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Formulation, In-Vitro Characterization, And Antifungal Efficacy of Itraconazole Topical Niosomal Gel: Development and Microbiological Evaluation

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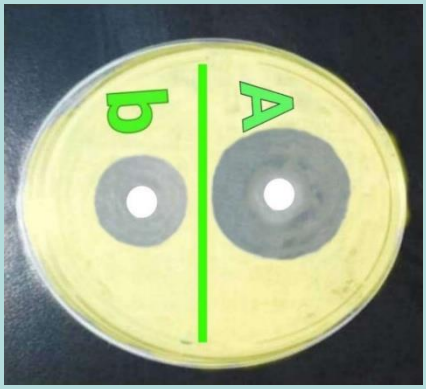
Abstract: **Background:** Itraconazole is an effective antifungal agent; however, improving its formulation as a topical agent is necessary due to its limited skin penetration and duration of action. The present study aims to improve the efficacy of antifungal treatments for skin infections by developing and studying itraconazole (TCZ) in a prolonged-release niosomal gel (NSM-Gel) formulation. **Methods:** TCZ-loaded NSM-Gel was formulated using a thin film hydration technique utilizing various non-ionic surfactants (tweens and spans). The TCZ niosomal gels' different formulae were assessed for pH, entrapment efficiency (EE%), particle size (PS), and polydispersity Index (PDI). Formula (TN4), which was selected as the best formula, was further examined for zeta potential (ZP), in-vitro release, scanning electron microscopy (SEM), physical stability study, and in-vitro antifungal efficacy. **Results:** the EE% for the developed NSM-Gel is satisfactory (86.41 – 98.44%), PS between 4.12 and 8.17 µm, and PDI between 0.22 and 0.39. The release of TCZ from the NSM-Gel provided an appropriate prolonged release effect. TN4 had an elevated EE% with a delayed release profile (100.00% ± 1.25% after 18hrs). The results revealed the existence of vesicles characterized by a spherical morphology. Physical stability studies of formula (TN4) showed good physical characteristics. Furthermore, TN4 demonstrated superior antifungal activity against Candida albicans, as evidenced by a larger inhibition zone than the commercial product Venzole® gel 1% (2.40 vs. 1.50 cm).
Conclusions: This investigation demonstrated the applicability of the NSM-Gel in achieving the expected prolonged release effect for transdermal TCZ administration in healing fungal infections.. (Style=ANU_Abstract)



Keywords: Antifungal drugs, Itraconazole, Niosomal gel, Sustained release action.

Introduction

Invasions caused by fungi manifest as superficial infections in the mucous membranes, nails, and skin. Candidiasis is one of the common forms of superficial fungus infections, and it can spread to deeper tissues in people with compromised immune systems. Intergluteal and underarm regions are commonly affected by candidiasis because they are damp, warm, and wrinkled (1). Topical therapy for fungal infections is recommended over systemic treatment owing to its direct delivery to the affected location, reduced adverse effects, and increased patient compliance (2). The stratum corneum, the outermost part of the skin, is the primary obstacle against drug penetration; enhanced stratum corneum penetration represents one of the main targets for antifungal drug delivery systems (3, 4).

Itraconazole (TCZ) is one of the antifungal medicines that belongs to triazoles first generation. It distinguishes itself from other azole antifungals, like miconazole, by having a triazole ring in its chemical structure rather than an imidazole-containing compound (5). Antifungals containing imidazole are most commonly used topically. On the other hand, systemic treatment involves using TCZ and some triazole antifungals due to their safety and effective oral absorption (6). Triazoles function by interfering with lanosterol 14α-demethylase, a cytochrome P450 enzyme that is important for transforming lanosterol to ergosterol, a component of fungi's cell membrane; when the synthesis of ergosterol is restricted, cellular permeability increases, releasing intracellular components (7).

Topical administration of TCZ is ineffective for treating cutaneous disorders due to inadequate penetration via the skin. Traditional formulations use greater dosages to compensate for their limited permeation. Vesicular drug delivery systems have gained popularity as topical medication carriers because they can cross the barrier formed by the skin (8).

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Niosomes (NSM) is a colloidal drug delivery system that utilizes non-ionic surfactant vesicles. NSMs are similar in structure to liposomes, consisting of a bilayer. Compared to liposomes, the materials utilized to develop NSM decrease the physical stability difficulties of liposomes, like leakage, fusing, and aggregation, while providing additional advantages in transportation, storage, and dosage (9-11). NSM can trap hydrophilic and lipophilic drugs in vesicular membranes while remaining non-ionic (12). Therefore, the current research aims to develop and characterize a prolonged-release niosomal gel (NSM-Gel) formulation of TCZ to enhance the anti-microbial activity for treating skin fungal infections.

Materials and Methods

2.1 Materials

Itraconazole was gifted by Sigma Pharmaceutical Industries, Egypt. Span40, Span60, and Tween80 were bought from Delta Pharma, Egypt. Cholesterol was bought from Sedico, Egypt. Carbopol934 and Triethanolamine was purchased from Penta Pharma, Egypt. Potassium dihydrogen orthophosphate and Disodium hydrogen orthophosphate were purchased from Zeta pharma, Egypt. Chloroform, Glycerol, and Methanol were purchased from Al-Rowad Pharma, Egypt. Analytical grade solvents were utilized and acquired from the El-Nasr factory for pharmaceuticals in Egypt.

2.2 Instruments

Rotary flash evaporator (RV8, IKA, USA), bath sonicator (DT255H, SONOREX, Germany), ultracentrifuge (Optima XPN, Beckman Coulter, California, USA), Syring filter (Millex-HV Syringe, Merck, USA), UV spectrophotometer (V-730, Jasco, USA), Malvern Mastersizer (X ver. 2.15, Malvern Instr., UK), zeta potential analyzer (Nanotrac wave II, Microtrac, Germany) and scanning electron microscope (SEM) (JCM-7000, Jeol, USA).

2.3 Methods

2.3.1 Preparation of itraconazole niosomes

Itraconazole niosomes (TCZ-NSM) were formulated by applying a thin-film hydration technique utilizing a lipid blend consisting of surfactants (tween 80, span 40, and span 60) and cholesterol (CSL) (13), as indicated in Table 1.

Table 1: Preparation of TCZ-NSM different formulae

Ingredients (mg)	Formulae No.								
	TN1	TN2	TN3	TN4	TN5	TN6	TN7	TN8	TN9
Itraconazole	10	10	10	10	10	10	10	10	10
Tween 80	-	-	90	45	45	60	30	60	30
Span 40	-	90	-	-	45	-	-	30	60
Span 60	90	-	-	45	-	30	60	-	-
Cholesterol	10	10	10	10	10	10	10	10	10

CSL, surfactants, and TCZ have been dissolved in 20 ml of chloroform. The obtained lipid mixture was added to a 100 ml glass flask, and the chloroform was completely evaporated under negative pressure at 50 – 60℃ using a rotary flash evaporator (RV8, IKA, USA) until a thin lipid film was formed. The film was hydrated by adding 25 ml of phosphate buffer (7.4 pH). The hydration process was maintained for 1 hr, with the flask undergoing rotational movement at a temperature range of 55 – 65 ℃ within the rotary evaporator. The hydrated TCZ-NSM were sonicated for 15 min by using a bath sonicator (DT255H, SONOREX, Germany) and then centrifuged at 9,000 rpm for 20 min and 25℃ (Optima XPN Ultracentrifuge, Beckman Coulter, California, USA) which resulted in the formation of NSM suspension that contains both entrapped and free TCZ of different sizes. Different formulae were filtered through a 0.45 µm syringe filter (Millex-HV Syringe, Merck, USA) to remove free TCZ and retained NSM entrapped TCZ (14, 15).

2.3.2 Preparation of niosomes entrapped itraconazole gel

Nine distinct formulations (TN1 – TN9) of NSM entrapped TCZ, each containing TCZ at a concentration of 1%w/w, were then incorporated into a gel matrix made of 100 mg carbopol934, 200 mg glycerol, triethanolamine (in adequate quantity), and distilled water (DW) to make a total weight of 15 g (16, 17).

2.3.3 Evaluation of gel different formulae

Organoleptic characteristics

The NSM-Gel different formulae were assessed for their appearance, homogeneity, and color through visual examination.

pH measurement

The pH of the NSM-Gel different formulae was measured by an electronic pH meter (PHS-3C, Labohub, China). A 0.1g of each gel formula was solubilized in 10ml of DW. The electrode was immersed in the gel formulation, and the results were recorded (18). The result was an average of three recorded readings.

Determination of itraconazole entrapment efficiency (EE%)

The EE% of different formulations were determined by taking 0.2g of each formula in a glass test tube diluted with 15ml phosphate buffer at a pH of 7.4. The aqueous slurry was subjected to sonication using a bath sonicator at 10,000rpm for 20min. The supernatant was taken and analyzed using a UV spectrophotometer (V-730, Jasco, USA) to determine the free amount of itraconazole at a wavelength of 262 nm (19). The following equation determined Itraconazole EE%:

$$EE\% = \frac{\text{The total amount of TCZ} - \text{free amount of TCZ}}{\text{Total amount of TCZ}}\ x\ 100$$

2.3.4 In-vitro release experiment

The membrane diffusion technique assessed the release of TCZ from NSM-Gel different formulae and Venzole® gel 1%

(Moderik Healthcare, India) as a commercial product. The niosomal gel equivalent to 12.5 mg of TCZ was installed in a donor compartment consisting of a glass tube 10 cm long and 2.5 cm in diameter. The tube had previously been sealed with soaking cellulose membrane with a molecular weight cut-off of 12,000 Daltons. The glass tube was immersed in a beaker that contained phosphate buffer (100 ml, 5.5 pH), which served as the receptor compartment. The bottom portion of the gel tube only touched the diffusion medium's surface (1-2 mm deep). The receptor medium was kept at 37±1°C and stirred at 100 rpm with a magnetic stirrer; 3ml aliquots were taken away and refilled with equal volume at regular intervals to keep the receptor's phase volume constant. The gathered samples were examined using a UV spectrophotometer to detect the amount of TCZ at a wavelength of 262 nm (19).

2.3.5 Particle size analysis of TCZ-NSM

The PS of TCZ-NSM and Polydispersity Index (PDI) were determined using Malvern Mastersizer (X ver.2.15, Malvern Instr., UK). Before measurements, the NSM-Gel formulations were diluted with 10ml DW. The PDI was calculated as an indicator of homogeneity. If the PDI value is around 0.1, the sample is homogenous; if it is close to 0.5, it is heterogeneous (20).

2.3.6 Zeta potential (ZP) analysis

Charge on the TCZ-NSM vesicle surface was detected by using a ZP analyzer (Nanotrac wave II, Microtrac, Germany). Formula (TN4) was diluted with 10ml DW before measurement, and the result was repeated in triplicate (21).

2.3.7 Scanning electron microscope (SEM)

A scanning electron microscope (SEM) (JCM-7000, Jeol, USA) was used to examine the surface characteristics of TN4 (22).

2.3.8 Physical Stability Studies

The stability of the chosen TCZ niosomal gel formula (TN4) was assessed by storing it in tightly sealed glass containers in a refrigerator at a temperature of 4±1°C for six months. The stability study was conducted based on ZP, PS, EE%, and physical appearance; their variations during storage were recorded (23).

2.3.9 In-vitro antifungal activity

In-vitro antifungal activity of the chosen TCZ niosomal gel formula (TN4) was tested against Venzole® gel 1% (as positive control). The antifungal activity was tested by agar diffusion technique (24). This technique utilized Candida albicans (0.1%) culture on Sabouraud dextrose media. The strain was placed in a sterile NaCl (0.85%) solution at a ratio of 1:9. The media was diluted to 100 CFU/ml using sterile NaCl (0.85%) solution. An aseptic swab has been immersed in the culture dispersion media and positioned on the periphery of the agar petri dish, moving to the opposite side. Cups were fabricated within the implanted agar plates with a diameter of 6 mm (25). The cups were then infused with 0.5ml of the tested NSM-Gel (1 gm of TN4 was solubilized in 100 ml water for injection) and an equal quantity of the standard commercial gel. Subsequently, the Petri plates were placed in an incubator set at a temperature of 37°C. The antifungal efficacy of the formulated NSM-Gel was compared to the standard commercial formula (26). The extent of growth inhibition was evaluated for standard and tested formulations. Every group of samples was performed three times. Following 48 h, the centimeter-measured growth inhibition zone of Candida albicans was calculated (27).

2.4 Statistical analysis

Continuous data was presented using mean ± standard deviation; the results were analyzed using one-way ANOVA with post hoc Tukey test for pair-wise comparison. P-values ≤0.05 were regarded as significant.

Results and Discussion

3.1 Evaluation of gel different formulae

3.1.1 Organoleptic Characteristics and pH

All the formulated gels were homogenous and creamy white. Most formulae (TN4, TN5, TN7, TN8, and TN9) had pH suitable for topical application (4.0 – 6.0) (28), as indicated in Table 2. The reference product Venzole® gel 1% (Moderik Healthcare, India) was white homogenous with pH 6.1.

Table 2: Characterization of different formulae*

Formula No.	pH	EE%	PS (µm)	PDI
TN1	6.30	94.63 ± 1.02	4.12 ± 1.08	0.35 ± 0.031
TN2	6.70	92.30 ± 1.43	7.13 ± 1.25	0.22 ± 0.025
TN3	6.40	88.34 ± 1.47	8.17 ± 0.16	0.39 ± 0.043
TN4	5.90	98.44 ± 1.32	5.00 ± 1.23	0.28 ± 0.036
TN5	5.70	90.23 ± 1.24	6.76 ± 1.12	0.33 ± 0.025
TN6	6.80	91.74 ± 0.87	5.88 ± 1.47	0.31 ± 0.012
TN7	5.90	93.72 ± 1.44	4.65 ± 0.26	0.24 ± 0.022
TN8	5.30	86.41 ± 1.06	5.34 ± 0.15	0.34 ± 0.011
TN9	5.60	90.55 ± 1.22	7.19 ± 0.24	0.32 ± 0.014

*Data are mean ± SD, n=3

3.1.2 Entrapment efficiency of different gel formulations

The EE% of TCZ in different NSM-Gel formulations are shown in Table 2. The highest EE% is seen in both TN4 and TN1 (98.44% and 94.63%, respectively) ; both were formulated using tween80 and span60, in which TN4 had a 1:1 ratio of tween80 and span60, while TN1 contained only span60. Relatively speaking, formulations comprising span60 (TN1, TN 4, TN 6, and TN 7) had higher EE% than formulations comprising tween80 and span40. The formulation comprising tween80 only (TN 3) showed the least EE%.

Several facts explain these findings: to achieve NSM with low leakage and high EE%, for most systems, a hydration temperature greater than the gel-to-liquid phase transition

temperature is necessary (29); span60 has the highest phase transition temperature (50°C) and EE% compared to span40 and tween80 (30). The increased length of the alkyl chain directly affects the surfactants' hydrophilic-lipophilic balance (HLB) value, directly impacting the drug EE% (31). The drug EE% and stability of NSM generated using span60 will increase when the HLB of the surfactant decreases (32). This phenomenon can also be ascribed to the surfactants' alignment, arrangement, and compactness. Among the used surfactants, Span60 exhibits the largest saturated chain and the greatest EE% (33).

3.2 Zeta potential and particle size findings

Formula (TN4) composed of TCZ, CSL, tween80, and span60 (1:1) was diluted with 10ml DW before ZP measurement with -33.2mV ZP. Owodeha-Ashaka et al. found that colloidal particles were electrically stabilized and exhibited less flocculation or aggregation when the ZP value was high (>30mV) (34). According to Uchegbu and Florence, the vesicles prepared from non-ionic surfactants have a negatively charged surface because hydroxyl ions are adsorbed on their surface (35). The current study results demonstrated that the TCZ exhibited excellent physical stability when incorporated into the formed NSM-Gel, evidenced by the increase in the absolute value of ZP, which raised the repulsion between the vesicles; this prevented their reaggregation and ensured the electrical stability of NSM (21).

As shown in Table 2, the PS of all the formulated NSM-Gel was within the micro size range (4.12 to 8.17 µm). Formula containing span60 only has the lowest PS (TN1) while TN3, which contains solely tween80, had the largest PS, of more as the proportion of span60 grew in the formulations (TN1, TN7, TN4, and TN6, from highest to lowest), the size of the vesicles reduced (4.12, 4.65, 5.00, and 5.88 µm). According to Junyaprasert et al., spans have a higher degree of hydrophobicity, which means they have a lower surface free energy. As a result, they produce smaller vesicles. On the other hand, tweens are hydrophilic, meaning they have an affinity for water; this leads to a rise in water absorption by these bilayers, resulting in larger NSM (36).

3.3 In-vitro release of TCZ from niosomal gel formulations

Figure 1 demonstrated that the rate at which TCZ is released from NSM-Gel formulated with span40 (TN2) was slower than NSM-Gel formulated with span60 (TN1). However, NSM formulated with a combination of tween80 and span60 exhibited a greater reduction in the release of TCZ.

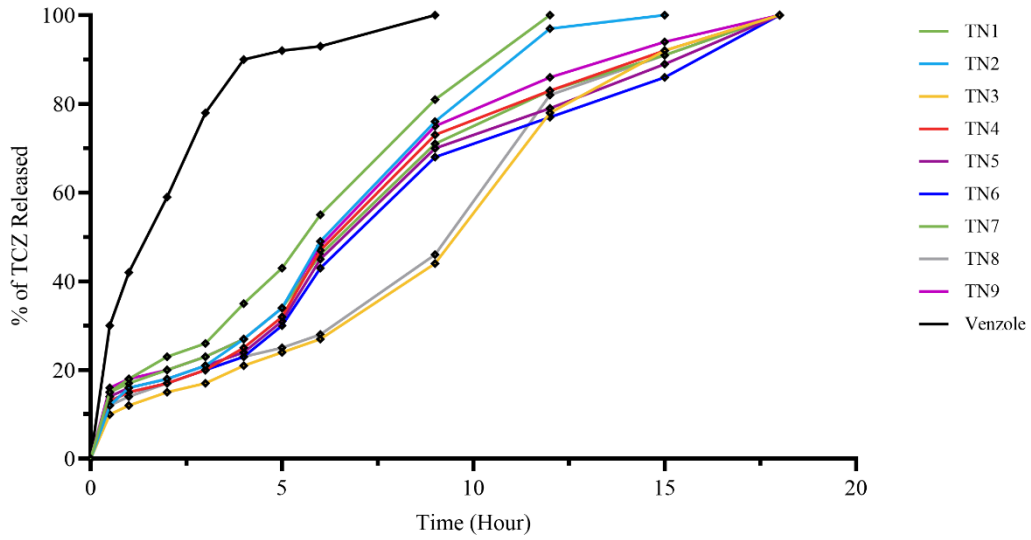

Figure 1: In-vitro release of TCZ niosomal gel different formulations and Venzole® gel 1%.

The NSM-Gel formula, comprising only tween80 (TN3), exhibited the lowest release of TCZ, which the hydrophilic properties of the surfactant can explain. The NSM-Gel formulation (TN4), formulated by using a combination of tween80 and span60 (1:1) and CSL, had the maximum EE% and demonstrated a significant *in-vitro* sustained release of TCZ at a significance level of P ≤0.05. Consequently, it was selected as the best formula.

The delayed release of TCZ from NSM-Gel different formulae, compared to Venzole® gel 1%, could be attributed to the encapsulation of TCZ into vesicles, which allows for a prolonged rate of TCZ release. The NSM-Gel (TN4) chosen as the best formula exhibited a prolonged release of the TCZ at a significance level of P <0.001 compared to Venzole® gel 1%. After 6hr, the NSM-Gel released 40.52±1.25% of the TCZ, whereas Venzole® gel 1% released 99.4±4.22% of TCZ after 4hr, as shown in Figure 1. This phenomenon may be attributed to the compact vesicular arrangement consisting of tween80, span60, and CSL, which is a significant obstacle to the diffusion of TCZ and delays its release. Palak et al. concluded that the prolonged release of NSM-Gel different formulae is caused by the gradual release of medicine from NSM. This delay is likely due to the time required for NSM to become hydrated and form niosomal vesicles before the TCZ can cross the cellophane membrane (37).

Consequently, throughout this time, the formulation showed zero-order release; this was explained by Nagalakshmi et al., who found that at *in-vitro* permeation conditions of 25°C, the formulae that contain spans form an organized gel (38).

3.4 Morphological characterization and scanning electron microscope

Further examination of surface characteristics of formulation (TN4) by SEM showed spherical vesicles with smooth surfaces. The addition of CSL into NSM-Gel leads to a variation in size. The vesicles exhibited distinct and independent characteristics,

with no signs of clumping or clustering, as illustrated by Figure 2.

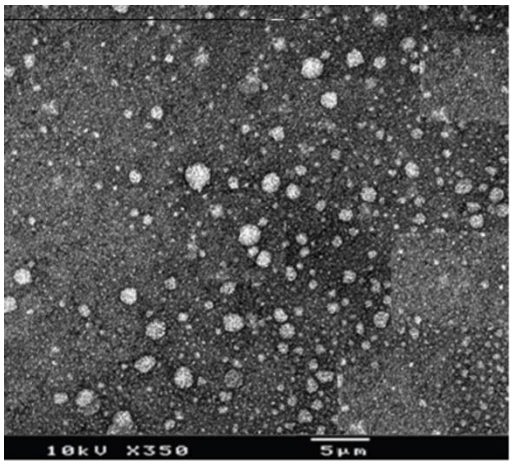

Figure 2: SEM image of TN4 formulation.

3.5 Physical stability studies

The chosen formulation (TN4) underwent stability testing in the current study. The sample was kept at a temperature of 4±1°C for six months. PS, EE%, and ZP changes were monitored and recorded during this time. No changes were noticed in the physical appearance, formulation uniformity, or vesicle clustering, as shown in Table 3. The results of our research aligned with the work conducted by Javani et al., which elucidated the impact of span60-based niosomal formulation's elevated phase transition temperature and diminished permeability on its EE% and size change (39). The outcome demonstrated the excellent stability and appropriateness of NSM-Gel for delivering TCZ topically.

Table 3: Stability studies of selected TCZ niosomal gel (TN4) at 4±1°C *

Time	PS (µm)	EE%	ZP (Mv)
Freshly prepared	5.00 ± 1.23	98.44 ± 1.32	-33.20 ± 2.34
One month	5.23 ± 0.66	98.12 ± 2.43	-32.87 ±2.64
Three months	6.43 ± 1.02	96.22 ± 1.89	-30.89 ± 2.78
Six months	7.87 ± 1.78	95.45 ± 2.21	-29.88 ± 1.65

*Data are mean ± SD, n=3

3.6 In-vitro antifungal activity

The *in-vitro* antifungal activity of TCZ niosomal gel was tested using the agar diffusion technique (40). The antifungal study was performed for (TN4) formulation compared with the commercial product Venzole® gel 1%. The diameter of the inhibition zone (IZ) is shown in Figure 3, and the study was repeated in triplicate.

Formulation TN4 (A) exhibited an IZ measuring 2.40cm, whereas Venzole® gel 1% (B) yielded an IZ of 1.50cm after 48h. The notable rise in IZ at ($P \leq 0.05$) of TN4 is that IZ is primarily influenced by the dissolution and diffusion of TCZ via the agar media. TCZ exerts its antifungal activity against *C. albicans* by preventing the cytochrome P450-dependent enzyme lanosterol demethylase, which is necessary for transforming lanosterol to ergosterol (41). The findings of IZ showed that the NSM-Gel (TN4) was more effective and had higher antifungal activity compared to Venzole® gel 1% after 48 h. The antifungal activity is produced by the TCZ that is incorporated into the NSM-Gel, and the NSM-Gel itself serves as a carrier for the transportation of the TCZ to the affected area.

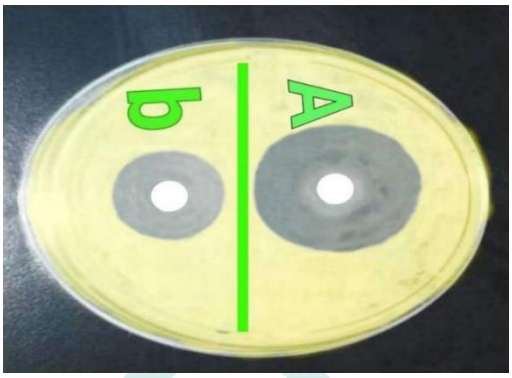

Figure 3: Inhibition zone for formulation TN4 (A) and Venzole ® gel 1% (B) after 48h.

4 Conclusion

Different formulae for itraconazole-loaded NSM-Gel were prepared using a thin film hydration technique. The formulated NSM-Gel different formulae exhibited an EE% ranging from 86.41 to 98.44%, and PS varied from 4.12 to 8.17 µm. The chosen best formula, TN4, determined by PS, EE%, and in-vitro release study, was discovered to have a ZP of -33.20 mV. This ZP value indicates the physical stability of TN4. Photomicrographs of formula (TN4) revealed the formation of spherical niosomal vesicles. It was shown that formulation (TN4) exhibits more elevated antifungal activity compared to Venzole® gel 1%, confirmed by the diameter of the inhibition zone of both formulations after 48 h. of study. It was shown that formula (TN4), composed of a blend of tween 80 and span 60 (1:1) as a surfactant mixture, is the best blend for formulating NSM-Gel with sustained release action. The in-vitro release studies have demonstrated that the formulated NSM-Gel (TN4) is an effective topical drug delivery system that provides a prolonged release of the encapsulated TCZ. Upcoming in-vivo studies can further support this finding. After conducting the stability study for six months, the TCZ niosomal gel (TN4) showed no significant changes in PS, ZP, or EE%.

Ethics approval and consent to participate

"Not applicable"

Consent for publication

"Not applicable"

Availability of data and materials

"The raw data required to reproduce these findings are available in the body and illustrations of this manuscript."

Author's contribution

The authors confirm their contributions to the paper as follows: study conception and design: Kaoud RM, Khalid AA, Ghazi YAF, Talip AM, Fawzi HA; theoretical calculations and modeling: Kaoud RM, Fawzi HA; data analysis and validation: Kaoud RM, Fawzi HA; draft manuscript preparation: Kaoud RM, Khalid AA, Ghazi YAF, Talip AM, Fawzi HA. All authors reviewed the results and approved the final version of the manuscript.

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Conflicts of interest

"The authors declare that there is no conflict of interest regarding the publication of this article."

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