Novel Emulsion Base for Vaginal Yeast Infection with Half Drug Concentration

Rigved Nagarkar, Manan Patel, Almas Babar

ABSTRACT

The dissolution of the drug in the vaginal cavity strongly influences the efficacy of the product due to insufficient moisture at the vaginal site. This study was undertaken to develop semi-solid dosage forms of miconazole nitrate to optimize its release. Formulations containing miconazole nitrate at 2% were developed using hypromellose gel, non-ionic emulsion, and cationic emulsion. The effect of penetration enhancers such as propylene glycol, dimethyl sulfoxide (DMSO), and diethylene glycol monoethyl ether at various concentrations was studied. Diffusion studies were carried out to evaluate the drug release and compared it against a commercial product. Formulation with the highest drug release was further evaluated at half (1%) drug concentration. Formulation with reduced drug levels along with the commercial product was evaluated for drug release for an extended time using human cadaver skin. The general order of average cumulative drug release from three bases was observed to be cationic emulsion > hydroxypropyl methylcellulose > non-ionic emulsion. Among all samples, the cationic emulsion with 5% DMSO gave a maximum drug release of 7.27 ± 0.2 mg/cm² with a flux of 0.70 mg/cm²/min compared to only 3.09 ± 0.1 mg/cm² drug release with 0.51 mg/cm²/min flux for brand product. The average cumulative drug release for formulation with half (1%) drug and brand (2%) drug over a period of 12 h through human cadaver skin was observed to be 8.28 ± 0.9 mg/cm² and 8.71 ± 0.9 mg/cm², respectively. This observation was in conformance with the in vitro antifungal studies showing an equivalent zone of inhibition.

Keywords: Cationic emulsion, Drug release, Dimethylsulfoxide, In vitro antifungal study.

INTRODUCTION

In recent years, the pharmaceutical industry is investing a maximum of their R&D efforts into the development of delivery systems, rather than pouring millions of dollars in inventing new molecules. This approach of new drug delivery systems is a potential pathway through which companies can optimize drug-formulation systems to have a maximum therapeutic benefit with minimum toxic effects. To achieve this goal, the area in focus is the improvement of formulation characteristics to improve or enhance its clinical efficacy. Drugs are delivered and marketed in various drug delivery systems, namely, oral, parenteral, sublingual, and topical. Topical drug delivery has taken a noticeable growth in recent years due to its obvious advantages such as avoiding first-pass metabolism, patient compliance, and others.[1] Topical dosage form includes creams, ointments, gel, solution, lotion, and spray.[2] Creams are mostly emulsion-based products which are either water in oil or oil in water type. Emulsions are a mixture of the oil phase and water phase stabilized by surfactant(s).[3] The nature and concentration of surfactants play an important role in the emulsion type and stability. For instance, a surfactant with a high hydrophilic-lipophilic balance (HLB) will form an oil-in-water emulsion, whereas a low HLB value surfactant will form a water in oil emulsion. Surfactants, based on the charge on the molecule, can be classified as anionic, cationic, and non-ionic. Permeation enhancers are a class of substances which stimulate the absorption of the drug through the skin.[4] Permeation enhancers or permeability enhancers increase the absorption of the drug by two different techniques. Some group of enhancers increases
the solubility of the drug, therefore, increasing the absorption. The second type of enhancers alters the skin structure permanently or temporarily, thus increasing the flux of drug across the skin.[6]

Topical drug delivery includes two basic types of products, one which is externally applied the second type is internally applied to the mucous membrane.[6,7] Recently, mucosal delivery is gaining a big popularity as drug delivery route. Specifically, vaginal delivery is attracting a lot of interest from the scientific community. Vagina is S-shaped with its folded walls.[8-10] The vaginal wall consists of a plethora of blood vessels which can be used for systemic delivery.[11] Vaginal drug delivery has been normally used for local administration of drugs rather than systemic route. There are still some challenges faced with the vaginal delivery of drugs. Delivery of drugs in the vagina depends not only on the formulation factors but also on patient factors such as humidity, secretions, and pH. Any biological or formulation factor affecting these parameters can affect drug absorption.

A vaginal yeast infection is inflammation of the vagina, which causes vaginal irritation, intense itchiness, and vaginal discharge.[12] A vaginal yeast infection affects the vagina and the tissues at the opening to vagina (vulva). Candida and the many other germs that normally live in the vagina keep each other in balance.[13] However, sometimes, the number of Candida albicans increases due to the external and physiological factors, leading to a yeast infection. Miconazole is the drug of choice for such infections. In addition to its antifungal and anti-parasitic action, it also has some antibacterial properties. In vitro studies suggest that it inhibits ergosterol synthesis. Ergosterol is a critical component in the fungal cell membrane.[14]

A drug to show its activity needs to be in a molecular form; in other words, needs to be soluble. It was extending this concept for a topical product applied onto the skin, drug which in the dissolved state will show its effect while undissolved drug would remain on the skin. This general principle was taken into consideration to enhance the drug solubility in the vehicle to provide optimum release of the drug. An increase in solubility was also accompanied by penetration enhancer ability to further promote drug absorption. Formulation with enhanced drug release due to the double impact of solubility and penetration enhancer was evaluated for efficacy at half the drug level.

**MATERIALS AND METHODS**

Miconazole nitrate was purchased from Letco Medical (Decatur, AL), Hydroxypropyl methylcellulose hydroxypropyl methylcellulose (HPMC) (Methocel® K 100M premium) from Colorcon (Mumbai, India), Catemol WPC from Phoenix Chemicals Inc (Somerville, NJ), Cetyl alcohol from Amend Drugs and Chemicals (New Jersey), Glycerol monostearate (SE) from Hallstar (Chicago, IL), 95% Ethanol from Pharmaco-Aaper (Toronto, Canada), dimethyl sulfoxide (DMSO) from Fagron (St. Paul, MN), Mineral Oil, Polysorbate 80, propylene glycol (PG), diethylene glycol monoethyl ether (DGME), Dextrose, Peptone, Agar and Cellulose Spectra/ pro seven dialysis membrane from Spectrum Chemicals and Lab Products (Henderson, NV), and Human Cadaver Skin was obtained from The New York Fire Fighter Skin Bank (New York, NY).

**Preparation of Standard Curve for Miconazole Nitrate**

A 100 mg of miconazole nitrate was dissolved in 100 mL of 95% ethanol. This was labeled as “stock solution A.” 10 mL of “stock solution A” was taken in another 100 mL volumetric flask. The volume was made up to 100 mL, with 95% ethanol. This solution was labeled as “stock solution B.” From “stock solution B,” 2 mL, 4 mL, 6 mL, 8 mL, and 10 mL solutions were withdrawn into different 100 mL volumetric flasks. Volume was made up to 100 mL using deionized water. Hence, the resulting concentrations in the flasks were 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL, and 10 µg/mL, respectively.[15] Sample with 10 µg/mL concentration was scanned using an ultraviolet (UV) spectrophotometer primarily for determining the maximum absorption (λmax) of the drug. Miconazole nitrate showed a peak at 271 nm, which was similar to the literature value of maximum absorbance at 272 nm.[16] A calibration curve was established using miconazole nitrate dilutions. A linear regression was performed, and the equation obtained was used to calculate the concentration of unknown samples.

**Preparation of Formulation Bases**

**Non-ionic gel base**

The required amount of HPMC was thoroughly dispersed in one-third of the required total amount of water at a temperature of 80–90°C. The agitation was continued until all the particles were wetted, and a consistent dispersion was obtained. The remainder of the water was added as cold water while agitating until a homogenous solution was obtained.[17] The solution was cooled down at room temperature. Agitation was continued until room temperature was reached to form a uniform, transparent gel matrix. The composition of the gel is shown in Table 1.
Preparation of samples

The required amount of miconazole nitrate [Table 2] was dissolved in DMSO at pre-determined levels (5%, 10%, and 15% w/w). The required amount of deionized water [Table 2] was added to the drug-DMSO mixture. This mixture was then incorporated into the gel base with optimum mixing with a Lightnin mixer (Palo Lab Supplies, New York) to ensure the drug-penetration enhancer mixture is uniformly distributed throughout the gel base. The same process was used to formulate samples using different penetration enhancers (PG and DGME) at the same levels as DMSO [Table 2].

Non-ionic emulsion base

A mixture of the calculated amount of oil phase ingredients [Table 3], namely, mineral oil, cetyl alcohol, and glyceryl monostearate self-emulsifying (GMS-SE) was melted in a beaker by heating at 75–80°C. Simultaneously, in second beaker water phase ingredients, i.e., glycerin, polysorbate 80, and water were heated while mixing with Lightnin’ mixer at 75–80°C. Oil phase ingredients were added to the water phase ingredients (stirring constantly) at the temperature of 75–80°C. Stirring was continued for 10–15 min at this temperature. The emulsion was then cooled down at room temperature while stirring.[18]

Preparation of samples

The samples were prepared similar to the gel base, where the required amount of miconazole nitrate [Table 4] was dissolved in DMSO, PG, and DGME separately at pre-determined levels (5%, 10%, and 15% w/w) with the application of heat (40–45°C). The calculated amount of water [Table 4] was added to the drug-enhancer mixture, which was then incorporated into the non-ionic emulsion base with mixing.

Cationic emulsion base

A mixture of the calculated amount of oil phase ingredients [Table 5], namely, mineral oil, cetyl alcohol, and glyceryl monostearate was melted in a beaker by heating at 75–80°C. Simultaneously, in second beaker water phase ingredients, i.e., glycerin, Catemol WPC, polysorbate 80, and water were heated while mixing with Lightnin’ mixer at 75–80°C. Oil phase ingredients were added to the water phase ingredients (stirring constantly) at the temperature of 75–80°C. Stirring was continued for 10–15 min at this temperature. The emulsion was then cooled down to room temperature while stirring.

Preparation of samples

The sample preparation method similar to the previously mentioned gel and emulsion bases was followed, where required amount of miconazole nitrate [Table 6] was dissolved in DMSO, PG, and DGME separately at pre-determined levels (5%, 10%, and 15% w/w) with the application of heat (40–45°C). The calculated amount of water [Table 6] was added to the drug-enhancer mixture, which was then incorporated into the cationic cream base with mixing.

In vitro Diffusion Studies

Using cellulose membrane

The delivery of drugs through the skin for local, targeted, or systemic delivery can be measured by in vitro and in vivo techniques.[19] Frequently, this has been done by in vitro technique because of the simplicity of the experimental design and conditions [Figure 1].

One-gram sample was placed in the donor compartment over the cellulose membrane and spread uniformly without entrapment of air bubbles.[20] The

Table 1: Composition of non-ionic gel base

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxypropyl methylcellulose</td>
<td>2.0</td>
</tr>
<tr>
<td>Purified water</td>
<td>78.0</td>
</tr>
<tr>
<td>Total</td>
<td>80.0</td>
</tr>
</tbody>
</table>

Table 2: Formulations using non-ionic gel base

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control Formulations (grams)</th>
<th>DMSO</th>
<th>Propylene glycol</th>
<th>DGME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
<td>5%</td>
<td>10%</td>
<td>15%</td>
</tr>
<tr>
<td>Hydroxypropyl methylcellulose gel base</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Miconazole nitrate</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DGME</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Purified water q.s.</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

DMSO: Dimethyl sulfoxide, DGME: Diethylene glycol monoethyl ether
receptor compartment has 13 mL of deionized water, which served as the diffusion medium and was stirred using a magnetic stirrer at a speed of 600. The limited solubility of miconazole nitrate in deionized water avoids dose dumping and will ensure constant release across the membrane. High, stirring speed helps in preventing any formation of any diffusion layer barrier resistance for the permeation of drugs. Aliquots (0.5 mL) from the receptor medium were withdrawn through the sampling port of the receptor compartment at pre-determined time intervals and replaced with fresh medium (0.5 mL) at each time interval. Collected samples were analyzed using a UV spectrophotometer at 271 nm. Each experiment was performed in triplicate for each formulation. Brand formulation Monistat (2%) was used as a control.

Optimizing the drug quantity in the base of choice

The formulation with the highest release was further evaluated for the optimization of drug concentration. The concentration of drug was reduced to half, i.e., 1%. The formulation will be denoted as Formulation IV.

Extended release studies using a cellulose membrane

The selected formulation (Formulation IV) was evaluated for drug release using cellulose membrane as a diffusion barrier against brand formulation for extended time period (12 h).

Using human cadaver skin

Full-thickness dermatome human cadaver skin from the right posterior leg of a male subject from “The New York Fire Fighters Skin Bank” was taken as a membrane. The cadaver skin was immersed in 100 mL of normal saline solution at room temperature for about 12 h before the experimentation. The skin was then grafted into pieces of size of approximately 2 cm$^2$. The integrity of the skin was checked by visual inspection to ensure the intactness of the skin and the presence of holes or imperfections (if any).

After thawing, the cadaver skin was mounted in between donor and receptor compartment. The skin was placed on the receiver chamber with stratum corneum facing toward the donor compartment. The experimental setup was the same as studies with a cellulose membrane. The experiments were performed in triplicate for each formulation.

In vitro Antifungal Activity

The antifungal efficacy studies were carried out using Sabouraud’s dextrose agar to ascertain the

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral oil</td>
<td>4.00</td>
</tr>
<tr>
<td>Cetyl alcohol</td>
<td>2.00</td>
</tr>
<tr>
<td>Glyceryl monostearate self-emulsifying</td>
<td>4.00</td>
</tr>
<tr>
<td>Glycerin</td>
<td>5.00</td>
</tr>
<tr>
<td>Polysorbate 80</td>
<td>2.00</td>
</tr>
<tr>
<td>Water</td>
<td>q.s. 80.00</td>
</tr>
</tbody>
</table>

**Table 4: Formulations using non-ionic emulsion base**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>DMSO</th>
<th>Propylene glycol</th>
<th>DGME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-ionic emulsion base</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Miconazole nitrate</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>DGME</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Purified water q.s</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

DMSO: Dimethyl sulfoxide, DGME: Diethylene glycol monoethyl ether

**Figure 1: Franz cell diffusion**

**Table 3: Composition of non-ionic emulsion base**
biological activity of the lead formulation of miconazole nitrate in comparison with the brand formulation against yeast micro-organism.

**Cup plate method**

The Sabouraud’s dextrose agar medium was taken into a 250 ml conical flask and was dissolved in 100 ml of distilled water. The pH of the medium was adjusted to 5.6, and it was sterilized in an autoclave at 121°C for 20 min. After the sterilization process, the medium was cooled at 40–45°C. A layer of the medium (~20 ml) was then poured into a sterilized Petri dish to give a depth of 3–4 mm. Cultures of yeast micro-organisms were dispersed in freshly prepared in yeast growth medium, and 0.5 ml of this was added and spread evenly on the solidified agar medium. The samples of miconazole nitrate creams – Formulation IV and Brand (0.1 g each) were carefully placed in the center of separate Petri dish and were incubated at room temperature for 48 h. The zone of inhibition was measured for both formulations.

**RESULTS**

**Preparation of Standard Curve**

A standard curve is plotted with absorbance on the Y-axis and concentration of the standard in µg/mL on X-axis, as shown in Figure 2.

**Table 5: Composition of cationic emulsion base**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral oil</td>
<td>6.00</td>
</tr>
<tr>
<td>Cetyl Alcohol</td>
<td>1.50</td>
</tr>
<tr>
<td>Glyceryl Monostearate</td>
<td>4.00</td>
</tr>
<tr>
<td>Catemol WPC</td>
<td>0.80</td>
</tr>
<tr>
<td>Glycerin</td>
<td>5.00</td>
</tr>
<tr>
<td>Polysorbate 80</td>
<td>1.00</td>
</tr>
<tr>
<td>Water q.s.</td>
<td>80.00</td>
</tr>
</tbody>
</table>

**Table 6: Formulations using cationic emulsion base**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulations (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 0%</td>
</tr>
<tr>
<td>Cationic emulsion base</td>
<td>80</td>
</tr>
<tr>
<td>Miconazole nitrate</td>
<td>2</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>-</td>
</tr>
<tr>
<td>DGME</td>
<td>-</td>
</tr>
<tr>
<td>Purified water q.s.</td>
<td>100</td>
</tr>
</tbody>
</table>

DMSO: Dimethyl sulfoxide, DGME: Diethylene glycol monoethyl ether

The standard curve for miconazole nitrate depicted a linear relationship and regression analysis with a very high correlation coefficient of 0.99.

**In vitro Release Data**

The drug release of miconazole nitrate from various semi-solid bases over a period of 2 h is shown in Figure 3. As per the data shown below, the overall order of release from the selected bases is seen to follow the order as follows:
Cationic emulsion system > Gel base system > Non-ionic emulsion system.

The average cumulative drug release from cationic emulsion, gel base, and non-ionic emulsion is observed to be 5.29 ± 0.3 mg/cm², 4.46 ± 0.1 mg/cm², and 2.36 ± 0.6 mg/cm², respectively, for a period of 2 h through a cellulose membrane, while brand formulation (Monistat® 2%) showed 3.10 ± 0.4 mg/cm² [Figure 3].

**HPMC Gel Base**

The *in vitro* release of drugs from HPMC gel base in the presence of penetration enhancers DMSO, PG, and DGME is shown in Figures 4-6, respectively.

**Table 7: Composition of Sabouraud’s dextrose agar**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>(%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>4.00</td>
</tr>
<tr>
<td>Peptone</td>
<td>1.00</td>
</tr>
<tr>
<td>Agar</td>
<td>2.00</td>
</tr>
<tr>
<td>Distilled water (q.s)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**Non-ionic emulsion based cream**

The *in vitro* release of drug from non-ionic emulsion base in the presence of penetration enhancers DMSO, PG, and DGME is shown in Figures 7-9, respectively.
Cationic emulsion based cream

The in vitro release of drug from cationic emulsion base in the presence of penetration enhancers DMSO, PG, and DGME is shown in Figures 10-12, respectively.

Optimizing the drug quantity in base of choice

Formulation IV with cationic emulsion with 5% DMSO (1% drug) showed an average cumulative drug release of 3.87 ± 0.4 mg/cm² [Figure 13].

Extended release studies using cellulose membrane

The average cumulative drug release after 12 h was found to be 8.58 ± 0.4mg/cm² and 7.74 ± 0.3mg/cm² for the brand and Formulation IV, respectively [Figure 14].
In vitro release of miconazole nitrate using human cadaver skin

The average cumulative drug release after 12 h across a human cadaver skin for brand product and Formulation IV was found to be 8.71 ± 0.9 mg/cm$^2$ and 8.28 ± 0.9 mg/cm$^2$, respectively [Figure 15].

In vitro Antifungal Studies

The results for in vitro antifungal studies is shown in Figure 16a and shows 0 h incubation with no growth of fungus, while Figure 16b shows 48 h incubation with an equal zone of inhibition for both brand and Formulation IV.

DISCUSSION

The standard curve for miconazole nitrate depicted a linear relationship and regression analysis with a very high correlation coefficient of 0.99, which confirms that plot obeys Beer-Lambert's law. A significant data are available on the spectrophotometric analysis of miconazole nitrate. Some researchers have analyzed individual molecules while others have worked with a mixture of multiple molecules. The standard curve of miconazole nitrate came out as expected with a linear relationship between concentration and absorbance.

Miconazole nitrate is a salt of weak base. The drug belongs to the Biopharmaceutics Classification System (BCS) Class II. One of the ways to increase the drug release from a base for an insoluble/partially soluble active is to increase the solubility of the drug in the base. This theory is in accordance with Fick’s first law of diffusion, which states that drug diffusion is a function of the concentration gradient. More the concentration gradient higher is the rate of drug diffusion. The concentration gradient is based on the amount of drug dissolved in the base, hence, available to diffuse. A wide range of techniques is available to increase drug solubilities such as salt formation, particle size reduction, polymorphs, use of cosolvents, and pH changes.

The pKa of miconazole is 6.77. Based on the Henderson-Hasselbach equation for salt of weak base (Miconazole Nitrate), lower the pH (acidic) higher will be the ionization state of miconazole, thus increasing its solubility. Release from different topical bases was evaluated, where the highest drug release was shown by cationic emulsion, followed by gel base and lastly non-ionic emulsion. The term “cationic” and “non-ionic” emulsions were used to signify the nature of the surfactant used in the emulsions. N-Stearoyl Cinnamoiniformyl Methyl Pyridinium Chloride, commonly known as Catemol WPC, is a cationic surfactant used as an emulsifier. GMS-SE is a well-known non-ionic emulsifier used in many topical formulations. Highest release was observed from cationic base when compared to other topical bases. It can be attributed to the higher solubility of miconazole nitrate in the cationic emulsion base. The pH of cationic emulsion is on the acidic side, mainly due to Catemol. Catemol shows a pH of 3.0 at 1% w/v solutions. The least amount of drug release from non-ionic emulsion base could be attributed to the complex structure of emulsion. On the other hand, HPMC is known to swell. The swelling of HPMC occurs.
by influx of surrounding water.[38] This influx can increase the solubility of miconazole nitrate as more solvent is now available for drugs; hence, a higher release for HPMC gel formulation is seen than non-ionic emulsion. Brand formulation Monistat® 7 uses cetyl and stearyl alcohols as emulsifier. These excipients are non-ionic in nature. Therefore, a release profile and average cumulative drug release are similar and closer to non-ionic emulsion.

A slightly higher release for Monistat® 7 was observed than a non-ionic emulsion. The higher release could be due to the presence of solubilizer PG and penetration enhancer isopropyl myristate. Penetration enhancers play a critical role in the diffusion process. Penetration enhancers are mainly divided into two categories depending on their mechanism of action. The first type is where the solubility of the active is increased, thus increasing its permeation, while second type of enhancers act reversibly or irreversibly on the skin, thus increase permeation of drug through the skin. PG, DMSO, and DGME were selected as penetration enhancers based on their ability to solubilize API and their mechanism of action on the skin. DMSO is used as penetration enhancer in formulations at concentrations as high as 15%. DMSO interacts with intercellular lipids present in the stratum corneum. They form aprotic interactions, which cause a reversible alteration of lipids. This way it disrupts the barrier function of stratum corneum. PG and DGME exert its mechanism of action as a penetration enhancer by dissolving lipids. This results in pore formation in the stratum corneum. The dissolution of skin lipids increases the cutaneous hydration. In addition to their ability to disrupt the skin structure, they also increase the solubility of miconazole nitrate. Miconazole nitrate, as discussed earlier, is a BCS Class II drug with water solubility of 26.3 µg/mL. PG increases the solubility of miconazole nitrate significantly, as high as 44.38 mg/mL. DMSO also has shown to increase the solubility of the drug at a staggering approximately 1000 times (25 mg/mL).

An increase in drug release with the addition of penetration enhancer was expected due to the increased solubility of the miconazole nitrate in the formulations. This observation was accurate for all the formulations. Although, the drug release did not necessarily increase with an increase in the level of penetration enhancer. In fact, a reverse action was observed for prototypes with gel base with PG, non-ionic emulsion with PG, and non-ionic emulsion with DGME where the drug release decreased with an increase in the level of penetration enhancer. Some formulations showed an increase in drug release at high enhancer concentrations. Formulations such as gel base with DMSO and DGME showed increased drug release at 15% and 10% enhancer concentrations, respectively. While non-ionic emulsion with DMSO did not show any difference with increase in enhancer level, but the release was higher compared to enhancer free formulation. The important factors influencing the penetration of the drug into the skin are not only the concentration of drugs but also the partition coefficient between the membrane and the base. The partition coefficient reflects the affinity of the drug for the membrane with respect to its formulation base.[46] Therefore, as the solubility of the drug in the base in significantly increased by PG, DMSO, and DGME, miconazole nitrate’s relative affinity in the formulation base increases compared to the membrane. Therefore, we do not see an increase in the drug release with an increase in the concentration of penetration enhancers. The same theory could be applied to the cationic emulsion with DMSO and PG. The highest release was observed with 5% DMSO and 10% PG for the two prototypes, respectively.

Reducing the drug quantity showed the same release characteristics for cellulose membranes and human cadaver skin. The average cumulative drug release (mg/cm²) was found to be similar to the brand. This comparable release of drugs from 1% formulation to a 2% Monistat is due to the presence of penetration enhancer DMSO at 5% concentration and an optimum base providing an increased solubility into the base. This comparable release from in vitro diffusion studies on cadaver skin was coupled with in vitro antifungal studies. Miconazole Nitrate inhibits ergosterol synthesis. Ergosterol is a chemical in the fungal cell membrane, inhibition of which leads to ruptured cell wall causing fungal cell death.[43] When Monistat was compared against Formulation IV on a Petri dish, a same zone of inhibition was observed. This matches with the drug release data showing that an equal release and equal efficacy in vitro was observed with the half concentration of drug compared to the brand formulation.

**CONCLUSION**

The in vitro diffusion studies were designed to evaluate the release of miconazole nitrate from various bases, namely, HPMC gel base, non-ionic emulsion base and a cationic emulsion base. This proved to be a useful method in screening formulation for the relative availability of the active ingredient. The in vitro data obtained from the diffusion studies indicated a general rank order of miconazole nitrate from the bases as: Cationic Emulsion Base >HPMC gel base >Non-ionic Emulsion base. In addition, the effect of various additives such as DMSO, PG, and DGME at different concentrations, on drug release was evaluated. The highest drug release was
exhibited by the cationic emulsion system. The effect of penetration enhancers was also seen, where 5% DMSO showed the best effect on drug release. This formulation with best release was selected for optimizing the drug concentration and was evaluated for extended study of 12 h through human cadaver skin. A comparable release was observed with the same zone of inhibitions with half drug level compared to the brand formulation. With a strong understanding of the nature of the molecule, its solubility characteristics, formulation base chemistry, it is possible to reduce the level of drug in a formulation without impacting its efficacy.

ACKNOWLEDGMENTS

Financial supports from Arnold and Marie Schwartz College of Pharmacy, Long Island University are gratefully acknowledged.

REFERENCES

26. Abou-Elkheir A. Spectrophotometric determination of miconazole nitrate and betamethasone valerate in bulk


