

Development and Evaluation of Eberconazole-Loaded Niosomes [†]

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Abstract: Invasive fungal infections require a long treatment schedule; however, treatment has become more cumbersome due to the development of resistance. Most antifungal moieties show systemic toxicity upon oral administration, leading to delivery of antifungal moieties via a topical route. Eberconazole (EBZ) is a BCS class II drug that has poor solubility and high permeability. It is a broad-spectrum imidazole derivative, which acts as a both fungicidal and fungistatic drug by inhibiting ergosterol synthesis. Various topical creams of EBZ are available in the market, but the lack of a proper dosing schedule and rapid removal lead to poor bioavailability. Niosomes are vesicular carriers that can entrap both hydrophilic and lipophilic drugs. Niosomal formulations have been prepared using Span20 (nonionic surfactant) and cholesterol by thin-film hydration (TFH) technique. During preformulation studies, the purity of EBZ was ascertained using FT-IR and melting point studies, while the standard calibration curve was prepared using UV-visible spectroscopy. The prepared niosomal formulations were characterized for their morphology, entrapment efficiency, particle size, and zeta potential. The formulation has shown $86 \pm 0.85\%$ entrapment efficiency, while the niosome appeared in a ring-like structure during its microscopic evaluation. Further evaluations of in vitro and in vivo release studies will be performed in the future for its efficacy and antifungal activity.

Keywords: eberconazole; niosomes; sustained release; antifungal



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1. Introduction

Fungal infections or mycosis are prevalent infections, but few systemic infections are lethal to hosts. Every year around one billion people become affected by fungal infections worldwide. They mainly manifest as either superficial (pityriasis Versicolor), subcutaneous (eumycetoma), or systemic (cryptococcosis) infections in different regions of the body [1]. These infections spread via air, such as by breath through fungal spores and via direct contact with skin or open wounds or cuts on the host's skin. It has also been observed that patients with weak immunity are highly susceptible to fungal infections. Many people with illnesses such as HIV/AIDS and people taking chemotherapy or steroids have decreased immunity and show an increased susceptibility to opportunistic fungal infections [2]. In the current context, the COVID-19 pandemic has shaken the world. Ongoing treatment has led to high demand for steroids, which directly decreases immunity and increases opportunistic fungal infections such as black fungus (mucormycosis) and white fungus (candida infections), etc. [3]. Some common symptoms of fungal infections are skin rash, itching, lumps, and discoloration of the skin. An antifungal agent, also known as an antimycotic agent, is a fungicide or fungistatic used to treat and prevent mycosis, such as ringworm and candidiasis, etc. Different classes of antifungal agents are polyenes (Amphotericin B, Nystatin, etc.), azoles, imidazoles (Bifonazole, Butoconazole, Clotrimazole, etc.), triazoles (Fluconazole, Itraconazole, Posaconazole, etc.), echinocandins (Caspofungin, Micafungin, etc.), and other agents. The significant side effects of antifungal agents are hepatotoxicity

and anaphylaxis, etc. However, a long-term treatment regimen leads to resistance toward antifungal agents [4–6].

Niosomal formulations are vesicular bilayer carriers that can entrap hydrophobic and hydrophilic drugs in their outer hydrophobic layer and inner aqueous core [7]. In vesicular systems, liposomes and niosomes are unilamellar or multilamellar spheroidal structures composed of amphiphilic molecules assembled into bilayers. They are considered primitive cell models, cell-like bioreactors, and matrices for encapsulation. Niosomes receive greater attention than liposomes due to their high physical and chemical stability, with low-cost nonionic surfactants compared with phospholipids [8]. These vesicles are composed of nonionic surfactants, cholesterol, and charge-inducing agents. These vesicles are nonimmunogenic, biocompatible, and biodegradable moieties, which may be considered as suitable drug-delivery carriers for sustained and controlled release applications. The unique bilayer structure helps to enhance the solubility and permeability of poorly soluble therapeutic moieties [9].

The outer layer provides an amenable surface for modification with different ligands, which helps in targeted delivery. Many different class of nonionic surfactants are available, such as alkyl ethers and alkyl glyceryl ethers, sorbitan fatty acid esters, polyoxyethylene fatty acid esters, and block copolymers. An ample number of methods are available to prepare niosomes, among which thin-film hydration is the most common technique [7,10].

Eberconazole (EBZ) is a topical, broad-spectrum imidazole derivative, effective in dermatophytosis, candidiasis, and pityriasis treatment. It was first introduced in Spain by Salvat Laboratories in 2005 for cutaneous fungal infections [11]. It also shows high activity against most triazole-resistant yeasts (*Candida krusei* and *Candida glabrata*) and fluconazole-resistant *Candida albicans*. It has also been shown to be effective against Gram-positive bacteria. Thomas et al. have reported its anti-inflammatory properties, making it unique among other imidazole derivatives and treating inflamed dermatophytic infections. It is a BCS class II drug and, due to its poor solubility, it requires high dosing, leading to toxicity. The available formulation for EBZ is a topical cream that has shown significantly high activity against superficial fungal infections [12].

In this study, EBZ-loaded niosomal formulations were developed for sustained delivery against fungal infections. EBZ-loaded niosomes were formulated using Span20 and cholesterol by the thin-film hydration technique. The prepared niosomal formulations were characterized for their morphology, particle size, zeta potential, entrapment efficiency, and functional group analysis through FTIR studies.

2. Materials and Methods

2.1. Materials

Span20, cholesterol, and Triton-X were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Eberconazole (EBZ) was provided as a gift sample from Mylan labs (Telangana), dialysis bag (MWCO 12000DA) was purchased from hi-media. A solvent such as ethanol and chloroform were purchased from Qualigens. Millipore water was used throughout the experimental study with a molar conductivity of 18 mΩ.

2.2. Methods

2.2.1. Preformulation Studies

Preformulation studies were performed for the identification of EBZ using melting point and FTIR (Fourier transform infrared) spectroscopy.

2.2.2. Preparation of Blank and Drug-Loaded Niosomal Vesicles

The niosomes were prepared by a thin-film hydration technique. Accurately weighed cholesterol and Span20 were taken in a 3:1 molar ratio and dissolved in 10 mL chloroform and methanol mixture (8:2). The above solution was subjected to the rotary evaporator (IKA-HB10) at 80 rpm and 40 °C. The thin film was formed after the complete evaporation of the organic solvent. Then, the film was hydrated with 10 mL of Milli-Q water above gel liquid

transition temperature at 70 °C by vortex mixing for 25 min. The drug-loaded niosome was also prepared by mixing EBZ in chloroform and methanol, along with cholesterol and Span20 [13].

2.3. Characterization Methods

Morphological Characterization using Leica Microscope: This study was carried out to visualize the morphological structure and images of the prepared niosome. The prepared niosome was taken over a glass slide, and a coverslip was mounted over it. The prepared slide was finally visualized under 10× and 40× magnification using a Leica microscope [14].

Particle size analysis (PSA): Particle size analysis was carried using Malvern Zetasizer nano series (Nano-S90) instrument based on the principle of photon correlation spectroscopy. Approximately 3 mL of prepared niosome was taken in a quartz cuvette and exposed to laser light diffraction at an angle of 90° for particle size analysis [15].

Zeta potential: To measure the zeta potential, prepared niosomal solution was diluted, injected into folded capillary supported with platinum electrodes, and placed in the sample holder of Zetasizer (Malvern) for analysis [15].

FT-IR analysis: FT-IR studies of different niosomal systems were performed using the KBr disc method. Furthermore, this study was carried out to analyze the various functional groups present in EBZ, blank niosomes, and EBZ-loaded niosomes [16].

Entrapment efficiency: The entrapment efficiency of EBZ-loaded niosomal formulation was determined by lysis of niosomal vesicles using Triton-X. After the lysis, EBZ-loaded niosomal formulations were centrifuged at 10,000 × *g* for 30 min at 4 °C using a refrigerated centrifuge (Eppendorf, 5415 R, Hamburg, Germany) to analyze the entrapment efficiency. After the centrifugation, the free-drug concentration in the supernatant was determined by measuring absorbance at 208 nm using a UV-visible spectrophotometer (Shimadzu, UV 2450 PC, Kyoto, Japan). The percentage of drug entrapment in niosomes was calculated using the following formula [17]:

$$\%Drug\ Entraped = \frac{(Total\ drug - Drug\ in\ supernatant)}{Total\ Drug} \times 100$$

3. Results and Discussion

3.1. Preformulation Studies

A melting point experiment was carried out to determine the purity of the sample. The smaller melting point range generally governs the purity of the sample. Here, the melting point determined by the OptiMelt (automated melting point system) instrument was found to be 183.4 °C (Figure 1). Reports are available for the melting point, which appears in the range of 183–184.5 °C. The result of melting point study appeared within the defined range, and hence it may be concluded that the drug is pure [18].

3.2. FTIR Study

FTIR of EBZ shows two characteristic peaks at 1284 cm⁻¹ and 632 cm⁻¹ for the alkyl halide and halo compound (chloro compound). The IR peak at 2110 cm⁻¹ corresponds to R-N=C stretching vibration, while the IR peak at 1963 cm⁻¹ corresponds to C=C stretching vibration for unsaturation of carbon in an aromatic ring. Multiple small characteristic peaks in the region of 1030–1230 cm⁻¹ may be due to C-N stretching [18].

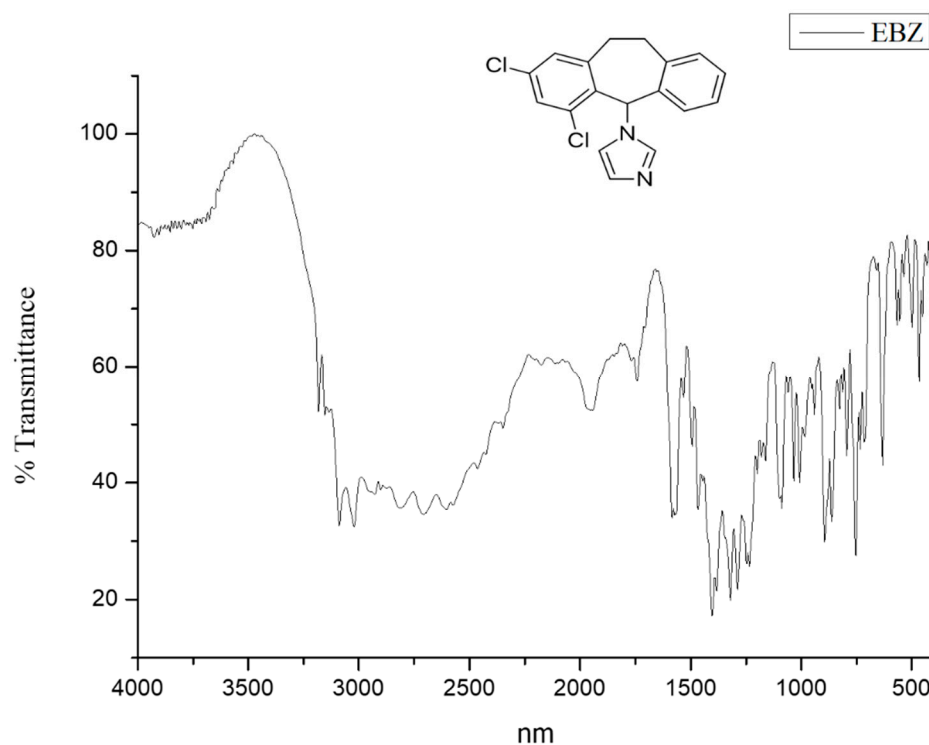


Figure 1. FTIR graph of eberconazole.

3.3. Standard Plot

The standard stock solution of EBZ was prepared by dissolving 10 mg of EBZ in 10 mL of the water–methanol system (7:3). To prepare standard calibration curve, different concentrations of EBZ were prepared in the range of 2–12 $\mu\text{g}/\text{mL}$ by diluting with a water–methanol system (7:3). The absorbance of the various dilutions of EBZ was measured at 208 nm using a UV-visible spectrophotometer (Shimadzu UV-2450). The regression coefficient of the EBZ under the selected concentration range was found as $r^2 = 0.9959$. The graph of the standard plot has been depicted in Figure 2.

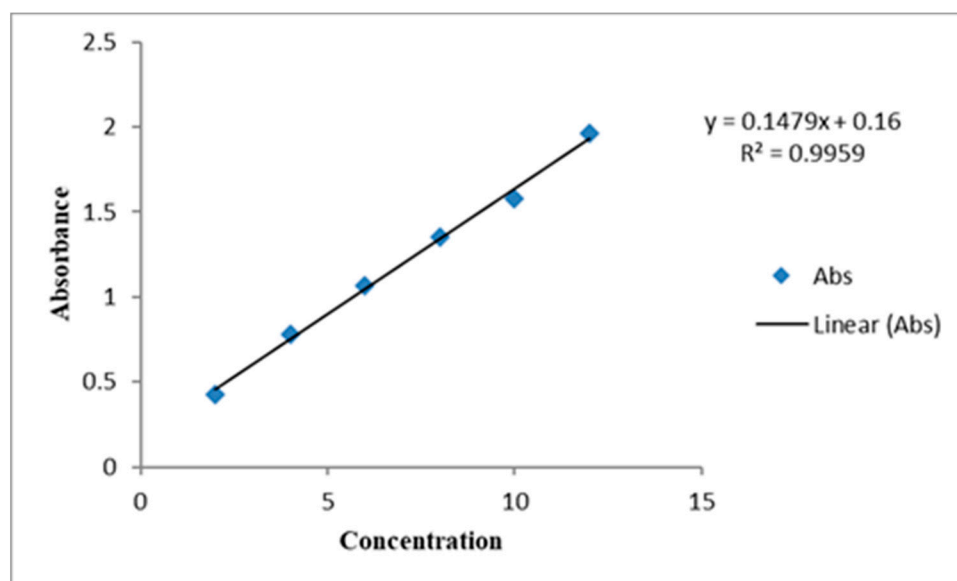


Figure 2. Standard calibration curve of EBZ.

3.4. Morphological Evaluation

Morphological evaluation of developed niosomal formulation was carried out using Leica microscopy (10× and 40×), which reveals the circular, ring-like structure of niosomes. Morphological images of developed niosomes have been depicted in Figure 3 [19].

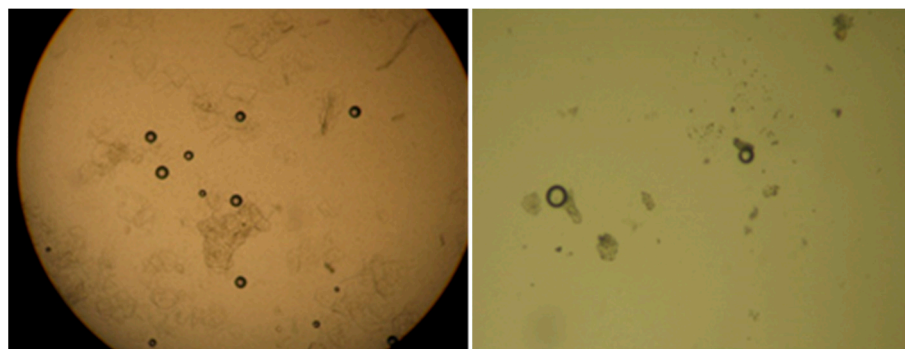


Figure 3. Leica microscopic image of niosomes at 10× and 40×.

3.5. Particle Size and Zeta Potential

The particle size and zeta potential were calculated for EBZ-loaded niosomal formulation with the help of Malvern Zetasizer. The obtained particle size appeared in the range of 550–620 nm, and zeta potential appeared in the range from -23.5 ± 1.89 mV to -25.5 ± 1.63 mV, respectively. The pictograph of particle size and zeta potential has been depicted in Figures 4 and 5, respectively [10].

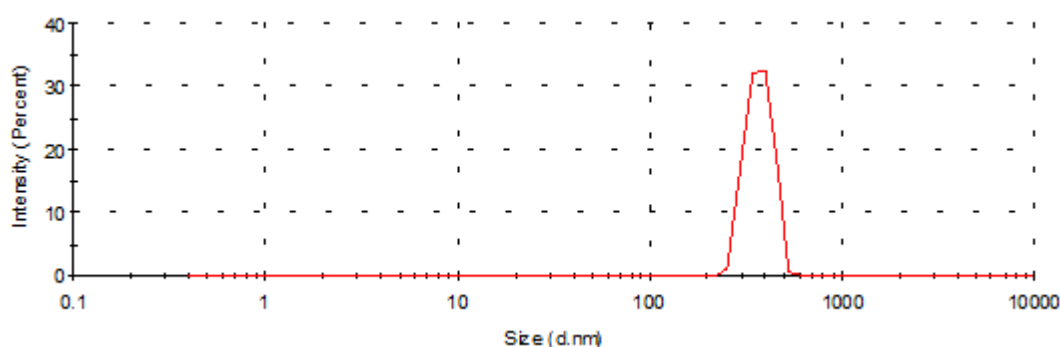


Figure 4. Graph representing particle size distribution of EBZ-loaded niosomes.

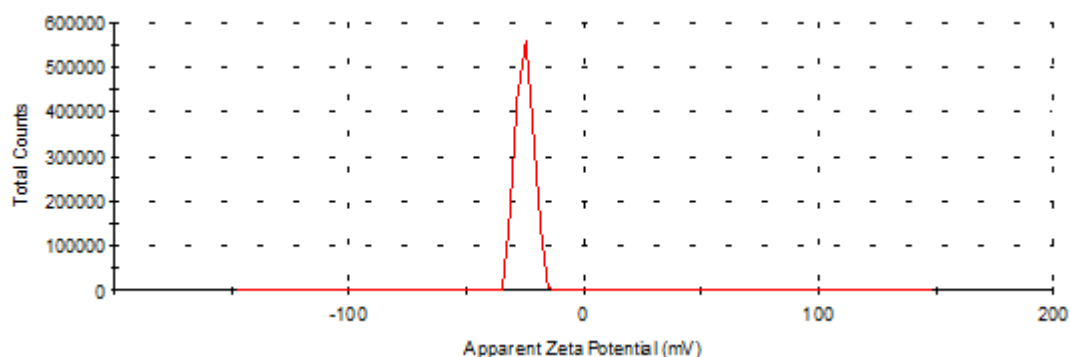


Figure 5. Graph representing zeta potential of EBZ-loaded niosomes.

3.6. Entrapment Efficiency

The entrapment efficiency was performed using centrifugation method, and the obtained entrapment efficiency of EBZ in EBZ-loaded niosomes was found to be 86%. Entrapment efficiency plays a significant role in the determination of the dose frequency of a

particular formulation. It is generally assumed that the greater the loading efficiency of a particular therapeutic moiety in a given formulation, then, the dosing schedule may be effectively decreased provided if the therapeutic moiety is released slowly in sustained or controlled manner from the reservoir.

3.7. FTIR Studies

The drug loading was analyzed by comparing the functional group present among the EBZ, blank niosomes, and EBZ-loaded niosomes. The combined FTIR spectra of EBZ, EBZ-loaded niosome (EBZ-NS), and blank niosome (Blank-NS) has been shown in Figure 6.

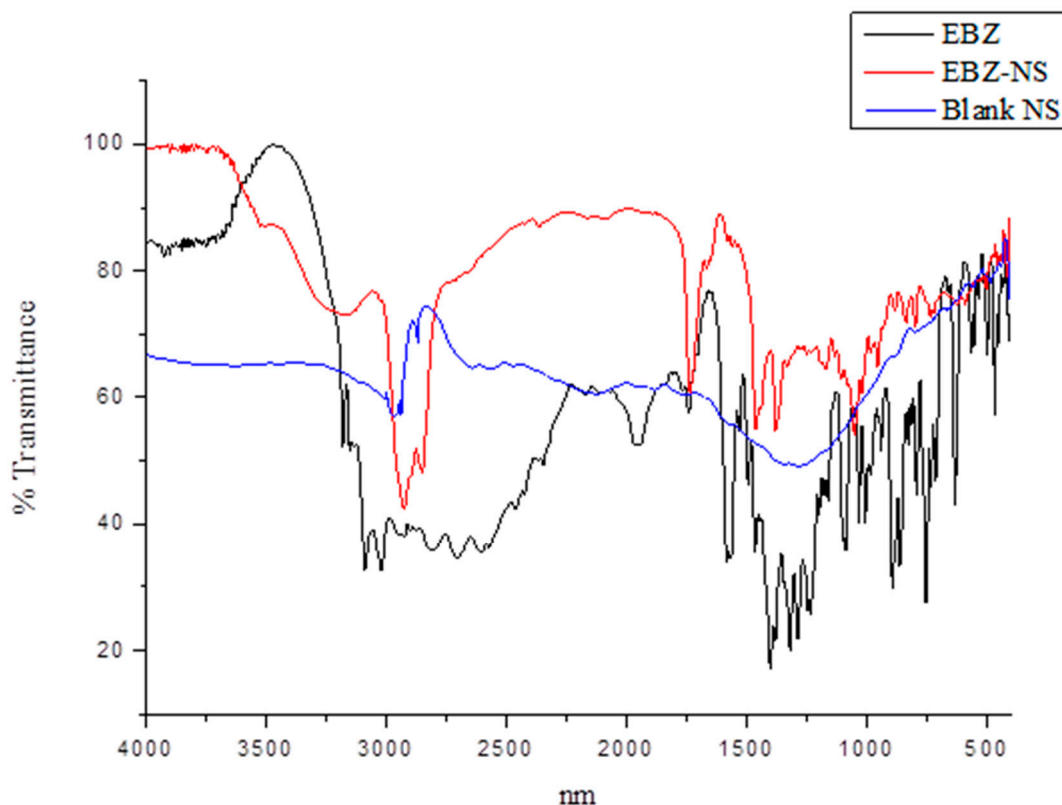


Figure 6. Combined spectra of EBZ, EBZ-loaded niosomes, and blank niosomes.

The obtained results have been analyzed that there has been broadening of peak in EBZ-loaded niosome at 3264 cm^{-1} which is very steep in EBZ and absent in blank niosomes. These results due to OH stretching of Span20, which is absent in EBZ. Few multiple peaks of EBZ appeared in the range from 2610 to 2805 cm^{-1} , but the same peak disappeared in EBZ-loaded niosomes, which may be strong amine salt stretching. One broad peak at 1915 cm^{-1} may be due to aromatic C–H bending, which disappears in EBZ-loaded niosomes. The intensity of the C–N stretching peak at 1340 cm^{-1} in EBZ has been reduced in EBZ-loaded niosomes, while it is absent in the blank niosome, which may suggest the entrapment of EBZ in niosomes. While multiple peaks in the range of 550 cm^{-1} to 850 cm^{-1} were characterized due to presence of C–Cl and it was also found to be reduced in EBZ-loaded niosomes as compared with EBZ alone [18].

4. Conclusions

An EBZ-loaded niosomal formulation was developed using thin-film hydration technology. These niosomal vesicles were then characterized based on particle size, zeta potential, and drug entrapment efficiency. These vesicles were also observed under Leica microscopy, which revealed the spherical, ring-like niosomal vesicles. Its negative zeta potential of -25 mV suggests its better stability due to interionic repulsion, while its nano-

sized range may help in better penetration. Its high entrapment efficiency helps in dose reduction. EBZ-loaded niosomal formulations are highly suitable for drug delivery in sustained, controlled release applications, and may be a better option compared with the currently available marketed preparations.

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