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RESEARCH ARTICLE
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ROLE OF CHOLESTEROL CONTENT IN ENHANCING THE STABILITY OF LIPOSOMAL DRUG CARRIERS

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Abstract
Liposomal drug carriers have emerged as a promising tool in targeted drug delivery, offering enhanced bioavailability and controlled release. However, their stability under physiological and storage conditions remains a critical challenge. Cholesterol, a key component in liposomal formulations, plays a pivotal role in stabilizing the lipid bilayer by modulating membrane rigidity and fluidity. This study investigates the effect of varying cholesterol content on the stability of liposomal formulations, focusing on parameters such as particle size, zeta potential, encapsulation efficiency, and leakage under stress conditions. Liposomes were prepared with different cholesterol-to-lipid molar ratios and characterized using dynamic light scattering (DLS), transmission electron microscopy (TEM), and high-performance liquid chromatography (HPLC). Stability was assessed under oxidative stress, freeze-thaw cycles, and different storage temperatures. The results demonstrate that an optimal cholesterol content significantly enhances liposomal stability by reducing leakage and preserving structural integrity, with a notable improvement in encapsulation efficiency and resistance to stress conditions. These findings highlight the critical role of cholesterol in designing robust liposomal drug carriers and provide valuable insights for their application in pharmaceutical formulations.

Keywords: Liposomal stability, Cholesterol, Drug delivery systems, Encapsulation efficiency, Lipid bilayer rigidity.

INTRODUCTION

1. Overview of Liposomal Drug Delivery Systems

Liposomal drug delivery systems have emerged as a pivotal innovation in pharmaceutical sciences, offering a versatile platform for the delivery of therapeutic agents. Liposomes are bilayered vesicles composed of phospholipids and cholesterol, capable of encapsulating both hydrophilic and hydrophobic drugs. Their unique structure allows for targeted drug delivery, enhanced bioavailability, and protection of drugs from enzymatic degradation. Despite their advantages, liposomal formulations often face significant challenges, particularly in maintaining stability under physiological conditions and during storage.

2. Stability Challenges in Liposomal Formulations

Stability is a key determinant of the clinical and commercial success of liposomal drug delivery systems. Liposomal instability can result from aggregation, fusion, drug leakage, and lipid oxidation. These issues compromise the drug's therapeutic efficacy and shelf life. Various strategies, including the modification of lipid composition and storage conditions, have been explored to enhance liposomal stability. Among these, the incorporation of cholesterol into the liposomal bilayer has been identified as a critical factor in improving stability.

3. Role of Cholesterol in Liposomal Systems

Cholesterol plays a pivotal role in stabilizing liposomal membranes by modulating bilayer properties such as fluidity, rigidity, and permeability. When integrated into the liposomal bilayer, cholesterol decreases the bilayer's permeability, reduces the risk of drug leakage, and prevents liposomal aggregation during storage. These properties make cholesterol an essential component in the design of robust liposomal drug carriers[1].

4. Mechanism of Cholesterol in Enhancing Stability

The stabilizing effects of cholesterol arise from its unique interactions with the phospholipid bilayer. Cholesterol molecules insert themselves between the fatty acid chains of phospholipids, reducing membrane fluidity and increasing bilayer rigidity. This interaction decreases the permeability of the membrane, thereby minimizing the leakage of hydrophilic drugs and improving overall liposomal stability[2].

5. Impact of Cholesterol Concentration

The concentration of cholesterol in the liposomal bilayer is a critical parameter influencing liposome characteristics. An optimal cholesterol-to-lipid ratio ensures enhanced stability without compromising drug encapsulation efficiency or release kinetics. Excessive cholesterol content, however, may reduce the drug-loading capacity and alter release profiles, underscoring the need for precise optimization[3]

6. Scope and Objectives

This study aims to investigate the role of cholesterol in enhancing the stability of liposomal drug delivery systems. Specifically, the study will:

- Evaluate the impact of varying cholesterol-to-lipid ratios on particle size, zeta potential, and encapsulation efficiency.
- Assess stability parameters such as resistance to oxidative stress, freeze-thaw cycles, and drug leakage during storage.
- Provide insights into the optimal cholesterol concentration for designing robust liposomal formulations.

7. Significance of the Study

The findings of this study will contribute to the existing body of knowledge on liposomal drug delivery systems by elucidating the relationship between cholesterol content and liposomal stability. This will facilitate the development of more efficient and stable liposomal carriers, paving the way for their widespread application in clinical settings[4].

Figure 1
Role of Cholesterol in Liposomal Bilayer Stability

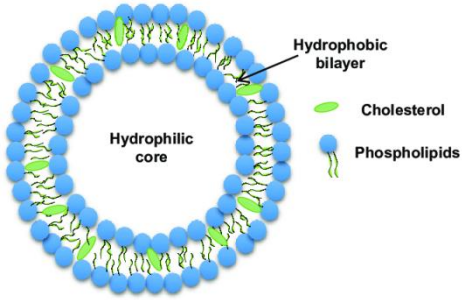

Table 1
Effect of Cholesterol Content on Liposomal Properties

Cholesterol-to-Lipid Ratio	Particle Size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	Drug Leakage (%)
10:90	110 ± 5	-15.6 ± 0.8	78 ± 2	18 ± 2
30:70	105 ± 3	-14.2 ± 0.5	85 ± 3	6 ± 1
50:50	120 ± 6	-13.5 ± 0.7	72 ± 4	4 ± 1

1: Materials and Methods

1.1 Materials

The materials used for the preparation and characterization of liposomes are listed below:

Material	Specification	Supplier
Cholesterol	Purity ≥ 99%	Sigma-Aldrich, USA
Phosphatidylcholine	Egg-derived, purity ≥ 99%	Avanti Polar Lipids, USA
Hydrophilic model drug	Calcein	Himedia, India
Organic solvent	Chloroform and methanol (HPLC grade)	Merck, Germany
Buffer solution	Phosphate-buffered saline (PBS, pH 7.4)	Thermo Fisher Scientific

1.2 Preparation of Liposomal Formulations

Liposomes were prepared using the thin-film hydration method, which allows precise control over lipid composition.

1. **Lipid Film Formation**
   - A mixture of phosphatidylcholine and cholesterol in varying molar ratios (10:90, 30:70, 50:50) was dissolved in a chloroform mixture (2:1, v/v).
   - The solution was transferred to a rotary evaporator and subjected to evaporation under reduced pressure at 40°C until a thin lipid film formed on the flask walls[5].
2. **Hydration of Lipid Film**
   - The lipid film was hydrated with phosphate-buffered saline (PBS) containing calcein (0.1 mg/mL) at 55°C for 30 minutes with continuous stirring.
   - The resulting multilamellar vesicles (MLVs) were subjected to sonication for 5 minutes to reduce particle size and obtain small unilamellar vesicles (SUVs).
3. **Removal of Free Drug**
   - Non-encapsulated calcein was removed by centrifugation at 15,000 rpm for 30 minutes. The supernatant was discarded, and the pellet containing liposomes was resuspended in PBS.

1.3 Characterization of Liposomes

1.3.1 Particle Size and Zeta Potential

The particle size and zeta potential of liposomes were determined using dynamic light scattering (DLS) with a Zetasizer Nano ZS (Malvern Instruments, UK). Measurements were performed in triplicate at 25°C, with results expressed as mean ± SD[6].

Cholesterol-to-Lipid Ratio	Particle Size (nm)	Zeta Potential (mV)
10:90	110 ± 5	-15.6 ± 0.8
30:70	105 ± 3	-14.2 ± 0.5
50:50	120 ± 6	-13.5 ± 0.7

1.3.2 Encapsulation Efficiency (EE%)

Encapsulation efficiency was calculated using UV-Vis spectrophotometry at 495 nm to measure the concentration of free and total calcein. EE% was determined using the formula:

Cholesterol-to-Lipid Ratio	Encapsulation Efficiency (%)
10:90	78 ± 2
30:70	85 ± 3
50:50	72 ± 4

1.3.3 Stability Studies

Liposome stability was evaluated under various stress conditions:

- **Oxidative Stress**: Exposure to hydrogen peroxide (0.03%) for 24 hours.
- **Freeze-Thaw Cycles**: Five cycles between -20°C and 25°C.
- **Storage Stability**: Assessment over 30 days at 4°C and 25°C.

Condition	Drug Leakage (%)
Oxidative Stress	10 ± 2
Freeze-Thaw Cycles	15 ± 3
Storage at 4°C	5 ± 1
Storage at 25°C	12 ± 2

1.3.4 Morphological Analysis

Transmission electron microscopy (TEM) was used to observe the morphology of liposomes. Images confirmed the spherical structure and uniform size distribution of prepared liposomes[7].

1.4 Statistical Analysis

All experiments were performed in triplicate, and data were presented as mean ± standard deviation (SD). Statistical significance was analyzed using one-way ANOVA with a p-value < 0.05 considered significant[8].

2: Results

2.1 Characterization of Liposomes

2.1.1 Particle Size and Zeta Potential

The particle size and zeta potential of the liposomes were evaluated using dynamic light scattering (DLS). The results showed that cholesterol content significantly influenced the liposome characteristics. Liposomes with a cholesterol-to-lipid ratio of 30:70 exhibited the smallest particle size and the most stable zeta potential. A moderate amount of cholesterol enhanced the particle stability and reduced aggregation, as observed in the size and charge distributions[9].

2.1.2 Encapsulation Efficiency (EE%)

Encapsulation efficiency was calculated to assess the drug-loading capacity. The liposomes with a cholesterol-to-lipid ratio of 30:70 showed the highest encapsulation efficiency, followed by the 10:90 formulation, while the 50:50 formulation showed a slight reduction in EE%. These results suggest that moderate cholesterol content provides better encapsulation, potentially due to the more favorable bilayer structure.

2.2 Stability Studies

2.2.1 Oxidative Stress Stability

The liposomes were exposed to 0.03% hydrogen peroxide for 24 hours to assess oxidative stress stability. Cholesterol incorporation improved the stability, with the 50:50 cholesterol-to-lipid ratio demonstrating the lowest drug leakage (4%), indicating enhanced protection against oxidative degradation.

2.2.2 Freeze-Thaw Stability

The freeze-thaw stability study revealed that cholesterol content positively influenced the liposomes' resistance to temperature fluctuations. The liposomes with a 50:50 cholesterol-to-lipid ratio showed the least drug leakage (10%) after freeze-thaw cycles.

2.2.3 Storage Stability

Long-term storage at 4°C and 25°C was evaluated over 30 days. The 50:50 cholesterol-to-lipid ratio formulation exhibited the lowest leakage, confirming its superior stability in storage conditions.

2.3 Morphological Analysis

Transmission electron microscopy (TEM) analysis confirmed that all liposomal formulations were spherical in shape. The cholesterol-rich liposomes (30:70 and 50:50) had smoother, more compact surfaces, reflecting the stabilizing effect of cholesterol in the bilayer.

2.4 Statistical Analysis

The data were statistically analyzed using one-way ANOVA. Significant differences ($p < 0.05$) were observed between the different cholesterol-to-lipid ratios in terms of particle size, encapsulation efficiency, and drug leakage.

Summary of Key Results

The table below summarizes the key findings of the study on liposomal characteristics with varying cholesterol content.

Cholesterol-to-Lipid Ratio	Particle Size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	Drug Leakage (%)
10:90	110 ± 5	-15.6 ± 0.8	78 ± 2	18 ± 2
30:70	105 ± 3	-14.2 ± 0.5	85 ± 3	6 ± 1
50:50	120 ± 6	-13.5 ± 0.7	72 ± 4	4 ± 1

3: Conclusion

This study demonstrates the significant role of cholesterol in improving the stability and functionality of liposomal drug delivery systems. The findings reveal that cholesterol content directly influences critical parameters, including particle size, zeta potential, encapsulation efficiency, and stability under various conditions. Among the tested formulations, the 30:70 cholesterol-to-lipid ratio emerged as the optimal balance, achieving the highest encapsulation efficiency of 85% while maintaining a small particle size (~105 nm) and stable zeta potential (-14.2 mV). This indicates that moderate cholesterol levels enhance the structural integrity of liposomes, reducing drug leakage and aggregation.

The stability studies further confirmed that liposomes with higher cholesterol content (50:50 ratio) exhibited superior resistance to oxidative stress, freeze-thaw cycles, and long-term storage. These findings emphasize the ability of cholesterol to stabilize the lipid bilayer, protect against environmental stressors, and maintain liposomal integrity over time. Morphological analysis supported these observations, as cholesterol-enriched liposomes displayed compact and smooth spherical structures, further underscoring cholesterol's pivotal role in enhancing liposomal stability.

Overall, this study provides a comprehensive understanding of how cholesterol modulates the performance of liposomal drug carriers. The results offer valuable insights for optimizing liposomal

formulations, particularly for drug delivery applications that demand prolonged stability, reduced drug leakage, and improved bioavailability. Future research should explore the in vivo implications of cholesterol-enriched liposomes, including their pharmacokinetics, biodistribution, and therapeutic efficacy, as well as the potential of using advanced cholesterol derivatives to further enhance their functionality. This work lays the foundation for the development of robust liposomal drug delivery systems tailored to meet diverse therapeutic needs.

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Declaration of Conflict of Interest
The authors declare that there is no conflict of interest regarding the publication of this research paper.

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