AAPS PharmSciTech (2022) 23:34
DOI: 10.1208/s12249-021-02189-2

Research Article

Formulation and Evaluation of Topical Biodegradable Films Loaded with Levofloxacin Lipid Nanocarriers

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Received 7 September 2021; accepted 29 November 2021

Abstract. Skin ulcers have increased sharply due to rise in the incidence of obesity and diabetes. This study investigated lipid nanocarriers as a strategy to improve the efficacy of levofloxacin (LV) in penetrating skin. Two surfactant types and different lipid mixtures were used in preparation of lipid nanocarriers. Mean particle size, percentage entrapment efficiency (%EE), *in vitro* release, and antimicrobial activity were examined. The selected formula was incorporated into a chitosan (CS) film that was subjected to physic-chemical characterization and *ex vivo* permeation study. The selected formula showed particle size, PDI, and ZP: 80.3 nm, −0.21, and −26 mV, respectively, synchronized with 82.12 %EE. *In vitro* release study showed slow biphasic release of LV from lipid nanocarriers. The antimicrobial effect illustrated statistically significant effect of lipid nanocarriers on decreasing the minimum effective concentration (MIC) of LV, particularly against *E. coli*. The optimized nanocarriers' formula loaded into CS film was clear, colorless, translucent, and smooth in texture. Based on the release profiles, it could be speculated that the CS film loaded with LV nanocarriers can maintain the antibacterial activity for 4 consecutive days. Thus, the local delivery of the drug in a sustained release manner could be predicted to enhance the therapeutic effect. Further clinical studies are strongly recommended.

KEY WORDS: levofloxacin; lipid nanocarriers; chitosan; biodegradable film; skin ulcers.

INTRODUCTION

Over time, patients suffering from chronic wounds and skin ulcers have increased sharply due to rise in the incidence of obesity, embolism, and treatment with chemotherapy (1, 2). Pressure ulcers are also difficult clinical problems that appear at localized areas of skin and mainly affect patients that require bed rest (3). Chronic and refractory ulcers Diabetic wounds are commonly related to Diabetics, where the healing progression is hindered by inhibiting the synthesis of various cells, cytokines, proteins, and growth factors. Diabetes also affects proliferation and migration of fibroblast and keratinocytes presenting a major risk for limb amputation (4).

The effective treatment of microbial skin infections is an inevitable step for treatment of ulcers. In injured skin, bacteria can reach the endodermal tissues causing inflammation, which leads to the formation of proteases and reactive oxygen species in inflammatory cells (5). The ulcers can be evolved by the existence of a biofilm which is an organized aggregation of bacteria encapsulated in an extracellular polymeric substance (6). Biofilms have been recognized in 60% of biopsy samples from chronic wounds and 6% of biopsy samples from acute wounds. These biofilms are considered the greatest barriers to curing of ulcers due to resistance to conventional antimicrobial therapies (7).

Levofloxacin (LV) is a broad spectrum third-generation fluoroquinolone with efficient microbiological activity against skin infections (8). Bioavailability and therapeutic effectiveness of LV and other transdermal delivering antibiotics had been developed through application of different nanocarriers' strategies. Nanotechnology is considered an excellent approach to conquer the limitations correlated with antibiotic therapy as poor dissolution; sensitivity to temperature, photolysis, and enzymatic degraded and in turn fills the gaps that conventional routes failed to occupy (9–11).

Nanotechnology is a revolutionary field that could be exploited to protect medical devices from drug-resistant biofilm infections and to create a new generation of biomedical and industrial applications (12). Several studies revealed the efficiency of nanocarriers' delivery vectors for encapsulating antibiotics, enhancing antimicrobial activity against

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Published online: 23 December 2021

resistant bacterial strains, and controlling the drug release to decrease frequency of administration (13–18).

Lipid nanoformulations provide various benefits that enhance the permeation of drugs through the skin layer. They are highly considered as topical drug delivery systems such as lipid nanoparticles (SLNs and NLCs) and vesicular nanocarriers such as liposomes, niosomes, and transferosomes. Nanostructured lipid carriers (NLCs) and solid lipid nanoparticles (SLNs) are widely utilized in cosmetics and dermatological treatments (19, 20). SLNs are composed of biocompatible lipid matrix dispersed in an aqueous surfactant phase as stabilizing agent. SLNs were easy to scale up but showed low entrapment efficiency and rapid drug release. In contrast, NLCs formed from mixture of solid and liquid lipids with high loading, entrapment efficiency, and low gradual release. Both are characterized by their small size which is an important constraint that affects the depth of penetration and location of deposition. Minor particle size associated with large specific surface area permits adhesion of lipid nanoparticles to the skin as thin film increasing contact time and subsequent drug absorption from nanocarriers (21–25). Furthermore, SLNs and NLCs are more suitable for targeted delivery to skin organelles such as hair follicles but they are not the first choice nano-delivery systems for transdermal purposes

Compared to other nanocarriers, NLCs showed high solubility, high drug encapsulation, and stability, which made them our candidate for the delivery of levofloxacin (LV)(26).

For further development of the delivery system, biodegradable films were chosen as vector for the LV nanocarriers. The polymeric biodegradable films have shown promising drug delivery activity across the skin and wound healing properties. They adhere to the body as a thin transparent film for delivering the active pharmaceutical ingredients to the target spot (27).

In the current study, chitosan (CS) was selected as the polymer to be used for preparation of biodegradable films, because it was previously reported for being biocompatible, biodegradable, and non-toxic(28, 29). It was applied as synthetic cellular biomaterial for replacing skin and has recently been utilized for the treatment of skin lesions (2). Moreover, CS has shown mucoadhesive and antimicrobial properties with good potential in different applications of drug delivery (30).

The aim of this study was to investigate lipid nanocarriers as a strategy to improve the efficacy of LV in penetrating skin biofilms. Two surfactant types (Tween 80 and polyvinyl alcohol) were evaluated for their effect on nanocarriers: particle size, entrapment efficiency, *in vitro*, *ex vivo*, and antimicrobial activity. For sake of evaluation, the nanoparticle selected formula was incorporated into CS film that was subjected to physic-chemical characterization and *ex vivo* skin permeation study.

MATERIALS AND METHODS

Materials

Levofloxacin (LV) was generously donated by Amoun Pharmaceutical Co. Low molecular weight chitosan (CS) (MW50000-190000D, degree of deactylation = 90%) and Glyceryl monostearate (GMS) were purchased from Sigma, USA. Glacial acetic acid, stearic acid, and oleic acids were obtained from Fluka Chemika-Biochemika, Switzerland. Polyvinyl alcohol (PVA), Propylene glycol, glycerol, methanol, and buffer system (KH_2PO_4 and Na_2HPO_4) were purchased in HPLC analytical grade from El Hekma Co. (Cairo, Egypt).

Preparation of LV Lipid Nanocarriers

Different formulations of LV-loaded lipid nanocarriers were formulated by hot homogenization technique followed by ultra-sonication according to previously published methods (26, 31). Weighed amounts of lipids were melted in water bath at 80°C, and then 100 mg of drug was dissolved in the molten lipid. The mixture of molten lipid/drug was dispersed into 30 mL of hot aqueous solution (85°C) containing surfactant (1% w/v PVA or 1% w/v Tween 80) under magnetic stirring (IKA Works, Asia Sdn. Bhd., Malaysia) at speed 2000 rpm for 5 min. The produced primary emulsion was ultrasonicated (UP50H, Hielscher, Germany) for 4 min, followed by the addition of 20 mL of cold distilled water. The composition of prepared nanocarriers' formulations is presented in Table I.

Characterization of Lipid Nanocarriers

Particle Size, Polydispersity Index, and Zeta Potential

The average nanocarriers size, polydispersity, and Zeta potential of the LV lipid nanocarriers were measured using dynamic light scattering integrated in a zeta-sizer Nano-ZS (Malvern Instruments Ltd., Worcestershire, UK). Fixed volumes of formulations were mixed with an amount of purified water to achieve a suitable scattering intensity. Triplicates were implemented for each sample at an angle of 90° at room temperature (25°C). Polydispersity index (PDI) was determined for assessing the particle size distribution.

Drug Load (%DL) and Entrapment Efficacy (%EE)

Drug loading (%DL) and entrapment efficiency (%EE) of LV in the prepared lipid nanocarriers were determined indirectly. Concentration of free drug was measured in aqueous supernatant solution after separation of lipid nanoparticles by centrifugation at 13,000 rpm for 30 min at 4°C in high-speed refrigerated centrifuge. The supernatant was filtrated through 0.22 μm membrane filter and the amount of LV entrapped was analyzed spectrophotometrically at λ_{max} 280 nm after suitable dilution with methanol. Each test was performed triplicate and the mean value was deduced. The %DL and %EE were calculated according to the following mathematical equations:

Compatibility Study by Differential Scanning Calorimetry (DSC)

The changes in the physical state of LV loaded in lipid nanocarriers were assessed using a thermal analysis system (DSC60, Shimadzu, Japan). DSC was performed on pure LV,

Table I. Composition of the Formulated Levofloxacin-loaded Lipid Carriers

Formula code	Stearic acid (gm)	Oleic acid (gm)	GMS (gm)	Surfactant type (w/v)		Lipid nanocarriers type
				Tween 80	PVA	
F1	1.8	0.2	1	———	1%	NLC
F2	1	———	———	———	1%	SLN
F3	1.8	0.2	1	1%	———	NLC
F4	1	———	———	1%	———	SLN

oleic acid, stearic acid, and LV-loaded nanocarriers (F3). Each sample (5 mg) was packed in flat bottomed aluminum pan with crimped on lid. The samples were heated at a constant rate of 10°C/min, under nitrogen flow over temperature range 50–400°C. An empty pan at the same conditions was used as the reference.

Transmission Electron Microscopy (TEM)

The shape of lipid nanocarriers was inspected using TEM (Jeol, JEM-Japan). The freshly prepared sample was deposited onto the surface of carbon-coated copper grids, natively stained by phosphotungstic acid, and dried at room temperature. At certain magnification, the sample was probed and visualized.

In Vitro Release Study for Prepared LV Nanocarriers

The diffusion of LV from the lipid nanoparticles in phosphate buffer (pH = 7.4) solution was investigated by dialysis bag method (32). The dialysis bag (molecular weight cut off: 12–14 kDa, Livingstone, NSW, Australia) was first immersed in PBS overnight prior use. Two mL aliquot of the selected LV nanocarriers formula was placed inside the dialysis bag, tied at both ends, and immersed in 50 mL of PBS release medium. The solution was stirred at 100 rpm with the help of the magnetic stirrer at 37±0.5°C. Sinking conditions were considered during LV dissolution test. At scheduled time intervals, 3 mL of dissolution medium was removed and replaced immediately with fresh medium. The cumulative percentage of drug released was determined spectrophotometrically at λ_{max} 280 nm. For the sake of comparison, the release of free LV was also conducted by similar criteria.

Antimicrobial Activity of the Nanoparticles

The antimicrobial effect of the chosen LV-loaded nanocarriers formula was estimated in comparison to that of the free drug using well diffusion method (33). The activated *E. coli* and *S. aureus* were cultivated in Soya Bean Casein Digest Agar (24 h before studies). Different concentrations of free LV and selected LV nanocarriers ranging between 0.025 mg/5 mL and 20 mg/5 mL were prepared. Aliquots of 100 μL of each sample were put into the wells. The culture media were incubated at 37°C (body temperature) for 24 h, and the inhibition zones surrounding the wells were calculated using a zone reader. To estimate the minimum inhibition concentration (MIC) they were serially diluted. The tubes were inoculated with a 1 mL of inoculums of tested bacterium (final concentration of 105–106 CFU/mL). At the end of incubation period, the tubes were examined for any evidence of bacterial growth. Lipid materials used in selected formula and free LV were applied as positive and negative controls, respectively.

Preparation of CS Films Loaded with LV Nanocarriers

Based on the results of previous examinations, the selected LV lipid nanocarriers' formula was utilized for loading into biodegradable CS film. Chitosan (CS) films loaded with either pure LV or LV lipid nanocarriers were prepared by solvent casting technique (Table II)(34). A cryoprotectant, namely sucrose 5% w/v was dispersed in prepared LV lipid nanocarriers suspension system before freezing at −20°C followed by freeze drying (Freeze drier Alpha 1e2 LD Martin-Christ-Germany) (26). Briefly, 50 mg pure drug LV or equivalent amount of freeze-dried LV-lipid nanoparticles were added to CS in 1% v/v acetic acid aqueous solution under continuous stirring 1000 rpm for 24 h. The plasticizers 2% v/v of propylene glycol and 0.5% v/v of glycerol were added into CS solution with stirring for 2 h at 200 rpm. The mixture was vacuum degasified for 1 h to remove air bubbles. Films were prepared by casting about 14 mL of drug/CS solution over a clean glass petri-dish (diameter of 15 cm) and left to dry at 25°C for 48 h. After complete drying, casted films were cut into pieces, wrapped into aluminum foil, and stored in desiccator at room temperature till further evaluation studies. The formula coded F5 represented plain film, while F6 and F7 presented CS film loaded with pure LV and CS film loaded with LV nanoparticles, respectively (Table II).

Characterization of the CS Films Loaded with LV Nanocarriers

Physical Examination

The drug-loaded films were subjected to visual inspection to evaluate the color (transparency), shape, and surface texture to make sure of the absence of any blocking or precipitating substance (35).

Thickness and Weight Variation

The thickness of different prepared films was detected using digital micrometer. It was measured at different positions of the films and the mean was then calculated

Table II. Composition of the CS Prepared Films

Formula code	CS	Propylene glycol (g)	Glycerol	LV	LV-LCs
F5	1 gm	2 gm	0.5 gm	———	———
F6	1 gm	2 gm	0.5 gm	50 mg	———
F7	1 gm	2 gm	0.5 gm	———	2.6 gm

(36). For weight determination, each formulation was cut from 10 different places into equal-sized pieces (2 cm^2) using a standard paper cutting knife. The pieces were weighed separately on an electronic balance and the average weight was calculated (37). The individual values can be varied within ± 5% of the mean.

Drug Content Uniformity

The prepared films were analyzed for drug content in order to ensure their therapeutic activity. Each film was kept individually in 100 mL methanol for 2 days in a dry place till complete solubility and complete drug release. Take 5 mL from the drug-methanol solutions to be filtered through 0.22 μm membrane filter. One mL of the filtrate was diluted to 50 mL with methanol and the drug concentrations were determined using a UV visible spectrophotometer at λ_{max} 280 nm. The polymeric solution without drug was served as blank. The mathematical average of three readings was taken.

Folding Endurance

This test is a good indicator for film brittleness as well as flexibility. The folding feasibility was determined by repeatedly folding the films up to100 times from the midpoint, between the fingers and the thumb, and then opened till the film showed rupture or cracks. The total number of foldings without breaking is considered as folding endurance value. This test was carried out for three times and the mean was recorded (35).

Swelling Index

The hydration of the prepared films was considered by calculating its swelling index. Briefly, the films were dried, weighed (given symbol W_1), and allowed to swell in petri-dish containing 5 mL of PBS 7.4. The swollen films were subsequently reweighed (given symbol W_2) after removing the excess water using filter paper (38). Finally, the index was estimated using the following:

Effect of Storage

Effect of Storage on LV-Loaded Nanocarriers

The stability of selected LV-loaded nanocarriers formula was studied by storage of 3 samples in closed containers at shelf temperature 25°C for 3 months. Along this period, the particle size and PDI were evaluated. At the end of the storage period, particle size and percent entrapment efficiency (%EE) were calculated.

Effect of Storage on CS-Film Loaded with LCs

The stability of chosen film was conducted by storage of 3 samples at 40 ± 2°C and 75 ± 5% RH for 3 months according to ICH guidelines. Along this period, films were inspected for the appearance, folding endurance, and drug content (35).

Ex Vivo Skin Permeation Study

The *ex vivo* skin permeation studies are used to predict the permeation characteristics of drug *in vivo*(39). The experimental procedure was approved ethically by the Animal Research ethical Committee, Faculty of Pharmacy, Helwan University. Franz diffusion cell was used to investigate the release profile of the drug from the prepared film system. It is carried out by using a cell that is composed of a donor, acceptor compartments, and a freshly prepared rat skin attached between them (40). The rabbit skin was prepared by cleaning 5 times with cotton wetted in ethanol. The vertical Franz-type diffusion cell was used with an effective permeation area of 1.5 cm^2 and with the stratum corneum facing the donor compartment. The receptor solution consisted of 200 mL of phosphate buffer pH 7.4 maintained at 37±0.5°C and constantly stirred with a magnetic bar at 75 rpm. Predetermined quantities of prepared CS films loaded with LV nanocarriers were placed on the donor compartment. Samples were withdrawn from the solution at time intervals (1, 2, 4, 6,8,12, 24, 48, 72, and 96 h) to determine the amount of the drug in the solution. The suspensions were separated using centrifugation at 4000 rpm for 15 min and filtered through 0.22 μm filter and the filtrates were spectrophotometrically measured at 280 nm. The withdrawn samples were replaced by the same volume of PBS 7.4 and maintained at body temperature (37±0.5°C). For sake of comparison, the release of levofloxacin from stock solution was conducted at the same conditions.

Statistical Analysis

All the experimental data were analyzed using SPSS 16.0 program (SPSS Inc., Chicago, IL, USA) with paired sample *T*-test at least significant difference at $P < 0.05$.

RESULTS AND DISCUSSION

Drug Loading (DL) and Entrapment Efficiency (EE %) of Prepared of LV Lipid Nanocarriers

The DL and EE % of levofloxacin within different formulations were presented in Table III. The % DL was

extended between 15.1 ± 0.65 and 22.7 ± 0.79 and the % EE was within the range of 63.2±3.1 to 82.12± 3.72. In this study, stearic acid was used as the main solid lipid due to its abundance, biocompatibility, and its ability to penetrate cells and accumulate in the cytoplasm (41–43). The loading capacity of drug in the nanoparticles is related to the lipid phase, drug solubility, and the chemical or physical structure of solid lipid matrix. In formulations coded F1 and F3, Glyceryl monostearate (GMS) was added for partial replacement of stearic acid in the prepared SLNs for sake of improvement of emulsification process of lipid phase in the aqueous phase and enhancing the drug encapsulation efficacy. The results in Table III also revealed statistically significant increase in both %DL and %EE in F1 and F3, indicating that the NLCs system showed more efficient capabilities for encapsulation for LV owing to its exclusive matrix structure. This harmony is expected due to the existence of great imperfections in the crystal lattice of NLCs leaves enough space to accommodate drug molecules and thus leads to improvement in drug loading capacity and drug entrapment efficiency (44–46). Moreover, the higher loading capacity of NLCs could be owing to the higher LV solubility in the oil section, which enclosed within the solid matrix (47). On studying the effect of surfactant, both Tween 80 and PVA are considered safe water-soluble surfactants with higher HLB. The results showed that Tween 80 caused slight increase in %DL and EE for both SLNs and NLCs formulations. These results agreed with literature that stated that Tween 80 is highly suitable to be used with isopropyl myristate, glycerol monostearate, stearic acid, and oleic acid (48).

Particle Size, PDI, and Zeta Potential

Particle size, zeta potential, and polydispersity of LV lipid nanocarriers were displayed in Table III. The measured particle size was in the nanometric size range lower than 100 nm; which is preferred for drug delivery to biological cell (46). The particle size ranged from 69.5 to 95.2 nm. The formulations F1 and F3 showed lower particle size than the other formulae owing to the presence of oleic acid with its low viscosity, which reduce the inside viscosity of LCs and consequently, reduced the surface tension to create smaller and smoother particles (49). Oleic acid at concentration 10% was used because of its effect on both lipid particle size and to allow massive crystal order disturbance. Furthermore, the surfactant also greatly influences the particle size of formulation by causing droplets stabilization. The results also have shown a significant difference between Tween 80 and PVA. This could be referred to the ability of Tween 80 to produce smaller particles by stabilizing the system formation more efficiently (50). Although PVA is a widely used polymeric surfactant in the external aqueous phase as an emulsifier, it was also reported that PVA exhibits non-linear behavior on particle size with high concerns on leaving unsafe residues in nanocarriers (51). The fabricated nanocarriers had PDI values extended from 0.21–0.55 indicating narrow size distribution and excellent sample size homogeneity. The zeta potential of all LCs formulations was ranged between −19 and −31 mV and displayed negative charge owing to the carboxylic molecules from the lipid structure. Moreover, the high zeta potential revealed the formulation stability owing to the charge's repulsion towards the natural tendency of aggregation (52).

Differential Scanning Calorimetry (DSC)

Thermodynamic variations owing to morphological changes during and after formulation steps can be detected by DSC. Fig. 1 displays the DSC thermograms of pure LV, oleic acid, stearic acid, GMS, and LV-loaded nanocarriers (F3). According to the DSC results, pure LV exhibited an endothermic characteristic peak at 229°C corresponding to its melting point (53). However, the melting characteristic peak of LV completely disappeared in DSC thermogram of F3, showing that LV might be molecularly dispersed in lipid nanocarriers or changed to its amorphous state.

Transmission Electron Microscope (TEM)

A representative TEM photomicrograph of LV nanocarriers' selected formula (F3) was illustrated in (Fig. 2). The particles looked like a dark circular disc. The TEM examination also revealed that the optimized formula (F3) had an almost homogeneous small-sized spherical appearance with a narrow size distribution. The resulted particle size matched with that determined previously by zeta sizer.

In Vitro Release Study

In vitro release of LV from prepared LCs compared to pure LV was assessed by dialysis bag in PBS (pH 7.4) containing 0.1% Tween 80 at 37°C as release medium to simulate effect of body fluids. The rate of LV release from its LCs versus time was constructed in (Fig. 3). The pure dug revealed a quicker complete release within the first 2 h, followed by a plateau pattern. However, encapsulated LV showed slow release from LCs formulations over a period up to 24 h. This may be attributed to the slow release of solubilized or dispersed drug from the lipid matrices. From the results, both F3 and F4 showed a biphasic drug release profile; displayed an initial rapid %drug release of 37.9 and 30.8, respectively in the first 4 h, followed by more sustained drug release that reached 72.5 and 65.1%, respectively up to 24 h. Firstly, the burst effect could be ascribed to rapid release of drug fixed at the surface of the LCs, while the sustained release correlated to the depth of entrapped levofloxacin in the core matrix of the LCs (54). The sustained release of drug is forecasted to enhance drug residence eliminating the adverse effects associated with repeated drug administration. Based on the previous results, F3 was chosen to be subjected for further investigations.

Selection of the Best Formula

Our criteria of selection were based on obtaining (Least particle size, PDI, ZP, and maximum EE% with more controlled release profile. Based on the previous results, it was concluded that the formula coded F3 showed the least particle size, PDI, and ZP: 80.3 nm, −0.21, and −26, respectively that synchronized with high %EE (82.12). This could be attributed to the effect of physical nature of NLCs as a unique type of lipid nanocarriers that is characterized with

Table III. Characterization of Prepared LV Lipid Nanocarriers (Data Represented as Mean ± SD, $n = 3$)

Formula code	DL (%)	EE (%)	Particle size (nm)	PDI	Zeta potential (mV)
F1	21.1± 0.80	73.1 ±3.10	73.1 ± 0.80	0.32	−31 ±3.10
F2	15.1 ± 0.65	63.21 ±4.80	95.2 ± 0.65	0.42	−19 ±4.80
F3	22.7 ± 0.79	82.12 ±3.72	69.5 ± 0.72	0.21	−26 ±5.21
F4	19.2 ± 0.72	68.40 ±5.21	80.32 ± 4.5	0.55	−20 ±3.72

high space capacity for drug encapsulation and low viscosity owing to liquid lipid portion exhibiting low particle size with minimal size diversity (43).

Antimicrobial Activity of the Optimized LV Nanocarriers

Since bacterial biofilms are associated with most non-healing wounds infections, the threat of infection, contamination, or colonization must be addressed through the use of broad-spectrum antibacterial agent to ensure that wounds do not become infected (55). Bessa *et al.*(56) found out that *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli* (*E. coli*), and *Corynebacterium spp* were the most common pathogens found in polymicrobial infections together with high resistance to majority of antibiotics. In this study, the disc diffusion (clear zone) method was used to evaluate the antibacterial activity of selected prepared lipid nanocarriers' formula (F3) against gram-negative bacteria, *E. coli*, and gram-positive bacteria, *S. aureus*. The role of the control was played by prepared plain lipid nanocarriers. In this case, there was no inhibition zone, as expected and previously reported (57). On the other hand, the concentrations ranged from 0.025 to 0.05 mg/5 mL in both F3 and free LV showed no inhibition zones, which may be attributed to the low concentration of LV. Table IV also shows that minimum inhibitory concentration (MIC) of LV was significantly reduced upon loading to LCs. For Staphylococcus aureus (ATCC 25923), free LV started to cause inhibition of bacterial growth at 0.625 compared to 0.30 mg/5 mL for LV-LCs (F3). The results also illustrated the more pronounced effect of LV against *E. coli*(gram-negative) in both free and encapsulated forms. At concentration 20 mg/5 mL, *S. aureus* and *E. coli* showed zone inhibition measured as 4.3 mm and 5.5 mm, respectively which expressed statistical significance compared to free LV. These results reinforce the hypothesis of using nanoformulations for controlled delivery of anti-infective agents in treatment of infections caused by resistant bacteria. Furthermore, according to the MIC (minimum inhibitory concentration) analysis, the preferential effect of these formulations in gram-negative strains indicates a possible interaction of delivery system with a membrane of these bacteria in 53.3% of the strains when compared with LV pure. The results can also be justified by the lipophilic nature of the delivery system that could improve the cellular uptakes of LV into the bacterial membrane and the small size of the particles.

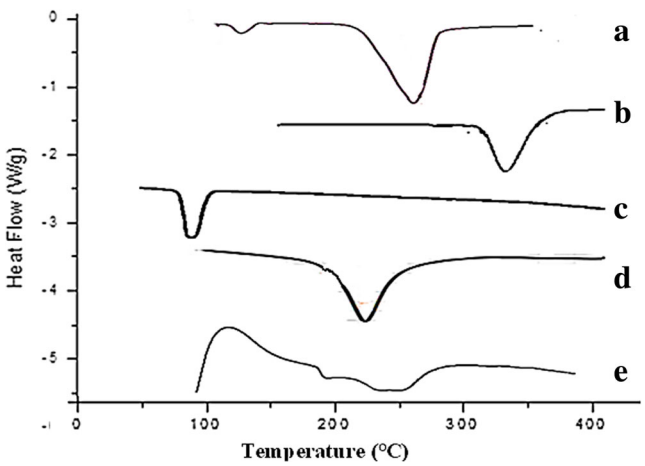

Fig. 1 DSC thermograms of **a** pure levofloxacin, **b** Oleic acid, **c** Stearic acid, **d** GMS, and E: optimized LV nanocarriers (F3)

Levofloxacin-Loaded Nanocarriers' Biodegradable Film

Numerous methods have been employed to prepare CS film delivery systems. Among these methods, solvent casting method was nominated owing to its inherent simplicity and robustness. Moreover, it warrants greater commercial viability as it is a feasible, straight forward process and cost-effective technique (33). Low MW chitosan was ideal choice for film preparation owing to its inherent low viscosity and mucoadhesion properties as well as its antimicrobial activity (58).

In Vitro Characterization of the Prepared Films

Physical Examination

Fig. 4a, b, c, and d, displays the visual appearance of the prepared films (formulations coded F5, F6, and F7) respectively. The films were photographed using a digital camera

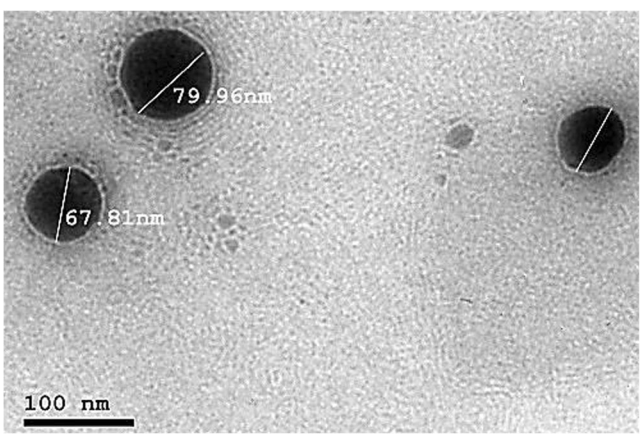

Fig. 2 TEM images of optimized LV nanocarriers (F3)

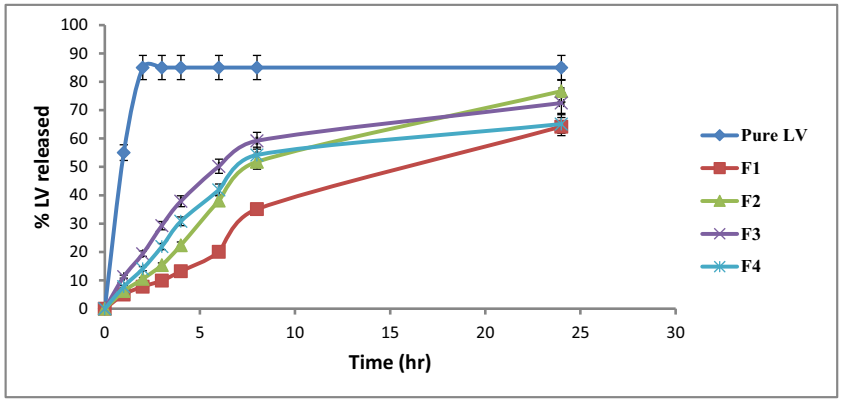

Fig. 3 *In vitro* release profiles of levofloxacin solution and different levofloxacin-loaded LCs formulations in phosphate buffer saline (pH 7.4) containing 0.1% Tween 80 at 37°C ±0.5°C

with 44 megapixels resolution. They appeared as clear, colorless, translucent, and smooth in texture. Moreover, they were flexible without any cracks, pores, and bubbles on its surface.

Thickness, Weight Variation, and Folding Endurance

Thickness, weight variation, and folding endurance of the prepared films were illustrated in Table V. The uniformity and accuracy of the drug dose among the films are mainly accompanying with the thickness and weight variation. The films were thin, with a thickness ranged from 0.32 ± 0.05 mm to 0.45 ± 0.06 mm. There was no significant difference between the thicknesses within the films. Weight variation was 7.01 ± 0.1 mg and 7.31 ± 0.08 mg for LV film and LV/LCs film, respectively. The similarity in the weight results indicates the homogeneity and uniformity of drug dosing. The films also displayed folding endurance values more than 50 folds. The higher folding values were a good pointer for the films flexibility and elasticity which may owe to the high % of plasticizers (35).

Drug Content Uniformity

The content uniformity was 89.5 ± 1.769 and 93.5 ± 2 for LV film and LV/LCs film, respectively. Both films were found to have drug uniform content, indicating evenly drug distribution and assuring their therapeutic efficiency.

Effect of Storage

Effect of Storage on LV-Loaded Nanocarriers

The optimized formula (F3) kept its physicochemical properties with no observed change in the particle size, zeta potential, or EE% after 3 months of storage at 25°C (Table VI).

Effect of Storage on LV/LCs Films

After 3 months of storage of the film coded (F7) at 40 ± 2°C and 75 ± 5% RH, the folding endurance and drug content were 90 and 89.7 ± 3.1, respectively. These

Table IV. Zone of Inhibition (mm) of LV-loaded Lipid Nanocarriers Formula (F3) Compared to Plain Lipid Nanocarriers and Free LV After 24 h for *E. Coli* and *S. Aureus* Bacterial Strains

Conc mg/5 mL	*E. coli* (ATCC 11229)		*Staphylococcus aureus* (ATCC 25923)	
	LV	F3	LV	F3
(mm)				
20	3.90 ± 0.00	5.50 ± 0	3.8 ± 0.13	4.3 ± 0.21
10	3.50 ± 0.00	4.75 ± 0.07	3.5 ± 0.07	3.9 ± 0.06
5	2.9 ± 0.07	4.35 ± 0.07	2.9 ± 0.14	3.3 ± 0.14
2.5	2.34 ± 0.07	3.86 ± 0.07	2.55 ± 0.07	2.7 ± 0.07
1.25	1.9 ± 0.14	3.41 ± 0.141	1.4 ± 0.14	2.3 ± 0.10
0.625	1.35 ± 0.12	2.95 ± 0.07	0.98± 0.04	1.9 ± 0.01
0.3	1.08 ± 0.03	2.34 ± 0.07	0.00	1.2± 0.02
0.15	0.00	1.8 ± 0.141	0.00	0.00
0.05	0.00	0.00	0.00	0.00
0.05	0.00	0.00	0.00	0.00
0.025	0.00	0.00	0.00	0.00

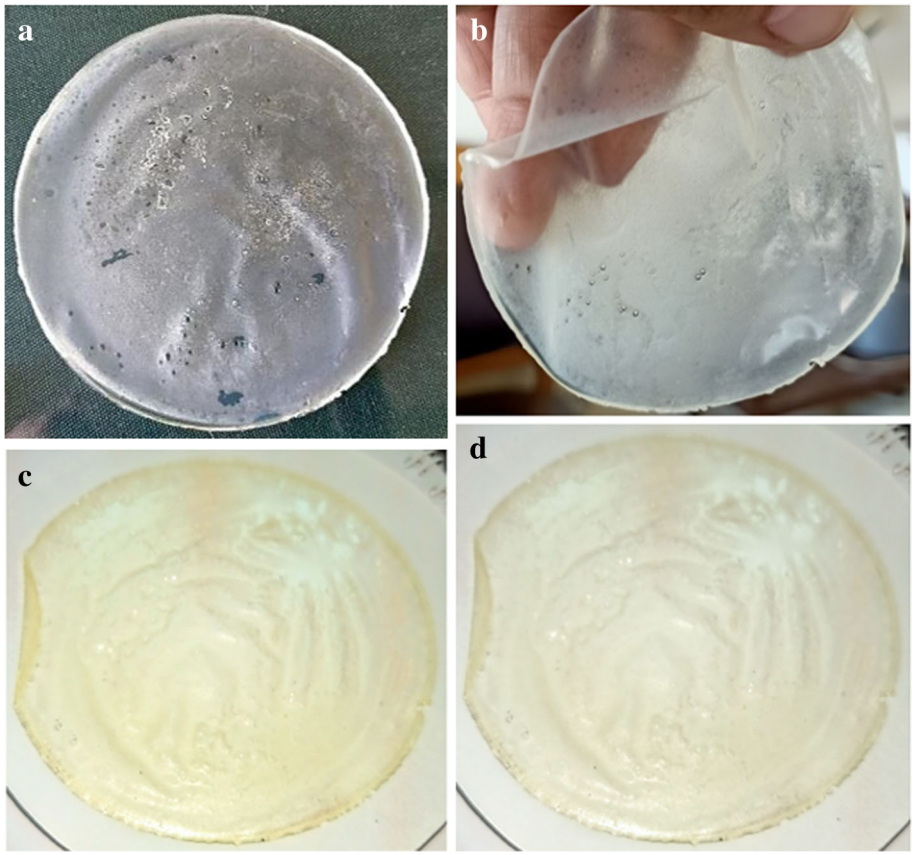

Fig. 4 Photographs of prepared films taken with digital camera 44 megapixels: **a** CS control film, **b** F5, **c** F6, **d** F7

results were found to be statistically insignificant ($P > 0.05$, paired *t*-test) with those obtained before storage, indicating the stability of the prepared film. It also kept its physical appearance with no observed change in color and texture. From the previous results of LV/LCs, it was observed that the content of the film was higher than lipid nanocarriers after storage confirming the stability of the prepared film and it as ability to keep the entrapped amount from leakage outside.

Ex Vivo Skin Permeation Study

There are many reports in literature that confirmed the Usefulness of Rabbit Skin as a Substitute for Human Skin in the *in vitro* Skin Permeation Study (59–61).

In vitro diffusion of LV from prepared films (F6 and F7) was assessed comparing to selected LV-loaded nanocarriers (F3)(Fig. 5). The study revealed that LV release pattern from the prepared films was slower than from LCs. The release of LV from prepared films coded F6 and F7 showed biphasic manner like the release from NLCs. They presented initial rapid release of 60.5 and 50% for F6 and F7, respectively in the first 24 h followed by sustained release up to 4 days. The rapid release is contributed by the leakage of free drug particles on the film superficial layer upon coming with immediate contact to the dissolution media (62). The sustained release may be attributed to the high CS concentration where high quantity of drug entrapped inside in its highly dense matrix. This manner of biphasic release is valuable for antibiotics to achieve high antimicrobial therapy by providing immediate effect and attain MIC then followed by sustained release keeping drug residence and avoiding repeated drug administration. Based on more slow and controlled release profile, the film loaded with LV/LCs(F7)

Table V. Thickness, Weight Variation and Folding Endurance for the Prepared Films (Data Represented as Mean ± SD, n = 3)

Code	Thickness (mm)	Weight variation (mg)	Folding endurance
F5	0.32± 0.05	————	75
F6	0.4 ± 0.1	7.01± 0.1	82
F7	0.45± 0.06	7.31 ± 0.08	92

Table VI . Effect of Storage on EE%, Particle Size, and Zeta Potential of Selected LV-loaded Nanocarriers (F3)

Parameter	Storage periods (months) at 25 ± 2°C	
	0	3
EE%	78.5 ± 3.21	82.12 ± 5.21
Particle Size (nm)	66.8 ± 3.5	69.5 ± 4.5
Zeta potential (mV)	−28 ± 3.21	−26 ± 5.21

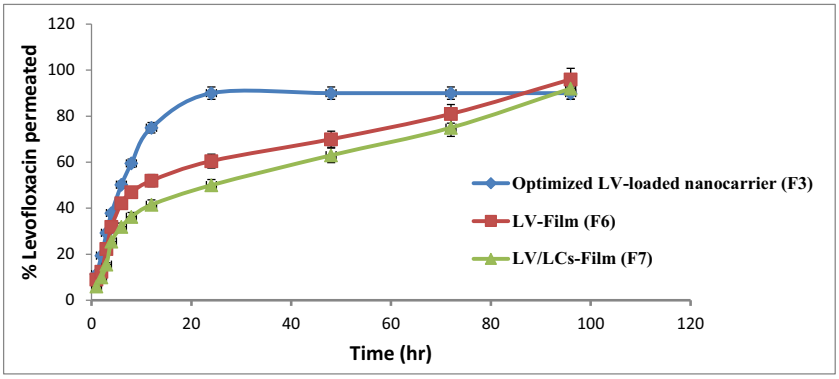

Fig. 5 *Ex vivo* skin permeation profiles of levofloxacin from prepared films (F6 and F7) comparing to selected LV-loaded nanocarriers (F3)

was elected as the formula, which could maintain the antibacterial activity for 4 days. Thus, the local delivery of the drug in a sustained release manner is predicted to enhance the therapeutic effect.

CONCLUSIONS

In this paper, it was concluded that CS film loaded with levofloxacin lipid nanocarriers (LV/LCs) could be simply prepared in satisfactory and cost-effective way. It was prepared from muchoadhesive CS polymer and mix of propylene glycol and glycerol as plasticizers. The film obviously showed good appearance, high homogeneity, and tolerable physical characteristics and smooth surface structure with good flexibility. The film also attained biphasic release manner for LV, which is valuable for antibiotics use to achieve high antimicrobial therapy keeping drug residence and avoiding repeated drug administration. Moreover, it showed effective antimicrobial performance, fast-acting, and low cytotoxicity and therefore can achieve selectivity and delivery to specific targets. Thus, local delivery of the drug in a sustained release manner is a promising technique to deliver the LV through the skin, enhancing its therapeutic effect and be preferred as patient-compliant drug therapy.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Helwan University for extended instrumental facility in successful accomplishment of the present work.

AUTHOR CONTRIBUTION

All authors conceived and designed the experiments; Rania S. Abdel-Rashid: performed the formulations and experiment, supervision, contribution in data analysis, and writing; Eman S. El-leithy: supervision, funding, data analysis, editing; Raghda Abdel-monem: methodology, supervision, contribution in data analysis, writing and drafting manuscript to appear in its final form.

DECLARATIONS

Ethics Approval and Consent to Participate Animals experimental protocol was ethically approved by the Animal Research Ethical Committee, Faculty of Pharmacy, Helwan University.

Conflict of Interest The authors declare no competing interests.

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