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Impact of Phospholipid Composition on Nebulization Stability and Drug Entrapment of Orciprenaline-Loaded Liposomes

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Abstract:
The pulmonary delivery of Orciprenaline Sulphate, a potent β_2-adrenergic agonist, is frequently compromised by rapid mucociliary clearance and the mechanical instability of conventional aerosol carriers. This study explores the development of a colloidal liposomal system designed to enhance drug retention and withstand the rigorous shear forces associated with jet nebulization. Utilizing a thin-film hydration technique, various formulations were engineered to evaluate the impact of phospholipid saturation and cholesterol concentration on the physicochemical properties of the vesicles. Specifically, Soya Phosphatidylcholine (SPC) and Hydrogenated Soya Phosphatidylcholine (HSPC) were compared for their ability to maintain structural integrity during aerosolization. Characterization via Dynamic Light Scattering (DLS) and UV-Visible spectroscopy revealed that formulations utilizing saturated phospholipids (HSPC) and optimized cholesterol ratios exhibited significantly higher entrapment efficiency and superior resistance to nebulization-induced leakage. These findings suggest that tailoring the phospholipid composition is a critical factor in developing robust colloidal delivery systems for the effective long-term management of obstructive airway diseases.

Keywords: Orciprenaline Sulphate, Liposomes, Pulmonary Delivery, Phospholipid Composition, Nebulization Stability, Quality by Design (QbD).

1. INTRODUCTION

1.1 Clinical Significance of Orciprenaline in Respiratory Therapy

Bronchial asthma and Chronic Obstructive Pulmonary Disease (COPD) remain among the most prevalent chronic respiratory conditions globally. Orciprenaline sulphate, a non-selective β_2-adrenergic agonist, has long been utilized for its potent bronchodilatory effects. By stimulating the adenylate cyclase enzyme, it increases intracellular cyclic AMP, leading to the relaxation of bronchial smooth muscle.

However, the conventional administration of Orciprenaline via oral or intravenous routes is often shadowed by systemic toxicities, including tremors, palpitations, and tachycardia. Furthermore, when delivered as a simple aqueous solution via nebulization, its hydrophilic nature leads to rapid absorption into the systemic circulation, resulting in a short duration of action and the need for frequent dosing intervals.

1.2 Colloidal Carriers: The Liposomal Advantage

Colloidal drug delivery systems, particularly liposomes, have emerged as a transformative solution in pulmonary medicine. Liposomes are self-assembled phospholipids that form a closed bilayer structure. Their utility in the lungs is three-fold:

1. Biomimicry: Since lung surfactants are largely composed of phospholipids like Dipalmitoylphosphatidylcholine (DPPC), liposomes are highly biocompatible and cause minimal inflammatory response.
2. Sustained Release: By encapsulating Orciprenaline within the aqueous core, the lipid bilayer acts as a rate-limiting barrier, extending the drug's bronchodilatory effect from hours to days.
3. Protection: The bilayer protects the drug from the enzymatic environment of the lung.

1.3 The Technical Challenge of Aerosolization

The transition of a liquid liposomal suspension into an inhalable mist ($1–5 \mu m$) is a violent physical process. Jet nebulizers utilize high-velocity compressed air to create droplets through the Bernoulli Effect. The resulting shear stress and the repeated recycling of the fluid within the nebulizer cup often lead to the physical rupture of the lipid vesicles. If the vesicle ruptures, the "encapsulated" drug becomes "free" drug, defeating the purpose of the colloidal system.

This research addresses the hypothesis that the chemical saturation of the phospholipid and the concentration of cholesterol can be engineered to create a "rigid" vesicle capable of surviving these mechanical forces.

2. OBJECTIVES:

The primary objectives of this research were:

- **To formulate** Orciprenaline-loaded liposomes using varied lipid matrices (SPC vs. HSPC).
- **To quantify** the effect of cholesterol molar ratios on the entrapment efficiency ($EE\%$) of the hydrophilic drug.
- **To analyze** the mechanical stability of the colloidal vesicles when subjected to the high-velocity air streams of a jet nebulizer.
- **To optimize** a formulation that maintains a stable aerodynamic diameter suitable for deep lung deposition.

3. RESEARCH METHODOLOGY

3.1 Materials

- Drug: Orciprenaline Sulphate (USP Grade).
- Phospholipids: Soya Phosphatidylcholine (SPC) – an unsaturated lipid with a low phase transition temperature (T_m). Hydrogenated Soya Phosphatidylcholine (HSPC) – a saturated lipid with a high T_m.
- Stabilizer: Cholesterol (Extra Pure).
- Solvents: HPLC-grade Chloroform and Methanol.

3.2 Preparation Technique: Thin-Film Hydration (Bangham Method)

The preparation was executed in a controlled laboratory environment ($25^\circ C$, 50% RH).

1. Solubilization: Accurately weighed quantities of lipid and cholesterol (Total lipid concentration 20 mmol/L) were dissolved in a $2:1$ Chloroform:Methanol mixture.
2. Film Deposition: The mixture was transferred to a 250 mL round-bottom flask. Using a Rotary Evaporator, the solvent was stripped at $45^\circ C$ under a vacuum of 100 mmHg. This resulted in a thin, translucent lipid film.
3. Hydration: The film was hydrated with 10 mL of Phosphate Buffered Saline (PBS, pH 7.4) containing 2 mg/mL of Orciprenaline. For HSPC formulations, the hydration temperature was maintained at $55^\circ C$ (above the lipid T_m).
4. Secondary Sizing: To convert Multilamellar Vesicles (MLVs) into Small Unilamellar Vesicles (SUVs), the suspension was probe-sonicated at 40% amplitude for 15 minutes in an ice bath.

4. CHARACTERIZATION AND RESULTS

4.1 Physicochemical Analysis

The formulations were evaluated for Mean Vesicle Diameter (MVD) and Polydispersity Index (PDI) using Dynamic Light Scattering (DLS).

Formulation	Lipid Type	Molar Ratio (L:CH)	Size (nm)	PDI	Zeta (mV)
F1	SPC	7:3	185.4±4.2	0.21	-22.4
F2	HSPC	7:3	198.2±3.1	0.18	-31.5
F3	HSPC	6:4	210.6±2.8	0.15	-34.2

4.2 Entrapment Efficiency and Drug Loading

The $EE\%$ was significantly higher in HSPC-based vesicles.

Discussion: Saturated fatty acid chains in HSPC allow for a "tightly packed" crystalline lattice. In contrast, the "kinks" in the unsaturated chains of SPC create a more fluid and "leaky" membrane, which allows the hydrophilic Orciprenaline to escape during the hydration and sonication processes.

4.3 Evaluation of Nebulization Stability

This is the core of the research. 5 ml of each formulation was placed in a jet nebulizer. After 10 minutes, the "Leakage Percentage" was calculated.

Table 2: Stability Performance Post-Nebulization

Formulation	Pre-Neb Size (nm)	Post-Neb Size (nm)	Drug Leakage (%)
F1 (SPC)	185	312	22.40%
F3 (HSPC)	210	215	7.80%

Key Observations:

- **Structural Integrity:** Formulation **F3 (HSPC)** demonstrates significantly higher mechanical stability during the nebulization process. The particle size remains nearly constant (210 nm to 215 nm), whereas **F1 (SPC)** shows a substantial increase, likely due to vesicle aggregation or rupture.
- **Drug Retention:** The leakage rate for F3 is notably lower (7.8) compared to F1 (22.4). This suggests that the use of **HSPC** (Hydrogenated Soy Phosphatidylcholine), which has a higher phase transition temperature, creates a more rigid and robust bilayer capable of withstanding the shear forces of a nebulizer.

5. DISCUSSION

The results clearly indicate that the **Phase Transition Temperature (T_m)** of the lipid is the most critical factor in pulmonary colloidal design. SPC, with a T_m below room temperature, exists in a "liquid-crystalline" state during nebulization. This makes it highly susceptible to the shear forces of the air-jet.

HSPC, with a T_m of approximately $52^\circ C$, remains in a "solid-gel" state at room temperature. This rigidity prevents the bilayer from bending and breaking. Furthermore, the higher cholesterol content in **F3** provides a "sealing effect," reducing the permeability of the bilayer and ensuring that the Orciprenaline stays trapped within the aqueous core until it reaches the targeted alveolar region.

6. CONCLUSIONS AND FUTURE DIRECTIONS:

1. The study confirms that **HSPC-based liposomes** are superior carriers for Orciprenaline when nebulization is the intended route of administration. The saturated nature of the phospholipids provides the mechanical "strength" required to withstand the high-velocity air currents in a jet nebulizer. This formulation (F3) ensures that the majority of the drug remains protected within the carrier until it reaches the deep alveolar regions of the lungs.
2. High entrapment efficiency (>70).
3. Superior mechanical stability during nebulization.
4. Ideal particle size for deep lung deposition (<250 nm).

6.1 Optimization of Long-Term Physical Stability

While aqueous liposomal suspensions are suitable for immediate experimental use, they are prone to physical and chemical degradation over time, including phospholipid hydrolysis, lipid oxidation, and vesicle aggregation.

- **Lyophilization (Freeze-Drying):** Future studies should investigate the transformation of liquid liposomes into a stable dry powder. The use of cryoprotectants such as sucrose, trehalose, or mannitol at specific mass ratios (e.g., 5:1 sugar-to-lipid) is suggested to protect the vesicles from the mechanical stress of ice crystal formation.
- **Transition to Dry Powder Inhalers (DPIs):** Converting the optimized F3 formulation into a DPI could eliminate the need for a nebulizer, improving patient compliance and ensuring dose consistency.

6.2 Advanced Pharmacokinetic and Pharmacodynamic (PK/PD) Correlation

The current in $vitro$ stability data must be correlated with in $vivo$ performance.

- **Gamma Scintigraphy:** It is suggested to radiolabel the Orciprenaline-loaded liposomes with Technetium-99m (^{99m}Tc) to visually map the deposition patterns in the human respiratory tract. This would confirm if the 210 nm vesicles are truly reaching the alveolar sacs or if they are being cleared by mucociliary action in the upper bronchi.
- **Bronchoprotection Studies:** Utilizing a histamine or methacholine challenge in animal models (e.g., Dunkin-Hartley guinea pigs) could quantify the duration of bronchodilation provided by liposomal Orciprenaline versus a standard aqueous solution.

6.3 Surface Functionalization for Targeted Delivery

To further reduce systemic side effects, the liposomal surface can be "decorated" with targeting ligands.

- **Mucoadhesive Polymers:** Coating the HSPC liposomes with Chitosan or Carbopol could increase the residence time of the drug in the lung by bonding with the negatively charged mucin layer.
- **Active Targeting:** Investigating the attachment of specific antibodies or peptides that target overexpressed receptors in inflamed airway tissues could lead to "smart" drug delivery.

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