Quality by Design Validated Formulation Development and Optimization of Controlled Release Metronidazole Benzoate Gel for Local Periodontal

Keywords: Box-Behnken design, Periodontal pocket, Controlled drug delivery, Kinetic Model

ABSTRACT

The objective of this research was to develop and optimize a controlled release gel (CRG) system for metronidazole benzoate (MZB), poorly water soluble drug using methylcellulose (MC) and hydroxypropyl methyl cellulose (HPMC E15) as a polymer for the treatment of periodontal diseases. The composition was optimized using a Box-Behnken design and 15 formulations were prepared. Gels were characterized by mechanical testing, UV, FTIR, DSC, TEM, appearance, pH, viscosity, clarity, homogeneity, extrudability and antibacterial performance against Staphylococcus aureus (SA) and Bacillus subtilis (BS). In vitro release of the gel formulations was compared with marketed preparation of metronidazole (Metrogyl® gel) using modified Franz diffusion cell. The results indicate that the MZB gel sustained the release of drug. Kinetics of drug release from the gels was studied by fitting the data to six kinetic models. The results showed Korsmeyer-Peppas, Weibull and Higuchi to be the most appropriate models to describe the release kinetics of MZB from 15 gel formulations.
INTRODUCTION

Periodontitis consists of inflammation affecting the supporting tissues around the teeth. There is progressive bone loss around the teeth consequently leading to loosening and loss of teeth and formation of periodontal pocket (1). This happens due to pre-existing gingivitis (inflammation of gingiva), then severe inflammation of adjoining structures with destruction of alveolar bone. Periodontitis is mainly caused by microorganisms which are observed on teeth surfaces. Inflammation may be as a result of exaggerated immune response against this infection. It is caused due to presence of number of microorganisms, therefore, is of complex nature (2). The treatment of periodontitis begins with reduction of total microbes through surgical and non-surgical means consisting of mechanical scaling, root planning (3), and often accompanied by antibiotics (4). Therefore these treatments eliminate entire microbial flora by keeping prolonged drug–microbial contact (5, 6).

Metronidazole has been used for the treatment of infections for nearly 145 years and is still successfully used for the treatment of trichomoniasis, amoebiasis, and giardiasis. Anaerobic bacterial infections caused by bacteroids species, fusobacteria and clostridia respond favorably to metronidazole therapy (7). Metronidazole is a nitroimidazole derivative that has been synthesized in various laboratories throughout the world (8). It was introduced as an antiprotozoal agent, but it is also active against anaerobic bacteria (9). Metronidazole is chemically (2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol). Metronidazole Benzoate (MZB) is chemically (2-(2-methyl-5-nitro-imidazol-1-yl) ethyl benzoate) (10). MZB is indicated in the treatment of infections caused by a wide range of anaerobic bacteria, protozoa and bacteroides (11, 12). Metronidazole is often replaced by metronidazole benzoate because latter has bland taste due to the ester compared to the bitter taste of the former therefore MZB is preferred in pediatric oral preparations. On prolonged use metronidazole causes leucopenia, neutropenia, increased risk of peripheral neuropathy and/or CNS toxicity (13).

A variety of specialized local delivery (intrapocket) includes introduction of antibiotics into the pocket by means selection of a suitable dosage form from a number of modern dosage formulations which include mucoadhesive tablets(14), dental gels, fibers, injectable semisolid systems (15), irrigation devices (16), films (17,20), inserts (18) and microspheres (19), microemulsion gel (21). Thereby higher concentration of antibiotics can be achieved in gingival

crevicular fluid than that achieved by systemic use of the same (22,23) subsequently eradicating infectious microbes (24,25). These devices generally control the release of an active medicament at the periodontal pocket (26).

Hydrogels are polymeric substances forming networks of hydrophilic nature, capable of absorbing excessive amounts of water. They have such physicochemical characteristics, which make them suitable for contact with human tissue without causing any harm (27). These polymers have been extensively employed in periodontal drug delivery devices because of their abundance, non toxic nature and tissue compatibility (28). Antibiotics utilized as site-specific dental formulations against periodontitis have become a viable alternative to conventional medication (29). By entrapping MZB in the network of a gel increases patient compliance and sustaining drug action is obtained. Reducing frequency of dosing overcomes undesirable side effects of MZB.HPMC E15 is the dominant hydrophilic carrier material used for the preparation of oral controlled drug delivery systems. One of its most important characteristics is the high swellability, which has a significant effect on the release kinetics of a drug (30,31).

MC is a natural carbohydrate polymer containing a vast number of hydroxyl groups (31) due to which it is water soluble. It can be used as thickener in the food industry, as admixture for concrete in civil construction, due to its viscous nature in water it can also be employed in the recovery of heavy oils in petroleum industries and as a matrix for the controlled release of drugs in the pharmaceutical industry (33,34).

In this work Box-Behnken design was considered. It requires fewer experimental runs and less time and thus provides a far more efficient and cost-effective technique than the conventional processes of formulating and optimization of dosage forms. Box-Behnken design is an independent quadratic design where designed combinations are at the midpoints of edges of the designed space and at the center. This design is rotatable and requires 3 levels of each factor (35, 36).

The quadratic-model could be described by the following equation

\[ Y_0 = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \] (1)
MATERIALS AND METHODS

Materials

Metronidazole was procured from Lincon Pharmaceuticals, Ahmedabad, India. HPMC E15 and Methyl Cellulose were obtained from The Dow Chemical Company, Mid Land, USA. Distilled Water was prepared in our laboratory.

Experimental Methods

Preliminary Testing

MC below 3% w/w produced gels with high mobility and above 5% w/w highly viscous form which showed slow release of drugs. HPMC E15 (4% w/w) and water (60% w/ v) increase the drug release from the gels.

Preparation of Metronidazole gels:

MZ gels were prepared by dispersing HPMC E15, MC in water and stirring was continued for 5 minutes then the samples were left for overnight to hydrate, and then centrifuged for 15 minutes to remove bubbles, MZ was dispersed in the formula and was mixed till a homogeneous gel formulation was obtained after adding the vehicle to make up the desired weight. 15 gel formulations containing 750 mg MZ were prepared using the following excipients, HPMC E15, Methyl Cellulose (MC) and water.

The design involves 3 factors and 3 levels. Design Expert R (version 7.1.3, Stat-Ease Inc., Minneapolis, Minnesota) was used to construct second order polynomial equation and draw quadratic response surfaces. Both types of variables are listed in Table I. Observed and anticipated responses of F1-F15 batches are given in Table II.
Table I. Variants in Box-Behnken Design

<table>
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<tr>
<th>Independent Variables</th>
<th>Levels</th>
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<tr>
<td></td>
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<tr>
<td>X1 HPMC E15 (% W/W)</td>
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<td>5</td>
</tr>
<tr>
<td>X2 MC (% W/W)</td>
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<td>4</td>
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<td>X3 Water (% W/V)</td>
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<table>
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<td>Time for 50% drug release [T50%] (Y2)</td>
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<tr>
<td>Time for 90% drug release [T90%] (Y3)</td>
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Table II. Results of F1 – F15 as per Box-Behnken design

<table>
<thead>
<tr>
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<th>Dependent Variables</th>
<th>Zone of Inhibition (mm)</th>
<th>pH</th>
<th>Drug Content % w/w</th>
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<td></td>
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<td>X1 X2 X3</td>
<td>Y1</td>
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<tr>
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<td>2</td>
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<td>4.15</td>
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<td>1784</td>
<td>4.15</td>
<td>11.98</td>
<td>37.38</td>
</tr>
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</table>

Staphylococcus aureus (SA) and Bacillus subtilis (BS). Viscosity in cPs (Y1), Time for 50% drug release [T50%] (Y2), Time for 90% drug release (hours) [T90%] (Y3)

Evaluation of pH of MZB gel

100 mg gel was kept in a 50 mL volumetric flask and then made up to volume with water to 50 ml. The pH of all gel formulations were measured with a glass microelectrode (Mettler Instruments, Giessen, Germany).
Viscosity Measurement

Viscosity measurements of all batches were carried out at room temperature using Brookfield viscometer by using spindle number 3 at 60 revolutions per minute.

Extrudability and Spreadability

A suitably modified method was adopted to test extrudability and spreadability of gel formulation. For measurement of spreadability excess amount of gel was put between two glass slides and compressed it till equal thickness of gel between the slide, and this was done by using force for a period of time. The time in which upper glass side moves over to the lower plate was taken as spreadability (S).

\[ S = \frac{ML}{T} \]

Where M=Weight applied to upper slide, L=Length moved on the glass slide and T=Time taken

Test for In vitro diffusion of drug

Modified Franz Diffusion Cell was used for permeation study. Cellophane membrane was tied to one end of donor compartment. Gel was accurately weighed containing 1mg of drug and was taken in donor compartment. The receptor compartment contains 40 ml of phosphate buffer (pH 6.6) and temperature 37±1°C was maintained throughout the study. 5 ml aliquots were withdrawn periodically for 12 hrs and amount of drug was estimated by UV spectroscopy at 319 nm.

Antibiotic Assay

All 15 batches containing MZB were used to conduct antibiotic assays in an aseptic area. The formulated gels and pure MZB in solution form were placed in the cups of nutrient agar plates containing Staphylococcus aureus and Bacillus subtilis respectively and were placed in an incubator at 37°C for 18 hrs.
Estimation of drug in formulations

Formulations containing 250 mg of drug was taken in 10 ml volumetric flask, dissolved in pH 6.6 phosphate buffer made up the volume to 10 ml and filtered. Absorbance values were measured at respective $\lambda_{\text{max}}$ (319 nm) for drug. Concentrations of drug were calculated from the standard calibration curve prepared in pH 6.6 phosphate buffer.

Syringeability study

The study was performed on F1- F15 batches for their ease in Syringeability using a 22 gauge needle.

FT-IR Study

Study was performed on the powdered samples of MZB, and its mixtures by FT-IR spectrophotometer Shimadzu 8400S, Japan. (Scanning range was 4000 - 400 cm$^{-1}$).

DSC Study

The thermal property of the MZB formulation was investigated by DSC on a simultaneous thermal analyzer (Perkin Elmer Pyris1 DSC). Sample weighing between 6 and 10 mg were used. Sample was heated from room temperature from 45°C to 400°C at a rate of 10°C/min under nitrogen.

TEM Study

EM grid preparation:

Sample was gently shaken and when the froth settled was filtered using the filter supplied with the vials. All grids were prepared within a few hours of sample mixing. The samples were prepared by applying a drop of sample suspension to a cleaned 300 mesh copper grid, blotting away with filter paper and immediately transferred on grid holder for imaging on electron microscope.
EM imaging:

Electron microscopy was performed using a Philips Tecnai T20 electron microscope, operating at 200K KeV equipped with a keen view soft imaging system CCD camera. Images of each grid were acquired at multiple scales to assess the overall distribution of the specimen. After identifying potentially suitable target areas for imaging at lower magnification from 19,000x to 50,000x.

Kinetic data analysis: Drug release models

Drug release rate from hydrophilic matrix systems depends on swelling behavior of the polymer, shape of the matrices, and diffusion properties of the polymer and dissolution characteristics of the drug. Dose and solubility of the drug, type and quantity of the polymer characteristics influence the mechanism of the drug release (37). Data obtained from the drug release studies were fitted into zero order, first order, Higuchi and Korsmeyer–Peppas models as shown in Table III.

Table III. Kinetic model Equation

<table>
<thead>
<tr>
<th></th>
<th>Equation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Zero-order: ( F = k_0 \cdot t )</td>
<td>(-k_0, k_1, k_H = ) the released constant in the applied model</td>
</tr>
<tr>
<td>2.</td>
<td>First-order: ( F = 100 \cdot [1 - \exp(-k_1 \cdot t)] )</td>
<td>(-k_H = ) the release constant incorporating structural and geometrical characteristics of the drug-dosage form</td>
</tr>
<tr>
<td>3.</td>
<td>Higuchi: ( F = k_H \cdot t^{1/2} )</td>
<td>(-n = ) the diffusional exponent indicating the drug-release mechanism</td>
</tr>
<tr>
<td>4.</td>
<td>Korsmeyer-Peppas: ( F = k_{KP} \cdot t^n )</td>
<td>(-k_{KP} = ) the release constant in Hixon-Crowell model</td>
</tr>
<tr>
<td>5.</td>
<td>Hixon-Crowell: ( F = 100 \cdot [1 - (1 - k_{HC} \cdot t)^2] )</td>
<td>(k_{HC} = ) the combined constant, ( k_{HC} = \varphi(C_0 \times n_0) ), where ( k_0 ) is the erosion rate constant, ( C_0 ) is the initial concentration of drug in the matrix, ( n_0 ) is the initial radius for a sphere or cylinder or the half-thickness for a slab; ( n ) is 1, 2, and 3 for a slab, cylinder, and sphere, respectively</td>
</tr>
<tr>
<td>6.</td>
<td>Hopfenberg: ( F = 100 \cdot [1 - (1 - k_{HB} \cdot t)^3] )</td>
<td>(k_{HB} = ) the constant related to the Fickian kinetics</td>
</tr>
<tr>
<td>7.</td>
<td>Peppas-Sahlín-1: ( F = k_1 \cdot t^m + k_2 \cdot t^{(m)} )</td>
<td>(k_1 = ) the constant related to Case-II relaxation kinetics</td>
</tr>
<tr>
<td>8.</td>
<td>Peppas-Sahlín-2: ( F = k_1 \cdot t^{0.5} + k_2 \cdot t )</td>
<td>(m = ) the diffusional exponent for a device or any geometric shape which inhibits controlled release</td>
</tr>
<tr>
<td>9.</td>
<td>Weibull-1: ( F = 100 \cdot [1 - \exp(\frac{-((t - T_i)\alpha)}{\beta})] )</td>
<td>(T_i = ) the location parameter which represents the lag time before the onset of the dissolution or release process, ( T = ) the time scale of the process</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(\alpha = ) the scale parameter which defines the time scale of the process</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(\beta = ) the shape parameter which characterizes the curve</td>
</tr>
</tbody>
</table>
Optimization after analysis of response data

Polynomial equation was statistically validated using Design Expert R_ on the basis of ANOVA. Evaluations of models were done by calculating statistically significant coefficients and $R^2$ values. Then they were compared with that of predicted values. MS-Excel was utilized for providing linear regression plots between existent and anticipated values and from the optimized region of overlay plot checkpoint batch was prepared.

RESULTS AND DISCUSSION

Physical characterization of gels

Fig. 1. Appearance of Gel

Color: Transparent, Texture: Smooth- homogeneous as shown in (Fig. 1), Spreadability: 5.02–6.61 (g cm)/s(38,39).

Measurement of viscosity

The results of viscosity for F1- F15 formulations were in Table II. As the concentration of methyl cellulose increases, there is an increase in the viscosity of the gel and the relation between the viscosity of gel and percent concentration of HPMC E15 inversely proportional (40).

Drug content

The formulations F1- F15 showed the following drug content (Table II). (41).
In Vitro Release of MZB from gels

The cumulative drug content permeated from the membrane for all F1- F15 was calculated. In Vitro release profiles of MZB across the cellophane membrane from the gel system were investigated (Fig. 2). Y1, Y2 and Y3 from F1- F15 gel formulations were quantitatively calculated and are shown in Table II. The cumulative amount of drug permeated through the cellophane membrane from different gel formulations was calculated. Almost complete drug release (90–100%) was obtained within 8 hrs in comparison with the Metrogyl® (a marketed preparation) showed a burst release; approximately 39.21% release was observed within 1 hr. In contrast, F1- F15 gels released nearly 30-34% Thus, F1- F15 gels appears to be more suitable for obtaining a long-term release kinetic (Fig. 2)(42).

Fig 2. In Vitro diffusion profile of batches F1 to F15 and marketed Metrogyl®
The test of Syringeability

The results indicate that all formulations F1- F15 showed proper syringeability through 22 gauge needle.(43,44).

Antibiotic assay

The results of the antimicrobial studies of all the gel formulations against the *Staphylococcus aureus* and *B. Subtilis* indicate satisfactory zone of inhibition. While comparing the zone of inhibition of 0.4 microgram/ml MZB powder and equivalent concentration of 15 gel formulations, the results of zone of inhibition in Table VI indicate that the diffusion of drug from gel is almost 1.5 times higher than the MZB powder as shown in Table II(45).

FTIR Study

FT-IR spectral data shows that MZB is stable in gel formulation(FT-IR spectra in Fig.3).

![Fig. 3. FTIR spectra a)MZB, b)MZB Gel](image)

FTIR study was performed to evaluate compatibility between drug and polymer utilized in study. IR spectrum of MZB and physical mixture of drug and polymers are characterized by principal absorption peaks at 1480.01 cm\(^{-1}\) and 719.40 cm\(^{-1}\)(Aromatic ring), 1801.39 cm\(^{-1}\)(Ester is...
characterized by the strong absorption band of the carbonyl C=O stretching vibration), 1259.43 cm\(^{-1}\) (C–N stretch, secondary aromatic amine), 1184.21 cm\(^{-1}\) (Ester is characterized by the control band of the C-O-single-bond vibrations), 1271.00 cm\(^{-1}\), and 1072.35 cm\(^{-1}\) (Benzoate ester is characterized by the C-O-group bands), 972.06 cm\(^{-1}\) (C-N stretching vibrations), 902.82 cm\(^{-1}\) and 829.33 cm\(^{-1}\) (Aliphatic C-N vibrations), 3100 cm\(^{-1}\)-3200 cm\(^{-1}\) (OH stretching, hydrogen bonds), 1306 cm\(^{-1}\)-1400 cm\(^{-1}\) (are associated with intermolecular hydrogen bonds at the C group and the O—H in plane bending vibration, respectively) as shown in Figure 3. The interaction between the drug and the polymers often leads to identifiable changes in the FTIR profile of solid systems. The spectrum of physical mixture of drug and polymers was equivalent to the addition spectrum of pure drug, indicating no interaction occurring in the simple physical mixture of drug and polymer. (46,47).

**DSC**

DSC was carried out for determination of the probable chemical interaction of the polymer and drug. As shown in Fig.4 MZB, MC and HPMC E15 show melting points at 161.13\(^{0}\)C, 277\(^{0}\)C, 285\(^{0}\)C respectively. [53,54].

TEM

**Fig. 5. Transmission electron micrographs MZB hydrogel**

TEM as shown in Fig.5 shows typical spherical structures of physical mixtures of MC and HPMC E15 (size ranges between 100-500 nm) in swollen state in water. MZB is shown in insoluble form (solid particle) entrapped within these polymer coats. The drug release profiles showed a characteristic behavior in which there was an initial burst of drug followed by a slow, sustained release. In this study, the hydrogels were used in their prepared state, without drying. Therefore, it is likely that partitioning to the surface of the gel caused the observed initial burst in our system. We propose that the observed drug release profiles are explained by the consistency of the coats surrounding the drug particles and the concentration of HPMC E15 and MC as discussed later.

**Determination of mechanism of release from Diffusion exponent (n)**

The responses of all fifteen formulations were observed and fitted to a variety of models simultaneously namely, Zero-Order, First-Order, Higuchi, Hixon-Crowell, Korsmeyer-Peppas, and Weibull using DD Solver software. The selection of best fit was done by showing high correlation coefficient values, and least F values. All statistically significant (p < 0.05) coefficients are included in the equations. A positive value represents an effect that favors the optimization, while a negative value indicates an inverse relationship between the factor and the response. (48,49). The release kinetics data indicated that the release of drug from all batches of gel best fit to different models. Correlation coefficient (r²) of all batches is shown in Table IV. The value of diffusion exponent (n) for the batches F1- F15 between 0.5 > n < 1.0 indicated anomalous nonfickian diffusion of drug from gel. The kinetics of release is affected by the
viscosity of swollen polymers. This process is evident from the in vitro drug release data of gel that is, the release of MZB decreased with increase in MC concentration.

Table IV. Dissolution data showing release kinetics and mechanism

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<th>Batch code</th>
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Response Surface Analysis by showing Contour Plots

We performed regression analysis of variable X and variable Y with the help of Microsoft Excel. Equations (1), (2), and (3) show fitted results. Table V show regression analysis for X and Y.

Table V. Regression analysis for the responses Y1, Y2 and Y3

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<td>Y2</td>
<td>4.15</td>
<td>0.19875</td>
<td>0.4271</td>
<td>-0.17536</td>
<td>1.05</td>
<td>-0.037</td>
<td>0.429</td>
<td>0</td>
<td>0.18</td>
<td>-0.97</td>
<td>-0.77</td>
</tr>
<tr>
<td>p</td>
<td>7.08E-09</td>
<td>0.00183</td>
<td>0.00011</td>
<td>0.006797</td>
<td>0.000003</td>
<td>0.459</td>
<td>0.001</td>
<td>-</td>
<td>0.018</td>
<td>0.000009</td>
<td>2.85 E-05</td>
</tr>
<tr>
<td>Y3</td>
<td>11.98</td>
<td>0.5375</td>
<td>0.01</td>
<td>0.52</td>
<td>0.135</td>
<td>-0.54</td>
<td>-1.12</td>
<td>0</td>
<td>-0.94</td>
<td>-0.85</td>
<td>-0.95</td>
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<tr>
<td>p</td>
<td>9.66E-08</td>
<td>0.02065</td>
<td>0.9606</td>
<td>0.042802</td>
<td>0.57939</td>
<td>0.064</td>
<td>0.012</td>
<td>-</td>
<td>0.014</td>
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Use of Polynomial Equations for Response Analysis

Y1: Viscosity

Following polynomial equation (2) for Viscosity

\[ Y1 = 1784 - 139.5X1 + 416.7X2 - 675.21X3 - 2.5X1X2 - 4.5X1X3 - 3.92X2X3 + 1.96X11 - 4.46X22 - 3.46X33 \]  

(2)

Where Y1 is the Viscosity, X1 and X2 is the polymer concentration, and X3 is the amount of vehicle. The model F-value of 1915.70 implicates that the model is significant (p < 0.0001). X2 which is concentration of MC had a more pronounced effect on viscosity than X1 and X3. The predicted \( R^2 \) is 0.9997 is almost in agreement with the adjusted \( R^2 \) of 0.7991. Fig.6. (a, b) show the effect of different independent variables on viscosity (Y1).

We have observed here that by doubling the concentration of methyl cellulose in the system raised the viscosity of the system almost three times. The change in % of HPMC from 5 to 8% did not increase the viscosity (only to the extent of 17-27%)(50).

X1, X2, X3 were having significant effect on viscosity but no significant effect was observed in case of X12, X13, X23, X123, X1X1, X2X2, X3X3 on viscosity.

Y2: Effect on 50% drug release (hr)

Following polynomial equation (3) for \( t_{50} \)

\[ Y2 = 4.15 + 0.19X1 + 0.42X2 - 0.17X3 + 1.05X1X2 - 0.037X1X3 - 0.42X2X3 + 0.18X11 - 0.97X22 - 0.77X33 \]  

(3)

Where Y2 is the time for 50% drug release (hr). The model was found to be significant (F-value = 112.96; p < 0.0001). X1, that is, polymer concentration and X3 that is amount of vehicle had a more pronounced effect on 50% drug release than X1 and X2. The predicted \( R^2 \) 0.9951 and adjusted \( R^2 \) 0.7862 are in good agreement with each other. Fig.7(a, b) which show the effect of different independent variables on 50% drug release (Y2).

Changes in factor X1 (HPMC) and factor X2 (MC) influenced the drug release rate. The drug
release rate decreased with increasing proportion of MC in matrix. An increase in polymer proportion increases the viscosity of gel layer and also results in gel layer with longer diffusional path length resulting in greater retardation of drug release.

Siepmann and coworkers have found that the drug diffusion coefficients are strongly dependent on the water content of the system (51).

In our study, increase in proportion of water resulted faster drug release. This can be ascribed to entry of vehicle into the pores in the matrix. This results in the formation of channels through which the medium can enter the swollen polymer matrix and release the drug. We have observed in our study that as the concentration of HPMC increased (4-6%), T_{50} for drug release decreases (3.13-2 hrs.). When as an increase in concentration of MC (3-% - 5% w/w) brought changes in T50 (3.13- 5 hrs.)

All the parameters, namely, X1, X2, X3, X12, X13, X23, X123, X1X1, X2X2, X3X3 were having significant effect on 50% drug release.

**Y3: Effect on 90% drug release (hr)**

Following polynomial equation (4) for T_{90}

\[
Y_3 = 11.98 + 0.53X_1 - 0.01X_2 - 0.52X_3 - 0.13X_1X_2 - 0.54X_1X_3 - 1.12X_2X_3 - 0.94X_1^2 - 0.85X_2^2 - 0.95X_3^3
\]  

(4)

Where Y3 is the time for 90% drug release, X1 and X2 show the polymer concentration and X3 amount of water. Among the independent factors, polymer concentration showed higher positive effect on the 90% drug release which is evident from the positive value for its coefficient. The negative effect of water is found to be more than that of HPMC E15. The interaction between the independent factors is also found to be significant. The model is significant (F-value = 9.444; p <0.022). Values for predicted (0.9444) and adjusted (0.6444) R-squared values are in reasonable agreement. Fig.8 (a, b) which show the effect of different independent variables on 90% drug release (Y3).

As per the results T_{90} (Y3) of drug increased with increase in X2 concentration of MC. Also diffusion study revealed that by increasing concentration of HPMC and MC amount of drug
release was lowered. This indicates the role of structure of gel forming a barrier for the release along with the increase in viscosity of the system. (51,52).

X1, X3, X23, X1X1, X2X2, and X3X3 were having significant effect on 90% drug release. Whereas X2, X12, X13, and X123 were insignificant on 90% drug release.

Figure 6. (a) Contour plot showing effect of Viscosity of variables [HPMC (X1), MC (X2) and Water (X3)] (b) the corresponding Response surface plot

Figure 7. (a) Contour plot showing effect of T50% of variables [HPMC (X1), MC (X2) and Water (X3)] (b) the corresponding Response surface plot
Figure 8. (a) Contour plot showing effect of T90% of variables [HPMC (X1), MC (X2) and Water (X3)] (b) the corresponding Response surface plot

Data analysis and Optimization

Values for the response Y1, Y2, and Y3 were found to be in the range Y1 960 ≤ Y1 ≥ 2600 cP, Y2 2 ≤ Y2 ≥ 6 Hrs. and Y3 8 ≤ Y3 ≥ 12 Hrs. Point prediction of the design expert software was used to determine the composition (F1-F15). The R² value for response value for response Y1, Y2 and Y3 was observed to be in the range 0.9997, 0.9951 and 0.9444, respectively. It indicates the excellent goodness of fit as p < 0.0001. Thus the low magnitudes of error as well as the significant values of R² in the present investigation prove high predictive ability of the RSM. From this analysis the formulation (F1- F15) were subjected to in vitro release studies. From the cumulative percentage release graph (Fig. 2), it is observed that drug is released in controlled manner till 12 hrs. To understand the release kinetics of MZB from gel compositions F1-F15, the diffusion data studied in vitro was applied to a variety of models mentioned earlier , namely, Zero-Order, First-Order, Higuchi, Hixon-Crowell, Korsmeyer-Peppas, and Weibull and drug release mechanism was obtained from data in table IV that the F1- F4 batches follow Korsmeyer-Peppas, F5 and F9- F12 follow Weibull, F13-F15 follow Higuchi model. The diffusional exponent (n) was found to between 0.5936- 0.703 for F1--F15 Batch which indicate anomalous non fickian transport.
Higuchi Model

Batches F13, F14, F15 followed Higuchi model. Based on the model one can conclude that MZB controlled release gel was designed on the principle of embedding MZB in a mixture of porous matrix of HPMC E15 and MC in which fluid penetrates the matrix, gel swells and forms a swollen matrix through which the drug diffuses out (53). Amount of MZB released is proportional to the square root of time multiplied with Higuchi dissolution constant obtained from slope of regression line. Higuchi has described drug release as diffusion process based on Fick’s law, square root of time dependent. For diffusion controlled process a plot of $Q \rightarrow \sqrt{T}$ is linear as shown in (Fig. 9).

Korsmeyer-Peppas Model

Batches F1- F4 and F6-F8 followed Korsmeyer-Peppas Model as shown in (Fig.10). All the batches showed drug release based on diffusion and erosion controlled mechanism in case of swollen matrices (54).
Weibull model

Batches F5, F9- F12 have been observed to have followed Weibull model, which expresses the accumulated fraction of drug in solution at time. We observed that all the curves obtained for above mentioned batches are half parabolic significantly showing b value<1 as shown in (Fig. 11). We obtained Td, which is time interval necessary to release 63.2% of drug present in MZB controlled release gel (54).

![Fig. 11. Weibull Model](image)

Checkpoint Batch

From the overlay plot we have made a checkpoint batch which follows Weibull kinetic model (as shown in Fig. 12- b).

![Fig. 12. a) Overlay Plot, b) Weibull Kinetic Model F5 batch](image)
CONCLUSION

Controlled release gel formulation of MZB was developed and optimized using a 3-factor, 3-level Box-Behnken design. Our method of formulation is simple and inexpensive. No sign of incompatibility was detected in the drug-excipient mixture subjected to studies such as FT-IR and DSC. This gel appeared clear, homogeneous, transparent, odorless, and tasteless. It can be filled in a tube with narrow orifice, therefore, ease of administration for patients, due to the viscous nature of the gel; MZB would be able to reside in the pocket for a longer time and would diffuse in a controlled manner eliciting a uniform response for an extended period of time. TEM as shown in Fig. 6 shows typical spherical structures of physical mixtures of MC and HPMC E15 (size ranges between 100-500 nm) in a swollen state in water. MZB is shown in insoluble form (solid particle) entrapped within these polymer coats. Batches F5, F9-F12 follows Weibull model, which signifies that increase in concentration of MC from 6 to 8% w/w decreased drug release, but increase in HPMC E15 from 5 to 8% w/w increased release of first 63% from 12-19 hrs to 5 hrs, also it was also observed that when the concentrations of MC and HPMC E15 were equal, the batches followed Weibull. The purpose of using methyl cellulose as an adjunct with HPMC E15 was to develop a sustained gel preparation of MZB. The combination of above mentioned excipients has not been employed by many researchers. It was investigated that while doubling the concentration of MC in the system, raised the viscosity of the system almost 3 times. The drug release decreased with increasing concentration of MC in the matrix (3-5% w/w). Concentration of MC at 4% w/w in combination with HPMC E15 at concentration of 6.25%w/w followed Higuchi model. (F13, F14, F15). When the concentration of MC was lower in comparison to concentration of HPMC, the drug release in those batches followed Korsmeyer peppas (F1- F4 and F6- F8). From above data, it can be concluded that concentration of methyl cellulose plays a vital role in controlled release kinetic of MZB in comparison to concentration of HPMC E15. Increase in proportion of water as vehicle resulted in faster diffusion of drug from gel matrices because water entered into the pores of gel matrix. This results in a formation of channels through which the media can enter the swollen polymer matrix and release the drug. While comparing the zone of inhibition of MZB powder and equivalent 0.4 µg/ml concentration of 15 gel formulations, the results indicate that the diffusion of drug from gel is almost 1.5 times higher than that in MZB powder.
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