Research Article

Formulation and Evaluation of Floating Microsphere Containing Anti Diabetic Drug

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ABSTRACT

The purpose of the present investigation was the development and characterization of gastro-retentive floating drug delivery system for anti-diabetic drug Metformin Hydrochloride that would retain the drug in stomach and continuously release the drug in controlled manner up to a predetermined time leading to improved bioavailability. Different formulations of Metformin Hydrochloride were prepared as the floating microspheres using hydroxy propyl methyl cellulose (HPMC) and Eudragit RS100 polymers by emulsion solvent evaporation technique. The dried floating microspheres were evaluated for micromeritics properties, flow properties, densities, particle size determination, scanning electron microscopy, floating behaviour, *in vitro* drug release studies, *in vivo* and stability studies. The kinetic study of prepared microspheres showed controlled drug release by matrix diffusion Process with zero order release rate kinetics with good stability. The developed gastro retentive floating drug delivery systems of Metformin Hydrochloride showed excellent physicochemical properties, stability and controlled drug release pattern, thereby improving the bioavailability of the drug and also manage the complicacy of the diabetes in a better manner.

Keywords: Floating microspheres, Metformin Hydrochloride, *In vitro* release, Bioavailability.

INTRODUCTION

Diabetes is one of the major causes of death and disability in the world. The latest, WHO estimate for the number of people with diabetes worldwide, in 2000, is 171 million, which is likely to be at least 366 million by 2030. The focus of medical community is on the prevention and treatment of the disease, as is evident from the rising number of research papers every year on the subject[1].

Floating Drug Delivery Systems (FDDS) or Hydrodynamically Balanced Systems (HBS) are among the several approaches that have been developed in order to increase the gastric residence time (GRT) of dosage forms. Both single and multiple unit systems have been developed. The single-unit floating systems are more popular but have a disadvantage owing to their 'all-or-nothing' emptying process leading to high variability of the gastro intestinal transit time. Still, the multiple-unit dosage forms may be better suited because they are claimed to reduce the inter subject variability in absorption and lower the probability of dose dumping. Such a dosage form can be distributed widely throughout the gastrointestinal tract (GIT), affording the possibility of a longer lasting and more reliable release of the drug from the dosage form[2].

Drugs that are easily absorbed from the GIT and have a short half-life are eliminated quickly from the blood circulation, so they require frequent dosing. To avoid this drawback, the oral sustained-controlled release formulations have been developed in an attempt to release the drug slowly into the GIT and maintain an effective drug concentration in the serum for longer period of time. However, such oral drug delivery devices have a physiological limitation of

GRT[3], variable and short gastric emptying time can result in incomplete drug release from the drug delivery system (DDS) in the absorption zone (stomach or upper part of small intestine), leading to diminished efficacy of the administered dose[4,5]. To overcome these limitations, approaches being proposed to prolong the GRT include: floating drug dosage systems (FDDS)[6-9], swelling or expanding systems[10-11], mucoadhesive systems[12], high-density systems[13], modified-shape systems[14], and other delayed gastric emptying devices[15].

Floating drug delivery is of particular interest for drugs that act locally in the stomach; are primarily absorbed in the stomach; are poorly soluble at an alkaline pH; have a narrow window of absorption; and are unstable in the intestinal or colonic environment. To provide good floating behavior in the stomach, the density of the device should be less than that of the gastric contents (≈1.004 g/cm^3)[16].

Microspheres have been widely accepted as a means to achieve oral and parenteral controlled release drug delivery system. The microsphere requires a polymeric substance as a carrier and a core material. Among the various methods developed for formulation of microspheres, the non-aqueous solvent evaporation method has gained much attention due to its ease of fabrication without compromising the activity of drug. Eudragit ® RS 100 and Eudragit® RL 100 are referred to as ammoniomethacrylate copolymers, with the former having 5% functional quaternary ammonium groups and the latter having 10% functional quaternary ammonium groups. Eudragit® RS 100 is a water-insoluble polymer that is widely used as a wall material for sustained release microcapsules due to its biocompatibility, good stability, easy fabrication and low cost[17].

Metformin is an insulin-sensitizing, anti-diabetic drug from the Biguanide class of oral anti-hyperglycemic agent. It was chosen as a model drug since it has a very short half life (1.5-3 h) and low bioavailability (50±10 %). The objective of the present study was to prepare floating microsphere of Metformin hydrochloride in order to maintain a sustained drug concentration in serum for longer period of time, which may result in enhanced absorption and thereby improved bioavailability.

MATERIALS AND METHODS

Materials

The polymer Eudragit RS 100 and HPMC was purchased from the Ponmani labs, Coimbature (India). The anti-diabetic drug Metformin Hydrochloride supplied as a gift sample by Cipla, Mumbai (India). All other chemicals were of analytical reagent grade and were used as received.

Methods

Preparation of floating microspheres

Microspheres containing anti-diabetic drug as a core material were prepared by a non-aqueous solvent evaporation method[1-18]. Briefly, drug (Metformin hydrochloride) and polymers (Eudragit RS100 and HPMC) were mixed in acetone at various ratios. The slurry was slowly introduced into 40 ml of liquid paraffin while being stirred at 1200 rpm by a mechanical stirrer equipped with a three bladed propeller at room temperature (27± 0.5°C). The solution was stirred for 2 h to allow the solvent to evaporate completely and the microspheres were collected by filtration. The microspheres were washed repeatedly with petroleum ether (40-60°C) until free from oil. The collected microspheres were dried for 1 hour at room temperature and subsequently stored in desiccators over fused calcium chloride (Table 1).

Table 1: Formulation of the floating microspheres prepared

Formulation Code	Metformin HCl (mg)	Eudragit Rs 100 (mg)	HPMC (mg)
F_1	250	800	100
F_2	250	700	200
F_3	250	600	300
F_4	250	500	400
F_5	250	400	500
F_6	250	300	600
F_7	250	200	700
F_8	250	100	800

Drug content

The drug content of floating microspheres was determined by dispersing 50 mg formulation (accurately weighed) in 10 ml ethanol followed by agitation with a magnetic stirrer for 12 h to dissolve the polymer and to extract the drug. After filtration through a 5 µm membrane filter (Millipore), the drug concentration was determined spectrophotometrically at 233 nm (GBC Cintra UV-spectrophotometer). The percentage drug entrapment and yield were calculated as follows:

$$\% \text{ Drug entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

Particle size analysis

The size of 300 particles of each batch was measured by using a calibrated micrometer attached[19] with a microscope and the average diameter was calculated.

Floating behavior

100 mg of the floating microsphere were placed in 0.1 N HCl (300 ml) containing 0.02% Tween 20[1,20]. The mixture was stirred with paddle at 100 rpm in a magnetic stirrer. The layer of buoyant floating microsphere was taken and separated by filtration at 1, 2, 4 and 6 h. Particles of both types were dried in a desiccator until constant weight. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles. The percentage of floating microsphere was calculated by the following equation:

$$\% \text{ floating microsphere} = \frac{\text{Weight of floating microsphere}}{\text{Initial weight of floating microsphere}} \times 100$$

Percentage compressibility index

The same tapping method was used to determine percentage compressibility index[20]. The percentage compressibility index was calculated according to following formula.

$$\% \text{ Compressibility index} = \left[1 - \frac{V}{V_0}\right] x100$$

Where, V and V_0 are the volumes of the sample after and before the standard tapping respectively.

Scanning electron microscopy

Morphological examination of the surface and internal structure of the dried floating microspheres was performed by using a Scanning electron microscope (model-6360 A0, Jeol, Japan) using platinum sputter technique. The working distance is 50 micrometer. Photographs were taken with 100× magnifications (Fig. 1).

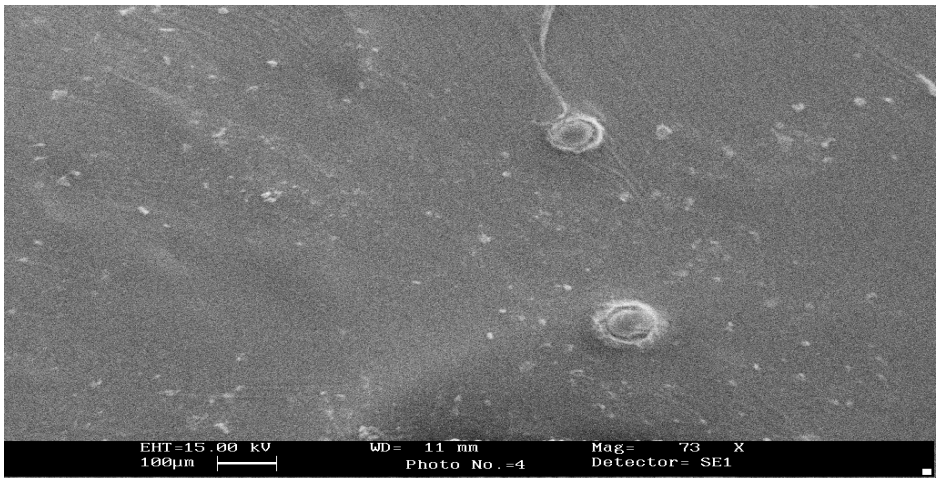

Fig. 1: SEM photograph of floating microspheres

In vitro release studies

The in vitro release of drug from the different formulations was examined using USP XXIII basket type dissolution apparatus[21]. The amount of floating microspheres equivalent to 100 mg drug was placed in the basket. Simulated gastric fluid (pH 1.2) (900ml) was used as the dissolution medium and maintained at 37±0.5°C at a rotation speed of 100 rpm. An aliquot of 1 ml of the solution was withdrawn at predetermined time intervals and replaced by 1 ml of fresh dissolution medium. Samples were assayed spectrophotometrically at 233 nm after filtration through a 0.45 µm membrane filter (Millipore). The dissolution studies were repeated using phosphate buffer pH 6.8 as dissolution medium (Fig. 2 and 3).

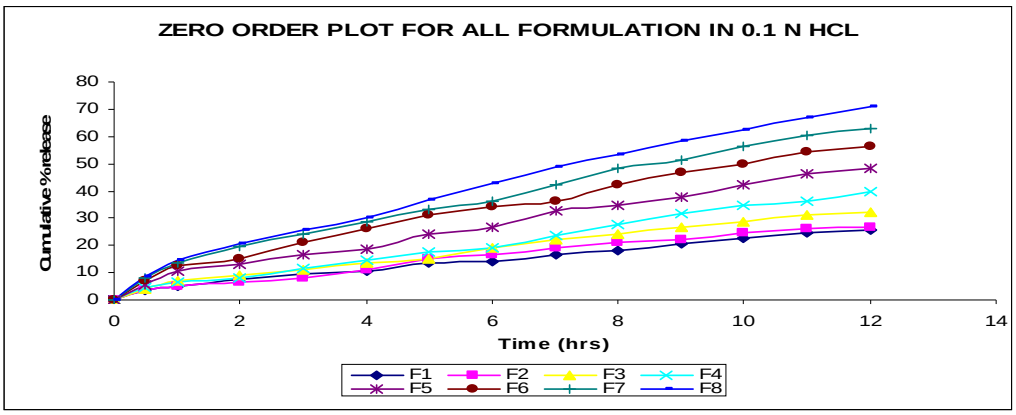

Fig. 2: *In vitro* drug release of metformin in 0.1 N HCl from different formulations

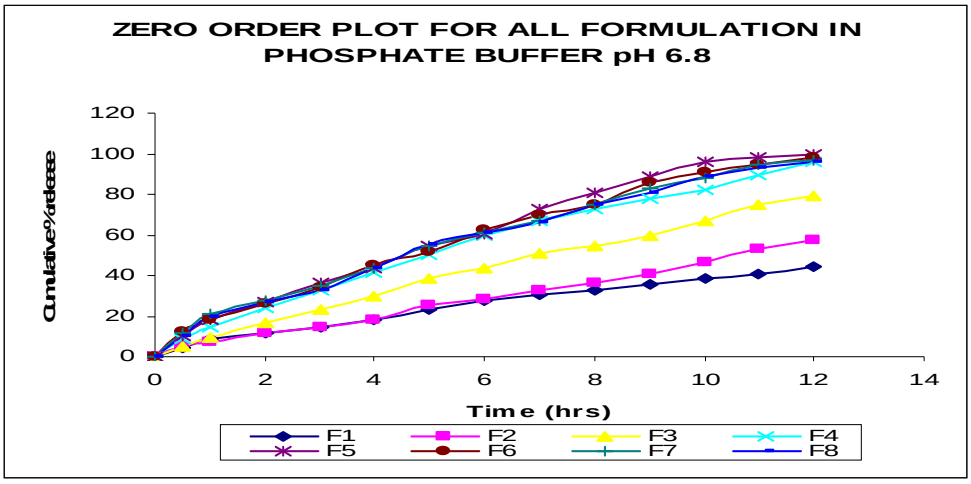

Fig. 3: *In vitro* drug release of metformin in PBS (pH 6.8) from different formulations

In vivo anti-hyperglycemic study

All the animal studies were conducted in accordance with the protocol approved by the Institutional Animal Ethical Committee (registration no. 688/02/C-CPCSEA; 10/87, dated 21.02.2002). Two groups of Wistar rats (5 in each group) that were fasted (with water) at least 12 h before the experiments were used for the study. Before drug administration, a blood sample as a control was taken from each rat from behind the eyeball through the angle of ocular cavity using small capillary tubes. The blood glucose level for the control and test samples was determined using the glucose-measuring instrument one touch ultra (lifescan, inc. milpitas, ca 95035 U.S.A.). The instrument was self-calibrated[22]. Pure Metformin and microsphere of Metformin were administered orally to each group using stomach intubations. A dose of 50 mg/kg of Metformin Hydrochloride was administrated in a suspension form (freshly prepared) for each rat. Blood samples were collected at predetermined time at 1-hour intervals up to 12 h, and the blood glucose level was performed as per method described earlier (Table 2; Fig. 4).

Stability study

From the prepared floating microspheres F_4 which showed appropriate balance between the buoyancy and the percentage release was selected for stability studies[23]. The prepared formulation (F_4) were placed in borosilicate screw capped glass containers and stored at room temperature (30 ± 2° C), oven temperature (40±2° C) and in refrigerator (5-8° C) for a period of 60 days. The samples were assayed for drug content at regular intervals of two week. All the determinations were made in triplicate. (Table 3).

Table 2: Result of anti-hyperglycemic activity after administration of F4

Time (h)	Dose mg/kg	Glucose Level mg/dl (Standard sol.)	Glucose Level mg/dl (microsphere Suspension)	% Reduction in glucose level	
				Standard solution	microsphere suspension
0		80	80	00	00
1		49	56	39.4	30
2		35	42	56.3	47.7
4	50 mg/kg	68	36	15	55
6		75	43	6.25	46.3
9		77	48	3.75	40
12		79	59	1.3	26.3

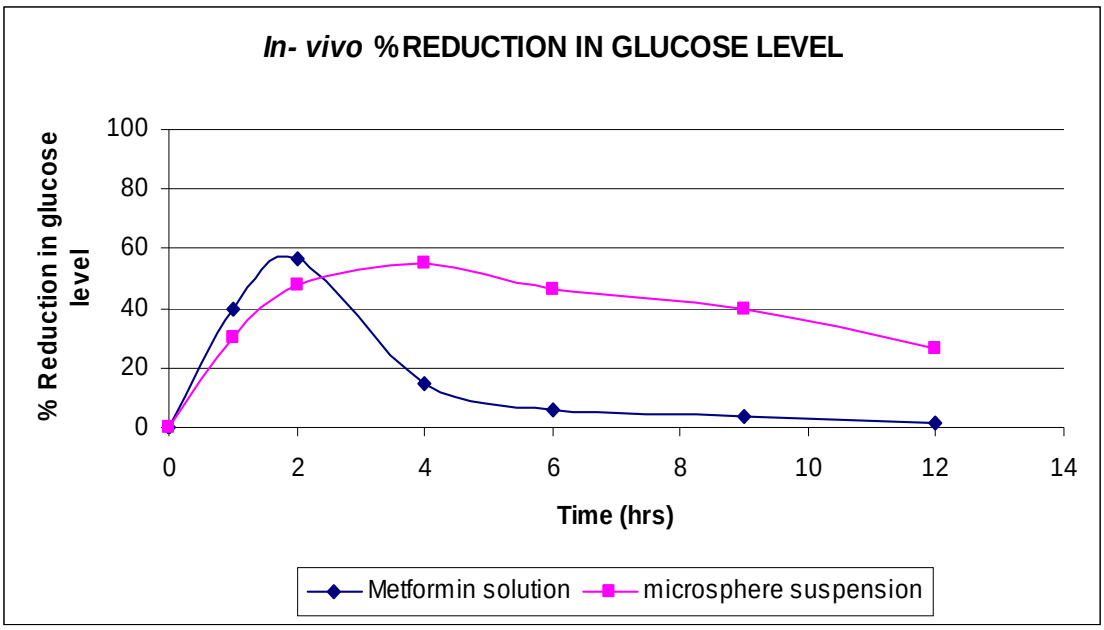

Fig. 4: *In vivo* percentage reduction in glucose level after the administration of F4 formulation

Table 3: Stability study data for f_4 formulation

Days	% Drug Remaining 5-8°C	% Drug Remaining 27±2°C	% Drug Remaining 42±2°C
0	100 ± 00	100 ± 00	100 ± 00
14	99.52 ± 0.014	99.89 ± 0.002	99.39 ± 0.039
28	99.43 ± 0.012	99.78 ± 0.021	99.14 ± 0.035
45	99.35 ± 0.016	99.56 ± 0.014	99.08 ± 0.03
60	99.24 ± 0.015	99.38 ± 0.016	99.01 ± 0.06

* Values are mean ± S.D.

RESULT AND DISCUSSION

Drug content

Drug content of all formulation was found in range of 42.64 to 73.62% and its efficiency slightly decreases with increasing the HPMC content (data not shown). The high entrapment efficiency of Metformin Hydrochloride is believed to be due to its poor aqueous solubility. The extent of loading influenced the particle size distribution of microspheres. When the distribution coefficient was high, efficiency of drug entrapment into microspheres was elevated. It is already reported that the size of microspheres depends upon various factors such as viscosity of the dispersed phase and dispersion medium, temperature, speed of string, amount and size of porous carrier, etc. So microspheres of desired size can be obtained by varying these factors.

Particle size analysis

It was already cleared that if the size of microspheres is less than 500 μm, release rate of drug will be high with reduced floating ability, while microspheres ranging between 500-1000 μm, the floating ability will be more and release rate will be in sustained manner. The average particle sizes of microspheres were between 608 and 864 μm. It was observed that the mean particle size of the microspheres was significantly decreased with increase in the concentration of HPMC and reduces in the concentration of Eudragit RS100. It may be attributed to the forming of a thicker Eudragit RS100 layer with the increase of concentration of Eudragit RS100 in the medium.

Floating behavior

When floating microspheres are dispersed in simulated gastric fluid without enzymes, due to high water solubility, Eudragit RS100 goes into solution forming pores on microspheres due to matrix erosion. This phenomenon makes the microspheres to float. The percentage buoyancy for different formulation was found in the range of 25.68-98.64%. Eudragit RS100 microspheres prepared with HPMC showed good floating properties. As the ratio of HPMC increased the floating behavior get reduced.

Percentage compressibility index

The compressibility index of developed microspheres was found in the range of 7.84-16.95%. It was clearly observed that as the ratio of HPMC increased, compressibility index was also increased.

Scanning electron microscopy

The microspheres were spherical, discrete and having a rough surface as evidenced by Fig. 1. The surface of HPMC/ Eudragit RS100 microspheres did not show any pores on the surface. SEM of floating microspheres collected after dispersion in simulated gastric fluid revealed the presence of pores on the surface which is responsible for floating behavior. The pores found in formulation F4 microspheres are shown in the Fig. 1. This clearly indicated that the floating nature of microspheres is due to matrix erosion resulted by solubilization of HPMC/ Eudragit RS100 from microspheres when they were dispersed in gastric fluid without enzymes.

***In vitro* release studies**

Ideal property of floating microspheres includes high buoyancy and sufficient sustained release of drug in pH 6.8. Percent drug release rate of F_1, F_2, F_3 formulations in 12 h, which is slow and incomplete drug release. In order to increases the percent drug release rate, the ratio of Eudragit and HPMC is decreased and increased respectively. F_4, F_5, F_6 formulations showed high release rate and F7, F_8 formulations showed high release rate, with less buoyancy. F_4 formulation showed appropriate balance between buoyancy and drug release rate, it may consider as a best formulation.

Drug release pattern was evaluated in 0.1 N HCl and phosphate buffer pH 6.8. Release rate of F_1, F_2, F_3 formulations were found to be slow and incomplete in both dissolution medium. It was found that drug release rate increased by decreasing and increasing the ratio of Eudragit and the HPMC respectively. Kinetics and mechanism of drug release from all formulation was evaluated on the basis of zero order, Higuchi equation and Peppas model. Correlation coefficient (r^2) and slope value for each equation in the range of (r^2=0.835-0.920 and n=0.746-0.857 for Peppas model. Zero order plots for all formulations were found to be linear in acidic and buffer solution of pH 6.8.which indicates that it may follow zero order kinetics (Fig. 2 and 3).

Higuchi plot was found to be linear, which indicates diffusion may be the mechanism of drug release for each formulation. Peppas plot was found with good linearity, its n > 0.5 for all formulations, indicating that drug release may follow anomalous diffusion (range=0.962-0.989).

Zero order plots for F_4 formulation was found to be linear in both dissolution medium, and is considered as a best fit for drug release. That indicates it may follow zero order mechanism. The *in vitro* release data was applied to various kinetic models to predict the drug release kinetic mechanism.

***In vivo* anti-hyperglycemic study**

In vivo efficiency of the optimized formulation F_4 was performed in healthy normal Wistar rats by measuring the hypoglycemic effect produced after oral administration using the glucose-measuring instrument. The drug was administered at a dose equivalent to 50 mg/kg pure Metformin hydrochloride, and drug loaded microspheres were used for the study. Pure

Metformin hydrochloride drug was administered in a suspension form at the same dose. A rapid reduction in blood glucose levels was observed and maximum reduction of (43%) was observed within 2 h after oral administration. Blood glucose levels were recovered rapidly to the normal level within 8 h. In the case of Metformin hydrochloride microsphere, the reduction in blood glucose level was slow and reached maximum reduction within 4-5 h after oral administration. This reduction in blood glucose level was sustained over longer periods of time (12 h). It was suggested that a 25% reduction in blood glucose levels is considered a significant hypoglycemic effect. This hypoglycemic effect (25%) was maintained only for 2-3 h after oral administration of the drug Metformin hydrochloride, whereas in the case of microsphere of Metformin hydrochloride, significant hypoglycemic effect (25%) was maintained for a period of 2 to 12 h. The sustained hypoglycemic effect observed over a longer period of time in the case of floating microsphere is due to the slow release and absorption of Metformin over longer periods of time (Table 2; Fig. 4).
Metformin hydrochloride sustained release formulation is significantly more effective than the immediate release formulation of Metformin in reducing fasting plasma glucose levels and side effects. Formulation of Metformin as floating microsphere sustained release dosage form may also exhibit a decrease in side effects.

Stability study

Stability study was carried out for the F4 formulation by exposing it to different temperature 5-8°C, 27°C and 42°C for 45 days. The sample was analyzed for drug content at the regular intervals. In stability study, there was no remarkable change in content of F4 formulation during 90 days in which it was stored at various temperatures revealed that microspheres are highly stable over reasonable period of time (Table 3).

CONCLUSION

Drug absorption in the GIT is a highly variable process, prolonging gastric retention of the dosage forms and extends the time of drug absorption. Floating floating microspheres are prepared with enteric coated polymer (Eudragit RS 100) successfully by the non-aqueous solvent evaporation technique. Upon incorporation of the hydrophilic polymer such as HPMC in the shell of microballoons, the amount of drug released from microspheres could be enhanced. In vitro data obtained from floating microspheres of Metformin Hydrochloride showed excellent floatability, good buoyancy and prolonged drug release. Microspheres of different size and drug content could be obtained by varying the formulation variables. Diffusion (Anomalous transport diffusion) was found to be the main release mechanism. Thus the prepared floating microspheres may prove to be potential candidates for multiple-unit delivery devices adaptable to any intra gastric condition.

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