International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 3, 2016

Original Article

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

ABEER KHATTAB, SOHA ISMAIL*

*Pharmaceutics Department, National Organization for Drug Control and Research, Giza, Egypt
Email: Abeer_Khattab75@yahoo.com

Received: 13 Nov 2015 Revised and Accepted: 13 Jan 2016

ABSTRACT

Objective: The aim of the present study was to formulate and evaluate nanoemulsion-based gel of oxiconazole nitrate (OXZ) for the treatment of vaginal infection.

Methods: The solubility of Oxiconazole nitrate in various oils, surfactant and co-surfactant, were done to identify the components of the nanoemulsion. Based on the solubility studies, isopropyl myristate (IPM) as oil, Cremophore EL as surfactant and ethanol as co-surfactant were selected for preparing nanoemulsion. Various nanoemulsions were prepared by using the spontaneous emulsification technique. The nanoemulsion area was determined by constructing of pseudo ternary phase diagrams. The formulated nanoemulsions were subjected to accelerated stability test. The nanoemulsion formulae that passed the stability test were characterized for its morphology, droplet size, conductivity and zeta potential. These optimized formulae were incorporated into the polymeric gel of carbopol 934 (CRB), hydroxypropyl methylcellulose (HPMC), sodium carboxymethylcellulose (NaCMC) and xanthum gum (XGUM), then evaluated for pH, viscosity, bio-adhesion properties, spreadability and drug content. The *in vitro* release and antifungal activity of the nanoemulsion gel formulae compared with the commercial available product, tinox® cream, was carried out to judge their efficiency.

Results: The optimal nanoemulsion formulae which composed of oil, surfactant: cosurfactant mixture (1:1 or 2:1), and water in ratio 5:45:50 showed the highest stability of nanoemulsion. All the nanoemulsion gel formulae showed higher release and antifungal activity than the marketed cream.

Conclusion: The Oxiconazole nitrate nanoemulsion-based gel could be successfully promising formulation for the topical treatment of fungal vaginal candidiasis.

Keywords: Nanoemulsion, Oxiconazole nitrate, Nanoemulsion-based gel, Vaginal candidiasis, Mucoadhesion

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. Imidazole antifungal agents [6, 7] are prepared in different dosage forms such as vaginal creams, pessaries, and oral tablets. The local (vaginal) delivery provides site-specific treatment and avoids toxic side effects of 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=1.8) 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 (Cremophore 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 for 72 h 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 232 nm, temperature at 40 °C, analytical column Lichrosphere 4.6 mm × 150 mm, and the mobile phase consists of methanol and phosphate buffer pH6.8 (75:25) delivered at 2.0 ml/min[19], injection volume of 20 µl, the measurements were repeated three times. The assay was linear (r^2=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, intraday and interday 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; Cremophore EL as surfactant and ethanol as a co-surfactant. The aqueous phase was doubled 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/CO mass ratio, the ratios of oil to the mixture of S/CO 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 aqueous phase; the second one corresponds the oil phase and the third one represents S/CO mixture at a fixed mass ratio [21].

Formulation of oxiconazole nitrate nanoemulsions (OXZ-NE)

Depending on the phase diagrams, OXZ loaded nanoemulsions were prepared at the different component ratio using the spontaneous emulsification method. Appropriate quantities of oil (IPM), surfactant (Cremophore EL) and cosurfactant (Ethanol) were weighed and mixed well. OXZ was accurately weighted to represent 1%w/w of the total weight of the nanoemulsion formulation, and then added to the previous mixture and stirred with a magnetic stirrer (100 rpm), at room temperature (25 °C±0.5) until the drug is completely dissolved. The weighed amount of water then added dropwise to the oil phase with continuous mixing using magnetic stirring for 30 min.

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 formulae 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

PH of nanoemulsion was measured by using a digital pH meter (Jenway 3510, UK), standardized using buffer pH 4.0 and 7.0 before use.

Viscosity determination

The rheological properties of the prepared NE were determined by means of Viscometer (Brookfield digital remaster DV III fitted with a CP-40 cone and plate spindle). A certain volume of each formula was put in a suitable container. The RPM was increased gradually in a suitable range to give torque values between 10-100 units, at 37±2°C, with 15 seconds between each two successive speeds. The rheological behavior of each system was investigated by plotting the shear stress versus the shear rate values and viscosity values were determined.

Refractive index

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 Triethanol-amine 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.

Spreadability measurement

Spreadability of the nanoemulsion-based gel was measured in terms of the diameter of gel circle produced when placed between two glass plates of definite weight. A weighed quantity of gel (0.5 g) was placed within a circle (1 cm diameter) drawn on a glass plate, and then a second glass plate were placed over the gel. A weight of 50 gm was left on the upper glass plate for 5 min. The increase in the diameter due to the spreading of the gels was measured [28-30].

***In vitro* 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 x1 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 attached to the arm of USP disintegration test apparatus and moved up and down in pH 4.5 citrate phosphate buffer [32] at 37 ± 1 °C. The sample on 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 dissolution 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 covered with a plastic wire screen (350 µ mesh size sinker). The dish was then immersed in 500 ml citrate phosphate buffer (pH 4.5) that placed in the vessel of USP dissolution test apparatus [32]. During the study, 2 ml of the aliquots was removed at different time intervals (0.5, 1, 2, 3, 4, 6, 8, 10, and 12 h) from the dissolution medium and replaced with fresh media. Samples were filtered through 0.45 µ syringe filter. The amount of OXZ released from the formula to the dissolution medium was determined by HPLC method described earlier.

***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-NBG was tightly packed in 10-g 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+10.98 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.

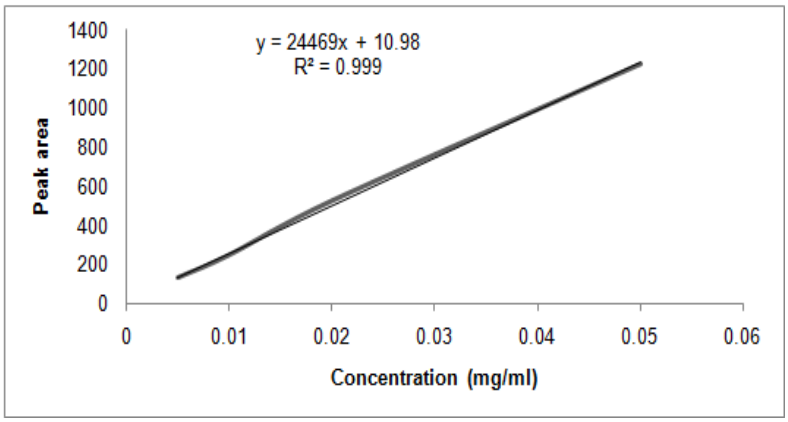

Fig. 1: Calibration curve of oxiconazole nitrate in citrate phosphate buffer pH 4.5

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

Vehicle type	Vehicle	Solubility (mg/ml)
	Water	$1.91X10^{-3}$
oil	Isopropyl myristate	5.65
	Castor oil	0.75
	Vitamin E	0.85
Co-surfactant	Ethanol	35
	Isopropyl alcohol	15.2
Surfactant	Cremophor EL	14.5
	Triacetin	9.9
	Span 80	6.2
	Span 85	5.5

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 based on 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 successful 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 rich apex. The translucent nanoemulsion region is represented in phase diagrams with no observation of distinct conversion from water-in-oil (W/O) to oil-in-water (O/W) nanoemulsions. On the phase diagram, the white region represents the turbid and conventional emulsions based on visual observation. Cremophore EL, which showed the highest solubility of the drug was selected as surfactant, ; it also has hydrophilic-lipophilic balance (HLB) value greater than 10 which allowed the formation of a stable o/w nanoemulsion upon dilution with water[34, 35]. Transient negative interfacial and fluid interfacial film are rarely achieved by the use of single surfactant [36]; usually, the addition of co-surfactant is necessary. The importance of co-surfactant is due to its ability to decrease the bending stress of interface and allow the interface film to be sufficiently flexible to take up different curvatures required to form nanoemulsions 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].

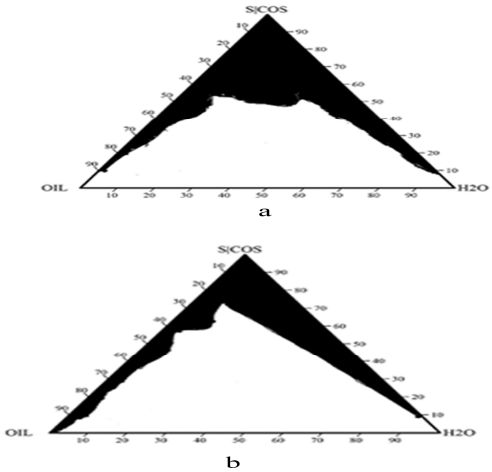

Fig. 2: (a) Pseudo-ternary phase diagrams of the oil (IPM), S/COS mixture (chromophore EL: ethanol) at mass ratio 2:1 and water system at 25 °C, (b) Pseudo-ternary phase diagrams of the oil (IPM), S/COS mixture (chromophore EL: ethanol)at mass ratio1:1 and water system at 25 °C

The formulations were subjected to thermodynamic stability studies to exclude metastable formulations, including centrifugation, heating-cooling cycle and freeze-thaw cycle tests. Only two formulae 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

Formulation No	S mix (ratio)	Oil/S mix (ratio)	%w/w of components in Nanoemulsion formulation			Drug %w/w	Thermodynamic stability
			Oil	S mix	Water		
NEF1	1:1	1:9	5.00	45.00	50	1	Pass
NEF2	1:1	1:8	5.55	44.44	50	1	Fail
NEF3	1:1	1:7	6.25	43.75	50	1	Fail
NEF4	2:1	1:9	5.00	45.00	50	1	Pass
NEF5	2:1	1:8	5.55	44.44	50	1	Fail
NEF6	2:1	1:7	6.25	43.75	50	1	Fail

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

Table 3: Composition of oxiconazole nitrate nanoemulsion-based gel

Formula code	OXZ W/W%	IPM W/W%	S mix W/W%	Water W/W%	S mix ratio	Gelling agent W/W%
NEF1-A	1	5	45	50	1:1	HPMC (4%)
NEF4-A	1	5	45	50	2:1	HPMC (4%)
NEF1-B	1	5	45	50	1:1	NaCMC(1%)
NEF4-B	1	5	45	50	2:1	NaCMC(1%)
NEF1-C	1	5	45	50	1:1	CRB (1%)
NEF4-C	1	5	45	50	2:1	CRB (1%)
NEF1-D	1	5	45	50	1:1	XGUM (1%)
NEF4-D	1	5	45	50	2:1	XGUM (1%)

NEF1and 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).

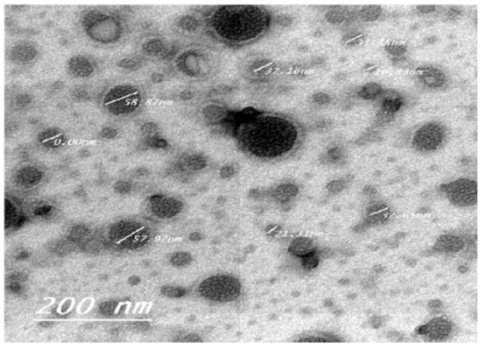

Fig. 3: Transmission electron monograph of OXZ-loaded nanoemulsion of NEF1 (5%IPM, 45% S mix and 50% water) at S: CoS mass ratio 1:1

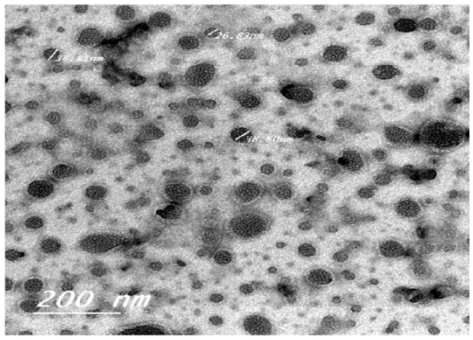

Fig. 4: Transmission electron monograph of OXZ-loaded nanoemulsion of NEF4 (5%IPM, 45% S mix and 50% water) at S: CoS mass ratio 2:1

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 formulae 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 [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).

Table 4: Physical characterization of selected nanoemulsion formulae with or without oxiconazole nitrate

Characterization	NEF1	Medicated NEF1	NEF4	Medicated NEF4
pH*	6.33±0.11	3.85±0.12	6.45±0.09	3.82±0.13
Conductivity* µs/cm	233.6±1.1	233.5±1.2	187.6±0.94	187.4±1.4
Particle size (nm)*	26.31±2.1	26.31±2.4	23.4±1.4	23.4±1.8
Polydispersity index (PDI)	0.55	0.55	0.58	0.58
Viscosity (cp)*	38.2±2.5	35.6±3.1	151±4.2	159.4±3.5
Zetapotential(mv)	-33.5	-33.5	-30.7	-30.7
Refractive index	1.39	1.41	1.38	1.40

*values are mean of triplicate±SD

Characterization of oxiconazole nitrate nanoemulsion-based gel

The pH of nanoemulsion-based gel was ranged from 6.5 to 7.09 which are within the limit of the semisolid specification and at this pH the gel will be not irritant to vagina [47]. Spreadability is an important parameter for ease of application of topical formulation from a patient compliance point of view. The gel containing HPMC showed the highest spreadability while gel containing XGUM showed the lowest spreadability as shown in table 4. It was found that formulae showed pseudoplastic rheological behavior and the viscosity of the prepared gels at 50 rpm was shown in table (5). The OXZ content of the NEBG was found to be ranged from 98.66±1.47 % to 99.78±0.88%.

***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. Formulae containing HPMC as gelling agent showed higher residence time. This is in agreement with Mortazvi *et al.* [48] who mentioned that although HPMC hydrogel has the slowest swelling index, but has

longer residence time since it has a good gelling force which is responsible for adhesion and the mechanical strength [49].

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 anti-fungal 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 cyctochrome 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 namoemulsion based gel

Formula code	pH* (10%w/v in water)	Viscosity* (cp) at 50 rpm	Spreadability* (cm)	Drug* content %	Adhesion* time (min)	Inhibition* zone (mm)
NEF1-A	6.90±0.02	8429±111.2	3.4±0.06	98.92±2.36	95±2.3	38±1.2
NEF4-A	6.95±0.04	1333±115.2	3.6±0.012	99.32±2.23	92±3.6	35±1.5
NEF1-B	6.5±0.03	8310±170.5	3.2±0.03	99.45±1.45	45±2.1	40±0.8
NEF4-B	6.70±0.06	5222±125.4	3.1±0.11	99.65±2.14	40±1.8	40±2.3
NEF1-C	6.60±0.04	8334±135.1	3.0±0.05	98.66±1.47	46±1.5	35±1.3
NEF4-C	7.09±0.01	4270±117.4	2.7±0.04	99.78±0.88	45±2.5	36±2.1
NEF1-D	7.03±0.04	1730±123.2	2.6±0.05	98.58±1.25	33±1.1	37±1.8
NEF4-D	6.90±0.02	1031±100.2	2.0±0.03	99.57±2.15	35±1.3	38±1.6
Tinox cream					15±.09	32±2.3
OXZ standard						34±2.4

*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 R^2 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.

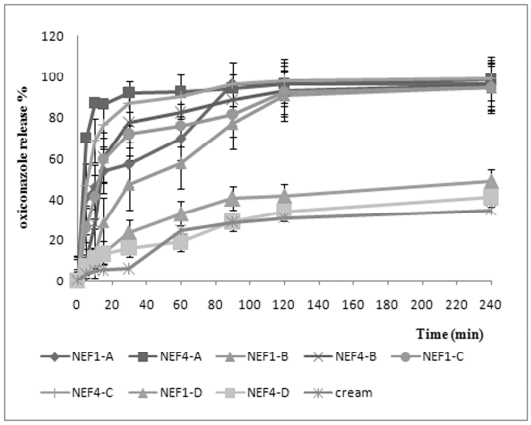

Fig. 5: Release profile of oxiconazole nitrate in citrate phosphate buffer pH 4.5

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

	Storage condition							
	25°C/60%RH±5%				45°C/65%RH±5%			
	0	1	2	3	0	1	2	3
Clog	NO	NO	NO	NO	NO	NO	NO	NO
PhaseSeparation	NO	NO	NO	NO	NO	NO	NO	NO
pH	6.9	6.9	6.9	6.9	6.8	6.8	6.9	6.9
Consistency	G*	G	G	G	G	G	G	G
Viscosity	8429	8430	8424	8429	8426	8429	8427	8430
% Drug content	98.92	98.91	98.92	98.92	98.81	98.92	98.93	98.91

*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.

ACKNOWLEDGEMENT

We are thankful to the National organization for drug control and research for the financial support. Authors are thankful to Eva pharma for the gift samples of drug and excipients.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Lanchares JL, Hernandez ML. Recurrent vaginal candidiasis changes in etiopathogenic patterns. Int J Gynecol Obstet 2000;71: S29–S35.
2. Ferrer J. Vaginal candidosis: epidemiological and etiological factors. Int J Gynecol Obstet 2000;71: S21–S27.
3. Sobel JD. Candidal vulvovaginitis. Clin Obstet Gynecol Mar 1993;1:153–65.
4. Horowitz BJ, Giaquinta D, Ito S. Pathogens in vulvovaginal candidiasis: implications for patient care. J Clin Pharmacol 1992;3:248–55.
5. Nencioni T, Pamparana F, Jacobellis M. Mycotic vulvovaginitis and topical cyclopiroxolamine. Minerva Ginecol 1988;12:719-22.
6. Matsui H, Sakanashi Y, Oyama TM, Oyama Y, Yokota S, Ishida S, *et al.* Imidazole antifungals, but not triazole antifungals, increase membrane Zn2+permeability in rat thymocytes possible contribution to their cytotoxicity. Toxicology 2008;2-3:142-50.
7. Fromtling RA: Overview of medically important antifungal azole derivatives. Clin Microbiol Rev 1988 Apr; 2:187-217.
8. Polak A. Antifungal activity of four antifungal drugs in the cutaneous retention time test. *Sabouraudia* 1984;22:501-3.
9. Ramelet AA, Walker-Nasir E. One daily application of oxiconazole nitrate cream is sufficient for treating dermatomycoses. *Dermatologica* 1987;175:293-5.
10. Gouveia DC, Jones DA, Silva C. Oxiconazole nitrate in the treatment of vaginal candidiasis: single dose versus 3-day treatment with econazole. Pharmatherapeutica 1984;10:682-5.
11. Gip L. Comparison of oxiconazole nitrate (Ro 13-8996) and econazole in dermatomycoses. *Mykosen* 1984;27:295-302.
12. Wagner W, Reckers-Czaschka R. Oxiconazole nitrate in dermatomycosis: a double-blind, randomized comparison with bifonazol. *Mykosen* 1987;30:484-92.
13. Sharma G, Jain S, Tiwary K, Kayr G. once daily bioadhesive vaginal clotrimazole tablets: design and evaluation. Acta Pharm 2006;56:337-45.
14. Adnan A, Mohammad R, Farhan JA, Zeenat I, Roop KK, Aqil M, Sushama T. Nanoemulsion components screening and selection: a technical note. AAPS Pharm Sci Tech 2009;1:69-76.
15. AzeemA, Rizwan M. Nanoemulsion components screening and selection: A technical note. AAPS Pharm Sci Tech 2009;10:69-76.
16. Patel MR, Patel RB, Parikh JR, Solanki AB, Patel BG. Effect of formulation components on the *in vitro* permeation of microemulsion drug delivery system of fluconazole. AAPS PharmSci Tech 2009;10:917-23.
17. Kantarci G, Ozguney I, Karasulu HY, Arzik S, Guneri T. Comparison of different water/oil microemulsions containing diclofenac sodium Preparation, characterization, release rate, skin irritation studies. AAPS Pharm Sci Tech 2007;8:E91.
18. Mohamed MI. Optimization of chlorphenesin Emulgel Formulation. AAPS 2004;3:e26.
19. Yogeshwar G. Bachhav, Vandana B. Patravale. The microemulsion-based vaginal gel of clotrimazole: Formulation, *in vitro* evaluation, and stability studies. AAPS Pharm Sci Tech June 2009;2:476-81.
20. Ghada H. Formulation and In-vitro evaluation of nyatatin nanoemulsion-based gel for topical delivery. J Am Sci 2012;12:541-8.
21. Shafiq S, Shakeel F, Talegaonkar S, Ali J, Baboota S, Ahuja A, Khar RK, Ali M. Formulation development and optimization using nanoemulsion technique: A technical note. AAPS Pharm. Sci. Tech. 2007;2: E1-E6.
22. Sushama T, Mohd T and Raid MA. Design and development of o/w nanoemulsion for the transdermal delivery of ondansetron. Bulletin of pharmaceutical Research 2011;3:18-30.
23. Shakeel F, Baboota S, Ahuja A, Ali J, Aquil M, Shafiq S. Nanoemulsions a Vehicle for Transdermal Delivery of Aceclofenac. AAPS Pharm Sci Tech 2007;4: E1-E9.
24. Yashpal S, Tanuj H, Harsh K. Nanoemulsions: A pharmaceutical review. Nanoemulsions — Advances in Formulation, Characterization, and Applications in Drug Delivery. Int J Pharma Prof Res 2013;2:928-35.
25. Faiyaz S, Wafa R, Musa A. Investigation of true nanoemulsions for the transdermal potential of indomethacin: Characterization, rheological characteristics and ex vivo skin permeation studies. J Drug Target 2009;17:435-41.
26. Singh B, Kumar B, Jain SK, Shafaat K. Development and characterization of A nanoemulsion gel formulation for transdermal delivery of carvedilol. Int. J. Drug Dev Res Jan-March 2012;1:151-61.
27. Sripriya V, Jun S. Self-nano emulsifying drug delivery systems (SNEDDS) for oral delivery of protein drugs I. Formulation development Int J Pharm October 2008;1-2:2-9.
28. Pradhnya W, Swapnil W, Ashwini Y. Development of isotretinoin gel for the treatment of acne vulgaris. Research Journal of Pharmaceutical, Biological and Chemical Sciences January–March 2011;1:220-30.
29. Desai KG. Enhanced Skin Permeation of rofecoxib using topical microemulsion gel. Drug Dev Res 2004;63:33–40.
30. Bachhav YG, Patravale VB. The microemulsion based vaginal gel of fluconazole: Formulation, *in vitro* and *in vivo* evaluation. Int J Pharm 2009;365:175–9
31. Nakamura F, Ohta R, Machida Y, Nagai T. *In vitro and in vivo* nasal mucoadhesion of some water-soluble polymers. Int J Pharm 1996;134:173–81.
32. Shiva KY, Naveen KN, Sharada Goranti, Sambit KD. Development of intravaginal metronidazole gel for the treatment of bacterial vaginosis: Effect of Mucoadhesive natural polymers on the release of Metronidazole. Int J PharmTech Res 2010;3:1746-50.
33. Chudasama A, Patel V, Nivsarcar M, Vasu K, Shishoo C. Investigation of microemulsion system for transdermal delivery of itraconazole. J Adv Pharm Technol Res 2011;2:30-8.
34. Faiyaz S, Sanjula B, Alka A, Javed A, Mohammed A, Sheikh S. Nanoemulsions as vehicles for transdermal delivery of aceclofenac. AAPS Pharm Sci Tech 2007;8:4.
35. Kommuru TR, Gurley B, Khan MA, Reddy IK. Self emulsifying drug delivery systems) (SEDDS) of coenzyme Q_{10}: Formulation development and bioavailability assessment. Int J Pharm 2001;2:233-46.
36. Lawrence MJ. Surfactant systems (SEDDS): microemulsions and vehicles as vehicles for drug delivery. Eur J Drug Metab Pharmacokinet 1994;3:2257-69.
37. Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. Adv Drug Deliv Rev 2000;1:89-121.
38. Chhabra G, Chuttani K, Mishra AK, Pathak K. Design and development of nanoemulsion drug delivery system of amlodipine besilate for improvement of oral bioavailability. Drug Dev Ind Pharam 2011;8:907-16.
39. Aboofazeli R, Lawrence CB, Wicks SR, Lawrence MJ. The investigation into formation and characterization of phospholipid microemulsions III. Pseudo-trnary phase diagrams and either an alkanoic acid, amine alkane diol, polyethylene glyol alkyl ether or alcohol as cosurfactant. Int J Pharm 1994;1:63-72.
40. Ghosh PK, Murthy RSR. Microemulsions: a potential drug delivery system. Curr Drug Delivery 2006;2:167-80.
41. Kreilgaard M. Influence of microemulsions on cutanteous drug delivery. Adv Drug Delivery 2002;54: S77-98.
42. Hua L, Weisan P, Jiayu L, Ying Z. Preparation, evaluation, and NMR characterization of vinpocetine microemulsion for transdermal delivery. Drug Dev Ind Pharm 2004;6:657-66.

43. Gao ZG, Choi HG, Shin HJ, Park KM, Lim SJ, Hwang KJ, *et al*. Physicochemical characterization and evaluation of microemulsion system for oral delivery of cyclosporine A. Int J Pharm 1998;161:75-86.
44. Mruali RP, Rashim BP, Jolly RP. Effect of formulation components on the *in vitro* permeation of microemulsion drug delivery system of fluconazole. AAPS Pharm Sci Tech 2009;10:917-23.
45. Gulam M, Khan, Zeenat I, Bansal T, Talegaonkar S. Preparation and characterization of oil in water nano-reservoir systems for improved oral delivery of atorvastatin. Curr Nanosci 2009;13:428-40.
46. Hasse A, Keipert S. Development and characterization of microemulsions for ocular application. Eur J Pharm Biopharm 1997;43:179-83.
47. Farhan JA, Mohd AA, Zeenate IK, Roop KK, Mushir A. Development and *in vitro* evaluation of an acid-buffering bioadhesive vaginal gel for mixed vaginal infection. Acta Pharm 2008;58:407-19.
48. Mortazavi SA, Smart JD. Factors influencing gel-strengthening at the mucoadhesive-mucus interface. J Pharm Pharmacol 1994;46:86-90.
49. Mortazavi SA. Investigation of various parameters influencing the duration of mucoadhesion of some polymers containing discs. Daur 2002;10:98-104.
50. Anita P, Jayvadan P. Mucoadhesive microemulsion based prolonged release vaginal gel for the anti-fungal drug. Am J PharmTech Res 2012;2:4.