### MATERIALS

List of Materials/Excipients used in the study

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Name of Materials</th>
<th>Grade</th>
<th>Name of manufacture or Suppliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Metformin HCl</td>
<td>Pharma</td>
<td>Zydus Research Centre</td>
</tr>
<tr>
<td>2.</td>
<td>Paracetamol</td>
<td>Pharma</td>
<td>Piramal Healthcare Ltd. Baddi</td>
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<tr>
<td>3.</td>
<td>Diclofenac sodium</td>
<td>Pharma</td>
<td>BPRL, Bangalore</td>
</tr>
<tr>
<td>4.</td>
<td>Olsalazine sodium</td>
<td>Pharma</td>
<td>Karnataka Chemsyn Limited, Bangalore</td>
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<tr>
<td>5.</td>
<td>Ranitidine HCL</td>
<td>Pharma</td>
<td>Orchev Pharma Pvt. Ltd., Gujarat, India.</td>
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<tr>
<td>6.</td>
<td><em>Borassus flabellifer</em> endosperms</td>
<td>---</td>
<td>Local market, Udupi district</td>
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<tr>
<td>7.</td>
<td>Lactose – DCL 15</td>
<td>IP</td>
<td>Zydus Research Centre, Ahmadabad</td>
</tr>
<tr>
<td>8.</td>
<td>Croscarmellose sodium</td>
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<td>Zydus Research Centre, Ahmadabad</td>
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<td>9.</td>
<td>Microcrystalline cellulose</td>
<td>IP</td>
<td>Zydus Research Centre, Ahmadabad</td>
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<td>IP</td>
<td>S.D fine chemicals Ltd. Mumbai</td>
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<td>Gum Acacia</td>
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<td>Propyl paraben</td>
<td>LR</td>
<td>S.D fine chemicals Ltd. Mumbai</td>
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<td>13.</td>
<td>Methyl Paraben</td>
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<td>15.</td>
<td>Magnesium stearate</td>
<td>IP</td>
<td>Spectrochem Pvt. Ltd., Mumbai</td>
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<td>17.</td>
<td>Talc</td>
<td>IP</td>
<td>Lincoln Pharmaceutical Ltd, khatraj</td>
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<td>18.</td>
<td>Isopropyl alcohol</td>
<td>LR</td>
<td>Ranbaxy Fine Chemical Ltd., New Delhi</td>
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<td>19.</td>
<td>Acetone</td>
<td>LR</td>
<td>Ranbaxy Fine Chemical Ltd., New Delhi</td>
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<td>20.</td>
<td>Potassium Dihydrogen phosphate</td>
<td>LR</td>
<td>Ranbaxy Fine Chemical Ltd., New Delhi</td>
</tr>
<tr>
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<td>Hydrochloric acid</td>
<td>LR</td>
<td>Ranbaxy Fine Chemical Ltd., New Delhi</td>
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<td>22.</td>
<td>Ethanol</td>
<td>LR</td>
<td>Ranbaxy Fine Chemical Ltd., New Delhi</td>
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<td>23.</td>
<td>Chloroform</td>
<td>LR</td>
<td>Ranbaxy Fine Chemical Ltd., New Delhi</td>
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<td>24.</td>
<td>Citric acid monohydrate</td>
<td>LR</td>
<td>Ranbaxy Fine Chemical Ltd., New Delhi</td>
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<tr>
<td>25.</td>
<td>Sodium bicarbonate</td>
<td>LR</td>
<td>Spectrochem Pvt. Ltd., Mumbai</td>
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<td>26.</td>
<td>Glycerol</td>
<td>LR</td>
<td>S.D fine chemicals Ltd. Mumbai</td>
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<tr>
<td>27.</td>
<td>Disodium hydrogen phosphate</td>
<td>LR</td>
<td>S.D fine chemicals Ltd. Mumbai</td>
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</table>
## Table 7: Instruments/ Equipments used in present investigation

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Name of the instruments/ Equipments</th>
<th>Model and company name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>UV Spectrophotometer</td>
<td>UV 1601, Shimadzu</td>
</tr>
<tr>
<td>2.</td>
<td>FTIR spectrophotometer</td>
<td>FTIR 8300, Shimadzu, Japan</td>
</tr>
<tr>
<td>3.</td>
<td>Tablet compression machine</td>
<td>Cemach tablet press, India (12 station)</td>
</tr>
<tr>
<td>4.</td>
<td>Dissolution test apparatus</td>
<td>Electro Lab, Mumbai, India</td>
</tr>
<tr>
<td>5.</td>
<td>Electronic balance</td>
<td>CPA 225D, Sartorius</td>
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<tr>
<td>6.</td>
<td>Roche Friabilator</td>
<td>Electro Lab, Mumbai, India</td>
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<tr>
<td>7.</td>
<td>Hardness tester</td>
<td>Monsanto hardness tester</td>
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<td>8.</td>
<td>Tablet disintegration test machine</td>
<td>Electro Lab, Mumbai, India</td>
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<td>9.</td>
<td>Tapped density tester (USP)</td>
<td>ETD-1020, Electro lab</td>
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<td>10.</td>
<td>Differential scanning calorimetry</td>
<td>Shimadzu limited (DSC- 60), Japan</td>
</tr>
<tr>
<td>11.</td>
<td>Stability chamber</td>
<td>EIE instrument Pvt Ltd</td>
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<td>12.</td>
<td>Thermostat water bath</td>
<td>Thermo lab, Mumbai.</td>
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<td>13.</td>
<td>Tray dryer</td>
<td>EIE instrument Pvt Ltd</td>
</tr>
<tr>
<td>14.</td>
<td>Ultrasonifier</td>
<td>EIE instrument Pvt Ltd</td>
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<td>15.</td>
<td>Vernier caliper</td>
<td>Yuri measuring instrument</td>
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<td>16.</td>
<td>Stability chamber</td>
<td>EIE instrument Pvt Ltd</td>
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<td>17.</td>
<td>Brookfield DV-111+ Rheometer</td>
<td>Brookfield Engineering Laboratories Inc., USA</td>
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<td>18.</td>
<td>Scanning Electron Microscope (SEM)</td>
<td>Jeol, Japan</td>
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<td>19.</td>
<td>High Performance Liquid Chromatography</td>
<td>Shimadzu, Japan</td>
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<td>20.</td>
<td>Digital pH meter</td>
<td>Elico India Systronics, Ahmedabad</td>
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<td>21.</td>
<td>Autoclave</td>
<td>M.K. Laboratories, Bangalore</td>
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<td>22.</td>
<td>Water Distillation Unit</td>
<td>Bhanu Scientific Industries, Co. Pvt. Ltd., Bangalore</td>
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<td>23.</td>
<td>Hot air oven</td>
<td>PSM Industries, Bangalore</td>
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<td>24.</td>
<td>0.2 µm filter units</td>
<td>Minisart, Germany.</td>
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<td>25.</td>
<td>Centrifuge</td>
<td>Remi Equipments, Mumbai, India</td>
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<td>26.</td>
<td>Orbital Shaker Incubator</td>
<td>Plastocrafts, Bangalore</td>
</tr>
<tr>
<td>27.</td>
<td>Magnetic Stirrer 2MLH</td>
<td>Remi Equipments, Mumbai, India</td>
</tr>
<tr>
<td>28.</td>
<td>Test Sieve (no.)</td>
<td>Filterwel, India.</td>
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</tbody>
</table>
**METHODS**

**Preformulation studies on Borassus flabellifer mucilage (BFM)**

Preformulation studies were performed on the BFM, which included isolation, purification, physiochemical, phytochemical characterization and toxicity study of the mucilage.

**Collection of Borassus flabellifer endosperms**

*Borassus flabellifer* endosperms were purchased from local market of Udupi district, Karnataka, India.

**Authentication of Borassus flabellifer endosperm**

The authentication of the *Borassus flabellifer* endosperm was done by the scientific officer, Science center, Pilikula Nisargadhama, Vamanjoor, Mangalore, a herbarium of the selected part was done and reserved it for future reference.

**Isolation and purification of mucilage from Borassus flabellifer endosperm**

The endosperm of *Borassus flabellifer* fruit contains mucilage. To increase the yield of the mucilage, the endosperm of *Borassus flabellifer* fruit were extracted by different solvents.

The endosperm of *Borassus flabellifer* were collected, cut into small pieces and dried using tray dryer at 37°C for 24 h at room temperature, made fine powder by crushing in a mixer. The fine powder was soaked in different solvents such as water, hot-water, phosphate buffer solution (PBS) of pH 4.0, 6.8, 9.2, separately for 2-3h and heated up to 80-90°C for 30-45 min to release the mucilage into the solvents. The separated mucilage was then extracted by using muslin cloth to remove the marc and concentrated viscous solution under reduced pressure at 60-70°C. Acidified ethanol (5% HCl in 75% ethanol) was added to the concentrated viscous solution with constant stirring. The gel like precipitate was formed and separated by filtration. The precipitate was washed 2-3 times with 75% and 95% ethanol. After complete washing of the precipitate with ethanol 95%, brownish white powder was obtained. The powder was dried in an oven at 37°C, collected, powdered, passed through a sieve no.80 and stored in a desiccator till use. The brownish white powder was considered as mucilage for pharmaceutical use.

**Acute toxicity study on BFM**

The isolated *Borassus flabellifer* mucilage (BFM) was subjected to acute oral toxicity studies in rats according to OECD guidelines (no.423) to evaluate its toxicity and median lethal dose (LD$_{50}$). The animals used in the toxicity studies were sanctioned by the Institute Animal Ethical Committee (Approval No:KCP/IAEC/Ph.Ceutics/05/2011-2012).
Characterization of BFM

Percent yield

The percentage yield was calculated based on the amount of endosperm of *Borassus flabellifer* fruit used for the extraction process and the amount of dry water soluble mucilage obtained individually depends upon the solvents used. The percentage yield was calculated using the formula mentioned below,

\[
\text{% yield} = \frac{\text{Wt. of dried mucilage obtained}}{\text{Wt. of endosperm powder taken}} \times 100
\]

Organoleptic evaluation

The isolated mucilage was subjected for various organoleptic evaluations which included evaluation of color, odour, shape, taste and special features like touch and texture. The majority of information on the identity, purity and quality of the material can be drawn from these observations.

Physicochemical characterization of BFM

Solubility test

The extracted BFM was subjected for solubility test in different solvents as per British Pharmacopoeia specifications.

Loss on drying

An accurately weighed quantity of about 1 to 2 g of extracted BFM was taken in tarred petri dish. The powder was then evenly distributed and it was kept in a hot air oven at 105°C till a constant weight was recorded. The percentage loss of moisture on drying was calculated using the formula and expressed as a percentage.

\[
\text{% Loss on drying} = \left(\frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}}\right) \times 100
\]

Swelling index

Swelling index of BFM was determined by using reported method. 1 g of BFM powder was accurately weighed and carefully transferred into a 50 mL measuring cylinder. The volume was made up to 50 mL with distilled water. The cylinder was firmly closed and shaken vigorously every 10 min for 1 h and then allowed to stand undisturbed for 24 h. The volume occupied by the material under test after the entire 24 h was measured. The mean of triplicate determinations was recorded as the swelling index. Swelling index (SI) is expressed as a percentage and calculated according to the following equation.

\[
\text{% swelling Index} = \left(\frac{\text{Initial volume of powder in measuring cylinder}}{\text{Final volume of powder in measuring cylinder}}\right) \times 100
\]
The same procedure was repeated using different media such as 0.1 N hydrochloric acid and pH 7.4 phosphate buffer.

**pH determination**

pH of the 1\%w/v solution of the BFM was determined using a digital pH meter.

**Determination of surface tension of BFM**

The surface tension of the BFM was determined by drop count method, using a stalagmometer. The stalagmometer was filled with purified water above the upper mark. Using the screw pinch cork, the flow rate was adjusted to 10-15 drops/min. Then, number of drops of water was counted between the marks of the stalagmometer ($n_1$). The water was removed and the stalagmometer was filled with the BFM solution (0.1\%w/v) and number of drops was counted ($n_2$). The surface tension of the BFM was determined using formula given below.

$$\text{Surface tension } (\gamma_2) = \frac{n_2 \rho_1 \gamma_1}{n_1 \rho_2}$$

Where, $n_1$=number of drops of water  
$n_2$=number of drops of sample  
$\rho_1$=density of water (0.9956 g/mL)  
$\rho_2$=density of sample  
$\gamma_1$=surface tension of water (71.18 dynes/cm)

**Determination of Zeta potential**

Zeta potential was measured by using a Zeta master (Malvern, UK). The BFM powder was dispersed in Phosphate buffer pH 6.8 by using ultrasonication for 30min, and the cell was filled with the BFM suspension and zeta potential was measured.

**Ash values**

Ash values such as total ash, acid insoluble ash and water-soluble ash were determined according to Indian Pharmacopoeia. The following procedures were used for determination of ash values.

**Total Ash**

About 2 g of the powdered BFM was accurately weighed in a tarred silica crucible. Then it was incinerated at a temperature not exceeding 450ºC for 4 h, until free from carbon, cooled and weighed. The percentage of total ash was calculated with reference to air dried sample.

$$\% \text{ Total ash value} = \left(\frac{\text{Weight of total ash}}{\text{Weight of the sample taken}}\right) \times 100$$
Acid Insoluble Ash

The ash obtained from the above step was boiled for 5 min with 25 ml of 2 M HCl. Filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited in a tarred crucible at a temperature not exceeding 450°C for 4 h. Cooled in a desiccator and weighed. Calculated the percentage of acid insoluble ash with reference to the air-dried drug using following formula,

\[
\text{% Acid insoluble ash value} = \left( \frac{\text{Weight of acid insoluble ash}}{\text{Weight of the sample taken}} \right) \times 100
\]

Water-soluble Ash

The ash obtained from the above step was boiled with 25 ml of water. Filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited in a tarred crucible at a temperature not exceeding 450°C for 4 h. Cooled in a desiccator and weighed. Subtracted the weight of insoluble matter from the total weight of ash. The difference in weight represents the weight of water soluble ash. Calculated the percentage of water soluble ash with reference to the air-dried drug using the following formula,

\[
\text{% Water soluble ash value} = \left( \frac{\text{Weight of total ash} - \text{Weight of water insoluble ash}}{\text{Weight of the sample taken}} \right) \times 100
\]

Melting point determination

The powdered sample of BFM was transferred into a capillary tube and by using melting point apparatus melting point was determined.

Viscosity determination

Rheological studies of BFM were carried out using varying concentrations (0.1–0.5% w/v) prepared in distilled water. The viscosities were measured using a Brookfield viscometer.

Microbial Count

Specified amount (10 g) of the sample was dissolved in a suitable medium to have no antibacterial activity under conditions of test and the volume was adjusted to 100 mL with the same medium. The pH was adjusted to 7.

Examination for Bacteria and Fungi

To a petri dish of 10 cm diameter, 20 mL of nutrient agar was added at temperature not more than 45°C. The sample solution was spread on the surface of the solidified medium. The Petri dishes of required number were prepared and incubated at 37°C for 24 h. but, sabouraud dextrose agar medium was used for fungi and the plate was incubated at 28°C for 48 h. The number of colonies formed was counted.
Thermal stability
A sufficient quantity of the powdered mucilage was taken in a petridish and exposed to successive higher temperatures (30°C, 40°C, 50°C, etc.). The temperature at which the product showed a change in color was noted. For thermal stability under liquid conditions, 1% solution of mucilage was exposed to successive higher temperatures (30°C, 40°C, 50°C, etc...) and the temperature at which the product showed a change in viscosity was noted.

Thermo gravimetric analysis
Thermo gravimetric analysis (TGA) of BFM was performed in a Shimadzu TGA apparatus Japan.

Differential scanning calorimetry study
Thermal properties of BFM were characterized using a DSC-60, Shimadzu limited, Japan.

Fourier transform-infra red spectral study
The Fourier transform-infra red (FT-IR) spectrum of the BFM was recorded by using FTIR 8300, Shimadzu, Japan, using potassium bromide (KBr) discs.

X-ray powder diffraction study
X-ray diffraction (XRPD) patterns of the BFM were analyzed using a Siemens D5000 X-ray diffractometer, Germany.

Microstructure studies
The morphological features of the BFM were studied with scanning electron microscope (SEM, JEOL, Japan).

Elemental analysis
Elemental analysis of carbon, hydrogen and nitrogen was carried using a Thermo Finnegan FLASH EA 1112 CHNS-2000 analyser.

Phytochemical screening of BFM
The extracted BFM was tested for chemical characteristics for identification, Ruthenium red test, Molisch’s test, test for reducing sugars, test for Tannins, test for chlorides, Test for sulphates, test for Uronic acid, Test for flavanoids, Test for steroids, Test for saponins, Test for tannins, Test for phenols and test for alkaloids. The BFM was also tested for unwanted chemicals viz; foreign matter, heavy metal and arsenic.

Micrometric characterization of BFM
Particle size
The particle size of the BFM was determined by the microscopic method.
Angle of repose

Angle of repose was determined by the fixed funnel method. The height and mean diameters of the powder cones were determined and the tangent of the angle of repose calculated using the equation:

\[ \tan \theta = \frac{h}{r} \]

Bulk Density and Tapped Density

The bulk density of BFM was determined by the three-tap method. Weighed quantity of BFM powder was carefully introduced into a 100 mL graduated cylinder. The cylinder was dropped onto a hard wood surface 3 times from a height of 2.5 cm at an interval of 2 seconds. The bulk density was calculated by using the equation

**Bulk density = Mass of the dry powder/Bulk volume**

Tapped density

The weighed quantity of dry powder was taken in a graduated cylinder. The cylinder was placed on the tap density tester and subjected to 250 drops per minute and drop height is 3mm±10%. The volume of powdered bed is measured after each increment of 250 drops until the difference of last two volume measurement is zero and the tapped volume was recorded.

The tapped density was calculated by using the equation

**Tapped density = Mass of the dry powder/Tapped volume**

Carr’s Consolidation Index/ Compressibility Index

It is a simple test to evaluate the LBD and TBD of a powder and the rate at which it packed down. Carr’s Index can be calculated by following equation

**Carr’s Index (%) = \([(TBD-LBD) x 100]/TBD] **

Hausner Ratio

It was determined by using the Following formula

**Hausner Ratio= Tapped bulk density/ Loose bulk density**

True density

True density was determined by liquid displacement method at 25°C. The weight \((W_1)\) of the clean, dry 50mL density bottle was determined. The bottle was filled with water and the top of the bottle was dried with filter paper and weighed as \((W_2)\). The procedure was repeated using benzene to obtain the weight of the bottle plus benzene \((W_3)\). Benzene was used as the displacement liquid. About 3g of the BFM powder was transferred to dried
density bottle and weighed as \(W_4\). The bottle was filled with benzene and the weight \(W_5\)
was measured. The density of the benzene used was calculated using the formula.

\[
\text{Density of benzene } (\rho) = \frac{(W_5 - W_1)}{(W_2 - W_1)} \times 0.9971
\]

\((\text{Density of water at } 25^\circ C = 0.9971 \text{ g/cc})\)

The true density of BFM was calculated from the following formula,

\[
\text{Density of sample} = \frac{\left(\frac{W_4 - W_1}{\rho}\right)}{\left(\frac{W_2 - W_1}{\rho}\right) - \left(\frac{W_5 - W_4}{\rho}\right)}
\]

**Accelerated stability study**

BFM was subjected to accelerated stability studies according to ICH guidelines to
predict the stability of mucilage. The samples were analyzed at regular intervals as per the
stability protocol.

**Stability protocol for Mucilage**

**Mucilage: Borassus flabellifer mucilage**

**Date of starting:** 01-12-2011

**Quantity loaded:** 20 g approx. in each petri dish

**Quantity Sample:** 2 g approx.

**Table 8: Accelerated stability study protocol of BFM**

<table>
<thead>
<tr>
<th>Duration</th>
<th>40°C±2/75% RH</th>
<th>Test</th>
</tr>
</thead>
<tbody>
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<td>Initial</td>
<td>01-12-2011</td>
<td>1. Organoleptic evaluation</td>
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<tr>
<td>6 Month</td>
<td>01-05-2012</td>
<td>2. pH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Moisture content</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Microbial count</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5. FTIR</td>
</tr>
</tbody>
</table>
Figure 5: Dried crude BFM

Figure 6: Trituration of crude BFM

Figure 7: Cloudy mass of isolated BFM

Figure 8: Isolated BFM

Figure 9: Dried isolated BFM

Figure 10: Powdered isolated BFM
EXPLORATION OF THE DISINTEGRATING PROPERTY OF BFM

Preformulation studies on Metformin HCl

Description/Appearance
Description and appearance were tested by physical observation to see if it matches with the specifications in the Pharmacopoeia.

pH of the solution
The pH of the 1% w/v of drug solution in water was measured using pH meter.

Solubility Profile
Solubility of Metformin HCl was determined in different solvents. Solubility studies were performed by taking excess amount of Metformin HCl in different beakers containing the solvents. The mixtures were shaken for 24 hrs at regular intervals. The solutions were filtered by using whatmann’s filter paper grade no. 41. The filtered solutions are analyzed spectrophotometrically at 233nm.

Analytical method for estimation of the drug
Weighed quantity of Metformin HCl (100mg) was dissolved in minimum quantity of distilled water and the volume made up to 100ml with simulated intestinal fluid (pH 6.8) to give a concentration of 1000mcg/ml. This was further diluted 10 times to get a concentration of 100 mcg/ml. Aliquots of 0.2, 0.4, and 0.6 up to 1.4 ml of stock solution were pipetted into 10ml volumetric flask and the volume was made up to 10ml with SIF (pH 6.8) to get concentrations of 2, 4, and 6 up to 14 mcg/ml. The absorbance was measured at 233 nm against reagent blank (SIF, pH 6.8).

Drug-excipient compatibility studies

Fourier Transform Infrared (FTIR) Spectroscopy
The FTIR spectra of Metformin hydrochloride and physical mixture of drug with other excipients were obtained by using a FTIR spectrophotometer.
Differential Scanning Calorimetry (DSC) analysis

DSC analysis of drug and different excipients were performed using Shimadzu DSC-60, Japan.

Formulation of fast dissolving tablets of Metformin HCl

Fast dissolving tablets of Metformin HCl were prepared by using direct compression method using BFM powder and Ac-Di-Sol® at concentrations of 0.5, 1, 1.5, 2 and 2.5 % w/w. All the required ingredients as per the formulation table were weighed and passed through size 40# sieve. The Mixture was then blended in a double cone blender for 15 min. The powder blend was evaluated for flow properties. The composition of each formulation is given in table 9.

Table 9: Composition of different batches of Metformin HCl FDT’S

<table>
<thead>
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<th>Ingredients( mg/ tablet)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
<th>F10</th>
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<tbody>
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<td>500</td>
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<tr>
<td>BFM*</td>
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<td>6</td>
<td>9</td>
<td>12</td>
<td>15</td>
<td>--</td>
<td>--</td>
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</tr>
<tr>
<td>Cross Carmellose Sodium</td>
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<td>3</td>
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<td>3</td>
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</tr>
<tr>
<td>Aerosil</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Flavor (Orange)</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Avicel</td>
<td>73</td>
<td>70</td>
<td>67</td>
<td>64</td>
<td>61</td>
<td>73</td>
<td>70</td>
<td>67</td>
<td>64</td>
<td>61</td>
</tr>
<tr>
<td>Total weight of tablet</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
</tr>
</tbody>
</table>

BFM*: Borassus flabellifer mucilage

Evaluation of Metformin HCl powder Blend

The angle of repose, bulk density, tapped density, compressibility index, Hausner ratio of Metformin HCl powder blend were determined as per the method described in the preformulation studies on BFM.

Compression of tablet

After evaluation of powder blend it was then blended with talc, magnesium stearate, aerosil and compressed into tablets using Cemach rotary tablet punching machine.
Evaluation of Metformin HCl FDT’s\textsuperscript{80-84}

All the tablets were evaluated for following different parameters which includes;

**General appearance**

Five tablets from different batches were randomly selected and organoleptic properties such as color, odor, taste, shape, were evaluated.

**Thickness and diameter**

Thickness and diameter of five tablets from each batch were determined using Vernier caliper.

**Hardness**

The hardness of five tablets from each batch was determined using the Monsanto hardness tester.

**Friability**

The friability of the tablet was determined using Roche Friabilator. 10 tablets from each batch were subjected for friability test. The tablets were weighed again ($W_{\text{final}}$). The % friability was then calculated by

$$F = \frac{W_{\text{initial}} - W_{\text{final}}}{W_{\text{initial}}} \times 100$$

**Weight Variation**

Twenty tablets from each batch were randomly selected and their individual weight was noted and the average weight and standard deviation of 20 tablets were determined.

**In vitro dispersion test**

*In vitro* dispersion time was measured by placing a tablet in a petridish containing 6 ml of phosphate buffer pH 6.8 and the time taken for the tablet to completely disintegrate into fine particles was noted.

**In vitro disintegration test**

The *in-vitro* disintegration time of a tablet was determined using disintegration test apparatus using phosphate buffer pH 6.8 maintained at 37±2°C. The time taken for the tablet to disintegrate completely with no palpable mass remaining in the apparatus was measured and recorded.

**Drug content**

20 tablets from each batch were analyzed for drug content at 233 nm using UV-Visible spectrophotometer.
**Wetting time**

A piece of tissue paper folded twice was placed in a petri dish containing 6 ml of simulated saliva pH 6.8. A tablet having amaranth powder on the upper surface was placed on the tissue paper. Time required to develop red color on the upper surface of tablet was recorded as wetting time.

**Water absorption ratio**

A piece of tissue paper folded twice was placed in a small petri dish containing 6 ml of simulated intestinal fluid (pH 6.8). A tablet was put on the paper and the time required for complete wetting was measured. The wetted tablet was then weighed.

Water absorption ratio R, was determined using following equation,

\[ R = \frac{W_a - W_b}{W_b} \times 100 \]

Where \( W_a \) = weight of tablet after absorption; \( W_b \) = weight of tablet before absorption

*In vitro dissolution study*

The *in vitro* release of drug from fast dissolving tablets was determined using USP Dissolution Testing Apparatus II. The dissolution test was performed using 900 ml of SIF (pH 6.8), at 37±0.5°C at 50 rpm. At regular interval 5ml of the sample was withdrawn and samples were filtered through Whatmann’s filter paper no. 41. Absorbance of these solutions was measured at 233 nm using UV spectrophotometer.

*Water uptake study*

Ten tablets from each formulation were kept in a desiccator, over calcium chloride, at 37°C for 24 hours. This was done to remove maximum amount of moisture as possible from the tablets. The tablets were weighed and exposed to 82.5% RH at room temperature for a week. One batch of control tablets was kept to assess the moisture uptake due to other excipients. The tablets were weighed and the increase in weight was reported.

*In vivo disintegration time*

Six healthy human volunteers were selected and their written consent was obtained. Each volunteer randomly took one tablet and kept on the tongue. The time taken for complete disintegration of the tablet on the tongue was noted. It is expressed in seconds.

*Mouth feel*

The same human volunteers participated in taste evaluation test, were asked to give their opinion about the feeling of smoothness or grittiness of the dispersion soon after the tablet got disintegrated.
Drug release kinetics

To examine the drug release kinetics and mechanism, the release kinetics of the developed formulations were analyzed according to different kinetic models.

Scanning Electron Microscopy (SEM)

The surface morphology of the optimized batch was examined by using Jeol- JSM-5600 LV, Japan at 35 X magnification.

Stability studies

The optimized formulations were tested for stability study as per ICH guidelines. The tablets were stored at 25±2°C/60 ±5% RH and 40±2°C/75 ±5% RH test conditions for three months. After an interval of one month samples were withdrawn and tested for disintegration time, hardness, friability, drug content and in vitro drug release.

EXPLORATION OF THE BINDING PROPERTY OF BFM

Preformulation studies on paracetamol

Solubility

Solubility of paracetamol was determined in ethanol (95%), and acetone. Solubility studies were performed by taking excess amount of paracetamol in different beakers containing the solvents. The mixtures were shaken for 24 hrs at regular intervals. The solutions were filtered by using whattmann’s filter paper grade no. 41. The filtered solutions are analyzed spectrophotometrically at 243nm.

Loss on drying

Loss on drying was determined by accurately weighing 1 gm of the drug and drying at 105°C for three hours.

Determination of melting point

Melting point of paracetamol was determined by capillary method. The powder at what temperature it melts was noticed.

Determination of \( \lambda_{\text{max}} \)

A solution of paracetamol containing the concentration 10µg/ml was prepared in acetone and UV spectrum was taken using Shimadzu 1601UV-Visible double beam spectrophotometer. The solution was scanned in the range of 200-400 nm.

Preparation of standard calibration curve of paracetamol

100 mg of paracetamol was accurately weighed and transferred into 100 ml volumetric flask. It was dissolved and made up to the volume with 0.1N HCL to give stock
solution containing 1000µg/ml. From the standard stock solution, 10 ml solution was diluted with 100 ml 0.1N HCl (100µg/ml). Appropriate aliquots were taken into different volumetric flask and made up to 10 ml with 0.1 N HCl so as to get six different concentrations (2, 4, 6, 8, 10, µg/ml). The UV absorbance readings were taken at 243 nm using UV/visible spectrophotometer.

**Drug-excipient compatibility study**

**Fourier Transform Infrared (FTIR) Spectral analysis**

The FTIR spectra of pure drug and physical mixture of drug with other excipients were obtained by using a FTIR spectrophotometer.

**Differential Scanning Calorimetry (DSC) analysis**

DSC analysis of drug and different excipients were performed using Shimadzu DSC-60 thermal analyser.

**Formulation of paracetamol tablets**

Wet granulation method was used to prepare paracetamol granules. The binder concentrations used were 2, 4, 6, 8 and 10% w/w. The compositions of tablets for each batch were given in table 10. Paracetamol and lactose was thoroughly mixed and the binder solution of specified concentration was added to moisten the powder blend. The wet coherent mass was granulated by passing them through sieve number 12. The granules were dried at 60°C for 1 to 2 hrs in hot air oven. The dried granules were passed through sieve no.22/44. Similar procedure was used for the preparation of paracetamol tablets using 10% w/w starch paste as a standard binder.

**Table 10: Composition of different batches of paracetamol tablets containing BFM**

<table>
<thead>
<tr>
<th>Ingredients (mg/tablet)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol IP</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Starch paste</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>60</td>
</tr>
<tr>
<td>Starch powder</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>BFM*</td>
<td>12</td>
<td>24</td>
<td>36</td>
<td>48</td>
<td>60</td>
<td>--</td>
</tr>
<tr>
<td>Orange flavour</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Talc</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Aerosil</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Aspartame</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Lactose</td>
<td>49</td>
<td>37</td>
<td>25</td>
<td>13</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total weight of tablet</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
</tr>
</tbody>
</table>

BFM* -- *Borassus flabellifer* mucilage; F1 to F5 – granulated using BFM; F6 – granulated using starch paste (10%w/w)
Pre-compression evaluation of paracetamol granules

The angle of repose, bulk density, tapped density, compressibility index, Hausner ratio of paracetamol granules were determined as per the method described in the exploration of disintegrating property of BFM.

Surface analysis by SEM

The morphological feature of the paracetamol granules prepared with 8% concentration of the BFM was studied with scanning electron microscope of JEOL, Tokyo, Japan.

True density

True (particle) densities were determined by pycnometer method, with xylene as the displacement fluid. The formula for calculating true density is as below:

\[
\text{True density} = \frac{W_2 - W_1}{W_4 - W_3}
\]

Where,

- \(W_1\) = Weight of pycnometer
- \(W_2\) = Weight of xylene
- \(W_3\) = Weight of sample
- \(W_4\) = Weight of pycnometer, xylene and sample

Compression of tablet

After performing precompression test on granules the dried granules were lubricated with magnesium stearate, talc and aerosil and then compressed into tablets by using 12 mm flat face round tooling on a Cemach rotary tablet machine. The batch size of 100 tablets was prepared.

Evaluation of paracetamol tablets

The prepared tablets were evaluated for general appearance, hardness, friability, weight variation, thickness, diameter as per the method described in the exploration of disintegrating property of BFM.

The other evaluation tests were carried out on formulated tablets which includes;

Drug content

Drug content of different batches of paracetamol tablets were estimated by using UV-VIS spectrophotometer at 243 nm.

Disintegration test

The test was performed using disintegration test apparatus by using 0.1N HCL maintained at 37°C.
In vitro dissolution testing

The release rate of paracetamol from tablets was determined using USP Dissolution Testing Apparatus II. The dissolution test was performed using 900 ml of 0.1NHCL, at 37±0.5°C at 50 rpm. At regular interval 10 ml of the sample was withdrawn and filtered through and absorbance of these solutions was measured at 243 nm using UV spectrophotometer.

Surface analysis by SEM

The morphological feature of the optimized paracetamol tablet formulation F4 and tablets without BFM was studied with scanning electron microscope of JEOL, Tokyo, Japan.

Stability studies

Stability studies were carried out on satisfactory formulation F4 as per ICH guidelines for three month and various parameters were studied during stability study.

EXPLORATION OF MATRIX FORMING PROPERTY OF BFM

Preformulation Studies of diclofenac sodium

Melting point of diclofenac sodium and solubility of diclofenac sodium in different solvents were determined as per the methods described in the exploration of the binding property of BFM.

Determination of \( \lambda_{\text{max}} \)

A solution of diclofenac sodium containing the concentration 10 µg/ ml was prepared in acetone and UV spectrum was taken using Shimadzu 1601 UV/Vis double beam spectrophotometer. The solution was scanned in the range of 200-400 nm.

Preparation of standard calibration curve of diclofenac sodium

100 mg of diclofenac sodium was accurately weighed and transferred into 100 ml volumetric flask. It was dissolved and made up to the volume with 0.1N HCL to give stock solution containing 1000µg/ml. From the standard stock solution, 10 ml solution was diluted with 100 ml 0.1N HCl (100µg/ml). Appropriate aliquots were taken into different volumetric flask and made up to 10 ml with 0.1 N HCl so as to get six different concentrations (4, 6, 8, 10 and 12 µg/ml). The UV absorbance readings were taken at 276 nm using UV/visible spectrophotometer. Similarly the standard curve of Diclofenac sodium in phosphate buffer pH 6.8 was prepared by same method as described earlier.
Drug-excipient compatibility study

Fourier Transform Infrared (FTIR) Spectral analysis

FTIR spectra of pure drug and physical mixture of drug and excipients were recorded by using a FTIR Spectrophotometer.

Differential Scanning Calorimetry (DSC) analysis

DSC analysis of drug and excipients were performed using Shimadzu DSC-60, Japan.

Formulation of diclofenac sodium matrix tablets

Sustained release matrix tablets of diclofenac sodium with BFM were prepared using different concentration of BFM viz., 2.5, 5, 7.5, 10 and 12.5% w/w. Wet granulation method was used to prepare granules of drug using IPA: water (3:1) as binder solvent. Required quantity of BFM, diclofenac sodium and lactose were mixed, granulated with IPA: water (3:1). The wet mass was granulated by passing them through sieve number 12. The granules were dried at 60°C for 1 to 2 hrs in hot air oven. The dried granules were passed through sieve no.22/44 blended with a mixture of talc and magnesium stearate and compressed into tablets using rotary tablet press. Similar procedure was used for preparation of diclofenac tablets using 12.5% w/w guar gum as a known matrix polymer. The compositions of each formulation were shown in Table 11.

Table 11: Composition of different batches of diclofenac sodium matrix tablets

<table>
<thead>
<tr>
<th>Ingredients (mg/tablet)</th>
<th>Formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>100</td>
</tr>
<tr>
<td>BFM*</td>
<td>6.25</td>
</tr>
<tr>
<td>Guar gum</td>
<td>--</td>
</tr>
<tr>
<td>Lactose</td>
<td>136.25</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>5</td>
</tr>
<tr>
<td>Talc</td>
<td>2.5</td>
</tr>
<tr>
<td>Total weight of tablet</td>
<td>250</td>
</tr>
</tbody>
</table>

BFM: *Borassus flabellifer* mucilage; # All quantities are in milligrams; # all the batches contained 1% w/w talc and 2% w/w magnesium stearate

Evaluation of diclofenac sodium granules

The angle of repose, bulk density, tapped density, compressibility index, Hausner ratio and total porosity of diclofenac sodium granules were determined as per the method described in the exploration of binding property of BFM.
Evaluation of diclofenac sodium matrix tablets

The prepared tablets were evaluated for general appearance, hardness, friability, weight variation, thickness, diameter as per the method described in the exploration of disintegrating property of BFM. The other evaluation tests were carried out on formulated tablets which includes;

Drug content

Drug content of each batch of diclofenac sodium matrix tablets were determined by measuring the absorbance at 276 nm using a Shimadzu UV-Vis double beam spectrophotometer 1601.

Swelling index

One tablet from each formulation was kept in a petri dish containing pH 6.8 phosphate buffers. At the end of 2, 4, 6, 8, 10 and 12 hrs tablets were withdrawn, soaked on tissue paper and weighed and then percentage weight gain by the tablet was calculated by formula;

\[
SI\% = \frac{(\text{weight of swollen tablet} - \text{initial weight of tablet})}{\text{(initial weight of tablet)}} \times 100
\]

Disintegration test

The test was performed using disintegration test apparatus by using pH 6.8 phosphate buffer maintained at 37°C.

In vitro drug release study

The release rate of diclofenac sodium from tablets was determined using USP Dissolution Testing Apparatus II. The dissolution test was performed using 900 ml of 0.1N HCl for 2 h, at 37±0.5°C at 50 rpm and then it was replaced with 900 ml of pH 6.8-phosphate buffer and study was continued upto 12 h. At regular interval 5 ml of the sample was withdrawn, filtered and absorbance of these solutions was measured at 276 nm using UV spectrophotometer.

Kinetics of drug release

To examine the drug release kinetics and mechanism, the in vitro release data were fitted to different kinetic models.

Scanning Electron Microscopy

The optimized formulation (F5) was selected for SEM analysis. The tablet surface morphology was taken before dissolution and 12th hour of dissolution.
Stability studies

Stability studies were carried out on satisfied formulation F5 as per ICH guidelines for three month and various parameters were studied during stability study.

**EXPLORATION OF SUSPENDING PROPERTY OF BFM**

Preformulation studies of paracetamol were conducted as per the methods given in exploration of binding property of BFM in paracetamol tablets.

**Drug-excipient compatibility study**

**Fourier Transform Infrared (FTIR) Spectral analysis**

FTIR spectra of pure drug and physical mixture of drug and excipients were recorded by using a FTIR spectrophotometer.

**Differential Scanning Calorimetry (DSC) analysis**

DSC analysis of drug and excipients were performed by using Shimadzu DSC-60, Japan.

**Formulation of paracetamol suspension**

Paracetamol suspension was prepared according to formula given in table 12. Paracetamol suspension was prepared using different concentration of BFM in the concentration 1, 1.5, 2.0 and 2.5%w/v. The required quantity of BFM powder was added to the powdered drug and little quantity of water was added and triturated until homogeneous paste was obtained. To these required quantity of preservatives like methyl and propyl paraben, then citric acid, vanillin flavor, aspartame, amaranth powder was added and further triturated to form a homogenous mixture. The mixture was transferred to a 100 ml calibrated bottle and the volume was made up using sufficient quantity of distilled water. The suspensions were stored in stoppered glass bottles. All the prepared suspensions were deflocculated. Flocculated suspensions were prepared using magnesium aluminium silicate (0.04mol) as flocculating agent. The procedure was repeated for the preparation of suspensions containing 1, 1.5, 2.0 and 2.5%w/v of tragacanth powder.

**Evaluation of paracetamol suspension**

**Physical test**

The prepared suspension was subjected for physical test. At weekly intervals, for a period of 4 weeks, the prepared suspensions were observed for physical changes such as aggregation, caking and crystal growth formation.
TABLE 12: Composition of different batches of paracetamol suspension

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol (g)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>BFM* (g)</td>
<td>1</td>
<td>1.5</td>
<td>2.0</td>
<td>2.5</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Gum tragacanth (g)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1</td>
<td>1.5</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Methyl paraben (mg)</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Propyl paraben (mg)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Citric acid (mg)</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Vanillin flavour (mg)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Aspartame (mg)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Amaranth powder (mg)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Purified water q.s. to ml</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

BFM* *Borassus Flabellifer* mucilage

**Sedimentation volume and rate**

The sedimentation volume of the suspensions were determined by keeping 50 ml of each suspensions in stoppered measuring cylinder and stored undisturbed at room temperature. The volume of the sediments in the suspension was noted on daily basis for 7 days and thereafter weekly for 49 days (7 weeks). From the values of F obtained, Graphs of sedimentation volume (Vu/Vo) against time were plotted, from which sedimentation rate was calculated.

**Redispersibility**

Fixed volume (50 ml) of the each suspension was kept in calibrated tubes which were then stored at room temperature for various time intervals (5, 15, 25 and 30 days). At regular interval of 5 day, one tube was removed and shaken vigorously to redistribute the sediment and the presence of deposit if any is recorded.

**Rheological assessment**

The time required for each suspension sample to flow through a 10 ml pipette was determined and the apparent viscosity (ηα in ml⁻¹) was calculated using the equation:

\[
\text{Flow rate} = \eta_\alpha = \frac{\text{Volume of pipette (ml)}}{\text{Flow time (seconds)}}
\]

The viscosity (in poise) of the samples was determined at 25°C using Brookfield viscometer at 50 rpm by using spindle no.3.

**Microscopical examination**

Samples of the suspensions formulated using the BFM and gum tragacanth as suspending agents were microscopically examined for crystal growth under a metallurgical microscope (Model: NJF-120A, Japan). A drop of each sample was put on a slide and placed
on the stage of the microscope. The objects were viewed at X100 magnification from the screen attached. The photomicrographs were printed out.

**Particle size analysis**

The particle size distribution of paracetamol in the suspension was determined using optical microscope. A total of 200 particles were counted and their size was determined.

**pH**

The pH of the suspensions was determined at intervals of one week for 21 days using pH meter.

**Drug content**

Drug content of each batch of paracetamol suspension were determined after suitable dilution by using UV-Visible spectrophotometer at 243 nm.

**In vitro drug dissolution study**

The release rate of paracetamol from paracetamol suspension was determined using USP Dissolution Testing Apparatus II. The dissolution test was performed using 900 ml of 0.1NHCL at 37±0.5°C at 50 rpm. At regular interval 2 ml of the sample was withdrawn, filtered and absorbance of these solutions was measured at 243 nm using UV spectrophotometer.

**Microbiological evaluation**

The microbial loads of formulations F3 and F7 were determined according to the BP 2010. This was done on day 0 of formulation and on day 14 following storage under ambient conditions.

**Stability study**

Stability studies were carried out on optimized formulation as per ICH guidelines for three month and various parameters were studied during stability study.

**EXPLORATION OF GELLING PROPERTY OF BFM**

Preformation studies of diclofenac sodium were performed as per the methods mentioned in the exploration of matrix forming property of BFM in diclofenac tablets.

**Preparation of diclofenac gel**

Required quantity of BFM was dispersed in hot water using a magnetic stirrer to achieve complete dispersion, to this required quantity of glycerin, drug and methyl paraben were added and stirred well. Finally it was made upto the required weight with distilled water. Eight batches of diclofenac gels were prepared and stored in cool place until further use. The composition of each batches of diclofenac gels were presented in table 13.
TABLE 13: Formulation of different batches of diclofenac gel containing BFM

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
</tr>
</thead>
<tbody>
<tr>
<td>BFM*</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Tragacanth</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Glycerin</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Purified water q.s. to (g)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

BFM* *Borassus flabellifer* mucilage

Evaluation of diclofenac gels

Viscosity determination

Viscosity of diclofenac gels were determined using Brookfield viscometer with a shear rate of 5 rpm for 5 min.

pH Determination

Two grams of prepared gel was dissolved in the 100 ml of phosphate buffer solution and pH of the resulted solution was measured by Elico digital pH meter at 25±1°C.

*In vitro diffusion profile*

One gram of the prepared gel was taken in the permeation cell and the permeation cell was immersed in a beaker containing 100 ml of phosphate buffer of pH 6.8. The cell was immersed in to a depth of 1 cm below the surface of buffer, which was agitated by a magnetic stirrer and the temperature was maintained at 37°C±1°C. Specified volume of the sample was withdrawn from the receptor compartment periodically. The drug concentration was determined spectrophotometric method at 276nm.

Skin irritation study

Guinea pigs (400-500 g) of either sex were used for testing of skin irritation. The animals were obtained from the animal houses of Karavali College of Pharmacy, Mangalore. Hairs were depleted from the back of guinea pigs and area of 4cm² was marked on both the sides, one side served as control while the other side as test. Gel was applied (500mg/ guinea pig) twice a day for 7d and the site was observed for any sensitivity and the reaction if any, was graded as

0 : No reaction
0.5 : Slight patchy erythema
1 : Slight but confluent or moderate but patchy erythema
2 : Moderate erythema
3 : Severe erythema with or without edema
Homogeneity

All developed gels were tested for homogeneity; appearance and presence of any aggregate by visual inspection after the gels have been set in the container.

Spreadability

It was determined by wooden block and glass slide apparatus. Spreadability was then calculated by using the formula:

\[ S = \frac{M \times L}{T} \]

Where; \( S \) = Spreadability; \( M \) = Weight tied to upper slide; \( L \) = Length of glass slide; \( T \) = Time taken to separate the slide completely from each other.

Consistency

The measurement of consistency of the prepared gels was done by dropping a cone attached to a holding rod from a fix distance of 10 cm. The distance traveled by cone was noted down after 10 sec.

Drug content

The drug content of all the developed formulations and marketed gel formulation were determined by using UV-spectrophotometer at 276 nm using phosphate buffer pH 6.8 as blank.

Stability testing

Stability studies were carried out on optimized formulation as per ICH guidelines for three month and various parameters were studied during stability study.

*In vitro* anti-inflammatory activity\(^{92-93}\)

The *in vitro* anti-inflammatory activity of the gel formulation was performed using carrageenan induced rat hind paw edema model. Albino rats of Wistar strains of either sex between 140-170 grams were selected for the studies. The animals were divided into three groups comprising six animals in each group.

Group 1:- Control, received placebo gel
Group 2:- Received 1.2 mg/kg equivalent to diclofenac in gel formulation
Group 3:- Marketed gel Received 1.2 mg/kg equivalent to diclofenac in gel formulation

Immediately after drug administration 0.05 ml of 1% w/v solution of carrageenan was injected into the planter surface of the hind paw. The hind paw volume was measured at different time intervals for 4 h after carrageenan treatment using a plethysmograph.

\[ \text{Anti-inflammatory activity (\%)} = \left(1 - \frac{A}{B}\right) \times 100 \]

Where \( A \) is the change in paw volume in the treated group and \( B \) is the change in paw volume in the control group.
EXPLORATION OF BFM AS A CARRIER FOR COLON DRUG DELIVERY

Preformulation studies of olsalazine sodium

Melting point of olsalazine sodium and solubility of olsalazine sodium in different solvents were determined as per the methods described in the exploration of the binding property of BFM.

Determination of $\lambda_{\text{max}}$

Stock solution (100µg/ml) of olsalazine sodium was prepared in methanol. This solution was appropriately diluted with 0.1N HCl to obtain a concentration of 30µg/ml. The UV spectrum was recorded in the range of 200-400 nm on Shimadzu UV-visible spectrophotometer. The same procedure was carried out in the solvents such as pH 6.8 phosphate buffer and pH 7.4 phosphate buffer solutions.

Preparation of standard calibration curve

100 mg of olsalazine sodium was weighed accurately and transferred to a 100 ml volumetric flask. This was dissolved in 0.1N HCl (pH=1.2) and volume was made upto 100 ml. This solution was treated as the stock solution and contained 100 µg/ml of olsalazine sodium solution. From this stock solution concentrations of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10µ g/ml were obtained. Absorbance of these solutions were measured at 245 nm against blank solution i.e., 0.1N HCl. Same procedure was followed for the preparation of standard curve in pH 6.8 Phosphate buffer and pH 7.4 phosphate buffer solution.

Drug-excipient compatibility study

Fourier Transform Infrared (FTIR) Spectral analysis

FTIR spectra of pure drug and physical mixture of drug and excipients were recorded by using a FTIR spectrophotometer.

Differential Scanning Calorimetry (DSC) analysis

DSC analysis of drug and excipients were performed using Shimadzu DSC-60, Japan.

Biodegradation studies of BFM

The in vitro biodegradation studies of BFM were carried out in presence and absence of 2%w/v and 4%w/v rat caecal contents (RCC) before and after enzyme induction by using 1% w/v dispersion of BFM after incubation at 37°C for 24 h.

Formulation of colon targeted tablets of olsalazine sodium

The colon specific tablets were prepared by direct compression method. Five formulations were prepared using different concentrations of BFM (30% to 70%) as shown in table 14. Olsalazine sodium, mixed properly with BFM, microcrystalline cellulose and
other excipients like talc and magnesium stearate, slugged and these powder blends were evaluated for pre-compression characteristics prior to compression. After determining precompression characteristics powder blends were compressed in 12 station rotary tablet Mini press.

Table 14: Formulation of colon targeted olsalazine sodium matrix tablets

<table>
<thead>
<tr>
<th>Ingredients*</th>
<th>Formulation code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Olsalazine sodium</td>
<td>100</td>
</tr>
<tr>
<td>*BFM</td>
<td>120</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>171</td>
</tr>
<tr>
<td>Magnesium stearate (1%)</td>
<td>3</td>
</tr>
<tr>
<td>Talc (2%)</td>
<td>6</td>
</tr>
<tr>
<td>Total weight of tablet</td>
<td>400</td>
</tr>
</tbody>
</table>

BFM* = *Borassus flabellifer* mucilage; All the quantities are in mg

Evaluation of precompression characteristics of olsalazine sodium powder blend

The prepared olsalazine sodium powder blends were evaluated for angle of repose, tapped, bulk density, compressibility index and Hausner ratio as per the reported methods.

Evaluation of olsalazine sodium colon targeted tablets

The prepared tablets were evaluated for general appearance, hardness, friability, weight variation, thickness, diameter as per the method described in the exploration of disintegrating property of BFM.

The other evaluation tests were carried out on formulated tablets which includes:

Drug content uniformity

Drug content of each batch of olsalazine sodium colon targeted matrix tablets were determined by measuring the absorbance at 245 nm by using a Shimadzu UV-Vis spectrophotometer.

Swelling index

One tablet from each formulation was randomly selected, weighed individually ($W_1$) and placed separately in a wire basket which was placed in a 100 ml beaker containing 0.1 N HCL for first 2h and in pH 6.8 phosphate buffer for remaining 22h. At the end of 2, 4, 6, 8 and 24 hrs tablets were withdrawn from wire basket and excess water was removed using tissue paper. The swollen tablets were reweighed ($W_2$) and swelling index of each tablet was calculated by using the formula;
Chapter-II

Methodology

\[
\text{Swelling index (\%) } = \frac{W_2 - W_1}{W_1} \times 100\%
\]

**Disintegration test**

The disintegration time of tablet was determined by disintegration apparatus, using pH 1.2 buffer solution maintained at 37±0.5°C. Then the disintegration time of tablet is recorded.

**In vitro drug release studies**\(^9\)

The dissolution studies were carried out by using USP dissolution apparatus II in 0.1N HCl (SGF) for first 2 h, then in 7.4 pH Phosphate buffer (SIF) for next 3 h and for remaining hours in simulated colonic fluid (SCF) prepared in pH 6.8 saline phosphate buffer to mimic colonic pH. The apparatus was maintained at 37±0.5°C and at 100 rpm. At regular intervals specified volume of sample was withdrawn suitably diluted and drug concentrations in samples were measured by using UV-spectrophotometer at 245 nm.

**In vitro drug release study with 4\% (w/v) rat caecal matter**\(^9\)

In order to check the susceptibility of mucilage for the colonic bacteria, drug release studies was also carried out in presence of rat caecal content due to the similarity of human and rodent colonic micro flora. Initial studies were carried out at pH 1.2 for 2 h, after this, the dissolution medium was changed to pH 7.4 for 3 h followed by pH 6.8 containing 4\% (w/v) rat caecal matter and the dissolution was continued until the completion of 24 h. At regular intervals specified volume of sample was withdrawn suitably diluted and drug concentrations in samples were measured by using UV-spectrophotometer at 245 nm. The experiments were carried out with continuous CO\(_2\) supply into the beakers to simulate anaerobic environment of the caecum.

**Mathematical treatment of the in vitro release kinetics**\(^1\)

To examine the drug release kinetics and mechanism, the best one formulation was analyzed for various kinetic models.

**Scanning Electron Microscopy studies (SEM)**

In order to confirm the mechanism of drug release from optimized formulation F5, the surface morphology of F5 were taken before and after dissolution studies using SEM.

**Stability testing**

Stability studies were carried out on optimized formulation as per ICH guidelines for three month and various parameters were studied during stability study.
In vivo targeting efficiency

The evaluation of dosage form in animal model renders support to the in vitro studies. In this study, healthy rabbits were fasted overnight. Roentgenography study; a comparatively safer technique was carried out in healthy male albino rabbits to access the in vivo performance of the selected batch. The study was carried out using barium sulphate as X-ray opaque material. Tablets containing barium sulphate (15%) for the selected batch was formulated and administered to rabbits with a glass of water. After the administration of the formulation, X-ray images were taken under the supervision of a radiologist, to follow the movement, location and the integrity of the tablets in different parts of GIT.

EXPLORATION OF FILM FORMING POTENTIAL OF BFM

Preformulation studies on diclofenac were carried out as per the methods reported in the exploration of matrix forming property of BFM in diclofenac tablets.

Drug- polymer interaction studies

Differential Scanning Calorimetry

DSC curves of pure diclofenac and combination of drug and excipients were recorded by using differential scanning calorimeter.

Fourier Transform Infra-Red (FT-IR) spectral analysis

FTIR spectrums of pure diclofenac and combination of drug and excipients were obtained by a Fourier-Transform Infrared spectrophotometer.

Preparation of transdermal films

Transdermal films of diclofenac were prepared by solvent evaporation method. A mould of 5 cm length and 5 cm width with a total area of 25 cm$^2$ as fabricated was used. The bottom of the mould was wrapped with aluminium foil. Various proportions of BFM was taken in a beaker add propylene glycol and glycerin, Span-80, Propyl paraben, Methyl paraben and diclofenac (100 mg) were added with continuous stirring using magnetic stirrer for 30 min at 500 rpm. The resulted uniform solution was casted on the aluminium foil and dried at 40°C in the hot air oven for 24 h. An inverted funnel was placed over the mould to prevent fast evaporation of the solvent. After 24 h the dried films were taken out and stored in a desiccator for further studies. Compositions of different formulations are represented in table 15.
Table 15: Composition of different batches of diclofenac transdermal patches

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Diclofenac (mg)</td>
<td>100</td>
</tr>
<tr>
<td>BFM* (%)</td>
<td>3</td>
</tr>
<tr>
<td>Glycerin (ml)</td>
<td>0.3</td>
</tr>
<tr>
<td>Propylene Glycol (ml)</td>
<td>0.18</td>
</tr>
<tr>
<td>Span-80 (ml)</td>
<td>0.06</td>
</tr>
<tr>
<td>Methyl paraben (g)</td>
<td>0.025</td>
</tr>
<tr>
<td>Propyl paraben (g)</td>
<td>0.015</td>
</tr>
<tr>
<td>Water up to (ml)</td>
<td>20</td>
</tr>
</tbody>
</table>

BFM* = Borassus flabellifer mucilage

Evaluation of transdermal patch

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Thickness of the film

The thickness of the formulated film was measured at 3 different points using a digital caliper and average thickness of three readings was calculated.

Weight uniformity

The films of different batches were dried at 60°C for 4 hours before testing. Three patches from each batch were accurately weighed in a digital balance.

Folding endurance

A strip of film (5x5 cm) was cut and repeatedly folded; the number of times the film could be folded at the same place without breaking/cracking gave the value of folding endurance.

Percentage moisture absorption

The films were weighed accurately and placed in the desiccators containing 100 mL of saturated solution of potassium chloride, which maintains 80-90% RH. After 3 days, the films were taken out and weighed. The percentage moisture absorption was calculated using the formula:

\[
\text{Percentage moisture absorption} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100
\]

Percentage moisture loss

The films were weighed accurately and kept in a desiccators containing anhydrous calcium chloride at room temperature for 24 hours. After 3 days, the films were taken out and weighed. The percentage moisture content was calculated using the formula:
Chapter-II

Methodology

\[
\% \text{ moisture loss} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100
\]

**Moisture content**

The prepared films were weighed individually and kept in desiccator containing activated silica at room temperature for 24h. The films were weighed again, until constant weight is achieved. The % moisture content was calculated as a difference between initial and final weight with respect to final weight.

\[
\% \text{ Moisture content (MC)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100
\]

**Water vapour transmission rate**

Glass vials of 5 mL capacity were washed thoroughly and dried to a constant weight in an oven. About 3 g of fused calcium chloride was taken in the vials and the polymer films of 2.25 cm\(^2\) were fixed over the brim with the help of an adhesive tape. Then the vials were weighed and stored in a humidity chamber of 80-90% RH condition for a period of 24 h. The vials were removed and weighed at 24 h time intervals to note down the weight again.

\[
\text{Transmission rate} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Time} \times \text{Area}} \times 100
\]

**Tensile strength**

Tensile strength was determined by using computerized Precisa bottom-loading balance, with necessary modifications. A 1X1 cm patch was taken and subjected to studies.

**Drug content uniformity of films**

The drug content in each batch of formulated transdermal films was determined as per the reported method by using UV-spectrophotometer at 276 nm.

**Skin irritation test**

The skin irritation studies were carried out on formulated transdermal formulations using modified Draize test on Wistar rats. The hairs of the dorsal portion were removed physically with the help of sharp surgical scissors and the skin was washed properly one day prior to use. Group one was supplied with control formulation and group second were supplied with medicated formulation. Medicated formulation was secured on experimental side using an adhesive tape and non-medicated patch was adhered on the control side of rats. These were covered with occlusive covering to approximate the condition of use. The patches were removed after 7 days and each of the area was observed for any sign of erythema or edema.
**In vitro skin permeation study**

*In vitro* skin permeation studies were performed by using a modified Franz diffusion cell. The rat dorsal skin was mounted between the donor and receptor compartment of the diffusion cell. A 1cm$^2$ film was placed over donor membrane and the receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. The whole assembly was kept on a magnetic stirrer and the receptor compartment solution was stirred continuously at 50 rpm and temperature was maintained at 37±0.5°C. At regular time intervals 1 ml of the sample was withdrawn and analyzed for drug content by using UV-spectrophotometer at 276 nm.

**Scanning electron microscopy**

Film morphology of optimized formulation F5 was characterized before permeation and after permeation study by using scanning electron microscopy.

**Drug release kinetic study**

To examine the drug release kinetics and mechanism, the drug release data of optimized batch F5 was fitted to various kinetic models.

**Stability studies**

Stability studies were carried out on optimized formulation F5 as per ICH guidelines for two months and various parameters were studied during stability study.

**EXPLORATION OF BFM AS A CARRIER FOR FLOATING DRUG DELIVERY**

**Preformulation studies on Ranitidine HCl (RHCL)**

Melting point of RHCL and solubility of RHCL in different solvents were determined as per the methods described in the exploration of the binding property of BFM.

**Determination of $\lambda_{\text{max}}$ of RHCL using 0.1N HCL**

A solution of RHCL containing the concentration 10µg/ ml was prepared in 0.1 N HCl and UV spectrum was taken using Shimadzu 1601 UV/Vis double beam spectrophotometer. The solution was scanned in the range of 200 – 400 nm.

**Standard calibration curve of RHCL using 0.1NHCL**

100 mg of RHCL was accurately weighed and transferred into 100ml volumetric flask. It was dissolved and diluted to volume with 0.1N HCL to give stock solution containing 1000µg/ml. The standard stock solution was then serially diluted with 0.1N HCL to get 1 to 10µg/ml of RHCL. The absorbance of the solution was measured against 0.1 N HCL as blank at 314 nm using UV spectrophotometer. The absorbance values were plotted against concentration (µg/ml) to obtain the standard calibration curve.
Drug and excipient compatibility study

By TLC

Method

Drug and excipients in 1:2 ratios were mixed and stored in glass vials at 50°C. The samples were analyzed for compatibility by thin layer chromatography after one, three and seven weeks as per the method described below.

Procedure

10 ml of reference and test solutions were applied as spots on the dry activated plate. The solvent system was allowed to run up to a desired height; the plates were removed and allowed to dry. The dry plates were then exposed to iodine vapors in a chamber to observe the spots. The plates were then removed and the Rf values calculated.

Solvent systems used for TLC for compatibility studies of RHCL

<table>
<thead>
<tr>
<th>Stationary phase</th>
<th>Thin plates of Silica gel G having thickness of 0.25cm the plates were activated at 110°C for 30min prior to use.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>Dioxane : Methanol: Dimethyl formamide (6:3:2)</td>
</tr>
<tr>
<td>Separation technique</td>
<td>Ascending.</td>
</tr>
<tr>
<td>Reference solution</td>
<td>5 mg of Ranitidine hydrochloride was shaken with 5ml of mobile phase, decanted and used for spotting.</td>
</tr>
<tr>
<td>Test solution</td>
<td>Drug-Excipient mixture equivalent to 5mg of RHCL was shaken with 5ml of mobile phase, decanted and used for spotting.</td>
</tr>
</tbody>
</table>

By FTIR spectroscopy

Compatibility of pure drug and drug along with excipients were studied by using FTIR spectrophotometer.

Preparation of RHCL floating tablets

RHCL effervescent floating tablets were formulated by direct compression method using different concentrations of BFM with sodium bicarbonate and citric acid. All the ingredients were accurately weighed and blended uniformly in glass mortar. After sufficient mixing of drug as well as other components, then the powder mixture was lubricated with magnesium stearate and talc, and further mixed for additional 2-3 minutes. The tablets were compressed using multi station tablet press. The composition of each formulation of RHCL floating tablets is given in table 16.
Table 16: Composition of different batches of RHCL floating tablets

<table>
<thead>
<tr>
<th>Ingredients (mg)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHCL</td>
<td>336</td>
<td>336</td>
<td>336</td>
<td>336</td>
<td>336</td>
<td>336</td>
<td>336</td>
<td>336</td>
</tr>
<tr>
<td>BFM</td>
<td>40</td>
<td>80</td>
<td>120</td>
<td>160</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>HPMC K4M</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>40</td>
<td>80</td>
<td>120</td>
<td>160</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Citric acid (Anhydrous)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Talc</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Avicel</td>
<td>152</td>
<td>112</td>
<td>72</td>
<td>32</td>
<td>152</td>
<td>112</td>
<td>72</td>
<td>32</td>
</tr>
<tr>
<td>Total weight of tablet</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
</tr>
</tbody>
</table>

*HPMC K4M: indicates hydroxypropyl methylcellulose. All batches contained 336 mg RHCL equivalent to 300 mg daily dose, 1% w/w talc and 1% w/w magnesium stearate.

Pre-compression evaluation of RHCL powder blend

The flow properties of powders (before compression) were characterized in terms of bulk density, tapped density, Hausner’s ratio, angle of repose and Carr’s index as per the reported procedure.

Post-compression evaluation of RHCL floating tablets

The prepared tablets were evaluated for general appearance, hardness, friability, weight variation, thickness, diameter as per the method described in the exploration of disintegrating property of BFM.

The other evaluation tests were carried out on formulated tablets which includes:

Drug content

Drug content of each batch of RHCL tablets was determined by using UV-Vis spectrophotometer after suitable dilution at 314 nm.

In vitro buoyancy studies

The In vitro buoyancy studies were performed for all the formulations by placing the tablet in a 250 ml glass beaker, containing 200 ml of 0.1N HCl, pH 1.2, maintained at 37 ±0.5°C in a water bath. Their physical state was observed for 12 h. The buoyancy lag time and the total buoyancy time were determined visually.

In vitro drug release studies of RHCL floating tablets

The release rate of RHCL from floating tablets was determined using USP Dissolution Testing Apparatus II. The dissolution test was performed using 900 ml of 0.1NHCL at 37±0.5°C and 75 rpm. At regular interval 10 ml of the sample was withdrawn and samples were filtered through Whatmann’s filter paper no. 41. Absorbance of these solutions was measured at 314 nm using UV spectrophotometer.
Swelling index of RHCL floating tablets

Floating tablet of RHCL was weighed individually (designated as \( W_1 \)) and placed separately in glass beaker containing 200 ml of 0.1N HCl and incubated at 37ºC ±1ºC. At predefined time intervals until 12h, the tablet was removed from beaker, and the excess surface liquid was removed carefully using the filter paper. The swollen tablet was then re-weighed (\( W_2 \)) and swelling index (SI) was calculated using the following formula.

\[
SI = \frac{(W_2 - W_1)}{W_1} \times 100
\]

Scanning Electron Microscopy

In order to confirm the mechanism of drug release from optimized formulation F3, the surface morphology of F3 were taken before and after dissolution studies using SEM.

Drug release kinetics

To examine the drug release kinetics and mechanism, the best one formulation was analyzed for various kinetic models.

Stability studies

Stability studies were carried out on optimized formulation F5 as per ICH guidelines for three months and various parameters were studied during stability study.

Evaluation of gastric retention using X-Ray imaging\(^{115-118}\)

The selected tablet formula (F3) for in vivo investigation was reformulated with 15% BaS\(_4\) as opaque agent. In vivo study was performed in rabbits by using X-ray imaging technique. This X-ray study was performed in 6 healthy rabbits of either sex, weight 2kg-2.5kg. The rabbit was administrated with selected formulation F3. A radiograph was made just before the administration of the BaS\(_4\) loaded tablet to ensure the absence of radiopaque material in the stomach. Then the tablet was administered orally by placing them in hollow polyethylene tube. The tube was inserted into the mouth of rabbit and blown using rubber bulb. Rabbit was placed upright posture for checking the position of tablet in gastric region by using X-ray machine. X-rays were taken at different time intervals like 1hr, 2 hr, 4hr, 8hr and 12hr.
EXPLORATION OF BFM AS A CARRIER FOR MUCOADHESIVE DRUG DELIVERY

Mucoadhesive characterization of BFM with existing polymer

The mucoadhesive characterization of synthetic, semi synthetic or natural gum/mucilage involves various evaluation techniques with different methods. To confirm the mucoadhesive character of the selected natural mucoadhesive agent (BFM) it was compared to other existing mucoadhesive polymer like hydroxy propyl cellulose (HPC)\textsuperscript{119}.

Mucoadhesive studies

Shear stress measurement

The test was performed by using different concentrations viz; 1, 2 and 3%w/v of HPC and BFM. Three glass plates were taken, to each of the glass plates a specified weight of prepared solution was applied. Another clean slide was placed over the first plate and applied solution was made to spread between the two glass plates by placing weight on the glass plates. It was kept undisturbed for specified period of time viz; 15, 30 and 60 min, then one side of glass plate was fixed to a hook and the other end was connected to a twin passing over a pulley and at the end of pan weight was attached, after a specified period of time viz; 15, 30 and 60 min, weight was placed in an increasing manner till the plates attached with polymer got detached. The weight which just detaches, were noted\textsuperscript{120-121}.

Wihelmy’s method

To characterize the mucoadhesive strength, Wihelmy’s method was used. A small glass plate (2x5 cm) was coated by dipping into a 1%w/v solution of test materials HPC and BFM. The mucus gel was taken from goat intestine kept in a suitable container, where the above-mentioned glass plate can be kept in contact with gel in a balanced condition and the temperature was maintained at 30°C. Nylon thread was attached at one end of the glass plate. Provision was given to raise the weight at the other end. At specified intervals 5, 10, 15 and 30min, weight was added to detach the coated glass plate from gel and the force required to pull the plate out of the gel was determined under experimental condition. Six plates were tested for each material and the average weights required were calculated\textsuperscript{122-123}.

Falling sphere method

To characterize the mucoadhesive strength, the falling sphere method was used. A clean burette was taken and filled with 10% mucus solution and fixed in a stainless steel stand. Mustard grain were taken and dipped in polymer solutions of HPC.
and BFM of various concentrations viz; 0.5, 1.0 and 3.0% w/v and then each grain were slowly placed at the top of the mucus layer. Time taken by the grain to cover 50 divisions in the burette was noted and values were tabulated\(^{124-125}\).

**Detachment force measurement**

This method was used to assess the tendency of mucoadhesive materials to adhere to the esophagus. Immediately after slaughter, the intestines were removed from the sheep and transported to the laboratory in tyrode solution. During the experiment the solution was aerated with pure oxygen and kept at 37\(^{0}\)C. Segments of 6-7 cm long were cut from the intestine and placed it on one glass slide and it was tied. The glass slide with the intestine was affixed on one side of the floor, below the modified physical balance. Plain mucoadhesive tablet prepared by using HPC and BFM, was pasted on another glass slide and it was balanced on the assembled physical balance with a beaker on the other side, which was used to hold the water. Now the balance was calibrated. At specific intervals, applied weight and the force required to detach measured for mucoadhesive strength.

**Recording of adherence**

To characterize the mucoadhesive strength, the recording of adherence method was used. The mucoadhesive tablet of sumatriptan succinate was kept on the intestine segment side and pressed lightly to the intestine segment with the help of forceps. The assembly was kept undisturbed for different time intervals viz; 5, 10, 15 and 30 minutes. Then water was added with help of burette slowly drop by drop into the beaker, the water required to pull out the tablet from the intestinal segment represents the force required to pull the tablet against the adhesion.

The force in Newton's is calculated by the equation, \(F = 0.00981 \frac{W}{2}\)

Where \(W\) is the amount of water.

**Preformulation studies**

The following preformulation studies were performed for Sumatriptan Succinate

1. Determination of pH
2. Drug-excipients compatibility studies
3. Determination of solubility.

**Determination of pH**

The pH of 1% w/v concentration of sumatriptan succinate in water was tested by using calibrated Elico pH meter.
Drug-excipients compatibility study

**Differential Scanning Calorimetry**

DSC curves of pure sumatriptan succinate and combination of drug and excipients were recorded by using differential scanning calorimeter (DSC-60, Shimadzu, Japan).

**Fourier Transform Infra-Red (FT-IR) spectral analysis**

FTIR spectrums of pure sumatriptan succinate and combination of drug and excipients were obtained by a Fourier-Transform Infrared spectrophotometer.

**Analytical Methods**

**Determination of λ\text{max} of sumatriptan succinate**

**Preparation of stock solution**

Stock solution (100µg/ml) of sumatriptan succinate was prepared in phosphate buffer 6.2. This solution was appropriately diluted with phosphate buffer of pH 6.2 to obtain a concentration of 10µg/ml. The resultant solution was scanned in range of 200-400nm on Shimadzu UV-Visible spectrophotometer.

**Standard calibration curve of sumatriptan succinate in phosphate buffer of pH 6.2**

100mg of sumatriptan succinate was accurately weighed and dissolved in 100ml of phosphate buffer 6.2 to obtain a concentration of 1000µg/ml. From the above solution 10ml was withdrawn and diluted to 100ml to obtain a concentration of 100µg/ml. From this stock solution aliquots of 1ml, 2ml, 3ml, 4ml, 5ml and 6ml were diluted in 50ml volumetric flask with phosphate buffer to give concentrations in range of 2µgm/ml to 12µgm/ml respectively, absorbance was measured at 265nm.

**Preparation of sumatriptan succinate mucoadhesive tablets**

Sumatriptan succinate mucoadhesive tablets were prepared by using wet granulation method. The drug and BFM were used in various ratios viz; 1:0.25, 1:0.5, 1:0.75, 1:1 and 1:1.25. All the ingredients were accurately weighed and mixed in a glass mortar and the required quantity of warm water was added to the powder mixture and mixed thoroughly. The wet mass was granulated by passing them through sieve number 16. The granules were dried at 60°C for 30 minute in hot air oven. The dried granules were passed through sieve.no.22/44 blended with a mixture of talc and magnesium stearate and compressed into tablets using rotary tablet press. 50 tablets were prepared per batch. The composition of each formulation of sumatriptan succinate mucoadhesive tablets is presented in table 17.
Table 17: Composition of different batches of sumatriptan mucoadhesive tablets

<table>
<thead>
<tr>
<th>Ingredients (mg/tablet)</th>
<th>Formulations (Drug: BFM ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:0.25</td>
</tr>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Sumatriptan Succinate</td>
<td>25</td>
</tr>
<tr>
<td>BFM</td>
<td>6.25</td>
</tr>
<tr>
<td>MCC</td>
<td>66.75</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>1</td>
</tr>
<tr>
<td>Talc</td>
<td>1</td>
</tr>
<tr>
<td>Total weight of tablet</td>
<td>100</td>
</tr>
</tbody>
</table>

Evaluation of sumatriptan succinate granule characteristics

Sumatriptan succinate granule characteristics such as flow property, bulk density, tapped density, compressibility index, and Hauser's ratio were analyzed for the prepared sumatriptan succinate granules.

Evaluation of sumatriptan succinate mucoadhesive tablets

The prepared tablets were evaluated for general appearance, hardness, friability, weight variation, thickness, diameter as per the method described in the exploration of disintegrating property of BFM.

The other evaluation tests were carried out on formulated tablets which includes;

Drug content

Drug content of each batch of sumatriptan succinate mucoadhesive tablets was determined by using UV-Vis spectrophotometer after suitable dilution at 265 nm.

Swelling study

The swelling index of formulated mucoadhesive tablets of sumatriptan succinate was carried out as per the method mentioned in the exploration of BFM as a carrier for floating drug delivery system by using phosphate buffer pH 6.2.

Microenvironment pH

The tablet was allowed to swell by keeping it in contact with 10 mL of distilled water (pH 6.8±0.05) in small beakers and the pH was measured at different time intervals viz; 1, 2, 3, 4 up to 8 hrs by using pH meter.

Ex vivo mucoadhesive strength and mucoadhesion time

Ex vivo adhesion strength is the force in grams required to pull out a tablet from sheep buccal mucosa. Time required to detach or erode sumatriptan succinate oral mucoadhesive tablets from the sheep buccal mucosa was taken as ex-vivo mucoadhesion time in hours.
**In vitro dissolution studies**

The release rate of sumatriptan succinate from oral mucoadhesive tablets was determined using USP Dissolution Testing Apparatus II. The dissolution test was performed using 900 ml of phosphate buffer pH 6.2 at 37±0.5°C and 50 rpm. At regular interval 5 ml of the sample was withdrawn, filtered and absorbance of these solutions was measured at 265 nm using UV spectrophotometer.

**Release kinetic studies**

To examine the drug release kinetics and mechanism, the best one formulation was analyzed for various kinetic models.

**Stability Studies**

Stability studies were carried out on optimized formulation F5 as per ICH guidelines for two months and various parameters were studied during stability study.

**In vivo mucoadhesive study**

**Preparation of barium sulphate tablet (mucoadhesive tablet)**

100mg tablet was prepared in ratio of 1:1 with barium sulphate and BFM by the addition of 5% w/v gelatin solution to make dough mass and this mass was passed through sieve #10. Thus obtained granules were passed through the sieve # 18 to remove fines. Two healthy male rabbits weighing 2.5 kg were selected and administered orally with the tablet and X-ray photograph was taken at different time intervals.