Original Article

Design Formulation and *in vitro* Evaluation of Gastroretentive Microspheres of Selegiline Hydrochloride for Parkinson's Disease by Design Expert

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ABSTRACT

Introduction: Selegiline hydrochloride is primarily used to treat Parkinson's disease. Selegiline hydrochloride mucoadhesive microspheres were prepared to improve the bioavailability of the drug and its appropriate therapeutic performance. **Materials and Methods:** The ionic gelation process was used to formulate the gastroretentive mucoadhesive microspheres using different polymers such as gum kondagogu (150 to 450 mg), karaya gum (10 to 70 mg), and carbopol 940P (150 to 450 mg) of different concentrations in preliminary trial formulations (SM1-SM14) after performing preformulation studies. Optimisation of selegiline hydrochloride mucoadhesive microspheres (SHM1 to SHM11) was done by optimizing. The study employed independent variables-Karaya gum concentration (10, 40, and 70 mg) and stirring speed (500, 1000, and 1500 rpm)-alongside dependent variables: percentage entrapment efficiency, particle size, and cumulative drug release. Design Expert 13 software employing Central Composite Design facilitated optimization. ANOVA elucidated the influence of these variables on the dependent ones, providing insights into the interplay between polymer concentration, stirring speed, and the measured outcomes. For optimised formulation, SEM was done to determine structural features. **Results:** Initial investigations revealed that, aside from gum kondagugu, formulations containing carbopol 934P demonstrated superior mucoadhesion and drug release characteristics. The optimised formulation SHM12 (given by Design Expert 13 software in Overlay Plot) having 64.31 mg of Karaya Gum at stirring speed 1500 rpm showed 84.84% entrapment efficiency, 450 μm particle size, and 96.53 cumulative percent drug release. Results were confirmed experimentally. **Conclusion:** The study concluded that the developed mucoadhesive microspheres for selegiline hydrochloride exhibited enhanced cumulative drug release, ultimately enhancing bioavailability.

Keywords: Selegiline hydrochloride, Parkinson's disease, Mucoadhesion, Microspheres, Ionic gelation method, Design Expert 13, Response Surface Methodology.

INTRODUCTION

Oral drug administration is the most desirable and feasible drug delivery method, but this route of administration is limited due to poor bioavailability. Microspheres are one of the multiparticulate drug delivery systems that are used for controlled drug delivery. This dispenses the medications more uniformly in the gut. Gastroretentive dosage forms in the form of mucoadhesive microspheres help to retain the drug in the stomach for a longer period, improving bioavailability.[1]

Gastroretentive microspheres are widely used to delay and modify the characteristics of drug release. The ionic gelation method that was used for this preparation has reproducibility in results and also avoids local irritation as the formulation is free from organic solvents.[2,3]

Parkinson's disease causes trembling, stiffness, and difficulty walking. Most widely used medication is carbidopa, levodopa, dopamine agonists such as pramipexole and apomorphine, monoamino oxidase inhibitors such as selegiline and rasagiline, anticholinergics such as benztropine. Selegiline hydrochloride is an invariable MAOB inhibitor and is used for Parkinson's disease, dementia, and depression. Selegiline hydrochloride has a low biological half-life (1.5 to 3.5 hr). The release can be extended by developing micro particulate drug delivery systems that can deliver a drug over extended periods compared with conventional delivery systems. That minimises drug-related side effects and

variations in drug concentration in the blood.[4] This study aims to increase the stomach residence time of microspheres by direct contact with gastric mucosa and control the first pass effect of the drug as it is absorbed in the stomach itself.[5,6]

MATERIALS AND METHODS

Materials

Selegiline hydrochloride was acquired as a gift from Intas Pharmaceuticals Ltd., Ahmedabad, India. Gum Kondagugu and Karaya gum were obtained from MSN Labs Pvt. Ltd., India., Carbopol 940P, sodium alginate, glacial acetic acid, and calcium chloride were procured from SD Fine Chemicals Ltd., Mumbai, India. Marketed product: Selgin 5 mg tablet, from Intas Pharmaceuticals Ltd., Ahmedabad, India.

Methods

Determination of λ_{max} of drug solution

A 10 μg/mL drug solution was made and scanned with UV spectrophotometer by using 0.1 N HCl (blank solution) in the wavelength range of 200 to 400 nm. Selegiline hydrochloride has a maximum wavelength of 281 nm.[7,8]

Preparation of standard curve with 0.1 N HCl

10 to 60 μg/mL concentrations of drug solutions in 0.1 N HCl were prepared and scanned at Selegiline hydrochloride maximum wavelength of 281 nm by using 0.1 N HCl as a blank solution using a UV spectrophotometer.[9,10]

Preparation of Selegiline hydrochloride mucoadhesive microspheres

The ionic gelation technique was used to prepare mucoadhesive microspheres containing selegiline hydrochloride as a drug. To make a homogenous polymer mixture, sodium alginate was mixed with mucoadhesive polymers like gum kondagugu or carbopol 940P, and karaya gum. To make a homogeneous dispersion, the selegiline hydrochloride drug was added in polymer dispersion and well stirred by magnetic stirrer. The calcium chloride was dissolved in a 2% glacial acetic acid solution (glacial acetic acid stabilises the shape of microspheres) to make the gelation medium. A 21-gauge syringe needle was used to extrude the homogeneous alginate solution into the medium distance maintained as 10 cm. To improve mechanical strength, the gel microspheres were allowed to stay in the mixture for 30 min while being stirred on magnetic stirrer at room temperature. The microspheres were then filtered, collected, and rinsed twice with distilled water. These were dried at room temperature for 24 hr, and stored. Advantage of ionic gelation method is that; it does not require any specific equipment. The composition of prepared microspheres in preliminary trial formulations is presented in Table 1.[11-15]

Preformulation trials

Preformulation studies offer the required details to determine the nature of the drug substance and provide a framework for combining the drug with pharmaceutical excipients to develop a dosage form.[16-18]

Drug authentication by Fourier transform infra red spectroscopy

The purpose of the FTIR investigation was to see whether the drug was compatible with the excipients or not. The FTIR spectra of selegiline hydrochloride were obtained using the potassium bromide dispersion method with a drug-to-potassium bromide ratio 1:100 (by weight). Dry potassium bromide was used as a blank pellet and scanned in the IR region between 4000 and 400 cm[1]. The spectra of the drug with potassium bromide pellets were made and run in the same IR region. Both spectra were compared.[19,20]

Experimental Design- Surface Response Methodology

Central Composite Design (CCD) from surface response methodology in Design Expert 13 software was used for the optimisation of selegiline hydrochloride mucoadhesive microspheres. Surface response methodology explores the relationship between variables (product/process) and responses. It is a statistical and mathematical tool for designing and optimizing the variables. The eleven formulations, including three repetitive centers points (SHM1 to SHM11), were given by software using independent variables-Karaya gum concentration (10, 40, and 70 mg) and stirring speed (500, 1000 and 1500 rpm)- alongside dependent variables: percent cumulative drug release, percentage entrapment efficiency, particle size. From the results of preliminary trials, the concentrations of sodium alginate (2.25%), calcium chloride (10%), and carbopol 940P (400 mg) were kept constant in further optimization. The selegiline hydrochloride concentration is 5 mg. Independent variables and dependent variables in the central composite design were shown in Table 2.

Characterization

Particle Size Analysis

Particle size analysis of the microspheres was done using the optical microscopy. The eyepiece micrometre was calibrated using a standard stage micrometre. A small quantity of microspheres was added to 10 mL of water. A small drop of drug containing microsphere suspension was kept on a clean glass slide. Then the stage micrometre was replaced with a microsphere formulation slide, and the diameter of at least 300 particles was measured with that calibrated eye piece.[21]

Table 1: Formulation trials for Selegiline hydrochloride mucoadhesive microspheres for preliminary trials.

Formulation Code	Selegiline hydrochloride (mg)	Sodium alginate %	Calcium chloride %	Gum Kondagogu (mg)	Carbopol 940 P (mg)	Karaya Gum (mg)
SM1	5	1.0	10	150	-	10
SM2	5	1.25	10	200	-	20
SM3	5	1.5	10	250	-	30
SM4	5	1.75	10	300	-	40
SM5	5	2.0	10	350	-	50
SM6	5	2.25	10	400	-	60
SM7	5	2.5	10	450	-	70
SM8	5	1.0	10	-	150	10
SM9	5	1.25	10	-	200	20
SM10	5	1.5	10	-	250	30
SM11	5	1.75	10	-	300	40
SM12	5	2.0	10	-	350	50
SM13	5	2.25	10	-	400	60
SM14	5	2.5	10	-	450	70

Table 2: Independent variables (Factors) and dependent variables (Responses) in Central Composite Design.

Run	Factor 1 A:Polymer Concentration	Factor 2 B:Stirring Speed	Response 1 % Entrapment Efficiency	Response 2 Particle Size	Response 3 % Cumulative Drug Release
	mg	Rpm	%	µg	%
SHM1	40	1500	79.12	381.31	91.48
SHM2	70	1000	87.94	481.3	96.53
SHM3	10	1000	73.59	331.95	82.78
SHM4	40	1000	81.16	394.63	90.32
SHM5	70	500	89.89	494.09	95.58
SHM6	10	500	75.44	346.02	81.38
SHM7	10	1500	71.7	317.99	84.04
SHM8	40	1000	81.12	394.85	90.26
SHM9	40	500	82.99	408.28	89.13
SHM10	70	1500	86.15	468.29	97.63
SHM11	40	1000	81.09	394.74	90.23

Code value	Actual value		Level of variables		
	X1	X2			
-1	5	500	LOW		
0	20	1000	MEDIUM		
+1	35	1500	HIGH		

Ex vivo wash off test

The microspheres mucoadhesive properties were assessed by using an *ex vivo* adhesion test known as the wash off test. USP disintegration test apparatus was used for performing *ex vivo* wash off test. Elastic bands were used to mount stomach mucosal layer (2×2 cm) onto 3×1″ glass slide. That glass slide was tied with thread to hang to the arm of disintegration apparatus. Each moist tissue specimen was covered with microspheres, and the supporting thread was hung to the arm of a USP disintegration apparatus. Up and down movements in a test fluid at 37ºC obtained in a beaker were adjusted on the disintegration machine

holding tissue samples. At fixed hourly intervals up to 8 hr, the number of microspheres stick to the tissue was counted. The test was carried out in stomach fluid with a pH of 1.2 (0.1N HCl).

The adherent percent was calculated using the equation below.

[Mucoadhesion Number of microspheres adhered $\times$ 100] $\div$

Number of microspheres applied

In vitro drug-release studies of microspheres

Microspheres were tested for *in vitro* drug release by means of a USP dissolution apparatus II (paddle type) at 100 rpm in 900 mL (dissolution fluid-0.1 N HCl) for 12 hr at 37±0.5ºC. The microsphere formulations containing the equivalent of 5 mg of selegiline hydrochloride drug were placed in a No. "0" size hard gelatin capsule and then placed in 0.1 N HCl. At regular intervals of time, 5 mL samples were taken and replaced by an equal fresh dissolution fluid. The samples examined using a UV spectrophotometer at 281 nm. From these results, the percent drug release was calculated. The optimized formulation SHM 12 drug release was compared with marketed product, Selgin 5 mg tablet.[23,24]

Statistical Analysis

An evaluation utilized a statistical model featuring interactive polynomial terms (two-factor) to analyze the system's response.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4X_1^2 + b_5X_2^2 + b_6X_1X_2^2 + b_7X_1^2X_2 + b_8X_1^2X_2^2$$

In the analysis, Y represented the dependent variable. The intercept, b0, indicated the mean response across eleven runs. Coefficients b1 and b2 were assigned to factors X_1 and X_2. Employing Design Expert 13. Correlations between variables were examined, presented as correlation coefficients.

Differential Scanning Calorimetry

Utilizing a Perkin-Elmer-DSC/7 (CT, USA), DSC tests was conducted on drug, polymer, mixture, and microsphere samples. The device was calibrated with iridium for melting point and heat of fusion. Samples in aluminum pans were heated (by 10ºC/min) starting from 30 to 400ºC, referencing an empty pan. Nitrogen gas (40 mL/min) served as purge gas in DSC analysis.[25-27]

RESULTS

Standard curve of Selegiline hydrochloride with 0.1 N HCl

From the UV spectrophotometric analysis, the λ_{max} of selegiline hydrochloride was found at 281 nm. Linearity was detected in range of 10-60 μg/mL concentration. The standard curve showed an R^2 value>0.999 at pH 1.2, which suggests that, it obeys the Beer-Lambert law (Figure 1).

Preliminary trials

Preliminary trial results for the prepared microspheres were presented. Optical microscopy used to measure particle size of mucoadhesive microspheres. All the formulations from SM1 to SM14 were found to be in the range of 325.61±2.32 to 446.62±1.62 μm. The surface area of the particle can be increased by making smaller particles, which will effectively change the release pattern of the drug.

The drug entrapment efficiency of all the formulations from SM1 to SM14 was found to be in the range of 72.15±2.44 to 86.22±1.90%. More entrapment efficiency indicates more drug loading, which in turn leads to higher rates of drug release at the site of absorption.

The mucoadhesion of formulations SM1 to SM14 was found to be in the range of 81.34±1.12% to 95.39±1.67%. The mucoadhesion of microspheres is governed by polymers, which enable the microspheres to adhere for a longer time so that the entire drug will release at the site of adhesion, thereby enhancing bioavailability and therapeutic activity. The formulation SM13 showed maximum adhesion.

In vitro drug-release studies of mucoadhesive microspheres of Selegiline hydrochloride (SM1-SM14).

The developed formulations of mucoadhesive microspheres of selegiline hydrochloride (SM1-SM14) were kept through *in vitro* dissolution tests using a USP II dissolution apparatus in dissolution medium-0.1 N HCl for 12 hr. All the formulations released more than 70% of the drug, and they are in the range of 80.99±1.14 to 99.49±1.52 % after 12 hr. Microspheres of selegiline hydrochloride were prepared by using two polymers, such as gum kondagogu (SM1-SM6) and carbopol 940P (SM7-SM14), in different concentrations. Among all, SM13 formulation showed high release (Figure 1).

Optimization by Central Composite Design
Entrapment efficiency

The percentage entrapment efficiency (%EE) was from 71.70±0.19 to 89.89±0.35 % in SHM1 to SHM11 formulations. The maximum was found in formulation SHM5 at 89.89±0.35% which had the highest Karaya gum concentration (70 mg) and lowest stirring speed (500 rpm). A 3D surface response graph was shown in Figure 2 a.

Particle size

The particle size 494.09 μm, highest was got in formulation SHM5, which had the highest Karaya gum concentration (70 mg) and 500 RPM was the lowest stirring speed. In all formulations, the smallest particle size at 317.99 μm in SHM7, which had the lowest Karaya gum concentration (10 mg) and highest stirring

Table 3: ANOVA for Cumulative percent drug release.

Source	Sum of squares	d_f	Mean Square	*F*-value	*p*-value	
Model	297.07	4	74.27	35100.69	<0.0001	Significant
A-Polymer Concentration	287.60	1	287.60	1.359E+05	<0.0001	
B-Stirring Speed	8.31	1	8.31	3926.24	<0.0001	
AB	0.0930	1	0.0930	43.97	0.0006	
A²	1.07	1	1.07	507.28	<0.0001	
Residual	0.0127	6	0.0021			
Lack of Fit	0.0085	4	0.0021	1.01	0.5522	Not significant
Pure Error	0.0042	2	0.0021			
Cor Total	297.08	10				

Table 4: Confirmation table.

Analysis	Predicted Mean	Predicted Median	Standard Deviation	n	SE Predicted	95% PI low	Data Mean	95% PI high
% Entrapment Efficiency	84.8419	84.8419	0.0510415	3	0.0428541	84.7405	84.8233	84.9432
Particle Size	450	450	0.0805416	3	0.0750858	449.816	450.067	450.184
% Cumulative Drug Release	96.5359	96.5359	0.0459982	3	0.0428824	96.4309	96.4533	96.6408

SE: Standard error; PI: Predicted interval.

speed (1500 RPM) observed. A 3D surface response graph was shown in Figure 2 b.

Cumulative percent drug release

At the 12th hr, total drug release observed from 81.38±0.12 to 97.63±0.15%. The highest was observed as 97.63±0.15 % at 12 hr in the SHM10 batch, which had 70 mg of Karaya gum and a stirring speed of 1500 rpm. The optimised formulation SHM 12 (given by Design Expert 13 software) drug release was compared with marketed product, Selgin 5 mg tablet. Which was shown in Figure 1b. Statistical significance shown in Table 3, 3D surfaces response graph in Figure 2 c.

STATISTICAL ANALYSIS

Entrapment efficiency

The model's *F*-value is 42669.76. A *p*<0.0500 shows that model terms were considered significant. A, B, and A² were important model terms. The lack of Fit *F*-value was 2.56. A large lack of fit *F*-value has a 30.46% chance of cause by noise. Positive was a minor lack of fit. The predicted R^2 and the adjusted R^2 difference was less than 0.2, which was in equitable agreement. A ratio of 591.308 indicates an adequate signal (signal-to-noise ratio minimum 4 was preferred).

Particle size

The model *F*-value is 1344270.88, which is significant. A, B, AB, and A² were model terms in this situation. Lack of fit, 0.30 F-value. F-value was 85.69% (caused by noise). Predicted R^2, 1.0000 and the adjusted R^2, 1.0000 difference was <0.2, which was a reasonable agreement. The signal-to-noise ratio was 3244.37, which was good.

Cumulative percent drug release

The model's F-value was 35100.69. A, B, AB, and A² were model terms. The F-value for the lack of fit, 1.01, indicates that the lack of fit was not significant in comparison to the pure error. Lack of Fit F-value was 55.22%, which was not statistically significant. The gap between the predicted R^2 of 0.9998 and the adjusted R^2 of 0.9999 was less than 0.2, which was considered as reasonable agreement. The signal-to-noise ratio of 522.379 was satisfactory as it was higher than 4.

Coded Equations

Factor coefficients can be compared and relative importance of the factors can be determined with coded equation.

% Entrapment efficiency=$81.10+7.21(X_1)-1.89(X_2)-0.3110(X_{12})$
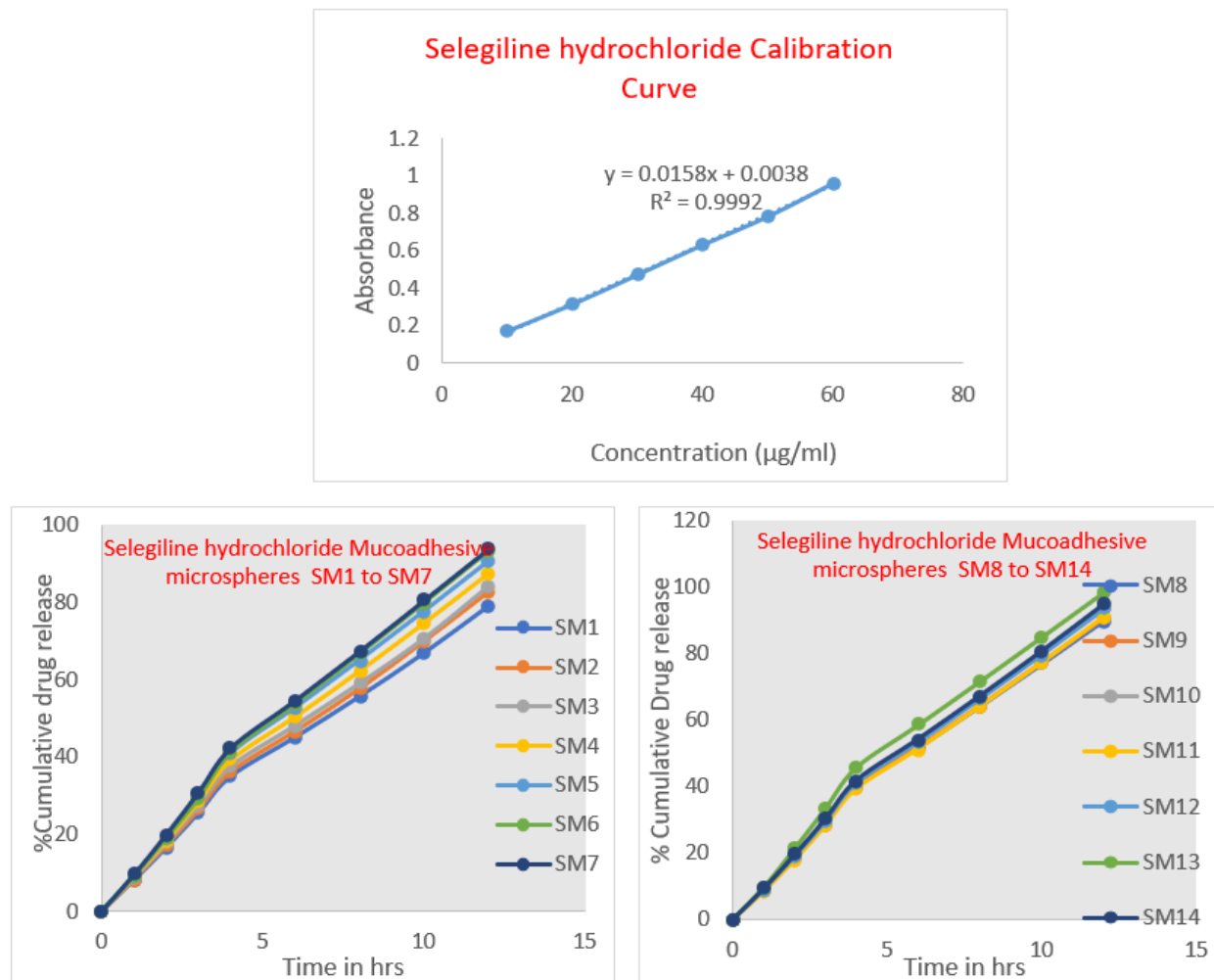
Figure 1a: Calibration curve and Comparative *in vitro* dissolution studies of Seleginine hydrochloride mucoadhesive microspheres SM1-SM14.
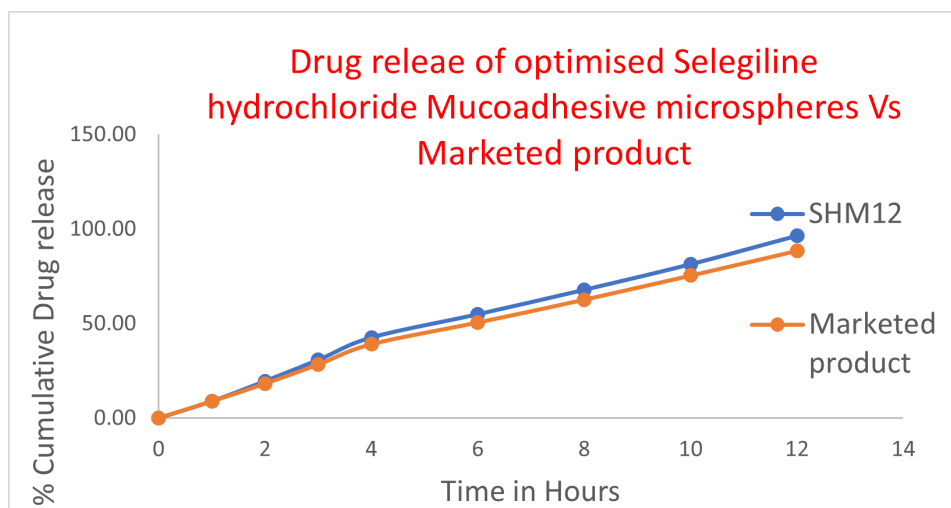

Figure 1b: Comparative *in vitro* dissolution studies of optimised Seleginine hydrochloride mucoadhesive microspheres formulation with marketed product.

$$Particle\ size = 394.76 + 74.62(X_1) - 13.47(X_2) + 0.5575(X_1X_2) + 11.84(X_{12})$$

$$Cumulative\ percent\ drug\ release = 90.28 + 6.92(X_1) + 1.18(X_2) - 0.1525(X_1X_2) - 0.6273(X_{12})$$

The ideal variable setting was used to create an optimised formulation of selegiline hydrochloride microspheres. The optimised microsphere responses displayed in the overlay plot were 96.53% of cumulative drug release, 84.84% of entrapment efficiency, and 450 μm of particle size. Which were shown in the overlay plot in (Figure 2 d). A combination of factors suggested with a desirability function of 0.926.

Confirmation

Factors taken from the overlay plot were tested experimentally and confirmed that the mean responses were in between the values of 95% PI low and 95% PI high (Table 4).

Characterization of Selegiline hydrochloride mucoadhesive microspheres

Drug-excipient compatibility

FTIR Studies

Studying the FTIR spectra of pure drug and formulation, as observed by the absence of significant changes in the peaks of both spectra. The FTIR spectra of drugs, polymers, and their physical mixture were recorded from 400 to 4000 cm^{-1} using the potassium bromide press pellet technique. Figure 3 shows a comparison of the FTIR spectra of the pure drug selegiline hydrochloride, sodium alginate, carbopol, karaya gum, physical mixture, and final formulation. The FTIR stretching frequencies of selegiline hydrochloride were observed at 1315.50 cm^{-1} (C-N stretch), 1444.73 cm^{-1} (C=C), 1609.9 cm^{-1} (N-H), 2065.83 cm^{-1} (C≡C). All these characteristic bands were also retained in the physical mixture (Figure 3).

Differential scanning calorimetry

From ambient to 400ºC (a rate of 10ºC/min) the DSC thermogram of selegiline hydrochloride, polymers, physical mixture, and formulation was recorded. Selegiline hydrochloride has a melting transition temperature range of 140.61 to 148.65ºC, peak at 145.29ºC. The drug and polymer melting points did not vary much in the physical mixing, as demonstrated by the melting peak of selegiline hydrochloride at 144.32ºC. It could be linked to drug disintegration in molten polymer before the drug reaches its melting point shown in (Figure 4).

Scanning electron microscopy (SEM)

The microspheres were found distinct, spherical, and contained drug and polymer connections on their exterior surfaces, according to a SEM scan (Figure 5).
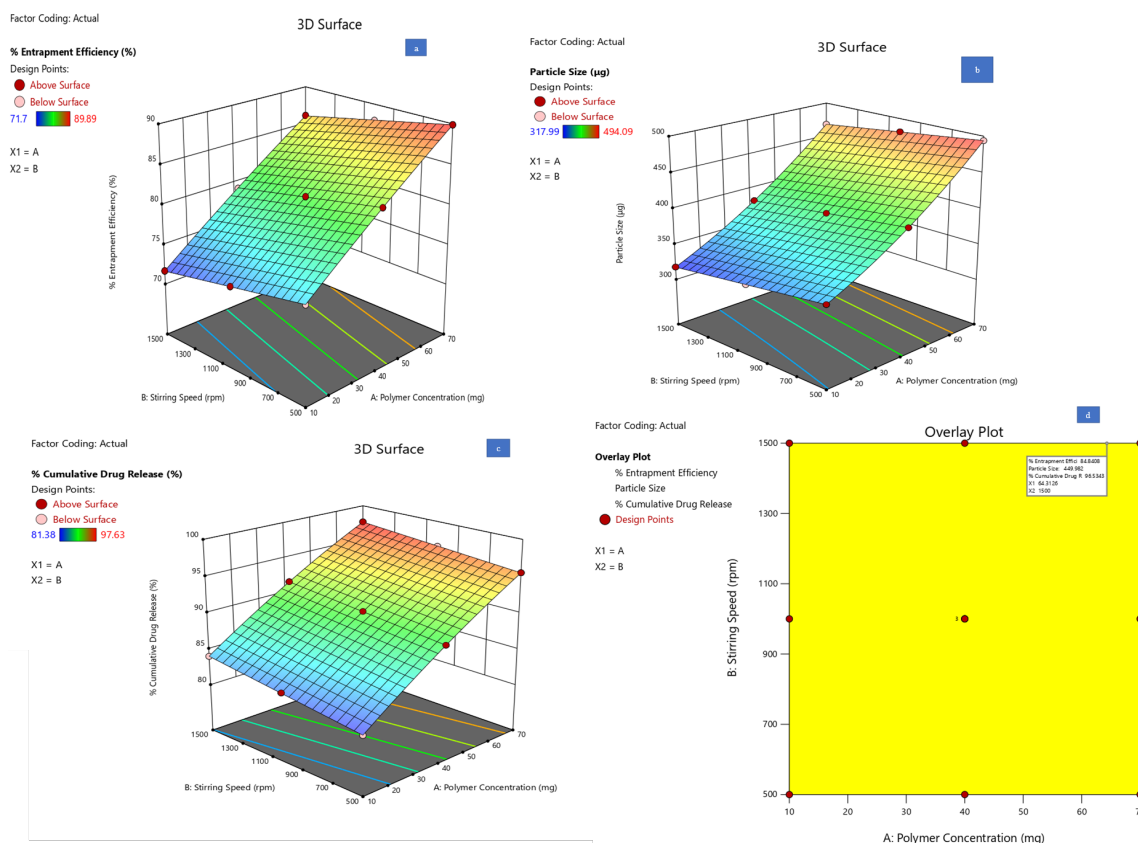

Figure 2: 3D Surface response graph of (a) Entrapment efficiency, (b) Particle size, (c) Cumulative percent drug release, (d) Overlay plot.
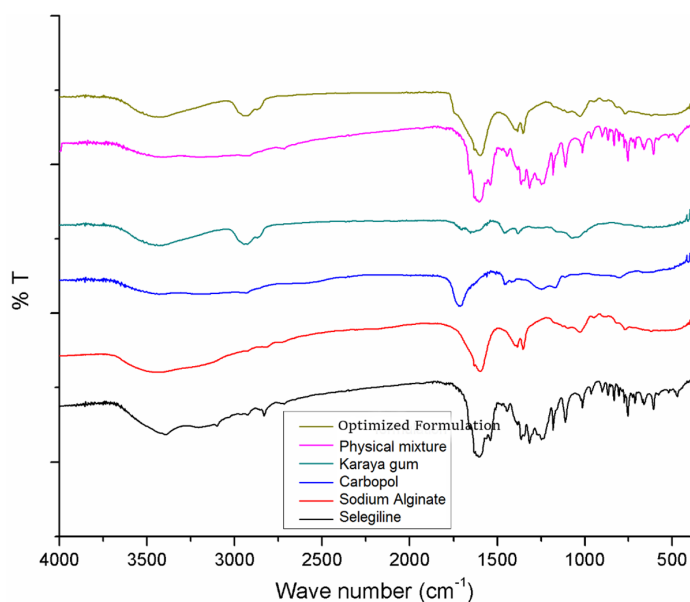
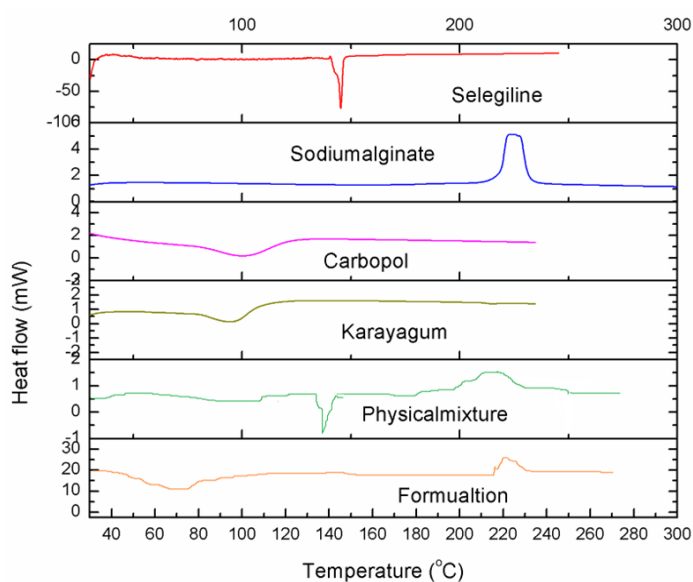**Figure 3:** FTIR studies of Selegiline hydrochloride pure drug, optimized formulation, physical mixture and excipients.

Figure 4: DSC studies of Selegiline hydrochloride pure drug, optimized formulation, physical mixture and excipients.
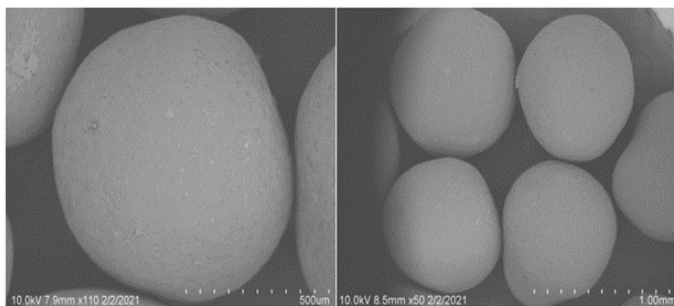

Figure 5: Scanning electron micrographs of mucoadhesive microspheres.

DISCUSSION

Varying stirring speeds influenced microsphere size, with higher speeds yielding smaller particles. increased viscosity, however, hampered propeller mixing efficiency, leading to larger microspheres. A near-unity correlation coefficient underscored a well-fitting model. optimized microsphere parameters indicated potential for effective formulation enhancement. Notably, integrity of the drug remained intact, as evident from minimal chemical alterations. Further, drug-polymer interactions were absent in drug excipient compatibility studies. this lack of interaction was also reflected in peaks of the drug in ftir spectra of selegiline hydrochloride-loaded microspheres. The drug's slight melting peak shift, observed in DSC thermograms of optimized formula, was attributed to drug dissolution in molten polymer. Consequently, physical combination yielded no new chemical entities or adducts. Microsphere surface pores, as revealed by SEM imagery, facilitated diffusion processes. Confirmation of selegiline hydrochloride's amorphous state emerged from XRD patterns, which lacked distinguishing peaks in loaded microspheres. *In vitro* dissolution studies of optimised formulation and marketed product were compared and found that optimised microsphere formulation was having better bioavailability intern it indicates more therapeutic efficiency.

CONCLUSION

Selegiline hydrochloride mucoadhesive microspheres were formulated after an extensive literature survey. In preliminary trials, different formulations of selegiline hydrochloride microspheres (SM1-SM14) were formulated by the ionic gelation method. In earliest trials, when compared to gum kondagogu, the formulations with carbopol 940P showed the best mucoadhesion and drug release, and the SM13 formulation showed the best drug release and mucoadhesion. Further optimization of selegiline hydrochloride mucoadhessive microspheres (SHM1 to SHM11) was done by optimizing independent variables by estimating dependent variables by using central composite design in Design Expert software 13 in Response Surface methodology. Desirability function found to be 0.926, which was satisfactory as reaching unity. The optimised formulation (SHM12) displayed a %CDR of 96.53 with a %EE of 84.84, a particle size of 450 μm in the overlay plot. Generated model work fit for microsphere optimization, and it is confirmed experimentally. In comparison of *in vitro* dissolution studies of optimised formulation and marketed product, optimised microsphere formulation was having better bioavailability. In future directions the work can be continued with *in vivo* studies to confirm bioavailability and therapeutic efficiency of optimised selegiline hydrochloride microspheres.

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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; **%EE:** Percent Entrapement Efficiency; **%CDR:** Percentage Cumulative Drug Release, **PI:** Prediction interval.

SUMMARY

Mucoadhesive microspheres containing selegiline hydrochloride were developed following a thorough review of the literature. Using the ionic gelation method, several formulations of selegiline hydrochloride microspheres (SM1–SM14) were created in preliminary trials. The formulations containing carbopol 940P demonstrated the best mucoadhesion and in vitro drug release in the preliminary trials, while the SM13 formulation demonstrated the best mucoadhesion and drug release. Selegiline hydrochloride mucoadhesive microspheres (SHM1 to SHM11) underwent additional optimisation by utilising central composite design in Design Expert software 13 in Response Surface methodology to estimate dependent variables and optimized microsphere formulation was having better *in vitro* drug release.

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