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Formulation Development and Characterization of Lovastatin Nanogel for the Treatment of Hyperlipidemia

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ABSTRACT

Lovastatin (LS) is the cholesterol-lowering drug in the statin class, but it has poor oral bioavailability due to its high metabolism and low solubility, which affect its clinical efficacy. To overcome limitations associated with LS, the current study sought to develop a transdermal nanoemulsion using linseed oil and finally convert it into a nanogel formulation. Nanoemulsion (NE) was prepared using the spontaneous titration method. Different components of NE were selected based on solubility study and pseudo ternary phase diagrams were constructed using the titration method to determine the concentration range of components. Carbopol 934 was used to convert NE to nanogel (NG). The NE was selected based on the stability study and the composition of optimized NE consists of oil phase as 10%w/w linseed oil, 35%w/w Tween 80, and Polyethylene glycol 400 in 1:1 as S_{mix}, and 55%w/w aqueous phase as water. The optimized NE (NE3) was characterized for various parameters and the formulation NE3 was found with desired globular size (108.9 ± 3.12 nm), polydispersity index 0.257 ± 0.015, zeta potential (–16.93 ± 1.12 mV), and spherical morphology. NE3 was combined with carbopol 934 to convert into NG and further characterized for pH, rheological behavior, and *in vitro* permeation study. The *in vitro* drug permeation study showed that the NG (33.69 ± 0.75 µg/cm^2/h) and NE (36.63 ± 0.55 µg/cm^2/h) have maximum permeation flux rate as compared to LS suspension (6.41 ± 1.13 µg/cm^2/hours). These results conclude that the NG formulation of LS can be a safe and effective alternative to an oral formulation of LS with enhanced permeation characteristics for transdermal delivery.

Keywords: Lovastatin, Nanoemulsion, Nanogel, Solubility, Stability, Permeation.

INTRODUCTION

Hyperlipidemia is the main risk factor for the development of atherosclerosis, which eventually results in cardiovascular disease. The increased prevalence of dyslipidemia is mainly due to food habits and lifestyle factors brought about by the Westernization of food in many developing nations including Saudi Arabia[1]. Lovastatin (LS) is one of the most effective drugs for lowering cholesterol in the statin class of drugs. The drug LS inhibits the HMG-CoA reductase enzymes and prevents the

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formation of cholesterol[2]. Moreover, LS showed the ability to lower LDL levels while not affecting HDL levels. LS has been shown in numerous studies to reduce the death rate from coronary heart disease[3,4]. The Biopharmaceutical Classification System (BCS) classifies LS as a class II drug that has low solubility and high solubility. LS is available in both immediate-release and extended-release tablet dosage forms, however, due to its high metabolism in the liver and gut and its low solubility (0.0004 mg/mL in water), it has a poor oral bioavailability (<5%)[4]. Due to these characteristics, LS is a potential drug candidate for transdermal delivery in an attempt to increase drug bioavailability. A greater amount of the drug can enter into the systemic circulation when LS is delivered transdermally since it avoids the effects of hepatic metabolization.

Transdermal drug delivery system (TDDS), is a cutting-edge technique for drug delivery that can be utilized to increase absorption and prevent hepatic first-pass metabolism for systemic effects. Antihyperlipidemic drugs can be applied topically or systemically via transdermal delivery[5]. Due to the first pass effect being avoided, bioavailability can be increased. The gastrointestinal discomfort associated with statin drugs can also be prevented by using TDDS. For the transdermal delivery of drugs, nanoemulsions (NEs) are being investigated extensively. NEs are isotropically transparent, thermodynamically stable mixtures of oils, surfactants, co-surfactants, and water in the appropriate quantities that have globule sizes from 5 to 200 nm[6]. They have nanoscale globules, which make them optically transparent. Apart from these, they provide many benefits, including greater ability to dissolve, improved efficiency of drug-loading for both hydrophilic and lipophilic molecules, and high thermodynamic stability. NEs are preferable in TDDS as they can penetrate into deeper layers of skin[7]. However, their low viscosity, makes the composition difficult to apply transdermally. To avoid this problem, different gelling agents are added in NEs which increase formulation viscosity and make transdermal application easier. Gelling agents help in transdermal application by creating a three-dimensional hydrogel network that traps nanoemulsion globules[8].

Transdermal administration of LS was also investigated by some researchers to increase the bioavailability of the drug. Soujanya *et al.,* 2018 developed proniosome-based transdermal formulation of LS by the coacervation phase separation method. LS-loaded proniosomal formulation showed high percentage entrapment efficiency and controlled drug release[9]. Gupta *et al.,* 2022 developed LS-loaded solid lipid nanoparticles (SLN) for transdermal delivery using glyceryl monostearate as lipid using solvent emulsification diffusion method[10]. Spoorthy *et al.,* 2023 prepared polymeric nanoparticles and SLN of LS and then included them in a transdermal patch to overcome the problem associated with oral delivery of LS[11]. Very few articles have been published related to the NE gel formulation of LS. Kaur *et al.,* 2019 prepared the NEs gel with tween 80, labrafac PG, and transcutol and evaluated its potential for osteoporosis[8]. Linseed oil has cardioprotective effect. It has rich sources of ω-3 fatty acid, which primarily improve lipid profile and act on the cardiovascular system[12]. There is no reported NEs gel formulation of LS prepared using linseed oil as the oil phase. Therefore, this research aims to formulate a nanoemulsion-based nanogel formulation using linseed oil for transdermal delivery of LS which can bypass the liver metabolism of the LS and increase the permeability of the LS which ultimately increases the bioavailability of LS. Therefore, the prepared nanogels (NG) formulation would increase the medication's efficacy.

MATERIAL AND METHOD

Materials

The drug (LS) was obtained from Jamjoom Pharmaceutical Co. Ltd., Saudi Arabia as a gift sample. The oils, surfactants, and co-surfactants were bought from SD Fine Chemicals, India, and Sigma-Aldrich, India. Analytical grade chemicals and reagents were used in this study.

Methods

Screening of components for nanoemulsions

Screening on different components for NEs preparation is based on an equilibrium solubility study[13]. The solubility of the drug was examined by mixing an excess of the drug into two milliliters of various oils (Linseed oil, Sunflower oil, Eucalyptus oil and Olive oil), surfactants such as Tween 20, Tween 60, Tween 80 and Cremophor RH 40), and co-surfactants such as Myoglyol, Isopropyl myristate, PEG 400, and Propylene glycol. The mixtures were mixed on an isothermal orbital shaker for 72 h at a

temperature of 25 ± 2°C for equilibrium. After that, samples were obtained, and centrifugation was done for 15 min at 3000 rpm. Supernatants were collected and passed through membrane filters with a 0.22 µ pore size which were further analyzed at 238 nm using UV spectrophotometer[14].

Formulation of nanoemulsions

Linseed oil was selected as the oil phase, whereas, PEG 400 as a co-surfactant, and Tween 80 as a surfactant based on the solubility investigation as it showed the highest solubility of the LS (Table 1). Different weight ratios of 1:1, 1:2, and 2:1%w/w of surfactant and co-surfactant were employed to make S_{mix}. To determine the optimal ratio of S_{mix}, we created ternary phase diagrams for each S_{mix}. The optimum S_{mix} was chosen for the nanoemulsion preparation process. In the oil phase (linseed oil) the drug was mixed by selecting formulations from the NEs regions of the prepared ternary phase diagram. The formulation was chosen based on the need to emulsify the most oil with the least amount of S_{mix}. The resulting mixture was gradually mixed with double-distilled water and stirred gently until equilibrium was reached. Five NEs formulations (NE1-NE5) were prepared with different weights of oil, S_{mix}, and water phase (Table 2). The NEs were vortexed and monitored for up to 24 h to detect any phase separation. Different physical stability tests such as the freeze-thaw cycle and centrifugation study were performed on the formulations[6].

Physical stability testing

The stability tests of the NEs (NE1-NE5) were performed to solve the metastable formulation issue. The formulations chosen from pseudo-ternary phase diagrams underwent additional testing, including centrifugation, freeze-thaw, and heating-cooling cycles. For the centrifugation study, the drug-loaded NE formulations (NE1–NE5) underwent centrifugation for 30 min at 5000 rpm. Further, the NEs formulations were exposed to 6 cycles of heating and cooling at 45°C and 4°C respectively for 48 hours. Freeze-thaw cycle: the storage cycles (-21°C and +25°C) were applied to the NEs formulations for 48 hours[15,16]. visual observation was conducted to assess how the appearance of NEs has changed. Phase separation and creaming were seen in NEs and the selected formulations were discarded. For further analysis, the thermodynamically stable NE formulation was selected.

Physicochemical characterization of NEs formulation

Droplet size (DS), polydispersity index (PDI), and zeta potential (ZP) analysis

Using photon correlation spectroscopy, DS, PDI, and ZP of NE formulation were determined[17]. The DS, PDI, and ZP measurements were performed through Malvern Zetasizer Nano-ZS90 (Malvern Analytical Ltd., United Kingdom). Appropriate dilution of the samples was done and the diluted samples were analyzed at a detection angle of 90° and temperature of 25°C for PS and PDI measurement. The PDI was determined as it represents particle size distribution. The diluted sample was also used for ZP measurement with the same instrument using a second electrode to measure the ZP. ZP is used to determine the dispersion ability of globules in NEs. All measurements were done in triplicate and expressed as mean ± SD.

Transmission electron microscopy analysis

The shape of dispersed globules in NEs was examined through TEM (JEOL JEM1010, Tokyo, Japan) operated at 100 kV accelerated voltage. On the carbon-coated grid, diluted NE with water (1:10) was applied which was further treated with 2% phosphotungstic acid droplets. Then the coated grid was kept for drying at room temperature after that it was observed under a microscope and the image was taken on the AMT image–capture engine[18].

Preparation of nanogel

The thermodynamically stable formulation (NE3) was chosen for conversion into nanogel (NG) using the previously published method[15]. To prepare the NG, the stable nanoemulsion (NE3) was mixed with the carbopol 934 polymer dispersion. 1 g of carbopol 934 was mixed with 100 milliliters of distilled water in a beaker, and the mixture was allowed to completely dissolve. 0.5 g of triethanolamine was added dropwise until a clear gel composition was achieved following full dispersion. The gel base and nanoemulsion were mixed 1:1 while being continuously stirred. The prepared NG was subjected to observation for different parameters. The viscosity, pH, homogeneity, and spreadability of the NG were determined. The content uniformity was tested to ensure that the amount of drug in each portion of NG is consistent.

Characterization of nanogel

pH, Spreadability, and Rheological evaluation

The physical homogeneity, color, and consistency of the NG were determined. The pH of NG was measured before use to ensure stability and skin comfort. The pH of the prepared NG was determined at a temperature of 25 ± 2°C using a pH meter. The viscosity of NG formulation was measured with a Brookfield viscometer (GallenKamp, England), and the results were given as mean ± SD. The spreadability of the NG formulation was determined by the previously described method[16]. The sample was placed between two glass slides to test the spreadability of the NG. 1 g of NG was placed on the pre-marked glass slide had a circle with a diameter of 1 cm on its lower side, and the upper glass slide was covered on it. The weight of 200 g was placed on the upper glass slide for five minutes and the spreading of NG was measured using equation 1.

Spreadability (%) = Increase in diameter/Initial diameter × 100 (1)

Drug content determination

Drug content determination was performed by dissolving the NG sample in 10 mL of ethanol and then diluting it with distilled water q.s 100 mL. Using 0.22 µ membrane filters, the sample was filtered and thereafter subjected to analysis at 238 nm using a UV spectrophotometer[13]. Equation 2 was used to calculate the %drug content:

%Drug content = Observed drug content/Total amount of drug taken x 100 (2)

***In vitro* drug permeation study**

Franz diffusion cells were used to measure the *in vitro* drug permeation[19]. The cellophane membrane (MW 12-14000 Da) was used with a surface area of 4.9 cm^2. The membrane was initially treated with pH 6.8 phosphate buffer for 30 min at 25°C. The treated cellophane membrane was kept between the donor and receptor compartments of the Franz diffusion cell. Then 1 g of NG formulation was placed in the donor compartment whereas, pH 6.8 phosphate buffer was placed in the receptor compartment which was kept at 37 ± 0.5°C. 500 µL of aliquots were removed through the sampling port of the diffusion cell at predetermined intervals (0.5, 1, 2, 3, 4, 5, and 8 h) and replenished with the same quantity of fresh receptor solution to maintain the sink condition. The aliquots were filtered and analysis was done at 238 nm using a UV spectrophotometer[8].

Statistical analysis

The data are shown as mean ± SD. Graph Pad Prism version 5.0 (Graph Pad Software Inc., USA) was used to analyze all the data using one-way ANOVA.

RESULTS AND DISCUSSIONS

Equilibrium solubility study

Linseed oil showed the highest solubility of LS (26.09 ± 0.234 mg/mL) among the various oils used for the solubility analysis. Similarly, the drug solubility was found 26.84 ± 1.23 mg/mL in the surfactant tween 80 and 30.59 ± 2.067 mg/mL in the co-surfactant PEG 400. Based on solubility analysis, linseed oil, Tween 80, and PEG 400 were used as the oil phase, surfactant, and co-surfactant to produce pseudo-ternary phase diagrams, as shown in Table 1.

Table 1: Solubility analysis of LS

Oil	Solubility of LS (mg/mL) ± SD (n=3)
Almond oil	12.44 ± 0.123
Sunflower oil	11.29 ± 0.32
Eucalyptus oil	12.48 ± 0.513
Linseed oil	26.09 ± 0.234
Olive oil	12.28 ± 1.09
Surfactants	Solubility of LS (mg/mL) ± SD (n=3)
Cremophor RH 40	19.29 ± 0.321
Tween 20	16.37 ± 0.34
Tween 60	14.06 ± 1.01
Tween 80	26.84 ± 1.23
Co-surfactants	Solubility of LS (mg/mL) ± SD (n=3)
Myoglyol	12.46 ± 0.147
Isopropyl myristate	12.74 ± 0.198
PEG 400	30.59 ± 2.067
Propylene glycol	15.54 ± 0.201

Formulation of nanoemulsions

To identify the NE region that resulted from combining the S_{mix} and oil phases, ternary phase diagrams were prepared. When titrating the oil with water, S_{mix} emulsifies it at specific ratios that cause NE production. Here, S_{mix} was prepared by combining Tween 80 and PEG 400 at various ratios of 1:1, 1:2, and 2:1. As shown in Fig. 1, a broader region of the NE was produced when we used S_{mix} at a 1:1 ratio. The larger area in the ternary phase diagram suggested that the nanoemulsifying effectiveness of formulation is

better at the selected ratio (1:1) and better interaction between the aqueous phase, S_{mix}, and the oil phase. The formulation should contain the minimum amounts of surfactants and co-surfactants because it has been documented that using too much surfactant can induce skin irritation and toxicity-related problems[20,21]. However, as co-surfactant concentration increased, the nanoemulsion zone reduced significantly. Whereas the nanoemulsion region in the ternary phase diagram increases as the surfactant concentration in the S_{mix} increases. Since a high surfactant concentration irritates the skin[18], therefore, we chose S_{mix} in a 1:1 ratio. Table 2 represents the composition of different formulations at the S_{mix} of 1:1 ratio.

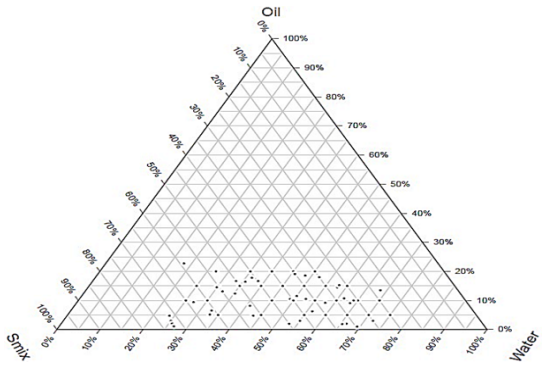

Fig. 1. Ternary phase diagram prepared using S_{mix} in 1:1

Table 2: Composition of various nanoemulsion formulations having S_{mix} (1:1)

Formulations	Oil	S_{mix}	Distilled water	LS
NE1	5	20	75	2.5
NE2	5	35	60	2.5
NE3	10	35	55	2.5
NE4	15	30	55	2.5
NE5	15	35	50	2.5
NE6	20	30	50	2.5

*Weight taken as %w/w

Physical stability of nanoemulsion formulations

The physical stability of the NEs (NE1-NE6) during centrifugation testing, freeze-thaw cycles, and heating-cooling cycles is shown in Table 3. When subjected to the centrifugation test and the freeze-thaw cycles, NE1 did not pass the physical stability study. When subjected to centrifugation, NE2 was not stable. When subjected to the freeze-thaw and heating-cooling cycles, NE4 and NE6 failed the stability investigation. However, when the NE5 formulation was subjected to the freeze-thaw cycle, its stability failed. The single formulation, NE3, is the one that passes all of the thermodynamic stability tests and was used for further characterization.

Table 3: Thermodynamic stability of selected formulation of nanoemulsion

Formulations	Heating-cooling cycles	Centrifugation cycles	Freeze-thaw cycles	Inferences
NE1	√	x	x	Failed
NE2	√	x	√	Failed
NE3	√	√	√	Passed
NE4	x	√	x	Failed
NE5	√	√	x	Failed
NE6	x	√	x	Failed

Characterization of nanoemulsions

DS, PDI, and ZP analysis

DS is an important characteristic of NE as it shows how the NE droplet behaves. Droplet penetration will occur more quickly and deeply in the skin when droplets are smaller in size[22]. The optimized LS-loaded NEs (NE3) have an average DS of 108.9 ± 3.12 nm with the PDI value of 0.257 ± 0.015 (Fig. 2). NE droplet size is within the intended range i.e. less than 200 nm. PDI indicates the uniformity of droplet size in the NEs formulation. A low value of PDI (≤ 0.5) indicates the higher stability of NE formulation[23]. It was possible to identify the homogeneous droplet size distribution in the NE as the PDI of the NE3 formulation was less than 0.5. ZP quantifies the charge on the NE droplets' surface; the prepared NE3 formulation had a negative ZP of –16.93 ± 1.12 mV (Table 4).

Table 4: DS, PDI, and ZP of NE3 (n = 3)

Optimized Formulation Code	DS(nm)	PDI	ZP (mV)
NE3	108.9 ± 3.12	0.257 ± 0.015	–16.93 ± 1.12

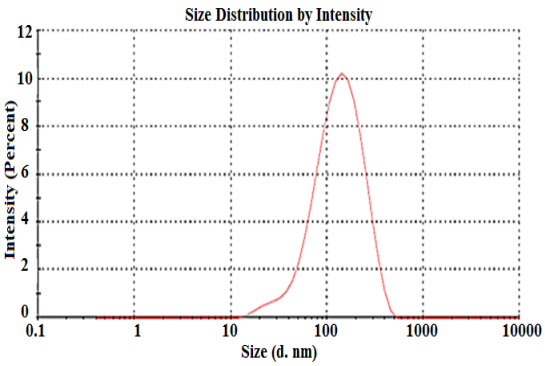

Fig. 2. Size distribution of NE3 formulation

TEM analysis

The morphology and shape of the NE's nanoscale globules are visible in TEM photomicrographs. The obtained NE3 formulation photomicrographs show that the sphere-shaped globules and their nano size were confirmed as shown in Fig. 3. Additionally, there was no evidence of globule coalescence, demonstrating the formulation's physical stability[24].

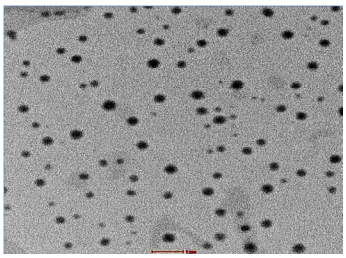

Fig. 3. TEM images of optimized NE3 batch NE

Preparation and characterization of nanogel

Thermodynamic stability and nanosize range of NE3 nanoemulsion formulations led to their inclusion in the gel matrix to prepare NG. To prepare the NG, the NE3 nanoemulsion formulation was mixed in a 1:1 ratio with the carbopol 934 gel base. At room temperature, the mixture was stirred until the nanogels became transparent and uniform. For transdermal preparations, pH is a crucial parameter since the formulation's pH should be close to the skin's pH. The prepared NG formulations had a pH of 6.6 ± 0.11. Further, the viscosity of the NG formulations was found 23286 ± 3.85 cp. In the NG formulation, the drug content of LS was 98.78 ± 1.39% which showed that the drug was uniformly distributed throughout the NG system. The gel extrusion test indicates how easily the formulation can be extruded from the tube. Extrudability was found 15.46 ± 1.82 g/cm^2 which shows that the NG formulation can extrude out of the tube. (Table 5) shows the results of drug content, pH, viscosity, and extrudability of developed NG.

Table 5: pH, viscosity, drug content, and extrudability of the NE gel formulation (n= 3)

Parameters	NE gel formulation
pH	6.6 ± 0.11
Viscosity (cP)	23286 ± 3.85
Drug content uniformity (%)	98.78 ± 1.39
Extrudability (g/cm^2)	15.46 ± 1.82

***In-vitro* drug permeation study**

Figure 4 shows the %drug penetration from NE, NGs, and drug suspension during the *in vitro* permeation study. It was observed that the formulation of NE and NG has a high permeation rate as compared to drug suspension. The formulation with the highest flux value was NE (36.63 ± 0.55 µg/cm^2/h), followed by NGs (33.69 ± 0.75 µg/cm^2/hours). Compared to the formulations, the drug suspension permeation flux value (6.41 ± 1.13 µg/cm^2/h) was much lower. It is clear from the flux value that LS permeation has been greatly increased by nanosized droplets. Therefore, the formulation exhibited improved permeation capabilities due to the addition of nano-sized droplets of NE, which aid in crossing different stratum corneum barriers.

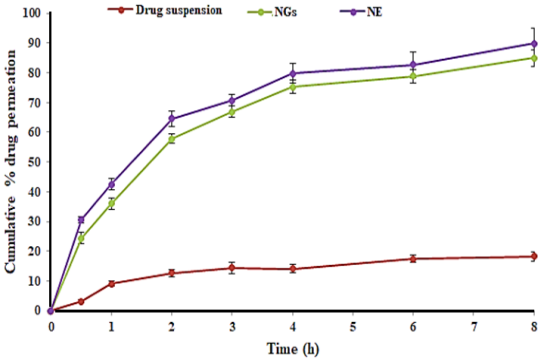

Fig. 4. Cumulative % of LS permeated from NE and NGs formulations in 8 hours

CONCLUSION

LS-loaded NG formulation was developed using linseed oil, tween 80 along with PEG 400 as surfactant, co-surfactant (S_{mix} in 1:1), and cabopol 934 as a gelling agent for transdermal delivery of LS to improve its bio-performance and overcome the drawback associated with its oral delivery. Different components of the formulation were selected based on solubility and the NEs were prepared by spontaneous emulsification method. The thermodynamically stable NE was selected and characterized for various parameters. The NEs formulation was found in the nano range (<200 nm) which is suitable for transdermal delivery of LS. Finally, NE3 formulation was converted into NG using cabopol 934. *In vitro* permeation study was performed for NE, NG, and pure drug suspension. The NG and NE formulation demonstrated a more than 5-fold higher permeation flux rate than that of pure LS suspension in the permeation study. Therefore, the LS-loaded NG formulation was found effective in terms of *in vitro* permeation performance. So, it is concluded that the NG-based formulation of LS is a potential approach for treating hyperlipidemia and could be an alternative to oral

formulation. Moreover, detailed pharmacokinetic and pharmacodynamic studies are required for the clinical outcomes of this investigation.

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Conflict of interest

The authors report no conflicts of interest.

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