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Formulation, evaluation and optimization of stomach specific *in situ* gel of clarithromycin and metronidazole benzoate

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Abstract

The present investigation deals with the formulation, optimization and evaluation of sodium alginate based *In situ* gel of Clarithromycin and Metronidazole Benzoate. Sodium alginate used as a polymer and $CaCO_3$ was used as a cross-linking agent. The *In situ* formulation exhibited well, viscosity, drug content and sustained drug release; this study reports that oral administration of aqueous solutions containing sodium alginate results in formation of *In situ* gel, such formulations are homogenous liquid when administered orally and become gel at the contact site. The results of a 3^2 full factorial design revealed that the concentration of sodium alginate and concentration of $CaCO_3$ significantly affected the dependent variables Q_1, Q_{12} and T_{80}. These *In situ* gels are, thus, suitable for oral sustained release of Clarithromycin and Metronidazole Benzoate.

Keywords: *In situ* gel; Stomach specific; Gastric residence time.

Introduction

Helicobacter pylori are also the first bacterium to be classified as a definite carcinogen by the World Health Organization's. H. pylori are the only known organism capable of colonizing the harsh environment of the human stomach. It is associated with the development of serious gastro duodenal disease—including peptic ulcers, gastric lymphoma and acute chronic gastritis. And also single antibiotic therapy is not effective for the eradication of *H. pylori* infection in vivo. This is because of the low concentration of the antibiotic reaching the bacteria under the mucosa, instability of the drug in the low pH of gastric fluid and short residence time of the antibiotic in the stomach. Triple therapy for treatment of *H. pylori* includes Proton pump inhibitor, Clarithromycin (500 mg), and Metronidazole (400 mg) or amoxicillin (1 g) twice a day. Metronidazole is broad spectrum of antiprotozoal and anti-bacterial activity. It absorbed completely and promptly after oral intake Clarithromycin is semisynthetic macrolide antibiotic derived from erythromycin .that is active against a variety of microorganisms. It is effective against Mycobacterium avium complex (MAC) and is used for the treatment of *H. pylori*-associated peptic ulcer disease One way to improve the efficacy in eradicating the infection is to deliver the antibiotic locally in the stomach. Better stability and longer residence time will allow more of the antibiotic to penetrate through the gastric mucus layer to act on *H. pylori*[1].

In situ gel forming drug delivery is a type of mucoadhesive drug delivery system. *In situ* gel forming drug delivery systems are a revolution in oral drug delivery. These hydrogels are liquid at room temperature but undergo gelation when in contact with body fluids or change in pH. These have a

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characteristic property of temperature dependant and cation induced gelation.

The main objectives are preparation and evaluation of *In situ* gelling system of Clarithromycin and Metronidazole Benzoate based on sodium alginate that retains in the stomach by adhere with gastric wall. Provides an increased gastric residence time resulting in prolonged drug delivery in gastrointestinal tract.

Experimental Methodology

a) Materials

Metronidazole benzoate (MTB) and Clarithromycin (CLR) were gifted by Lincoln Pharmaceuticals Ltd. (India). HPMC was gifted by K100M Shin-Etsu Chemical Corporation Ltd. Sodium alginate and Calcium carbonate was purchased from S. D. Fine Chemicals LTD. Mumbai, India. Xanthan gum, Aerosil, Sodium Methylparaben, Sodium Propylparaben and Sorbitol were purchased from Shital Chemicals Ltd.

b) Differential Scanning Calorimetry (DSC) studies

DSC study was carried out using DSC-60 instrument (M/s Shimadzu) to check the matrix formation as well as the compatibility of ingredients. DSC thermograms of pure drugs (CLR & MTB) and excipients were taken for their identical endothermic reaction. Further their physical mixtures of drugs and polymers were also studied for their interactions[2]**.** Finally, physical mixture of all above ingredients was scanned for DSC. DSC thermograms were shown in figure 1.

c) Preparation of *In situ* Gel

First of all, active material (CLR and MTB) were passed from 60# sieve while other inactive ingredients were passed form 40# sieve. In around 35% water, dissolve HPMC K 100M. Then add calcium carbonate and active material to it while stirring so that there was proper and homogenous dispersion of the drug. Take around 30% water in other beaker and heat to NMT 60°C on hot plate and to it dissolve sodium alginate. Then add Xanthan gum to dissolved sodium alginate and make it dissolve. Cool it to 40°C.Add step 1 to step 2 or vice-versa. Mix well. In around 5% water, dissolve sodium methyl paraben, sodium propyl paraben and sweetener and after cooling to 40°C add to above mixture of step 3 and mix well.

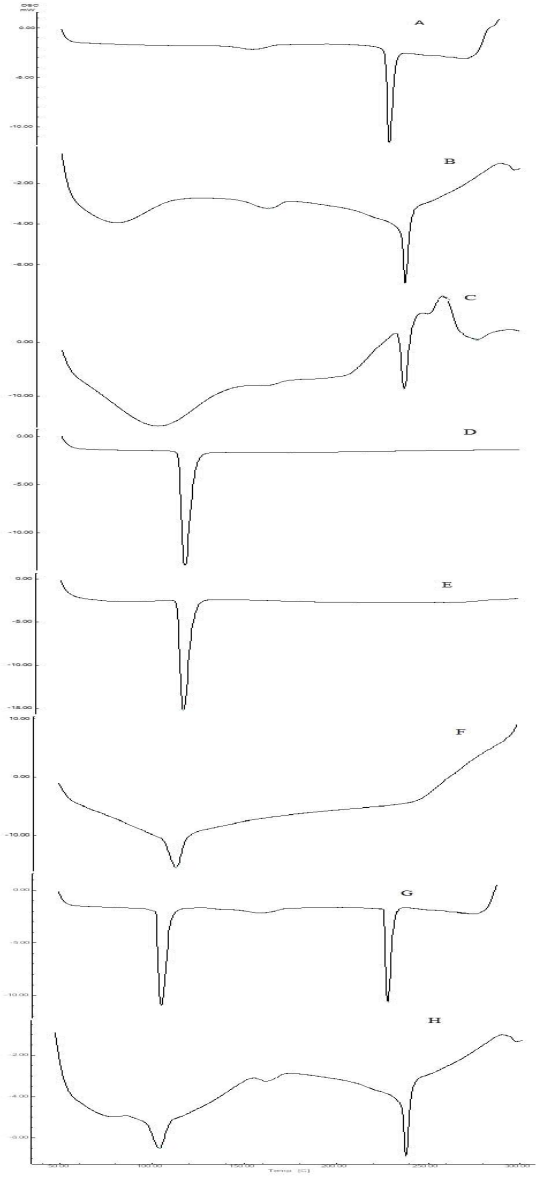

Figure 1. Results of Differential Scanning Calorimetry (DSC) Analysis Drug (CLR) (A), CLR + HPMC K100M (B), CLR+ **Sodium alginate (C), Metronidazole benzoate (MTB) (D), MTB+ HPMC K100M (E), MTB+ Sodium alginate (F), CLR+ MTB(G) and Drugs(CLR +MTB) + Polymers + $CaCO_3$ (H).**

In around 5% of water, make slurry of Aerosil and add to above mixture of and mix well. Add 10% Sorbitol with constant stirring to above mixture of

step 5. In around 0.6% water, dissolve color and filter this color solution through muslin cloth and add to above mixture and mix well. Dissolve menthol in flavor and add to above mixture and mix well. Make up volume to 100% with distilled water. Finally, stir well.

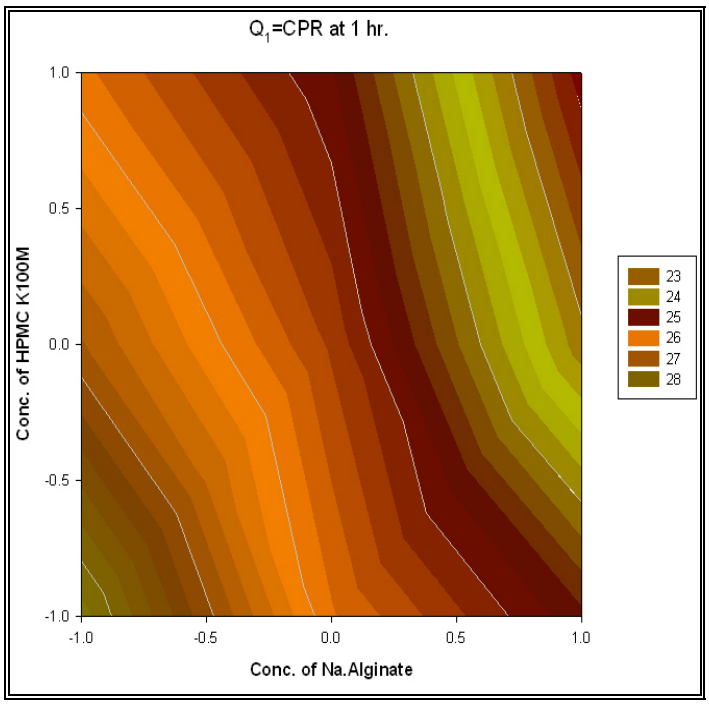

Figure 2: Contour plot showing effect of X_1 and X_2 on Q_1 for CLR

d) Optimization by using 3^2 full factorial designs

It is desirable to develop an acceptable pharmaceutical formulation in shortest possible time using minimum number of man-hours and raw materials. Traditionally pharmaceutical formulations are developed by changing one variable at a time approach. The method is time consuming in nature and requires a lot of imaginative efforts. Moreover, it may be difficult to develop an ideal formulation using this classical technique since the joint effects of independent variables are not considered. It is therefore very essential to understand the complexity of pharmaceutical formulations by using established statistical tools such as factorial design. In addition to the art of formulation, the technique of factorial design is an effective method of indicating the relative significance of a number of variables and their interactions.

The number of experiments required for these studies is dependent on the number of independent variables selected. The response (Y_i) is measured for each trial.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad \text{..... (1)}$$

Where Y is the dependent variable, b_0 is the arithmetic mean response of the nine runs and b_i is the estimated coefficient for the factor X_i. The main effects (X1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed[3].

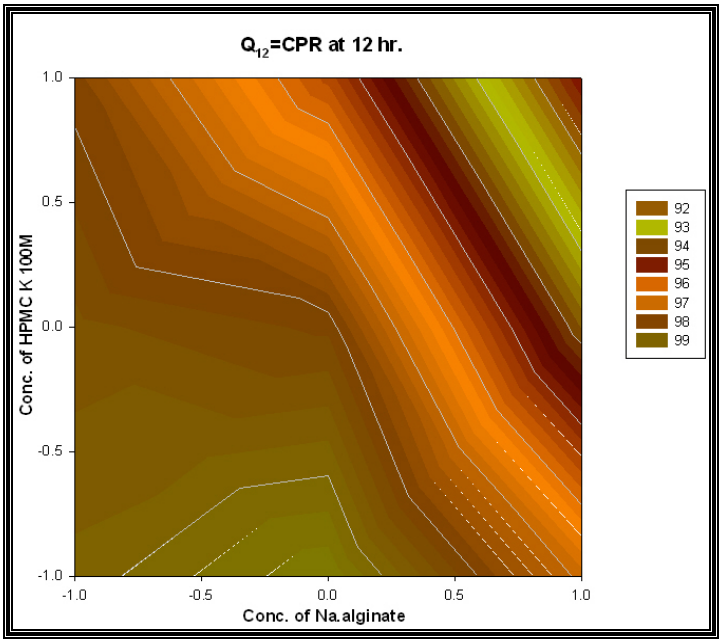

Figure 3: Contour plot showing effect of X_1 and X_2 on Q12 for CLR

A 3^2 randomized full factorial design was utilized in the present study. In this design two factors were evaluated, each at three levels, and experimental trials were carried out at all nine possible combinations. The design layout and coded value of independent factor is shown in Table 1. The factors were selected based on preliminary study. The concentration of Sodium alginate (X_1) and concentration of HPMC K-100M (X_2) were selected as independent variables**.**

The selected dependent variables are given below:

Y_1 = Cumulative percentage release (CPR) at 1 hr

Y_2 = Cumulative percentage release (CPR) at 12 hr

Y_3 = Time required for 80% of drug release ($T_{80\%}$)

e) Evaluations

1. pH measurement

The pH was measured in each of the solution of sodium alginate based *In situ* solutions, using a calibrated digital pH meter at 27°C. The measurements of pH of each data were in triplicate and the average values are given in Table 1.

Table 1: Optimization of SR suspension formulation using 3^2 full factorial designs

3^2 full factorial design for CLR

Formulation codes	Independent variable		Dependent variable		
	X_1	X_2	Q_1	Q_{12}	T_{80}
F1	-1	-1	28.3	98.87	8.6
F2	-1	0	26.82	98.46	9
F3	-1	+1	25.86	97.89	9.2
F4	0	-1	25.83	99.57	9.1
F5	0	0	25.36	98.15	9.3
F6	0	+1	24.82	95.52	9.6
F7	+1	-1	24.66	96.89	9.3
F8	+1	0	23.08	93.78	9.6
F9	+1	+1	22.29	91.2	9.8

3^2 full factorial design for MTB

Formulation codes	Independent variable		Dependent variable		
	X_1	X_2	Q_1	Q_{12}	T_{80}
F1	-1	-1	35.38	98.91	8.3
F2	-1	0	33.2	98.51	8.8
F3	-1	+1	32.33	97.91	9.1
F4	0	-1	32.29	99.62	9
F5	0	0	31.7	98.13	9.1
F6	0	+1	31.02	95.42	9.4
F7	+1	-1	30.82	96.6	9.2
F8	+1	0	28.85	93.5	9.4
F9	+1	+1	27.86	91.33	9.7

Translation of coded levels in actual units

Independent Variables	Real Value		
	Low (-1)	Medium (0)	High (+1)
Sodium alginate (X_1)	1.25 %	1.5 %	1.75 %
HPMC K100 M (X_2)	0.4 %	0.6 %	0.8 %

Independent Variables	Real Value		
	Low (-1)	Medium (0)	High (+1)
Sodium alginate (X_1)	1.25 %	1.5 %	1.75 %
HPMC K100 M (X_2)	0.4 %	0.6 %	0.8 %

2. Determination of viscosity

Viscosity of the samples was determined using a Brookfield digital viscometer (Model no LVDV 2P230) with spindle number 1. The sample temperature was controlled at 25±1°C before the each measurements. The viscosity of the solutions prepared in water was determined at ambient condition using 2 ml aliquot of the sample. Increasing the concentration of a dissolved or dispersed substance generally gives rise to increasing viscosity (*i.e*. thickening), and also as molecular weight of a solute increases viscosity increases[4].

3. Determination of drug content

Standard preparation

(A) For CLR

Weigh accurately about 50mg of CLR Reference Standard & transfer it to 25ml volumetric flask. Add about 10ml of methanol & sonicate to dissolve. Make up the volume with methanol. Take 10ml of this stock solution to a 50-ml volumetric flask, dilute with Mobile phase to volume, and mix. Pass a portion of this solution through a 0.5mvm or finer porosity, and use the filtrate as the standard preparation.

(B) For MTB

Weigh accurately about 64 mg of MTB Reference Standard & transfer it to 25ml volumetric flask. Add about 10ml of methanol & sonicate to dissolve. Make up the volume with methanol. Take 10ml of this stock solution to a 50-ml volumetric flask, dilute with Mobile phase to volume, and mix. Pass a portion of this solution through a 0.5mcm or finer porosity, and use the filtrate as the standard preparation.

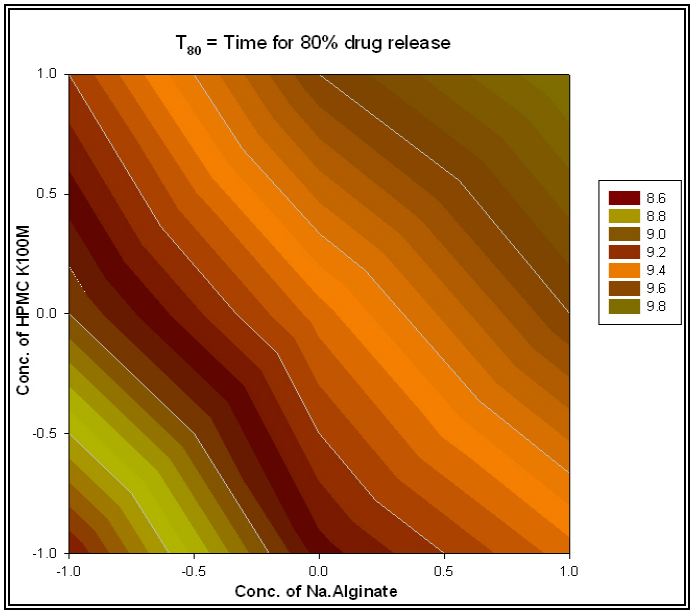

Figure 4: Contour plot showing effect of X_1 and X_2 on $T_{80\%}$ for CLR

Sample preparation

Transfer an accurately measured 40ml of the suspension, with the aid of about 330ml of 0.067 M dibasic potassium phosphate to a 1000-ml volumetric flask containing about 50ml of 0.067M dibasic potassium phosphate. Shake by mechanical means for 30minutes, dilute with methanol to volume, and mix. Sonicate for about 30 minutes, and allow cool. Dilute with methanol to volume, add a magnetic stirrer bar, and stir for 60 minutes, allow settle and transfer an accurately measured 10ml of the clear supernatant to a 50-ml volumetric flask, dilute with mobile phase to volume, mix and pass through a filter having a 0.5-mcm or finer porosity. Use the filtrate as the Sample solution.

4. *In-vitro* gelling capacity

To evaluate the formulations for their *in-vitro* gelling capacity by visual method, colored solutions of *in situ* gel forming drug delivery system were prepared. The *in-vitro* gelling capacity of prepared formulations was measured by placing five ml of the gelation solution (0.1N HCl, pH 1.2) in a 15 ml borosilicate glass test tube and maintained at 37±1ºC temperature. One ml of colored formulation solution was added with the help of pipette. The formulation was transferred in such a way that places the pipette at surface of fluid in test tube and formulation was slowly released from the pipette. As the solution comes in contact with gelation solution, it was immediately converted into stiff gel like structure. The gelling capacity of solution was evaluated on the basis of stiffness of formed gel and time period for which the formed gel remains as such. Color was added to give visualized appearance to formed gel. The *in-vitro* gelling capacity was graded in three categories on the basis of gelation time and time period for which the formed gel remains[5].

(+) Gels after few minutes, dispersed rapidly
(++) Gelation immediate remains for few hours
(+++) Gelation immediate remains for an extended period

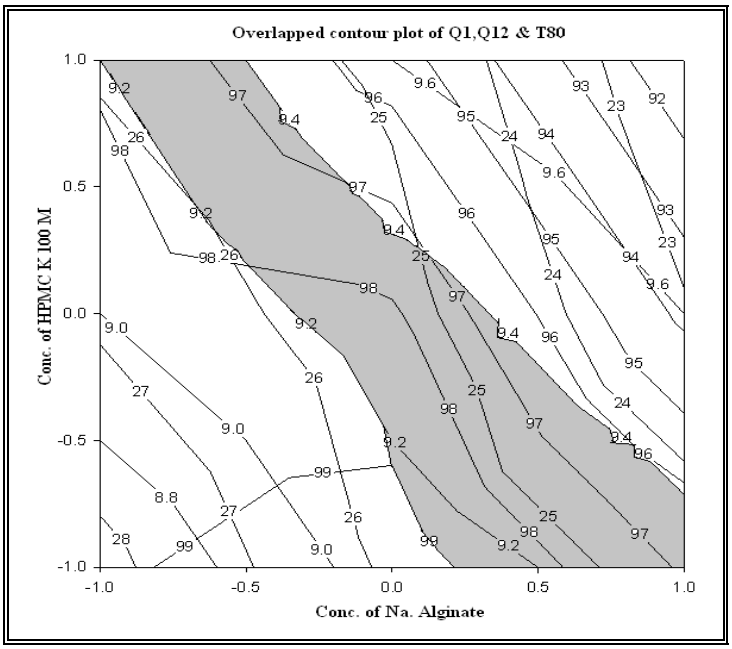

Figure 5: Overlapping spectra of Q_1, Q_{12} & $T_{80\%}$ for CLR

5. *In-vitro* floating ability

The *in-vitro* floating study was carried out using 0.1N HCl, (pH 1.2) .The medium temperature was kept at 37°C. Ten milliliter formulation was introduced into the dissolution vessel containing medium without much disturbance. The time the formulation took to emerge on the medium surface (floating lag time) and the time the formulation constantly floated on surface of the dissolution medium (duration of floating) were noted[6].

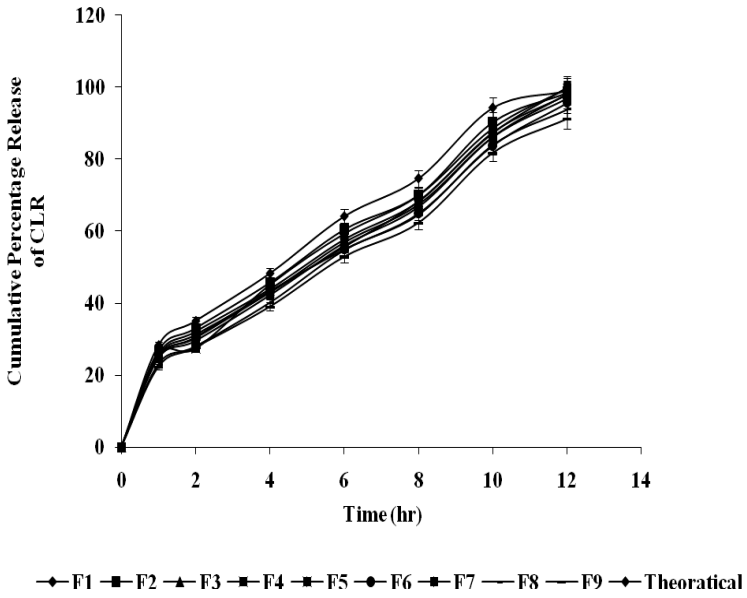

Figure 6: *In-vitro* release profile of CLR of Batches F1 to F9

6. *In-vitro* drug release study

The release rate of CLR & MTB from sustained release suspension was determined using USP XXIV dissolution testing apparatus I (basket covered with muslin cloth) at 50 rpm. This speed was slow enough to avoid the breaking of gelled formulation and was maintaining the mild agitation conditions believed to exist in vivo. The dissolution medium used was 900 ml of 0.1 N HCl, and temperature was maintained at 37°C. A sample (five ml) of the solution was withdrawn from the dissolution apparatus at 1,2,4,6,8,10 &12 hrs of dissolution. The samples were filtered through 0.45µ membrane filter and analyzed using HPLC method. Cumulative % of drug release was calculated & observations are shown in tables[7-9].

7. Accelerated stability study of Check Point batch

Clarithromycin & Metronidazole benzoate SR suspension were first packed in glass bottles (well stoppered) and then packing forms were kept for three months and the stability of the suspension was monitored up to 3 months at accelerated stability conditions (45 ºC temperature and 75 ± 5% RH). Periodically (initial, 1, 2 and 3 months interval) samples were removed and characterized by pH, viscosity, %assay, in-vitro gelling capacity, floating lag time, total floating time and *in-vitro* drug release study. The similarity factor (f_2) was applied to study the effect of storage on check point batch[10,11].

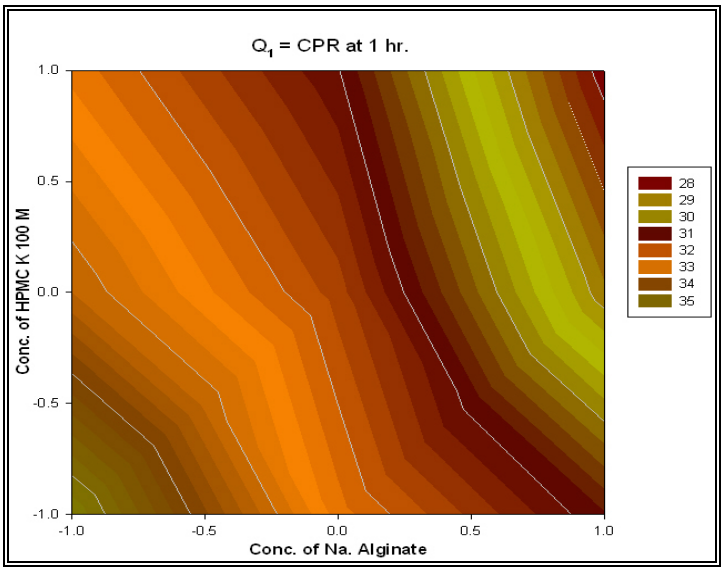

Figure 7: Contour plot showing effect of X_1 and X_2 on Q_1 for MTB

Result & discussion

1. DSC study

From the above DSC Study and physical observation we have concluded that there was no significant Drug- Excipient interaction was observed. From DSC study, we can show that there is no change in drug's melting peak. So we can conclude that drugs and other excipients are compatible which each other as per shown in Figure 1.

2. Optimization of CLR & MTB SR Suspension using 3^2 full factorial designs

2.1 For CLR

(A) Effect of formulation variable on CPR at 1 hr (Q_1)

Concerning Q_1, the results of multiple linear regression analysis showed that both the coefficients b_1 and b_2 bear a negative sign. The fitted equation relating the response Q_1 to the transformed factor is shown in following equation,

$$\mathbf{Q_1 = 25.198 - 1.825\ X_1 - 0.017X_2 + 0.0175\ X_1X_2 - 0.168\ X_1^2 + 0.206\ X_2^2}$$

$$\mathbf{(R^2 = 0.9730) \qquad \ldots\ldots\ldots (2)}$$

The Q1 for all batches F1 to F9 shows good correlation co-efficient of 0.9730. From table 2, Variable X_1 has p value 0.002743($p<0.05$) & variable X_2 has p value 0.0165($p<0.05$). Variables which have p value less than 0.05, significantly affect the release profile. It is possible that at higher polymers concentration, drug is trapped in smaller polymer cells and it is structured by its close proximity to the polymer molecules. So, increasing the amount of the polymer in the formulations increased the time it took for the drug to leave the formulation and retard release of drug into the medium.

Table 2: Summary output of Regression analysis of CLR for effect of X_1 & X_2 on Q_1, Q_{12} and $T_{80\%}$

		Q_1		Q_{12}		$T_{80\%}$	
Regression Statistics	**Multiple R**	0.986445		0.996427		0.995913	
	R^2	0.973074		0.992867		0.991842	
	Adjusted R^2	0.928197		0.980979		0.978246	
	Standard error	0.487563		0.376965		0.053576	
	Observations	9		9		9	
		Coefficient value	**P-value**	**Coefficient value**	**P-value**	**Coefficient value**	**P-value**
Coefficients	**b_0**	25.19889	6.61E-06	97.84	5.22E-08	9.355556	1.71E-07
	b_1	-1.825	0.002743	-2.225	0.000717	0.316667	0.00072
	b_2	-0.97	0.016513	-1.78667	0.001373	0.266667	0.00119
	b_{12}	0.0175	0.94729	-1.1775	0.008274	-0.025	0.41953
Equations		$Q_1 = 25.198 - 1.825(X_1) - 0.017(X_2) + 0.0175X_1X_2$		$Q_{12} = 97.84 - 2.225(X_1) - 1.786(X_2) - 1.177X_1X_2$		$T_{80\%} = 9.355 + 0.316(X_1) + 0.266(X_2) - 0.025X_1X_2$	

The relationship between formulation variables (X_1 and X_2) and Q_1 was further elucidated using contour plot. The effects of X_1 and X_2 on Q_1 are given in Figure 2. At highest levels of X_2, Q_1 decreased from 28.3% to 22.29% when X_1 was increased from -1 level to the +1 level.

Table 3: Summary output of Regression analysis of MTB for effect of X_1 & X_2 on Q_1, Q_{12} and $T_{80\%}$

		Q_1		Q_{12}		$T_{80\%}$	
Regression Statistics	**Multiple R**	0.985212		0.993711		0.98622	
	R^2	0.970643		0.987462		0.972629	
	Adjusted R^2	0.921716		0.966566		0.927011	
	Standard error	0.627682		0.504519		0.10844	
	Observations	9		9		9	
		Coefficient value	**P-value**	**Coefficient value**	**P-value**	**Coefficient value**	**P-value**
Coefficients	**b_0**	31.42556	7.27E-06	97.77778	1.25E-07	9.155556	0.00152
	b_1	-2.23	0.003194	-2.31667	0.001507	0.35	0.00422
	b_2	-1.21333	0.017858	-1.745	0.003452	0.283333	0.00773
	b_{12}	0.0225	0.947359	-1.0675	0.024145	-0.075	0.26055
Equations		$Q_1 = 31.425 - 2.23(X_1) - 1.213(X_2) + 0.0225X_1X_2$		$Q_{12} = 97.777 - 2.316(X_1) - 1.745(X_2) - 1.067X_1X_2$		$T_{80\%} = 9.155 + 0.35(X_1) 0.283(X_2) - 0.075X_1X_2$	

The amount of drug released at the end of 12 hrs is also important parameter for prominent drug release from sustained release matrix formulation. Concerning Q_{12}, the results of multiple linear regression analysis showed that, coefficients b_1 and b_2, as well as interaction term b_{12} bear a negative sign. The fitted equation relating the response Q_{20} to the transformed factor is shown in following equation,

$$\mathbf{Q_{12} = 97.84 - 2.225\ X_1 - 1.786\ X_2 - 1.177\ X_1X_2 - 1.565\ X_1^1 - 0.14\ X_2^2}$$

$$\mathbf{(R^2 = 0.9928)} \qquad \mathbf{\ldots\ldots. (3)}$$

The Q_{12} for all batches F1 to F9 shows good correlation co-efficient of 0.9928. From table 2, Variable X_1 has p value 0.0007 ($p<0.05$), variable X_2 has p value 0.0013 ($p<0.05$) & the interaction term b_{12} has p value 0.0082 ($p<0.05$). Variables which have p value less than 0.05, significantly affect the release profile.

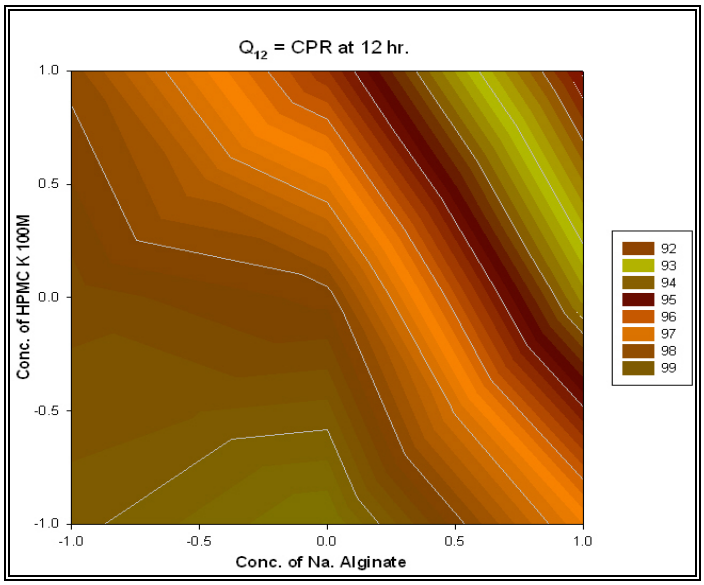

Figure 8: Contour plot showing effect of X_1 and X_2 on Q_{12} for MTB

(B) Effect of formulation variable on CPR at 12 hr (Q_{12})

The relationship between formulation variables (X_1 and X_2) and Q_{12} is further elucidated using contour plot. The effects of X_1 and X_2 on Q_{12} are given in Figure 3 at highest levels of X_2, Y_2 decreased from 98.87% to 91.2% when X_1 was increased from -1 level to the +1 level.

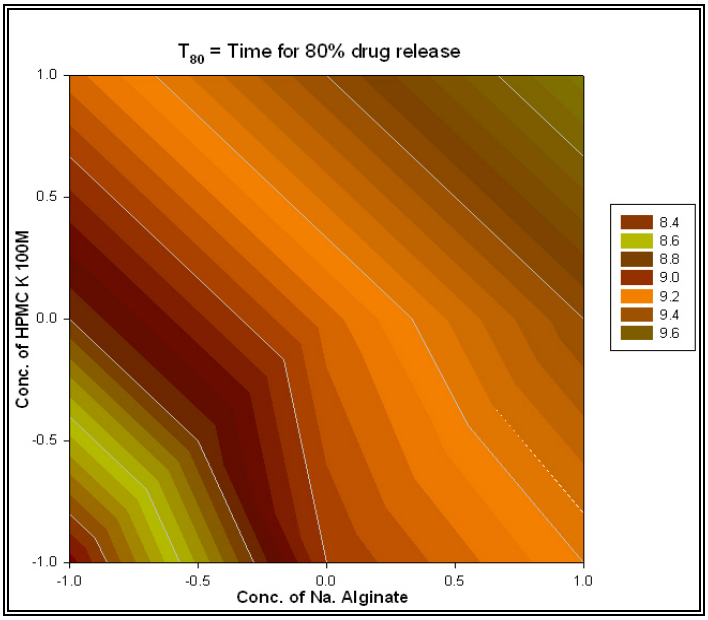

Figure 9: Contour plot showing effect of X_1 and X_2 on $T_{80\%}$ for MTB

(C) Effect of formulation variable on $T_{80\%}$

The time required for 80% of the drug release is an important parameter, for prominent drug release from sustain release matrix formulation.

In the case of $T_{80\%}$, Variable X_1 & X_2 are found to be significant based on its P-value ($p<0.05$), from Table 2. The results showed in Table 2 revels that, when the concentration of Sodium alginate (X_1) & concentration of HPMC K 100M (X_2) was increased, T_{80} was increased.

$$\mathbf{T_{80\%} = 9.355 + 0.316\ X_1 + 0.266\ X_2 - 0.025\ X_1X_2 - 0.083\ X_1^1 - 0.033\ X_2^2}$$

$$\mathbf{(R^2 = 0.9918)} \qquad \mathbf{\ldots\ldots. (4)}$$

The relationship between formulation variables (X_1 and X_2) and T_{80} was further elucidated using contour plot. The effects of X_1 and X_2 on T_{80} are given in Figure 4. At highest levels of X_2, T_{80} increased from 8.6 hr. to 9.8 hr. when X_1 was increased from -1 level to the +1 level.

2.2. For MTB

(A) Effect of formulation variable on CPR at 1 hr (Q_1)

Concerning Q_1, the results of multiple linear regression analysis showed that both the coefficients b_1 and b_2 bear a negative sign. The fitted equation relating the response Q_1 to the transformed factor is shown in following equation,

$$Q_1 = 31.425 - 2.23 X_1 - 1.213 X_2 + 0.022 X_1X_2 - 0.263 X_1^2 + 0.366 X_2^2$$

$$(R^2 = 0.9706) \quad \ldots\ldots\ldots (5)$$

The Q_1 for all batches F1 to F9 shows good correlation co-efficient of 0.9706. From table 3, Variable X_1 has p value 0.00319($p<0.05$) & variable X_2 has p value 0.0178($p<0.05$). Variables which have p value less than 0.05, significantly affect the release profile. This finding was probably due to the increased strength of the formed gel structure. It is worthwhile to remember that the drug diffusion is controlled by the penetration of liquid through the gel structure

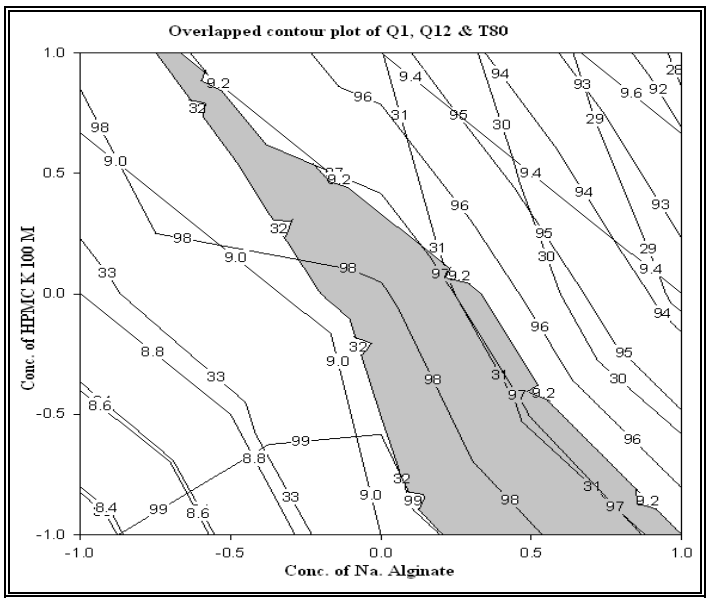

Figure 10: Overlapping spectra of Q_1, Q_{12} & $T_{80\%}$ for MTB

It is possible that at higher polymers concentration, drug is trapped in polymer cells and it is structured by its close proximity to the polymer molecules. So increasing the amount of the polymer in the formulations increased the time it took for the drug to leave the formulation and retard release of drug into the medium.

(B) Effect of formulation variable on CPR at 12 hr (Q_{12})

The relationship between formulation variables (X_1 and X_2) and Q_1 was further elucidated using contour plot. The effects of X_1 and X_2 on Q_1 are given in Figure 7. At highest levels of X_2, Q_1 decreased from 35.38% to 27.86% when X_1 was increased from -1 level to the +1 level.

The amount of drug released at the end of 12 hrs is also important parameter for prominent drug release from sustained release matrix formulation. Concerning Q_{12}, the results of multiple linear regression analysis showed that coefficients b_1 and b_2, as well as interaction term b_{12} bear a negative sign. The fitted equation relating the response Q_{12} to the transformed factor is shown in following equation,

$$Q_{12} = 97.77 - 2.316 X_1 - 1.745 X_2 - 1.067 X_1X_2 - 1.596 X_1^1 - 0.081 X_2^2$$

$$(R^2 = 0.9874) \quad \ldots\ldots. (6)$$

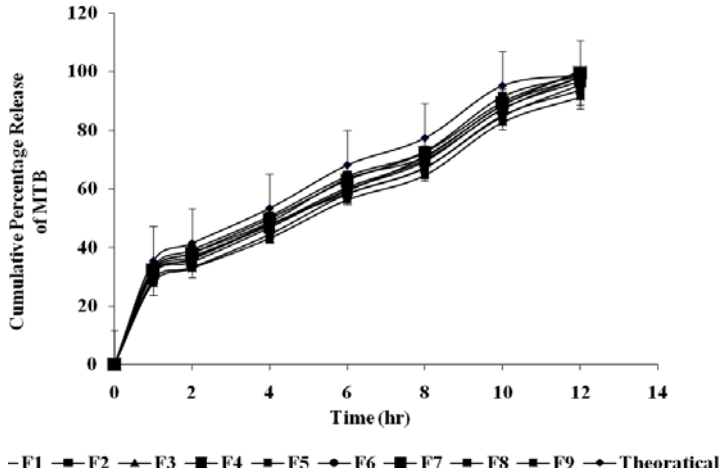

Figure 11: *In-vitro* release profile of MTB of Batches F1 to F9

The Q_{12} for all batches F1 to F9 shows good correlation co-efficient of 0.9874. From table 3, Variable X_1 has p value 0.0015 ($p<0.05$), variable X_2 has p value 0.0034 ($p<0.05$) & the interaction term b_{12} has p value 0.024 ($p<0.05$). Variables which have p value less than 0.05, significantly affect the release profile.

Table 4: Evaluation of Batches F1 to F9

Batch Code	Viscosity (cps)	pH	% Assay of CLR	% Assay MTB	Gelling in 0.1N HCL	Duration of floating (hours)
F1	148	7.2	97.89±0.63	97.74±0.63%	+	< 9
F2	151	7.2	98.03±0.42	98.01±0.31%	++	< 11
F3	154	7.3	98.01±0.15	97.87±0.13%	+++	> 12
F4	158	7.4	98.07±1.05	98.12±1.3%,	++	> 12
F5	162	7.6	99.52±0.42	101.43±0.41%	+++	> 12
F6	168	7.7	100.06±0.12	99.27±0.24%	+++	> 12
F7	176	7.8	99.85±0.21	100.14±0.11%	+++	> 12
F8	187	7.5	99.63±0.17	99.36±0.32%	+++	> 12
F9	194	7.8	98.84±0.63	98.85±0.62%	+++	> 12

Note: Spindle LV1, Speed: 10 RPM, Temperature: 25 ± 1°C, (+), gels after few minutes, dispersed rapidly; (++), gelation immediate, remains for few hours; (+++), gelation immediate, remains for an extended period

The relationship between formulation variables (X_1 and X_2) and Q_{12} is further elucidated using contour plot. The effects of X_1 and X_2 on Q_{12} are given in Figure 8. At highest levels of X_2, Y_2 decreased from 98.91% to 91.33% when X_1 was increased from -1 level to the +1 level.

Table 5: Evaluation of Accelerated Stability study of Check point batch

Evaluation parameters	Time period for sampling			
	Initial	1 month	2 month	3 month
pH	7.8	7.8	7.78	7.78
Viscosity (cps)	167	167	167	168
In-vitro gelling capacity	+++	+++	+++	+++
Floating lag time (min)	<1	<1	<1	<1
Total floating time (hr)	>12	>12	>12	>12
% assay (Clarithromycin)	99.27±0.23	99.21±0.14	99.23±0.25	99.2±0.41
%assay (Metronidazole benzoate)	99.56±0.21	99.54±0.19	99.53±0.31	99.51±0.36

(C) Effect of formulation variable on $T_{80\%}$

The time required for 80% of the drug release is an important parameter for prominent drug release from sustained release matrix formulation.

In the case of $T_{80\%}$, Variable X_1 & X_2 are found to be significant based on its P-value ($p<0.05$) (Table 3). The results showed in Table 1 revels that, when the concentration of Sodium alginate (X_1) &

concentration of HPMC K 100M (X_2) was increased, T_{80} was increased.

$$T_{80\%} = 9.155 + 0.35\ X_1 + 0.283\ X_2 - 0.075\ X_1X_2 - 0.083\ X_1^1 + 0.0167\ X_2^2$$

$$(R^2 = 0.9726) \qquad \ldots\ldots. (7)$$

The relationship between formulation variables (X_1 and X_2) and T_{80} was further elucidated using contour plot. The effects of X_1 and X_2 on T_{80} are given in Figure 9. At highest levels of X_2, T_{80} increased from 8.3 hr. to 9.7 hr. when X_1 was increased from -1 level to the +1 level.

3. Evaluation of 3^2 full factorial design formulation batches

3.1 Viscosity profile

The results of viscosity measurement of the formulations of batches F1 – F9 are shown in Table 4. The order of viscosity of all formulations was F9 > F8 > F7 > F6 > F5 > F4 > F3 > F2 > F1 respectively. The formulations showed a marked increase in viscosity with increasing concentration of sodium alginate and HPMC k100M.

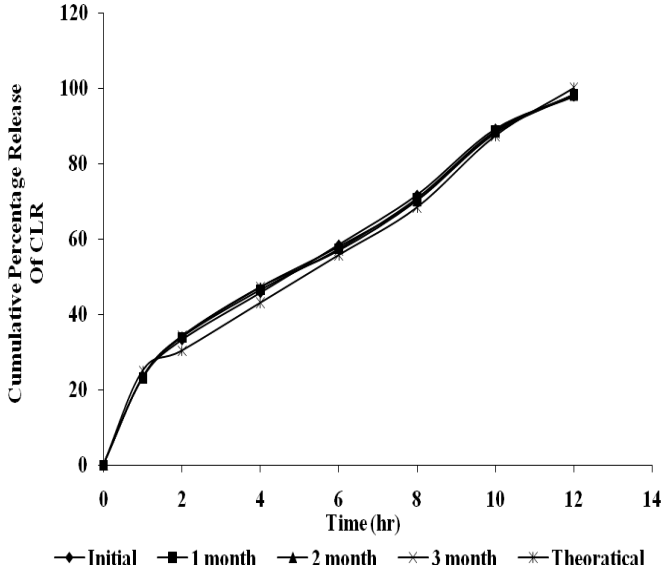

Figure 12: *In-vitro* release profile of CLR before and after stability

3.2 pH Measurement

The pH of all the formulations was observed in the range of 7.2 – 7.8 (Table 4). It is well documented that within this pH range Metronidazole benzoate as well as preservatives (Methyl paraben and Propyl paraben) retain their activity. Therefore, there was no need for pH adjustment by any external alkalizing agent.

3.3 Drug content

This is one of an important requirement for any type of dosage form. Amount of the drug present in the formulation should not deviate beyond certain specified limits from the labeled amount (Table 4).

3.4 *In-vitro* gelling capacity

The two main pre-requisites of in situ gelling systems are optimum viscosity and gelling capacity (speed and extent of gelation). The formulation should have an optimum viscosity that will allow easy swallowing as a liquid, which then undergoes a rapid sol–gel transition due to ionic interaction. Moreover, the *in situ* formed gel should preserve its integrity without dissolving or eroding for prolonged period to facilitate sustained release of drugs locally. Sol to gel transformation of sodium alginate occurs in the presence of either monovalent or divalent cations in contact with the gastric fluids. The calcium carbonate present in the formulation as insoluble dispersion is dissolved and releases carbon dioxide on reaction with acid, and the *in situ* released calcium ions results in formation of gel with floating characteristics. It is established that formulations containing calcium carbonate produce containing sodium bicarbonate. This is due to the internal ionotropic gelation effect of calcium on sodium alginate.

3.5 *In-vitro* floating ability

The time taken by the formulation to emerge on the medium surface (floating lag time) and the time for which the formulation constantly floated on the dissolution medium surface (duration of floating) are shown in Table 4.

The released carbon dioxide is entrapped in the gel network producing buoyant formulation and then calcium ion reacted with sodium alginate produced a cross-linked three-dimensional gel network and swelled structure of HPMC K100M that might restrict the further diffusion of carbon dioxide and drug

molecules and has resulted in extended period of floating and drug release respectively.

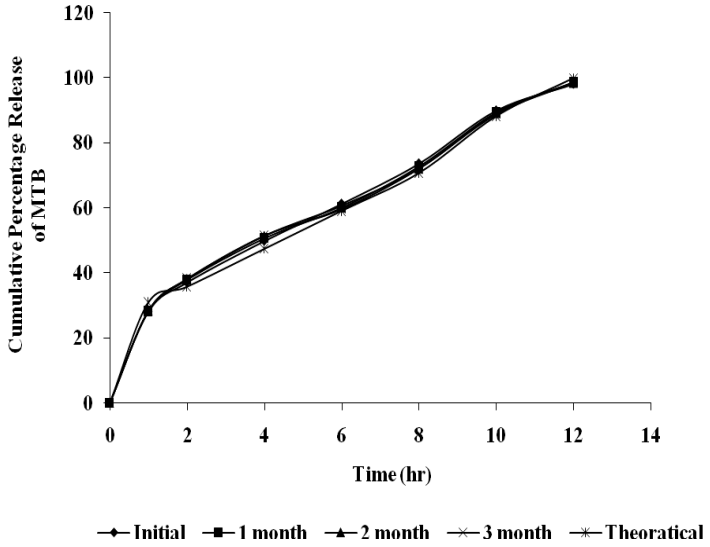

Figure 13: *In-vitro* release profile of MTB before & after stability

3.6 *In-vitro* drug release

The effect of polymer concentration on *in-vitro*drug release from *in situ* gels is shown in Figure 6 & Figure 11. A significant decrease in the rate and extent of drug release was observed with the increase in polymer concentration and is attributed to increase in the density of the polymer matrix and also an increase in the diffusional path length which the drug molecules have to traverse. The relsease of drug from these gels was characterized by an initial phase of high release (burst effect). However, as gelation proceeds, the remaining drug was released at a slower rate followed by a second phase of moderate release. This bi-phasic pattern of release is a characteristic feature of matrix diffusion kinetics. The initial burst effect was considerably reduced with increase in polymer concentration.

7. Stability study

Sample withdrawn at the interval of one month for three months showed no change in *in-vitro* drug release profile (Figure 12 & 13). Results of the stability study show no remarkable change in the release profile, assay and other evaluation parameters of the CLR & MTB SR suspension after the stability.

Conclusion

In formulation CLR & MTB SR suspension, a 3^2 full factorial design was employed for preparation of suspension possessing optimized characteristics (batches F_1 to F_9). The amount of Sodium alginate (X_1) and HPMC K-4M (X_2) were selected as independent variables. Cumulative % drug release selected as dependent variable (response; Y). Based on result of multiple linear regression analysis, it was concluded that both variables significantly affect the release profile at Q_1, Q_{12} and T_{80}. So role of polymer concentration is very important in this formulation. From DSC study, we can show that there is no change in drug's melting peak. So, we can conclude that drug and other excipient are compatible which each other. Stability study of Check point batch after three month showed no change in in-vitro drug release profile, % assay and other evaluation parameters. It was concluded that by adopting a systematic formulation approach, an optimum point could be reached in the shortest time with minimum efforts.

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