# Development And Characterization of Rivastigmine-Loaded Transferosomal Vesicles for Enhanced Transdermal Delivery

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***Abstract*—The present study was aimed at the development and characterisation of rivastigmine-loaded Transferosomal vesicles for enhanced transdermal drug delivery. Transferosomes were formulated using soya lecithin, cholesterol, and different edge activators (Tween 80 and Span 80) by the thin film hydration technique. The prepared formulations (F1–F8) were evaluated for compatibility, vesicle morphology, particle size, zeta potential, percentage yield, drug entrapment efficiency, in vitro drug release, release kinetics, and stability. FTIR studies confirmed the compatibility of rivastigmine with formulation excipients, indicating the absence of chemical interactions. Particle size analysis revealed nano-sized vesicles ranging from 175 to 189 nm with acceptable zeta potential values (–22 to –28 mV), ensuring formulation stability. SEM analysis showed spherical vesicles with smooth surfaces and uniform morphology. Among all formulations, F6 exhibited the highest yield (85.96%) and drug entrapment efficiency (88.15%). In vitro drug release studies demonstrated sustained release of rivastigmine up to 12 hours, with the optimised formulation F6 showing maximum cumulative drug release (98.80%). Kinetic analysis indicated that the drug release followed first-order kinetics and the Higuchi diffusion model. Stability studies conducted as per ICH guidelines showed no significant changes in drug release and physical characteristics over three months. Overall, the results suggest that transferosomal vesicles are a promising carrier system for the transdermal delivery of rivastigmine, offering sustained release and improved therapeutic potential.**

***Index Terms*—Rivastigmine, FTIR Studies, Thin film hydration method, Transferosomes, In vitro drug release studies**

## I. INTRODUCTION

Transdermal drug delivery systems (TDDS) have emerged as a promising alternative to conventional oral therapy by offering controlled drug release, avoidance of hepatic first-pass metabolism, reduced systemic side effects, and improved patient adherence. However, the effectiveness of transdermal delivery is significantly limited by the highly organised structure of the stratum corneum, which acts as the principal barrier to drug permeation.[1] To overcome this limitation, various novel vesicular carrier systems such as liposomes, niosomes, Ethosomes, and transferosomes have been explored for enhanced skin permeation.[2] Transferosomes are ultra-deformable lipid vesicles composed of phospholipids and edge activators, typically surfactants, which impart exceptional elasticity to the vesicular membrane.[3] This unique deformability enables transferosomes to penetrate intact skin by squeezing through narrow intercellular pathways of the stratum corneum without vesicle rupture.[4] Rivastigmine, a reversible inhibitor of both acetylcholinesterase and butyryl cholinesterase, is widely prescribed for the treatment of mild to moderate Alzheimer's disease. Rivastigmine possesses physicochemical properties favorable for transdermal delivery, including low molecular weight and suitable lipophilicity; however, its skin permeation remains inadequate when delivered through conventional topical formulations.[5] Incorporation of rivastigmine into transferosomal vesicles is expected to enhance its transdermal flux, provide sustained drug release, and minimize systemic side effects associated with oral

administration.[6] Furthermore, transdermal delivery of rivastigmine may offer a non-invasive and patient-friendly therapeutic approach, particularly beneficial for elderly patients suffering from Alzheimer's disease. In this context, the present study aims to develop and characterize rivastigmine-loaded transferosomal vesicles for enhanced transdermal delivery.[7]

## II. MATERIALS

Rivastigmine 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

Fourier transform infrared spectroscopy:

Fourier transform IR spectra were obtained on a Shimadzu FT-IR spectrometer. Samples were prepared in KBr disks (2mg sample in 200mg KBr). The scanning range was 450-4000 $cm^{-1,}$ and the resolution was 4 $cm^{-1}$.[10]

Formulation development

Table-1: Formulation table

| Ingredients | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|
| Rivastigmine | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Soya lecithin | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Cholesterol | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Tween 80 | 5 | 10 | 15 | 20 | - | - | - | - |
| Span 80 | - | - | - | - | 5 | 10 | 15 | 20 |
| Chloroform | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Methanol | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 7.4 Phosphate buffer | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s |

Preparation method trasferosomes

In a round-bottom flask, dissolve Phosphatidylcholine and edge activator and cholesterol and drug (5mg) in chloroform: methanol (2:3mL) until complete dissolution. Place flask on rotary evaporator at 40–45 °C under reduced pressure and rotate until a dry, uniform thin lipid film forms on the flask wall (30–45 min). If no rotavapor, use a gentle $N_2$ stream in a round flask and dry in vacuum desiccator. After forming the film, leave under vacuum for 1–2 h to remove trace solvents. Hydrate the film with pre-warmed (room temperature or 37 °C) phosphate buffer saline pH 7.4. Add buffer slowly while rotating the flask to detach the film, vortex for 10–15 min to yield a milky transferosomal suspension. Hydration volume and time influence vesicle size & entrapment. Centrifuge at 10,000–15,000 rpm for 30 min to separate unentrapped Rivastigmine. Collect pellet (transferosomes) and re-disperse in fresh buffer to known volume. Save supernatant for free-drug assay. [11]

## IV. CHARACTERIZATION

Particle Size

The particle size of the Transferosomes was determined using a Particle Size Analyser (PSA) with the dynamic light scattering (DLS) method. The measurements were performed using Horiba Scientific SZ-100, with the sample diluted 10 times in aqueous medium at room temperature.[13]

Zeta-potential:

The sample was diluted with distilled water (1:100 (V/V)), and zeta potential was determined using Malvern zetasizer (Nano ZS, Malvern Instruments, United Kingdom). Measurement was based on the

electrophoretic mobility of the particles, which was converted to the zeta potential by in-built software based on the Helmholtz-Smoluchowski equation [14]

SEM analysis

The shape, surface characteristics, and size of the Transferosomes were observed by scanning electron microscopy. Once again, 0.2 g of the Transferosomes in a glass tube was diluted with 10 ml of pH 7.4 phosphate buffer. The Transferosomes were mounted on an aluminium stub using double-sided adhesive carbon tape. Then the vesicles were sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope (Hitachi 3700N, Germany) equipped with a digital camera, at 10 kV accelerating voltage.[15]

Drug entrapment efficiency

The entrapment efficiency (EE%) of rivastigmine in transferosomal vesicles was determined by separating the free (unentrapped) drug from the vesicles using centrifugation at 15,000 rpm for 1 hour at 4 °C. The supernatant containing the unentrapped drug was collected, and the rivastigmine content was quantified using a UV-visible spectrophotometer at the λmax of 225 nm. The entrapment efficiency was calculated using the formula:

EE% = Total drug – Free drug/Total drug×100

In vitro diffusion profile[19]

For in vitro drug release studies, the optimized rivastigmine-loaded transferosomal vesicles were evaluated using a Franz diffusion cell with a dialysis membrane. The receptor compartment was filled with phosphate buffer pH 7.4, maintained at 37 ± 0.5 °C, and stirred continuously. A known quantity of vesicular formulation was placed in the donor compartment, and samples were withdrawn from the receptor compartment at predetermined time intervals, replacing the withdrawn volume with fresh buffer. The samples were analyzed spectrophotometrically at 225 nm to determine the cumulative percentage of rivastigmine released over time. The release data were further fitted to kinetic models to understand the drug release mechanism and confirm sustained delivery behavior.

Drug release kinetics[20]

The models used were zero order (equation 1) First order (equation 2) and Higuchi model (equation 3) and Koresmeyer Peppas model (equation 4).

i) Zero order kinetics:

$R = K_o t$ -- (1)

R=cumulative percent drug

Ko=zero order rate constant

ii) First order kinetics

$\log C = \log C_o - K_1 t / 2.303$ -- (2)

where C = cumulative percent drug

$K_1$ = first order rate constant

iii) Higuchi model

$R = K_H t^{0.5}$ -- (3)

Where R = cumulative percent drug

$K_H$ = higuchi model rate constant

iv) Korsmeyer peppas model:

$M t / M \alpha = K_k t^n$

$\log M t / M \alpha = \log K_k + n \log t$ -- (4)

where $K_k$ = korsermeyer peppas rate constant

'M t / M α' is the fractional drug , n = diffusional exponent, which characterizes the mechanism of drug.

The obtained regression co-efficient (which neared 0.999) was used to understand the pattern of the drug from the Transferosomes.

Stability studies [21]

The main objective of the stability testing is to provide evidence on how the quality of the drug product varies with time under the influence of temperature and humidity. The stability study for the Transferosomal formulation was done as per ICH guidelines in a stability chamber for a period of 3 months.

## V. RESULTS AND DISCUSSION

Compatibility study (IR spectroscopy)

FTIR analysis was performed in order to study the compatibility of ingredients used in the preparation of transferosmes, using a Shimadzu FTIR spectrophotometer (Bruker). Rivastigmine and Excipients their mixture with ratio (1:1) was evaluated using FTIR spectrophotometer using potassium bromide disc technique where 1mg of the sample is mixed with 100 mg of dry powdered KBr, the mixture is pressed into a transparent disc and was inserted in the apparatus for IR scan.

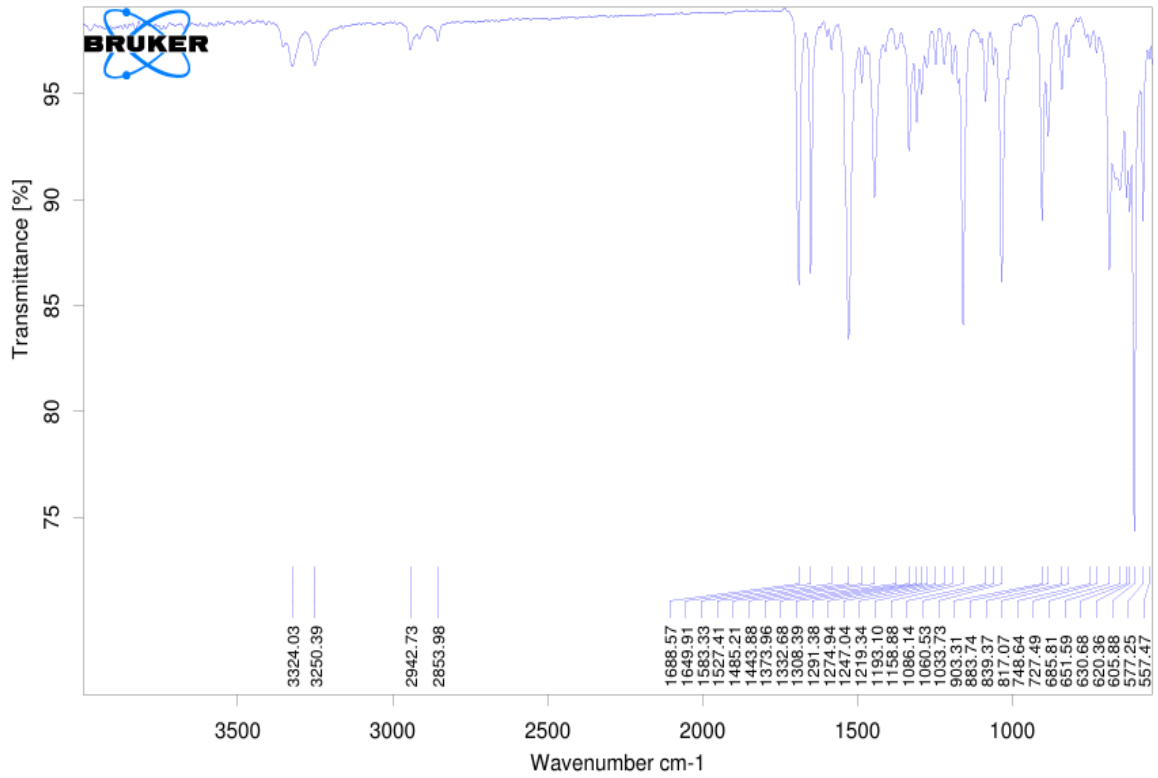


Fig-1: FT-IR Sample for Rivastigmine

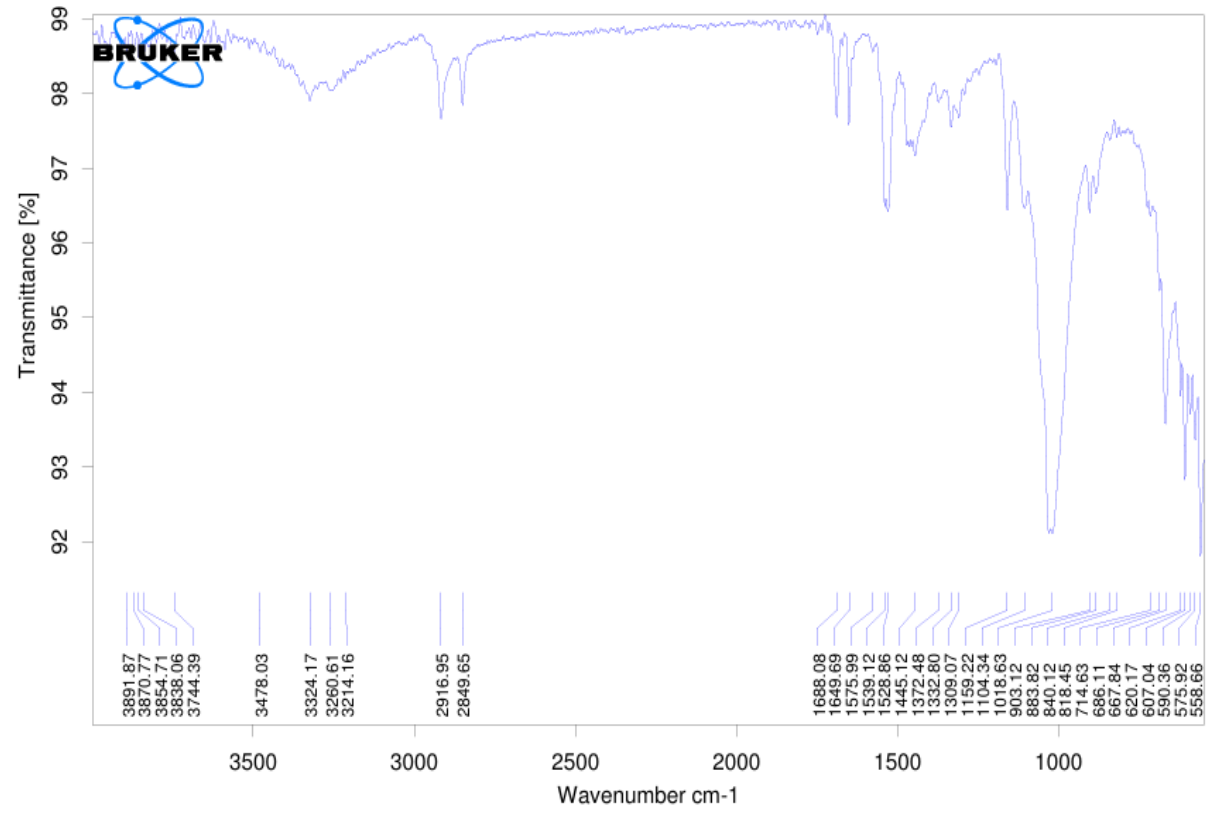


Fig-2: FT-IR Sample for Optimized formulation

Discussion: The FTIR study confirms that rivastigmine is compatible with the excipients used in the transferosomal formulation and is physically entrapped within the lipid bilayer rather than chemically interacting with formulation components. This compatibility is essential for maintaining drug stability, ensuring effective entrapment, and achieving controlled drug release from the transferosomal vesicles

Determination of Vesicle morphology and Size

The morphological characteristics of formulated transferosomes were carried by using Scanning electron microscopy (SEM). A small drop of Transferosomal suspension was placed between two rivets fixed on a gold plated copper sample holder. The whole system was slushed under vacuum in liquid nitrogen. The sample was heated to -85$^0$C for 30 min to sublime the surface moisture. Finally the sample was coated with gold and allowed the SEM to capture the images at a temperature of - 120$^0$c and voltage of 5kV.

Particle size Analysis

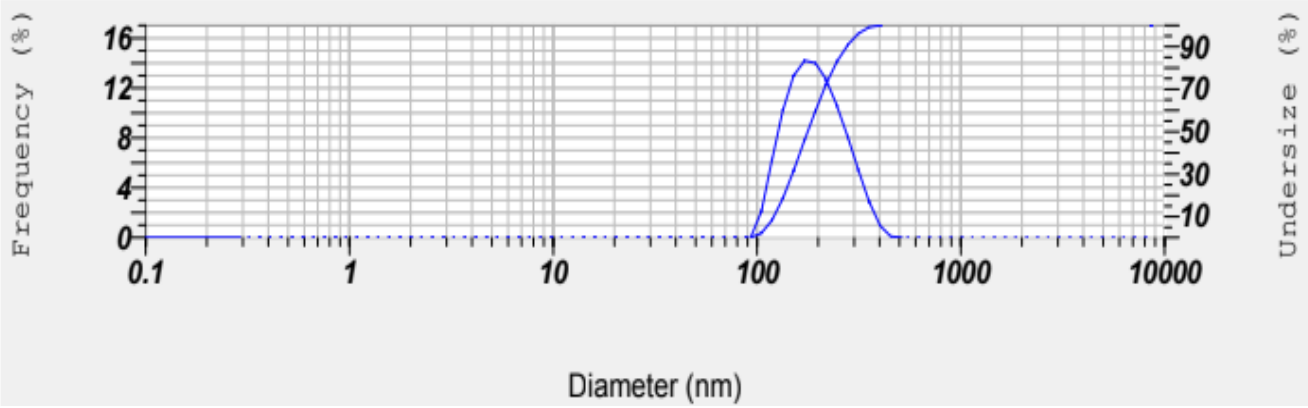


Fig-3: Particle size Analysis of Transferosomes

Discussion: Among the formulations, F6 exhibited the smallest particle size (175 nm), indicating efficient vesicle size reduction during formulation. The smaller particle size of F6 may be attributed to an optimal concentration of edge activator, which reduces interfacial tension and promotes the formation of uniform and highly deformable vesicles. In contrast, F8 showed the largest particle size (189 nm), possibly due to vesicle aggregation or increased bilayer fluidity at higher surfactant concentrations.

SEM Analysis

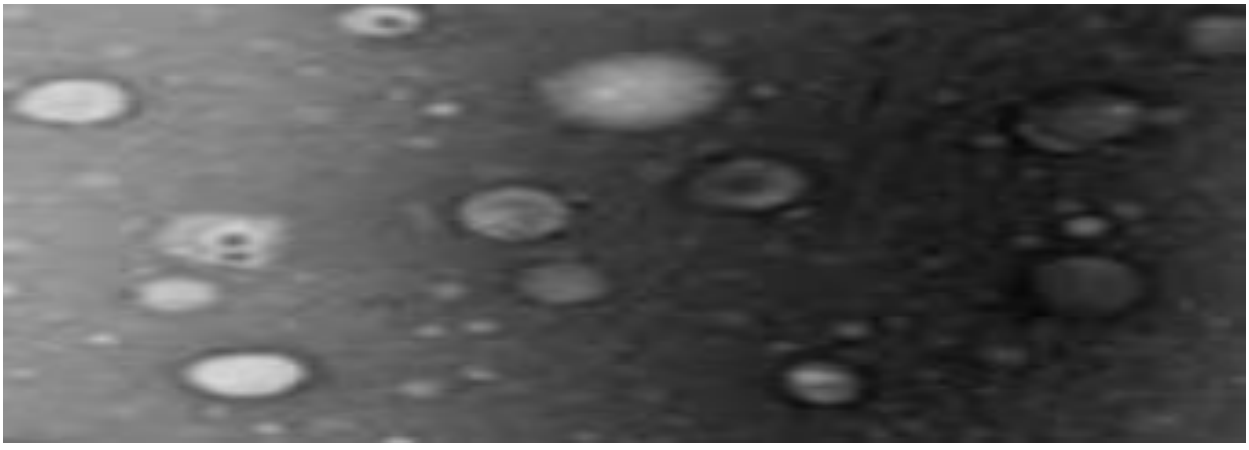

Fig-4: SEM analysis of transferosomes

Discussion: Scanning Electron Microscopy images confirmed the formation of discrete, nearly spherical vesicles with a smooth surface. No crystalline drug particles were visible, suggesting that Rivastigmine was successfully incorporated into the lipid bilayers of the transferosomes. The absence of surface cracks or irregularities reflects good structural integrity.

Zeta potential

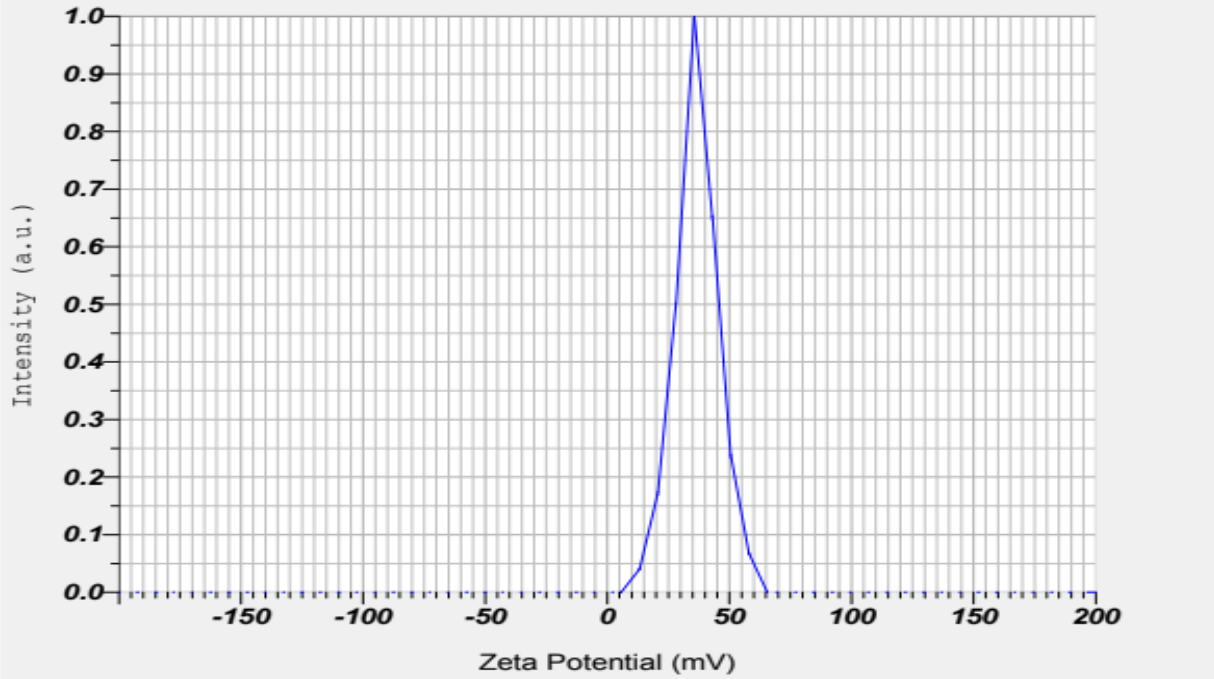


Fig-5: Zeta potential of Transferosomes

Discussion: The zeta potential values of all formulations ranged from –22 mV to –28 mV, indicating that the vesicles possessed a moderately high negative surface charge. This negative zeta potential contributes to electrostatic repulsion between vesicles, thereby preventing aggregation and enhancing physical stability during storage.

Table-3: Evaluation Studies of particle size and Zeta potential Transferosomes

| F. No | Particle size (nm) | Zeta potential(mV) |
|---|---|---|
| F1 | 184 | -24 |
| F2 | 183 | -25 |
| F3 | 180 | -23 |
| F4 | 179 | -27 |
| F5 | 181 | -25 |
| F6 | 175 | -22 |
| F7 | 186 | -26 |
| F8 | 189 | -28 |

Yield of Transferosomes

Table-4: Yield of Transferosomes

| F. No | Yield (%) |
|---|---|
| F1 | 76.39 |
| F2 | 75.34 |
| F3 | 77.56 |
| F4 | 78.89 |
| F5 | 81.10 |
| F6 | 85.96 |
| F7 | 82.34 |
| F8 | 80.67 |

Discussion: F6 exhibited the highest yield (85.96%), suggesting that the optimised ratio of phospholipid and edge activator in this formulation contributed to improved vesicle formation and reduced processing losses.

Entrapment Efficiency:

Table-7: Drug entrapment efficiency

| F. code | Drug entrapment efficiency |
|---|---|
| F1 | 80.20 |
| F2 | 83.66 |
| F3 | 84.21 |
| F4 | 79.81 |
| F5 | 76.93 |
| F6 | 88.15 |
| F7 | 83.55 |
| F8 | 80.10 |

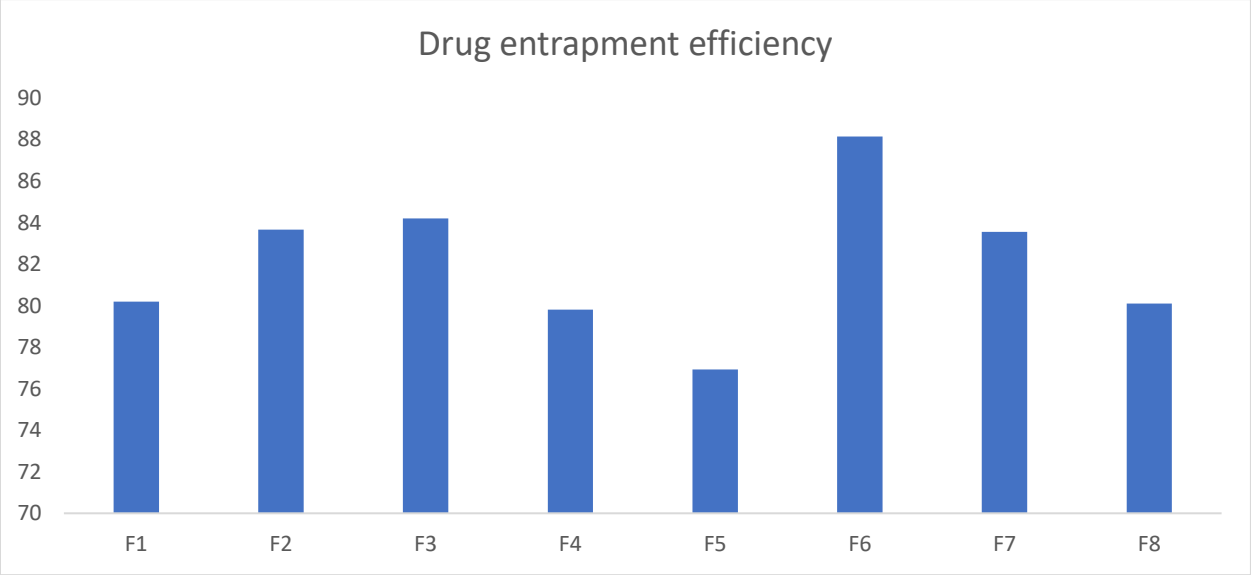


Fig-6: Drug entrapment efficiency of all formulations

Discussion: Among the formulations, F6 exhibited the highest entrapment efficiency (88.15%), indicating optimal compatibility between the drug, phospholipid, and edge activator. The superior DEE of F6 may be attributed to an ideal surfactant concentration that enhanced vesicle elasticity without compromising membrane integrity, thereby reducing drug leakage during formulation and processing.

*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. The drug release profiles of Transferosomes containing different ratios of lipids.

Table-8: *In vitro* drug release profiles of Transferosomal gel (F1-F8)

| Time (hr) | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_6$ | $F_7$ | $F_8$ |
|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 22.39 | 25.78 | 22.59 | 26.39 | 24.85 | 28.59 | 21.25 | 23.36 |
| 2 | 32.35 | 33.89 | 31.25 | 35.98 | 37.20 | 39.87 | 34.53 | 35.82 |
| 3 | 44.51 | 43.38 | 44.58 | 45.98 | 48.96 | 49.98 | 47.50 | 46.33 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 4 | 51.98 | 53.24 | 55.82 | 57.46 | 58.22 | 59.76 | 53.36 | 58.98 |
| 6 | 60.24 | 64.69 | 65.89 | 63.22 | 66.98 | 67.83 | 61.43 | 65.52 |
| 8 | 71.43 | 72.50 | 70.15 | 73.25 | 74.53 | 76.52 | 74.95 | 73.56 |
| 10 | 82.53 | 80.21 | 82.36 | 84.53 | 85.16 | 86.36 | 85.34 | 84.31 |
| 12 | 92.35 | 93.36 | 94.12 | 95.63 | 97.30 | 98.80 | 96.42 | 97.85 |

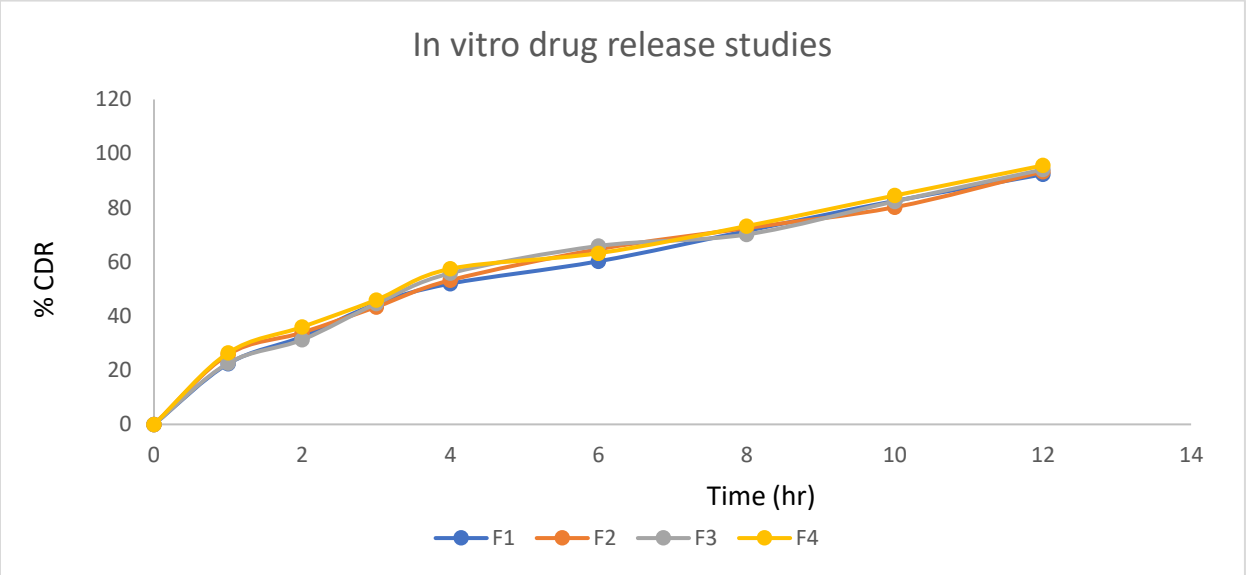


Fig-7: In vitro drug release studies of F1-F4 formulations

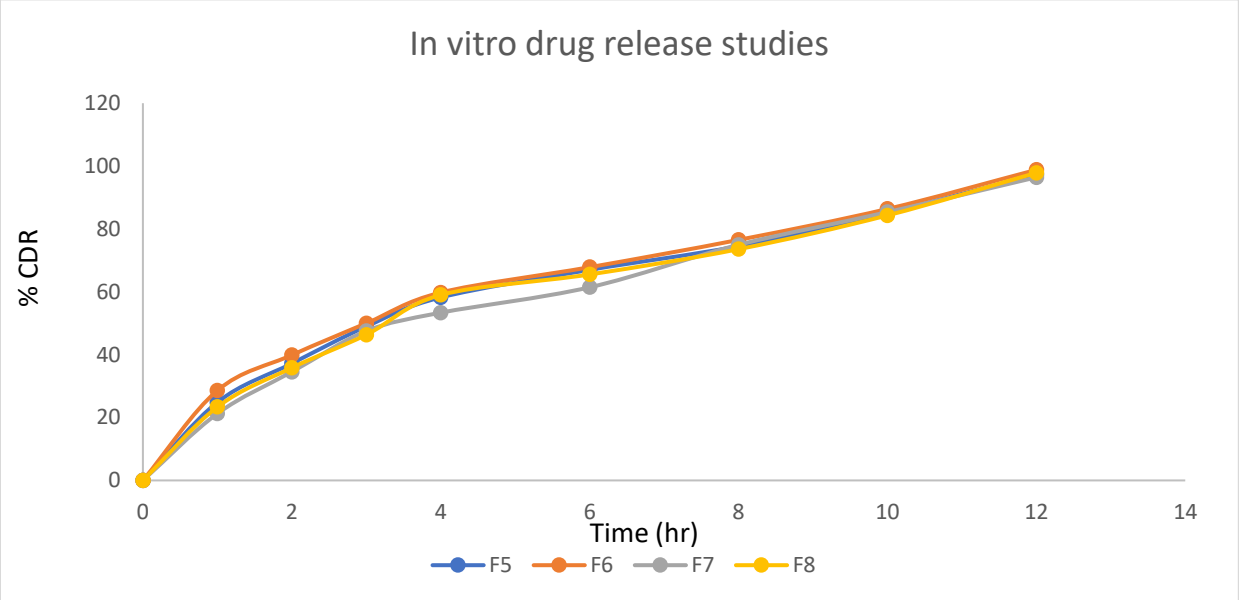


Fig-8: In vitro drug release studies of F5-F8 formulations

Discussion: All formulations exhibited a sustained release profile in phosphate buffer pH 7.4, with cumulative drug release reaching 92.35–98.80 % over 12 h. Among them, F6 achieved the highest release (98.80 %) and demonstrated consistent release behaviour. The rate and extent of drug release were found to depend on the polymer type and its concentration.

Kinetic modelling of drug release

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

Zero-order kinetics

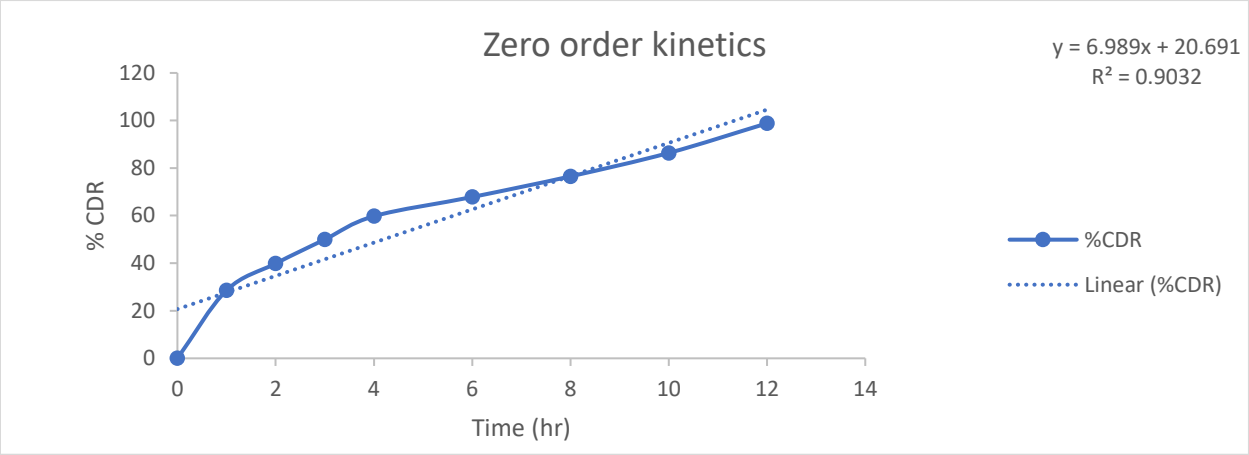


Fig-9: Zero-order kinetics of optimised formulation

First order kinetics

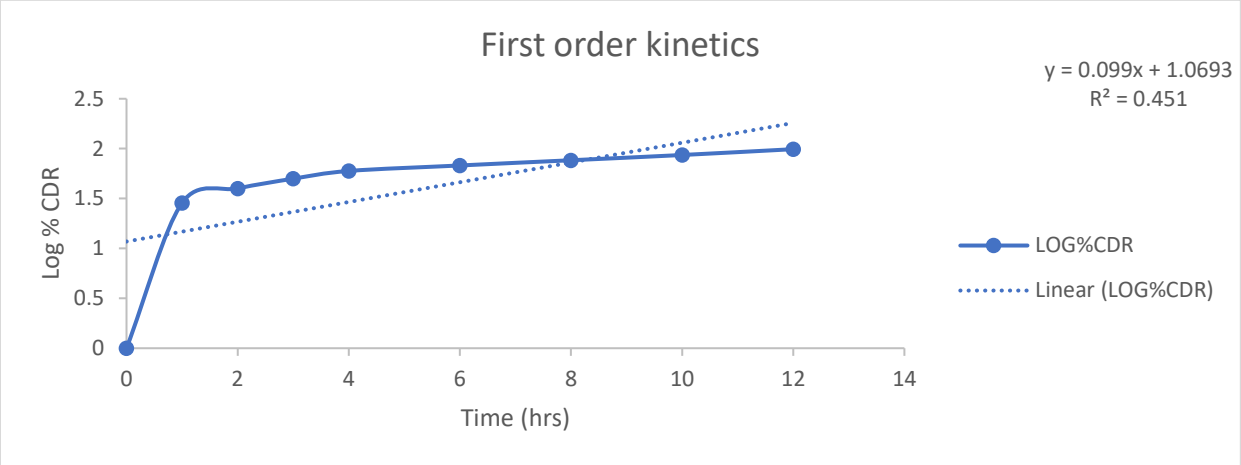


Fig-10: First order kinetics of optimised formulation

Higuchi model

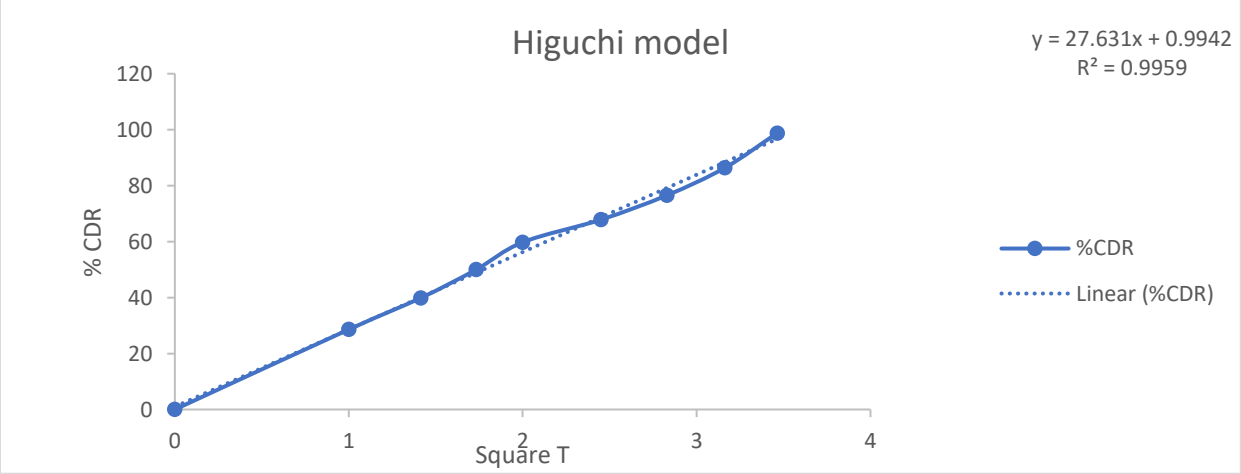


Fig-11: Higuchi model of optimized formulation

Korsmeyer peppas

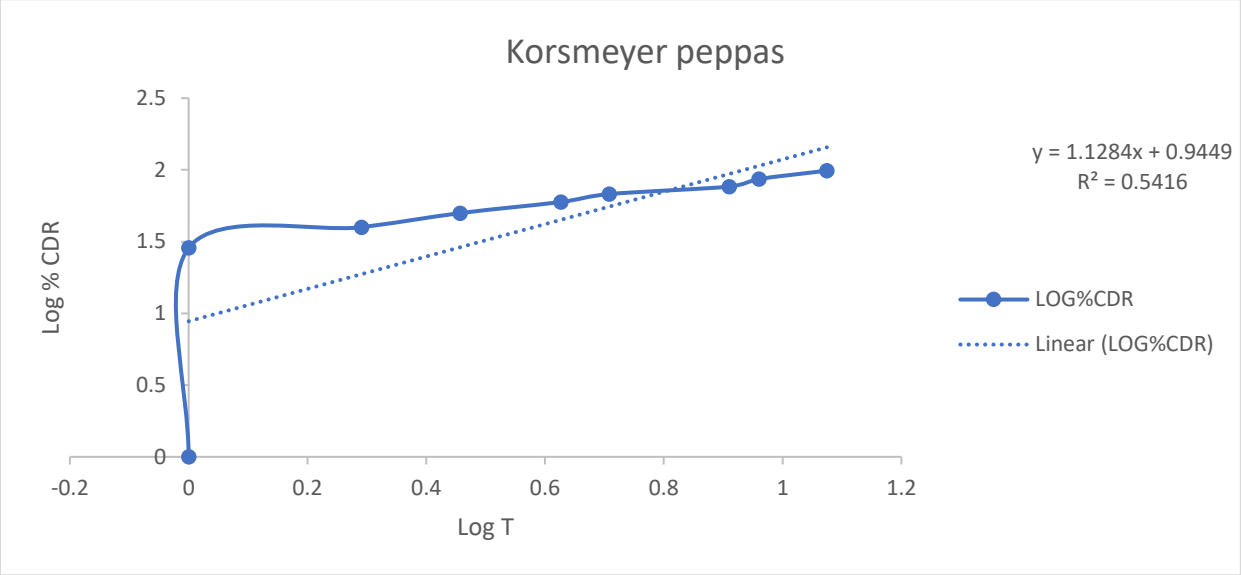


Fig-12: Korsmeyer peppas of optimized formulation

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

Regression values are higher with First order release kinetics. Therefore, Rivastigmine optimized formulation follows Higuchi model release mechanism.

Discussion: Kinetic modelling of the release data for the optimized batch (F6) showed that the release profile fitted First-order kinetics with a good correlation coefficient, indicating that the release rate was concentration-dependent. The release mechanism followed the Higuchi diffusion model, suggesting that the predominant mechanism of drug liberation from the gel was diffusion through the hydrated matrix.

Stability studies

Table-9: Stability studies of optimized formulations at 40 ± 2 $^0$C and 75 ± 5% RH for 3 months

| F. no | Parameters | Initial | 1st Month | 2nd Month | 3rd Month | Limits as per Specifications |
|---|---|---|---|---|---|---|
| F-6 | 25$^0$C/60%RH | 98.80 | 97.56 | 96.35 | 95.73 | Not less than |
| F-6 | 30$^0$C/75% RH | 98.80 | 97.25 | 96.20 | 95.40 | Not less than |
| F-6 | 40$^0$C/75% RH | 98.80 | 97.13 | 96.18 | 95.50 | Not less than |

Discussion: Accelerated stability testing of the optimized formulation (F4) at 40 ± 2 °C/75 ± 5 % RH for 3 months demonstrated negligible changes in cumulative drug release (remaining above 94 %) and no significant alterations in physical appearance or pH. These results indicate that the transferosomal gel retained its physicochemical properties and drug content during the storage period.

## VI. CONCLUSION

In conclusion, the developed transferosomal system represents a promising and patient-friendly approach for the transdermal delivery of rivastigmine, potentially improving therapeutic efficacy, reducing dosing frequency, and minimizing systemic side effects associated with conventional oral administration. This study supports the suitability of transferosomes as an advanced vesicular carrier for the management of Alzheimer's disease.

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