Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
NLM (National Library of Medicine): ID: (101671502)
Index Copernicus Value 2023: 85.86
Volume 14, Issue 6; 2025, 138-146

ISSN (Online): 2279-0594
ISSN (Print): 2589-8752

Research Article

Optimization of Rasagiline Mesylate-Loaded Polycaprolactone Nanoparticles for Intranasal Delivery Using Box–Behnken Design

Gulshan Kumar[1], Mayank Bansal[2], Jayesh Gadhiya[3]

[1]Research Scholar, Jaipur College of Pharmacy, Jaipur
[2]Principal & Professor, Jaipur College of Pharmacy, Jaipur
[3]Research Scientist, Ortiv Q3 Pvt Ltd

Article Info: Received: 18-09-2025 / Revised: 16-10-2025 / Accepted: 20-11-2025
Corresponding Author: Gulshan Kumar
DOI: https://doi.org/10.32553/jbpr.v14i6.1395
Conflict of interest statement: No conflict of interest

Abstract:
Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic neuronal loss, leading to impaired motor and cognitive functions. Rasagiline Mesylate, although therapeutically effective, suffers from low oral bioavailability due to extensive first-pass metabolism and short half-life. The present study aimed to develop and optimize Polycaprolactone (PCL) nanoparticles loaded with Rasagiline Mesylate for intranasal delivery to enhance brain targeting and prolong therapeutic action. Nanoparticles were prepared using a modified nanoprecipitation technique and optimized through Box–Behnken Design (BBD) using Design Expert® 7.0 software. Polymer concentration (A), organic phase volume (B), and surfactant concentration (C) were selected as independent variables, while particle size and entrapment efficiency served as response parameters. ANOVA confirmed the significance of the quadratic model for both responses, with minimal lack of fit. The optimized formulation (Polymer: 232.6 mg; Organic phase: 9.2 ml; Surfactant: 0.59%) exhibited a desirability of 1.0, demonstrating excellent agreement between predicted and experimental responses. Results suggest that intranasal Rasagiline-loaded PCL nanoparticles provide a promising strategy for enhanced brain delivery in PD management by bypassing hepatic metabolism and sustaining drug release.
Keywords: Rasagiline Mesylate, Polycaprolactone (PCL) Nanoparticles, Intranasal Delivery, Box–Behnken Design, Optimization, Entrapment Efficiency, Particle Size, Parkinson's Disease.

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the depletion of dopaminergic neurons and the consequent decline in motor coordination and cognitive stability [1]. Conventional oral therapy with Rasagiline Mesylate suffers major drawbacks such as extensive first-pass metabolism, short half-life, and limited brain delivery, resulting in reduced therapeutic efficiency and poor patient compliance [2]. Intranasal delivery has emerged as a promising alternative since the nasal cavity provides a direct connection to the brain via olfactory and trigeminal pathways, bypassing the blood–brain barrier and significantly enhancing drug bioavailability [3]. Nanoparticle-based systems, particularly those formulated using biodegradable polymers like Polycaprolactone (PCL), offer additional advantages such as sustained drug release, protection from

enzymatic degradation, and improved permeability across biological membranes [4].
The preparation of Rasagiline Mesylate–loaded PCL nanoparticles typically involves nanoprecipitation, enabling the formation of uniformly dispersed nanocarriers with high entrapment efficiency and controlled release characteristics. Optimization of formulation parameters—including polymer concentration, surfactant level, solvent–antisolvent ratio, and stirring speed—is critical to achieve minimal particle size, narrow polydispersity, and maximum drug loading efficiency. Systematic optimization enhances not only the nasal retention and mucoadhesion of the nanoparticles but also facilitates improved drug transport to the brain, ultimately offering prolonged therapeutic action with reduced dosing frequency. Thus, developing and optimizing intranasal nanoparticle formulations presents a scientifically sound and clinically meaningful strategy for effective PD management [5,6].

Method of Preparation

Preparation PCL Nanoparticles

Nanoparticles were prepared using modified nanoprecipitation method. In brief, prepare solution-A by dissolving PCL in acetone at 40°C and Solution-B by dissolving Drug in ethanol. Now add solution-B into Solution-A to prepared organic phase. The organic phase added drop wise manner under vigorous stirring followed by magnetic stirring at room temperature into the Borate buffer solution PH 9.0 which is used instead of aqueous phase containing dissolved stabilizing agent (aqueous phase) using syringe equipped with needle at the rate of 2.5-3ml/min. Later the dispersion was kept for magnetic stirring for 6 hours at room temperature to evaporate the organic solvent. The obtained nanosuspension was stored at room temperature until further use.

Optimization by Design Expert 7.0 Software

Introduction to Box–Behnken Design

The Box–Behnken Design (BBD) is a widely used response surface methodology (RSM) tool introduced by Box and Behnken in 1960. It evaluates the effects of multiple formulation variables efficiently by studying them at three coded levels (−1, 0, +1). The design is suitable for fitting quadratic models and provides reliable estimation of responses with minimal experimental runs. A key advantage of BBD is its reduced number of experiments compared to other RSM designs, making it cost-effective while still ensuring accurate optimization. It also avoids extreme factor combinations because trials are positioned at the midpoints of the edges of the experimental domain and the design center. For valid application, a minimum of three independent variables is required. The figure below typically represents the experimental layout of a three-factor Box–Behnken Design, where each point corresponds to a specific formulation run [7].

Preliminary investigation

After identifying the variables that might affect the product Quality Attributes, preliminary investigation of variables was carried out. The effects of selected variables on the particle size and entrapment efficiency were studied to calculate their optimal values for final optimization of formulation using response surface experimental design study [8].

Table 1: Variables and Response Parameters

Independent Variables	
Factor 1: A	Polymer Concentration (mg)
Factor 2: B	Organic Phase (ml)
Factor 3: C	Surfactant Concentration (%)
Response Parameters	
Response Y1	Size (nm)
Response Y2	Entrapment efficiency

Table 2: Values for Independent Variables

Values	A: Polymer Conc. (mg)	B: Organic Phase (ml)	C: Surfactant Conc. (%)
Low Actual	50	5	0.25
High Actual	300	10	1
Low coded	-1	-1	-1
High coded	1	1	1

Response Surface Methodology was applied using comprehensive software, Design-Expert 7.0.0 to fit second order polynomial equations, obtained by multiple linear regression analysis (MLRA) approach.

A full and reduced model for all variables was established by putting the values of regression coefficients in polynomial equation. Statistical soundness of the polynomial equations was established on the basis of ANOVA statistics. Three-dimensional response surface plots were established by varying levels of two factors and keeping the third factor at fixed levels at a time. In this way they are more helpful in understanding the actual interaction amongst the varying factors on the response parameter and are more meaningful [9,10].

The 3-D response surface graphs were constructed using Design Expert software. The experimental design and the derived polynomial equation for the optimization of nanosuspension formulations were validated for their utility by performing check point analysis. Eight optimum checkpoints were selected, prepared and evaluated for response parameters. Statistical comparison between the predicted values and average of three experimental values of the response parameters was performed to derive percentage error and to evaluate significant difference between these values. Optimized formulation was derived by specifying goal and importance to the formulation variables and response parameters [11,12]. Results obtained from the software are further verified by actual preparation of the batches and comparing the predicted and actual results.

Results and discussion

Box-Behnken Design

Table 3: Box-Behnken Design

Std	Run	Block	A: Polymer conc (mg)	B: Organic phase (ml)	C: Surfactant (%)	Response 1: Size (nm)	Response 2: EE (%)
2	1	Block 1	300	5	0.63	277.3	21.3
14	2	Block 1	175	7.5	0.63	242.5	44.69
10	3	Block 1	175	10	0.25	271.8	42.27
3	4	Block 1	50	10	0.63	174.9	45.3
11	5	Block 1	175	5	1	253	37.88
15	6	Block 1	175	7.5	0.63	275.3	52.52
7	7	Block 1	50	7.5	1	169.4	22.31
12	8	Block 1	175	10	1	235	61.51
6	9	Block 1	300	7.5	0.25	277.7	20.24
4	10	Block 1	300	10	0.63	278.2	27.27
1	11	Block 1	50	5	0.63	176.2	27.5
9	12	Block 1	175	7.5	1	250.4	37.82
13	13	Block 1	175	7.5	0.63	267.4	51.69
5	15	Block 1	50	7.5	0.25	175.9	26.98

ANOVA Analysis of Experimental Design for Size (nm)

To identify the significant parameters and their interactions, ANOVA was performed for each parameter. The value of the coefficients of A, B and C are related to the effect of these variables on the response. A positive sign of coefficient indicates a synergistic effect while a negative term indicated an antagonistic effect upon the response. Larger coefficients mean the independent variables has more potent influence on the responses.

Coefficients with p-value less than 0.05 had a significant effect on the measured response.

Response 1 Size

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	24461.88	9	2717.99	20.94	0.0019	significant
A-Polymer conc	*18798.60*	*1*	*18798.60*	*144.86*	*< 0.0001*	
B-Organic phas	*1.44*	*1*	*1.44*	*0.011*	*0.9201*	
C-surfactant co	*581.40*	*1*	*581.40*	*4.48*	*0.0879*	
AB	*1.96*	*1*	*1.96*	*0.015*	*0.9070*	
AC	*108.16*	*1*	*108.16*	*0.83*	*0.4031*	
BC	*384.16*	*1*	*384.16*	*2.96*	*0.1460*	
A^2	*4418.15*	*1*	*4418.15*	*34.05*	*0.0021*	
B^2	*0.43*	*1*	*0.43*	*3.321E-003*	*0.9563*	
C^2	*285.39*	*1*	*285.39*	*2.20*	*0.1982*	
Residual	648.86	5	129.77			
Lack of Fit	*62.77*	*3*	*20.92*	*0.071*	*0.9699*	*not significant*
Pure Error	*586.09*	*2*	*293.04*			
Cor Total	25110.74	14				

Figure 1: ANOVA Analysis of Experimental Design for Size (nm)

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, A2 are significant model terms.

Values greater than 0.1000 indicate the model terms are not significant.

If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Lack of Fit F-value" of 0.07 implies the Lack of Fit is not significant relative to the pure error.

There is a 96.99% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Parameters derived from ANOVA analysis of Size

Table 4: Parameters derived from ANOVA analysis of Size

Parameter	Value
Std. Dev.	11.39
Mean	238.41
C.V. %	4.78
PRESS	2323.09
R-Squared	0.9742
Adj R-Squared	0.9276
Pred R-Squared	0.9075
Adeq Precision	12.256

The "Pred R-Squared" of 0.9075 is in reasonable agreement with the "Adj R-Squared" of 0.9276 as one might normally expect. This may indicate a large is a block effect or a possible problem with your model and or data.

Mathematical Model for Size (nm) Actual Equation:

Size= +261.73+48.47 * A+0.43 * B-8.53 * C+0.70 * A * B-5.20 * A * C-9.80 * B * C-34.59* A2-0.34 * B2-8.79 * C2

From mathematical model for particle size, it was found that with the increase in variables A, particle size Increases.

Factor B and C show inverse proportional effect with particle size, as we increase concentration of Factor B and C particle size decreases.

Response surface curve showing combined effect of independent variables on size

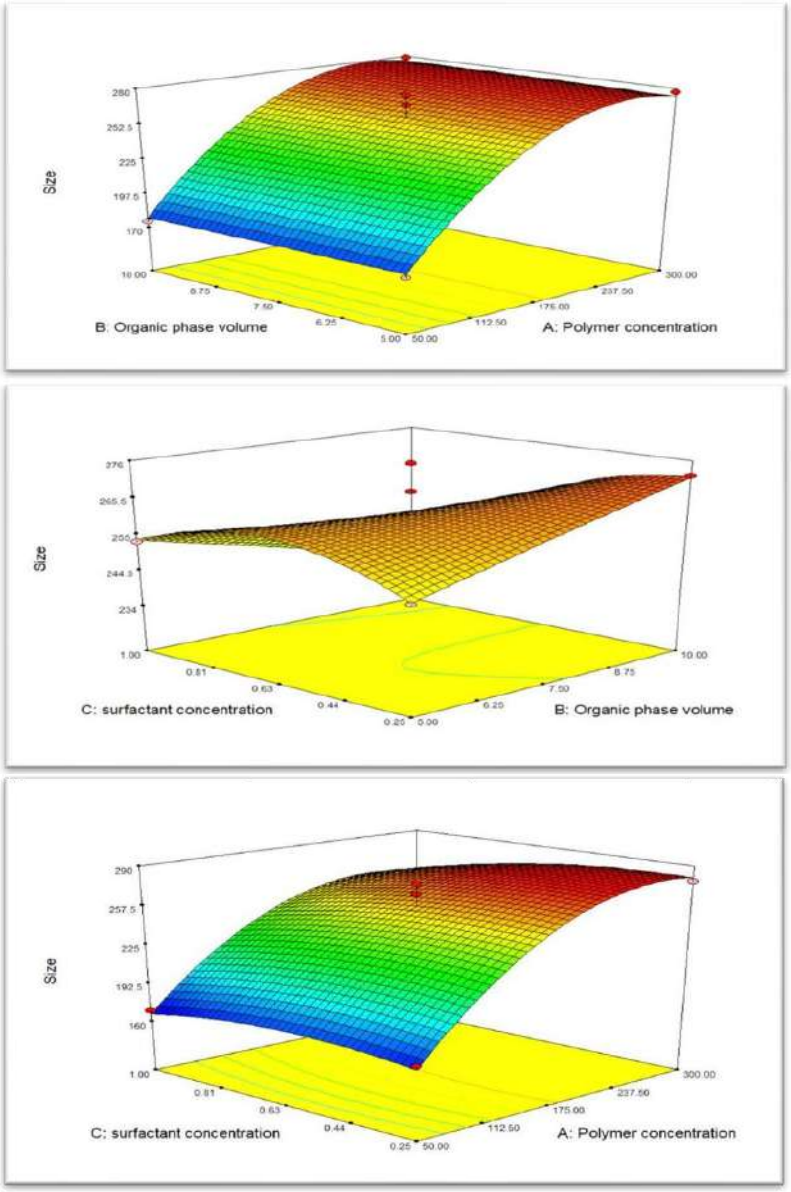

Figure 2: Response surface curve showing combined effect of independent variables on size

ANOVA Analysis of Experimental Design

Response 2 EE

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2290.23	9	254.47	9.30	0.0122	significant
A-Polymer conc	*114.23*	*1*	*114.23*	*4.17*	*0.0965*	
B-Organic phas	*336.05*	*1*	*336.05*	*12.28*	*0.0172*	
C-surfactant co	*38.02*	*1*	*38.02*	*1.39*	*0.2916*	
AB	*34.99*	*1*	*34.99*	*1.28*	*0.3095*	
AC	*13.99*	*1*	*13.99*	*0.51*	*0.5066*	
BC	*91.97*	*1*	*91.97*	*3.36*	*0.1262*	
A^2	*1552.89*	*1*	*1552.89*	*56.74*	*0.0007*	
B^2	*5.47*	*1*	*5.47*	*0.20*	*0.6735*	
C^2	*132.06*	*1*	*132.06*	*4.83*	*0.0794*	
Residual	136.83	5	27.37			
Lack of Fit	*99.83*	*3*	*33.28*	*1.80*	*0.3768*	*not significant*
Pure Error	*37.00*	*2*	*18.50*			
Cor Total	2427.06	14				

Figure 3: ANOVA Analysis of Experimental Design for Entrapment efficiency (%)

The Model F-value of 9.30 implies the model is significant.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case B, A2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), mode reduction may improve your model.

The "Lack of Fit F-value" of 1.80 implies the Lack of Fit is not significant relative to the pure error.

There is a 37.68% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit. n for Entrapment efficiency (%).

Parameters derived from ANOVA analysis of Entrapment efficiency

Table 5: Parameters derived from ANOVA analysis of Entrapment efficiency

Parameter	Value
Std. Dev.	5.23
Mean	36.16
C.V. %	14.47
PRESS	1680.56
R-Squared	0.9436
Adj R-Squared	0.8421
Pred R-Squared	0.3076
Adeq Precision	10.069

The "Pred R-Squared" of 0.3076 is not as close to the "Adj R-Squared" of 0.8421 as one might normally expect.

This may indicate a large block effect or a possible problem with your mode and/or data. Things to consider are model reduction, response tranformation, outliers, etc."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 10.069 indicates an adequate signal. This model can be used to navigate the design space.

Mathematical Model for EE

Actual Equation:

EE= +49.63-3.78

20.51 * A2+1.22* A+6.48 * B+2.18* B2-5.98* C2 * C-2.96 * A * B+1.87 * A * C+4.79 * B * C-

From mathematical model for entrapment Efficiency, it was found that with the increase in variables A and B, entrapment efficiency Increases. But effect of Factor A limited up to certain value after that EE efficiency decreases. Factor can't show significant effect on entrapment Efficiency.

Response surface curve showing combined effect of independent variables on EE (entrapment efficiency)

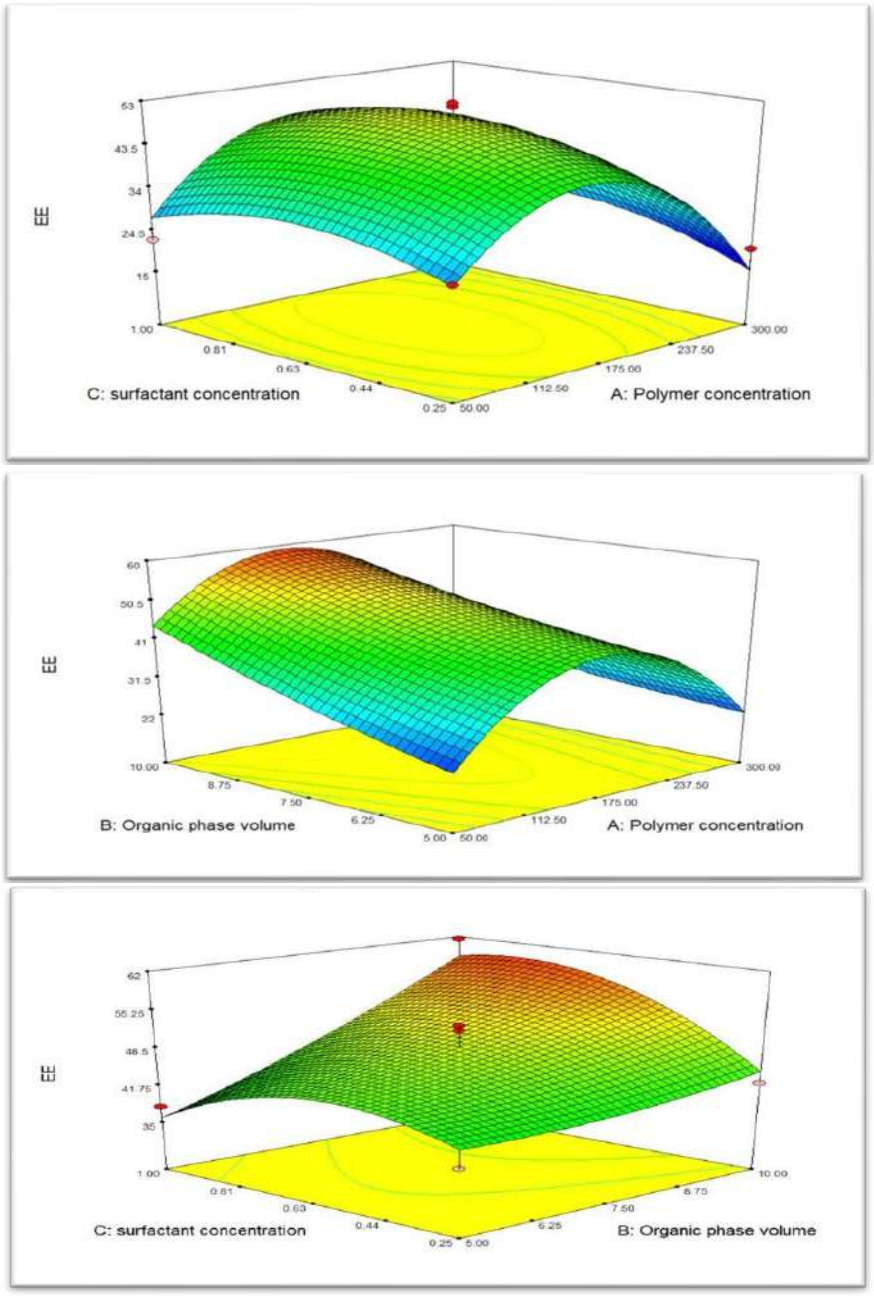

Figure 4: Response surface curve showing combined effect of independent variables on EE (entrapment efficiency)

ANOVA Analysis for Design Space

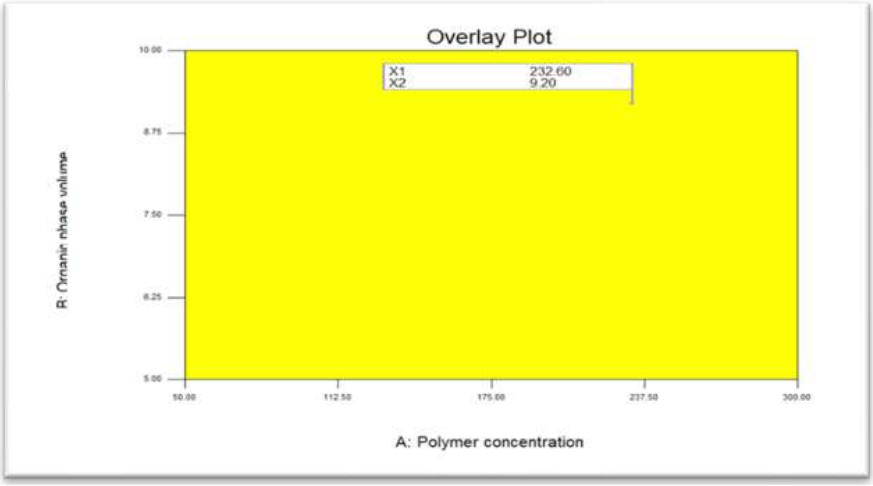

Figure 5: ANOVA Analysis for Design Space

Desirability Study for Optimization

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Polymer concen	is in range	50	300	1	1	3
Organic phase v	is in range	5	10	1	1	3
surfactant conc	is in range	0.25	1	1	1	3

Figure 6: Desirability Study for Optimization

Final optimized formulation of Nanoparticles predicted from BBD/ Desirable Batch Obtained

Table 6: Desirable Batch Obtained

Number	Polymer Concentration (mg)	Organic Phase (ml)	Surfactant Concentration (%)	Desirability	Status
1	232.6	9.2	0.59	1	Selected

The amount of drug (50mg) were constant for each batch.

Conclusion

The present study successfully formulated and optimized Rasagiline Mesylate-loaded PCL nanoparticles using a modified nanoprecipitation method and Box–Behnken experimental design. Statistical evaluation through ANOVA confirmed that the selected factors—polymer concentration, organic phase volume, and surfactant concentration—significantly influenced particle size and entrapment efficiency. The obtained optimized batch (A = 232.6 mg, B = 9.2 ml, C = 0.59%) achieved a perfect desirability index (1.0), indicating the robustness and predictive accuracy of the design model. The optimized nanoparticles demonstrated a favorable particle size range suitable for nasal deposition and an improved entrapment efficiency conducive for sustained release. The intranasal delivery approach offers notable advantages, including bypassing first-pass metabolism, rapid onset,

and direct nose-to-brain transport, which is beneficial for chronic conditions like Parkinson's disease.

Overall, the study establishes Rasagiline-loaded PCL nanoparticles as a promising alternative to conventional oral therapy, potentially enhancing therapeutic efficacy and patient compliance.

References

1. Wirdefeldt K, Adami HO, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson's disease: a review. Neuroepidemiology. 2011;37(1):1–11.
2. Tripathi KD. Essentials of Medical Pharmacology. Jaypee Brothers Medical Publishers; 2019.
3. Olanow CW, Stern MB, Sethi K. The scientific and clinical basis for the treatment of Parkinson disease. Neurology. 2009;72(21 Suppl 4):S1–136.
4. Mittal D, Ali A, Pasha S, et al. Role of oxidative stress in neurodegeneration and therapeutic strategies. Neurobiology of Disease. 2014;65:168–177.
5. Pangeni R, Sharma G, Pathak K. Nose-to-brain drug delivery systems: a promising approach for targeting neurodegenerative disorders. Journal of Pharmacy and Pharmacology. 2014;66(10):1390–1400.
6. Mygind N, Dahl R. Anatomy, physiology and function of the nasal cavities. Allergy. 1998;53(56 Suppl):5–12.
7. Box GEP, Behnken DW. Some new three-level designs for the study of quantitative variables. Technometrics. 1960;2(4):455–475.
8. Reddy Dumpa RK, Kumar R, Akhila M. Design and optimization of intranasal Rasagiline Mesylate gel using statistical models. International Journal of Pharmaceutical Sciences Review and Research. 2025; 72(1):45–52.
9. Hala N, Ibrahim M, El-Sisi A. Intranasal Rasagiline Mesylate-loaded transferosomal in situ gel for improved brain targeting. Drug Delivery. 2023;30(1):1120–1134.
10. Paul A, Das S, Ghosh S. Nanoparticles for therapeutic applications in Parkinson's disease: current and future perspectives. Journal of Controlled Release. 2020; 324:217–232.
11. Barcia E, Boeva L, García-García L, et al. Nanotechnology-based drug delivery of ropinirole for Parkinson's disease. European Journal of Pharmaceutics and Biopharm aceutics. 2017;119:262–271.
12. Aditya N, Khurana N, Sharma G. Intranasal thermosensitive gels of Rasagiline Mesylate: improvement in bioavailability and brain targeting. AAPS PharmSciTech. 2013;14 (3):1073–1082.