MEDICATED CHOCOLATE FORMULATION BY FACTORIAL DESIGN

To study all possible combination of all levels at all factors three level two factor full factorial design constructed and conducted in fully randomize order the dependent variables work %CDR of Clarithromycin and Hardness.

ANOVA test for %CDR of Clarithromycin

ANOVA test was performed to evaluate the level of significance of the tested factors on the %CDR of Clarithromycin as well as the interaction between the factors.

Table 16: Anova table for %CDR of Clarithromycin.

Source	Sum of Squares	Df	Mean Square	F Value	p-value
Model	80.9894	5	16.19856	14.42450	0.0262
X1-Amt of chocolate base	27.52044	1	27.52044	24.5066	0.0159
X2-Conc. of SSG	31.14485	1	31.14486	27.73399	0.0135
X1X2	9.0902344	1	9.090226	8.094705	0.0656
$X1^2$	12.0981	1	12.08683	10.76313	0.0465
$X2^2$	1.150145	1	1.150141	1.024183	0.3864
Residual	3.36965	3	1.122987		
Cor Total	84.3615	8			

Influence of concentration of SSG on %CDR of Clarithromycin

ANOVA results had shown that concentration of SSG had significant effect on %CDR of Clarithromycin ($P < 0.05$).

Influence of amount of chocolate base on %CDR of Clarithromycin

ANOVA results has shown that amount of chocolate base had significant effect on %CDR of Clarithromycin ($P < 0.05$).

Table 17: Full polynomial equation.

Coefficient	%CDR (Clarithromycin)
Intercept	85.81
X1 (Amount of chocolate base)	-2.15
X2 (Concentration of SSG)	+2.27
X1*X2	-1.52
$X1^2$	+2.47
$X2^2$	+0.77
R^2	0.97
Adjusted R^2	0.88
Press value	38.87

Equation proved that there was significant effect of X1 and X2 on %CDR of Clarithromycin. From the regression

analysis for %CDR of Clarithromycin shown in ANOVA table it was shown that X1, X2, X1X2 and $X1^2$ were significant. So, polynomial equation was reduced to the form shown in below.

$Y= -2.15X1 + 2.27X2 – 1.52X1X2 + 2.47X1^2$

Contour plot of % CDR of Clarithromycin

The best way to look at the output is to draw 2D contour plot. The contour plot of %CDR of Clarithromycin revealed non-linearity (curved lines). **Figure no.12.**

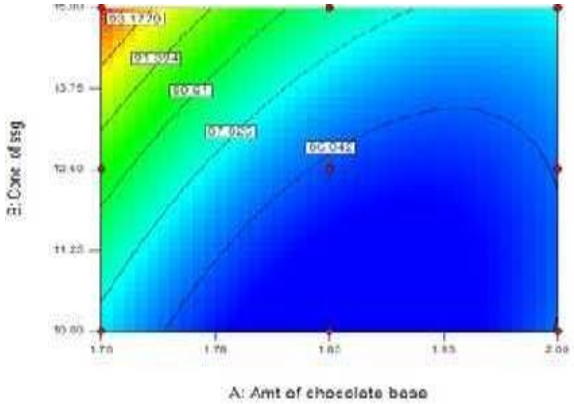

Figure 12 2D contour plot of %CDR of Clarithromycin.

3D surface plot shown in figure. The 3D surface plot shows that the relationship between X1 and X2 were non-linear in nature and highest response were seen with high(+1) level of concentration of SSG and amount of chocolate base and low response was shown with low level(-1) of concentration. Hence, it was concluded that with the change in concentration of SSG and amount of chocolate base has shown significant change in %CDR of Clarithromycin.

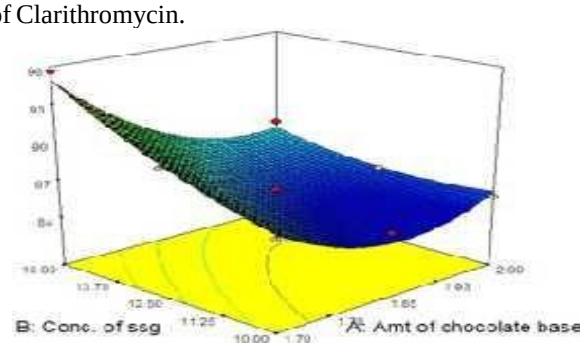

Figure 13: 3D Contour plot of % CDR of clarithromycin.

ANOVA TEST FOR HARDNESS

ANOVA test was carried to evaluate the level of significance of the tested factors on the hardness as well as the interaction between the factors.

Table 28: ANOVA table for Hardness.

Source	Sum of Squares	Df	Mean Square	F Value	P value
Model	2.877	5	0.576	12.042	0.0335
X1-Amt of chocolate base	2.533	1	2.534	53.057	0.0052
X2-Amount of SSG	0.007	1	0.007	0.138	0.7337
X1X2	0.24	1	0.24	5.231	0.1063
$X1^2$	0.004	1	0.004	0.101	0.7677
$X2^2$	0.07	1	0.07	1.673	0.2864
Residual	0.144	3	0.0478		
Cor Total	3.01	8			

Influence of concentration of SSG on Hardness

ANOVA results showed that amount of SSG had no significant effect on Hardness (P>0.05).

Influence of amount of chocolate base on Hardness

ANOVA results showed that amount of chocolate base had significant effect on Hardness (P < 0.05).

Table 19: Full polynomial equation.

Coefficient	Hardness
Intercept	+3.78
X1 (Amount of chocolate base)	+0.64
X2 (Amount of SSG)	+0.023
X1*X2	-0.24
$X1^2$	-0.051
$X2^2$	-0.21
R^2	0.94
Adjusted R^2	0.88
Press value	1.71

Above equation gives significant effect of X1 on Hardness. From the regression analysis for Hardness shown in ANOVA table. It was shown that X1 and X1X2 are significant. So, polynomial equation was reduced to the form shown in equation.

$Y= 0.64X1 – 0.24X1X2$

Contour plot of Hardness

The best way to look at the output is to draw 2D contour plot. The contour plot of Hardness revealed non-linearity. (curved lines). **Figure no. 14**.

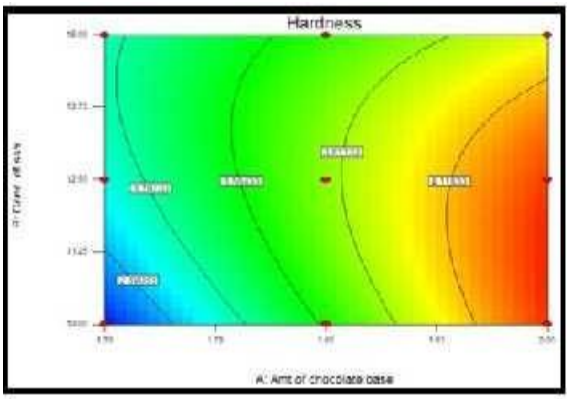

Figure 14: 2D contour plot of Hardness.

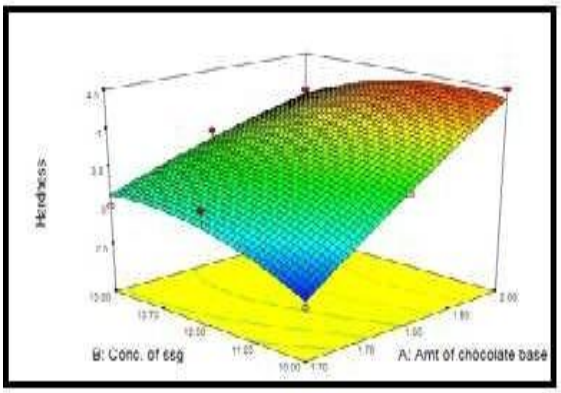

Figure 15: 3D contour plot of Hardness.

3D surface plot is shown in **figure no.15**. The 3D surface plot also showed that the relationship between X1 and X2 is non-linear in nature and highest response was seen with high(+1) level of amount of chocolate base and low response was shown with low level(-1) of concentration. Lowest response was seen with high (+1) level of amount of SSG. Hence, it was concluded that with the change in amount of chocolate base showed significant change in hardness.

Optimization of parameters and Validation of 3^2 full factorial design

After generating the polynomial equations relating the dependent and independent variables were optimized for the responses. The optimum values for the variables were obtained by graphical and numerical analysis using the Design-Expert software which was based on criterion of desirability. Percentage error was measured so as to find out the procedure. As shown in **Table 22** the observed value was found quite closer to the predicted value. Linearity was observed between actual and predicted values of response variable showed excepted ability of RSM as the percentage error was obtained less than 2%. So formulation was validated by experimental design.

Table 22: Predicted and observed values of response variables and percentage predicted error for the check point batch (validation of model).

Check point batches	X1 (Amount of chocolate base)	X2 (Amount of SSG)	Predicted (% CDR of Clarithromycin)	Actual (% CDR of Clarithromycin)	% Error
Batch 1	1.75	14.02	91.03	89.98	1.15
Batch 2	1.74	13.98	91.41	90.25	1.26
Batch 3	1.73	13.67	91.53	90.50	1.12

Check point batches	X1 (Amount of Chocolate base)	X2 (Amount of SSG)	Predicted Hardness (Kg/cm^2)	Actual Hardness (Kg/cm^2)	% Error
Batch 1	1.75	14.02	3.27	3.4	-0.4
Batch 2	1.74	13.98	3.27	3.4	-0.4
Batch 3	1.73	13.67	3.27	3.4	-0.4

SHORT TERM STABILITY STUDY OF OPTIMIZED BATCH (F9).

Short term stability study was done on the optimized for 1 month at room temperature and refrigerating temperature. The sample was evaluated for hardness, in vitro drug release, and melting point. Values obtained for parameters were found to be within ±5% of initial values. Stability study did not reveal any degradation of the Clarithromycin also no changes in release profile of the optimized formulation.

Table 21: stability study of optimized batch (F9).

Sr. No.	Stability Condition	Interval of testing (Day)	Hardness	%CDR of Clarithromycin	Appearance
1	Room temperature	Initial 15 days 30 days	3.4 3.3 3.4	97% 96.05% 96.11%	Acceptable Acceptable Acceptable
	Refrigerated	Initial	3.4	96.04%	Acceptable
2	condition	15 days	3.3	97.01%	Acceptable
	(2˚C-8˚C)	30 days	3.4	96.09%	Acceptable

CONCULSION

3^2 full factorial design showed that the formulation containing 1.70 gm of chocolate base and 15 mg of SSG was optimized batch. Solubility study with β-CD showed that the basic objective of solubility enhancement was achieved. As the formulation is cost effective it may show better marketed business in pharmaceutical industry. Hence, the newer approach for preparation of medicated chocolate formulation will be cost effective from industrial point of view and also it may show better marketing potential along with improved patient compliance.

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