FORMULATION AND EVALUATION OF OXICONAZOLE NITRATE MUCOADHESIVE NANOEMULSION BASED GEL FOR TREATMENT OF FUNGAL VAGINAL INFECTION

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INTRODUCTION

Candidiasis is one of the most common vaginal disorders. It was found that approximately 75% of women suffer from vulvovaginal candidiasis during their life and about 40% to 50% of them suffer from multiple incidences [1, 2]. These episodes of vulvovaginal candidiasis are often very uncomfortable and painful and can include itching, irritation, continuous vaginal discharge and dysuria [3-5]. The most commonly antifungal drugs used for the treatment of vulvovaginal candidiasis in recent years were the imidazole antifungal agents [6, 7]. The most commonly antifungal agents that are administrated orally. OXZ has a broad-fungicidal or fungistatic activity against a number of pathogenic fungi including candida albicans. Its antifungal activity is due to the inhibition of the ergosterol biosynthesis, which is critical for cellular membrane integrity. A cure rate of 92% was achieved in the treatment of vaginal candidiasis by OXZ tablet (600 mg). OXZ is available in various conventional dosage forms such as creams and lotions. However, these dosage forms do not offer a prolonged duration of action which improves the efficacy of OXZ. Furthermore, the poor water solubility of OXZ [1.91e+03 g/l] caused low bioavailability that limits its antifungal efficiency [8-13]. For improving the solubility of OXZ, nanoemulsion appeared to be a suitable approach. Nanoemulsions are thermodynamically stable dispersions of two immiscible liquids (oil and water) which is stabilized using a surfactant and cosurfactant molecules. They may be either transparent or translucent and have a droplet size of 5-200 nm [14, 15]. They are well tolerated orally, on the skin and mucous membrane when used to deliver topically active drugs. Nowadays, increasing drug loading, enhancing drug solubility and bioavailability are the most important advantages encouraging the usage of nanoemulsion as drug delivery carriers. The very small size of nanoemulsion provides a large surface area which enhances the solubilization of the drug and facilitates the penetration through the skin and epithelial layer [16-18]. The aim of this present study is to formulate mucoadhesive nanoemulsion gel of OXZ to increase its solubility and increase the time of contact with epithelial tissue. The increased solubility of OXZ in nanoemulsion could improve its activity against vaginal fungal infection. Also, it is very important to use a dosage form which can adhere to the vaginal mucosa to increase the residence time of OXZ in the vagina. This purpose can be achieved by gelling of the OXZ using bioadhesive gelling agent. A gel can be described as the cross-linked material that retains a large amount of solvent inside its medium and if the solvent retained in organic one, such material is known as organogels. Thus, in the present inquiry, the effectiveness of nanoemulsion-based mucoadhesive gel of OXZ was investigated for vaginal delivery. The developed nanoemulsion-based mucoadhesive gel of OXZ was evaluated for in vitro antifungal activity and in-vitro retention study.

MATERIALS AND METHODS

Materials

Oxiconazole nitrate (OXZ) and Tinox cream® were kindly gifted from Eva-pharm Company, Egypt. Isopropyl myristate (IPM), Castor oil, Vitamin E, Ethyl alcohol; Isopropyl alcohol was purchased from Fluka, Biochemical, Switzerland. Span 80 (HLB=4.3), Span 85 (HLB=18) and Triacetin was purchased from Sigma-Aldrich, USA. Cremophore EL (HLB=12-14) was purchased from BASF. All other chemicals used were of analytical grade.

Methodology

Determination of saturated solubility of oxiconazole nitrate

The solubility of OXZ in various oils (isopropyl myristate, castor oil,
and vitamin E), surfactant (Cremophor EL, span 80, and span 85) and co-surfactants (ethyl alcohol and isopropyl alcohol) was determined by dissolving an excess amount (500 mg) of OXZ powder in 3 ml of each vehicle in 10 ml screw-capped test tubes. The mixtures were vortexed using a vortex mixer for 10 min and kept at 37±1°C in an isothermal shaker (250 rpm) until reaching the equilibrium. After that, the samples were centrifuged at 10000 rpm for 15 min and the supernatant was filtered through a 0.45 µm syringe driven filter. The supernatant was diluted with methanol and quantified by a validated HPLC method. OXZ samples were analyzed by using Agilent 1200 HPLC system, Agilent, USA, with the detection wavelength of 242 nm, temperature at 40 °C, analytical column Lichsphere 4.6 mm × 150 mm, and the mobile phase consists of methanol and phosphate buffer pH 6.8 (75:25) delivered at 2.0 ml/min[19], injection volume of 20 µl, the measurements were repeated three times. The assay was linear (r²=0.999) in the concentration range 5-250 µg/ml with a lowest detection limit of 1.35µg/ml of OXZ. The procedure was validated in terms of accuracy, intra-day and inter-day precision and the relative deviation in both cases was calculated (less than 2%).

Construction of pseudo-ternary phase diagrams

Based on the solubility study, IPM was chosen as oil; Cremophor EL as surfactant and ethanol as a co-surfactant. The aqueous phase was dispersed distilled water. Water titration method was used to construct pseudo-ternary phase diagrams at ambient temperature (25°C)[20] to determine the components concentration range for the existing range of nanoemulsion. Two phase diagrams were constructed in 1:1 and 2:1 mass ratios of Cremophore: Ethanol, respectively. For each phase diagram at a specific S/O mass ratio, the ratios of oil to the mixture of S/O were varied from 1.9 to 9.1. Water was added drop by drop under gentle stirring to each oily mixture until the onset of turbidity or phase separation. The nanoemulsion phase was recognized as the region in the phase diagram where transparent, easily flowable and clear formulations are obtained. One axis of the pseudo-diagram represents the phase diagram where transparent, easily flowable and clear formulations are obtained. The average droplet size, polydispersity index (PDI) and zeta potential measurement was made at a constant frequency of 1 Hz at ambient temperature.

Thermodynamic stability studies of drug loaded nanoemulsion

In order to find out the stable nanoemulsion and to discard the unstable or metastable nanoemulsion, formulated nanoemulsions were subjected to thermodynamic stability testing, which comprises of various parameters. Physical stability of nanoemulsions was continuously monitored over a period of time, whereas phase separation and turbidity were observed at room temperature [22]. Selected formulations were centrifuged at 3500 rpm for 30 min. Formulations, which did not show precipitation or any phase separations were subjected to three heating-cooling cycles between refrigerator temperature 4 °C and 45 °C, at each temperature, the formulation were stored for not less than 48hr. The formulations, which were found to be stable at these temperatures, were subjected to freeze-thaw cycle test. Three freeze-thaw cycles were carried out for the formulations between 21 °C and 25 °C. The formulations that survived thermodynamic stability tests were carried out for characterization [23].

Characterization of nanoemulsion formulations

Droplet size analysis and zeta potential measurement

The average droplet size, polydispersity index (PDI) and zeta potential of the prepared nanoemulsion were determined using Malvern Zetasizer (Nano ZS90, Malvern instrument Ltd., UK) with a 50 mV laser. The measurements were performed at 25 °C at a fixed angle of 90 °. The measurement time was 2 min. 1 gram of each formula was dispersed in 100 ml of double distilled water under gentle stirring in a glass beaker. Then 1 ml aliquot was withdrawn and placed in square glass cuvettes for measurement. Each droplet size value was mean of triplicate samples±SD [24].

Transmission electron microscopy studies

Morphology and structure of the nanoemulsion globules were performed using transmission electron microscopy (TEM) (Joel Co, 2100 HRT, Japan) operating at 40 kV. To perform the TEM observations of the nanoemulsion the formulation was first diluted with water (1:10) [25]. A drop of diluted nanoemulsion was then directly deposited on a carbon-coated copper grid, stained by 2% of uranyl acetate and observed after drying by TEM.

pH determination

The refractive index of nanoemulsion formulations was determined at 25 °C±0.5 using Abbe refractometer, Germany. Standardization was performed using castor oil [26].

Electroconductivity study

The electrical conductivity (σ) of the prepared nanoemulsions was determined using digital conductometer, (HANNA instrument H1255, Romania) to assess the nanoemulsion structure [27]. The measurement was made at a constant frequency of 1 Hz at ambient temperature.

Dilutability

The prepared nanoemulsions were diluted in 1:10 and 1:100 ratios with double distilled water to check if the system shows any signs of separation.

Formulation of oxiconazole nitrate nanoemulsion-based gel (OXZ-NEBG)

Nanoemulsion has low viscosity and difficult to apply, so it should be filled with a suitable gelling agent. Various gelling agents, namely, xanthan gum (XGUM), sodium carboxymethylcellulose (NaCMC), hydroxypropylmethylcellulose (HPMC) and Carbopol 934 (CRB) were evaluated for their ability to gel OXZ nanoemulsion. Gelling agent was dispersed or dissolved slowly in 10g of the OXZ nanoemulsion with the help of stirrer at 1,000 RPM. The selection of gelling agents depends on their compatibility with nanoemulsion structure and the ease of spreadability. The optimized composition of OXZ nanoemulsion-based gel is shown in table 2. In the case of NEBG containing Carbopol 934, a suitable amount of Triethanolamine was used as a neutralizer.

Characterization of nanoemulsion-based gel

Determination of pH, viscosity and oxiconazole nitrate content

pH and viscosity of the gel were determined as mentioned before. For determination of OXZ content, approximately 0.1 g of the prepared gel was dissolved in 25 ml of methanol. The drug content was determined using the above-mentioned HPLC method.
Surfactant

Vehicle type

In vitro
described earlier.

formula to
medium and replaced with fresh media.
The study, 2 ml of the aliquots was
was then immersed in 500 ml
speed of 50 rpm. A watch dish containing 0.5
using USP dissolution test apparatus II at 37 ±

°C. The sample on the plate was immersed into
plate was attached to the arm of USP
dissolution test apparatus [32]. [31]. Briefly, a vaginal piece (dimension 1 cm x 1 cm),
procured from a slaughterhouse, was fixed on a
glass plate using glue
and 0.5 mg of the gel was added to the vaginal surface. After 5 min, the
plate was immersed into the solution when the arm moved down and was out of the solution when the arm moved up. The residence time of the samples on the plate was detected visually. The experiments were performed in triplicate.

bio-adhesion study (wash off test)
The bioadhesive potential of the OXZ-NEBG was evaluated in comparison with the marketed Oxiconazole nitrate cream (Tinox® cream) by an in vitro bio-adhesion method that was reported by Nakamura et al. [31]. Briefly, a vaginal piece (dimension 1 cm x 1 cm), procured from a slaughterhouse, was fixed on a glass plate using glue
and 0.5 mg of the gel was added to the vaginal surface. After 5 min, the
plate was immersed into the solution when the arm moved down and was out of the solution when the arm moved up. The residence time of the samples on the plate was detected visually. The experiments were performed in triplicate.

in vitro drug release study
In vitro release profiles of OXZ-MBG and Tinox cream® were studied using USP disintegration test apparatus II at 37 ± 0.5 °C with a rotating speed of 50 rpm. A watch dish containing 0.5 g of the gel was tightly packed in a vaginal piece (dimension 1 cm x 1 cm), procured from a slaughterhouse, was fixed on a glass plate using glue
and 0.5 mg of the gel was added to the vaginal surface. After 5 min, the
plate was immersed into the solution when the arm moved down and was out of the solution when the arm moved up. The residence time of the samples on the plate was detected visually. The experiments were performed in triplicate.

In vitro antifungal activity
The antifungal activity of OXZ from the NEBG as well as 1% w/v OXZ standard and marketed formulation (Tinox® cream) was evaluated using Candida albicans as a representative fungus, adopting the cup plate method. The mean inhibition zone was calculated for each plate, and this value was taken as an indicator for the antifungal activity. The concentration of C. albicans (ATCC 10231) in inocula was equivalent to 5 × 10[15] CFU/ml [33]. 0.5 g of OXZ-NEBG, Tinox cream (equivalent to 5 mg OXZ), and 1 ml of OXZ suspension (5 mg/ml) was placed in an agar plate and plates were kept at room temperature for 48 h in dark conditions. The mean zone of inhibition after incubation was recorded for all the test samples (n = 3).

Stability studies
Stability of the best formula showed good bio-adhesion and fungal activity was assessed at various storage conditions viz. 25 °C/60% relative humidity (RH) and 40 °C/65% RH for a period of 3 mo. OXZ-NEBG was tightly packed in a plastic container. Samples (n = 3) were removed in 0, 30, 60, and 90 d and were assessed for OXZ content as well as clog formation, phase separation and viscosity.

RESULTS AND DISCUSSION
Solubility of oxiconazole nitrate
Linearity was studied for a drug by the proposed HPLC method. A Linear relationship was obtained between the area under the peak and drug concentration. A first order equation was used for calibration, where 24469x+1.098 is the regression equation and r = 0.999. The line range was from 5-50µg/ml. Calibration curve was shown in (Fig.1). The solubility studies of OXZ in different excipients at 25°C were represented in table (1). The results cleared that among the oil used; IPM exhibited the highest solubilizing potential for OXZ. The highest OXZ solubility was found in Cremophore EL and ethanol as surfactant and co-surfactant, respectively. Therefore, IPM, Cremophore EL and Ethanol were chosen for the construction of the phase diagram.

Table 1: Saturated solubility of oxiconazole nitrate (OXZ) in different vehicles for 72 h at 25°C

<table>
<thead>
<tr>
<th>Vehicle type</th>
<th>Vehicle</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>oil</td>
<td>Water</td>
<td>1.91x10⁻⁸</td>
</tr>
<tr>
<td></td>
<td>Isopropyl myristate</td>
<td>5.65</td>
</tr>
<tr>
<td></td>
<td>Castor oil</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>0.85</td>
</tr>
<tr>
<td>Co-surfactant</td>
<td>Ethanol</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Isopropyl alcohol</td>
<td>15.2</td>
</tr>
<tr>
<td>Surfactant</td>
<td>Cremophor EL</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>Triacetin</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>Span 80</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>Span 85</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Fig. 1: Calibration curve of oxiconazole nitrate in citrate phosphate buffer pH 4.5
Construction and characterization of phase diagram

The purpose of pseudo-ternary phase diagram was to find out the concentration range of oil, surfactant, and co-surfactant, and water uptake and the transparency of the formulation. Fig. 2 showed the phase diagrams at surfactant: co-surfactant (S: COS) ratio (2:1 and 1:1), respectively. It was found that low percentage of oil (5%) was selected as the highest concentration to form stable nanoemulsion. 45% of S: COS mixture at a ratio (2:1) or (1:1) was selected as the minimum concentration for stable and succulent nanoemulsion and as maximum safe concentration to prevent toxicity and irritation. It was reported that the highest flux and permeability coefficient was observed for a formulation that contains the maximum amount of water, so 50% of the water was selected. Nanoemulsion area was obtained towards the surfactant contains the maximum amount of water, so 50% of the water was selected as the minimum concentration for stable and successful nanoemulsion. 45% of S: COS mixture at a ratio (2:1) or (1:1) was selected as the highest concentration to form stable nanoemulsion over a wide range of composition [37-40]. Co-surfactant is added to achieve nanoemulsion systems at low surfactant concentrations [41]. The area of the nanoemulsion isotropic region decreased when the ratio of S: COS mixture changed from 2:1 to 1:1. High concentration of co-surfactant appeared to have a destabilizing effect that could be a probable factor for the substantial reduction of nanoemulsion area. The surfactant and the co-surfactant mass ratio were found to have pronounced effect on phase properties [size and position of nanoemulsion zone] [42].

Preparation of oxiconazole nanoemulsion (NE) and oxiconazole nanoemulsion-based gel (NEBG)

Nanoemulsions containing 1% OXZ using IPM as oil phase, Cremophore EL as surfactant and ethanol as cosurfactant were prepared using the spontaneous emulsification method (table 2). No change was observed in pseudo-ternary phase behavior when OXZ was incorporated in the formulation, showing the desirable stability of nanoemulsions consisting of non-ionic surfactant, which was not affected by a change in the pH or ionic strength [37].

![Fig. 2: (a) Pseudo-ternary phase diagrams of the oil (IPM), S/COS mixture (chromophore EL: ethanol) at mass ratio 2:1 and water system at 25 °C, (b) Pseudo-ternary phase diagrams of the oil (IPM), S/COS mixture (chromophore EL: ethanol) at mass ratio 1:1 and water system at 25 °C](image)

The formulations were subjected to thermodynamic stability studies to exclude metastable formulations, including centrifugation, heating-cooling cycle and freeze-thaw cycle tests. Only two formulations NEF1 containing 5% oil, 45% S: COS mixture at ratio (1:1) and water 50% and NEF4 containing 5% oil, 45% S: Cos mixture at ratio (2:1) and water 50% showed no phase separation, creaming, cracking or turbidity, so they were used as vehicle for preparing of different gel using HPMC 4%, NaCMC, 1% CRB 934 1% and XGUM 1% as gelling agent, the composition of nanoemulsion gel formulae were shown in table (3).

### Table 2: Composition of different formulations of oxiconazole nitrate nanoemulsions and their stability evaluations

<table>
<thead>
<tr>
<th>Formulation No</th>
<th>S mix (ratio)</th>
<th>Oil/S mix (ratio)</th>
<th>%w/w of components in Nanoemulsion formulation</th>
<th>Drug %w/w</th>
<th>Thermodynamic stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEF1</td>
<td>1:1</td>
<td>1:9</td>
<td>5.00</td>
<td>45.00</td>
<td>50</td>
</tr>
<tr>
<td>NEF2</td>
<td>1:1</td>
<td>1:8</td>
<td>5.55</td>
<td>44.44</td>
<td>50</td>
</tr>
<tr>
<td>NEF3</td>
<td>1:1</td>
<td>1:7</td>
<td>6.25</td>
<td>43.75</td>
<td>50</td>
</tr>
<tr>
<td>NEF4</td>
<td>2:1</td>
<td>1:9</td>
<td>5.00</td>
<td>45.00</td>
<td>50</td>
</tr>
<tr>
<td>NEF5</td>
<td>2:1</td>
<td>1:8</td>
<td>5.55</td>
<td>44.44</td>
<td>50</td>
</tr>
<tr>
<td>NEF6</td>
<td>2:1</td>
<td>1:7</td>
<td>6.25</td>
<td>43.75</td>
<td>50</td>
</tr>
</tbody>
</table>

NE= nanoemulsion, Pass= no phase separation, creaming, cracking or turbidity

### Table 3: Composition of oxiconazole nitrate nanoemulsion-based gel

<table>
<thead>
<tr>
<th>Formula code</th>
<th>OXZ W/W%</th>
<th>IPM W/W%</th>
<th>S mix W/W%</th>
<th>Water W/W%</th>
<th>S mix ratio</th>
<th>Gelling agent W/W%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEF1-A</td>
<td>1</td>
<td>5</td>
<td>45</td>
<td>50</td>
<td>1:1</td>
<td>HPMC (4%)</td>
</tr>
<tr>
<td>NEF1-B</td>
<td>1</td>
<td>5</td>
<td>45</td>
<td>50</td>
<td>1:1</td>
<td>NaCMC (1%)</td>
</tr>
<tr>
<td>NEF1-C</td>
<td>1</td>
<td>5</td>
<td>45</td>
<td>50</td>
<td>1:1</td>
<td>CRB (1%)</td>
</tr>
<tr>
<td>NEF1-D</td>
<td>1</td>
<td>5</td>
<td>45</td>
<td>50</td>
<td>1:1</td>
<td>XGUM (1%)</td>
</tr>
</tbody>
</table>

NEF1 and NEF4 = selected NE, A, B, C and D indicated type of gelling agent, A=HPMC, B=NaCMC, C=CRB, D=XGUM
Characterization of nanoemulsions

TEM, droplet size and size distribution

Images by TEM revealed that the nanoemulsion droplets were almost spherical in shape, dark and have an amorphous core as shown in Fig.3 and 4. All the systems were clear, isotropic liquid and capable of maintaining their isotropic nature with no formation of the liquid crystal when water content increased up to 100%. The micrograph exhibits, the droplet size of the sample were in the range of nanoemulsion and in agreement with results obtained from droplet size analysis by Zetasizer. The average droplet size of NEF1 and NEF4 was 26 and 23 nm, respectively (Table 4).

The small average diameter of droplets was expected to be due to the penetration of the cosurfactant molecules to the surfactant film, lowering the fluidity and the surface viscosity of the interfacial film, decreasing the radius of curvature of the microdroplets and forming transparent systems [43]. The polydispersity index (PDI) indicates uniformity of droplet size within the formulation and its stability. The values of PDI were found to be 0.55 and 0.54 for NEF1 and NEF4, respectively. The low value of PDI indicated the uniform distribution of nanodroplets within the formulation. No statistical difference was found between plain nanoemulsion and medicated nanoemulsion at p<0.5, that may be due to the higher solubilization effect of Cremophor EL and the low loaded amount of oxiconazole nitrate, table (4).

The gel containing HPMC as gelling agent showed higher residence time. It was found that HPMC hydrogel has the slowest swelling although HPMC hydrogel has the slowest swelling.

Conductivity measurements are highly useful in determining the nature of the continuous phase [44] and check the stability of nanoemulsion. Higher conductivity values of nanoemulsion are attributed to a larger percentage of water which allows more freedom of mobility of ions [45]. The higher values also confirm the o/w type of nanoemulsion and its stability without conversion [46]. Zeta potential of NEF1 and NEF4 was high which indicates the stability of the system. The incorporation of the drug had no effect on the conductivity or zeta potential of the nanoemulsion as shown in the table (4).

Physical characterization of plain and medicated nanoemulsion

pH of NE affected by adding of OXZ, since pH of NEF1 and NEF4 were 6.33±0.11 and 6.45±0.09, respectively, while the pH of their medicated formule was 3.85±0.12 and 3.82±0.13, respectively. The decrease in pH of the nanoemulsion after addition of the drug may be due to the acidic nature of oxiconazole nitrate since pH of 10 mg/ml suspension of oxiconazole nitrate in water ranged 3.4.

Rheological behavior of NEF1 and NEF4 was Newtonian, decreasing the viscosity values of NEF1 than NEF4 was due to the decrease in the amount of Cremophor EL in NEF1 than the NEF4, table (4).

The refractive index represents the net value of the components of nanoemulsion and indicates the isotropic nature of the formulation. The mean values of RI for both formulae either, plain or medicated were shown in the table (4).

Electrical conductivity measurements are highly useful in determining the nature of the continuous phase and check the stability of nanoemulsion. Higher conductivity values of nanoemulsion are attributed to a larger percentage of water which allows more freedom of mobility of ions. The higher values also confirm the o/w type of nanoemulsion and its stability without conversion. Zeta potential of NEF1 and NEF4 was high which indicates the stability of the system. The incorporation of the drug had no effect on the conductivity or zeta potential of the nanoemulsion as shown in the table (4).

In vitro bioadhesive study

The bioadhesive potential of OXZ-NEBG and Tinox cream® was evaluated by in vitro method. The nanoemulsion gel showed significantly higher residence time as compared to Tinox cream® (P < 0.05) as shown in the table (5). This clearly indicates that the mucoadhesive polymer used in the formulation of the gel can prevent the gel leaching from the vaginal tissue as compared to cream. The stronger the mucoadhesive property of the polymer depends on the nature and concentration of the polymer. Formuale containing HPMC as gelling agent showed higher residence time. This is in agreement with Mortazavi et al. [48] who mentioned that although HPMC hydrogel has the slowest swelling index, but has

<table>
<thead>
<tr>
<th>Characterization</th>
<th>NEF1</th>
<th>Medicated NEF1</th>
<th>NEF4</th>
<th>Medicated NEF4</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.3±0.11</td>
<td>3.85±0.12</td>
<td>6.45±0.09</td>
<td>3.82±0.13</td>
</tr>
<tr>
<td>Conductivity* μS/cm</td>
<td>23.3±1.1</td>
<td>23.5±1.2</td>
<td>187.6±0.94</td>
<td>187.4±1.4</td>
</tr>
<tr>
<td>Particle size (nm)*</td>
<td>26.3±2.1</td>
<td>26.3±2.4</td>
<td>23.4±1.4</td>
<td>23.4±1.8</td>
</tr>
<tr>
<td>Polydispersity index (PDI)</td>
<td>0.55</td>
<td>0.55</td>
<td>0.58</td>
<td>0.58</td>
</tr>
<tr>
<td>Viscosity (cp)*</td>
<td>38.2±2.5</td>
<td>35.6±3.1</td>
<td>151±4.2</td>
<td>159.4±3.5</td>
</tr>
<tr>
<td>Zetapotential (mv)</td>
<td>-33.5</td>
<td>-33.5</td>
<td>-30.7</td>
<td>-30.7</td>
</tr>
<tr>
<td>Refractive index</td>
<td>1.39</td>
<td>1.41</td>
<td>1.38</td>
<td>1.40</td>
</tr>
</tbody>
</table>

*values are mean of triplicate±SD

In vitro bioadhesive study

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In vitro antifungal activity

The OXZ nanoemulsion-based gel formulation showed antifungal activity when tested microbiologically by cup plate technique. The values of the zone of inhibition produced by OXZ-NEBG, OXZ standard, and Tinox cream were shown in the table (5). It is obviously clear that antifungal activity OXZ-NEBG is higher than the marketed Tinox® cream and OXZ standard (P<0.05). The enhanced in vitro antifungal activity of OXZ-NEBG may be due to the higher penetration of oil globules containing OXZ through fungal cell walls to inhibit ergosterol. Fungistatic effect of Oxiconazole nitrate may result from interference with ergosterol synthesis, which is required for cytoplasmic membrane integrity of fungi. It acts to destabilize the fungal cytochrome P450 51 enzyme (also known as Lanosterol 14-alpha demethylase). This is vital in the cell membrane structure of the fungus. Its inhibition leads to cell lysis. Oxiconazole nitrate has also been shown in inhibiting DNA synthesis and suppress intracellular concentrations of ATP [19].

Table 5: Physical characterization of oxiconazole nitrate nanoemulsion based gel

<table>
<thead>
<tr>
<th>Formula code</th>
<th>pH* (10%w/v in water)</th>
<th>Viscosity* (cp) at 50 rpm</th>
<th>Spreadability* (cm)</th>
<th>Drug* content %</th>
<th>Adhesion* time (min)</th>
<th>Inhibition* zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEF1-A</td>
<td>6.90±0.02</td>
<td>8249±111.2</td>
<td>3.4±0.06</td>
<td>98.92±2.36</td>
<td>95±2.3</td>
<td>38±1.2</td>
</tr>
<tr>
<td>NEF1-B</td>
<td>6.5±0.03</td>
<td>8310±170.5</td>
<td>3.2±0.03</td>
<td>99.48±1.45</td>
<td>45±2.1</td>
<td>40±0.8</td>
</tr>
<tr>
<td>NEF1-D</td>
<td>6.70±0.06</td>
<td>5222±125.4</td>
<td>3.1±0.11</td>
<td>99.65±2.14</td>
<td>40±1.8</td>
<td>40±2.3</td>
</tr>
<tr>
<td>NEF1-C</td>
<td>6.60±0.04</td>
<td>8334±135.1</td>
<td>3.0±0.05</td>
<td>98.66±1.47</td>
<td>46±1.5</td>
<td>35±1.3</td>
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<tr>
<td>NEF1-D</td>
<td>7.09±0.01</td>
<td>4270±117.4</td>
<td>2.7±0.04</td>
<td>99.78±0.88</td>
<td>45±2.5</td>
<td>36±2.1</td>
</tr>
<tr>
<td>NEF1-D</td>
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<td>1730±123.2</td>
<td>2.6±0.05</td>
<td>98.58±1.25</td>
<td>33±1.1</td>
<td>37±1.8</td>
</tr>
<tr>
<td>NEF1-D</td>
<td>6.90±0.02</td>
<td>1031±100.2</td>
<td>2.0±0.03</td>
<td>99.57±2.15</td>
<td>35±1.3</td>
<td>38±1.6</td>
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<td>Tinox cream</td>
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<td></td>
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<td></td>
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<tr>
<td>OXZ standard</td>
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</table>

*values are mean of triplicate±SD

In vitro release study

In vitro release profile of the prepared gel and Tinox® cream is shown in Fig 5. More than 95% of OXZ were released from NEBG containing Carbopol, Na CMC and HPMC over 4 h, while only 34.75, 48.97 and 40.97% released from cream, NEF1/XGUM, and NEF4/XGUM, respectively which indicates that the drug release from gels can be controlled by polymer type. The enhanced dissolution rate of OXZ from the most of the gel could be attributed to the small size of nanoemulsion incorporated in the gel, which permitted a faster rate of drug dissolution into the aqueous phase, much faster than that of OXZ cream. Curve fitting of in vitro release data of all the formulation was compared with different release model. The correlation coefficient R² indicated that drug release followed diffusion mechanism from nanoemulsion-based vaginal gels as the values of the correlation coefficient higher in case of zero-order equation. This indicates that drug release depends on swelling, relaxation and erosion of polymer with zero order release kinetics [50].

Stability studies

The formulation has to be remained stable for a sufficient period of time, even if exposed to variable conditions of temperature and humidity. Results of stability study of the selected formula NEF1-A are shown in the table (6). There was no significant change in viscosity and drug content. Also, no clog or phase separation was observed during 3 mo at variable temperature condition.

Table 6: Stability studies of optimized nanoemulsion-based gel NEF1-A

<table>
<thead>
<tr>
<th>Storage condition</th>
<th>25°C/60%RH±5%</th>
<th>45°C/65%RH±5%</th>
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<tbody>
<tr>
<td>0</td>
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<td>Clog</td>
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<tr>
<td>Phase Separation</td>
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<td>NO</td>
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<tr>
<td>pH</td>
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<td>6.9</td>
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<td>Consistency</td>
<td>G*</td>
<td>G</td>
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<tr>
<td>Viscosity</td>
<td>94.29</td>
<td>84.39</td>
</tr>
<tr>
<td>% Drug content</td>
<td>98.92</td>
<td>98.91</td>
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</table>

*G=Good

CONCLUSION

The nanoemulsion-based gel could be successfully alternative dosage form to deliver poorly soluble OXZ through the vagina. OXZ nanoemulsion gel with mucoadhesive properties is promising for prolonging the vaginal residence time and thereby better therapeutic effects. In addition, they provide intimate contact between dosage form and vaginal mucus, which may result in high
drug concentration in the local area. The Oxiconazole nitrate nanoemulsion-based vaginal gel could be successfully developed for the topical treatment of vaginal candidiasis. The developed oxiconazole nitrate nanoemulsion-based vaginal gel showed good in vitro antifungal activity against Candida albicans when compared with standard and capable of loading therapeutics dose of oxiconazole nitrate, to control its release for 4 h.

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CONFLICT OF INTERESTS

Declared none

REFERENCES


