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Transdermal Delivery System of Doxycycline-Loaded Niosomal Gels: Toward Enhancing Doxycycline Stability

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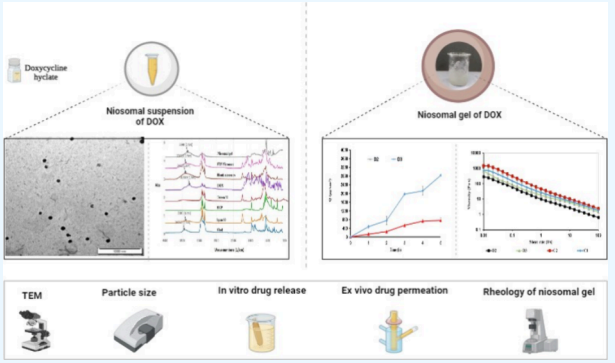
ABSTRACT: Doxycycline (DOX) is an antimicrobial agent that is susceptible to photosensitivity and thermal degradation. It addition, it causes gastrointestinal side effects when taken orally. Therefore, the development of alternative formulations is necessary to improve drug stability and promote patient compliance. The aim of the present study was to encapsulate DOX in niosomes as a nanocarrier to deliver DOX transdermally and enhance its stability in the formulation. DOX niosomes were prepared using nonionic surfactants, cholesterol, and dihexadecyl phosphate (DCP). After that, niosomes were characterized in terms of practical size (PS), zeta potential (ZP), morphology, and entrapment efficacy (EE%). DOX niosomal gels were then prepared using Carbopol and penetration enhancers (poly(ethylene glycol) 400 (PEG 400) and propylene glycol (PG)). The flux of DOX from the optimized formula was 322.86 $\mu g/cm^2/h$ over 5 h, which equates to 71.2% of DOX. Furthermore, neither the DOX niosomal gel (D3) nor the comparable blank niosomal gel had a negative influence on human dermal fibroblast (HDF) cells. The findings of the antimicrobial effectiveness of DOX niosomes indicated that the niosomal formulation improved the antibacterial activity of DOX against *E. coli*. Permeation studies demonstrated significantly higher DOX permeation when the niosomal gel was applied to rat skin, compared to the conventional gel. Permeability parameters such as flux and the permeability coefficient increased more than 10-fold using the niosomal gels compared with those of conventional gels. In conclusion, a new niosomal gel formulation could serve as an effective alternative for the commercially available form of DOX.



1. INTRODUCTION

Transdermal drug delivery (TDD) is one of the delivery methods capable of delivering a significant amount of drugs into systemic circulation through the intact skin.[1] TDD has specific advantages over other drug delivery routes, such as its nonpainful and its simple application.[2] In addition, TDD has a more consistent drug profile, decreasing adverse reactions and avoiding the presystemic metabolism which is typically associated with oral administration.[2,3] Specific requirements for successful TDD must be met, including a drug molecular weight of around 500 Da and the lipophilicity within a log *P* range of 1−3.[4] Because stratum corneum is a highly effective membrane that can hinder drug penetration,[5] both passive and active techniques could be used to improve drug delivery to the skin. Passive approaches play an important role in the transdermal formulation by modifying the structure of the stratum corneum, which may facilitate the penetration of drugs across the skin barrier.[5] Active approaches involve strategies that use external technologies to improve drug delivery through the skin barrier such as microneedles and lasers.[4]

Nanotechnology presents an innovative method of delivering drugs to specific parts of the body via various systems such as liposomes and niosomes.[6] Niosomes are more stable than liposomes, making them an excellent candidate for drug nanocarriers.[7] Nonionic surfactants are the main component of niosomes' structure in which the drugs could be entrapped in the inner core or bilayer of niosomes, depending on their lipophilicity.[8] Moreover, niosomes offer several advantages over liposomes, including less irritation, scalability, storage convenience, low cost, and biocompatibility.[6,8] Furthermore, this technique enhances drugs permeation across the skin, which make it an efficient drug delivery system.[7,9]

Doxycycline hyclate (DOX) is an antibiotic with a broad spectrum which inhibits and kills a wide range of bacteria.[10−12] Gastrointestinal side effects are always reported by using DOX.[13] DOX is available for oral administration in two salt forms: monohydrate and hyclate. In addition, it is available as enteric-coated tablets.[13] DOX monohydrate is more soluble in

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Table 1. Composition, HLB Value, and Method of Preparation of DOX Niosomal Formulations

Formulation	Span 60 (mg)	Tween 80 (mg)	Tween 60 (mg)	Span 40 (mg)	Chol (mg)	DCP (mg)	DOX (mg)	HLB value[a]	Method of preparation[b]
F1	50	-	-	-	50	2	100	4.7	Ether inj.
F2	30	-	10	-	20	2	100	7.1	Ether inj.
F3	10	-	30	-	20	2	100	12.3	Ether inj.
F4	20	-	20	-	40	2	100	7.5	Ether inj.
F5	20	-	20	-	20	2	100	7.5	Ether inj.
F6	40	-	40	-	80	2	100	7.5	Ether inj.
F7	40	-	40	-	40	2	100	7.5	Ether inj.
FT1	40	-	40	-	40	2	100	7.5	Thin film
FT2	40	-	40	-	80	2	100	7.5	Thin film
FT3	-	15	25	25	65	2	100	12.6	Thin film
FT4	-	15	25	25	130	2	100	12.6	Thin film
FT5	100	-	100	-	50	2	100	7.5	Thin film

[a]Hydrophilic−lipophilic balance (HLB). [b]Ether injection (Ether inj.).

water than hyclate and has a more basic pH. There have been reports of up to 40% GI upset with DOX hyclate and 18% with enteric-coated DOX hyclate.[13] Unlike tetracycline, DOX can be used with food to decrease GI distress such as nausea. Moreover, the hyclate form causes esophagitis more frequently than the DOX monohydrate because of the acidic form of this drug. Patients are frequently instructed to take DOX with a full glass of water and to avoid lying down for 1 h after taking the medication to reduce the risk of developing esophagitis.[13] Moreover, due to its high photosensitivity and tendency to undergo thermal degradation at high temperatures,[14] DOX should be formulated using different formulation strategies to improve its stability.

DOX is primarily used in the treatment of acne vulgaris for a long time.[15] The duration of DOX treatment for acne might last from a few weeks to several months.[15] Therefore, it becomes essential to address the limitations associated with conventional oral systems by developing an alternative dosage form. This is especially crucial for patients with acne or those who require DOX for other indications because this approach aims to improve patient compliance. Consequently, the primary purpose of this study is to develop and assess a niosomal gel as an alternative way of delivering DOX transdermally.

Materials. Doxycycline hyclate (DOX) was a gift from Dar AlDawaa (Amman, Jordan). Triethanolamine (TEA), isopropyl alcohol, diethyl ether, and methanol were purchased from Tedia (Fairfield, OH, USA). Propylene glycol (PG) and poly(ethylene glycol) 400 (PEG400) were provided from GCC Diagnostics (Flintshire, U.K.). Dihexadecyl phosphate (DCP) and polyvinylpyrrolidone (molecular weight 40 000 Da) were purchased from Sigma-Aldrich (Dorset, U.K.). Span 60, Tween 60, Tween 80, phosphate-buffered saline (PBS) tablets, and cholesterol (Chol) were purchased from Sigma-Aldrich (Dorset, U.K.). All other chemicals used in this study were of analytical grade.

2. METHODS

2.1. Preparation of Niosomes. Two methods, ether injection and thin film hydration, were employed to prepare niosomes. Because of its simplicity and low cost, the ether injection method was initially chosen. In the ether injection method, various nonionic surfactants including Span 60, Tween 60, and Tween 80 were dissolved in 16 mL of diethyl ether while 100 mg of DOX was dissolved in 4 mL of methanol, and then the two solutions were sonicated and mixed in the ether injection procedure as shown in Table 1. Using a syringe pump, the resultant mixture was injected over hot phosphate-buffered saline (PBS, pH 7.4) at 40 °C, followed by cooling and refrigerating. In the thin film hydration method, the nonionic surfactants were dissolved in 16 mL of diethyl ether, and 100 mg of DOX was dissolved in 4 mL of methanol as shown in Table 1. These two solutions were sonicated for 5 min at room temperature and mixed to form one clear solution, and then this solution was placed in a 200 mL amber round-bottomed flask and the thin film was obtained by evaporating the organic solvent in a rotary evaporator under reduced pressure at 40 °C and at a speed of 120 rpm for 30 min. After the thin film was formed, the amber round-bottomed flask was inserted into a desiccator overnight. A rotary evaporator was used to evaporate this solution, resulting in a thin layer that was subsequently hydrated with PBS (pH 7.4) for 45 min. The niosomal solution that resulted was kept at 4 °C.

2.2. Characterization of Niosomal Formulations.

2.2.1. Particle Shape and Morphology. The morphology of the DOX niosomes was determined using a transmission electron microscope (TEM, FEI Morgani 268, operating voltage of 60 kV, Holland) linked to a Mega View II digital camera. Before imaging, one drop of niosomes was diluted with distilled water (1:2 *v*/*v*) and deposited on a carbon-coated copper grid. ImageJ software was used to estimate the shape of the niosomes.

2.2.2. Particle Size (PS) and Polydispesity Index (PDI). The particle size (PS) and polydispersity index (PDI) of the niosomal formulas were evaluated using a Brookhaven 90 Plus particle size analyzer (Brookhaven 90 plus, Holtsville, NY, USA), utilizing a dynamic light scattering methodology. Each formula was assessed in triplicate, and the resulting data were used to calculate the mean and standard deviation (SD).

2.2.3. Zeta Potential (ZP). The ZP of the niosomes was measured by a particle Zetasizer analyzer (Brookhaven 90 plus, USA) using an electrophoretic light scattering (ELS) technique. Each formula was diluted and sonicated until the solution became clear, and then ZP was measured in triplicate and the mean and SD were calculated.

2.2.4. Drug Entrapment Efficiency. Using centrifugation, niosomes containing DOX were separated from the unencapsulated DOX. The niosomal suspension was centrifuged for 1 h at 4 °C and 16000 rpm. Then, the supernatant

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Table 2. Composition of the Niosomal and Control Gels

Ingredients	Niosomal gels			Conventional gels	
	D1	D2	D3	C1	C2
Carbopol974 (1%)	55% (*w*/*w*)	55% (*w*/*w*)	55% (*w*/*w*)	55% (*w*/*w*)	55% (*w*/*w*)
DOX	6.5% (*w*/*w*, niosomal DOX from FT1)	6.5% (*w*/*w*, niosomal DOX from FT5)	6.5% (*w*/*w*, niosomal DOX from FT5)	6.5% (*w*/*w*, free drug)	6.5% (*w*/*w*, free drug)
PG	0.1% (*v*/*w*)	0	0.1% (*v*/*w*)	0	0.1% (*v*/*w*)
PEG 400	0	0.1% (*v*/*w*)	0	0.1% (*v*/*w*)	0
Water	q.s	q.s	q.s	q.s	q.s

was removed, and fresh PBS (pH 7.4) was added before another cycle of centrifugation, under the same conditions, ensuring free drug removal. The obtained niosomes were then dissolved in 1 mL of isopropanol and sonicated for 10 min at room temperature. The volume was then diluted by a factor of 100 and adjusted to 10 mL with PBS (pH 7.4) and analyzed at a wavelength of 360 nm using UV−vis spectroscopy to determine the drug quantity, which was then used in Equation 1 to calculate the entrapment efficiency (EE%)

$$\text{EE\%} = \frac{\text{Entrapped amount of drug}}{\text{Total amount of drug loaded}} \times 100\% \quad (1)$$

2.2.5. In vitro Drug Release. To assess the release of DOX from niosomes, formulas FT1, FT3, and FT5, which exhibited higher entrapment efficiencies, were selected. After the unentrapped drug was removed, the niosomes were reconstituted by 2 mL of PBS (pH 7.4) and placed in a cellulose dialysis bag with a cutoff of 12−14 kDa. The releasing medium was 20 mL of PBS (pH 7.4) containing 20% 2-propanol at 32 °C and 150 rpm. Then a volume of 3 mL was taken after 1, 2, 3, 4, and 5 h from the samples and replaced directly with a fresh releasing medium. UV−vis spectroscopy was used to measure the amount of DOX released at 360 nm. For each formula, the test was performed three times, and the mean and SD were calculated.

2.2.6. Antimicrobial Test. The antimicrobial effectiveness of DOX niosomes was evaluated against Gram-negative bacteria, specifically *Escherichia coli* (*E. coli*).[16] An agar well plate diffusion method was used to comply to established standards for testing bacteria and yeasts, as supported by the Clinical and Laboratory Standards Institute (CLSI).[17] The wells (9 mm in diameter) were made using a sterile cork borer, and subsequently a sterile cotton swab was used to uniformly distribute an overnight bacterial inoculum over a sterile Mueller-Hinton agar plate. The following solutions were prepared to evaluate the antibacterial activity of PBS containing DMSO as a negative control (NC), DOX 16 μg/100 μL in DMSO as a positive control (PC), and DOX niosome FT5 equiv to DOX 16 μg/100 μL in DMSO.

2.2.7. Preparation of Niosomal Gels. Niosomal gels were formulated using FT1 and FT5 niosomal formulations due to their high entrapment efficiency (EE%) and release characteristics. Different compositions and ratios of the gel base (Carbopol 974) were prepared, as detailed in Table 2. To prepare a niosomal gel, 1% Carbapol 974 was prepared by weighting 1 g and adding up to 100 mL of double-distilled water with constant stirring using a magnetic stirrer for 2−3 h until a homogeneous mixture was obtained. Then the gel was kept for 24 h for adequate swelling of the polymer at room temperature. Subsequently, 5 g of 1% Carbopol gel was taken, and different penetration enhancers (PG and PEG 400) were added to the gel base. The selected DOX-loaded niosomes (FT1 and FT5) were gradually incorporated into the hydrated Carbopol 974 to formulate niosomal gels with a final DOX concentration of 6.5% *w*/*w* for all formulas. TEA was employed to induce gel formation and adjust the pH of gels to the range of 6.5−7.4, monitored with a pH meter. Similarly, control gels were formulated by incorporating free DOX instead of niosomes, as described in Table 2.

2.2.8. Characterization of the Niosomal Gels. The niosomal gels were characterized for organoleptic, homogeneous, pH, and rheological properties (viscosity and viscoelastic properties).

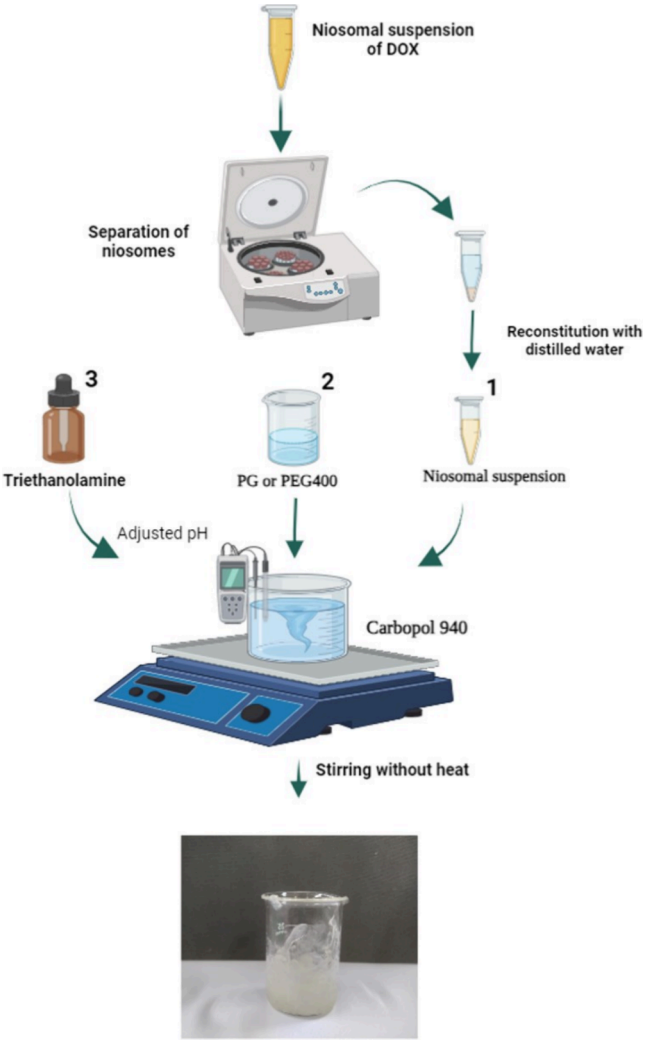

Figure 1. Schematic illustration outlining the process of niosomal gel preparation.

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2.2.8.1. Appearance and Color. The selected niosomal gels were evaluated for any change in gel clarity and color, ratifying the physical stability of the prepared gels and comparing these characteristics with the conventional gels

2.2.8.2. pH. A sample of 2 g of each niosomal gel was dispersed in 25 mL of distilled water. A digital pH meter was used to determine the pH of the dispersions.

2.2.8.3. Spreadability. The spreadability test was carried out as described in ref 18. Briefly, 0.5 g of niosomal gel was weighed and placed on a flat plate, with another plate placed over the gel. After a half kilogram of weight was allowed to sit on the upper plate for 5 min, the diameter of the gel circle after spreading was measured.

2.2.8.4. Drug Content. To measure the drug content, 1 g of niosomal gel was dissolved in 40 mL of PBS containing 50% methanol and agitated for 30 min, after which a certain volume of the sample was extracted. The amount of DOX was measured using UV−vis spectroscopy, and a calibration curve of DOX was prepared over a concentration range of 16.50−36.30 μg/mL at a wavelength of 360 nm

2.2.9. Rheological Properties. Measurements of the rheological properties (viscosity and viscoelastic measurements) of DOX-loaded niosomal gels (D1 and D2) and DOX conventional gels (C1 and C2) were performed in triplicate at 32 °C using a controlled-stress rheometer (CSR) (Anton Paar, MCR 302; Graz, Austria) with cone−plate geometry (gap of 0.1 mm, cone diameter of 25 mm, and cone angle of 1°), as described in ref 19.

2.2.9.1. Viscosity. Gel samples of 0.5 g were loaded onto the plate and left to relax for ∼1 min prior to testing. The viscosity of each gel was determined by applying a shear rate ranging from 0.1 to 100 s^{-1}. The flow curves of gels (viscosity vs shear rate) were obtained and evaluated.

2.2.9.2. Viscoelastic Properties. The strain-sweep measurements for all gels were performed to determine the linear viscoelastic (LVE) region, as described in ref 20. In this region, the elastic (G') and viscous (G'') moduli of gels must remain constant and independent of strain.[21] In addition, the frequency-sweep measurement of gels must be performed within this region.[21] To determine the LVE region, gel samples of 0.5 g were loaded onto the lower plate and left to equilibrate at 32 °C for 1 min. Then, the upper cone was lowered until the gap between the cone and plate was 0.1 mm. The cone was oscillated at a frequency of 6.28 rad/s during the strain-sweep measurement. The G' and G'' moduli were probed as a function of oscillatory strain ranging between 0.01 and 100%. The frequency-sweep measurement was conducted to determine the viscoelastic behavior (G' and G'') of the gels based on the strain-sweep measurement. Gels were exposed to dynamic oscillation over a frequency range of 0.1−100 rad/s and at fixed strain selected from the previously determined LVE region.

2.2.10. In Vitro Release. To perform the *in vitro* release study, the niosomal gels (D1, D2, and D3) were carefully placed inside cellulose dialysis bags with a cutoff of 12−14 kDa and immersed in a releasing medium consisting of 20 mL of PBS (pH 7.4) containing 20% isopropanol. The entire system was maintained at a temperature of 32 °C and agitated at a constant speed of 150 rpm. The amount of DOX released from the gels was determined by UV−vis spectroscopy at 360 nm. The experiment was repeated three times for each gel, and the mean and standard deviation (SD) were calculated.

2.2.11. Kinetics of Drug Release. Model-dependent methods are very useful for describing the release profiles of drugs. The zero-order, first-order, Higuchi, and Korsmeyer-Peppas models were applied to determine the kinetics of DOX release from the niosomal gels.

2.2.12. Ex Vivo Release. The permeation study of niosomal gels (D2 and D3) was investigated using Franz diffusion cells with a 12 mL volume of receiving medium (PBS pH 7.4) containing 20% isopropanol, an orifice diameter of 15 mm, and a diffusion surface area of 1.76 cm^2. The Ethics Committee for Scientific Research granted approval (approval number 4/2335/27, dated September 2022) for the rat skin preparation protocols. Rat skin was used as a membrane, where subcutaneous tissue and hair were removed, and then rinsed with water and cut into appropriately sized pieces. The amount of DOX that permeated the rat skin was calculated by taking 3.0 mL aliquots from the receptor compartments at various time points (1, 2, 3, 4, and 5 h) and immediately replacing them with 3 mL of freshly prepared PBS (pH 7.4) containing 20% isopropanol. The samples were analyzed using UV−vis spectroscopy at 360 nm. After 5 h, the skin was taken to assess skin retention with DOX. The skin was rinsed twice with distilled water and cut into small pieces. Then 20 mL of methanol was added to remove any amount of DOX from the skin layers and sonicated for 30 min at room temperature. The skin was centrifuged for 30 min at 12,000 rpm. The supernatant was taken and centrifuged again for 30 min at 12,000 rpm to remove any hair or parts of skin that had dispersed in the solution. The supernatant was collected and diluted and then measured by UV−vis spectroscopy at 360 nm.[22] The cumulative amount of DOX that permeated the rat skin per unit area (Q/A) was plotted versus time (t). Q/A was calculated as described previously in ref 23.

2.2.13. Attenuated Total Reflectance−Fourier Transform Infrared Spectroscopy (ATR−FTIR). The spectrum of different components of the optimal niosomal formulation (FT5) was investigated by using attenuated total reflectance−Fourier transform infrared spectroscopy (ATR−FTIR) spectroscopy. A comprehensive spectrum encompassing a range of components including Span 60, Tween 60, Chol, DCP, DOX, a physical mixture of DOX niosomes, blank niosomes, and FT5 niosomes was generated in the wavenumber range of 4000−400 cm^{-1} at 2 cm^{-1} resolution with 32 scans per spectrum. The spectral data collected was saved in CSV format and processed with Ira version 1.[24]

2.2.14. Stability Study. A short-term stability study of the niosomal gels (D2 and D3) was performed after 1 month of preparation. The niosomal gels were stored in the refrigerator at 4 °C as described in ref 19. The physical and organoleptic properties, such as color, appearance, pH, spreadability, and drug content, were evaluated for both gels.

2.2.15. Cytotoxicity Assay. The cytotoxicity evaluation of the DOX niosomal gel D3 and blank gel niosomes was performed by utilizing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay, as described in ref 25. The primary human dermal fibroblasts (HDF) were generously provided by Wulhan Al-Shaer of the Center for Cell Therapy at the University of Jordan. These HDF cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, maintaining a controlled environment at 37 °C with 5% carbon dioxide (CO_2). The experimental approach involved seeding HDF cells in a 96-well plate at a density of 5000 cells per well. The cells were allowed 24 h to adhere and

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Table 3. PS, PDI, ZP, and EE% for DOX Niosomes[a]

Formula	PS (nm)	PDI	ZP (mV)	EE%
F1	826.70 ± 8.90	0.05 ± 0.00	−28.42 ± 2.11	27.00 ± 5.03
F2	464.77 ± 7.55	0.05 ± 0.00	−48.96 ± 3.15	37.50 ± 0.50
F3	374.17 ± 8.00	0.05 ± 0.00	−36.58 ± 2.32	15.20 ± 0.43
F4	344.10 ± 5.31	0.05 ± 0.00	−51.20 ± 1.81	43.00 ± 2.17
F5	362.20 ± 15.50	0.05 ± 0.00	−47.15 ± 4.01	50.39 ± 2.11
F6	208.80 ± 18.90	0.05 ± 0.00	−49.59 ± 4.29	25.40 ± 3.10
F7	264.50 ± 13.60	0.05 ± 0.00	−45.04 ± 3.23	31.20 ± 1.90
FT1	358.20 ± 15.80	0.05 ± 0.00	−15.42 ± 1.14	42.30 ± 3.10
FT2	509.00 ± 16.30	0.05 ± 0.00	−19.34 ± 2.40	32.20 ± 2.90
FT3	674.10 ± 14.90	0.09 ± 0.07	−54.27 ± 2.10	41.16 ± 0.70
FT4	657.00 ± 20.00	0.03 ± 0.02	−39.53 ± 3.44	28.07 ± 6.30
FT5	270.90 ± 19.50	0.05 ± 0.00	−32.5 ± 4.00	59.20 ± 1.18

[a]Mean ± SD (n = 3).

recover. The following day, various serial dilutions of the D3 formulation, ranging from 360, 180, 90, and 45 μM, along with the corresponding blank dilutions, were applied to the wells. In addition, a positive control containing DOX at a concentration of 10 μM was included. Following a 72 h period of growth at a temperature of 37 °C, a volume of 20 μL of a solution containing 5 mg/mL MTT was added to each well, and the mixture was incubated for 4 h at the same temperature. After the incubation period, the liquid containing the culture was removed, and the resulting purple crystals of formazan were dissolved by adding DMSO. Afterward, the samples were analyzed for absorbance at wavelengths of 450 and 560 nm using a microplate reader (GloMax) multidetection system. The data were then used to plot cell survival (%) against the drug concentration (μM) in Excel files. The experiment was independently replicated three times, and the results are presented as means ± (SD).

2.2.16. Statistical Analysis. A *t*-test analysis was used to statistically analyze stability studies and permeation studies whereas one-way analysis of variance (ANOVA) was used to statistically analyze the results of PS, PDI, ZP, and EE% In each case, a statistically significant difference was defined as $p < 0.05$ using GraphPad Prism software (ver. 6; GraphPad, Inc., San Diego, CA, USA).

3. RESULTS AND DISCUSSION

3.1. Preparation and Characterization of DOX Niosomes. DOX niosomes, prepared by the two methods, the ether injection method and the thin film hydration method, were further characterized for PS, PDI, ZP, and EE%, as illustrated in Table 3. It was found that the size of niosomes depends on different factors including the HLB value, Chol to surfactant ratio, composition of niosomes, and method of preparation (Karim et al., 2010). The PS of DOX niosomes ranged from 270.90 ± 19.50 to 826.70 ± 8.90 nm. A size comparison between F7 (264.50 ± 13.60 nm) and FT1 (358.20 ± 15.80 nm), which have the same components and amount of composition but different methods of preparation, showed that FT1, prepared by the thin film hydration method, was significantly ($p < 0.05$) larger than F7, prepared by the ether injection method. The discrepancy in the niosomal size is related to the fact that the ether injection method produced smaller niosomes than the thin film hydration method, with ether injection producing SUVs and thin film hydration producing MLVs.[26]

The size of the niosomes can be changed by controlling their composition, as displayed by examining DOX niosomes (F1) and others produced via the ether injection method. The results showed a considerable increase in PS for F1 ($p < 0.05$) composed of Span 60 as a primary component, compared with other formulas (F2−F7) composed of a combination of Span 60 and Tween 60, resulting in smaller niosomes. The difference in the niosomal size is due to the characteristics of these surfactants. Span 60, with its single alkyl tail, larger hydrophobic part, and low HLB (4.7), produces larger vesicles, whereas Tween 60, with its large hydrophilic headgroup and high HLB (14.9), produces smaller niosomes.[27] This finding is consistent with Bayindir et al.,[27] who reported that paclitaxel niosomes prepared with various nonionic surfactants such as Tweens, Brij 78, and Span 20 exhibited smaller sizes compared to those prepared with surfactants having smaller headgroups and longer alkyl chains. Another factor that may affect the size of niosomes is the Chol to surfactant ratio, where FT5 (1:4) showed a significant reduction in PS ($p < 0.05$) when compared to FT1 (1:2) and FT2 (1:1) and all were prepared using Span 60 and Tween 60. The smaller size of FT5 could be attributed to the lower Chol to surfactant ratio when compared to FT1 and FT2 formulas. This result aligns with Mali et al.,[28] who prepared minoxidil-loaded niosomes, wherein modifying the Chol to surfactant ratio while using the same type of surfactant led to an increase in PS. This tendency was also obvious in F4 and F5, which had similar compositions but varied Chol to surfactant ratios as shown in Table 3. When incorporated in formulations, Chol acts as a membrane stabilizer. Gradual inclusion of Chol causes the gel-to-liquid phase transition in lipid bilayers to broaden and eventually disappear. Chol alters the fluidity of chains in bilayers, improving orientational order while decreasing permeability.[27]

PDI is used to illustrate the size distribution within a given sample. The PDI values ranged from 0.02 to 0.08, which is within the predicted range for stable and aggregation-resistant systems, indicating a narrow particle size distribution.[6] In addition, ZP plays an important role in the stability of niosomes.[29] The range of ZP of niosomes was between −15.42 and −53.3 mV. These ZP values are sufficient to produce vesicle repulsion, preventing aggregation and providing stable niosomes.[30]

F5 showed a significantly higher EE% compared to F4 ($p < 0.05$). This trend was obvious throughout other pairs of formulas, including F6 and F7, FT1 and FT2, and FT3 and FT4, where lowering the Chol to surfactant ratio resulted in a

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significant increase in EE% ($p < 0.05$). In particular, the most significant improvement in EE% ($p < 0.05$) was observed when the Chol to surfactant ratio was lowered to 25% in FT5, with a Chol-to-surfactant ratio of 1:4, leading to the highest EE% of 59.20 ± 1.18%. It is important to note that high Chol levels can disrupt the typical bilayered structure of vesicular membranes, resulting in a decline in the drug encapsulation level. A previous study on atenolol-loaded niosomes, exploring various Chol to surfactant ratios, showed similar findings, with a significant reduction in EE% ($p < 0.05$) as the Chol content increased.[31]

The hydrophilic−lipophilic balance (HLB) value has also a significant impact on the EE% of niosomes. The HLB values in the range of 14−17 are not favorable to niosomal formation, whereas a lower HLB value of 8.6 resulted in the highest EE %,[32] in agreement with our findings. For instance, F2 had a lower HLB value of 7 while F3 had a higher HLB value of 12. The EE% values of F2 and F3 differed significantly ($p < 0.05$), based on the HLB values, which were 37.50 ± 0.50 and 15.20 ± 0.43%, respectively.

DOX is a highly unstable drug that rapidly degrades and oxidizes. Several factors may induce the oxidation of DOX, including exposure to water, light, and high temperatures.[14,33] The oxidation of DOX may occur even at room temperature.[33] Two different methods were used in the preparation of DOX niosomes: the ether injection method and the thin film hydration method. Initially, the ether injection method was used to prepare the DOX niosomes. However, due to stability difficulties with the ether-injected niosomes including the visible color change within 3 days of preparation (Figure 2A,B) and low EE%, the thin film hydration approach was subsequently used to prepare DOX-loaded niosomes. The purpose of this method transition was to address the issues related to the low EE% and formulation instability that were seen when using the ether injection method.

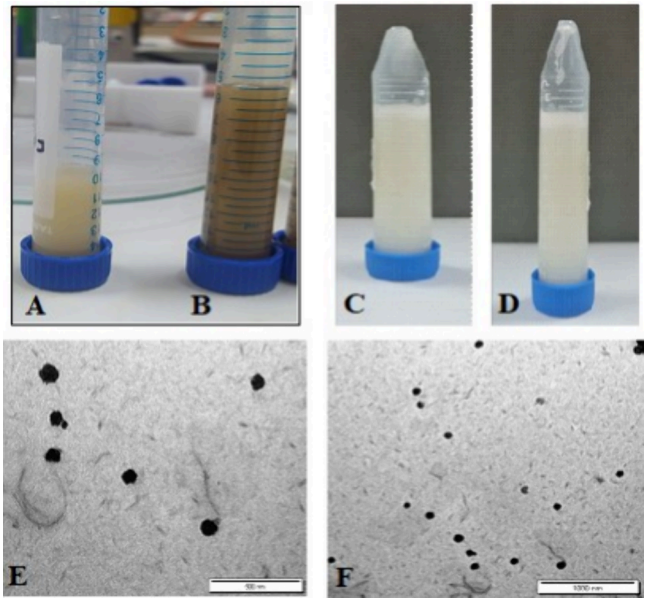

Figure 2. (A) Color of DOX niosomes (F2) on the day of preparation and (B) DOX niosomes (F2) after 3 days of preparation by the ether injection method. (C) Color of DOX niosomes for FT5 formulation on the day of preparation and (D) after 5 days of preparation by the thin film hydration method. TEM micrograph of DOX niosomes (FT5) at different magnifications: (E) 500 nm scale bar and (F) 1000 nm scale bar.

The color change in the niosomal suspension to a brown is an indication of DOX degradation.[14] DOX was loaded into niosomes to protect it from light exposure before being incorporated into a gel matrix. However, it is anticipated that when niosomes were produced by the ether injection method, a significant fraction of DOX was leaked from these vesicles. This leakage left the free drug exposed to light, leading to the degradation of DOX. This result emphasizes the need for more stable niosomal preparation method in order to overcome such anticipated degradation of DOX. Moreover, niosomes manufactured by the ether injection method are classified as small unilamellar vesicles (SUVs) which have a limited capacity for loading drugs as reported in a study published by Chen et al.[26] Hydrophilic drugs tend to experience challenges related to the leakage of the drug into the external medium, as described by Barichello et al.[34] On the contrary, niosomes prepared using the thin film hydration method are multilamellar vesicles (MLVs), which have been pinpointed for their improved stability and higher loading capacity.[26,34,35] Figure 2C,D shows DOX niosomes (FT5) prepared by the thin film method after 5 days of preparation, with no difference in color or texture. Because of stability issues noticed within 3 days after preparation, all niosomal formulations prepared by the ether injection method were excluded from the study. Figure 2E,F shows the shape and morphology of DOX niosomes (FT5) studied by TEM. All niosomes had a spherical shape and were free of aggregation.

3.2. *In Vitro* Release. Based on the EE% values for DOX niosomes, FT1, FT3, and FT5 were chosen for the *in vitro* drug release studies (Figure 3A). The *in vitro* release study was performed for 5 h. This is related to the cosmetics field that aims to serve the consumer with the best results in a short period of time to increase patient compliance.

The majority of previous research has shown that niosomal formulations have the ability to control the rate of drug release.[36−38] The results showed that DOX was gradually released from the niosomal dispersion. Within the 5 h period of time, the cumulative release for FT1, FT2, and FT3 was around 26, 24, and 39%, respectively, as shown in Figure 5. According to the obtained release patterns, all investigated niosomal formulations tend to release the encapsulated DOX in a controlled manner for up to 5 h. Our results were in agreement with Gugleva et al.,[39] who reported that the *in vitro* release studies showed a sustained release of DOX from niosomes. FT5 had the highest cumulative release, attaining 39.00 ± 5.80% over 5 h. This is attributed to the reduced Chol content in FT5, which was 25% compared with 50% in FT1 and FT3. Chol content is important in determining the release of DOX from niosomal formulations.[6,8] This is because Chol effectively seals the pores in the vesicular bilayer, reducing the level of drug leakage. In addition, FT1 and FT3 showed no significant difference ($p > 0.05$) in % release of DOX. Based on the *in vitro* release studies and EE%, DOX niosome FT5 was used in the preparation of the niosomal gels.

3.3. Antimicrobial Test. Figure 3B shows the zones of inhibition of DOX niosomes (FT5), the positive control of the DOX solution (16 μg100 μL, PC), and the negative control (NC) consisting of DMSO and PBS (pH 7.4). The findings clearly indicated that the niosomal formulation improved the antibacterial activity against *E. coli*. This improvement was shown by the higher inhibitory zone diameter of 2.6 mm for

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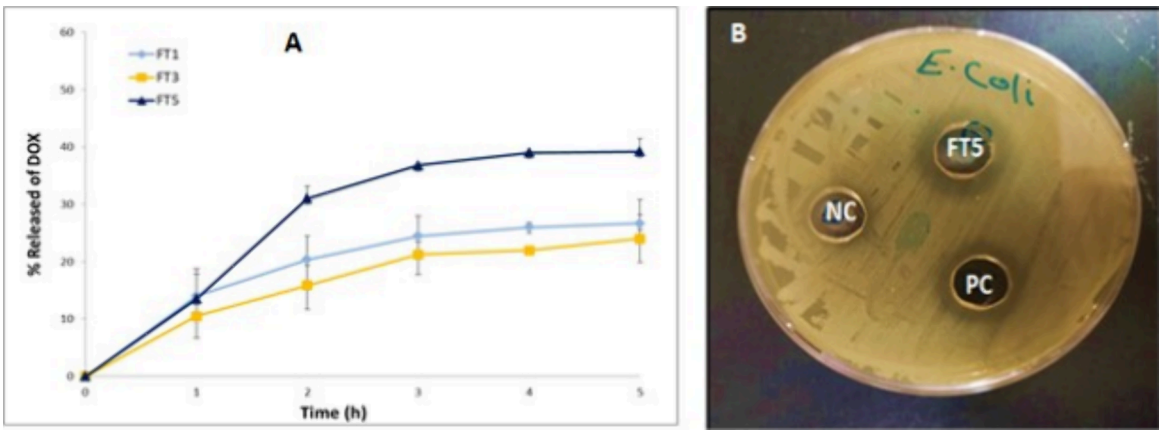

Figure 3. (A) *In vitro* release studies of DOX from FT1, FT3, and FT5 niosomal formulations (n = 3) and (B) zones of inhibition of FT5, positive control (PC) (DOX 16 μg100 μL), and negative control (NC) against *E. coli*.

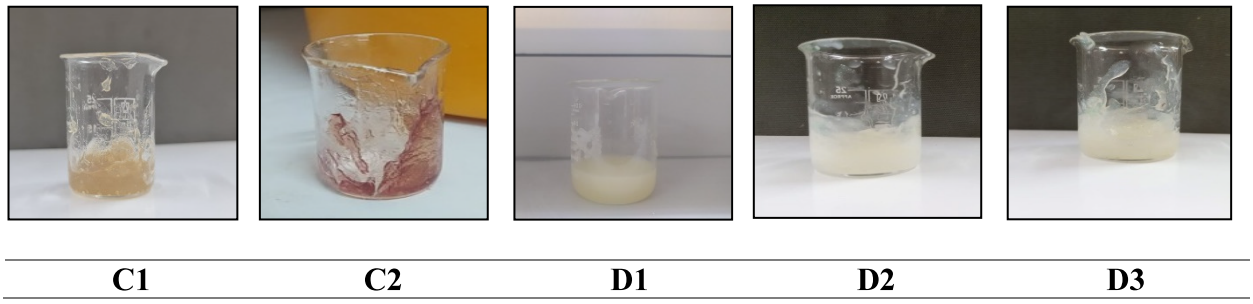

Figure 4. Appearance and color of conventional and niosomal gels.

FT5, which exceeds the 2.3 mm diameter obtained for the positive control (PC). Previously, Kashef et al.[40] investigated the antibiofilm effects of ciprofloxacin-loaded niosomes against an *S. aureus* biofilm and demonstrated that niosome encapsulation reduced the minimum biofilm eradication concentration of ciprofloxacin by 2−4-fold compared to that of free ciprofloxacin.

3.4. Preparation and Characterization of Niosomal Gels. Conventional gels (C1 and C2) were formulated using free DOX to serve as controls, whereas niosomal gels (D1, D2, and D3) were developed using DOX niosomes (FT5) with Carbopol gel matrices, as shown in Table 2. The niosomal gels were evaluated for appearance, spreadability, rheology, *in vitro* drug release, drug content, kinetics release, *ex vivo* drug permeation, deposition study, and stability study.

3.4.1. Appearance. The niosomal gels had a white or slightly yellow color, in contrast to the conventional gels, which had a brown to dark-yellow color (Figure 4). DOX is gradually degraded when exposed to humidity or light, with decomposition intensified at elevated temperatures.[14,33] Therefore, the difference in color indicated that the drug had been incorporated within the prepared niosomes, thereby effectively protecting it from light exposure.[41]

3.4.1.1. pH. The pH values of the niosomal gels (D1, D2, and D3) were 8.20 ± 0.28, 6.71 ± 0.68, and 6.51 ± 0.40, respectively. The pH of the niosomal gels (D2 and D3) closely resembled that of normal skin.[42] These findings give clear evidence that the niosomal gels are well suited for transdermal administration, whereas D1 with a pH of 8.20 ± 0.28, made it inappropriate for skin application. The higher pH in D1 can be attributed to the necessity of using more triethanolamine (TEA) to obtain the appropriate gel texture. Therefore, it was excluded from further characterization.

3.4.1.2. Drug Content. The drug content of the niosomal gels (D2 and D3) was 92.50 ± 2.12 and 99.95 ± 0.07%, respectively. The high DOX content indicated the incorporation of DOX into niosomes, which were further loaded into gels to stabilize DOX and prevent its degradation.

3.4.2. Spreadability. In terms of patient compliance, spreadability is one of the critical parameters of any skin formulation.[43] Spreadability refers to how easily a gel may spread uniformly with little shear stress.[18] Consistent spreading of the topical formulation ensures even distribution of the gel and consistent delivery of the recommended drug dose.[44] The final diameters for D2 and D3 were found to be 1.25 and 1 cm, respectively, indicating good spreadability. Gels also adhere well on skin. The results clearly showed that D2 and D3 had comparable levels of spreadability. This can be related to the gel's viscosity, as gels with a reduced viscosity tend to facilitate smoother and easier spreading.[45]

3.4.3. Rheology of Niosomal and Conventional Gels. Gels display rheological behavior that may influence the release of loaded drugs, affecting their therapeutic efficacy and bioavailability.[46] In addition, the rheological nature of polymer gels plays an important role in understanding how well gels adhere and spread.[45]

Figure 5A shows the flow curves (viscosity vs shear rate) of the niosomal (D2 and D3) and conventional (C1 and C2) gels. Gels exhibited shear-thinning (pseudoplastic) behavior, where the viscosity decreased with increasing shear rate.[47] The niosomal gel D3 had a considerably higher viscosity than D2. The difference in the viscosity of the niosomal gels is most likely due to the type of penetration enhancers used in the gel

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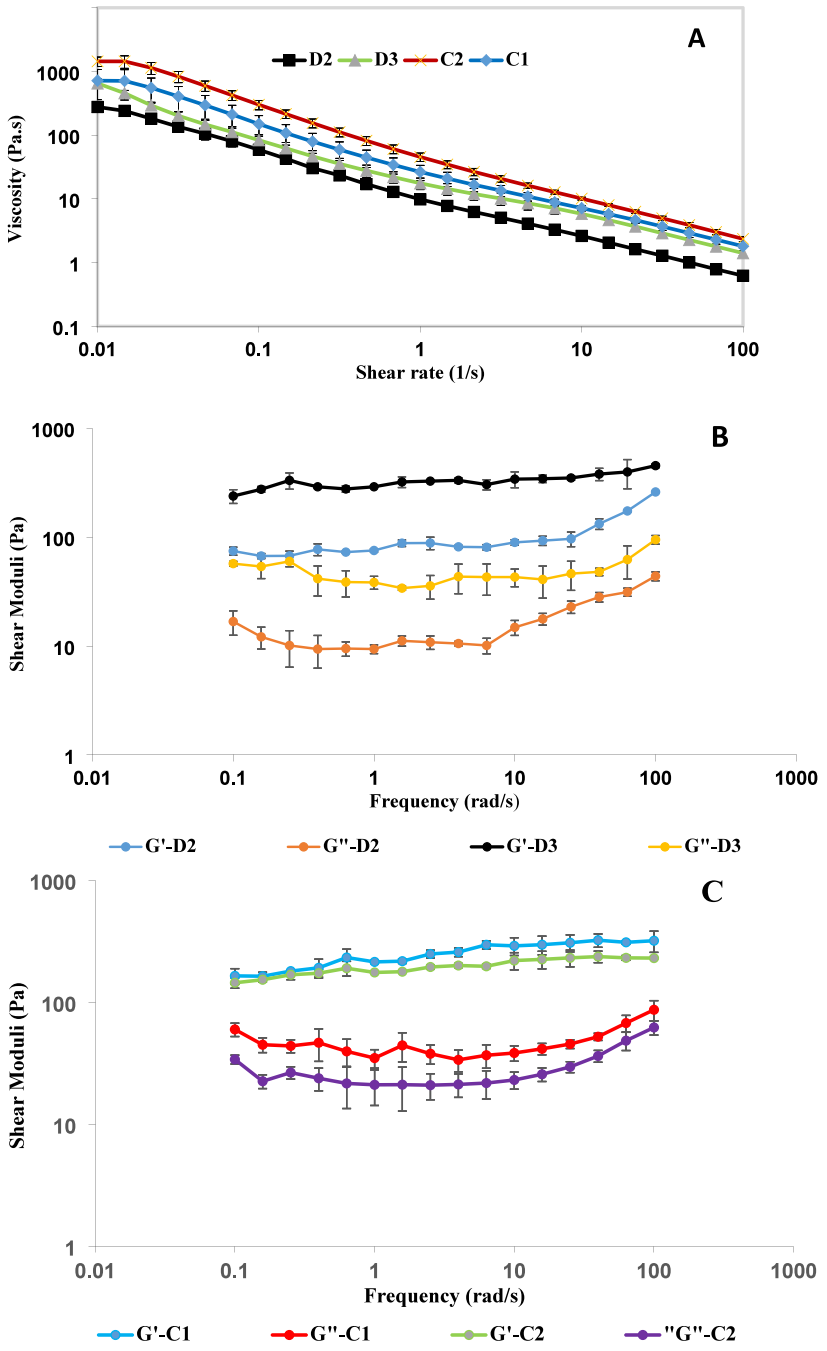

Figure 5. (A) Viscosity versus shear rate for niosomal gels (D2 and D3) and conventional gels (C1 and C2). Frequency sweep of (B) niosomal gels and (C) conventional gels. Data are presented as the mean ± SD (n = 3).

composition. In particular, the addition of PEG 400 to gels resulted in reduced viscosity. This result is consistent with Chessa et al.,[48] who used PG and PEG as penetration enhancers and found that gels containing PEG had lower viscosity than those containing PG.[48] This observation is attributed to the role of PEG within the gel matrix, where Carbopol forms a three-dimensional network with spaces capable of accepting PEG. PEG 400 as a cosurfactant with low molecular weight can be easily accommodated within the cross-linked Carbopol matrix, resulting in more linear behavior. Hydrogen-bonded interpolymer complexes are developed between Carbopol with its proton donor groups (carboxylic acids) and those with proton acceptor groups such as PEG.[49]

Conventional gels (C1 and C2), loaded with free DOX, displayed pseudoplastic (thinning) behavior with high viscosity at low shear rates and lower viscosity at higher shear rates. This indicated that the addition of niosomes maintained the shear-thinning behavior of the niosomal gels, in agreement with our previous study.[19] In addition, the viscosity of C1, containing PEG 400, was lower than that of C2, containing PG, in agreement with observations of D2 and D3. The shear-thinning behavior of the niosomal and conventional gels is crucial for topical application for easily spreading the gels over human skin when rubbed.[19]

The flow curves showed that the niosomal gels (D2 and D3) had lower viscosity values than their corresponding control gels

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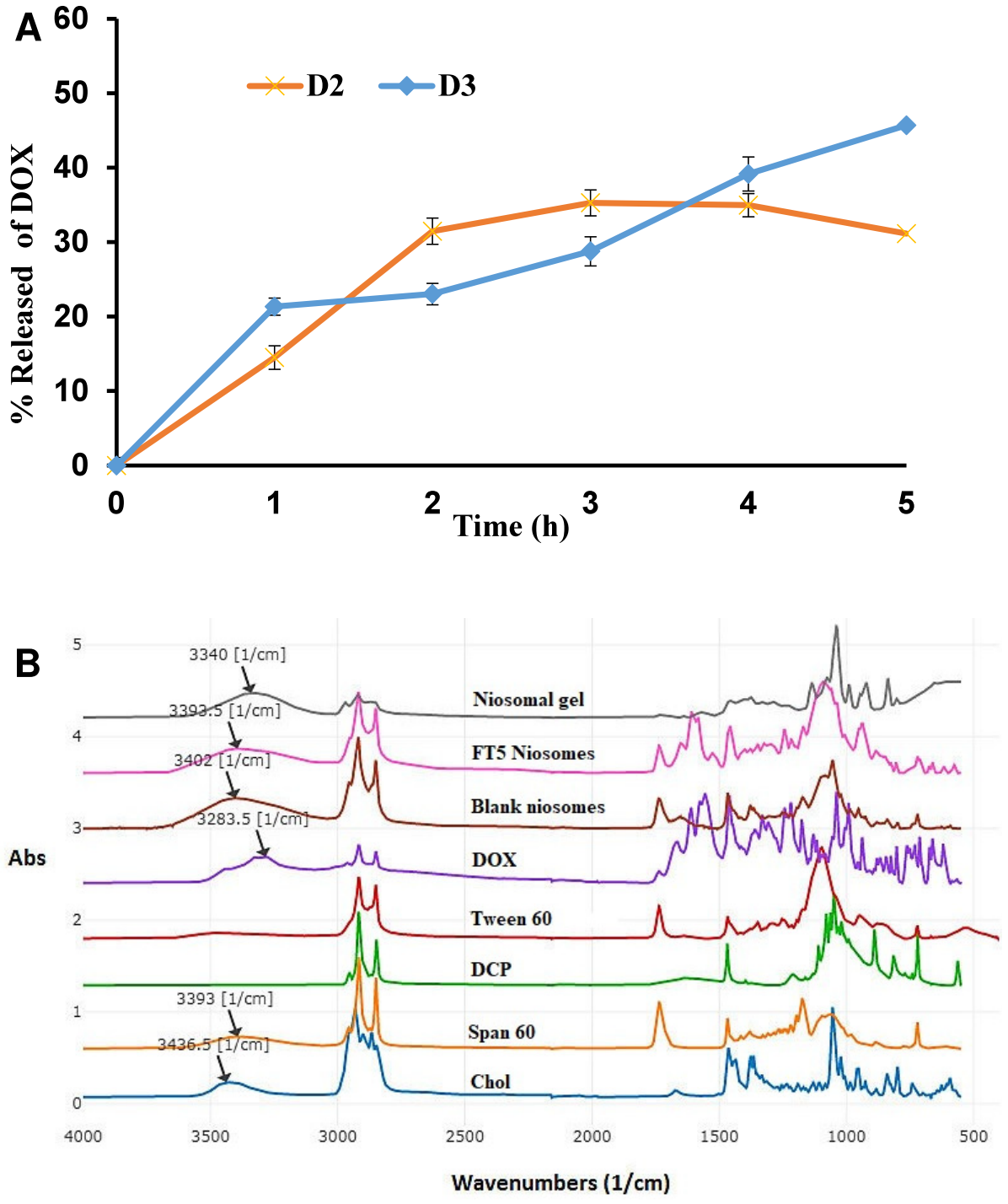

Figure 6. (A) Cumulative release of DOX from D2 and D3 gels. Data are presented as the mean ± SD, $n = 3$. (B) FTIR spectra of Chol, Span 60, DCP, Tween 60, DOX, blank niosomes, FT5, and niosomal gel.

(C1 and C2), which is attributed to the incorporation of DOX niosomes into D2 and D3 gels that may interfere with the Carbopol network, weakening the interactions between the interpolymer connections and decreasing their thickening effectiveness. These findings are in alignment with Zaid Alkilani et al.[19] and Kumbhar et al.,[44] who prepared shear-thinning niosomal gels of azithromycin and lornoxicam, respectively, and found that the viscosity of the niosomal gels was lower than that of the conventional gels. In addition, Kumbhar et al.[44] reported that the lower viscosity of the niosomal gels provides better spreadability compared to that of conventional gels.

The amplitude sweep test was performed on the niosomal (D2 and D3) and conventional (C1 and C2) gels to determine the elastic (storage, G') and viscous (loss, G'') moduli when the strain is in the linear viscoelastic region. In this test, G' and G'' of the gels are measured by applying an oscillatory strain and observing the response of the gel.[20] The LVE region of D2, D3, C1, and C2 was in the strain range of 0.010−0.631%.

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In this region, gels remained unbroken over a broad strain range.

The frequency-sweep measurements were conducted on the niosomal (D2 and D3) and conventional (C1 and C2) gels using a constant strain value of 0.1%, selected within the LVE region, to determine the viscoelastic properties (G' and G'') of gels. The viscoelastic properties of gels are shown in Figure 5B,C. In the investigated frequency range (0.1−100 rad/s), the four gels showed a dominance of the elastic modulus (G') over the viscous modulus (G''), suggesting stable gels with more solid-like behavior. The higher G' and G'' of D3 compared to those of D2 indicated that D3 was more elastic (Figure 5B) and thereby could withstand stress when applied to the skin. In addition, G' and G'' of C1 were slightly higher than those of C2. These results showed that the addition of niosomes maintained the viscoelastic characteristics of gels with G' dominating G'', in agreement with ref 19. However, based on the composition of the gels, the effect of niosomes on the viscoelastic characteristics of D2 and D3 gels varied.

3.4.4. In Vitro Drug Release Study of Niosomal Gels. Figure 6A shows the release profiles of DOX from niosomal gels D2 and D3. The differences in DOX release profiles between niosomal gels D2 and D3 are due to compositional differences. D3, which contains propylene glycol (PG) but lacks polyethylene glycol (PEG 400), has a larger initial release of DOX than D2, which lacks PG. This difference could be due to PG's possible effect on drug solubility and gel matrix characteristics. The cosolvent utilized in the semisolid preparation must be carefully chosen to provide effective skin delivery of the loaded drug. PG is a more effective absorption enhancer than PEG 400 as previously described.[50]

The results indicated that the release of DOX from these gels exhibited a biphasic profile. Initially, there was a rapid release of the drug, where approximately 14.50 and 21.33% of the encapsulated drug was released within the first hour from D2 and D3, respectively. The rapid drug release can be attributed to the fact that the encapsulation of hydrophilic drugs such as DOX in niosomes can occur via two primary ways. These drugs can either be contained within the niosomal internal aqueous core or adsorbed onto the lipid bilayer's surface.[51] Therefore, the DOX adsorbed onto the lipid bilayer's surface was released within the first hour. Consequently, when these vesicles were dispersed in the buffer solution, the drug gradually released from the niosomal internal core. Over the subsequent 5 h, 31.13 and 45.69% of DOX were released from D2 and D3, respectively. The cumulative release of DOX from D3 was significantly higher than that from D2 ($p < 0.05$). Following this period, the entrapped drug exhibited a sustained release profile. Our findings are consistent with those presented by El Ridy et al.,[52] who showed that lornoxicam release from niosomal gels was biphasic. It is worth noting that the variations in the *in vitro* release pattern could be influenced by many factors such as lamellarity, vesicle size, and membrane fluidity.[39] These factors, in turn, are dependent on the chain length of the surfactant used in the preparation of the niosomes.

3.4.5. Fourier Transform Infrared Spectroscopy (FTIR). Samples of Span 60, Tween 60, Chol, DCP, DOX, blank niosomes, FT5, and niosomal gel were examined by FTIR (Figure 6B). The OH stretching peak of Span 60 was identified in the blank niosome spectrum at 3402 cm^{-1}. The carbonyl dimer was observed to shift to 2918 cm^{-1}, whereas the C═O stretching peak changed to 1737 cm^{-1}. The changes in the peaks corresponding to the carbonyl groups could be attributed to Span−cholesterol interactions, specifically hydrogen bonding, which is indicative of the formation of niosomes.[19] The majority of the distinctive peaks related to Span 60, Tween 60, and Chol can be identified in the FTIR spectra of the blank and FT5 niosomes. In the spectrum of FT5 niosomes, strong peaks at 3392, 2918, and 1737 cm^{-1} were observed, and the peaks are most likely due to OH stretching, the carbonyl dimer, and C═O stretching, respectively. Nonetheless, the characteristic peaks of DOX and excipients were presented in niosomes and niosomal gel, with a shifting at a wavenumber of 3340 cm^{-1} in niosomal gel attributed to a strong hydrogen bond between formulation components.[53] The hydrogen bond is most likely formed due to the interaction between the glycerol oxygen in Span 60 and the β-OH group in Chol as described in ref 54. The similarity noticed between the FTIR spectra of FT5 niosomes and blank niosomes serves as strong evidence indicating the efficient trapping of DOX within the niosomal structure as seen in Figure 6B, which is consistent with the findings reported in previous studies.[55,56]

3.4.6. Kinetics Release. The mathematical kinetic models were employed to determine the mechanism of DOX release from the niosomal gels (D2 and D3), namely, first-order and Korsmeyer-Peppas models (Table 4).

Table 4. Kinetic Analysis of the Release Data of DOX from Niosomal Gels (D2 and D3)

	Korsemeyer-Peppas		First order
Niosomal gel	Coefficient of correlation (R^2)	n values	Coefficient of correlation (R^2)
D2	0.9400	0.72	0.8440
D3	0.9020	0.56	0.9790

To release a drug from a delivery system, it is important to understand the precise mass transport mechanisms involved in drug release and to quantitatively anticipate the subsequent drug release kinetics.[57] The Korsmeyer-Peppas model is a more complicated kinetic model compared with a first-order model that is frequently used to predict drug release from polymeric matrixes. Unlike the first-order model, the Korsmeyer-Peppas model incorporates both Fickian diffusion and non-Fickian mechanisms which enable drug release from such matrixes.[58] In addition, the first-order model is better suited to dosage forms containing water-soluble drugs in a porous matrix, while the Korsmeyer-Peppas model is suitable for systems with a polymeric matrix or a nonuniform drug distribution suh as hydrogels.[59,60] The correlation coefficients (R^2) of the kinetic models was used to determine the best model that fits the release data.[46] The release of DOX from D2 perfectly followed the Korsmeyer-Peppas model, where the trend line or regression line showed the highest value of R^2. In the Korsmeyer-Peppas model, the value of n describes the release mechanism of the drug,[61] where the slope of the plot represents the release exponent (n). $n < 0.45$ corresponds to Fickian diffusion, $0.45 < n < 0.89$ corresponds to non-Fickian transport, and $n > 0.89$ corresponds to super case II transport.[61] For D2, n was found to be 0.72, implying that the drug release from D2 followed non-Fickian (anomalous) transport that is coupled with diffusion and polymer relaxation mechanisms.[36] The Korsmeyer-Peppas model appears to best explain the drug release from niosomal formulations, in

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accordance with a previous study which investigates niosomes as a drug delivery platform.[36,62−64] For D3, it was found that the best fit model was the first-order model (R^2 = 0.9790), indicating that the rate of drug release is proportional to the residual drug concentration in the delivery system.[65] Similar findings have been seen by Saafan et al.,[66] who investigated the release kinetics of chloroquine-loaded niosomes and showed that the first-order model was the most appropriate model.

3.4.7. Ex Vivo Drug Permeation and Deposition Studies. A permeation study was performed to investigate the best formulation that permeated the skin. Figure 7 illustrates the

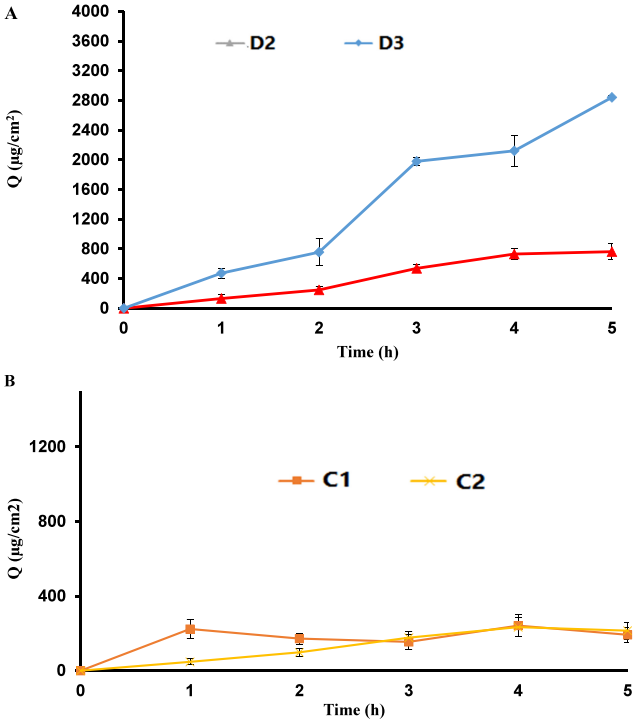

Figure 7. Cumulative amount of DOX permeating through rat skin per unit surface area (Q) vs time (t) for (A) D2 and D3 and (B) C1 and C2. Data are presented as the mean ± SD (n = 3).

permeation profiles of DOX permeating across full thickness rat skin (700 μm) following the application of the niosomal (D2 and D3) and conventional (C1 and C2) gels. The permeation studies for all gels were conducted for 5 h. The cumulative amount (Q) of DOX permeating rat skin per unit area (μg/cm^2) was plotted versus time (t) for all gels. The results demonstrated that D2 and D3 delivered 433.49 ± 8.04 and 1614.33 ± 14.23 μg/cm^2 of DOX, respectively, within a 5 h period. These values corresponded to 42.38 ± 1.06 and 71.2 ± 0.6% of DOX released from D2 and D3, respectively. The steady-state flux (J_{ss}), determined by calculating the slope of Q/A versus time[47] was 86.69 ± 0.91 and 322.86 ± 2.08 μg/cm^2/h for D2 and D3, respectively. Notably, when comparing J_{ss} of the two niosomal gels, it was found that D3 exhibited a significantly ($p < 0.05$) higher J_{ss} than D2 as summarized in Table 5.

The D3 formulation has the highest release amount (Q/A), 1614.31 g/cm^2, followed by D2, C2, and C1. This suggests that the D3 formulation has significant drug release. The steady-state flux (J_{ss}), which represents the rate of drug release over time, has a similar pattern, with D3 having the highest value at 322.86 g/cm^2/h. In comparison to the conventional gel (C1 and C2), permeability parameters such as J_{ss} and the permeability coefficient increased more than 10-fold using the niosomal gel (D3).

Table 5. Drug Permeation Parameters for the Niosomal and Conventional Gels f in PBS (pH 7.4) at 32 °C[a]

Gel Formulation	(Q/A) μg/cm^2	(J_{ss}) (μg/cm^2/h)	(P) (cm/h) × 10^{-4}
D2	433.49 ± 8.04	86.69 ± 0.91	0.2 ± 0.03
D3	1614.31 ± 14.23	322.86 ± 2.08	0.6 ± 0.02
C1	112.61 ± 15.20	22.52 ± 0.31	0.04 ± 0.00
C2	174.44 ± 4.20	34.88 ± 0.13	0.06 ± 0.00

[a]Data are presented as the mean ± SD (n = 3).

The different penetration enhancers, used in the preparation of niosomal gels, played a vital role in niosome permeation of the skin. For instance, PG may boost drug flux due to the possible occupation of its hydrogen bonding sites, leading to increased drug partitioning and penetration.[67] The mechanism of PG in enhancing drug permeation involves the extraction of lipids from the SC,[68] whereas PEG is thought to be an effective skin permeability enhancer due to its solubilizing capabilities.[67] These results are in agreement with Zhang et al.,[69] where niacinamide was mixed with different penetration enhancers (PG and PEG) for trandermal delivery systems. The results showed that PEG increased the skin retention of niacinamide whereas PG increased the drug's penetration of the skin layer to obtain a successful transdermal delivery system.[69] In addition, Shah et al.[70] evaluated the *in vitro* permeation behavior of tramadol lotion containing PG and PEG as permeation enhancers. The results showed that PG was the best penetration enhancer for the transdermal delivery of tramadol compared with other penetration enhancers.

Furthermore, the remaining DOX was used to determine the DOX retention in the skin. Table 6 shows no statistical

Table 6. %Release and %DOX Remaining in the Skin for the Niosomal Gels (D2 and D3)[a]

Niosomal gels	%Release	%DOX remaining in the skin
D2	42.38 ± 1.06	32.67 ± 7.29
D3	71.20 ± 0.62	25.15 ± 1.50

[a]Data are presented as the mean ± SD (n = 3).

difference ($p > 0.05$) in the percentage of DOX remaining in the skin between D2 and D3. As a result, for acne patients, both niosomal gels (D2 and D3) are useful because of their ability to retain DOX in the skin and target hair follicles beneath the skin layers, thereby limiting the growth of bacteria responsible for acne vulgaris.

When the findings of the *ex vivo* study were combined with the viscosity data and the viscoelastic properties, the effect of gel viscosity on skin penetration became a point of debate. According to the literature, the higher viscosity may retard the drug's ability to permeate the skin,[71] whereas other studies claimed that increased viscosity has no major impact on the skin penetration ability.[72] Furthermore, maintaining an appropriate level of viscosity is thought to be beneficial to preventing the formulation from "running off" and, as a result, prolonging its residence time on the skin. As a result, when developing new products, it is best to aim for a moderate viscosity.[72] In our study, we observed that the viscosity of the system had no influence on the permeation of DOX, which is

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consistent with previous studies which revealed that the viscosity in specific formulations had no detectable effect on the permeation behavior of the system.[72]

3.4.8. Stability Study. After 1 month of preparation, niosomal gels (D2 and D3) showed no significant change in gel clarity and color, as shown in Figure 8. Furthermore, there

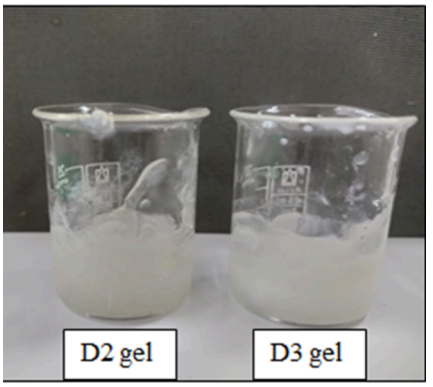

Figure 8. Color and appearance of niosomal gels (D2 and (D3) after 1 month of preparation

was no odor from the gel. Physical changes were not observed, based on the visual examination, after 1 month of storage at 4 °C, where the color, appearance, pH, and %recovery exhibited no significant differences ($p > 0.05$) when compared to the freshly prepared gels as summarized in Table 7.

3.4.9. Cytotoxicity Study. Previous studies have demonstrated that DOX can be toxic to normal cells when its concentration exceeds 200 μM, limiting its utilization at these higher levels.[73,74] Therefore, an assessment of the toxic effect of the DOX niosomal gel was performed using HDF in an MTT assay. The findings of this study suggested that, at the concentrations investigated, neither the DOX niosomal gel (D3) nor the corresponding blank niosomal gel showed a notable harmful effect on HDF cells, as demonstrated in Figure 9. The implications of these findings are noteworthy in relation to the possible applications of the DOX niosomal gel, such as its potential use in treating acne and promoting wound healing.

4. CONCLUSIONS

Niosomes offered an alternative approach to delivering DOX in patients using a stable, low-cost method. Niosomes have been developed and evaluated as a transdermal delivery vehicle for DOX in order to increase drug penetration and improve its stability. The thin film hydration method was used to prepare a number of DOX niosomal formulations, which were examined for shape, PS, PDI, ZP, and EE%. Vesicles observed under a TEM microscope were free of aggregation, with a uniform spherical shape. The niosomal formulation (FT5) showed the highest EE% of DOX (59.2 ± 1.18%). Niosomal gels were developed in an effort to increase DOX stability and avoid the side effects associated with the conventional dosage form of

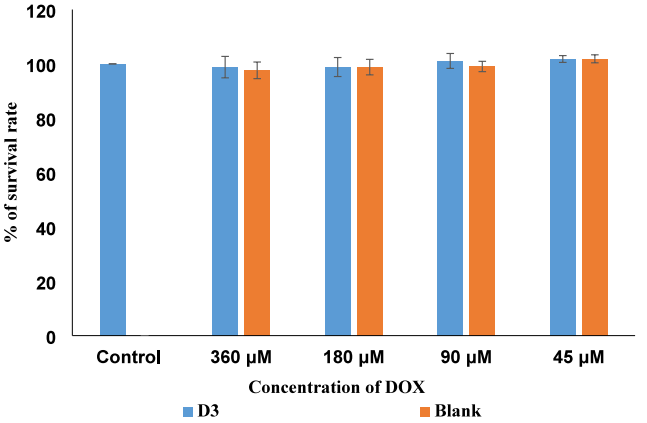

Figure 9. MTT assay of the niosomal gel (D3) and blank gel on HDF cells after 72 h of incubation at different DOX concentrations. Data are presented as the mean ± SD ($n = 3$).

DOX by delivering the gel via a transdermal route. The niosomal formulation (FT5) with the highest percentage of EE, best release percentage, and best stability outcomes was selected for the gel formulation. Niosomal gel loaded with DOX demonstrated a sustained release pattern and higher stability when compared with conventional gel formulations loaded with free DOX. Furthermore, neither the DOX niosomal gel (D3) nor the comparable blank niosomal gel had a negative influence on HDF cells. Permeation studies demonstrated significantly higher DOX permeation when the niosomal gel was applied to rat skin compared to the conventional gel. In comparison to the conventional gel, permeability parameters such as flux and the permeability coefficient increased more than 10-fold when using the niosomal gel. In conclusion, a new niosomal gel formulation could serve as an effective alternative for the currently available commercial form of DOX.

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Table 7. Parameters of D2 and D3 Gels after 1 Month of Storage at 4 °C

	Appearance		pH		%Recovery	
Niosomal gel	Freshly prepared	1 month	Freshly prepared	1 month	Freshly prepared	$_{10}$ month
D2	Clear	Clear	6.71 ± 0.68	7.20 ± 0.14	92.50 ± 2.12%	95.00 ± 1.70
D3	Clear	Clear	6.51 ± 0.40	6.23 ± 0.32	99.95 ± 0.07%	100.00 ± 0.52

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Notes

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