Chapter 5
Floating in situ gel based on alginate as carrier for stomach-specific drug delivery of famotidine
5.1 Aim of present investigation

Famotidine is a histamine H2-receptor antagonist. It is widely prescribed in gastric ulcers, duodenal ulcers, Zollinger-Ellison syndrome and gastroesophageal reflux disease. In the management of benign gastric and duodenal ulceration the dose is 40 mg daily by mouth at bedtime, for 4 to 8 weeks. In gastroesophageal reflux disease the recommended dose is 20 mg by mouth twice daily for 6 to 12 weeks; where gastroesophageal reflux disease is associated with esophageal ulceration, the recommended dosage is 40 mg twice daily for a similar period. For the short term symptomatic relief of heartburn or non-ulcer dyspepsia a dose of 10 mg up to twice daily is suggested. In the Zollinger-Ellision syndrome the initial dose by mouth is 20 mg every 6 hrs, increased as necessary; dose up to 80 mg daily have been employed. Its low bioavailability (40-45%) and short biological half-life (2.5-4.0 hrs) following oral administration favors development of a sustained release formulation.

The gastroretentive drug delivery systems can be retained in the stomach and contribute in improving the oral sustained delivery of drugs that have an absorption window in a particular region of the gastrointestinal tract. These systems help in continuously releasing the drug before it reaches the absorption window, thus ensuring optimal bioavailability. It has been reported that the oral treatment of gastric disorders with an H2 receptor antagonist like famotidine or ranitidine used in combination with antacids promotes local delivery of these drugs to the receptor of parietal cell wall. Local delivery also increases the stomach wall receptor site bioavailability and increases efficacy of drugs to reduce acid secretion. Hence this principle may be applied for improving systemic as well as local delivery of famotidine, which would efficiently reduce gastric acid secretion.

There are a number of approaches that can be used to prolong gastric retention time, like these include floating drug delivery systems, also known as hydrodynamically balanced systems, swelling and expanding systems, polymeric bioadhesive systems, modified-shape systems, high-density systems, and other delayed gastric emptying devices. A floating drug delivery system, being less
dense than gastric juice due to the incorporation of at least one porous structural element was described. Recently, research has been done using famotidine as an effervescent type drug delivery system. Also, a new type of multiparticulate floating drug delivery system consisting of a highly porous carrier material (foam powder), drug and polymer: low density microparticles have been proposed.

In situ gel, or *in-vivo* gel, environment sensitive gel is a new dosage form which has been used in stomach-specific drug delivery recently. Oral administration of in situ gels as low viscosity solution and upon contact with the simulated gastric fluid, the polymer changes conformation producing a gel, so it cannot only prolong the contact time between the drug and the absorptive sites at the stomach, but also release drug slowly and continuously. The alginate based in situ gelling liquid formulation containing calcium ion in complexed form gets converted into gel when reaches to acidic environment of stomach and make the formulation to float for prolong period of time. The optimum quantity of sodium citrate was added to the above formulation to maintain its fluidity at room temperature before administration. The proposed alginate based floating in situ gelling systems of famotidine (FIGF), would have the advantage of ease of administration, as being a liquid, and is more patient compliance.

It is our intention to develop floating in situ gelling systems of famotidine that:

1. Formation of gel in gastric fluid and float to the stomach for prolonged period of time.
2. Provide an increased gastric residence time resulting in prolonged drug delivery in gastrointestinal tract.
3. Delivers a drug at a controlled rate over a period of time, which is same as or less than the residency period of the delivery system in the gastrointestinal tract.
4. Shows better *in-vivo* performances than conventional dosage forms.
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In context to above intention, following criteria were aimed to achieve:

1. The proposed alginate based floating in situ gelling systems of famotidine (FIGF), would have the advantage of ease of administration, as being a liquid, and is more patient compliance.
2. Floating time of in situ gel should be more than 8 hrs.
3. Drug entrapment should be more than 50 %.
4. More than 90 % of drug should be released within 10 hrs.
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5.2 Experimental

5.2.1 Estimation of famotidine

A solution of Famotidine was prepared in 0.1 N HCl and Phosphate buffer pH 4.5 and UV spectrum was taken using Shimadzu UV-1601 UV/Vis double beam Spectrophotometer (Kyoto, Japan). The UV maxima of Famotidine was found to be 265 nm in 0.1 N HCl and also same in Phosphate buffer pH 4.5

5.2.1.1 Preparation of standard curve of famotidine in 0.1 N HCl

Famotidine (10 mg) was dissolve in 0.1 N HCl and volume is made up to 100 mL in volumetric flask. 1 mL of stock solution (100 µg/mL) was further diluted with 0.1 N HCl to obtained solution of 10 µg/mL to 25 µg/mL. Absorbance of each solution was measured at 265 nm using Shimadzu UV-1601 UV/Vis double beam Spectrophotometer (Kyoto, Japan) and 0.1 N HCl as a reference standard. The standard curve was generated for entire range from 10 to 25 µg/mL. The experiment was performed in triplicate and based on average absorbance; the equation for the best line was generated. The results of standard curve preparation are shown in Figure 5.1.
Table 5.1: Standard curve of famotidine in 0.1 N HCl

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Concentration (µg/mL)</th>
<th>Absorbance 1</th>
<th>Absorbance 2</th>
<th>Absorbance 3</th>
<th>Average Absorbance</th>
<th>Calculated Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>0.180</td>
<td>0.182</td>
<td>0.181</td>
<td>0.181</td>
<td>0.184</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0.356</td>
<td>0.360</td>
<td>0.358</td>
<td>0.358</td>
<td>0.354</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>0.523</td>
<td>0.523</td>
<td>0.521</td>
<td>0.521</td>
<td>0.531</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>0.702</td>
<td>0.710</td>
<td>0.706</td>
<td>0.706</td>
<td>0.714</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>0.884</td>
<td>0.892</td>
<td>0.889</td>
<td>0.889</td>
<td>0.890</td>
</tr>
</tbody>
</table>

Correlation Coefficient = 0.9995
Absorption = 0.0353x + 0.0018

Figure 5.1: Standard curve of famotidine in 0.1 N HCl
5.2.1.2 Preparation of standard curve of famotidine in phosphate buffer (pH 4.5)

Famotidine (10 mg) was dissolve in phosphate buffer (pH 4.5) and volume is made up to 100 mL in volumetric flask. 1 mL of stock solution (100 µg/mL) was further diluted with phosphate buffer (pH 4.5) to obtained solution of 10 µg/mL to 25 µg/mL. Absorbance of each solution was measured at 265 nm using Shimadzu UV-1601 UV/Vis double beam Spectrophotometer (Kyoto, Japan) and phosphate buffer (pH 4.5) as a reference standard. The standard curve was generated for entire range from 10 to 25 µg/mL. The experiment was performed in triplicate and based on average absorbance; the equation for the best line was generated. The results of standard curve preparation are shown in Figure 5.2.
Table 5.2: Standard curve of famotidine in phosphate buffer (pH 4.5)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Concentration (µg/mL)</th>
<th>Absorbance</th>
<th>Average Absorbance</th>
<th>Calculated Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0.188</td>
<td>0.191</td>
<td>0.190</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0.331</td>
<td>0.339</td>
<td>0.335</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>0.499</td>
<td>0.504</td>
<td>0.502</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>0.654</td>
<td>0.646</td>
<td>0.650</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>0.803</td>
<td>0.813</td>
<td>0.808</td>
</tr>
</tbody>
</table>

Correlation Coefficient = 0.9996
Absorption = 0.031x + 0.0317

Figure 5.2: Preparation of standard curve of famotidine in phosphate buffer (pH 4.5)
5.2.2 Preparation of in situ gelling solution

Sodium alginate solutions of concentrations 0.25, 0.5, 1.0 and 1.5 % (w/v) were prepared by adding the alginate to ultrapure water containing 0.25% (w/v) sodium citrate and 0.075% (w/v) calcium chloride and heating to 60 °C while stirring. Famotidine (40 mg) was then dissolved in 10 mL of 0.1N hydrochloride acid solution (pH 1.2) and added in the resulting solution after cooling to below 40 °C. The solution was neutralized by 0.1N sodium hydroxide. A 1% (w/v) control solution (for use in the in vitro release experiments) was prepared by dissolving famotidine in a 0.6% (w/v) aqueous solution of sodium alginate. A 1% (w/v) solution of famotidine was prepared in ultrapure water. The resulting alginate in situ gel solution containing famotidine was checked for viscosity and gelling property (Figure 5.3) and finally stored in amber color narrow mouth bottles until further use. In the preliminary batches J1 to J12 the concentration of calcium chloride and sodium citrate were kept constant at 0.075 and 0.25 % w/v, respectively. The concentration of the alginate was varied in batches J1 to J12 from 0.25 to 1.5 % w/v. The effect of formulation variables on characteristics of the sodium alginate based in situ gel of famotidine are summarized in Tables 5.2.3 and 5.2.4. In factorial design batches F1 to F9, the concentration sodium alginate (X₁) and the concentration of calcium chloride (X₂) were varied from 0.5 to 1.5 % w/v and 0.05 to 0.1 % w/v respectively, as shown in Table 5.4.

5.2.3 Optimization by using 3² full factorial design

On the basis of the preliminary trials in the present study a 3² full factorial design was employed to study the effect of independent variables, i.e. concentration of sodium alginate (X₁) and the concentration of calcium chloride (X₂) on dependent variables; drug content, viscosity, % drug released at 4 hrs (Q₅₀) and 8 hrs (Q₈₀). A statistical model (see equation) incorporating interactive and polynomial terms was utilized to evaluate the responses.

\[ Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_{12} + b_{22}X_{22} \]  \[5.1\]
Where, $Y$ is the dependent variables, $b_0$ is the arithmetic mean response of the nine runs, and $b_1$ is the estimated coefficient for the factor $X_1$. The main effects ($X_1$ and $X_2$) represent the average result of changing one factor at a time from its low to high value. The interaction terms ($X_1X_2$) show how the response changes when two factors are simultaneously changed. The polynomial terms ($X_{12}$ and $X_{22}$) are included to investigate non-linearity. The polynomial equation can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative. The high values of correlation coefficient for the dependent variables indicate a good fit. The equation may be used to obtain estimate of the response because small error of variance was noticed in the replicates.

5.2.4 Physical appearance and pH

All the prepared alginate based in situ solutions of famotidine were checked for their clarity and the time required for gel formation. The pH was measured of in situ solutions of famotidine using a calibrated digital pH meter at 25°C. All measurements of pH were made in triplicate and the results are given in Table 5.3.

5.2.5 In vitro gelation study and viscosity measurement of in situ gels

Famotidine in situ solution (5 mL) and artificial simulated gastric fluid (100 mL) were mixed (1:20, v/v) and gelation was observed by visual examination. The viscosity of the sodium alginate solution either in solution or in gel made with artificial simulated gastric fluid were determined with a Brookfield viscometer (Model no RVT 81990) using a 20 mL aliquot of the sample. Measurements were performed using suitable spindle number at 6, 12, 30, 60 rpm, and the temperature was maintained at 25±1°C. The viscosity was read directly from the viscometer display. Gelation was also checked in collected gastric juice from the rats. All measurements were made in triplicate and the results are given in Tables 5.3 and 5.4.

5.2.6 Determination of drug content

The amount of famotidine in each sample was determined by spectrophotometer (UV-1601, Shimadzu Co Ltd., Kyoto, Japan). The UV absorbance of the sample
was determined at a wavelength of 265 nm. The drug content for batches J1 to J12 and F1 to F9 are depicted in Tables 5.3, 5.4 and Figure 5.4.

5.2.7 Measurement of in vitro drug release
The release of famotidine from floating in situ gel were determined as described by Zatz and Woodford with some modification using USP dissolution test apparatus (USP 24) with a paddle stirrer at 50 rpm. This speed was slow enough to avoid the breaking of gelled formulation and was maintaining with the mild agitation conditions believed to exist in-vivo. The dissolution medium used was 500 mL of 0.1N HCL (pH 1.2), and temperature was maintained at 37 ± 0.2 °C. Ten mL of formulation was drawn up using disposable syringe, the needle was wiped clean and excess formulation was removed from the needle end. The syringe end was then placed into the Petri dish (4.5 mm internal diameter) and the syringe plunger depressed slowly to extrude 10 mL and finally Petri dish containing formulation was kept in the dissolution vessel containing dissolution medium without much disturbance. At each time interval, a precisely measured sample of the dissolution medium was removed and replenished with prewarmed (37°C) fresh medium. Samples were withdrawn at predetermined time intervals, filtered through a 0.45 µm membrane filter, dilute suitably and analyzed spectrophotometrically. The experiments were conducted in triplicate. The amount of drug released at 4 hrs ($Q_{50}$) and 8 hrs ($Q_{80}$) were calculated. The average value of $Q_{50}$ and $Q_{80}$ for batches F1 to F9 is mentioned in Tables 5.4 and Figure 5.5.

5.2.8 Measurement of water uptake by the gel
The water uptake by the gel was determined using a Thermogravimetric Analyzer (TGA-50, Shimadzu, Kyoto, Japan). The in situ gels formed in 40 mL of Sorensen’s phosphate buffer were used for this study. At periodic time intervals a portion of the gel was carefully removed. The sample was immediately loaded onto a TGA pan after removal of surface water by an absorbing tissue. The sample was subjected to a controlled temperature program (10 °C/min). The weight loss (% (w/w)) on heating was measured over 30–200 °C. Water uptake
of in situ gels containing various cross-linker concentrations and different reaction times was examined over 6 hrs. All studies were carried out in triplicate.

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Concentration of Sodium alginate (%)</th>
<th>pH</th>
<th>Viscosity (cp)</th>
<th>Drug content (%)</th>
<th>Characteristic of In situ gels</th>
</tr>
</thead>
<tbody>
<tr>
<td>J1</td>
<td>0.25</td>
<td>7.4</td>
<td>90</td>
<td>83.25</td>
<td>Gel is not form properly</td>
</tr>
<tr>
<td>J2</td>
<td>0.25</td>
<td>7.4</td>
<td>92</td>
<td>86.22</td>
<td></td>
</tr>
<tr>
<td>J3</td>
<td>0.25</td>
<td>7.3</td>
<td>91</td>
<td>84.55</td>
<td></td>
</tr>
<tr>
<td>J4</td>
<td>0.5</td>
<td>7.1</td>
<td>150</td>
<td>91.92</td>
<td>Gel formation</td>
</tr>
<tr>
<td>J5</td>
<td>0.5</td>
<td>7.1</td>
<td>153</td>
<td>93.80</td>
<td></td>
</tr>
<tr>
<td>J6</td>
<td>0.5</td>
<td>7.2</td>
<td>155</td>
<td>92.35</td>
<td></td>
</tr>
<tr>
<td>J7</td>
<td>1</td>
<td>7.0</td>
<td>236</td>
<td>97.87</td>
<td>Gel formation</td>
</tr>
<tr>
<td>J8</td>
<td>1</td>
<td>6.9</td>
<td>238</td>
<td>98.98</td>
<td></td>
</tr>
<tr>
<td>J9</td>
<td>1</td>
<td>7.0</td>
<td>235</td>
<td>98.25</td>
<td></td>
</tr>
<tr>
<td>J10</td>
<td>1.5</td>
<td>6.8</td>
<td>331</td>
<td>96.56</td>
<td>Gel formation</td>
</tr>
<tr>
<td>J11</td>
<td>1.5</td>
<td>6.7</td>
<td>299</td>
<td>98.11</td>
<td></td>
</tr>
<tr>
<td>J12</td>
<td>1.5</td>
<td>6.8</td>
<td>332</td>
<td>97.12</td>
<td></td>
</tr>
</tbody>
</table>

*All the batches were prepared using 0.075% (w/v) calcium chloride and 0.25% (w/v) sodium citrate.
### Table 5.4: $3^2$ full factorial design layout*

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Variables levels in coded form</th>
<th>Viscosity (cp)</th>
<th>Drug content (%)</th>
<th>% Drug release ($Q_{50}$)</th>
<th>% Drug release ($Q_{80}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_1$ $X_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>-1 -1</td>
<td>98</td>
<td>92.12</td>
<td>98.18</td>
<td>98.18</td>
</tr>
<tr>
<td>F2</td>
<td>-1 0</td>
<td>134</td>
<td>93.65</td>
<td>97.66</td>
<td>97.66</td>
</tr>
<tr>
<td>F3</td>
<td>-1 +1</td>
<td>155</td>
<td>94.78</td>
<td>91.39</td>
<td>99.23</td>
</tr>
<tr>
<td>F4</td>
<td>0 -1</td>
<td>192</td>
<td>95.92</td>
<td>75.76</td>
<td>98.22</td>
</tr>
<tr>
<td>F5</td>
<td>0 0</td>
<td>236</td>
<td>98.72</td>
<td>54.81</td>
<td>92.40</td>
</tr>
<tr>
<td>F6</td>
<td>0 +1</td>
<td>266</td>
<td>95.54</td>
<td>50.29</td>
<td>81.26</td>
</tr>
<tr>
<td>F7</td>
<td>+1 -1</td>
<td>296</td>
<td>96.22</td>
<td>46.17</td>
<td>78.66</td>
</tr>
<tr>
<td>F8</td>
<td>+1 0</td>
<td>335</td>
<td>97.95</td>
<td>43.10</td>
<td>75.43</td>
</tr>
<tr>
<td>F9</td>
<td>+1 +1</td>
<td>365</td>
<td>95.75</td>
<td>37.71</td>
<td>71.21</td>
</tr>
</tbody>
</table>

**Translation of coded levels in actual units**

<table>
<thead>
<tr>
<th>Variables level</th>
<th>Low (-1)</th>
<th>Medium (0)</th>
<th>High (+1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of sodium alginate ($X_1$)</td>
<td>0.5%</td>
<td>1%</td>
<td>1.5%</td>
</tr>
<tr>
<td>Concentration of Calcium chloride ($X_2$)</td>
<td>0.05%</td>
<td>0.075%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

*All the batches contain 40 mg famotidine, viscosity measured at 150 rpm.
Figure 5.3: Gel formation of alginate based in situ gel in simulated gastric fluid (batch F5)
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Figure 5.4: Drug content of alginate based in situ gel batches F1-F9

Figure 5.5: Percent drug release from in situ gel batches F1-F9
Table 5.5: Summary of results of regression analysis

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>B0</th>
<th>B1</th>
<th>B2</th>
<th>B11</th>
<th>B22</th>
<th>B12</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity</td>
<td>236.381</td>
<td>102.73</td>
<td>34.57</td>
<td>1.14</td>
<td>-2.07</td>
<td>-7.57</td>
<td>0.9996</td>
</tr>
<tr>
<td>Drug content (%)</td>
<td>98.02</td>
<td>1.78</td>
<td>0.52</td>
<td>-1.11</td>
<td>-1.87</td>
<td>-1.94</td>
<td>0.8361</td>
</tr>
<tr>
<td>Q₅₀ (%)</td>
<td>56.84</td>
<td>-30.47</td>
<td>-10.5</td>
<td>-5.23</td>
<td>12.5</td>
<td>5.16</td>
<td>0.9853</td>
</tr>
<tr>
<td>Q₈₀ (%)</td>
<td>90.11</td>
<td>-13.09</td>
<td>-5.35</td>
<td>0.07</td>
<td>-2.43</td>
<td>0.76</td>
<td>0.9416</td>
</tr>
</tbody>
</table>

5.2.9 Stability studies

Stability studies were carried out on gel formulation according to ICH (International Conference on Harmonization) guidelines. A sufficient quantity of in situ gel in glass bottles was stored in desiccator containing saturated solution of sodium chloride, which gave a relative humidity of 75±5%. The desiccator was placed in a hot air oven maintained at 40±2 °C and samples were withdrawn at 0, 30, 60, and 90 days. The physical stability of gel was observed periodically the occurrence of turbidity or gelation. The drug content remaining and the viscosity of formulation were measured at predetermined time interval. The results of the stability study for the selected batch of alginate based in situ formulation is given in Figure 5.6.
5.2.10 Data fitting

An attempt was made to fit the dissolution data into Zero order\textsuperscript{19} release kinetics represented:

\[ m = k \times t \] \hspace{1cm} [5.2]

Where, \( k \) is zero-order constant, \( m \) is the % drug unreleased and \( t \) is the time. The plot of % drug unreleased (released) versus time is linear.

The data was treated with the First order\textsuperscript{20} release kinetics to characterize the mechanism of drug release:

\[ m = ea \times e^{-bt} \] \hspace{1cm} [5.3]

Where, \( a \) is the intercept and \( b \) is the slope. It assumes that the drug molecules, diffuses out through a gel like layer formed around the drug during the dissolution process. A plot of log % drug release versus time is linear.
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The data was treated with the Higuchi\textsuperscript{21} model to characterize the mechanism of drug release:

$$m = 100 - q \times \text{square root of time}$$

[5.4]

Where \( q \) is the Higuchi constant (% per square root of time). In this model, a plot of % drug unreleased (released) versus square root of time is linear.

The dissolution data was also analyzed using the Krosmeyer-Peppas model\textsuperscript{22} to determine the kinetic of drug release from different batches of in situ gel:

$$\frac{M_t}{M_\infty} = Kpt^n$$

[5.5]

Where, \( \frac{M_t}{M_\infty} \) represent the fraction of drug released at time \( t \) and \( Kp \) is the kinetic constant characterizing the polymeric system and \( n \) stands for the diffusion exponent.

The results of \( F \)-statistics were used for the selection of the most appropriate model. Results of summary of results of regression analysis and data fitting are shown in Tables 5.5 and 5.6, respectively. The curve fitting, simulation and plotting was performed in Excel (Microsoft Software Inc., USA) and Sigma plot version 10.0 (Sigma plot software, Jangel Scientific Software, San Rafael, CA).

The effects of independent variables on the response parameters were visualized from the contour plots. Numerical optimization using the desirability approach was employed to locate the optimal settings of the formulation variables so as to obtain the desired response\textsuperscript{23}. An optimized formulation was developed by setting constraints on the dependent and independent variables. The formulation developed was evaluated for the responses and the experimental values obtained were compared with those predicted by the mathematical models generated. Counter plots showing the effect of the concentration of sodium alginate \( (X_1) \) and the concentration of calcium chloride \( (X_2) \) on drug content, viscosity, % drug released at 4 hrs \( (Q_{50}) \) and 8 hrs \( (Q_{80}) \) appear in Figure 5.7.
### Table 5.6: Results of models fitting of batches F1-F9

<table>
<thead>
<tr>
<th>Batch no.</th>
<th>Regression</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero order kinetic</td>
<td>First order kinetic</td>
<td>Higuchi kinetic</td>
<td>Krosmeyer peppas</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>0.7068</td>
<td>0.4951</td>
<td>0.8299</td>
<td>0.9124</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>0.7491</td>
<td>0.5110</td>
<td>0.8637</td>
<td>0.9314</td>
<td></td>
</tr>
<tr>
<td>F3</td>
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<td>0.9812</td>
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Figure 5.7: Counter plots showing the effect of the concentration of sodium alginate ($X_1$) and the concentration of calcium chloride ($X_2$) on viscosity (a), drug content (b), $Q_{50}$ (c) and $Q_{80}$ (d)
5.11 In-vivo study

*In-vivo* evaluation studies for optimized formulation were performed on normal healthy Wistar rats weighing 200-250 gm each as per pylorus ligation method\(^\text{24}\). The approval of the Institutional Animal Ethics Committee was obtained before starting the study. The study was conducted in accordance with standard institutional guidelines. Three groups of Wistar rats (5 in each group) that were fasted (with water) at least 24 hrs before experiments were used for the study and divided first group as control, second as control plus immediate treatment and third as treated (in situ famotidine gel). Wistar rats were anaesthetized with ether and a portion of the abdomen was opened by a small midline incision below the xiphoid process. The pylorus portion of the stomach was lifted and ligated. During this process, care was taken to avoid the traction to the pylorus or damage to its blood supply the stomach was closed by interrupted sutures. In first group after 5 hrs the animals were sacrificed and the stomachs were removed, cut along the greater curvature and subjected to measurement of ulcer index. In second group alginate based in situ gel of famotidine were administered orally after 5 hrs of ligation, after 20 min the animals were sacrificed and the stomachs were removed, cut along the greater curvature observed whether gel is form or not and subjected to measurement of ulcer index. And in third group the alginate based in situ gel of famotidine were administered orally 30 min before starting the experiment in 24 hrs fasted rats and after 8 hrs of animals were sacrificed and observed for the effect of drug by counting the ulcer index. The ulcer index was determined using the formula: \(^{24-26}\) Ulcer index = \(10/X\), Where \(X\) = Total mucosal area/Total ulcerated area. The results of *in-vivo* study are depicted in Figures 5.8 to 5.11.
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Figure 5.8: Group 1 as a control

Figure 5.9: Group 2 as control + immediate treatment by alginate based in situ gel
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Figure 5.10: Group 3 as treated + alginate based in situ gel

![Figure 5.10: Group 3 as treated + alginate based in situ gel](image)

Figure 5.11: Ulcer index of alginate based in situ of famotidine

![Figure 5.11: Ulcer index of alginate based in situ of famotidine](image)
5.3 Results and Discussion

5.3.1 Preliminary trials

The floating in situ gels of famotidine were prepared by ion activation technique, dissolving varying concentrations of alginate in deionized water containing sodium citrate, to which varying concentrations of drug and calcium chloride was added. In preliminary trial batches of J1 to J12 (Table 5.3) were prepared using different concentration of sodium alginate to see the effect on the viscosity of the solution, drug content, pH and the physical properties of the gel in simulated gastric fluid (pH 1.2). The concentration of sodium alginate was varied from 0.25 to 1.5 % w/v. In the batches J1 to J3 (0.25 % w/v) improper gelation was observed which leads the rapid flow of the formulation. Also the time required for gelation and drug content was very low. Batches J4 to J6 prepared using 0.5 % w/v of sodium alginate the gelation, time required for gelation and drug content were slightly better than batches J1 to J3. While in the batches J7 to J12 all the characteristics of the gels were good but, in the batches of J10 to J12 the viscosity of the solutions were very high because of the higher concentration of sodium alginate which was difficult to pour while it was not observed in batches J7 to J9. Thus, we can conclude that 1 % w/v sodium alginate was the optimum concentration. The concentration of sodium citrate was constant in all the batches (0.25 % w/v) and observed no significant effect.

On the basis of the preliminary trials in the present study a $3^2$ full factorial design was employed to study the effect of independent variables, i.e. concentration of sodium alginate ($X_1$) and concentration of calcium chloride ($X_2$) on dependent variables viscosity, drug content, drug released at 4 hrs ($Q_{50}$) and 8 hrs ($Q_{80}$). The results summarized in Table 5.4 clearly indicate that all the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among the nine batches (F1 to F9). Fitted equations (full models) relating the responses i.e. viscosity, drug content, $Q_{50}$ and $Q_{80}$ to the transformed factor are shown in Table 5.5. The polynomial equation can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative. The high values of
correlation coefficient (Table 5.5) for the dependent variables indicate a good fit. The equation may be used to obtain estimate of the response because small error of variance was noticed in the replicates.

### 5.3.2 Factorial equation for viscosity

The viscosity is an important variable because it affects the gelation of the solutions, the flow of the formulation and time required for the gelation. The viscosity is dependent on the concentrations of the polymer and calcium chloride. The linear model generated for viscosity was found to be significant with an $F$-value of 590.35 ($p<0.0001$) and $R^2$ value of 0.9993:

$$\text{Viscosity (cp)} = 236.381 + 102.73X_1 + 34.57X_2 + 1.14X_1X_2 - 2.07X_1^2 - 7.57X_2^2 \quad [5.6]$$

The counter plot (Figure 5.7a) shows that the viscosity of solution increased from 98 to 155 cp and 296 to 365 cp at lower and higher levels of concentration of sodium alginate, respectively, as concentration of calcium chloride increased. The results of the equation indicate that the effect of $X_1$ (concentration of sodium alginate) is more significant than $X_2$ (concentration of calcium chloride). Moreover, amount of calcium chloride had a positive effect on the viscosity, i.e. as the volume of cross-linking agent increase, the viscosity increases.

### 5.3.3 Factorial equation for drug content

The linear model generated for drug content was found to be significant with an $F$-value of 2.041 ($p<0.0001$) and $R^2$ value of 0.8361:

$$\text{Drug content} = 98.02 + 1.78X_1 + 0.52X_2 - 1.11X_1X_2 - 1.87X_1^2 - 1.94X_2^2 \quad [5.7]$$

The counter plot (Figure 5.7b) shows that the drug content increased from 92.12 to 94.78 % at lower levels of concentration of sodium alginate and decreased from 97.75 to 96.22 % at higher levels of concentration of sodium alginate as concentration of calcium chloride increased. The results of the equation indicated that both the concentration of the $X_1$ and $X_2$ were responsible for the drug content of the in situ formulations but the effect of $X_1$ (concentration of sodium alginate) is
more significant than $X_2$ (concentration of calcium chloride), the effect of the $X_2$ was very less so, it was considered non significant compared to the concentration of sodium alginate.

**5.3.4 Factorial equation for $Q_{50}$**

The amount of drug released in an important parameter for sustained release action of the in situ gel of famotidine. The linear model generated for drug released at 4 hrs was found to be significant with an $F$-value of 26.97 ($p<0.005$) and $R^2$ value of 0.9853:

$$Q_{50} = 56.84 - 30.47X_1 - 10.55X_2 + 5.23X_1X_2 + 12.517X_1^2 + 5.16X_2^2 \quad [5.8]$$

The counter plot (Figure 5.7c) shows that the drug release at 4 hrs ($Q_{50}$) decreased from 98.18 to 91.39 at lower and 46.17 to 37.71 at higher levels of concentration of sodium alginate, respectively, as concentration of calcium chloride increased. The results depicted in Table 5.4 indicate that the percentage drug released in vitro is highly depended on the concentration of sodium alginate ($X_1$) and the concentration of calcium chloride ($X_2$). The concentration of calcium chloride ($X_2$) has a negative effect on $Q_{50}$, while the concentration of sodium alginate had lower effect on $Q_{50}$.

**5.3.5 Factorial equation for $Q_{80}$**

The linear model generated for drug released at 8 hrs found to be significant with an $F$-value of 6.45 ($p<0.005$) and $R^2$ value of 0.9416:

$$Q_{80} = 90.11 - 13.09X_1 - 5.35X_2 + 0.07X_1X_2 - 2.43X_1^2 + 0.76X_2^2 \quad [5.9]$$

The counter plot (Figure 5.7d) shows that the drug release at 8 hrs ($Q_{80}$) increased from 98.18 to 99.23 at lower and decreased from 78.66 to 71.21 at higher levels of concentration of sodium alginate, respectively, as concentration of calcium chloride increased. The results depicted in Table 5.5 indicate that the percentage drug released in vitro is highly depended on the concentration of sodium alginate ($X_1$) and the concentration of calcium chloride ($X_2$). The
concentration of calcium chloride ($X_2$) has a negative effect on $Q_{80}$, while the concentration of sodium alginate had lower effect on $Q_{80}$.

**5.3.6 Release mechanism**

Release of the drug from a polymeric matrix depends on the amount of water associated with the system. The release of the drug may involve the penetration of water into the matrix and simultaneous release of the drug via diffusion or dissolution as governed by Ficks law $^{27-28}$. The results of curve fitting of factorial batches into different mathematical models are given in Table 5.6. The mechanism of drug release from the in situ gel was found to be diffusion controlled because plots of percentage cumulative drug release vs square root of time were found to be linear with the regression coefficient ($R^2$) values ranging from 0.9124–0.9986 for the factorial batches. The release profile of batch F5 fitted to Korsmeyer-Peppas equation, $F$-value was found to be 10.16. The value of correlation coefficient was found to be 0.9986. The values of slope and intercept were found to be 0.8621 and -1.42, respectively. The results of $F$-statistics were used for the selection of the most appropriate model, thus it was concluded that the release profile fitted best to Korsmeyer-Peppas equation ($F=10.16$).

**5.3.7 Optimized batch**

A numerical optimization technique using the desirability approach was employed to develop a new formulation with the desired responses. Constraints like maximizing drug content, minimizing the viscosity and release at the end of 10 hrs in addition to minimizing the $Q_{50}$ and $Q_{80}$ were to set as goals to locate the optimum setting of independent variables in the new formulation. The optimized in situ gel formulation (J10) was developed using a 0.75 % w/v of sodium alginate and 0.0625 % w/v of calcium chloride. The optimized formulation was evaluated for percentage viscosity, drug content, $Q_{50}$ and $Q_{80}$. The results of experimentally observed responses and those predicted errors for the response parameters ranged between 0.46-1.95 percent, with the value of absolute error of 1.25±0.56 %. The low value of error indicates the high prognostic ability of factorial equation and counter plot methodology. The drug content from the
optimized formulation was found to be low 96.5% and viscosity of 208 cp, thus batch F5 was selected for further study, which exhibited a high drug content of 98.72 and the viscosity of 236 cp which is easy for swallowing and good ability for gelation immediately after oral administration.

The water associated with the formulation at any point in time in the release medium was studied by TGA. The percentage of weight loss was thought to be due to water loss during heating. TGA was also used to study the effect of cross-linking on water uptake by the gels. The result of the water uptake by the sodium alginate based in situ gel of famotidine at 8 hrs was 71.72% and the good correlation coefficient (0.9983). There was a sudden increase in water uptake followed by a decrease. This decrease is particularly prominent for gels without cross-linker and has been observed in lower concentrations of cross-linker. This decrease in water uptake can be explained by the collapsing of gels with time.

There was also a decrease in water uptake by the gels with cross-linker. The formation of cross-linked networks provided an additional barrier to water penetration. As the concentration of the cross-linker in the delivery system increased, the time taken to reach maximum water uptake increased. At a higher cross-linker concentration the collapsing of the gel was negligible compared to gels without a cross-linker.

Based on visual identification, the in situ gel has remained as liquid for a period of 3 months without the occurrence of turbidity or gelation at 40±2°C. As illustrated in Figure 4, the viscosity of the gel slightly changed from 236 cp at 0 month to 241 cp at the 3rd month. The samples also were analyzed for famotidine content by spectrophotometer. The results showed that about 2.32% content decrease was found when the in situ gel was kept at 40±2°C for 3 months. Since the overall degradation is <5%, a tentative shelf life of 2 years may be assigned to the formulation.

5.3.8 Results of in-vivo study

The in-vivo study was carried out by pylorus ligation method in rats to see whether the gel was formed or not in the stomach of the rats and also checked the effect of the drug by counting the ulcer index. Results of group 1 showed
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ulcer (Figure 5.8) while results of group 2 showed gel was formed but the ulcers was also identified (Figure 5.9). Results of group 3 showed gel was after 5 hrs of the treatment and an ulcer was reduced (Figure 5.10). The gel formation was checked in collected gastric juice of the rats and results showed immediately formation of gel in gastric juice of the rats. The ulcer index of group 1, 2 and 3 were 2.25, 2.26 and 0.5995, respectively (Figure 5.11). Thus we concluded that the gel formation in the stomach of the rats and significant reduction of ulcers were also observed.
5.4 Conclusion

This study reports that oral administration of aqueous solutions of famotidine containing sodium alginate results in formation of in situ gel at the stomach site. The results of a $3^2$ full factorial design revealed that the concentration of sodium alginate and concentration of calcium chloride significantly affected on the dependent variables like viscosity, drug content, $Q_{50}$ and $Q_{80}$. The *in-vivo* study demonstrated the excellent gel formation in the stomach of the rat and significant anti-ulcer effect of alginate based in situ gel of famotidine over longer period of time.
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