Ind. J. Pharm. Edu. Res., 2023; 57(2s):s262-s273.
https://www.ijper.org

Original Article

Design Formulation and Statistical Evaluation of Gastroretentive Microspheres of Rasagiline Mesylate for Parkinson's Disease Using Design Expert

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Received: 23-11-2022;
Revised: 13-01-2023;
Accepted: 17-02-2023.

ABSTRACT

Introduction: Rasagiline mesylate is primarily prescribed to treat the symptoms of idiopathic Parkinson's disease works as irreversible inhibitor of mono amino oxidase. The microspheres were designed for extended retention of drug in gastrointestinal tract, resulting in superior absorption and enhanced bioavailability by oral route. **Materials and Methods:** The ionotropic gelation method was used to prepare the formulations RM1 to RM14 mucoadhesive microspheres with Sodium alginate, Calcium chloride, Carbopol 934, Xanthan gum, Chitosan of different concentrations were formulated in preliminary trials after performing preformulation studies such as FTIR, DSC. Optimization of Rasagiline mesylate mucoadhesive microspheres (RMS1 to RMS11) were done by optimizing independent variables such as polymer concentration i.e. Xanthan Gum (5 mg, 20 mg and 35 mg), a Stirring speed (500, 1000 and 1500 rpm) and dependent variables such as percentage entrapment efficiency, particle size and cumulative percent drug release. Optimization was done by using Design export 13 software by Central composite design from Response surface methodology. ANOVA explains the impact of independent variables on the dependent variables. For optimized formulation structural features determined by SEM and XRD. **Results:** In preliminary studies it was found that, apart from Chitosan, the formulations with Carbopol 934P had shown best mucoadhesion and drug release. The optimized formulation RMS12 (given by Design expert software) having 32.12 mg of Xanthan gum at 1500RPM showed 86.84% entrapment efficiency, 440µm particle size and 96.43 Cumulative percent drug release. **Conclusion:** It was concluded that the formulated Gastro retentive mucoadhesive microspheres of Rasagiline mesylate was found to be having best *in vitro* drug release.

Keywords: Rasagiline mesylate, Parkinson's disease, Mucoadhesion, Microspheres, Design expert, Response surface methodology.

INTRODUCTION

The diameter of a microsphere ranges between 1 µm and 1000 µm. The particles are spherical free-flowing made up of proteins or polymers.[1] In addition to natural polymers, waxes, biodegradable synthetic polymers are also used to make them. As a strategy for controlling drug delivery, mucoadhesive microspheres were designed to extend the duration of the dosage form remains at the site of absorption, thus improving and enhancing the bioavailability of the drug.[2]

Rasagiline mesylate is a irreversible, selective second-generation monoamine oxidase type B inhibitor which is primarily responsible for inactivating dopamine in the central nervous system. Rasagiline mesylate has been used to treat motor complications caused by Parkinson's disease. However, the Rasagiline mesylate undergoes first-pass metabolism, has low bioavailability (36%) and short half-life of 1.5 to 3.5 hr. So, there is a need to make gastro retentive formulation due to the short half-life, poor bioavailability and to maintain therapeutic levels of the drug.[3-5]

Design of Experiments (DOE) is an active means in order to optimise the formulation with the fewest possible runs and identify the factors that have the greatest influence on the formulated microspheres. The relationship between factors (independent variables) selected and the responses (dependent variables) noticed by DOE and the variability in responses were notified.[6,7]

DOI: 10.5530/ijper.57.2s.30

This study aims to improve oral bioavailability of Rasagiline mesylate by formulating into mucoadhesive gastroretentive microspheres. Gastroretentive formulation will reduce high first pass metabolism for the drug by prolonging residence time in stomach.

MATERIALS AND METHODS

Experimental investigation

Rasagiline mesylate was gifted by Sun Pharma, Mumbai, India. Xanthan gum, Chitosan were purchased from MSN Laboratories Pvt. Ltd., Hyderabad, India. Carbopol 934P, Sodium alginate, Calcium chloride and Glacial acetic acid were purchased from SD Fine Chem., Mumbai.

Different excipients were used like Sodium alginate, Calcium chloride for the formation of outer shell of microspheres. In order to control the drug release, Xanthan gum was used. Mucoadhesive polymers like Carbopol 934P and Chitosan were selected and compared.[8] In preliminary trails it was observed that 2.25% of Sodium alginate, 10% of Calcium chloride had shown good gelation and 400 mg of Carbopol 934P had shown best mucoadhesion. Therefore, 400mg of Carbopol 934P, 10% of calcium chloride, and 2.25% of Sodium alginate were regarded as fixed attributes for further optimization. Further optimization of Rasagiline mesylate mucoadhesive microspheres (RMS1 to RMS11) was done by optimizing independent variables in three levels such as Xanthan Gum (5, 20 and 35 mg) and rpm (500, 1000 and 1500) done by estimating dependent variables as percentage entrapment efficiency, particle size and cumulative percent drug release by using Central Composite Design from Response surface methodology using Design Export 13 software. As per the Central composite design, Rasagiline mesylate loaded microspheres were prepared with eleven possible compositions. This explained how the dependent variables effected by the independent variables by ANOVA.[9] These responses were evaluated using a statistical model that includes two-factor interactive polynomial term.

Methods

The orifice ionic gelation method was used to produce mucoadhesive microspheres containing Rasagiline mesylate. In the purified water, Sodium alginate was mixed with mucoadhesive polymers such as Chitosan, Carbopol 934P and Xanthan gum. Rasagiline mesylate was added to the polymer dispersion and thoroughly mixed on a magnetic stirrer. The gelation medium was made by dissolving 10% Calcium chloride in 2% Glacial acetic acid solution (It helps in the formation of stable microspheres). The homogeneous alginate solution was extruded into the gelation medium using a 21G syringe needle with stirring. The distance between the needle's edge and the gelation medium's surface was maintained around 10 cm[10-13] The formulation of microspheres for preliminary trials was showed in (Table 1). The formulation of microspheres for optimized formulation was showed in (Table 2).

Characterization and Evaluation of Mucoadhesive Microspheres

Entrapment efficiency

Rasagiline Mesylate loaded microspheres were washed with 10 mL of 0.1N HCl to get rid of unentrapped drug on the surface. After washing, microspheres were digested in 10 mL of 0.1N HCl for 24 hr at room temperature and estimated the drug content using UV-visible spectrophotometer (Elico SL–244).[14]

Particle size

An optical microscope was used to measure the microspheres' particle size. The ocular micrometre which was previously calibrated with stage micrometre was used to find the particle size.[15,16]

Shape and Surface morphology

Using a scanning electron microscope, prepared microspheres' surface morphology and shape were evaluated. (Hitachi S-3700N).

Ex vivo Wash-off test

The mucoadhesive capabilities of the microspheres were evaluated using the *ex vivo* wash-off test. With the help of elastic bands, a piece of the stomach mucosal layer (2 x 2 cm) was mounted onto 3 x 1 inch glass slides. Glass slide was attached to an appropriate support. Each wet mounted tissue was applied with approximately 100 microspheres before the support was hung from the arm of a disintegrating test apparatus USP. The apparatus was set up for a slow, regular up and down movement in a 0.1N HCl taken in a beaker at 37 ± 0.5°C. The machine was stopped after 1 hr and then for every 1 hr up to 8 hr, and counted the number of microspheres still adhered to tissue. The following equation was used to calculate adhering percent.

$$\% \text{ Mucoadhesion} = [\text{Number of microspheres adhered} \div \text{Number of microspheres applied}] \times 100$$ [17,18]

In vitro drug release

The USP dissolution test apparatus II (Electro Labs TDT-06P) using 900mL of 0.1N HCl at 100 rpm for 12 hr at temperature 37±0.5°C were used. The dissolution test was performed by taking microspheres containing equivalent to 1 mg of drug and placed in capsule "0" size. The samples were taken out and replaced with fresh 0.1N HCl each time at predetermined intervals. The absorbance of collected samples was estimated at 265nm using a UV-Visible spectrophotometer (Elico SL – 244). From this the *in vitro* drug release was calculated.[19]

Statistical Analysis

To assess the response, a statistical model with two-factor interactive polynomial terms in the system was used.

Table 1: Formulation trials for Rasagiline Mesylate mucoadhesive microspheres in preliminary trials.

Formulation Code	Rasagiline Mesylate (mg)	Sodium Alginate %	Calcium Chloride %	Chitosan (mg)	Carbopol 934 P (mg)	Xanthan Gum (mg)
RM1	1	1.0	10	150	-	5
RM2	1	1.25	10	200	-	10
RM3	1	1.5	10	250	-	15
RM4	1	1.75	10	300	-	20
RM5	1	2.0	10	350	-	25
RM6	1	2.25	10	400	-	30
RM7	1	2.5	10	450	-	35
RM8	1	1.0	10	-	150	5
RM9	1	1.25	10	-	200	10
RM10	1	1.5	10	-	250	15
RM11	1	1.75	10	-	300	20
RM12	1	2.0	10	-	350	25
RM13	1	2.25	10	-	400	30
RM14	1	2.5	10	-	450	35

Table 2: Central Composite Design Formulations of Rasagiline mesylate Loaded Mucoadhessive Microspheres.

Formulation Code	Rasagiline mesylate (mg)	Sodium Alginate (%)	Calcium Chloride (%)	Carbopol 934 P (mg)	Xanthan Gum mg
RMS1	1	2.25	10	400	20
RMS2	1	2.25	10	400	5
RMS3	1	2.25	10	400	35
RMS4	1	2.25	10	400	5
RMS5	1	2.25	10	400	35
RMS6	1	2.25	10	400	20
RMS7	1	2.25	10	400	20
RMS8	1	2.25	10	400	20
RMS9	1	2.25	10	400	35
RMS10	1	2.25	10	400	5
RMS11	1	2.25	10	400	20

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_1 X_2 + b_4 X_1^2 + b_5 X_2^2 + b_6 X_1 X_2^2 + b_7 X_1^2 X_2 + b_8 X_1^2 X_2^2$$

Where Y-dependent variable, b_0 (intercept) is the average response over eleven runs, b_1 and b_2 are the assessed coefficients for factors (X_1 and X_2). Since the experiment consist of more than one independent variable therefore the statistical analysis by multiple regression analysis of the factorial design was performed using Design Expert 13. Before the treatment of regression, correlation between all independent and dependant variable has been checked and expressed in terms of correlation coefficient.

Differential scanning calorimetry

A differential scanning calorimeter was utilized to conduct DSC studies on pure drug, polymers, physical mixtures, and optimised microsphere formulation. Samples were removed and replaced with new ones at predetermined intervals. The melting point and heat of fusion of the instrument were calibrated with indium. Each sample was weighed into a standard aluminum pan, which was heated at a rate of 10°C/min starting from 30°C to 400°C. An empty pan served as the starting point. Nitrogen gas was used as a purge gas in the DSC analysis at a flow rate of 40 mL/min. (Perkin Elmer DSC/7).[20]

X-ray diffractometery

Using the X-ray powder diffraction (XRD) technique, the crystallisation properties of pure drug and drug loaded microsphere of optimised formulation were examined. The temperature range for powder diffractometers is 3 to 50°C. (D/MAX-2500PC, Rigaku, Japan).

RESULTS

Standard curve of Rasagiline Mesylate

The UV-spectrophotometric analysis revealed that Rasagiline mesylate had showed maximum absorption at 265 nm. The linearity in the standard graph was seen between the concentration range of 2 to 12 µg/mL and showed R^2 value of 0.999 in 0.1 N HCl at pH 1.2. Shown in Figure 1.

Preliminary trials

The particle size of Mucoadhesive microspheres was measured by using Optical Microscopy. All the formulations from RM1 to RM14 were found to be in the range of 322.51 ± 2.54 µm to 472.24 ± 2.41 µm. The formulation RM14 showed the particle size of 472.24 ± 2.41 µm. The Entrapment Efficiency of formulations RM1 to RM14 was found to be in the range of 67.48 ± 2.36 to 90.34 ± 1.79%. The amount of drug loaded in microspheres can be known from the entrapment efficiency.

The Percentage Mucoadhesion of all the prepared formulations from RM1 to RM14 was found to be in the range from 79.56±1.75 to 95.85±1.89%. The formulation RM13 showed the maximum percentage mucoadhesion 95.85±1.89. The results of the various evaluation tests of the microspheres in preliminary trails were showed in (Table 3).

The *in vitro* studies of mucoadhesive microspheres of Rasagiline mesylate (RM1-RM14) were found in the range of 81.72±1.14 to 97.04±1.52% after 12 hr. Among all formulations, RM13 showed the highest drug release. The *in vitro* drug release studies shown in Figure 2.

Optimization by Central Composite Design

Independent variables and dependent variables are given in (Table 4).

Entrapment efficiency

The Entrapment efficiency range was found to be from 74.94±0.19 to 90.58±0.35% among RMS1 to RMS11 formulations. The maximum drug entrapment was found in formulation RMS3 as 90.58±0.35% that had highest Xanthan gum concentration (35 mg) and lowest stirring speed (500 RPM).

The model is significant, as evidenced by the 1366.45 model *F*-value. This could happen due to noise only 0.01% of the time. Significant model terms have *P*-values < 0.0500. A, B, and A^2 are important model terms. The lack of fit is not significant in comparison to the pure error, as indicated by the lack of fit *F*-value of 0.34. A large Lack of Fit *F*-value has an 85.75% chance of being caused by noise. Positive is a minor lack of fit. The adjusted R^2 of 0.9976 and the predicted R^2 of 0.9966 are reasonably in agreement; the difference is < 0.2. Adequate precision is 102.882. A ratio > 4 is desirable which can be used to direct the design space. Statistical significance was shown in Table 5. 3D Surface response graphs were shown in Figure 3.

Particle size

The largest particle size, 485.48 µm, was found in formulation RMS3 that had lowest stirring speed (500 RPM) and having highest Xanthan gum concentration (35 mg). Out of all eleven formulations, the smallest particle size was observed as 313.44 µm in RMS4 that had lowest level of Xanthan gum (5 mg) and highest level of Stirring speed (1500 RPM).

The model *F*-value of 9267.74 denotes the model is significant. *P*-values < 0.0500 indicate the model terms are significant. Terms A, B, AB and A^2 are significant. The *F*-value of 0.57 indicates the Lack of Fit is relative to the pure error not significant. The Lack of Fit *F*-value of 71.82% was shown largely due to noise. The predicted R^2 of 0.9995 and adjusted R^2 of 0.9997; showed the difference < 0.2. The signal-to-noise ratio was found to be 263.251 with precision, which denotes a strong signal. Statistical significance was shown in Table 6 and 3D Surface response graphs were shown in Figure 4.

Cumulative percent drug release

Cumulative percent drug release at 12th hr was selected as a response in the design of this experiment. At the 12th hr, total drug release in all the formulations was found to be 80.15±0.12 to 98.07±0.15%. The highest drug release was found to be 98.07±0.15%. at 12th hr in RMS5 batch having 35 mg of Xanthan gum and 1500RPM.

The model is significant, as indicated by the Model *F*-value of 1839.56. Model terms are significant when the *P*-value is < 0.0500. A, B, and A2 are important model terms. The *F*-value

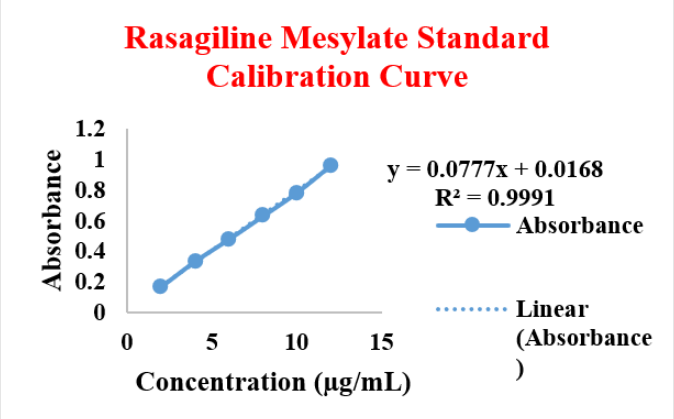

Figure 1: Rasagiline Mesylate Standard Calibration Curve.

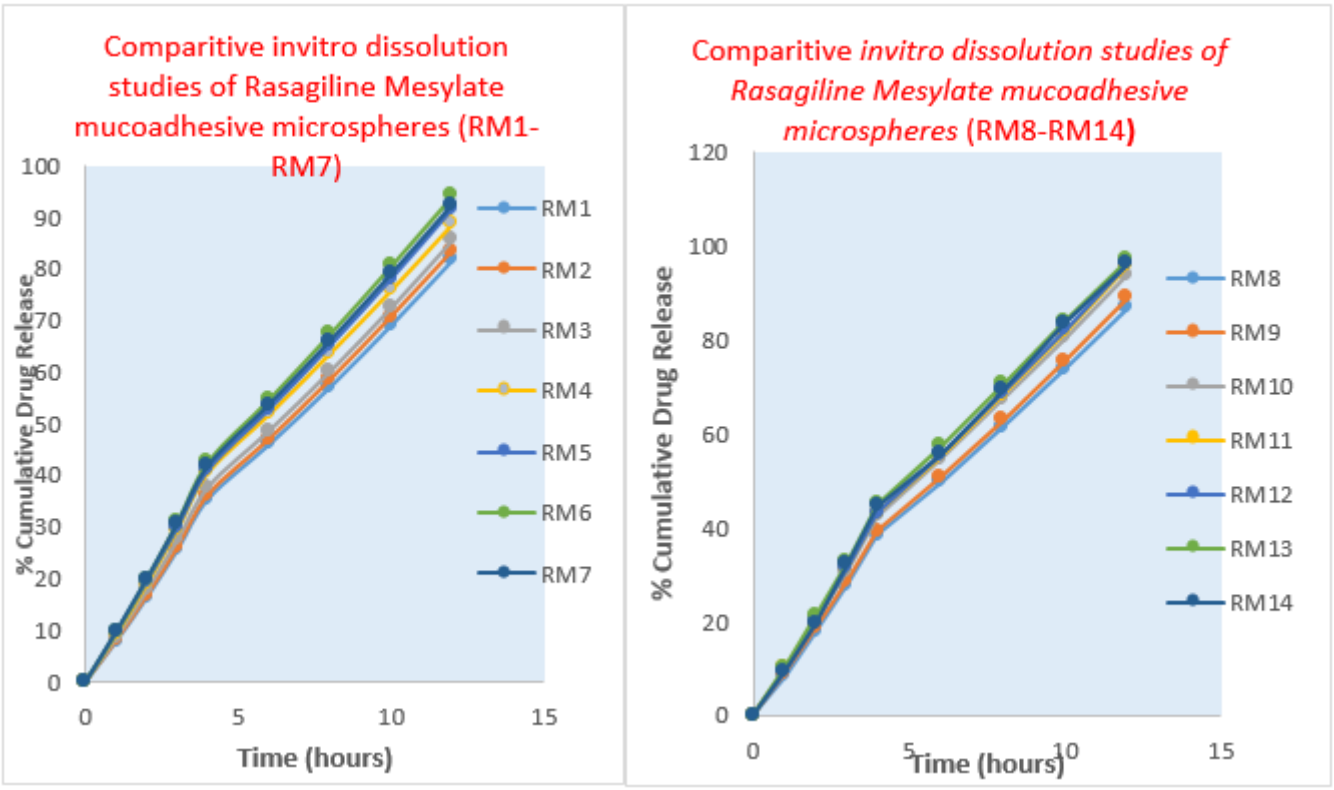

Figure 2: Comparative *in vitro* Dissolution studies of Rasagiline Mesylate Mucoadhesive Microspheres (RM1-RM7) and (RM8-RM14).

Table 3: Evaluation of Rasagiline mesylate mucoadhesive microspheres (RM1-RM14) in preliminary trials.

Formulation	Particle Size (µm)	Drug Entrapment Efficiency (%)	Mucoadhesion (%)
RM1	322.51 ± 2.54	67.48 ± 2.36	79.56±1.75
RM2	331.34 ± 1.68	72.87 ± 2.12	82.16±1.98
RM3	350.47 ± 1.73	75.45 ± 1.77	86.65±1.23
RM4	372.56 ± 2.32	78.89 ± 1.64	84.45±1.61
RM5	394.23 ± 1.41	83.37 ±1.21	87.52±1.77
RM6	411.65 ± 1.54	85.12 ± 1.85	94.74±1.09
RM7	445.24 ± 2.32	88.47 ± 1.16	90.99±1.21
RM8	316.71 ± 1.24	69.23 ± 1.59	87.66±1.32
RM9	329.15 ± 1.26	72.69 ± 1.37	82.81±1.38
RM10	346.27 ± 1.68	76.12 ± 1.14	85.78±1.25
RM11	371.56 ± 1.51	82.56 ± 2.71	87.65±1.51
RM12	405.23 ± 1.49	86.84 ± 1.63	90.12±1.38
RM13	437.65 ± 1.54	90.34 ± 1.79	95.85±1.89
RM14	461.24 ± 2.41	90.06 ± 1.48	95.37±1.45

Above parameters are communicated as Mean ± SD; (n=3)

Table 4: Independent variables and Dependent variables.

Batch Code	Rasagiline mesylate (mg)	Independent Variables		Dependent Variables		
		Polymer Concentration (X_1)	RPM (X_2)	% Entrapment Efficiency (Y_1)	Particle Size (µm) (Y_2)	Cumulative% Drug released at end of 12hr (Y_3)
RMS1	1	20	500	84.67	394.78	88.07
RMS2	1	5	1000	76.64	321.69	81.85
RMS3	1	35	500	90.58	485.48	96.06
RMS4	1	5	1500	74.94	313.44	82.82
RMS5	1	35	1500	87.76	459.53	98.07
RMS6	1	20	1000	83.15	380.56	88.54
RMS7	1	20	1000	83.75	382.56	88.69
RMS8	1	20	1500	81.89	371.26	89.65
RMS9	1	35	1000	89.42	472.35	97.14
RMS10	1	5	500	77.68	332.58	80.15
RMS11	1	20	1000	83.75	382.56	88.76
Code Value	**Actual Value**		**Level of Variables**			
	X_1	X_2				
-1	5	500	LOW			
0	20	1000	MEDIUM			
+1	35	1500	HIGH			

Table 5: ANOVA Results for Predicting % Entrapment Efficiency (Y1).

Source	Sum of Squares	d_f	Mean Square	*F*-value	*p*-value	
Model	259.63	3	86.54	1366.45	< 0.0001	Significant
A-Polymer Concentration	247.04	1	247.04	3900.54	< 0.0001	
B-RPM	11.59	1	11.59	183.04	< 0.0001	
A^2	0.9994	1	0.9994	15.78	0.0054	
Residual	0.4433	7	0.0633			
Lack of Fit	0.2033	5	0.0407	0.3389	0.8575	Not significant
Pure Error	0.2400	2	0.1200			
Cor Total	260.08	10				

for the lack of fit of 6.97 indicates not significant in comparison to the pure error. The predicted R^2 and adjusted R^2 difference is 0.9982 < 0.2. Adequate precision > 4 is desirable. *t* value 112.965 specifies an adequate signal which can be used to path the design space. The statistical significance was reported in Table 7, 3D Surface response graphs, overlay plot were shown in Figure 5 and 6 respectively.

Statistical Analysis

The value of the correlation coefficient near to unity indicates a good fit. Fitted linear regression equation connecting the responses Entrapment efficiency, Particle size, and Cumulative percent drug release to the transformed factor was showed below. Coefficient values given in Table 8.

Combination of variables was suggested by the software with a desirability function of 0.834, which is satisfactory as reaching 1 shown in Figure 7. The ideal "variables" settings were used

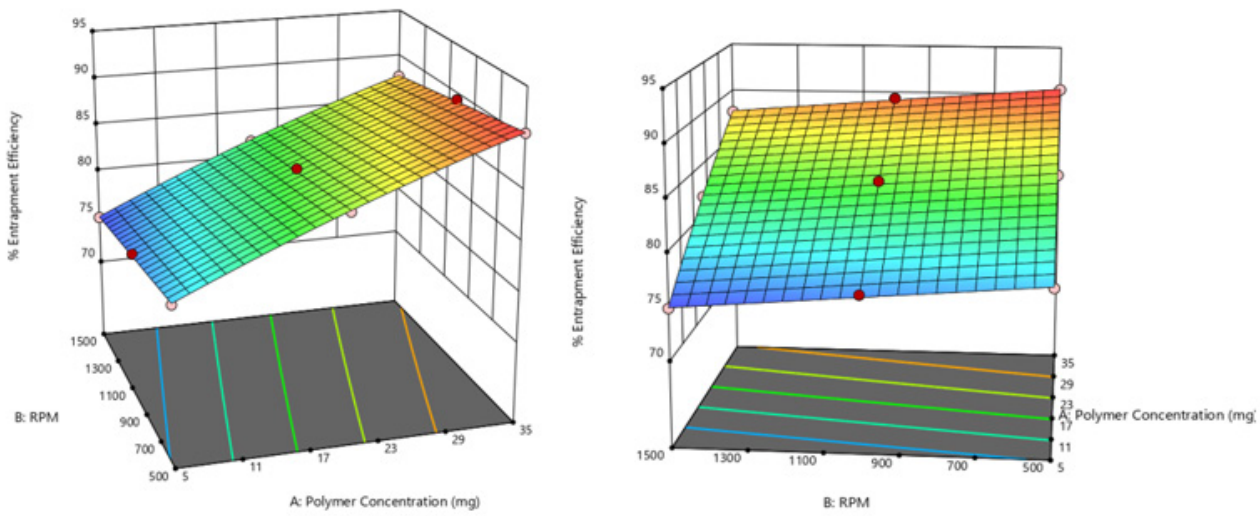

Figure 3: Polymer Concentration and stirring speed in relation to percent entrapment efficiency were shown in 3D Surface response graphs.

Table 6: ANOVA Results for Predicting Particle size (Y2).

Source	Sum of Squares	d_f	Mean Square	*F*-value	*p*-value	
Model	35121.10	4	8780.28	9267.74	< 0.0001	Significant
A-Polymer Concentration	33697.52	1	33697.52	35568.35	< 0.0001	
B-RPM	784.56	1	784.56	828.11	< 0.0001	
AB	11.59	1	11.59	12.24	0.0129	
A^2	627.43	1	627.43	662.27	< 0.0001	
Residual	5.68	6	0.9474			
Lack of Fit	3.02	4	0.7544	0.5658	0.7182	Not significant
Pure Error	2.67	2	1.33			
Cor Total	35126.79	10				

to create Rasagiline mesylate microspheres, which were then tested for their responses. The optimised microspheres displayed cumulative percent drug release of 96.43 with a percent entrapment efficiency of 86.84, particle size of 440 µm.

Characterization of Rasagiline Mesylate Mucoadhesive Microspheres

Drug excipient compatibility by FTIR

Figure 8 compares the FTIR-spectra of the pure drug Rasagiline Mesylate with those of the physical mixture, Sodium alginate, Carbopol, Xanthan gum, and the optimized formulation (RMS12). Pure Rasagiline mesylate's distinctive FTIR stretching frequencies were seen, with bands at 2934 cm^{-1} and 2849 cm^{-1} corresponding to the stretching of aromatic C-H group and the aliphatic C-H group, respectively. Various bands Peaks at 1192 cm^{-1} and 3219 cm^{-1} (secondary amine N-H). All of these traits were also present in the physical combination. Shown in (Figure 8).

Differential scanning calorimetry

Rasagiline Mesylate, polymers, the physical mixture, and the formulation's DSC thermogram were recorded starting from 30 to 400°C (10°C/min is heating rate). Rasagiline Mesylate demonstrated a melting transition between 128.43°C and 138.65°C, with a peak at 131.29°C, according to a comparison of thermal transitions. The melting peak for Rasagiline Mesylate in physical mixture at 126.32°C indicates that the physical mixture has not significantly changed the melting temperatures of the drug and polymer as shown in Figure 9.

Scanning electron microscopy

Scanning electron microscopic studies of optimized mucoadhesive microspheres RMS12, A SEM image showed that

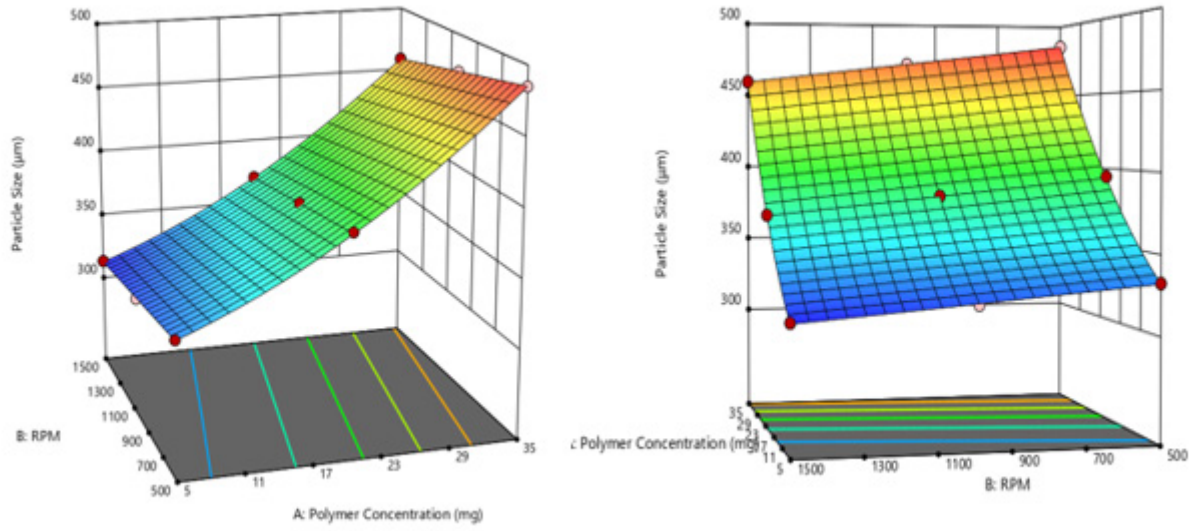

Figure 4: Polymer Concentration and stirring speed over particle size were shown 3D Surface response graphs.

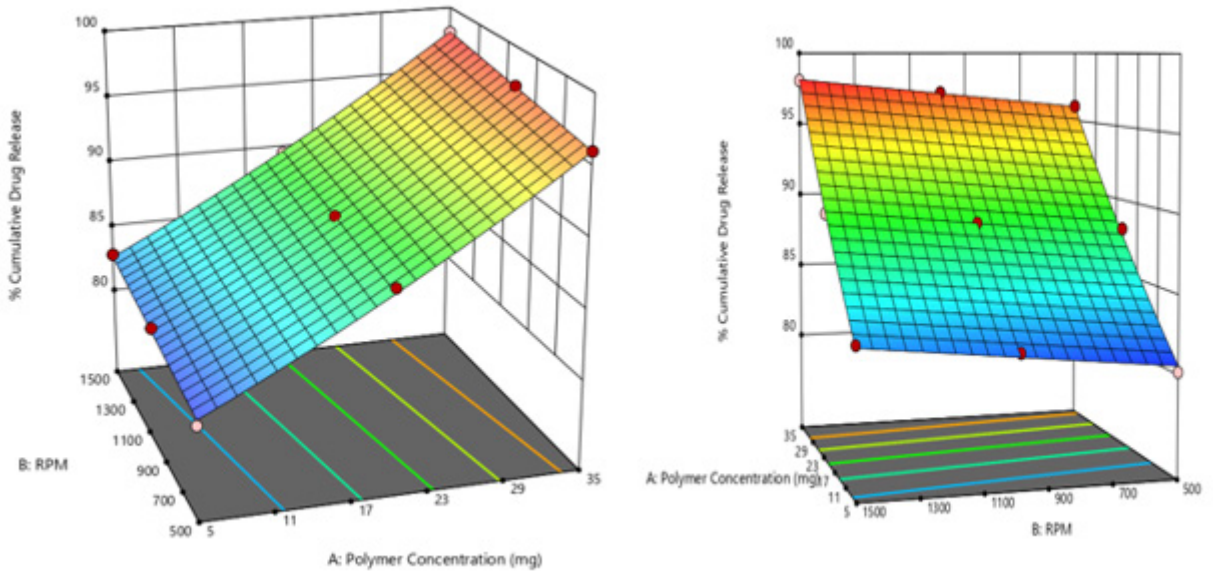

Figure 5: Polymer concentration and stirring speed in relation to cumulative percent drug release were shown in 3D Surface response graphs.

Table 7: ANOVA Results for Cumulative Percent Drug Release (Y3).

Source	Sum of Squares	d_f	Mean Square	*F*-value	*p*-value	
Model	367.13	3	122.38	1839.56	< 0.0001	Significant
A-Polymer Concentration	359.60	1	359.60	5405.43	< 0.0001	
B-RPM	6.53	1	6.53	98.18	< 0.0001	
A^2	1.00	1	1.00	15.07	0.0060	
Residual	0.4657	7	0.0665			
Lack of Fit	0.4404	5	0.0881	6.97	0.1302	Not significant
Pure Error	0.0253	2	0.0126			
Cor Total	367.60	10				

Table 8: Coefficients Table.

	Intercept	A	B	AB	A^2
% Entrapment Efficiency	83.442	6.41667	-1.39		-0.605333
p-values		< 0.0001	< 0.0001		0.0054
Particle Size	382.344	74.9417	-11.435	-1.7025	15.1677
p-values		< 0.0001	< 0.0001	0.0129	< 0.0001
% Cumulative Drug Release	88.742	7.74167	1.04333		0.606333
p-values		< 0.0001	< 0.0001		0.0060

% Entrapment efficiency = 83.44 + 6.42(X_1) -1.39(X_2) - 0.6053(X_1^2)Particle size = 382.34 +74.94(X_1) - 11.43(X_2) - 1.70(X_1X_2) +15.17(X_1^2) Cumulative percent drug release = 88.74 +7.74(X_1) +1.04(X_2) + 0.6063(X_1^2)

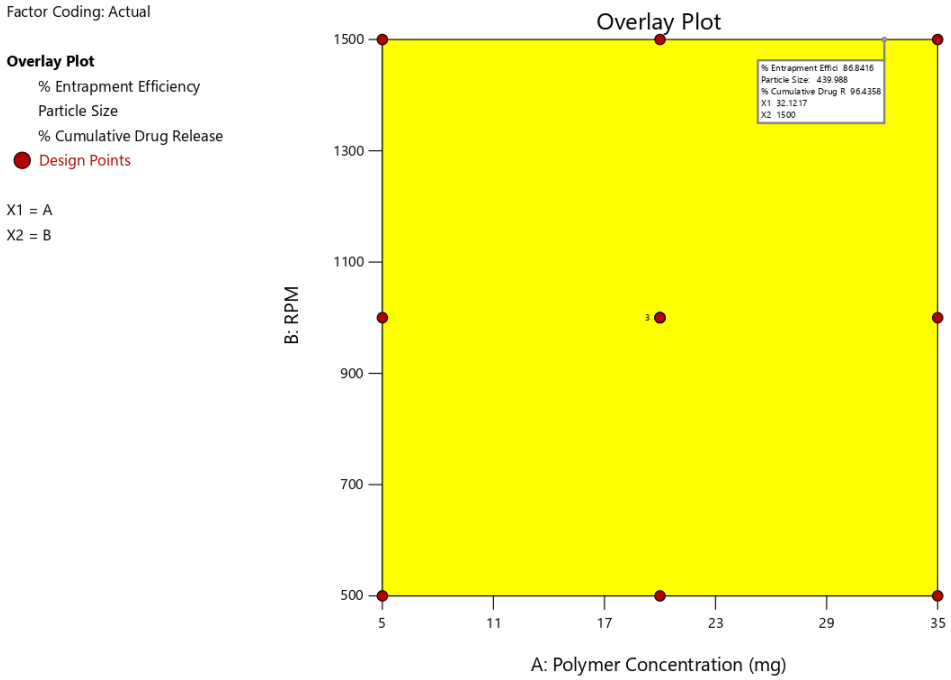

Figure 6: Overlay Plot.

the microspheres were distinct, spherical, and had drug and polymer associations on their outer surfaces (Figure 10).

X-ray diffraction

The pure drug Rasagiline mesylate powder underwent XRD analysis to show the presence of numerous diffraction bands, which are indicative of crystalline materials (Figure 11).

DISCUSSION

Higher the stirring speed, leads to smaller the particle size of microspheres. Increase in viscosity leads to decrease in the mixing efficiency of propellers which results in larger particle size of the microspheres. The value of the correlation coefficient near to unity indicates a good fit. The optimised microspheres parameters were suggested that the generated models would work well for microsphere optimization. The compatibility of the drug with the excipients used in the formulation was demonstrates that the drug's chemical integrity has not changed materially. It was determined that there was no physical interaction between the drug and the polymers. The drug's distinctive peaks in the FTIR-spectra of Rasagiline mesylate-loaded microspheres served as a sign that the drug had been completely encapsulated within the internal structure of the microspheres. From DSC thermogram it is known that the drug's melting peak in a physical mixture was slightly shifted; this may be because the drug dissolved in molten polymer before it melted. It can be concluded that neither an adduct nor a new chemical entity can be created by the physical combination of the drug and polymer. Drug and polymer physical mixture showed no signs of interaction. In the prepared microspheres, the drug's endothermic peak is missed. It was clear that the pure drug was not present in the microspheres' melting endotherm peak. These facts alone allow us to draw the

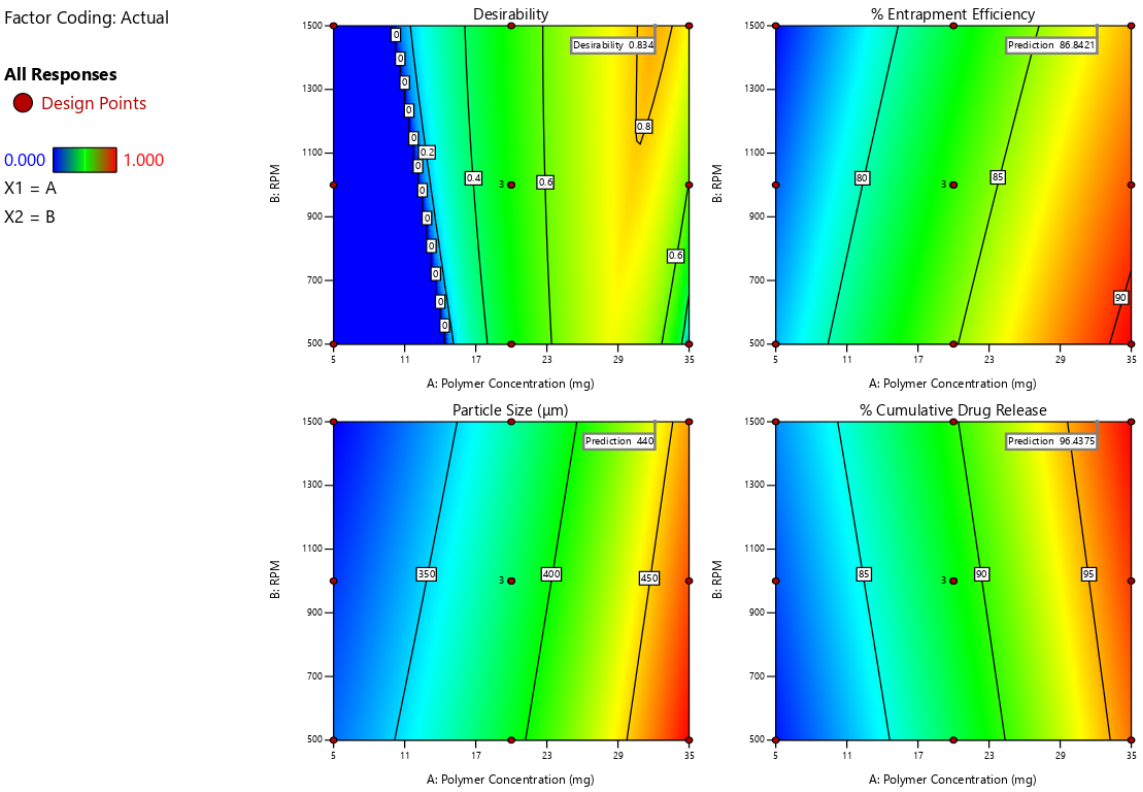

Figure 7: Numerical counter plot with Desirability and Prediction.

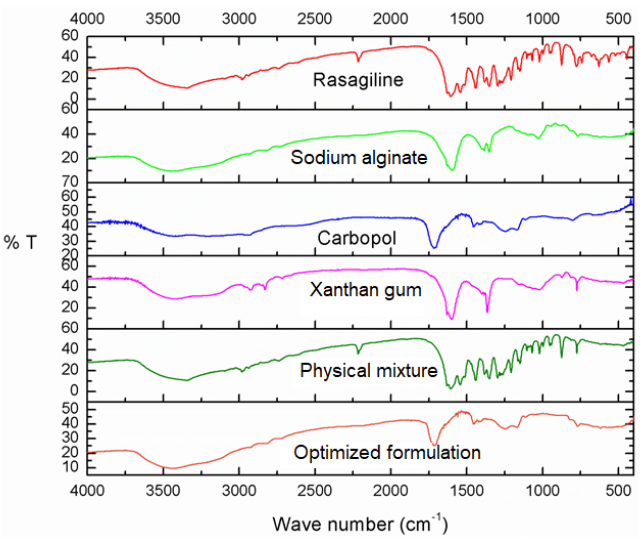

Figure 8: FTIR studies of Rasagiline Mesylate pure drug, optimized formulation RMS12, physical mixture and excipients.

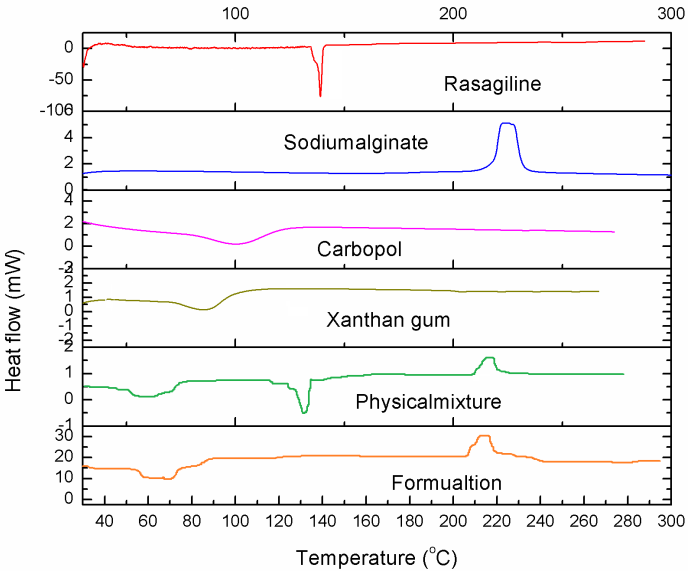

Figure 9: DSC thermogram of Rasagiline Mesylate pure drug, optimized formulation RMS12, physical mixture and excipients.

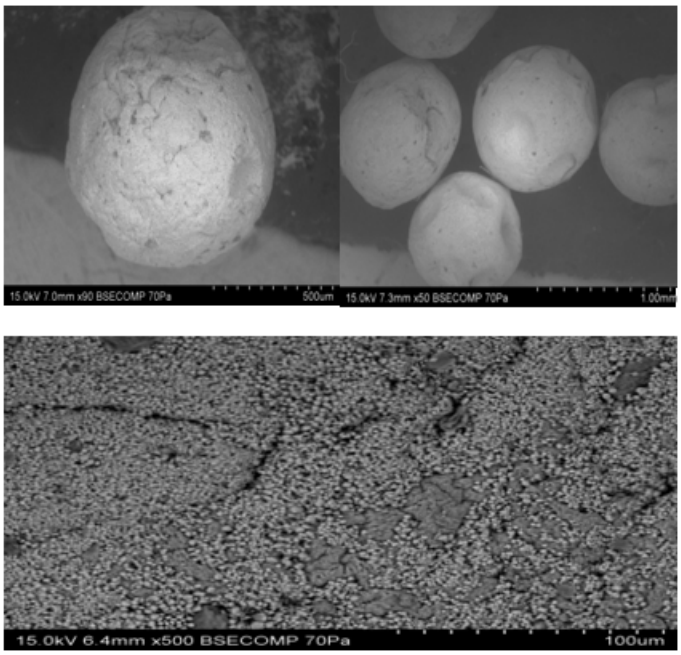

Figure 10: Scanning electron micrographs of Mucoadhesive Microspheres.

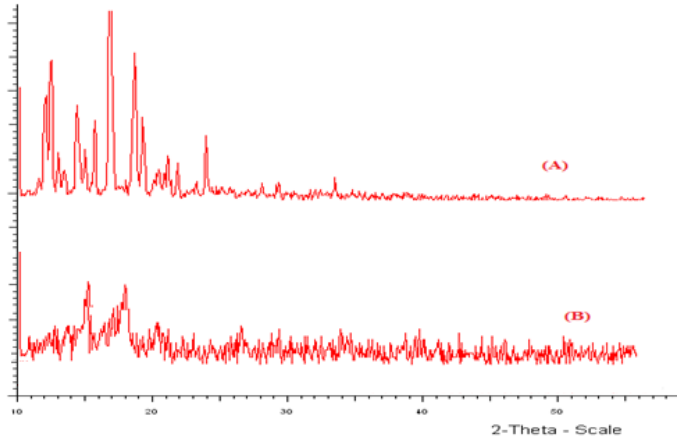

Figure 11: X-Ray powder diffractograms of (A) Rasagiline mesylate pure drug, and (B) Rasagiline mesylate loaded optimized microsphere formulation.

conclusion that the drug in the microspheres has an amorphous structure because its melting point (126.32°C) is visible in the physical mixture. A SEM image showed that the pores on the surface of microspheres aid in the diffusion mechanism. Rasagiline mesylate amorphous state was confirmed by the lack of distinguishing peaks in the XRD pattern of Rasagiline Mesylate-loaded microspheres

CONCLUSION

Rasagiline mesylate mucoadhesive microspheres were developed by doing extensive literature survey. Different formulations of Rasagiline mesylate microspheres (RM1-RM14) were prepared by ionic gelation method in preliminary trials. In preliminary trials it was found that, apart from Chitosan, the formulations with Carbopol 934P had shown best mucoadhesion and drug release. Further optimization of Rasagiline mesylate mucoadhesive microspheres (RMS1 to RMS11) done by optimizing independent variables such as polymer concentration, Xanthan Gum (5mg, 20mg and 35mg) and rpm (500, 1000 and 1500) and estimating dependent variables such as percentage entrapment efficiency, particle size and cumulative percent drug release by using Central composite design from Response surface methodology. Combination of variables was suggested by the software with a desirability function of 0.834 which is satisfactory as reaching 1. The optimized formulation (RMS12) displayed a Cumulative percent drug release of 96.43 with a percent entrapment efficiency of 86.84, particle size of 440 µm. It was suggested that the generated models would work well for microsphere optimization.

ACKNOWLEDGEMENT

We would like to thank management of Sarojini Naidu Vanita Pharmacy Maha Vidyalaya for providing access to the research facilities and QbD Expert.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DOE: Design of experiments; **UV:** Ultraviolet; **ANOVA:** Analysis of variance; **HCl:** Hydrochloric acid; **RPM:** Rotation per minute; **FTIR:** Fourier-transform infrared spectroscopy; **DSC:** Differential scanning calorimetry; **SEM:** Scanning electron microscopy; **XRD:** X-ray diffraction.

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Cite this article: Prasanthi R, Haarika B, Selvamuthukumar S. Design Formulation and Statistical Evaluation of Gastroretentive Microspheres of Rasagiline Mesylate for Parkinson's Disease Using Design Expert. Indian J of Pharmaceutical Education and Research. 2023;57(2s):s262-s273.