DEVELOPMENT AND OPTIMIZATION OF SUSTAINED RELEASE IN SITU GEL FOR THE TREATMENT OF ULCERATIVE COLITIS

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ABSTRACT

The objective of the research work was focused on optimization and evaluation of Mesalamine rectal in-situ gel of controlled/sustained release. This is used as anti-ulcerative colitis drug, for pediatric dose. The in-situ gel formulation were prepared by simple mixing method it’s a cooling technique based on the temperature triggering system by incorporation of various polymers like Poloxamer 407 and HPMC E-50 in different proportions. The in-situ gel characteristics were evaluated for clarity, gelation time, gelation temperature, gel strength, and in-vitro drug release studies. The formulations gave an initial burst effect and followed by sustained release for 12 h. Optimized formulation showed release of drug up to 92.860% in 12 h. Optimization was done by using design expert software. The optimized formula (F3) showed no significant changes on stability studies when stored at 40°C/75% RH for one month according to ICH guidelines.

KEYWORDS: Rectal In-situ gel, Mesalamine, Pluronic F-127, HPMC E50, Pediatric dose.

INTRODUCTION

Rectally administered Mesalamine formulations, which include suspensions, suppositories, gels and foams, offer the advantage of delivering a known amount of Mesalamine topically to the distal colon. Earlier reported studies suggest that topical preparations result in the better responses in mild to moderate distal Ulcerative Colitis when compared with oral therapy.[1] The in situ gel system offers ease of administration, improved patient compliance and comfort. Also the formulation is less complex which lowers the investment and manufacturing cost. There are several possible mechanisms that lead to in situ gel formation: solvent exchange, UV irradiation, Ionic cross-linkage, pH change, and temperature
Ulcerative colitis is a disease that causes inflammation and sores (ulcers) in the lining of the large intestine (colon). It usually affects the lower section (sigmoid colon) and the rectum. But it can affect the entire colon. In general, the more of the colon that’s affected, the worse the symptoms will be. The disease can affect people of any age. But most people who have it are diagnosed before the age of thirty. Mesalamine (Mesalazine) is a 5-aminosalicylic acid compound and used as the first-line treatment for patients with mild-to-moderate UC. There are multiple formulations of Mesalamine available, primarily differentiated by their means of delivering active Mesalamine to the colon. Mesalamine has been demonstrated in randomized controlled trials to induce both clinical response and remission, and maintain clinical remission. Poloxamers are a family of triblock copolymers consisting of two hydrophilic blocks of polyoxyethylene separated by a hydrophobic block of polyoxypropylene, which form micelles at low concentrations and form clear thermally reversible gels at high concentrations. Hydroxypropyl Methyl Cellulose (HPMC) E50 was added to in situ gels to improve the mucoadhesive and mechanical properties of formulations and to prolong the residence time. Rectally administered preparations of 5-aminosalicylic acid (5-ASA) should be the preferred treatment for mildly to moderately active left-sided or distal ulcerative colitis. Administering of Mesalamine rectally offers the advantage of delivering the treatment directly to the site of maximal inflammation while potentially minimizing the frequency of systemic adverse effects.

**MATERIALS AND METHODS**

<table>
<thead>
<tr>
<th>Materials</th>
<th>Source</th>
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<tbody>
<tr>
<td>MESALAMINE</td>
<td>Yarrow Chem. Products. Mumbai</td>
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<tr>
<td>HPMC E50</td>
<td>Yarrow Chem. Products. Mumbai</td>
</tr>
<tr>
<td>POLOXAMER 407</td>
<td>Yarrow Chem. Products. Mumbai</td>
</tr>
<tr>
<td>PEG 6000</td>
<td>Yarrow Chem. Products. Mumbai</td>
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**PREFORMULATION STUDIES**

**Solubility studies**

The solubility study of the drug was carried out using different solvents; water, ethanol, PBS 7.4, dilute Hydrochloric acid, 10% Sodium hydroxide.

**FT-IR spectral studies**

Bruker Spectrophotometer was used to analyze the pure drug and their physical mixtures which was stored at 40±2°C/75±5%RH for 2 months.
Preparation of solid dispersion
The Kneading method is adopted to prepare to solid dispersion of Mesalamine. The physical mixtures were prepared by mixing accurate weight of Mesalamine with PEG 6000 in drug: polymer ratio of 1:2 and 1:4 for 5 mins using mortar and pestle. The physical mixture was trituriated using a small volume of ethanol-water (1:1) solution to give thick paste, which was kneaded for 20 minutes, and then slurry prepared transferred to tubing glass vials. Then sample is allowed to freeze and then lyophilization process is carried to dry the resultant product. The obtained sample is then scrapped and powdered using mortar and pestle and stored in a tightly closed container.

Standardization Procedure for estimation of Mesalamine
Preparation of Buffer (Phosphate buffer 7.4)
Dissolve 2.38 g of Disodium Hydrogen Phosphate, 0.19 g of Potassium Dihydrogen Phosphate and 8.0 gm of Sodium Chloride in sufficient distill water to produce 1000mL. pH is adjusted.

Preparation of standard stock solutions
100 mg of Mesalamine was accurately weighed and transferred to 100ml clean and dry volumetric flask and 70 ml of pH 7.4 phosphate buffer added to the flask and sonic ate to dissolve the drug completely and make up the volume. From which second stock solution was prepared of concentration 100mcg/mL. From this dilution other serial stock solution was prepared of standard concentrations of 5mcg/mol to 25mcg/mL. The calibration curve was constructed by plotting concentration against Absorbance at 330 nm.

METHODOLOGY
Preparation of Mesalamine In situ gel
The matrix-type in-situ gel formulations containing were prepared by simple mixing method (cooling technique). Poloxamer 407 and HPMC E-50 were used as polymers in the preparation of in-situ gel formulation. Cold Phosphate Buffer Saline (PBS) of pH 7.4 was used as a solvent. Weighed required quantity of polymers (Poloxamer 407, HPMC E-50) and drug were dissolved in 5 ml of cold PBS 7.4.
FORMULATION CHART OF MESALAMINE IN SITU GEL BY USING FACTORIAL DESIGN

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Amount of Drug (mg)</th>
<th>Amount of Poloxamer 407 (gm)</th>
<th>Amount of HPMC E50 (mg)</th>
<th>Amount of Solvent (mL)</th>
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<td>50</td>
<td>5</td>
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<tr>
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<td>F8</td>
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<td>1.6</td>
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EVALUATION OF MESALAMINE INSITU GEL

Clarity
All the in-situ gels were visually inspected for color and clarity.

Determination of flow behavior of sols
Sample tubes containing 10ml of polymer solutions initially at 20°C were incubated for 10 min at 37°C in a water bath and their flow properties observed visually. Gel formation was indicated by a lack of movement of the meniscus on tilting the tube.

Sol-Gel transition time and temperature
Gelation temperature is defined as the temperature at which the liquid phase makes a transition to gel. A 20 mL beaker containing a magnetic bar and each formulation were placed in a magnetic stirrer. Heated at a constant rate while stirring. The gelation temperature was measured when the magnetic bar stopped moving due to gelation. Each preparation was tested thrice to control the repeatability of the measurement.

Gel-Strength
A sample of 50 gm of gel was placed in a 100 ml graduated cylinder and gelled in a thermostat at 37°C. Weight of 20 gm was allowed to penetrate into the gel. The gel strength, which means the viscosity of the gels at physiological temperature was determined by the time (in seconds), the apparatus took to sink 5cm down through the prepared gel.
Drug content
5mL of solution were taken in a 100 mL volumetric flask, dissolved in small quantity of Phosphate buffer 7.4. After the solution is completely dissolved the volume was made up to 100 mL with Phosphate buffer 7.4.

In-Vitro Drug Dissolution Studies
In vitro dissolution studies were performed by using a dissolution apparatus with a paddle type of 1000 mL capacity. PBS 7.4 prepared and 900 mL of the solution is taken and in-situ gel has to be inserted into it. The apparatus is operated at 100 rpm, external and bath temperature maintained at 37 °C ± 0.5°C and samples were withdrawn at for 12 consecutive hours of at 1h time interval and analyzed for drug content spectrophotometrically at 330 nm. The dissolution chamber was replenished with an equal volume of buffer solution at each sample withdrawal.

Data Analysis (Curve Fitting Analysis)
- Higuchi release model.
- Korsmeyer and peppas release model.
- Zero order release rate kinetics.
- Regression analysis.

RESULTS AND DISCUSSION
Preformulation Studies
Solubility studies
The Solubility of Mesalamine was found that it is soluble in hot water, dil. HCl and dil. Sodium Hydroxide, slightly soluble in water, Ethanol and PBS 7.4.

Melting point
The melting point of Mesalamine was found to be 183 °C. It complies with the standards thus indicating the purity of drug sample.
FT-IR spectral studies

Figure 3: FT-IR peaks of Mesalamine

Figure 4: FT-IR graph of Solid Dispersion of Mesalamine with PEG 6000, Poloxamer 407 and HPMC E50.

Spectrophotometric Analysis

Spectrophotometric data construction of standard graph of Mesalamine using Phosphate buffer 7.4.
GELATION TIME
The value for the Gelation time of the formulations from F1-F8 ranges from 85 sec to 130 sec as shown in table below. The formulation F8 takes least time to form (85 sec) because it possesses stronger concentration of Poloxamer 407 as well as HPMC E 50.

Comparison of Gelation Time of all Formulations (F1-F8)

GELATION TEMPERATURE
The Gelation Temperature of the formulations F1-F8 ranges from 28 °C to 32 °C as shown in table below. The formulation F8 starts to form gel at temperature of 28 °C which reflects the high concentration of Poloxamer and HPMC E50 in it. This suggests that the higher the concentration of polymers lowers the temperature that requires forming gel.

Figure 7: Comparison of Gelation Temperature of all formulations (F1-F8)

GEL STRENGTH
The gel strength of all the formulation in term of time ranges from 30 sec to 51 sec as shown in table below. This parameter suggests the viscosity of the preparations at physiological temperature. The formulation F1 has least value for the time require penetrating the gel while
the formulation F2 acquires more duration. Thus the F2 is more viscous than others 7 formulations which mean it have longer retention time when applied rectally inside the colon.

![Figure 8: Comparison of Gel Strength of all formulations (F1-F8)](image)

**DRUG CONTENT**

The percentage drug content of all the formulations was found to be in the range of 96.91 % to 99.70 % as shown in the table below. Thus the amount of drug present in all the formulations is nearly uniform. Hence every single dose from either of the formulations exhibit almost same percentage of the drug.

![Comparison of Drug Content of all formulations (F1-F8).](image)

**IN VITRO DISSOLUTION STUDY**

In vitro dissolution studies of Mesalamine in-situ gels were carried out in dissolution apparatus in the Phosphate Buffer 7.4. The release data were given in the table below respectively for formulation F1 to F8.
Comparative In vitro dissolution study of formulations F1-F8.

In vitro dissolution studies of all the formulations (F1 to F8) was carried out in USP type 2 dissolution chamber for about 12 h. All the formulations showed different In vitro release pattern that ranged from 90.903575 % to 94.86071 % based upon the concentration of the polymers used. The formulation F1 showed the maximum drug release after 12th h of release while the formulation F8 shows the least value. This may be because of the presence of least quantity of the Poloxamer 407 and HPMC E50 present in the formulation F1 and for the formulation F8 has the highest value of them. Whilst the other formulation showed in between released values.

KINETICS OF DRUG RELEASE

Zero order release

Comparison of zero order Kinetics of In vitro drug release of formulations (F1-F8).
First order release

Comparison of first order Kinetics of In vitro drug release of formulations (F1-F8).

Higuchi model

Comparison of Higuchi model of In vitro drug release Kinetics of F1-F8

Korsmeyer-Peppas equation

Comparison of Korsmeyers-Peppas equation of In vitro Drug release F1-F8
CONCLUSION
In the present work, an attempt has been made to develop rectal in situ gels of Mesalamine. The FT-IR spectra revealed that, there was no interaction between polymers and drug. From the data obtained, it is observed that amongst the various combinations of the polymers used in the study, in situ gels that were formulated (cooling technique) using Poloxamer 407 (1.4 gm) and HPMC E-50 (100mg) exhibited better results. The effectiveness of polymers (Poloxamer 407 and HPMC E-50) on the drug release was explained.

The factorial design was used to find out the effect of independent variables on the dependable variables. The result of factorial design revealed that the Poloxamer 407 and HPMC E-50 have significant effect on the gelation time, gelation temperature, gel strength, the drug release at 1st h, the drug release at 6th h and the drug release at 12th h. The observed independent variables were found to be very close to predicted values of optimized formulation which demonstrates the feasibility of the optimization procedure in successful development of rectal in situ gel containing Mesalamine by using Poloxamer 407 and HPMC E-50. The drug release form the optimized formula was found to be following the zero order kinetics and n value range of the korsmeyer-Peppas equation is 0.71 to 0.79, which indicates non-fickian diffusion mechanism. Thus the release of drug from the dosage form was found to be time dependent.

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REFERENCES


