Enhanced Permeability of Rasagiline Mesylate through Nasal In-situ Gel: A Central Composite Design Study

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Abstract:

Transmucosal nasal drug delivery has become a major player in the field of drug delivery technology during the past few decades as a non-invasive method. “Rasagiline mesylate (RM) is a MAO-B inhibitor used in the treatment of idiopathic Parkinson's disease”, currently available in oral solid dose formulations with an absolute oral bioavailability of 36%. This study focuses on formulating a RM “nasal in-situ gel to enhance” drug availability at the site of action. Poloxamer 407 and N-dodecyl-β-D-maltoside (DDM) were selected as independent variables in the formulation process. A two-level, “two-factor Central Composite Design (CCD) was employed to investigate the impact of polymer concentrations on the dependent variables”. HPMC was used as a viscosity modifier. Statistical analysis identified the most effective formulation within the design space. The optimized formulation was evaluated using ATR and DSC studies, and for pH, viscosity, gelation, drug content, and spreadability. The results indicated that increasing “the concentration of Poloxamer 407 increased gelation time” and mucoadhesive strength without significantly affecting drug permeability. Conversely, variations in DDM concentration did not notably impact gelation time and mucoadhesive strength but had a direct effect on the permeability coefficient. This study underscores the crucial roles of Poloxamer 407 and DDM in optimizing RM nasal in-situ gel formulations for improved permeation of RM drug delivery to the site of action.

Keywords: Rasagiline mesylate (RM), MAO-B inhibitor, Parkinson's disease, Nasal in-situ gel, Drug availability, Poloxamer 407, N-dodecyl-β-D-maltoside (DDM), Central Composite Design (CCD), Mucoadhesive strength, Permeability coefficient.

1. Introduction:

The nasal route for medication administration has become a significant substitute for the parenteral route within the last ten years [1, 2]. This approach makes the patient more tractable and offers a greater level of patient compliance. Additionally, patients may self-administer medications without experiencing any pain, which contributes to the popularity of this method. Because of the nasal cavity's rich vasculature and extremely permeable membranes, drugs delivered through this route absorb swiftly and reach therapeutically effective plasma levels quickly. Additionally, “the nasal route has an advantage over the oral route, especially for medications that have low oral bioavailability because to gastrointestinal tract (GIT) enzyme breakdown and pH instability”. Additionally, the nasal route of administration is advantageous for medications that undergo gut wall metabolism, P-glycoprotein efflux, and significant hepatic first-pass metabolism [3]. Its ability to deliver a medication to “the central

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nervous system" and systemic circulation is the reason for nasal drug delivery. offers a potential drug delivery route; the nasal mucosa increases bioavailability and has a rapid beginning of action. However, the nasal route has a drawback in that drug particles are removed from the nose before being fully absorbed by the nasal mucosa, a process known as mucociliary clearance [4]. The process of gel formation at the site of action upon application of the formulation is known as "in-situ" gelation. A strong inhibitor of the monoamine oxidase (MAO) type B enzyme, rasagine mesylate (RM) plays a key part in "the central nervous system's" inactivation of biogenic amines. In the central nervous system, MAO-B is principally in charge of dopamine inactivation [5]. Since dopamine insufficiency is the main cause of PD's clinical symptoms, inhibiting MAO-B tends to bring dopamine levels back to normal, which lessens PD symptoms. It was anticipated that a new dosage form for RM would be developed in order to overcome its limited bioavailability and incapacity to achieve a high concentration in brain tissue following oral treatment [6]. Developing and testing in-situ gels for RM administration was the aim of this work.

2. Materials and Methods:

Rasagiline mesylate obtained as a free sample from Optimus, Poloxamer-407, HPMC E50 LV and DDM (N-dodecyl-beta-D-maltose) obtained from Sigma Aldrich.

2.1. Method of preparation of In-situ gel: A CCD design was used to develop nasal in situ gel loaded with Rasagiline mesylate. "The composition of different formulations of RM in situ nasal gels is shown in Table 1". "The drug (0.3 g) was dissolved in" ethanol (0.05 g) and purified water (20 g) was added under continuous stirring [7]. In another glass beaker the polymeric solution was prepared where to the 40 g purified water HPMC E50 LV (0.20 g), DDM and poloxamer 407. DDM and poloxamer concentrations varies which is captured in Table 1 were added under continuous stirrer, stirring continued till clear solution obtained. To the polymeric mixture drug solution was added under continuous stirring. For fifteen minutes, the resulting mixture was mixed. Purified water was used to get the final volume up to 100 g.

In the current study, the impacts of specific independent factors on the answers were assessed using a two-level, two-factor CCD. Poloxamer 407 (A) and DDM (B), two independent variables, were taken into account. The experiment measures the permeability coefficient, mucoadhesive strength, and gelling time. The polynomial equation was fitted and analyzed mathematically. "A graphical optimization methodology" and a numerical method utilizing the alpha 0.05 confidence interval value were used to solve the optimized formula [8]. Table 1 lists a total of 11 experimental runs for the two level two-factor CCD.

2.2. Mucoadhesive strength: With the use of a sophisticated chemical balance, the amount of force needed to break the in-situ gel positioned between the two nasal mucosae was ascertained. The study uses the nasal mucosa, primarily the bovine mucosa. With the use of a rubber band, the nasal mucosa is secured to a transparent glass surface on one side of the balance, and another mucosa is positioned such that its surfaces face each other in an inverted orientation relative to the first [9]. The two mucosae are separated by a tiny amount of the produced in-situ gel (about 50 mg), which is then let to come into touch with one another for a few minutes. Different mucosa were used for each measurement with a different formulation. Weight was applied to the opposite side of the pan until the two mucosae separated. The force or

stress separation per centimeter square of the mucosa area employed is the measure of mucoadhesive strength.

2.3. Gelling time: The methods outlined by Miller and Donovan were used to calculate the gelling time of formulas. Prior to administration, the system is in the sol form; upon administration, it transforms into the gel form. The amount of time needed for sol to change into gel is known as the gelling time. By adding a small amount of simulated nasal fluid to 2 milliliters of prepared solution in a test tube, “keeping the temperature at 37 degrees Celsius, and visually observing the gel formation (the test tube was positioned horizontally to assess the gelation and observe the sample condition)”, the time for gelation is measured in seconds [10].

2.4. In-vitro permeation study: The experiment employed goat nasal mucosa and the Franz diffusion cell apparatus. Fresh nasal mucosa was supplied by the slaughterhouse [12]. The tissue is cleaned with a pH 6.8 phosphate buffer and then “placed between the donor and receptor compartments”. While 5 mL of the formulation was put on the donor compartment, “the receptor compartment was filled with a pH” 6.8 phosphate buffer. The temperature was maintained at 37 ± 0.5 °C throughout the research using a 500rpm magnetic stirrer. To examine drug penetration, a 1 mL sample was taken at a predetermined time and left for 6 hours using UV-visible spectrophotometry.

2.5. ATR study: When combined with “infrared spectroscopy, the sampling method known as attenuated total reflection (ATR)” allows samples to be analyzed straight from the liquid or solid state without the need for further preparation [13]. ATR spectroscopy is very helpful for tracking the composition of polymers live. “IR can identify the components of a chemical process because of its capacity to fingerprint chemical components. Germany's ATR Bruker Opus 7.0” carried out the investigation.

2.6. DSC study: Temperature and changes in a sample's physical properties over time are measured by a thermal analysis tool called DSC. “In other words, the device is a thermal analysis instrument that determines the temperature and heat transfer associated with material transitions as opposed to time and temperature”. DSC-60, Shimadzu, USA, performed DSC. The drug, polymer and final formulation DSC thermogram were evaluated for the compatibility and to observe any form transition upon formulation.

2.7. XRD of study: A non-destructive test technique for examining the structure of crystalline materials is X-ray diffraction, commonly shortened to XRD. By examining the crystal structure, XRD analysis may determine which crystalline phases are present in a material and, consequently, provide information about its chemical composition. Thermo Scientific, India's ARL EQUINOX 100 conducted the XRD research [15].

2.8. Characterization of In-situ gel:

2.8.1. Gelling temperature: The temperature at which the formulation's meniscus would stop moving when the container was slanted 90 degrees is known as the gelling temperature. The gelation studies were determined using “Miller and Donovan's” approach [16]. “The gelling temperature was determined by placing the test tube containing a sufficient volume of the generated solutions in a water bath at 4 °C. The temperature of the water bath was steadily increased by 1 °C every two minutes”.

2.8.2. Viscosity: Brookfield viscometer (model DV-II+Pro, USA) was used to measure the viscosity of prepared gels. For this purpose, spindle No. 64 was used. Viscosity was recorded by rotating the spindle at 50 rpm [17]**.**

2.8.3. Determination of pH: Purified water was used to dilute "the solution after one milliliter of the produced gels was transferred to a 10-milliliter volumetric flask [18]. A digital pH meter (Shambhavi Impex, India) that had been calibrated using phosphate buffers at pH 4 and pH 7 was used to measure the pH of the resultant solution".

2.8.4. Drug content assay: One ml of the prepared formulation was dispersed in 10 ml of methanol for 2–3 min with occasional shaking. The resulting solution was filtered through a 0.45 µm filter paper and was "diluted with methanol" [19]. "The quantity of RM in the formulation was measured using a Shimadzu 1800 spectrophotometer at 264 nm".

2.8.5. Spreadability: About 500 mg of produced gel was spread out on glass-covered graph paper to gauge the gels' spreadability. We then topped the gel pile with a second glass. The gel diameter was calculated by measuring the diameter length of several slides [20].

3. Results and Discussion:

Table 1: Formulation table of in-situ gels as suggested by CCD

	Factor 1	Factor 2	Response 1	Response 2	Response 3
Run	**A: Poloxamer 407**	**B: DDM**	**Gelling time**	**Mucoadhesive strength**	**permeability coefficient**
	%	%	S	Dyne/cm^2	cm/s
1	22.5	2.2	38.5	1765.19	0.00241
2	22.5	1.5	37.1	2026.33	0.00203
3	30.0	1.0	41.6	2451.66	0.00169
4	15.0	2.0	29.7	1557.13	0.00284
5	11.9	1.5	26.1	1276.79	0.0034
6	22.5	0.8	37.5	1716.16	0.00169
7	22.5	1.5	36.9	1961.33	0.00201
8	22.5	1.5	36.7	2157.46	0.00205
9	33.1	1.5	45.4	2745.86	0.00156
10	30.0	2.0	43.9	2353.59	0.00168
11	15.0	1.0	31.2	1470.99	0.0024

In-vitro permeation study: The in-vitro permeation study was performed while the impact of poloxamer 407 and DDM on permeation was assessed. A permeation comparison was between "F6" which contained 0.79% and "F7" which contained "1.5%" of DDM. The "F7" exhibited 99.97 % whereas "F6" exhibited 99.18 % permeation of the drug. This indicates a good amount of drug permeated in the presence of a greater amount of DDM. It also noted that a greater amount of poloxamer 407

hindered the drug permeation as can be seen from “F9” which permeated 99.11% at 14h whereas “F8” permeated 99.94% at 10 h Figure 1 and 2.

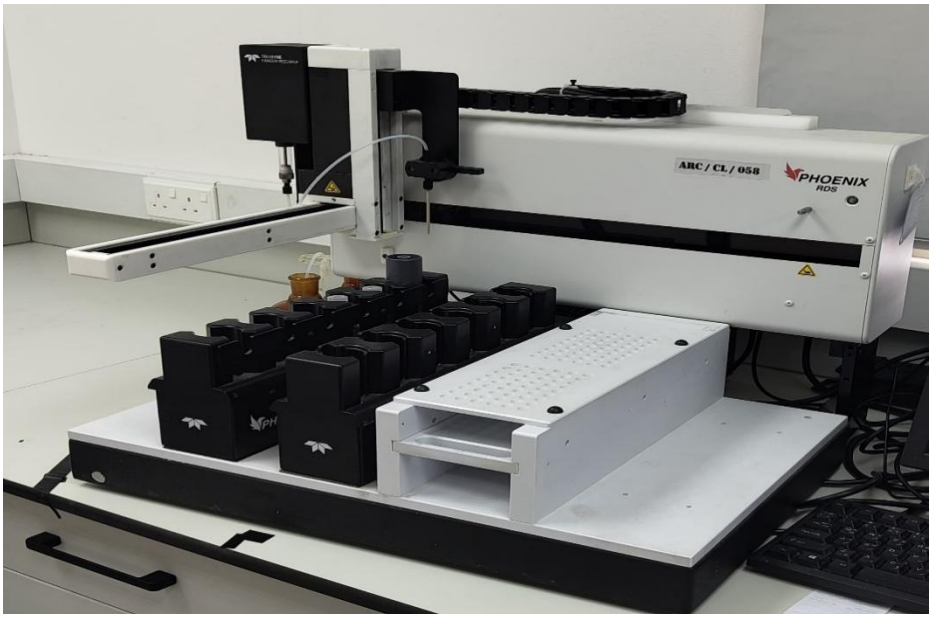

Figure 1: Franz Diffusion Cell Apparatus

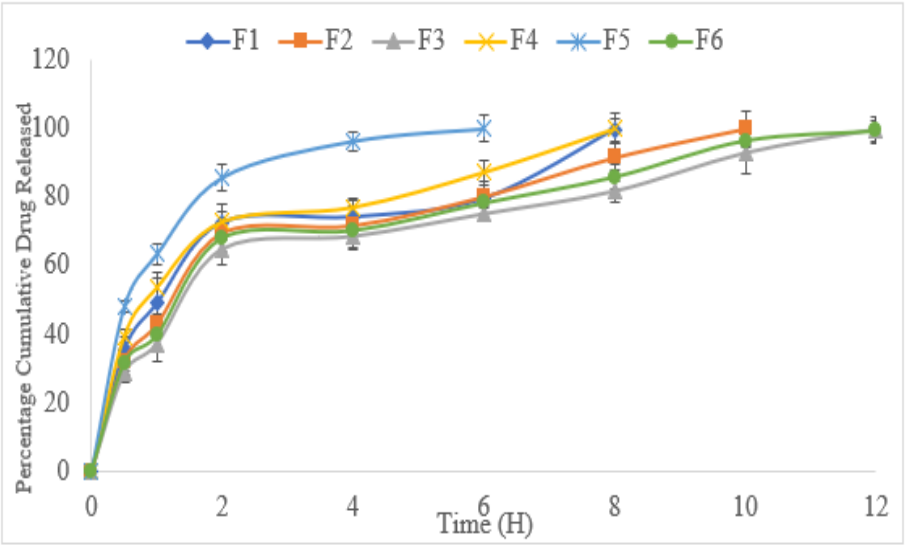

Figure 2: In-vitro permeation study of F1-F6 as suggested by CCD

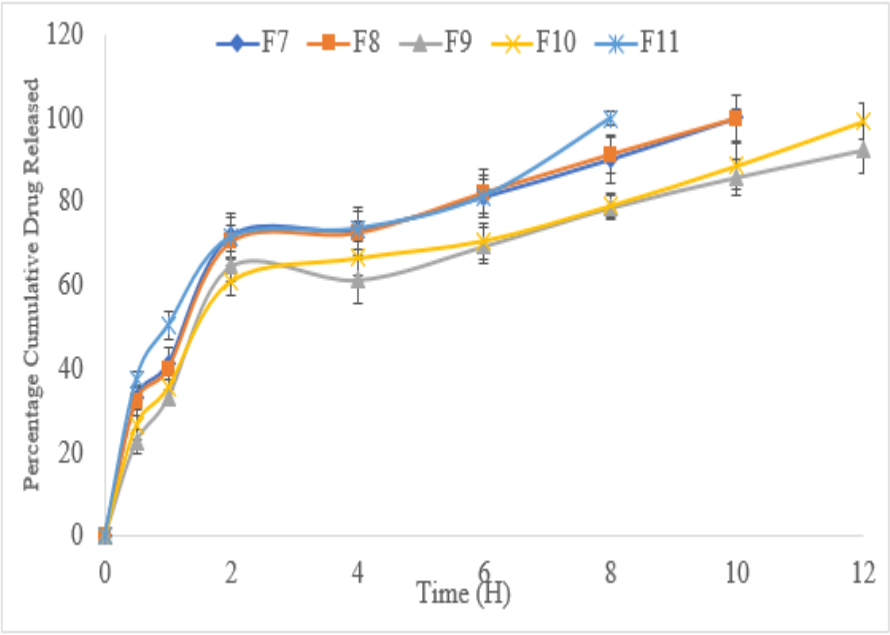

Figure 3: In-vitro permeation study of F7-F11 as suggested by CCD

3.1. Response surface methodology (RSM):

Response 1 [Gelling time] =36.9+**6.486A**-0.276B+**0.95AB 0.643A²** + 0.481B² Figure 3.

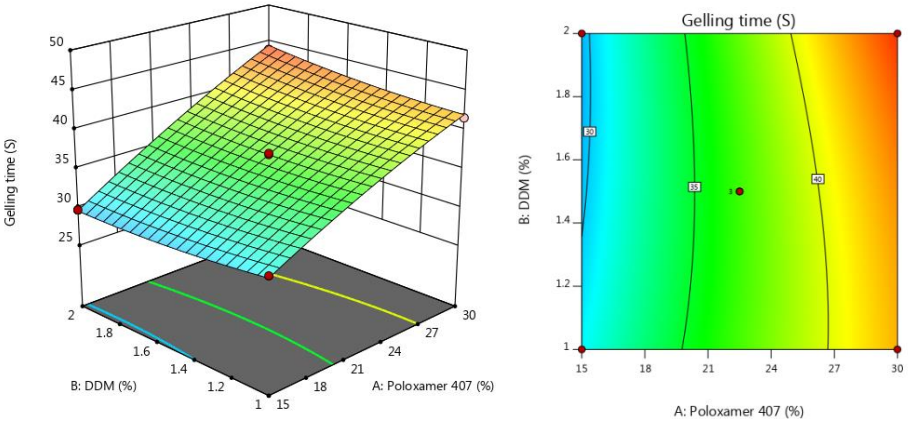

Figure 4: 3D & 2D response surface plot for Response 1 (Gelling time)

Response 2 [Mucoadhesive strength] =2048.37+**481.83A**+7.17B-46.05AB+2.061A²-**136.26B²** from Figure 4.

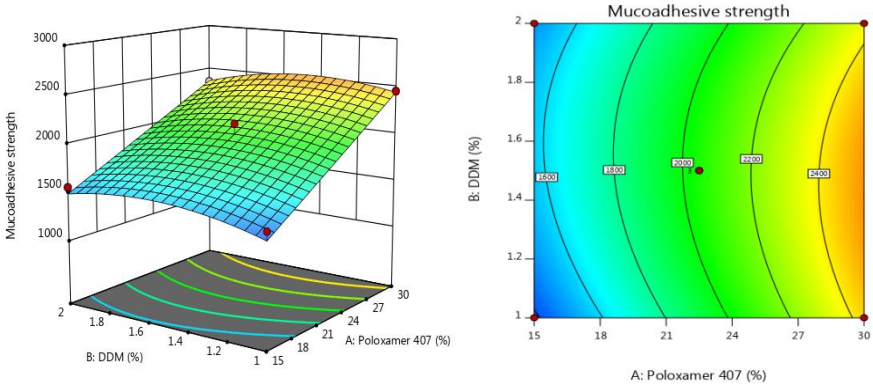

Figure 5: 3D & 2D response surface plot for Response 2 (Mucoadhesive strength)

Response 3 [Permeability coefficient]= 0.002-**0.0005A+0.0001B**-0.00011AB+**0.00019A^2**-1.81B^2 from Figure 5.

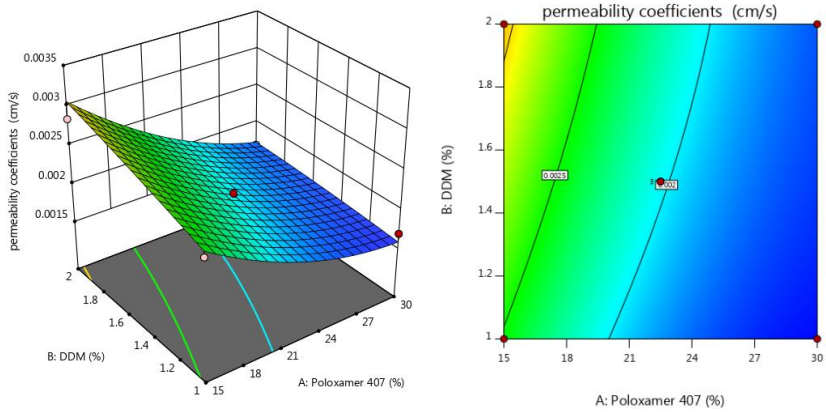

Figure 6: 3D &2D response surface plot for Response 3 (Permeability coefficient)

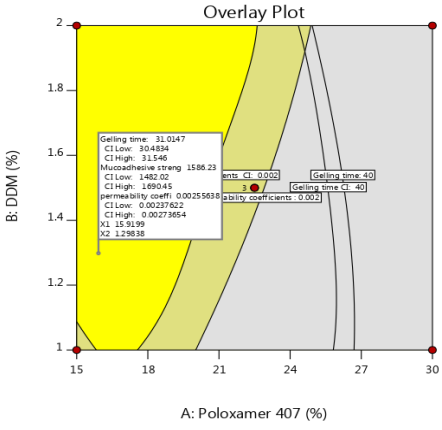

Figure 7: Overlay plot of region highlighting the optimized space and value

From the mathematical and graphical method of optimization; the best and optimized formulation developed and the design space (Yellow color region) is mentioned in Figure 6 and 7. Prior to identifying the optimal formulation, the literature survey's goal ranges (for answers) were set. Table 2 mentions the improved formulation.

Table 2: Optimization formulation table

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	Poloxamer 407	15.92	15.00	30.00	0.0000	Actual
B	DDM	1.30	1.0000	2.00	0.0000	Actual

Table 3: Point Prediction table of optimized formulation

Response	Predicted Mean	Predicted Median	Observed	Std Dev	SE Mean	95% CI low for Mean	95% CI high for Mean	95% TI low for 99% Pop	95% TI high for 99% Pop
Gelling time	31.02	31.02	30.21	0.49	0.26	30.3383	31.6937	27.864	34.168
Mucoadhesive strength	1586.36	1586.36	1615.8	95.53	51.7136	1453.43	1719.3	968.044	2204.68
permeability coefficients	0.002	0.003	0.003	0.0002	8.93984E-05	0.00232645	0.00278606	0.00148735	0.00362516

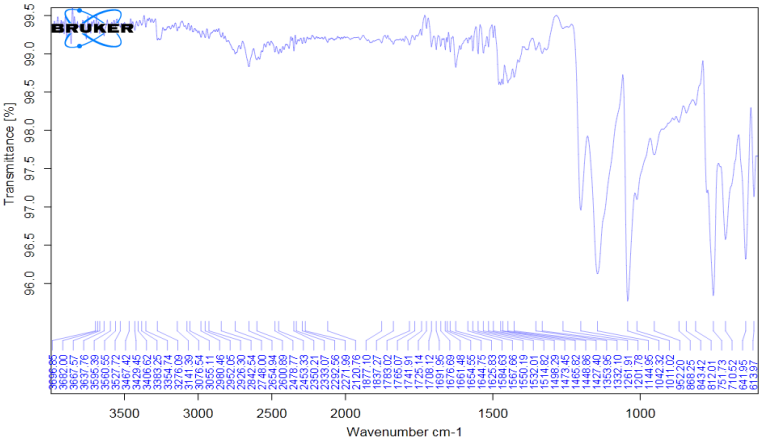

Figure 8: ATR spectra of pure Rasagiline mesylate

The ATR spectrum of Rasagiline mesylate Figure 8 shows typical characteristic peaks at a band of 2926.30 $cm^{-1}$and "2842.54 cm^{-1} owing to aromatic C-H stretching and aliphatic C-H stretching groups. The other bands Peaks at 3276.09 cm^{-1} (Secondary amine N-H) and 1042.32 cm^{-1} in (aliphatic amino C-N) respectively". It also noted a strong stretching of C-O at place 1142.95 cm^{-1} in table 3.

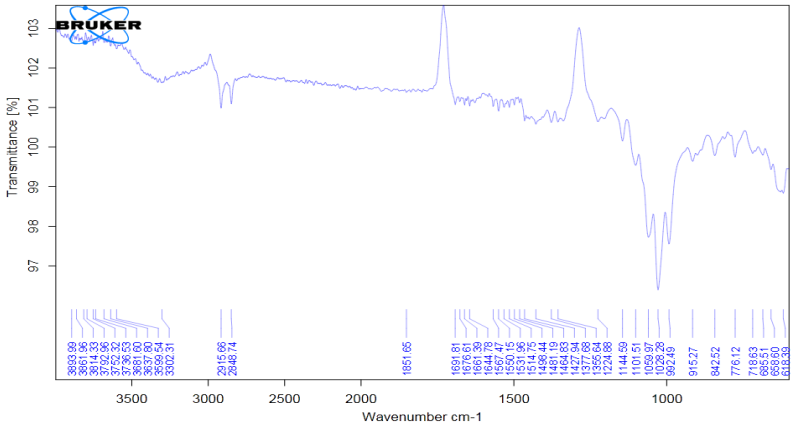

Figure 9: ATR spectra of DDM

The FTIR spectrum of DDM Figure 9 shows typical characteristic peaks which are analyzed carefully. It should be noted the presence of a characteristic band corresponding to the stretching band of 1028.28 cm^{-1} by C-N stretching. The characterized peak at 1224.88 cm^{-1} was noted by C-O stretching. Similarly, a prominent band at 2915.66 cm^{-1} was exhibited by OH stretching.

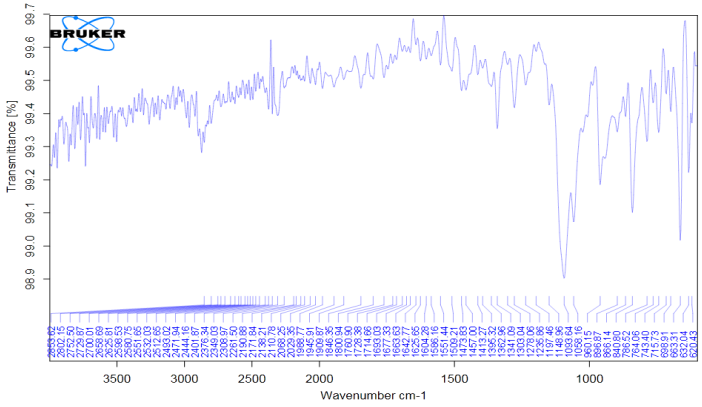

Figure 10: ATR spectra of Poloxamer-407

"The IR spectra of poloxamer 407 is characterized by primary absorption peaks at 2171.84 cm–1 (C-H stretch aliphatic), 1347.09 cm–1 (in-plane O-H bend), and 1093.64

cm–1 (C-O stretch)", as seen in Figure 10. Absorption is detected and verified thoroughly in the sample.

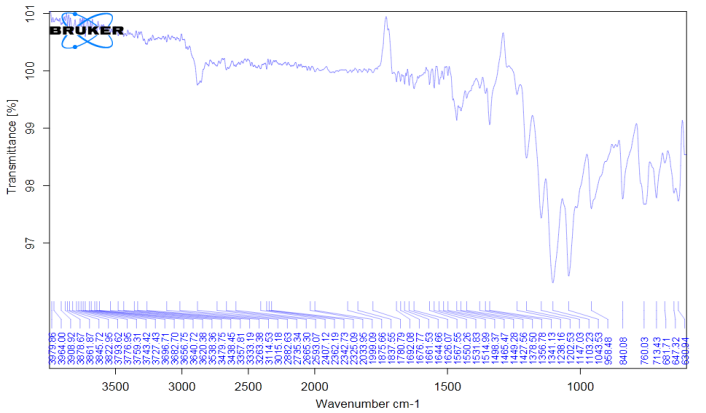

Figure 11: ATR spectra of optimized formulation

The optimized formulation underwent for ATR analysis for group position and identifications. It noted there was no such major changes took place and peaks were at characteristic position. The ATR spectrum of optimized formulation Figure 11 shows typical characteristic peaks at a band of 2882.63 $cm^{-1}$and "2735.34 cm^{-1} owing to aromatic C-H stretching and aliphatic C-H stretching groups. The other bands Peaks at 3263.38 cm^{-1} (Secondary amine N-H) and 1043.53 cm^{-1} in (aliphatic amino C-N) respectively". It also noted a strong stretching of C-O at place 1147.03 cm^{-1}.

3.2. DSC study:

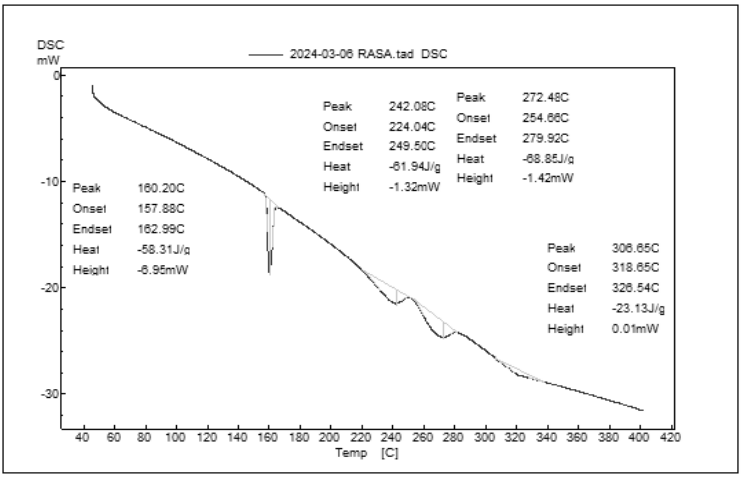

Figure 12: DSC of Rasagiline mesylate

According to the literature review, it found a strong endothermal peak Figure 12 at 160.20 °C with a heat of energy of -58.31 mJ. Two further thermal degradation peaks were also observed at 242.08 and 272.48 °C.

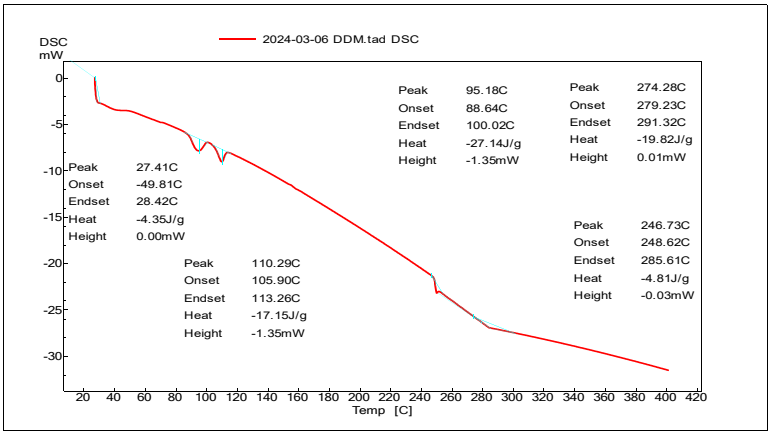

Figure 13: DSC thermogram of DDM

DDM exhibited a characteristic melting point peak at 110.29°C as well as another peak noted at 27.41°C which is as per the literature review information provided. The characteristic peak indicated the purity of the sample.

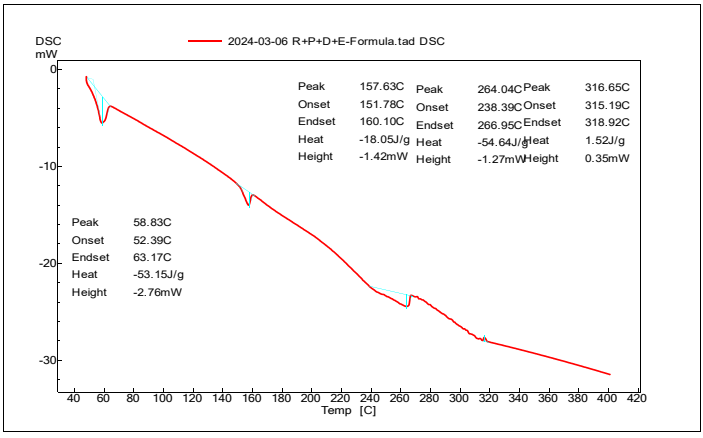

Figure 14: DSC thermogram of Optimized formulation (In-situ) gel

A significant endothermal peak at 157.63 °C was seen in the DSC research Figure 14, this value is close to pure rasagine mesylate, as previously documented at 160.20 °C. It also inferred thermal stability of pure drug in formulation. We also noted a prominent peak appeared at 58.83 °C corresponding to poloxamer 407.

3.3. XRD of study:

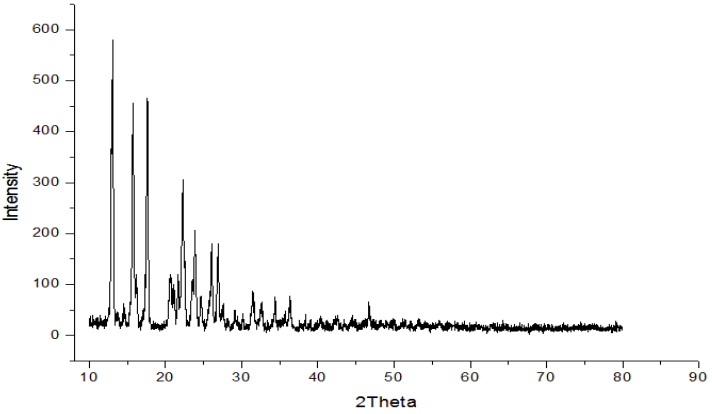

Figure 15: XRD of Rasagiline mesylate

Peaks with intensities of 590 (2Theta), 480 (2Theta), and 490 (2Theta) with an intensity of 600 were seen at positions 13 (2Theta), 16 (2Theta), and 18 (2Theta). With intensities below 300, a few additional distinctive peaks emerged at 22 (2Theta), 24 (2Theta), and 26 (2Theta) Figure 15.

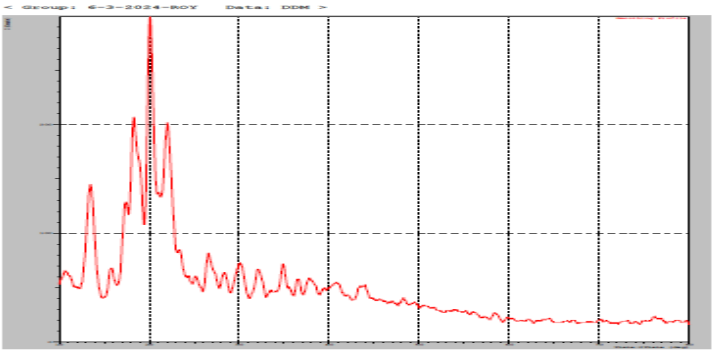

Figure 16: XRD spectra of DDM

While observing XRD spectra of DDM, the peaks appeared to be more prominent and sharp. Sharp peaks appeared at 9.247, 18.076, 20.248, 22.874 and 16.036 (2Theta) with intensity below 300. Few more non prominent peaks appeared at below 100 intensity count Figure 16.

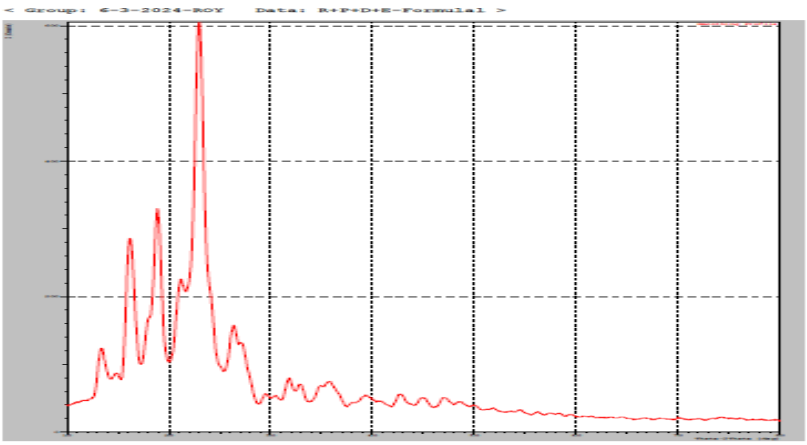

Figure 17: XRD spectra of Optimized formulation (In-situ) gel

The purpose of XRD study was to trace pure drug in in-situ formulation". It ascertained in the XRD study Figure 17, highlighted peak as well lesser number of significant characteristic peak at position 11.75, 19.16 and 23.85 (2Theta); Those highlighted peaks were identical with pure Rasagiline mesylate. Along with a larger number of peaks corresponding to pure drug disappeared or reduced their intensity in our optimized formulation. This indicated presence of Rasagiline in partial crystalline arrangement. However, the few more characteristic peaks disappeared; "which could be due to presence of solvent and reduced crystallinity during formulation development.

4. Characterization of In-situ gel:

Table 4: Formulation table of in-situ gels as suggested by CCD

	Factor 1	Factor 2	Characterization				
Run	**A:Poloxamer 407**	**B:DDM**	**Gelling temperature**	**Viscosity**	**pH**	**Drug content**	**Spreadibility**
	%	**%**	**°C**	**Cp**	**-**	**%**	**mm**
1	22.5	2.2	34.5	512.5±3.7	7.2±0.1	97.54±2.1	84.37±4.2
2	22.5	1.5	35.7	508.1±4.1	7.3±0.1	97.86±1.9	87.33±5.1
3	30.0	1.0	32.4	645.2±6.9	7.2±0.2	96.39±3.2	79.06±3.7
4	15.0	2.0	37.2	477.4±4.6	7.3±0.2	98.22±1.5	91.28±2.5
5	11.9	1.5	40.3	418.6±11.5	7.2±0.1	97.95±4.6	96.11±3.9
6	22.5	0.8	34.9	501.3±7.5	7.4±0.3	98.15±2.9	90.25±1.7
7	22.5	1.5	35.8	509.4±8.4	7.1±0.2	98.07±3.6	88.12±2.7

8	22.5	1.5	35.9	503.8±9.3	7.2±0.1	98.31±4.5	88.06±1.9
9	33.1	1.5	29.7	745.2±10.2	7.3±0.2	97.85±1.8	73.21±5.5
10	30.0	2.0	31.8	651.7±12.5	7.2±0.1	98.06±2.7	75.85±3.8
11	15.0	1.0	37.6	468.4±8.5	7.40.1	97.93±5.2	92.53±2.6

From table 4, the gelling temperature decreases as the concentration of Poloxamer 407 increases. This is evident from the comparison between runs with higher Poloxamer 407 concentrations (Runs 9 and 10) which have lower gelling temperatures (29.7°C and 31.8°C), compared to runs with lower Poloxamer 407 concentrations (Runs 5 and 11) which have higher gelling temperatures (40.3°C and 37.6°C). This trend is consistent across various DDM concentrations, indicating that DDM also influences the gelling temperature but to a lesser extent. The viscosity increases as the concentration of Poloxamer 407 increases. The DDM concentration also impacts the viscosity to a lesser extent than Poloxamer 407. The combined effect of Poloxamer 407 and DDM suggests that while Poloxamer 407 concentration is the primary factor influencing viscosity, DDM modulates this effect. Higher DDM concentrations tend to increase the viscosity slightly.

The formulations' viscosity is primarily governed by the concentration of Poloxamer 407, with higher concentrations resulting in higher viscosities. DDM also influences the viscosity, with higher concentrations tending to increase it, though its effect is less pronounced than Poloxamer 407.

The spreadability decreases as “the concentration of Poloxamer 407 increases”. This is evident from the comparison between runs with higher Poloxamer 407 concentrations (Runs 9 and 10) which have lower spreadability (72.53 and 75.85), compared to runs with lower Poloxamer 407 concentrations (Runs 5 and 11) which have higher spreadability (96.15 and 92.53). In conclusion, the spreadability of the formulations is primarily governed by the concentration of Poloxamer 407, with higher concentrations resulting in lower spreadability.

The drug content appears to slightly increase with higher concentrations of Poloxamer 407. The DDM concentration also impacts the drug content but to a lesser extent compared to Poloxamer 407. The combined effect of Poloxamer 407 and DDM suggests that while Poloxamer 407 concentration is the primary factor influencing drug content, DDM also modulates this effect. Higher DDM concentrations tend to result in slightly higher drug content. DDM also influences the drug content, with higher concentrations tending to slightly increase it.

5. Conclusion:

The goal of this study is to design and optimize the in-situ gel of Rasagiline Mesylate. To assess the impact of specific independent factors on the replies, a two-level, two-factor Central Composite design (CCD) was employed. Polymers such as HPMC E50 LV, Poloxamer-407, and N-dodecyl-beta-D-maltose (DDM) were used in combination to create nasal gels. The mathematical and graphical approaches of optimization are used to build the best and most optimal “formulation from the design space”. The pH of the enhanced formulation, viscosity, gelation, drug content, and spreadability are assessed for ATR and DSC studies. As Poloxamer 407 concentration rises, the gelling

temperature and spreadability fall. Higher doses of Poloxamer 407 seem to marginally increase the viscosity and drug content.

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