Formulation And In Vitro Evaluation of Donepezil Ethosomal Drug Delivery System

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Abstract—**The present study aimed to design, prepare, and evaluate Donepezil-loaded ethosomal vesicles for improved transdermal drug delivery. Eight formulations (F1–F8) were prepared and characterised for particle size, zeta potential, and drug entrapment efficiency (EE). The particle size ranged from 72 to 98 nm, with negative zeta potentials of -20 to -25 mV, indicating stable nanosized vesicles. Drug entrapment efficiency varied between 76.98% and 85.10%, with formulations F6 and F4 showing the highest EE, attributed to optimised lipid-to-drug ratios and favourable vesicle size. SEM analysis confirmed spherical and uniform vesicle morphology. In vitro release studies demonstrated sustained drug release, and kinetic modeling indicated diffusion-controlled release. The optimized formulations remained stable under accelerated conditions for three months. Overall, ethosomal carriers were shown to be an effective and stable system for transdermal delivery of Donepezil, potentially improving bioavailability and patient compliance.**

Index Terms—**Donepezil, Ethosomes, FTIR Studies, In vitro drug release studies, Stability studies**

I. INTRODUCTION

Among various vesicular carriers, Ethosomes have emerged as a promising transdermal drug delivery system. Ethosomes are soft, malleable vesicles composed mainly of phospholipids, a high concentration of ethanol (20–45%), and water. The presence of ethanol imparts flexibility to the lipid bilayer and enhances skin permeability by fluidizing the stratum corneum lipids.[1] This unique composition allows ethosomes to penetrate deeply into the skin layers and facilitate systemic drug delivery, making them particularly suitable for drugs requiring controlled and sustained release.[2] Donepezil is a centrally acting, reversible acetylcholinesterase inhibitor widely prescribed for the symptomatic management of mild to moderate Alzheimer's disease.[3] By inhibiting acetylcholinesterase, donepezil increases acetylcholine concentration in the synaptic cleft, thereby improving cholinergic neurotransmission and temporarily enhancing cognitive function.[4] Despite its clinical effectiveness, conventional oral administration of donepezil is associated with certain limitations such as gastrointestinal side effects (nausea, vomiting), first-pass hepatic metabolism, fluctuating plasma drug levels, and reduced patient compliance, particularly in geriatric populations who often experience swallowing difficulties.[5]The present study focuses on the formulation and in vitro evaluation of donepezil-loaded ethosomal vesicles as a novel transdermal drug delivery system. The research aims to develop a stable, optimized ethosomal formulation and to characterize it with respect to vesicle size, zeta potential, entrapment efficiency, morphology, and in vitro drug release profile.

II. MATERIALS

Donepezil was procured from Hetero Labs, Hyderabad.Soya lecithin, Tween 80 and Cholesterol was obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

III. METHODOLOGY

A. Compatibility study (IR spectroscopy)

Fourier Transform Infrared (FTIR) spectroscopy was carried out to study the drug excipient compatibility in the Donepezil ethosomal formulation. A small quantity (about 1–2 mg) of pure Donepezil,

individual excipients, and their physical mixture was accurately weighed and mixed thoroughly with 100–200 mg of dry, IR-grade potassium bromide (KBr) to obtain a uniform powder. The mixture was then compressed using a hydraulic press to form a transparent KBr pellet. The prepared pellet was placed in the sample holder of the FTIR spectrophotometer, and the spectrum was recorded over a wavenumber range of 4000–400 cm^{-1} at room temperature after background correction using a blank KBr pellet. The FTIR spectra of the pure drug and physical mixture were compared to identify characteristic functional group peaks and to detect any possible shifts or disappearance of peaks, thereby assessing the compatibility of Donepezil with the selected excipients used in the ethosomal drug delivery system.[6]

B. Formulation development

Table-1: Composition of Donepezil Ethosomes (F1 to F4)

S. No.	Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8
1	Donepezil	5	5	5	5	5	5	5	5
2	Phosphatidyl choline	100	200	300	400	500	600	700	800
3	Ethanol	20	20	20	20	20	20	20	20
4	Purified Water	10	10	10	10	10	10	10	10

C. Procedure

Donepezil liposomes prepared by using thin film hydration technique. The required quantity of phospholipid (soya lecithin) was dissolved in ethanol under continuous stirring at room temperature. Donepezil hydrochloride was then added to the alcoholic phase and stirred until a clear solution was obtained. Purified water was heated separately to 30–40°C.The aqueous phase was slowly added to the alcoholic phase in a fine stream under constant stirring using a magnetic stirrer. Stirring was continued for 30–45 minutes, resulting in the formation of a milky ethosomal dispersion. The formed ethosomal dispersion was subjected to probe sonication or bath sonication for 5–10 minutes to reduce vesicle size and obtain a uniform distribution. The prepared ethosomal suspension was allowed to stand at room temperature for 30 minutes to ensure complete vesicle formation. The final Donepezil ethosomal formulation was transferred into a clean, amber-colored glass container and stored at 4°C until further evaluation.[7]

IV. CHARACTERIZATION[8,9,10,11,12]

1.Vesicle Size and Size Distribution

The average vesicle size and polydispersity index (PDI) of the ethosomal formulation were determined using a dynamic light scattering (DLS) particle size analyzer after suitable dilution with distilled water.

2. Zeta Potential

Zeta potential was measured using a zeta sizer to assess the surface charge and physical stability of the ethosomal vesicles.

3. Entrapment Efficiency (%)

Entrapment efficiency of Donepezil in ethosomes was determined by separating the unentrapped drug using centrifugation followed by estimation of drug content in the supernatant using a suitable analytical method.

4. Surface Morphology

The shape and surface morphology of ethosomal vesicles were examined using Scanning Electron Microscopy (SEM).

5. pH of Ethosomal Suspension

The pH of the ethosomal dispersion was measured using a digital pH meter to ensure suitability for transdermal application.

6. Drug Content

Drug content was estimated by lysing the ethosomal vesicles with a suitable solvent and analyzing the sample using UV–Visible spectrophotometry.

7. In-Vitro Drug Release Study

In-vitro drug release was carried out using a Franz diffusion cell with an appropriate dialysis membrane and receptor medium maintained at 37 ± 0.5°C.

8. Release Kinetics

The drug release data were fitted to different kinetic models such as zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to determine the release mechanism.

9. Stability Studies

Stability studies were conducted on the optimized ethosomal formulation under different storage conditions as per ICH guidelines, and changes in vesicle size, entrapment efficiency, and drug content were evaluated.

V. RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR):

The compatibility between the drug and the selected excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the extract and excipients mixture, which confirmed the absence of any chemical interaction between the extract and various excipients.

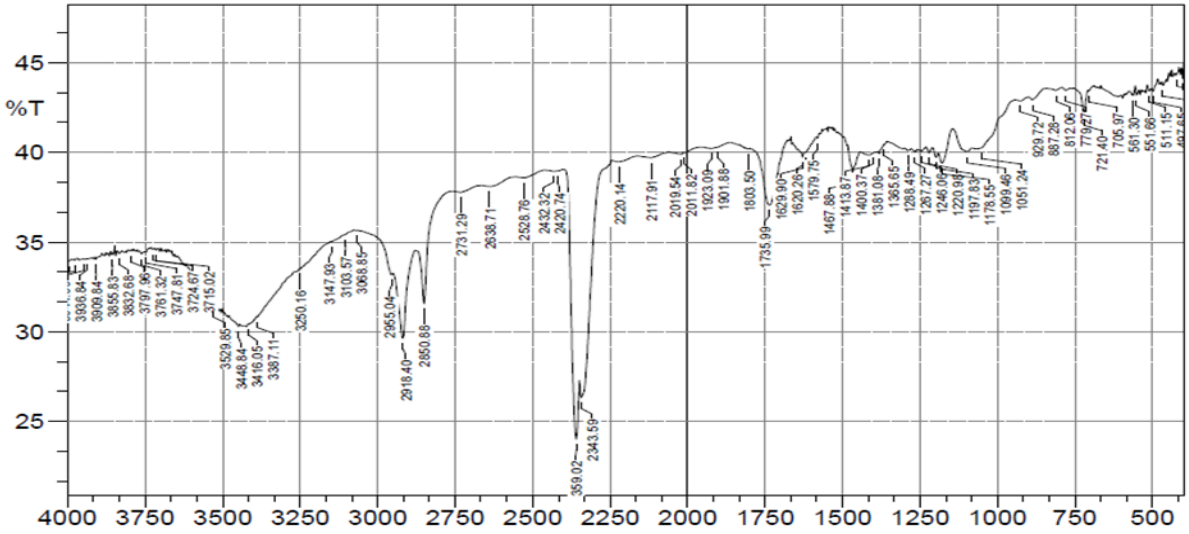

Fig-1: FTIR Studies of Donepezil

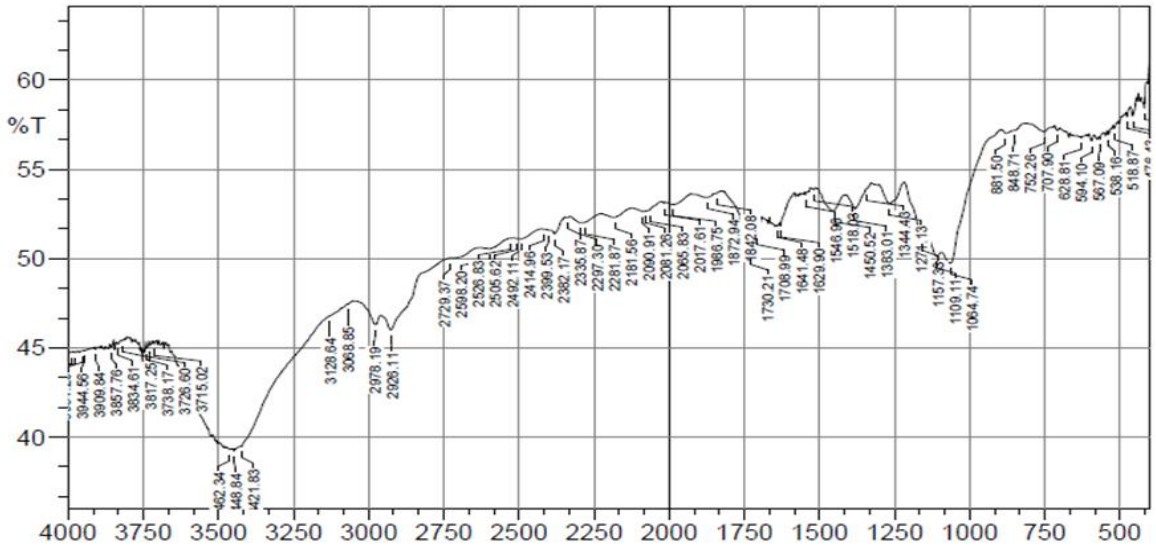

Fig-2: FTIR Spectra of physical mixture of drug and excipients

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of Puredrug and physical mixture of drug and excipients were studied. The characteristic absorption of peaks was obtained as above and as they were in official limits (±100 cm^{-1}) the drug is compatible with excipients.

Determination of Particle size

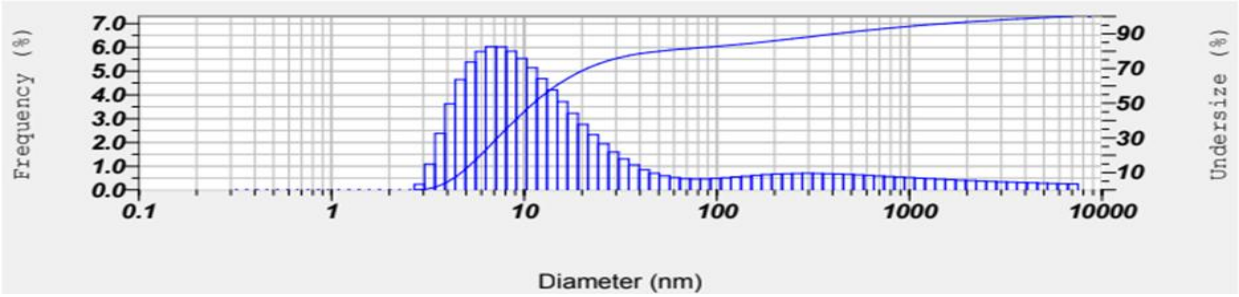

Fig-3: Particle size analysis of Optimized Ethosomes

Zeta potential

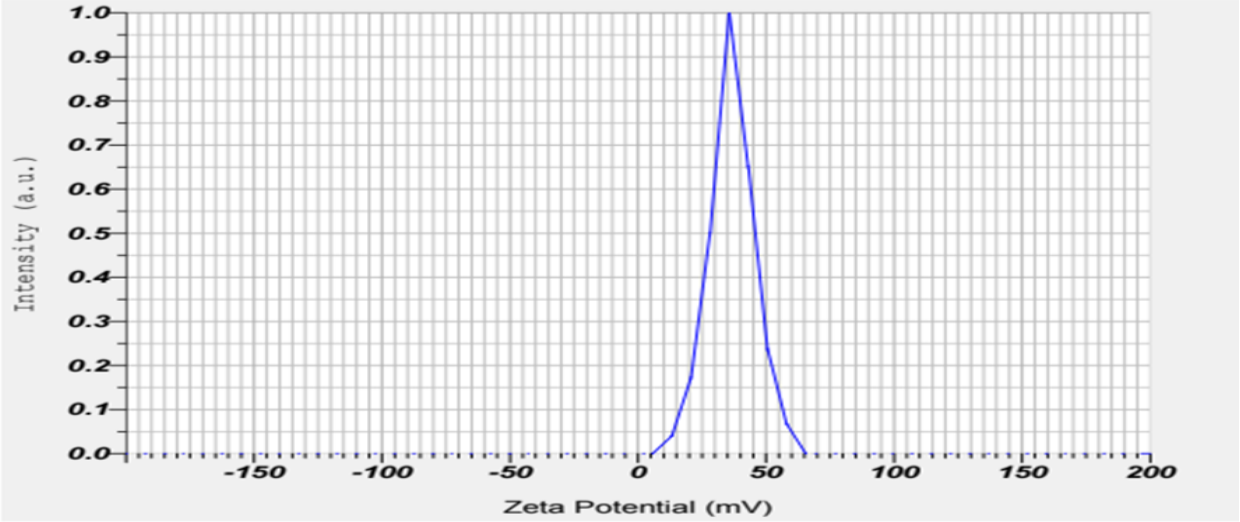

Fig-4: Zeta potential of Optimized formulation

Table-2: Evaluation Studies of particle size and Zeta potential Ethosomes

F. No	Particle size (nm)	Zeta potential
F1	76	-21
F2	90	-22
F3	86	-25
F5	97	-24
F6	72	-20
F7	83	-23
F8	98	-25

SEM Analysis

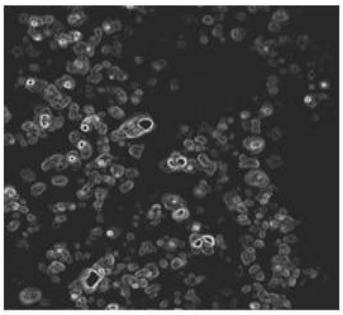

Fig-5: SEM Analysis of Ethosomes

- SEM images confirm the spherical morphology of ethosomes.
- Smooth surfaces suggest uniform vesicle formation without significant aggregation.
- The vesicle sizes observed under SEM are consistent with particle size measurements, confirming reliability of results.

Entrapment Efficiency:

Table-3: Drug entrapment efficiency

F. code	Drug entrapment efficiency
F1	76.98
F2	81.25
F3	79.80
F4	83.26
F5	77.58
F6	85.10
F7	82.20
F8	83.25

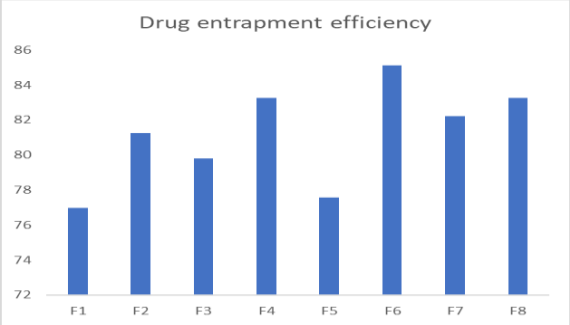

Fig-6: Drug entrapment efficiency of all formulation

- Drug entrapment efficiency (EE) ranged from 76.98% (F1) to 85.10% (F6).
- Higher EE indicates effective incorporation of Donepezil into the lipid bilayer of ethosomes.
- F6 and F4 (85.10% and 83.26%) showed the highest entrapment, possibly due to optimal lipid-to-drug ratio.
- Formulations with smaller particle size (like F6) also tend to have higher surface area, which can increase EE.

In vitro release study:

Phosphate buffer pH 7.4 was used as medium for the release studies and good linearity was observed in the plotted standard graph with a correlation coefficient of 0.998.

Table-4: In vitro drug release profiles of Donepezil transdermal patches (F1-F8)

Time	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	17.56	16.35	16.39	17.94	18.63	19.68	17.52	17.81
2	25.36	25.31	27.15	23.69	27.82	28.92	26.53	27.46
3	32.10	34.97	35.69	35.40	34.79	36.98	32.57	35.91
4	41.15	44.10	45.68	44.15	45.69	48.19	44.20	47.42
6	55.91	51.18	56.19	57.98	58.10	60.12	57.16	59.72
8	69.81	66.53	67.48	69.81	70.52	72.35	68.92	73.56
10	80.25	82.20	81.26	82.20	81.36	83.65	80.16	82.10
12	92.36	94.58	95.63	96.89	94.56	98.50	93.69	97.58

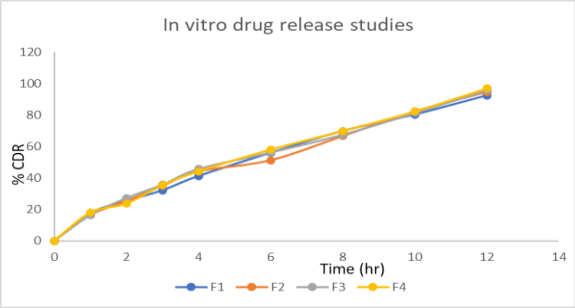

Fig-7: In vitro drug release studies of F1-F4 formulations

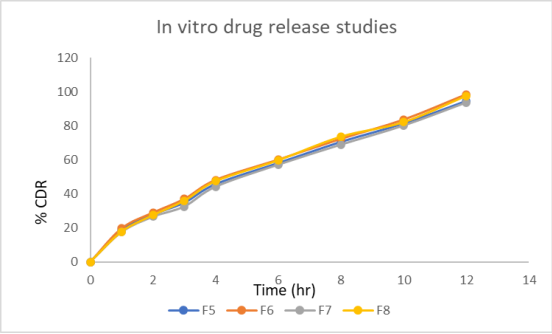

Fig-8: In vitro drug release studies of F5-F8 formulations

Kinetic modelling of drug release

All the 8 formulations of prepared ethosomes were subjected to in vitro release studies these studies were carried out using diffusion apparatus.

The dissolution medium consisted of 900 ml of Standard buffer pH 7.4 period of time.

The results obtaining in vitro release studies were plotted in different model of data treatment as follows:

1. Cumulative percent drug released vs. time (zero order rate kinetics)
2. Log cumulative percent drug retained vs. time (First Order rate Kinetics)

Cumulative percent drug released vs. square root of time (Higuchi's

Classical Diffusion Equation)

Log of cumulative % release Vs log time (Pappas Exponential Equation)

Zero order kinetics

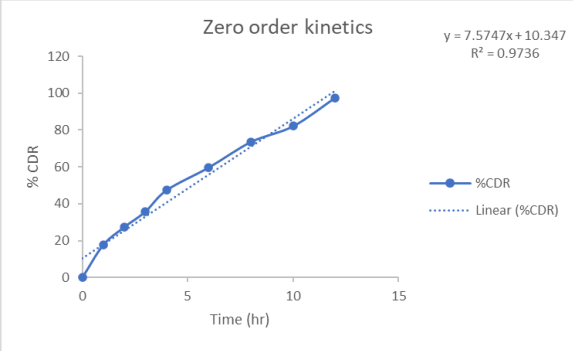

Fig-9: Zero order kinetics of optimized formulation

First order kinetics

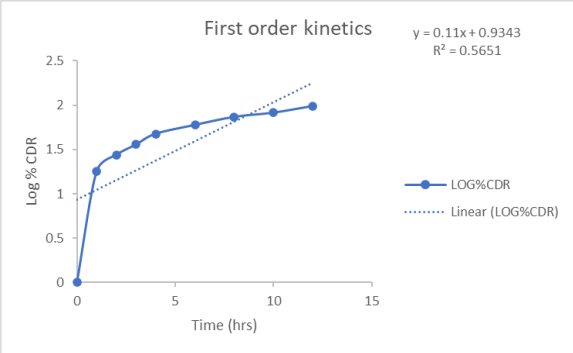

Fig-10: First order kinetics of optimized formulation

Higuchi model

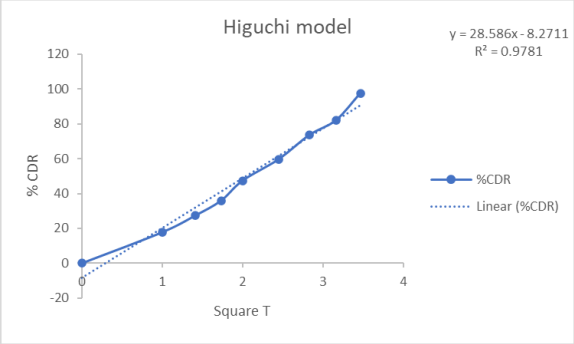

Fig-11: Higuchi model of optimized formulation

Korsmeyer peppas

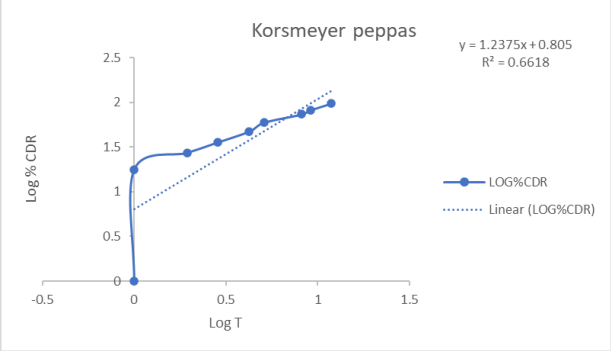

Fig-12: Korsmeyer peppas of optimized formulation

The kinetic values obtained for formulation F6 were shown. The values of in vitro release were attempted to fit into various mathematical models.

Stability studies

Optimized formulations F6 was selected for accelerated stability studies as per ICH guidelines.

Table-5: Stability studies of optimized formulations at 40 ± 2 ^{0}C and 75 ± 5% RH for 3 months

Formulation Code	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-6	97.58	96.53	95.72	94.71	Not less than 85 %
F-6	97.58	96.50	95.43	94.26	Not less than 85 %
F-6	97.58	96.42	95.20	94.10	Not less than 85 %

VI. CONCLUSION

Donepezil-loaded ethosomal formulations were successfully prepared and optimised for transdermal delivery. These results suggest that ethosomal carriers are a promising strategy for transdermal delivery of Donepezil, potentially improving bioavailability, therapeutic efficacy, and patient compliance in the management of Alzheimer's disease.

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