RESEARCH ARTICLE

Design, Preparation, and *In-vitro* Evaluation of Novel Ocular Antifungal Nanoemulsion Using Posaconazole as a Model Drug

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Received: 10th March, 2021; Revised: 9th April, 2021; Accepted: 25th May, 2021; Available Online: 25th September, 2021

ABSTRACT

Fungal eye infection is considered extremely rare and can be very serious. This study aims to prepare and evaluate eye drop in nano-emulsion form to treat fungal keratitis resistant to old-type anti-fungal drugs. Posaconazole is an anti-fungal drug its mechanism is deemed to be extremely similar to that of other anti-fungal agents of the azole. Depending on drug solubility results in nano-emulsion composition, pseudo-ternary phase diagrams were constructed and nano-emulsion region detected that help to prepare 3 posaconazole nano-emulsion formulas by using isopropyl myristate as oil mixed with different ratios of surfactant labrasol) and co-surfactant transcutol p in 1:2, 1:1 and 2:1 ratios. All the prepared formulas subjected to different characterization including accelerated physical stability study, light transmittance measurement, droplet size and polydispersity index determination, Zeta potential measurement, pH evaluation, and estimation of drug content. Selected formula (F2) which consist of (4%c IPM oil, 36% of (2:1 labrasol:transcutol – p) as s-mix ratio, 59% DW and 1% POZ), shows a good drug release (92.17%), drug content equal to (98.05 ± 0.012) and its particle size, PDI and zeta potential was 15.3 nm, 0.154 and 53.72 Mv, respectivly. So it was selected as the optimum nano-emulsion formula to be used as an ocular dosage form, and it undergoes further investigation. Osmolarity, permeability, irritation and microbiological studies were done, and results of all these studies indicating that F2 was nonirritant, permeable and gave a significant improvement in antimicrobial activity. The study concluded that nano-emulsion is considered an advance technique for the ocular preparation and improves solubility and permeability of insufficient water-soluble medications through the cornea.

Keywords: Anti-fungal, Eye drop, Nano-emulsion, Posaconasole.

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.3.71

How to cite this article: Nasser ST, Abdulrassol AA, Ghareeb MM. Design, Preparation, and *In-vitro* Evaluation of Novel Ocular Antifungal Nanoemulsion Using Posaconazole as a Model Drug. International Journal of Drug Delivery Technology. 2021;11(3):1058-1064.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The eye is one of the human body's most restrained and complicated organ. It is partly separated by various barriers from the rest of the body. The drug delivery system to eye ball is one of the most complicated mechanisms in pharmaceutical sciences.[1] Less than 5% of the drugs present in eye drops, however, infiltrate the corneal membrane and enter the intraocular tissues; and the remaining dosage typically undergoes transconjunctival absorption or transnasal absorption or drainage into the nasolacrimal channel. There are many explanations for this poor bioavailability, such as the anatomically complex eye shape, narrow surface for absorption and poor corneal clarity, corneal epithelium lipophilicity, metabolism, enzymolysis, protein bonding in tear fluid, and defense mechanisms, i.e., tear forming, blinking, and liquid drainage through the estimated nasolacrimal duct. Approximately 30 µL is the blinking-free conjunctival sac volum potential which is known to be low; all of these above defense measures should result in a decrease in the concentration of drugs on the application site (eye surface). And so it would take quicker for the drug to be in contact with the eye tissue than necessary for proper absorption. The key goal for the development of ocular preparation is to obtain the optimal concentration of substance at the absorption site and to sustain it for a sufficiently long period of time, which in turn contributes to lower application times.[2]

A major upgrade to traditional forms regarding ocular preparations is the introduction of polymers allowing long contact time of corneal surface and active ingredient, thus; causing an increase in its bioavailability. Also, the next option for changing the ophthalmic active ingredient bioavailability involves adding excipients to formulation and enhancing the drugs' penetration into the eye tissue. These excipients involved surfactants, chelating agents and cyclodextrins, which in addition to active ingredients, forming complexes of inclusion causing an increase in the permeability and solubility as well as the bioavailability of poorly soluble drugs.[3]

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Fungal eye infection extremely rare, but they can be very serious and if left unchecked clinically may lead to blindness and loss of vision. Those ocular infections might happen because of many causes like eye damage, eye surgery, contamination and/or improper use of ocular products, systemic fungal infections and immunocompromised morbidity, were majorly located in the ocular tissues like aqueous and/or vitreous humour, cornea, sclera, and other eye coverings. These fungal infections were mainly resulting from filamentous fungal species like Fusarium and Aspergillus, also non fungal species like Candida.[4]

Posaconazole(POZ) a broad-spectrum triazole anti-fungal agent, The US Food and Drug Administration has approved the treatment of oropharyngeal candidiasis for patients at high risk of developing such infection due to extreme immune compromising, such as hematopoietic stem cell transplantation in patients at high risk of itraconazole and/or fluconazole refractories and/or as a prophylaxis of invasive Aspergillus and Candida infections. Furthermore, posaconazole as a therapeutic agent for infections by some filamentous fungi is promising. posaconazole is a class II drug according to the biopharmaceutical classification system that means low solubility and high permeability. Anti-fungal mechanism of POZ is extremely similar to that of other anti-fungal azole agents, primarily inhibiting CYP-dependent 14-α demethylase in the ergosterol biosynthetic pathway, a key component of the fungal cell membrane. The accumulation of toxic 14-a methyl sterols and ergosterol depletion occurs as a result of enzyme inhibition, resulting in disruption of the function of the fungal cell membrane, and cell growth and division blockage. Half-life (t0.5) of POZ is in the range of 20-66 hours (mean t0.5 about 35 hours). 32L/hour is POZ total body clearance. The major elimination pathway is via feces (mainly parent drug), While minor elimination occurring by renal clearance (about 13% of radiolabeled taken dose).[5] This study aims to prepare and evaluate POZ eye drop in nano-emulsion form to be used in treatment of fungal keratitis that resistant to old type of anti-fungal drugs and improve its solubility and increase permeability through the cornea.

MATERIALS

Posaconazole (POZ), isopropyl myristate, labrasol, and transcutol-p were purchased from Hyperchem, China. Methanol was purchased from Chemlab, Belgium. Dialysis membrane; MwCO: 8000 -14000 D product of USA. Phosphate buffer pH 7.4 was obtained from HiMedia laboratories, India.

METHODS

Construction of Pseudo-ternary Phase Diagrams

Depending on results of POZ solubility in different oils, surfactants and co-surfactants, the pseudo-ternary phase diagram has been advanced by the aqueous titration process. Deionized water (DW) has been employed as an aqueous medium, mixed with different mixtures of surfactant (labrasol) and co-surfactant (transcutol p) in 1:2, 1:1 and, 2:1 ratios, on the basis of rising surfactant levels, as well as rising co-surfactant concentration. Chosen oil was (Isopropyl myristate (IPM)) with S-mix were combined progressively at varying ratios for each phase diagram in different vials of glass in ratios of (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9) (w/w).[6] A clear and uniform mix by a vortex blend for 5 minutes was applied to the volumes in each surfactant and co-surfactant mixture (Smix). Every mixture was then titrated under a gentle magnetic stirrer without heating it with DW. The concentration of water at the end of the titration was measured in which clear to turbid changes occurred. These mixtures were then used to determine the limits on the nano-emulsion region that fit the value of the oils selected. In the presence of drug enriched oil, also phase diagrams were built as the hydrophobic portion to determine the effect of adding medicines on their nano-emulsion frontier. In each diagram, the greater the spectrum of emulsion indicates higher hydration. The optimal percentage w/w of the emulsion composition for further studies is chosen according to the results obtained.[7] CHEMIX software was used to create the pseudo-ternary phase diagrams.[8]

Preparation of POZ Nanoemulsion

According to pseudo-ternary phase diagrams, different O/W nano-emulsion formulations as listed in Table 1, have been produced by the water titration approach, using the surfactant and co-surfactant mixture (S-mix) and oil concentrations. To prepare 10 gm of 1% POZ nanoemlsion, the primary emulsion was produced by dissolving 100 mg of POZ in IPM oil using a vortex mixer for ten minutes, followed by adding the selected S-mix in a fixed proportion until a clear solution was achieved. DW was then added into the clear solution with continuous stirring by magnetic stirrer at room temperature (~500 rpm) until a clear emulsion was formed. In order to achieve very small droplet sizes, the prepared emulsions were subjected to ultra-sonication using a 25 kHz sonicator for 1 minute. Extended use of ultrasonication leads to the production of heat. The NE formula container with the Ice Breakers has been remedied to resolve this issue.[9] All these steps are shown in Figure 1. Nano-emulsions were moved to the new vial and placed in the refrigerator at 4°C. The final formulas (F1-F3) obtained was subject to characterization.

Characterization of Prepared POZ Nanoemulsion:

Accelerated Physical Stability Study[10]

Accelerated storage experiments using centrifugation and thermal stress tests, including freeze-thawing and heating

Table 1: Posaconazole nanoemulsuin composition as W/W%

Formula no.	*Oil type*	*Oil %*	*S.A.A*	*Co.S.A.A*	*S-mix ratio*	*S-mix %*	*DDW*	*Drug %*
F1	IPM	4	labrasol	trascutol-p	1:1	36	59	1
F2	IPM	4	labrasol	trascutol-p	2:1	36	59	1
F3	IPM	4	labrasol	trascutol-p	1:2	36	59	1

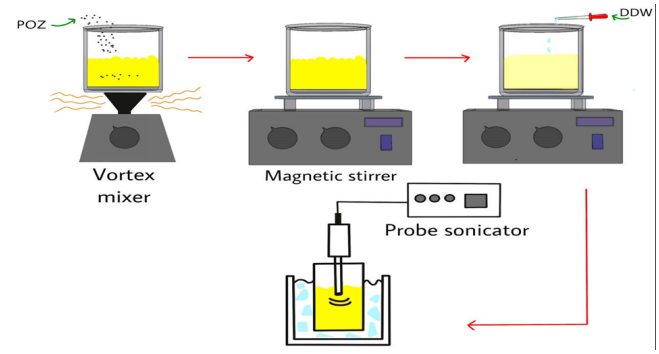

Figure 1: Schematic diagram describing the preparation of Posaconazole nano-emulsion by water titration method

cooling cycles have been done to determine physical stability of nano-emulsions. Formulations overcome these thermodynamic stress tests have been taken for further studies.

- *Centrifugation:* The centrifugation was conducted for 30 minutes at 3500 rpm and the cracking, creaming, phase separation, and precipitation were inspected and heating-cooling cycle test was done for the selected stable formulas.
- *Heating-cooling cycle:* Six cycles were carried out between the refrigerator degree of temperature 4ºC and 45ºC, with storage at every temperature not lower than 48 hours, and the formulations have been examined at those temperature degrees for stability.
- *Freeze-thaw cycle:* For all prepared nano-emulsion formulations, 3 freeze-thaw cycles between -21ºC and +25º C with storage at every one of the temperatures for at least 48 hours were performed.

Light Transmittance Measurement (%T)

This test was done to measure nano-emulsion transperancy. The percent of light transmittance was measured for all prepared nano-emulsions. The measurement was made by using UV- vis spectrophotometer at 650 nm keeping distilled water as a blank.[11]

Zeta Potential Droplet Size and Polydispersity Index Determination

The potential of Zeta for all formulas Using the 'Nano Brook 90Plus-zeta seizer' (Brookhaven Instruments USA) nano-emulsion was measured. The diluted sample of each formula was put in the electrophoretic cell, measured at 25 ± 1°C, and the average values were determined. The determination of the particle size was carried out using the Nano Brook 90Plus particle size analyzer (Brookhaven instruments the USA). This particle size analyzer offers different options. The essential is to determine an average diameter and calculate the polydispersity that is required for many applications. Until measurement nano-emulsion was diluted with 100-fold distilled water and stirred gently (to improve homogeneity).[12]

pH Evaluation

The pH of all prepared nano-emulsions was measured using a digital pH meter.[13]

Estimation of Drug Content

UV spectroscopic approach has measured the amount of POZ in prepared nano-emulsions. The drug content was represented to the theoretical quantity added as a percentage of drugs inside the system. An estimated 1 ml of nano-emulsion was dissolved in methanol and the resulting solution was analyzed at 288 nm in the UV-visible Spectrophotometer.[14]

In-vitro Drug Release Studies

USP type II (Campbell Electronics, India) 300 mL of (pH 7.4) phosphate buffer with 0.5% sodium lauryl sulfate used as a dissolution media at 37°C, dialysis bags (8000–14000D) used as a donor part this dialysis membrane was soaked in phosphate buffer overnight before the experiment and filed with 1 gm of prepared POZ nano-emulsion (10 mg of posaconazole). The release study was done for (F1-F3) in comparison with 1% POZ suspension at 50 rpm, and five mL of dissolution medium was withdrawn at each time interval (0.5, 1, 1.5, 2, 2.5, 3.5, 4, 5,6,7,8 hours) and replaced with 5 mL of a freshly prepared buffer. The measurements were performed in triplicate, and values were the mean ± SD. Spectrophotometrically, the samples obtained were analyzed at λ_{max} 288 nm.

Examination of Selected POZ Nanoemulsion as an Eye Drop

Determination of Osmolarity

The osmolarity was measured using OSMOMAT 030, which can be described as an automatic cryoscopic osmometer, measuring the freezing point depression to determine aqueous solutions' total osmolality.[15]

Permeability Study

The permeation along the corneal membrane was studied using goat corneas. The goats' entire eyeballs from a slaughtering house were procured and carried in normal saline, at 4ºC to the laboratory. The corneas and the 5–6 mm scleral tissues were carefully removed and treated with cold saline. The washed corneas are maintained in a simulated tear fluid (STF) solution of a pH of 7.40 cold, newly prepared with 0.5 % lauryl sodium, at 37°C. In order to make intimate contact with the formulation on the donor compartment, the research was done using Franz-diffusion cells. The diffusion area of the cornea was 0.85 cm^2. At 37°C 0.5ºC, the receptor compartment was filled by the STF pH of 7.40. At 50 rpm, the receptor medium was stirred. At each time interval, the samples were withdrawn and replenished with an equal volume of the STF pH of 7.40. The permeation study lasted 12 hours, and samples were analyzed spectrophotometrically for POZ at 288 nm. The results were expressed as the mean of three experiments plus standard deviation. The amount of drug permeated per unit area through excised cornea (ug/cm2) was plotted against time (h), and the permeation parameters of POZ from selected formulae and POZ suspension were computed. The steady state flux (J) values across excised cornea were calculated using the relationship between the linear slopes of permeation graphs.

$$J = dQ /dt\ A\ (\mu g/cm^2\ hr) \quad (1)$$

The permeation coefficient P was calculated as:

$$P = J / C0 \times 60 \times 60 \text{ (cm/s)} \quad (2)$$

Where C_0 represents the initial concentration of the drug in the donor compartment (μg/cm^3). The values of the x-axis intercept of regression lines were used to determine drug absorption lag times (the time it takes for the medication to saturate cornea and reach receiving compartment).[16]

Irritation Test

In-vivo monitoring of ocular irritation of a POZ selected formula as an eye drop was achieved using a modified Draize test. The New Zealand albino rabbits that weigh 2–3 kg were utilized in a group of 6 adult males. All the rabbits had no abnormalities and were stable. The standard moisture, light, air, temperature, standard food and water conditions were maintained. All experimentations were conducted in compliance with the Guide for Treatment and Use of Lab animals by National institutes of health and authorized by the Animal Ethics Committee of the College of Pharmacy, University of Baghdad, Iraq. To the right eye, each rabbit had 0.5 μL of a chosen formula inserted to lower cul-de-sac of the right eye (this formula has been sterilized through filtration via a .022 μm membrane filter), with the left eye acting as a control. A separated group of two rabbits considers as control received normal saline. With intervals of 0, 1, 2, 3, 4, 5, 6, 24, 48, and 72 hours after installation, every rabbit eye was examined and studied for ocular discomfort parameters like the conjunctival chemosis, discharge and redness. Then, at each time, a score was assigned based upon the observed irritation of every one of the rabbit eyes, which ranges between 0 (no irritation) and 3 (highest irritation), as shown in Table 4. A significant irritant was described as a score of 2 or 3 in any item or a total ocular index of irritation (summation of total clinical assessment scores) of more than 4 over the investigation period.[17]

Microbiological Studies

The biological effect of optimized POZ nano-emulsion formulations was determined by the anti-microbiological studies. Diffusion methods determined the microbial efficiency (well diffusion technique). The norm was pure drug sterile suspension and plain nano-emulsion. In sterile potato dextrose agar medium, previously seeded with test organism *Candida albicans* and *Aspergillus niger*, inoculation plates allowed to spread the solution for 2 hours and incubated at 37ºC for 24–48 hours, 4% POZ suspension, plain nano-emulsion and optimized formulation were inoculated. The formulation's inhibitory zone effect was compared to that of the POZ suspension and plain nano-emulsion.[18]

RESULTS AND DISCUSSION

Constructing Pseudo-ternary Phase Diagram

The goal was to define the current range of nano-emulsions regions. The region of translucent nano-emulsion is seen in any phase diagram. The remainder of the area on phase diagram is based on visual observations of turbid and traditional emulsions. The selected S-Mix ratio was (1:1, 1:2, and 2:1) that helps to study the effect of these ratios on particle's nanosize and polydispersibility index.The ratios of (1:3 and 3:1) were also examined but it seems that there was no remarkable effect of these changes so they were neglected.The results indicated that nano-emulsion area extended with increasing in S-Mix ratio and that is because of increasing in HLB value so leading to formation of O/W nano-emulsion. The results showed that a combination of two non-ionic surfactants with great differences in HLB values of them could yield stable preparations between them. This may be due to the dissolving in the oil process of low HLB surfactants and the dissolution of a high HLB in the water allowing them to work together well enough to have a stronger effect than the surfactant and co-surfactant mixture with closer HLB values.[19]

Preparation of POZ Nano-emulsion

Low water solubility of POZ needs an adequate amount of oil to solubilize the POZ dose (10 mg) in 1-mL nano-emulsion formulations. This is important for the consistency of the formulation and the high permeability of the medication since only the groups of drugs that are present in the formulation in the dissolved form can penetrate. POZ saturated solubility studies in IPM is approximately 213.08 ± 1.09 mg/mL. With a concentration of 4% IPM represent 0.4 mL in 10 mL formulation, so POZ can be solubilized.

Characterization of Prepared POZ Nanoemulsion

Accelerated Physical Stability Study: After completion of the centrifugation procedure, heating-cooling, and freezing–thaw cycles all the prepared nano-emulsions show no signs of instability like that (phase separation, creaming, and cracking)

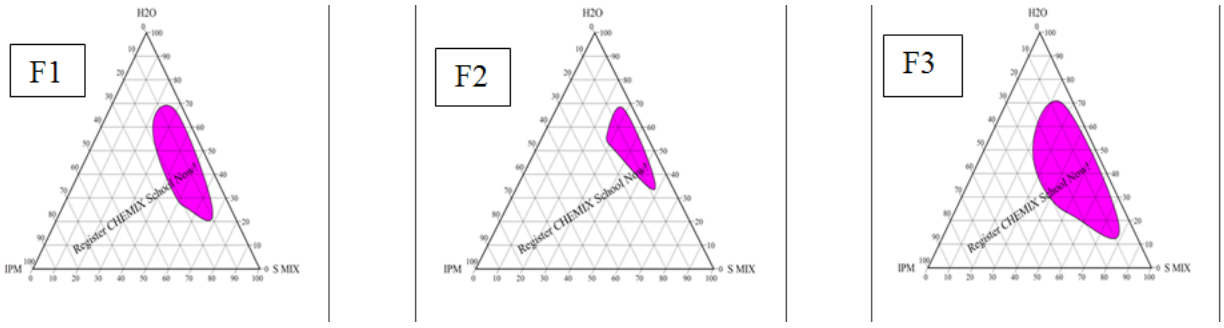

Figure 2: Pseudo ternary phase diagrams used to prepare (F1-F3)

because assessment of long-term stability of prepared nano-emulsions shelf life under environmental storage conditions can be very tedious and time-consuming which is considered uneconomical.

Light Transmittance Measurement (%T): Percentage transmittance values close to 100 percent showed that all formulations were clear, transparent and easy to transmit light. The highest transmittance percentage value (99.21 ± 0.01 percent) was found with (F2) while (F1) was found to have the lowest transmittance percentage value (98.55 ± 0.08%). Their transparency is due to their small size, which is less than 25% of the light's wave length. There is no major difference between all NE formulations in transmittance.

Zeta Potential Droplet Size and Polydispersity Index Determination: The observed zeta potential values of F1, F2 and F3 were listed in Table 2 According to the classical electrical double layer theory, a zeta potential value above ± 30 mV demonstrates moderate repulsion between similarly charged particles, thereby decreasing flocculation or aggregation and potentially stabilizes the dispersion. It was found that F3 formula have a zeta potential ≤ 10 mV (9.38) which indicate instability of this formula. Also from Table 2, we can see that F2 formulas out of three less than 100nm in size, was prepared by using a 2:1 smix ratio which causes in improvement in the co-surfactant molecules are penetrated the surfactant film. This would reduce the fluidity and surface viscosity of the interface film, reduce the curvature radius of the droplets and thereby create transparent systems. The low polydispersibility values observed for all the formulations indicated uniformity of droplet size within each formulation.[20]

pH Evaluation: The pH values of the prepared POZ nano-emulsions were within the appropriate range (6.82 ± 0.02–7.64 ± 0.01) as given in Table 2. The obtained values were also able to maintain drug stability as POZ is stable at a neutral pH; since tears have a large buffering capability, no pH adjustment was made.[21]

Estimation of Drug Content: The drug content of prepared POZ nano-emulsions was in the range of (97.05 ± 0.14z–99.65 ± 0.31 w/v) as shown in Table 2. This high drug content which falls within the range listed by BP and USP indicates the stability of drugs during the preparation or nanoemulsification process which is an important need for good nano-emulsion without any precipitation.[22]

In-vitro Drug Release Studies: It was noticed that the release profile of nano-emulsion was significantly ($p < 0.05$) improved, i.e. the drug release was increased dramatically when prepared as a nano-emulsion. Cumulative percents of drug released were (F2 (92.17) >F1 (85.08)>F3(81.28)) while the prepared POZ suspension release was only 7.3% at the end of 8 hours. The higher nano-emulsion releasing profile may be caused by the smaller particle size of the medicinal products so that the exposed surface area increases to the dissolution medium and the higher drug solubilization potential.[23]

The POZ nanoemulsion formula (F2) which consists of (4%c IPM oil, 36% of (2:1 labrasol:transcutol–p) as s-mix ratio,59% DW and 1% POZ),shows a good drug release (92.17%), drug content equal to (98.05 ± 0.012) and its particle size, PDI and zeta potential was 15.3 nm, 0.154 and 53.72 Mv, respectivly. So it was selected as the optimum nano-emulsion formula to be used as an ocular dosage form, and it undergoes further investigation.

Determination of Osmolarity: The osmolality of this nano-emulsion formula ranged from 298 to 300 mOsm/kg after fixation of F2 tonicity. On waking, to a report on human tear osmotic pressure. When eyes are open, the osmolality can range from 231 mOsm/kg to 446 mOsm/kg due to evaporation. Solutions that have osmolality less than 100 mOsm/kg or over 640 mOsm/kg are irritant depending on the drop size.[24]

Permeability Study: By using Franz-diffusion cells goat corneal membrane the permeation was studied POZ is a medication class II in BCS that is water insoluble and lipid soluble. Its high lipid solubility allows it to pass through the cornea endothelium and epithelium, but its low aqueous solubility prevents it from passing through the hydrophilic stroma.[5]

The analysis of data indicates (Figure 4) that F2 was the most efficient in drug permeation according to the cumulative amount permeated per unit area, steady state flux, and permeability coefficient values. The Jss of F2 and POZ susp were (33.267 ± 0.092 ug/cm^2.h) and (13.733 ± 0.15 ug/cm^2.h), respectively. A 2.5 fold increase in POZ permeability coefficient was obtained in case of F2 ($1.8*10^{-5}$ ± 0.85 cm/s) as compared with permeability coefficient of POZ susp ($0.76*10^{-5}$ ± 0.23 cm/s). Thus, it could be concluded that F2 nano-emulsion were significantly better than POZ susp in enhancing penetration of POZ through eye cornea.

Table 2: values of the zeta potential, particle sizes, and poly-dispersit index of prepared poz nanoemulsions

F. No.	*Zeta potentials (My)*	*Particle sizes (nm)*	*PDIs*	*pH+SD*	*% of drug content*
Fl	23.33	166.5	0.350	7.56 ± 0.05	98.07 ± 0.13
F2	53.72	15.3	0.154	6.86 ± 0.07	98.05 ± 0.12
F3	9.38	324.1	0.306	6.91 ± 0.15	99.83 ± 0.15

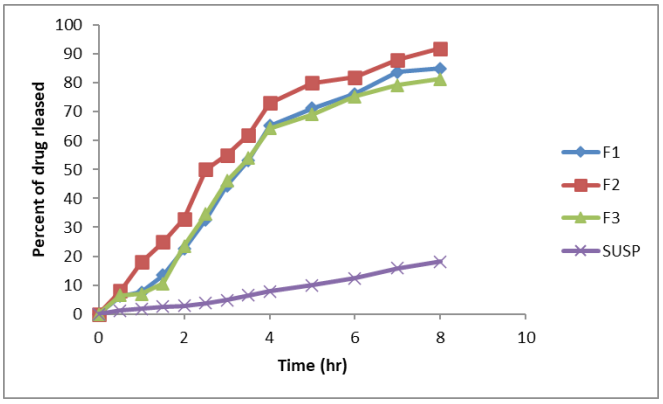

Figure 3: Release profile of six selected formula prepared by IPM as oil in comparasion with 1% POZ suspension

Table 3: Scores of draize irritation test of F32 (Optimum Formula).

	Observation time (hours)							
Parameter to be observed	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Conjunctival discharge	0	0	0	0	0	0	0	0
Conjunctival chemoses	0	0	0	0	0	0	0	0
Conjunctival redness	0.5	0	0	0	0	0	0	0

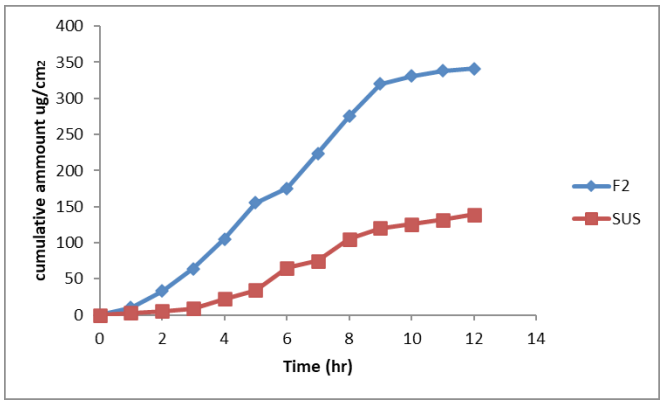

Figure 4: POZ permeation through goat corneas from F2 and POZ suspension.

Irritation Test: To ensure that the eye drop does not irritate the patient's eye and that the rabbits are not aggressive, they are easy to treat, test and observe. Irritation experiments of rabbit eyes are more cost-effective because the animal is readily available, the eyes are identical in size to human eyes, and there is a vast collection of knowledge to compare. At the first moment of instillation, the application of the optimal formulation in the eyes of six rabbits showed mild conjunctival redness in one of the rabbits and no conjunctival discharge or conjunctival chemoses. A discomfort score of F2 in rabbits' eyes did not reach 0.5 at any point during the test as given in table 3 based on modified Draize scores. After 3 hours of instillation of optimum solution, conjunctive redness was observed to decrease and disappear slightly. Based on these findings and given that the eye of the rabbit is more irritable compared with the human eye, the best formulation has been found non-irritant and well tolerable.[25]

Microbiological Studies: In vitro antimycotic inhibitory activity of 1% POZ nano-emulsion (F2), 4% POZ suspension and plain nano-emulsion were examined on the test organism *Candida albicans* and *A. niger* to calculate the zone of inhibition. Table 4 shows the values of the zone of inhibition for the test and the 2 controls in a separate manner. F2 appeared to be radically different from two other controls. The values of the inhibition zone of the test have been compared to the values of the inhibition zone of the 2 controls, and the obtained result indicates that the F2 formula had the maximum inhibition zone. As a result, it is possible to infer that the prepared solution significantly ($p<0.05$) outperformed both controls in terms of antimicrobial activity. This could be as POZ presence in a soluble form that made it easily diffuse to the medium of agar and affect on fungus.[26]

Table 4: Antibacterial Activity of 1% POZ Nanoemulsion (F32),4% POZ Suspension and Plain Nanoemulsion Represented by Zone of Inhibition in (mm)

	Zone of inhibition (mm)		
Tested organisms	*F32*		Plain
C. albicans	13 ± 0.90	5 ± 1.00	4 ± 1.20
Aspergillus niger	22 ± 1.08	4 ± 0.92	3 ± 0.93

CONCLUSION

The conclusion from this work was that a substantial dosage type for water-insoluble drugs was presented by nano-emulsion. Prepared with Capmul Mcm, Cremophor and Transcutol-p, a nano-emulsion formula was promising method to increase dissolution rate and posaconazole solubility. This research can be used as a model approach for the creation of other nano-emulsion drug systems.

The Posaconazole nano-emulsion was effectively formulated by developing pseudoternary phase diagram of different proportions of IPM oil, labrasol, transcutol-P and water. They show transmittance value >98% which proves the transparency of the system and the droplets are in nanometer dimensions. The optimized nano-emulsion (F2) showed adequate anti-fungal effect of the posaconazole and is safe for ocular application and its permeability through the eye cornea was significantly better than POZ suspension. POZ nano-emulsion was found to be non-irritant in an in-vivo irritation test. In a nutshell, the optimized nano-emulsion formulation (F2) could be a promising and viable drug delivery system for effective delivery of posaconazole for treatment of various fungal eye infections.

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