Research Article

Formulation and In-Vitro Evaluation of Floating Microspheres of Anti-Diabetic Drug Prepared by Solvent Evaporation Method

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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 Sitagliptin Phosphate 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 Sitagliptin Phosphate were prepared as the floating microspheres using Hydroxypropyl methylcellulose (HPMC) and Eudragit RS100 polymers by emulsion solvent evaporation technique. The dried floating microspheres were evaluated for drug content, particle size analysis, incorporation efficiency, floating behavior and in-vitro drug release studies. The developed gastro retentive floating drug delivery system of Sitagliptin Phosphate showed excellent physicochemical properties 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, Sitagliptin Phosphate, 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]. Gastro-retentive Floating Drug Delivery Systems (GFDDS) 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: Gastro-retentive Floating Drug

Delivery Systems (GFDDS) [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/cm3)[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].

Sitagliptin is an insulin-sensitizing, antidiabetic drug. It was chosen as a model drug since it has a half life (8-14 h) and bioavailability (87 %). The objective of the present study was to prepare floating microsphere of Sitagliptin Phosphate 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 gift from the Nirlife healthcare, Ahmedabad (India). The anti-diabetic drug Sitagliptin Phosphate supplied as a gift sample by Hangzhou Longshine Bio-tech (China). 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 nonaqueous solvent evaporation method [1-17]. Briefly, drug (Sitagliptin Phosphate) 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 desiccator over fused calcium chloride (Table 1).

Table 1: Formulation of the floating microspheres prepared

Formulation Code	Sitagliptin Phosphate (mg)	Eudragit RS (mg)	HPMC (mg)
F1	100	300	100
F2	100	250	150
F3	100	200	200
F4	100	150	250
F5	100	100	300

Preformulation

For the drug preformulation characteristics like description, solubility, melting point, bulk density, tapped density, angle of repose, Hausner's ratio and compressibility index were performed.

UV absorbance maximum for Sitagliptin Phosphate was determined by dissolving the pure drug sample in 0.1 N HCl (pH 1.2) and scanned in the wave length range of 400 – 200 nm in spectrum mode by using labindia UV spectrophotometer.

Assay was performed for the drug by method given in the pharmacopoeia. Calibration curve of Sitagliptin Phosphate was constructed by measuring the absorbance of different concentrations of drug (10, 20, 30, 40.....80 µg/ml) in 0.1 N HCl at 267 nm. A graph was plotted by taking concentration on X axis and absorbance on Y axis. (Fig.2, 3)[18, 19, 20]

Drug	Angle of Repose (Θ)	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Compressibility Index (%)	Hausner's Ratio
Sitagliptin Phosphate	36	0.53	0.62	16.98	1.21

Drug content

The drug content of floating microspheres was determined by dispersing 100 mg formulation (accurately weighed) in 100 ml distilled water 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 267 nm (UV-spectrophotometer).

The percentage drug entrapment and yield were calculated as follows:

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

Incorporation efficiency

Floating microspheres were dissolved in a minimum amount of methanol and drug was extracted into suitable aqueous media (0.1 N HCL) by evaporating methanol. The solution was filtered through whatman filter paper, diluted suitably and analyzed for drug content spectrophotometrically at 267 nm using 0.1N hydrochloric acid as blank [21, 22].

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

Floating microspheres (equivalent to 100 mg) were dispersed in 900 ml of 0.1 N hydrochloric acid (pH 1.2) containing tween 20 (0.02 w/v %) to simulate gastric fluid at 37°C. The mixture was stirred with a paddle at 100 rpm and after 12 hr, the layer of buoyant microspheres (W_f) was pipetted and separated by filtration simultaneously sinking microspheres (W_s) was also separated. Both types of microspheres were dried at 40°C overnight. Each weight was measured and buoyancy was determined by the weight ratio of the floating microspheres to the sum of floating and sinking microspheres. [23, 24, 25]

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

In-vitro Drug release

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) (900 ml) 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 267 nm after filtration through a 0.45 µm membrane filter (Millipore). The dissolution studies were repeated using phosphate buffer pH 7.4 as dissolution medium (Fig. 3).

RESULT AND DISCUSSION

Drug content

Drug content of all formulation was found in range of 42.64 % to 78.62 % and its efficiency slightly decreases with increasing the HPMC content (data not shown). 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 stirring, amount and size of porous carrier, etc. So microspheres of desired size can be obtained by varying these factors.

Incorporation efficiency

The incorporation efficiency of formulation F1 to F5 was carried out and found to be in a range 75.4 % to 48.1 %.

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, the average particle sizes of microspheres were between 380 and 575 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 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 90.5 % to 60.23 %. Eudragit RS100 microspheres prepared with HPMC showed good floating properties. As the ratio of HPMC increased the floating behavior get reduced.

In-vitro Drug release

The drug release from formulation F1 to F5 (Fig.2) was as follows. F1, F2, F3, F4 and F5 show percentage drug release 65.12 %, 72.45 %, 81.67 %, 87.4 % to 95.26 % at end of 8 hour. In order to increase the percent drug release rate, the ratio of Eudragit and HPMC is decreased and increased respectively.

CONCLUSION

Drug absorption in the GIT is a highly variable process, prolonging gastric retention of the dosage form and extends the time of drug absorption. Floating microspheres are prepared with enteric coated polymer (Eudragit RS100) 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. From the results it was observed that drug: polymer ratio influences the particle size, drug content, incorporation efficiency, floating behavior and in-vitro drug release of floating microspheres.

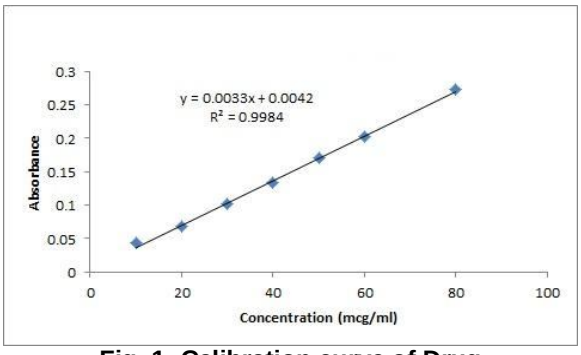

Fig. 1: Calibration curve of Drug

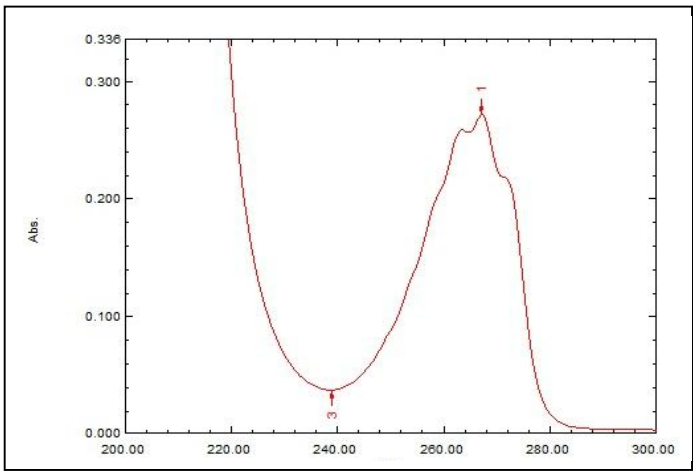

Fig. 2: λ_{max} of Sitagliptin Phosphate

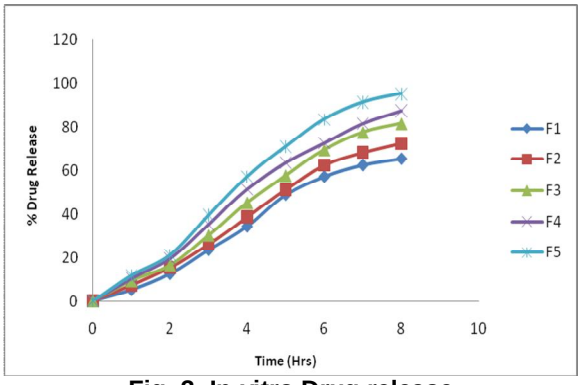

Fig. 3: In-vitro Drug release

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