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Formulation and Evaluation of Nano Ethosomal Gel as A Transdermal Drug Delivery System Containing Antiparkinsonism Drug-Selegiline Hydrochloride

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ABSTRACT:

Objective: Ethosomes play an important role in the transdermal treatment due to its high deformability and transport of active substances more efficiently through the stratum corneum into the deeper layers of the skin than conventional liposomes because of their high ethanol concentration. Selegiline hydrochloride belongs to BCS class III drughas an oral bioavailability of about 10%, which increases when ingested together with a fatty meal, as the molecule is fat soluble. Its metabolites bind extensively to plasma proteins (at a rate of 94%) and half-life 1.2-2 hours.

The main aim of the work was to prepare ethosomes containing selegiline hydrochloride in order to improve permeability and sustainability of the drug release and minimize associated side effects

METHODS: Ethosomes were prepared by cold method by applying central composite. From the preliminary trials, the constraints for independent variables A (soya lecithin), and B (ethanol), were set and experimental runs were generated to prepare ethosomes. The prepared ethosomes were evaluated for particle size and shape by scanning electron microscopy, entrapment efficiency and in-vitro release studies. Optimized selegiline hydrochloride ethosomes were then incorporated into a gel. The prepared gel was evaluated for pH, viscosity, drug content, in-vitro drug permeation study. Stability studies were carried for the gel according to ICH guidelines.

RESULTS: selegiline hydrochloride loaded ethosomes were prepared and evaluated. Ethosomes were spherical in shape and had particle size ranging from 159.33-454.26nm. The entrapment efficiency of formulated ethosomes were found ranging from 65.09-95.46% .Further the ethosomes formulations were showed controlled release of drug for 8hours. Upon fitting of in-vitro drug release data into various kinetic models, the release was found to follow first order model.

CONCLUSION: The research work can be continued using various natural or synthetic polymers by different formulation methods for the formulation of ethosomes. Method for preparing ethosomal drug delivery can be utilized for others drugs with more adverse effects, low permeability and low oral bioavailability.

KEY WORDS: Parkinsonism, permeability, bioavailability, entrapment efficiency, optimization.

I. INTRODUCTION:

PARKINSONISM:

Parkinsonism is the second most common progressive neurodegenerative movement disorder, which affecting 1-2% of people over 65 people in the world[(1)]. After the Alzheimer disease it is the next second most neurodegenerative disease which affecting patients in large numbers throughout the world[(2)]. Parkinson's characterized by early prominent death of dopaminergic neurons in the substantia nigra pars compacta (snpc) and wide spread presence of alpha-syncline (asyn), an intra cellular protein. The autonomic features of PD are seborrhea, hyperhidrosis, bladder dysfunction, orthostatic syndrome and neuropsychiatric symptoms such as depression, sleep disorder, psychosis, and cognitive dysfunction with impaired execution[(3)].In 2016, 6·1 million individuals had Parkinson's disease globally, compared with 2·5 million in 1990. This increase was not solely due to increasing numbers of older people, because age-standardized prevalence rates increased by 21·7% over the same period. Parkinson's disease caused DALYs and 211296 deaths in 2016[(4)].

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MONOAMINOOXIDASE B (MAO-B) INHIBITION:

MAO-B-I stabilize the dopamine levels in the synaptic cleft. Two compounds of the propargylamine group, selegiline and rasagiline, both irreversible MAO-B inhibitors, have demonstrated a symptomatic effect in PD patients. MAO-B-inhibition catalyses the oxidative deamination of active amines and therefore causes prolong dopamine activity[6].

SELEGILINE HYDROCHLORIDE

Selegiline, also known as L-diphenyl and sold under the brand names Elderly and Emsam among others, is a medication which is used in the treatment of Parkinson's disease and major depressive disorder[7].It is belongs to bcs class III drughas an oral bioavailability of about 10%, which increases when ingested together with a fatty meal, as the molecule is fat soluble. Its metabolites bind extensively to plasma proteins (at a rate of 94%) and half-life 1.2-2 hours. The dosage of selegiline hydrochloride for oral and transdermal patch were 5mg/12 hour and 9-12mg/24hrs respectively[8]. Transdermal administration of selegiline hydrochloride results in 5-fold higher bioavailability when compare to oral route of administration. Selegiline hydrochloride is administered though transdermal system is developed to reduce the dietary tyramine safety issue (“cheese reaction”) of orally administration. Transdermal dosing results in significantly higher exposure to selegiline and lower exposure to all metabolites when compared to oral dosing, this is due to the extensive first-pass metabolism of the pill form and low first-pass metabolism of the patch or gel form[9]. The site of application is not a significant factor in how the drug is distributed. In humans, selegiline hydrochloride does not accumulate in the skin, nor is it metabolized there, selegiline hydrochloride has low oral bioavailability due to its high first pass metabolism and scarce oral absorption[10]. In the present study, preparation of selegilineethosomal gel vai transdermal route of administration is to improve the permeability and therapeutic efficiency of the anti-parkinsonism drug-selegiline hydrochloride.

TRANSDERMAL DRUG DELIVERY SYSTEM:

Transdermal drug delivery is growing importance due to its non-invasive procedure for administration of many drugs. The transdermal drug delivery reduces a number of limitations of oral drug delivery such as degradation of drugs by digestive enzymes, irritation of gastrointestinal mucosa and first pass effect. Also due the pain on administration associated with parenteral route, patients highly prefer transdermal route. Hence transdermal dosage forms enjoy being the most patient compliant mode of drug delivery. Transdermal drug delivery system (TDDS) showed promising result in comparison to oral drug delivery system as it eliminates gastrointestinal interferences and first pass metabolism of the drug [11].

THE SKIN

Human skin is an important target site for the application of drug especially in the local disease. The skin is the largest organ of the body, accounting for about 15% of the total adult body weight and is having a total surface area of approximately 1.8m^2. The skin is composed of three layers; epidermis, dermis and subcutaneous tissue. The outermost level is epidermis, consists of a specific constellation of cells known as keratinocytes. The middle layer is dermis; it is fundamentally made up of the fibrillar structural protein known as collagen. The dermis lies on the subcutaneous tissue. which contains small lobes of fat cells known as lipocytes[13].

Stratum corneum is composed of 10-15 layers of keratinocytes. Keratinocytes synthesize keratin, a long thread like protein acts as major barrier and behaves like a rate limiting membrane for permeation of drug. The corneocyte are entirely surrounded by crystalline lamellar lipid region and are very densely cross linked by protein structure, which reduces absorption of drugs into the cells. The compounds with a molecular weight 500 Dalton cannot cross the skin[14].

ETHOSOMES:

Ethosomes are non-invasive, soft and malleable ethanolic vesicles, which is invented by Touitou and it was named as ethosomes, because of the presence of ethanol in the vesicular structure. Vesicular framework is most generally examined approach for transdermal medication conveyance nowadays [17]. Ethosomes are mainly composed of phospholipids, (phosphatidylcholine, phosphatidylserine, phosphatidic corrosive), plenty of ethanol and water. The high concentration of ethanol makes the ethosomes special, as ethanol is known for its Disturbance of skin lipid bilayer organization; therefore, when drug entrapped into a lipid vesicle membrane, it gives that vesicle the ability to penetrate the stratum corneum. Also,

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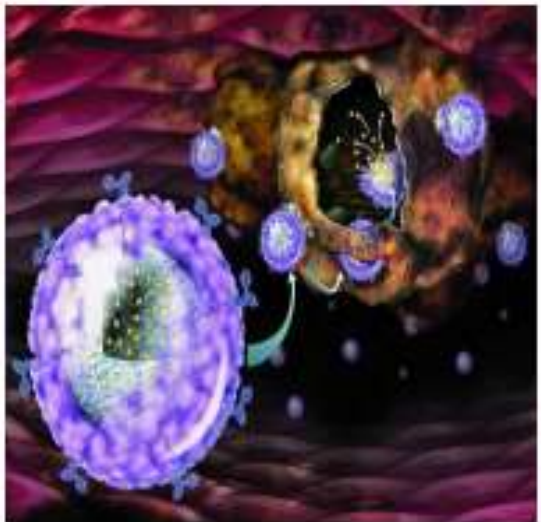
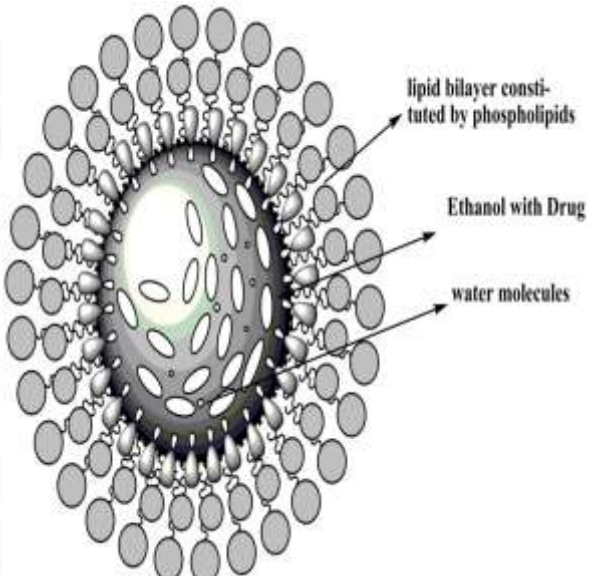
because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure and improves drug distribution ability in stratum corneum lipids [18], [19].





STRUCTURE OF ETHOSOMES

Composition of ethosomes[20], [21]:

The ethosomes are mainly compose of hydro alcoholic or hydro/alcoholic/glycolic phospholipid, high concentration of ethanol and water. Ethosomes may contain phospholipids with various chemical structure like phosphatidylcholine (PC), hydrogenated pc, phosphatidic acid (PA), alcohol (ethanol or isopropyl alcohol), and water. Drug delivery can be modulated by altering alcohol: water or alcohol-polyol: water ratio.

II. MATERIALS AND METHODS

Materials

Selegiline hydrochloride was gifted by embio-pharma Mumbai, Soya lecithin, Cholesterol, Chloroform, n-Hexane

Instruments

Shimadzu UV-vis spectrophotometry model, FTIR, Magnetic stirrer, Dissolution apparatus.

ANALYTICAL METHODS

UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF SELEGILINE HYDROCHLORIDE

A precise sensitive and accurate method for estimating selegiline hydrochloride was done using UV visible spectrophotometer Procedure for UV spectroscopic method

PREPARATION OF PHOSPHATE BUFFER pH 6.8

To prepare phosphate buffer ph-6.8, 28.80gm of disodium hydrogen phosphate and 11.45gm of potassium dihydrogen phosphate was dissolved in sufficient water to produce 1000ml.

DETERMINATION OF ΛMAX IN PHOSPHATE BUFFER (PH 6.8)

The absorption maxima (ג-max) for selegiline hydrochloride in buffer was determined 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 206nm shown in fig no 7.

PREPARATION OF STANDARD CALIBRATION CURVE OF SELEGILINE HYDROCHLORIDE IN PHOSPHATE BUFFER (PH 6.8)

PREPARATION OF STANDARD SOLUTION:100 mg of the drug was accurately weighed into 100 ml volumetric flask which was dissolved and made up to mark with buffer to get 1000 µg/ml solution and was used as a standard stock solution (SS-I)

Working standard- i:

from SS-I 10ml was pipetted out into a 100 ml volumetric flask and further diluted up to mark with buffer pH6.8 to get 100 µg/ml solution (WS-I) and was used to prepare further dilutions. From working solution (WS-I) 0.5, 1, 1.5, 2, and, 2.5, were pipetted out into 10 ml volumetric flask and were diluted up to the mark with buffer to get concentration range 5 to 25 µg/ml. These solutions were scanned and the absorbance was measured at

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206nm against the blank (buffer). The absorbance values thus obtained were plotted against the respective concentration to obtain the standard calibration graph. The procedure was repeated three times and average values of the absorbance was calculated. The data obtained were statistically evaluated to obtain the standard deviation of the said values and regression coefficients were calculated. The results are shown in the table no: 8 and graph represented in the fig no: 12

THE LINEAR REGRESSION ANALYSIS:
The linear regression analysis was done on the absorbance points. The results obtained are as follows:
Standard calibration curve in phosphate buffer 6.8pH
The slope=0.0431
The intercept=0.0009
The regression coefficient=0.999
A straight line equation (y= mx+c) was generated to facilitate the calculation pf amount of drug. The equation is as follows
Absorbance =0.0431 × concentration + 0.0009

DRUG-EXCIPIENTS COMPATIBILITY STUDIES
FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR)

In order to check the integrity (Compatibility) of drug in the formulation, FT-IR spectra of the formulations along with the drug and other excipients were obtained and compared with that of pure drug and excipients using Shimadzu FT-IR 8400 spectrophotometer. In the present study, Potassium bromide (KBr) pellet method was employed. The samples were thoroughly blended with dry powdered potassium bromide crystals. The mixture was compressed to form a disc. The disc was placed in the spectrophotometer and the spectrum was recorded. The FT-IR spectra of the formulations were compared with the FT-IR spectrum of the pure drug and polymers.

EXPERIMENTALDESIGN

Central composite design was used 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 and ethanol concentration. The factors and levels are mentioned in the table no: 3.The lower and higher levels of independent factors were selected based on the reported literature and initial screening experiments conducted.

SL. NO	INDEPENDENT VARIABLES	UNITS	LOW LEVEL	HIGH LEVEL
1	Soya lecithin	g	2	4
2	Ethanol	ml	30	50

SELECTED INDEPENDENT VARIABLES FOR OPTIMIZATION.

METHOD OF PREPARATION OF NANO ETHOSOMES CONTAINING SELEGILINE HYDROCHLORIDE

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The selegiline hydrochloride loaded ethosomal formulations were prepared classical cold method introduced by Touitou et al., with slight modifications.

Std	Run	X_1:soya lecithin Conc in (%w/v)	X_2:Ethanol (%v/v)
4	1	2	40
9	2	4	50
8	3	3	50
6	4	4	40
7	5	2	50
3	6	4	30
1	7	2	30
2	8	3	30
5	9	3	40

Table.4: Formulation design for preparation of selegiline hydrochloride ethosomes

The ethosomal system of selegiline hydrochloride composed of 1-4 % phospholipids, 20-50 % ethanol, 6mg of selegiline, 5mg of cholesterol and aqueous phase to 100 % w/w. Phospholipid, cholesterol drug were dissolved in ethanol by vigorous stirring followed by heating at 30°C in water bath. Then water is heated at 30°C in a separate vessel, both mixtures are mix together following 5 min stirring in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desire extend using sonication (bath sonication) and homogenization method.

EVALUATION OF ETHOSOMES

PARTICLE SIZE ANALYSIS

The particle size of ethosomes were measured by using Malvern zeta sizer nano ZS-90. Before analysis the ethosomes dispersion was diluted with HPLC grade water followed by sonication for 30 minutes. The average particle size was determined form the particle size distribution data.

SCANNING ELECTRON MICROSCOPY (SEM)

SEM micrographs were taken for the prepared nano particles using scanning electron microscopy (Carl zeisus FESEM model number: Ultra 55USA) at different required magnification at room temperature. The micrographs were analyzed for micro graphical characteristics.

DRUG ENTRAPMENT EFFICIENCY

The prepared ethosomal dispersion was centrifuged at 6000 rpm for 30 min at 4^0 C using REMI cooling centrifuge. The supernatant is analyzed for the free drug content. The entrapment efficiency (%) of the drug was calculated by using the following equation

$$\%\text{entrapment efficiency} = \frac{\text{total drug load} - \text{free drug content}}{\text{total drug load}} \times 100$$

IN VITRO DIFFUSION STUDY FOR SELEGILINE HYDROCHLORIDE LOADED ETHOSOMES

The in-vitro drug release profile of the selegiline hydrochloride loaded ethosomes were studied by using Franz diffusion cell. The cellophane membrane 60 (pore size= 2.4nm) was soaked overnight in the p^H 6.8 phosphate buffer. Then calculated amount of the product was kept in the donor compartment above the dialysis membrane 250 ml of the phosphate buffer of pH 6.8 was taken in 250 ml beaker. The beaker was placed over the magnetic stirrer, the temperature and rpm were maintained at 34 ± 0.5^0 C and 100 rpm throughout the study. The sample 2 ml was withdrawn at predetermined interval of time (1, 2, 3, 4, 5, 6, 7 and 8 hr) and replaced with equal amount of fresh buffer. After the suitable dilution

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the sample was analyzed for the drug concentration by UV spectrophotometer at 206 nm.

PREPARATION OF ETHOSOMAL HYDROGEL

About 1g of the Carbopol 940 P was weighed and transferred slowly into the 100ml distilled water taken in the beaker .this solution was stirred at 200 rpm for 3 hrs under magnetic stirring. To this10g of base gel calculated amount of ethosomal preparation was added and stirred at 100rpm for 2 h followed by the addition of methyl paraben and propyl paraben. Triethanolamine about 1-3 drops was added to get the proper gel consistency as mentioned in the table below

Si no	Ingredients	Quantity taken
1	Carbopol 940P	1g
2	Methyl paraben	0.2g
3	Propyl paraben	0.3g
4	Optimized selegiline loaded ethosomes	10 ml
5	triethanolamine	q.s
6	Distilled water	q.s

FORMULATION TABLE FOR PREPARATION OF ETHOSOMAL GEL.

EVALUATION OF ETHOSOMAL HYDROGEL

SPREADABILITY

The spreadability of the formulations was determined by using the horizontal plate method. A standard weight 5g was tied to the upper glass plate and was about 1g of selegiline hydrochloride hydrogel was placed between two horizontal plates. The whole set was held in a vertical position the time required for the plate to slide off from the other plate was noted and spreadibility was calculated from the formula

Spreadability =x $= \frac{M \times L}{T}$

M= weight tied to upper slide (g)

L=length of glass slide (cm)

T=time taken (sec)

P^H DETERMINATION

The p^H of the gel was measured using the p^H meter. Before the analysis of the p^H meter was calibrated using p^H 4, 7 and 9.2 standard solutions. After the calibration the glass electrode was immersed in the gel (1g) and the p^H was noted.

VISCOSITY

Viscosity of the gel was determined by using the Brookfield viscometer. The temperature was maintained at 25^0 C. Helipath T bar spindle no 96F was fixed to viscometer and immersed in the beaker containing 10g of the selegiline hydrochloride loaded ethosomal hydrogel. The viscometer was operated at the different rpm and reading was noted in centipoise (cps)

DRUG CONTENT ESTIMATION

Accurately weighed 1g of the gel was transferred to the 100ml of volumetric flask containing 20 ml of phosphate buffer pH 6.8. The volumetric flask was shaken for 30min and volume was made up to 100ml using the phosphate buffer. After the suitable dilution the sample was analysed using the UV Visible spectrophotometer at 206nm.

$$\text{drug content} = \frac{\text{absorbance} \times \text{dilution factor}}{\text{slope} + \text{intercept}}$$

INVITRO DRUG RELEASE FOR SELEGILINE HYDROCHLORIDE HYDROGEL

The in-vitro drug release profile of drug loaded hydrogel were studied using the Franz diffusion cell. About 0.1g of gel was kept in the dialysis membrane was mounted over the donor compartment and fixed on it. The dialysis membrane was soaked overnight in p^H 6.8 phosphate buffer. The receptor compartment is filled with buffer. The beaker was placed on the magnetic stirrer, the temperature and rpm were maintained at 37+/- 0.5^0 C and 100rpm throughout the study. The sample 2ml was withdrawn at predetermined interval of time (1, 2, 3, 4, 5, 6, 7, 8, and12h) respectively and replaced with equal volume of fresh buffer. After the suitable dilution the sample was analyzed for drug concentration by using UV visible spectrophotometer at 206 nm.

KINETIC MODELLING OF DRUG RELEASE PROFILES

To analyse the drug release rate kinetics and mechanism of the drug release from ethosomal gel and in vitro diffusion studies data was fitted into zero order, first order, higuchi and korsmeyerpappas model. Kinetic studies involve

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comparing the regression R^2 values and n values (slope) in case of first order kinetic model obtained to select the best fit model.

STABILITY STUDIES

In any rational design and evaluation of dosage form for drug, the stability of the active compound must be a major criterion in determining their acceptance or rejection. Stability of a drug can be defining as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications. Formulation was selected and kept for stability studies.it was packed in an aluminium foil and sealed tightly and studies were carried out for 30 days by keeping at

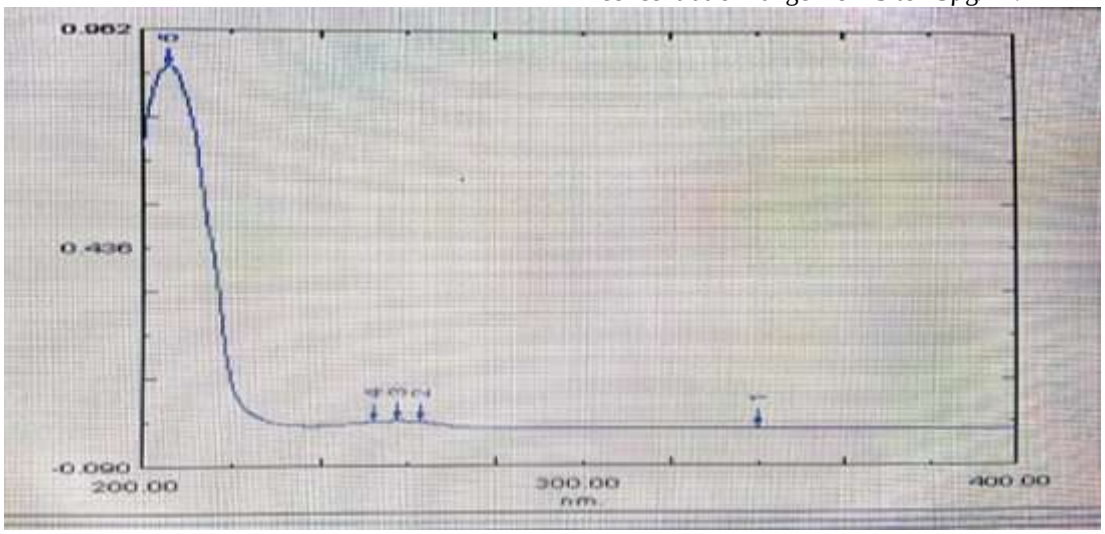
- 15.0± 2^0 C and 45.0 ± 5.0 %RH
- 30.0± 2^0 C and 65.0 ± 5.0 %RH
- 40.0± 2^0 C and 75.0 ± 5.0 %RH

Sample was withdrawn on 0^{th}, $15^{th,}$ 30^{th} day and was analyzed for drug content, entrapment efficiency.

III. RESULTS:

STANDARD CURVE OF SELEGILINE HYDROCHLORIDE IN PHOSPHATE BUFFER pH6.8:

Calibration curve of selegiline hydrochloride in Phosphate buffer pH 6.8 at 206 nm is shown in table 16. Figure 18 shows the standard curve with a regression value of 0.999 and slopeof 0.0431 in Phosphate buffer pH 6.8. Beer's range was obeyed in the concentration of 5 to 25 µg/ml. The curve was found to be linear in the concentration range from 5 to 25µg/ml.



UV SPECTRUM OF SELEGILINE HYDROCHLORIDE IN BUFFER PH 6.8

Sl. No	Volume of SS-2 (ml)	Volume made up to (ml)	Conc (µg/ml)	Absorbance			concurrentAbsorbance
				Ttrial 1	Trial 2	TTrial 3	
1	0	10ml	0	0	0	0	0
2	0.5		5	0.219	0.220	0.211	0.213
3	1		10	0.448	0.427	0.450	0.451
4	1.5		15	0.639	0.608	0.621	0.634
5	2		20	0.850	0.838	0.852	0.849
6	2.5		25	1.007	1.002	1.009	1.004

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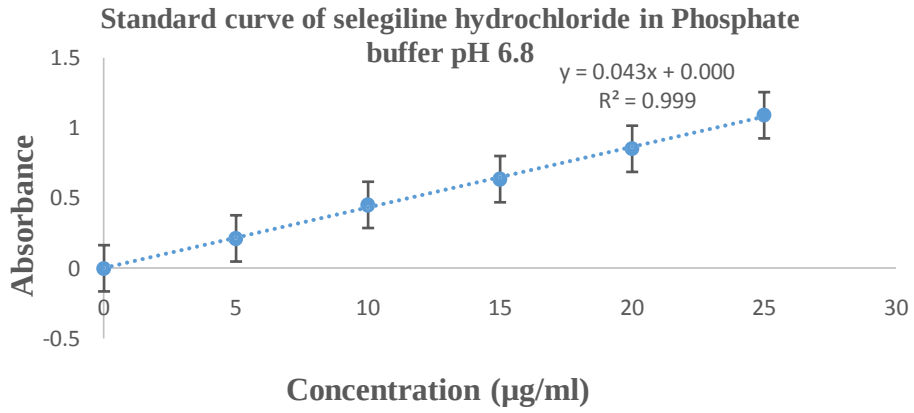
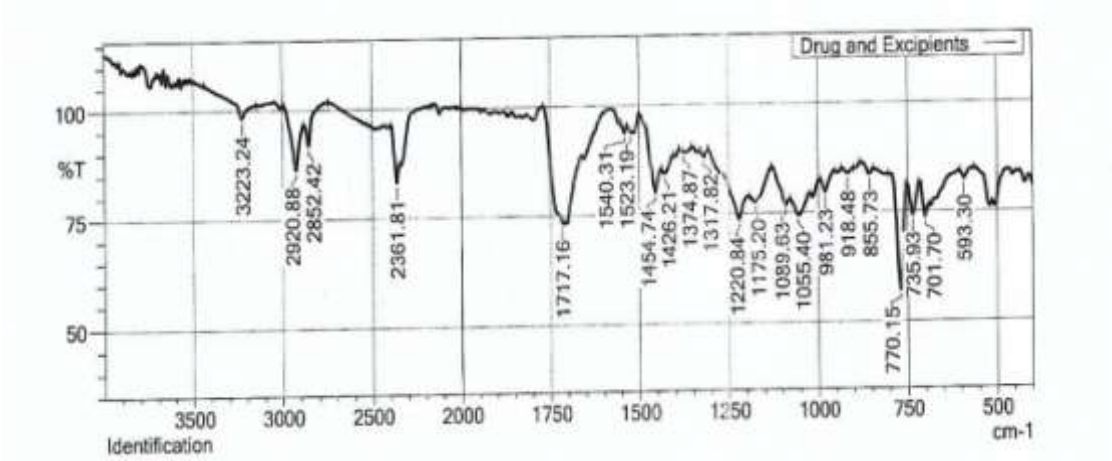
SPECTROMETRIC DATA FOR THE ESTIMATION OF SELEGILINE HYDROCHLORIDE IN 6.8PH BUFFER



STANDARD CALIBRATION CURVE OF SELEGILINE HYDROCHLORIDE IN BUFFER PH 6.8
Regression analysis:
Slope: 0.0431
Intercept: 0.0009
Regression coefficient: 0.999
COMPATIBILITY STUDIES
FTIR SPECTROSCOPIC STUDIES:



FT-IR SPECTRA OF DRUG SELEGILINE HYDROCHLORIDE + EXCIPIENTS

Functional groups	Wave number (cm^{-1})				
	Selegiline	Mixture	SL+lecithin	SL+cholestrol	SL+carbopol
N–H	1640.21	1635.74	1641.43	1632.01	1625.47
C–H and O–H	2869.4	2853.3	2861.90	2854.63	2852.88
C–H and O–H	1625.72	1625.77	1632.14	1622.3	1660.11

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C=N and C–N	3200.04	3223.24	3213.6	3222.18	3250.01

FUNCTIONAL GROUPS PRESENT IN INFRA-RED SPECTRUM OF DRUG AND WITH EXCIPIENTS

PREPARATION OF SELEGILINE LOADED ETHOSOMES

Run	Drug in (mg)	soya lecithin Conc in (%w/v)	Ethanol (%v/v)	Cholesterol in(mg)	Water in (ml)
1	6mg	2	40	5mg	q.s
2		4	50		
3		3	50		
4		4	40		
5		2	50		
6		4	30		
7		2	30		
8		3	30		
9		3	40		

FORMULATION OF SELEGILINE HYDROCHLORIDE LOADED ETHOSOMES
CHARACTERIZATION

Formulations	Particle size (µm)	Entrapment Efficiency (%)
1	159±12	65±0.09
2	249±10	94±0.24
3	386±02	71±0.9
4	175±08	79±0.67
5	226±17	71±0.54
6	255±09	82±0.81
7	409±04	95±0.46
8	164±18	92±0.74
9	454±03	86±0.34

PARTICLE SIZE, AND ENTRAPMENT EFFICIENCY OF SELEGILINE HYDROCHLORIDE LOADED ETHOSOMES.

SCANNING ELECTRON MICROSCOPY

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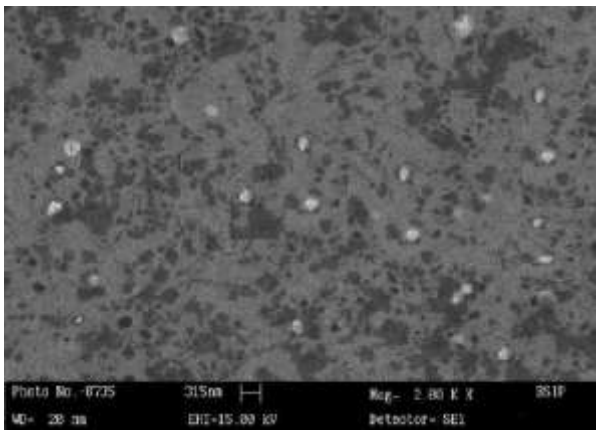

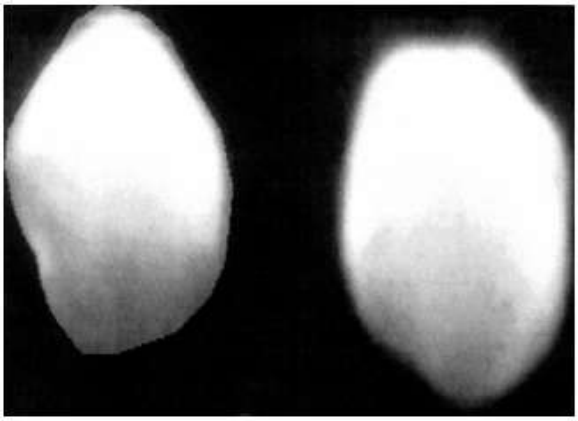

SEM IMAGE OF OPTIMIZEDFORMULATION

OPTIMIZATION:

SEQUENTIAL MODEL SUM OF SQUARES [Type I]

Response 1: particle size

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	6.817E+05	1	6.817E+05			
Linear vs Mean	96128.33	2	48064.17	52.98	0.0002	
2FI vs Linear	676.00	1	676.00	0.7090	0.4382	
Quadratic vs 2FI	4582.78	2	2291.39	37.27	0.0076	Suggested
Cubic vs Quadratic	77.67	2	38.83	0.3637	0.7609	Aliased
Residual	106.78	1	106.78			
Total	7.833E+05	9	87033.00			

OPTIMIZATION RESPONSE OF PARTICLE SIZE

ANOVA FOR QUADRATIC MODEL

Response 1: particle size

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.014E+05	5	20277.42	329.81	0.0003	significant
A-lecithin	94000.17	1	94000.17	1528.92	< 0.0001	
B-ethanol	2128.17	1	2128.17	34.61	0.0098	
AB	676.00	1	676.00	11.00	0.0452	
A^2	4576.06	1	4576.06	74.43	0.0033	
B^2	6.72	1	6.72	0.1093	0.7626	
Residual	184.44	3	61.48			
Cor Total	1.016E+05	8				

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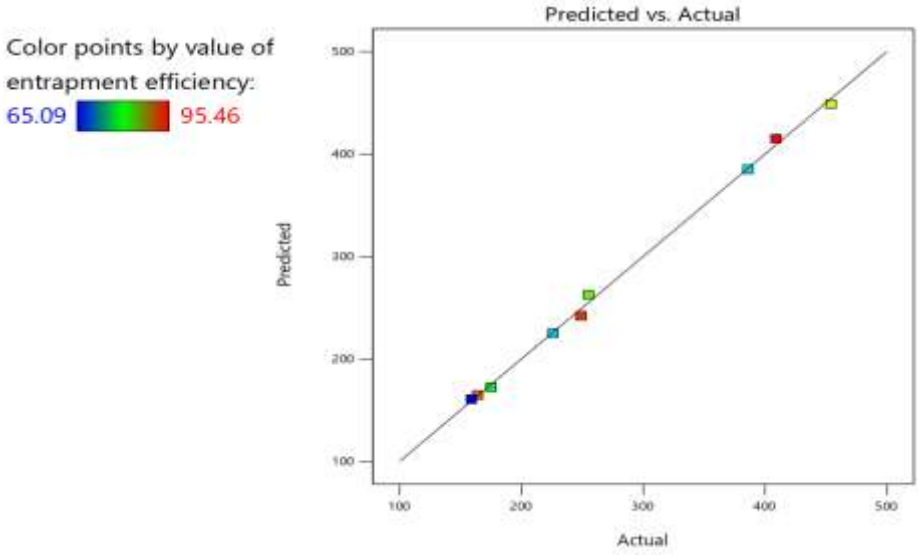
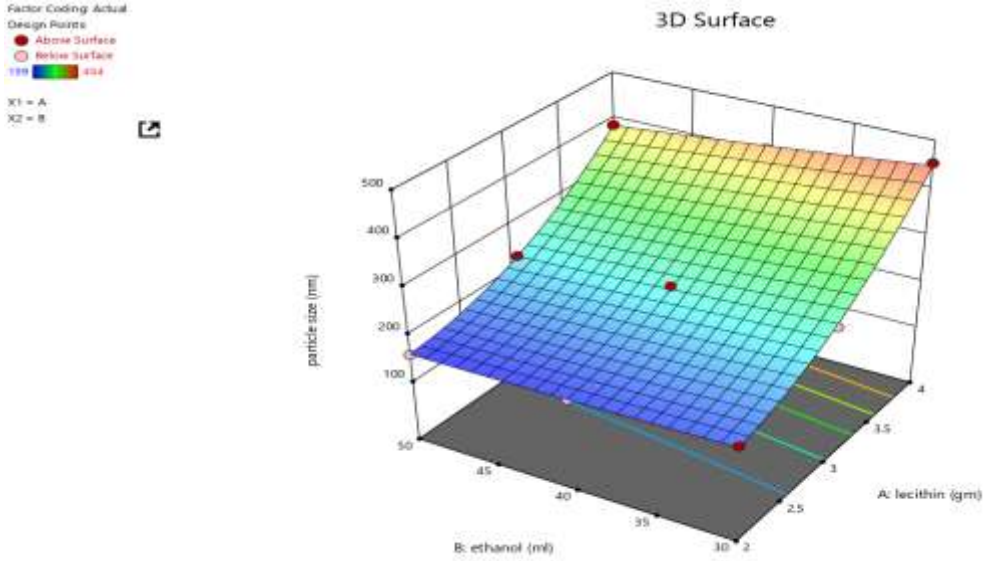
ANOVA QUADRATIC MODEL FOR RESPONSE PARTICLE SIZE



PREDICTED V/S ACTUAL CORRELATION OF PARTICLE SIZE



3D RESPONSE GRAPH OF ETHANOL AND LECITHIN AGAINST PARTICLE SIZE

FIT SUMMARY

Response 2: entrapment efficiency

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.3081		0.0994	-0.4286	
2FI	0.9954		-0.0807	-1.7350	
Quadratic	0.0018		0.9735	0.8825	Suggested
Cubic	0.2762		0.9939	0.8619	Aliased

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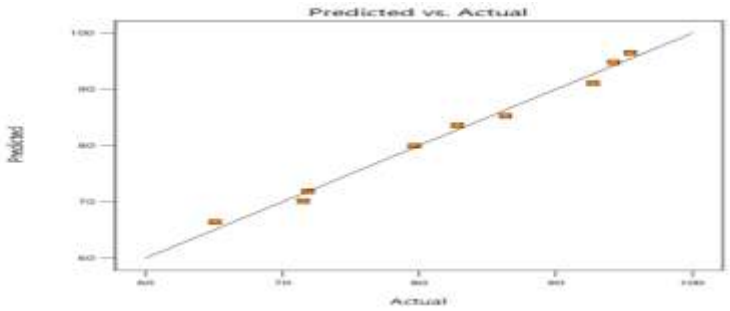
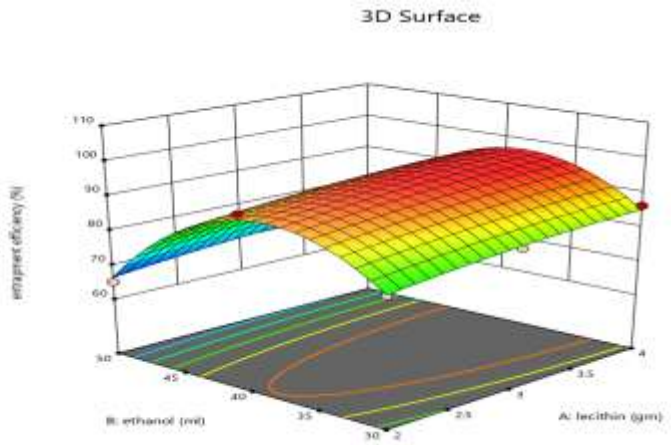
FACTORIAL MODEL FOR RESPONSE ENTRAPMENT EFFICIENCY
ANOVA FOR QUADRATIC MODEL
Response 2: entrapment efficiency
ANOVA QUADRATIC MODEL FOR RESPONSE ENTRAPMENT EFFICIENCY



Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	958.65	5	191.73	59.79	0.0033	significant
A-lecithin	43.74	1	43.74	13.64	0.0344	
B-ethanol	270.55	1	270.55	84.37	0.0027	
AB	0.0049	1	0.0049	0.0015	0.9713	
A^2	1.99	1	1.99	0.6196	0.4887	
B^2	642.37	1	642.37	200.33	0.0008	
Residual	9.62	3	3.21			
Cor Total	968.27	8				

PREDICTED V/S ACTUAL CORRELATION OF ENTRAPMENT EFFICIENCY



3D RESPONSE CURVE OF ETHANOL AND LECITHIN AGAINST ENTRAPMENT EFFICIENCY
ACTUAL AND PREDICTED VALUES:

Variables	Predicted	Actual

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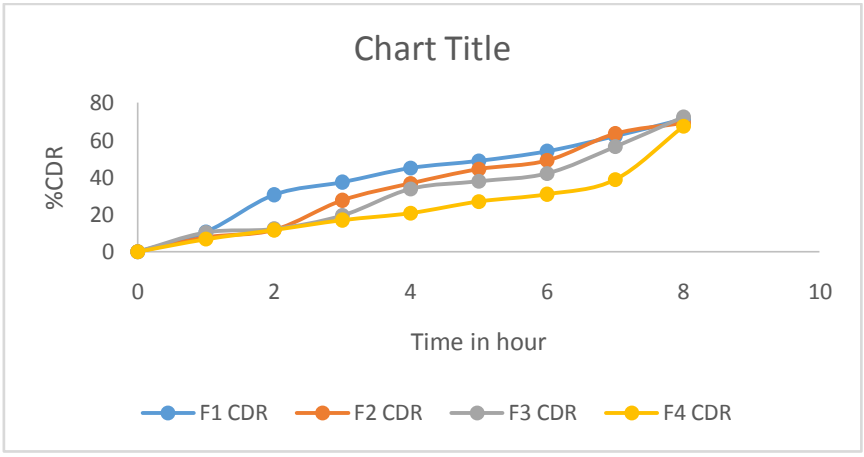
Particle size (nm)	171.345	167.14
Entrapment efficiency (%)	83.136	82.02

ACTUAL AND PREDICTED VALUES
INVITRO DRUG RELEASE OF ETHOSOMES

TIME (hr)	Percentage cumulative drug release (%) mean ± SD			
	F1	F2	F3	F4
1	10.66±0.18	7.73±0.33	10.33±0.22	6.66±0.62
2	21.53±0.14	11.72±0.67	12.18±0.18	11.66±0.19
3	37.28±0.21	27.62±0.14	19.43±0.33	16.92±0.92
4	44.86±0.11	36.68±0.28	33.68±0.16	20.70±0.12
5	48.68±0.23	44.34±0.19	37.76±0.9	36.41±0.15
6	53.82±0.35	48.94±0.43	41.97±0.64	45.92±0.83
7	61.94±0.17	63.33±0.83	56.30±0.30	52.33±0.37
8	71.03±0.25	69.09±0.21	62.26±0.18	59.28±0.26

%CDR FOR F1-F4 ETHOSOMAL FORMULATION.



%CDR GRAPH FOR F1-F4

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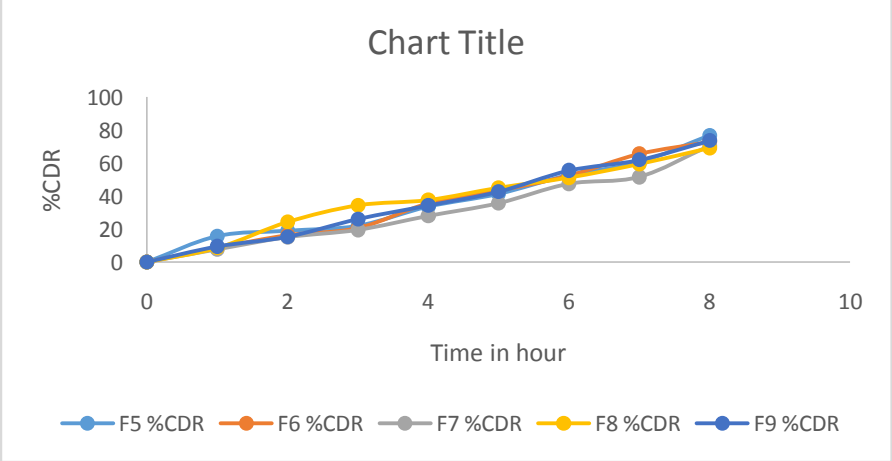
TIME (Hour)	Percentage cumulative drug release (%) mean± SD				
	F5	F6	F7	F8	F9
1	15.66±0.62	8.58±0.26	7.67±0.54	8.58±0.12	9.4±0.28
2	18.95±0.14	16.26±0.33	15.05±0.42	24.17±0.36	15.32±0.63
3	21.94±0.27	20.63±0.71	27.86±70	34.27±0.47	26.04±0.27
4	33.53±0.75	35.57±0.41	35.57±53	44.87±0.37	34.28±0.49
5	41.24±0.58	43.33±0.47	47.33±0.17	51.04±0.76	42.66±0.55
6	52.66±0.33	52.43±0.34	51.45±0.55	59.26±0.21	55.46±0.26
7	60.33±0.33	59.57±0.07	51.45±0.66	61.98±0.86	59.27±0.16
8	68.66±0.67	61.87±0.83	67.24±0.27	68.73±0.18	64.29±0.71

%CDR FOR F5-F9



%CDR GRAPH FOR F5-F9

OPTIMIZED FORMULA

Number	lecithin	ethanol	particle size	StdErr(particle size)	entrapment efficiency	StdErr(entrapment efficiency)	Desirability	
1	2.000	43.184	171.345	6.386	83.136	1.458	0.613	Selected

SELECTED OPTIMIZED FORMULA

IN-VITRO DRUG RELEASE STUDIES:

sl no	time	abs	con	c/5ml	c/100ml	L0SS	CLA	CDR	%CDR
1	1	0.0928	4.64	0.0232	0.464	0	0	0.464	7.733333
2	2	0.136	6.8	0.034	0.68	0.0232	0.0232	0.7032	11.72
3	3	0.32	16	0.08	1.6	0.034	0.0572	1.6572	27.62
4	4	0.4128	20.64	0.1032	2.064	0.08	0.1372	2.2012	36.68667
5	5	0.484	24.2	0.121	2.42	0.1032	0.2404	2.6604	44.34
6	6	0.515	25.75	0.12875	2.575	0.121	0.3614	2.9364	48.94
7	7	0.662	33.1	0.1655	3.31	0.12875	0.49015	3.80015	63.33583
8	8	0.674	33.7	0.1685	3.37	0.1655	0.65565	4.02565	67.09417

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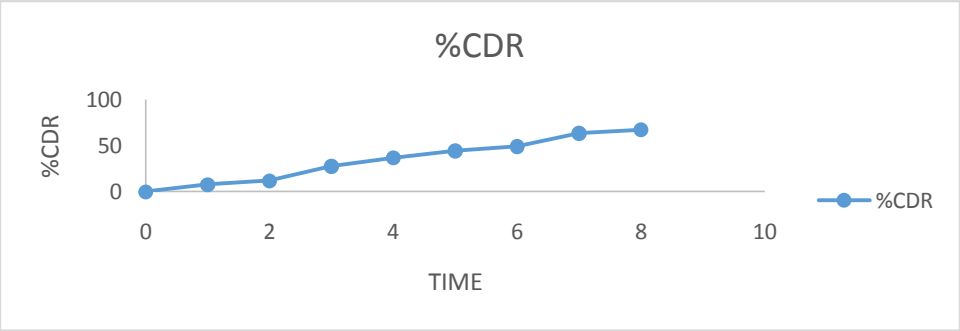
% CDR OF OPTIMIZED ETHOSOMAL FORMULATION



% CDR FOR OPTIMIZED ETHOSOMAL FORMULATION

EVALUATED PARAMETERS FOR THE PREPARED GEL CONTAINING SELEGILINE HYDROCHLORIDE ETHOSOMES:

Parameters	Results
Viscosity	721.29 ± 0.99 cps
pH	6
Drug content	99.11%

EVALUATED PARAMETERS OF GEL

sl no	time	abs	con	c/5ml	c/100ml	L0SS	CLA	CDR	%CDR
1	1	0.0825	4.125	0.020625	0.4125	0	0	0.4125	6.875
2	2	0.126	6.3	0.0315	0.63	0.020625	0.020625	0.650625	10.84375
3	3	0.271	13.55	0.06775	1.355	0.0315	0.052125	1.407125	23.45208
4	4	0.395	19.75	0.09875	1.975	0.06775	0.119875	2.094875	34.91458
5	5	0.407	20.35	0.10175	2.035	0.09875	0.218625	2.253625	37.56042
6	6	0.483	24.15	0.12075	2.415	0.10175	0.320375	2.735375	45.58958
7	7	0.518	25.9	0.1295	2.59	0.12075	0.441125	3.031125	50.51875
8	8	0.548	27.4	0.137	2.74	0.1295	0.570625	3.310625	55.17708

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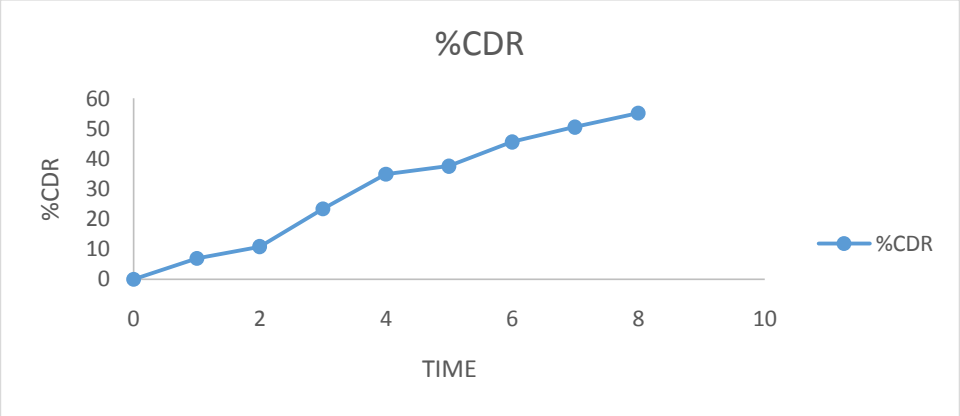
% CDR OF OPTIMIZED ETHOSOMAL HYDROGEL FORMULATION



% CDR OF OPTIMIZED ETHOSOMAL GEL

DRUG KINETIC STUDY:

Time (Hr)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	log Cumu % drug released	% Drug released	Cube Root of % drug Remaining(Wt)	Wo-Wt
0	0	100	0.000	2.000	0.000	0.000	100	4.642	0.000
1	6.88	93.125	1.000	1.969	0.000	0.837	6.875	4.533	0.109
2	10.84	89.1563	1.414	1.950	0.301	1.035	3.9687	4.467	0.175
3	23.45	76.54792	1.732	1.884	0.477	1.370	12.60838	4.246	0.396
4	34.91	65.0855	2.000	1.813	0.602	1.543	11.46242	4.022	0.620
5	37.56	62.43958	2.236	1.795	0.699	1.575	2.64592	3.967	0.675
6	45.59	54.4125	2.449	1.736	0.778	1.659	8.02708	3.789	0.853
7	50.52	49.4823	2.646	1.694	0.845	1.703	4.9302	3.671	0.971
8	55.18	44.823	2.828	1.652	0.903	1.742	4.6593	3.552	1.090

DRUG KINETIC MODEL

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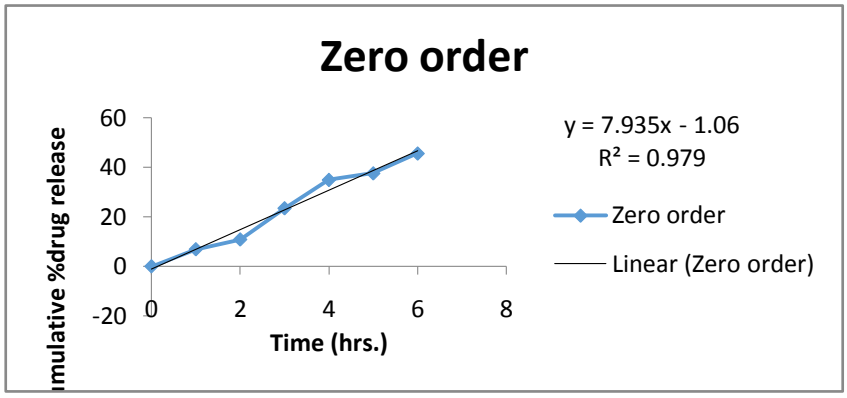
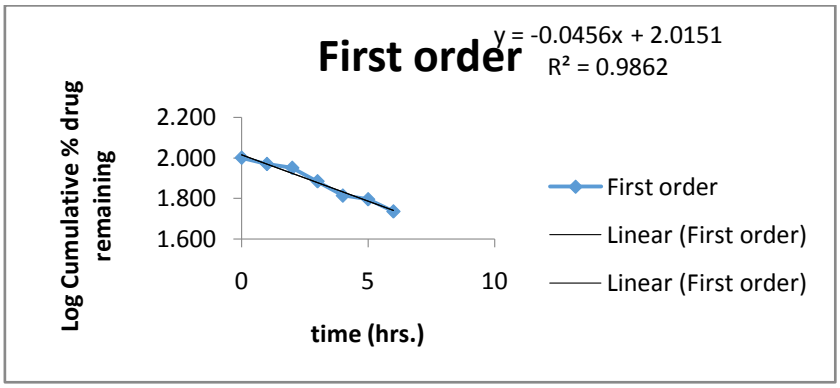
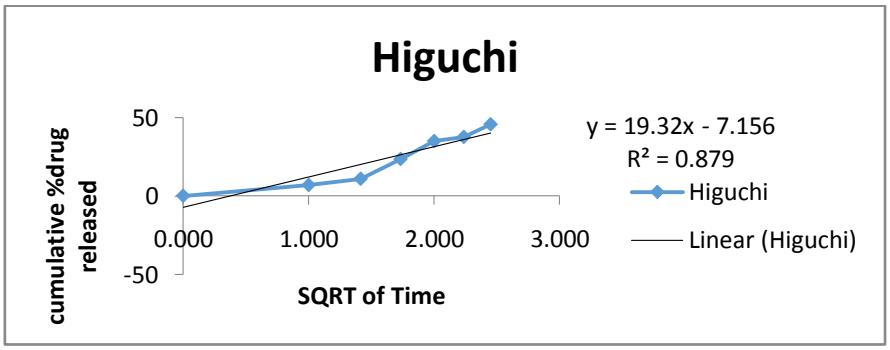


ZERO ORDER MODEL



FIRST ORDER MODEL



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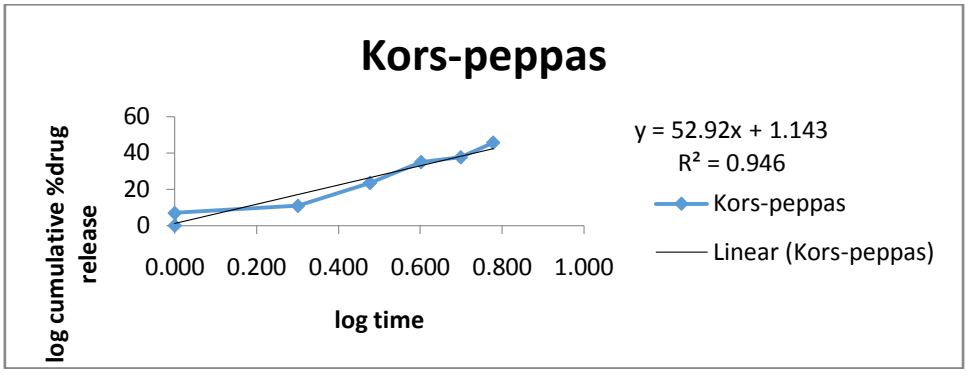
HIGUCHI MODEL



KORS- PEPPAS MODEL

	Zero order	Korsmeyer-peppas	Higuchi	First order
K0(slope)	7.935	52.9260	19.325	-0.0456
R^2	0.9793	0.9465	0.8791	0.9682
	7.935		K	0.313

DATA FOR IN-VITRO RELEASE KINETIC MODELS.

STABILITY STUDIES

Storage condition $40^0C±2^0C/75\%RH±5\%RH$			
Sample type	Sampling interval	% drug content	Entrapment efficiency
Ethosomal formulation(opt)	0 day	93.33	76±3.4
	30 day	90.05	75±2.9

IV. DISCUSSION

UV SPECTROPHOTOMETRIC METHOD:

UV spectrophotometric studies showed that the λmax for selegiline hydrochloride was found to be 206 nm in phosphate buffer ph 6.8.

The standard calibration curve of selegiline hydrochloride in buffer pH6.8 solution was performed.

Slope: 0.0431, Intercept: 0.0009, Regression coefficient: 0.999 with linearity of 5-25 µg/ml was obtained in methanol. Method is simple, robust and suitable for estimation of drugs and its dosage forms.

COMPATIBILITY STUDIES:

FT-IR SPECTROSCOPIC STUDIES:

The FT-IR spectra of pure selegiline hydrochloride, soya lecithin, cholesterol and carbopol were taken and compared with each other. Selegiline hydrochloride ethosomes gave the same principle peaks as that of pure selegiline hydrochloride and some of the peaks of soya lecithin, cholestrol and carbopol. Thus, the polymers used in the formulation depicted no major changes in the standard peaks of drug which demonstrated the compatibility of excipients with pure selegiline hydrochloride.

CHARACTERIZATION OF SELEGILINE HYDROCHLORIDE ETHOSOMES

OPTIMIZATION:

PARTICLE SIZE:

The particle size was increased with increase concentration of lecithin (observed for 2-5 w/v), which was explained by polynomial equation.

Particle size=+242.11+125.17lecithin-18.83ethanol-13.00lecithin*ethanol+47.83lecithin^2 + 1.83ethanol^2

The response was analyzed by a factorial model which showed F values of 329.81 and P value of 0.0003 (table 13) indicating a non-significant response. The actual and predicted values of the particle size were correlated.

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Average particle size of ethosomes were found in the range of 159.21nm. It showed that the particles were in 159.01-454.23nm nanometer range.

ENTRAPMENT EFFICIENCY:

Entrapment efficiency was found to increase with increase in lecithin concentration and further it was explained by the following polynomial equation

Entrapment efficiency = +94.81+2.70lecithin - 6.72ethanol+ 0.0350lecithin×ethanol-0.9967lecithin^2-17.92ethanol^2

Based on the optimized results the entrapment efficiency of selected formulation (94.81%) was found as the lecithin concentration shows positive effect (+2.701) on entrapment efficiency (increases with increase concentration) and ethanol shows negative effect on (decrease with increase in ethanol concertation).

The response was analyzed by a factorial model which showed F value of 59.79 and P value of 0.0033 (table 15) indicate the significant response. The actual and predicted values of entrapment efficiency was correlated.

IN VITRO DIFFUSION STUDIES:

In-vitro drug release studies were carried out for 8 h for optimized formulation. The cumulative percentage drug release after 8 hours was found. The percentage drug release was found to be 80%. The decrease in the percentage drug release was found with increase in the concentration of the lipid, this may be due to the high viscous and thickness of lipid layer.

REGRESSION ANALYSIS

The formulation were optimized using desirability method of numerical optimization. The optimum settings for dependent and independent variables were defined. The set criteria for all independent variables and dependent variables are shown in the table. All the response variables are subjected to the regression analysis to determine the regression coefficients and all the dependent variables were found to be significant except particle size and entrapment efficiency. The optimized formulation was prepared in accordance with the predicted model and studied for responses. The results clearly indicated that all the independent factors had an important role in the preparation of selegiline hydrochloride ethosomal gel. The actual and predicted values of optimized formulation were compared as shown in table and are in close agreement with eachother.

EVALUATION OF SELEGILINE HYDROCHLORIDE HYDROGEL

SPREADABILITY

The spreadbility of the ethosomal hydrogel formulation was found to be 0.346±0.07441 g.cm/ sec.

P^H STUDIES

P^H of ethosomal loaded with selegiline hydrochloride was found to be 6.8±1.2 respectively. This were found be near to skin p^H

VISCOSITY STUDIES

Viscosity of the selegiline hydrochloride gel is found to be 740.42 ± 0.81 cps at 50 rpm. The viscosity of ethosomal gel was high due to high concentration of lipids present.

IN VITRO DIFFUSION STUDY OF ETHOSOMAL GEL

The cumulative percentage drug release from the ethosomal hydrogel was found to be 55.177after 8 hour of diffusion study this may be because of the ethosomal gel containing the drug loaded lipid particle the penetration of the drug concentrates on the skin and remains localized for a longer period of time thus enabling drug targeting to the skin.

KINETIC MODELLING

Different models were adopted for fitting the drug release data in kinetic modelling and result for the optimized formulation showed that in in vitro drug release follows first order kinetics which defines the rate drug release is dependent on drug remaining in the dosage form.

STABILITY STUDIES

Ethosomal hydrogel of selegiline this studies reveled that gel of selegiline hydrochloride was stable in terms of spreadability, p^H, viscosity and cumulative percent drug release after storage for 1 months at 40±2^0 C 75±5% RH. There was no significant difference in drug content and entrapment efficiency of ethosomal formulation after 1 month of stability study.

V. CONCLUSION

In the present work, selegiline Hydrochloride ethosomes were prepared using soya lecithin, cholesterol and ethanol by cold method.

The following conclusions can be drawn from the study:

- The main aim of this preparation is to improve the permeability, bioavailability and sustainability of the drug by incorporate into the ethosome vesicle carrier.
- Cold method can be employed successfully to formulate ethosomes containing

selegilinehydrochloride.

- Identification of the absorption maxima of the selegiline by using 6.8 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 its shows drug and excipients were compatibility.
- SEM analysis of the selegiline hydrochloride revealed that the prepared ethosomes were porous, smooth and sphericalparticles.
- Using optimal polymer concentration led to increase in % yield and % entrapmentefficiency.
- Analysis of drug release mechanism showed that the drug release followed first order kinetic model.
- The in-vitro activity shows that the product efficiency was improved by formulated as ethosomal drugdelivery.
- Stability studies revealed there was no significant change in percentage drug content and % in-vitro drug release and found to be stable at the end of storage period.

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