Metformin Hydrochloride Floating Microspheres: Design and In Vitro and In Vivo Evaluation for Oral Controlled Release

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ABSTRACT

To create once-daily composition, the floating microspheres were used to provide extended and uniform release in the stomach. Short processing times, little exposure of materials to high temperatures, and high encapsulation effectiveness are main benefits of this technique. The manufacture of metformin hydrochloride floating microspheres, assessment of the in vitro release from the Floating Drug Delivery System, and adjustment of the floatation and drug release pattern to meet the desired release profile were all examined in the current work. Using the Eudragit RS100 controlling polymer and 250 mg of metformin hydrochloride per batch, floating microspheres were created. The in vitro release performance of these microspheres was then assessed using the standard pharmacopoeial tests, including yield (%), particle size analysis, floating ability, drug entrapment efficiency, surface topography, and release studies. The result showed that mixing ratio of components in organic phase affected the size distribution (206.3-469.8 µm), drug entrapment efficiency (58.27-40.06 (% wt/wt),% yield (78.27-75.44% wt/wt), floating ability (82.67-60.84%) as well as drug release of floating microspheres (53.06-74.84% after 12 hrs), floating duration (8-12 hrs) and the most effective results were obtained at the ratio of drug: polymer, stirring speed, droplet stabilizer(1:5, 700rpm, 10%wt/wt). The decline in glucose levels was slower and was discovered to be 106.000.54mg/dl attained 8 hours after oral treatment in the case of floating microspheres of metformin hydrochloride. Because metformin hydrochloride was released and absorbed slowly over a longer length of time, a sustained hypoglycemia impact was seen over longer durations of time in the case of floating microspheres.

Keywords: *In-vivo, Floating Microspheres, Metformin Hydrochloride, Controlled Release.*

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INTRODUCTION

In order to increase the bioavailability of medications and enhance the residence time of the dosage form at the site of application or absorption, floating drug delivery systems have been studied [1,2]. These systems also make it easier for the dosage form to make intimate contact with the underlying absorption surface. For oral routes, floating drug delivery systems in the form of tablets, capsules, beads, and granules have been described in a number of studies; however, there are surprisingly few publications on floating microspheres.

The goal of this project is to create, describe, and assess metformin hydrochloride floating microspheres using low density base polymers for extended gastrointestinal absorption. The heart of microencapsulation was metformin hydrochloride, an efficient antidiabetic that requires regulated release due to its brief biological half-life of 5.4 0.6 hours [3,4]. The floating microspheres were tested for controlled release using in vitro and in vivo techniques.

MATERIALS AND METHODS

Materials

Metformin hydrochloride sample was taken from Lifecare Neuro Products Limited (Baddi, Himanchal Pradesh, India). Eudragit RS 100 sample was taken from Evonik Industries. Magnesium stearates were procured from commercial sources. All other reagents used were of analytical grade.

Methods

Single Emulsion Solvent EvaporationMethod

The active ingredient metformin hydrochloride (250 mg) was dispersed in ethanol (50 ml) and stirred well with a magnetic stirrer to make a transparent solution. The microspheres containing metformin hydrochloride were made by single emulsion solvent evaporation technique (Eudragit RS 100 1250 mg).

In this method the drug – metforminhydrochloride and polymer- Eudragit RS100 into 1:5 ratios were dispersed in 50ml ethanol. Magnesium stearate (10%wt/wt) was dispersed in the drug and polymer solution which acted as a droplet stabilizer.

With constant mechanical stirring at a speed of 700 rpm, the dispersion was added to 250 ml of light liquid paraffin containing 30 ml n- hexane. The ethanol was stirred continuously for a further two hours until it fully evaporated.

The microspheres formed were collected by filtration in vacuum, washed repeatedly with petroleum ether each and dried at 30±2°C for 24 hours. The microspheres prepared along with their drug: polymerare listed in Table 1. [5,6,8].

Optimization of Drug and Polymer Ratio

Various batches F1, F2, F3, F4, F5, F6, F7, F8, F9 and F10 with different drug: polymer ratio i.e.1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, and 1:10 were prepared by single emulsion solvent evaporation method. Keeping stirring speed 700rpm, droplet stabilizer magnesium stearate(150mg) and solidifying agent n-hexane.

Table 1. Optimization of Drug and Polymer Ratio

Batch	Drug: Polymer	an ± S. D.,n=10	Mean ± S. D., n=3				
		Average Diameter (µm)	Floating Duration (Hrs)	Floating Ability(%)	Yield (%wt/wt)	DEE (%wt/wt)	Drug Loading (%wt/wt)
F1	1:1	206.3(±2.497)	11	82.673 (±0.577)	78.27 (± 0.015)	58.27 (±0.020)	25.32 (±0.010)
F2	1:2	223.7(±2.946)	11	84.83 (±0.763)	79.02 (± 0.009)	62.36 (±0.025)	18.88 (±0.015)
F3	1:3	259.8(±4.315)	12	86.01 (±0.215)	79.05 (± 0.040)	74.22 (±0.015)	17.24 (±0.010)
F4	1:4	259.1(±4.818)	12	89.60 (±0.360)	81.18 (± 0.042)	77.51 (±0.015)	14.60 (±0.020)
F5	1:5	255.1(±2.726)	12	95.47 (±0.416)	82.56 (± 0.035)	79.38 (±0.015)	12.61 (±0.010)
F6	1:6	293.5(±2.838)	10	79.78 (±0.503)	80.99 (± 0.100)	61.32 (±0.010)	8.38 (±0.010)
F7	1:7	314.7(±7.040)	10	73.72 (±0.249)	79.23 (± 0.035)	53.32 (±0.015)	6.42 (±0.005)
F8	1:8	354.5(±5.949)	9	68.47 (±0.551)	77.82 (± 0.020)	42.73 (±0.026)	4.58 (±0.017)
F9	1:9	375.1(±8.252)	8	64.90 (±0.360)	74.55 (± 0.026)	41.04 (±0.051)	3.97 (±0.005)
F10	1:10	469.8(±5.978)	8	60.84 (±0.764)	75.44 (± 0.210)	40.06 (±0.065)	3.53 (±0.005)

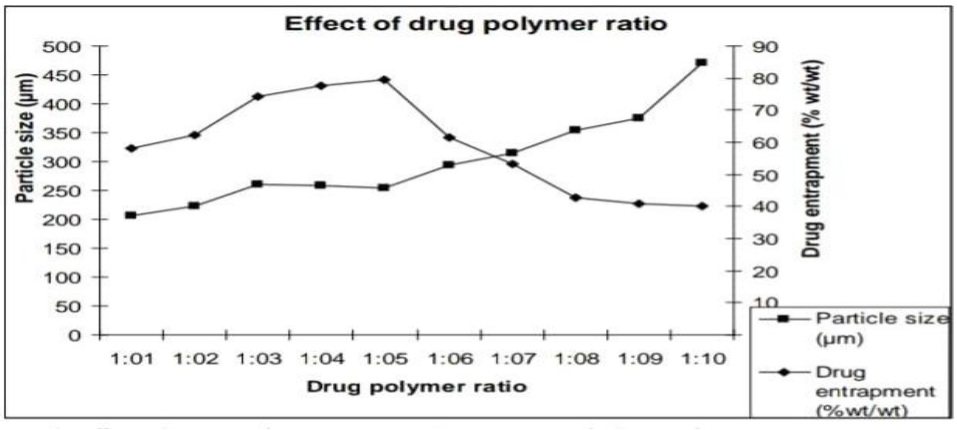

Fig. 1. Effect of Drug-Polymer Ratio on Average Particle Size and Percent Drug EntrapmentEfficiency

Optimization of Stirring Speed

Floating microspheres were prepared by single emulsion solvent evaporation method with optimized ratio of drug and polymer (1:5) keeping the quantity of droplet stabilize and hardening agent constant, utilizing four different speeds i.e.500, 700, 1000 and 1300 rpm.

Table 2. Optimization of Stirring Speed

Batch	RPM	an ± S. D.,n=10	Mean ± S. D., n=3				
		Average	Floating	Floating	Yield	DEE	Drug Loading

		Diameter(µm)	Duration (Hrs)	Ability (%)	(%wt/wt)	(%wt/wt)	(%wt/wt)
F5E1	500	294.5 (±2.89)	11	81.67 (±0.432)	77.27 (±0.028)	67.51 (±0.020)	10.71 (±0.015)
F5E2	700	255.1 (±2.72)	12	95.47 (±0.416)	82.56 (± 0.035)	79.38 (±0.015)	12.61 (±0.010)
F5E3	1000	208.3 (±2.57)	10	80.56 (±0.341)	75.27 (±0.020)	63.32 (±0.027)	10.02 (±0.013)
F5E4	1300	159.0 (±1.63)	10	78.23 (±0.540)	73.27 (±0.040)	59.30 (±0.022)	9.41 (±0.011)

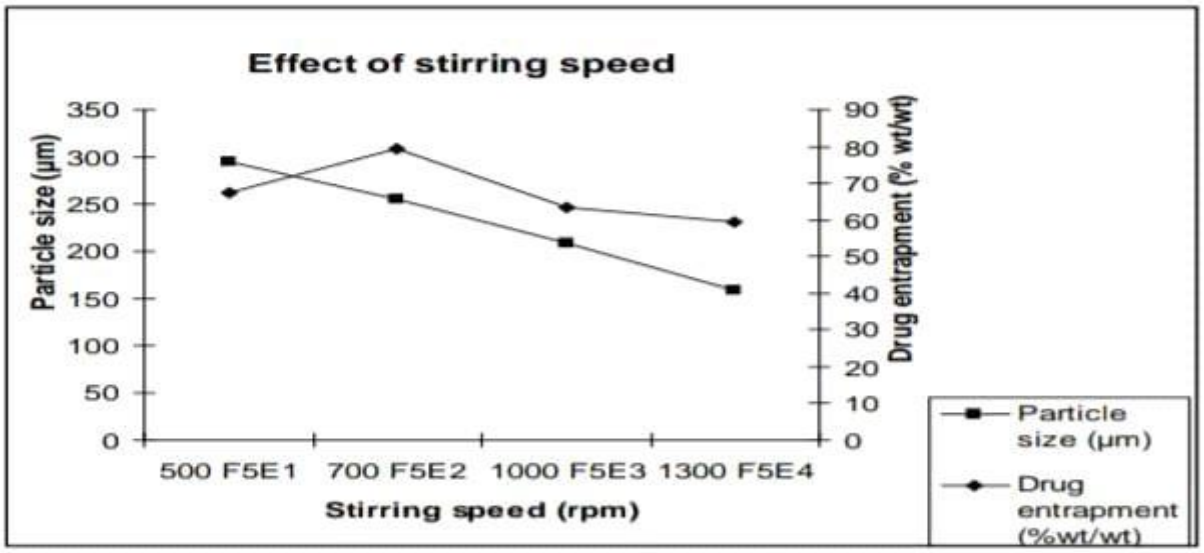

Fig 2. Effect of Stirring Speed on average particle size and percent drug entrapment

Optimization of Quantity of DropletStabilizer

Floating microspheres were prepared by keeping drug polymer ratio (1:5) and stirring speed (700) constant taking 5, 10and 15% wt/wt of magnesium stearate.

Table 3. Optimization of Quantity of Droplet Stabilizer

Batch	Droplet stabilizer (%wt/wt)	n ± S. D.,n=10	Mean ± S. D., n=3				
		Average Diameter(µm)	Floating Duration (Hrs)	Floating Ability (%`)	Yield (%wt/wt)	DEE (%wt/wt)	Drug Loading (%wt/wt)
F5E1	5	325.5 (±7.255)	10	80.59 (±0.344)	78.20 (±0.039)	76.21 (±0.014)	12.05 (±0.012)
F5E2	10	255.1 (±2.726)	12	95.47 (±0.416)	82.56 (± 0.035)	79.38 (±0.015)	12.61 (±0.010)
F5E3	15	210.0 (±2.595)	10	78.25 (±0.544)	80.20 (±0.042)	72.05 (±0.014)	11.43 (±0.011)

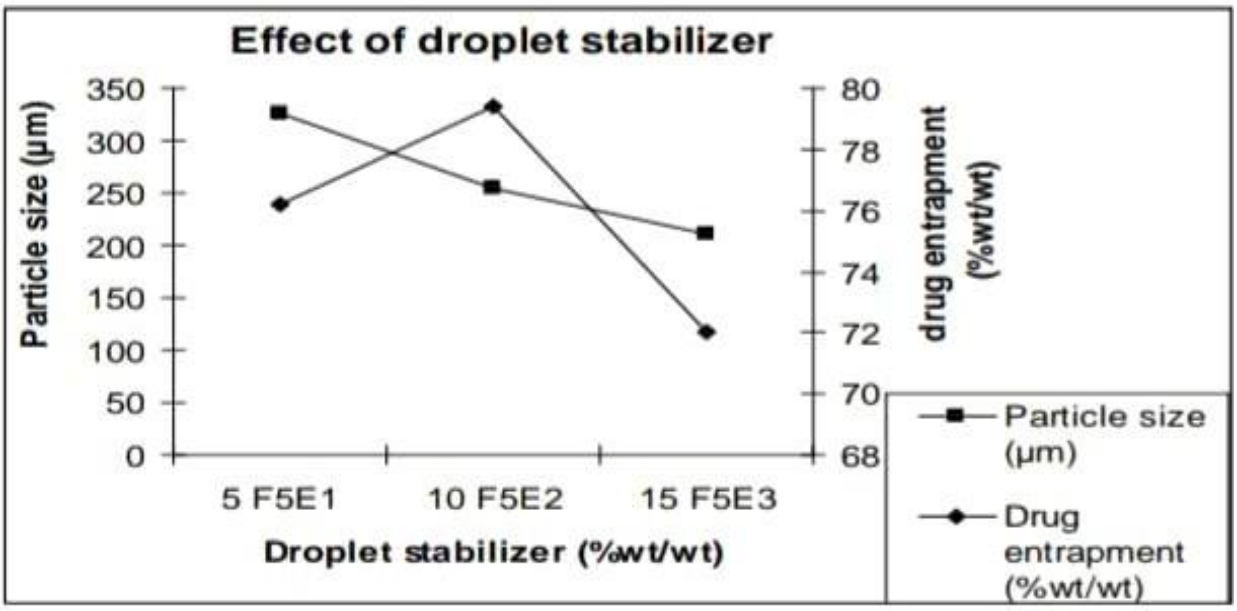

Fig. 3. Effect of Droplet stabilizer on Average Particle Size and Percent Drug Entrapment

Characterization and Evaluation ofFloating Microspheres

Scanning Electron Microscopy

The microspheres were observed under a scanning electron microscope. They were mounted directly onto the SEM sample stub using double-sided sticking tape and coated with gold film under reduced pressure (0.001mm of Hg).

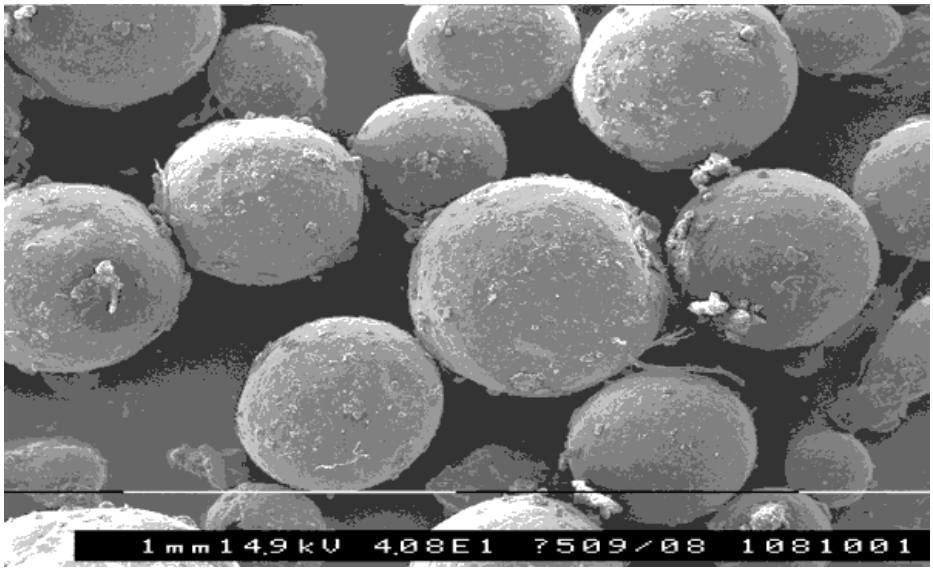

Fig. 4. SEM photograph of Metformin Hydrochloride Floating Microspheres

Drug loading, Drug Entrapment Efficiency

A quantity of microspheres containing equivalent to 50mg of metformin hydrochloride was taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots 100ml of 0.1 N HCl repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 0.1N HCl. The

solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically at 232nm against appropriate blank.[8]

The amount of drug entrapped in the microspheres was calculated using the following formula:

Drug Entrapment Efficiency =

(Amount of drug actually present/ Theoretical drug load) X 100

Drug loaded on to the microspheres was estimated using the following formula:

Drug loading =

(Amount of drug actually present/ total weight of microspheres) X 100

***In-Vitro* Evaluation of Floating Ability** The in vitro floating ability studies were carried out using Dissolution apparatus[USP XXIII apparatus no. 2 (paddle)].

In vitro floating ability of microspheres was studied simulated gastric fluid containing 1% Tween 80 as a dispersing medium. Microspheres were spread of over the surface of 900 ml of dispersion medium at 37±0.5°C and agitated by a paddle rotating at 100rpm. Each fraction of microspheres floating on the surface and those settled down were collected at a predetermined time point. The collected samples were weighted after drying.

% floating microspheres = (Weight of floating microspheres/initial weight of floating microspheres) X 100

***In-Vitro* Drug Release Study**

The in vitro drug release studies were carried out by paddle method using Dissolution apparatus [USP XXIII apparatus no. 2 (paddle)]. A quantity of microspheres equivalent to 100 mg of the drug was used. The 900ml of 0.1N HCl was used as dissolution fluid. The paddle was rotated at a speed of 100 rpm and the whole system was thermally controlled at 37±1ºC.

Five ml of the aliquots were withdraw at predetermined time intervals and filtered through whatmann filter paper. The samples were suitable diluted with 0.1N HCl and the solutions were analyzed for the drug content spectrophotometrically at

232 nm against reagent blank. The dissolution medium was replaced with same volume of 0.1N HCl to maintain sink condition [8,9]. From this percentage drug release is calculated and plotted against function of time study the pattern of drug release. The drug release experiments wereconducted in triplicate (n=3).

Table 4. Cumulative % release of Metformin Hydrochloride from Floating Microspheres in 0.1N HCl.

Time(h)	Cumulative % drug release									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
01	21.45	20.79	22.76	21.45	24.33	21.58	11.74	8.46	7.28	6.10
02	23.94	23.15	27.33	26.43	31.15	24.46	18.3	18.82	11.47	10.03
03	33.12	29.58	34.43	35.75	38.63	32.33	27.87	22.5	21.31	18.03
04	41.91	33.12	39.68	40.99	44.14	37.19	34.3	27.48	26.04	23.41
05	44.67	36.27	45.59	47.55	49	44.54	38.5	31.94	30.63	27.09
06	46.77	44.01	55.56	55.95	56.74	47.55	42.31	37.84	35.35	32.2
07	51.87	47.55	64.74	66.97	67.5	51.1	46.37	42.83	40.86	36.14
08	59.23	57.39	68.2	75.63	76.68	57.26	49.39	45.72	45.06	40.73
09	66.31	67.5	71.96	81.4	81.66	62.12	53.59	47.82	47.29	45.32
10	70.91	72.48	73.96	83.5	84.29	68.81	57	53.06	51.1	47.55
11	73.79	74.71	74.45	84.42	85.34	72.74	60.54	56.34	53.59	49.91
12	74.84	75.37	76.68	85.21	85.6	73.79	63.69	60.41	58.31	53.06

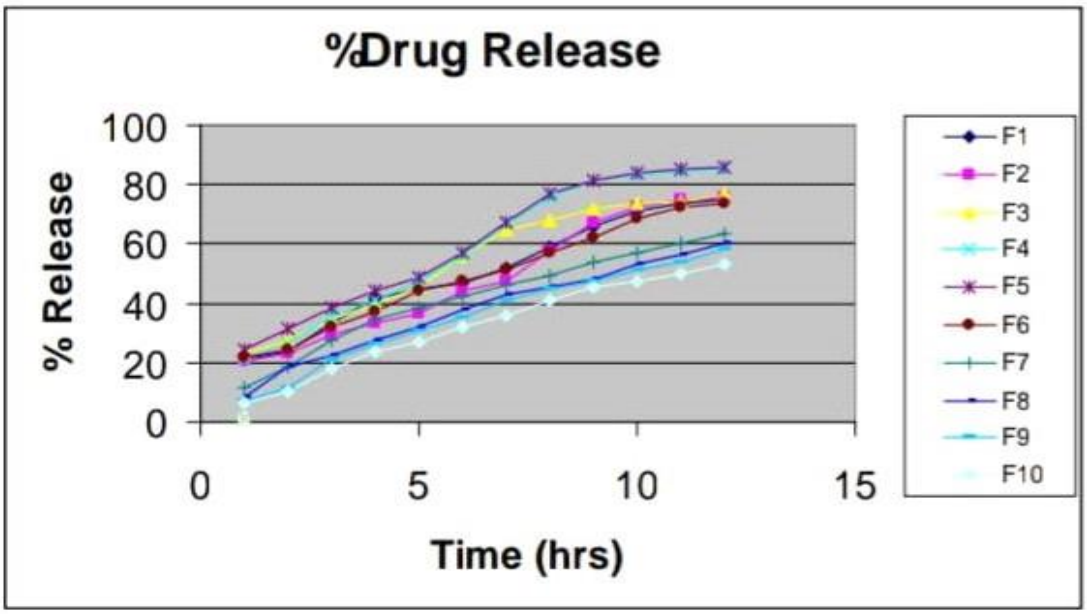

Fig. 5. In-vitro Release Profile of Floating Microspheres of Metformin Hydrochloride in 0.1N HCl.

Kinetic Modeling of Drug Release

The dissolution profile of all the batches was fitted to zero order, first order, Higuchi, Hixon-Crowell, Korsmeyer and Peppas to ascertain the kinetic modeling of drug release and the model with the highest correlation coefficient was considered to be the best model [12,13,14].

Stability Study

The floating microspheres of optimized formulation (F5) were put on short term stability study at 4^0C, 40^0C/75% RH and room temperature condition for the periods of three months. The floating microspheres were evaluated for residual drug content at after a period of 10, 20, 30, 60, and 90 day
[15].

***In Vivo* Evaluation**

The *in vivo* activity was required for controlled released action of floating microspheres over on conventional dosage form. The *in vivo* activity has been approved by Institutional Ethical Committee.

The animals used for experiment were adult male Albino rats (Wistar strain150- 250g). Total of 24 rats were taken, 6 rats in each group, and were fasted 16 hours before the day of experiment with free access to water. Diabetes was induced in all rats by intraperitonial injection of alloxan monohydrate. The dose of alloxan monohydrate was 125mg/kg body weight [1,3]. The vehicle used for preparation of alloxan monohydrate was normal saline [0.9% w/v].

A blunt end Cannula fitted with plastic syringe was used to administer orally the optimized formulation (floatingmicrospheres of metformin hydrochloride), and standard drug (metformin hydrochloride).

Animals of all the groups were treated with an oral D-glucose load of 2gm/kg by means of Cannula. Group I was a normal group and group II was diabetic control group. Group III, IV

were treated orally with floating microspheres of metformin hydrochloride at doses level of 144mg and standard drug (Metformin hydrochloride) 300 mg/kg b.w. solution.

Blood samples were withdrawn from the tail of each rat using sharp sterile blade under light ether anesthesia after 0min, 1hrs, 2hrs, 4hrs and 8 hours.and determined blood glucose concentration glucometer (Accu-Check) [16,17].

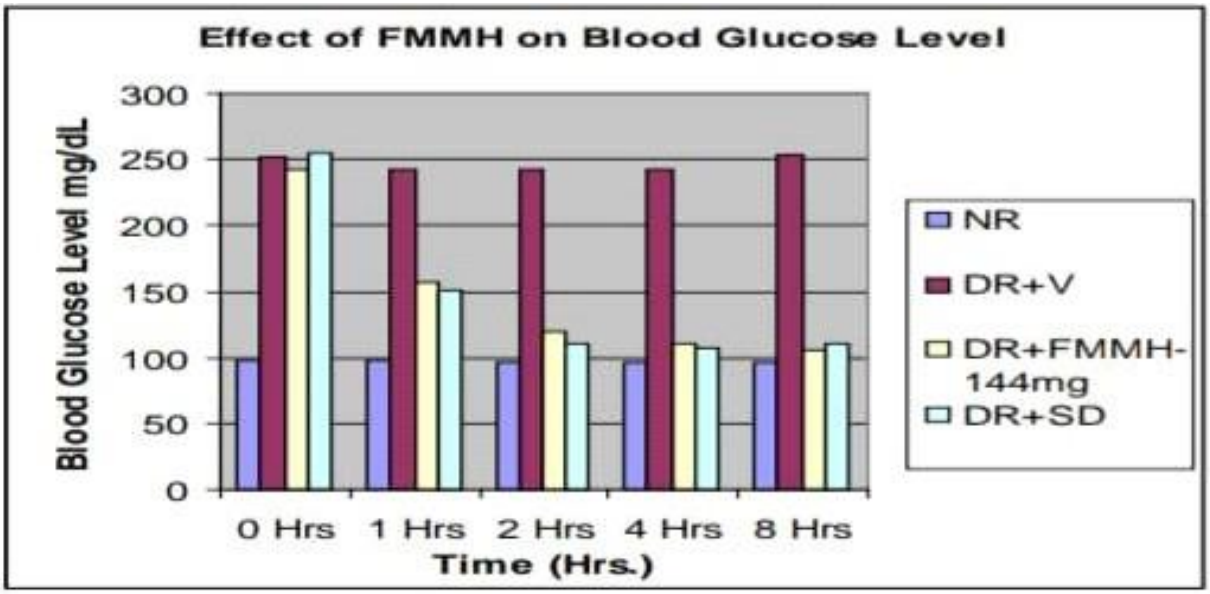

Fig. 6.Effect of FMMH on blood glucose level

Statistical Analysis

Analysis of variance (ANOVA) was preformed to find out significant difference among the formulations having optimum floating and drug release using Prism software. ANOVA was applied on

% cumulative drug release of metformin hydrochloride followed by Newman-Keuls Multiple comparison test.

Results and Discussion

The long term therapy of metformin hydrochloride is required for proper management of type-2 diabetes mellitus. Hence controlled drug delivery is demanded. Therefore, it was decided to formulate a therapeutic system bearing metformin hydrochloride, which delivers the drug through the oral route for prolonged period of time, decrease the dose related side effects and improve patient compliance.

The floating microspheres were prepared by single emulsion solvent evaporation technique by dissolving Eudragit RS 100 and metformin hydrochloride in ethanol under magnetic stirring. Magnesium stearate was selected as the droplet stabilizer. The process parameters which were optimized included drug: polymer ratio, stirring speed (rpm) and quantity of droplet stabilizer.

The mean diameter of floating microspheres of metformin hydrochloride was found to increase from 206.30µm to 469.80µm with increase in drug to polymer ratio from 1:1 to 1:10 (Table1, Figure 1). This increase in particle size can be attributed to an increase in viscosity with increasing polymer concentration, which resulted in larger emulsion droplets and finally larger microspheres size.

The mean diameters of floating microspheres prepared using various agitation speeds (i.e. 500, 700, 1000 and 1300rpm) were 294.50, 225.10, 208.30 and 159.0µm respectively. It was found that particle size decreased with increase in speed of agitation. It was found that at 700rpm stable microspheres formulation was achieved at 700rpm with average particle size, maximum drug entrapment and maximum floating ability of 255.1µm, 79.38 % wt/wt and 95.47 % respectively. (Table2, Figure 2. Figure 4).

The mean particle diameter of microspheres prepared using various quantity of droplet stabilizer (*i.e.* 5, 10 and 15% wt/wt) was 325.5, 255.1 and 210µm respectively. The mean particle size of floating microspheres was found to increase with decreasing amount of magnesium stearate. As the droplet stabilizer amount increased, the shape of microspheres became irregular and the sizeof the microspheres was reduced from
325.5 µm to 210 µm. The size of microspheres was found to increase because low magnesium stearate content failed to prevent droplet coalescence in the oil medium. On the other hand the mean particle size decreased on increasing the amount of magnesium stearate (15% wt/wt). This was a consequence of stabilization of the oil droplets with magnesium stearate. Spherical, discrete microspheres were formed when magnesium stearate content was maintained at (10 % wt/wt). (Table 3, Figure 3.) Percent drug entrapment and loading were found to be 79.38 % wt/wt and 12.61 % wt/wt respectively.

In vitro drug release study for all the batches was carried out using USP type II apparatus using 0.1N HCl as dissolution medium, at 100rpm and 37±1ºC. All the batches showed an initial burst release (21.40-6.10)% in 1 hour which is attributed to surface associated drug, followed by a slower release phase as entrapped drug slowly diffuses out into the release medium. After 12 hrs 74.84- 56.06% of drug was released for formulations F1 to F10. (Table 4, Figure5.)

The optimized batch F5 showed an initial burst release (24.33%) in 1 hour attributed to surface associated drug, followed by a slower release phase as entrapped drug slowly diffuses out into the release medium, 85.6% drug was released after 12 hours. There was a sustained release of drug at a constant rate. The release of the drug at controlled rate from microparticles was due to the formation of pores. Kinetic models further support the above statements. The n value and r^2 value of the optimized batch was found to be 0.5641 and 0.9741 respectively, which shows that the formulation released the drug by the process of diffusion.

The $t_{10\%}$ obtained in case of formulation stored at 4±1C and 40±1˚C were found lower in comparison with the formulationstored at room temperature which

indicated that the formulation tend todegrade faster at lower and higher temperatures. The results of stability studies suggest that for adequate shelf life of floating microspheres formulation theideal storage temperature is roomtemperature.

In case of floating microspheres of metformin hydrochloride, the reduction in glucose levels was slower and found to be 106.00±0.54mg/dl reached 8 hours after oral administration. The sustain hypoglycemic effect observed over longer periods of time in the case of floating microspheres was due to the controlled release and absorption of metformin hydrochloride over longer period of time (Figure 6).

CONCLUSION

Thus, spherical microspheres consisting of Eudragit RS100 containing metformin hydrochloride could be prepared by single solvent evaporation method. The microspheres exhibited good floating properties in an in vitro test. Metformin hydrochloride release from these floating microspheres slow and extended over longer periods of time. Drug release was diffusion controlled followed zero, Korsmeyer-Peppas, order kinetics after a long period of 12 hrs. in the in vivo evaluation Eudragit RS 100 floating microspheres could sustained the hypoglycemic effect of metformin hydrochloride over a 8 hrs period. These floating microspheres are thus, suitable forcontrolled release of metforminhydrochloride.

Results of present study provided an insight into the significance of floating microspheres as an oral delivery device forantidiabetic drug metformin hydrochloride. The investigated system has potential and is capable of maintaining constant drug concentration over prolonged period due to its controlled action. This can be expected to reduce the frequency of administration seen with repeated administration of conventional metformin hydrochloride loaded dosage forms, which ultimately improves the patient compliance.

It is concluded that a controlled release floating microspheres metformin hydrochloride formulation with satisfactory in vitro release characteristics and in vivo antidiabetic effect was successfully prepared.

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