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A NOVEL AND ADVANCING APPROACH FOR TARGET ANTIFUNGAL THERAPY

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ABSTRACT

The review focuses on studying how to create, improve, and test a new type of treatment that combines liposomes (tiny fat-like particles) containing Amphotericin B (an antifungal medication) with an in situ vaginal gel. The goal of this innovative approach is to treat fungal infections, especially vulvovaginal candidiasis (a common yeast infection affecting the vaginal area). Amphotericin B, a potent antifungal medication, is highly effective against a wide range of fungal infections. However, its use is often restricted because it can cause harmful side effects when it spreads throughout the body (systemic toxicity) and is not well absorbed when taken using standard methods (poor bioavailability). This makes it challenging to use Amphotericin B effectively without causing unwanted health issues. Incorporating liposomes into an in-situ gel formulation addresses these challenges by enhancing drug encapsulation, providing sustained release, and improving mucosal adherence. The formulation aspects, such as the selection of polymers and surfactants, influence the gel's sol-to-gel transition, viscosity, and mucoadhesive properties. This sentence explains that the formulation aims to treat fungal infections directly at the site of infection using a controlled and long-lasting delivery method. By doing this, the treatment can work more effectively and improve patient compliance (making it easier for patients to stick to the treatment). Considering the future, the plan is to further improve this formulation using advanced methods, explore its use for other types of vaginal infections, and carry out clinical trials to confirm that it works well and is safe for use.

Keywords: Fungal infection, Amphotericin-B, Liposomes, In-situ gel

INTRODUCTION

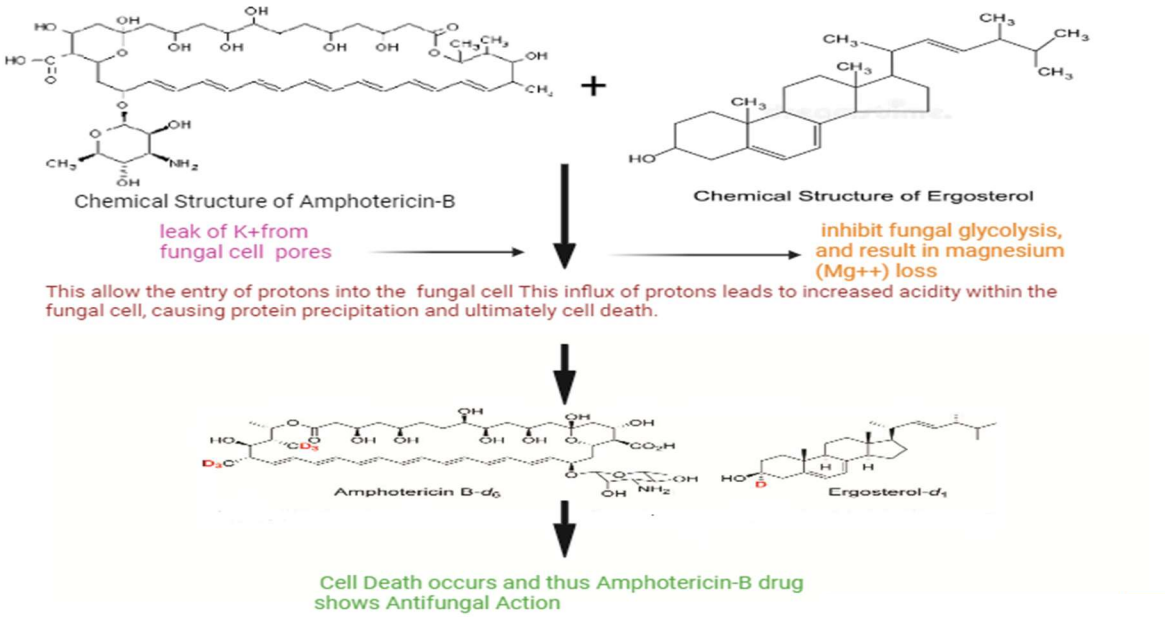
Developing an in-situ gel spray containing Amphotericin B liposomes offers an innovative approach for antifungal therapy. This formulation improves drug delivery, stability, and patient compliance, leading to better outcomes. Amphotericin B effectively treats severe fungal infections, but its toxicity and low solubility limit its use, prompting exploration of safer, more effective delivery methods **[1]**. Liposomal formulations of Amphotericin B reduce toxicity by encapsulating the drug, limiting tissue interaction, and enabling controlled, targeted release **[2]**. In-situ gels enhance liposomal Amphotericin B delivery, transforming from liquid to gel upon application, ensuring localized, controlled drug release **[3]**. This feature enables prolonged drug release, enhancing efficacy and retention. The spray formulation ensures easy application and uniform topical distribution **[4]**. This method boosts patient comfort and drug absorption at the infection site, minimizing systemic exposure and side effects. Optimizing liposome size, charge, gelation temperature, spray properties, and drug release rate is essential **[5]**. Optimizing these factors enhances Amphotericin B's therapeutic effects while reducing toxicity. Advanced nanoparticle and polymer technologies improve stability and efficacy, making the liposomal in-situ gel spray a safer, more effective option for severe fungal infections compared to conventional treatments **[6]**.

Female Genital Routes: Potent Drug Distribution: The vagina itself does not have any glands. Vaginal fluids originate from sources like Bartholin's and Skene's glands, cervical mucus, and secretions from the endometrium. They contain urea, proteins, fatty acids, and carbohydrates. Daily, the vagina produces around 6 grams of fluid, with 0.5–0.75 grams usually present inside **[7]**. This route offer consistent drug levels with fewer doses, avoids the first-pass effect, isn't influenced by gastrointestinal issues, and allows for discreet, efficient drug absorption with lower doses **[8]**. The vagina's rich blood supply and large surface area make it effective for both local and systemic drug delivery. Various formulations, like gels, foams, and IUDs, can provide controlled release, enhancing patient comfort and adherence. Additionally, adjusting pH and enzymes in these formulations boosts effectiveness **[9]**.

Unveiling the Mechanism of Action: How Amphotericin B Targets and Disrupts Fungal Cell Integrity:

AmB works by binding to ergosterol, a crucial component of fungal cell membranes. This

binding happens because of two regions of the drug: one that is hydrophobic (repelling water, known as the polyene hydrocarbon) and another that is hydrophilic (attracting water, called the polyhydroxyl chain). AmB forms complexes with ergosterol in the fungal membrane, creating pores that leak potassium and magnesium, disrupting glycolysis. Proton influx increases acidity, leading to protein precipitation and fungal cell death **[10]**. Amphotericin B removes ergosterol, weakening the fungal membrane and causing cell destruction, making it a potent antifungal. It also generates free radicals and activates the immune system to help eliminate infections **[11]**. Recent studies suggest that amphotericin B mainly disrupts cell membranes by interacting with sterols like ergosterol and cholesterol, rather than forming ion channels. Most of the drug accumulates on the membrane surface, extracting sterols, compromising integrity, and causing cell death **[12]**.



METHOD FOR PREPARING LIPOSOMES:

1) **Thin Film Hydration-Bridging the Gap Between Lipid Composition and Therapeutic Efficacy:** The thin-film hydration method is among the most straightforward techniques for synthesizing liposomes in a laboratory environment **[13]**. To achieve a homogeneous mixture, phospholipids are dissolved in an organic solvent, which is then evaporated using vacuum or gas (nitrogen/argon) for

small volumes, or rotary evaporation for larger volumes. The resulting lipid film is hydrated with water or a phosphate-buffered solution, forming bilayers **[14]**. Hydration occurs for 1–2 hours at 60–70 °C to maintain temperatures above the lipids' phase-transition point. This ensures proper hydration, promoting the formation of liposomal structures by keeping the lipid components in their optimal state **[15]**.

2) **Loding of drug molecules in Liposomes:** Nanoparticles like liposomes enhance drug therapy by delivering the correct dosage directly to the target site, minimizing exposure to healthy tissues and reducing side effects. This targeted approach improves the overall effectiveness of the treatment **[16]**. Drugs can be loaded into liposomes through passive or active methods. In passive loading, a solvent is evaporated after dissolving the drug and phospholipids, allowing for drug incorporation into the liposomes. This method enhances treatment effectiveness by ensuring targeted delivery, with hydrophobic drugs embedded in the bilayer and hydrophilic drugs in the aqueous core. While it's user-friendly and efficient for various medications, it offers less control over drug placement. In contrast, active loading uses a pH gradient to enable drugs to cross the lipid bilayer and enter the aqueous core **[17]**. Drugs are incorporated into liposomes using a pH gradient between the liposome's internal environment and the external surroundings. This gradient creates an acidic or basic interior, facilitating drug uptake and enhancing encapsulation. The amphipathic drugs can traverse the lipid bilayer, concentrating within the aqueous core, ensuring effective loading for targeted delivery. This method typically achieves higher encapsulation efficiency compared to passive loading and is particularly beneficial for ionizable drugs, enhancing their stability and retention. The physicochemical properties of these drugs act as "trapping" agents, aiding in controlling the rate of drug release. However, this approach can be challenging for drugs that are insoluble or weakly soluble in water **[18]**. Remote loading technology enhances liposome-based drug

delivery by improving drug accumulation, reducing leakage, and increasing loading efficiency compared to passive methods. The counterion of the trapping agent influences the aggregation or crystallization of drug-counterion salts in the liposome's aqueous phase **[19]**.

3) **Amphotericin-B loading in liposomes:** Loading amphotericin B into liposomes involves encapsulating this antifungal agent within lipid bilayers to improve its solubility and reduce toxicity. Traditional administration can cause nephrotoxicity due to the drug's poor water solubility. Liposomal formulations enhance pharmacokinetics and therapeutic index. Amphotericin B can be incorporated through passive loading during liposome formation or active loading using a pH gradient. Encapsulation offers benefits such as controlled drug release and targeted delivery. Additionally, the liposomal environment protects amphotericin B from degradation, enhancing its efficacy and minimizing side effects in fungal infection treatment **[20]**.
4) **Forming of insitu gel:** Choosing the right polymers significantly impacts the development of in-situ gelling technologies. The process involves dissolving copolymers in a polymer solution, mixing it with a drug aqueous solution, adding excipients, and finally, incorporating distilled water to reach the desired volume. To prepare the gel using the cold method, Poloxamer is dissolved in distilled water at 10-20% (w/v) concentrations, maintaining the temperature below 5 °C for a 50 mL solution. The mixture is refrigerated for 24 hours for full dissolution. For gel testing, 20 mL of the Poloxamer solution is placed in a beaker with a magnetic stirrer and thermometer in a water bath. The solution is stirred at 100 rpm while the temperature rises by 2 °C per minute. Gel formation is noted when the magnetic bead stops, and this temperature is recorded. Concentrations that gel around 35-37 °C are chosen for further optimization **[21]**.

EVALUATION PARAMETERS:

1. **Particle Size Analysis:** Another name for dynamic light scattering (DLS) is photon correlation spectroscopy (PCS) **[22]**. This method estimates particle sizes and their distribution by

observing changes in scattered light intensity over time. Fluctuations arise from the random movement of particles in a liquid, enabling the assessment of particle size and the uniformity of size distribution (polydispersity) **[23]**. A 780 nm laser was used at room temperature to measure particle sizes, with 40 µL sample volumes and a minimum of three measurements for accuracy. In methanol-free conditions, the mean particle size was 112 nm, with a polydispersity index (PDI) of 0.329, indicating moderate size variation. The Stokes-Einstein equation, based on diffusion coefficient, temperature, solvent viscosity, and Boltzmann's constant, facilitated size calculations using DLS. Silica nanoparticles (82 nm) served as a control due to solvent viscosity's influence on measurements. Adding methanol increased the size of both silica and other particles by about 70%, likely due to higher viscosity. After dilution, the final methanol concentration of around 13% led to an additional 30% increase in particle size, emphasizing the importance of considering solvent viscosity in DLS evaluations **[24]**.

2. **Zeta Potential:** One important measure of the stability of colloidal dispersions, such as liposomal formulations, is zeta potential **[25]**. The instrument measures the surface charge of Amphotericin B liposomes, influencing their stability and interactions within biological systems **[26]**. Typically, a high absolute zeta potential value (positive or negative) suggests powerful electrostatic repulsion between particles, reducing the likelihood of aggregation and ensuring better stability **[27]**. The zeta potential can be affected by the composition of the lipids utilized in the formulation, such as cholesterol and phospholipids **[28]**. A negatively charged lipids aid to inhibit aggregation, a negative zeta potential is frequently found **[29]**. Stable AmB liposomal formulations generally exhibit zeta potentials within the range of ±30 mV, based on the formulation components and preparation methods **[30]**. Zeta potential also affects how the liposomes engage with biological membranes, have an effect how well drugs are delivered and how long the liposomes stay in the body's circulation **[31]**.

3. **In- vitro Drug Release Study:** The closed-loop USP Apparatus 4 system was employed for testing, featuring a dissolution apparatus, multi-channel dispenser, and UV-Visible spectrophotometer. The dissolution tester could analyze seven samples simultaneously, with two flow-through cells designated for the reference formulation (AmBisome) and the control (free Amphotericin B). This setup enabled effective measurement of the maximum expected concentrations for accurate comparisons in the dissolution study **[32]**. Solubilizers ensure optimal conditions for drug release, especially for complex injectable drugs. Hydroxyl propyl cyclodextrin improves doxorubicin release, while solvents like acetonitrile and ethanol aid drug release from polymer systems. The USP-4 IVR assay assesses Amphotericin B liposomal formulations, revealing initial burst release differences despite non-physiological conditions (55°C and 5% w/v γ-cyclodextrin) used for 24-hour release without damaging liposomes **[33]**.

4. **Stability Testing:** Stability studies in pharmaceuticals ensure that drug products maintain their safety, efficacy, and quality throughout their shelf life. These tests, crucial for regulatory approval, evaluate how storage conditions like temperature, humidity, light, and time affect drug performance and stability **[34]**. Liposomes are inherently thermodynamically unstable, facing physical issues like fusion and aggregation, as well as chemical degradation, such as oxidative damage during storage. Stability testing is crucial for liposomal drug products to prevent breakdown and aggregation. Techniques for stabilization include steric and electrostatic methods to reduce particle clumping. Additionally, processes like freeze-drying and the addition of antioxidants can improve chemical stability and decrease degradation risk. Pegylation, which adds polyethylene glycol (PEG) to liposomes, is emphasized as a strategy to enhance stability in biological environments, prolonging their effectiveness **[35]**. Stability studies for in situ gels are essential to ensure their physical, chemical, and biological

integrity over time. These evaluations assess parameters such as pH level, viscosity, drug content, gelation time, and drug release profiles under various storage conditions. Key factors like temperature, humidity, and light exposure are tested to determine the gel's shelf life and stability. The aim is to confirm that the in-situ gel maintains its therapeutic efficacy, safety, and gel-forming properties throughout its intended shelf life **[36]**.

5. **Assessment of Gel Viscosity Under Controlled Conditions:** The viscosity of the in-situ gel was measured using a viscometer. Gel samples were placed in a beaker at 37 ± 0.5 °C, with a spindle rotating at 100 rpm. Viscosity readings were taken as the gel cooled, ensuring accuracy through triplicate measurements **[37]**.
6. **Gelation temperature:** The experimental setup used to determine the gelation temperature of a formulation. A beaker containing 20 mL of a cold solution of the formulation is placed in a water bath for controlled heating. A magnetic bead is added to the beaker to help stir the solution, and a thermometer is carefully positioned in the solution to measure the temperature without touching the bottom of the beaker, which could disrupt the stirring. As the solution is stirred at a speed of 100 rpm, the temperature is gradually increased by 2 °C per minute. The moment the magnetic bead stops spinning indicates that the gel has formed, and this temperature at which the gelation occurs is recorded **[21]**.
7. **pH of the formulation:** The gel formation in pH-sensitive formulations relies on the pKa of the polymers, which indicates the pH where their ionization changes. Polymers with weak acidity gel below their pKa, while weak bases do so above **[38]**. Liposomes encapsulate Amphotericin B, improving stability and solubility. pH adjustment in an in-situ gel spray ensures optimal gelation and comfort upon contact with body fluids **[39]**. Maintaining the appropriate pH is crucial for drug stability, solubility, and effective absorption. It also ensures proper gel formation, promoting controlled drug release and minimizing irritation, thereby enhancing user comfort and improving the overall effectiveness of the treatment **[40]**.

8. **Thermoreversible studies:** Thermoreversible studies were performed using a constant temperature bath. The in-situ gel formulations were immersed in a water bath that was initially chilled to a temperature range of 4 to 5°C. This low temperature is likely used to maintain the gel's properties and stability before further processing or testing. the process of heating the formulations, where the temperature is raised steadily by 2°C each minute. During this period, the transition from a liquid state (sol) to a solid state (gel) is carefully monitored, including any changes in viscosity. This tracking helps identify when formulations reach their gel point, confirming the successful transformation. The temperature is then lowered to observe the reverse transition from gel to sol, recording viscosity at various temperature points. Viscosity measurements for all formulations were taken using a viscometer with spindle no. 62, operating at 10 rpm. Additionally, viscosity was assessed at different speeds of 10, 50, and 100 rpm to evaluate the formulations' rheological properties **[21]**.
9. **Microbiological Activity:** The effectiveness of drug-loaded formulations against *E. coli* was evaluated using the agar well diffusion method to measure their antibacterial activity. Testing occurred at various intervals over 12 weeks: on day 1, and then at weeks 3, 6, 9, and 12. Formulations were stored in glass and plastic containers at 25°C and 4°C to assess the impact of storage conditions on their antimicrobial efficacy. Throughout the study, the formulations retained their original texture, homogeneity, color, and appearance, with no signs of instability. Consistent activity was observed across all storage conditions for the entire 12-week period **[41]**.

CONCLUSION:

This innovative formulation enhances drug retention at the target site, facilitates extended medication release, and delivers treatment precisely where required. It also minimizes the risk of side effects and systemic toxicity, leading to fewer adverse effects on the body while effectively addressing the infection. The liposomal formulation embedded in an in-situ gel matrix offers a synergistic strategy, merging the advantages of both systems to enhance patient compliance and therapeutic

effectiveness. Future advancements may focus on incorporating stimuli-responsive polymers to achieve on-demand drug release, integrating bioadhesive properties for extended retention at the infection site, and exploring nanoparticle-based modifications to enhance cellular uptake and penetration. Additionally, clinical investigations and pharmacokinetic studies will be essential to establish its safety and efficacy profile, potentially expanding its application to other vaginal infections and incorporating combination therapies to tackle resistant fungal strains. This novel formulation could pave the way for a new generation of localized antifungal therapies with broad-spectrum applications.

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