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# Ionotropically emulsion gelled polysaccharides beads: Preparation, *in vitro* and *in vivo* evaluation

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ABSTRACT

Floating famotidine loaded mineral oil-entrapped emulsion gel (MOEG) beads were prepared by the emulsion–gelation method. Different polysaccharides (sodium alginate and pectin), oil concentrations (10%, 20% and 30% w/w) and drug:polymer (D:P) ratios (1:1, 2:1 and 3:1) were used and their influence on beads uniformity, drug entrapment efficiency (DEE) and *in vitro* drug release, was studied. The results clearly indicated that retardation of drug release for 4 h was achieved by the oil hydrophobic diffusional barrier, especially in the presence of the compact network of alginate beads. Calcium alginate beads containing 20% oil and 2:1 D:P ratio, showed an optimum DEE of 88.32%. When evaluated *in vivo*, this formula displayed superior antiulcer activity (>2) over drug suspension or marketed conventional tablets.



## 1. Introduction

Gastroretentive systems could lengthen the gastric residence time of a dosage form for a period of time over which the drugs may be released gradually. These systems may delay the arrival of some drugs to their absorption site but are beneficial as carriers for drugs that act locally in the stomach (Murata, Sasaki, Miyamoto, & Kawashima, 2000), drugs that are poorly soluble at an alkaline pH (Machida, Inouye, & Tokumura, 1989) and drugs with a narrow window of absorption (Chungi, Dittert, & Smith, 1979). Among the various ways of increasing the retention time in the stomach, the low density systems are capable of floating on the gastric contents for a prolonged period of time allowing slow release of drugs at a desired rate (Singh & Kim, 2000). Numerous research efforts have been concentrated to develop multiple unit systems such as floating gel beads which showed superiority over single ones in terms of uniform distribution along the gastrointestinal tract, reduction of the intersubject variability in absorption and lowering the probability of dose-dumping characteristics (Rouge, Leroux, Cole, Doelker, & Buri, 1997). The tendency of multiple unit gel beads to possess floating nature as a result of oil incorporation was recently reported in the literature (Sriamornsak, Thirawong, & Puttipipatkhachorn, 2004; Choudhury & Kar, 2005).

As a dispersed phase, oil generates uniform emulsion creating multiple tiny chambers in the bead matrix for better buoyancy. The formed emulsion is stabilized by the surface active ability of alginate and pectin (Choudhury & Kar, 2005; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003). The inclusion of oil provides a diffusional barrier towards drug escape from porous beads. In addition, the hydrophobic nature of oil reduces drug loss during entrapment processes. Because of their high volatility and porosity, volatile oils have major drawbacks of uneven sphere production, great loss of original size of beads and very rapid drug release. Furthermore, as compared to fixed oils, mineral oil has a relative lower density that reduces the amount required to give buoyancy (Sriamornsak et al., 2004), added to a more prolonged drug release characteristics (Choudhury & Kar, 2005).

In this regard, the present work deals with the formulation, *in vitro* characterization and *in vivo* evaluation of mineral oil-entrapped emulsion gel (MOEG) beads as a delivery system of famotidine. Famotidine, an $H_2$ receptor antagonist, suffers from incomplete and variable oral absorption (Hui, Kolars, Hu, & Fleisher, 1994) which occurs mainly in the proximal small intestine (Mehta, Doshi, & Joshi, 2003). Gastroretentive controlled release gel beads could continually supply famotidine in solution form to its most efficient site of absorption, where its bioavailability would be improved. Furthermore, local delivery of famotidine might increase the stomach wall receptor site availability, increasing its efficacy in reduction of acid secretion (Coffin & Parr, inventors, 1995). Moreover, an additional valuable advantage could be achieved after filling such beads in capsules, for providing light stability to this light sensitive drug.

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## 2. Materials and methods

### 2.1. Materials

Famotidine and sodium alginate were kindly supplied by Memphis Co. (Cairo, Egypt). Low methoxy (LM) pectin LM104 was obtained as gift sample from CP Kelco Co. (Denmark). Light mineral oil and anhydrous calcium chloride ($CaCl_2$) were obtained from El Nasr pharmaceutical chemicals (ADWIC) Co. (Cairo, Egypt). All other chemicals and reagents were of the highest purity available from local sources.

### 2.2. Methods

#### 2.2.1. Preparation of famotidine loaded calcium alginate and calcium pectinate MOEG beads

Famotidine loaded MOEG beads were prepared by the emulsion–gelation method (Sriamornsak et al., 2004). In this method, a pre-gelation liquid of either 50 ml of 1% w/w sodium alginate solution or 25 ml of 2.5% w/w pectin solution was prepared. Mineral oil, in concentrations (10%, 20% and 30% w/w), was then added to the polymer solution to make 100-g mixtures. To ensure emulsion stabilization, the mixtures were homogenized at 10,000 rpm using a homogenizer (Erweka, type 4R401, Germany) for 10 min. Famotidine was then dispersed in the formed emulsion in different drug:polymer (D:P) ratios (1:1, 2:1 and 3:1 w/w). The bubble-free emulsion was extruded, using a 16G syringe needle into 250 ml gently agitated 0.1 M $CaCl_2$ solution at room temperature. The emulsion gel beads were allowed to stand in the solution for 20 min before being separated and washed with 3× 100 ml distilled water. The beads were air-dried at room temperature. The composition of different calcium alginate (A1–A9) and calcium pectinate (P1–P9) MOEG beads is shown in Table 1.

#### 2.2.2. Characterization of MOEG beads

*2.2.2.1. Study of the homogeneity and uniformity of beads.* In order to prepare uniform beads (i.e. of the same size and density) it is essential that synthesis conditions such as viscosity, rate of falling of drops, stirring rate and distance between syringe and gelation medium, be maintained constant during the course of the formation of beads. Variation in any of these parameters during the bead formation process may result in the production of non-homogenous and non-uniform beads, affecting the overall results to an appreciable extent (Bajpai & Tankhiwale, 2008). Also, process homogeneity was greatly influenced by emulsion homogenization which yields fine dispersion of oil and water with size uniformity. Without homogenization, the oil might separate out from the solution and uneven sized beads were formed (Choudhury & Kar, 2005). In order to test the product uniformity, the individual diameters of 20 dried MOEG beads were measured with a calliper (Cole-Parmer instrument Co.) as reported (Murata et al., 2000). The results are expressed as the mean diameter (mm) ± standard deviation. The sphericity of the beads was also determined by axial and diametral measurements.

*2.2.2.2. Density measurements.* The mean weight and diameter of the beads were measured and used to mathematically calculate the densities of the spherical calcium alginate and pectinate beads using the following equations:

$$D = \frac{M}{V} \quad (1)$$

$$V = \frac{4}{3}\pi r^3 \quad \text{(for a typical sphere)} \quad (2)$$

where $D$ is the density of the beads; $M$ is the weight of the beads; $V$ is the volume of the beads; $r$ is the radius of the beads.

*2.2.2.3. Determination of the beads buoyancy and integrity.* The MOEG beads ($n = 20$) were soaked in beakers filled with 50 ml of 0.1 N HCl (pH 1.2). The floating ability of the beads was measured by visual observation for an overall duration of 6 h. The preparation was considered to have buoyancy in the test solution only when all of the beads floated (Cooreman, Krausgrill, & Hengels, 1993). The integrity of the beads was also observed visually during the buoyancy test.

*2.2.2.4. Determination of drug entrapment efficiency (DEE).* An accurately weighed amount of 50 mg of famotidine loaded MOEG beads was dissolved in 250 ml (in case of alginate beads) or 500 ml (in case of pectinate beads) of phosphate buffer pH 7.4 by stirring for 6 h using magnetic stirrer. The resulting solution was then filtered using 0.45 μm Millipore filter (Sartorius, GmbH, Germany). Famotidine content was determined spectrophotometrically (Shimadzu UV–visible 1601 PC, Kyoto, Japan) at the predetermined $\lambda_{max}$ (286 nm). The determinations were made in triplicate and DEE was calculated according to the following equation:

$$\text{DEE}(\%) = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \quad (3)$$

*2.2.2.5. Scanning electron microscopy (SEM).* Morphological examination of the surface and internal structure of the dried MOEG beads was carried out using a scanning electron microscope (JEOL JEM-1200 EX II, Japan) equipped with secondary electron detector at an accelerating voltage of 10 kV. The samples were coated with gold to a thickness of about 30 nm in a vacuum evaporator. The internal structure of beads was examined by cutting them with a steel blade.

#### 2.2.3. In vitro famotidine release studies

The *in vitro* release studies were carried out using USP rotating basket apparatus-Pharma test, type PTW-2, Germany (apparatus I). Amounts of beads equivalent to 40 mg famotidine were introduced into the baskets which were rotated at 50 rpm in 900 ml 0.1 N HCl (pH 1.2), maintained at 37 ± 0.5 °C. Aliquots of 5 ml of the solution were withdrawn at predetermined time intervals and replaced by fresh dissolution medium. The withdrawn samples were analyzed for famotidine content spectrophotometrically at $\lambda_{max}$ (265 nm). The release of famotidine plain powder and marketed conventional famotidine tablet were studied as well. None of the ingredients used in the bead formulations interfered with the assay. The results were expressed the mean of three experiments.

*2.2.3.1. Analysis of the in vitro release studies.* Mathematically, the release profiles of the various formulae in 0.1 N HCl were compared using the similarity factor ($f_2$) defined in FDA Scale-up and Post-Approval Change (SUPAC) guidelines for solid dosage forms (US Department of Health and Human Sciences, 1997) using the following equation:

$$f_2 = 50\log\{[1 + 1/n\sum(R_t - T_t)^2]^{-0.5} \times 100\} \quad (4)$$

where n is the number of time points and $R_t$ and $T_t$ are the percent drug released at each time point for the reference and the test. For comparison between each set of two formulae, the formula containing the lower D:P ratio and the lower oil concentration were taken as references to elucidate the effect of oil concentration and D:P on the *in vitro* drug release. Also, the marketed conventional tablet was considered as a reference for comparative pur-

poses with different MOEG beads formulae. A similarity factor ($f_2$) between 50 and 100 suggests that the two release profiles are similar (Tang & Gan, 1998).

*2.2.4. Statistical analysis of data*

Statistical treatment of data using the Student's *t*-test and analysis of variance (ANOVA) with $P = 0.05$ as a minimum level of significance was performed with the GraphPad Instat Version 3.0 (GraphPad software, San Diego, CA, USA).

*2.2.5. In vivo evaluation of famotidine loaded MOEG beads*

*2.2.5.1. Induction of gastric antral ulcers into a rat model.* A number of 48 albino rats of either sex weighing 150–250 g were divided into eight groups each of six. Animals were used for the study, following the approval of the experimental protocol by the Ethics Committee of EAPRU (Experimental and Advanced Pharmaceutical Research Unit, Faculty of Pharmacy, Ain Shams University). The animals were deprived of food but allowed free access to water for 24 h before the day of doing the experiment. The anti-ulcerogenic effect of famotidine was investigated using the ethanol-induced ulcer model. The study was performed on the eight groups of animals as follows: group I, control group (received normal saline); group II, untreated group (received absolute ethanol "6.6 ml/kg orally"); group III,[1] received 30 mg/kg (Doi et al., 1999) plain famotidine powder in suspension form 1 h before ethanol administration; group IV, received dispersed marketed conventional tablet in suspension form (equivalent to 30 mg/kg) 1 h before ethanol administration; group V, received selected beads formula (equivalent to 30 mg/kg) 1 h before ethanol administration; group VI, received 30 mg/kg plain famotidine powder in suspension form 3 h before ethanol administration; group VII, received dispersed marketed conventional tablet in suspension form (equivalent to 30 mg/kg) 3 h before ethanol administration; group VIII, received selected beads formula (equivalent to 30 mg/kg) 3 h before ethanol administration.

Following a 60 min period, groups (III, IV and V) were given ethanol by gavage. On the other hand, groups (VI, VII and VIII) were given ethanol after 3 h from drug administration. One hour after ethanol administration, rats were sacrificed for gastric ulcers evaluation.

*2.2.5.2. Gross morphological evaluation of gastric antral ulcers.* The freshly excised stomachs were examined macroscopically for hemorrhagic lesions in the glandular mucosa. Immediately after the animals were sacrificed, their stomachs were dissected out, cut along the greater curvature and the mucosae were rinsed with normal saline to remove blood contaminant, if any, and stretched on a feline board. Gross mucosal lesions were recognised as hemorrhage or linear breaks (erosions) with damage to the mucosal surface. The recently published, Pauls index (Zueva, Reikhart, & Krylova, 2003), was used to assess ulcerogenic effect. It is the integral indicator of the number of lesions induced per formula and is calculated by multiplying the mean number of ulcers and % of animals with ulcers and then divided by 100%.

Moreover, the antiulcer activity (AA) of the preparations was evaluated as follows (Zueva et al., 2003): the untreated group Pauls index was divided by the experimental group Pauls index. The test preparation was considered active if AA was at least of two units (Krylova et al., 2006).

*2.2.5.3. Histopathological examination of gastric antral ulcers.* Histopathological examination was done for stomach specimens preserved in 10% formalin and were ranked according to the severity of the inflammatory reaction as follows: severe reaction (+++), moderate reaction (++), mild reaction (+), almost normal tissue (±), and tissue totally free from any inflammatory reaction (−).

[1] A volume of 0.5 ml of 1% Na CMC suspension was administered by gavage through an intragastric tube.

## 3. Results and discussion

*3.1. Preparation of famotidine loaded calcium alginate and calcium pectinate MOEG beads*

A preliminary study was carried out according to the method adapted by Murata et al. (2000) and Won, Kim, Kim, Park, and Moon (2005) where 1% sodium alginate concentration yielded mechanically strong and spherical beads. Compared to calcium alginate beads, the calcium pectinate beads of the same concentration showed weaker mechanical strength with failure of beads formation. Several concentrations were tried and the 2.5% pectin was found suitable to produce beads with acceptable mechanical strength. However, these two concentrations were lower than the concentration usually used by many researchers (Pillay & Fassihi, 1999; Sriamornsak, Sungthongjeen, Nunthanid, & Puttipipatkhachorn, 2007; Talukder & Fassihi, 2004; Whitehead, Collett, & Fell, 2000).

*3.2. Characterization of MOEG beads*

*3.2.1. Study of the homogeneity and uniformity of beads*

The mean diameters of the famotidine-loaded calcium alginate and calcium pectinate beads are shown in Table 1. In fact, small values of standard deviation, revealed in Table 1, confirmed high process uniformity regarding homogenization efficiency and low variability in processing conditions.

Further inspection of the results (Table 1) reveals that the mean diameter of the calcium alginate beads ranged between 2.05 and 3.08 mm whereas those of calcium pectinate spherical beads were slightly larger varying from 2.72 to 3.67 mm. Moreover, whatever the polysaccharide used, the increase in both oil concentration and D:P ratio led to an increase in beads size. This might be due to the increased droplet viscosity by higher oil content or drug concentrations. Droplets of lower viscosity were efficiently stirred, with a reduction in emulsion droplet size leading to smaller bead formation. Similar results were reported (Sriamornsak, Thirawong, & Puttipipatkhachorn, 2005). It should be pointed out that, by visual inspection; the dried MOEG beads of either type were of spherical geometry. Hence, their diametral and axial measurements were identical except for formulae (P1–P3). These formulae had a disc-like geometry with axial measurements 1.21- to 1.42-folds higher than diametral measurements.

*3.2.2. Density measurements*

Table 1 shows that the calculated densities of all the prepared beads were less than the density of 0.1 N HCl (i.e. 1.004 g $cm^{-3}$) imparting their flotation. Their values ranged from 0.119 to 0.146 and 0.066 to 0.129 g $cm^{-3}$ in case of calcium alginate and calcium pectinate beads, respectively. It is to be noted that formulae prepared with pectin displayed lower beads-densities values compared to those prepared with alginate. A matter which could be attributed to the molecular structure of pectin. The galacturonic acid units in pectin are similar to the guluronic acid units in alginate, and the gel cross-linking mechanisms are generally thought to be very similar. Although there have been many parallels drawn between the calcium-induced gelation of alginate and pectin, there are some notable differences, including the insertion of rhamnose residues, which interrupt the pectin chains that would probably cause differences in gel formation and properties (Rolin, 1993).

**Table 1**
Composition, mean diameter, density and drug entrapment efficiency of famotidine oil-entrapped calcium alginate and calcium pectinate MOEG beads

| Formula code | Oil concentration (% w/w) | Drug:polymer ratio (% w/w) | Mean diameter[c] (mm) (mean ± SD) | Density (g/cm$^3$) | Drug entrapment efficiency (%) (mean ± SD) |
|---|---|---|---|---|---|
| A1[a] | 10 | 1:1 | 2.05 ± 0.17 | 0.146 | 47.31 ± 3.30 |
| A2 | | 2:1 | 2.33 ± 0.09 | 0.127 | 77.07 ± 0.94 |
| A3 | | 3:1 | 2.39 ± 0.14 | 0.117 | 72.02 ± 1.58 |
| A4 | 20 | 1:1 | 2.50 ± 0.13 | 0.139 | 52.26 ± 6.55 |
| A5 | | 2:1 | 2.61 ± 0.13 | 0.140 | 88.32 ± 3.33 |
| A6 | | 3:1 | 2.66 ± 0.19 | 0.126 | 88.29 ± 2.87 |
| A7 | 30 | 1:1 | 2.84 ± 0.17 | 0.142 | 67.76 ± 4.64 |
| A8 | | 2:1 | 2.96 ± 0.18 | 0.128 | 75.13 ± 3.43 |
| A9 | | 3:1 | 3.08 ± 0.19 | 0.122 | 78.43 ± 2.97 |
| P1[b] | 10 | 1:1 | N/A[d] | N/A | 62.77 ± 4.41 |
| P2 | | 2:1 | N/A | N/A | 84.80 ± 2.40 |
| P3 | | 3:1 | N/A | N/A | 86.88 ± 3.87 |
| P4 | 20 | 1:1 | 2.78 ± 0.17 | 0.129 | 72.57 ± 1.48 |
| P5 | | 2:1 | 2.96 ± 0.18 | 0.112 | 88.95 ± 2.86 |
| P6 | | 3:1 | 3.05 ± 0.26 | 0.117 | 90.08 ± 1.72 |
| P7 | 30 | 1:1 | 3.20 ± 0.29 | 0.083 | 75.54 ± 2.92 |
| P8 | | 2:1 | 3.35 ± 0.18 | 0.066 | 84.52 ± 1.86 |
| P9 | | 3:1 | 3.67 ± 0.14 | 0.067 | 89.72 ± 3.86 |

[a] Calcium alginate MOEG beads.
[b] Calcium pectinate MOEG beads.
[c] $n = 20$.
[d] N/A, non-applicable.

The pectin gel may not form as densely as the alginate gel (Sriamornsak & Kennedy, 2007), a geometry which will favor more oil entrapment hence, lower densities are obtained especially in the presence of higher oil concentrations.

*3.2.3. Determination of the beads buoyancy and integrity*

A preliminary study showed that the amount of oil has a great influence on beads buoyancy: oil concentrations lower than 10% w/w yielded non-floating MOEG beads. However, the emulsifying property was limited when the oil concentration was increased and oil began to leak from the beads at 40% w/w even with thorough homogenization.

Instantaneous *in vitro* floating behavior was observed for either calcium alginate or calcium pectinate MOEG beads and lasted for at least 6 h except for P2 and P3 which sank first then gradually float. These formulae (P2 and P3) were non-spherical in shape with a disc-like geometry. This might have led to unequal forces of the release medium (0.1 N HCl) on their surfaces. Furthermore, it was obvious that, visually, none of the formulations swelled or disintegrated visibly in 0.1 N HCl. They remained firm and intact throughout the test. This is due to the stability of alginate and pectinate hydrogels at lower pH (Sriamornsak et al., 2007; Yotsuyanagi, Ohkubo, Ohhashi, & Ikeda, 1987). The protonated –COOH groups in the polymers (insoluble alginate or pectinate salts) do not ionize to induce chain-relaxation processes.

*3.2.4. Determination of drug entrapment efficiency (DEE)*

The DEE of calcium alginate beads ranged between 47.31% and 88.32% whereas that of calcium pectinate beads varied from 62.77% to 90.08% as shown in Table 1. From the same table, it is clear that, irrespective of the polysaccharide type, at a given oil concentration, increasing the initial drug loading resulted in a significant increase in DEE up to 2:1 D:P ratio (Student's *t*-test, $P < 0.05$). Higher D:P ratio (3:1) did not affect DEE significantly (Student's *t*-test, $P > 0.05$) except for A3 where a significant reduction in DEE amounting to 72.02% was observed. The corresponding formula in calcium pectinate beads (P3) did not show such observation. This might be due to the previously mentioned difference between cross-linked networks of both polysaccharides.

Further inspection of the results (Table 1) reveals that for calcium alginate beads, on keeping D:P ratio constant, DEE reached its maximum by increasing oil concentration up to 20% except for 1:1 D:P ratio where a significant increase in DEE was observed on further increase of oil concentration (30%) amounting to 67%. On the other hand, for calcium pectinate beads, at the same D:P ratio, increasing oil concentration had no influence on DEE (one-way ANOVA, $P > 0.05$) except for 1:1 D:P ratio where a significant increase in DEE (Student's *t*-test, $P < 0.05$) reaching a value of 72% on increasing the oil concentration to 20% compared to 62% for the 10% oil.

From these findings, it could be concluded that 2:1 D:P ratio has the optimum drug loading with both types of polymers at 10% and 20% oil concentrations for calcium pectinate and calcium alginate, respectively, except for 1:1 D:P ratio where 20% and 30% oil showed the highest DEE for the two types of beads, respectively.

Finally, a direct correlation was found to exist between particle size (diameter) and the corresponding DEE at the same oil concentration. Al-Kassas, Al-Gohary, and Al-Faadhel (2007) reported similar relationship between particle size and DEE.

*3.2.5. Scanning electron microscopy (SEM)*

The external surface and cross-sectional morphologies of calcium alginate and pectinate MOEG beads, containing 30% mineral oil and D:P ratio 3:1, beads were examined by SEM. The external surface of the spherical shape MOEG beads appeared rough, irrespective of the polymer type, as displayed in Fig. 1, A1 and A2 (150×). The surface was covered with irregular clusters of famotidine crystals which were probably formed as a result of their migration along with water and hence, leaching out onto the surface during drying and subsequent shrinkage. Due to the higher amounts of drug used, calcium pectinate beads showed more rough surface than their counterpart calcium alginate beads. The cross-sectioned MOEG beads, Fig. 1, B1 and B2 (30×) and B3 and B4 (1000×), shows that calcium alginate beads seemed more compact and rigid with ordered network in comparison to more porous and less rigid random fibrillar network seen in calcium pectinate beads. This was in accordance with density measurements.

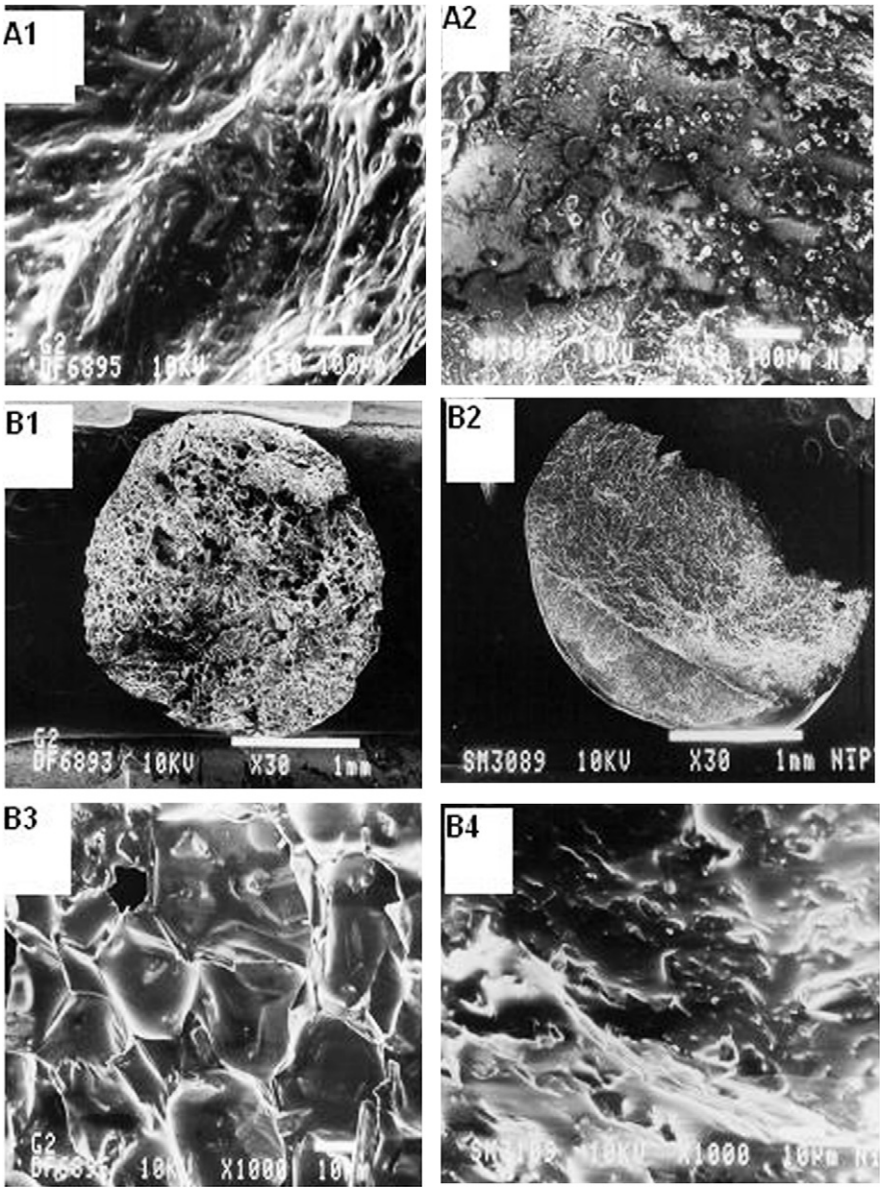


**Fig. 1.** SEM micrographs of oil-entrapped emulsion gel beads. Group A: the surface of beads, group B: the section of beads, A1, B1 and B3 (calcium alginate beads), A2, B2 and B4 (calcium pectinate beads).

### 3.3. In vitro famotidine release studies

The release profiles of famotidine loaded calcium alginate and pectinate MOEG beads are illustrated in Figs. 2 and 3. The release of famotidine from these MOEG beads was obviously slower than the dissolution of famotidine powder and marketed conventional tablet which occurred within 10 and 30 min, respectively (figure not shown).

Being a weak base and highly soluble in 0.1 N HCl, famotidine showed an initial burst effect within the first 10 min. This might be due to the presence of surface deposited drug along with rapid water infiltration creating aqueous channels for famotidine to permeate out.

Further inspection of Figs. 2 and 3 reveals that famotidine was released immediately from calcium alginate MOEG beads containing 10% oil concentration (A1–A3). Increasing oil concentration resulted in a decrease in famotidine release rate and consequently, prolonged drug release at all D:P ratios except for A6. On the other hand, calcium pectinate beads showed only a slight prolongation of

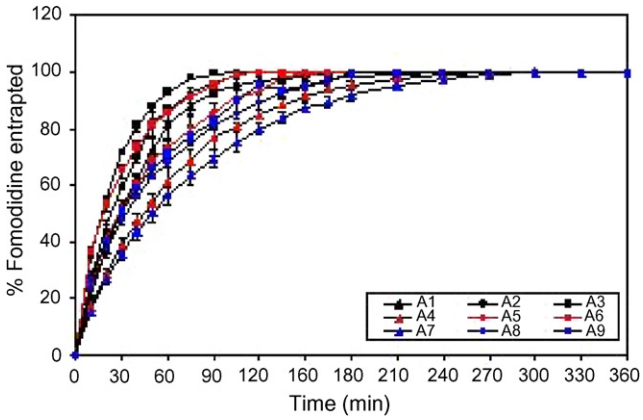


**Fig. 2.** Release of famotidine from oil-entrapped calcium alginate emulsion gel beads in 0.1 N HCl (pH 1.2).

drug release in case of formulae containing 30% oil, whatever D:P ratio (P7–P9). Similar results were previously found for pectin-coated pellets as compared to alginate-coated pellets (Sriamornsak & kennedy, 2007).

These previous findings could be explained based on the following hypothesis: as long as famotidine was found, experimentally in our laboratory, to be totally insoluble in mineral oil but freely soluble in 0.1 N HCl, it will diffuse easily out of the beads increasing the rate of penetrant entry into the beads. Therefore, for the basic famotidine, the hydrophobic diffusional barrier, offered by oil inclusion, was essential for retarding drug release. This latter effect was added to the compact and dense nature of the calcium alginate beads. Unfortunately, the flexible and less compact structure of calcium pectinate beads interfered with hydrophobic barrier promoting penetrant attack to the matrices, thus, proved to be less effective in retarding drug release.

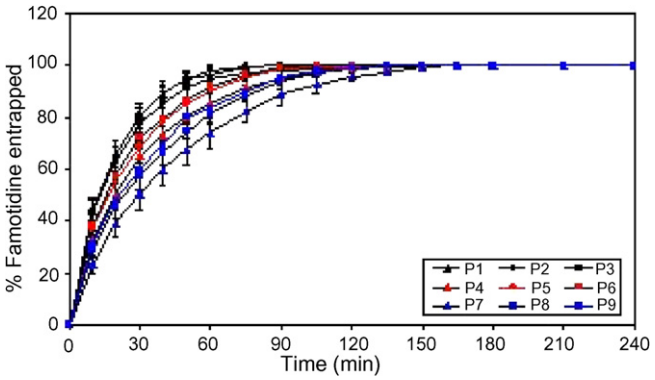


**Fig. 3.** Release of famotidine from oil-entrapped calcium pectinate emulsion gel beads in 0.1 N HCl (pH 1.2).

Applying the similarity factor ($f_2$) measurements for comparative purposes, reveals that only calcium alginate beads containing 10% oil and 1:1 D:P ratio (A1) was significantly different ($f_2 < 50$) from those containing 20% oil concentration and the same D:P ratio (A4). The same trend of $f_2$ value was obtained from calcium pectinate beads containing the same oil concentrations and D:P ratios (P1 and P4). Moreover, the results of comparison between calcium pectinate beads containing higher oil concentrations (P4 and P7) appeared significantly different. Although formulae P1 and P3 were dissimilar compared to P4 and P6, according to similarity factor data, yet both of them displayed no drug release retardation. Finally, when marketed conventional tablet was considered as a reference, the dissimilarity with all the tested formulae, including those of rapid drug release, was depicted which affirmed the effect of hydrophobic oil barrier.

Fitting the *in vitro* release results to Higuchi, zero-order and first-order models denoted Fickian diffusion of famotidine release for these MOEG beads. Similar finding was reported by several authors (Bajpai & Tankhiwale 2008; Talukder & Fassihi, 2004; Tang, Venkatraman, Boey, & Wang, 2007).

From the above results, it is clear that calcium alginate MOEG beads containing 20% oil concentration and 2:1 D:P ratio, showed an optimum DEE (88.32%) and extended drug release over 4 h. Hence, they were evaluated *in vivo*.

### 3.4. In vivo evaluation of famotidine loaded MOEG beads

Intragastric ethanol administration in rat models caused marked changes in cellular levels, membrane damage, cell death, exfoliation and epithelial erosion (Sener, Paskaloglu, & Ayanoglu-dulger, 2004).

The stomach of rats in group I (control group) revealed no inflammation, no hemorrhage or any patches of hyperemia, Fig. 4a. On the contrary, the mucosa of rat's stomach in group II, Fig. 4b, showed marked red elongated patches of ulcers. This finding

**Table 2**
Pauls index, antiulcer activity and severity of inflammatory reaction of different rat groups

| Animal group | Average number of ulcers | % Incidence of animals | Pauls index | Antiulcer activity (AA) | Severity of inflammatory reaction |
|---|---|---|---|---|---|
| I | Zero | Zero | Zero | – | – |
| II | 13.66 ± 4.54 | 100 | 13.66 | – | +++ |
| III | 4.66* ± 3.14 | 83.33 | 3.88 | 3.52 | + |
| IV | 6.508 ± 3.50 | 83.33 | 5.41 | 2.52 | ++ |
| V | 2.66* ± 2.16 | 66.66 | 1.77 | 7.71 | ± |
| VI | 11.16 ± 1.96 | 100 | 11.16 | 1.22 | +++ |
| VII | 11.83 ± 2.31 | 100 | 11.83 | 1.15 | +++ |
| VIII | 6.66* ± 3.55 | 83.33 | 5.54 | 2.46 | ++ |

* $P < 0.05$ compared to group II (untreated group).

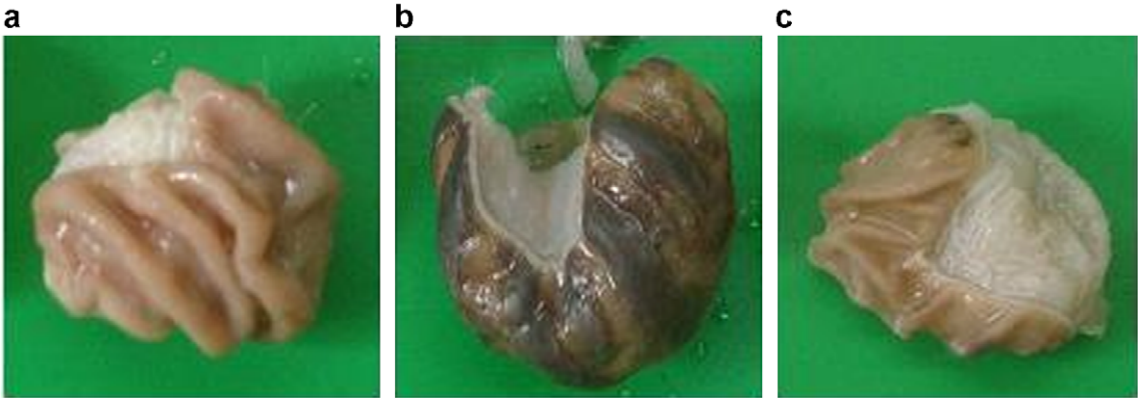


**Fig. 4.** Gross appearance of preserved stomach tissue (in 10% formalin) (a) group I (control), (b) group II (untreated) and (c) group V (treated with MOEG beads).

was manifested by the presence of high number of ulcers and high % incidence of animals with ulcers (100%) showing the highest Pauls index reaching a value of 13.66 (Table 2). When given 1 h before ethanol administration, all famotidine containing formulae (suspension, dispersed marketed conventional tablet and floating beads) (groups III, IV, V "Fig. 4c"), showed significantly smaller numbers of ulcers (Student's *t*-test, $P < 0.05$) and smaller values of Pauls index in comparison with group II (untreated group) (3.88, 5.41, 1.77 *versus* 13.66), respectively. They also displayed a satisfactory antiulcer activity (AA > 2). It is to be pointed out that the AA of famotidine was prolonged for 4 h only when it was administered as alginate MOEG beads (AA = 2.46).

*3.4.1. Histopathological examination of gastric antral ulcers*

According to the severity of inflammatory reaction in the rat's stomach (Table 2), the different rat's groups could be arranged in a descending order as follows: Table 2, group II (no treatment) ≈ group VI ≈ group VII > group III ≈ group IV > group VIII > group V > group I (control group, "Fig. 5"). The stomach of the rats of group II, Fig. 6, showed focal ulceration with hemorrhages detected in the lining mucosal epithelium and underlying tissues in association with oedema, inflammatory cells infiltration and congested blood vessels in submucosal layer.

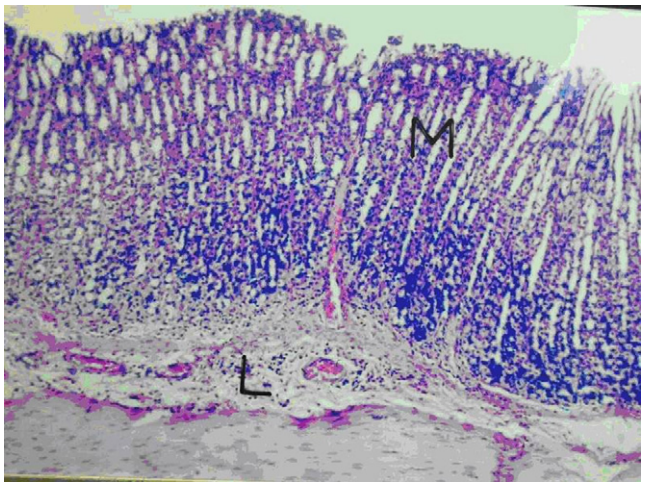


**Fig. 7.** Histopathological examination of glandular stomach of rats in group V (treated with MOEG beads) mild oedema and few inflammatory cells infiltration in submucosa (L) in focal manner.

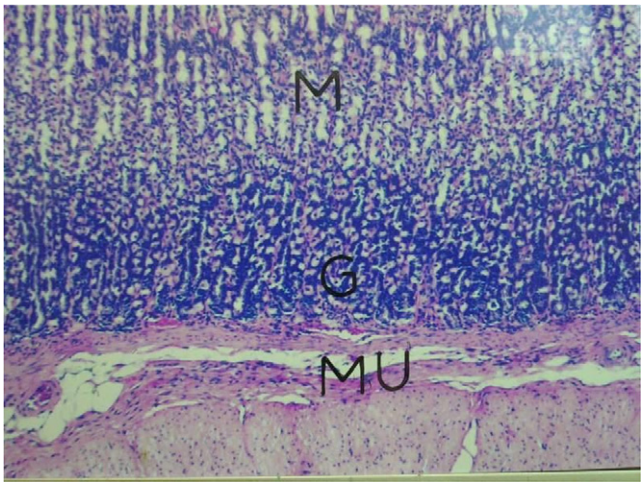


**Fig. 5.** Histopathological examination of glandular stomach of rats in group I (control) showing the normal histological structure of the mucosa (M), glands (G) and musculature (MU).

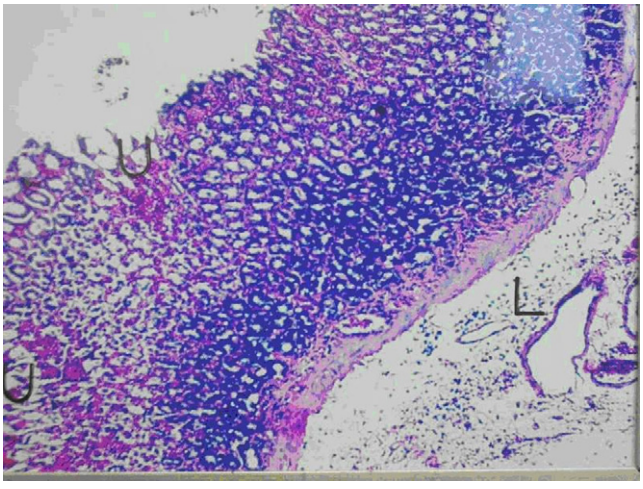


**Fig. 6.** Histopathological examination of glandular stomach of rats in group II (untreated) showing focal hemorrhagic areas in the mucosa with destruction of the lining epithelium (hemorrhagic ulcer) (U).

On the other hand, the tissue of the stomach of rats, receiving drug suspension (group III) and commercial tablet (group IV) 1 h before alcohol administration showed moderate reaction where oedema with inflammatory cells infiltration with dilatation and congestion of the blood vessels were detected in submucosal layer. Meanwhile, the severity of reaction, in group V, was within the normal limit as the submucosal layer showed mild oedema with few inflammatory cells infiltration as presented in Fig. 7. The lasting of the AA with famotidine floating MOEG beads, given 3 h before ethanol administration, was also seen, from histopathological studies, where only few ulceration in the lining epithelium was noticed. Groups VI and VII, receiving other famotidine dosage forms, showed rather severe reactions to ethanol.

Gastric retention of basic drug, famotidine, through loading to floating MOEG beads, prolonged its residence in the stomach where it is more soluble. The retention of the beads for an extended period of time was confirmed visually upon excision of the rat's stomachs. For a drug with a narrow absorption window, this resulted in a higher bioavailability with a prolonged gastroprotective effect.

## 4. Conclusion

In conclusion, the emulsion–ionotropic gelation of alginate and pectinate polymers offers a flexible, easily controllable and consistent process for achieving the homogeneity and uniformity of famotidine-loaded beads formation. Inclusion of mineral oil droplets confers buoyancy for at least 6 h. The results demonstrated the superiority of alginate matrices over pectinate matrices to sustain the drug release.

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