ejpmr, 2022,9(10), 536-551

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

FORMULATION AND DESIGN OPTIMIZATION OF NANO ETHOSOMAL GEL FOR DELIVERY OF RASAGILINE MESYLATE: IMPROVEMENT IN BRAIN LOCALIZATION AND BIOAVAILABILITY

Navaneethan S.*, **Somashekhar C.N. and Harshitha V.Y.**

*Department of Pharmaceutics, Bharathi College of Pharmacy, Bharathinagar-571422, Maddur Taluk, Mandya District, Karnataka, India.

***Corresponding Author: Navaneethan S.**

Department of Pharmaceutics, Bharathi College of Pharmacy, Bharathinagar-571422, Maddur Taluk, Mandya District, Karnataka, India.

Article Received on 21/08/2022 **Article Revised on 11/09/2022** **Article Accepted on 01/10/2022**

ABSTRACT

To Improve the developed of nano ethosomes containing Rasagiline mesylate for effective treatment of Parkinson's disease. Nano ethosomes were prepared by the cold method. D- for applied optimal design for formulation optimization. Ethanol, propylene glycol, and phospholipids were selected as independent variables, while encapsulation efficiency (EE) of the nano ethosomes was a dependent variable. In the optimum formulation of RM ethosomes, in which ethanol (30.0%), propylene glycol (20.0%), and phospholipids (2.0%) have a higher EE of 83.14% with spherical bilayered structure revealed from SEM analysis, the average particle size range of 153.7 nm and zeta potential values obtained as -31.4mV. Further, the nano ethosomes formulations showed controlled release of drug for 12hours. After fitting in-vitro drug release data into various kinetic models, to found the release to follow the first-order model. These results confirmed that nano ethosomes containing Rasagiline mesylate have the potential for the treatment of Parkinson's disease.

KEYWORDS: Nano ethosomes, Parkinson's disease, Rasagiline mesylate, D- optimal design, Entrapment efficiency.

INTRODUCTION

Parkinson's disease is a chronic neurodegenerative disorder in which loss of dopamine neurons leads to deterioration of motor and non-motor functions, affecting the quality of life. Motor symptoms associated with Parkinson's disease are tremors, stiffness, bradykinesia, impaired balance, and a shuffling walk. In the later stage, non-motor symptoms such as dementia, anxiety, and depression were also noticed.[1] Dr. James Parkinson described Parkinson's disease in 1817 as shaking palsy, named after him. Parkinson's disease is a chronic degenerative disorder in which, with time, degeneration of both motor functions such as immobility of muscles and non-motor functions such as memory gets affected due to which living quality of the patient, its family members, and its caretaker get compromised.[2]

Parkinson's disease has been recognized as the second most common neurodegenerative disorder and most common movement-related disorder worldwide. Initially, Parkinson's disease was understood as just a movement disorder. However, after so many years of the progression of the disease, it was found that it is not just a movement disorder. However, other symptoms such as postural instability and memory loss were also associated.[3] Only 10 % of Parkinson's disease cases are found to have a genetic basis; the rest, 90%, are sporadic.[4] The foremost pathological characteristic of Parkinson's disease is an accumulation of protein α-synuclein in Lewy bodies.[5] The exact reason for the occurrence of Parkinson's disease is still unclear; however, certain risk factors such as age, brain injury/infection, exposure to toxins/pesticides, and genetic reasons are the cause of Parkinson's disease.[6]

The main risk factors for Parkinson's disease are elevated cholesterol level, head injury, high calories intake, exposure to environmental toxins such as carbon disulfide, cyanides, herbicides, pesticides, some organic solvents, oxidative stress, mitochondrial dysfunction, and nitric oxide toxicity.[7,8,9]

Rasagiline mesylate is an irreversible selective monoamine oxidase inhibitor with neuroprotective effects by up-regulation of protein kinase C (PKC) alpha, PKCepsilon, the anti-apoptotic Bcl-2, Bcl-xL, and Bcl-w and induction of neurotrophic factors which is mainly used for the dealing with Parkinson's disease. In general, monoamine oxidase inhibitors prevent the oxidative deamination of monoamines. After chronic administration, Rasagiline mesylate has shown neurorescue/neurogenesis action in mice dopaminergic

neurons. This was supposed to occur because of activation of specific cell signaling mediators related to neurotrophic factors responsive-tyrosine kinase receptor (Trk) pathway and Akt/PKB. The Propargylamine group in the drug's structure augments the neurotrophic factors' expression. MAO, a flavin-containing enzyme, is a mitochondrial enzyme that can be stratified into molecular species A and B and is mainly confined to mitochondrial membranes in the nerve extensions, brain, liver, and intestinal mucosa. Both MAO differs in substrate specificity and their localization, i.e., the area where these are majorly located. MAO-A maintains the metabolism of catecholamines and serotonin in the secondary tissues and is majorly found in the gastrointestinal tract and liver. MAO-B is the chief variety in the individual brain. In ex vivo studies in various tissues, rasagiline mesylate was revealed to be an effective, irreversible monoamine oxidase B (MAO-B) inhibitor.

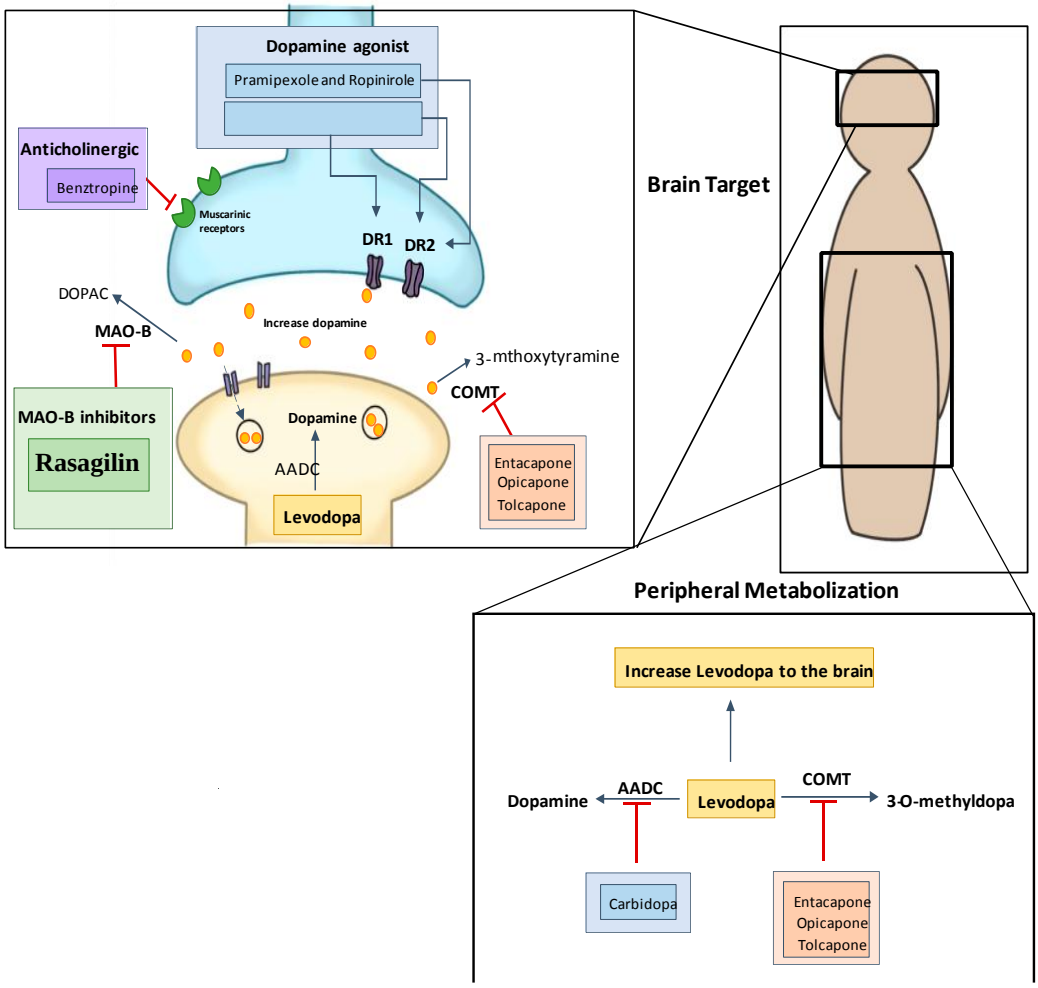


Figure 3.1.2: Mechanism of action and sites of action of Rasagiline mesylate.

Transdermal delivery is a convenient, painless, and non-invasive approach that enables the self-administration of drugs by the patients. It Enables bypass of the first-pass effect, maintains constant drug levels over a prolonged period, and reduces gastrointestinal-related side effects.

Nowadays, the transdermal route is viewed with oral treatment as the most successful innovative research area in drug delivery. "Transdermal delivery is an important delivery route that delivers precise amount of drug through the skin for systemic action. Improved methods of drug delivery for biopharmaceuticals are important for two reasons; these drugs represent rapidly growing portion of new therapeutics, and are most often given by injection. Discovery of new medicinal agents and related innovation in drug delivery system have not been only enabled the successful implementation of novel pharmaceutical, but also permitted the development of new medical treatment with existing drugs."

Nano Ethosomes are a modified form liposome, which has proved to be suitable carriers in the transdermal area. Nano Ethosomes are mainly lipid vesicles made of phospholipids, ethanol, and water. Nano Ethosomes have an aqueous core that contains the ethanolic solution of the drug, and the outer layer comprises the lipid bilayer (Fig. 1). The effect of ethanol fluidizing the bilayers of phospholipids.

It contributes to creating blisters with a malleable structure that enables to attain molecules (drugs, pharmaceuticals, or active agents) to deeper layers of the skin. Due to its unstable nature and low permeability, the medicine delivery from liposomes has limited results in the transdermal formulations. Due to the concern owing to the stability of liposomes, a new vesicular carrier, niosomes, was developed to fight the problems of low stability. Despite that, liposomes and niosomes couldn't resist the problems of poor skin permeability. Hence, ethanolic vesicles have been developed to enhance the saturation of medicines across the skin. The size range of Nano Ethosomes ranges from tens of nanometres, the transdermal flux of Nano Ethosomes is more, and their skin permeability.[46]

MATERIALS AND METHODS

Rasagiline Mesylate was a gift sample from Bangalore. 1.561 mg of Rasagiline mesylate is equivalent to 1 mg Rasagiline. Each 1gm contain 1mg of Rasagiline. Therefore, the drug to be incorporated was calculated such that each 1gm of gel contains 1.561 mg of Rasagiline mesylate. Soya lecithin was procured from Sigma Aldrich, Bangalore. Cholesterol Central Drug House (P) Ltd Delhi purchased ethanol, Propylene glycol, Potassium dihydrogen orthophosphate, and Sodium hydroxide pellets from S.D. Fine chemicals, Mumbai. All the chemicals used in the preparations are in analytical grade.[15,16]

METHOD OF PREPARATION

The nano ethosomal formulation was prepared according to the method reported by Touitou et al. The nano ethosomes system designed here comprised 2-4% phospholipids, 20-40% ethanol, drug (Rasagiline mesylate), 5-20%propylene glycol, and water to 100% w/w. First, phospholipids and drugs were dissolved in an ethanol propylene glycol mixture. Next, the mixture was heated to 30^0C in a water bath. The double-distilled water heated to 30^0C was added slowly with constant mixing at 700 rpm in a closed vessel. Mixing was continued for an additional 15mins to keep the system at 30^0C throughout the preparation. Next, the formulation was sonicated at 40^0C using a bath sonicator for 20 mins. Then again, preparation was sonicated at 40^0C using a probe sonicator in 3 cycles of 5min with 5min rest between cycles at 40W.[17,18,19]

PREFORMULATION STUDIES[20-22]

a) **Identification of drug:** The identification of Rasagiline mesylate was done by UV, DSC, and FTIR and confirmed as per monographs.

b) **Solubility analysis:** Solubility analysis of Rasagiline mesylate was carried out in various solvents and Phosphate buffer. As a result, 10 mg of Rasagiline mesylate was dissolved in, i.e., water, ethanol, methanol, acetone, chloroform, phosphate buffer, pH 1.2, pH 4.4, pH 6.8, pH 7.4 isopropanol, propylene glycol, PEG 400.

c) **Melting point determination:** The capillary method has used the determination of the melting point of Rasagiline mesylate. A little amount of compound was placed in a thin-walled capillary tube of about 10-15 cm long and 1 mm inside diameter and closed at one end. The capillary containing the sample and a thermometer is then suspended in an oil bath containing liquid paraffin. So, they can be heated slowly and evenly. The temperature range over which the sample is observed to melt is taken as the melting point.

d) **Drug-excipients interactions studies by Fourier Transform Infrared (FTIR):** The FT-IR spectrum of Rasagiline mesylate has been recorded with a Fourier-Transform IR spectrophotometer within the range 4000-400 cm-1 by utilizing the method of KBr pellet. This method took a small amount of drug (approx. 1mg) in a mortar, and KBr was added to it in the ratio of 1:10, followed by trituration with a pestle. Then the mixture was put in a dye cavity and pressed with KBr press under a pressure of 4-5 tons, leading to a thin film formation. This film was placed in the sample compartment, and the FT-IR was performed in the mentioned range to obtain the spectrum of the drug and the physical mixture of the Excipients.

e) **Thermal analysis by DSC:** DSC was employed in a thermal analytical process to get the thermogram Rasagiline mesylate by using aluminium pans. In this technique, an accurate weight of the sample was taken in the aluminium pans and sealed tightly. To determine the thermal behavior of the drug, the aluminium pans containing the sample were heated at a temperature range of 40-300^0C, with a scanning rate of 20^0C/min. Nitrogen gas was purged continuously at a 40ml/min flow rate to provide an inert atmosphere.

f) DETERMINATION OF λ MAX[23-24]

UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF RASAGILINE MESYLATE

A precise, sensitive and accurate method for estimating Rasagiline mesylate was done using UV visible spectrophotometer Procedure for UV spectroscopic method.

PREPARATION OF PHOSPHATE BUFFER pH 7.4

To prepare phosphate buffer ph-7.4, 50ml of Potassium dihydrogen orthophosphate and 22.4ml of Sodium hydroxide make up to 200 ml of distill water.

DETERMINATION OF ΛMAX IN PHOSPHATE BUFFER (PH 7.4)

To determine the absorption maxima (ג-max) for Rasagiline mesylate in the buffer by scanning the drug solution in the range of 200 nm to 400 nm using a UV visible spectrophotometer. The drug exhibits a ג-max at 271nm shown in fig no.

PREPARATION OF STANDARD CALIBRATION CURVE OF RASAGILINE MESYLATE IN PHOSPHATE BUFFER (PH 7.4)

- 100 mg of accurately weighed Rasagiline mesylate was dissolved and made up to 100ml with pH 7.4 buffer solution.
- From the above solution, 50, 100, 150, 200, and 250 µg/ml were prepared and analyzed by UV spectrophotometer at λmax 271nm.
- Then plotted, the graph of absorbance v/s concentration in µg/ml and to calculated the r2 value of this graph.

g) Experimental design: Central composite design was inculcated to get different experimental runs using design expert software version 13 which analyzes main effects, certain interactions and also helps to identify the significant factors from those which are not important. The independent variables selected were soya lecithin concentration, Ethanol concentration and Propylene Glycol concentration. The factors and levels are mentioned in the table no:4.3. the lower and higher levels of independent factors were selected based on the reported literature and initial screening experiments conducted.

Table 1: Selected independent variables for optimization.

SL. NO	Independent Variables	Low Level	Intermediate Level	High Level
1	Soya lecithin	2	-	4
2	Ethanol	20	30	40
3	Propylene Glycol	5	10	20

h) EVALUATION OF NANO ETHOSOMES[25-28]

1. **PARTICLE SIZE ANALYSIS:** To quantify the mean or average size of the particle and zeta potential by using the quasi-elastic light scattering method by using a Zeta sizer or particle size analyzer (Malvern instrument ZA-90) at a scattering angle of 90º. To estimate those parameters, dilutions of the sample were done with milli-Q water in a test tube. Using a disposable cuvette, the diluted samples were measured in triplicate at 25±1ºC. For zeta potential, a zeta cell was used.
2. **SCANNING ELECTRON MICROSCOPY (SEM):** To determine the surface morphology of the specimen by using a scanning electron microscope. The sample is dried thoroughly in a Vaccum desiccator before mounting on brass specimen studies, using double-sided adhesive tape. Gold palladium alloy of 120°A Knees was coated on the sample sputter coating unit (Microtrac ltd) in Argon at an ambient of 8-10°C with a plasma voltage of about 20mV. The sputtering was done for nearly 5 minutes to obtain a uniform coating on the sample to enable good quality SEM images.
3. **DRUG ENTRAPMENT EFFICIENCY:** The prepared Nano Ethosomal dispersion was centrifuged at 6000 rpm for 30 min at 40 C using a REMI cooling centrifuge. The supernatant is analyzed for the free drug content. To calculate the drug's entrapment efficiency (%) by using the following equation.

$$\%\text{Entrapment efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100$$

4. ***In-vitro* diffusion study:** In the in-vitro diffusion study, the diffusion medium used was phosphate buffer pH 7.4. Assembly of diffusion cell for in-vitro diffusion studies the diffusion cell was designed as per the dimension given. A diffusion cell with an effective diffusion area of 3.14 cm^2 was used for in-vitro permeation studies. The egg membrane was mounted on the cell carefully to avoid the entrapment of air bubbles under the egg membrane. Ensure intimate contact of egg membrane with receptor fluid by placing it tightly with a clamp. The speed of the stirring was kept constant throughout the experiment. The donor compartment consists of 1 ml of nano Ethosomes containing Rasagiline mesylate. Then, the receptor compartment consisted of 100 ml of phosphate buffer of pH 7.4 in a 250 ml beaker. And placed the beaker over the magnetic stirrer and maintained the temperature and rpm at 34+/- 0.5^0 C and 100 rpm throughout the study. The sample of 3 ml was withdrawn at a predetermined interval of time (1, 2, 3, 4, 5, 6, 7, 8,9,10,11 and 12 hr) and replaced with an equal amount of fresh buffer. After the suitable dilution of the sample and absorption under UV spectroscopy.

5. **Drug release kinetics[29-30]**

Various equations were used to describe the drug's release kinetic process in all formulations, such as the zero-order rate equation, which represents the system where the release rate is independent of the concentration of the dissolved species. The first-order equation describes the release from the scenarios where the dissolution rate depends on the concentration of dissolving species. The Higuchi equation describes the departure from the system where the solid drug is dispersed in an insoluble matrix, and the drug release rate is related to the diffusion rate. The Korsmeyer-Peppas equation is used to analyze whether the release mechanism is Fickian diffusion or non-Fickian diffusion. 'n' value can be used to characterize different release mechanisms.

I. Zero-order kinetics: In pharmaceutical dosage forms, following this profile release the same amount of drug by a unit of time, and it is the ideal drug release method to achieve a prolonged

pharmacological action. The following relation can, in a simple way, express this model

$$Qt = (QoC + Kot)$$

Where,
Qt = the amount of drug dissolved in time t.
Qo = the initial amount of drug in the solution (most times, Qo=0) and
Ko = the zero-order release constant.

II. First-order kinetics: The release rate data were fitted to the following equation to study the first-order release rate kinetics.

$$LogQt = logQo + Kt/(2.303)$$

Where,
Qt = amount of drug dissolved in time t.
Qo = the initial amount of drug in the solution and
K =the zero-order release constant.

In this way, a graphic of the decimal logarithm of the released amount of drug versus time will be linear. The pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in porous matrices, release a drug in a way that is proportional to the amount of drug remaining in its interior, in such a way that the amount of drug released by a unit of time diminishes.

III. Higuchi model: Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs in semi-solid and solid matrices. The mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. The simplified Higuchi model is expressed as:

$$Qt = KH.t\ ½$$

Where,
Qt = Amount of drug released in time t and,
KH = Higuchi dissolution constant.

IV. Korsmeyer-Peppas model: This model is used to simplify the empirical equation to describe general solute release behavior from controlled-release polymer matrices:

$$Mt/M\infty = K.tn$$

Where,
Mt/ M∞ = Fraction release, of drug
t = Drug release time and
n = Diffusional exponent for the drug release that is dependenton the shape of the matrix dosage form.

The results obtained from in-vitro drug release studies were plotted adopting four different mathematical models of the data treatment as follows:

- % Cumulative Drug Release v/s Time (Zero-order rate kinetics).
- Log % Cumulative Drug Retained v/s Time (First-order rate kinetics).
- % Cumulative Drug release was plotted against T (root time). (Higuchi model)
- Log % Cumulative Drug Release v/s Log Time (Peppas exponential equation).

f) STABILITY STUDIES[30]

The stability of a drug has been defined as the stability of a particular formulation in a specific container to remain within its physical, chemical, therapeutic, and toxicological specifications throughout its shelf life.

PROCEDURE

All the formulations were packed in the Glass vessel, wrapped with aluminum foil, and kept at 5º C ± 3ºC, 30º C ± 2ºC (65±5%RH) and 40º± 2ºC/75% RH for 30, 60 days in a stability chamber and evaluated for their drug entrapment efficiency and particle size.

RESULTS and DISCUSSION

a. Solubility analysis

Table no 3: Solubility analysis of Rasagiline mesylate.

S. no.	Solvents	Solubility (mg/ml)
1	Water	58.46±0.16
2	pH 7.4 PBS	66.58±0.27
3	Ethanol	72.57±0.17
4	pH 6.8	93.14±0.27
5	pH 4.5	118.27±0.38
6	Methanol	306.26±0.75

b. DETERMINATION OF Λ MAX STANDARD CURVE OF RASAGILINE MESYLATE IN PHOSPHATE BUFFER (pH 7.4)

The calibration curve of Rasagiline mesylate in Phosphate buffer pH 7.4 at 271 nm is shown in table 4. Figure 4 shows the standard curve with a regression value of 0.9987 and a slope of 0.0036 in Phosphate buffer pH 7.4. The curve is linear in the concentration range from 50 to 250µg/ml.

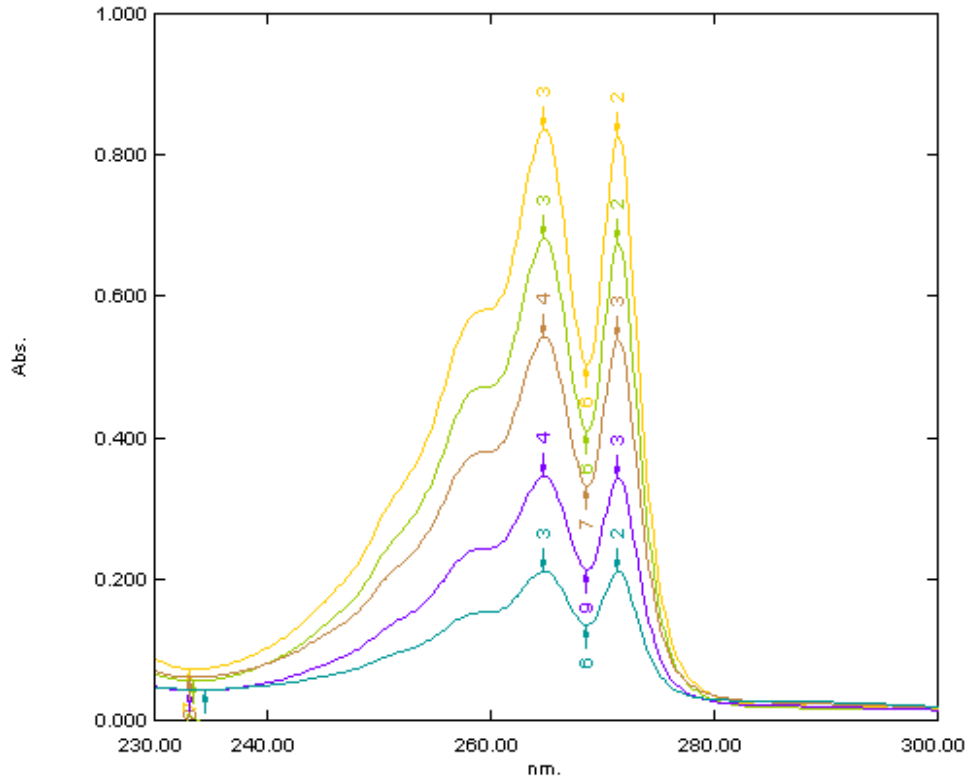
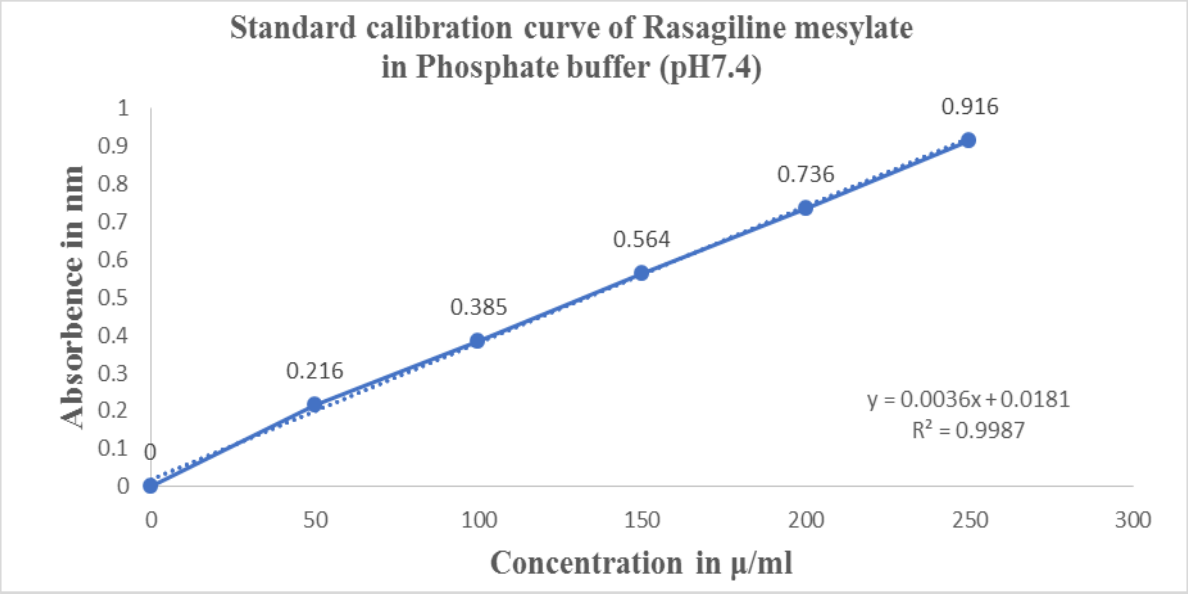


Fig 3: UV Spectrum of Rasagiline mesylate in phosphate buffer (pH7.4).



Graph 1: Plot of standard calibration curve of rasagiline mesylate.

Table no 4: Spectrometric data for the estimation of rasagiline mesylate in 7.4ph buffer

Sl. No	Conc (µg/ml)	Absorbance			Standard deviation (SD)
		Trial 1	Trial 2	Trial 3	
1	0	0	0	0	0
2	50	0.218	0.224	0.208	0.216±0.008
3	100	0.384	0.395	0.376	0.385±0.0095
4	150	0.563	0.574	0.556	0.564±0.009
5	200	0.737	0.751	0.722	0.736±0.014
6	250	0.916	0.926	0.906	0.916±0.01

c. Compatibility studies by FTIR

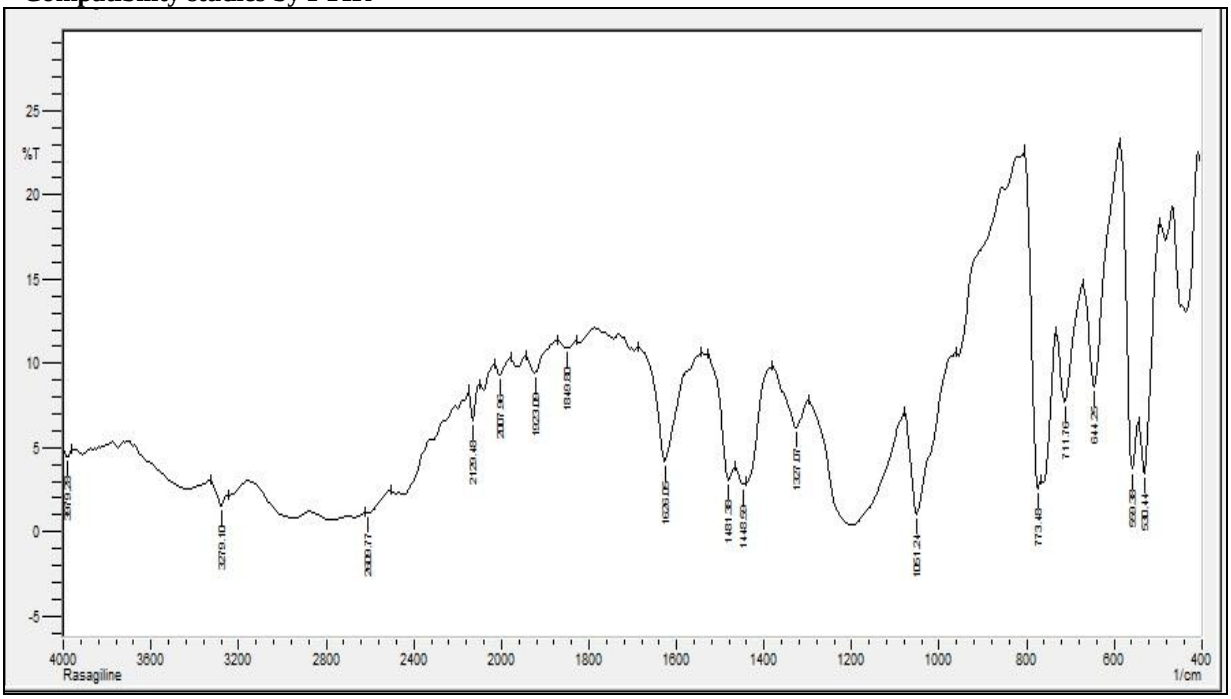


Figure 4: FT-IR spectra of drug of rasagiline mesylate.

Functional group	Wave number (cm^{-1}) of pure drug	Wave number (cm^{-1}) RM + soya lecithin	Wave number (cm^{-1}) RM +Cholesterol	Wave number (cm^{-1}) RM+Carbopol 934
O-H (Stretching)	3279.10	3454.32	3279.10	3223.56
N-H (Bending)	1626.06	1620.26	1626.05	1627.97
C-H Ar (Stretching)	3058.14	3095.27	3219.30	3062.17
C=O (Stretching)	-	1713.12	-	1720.56
C-H(Bending)	1327.07	1482.09	1477.52	1444.73
C ≡ C (Stretching)	2129.48	2099.09	2125.63	2125.63
S=O (Bending)	1051.21	1032.21	1051.24	1047.38
CH Alkane (Stretching)	2980.24	2974.14	2985.10	2936.76

d. PREPARATION OF RASAGILINE MESYLATE LOADED NANO ETHOSOMES

Table 5: The main compositions and physical characteristics of nano ethosome.

No.	Composition			Vesicle size (nm)	Entrapment efficiency (%)
	Ethanol(%v/v)	Soya lecithin(%w/v)	Propylene Glycol (%v/v)		
EF-1	2	20	10	286.0	66.48
EF-2	2	30	10	281.4	69.07
EF-3	2	40	10	324.1	60.55
EF-4	2	30	5	228.1	71.11
EF-5	2	30	10	220.9	76.57
EF-6	2	30	20	153.7	83.14
EF-7	4	20	10	224.4	73.05
EF-8	4	30	10	106.2	91.11
EF-9	4	40	10	360.2	57.96
EF-10	4	30	5	197.9	79.81
EF-11	4	30	10	206.9	78.42
EF-12	4	30	20	127.7	85.92

Each data represents the mean±S.D. (n = 3).

❖ **Response 1: particle size**

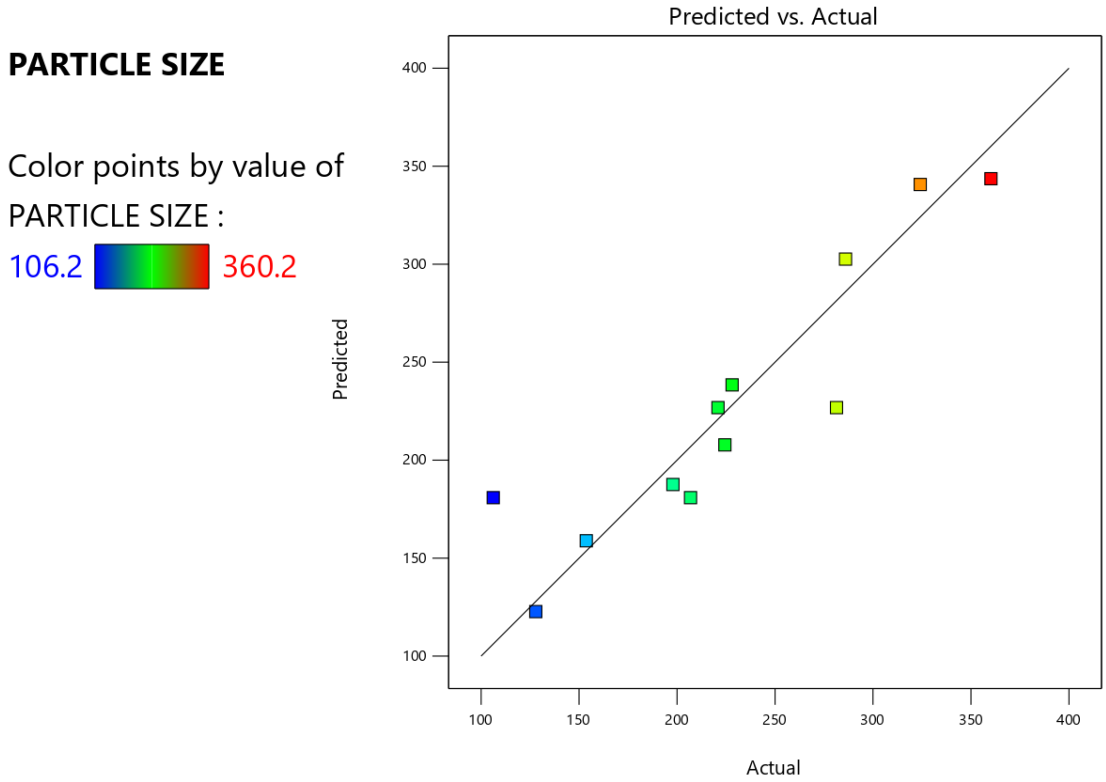


Figure 5.10: predicted v/s actual correlation of particle size.

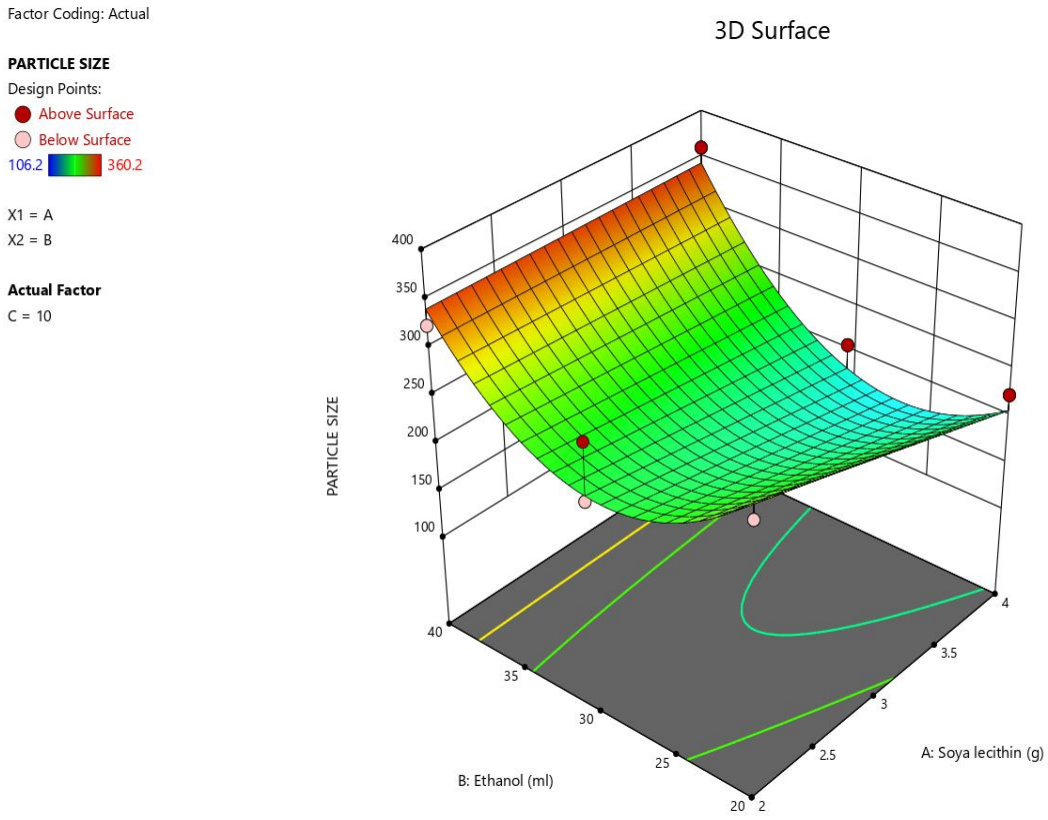


Figure -5.11: 3D response graph of ethanol and lecithin against particle size.

❖ **Response 2: Entrapment efficiency**

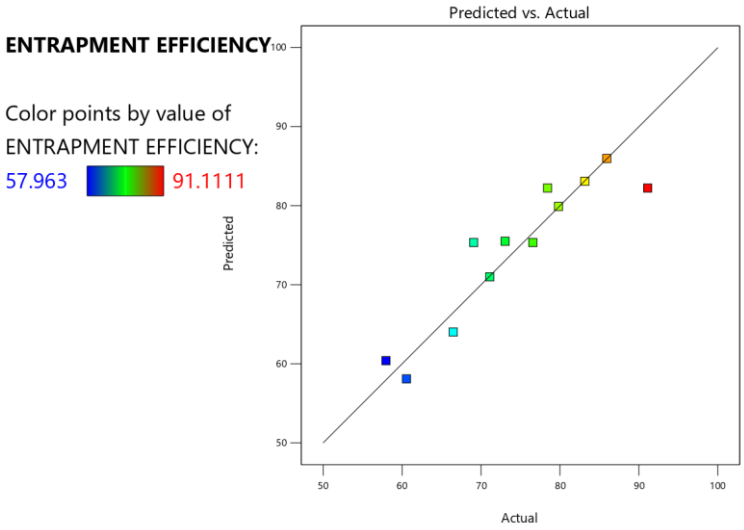


Figure 5.12: predicted v/s actual correlation of entrapment efficiency.

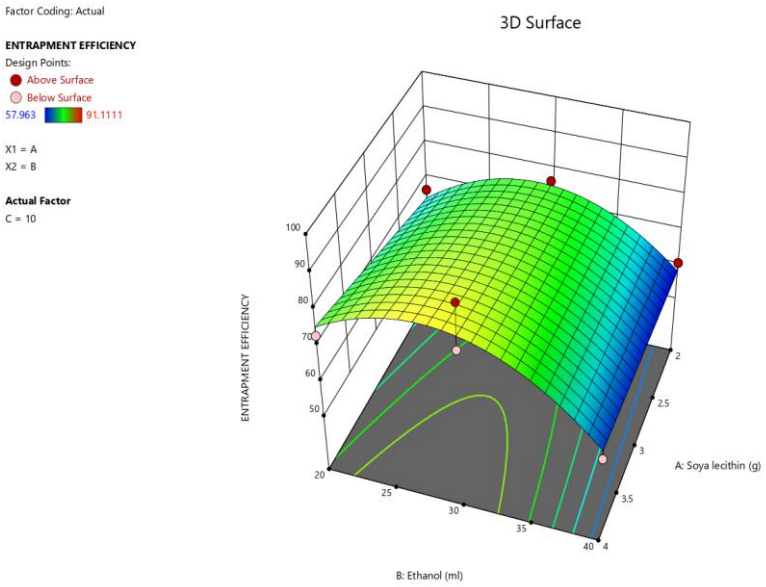


Figure 5.13: 3D response curve of ethanol and lecithin against entrapment efficiency.

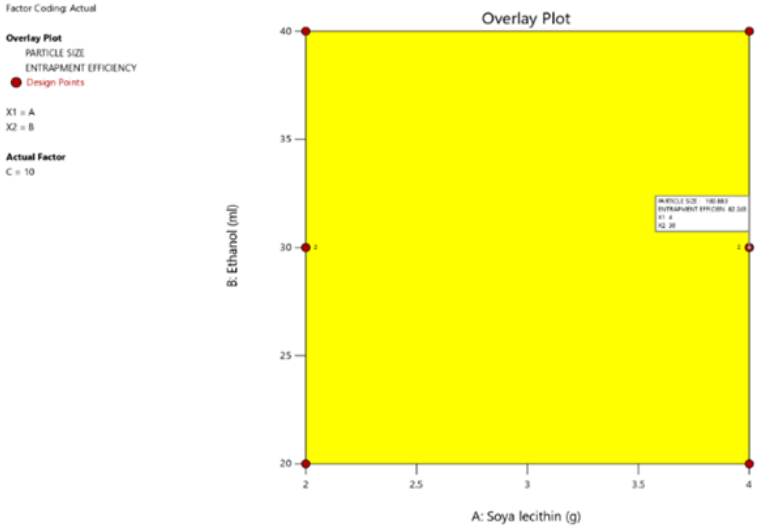


Figure 5.14: Overlay plot for optimized Formulation.

Table 5.6: Check point analysis of optimized RM-nano ethosomes (EF 8).

Formulation EF8	Soya lecithin	Ethanol	Propylene Glycol	Particle size (nm)	Entrapment efficiency (%)	Desirability
Predicted	4	29.663	10	128.7	87.481	0.874
Observed	4	30	10	106.2	91.111	
Relative error	0	0..337	0	22.7	3.63	

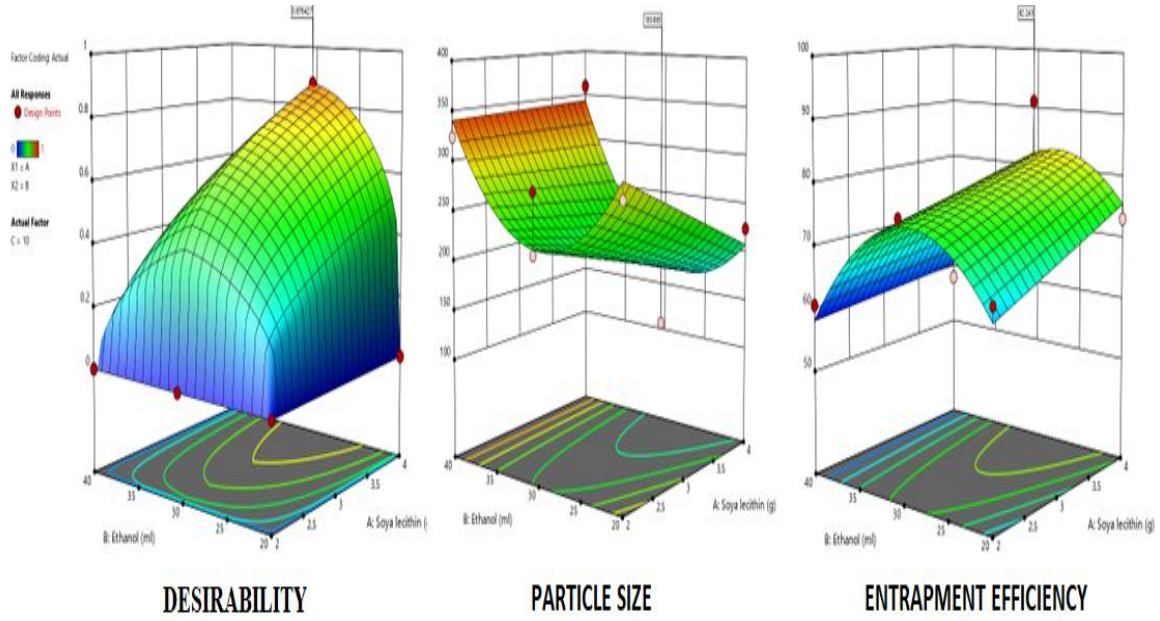


Figure 5.15: Desirability plot for RM-nano ethosomes.

i. PARTICLE SIZE AND ZETA POTANCIAL

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	153.7	Peak 1:	184.5	100.0	79.24
PdI:	0.155	Peak 2:	0.000	0.0	0.000
Intercept:	0.899	Peak 3:	0.000	0.0	0.000

Result quality : Good

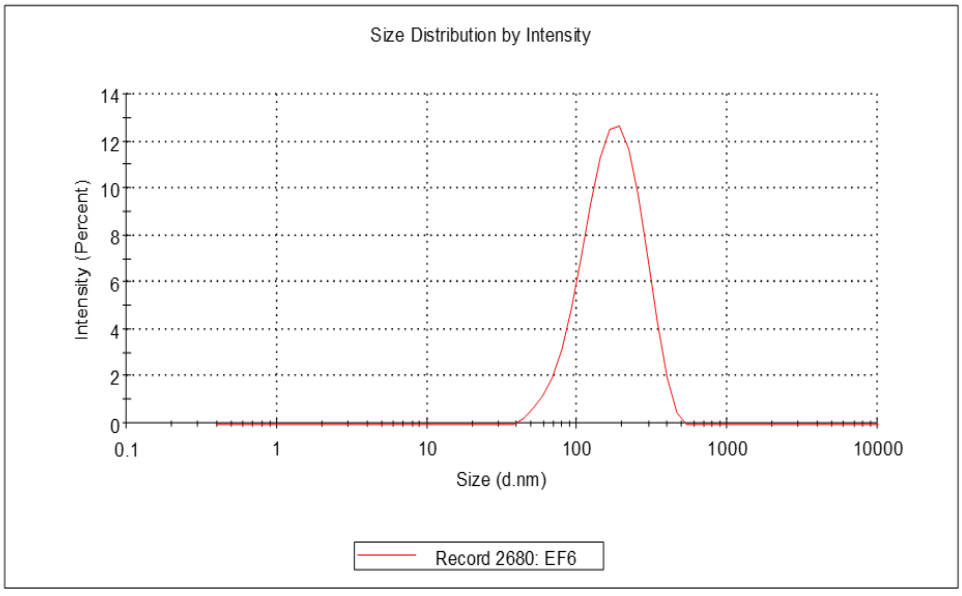


Figure 5: Particle size analysis of EF6.

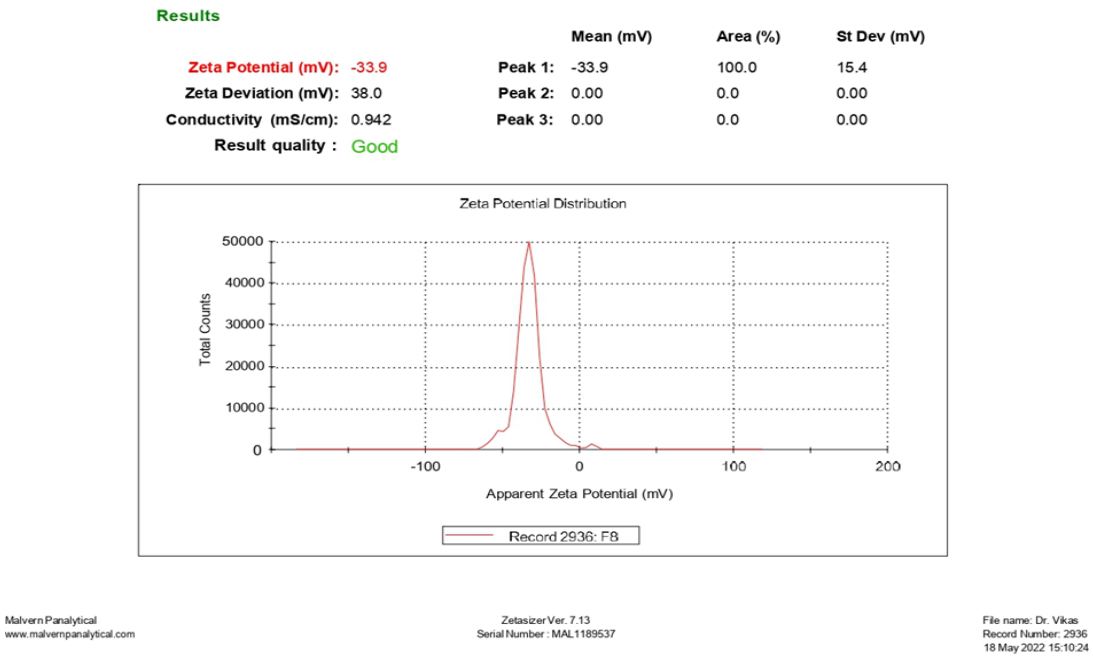


Figure 6: Zeta Potential analysis of EF6.

ii. SCANNING ELECTRON MICROSCOPY

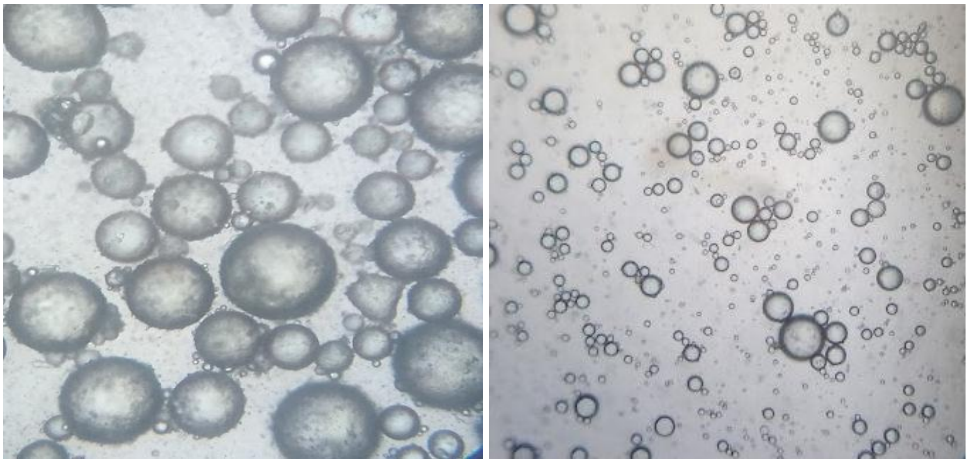


Figure 5.22: Microscopic image of RM-EF8 Before Sonication.

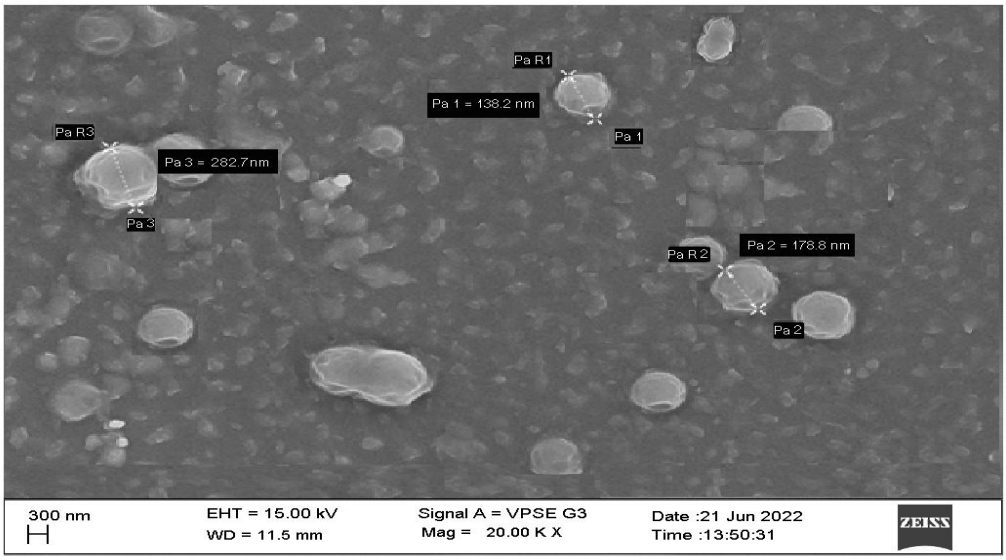


Figure 5.23: SEM image of RM-EF8.

iii. *In-vitro* diffusion study

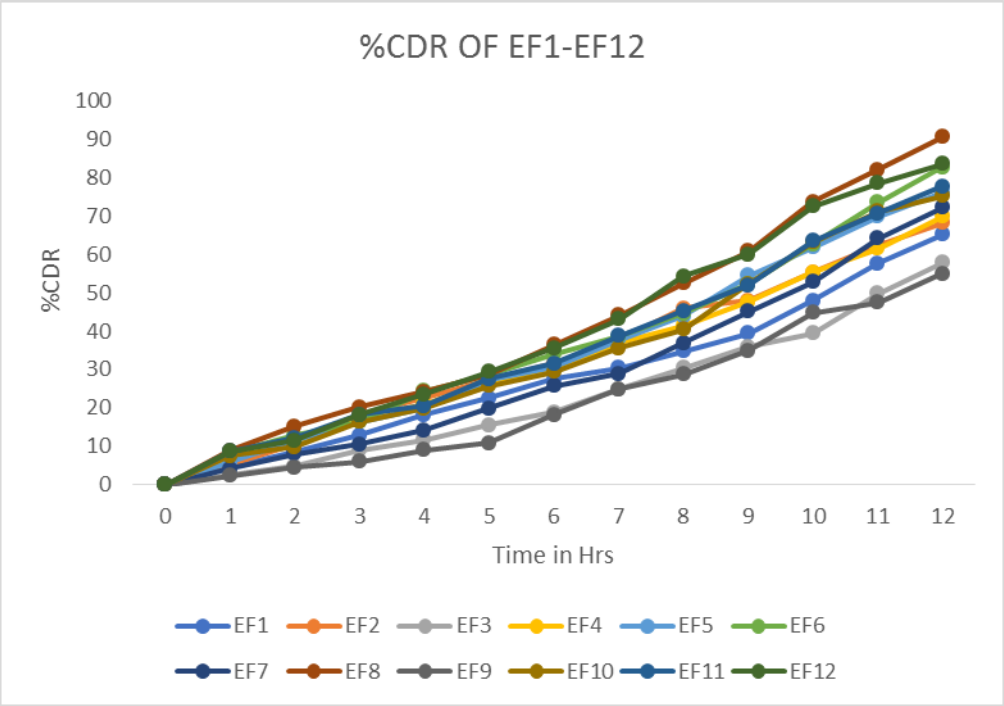


Fig no 6: Percentage cumulative drug release (%) F1-F6 nano ethosomal formulation.

DRUG KINETIC STUDY

Table no 7: Drug Kinetic Study For Ef1-Ef12 Formulation.

Formulation code	%CDR	Zero order	First order	Higuchi	Peppas	n values
EF-1	65.185	0.9784	0.9154	0.8629	0.9113	1.2963
EF-2	68.055	0.9969	0.964	0.9138	0.8904	1.3097
EF-3	57.777	0.966	0.9115	0.8196	0.9715	1.3966
EF-4	69.814	0.9932	0.9415	0.8971	0.8639	1.2539
EF-5	76.018	0.9873	0.9192	0.8719	0.8895	1.3247
EF-6	82.592	0.9784	0.8633	0.8643	0.8456	1.2669
EF-7	72.129	0.9627	0.8783	0.8147	0.9332	1.3722
EF-8	90.555	0.9813	0.8383	0.8639	0.8397	1.2948
EF-9	54.90	0.9572	0.8383	0.7971	0.9694	1.4339
EF-10	75.185	0.9742	0.8985	0.8501	0.8672	1.286
EF-11	77.592	0.9837	0.9059	0.8713	0.8404	1.2523
EF-12	83.518	0.9887	0.9096	0.8774	0.8576	1.3158

5.4.7 Stability studies

Table 5.23: Intermediate stability studies after three months of Storage at 5º± 3ºC, 30º ± 2º C/65% RH and 40º± 2ºC/75% RH.

Parameter	Duration in months		
	EF8		
	At 5^0C±3^0C for three months	At 30º ± 2º C/65% RH for three months	At 40º± 2ºC/75% RH for three months
Particle size(nm)	112.4	124.1	154.8
Entrapment Efficiency (%)	88.28	84.62	79.17
% CDR	86.56	82.24	76.39

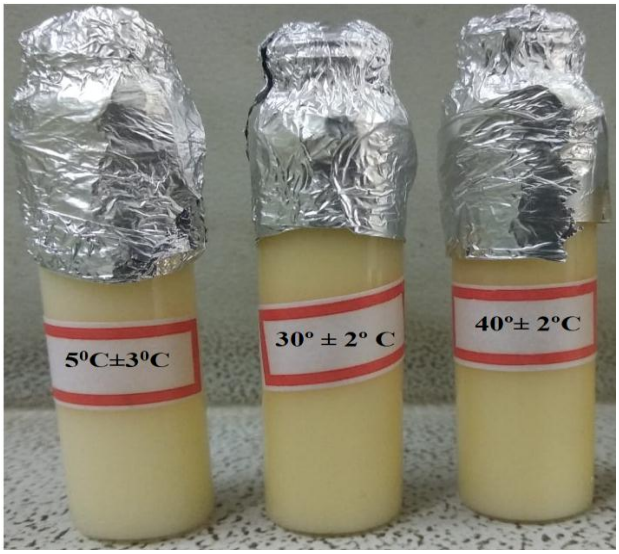


Figure 5.40: Stability studies of Optimized formulation after three months of Storage at 5°± 3°C, 30° ± 2° C/65% RH and 40°± 2°C/75% RH.

Evaluation of RM - Loaded nano Ethosomal Gel

The optimized nano ethosomes EF-6 G1, EF-8 G2, and EF-12 G3 were formulated into a gel using Carbopol 934. the 2% w/w Carbopol was found suitable for gelling the Nano ethosomes because of their desired consistency.

5.5.1 Spreadability

Table 5.25: Spreadability of nano ethosomal gel.

Formulation code	Mass (gm)	Radius (cm)	Time (sec)	Spreadability
EF-6 G1	500	2.5	60	20.83 ± 0.13
EF-8 G2	500	2.9	60	24.16 ± 008
EF-12 G3	500	2.6	60	21.66 ± 0.04

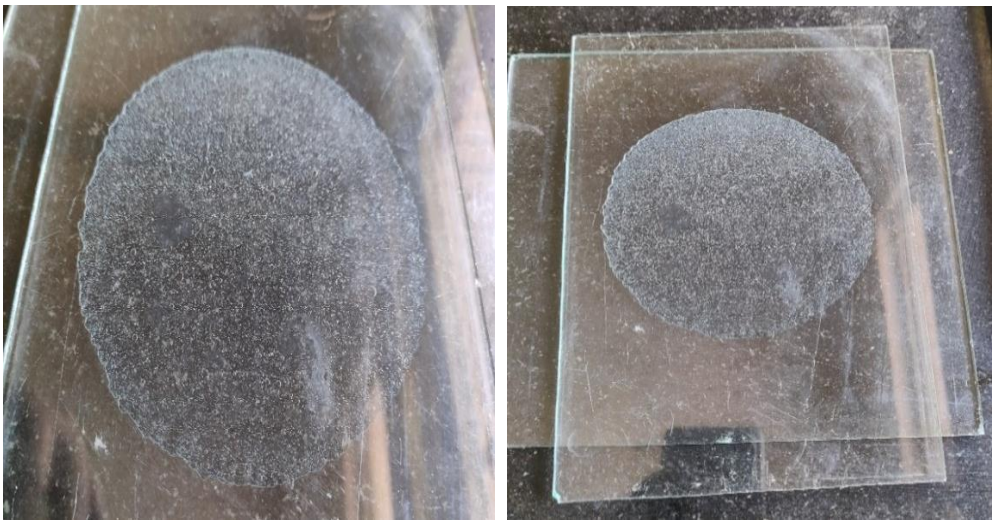


Figure 5.41: this shows the wide spread of the nano Ethosomal gel which determines the spreadability with the viscosity of the gel.

5.5.2. Determination of pH and Drug content

Table 5.25: pH and Drug content of nano ethosomal gel

Formulation code	pH	% Drug content
EF-6 G1	7.08±0.024	89.166
EF-8 G2	7.30±0.042	96.667
EF-12 G3	6.81±0.002	92.778

5.5.3 Viscosity

Table 5.25 Viscosity of nano ethosomal gel

Formulation code	Viscosity (cP)	Speed (rpm)	% Torque	Model	Temp°c	Spindle
EF-6 G1	1868.16	10	20.1	LV	27.0	T-F
EF-8 G2	2122.41	10	32.2	LV	27.0	T-F
EF-12 G3	2016.45	10	27.6	LV	27.0	T-F

***In-vitro* drug release study**

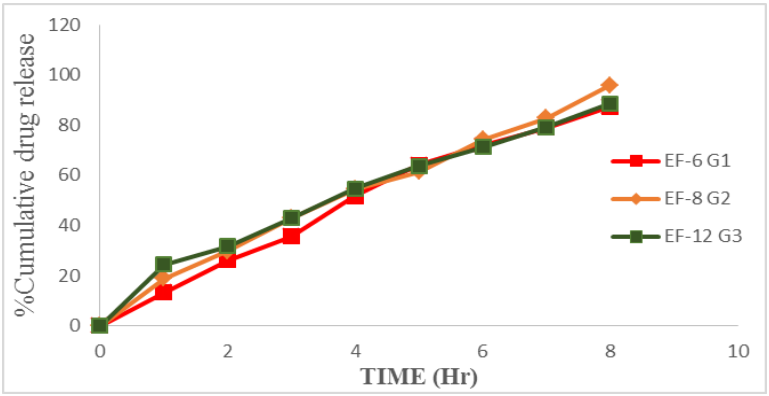


Figure 5.42: % cumulative drug release of EF-6 G1, EF-8 G2 and EF-12 G3.

5.5.4 Stability studies

Table no 5.31: Intermediate stability studies for optimized RM-Loaded Nano ethosomal gel at 5º± 3ºC, 30º ± 2º C/65% RH and 40º± 2ºC/75% RH.

Parameter	30 days EF-8 G2			60 days EF-8 G2		
	At 5º± 3ºC	At 30º ± 2º C	At 40º± 2ºC	At 5º± 3ºC	At 30º ± 2º C	At 40º± 2ºC
Drug content	96.54	95.75	93.24	94.27	92.85	89.14
%CDR	94.87	92.87	91.61	92.14	89.42	83.84
pH (range)	7.3	7.3	7.2	7.3	7.2	7.1
Colour	NCC	NCC	NCC	NCC	NCC	NCC
Spreadability	No change change	No change	No change	No change	No change	No change
Appearance	No change	No change	No change	No change	No change	No change

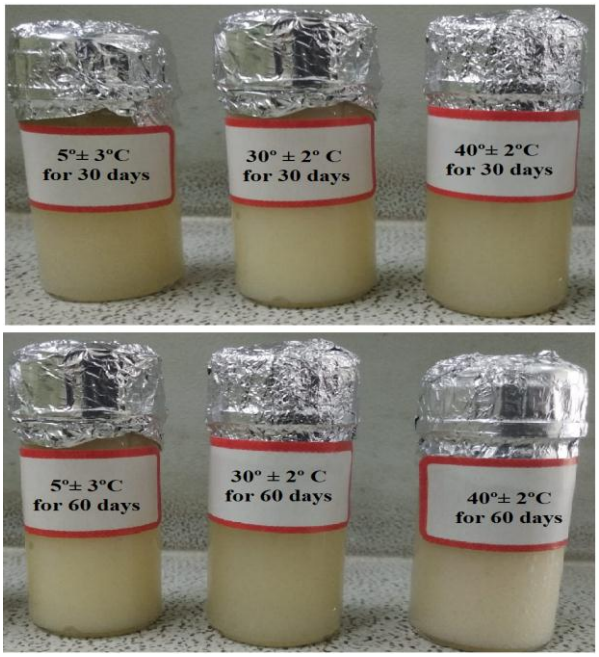


Figure 5.47: Stability studies of Optimized Gel formulation After 30 days and 60 days at 5º± 3ºC, 30º ± 2º C/65% RH and 40º± 2ºC/75% RH.

CONCLUSION

In the present work, the study of formulation and evaluation of topical gel containing nano Ethosomes of Rasagiline mesylate were successfully carried out, and by the reproducible results of the executed experiments, it can be concluded that,

- Rasagiline mesylate is a propargylamine derivative, and it is used to treat the symptoms of Parkinson's disease. The bioavailability of Rasagiline mesylate is 35%and the half-life is 1.5–3.5 h. In the present study, an attempt was made to formulate nano Ethosomes of Rasagiline mesylate for efficient drug delivery through the skin.
- A suitable method of analysis of drugs by UV spectrophotometry was developed. Rasagiline mesylate showed maximum absorption at a wavelength of 271nm in phosphate buffer pH 7.4. The correlation coefficient was found to be 0.9987, which showed a linear relationship between concentration and absorbance. Thus, it can be concluded that Beer's law was obeyed.
- Preformulation study for drug-excipients compatibility by FT-IR and DSC confirmed the purity of the drug. FT-IR studies showed no significant interaction between drug and polymer. The principal peaks of the drug in the spectrum obtained for the formulation were not altered.
- Nano Ethosomes of Rasagiline mesylate were prepared by the cold method.
- Lecithin and Ethanol is chosen as vesicle forming component, providing rigidity and stearic acid to provide the negative charge to the bilayer.
- The results showed that the particle size also tends to increase with an increase in the concentration of phospholipids. Since the coefficient of lecithin is considerably more than cholesterol, the effect of lecithin is found to be more than that of cholesterol.
- Best formulation was chosen based on entrapment efficiency and particle size, and in vitro drug release that Formulation was used to prepare gel.
- Zeta potential of optimized Formulation was -33.9 mV indicating moderately stable.
- The SEM results of nano Ethosomes of Rasagiline mesylate have shown smooth and spherical shape.
- 2% w/w Carbopol 934P was chosen as a gelling agent, and Formulation EF6, EF8, and EF12 were prepared gel, and the effect of it was studied for better Formulation.
- The study was carried out to see the effect of three Formulations by pH, viscosity, Drug content, Spreadability, and %CDR.
- The EF8 shows good results compare to another two formulations. They were found to be in pH (7.30), viscosity (2122.41), Drug content (96.66), Spreadability (24.16), and %CDR (95.83), respectively.
- The best fit model was found to be Zero order for all the formulations and followed the non-fickian mechanism of release.
- The optimized Formulation was tested for stability for 60 days at 5°C ± 3°C, Room temperature and 40º± 2ºC/75% RH. The results show the formulations did not undergo any physiological modifications or interactions and no significant change in Colour, PH, Spreadability, appearance, percentage of drug content, and % *in-vitro* drug release and were found to be stable at the end of the storage period.

REFERENCES

1. Patel D, Bhargava P. Ethosomes-a Phyto Drug Delivery System. Adv Res Pharm Biol., 2012; 2: 1-8.
2. Jain H, Patel J, Joshi K, Patel P, Upadhyay UM. Ethosomes: A novel drug carrier. Int J. Clin Pract., 2011; 7(1): 1-4.
3. Gangurde PA, Saudagar RB. Ethosomes novel drug delivery. Int J pharma chem Res., 2017; 3: 25-31.
4. Pakhale NV, Gondkar SB, Saudagar RB. Ethosomes: Transdermal Drug Delivery System. J drug deliv ther., 2019; 9(3): 729-33.
5. Cuenca L, Gil-Martinez AL, Cano-Fernandez L, Sanchez-Rodrigo C, Estrada C, Fernandez-Villalba E, Herrero MT. Parkinson's disease: a short story of 200 years. Histology and histopathology, 2018; 34(6): 573-91.
6. Lee TK, Yankee EL. A review on Parkinson's disease treatment. Neuroimmunology and Neuroinflammation. 2021 Jan 25;8. Surathi P, Jhunjhunwala K, Yadav R, Pal PK. Research in Parkinson's disease in India; A review. Annals Indian Acad Neurol, 2016; 19(1): 9-20.
7. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, Schrag AE, Lang AE. Parkinson disease. Nat. Rev. Dis. Primers, 2017; 3(1): 1-21.
8. Müller T. Catechol-O-methyltransferase inhibitors in Parkinson's disease. Drugs, Feb, 2015; 75(2): 157-74.
9. Dorsey ER, Elbaz A, Nichols E, Abd-Allah F, Abdelalim A, Adsuar JC, Ansha MG, Brayne C, Choi JY, Collado-Mateo D, Dahodwala N. Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet Neurology, Nov 1, 2018; 17(11): 939-53.
10. Thebault JJ, Guillaume M, Levy R. Tolerability, safety, pharmacodynamics, and pharmacokinetics of rasagiline: a potent, selective, and irreversible monoamine oxidase type B inhibitor. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 2004; 24(10): 1295-05.
11. Bali NR, Salve PS. Impact of rasagiline nanoparticles on brain targeting efficiency via gellan gum based transdermal patch: A nanotheranostic perspective for Parkinsonism. Int J Biol Macromol, 2020; 64: 1006-24.
12. Mandel S, Weinreb O, Amit T, Youdim MB. Mechanism of neuroprotective action of the anti-

Parkinson drug rasagiline and its derivatives. Brain research reviews, Apr 1, 2005; 48(2): 379-87.
13. Lecht S, Haroutiunian S, Hoffman A, Lazarovici P. Rasagiline–a novel MAO B inhibitor in Parkinson's disease therapy. Therapeutics and clinical risk management, 2007; 3(3): 467-74.
14. Wang M, Zhou W, Zhang Q, Zong S, Lv C. Pharmacokinetics, Pharmacodynamics, and Safety of a Single Escalating Dose and Repeated Doses of Rasagiline Transdermal Patch in Healthy Chinese Subjects. Clinical pharmacology in drug development, 2020; 9(5): 602-09.
15. Tansey MG, Goldberg MS. Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. Neurobiology of disease, Mar 1, 2010; 37(3): 510-8.
16. Chan LL, Rumpel H, Yap K, Lee E, Loo HV, Ho GL, Fook-Chong S, Yuen Y, Tan EK. Case control study of diffusion tensor imaging in Parkinson's disease. Journal of Neurology, Neurosurgery & Psychiatry, Dec 1, 2007; 78(12): 1383-6.
17. Mishra N, Tiwari DK, Mishra K. Development of Intranasal Deformable Ethosomes of Rasagiline Mesylate for The Effective Management of Parkinsonism.
18. Shelke S, Shahi S, Jalalpure S, Dhamecha D. Poloxamer 407-based intranasal thermoreversible gel of zolmitriptan-loaded nanoethosomes: formulation, optimization, evaluation and permeation studies. Journal of liposome research, Oct 1, 2016; 26(4): 313-23.
19. Avadhani KS, Manikkath J, Tiwari M, Chandrasekhar M, Godavarthi A, Vidya SM, Hariharapura RC, Kalthur G, Udupa N, Mutalik S. Skin delivery of epigallocatechin-3-gallate (EGCG) and hyaluronic acid loaded nano-transfersomes for antioxidant and anti-aging effects in UV radiation induced skin damage. Drug delivery, Jan 1, 2017; 24(1): 61-74.
20. Jiang Y, Zhang X, Mu H, Hua H, Duan D, Yan X, Wang Y, Meng Q, Lu X, Wang A, Liu W. Preparation and evaluation of injectable Rasagiline mesylate dual-controlled drug delivery system for the treatment of Parkinson's disease. Drug delivery, Jan 1, 2018; 25(1): 143-52.
21. Viveksarathi K, Kannan K. Effect of the moist-heat sterilization on fabricated nanoscale solid lipid particles containing rasagiline mesylate. International Journal of Pharmaceutical Investigation, Apr, 2015; 5(2): 87.
22. Wake PS, Kshirsagar MD. Compatibility study In-vitro drug release study of solid lipid nanoparticle based transdermal drug delivery system for rasagiline mesylate. Evaluation, 2017; 10: 5.
23. Shaikh T. Development and Validation of UV-Spectrophotometric method for determination of Palladium Content in Rasagiline Mesylate.
24. Bali NR, Shinde MP, Rathod SB, Salve PS. Enhanced transdermal permeation of rasagiline mesylate nanoparticles: design, optimization, and effect of binary combinations of solvent systems across biological membrane. International Journal of Polymeric Materials and Polymeric Biomaterials, Feb 11, 2021; 70(3): 158-73.
25. Sundar VD, Divya P, Dhanaraju MD. Design Development and Characterisation of Tramadol Hydrochloride Loaded Transethosomal Gel Formulation for Effective Pain Management.
26. Pawar AY, Jadhav KR, Naikwade K, Mahajan TP. Formulation & Evaluation of Naringin Nanoethosome by Cold Method. ArticleGet, Aug 31, 2021: 139-52.
27. El-Shenawy AA, Abdelhafez WA, Ismail A, Kassem AA. Formulation and characterization of nanosized ethosomal formulations of antigout model drug (febuxostat) prepared by cold method: In vitro/ex vivo and in vivo assessment. Aaps Pharmscitech, Jan, 2020; 21(1): 1-3.
28. Bajaj KJ, Parab BS, Shidhaye SS. Nano-transethosomes: A Novel Tool for Drug Delivery through Skin. INDIAN JOURNAL OF PHARMACEUTICAL EDUCATION AND RESEARCH, Jan 1, 2021; 55(1): S1-0.
29. Kunasekaran V, Krishnamoorthy KA. Kinetic modeling of Rasagiline mesylate from nanoscale solid lipid particles. Int. J. Pharm. Pharm. Sci., Nov 1, 2015; 7(11): 300-5.
30. Khan P, Akhtar N. Phytochemical investigations and development of ethosomal gel with Brassica oleraceae L.(Brassicaceae) extract: An innovative nano approach towards cosmetic and pharmaceutical industry. Industrial Crops and Products, Sep 1, 2022; 183: 114905.