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Original Article

NANOEMULSION GEL OF NUTRACEUTICAL CO-ENZYME Q_{10} AS AN ALTERNATIVE TO CONVENTIONAL TOPICAL DELIVERY SYSTEM TO ENHANCE SKIN PERMEABILITY AND ANTI-WRINKLE EFFICIENCY

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ABSTRACT

Objective: The object of our investigation was to develop and characterize nanoemulsion gel (NEG) as transdermal delivery systems for the poorly water soluble drug, Co-enzyme Q_{10} (Co Q_{10}), to improve its solubility and skin permeability and thus improving its anti-wrinkle efficiency.

Methods: An optimized nanoemulsion (NE) formula was chosen according to its particle size and stability and converted into nanoemulsion gel using different gelling agents, including; carbopol 934 (1%), xanthan gum (2%) and sodium carboxymethyl cellulose(NaCMC) (2%). Drug loaded nanoemulsion gels were characterized for particle size, zeta potential, viscosity and rheological behavior, conductivity, spreadability, drug content and permeation studies using Franz diffusion cell.

Results: NEG containing 10% w/v isopropyl myristate (IPM) as oil, 60% w/v tween 80 and transcutol HP as surfactant/co-surfactant mixture (S/CoS), 30%w/v water, 2%w/v drug, and 1% w/v carbopol 934 as gelling gent was concluded as an optimized NEG formula. It exhibited pH, viscosity, drug content, particle size, zeta potential, polydispersity index(PDI) and spreadability, as 5.4±0.011, 27588±2034.34 cps,101.51±0.93%,120.5±1.19 nm,-29.8±1.46, 0.273 and 6.16±0.28 cm, respectively. Also, it showed significantly higher cumulative amount of drug permeated through dialysis membrane (281.71±0.97µg/cm^2) and through rat skin (20.73±2.5 µg/cm^2) than the other formulae and marketed formulation ($P<0.001$). In addition, its permeability parameters like drug flux (Jss), enhancement ratio (Er) and permeability coefficient (Kp) exhibited the highest values; 12.79µg/cm^2/h, 95.92×10^{-4} cm^2/h and 57.35, respectively for *in vitro* permeation study and 0.968µg/cm^2/h, 7.26×10^{-4} cm^2/h and 1.183, respectively for *ex-vivo* permeation study.

Further histopathological evaluation test showed that CoQ_{10} NEG has a good anti-wrinkle efficacy compared to the conventional topical dosage form.

Conclusion: These results judged NEG to be a promising alternative carrier for topical delivery of Co Q_{10} to enhance its solubility, skin permeability and thus anti-wrinkle efficiency.

Keywords: Coenzyme Q_{10}, Nanoemulsion gel, Topical delivery, Skin wrinkles, Permeability

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INTRODUCTION

Topical delivery systems (TDSs) are self-contained discrete systems applied to the intact skin to deliver drugs at a controlled or sustainable rate [1]. Additionally, they avoid GIT side effects, inactivation of the drug by GIT enzymes, the interaction of the drug with food and first-pass metabolism of drugs in GIT [2].

Conventional topical dosage forms such as cream, ointments, and pastes have the disadvantage of being sticky for applying to the skin and having a low permeability for the drug. For this reason, transparent gels are widely used in cosmetics and pharmaceutical preparations. Gels are formed by incorporation of drugs in the aqueous or hydrophilic liquid of the gelling agent network. They are mainly appropriate for water-soluble drugs rather than hydrophobic drugs, which are difficult to incorporate into aqueous gel bases [3]. To overcome this disadvantage, nanoemulgel (NEG) is developed. NEG is a novel pharmaceutical drug delivery system that is widely used because it is a stable and better vehicle for hydrophobic or poorly water soluble drugs oil in water (o/w) emulgel as well as hydrophilic drugs water in oil (w/o) emulgel. NEG is nanoemulsion (NE), (either o/w or w/o), that are gelled by mixing with a gelling agent. This NE is thermodynamically stable, transparent with mean droplet diameters ranging from 20 to 200 nm (typically below 100 nm) [4]. Its vast network structure allows better drug loading capacity compared to other novel approaches like niosomes and liposomes consequently, avoiding the drug leakage and lesser entrapment efficiency. They possess various advantages over emulsions and microemulsions, such as higher surface area (due to the smaller droplet size of the NE), higher skin permeation, higher retention potential and improved physical stability [5-9]. In addition, controlling drug release and prolonging the effect of drugs having shorter $t_{1/2}$. No intensive production mechanisms are used in its preparation and no need intensive sonication which may result in drug degradation [10]. Nanoemulsification is recommended to be a potential method for improving drug permeation through the skin without the using permeation enhancers, since their components (oils, surfactants, and co-surfactants) could act as permeation enhancers [11]. Co Q_{10} is an endogenous free radical scavenger and exists in mitochondria in all parts of the body as a skin. It acts as an antioxidant like vitamins A, C and E. Co Q_{10} is used in the skin care cosmetic products as it can provide the skin with energy, protect it against skin aging and wrinkles, and repair skin. The aqueous solubility of Co Q_{10} is very low, causing a low oral bioavailability and permeability [12]. This study aimed to formulate one of the poorly soluble drugs Co Q_{10} in new dosage form (nanoemulsion gel (NEG) drug delivery system) for enhancement of solubility and permeability of Co Q_{10} through topical applications as well as improvement of its anti-wrinkle efficacy. The optimized NE formula was incorporated into gel matrix bases to obtain different (NEG) formulae. These NEG formulae were prepared using three types of hydrophilic polymers namely; xanthan gum, carbopol 934 and sodium carboxymethylcellulose (NaCMC).

MATERIALS AND METHODS

Materials

Co Q_{10} was kindly donated as a gift from Mebaco Company, Cairo, Egypt. Tween80, span 20, olive oil, ethanol and isopropanol (HPLC grade) were purchased from El-Nasr Pharmaceutical Chemical Company, Cairo, Egypt. The avocado oil was obtained as a gift from (Jan Dekker Nederland B. V, Wormerveer, and Holland). Caproyl 90 (polypropylene glycol monocaprylate), Labrasol (capryl-caproyl macrogol-8-glyceride), labrafil M1944 CS (polyoxyethylene kernel oil) and transcutol HP (diethylene glycol monoethyl ether) were given as a gift from Gattefosse (Cedex, France). Cremophor EL (polyoxyl 35 hydrogenated castor oil), isopropyl myristate (IPM) and propylene glycol (PG) were purchased from Sigma-Aldrich, Germany. Disodium hydrogen phosphate and sodium dihydrogen phosphate were purchased from El-Gomhouria Company, Cairo; Egypt. Triethanolamine was purchased from Sigma-Aldrich, Cairo, Egypt. xanthan gum, carbopol 934 and (NaCMC) were kindly donated as a gift from Luna Company, Cairo, Egypt. Formaldehyde was purchased from El-Nasr Pharmaceutical Chemical Company, Cairo, Egypt. Xylene, paraffin bees wax, hematoxylin, and eosin were purchased from Sigma Aldrich Chemical Company, Cairo, Egypt.

Pre-formulation study of nanoemulsion

Solubility study

The solubility of Co Q_{10} in different oils namely; (oleic acid, IPM: oleic acid at ratio (3:1), IPM, avocado oil, caproyl 90, labrafil M1944 CS and olive oil), surfactants namely; (tween 80, cremophor EL and labrasol) and co-surfactants namely; (PG, span 20 and transcutol HP) were determined following method described by Rania *et al.*[4]. An excess amount of Co Q_{10} was allowed to dissolve in 1g of each oil, surfactants, and co-surfactants in 10-ml capacity stoppered vials separately. The mixtures were vortexed to facilitate drug solubilization (Stuart vortex mixer, U. K) and kept at 37˚C±1.0 in an isothermal shaker (Shaking water bath, B S-11, Isothermal shaker, Korea) for 72h to reach equilibrium. These mixtures were rotated in the centrifuge at 3500 rpm for 15 min. The supernatant was filtered through a 0.45-µm membrane filter and diluted with the mobile phase (ethanol: isopropanol) at a ratio (75:25). The concentration of Co Q_{10} was determined by HPLC method reported by Joe and Sunil, 2005 [13].

Surfactant/co-surfactant screening

Various S/CoS combinations with hydrophilic/lipophilic balance (HLB) values in the range (10-13) were prepared and screened to emulsify o/w mixture and form o/w NE. Briefly, the selected oil, and water were mixed in the ratio 1:1; then the S/CoS combination was added drop wise to o/w mixture. During the titration, the samples were sonicated to reach the equilibrium quickly. The amounts of S/CoS mixture required to completely emulsify and homogenize the equal quantities of oil and water were determined following the method developed by Ljiljana and Marija [14]. The S/CoS mixtures of low concentration (<60%) that succeed to emulsify o/w mixture were selected for further study.

Construction of pseudoternary systems

Based on the solubility and screening studies, IPM and IPM: oleic acid at a ratio (3:1) were selected as the oil phase. Labrasol, cremophor EL and tween 80 selected as a surfactant (S), PG and transcutol HP as co-surfactant (CoS), and distilled water as the aqueous phase. Six blends of three surfactants and two co-surfactants were prepared by mixing each surfactant with each co-surfactant at different mass ratios (4:1, 3:1, and 2:1). Different phase diagrams were constructed using aqueous phase titration methods. Combinations of different weight ratios of oil and S/CoS mixtures from 1:9 to 9:1 were made to delineate boundaries of phases precisely formed in the phase diagrams. Water was added drop wise to the specified weights of oils: S/CoS mixtures under magnetic stirring at 25˚C till the first turbidity. The weights of the components of each NE system were recorded as percentages to be presented in tri-phase diagrams.

Preparation and optimization of Co Q_{10} nanoemulsion

Depending on the phase diagrams, Various Co Q_{10} loaded NEs from the constructed phase diagrams were prepared at the different component ratio using aqueous titration method. Appropriate quantities of oil, surfactant, and co-surfactant were weighed and mixed well. Co Q_{10} was accurately weighted to represent 2%w/w of the NE formulation total weight and then mixed with the previous mixture at room temperature using a magnetic stirrer (100 rpm) until complete dissolving of the drug. The specified weight of water added drop by drop to the oil/surfactant mixture with continuous stirring using magnetic stirring for 30 min. To optimize the NE formula, 1g of each formula was diluted with 100 ml distilled water. The formulae that successfully passed the dilution test that showed transparent and homogenous NEs were subjected to further investigation namely; thermodynamic stability test.

Thermodynamic stability tests

NE formulae were evaluated for their stability under different stress conditions. Firstly, the formulae were centrifuged at 3500 rpm for 30 min. The formulations that would not exhibit any phase separations were subjected to heating-cooling cycle test. The samples were stored for six cycles at a temperature between 4 °C and 45 °C for 48 h at each temperature. Formulations showed good stability under these circumstances were exposed to freeze-thaw cycle test. This cycle was performed between-21 °C and 25 °Cand repeated 3 times [15]. The formulation that survived thermodynamic stability tests were selected and incorporated into the gel.

Preparation of NEGs

The optimized NE formula was incorporated into gel matrix bases to obtain different NEGs. These NEG formulae were prepared using three types of hydrophilic polymers namely; xanthan gum (2% w/w) [16], carbopol 934 (1% w/w) of the total weight formula [17] and sodium carboxymethylcellulose (NaCMC) (2%w/w) [18] by the use of two different methods namely; emulsification method and geometric dilution method.

Emulsification method (A)

In this method, the oil phase was prepared by dissolving the specified amount of drug (2 %) by the specified weight of IPM (10%), tween 80 (40%) and transcutol (20%) mixture using magnetic stirring until the formation of a clear solution. Aqueous gel phase was prepared by dispersing the specified amount of gelling agent (carbopol-934, NaCMC or xanthan gum) in sufficient quantity of distilled water using stirring. This dispersion was kept in the refrigerator for 24 h for complete swelling of the gelling agents. The oil phase was transferred slowly portion wise to the formed gel and mixed well, then water was added to get the final preparation of 100%w/w. The sample was stirred continuously at 1100 rpm to ensure the emulsification and the homogeneous distribution of drug [19].

Geometric dilution method (B)

In this method, NE is firstly prepared. Briefly, the drug was dissolved in IPM, tween 80 and transcutol mixture using a magnetic stirrer. After complete homogenization of the mixture, the specified weight of the water was added dropwise with continuous stirring to form NE. The aqueous gel phase was prepared as mentioned above. By using the geometric dilution method, one part of the gel was added to two parts of NE under mixing to obtain the NEG. The final preparation was 100% w/w.

Triethanolamine (TEA) was added in a concentration (0.5% w/w) to the formula containing carbopol-934 for neutralization and regulation of pH in the range of (6-9) [17]. Glycerol was added in a concentration (5%w/w) to each formula to act as humectants [16].

Evaluation of Co Q_{10} NEG

Percentage of drug content

This study was carried out by taking (0.1 g) of each formula in a volumetric flask (10 ml capacity) and dissolving the drug using 10 ml of the mobile phase (ethanol: isopropanol at ratio 75:25). These solutions were sonicated for 5 min to break the lumps of gel, and filtered through a 0.45 µm membrane filter. The drug content was determined by the mentioned HPLC method [20]. The percentage of drug content was determined following the given equation (1) [21]. The results were done triplicate and represented as mean±SD

$$\% \text{ Drug content } = \frac{\text{Actual content}}{\text{Theoretical content}} \times 100 \text{ Equation (1)}$$

pH determination

The pH measurement of the formulae was determined in triplicate at 25 °C with a digital pH meter (pH meter, Hanna instrument, USA) (human skin ranges 5-7). The pH meter was adjusted previously using a buffer solution of pH 4, 7 and 10.

Viscosity measurements and rheological properties

The viscosity and rheological properties of the formulae were determined by using viscometer (Brookfield digital rheometer, DV III ultra-programmable rheometer, USA) using a cone plate method. A 0.5 g of each NEG was put in the lower plate of the viscometer. The spindle cone (CP-40) was rotated at gradual rpm ranged from 0.01 to 3 to obtain a suitable torque from 10 to 100, at 25±1 °C, with the 30s between each measurement. The rheological behavior of NEG was studied by plotting the log values of shear stress τ (dyne. cm^2/s) versus the log values of the shear rate γ (s^{-1}) [18].

The flow index (n) and consistency (k) were determined from the following power law equation:

$$\tau = k\,\gamma \text{ or } \log \tau = \log k + n \log r \text{ Equation (2)}$$

n=1 for Newtonian flow, for n>1 indicates shear thickening flow, for n<1 refers to shear thinning flow. The lower the (*n*) value the more shear thinning the formulation [22].

Transmission electron microscope (TEM), Globule size and distribution analysis

The average droplet size and polydispersity index (PDI) of prepared NE and NEG formulae were determined using Malvern Zeta-Sizer 90 instrument (UK) using a laser beam of 50 mv. The measurement time was 3 min. One gram of each formula was dispersed into 100 ml of distilled water with gentle stirring in a glass beaker. An aliquot of diluted dispersion was transferred into the cell sample holder for droplet size analysis. The data were triplicate samples±SD [23].

Morphology and structure of NE globules were performed using transmission electron microscope (TEM, Tecnai G20, Super twin, double tilt, FEI, Netherland.) operating at 200 kv. An aliquot of the above-diluted dispersion was directly deposited on the holey film grid, stained by 1%w/v aqueous solution of phosphotungstic acid, and observed after drying.

Zeta potential measurement

Zeta potential was measured using a zeta potential analyzer ((NanoZS90, Zeta plus, Malvern instrument Ltd., UK). The diluted NE and NEG at ratio 1:100were exposed to an electric field (1v). The average±SD of the three independent measurements was recorded.

Electroconductivity study

The electrical conductivity (σ) of the formulated NE and NEG was determined using Hanna digital conductometer, (Model: H1255, Romania). The formulae were dispersed in distilled water at ratio 1:100 and 1:50 and the average±SD of the three independent measurements were recorded [24].

Spreadability

Spreadability of the NEGs was represented as the diameter of gel circle, obtained when the gel is compressed between two glass plates using a definite weight. A sample of (0.5 g) of each formula was placed inside a circle (1 cm diameter) drawn on a glass plate and covered with another glass plate. A weight of 100 g was placed on the cover glass plate for 5 min, after that the diameter of spreadability of the gel was measured. The results obtained are an average of three measurements [22].

Permeation studies

***In vitro* permeation, Ex-vivo skin permeation and skin retention studies**

In vitro permeation of CoQ_{10} from NEG formulae was investigated through a semipermeable membrane (Sigma, molecular weight cut off is 12,000 daltons) using Franz diffusion cells. The *ex-vivo* permeation studies were carried out using excised skin of wistar rats, the skin was prepared following method reported by Songkro, *et al.* [25] and Junyaprasert, *et al.* [26]. The cellulose membrane or skin pieces were clamped between the donor and receptor compartments of a locally fabricated Franz diffusion cells (diffusion area of 1.77 cm^2; receptor volume of 7.5 ml, stirring at 500 rpm). Phosphate buffer at pH 7.4 containing 5% w/v labrasol and 5% w/v isopropyl alcohol was used as the receptor medium for solubilization of Co Q_{10} and maintained at 37±0.5 °C. 0.5 g of NEG formulae was applied evenly on the surface of the membrane or skin in the donor compartment. The aliquots (1 ml) from the buffer media were withdrawn at time intervals (0.5, 1, 2, 4, 6, 8, 10, 12, 14, 16, 24 h.) and replaced immediately with a similar volume (1 ml) of fresh medium. The withdrawal samples were filtered using membrane filter (0.45 μm) and the drug content was determined using HPLC [27].

The cumulative amounts of permeated Co Q_{10} through the cellulose or skin membrane per unit surface area (cm^2) were calculated using the given equation (3) and subsequently used for plotting the profiles of the drug permeation per unit time.

$$\text{Cumualtive amount of drug permeated} = \frac{\text{Concentration } (\mu g/ml) \times \text{ dilution factor}}{\text{surface area of skin}}$$

Equation (3)

The rate of drug permeation at steady state (drug flux) (Jss) was presented by the slope of the linear regression of permeation-time curve. The permeability coefficient (Kp) and enhancement ratio (Er) were calculated from the following equations (4) and (5) respectively [28].

$$Kp = Jss/C_o \text{ Equation (4)}$$

$$Er = Jss\ of\ formulation/Jss\ of\ control \text{ Equation (5)}$$

Our experimental study was extended for investing the possibility of drug accumulation beneath the skin layers through the application of skin extraction process [16]. The skin pieces were collected, rinsed with distilled water, gently dried with a cotton swab, cut into small pieces and soaked in a 10 ml mixture of (ethanol: isopropyl alcohol) at a ratio (75:25 w/w %) for 12h in closed tubes. The tubes were sonicated for three cycles each 15 min to avoid the rise of the temperature during the extraction process. The supernatant was filtered 0.45um and the Co Q_{10} content was estimated using HPLC.

Comparative *in vitro and ex-vivo* permeation studies between the optimized NEG, marketed Co Q_{10} cream and a conventional Co Q_{10} gel

A comparative *in vitro* and *ex-vivo* permeation study between the optimized NEG formula and (Nivea Q_{10} anti-wrinkle cream as a market cream), or a conventional Co Q_{10} gel were carried out. The conventional Co Q_{10} gel was prepared by dispersing 1 g of carbapol 934 inadequate amount of water with continuous stirring to form gel. 2 g of the drug was dissolved in 20 g of labrasol using a sonicator at 60 °C and added portion wise to the carbapol gel with continuous stirring, water was added to obtain 100 g of the gel. The gel pH was adjusted to 6.5±0.5 with TEA. The same above permeation parameters were estimated.

Statistical analysis

The experimental *in vitro* and ex-vivo permeation data were further exposed to statistical study using one way ANOVA test "SPSS version 17.0 for windows" (SPSS Inc., USA) followed by post HOC LSD alpha at 95%confidence intervals. Variances were assumed to be significant* when P<0.05, highly significant** when P<0.01 and very highly significant*** when P<0.001. If P>0.05, refer to no significant difference.

***In vivo* anti-wrinkle evaluation**

The experimental protocol was ethically approved by the Animal Care Committee, Faculty of Pharmacy, Helwan University No (3) for 14/6/2016. Female rats (12 to 18 mo-old, weighing 200–240 g)

were got from the animal house of National Organization for Drug Control and Research. Rats had free access to food and water and were adapted to the following conditions (23±2 °C and 50±10% humidity with a 12 h light/12 h dark cycle) in an air-conditioned room for 1 w before the *in vivo* anti-wrinkle study.

Firstly, the hairs on the dorsal side of the rats were removed using hair removal cream then the animals were divided into four groups; each group contains three rats. The first group (un-treated control group), the second group was treated with NEG F3, the third group was treated with Co Q_{10} gel, the fourth group was treated with marketed Co Q_{10} cream. The animals in each group were delivered an amount of the treatment equivalent to 20 mg of Co Q_{10} twice a day for one month. The formulae were applied topically to the skin at square area 10×5 cm of the dorsal part. After 0, 7, 14, 21 and 30 d of treatment, photographs of the skin were taken before and after the treatment [29]. At the end of the study (30 d), the rats were sacrificed and the skin specimens (marked area) were cut. The skin specimens were fixed in 10% formalin solution for 24h, then the skin was washed with tap water followed by serial dilutions in alcohols (methyl, ethyl and absolute) for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degrees in hot air oven for 24h. Paraffin bees wax tissue blocks were prepared through sectioning at 4 microns thickness by a microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained with hematoxylin and eosin stains for histopathological examination under the light electric microscope [30].

RESULTS AND DISCUSSION

Preparation of Co Q_{10} loaded nanoemulsion

The solubility study of the drug in the different vehicle was done to select the suitable one of the maximal solubilizing potential to reach the optimum loading capacity of the drug and ensure no drug precipitation. Fig. 1 illustrates the solubility of Co Q_{10} in various oils, surfactants and co surfactants. The choice of excipients used in NE preparation was based on the solubility studies.

Co Q_{10} exhibited high solubilization in IPM: oleic mixture and IPM as oil phases, labrasol and tween 80 as surfactant and span 20 and transcutol HP as co-surfactant (fig. 1). In the S/CoS screening test, it was found that the system containing span 20 exhibited bad (turbid) NE or gave NE with a high concentration of S/CoS (>60%) that made the risk of skin irritation, so it was excluded. Six phase diagrams for IPM (fig. 2) and six phase diagrams for IPM: oleic acid (fig. 3) was constructed. A number of exhaustive NE formulae (18 formulae) were selected from the constructed phase diagrams, prepared by aqueous phase titration method and loaded with a drug in concentrations 2%, then, subjected to dilution test followed by thermodynamic stability tests (un-tabulated results). It was found that all formulae prepared with IPM: oleic acid mixture or containing labrasol as surfactant showed precipitation of the drug upon subjected to stability test. The stable formula which showed lower particle size was selected to be incorporated in a gelling agent to form NEG. This formula is composed of 10% w/v IPM as oil, 60% w/v tween 80 and transcutol HP as surfactant/co-surfactant mixture (at ratio 2:1), 30% w/v water, 2% w/v drug.

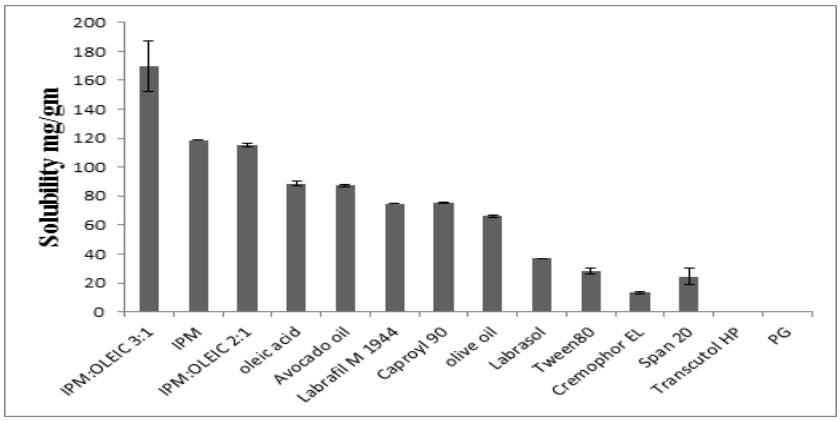

Fig. 1: Solubility of Co Q_{10} in different oils, surfactants, and co-surfactants, IPM: isopropyl myristate, PG: Propylene glycol, mean±SD (n=3)

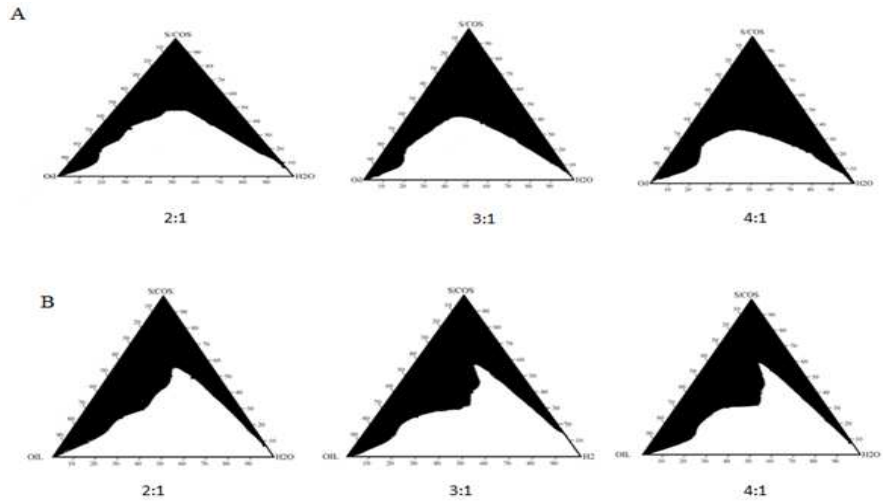

Fig. 2: A) Pseudoternary phase diagrams of IPM (oil), tween80: transcutol HP (S/CoS) at ratio 2:1, 3:1 and 4:1. B) Pseudoternary phase diagrams of IPM (oil), labrasol: transcutol HP (S/CoS) at ratio 2:1, 3:1 and 4:1

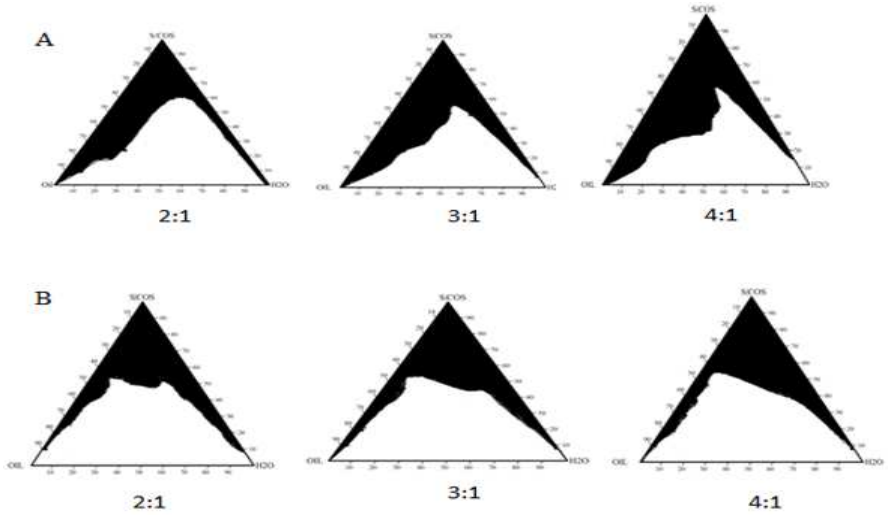

Fig. 3: A) Pseudoternary phase diagrams of IPM: oleic acid 3:1(oil), tween80: transcutol HP (S/CoS) at ratio 2:1, 3:1 and 4:1. B) Pseudoternary phase diagrams of IPM: oleic acid 3:1 (oil), labrasol: transcutol HP (S/CoS) at ratio 2:1, 3:1 and 4:1

Preparation of Co Q_{10} NEG formulae

Due to the small viscosity of the prepared NE, its applicability for dermal use would be difficult. Hence, the viscosity was increased by incorporating NE into a gel matrix resulting into NEG, which was found to be consistent, uniform and having a suitable viscosity to be applied dermally. The optimum NE formula was formulated into NEG by using three different gelling agents as (carbopol 934, xanthan gum and NaCMC), the composition of the NEG is shown in table 1.

Table 1: Composition of Co Q_{10} NEG formulae

Formula code	%IPM	% S/CoS*	% water	Gelling agent	Method of preparation
NEGF1	10	60	30	XGum (2%)	A
NEGF2	10	60	30	XGum (2%)	B
NEGF3	10	60	30	CP (1%)	A
NEGF4	10	60	30	CP (1%)	B
NEGF5	10	60	30	NaCMC (2%)	A
NEGF6	10	60	30	NaCMC (2%)	B

IPM: isopropyl myristate, XGum: xanthan gum, Cp: carbapol 934, NaCMC: sodium carboxymethylcellulose *S/CoS: (tween 80: transcutol HP at ratio (2:1)), NEGF: nanoemulsion gel formula

Evaluation of NEG formulae

Drug content

The drug content of the NEG formulae (F1to F6) ranged from 99.35±9.14 to 101.88±3.27 and 101.58±0.142 for F2 NE, table 2. The results showed that the percentage drug contents of the formulae prepared by the method (A) were slightly higher than those prepared by method (B). In addition, the drug was evenly dispersed all over the formulation. Uniformity of the drug content indicated the efficacy of the preparation procedure and the homogeneity of the formulation with the absence of gel lumps [21].

Determination of pH

The pH values of the NEG formulae (F1-F6) were ranged from 5.38±0.046 to 7.15±0.055which were near to the skin pH (5-7), table 2. The pH measurements were considered to be an acceptable limit for topical application to avoid the risk of any skin damage or irritation upon application.

Viscosity measurements and rheological properties

The viscosities of NEG formulae at 0.5 rpm were shown in table 2. The rheological behavior of Co Q_{10} NEG displayed non-Newtonian flow behavior (shear thinning system), as the flow index n<1 with thixotropic character, while NE presented Newtonian flow behavior, n=1, the results were shown in table 3, [31, 32]. This thinning flow is important for the percutaneous application of NEG formulae as a thick product become thinner under a shear stress so, it becomes easily spreadable on the skin [33].

Transmission electron microscope (TEM), globule size and distribution analysis

The average droplet size of the six NEG formulae (F1-F6) ranged from 10.51±0.88 to 150±0.96 nm, table 2. The results reveal no significant difference in the droplet size of (F1, F2) and (F5, F6) containing xanthan gum and NaCMC, respectively, compared to NE formula (11.76±1.100 nm), as shown in table 2. The results of the particle size analysis were similar to those reported by Kesavan, *et al.* [34] that incorporation of NE into gelling agent did not lead to droplet aggregation and no change in the particle size of NE for the formulae (F1, F2, F5 and F6 NEG). The small size of the droplets formed may be attributed to the penetration of co-surfactant molecules in the surfactant film, and its ability to lower the surface viscosity and the fluidity of the film, reducing the radius of curvature and forming transparent system [18]. On the other hand, the incorporation of NE in carbopol gel (F3 and F4) resulted in an increase in the particle size compared to the NE, This may be attributed to surfactant/polymer interaction that occurs mainly through a hydrogen-bond formation and leads to promoting the bridging of polymer and particle aggregation [35]. PDI of all formulae remained lower than 0.5, this reflects relatively low differences between particle sizes within the formulae and reflect the uniformity of particle diameter and monomial size distribution of NE population. Fig. 4a, 4b showed the particle size distribution and TEM of NEGF3, respectively.

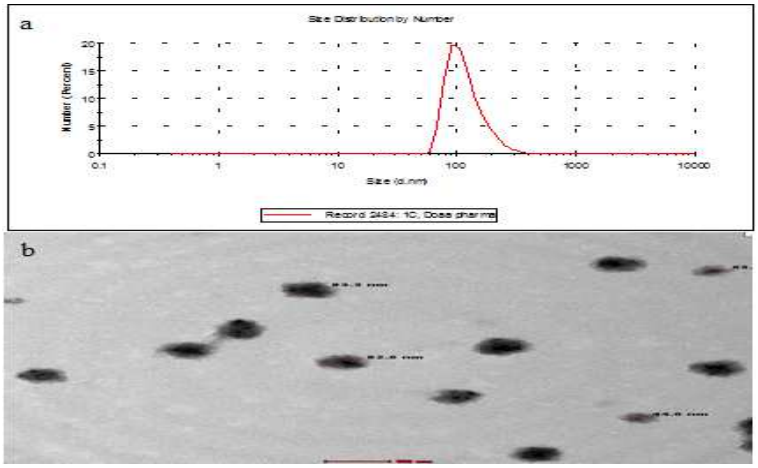

Fig. 4: a) Particle size distribution and b) TEM of NEGF3

Zeta potential measurement

The zeta potential values of the all NEG formulae (F1-F6) and NE were negative values ranged from (-20.6±1.7 to-40.7±4.64) as shown in table 2. The high negative zeta potential values indicate a deflocculated system in which repulsive forces exceed the attractive forces, thereby keeping the particles dispersed [22]. These values exhibited a significant increase in the zeta potential compared to the value of the optimum NE formula (-14.70±1.230). The high zeta potential in case of formulae containing NaCMC could be due to its anionic polysaccharide structure that consists of amphipathic anhydrous glucopyranose and hydrophilic carboxymethyl units 18. Thus, the repulsion of negative charge and ionized carboxymethyl groups could cause an increase in zeta potential [36]. Also, the negatively-charged carboxylate groups of carbapol and the negatively-charged carboxylate and sulphate groups of xanthan gum on the surface of NEG could result in an increase in zeta potential [37, 38].

Electroconductivity study

The conductivity values of the NEG formulae with different dilution ratio (1:50 and 1:100) with distilled water were ranged from 48.8±3.3to 112.4±2.23 µS/cm, table 2. From the results, it can be concluded that the formulae NEGF1, NEGF2, NEGF3 and NEGF4 exhibited slight increase in the conductivity values in the both dilution ratios more than the NE formula. The formulae F5 and F6 exhibited the highest conductivity value compared with other NEG formulae at both dilution ratios. Also, It exhibited 1.6 and 2.3 fold increase in conductivity values in dilution ratio 1:50 and 1:100 respectively, compared to NE formula. Conductivity represents the ion concentration in solution. When the ion concentration increases the conductivity measurement increases [39]. From this fact, the high conductivity of NEGF1 and NEGF2 containing xanthan gum could be because xanthan gum polymer is an anionic polysaccharide, increase the surface charge of emulsion droplets and thus increase the conductivity [40]. The incorporation of NE into carbopol 934 polymer as in NEGF3 and NEGF4 increases the conductivity because carbapol is an anionic polymer in neutral pH and its side chain will lose their proton and acquire a negative charge that makes carbopol weak anionic polyelectrolyte that ionized in high PH [39]. While NEGF5 and NEGF6 exhibited the highest conductivity due to the increase in the number of mobile ions H+or Na^+resulted from ionization of NaCMC polyelectrolyte chain in aqueous solution [41].

Spreadability

The spreadability values of all NEG formulae were found to be in the range of 4.7 cm±0.28 to 5.2 cm±0.53, as shown in table 2. These results indicated the ease of spreadability of the gel by low shear. The ease spreadability means ease of application and patient compliance. NEGF3 and NEGF4 containing carbopol 934 showed the highest spreadability than other formulae as they had the largest diameter of the spread circle [22].

Table 2: Characterization of NEG formulae compared to NE

Characterization	NE	NEGF1	NEGF2	NEGF3	NEGF4	NEGF5	NEGF6
pH	7.06±0.051	6.09±0.01	6.19±0.04	5.4±0.01	5.38±0.05	7.15±0.06	7.13±0.26
Spreadability (cm)	---------	5.5±0.5	5.0±1.0	6.2±0.3	5.7±1.0	4.3±0.6	4.7±0.6
Viscosity (cp) atrpm (0.5)	---------	27834±961.66	29015±3255.51	27588±2034.34	28038±296.27	31313.5±521.63	37123±1439.66
Flow index (n)	0.996	0.23	0.48	0.43	0.28	0.46	0.43
Droplet size (nm)	11.76±1.1	12.44±1.1	10.51±0.88	120.5±1.2	150±0.96	20.1±2.00	11.02±0.76
PDI	0.228	0.371	0.394	0.273	0.191	0.313	0.321
ZP (mv)	-14.7±1.23	-36.8±2.97	-40.7±4.64	-29.8±1.46	-20.6±1.7	-38±3.72	-29.7±2.93
Drug content (%)	101.58±0.142	101.71±7.31	99.35±9.14	101.51±0.93	99.62±3.47	101.88±3.27	99.91±1.68
EC (µS/cm) at ratio 1:50	42.5±2.29	59.36±0.55	55.73±1.56	48.8±3.3	50.23±6.56	72.36±1.66	78.76±3.3
EC (µS/cm) at ratio 1:100	48.03±0.55	65.8±1.63	66.83±1.8	62.63±2.47	55.2±4.96	112.4±2.23	110.06±3.05

NE: nanoemulsion, NEG: nanoemulsion gel, ZP: zeta potential, EC: electroconductivity, PDI: polydispersity index mean±SD (n=3)

Permeation studies

***In vitro and Ex-vivo* permeation study**

Fig. 5 showed the *in vitro* cumulative amounts of Co Q_{10} permeated from the different NEG formulae (NEGF1-NEGF6) at different time intervals. It was observed that the permeation of the drug from the different formulae through cellulose membrane can be ordered as follow: NEGF3>NEGF4> NEGF1>NEGF2>NEGF5>NEGF6; where the cumulative amounts of the drug permeated after 24 h were: 281.71±0.97, 126.8±6.06, 50.9±0.97, 44.79±0.744, 19.07±0.657 and 12.77±0.27 µg/cm^2, respectively. Statistical analysis of the *in vitro* permeation data revealed that the formulae F3 and F4 exhibited a significant increase in the cumulative amount of the drug permeated at ($p<0.001$) compared to other formulae.

The permeability parameters of the different NEGs were shown in table 3. NEGF3 showed the highest permeability parameters and their values were 12.79 µg/cm²/h and 127.9×10⁻⁵ cm²/h for Jss, and Kp, respectively.

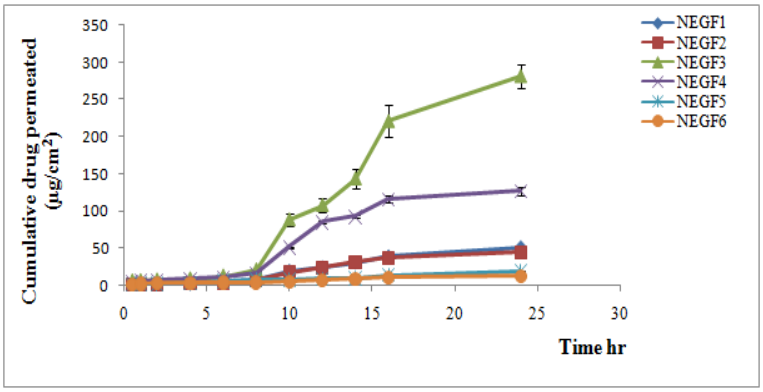

Fig. 5: *In vitro* drug permeation of the NEG formulae (mean±SD (n=3)

Table 3: *in vitro* and *ex-vivo* permeability parameters of Co Q10 NEG formulae

	***In vitro* permeation**		***Ex-vivo* permeation**		
Formula code	**J_{ss} (µg/cm²/h)**	**K_p (cm²/h)×10⁻⁵**	**J_{ss} (µg/cm²/h)**	**K_p (cm²/h)×10⁻⁵**	**Accumulated drug per area (µg/cm²)***
NEGF1	2.335	23.5	0.411	4.11	45.18±3.9
NEGF2	2.183	21.8	0.376	3.76	30.32±1.3
NEGF3	12.79	127.9	0.681	6.81	164.08±9.42
NEGF4	6.259	62.5	0.589	5.89	155.57±6.01
NEGF5	0.742	7.42	0.394	3.94	32.61±5.32
NEGF6	0.472	4.72	0.413	4.13	27.97±1.66

*mean±SD (n=3), Jss: drug flux, Kp: permeability coefficient

The *ex-vivo* results confirmed that F3 displayed the maximum cumulative quantity of Co Q_{10} permeated through the skin after 24 h than other formulae (P<0.001). Also, NEGF3 showed the highest accumulated amount of the drug (164.08±9.42µg/cm²). The cumulative amount of the drug permeated after 24 h can be arranged in descending order as follow F3>F4>F1>F5>F2>F6; where the amount of the drug permeated was 20.73±2.5, 17.74±4.33, 15.53±3.45, 10.82±2.32, 10.53±1.79and 9.51± 1.4µg/cm², respectively, fig. 6.

The rate and extent of the drug release depend on the viscosity, thickness, swelling, and erosion of the hydrated polymer as well as polymer-drug interactions [42]. The results revealed the effect of the polymer type on drug permeation since the permeation of drug from formulae F3 and F4 containing carbopol 934 showed the highest cumulative drug permeated after 24 h than the other formulae containing xanthan gum or NaCMC.

These results were consistent and similar with previous works [43-45] that supposed the possibility of formation of an ionic complex (without affecting the gel structure) between this anionic polymer (xanthan gum or Na CMC) and cationic drug such as Co Q_{10} resulting in hindering the drug release [46-48].

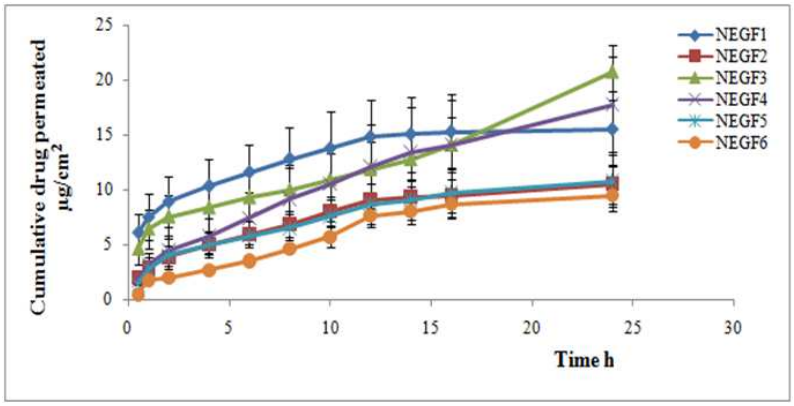

Fig. 6: E*x-vivo* drug permeation of the NEG formulae mean±SD (n=3)

Comparative ex-vivo permeation study

Fig. 7 showed the comparative *ex-vivo* permeation profile of NEGF3, Co Q_{10} cream, and Co Q_{10} conventional gel. The cumulative amount of drug permeated was ranked in the following descending order; NEG F3>Co Q_{10} Gel>Co Q_{10} cream**.** The amount of drug permeated from NEGF3 Co Q_{10} was significantly higher than Co Q_{10} conventional gel and Co Q_{10} cream (P<0.001) and their values were 20.73±2.5, 6.04±0.0212 and 1.16±0.014 (µg/cm²), respectively. The E_r of NEGF3 respects to Q_{10} gel was 6.81and 17.025respects to Q_{10} cream, table 4.

These results could be attributed to the lower particle size of NEGF3 (120.5±1.19 nm) than those of Co Q_{10} cream (2.69 µm±0.17) and Co Q_{10} gel (1.518 µm±0.65). It was apparent that smaller droplet size

provides a larger surface area for drug release through the membrane or the skin at the donor compartment, which resulted in a higher concentration gradient that acts as the driving force for drug release. While the larger particle leads to slower particle movement from the inner to the outer phase consequently, the release was delayed.

The superior drug permeated from NEG compared to Co Q_{10} cream and Co Q_{10} gel might be also due to the higher lipophilic drug solubilizing capacity of the nanosystem that leads to increasing the drug dissolution and improving the drug release [49, 50].

Estimation of the drug accumulated in rat skin

In comparing the amount of drug accumulated for NEGF3, Co Q_{10} cream and Co Q_{10} gel, table 4, it was found that NEGF3 exhibited a significantly higher amount of the accumulated drug than Q_{10} cream and gel ($p<0.001$), and their values were 164.08±9.42, 3.3±0.49 and 14.69±6.53 (µg/cm²) respectively. This higher amount may be attributed to the interaction between the components of NEG and the lipid bilayers of the stratum corneum (SC), thus enhancing the drug penetration [51].

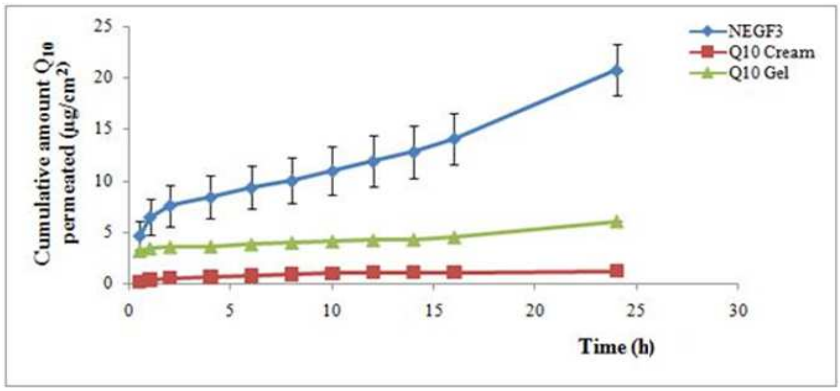

Fig. 7: Comparative *ex-vivo* drug permeation between NEGF3 and Co Q_{10} gel and Co Q_{10} cream (mean±SD (n=3))

Table 4: *Ex-vivo* comparative study between NEGF3, Co Q_{10} gel and Co Q_{10} cream

Formula code	J_{ss} (µg/cm²/h)	K_p (cm²/h)×10^{-5}	E_r (respect to Q_{10} cream)	E_r (respect to Q_{10} gel)	accumulated drug per area (µg/cm²)*
NEGF3	0.681	6.81	17.025	6.81	164.08±9.42
Q_{10} Gel	0.1	1.0	----------	----------	14.69±6.53
Q_{10} Cream	0.04	0.4	----------	----------	3.300±0.49

*mean±SD (n=3), Jss: drug flux, Kp: permeability coefficient, Er: enhancement ratio, Q_{10}: Co Q_{10}

Anti-wrinkle efficiency via skin histopathological evaluation

Photographs of surface morphology of the four groups after 30 d treatment were shown in fig. 8. It could be seen multiple numbers of wrinkle folds appeared on the surface of the skin in the case of the untreated group (fig. 8a). In the case of rat treated with NEGF3, the skin showed the virtual disappearance of wrinkles and appearance of smooth skin (fig. 8b). The groups treated with Co Q_{10} gel (fig. 8c) and Co Q_{10} cream (fig. 8d) showed few wrinkles on the rat skin.

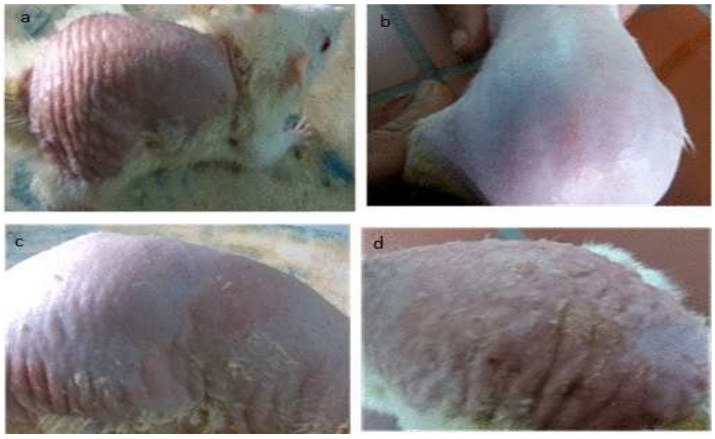

Fig. 8: Photographs of rat skin for (a) untreated group showed multiple wrinkles, (b) NEGF3 group showed disappearance of wrinkles and appeared of smooth skin, Co Q_{10} gel group (c) and Co Q_{10} cream group (d) showed few wrinkles after 30 d of treatment

The histopathological examination of the skin of the four groups was shown in fig. 9. The untreated rat skin showed hyperkeratosis (thickening of the stratum corneum) and parakeratosis (nucleated keratinocytes in the stratum corneum) in the epidermal layer with flattening thick horny layer [52, 53], a decline in sebaceous gland size and hair follicles with narrow lumen and pyknotic nuclei. The collagen, elastin fibers of the dermis and hypodermis showed poorly-defined histological structure (well defined degenerated

neoplastic smooth muscle cells). The collagen fibers lost their details and appeared fused with a homogeneous, cellular glassy eosinophilic appearance (hyalinization), hyalinization was also noticed in the skeletal muscle underneath the dermis and hypodermis layers (fig. 9a). The treated group with NEGF3 showed very less hyperkeratosis and parakeratosis with the appearance of the thick prickle cell layer and well retained healthy cells with obvious nuclei. Hypertrophy and hyperplasia appeared in the sebaceous gland cells. The hair follicles became with the wide lumen. The collagen elastic fibers of dermis and skeletal muscle showed a well differentiated fibrillar structure with less appearance of hyalinization (fig. 9b). In the case of Co Q_{10} gel (fig. 9c) and Co Q_{10} cream (fig. 9d) groups, a moderate hyperkeratosis, moderate parakeratosis, hypertrophy of the sebaceous gland and moderate hyalinization were observed. The superiority of the NEG of Co Q_{10} than Co Q_{10} gel and cream could be attributed to the higher amount of the drug delivered by nanogel as revealed in the *ex-vivo* permeation study. Co Q_{10} stimulates the propagation of fibroblasts through enhancing the elastin gene expression and conquers collagenase expression, thus improved the expression of type IV and VII collagen. Co Q_{10} can decrease metalloproteinases that induced by UVA and UVB radiation. These metalloproteinase enzymes cleave collagen, basement membrane components, and elastic fibers, leading to wrinkling formation [54, 55]. So it was expected the improvement of the efficiency of the Co Q_{10} action on skin wrinkles with increasing the amount of the drug targeted to the skin.

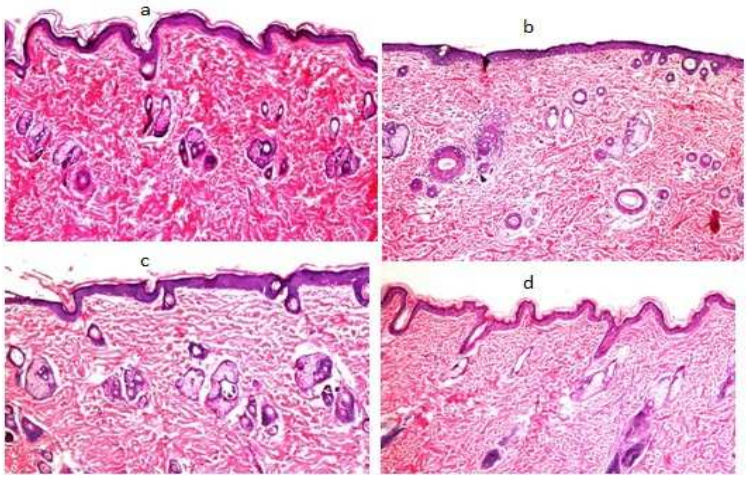

Fig. 9: Showed the histopathological examination of rat skin of untreated group and treated rat skin. The untreated rat skin showed hyperkeratosis and parakeratosis in the epidermal layer with flattening thick horny layer, a decline in sebaceous gland size and hair follicles with a narrow lumen and pyknotic nuclei. The collagen, elastin fibers of the dermis and hypodermis of the untreated group showed poor-defined structure (well defined degenerated neoplastic smooth muscle cells). The collagen fibers lost their details and appeared fused with a homogeneous, cellular glassy eosinophilic appearance (hyalinization). Hyalinization was also noticed in the skeletal muscle underneath the dermis and hypodermis layers (fig. 9a). While the treated group with CoQ_{10} NEGF3 (fig. **9b) showed less hyperkeratosis and parakeratosis with the appearance of the thick prickle cell layer and well retained healthy cells with obvious nuclei. Also, hypertrophy and hyperplasia appeared in sebaceous gland cells and hair follicles with a wide lumen. The collagen elastic fibers of the dermis and skeletal muscle showed a well differentiated fibrillar structure with less appearance of hyalinization. In the case of Co Q_{10} gel group (**fig. **9c) and CoQ_{10} cream groups (**fig. **9d), a moderate hyperkeratosis and moderate parakeratosis, moderate hypertrophy of the sebaceous gland and a moderate hyalinization were observed**

CONCLUSION

Our investigation suggested the sufficient manipulation and superiority of NEG matrix than conventional gel and cream to enhance the permeation of the nutraceutical Co Q_{10} through the stratum corneum barrier. The NEGF3 containing 10% w/v isopropyl myristate as oil, 60% w/v tween 80 and transcutol HP as S/CoS mixture, 30% w/v water, 2% w/v drug, and 1% carbopol 934 gelling agent was concluded as optimized formula. It showed 3.4 fold increases in the amount of Co Q_{10} permeated than Co Q_{10} conventional gel and 17.8 fold increases than Co Q_{10} cream. Therefore, NEG showed the highest anti-wrinkle efficiency than the other dosage forms, as skin appeared with fewer wrinkles, smooth surface, less parakeratosis and hyperkeratosis in the epidermal layer, Well-defined fibrillar structure without hyalinization in the elastic and collagen fibers of the dermis and skeletal muscles. These results judged NEG to be a promising alternative carrier for topical delivery of Co-enzyme Q_{10}.

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AUTHORS CONTRIBUTION

The authors state that no conflict of interest and have not any received payment in the preparation of this manuscript. Prof. Dr. Eman Saddar and Prof. Dr. Amna Makky chose the topic and reviewed the scientific paper. Dr. Abeer Khattab and Dr. Doaa Galaa implemented the idea in the lab and wrote the scientific paper.

ANIMAL RIGHTS

Approval to carry out studies on animals was reviewed and obtained from The Ethics Committee (No.3 for 14/6/2016), Helwan University, Cairo, Egypt and their guidelines were followed for these studies.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Adnan A, Sushama T, Lalit MN, Farhan JA, Roop KK, Zeenat I. Oil based nanocarrier system for transdermal delivery of ropinirole: a mechanistic, pharmacokinetic and biochemical investigation. Int J Pharm 2012;422:436-44.

2. Sunil KY, Manoj KM, Anupamaa T, Ashutosh S. Emulgel: a new approach for enhanced topical drug delivery. Int J Curr Pharm Res 2017;9:15-9.
3. Panwar AS, Upadhyay N, Bairagi M. Emulgel: a review. Asian J Pharm Life Sci 2011;1:333-4.
4. Rania H, Marwa, Tamadur A, Suhair S, Ola T. Nanoemulsion-based gel formulation of diclofenac diethylamine: design, optimization, rheological behavior and *in vitro* diffusion studies. Pharm Dev Technol 2015:1-10. Doi:10.3109/10837450.2015.1086372
5. Baboota S, Shakeel F, Ahuja A. Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib. Acta Pharm 2007;57:315–32.
6. Koteswari P, Krishna SR, Reddy VP, Narasu LM. Formulation and preparation of felodipine nanoemulsions. Asian J Pharm Clin Res 2011;4:116-7.
7. Gupta PV, Pandit JK, Narain PJ. Design, optimization and evaluation of nanoemulsion formulations by ultrasonication as vehicle for transdermal delivery of diclofenacdiethylamine. Pharmbit 2011;23:26–39.
8. Shah P, Bhalodia D, Shelat P. Nanoemulsion: a pharmaceutical review. Sys Rev Pharm 2010;1:24–32.
9. Singh M, Jain S. Nanoemulsion for skin targeting: present status and future prospects. Drug Delivery Lett 2011;1:159–70.
10. Ambade K, Jadhav SL, Gambhire MN, Kurmi SD, Kadam VJ, Jadhav KR. Formulation and evaluation of flurbiprofen microemulsion, Curr Drug Delivery 2008;5:32–41.
11. Shokri J, Azarmi S, Fasihi Z. Effects of various penetration enhancers on percutaneous absorption of piroxicam from emulgels. Res Pharm Sci 2012;7:225–34.
12. Veerawat T, Eliana BS, Varaporn BJ, Rainer HM. Cetyl palmitate-based NLC for topical delivery of coenzyme Q10–development, physicochemical characterization and *in vitro* release studies. Eur J Pharm Biopharm 2007;67:141–8.
13. Joe V, Sunil A. Comparative topical absorption and antioxidant effectiveness of two forms of coenzyme Q10 after a single dose and after long-term supplementation in the skin of young and middle-aged subjects. IFSCC Magazine 2005;8:1-6.
14. Ljiljana D, Marija P. The influence of co-surfactants and oils on the formation of pharmaceutical microemulsions based on PEG-8 caprylic| capric glycerides. Int J Pharm 2008;352:231-9.
15. Singh BP, Kumar B, Jain SK. Development and characterization of a nanoemulsion gel formulation for transdermal delivery of carvedilol. Int J Drug Devand Res 2012;4:151-6.
16. Varaporn BJ, Veerawat T, Eliana BS, Prapaporn B, Rainer HM. Q10-loaded NLC versus nanoemulsions: stability, rheology and *in vitro* skin permeation. Int J Pharm 2009;377:207–14.
17. Sheela AY, Sushilkumar SP. Formulation, *in vitro* and *in vivo* evaluation of nanoemulsion gel for transdermal drug delivery of nimodipine. Asian J Pharm Clin Res 2015;8:119-24.
18. Abeer K, Soha I. Formulation and evaluation of oxiconazole nitrate mucoadhesive nanoemulsion based gel for treatment of fungal vaginal infection. Int J Pharm Pharm Sci 2016;8:33-40.
19. Sajid AMD, Sarfaraz AMD, Nawazish A, Syed A, Shah Q, Intakhab AMD. Formulation, characterization and *in vivo* assessment of topical nanoemulsion of betamethasone valerate for psoriasis and dermatose. Int J Pharm 2013;3:186-99.
20. Mahesh B, Vasanth KP, Gowda DV, Atul S, Raghundan HV, Chetan SG. Enhanced permeability of cyclosporine from a transdermally applied nanoemulgel. Der Pharm Sinica 2015;6:69-7.
21. Elnaggar YSR, Talaat SM, Bahey-El-Din M, Abdallah OY. Novel lecithin-integrated liquid crystalline nanogels for enhanced cutaneous targeting of terconazole: development, *in vitro* and *in vivo* studies. Int J Nanomed 2016;11:5531-47.
22. Doaa AH, Dalia AE, Sally AA, Mohamed AE. Formulation and evaluation of fluconazole topical gel. Int J Pharm Pharm Sci 2012;4:176-83.
23. Zhao YI, Changguang W, Albert HL, Ren K, Tao G, Zhirong Z. Self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of zedoary essential oil: formulation and bioavaliability studies. Int J Pharm 2010;383:170-7.
24. Sripriya VR, Payal A, Jun S. Self-nanoemulsifying drug delivery systems (SNEDDS) for oral delivery of protein drugs II. *In vitro* transport study. Int J Pharm 2008;362:10-5.
25. Songkro S, Purwo Y, Becket G, Rades T. Investigation of newborn pig skin as an *in vitro* animal model for transdermal drug delivery. STP Pharma Sci 2003;13:133–9.
26. Junyaprasert VB, Boonme P, Songkro S, Krauel K, RadesT. Transdermal delivery of hydrophobic and hydrophilic local anesthetics from o/w and w/o brij97 based microemulsions. J Pharm Pharm Sci 2007;10:288–9.
27. Sandig AG, Calpena CAC, Fernandez CF, Martin VMJ, Clares NB. Transdermal delivery of imipramine and doxepin from newly oil-in-water nanoemulsions for an analgesic and anti-allodynic activity: development, characterization and *in vivo* evaluation. Colloids Surf B 2013;103:558–65.
28. Baboota S, Faiyaz S, Alka A, Javed A, Sheikh S. Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib. Acta Pharm 2007;57:315–32.
29. Duraivel S, Asma SS, Rabbani BS, Eesaf PS, Jilani S. Formulation and evaluation of anti-wrinkle activity of cream and nanoemulsion of moringaoleifera seed oil. IOSR J Pharm Biol Sci 2014;9:58-73.
30. Banchroft JD, Stevens A, Turner DR. Theory and practice of histological techniques. Fourth Ed. Churchil Livingstone, 1996, New York, London, San Francisco, Tokyo. 4th ed. Ch 6; 1996. p. 99-112.
31. Khurana S, Jain NK, Bedi PMS. Nanoemulsion based gel for transdermal delivery of meloxicam: physico-chemical, mechanistic investigation. Life Sci 2013;92:383–92.
32. Marta PA, Renata PR, Solange BF. Rheological behavior of semisolid formulations containing nanostructured systems. Nanocosmetics and nanomedicine; 2011. p. 37-45.
33. Alina O, Cristina DP, Mihaela VG, Lidia MP, Lucian I. Rheological study of a liposomal hydrogel based on carbopol. Romanian Biol Lett 2011;16:47-54.
34. Kesavan B, Jayaraman A, Velayutham R, Vobalaboina V, Yamsani MR. Lipid nanoparticles for transdermal delivery of flurbiprofen: formulation, *in vitro, ex vivo* and *in vivo* studies. Lipids Health Disease 2009;8:1-15.
35. Carla V, Luı´s A, Filipe EA, Joao JS, Alberto ACC. Design of a dual nanostructured lipid carrier formulation based on physicochemical, rheological, and mechanical properties. J Nanopart Res 2013;15:1993.
36. Hamed M, Chin PT. Effect of various hydrocolloids on physicochemical characteristics of orange beverages emulsion. J Food Agric Environ 2010;8:308-13.
37. Leila A, Shekoufeh B, Hamed H. The impact of variables on particle size of solid lipid nanoparticles and nanostructured lipid carriers; a comparative literature review. Adv Pharm Bull 2016;6:143–51.
38. Saharudin SH, Ahmad Z, Basri M. Role of xanthan gum on physicochemical and rheological properties of rice bran oil emulsion. Int Food Res J 2016;23:1361-6.
39. Pensak J, Warintorn R. Carbopol-guar gum gel as a vehicle for topical gel formulation of pectin beads loaded with rutin. Asian J Pharm Clin Res 2014;7:231-6.
40. Cheng LN, Mahiran B, Minaketan T, Roghayeh AK, Emilia A. Physicochemical characterization and thermodynamic studies of nanoemulsion-based transdermal delivery system for fullerene. Sci World J 2014:1-12. http://dx.doi.org/10.1155/2014/219035
41. Mohd S, Asmal R, Siti R, Azizan A, Nor SM. Biopolymer electrolyte based on derivatives of cellulose from kenaf bast fiber. Polymers 2014;6:2371-85.
42. Hanan R, Randa T. Development and characterization of sponge-like acyclovir ocular minitablets. Drug Delivery 2011;18:38-45.
43. Sareen R, Kumar S, Gupta GD. Meloxicam carbopol-based gels: characterization and evaluation. Curr Drug Delivery 2011;8:407–15.
44. Gaikwad VL, Yadav VD, Dhavale RP, Choudhari PB, Jadhav SD. Effect of carbopol 934 and 940 on fluconazole release from topical gel formulation: a factorial approach. Curr Pharma Res 2012;2:487–93.
45. Bhalekar MR, Madgulkar AR, Kadam GJ. Evaluation of gelling agents for Clindamycin phosphate gel. World J Pharm Pharm Sci 2015;4:2022–33.

46. Vinod KR, Santosh V, Sandhya S, David B, Otilia B, Yamsani MR. Comparative study of mucoadhesive polymers carbopol 974p and sodium carboxymethyl cellulose for single unit dosage of imatinib mesylate. Malaysian J Pharm Sci 2012;10:61–77.
47. Gautam S, Abhishek S, Nilesh Y, Ranendra NS. Study the effect of formulation variables on drug release from hydrophilic matrix tablets of milnacipran and prediction of *in vivo* plasma profile. Pharm Dev Technol 2013;13:1–9.
48. Elahe T, Zahra JA, Seyed AM. Development and *in vitro* evaluation of a contraceptive vagino-adhesive propranolol hydrochloride gel. Iran J Pharm Res 2012;11:13–26.
49. Siang YL, Yeong YP, Boon KK, Wei EK, Cai TT, Siew YT. Lipid-based delivery system for topical phenytoin. J Appl Pharm Sci 2016;6:14-20.
50. Yin Z, Wu-Qing O, Yun-Peng W, Shahid FS, Chao-Shuang H, Bo-Zhen W, *et al.* Effects of carbopol® 934 proportion on nanoemulsion gel for topical and transdermal drug delivery: a skin permeation study. Int J Nanomed 2016;11:5971–87.
51. Gaur PK, Mishra S, Purohit S, Dave K. Transdermal drug delivery system: a review. Asian J Pharm Clin Res 2009;2:14-20.
52. Marks R, Knight A, Laidler P. Atlas of skin pathology. Curr Histopathol 2015;11:9.
53. Jie S, Christopher RS. Benign versus malignant parakeratosis: a nuclear morphometry study. Modern Pathol 2010;23:799–803.
54. Chung JH, Seo JY, Choi HR, Lee MK, Youn CS, Rhie G, *et al.* Modulation of skin collagen metabolism in aged and photoaged human skin *in vivo*. J Invest Dermatol 2001;117:1218–24.
55. Zhang M, Dang L, Guo F, Wang X, Zhao W, Zhao R. Coenzyme Q10 enhances dermal elastin expression, inhibits IL-1a production and melanin synthesis in vitro. Int J Cosmet Sci 2012;34:273–9.