Formulation Development and Evaluation

of Sulfasalazine Pulsincap

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ABSTRACT
The aim of present study was to develop and evaluate the pulsincap formulation on the principle based on the pulsatile drug delivery system using sulfasalazine drug. Sulfasalazine act by scavenging the toxic oxygen metabolites produced by nutrophils. It is split into its component parts by bacteria in the colon, the 5-amino salicylic acid being the putative radical scavenger. Formalin treatment has been employed to modify the solubility of gelatin capsules. Quantitative test for formaline residue was carried out. FTIR and DSC study shown absence of chemical and physical interaction. Hydrogel plug of various components are prepared and used in pulsincap formulation. The drug content of the formulated pulsincap was found to be in the range 94.27 to 103.22. In vitro drug release study in pH 1.2 buffer was zero percent and capsule was intact for first 2 hrs. F4 formulation batch was selected as best formulation as F4 batch released maximum drug in pH 7.4 phosphate buffer than other formulation batches. In the present work an attempt has been made to develop and evaluate a time or site specific pulsatile drug delivery system of drug sulfasalazine.

Keywords: Chronotherapeutics, PDDS, Pulsincap, sulfasalazine, etc.

1. INTRODUCTION
Sulfasalazine drug is combination of sulfonamides (Sulfapyridine) with a salicylate. It act by scavenging the toxic oxygen metabolites produced by nutrophils. It is split into its component parts by bacteria in the colon, the 5-amino salicylic acid being the putative radical scavenger. It is poorly absorbed after oral administration. Therapeutic uses of Sulfasalazine are crohn's disease, ulcerative colitis, rheumatoid arthritis. Sulfasalazine is slightly soluble in alcohol & practically insoluble in water, benzene, chloroform, ether. Pulsatile delivery is defined as the rapid and transient release of certain amount of drug molecule within a short time period immediately after a predetermined off released period, i.e, lag time. This system provides spatial and temporal delivery of the drug. This system is designed according to circadian rhythm or biological clock. These deliver the drug at the right time and at the right place and in the right amount thus increasing patient compliance. Pulsatile system is beneficial for drug where night time dosing is required, such as anti-asthmatic and antiarrythmatic drug where the disease severity is time dependant. The principal rationale for the use of pulsatile release is for the drugs where a constant drug release i.e a zero order release is not desired.

Pulsincap system comprises of a water-insoluble capsule enclosing the drug reservoir. Seal the drug contents into the capsule body, a swellable hydrogel plug was used. It swelled, when this capsule came in contact with the dissolution fluid and after a lag time, the plug pushed itself outside the capsule and rapidly released the drug.

The aim of present study was to develop and evaluate the pulsincap formulation on the principle based on the pulsatile drug delivery system with the objectives was concluded to study the physical parameters of the dosage form, to estimate the drug content in the formulation, to study the drug release pattern using suitable In vitro method, to retard the release of drug over extended period of time, to develop the effective dosage form to avoid patient compliance, to decrease frequency of dosing for better utilization of drug.

2. MATERIALS AND METHODS
Sulfasalazine drug & Hard gelatin capsule shells were obtained as gift sample from Wallace pharmaceutical, Goa. HPMC, Ethyl cellulose, microcrystalline cellulose were used of analytical grade.
Method

2.1 Formaldehyde treatment
Formalin treatment has been employed to modify the solubility of gelatin capsules. Exposure to formalin vapours results in an unpredictable decrease in solubility of gelatin owing to the cross linkage of the amino group in the gelatin molecular chain aldehyde group of formaldehyde by Schiff’s base condensation. Hard gelatin capsule of size 0 was selected.

2.2 FTIR Study
The compatibility between drug (sulfasalazine) and polymers were detected by IR spectra (IR-Affinity, SHIMADZU). A few mg of the physical mixture were ground together in a mortar with about 100 times quantity of KBr. The finely ground powder was introduced into a stainless steel die. The spectra were recorded over the range of 4000 -2000- 400cm⁻¹

2.3 DSC Study
Thermograms of drug sulfasalazine, polymers like HPMC K4M, Methocel and physical mixture were obtained by using a differential scanning calorimeter (DSC Q20 V24.4 Build 116, Japan) at a heating rate 10° C/min over a temperature range of 0-300° C. The sample was hermetically sealed in an aluminium crucible. Nitrogen gas was purged at the rate of 10 ml/min for maintaining inert atmospheres and obtained graphs were studied.

2.4 Preparation of Hydrogel Plug
The Hydrogel plug in the Pulsincap dosage form should have the property to swell and get ejected in the intestinal fluid. Hydrogel plug was prepared by direct compression followed by placing in the capsule opening. Table no.1 indicate the ingredient used in formulation of hydrogel plug.

Table 1: Formulation of hydrogel plug

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Ingredient (mg)</th>
<th>Formulation</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HPMC K4M</td>
<td>100</td>
<td>60</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Lactose</td>
<td></td>
<td>40</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Methocel</td>
<td></td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

2.5 Formulation of pulsincap
The composition of different formulation was prepared given in table no.2. Accurately weighed quantity of drug sulfasalazine and mixed with viscosity increasing agent HPMC K4M separately. The powders were blended with PVP K30 (polivinyl pyrrolidone K30), methocel to controlled drug release and magnesium stearate as lubricant. Added talc as a glidant/flow promoter, ethyl cellulose as disintegrant and mixed properly. The final powder blend was passed through sieve 40 mesh. Powder blend was filled into formaldehyde treated capsules and optimize hydrogel plug was used to pack the content to formulate pulsincap.

Table 2: Formulation of pulsincap

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Ingredients (mg)</th>
<th>Formulation Batches</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sulfasalazine</td>
<td>250 250 250 250 250</td>
</tr>
<tr>
<td>2</td>
<td>Polyvinyl Pyrrolidone</td>
<td>16 16 16 16 16</td>
</tr>
<tr>
<td>3</td>
<td>HPMC K4M</td>
<td>6 9 12 - - -</td>
</tr>
<tr>
<td>4</td>
<td>Methocel</td>
<td>- - - 6 9 12</td>
</tr>
<tr>
<td>5</td>
<td>Microcrystalline Cellulose</td>
<td>20.5 17.5 14.5 20.5 17.5 14.5</td>
</tr>
<tr>
<td>6</td>
<td>Magnesium stearate</td>
<td>3 3 3 3 3</td>
</tr>
<tr>
<td>7</td>
<td>Ethyl cellulose</td>
<td>4.5 4.5 4.5 4.5 4.5</td>
</tr>
</tbody>
</table>

3 Evaluation Test

3.1 Formaldehyde treatment
The solubility test was carried out for formalin treated body capsule shell. The randomly selected 10 capsules shell was subjected to 100 ml different solvent like water, buffer of pH 1.2, 7.4 and 6.8 at room temperatures over period of 24 hrs. The solution was continuously observed for dissolution time of treated and untreated capsule shell.

3.2 Qualitative test for free formaldehyde
Standard formaldehyde solution used is formaldehyde solution (0.002 w/v) and sample solution is formaldehyde treated bodies (about 25 in number) were cut into small pieces and taken into a beaker containing distilled water. This was stirred for 1 hrs with a magnetic stirrer, to solubilize the free formaldehyde. The solution was then filtered into a 50 ml volumetric flask, washed with distilled water and volume was made up to 50 ml with the washings. 1ml of sample solution, 9ml of water was added. One milliliter of resulting solution was taken into a test tube and mixed with 4ml of water and 5ml of acetone reagent. The test tube was warmed in a water bath at 40 °C and allowed to stand for 40 min. The solution was not more intensely colored than a standard solution prepared at the same time and in the same manner using 1ml of standard solution in place of the sample solution. The comparison should be made by examining tubes down their vertical axis.

3.3 Micromeretic evaluation
Various micromeretic evaluation tests were carried out for powder blend and result are shown in table no.4

3.4 Evaluation of pulsincap
Weight variation
10 capsules were selected randomly from each batch and weight individually for weight variation. Result was noted.

Drug content
From each batch of the prepared pulsincap of sulfasalazine, powdered blend equivalent to 250 mg of sulfasalazine was accurately transferred into 100ml volumetric flask. Added 5ml of methanol to dissolve sulfasalazine. The solution was made up to volume with pH 7.4 phosphate buffer. The resultant solution was filtered through whatman filter paper and suitably diluted to obtain a stock solution of 1000μg/ml. 1ml was pipette out and diluted up to 100ml with pH 7.4 phosphate buffer to obtain 10μg/ml. The solution was assayed for drug content using UV-spectrophotometer method by measuring the absorbance at 350.5 nm. The drug content was determined by following equation using standard absorbance.

\[ \text{Drug content} = \frac{\text{Standard absorbance}}{\text{Test absorbance}} \times 100 \]

In Vitro Dissolution Studies of Pulsincap Formulation
Dissolution studies were carried out by using USP dissolution 2 apparatus (paddle method). In order to simulate the pH changes along the GI tract, two dissolution media with phosphate buffer pH 1.2, 7.4 were sequentially used. When performing experiments, the pH 1.2 medium was first used for 2h (since the average gastric emptying time is 2hour), then removed and the fresh pH 7.4 phosphate buffer was added.
A total of 900 ml of the dissolution medium was used at each time. Rotation speed was 50rpm and temperature was maintained at 37°C. 5ml of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analyzed at 358 nm, by double beam UV visible spectrophotometer.

4. RESULT AND DISCUSSION
4.1 Formaldehyde treatment
Among formaldehyde treated capsules, only the cap dissolved within thirty minutes, while the body of capsule shell remained intact for about 24 hour in water, phosphate buffer of pH 1.2, 7.4 & 6.8. Formaldehyde reacts with gelatin forming an irreversible complex. The primary amine group present in gelatin reacts with formaldehyde making it irreversibly bound.

4.2 Qualitative test for free formaldehyde
The formaldehyde capsules were tested for the presence of free formaldehyde. The sample solution was not more intensely colored than the standard solution inferring that less than 15μg free formaldehyde is present in 10 capsule.

4.2 FTIR Study
The drug-polymer compatibility was assessed by I. R spectra of drug, polymer and drug-polymer by IR spectra (IR- Affinity, SHIMADZU). From the interpretation it is found that there is no worth change in the wave numbers of the drug and drug-polymer combination. Hence the drug and polymer are compatible with each other. (fig. No. 1)

![Fig. 1: IR spectra of sulfasalazine, HPMC K4M, methocel, and mixture](image)

4.3 DSC Study
DSC thermogram of sulfasalazine drug, polymer and formulation was studied and it was found that drug sulfasalazine shown endothermic peak at 262.43\(^{0}\)C, HPMC K4M showed endothermic peak at 58-71\(^{0}\)C and methocel showed endothermic peak at 75\(^{0}\)C, 145\(^{0}\)C, 187.37\(^{0}\)C, 250.05\(^{0}\)C, 258\(^{0}\)C. The thermogram of mixture showed the absence of sulfasalazine peak suggesting that sulfasalazine and HPMC K4M is completely soluble in the liquid phase with excipient. The melting point of drug in mixture thermogram was observed at slightly lower temperature 258\(^{0}\)c. There is no difference in the melting point of pure and formulation. It indicates the absence of chemical interaction between the drug and polymer. (fig. no. 2)

![Fig. 2: DSC thermogram of (A) sulfasalazine, (B) HPMC K4M, (C) Mthocel and (D) Mixture](image)

4.4 Evaluation of Hydrogel Plug - The prepared hydrogel plug formulation were subjected to different evaluation test and the results were shown in table no.3
Table 3: Evaluation of Hydrogel Plug

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Formulation Batch</th>
<th>Swelling Time (hr)</th>
<th>Weight (mg)*</th>
<th>Hardness (kg/cm²)*</th>
<th>Thickness (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P1</td>
<td>24</td>
<td>100±0.00</td>
<td>4.2±0.047</td>
<td>3±0.047</td>
</tr>
<tr>
<td>2</td>
<td>P2</td>
<td>18</td>
<td>100±0.00</td>
<td>4.1±0.038</td>
<td>3.1±0.047</td>
</tr>
<tr>
<td>3</td>
<td>P3</td>
<td>8</td>
<td>100±0.00</td>
<td>4±0.029</td>
<td>3±0.038</td>
</tr>
</tbody>
</table>

n=3

From the observation, it was found that the formulation P3 bears all good properties require to control the drug release. Hence formulation P3 selected as optimized.

4.5 Evaluation of powder blend
Various micromeretic tests were perform as per the procedure and the result as follows (table no. 4). All the formulations exhibited the angle of repose values of 22.56 – 24.86, which was further supported by good compressibility index value of 12.1 – 24.12% and Hausner’s ratio of 1.11 – 1.29, thus indicating the suitability of powder blend for formulation.

Table 4: Micromeretic properties of formulation

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Angle of repose(°)</th>
<th>Bulk density (g/cm³)</th>
<th>Tapped density* (g/cm³)</th>
<th>Carr’s Index * (%)</th>
<th>Hausner’s ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22.56±0.041</td>
<td>0.32±0.013</td>
<td>0.37±0.009</td>
<td>12.1±0.25</td>
<td>1.11±0.011</td>
</tr>
<tr>
<td>2</td>
<td>23.20±0.232</td>
<td>0.31±0.019</td>
<td>0.34±0.017</td>
<td>12.3±0.33</td>
<td>1.10±0.017</td>
</tr>
<tr>
<td>3</td>
<td>23.50±0.136</td>
<td>0.35±0.025</td>
<td>0.41±0.014</td>
<td>14.7±0.21</td>
<td>1.14±0.019</td>
</tr>
<tr>
<td>4</td>
<td>23.56±0.220</td>
<td>0.33±0.009</td>
<td>0.40±0.011</td>
<td>17.1±0.11</td>
<td>1.19±0.019</td>
</tr>
<tr>
<td>5</td>
<td>24.45±0.062</td>
<td>0.22±0.007</td>
<td>0.27±0.005</td>
<td>24.12±0.435</td>
<td>1.25±0.081</td>
</tr>
<tr>
<td>6</td>
<td>24.86±0.041</td>
<td>0.22±0.008</td>
<td>0.28±0.011</td>
<td>21.43±0.032</td>
<td>1.29±0.02</td>
</tr>
</tbody>
</table>

4.6 Drug content
The drug content of the formulated pulsincap was found to be in the range 94.27 to 103.22. The drug content values for all the capsules were within the acceptable limits indicating that there was no loss of drug during the process of filing.

4.7 In-vitro release studies
The in-vitro drug release study of sulfasalazine from prepared microcapsules was carried first in pH 1.2, and found the capsules were intact for first two hrs. When this capsule came in alkaline media, hydrogel plug which absorb surrounding fluid and release the drug through the capsule. After complete wetting of the plug it forms a soft mass which was then easily ejected from the capsule body releasing the drug in pH 7.4 phosphate buffer. The formulation batches from F1 to F6 release drug as follows: 89.56%, 81.32%, 72.33%, 92.74%, 90.12%, 88.36% at the end of 12 hours. So from % drug release from various formulation batches, F4 formulation batch was selected as best formulation as F4 batch released maximum drug in pH 7.4 phosphate buffer than other formulation batches. The drug release from the formulation decrease with increase in the amount of polymer added in each formulation. The graphical representation (fig. no. 3) revealed that the drug released from the capsule following lag time of 3 to 4 hrs in order to swelling of hydrogel plug.
5. CONCLUSION
In the present study, an attempt has been made to prepare pulsincap to deliver sulfasalazine drug to colon region in treatment of ulcerative colitis. For this different formulation were prepared by taking different concentration of drug and polymer. Hydrogel plugs of 6mm were prepared by using HPMC K4M, Lactose and Methocel of 100 mg and P3 was selected for the development of pulsincap in respect to achieve maximum swelling for the pulsatile release of drug. *In-vitro* release studies were carried out for formulated pulsincap. From the above observations, we conclude that the drug release from the formulated pulsincap with hydrogel plug of HPMC K4M, Lactose and Methocel shows efficient drug release.

6. REFERENCES
1. USP United state pharmacopeia 24 and National formulary 19, Asian edition, Jan 2000. 1576-1577