

Original Article

Fabrication and characterization of Metformin loaded floating microspheres

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ABSTRACT

The aim of this study was to fabricate and characterize Metformin hydrochloride floating microspheres using Eudragit RS100 as a polymer. Microspheres were prepared by non-aqueous solvent evaporation method using ethanol/liquid paraffin system. The influence of formulation factors (drug: polymer ratio, stirring speed, concentration of surfactant) on particle size, encapsulation efficiency and in vitro release characteristics of the microspheres were investigated. The yields of preparation and encapsulation efficiencies were high for all formulations obtained. Mean particle size changed by changing the drug: polymer ratio or stirring speed. Although Metformin hydrochloride release rates from Eudragit RS100 microspheres were decreased as the concentration of Eudragit RS100 increased. By applying one way ANOVA followed by Newman-Keuls Multiple Comparison value obtained ($p < 0.05$) was considered to be statistically significant.

1, INTRODUCTION

Globally, as of 2010, an estimated 285 million people had diabetes, with type 2 making up about 90% of the cases. In 2013, according to the International Diabetes Federation, an estimated 381 million people had diabetes. Its prevalence is increasing rapidly, and by 2030, this number is estimated to almost double. Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries [1]. A plethora of antidiabetic drugs are used in the clinic, of which Metformin hydrochloride is a very widely accepted drug. Unlike other antidiabetic, Metformin hydrochloride does not induce hypoglycemia at any reasonable dose. In spite of its favorable clinical response and lack of significant drawbacks, chronic therapy with Metformin hydrochloride suffers from certain specific problems of which, the most prominent being the high dose (1.5-2.0 g/day), and low bioavailability (60%). Therefore, there are continued efforts to improve the pharmaceutical formulation of Metformin hydrochloride in order to achieve an optimal therapy [2, 3, 4].

The purpose of the present investigation was fabrication and characterization of floating drug delivery system for anti-diabetic drug Metformin Hydrochloride to improve gastro retention of drug in the stomach. The gastroretentive drug delivery system

can be retained in the stomach and assist in improving the oral sustained delivery of drugs that have an absorption window in a particular region of GIT [5]. Several approaches are currently used to prolong gastric retention time. These include floating drug delivery system, also known as hydrodynamically balanced systems, swelling systems and expanding systems, polymeric bioadhesive systems, high-density systems, and other delayed gastric emptying devices. Floating microspheres is one of the most feasible approach to obtain prolonged or controlled drug delivery, to improve bioavailability and to target drug to specific sites. Floating microspheres can also offer advantages like reducing plasma fluctuation, reducing side effects, decreasing dosing frequency, and improving patient compliance [6,7].

Eudragit RS 100 is referred to as amino methacrylate polymer, with having 5% functional quaternary ammonium groups. Eudragit RS 100 is a water-insoluble polymer that is widely used as a coating material for sustained release microspheres of highly water soluble drugs like Metformin hydrochloride. It is widely acceptable due to its biocompatibility, good stability, easy fabrication and low cost. The aim of this study was to prepare Eudragit RS 100 floating microspheres containing Metformin hydrochloride to achieve a controlled release profile suitable for oral administration [8,9]. Firstly, we investigated some formulation variables (polymer: drug ratio, stirring

speed, concentration of surfactant) to obtain spherical particles. Then yield of production, particle size analysis, encapsulation efficiency, surface properties, and Metformin hydrochloride release rate from microspheres were investigated. The influences of formulation variables on the microspheres properties were examined and the microsphere formulations suitable to achieve our goal were determined [10].

2. MATERIALS AND METHODS

Metformin hydrochloride was obtained from Alkem laboratories, Mumbai as a gift sample. Eudragit RS 100, Span 20, liquid paraffin, and n-hexane were purchased from S.D. Fine chemicals, Mumbai. Other chemicals used were all of analytical grade.

2.1 Preparation of microspheres

Metformin hydrochloride floating microspheres were prepared by non-aqueous solvent evaporation technique. Different amounts of polymer (250, 500, 750, 1000, and 1250 mg) was dissolved in 25 ml of ethanol by using a magnetic stirrer (Popular India Limited, Mumbai). Powdered Metformin hydrochloride (250 mg) was dispersed in polymer solution.

The resulting dispersion was then poured into a vessel of 1000 ml containing the mixture of 270 ml liquid paraffin and 30 ml n-hexane while stirring. Span 20 was added drop by drop into vessel during stirring. A mechanical stirrer with a blade (6 cm) diameter was used. Stirring was continued for two hours, until ethanol evaporated completely. Drug: polymer ratio (1:1, 1:2, 1:3, 1:4, and 1:5 w/w), span 20 (0.2, 0.3, 0.4, 0.5 %) and stirring speed (500, 750, 1000 rpm) of the system were changed to obtain spherical particles. After evaporation of ethanol, the microspheres formed were collected by filtration, washed 4-5 times with 50 ml n-hexane each and dried at room temperature for 24 hours.

Table 1. Optimization of drug polymer ratio

Batch	Drug: Polymer ratio	Average diameter (µm)	DEE (%w/w)	Buoyancy (%)
ES 1	1:1	209(±2.36)	72.68(±1.53)	91
ES 2	1:2	237(±2.15)	76.47(±2.24)	92
ES 3	1:3	248(±1.56)	79.12(±2.16)	95
ES 4	1:4	271(±2.47)	78.57(±2.96)	94
ES 5	1:5	284(±1.98)	78.78(±3.05)	94

2.2 Optimization of stirring speed

Stirring speed plays an important role in the microspheres size distribution and drug loading. Microspheres were prepared by the method described above with optimized ratio of drug and the polymer (1:3), keeping surfactant concentration (0.2%) constant, utilizing three different speeds i.e. 500, 750, and 1000 rpm [11].

Table 2. Optimization of stirring speed

Batch	RPM	Average diameter (µm)	DEE (%wt/wt)
ES 3	500	320 (± 2.5 mm)	72.59 (± 2.7)
ES 3	750	265 (± 1.3 mm)	79.18 (± 1.5)
ES 3	1000	257 (± 2.2 mm)	75.23 (± 2.8)

2.3 Optimization of emulsifier (span 20)

Concentration of emulsifier is an important parameter which needs to be optimized for optimum particle size and stability of the microspheres. Span 20 was used as an emulsifier and various concentrations of span 20 were taken. Microspheres were prepared according to method described above with optimized drug polymer ratio i.e. 1:3 and stirring speed 750 rpm with various concentrations i.e. 0.2 %, 0.3 %, 0.4 % and 0.5 % v/v of span 20.

Table 3. Optimization of emulsifier (span 20)

Batch	Span 20 (%V/V)	Average diameter (µm)	DEE (%w/w)
ES 3	0.2	310 (±2.11 mm)	70.43 (±2.25)
ES 3	0.3	305 (±2.45 mm)	72.68 (±1.86)
ES 3	0.4	270 (±3.15 mm)	77.43 (±2.25)
ES 3	0.5	267 (±2.27 mm)	71.63 (±1.26)

Microspheres dried at room temperature were then weighed and yield of microsphere preparation was calculated using the formula:

$$\text{Percentage yield} = \frac{\text{The amount of microspheres obtained (gm)}}{\text{The theoretical yield (gm)}} \times 100$$

Scanning electron microscopy

Shapes and surface characteristics of the microspheres were investigated and photographed using scanning electron microscopy.

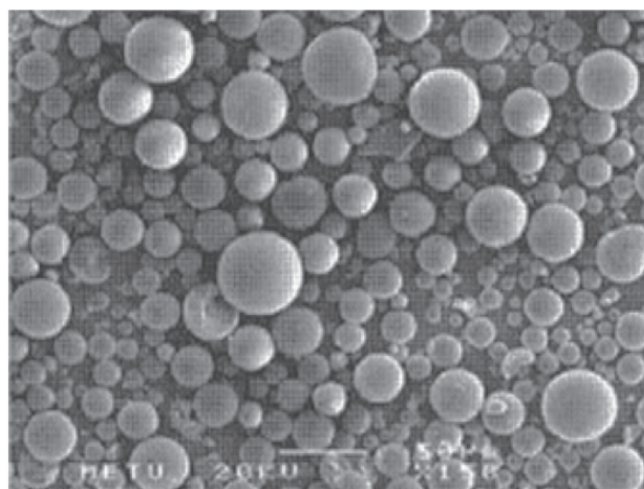


Fig. 1. Scanning Electron Microscopy of Metformin loaded floating microsphere

2.4 Determination of mean particle size

Mean particle size of microspheres was determined by using optical microscopy (Table 1).

2.5 Drug entrapment efficiency

A quantity of microspheres containing 100 mg equivalent of Metformin hydrochloride were incubated in 0.1 N HCl for 24 hours to determine drug entrapment efficiency. Metformin hydrochloride concentration was determined by measuring absorbance at 233 nm against reagent black (Table 1).

2.6% Buoyancy

% buoyancy was carried out using 0.1 N HCl containing 1% span 20 as a dispersing medium. Microspheres were spread over the surface of 500 ml of dispersing medium at 37± 0.5°C. A paddle rotating at 100 rpm agitated the medium. Each fraction of

microspheres floating on the surface and those settled down were collected at a predetermined time point. The collected samples were weighed after drying [12].

Percent buoyancy =

$$\frac{\text{Weight of microspheres floating on surface}}{\text{Weight of microspheres floating on surface}} \times 100$$

2.7 In-vitro release studies

In vitro drug release study of all the batches were carried out by paddle method using USP type 2 apparatus using 900 ml of 0.1 N HCl as dissolution medium at 100 rpm and 37±0.5°C. A quantity of microspheres containing 100 mg equivalent of Metformin hydrochloride was placed in the dissolution medium. The samples were withdrawn at a predetermined time interval, diluted approximately and were analyzed spectrophotometrically at 233 nm against reagent blank.

Table 4. In-vitro release profile of all formulations in 0.1 N HCl

Time (hrs)	Percent cumulative drug release ± S.D.				
	ES 1	ES 2	ES 3	ES 4	ES 5
01	20.15 (± 1.0)	19.20 (± 1.10)	19.23 (±0.68)	19.21 (±0.75)	20.63 (±0.68)
02	27.75 (± 1.2)	24.91 (± 1.19)	23.49 (±1.17)	26.80 (±1.34)	31.53 (±1.22)
03	33.91 (± 2.1)	32.95 (± 2.14)	31.52 (±2.12)	34.85 (±2.35)	41.44 (±2.32)
04	41.46 (± 1.8)	41.01 (± 1.84)	37.68 (±1.95)	43.38 (±2.9)	51.44 (±2.09)
05	47.17 (± 2.2)	50.49 (± 2.16)	44.33 (±2.07)	50.12 (±1.98)	58.06 (±2.01)
06	54.25 (± 2.2)	58.53 (± 2.23)	52.34 (±2.29)	56.17 (±2.47)	65.17 (±2.37)
07	59.91 (± 2.8)	65.17 (± 2.08)	59.46 (±2.36)	63.27 (±2.42)	69.42 (±2.84)
08	67.50 (± 2.7)	69.90 (± 2.81)	65.86 (±2.08)	69.42 (±2.38)	73.22 (±2.18)
09	74.12 (± 3.2)	74.64 (± 3.14)	73.22 (±3.11)	73.69 (±3.05)	74.64 (±3.10)
10	78.40 (± 3.4)	78.91 (± 3.4)	77.49 (±3.3)	76.53 (±3.05)	76.53 (±3.40)
11	82.59 (± 3.1)	80.33 (± 3.01)	79.84 (±2.10)	78.42 (±3.05)	77.95 (±3.10)
12	85.11 (± 3.2)	84.08 (± 3.10)	83.27 (±3.0)	81.33 (±3.15)	80.91 (±2.55)

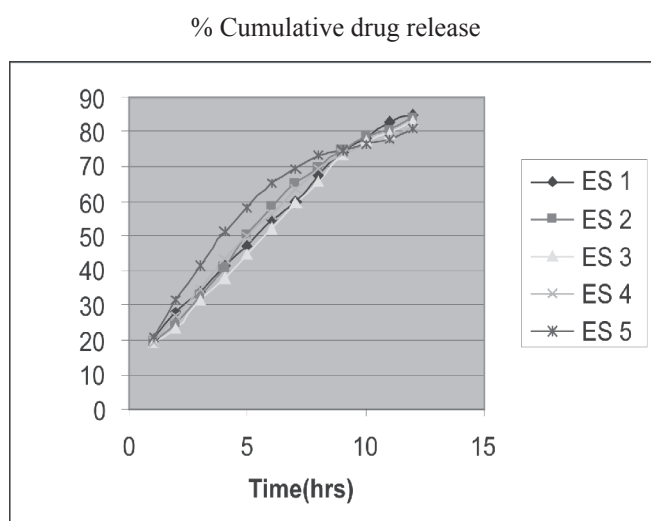


Fig. 2. In vitro release curve of Metformin Hydrochloride in 0.1 N HCl

Table 5. Correlation coefficient of optimized batch (ES3)

No.	Zero order	First order	Hixon crowell plot	Higuchi plot	korsmeyer peppas
1	0.9669	0.9942	0.916	0.9912	0.8779

2.8 Statistical analysis

The data obtained release rate determination studies of Metformin hydrochloride microspheres were analyzed statistically with one-way ANOVA followed by Newman-Keuls Multiple Comparison value obtained ($p < 0.05$) was considered to be statistically significant [13].

3. RESULT AND DISCUSSION

Non-aqueous solvent evaporation method was used to prepare Metformin hydrochloride loaded floating microspheres of Eudragit RS100. Ethanol was used as a solvent to dissolve the

drug and polymer whereas liquid paraffin along with n-hexane in a ratio of (9:1) is used as dispersing medium. Various formulations with different drug: polymer ratios were tried, stirring speed was also changed to obtain spherical particle. When drug: polymer ratio was too low (1:1, w/w) no spherical particle were obtained independent of stirring speed of the system. This shows that the viscosity of the inner phase is an important factor for the preparation of microspheres. Keeping the drug amount and the solvent volume constant, spherical particles were obtained as the amount of polymer increased to give a polymer drug ratio (3:1) (stirring speed 750 or 500 rpm) or 4:1 (stirring speed 750 rpm). However, when polymer : drug ratio was (3:1), the shape of particles were irregular at 500 rpm, because for this high polymer concentration, this stirring speed was not fast enough to disperse inner phase in outer phase. When stirring speed was 750 rpm the best spherical particles with good surface characteristics were obtained with the polymer: drug ratio of 3:1. Scanning electron micrographs of the microspheres prepared are shown (Fig. 1).

Drug entrapment efficiency was found to increase with increase in polymer concentration. At 3:1 polymer: drug ratio (ES 3) drug entrapment efficiency was found to be maximum 79.12(±2.16). % buoyancy of optimized formulation (ES 3) found to be 95% (Table 3).

Most of the microspheres obtained were collected in the size range of 200-300 μm by all formulation (Table 1). Increasing the polymer: drug ratio caused the mean particle size to shift towards a higher particle size. Higher concentration of polymer produced a more viscous dispersion which formed larger droplet and consequently larger microspheres.

Increasing the stirring speed decreased the particle size of microspheres. The yield of preparation and Metformin hydrochloride entrapment efficiencies were high for all formulations and maximum for optimized formulation (ES 3) (Table 2.)

The drug release rate from microspheres were studied at pH 1.2 or (0.1 N HCl) using the USP type 2 paddle method. The in vitro drug release profile was biphasic with an initial burst release (19.23 %) in 1 hour attributed to surface associated drug, followed by a slower release phase as the entrapped drug slowly diffuse out into the release medium. 83.27 % drug release after 12 hours there was a sustain release of drug at a constant rate (Fig. 2). The diffusion of drug, the erosion and degradation of polymer are the main mechanism for the drug release (Table 4).

Kinetics model further support the above statement. Zero order, first order, hixon crowell cube root plot, korsmeyer peppas, higuchi plot were applied on optimized formulation. The n value and r^2 value show that the formulation releases the drug by erosion as well as diffusion and optimized batch follow this release kinetic model (Table 5).

Statistical analysis was carried out by applying one way ANOVA followed by Newman Keuls Multiple Comparison, value obtained ($p < 0.05$) was considered to be statistically significant. The studied showed that drug release from all formulations was not found to be statistically significant. But on the basis of required size, shape, drug entrapment efficiency of floating microspheres ES 3, drug: polymer ratio (1:3) was found to be optimum batch.

4. CONCLUSION

Metformin hydrochloride loaded floating microspheres were prepared successfully using non-aqueous solvent evaporation method. Polymer: drug ratio and stirring speed of the system were important to obtain spherical particles. The yield of preparation and entrapment efficiency were high for all formulations. Metformin hydrochloride is highly water soluble drug which gives a controlled release from EudragitRS100 microspheres. Thus gastroretentive floating microspheres of Metformin hydrochloride supposed to remain in the stomach for longer period of time and give controlled release. These formulations can reduce dosing frequency, decrease side effects and improve patient compliance.

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