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ORIGINAL RESEARCH PAPER

# Preparation and Evaluation of Floating Beads as a Chronotherapeutic Approach for The Treatment of Ulcer

Praveen Golasangi, Sangeeta SS, Vijaya G Joshi*

Government of College of Pharmacy, P. Kalinga Rao Road, Subbaiah Circle, Bengaluru 560 027 (India)

* For correspondence

Email: vijay.joshi67@gmail.com

**Key words**
Ulcer; Chronotherapy; Famotidine; Sodium alginate; Calcium silicate

**Abstract**
The objective of the present work is to conceptualize a specific technology, based on a blend of floating and pulsatile principles to develop drug delivery system, intended for chronotherapy of ulcer. In the present study, floating calcium alginate beads were prepared employing ionotropic gelation method. The prepared beads were evaluated for size analysis, bulk density, porosity, drug content and entrapment efficiency, crushing strength, moisture content, scanning electron microscopy, stability studies, dissolution studies, drug release kinetics and their values found in acceptable range. The cumulative release of all the batches was found to be in the range of 90.35 to 97.99%. In-vitro dissolution studies revealed that 1 to 10% of drug was released in simulated gastric fluid (SGF) followed by pulse release in simulated intestinal fluid USP, without enzymes (SIF). The kinetic data was fitted to Korsemeyer-Peppas model. Hence, a pulsatile release of famotidine by a simple drug delivery system could be useful in chronopharmacotherapy of ulcer.

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## INTRODUCTION

Normal gastric acid secretion follows a circadian rhythm with a sudden surge of gastric acidity when pH level goes far below 4 in the midnight.[1] Heartburn, coughing or choking, breathlessness, wheezing and morning phlegm are common symptoms frequently reported during this time. These nocturnal symptoms not only adversely affect sleep, but also impair functional abilities during the day.[2,3] While gastric, small bowel motility and gastric emptying are all slower at night. A circadian rhythm has been found overall that was reproducible and fairly stable across seasons, decades, and days of the week. In peptic ulcer patients, gastric acid secretion is highest during the night. Suppression of nocturnal acid is an important factor in duodenal ulcer healing. Therefore, for active duodenal ulcer, once daily at bedtime is the recommended dosage regimen for H2 antagonists. Bedtime H2-receptor blockade using Chronotherapy overcome problems of sustained or profound decrease of 24 h intragastric acidity including the threat of enteric infection and infestation, potential bacterial overgrowth with possible N-nitrosamine formation.[4-8]

The present study is aimed to prepare floating pulsatile drug delivery system containing famotidine in the form of calcium alginate beads by employing ionotropic gelation method. Calcium silicate in different concentrations will be used as a floating agent. The objective of the study is to formulate ideal floating beads which are intended to release drug, famotidine to circadian rhythm i.e., time in between midnight and 2 am for the treatment of peptic ulcer. In this study, investigation of functionality of the polymer and floating agent to predict lag time and drug release will be analyzed. Lag time prior to drug release and cumulative percentage drug release in 3 to 4 h was identified.

## MATERIALS AND METHODS

### Materials

Famotidine was a gift sample from Strides Arcolab Pvt Ltd., calcium silicate was purchased from Leochem, sodium alginate (Himedia Lab) and other chemicals used in the study were of analytical grade.

### Estimation of Famotidine

A solution of famotidine was prepared in 0.1 N HCl and Phosphate buffer pH 6.8 and UV maximum was determined using Shimadzu UV-Visible Spectrophotometer.[9]

### Standard Curve of Famotidine in 0.1 N HCl (pH 1.2)

Famotidine (100 mg) was dissolved in 0.1 N HCl and volume was 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 obtain solution of 5 µg/mL to 25 µg/mL. Absorbance of each solution was measured at 265 nm using Shimadzu UV-Visible Spectrophotometer and 0.1 N HCl as a reference standard.

### Standard Curve of Famotidine in Phosphate Buffer (pH 6.8)

Famotidine (100 mg) was dissolved in phosphate buffer (pH 6.8) and volume was made up to 100mL in volumetric flask. 1mL of stock solution (100µg/mL) was further diluted with phosphate buffer (pH 6.8) to obtained solution of 5µg/mL to 25µg/mL. Absorbance of each solution was measured at 265 nm using Shimadzu UV-1601 UV-Visible Spectrophotometer and phosphate buffer (pH 6.8) as a reference standard.

### Compatibility Studies

IR studies were carried to study drug and polymer compatibility. The infrared spectra of famotidine, sodium alginate, calcium silicate and drug-loaded porous calcium alginate beads were recorded on FTIR (JASCO-FTIR 5300). The samples were prepared on KBr press.

### Preparation of Calcium Alginate Beads of Famotidine

The beads were prepared by the ionotropic gelation method.[10] Pulsatile floating beads were prepared by adding famotidine and floating agent, calcium silicate to 10 mL of sodium alginate solution in different concentrations as shown in Table 1 and stirred for 5 min to form a uniform dispersion. Prepared dispersion was extruded through an 18 G (1.2 mm diameter) needle drop wise into 100 ml of 2% calcium chloride solution. The gel beads formed were allowed to remain in the calcium chloride solution for 10

min. Resultant beads were then filtered, washed twice and dried at 40ºC for 24 h. A control batch using calcium silicate without famotidine was also prepared using same procedure with 2% w/v sodium alginate solution.

Table 1. Formulation chart of calcium alginate beads of famotidine

| Batch Formula | Famotidine (mg) | Sodium alginate (mg) | Calcium silicate (mg) |
|---|---|---|---|
| **BF1** | 100 | 100 | 200 |
| **BF2** | 100 | 150 | 200 |
| **BF3** | 100 | 200 | 200 |
| **BF4** | 100 | 100 | 600 |
| **BF5** | 100 | 150 | 600 |
| **BF6** | 100 | 200 | 600 |
| **BF7** | 100 | 100 | 1000 |
| **BF8** | 100 | 150 | 1000 |
| **BF9** | 100 | 200 | 1000 |

## Buoyancy Test of Prepared Calcium Alginate Beads

The prepared beads were studied for buoyancy time using USP XXIII Type 2 dissolution test apparatus (Electrolab TDT-08L).[11] Fifty beads of each batch were placed in 900 mL of 0.1 N HCl containing 0.02% w/v Tween 80 and agitated at 100 rpm and the temperature was maintained at 37±2ºC. Number of sinking beads was observed visually.

## Size Analysis

About 50 beads were selected for particle size determination, using digital Vernier calipers.[12]

## Bulk Density (Db)

It is the ratio of total mass of beads to the bulk volume of beads.[13] It was measured by pouring the 10 g of weighed beads into a measuring cylinder and the volume was noted. It is expressed in g/mL and is given by:

$$\mathrm{D}\boldsymbol{b} = \frac{\boldsymbol{M}}{\boldsymbol{Vb}}$$

Where $M$= is the mass of beads; $V_b$= is the bulk volume of the beads.

## Porosity of Beads

The porosity of the beads was determined from bulk and true beads volume.
And the porosity of the beads was calculated by using the formula:[14]

$$\varepsilon = 100[1 - \frac{vt}{vb}]$$

Where, $\varepsilon$= Porosity of the beads; Vt = true volume; Vb = Bulk volume.

## Drug Content and Entrapment Efficiency

Famotidine content in the weighed floating beads was estimated by a UV-Spectrophotometric method.[11] Accurately weighed quantity of floating beads equivalent to 50 mg of drug were suspended in 100 mL of phosphate buffer (pH 6.8). It was kept stirring with the help of magnetic stirrer for 5 h. The solution was filtered and after suitable dilution, Famotidine content in the filtrate was analyzed at 265nm using UV-Visible spectrophotometer. The drug entrapment efficiency was calculated using following equation:

$$\text{Drug Entrapment Efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

## Crushing Strength

The crushing strength of floating beads was determined using Pfizer hardness tester. The beads were selected randomly from each batch. The experiment was carried out in triplicate.

## Moisture Content

The total moisture content was measured by keeping one gram of beads in hot air oven at 60ºC till constant weight of beads observed. Then, the moisture content was determined by using the formula:

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{weight after drying}}{\text{weight after drying}} \times 100$$

## Scanning Electron Microscopy (SEM)

The shape and surface characteristics of the prepared beads were evaluated by means of SEM (Ultra-55 Carl Zeiss Field emission scanning electron microscope). The samples for SEM were prepared by gently sprinkling the beads on a double adhesive tape, which is stuck to an Aluminium stub. The stubs were then coated with gold using a sputter coater under high vacuum and high voltage to achieve a film thickness of 30nm. The samples were then imaged using a 3 KV electron beam.

## Dissolution Studies

The dissolution studies of the beads equivalent to 50 mg of famotidine were performed using USP XXIII Type 2 dissolution test apparatus (Electrolab TDT-08L). The drug release study was carried out in 0.1 N HCl for initial 2 or 4 h depending upon floating characteristics of beads, followed by dissolution in phosphate buffer, pH 6.8, each 900 mL, maintained at 37±0.5ºC and agitated at 100 rpm (n = 3). Periodically samples were withdrawn followed by replacement with fresh dissolution media and filtered through Whatman filter paper 41. Then, the concentration of famotidine was measured spectrophotometrically (UV spectrophotometer) at 265 nm for both acidic and basic media.

## Drug Release Kinetics

Data obtained from *in-vitro* drug release studies were fitted to various kinetic models like zero-order, first order, Higuchi, Korsemeyer and Peppas using PCP Disso V2 to predict the drug release mechanism.

# RESULTS AND DISCUSSION

## Estimation of Famotidine

The UV maximum of famotidine was found to be 265 nm in 0.1 N HCl and 265.4 nm in Phosphate buffer pH 6.8.

## Standard Curve of Famotidine in 0.1 N HCl

Calibration curve data of famotidine in 0.1 N hydrochloric acid at 265.4 nm is mentioned in Table 2. Fig 1 shows the standard calibration curve with a regression value of 0.9997, slope of 0.0326. The curve was found to be linear in the concentration range of 5 – 25 µg/mL.

Table 2. Absorbance values of famotidine in 0.1 N HCl (pH 1.2)

| S. No. | Concentration (µg/mL) | Absorbance Mean± S.D* |
|---|---|---|
| **1.** | 0 | 0 |
| **2.** | 5 | 0.181±0.07 |
| **3.** | 10 | 0.358±0.03 |
| **4.** | 15 | 0.521±0.12 |
| **5.** | 20 | 0.706±0.04 |
| **6.** | 25 | 0.889±0.06 |

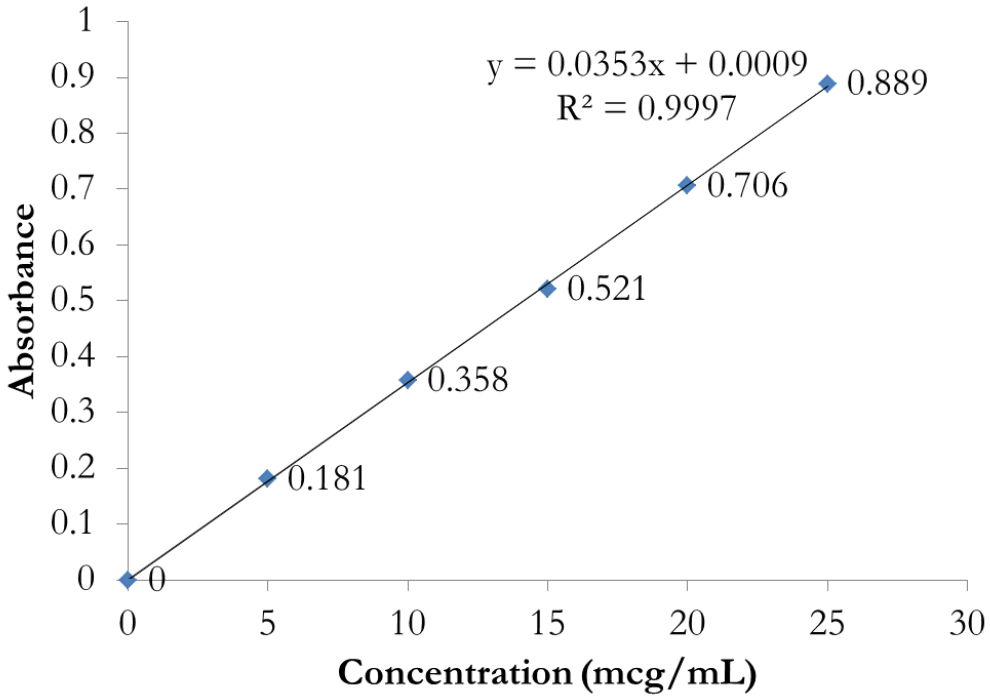


Fig 1. Standard Curve of Famotidine in 0.1 N HCl (pH 1.2)

## Standard Curve of Famotidine in Phosphate Buffer (pH 6.8)

Table 3 shows the calibration curve data of famotidine in phosphate buffer pH 6.8 at 265.4 nm. Fig 2 shows the standard calibration curve with a regression value of 0.9982, slope of 0.0364 in phosphate buffer (pH 6.8). The curve was found to be linear in the concentration range of 5 – 25 µg/mL. The result of this calibration curve was used to identify the concentration of drug in the drug content and dissolution studies.

Table 3. Absorbance values of famotidine in phosphate buffer (pH 6.8)

| S. No. | Concentration (µg/mL) | Absorbance Mean± S.D* |
|---|---|---|
| **1.** | 0 | 0 |
| **2.** | 5 | 0.183±0.07 |
| **3.** | 10 | 0.363±0.13 |
| **4.** | 15 | 0.544±0.08 |
| **5.** | 20 | 0.756±0.05 |
| **6.** | 25 | 0.893±0.15 |

## Compatibility Studies

FTIR was performed for the famotidine, sodium alginate, calcium silicate and the best formulation to detect any sign of interaction which would be reflected by a change in the position or disappearance of any characteristic peaks of the compound.

The studies on IR spectra showed N-H group peak at 3398.69 $cm^{-1}$ and peaks at 1286.55 $cm^{-1}$ and 1170.83 $cm^{-1}$ due to S-O stretching for famotidine. In comparision with pure drug, the absorption peak of the spectra for famotidine loaded beads (BF6) showed no shift and no disappearance of characteristic peaks suggesting that there is no interaction between drug and polymers as shown in Fig 3 to 6.

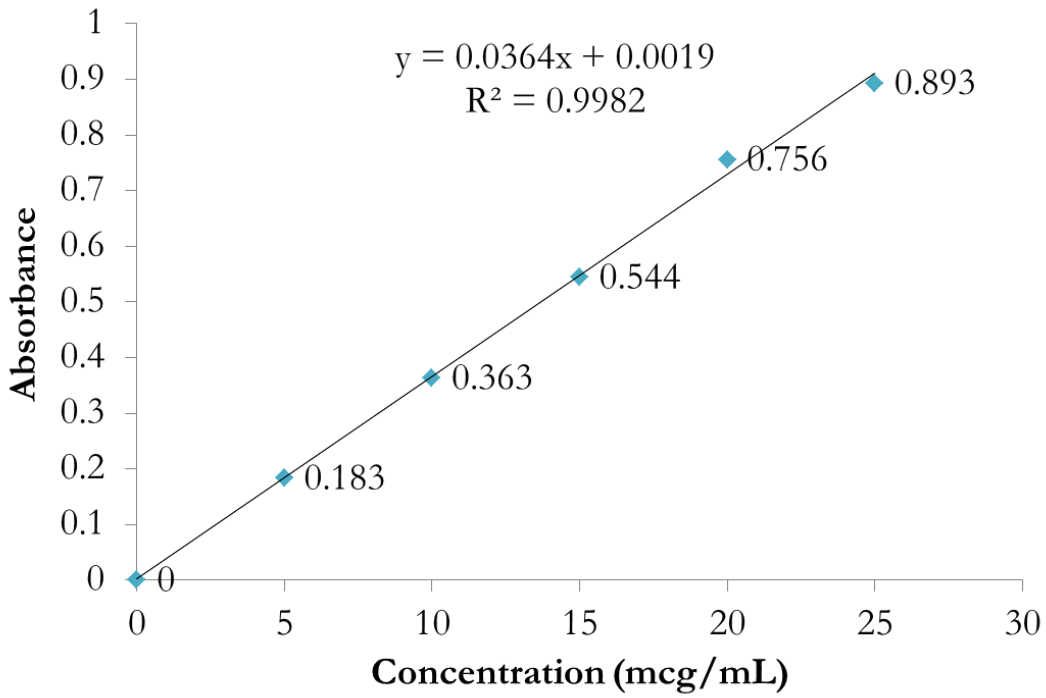


Fig 2. Standard Curve of Famotidine in phosphate buffer (pH 6.8)

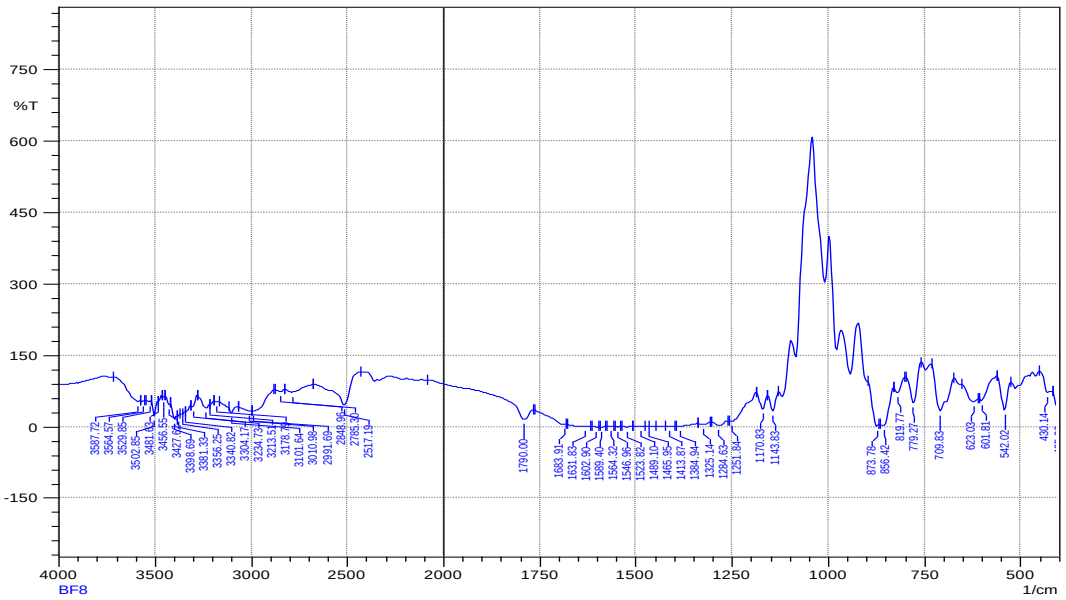


Fig 3. FTIR spectrum of famotidine

## Preparation of Calcium Alginate Beads of Famotidine

Floating calcium alginate beads were successfully prepared aiming the pulsatile delivery of famotidine according to circadian rhythm. All the prepared beads were spherical in nature with slightly rough surface. Increased concentration of floating agent increased the roughness of the surface. The color of the beads was white.

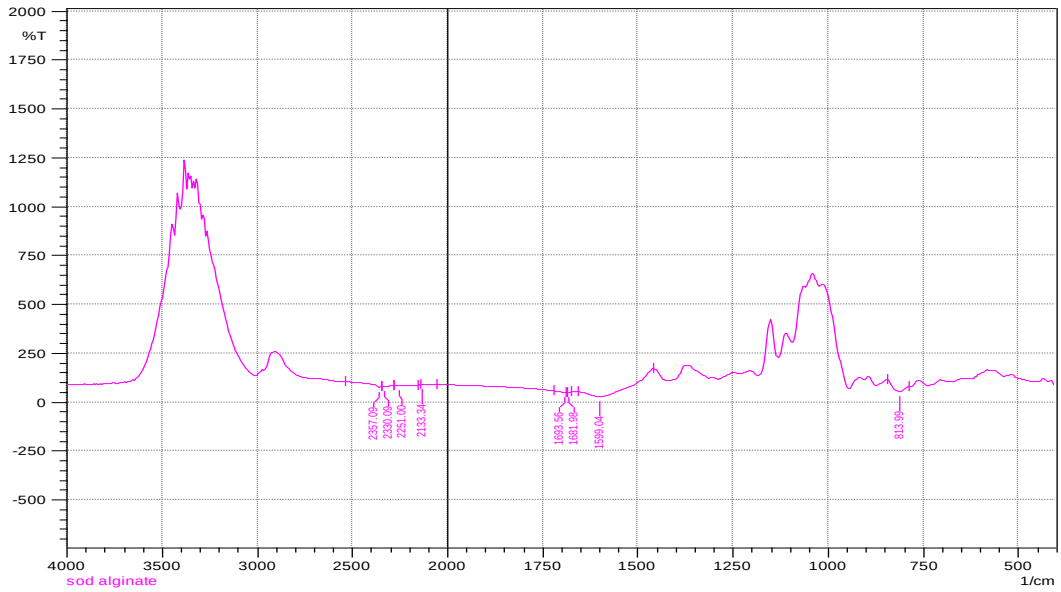


Fig 4. FTIR spectrum of sodium alginate

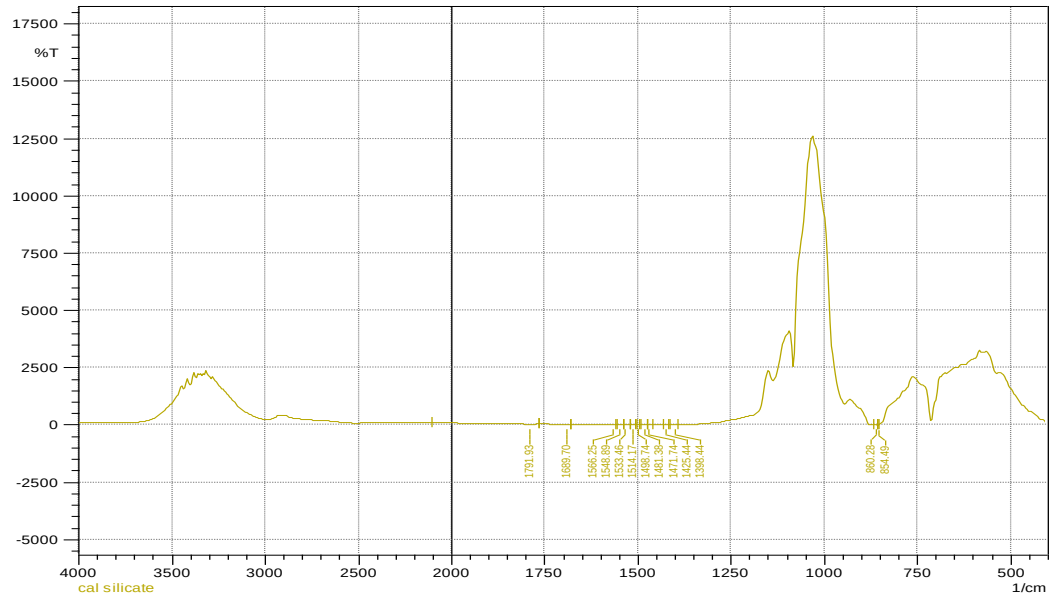


Fig 5. FTIR spectrum of calcium silicate

## Buoyancy Test

Floating properties of beads was studied by buoyancy test and time required for sinking all the beads was observed. The surfactant (Tween 80) was used in medium to simulate surface tension of human gastric juice (35-50mN/m$^2$). Floating time for different batches is given in Table 4. It was observed that with varying amount of calcium silicate fine tuning of floating time is achieved. Increase in ratio of calcium silicate in polymer has increased the floating time. Further desired floating time of 3 h was achieved by using 600 mg of calcium silicate, 200 mg sodium alginate and 100 mg of Famotidine.

Buoyancy of beads is directly related to performance of floating-pulsatile drug delivery system since lag time is equivalent to their floating time. Instantaneous in vitro floating behavior was observed for batches with calcium silicate due to low bulk density provided by porous nature of calcium silicate. Floating time is the time till all of the beads floated on medium. Floating time was primarily controlled by bulk density of beads, which in turn is affected both quantity of calcium silicate and concentration of sodium alginate. Floating behavior of beads could be explained by the polymer which forms liquid bridges over pores

present on surface of calcium silicate and did not intrude completely into the pores which results into air entrapped granules.

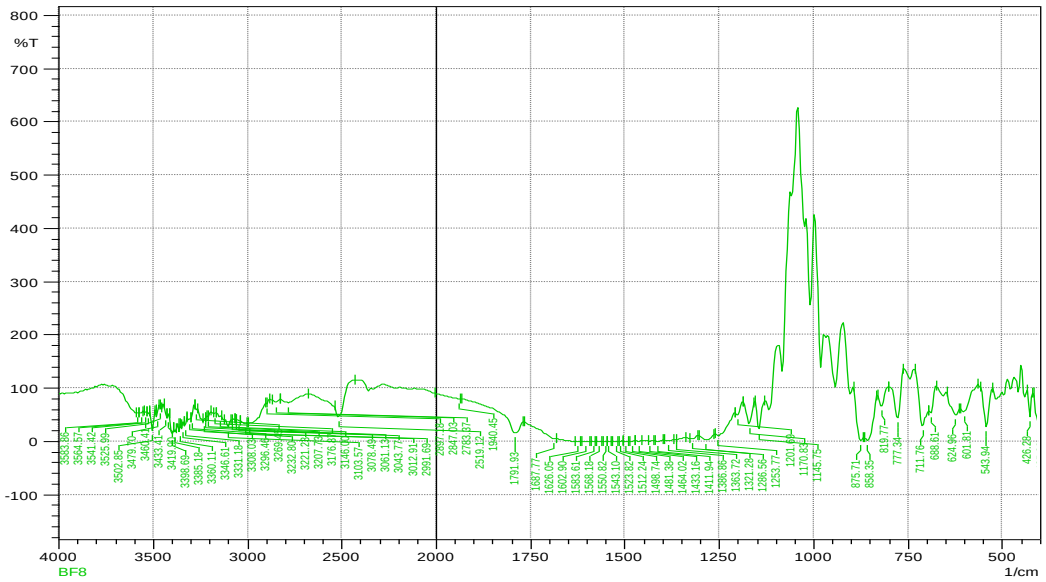


Fig 6. FTIR spectrum of Formulation BF6

Table 4. Buoyancy time of different batches of calcium alginate beads

| Batch Formula | Floating time (min) Mean± S.D* |
|---|---|
| **BF1** | 250±0.654 |
| **BF2** | 140±0.328 |
| **BF3** | 100±0.845 |
| **BF4** | 420±0.812 |
| **BF5** | 325±0.164 |
| **BF6** | 180±0.745 |
| **BF7** | 485±0.095 |
| **BF8** | 350±0.432 |
| **BF9** | 250±0.584 |
| **Control** | 150±0.184 |

## Size Analysis

Sizes of the beads were found to be in the diameter range of 1.34±0.09 to 2.49±0.05 mm for different batches and are tabulated in Table 5.

## Bulk Density (Db)

The bulk density of beads was found to be in the range of 0.481±0.002 to 0.656±0.003 for different batches and is given in Table 5. The decrease in bulk density was observed with increase in concentration of calcium silicate. The reduction in bulk density was due to the increase in size and porosity.

## Beads Porosity (%)

The % porosity for different batches was found to be in the range of 28.24±0.69 to 55.04±0.85 and is given in Table 5. It was noticed that, as the ratio of calcium silicate increased, the porosity also increased.

## Drug Content and Entrapment Efficiency

The drug content and entrapment efficiency of beads was found to be varied from 41.67±0.192 to 46.65±0.105 mg and 83.35±0.389 to 93.32±0.212 % for different batches, respectively and are mentioned in Table 5. The increased entrapment efficiency was observed due to the adsorption of famotidine over calcium silicate. Thus, drug entrapment efficiency was higher for the formulation BF9.

Table 5. Physicochemical properties of developed beads

| Batch Formula | Drug content (mg) | Entrapment efficiency (%) | Bead size (mm) | Bulk density (g/cc) | Porosity (%) |
|---|---|---|---|---|---|
| **BF1** | 41.67±0.192 | 83.35±0.389 | 1.48±02 | 0.605±0.003 | 33.2±0.16 |
| **BF2** | 42.25±0.122 | 84.51±0.249 | 1.61±0.01 | 0.631±0.003 | 36.52±0.61 |
| **BF3** | 42.8±0.226 | 85.60±0.447 | 1.78±0.01 | 0.656±0.003 | 35.63±0.81 |
| **BF4** | 42.05±0.110 | 84.11±0.215 | 1.65±0.04 | 0.511±0.001 | 41.51±0.73 |
| **BF5** | 43.63±0.156 | 87.27±0.307 | 1.81±0.03 | 0.556±0.001 | 38.29±0.87 |
| **BF6** | 45.52±0.085 | 91.06±0.170 | 1.92±0.07 | 0.594±0.002 | 41.54±0.58 |
| **BF7** | 44.23±021 | 88.46±0.422 | 2.12±0.03 | 0.481±0.001 | 55.04±0.85 |
| **BF8** | 44.70±0.11 | 89.42±0.233 | 2.31±0.07 | 0.514±0.002 | 54.16±0.15 |
| **BF9** | 46.65±0.105 | 93.32±0.212 | 2.49±0.05 | 0.544±0.002 | 57.07±0.83 |
| **Control** | - | - | 1.34±0.09 | 0.573±0.003 | 36.29±0.75 |

## Crushing Strength

The beads of all batches showed resistance to crushing when subjected to 3 to 4 kg probably due to stronger bonding between solid particles and alginate solution (Table 6). Higher mechanical strength of beads is imperative for avoiding breaking and distortion of beads during capsule filling or normal handling especially at the time of large batch production.

## Moisture Content

Low moisture content in all floating beads indicated the effectiveness of the adopted drying conditions. Low moisture level ensures better stability of famotidine in the beads. The moisture content for all batches was shown in Table 6.

## Scanning Electron Microscopy (SEM)

The SEM's of alginate beads were shown in Figure 7. The shape of the calcium alginate beads of BF6 was nearly spherical, porous and having rough surface since calcium silicate was layered over beads.

## Dissolution Studies

The cumulative release of all the batches was found to be in the range of 90.35 to 97.99%. Release studies in simulated gastric fluid (SGF) showed 1 to 10% drug release from beads of different batches because of improper coating of polymer during crosslinking. Comparatively lesser drug release at acidic pH was due to lack of disintegration and swelling of alginate beads. Sodium alginate shows hasty swelling and gel relaxation in pH 6.8 and showed burst release of drug from the beads (i.e., 80 to 85% of the drug should be released within 40-45 min from the dosage form) in alkaline pH. Consequently, the porous beads showed lag time in acidic media due to low solubility of polymer and then immediate pulse release in alkaline media. Drug dissolution of 85 to 90% from beads of each batch occurred within 45 min in simulated intestinal fluid (SIF), which would be worthwhile in in-vitro drug absorption from substantial surface area of small intestine. By reducing the concentration of alginate, the cumulative drug release was augmented (Table 6).

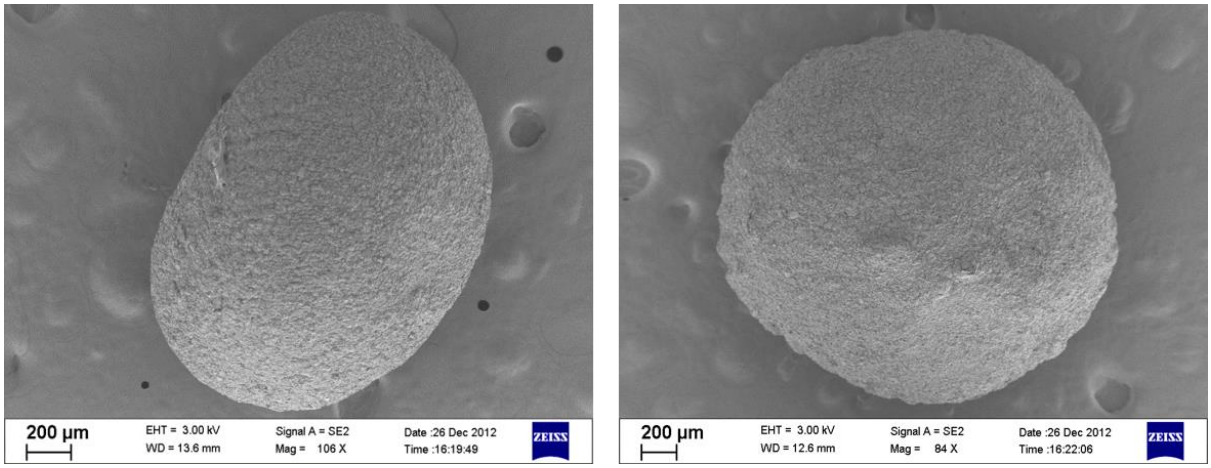


Fig 7. SEM photomicrographs of developed beads (Batch BF6)

Table 6. Crushing strength, moisture content and dissolution profiles of developed beads

| Batch Formula | Crushing Strength (Kg/cm²)* | Moisture Content (%)* | Dissolution Profile | |
|---|---|---|---|---|
| | | | Time (min) | Cumulative Drug Release (%) |
| **BF1** | 3.0 ±0.56 | 4.52±0.63 | 290 | 96.34 |
| **BF2** | 3.5 ±0.63 | 2.39±0.60 | 190 | 93.19 |
| **BF3** | 3.6 ±0.78 | 3.44±0.61 | 160 | 92.00 |
| **BF4** | 3.0 ±0.06 | 4.89±0.63 | 460 | 97.99 |
| **BF5** | 3.5 ±0.81 | 4.52±0.63 | 370 | 93.31 |
| **BF6** | 3.5 ±0.43 | 3.09±0.60 | 220 | 91.04 |
| **BF7** | 3.5±0.32 | 4.87±0.63 | 540 | 94.79 |
| **BF8** | 4.0±0.67 | 3.09±1.06 | 400 | 93.79 |
| **BF9** | 4.5±0.37 | 3.80±0.61 | 300 | 90.35 |
| **Control** | 3.5 ±0.13 | 2.39±0.60 | -- | -- |

## Drug Release Kinetics

The values of coefficient of correlation (r) were calculated and found to be more linear for first order release. It was concluded that release of drug from formulation batches BF1 to BF9 followed first order kinetics. The kinetic data was fitted to Korsemeyer-Peppas model. The values of diffusion coefficient (n) of all batches indicated that the release of drug occured by diffusion following non-Fickian transport mechanism (data not shown).

## CONCLUSION

From the prepared formulations, batch formula BF6 was found to be ideal with floating time of 3 h to maintain lag period. BF6 beads were having size range of 1.92±0.03 mm with entrapment efficiency of 91.06%. Cumulative drug release was found to be 91.06% at the end of 40 min after the lag period of 180 min. The present study demonstrates that famotidine could be successfully delivered to provide night-time relief of gastric acidity by the design of a floating pulsatile chronopharmaceutical formulation. The formulation is to be taken after meal; where timed pulsatile floating beads with delayed "burst" release will attenuate midnight acidity. This will provide an ideal therapeutic regimen with enhanced patient compliance. The developed system offers a simple and novel technique for pulse release of drugs in upper part of small intestine. The effect may be further confirmed by *in vivo* studies. Thus the fabricated outfit can be considered as one of the promising formulation technique for preparing floating pulsatile drug delivery systems. Hence, chronotherapeutic management of nocturnal acid breakthrough using an existing drug molecule, famotidine can be hopefully accomplished for the treatment of ulcer.

## DECLARATION OF INTEREST

It is hereby declared that this paper does not have any conflict of interest.

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