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Formulation and characterization of proniosomal gels loaded with levofloxacin for dermal drug delivery

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ABSTRACT

Formulation of proniosomal gels and evaluation of their potential in dermal drug delivery of levofloxacin, an antibacterial drug used to treat complicated bacterial infections. Levofloxacin-loaded proniosomal gels were prepared using coacervation phase separation using nonionic surfactants (spans and tweens). Different parameters of the proniosomal gels were evaluated, including particle size (PS), zeta potential (ZP), drug entrapment efficiency percentage (EE%), *in vitro* drug release, and ex vivo permeation studies. Based on the experimental results, the EE% for the prepared formulas ranged from 32.22±0.86 to 54.83±1.17%. Comparatively to others, levofloxacin could be best encapsulated using span 20. The particle size of the proniosomes ranged from 447±204 nm to 1089±17 nm. Proniosomal gel prepared with span 20 had the smallest vesicle size. The zeta potential range of prepared proniosomes was from 20.95±0 mV to 60.92±0.09 mV. The prepared formulations were found to have a polydispersity index ranging from 0.198±3.23 to 0.967±0.36. Almost all of the formulas displayed a linear release profile ranging from 33.028 to 97.56 percent over 4 hours. A higher level of drug deposition was observed with span 80 compared to tween 80 after 6 h: 18.296% versus 9.44%. The stability study showed that there was no significant change in EE%, PS, or ZP of levofloxacin proniosomal gels after 3 months of storage. In conclusion, the dermal application of the investigated proniosomal gel formulations demonstrated promising results as nanocarriers for levofloxacin.

Keywords: *Levofloxacin; spans, tweens; proniosomal gels; dermal drug delivery.*

1. INTRODUCTION

Levofloxacin is a member of the third generation of fluoroquinolones with a potent antibacterial effect and broad-spectrum activity against a wide range of bacterial strains. Levofloxacin is a chiral fluorinated carboxyquinoline, which is the L isomer of ofloxacin. It exerts its antibacterial effect by inhibiting DNA GYRASE and DNA IV topoisomerase resulting in a lethal effect for the cells. Levofloxacin is the drug of choice for treating many complicated infectious diseases. It can effectively treat respiratory tract infections, such as acute sinusitis, acute bronchitis, and pneumonia. In addition, it can be used effectively to treat complicated urinary tract infections caused by resistant strains. Thus, it is an interesting candidate for the treatment of human infectious diseases, but it has several adverse

effects when taken orally. Levofloxacin's most common adverse effects are gastrointestinal (GI) disturbances and stimulation of the central nervous system (CNS). The hydrophobic properties of levofloxacin present another challenge to its clinical use. Through dermal delivery of levofloxacin, both GI and CNS side effects can be eliminated, decreasing the dose required at the same time **[1, 2, 3]**.

There have been numerous trials in the field of nanotechnology to develop vesicular drug delivery systems. Liposomes and niosomes are the prototypes of the vesicular systems. Despite their multiple advantages as vesicular delivery systems, liposomes still have major drawbacks in terms of chemical stability issues. The associated issues paved the way for the exploration of the non-ionic surfactant vesicles which are known as niosomes. Even after the niosomes overcome the complications associated with the chemical stability of phospholipids, they still have some physical issues such as aggregation, fusion, or leakage of drug molecules from the vesicles during storage. **[4]**.

Proniosomes are a more stable version of niosomes. They are dry powders that form liposomal dispersion on brief agitation in hot aqueous media. This dry, free-flowing output is more suitable for long-term storage and sterilization. Proniosomes are considered to be an alternative to liposomes and other vesicular systems for entrapping hydrophobic and hydrophilic drugs. Comparing them to other vesicular carriers, they show better cost productivity, chemical and physical stability than liposomes, lower toxicity due to their non-ionic nature, and an easy formulation, which made them a promising industrial product **[5]**. Proniosomal gel preparations are semisolid liquid crystals made from non-ionic surfactants. In addition, they contain cholesterol and lecithin in lower concentrations. On top of that, cholesterol

and lecithin are also present in low concentrations. A minimum amount of organic solvent, like ethanol, and aqueous phase, like water, were needed for formulation. These structures are considered to be liquid crystalline compact niosomes hybrids that can be converted into niosomes in situ after hydration **[6]**.

The semisolid consistency of the proniosomal gel made them easy to be applied directly to the skin. In addition to the penetration-enhancing properties of the added surfactants, proniosomal gels could also serve as an effective vehicle for delivering many medications through the dermal route **[7]**.

2. MATERIALS AND METHODS

2.1. Materials

Levofloxacin powder sample was provided by Amoun pharmaceuticals, [Egypt]. Ethanol, Propylene glycol, Sodium dihydrogen monophosphate, and Disodium hydrogen diphosphate powders were provided from Nasr pharmaceutical company, Egypt. Pigskin was obtained from the local slaughterhouse. Cholesterol was purchased from the Sigma pharmaceutical company, Egypt. Spectra/Por® dialysis membrane, molecular weight cut-off 12,000-14,000 Da was purchased from Spectrum Laboratories Inc., Rancho Dominguez, and Canada. Span 20, Span 40, Span 60, Span 80, tween 20, Tween 40, Tween 60, Tween 80 were purchased from ADWIA, El-Nasr Pharmaceutical Co (Cairo, Egypt). Solutol® HS 15, Soybean Lecithin was provided as a sample from BASF (Ludwigshafen, Germany).

2.2. Methods

2.2.1. The preparation of proniosomal gels using coacervation phase separation method

Using the coacervation phase separation technique, preliminary experiments were conducted to select the ideal surfactants, their

concentration, lecithin, and cholesterol concentrations for the preparation of proniosomal gels. Nine diverse surfactants were screened, namely spans (span 20, 40, 60, and 80), tweens (tween 20, 40, 60, and 80), and solutol. In these formulations, different concentrations of surfactant were blended with levofloxacin, lecithin, and cholesterol.

As a way of examining the effect of cholesterol amount, different aqueous phases, and surfactants, various quantities of span 20 (250, 300, 350 mg) and a variety of phases (distilled water, phosphate buffer, and glycerol) were used.

The coacervation phase separation system was the most prevalent utilized strategy for the preparation of proniosomal gels. In a beaker: lecithin, levofloxacin, cholesterol (25 mg for each), and the surfactants (250 mg each) were dissolved in 0.5 mL of absolute isopropanol. The beaker was covered with a lid to avoid evaporation of the solvent and all the ingredients were warmed over a water bath at 60-70 °C till the surfactant mixture was dissolved completely. At last, the aqueous phase (0.16 mL of distilled water) was added to the blend. At that point, the preparation was warmed again over a water bath followed by cooling the solution overnight at room temperature until the dispersion was converted into gel [7, 8]. The composition of the proniosomal gels integrated with different quantities of the surfactants, lecithin, and cholesterol was enlisted in **Table 1**.

2.2.2. Characterization of levofloxacin loaded proniosomal gels

2.2.2.1 Characterization of particle size and zeta potential of levofloxacin loaded proniosomal gels

In a small glass vial, 100 mg from the proniosomal gel were accurately weighed. The proniosomal gels were appropriately diluted with distilled water before the measurements of the particle size. Average zeta potential and charge on the proniosomal preparations were determined after hydration with phosphate buffer PH (7.4) at 25 °C and three runs were carried out. The mean particle size and zeta potential for formulations were determined using a zeta sizer instrument (Malvern instruments, Nano ZS) [9, 10].

2.2.2.2.   Characterization of the drug entrapment efficiency

The percentage of the entrapment efficiency in hydrated proniosomes was determined by the centrifugation technique. 100 mg of the proniosomal gel was taken in a dry clean glass tube, and 10 mL of phosphate buffer (PH 7.4) was added. The fluid suspension was then sonicated in a sonicator bath (Copiague, N, Y, model number SC,52) for almost 30 min. At that point, the hydrated proniosomes were centrifuged at 15,000 rpm at 25 °C for 30 min. Where levofloxacin-containing niosomes were separated from the unentrapped drug by centrifugation technique. The supernatant containing unentrapped drug was withdrawn and investigated utilizing Shimadzu UV-visible spectrophotometer at 293 nm with the aid of utilizing phosphate buffer at PH (7.4) as a blank. The percentage of the entrapment efficiency can be calculated as follows by using this equation: $EE = [(C_t - C_r)/ C_t] * 100$ Where, C_t: the concentration of total levofloxacin, C_f: the concentration of free levofloxacin [11].

2.2.2.3 In vitro release of levofloxacin-loaded proniosomal gels.

By utilizing the dialysis method, the in vitro release profile of levofloxacin-loaded proniosomal gel was determined. The two ends of the dialysis bag were tightly closed with clips. Then, the dialysis bag was soaked in warm water for 10 min [9].

100 mg of the proniosomal gel was placed in a previously-swelled dialysis sheet and immersed

in 100 mL phosphate buffer which acts as a receptor compartment. Over a magnetic stirrer (Falc), the outer phase was stirred continuously. The heat was provided using a thermostatic hot plate with a magnetic stirrer to maintain the temperature at 37 °C. The samples were withdrawn at previously set time intervals of 0.25, 0.5, 1, 1.5, 2, 3, and 4 h followed by replacement with 2 mL fresh phosphate buffer to preserve the same total volume. The supernatant was then analyzed spectrophotometrically at 293 nm [12].

Table 1. The composition of the proniosomal gels integrated with different quantities of the surfactants, lecithin, and cholesterol

Formulation code	Surfactant quantity (mg)	Lecithin quantity (mg)	Cholesterol quantity (mg)	Drug quantity (mg)	Isopropanol (mL)	Distilled water (mL)
PN1-S20	250	25	25	25	0.5	0.16
PN2-S80	250	25	25	25	0.5	0.16
PN3-T60	250	25	25	25	0.5	0.16
PN4-T80	250	25	25	25	0.5	0.16
PN5-sol	250	25	25	25	0.5	0.16
S20 High	500	25	25	25	0.5	0.16
S80 High	500	25	25	25	0.5	0.16
T60 High	500	25	25	25	0.5	0.16
T80 High	500	25	25	25	0.5	0.16
Solutol High	500	25	25	25	0.5	0.16
Lecithin High	250	50	25	25	0.5	0.16
Lecithin V. High	250	75	25	25	0.5	0.16
Cholesterol high	250	25	50	25	0.5	0.16
Cholesterol V. High	250	25	75	25	0.5	0.16

The PN1-S20:proniosomal gel containing span 20, PN2-S80: proniosomal gel containing span 80, PN3-T60: proniosomal gel containing tween 60, PN4-T80: proniosomal gel containing tween 80, PN5-sol: proniosomal gel containing solutol, S20 High.

2.2.2.4 Ex vivo permeation of proniosomal gels loaded with levofloxacin

Fresh pig ears were provided from a nearby butcher house directly after the animal sacrifice. Full-thickness skin of the back of pig ears was carefully removed from the underlying subcutaneous lipids and cartilages using a scalpel. The extracted skin was cut into suitable pieces and soaked in phosphate buffer (PH 7.4) for one hour before utilization. Skin permeation tests were done utilizing Franz diffusion cells (diffusion area= 2.7 cm^2 and total receptor volume = 25 mL). The skin was then mounted in open two-chamber Franz-type diffusion cells loaded with phosphate buffer (PH 7.4). The total receptor volume which is equal to 25 mL of phosphate buffer was taken in the receptor compartment [13].

By utilizing clips, the receptor and the donor compartments were held and kept up at a temperature of 37±0.5 °C with the utilization of a water bath. The samples were withdrawn at predetermined time intervals of 0.25, 0.5, 1, 1.5, 2, 3, 5, and 6 h from the receptor compartment and immediately substituted with an equivalent volume of new receptor solution [11].

The extent of the drug deposited in the epidermal layers was determined. After the completion of the ex-vivo diffusion study, the skin was isolated from the Franz-diffusion cell. The skin was finely partitioned into three definite layers (stratum corneum, epidermis, and dermis). Using adhesive tape, stripping of the skin was performed firmly for successive 15 times in which firm squeezing was finished. By the utilization of surgical blades and forceps, the epidermis was separated from the dermis layer into two separate layers. Tape strips containing stratum corneum and skin layers of both epidermis and dermis were put separately in methanol. Then, they were left to be soaked overnight followed by sonication to extract the deposited drug molecules. The amount of levofloxacin deposited into the stratum corneum, epidermis, and dermis was expressed as a percentage of the total drug amount and was determined utilizing a UV spectrophotometer and there was no interference between different skin components and the UV measurement of the drug [14].

2.2.2.5 Stability studies

Assessment of the drug encapsulation percentage was done by the storage of the proniosomal gels at refrigeration temperature (4 °C-8 °C). Different samples were taken after a period of 3 months and leakage of drug from the formulations was analyzed for drug content using a UV spectrophotometer at 293 nm [15].

2.3. Statistical analysis

All experiments were performed in triplicates and average values were calculated. ANOVA and paired t-tests were used for statistical analysis using Graphpad® software. Differences were considered significant if the p values were less than 0.05.

3. RESULTS AND DISCUSSION

3.1. Preparation of levofloxacin loaded proniosomal gels

Several surfactants such as spans and tweens were screened to choose a convenient surfactant for producing gel formulations. Higher amounts of the selected surfactant, lecithin and cholesterol were examined to verify their effects on gel formulation success. As a result of several series of preliminary investigations with different aqueous and organic phases, isopropanol and distilled water were selected as the most suitable solvents for the subsequent experiments. After then, choosing the appropriate surfactants was easy: either spans (span 20 and span 80) or tweens (tween 60 and tween 80) or solutol [16].

Nonionic surfactants are the most common

type of surface-active agents used in preparing vesicles due to their superior benefits concerning stability, compatibility, and toxicity. By adding cholesterol, more hydrophobic surfactants can form vesicles, the tendency to an aggregate of the surfactant is suppressed, and the gel liquid transition temperature of the lipid bilayer is promoted providing more stability to the vesicle. Phosphatidylcholine is a major component of lecithin. Lecithin plays several crucial roles within the vesicular system: [A] It enhances permeation; [B] It enhances drug entrapment due to its high phase transition temperature [Tc] [C] The high phase transition temperature enhances the compactness of the bilayers with the minimum leakage of the medication molecules out of the vesicles. [D] It aids in producing smaller vesicles as a result of an increase in hydrophobicity. Lastly, the addition of water leads to swelling of the bilayer as a result of the interaction between the water and the polar groups of the surfactants, providing multivesicular, multilamellar, and spherical-shaped structures [17].

3.2. Determination of particle size and zeta potential of proniosomal gel loaded with levofloxacin

For physicochemical characterization of proniosomes, the particle size, polydispersity index, and zeta potential were measured. The particle size ranged from 447±204 nm to 1089 ±17 nm. Zeta potential plays an important role in determining the physical stability of colloidal vesicular carriers such as pronisomes. The electrokinetic potential of prepared proniosomes ranged from -20.95± 0 mV to -60.92 ±0.098 mV. High ZP values contribute to the stability of the dispersions, while negative values are due to the ionization of the free hydroxyl groups within surfactants and cholesterol. Additionally, lecithin is composed of phospholipids that, when ionized at neutral PH, contribute to its negative charge.

The prepared formulations have a polydispersity index ranging from 0.198±3.23 to 0.967±0.366. The polydispersity index was not influenced by the formulation composition studied [18]. The results of the particle size, zeta potential, and polydispersity index of the proniosomal gels loaded with levofloxacin were shown in **Table 2.**

3.2.1. Effect of surfactant type

The largest vesicle size was found in tween 80, while the smallest was found in the span 20 proniosomal gel formulation. This could be explained by the difference in entrapment efficiencies. Where entrapment efficiency of T80 was lower than that of S20. As a result of increasing hydrophobicity of the proniosomal bilayers in span-containing formulations, water intake by niosomes and surface free energy was reduced, resulting in smaller vesicles. Study results suggested that the use of different lipids did not increase the hydrophobicity of tween-containing formulations, as well as the particle size, remained large. There has been a suggestion that the size of a vesicle is associated with an alkyl chain length of the surfactant, whereby a longer alkyl chain produces larger vesicles seen in tween-containing formulations [19]. Solutol HS 15 is a recently developed non-ionic surfactant. Solutol proniosomal formulations exhibited relatively large particles as tweens. As it has hydrophilic-lipophilic balance (HLB) value nearly to tweens equal to 15 [20].

3.2.2. Effect of lecithin amount

The results suggested that the addition of lecithin led to an increase in particle size. A higher surfactant/lipid ratio increased in vesicle size and was related to an overall increase in hydrophilicity [17].

3.2.3. Effect of surfactant amount

By adding surfactants to lipid-based vesicles, the size of the vesicles was affected.

Mohamed et al., Arch Pharm Sci ASU 5(2): 288-303

Several factors influence vesicle size, including the concentration of surfactant, the number of carbon chains, and the length of the carbon chains. Due to competition occurring between surfactant molecules and other molecules in the lipid bilayer, the lipid vesicle size increases. Particle size was positively influenced by the amount of surfactant. A possible explanation for this might be the increment in entrapment of the drug resulting in larger vesicles **[18]**.

Table 2. The particle size polydispersity index and zeta potential of the proniosomal gels loaded with levofloxacin

Formulation code	Particle size (nm) mean±S.D	Polydispersity index mean±S.D	Zeta potential mean±S.D
PN1-S20	595 ± 13	0.226± 0.78	−29.86 ± 1.56
PN2-S80	766 ± 66	0.413± 0.23	−25.66±0.73
PN5-T60	934 ± 27	0.677± 2.5	−36.23± 0.12
PN5-T80	988 ± 20	0.555± 1.1	−42.33± 3.56
PN5-Sol	807 ± 9	0.894±0.66	−34.67± 0.32
S20 High	665±48	0.444±0.14	−37.23± 5.56
S80 High	911±32	0.676±0.87	−32.19±0.45
T60 High	1249±5	0.822±0.45	−40.14±8.35
T80 HIGH	1089 ±17	0.923±1.56	−60.92±0.09
Solutol High	988± 46	0.967±0.36	−40.14±0.13
Lecithin High	674± 65	0.334±0.777	−49.45±1.56
Lecithin V. High	835 ± 11	0.198±3.23	−52.19±3.78
Cholesterol High	882±76	0.888±0.99	−56.34±0.84
Cholesterol very high	447±204	0.302±0.147	−20.95± 0

3.2.4. Effect of cholesterol amount

An increase in the mean vesicle size of the produced proniosomes by increasing cholesterol quantity could be attributed to the increase in the drug load and to the drug being entrapped in the hydrophobic domain of the vesicle, which causes the bilayer molecules in the vesicle to separate, causing the vesicle to become larger in size. In contrast, using a higher concentration of cholesterol resulted in smaller particle sizes and increased hydrophobicity. As the cholesterol levels increased, the hydrophilicity of bilayers

decreased, limiting the water intake into the core of the vesicles and thereby increasing their size [9].

3.3. Characterization of the drug entrapment

efficiency

The entrapment efficiency values of proniosomal gels ranged from 32.22% to 54.83% as shown in **Table 3.**

Table 3. The drug entrapment efficiency of proniosomal gels

Formulation name	Used surfactant	Percentage of drug entrapment efficiency
PN1-S20	Span 20	54.83±1.17
PN2-S80	Span 80	51.08±0.86
PN5-T60	Tween 60	40.33±0.77
PN5-T80	Tween 80	49.44±1.603
PN5-Sol	Solutol	32.22±0.86

3.3.1. Effect of the surfactant type

The entrapment efficiency of proniosomal gels prepared with spans was observed to be high in comparison to the proniosomes prepared from various types of the tween.

This can be attributed to the following reasons:

Since the highly lipophilic segment of the drug is entirely encapsulated in the lipid bilayer, this leads to an increase in the drug's entrapment efficiency percent.

The alkyl chain length, phase transition temperatures, and the HLB estimation of the surfactant majorly affect the permeability of proniosomal gels [21].

The EE% of span 20 was significantly higher than that of span 80. Even though span 80 contains more extended alkyl chains. However, it has unsaturation in its structure, reducing its EE%. For tween proniosomal gels, tween 80 has a longer saturated alkyl chain than tween 60.

Also, tween 80 has a lower HLB than tween 60. This resulted in making the EE% of tween 80 significantly higher than that of tween 60. T80 being hydrophilic surfactant with a high HLB value [15] compared to 4.3 in the case of the hydrophobic span 80, this probably explained the lower entrapment efficiency of T80 compared to span 80 formulations [17]. The results were found to agree with the previous data reported by Shaji J et al. 2016 and Taymouri S et al. 2016 which indicated that the lower the HLB of the surfactant; the higher will be the entrapment efficiency [22]. Span 20 proniosomal formulations exhibited the highest entrapment efficiency. Solutol proniosomal formulations showed relatively low EE% relatively near to tweens. As it has HLB value nearly to tweens.

It is important to note, that the results relied on present evidence that indicated that the lower the HLB of the surfactant, and the higher the phase transition temperature, the higher will be the entrapment efficiency [20]. Finally, it was concluded that levofloxacin was the best

encapsulated using span 20 that exhibited the highest entrapment efficiency in comparison to others prepared by tweens [23].

3.3.2. Effect of surfactant amount

There was a positive significant effect of the surfactant amount on EE% for tween 60, tween 80, and span 20. It could be explained by the increased number of vesicles formed by the increased amount of surfactant. Conversely, EE% was decreased with the increase in surfactant amount in the case of span 80 and solutol. This could be due to the formation of mixed micelles together with niosomes, which might lead to lower EE%. Additionally, the permeability of the membrane of vesicles was increased by the surfactant molecules arranged within the lipid bilayer structure. This might introduce pores into the membrane and increase its fluidity, which led to lower EE% [18].

3.3.3. Effect of the amount of cholesterol

As cholesterol concentration increased in proniosomal gels containing levofloxacin, the entrapment efficiency percent increased significantly. This was a consequence of the simultaneous arrangement of the steroidal skeleton with surfactant molecules that resulted in increased rigidity. As cholesterol concentration increased further, entrapment efficiency also decreased. A potential explanation was that drug molecules and cholesterol compete for space in the limited sites of the bilayer structure of niosomes [24].

3.3.4. Effect of lecithin amount

The increase in the concentration of lecithin caused a significant increase in the EE%. On the other hand, a further increase in the concentration of lecithin caused a decrease in the EE%. Nevertheless, a detailed examination of the structure of lecithin showed that double bonds in nonhydrogenated phosphatidylcholine allow the chains to bend. The niosomal membrane is formed when they are assembled. Due to that, the membrane permeability increased, resulting in a decrease in gel encapsulation. However, the saturation of the double bond and the presence of hydrogenated lecithin causes bilayer molecules to be assembled resulting in increased rigidity and a reduction in permeability, consequently increasing EE%. The effect of using different concentrations of surfactants, cholesterol, and lecithin on the entrapment efficiency of the proniosomal gels was shown in **Fig. 1.**

3.3.4. *In vitro* release of levofloxacin loaded proniosomal gels

A sustainable drug release profile for approximately 4 h was achieved by incorporating the drug molecules into proniosomal gels. It was evident that levofloxacin was released from the proniosomes with an initial phase release pattern followed by a sustained release pattern. The initial rapid discharge might be due to the desorption of the drug molecules that were present on the niosome surfaces. Additionally, it has been reported that this drug explosion occurred because the highly ordered lipid particles could not accommodate large amounts of drugs. [12].

The release profile of proniosomal gels showed an increase in drug release accompanied by the use of tweens due to their high HLB value. It could be explained by the fact that these hydrophilic surfactants have better solubilizing power towards hydrophobic solutes as compared to hydrophobic surfactants as spans. As such, this idea was a reasonable clarification for the lower drug release rates observed with span formulations in comparison to tween formulations. Solutol proniosomal formulations showed high release values relatively near to tweens. As it has an HLB value nearly to tweens [25]. The release data for the proniosomal gels was illustrated in **Fig. 2.**
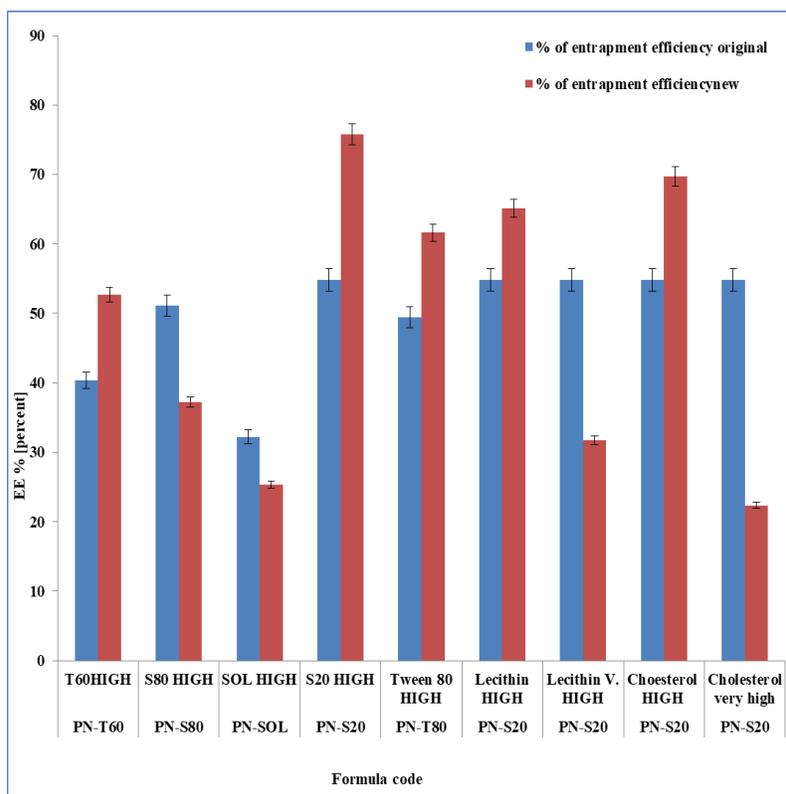
Fig. 1. The effect of using different concentrations of surfactants, cholesterol, and lecithin on the entrapment efficiency of the proniosomal gels
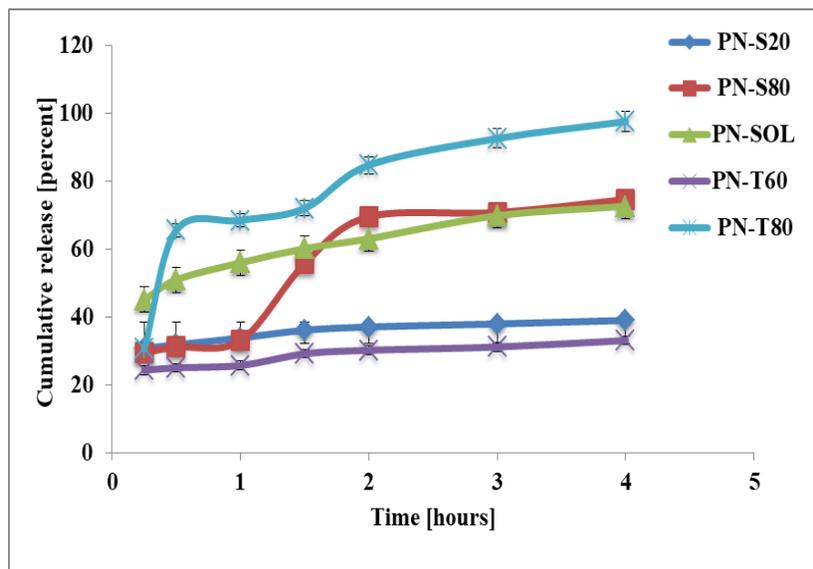

Fig. 2. The *in vitro* drug release data of the proniosomal gel formulations

3.3.4.1. Type of surfactant

There was a linear release of drug in the majority of formulas from 33.028% at the minimum to 97.56% at the maximum over 4 hours. Proniosomes prepared from tween 60 and 80 exhibited alkyl chain length-dependent release. As a result of the unsaturation in the alkyl chains in span 80, the release from the proniosomal gel of span 80 was higher than that of span 20 [26].

3.3.4.2. The effect of surfactant amount

The release of the drug from the proniosomal gel was increased by increasing the concentration of span 20 and span 80. In contrast, the release of medication from the proniosomal gel was significantly diminished by increasing the concentrations of tween 60, tween 80, and solutol.

Surfactants with variable concentrations could greatly affect the release patterns of proniosomal gels. This could be clarified depending on the different properties of the surfactants. In addition to their variable bilayer flexibility, they also exhibit a diverse affinity to drug molecules, which might explain this observed differentiation in the results [26].

The effect of the amount of the surfactant on the in vitro release of the proniosomal gels was shown in **Fig. 3**.
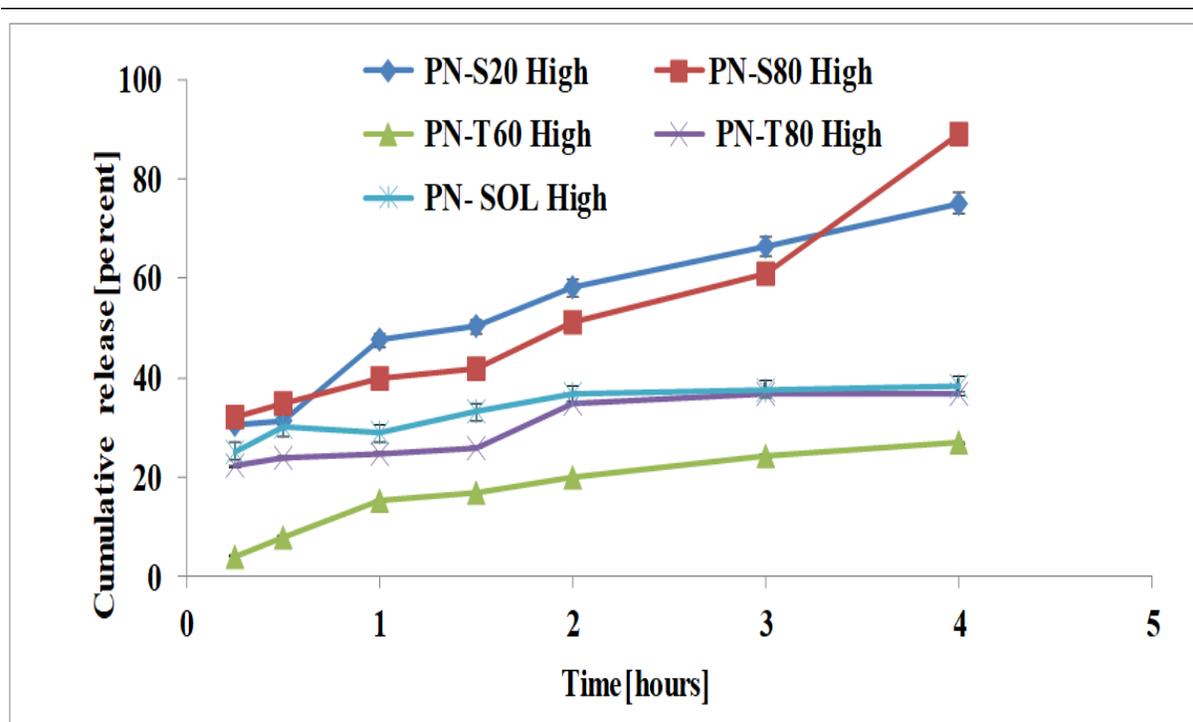

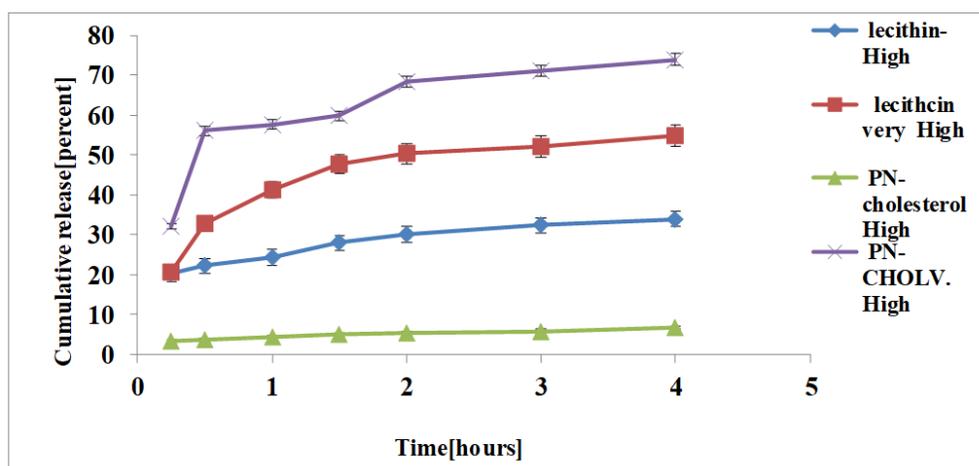**Fig. 3.** The drug release data of the proniosomal gel using a higher quantity of surfactants

Fig. 4. The drug release data of the proniosomal gel using a higher quantity of lecithin and cholesterol
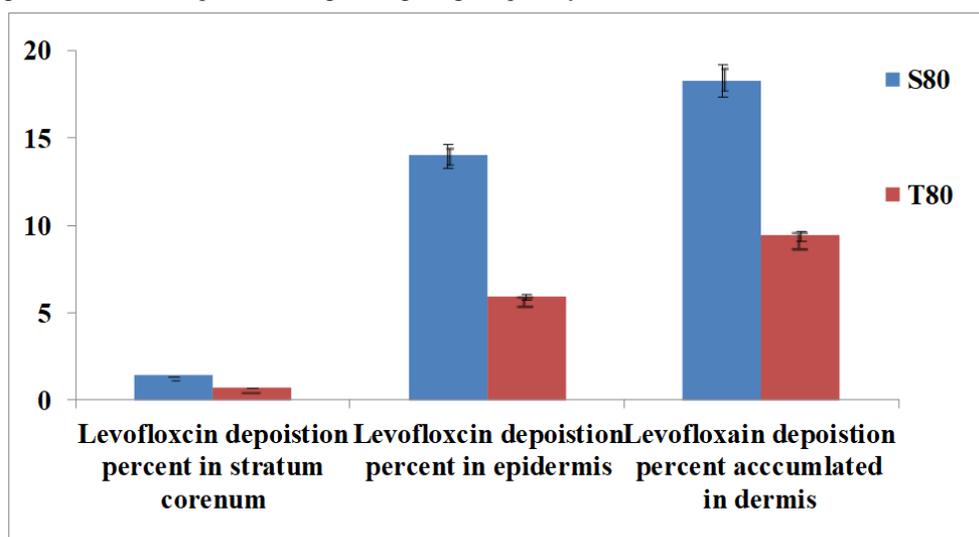

Fig. 5. The percent of the drug deposition in stratum corneum, epidermis, and dermis reflecting the ex vivo deposition for the selected formulae

3.3.4.3. Effect of lecithin amount

Varying the concentration of lecithin had a great effect on the proniosomal gel release rates. This could be further clarified by the fact that lecithin functions as a cosurfactant that aids in the formation of proniosomal vesicles and subsequently enhances the release of drug molecules from vesicles. It increases the hydrophobicity of the vesicles so And increases the permeation of the medications. Lecithin also acts as a penetration enhancer. As a result, increasing its concentration might enhance

medication release [27].

3.3.4.4. Effect of cholesterol amount

The elevated cholesterol content resulted in a decreased drug release effect in comparison to the other proniosomal gel containing higher cholesterol amounts. A potential explanation could be that an initial increment in cholesterol amounts resulted in a thicker lipid bilayer that acts as a barrier to drug release In contrast, cholesterol concentrations that exceeded a certain level might disrupt the regular linear structure of

the vesicular membrane, causing increased drug release **[21]**. The effect of increasing the amount of cholesterol and lecithin on the in vitro release of proniosomal gels was shown in **Fig. 4.**

3.4. Ex vivo/ permeation studies

In comparison to tween 80, span 80 showed a higher percentage of drug penetration across various skin layers after six hours. Tween's lower lipophilic nature compared with span might contribute to that. Nonionic surfactants improve penetration by acting as penetration-enhancing agents. Where they interact with the intercellular lipophilic barrier of the stratum corneum, thereby barring its function **[11]**.

The more the drug molecules are deposited in skin layers with proniosomal gels, the greater their potential to bypass the stratum corneum's barrier function and to deliver the drug effectively. This was also because isopropanol act as a penetration enhancer and its branched structure acts as a cosurfactant, allowing the drug to penetrate more deeply into the bilayer **[12]**. The extent of the drug deposited in the skin layers was shown in **Fig. 5.**

3.5. Stability studies

In the present study, stability studies were conducted on all formulations. The proniosomal gels were stored at 4 °C for 3 months to observe changes in %EE, PS, and ZP. After storage, there have been slight changes in entrapment efficiency and physicochemical characteristics **[10]**. The effect of the storage conditions on the entrapment efficiency and the particle size of proniosomal gels loaded with levofloxacin was shown in **Figs. 6&7.**

Conclusion

Using the coacervation phase separation method, proniosomal gels were prepared with different nonionic surfactants, including spans and tweens, combined with cholesterol and

lecithin measured in determining amounts. The experimental results showed that the EE% for the prepared formulae ranged from 32.22 ± 0.86 to $54.83\pm1.17\%$. The smallest vesicle size was found in proniosomal gels prepared with span 20. A significant increase in entrapment efficiency percent was observed for proniosomal gels containing levofloxacin after the initial increase of lecithin and cholesterol concentrations. While the further increase in their concentration resulted in a decrease in EE%. There was a positive significant effect of using high surfactant concentration on the entrapment efficiency percent in tween 60, tween 80, and span 20. Conversely, the EE% was significantly decreased with an increase in surfactant amount in the case of span 80 and solutol. The particle size of the proniosomes ranged from 447 ± 204 nm to 1089 ± 17 nm. Proniosomal gels prepared with span 20 had the smallest vesicle size. A positive effect was seen on the particle size of the produced proniosomes when the concentration of surfactant and lecithin was increased. The initial increase in cholesterol quantity led to an increase in the mean size of the proniosomes produced. By contrast, higher concentrations of cholesterol resulted in an increase in hydrophobicity and smaller vesicles. The zeta potential of prepared proniosomes ranged from -20.95 ± 0 mV to -60.92 ± 0.09 mV. The prepared formulations had a polydispersity index ranging from 0.198 ± 3.23 to 0.967 ± 0.36. A sustained drug release profile was evident after the incorporation of the drug molecules into the proniosomal gels for approximately four hours. According to the results, most formulas had a linear release profile ranging from 33.028% to 97.56%. The release rate of the drug from the proniosomal gel was increased by increasing span 20 and spans 80 concentrations; however, it was decreased by increasing the concentrations of tween 60, tween 80, and solutol. By varying the concentration of lecithin, the release rate of the proniosomal gels

increased, while the elevated cholesterol content resulted in a reduced release of the drugs in contrast with the other proniosomal gel with a higher concentration of cholesterol. Span 80 showed a higher percentage of drug deposition (18.296%) across different skin layers compared to tween 80 (9.44%) after six hours. The stability study did not reveal any significant changes in levofloxacin EE%, PS, or ZP.

Study results reported that proniosomal gels could be potentially promising carriers for dermal delivery of levofloxacin to improve the therapeutic effectiveness and increase patient compliance.
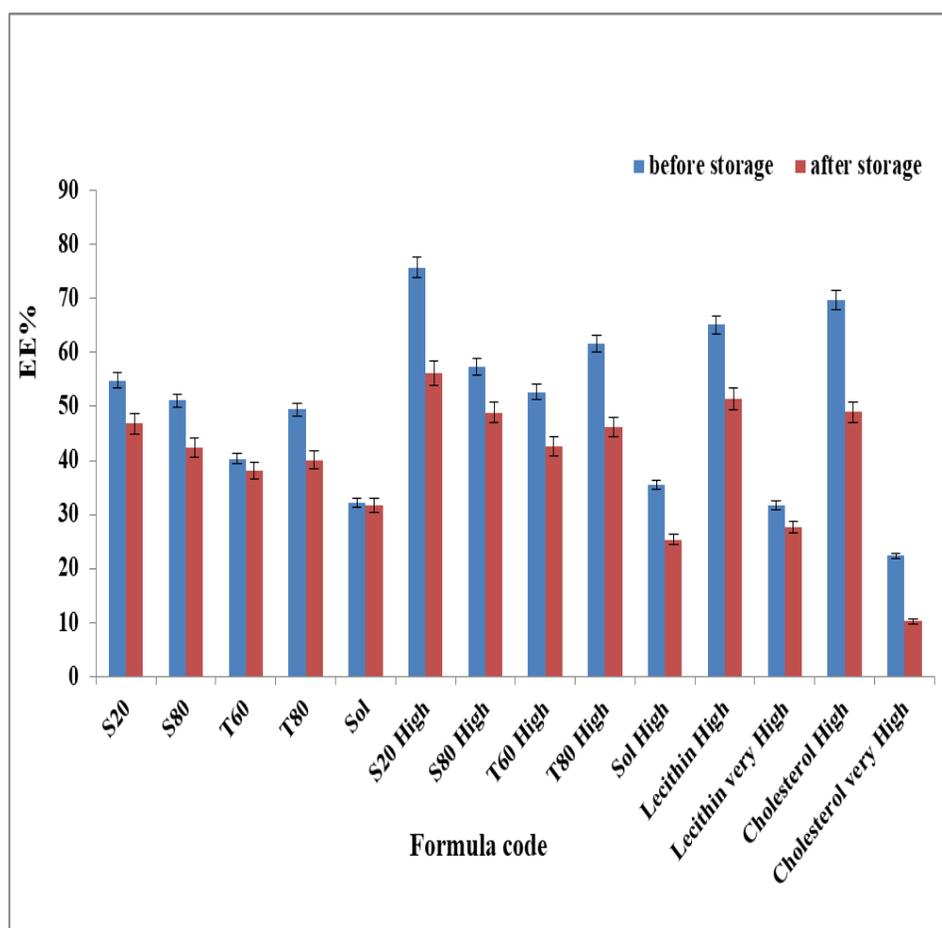

Fig. 6. The effect of the storage conditions on the entrapment efficiency of proniosomal gels loaded with levofloxacin
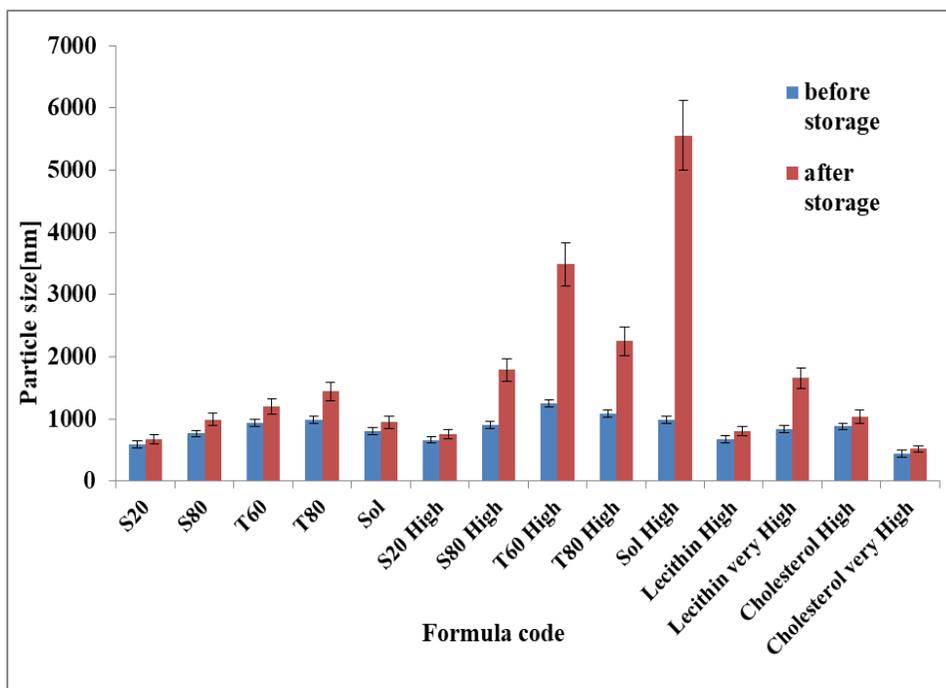
Fig. 7. The effect of storage conditions on the particle size of the proniosomal gels

Declarations

Ethics approval and consent to participate

Not applicable

Consent to publish

All authors have read and agreed to the published version of the manuscript.

Availability of data and materials

Data analyzed during this study are all included in the main manuscript.

Competing interests

No competing interests were declared by the authors.

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