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 CaCO3 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 CaCO3 significantly affected the dependent variables Q1, Q12 and T80. 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.

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
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. 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 + CaCO3 (H).](image-url)
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.

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.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_1b_2X_1X_2$$

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 ($X_1$ 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.

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.

Figure 3: Contour plot showing effect of $X_1$ and $X_2$ on $Q_{12}$ 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\( _{80}\% \))

The pH was measured in each of the solution of sodium alginate based \textit{In situ} solutions, using a calibrated digital pH meter at 27\(^\circ\)C. The measurements of pH of each data were in triplicate and the average values are given in Table 1.

e) Evaluations

1. pH measurement

\[
\text{Table 1: Optimization of SR suspension formulation using } 3^2 \text{ full factorial designs}
\]

<table>
<thead>
<tr>
<th>Formulation codes</th>
<th>Independent variable</th>
<th>Dependent variable</th>
<th>Formulation codes</th>
<th>Independent variable</th>
<th>Dependent variable</th>
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<tr>
<td></td>
<td>X(_1)</td>
<td>X(_2)</td>
<td>Q(_1)</td>
<td>Q(_{12})</td>
<td>T(_{80})</td>
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<tr>
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<tr>
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<td>-1</td>
<td>24.66</td>
<td>96.89</td>
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<tr>
<td>F8</td>
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<td>0</td>
<td>23.08</td>
<td>93.78</td>
<td>9.6</td>
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<tr>
<td>F9</td>
<td>+1</td>
<td>+1</td>
<td>22.29</td>
<td>91.2</td>
<td>9.8</td>
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</tbody>
</table>

Translation of coded levels in actual units

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Low (-1)</th>
<th>Real Value</th>
<th>High (+1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium alginate (X(_1))</td>
<td>1.25 %</td>
<td>1.5 %</td>
<td>1.75 %</td>
</tr>
<tr>
<td>HPMC K100 M (X(_2))</td>
<td>0.4 %</td>
<td>0.6 %</td>
<td>0.8 %</td>
</tr>
</tbody>
</table>

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\(\pm\)1\(^\circ\)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 (\textit{i.e.} thickening), and also as molecular weight of a solute increases viscosity increases\(^4\).

3. Determination of drug content

\textbf{Standard preparation}

\textbf{(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.
A. 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 X1 and X2 on T80% 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.

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

Figure 5: Overlapping spectra of Q1, Q12 & T80% 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.

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. 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 °C 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 (f2) was applied to study the effect of storage on check point batch.

Figure 7: Contour plot showing effect of X1 and X2 on Q1 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 (Q1)

Concerning Q1, the results of multiple linear regression analysis showed that both the coefficients b1 and b2 bear a negative sign. The fitted equation relating the response Q1 to the transformed factor is shown in following equation,

\[ Q1 = 25.198 -1.825 X_1 -0.0175 X_1X_2 + 0.0175 X_1X_2^2 \]

\[ R^2 = 0.9730 \]  

The Q1 for all batches F1 to F9 shows good correlation co-efficient of 0.9730. From table 2, variable X1 has p value 0.002743(p<0.05) & variable X2 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 X1 & X2 on Q1, Q12 and T80%

<table>
<thead>
<tr>
<th></th>
<th>Q1</th>
<th>Q12</th>
<th>T80%</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Multiple R</td>
<td>0.986445</td>
<td>0.996427</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.973074</td>
<td>0.992867</td>
</tr>
<tr>
<td></td>
<td>Adjusted R²</td>
<td>0.928197</td>
<td>0.980979</td>
</tr>
<tr>
<td></td>
<td>Standard error</td>
<td>0.487563</td>
<td>0.376965</td>
</tr>
<tr>
<td></td>
<td>Observations</td>
<td>9</td>
<td>9</td>
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<tr>
<td>Coefficients</td>
<td>Coefficient value</td>
<td>P-value</td>
<td>Coefficient value</td>
</tr>
<tr>
<td>b₀</td>
<td>25.19889</td>
<td>6.61E-06</td>
<td>97.84</td>
</tr>
<tr>
<td>b₁</td>
<td>-1.825</td>
<td>0.002743</td>
<td>-2.225</td>
</tr>
<tr>
<td>b₂</td>
<td>-0.97</td>
<td>0.016513</td>
<td>-1.78667</td>
</tr>
<tr>
<td>b₁₂</td>
<td>0.0175</td>
<td>0.94729</td>
<td>-1.1775</td>
</tr>
<tr>
<td>Equations</td>
<td>Q₁ = 25.198 – 1.825(X₁) - 0.0175(X₁X₂) + 0.0175X₁X₂²</td>
<td>Q₁₂= 97.84 – 2.225(X₁) – 1.78667(X₂) – 1.177X₁X₂</td>
<td></td>
</tr>
</tbody>
</table>

The relationship between formulation variables (X₁ and X₂) and Q₁ was further elucidated using contour plot. The effects of X₁ and X₂ on Q₁ are given in Figure 2. At highest levels of X₂, Q₁ decreased from 28.3% to 22.29% when X₁ was increased from -1 level to the +1 level.

### Table 3: Summary output of Regression analysis of MTB for effect of X1 & X2 on Q1, Q12 and T80%

<table>
<thead>
<tr>
<th></th>
<th>Q1</th>
<th>Q12</th>
<th>T80%</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Multiple R</td>
<td>0.985212</td>
<td>0.993711</td>
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<tr>
<td></td>
<td>R²</td>
<td>0.970643</td>
<td>0.987462</td>
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<td></td>
<td>Adjusted R²</td>
<td>0.921716</td>
<td>0.966566</td>
</tr>
<tr>
<td></td>
<td>Standard error</td>
<td>0.627682</td>
<td>0.504519</td>
</tr>
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<tr>
<td>Coefficients</td>
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<td>P-value</td>
<td>Coefficient value</td>
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<td>b₀</td>
<td>31.42556</td>
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<td>97.77778</td>
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<tr>
<td>b₁</td>
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<td>0.003194</td>
<td>-2.31667</td>
</tr>
<tr>
<td>b₂</td>
<td>-1.21333</td>
<td>0.017858</td>
<td>-1.745</td>
</tr>
<tr>
<td>b₁₂</td>
<td>0.0225</td>
<td>0.947359</td>
<td>-1.0675</td>
</tr>
<tr>
<td>Equations</td>
<td>Q₁ = 31.425 – 2.23(X₁) - 1.21333(X₂) - 0.0225X₁X₂</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ Q₁ = 1.213(X₂) + 0.0225X₁X₂ \]
\[ Q₁₂= 1.745(X₂) – 1.067X₁X₂ \]
\[ T_{80\%}= 0.283(X₂) – 0.075X₁X₂ \]
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,

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

(R$^2$= 0.9928) 

…… (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.

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

(R$^2$= 0.9918) 

…… (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 (Q1)**

Concerning Q1, the results of multiple linear regression analysis showed that both the coefficients b1 and b2 bear a negative sign. The fitted equation relating the response Q1 to the transformed factor is shown in following equation,

\[ Q_1 = 31.425 -2.23 X_1 - 1.213 X_2 + 0.022 X_1X_2 - 0.263 X_1^2 + 0.366 X_2^2 \]

\[ (R^2 = 0.9706) \]

The Q1 for all batches F1 to F9 shows good correlation co-efficient of 0.9706. From table 3, Variable X1 has p value 0.00319 (p<0.05) & variable X2 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.

**B) Effect of formulation variable on CPR at 12 hr (Q12)**

The relationship between formulation variables (X1 and X2) and Q1 was further elucidated using contour plot. The effects of X1 and X2 on Q1 are given in Figure 7. At highest levels of X2, Q1 decreased from 35.38% to 27.86% when X1 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 Q12, the results of multiple linear regression analysis showed that coefficients b1 and b2, as well as interaction term b12 bear a negative sign. The fitted equation relating the response Q12 to the transformed factor is shown in following equation,

\[ Q_{12} = 97.77 - 2.316 X_1 - 1.745 X_2 - 1.067 X_1X_2 - 1.596 X_1^2 - 0.081 X_2^2 \]

\[ (R^2 = 0.9874) \]

The Q12 for all batches F1 to F9 shows good correlation co-efficient of 0.9874. From table 3, Variable X1 has p value 0.0015 (p<0.05), variable X2 has p value 0.0034 (p<0.05) & the interaction term b12 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

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

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

<table>
<thead>
<tr>
<th>Evaluation parameters</th>
<th>Time period for sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>pH</td>
<td>7.8</td>
</tr>
<tr>
<td>Viscosity (cps)</td>
<td>167</td>
</tr>
<tr>
<td>In-vitro gelling capacity</td>
<td>+++</td>
</tr>
<tr>
<td>Floating lag time (min)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Total floating time (hr)</td>
<td>&gt;12</td>
</tr>
<tr>
<td>% assay (Clarithromycin)</td>
<td>99.27±0.23</td>
</tr>
<tr>
<td>% assay (Metronidazole benzoate)</td>
<td>99.56±0.21</td>
</tr>
</tbody>
</table>

(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 reveals that, when the concentration of Sodium alginate \((X_1)\) &
concentration of HPMC K 100M ($X_2$) was increased, $T_{80}$ was increased.

$$\text{T}_{80\%} = 9.155 + 0.35 \times X_1 + 0.283 \times X_2 - 0.075 \times X_1 X_2 - 0.083 \times X_1^2 + 0.0167 \times X_2^2$$

($R^2 = 0.9726$) \hspace{1cm} ....... (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.

3.2 $\text{pH}$ 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 release 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 F1 to F9). 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 $Q_{11}, Q_{12}$ 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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References


