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Original Article

PREPARATION AND EVALUATION OF ONCE-DAILY FLOATING GMO-ALGINATE MICROSPHERES CONTAINING FAMOTIDINE

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ABSTRACT

Objective: In this work, a gastro-retentive floating microsphere delivery system composed of Drug/Glyceryl mono-oleate (GMO) embedded in a Ca-alginate gas-generated matrix was designed to improve the bioavailability of a slightly-soluble model drug Famotidine.

Methods: The water/Oil emulsion method was used to prepare Famotidine floatable microspheres, and formulation variables such as Alginate: GMO ratio, gas-generated bicarbonates concentration, and loading drug concentration were investigated. Conventional techniques, including DSC, XRD and FTIR were performed to confirm Famotidine compatibility with GMO and Alginate polymers. Real-Time X-ray Radiography was used for *in vivo* imaging of Famotidine floatable microspheres using rabbits as an animal model. HPLC spectroscopic technique was used to determine Famotidine plasma concentration after oral administration of Alginate-GMO loaded microspheres.

Results: Floating Famotidine Alginate-GMO microspheres (0.75:1:0.25) w/w/w showed a remarkable entrapment efficiency (>98%), good buoyancy (>84) and prolonged *in vitro* drug release properties (>24 hours). DSC, XRD, and FTIR techniques showed no evidence of interaction between Famotidine and Alginate or GMO. *In vivo* Imaging of Famotidine floatable microspheres showed that capsules containing Famotidine-Alginate microspheres were not detected after 3 h of administration, while capsules containing Famotidine-GMO-Alginate microspheres can be detected for more than 12 h, indicating superior gastric retention properties. The pharmacokinetic parameters were calculated for Famotidine: GMO-Alginate, and Famotidine: alginate and compared with the plain drug over 24 h period. Famotidine: GMO-Alginate microspheres exhibited controlled and prolonged absorption Tmax of 6.0 vs. 3.0 and 2.0 h; Cmax of 124.9±0.9 vs. 323.7±0.4and 458.6±0.5 ng/ml; AUC0-24 of 2153.025±6.7 vs. 1650.4±1.9 and 1110.725±2.1 ng/ml for Famotidine: alginate and plain drug, respectively, reflecting the increase in the bioavailability of the drug in the floating formulations compared to the free drug.

Conclusion: Prolonged gastric retention time and sustained release properties of floating GMO-alginate microsphere suggest that it could provide a valuable sustained release dosage form of slightly-soluble drugs.

Keywords: Famotidine, Slightly-soluble drug, Water/Oil emulsion method, Glyceryl mono-oleate (GMO), Ca-alginate, Gastro-retentive floating microspheres

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INTRODUCTION

Famotidine is a histamine H2 receptor antagonist medication that decreases stomach acid production. It is used to treat peptic ulcer disease, gastroesophageal reflux disease, and Zollinger-Ellison syndrome. It is taken by mouth or by injection into a vein [1]. Famotidine is probably the first choice not only for its lower cost but also for its more potent stability compared to cimetidine and ranitidine [2]. Famotidine (fig. 1) is N-(aminosulfonyl)-3-[2-[diaminomethylene)amino]-4-thiazolyl]methyl]thio]
propanimidamide, a white to pale yellow crystalline compound, freely soluble in glacial acetic acid, slightly soluble in methanol, very slightly soluble in water, and practically insoluble in ethanol [3]. At 20 ° C, famotidine dissolves in water at 0.1% [4]. It is effect onset occurs within 1 hour, peak serum concentration normally sets within 1 to 3 h, and half time of approximately 2.5 to 3.5 h [5]. Famotidine undergoes minimal first-pass metabolism [6], and a large part of famotidine is excreted in urine to eliminate as an unchanged drug [7]. Famotidine is poorly water soluble, having a slow dissolution rate in the gastrointestinal tract resulting in low oral bioavailability (40-45%) and incomplete absorption after oral administration [5]. Famotidine is less soluble at higher pH; therefore increasing gastro-retention time can improve oral bioavailability. Many researchers are developing various formulations that increase drug gastro-retention time and thus promote local delivery of drugs to receptors in the parietal cell membrane and increase bioavailability [8-10].

Monoglycerides are used in many food applications, such as bread and cake production for the improvement of shelf life and flavor retention [11]. The particular properties of monoglyceride-based cubic phases, temperature stability, bicontinuous structure, high internal surface area, and low-cost raw materials make them desirable for personal cure products and pharmaceutical industry applications [12]. In addition, the stiffness and high viscosity make the GMO–water cubic phase an excellent in situ forming biodegradable matrix-type drug delivery system with varying molecular weights and solubilities in water, such as Aspirin, vitamin E, Oxybutynine hydrochloride, Metronidazole, Tetracycline, Timolol maleate, Chlorpheniramine maleate, Propranolol HCl, Melatonin, and Haemoglobin [13].

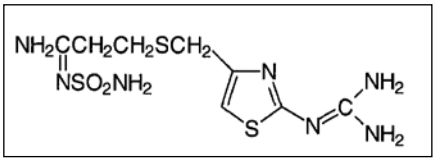

Fig. 1: Chemical structure of famotidine

Sodium alginate is a sodium salt of alginic acid, a naturally occurring, anionic, linear non-toxic polysaccharide found in brown algae consisting of varying ratios of Guluronic and Mannuronic acid units. Alginate has been widely used as food and pharmaceutical additives, such as a tablet disintegrate and gelling agent [14]. Alginate delivery systems are formed when monovalent, water-soluble salts of Guluronic and Mannuronic acid residues undergo cross-linking

gelation with divalent cations, such as Ca^{++}into the water-insoluble gel matrix. Each Ca^{++}ion takes part in nine coordination links with an oxygen atom, resulting in a three-dimensional network of calcium-alginate, the so-called "egg-box" structure [15, 16]. Alginate polymers have been widely used in biomedical applications as they are biodegradable and biocompatible, but suffer from the limitation of rapid drug release in physiologic salt concentration. In the presence of monovalent (e. g. sodium) salts, insoluble calcium alginate gets converted into a soluble form (sodium alginate), resulting in rapid disintegration of the delivery system and drug release [12, 17]. Most drugs that are administered via the alimentary canal can be absorbed through passive diffusion, convective transport or aqueous channels, active transport, facilitated transport, ion-pair transport, and endo-or pinocytosis pathway. However, these pathways are not available in all segments of the alimentary canal except the small intestine [18]. The bioavailability and absorption of nutrients and drugs in the GI tract are regulated by the following factors: blood and GI tract pH, gastric emptying, intestinal motility, and blood flow to the GI tract [19]. The stomach is a capacious organ with about 1.5 L of capacity and approximately 0.11 m^2 of the absorbing surface area [18]. For gastro-retentive delivery of drugs relatively long residence time of the delivery system in the stomach plays a key role. There are several approaches that have been studied and developed to prolong the gastric retention time [19] such as: (1) Incorporation and use of lipid vehicles, fatty acids, or even gastric emptying delaying drugs; (2) Combination of bio-adhesive polymers that can attach to the gastric tissue and increase the residence time; (3) Intra-gastric retention shapes or swelling that prolong the retention in stomach via shape or size of dosage form; (4) High density units that position in the lower part of antrum to remain in the stomach; (5) Hydro-dynamically balance system (HBS), which contains high proportion of one or more hydro-colloids to give a bulk density lower than that of gastric juice so that it can stay buoyant on the gastric liquid without being affected by gastric emptying; (6) Gastro-inflatable delivery device (GIDD), which contains one or more inflatable devices that may stay buoyant on the top surface of the stomach contents by either gas-filled chamber at body temperature or gas-generated matrix when bicarbonates and carbonates are exposed to gastric juice.

In this work, a gastro-retentive floating microsphere delivery system composed of drug/GMO embedded in a Ca-alginate gas-generated matrix was designed to improve the bioavailability of a slightly-soluble model drug Famotidine.

MATERIALS AND METHODS

Materials

Famotidine was obtained from Sedico Pharm Co. (Cairo, Egypt). Sodium Alginate was purchased from Sas chemicals (Mumbai, India). Monoolein was obtained from Danisco Emulsifiers (DIMODAN® MO 90/D, Denmark). Sorbitan Monooleate (Span80) was obtained from Loba Chemie (Mumbai, India). Pluronic F127, with an average molecular weight of 12,500, was purchased from BASF (Ludwigshafen, Germany). Sodium bicarbonate, Dichloromethane (DCM), Methanol, Iso-propyl alcohol, Conc HCl, Sodium Chloride, Glacial acetic acid, Disodium hydrogen phosphate, Ethylacetate, Acetonitrile (HPLC analytical grade), and Calcium chloride dehydrate were purchased from El-Nasr chemicals Co. (Cairo, Egypt).

Methodology

Preparation of floatable Ca-alginate microspheres

i. Preparation and characterization of blank GMO-Ca-alginate microspheres

Blank dispersions of GMO were prepared by the conventional fragmentation method, which involves mechanical dispersion of bulk cubic gel in the presence of pluronic as a stabilizer [12,20]. In detail; 300 mg GMO was weighed into a 5 ml glass vial, heated to 45 °C in a water bath, then water (0.7 ml) was gently dropped on the surface of lipids and finally incubated at room temperature 24 h to allow formation and equilibration of the cubic gel phase. The resultant viscous cubic gel was mixed with 1.5 ml of 2% Pluronic F127 solution (10:1, GMO: F127 w/w) to form a coarse dispersion. This dispersion was subsequently homogenized using a microtip probe sonicator (4 mm diameter tip, 25KHz frequency, and 30 Watts power output, VCX series, Sonics, USA) for 2 min at 25 °C. Different volumes of blank GMO-dispersion were emulsified into 10 ml of 4% (w/v) sodium alginate solution by vortexing at 1000 rpm (IKA Labortechnik, Germany) for 2 min to get a final Alginate: GMO w/w ratios of 1:1, 1:0.5, and 1:0.25. These primary emulsions were further emulsified into 50 ml DCM/2 ml Span 80 and then emulsified for 2 min at 15200 rpm using a homogenizer. A 2 cm magnetic bar was placed into the emulsion and the glass container was tightly closed by a rubber closer. Microspheres were prepared by adding 8 ml of 5% (w/v) CaCl2 (dissolved in a 1:2 mixture of methanol and isopropyl alcohol) to the emulsion drop by drop via 10 ml syringe at1000 rpm and stirring for 60 min to assure efficient cross-linking. In this step, microspheres were formed in suspension. Microsphere suspension was allowed to stabilize on ice for about 10 min, and the microspheres were collected by filtration in a vacuum, washed with isopropyl alcohol twice, and finally dried at room temperature. The morphology and average particle diameter were characterized by a Scanning electron microscope (SEM), (Phnom-ProG2, Netherlands) after samples drying under vacuum and observed at 5 KV. For particle size analysis, the diameter of 50-100 particles was measured and averaged from photos by computer software (Particlemetric, Phenomworld) came together with SEM equipment.

ii. Preparation and characterization of floatable blank GMO-Ca-alginate microspheres

Using the optimum GMO: Alginate ratio obtained before, the floatable GMO-Alginate microspheres were prepared by mixing 100, 200, and 300 mg sodium bicarbonate into the 10 ml of 4% (w/v) sodium alginate solution before adding GMO-dispersion, then vortex at 1000 rpm for 2 min to get GMO: Alginate emulsion that further emulsified into 50 ml DCM/2 ml Span 80 as mention before. The buoyancy of microspheres was determined by placing 100 mg microspheres in a 100 ml beaker containing 0.1N HCL for 12 hr. The time required for the microspheres to rise to the surface and float was determined as floating lag time (FLT). The floated and sinking microspheres were collected and dried under vacuum for 12 h and weighed. Buoyancy was determined by the weight ratio of the floating microspheres to the sum of floating and sinking microspheres after 12 hr in 0.1N HCL [21].

iii. Preparation of drug-loaded floatable Ca-alginate microspheres

Using the optimum sodium bicarbonate concentration obtained before microspheres containing 1:0.025, 1:0.05, 1:0.075, 1:0.01 w/w Alginate: Famotidine ratios were prepared by dissolving 10, 20, 30, and 40 mg Famotidine into 2 ml glacial acetic acid then added to 10 ml of 4% (w/v) sodium alginate solution containing sodium bicarbonate and vortex for 2 min then further emulsified into 50 ml DCM and $CaCl_2$ was added as discussed before. Microsphere buoyancy and the percentage of drug entrapment efficiency were determined for each drug ratio.

iv. Preparation of drug-loaded floatable GMO-alginate microspheres

Microspheres containing 1:0.025, 1:0.05, 1:0.075, 1:0.01 w/w Alginate: Famotidine ratios were prepared by dissolving 10, 20, 30, and 40 mg Famotidine into 2 ml glacial acetic acid then added to the molten GMO heated to 45 °C in a water bath. The resultant viscous cubic gel was incubated at room temperature 24 h, mixed with 1.5 ml of 2% Pluronic F127 solution, and homogenized using a sonicator for 2 min at 25 °C. Drug-GMO dispersions were emulsified into 10 ml of 4% (w/v) sodium alginate solution containing sodium bicarbonate by vortexing for 2 min and further emulsified into 50 ml DCM. Microspheres were prepared by adding 8 ml of 5% (w/v) $CaCl_2$, stabilize on ice, collected by filtration, washed with isopropyl alcohol, and dried at room temperature. Microsphere buoyancy and drug content were determined for each drug ratio.

v. Estimation of famotidine by HPLC

Quantitative estimation of the drug in tested microspheres was performed using a laboratory HPLC reference method, using Agilent 1100 Series HPLC (Agilent Technologies, Waldron, Germany). The separation was carried out on C_{18} (4.6 mmX150 mm) column filled

with 5µ Equisil BDS, and the elution solvent consisted of a filtered and degassed mixture of methanol and 1% acetic acid aqueous solution in the ratio of 30:70 (v/v), at a flow rate of 0.4 ml/min [22]. For the calibration curve, serial concentrations of Famotidine in methanol containing 2, 4, 8, 16, 20, 32, 40, and 48 µg/ml were prepared. The absorbance of the prepared solution was measured by HPLC at the predetermined λ_{max} (254 nm).

vi. Determination of drug entrapment efficiency

Accurately weighed 10 mg Famotidine loaded microspheres were added to a mixture of 10 ml phosphate buffer (pH 7.4) and 10 ml glacial acetic acid in 50 ml stoppered flask and left for 24 h under orbital shaking 150rpm at 37 °C [23]. The dispersion obtained was transferred to a 15 ml falcon tube and centrifugation at 6000 rpm for 60 min, the supernatant liquid was collected and the concentration of Famotidine was analyzed by an HPLC. The characteristic absorbance of Famotidine at λ_{max} 254 nm was recorded and compared with a standard curve generated from the Famotidine concentrations varying from 0 to 48 µg/ml. The ratio of the actual to the theoretical drug contents in microspheres was termed entrapment efficiency. 1 ml porcine pancreatic lipase (1500 IU/ml) digestion medium was added into phosphate buffer pH 7.4 in the case of Famotidine-GMO-alginate microspheres to assure complete drug recovery.

Physico-chemical analysis

i. Differential scanning calorimetry (DSC) thermal analysis

Samples weighing 5-10 mg of drug alone, Famotidine-Alginate microspheres, and Famotidine-GMO-alginate microspheres were sealed in flat-bottomed aluminum pans and heated under an atmosphere of nitrogen. A heating rate of 10 °C min^{-1} was employed over a temperature range of 30-400 °, using Differential Scanning Calorimeter (Perkin Elmer, Germany). An empty aluminum pan was used as a reference.

ii. X-ray diffraction crystallography (XRD)

The samples (Famotidine, Famotidine-Alginate microspheres, and Famotidine-GMO-alginate microspheres) were scanned over a range of 2Θ angles from 2 °-50 °, with an angular speed of 0.02 ° per second, using Philips X-ray diffraction equipment model PW/1710 with Cu tube anode, 40 Kv voltage, and 35 mA current generator. The instrument utilized a special software program to analyze peak position and intensities.

iii. Fourier transform infrared (FTIR) spectral analysis

Samples of the drug alone, drug-Alginate microspheres, and drug-GMO-alginate microspheres were mixed with KBr and compressed into a disc using a hydraulic pump under a pressure of about 5 tons. The spectra were recorded over a range of 4000-500 cm-1, using an FT-IR spectrometer (Shimadzu, Tokyo, Japan). Each FTIR spectrum was obtained using the averAlginatee of 16 scans at a resolution of 4 cm^{-1}.

***In vitro* drug release studies**

In vitro drug release from the optimum formulation, microspheres were studied using the Dialysis bag method [24], 6 cm long, 1 cm wide dialysis bag molecular weight cut off 12000–14000 (Cellulose tubing membrane, Sigma-Aldrich, USA) was soaked in de-ionized water for 12 h before use. Then, accurately weighed amounts (10 mg) of microspheres were filled into the dialysis bag with the two ends fixed by a thread and suspended into 500 ml Stoppered Flask containing 250 ml buffer pH 1.2 USP simulated gastric fluid without enzyme (2 gm NaCl and 7 ml conc. HCl per 1000 ml de-ionized water).

The Flask was placed on a magnetic stirrer (100 rpm) at 37 °C, five-milliliter samples were withdrawn at predetermined time intervals for 24 h, and 5 ml of the fresh medium, kept at the same temperature, was replaced. Floating Famotidine GMO-Alginate microspheres were carried out in the presence and absence of 1 ml porcine pancreatic lipase (1500 IU/ml) digestion medium. The samples were diluted and analyzed at 254 nm by HPLC. The dissolution profiles were obtained by plotting the cumulative percentage of drug released on the y-axis and time (in hours) on the x-axis.

Kinetic model analysis of the drug release from prepared floatable microspheres

To know the mechanism of drug release from the floatable microspheres, the experimental cumulative release data were fitted on various release models commonly used to describe the release kinetics from microspheres viz. zero order, first order, Higuchi kinetic and Hixon-Crowell models at pH 1.2 using multiple linear regression analysis programs (Kinet DS3 software). The correlation coefficient (r^2) close to unity was taken as order of release, the following models were fitted: cumulative % drug release versus time (zero-order kinetic model), log cumulative % drug remaining versus time (first-order kinetic model), cumulative % drug release versus square root of time (Higuchi model) [25]. Hixon–Crowell equation describes the release from systems where a change in the surface area and particle diameter [26].

***In vivo* evaluation of famotidine floatable microspheres**

i. Animal preparation

Male albino rabbits (New Zealand) weighing 2.0-2.5 Kg were selected for this study, all animals were healthy during the period of the experiment. The animals were obtained from the National Veterinary Hospital Pharm, Cairo, Egypt. All efforts were made to maintain the animals under controlled environmental conditions (Temperature 25 °C, Relative Humidity 45%, and 12 h alternate light and dark cycle) with 100 % fresh air exchange in animal rooms. Rabbits were fed a standard diet with free access to water throughout the experiment. The study was carried out in the Military Veterinary Hospital, Cairo, Egypt. The study was performed according to ethics coded for experimental and clinical studies at the Faculty of Pharmacy, Cairo University (Cairo, Egypt), PI (1225). The animal care and handling were done according to the guidelines set by the World Health Organization, Geneva, Switzerland and the experimental protocol of the animal study was performed according to the guidelines issued by the ethical committee of the Faculty of Pharmacy, Cairo University, Egypt.

ii. *In vivo* imaging of famotidine-alginate microspheres by real-time X-ray radiography

Optimum *in vitro* formulations were prepared as discussed before with the addition of 10 mg $BaSO_4$ (as radiopaque contrast). Rabbits were classified into two different groups (n = 6/group). Rabbits belonging to group 1 were given a single oral capsule loaded with 200 mg Famotidine-Alginate microspheres. Rabbits belonging to group 2 were given capsules containing 100 mg Famotidine-GMO-alginate microspheres. Behind the back of the tongue, capsules were inserted to avoid them to become destructed. Rabbits were retained in rabbit restrainers during the experiment time. To determine gastric targeting efficiency, contrast generated by BaSO4 radiography was assessed using a villa X-ray medical system (Buccinasco, MI, Italy) [27].

iii. *In vivo* famotidine release analysis by HPLC assay

Rabbits were divided into 3 groups, each consisting of 6 animals, first group received a conventional hard gelatine capsule containing 6 mg/kg of free Famotidine. The second and third groups received a capsule containing the formulated drug/calcium alginate and drug/GMO-alginate floating microspheres equivalent to 6 mg/kg Famotidine, respectively. All capsules were administered orally as a single dose to all groups. The capsules were put behind the tongue to avoid their destruction due to biting. Food was withdrawn from the rabbits 12 h before drug administration and until 24 h post-dosing. Blood samples (1 ml) were collected in heparinized tubes before the drug administration (zero time), and at 0.5,1, 1, 1.5, 2, 3, 4, 6, 8, 10, 14, and 24 h after dosing. All blood samples were centrifuged at 2000 rpm for 20 min, and the serum was stored in a freezer at-20 °C until the HPLC assay. Sample preparation (1 ml plasma) was done by a single-step liquid-liquid extraction with methanol. The resulting clear organic layer was separated through centrifuging at 2000 *x* for

20 min at–2 °C. Samples were protected from light and stored at 4 °C until injection. For injection, samples were reconstituted with 200 µl mobile phase consisting of A mixture of (buffer: Acetonitrile: Methanol) (500:120:20) (V: V) consecutively, and an aliquot of 100 µl was injected onto the chromatographic system [28]. Detection was carried out at λ_{max} 254 nm and the chromatogram quantified the area.

iv. Pharmacokinetic analysis

Plasma level data obtained from the individual rabbit per each group were used to estimate the main pharmacokinetic parameters; maximum plasma concentration (C_{max}, ng/ml), the time required to reach maximum plasma concentration (T_{max}, h.), and area under the plasma concentration-time curve from time 0 to 24 h (AUC 0-24, ng/ml/h) were calculated by the use of PK Solution 2.0 software package (Summit Research Services, Montrose, USA). The results were expressed as mean±standard deviation (SD).

v-Statistical analysis

Statistical analysis Results of the amount of Famotidine as a model drug in the case of free drug, Famotidine-Alginate microspheres, and Floating Famotidine-GMO-Alginate microspheres delivered to plasma were calculated for each rabbit and a one-way analysis of variance (ANOVA) used to evaluate treatment differences. All statistical analyses were performed with the SPSS software package (SPSS for Windows 14.0, SPSS, USA).

RESULTS AND DISCUSSION

Preparation of floatable Ca-alginate microspheres

i. Preparation and characterization of blank GMO-Ca-alginate microspheres

In the preparation of Ca-alginate gel microspheres by w/o emulsion method, the aqueous emulsion droplets, containing polymer, transformed into solid microspheres upon the addition of CaCl2. The divalent calcium ions are bound in a highly cooperative manner to the guluronic acid units of the alginate (cross-linking), leading to the formation of water-insoluble gel particles. The microspheres prepared with GMO/Alginate were examined as shown in SEM photographs (fig. 2). Alginate: GMO ratio significantly affected the particle shape and morphology. Particles with low GMO contents (1:0.25) were spherical with a white-doted surface and an average diameter of 38.7 µm±3.5 µm (fig. 2a,b). With increasing GMO contents (1:0.5and1:1), microparticles become more flattened, collapsed, and larger in size (46-69 µm average diameter) (fig. 2c,d). This size increase may be related to higher alginate network density produced by increased GMO concentration and, thus reduced homogenization efficiency. Particles with low GMO contents (1:0.25) were selected for buoyancy experiments.

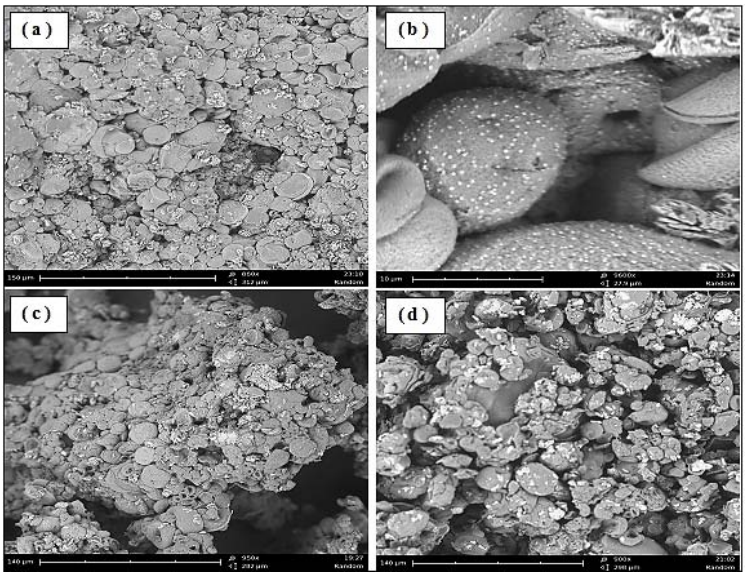

Fig. 2: SEM photographs of; (aandb) 1:0.25 w/w Alginate: GMO microspheres, (c) 1:0.5 w/w Alginate: GM O microspheres, (d) 1:1 w/w Alginate: GMO microspheres

ii. Preparation and characterization of floatable blank GMO-Ca-alginate microspheres

Sodium bicarbonate was added as a gas-generating agent. Sodium bicarbonate induced carbon dioxide generation in the presence of a dissolution medium (0.1 N HCl). The gas generated is trapped and protected within the gel, formed by the hydration of the polymer, thus decreasing the density of the microspheres. As the density falls below 1g/ml, the microspheres become buoyant [29]. The buoyancy results are shown in table (1); at a lower concentration of sodium bicarbonate (100 mg) more time was required to ditch the microspheres from their aggregates with a lag time of 82±2 sec and buoyancy of 75%. But with the increase in sodium bicarbonate amount (200 and 300 mg) the microspheres were detached early and became more buoyant at 79 and 85%. Further, the entrapment of CO_2 bubbles within the microsphere's matrix resulted in a longer floating duration of>12hr. Formulation with 300 mg Sodium bicarbonate contents (1:0.25) was selected for drug loading experiments.

iii. Estimation of famotidine by HPLC

Fig. 3 shows a linear relationship between peak area and drug concentration at the concentration range 1-48 µg/ml (Famotidine/methanol) and λmax of 254 nm. The correlation coefficient was found to be 0.999 and the regression equation of y = 199504x–1782.5.

Table 1: Buoyancy and floating lag time of the prepared microspheres (Values represent mean, n=3)

Sod. Alginate (mg)	GMO-dispersion (mg)	Sodium bicarbonate (mg)	% Buoyancy	Lag time	Floating time
400	100	100	75	82 sec	9 hr.>
400	100	200	7	54 sec	12 hr.>
400	100	300	85	48 sec	12 hr.>

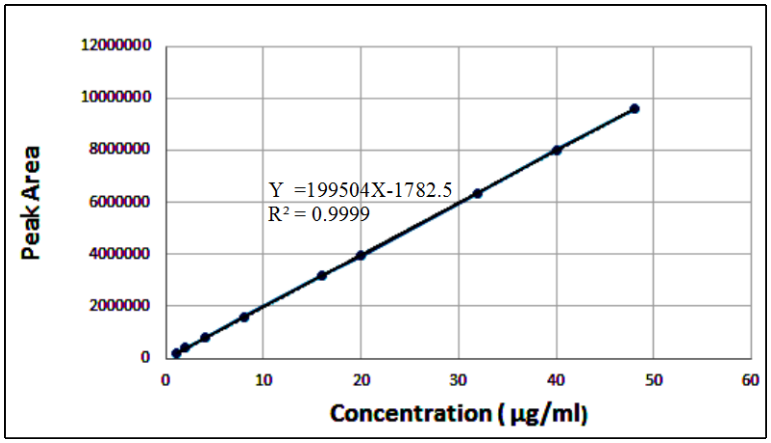

Fig. 3: Standard calibration curve of Famotidine at λ_{max} 254 nm

iv. Preparation and evaluation of famotidine floatable microspheres

The values of entrapment efficiency of Famotidine-Alginate-and Famotidine-GMO-Alginate microspheres were shown in table 2. High drug entrapment efficiency was noticed for all Famotidine microspheres with good floatable properties. The drug contents were in the range of 92.3–95.1 % for Famotidine/Ca-alginate microspheres and in the range of 97.9–98.7 % for Famotidine/GMO-alginate microspheres, which related to the low water solubility of Famotidine. By increasing drug ratios (1:0.025, 1:0.05, 1:0.075, 1:0.01 w/w), a minor decrease in the entrapment efficiency was observed, suggesting that both polymers used are sufficient to entrap the hydrophobic drug. The buoyancy of GMO-alginate microspheres was significantly lower than those of conventional alginate microspheres at all Famotidine-drug ratios. This result indicated that the addition of GMO highly increases the internal structure density of the alginate matrix, smaller pore channels, and thus more lag time. Formulation with 40 mg Famotidine contents (fig. 4) was selected for *in vitro* and *in vivo* drug Studies.

Table 2: Buoyancy and percentage drug entrapment efficiency of the prepared famotidine floatable microspheres (Values represent mean, n=3)

Sod. alginate (mg)	GMO-dispersion (mg)	Sodium bicarbonate (mg)	Famotidine (mg)	Drug entrapment %	Buoyancy %	Lag time	Floating time
400	----	300	10	95.1	96	36 sec	12 hr.>
400	----	300	20	94.8	95.8	36 sec	12 hr.>
400	----	300	30	93.6	95.6	36 sec	12 hr.>
400	----	300	40	92.3	95.5	37 sec	12 hr.>
400	100	300	10	97.9	85	48 sec	12 hr.>
400	100	300	20	98.2	84.9	48 sec	12 hr.>
400	100	300	30	98.4	84.7	49 sec	12 hr.>
400	100	300	40	98.7	84.6	49 sec	12 hr.>

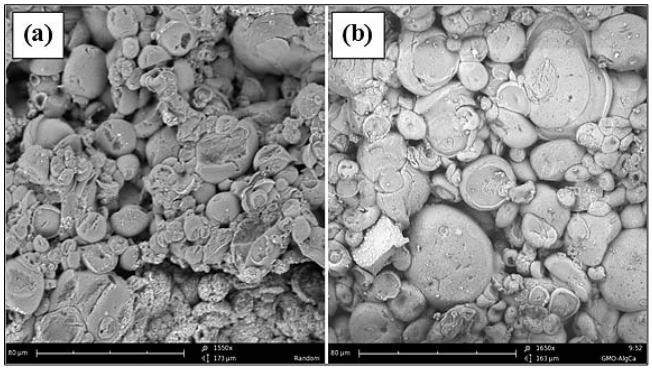

Fig. 4: SEM photographs of 40 mg loading famotidine floatable microspheres; Drug: alginate microspheres, and (b) Drug: alginate: GMO microspheres

Physico-chemical analysis

i. DSC thermal analysis and X-ray diffraction

DSC studies were performed on Famotidine alone, Famotidine-Alginate microspheres, and Famotidine-GMO-Alginate microspheres. Fig. 5 shows a thermogram of pure powder Famotidine that has two endothermic transitions. A Characteristic sharp endothermic peak around 165.31° mainly related to Famotidine melting point temperature (Tm), and another endothermic peak appeared at 200.61° which may be attributed to drug decomposition temperature (Td) [30, 31]. The DSC results of Famotidine-Alginate and Famotidine-GMO-Alginate microspheres show the dis-

appearance of the characteristic Tm peak of the drug, which can be explained by the transformation of Famotidine from crystalline form to its amorphous form during the dissolving and re-precipitation processes [32]. Fig. 6 shows an X-ray pattern of pure Famotidine with numerous sharp distinct peaks at 2θ angles of 11.7, 15.2, 19.5, 22.5, 24.18, 30.3, and 35.4, indicating the crystalline nature of the drug. Famotidine-Alginate and Famotidine-GMO-Alginate microspheres show an absence of crystalline peaks of the drug and all peaks appeared in low intensities while others disappeared. These results indicate the transformation of Famotidine from crystalline form to amorphous form as confirmed by the DSC results [32].

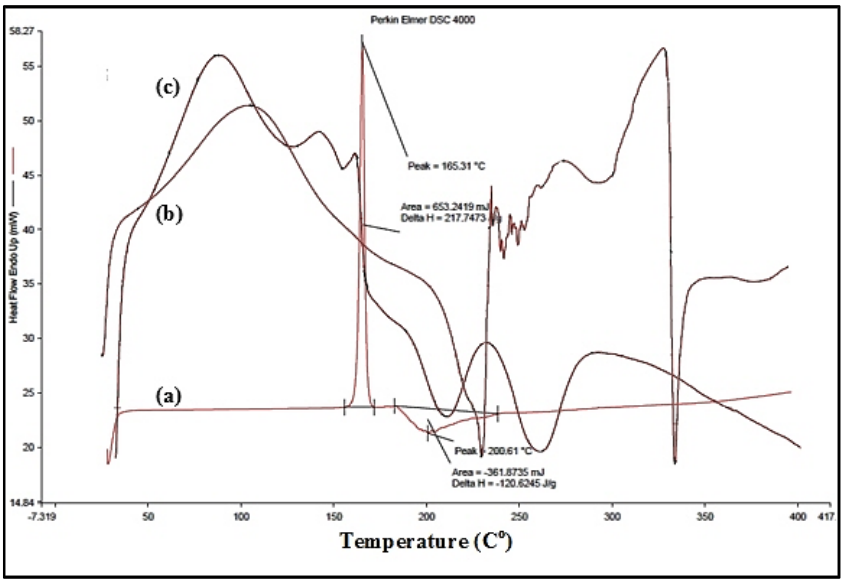

Fig. 5: DSC curves of (a) pure famotidine; (b) Famotidine-alginate microspheres; (c) Famotidine-GMO-alginate microspheres

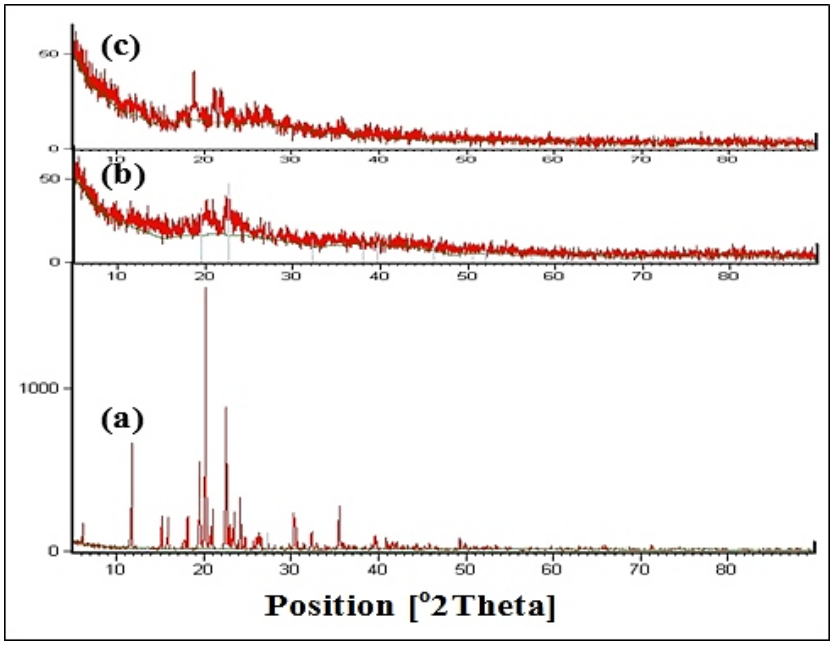

Fig. 6: X-ray curves of (a) pure famotidine; (b) Famotidine-Alginate microspheres; (c) Famotidine-GMO-Alginate microspheres

ii. Fourier transform infrared (FTIR) spectral analysis

FTIR analysis studies were performed on Famotidine alone, Famotidine-Alginate microspheres, and Famotidine-GMO-Alginate microspheres. Famotidine molecule consists of guanidine, thiazole, thioether, and sulfamoyl parts. Fig. 7 and table 3 shows the IR spectrum of pure Famotidine that shows peaks at 3501 cm^{-1},1587 cm^{-1} due to NH^2 stretching, at 2934 cm^{-1}, 1632 cm^{-1} due to C=N stretching, at 1316 cm^{-1}, 1136 due to C-N stretching in guanidine part, at 3347 cm^{-1} due to C-N stretching at 1420 due to C-N stretching in thiazole part, at 1416 cm^{-1} due to C-C-C stretching, at 1276 cm^{-1} due to C-C stretching in thioether part, at 3051 cm^{-1}, at 3347 cm^{-1}, 1525 cm^{-1} due to NH_2 stretching at 2934 cm^{-1}, at 1130 cm^{-1} due to C=N stretching at 1136 due to N-C-N stretching in sulfonyl part. There is no absence of any functional peaks in all spectra, thus it revealed that there is no significant physicochemical interaction between the drug and Alginate or GMO. In addition, there were no new bands observed in all drug microspheres, which confirms that no new chemical bonds were formed between Famotidine and both polymers studied [33, 34].

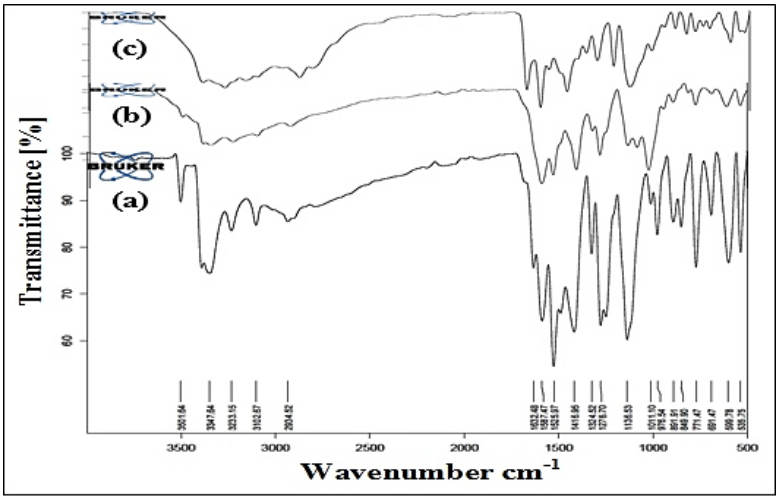

Fig. 7: The FTIR of (a) pure famotidine; (b) Famotidine-alginate microspheres; (c) Famotidine-GMO-alginate microspheres

Table 3: Most effective FTIR bands of famotidine, famotidine-alginate-alginate, and famotidine-GMO-alginate microspheres (cm^{-1})

Main function gps	Assignment	Free famotidine	Famotidine-alginate microspheres	Famotidine-GMO-alginate microspheres
Guanidine gp	NH^2 Stretching	3501,1587	1587	1585
	C=N Stretching	2934,1632	1632	1629
	C-N Stretching	1316,1136	1316,1136	1305,1129
Thiazole gp	C-H Stretching	3347	3347	3345
	C-N Stretching	1416	1416	1414
Thioether gp	C-C-C Stretching	1420	1416	1412
	C-C Stretching	1276	1276	1271
Sulfamoyl gp	NH^2 Stretching	3501,3347,1525	3356,1525	3348,1519
	C=N Stretching	2934,1130	1136	1130
	N-C-N Stretching	1136	1136	1136

In vitro drug release studies

Fig. 8 shows the release profiles of Famotidine pure drug, optimum formulation of Alginate: Famotidine (1:0.1), and optimum formulation of Alginate: GMO: Famotidine (1: 0.25:0.1) microspheres using the dialysis bag method (at pH 1.2 NaCl/HCl buffer). Being very slightly soluble in water, Famotidine pure drug showed approximately total release (99.7%) within 3 h while the conventional floatable calcium alginate microspheres showed 73.5% release within 3 h. Many reports provided that Calcium alginate hydrogel shrinks at low pH, leading to a much narrower pore size network for the drug to diffuse out through [33, 35]. The addition of GMO dispersion was shown to sustain drug release for a longer period (only 20.43% cumulative release within 3 h), which was attributed to water uptake of GMO and the formation of cubic phase that is highly viscous and acts as a rate-limiting factor in drug release. These releases could be attributed to the removal of the cross-linker bivalent cation, calcium, from the alginate microspheres by monovalent cations, sodium contained in the buffer solution [33, 35]. Lipase digestion medium has a major effect on the release profile from GMO-alginate microspheres; a complete drug released (99.8%) was reached within 12 h in the presence of lipase enzyme compared to 99.71% cumulative release after 23 h without lipase, which concluded that digestive enzymes can affect the drug release from our designing GMO-alginate microspheres.

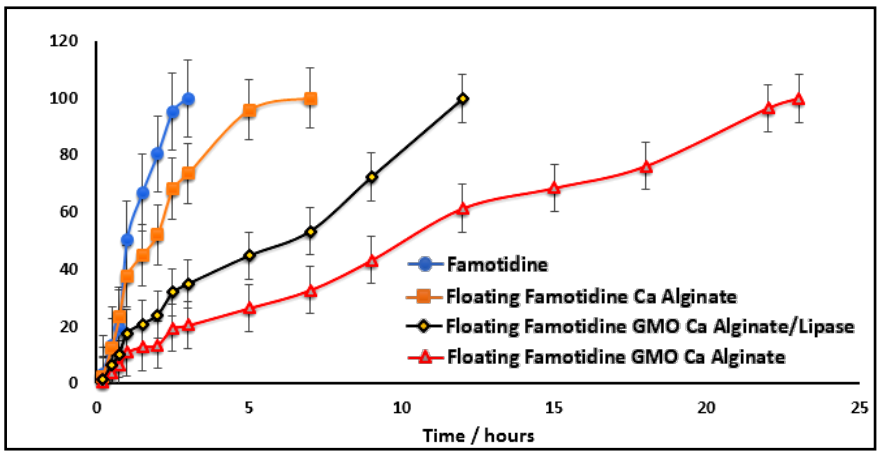

Fig. 8: Release profiles of famotidine from free famotidine, floating famotidine-alginate, and famotidine-GMO-alginate microspheres. (Values represent mean±SD, n=3)

Kinetic model analysis of the drug release from prepared floatable microspheres

Various mathematical drug release models viz. zero order, first order, Higuchi and Hickson-Crowell kinetic models were adopted to predict the nature of drug release from microspheres table 4. For free drugs, the release kinetic was Zero-Order (r^2=0.9706 and 0.8963); this indicates that drug release does not depend on concentration. For Famotidine calcium alginate microspheres, the release was governed by Zero-Order (r^2 =0.9237, 0.9996 and0.9358), and Hickson-Crowell equation (r^2 =0.9997) indication that drug release does not depend on the concentration and drug release may relate to the change in surface area, particles diameter that occurred with time [36]. For floating Famotidine GMO alginate microspheres, in the first 2 h the better fits were found for Zero-Order (r^2 =0.9227), indicating that drug release does not depend on the concentration and still pH is low and alginate would be protonated into the insoluble but swelling form, and the presence of GMO increase network density of alginate and control pore size of alginate so not allow the drug to diffuse, that explains the low aliquot of the release [37]. In the second 2 h, better fits were found for the Hickson-Crowell equation (r^2 =0.9997), indicating that drug release may be due to a change in surface area and particle size diameter and also due to the presence of lipase enzyme, which degrades GMO. Finally, after 4 h, the better fits were found for First-Order (r^2 =0.9818) indicating that drug release is concentration dependent and parameters such as porosity and diffusion path length are varying during the release process and the presence of lipase enzyme that allows the drug to diffuse and zero-order was not observed.

Table 4: Coefficients of release rate for free famotidine, famotidine-alginate, and famotidine-GMO-alginate floating microspheres, release data fitted with different models (bold values indicate the best fits)

Release models	Free famotidine			Floating Famotidine-alginate microspheres			Floating famotidine-GMO-alginate microspheres/lipase		
	0-2 h pH=1.2	2-4 h pH=1.2	4-24 h pH=1.2	0-2 h pH=1.2	2-4 h pH=1.2	4-24 h pH=1.2	0-2 h pH=1.2	2-4 h pH=1.2	4-24 h pH=1.2
Zero-order r^2	0.9706	0.8963	-	0.9237	0.9996	0.9358	0.9217	0.9993	0.9669
First-order r^2	0.7192	0.8953	-	0.6997	0.9993	0.9302	0.6972	0.9994	0.9818
Higuchi-model r^2	0.4751	0.6815	-	0.5032	0.9971	0.7116	0.5021	0.9967	0.7020
Hixon Crowell r^2	0.8369	0.8956	-	0.8005	0.9997	0.9321	0.7974	0.9997	0.9786

***In vivo* evaluation of famotidine floatable microspheres**

i. *In vivo* imaging of famotidine-alginate microspheres by real-time X-ray radiography

The X-ray imaging of oral capsules coating floating Famotidine/$BaSO_4$ microspheres**;** Famotidine-Alginate, and Famotidine-GMO-Alginate microspheres after different times (0-12 h) were shown (fig. 9). Capsules containing Famotidine-Alginate, and Famotidine-GMO-Alginate microspheres were detected by X-ray at 5 min post administration fig. (7b, candd). After 3 h, capsules containing Famotidine-GMO-Alginate microspheres can be detected by X-ray, while capsules containing Famotidine-Alginate microspheres were not detected (fig. 7e,f), indicating low floating properties of Famotidine-Alginate microspheres. This is the reason why no further assessment of capsules loaded Famotidine-Alginate microspheres was performed after this time point [3 h]. At 6and12 h post-administration, capsules containing Famotidine-GMO-Alginate microspheres can be easily detected in the stomach by X-ray (fig. 7g,h), indicating superior gastric retention properties of Famotidine-GMO-Alginate microspheres.

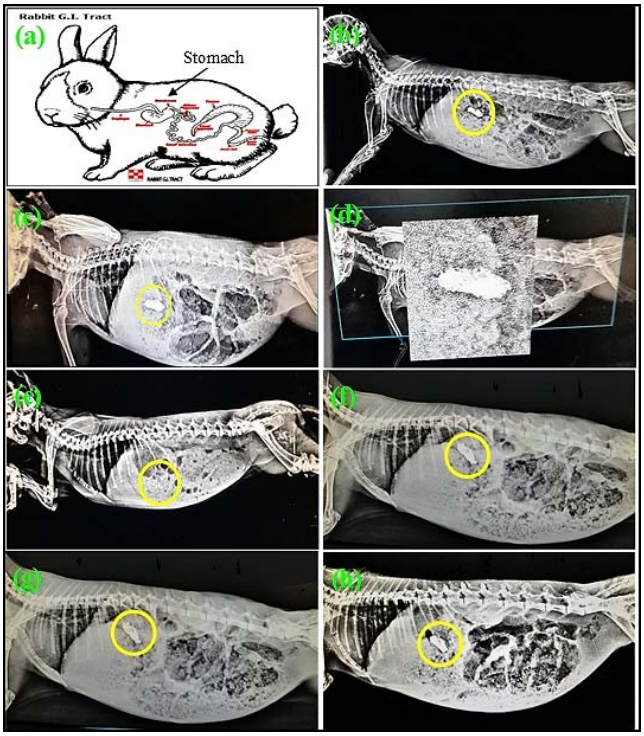

Fig. 9: X-ray imaging of Capsules coating the Floating Famotidine microspheres; (a) Rabbit GIT diagram, (b) Famotidine-Alginate [5 min], (c) Famotidine-GMO-Alginate [5 min], (d) Famotidine-GMO-Alginate [5 min]5X magnification, (e) Famotidine-Alginate [3 h], (f) Famotidine-GMO-Alginate [3 h], (g) Famotidine-GMO-Alginate [6 h], and (h) Famotidine-GMO-Alginate [12 h]. $BaSO_4$ was used as radiopaque contrast in all microspheres prepared

ii. *In vivo* famotidine release analysis, pharmacokinetic studies, and statistical analysis

The biological half-life of Famotidine is approximately 2.5 to 3.5 h, thus necessitating frequent administration (3 to 4 times a day) to maintain constant therapeutic drug levels [5]. Ca-alginate matrix is one of the commonly used biodegradable microspheres, but the resulting calcium alginate bead is usually very permeable, making it very difficult to control drug release for a prolonged period [35]. This problem can be minimized by mixing alginate with glyceryl mono-oleate (GMO), providing a prolonged *in vitro* release (>12 h) formulations [17]. The *in vivo* extended investigation of such a combination was carried out in this work using HPLC analysis. The pharmacokinetic data of Famotidine pure drug, Alginate: Famotidine (1:0.1), and Alginate: GMO: Famotidine (1:0.25:0.1) microspheres were shown in fig. 10 and table 5. From the obtained data, it was observed that the absorption of free Famotidine reached its peak plasma concentration in (2hr), whereas the T_{max} for the Alginate: Famotidine and GMO: Alginate: Famotidine formulations were 3.0 and 6.0 h, respectively. The mean peak plasma concentrations (C_{max}) were 323.7±0.4 ng/ml for Alginate: Famotidine formulation, 124.9±0.9 ng/ml for formulation GMO: Alginate: Famotidine compared to 458.6±0.5 ng/ml for the free drug. The area under the curve over 24 h (AUC_{0-24}) is lowest for the free drug (1110.725±2.1 ng/ml) and highest for the GMO: Alginate: Famotidine (2153.025±6.7 ng/ml), reflecting the increase in the bioavailability of the drug in the floating formulations compared to the free drug. The increase in the T_{max} and the decrease in the mean C_{max} of the test formulation compared to the plain drug indicated the sustained release effect of the floating microsphere formulations. The higher T_{max} and the lower C_{max} of the GMO: Alginate: Famotidine formulation compared to the Alginate: Famotidine formulation reflects the positive effect of GMO on the sustained release properties of alginate microspheres. From obtained data, the calcium alginate matrix alone could not control the Famotidine release effectively for 24 h, while the microsphere matrix prepared with floating alginate-GMO (0.75:1:0.25 w/w/w) is a better system for once daily extended release of Famotidine. The slow release of drugs from composite microspheres may be due to the greater viscosity of the gel matrix resulting in a slower release of the drug [24]. Table 5 showed the result of Famotidine as a model drug in the case of free drug, Famotidine-Alginate microspheres, and Floating Famotidine-GMO-Alginate microspheres delivered to plasma indicating a significant difference between Famotidine as a free drug, Famotidine-Alginate microspheres and Floating Famotidine-GMO-Alginate microspheres ($p<0.05$)

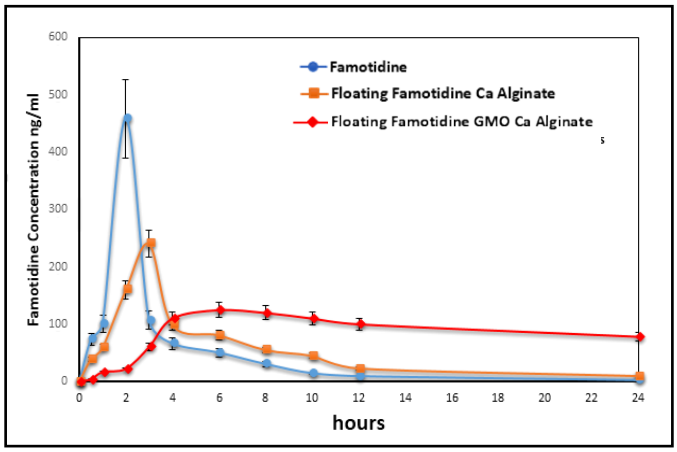

Fig. 10: Famotidine plasma concentration-time curves following oral administration of the three formulations in rabbits; Free drug, Alginate: Famotidine, and GMO-Alginate: Famotidine (Values represent mean±SD, n=6)

Table 5: Pharmacokinetic parameters of famotidine formulations (Values represent mean±SD, n=6) All data significant at $p<0.05$

Parameter	Free famotidine	Floating Alginate: famotidine	Floating GMO-alginate famotidine
Famotidine dose (mg)	6 mg/kg	Microspheres equivalent to 6 mg/kg	microspheres equivalent to 6 mg/kg
T_{max} (h)	2	3	6
C_{max} (ng/ml)	458.6±0.5	323.7±0.4	124.9±0.9
AUC_{0-24} (ng/ml)	1110.725±2.1	1650.4±1.9	2153.025±6.7

CONCLUSION

In the present study, a floating GMO-Alginate microsphere was developed to provide a dosage form with improved gastric retention time and sustained release properties compared to conventional alginate microspheres and thus increase the bioavailability of Famotidine. Using HPLC, the pharmacokinetics parameters of both formulations were deduced in this study. It could be concluded that floating gastro-retentive Alginate-GMO (0:75:1:0.25 w/w/w) is a better system for once daily extended release of the water-insoluble drug as Famotidine compared to Ca-alginate matrix alone. Prolonged gastric retention time and sustained release properties of floating GMO-alginate microsphere suggest that it could provide a valuable sustained release dosage form of poorly water-soluble drugs.

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ETHICS APPROVAL

The study was performed according to ethics coded for experimental and clinical studies at the Faculty of Pharmacy, Cairo University (Cairo, Egypt), PI (1225).

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

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