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Development and *in vivo* characterization of gastroretentive drug delivery system for the treatment of gastro esophageal reflux disease

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ABSTRACT

Gastroretentive drug delivery system i.e. floating microspheres of an H_2 receptor antagonist drug 'famotidine' was successfully prepared using combination of polymer as hydroxyl propyl ethyl cellulose K15M (HPMC K15M) and cellulose acetate via non-aqueous solvent evaporation (oil-in-water) technique. Famotidine loaded floating microsphere formulations were prepared by dissolving polymer in solvent mixture of acetone and ethyl acetate in which oil phase was slowly introduced and stirred well to obtain microspheres. The physicochemical properties of formulation was extensively studied such as surface morphology, particle size, percentage yield, percentage drug entrapment efficiency, swelling index, percent buoyancy and in vitro drug release studies. Anti ulcer activity of famotidine floating microspheres on swiss albino rats was found to be quite convincing regarding significant decrease in ulcer index and total acid volume as compared with standard and control group. The pH of stomach was found to be increases with a decrease in gastric acidity. It was concluded that drop in ulcer index resulting from floating microspheres of famotidine might contribute better for the gastro esophageal reflux disease (GERD) treatment.

Keywords: Floating microspheres, Anti ulcer activity, Ulcer Index, Total acid volume, gastric residence time

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INTRODUCTION

Oral administration is the most convenient and preferred means of drug delivery to the systemic circulation. Many attempts have been made to develop sustained-release preparations with extended clinical effects and reduced dosing frequency. In order to develop oral drug delivery systems, it is necessary to optimize both the release rate of the drug from the system and the residence time of the system within the gastrointestinal tract [1,2]. Various approaches have been used to retain the dosage form in the stomach as a way of increasing the gastric residence time (GRT) including floating systems. Floating systems allow prolonged residence time of dosage forms in the stomach and the achievement of constant plasma levels [3]. These have a bulk density lower than the gastric content. They remain buoyant in the stomach for a prolonged period, with the potential for continuous release of drug. Eventually, the residual system is emptied from the stomach. These systems can be classified in to the following types: First one is hydrodynamically balanced systems: These are single-unit dosage forms, containing one or more gel-forming hydrophilic polymers. Hydroxypropylmethylcellulose (HPMC) is the most common used excipients although hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC), sodium carboxymethylcellulose (NaCMC), agar, carrageenans or alginic acid are also used [2]. Second one is gas-generating system: Floatability can also be achieved by generation of gas bubbles. CO_2 can be generated in situ by incorporation of carbonates or bicarbonates, which react with acid- either the natural gastric acid or co-formulated as citric or tartaric acid. The optimal

stoichiometric ratio of citric acid and sodium bicarbonate for gas generation is reported to be 0.76:1. [4]. Third one is raft-forming system: Gel-forming solution (e.g. sodium alginate solution containing carbonates or bicarbonates) swells and forms a viscous cohesive gel containing entrapped CO_2 bubbles on contact with gastric fluid [5]. Last one is low density systems: Gas-generating systems inevitably have a lag time before floating on the stomach contents, during which the dosage form may undergo premature evacuation though the pyloric sphincter. Low-density systems ($<1 g/cm^3$) with immediate buoyancy have therefore been developed. They are made of low-density materials, entrapping oil or air. Most are multiple unit systems, and are called ‘‘microballoons’’ because of the low-density core [6,7].

Gastro esophageal reflux disease (GERD) is a most common severe problem amongst the wide range of population across the nation due to unhealthy food habits and life style. The most frequent symptom of GERD is heartburn for which antacids and H_2-receptor antagonist are prescribed. The effect of H_2 blockers is very short i.e. it requires dosing several times per day and also associated with undesirable fluctuations in gastric acid levels [8].

Famotidine is a histamine H_2-receptor antagonist that inhibits stomach acid production, and it is commonly used in the treatment of GERD. It also treats conditions in which the stomach produces too much acid, such as Zollinger-Ellison syndrome and other conditions in which acid backs up from the stomach into the esophagus, causing heartburn [8].

The purpose of this study was to develop and evaluate gastroretentive drug delivery system of an H_2-receptor blocking drug so to increase the gastric residence time of drug in stomach and obtain a sustained drug delivery. This also reduces the dosing frequency of the drug.

MATERIALS AND METHODS

Materials

Famotidine was obtained as gift sample from Cadila Pharmaceuticals Limited, Ahmedabad, India. HPMC K15M was obtained as gift sample from Sun Pharmaceuticals, Baroda, India. Cellulose Acetate, Acetone, Ethyl Acetate, Liquid Paraffin, and Petroleum ether were purchased from Central Drug House (P) Ltd. New Delhi, India. All other chemicals used were of analytical grade.

Preparation of Floating Microspheres

Floating microspheres containing famotidine were prepared by non-aqueous solvent evaporation (oil-in-water) technique. The drug and polymer in different proportions are weighed (as shown in table 1), the polymer was co-dissolved into previously cooled mixture of acetone and ethyl acetate at room temperature. The slurry of liquid paraffin while was slowly introduced and stirred at 500 RPM with the help of mechanical stirrer which is equipped with three bladed stirrer at room temperature. The solution was stirred for 2 h, so that, the solvent to evaporate completely and microspheres were collected by filtration. The microspheres were washed repeatedly with petroleum ether (40-60°C) until free from oil. The collected microspheres were dried for 1 h at room temperature and subsequently stored in desiccators over fused calcium chloride [9]

Characterization of Famotidine loaded Floating Microspheres

Particle size analysis

Size distribution was determined by optical microscopy using stage micrometer slide and calibrated eye piece by counting at least 100 microspheres per batch. [9]

Surface morphology

Shape and surface morphology of drug loaded floating microspheres was visualized by scanning electron microscopy (LEO-430 Cambridge and U.K). Samples were prepared by lightly sprinkling nanoparticles on a double adhesive tape, on an aluminum stub. The stubs were then coated with gold to a thickness of 200 to 500 A^0 under an argon atmosphere using gold sputter module in a high vacuum evaporator. The samples were then randomly scanned and photomicrographs taken at different magnifications with SEM [11]

Percentage Yield

The prepared floating microspheres of famotidine were collected and weighed for determining the percentage yield of microspheres. The measured weight was divided by total amount of all non-volatile components which were used for the preparation of microspheres [12]. The yield of microspheres was calculated by the formula given below:

% Yield = (Actual weight of product / Total weight of excipients and drug) × 100

Percentage Drug Entrapment Efficiency (%DEE)
To determine the incorporation efficiency, 10 mg of microspheres were taken. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1N HCl repeatedly. The suspension was filtered to separate shell fragments. Drug contents were analyzed spectrophotometrically at 217 nm [12]. The amount of drug entrapped in the microspheres was calculated by the following formula.

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

Swelling index
For estimating the swelling index, the microspheres were suspended in 5 mL of simulated gastric fluid USP (pH 1.2). The particle size was monitored by microscopy technique every 1 h using an optical microscope (Labomed CX RIII). The increase in particle size of the microspheres was noted for up to 8 h, and the swelling index was calculated [13]. The swelling index for the microspheres of Formulations F1 to F9 is reported in the Table.

***In vitro* evaluation of floating ability (% Buoyancy)**
Microparticles (0.3g) were spread over the surface of a USP XXIV dissolution apparatus (type II) filled with 900 ml 0.1 mol- HCl containing 0.01% Tween 80. The medium was agitated with a paddle rotating at 100 RPM for 12 h. The floating and the settled portion of microspheres were recovered separately. The microspheres were dried and weighed. Buoyancy percentage was calculated as the ratio of the mass of the microspheres that remained floating and the total mass of the microspheres [14].

% Buoyancy = (Weight of floating microspheres/ Initial weight of floating microspheres) × 100

***In-vitro* Drug Release Studies**
The *in-vitro* dissolution studies were carried out by using USP II paddle type dissolution apparatus. Weighed amount of drug loaded floating microspheres was introduced into 900 ml 0.1 N HC1, used as a dissolution medium, maintained at 37 ±0.5˚C at a rotation speed of 100 RPM. The samples were withdrawn at predetermined time intervals. First two samples were withdrawn at 30 min. interval and next five samples were withdrawn at 1 h interval. The samples were analyzed spectrophotometrically at 217 nm to determine the concentration of drug present [15, 16].

Release Kinetics Studies
The data obtained for *in-vitro* release were fitted for zero-order, first-order, and Higuchi release models for F9 formulation. The interpretation of data was based on the value of the resulting regression coefficients. The *in-vitro* drug release showed the highest regression coefficient values for Higuchi's model, indicating diffusion to be the predominant mechanism of drug release [17].

***In vivo* Studies:**
The Swiss albino rats weighing between 150-250 gm, were divided into 5 groups, in which each group contain 6 rats [18]. The care and maintenance of animals were as per the approved guidelines of the "Committee for the purpose of control and supervision of experiments on animals" (CPCSEA), India (Reg. No. 837/ac/04/CPCSEA). All animal procedure was approved by the Institutional Animal Ethical Committee.

Several groups divided are describes as follows:
Group-1: Served as Control (Glacial Acetic Acid)
Group-2: Served as Standard (Omiprazole 20mg/kg)
Group-3: Served as treated Test I (Famotidine floating microspheres)
Group-4: Served as treated Test II (Famotidine floating microspheres)
Group-5: Served as treated Test III (Famotidine floating microspheres)

RESULTS AND DISCUSSION

The gastroretentive drug delivery system has been successfully prepared by non-aqueous solvent evaporation (oil-in-water) technique. Microspheres were chiefly spherical in appearance. The percentage yield of floating microspheres was greater than 70% for all the formulations as shown in Fig. 1. To observe the effect of polymer concentration on the percentage yield of the resulting microspheres, formulation were prepared using varying concentration of cellulose acetate and HPMC K 15 M with respect to total amount of polymers. The percentage yield of the microspheres was found to be increased with increasing cellulose acetate concentration (Table 2). The particle size of floating microspheres formulation F1 to F9 was found to be between 220±1.78 to 290 ± 1.98 (Table 2). The effect

of polymer concentration on the particle size of floating microspheres was determined. The particle size of the microspheres was found to be increased with increasing cellulose acetate concentration (as shown in Table 2). The size of microspheres was determined using a microscope fitted with an ocular micrometer and stage micrometer. Scanning electron microscopy was performed to characterize the surface of formed microspheres (Fig. 2). The effect of the combination of the polymers over encapsulation efficiency was convincing. The encapsulation efficiency was found to be abruptly increasing when both polymers were used together as shown in Fig. 3. Encapsulation efficiencies of formulation F1-F9 ranged from 75.28±2.67% to 90.65±0.13%. Maximum encapsulation efficiency was observed in the formulation consist of cellulose acetate and HPMC K 15 M. *In vitro* drug release studies revealed a sustained release upto 24h. Formulation F9 shows the maximum release as shown in Fig 4. *In-vivo* studies were performed for evaluation of anti-ulcer activity of Famotidine floating microspheres as shown in Fig. 5 [19]. On increasing dose of Famotidine floating microspheres (3mg/kg, 6mg/kg, 12mg/kg) ulcer-index was found in Control group, Standard group (Omiprazole) 20 mg/kg, Test group I, Test group II, Test group III as 132.17±0.57, 23.67±0.34, 67.33±0.24, 39.00±0.94, 29.66±0.12 respectively (Table 4, Fig. 6), pH was determined to be 1.6±0.45, 4.8±0.51, 2.5±0.22, 3.2±0.35, 4.1±0.03 in Control group, Standard group (Omiprazole) 20 mg/kg, Test group I, Test group II, Test group III respectively, (Table 3, Fig. 7), total acid volume was determined to be 1.8±0.2, 2.4±0.45, 2.0.9±0.2, 2.9.4±0.5, 1.7±0.62 in Control group, Standard group (Omiprazole) 20 mg/kg, Test group I, Test group II, Test group III respectively (Table 4, Fig. 8) and gastric acidity (µEq/100g/h) was determined to be 67.83±2.70, 16.29±0.60, 32.17±0.54, 27.33±0.33, 18.33±0.66 in Control group, Standard group (Omiprazole) 20 mg/kg, Test group I, Test group II, Test group III respectively (Table 3, Fig. 9). A significant correlation ($p<0.0001$) was observed between the acidity and severity of the gastric damage (given as UI), demonstrating the effect of acidity in ulcer induced by pylorus ligation.

Table 1: Formulation Table of several Famotidine loaded Floating Microspheres

Formulations	Amount of Drug (mg)	HPMC K 15 M (mg)	Amount of Cellulose acetate (mg)	Acetone and Ethyl acetate (1:1) (ml)	Amount of liquid Paraffin (ml)
F1	30	150	0	30	30
F2	30	300	0	30	30
F3	30	450	0	30	30
F4	30	120	30	30	30
F5	30	240	60	30	30
F6	30	360	90	30	30
F7	30	100	50	30	30
F8	30	200	100	30	30
F9	30	300	150	30	30

Table 2: Physico-chemical Parameters of Famotidine loaded Floating Microspheres Formulations

Formulations	Particle Size ±S.D (µm)	Percentage Yield ±S.D	Percentage Drug Entrapment Efficiency ±S.D	Percentage Buoyancy ±S.D	Swelling Index
F1	220±1.78	70.56±2.42	80.74±2.34	88.71±1.67	0.866
F2	240±0.23	76.14±1.37	79.17±2.56	83.90±1.32	0.819
F3	235±0.89	79.37±2.45	75.28±2.67	82.21±1.09	1.172
F4	184±1.02	72.11±1.67	76.61±1.37	87.56±2.34	1.113
F5	244±2.78	80.90±2.23	77.09±1.89	89.95±2.67	0.982
F6	210±0.19	85.19±1.89	80.49±2.70	84.21±2.89	1.423
F7	271±2.54	92.71±2.67	90.65±0.13	97.05±2.76	1.282
F8	277±2.87	93.94±1.90	86.97±0.89	80.26±0.34	1.22
F9	290±1.98	98.21±1.85	81.89±0.36	91.12±1.42	1.45

Table 3: Gastric acidity and pH of various Animal Groups treated with Famotidine floating Microspheres

S. No.	Treatment	Number of Animals	Gastric Acidity (µEq/100g/h)	pH
1.	Control group	06	67.83±2.70	1.6±0.45
2.	Standard Group (Omiprazole) 20 mg/kg	06	16.29±0.60	4.8±0.51
3.	Test group I	06	32.17±0.54	2.5±0.32
4.	Test group II	06	27.33±0.33	3.2±0.05
5.	Test group III	06	18.33±0.66	4.1±0.03

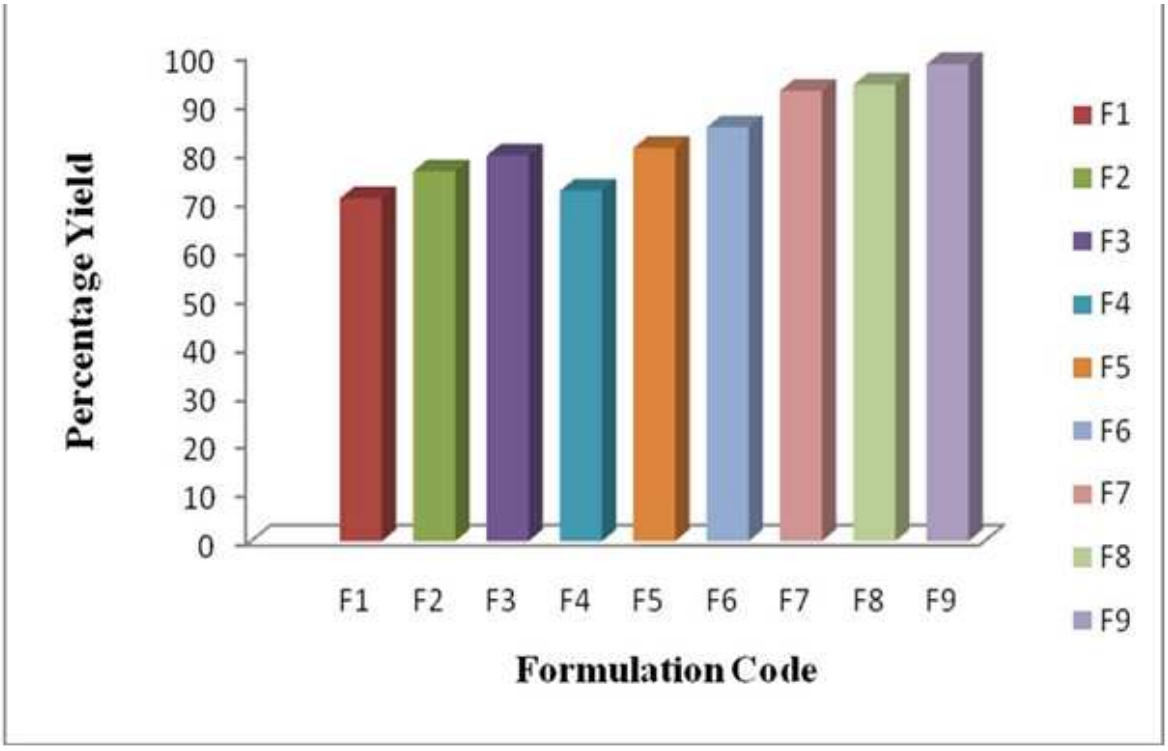

Fig. 1: Percentage Yield of different Famotidine loaded floating microspheres formulations

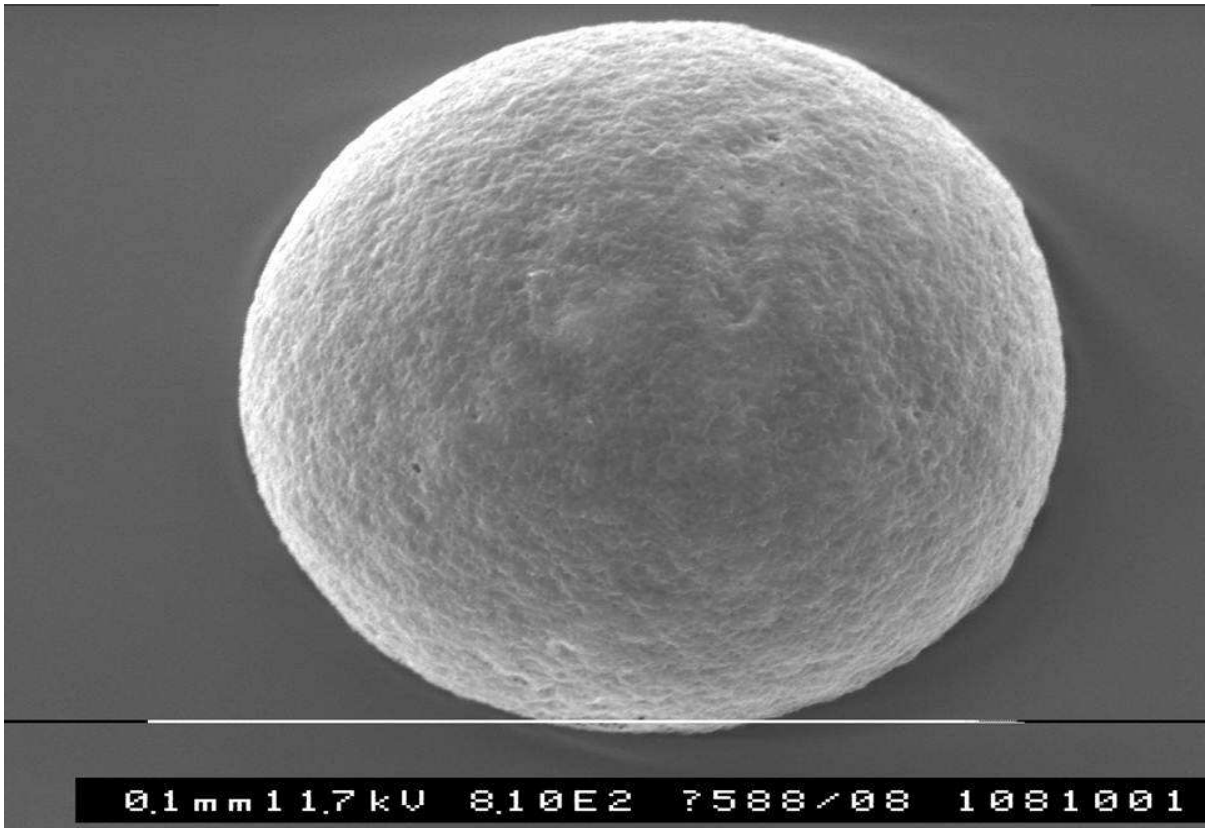

Fig 2: Scanning Electron Microscopy SEM Photograph of Floating Microspheres of Famotidine (F9)

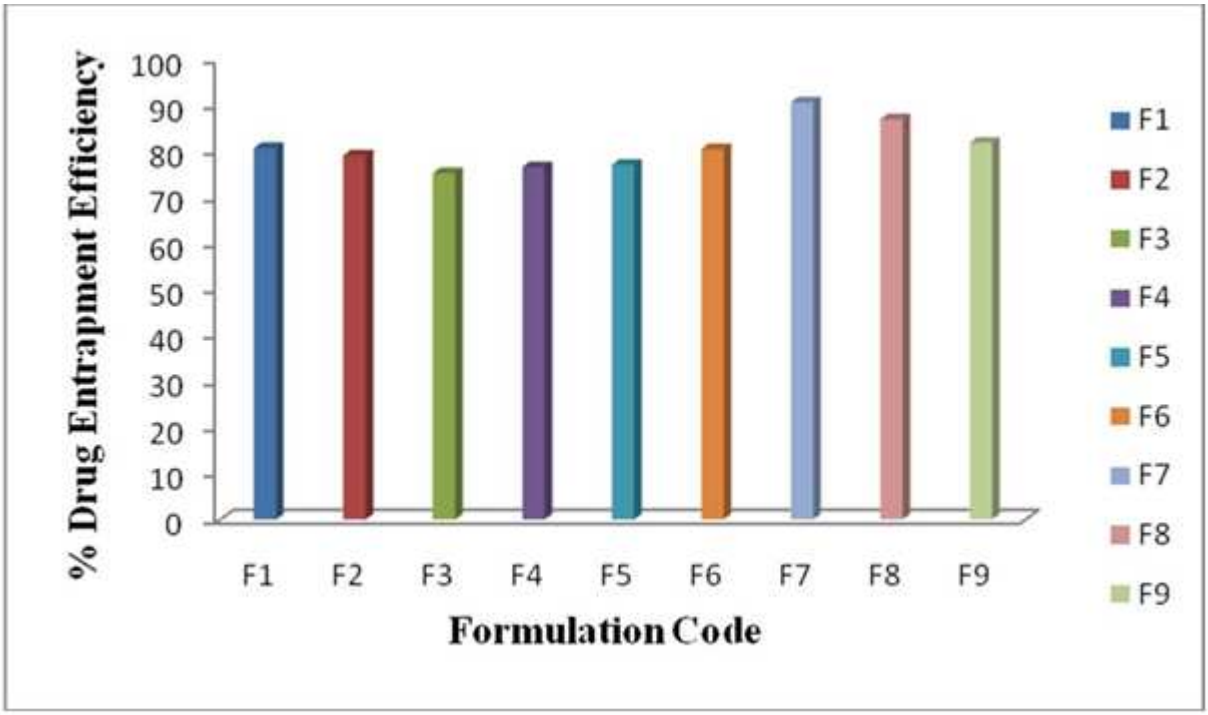

Fig. 3: Percent Drug entrapment efficiency of different formulations of Famotidine floating microspheres

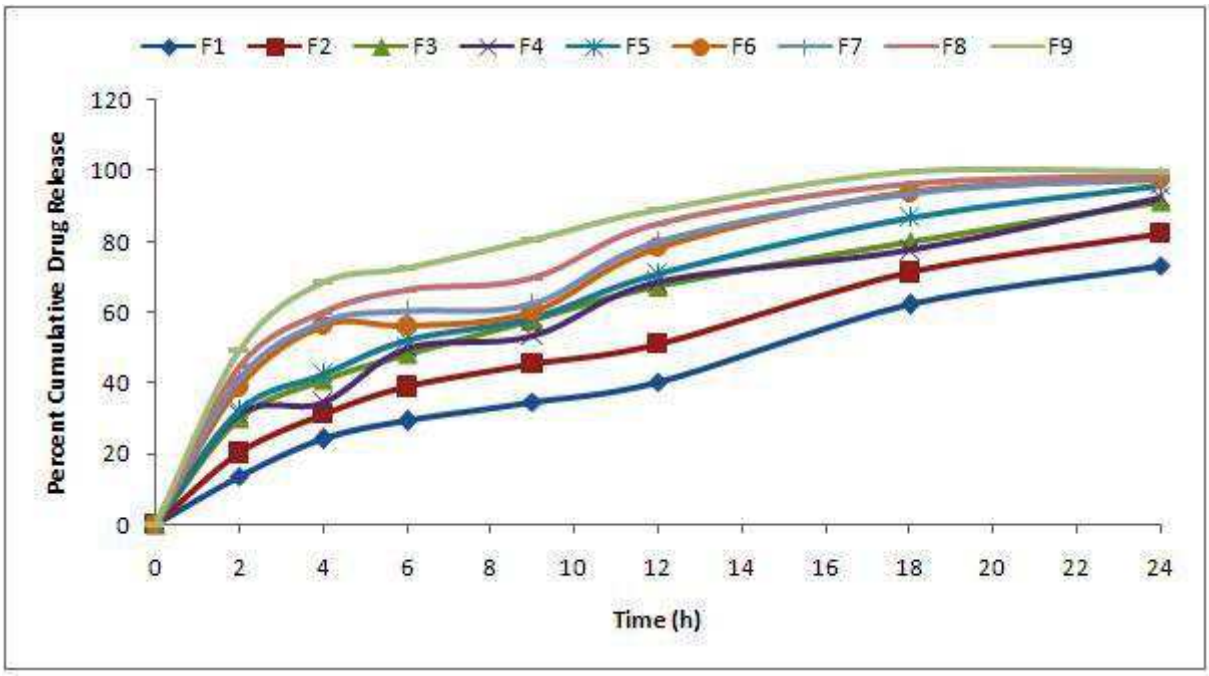

Fig 4. *In vitro* drug release profiles of all formulations (F1-F9)

Table 4: Ulcer-Index (mm) and Total acid volume (ml) of various Animal Groups treated with Famotidine microspheres

S. No.	Treatment	Animals	Ulcer Index (mm)	Total acid volume (ml)
1.	Control group	06	132.17±0.57	1.8±0.26
2.	Standard Group (Omiprazole) 20 mg/kg	06	23.67±0.34	2.4±0.45
3.	Test group I	06	67.33±0.24	2.0±0.23
4.	Test group II	06	39.00±0.94	2.9±0.52
5.	Test group III	06	29.66±0.12	1.7±0.62

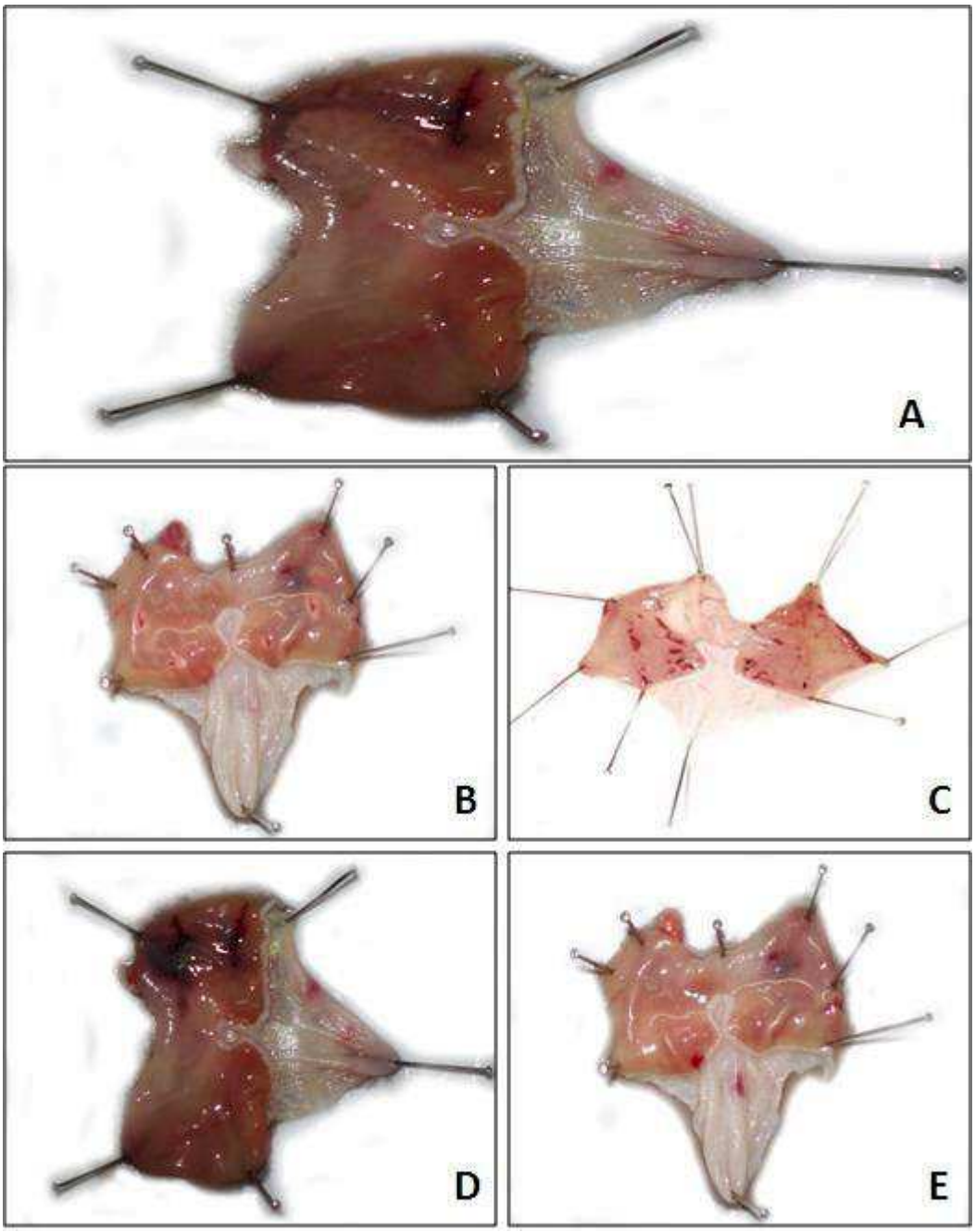

Fig 5: Stomach of rat (A) Standard group (Omiprazole 20mg/kg); (B) Drug dose (3 mg/ml) of test group I; (C) Control group (Glacial Acetic Acid); (D) Drug dose (6 mg/ml) of test group II; (E) Drug dose (12 mg/ml) of test group III

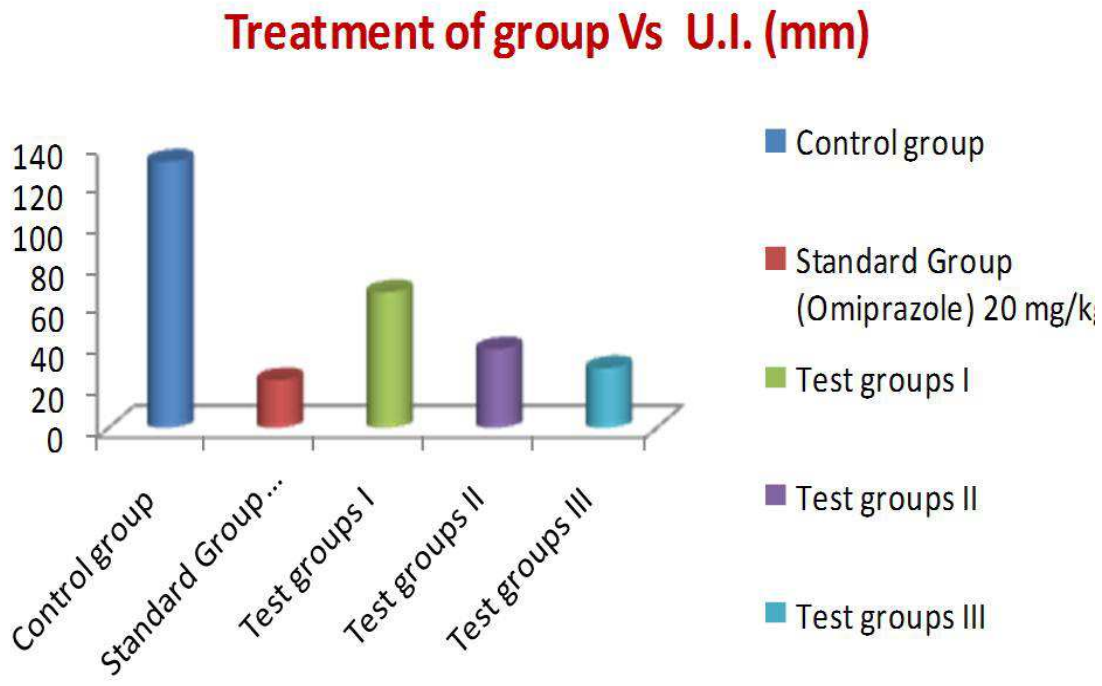

Fig. 6: Ulcer-index of various groups after increasing dose of Famotidine Floating microspheres

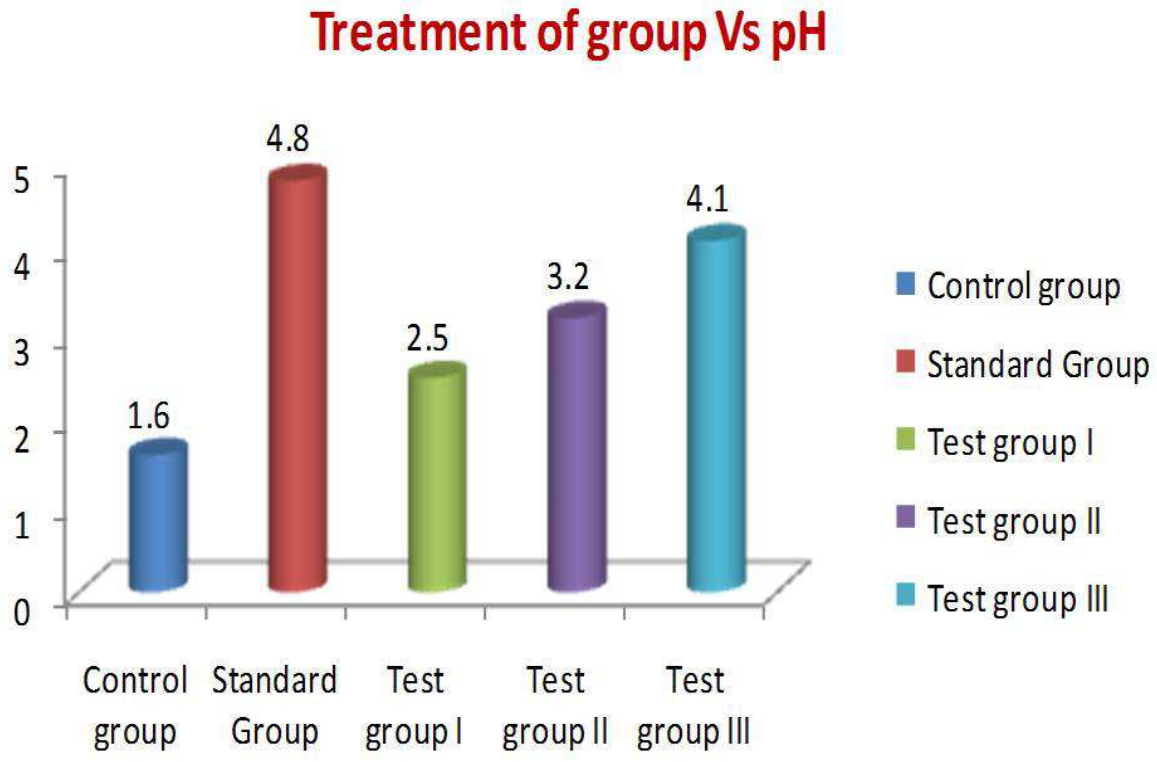

Fig. 7: Effect of increasing dose of Famotidine on the pH of different groups of animal

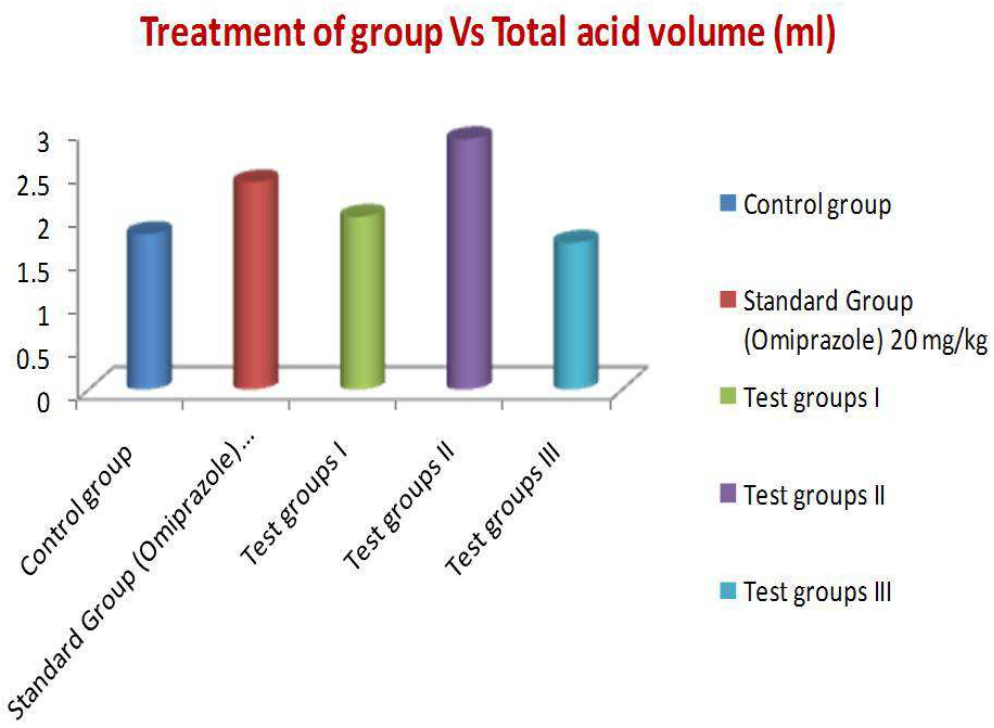

Fig. 8: Total acid volume secreted in stomach of various groups after increasing dose of Famotidine floating microspheres

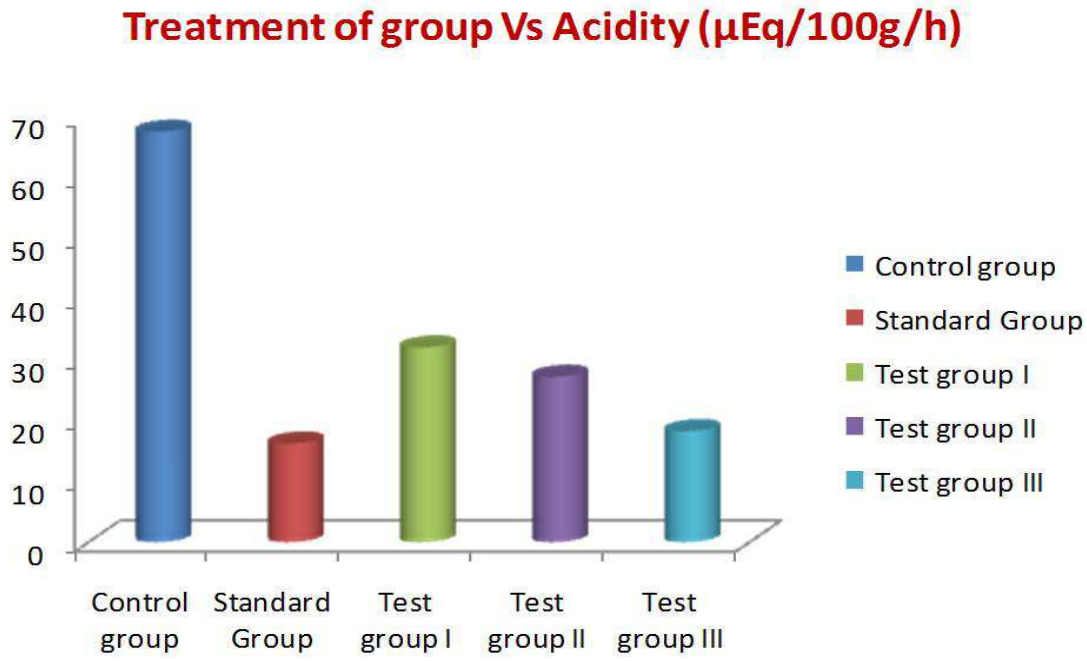

Fig 9: Effect of increasing dose of Famotidine on the Gastric acidity of different groups of animal

CONCLUSION

Oral administration is the most convenient and preferred means of drug delivery to the systemic circulation. These systems allow prolonged residence time of dosage forms in the stomach and the achievement of constant plasma levels. Gastric retention drug delivery system can be retained in the stomach for a long time. Such retention systems are important for drugs that are degraded in intestine or for drugs like antacids or certain antibiotics, enzyme that should act locally in the stomach. If the drugs are poorly soluble in intestine due to alkaline pH and then its retention in gastric region may increase the solubility before they are emptied, resulting in increased bioavailability. Floating

microspheres of famotidine had shown promising results and could be used to deliver the drug in a controlled manner.

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