Research Paper

Floating In Situ Gel based on Alginate as Carrier for Stomach-Specific Drug Delivery of Famotidine

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ABSTRACT: Alginate based floating in situ gelling systems of famotidine (FIGF) were prepared by dissolving varying concentrations of alginate in deionized water containing sodium citrate, to which varying concentrations of drug and calcium chloride was added and dissolved by stirring. Results of preliminary trials indicate that concentrations of sodium alginate, calcium chloride and sodium citrate affected the characteristics of in situ gel. A $3^2$ full factorial design was employed to study the effect of independent variables, concentration of sodium alginate ($X_1$) and concentration of calcium chloride ($X_2$) on dependent variables, i.e. viscosity, drug content, drug release at 4 hrs ($Q_{50}$) and drug release at 8 hrs ($Q_{80}$). A sustained drug release was obtained for more than 8 hrs. In vivo testing of FIGF to albino Wistar rats demonstrated significant anti-ulcer effect of famotidine.

KEY WORDS: Alginate; floating; in situ gel; famotidine; ulcer index

Introduction

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 reflux 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-Ellison syndrome the initial dose by mouth is 20 mg every six hrs, increased as necessary; dose up to 80 mg daily have been employed (Reynolds J.E.F, Martin, 1996). 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 (Singh BN and Kim KH, 2000). 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 (Coffin M and Parr A, 1995).

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 (Li S et al., 2001; Li S et al., 2003; Kedzierewicz F et al., 1999; Davis SS et al., 1986; Groning R and Heun G, 1989; Arora S et al., 2005 and Chawla G and Bansal A, 2003). A floating drug delivery system, being less dense
than gastric juice due to the incorporation of at least one porous structural element was described (Müller W and Anders E, 1989). Recently, research has been done using famotidine as an effervescent type drug delivery system (Jaimini M et al., 2007). 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 (Streubel A et al., 2002 and Streubel A et al., 2003).

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 (Shi-lei C et al., 2009 and Cao SL et al., 2007). The alginate based in situ gelling solution 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. Kubo and group (2003) prepared in situ gelling system for the oral sustained delivery of paracetamol. Results showed diffusion-controlled release of paracetamol from the gels over a period of 6 hrs and bioavailability of paracetamol from the gels formed in situ in the stomachs of rabbits following oral administration of the liquid formulations was similar to that of a commercial available suspension containing an identical dose of paracetamol. Bioavailability of theophylline from alginate gels formed by in situ gelation in the rat stomach was increased by 1.3-2-fold in rats for alginate concentrations of 2.0 to 1.0%, respectively as shown in Table 2. 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 compliant.

**Materials and Methods**

**Materials**

Famotidine was gifted by Torrent Pharmaceuticals Pvt. Ltd (Chhatral, India). Alginate and sodium citrate were purchased from Sigma Chemicals Ltd (New Delhi, India). Calcium chloride was purchased from Muby Chemicals (Mumbai, India). All other reagents were of analytical grade.

**Animals**

Six-week-old male specific pathogen free Wistar rats (Body weight 200-250 gm) were gifted from Torrent Research Center (Ahmedabad, India) and maintained under standard laboratory conditions (room temperature, 23° ± 2°C; relative humidity, 55% ± 5%; 12/12 hours light/dark cycle) with free access to a commercial rodent diet and tap water.

**Methods**

**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 ultra pure water containing 0.25% (w/v) sodium citrate and 0.075% (w/v) calcium chloride and heating to 60 °C while stirring. Famotidine 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 ultra pure water. The resulting alginate in situ gel solution containing famotidine was checked for viscosity and gelling property (Figure 1) 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 1 and 2. In factorial design batches F1 to F9, the concentration sodium alginate (X1) and the concentration of calcium chloride (X2) were varied from 0.25 to 1.0 % w/v and 0.05 to 0.1 % w/v respectively, as shown in Table 2.

**Experimental design**

A factorial design experiment was conducted to study the effect of two factors, namely; the sodium alginate (X1) and calcium chloride (X2) concentration, each at three levels (0.5, 1.0 and 1.5%), and (0.05, 0.075 and 1.0%), respectively. This gave 3² =9 formulae. The responses were the drug content, viscosity, percentage drug released at 4 hrs (Q40) and 8 hrs (Q80). The assigned number for each formula and the typical design of the factorial experiment are shown in Table 2. All experimental data were analyzed statistically according to the established factorial design using Excel (Microsoft Software Inc., USA) computer program by which the analysis of variance (ANOVA) was performed and the main effects and
interactions were calculated. 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 3 and 4, respectively. The plotting was performed in Sigma plot version 10.0 (Sigma plot software, Systat 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 to obtain the desired response (Karasulu E et al., 2003). 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.

### Table 1 Results of preliminary trial batches*

<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 ± 1.0</td>
<td>83.25 ± 1.2</td>
<td>Gel is not form properly</td>
</tr>
<tr>
<td>J2</td>
<td>0.25</td>
<td>7.4</td>
<td>92 ± 2.0</td>
<td>86.22 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>J3</td>
<td>0.25</td>
<td>7.3</td>
<td>91 ± 3.27</td>
<td>84.55 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>J4</td>
<td>0.5</td>
<td>7.1</td>
<td>150 ± 2.5</td>
<td>91.92 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>J5</td>
<td>0.5</td>
<td>7.1</td>
<td>153 ± 4.6</td>
<td>93.80 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>J6</td>
<td>0.5</td>
<td>7.2</td>
<td>155 ± 1.73</td>
<td>92.35 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>J7</td>
<td>1</td>
<td>7.0</td>
<td>236 ± 2.64</td>
<td>97.87 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>J8</td>
<td>1</td>
<td>6.9</td>
<td>238 ± 2.0</td>
<td>98.98 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>J9</td>
<td>1</td>
<td>7.0</td>
<td>235 ± 2.64</td>
<td>98.25 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>J10</td>
<td>1.5</td>
<td>6.8</td>
<td>331 ± 0.86</td>
<td>96.56 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>J11</td>
<td>1.5</td>
<td>6.7</td>
<td>299 ± 1.0</td>
<td>98.11 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>J12</td>
<td>1.5</td>
<td>6.8</td>
<td>332 ± 1.32</td>
<td>97.12 ± 1.6</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. | Mean ± SD (n=3)

### Table 2 $3^2$ 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>$X_1$</td>
<td>$X_2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>-1</td>
<td>-1</td>
<td>98 ± 1.80</td>
<td>92.12 ± 1.2</td>
<td>98.18 ± 0.81</td>
</tr>
<tr>
<td>F2</td>
<td>-1</td>
<td>0</td>
<td>134 ± 1.32</td>
<td>93.65 ± 0.5</td>
<td>97.66 ± 1.2</td>
</tr>
<tr>
<td>F3</td>
<td>-1</td>
<td>+1</td>
<td>155 ± 2.59</td>
<td>94.78 ± 0.8</td>
<td>91.39 ± 2.2</td>
</tr>
<tr>
<td>F4</td>
<td>0</td>
<td>-1</td>
<td>192 ± 1.73</td>
<td>95.92 ± 0.5</td>
<td>75.76 ± 1.4</td>
</tr>
<tr>
<td>F5</td>
<td>0</td>
<td>0</td>
<td>236 ± 2.64</td>
<td>98.72 ± 1.2</td>
<td>54.81 ± 0.6</td>
</tr>
<tr>
<td>F6</td>
<td>0</td>
<td>+1</td>
<td>266 ± 2.29</td>
<td>95.54 ± 1.4</td>
<td>50.29 ± 1.6</td>
</tr>
<tr>
<td>F7</td>
<td>+1</td>
<td>-1</td>
<td>296 ± 2.0</td>
<td>96.22 ± 0.4</td>
<td>46.17 ± 0.6</td>
</tr>
<tr>
<td>F8</td>
<td>+1</td>
<td>0</td>
<td>335 ± 1.0</td>
<td>97.95 ± 1.3</td>
<td>43.10 ± 1.1</td>
</tr>
<tr>
<td>F9</td>
<td>+1</td>
<td>+1</td>
<td>365 ± 2.5</td>
<td>95.75 ± 0.6</td>
<td>37.71 ± 0.5</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, the floating lag time was less than 5 sec and duration of floating was $> 15$ hrs, viscosity measured at 60 rpm.

* Mean ± SD (n=3)
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 37°C. All measurements of pH were made in triplicate and the results are given in Table 1.

In vitro gelation study and viscosity measurement of in situ gels
Famotidine in situ solution (5 ml) and artificial simulated gastric fluid (SGF, 100 ml) were mixed (1:20, v/v) and gelation was observed by visual examination (Fig. 1). The viscosity of the sodium alginate solution either in solution or in gel made with artificial simulated gastric fluid were determined with a Brookfield digital viscometer (Model no LVDV 2P30) using a 20ml aliquot of the sample. Measurements were performed using suitable spindle number at 6, 12, 30, 60 rpm, and the temperature was maintained at 37±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 1 and 2.

Fig. 1 Gel formation of alginate based in situ gel in simulated gastric fluid (batch F5).

Determination of drug content
The amount of famotidine in each sample was determined by spectrophotometer (UV-1700, Shimadzu Co Ltd., Kyoto, Japan). The UV absorbance of the sample was determined at a wavelength of 266 nm. The drug content for batches J1 to J12 and F1 to F9 are depicted in Tables 1 and 2.

In vitro floating study
The in vitro floating study was determined using USP dissolution apparatus having 500 ml of simulated gastric fluid (pH 2.0). The Petri dish containing 10 ml of in situ gelling solution was immersed into dissolution apparatus (USP 24) containing 500 ml of SGF (pH 2.0) at 37°C. The time the formulation took to emerge on the medium surface (floating lag time) and the time the formulation constantly floated on the dissolution medium surface (duration of floating) were noted by visual observation.

Measurement of in vitro drug release
The release of famotidine from floating in situ gel were determined as described by Zatz and Woodford (1987) 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. 10 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 pre warmed (37°C) fresh medium. Samples were withdrawn at predetermined time intervals, filtered through a 0.45 µ membrane filter, dilute suitably and analyzed spectrophotometrically. The experiments were conducted in triplicate. The amount of drug released at 4 hrs (Q50) and 8 hrs (Q80) were calculated (Paulo Costa et al., 2001 and Wagner JG, 1969). The average value of Q50 and Q80 for batches F1 to F9 is mentioned in Table 2 and Fig. 2.

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 simulated gastric fluid 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 six hrs. All studies were carried out in triplicate.

**In vitro bioadhesion tests**

The in vitro bioadhesive property of in situ gel (batch F 5) was assessed on rat stomach mucosa, using a TAXT2i Texture Analyzer (Stable Micro Systems, Ltd., UK). The test parameters were: pretest speed 0.6 mm s⁻¹, test speed 0.1 mm s⁻¹, contact time 3.5 min, preload 1 N, load cell 500 N, diameter of upper probe 30 mm. The rat stomach mucosal tissue was cleaned, washed and stored at −20 °C. Preserved, cleaned and thawed rat stomach mucosa was incised longitudinally just before the experiment. Rat stomach mucosa was mounted on the platform below the texture analyzer probe. A cellophane membrane, equilibrated with simulated gastric fluid at 37±1 °C for 24 hrs was tied to the upper probe. Surface of rat stomach mucosa was moisturized with simulated gastric fluid and gel was applied. The test was run after completing the pre-test requirements.

**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 ºC, 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 Fig. 3.

![Fig. 2 Cumulative percentage of famotidine release from in situ gel batches F1-F9.](image-url)
In-vivo study

Male Wistar rats (200-250 gm each) were utilized for in vivo experiment study. All the animal studies were conducted in accordance with the protocol approval by the Institutional Animal Ethics Committee. The ulcer protective efficiency of famotidine in situ gel was compared with plain famotidine solution dissolved in PBS (pH 7.4). The animals were divided into five groups, each group containing five animals. The first group was treated as control and was fed with PBS (pH 7.4) by oral route. Second, third and fourth group was treated with immediate treatment of in situ gel, plain famotidine solution (equivalent to 30 mg/kg) (Doi Y et al., 1999) and famotidine in situ gel, respectively. The fifth group was fed with PBS (pH 7.4) and treated as blank.

One milliliter of 80% ethanol was used orally to induce gastric ulcer (Narayan S et al., 2004) after 5 hrs except fifth group. The alcohol was given to dissolve the mucus coat of the stomach and so the condition was made to allow gastric acid to act on gastric walls. After 8 hrs, the animals were sacrificed (second group the animals were sacrificed after 20 min to observed whether gel is form or not) and stomachs were removed and dissected carefully to observe the ulcer protective function of famotidine in situ gel as compared to plain famotidine solution. The incised stomachs were first washed with running tap water and placed on the watch glass and examined for severity of ulceration. The ulcer index was determined using the formula: (Shay H et al., 1945; Kulkarni SK, 1999 and Ganguly AK, 1969) Ulcer index = 10/X, Where X = Total mucosal area/Total ulcerated area.

Results and Discussion

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 1) 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. In addition, 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 then 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. The floating ability of the prepared formulations was evaluated in SGF pH 2.0 (Fig. 1).
and the time the formulation constantly floated on the
dissolution medium surface (duration of floating) were
evaluated. All batches showed the floating lag time less
than 5 sec and duration of floating was more than 15 hrs.
Formulations containing calcium chloride demonstrated
excellent floating ability. Upon contact with an acidic
medium, gelation and cross linking by Ca\(^{++}\) ions occurred
to provide a gel barrier at the surface of the formulation.

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 2 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 3.
The significance of the estimated effects was tested by
analysis of variance. The accuracy of the statistical model
used was described by the determination coefficient R\(^2\). R\(^2\)
was the fraction of the data explained by the model, and
value close to 1 indicated a good model (Table 3). The
factorial design batches F1 to F9 also showed the floating lag time less than 5 sec and duration of
floating was more than 15 hrs.

**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 solutions showed a marked increase in viscosity with
increasing concentration of alginate as shown in Tables 1
and 2. The observed increase in viscosity with increase in
concentration has been noted previously for alginate and
was attributed to a consequence of increasing chain
interaction with polymer concentration. Increasing the
calcium chloride content in the formulation simultaneously
increased the viscosity at all polymer concentrations
studied. Since the calcium chloride is present in the
formulations as insoluble dispersion, an increase in its
concentration proportionally increased the number of
particles dispersed, thus contributing to increased viscosity
(Tables 1 and 2).

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:

\[
\text{Viscosity (cp)} = 236.381 + 102.73X_1 + 34.57X_2 + 1.14X_1X_2 - 2.07X_1^2 - 7.57X_2^2 \quad \ldots [1]
\]

The contour plot [Figure 4 (a)] 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.

**Factorial equation for drug content**

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:

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

The contour plot [Figure 4 (b)] shows that the drug
content increased from 92.12 to 94.78 % at lower levels of
concentration of sodium alginate and decreased from 96.22
to 97.75 % 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). 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.

**Table 3 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>Q(_{50}) (%)</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>Q(_{80}) (%)</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>
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. 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:

$$Q_{50} = 56.84 - 30.47X_1 - 10.55X_2 + 5.23X_1X_2 + 12.517X_1^2 + 5.16X_2^2$$ ....[3]

The contour plot [Figure 4 (c)] 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 3 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 ($X_1$) had a greater negative effect on $Q_{50}$.

Factorial equation for $Q_{80}$

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:

$$Q_{80} = 90.11 - 13.09X_1 - 5.35X_2 + 0.07X_1X_2 - 2.43X_1^2 + 0.76X_2^2$$ ....[4]

Fig. 4 Contour 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).
The contour plot [Figure 4 (d)] 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 3 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 ($X_1$) had a greater negative effect on $Q_{80}$.

**Release mechanism**

The release data from floating in-situ gels over the whole period were analyzed according to the treatment proposed by Higuchi (1962) for drug release from semisolid vehicles containing dissolved drug. In order to investigate the mode of drug release from floating in situ gels the release data were analyzed with the following mathematical models: zero-order kinetic ($m = k \cdot t$); first order kinetic ($m = e^{-bt}$); Higuchi equation ($m = 100 - q \cdot \sqrt{t}$). The examination of the coefficient of determination ($r^2$) indicated the drug release followed diffusion controlled mechanism from the FIGFs, as the $r^2$ values for Higuchi’s square root of time ranged between 0.8299 to 0.9858 was always higher in comparison to zero (ranged between 0.7068 to 0.9859) as well as first order (ranged between 0.4951 to 0.9797). Further, to understand the drug release mechanism, the data were fitted to Korsmeyer-Peppas exponential model (Korsmeyer RW et al., 1983) $Mt/M_{\infty} = K t^n$, where $Mt/M_{\infty}$ is fraction of drug released after time ‘$t$’ and ‘$K$ ’ is kinetic constant and ‘$n$’ is release exponent which characterizes the drug transport mechanism. The values ‘$n$’ were in the range of 0.3735-0.6678, which was further indicative of the drug, releases following diffusion control mechanism. The release profile of batch F5 fitted to Korsmeyer-Peppas equation, $F=10.16$. 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 ($F^2=10.16$). The results of release data of factorial batches into different mathematical models are given in Table 4.

**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 contour 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.

<table>
<thead>
<tr>
<th>Batch no.</th>
<th>Zero order kinetic</th>
<th>First order kinetic</th>
<th>Higuchi kinetic</th>
<th>Korsmeyer-peppas</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.7068</td>
<td>0.4951</td>
<td>0.8299</td>
<td>0.9124</td>
</tr>
<tr>
<td>F2</td>
<td>0.7491</td>
<td>0.5110</td>
<td>0.8637</td>
<td>0.9314</td>
</tr>
<tr>
<td>F3</td>
<td>0.8128</td>
<td>0.5550</td>
<td>0.9048</td>
<td>0.9505</td>
</tr>
<tr>
<td>F4</td>
<td>0.9149</td>
<td>0.9300</td>
<td>0.9651</td>
<td>0.9848</td>
</tr>
<tr>
<td>F5</td>
<td>0.9859</td>
<td>0.8790</td>
<td>0.9858</td>
<td>0.9986</td>
</tr>
<tr>
<td>F6</td>
<td>0.9841</td>
<td>0.9679</td>
<td>0.9804</td>
<td>0.9929</td>
</tr>
<tr>
<td>F7</td>
<td>0.9872</td>
<td>0.9731</td>
<td>0.9879</td>
<td>0.9972</td>
</tr>
<tr>
<td>F8</td>
<td>0.9853</td>
<td>0.9680</td>
<td>0.9773</td>
<td>0.9927</td>
</tr>
<tr>
<td>F9</td>
<td>0.9853</td>
<td>0.9797</td>
<td>0.9651</td>
<td>0.9812</td>
</tr>
</tbody>
</table>
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.

The force required to detach the formulation from the surface of tissue was determined as the peak value in the resultant force-time plot. The bioadhesive strength of F5 formulation was found to be 0.5 N, pointing to good bioadhesion. In vitro bioadhesive test showed that famotidine in situ gel adhered more strongly to gastric mucous layer and could retain in gastrointestinal tract for an extended period.

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 3, 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.

**Results of in-vivo study**

Gastro retentive floating drug delivery systems greatly improve stomach pharmacotherapy through local drug release, which leads to high drug concentrations at the gastric mucosa (eradicating *H pylori* from sub mucosal tissue of the stomach), making it possible to treat duodenal ulcers, gastritis and oesophagitis, and reduce the risk of gastric carcinoma (Bardonnet PL et al., 2006). Elmowafy et al (2009) prepared floating famotidine loaded mineral oil-entrapped emulsion gel (MOEG) beads by the emulsion–gelation method. The results clearly indicated that retardation of drug release for 4 hrs was achieved by the oil hydrophobic diffusion barrier, especially in the presence of the compact network of alginate beads. When evaluated in vivo, this formula displayed superior anti-ulcer activity (>2) over drug suspension or marketed conventional tablets. The ulcer index after 5 h of treatment with different formulations was found to be 4.5 ± 0.28 in case of plain famotidine solution, while ulcer index was significantly reduced to 0.5 ± 0.13 in case of famotidine loaded PEGylated PPI 5.0G dendrimers, indicating sustained release of the drug from drug-PEGylated dendrimer complex (Gajbhiye VP et al., 2009).

The present in vivo investigations demonstrated that there was a marked difference in the reduction of ulcer index from the famotidine in situ gel (batch F5 drug content of 98.72 and the viscosity of 236 cp) when compared with the plain famotidine solution (P < 0.05). It was observed that the formulation under study not only decreased the ulcer index to a significant larger magnitude but also sustained this magnitude (Table 5). In case of ethanol treated group, the ulcer index was found to be 2.25 ± 0.15 (Fig. 5a). In case of immediate treatment group, the gel was formed but the ulcers was also identified to be 2.26 ± 0.2 (Fig. 5b). While in case of famotidine in situ gel, the ulcer index was found to be only 0.59 ± 0.04 after 8 hrs of dosing (Figure 5c). However, for plain drug the ulcer index was found to be 1.82 ± 0.08 after 8 hrs of dosing (Figs. 5d and 6). The possible reason for this result may be the drug concentration in the body that was maintained for a longer duration in case of famotidine in situ gel as compared with that of plain famotidine. 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.

**Table 5** Effect of famotidine in situ gel on ulcer index.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Ethanol treated</th>
<th>Immediate treatment</th>
<th>Plain famotidine</th>
<th>Famotidine in situ gel</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer index</td>
<td>2.25 ± 0.15</td>
<td>2.26 ± 0.2</td>
<td>1.82 ± 0.08</td>
<td>0.59 ± 0.04</td>
<td>Not detectable</td>
</tr>
</tbody>
</table>
Fig. 5 (a) Ethanol treated group (b) Immediate treatment of alginate based in situ gel group (c) Famotidine in situ gel treated group (d) Plain famotidine treated group.

Fig. 6 Ulcer index of alginate based in situ of famotidine.
Conclusion

This study reports that oral administration of aqueous solutions 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, Q50 and Q80. 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.

References


