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\textsubscript{10} (CoQ\textsubscript{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 (5%/60%) showed the highest values; 12.79µg/cm\textsuperscript{2}, 95.92×10\textsuperscript{-4} cm/s, 25.85×10\textsuperscript{-4} cm\textsuperscript{2}/h and 7.26×10\textsuperscript{-7} cm\textsuperscript{2}/h for permeation through dialysis membrane, 2(0.73±2.5 µg/cm\textsuperscript{2}) and through rat skin (20.73±2.5 µg/cm\textsuperscript{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\textsuperscript{2}/h, 95.92×10\textsuperscript{-4} cm\textsuperscript{2}/h and 7.26×10\textsuperscript{-7} cm\textsuperscript{2}/h and 57.35, respectively for in vitro permeation study and 0.968µg/cm\textsuperscript{2}/h, 7.26×10\textsuperscript{-7} cm\textsuperscript{2}/h and 1.183, respectively for ex-vivo permeation study.

Further histopathological evaluation test showed that CoQ\textsubscript{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 CoQ\textsubscript{10} to enhance its solubility, skin permeability and thus anti-wrinkle efficiency.

Keywords: Coenzyme Q\textsubscript{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 hydrophobic 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. 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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{10} in new dosage form (nanoemulsion gel (NEG) drug delivery system) for enhancement of solubility and permeability of Co Q\textsubscript{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) formulations. These NEG formulations were prepared using three types of hydrophilic polymers namely; xanthan gum, carbopol 934 and sodium carboxymethyl cellulose (NaCMC).
Preparation and optimization of CoQ10 diagrams. Water was added drop wise to the specified weights of oils to delineate boundaries of phases precisely formed in the phase each surfactant with each co-surfactant at different mass ratios (4:1, of three surfactants and two co-surfactants were prepared by mixing surfactant (CoS), and distilled water as the aqueous phase. Six blends and tween 80 selected as a surfactant (S), PG and transcutol HP as co-surfactants at a ratio (3:1) were selected as the oil phase. Labrasol, cremophor EL (polyoxyethyl macrogol-8-glyceride), Labrafil M1944 CS (polyoxyethylene kernel oil) and transcutol HP (diethylene glycol monoethyl ether) were given as a gift from Gattefosse (Gedex, France). Cremophor EL (polyoxy 35 hydrogenated castor oil), propylene glycol (PG) were purchased from Sigma-Aldrich, Germany. Disodium hydrogen phosphate and sodium dihydrogen phosphate were purchased from El-Nasr Pharmaceutical Chemical Company, Cairo, Egypt. Triethanolamine was purchased from Sigma-Aldrich, Cairo, Egypt, xylene, paraffin wax, hematoxylin, and eosin were purchased from Sigma Aldrich Chemical Company, Cairo, Egypt.

Pre-formulation study of nanoemulsion

Solubility study
The solubility of CoQ10 in different oils namely; oleic acid, IPM; oleic acid at ratio (3:1), IPM, avocado oil, capryol 90, labmfil M1944 CS and oil) surfactants 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 CoQ10 was allowed to dissolve in 1g of each oil, surfactants, and co-surfactants in 10-ml capacity stopped vials separately. The mixtures were vortexed to facilitate drug solubilization (Stuart vortex mixer, U. K) and kept at 37 °C 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 CoQ10 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 surfactant/co-surfactant combination was emulsified by sonication to reach the equilibrium quickly. The amounts of water were mixed in the ratio 1:1; then the S/CoS combination was vortexed to facilitate drug solubilization (Stuart vortex mixer, U. K) and stirred continuously at 1100 rpm to ensure the emulsification and the complete swelling of the gelling agents. The oil phase was transferred to two parts of NE under mixing to obtain the NEG. The final 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 sterrer. 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.

Preparation and optimization of CoQ10 nanoemulsion

Depending on the phase diagrams, Various CoQ10-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. CoQ10 was accurately weighed 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 formulation 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 formulæ were evaluated for their stability under different stress conditions. Firstly, the formulæ 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 °C and 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 formulæ 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.

Evaluation of CoQ10 NEG
Percentage of drug content
This study was carried out by taking (0.1 g) of each formulæ in a volumetric flask (10 ml capacity) and dissolving the drug using 10 ml of the mobile phase (methanol: isopropanol at ratio 75:25). These solutions were sonicated for 5 min to break the humps 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.
% Drug content = \frac{\text{Actual content}}{\text{Theoretical content}} \times 100 \text{ Equation (1)}

\text{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.

\text{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 \( \tau \) (dyne. cm\(^{-3}\)) versus the log values of the shear rate \( \gamma \) (s\(^{-1}\)) [18].

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

\[ \tau = k \gamma^n \quad \text{or} \quad \log \tau = \log k + n \log \gamma \text{ Equation (2)} \]

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].

\text{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, Netherlands) 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.

\text{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 1:100 were exposed to an electric field (1V). The average±SD of the three independent measurements was recorded.

\text{Electroconductivity study}

The electrical conductivity (\( \sigma \)) of the formulated NE and NEG was determined using Hanna digital conductometer, (Model: HI 255, 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].

\text{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].

\text{Permeation studies}

\text{In vitro permeation, Ex-vivo skin permeation and skin retention studies}

In vitro permeation of CoQ10 from NEG formulae was investigated through a semi-permeable 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 Q10 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 Q10 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.

\[ \text{Cumulative amount of drug permeated} = \frac{\text{Concentration (µg/ml)} \times \text{dilution factor}}{\text{surface area of skin}} \quad \text{Equation (3)} \]

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].

\[ Kp = \frac{Jss}{C_o} \quad \text{Equation (4)} \]

\[ Er = \frac{Jss}{f_{formulation}} \quad \text{Equation (5)} \]

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 CoQ10 content was estimated using HPLC.

\text{Comparative in vitro and ex-vivo permeation studies between the optimized NEG, marketed Co Q10 cream and a conventional Co Q10 gel}

A comparative in vitro and ex-vivo permeation study between the optimized NEG formula and (Nivea Co Q10 anti-wrinkle cream as a market cream), or a conventional Co Q10 gel were carried out. The conventional Co Q10 gel was prepared by dispersing 1 g of carcapol 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 carcapol 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.

\text{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.

\text{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 wk 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 CoQ10 gel, the fourth group was treated with marketed CoQ10 cream. The animals in each group were delivered an amount of the treatment equivalent to 20 mg of CoQ10 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 CoQ10 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 CoQ10 in various oils, surfactants and co-surfactants. The choice of excipients used in NE preparation was based on the solubility studies.

CoQ10 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.

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![Graph](https://via.placeholder.com/150)

Fig. 1: Solubility of CoQ10 in different oils, surfactants, and co-surfactants, IPM: isopropyl myristate, PG: Propylene glycol, mean±SD (n=3)

![Graph](https://via.placeholder.com/150)

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

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210
Preparation of CoQ10 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 CoQ10 NEG formulae

<table>
<thead>
<tr>
<th>Formula code</th>
<th>%IPM</th>
<th>% S/CoS*</th>
<th>% water</th>
<th>Gelling agent</th>
<th>Method of preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEG F1</td>
<td>10</td>
<td>60</td>
<td>30</td>
<td>XGum (2%)</td>
<td>A</td>
</tr>
<tr>
<td>NEG F2</td>
<td>10</td>
<td>60</td>
<td>30</td>
<td>XGum (2%)</td>
<td>B</td>
</tr>
<tr>
<td>NEG F3</td>
<td>10</td>
<td>60</td>
<td>30</td>
<td>CP (1%)</td>
<td>A</td>
</tr>
<tr>
<td>NEG F4</td>
<td>10</td>
<td>60</td>
<td>30</td>
<td>CP (1%)</td>
<td>B</td>
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<td>NEG F5</td>
<td>10</td>
<td>60</td>
<td>30</td>
<td>NaCMC (2%)</td>
<td>A</td>
</tr>
<tr>
<td>NEG F6</td>
<td>10</td>
<td>60</td>
<td>30</td>
<td>NaCMC (2%)</td>
<td>B</td>
</tr>
</tbody>
</table>


Evaluation of NEG formulae

Drug content

The drug content of the NEG formulae (F1 to 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 to F6) were ranged from 5.38±0.046 to 7.15±0.055 which 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 CoQ10 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 becomes 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 formulæ 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 NEG F3, respectively.
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±4.6) 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 amphiphatic 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 carbopol 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±3.3 to 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 carbopol 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±0.3, 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].
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 CoQ10 NEG formulae**

<table>
<thead>
<tr>
<th>Formula code</th>
<th>In vitro permeation</th>
<th>Ex-vivo permeation</th>
<th>Accumulated drug per area (µg/cm²)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jss (µg/cm²/h)</td>
<td>Kp (cm²/h)×10⁻⁵</td>
<td></td>
</tr>
<tr>
<td>NEGF1</td>
<td>2.335</td>
<td>23.5</td>
<td>0.411</td>
</tr>
<tr>
<td>NEGF2</td>
<td>2.183</td>
<td>21.8</td>
<td>0.376</td>
</tr>
<tr>
<td>NEGF3</td>
<td>12.79</td>
<td>127.9</td>
<td>0.681</td>
</tr>
<tr>
<td>NEGF4</td>
<td>6.259</td>
<td>62.5</td>
<td>0.589</td>
</tr>
<tr>
<td>NEGF5</td>
<td>0.742</td>
<td>7.42</td>
<td>0.394</td>
</tr>
<tr>
<td>NEGF6</td>
<td>0.472</td>
<td>4.72</td>
<td>0.413</td>
</tr>
</tbody>
</table>

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

The ex-vivo results confirmed that F3 displayed the maximum cumulative quantity of CoQ₁₀ 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±1.79 and 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 CoQ₁₀ resulting in hindering the drug release [46-48].

![Fig. 6: Ex-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 Q₁₀ cream, and Co Q₁₀ conventional gel. The cumulative amount of drug permeated was ranked in the following descending order; NEG F₃>Co Q₁₀ Gel>Co Q₁₀ cream. The amount of drug permeated from NEGF3 Co Q₁₀ was significantly higher than Co Q₁₀ conventional gel and Co Q₁₀ 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 Eᵣ of NEGF3 respects to Q₁₀ gel was 6.81 and 17.025 respects to Q₁₀ cream, table 4. These results could be attributed to the lower particle size of NEGF3 (120.5±1.19 nm) than those of CoQ₁₀ cream (2.69 µm±0.17) and CoQ₁₀ 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 Q10 cream and Co Q10 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 Q10 cream and Co Q10 gel, Table 4, it was found that NEGF3 exhibited a significantly higher amount of the accumulated drug than Q10 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 Q10 gel and Co Q10 cream (mean±SD (n=3))**

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Jₚ (µg/cm²/h)</th>
<th>Kₚ (cm²/h)×10⁻⁵</th>
<th>Eₑ (respect to Q10 cream)</th>
<th>Eₑ (respect to Q10 gel)</th>
<th>accumulated drug per area (µg/cm²)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEGF3</td>
<td>0.681</td>
<td>6.81</td>
<td>17.025</td>
<td>6.81</td>
<td>164.08±9.42</td>
</tr>
<tr>
<td>Q10 Gel</td>
<td>0.1</td>
<td>1.0</td>
<td>---</td>
<td>---</td>
<td>14.69±6.53</td>
</tr>
<tr>
<td>Q10 Cream</td>
<td>0.04</td>
<td>0.4</td>
<td>---</td>
<td>---</td>
<td>3.300±0.49</td>
</tr>
</tbody>
</table>

*mean±SD (n=3), Jₚ: drug flux, Kₚ: permeability coefficient, Eₑ: enhancement ratio, Q₁₀: Co Q₁₀

**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 Q₁₀ gel (Fig. 8c) and Co Q₁₀ 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 Q₁₀ gel group (c) and Co Q₁₀ 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 Q10 gel (fig. 9c) and Co Q10 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 Q10 than Co Q10 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 Q10 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 Q10 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 Q10 action on skin wrinkles with increasing the amount of the drug targeted to the skin.

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 Q10 through the stratum corneum barrier. The NEG3 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 Q10 permeated than Co Q10 conventional gel and 17.8 fold increases than Co Q10 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 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 CoQ10 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 Q10 gel group (fig. 9c) and CoQ10 cream groups (fig. 9d), a moderate hyperkeratosis and moderate parakeratosis, moderate hypertrophy of the sebaceous gland and a moderate hyalinization were observed.

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AUTHORS CONTRIBUTION

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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


