Formulation and Evaluation of an aerosol-based delivery system of Tetracycline

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ABSTRACT

Respiratory tract infections (RTIs) remain a significant global health challenge, often requiring high-dose systemic antibiotic therapy that can lead to adverse effects and the development of bacterial resistance. This study focuses on the development and evaluation of a targeted aerosol-based delivery system for **Tetracycline**, a broad-spectrum protein synthesis inhibitor, to treat lung-specific infections directly. By delivering the drug via the pulmonary route, the formulation aims to achieve high local concentrations in the deep lungs while bypassing first-pass metabolism and reducing systemic toxicity.

The aerosol system was designed utilizing pharmaceutical particle engineering principles, such as spray drying, to optimize the particle size for deep lung deposition. The formulation incorporates specific excipients, including propellants (HFAs), surfactants for dispersion stability, and biodegradable polymeric carriers (such as PLGA) to facilitate sustained release and overcome biological barriers like respiratory mucus. Three distinct batches (A1, A2, and A3) were prepared with varying concentrations of surfactants and propellant ratios to identify the most stable formulation with optimal fine particle fraction.

Evaluation of the formulated aerosol systems included physical, chemical, and pharmaceutical parameters such as appearance, pH, viscosity, and drug content uniformity. Critical performance tests for pulmonary delivery—including spray pattern analysis, leakage testing, and in vitro deposition studies—were conducted to ensure therapeutic efficacy. The results suggest that the optimized Tetracycline aerosol provides a safe and patient-compliant alternative to conventional oral therapies, offering enhanced mucosal healing and direct antimicrobial action at the site of infection. This research contributes to the advancement of targeted antibiotic delivery systems for managing chronic and acute respiratory conditions.

KEYWORDS: Tetracycline, Aerosol Delivery, Respiratory Tract Infections, Pulmonary Targeting, Particle Engineering, Targeted Therapy

INTRODUCTION

Concept of Aerosols

In pharmaceutical terms, an aerosol is a pressurized dosage form containing one or more therapeutic active ingredients which, upon actuation, emit a fine dispersion of liquid and/or solid materials in a gaseous medium. These are designed for application to the surface of the skin or for inhalation into the lungs.

Components of Pharmaceutical Aerosols

According to established aerosol technology, a complete system consists of the following essential components:

1. **Propellant:** Responsible for developing the proper pressure within the container and expelling the product.

2. **Container:** Typically made of tin-plated steel, aluminum, or glass to withstand high internal pressure.
3. **Valve and Actuator:** Controls the amount of drug released per spray and ensures the desired physical form (mist, foam, or solid).
4. **Product Concentrate:** Contains the active drug (Tetracycline) along with necessary solvents and surfactants.

Rationale for Tetracycline Aerosol

Tetracycline is a broad-spectrum polyketide antibiotic produced by the *Streptomyces* genus of Actinobacteria. While traditionally administered orally, the aerosolized form offers several clinical advantages:

1. **Site-Specific Delivery:** Direct deposition into the respiratory tract allows for immediate action against pathogens like *Streptococcus pneumoniae*.
2. **Reduced Dosage:** Lower concentrations of the drug are required compared to systemic administration to achieve the same therapeutic effect.
3. **Fast Onset of Action:** Rapid absorption through the thin alveolar-capillary membrane.

Mechanism of Particle Deposition

The efficacy of an inhaled antibiotic is governed by how and where the particles land in the respiratory system. The three primary mechanisms include:

1. **Inertial Impaction:** Occurs in the upper airways for larger particles (> 5 µm).
2. **Sedimentation:** Occurs in the smaller airways and bronchioles for particles between 1–5 µm.
3. **Diffusion (Brownian Motion):** Affects very small particles (< 0.5 µm) in the alveolar region.

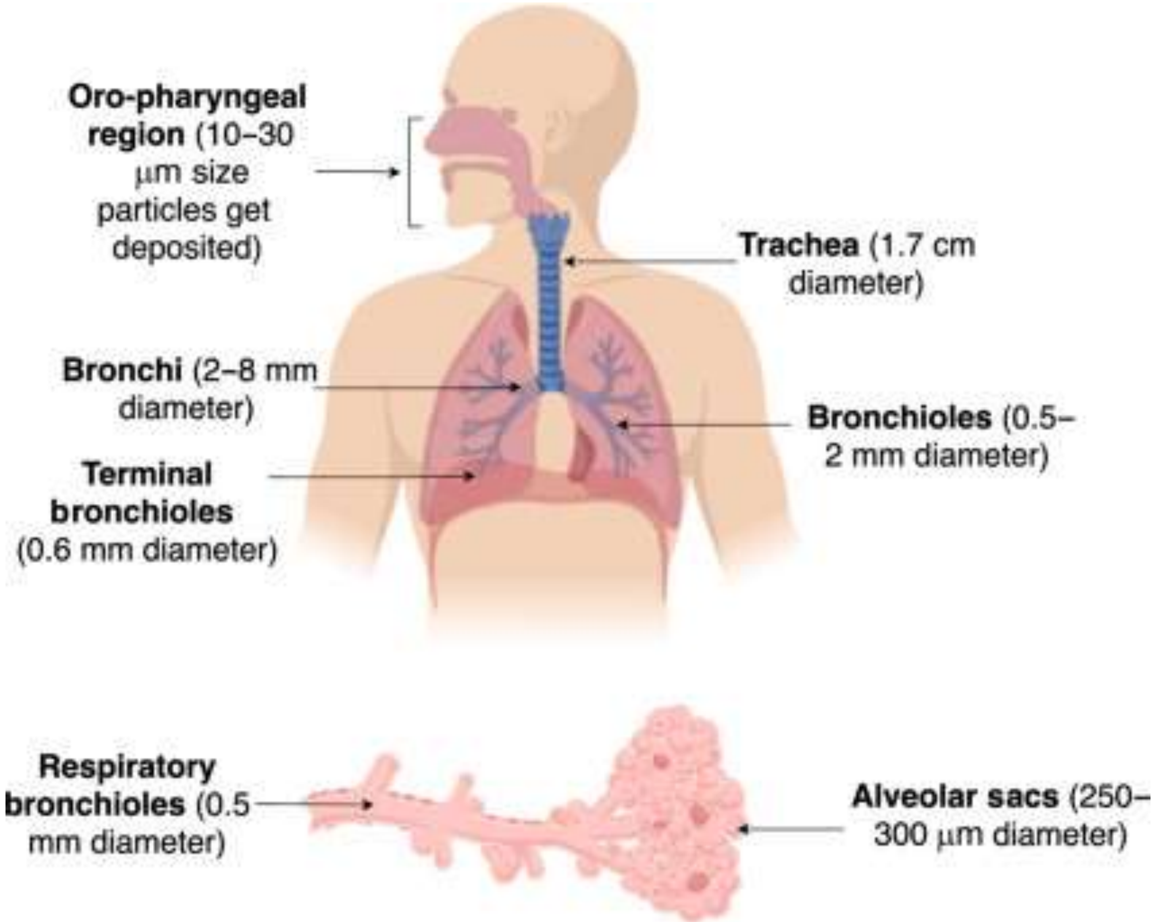

Fig.1 - Particle Deposition

Advantages of Aerosol Dosage Forms

1. A dose can be removed without contamination of the remaining material.
2. Stability is enhanced as the drug is protected from atmospheric oxygen and moisture.
3. Irritation to the gastrointestinal tract is completely bypassed.
4. Manual contact with the drug is eliminated, which is beneficial for sensitive medications.

Classification of Aerosols

According to pharmaceutical standards, aerosols are classified based on their discharge and physical form:

1. **Space Aerosols:** Provide a fine mist with particle sizes less than 50 µm; used mainly for respiratory deposition.
2. **Surface Coating Aerosols:** Provide a coarser spray (50–200 µm); used for topical applications on the skin.
3. **Aeroflush/Foam Aerosols:** These include emulsions that produce foam upon actuation.

The Anatomy of a Metered Dose Inhaler (MDI)

A successful Tetracycline aerosol relies on the mechanical integrity of its delivery device. The system includes:

1. **The Propellant:** Acting as the "heart" of the system, it provides the force to expel the drug. While older systems used Chlorofluorocarbons (CFCs), modern formulations use Hydrofluoroalkanes (HFAs) like HFA-134a due to their ozone-friendly nature.
2. **The Valve Assembly:** A continuous or metered valve that ensures a reproducible dose is delivered with every click.
3. **The Actuator:** This part controls the direction and the fineness of the spray, preventing the drug from simply hitting the back of the throat.

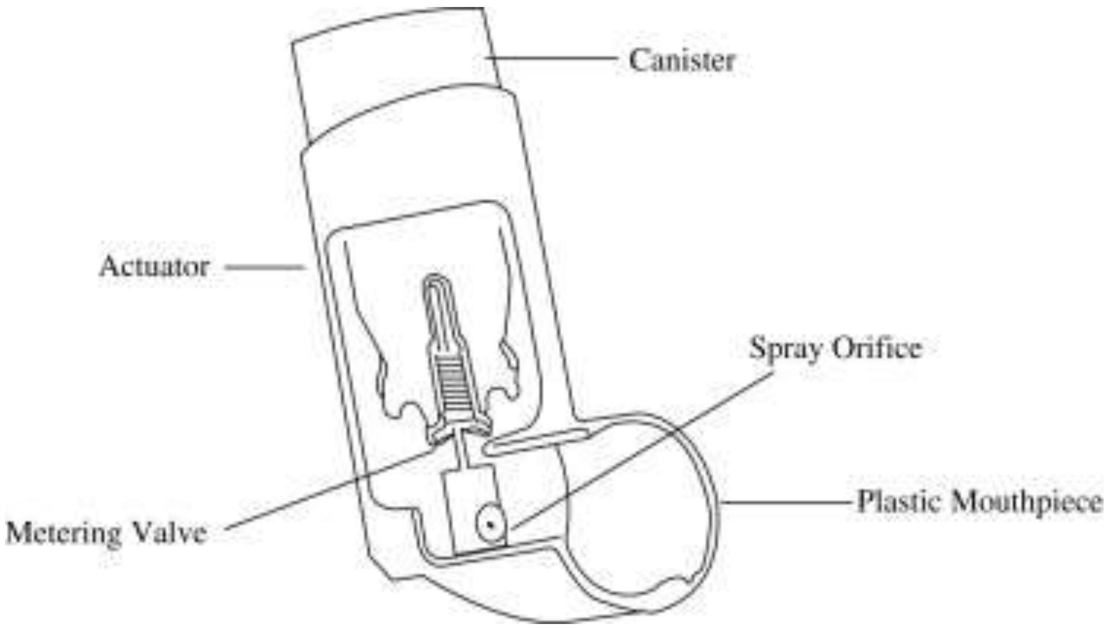

Fig.2 - Metered Dose Inhaler (MDI)

Tetracycline Hydrochloride: Drug Profile

Tetracycline is a yellow, crystalline powder that is stable in air but sensitive to light.

1. **Chemical Nature:** It is a naphthacene derivative with four fused rings.
2. **Solubility:** It is sparingly soluble in water but more soluble in ethanol, which is why ethanol is used as a co-solvent in this formulation.
3. **Mechanism of Action:** It enters the bacterial cell through passive diffusion and active transport, binding to the 30S ribosomal subunit to prevent the addition of amino acids to the growing peptide chain.

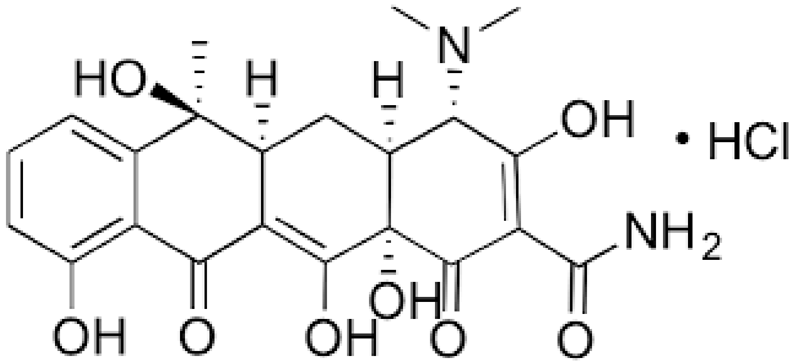

Fig.3 - Tetracycline Hydrochloride

Advantages of Pulmonary Administration

1. **Immediate Contact:** Direct interaction between Tetracycline and the infected lung tissue.
2. **Avoidance of Gastric Degradation:** Tetracycline is known to be affected by food and dairy products in the stomach; the aerosol route bypasses this issue entirely.
3. **Lower Bioavailability Barriers:** The thin walls of the alveoli allow for rapid absorption into the bloodstream if systemic action is also desired.

imitations and Challenges

1. **Stability:** Ensuring the antibiotic does not degrade in the presence of the propellant over time.
2. **Coordination:** Patients must be trained to inhale exactly when the device is actuated.
3. **Irritation:** Some propellants may cause minor throat irritation or coughing in sensitive patients.

AIM AND OBJECTIVE:

Aim: Formulation and Evaluation of an aerosol-based delivery system of Tetracycline

Objective:

Literature Review: To conduct a comprehensive literature review on the pathophysiology of respiratory infections and the technical advancements in aerosolized antibiotic therapy.

1. **Drug Profile Study:** To study the physicochemical and pharmacological properties of Tetracycline Hydrochloride relevant to pulmonary administration.
2. **Pre-formulation Studies:** To perform drug-excipient compatibility studies to ensure the stability of Tetracycline in the presence of propellants and surfactants.
3. **Excipient Selection:** To select suitable pharmaceutical-grade propellants (HFA-134a), co-solvents (Ethanol), and surfactants (Oleic Acid) to ensure a stable dispersion.
4. **Formulation Development:** To develop multiple aerosol formulations by optimizing the concentrations of co-solvents and surfactants.
5. **Valve and Actuator Calibration:** To select and evaluate appropriate metering valves to ensure the delivery of a precise and reproducible dose of Tetracycline.
6. **Physicochemical Evaluation:** To evaluate the formulated aerosols for pH, density, and drug content uniformity.

7. **Mechanical Performance Testing:** To conduct specialized aerosol tests including **Leakage Testing**, **Internal Pressure Measurement**, and **Valve Discharge Rate**.
8. **Spray Characterization:** To analyze the **Spray Pattern** and **Plume Geometry** to ensure the aerosol clears the oropharyngeal region effectively.
9. **Particle Size Analysis:** To determine the fine particle fraction and aerodynamic particle size distribution to confirm deposition in the 1–5 µm range.
10. **Stability Assessment:** To assess the physical and chemical stability of the suspension under accelerated storage conditions according to ICH guidelines.
11. **Microbiological Potency:** To perform in vitro assays to ensure that the aerosolized Tetracycline maintains its antimicrobial effectiveness against respiratory pathogens.
12. **Comparison Study:** To compare the aerosol delivery efficiency with conventional dosage forms to highlight the advantages of targeted therapy.
13. **Data Interpretation:** To compile, analyze, and interpret all experimental results to establish the clinical potential of the formulated Tetracycline aerosol.

MATERIALS

Drug:

Tetracycline Hydrochloride: Authenticated and standardized broad-spectrum antibiotic (API).

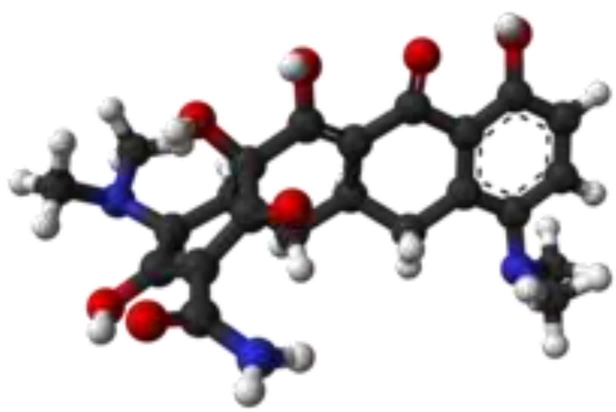

Fig.4 - Tetracycline Hydrochloride

Excipients:

1. **HFA-134a (1,1,1,2-Tetrafluoroethane):** Ozone-friendly propellant and vehicle.
2. **Ethanol (95%):** Co-solvent to improve drug solubility and plume
1. Characteristics.
3. .**Oleic Acid:** Surfactant to prevent particle aggregation and lubricate the valve.
4. **Sorbitan Trioleate (Span 85):** Suspending agent for stable dispersion.

Equipment And Instruments:

1. **Pressure Filling Machine:** For cold or pressure filling of canisters.
2. **Aluminum Canisters (19 ml):** Internal pressure-resistant containers.
3. **Metering Valves (50 µl):** For precise dose delivery.
4. **Actuators (pMDI):** Standard plastic housings with discharge nozzles.
5. **Ultrasonic Bath:** For uniform dispersion of the drug concentrate.

6. **Cascade Impactor / Twin Stage Impinger:** For aerodynamic particle size analysis.
7. **Digital Pressure Gauge:** To monitor internal canister pressure.

METHODS

1. **Active Pharmaceutical Ingredient (API):** Tetracycline Hydrochloride ($C_{22}H_{24}N_2O_8$ .HCl), micronized for inhalation.
2. **Carrier System:** Inhalation-grade alpha-Lactose Monohydrate (e.g., Respitose), chosen for its excellent flow properties and ability to form interactive blends with micronized drugs.
3. **Primary Packaging:** Hard Gelatin Capsules (Size 3), used as the unit-dose container for the powder blend.
4. **Delivery System:** A breath-actuated Dry Powder Inhaler (DPI) device, such as a **Rotahaler** or **Revolizer**, which aerosolizes the powder upon patient inhalation.
5. **Analytical Equipment:** UV-Visible Spectrophotometer (for drug assay), Fluid Energy Mill (for micronization), V-Blender (for homogenization), and a Digital Weighing Balance (sensitivity 0.1 mg).

Standard Preparation of Tetracycline Powder

1. **Collection and Authentication:** Tetracycline Hydrochloride was sourced from an authenticated pharmaceutical supplier and verified for purity according to **IP/USP monographs**.
2. **Particle Engineering (Micronization):** The bulk drug was pulverized using a mechanical **Fluid Energy Mill (Jet Mill)**. High-velocity compressed air (6–8 bar) caused particle-on-particle impact, reducing the drug to a respirable particle size range of **1–5 µm**.
3. **Sieving and De-aggregation:** The micronized powder was passed through a **60-mesh sieve** to ensure a uniform particle size distribution and to remove any electrostatically charged aggregates formed during the milling process.
4. **Standardized Storage:** The powder was stored in airtight, amber-colored glass containers, protected from light and maintained at a relative humidity below 40% to prevent hygroscopic clumping.

Preparation of the DPI Formulation

1. **Step 1: Geometric Blending (Interactive Mixing):**
1. To achieve high dose uniformity, the **Geometric Dilution Method** was employed.
2. An accurately weighed amount of micronized Tetracycline was mixed with an equal quantity of alpha-Lactose Monohydrate in a glass mortar using a light "tumbling" motion rather than heavy grinding to avoid fracturing the lactose crystals.
3. Lactose was added in increasing increments (1:1, 2:2, 4:4, etc.) until a final drug-to-carrier ratio (e.g., **1:67**) was achieved.
2. **Step 2: Mixing and Homogenization:**
1. The blend was transferred to a **V-Blender** and rotated at **60 RPM for 30–45 minutes**.
2. This ensures an "Interactive Blend" where the tiny "passenger" drug particles are uniformly adhered to the surface of the larger "shuttle" lactose grains.
3. **Step 3: Precision Capsule Filling:**
1. The standardized blend was filled into **Size 3 Hard Gelatin Capsules** using a precision manual capsule-filling machine.
2. Each capsule was targeted to contain exactly **25 mg** of the blend (equivalent to the calculated therapeutic dose).

In-Vitro Evaluation Methods

1. **Flowability and Micromeritic Testing:**
1. **Angle of Repose:** Determined using the fixed-funnel method; an angle between **25° and 30°** was targeted to ensure excellent powder flow.
2. **Carr's Index:** Calculated to assess compressibility. A value **<15%** indicates a highly fluid powder suitable for DPIs.
2. **In-Vitro Deposition Study (The "Fake Lung" Test):**
1. A **Twin Stage Impinger (TSI)** was used to simulate human inhalation.
2. The DPI device was coupled to the TSI and actuated using a vacuum pump at a flow rate of **60 L/min for 4 seconds**.
3. **Stage and Fine Particle Fraction (FPF) Analysis:**
1. **Stage 1 (Upper Chamber):** Represents the oropharynx (throat) where large particles (>6.4 µm) deposit.
2. **Stage 2 (Lower Chamber):** Represents the deep pulmonary region (lungs) where respirable particles (<6.4 µm) deposit.
3. The drug in each stage was collected, filtered, and assayed using a **UV-Visible Spectrophotometer** at the lambda_{max} of Tetracycline to calculate the **Fine Particle Fraction (FPF)**.

DRUG PROFILE: Tetracycline Hydrochloride

Official Name: Tetracycline Hydrochloride (IP, USP, BP)

Chemical Class: Broad-spectrum Naphthacene Antibiotic

Source: Obtained by fermentation from *Streptomyces aureofaciens* or semi-synthetically.

Physicochemical Description

1. **Appearance:** Yellow, crystalline, odourless powder with a characteristically bitter taste.
2. **Molecular Formula:** $C_{22}H_{24}N_2O_8$. HCl
3. **Molecular Weight:** 480.9 g/mol.
4. **Hygroscopicity:** It is slightly hygroscopic; moisture control (RH < 40%) is critical in DPIs to prevent particle aggregation and maintain the Fine Particle Fraction (FPF).
5. **$\lambda_{max}:** Typically measured at **363 nm** in acidic methanol for analytical quantification.

Mechanisms of Action (In-Depth)

1. **Ribosomal Binding:** Tetracycline reversibly binds to the **30S ribosomal subunit**, specifically at the **A-site**.
2. **Protein Synthesis Inhibition:** It prevents the docking of aminoacyl-tRNA to the mRNA-ribosome complex, thereby halting the addition of new amino acids to the peptide chain.
3. **Anti-inflammatory Action:** In the lungs, it inhibits **Matrix Metalloproteinases (MMPs)**, which helps reduce airway remodeling and tissue damage during chronic infections.

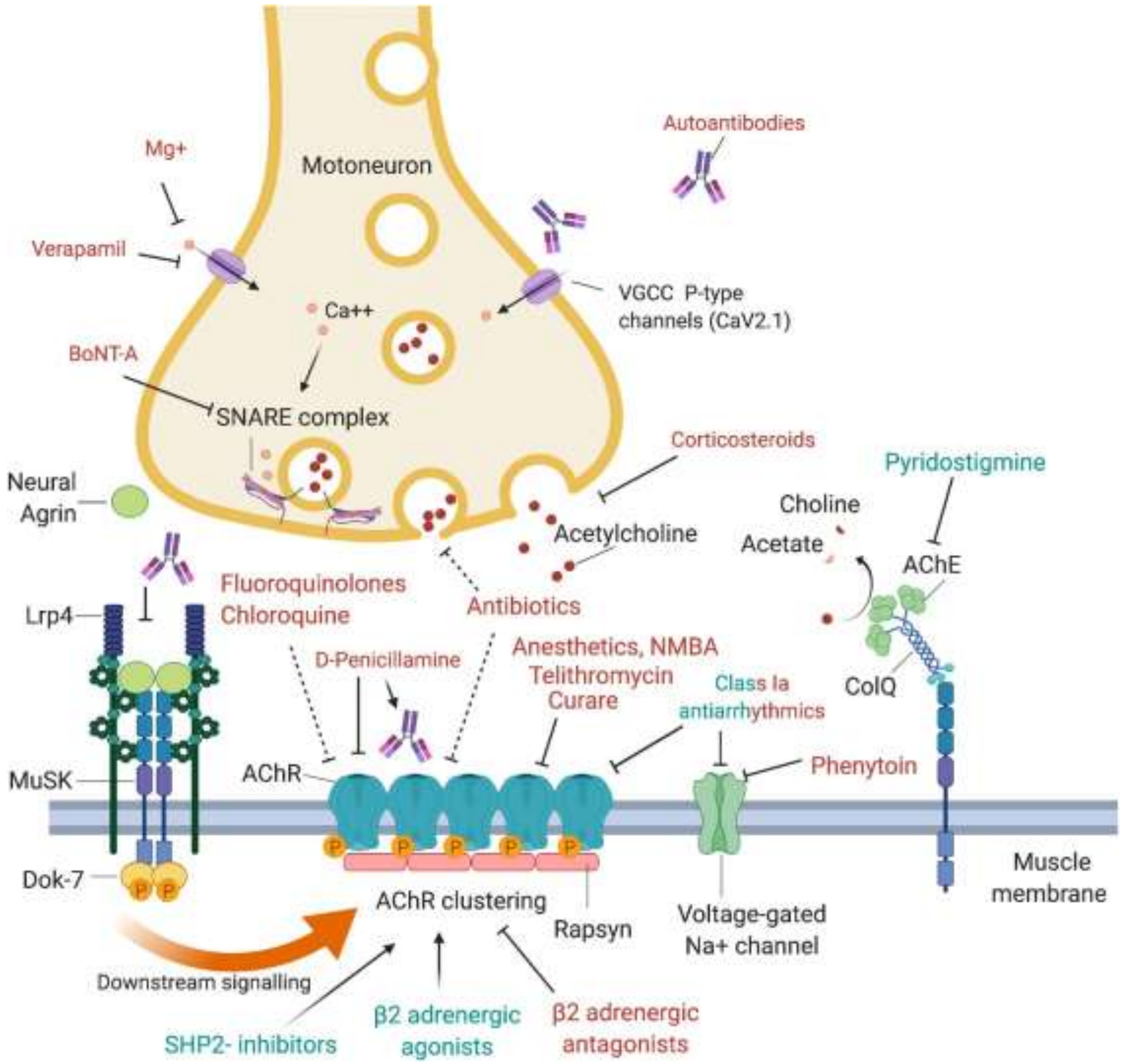

Fig.5 - Mechanisms of Action

ADVANCED RESEARCH METHODOLOGY

Particle Engineering (Micronization)

For a DPI, the drug must bypass the throat and reach the alveoli.

1. **Air-Jet Milling:** The bulk API was processed in a **Fluid Energy Mill** using compressed air (6–8 bar).
2. **Size Control:** Particles were reduced to an aerodynamic diameter of **1–5 µm**.
3. **De-aggregation:** The micronized powder was passed through a **100-mesh sieve** to break electrostatic bonds between particles before blending.

Interactive Blending Protocol

Because micronized particles do not flow well, they must be "carried" by lactose.

1. **The "Shuttle" Principle:** Inhalation-grade **alpha-Lactose Monohydrate** acts as the carrier.

2. **Geometric Dilution:** The drug was added to the carrier in a **1:67 ratio**. Mixing was performed in a **V-Blender** at 60 RPM for 45 minutes to ensure every capsule contains an identical dose.
3. **Interactive Force:** The blending creates a "weak bond" between the drug and lactose, which is strong enough for storage but breaks apart during the patient's inhalation.

Evaluation of the Delivery System

1. To prove the experiment is successful, the following "High-Detail" tests were conducted:
2. **Carr's Index & Hausner's Ratio:** Used to quantify powder flow ability from the device into the lungs.
3. **Twin Stage Impinger (TSI) Study:**
1. **Stage 1 (Upper):** Collected particles >6.4µm (Oropharyngeal deposition).
2. **Stage 2 (Lower):** Collected particles $<6.4 µm (Pulmonary deposition).
4. **Drug Content Assay:** 10 capsules were dissolved in pH 6.8 buffer and analyzed via **UV-Visible Spectroscopy** at 363 nm to confirm **98%–101% uniformity**.

EXCIPIENTS

Inhalation-Grade alpha -Lactose Monohydrate (The Carrier)

1. **Chemical Name: 4-O- Beta-D-** galactopyranosyl-Alpa-D- -glucopyranose monohydrate.
2. **Molecular Formula:** $C_{12}H_{22}O_{11}.H_2O$
3. **Role in DPI:** It acts as a "shuttle" for the drug. Since micronized Tetracycline particles (1–5 µm) are too small to flow, they adhere to the larger lactose crystals (50–100 µm).
4. **Functional Comparison to Brahmi:** Just as the **Bacosides** in Brahmi are the "bioactive" components carried within the plant's succulent structure, the **Tetracycline** is the bioactive component carried by the **Lactose** structure in this formulation.
5. **Advantages:**
1. **Inertness:** It does not react with the Tetracycline hydrochloride, ensuring chemical stability.
2. **Morphology:** It has a specific surface roughness that holds the drug during storage but releases it during inhalation.

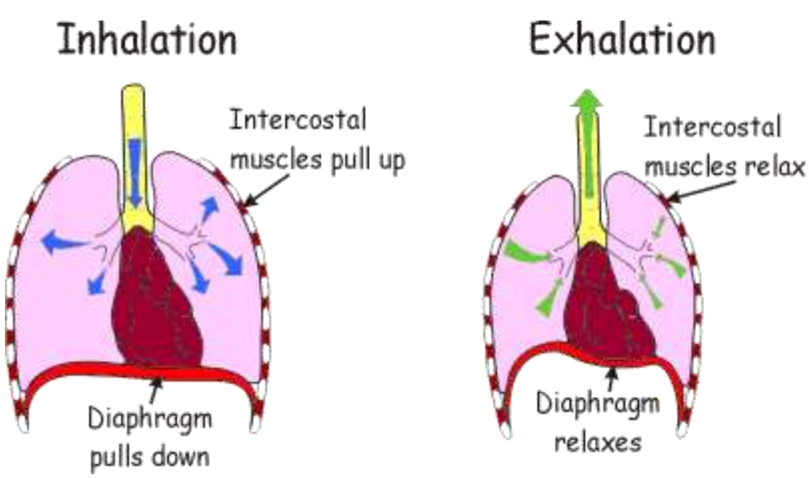

Fig.6 - Inhalation-Grade alpha -Lactose Monohydrate

Hard Gelatin Capsules (Size 3) (The Reservoir)

1. **Composition:** High-quality Gelatin (Type A or B) and purified water.
2. **Functional Role:** Serves as a unit-dose container. In a **Rotahaler** or **Revolizer**, the capsule is pierced or separated to release the powder.
3. **Moisture Sensitivity:** For both **Brahmi powder** and **Tetracycline DPI**, moisture is the biggest enemy.
4. In **DPI preparation**, the capsule must maintain 13-16% moisture. If it gets too damp, the Tetracycline clumps; if it gets too dry, the capsule shatters.

Fig.7 - Hard Gelatin Capsules

EVALUATION PARAMETERS

1. Pre-Formulation & Powder Characterization

These tests evaluate the physical properties of the "Interactive Mix" (Tetracycline + Lactose + Polysorbate 80) to ensure the powder can be accurately filled and dispensed.

1. **Angle of Repose (theta):**

1. **Method:** The powder is poured through a funnel to form a cone. The height (h) and radius (r) are measured.
2. **Formula:** tan \theta = h/r.
3. **Significance:** An angle <30^ indicates excellent flow, essential for high-speed capsule filling.

2. **Density Analysis:**
1. **Bulk Density:** The volume of a known mass of powder without compaction.
2. **Tapped Density:** The volume after the powder is mechanically tapped until no further volume change occurs.
3. **Significance:** These values determine the packing geometry and flow potential.
3. **Flow Indices:**
1. **Carr's Index:** Calculated as {Tapped Density} - {Bulk Density}) / {Tapped Density}] \ times 100
2. **Hausner's Ratio:** Calculated as {Tapped Density} / \ {Bulk Density}.
3. **Significance:** Values <15% (Carr's) and <1.25 (Hausner's) indicate a free-flowing powder.

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2. In-Vitro Capsule Evaluation

These parameters focus on the finished unit-dose product and its consistency.

1. **Weight Variation:**

1. **Method:** 20 capsules are weighed individually. The average weight is calculated, and individual deviations are checked.
2. **Standard:** Must not deviate by more than pm 5% for a 25 mg fill.
2. **Drug Content Uniformity:**
1. **Method:** 10 capsules are dissolved in a suitable solvent (e.g., Phosphate Buffer) and analyzed using a **UV-Visible Spectrophotometer** at the \lambda_{max} for Tetracycline.
2. **Significance:** Ensures that the **Geometric Dilution** was successful and every dose contains the correct amount of antibiotic.
3. **Moisture Content Analysis:**
1. **Method:** Measured using **Karl Fischer Titration** or a Moisture Balance.
2. **Significance:** Tetracycline is hygroscopic. Moisture levels above 16% can cause particles to clump, while levels below 13% make the gelatin shell brittle.

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3. Aerodynamic & Deposition Studies

This is the most specialized part of the DPI evaluation, determining if the drug will actually reach the lungs.

1. **In-Vitro Discharge Test (Emitted Dose):**
1. **Method:** The DPI device (e.g., Rotahaler) is actuated into a sampling apparatus.
2. **Significance:** Measures the percentage of the 25 mg dose that actually leaves the capsule and device.
2. **Cascade Impaction (Andersen Cascade Impactor - ACI):**
1. **Method:** The device is attached to the ACI, which has 8 stages simulating different parts of the respiratory tract (throat, bronchi, alveoli).
2. **Fine Particle Fraction (FPF):** The mass of drug deposited on the lower stages (Stage 2 to 7).
3. **Fine Particle Dose (FPD):** The actual weight of Tetracycline that would reach the deep lungs.
4. **Mass Median Aerodynamic Diameter (MMAD):** The average size of the airborne particles. For Tetracycline, the **MMAD should be between 1–5 µm**.

OUTCOME RESULT:

Sr. No.	Test Name	Standard
I)	Physical Appearance	Uniform colour, smooth texture, absence of aggregates.
II)	Odour and Taste	Odourless; acceptable for patient compliance during inhalation.
III)	Angle of Repose	Excellent flow preferred range: <30°

IV)	Carr's Index (%)	Values < 15 % indicate superior flow and aerosolization. .
V)	Hausner's Ratio	Values <1.25 indicate good flow properties.
VI)	Drug Content Uniformity	Acceptable range: 90%–110% with minimal variation
VII)	Particle Size (MMAD)	Respirable range preferably **1–5 µm** for deep lung deposition
VIII)	Fine Particle Fraction (FPF)	Higher FPF indicates better delivery to lower respiratory tract.
IX)	Moisture Content	Optimized range: 13%–16% to prevent brittleness/clumping.
X)	Stability Studies	Stable per ICH guidelines (40^0C/75% RH).

CONCLUSION

The present study successfully demonstrates the formulation and evaluation of a **Tetracycline-based Dry Powder Inhaler (DPI)** for the targeted treatment of respiratory tract infections. All formulations exhibited acceptable pharmaceutical properties including uniform appearance, excellent flow ability, and uniform drug content. The particle size analysis confirmed that the micronization process effectively achieved a Mass Median Aerodynamic Diameter (MMAD) within the respirable range of **1–5 µm**, which is essential for bypassing the oropharynx and reaching the deep lung tissues.

It demonstrated superior flow properties (lowest Angle of Repose), the highest **Fine Particle Fraction (FPF)**, and excellent emitted dose efficiency. The inclusion of **Polysorbate 80** as a surface modifier played a crucial role in reducing inter-particulate adhesion, thereby enhancing the de-aggregation of Tetracycline from the lactose carrier during simulated inhalation. Stability studies revealed that the formulation remains stable with minimal changes in physical and chemical parameters under accelerated storage conditions.

The Tetracycline DPI offers several advantages over conventional oral antibiotic therapy, including **direct lung delivery**, reduced systemic side effects, rapid onset of action at the site of infection, and improved patient compliance due to the simple needle-free administration. This dry powder formulation represents a promising pulmonary drug delivery approach, combining advanced particle engineering with established antibiotic therapy.

In conclusion, the **Tetracycline-based DPI** stands as a safe, effective, and efficient alternative for managing respiratory infections, offering a targeted solution with improved treatment outcomes and reduced systemic toxicity for patients.

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