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Formulation and Evaluation of Nano Ethosomes Contaning Rasagiline Mesylate for the Effective Management of Parkinsonism

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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: Nanoethosomes, Parkinson's disease, Rasagiline mesylate, D- optimal design, Entrapment efficiency.

I. INTRODUCTION

Nano ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and the systemic circulation. It is a soft lipid vesicle containing phospholipids (phosphatidylcholine, phosphotidylserine, phosphatitidic acid) and relatively high concentrations of alcohol (ethanol and isopropyl alcohol) and water. The high ethanol concentration makes the nano Ethosomes unique and valuable for transcellular delivery; Ethanol is known for its disturbance of skin lipid bilayer. In addition, because of their high ethanol concentration, it gives the ability to penetrate the stratum corneum. The nano Ethosomes size range varies from 10nm to microns. **(1,2)**

The skin is one of the human body's most extensive and readily accessible organs. One of the most significant disadvantages to transdermal drug delivery is the skin's low permeability, limiting the number of drugs that can deliver. Nano ethosomes as novel vesicles in transdermal drug delivery show significant effects on drug penetration through the biological membrane. Nano Ethosomes are conceptually sophisticated. They are manageable preparation and safe for use. The transdermal route is a promising alternative to drug delivery for systemic effect. An attempt was made to Prepare a highly efficient nano ethosomal drug delivery system. Nano Ethosomes have a higher penetration rate through the skin than liposomes; hence these can be used widely in place of liposomes. Nano Ethosomes enhanced skin permeation, improved drug delivery. **(3,4)**

PARKINSONISM:

Alzheimer's disease or Parkinson's disease is a prevalent and incidental neurodegenerative disorder, affecting more than 2% of the population around the age of 65 years and above. **(5)** In 2015, 6.2 million people had Parkinson's disease globally, and approximately 11 lakh people died due to Parkinson's disease. There is progressive development of both motor and non-motor symptoms. **(6)** PD disorder is characterized by early prominent death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and widespread presence of alpha-synuclein (aSyn), an intracellular protein. **(7)** PD symptoms include abnormal movements, such as tremors, rigidity, bradykinesia, and postural instability. PD is characterized by progressive loss of dopaminergic

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neurons in the substantia nigra of the midbrain region, which results in an imbalance between the dopaminergic and cholinergic systems. Dopamine functions as a neurotransmitter coordinate movement and control muscle tone. (**8**) PD treatment includes symptomatic and is predominantly focused on the dopaminergic pathway such as dopamine and levodopa agonists. The highly potent selective irreversible monoamine oxidase (MAO) B inhibitor. the MAO inhibitors are Rasagiline, Selegiline, cabergoline, etc. (MAO) B inhibitor catalyzes the oxidative deamination of active amines and causes prolonged dopamine activity. **(9)**

RASAGILINE MESYLATE

Rasagiline mesylate is used as a first-line agent for the early management of Parkinson's disease, but its water-soluble nature creates hurdles to cross the blood-brain barrier. Also, its low oral bioavailability and rapid elimination require frequent dosing. (**10**) It is a potent, selective, irreversible, second-generation inhibitor of monoamine oxidase type B (MAO-B) and has neuroprotective activity not related to its MAO-inhibitory activity. The theoretical basis for using MAO-B inhibitors in Parkinson's disease is that inhibition of MAO-B in the human brain decreases the oxidative deamination of both endogenous dopamine and dopamine produced from exogenous levodopa. Thus, dopamine levels are increased, and dopaminergic function is restored. (**11**)

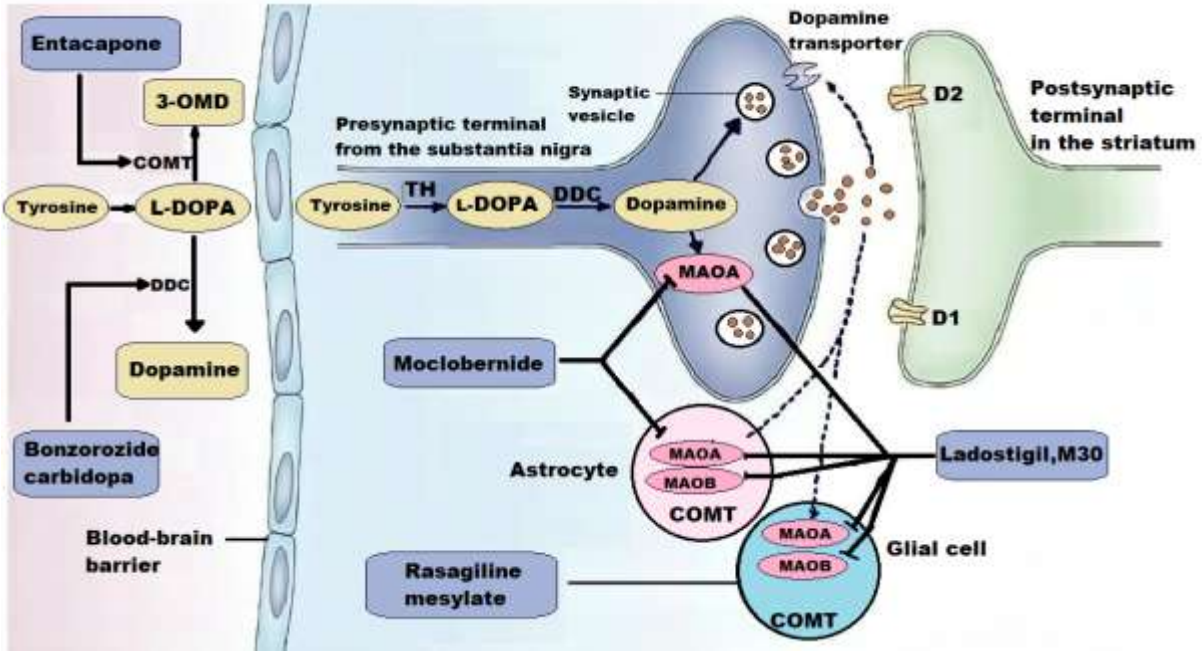

FIG 1: - MECHANISUM OF ACTION DRUG RASAGILINE MESYLATE

Rasagiline mesylate shows high solubility and low permeability; hence it belongs to the BCS class III drug. It is used in the treatment of PD as oral tablets. The oral bioavailability of rasagiline is 35%, it reaches Tmax after 0.5–1 hour, and its half-life is 1.5–3.5 hours. It undergoes extensive hepatic metabolism primarily by cytochrome P450. (**12**) It exerts linear absorption at doses of 1–10 mg/day. It reaches Cmax after 1 and 2 mg; the oral dose is 2.5 ng/mL and 4.9 ng/ml. High-fat meal decreases the area under the rasagiline curve (AUC) by 20%. The volume of distribution (Vd) of rasagiline varies between 87 to 243 L. Oral clearance of the drug is 94.3 L/hour, and since the liver blood flow is about 90 L/hour. (**13**)

A Rasagiline transdermal system can be used to offer continuous rasagiline to patients with Parkinson's disease who cannot take oral medications. The median value of tmax was the same at the 1.2- mg and 2.5-mg transdermal doses. The mean values of t½ were similar at the 1.25-mg and 2.5-mg transdermal doses; both are longer than that of the oral tablet. After normalization by dose, the mean Cmax norm(dose) of the 1-mg tablet dose was 2-fold higher than that of the transdermal patch dose. The mean AUC, norm(dose) of 1.25 mg and 2.5 mg for the transdermal patch doses were 3-fold and 4-fold higher than that of the 1-mg tablet dose. (**14**)

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MATERIALS AND METHODS: Rasagiline Mesylate was a gift sample from Bangalore. 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)**

AMOUNT OF DRUG TO BE ADDED: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.

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.

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

- 100mgofaccuratelyweighedRasagiline mesylatewasdissolvedandmadeupto100mlwithpH7.4buffersolution.
- 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: The central composite face-centered design was taught to get different experimental runs using design expert software version 13, which analyzes the main effects and specific interactions and helps identify the significant factors from those that are not important. The independent variables selected were soya lecithin concentration, Ethanol concentration, and Propylene Glycol concentration. The factors and levels are mentioned in table no: -1. The lower and higher levels of independent factors were selected based on the reported literature and initial screening experiments conducted.

SL. NO	INDEPENDENT VARIABLES	LOW LEVEL	INTERMEDIATE LEVEL	HIGH LEVEL
1	Soya lecithin	2	-	4
2	Ethanol	20	30	50
3	Propylene Glycol	5	10	20

Table-1: Selected independent variables for optimization.

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.

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

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

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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. Drugrelease 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

$$\mathbf{Qt = (QoC + Kot)}$$

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.

$$\mathbf{LogQt = logQo + Kt/(2.303)}$$

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:

$$\mathbf{Qt = KH.t^{½}}$$

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:

$$\mathbf{Mt/M\infty = K.t^{n}}$$

Where,
Mt/ M∞ = Fraction release, of drug

t=Drugreleasetime and

n =Diffusionalexponentforthedrugreleasethatisdependenton theshapeofthematrixdosageform.

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 plastic vessel, wrapped with aluminum foil, and kept at 30º C ± 2ºC and (65±5%RH) for 30, 60 days in a stability chamber at 5º C ± 3ºC temperature in a refrigerator and evaluated for their drug entrapment efficiency and particle size.

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II. RESULTS AND DISCUSSION:

a. Melting point determination

Reported	Method	Observed
152-155 ºC	Thiel's tube method	153 ºC
	DSC	154.35 ºC

Table no-2: - Melting point of Rasagiline mesylate

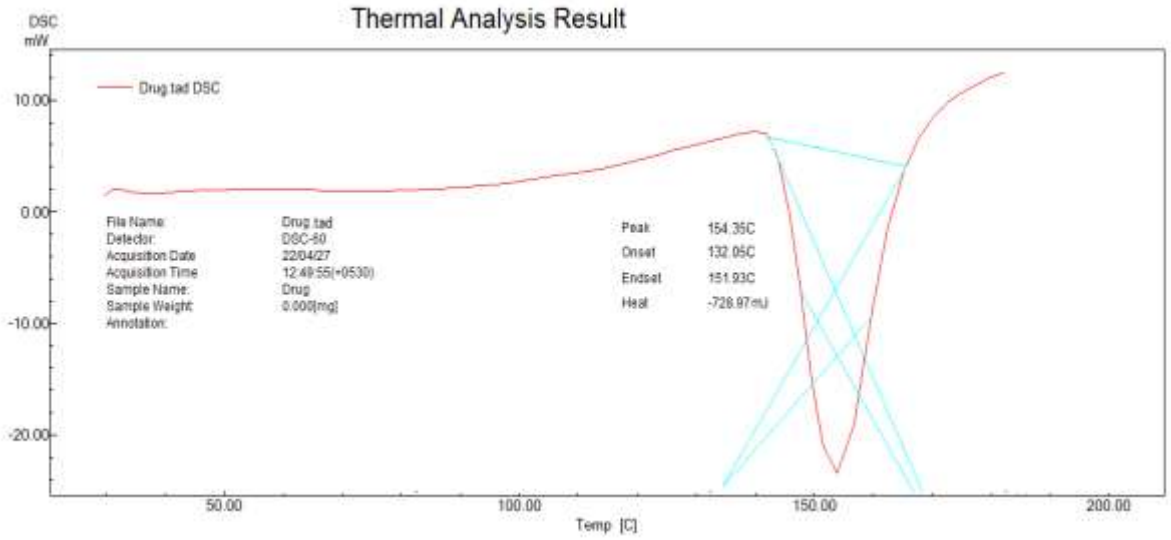

Figure 2: DSC Thermograph of Rasagiline mesylate

b. Solubility analysis

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

Table no 3: Solubility analysisof Rasagiline mesylate

c. DETERMINATION OF Λ MAX:

STANDARD CURVE OF RASAGILINE MESYLATE IN PHOSPHATE BUFFER (pH 7.4):

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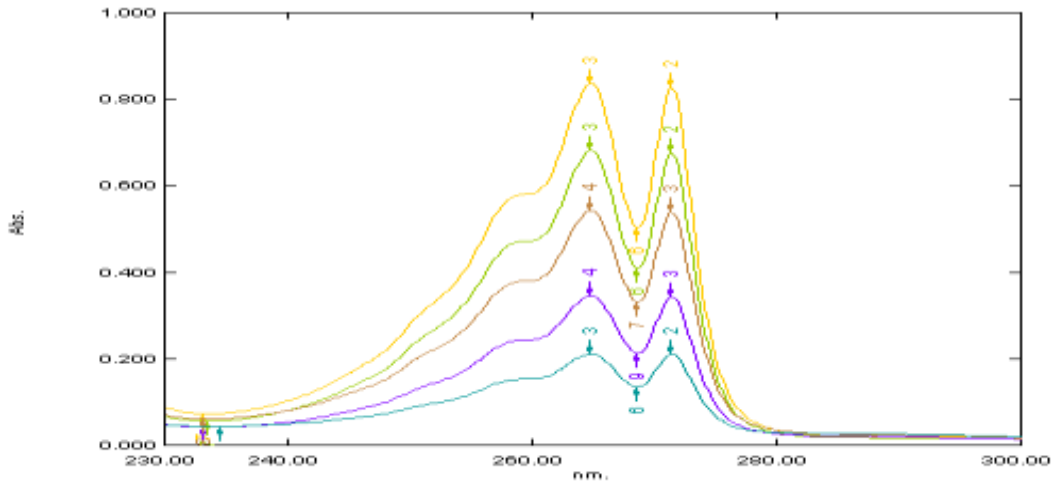
The calibration curve of Rasagiline mesylate in Phosphate buffer pH 7.4 at 271 nm is shown in table 4. Figure 4 shows the standard curve with a regression value of 0.9987 and a slope of 0.0036 in Phosphate buffer pH 7.4. The curve is linear in the concentration range from 50 to 250µg/ml.

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



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

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

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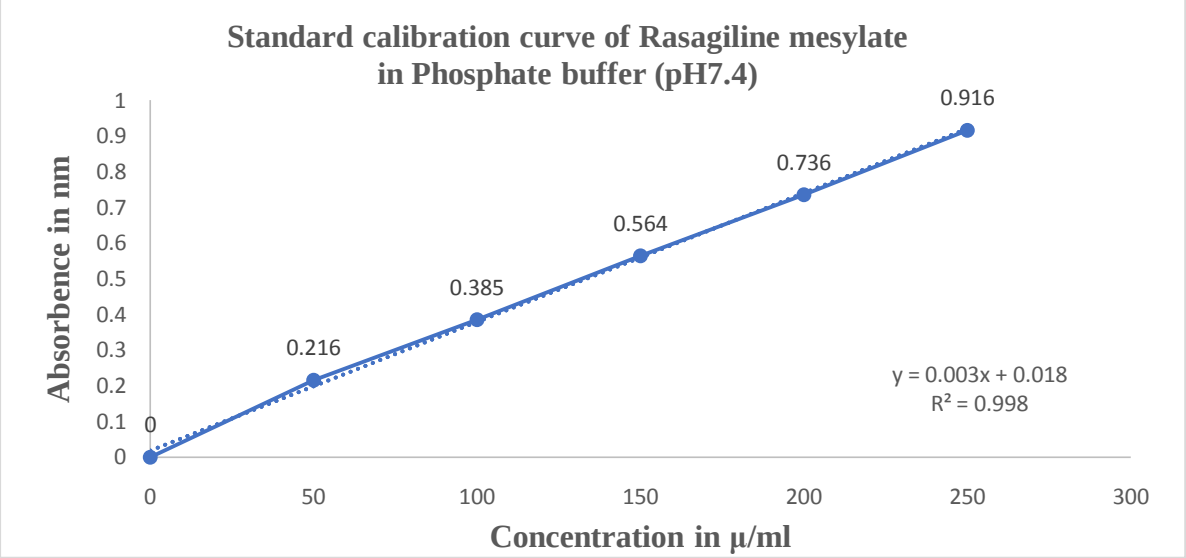

Graph1: Plot of standard calibration curve of rasagiline mesylate

d. Compatibility studies by FTIR

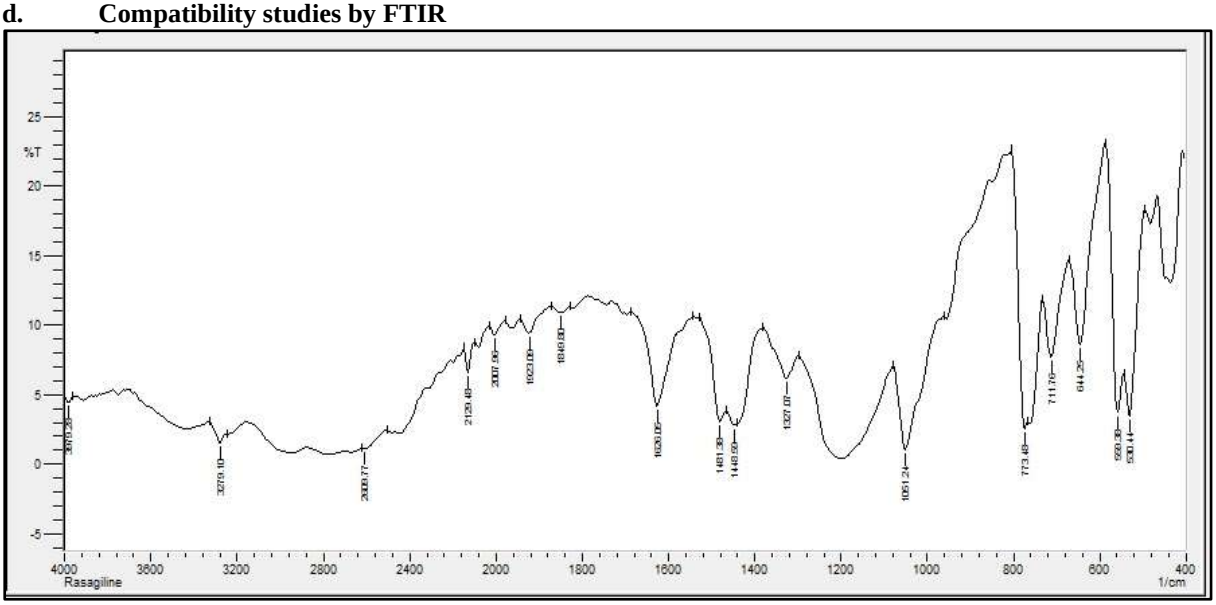

Figure 4: FT-IR spectra of drug of rasagiline mesylate

e. PREPARATION OF RASAGILINE MESYLATE LOADED NANO ETHOSOMES

Formulation code	Drug (%w/v)	Soya lecithine (%w/v)	Ethanol (%v/v)	Propylene Glycol (%v/v)	Cholesterol (%w/v)	Distill water (%v/v)
EF-1	1	2	20	10	5	q. s
EF-2	1	2	30	10	5	q. s
EF-3	1	2	40	10	5	q. s

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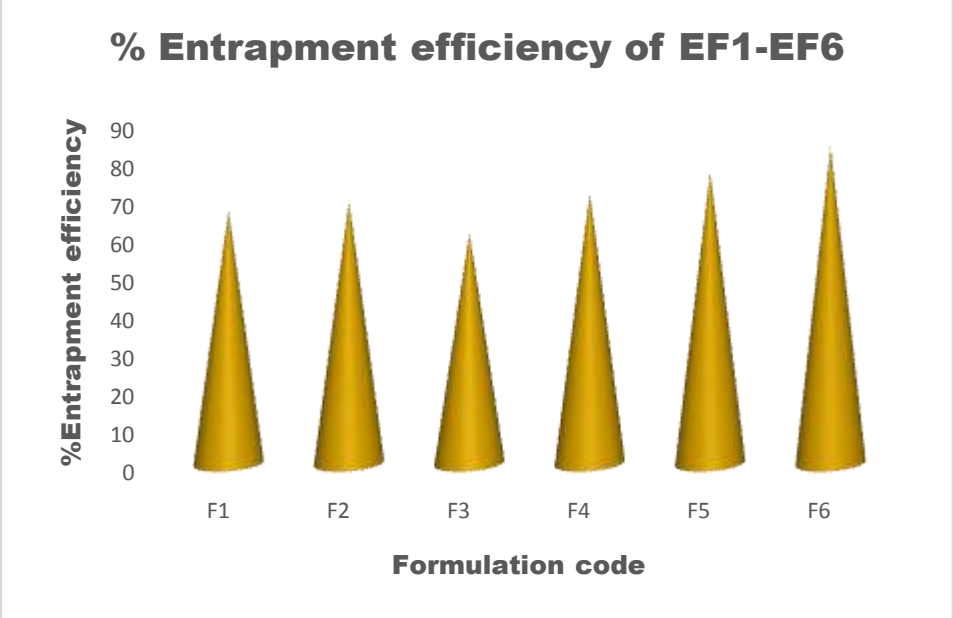
EF-4	1	2	30	5	5	q. s
EF-5	1	2	30	10	5	q. s
EF-6	1	2	30	20	5	q. s

Table 5: Composition of Different nano Ethosomal formulations prepared by Cold method

EVALUATION OF NANO ETHOSOMES

Formulations	Particle size (µm)	Entrapment Efficiency (%)
EF-1	286.0±6	66.48±0.14
EF-2	281.4±14	69.07±0.40
EF-3	324.1±24	60.55±0.55
EF-4	228.1±2	71.11±0.1
EF-5	220.9±17	76.57±0.41
EF-6	153.7±8	83.14±0.81

Table 5: Particle size and Entrapment Efficiency of Nano ethosomes



Graph2: Entrapment efficiency of EF1-EF6

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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

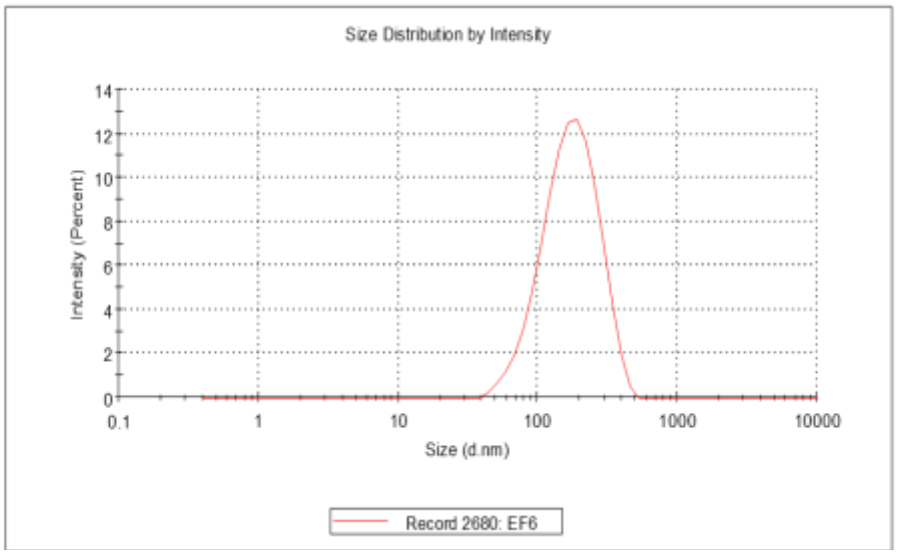

Figure 5: Particle size analysis of EF6

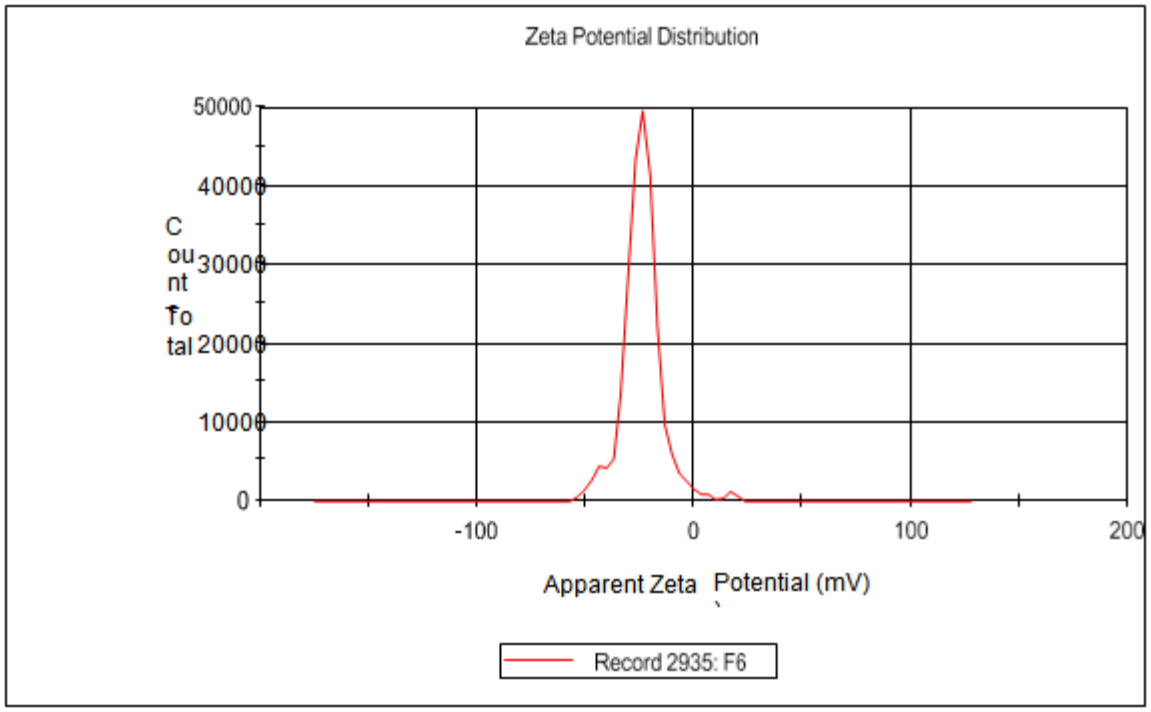

Figure 6: Zeta Potential analysis of EF6

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ii. SCANNING ELECTRON MICROSCOPY

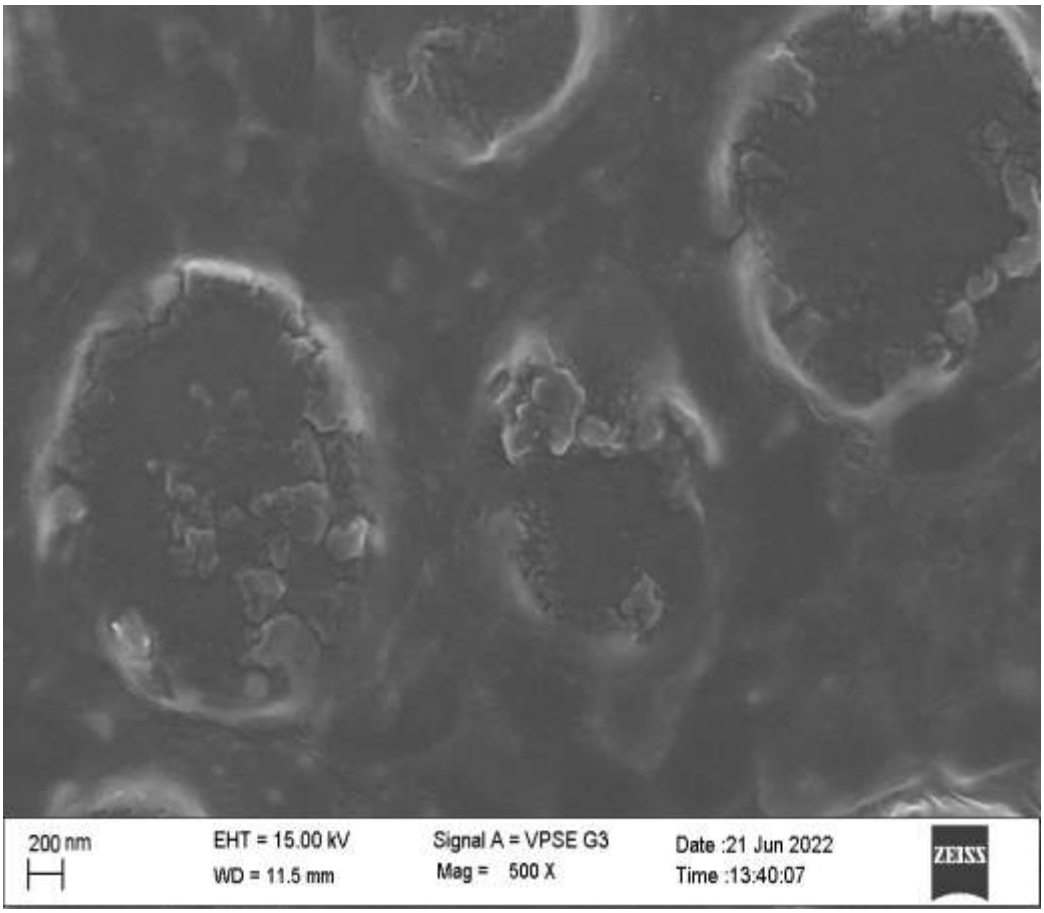

Figure 7: SEM IMAGE OF OPTIMIZEDFORMULATION OPTIMIZATION:

iii. In-vitro diffusion study:

TIME in (hrs)	Percentage cumulative drug release (%) mean± SD					
	EF-1	EF-2	EF-3	EF-4	EF-5	EF-6
0	0	0	0	0	0	0
1	4.72±0.22	5.18±0.51	2.59±0.25	6.85±0.18	5.92±0.59	8.42±0.26
2	8.51±0.85	10.55±0.55	4.81±0.45	10.92±0.53	11.48±0.18	12.77±0.78
3	12.77±0.77	18.24±0.74	9.07±0.47	17.22±0.22	16.29±0.63	16.29±0.63
4	18.24±0.74	22.68±0.51	11.75±0.92	21.11±0.11	19.81±0.41	24.44±0.44
5	22.59±0.25	27.22±0.22	15.55±0.56	26.48±0.18	27.22±0.22	28.51±0.52
6	27.59±0.25	31.57±0.40	18.79±0.63	30.18±0.59	30.64±0.85	33.98±0.18
7	30.37±0.37	37.77±0.77	24.81±0.41	36.85±0.15	37.77±0.78	38.51±0.52
8	34.72±0.22	45.92±0.59	30.37±0.37	41.48±0.18	43.98±0.18	44.53±0.04

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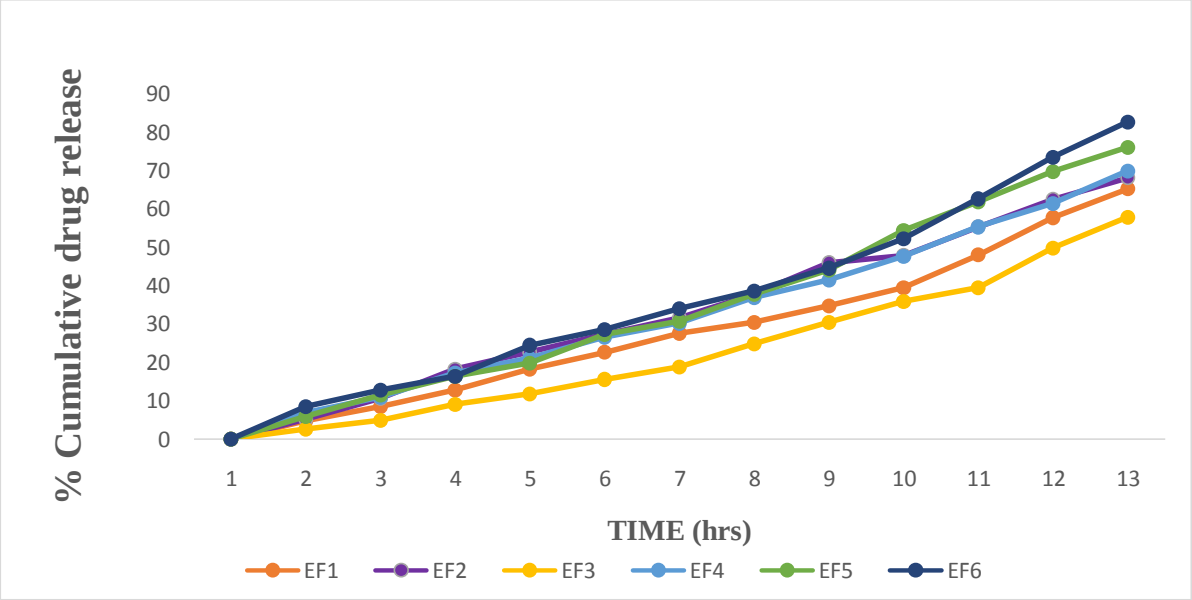
9	39.44±0.44	47.87± 0.37	35.83±0.33	47.59±0. 29	54.35±0.15	52.22±0.22
10	47.96±0.29	55.27± 0.77	39.44±0.44	55.27±0. 78	61.85±0.24	62.59±0.29
11	57.68±0.51	62.40± 0.74	49.81±0.41	61.38±0. 89	69.72±0.22	73.42±0.53
12	65.18±0.51	68.05± 0.51	57.77±0.18	69.81±0. 48	76.01±0.08	82.59±0.25

Table no 6: Percentage cumulative drug release (%) mean± SD



Graph 3: %CDR FOR F1-F6 NANO ETHOSOMAL FORMULATION

DRUG KINETIC STUDY FOR EF6 FORMULATION:

TIME(hr)	Log T	SQRT	%CDR	log%CDR	%Drug remaning	log% drug remaning
0	0	0	0	0	100	2
1	0	1	8.42	0.9253	91.58	1.9618
2	0.301	1.414	12.77	1.1061	87.23	1.9406
3	0.4771	1.732	16.29	1.2119	83.71	1.9227
4	0.602	2	24.44	1.3881	75.56	1.8782
5	0.6989	2.236	28.51	1.4549	71.49	1.8542
6	0.7781	2.449	33.98	1.5312	66.02	1.8196
7	0.845	2.645	38.51	1.5855	61.49	1.7888
8	0.903	2.828	44.53	1.6486	55.47	1.7440
9	0.9542	3	52.22	1.7178	47.78	1.6792
10	1	3.162	62.59	1.7965	37.41	1.5729
11	1.0413	3.316	73.42	1.8658	26.58	1.4245
12	1.0791	3.464	82.59	1.9169	17.41	1.2407

Table no 7: DRUG KINETIC STUDY FOR EF6 FORMULATION

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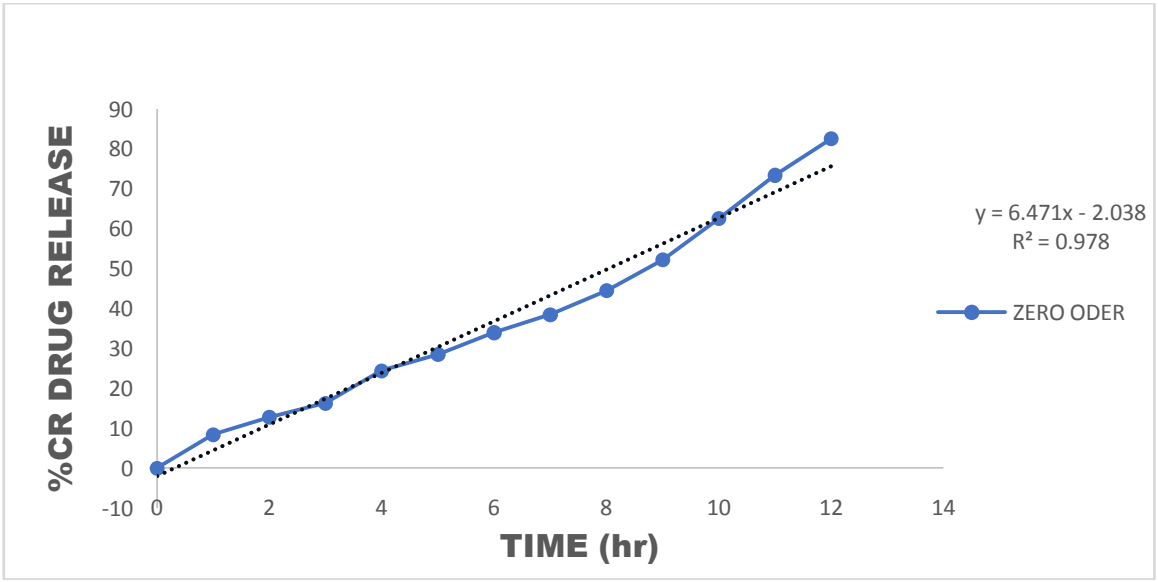
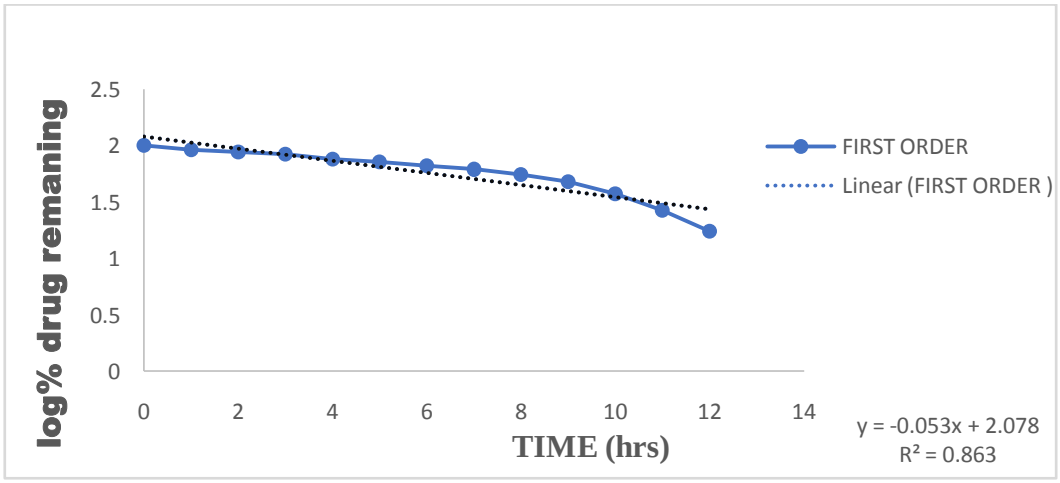
ZERO ORDER RELEASES KINETICS



Graph 4: ZERO ORDER RELEASES KINETICS

FIRST ORDER RELEASES KINETICS



Graph 5: FIRST ORDER RELEASES KINETICS

HIGUCHI RELEASE KINETICES

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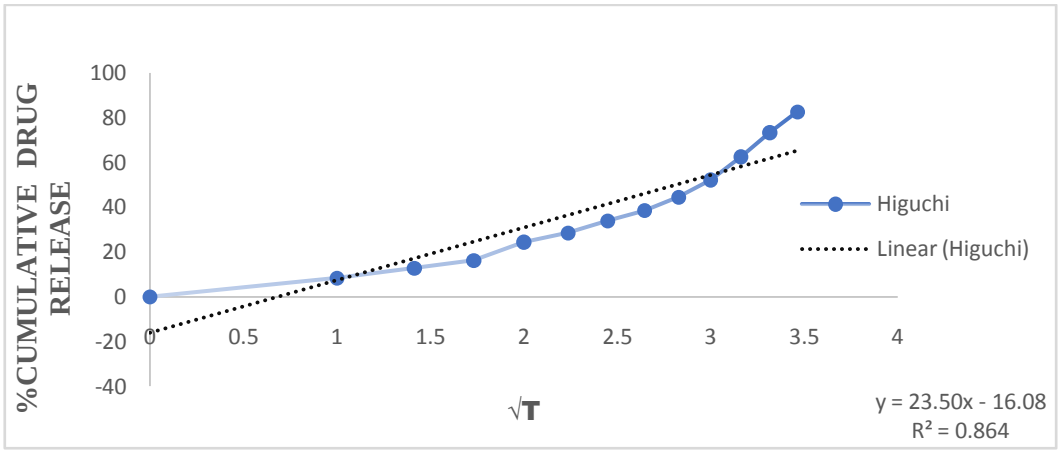
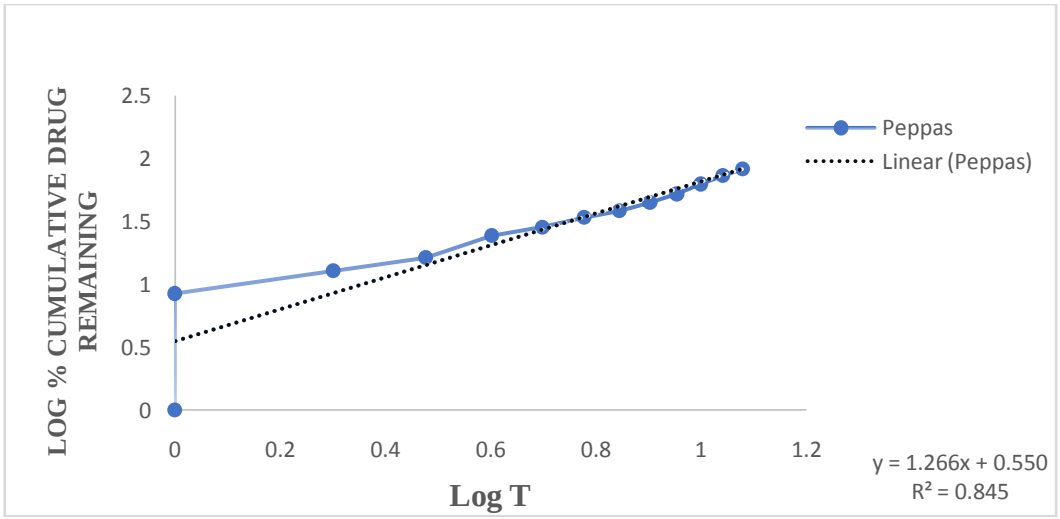


Graph 5: Higuchi release kinetices

KORS-PEPPAS RELEASE KINETICES



Graph 6: KORS-PEPPAS RELEASE KINETICES

MODEL NAME	ZERO ORDER	FIRST ORDER	HIGUCHI	KORS-PEPPAS
SLOPE	6.4714	-0.0537	23.504	1.2669
r^2	0.9784	0.8626	0.8643	0.8456

Table no 8: RESULT FOR IN-VITRO RELEASE KINETIC MODELS.

STABILITY STUDIES OF EF6:

The results of drug content of ideal formulation EF6 after 30 Days and 60 Days of stability testing under different storage conditions are shown in Table 3. In addition, in vitro release profiles for the same formulation are stored under other conditions.

Formulation	At Room Temp. for 30 days		At $5^0C±3^0C$ for 30 days.	
	Particle size(µm)	Entrapment Efficiency (%)	Particle size(µm)	Entrapment Efficiency (%)
Optimized batch	193±6	75.21±0.47	167.9±2	79.14±0.65

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Formulation	At Room Temp. for 60 days		At $5^0C\pm3^0C$ for 60 days.	
	Particle size(µm)	Entrapment Efficiency (%)	Particle size(µm)	Entrapment Efficiency (%)
Optimized batch	226.5±9	63.34±0.21	178.6±12	76.85±0.24

Table no 8: Stability studies of EF6 formulation was stored at $5^0c\pm3^0c$ and room temperature

On comparing both data with the EF6, it was observed that there was a slight decrease in Particle size and Entrapment efficiency when the formulation was stored at $5^0C\pm3^0C$ and Room temperature because there might be chances for drug degradation that decreased the drug release at the higher temperature.

III. CONCLUSION

In the present work, the cold method prepared Rasagiline mesylate nano ethosomes using soya lecithin, propylene glycol, cholesterol, and Ethanol. it can draw the following conclusions from the study:

- The main aim of this preparation is to improve the drug's permeability, bioavailability, and sustainability by incorporating it into the nano ethosome vesicle carrier.
- Cold method can be employed successfully to formulate nano ethosomes containing Rasagiline mesylate.
- Identification of the absorption maxima of the Rasagiline mesylate by using 7.4 pH buffer as the solvent in the range of 200-400nm.
- FT-IR spectra of the pure drug and drug excipients mixture indicated no drug-excipient interaction; hence it shows drug and excipients were compatible.
- SEM analysis of the Rasagiline mesylate revealed that the prepared nano ethosomes were porous, smooth, and spherical particles
- This study of rasagiline mesylate loaded nano ethosomes revealed amelioration in the encapsulation efficiency upon increasing the amount of Ethanol and phospholipids to a certain extent in preparation.
- Analysis of the drug release mechanism showed that the drug release followed the first-order kinetic model.
- Invitro permeation study through Transdermal drug delivery of RM loaded ethosomes containing Ethanol: propylene glycol: phospholipids (30%:20%:2%) showed superior permeation results as the presence of Ethanol in the aqueous compartment of the nano ethosomal vesicles favored the encapsulation of RM and enhanced its permeation through Transdermal route.
- Stability studies revealed there it was observed that there was a slight decrease in Particle size and Entrapment efficiency when the formulation was stored at $5^0C\pm3^0C$ and Room temperature because there might be chances for drug degradation that decreased the drug release at the higher temperature.

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