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Formulation and evaluation of Clopidogrel bisulphate floating Matrix Tablets

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Abstract:
The objective of the present research was to formulate clopidogrel bisulphate floating matrix tablets by direct compression method to prolong the gastric residence time and to improve bioavailability of the drug. Various grades of HPMC such as K4M, K15M, K100M, cellulose acetate and Ethyl cellulose N50 were used as drug release retarding polymers and sodium bicarbonate is used as gas generating agent. The formulated tablets were characterized for quality control tests like weight variation, hardness, friability, % drug content, lag time and total floating time. The effect of concentration and viscosity of polymer and combination of different polymers on in vitro drug release was studied including release kinetics, swelling and erosion index. Quality control tests results were found to be uniform within the pharmacopeial limits. In vitro drug release study showed that formulations containing HPMC K4M extended drug release upto 5hrs to 9 hrs, formulations containing HPMC K15M extended drug release upto 7 hrs to 11 hrs and formulation containing HPMC K100M extended drug release upto 9 hrs to 13 hrs. F9 showed optimum floating time and extended drug release upto 13 hrs. Combination of polymers also yielded better results when compared to the individual polymer. The drug release mechanism was observed to follow zero order kinetics and non Fickian diffusion mechanism. Drug excipient interaction of the prepared formulations was characterized by FTIR and DSC study and confirmed that no interaction was found. Surface topography, texture of the swollen tablet was studied by using SEM study.

Keywords: Anti platelet agent, clopidogrel bisulphate, direct compression, floating drug delivery, gastric residence time, Release Kinetics.

INTRODUCTION

The aim of formulating floating drug delivery system is to provide a therapeutic amount of drug at the proper site in the body and then to maintain the desired drug concentration throughout the delivery period. Drug delivery systems are becoming more sophisticated as pharmaceutical scientists are acquiring a better knowledge on the physicochemical and biological parameters that effect performance of delivery systems (1). Oral drug delivery is the most preferable route of administering the drugs due to ease of administration, patient compliance and flexibility in formulation and handling of these forms (2). Approximately 50% of the dosage forms available in the market are oral drug delivery systems (3). A major disadvantage in formulating oral controlled drug delivery systems is that, not all drug candidates are absorbed uniformly throughout the Gastrointestinal Tract (GIT) (4). The extent of GIT drug absorption depends on contact time of drug with the intestinal mucosa (5). Conventional drug dosage forms are taken several times a day to maintain the drug concentration within the therapeutically effective range needed for treatment (6). Success of any oral drug delivery system mainly depends on its degree of absorption through GIT. Thus, the idea of enhancing drug absorption necessitated in developing Gastro retentive drug delivery system (GRDDS) (7).
The controlled gastric retention of solid dosage forms may be achieved on the basis of the mechanism of mucoadhesion, floatation, and sedimentation which delay gastric emptying (8). Gastro retentive systems can remain in the gastric region for several hours and hence significantly prolong the gastric residence time of drugs. Prolonged gastric retention improves bioavailability of the drugs, reduces drug waste, and improves solubility for drugs which are poorly soluble in a high pH environment. GRDDS has applications in local drug delivery to the stomach and proximal small intestines. Gastro retentive drug delivery system helps provide new therapeutic possibilities and substantial benefits for patients by increasing bioavailability of new products (9,10).

Clopidogrel bisulphate is an antiplatelet agent used to inhibit blood clots in a variety of conditions such as peripheral vascular disease, coronary artery disease, and cerebrovascular disease (11). Clopidogrel a prodrug and requires CYP2C19 for its activation (12). It acts on the ADP receptor on platelet cell membranes. The drug irreversibly inhibits the P2Y12 subtype of adenosine diphosphate receptor, which is important step in activation of platelets and eventual cross-linking by the protein fibrin (13). Bioavailability of Clopidogrel bisulphate has not been found to be affected by food.

**METHOD**

**Formulation development of clopidogrel bisulphate floating matrix tablets**

Floating matrix tablets containing clopidogrel bisulphate was formulated by direct compression method using different viscosity grades of HPMC like K4M, K15M, K100M and Ethyl cellulose N50, Cellulose acetate. Sodium bicarbonate was used as a gas generating agent. All the ingredients were weighed according to the formula and blended in mortar and sifted through 30# sieve. The powder blend was mixed uniformly in polybag for 10 minutes. The blend was compressed into tablet using required mm of punch on 16 station rotary tablet compression machine (Cadmach, Ahmedabad).

**EVALUATION OF TABLETS**

Weight Variation (14,15) Test was done by randomly selecting twenty tablets from each batch and weighed individually using electronic balance (shimadzu). The crushing strength (15) kg/cm² of prepared tablets was determined by using Monsanto tablet hardness tester. The friability of tablets was determined using Roche friabilitator. Friabiliator (15) was revolved at 25 rpm for 4 mins (Sisco, India).

% Drug Content

Content uniformity of the tablets was done by collecting 10 tablets and crushed in mortar. A quantity of powder equivalent to 97.8 mg of clopidogrel bisulphate was accurately weighed.
and transferred into a 100 ml volumetric flask and dissolved in 0.1 N HCl and the volume was made with 0.1 N HCl (pH 1.2). The solution was filtered through Whatman filter paper. The stock solution was suitably diluted with 0.1 N HCl and the absorbance was measured at λmax 254 nm using UV / visible spectrophotometer (Labindia UV – 3092) against 0.1 N HCl as blank (16,17).

**In Vitro Buoyancy Test**

The prepared tablets were subjected to in vitro buoyancy test by placing them in 100 ml beaker containing 0.1N HCl (pH 1.2, temp. 37±0.5°C). The time taken for the tablet to rise to the surface and float was taken as lag time. Total floating time was determined by visual observation (18,19).

**In Vitro Dissolution Studies**

The dissolution study was carried out using USP type II (paddle method) apparatus in 900 ml of 0.1 N HCl (pH 1.2) until the complete drug was released from the matrix formulations. The temperature of the dissolution medium was kept at 37± 0.5 ºC. The paddle rotation was maintained at 50 rpm. 10 ml of sample solution was withdrawn at specified interval of time and filtered through Whatman filter paper. The stock solution was placed in wire mesh basket. The tablets were removed periodically (1hr, 3hr, 6hr, 9hr, 12hr) from dissolution medium. Excess water on the surface of the tablet was removed by blotting paper. Swelling characteristics were expressed in terms of percentage weight uptake. Weight gain by the tablet was measured by using the formula (22).

\[
\text{% Swelling Index} = \frac{W_t - W_0}{W_0} \times 100
\]

Where, SI = Swelling index, Wt = Weight of tablet at time ‘t’ and Wo = Weight of tablet at time ‘0’.

**% Erosion index**

The extent of erosion was determined in terms of percentage weight lost by the tablets. The erosion properties of matrix tablet containing drug were determined by placing the tablet in the dissolution test apparatus type I (basket), in 900 ml of 0.1N HCl at 37 ± 0.5°C at 50rpm. The tablets were removed periodically (1hr, 3hr, 6hr, 9hr, 12hr) from dissolution medium. Excess water on the surface of the tablet was removed by blotting paper. The tablets were placed in hot air oven and dry weight of tablet was measured. % Erosion Index was calculated using the formula (22).

\[
\text{% Erosion Index} = \frac{W_0 - W_t}{W_0} \times 100
\]

Wt = dry weight of tablet at time ‘t’ and Wo = Weight of tablet at time ‘0’.

**In vitro Drug Release Kinetics**

The release mechanism of clopidogrel bisulphate from floating matrix tablets was determined by following kinetic models, Zero-Order Kinetics, First order kinetics, Higuchi Model, Hixson Crowell equation, Korsmayer Peppas equations (23).

**CHARACTERIZATION OF DRUG AND EXCIPIENTS**

**Differential scanning calorimetry (DSC)**

Differential Scanning Calorimetry (DSC) studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Clopidogrel
bisulphate was mixed with the excipients and the DSC analysis of each sample under the analogous conditions of temperature range 40 – 300º C, heating rate 10ºC/min, nitrogen atmosphere (20ml/min) and alumina as reference. Differential Scanning Calorimetry (DSC) was performed on pure drug, excipients and composition of final formulation. DSC measurements were done on a Shimadzu DSC-60 and samples were heated at the rate of 10ºC min-1. The samples were heated in an aluminium cup up to 300ºC.

**Fourier transform infrared (FTIR)**

FTIR studies are very helpful in the evaluation of drug–polymer interaction studies. If there is any incompatibility between the drugs and excipients, these can be predicted by changes in the functional peaks (characteristic wave numbers). Diffuse reflectance technique was used(400 to 4000 cm-1), drug and various polymers were thoroughly mixed with 300mg of potassium bromide, compressed and the spectrum was obtained by placing the thin pellet in light path.

**Scanning electron microscopy (SEM)**

Scanning electron microscopy has been used to determine surface topography, texture, and to examine the morphology of fractured or sectioned surface. SEM is probably the most commonly used method for characterizing drug delivery systems, owing in large to simplicity of sample preparation and ease of operation. SEM studies were carried out by using JEOL JSM T-330A scanning microscope (Japan) (25).

**RESULTS AND DISCUSSION**

In the present study, clopidogrel bisulphate floating matrix tablets prepared by using different viscosity grades of HPMC (K4M, K15M, K100M) and in combination with cellulose acetate and ethyl cellulose N50. A total number of 15 formulations were prepared by direct compression technique.

**Quality control tests of formulated floating matrix tablets:**

Clopidogrel bisulphate floating matrix tablets were prepared with HPMC K4M, K15M and K100M and in combination with cellulose acetate and ethyl cellulose N50. The formulated tablets were subjected various quality control tests. All the formulated tablets passed weight variation test and the standard deviation values were within the pharmacopoeial limits. The hardness of tablets from each batch was ranged from 4.6±0.5 to 5.6±0.6 kg/cm². Friability of each batch was found to be in the range of 0.25 % to 0.73% which indicates good mechanical stability for handling and transportation of the prepared tablet formulation. % Drug content of all the formulations was found to be in the range of 99.2% to 100.2±1.8 % respectively.

**In vitro buoyancy studies:**

To maintain buoyancy, gas generating agent plays crucial role. The gas generating agents immediately produce carbon dioxide when it comes in contact hydrochloric acid solution to generate sufficient porosity which helps the tablet to float. Formulation F1 to F3 prepared with HPMC K4M showed lag time of 5 to 30 sec and remained buoyant for 6 to 9 hr. Formulation F4-F6 prepared with HPMC K15M showed lag time 15 to 20 sec and remained buoyant for 7 to 11 hr. Formulation F7-F9 prepared with HPMC K100M showed lag time 10 to 45 sec and remained buoyant for 9 to 13 hr. This might be observed due to high viscosity of polymer which maintains the integrity of the tablet for longer duration by reducing the effect of erosion thus resulting in increase in floating time. In the formulation F10-F12 prepared with different...
viscosity grades of HPMC in combination with cellulose acetate showed lag time 20 to 30 sec and remained buoyant for >12hr. Similar results were obtained in the formulation F13-F15 prepared with different viscosity grades of HPMC in combination with ethyl cellulose N50 showed lag time 25 to 45 sec and remained buoyant for >12hr respectively.

**In vitro Drug Release of Clopidogrel bisulphate from floating matrix tablets:**

The in vitro Drug release of Clopidogrel bisulphate from floating matrix tablets were determined by using USP dissolution testing apparatus II (paddle type) at 50 rpm using 0.1N HCl. The release of Clopidogrel bisulphate mainly depends upon the polymer concentration. In vitro Drug release of from the formulations F1 to F3 ranged from 59.5 to 32.4% during the first hour and after 5 hrs F1 showed complete drug release. F2 and F3 extended the drug release upto 7hrs and 9hrs. Burst release was observed only in formulations containing low polymer concentration. Drug release of from the formulations F4 to F6 ranged from 33.6 to 19.2 % during the first hour, and after 5 hrs it was between 77.3 and 53.3 %. Formulations F4, F5 and F6 showed complete drug release within 7hrs, 9hrs and 11 hrs respectively. The drug release from formulations F7, F8 and F9 ranged from 26.7 to 11.4 during 1hr and after 5hrs it was between 64.3 to 39.2%. In vitro drug release extended up to 9 h, 11 h and 13 h containing high polymer concentration. It was found that the release rate of the drug from the tablets was found to decrease with increase in polymer concentration. Formulations F10, F11 and F12 containing different viscosity grades of HPMC in combination with Cellulose acetate extended drug release upto 12hrs. Formulations F13, F14 and F15 containing different viscosity grades of HPMC in combination with Ethyl Cellulose N50 extended drug release upto 11hrs.

Based on the results with all polymers, the order of the drug release was dependent on the type of polymer and polymer proportion. HPMC K100M showed more retardation than HPMC K15 M than HPMC K4 M. Single polymer dosage form yielded more retardation compared with combination of polymers in a formulation. Combination of polymers also increases the weight of the tablet which may cause inefficiency in floating for prolonged time which leads to poor bioavailability of the drug from the dosage form.

**In vitro release kinetics:**

The highest correlation coefficient was found with zero order in all the formulations in the range of 0.992 to 0.998 which indicates that the drug release is time dependent. Formulations F2, F8, F10, and F11 to F15 showed Krosmeyer peppas model. The results showed that most of the release exponent ‘n’ values were in between 0.309 to 0.873. It shows that the drug release follows anomalous or non-Fickian diffusion.

**% Swelling index:**

The release of drugs from matrix formulations has been linked to the nature of matrix material, as well as complex processes such as swelling, diffusion and erosion. Swelling index study was done with selected formulations F3, F6, F9 which showed extended drug release. All the formulations showed good swelling behaviour up to 12h showing greatest water absorption. Maximum swelling was observed to be 326.21% in F9 containing HPMC K100M and least 197.19 % in F3 containing HPMC K4 M. It was found that % swelling index of the formulations increased as the polymer concentration and viscosity was increased.

**% Erosion index:**
Erosion index study was done with selected formulations F3, F6, F9 which showed extended drug release. Maximum erosion occurred in formulation F3 – 81% containing HPMC K4M and least in formulation F9 containing HPMC K100M at the end of 12 h. All the formulations achieved good erosion index that correlates to the drug release mechanism. Swelling and erosion of the polymer occurred simultaneously, and both of them contributed to the overall drug-release rate. **In vivo assessment of Gastro retention**

In vivo studies were conducted on 3 healthy male human volunteers to find the gastric residence time of the tablet. The studies were based on x-ray radiography. For the in-vivo studies, formulation F9 was taken in which drug is replaced with barium sulphate, for the purpose of radio opacity needed in tracking the floating tablet in GIT. All the physicochemical properties were within the range. The tablets were given to the volunteers with a glass of water and standard diet was provided. X-rays were taken at different time intervals such as 15 min and 8 hours. The x-ray images show the tablet residence in stomach for 8 hours clearly indicating good floating property.

<table>
<thead>
<tr>
<th>Table 1: Formulation development of clopidogrel bisulphate floating matrix tablets</th>
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<tbody>
<tr>
<td><strong>Ingredient</strong></td>
</tr>
<tr>
<td>Clopidogrel bisulphate</td>
</tr>
<tr>
<td>HPMC K4M</td>
</tr>
<tr>
<td>HPMC K15M</td>
</tr>
<tr>
<td>HPMC K100M</td>
</tr>
<tr>
<td>Cellulose acetate</td>
</tr>
<tr>
<td>Ethyl Cellulose N50</td>
</tr>
<tr>
<td>Sodium Bicarbonate</td>
</tr>
<tr>
<td>Lactose</td>
</tr>
<tr>
<td>Aerosil</td>
</tr>
<tr>
<td>Talc</td>
</tr>
<tr>
<td>Magnesium stearate</td>
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<tr>
<td>Total weight (Mg)</td>
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<tr>
<th>Table 2: Formula used for development of floating matrix tablets, for <em>In Vivo</em> study</th>
</tr>
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<tr>
<td><strong>S. No</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>Total weight</td>
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</table>
Figure 1: In vitro Drug Release of Clopidogrel bisulphate floating matrix tablets prepared with HPMC K4m.

Figure 2: In vitro Drug Release of Clopidogrel bisulphate floating matrix tablets prepared with HPMC K15m.

Figure 3: In vitro Drug Release of Clopidogrel bisulphate floating matrix tablets prepared with HPMC K100m.
Figure 4: In vitro Drug Release of Clopidogrel bisulphate floating matrix tablets prepared with different viscosity grades of HPMC and cellulose acetate.

Figure 5: In vitro Drug Release of Clopidogrel bisulphate floating matrix tablets prepared with different viscosity grades of HPMC and Ethyl cellulose N50.

Figure 6: % Swelling index of Clopidogrel bisulphate floating matrix tablets for the selected formulations F-3, F-6, F-9.
Figure 7: % Erosion index of Clopidogrel bisulphate floating matrix tablets for the selected formulations F-3, F-6, F-9

Table 3: Invitro drug release kinetics of Clopidogrel bisulphate floating matrix tablets

<table>
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<tr>
<th>Formulation codes</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi</th>
<th>Hixon Crowell</th>
<th>Korsmayer peppas</th>
<th>n</th>
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<tr>
<td>F1</td>
<td>0.993</td>
<td>0.879</td>
<td>0.989</td>
<td>0.801</td>
<td>0.978</td>
<td>0.309</td>
</tr>
<tr>
<td>F2</td>
<td>0.978</td>
<td>0.858</td>
<td>0.996</td>
<td>0.582</td>
<td>0.996</td>
<td>0.507</td>
</tr>
<tr>
<td>F3</td>
<td>0.992</td>
<td>0.886</td>
<td>0.985</td>
<td>0.796</td>
<td>0.978</td>
<td>0.531</td>
</tr>
<tr>
<td>F4</td>
<td>0.993</td>
<td>0.918</td>
<td>0.991</td>
<td>0.865</td>
<td>0.991</td>
<td>0.532</td>
</tr>
<tr>
<td>F5</td>
<td>0.998</td>
<td>0.865</td>
<td>0.973</td>
<td>0.796</td>
<td>0.974</td>
<td>0.594</td>
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<tr>
<td>F6</td>
<td>0.996</td>
<td>0.820</td>
<td>0.976</td>
<td>0.713</td>
<td>0.989</td>
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</tr>
<tr>
<td>F7</td>
<td>0.995</td>
<td>0.869</td>
<td>0.986</td>
<td>0.771</td>
<td>0.987</td>
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<tr>
<td>F8</td>
<td>0.990</td>
<td>0.840</td>
<td>0.987</td>
<td>0.579</td>
<td>0.997</td>
<td>0.760</td>
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<tr>
<td>F9</td>
<td>0.996</td>
<td>0.789</td>
<td>0.974</td>
<td>0.483</td>
<td>0.994</td>
<td>0.873</td>
</tr>
<tr>
<td>F10</td>
<td>0.978</td>
<td>0.937</td>
<td>0.993</td>
<td>0.875</td>
<td>0.993</td>
<td>0.646</td>
</tr>
<tr>
<td>F11</td>
<td>0.992</td>
<td>0.913</td>
<td>0.978</td>
<td>0.861</td>
<td>0.987</td>
<td>0.823</td>
</tr>
<tr>
<td>F12</td>
<td>0.991</td>
<td>0.823</td>
<td>0.982</td>
<td>0.518</td>
<td>0.993</td>
<td>0.830</td>
</tr>
<tr>
<td>F13</td>
<td>0.978</td>
<td>0.852</td>
<td>0.993</td>
<td>0.511</td>
<td>0.994</td>
<td>0.629</td>
</tr>
<tr>
<td>F14</td>
<td>0.995</td>
<td>0.851</td>
<td>0.987</td>
<td>0.744</td>
<td>0.996</td>
<td>0.761</td>
</tr>
<tr>
<td>F15</td>
<td>0.984</td>
<td>0.850</td>
<td>0.984</td>
<td>0.522</td>
<td>0.989</td>
<td>0.735</td>
</tr>
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Figure 8: In-vitro buoyancy study of Clopidogrel bisulphate floating matrix tablets
After 15 minutes

Figure 9: In-vivo confirmation of Buoyancy using radiographic studies at 15min and 8hrs

Figure 10: FTIR spectra of clopidogrel bisulphate pure drug

Figure 11: FTIR spectra of clopidogrel bisulphate floating matrix tablet prepared with HPMC K100M
Figure 12: DSC thermogram of clopidogrel bisulphate pure drug

Figure 13: DSC thermogram of clopidogrel bisulphate floating matrix tablet prepared with HPMC K100M

Figure 14: SEM photographs of samples of formulation F9 at 1hr and 8hrs
CHARACTERIZATION OF DRUG AND EXCIPIENTS:

Fourier Transform Infrared Spectroscopy (FT-IR) study:
The physicochemical compatibility of the drugs and polymers was established through FTIR studies. FTIR study was conducted on the selected formulation F9 prepared with polymer HPMC K100M. IR spectral analysis of pure Clopidogrel bisulphate showed the peaks at wave numbers confirming the purity of drug with standard. The spectrum peak points of the formulation were similar with that of the pure Clopidogrel bisulphate clearly indicating that there is no drug-polymer interaction.

Differential Scanning Calorimetric (DSC) study:
Selected formulations of Clopidogrel bisulphate floating matrix tablets were characterized for DSC. The pure Clopidogrel bisulphate showed a sharp endothermic peak at 217.7 °C. Similar endothermic peaks were observed at similar temperature in the prepared tablets with HPMC K100M at 219.9 °C. The above study confirms that there was no drug polymer interaction.

Scanning electron microscopy:
SEM study was done for formulation F9 which is selected as the best formulation. SEM showed the formation of pores on the surface of the tablet during the in vitro dissolution studies which confirmed the swelling and erosion behaviour of the tablets prepared with HPMC K100M.

CONCLUSION

The objective of present investigation was concerned with the development of Clopidogrel bisulphate floating matrix tablets by direct compression method. The post-compression evaluation parameters showed that prepared floating matrix tablets of Clopidogrel bisulphate possessed optimum hardness and friability. This indicates good mechanical strength for handling and transportation of the prepared tablet formulation. The results of quality control tests were within pharmacopoeial limits. In vitro drug release of the formulations prepared with HPMC K100M extended unto 13 hrs and is selected as the best formulation. In vitro drug release profile of the prepared formulations followed zero order kinetics. This may in turn reduce the dosing frequency, thereby improving patient compliance. The release mechanism follows non Fickian diffusion. % Swelling and Erosion index of the polymer occurs simultaneously, and both of them contribute to the overall drug-release rate. FTIR and DSC study showed that there was no interaction between the drug and selected excipients and are compatible in formulating into dosage form.

ACKNOWLEDGEMENT

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