Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 2, 2024, pp. 823-835.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

BULL. PHARM. SCI. Assiut Univ.

PREPARATION AND CHARACTERIZATION OF SOLID LIPID NANOPARTICLES PHOSPHOLIPID COMPLEX LOADED WITH FENTICONAZOLE NITRATE FOR TOPICAL MANAGEMENT OF *CANDIDA ALBICANS*

Sarmad dheyaa noori[1*], Hussein hamed hayal[2], Hayder A. Hammoodi[3], Amira amin[2,4]

[1]*Department of Pharmaceutics College of Pharmacy - Al-Ayen Iraqi University*
[2] *Department of Pharmacognosy, College of Pharmacy, Al-Ayen Iraqi University*
[3]*Department of Pharmacy, Mazaya University College, Thi-Qar, Iraq*
[4]*Department of Clinical Pharmacy and Pharmacy Practice. Faculty of Pharmacy. Damnhour University*

The current investigation aimed for loading Fenticonazole nitrate (FTN), an antifungal agent with low aqueous solubility, into solid lipid nanoparticles (SLNs) phospholipid complex for management of candida albicans topically. Fenticonazole nitrate is an antifungal medication which has substantial first pass metabolism results in low oral bioavailability. In order to prevent oral complications, the production of SLNs-phospholipid complex for improving Fenticonazole nitrate topical distribution was the focus of this study. By using a $2^1.3^1$ full factorial design and different solid lipids and surfactants, SLNs formulas were created. SLNs phospholipid complex were fabricated applying thin film hydration method. SLNs phospholipid complex were evaluated with regard to entrapment efficiency percent (EE%), particle size (PS), polydispersity index (PDI), and zeta potential (ZP). Further explorations were conducted on the optimum formulation. Utilizing Design Expert® software, the optimum formula (F2) was determined, revealing ZP of -23.50±1.11 mV, PDI of 0.41±0.005, PS of 137.05±0.45nm, and EE% of 77.44±0.94%. Further, the optimum formula showed spherical SLNs without aggregation under transmission electron microscope evaluation. In-vitro release study showed that the optimum formula was released more rapidly than FTN suspension. In addition, during storage the optimum formula was stable. The histological investigation verified the safety of the optimum SLNs

Keywords:*Fenticonazole nitrate; candida albicans; factorial design; solid lipid nanoparticles; histopathological study*

INTRODUCTION

Recently, there has been an increase in the global spread of skin infections caused by various fungal species, including *Trichophyton species* and *Candida albicans*. The rise in immunocompromised individuals from organ transplants, cancer chemotherapy, and human immunodeficiency virus infections has led to an increase in the frequency of fungal infections.[1] Antifungal medicines are mostly treated by topical application or systemic oral administration. While systemic antifungal medications administered orally are proven to be more effective, doing so typically carries a higher risk of drug-drug interactions and unpleasant side effects.[2] An antifungal imidazole derivative called Fenticonazole nitrate (FTN) works by preventing the formation of ergosterol and subsequently causing damage to the cytoplasmic membrane.[3] Additionally, it inhibits the particular release of protease acid by *Candida albicans*, which facilitates the yeast's adhesion to epithelial

Received : 21/8/2024 & Accepted : 14/10/2024

*Corresponding author: Sarmad dheyaa noori, E-mail: sarmaddheyaa@gmail.com

cells, as well as cytochrome oxidases and peroxidases.[1] FTN therefore has fungicidal and fungistatic effects on yeasts, fungi, and dermatophytes. Azole (such as FTN) exhibits both fungistatic and fungicidal effect as it acts as fungistatic at low concentration as they interfere the ergosterol formation, and fungicidal at high concentration resulted from the complete destruction of cell wall.[4] Furthermore, FTN has broad-spectrum antibacterial activity against Gram positive bacteria as well as bacteria that are frequently linked to vaginal infections and fungal skin infections. Consequently, FTN is regarded as the best topical treatment for combined bacterial and mycotic infections, as opposed to other multiagent regimens. FTN has been studied in previous research studies using several nanocarriers for topical management of *tinea corporis*[5] and *candida albicans*.[6]

Regrettably, FTN's poor solubility in water (≤0.1 mg/ml) necessitates the development of a novel vesicular delivery technology in order to efficiently administer FTN and force the treatment of fungal diseases.[5] Hydrophobic drug-loading solid lipid nanoparticles (SLNs) have been regarded as an appealing colloidal drug delivery vehicle. This is due to the potential to combine the benefits of other established colloidal carriers, including liposomes, fat emulsions, microemulsions, and nanoparticles.[7] In order to enhance drug absorption and distribution, encapsulating medications in SLNs will not only guarantee their controlled release but also shield them from enzymatic degradations.[8]

However, during storage, medicines with high crystalline structures exhibited a propensity to recrystallize from their amorphous state in the SLN, which could cause instability in the SLN in suspension form. Therefore, earlier research encouraged the addition of phospholipid to SLNs to create an SLNs-phospholipid complex, which inhibits the tendency of cooled melt to re-crystallize even at refrigerator temperatures.[9] Phospholipid complex-loaded nanoparticles have been shown in the literature to enhance the oral bioavailability of medications through a variety of mechanisms, including improving the solubility, dissolution, permeability, and absorption of medications from the gastrointestinal tract by examining the intestinal lymphatic pathways and avoiding their first pass metabolism.[10]

Thus, the goals of the present investigation were to evaluate the safety of SLNs-phospholipid complex applied topically as well as the possibility that it might raise Fenticonazole nitrate for topical use. In order to do that, many factors affecting the features of SLNs-phospholipid complex were investigated using full factorial $2^1.3^1$ design using Design Expert® software to determine the optimum formulation. Entrapment efficiency percentage (Y_1), particle size (Y_2), and polydispersity index (Y_3) were chosen as dependent factors, and solid lipid type (X_1) and SAA type (X_2) were examined as independent variables. The optimum SLNs-phospholipid complex was further assessed in terms of stability and shape. Furthermore, histopathology studies of Fenticonazole nitrate from the optimum SLNs-phospholipid complex were conducted using male Wistar rats.

MATERIALS AND

Fenticonazole nitrate (FTN), phospholipid from soya bean were purchased from Sigma Aldrich Chemical Co. (St. Louis, USA). Glyceryl dibehenate, and Geleol were gifted from gattefosse, France. Tween 20 (T20), Tween 80 (T80), Span 60 (S60), chloroform, and methanol were obtained from El-Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt).
Methods

Preparation of Fenticonazole nitrate loaded SLNs-phospholipid complex

By applying the thin film hydration method, three surfactants (100 mg) Tween 20, Tween 80, and Span 60 and different kinds of solid lipids (200 mg) Glyceryl dibehenate and Geleol were combined to create the SLNs-phospholipid complex. In a long-necked, round-bottom flask, the phospholipid (100 mg), surfactants, and solid lipids were first dissolved in 10 milliliters of chloroform together with 10 mg of fenticonazole nitrate. Using a rotatory evaporator, the organic phase was gradually evaporated at 60°C to produce a thin, transparent layer of particles (Rotavapor, Heidolph VV 2000, Burladingen, Germany) at 90 rpm while keeping pressure under vacuum for 30 minutes. For 45 minutes, the film was

hydrated with 10 mL of hydration media (bi-distilled water). To obtain mature vesicles, the vesicles dispersion was kept overnight at 4°C.[11]

Characterization and optimization of Fenticonazole nitrate loaded SLNs-phospholipid complex

Determination of entrapment efficiency percentage (EE%)

The SLNs-phospholipid complex dispersion for the developed formulations was centrifuged at 20,000 rpm for 1 hour at 4°C using a cooling centrifuge (Sigma 3K 30, Germany). The pellet was then lysed in methanol and assessed at λ_{max} 252 nm using a UV-Vis spectrophotometer (Shimadzu UV1650 Spectrophotometer, Koyoto, Japan).[5] EE% was found using the direct technique. [12]

Determination of particle size (PS), polydispersity index (PDI) and zeta potential (ZP)

Using a Malvern Zetasizer 2000 (Malvern Instruments Ltd., UK), the mean PS, PDI, and ZP of the SLNs-phospholipid complex's dispersions were estimated for the generated formulae. Following dilution, the measurements were carried out. By monitoring the particles' electrophoretic movement in the electrical field, the ZP assessment was accomplished. Every measurement was done three times.[13]

Assessment of the influence of different formulation parameters using $2^1.3^1$ full factorial design

Applying lowest experimental runs, a complete $2^1.3^1$ full factorial design was employed to determine the impact of several factors on the aspects of Fenticonazole nitrate loaded SLNs-phospholipid complex dispersions.[14] Two factors were assessed in the chosen design: one had two levels (X_1: solid lipid type), and the other had three levels (X_2: SAA type). As dependent variables, the EE% (Y_1), PS (Y_2), and PDI (Y_3), were identified **(Table 1)**. To create Fenticonazole nitrate loaded SLNs-phospholipid complex, all probable combinations were tested in the experiments **(Table 1)**. The experimental data were analyzed using Design Expert® software version 11 (Stat Ease, Inc., Minneapolis, Minnesota, USA) to independently source the impacts of these components, and then analysis of variance (ANOVA) was used to assess the significance.

Optimization of Fenticonazole nitrate loaded SLNs-phospholipid complex

To determine which formulation should be selected for further investigation, the desirability function was established. This function predicts the optimal levels of selected components. Selecting the optimal formulation required meeting specific criteria, including achieving the lowest PS and PDI and the maximum EE%.

Table 1: $2^1.3^1$ Full factorial design for optimization of Fenticonazole nitrate loaded SLNs-phospholipid complex.

Factors (independent variables)	**Levels**		
X_1: Solid lipid type	Glyceryl dibehante		Geleol
X_2: SAA type	T20	T80	S60
Responses (dependent variables)	**Constraints**		
Y_1: EE (%)	Maximize		
Y_2: PS (nm)	Minimize		
Y_3: PDI	Minimize		

Abbreviations: EE%; entrapment efficiency percent, PS; particle size, PDI; polydispersity index, T20; tween 20, T80; tween 80, SAA, surfactant, and S60, Span 60.

Determination of amount of drug release

The amount of medication released was measured for six hours at 37°C using the USP dissolution tester equipment II. The optimal SLNs-phospholipid complex of (2 mL samples) was added into plastic cylindrical as donor compartment and sealed with cellulose membrane with surface area of 3.14 cm^2, that contained 5 mg of FTN. The formulations were immersed in 50 milliliters of pH 5.5 phosphate buffer release medium.[15] In this volume, the sink state was preserved. At 1, 2, 3, 4, 5, and 6 hours, aliquots were removed. A UV spectrophotometer with a λ_{max} of 252 nm^5 was used to evaluate aliquots (1 ml) of Fenticonazole nitrate. Three experiments were carried out.

Transmission electron microscopy (TEM)

Using a Joel JEM 1230 transmission electron microscope (Tokyo, Japan), the optimal SLNs-phospholipid complex's morphology was examined. A thin layer of the nanodispersion was applied to a copper grid covered with carbon, dyed with 1.5% phosphotungstic acid, then observed, and photographed.[16]

Stability studies

For forty-five days, the ideal SLNs-phospholipid complex was kept at 4°C. At 0 and 45 days, samples from each formulation were taken out. Comparing the initial measurements with the values obtained after storage allowed for the evaluation of stability. As previously mentioned, measurements of the EE%, PS, PDI, ZP, and Q6h (%) from the SLNs-phospholipid complex were made. Statistical significance was analyzed by Student's t-test using SPSS® software 22.0. Difference at $p \leq 0.05$ was considered significant.[17]

pH assessment

The pH of the optimum SLNs-phospholipid complex was estimated, by a calibrated pH meter (Hanna, type 211, Romania).

Histopathological study

The study design was authorized by the ethical committee of the Mazaya University College, (reference number = (PI) 150). Six animals were divided into two groups where groups one behaved as control left untreated, while group two was treated with the optimum SLNs-phospholipid complex. The treatment duration lasted one day. After being fixed for 24 hours in 10% formol saline, skin samples were cleaned, and alcohol was used to dehydrate them. Following a 24-hours period at 56°C, the specimens were cleaned in xylene, embedded in paraffin wax blocks, and sectioned using a sledge microtome (Rotary Leica RM2245, USA) at a thickness of 4 mm for each skin sample. Using light microscopy, the specimens were deparaffinized and stained with hematoxylin and eosin stains for histological analysis. (Axiostar plus, Zeiss, New York, NY).[18]

RESULTS AND DISCUSSION

Results

Analysis of factorial design

Two independent variables were investigated: the solid lipid type (X_1) and SAA type (X_2); dependent variables included EE% (Y_1), PS (Y_2), and PDI (Y_3). The experimental results were evaluated using Design Expert® (Stat Ease, Inc., Minneapolis, MN) version 11 to source independently the main effects of these factors, which were then evaluated employing analysis of variance (ANOVA) to decide the significance of each factor. The two-factor interaction (2 FI) model was utilized, and it was seen that the predicted R^2 values were in reasonable agreement with the adjusted R^2 in all responses, with reference to the design analysis results in **Table 2**, all responses showed adequate precision with a ratio greater than 4.

The effect of formulation variables on EE%

It is possible to create multilayered nanosystems into an efficient drug delivery system for the targeted or controlled release of lipophilic medicines. Encapsulating bioactive inside phospholipid containing formulations offers the best distribution, enhanced stability, protection, and permeability, depending upon the lipid composition and characteristics.[19]

The effect of the independent variables, solid lipid type (X_1) and SAA type (X_2) on the EE% of Fenticonazole nitrate loaded SLNs-phospholipid complex is shown in **Table 2,3** and is graphically illustrated as 3-D surface

plots in **Fig. 1A-B**. EE% ranged from 65.35±0.46 to 81.66±0.83%.

The polynomial equation for EE% is: EE%=+73.44-4.62* A+6.00* B [1] +1.73* B[2] +2.39*AB[1]+2.34*AB[2]

It is observed that solid lipid type (X_1) had a significant effect on EE% ($p<0.0001$). In comparison to Geleol, Glyceryl dibehnate was found to produce greater EE% values. The previous results could be correlated to the fact that glyceryl dibehenate is a mixture of 13%–21% monoacylglycerols, 40%–60% diacylglycerols, and 21%–35% triacylglycerols of behenic acid. The mono- and diglycerides of glyceryl behenate possess surface active properties (Hydrophilic-Lipophilic Balance (HLB) = 2–5).[20] Hence imparts an amphiphilic character to the utilized system and enhanced Fenticonazale nitrate solubilization and entrapment inside the nano-system, the previous results agreed with previous literature.[21]

It is important to note that a high drug solubility in the lipid melt is a requirement for obtaining adequate drug loading. Following the cooling of the lipid melt, solubility often declines and may even be lower in the solid lipid. Drug solubilization is facilitated by the presence of mono and diglycerides in the lipid utilized as the matrix material. In addition, the chemical feature of the lipid is also significant as lipids that form highly crystalline particles with a perfect lattice led to drug expulsion. Lipids that are mixtures of mono-, di-, and triglycerides and lipids (such as Glyceryl dibehnate) composing of fatty acids of different chain lengths form fewer perfect crystals with various imperfections, offering space to load the drugs.[22] Further, the perfect structure of glyceryl monostearate would lead to drug expulsion.[23]

Considering SAA type (X_2), it has a significant effect on EE%, it was found that EE% increased by using more hydrophilic SAA in the subsequent order: Tween20>Tween80>Span 60 as the HLB values for the utilized SAA were 16.7, 15, and 4.6, respectively.[15] The aforementioned findings could be correlated to that high HLB of SAA improved the solubilization of Fenticonazole nitrate inside SLNs, hence improved EE% of the formed SLNs.[24]

Table 2: Output data of the $2^1.3^1$ full factorial analysis of SLNs-phospholipid complex formulations and predicted and observed values for the optimum SLNs-phospholipid complex (F2).

Responses	**EE%**	**PS (nm)**	**PDI**
Adjusted R^2	0.924	0.969	0.920
Predicted R^2	0.835	0.943	0.853
Adequate precision	15.08	16.94	10.59
Significant factors	X_1, X_2	X_1, X_2	X_1
Predicted value of optimum formula (F2)	77.44	137.05	0.410
Observed value of optimum formula (F2)	77.44	137.05	0.410

Abbreviations: EE%, entrapment efficiency percentage; PS, particle size; and PDI, polydispersity index.

Table 3: Experimental runs, independent variables, and measured response of the $2^1.3^1$ full factorial experimental design of SLNs-phospholipid complex.

	Solid Lipid type	**SAA type**	**EE%**	**PS (nm)**	**PDI**	**ZP (mV)**
F1	Glyceryl dibehnate	T20	81.66±0.833	225.10±3.33	0.273±0.01	-23.10±1.61
F2	Glyceryl dibehnate	T80	77.44±0.94	137.05±0.45	0.410±0.005	-23.50±1.11
F3	Glyceryl dibehnate	S60	75.06±2.56	121.85±5.05	0.393±0.009	-24.75±0.25
F4	Geleol	T20	77.21±0.28	322.55±25.55	0.572±0.033	-24.41±0.01
F5	Geleol	T80	72.90±2.90	190.55±5.45	0.491±0.059	-32.95±0.45
F6	Geleol	S60	65.36±0.46	149.70±0.25	0.351±0.001	-29.50±0.50

Note: Data represented as mean ± SD (n=3).

Abbreviations: EE%, entrapment efficiency percentage; T20, tween 20; T80, tween 80: S60, Span60: PS, particle size; PDI, polydispersity index and ZP, zeta potential.

- All formulae contained 100 mg SAA, and 200 mg solid lipid.

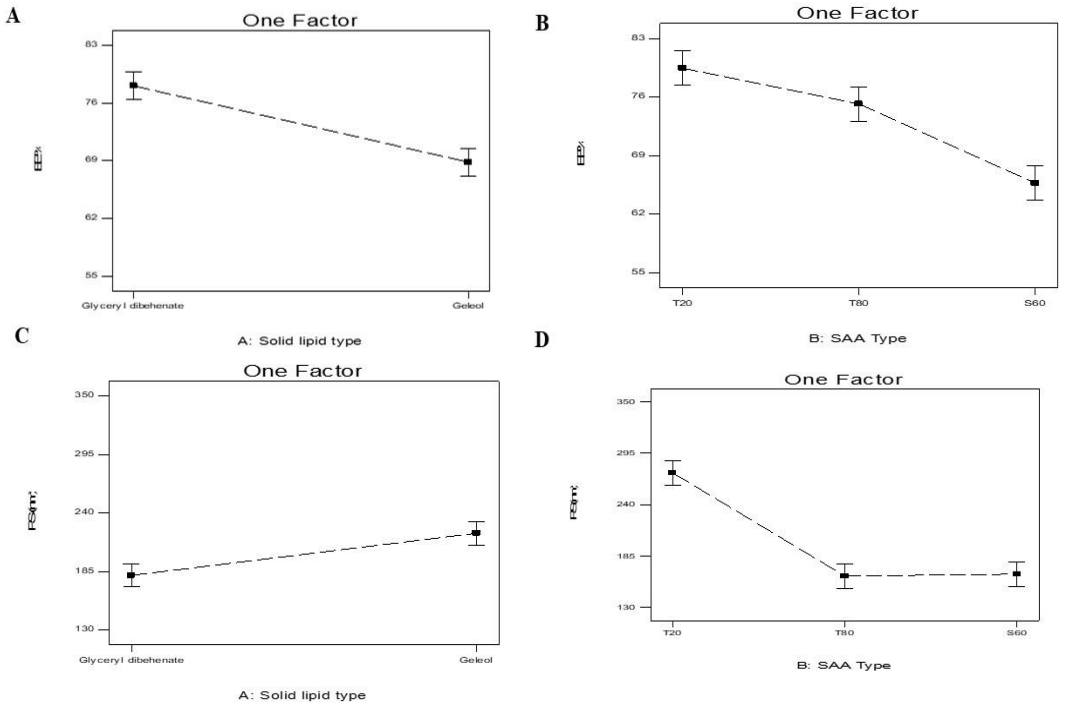

Fig. 1: Effect of solid lipid type (X_1) and SAA type (X_2) on EE%, and PS on SLNs-phospholipid complex.

The effect of formulation variables on PS

The mean hydrodynamic diameter of the particles is indicated by the z-average diameter[25] was measured and presented in **Table2,3** and graphically illustrated in 3-D surface plots **(Fig. 1C-D)**. The PS of the nanosystem might affect the degree of drug deposition as well as skin penetration.[26] PS ranged from 121.85±5.05 to 322.55±25.55 nm.

It is noticeable that both solid lipid type (X_1) and SAA type (X_2), influenced significantly ($p<0.0001$) the PS of the vesicles. The polynomial equation of PS was as following:

PS (nm) =+201.18+19.84* A+72.65* B [1]-37.38* B [2]+28.88* AB[1]+6.91* AB[2]

For solid lipid type (X_1), it was found that Geleol produced larger PS than Glyceryl dibehnate this was in accordance with previous literature.[27] In addition it was found that Glyceryl dibehnate produced larger EE% than Geleol, hence accommodates larger spaces for drug inclusion and subsequently increased PS.[15] Further, it was also observed from the results that the PS was in accordance with the amount of drug entrapped into the vesicles. Therefore, the increase in EE% would give another explanation for the larger PS of vesicle.[28]

For SAA type (X_2), it was observed that PS augmented in the subsequent order S60<T80<T20, this could be related to SAA hydrophilicity, as the hydrophilicity of SAA increased the PS increase. Aziz et al stated by increasing SAA hydrophilicity resulted in increasing the water uptake by the nanosytems with resultant increase in PS.[18]

The effect of formulation variables on PDI

The width of unimodal size distributions is measured by the PDI. Further, PDI was measured and presented in **Table 2,3** and graphically illustrated in 3-D surface plots **(Fig. 2 A-B)**. PDI ranged from 0.237±0.01 to 0.572±0.033. A homogeneous dispersion shows a value of 0, whereas a completely heterogeneous polydisperse population is indicated by a value of 1. A PDI that is considered acceptable should be less than 0.5. The produced vesicles' polydispersity indices were often minimal, as can be seen from the data, indicating strong homogeneity and a narrow size distribution. [29]

The polynomial equation for PDI is:

PDI= +0.42+0.056* A+7.417E-003* B [1] +0.03* B[2]+0.093* AB[1]-0.01* AB[2]

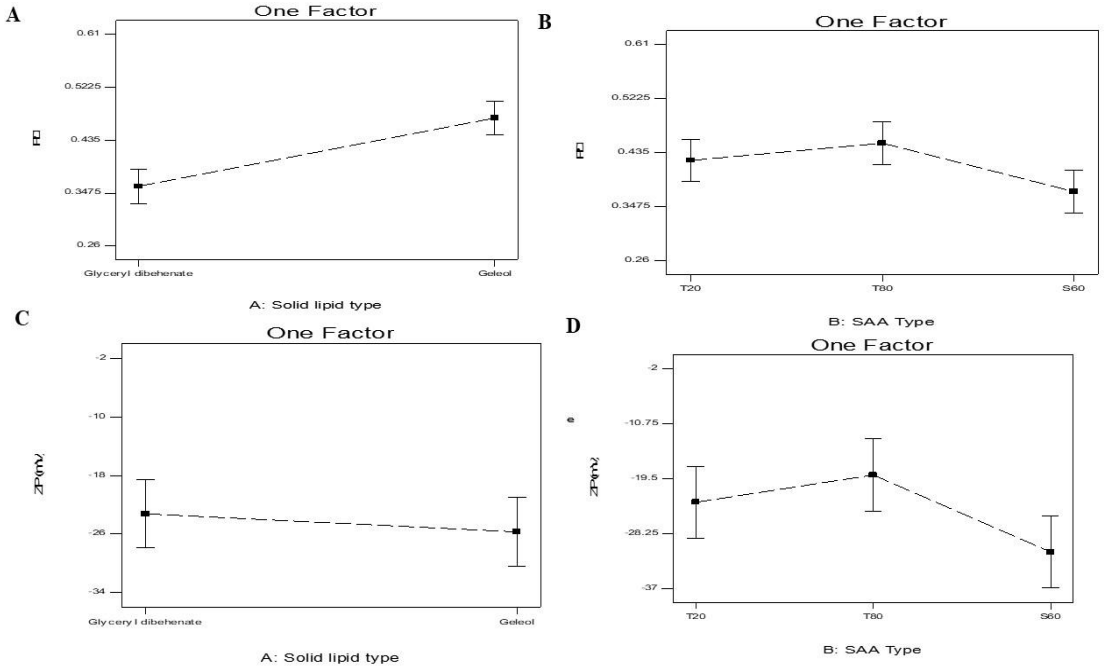

Fig. 2: Effect of solid lipid type (X_1) and SAA type (X_2) on PDI, and ZP on SLNs-phospholipid complex.

Factorial analysis of variance displayed that only solid lipid type (X_1) showed significant effect on PDI with p values of (p=0.0027), Geleol produced bigger PDI than glyceryl dibehnate. The previous outcomes showed agreement of PS results with PDI values, as the PS of SLNs-phospholipid complex increased PDI subsequently increased this was described previously in literature.[15]

ZP evaluation

ZP ranged from -23.10±1.61 to -32.95±0.45 mV. For electrostatic reasons, colloidal particles with (≥|15|mV) are expected to be stable due to steric reasons.[30]

The negative charge present in SLNs formulae was due to carboxylic groups present in solid lipids (Glyceryl dibehnate, and Geleol).[31]Both factors should no significant effect hence ZP was removed from SLNs optimization.

Selection of the optimized formulation

A set of criteria was first established in the Design Expert® software (Stat Ease, Inc., Minneapolis, MN) version 11 in order to choose the best formula. Particles having the highest EE%, and lowest PS and PDI were given preference according to these parameters. The primary effects of these parameters were separately found through the use of Design Expert® to examine the experimental data. An analysis of variance (ANOVA) was then performed to ascertain the importance of each element. The formula that satisfied the predetermined criteria was the best one. The optimum SLNs-phospholipid complex (F2) had a ZP of -23.50±1.11 mV, PDI of 0.41±0.005, PS of 137.05±0.45nm, and EE% of 77.44±0.94%. The expected and observed responses of were compared and are displayed in **Table 2** to verify the validity of our experiment. There was a strong correlation found between the actual and anticipated values for the optimum formula F2 that composed of glyceryl dibehnate and Tween 80.

Characterization of the optimum formula

***In-vitro* drug release**

When expecting a drug's *in-vivo* performance, the release profile is a crucial indicator. High surface area and a faster rate of disintegration are the outcomes of PS decrease.[32] Q6h for the optimum SLNs-phospholipid complex (F2) and Fenticonazole nitarte suspension is displayed in **Fig. 3**. Additionally, the results indicated that the optimum SLNs-phospholipid complex contains Tween 80 that provides a significant (P=0.001) higher release percent compared to Fenticonazole nitarte suspension. As the aqueous solubility of Fenticonazole nitarte

represents its main drawback as it is less than 0.10mg/mL, as it could significantly affect the drug activity, release rate or even results in microbial resistance. Further, the enhanced release rate from nanosystem is likely due to the fact that Tween 80 created the small PS particles, increasing the total surface area and, thus, the release rate.[33] Furthermore, the inclusion of Tween 80 led to the formation of mixed micelles with phospholipid and solid lipid hence, improved Fenticonazole Nitrate's aqueous solubility in the aqueous phase.[34]

Morphology of vesicles

TEM examination was used to examine the external morphology of the obtained optimum SLNs-phospholipid complex formulation. The morphological shape revealed that they were spherical and had a consistent size distribution **(Fig. 4)**. The Zetasizer-determined SLNs-phospholipid complex particle sizes correlated with TEM particle size evaluation

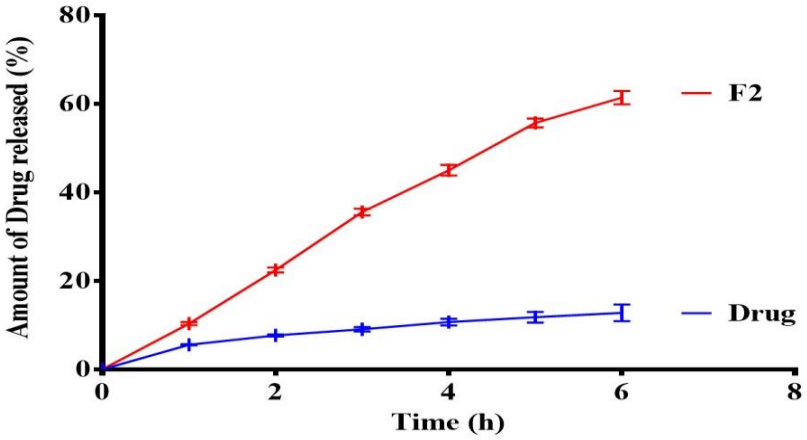

Fig. 3: *In-vitro* drug release for Fenticonazole nitrate and the optimum formula (F2).

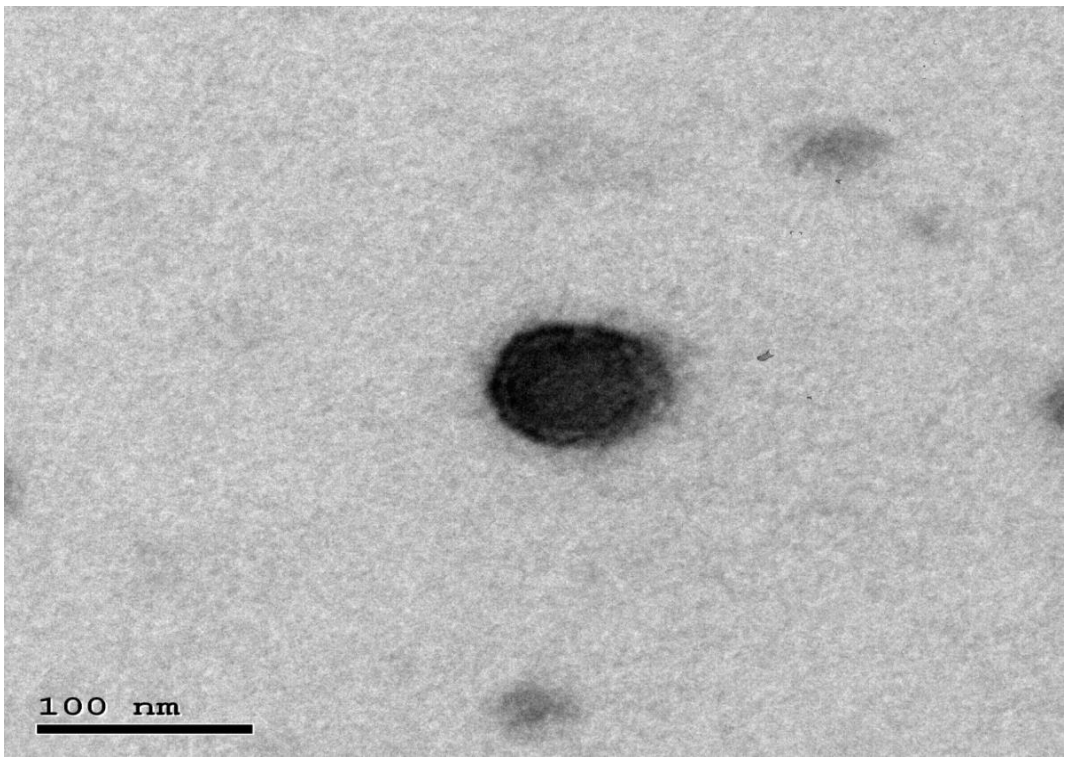

Fig. 4: Transmission electron micrograph for the optimum SLNs-phospholipid complex.

Stability study

During storage, lipid nanoparticles formulations have a tendency to fuse and disintegrate, changing PS, PDI, and ZP. Additionally, these modifications result in a decrease in the EE% and medication leakage from the vesicles.[35] SLNs-phospholipid complex (F2) were visually inspected for aggregation and appearance changes. Furthermore, it was determined what EE% of 76.34±0.71, PS of 140.00±2.00 nm, PDI of 0.39±0.01, ZP of -23.00±2.00 mV, and Q6h of 64.15±2.10%. After 45 days at 4°C, statistical analysis showed that there was no significant change between the fresh and preserved SLNs-phospholipid complex in terms of EE%, PS, PDI, ZP and Q6h with p values of 0.9, 0.76, 0.89, 0.56, and 0.5 respectively. These results suggest that the ideal optimum SLNs-phospholipid complex is stable.

pH measurement

The inspected pH assessments for optimum SLNs-phospholipid complex ranged from 4.70 ± 0.11 to 5.12 ± 0.14, which is regarded as suitable for topical application on skin. [36]

Histopathological study

Permeation enhancers are thought to be a key barrier to topical delivery due to skin irritation.[37] When compared to untreated skin sections (group I), light microscopy analysis of groups II which were treated with the optimum SLNs-phospholipid complex, respectively, revealed no histological alterations in epidermal and dermal cells **(Fig. 5)**. These results showed that the optimum SLNs-phospholipid complex formulation had a tolerable level of acceptability. Further, previous studies confirmed the tolerability of both glyceryl dibehnate and Tween 80.[38,39] The obtained results suggest that the optimized SLNs-phospholipid complex have an acceptable safety level and are not expected to cause skin irritation in clinical trials according to the fact that there were no clear signs of skin intolerability in the performed in the *in-vivo* histopathological study.

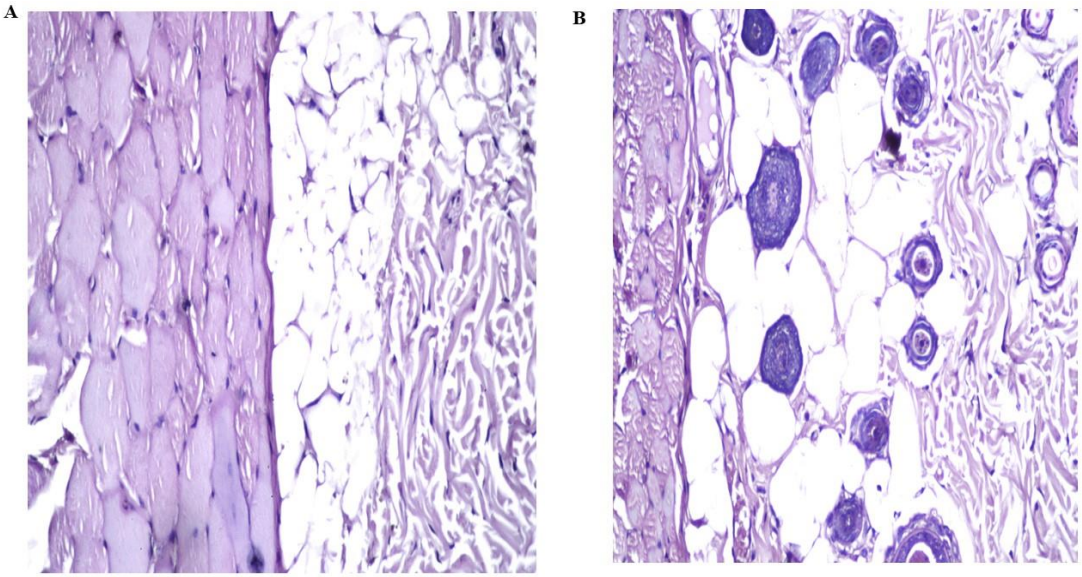

Fig. 5: Histopathological study for the optimum SLNs-phospholipid complex (F2) (b) compared to the negative control (a).

Conclusion

In this study, we developed SLNs-phospholipid complex as a topical Fenticonazole nitrate delivery drug. In accordance with the $2^1.3^1$ full factorial design, six formulations were created using the thin film hydration process. These formulations were then utilized to choose the best nanoformula, which had spherical morphology, a good drug EE%, minimal PS, and good ZP values. The optimum formula was stable during storage period. The *in-vitro* release study confirmed the enhancement of solubilization and release of fenticonazole nitrate from the optimum formula. Additionally, the *in-vivo* histological investigation verified that optimum formula did not cause irritation when applied to rat skin. The findings therefore indicated that, since SLNs-phospholipid complex applied topically without any irritation it could be regarded as a potential topical administration strategy. To prove that SLNs-phospholipid complex is therapeutically effective in humans, more research is required.

REFERENCES

1. S. Veraldi and R. Milani, "Topical fenticonazole in dermatology and gynaecology current role in therapy", ***Drugs,*** 68(15), 2183-2194 (2008).
2. W.H. Abd-Elsalam, S.A. El-Zahaby and A.M. Al-Mahallawi, "Formulation and in vivo assessment of terconazole-loaded polymeric mixed micelles enriched with Cremophor EL as dual functioning mediator for augmenting physical stability and skin delivery", ***Drug Deliv***, 25(1), 484–492(2018).
3. R. Campos, S.F. Bittencourt, J.A. Rojas-Moscoso, L. Pissinatti, L.S. Chen, M. Porto, R.A. Moreno, G.D. Mendes and G. De Nucci, "The rabbit vagina as an in vivo model for vaginal fenticonazole permeability and toxicity", ***J Pharmacol Toxicol Methods,*** 94, 14–18 (2018).
4. S. Ahmed, M.M. Amin and S. Sayed, "A comprehensive review on recent nanosystems for enhancing antifungal activity of fenticonazole nitrate from different routes of administration", ***Drug Deliv***, 30(1), 2179129 (2023).
5. R. Albash, M.H. Ragaie, M.A.E. Hassab, R. El-Haggar, W.M. Eldehna, S.T. Al-Rashood and S. Mosallam, "Fenticonazole nitrate loaded trans-novasomes for effective management of tinea corporis: design characterization, in silico study, and exploratory clinical appraisal", ***Drug Deliv,*** 29(1), 1100–1111 (2022).
6. R. Albash, C. Yousry, A.M. Al-Mahallawi and A.A. Alaa-Eldin, "Utilization of PEGylated cerosomes for effective topical delivery of fenticonazole nitrate: in-vitro characterization, statistical optimization, and in-vivo assessment", ***Drug Deliv***, 28(1), 1–9 (2021).
7. D. Liu, L. Chen, S. Jiang, S. Zhu, Y. Qian, F. Wang and R. Li, Q. Xu, "Formulation and characterization of hydrophilic drug diclofenac sodium-loaded solid lipid nanoparticles based on phospholipid complexes technology", ***J Liposome Res,*** 24(1), 17–26 (2014).
8. L. Wang, C.Y. Wang, Y. Zhang, H.J. Fu, Y. Gao and K.R. Zhang, "Preparation and characterization of solid lipid nanoparticles loaded with salmon calcitonin phospholipid complex", ***J Drug Deliv Sci Technol,*** 52, 838–845(2019).
9. K. Westesen and B. Siekmann, "Investigation of the gel formation of phospholipid-stabilized solid lipid nanoparticles", ***Int J Pharm,*** 151(1), 35-45(1997).
10. Q. Peng, Z.R. Zhang, X. Sun, J. Zuo, D. Zhao and T. Gong, "Mechanisms of phospholipid complex loaded nanoparticles enhancing the oral bioavailability", ***Mol Pharm,*** 7, 565–575(2010).
11. R. Albash, N.M. Badawi, M.I.A. Hamed, M.H. Ragaie, S.S. Mohammed, R.M. Elbesh, K.M. Darwish, M.O. Lashkar, S.S. Elhady and S. Mosallam, "Exploring the synergistic effect of bergamot essential oil with spironolactone loaded nano-phytosomes for treatment of acne vulgaris: in vitro optimization, in silico studies, and clinical evaluation", ***Pharmaceuticals,*** 16(1), 128 (2023).
12. H. Refai, A.A. El-Gazar, G.M. Ragab, D.H. Hassan, O.S. Ahmed, R.A. Hussein, S. Shabana, P. Waffo-Téguo, J. Valls, A.K. Al-Mokaddem, H.M.R.M. Selim,

E.M. Yousef, S.K. Ali, A. Salman, H.B. Abo-Zalam and R. Albash, "Enhanced wound healing potential of spirulina platensis nanophytosomes: metabolomic profiling, molecular networking, and modulation of HMGB-1 in an excisional wound rat model", ***Mar Drugs,*** 21(3), 149 (2023).

13. H.R.H. Mohamed, S. El-Shamy, S.S. Abdelgayed, R. Albash and H. El-Shorbagy, "Modulation efficiency of clove oil nano-emulsion against genotoxic, oxidative stress, and histological injuries induced via titanium dioxide nanoparticles in mice", ***Sci Rep,*** 14(1),7715 (2024).
14. R. Albash, M.M. Abdellatif, M. Hassan and N.M. Badawi, "Tailoring terpesomes and leciplex for the effective ocular conveyance of moxifloxacin hydrochloride (Comparative assessment): In-vitro, ex-vivo, and in-vivo evaluation", ***Int J Nanomedicine,*** 16, 5247-5263(2021).
15. R. Albash, A.A. Abdelbary, H. Refai and M.A. El-Nabarawi, "Use of transethosomes for enhancing the transdermal delivery of olmesartan medoxomil: In vitro, ex vivo, and in vivo evaluation", ***Int J Nanomedicine,*** 14, 1953-1968 (2019).
16. M.A. Eltabeeb, R.R. Hamed, M.A. El-Nabarawi, M.H. Teaima, M.I.A. Hamed, K.M. Darwish, M. Hassan, M.M. Abdellatif, "Nanocomposite alginate hydrogel loaded with propranolol hydrochloride kolliphor® based cerosomes as a repurposed platform for Methicillin-Resistant Staphylococcus aureus-(MRSA)-induced skin infection; in-vitro, ex-vivo, in-silico, and in-vivo evaluation", ***Drug Deliv Transl Res***, (2024).
17. M.H. Teaima, M.A. Eltabeeb, M.A. El-Nabarawi and M.M. Abdellatif, "Utilization of propranolol hydrochloride mucoadhesive invasomes as a locally acting contraceptive: in-vitro, ex-vivo, and in-vivo evaluation", ***Drug Deliv,*** 29(1), 2549-2560 (2022).
18. D.E. Aziz, A.A. Abdelbary and A.I. Elassasy, "Investigating superiority of novel bilosomes over niosomes in the transdermal delivery of diacerein: in vitro characterization, ex vivo permeation and in vivo skin deposition study", J Liposome Res, 29(1):7385(2019).https://doi.org/10.1080/08982104.2018.1430831.
19. A.C. Mendes, C. Gorzelanny, N. Halter, S.W. Schneider, I.S. Chronakis, "Hybrid electrospun chitosan-phospholipids nanofibers for transdermal drug delivery", ***Int J Pharm,*** 510(1), 48-56 (2016).
20. A.C. Silva, M.H. Amaral, E. González-Mira, D. Santos and D. Ferreira, "Solid lipid nanoparticles (SLN) - based hydrogels as potential carriers for oral transmucosal delivery of risperidone: preparation and characterization studies", ***Colloids Surf B Biointerf,*** 93, 241–248 (2012).
21. H. Abbas, R. Kamel and N. El-Sayed, "Dermal antioxidant, anti-inflammatory and anti-aging effects of Compritol ATO-based Resveratrol colloidal carriers prepared using mixed surfactants", ***Int J Pharm***, 541, 37–47 (2018).
22. V. Makwana, R. Jain, K. Patel, M. Nivsarkar and A. Joshi, "Solid lipid nanoparticles (SLN) of Efavirenz as lymph targeting drug delivery system: Elucidation of mechanism of uptake using chylomicron flow blocking approach", ***Int J Pharm,*** 495(1), 439-446(2015).
23. D.Z. Hou, C.S. Xie, K.J. Huang and C.H. Zhu, "The production and characteristics of solid lipid nanoparticles (SLNs)", ***Biomaterials,*** 24(10), 1781–1785 (2003).
24. A. Malzert-Fréon, G. Saint-Lorant, D. Hennequin, P. Gauduchon, L. Poulain and S. Rault, "Influence of the introduction of a solubility enhancer on the formulation of lipidic nanoparticles with improved drug loading rates", ***Eur J Pharm Biopharm,*** 75(2), 117-127(2010).
25. S.M. Ezzat, M.M. Salama, A.N. ElMeshad, M.H. Teaima and L.A. Rashad, "HPLC–DAD–MS/MS profiling of standardized rosemary extract and enhancement of its anti-wrinkle activity by encapsulation in elastic nanovesicles", ***Arch Pharm Res,*** 39, 912–925 (2016).
26. M.S. Roberts, Y. Mohammed, M.N. Pastore, S. Namjoshi, S. Yousef, A. Alinaghi, I.N. Haridass, E. Abd, V.R. Leite-Silva, H.A.E. Benson and J.E. Grice, "Topical and cutaneous delivery using

nanosystems", ***J Control Release***, 247, 86–105 (2017).
27. L. Martínez-Acevedo, M. de la L. Zambrano-Zaragoza, G. Vidal-Romero, S. Mendoza-Elvira and D. Quintanar-Guerrero, "Evaluation of the lubricating effect of magnesium stearate and glyceryl behenate solid lipid nanoparticles in a direct compression process", ***Int J Pharm,*** 545(1-2),170-175(2018).
28. R.M. Hathout and A.H. Elshafeey," Development and characterization of colloidal soft nano-carriers for transdermal delivery and bioavailability enhancement of an angiotensin II receptor blocker", ***Eur J Pharm Biopharm***, 82, 230–240(2012).
29. A.H. Salama and M.H. Aburahma, "Ufasomes nano-vesicles-based lyophilized platforms for intranasal delivery of cinnarizine: preparation, optimization, ex-vivo histopathological safety assessment and mucosal confocal imaging", ***Pharm Dev Technol***, 21, 706–715(2016).
30. Z. Sun, V. Nicolosi, D. Rickard, S.D. Bergin, D. Aherne and J.N. Coleman, "Quantitative evaluation of surfactant-stabilized single-walled carbon nanotubes: Dispersion quality and its correlation with zeta potential", ***J Phys Chem C,*** 112, 10692–10699 (2008).
31. F. Qazi, M.H. Shoaib, R.I. Yousuf, M.I. Nasiri, K. Ahmed and M. Ahmad, "Lipids bearing extruded-spheronized pellets for extended release of poorly soluble antiemetic agent - Meclizine HCl", ***Lipids Health Dis,*** 16(1), 75(2017).
32. M.M. Abdellatif, I.A. Khalil and M.A.F. Khalil, "Sertaconazole nitrate loaded nanovesicular systems for targeting skin fungal infection: In-vitro, ex-vivo and in-vivo evaluation", ***Int J Pharm,*** 527, 1–11(2017).
33. H.M. Aboud, A.A. Ali, S.F. El-Menshawe, A.A. Elbary, "Nanotransfersomes of carvedilol for intranasal delivery: formulation, characterization and in vivo evaluation", ***Drug Deliv,*** 23, 2471–2481(2016).
34. A.M. Al-Mahallawi, A.A. Abdelbary and M.H. Aburahma, "Investigating the potential of employing bilosomes as a novel vesicular carrier for transdermal delivery of tenoxicam", ***Int J Pharm,*** 485, 329–340(2015).
35. A. Zeb, O.S. Qureshi, H.S. Kim, J.H. Cha, H.S. Kim and J.K. Kim, "Improved skin permeation of methotrexate via nanosized ultradeformable liposomes", ***Int J Nanomedicine,*** 11, 3813-3824 (2016).
36. M.M. El-Naggar, M.A. El-Nabarawi, M.H. Teaima, M. Hassan, M.I.A. Hamed, A.A. Elrashedy and R. Albash, "Integration of terpesomes loaded Levocetrizine dihydrochloride gel as a repurposed cure for Methicillin-Resistant Staphylococcus aureus (MRSA)-Induced skin infection; D-optimal optimization, ex-vivo, in-silico, and in-vivo studies", ***Int J Pharm,*** 633,122621 (2023).
37. K. Priyanka and S. Singh, "A review on skin targeted delivery of bioactives as ultradeformable vesicles: overcoming the penetration problem", ***Curr Drug Targets***, 15(2), 184-98 (2014).
38. H. Abbas, R. Kamel and N. El-Sayed, "Dermal anti-oxidant, anti-inflammatory and anti-aging effects of Compritol ATO-based Resveratrol colloidal carriers prepared using mixed surfactants", ***Int J Pharm,*** 541(1-2), 37-47 (2018).
39. A. Ascenso, A. Salgado, C. Euletério, F.G. Praça, M.V.L.B. Bentley, H.C. Marques, H. Oliveira, C. Santos and S. Simões, "In vitro and in vivo topical delivery studies of tretinoin-loaded ultradeformable vesicles", ***Eur J Pharm Biopharm,*** 88(1), 48-55 (2014).

Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 2, 2024, pp. 823-835.

نشرة العلوم الصيدلية
جامعة أسيوط

BULL. PHARM. SCI. Assiut Univ.

تحضير وتوصيف متراكب الجزيئات النانوية الدهنية الصلبة مع الفوسفوليبيد محمل بنترات فينتيكونازول لعلاج الكانديدا موضعياً

سرمد ضياء نوري[1*] – حسين حامد حيال[2] – حيدر أ. حمودي[3] – أميرة أمين[2،4]

[1] قسم الصيدلانيات كلية الصيدلة – جامعة العين العراقية

[2] قسم العقاقير، كلية الصيدلة، جامعة العين العراقية

[3] قسم الصيدلة، كلية مزايا الجامعية، ذي قار، العراق

[4] قسم الصيدلة السريرية وممارسة الصيدلة ، كلية الصيدلة ، جامعة دمنهور

هدفت الدراسة الحالية إلى تحميل نترات فنتيكونازول (FTN)، وهو عامل مضاد للفطريات ذو قابلية ذوبان منخفضة في الماء، في مجمع فسفوليبيد الجسيمات النانوية الدهنية الصلبة (SLNs) لإدارة المبيضات البيضاء موضعيًا. نترات فنتيكونازول هو دواء مضاد للفطريات له نتائج ايض أولي كبيرة تؤدي إلى انخفاض التوافر البيولوجي عن طريق الفم. من أجل منع المضاعفات الفموية، كان إنتاج مجمع فسفوليبيد الجسيمات النانوية الدهنية الصلبة لتحسين توزيع نترات فنتيكونازول الموضعي هو محور هذه الدراسة. باستخدام تصميم عاملي كامل ٢١,٣١ والدهون الصلبة والمواد الخافضة للتوتر السطحي المختلفة، تم إنشاء صيغ SLNs. تم تصنيع مجمع فسفوليبيد الجسيمات النانوية الدهنية الصلبة (SLNs) باستخدام طريقة ترطيب الغشاء الرقيق. تم تقييم مجمع فسفوليبيد الجسيمات النانوية الدهنية الصلبة (SLNs) فيما يتعلق بنسبة كفاءة الاحتجاز (EE٪) وحجم الجسيمات (PS) ومؤشر تعدد التشتت (PDI) والجهد زيتا (ZP). تم إجراء المزيد من الاستكشافات على التركيبة المثلى. باستخدام برنامج Design Expert®، تم تحديد التركيبة المثلى (F2)، وكشفت عن ZP من −٢٣,٥٠±١,١١ mV، وPDI من ٠,٤١±٠,٠٠٥، وPS من ١٣٧,٠٥±٠,٤٥nm، وEE% من ٧٧,٤٤±٠,٩٤%. علاوة على ذلك، أظهرت التركيبة المثلى SLNs كروية بدون تكتل تحت تقييم المجهر الإلكتروني النافذ. أظهرت دراسة الإطلاق في المختبر أن التركيبة المثلى تم إطلاقها بشكل أسرع من معلق FTN. بالإضافة إلى ذلك، أثناء التخزين كانت التركيبة المثلى مستقرة. أكد التحقيق النسيجي التشريحي سلامة SLNs المثلى.