

## FORMULATION AND EVALUATION OF FAMOTIDINE MICRO BALLOONS WITH ENHANCED ANTI-ULCER ACTIVITY

RITESH KUMAR<sup>1</sup>, PAWAN KUMAR GAUTAM<sup>2</sup>, AMRISH CHANDRA<sup>3\*</sup>

<sup>1</sup>Department of Pharmacy, IFTM University, Moradabad, 244102, Uttar Pradesh, India, <sup>2</sup>Department of Pharmacy, S. N. Medical College, Agra, 282002, Uttar Pradesh, India, <sup>3</sup>Amity Institute of Pharmacy, Amity University, Noida, 201313, Uttar Pradesh, India  
Email: chandra.amrish@gmail.com

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### ABSTRACT

**Objective:** The aim of the present study was to formulate and optimize famotidine loaded micro balloons for enhancing bioavailability, increasing gastric residence time of drug and to achieve sustained release in the stomach.

**Methods:** Microballoons were prepared using emulsion solvent diffusion method using HPMC K4M as the polymer. All the formulated microspheres were subjected to various evaluation parameters such as % drug entrapment, micromeritics properties, % buoyancy and *in vitro* drug release studies. The formulation was optimized using 3<sup>2</sup> full factorial design. Optimized formulation was subjective to *in vivo* floating (X-ray) and *in vivo* antiulcer studies.

**Results:** The microballoons were smooth and spherical in shape and were porous in nature due to hollow cavity. Sustained/controlled release of drug was observed for more than 12 h. based on the results of % drug entrapment, *in vitro* drug release and % buoyancy studies, formulation F6 was selected as optimized formulation. The release kinetics of optimized formulation followed Higuchi model and mechanism of release was non-Fickian diffusion. Examination of the X-ray radiographic images taken during the study indicated that the optimized formulation remained buoyant and uniformly distributed in the gastric contents for a long period. In ethanol-induced ulcer model, drug-loaded microballoons treated group showed significant ulcer protection index of 83.26% as compared to the marketed brand of famotidine 76.09% and untreated control group.

**Conclusion:** Famotidine-loaded floating micro balloons were successfully prepared and prove to be useful for the prolonged gastric residence of the drug, better bioavailability, patient compliance and anti-ulcer activity.

**Keywords:** HPMC, Famotidine, *In vitro* release, Factorial design, % drug entrapment, *In vivo* floating

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### INTRODUCTION

Different types of ulcers such as duodenal ulcers, gastric ulcers, Zollinger-Ellison syndrome and gastroesophageal reflux disease are major side effects of many drugs and H<sub>2</sub>-receptor antagonists are used for their treatment. Famotidine is one such potent histamine H<sub>2</sub>-receptor antagonist with a short biological half-life (2.5-4 h). Famotidine is readily but incompletely absorbed with site-specific absorption in the upper part of the gastrointestinal tract (GIT) with an oral bioavailability of 43-50% due to narrow absorption window in GIT [1-2]. Famotidine is also stable in acidic pH and has low conventional dose (20-100 mg daily in divided dose) so it is able to allow incorporation of polymer and other formulation excipients. Famotidine shows site-specific action in the treatment of different types of ulcers thus providing drug continuously to its absorption sites in a controlled manner, extending absorption phase and increasing the magnitude of drug effect [3].

Therefore the development of controlled/sustained release formulation in the form of multiple units floating drug delivery system (FDDS) such as microballoons (floating microspheres) would be an ideal approach for oral delivery of famotidine. Microballoons are gastro retentive drug delivery systems based on non-effervescent approach. Microballoons are spherical empty particles without core ideally having a size less than 200 micrometres. Microballoons float and can be retained in the stomach due to their lower bulk density than the gastric contents and remain buoyant in the stomach for a prolonged period of time without affecting the gastric emptying rate of other contents. Microballoons assist in improving the oral sustained delivery of drugs that have an absorption window in a particular region of the gastrointestinal tract.

Microballoons are considered as one of the most promising buoyant systems, as they possess the unique advantages of multiple unit systems as well as better floating properties, because of central hollow space inside the microsphere [4-6]. The aim of present study was to

formulate and optimize gastro-retentive microballoons of famotidine for increasing gastric residence time of formulation thereby giving controlled release of famotidine in the gastric fluid for site-specific treatment of peptic ulcer, increasing the magnitude of drug effect, improving the oral bioavailability of the drug and patient compliance.

### MATERIALS AND METHODS

#### Materials

Famotidine was obtained as gift sample from Sun Pharmaceuticals Pvt Ltd, Gujarat, India. Hydrochloric acid was purchased from qualigens fine chemicals, Mumbai, India. HPMC K4M was procured from Titan Biotech Ltd, India. All the ingredients used were of pharmaceutical grade. Solvents of reagent grade were used in all experiments.

#### Methods

##### Drug-excipient compatibility study

Drug-excipient compatibility study was carried out to check the compatibility of the drug with the polymer in preparation of microspheres of famotidine. The drug and polymer mixture of quantity 10 mg and 400 mg of KBr were acquired in a mortar and were triturated. A little volume of the triturated sample was seized and kept on to the sample holder and examined from 4000 cm<sup>-1</sup> to 400 cm<sup>-1</sup> in Shimadzu FTIR 8400 spectrophotometer. The spectra obtained were matched with that of the peaks obtained from the FTIR study of the pure drug sample and interpreted for the interaction of drug and polymers if any [7-9].

##### Preparation of famotidine loaded floating microballoons

Floating microballoons were prepared by the emulsion solvent diffusion method. Nine formulations of floating microballoons were prepared. Different ratios of famotidine and HPMC K4M were mixed in a mixture of ethanol and dichloromethane (DCM) in the ratio of 2:1.

The resulting suspension was added slowly into stirring to 0.50% w/v 400 ml solution of polyvinyl alcohol (PVA) at room temperature. The emulsion formed was stirred continuously for 2 h using a propeller-type agitator at different rpm (900, 1200 and 1500 rpm). The temperature was maintained at 40°C. The finely dispersed droplets of the polymer solution of the drug were solidified in an aqueous phase via the diffusion of the solvent, leaving the cavity of microspheres filled with water. Microballoons formed were filtered using a nylon cloth and washed repeatedly with distilled water. The collected floating microballoons were dried at room temperature and stored in desiccators [10].

### Evaluation of famotidine loaded floating microballoons

#### Micrometrics properties of microballoons

##### Angle of repose

The angle of repose is the angle a pile forms with the ground. Angle of repose was determined using fixed funnel method. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the heap of the blends. The accurately weighed blend is allowed to pass through the funnel freely onto the surface. The height and diameter of the powder cone were measured and angle of repose was calculated using the following equation.

$$\theta = \tan^{-1}(h/r)$$

Where, h = height of pile, r = radius of pile, and  $\theta$  = angle of repose

##### Bulk density and tapped density

Bulk density is defined as the mass of powder divided by the bulk volume. Bulk density is determined by pouring a known quantity of microspheres into the measuring cylinder without compacting and read the unsettled apparent volume.

Bulk density is the ratio between a given mass of a microsphere and its bulk volume.

Bulk Density = Mass of powder/Bulk volume of the powder

Tapped density is determined by transferring a known quantity of microballoons (2 gm) into a measuring cylinder (10 ml) and is tapped mechanically till a constant volume is obtained. This volume is the bulk volume and it includes the true volume of the powder and the void space among the microballoons. Tapped density is calculated by using the formula.

Tapped density = Weight of powder/Tapped volume of the powder

##### Carr's index

Carr's compressibility index CI (Carr, 1965) is defined as follows:

$$CI = \frac{\rho_t - \rho_a}{\rho_t} = \frac{V_t - V_a}{V_t}$$

Where  $\rho_t$  and  $\rho_a$  -tapped and poured bulk density; and  $V_t$  and  $V_a$  -tapped and poured bulk volume respectively.

##### Hausner's ratio

A similar index has been defined by Hausner [11].

Hausner's ratio = Tapped density/Poured density

##### Determination of particle size

The particle size of microballoons was measured by using an optical microscope fitted with an eyepiece micrometre and a stage micrometre. The mean particle size was determined by measuring 200 particles with the help of a calibrated eyepiece micrometre [12].

##### Surface morphology

The surface characteristics and internal textures of the famotidine loaded floating microballoons were studied by scanning electron microscope. The samples were mounted on double-sided tape that has previously been secured on copper stubs and then analyzed at different magnifications. Photomicrographs were captured randomly using scanning electron microscope.

### Drug entrapment efficiency

Estimation of drug entrapment efficiency (DEE) in floating microballoons was carried out by dissolving the weight amount (100 mg) of crushed microballoons in required quantity of 0.1N HCl, pH 1.20 and analyzed spectrophotometrically at a wavelength of 266 nm using the calibration curve. The polymer debris was removed by filtering through Whatman filter paper (No. 40). Each batch should be examined in a triplet manner. The entrapment efficiency of floating microballoons was calculated by dividing the actual drug content by the theoretical drug content of microballoons [13]. The % DEE of floating microballoons was calculated using this following formula:

$$DEE (\%) = \left[ \frac{\text{Actual drug content of microballoons}}{\text{Theoretical drug content of microballoons}} \right] \times 100$$

### In vitro buoyancy study

This study was carried out by USP type II dissolution test apparatus by spreading the microballoons on a simulated gastric fluid (900 ml of 0.1 N HCl, pH 1.20) containing 0.02% w/v Tween 20 as a surfactant. The media is stirred at 100 rpm at 37±0.5°C for 12 h. After a specific interval of time, both the fractions of microballoons (floating and settled microballoons) were collected and buoyancy of the floating microballoons was determined by using formula [14].

$$\text{Buoyancy (\%)} = \frac{Q_f}{Q_f + Q_s} \times 100$$

Where  $Q_f$  is the weight of floating microballoons and  $Q_s$  is the weight of settled microballoons respectively.

### In vitro dissolution study

The *in vitro* dissolution studies were carried out using USP type II dissolution apparatus (Paddle Type). The study was carried out in 900 ml of 0.1N HCl (pH 1.20). The dissolution medium was kept in a thermostatically controlled water bath, maintained at 37±0.5°C. Microballoons containing drug equivalent to 40 mg were spread over the surface of 900 ml of dissolution media (0.1N HCl, pH 1.20). The paddle was rotated at 50 rpm. At predetermined time intervals i.e. 1, 2, 4, 6, 8, 10, 12 and 15 h 5 ml of sample was withdrawn from the dissolution apparatus and replaced with fresh media to maintain sink conditions. The drug concentration was analyzed by using UV spectrophotometer (Shimadzu 1800, Japan) at 266 nm [15-17].

### Optimization and validation of experimental design

Optimization technique based on response surface methodology was utilized. It is generally used to determine the optimum combination of factors that yield the desired response and describes the response near the optimum. The runs or formulations, which are designed based on 3<sup>2</sup> full factorial designs, are evaluated for the response variables. The response values are subjected to multiple regression analysis to find out the relationship between the factors used and the response values obtained. Independent variables or factors studied were a drug: polymer ratio ( $X_1$ ) and stirring speed ( $X_2$ ). The response values or dependent variables subjected for this analysis were % entrapment efficiency, % drug release at 12 h and % Buoyancy. The effect of formulation variables on the response variables was statically evaluated by applying one-way ANOVA at 0.05 level using a commercially available software package Design Expert® 10.0.3 (Stat-Ease, USA). The design was evaluated by polynomial cubic model, which bears the form of the following equation.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_1^2X_1^2 + b_2^2X_2^2 + b_{12}^2X_1^2X_2 + b_{12}^2X_1X_2^2$$

Where Y, is the dependent variable;  $b_0$  is the arithmetic mean response of the 9 runs; and  $b_1$  and  $b_2$  are the estimated coefficients for the independent factors  $X_1$  and  $X_2$ , respectively. 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 term ( $X_1X_2$ ) shows how the response changes when 2 factors are simultaneously changed. The polynomial terms ( $X_1^2$  and  $X_2^2$ ) are including investigating nonlinearity. The student t-test was conducted to examine the probability of each coefficient being equal to zero. All tests were performed at a 95% level of significance. In the final

reduced model of polynomial equation, only the significant factors and coefficients were included. The reduced polynomial equation was applied to predict the theoretical responses (predicted responses). Once the predicted response was established, the extra design checkpoint formulation was formulated and evaluated to validate the polynomial equation model.

The extra design checkpoint formulation (F10) was obtained from transformation of each of two variables at three levels. In general, the formula for transformation is as follow [18].

$$\frac{\text{"X-the average of the three levels"}}{\text{"1/2 of the difference of the levels"}}$$

The values for the variables obtained after transformation were incorporated into the extra design checkpoint formulation designated as F10.

The model, a comparison between the experimental and predicted values of the responses is also presented in terms of % Bias.

% Bias was calculated by the following equation:

$$\% \text{ Bias} = \frac{\text{Predicted value} - \text{Experimental value}}{\text{Predicted value}} \times 100$$

To demonstrate graphically the influence of each factor on responses and to indicate the optimum level of factors, the contour and response surface plots were generated using Design-Expert 10.0.3 software,  $p < 0.05$  was considered significant.

#### Drug release kinetics of optimized formulation

*In vitro* drug release data of optimized formulation (F6) was subjected to various mathematical models such as zero order, first order, Higuchi and Peppas kinetics models to understand the mechanism from the floating microballoons of famotidine. All curve fitting and plotting were performed using Microsoft excel software and regression coefficient ( $r^2$ ) values were calculated. In this by comparing the regression coefficient ( $r^2$ ) values obtained, the best fit model was selected [19-23].

#### *In vivo* floating behaviour study

Healthy rabbits weighing approximately 2 kg were used to assess *in vivo* floating behaviour. Ethical clearance for the handling of experimental animals was obtained from the institutional animal ethical committee (Registration No. 1321/PO/ReBi/S/10/CPCSEA) constituted for the purpose. The animals were housed in individual cages, and the experiments were carried out in a sanitized room. Rabbits fasted for 12 h and the first X-ray photograph was taken to ensure the absence of radio-opaque material in the stomach. Barium sulphate was incorporated into the microballoons to make the microballoons X-ray opaque. The amount of barium sulphate was kept 10 mg per g of microballoons to ensure visibility by X-ray and to enable the microballoons to float. Rabbits were made to swallow barium sulphate loaded microballoons with sufficient quantity of water. During the experiment, rabbits were not allowed to eat but the water was provided *ad libitum*. At predetermined time intervals (0, 6 and 12 h) the radiographs of the abdomen were taken using an X-ray machine [24-25].

#### Antiulcer activity

Albino Wistar rats of either sex weighing between (150-200 gms) were used to determine antiulcer activity. Ethical clearance was obtained from the institutional animal ethical committee (Registration No. 1321/PO/ReBi/S/10/CPCSEA) constituted for the purpose. Albino Wistar rats were housed in individual cages, and the experiments were carried out in a sanitized room. Albino Wistar rats were divided into four groups of six animals in each group. Group I served as control rats, administered 80% v/v ethanol (1 ml/200 gm, p. o.); Group II administered optimized formulation (20 mg/kg, p. o.); Group III administered optimized formulation modified with addition of 25 mg of gallic acid (20 mg/kg, p. o.) to study the effect of gallic acid (natural antioxidant); Group IV administered famotidine marketed drug (20 mg/kg, p. o.). The animals fasted for 24 h with free access of water. The animals were treated with formulations or control according to the various groups as designed. 1 hour later 80% v/v alcohol was administered p. o. to each animal. Animals were sacrificed after 1 hour of alcohol administration, stomachs were isolated, cut and opened along the greater curvature and pinned on a soft board. Ulcer index parameters were studied [26, 27].

Erosions formed on the glandular portion of the stomach were counted and each was given a severity rating on 1-3 scale, based on the diameter of the ulcer. Scoring of ulcers for normal stomach, red coloration, spot ulcer, hemorrhagic streak, ulcers and perforation were carried out as 0, 0.5, 1, 1.5, 2 and 3 respectively.

Mean ulcer score for each animal will be expressed as ulcer index. % Ulcer protection can be calculated by using this formula.

$$\% \text{ Ulcer protection} = \frac{\text{Mean ulcer in control} - \text{mean ulcer in test}}{\text{Mean ulcer in control}} \times 100$$

Calculation of ulcer index can be carried out using this formula.

$$UI = (UN + US + UP) \times 10^{-1}$$

UI = Ulcer index

UN = Average of number of ulcer per animal

US = Average of severity score

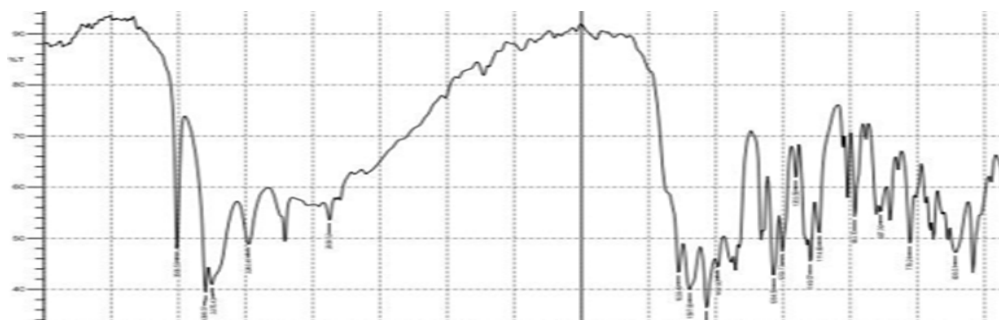
UP = Percentage of an animal with ulcer

The Dunnett's test was employed for statistical comparison. In all the cases, values of  $P < 0.05$  were considered significant. All values were presented as mean  $\pm$  SEM.

## RESULTS AND DISCUSSION

#### Drug-excipient compatibility study

After performing FTIR of the famotidine, HPMC and microballoons, it was found that the peaks obtained in the formulation were in between the range of main principle peaks and were found to be very near to previously performed FTIR of pure drug famotidine. No major deviation in peaks was obtained in IR spectra, hence this indicates that drug was compatible with excipients without any significant interaction between famotidine and HPMC. The results of IR spectra suggest that selection of excipient for preparation of microballoons were suitable. Hence it cannot alter the therapeutic efficacy of famotidine, and it also supports to continue further research works.



(a)

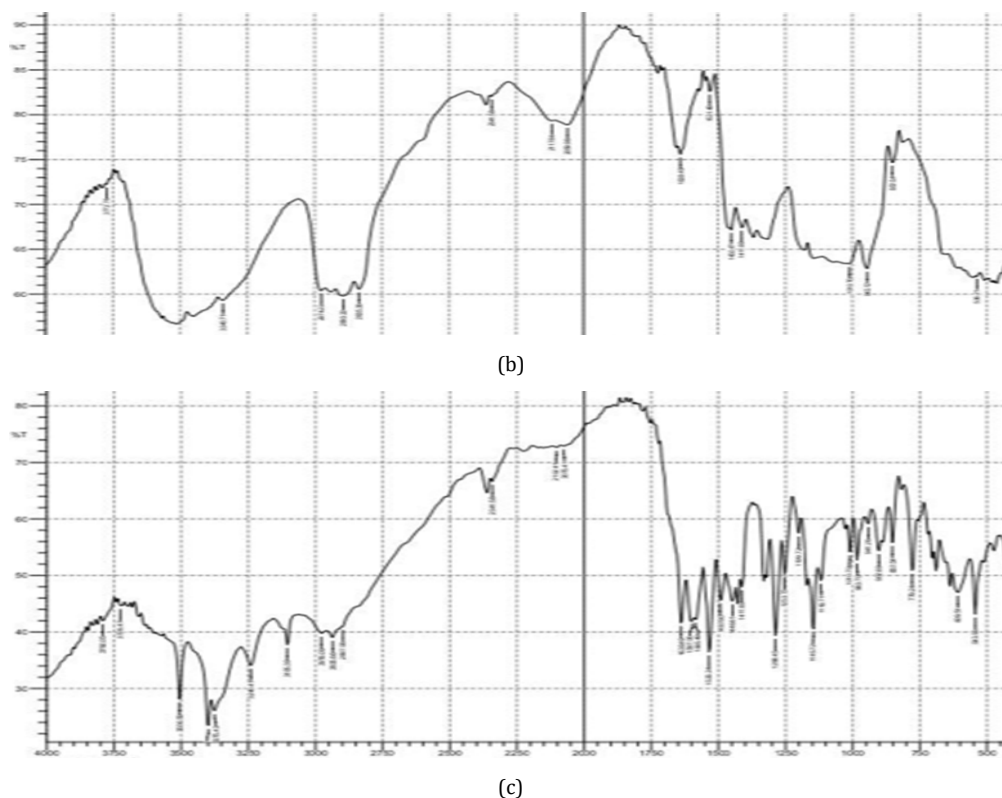


Fig. 1: FTIR spectra of (a) Famotidine (b) HPMC (c) Famotidine loaded microballoons

#### Preparation of famotidine loaded floating microballoons

The floating famotidine loaded microballoons were prepared using emulsion solvent diffusion method as discussed in preparation of floating microballoons of famotidine. The polymer used was HPMC K4M and its composition was tabulated in table 1. Further, the stirring speed was varying from 900 to 1500 rpm. The finely

dispersed droplets of the polymer solution of the drug were solidified in an aqueous phase via the diffusion of the solvent, leaving the cavity of microspheres filled with water. The benefits of preparation technique include low processing time, lack of exposure of drug to high temperature due to which stability of drug increased during the processing leading to high % entrapment efficiency of the drug in microballoons.

Table 1: Composition of floating microballoons using 3<sup>2</sup> full factorial experimental design

Formulations code	Variable level in the coded form	
	X <sub>1</sub>	X <sub>2</sub>
F1	-1	-1
F2	0	-1
F3	1	-1
F4	-1	0
F5	0	0
F6	1	0
F7	-1	1
F8	0	1
F9	1	1
Concentration/value of independent variables		
Level	Concentration of HPMC K4M (mg)	Stirring Speed (rpm)
-1	100	900
0	200	1200
1	300	1500
Response variables		
Y1	% Entrapment Efficiency	
Y2	% Drug Release in 12 h	
Y3	% Buoyancy	

#### Micromeritic properties of microballoons

All the prepared batches of famotidine microballoons were evaluated for micromeritic properties such as bulk density, tapped density, carr's compressibility index, hausner's ratio and angle of repose as shown in table 2.

#### Angle of repose

The values for angle of repose of all prepared nine formulation batches were determined in the range of 22.14°±0.67 to 27.19°±0.62 showing that the different batches of microballoons were free-flowing with good flow property. In case of pure drug angle of repose was 39.4

$\pm 1.34$  suggesting poor flow properties. All formulation batches possess smoother and regular surface of the particles.

#### Bulk density

The bulk density values for all nine formulation batches were determined. The result of poured bulk density ( $\text{g}/\text{cm}^3$ ) ranged from  $0.49 \pm 0.24$  to  $0.69 \pm 1.06$  as shown in table 2. The bulk densities of different formulations were found to be much less than the density of the gastric fluid ( $1.004 \text{ g}/\text{cm}^3$ ) and  $0.1 \text{ N HCl}$ , pH 1.20 ( $0.997 \text{ g}/\text{cm}^3$ ). Low density of microballoons increased the porosity of microballoons. Being less in density, the microballoons were expected to float immediately with less or no lag time.

#### Tapped density

The tapped density of microballoons of all nine formulation batches were found to be in the range of  $0.55 \pm 0.58$  to  $0.76 \pm 0.45 \text{ g}/\text{cm}^3$  as tabulated in table 2. Therefore, it was expected to be suitable for formulation of floating microballoons as they were having less density than  $0.1 \text{ N HCl}$ , pH 1.20.

#### Compressibility index

Carr's compressibility index was evaluated to determine compressibility characteristics of the microballoons. Carr's compressibility index of floating microballoons of all nine formulations ranged from  $7.935.40 \pm 0.0009$  to  $13.11 \pm 0.0003 \%$  indicating excellent flow properties of microspheres with good

compressibility while in case of pure drug Carr's compressibility index was  $24.86 \pm 0.0012 \%$  showed poor flow properties of the pure drug. Therefore, prepared floating microballoons showed improvement in flow properties as compared to pure drug and packability inside the capsules meant an ease of filling the microballoons inside the capsules.

#### Hausner's ratio

Hausner's ratio for all nine formulations was in the range of 1.08 to 1.15 ( $< 1.25$ ) indicating good flow properties of microballoons while in case of a pure drug, Hausner ratio was 1.33. It suggested that Hausner ratio of all formulations of microballoons were less than pure drug and the result of the study showed enhancement of flow properties of the pure drug in case of floating microballoons.

#### Determination of particle size

The mean particle size of all formulations was ranged from  $109.43 \pm 1.18 \mu\text{m}$  to  $160.58 \pm 0.73 \mu\text{m}$  as tabulated in table 2. Formulation batches F7 showed smallest and F3 showed the largest size of microballoons. Higher particle size was obtained when the proportion of HPMC K4M was increased in formulations. This was due to a significant increase in viscosity in a fixed volume of solvent, thus causing an increase in emulsion drop size and finally increases in size of particles. Increased speed of mixing (stirring) resulted in the formation of small-sized microballoons.

**Table 2: Micromeritics properties of different formulations of floating microspheres**

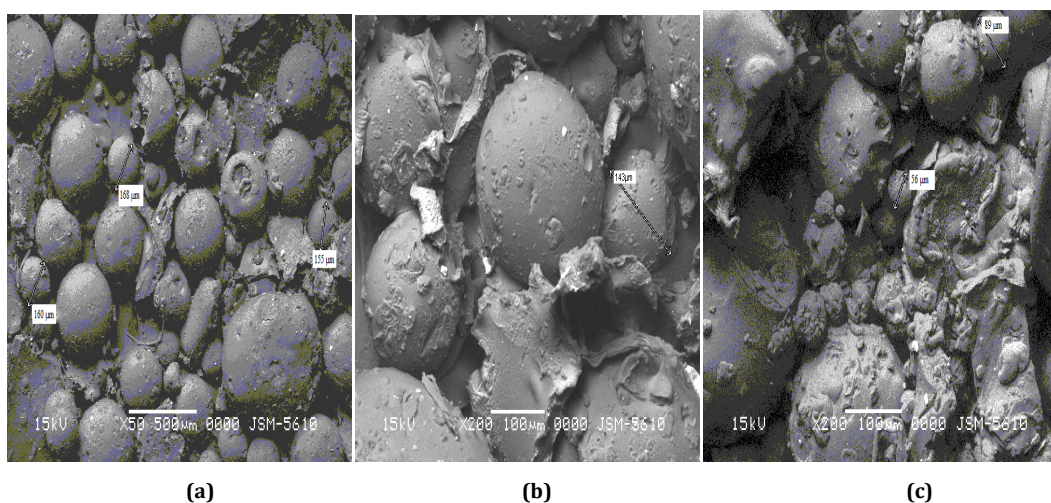
S. No.	Formulation code	Angle of repose ( $^\circ$ )	Poured Density ( $\text{g}/\text{cm}^3$ )	Tapped density ( $\text{g}/\text{cm}^3$ )	Carr's compressibility index (%)	Hausner's ratio	Particle size ( $\mu\text{m}$ )
1	F1	$22.14 \pm 0.67$	$0.49 \pm 0.24$	$0.55 \pm 0.58$	10.90	1.12	$151.25 \pm 0.25$
2	F2	$23.32 \pm 0.42$	$0.53 \pm 0.63$	$0.61 \pm 0.48$	13.11	1.15	$158.17 \pm 0.86$
3	F3	$25.28 \pm 0.61$	$0.61 \pm 0.72$	$0.67 \pm 0.35$	8.95	1.09	$160.58 \pm 0.73$
4	F4	$23.42 \pm 0.37$	$0.53 \pm 0.57$	$0.58 \pm 0.67$	8.62	1.09	$129.56 \pm 0.68$
5	F5	$23.84 \pm 1.74$	$0.59 \pm 0.39$	$0.65 \pm 0.53$	9.23	1.10	$135.28 \pm 1.15$
6	F6	$26.14 \pm 0.49$	$0.68 \pm 0.42$	$0.76 \pm 0.45$	10.52	1.11	$141.62 \pm 1.24$
7	F7	$23.68 \pm 0.88$	$0.58 \pm 0.82$	$0.63 \pm 0.58$	7.93	1.08	$109.43 \pm 1.18$
8	F8	$24.72 \pm 0.39$	$0.69 \pm 1.06$	$0.75 \pm 0.46$	8.00	1.08	$113.32 \pm 0.92$
9	F9	$27.19 \pm 0.62$	$0.62 \pm 0.76$	$0.68 \pm 0.73$	8.82	1.09	$120.14 \pm 0.78$

\* All values are expressed as mean  $\pm$  SD, n = 3

#### Surface morphology

The surface morphology of famotidine loaded floating microballoons was investigated by SEM. It was seen that microballoons were spherical in appearance and exhibit a range of sizes within each batch. The surface was observed to be smooth, dense and less

porous, whereas the internal core was highly porous. The less porous outer surface and highly porous internal surface supported controlled release of drug from the microballoons and good buoyancy. The porous nature and cavity formed in the microballoons would dictate the floating behaviour of famotidine loaded floating microballoons.



**Fig. 2: SEM photographs of (a) range of floating microspheres (b) smooth texture of floating microspheres (c) porous internal surface of floating microspheres**

**Drug entrapment efficiency**

The percent drug entrapment efficiency (% DEE) of all formulation batches were found to be in the range of 63.47±1.46% to 70.34±0.98% as shown in fig. 2. The % drug entrapment efficiency was more when the concentration of polymer (HPMC K4M) was increased in the drug: polymer ratio. Drug encapsulation efficiency was found to be increased due to increase in the concentration of gelling polymer leading to increase in crosslinking structure and viscosity of internal phase which leads to reduce migration of drug in aqueous phase. % DEE of formulations was found to decrease with an increase in stirring speed from 900 to 1500 rpm due to smaller size microspheres formed at a higher speed of rotation and leakage of drug from microspheres.

**In vitro buoyancy study**

*In vitro* buoyancy (%) for all the formulations batches, F1 to F9 were varied from 64.17±1.16% to 83.21±1.07%. It was observed that all the formulations were able to remain float in a continuous manner on the dissolution medium over a period of 12 h. The *in vitro* buoyancy of hollow microspheres of famotidine was due to the presence of pores, a hollow structure and low bulk density. It may also deduce that these microballoons can float in gastric fluid retarding the passage of the microballoons into the intestinal region and increasing their residing time in the stomach. The *in vitro* % buoyancy was found to increase with increasing concentration of polymer (HPMC K4M) while decreased with an increase in stirring speed.

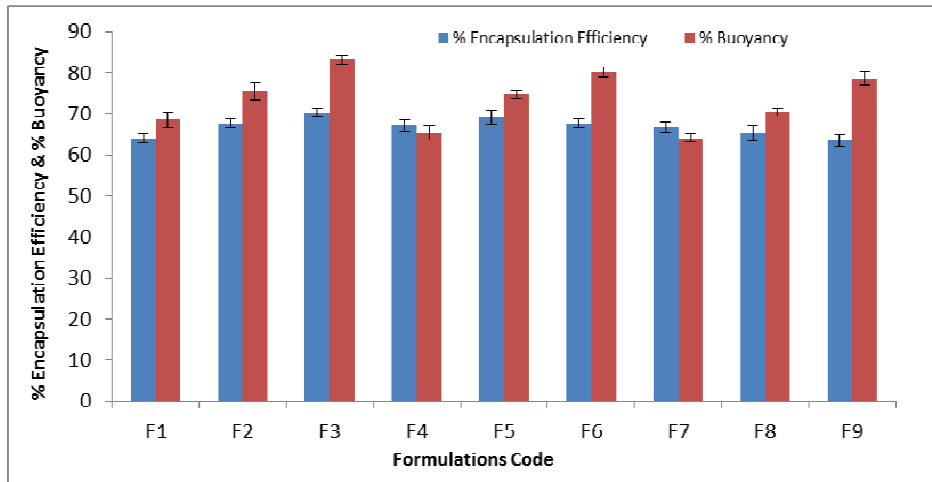


Fig. 3: Comparative chart of % encapsulation efficiency and % buoyancy of different formulations

**In vitro dissolution study**

*In vitro* drug release of famotidine from microballoons was performed in 0.1 N HCl, pH 1.20. The *in vitro* release profile showed initial burst release up to 1 h which may be due to the surface associated drug, followed by a sustained release phase as the entrapped drug slowly diffused into the dissolution medium. There was the sustained release of drug at a constant rate. The results of the study showed that on increasing the concentration of HPMC K4M it decreased drug release from microballoons. The high

concentration of HPMC K4M decreases the drug release from microballoons due to more stiffness of microballoons and increased diffusional path length. The other reason may be that smaller microballoons were formed at lower concentrations of HPMC K4M and have a large surface area exposed to the dissolution medium resulting in faster drug release. Higher stirring speed also lead to increase in drug release due to the breakdown of microballoons into small pieces or rupturing the surface of microballoons. Formulations with low stirring speed showed sustained release of drug from microballoons.

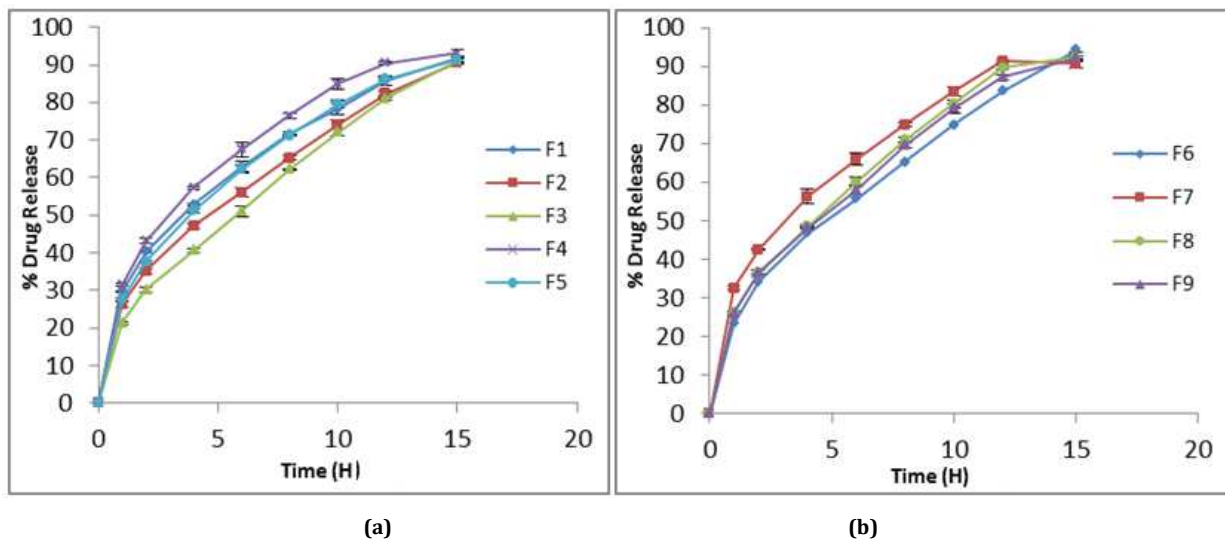


Fig. 4: *In vitro* drug release profile of floating microballoons for (a). F1 to F5 (b). F6 to F9

**Optimization and validation of experimental design**

The nine formulations of microspheres were prepared to study the effect of polymer concentration and speed of stirring speed.

The effect of formulation variables on the response variables was statically evaluated by applying ANOVA at 0.05 level using a commercially available software package Design Expert® 10.0.3 (Stat-Ease, USA). Mathematical polynomial cubic equations were generated for all the three dependent variables or response parameters such as % entrapment efficiency, % drug release at 12 h and % Buoyancy. The mathematical models were tested for significance. The response values are subjected to multiple regression analysis to find out the relationship between the factors used and the response value obtained.

As there were insignificant terms, the model reduction was required. After reduction, reduce cubic model was used and thus the data points were better fitted with the model and all the response models

were significant with the response parameters. It was found that quadratic model is best fitted to determine the effect of independent variables on response variables.

The quadratic equations generated for responses were given as

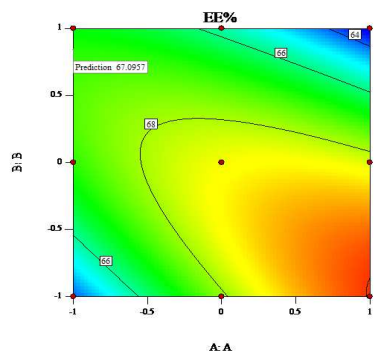
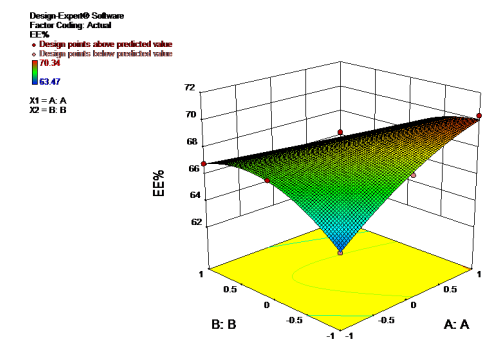
$$\% EE = +68.52 + 0.54X_1 - 1.08X_2 - 2.39X_1X_2 - 0.77X_1^2 - 1.72X_2^2$$

$$\% \text{ Drug release} = +86.38 - 2.61X_1 + 3.15X_2$$

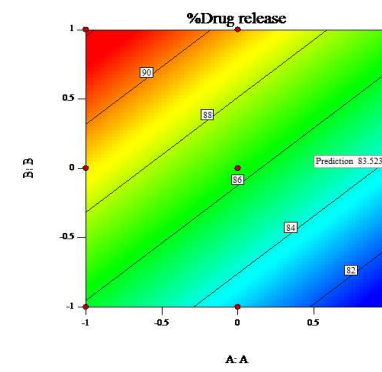
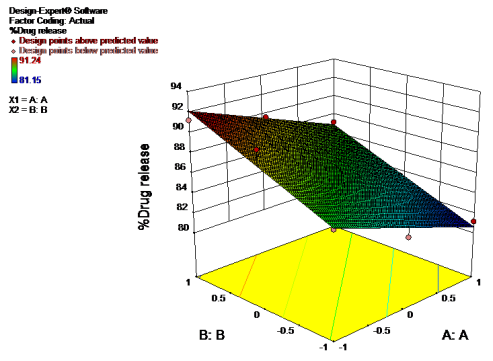
$$\% \text{ Buoyancy} = +73.41 + 7.31X_1 - 2.32X_2$$

Where  $X_1$  and  $X_2$  represent the coded values of the polymer concentration and stirring speed (rpm) respectively. The positive value of a factor in the above equations points out the enhancement of that response and vice versa.

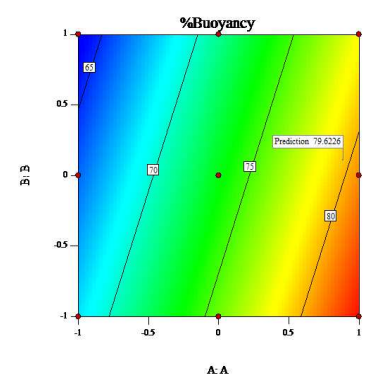
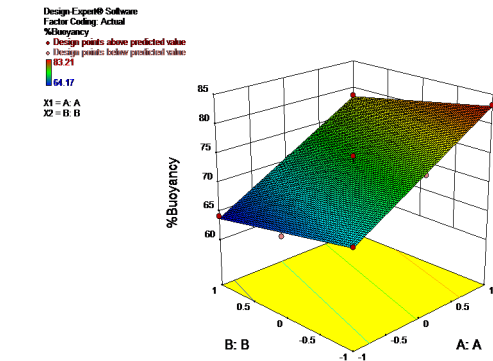
To demonstrate graphically the influence of each factor on responses and to indicate the optimum level of factors, the contour and response surface plots were generated using Design-Expert 10.03 software.



(a)



(b)



(c)

Fig. 5: Response surface and contour plot of effect of  $X_1$  and  $X_2$  on (a). % entrapment efficiency (b). % drug release at 12 h (c) % buoyancy

By calculating actual polymer concentration (225 mg) and stirring speed (1275 rpm) from transformed proportions of each variable, the extra design checkpoint formulation (F10) was designed.

The extra design checkpoint batch was observed to have % entrapment efficiency, % drug release and % buoyancy of 68.33%, 84.21% and 78.66% respectively. The statistical insignificance of the observed values for extra design checkpoint formulation (F10) was evaluated with the predicted value using Student's t-test.

It was found to non-significant with 95% confidence interval. This statistical insignificance of the difference between the predicted and observed responses of extra design checkpoint formulation not only

validate the design adopted for optimization but also confirmed the usefulness of a polynomial equation in predicting the % entrapment efficiency, % drug release and % buoyancy.

For the purpose of selecting the optimized formulation, the batch having maximum similarity when compared with the response variables of the extra design checkpoint batch and predicted values can be considered as the optimized batch.

Table 3 showed that the observed values of the prepared batch (F6) were very close to the predicted values, with low percentage bias, suggesting that the optimized formulation was trustworthy and rational.

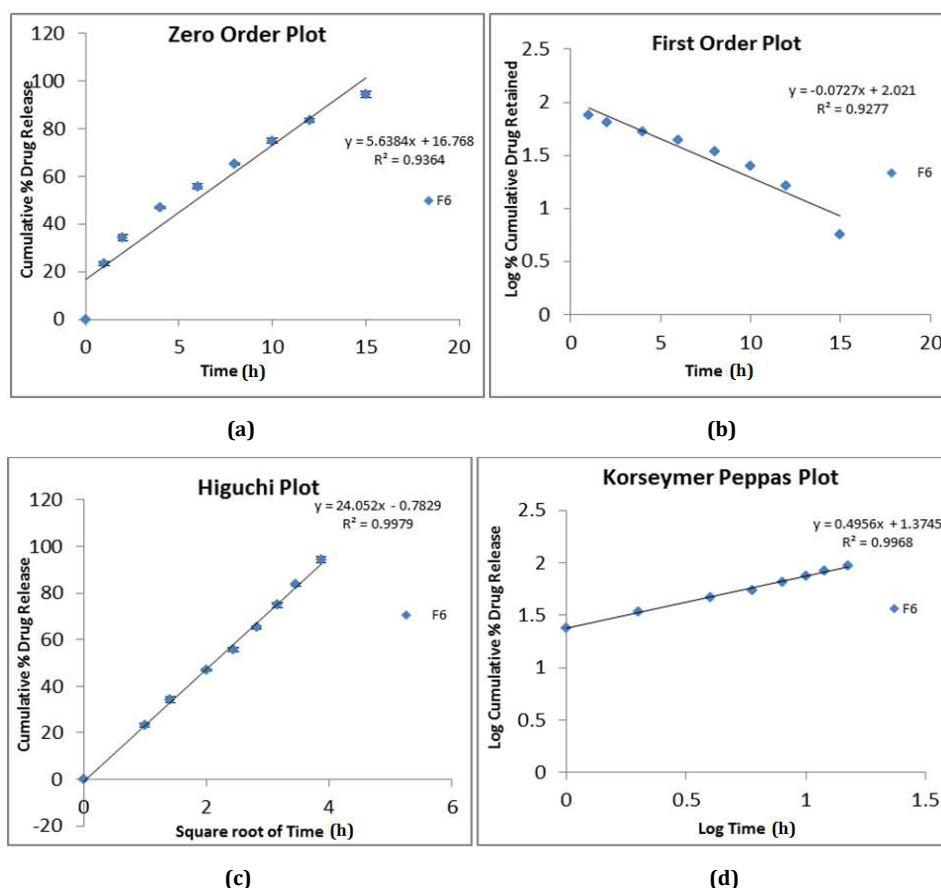
**Table 3: Comparison of the observed and predicted values in the microsphere**

Responses variable	Predicted value	Observed value	Bias %
% Entrapment Efficiency	67.0957	67.63	-0.796
% Drug Release	83.5238	83.52	0.0045
% Buoyancy	79.6226	80.14	-0.649

### Drug release kinetics of optimized formulation

The best selected *in vitro* drug release data of optimized formulation were fitted to various kinetics models such as zero order, first order, Higuchi and Peppas models. The highest regression coefficient ( $r^2$ ) values were obtained for Higuchi model (0.9979) followed by Korsymer-Peppas (0.9968), zero order (0.9364) and first order (0.9277) model using Microsoft excel software. It indicates diffusion

to be the predominant mechanism of drug release from floating microballoons. Release mechanism was studied by Peppas Equation and value of slope or diffusion exponent ( $n$ ) was determined. The value of diffusion exponent ( $n$ ) was found to be 0.4956 that indicates non-Fickian or anomalous diffusion mechanism which leads to the conclusion that drug release mechanism indicates a combination of diffusion and spheres erosion. Zero order, first order, Higuchi and Korsymer-Peppas plots were shown in fig. 6 (a), (b), (c) and (d).



**Fig. 6: Drug release kinetic profile of optimized formulation F6 (a) zero order (b) first order (c) Higuchi (d) Korsymer peppas**

### *In vivo* floating behavior study

The *in vivo* floating behavior of microballoons loaded with barium sulphate was investigated by radiographic images (X-ray

photographs) of rabbit's stomach at periodic time intervals. The radiographs obtained at 0, 6 and 12 h are shown in fig. which indicates a uniform distribution of formulation over the gastric fluid and *in vivo* duration of floating for more than 12 h.



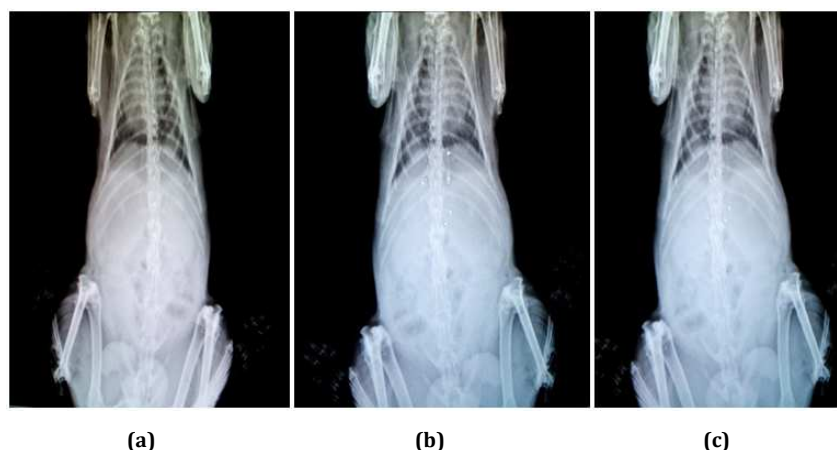


Fig. 7: X-ray photograph of rabbit stomach: (a) without drug loaded microballoons (b) showing buoyancy of microballoons after 6 h (c) showing buoyancy of microballoons after 12 h

#### Antiulcer activity

In control group of animals, oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, black and dark red lesions. The *in vivo* evaluation showed that animals treated with the optimized formulation, an optimized formulation with gallic acid and administered marketed brand of famotidine showed significant protection index of 83.26%, 89.04% and 76.09% respectively in

comparison to control. Gallic acid is a well-known natural antioxidant that is basically a secondary polyphenolic metabolite. Floating microballoons of an optimized formulation containing gallic acid showed high ulcer protection index of 89.04% as compared to 83.26% in the optimized formulation (F6). It may be due to the synergistic effect of famotidine with gallic acid. The combination of both may provide formulations with increased therapeutic effect. Macroscopical changes of ethanol-induced models are shown in fig. 8(a), (b), (c) and (d).

Table 4: Effect of various formulations on ethanol-induced gastric ulcer in rats

Treatment	Ulcer index	% Ulcer Protection	pH of gastric juice
Control	4.1±1.25	-	1.9±0.62
Optimized formulation	0.62±1.06*	83.26	4.3±0.39*
Formulation with gallic acid	0.49±1.35*	89.04	4.8±0.81*
Marketed brand of famotidine	0.98±10.83*	76.09	3.9±0.24*

Values expressed as mean±SEM; \*P<0.05 when compared with control group.

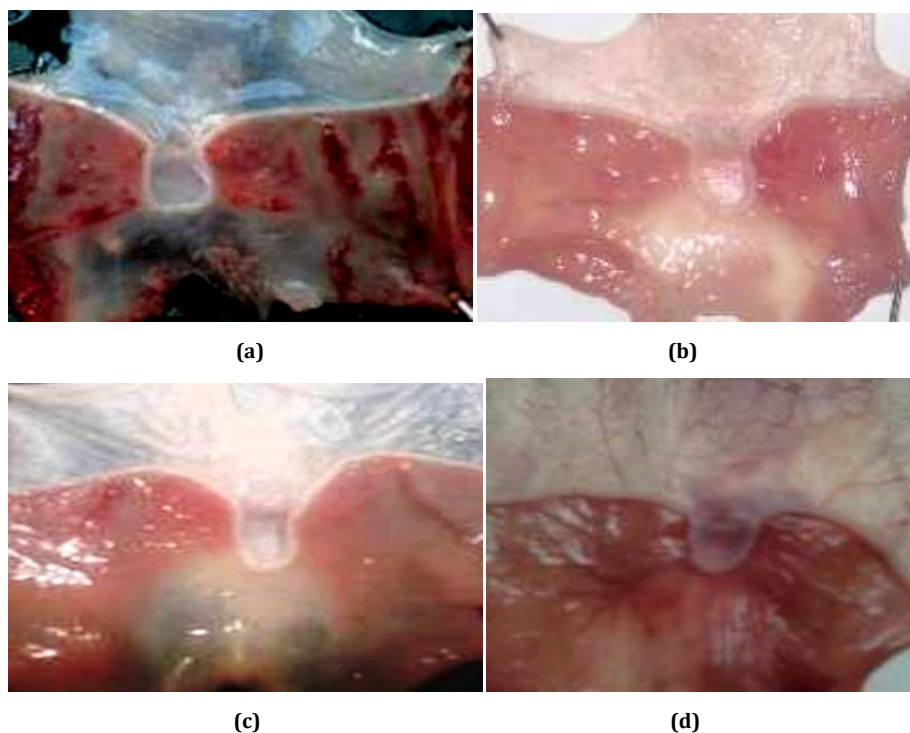


Fig. 8: (a) Ulcerated control stomach (b) Optimized formulation treated stomach (c) Optimized formulation with gallic acid treated stomach (d) Famotidine marketed drug treated stomach

## CONCLUSION

Floating microballoons of famotidine with swellable hydrophilic polymer were successfully prepared using emulsion solvent diffusion method, by applying  $3^2$  factorial design. The prepared microballoons had a different size and the % entrapment efficiency of the drug by varying the formulation variables such as polymeric concentration and stirring rate. The prepared formulations were further evaluated and based on the results of *in vitro* drug release studies, % entrapment efficiency and % buoyancy, F6 was chosen as the best formulation.

Optimized formulation (F6) followed Higuchi kinetics and the release mechanism was non Fickian diffusion ( $n=0.4956$ ). SEM study showed porous nature and cavity formed in the microballoons. *In vivo* radiographic images were helpful to locate the position of floating microballoons in the gastrointestinal tract and also confirmed that the prepared microballoons were able to retain in the stomach for a prolonged period of time and sustained release of famotidine. Thus, the prepared buoyant microballoons may prove to be potential candidates for a multiple-unit delivery system for intragastric drug delivery. *In vivo* anti-ulcer evaluation showed that animals treated with the optimized formulation, an optimized formulation with the gallic acid of famotidine showed significant ulcer protection of 83.26% and 89.04% respectively in comparison to control. Thus, such floating microballoons of famotidine prove to be useful for the prolonged gastric residence of the drug, better bioavailability and anti-ulcer activity.

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## AUTHORS CONTRIBUTIONS

All the author have contributed equally

## CONFLICT OF INTERESTS

There are no conflicts of interest

## REFERENCES

1. Tripathi KD. Essentials of medical pharmacology. 7<sup>th</sup> ed. New Delhi, India: Jaypee Brothers Medical Publishers; 2014.
2. Kumar R, Chandra A, Gautam PK. Development and validation of UV spectrophotometric method for quantitative estimation of famotidine in bulk and tablet dosage form. *Asian J Pharm Clin Res* 2017;10:381-5.
3. United States Pharmacopoeia 32, National Formulary 27, Rockville MD: United States Pharma copoeial Convention 2009;2:2342-5.
4. Kumar R, Kamboj S, Chandra A, Gautam PK. Microballoons: an advance avenue for gastro-retentive drug delivery system-a review. *UK J Pharm Biosci* 2016;4:19-30.
5. Kumar R, Gupta S, Chandra A, Gautam PK. Floating tablets: a realistic approach in gastro-retentive drug delivery system. *Int J Pharm Res Bio Sci* 2016;5:1-20.
6. Kumar R, Philip A. Gastroretentive dosage forms for prolonging gastric residence time. *Int J Pharm Med* 2007;21:157-71.
7. Skoog DA, Holler FJ, Crouch SR. Instrumental analysis. 11<sup>th</sup> ed. Delhi: Cengage Learning India Pvt Ltd; 2012.
8. Kalsi PS. Spectroscopy of organic compounds. 6<sup>th</sup> ed. New Delhi: New age International Publishers; 2012.
9. Sharma YR. Introduction of organic spectroscopy. 4<sup>th</sup> ed. New Delhi: CBS Publishers and Distributors; 1991.
10. Joshi VK, Jaimini M. Microballons drug delivery system: a review. *Asian J Pharm Res Dev* 2013;1:7-17.
11. Aulton ME. Pharmaceuticals: the science of dosage form design. 2<sup>nd</sup> ed. New York: Churchill Livingstone; 2002.
12. Martin A. Physical pharmacy. 4<sup>th</sup> ed. New Delhi: B I Waverly Pvt Ltd; 1996.
13. Kumar R, Chandra A, Saloni S, Gautam PK. Advanced multiple units controlled release floating beads: a review. *World J Pharm Res* 2017;6:238-59.
14. Mali AD, Bathe RS. An updated review on microballoons for a better approach in gastrotretnton. *AJP Sci* 2015;5:188-92.
15. Huber L. Validation of analytical methods and processes. In: *The Pharmaceutical codex, Principles and practice of pharmaceuticals*. 12<sup>th</sup> ed. London: The Pharmaceutical Press; 1994. p. 507-24.
16. Kumar R, Chandra A, Gupta S, Gautam PK. Development and validation of UV Method for quantitative estimation of lafutidine in bulk and tablet dosage form. *Int J Appl Pharm* 2017;9:75-9.
17. Kumar R. Development and *in vitro* evaluation of sustained release floating matrix tablets of metformin hydrochloride. *Int J Pharm Sci Res* 2010;1:96-101:13-7.
18. Bolton S. *Pharmaceutical statistics practical and clinical applications*. 4<sup>th</sup> ed. New York: Marcel Decker Inc; 2004.
19. Yang L, Fasshi R. Zero-order release kinetics from the self-correcting floatable configuration drug delivery system. *J Pharm Sci* 1996;85:170-3.
20. Mulye NV, Turco SJ. A simple model based on first-order kinetics to explain the release of highly water-soluble drugs from porous dicalcium phosphate dihydrate matrices. *Drug Dev Ind Pharm* 1995;21:943-53.
21. Higuchi T. Mechanism of sustained action medication. Theoretical analysis of the rate of release of solid drugs dispersed in solid matrices. *J Pharm Sci* 1963;52:1145-9.
22. Korsmeyer RW, Gurny R, Doelker EM, Buri P, Peppas NA. Mechanism of solute release from porous hydrophilic polymers. *Int J Pharm* 1983;15:25-35.
23. Kumar R, Chandra A, Garg S. Formulation and *in vitro* evaluation of sustained release gastro-retentive tablets of metformin hydrochloride. *Int J Thera Appl* 2012;7:13-7.
24. Gupta R, Prajapati SK, Pattnaik S, Bhardwaj P. Formulation and evaluation of novel stomach specific floating microspheres bearing famotidine for treatment of gastric ulcer and their radiographic study. *Asian Pac J Trop Biomed* 2014;4:729-35.
25. Sharma M, Kohli S, Dinda A. *In vitro* and *in vivo* evaluation of repaglinide loaded floating microspheres prepared from different viscosity grades of HPMC polymer. *Saudi Pharm J* 2015;23:675-82.
26. Sawant K, Patel M, Patel J, Mundada P. Formulation, optimization, characterization and *in vivo* anti-ulcer activity of esomeprazole magnesium trihydrate gastroresistant microspheres. *Int J Pharm Pharm Sci* 2017;9:273-82.
27. Sabry SA, Hasan AA, Abdallah MH. Gastroretentive nizatidine loading microballoons for treatment of peptic ulcer. *Int J Pharm Pharm Sci* 2015;7:220-5.