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Controlling Release of Metformin HCl through Incorporation into Stomach Specific Floating Alginate Beads

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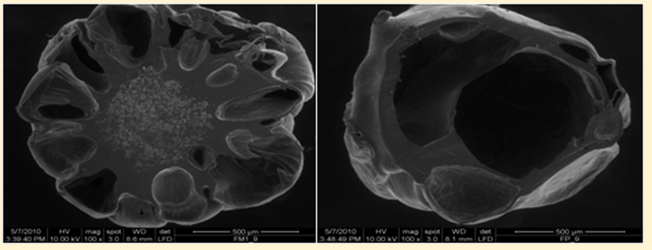
ABSTRACT: The aim of present study was to develop stomach specific floating beads of metformin hydrochloride for effective management of type 2 diabetes mellitus. The beads were evaluated for surface morphology, particle size, tapped density, true density, percent porosity, drug entrapment efficiency, percent yield, differential scanning calorimetry, *in vitro* floating ability and *in vitro* drug release. Stability studies were performed at 25 and 40 °C up to 45 days. Effectiveness of the formulations was evaluated *in vivo* by hypoglycemic response in both normal and diabetic albino rats. The beads were grossly spherical in shape, and average particle diameter of beads was found to be in the size range of 861.34 to 991.75 μm. Percent entrapment was found to be in the range of 77.61 to 82.48%. Beads demonstrated favorable *in vitro* floating ability. All the formulations followed a non-Fickian release mechanism. It was found that there was no significant effect on floating ability of aged beads since it floated up to an 8 h study period. *In vivo* studies on diabetic rats showed that the hypoglycemic effect induced by the metformin hydrochloride loaded alginate beads was significantly greater ($P < 0.05$) and more prolonged than that induced by the nonfloating beads. The results clearly demonstrated the ability of the formulation to maintain blood glucose level and improved the patient compliance by enhancing, controlling and prolonging the systemic absorption of metformin hydrochloride.



KEYWORDS: metformin hydrochloride, floating drug delivery, beads, aging effect, hypoglycemic activity

INTRODUCTION

Floating drug delivery systems, also known as hydrodynamically balanced systems, have been employed successfully to retain the drug in the stomach as single- or multiple-unit systems. Multiple-unit systems are advantageous due to lower chances of dose dumping and less intersubject variability. Further, they afford the possibility of a longer-lasting and more reliable release of the drug from the dosage form.[1] Jain et al.[2−4] has discussed *in vitro* and *in vivo* characterization of calcium silicate-based floating microspheres of repaglinide and orlistat. They have also reported preparation and evaluation of calcium silicate-based floating granular delivery system of repaglinide and ranitidine hydrochloride.[5,6] Our group demonstrated previously beads of Gelucire 43/01 for floating delivery of metformin hydrochloride.[7] The prepared formulations showed better controlled release behavior when compared with its conventional dosage form and comparable release profile with marketed sustained release product.

A plethora of antidiabetic drugs are used in the clinic, of which metformin hydrochloride (MH) is a very widely used and accepted drug. Unlike other antidiabetics, MH does not induce hypoglycemia at any reasonable dose, and hence it is usually called an antihyperglycemic (or euglycemic) rather than a hypoglycemic drug.[8] In spite of its favorable clinical response and lack of significant drawbacks, chronic therapy with MH suffers from certain specific problems, the most prominent being short biological half-life (1.5−3 h),[9] high dose (0.5−3.0 g/day), low bioavailability (50−60%) and high incidence of gastrointestinal (GI) side effects (30% cases).[8] Also the main site of its absorption is reported to be proximal small intestine.[10] Thus, the development of controlled-release dosage forms of MH in the form of a gastroretentive system would clearly be advantageous.

The aim of the present study was to develop a gastroretentive multiparticulate delivery system of MH for effective management of type 2 diabetes mellitus. To achieve this objective, floating beads of MH were prepared and characterized. Moreover, the hypoglycemic effect of prepared beads on normal and diabetic rats was investigated and compared with that of nonfloating beads.

MATERIALS AND METHODS

Materials. Metformin HCl was procured as gift sample from M/s Cadila Pharmaceuticals Pvt. Ltd., SIDCO Samba, Jammu and Kashmir, India. Hydroxypropyl methylcellulose (HPMC) and streptozotocin were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Calcium carbonate and calcium chloride were purchased from Qualigens Fine Chemicals, Mumbai, India. Sodium alginate and glacial acetic acid were purchased from S. D. Fine-Chem. Ltd., Mumbai, India. All other chemicals used were of analytical reagent grade.

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Preparation of Floating and Nonfloating Alginate Beads. Beads were prepared according to the procedure described by Choi et al.,[11] with minor modifications. Briefly, drug solution was prepared by taking 200 mg of drug and dissolving it in 5 mL of distilled water. This drug solution was mixed with previously prepared alginate solution (2% w/v) containing 30% HPMC with constant agitation for 2−3 h on a magnetic stirrer (Remi, India). Gas-generating agent, i.e. calcium carbonate, was dispersed in the solution, and mixing was continued until thorough dispersion was achieved. The resulting drug−polymer solution was added dropwise to the calcium chloride solution (2% w/v $CaCl_2$ in 5% v/v glacial acetic acid) using a syringe fitted with a needle (23 G, internal diameter 0.7 mm). The gelled beads were allowed to cure for 15 min with constant stirring. Beads were collected by filtration, washed with distilled water, and dried in desiccators over fused calcium chloride at 25 °C for 48 h.

Nonfloating alginate beads were prepared by same method described above except using gas generating agent, i.e. calcium carbonate and HPMC.

Drug Entrapment Efficiency (DEE) and % Yield. Entrapment efficiency was calculated by a method suggested by Basu and Rajendran,[12] with minor modifications. An accurately weighed amount (20 mg) of drug loaded beads was pulverized in a clean and dry glass mortar and incubated in 20 mL of simulated gastric fluid (SGF, pH 2.0, KCl/HCl buffer) at room temperature for 24 h for complete extraction of drug. The suspension was centrifuged (Remi, India) at 5000 rpm for 15 min. The clear supernatant solution was assayed spectrophotometrically (UV-1800 spectrophotometer, Shimadzu, Japan) for drug content at 233 nm after suitable dilution. All the experiments were performed in triplicate, and entrapment efficiency was calculated using the following formula:

$$\text{DEE}\ (\%) = \frac{L}{L_o} \times 100$$

where L is the actual drug content in the weighed quantity of the beads and L_o is the theoretical drug content in the weighed quantity of the beads.

The prepared beads were collected by filtration, dried in desiccators and weighed. The measured weight was divided by the total amount of all nonvolatile components, which were used for the preparation of the beads.[7] Percentage yield was calculated using following formula:

$$\%\ \text{yield} = \frac{\text{weight of beads collected}}{\text{weight of all nonvolatile components used in the preparation}} \times 100$$

Surface Morphology. The external and internal morphology of the selected formulations of floating and nonfloating beads was studied by scanning electron microscopy (SEM) (FEI Quanta-200 MK2, The Netherlands). The sample for SEM was prepared by sticking the beads on a piece of double adhesive tape, which was previously stuck to an aluminum stub. Then the samples were kept inside the vacuum chamber and randomly scanned, and photomicrographs were taken.

Micromeritic Studies. The average particle size of beads was determined with a micrometer (Mittotuyo Micrometer, NSK Co., Japan) and calculated as the average value of size of 100 beads. The beads were also characterized for tapped density (P_p), true density (P_t), and percent porosity. Tapped density of beads was determined using tapped density apparatus. Measuring cylinder was tapped until concurrent results in terms of volume obtained.

$$\text{tapped density} = \frac{\text{mass of beads}}{\text{volume of beads aftertapping}}$$

True density was determined using a benzene displacement method. Percent porosity (P) was calculated using the equation

$$P\ (\%) = \left[1 - \frac{P_p}{P_t}\right] \times 100$$

where P_t and P_p are the true density and tapped density, respectively.

Differential Scanning Calorimetry (DSC). The DSC analysis of pure drug, individual polymers, placebo and drug loaded formulations were carried out using a Diamond DSC (Perkin-Elmer, USA). Samples of 2−6 mg were placed in aluminum pans (Al-Crucibles, 40 Al) and sealed. The probe was heated from 30 to 270 °C at 10 °C/min under nitrogen atmosphere.

***In Vitro* Buoyancy Test.** The floating lag time and the duration of floating of the fabricated beads were determined in a rotating six paddle apparatus (dissolution rate test apparatus USP/IP/BP STD, Jyoti Scientific, India).[13] A known number of beads were placed on 900 mL of SGF (pH 2.0) with 0.02% w/v Tween 80. The paddle speed was maintained at 75 rpm, with temperature of the medium maintained at 37 ± 0.2 °C. At preidentified time intervals, the number of beads remaining buoyant was determined by visual inspection. Percentage buoyancy was calculated using the following formula:

$$\text{buoyancy}\ (\%) = \left[\frac{N_f}{N_f + N_s}\right] \times 100$$

where N_f and N_s are the number of floating and settled beads, respectively. The determinations were carried out three times to establish the reproducibility of the results.

***In Vitro* Drug Release Study.** Dissolution of the beads of each batch was carried out using USP type-II apparatus using a paddle. Nine hundred milliliters of SGF (pH 2.0) containing 0.02% w/v Tween 80 was used as the dissolution medium. The dissolution medium was maintained at 37 ± 0.5 °C. Beads equivalent to 100 mg of drug were placed in the dissolution vessel, and the rotational speed of the paddle was set at 75 rpm. The sample (5 mL) was withdrawn at predetermined time intervals for 8 h, and the same volume of fresh medium was replaced. Samples were filtered using Whatman filter paper (# 41) and then analyzed by using an UV double beam spectrophotometer at 233 nm against blank after suitable dilution. All the experiments were performed in triplicate.

Model Fitting of the Release Study. The *in vitro* release data were treated with five kinetic models, i.e. zero order (eq 1), first order (eq 2), Higuchi-matrix (eq 3), Peppas−Korsmeyer (eq 4) and Hixon−Crowell (eq 5) release equations, to find the equation in which the release data fits best.

$$R = k_1 t \quad (1)$$

$$\log \text{UR} = k_2 t / 2.303 \quad (2)$$

$$R = k_3 t^{0.05} \quad (3)$$

$$R = k_4 t^n \quad (4)$$

$$(\text{UR})^{1/3} = k_5 t \quad (5)$$

Table 1. Batch Specification of the Floating Beads

formulation code	sodium alginate (%)	alginate:calcium carbonate	calcium chloride (%)
FM-1	2	1:0.25	2
FM-2	2	1:0.5	2
FM-3	2	1:0.75	2
FM-4	2	1:1	2
FM-5	2	1:1	3
FM-6	2	1:1	4

where R and UR are the released and unreleased percentages, respectively, at time t; k_1, k_2, k_3, k_4 and k_5 are the rate constants of zero order, first order, Higuchi-matrix, Peppas−Korsmeyer model and Hixon−Crowell, respectively, and n is the release (diffusion) exponent.

Stability Studies. Prepared beads were kept in amber colored glass bottles at two temperatures, i.e. room temperature (25 °C) and accelerated temperature (40 °C), for 45 days and then were characterized for their morphology, residual drug content, *in vitro* floating ability and *in vitro* drug release.

***In Vivo* Study.** In the present work, *in vivo* study of the developed drug delivery system was performed on male albino rats (Sprague−Dawley strain) in an animal house of RKDF College of Pharmacy, Bhopal (MP) (Reg. No. 780/CPCSEA, Sept. 2006), after approval by the Institutional Animal Ethical Committee (IAEC). Rats weighing 250−300 g and age 7−8 weeks were included in this study. The animals were housed under standard conditions with free access to water and food (regular rat chow), with exception of food deprivation during the period of blood sampling throughout the experiment. An experimentally induced model of type-2 diabetes was produced in the albino rats by intraperitoneal injection of streptozotocin (once daily injection of 60 mg/kg body weight for 3 consecutive days) into rats that had been fasted overnight but with access to water *ad libitum*.[14] The degree of diabetes was assessed 4 days later by measurement of blood glucose levels using a glucometer (Accu-Check Active, Roche) and rats with blood glucose level above 300 mg/dL were considered diabetic and were included in the experiments.[15] Rats were randomly divided into six groups of six animals in each group as follows:

group I: diabetic control rats
group II: diabetic rats treated with nonfloating beads containing MH
group III: diabetic rats treated with optimized formulation of floating beads containing MH (FM-4)
group IV: normal control rats
group V: normal rats treated with nonfloating beads containing MH
group VI: normal rats treated with optimized formulation of floating beads containing MH (FM-4)

The control groups (groups I and IV) received only the normal saline solution (0.9% NaCl). Floating and nonfloating beads were administered at a dose equivalent to marketed formulation, i.e. 400 mg/kg.[16] Formulations were administered orally by force-feeding with 1 mL of water via a rubber tube under nonanesthesia. Rats were restrained in a supine position during administration and at each blood sampling. No food or liquid other than water *ad libitum* was allowed during the experimental period. Blood samples (approximately 0.1 mL) were withdrawn each time from the tail vein using an aseptic procedure at different time intervals (0, 1, 2, 3, 4, 5, 6, 7, 8, and 10 h) over the study period. The efficacy of the formulations was estimated by measuring the plasma glucose concentration using clinical glucometer (Accu-Check Active, Roche).

Table 2. Percentage Yield and Drug Entrapment (%) of Different Floating Formulations[a]

formulation code	yield (%)	drug entrapment (%)
FM-1	85.86 ± 0.62	82.48 ± 0.56
FM-2	88.40 ± 0.49	81.03 ± 0.40
FM-3	88.20 ± 0.53	79.67 ± 0.37
FM-4	88.15 ± 0.42	78.84 ± 0.56
FM-5	86.36 ± 0.47	77.61 ± 0.60
FM-6	88.67 ± 0.54	77.72 ± 0.49

[a] Values are average of readings (mean) ± standard deviation (SD) (n = 3).

RESULTS AND DISCUSSION

Preparation of Floating Alginate Beads. In the present investigation, formulations of MH were prepared with alginate in order to improve the gastric retention time of the drug and to provide controlled release. In this method calcium carbonate has been used as a gas-forming agent in order to form pores in the alginate matrix, which helps the beads to float in the gastric environment. Glacial acetic acid was used in the method to evolve CO_2 since the carbonate salts are insoluble at neutral pH; divalent ions were only released in the presence of acid.[11] The method of preparation of the beads was found to be simple, reproducible and economic. Six preparations were prepared using different concentrations of calcium carbonate and calcium chloride and assigned batch codes as FM-1 to FM-6 (Table 1).

Percentage Yield and Drug Entrapment Efficiency. Percentage yield of the prepared formulations were found to be in the range of 85.86 ± 0.62% to 88.67 ± 0.54% (Table 2). It was observed that the values of percentage yield were very slightly affected by increasing the concentration of gas-forming agent and calcium chloride concentration. The DEE of the prepared formulations was found to be in the range of 77.61 ± 0.60% to 82.48 ± 0.56% (Table 2). The high incorporation efficiencies are seen with lower concentrations of gas-forming agent, as gas-forming agents increase porosity and pore diameter in microparticles by virtue of their gas evolving nature, microparticles unable to retain the drug more effectively.[13]

Surface Morphology. The beads were found to be grossly spherical in shape. Figures 1A and 1B show SEM image of single floating bead and nonfloating bead, respectively. The cross-sectional morphology of drug loaded and placebo beads were also examined with SEM (Figures 1C and 1D). In the case of drug loaded beads, an inner core of alginate gel matrix is observed, which is absent in placebo beads. It may be suggested that the core may contain the drug entrapped in it.

Micromeritic Studies. The particle size of the prepared formulations ranged from 861.34 ± 2.54 μm to 991.75 ± 2.86 μm (Table 3). By keeping other factors constant, the bead size was found to increase with the increase in the concentration of gas-forming agent, which may be due to the pore formation. There is only slight increase in bead size with respect to increase in calcium chloride concentration. This may be due to higher porosity of the beads as evident from the porosity data.

The tapped density values range from 0.40 to 0.63 g cm^{-3} (Table 3). Almost similar results were obtained by Bajpai and Tankhiwale,[17] who obtained tapped density values between 0.40 and 0.71 g cm^{-3} for floating sodium alginate/dextran-based hydrogel beads. Obviously, these values are less than the density of SGF (i.e., 1.004 g cm^{-3}), thereby implying that the beads will have the propensity to exhibit an excellent buoyancy effect *in vivo*. The true density of beads was found to be in the range of 1.71 to 2.14 g.cm^{-3}.

Porosity was studied to determine the effects of gas-forming agent on pore structure of floating beads. The % porosity for the prepared formulations ranged between 67.90% and 77.89%. By increasing the ratio of gas-forming agent, the % porosity of the beads was increased. This was due to evolution of a high amount of gas generation during the preparation. The % porosity value for FM-5 and FM-6 was found to be higher than FM-4. It is assumed that when the alginate solution is dropped into 3% and 4% $CaCl_2$ solutions, the ionotropic gelation and CO_2 evolution take place simultaneously. Now, due to appreciably higher concentration of cross-linker calcium ions, the cross-linking of beads occurs to such a great extent and at such a fast rate that the gas bubbles produced remain almost entrapped within the beads, thus imparting to them a highly porous structure.[17]

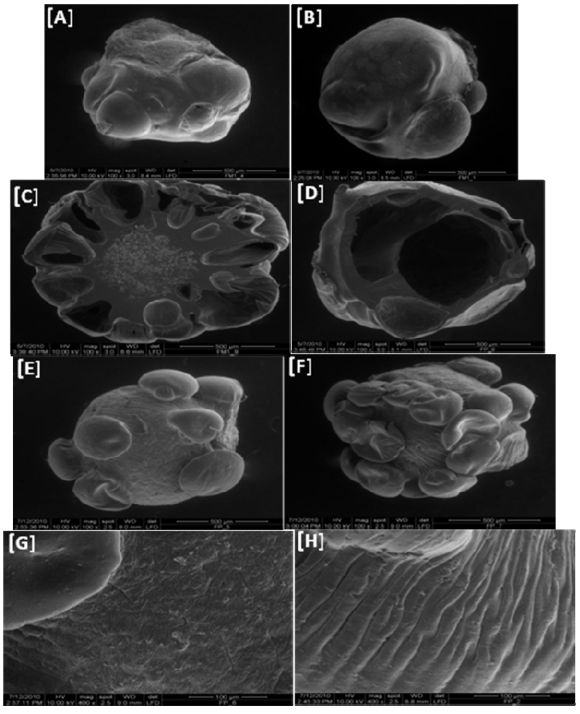

Figure 1. SEM image of [A] single floating bead, [B] single nonfloating bead, [C] cross-sectional morphology of drug loaded floating bead, [D] cross-sectional morphology of placebo floating bead, [E] floating bead stored at 25 °C, [F] floating bead stored at 40 °C, [G] surface morphology of floating bead stored at 25 °C and [H] surface morphology of floating bead stored at 40 °C.

Differential Scanning Calorimetry. Mixtures should be examined under nitrogen to eliminate oxidative and pyrrolytic effect at a standard heating rate (2, 5, or 10 °C/min) on DSC, over a temperature range, which will encompass any thermal changes due to both drug and excipient. Appearance and/or disappearance of one or more peaks in thermograms are considered indicative of interaction. Though interaction can be observed by TLC (thin layer chomatography) as well, the rapidity of DSC gives it an edge over TLC.[18]

Thermograms of MH, HPMC, sodium alginate, $CaCO_3$, placebo formulation (without drug) and optimized formulation (with drug) were recorded in a differential scanning calorimeter to characterize the solid state of the drug in the beads and to know any existing interaction between the polymer and the drug (Figure 2). Pure MH showed a sharp endothermic peak at 232.92 °C in the DSC thermogram. This transition is attributed to compound melting.[19] DSC scans of sodium alginate showed a wide endothermic peak at around 85 °C that has been attributed to the evaporation of water, and the appearance of exothermic behavior was detected at 259.55 °C, coinciding with the exothermic behavior of the sodium alginate as reported in several publications[20,21] as the decomposition of the polymer. There were no peaks observed for the HPMC and $CaCO_3$ in the DSC thermogram. Absence of alginate exothermic peak in the placebo thermogram may be due to interaction between alginate−HPMC−$CaCO_3$, as in the case reported with chitosan−alginate by Crcarevska et al.,[20] who prepared chitosan coated Ca−alginate microparticles loaded with budesonide. A very slight variation in melting endotherm of MH in drug loaded formulation but significantly varied in peak intensity was observed in the thermogram; this may be attributable to a homogeneous dispersion of the drug in the polymers and also may be due to increase in drug to polymer ratio.[12,22] This also indicates the possibility of transformation of its (MH) crystal to amorphous structure, which probably occurs during preparation of beads.[20]

***In Vitro* Buoyancy Behavior.** To mimic the *in vivo* gastric retention, the *in vitro* floating behavior of the prepared formulations was studied in SGF (pH 2.0). The surfactant (0.02% w/v Tween 80) was used in the medium to simulate the surface tension of human gastric juice (35−50 mN/m^2).[23] The time taken for the dosage form to emerge on the surface of the medium is called the buoyancy lag time, and the duration of time by which the dosage form constantly emerges on the surface of the medium is called the total floating time (TFT). During this

Table 3. Micromeritic Studies of the Prepared Floating Formulations[a]

formulation code	particle size (μm)	tapped density (g/cm^3)	true density (g/cm^3)	porosity (%)
FM-1	861.34 ± 2.54	0.63 ± 0.031	1.76 ± 0.081	67.90 ± 1.68
FM-2	908.42 ± 2.92	0.49 ± 0.032	1.71 ± 0.081	70.52 ± 2.57
FM-3	967.42 ± 2.75	0.45 ± 0.015	1.87 ± 0.11	73.54 ± 4.7
FM-4	967.39 ± 2.81	0.44 ± 0.011	2.01 ± 0.20	76.82 ± 3.04
FM-5	967.68 ± 2.89	0.44 ± 0.065	1.93 ± 0.10	77.40 ± 2.07
FM-6	991.75 ± 2.86	0.40 ± 0.055	2.14 ± 0.12	77.89 ± 1.70

[a] Values are mean ± SD (n = 3).

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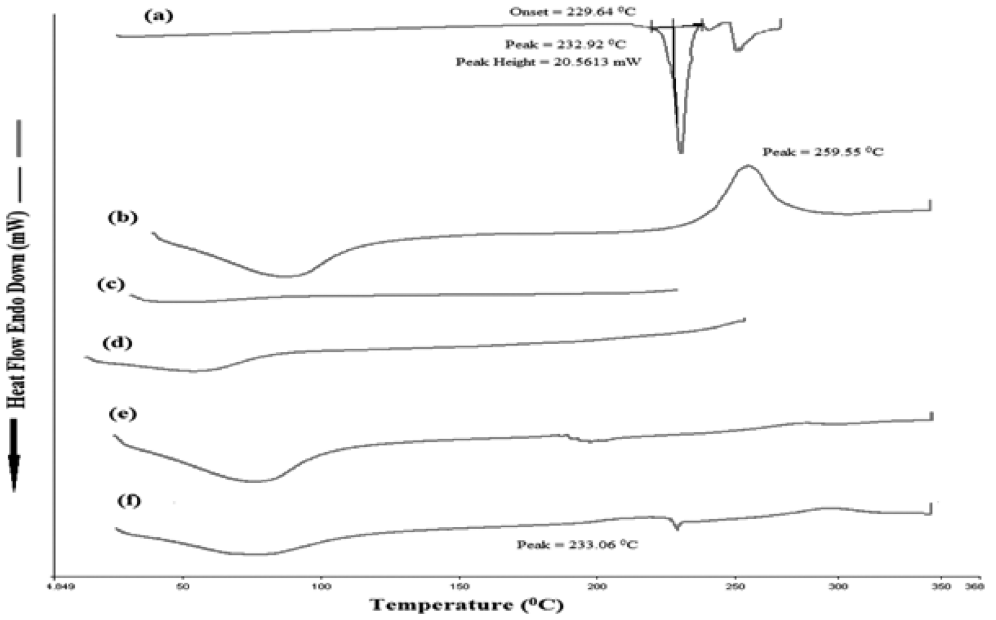

Figure 2. DSC thermogram of (a) pure MH, (b) sodium alginate, (c) $CaCO_3$ (d) HPMC, (e) placebo floating formulation, (f) drug loaded floating formulation.

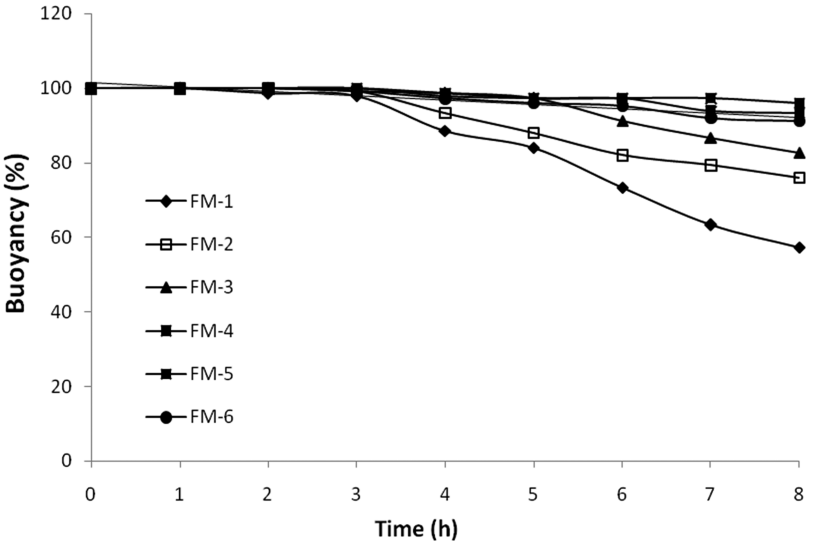

Figure 3. Percent buoyancy vs time profiles of the prepared floating formulations.

study it was found that the prepared formulations showed no buoyancy lag time.

A variable data of buoyancy (%) values ranging from 57.33% to 96% was found for the prepared formulations. After 1 h, all formulations except FM-1 showed 100% floating ability. Then, the floating ability of the formulations started to decrease. After 8 h it was found that FM-4 had highest % of buoyancy (96%) and FM-1 had lowest (57.33%). The floating ability of prepared formulations followed the order FM-1 < FM-2 < FM-3 < FM-6 < FM-5 < FM-4 (Figure 3).

Buoyancy of the beads was found to be directly related to the concentration of gas-forming agent. As the concentration of the gas-forming agent increases, the number of air trapped pores in beads increases, which makes the beads float.[13] Instantaneous *in vitro* floating behavior was observed for beads of all batches, which may be due to the low apparent density provided by the porous nature of beads as evident from the micromeritic studies.

***In Vitro* Drug Release Study.** Alginate is known not to swell in acidic media, as the protonated −COOH groups do not ionize to induce chain-relaxation processes. This was also observed visually, as the beads remained almost intact throughout the release studies. Therefore the nonswelling tendency of the beads reduces the matrix permeability and limits drug diffusion. However, due to the porous nature of the beads, a greater area is exposed to invading solvent and the drug continues to diffuse out slowly into the release medium.[17] Also the HPMC used in the formulation provides sustained release. The observed sustained release of drug from the fabricated alginate beads could be due to two reasons: partially coiled HPMC chains interpenetrate with

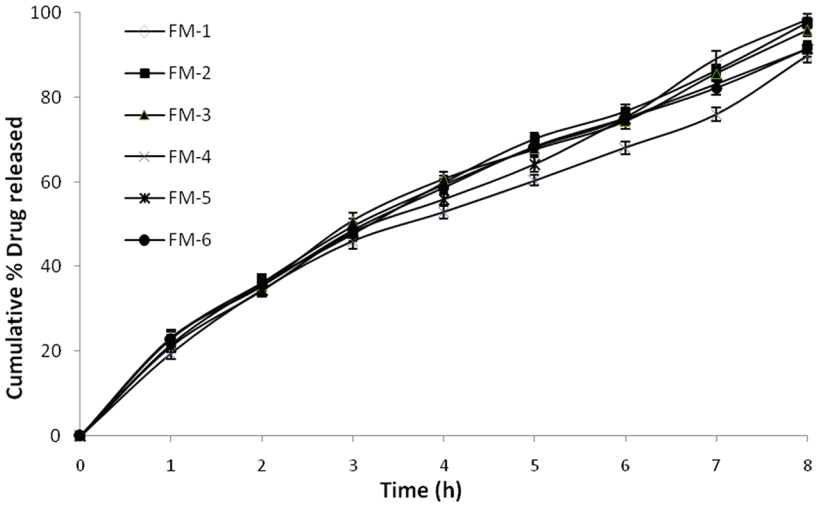

Figure 4. *In vitro* drug relase profile of MH from different floating formulations. Values are mean ± SD (n = 3).

sodium alginate network and the swelling of HPMC caused formation of viscous gelatinous layer that controls the drug release.[24]

The amount of drug released was studied in a USP type-II dissolution apparatus (paddle type) taking SGF (pH 2.0) as dissolution medium to simulate the gastric pH conditions. Within an hour, an initial release of MH (20−25%) from all prepared formulations was observed, except for FM-4 with 19.5% drug release within 1 h. Then a gradual release of MH was seen up to 8 h from all formulations. At the end of the release study, i.e. after 8 h, the MH release from all formulations was found to be more than 90% except for the case with FM-4, the formulation prepared with the highest concentration of incorporated gas-forming agent, which showed the cumulative drug release up to 89.70% after 8 h. The highest cumulative % drug release was observed from FM-1 (98.43%), the formulation with lowest concentration of incorporated gas-forming agent. The release of MH from the prepared formulations followed the order FM-1 > FM-2 > FM-3 > FM-5 > FM-6 > FM-4 (Figure 4).

The formulation FM-4 prepared with the highest amount of $CaCO_3$ showed the lowest release (89.70%) at the end of 8 h. It was also observed that, as the concentration of $CaCO_3$ increased, the release of MH from the beads decreased. The effect is observed in spite of increased bead porosity. This may be due to the internal ionotropic gelation effect of $CaCO_3$.[25,26] It is present as an insoluble dispersion in neutral pH aqueous alginate solution; however, in acidic media, the $CaCO_3$ becomes water-soluble. The ionized Ca^{2+} ions then promote internal gelation by cross-linking with alginate carboxyl group.[11]

In order to know the effect of cross-linking agent on the release profile of MH from the formulations, beads were prepared with increasing amount of cross-linking agent. The cumulative drug release of beads prepared with high concentration of cross-linking agent (FM-5 and FM-6) is slightly higher than that of beads prepared with low concentration of cross-linking agent (FM-4). This may be due to higher porosity of these beads in comparison to beads prepared with low concentration of cross-linking agent.

When plotted with Peppas−Korsmeyer's power law equation all the prepared formulations showed high correlation coefficient value (r > 0.99) (Table 4). Release exponent (n) value was determined from the slope of the straight line of this plot and was found to be within the range of 0.669 to 0.719. The diffusional exponent, n, specifies the mechanism of release, which depends on the release mechanism and the shape of the matrix tested. Exponent (n) for polymeric controlled delivery systems of spherical geometry has values of n = 0.43 are for Fickian diffusion; 0.43 < n < 0.85 is an indication of both diffusion controlled drug release and swelling controlled drug release (anomalous transport or non-Fickian transport); and n > 0.85 indicates case II transport which relates to polymer relaxation during gel swelling.[27,28] Therefore it is concluded that all the formulations followed a non-Fickian release mechanism, i.e. release was governed by both diffusion and swelling of polymer as described by the Peppas−Korsmeyer model.

Formulation FM-4 was assigned as optimized formulation due to its better buoyancy behavior and controlled release as compared to other prepared formulations, and it was selected for further stability and *in vivo* studies.

Stability Studies. The SEM images of the stored beads (Figures 1E and 1F) show that the pores of the beads are shrunken to some extent. This shrinking is marked in the case of beads stored at 40 °C. Also the surface morphology of the beads reveals that the surface has become rigidified, which is prominent in the case of beads stored at 40 °C (Figure 1H); in the case of beads stored at 25 °C the surface is both rigid and rough (Figure 1G).

After it was confirmed from the SEM analysis that the pores of the beads are shrunken to some extent, the particle size analysis was necessary to know the effect of aging on particle size of the beads. It was found that the particle size of the beads (966.24 ± 2.06 μm) stored at 25 °C does not differ significantly (P > 0.05) from the previously reported particle size (967.39 ± 2.81 μm) and that of beads stored at 40 °C (947.04 ± 2.43 μm) differs significantly (P < 0.05) from the previously reported particle size. This is due to slight shrinkage of the beads stored at 25 °C and marked shrinkage in the case of beads stored at 40 °C. The drug entrapment of the stored beads was found to have no significant change (P > 0.05) due to aging. The drug entrapment efficiency of the stored beads at 25 and 40 °C was found to be 78.12 ± 0.22% and 77.25 ± 0.12%, respectively. The pores of the beads

Table 4. Comparison of Different Dissolution Kinetics Models for the Release of MH from Different Floating Formulations in SGF (pH 2.0)

	r				Peppas−Korsmeyer	
formulation code	zero order	first order	Higuchi-matrix	Hixon−Crowell	*r*	*n*
FM-1	0.9856	0.8938	0.9857	0.9502	0.9933	0.697
FM-2	0.9870	0.8586	0.9918	0.9445	0.9958	0.677
FM-3	0.9931	0.9601	0.9936	0.9334	0.9991	0.698
FM-4	0.9919	0.7821	0.9909	0.9138	0.9979	0.719
FM-5	0.9912	0.9399	0.9954	0.9804	0.9981	0.705
FM-6	0.9865	0.9467	0.9977	0.9848	0.9982	0.669

r- Correlation coefficient, n- Diffusional exponent

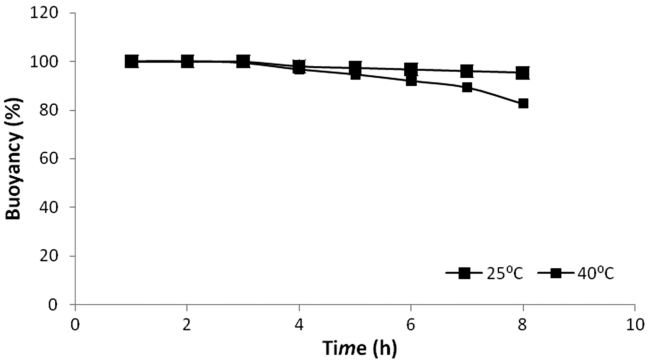

Figure 5. Buoyancy (%) vs time profile of floating bead stored at 25 and 40 °C.

have shrunken but the core is unaffected, therefore the drug content of the beads was unaffected in the stability conditions.

It was found that the beads stored at 25 °C showed no change in floating behavior due to aging. But the result of the beads stored at 40 °C varies from the reported results as there is more than 10% reduction of the floating ability of the beads (Figure 5). This may be due to shrinking of the pores of the beads, as the floating ability depends upon the number of air trapped pores in beads, making the beads float.[13] The release profile of MH from the stored beads was found to be 90.48 ± 1.17% in the case of beads stored at 25 °C and 91.36 ± 2.87% in the case of beads stored at 40 °C. Therefore it is concluded the drug release remained unaffected by aging as no significant decrease ($P > 0.05$) in the cumulative drug release was found after 8 h of study.

***In Vivo* Study.** Streptozotocin is well-known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms.[29] Intraperitoneal administration of streptozotocin (60 mg/kg) effectively induced diabetes in normal albino rats as reflected by glycosuria, hyperglycemia, polydipsia and body weight loss when compared with normal rats. This kind of diabetic model mimics type 2 diabetes mellitus.[14]

It is already reported that MH is usually not effective at low concentrations such as 200 mg/kg and therefore diabetic rats were treated with floating and nonfloating beads containing equivalent dose of 400 mg/kg.[16] Often large doses are used to affect measurable level of blood glucose in experimental animals. To know the effect of drug at the same dose level in normal animals, the study was also conducted with normal healthy albino rats.

The glucometer works on the principle of biamperometry. Glucose dehydrogenase present in the strip converts the glucose in the blood sample to gluconolactone. This reaction creates a harmless electrical current that the meter interprets for blood sugar.

When aqueous dispersion of nonfloating beads was administered, the plasma glucose level declined gradually within 1 h in both diabetic and normal rats. Figure 6 and Figure 7 show average serum glucose level vs time profiles after oral administration to diabetic rats and normal rats, respectively. The reduction in glucose level reached its maximum at 2 h and the glucose level was restored after 3 h in both cases. A 25% reduction in blood glucose levels is considered a significant hypoglycemic effect.[30] In the case of both floating and nonfloating formulations, the decline in blood glucose level was slower. The hypoglycemic effect was maintained during the period of 2 to 5 h when the nonfloating beads were administered to diabetic rats, but in the case of optimized floating formulation the hypoglycemic effect was maintained during the period of 3 to 9 h. The sustained hypoglycemic effect observed for longer period of time in the case of floating formulation is due to the slow release and absorption of MH over an extended period of time.

In the case of normal rats treated with nonfloating beads the hypoglycemic effect was maintained during the period of 3 to 5 h, although it was not significant. It was observed that the reduction in blood glucose level was not below the normal level, i.e. <25% of blood glucose level. This is because metformin is not a hypoglycemic agent as such but has the tendency to bring blood sugar level to the normoglycemic level.[16] In the case of floating formulation, it was observed that the blood glucose level was reduced below 25% from 3 to 4 h only, but the blood glucose level was reduced in a sustained manner though not significant up to 8 h. It is a good feature of the formulation that it does not reduce the blood glucose level significantly for a longer duration of time in normal rats.

One-way ANOVA followed by Dunnett's multiple comparison tests was used to analyze if the reduction in blood glucose level of the nonfloating beads and formulation significantly differs from the control or not. Blood glucose levels for diabetic rats in group II and III rats were significantly lower than those in group I ($P < 0.05$). A similar result was obtained in the case of normal rats as blood glucose levels in groups V and VI were significantly lower than in group IV. The results reveal that the blood glucose levels of the group treated with MH loaded

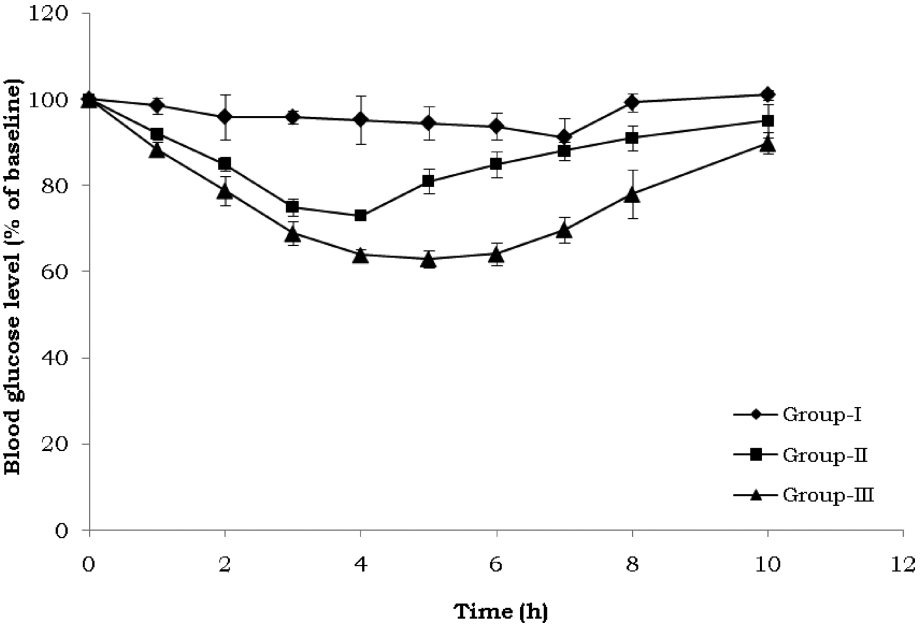

Figure 6. Hypoglycemic effect of floating beads containing metformin HCl administered orally in diabetic rats: group I, diabetic control rats; group II, diabetic rats treated with nonfloating beads containing metformin HCl; group III, diabetic rats treated with floating beads containing metformin HCl. Values are mean ± SD (n = 6).

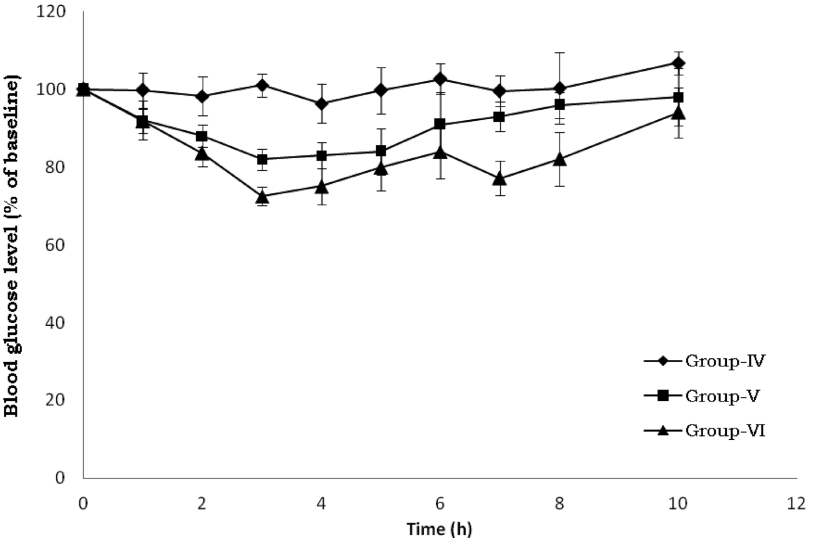

Figure 7. Hypoglycemic effect of floating beads containing metformin HCl administered orally in normal rats: group IV, normal control rats; group V, normal rats treated with nonfloating beads containing metformin HCl; group VI, normal rats treated with floating beads containing metformin HCl. Values are mean ± SD (n = 6).

alginate beads and the group treated with the nonfloating beads were lower than that of the control group.

CONCLUSION

The results clearly demonstrated the ability of the formulation to maintain blood glucose level and improved the patient compliance by enhancing, controlling and prolonging the systemic absorption of MH. Thus, it was concluded that the formulation was able to sustain the drug in an effective manner for prolonged duration of time, and able to maintain the reduced blood glucose level for a longer duration as compared to nonfloating beads.

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■ ABBREVIATIONS USED

MH, metformin hydrochloride; GI, gastrointestinal; UV, ultraviolet; DSC, differential scanning calorimetry; SEM, scanning electron microscopy; SGF, simulated gastric fluid; $CaCl_2$, calcium chloride; $CaCO_3$, calcium carbonate; HPMC, hydroxypropyl methylcellulose

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