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DESIGN AND STATISTICAL OPTIMIZATION OF METFORMIN HYDROCHLORIDE LOADED FLOATING MICROSPHERES: INFLUENCE OF NATURAL POLYMERS

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Keywords:

Floating microspheres, Metformin hydrochloride, Tamarind gum powder, Neem gum powder, Factorial design approach

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ABSTRACT: Floating microspheres have been showing high potential for gastro retention and provide an efficient means of enhancing bioavailability and controlling the release of drugs. Metformin hydrochloride is an effective antidiabetic that required controlled release due to its Short half-life of about 4.5-7 hours and bioavailability 50-60 %. The present research works aim is to develop, evaluate and statistically optimize the Metformin hydrochloride loaded floating microspheres for treatment of type 2 Diabetes mellitus. Six formulations were prepared by ionotropic gelation method by using various polymers such as HPMC K 100 M, Carbopol 934 P, Sodium bicarbonate etc and natural polymers such as tamarind gum powder and neem gum powder. The prepared microspheres were analyzed for particle size, surface morphology, % yield, % drug entrapment efficiency, *in-vitro* buoyancy, *in-vitro* drug release studies, *in-vitro* drug release kinetics, stability studies *etc*. From the preliminary trial, F4 Formulation was found to be the best formulation with Particle size of 820± 6.15 µm, *in-vitro* buoyancy of 95.6% and in vitro drug release of 76.33%. From statistically optimized data it was observed that R2 formulation was the best optimized formulation. Results clearly indicate that floating microspheres of Metformin hydrochloride offers a suitable, practical approach to achieve a prolonged gastric residence time and continuous release of the medication over an extended period of time thus oral bioavailability of the drug and subsequent efficacy is improved.

INTRODUCTION: Drug absorption in GIT is highly variable procedure and enhancing gastric retention of dosage form extends the time for drug absorption. Gastro retentive drug delivery system are those which retain in the stomach for prolonged period of time there by release the drug slowly from the delivery systems and maintain a constant drug concentration in the blood plasma. Multiple unit dosage form has proven the lower possibility of dose dumping as well as reduced inter and intra subject variability of the drug absorption Floating microspheres have been showing high potential for gastro retention and provide an efficient means of enhancing bioavailability and controlling the release of drugs [1, 2].

Metformin hydrochloride is an effective antidiabetic that required controlled release due to its Short half-life of about 1.5-4.5 hours and bioavailability 50-60 %. The side effects associated with Metformin Hydrochloride (HCl) is that it may cause GIT (Gastro Intestinal Tract) upset, nausea, vomiting, mild diarrhea, abdominal pain, anorexia *etc* [3]. It is slowly and incompletely absorbed from the GIT and well absorbed from the upper part of GIT.MH (Metformin Hydrochloride) does not bind

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to plasma proteins and is excreted unchanged in the urine [4]. Conventional oral sustained release (SR) formulations are designed to release the drug at a constant rate all along the GIT with a sizeable percentage of drug being released in the colon for absorption [5]. However in the case of Metformin HCl, its poor colonic absorption in healthy human subjects is reported to result in incomplete absorption in the conventional SR formulations. Therefore, this drug may be formulated as GRDDS in order to minimize frequent dosing with more uniform drug levels and to improve oral bioavailability [6]. The present work was undertaken to prepare metformin hydrochloride loaded floating microspheres for the treatment of type II diabetes mellitus and to determine the influence of natural polymers on the formulation. Preliminary trial batch were statistically optimized by fitting into 2^2 factorial design to select the best formulation by carrying out the evaluation.

MATERIALS AND METHODS:

Materials: Metformin hydrochloride was obtained as a gift sample True Care Pharmaceuticals, Pondicherry. Hydroxypropyl methyl cellulose K4M was obtained from Spectrum Reagents and Chemicals Cochin.Carbopol 934 P, calcium chloride and sodium alginate were purchased from Yarrow Chem Products, Mumbai. Tamarind gum powder was obtained from Nice Chemicals, Cochin and Neem gum powder was obtained from Neoteric, Coimbatore

Method of Preparation of Floating Microspheres: Metformin hydrochloride loaded floating microspheres were prepared by ionotropic gelation method. Six formulations were prepared with different ratio of HPMC K4M, Carbopol 934 and sodium alginate with Calcium chloride as counter ion. Tamarind gum powder and neem gum powder were used as the natural polymers. 3 % Sodium alginate was dissolved in 50 ml distilled water and stirred by using a mechanical stirrer (Remi Motors, Mumbai, India). To the resultant solution, 500mg Metformin hydrochloride and the different amount of HPMC K4M, Carbopol 934 P, sodium bicarbonate, tamarind gum powder and neem gum powder were added consecutively to the alginate solution. Mixed them thoroughly for 1hr with the magnetic stirrer (Remi Equipments, Mumbai, India). For microsphere formation, the drug-polymer solution was dropped through 21G needle into 100 ml 7.5%w/v aqueous solution of Calcium chloride.

The solution was continuously stirred at 100-rpm by using a magnetic stirrer at room temperature for 10 min. The resultant microspheres were filtered and washed 2 times with distilled water. Then the formulated microspheres were dried at room temperature for 24 hrs [7, 8]. **Table 1** Enlisted the different formulation batches prepared by using different concentrations of the HPMC K 100 M, sodium alginate, sodium bicarbonate, calcium chloride, tamarind gum powder and neem gum powder. Total six formulation batches were prepared (F1 to F6) and evaluated for percent yield, drug content, drug entrapment efficiency, particle size and surface morphology, *in-vitro* buoyancy, *in-vitro* drug release etc.

TABLE 1: FORMULATION DESIGN OF METFORMIN HCL FLOATING MICROSPHERES

Sl. no.	Ingredients	Formula (mg)					
		F1	F2	F3	F4	F5	F6
1.	Metformin hydrochloride	500	500	500	500	500	500
2.	HPMC K 100 M	500	200	200	-	200	200
3.	Carbopol 934	650	650	650	650	650	650
4.	Tamarind gum powder	-	100	200	250	300	-
5.	Neem gum powder	-	200	100	250	-	300
6.	Sodium bicarbonate	100	100	100	100	100	100
7.	Sodium alginate	3%	3%	3%	3%	3%	3%
8.	Calcium chloride	7.5%	7.5%	7.5%	7.5%	7.5%	7.5%

Statistical Optimization: Statistical optimization of the formulations was carried out by factorial design approach. In the present study was two factor two level Factorial Design was employed. The experimental trails were performed at 4 possible combinations and 2 independent formulation variables were evaluated. The independent variables were concentration of

HPMC K 100 M and concentration of tamarind gum powder. The dependent variables were in vitro drug release and in vitro buoyancy.

Characterization of Floating Microspheres

Percentage Yield: The percent yield of floating microspheres was calculated according to following formula:

% yield = (Mass of floating microspheres) / (Mass of drug+Mass of polymer) × 100

Drug Content and Drug Entrapment Efficiency: Microspheres equivalent to 50 mg of the drug were crushed and extracting with aliquots of 0.1N HCl (pH-1.2) repeatedly. The extract was transferred to a100 mL volumetric flask and the volume was made up using 0.1N HCl (pH-1.2). The solution was filtered and the absorbance was measured at 234 nm against appropriate blank [9]. The % of drug loaded and entrapped in the floating microspheres was calculated by the following formulas:

% Drug loading = Weight of the drug loaded in the microspheres (DC) / Total weight of the microspheres × 100

% Drug entrapment = Amount of drug actually present (DC) / Theoretical drug loaded expected × 100

(DC-Actual Drug Content)

Percentage *In-vitro* Buoyancy: To determine the *in-vitro* buoyancy, 50 mg formulated Metformin hydrochloride floating microspheres were placed in 0.1 N HCl containing 0.02 w/v% Tween 20. The medium was stirred at 100 rpm in a magnetic stirrer and the temperature controlled at 37ºC ± 0.5ºC. After 8 h, the layer of floating microspheres and sinking microspheres were separated by filtration. Both the fractions of microspheres were weighed and percentage of buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles [10].

Percentage buoyancy (%) = W_f / (W_f+Ws) × 100

W_f = weight of final microspheres; Ws= weight of sinking microspheres

Evaluation of Micromeritic Properties: Microspheres were characterized for micromeritic properties such as bulk density, tapped density, Hausner's ratio and Compressibility Index. Angle of repose were measured for the determination of flow property of microspheres [11, 12].

Particle Size Determination: The particle size of the microspheres was determined by using optical microscopy method. Approximately 100 floating microspheres were counted for particle size determination.

FT-IR Study: To study possible physical and chemical changes upon interaction between the drug and polymers. The Pure drug, polymers alone and drug loaded in individual polymers as well as in combinations were evaluated. KBr (IR Grade) pellet in a proportion of 1:100 sample and are analyzed in FTIR spectrophotometer over a range of 400 - 4000 per cm [13, 14].

TABLE 2: LAYOUT OF INDEPENDENT VARIABLES FOR OPTIMIZATION OF FLOATING MICROSPHERE

Factors (Independent)	Levels	
	-1	*+1*
X1(Conc. of HPMC K 100 M)	150	250
X2(Conc. of Tamarind gum powder)	150	250

TABLE 3: LAYOUT AND RESULTS OF DEPENDENT VARIABLES FOR OPTIMIZATION OF FLOATING MICROSPHERES

Run Order	Independent variables		Dependent variables	
	X1 (mg)	X2 (mg)	Y1 (%)	Y2 (%)
R1	-1	-1	77.92	92.64
R2	-1	+1	80.21	96.13
R3	+1	-1	78.46	94.84
R4	+1	+1	76.35	89.87

Note: X1 (Conc. of HPMC K 100 M), X2(Conc. of Tamarind gum powder), Y1 (*in-vitro* drug release), Y2 (*in-vitro* buoyancy).

Shape and Surface Morphology using SEM: The morphological study of floating microspheres was carried out by Scanning Electron Microscope (SEM). The sample was loaded on copper sample holder and sputter coated with carbon followed by Gold and the surface morphology is observed.

***In-vitro* Drug Release Study:** *In-vitro* drugrelease studies were carried out using USP Type I dissolution apparatus. Equivalent amount of microsphere containing 500 mg of drug was placed in dissolution medium (900 ml) of 0.1 N HCl. The temperature was maintained at temperature 37°C ±

0.5° C and was agitated by paddle at speed of 100 rpm. 5 ml of dissolution fluid was withdrawn at regular time intervals up to 8 h and a 5 ml of fresh dissolution fluid was added to replace the sample that was withdrawn. The samples were filtered, diluted and were analyzed by UV/Visible spectroscopy at 234 nm. The percentage of drug release from the floating microsphere was calculated.

Release Kinetics: The release rate kinetics and mechanism of drug release were studied by fitting the dissolution data in to zero order, first order, Higuchi- matrix and Korsmeyer-Peppas equations. The correlation coefficient values were calculated and used to find the fitness of the data [15, 16].

RESULT AND DISCUSSION: The results of percentage yield, drug content, drug entrapment efficiency and *in-vitro* buoyancy of prepared formulations batches were given in the table 4. All the prepared formulations F1 to F6 showed percent yield in the range 81.21% to 86.06 %. F2 shows higher percentage yield of 86.06%.

The drug content in the range of 38.05% to 45.14%. F1- F6 formulations drug entrapment efficiency was found to be in the range of 76.10% to 90.29%. F2, F3 & F4 formulation has the highest drug content and drug entrapment efficiency.

In-vitro buoyancy of floating microspheres F1-F6 was found to be in the range of 86 to 95.6%. The F4 formulation has the high *in-vitro* buoyancy than the other formulations due to higher concentration of floating polymers such as tamarind gum powder and HPMC K 100 M.

TABLE 4: PERCENTAGE YIELD, DRUG CONTENT, DRUG ENTRAPMENT EFFICIENCY, *IN-VITRO* BUOYANCY

Formulation code	Percentage yield (%)	Drug content (%)	Drug entrapment efficiency (%)	*In-vitro* buoyancy (%)
F1	84.51	38.99±0.32	77.98±0.20	86±0.26
F2	86.06	40.52±0.35	81.04±0.22	89±0.36
F3	82.58	39.80±0.25	79.61±0.14	94.8±0.34
F4	81.21	45.14±0.30	90.29±0.34	95.6±0.25
F5	85.71	38.93±0.29	77.87±0.41	88.9±0.31
F6	83.42	38.05±0.26	76.10±0.33	93.23±0.40

Mean ± SD, n = 3.

Micromeritic properties determined for formulation batches F1-F6 were shown in **Table 5**. The angle of repose for formulations F1, F2, and F4 was found to be in the range of 25-30, % Compressibility index was in the range of 11-18 for all the formulations.

The values specified in **Table 5** indicate that all the formulations showed good flow properties and compressibility may due to uniform and spherical shape of the microspheres. The size of the particle was determined by optical microscopy and ranges from 820±6.15 to 928±8.12 μm, the size increased with polymer concentration.

This may be due to increase in viscosity in a fixed volume of solvent, thus causing increase in droplet size and finally increase in size of particle. The SEM photography of floating microspheres indicates that they are spherical in shape **Fig. 1.** shows SEM image of optimized formulation.

TABLE 5: MICROMERITICS DATA AND AVERAGE PARTICLE SIZE

Formulation code	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle of repose (θ)	Carr's index (%)	Hausner's ratio	Average particle size (μm)
F1	0.76	0.84	26.13±0.23	14.60	1.171	839±0.51
F2	0.42	0.48	25.72±0.24	12.5	1.143	928±0.12
F3	0.68	0.79	32.19±0.17	13.92	1.161	916±0.56
F4	0.87	0.98	28.76±0.36	11.22	1.126	820±0.15
F5	0.52	0.61	33.61±0.16	14.75	1.173	851±0.45
F6	0.44	0.53	37.12±0.26	16.98	1.20	895±0.23

Mean ± SD, n = 3.

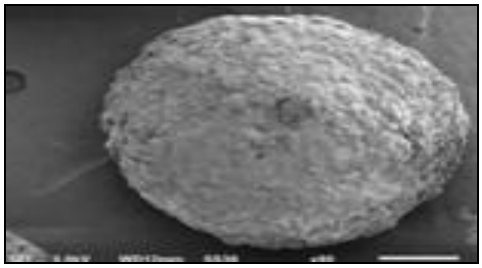

FIG. 1: SCANNING ELECTRON MICROGRAPH OF FLOATING MICROSPHERES

The FT-IR spectra of pure drug and as well as physical mixture of drug and excipients like HPMC K 100 M, tamarind gum powder and neem gum powder are shown in the **Fig. 2**. No significant shifts in the peak of pure drug and no new additional peak indicate the compatibility and stability of drug and excipients in the formulated floating microspheres.

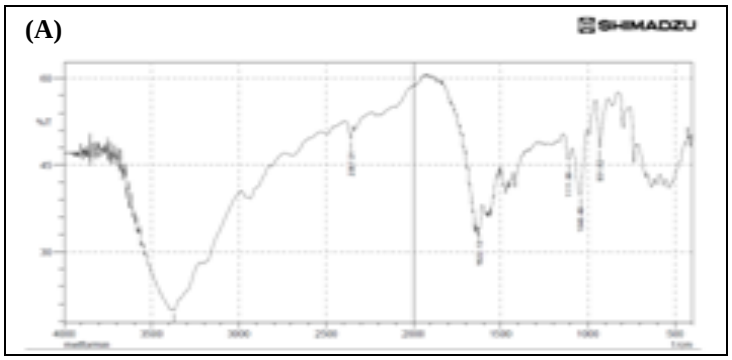

FIG. 2A: FTIR SPECTROSCOPY FOR PURE METFORMIN HYDROCHLORIDE

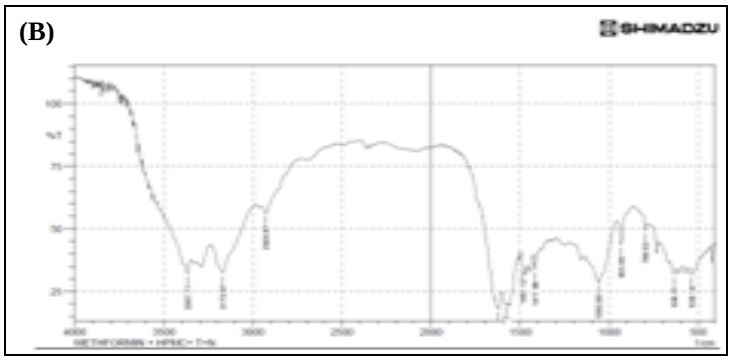

FIG. 2B: FTIR SPECTROSCOPY OF METFORMIN HYDROCHLORIDE, HPMC K 100 M, TAMARIND GUM AND NEEM GUM POWDER

The *in-vitro* drug release of all the formulations in 0.1N HCl are depicted in the **Fig. 3**. From the diffusion profiles it was evident that all the formulations (F1- F6), showed a cumulative percentage release of 61.8% to 76.33%, based on the dissolution profiles, F4 formulation shows highest drug release of 76.33% after 8 hours of study. And it was found that as the polymer concentration was increases the release rate was also increases. The *in-vitro* release data of the optimized formulation were fitted into various models to determine the mechanism of drug release from the floating microspheres. The Korsemeyer peppa's plot n value was found to be 0.47. Hence the optimized formulation follows Anomalous diffusion or Non - Fickian diffusion refers to combination of both diffusion and erosion controlled rate release.

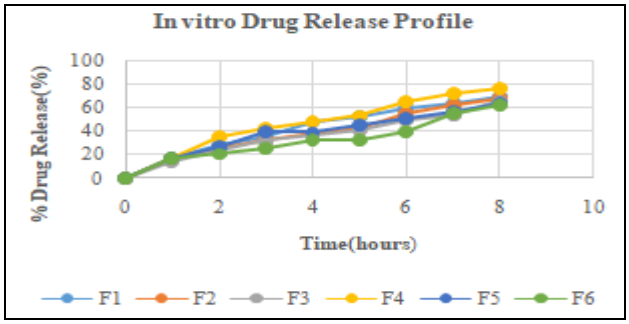

FIG. 3: *IN-VITRO* DRUG RELEASE OF ALL THE FORMULATIONS (F1-F6) IN 0.1N HCL

Statistical optimization Two Factor Two Level Factorial design: Metformin hydrochloride loaded floating microspheres were statistically optimized by using Minitab software. Based on the preliminary trial and previous studies F4 formulation was fitted into two factor two level factorial design approach [17, 18, 19].

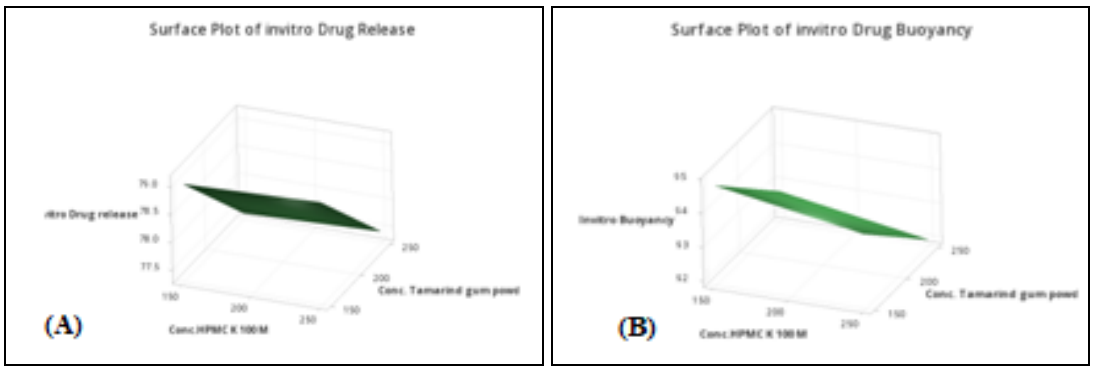

FIG. 4: SURFACE PLOT OF (A) Y1 - *IN-VITRO* DRUG RELEASE (B) Y2- *IN-VITRO* BUOYANCY

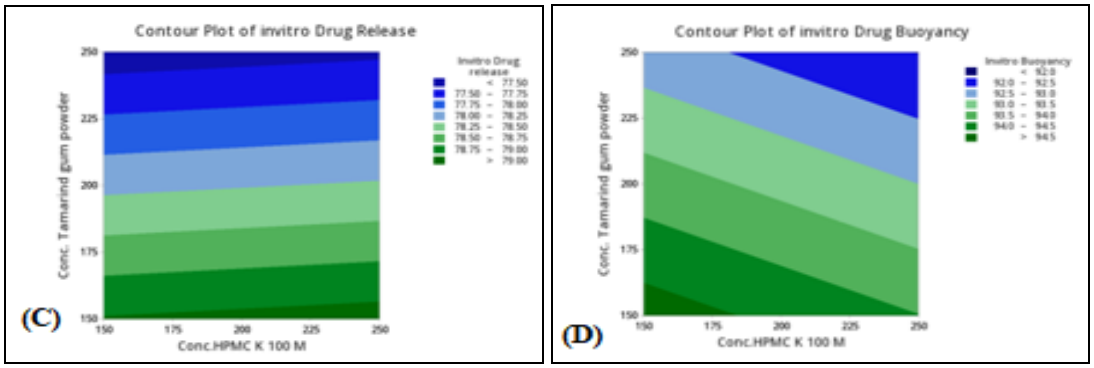

FIG. 4: CONTOUR PLOT OF (C) Y1 – *IN-VITRO* DRUG RELEASE (D) Y2- *IN-VITRO* BUOYANCY

Effect of Independent Variables on *In-vitro* Drug Release: The P value for X1 (conc. of HPMC K 100 M) was found to be 0.047 it means that if the concentration of HPMC K 100 M increases, *in-vitro* drug release also increases. In case of X2 (conc. of Tamarind gum powder) the P value was found to be is 0.127, it indicates that the concentration of tamarind gum powder does not influences the % *in-vitro* drug release.

Y1 = 92.33-0.08710 X1- 0.07140 X2 + 0.000440 X1*X2

Effect of Independent Variables on *In-vitro* Buoyancy: The P value for X1(conc. of HPMC K 100 M) was found to be 0.025 and for X2 (conc. of Tamarind gum powder) it is 0.041. It indicates that if the concentration of HPMC K 100 M and Tamarind gum powder increases the % *In-vitro* buoyancy also increases.

Y2 = 124.6 - 0.1766 X 1- 0.1489 X2 + 0.000846 X1*X2

From the 4 possible combination the constant regression values for *in-vitro* drug release was 80.21% and the *in-vitro* buoyancy was 96.13%. From the data it was observed that R2 formulation was the best formulation.

CONCLUSION: Metformin hydrochloride loaded floating microspheres with natural polymers were successfully prepared by ionotropic gelation method. Based on the preliminary trial F4 formulation was found to be the best and is statistically optimized by factorial design. From the statistical analysis R2 formulation is the optimized formulations having for *in-vitro* drug release of 80.21% and the *in-vitro* buoyancy of 96.13%. The use of tamarind gum powder (floating polymer) and neem gum powder (sustaining agent) along with HPMC proved to show better retarding ability which was clearly seen from the results. Therefore, tamarind gum powder and neem gum powder can be considered as promising materials for designing floating microspheres and further studies can be carried out. Thus, the prepared floating microspheres may prove to be potential candidates for multiple-unit delivery with enhanced bioavailability and patient compliances.

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