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RESEARCH ARTICLE

Design and Optimization of Gastro-Retentive Repaglinide Microspheres by Box-Behnken Design

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ABSTRACT

The current study involves development and optimization of microspheres based floating system containing Repaglinide by solvent evaporation method for gastro retentive delivery. Combination of polymer Ethyl cellulose and Eudragit RSPO were used to prepare microspheres having poly vinyl alcohol as an emulsifying agent where it sustain the drug delivery upto 12 hr. The effect of various process variables like drug polymer ratio, organic phase addition time and stirring speed on drug release at 2 hr (Q_2), drug release at 8 hr (Q_8) was optimized using box behnken design and analyzed using response surface methodology. The result of FT-IR shows no interaction between drug and polymer. There was an effect on mean particle size by altering drug polymer ratio and stirring speed. The observed responses were coincided well with the predicted values given by the optimization technique. All the batches of microspheres were evaluated for flow properties, % yield, % drug loading, particle size analysis, % buoyancy, *in vitro* drug release at 2 hr and at 8 hr. The optimized batch MS30 showed the highest % yield (98.34%), % drug loading (55.12%), % CDR at 2 hr (15.79 %) and %CDR at 8 hr (80.01%). The average particle size of optimized batch MS30 was 160 µm. The result of kinetic model of optimized batch MS30 shows non fickian diffusion kinetics. Stability study was performed on optimized batch MS30 as per ICH guidelines and no significant change was found in drug content on storage.

KEYWORDS

Repaglinide, Ethyl Cellulose, Eudragit RSPO, Solvent Evaporation Method

INTRODUCTION

Drugs that are easily absorbed from the gastrointestinal tract (GIT) and have a short half-life are eliminated quickly from the blood circulation, so they require frequent dosing. To avoid this drawback, the oral sustained-controlled release formulations have been developed in an attempt to release the drug slowly into the GIT and maintain an effective drug concentration in the serum for longer period of time.

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However, conventional drug delivery devices have a physiological limitation of gastric retention time (GRT), variable and short gastric emptying time can result in incomplete drug release from the drug delivery system (DDS) in the absorption zone (stomach or upper part of small intestine), leading to diminished efficacy of the administered dose. To overcome these limitations, approaches being proposed to prolong the GRT. The rate controlled oral drug delivery system has given impetus to significant advancements in the pharmaceutical engineering, of novel dosage forms such as microsphere, which are solid colloidal

polymeric carriers less than 100µm in size. These offer great advantages right from helping to increase the stability of drugs, proteins and up to controlled drug release properties. In addition to the inherent property of reduced cytotoxicity, biodegradable polymeric microparticles have been found to be extremely effective in controlled and targeted drug release, and time controlled drug delivery system. Microspheres have the advantages of passing through the GIT uniformly, which not only avoid the vagaries of gastric emptying but also provide an adjustable release and reduced inter-subject variability in absorption and risk of local irritation were achieved consequently. Diabetes mellitus is a major and growing public health problem throughout the world and is associated with increased cardiovascular mortality, so, the current research work is focused towards anti-diabetic treatments.

Repaglinide (Rg), a fast and short acting meglitinide analog, is chosen as the drug candidate for polymeric microsphere formulation. As far as the specific properties of the Repaglinide are concerned, though it possesses phenomenal anti-diabetic properties, it has only short half-life, say 1 hr, low bioavailability (50%) and poor absorption characteristics in the upper intestinal tract. Furthermore, it produces hypoglycemia after oral administration. Since these drugs are intended to be taken for a long period, patient compliance is also very important. Headache, gastrointestinal effects, and musculoskeletal pain are also been reported by repaglinide users. Microsphere with enclosed anti-diabetic drug, can improve the therapeutic efficacy of the drug and also the polymeric microsphere which releases the drug in a predetermined controlled manner for a prolonged duration. Thus, the adverse effects, as mentioned earlier, due to conventional dose can be surmounted.

The present investigation is carried out to develop and evaluate a stable microsphere based delivery system using polymer, which would deliver Repaglinide, an anti-diabetic drug, at a controlled rate for a prolonged period of time and enhance its gastric retention time in GIT to give sustained release action.[1, 2]

MATERIALS AND METHODS

Repaglinide was kindly gifted from Torrent Research Centre, Bhat. Polyvinyl alcohol and ethyl cellulose (EC) was obtained from Signet, mumbai and eudragit was gifted from Evonic industries. All the other chemicals and reagents used were analytical grade.

Experimental Work

Preparation Method[3, 4, 5, 6]

Microspheres containing EC, eudragit RSPO and combination of EC & eudragit RSPO as coating material were prepared by a solvent evaporation method in which drug and polymer in different proportion (Table 1) were mixed in the mixture of solvent system dichloromethane: ethanol: acetone (1:2:2). The clear solution was slowly introduced as a thin stream into 200 ml of 0.2% polyvinyl alcohol aqueous phase maintained at 30-40ºC. The resultant emulsion was stirred at 800 RPM for 2 hr to allow complete evaporation of solvent. The prepared microsphere were filtered with 0.5µm millipore and washed repeatedly with water.

Parameters Kept Constant are:

Stirring speed 800 RPM,

0.2% Poly vinyl alcohol,

12ml ethanol, 8ml DCM, 12ml acetone,

Temperature 30-40ºC

Optimized batch from above microspheres were prepared by a solvent evaporation method using different emulsifying agent (Table 2).

Application of Box Behnken Design[7, 8, 9]

Box behnken design as per Table 3.

Data Transformation

The data transformation simplifies the calculations for model development. The data generated by the experimental design was utilized for drawing contour plot, to obtain an optimized region within the factorial space, and thereby produce an optimized formulation.

Table 1: Composition of Repaglinide Microspheres

Batch no.	Drug : Polymer Ratio	Polymer	Emulsifying Agent
MS1	1:5	Ethyl Cellulose	0.2% PVA Solution
MS2	1:10	Ethyl Cellulose	0.2% PVA Solution
MS3	1:15	Ethyl Cellulose	0.2% PVA Solution
MS4	1:5	Eudragit RSPO	0.2% PVA Solution
MS5	1:10	Eudragit RSPO	0.2% PVA Solution
MS6	1:15	Eudragit RSPO	0.2% PVA Solution
MS7	1:10	EC: Eudragit RSPO(1:1)	0.2% PVA Solution
MS8	1:10	EC: Eudragit RSPO(1:4)	0.2% PVA Solution
MS9	1:10	EC: Eudragit RSPO(2:3)	0.2% PVA Solution

Table 2: Composition of Repaglinide Microspheres with Different Emulsifying Agents

Batch No.	Drug : Polymer Ratio	Type of Polymer	Solvent (Acetone: Ethanol: DCM)	Emulsifying agent
MS10	1:10	EC : Eudragit RSPO(2:3)	2:2:1	0.08% SLS Solution
MS11	1:10	EC: Eudragit RSPO(2:3)	2:2:1	Light Liquid Paraffin

Table 3: Selection of Factors, Levels and Responses for Box Behnken Design

Variable	Level		
Independent variables			
Coded value	**-1**	**0**	**+1**
Drug: Polymer ratio (A)	1:5	1:10	1:15
Organic Phase Addition Time(B)	5	10	15
Stirring Speed(C)	500	750	1000
Dependent variables			
Dissolution rate at 2 hr	-	-	-
Dissolution rate at 8 hr	-	-	-

Table 4: Design Layout and Data Transformation

Sr.no	Batch no	Drug: Polymer Ratio	Organic Phase Addition Time (min)	Stirring Speed
1	MS12	1.5	5	750
2	MS13	1.15	5	750
3	MS14	1.5	15	750
4	MS15	1.15	15	750
5	MS16	1.5	10	500
6	MS17	1.15	10	500
7	MS18	1.5	10	1000
8	MS19	1.15	10	1000
9	MS20	1:10	5	500
10	MS21	1:10	15	500
11	MS22	1:10	5	1000
12	MS23	1:10	15	1000
13	MS24	1:10	10	750
14	MS25	1:10	10	750
15	MS26	1:10	10	750

Statistical Analysis[10]

The statistical analysis of the box behnken design batches was performed by multiple quadratic regression analysis using design expert software trial version of 8.0.1.7. Data of two sets were evaluated by paired *t* test and one-way analysis of variance (ANOVA) was applied to check significant difference in drug release from different formulations. *p*values of less than 0.05 ($p < 0.05$) were considered to be significant.

Check Point Analysis

Validation of box behnken design was carried out using check point analysis. Check point analysis was performed by preparing three batches of different variables. After performing *in vitro* study of check point batches %CDR at 2hr (Q_2) and at 8 hr (Q_8) was measured. The predicted response of Q_2 and Q_8 was calculated using polynomial equation for 2 hr and for 8 hr respectively and the measured and predicted response was compared.

Optimization of Formulation by Box Behnken Design

The response variables were optimized using design expert software. The prognosis of optimum formulation was conducted using two stage technique in which first a feasible space was located and second an exhaustive grid search was conducted to predict the possible

solution. The region of optimality was also ratified using overlay plots. The microsphere of optimized batch was formulated using composition given in table 6 and evaluated for cumulative % drug release. The observed and predicted responses were critically compared.

Table 5: Check point batches MS27 to MS29

Batch No	Drug: Polymer Ratio	Organic Phase Addition Time	Stirring Speed
MS27	-0.5	+0.5	0
MS28	-0.5	0	+0.5
MS29	0	-0.5	-0.5

Table 6: Composition of Optimized Batch

Ingredients	MS30
Drug: Polymer Ratio	1:11
Organic Phase addition time	13.45 min
Stirring Speed	722
Continuous Phase	0.4% PVA solution
Solvent	Acetone: Ethanol: DCM (2:2:1)

Evaluation of Microspheres[11, 12]

Bulk Density and Tapped Density

The microspheres fabricated are weighed and transferred to a 10ml graduated glass cylinder. The volume was measured which are known as bulk density. The cylinder is tapped until the microsphere bed volume is stabilised.

Angle of Repose

The maximum angle which is formed between the surface of a pile of powder and horizontal surface is called the angle of repose.

$$\mathbf{Tan\ \theta = h/r}$$

Where, θ = angle of repose,

h = Height of the heap,

r = Radius of the heap.

% Yield

Microspheres after drying at 40ºC were weighed to calculate the percentage yield of microspheres using the following formula:

$$\text{Percentage yield} = \frac{\text{Total amount of microparticles}}{\text{total weight of drug and polymer}} \times 100$$

% Drug Loading

100 mg of microspheres were accurately weighed and crushed in mortar pestle. Crushed particles were soaked in 100 ml of distilled water. Solution was then sonicated and stirred for 24 hrs. The solution was then filtered and filtrate was appropriately diluted and measured the absorbance in UV visible spectrophotometer at λmax 243nm.

Particle Size Analysis

The particle size of microspheres was determined by optical microscopy method; approximately 100 microspheres were counted for particle size using a calibrated optical microscope. The microspheres were uniformly spread on a slide. The particle size of the microsphere was measured, along the longest axis and the shortest axis (cross shaped measurement). Average of these two readings was given as mean diameter of particles. The diameter of a minimum number of 100microspheres in each batch was calculated.

***In-vitro* Drug Release Study** [13]

Microspheres were evaluated for *in-vitro* release study in 0.1 N HCl. A weighed amount of floating microspheres equivalent to 10 mg drug was filled into a capsule and placedin basket. 0.1 N HCl at pH 1.2 containing tween 20 (0.02% w/v) was used as the dissolution medium and maintained at 37±0.5°C at a rotation speed of 100 RPM. 10 ml of sample was collect at each hour to measure UV absorbance and replaced with 10 ml of fresh media. The sample aliquots were collected up to

12 hrs. The absorbance of sample was then measured in UV visible spectrophotometer at λ_{max} 243.15 nm.

Floating Behavior

Fifty milligrams of the floating microparticles were placed in 0.1 N HCl containing 0.02 w/v% tween 20. The mixture was stirred at 100 RPM in a magnetic stirrer. After 12 hr, the layer of buoyant microparticles was pipetted and separated by filtration. Particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in a desiccator until constant weight. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles.

Surface Topography

Surface morphological study had been carried out by Scanning Electron Microscopy (SEM). The shape and surface morphology of Repaglinide microspheres were investigated using SEM. The samples for SEM study were prepared by lightly sprinkling the formulation on a double-adhesive tape stuck to an aluminium stub. The stubs were then coated with gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned at 15 accelerated voltage and different magnification of 35 and 100 times were taken with a scanning electron microscope.

Application of Kinetic Models[14]

The dissolution data of all controlled-release microparticles and control formulation was fitted to kinetics models i.e., Zero order, First order, Higuchi, and Korsmeyer–Peppas to find out drug release pattern and mechanism.

Stability Study of Optimized Batch[15]

Stability studies were performed according to ICH guideline. Optimized batch was packed in an airtight amber glass bottles. The bottles were kept at 25ºC ± 2ºC / 60% RH ± 5% RH and 40ºC ± 2ºC / 75% RH ± 5% RH tested after 6 month. The sample of microspheres was then evaluated for stability by determining drug loading, *in-vitro* dissolution study and physical appearance.

RESULTS AND DISCUSSION

% Yield and % Drug Loading

Results of % yield and % drug loading of Batches MS1 to MS9 are listed in table 7. From this results, it was observed that microspheres prepared with ethylcellulose have a good spherical shape but having very low drug loading (18.60% ± 1.98) and % yield (51.2% ± 2.98). While microspheres with eudragit RSPO have higher drug loading (20.66% ± 2.78) compared to ethylcellulose. The % drug loading was found to be increased when both polymers were used together so microspheres prepared using combination of ethyl cellulose and eudragit RSPO show highest drug loading(54.66% ± 0.91) and % yield (96.35% ± 1.43) in batch MS25.

Microspheres prepared using sodium lauryl sulphate and light liquid paraffin as an emulsifying agent (MS10 and MS12) show less drug loading compared to PVA as emulsifying agent. Microspheres prepared using liquid paraffin as an emulsifying agent show good drug loading and % yield then SLS but less than PVA.

All the batches were prepared using ethanol, acetone and DCM with ratio of 2:2:1. Here solvent with low water solubility results in slow polymer precipitation which facilitate complete partitioning of drug into aqueous phase.

Flow Property and % Buoyancy of Microspheres

The Hausner's ratio was used to access compressibility property of drug. Hausner's ratio of Repaglinide has indicated extremely poor flow property. From the table 8 it was observed that Hausner's ratio of all the batches of microspheres were in the range of 1.08± 0.06 to 1.55 ± 0.21 which indicates good flow property.

Table 7: % Yield and % Drug Loading of Microspheres

Batch code	% Yield	% Drug Loading
MS1	46.34±2.34	13.30±2.01
MS2	49.80±2.45	18.60±1.98
MS3	51.2±2.98	15.00±2.34
MS4	43.85±2.10	18.22±2.65
MS5	50.78±1.87	20.66±2.78
MS6	44.00±1.45	27.40±2.31
MS7	73.87±2.6	43.35±1.76
MS8	78.75±1.23	42.73±2.13
MS9	86.12±1.32	50.46±2.06
MS10	45.68±1.56	5.32±1.99
MS11	52.31±1.03	8.2±1.41
MS12	83.75±1.34	44.85±2.54
MS13	84.28±1.88	50.4±3.01
MS14	85.23±2.33	48.33±2.78
MS15	87.5±2.89	43.18±2.06
MS16	85.0±1.54	48.48±1.87
MS17	80.00±2.01	39.49±1.65
MS18	79.04±1.78	42.09±2.70
MS19	80.00±1.71	43.47±2.73
MS20	89.43±1.43	50.81±1.65
MS21	91.67±0.91	49.41±1.31
MS22	92.12±0.98	51.77±0.67
MS23	94.89±1.67	53.64±1.97
MS24	96.11±2.01	54.34±2.10
MS25	96.35±1.43	54.66±0.91
MS26	97.03±0.71	53.98±1.88

The value of angle of repose was used to understand flow property. Repaglinide powder could not pass through the funnel during experiment. The poor flow of Repaglinide could be due to its crystalline nature which posed hurdles in the uniform flow through funnel. While, all the prepared microspheres exhibit good flow property in the range of 22.87 ± 0.24 to 30.01 ± 0.54 which indicates free flowing nature of microspheres.

Particle Size Determination

Particle size analysis was performed by optical microscopy of microspheres. Microspheres of prepared batches have different mean particle size within range of 240µm to 380µm. Microspheres show spherical in shape as shown in figure 3.

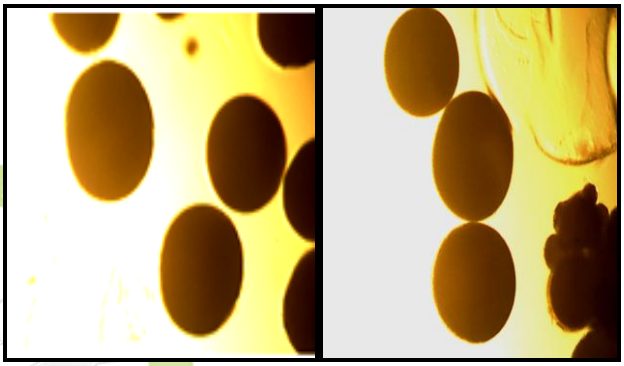

Figure 3: Repaglinide Microspheres in Optical Microscope

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

In-vitro drug release study was performed in USP apparatus II in 0.1 N HCl for 12 hrs and the graphs was plotted between Time Vs % Cumulative Drug Release (%CDR).

Different release profiles were observed with each combination of polymers. The effect of changes in polymer proportion in batches MS7 to MS9 has been shown in figure 4. When microspheres prepared by using combination of two polymer having polymer ratio Eudragit RSPO: EC (1:1), slow drug release was observed compare to polymer ratio Eudragit : EC (4:1). It was observed that EC gave comparatively slow drug release profile than Eudragit. The reason for this may be the insolubility in water and hydrophobicity of the ethyl cellulose. Microspheres prepared using polymer Eudragit RSPO: EC (1:1) showed drug release upto 83% to 86%.This batch was controlling drug release more than 12 hr.

Table 8: Flow Property and % Buoyancy of Microspheres

Batch no	Bulk Density* (gm/ml)	Tapped Density* (gm/ml)	Hausner's Ratio*	Angle of Repose*	% Buoyancy
MS1	0.54± 0.3	0.67± 0.7	1.23± 0.5	22.54± 0.4	74± 2.1
MS2	0.48± 0.2	0.53± 0.3	1.08± 0.1	28.67± 0.5	76± 3.5
MS3	0.51± 0.9	0.59± 0.4	1.41± 0.4	33.76± 0.1	77± 1.6
MS4	0.54± 0.1	0.61± 0.8	1.55± 0.1	26± 0.2	73± 2.1
MS5	0.46± 0.7	0.59± 0.6	1.35± 0.8	29.43± 0.8	76± 2.4
MS6	0.56± 0.6	0.72± 0.1	1.48± 0.4	30.01± 0.4	82± 1.5
MS 7	0.40 ± 0.4	0.57 ± 0.8	1.42 ± 0.2	28.51 ± 0.7	78±1.6
MS 8	0.42 ± 0.1	0.54 ± 0.9	1.28 ± 0.9	26.16 ± 0.3	78±4.01
MS 9	0.49 ± 0.1	0.58 ± 0.1	1.18 ± 0.1	29.40 ± 0.2	81±4.2
MS10	0.54± 0.3	0.61± 0.9	1.24± 0.5	22.56± 0.6	72±3.4
MS11	0.67± 0.2	0.72± 0.5	1.45± 0.5	19.99± 0.7	73±3.1
MS12	0.51± 0.3	0.57± 0.7	1.34± 0.2	26.54± 0.8	72±2.6
MS13	0.49± 0.5	0.59± 0.9	1.04± 0.7	29.01± 0.5	72±2.6
MS14	0.50± 0.8	0.63± 0.4	1.54± 0.3	25± 0.9	71±1.5
MS 15	0.51± 0.2	0.58± 0.1	1.39± 0.3	22.49± 0.9	74± 3.1
MS16	0.47 ± 0.1	0.57 ± 0.5	1.21 ± 0.1	25.36 ± 0.3	80±3.4
MS17	0.45 ± 0.4	0.52 ± 0.1	1.15 ± 0.5	24.12 ± 0.9	83±1.8
MS18	0.46 ± 0.3	0.50 ± 0.3	1.08 ± 0.6	26.38 ± 0.7	82±1.67
MS19	0.44 ± 0.6	0.53 ± 0.6	1.20 ± 0.2	22.87 ± 0.4	83±2.06
MS20	0.48 ± 0.2	0.59 ± 0.4	1.22 ± 0.3	25.49 ± 0.8	81±4.1
MS21	0.39 ± 0.6	0.51 ± 0.2	1.30 ± 0.1	23.83 ± 0.7	79±3.4
MS22	0.37 ± 0.1	0.53 ± 0.3	1.43 ± 0.4	24.76 ± 0.5	83±3.1
MS23	0.41 ± 0.1	0.51 ± 0.5	1.24 ± 0.2	26.59 ± 0.5	84±2.6
MS24	0.39 ± 0.6	0.51 ± 0.2	1.29 ± 0.1	21.83 ± 0.7	74±1.78
MS25	0.37 ± 0.1	0.53 ± 0.3	1.23 ± 0.4	21.76 ± 0.5	85±1.3
MS26	0.41 ± 0.1	0.51 ± 0.5	1.24 ± 0.2	22.59 ± 0.5	85±1.1

*All the reading were calculated as mean value and with standard deviation where n=3

While using polymer Eudragit RSPO: EC (4:1) show initial burst effect within 2 hr. Here total drug release observed merely after 8 h. Microspheres with combination of two polymer release profile in controlled manner with time period up to 12 hrs. Batch MS9 containing both polymer showed highest drug loading with spherical shape particles and have sustained action.

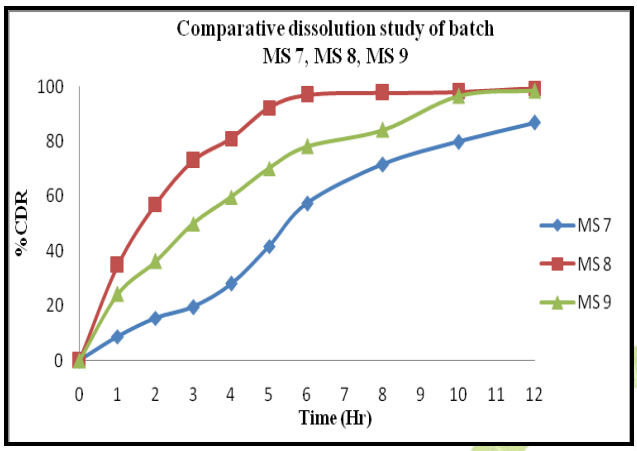

Figure 4: *In vitro* Dissolution study of batch MS7, MS8, MS9

Table 9 : Mean Particle Size of Repaglinide Microspheres

Batch Code	Mean Particle Size* (µm)
MS1	410±2.65
MS2	473±10.89
MS3	490±2.01
MS4	310±8.91
MS5	331± 2.88
MS6	387± 5.78
MS7	267 ± 9.48
MS8	258 ± 2.36
MS9	254 ± 8.62
MS10	567± 8.42
MS11	612± 3.67
MS12	156 ± 3.65
MS13	190 ± 7.44
MS14	132±2.0
MS15	175 ± 2.87
MS16	285 ± 8.42
MS17	307 ± 6.85
MS18	249 ± 6.16
MS19	271 ± 4.73
MS20	197 ± 2.62
MS21	173 ± 2.48
MS22	189 ± 3.28
MS23	140 ± 7.94
MS24	110 ± 2.48
MS25	117 ± 2.28
MS26	102 ± 7.94

Effect of Drug: Polymer Ratio

From the figure 5, it was observed that %CDR was higher in MS12, MS14, MS16, MS18 with drug: polymer ratio 1:5 compared to MS13, MS15, MS17, MS19 with drug: polymer ratio 1:15.So, it was studied that change in the polymer concentration alter the release rate profile. At lower concentration higher burst release effect was found but when increasing the polymer concentration there was formation of larger microspheres having small surface area which control the release rate.

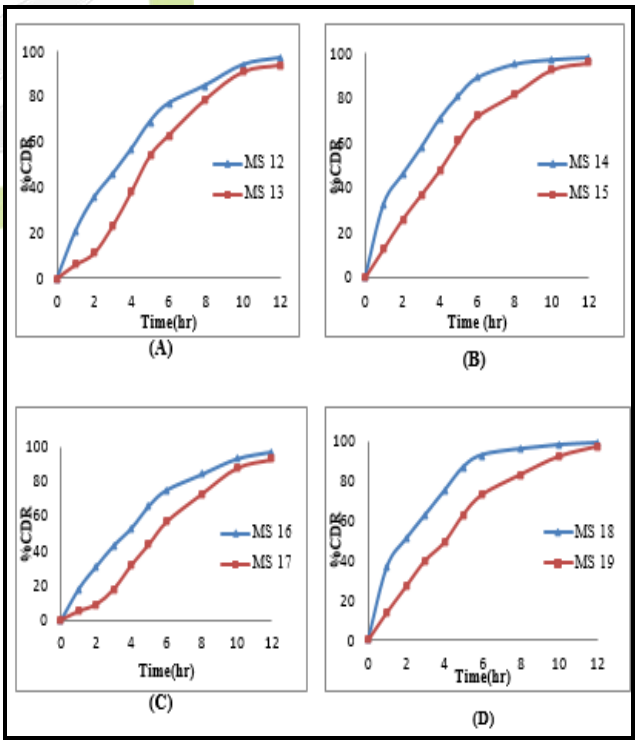

Figure 5: Effect of Drug: Polymer Ratio- Comparative dissolution profile of batch (A) MS12 and MS13 (B) MS14 and MS15 (C) MS16 and MS17(D) MS18 and MS19

Effect of Organic Phase Addition Time

The drug release profile has indicated that %CDR was higher in MS14, MS15, MS21, MS23 with organic phase addition time 15 min compared to MS12, MS13, MS20, MS22 with organic phase addition time 5 min. Addition of solvent at faster speed results in slow release rate due to solvent may diffuse into the aqueous phase before stable emulsion droplets developed and aggregation of microspheres droplets occurs which slow down the release rate.

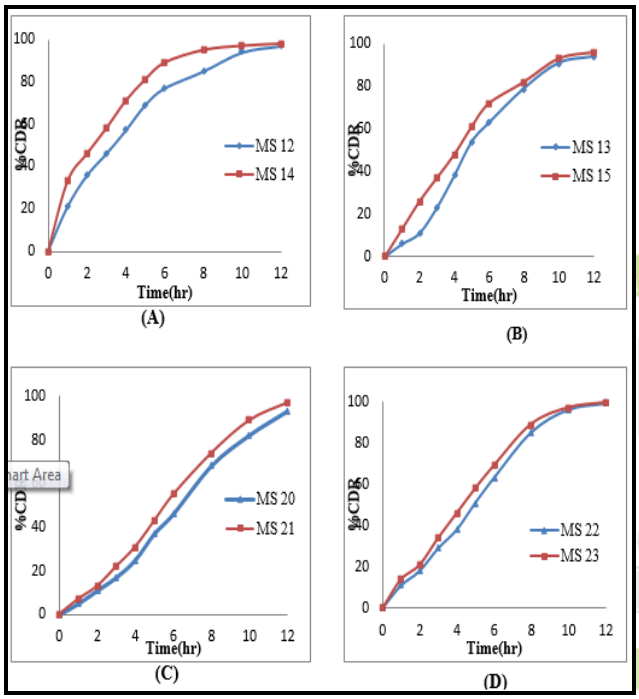

Figure 6: Effect of Organic Phase Addition Time-Comparative dissolution profile of batch (A) MS12 and MS14 (B) MS13 and MS15 (C) MS20 and MS21 (D) MS22 and MS23

Effect of Stirring Speed

Batches MS18, MS19, MS22 and MS23 with stirring speed 1000 has shown higher drug release compared to batches MS16, MS17, MS20 and MS21 with stirring speed 500.*In vitro* drug release profile of these batches is depicted in figure 7. From these, it was concluded that at higher stirring speed release rate is fast as there is a formation of smaller size particles and no agglomeration of particles and in case of lower RPM release rate is slow as there is a formation of larger particle compared to higher speed.

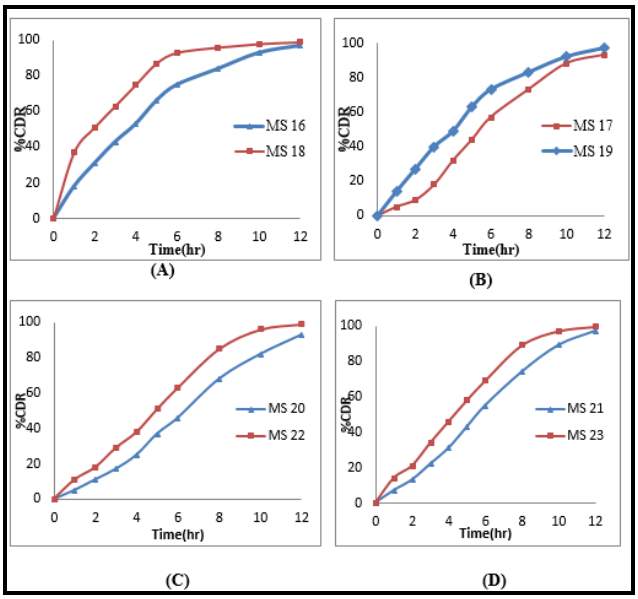

Figure 7: Effect of Stirring Speed- Comparative dissolution profile of batch (A) MS16 and MS18 (B) MS17 and MS19 (C) MS20 and MS22 (D) MS21 and MS23

In vitro Release Profile of Batch MS 24 to MS 26

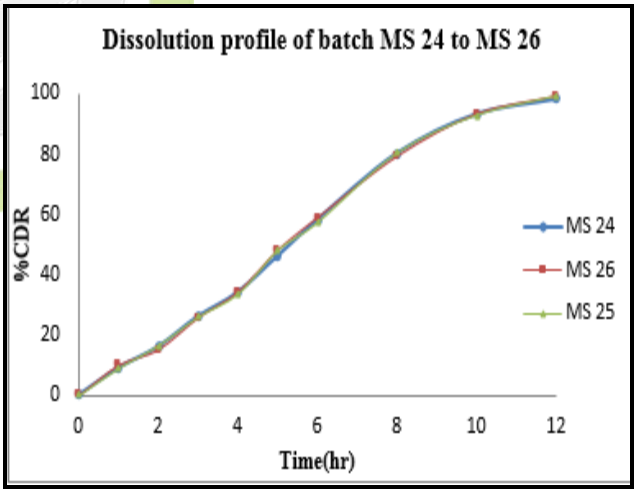

Figure 8: *In vitro* Dissolution study of batch MS 24 to MS 26

Here batch MS24 to MS26 were center point batches and all were prepared using Drug: Polymer Ratio 1:10, Solvent addition time 10 min, Stirring s peed 750 RPM. So, these batches were selected to optimize the parameter as they shown drug release in controlled manner with time.

Statistical Analysis

The statistical analysis of the box behnken design batches was performed by multiple quadratic regression analysis. The % drug release at 2 hr and 8 hr was selected as dependent variables. The values of % drug release at 2 hr and 8 hr for the 15 batches (MS12 to MS26) showed a wide variation, the results were shown in table 10. The data clearly indicate that the values of dependent variables were strongly dependent on the independent variables.

Table 10: Value of Dependent Variables Q_2 and Q_8

Batch No	% Cumulative Drug Release at 2 hr Q_2	% Cumulative Drug Release at 8 hr Q_8
MS12	35.56	85.23
MS13	11.93	79.32
MS14	46.43	95.38
MS15	26.05	82.67
MS16	31.98	84.91
MS17	9.43	73.05
MS18	51.77	96.69
MS19	27.67	83.43
MS20	11.98	68.91
MS21	13.43	74.05
MS22	18.77	85.69
MS23	21.67	89.43
MS24	15.10	80.91
MS25	16.56	80.21
MS26	15.22	79.12

Polynomial Equation for % Cumulative Drug Release at 2 hr (Q_2)

$Y_{120} = 15.62 - 10.62X_1 + 3.67X_2 + 5.92X_3 + 0.81X_1X_2 + 2.81X_1X_3 + 0.36X_2X_3 + 13.35X_1^2 + 1.01X_2^2 - 0.17X_3^2$, $R^2=0.9546$

The % CDR is an important parameter for extended release of microspheres. The % CDR at 2 hr of sustained release microspheres varied from 9.43% to 51.77% and showed good correlation coefficient as 0.9482. Result of the regression analysis indicate that variables A (drug: polymer ratio) and C (RPM) were significant.

Polynomial Equation for % Cumulative Drug Release at 8hr (Q_8)

$Y_{480} = 80.08 - 5.47X_1 + 2.80X_2 + 6.79X_3 - 1.70X_1X_2 - 0.78X_1X_3 - 0.35X_2X_3 + 5.29X_1^2 + 0.088X_2^2 - 0.85X_3^2$, $R^2=0.9738$

The % CDR is an important parameter for extended release of microspheres. The % CDR at 8hr of sustained release microspheres varied from 68.91% to 96.89% and showed good correlation coefficient as 0.9729. Result of the regression analysis indicate that variables A (drug: polymer ratio), B (organic phase addition time) and C (RPM) were significant.

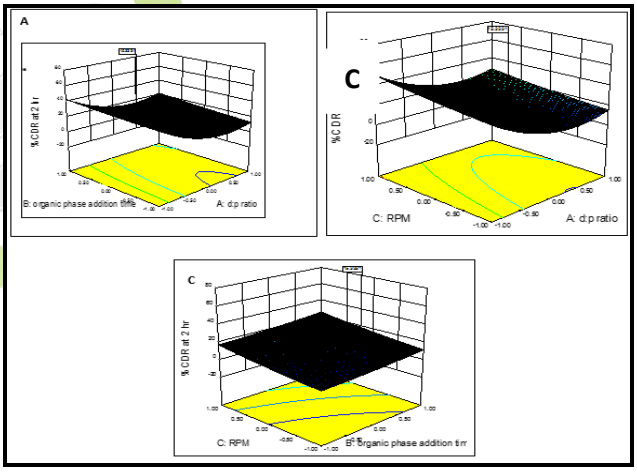

Figure 9: (A) Effect of Drug: Polymer Ratio and Organic Phase Addition Time, (B) Effect of Drug: Polymer Ratio and Stirring Speed, (C) Effect of Organic Phase Addition Time and Stirring Speed

ANOVA for % Cumulative Drug Release at 2 hr

From ANOVA results, p value less than 0.0500 indicate model terms are significant. Here, terms A and C are significant. Response surface plot indicate the augmentation of line toward the A factor in AB and AC factor interaction. So,

factor A (drug: polymer ratio) is more significant.

Table 11: ANOVA: Data of Dependent Variable at 2 hr

	SS	Df	MS	F value	p value
Regression					
Full Model	2238.8	9	248.7	11.7	0.007
Reduced Model	2110.8	3	703.6	33.0	8.48E-06
Residual					
Full Model	106.28	5	21.25	-	-
Reduced Model	234.22	11	21.29	-	-

Response Surface Plot for % Cumulative Drug Release at 8hr (Q_8)

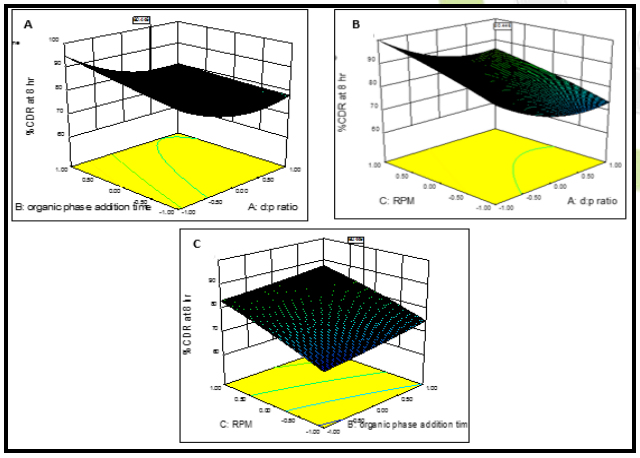

Figure 12: (A) Effect of Drug: Polymer Ratio and Organic Phase Addition Time (B) Effect of Drug: Polymer Ratio and Stirring Speed(C) Effect of Organic Phase Addition Time and Stirring Speed

ANOVA for % Cumulative Drug Release at 8hr

From ANOVA results, p values less than 0.0500 indicate model terms are significant. Here, terms A and C are significant. Response surface plot indicate the augmentation of line toward the A factor in AB and AC factor interaction. So, factor A (drug: polymer ratio) is more significant.

Table 12: ANOVA: Data of Dependent Variable at 8hr

	SS	Df	MS	F value	p value
Regression					
Full Model	792.82	9	88.09	26.65	0.001
Reduced Model	776.45	4	194.11	51.47	1.23E-06
Residual					
Full Model	21.32	5	4.26	-	-
Reduced Model	37.70	10	3.77	-	-

Check Point Analysis

Validation of box behnken design was carried out using check point analysis. The values of variables were fitted into polynomial equation as a predicted response. And predicted response was compared with measured. There is no significant change in predicted and measured response of Q_2 and Q_8 and equivalency of both responses proved robustness of polynomial equation.

Optimization of Box Behnken Design

Validation of box behnken design is necessary for confirmation of applied model. Optimized batch MS30 contains drug: polymer ratio 1:11, organic phase addition time 13.45 min, stirring speed 722 RPM, was formulated and evaluated for different physico chemical parameter to calculate the design. All the parameters of optimized batch are as per requirement.

Table 13: Check Point Batches Response at Q_2 and Q_8

Batch No	A	B	C	Predicted Response		Measured Response	
				At 2 hr	At 8 hr	At 2 hr	At 8 hr
MS27	-0.5	+0.5	0	25.85	86.03	24.02±2.43	85.31±1.3
MS28	+0.5	0	-0.5	9.33	75.15	10.56±1.88	76.92±2.01
MS29	0	-0.5	+0.5	16.16	82.02	15.33±1.95	81.98±2.79

With multiple responses it is necessary to find regions where requirements simultaneously meet the critical properties (the *sweet spot).* Graphical optimization displays the area of feasible response values in the factor space. Regions that do not fit the optimization criteria are shaded gray. The area that satisfies the constraints will be yellow, while the area that does not meet the criteria is gray.

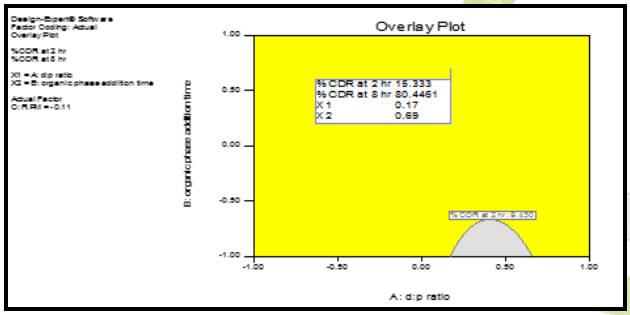

Figure 13: Overlay Plot for Optimized Batch

Table 14: Evaluation Parameters of Optimized Batch MS30

Evaluation Parameters	Batch MS30
% yield	98.34±0.87
% drug loading	55.12±0.73
Bulk density(gm/ml)	0.57±0.09
Tapped density(gm/ml)	0.59±0.14
Hauser's ratio	1.05±0.11
Angle of repose(°)	20.56±0.77
% Buoyancy	90.69±1.25
Mean particle size(µm)	180 ±2.54
In vitro drug release(Q_2)	15.79±2.78
In vitro drug release(Q_8)	80.01±2.11

In Vitro Drug Release Study of Optimized Batch MS30

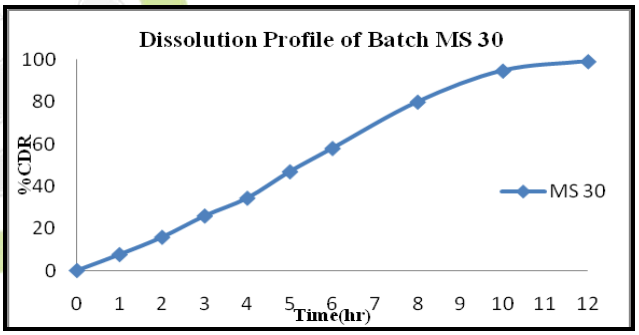

Figure 14: *In Vitro* Drug Release of Batch MS 30

Surface Topography of Optimized Batch

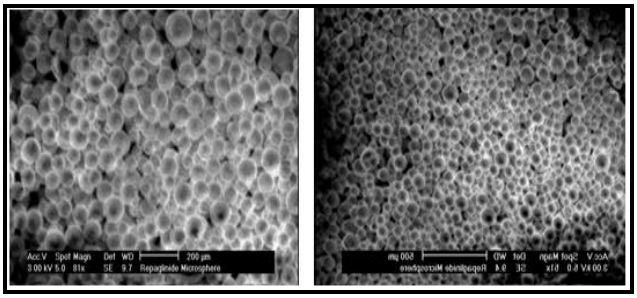

Figure 15: SEM of Repaglinide Microspheres

Surface morphology study was performed by Scanning Electron Microscope (SEM) for final formulation of Repaglinide microspheres.

Microspheres were observed at 50 and 80 times magnification. SEM of final formulation i.e. drug: polymer ratio of 1:1, Organic phase addition time (13.45 min), stirring speed (722RPM) batch MS30 microspheres shows particles in spherical shape. The smooth and even surface was because of highly plasticizing nature of Eudragit.

Application of Kinetic Model

The *in vitro* release data were kinetically analyzed for establishing kinetic of drug release. Zero-order, First-order, Higuchi, Korsmeyer-peppas and Hixoncrowell models were tested. Table 15 enlists the regression parameters obtained after fitting dissolution release profile to various kinetic models.

The curve fitting of optimized batch was best explained by zero order equation and korsmeyer-peppas model, based on highest goodness of fit (R^2 0.9916) and lowest value of SSR (Sum of Square of Regression) and AIC (Akaike Information Criterion) as the plots showed the highest linearity (r^2 = 0.9916) followed by Hixoncrowell (r^2 = 0.970), followed by first order equation (r^2 = 0.9748). Hence the drug release kinetics demonstrates that the concentration was nearly independent of drug release. n value of korsemeyer-peppas is 0.955 indicate that drug release observed by diffusion and erosion both mechanism and the model is non fickian (anomalous transport).

Stability Study of Optimized Batch

The selected optimized Formulation MS30 were evaluated for stability studies which were stored at 25ºC ± 2ºC / 60% RH ± 5% RH and 40ºC ± 2ºC / 75% RH ± 5% RH tested for 6 month, and were analyzed for their drug content at that interval. The residual drug contents of formulations were found to be within the permissible limits and the results of 6 month's duration are shown in the Table 16.

Table 15: Model Fitting for Optimized Batch MS30

Model	R^2	r	k	SSR	AIC
Zero-order	0.9833	0.9916	9.061	192.79	54.61
First-order	0.9099	0.9748	0.153	1041.62	71.48
Higuchi	0.8430	0.9541	24.707	1815.25	77.03
Hixoncrowell	0.9480	0.9862	0.044	601.2	65.99
Korsmeyer - peppas	0.9843	0.9924	9.97	181.13	3.55

Table 16: Stability Study of Optimized Batch MS30

Time	Initial drug Loading of batch MS 30	Batch MS30 stored at 25ºC ± 2ºC / 60% RH ± 5% RH		Batch MS30 stored at 40ºC ± 2ºC / 75% RH ± 5% RH	
		Physical appearance*	%Drug Loading	Physical appearance*	%Drug Loading
6 month	55.12%	+++	54.98%	+++	54.07%

*+++ = Same as on zero day

CONCLUSION

Repaglinide (Rg), a fast and short acting meglitinide analog, having shorter half life, is chosen as the drug candidate for polymeric microsphere formulation. Drug-Excipients compatibility was performed by FT-IR. Microspheres was prepared with two different polymer (ethyl cellulose and eudragit) and combination of both polymer using different emulsifying agents in which the combination of EC: Eudragit RSPO (2:3) polymer using PVA as emulsifying agent had shown highest drug loading and %yield. *In-Vitro* drug release studies were performed for Repaglinide microspheres for 12 hrs. By applying box behnken design other variables were optimized. Batch MS12 to MS26 were developed using three variables (drug: polymer ratio, organic phase addition time, stirring speed) with three level of them. It was concluded that as the concentration of polymer increases the release rate decreases as at higher concentration less chances of drug to diffuse away from polymer. It was observed that if the solvent addition time too fast, solvent may diffuse into the aqueous phase before stable emulsion droplets developed and aggregation of microspheres droplets occurs which slower the release rate. It was also concluded that at higher stirring speed release rate is fast because of formation of smaller size particles and no agglomeration of particles. After optimization validation of optimized batch MS30 was done using Drug: Polymer ratio 1:11, Organic phase addition time 13.45 min and stirring speed 722 RPM. Particle size analysis and scanning electron microscopy of optimized batch was done shows the particles are in range of 40µm to 100µm having spherical in shape. Zero order kinetic model for batch MS30 indicate that drug release was independent of concentration and follows non fickian mechanism as n= 0.955 of koysmeyer-peppas. Stability of Repaglinide microspheres was performed for 1 month at 25ºC ± 2ºC / 60% RH ± 5% RH and 40ºC ± 2ºC / 75% RH ± 5% RH. It shows good stability of microparticles.

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