Volume 7, Issue 5, 2017, 2123 - 2134 ISSN 2069-5837

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Original Research Article Open Access Journal

Received: 11.08.2017 / Revised: 15.09.2017 / Accepted: 25.09.2017 / Published on-line: 15.10.2017

Development and evaluation of a novel griseofulvin floating drug delivery systems

Amal El Sayeh F. Abou el Ela [1, 2, *], Mona Mohamed El Khatib [1, 3], Assil Najeeb AlAjmi [1]

[1] Department of Pharmaceutics, Faculty of Pharmacy, King Saud University, Female Section, Riyadh, Saudi Arabia
[2] Department of Pharmaceutics, Faculty of Pharmacy, Assiut University, Assiut, Egypt
[3] Department of Pharmaceutics, Faculty of Pharmacy, Cairo University, Cairo, Egypt

*corresponding author e-mail address: ***aelsaih@ksu.edu.sa***

ABSTRACT

The aim of the present study is to develop and evaluate gastro-retentive floating beads and floating *in situ* gels of griseofulvin (GF) in order enhance the dissolution rate and the bioavailability of GF. Different formulae of GF floating beads and floating *in situ* gels were prepared using sodium alginate, hydroxypropylmethyl cellulose (HPMC), carbopol, xanthan gum and pectin as polymers. The prepared formulae were evaluated for their physiochemical properties including the drug encapsulation efficiency, particle size, morphology, swelling, floating, density, gelation and viscosity behavior. Furthermore, the *in vitro* release pattern was examined. The results indicated that for GF floating beads; as the concentration of polymer increased, the encapsulation efficiency, particle size and floating lag time were increased. Swelling study showed that all GF floating formulae remained intact during the time of the experiment in buffered solution (pH, 1.2). Floating beads containing pectin showed rapid swelling rate, while the beads prepared with HPMC showed slower rate of swelling. For the floating *in situ* gels the results revealed that floating duration was more than 24 hours and all prepared floating *in situ* gels shown a decrease in viscosity with the increase in the shear rate. It was also found that the release rate of GF from all floating beads and floating in situ gels was significantly higher than its release from powder.

Keywords: *Floating delivery systems, alginate beads, in situ floating gels, gastric residence time, hydrophilic polymers, griseofulvin.*

1. INTRODUCTION

It is highly understandable in the recent research and patent literature that there is an increased interest in novel dosage forms that are retained in the stomach for a prolonged and predictable period of time and thereby improve the bioavailability of drugs that are favorably absorbed from the upper gastrointestinal tract [1]. After oral administration, such a drug delivery would be reserved in the stomach and releases the drug in a controlled manner, so that the drug could be delivered continuously to its absorption sites in the gastrointestinal tract. Several approaches have been proposed to increase gastric residence time of drug delivery systems in the upper part of the gastrointestinal tract. Low density systems or floating drug delivery systems (FDDS) are one of these approaches that have bulk density lower than that of the gastric fluid, and thus stay buoyant in the stomach for a lengthy period to get sufficient drug bioavailability [2]. This delivery system is looked for drugs with an absorption window in the stomach or in the upper small intestine [3].

Based on the mechanism of buoyancy two distinctly different technologies; non-effervescent and effervescent systems have been used for the development of the floating drug delivery system. Effervescent floating drug delivery systems are the systems which produce CO_2, thus decreasing the density of the system, stay buoyant in the stomach for a prolonged period of time and release the drug gradually at a desired rate. Effervescent systems used CO_2 as gas generating agents (e.g. sodium bicarbonate, citric acid or tartaric acid) to achieve floatability. The non-effervescent floating drug delivery system is grounded on the mechanism of swelling of polymer or bioadhesion to mucosal layer in the GI tract. Non-effervescent floating dosage forms used a gel forming or swellable polymers as cellulose kind of hydrocolloids, polysaccharides, and matrix forming polymers like polycarbonate, polyacrylate, polymethacrylates, and polystyrene. Also, chitosan, karaya gum, xanthan gum, and polyethylene oxide utilized as polymers. When these hydrophilic polymers interact with the gastric fluid, they hydrate and form a colloidal gel barrier around the surface of the particles. This gel barrier reins the rate of the fluid penetration into the particles and resulting release of the drug [4].

The alginate beads have been utilized for floating dosage forms containing amoxicillin [5], furosemide [6], meloxicam [7] sulindac [8], diclofenac [9], tiaramide [10] and ampicillin [11]. The alginate beads have been utilized to entrap sparingly water soluble drug as verapamil to successfully deliver the drug in stomach for prolonged period of time without using organic solvent [12].

In situ floating gelling systems are a novel approach in the FDDS and were used for the delivery of the drug for gastrointestinal infections and disorders. These systems are in solution forms when administered orally, and in contact with gastric fluids, they swell forming a viscous cohesive gel [13]. The

formed *in situ* gel floats on the gastric fluid because it has bulk density less than the gastric fluid. So the system stays buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time and increases gastric residence time resulting in prolonged drug delivery in gastrointestinal tract [14]. When the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system. After release of the drug, the residual system is vacated from the stomach. This results in an increased gastro retention time and a better regulator of the fluctuations in plasma drug concentration [15].

Griseofulvin (GF) is an antifungal agent with poor solubility and low bioavailability. To enhance the GF bioavailability, several trials were made in order to improve both its solubility and its dissolution rate such as micronization [16], complexation of GF with cyclodextrin [17], preparation of GF nanoparticles [18], preparation of GF nanosuspensions [19], liquisolid compact of GF particles [20]. Science GF has low oral bioavailability, it will be incorporated in a novel floating *delivery* systems to prolong the gastric residence time which will increase drug delivery with more patient compliance and decrease the frequency of the dosing.

The aim of this study is to develop and evaluate GF floating beads and GF *in situ* floating gels in order to enhance dissolution rate and improve bioavailability of GF. The prepared formulae will be evaluated for swelling, particle size, morphology, encapsulation efficiency, rheology, gelation and floating characterization. The *in vitro* drug release will be also carried out and the results will be analyzed according to different kinetic models.

2. EXPERIMENTAL SECTION

2.1. Materials. Ultra micronized griseofulvin (GF) was a gift from (Nile Pharm. Industry Company, Cairo, Egypt). Hydroxypropylmethyl cellulose, HPMC (Methocel 4000 cp Premium®) was obtained from (Colorcon, UK). Sodium alginate and carbopol 980 were obtained as gifts from (Al-Amriya Pharm. Industry Company, Alexandria, Egypt). Pectin and xanthan gum were purchased from (BDH, UK). Light Liquid Paraffin from (Lobachemi Company, India). Calcium carbonate, calcium chloride and sodium citrate were obtained from (BDH, UK). Tween 80 was obtained from (Avonchem Ltd., UK). All other chemicals and solvents were of analytical grade and were used as received.

2.2. Preparation of GF floating beads. Griseofulvin (GF) floating beads were prepared by emulsion gelation technique carried out by [12]. The composition of the prepared formulations is shown in Table 1. The polymeric solutions were composed of sodium alginate with one of the following polymers; hydroxypropylmethyl cellulose (HPMC), pectin, xanthan gum, and carbopol 980 in different ratios. The required weights of sodium alginate and polymer were dissolved separately in distilled water (60 and 30 ml), respectively and the two solutions were mixed thoroughly to obtain homogenous polymer solution. Light liquid paraffin was added to polymer solution and after that GF was added. The mixture was homogenized for 10 min at 9000 rpm and dropped through a syringe needle with a diameter of 0.8 mm into 100 ml of 2 % (w/v) calcium chloride solution from a constant height (7 cm) and constant rate (40 rpm) at room temperature. The dropping rate was 1ml/min. The formed beads were collected, washed with distilled water and dried in the oven at 30 ˚C for 48 h.

2.3. Preparation of GF floating *in situ* gels. Sodium alginates, at different concentrations (1.5, 2, and 2.5 % W/V), were dissolved in deionized water containing calcium chloride (0.075 % W/V) and sodium citrate (0.25 % W/V).

HPMC (0.5 % W/V) was added to formulae G7, G8 and G9 as shown in Table 2. The sodium alginate solution was heated to 50 ˚C while stirring. After cooling below 40 ˚C, calcium carbonate (1.5 or 2 % W/V), GF (0.5 % W/V), and tween 80 (0.5 % W/V) were dispersed well with continuous stirring for 5 min. The resulting sodium alginate floating *in situ* gels containing GF was finally stored until further use.

2.4. Characterization of GF floating beads.

24.1. The percentage of yield and encapsulation efficiency. The percentage of yield and encapsulation efficiency of GF floating beads were determined (n=3) [21].

Percentage yield = Weight of the beads/ (weight of drug+polymer weight) x 100.

Encapsulation efficiency was determined by weighing 30 mg of GF formulated beads, dissolved in 100 ml of buffer solution (pH, 1.2), centrifuged, filtered and the filtrate was analyzed at 296 nm using a UV/visible spectrophotometer (Genesys™ 5, Thermospectronic, USA).

Percentage encapsulation efficiency = (Actual amount of drug /Theoretical amount of drug) x 100.

2.4.2. Scanning electron microscope (SEM). GF formulated alginate beads were examined for morphology and surface properties using SEM (Metler Toledo, Tokyo, Japan).

2.4.3. Determination of particle size. The particle size of the formulated GF beads was determined using optical microscope calibrated with eye piece and stage piece micrometer (Micromaster®, Fisher Scientific, Germany). Thirty beads were randomized selected and the mean diameters were calculated [12].

2.4.4. Determination of swelling ratio. Floated beads (50 mg) was placed in 100 ml of buffered solution (pH, 1.2), at 37 ± 0.5 ˚C. The beads were taken out at various time intervals, dried and weighed to calculate the swelling ratio. The swelling ratio (SR) was calculated using the following equation [22].

Swelling Ratio (SR) = (Weight of swollen beads- Initial weight of beads)/Initial weight of beads.

The experiment was performed in triplicate and the mean values and standard deviation were calculated.

2.4.5. *In vitro* floating ability and density. The mean weight and diameter of prepared floating beads were measured and used to calculate their densities mathematically using the following equation [23].

Density = Weight of beads/Volume of beads.

The floating efficacy of GF beads was measured by suspending 50 mg of the beads in 100 ml of buffered solution (pH, 1.2). The solution was stirred at 100 rpm and the time required for the most of the beads to rise to the surface of the solution was recorded as the floating lag time. The duration of time by which the beads constantly float on the surface of the medium is the floating time. After 8 h, the layer of floated as well as the sinking layer were separately collected by filtration. Beads of both layers were dried at 40 ˚C until constant weight was achieved. Both fractions of beads were weighed and the buoyancy was determined by the weight ratio of floating beads to the sum of floating and sinking beads [22].

2.4.6. *In vitro* GF release study.

The *in vitro* dissolution of the prepared beads was carried using USP dissolution test apparatus II (Erweka DT-600 GmbH, Germany). Known weight of beads containing equivalent amount of 5 mg of GF was placed in the dissolution vessel containing 900 ml of buffered solution (pH, 1.2) maintained at 37 ± 0.5 ˚C, and 50 rpm. An aliquot of 5 ml of the solution was withdrawn at predetermined time intervals of 15, 30, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min and replaced with a fresh dissolution medium. The withdrawn samples were filtered and the amount of drug released was analyzed using UV spectrophotometer at 296 nm. The release rate of 5 mg of GF powder was also determined. The experiment was carried out in triplicate and the mean values were plotted versus time. The results were expressed as the percentage of cumulative amount of drug released as function of time.

2.4.7. Kinetic study. The *in vitro* release results were analyzed using different kinetic models; zero-order, first-order kinetic, Higuchi diffusion and Korsmeyer-Peppas models [24].

2.4.8. Stability study. The stability of the prepared floating beads (F5) was studied at 25 ± 2 ˚C for 1 year and at 40 ± 2 ˚C and 75 ± 2 % relative humidity (RH) in closed high-density polyethylene bottles for 3 months. Samples were withdrawn at different time intervals and tested for physical changes (color, texture), and drug content.

2.5. Evaluation of GF floating *in situ* gels.

2.5.1. Scanning electron microscope (SEM). An SEM (shimadzu DSC-60, Tokyo, Japan) was utilized to study the morphology of floating *in situ* gels. Five ml from GF formulated floating in situ gel solution was added to 100 ml of the buffered solution (pH, 1.2). The formed gel was lyophilized for 48 h using freeze dryer (alpha 1-2d plus, Germany) and the electron microphotographs were obtained. The specimens were coated under vacuum with gold in an argon atmosphere prior to the observation [25].

***2.5.2. In vitro* floating study.** *In vitro* floating ability was determined using a dissolution USP apparatus II (DT-600, ERWEKA, Germany) using 500 ml of the buffered solution (pH, 1.2) at 37 ± 0.5 ˚C. Five ml of the prepared floating *in situ* gelling solution was withdrawn and placed into a petri dish and the a petri dish was kept in the dissolution vessel. The time needed of the gel to emerge on the dissolution medium surface was considered as floating lag time and the time the gel constantly floated on the surface of the solution as duration of floating [26].

2.5.3. Density measurement of floating *in situ* gels. The prime requirement of the floating *in situ* gel formed is that it must have density lesser than gastric contents (~ 1.004 gm/cm^3). The density was measured by added 0.5 ml of the buffered solution (pH, 1.2) to 1 ml of floating in situ gelling solution. Known volume from formulated floating in situ gel was placed inside eppendorf. The density of this gel was calculated by subtracting the total weight of eppendorf and the forming gel from the weight of eppendorf. The weight of this gel was noted and accordingly density was reported [27].

***2.5.4. In vitro* gelling capacity.** The *in vitro* gelling capacity of prepared in situ gel formulations was measured by using 5 ml of the buffered solution (pH, 1.2) as gelation solution maintained at 37 ± 0.5 ˚C. One ml of the formulation GF in situ solution was added to the gelation solution with the help of a pipette. As the formulated GF solution comes in contact with gelation solution, it was immediately converted into a stiff gel like structure. The gelling capacity of solution was evaluated on the basis of stiffness of formed gel and time period for which the formed gel remains as such. The *in vitro* gelling capacity was graded in four categories on the basis of gelation time and time period for which the formed gel remains The four categories are; (+) gels after few min, dispersed rapidly, (+ +) gelation immediate remains for 12 h, (+ + +) gelation immediate remains for more than 12 h, and (+ + + +) gelation immediate remains for more than 24 h [28].

2.5.5. Rheological studies. The determination of the viscosity of prepared GF *in situ* gels was carried out at 30 ± 0.5 ˚C using a cone and plate viscometer with spindle 52 (Brookfield Engineering Laboratories, model HADV-II, Middleboro, MA). About 0.5 g of the formula to be examined was applied to the plate and left for equilibrium. The shear rate was increased from 100 to 800 s^{-1} with 120 s between each 2 successive speeds. The viscosity was determined from the flow curve obtained at different values of the shear rate and all measurements were made in duplicate.

***2.5.6. In vitro* release study.** The *in vitro* dissolution of GF from the floating *in situ* gel preparations was determined using USP dissolution apparatus II (Erweka DT-600 GmbH, Germany) at 50 rpm. The dissolution medium used was 500 ml of the buffered solution (pH, 1.2) at 37 ± 0.5 ˚C. Five ml from each GF prepared floating in situ gels equivalent to 25 mg GF was withdrawn and placed into a petri dish and the petri dish was kept in the dissolution vessel. At different time intervals, 5 ml of the dissolution medium was withdrawn and replenished with fresh medium. The samples were filtered and evaluated using UV spectrophotometer at 296 nm. Each study was conducted at triplicate for 8 h. The same procedure was applied to GF powder (25 mg) and various kinetic models were used to analyze the *in vitro* release data.

3. RESULTS SECTION

3.1. Evaluation of GF floating beads. The data of physicochemical characterization include percentage yield, drug encapsulation efficiency, floating lag time and density of all GF beads are represented in Table 3. The percentage yield ranged from 79.88 % ± 2.3 to 98.57 % ± 1.3 for F4 and F7, respectively. Results showed that percentage yield increased by increasing polymer concentration. With respect to polymer type, beads composed of sodium alginate/xanthan gum (F7) showed the

highest yield while those composed of sodium alginate/ pectin (F4) showed the lowest one.

The encapsulation efficiency represents the percentage of actual amount of the drug with respect to theoretical amount of drug encapsulated in the beads. The encapsulation efficiency % of the sodium alginate beads is shown in Table 3. The encapsulation efficiency was affected by the concentration as well as the type of the polymer used in the beads. The increase in the polymer concentration resulted in an increase in the percentage of drug encapsulation efficiency. As concentration of HPMC increased from 2 % to 3 % (W/W) for F1 and F5, respectively, the percentage of encapsulation efficiency increased from 89.06 ± 0.79 to 93.5 ± 0.41, respectively. The results are in agreement with that concluded by Abou el Ela et al., 2014 [29]. Moreover, the encapsulation efficiency of beads was dependent on the polymer type. The prepared beads using HPMC and carbopol had more encapsulation efficiency than those prepared with xanthan gum and pectin. The encapsulation efficiencies of the beads prepared with HPMC and carbopol (F1, F2, F5 & F6) were 89.06 $\pm$ 0.79, 81.97 $\pm$ 0.43 %, 93.50 ± 0.41 and 91.48 ± 2.59, respectively. While for the beads prepared with xanthan gum and pectin (F3, F4, F7 & F8) the percentage of the encapsulation were 78.6 $\pm$ 1.5, 65.97 $\pm$ 0.82, 79.48 ± 0.95 and 77.26 ± 0.96, respectively.

3.1.1. SEM of GF floating beads. The surface morphology of the beads was observed using scanning electron microscope (SEM). Figures 1 and 2 showed that beads had oval like shape in F3 and F4 using xanthan and pectin, respectively, and showed smooth surfaces. While the beads had spherical shapes in F1 and F2 using HPMC and carbopol, respectively. It was observed that the number of pores contributes to the production of small cracks which was evident on the surfaces of both the HPMC and carbopol beads. Such number of pores was evidently lesser on the surfaces of pectin and xanthan gum beads.

3.1.2. Particle size analysis. The particle size of the beads was studied to distinguish the effect of using different types and concentrations of polymer on the size of the beads. In order to formulate uniform beads, rate of dropping, stirring, curing time, and distance between syringe and gelation media were retained constant during the course of preparation of all formulae. It is observed from the results that mean particle size of the GF floating beads markedly increased with increasing polymer concentration and it was in the range from 1702 ± 19.90 µm in F1 containing 2 % of HPMC to 1995 ± 18.40 µm in F5 containing 3 % of HPMC (Table 3). This may be related to the increase in the viscosity of the dropping solution as concluded by Amal El Sayeh et al., (2014) [29]. The same observations were obtained for the other polymers used.

3.1.3. Swelling of GF floating beads. The polymeric hydrogels are three dimensional cross linked networks that have the capability to absorb water and swell without loosing and changing their shape [30]. They have a high swelling ability, which depend mainly on the external conditions (pH, temperature) and the parameters of the gel (i.e. mesh size). In order to study the swelling behavior of the beads, the swelling test was conducted for 8 h and the swelling ratios of the beads were determined. The swelling ratio describes the amount of water that contained within the hydrogel at equilibrium which is a function of the network structure, hydrophilicity and ionization of the functional groups. Figure 3 shows the effect of polymer type and polymer concentration on the swelling behavior of the prepared floating beads. Overall, it was evident that, the swelling ratios increased by increasing the polymer concentration, however this increase was not statistically noteworthy for all polymers.

The increase in the concentration of polymer from 2 % to 3 % (W/W) increased the swelling ratios. Formulated beads (F1) containing 2 % (W/W) HPMC had a swelling ratio of 1.9 ± 0.012, while F5 containing 3 % (W/W) HPMC had a swelling ratio of 2.4 ± 0.02 (Fig.3). The same results were obtained by using other polymers with different percentage. Floating beads F2 showed the lowest equilibrium swelling ratio of 1.67 ± 0.06 while F5 showed the highest swelling ratio of 2.4 ± 0.02 after 8 h.

Furthermore,, it was observed that formulae (F4 & F8) that contain pectin as polymer showed rapid swelling rate and reached equilibrium state within 5 h, while formulae (F1 & F5) that comprise of HPMC, showed lower rate of swelling and reached equilibrium within 7.5 h. This may be due to the differences between the polymers in hydrophilicity, water uptake, and nature of network of the swollen polymer. Beads comprising of HPMC showed higher swelling ratio than other beads due to the hydrophilicity nature of these cellulose derivative polymers, in addition to the existence of hydroxyl group in the molecules which play a significant role in water uptake and in matrix integrity of swollen polymer[31]. The polymers could be arranged according to their swelling ratios in the order of HPMC > pectin > xanthan $\geq$ carbopol.

3.1.4. Density and floating studies. The object remains afloat in the stomach when the specific density is less than that of 1.004gm/cm^3. The value of density ranged from 0.45 $\pm$ 0.2 to 0.76 $\pm$ 0.3 gm/cm³ for F4 with 2 % sodium alginate/pectin and F5 with 3 % sodium alginate/ HPMC, respectively (Table 3). It was observed that the density of the formulae changed by changing the polymer type and polymer concentration. The increase of polymer concentration led to an increase in the density of the floating beads. The density of the beads that contain pectin in F4 and F8 are 0.4 ± 0.2 gm/cm³ and 0.54 ± 0.2 gm/cm³, respectively. The density of the floating beads prepared with HPMC in F1 and F5 are 0.65 ± 0.3 and 0.76 ± 0.3 gm/cm³, respectively.

The GF floating beads showed different floating properties depending on the type and concentration of polymers. All the formulae were continued floated for more than 24 h and the difference between them was the floating lag time. A lag time in the range of 9 $\pm$ 1 s to 18 $\pm$ 2 s was detected for the GF floating beads of F1 and F5, respectively. It is observed that the increase in the concentration of the polymer resulted in a significant ($P < 0.05$) increase in the floating lag time. The polymers can be arranged on the following ranked order according to their floating lag time as HPMC > xanthan gum > carbopol > pectin.

3.1.5. *In vitro* GF floating beads release studies. The *in vitro* release of GF from the different prepared floating beads is presented in Figs. 4 and 5. GF was poorly released from its powder and within 8 h only 27.3 ± 0.81 % of the drug was released. Its release rate from the prepared floating beads was improved over a longer period of time (8 h) depending on polymer type and its concentration in each formula.

From the obtained results it was concluded that the release rate of GF from formulated beads was dependent on the type of polymer. F1, F2, F3 and F4 containing 2 % of HPMC, carbopol, xanthan gum and pectin, respectively had significantly ($p<0.05$) higher release rate of the drug as compared to its release from the powder. The percent release of GF after 8 h from F1, F2, F3 and F4 was 47.8± 0.7, 42.9± 1.9, 39.6 ± 0.3 and 42.1 ± 1.1, respectively. The same observation was obtained using 3 % of the polymers. The release rate of GF from the formulated beads in accordance with the polymer type was ranked in the order of HPMC > carbopol > pectin > xanthan gum. The highest percentage of the drug release was from floating beads (F1 & F5) congaing HPMC. This may be due to the formation of more pores and channels due to the presence of hydrophilic cellulose in nature polymer HPMC, held the drug in intimate contact with dissolution medium owing to its water retention potential thus, increased its wettability.

Furthermore, it was observed that there was a prolongation of the release rate of GF proportional to the increasing percent of polymer concentration. This can be seen in F1, F2, F3 and F4 containing 2 % of polymer and in F5, F6, F7 and F8 containing 3 % of polymer. The percent release of GF after 8 h from F1, F2, F3 and F4 was 47.8± 0.7, 42.9± 1.9, 39.6 ± 0.3 and 42.1 ± 1.1, respectively while the percent release from F5, F6, F7 and F8 was 44.6±1.9, 41.1±0.8, 36.5±0.8 and 40.1±0.7, respectively. The highest percentage of GF released from F1 and F5 containing HPMC in 2% and 3 % respectively was 47.77 and 44.62, respectively while the lowest amount released (39.56 and 36.72) was from F3 and F7 containing xanthan gum in 2 % and 3 %, respectively. The prolongation of the release rate from the sodium alginate hydrogel beads with an increase of polymer concentration reflects the simultaneous increase in gel strength which is a defining factor in this case. Since the drugs in beads matrices were dissolute through the pores of the polymers network, increasing the polymer concentration significantly reduces the size of the pores, thus in turn prolonging the drug dissolution [32].

3.1.6. *In vitro* release kinetic study. The *in vitro* release data of GF were fitted to zero, first-order, Higuchi diffusion and Korsmeyer-Peppas kinetic models as presented in Table 4. The best fit, with the highest determination coefficients (R^2) of GF formulations was achieved with Higuchi diffusion model followed by first-order model for F1 and F5. While the best fit with the highest determination coefficients (R^2) was shown with first-order model for F2, F3, F4, F6, F7 and F8. Higuchi diffusion model describes drug release from polymeric system by diffusion mechanism and non-Fickian release behavior ($n>0.5$).

3.1.7. Stability Study. GF beads formula (F5) was subjected to stability study at 25 and 40 ˚C with relative humidity (RH) of 75 % for a period of 12 and 3 months, respectively. After storage conditions, beads were analyzed for any change in the physical appearance and drug content. Storage of F5 at 40 ± 2 ˚C with RH of 75 ± 2 % resulted in appreciable changes in the physical properties and drug content. After 3 months of storage the color was darken, texture became sticky, and the drug content was 81.63 ± 0.14 %. The long term stability study of F5 at 25 ˚C with relative humidity (RH) of 75 % for 12 months did not result in a significant change in the physical appearance and the drug content was 91.39 ± 0.22 %. It was concluded that, GF beads were sensitive to high temperature and humidity and should be stored at ambient temperature.

3.2. Evaluation of GF floating *in situ* gels.

3.2.1. Scanning electron microscope (SEM). SEM examination of F5 and F9 GF floating *in situ* gels showed that G5 had more crystalline forms ranging from smaller to medium sizes as compared to G9 that has fewer crystalline forms (Fig. 6).

3.2.2. *In vitro* GF *in situ* gels floating study. The *in vitro* floating of the prepared formulations was evaluated in the buffered solution (pH, 1.2). The time needed for the formulation to emerge on the medium surface (floating lag time) and the time the formulation constantly floated on the dissolution medium surface (duration of floating) were determined and shown in Table 5. Upon contact with an acidic medium, the gelation and cross-linking by Ca^{++} ions occurred to provide gelation at the surface of the formulation. The calcium carbonate effervesced when come in contact to an acidic medium and releases carbon dioxide and calcium ions. The released carbon dioxide is entrapped in the gel network producing floating formulation. Then the calcium ion reacts with alginate producing a cross-linked three dimensional gel network that might restrict the further diffusion of carbon dioxide and led to the prolongation in the duration of drug floating and drug dissolution, respectively [33].

It was noticed that as the concentration of calcium carbonate increased from 1.5 % to 2 % (W/V), the floating lag time reduced from 22 $\pm$ 2 s (G1) to 19 $\pm$ 1 s (G2). Furthermore, the duration of GF floating *in situ* gels increased from 24 h to more than 48 h as the concentration of polymer increased from 1.5 % to 2.5 % (W/V). The same observation was obtained by increasing the concentration of calcium carbonate from 1.5 % to 2 % in G3, G4, G5 and G6, respectively. This is in agreement with the previously reported results that shown the floating capability were dependent on the calcium carbonate and polymer concentration [34].

3.2.3. Density of GF floating *in situ* gels. All formulae have density less than 1.004 gm/cm³ and the density ranged from 0.86 ± 0.02 gm/cm^3 to 0.97 ± 0.03 gm/cm^3 for G8 and G3, respectively (Table 5).

3.2.4. *In vitro* gelling capacity. Table 5 represents the gelling capacity of GF floating *in situ* gels. It was observed that the gelling lag time varied from 1 s to 4 s for G9 and G1, respectively. As the concentration of the polymer increased, the gelling lag time decreased while the gelling capacity increased. The floating *in situ* gel containing the highest concentration of polymer (2.5 % sodium alginate and 0.5 % HPMC) G9 had the lowest gelation time (1 s), whereas G1 containing the lowest polymer concentration had the highest gelation time (4s). This was attributed to the increase in crosslinking degree with the increase of polymer concentration.

3.2.5. Viscosity study. The flow behavior of all GF *in situ* gel formulations are presented in Figs. 7 and 8. It is clear that, all the prepared GF floating *in situ* gels exhibited non Newtonian pseudoplastic flow behavior (shear thinning systems) at 34 ±0.5 ^{0}C. As the shear rate was increased, the normally molecular structure of the gelling material is caused to align its long axes in the direction of a flow which in turns reduces the internal resistance of the material and hence decreases its viscosity. From

the figures it was observed that the viscosity increased from 480.3 ± 2.8 to 686.4 ± 3.9 (mPas) for G1 and G5, respectively, with an increase of sodium alginate concentration from 1.5 % (W/V) G1 to 2.5 % (W/V) G5 at shear rate of 100 s^{-1}. Furthermore, the viscosity was significantly increased to 1398 ± 4 as 0.5 % of HPMC was added to 2.5 % of sodium alginate (G9). The same observations were obtained for F1 and F7 using 1.5 % of sodium alginate and for F3 and F8 using 2 % of sodium alginate. This results was significantly ($P < 0.05$) obtained for sheer rate ranged from 100 to 500 S^{-1}, while the difference was not significant ($P > 0.05$) for sheer rate from 600 to 800 s^{-1}. This is in agreement with the previously results obtained [25].

In the formulae G3 and G4 containing 2 % of sodium alginate; but with different concentrations of calcium carbonate; 1.5 % and 2 %, respectively. It was evident that the viscosity of the formulae significantly ($P < 0.05$) increased from 546.3 ± 7.1 to 655.7 ± 4.4 (mPas) at shear rate 100 s^{-1}, respectively. This observation was also obtained for the other shear rates used. This change in viscosity may be due to that the increase in the amount of dispersed calcium carbonate led to an increase in the number of particles dispersed, thus contributing to increased viscosity. These results were also obtained from the other formulae (G1, G2, G5, and G6). The results are in agreement with previously data obtained [27].

3.2.6. *In vitro* GF floating *in situ* gel release study. The drug release pattern was studied for all floating *in situ* gel formulae for 8 h and the results are provided in Figs. 9 and 10. The rate of release of GF from its powder was very poor, approximately 27.3 ± 0.81 % released within 8 h relative to the release rate of the drug from all floating *in situ* gels. The rate and the extent of GF release was markedly enhanced from the floating *in situ* gel delivery systems. This may be attributed to that floating *in situ* gels change the properties of GF particles by simply dispersing the drug particles in the gel, which increase the wetting properties of drug particles, and subsequently may improve its release and bioavailability.

The release of GF from floating *in situ* gels was affected by the polymer concentration. As the concentration of sodium alginate increased from 1.5 % to 2.5% (W/V), there was a significant ($P<0.05$) decrease ($P<0.05$) in the rate and extent of drug released from *in situ* gels. It was observed that the percentage of GF released from G1 and G5 containing 1.5 % and 2.5 % of sodium alginate concentration were 65.06±4.47 and 55.12 ± 0.98, respectively. The same observation was obtained for G2 and G6. This could be attributed to the increase in polymer concentration is accompanied by the increase in the density of the polymer matrix and the diffusional path length, where the drug molecules have to traverse, is also increased.

Furthermore, it was observed that there was a non significant ($P>0.05$) increase in the rate and extent of the drug released with an increase in the concentration of calcium carbonate as shown in Figs. 9 and 10. These observations were obtained for Formulae G1 and G2, containing 1.5 % of sodium alginate with 1.5 % and 2 %, respectively of calcium carbonate; G3 and G4, containing 2 % of sodium alginate with 1.5 % and 2 %, respectively of calcium carbonate and different concentrations; and G5 and G6, containing 2.5 % of sodium alginate and 1.5 % and 2 % of calcium carbonate, respectively.

The effect of concentration of HPMC (0.5 %) as gelling polymer on the *in vitro* drug released from *in situ* gels was also studied. A significant decrease in rate and extent of drug released was obtained using 0.5 % HPMC as shown in G7, G8 and G9 in the comparison with G1, G3 and G5 without HPMC. The percentage of GF released after 8 h from formulae G5 and G9 was 55.12 ± 1.46 % and 44.08 ± 3.42 %, respectively. The same observation was obtained for G1 and G7; G3 and G8.This may be due to the increase in the viscosity and a decrease in the lag time to form stable *in situ* gels. The *in vitro* drug release for all GF *in situ* gels showed sustained drug release for a long period of time of the experiment (8h).

3.2.7. Kinetic study. The best fit with the highest determination coefficient (R^2) for all *in situ* gel formulations was shown with Higuchi model (Table 6). These results revealed that the release pattern was best fitted to Higuchi model which describes drug release from a polymeric system by diffusion mechanism. Fickian diffusion mechanism of release ($n<0.5$) was the drug release controlling mechanism for all formulae.

Table 1. Composition of different GF floating beads (% W/V).

Formulae Code	GF	Polymer conc.	Sodium alginate	HPMC	Carbopol	Xanthan gum	Pectin	Calcium chloride	Light liquid paraffin
F1	0.2	2	1.333	0.667	-	-	-	2	10
F2	0.2	2	1.333	-	0.667	-	-	2	10
F3	0.2	2	1.333	-	-	0.667	-	2	10
F4	0.2	2	1.333	-	-	-	0.667	2	10
F5	0.2	3	2.000	1	-	-	-	2	10
F6	0.2	3	2.000	-	1	-	-	2	10
F7	0.2	3	2.000	-	-	1	-	2	10
F8	0.2	3	2.000	-	-	-	1	2	10

Table 2. Composition of different GF floating *in situ* gels (% W/V).

Formulae code	GF	Sodium alginate	$CaCo_3$	HPMC	$CaCl_2$	Na citrate	Tween80 (% V/V)
G1	0.5	1.5	1.5	-	0.075	0.25	0.5
G2	0.5	1.5	2.0	-	0.075	0.25	0.5

G3	0.5	2.0	1.5	-	0.075	0.25	0.5
G4	0.5	2.0	2.0	-	0.075	0.25	0.5
G5	0.5	2.5	1.5	-	0.075	0.25	0.5
G6	0.5	2.5	2.0	-	0.075	0.25	0.5
G7	0.5	1.5	1.5	0.5	0.075	0.25	0.5
G8	0.5	2.0	1.5	0.5	0.075	0.25	0.5
G9	0.5	2.5	1.5	0.5	0.075	0.25	0.5

Table 3. Physicochemical characterization of different GF floating beads (mean ± SD, n=3).

Formulae code	Yield (%)	Drug encapsulation efficiency (%)	Density (g/cm³)	In vitro floating lag time (S)	Particle size (µm)
F1	86.48 ± 2.1	89.06 ± 0.79	0.63±0.3	9.0±1.0	1702 ± 19.90
F2	85.01 ± 1.5	81.97 ± 0.43	0.63±0.2	7.5±0.5	1862 ± 10.13
F3	86.83 ± 2.0	78.60 ± 1.50	0.60±0.4	8.0±1.0	1788 ± 22.60
F4	79.88 ± 2.3	65.97 ± 0.82	0.45±0.2	6.5±0.5	1724 ± 13.50
F5	97.73 ± 2.5	93.50 ± 0.41	0.76±0.3	18.0±2.0	1995 ± 18.40
F6	85.98 ± 1.7	91.48 ± 2.59	0.75±0.1	11.5±1.5	1926 ± 14.42
F7	98.57 ± 1.3	79.48 ± 0.95	0.67±0.4	12.0±2.0	1866 ± 16.80
F8	88.10 ± 1.8	77.26 ± 0.96	0.54±0.2	10.5±0.5	1947 ± 15.60

Table 4. The kinetic parameters of GF floating beads release data according to different kinetic models.

Formula Code	Zero-order		First-order		Higuchi		Korsmeyer - Peppas	
	R^2	K_0 (%min^{-1})	R^2	K_1 (min^{-1})	R^2	K (%min^{-1})	R^2	n
F1	0.868	0.111	0.936	0.001	0.981	2.031	0.991	0.592
F2	0.926	0.105	0.973	0.001	0.950	1.895	0.986	0.688
F3	0.954	0.093	0.983	0.001	0.929	1.669	0.986	0.754
F4	0.928	0.101	0.973	0.001	0.955	1.829	0.989	0.685
F5	0.906	0.102	0.953	0.001	0.970	1.856	0.992	0.639
F6	0.922	0.096	0.966	0.001	0.956	1.740	0.989	0.675
F7	0.969	0.086	0.989	0.001	0.916	1.537	0.989	0.800
F8	0.960	0.088	0.980	0.001	0.921	1.564	0.985	0.778

Table 5. Floating behavior, density and gelling capacity of GF floating *in situ* gels (mean ± SD).

Formulae code	In vitro floating lag time (s)	In vitro floating duration (h)	Density (gm/cm³)	In vitro gelation lag time (s)	In vitro gelation capacity *
G1	22 ± 2	> 24	0.93 ± 0.01	4 ± 1	+++
G2	19 ± 1	> 24	0.91 ± 0.02	4 ± 1	+++
G3	20 ± 2	> 24	0.97 ± 0.03	4 ± 2	+++
G4	17 ± 2	48	0.91 ± 0.01	3 ± 1	++++
G5	18 ± 2	> 48	0.96 ± 0.01	2 ± 1	++++
G6	15 ± 2	> 48	0.90 ± 0.02	2 ± 2	++++
G7	16 ± 1	> 48	0.91 ± 0.01	2 ± 1	++++
G8	15 ± 3	> 48	0.86 ± 0.02	1 ± 1	++++
G9	12 ± 1	> 72	0.88 ± 0.01	1 ± 1	++++

*+++ change during 24 h, ++++ not change during 24 h.

Table 6. The kinetic parameters of GF *in situ* gels release data according to different kinetic models.

Formula Code	Zero-order		First-order		Higuchi		Korsmeyer -Peppas	
	R^2	K_0 (% min^{-1})	R^2	K_1 (min^{-1})	R^2	K (% min^{-1})	R^2	n
G1	0.640	0.156	0.828	0.003	0.984	2.944	0.993	0.436
G2	0.570	0.169	0.826	0.003	0.981	3.168	0.997	0.413
G3	0.622	0.142	0.815	0.002	0.988	2.660	0.997	0.434
G4	0.677	0.143	0.852	0.002	0.994	2.673	0.998	0.458
G5	0.563	0.140	0.770	0.002	0.980	2.630	0.997	0.411
G6	0.640	0.139	0.820	0.002	0.990	2.593	0.997	0.441
G7	0.687	0.125	0.834	0.002	0.995	2.338	0.998	0.461
G8	0.654	0.122	0.807	0.002	0.992	2.278	0.998	0.447
G9	0.640	0.112	0.783	0.002	0.991	2.097	0.997	0.441

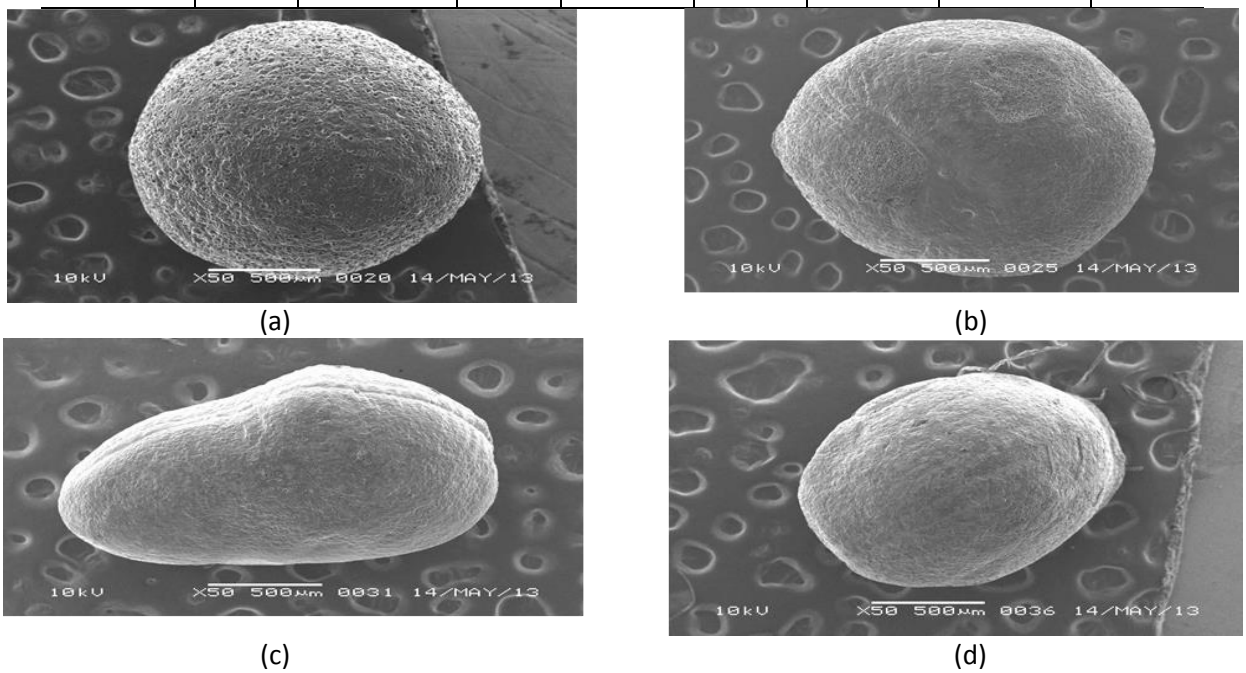


Figure 1. Scanning electron microphotographs of GF floating beads, F1 (a), F2 (b), F 3 (c) and F4 (d).

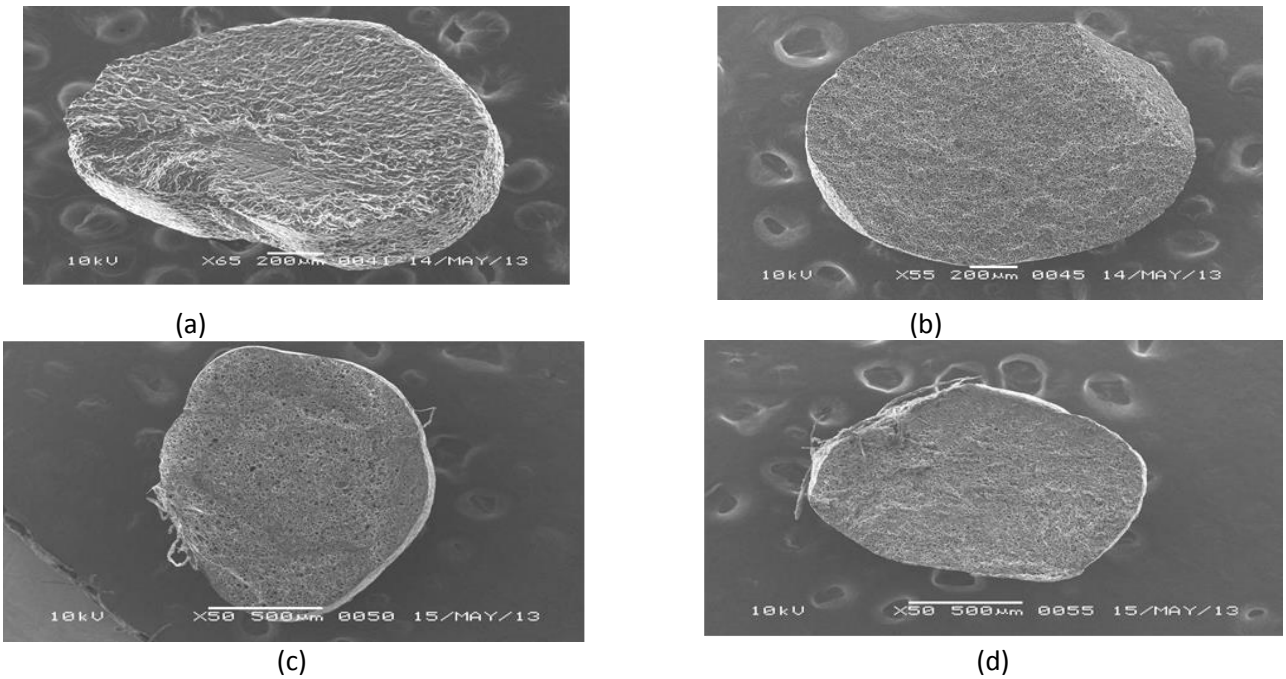


Figure 2. SEM (cross-sectional photographs) of GF floating beads, F1 (a), F2 (b), F 3 (c) and F4 (d).

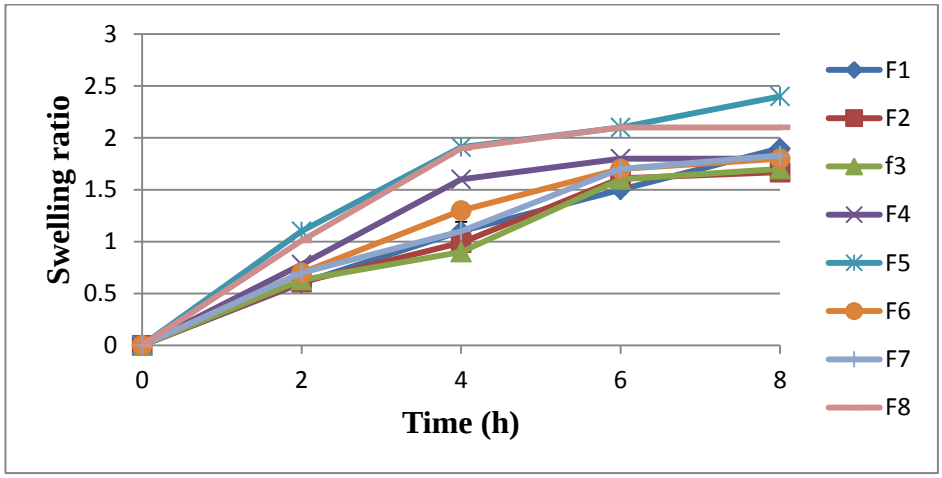


Figure 3. Swelling ratios of GF floating beads in the buffered solution (pH, 1.2), n=3, mean ± SD.

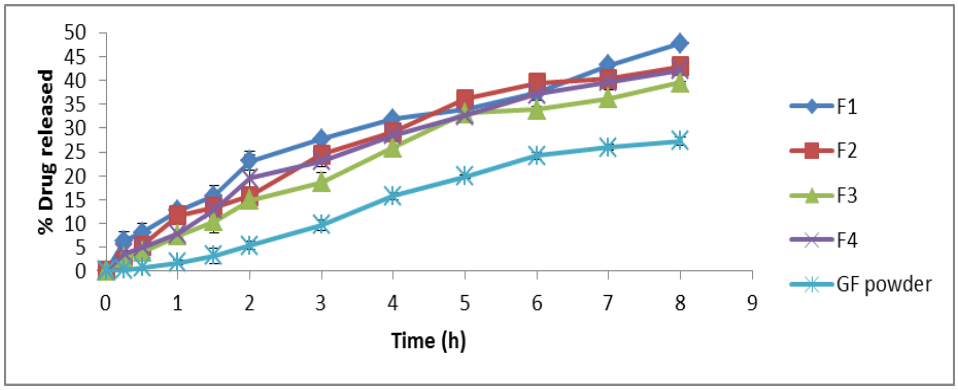
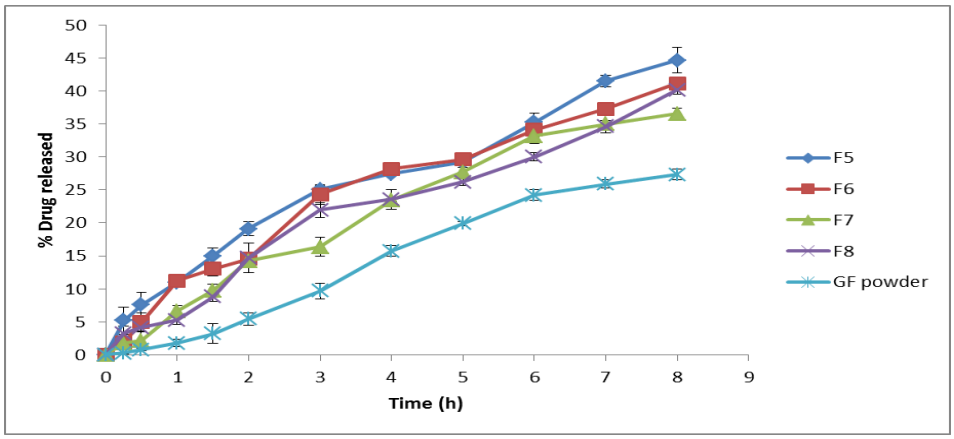


Figure 4. In vitro release profiles of GF (mean ±SD, n=3) from different floating beads and GF powder in buffered solution (pH, 1.2).



Figures 5. In vitro release profiles of GF (mean ±SD, n=3) from different floating beads, and GF powder in buffered solution (pH 1.2).

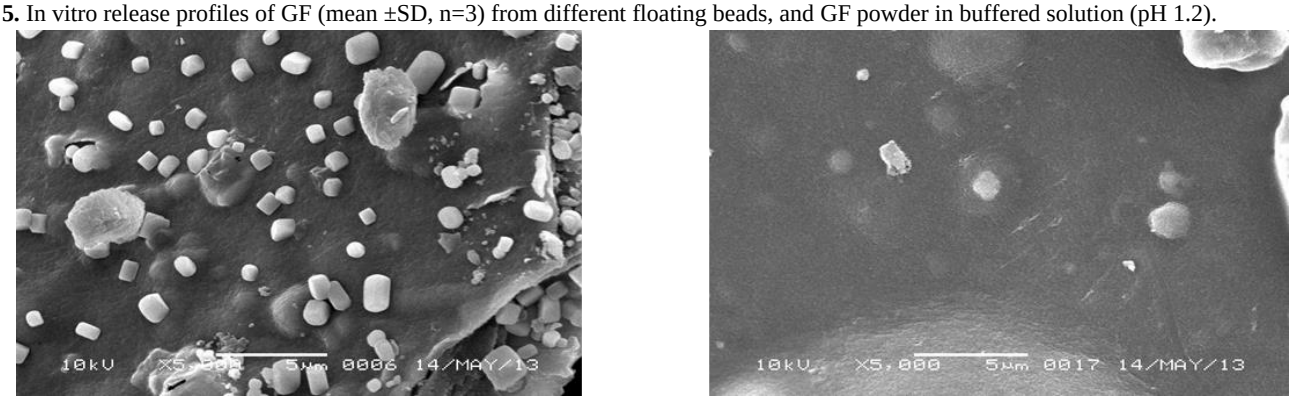


Figure 6. SEM of GF floating in situ gels, G5 (A) and G9 (B).

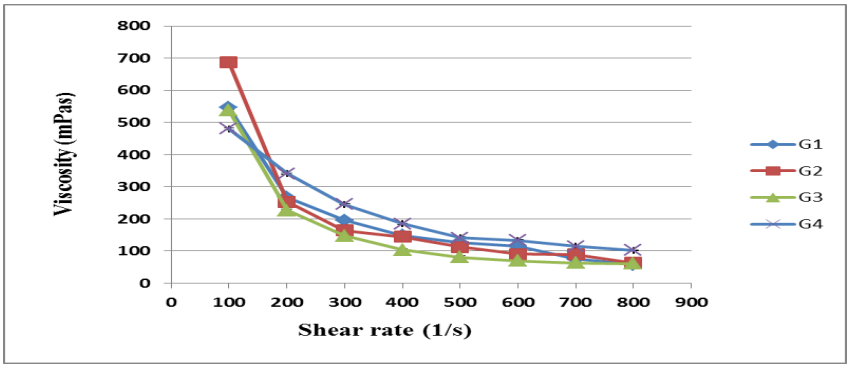


Figure 7. Viscosity-shear rate profiles of different GF floating *in situ* gels at 30 ± 0.5 °C (n=2, mean ± SD).

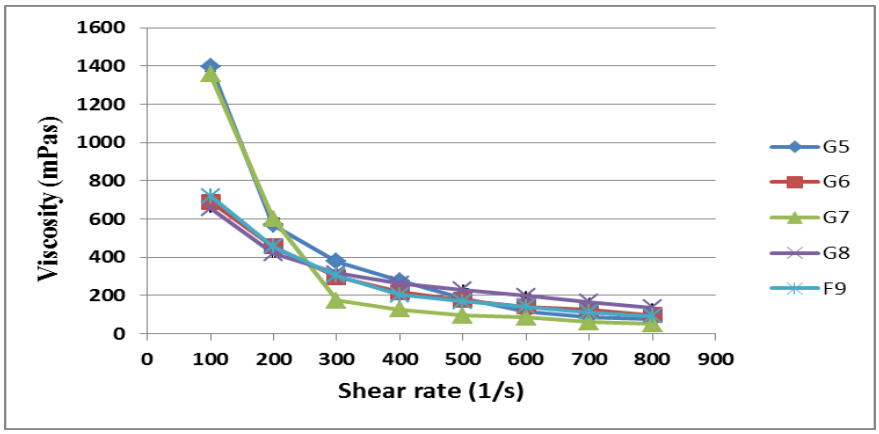


Figure 8. Viscosity-shear rate profiles of different GF floating in situ gels at 30 ± 0.5 °C (n=2, mean + SD).

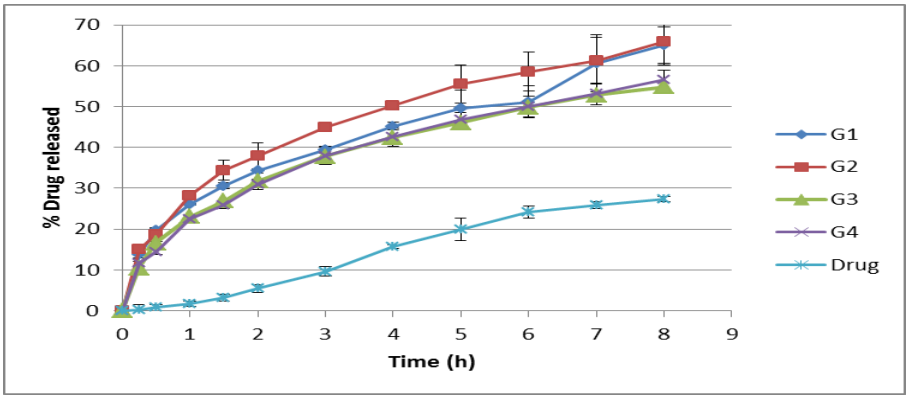


Figure 9 . *In vitro* release profiles of GF (mean ± SD, n=3) from different floating *in situ* gels and GF powder in buffered solution (pH, 1.2).

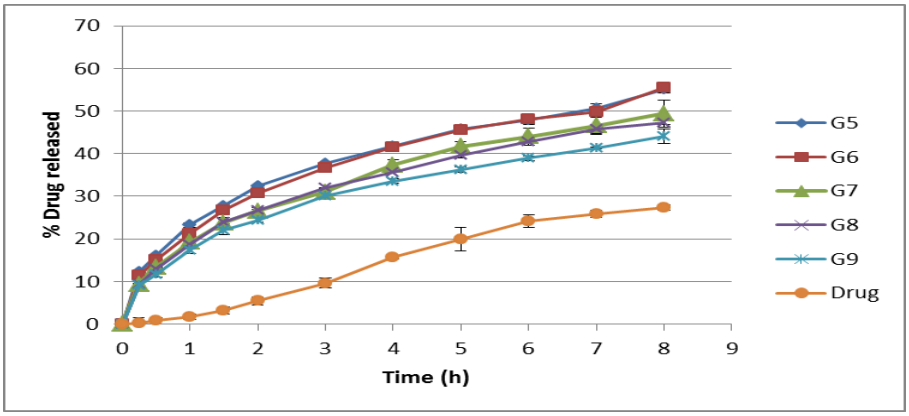


Figure10. In vitro release profiles of GF (mean ± SD, n=3) from different floating in situ gels and GF powder in buffered solution (pH, 1.2).

4. CONCLUSIONS

The present study aims to formulate gastroretentive floating beads and floating *in situ* gels of ultramicronized GF in order to enhance the dissolution rate and hence bioavailability of the drug. The results showed good physicochemical properties as floating, encapsulation efficiency, density, gelation and viscosity. Moreover, the dissolution rate of GF from floating beads and

floating *in situ* gels was significantly enhanced in the comparison with its dissolution rate from powder.

5. REFERENCES

[1]. Dixit N., Floating drug delivery system, *Journal of Current Pharmaceutical Research*, 7, 1, 6-20, **2011**.

[2]. Badoni A., Ojha A., Gnanarajan G, Kothiyal P., Review on gastro retentive drug delivery system, *The Pharma Innovation*, 1 , 8, 32-42, **2012**.

[3]. Singh B.N., Kim K.H., Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention, *Journal of Controlled Release*, 63, 3, 235-259, **2000**.

[4]. Yellanki S.K., Nerella N.K., Stomach specific drug delivery of riboflavin using floating alginate beads, *International Journal of Pharmacy and Pharmaceutical Sciences*, 2, 160-163, **2010**.

[5]. Whitehead L., Collett J.H, Fell J.T., Amoxycillin release from a floating dosage form based on alginates, I*nternational Journal of Pharmaceutics*, 210, 1-2, 45-49, **2000**.

[6]. Iannuccelli V., Coppi G., Leo E., Fontana F., Bernabei M.T., PVP solid dispersions for the controlled release of furosemide from a floating multiple-unit system, *Drug Development and Industrial Pharmacy*, 26, 6, 595-603, **2000**.

[7]. Sharma S., Pawar A., Low density multiparticulate system for pulsatile release of meloxicam, *International Journal of Pharmaceutics*, 313, 1-2, 150-158, **2006**.

[8]. Abd-Elmageed A., Preparation and evaluation of sulindac alginate beads, *Bulletin of Pharmaceutical Sciences Assiut University*, 22, 73-80, **1999.**

[9]. Mirghani A., Idkaidek N.M., Salem M.T.S., Najib N.M., Formulation and release behavior of diclofenac sodium in compritol 888 matrix beads encapsulated in alginate, *Drug Development and Industrial Pharmacy*, 26, 7, 791-795, **2000**.

[10]. Fathy M., Safwat S., El-Shanawany S., Tous S.S., Otagiri M., Preparation and evaluation of beads made of different calcium alginate compositions for oral sustained release of tiaramide, *Pharmaceutical Development and Technology*, 3, 3, 355-364, **1998**.

[11]. Torre M.L., Giunchedi P., Maggi L., Stefli R., Machiste E.O., Conte U., Formulation and characterization of calcium alginate beads containing ampicillin, *Pharmaceutical Development and Technology*, 3, 2, 193-198, **1998**.

[12]. Khan A.D., Bajpai M., Formulation and evaluation of floating beads of verapamil hydrochloride, *International Journal of PharmTech Research*, 3, 3, 1537-1546, **2011**.

[13]. Kumar A., Verma R., Purohit S., Bhandari A., Overview of gastro-retentive drug delivery system, *Journal of National Conscientia*, 2, 423-436, **2011**.

[14]. Shah J., In situ gel: A novel approach of gastroretentive drug delivery, *Asian Journal of Biomedical and Pharmaceutical Sciences*, 2, 8, 1-8, **2012**.

[15]. Ibrahim H.K. A novel liquid effervescent floating delivery system for sustained drug delivery, *Drug Discoveries and Therapeutics*, 3, 4, 168-175, **2009**.

[16]. Chaumeil J., Micronization: a method of improving the bioavailability of poorly soluble drugs, *Methods and Findings in Experimental and Clinical Pharmacology*, 20, 3, 211-216, **1998**.

[17]. Dhanaraju M., Kumaran K.S, Baskaran T., Moorthy M.S.R., Enhancement of bioavailability of griseofulvin by its complexation with β-cyclodextrin, *Drug Development and Industrial Pharmacy*, 24, 6, 583-587, **1998**.

[18]. Trotta M., Gallarate M., Carlotti M.E., Morel S., Preparation of griseofulvin nanoparticles from water-dilutable microemulsions, I*nternational Journal of Pharmaceutics*, 254, 2, 235-242, **2003**.

[19]. Trona M., Cavalli R., Chirio D., Griseofulvin nanosuspensions from triacetin-in-water emulsions, ***STP Pharma Sciences***, 13, 6, 423-426, **2003**.

[20]. Elkordy A.A., Essa E.A., Dhuppad S., Jammigumpula P., Liquisolid technique to enhance and to sustain griseofulvin dissolution: effect of choice of non-volatile liquid vehicles, I*nternational Journal of Pharmaceutics*, 434, 1-2, 122-132, **2012**.

[21]. Prasad S.K., Mukesh T., Ashok S., Monit S., Ashish S., Preparation and optimization of oral floating alginate gel beads of famotidine, *International Journal of Pharmaceutical Studies and Research*, 3, 2, 4-8, **2012**.

[22]. Ravindra K., Sabitha P., Effect of different co-polymers on sodium alginate microcapsules containing isoniazid. *International Journal of PharmTech Research*, 2, 4, 2198-2203, **2010**.

[23]. Soni S., Oil entrapped floating multiparticulate system of ciprofloxacin using sodium alginate by emulsion gelation technique, *Journal of Drug Delivery and Therapeutics*, 2, 5, 50-54, **2012**.

[24]. Siepmann J., Peppas N., Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC), *Advanced Drug Delivery Reviews*, 48, 2-3, 139-157, **2001**.

[25]. Patel R.P., Ladani R., Dadhaniya B., Prajapati B., In situ gel delivery systems for chlordiazapoxide using artificial neural network. *International Journal of Pharmaceutical Health and Sciences*, 1, 1, 10-22, **2010**.

[26]. Rajinikanth P., Balasubramaniam J., Mishra B., Development and evaluation of a novel floating in situ gelling system of amoxicillin for eradication of Helicobacter pylori, I*nternational Journal of Pharmaceutics*, 335, 1-2, 114-122, **2007**.

[27]. Gulecha B., Shahi S., Lahoti S., Floating in situ gelling drug delivery system of verapamil hydrochloride. *American Journal of PharmTech Research*, 2, 4, 954-969, **2012**.

[28]. Jayswal B.D., Yadav V.T., Patel K.N., Patel B.A., Patel P.A., Formulation and evaluation of floating in situ gel based gastro retentive drug delivery of cimetidine, International Journal of Pharmmceutical Research Scholars, 1, 2, 327-337, **2012**.

[29]. Amal El Sayeh F.A., Hassan M.A., El-Maraghy D.A., Ketorolac tromethamine floating beads for oral application: characterization and in vitro/in vivo evaluation, *Saudi Pharmaceutical Journal*, 2, 4, 349-359, **2014**.

[30]. Kulkarni R., Biswanath S., Electrically responsive smart hydrogels in drug delivery: a review, *Journal of Applied Biomaterials and Biomechanics*, 5, 3, 125-139, **2006.**

[31]. Borkar S., Suresh R., Sawant V., Shende V., Mokale V., Dama G., An approach to formulate bilayered gastroretentive floating drug delivery system of cefpodoxime proxetil, *International Journal of ChemTech Research*, 2, 2, 1229-1242, **2010**.

[32]. Ghareeb M.M., Issa A.A., Hussein A.A., Preparation and characterization of cinnarizine floating oil entrapped calcium alginate beads, *International Journal of Pharmaceutical Sciences and Research*, 3, 501-508, **2012**.

[33]. Miyazaki S., Aoyama H., Kawasaki N., Kubo W., Attwood D., In situ-gelling gellan formulations as vehicles for oral drug delivery, *Journal of Controlled Release*, 60, 2-3, 287-295, **1999**.

[34]. Rajinikanth P., Mishra B., Floating in situ gelling system for stomach site-specific delivery of clarithromycin to eradicate H. pylori, *Journal of Controlled Release*, 125, 1, 33-41, **2008**.

6. ACKNOWLEDGEMENTS

This research project was supported by a Grant from the''Research Center for Female Scientific and Medical Colleges'' Deanship of Scientific Research, King Saud University.