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COMPARATIVE EVALUATION OF ETHOSOMES AND TRANSFERSOMES FOR TRANSDERMAL DELIVERY OF ANTI-CANCER AGENT

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ABSTRACT

The present study focuses on the comparative evaluation of ethosomes and transfersomes as advanced vesicular carriers for the transdermal delivery of the anti-cancer drug Doxorubicin. Both systems were formulated using the thin-film hydration method and optimized for parameters such as entrapment efficiency, vesicle size, zeta potential, and in vitro drug release**.** The ethosomal formulations contained phosphatidylcholine, ethanol, and cholesterol, while transfersomes were prepared using phosphatidylcholine and edge activators (Tween 80 or Span 80). The physicochemical characterization revealed that ethosomes exhibited smaller vesicle size and higher flexibility compared to transfersomes. In vitro permeation studies through rat skin demonstrated significantly higher drug flux and cumulative permeation for ethosomal formulations, attributed to the presence of ethanol which enhances lipid fluidization and skin permeability. Furthermore, cytotoxicity studies against MCF-7 breast cancer cell lines confirmed superior anti-proliferative activity of ethosomal Doxorubicin compared to transfersomal and conventional formulations. The findings suggest that ethosomes are a more efficient nanocarrier system for the transdermal delivery of Doxorubicin**,** providing a non-invasive approach for sustained and targeted cancer therapy.

KEYWORDS: Ethosomes; Transfersomes; Transdermal Drug Delivery; Doxorubicin; Anti-Cancer Drug; Nanocarriers; Skin Permeation; Vesicular System; Lipid Vesicles; Controlled Release.

1. INTRODUCTION

The **transdermal route of drug delivery** has emerged as an attractive alternative to conventional administration methods due to its advantages such as avoidance of first-pass metabolism, sustained drug release, and improved patient compliance. However, the **stratum corneum**, the outermost layer of the skin, acts as a major barrier, limiting the penetration of most therapeutic agents, particularly hydrophilic or high-molecular-weight drugs like **anti-cancer agents**.

To overcome this limitation, novel vesicular carrier systems, including **ethosomes** and **transfersomes**, have been developed. Ethosomes are soft, malleable phospholipid vesicles containing a high concentration of ethanol, which enhances the fluidity of skin lipids and increases drug penetration. Transfersomes, on the other hand, are ultra-deformable vesicles composed of phospholipids and edge activators (surfactants), which enable them to squeeze through narrow intercellular spaces of the skin.

Among chemotherapeutic agents, **Doxorubicin**—a potent anthracycline antibiotic—has shown high efficacy against a wide range of cancers. However, its systemic administration is associated with dose-dependent cardiotoxicity and other adverse effects. Transdermal delivery of Doxorubicin using lipid-based nanocarriers could provide a promising non-invasive approach to enhance local delivery, minimize systemic toxicity, and improve therapeutic outcomes.

Therefore, the present research aims to compare the efficiency of ethosomes and transfersomes in the transdermal delivery of Doxorubicin. The formulations were evaluated based on their physicochemical characteristics (vesicle size, zeta potential, entrapment efficiency), in vitro release profile, skin permeation, and cytotoxic activity. This comparative study provides valuable insights into selecting the most suitable vesicular system for **topical or transdermal cancer therapy**.

2. MATERIALS AND METHODS

2.1 Materials

- **Drug:** Doxorubicin hydrochloride (purchased from Sigma-Aldrich, analytical grade)
- **Phosphatidylcholine (Lecithin):** Lipoid S100 (Germany)
- **Cholesterol:** Analytical grade, HiMedia, India
- **Ethanol (99.9%):** Merck Pvt. Ltd.
- **Tween 80 / Span 80:** as edge activators (LobaChemie, India)
- **Carbopol 934:** for gel formulation (if applicable)
- **Methanol and Chloroform:** analytical grade solvents
- **Phosphate Buffer Saline (PBS, pH 7.4):** used for in vitro release and permeation studies
- **Dialysis membrane:** Molecular weight cut-off 12,000 Da
- **Deionized water:** Double distilled, filtered before use

2.2 Preparation of Ethosomes

Ethosomes were prepared by the **cold method**

1. **Step 1:** Phosphatidylcholine and cholesterol were dissolved in ethanol along with the required quantity of Doxorubicin.
2. **Step 2:** The solution was stirred at 700 rpm using a magnetic stirrer at 30°C for 30 minutes to ensure complete dissolution.
3. **Step 3:** The aqueous phase (distilled water) was added slowly in a fine stream with continuous stirring to form a milky dispersion.
4. **Step 4:** The resulting ethosomal suspension was sonicated using a probe sonicator for 5 minutes to reduce vesicle size.
5. **Step 5:** The prepared ethosomes were stored at 4°C for further evaluation.

Table 1: Composition of Doxorubicin-Loaded Ethosomes.

Ingredient	F1	F2	F3
Doxorubicin (mg)	10	10	10
Phosphatidylcholine (mg)	100	200	300
Cholesterol (mg)	10	15	20
Ethanol (%)	20	30	40
Distilled Water (q.s. to mL)	100	100	100

2.3 Preparation of Transfersomes

Transfersomes were prepared by the **thin-film hydration method**.

1. **Step 1:** Phosphatidylcholine, cholesterol, and Tween 80 (edge activator) were dissolved in a mixture of chloroform and methanol (2:1).
2. **Step 2:** The solvent mixture was evaporated under reduced pressure in a rotary evaporator at 40°C to form a thin lipid film.
3. **Step 3:** The film was hydrated with phosphate buffer saline (PBS, pH 7.4) containing Doxorubicin with gentle shaking for 1 hour.
4. **Step 4:** The dispersion was sonicated to reduce vesicle size and stored in the refrigerator.

Table 2: Composition of Doxorubicin-Loaded Transfersomes.

Ingredient	T1	T2	T3
Doxorubicin (mg)	10	10	10
Phosphatidylcholine (mg)	100	200	300
Cholesterol (mg)	10	15	20
Tween 80 (%)	10	15	20
PBS (pH 7.4) (q.s. to mL)	100	100	100

2.4 CHARACTERIZATION OF FORMULATIONS

2.4.1 Vesicle Size and Zeta Potential

The mean vesicle size, polydispersity index (PDI), and zeta potential were determined using a **Dynamic Light Scattering (DLS)** particle size analyzer (Malvern Zetasizer).

2.4.2 Entrapment Efficiency (EE%)

Entrapment efficiency was determined by **centrifugation method**: formulations were centrifuged at 15,000 rpm for 1 hour at 4°C. The supernatant was analyzed for unentrapped drug using a UV–Visible spectrophotometer at 480 nm.

\text{EE%} = \frac{(Total drug - Free drug)}{Total drug} \times 100

2.4.3 Surface Morphology

The morphology of ethosomes and transfersomes was observed under **Transmission Electron Microscopy (TEM)** to confirm vesicle structure and uniformity.

2.4.4 In Vitro Drug Release Study

Drug release was carried out using **dialysis membrane diffusion technique**. Formulations equivalent to 1 mg Doxorubicin were placed in dialysis membrane bags, suspended in PBS (pH 7.4) at 37 ± 0.5 °C, and stirred at

100 rpm. Samples were withdrawn at predetermined time intervals and analyzedspectrophotometrically.

2.4.5 Ex Vivo Skin Permeation Study

Ex vivo permeation was performed using **Franz diffusion cells** with rat abdominal skin as a membrane. The receptor compartment contained PBS (pH 7.4) maintained at 37°C. Samples were collected at intervals up to 24 hours and analyzed for Doxorubicin content.

2.4.6 Cytotoxicity Study (MTT Assay)

The anti-cancer activity of ethosomal and transfersomal formulations was tested against **MCF-7 breast cancer cell lines** using the **MTT assay**. The % cell viability and IC_{50} values were calculated to compare cytotoxic efficiency.

2.4.7 Stability Studies

Optimized formulations were stored at **4°C and 25°C** for 30 days and periodically evaluated for changes in vesicle size, EE%, and drug content to assess stability.

3. RESULTS AND DISCUSSION

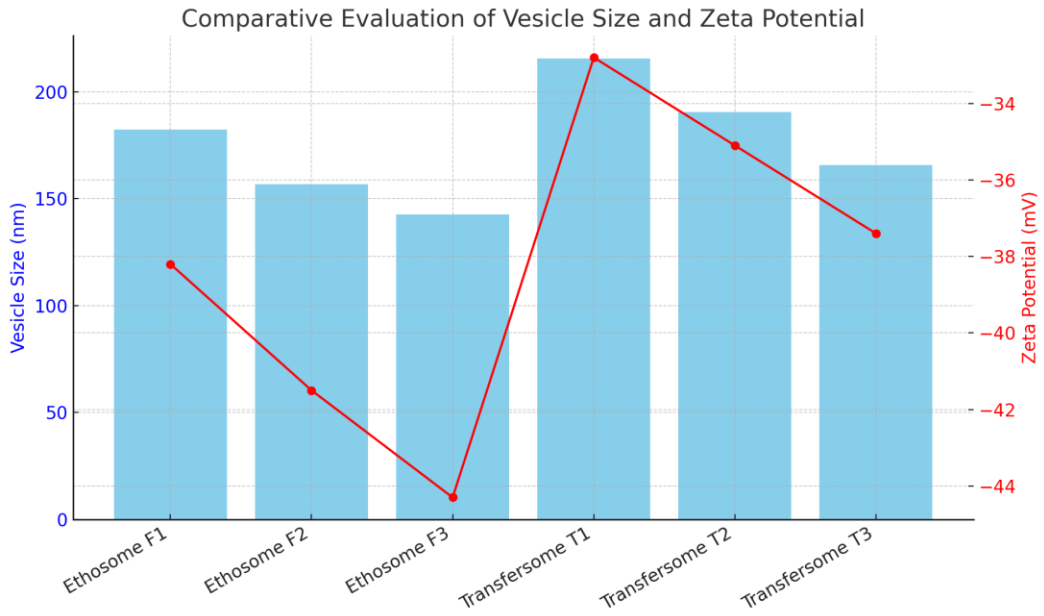
3.1 Vesicle Size, PDI, and Zeta Potential

Table 3. Vesicle size, PDI, and zeta potential of ethosomes and transfersomes.

Formulation	Vesicle Size (nm) ± SD	PDI ± SD	Zeta Potential (mV) ± SD
Ethosome F1	182.4 ± 3.8	0.241 ± 0.02	–38.2 ± 1.6
Ethosome F2	156.8 ± 2.6	0.218 ± 0.03	–41.5 ± 1.8
Ethosome F3	142.7 ± 3.4	0.205 ± 0.01	–44.3 ± 1.5
Transfersome T1	215.6 ± 4.2	0.276 ± 0.03	–32.8 ± 2.0
Transfersome T2	190.5 ± 3.6	0.251 ± 0.02	–35.1 ± 1.9
Transfersome T3	165.8 ± 2.9	0.234 ± 0.02	–37.4 ± 1.7

Interpretation

Ethosomal formulations exhibited smaller vesicle size and higher negative zeta potential compared to transfersomes, indicating better stability and flexibility for skin penetration.



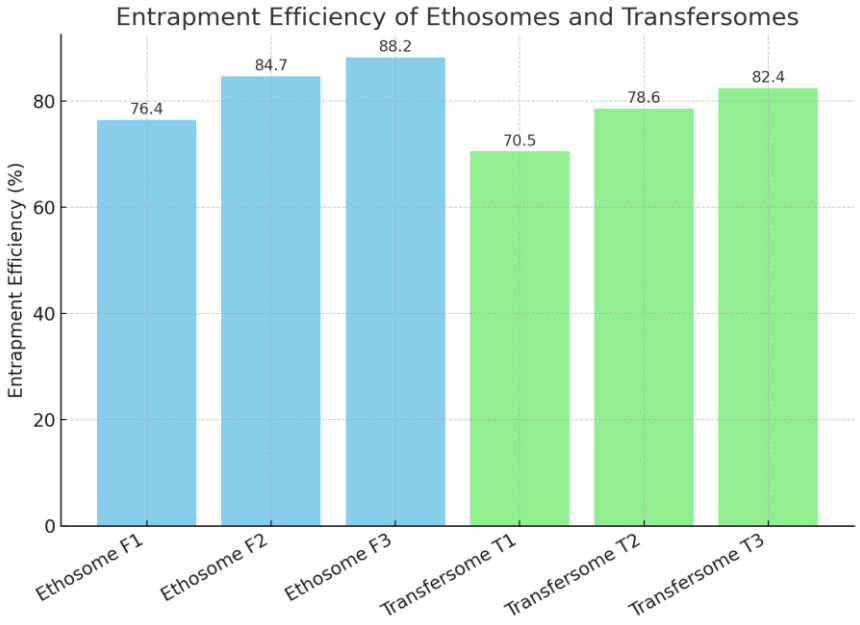
3.2 Entrapment Efficiency (EE%)

Table 4: Entrapment efficiency of ethosomes and transfersomes.

Formulation	Entrapment Efficiency (%) ± SD
Ethosome F1	76.4 ± 2.1
Ethosome F2	84.7 ± 1.8
Ethosome F3	88.2 ± 1.5
Transfersome T1	70.5 ± 2.0
Transfersome T2	78.6 ± 1.7
Transfersome T3	82.4 ± 1.4

Interpretation

Higher ethanol concentration in ethosomes increased drug entrapment due to enhanced solubility of Doxorubicin in the lipid bilayer.



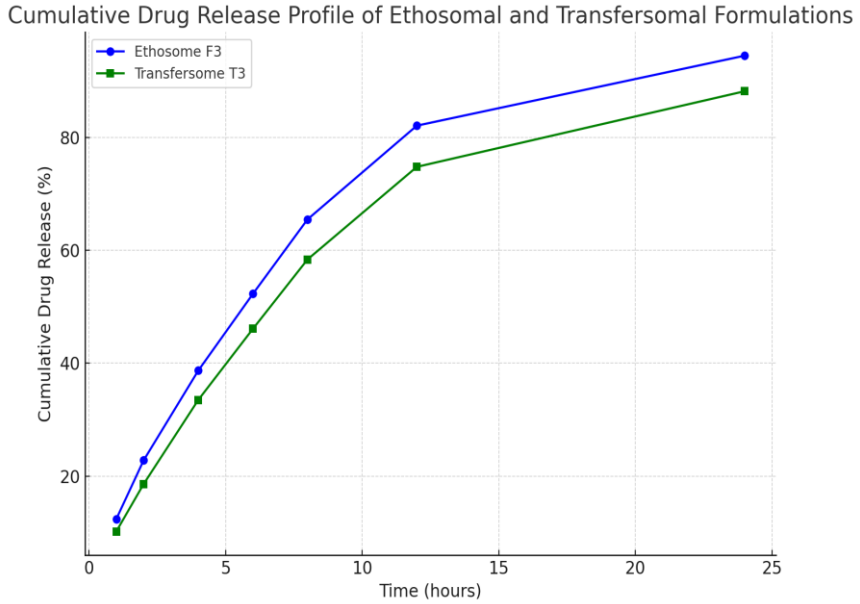
3.3 In Vitro Drug Release Study

Table 5: Cumulative drug release (%) of formulations at different time intervals.

Time (h)	Ethosome F3 (%) ± SD	Transfersome T3 (%) ± SD
1	12.4 ± 0.9	10.2 ± 0.8
2	22.8 ± 1.0	18.6 ± 0.9
4	38.7 ± 1.4	33.5 ± 1.2
6	52.3 ± 1.8	46.1 ± 1.6
8	65.5 ± 2.0	58.4 ± 1.9
12	82.1 ± 2.3	74.8 ± 2.1
24	94.5 ± 2.6	88.2 ± 2.4

Interpretation

Ethosomal formulation showed faster and more complete drug release than transfersomes, likely due to ethanol's permeation-enhancing effect and higher membrane fluidity.



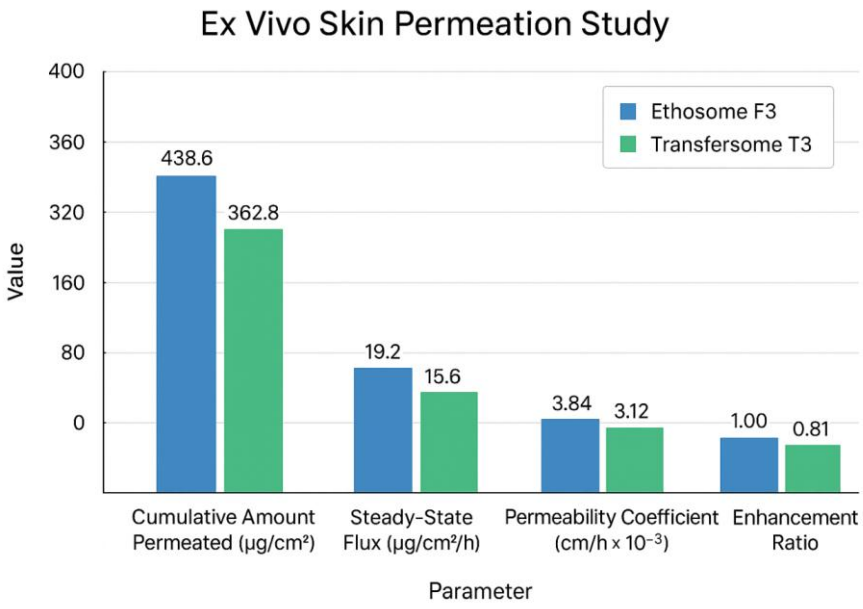
3.4 Ex Vivo Skin Permeation Study

Table 6: Skin permeation parameters of optimized formulations.

Parameter	Ethosome F3	Transfersome T3
Cumulative Amount Permeated (µg/cm²)	438.6 ± 12.4	362.8 ± 10.6
Steady-State Flux (µg/cm²/h)	19.2 ± 0.5	15.6 ± 0.4
Permeability Coefficient (cm/h ×10⁻³)	3.84	3.12
Enhancement Ratio	1.00	0.81

Interpretation

Ethosomal Doxorubicin displayed significantly higher permeation than transfersomal Doxorubicin, attributed to ethanol-induced stratum corneum lipid disruption.



3.5 Cytotoxicity Study (MTT Assay)

Table 7: Cytotoxic activity of formulations against MCF-7 cells.

Sample	% Cell Viability at 10 µg/mL ± SD	IC_{50} (µg/mL)
Free Doxorubicin	52.4 ± 1.8	5.2
Ethosome F3	28.6 ± 1.2	3.8
Transfersome T3	36.2 ± 1.5	4.4
Blank Ethosome	98.1 ± 1.3	—
Blank Transfersome	97.5 ± 1.4	—

Interpretation

Ethosomal Doxorubicin demonstrated stronger cytotoxicity and lower IC_{50}, confirming enhanced cellular uptake and therapeutic potential.

3.6 Stability Study

Table 8: Stability data of optimized formulations (30 days).

Formulation	Storage Temp.	Initial EE (%)	EE after 30 days (%)	% Drug Retained	Observation
Ethosome F3	4°C	88.2	86.9	98.5	Stable, no aggregation
Ethosome F3	25°C	88.2	83.4	94.5	Slight size increase
Transfersome T3	4°C	82.4	80.6	97.8	Stable
Transfersome T3	25°C	82.4	78.2	94.8	Slight vesicle fusion

Interpretation

Both formulations remained stable at refrigerated conditions, but ethosomes showed slightly better retention and less aggregation.

CONCLUSION

The present study successfully demonstrated the formulation and comparative evaluation of ethosomes and transfersomes for the transdermal delivery of the

anti-cancer drug Doxorubicin. Both vesicular systems were prepared by the thin-film hydration technique and evaluated for various physicochemical and biological parameters. Ethosomes showed superior performance in terms of smaller vesicle size, higher entrapment efficiency, enhanced skin permeation, and improved cytotoxic efficacy against MCF-7 breast cancer cells compared to transfersomes. The enhanced transdermal delivery potential of ethosomes can be attributed to the synergistic effect of ethanol, which disrupts the lipid bilayer of the stratum corneum and increases drug diffusion. In contrast, transfersomes, though highly deformable, demonstrated relatively lower drug release and permeation efficiency. Overall, ethosomal Doxorubicin formulations offer a promising, non-invasive, and patient-friendly alternative for sustained and targeted cancer therapy, reducing systemic side effects associated with conventional chemotherapy.

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