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# FORMULATION AND EVALUATION OF LORAZEPAM LOADED TRANSFEROSOMES FOR THE MANAGE NEURODEGENERATIVE DISORDERS

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## ABSTRACT

The present study was undertaken to develop and evaluate a Lorazepam-loaded transfersomal formulation for enhanced topical/transdermal drug delivery with the objective of improving skin permeation, providing sustained drug release, and enhancing therapeutic efficacy. Transfersomes were prepared by varying the concentrations of soya phosphatidylcholine, ethanol, drug, and processing conditions to obtain an optimized vesicular system. Nine formulations (F1–F9) were developed and evaluated for vesicle size and entrapment efficiency. The optimized formulation (F9) exhibited an average vesicle size of 165.58 nm, entrapment efficiency of 73.49%, and a zeta potential of −40.36 mV, indicating excellent colloidal stability. Transmission Electron Microscopy confirmed the formation of spherical, uniformly distributed vesicles with smooth surfaces. The in-vitro drug release study demonstrated a sustained release profile, with 98.12% cumulative drug release over 12 hours, suggesting efficient controlled drug delivery. Release kinetic analysis revealed that the formulation best fitted the Higuchi model ($R^2$ = 0.9902), indicating diffusion-controlled drug release, while the Korsmeyer–Peppas model ($R^2$ = 0.9899) suggested the contribution of both diffusion and matrix relaxation mechanisms. Stability studies performed for three months under refrigerated and room temperature conditions demonstrated superior stability under refrigerated storage (4.0 ± 0.2°C), with minimal reduction in drug assay and no significant changes in physical appearance, whereas formulations stored at room temperature exhibited slight turbidity and marginal drug degradation. The overall findings indicate that the optimized Lorazepam-loaded transfersomal formulation possesses desirable physicochemical characteristics, sustained drug release behavior, and satisfactory stability, making it a promising carrier system for topical/transdermal delivery of Lorazepam with the potential to improve therapeutic efficacy and patient compliance.

**Keywords:** Lorazepam, Transfersomes, Transdermal Drug Delivery, Phospholipid Vesicles, Entrapment Efficiency, Zeta Potential, In-vitro Drug Release, Higuchi Kinetics, Stability Study, Nanovesicular Drug Delivery.

## INTRODUCTION

Neurodegenerative disorders constitute a group of progressive and irreversible diseases characterized by the gradual degeneration and loss of neuronal structure and function within the central nervous system (Dugger and Dickson, 2017). Disorders such as Alzheimer's disease, Parkinson's disease,

Huntington's disease, multiple sclerosis, and other neurological conditions significantly impair cognitive, behavioral, and motor functions, leading to reduced quality of life and increased healthcare burden worldwide. The prevalence of these disorders continues to rise due to increased life expectancy and aging populations, making the development of effective and patient-friendly therapeutic strategies an important area of pharmaceutical research (Alqahtani *et al.,* 2023).

Lorazepam, a benzodiazepine derivative, is widely used in the management of anxiety, insomnia, epilepsy, muscle spasms, and acute neurological conditions. It is also employed as an adjunctive therapy in several neurodegenerative disorders to control anxiety, agitation, muscle rigidity, and seizure-related complications. Despite its therapeutic effectiveness, conventional oral administration of Lorazepam is associated with several limitations, including extensive first-pass metabolism, variable gastrointestinal absorption, fluctuations in plasma drug concentration, and the requirement for repeated dosing due to its relatively short duration of action. These drawbacks may reduce patient compliance, particularly among elderly individuals and patients with chronic neurological disorders (Committee on the Review of Medicines, 1980).

Transdermal drug delivery has emerged as an attractive alternative to conventional oral administration because it bypasses hepatic first-pass metabolism, provides sustained drug release, minimizes gastrointestinal side effects, and improves patient compliance. However, the skin, particularly the stratum corneum, acts as a highly efficient barrier that limits the permeation of most therapeutic agents. Therefore, the development of advanced vesicular carrier systems capable of overcoming this barrier has become an important focus in transdermal drug delivery research (Wong *et al.,* 2023).

Transfersomes are highly flexible and ultra-deformable lipid vesicles composed mainly of phospholipids and edge activators. Unlike conventional liposomes, transfersomes possess remarkable elasticity, enabling them to deform and pass through skin pores much smaller than their own diameter without losing structural integrity (Rai *et al.,* 2017). This unique characteristic enhances drug penetration into deeper skin layers and improves systemic absorption. In addition to enhanced permeation, transfersomes offer several advantages, including high drug-loading capacity, improved stability, protection of encapsulated drugs from degradation, controlled and sustained drug release, reduced systemic toxicity, and improved therapeutic efficacy. These properties make transfersomes promising carriers for delivering drugs intended for long-term management of neurological disorders (Matharoo *et al.,* 2024).

Several studies have demonstrated the successful application of transfersomal systems for enhancing the transdermal delivery of poorly permeable therapeutic agents. The incorporation of phospholipids with suitable edge activators improves vesicle deformability and facilitates efficient transport of drug molecules across the skin. Furthermore, nanosized transfersomal vesicles exhibit a high surface area-to-volume ratio, promoting intimate contact with the skin surface and improving drug permeation. The

sustained-release behavior of transfersomes also helps maintain therapeutic drug concentrations over extended periods while reducing dosing frequency (Akram *et al.*, 2021).

Considering the therapeutic importance of Lorazepam and the advantages offered by transfersomal vesicles, the present study was designed to develop and evaluate a Lorazepam-loaded transfersomal formulation for topical/transdermal drug delivery. Transfersomes were prepared by varying the concentrations of formulation components to optimize vesicle characteristics. The prepared formulations were evaluated for average vesicle size, entrapment efficiency, zeta potential, morphology using Transmission Electron Microscopy (TEM), in-vitro drug release, release kinetics, and stability under different storage conditions. The optimized formulation was identified based on its physicochemical properties and sustained drug-release performance (Jain *et al.*, 2024).

The successful development of a stable Lorazepam-loaded transfersomal system may provide an effective alternative to conventional dosage forms by improving skin permeation, prolonging drug release, enhancing therapeutic efficacy, reducing dosing frequency, and improving patient compliance. Therefore, this study contributes to the growing field of nanovesicular drug delivery systems and highlights the potential of transfersomes as promising carriers for the effective management of neurological disorders.

## MATERIALS AND METHODS

### Material

Lorazepam was obtained as a gift sample from a reputed pharmaceutical company. Soya phosphatidylcholine (Soya PC) was used as the primary phospholipid for the preparation of transfersomes, while ethanol served as the penetration enhancer and vesicle-forming component. Cholesterol was incorporated to improve membrane stability, and Tween 80 was used as the edge activator to enhance vesicle deformability. Chloroform and methanol were used as organic solvents during formulation development. Phosphate buffer (pH 7.4) was employed as the hydration medium and dissolution medium for in-vitro drug release studies. All chemicals and reagents used in the study were of analytical grade and were used without further purification. Double-distilled water was used throughout the experimental work.

### Methods

### Preparation of Lorazepam loaded Transfersomes

Lorazepam-loaded transfersomes were prepared by the ethanol injection method. Briefly, soya phosphatidylcholine (Soya PC) was accurately weighed and dissolved in the required quantity of ethanol in a closed glass vessel. The ethanolic lipid solution was maintained at 30 ± 1°C using a thermostatically controlled water bath. Lorazepam was dissolved in distilled water and preheated to the same temperature. The aqueous drug solution was then added slowly in a fine stream into the ethanolic lipid solution under continuous magnetic stirring at 900 rpm. Stirring was continued for 5–15 min depending on the formulation. The resulting vesicular dispersion was allowed to cool at 25 ± 1°C for 45 min to facilitate spontaneous vesicle formation and stabilization. The prepared transfersomal formulations were evaluated for vesicle size and entrapment

efficiency, and the optimized formulation was selected for further characterization. The preparation method was adopted with suitable modifications from the methods reported by Vieira et al. (2023) and Khan et al. (2022).

**Characterization of Lorazepam loaded Transfersomes**

**Surface charge and vesicle size**

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK). Zeta potential measurement of the Transfersomes was based on the zeta potential that was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, a Zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9 % NaCl adjusted to a conductivity of 50 lS/cm (Khan *et al.*, 2021).

**Entrapment efficiency**

One milliliter of Transfersomes suspension was centrifuged at 15.000 rpm for 1 h to allow the separation the entrapped drug from the un-entrapped drug. After removal of the supernatant, the sediment was lysed using methanol and then analyzed spectrophotometrically at 230nm using a UV spectrophotometer (Labindia 3000+) (Bnyan *et al.*, 2019). The EE% of Lorazepam in the prepared Transfersomes was calculated applying the following equation:

$$\% \ Entrapment \ Efficiency = \frac{Terotical \ drug \ content - Practical \ drug \ content}{\text{Therotical drug content}} \times 100$$

**Transmission electron microscopy**

Surface morphology was determined by TEM, for TEM a drop of the sample was placed on a carbon-coated copper grid and after 15 min it was negatively stained with 1% aqueous solution of phosphotungustic acid. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron microscopy (TEM Hitachi, H-7500 Tokyo, Japan) (Zhaoa *et al.*, 2013).

***In vitro* dissolution rate studies**

*In vitro* drug release of the sample was carried out using USP- type I dissolution apparatus (Basket type). The dissolution medium, 900 ml Phosphate buffer pH 7.4 was placed into the dissolution flask maintaining the temperature of 37±0.5°C and 75 rpm. 10 mg of prepared Transfersomes was placed in each basket of dissolution apparatus. The apparatus was allowed to run for 8 hours. Sample measuring 3 ml were withdrawn after every interval (30 min, 1 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, and 12 hrs.) up to 12 hours using 10 ml pipette. The fresh dissolution medium (37ºC) was replaced every time with the same quantity of the sample and takes the absorbance at 230 nm using spectroscopy (Zheng *et al.*, 2006).

**Stability Studies**

Stability study was carried out for drug loaded Transfersomes at two different temperatures i.e. refrigeration temperature (4.0±0.2°C) and at room temperature (25-28±2°C) for 3 weeks. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The formulations were analyzed for any physical changes and drug content (Barbalho *et al.*, 2024).

**RESULTS AND DISCUSSION**

Nine transfersomal formulations (F1–F9) were developed using different concentrations of soya phosphatidylcholine, ethanol, drug, and stirring time to optimize the vesicular

characteristics of Lorazepam-loaded transfersomes. The composition of all prepared formulations is presented in Table 1. The formulation variables were selected to investigate their influence on vesicle formation, particle size, drug entrapment, and overall stability of the transfersomal system.

Table 1 shows that the concentration of phospholipid, ethanol content, drug loading, and processing conditions significantly affected the physicochemical characteristics of the prepared vesicles. Increasing the phospholipid concentration initially improved vesicle formation and drug encapsulation; however, excessive lipid concentration resulted in larger vesicles because of increased membrane rigidity and vesicle aggregation. Ethanol played a vital role in improving membrane fluidity and deformability, thereby facilitating the formation of nanosized vesicles. Similarly, appropriate stirring conditions promoted uniform vesicle formation and prevented aggregation. Based on the preliminary evaluation, formulation F9 exhibited the most desirable characteristics and was selected as the optimized formulation for further characterization.

The average vesicle size and percentage entrapment efficiency of all formulations are summarized in Table 2. The prepared formulations exhibited vesicle sizes ranging from 145.65 to 356.65 nm, whereas entrapment efficiency varied between 68.85% and 78.98%, indicating that formulation variables had a significant influence on vesicle characteristics.

As shown in Table 2, formulation F1 exhibited the largest vesicle size (356.65 nm) with an entrapment efficiency of 75.65%, whereas F8 produced the smallest vesicle size (145.65 nm) but showed comparatively lower entrapment efficiency (68.85%), suggesting that extremely small vesicles possess limited internal volume for drug encapsulation. Formulation F2 exhibited the highest entrapment efficiency (78.98%); however, its vesicle size was comparatively larger than that of the optimized formulation.

Among all the formulations, F9 demonstrated the best balance between vesicle size and drug loading, exhibiting an average vesicle size of 165.58 nm with 73.49% entrapment efficiency. These results indicate that the selected formulation variables produced stable nanosized vesicles capable of enhancing skin permeation while maintaining adequate drug loading. The optimized formulation (F9) was further characterized for vesicle size, entrapment efficiency, and zeta potential. Characterization results are presented in Table 3.

As shown in Table 3, formulation F9 exhibited an average vesicle size of 165.58 nm, an entrapment efficiency of 73.49%, and a zeta potential of −40.36 mV. The nanosized vesicles are expected to penetrate efficiently through the stratum corneum because of their highly deformable nature. The satisfactory entrapment efficiency confirms efficient incorporation of Lorazepam into the phospholipid bilayer, while the high negative zeta potential indicates excellent colloidal stability by preventing vesicle aggregation through electrostatic repulsion. These characteristics suggest that formulation F9 possesses desirable physicochemical properties for topical and transdermal drug delivery.

The morphology of the optimized transfersomes was examined by Transmission Electron Microscopy, and the representative micrograph is presented in Figure 1.

As observed in Figure 1, the transfersomal vesicles were predominantly spherical with smooth surfaces and well-defined boundaries. The vesicles appeared uniformly dispersed without noticeable aggregation, confirming successful vesicle formation. Minor variation in vesicle size was observed, which is commonly encountered in lipid-based vesicular systems. The TEM findings were in close agreement with the particle size analysis presented in Table 3, confirming the successful preparation of stable nanosized transfersomes.

The in-vitro drug release profile of the optimized formulation F9 is presented in Table 4. As shown in Table 4, formulation F9 exhibited an initial drug release of 18.85% within the first 30 minutes, which can be attributed to the release of surface-associated drug molecules. Thereafter, drug release occurred gradually, reaching 29.95% after 1 hour, 38.85% after 2 hours, 49.98% after 4 hours, 68.85% after 6 hours, 82.25% after 8 hours, and 98.12% after 12 hours.

The sustained release profile indicates that the phospholipid bilayer effectively regulated drug diffusion, thereby prolonging drug release over an extended period. Such controlled release is highly desirable for topical drug delivery because it maintains therapeutic drug concentrations while reducing the frequency of administration. The nearly complete drug release after 12 hours confirms the suitability of formulation F9 for sustained topical delivery of Lorazepam.

The release kinetics of formulation F9 was analyzed using Zero-order, First-order, Higuchi, and Korsmeyer–Peppas models. The regression coefficient ($R^2$) values are presented in Table 5.

As shown in Table 5, the Higuchi model exhibited the highest regression coefficient ($R^2$ = 0.9902), followed closely by the Korsmeyer–Peppas model ($R^2$ = 0.9899), indicating that drug release predominantly occurred through diffusion from the phospholipid matrix with a minor contribution from matrix relaxation. The Zero-order model also demonstrated a high correlation ($R^2$ = 0.9696), suggesting a nearly constant drug release rate throughout the study period.

The comparatively lower $R^2$ value obtained with the First-order model (0.9065) indicates that the release mechanism was not primarily dependent on the remaining drug concentration. The kinetic analysis confirmed that formulation F9 follows a diffusion-controlled sustained-release mechanism, making it suitable for prolonged topical therapy.

The stability of the optimized transfersomal formulation was evaluated for three months under refrigerated (4.0 ± 0.2°C) and room temperature (25–28 ± 2°C) storage conditions. The results are presented in Table 6.

As shown in Table 6, the formulation stored under refrigerated conditions remained physically stable throughout the study period. The drug assay decreased only slightly from 98.12% to 97.72%, and no visible changes in physical appearance were observed, indicating excellent physicochemical stability.

The formulation stored at room temperature exhibited a gradual decline in drug assay from 97.68% to 95.96%, accompanied by

progressive changes in physical appearance from slight turbidity after one month to moderate turbidity after two months and high turbidity with slight agglomeration after three months. These changes are likely due to phospholipid oxidation, vesicle fusion, and partial drug leakage occurring at elevated temperatures. Despite these physical changes, the formulation retained more than 95% drug content after three months, indicating acceptable chemical stability. The findings clearly demonstrate that refrigerated storage conditions are more suitable for preserving vesicle integrity and maintaining the long-term stability of Lorazepam-loaded transfersomes.

**Table 1: Formulation development of Lorazepam-loaded transfersomes**

| Formulation Code | Soya PC (% w/v) | Ethanol (mL) | Drug (% w/v) | Stirring Time (min) |
|---|---|---|---|---|
| F1 | 0.5 | 10 | 1.0 | 5 |
| F2 | 1.0 | 10 | 1.0 | 5 |
| F3 | 1.5 | 10 | 1.0 | 5 |
| F4 | 1.0 | 5 | 1.0 | 5 |
| F5 | 1.0 | 10 | 1.0 | 5 |
| F6 | 1.0 | 15 | 1.0 | 5 |
| F7 | 1.0 | 10 | 1.5 | 5 |
| F8 | 1.0 | 10 | 1.0 | 10 |
| F9 | 1.0 | 10 | 1.0 | 5 |

**Table 2: Results of Average Vesicle Size and Entrapment Efficiency**

| F. Code | Average Vesicle Size (nm) | Entrapment Efficiency (%) |
|---|---|---|
| F1 | 356.65 | 75.65 |
| F2 | 285.65 | 78.98 |
| F3 | 310.24 | 69.98 |
| F4 | 245.85 | 76.65 |
| F5 | 179.98 | 74.45 |
| F6 | 265.85 | 71.12 |
| F7 | 198.85 | 69.98 |
| F8 | 145.65 | 68.85 |
| F9 | 165.58 | 73.49 |

**Table 3: Characterization of Optimized formulation of Transfersomes**

| Characterization | Average vesicle size (nm) | % Entrapment efficiency | Zeta Potential (mV) |
|---|---|---|---|
| F9 | 165.58 | 73.49 | -40.36 |

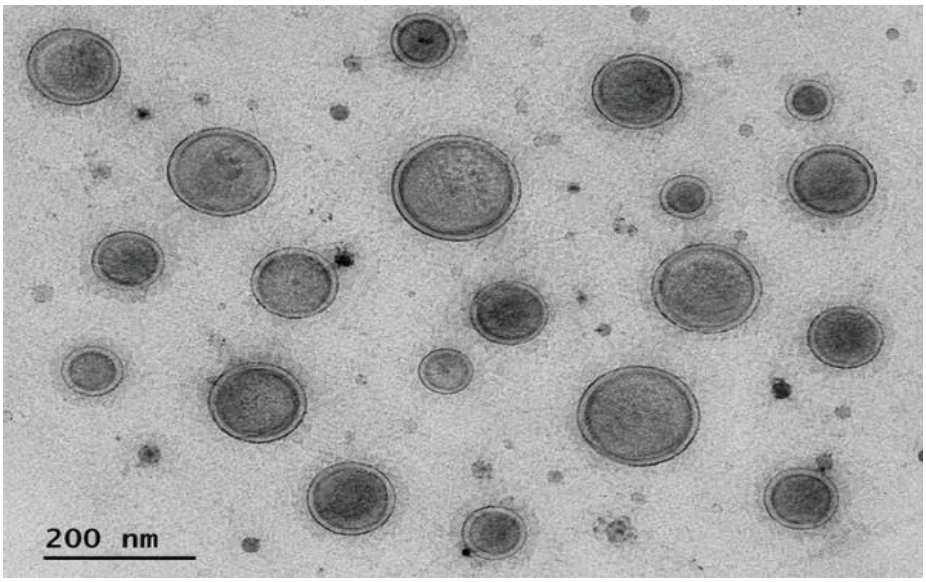


**Figure 1: TEM image of optimized transfersomes formulation F9**

**Table 4: *In-vitro* drug release data for optimized formulation F9**

| Time (h) | Square Root of Time(h)$^{1/2}$ | Log Time | Cumulative*% Drug Release | Log Cumulative % Drug Release | Cumulative % Drug Remaining | Log Cumulative % Drug Remaining |
|---|---|---|---|---|---|---|
| 0.5 | 0.707 | -0.301 | 18.85 | 1.275 | 81.15 | 1.909 |
| 1 | 1 | 0 | 29.95 | 1.476 | 70.05 | 1.845 |
| 2 | 1.414 | 0.301 | 38.85 | 1.589 | 61.15 | 1.786 |
| 4 | 2 | 0.602 | 49.98 | 1.699 | 50.02 | 1.699 |
| 6 | 2.449 | 0.778 | 68.85 | 1.838 | 31.15 | 1.493 |
| 8 | 2.828 | 0.903 | 82.25 | 1.915 | 17.75 | 1.249 |
| 12 | 3.464 | 1.079 | 98.12 | 1.992 | 1.88 | 0.274 |

**Table 5: Regression analysis data of optimized formulation F9**

| Batch | Zero Order | First Order | Higuchi | Korsmeyer Peppas |
|---|---|---|---|---|
| | $R^2$ | $R^2$ | $R^2$ | $R^2$ |
| F9 | 0.9696 | 0.9065 | 0.9902 | 0.9899 |

**Table 6: Stability study of optimized formulation of Transfersomes**

| Characteristic | 1 Month | | 2 Months | | 3 Months | |
|---|---|---|---|---|---|---|
| **Storage Temperature** | **4.0 ± 0.2°C** | **25–28 ± 2°C** | **4.0 ± 0.2°C** | **25–28 ± 2°C** | **4.0 ± 0.2°C** | **25–28 ± 2°C** |
| **Drug Assay (%)** | 98.12 | 97.68 | 97.94 | 96.85 | 97.72 | 95.96 |
| **Physical Appearance** | Normal | Slight turbidity | Normal | Moderate turbidity | Normal | High turbidity with slight agglomeration |

## CONCLUSION

The present study successfully developed and optimized a Lorazepam-loaded transfersomal formulation for topical/transdermal drug delivery. Among the nine formulations prepared, F9 was identified as the optimized formulation based on its desirable physicochemical characteristics, including a vesicle size of 165.58 nm, 73.49% entrapment efficiency, and a zeta potential of −40.36 mV, indicating good colloidal stability. The formulation exhibited spherical vesicles, sustained drug release (98.12% in 12 hours), and followed the Higuchi release model, confirming diffusion-controlled drug release. Stability studies demonstrated that the formulation remained stable under refrigerated conditions with minimal changes in drug content and physical appearance. The optimized transfersomal formulation showed excellent potential as a sustained transdermal drug delivery system for Lorazepam, offering improved stability, prolonged drug release, and the possibility of enhanced therapeutic efficacy and patient compliance

## DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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