Application of Box-Behnken Design for Formulation and Optimization of Novel Gastroretentive Microballoons by Solvent Evaporation Method.

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ABSTRACT:

The present study involves preparation and evaluation of floating microballoons of Repaglinide for improving the bioavailability. Microballoons promises to be a potential approach for gastric retention time in the gastrointestinal tract (GIT) they are designed to overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug by controlling release rate for drugs. The Microballoons were prepared by Solvent Evaporation technique using polymers such as Ethyl Cellulose, Hydroxy Propyl Methyl Cellulose along with solvents like alcohol, dichloromethane and Surfactant like Tween 80 to decrease the interfacial tension between fluids. The optimization of microballoons was done by using Design of Experiments (DoE) studies Box-Behnken design is utilised for the same.Prepared microballoons were analyzed for their Percentage Buoyancy (PB), Mean Geometric Diameter (DG) and Entrapment Efficiency (EE).*In vitro* drug release studies were performed and drug release kinetics was evaluated using the linear regression method.*In vitro* drug release of the optimised formulation was found to be 95.48 %. The prepared microballoons exhibited prolonged drug release i.e. Upto 12 hours. Stability studies was carried out at 40 ± 2°C Temp and 75 ± 5% Relative Humidity shows no significant change in microballoons of the optimized formulation after 06 months of storage.

Keywords: Floating Microballoons, Hydroxy Propyl Methyl Cellulose, Ethyl Cellulose, Repaglinide, Percentage Buoyancy (PB), Mean Geometric Diameter (DG) and Entrapment Efficiency (EE).

INTRODUCTION:

Oral dosage forms face several restrictions like inability to retain the dose in GIT (Gastro Intestinal Track) due to fluctuation in gastric emptying which will lead to non-uniform absorption ,inadequate medication & shorter residence time of dosage form in stomach these complications provoked to the development of control release dosage form with Gastro-retentive properties which lead to formulation of Microballoons with low density, sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period of time.[1,2] As the system floats over gastric contents the dug is released slowly at desired rate resulting in increased gastric retention with reduced fluctuations in plasma dug concentration. Microballoons hold potential approach for gastric retention due to significant increase in gastric residence time, enhancement in bioavailability of BCS Class II Drugs e.g., Repaglinide, Glibenclamide, Glibornuride, Gliclazide, Glipizide, Gliquidone, Glisoxepide and Glyclopyramide, improving patient compliance by reducing dosing frequency, enhance retention of medication which solubilize only in stomach, enhance solubility for drugs which are less soluble at higher pH.

Microballoons (Hollow microsphere) are drug delivery system that promises to be a potential approach for gastric retention. Microballoons are based on non-effervescent system containing empty particles of spherical shape without core. Microballoons drug delivery systems have shown to be of better significance in controlling release rate for drugs having site specific absorption. The floating microballoons showed gastro retentive controlled release delivery with efficient means of enhancing the bioavailability and promises to be a potential approach for gastric retention. Optimized hollow Microballoons will find the central place in novel drug delivery, particularly in safe, targeted and effective *in-vivo* delivery promises to be a potential approach for gastric retention. [3, 4]Floating microballoons can be potential carrier to achieve sustained delivery of drug in stomach by increasing residence time of drug and slowly releasing drug at its site of absorption. The drugs which are specifically absorbed from upper part of GIT or which are unstable at alkaline lower GIT can be formulated as floating microballoons to increase their absorption and thus bioavailability. Delivering drugs through microballoons can help maintain plasma concentration of drug without being fluctuated for longer time. In present study, Microballoons of Repaglinide were prepared, optimized and evaluated for in vitro performance and stability study.

MATERIALS AND METHODS:

Repaglinide was procured from Chempur Pharma, Mumbai, Hydroxy PropylMethyl Cellulose K4M and Hydroxy Propyl Methyl Cellulose K15M, Ethyl Cellulose, Tween 80 was purchased from Ranbaxy Fine chemicals, Mumbai. All other chemicals used were of analytical grade.

Preparation of Microballoons

Microballoons were prepared by solventevaporation technique. [3] Repaglinide (50 mg) and HPMCK15M and ECwere used in different ratios. The total weights of HPMC and EC per 100 mg of Repaglinide were keptconstant at 200 mg. The polymers weredissolved in a mixture of alcohol anddichloromethane (in varying ratios). The total amount of solvent mixturewas kept constant at 100 ml and theprocedure was carried out at roomtemperature. The resulting solution waspoured into 250 ml of distilled watercontaining 0.01 %v/v Tween 80, maintainedat different temperatures, and then stirred at varying agitationspeed (200 - 1600 rpm) for 20 min to allowthe volatile solvent to evaporate. Themicroballoons formed were filtered, washedwith distilled water and dried.

Selection of Polymers

Floating microballoonsprepared using polymers as Sodium Alginate; Methocels (HPMC)and Ethyl cellulose are being researched for controlled delivery of drug in stomach. For optimization of typeof polymers and their concentrations, various trials were performed. Otherparameters like stirring speed (500 rpm), temperature (28°C) and phase ratiowere kept constant during these selection studies. The selection criteria were; Percentage Buoyancy (PB), Mean Geometric Diameter (DG) and Entrapment Efficiency (EE).

Selection of processing conditions

1. **Selecting of stirring speed**

   Optimized batches of microballoonswith selected excipients were subjected to various stirring speeds (200-1600 rpm) at room temperature. All the batches of prepared microballoonswere analyzed for their PB, DG andEE.

2. **Selection of temperature**

   To study the effect of temperature, the optimized batches of microballoonswereprepared at various temperatures conditions. For all these batches, excipients andstirring speed were kept constant and evaluated for three constrains as PB, DG and EE.

3. **Selection of phase ratio**

   Different ratios of good solvent and poor solvent were utilized and the resultedmicroballoonswere evaluated for various parameters like; PB, DG and EE. For allthese batches, excipients, stirring speed and temperature were kept constant.

Optimization by Experimental Design

The optimizations of microballoons were further persisted by using Design ofExperiments (DoE). [4]On the basis ofpreliminary trials, experimental domains were determined and appropriateexperimental design was selected. Box-Behnken design was implemented for Repaglinidemicroballoons. [5]

Box-Behnken design for Repaglinide microballoons:-

Based on preliminary trials, independent variables (factors) were determined as;

Amount of EC(X_1), Amount ofMethocelK15M (X_2) and stirring speed (X_3) (Table 1).

Table 1: Design layout of Box-Behnken design batches

Batch Code	**Independent Variables**		
	Amount of EC (X_1)	Amount of MethocelK15M (X_2)	Stirring speed (X_3)
B1	-1	-1	0
B2	1	-1	0
B3	-1	1	0
B4	1	1	0
B5	-1	0	-1
B6	1	0	-1
B7	-1	0	1
B8	1	0	1
B9	0	-1	-1
B10	0	1	-1
B11	0	-1	1
B12	0	1	1
B13	0	0	0

In this design, three factors with three levels were probed to investigate the main and interaction effects on preferred responses. The design consisted a total 13runs (B1 to B13) and each of them was formulated in triplicates inorder to estimate reproducibility of the model. [5] A second order quadratic model incorporating interactive and polynomial termswas exercised to evaluate the responses.

$$Y_1 = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (1)$$

Where,

Y_1 was dependent variable,

b_0 was arithmetic mean response of 13 runs and

b_1 was the estimated coefficient for factor X_1.

The main effects (X_1,X_2 and X_3) signify average result of altering one factor at a time from its lowest to highest value. The interaction terms (X_1X_2,X_2X_3 and X_1X_3) prompt change in responses when two factors are simultaneously altered. The polynomial terms (X_1^2,X_2^2 and X_3^2) were added to investigate nonlinearity of the model. [6]

Data were further analyzed byMicrosoft Excelversion 2019 for regression analysis. Analysis of variance(ANOVA) was executed to assure no significant difference between developed fullmodel and reduced model. Contour, response surface and perturbation plots weregenerated to study response variations against independent variables using Design Expert®13 (Stat-Ease. Inc. Minneapolis, USA) software. Additionally the composition of optimized (check point) batch was derived by constructing overlay plot. The percentage relative error of each response was calculated using following equation in order to judge validity of the model. [7]

Evaluation of Microballoons

1. **Drug Content, Percent Yieldand Entrapment Efficiency:**
   Accurately weighed quantity of microballoonswas dissolved in sufficient quantity of a suitable solvent, in which it was easily soluble, in a volumetric flask. The volume was

made up to the mark with the same solvent. These solutions were appropriately diluted and drug content was determined by respective analytical methods. The experimental drug content was calculated using calibration equation as a mean of three independents determinations. The percent (%) yield and Entrapment Efficiency of sample was calculated using Equation 2 and 3. [8, 9]Average of three determinations was considered as result of the test carried out.

$$\% \text{ Yield} = \frac{\text{total weight of microballons}}{\text{total weight of drug and excipients}} \times 100 \qquad (2)$$

$$\text{Entrapment efficiency} = \frac{\text{Weight of repaglinide in microballoons}}{\text{Initial weight of Repaglinide}} \times 100 \qquad (3)$$

2. Size Analysis

Size analysis of prepared microballoonswas performed using optical microscopy method. The size of randomly selected microballoons(300) was measured and their mean geometric diameter (DG) was calculated. [10]

3. Dissolution method

In-vitro release study of prepared microballoons was carried out in USP type II (paddle type). The dissolution study was carried out in 900 mL dissolution media at 37 ± 0.5°C. Sample of 5 mL were withdrawn at specified time interval and replaced with fresh media. The samples were analyzed using suitable analytical method in order to find out amount of dissolved drug. All determinations were performed in triplicate. [11, 12]

4. Stability Study

According to the ICH guidelines, the optimized batches were placed in high density polyethylene (HDPE) bottles. The mouths of the bottles were closed tightly with aluminium foil to prevent the access of air from the atmosphere to the sample inside the bottles. Samples were stored at 40 ± 0.5°C and 75 ± 5% relative humidity (RH) for 6 months in programmable environmental test chamber. The samples were withdrawn at particular time period and statistically analyzed. [13]

Results and Discussion

Selection of excipients and processing conditions

1. Selection of Excipients

In the formulation of pharmaceutical dosage form various excipients are utilized to improve the functionality of dosage forms. All these excipients have a significant impact on their bioavailability, toxicity, stability and efficacy. [14, 15]Addition of small amounts of surfactants or polymers is the method usually applied to obtain the best spherical microballoons. Surfactants or polymers can decrease the interfacial tension between fluids, change the viscosity of phases and hence influences the droplets sphericity. The results of Repaglinide microballoons with all studied excipients have been summarized in

Table 2. Concentrations of excipients incorporated in formation of microballoons were within limits as per inactive ingredients guidelines (IIG) of FDA. [16]

Table 2: Selection of excipients for repaglinidemicroballoons

Batch	***Excipients***	***Amount (mg)***	***Results ****		
			PB (%)	***DG (µm)***	***EE (%)***
PR1	Sodium alginate	50	36.15 ± 2.64	247.33 ± 17.52	25.41 ± 2.12
PR2		100	33.45 ± 2.58	328.57 ± 25.24	28.34 ± 1.78
PR3		150	40.12 ± 3.45	385.56 ± 35.36	28.95 ± 3.29
PR4		200	42.57 ± 3.75	378.54 ± 37.51	35.93 ± 2.57
PR5		250	43.45 ± 5.56	453.87 ± 54.21	43.87 ± 2.42
PR6	Ethyl cellulose	50	40.52 ± 2.04	283.54 ± 24.56	29.27 ± 1.93
PR7		100	49.63 ± 3.52	278.71 ± 22.54	31.35 ± 2.15
PR8		150	51.54 ± 3.31	294.33 ± 25.57	35.54 ± 2.71
PR9		200	63.57 ± 3.84	328.89 ± 36.43	46.78 ± 3.15
PR10		250	64.56 ± 4.25	556.78 ± 34.25	57.12 ± 4.12
PR11	Methocel K15M	50	42.12 ± 3.54	184.54 ± 17.63	33.13 ± 3.58
PR12		100	49.56 ± 2.67	209.72 ± 31.57	40.57 ± 3.54
PR13		150	58.64 ± 4.56	234.31 ± 24.55	51.53 ± 2.87
PR14		200	70.17 ± 3.32	258.79 ± 31.14	62.48 ± 4.83
PR15		250	68.34 ± 5.51	394.45 ± 34.73	64.59 ± 4.43
PR16	MethocelK4M	50	27.41 ± 2.47	239.47 ± 34.69	24.54 ± 1.13
PR17		100	34.26 ± 2.54	312.63 ± 14.43	31.14 ± 2.06
PR18		150	37.52 ± 3.71	353.64 ± 40.15	35.72 ± 2.47
PR19		200	43.13 ± 3.54	369.28 ± 42.28	38.18 ± 3.14
PR20		250	51.25 ± 2.35	567.61 ± 41.26	37.84 ± 2.23

* Results are mean of three observation ± SD

For all the batches there was a significant improvement in the particle size of microballoons. This might be attributed to presence of polymers on the surface, which increases particle-particle interaction, may cause faster squeezing out of solvents (DCM and ethanol) to the surface, which results in particle size enlargement.Results showed that microballoonsobtained with EC (Ethyl Cellulose) and MethocelK15M at amount of 100 mg to 300 mg improved functionality of repaglinidemicroballoons. EC (Ethyl Cellulose) andMethocelK15M amount below 100 revealed microballoonswith poor characteristics and also required more time to grow. This might be due to the inability of low amount to start formation of microballoons. Further, higher amount (>200 mg) of EC and MethocelK15Mresulted in undesiredPB, DG and EE. This might be due to higher viscosity in the presence of polymers. In addition to these, microballoonswere carried out by utilizing different concentration of Sodium alginate (SA), Ethyl cellulose (EC), Methocel K15M (MK15M) and Methocel K4M (MK4M). However, results of SA and MK4M polymers the studied parameters were unfavourable. This might be attributed to the polymer structure, structure compatibility in term of free availability of hydrophobic

and hydrophilic group for interaction between polymer and drug, polymer molecular weight and viscosity.[6] Therefore, addition of SA and MK4M was not further considered for the preparation of Repaglinide microballoons. Furthermore, EC and MK15M were utilized for formation of repaglinidemicroballoons.

2. Selection of Stirring Speed

Different Repaglinidemicroballoons (PR21 to PR37) were prepared to select optimum speed of rotation (Table 3).

Table 3: Selection of stirring speed for repaglinidemicroballoons

Batch	*Speed (rpm)*	*Results **		
		PB (%)	*DG (µm)*	*EE (%)*
PR21	200	21.66 ± 0.98	931.33 ± 123.57	16.57 ± 0.85
PR22	400	23.24 ± 1.32	721.41 ± 97.25	24.42 ± 1.26
PR23	600	32.31 ± 2.42	552.92 ± 37.86	33.54 ± 2.91
PR24	800	59.75 ± 2.65	335.47 ± 20.14	42.38 ± 3.13
PR25	1000	68.45 ± 3.56	238.34 ± 23.78	49.26 ± 2.37
PR26	1200	70.21 ± 4.24	152.41 ± 22.57	57.57 ± 3.37
PR27	1400	68.26 ± 1.24	164.56 ± 18.35	28.24 ± 2.17
PR28	1600	69.24 ± 1.42	152.56 ± 21.51	25.17 ± 2.14

* Results are mean of three observation ± SD

When the agitation speed was reduced to 200-600 rpm, larger irregular microballoonswere obtained, where the shear energy may not be sufficient for the formation of spherical shape of microballoons. Microballoonsprepared between800 to 1200 rpm resulted in uniform size distribution. It appears to be clear that optimum shear force of the agitated liquid and collisions with equipment surfaces and other particles were squeezing and moulding the irregular microballoonsinto an almost perfect spherical shape. The impact of agitation speed on formation of microballoonswas such that on further increasing the agitation speeds beyond 1200 rpmmicroballoonswith undesired characteristics were produced with poor crushing strength. Therefore, it was determined that the speed between 800 to 1200 rpm was optimized by implementation of suitable design of experiment.

3. Selection of Temperature

Temperature is a very critical parameter in the formation microballoons. The results depicted that at low (8°C) temperature the microballoons were much bigger than at room temperature (28°C) (Table 4). Because of undesired properties of prepared microballoons of batch PR29 and PR31 they were not further considered. This might be attributed to less solubilisation of the drug and polymer would cause reduced wetting of drug particles and therefore, microballoons obtained with poor characteristics.

Table 4: Selection of temperature for repaglinidemicroballoons

Batch	*Temperature (°C)*	*Results **		
		PB (%)	*DG (µm)*	*EE (%)*
PR29	5	27.31 ± 2.51	824.65 ± 35.31	15.31 ± 2.10

PR30	28	63.45 ± 3.24	157.24 ± 12.24	69.97 ± 3.54
PR31	40	36.46 ± 3.41	142.31 ± 21.17	57.36 ± 1.58

* Results are mean of three observation ± SD

Results revealed that microballoons prepared at room temperature observed with good sphericity along with higher EE. While at higher temperature (40°C) microballoons were not formed with EE along with poor PB. This might be attributed to higher rate of evaporation of solvent at high temperature which leads to subsequent unavailability of bridging liquid to complete microballoons.Finally, it was decided that temperature for the preparation of microballoonswas most suitable at room temperature.

4. Selection of Solvent Ratio

Ratio of ethanol to dichloromethane (DCM) altered the fundamental properties of microballoons (Table 5).

Table 5: Selection of phase ratio for Repaglinide microballoons

Batch	***Solvent ratio (Ethanol : DCM)***	***Results ****		
		PB (%)	***DG (µm)***	***EE (%)***
PR32	1:3	43.52 ± 2.65	433.28 ± 38.68	53.65 ± 2.13
PR33	1:2	54.72 ± 5.61	322.75 ± 43.32	59.24 ± 3.41
PR34	1:1	71.43 ± 2.45	157.64 ± 23.64	71.23 ± 3.48
PR35	2:1	65.63± 2.34	293.68 ± 22.44	52.34 ± 2.64
PR36	3:1	54.14 ± 3.81	353.45 ± 32.76	47.42 ± 3.45

* Results are mean of three observation ± SD

In batch PR34 (solvent ratio, 1:1), microballoonswere obtained with desired characteristics like PB, DG and EE. Other batches show poor properties of microballoons and hence it was decided to use solvent ratio as 1:1 in further investigations.

5. Optimization by Experimental Design

From a Quality-by-Design perspective, the aim of optimization was to establish the applicability of a Bayesian statistical methodology to identify the Design Space (DS) of agglomeration process. Following the ICH Q8 guideline, the DS is defined as the ‘‘Multidimensional combination and interaction of input variables (e.g., materials attributes) and process parameters that have been demonstrated to provide assurance of quality’’.[18, 19] The majority of scientists now routinely use experimental design as part of scientific approach in order to: reduce costs, reduce waste, improve quality; reduce timelines of process investigation; obtain robust products and processes.[20] Response surface methodology (RSM) consists of a group of mathematical and statistical techniques used in the development of an adequate functional relationship between a response of interest and a number of associated control (or input) variables.[21, 22]. Thus, the present investigation was persisted with Box–Behnken design for optimization of Repaglinide microballoons.

The Box–Behnken design was specifically selected since it requires fewer treatment combinations than a Central Composite Design in case involving three or four factors.[23,24]On the basis of preliminary trials, solvent ratio of ethanol to DCM is 1:1 and

system temperature at 28 °C was fixed for all the experimental design batches. Further, EC and MK15Mwere optimized between 100 to 200mg along with rotational speed between 800 to 1200 rpm. There were three critical components [Amount of EC (X_1), Amount of Methocel K15M (X_2) and stirring speed (X_3)] significantly influenced various properties of repaglinidemicroballoonsand hence, they were utilized for systemic studies (Table 6).

Table 6: Actual levels of Box-Behnken design for Repaglinidemicroballoons

Coded Values	***Actual Values***		
	X_1[a]	X_2[b]	X_3[c]
-1	100	100	800
0	150	150	1000
1	200	200	1200

[a] X_1 – Amount of EC (mg), [b] X_2 – Amount of Methocel K15M mg), [c] X_3 –Stirring speed (rpm)For all experimental design batches of repaglinidemicroballoons, various critical quality attributes like percent buoyancy (PB, %), mean geometric diameter (DG, μm) and entrapment efficiency (EE, %) were evaluated (Table 7).

Table7: Results of Box–Behnken design batches of Repaglinide microballoons *

Batch Code	***PB*[a] *(%)***	***DG*[b] *(μm)***	***EE*[c] *(%)***
B1	69.98 ± 2.75	196.15 ± 8.15	32.56 ± 2.31
B2	55.35 ± 1.86	267.33 ± 10.14	71.56 ± 1.97
B3	74.53 ± 2.35	245.19 ± 9.31	51.42 ± 1.25
B4	84.36 ± 3.04	154.87 ± 2.35	45.75 ± 2.35
B5	55.14 ± 1.17	321.41 ± 12.35	37.56 ± 2.07
B6	56.67 ± 2.35	276.2 ± 10.64	57.25 ± 2.14
B7	71.35 ± 3.21	195.32 ± 9.45	40.01 ± 2.38
B8	65.18 ± 2.68	209.45 ± 8.45	63.12 ± 3.21
B9	59.35 ± 2.14	241.75 ± 7.19	54.26 ± 2.74
B10	58.34 ± 1.87	275.31 ± 10.38	46.58 ± 3.13
B11	64.15 ± 1.75	212.45 ± 7.25	75.56 ± 2.15
B12	75.42 ± 2.31	174.32 ± 5.45	54.61 ± 2.34
B13	67.35 ± 2.23	181.55 ± 6.57	56.72 ± 3.17

* Results are mean of three determinations ± SD; [a] Percent buoyancy;[b] Mean geometric diameter; [c]Entrapment efficiency

The values of the coefficients of Equation 1 were determined by multiple linear regression analysis. [23]They are shown in Table 8. The polynomial terms could be used to draw conclusions after considering magnitude of coefficients and mathematical sign it expresses either positive or negative. Main effects represent the average result of changing one factor from −1 to +1, and the interactions explained the result when any two

or all three factors were changed simultaneously. If none of the factors had any effect, then the responses (Y_1, Y_2, and Y_3) would be scattered randomly around their mean value (b_0).

Table 8: Regression analysis of Box–Behnken design batches of RepaglinideMicroballoons

Coefficients	*PB(Y_1)*		*DG (Y_2)*		*EE (Y_3)*	
	FM [a]	***RM*** [b]	***FM***	***RM***	***FM***	***RM***
b_0	67.35	65.936	181.55	209.018	56.72	52.84308
b_1	-1.18	–	-6.2775	–	9.51625	9.51625
b_2	5.4775	5.4775	-8.49875	–	-4.4475	–
b_3	5.825	5.825	-40.3913	-40.3913	4.70625	–
b_{12}	6.115	–	-40.375	-40.375	-11.1675	-11.1675
b_{23}	3.07	–	-17.9225	–	-3.3175	–
b_{13}	-1.925	–	14.835	–	0.855	–
b_{11}	0.7375	–	29.48625	–	-7.3325	–
b_{22}	2.9675	–	4.84875	–	0.935	–
b_{33}	-6.0025	–	39.55875	29.25825	0.0975	–

[a]FM, Full model; [b] RM, Reduced model; [c]Nonsignificant (P>0.05) coefficients for Y_1; [d]Nonsignificant (P>0.05) coefficients for Y_2; [e]Nonsignificant (P>0.05) coefficients for Y_3

Using 5% significance level, a model was considered significant if the *P*-value (significance probability value) was less than 0.05.[26] For percent buoyancy (Y_1), coefficient b_1,b_{12}, b_{23}, b_{13}, b_{11}, b_{22} and b_{33} were found to be insignificant (P>0.05) and therefore, these terms were separated from their full model in order to develop reduced model (Table 7). Similarly, the coefficients b_1, b_2, b_{23}, b_{13}, b_{11} and b_{22} for mean geometric diameter(Y_2);and b_2,b_3, b_{23}, b_{13} b_{11}, b_{22} and b_{33} for entrapment efficiency (Y_3); were found to be insignificant (P>0.05) and hence, these terms were removed from their respective full model in order to develop reduced model.[27]

The removal of insignificant terms was further justified by executing analysis of variance (ANOVA) test (Table 9). In order to evaluate the fit of model, values of explained variation (R^2), usually between 0 and 1, provide excellent guidance. Acceptable values were totally dependent on the nature of the data that were being examined. In this experiment, high value of correlation coefficients for all the selected dependent variables i.e., Y_1, Y_2, and Y_3 illustrated goodness of fit. This was an inference of validation of reduced model. [28]

Table 9: Calculation for testing the model in portions for Repaglinide microballoons

Model	*df* [e]	*SS* [d]	*MS* [e]	R^2
Percent buoyancy (Y_1)				
Regression				

FM[a]	9	901.35065	100.15007	0.92901361
RM[b]	2	511.46905	255.73452	0.527166319
Residual				Fcal = 2.4261
FM	3	68.87265	22.95755	Fcritical = 8.89
RM	10	458.7543	45.87543	df = (7, 3)
Mean Geometric Diameter (Y_2)				
Regression				
FM	9	27421.55	3046.839	0.966863412
RM	3	22206.17	7402.057	0.782972857
Residual				Fcal = 2.7747
FM	3	939.7986	313.2662	Fcritical = 8.94
RM	9	6155.184	683.9094	df = (6, 3)
Entrapment efficiency (Y_3)				
Regression				
FM	9	1787.664	198.6294	0.93457
RM	2	1223.324	611.6622	0.6395
Residual				
FM	3	125.1404	41.71348	Fcal = 1.9327
RM	10	689.4803	68.94803	Fcritical = 8.89
FM	3	0.1158	0.0386	df = (7, 3)
RM	6	0.2659	0.0443	

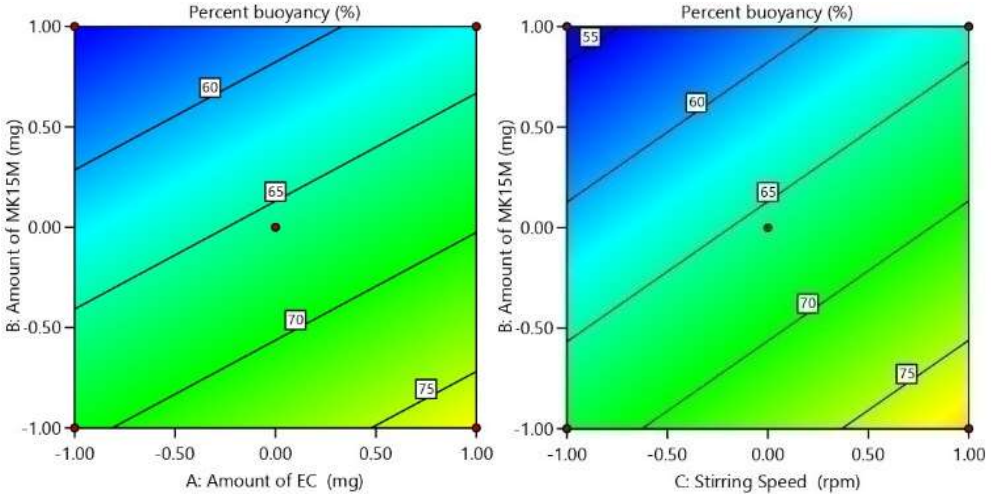
[b] RM, Reduced model; [c]df, Degree of freedom; [d] SS, Sum of squares; [e] MS, Mean of squares

The fitted model, for Y_1,was evaluated using priori linearity hypothesis test [29] and the results indicated that no evidence of lack of fit was observed in the 95% confidence interval, because calculated F value (Fcal) was 2.4261 and lower than their critical/tabulated value (Ftab) which was 8.89 [17,18]. Likewise, for response Y_2 and Y_3, calculated F values was less than their respective critical values (at $\alpha = 0.05$) which suggested insignificant difference amongst full and reduced model (Table 9). The data of all the 13 batches of experimental design were used to generate interpolated values with the assistance of contour and perturbation plots. [32]

A. Influence of formulation composition on percent buoyancy (Y_1):

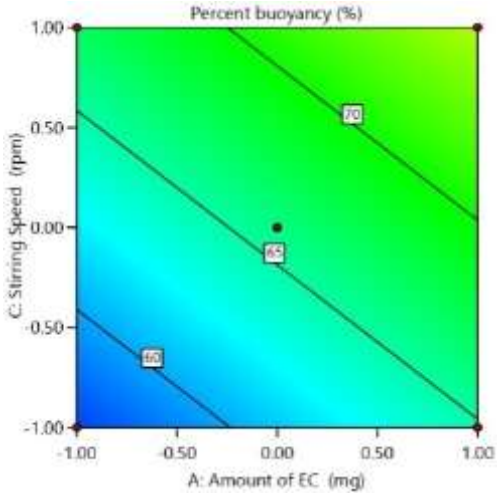
The results of regression analysis for the percent buoyancy (PB) depicted positive sign for regression coefficients b_2 and $b_3$whereas it offered negative sign for coefficients b_1. These suggested that with increase in amount of MK15M and stirring speed with decrease in amount of EC, the PB of repaglinidemicroballoons. A highest PB of 84.36% was observed in batch B4 with levels of X_1, X_2 and X_3 as 1, 1 and 0,

respectively. The results of contour plots are illustrated in Figure 1.



(a)

(b)



(c)

Figure 1: Influence of formulation variables X_1 and X_2(a), X_2 and X_3 (b) and X_1 and X_3 (c) on PB(Y_1) of repaglinidemicroballoons by Contour plot. From the contour plots it is clear that increased amount of MK15M and stirring speed resulted in increased the response, PB (Figure 1). Additionally, it was also found that stirring speed had little more positive effect on PB because of higher coefficient value (b_3 = 5.825) whereas comparative low magnitude of amount of MK15M (b_2 = 5.477) indicated the less positive effect on PB(Table 7). The value of negative coefficient (b_1 = –1.18) of amount of EC for PBsupported significant influence.

As the number of RSM factors increased, it became difficult to visualize the response surface with graphical tools. In this case it's helpful to view a special form of response plot called "perturbation" for RSM data. Perturbation plots compare the effect of all the factors at a particular point in the RSM design space. The response was plotted by changing only one factor over its range, while holding all other factors constant. On the perturbation plot, a steep slope or curvature in an input variable revealed a relatively high sensitivity of response. Perturbation plot in Figure 2 explained the effect of variables (X_1, X_2 and X_3) on the response, PB. The higher

negative slope of X_3 indicated significant reduction in PB when deviated from -1 to 1. Smilarly, positiveslope of X_1 and X_3 revealed negative impact on PB of X_1 from level -1 to 1. These outcomes validate the regreession analysis (Table 7) and also confirm the findings of counter plots (Figure 1).

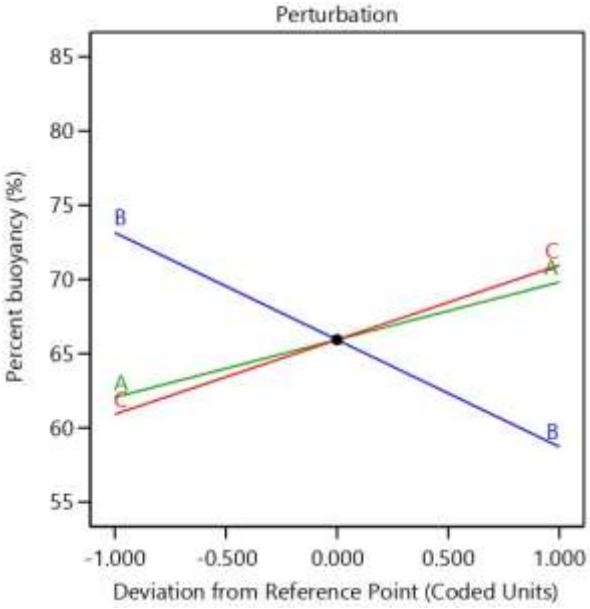
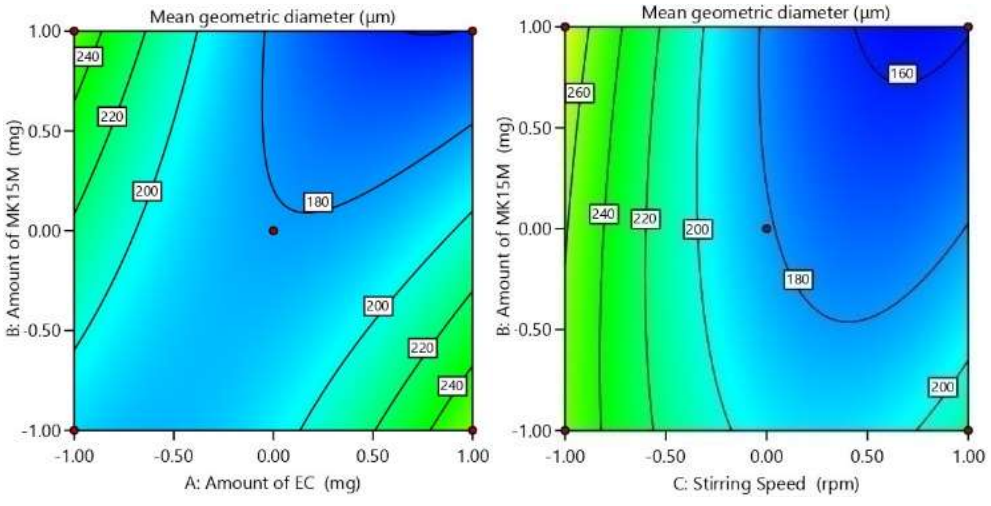
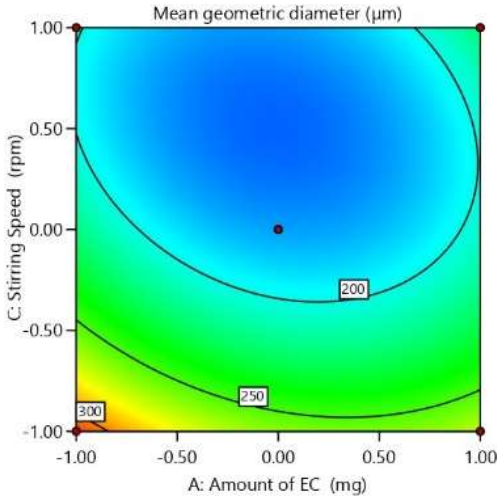


Figure 2: Perturbation plot showing effect of independent factors on PB of repaglinidemicroballoons while keeping other variables at their respective midpoint

B. **Influence of formulation composition factor on mean geometric diameter (Y_2):**Randomly selected microballoons from each batch were subject to mean geometric diameter(DG) determination. The results of regression analysis for DG (Y_2) depicted negative sign for regression coefficients b_1, b_2 and b_3 (Table 8). These finding revealed that with decreased in X_1, $X_2$and X_3, decreasesthe DG of repaglinidemicroballoons. Greater magnitude of coefficient b_3 indicated more impacts of stirring speed as compared to amount of EC and MK15M on DG. Influences of independent variables (i.e. X1, X2 and X3) on DG of microballoons are illustrated in Figure 3.



(a) (b)



(c)

Figure 3: Influence of formulation variables X_1 and X_2 (a), X_2 and X_3 (b) and X_1 and X_3 (c) on DG (Y_2) of repaglinidemicroballoons by Contour plot

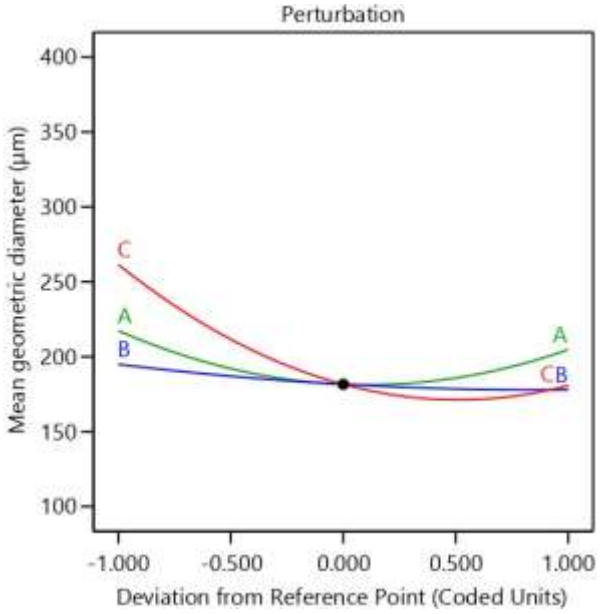


Figure 4: Perturbation plot showing effect of independent factors on DG of repaglinidemicroballoons while keeping other variables at their respective midpoint

The higher negative slope of X_3 indicated significant reduction in DG when deviated from -1 to 0 whereas from 0 to -1 slope remains almost constant. Smilarly, negative slope of X_1 revealed negative impact on PB of X_1 from level -1 to 0 whereas 0 onwards theres is slight increment in DG as slope become positive to this region. Additionally, it is observed that there in very little impact of X_2 on DG as slope is almost constant from level -1 to 1. These outcomes validate the regreession analysis (Table 8) and also confirm the findings of counter plots (Figure 4).

C. Influence of formulation composition factor on entrapment efficiency (Y_3):

The results of regression analysis for Y_3 depicted positive signs for regression coefficients $b_1$and b_3. This suggested that with increased amount of EC and stirring

speed the entrapment efficiency (EE) of repaglinidemicroballoonsincreased. A highest EE of 75.56% was observed in batch R11 with levels of X_1, X_2 and X_3 as 0, –1 and 1, respectively. The results of contour plots are illustrated in Figure 5.

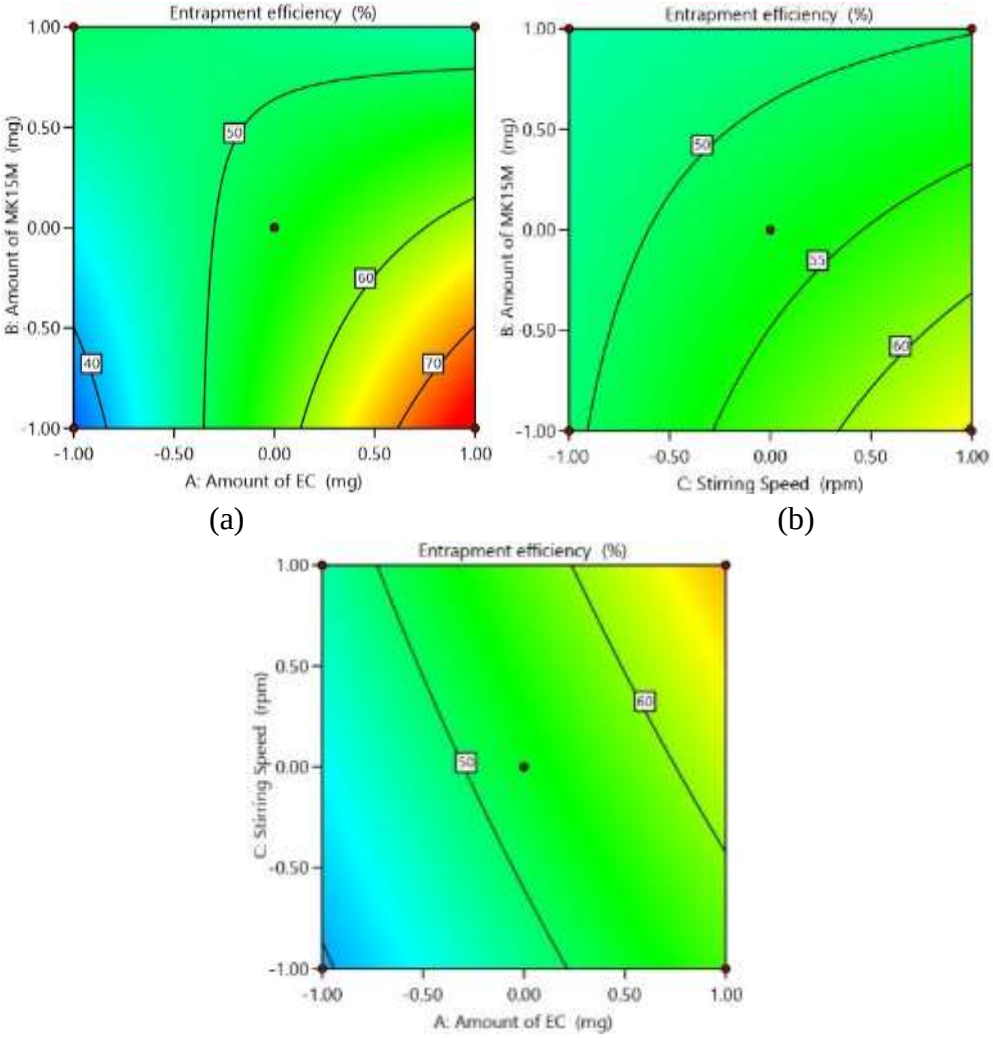


Figure 5: Influence of formulation variables X_1 and X_2 (a), X_2 and X_3 (b) and X_1 and X_3 (c) on EE (Y_3) of repaglinidemicroballoons by Contour plot

Perturbation plot (Figure 6) revealed that, amount of EC and stirring speed increased the EE at broad level from –1 to 1. Additionaly, as amount of MK15M increased from -1 to 1, the EE declinned. This observation was might be due to increased viscosity of external phase with addition of more amount of MK15M which altered the size and distribution of microballons. This finding also correlates the observation of counter plots (Figure 5).

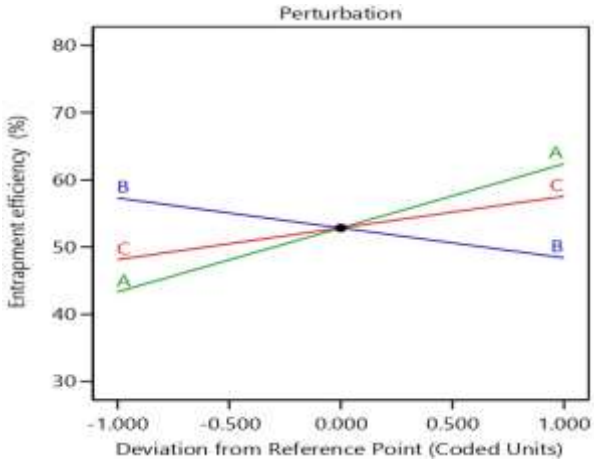


Figure 6: Perturbation plot showing the effect of each of the independent variables on EE of repaglinide microballoons

6. Optimization of repaglinidemicroballoons:

Optimized agglomerate in the form of check point batch was elected on the basis of the optimal solution as obtained by Design Expert®13 (Stat-Ease Inc. Minneapolis, USA) software. To optimize all the responses with different targets, a multicriteria decision approach (a graphical optimization technique by the overlay plot) was used.[31] This practice was performed within the context of the ICH Q8(R2)[34]note concerning pharmaceutical development.

The optimized formulation was obtained by applying constraints on dependent variable responses and independent variables. [35] The constraints were: maximum PB; minimal DG and maximum EE. These constrains were common for all the formulations. The recommended concentrations of the independent variables were calculated by the Design Expert® software from the overlay plot. The extensive grid and feasibility searches provided that the optimum formulations and the overlay plot is depicted in Figure 7, where one solution was found with a highest desirability. The optimum values of selected variables obtained were -0.74 (X_1; amount of EC), 0.51 (X_2; amount of MK15M) and 0.61 (X_3; stirring speed).

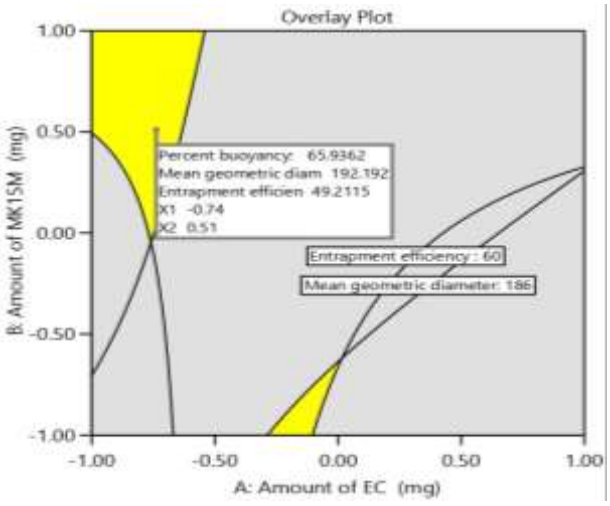


Figure 7: Overlay plot with optimized batch of repaglinidemicroballoons

Check point/optimized batch of repaglinidemicroballoons(RM) was prepared according to the levels of factors optimized. The results depicted nonsignificant ($P>0.05$) difference and lower magnitude of relative error (in percent) between experimentally obtained and theoretically computed data of PB, DG and EE[36]as well as significant values of R^2[37]suggested the robustness of mathematical model and high predictive ability of RSM applied (Table 10).

Table 10: Results of optimized batch (RM) of repaglinidemicroballoons

Response	***Predicted Value***	***Experimental Value [Mean ± SD (n=3)]***	***% Relative Error***
PB (%)	65.93	69.31 ± 2.12	4.88%
DG (μm)	192.19	181.83 ± 4.26	5.70%
EE (%)	49.21	52.47 ± 1.05	6.21%

Stability studies

Stability is defined as the ability of particular drug or dosage form in a specific container, sealed high density polyethylene bottles, to remain within its physical, chemical, therapeutic and toxicological specification. It is considered good practice to test the stability of drug substances and drug products according to the ICH Q1A(R2) (2003); Q1B (1996), Q1C (1996), Q1D (2002), Q1E (2003) and Q5C (1995) guidelines, [39,40,41,42,43,44] or the World Health Organization (WHO) Technical Report, "Stability testing of active pharmaceutical ingredients and finished pharmaceutical products" (WHO, 2009). The ICH document Q1A(R2) states a minimum of 6 months for accelerated, stability data is needed. [38]To interpretation of stability data, simple concept of "change-over-time" and "variability" determined in literature. [45]

In the present work, accelerated stability study was carried out for selected microballoons (RM) at 40 ± 2°C and 75 ± 5% RH for six months using a programmable environmental test chamber. Various evaluation parameters were determined during the stability period and results are given in Table 12. Results of the stability study of optimized microballoonsof repaglinide(RM) and exhibited no remarkable change in the all selected responses at the end of 3 and 6 months.

Table 11: Results of evaluation parameters of RM during stability periods

Parameters	***Storage Periods***		
	At initial time	***3 Months***	***6 Months***
PB (%)	69.31 ± 2.12	71.02 ± 2.51	70.24 ± 1.57
DG (μm)	181.83 ± 4.26	184.75 ± 5.04	183.02 ± 5.63
EE (%)	52.47 ± 1.05	51.98 ± 1.21	52.79 ± 0.97

*Results are mean of three observations ± SD

SUMMARY AND CONCLUSION:

- Microballoons of Repaglinide can be prepared successfully by using solvent evaporation technique. The prepared microballoons exhibited a good entrapment efficiency and % Buoyancy.The concentration of Ethyl Cellulose, Methocel, surfactant, and stirring speed play a crucial role in particle size, drug entrapment efficiency, and in vitro buoyancy.

- The formulation was optimized by the Box-Behnken design, the experimental design, regression analysis, and contour plots were used in optimizing formulation variables in the preparation of Repaglinide Microballoons. The optimized formulation prepared using the predicted levels of factors provided the desired observed responses with Y1, Y2 and Y3 for percent buoyancy (PB), geometric diameter (DG) and entrapment efficiency (EE), respectively.
- The developed floating microballoons of Repaglinide increase the gastric residence time and prolong the drug release in the stomach, which, in turn, improves the local availability of the drug. From the result it can be concluded that these variables had significant effect on the reponses.
- In vitro drug release studies showed that release from the microballoons get successfully retarded for over 12 h.The stability study shows no significant change in microballoons of the optimized formulation after 06 months of storage.
- Themultiparticulate delivery system can prove to be a better option as compared to other oral dosage forms.Finally, it is possible to conclude that microballoons drug delivery systems can be used as gastro-retentive drug delivery systems, reducing dosing frequency and improving patient compliance.

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