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RESEARCH ARTICLE

Influence of omega fatty acids on skin permeation of a coenzyme Q10 nanoemulsion cream formulation: characterization, *in silico* and *ex vivo* determination

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ABSTRACT

Objective: The aim of this study was to develop a coenzyme Q10 nanoemulsion cream, characterize and to determine the influence of omega fatty acids on the delivery of coenzyme Q10 across model skin membrane *via ex vivo* and *in silico* techniques.

Methods: Coenzyme Q10 nanoemulsion creams were prepared using natural edible oils such as linseed, evening primrose, and olive oil. Their mechanical features and ability to deliver CoQ10 across rat skin were characterized. Computational docking analysis was performed for *in silico* evaluation of CoQ10 and omega fatty acid interactions.

Results: Linseed, evening primrose, and olive oils each produced nano-sized emulsion creams (343.93–409.86 nm) and exhibited excellent rheological features. The computerized docking studies showed favorable interactions between CoQ10 and omega fatty acids that could improve skin permeation. The three edible-oil nanoemulsion creams displayed higher *ex vivo* skin permeation and drug flux compared to the liquid-paraffin control cream. The linseed oil formulation displayed the highest skin permeation (3.97 ± 0.91 mg/cm^2) and drug flux (0.19 ± 0.05 mg/cm^2/h).

Conclusion: CoQ10 loaded-linseed oil nanoemulsion cream displayed the highest skin permeation. The highest permeation showed by linseed oil nanoemulsion cream may be due to the presence of omega-3, -6, and -9 fatty acids which might serve as permeation enhancers. This indicated that the edible oil nanoemulsion creams have potential as drug vehicles that enhance CoQ10 delivery across skin.

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KEYWORDS
Nanoemulsion; omega-fatty acids; linseed oil; evening primrose oil; olive oil; permeation enhancer

Introduction

Topical drug delivery is considered the most ideal route of drug administration to obtain localized effect for skin-associated diseases. In dermatology, topical formulations are used to treat skin diseases with the intention to distribute the applied active agent to the affected tissue. A major concern in topical drug delivery is the permeation of drug through the stratum corneum (SC) of the skin in a controlled manner to obtain the desired therapeutic effect [1]. Topical delivery of drugs, and by extension their transdermal delivery, has many advantages compared to other routes of administrations [2,3] including increased patient compliance, reduced side effects, and the potential for immediate cessation of toxicity because of their easy removal. However, optimal delivery may not always be achieved because of the excellent barrier function in skin, which is mostly due to the highly organized structure of the SC in the outermost part of skin [4,5]. CoQ10 is a vitamin-like substance and lipophilic in nature (log $p > 10$). It shows antioxidant and skin-protective properties which prevent aging and photo-aging of skin [6–9]. *In silico* tools have become a close counterpart to more traditional experimental approaches in an effort to understand the molecular aspects of biological systems. Computational approaches, such as molecular docking and quantitative structure-activity relationships (QSAR), are widely employed in the search for novel therapeutic targets [10]. In recent years, molecular modeling has been used to investigate possible types of interactions between the fatty acids and drug complexes to understand their influence on drug permeation through the skin [11,12]

This study attempts to develop a suitable delivery vehicle that can be used to optimally deliver CoQ10 across the skin and to predict this effectiveness of permeation through molecular modeling. Nanoemulsions are thermodynamically stable and isotropically clear systems of two immiscible liquids. The dispersed phase typically is comprised particles with sizes less than 500 nm, has a low oil/water interface tension and is inherently stable against flocculation, creaming, and sedimentation. Nanoemulsions are formulated for a broad variety of topical and transdermal applications and offer many advantages such as low skin irritation, high drug loading, and the potential for skin hydration and permeation [9,13–15]. Nanoemulsions can penetrate through the skin surface because of their relatively small size, a characteristic that can be exploited to enhance the permeation of active ingredients [16].

Edible oils such as olive, linseed, and evening primrose oil are composed of various fatty acids such as α-linolenic acid (ALA, omega-3), linoleic acid (LA, omega-6), and oleic acid (OA, omega-9) were used in the study. Fatty acids are well recognized as drug permeation enhancer for delivery across skin [17], and functions also as endogenous compounds in human skin lipids, including SC. Thus, fatty acids can be utilized as adjuvant to exert the enhancement effects [11,18]. ALA is a polyunsaturated n-3

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(omega-3) fatty acid and could be found in linseed oil. It has properties that can help in the repair of skin cells by improving the skin tensile strength and augmentation of collagen content [19]. LA is an unsaturated omega-6 fatty acid found in abundance in evening primrose oil. LA has also been documented as being a dermal penetration enhancer that can significantly increase the permeation of drugs across skin [20]. OA is a non-essential monounsaturated omega-9 fatty acid and a primary component of olive oil. OA is reported to have skin nourishing properties, ability to moisturize the skin, and compared to the other fatty acids is more widely used as a penetration enhancer [20,21]. The aim of this study was to prepare a stable nanoemulsion cream carrier system for CoQ10 as an active ingredient utilizing different sources of omega fatty acids from edible oil. The formulations were characterized, and the delivery of CoQ10 from these vehicles investigated across the skin to determine whether the presence of fatty acids in nanoemulsion formulations would result in improved delivery.

Materials and methods

Materials

Linseed oil, evening primrose oil, olive oil, cetrimide, butylated hydroxyanisole (BHA), and propylene glycol (PG) were purchased from Sigma-Aldrich (St. Louis, MO). Coenzyme Q10 was purchased from Beijing Wisapple Biotech Co., Ltd. (Beijing, China). Cetostearyl alcohol, glyceryl monostearate, tween 80, span 80, and liquid paraffin were obtained from R&M chemicals (Selangor, Malaysia). Hexane, sodium methoxide, and cellulose acetate membrane 0.45 um were purchased from Sterlitech Corporation (Kent, WA). Methanol and ethanol absolute were purchased from Merck Group (Darmstadt, Germany). Ventral skin of rats was obtained from the animal house of the Faculty of Pharmacy, UKM.

Determination of omega-fatty acid composition in oils

Fatty acid analysis was conducted using gas chromatography (GC) 2010 (Shimadzu, Kyoto, Japan) with a flame ionization detector (FID). The injector temperature was maintained at 250 °C and the detector temperature was maintained at 275 °C. Initially, temperature of the column was maintained at 180 °C for 2 min and then gradually increased to 240 °C at the rate of 4 °C/min. Nitrogen was used as a carrier gas with flow rate of 60 cm/s. Aliquots of 1 μL FAME was injected into the highly polar cyanosiloxane column, SP-2380 (30 mm × 0.25 mm × 0.20 μm film thickness) from Supelco Inc. (Bellefonte, PA). To determine the composition of fatty acids in oils, samples were first treated to prepare fatty acids methyl esters (FAMEs). An aliquot of 0.5 mL of 0.5 M sodium methoxide solution (prepared by mixing sodium methoxide powder in anhydrous methanol) was added to the solution of 50 mg of sample in 1 mL of hexane, in 2 mL screw-capped vial. The vial was capped and screwed on tightly. The solution in the vial was homogenized by using Autovortex SA6 (Stuart Scientific, Stone, UK) for 1 min and was left to stratify until the upper layer becomes clear (5 min). The distinct upper layer of methyl ester was separated carefully into a capped vial and 1 μL sample was injected into GC for analysis.

Preparation of nanoemulsion cream

Three different types of nanoemulsion creams were prepared from linseed oil, evening primrose oil, and olive oil. Oil-in-water (o/w) nanoemulsions were prepared individually using linseed oil, evening primrose oil, and olive oil with the addition of cetyl alcohol and glyceryl monostearate in the oil phase while Tween 80/Span 80 in distilled water was used as the aqueous phase. CoQ10 was dissolved in the oil phase. Both oil and aqueous phase were gently heated in a water bath separately at 60 °C and stirred with glass rod to homogeneity. To prepare the nanoemulsion cream formulation both phases were mixed together and stirred at a rate of 1500 rpm for 15 min while keeping the temperature of the mixture below 60 °C. The final mixture was homogenized using a high shear mixer (IKA T25 basic; Ultra Turrax®, Karlsruhe, Germany) at 14,000 rpm for 1.5 min (Figure 1). All the formulations were kept in amber bottles and stored at 4 ± 2 °C. Control CoQ10 cream formulation was also prepared in the same manner as the edible oil creams, with the exception being the oil phase replaced with liquid paraffin. The composition of all the formulations is listed in Table 1.

Droplet size and zeta potential determination

Droplet size and zeta potential of CoQ10-loaded nanoemulsion creams were measured by photon correlation spectroscopy using Zetasizer Nano ZS (Malvern, Worcestershire, UK). The samples were diluted at 1:100 ratio with deionized water, and then ultrasonicated using Ultrasonicator (Branson 5510; Marshall Scientific, Hampton, NH) to homogenize the samples. The samples later were placed in folded capillary cells and results for size and zeta potential were recorded. The measurements were taken in fully automatic mode.

Determination of pH

The pH of all the formulations was determined by using a digital pH meter (Mettler-Toledo, Columbus, OH) calibrated with standard buffers of pH 4 and 7.

Rheological study

Rheological measurements of formulations were performed using a Bohlin Gemini HR nano-rheometer (Malvern Instruments Ltd, Worcestershire, UK) equipped with a cone and plate measuring system. The experiments were performed using a cone with a diameter of 20 mm and the angle of the cone was 2°. The shear rate was increased to 500 s^{-1} in 3 min, followed by a decrease to zero at the same time interval. The average apparent viscosity for each sample was obtained from the maximum shear rate of 500 s^{-1}. All the analysis was done in triplicates and the mean values formulation were reported and the linearity of viscoelastic properties was verified for all samples.

Texture analysis

Texture analysis was conducted using a Texture Analyzer Pro CT3 10 K (Brookfield Engineering Laboratories, Middleboro, MA) with a 10,000 g load cell. A back-extrusion test fixture (TA-DEC) with a cylindrical probe (TA4/100) with a 34-mm diameter was used to perform the compression tests. Samples of the formulations (25 g) were placed into the fixture base table (TA-BT-KIT) and the probe was then used to compress the cream until it penetrated to a depth of 5 mm and withdrawn at a speed of 2 mm/s (a 2-cycle test). Firmness, peak stress, and adhesiveness were calculated using the peak and the area under the curve of the force versus distance profile.

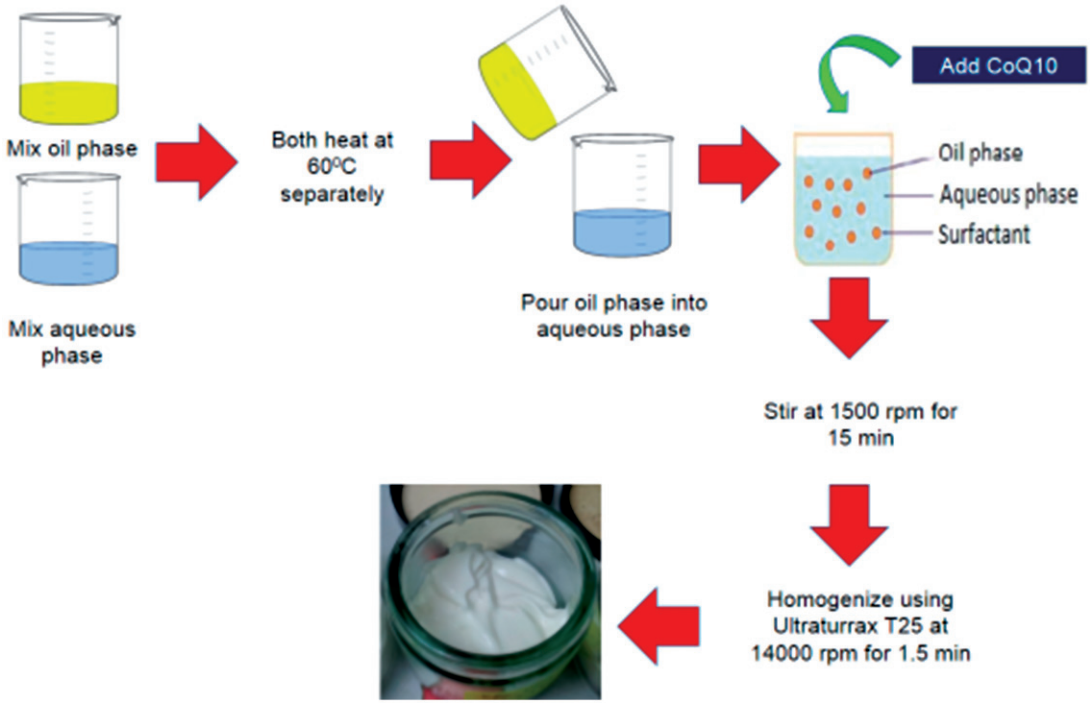

Figure 1. Schematic pathway of preparation of CoQ10-loaded nanoemulsion creams.

Table 1. Formulation of CoQ10-loaded nanoemulsion creams.

Formulation	Oil (g)	Cetyl alcohol (g)	Glyceryl monostearate (g)	Tween 80 (g)	Span 80 (g)	CoQ10 (g)	Distilled water (g)
LQ	12	1	0.5	7.7	18.3	1	59.5
EQ	12	1	0.5	7.7	18.3	1	59.5
OQ	12	1	0.5	7.7	18.3	1	59.5
LP	12	1	0.5	7.7	18.3	1	59.5

LQ: CoQ10-loaded linseed oil'; EQ: CoQ10-loaded evening primrose oil; OQ: Co-Q10-loaded olive oil; LP: Co-Q10-loaded liquid paraffin (control).

Drug content

The CoQ10 content was determined using a UV-1800 spectrophotometer (Shimadzu, Japan). Dilutions of the formulations (0.002 mg/mL) were prepared and analyzed at $\lambda = 275$ nm. The CoQ10 content was calculated against a standard CoQ10 calibration curve.

Molecular docking

Molecular docking studies of CoQ10 and the fatty acids were performed using open source programs, including AutoDock Vina of the PyRx Virtual Screening software and BIOVIA Discovery Studio 2017 (San Diego, CA). AutoDock Vina screening software was used for docking preparation, evaluation of the binding affinity, and energy (kcal/mol) calculations of the CoQ10 and fatty acids. BIOVIA Discovery Studio 2017 was used to perform the virtual analysis. Before docking, all water molecules and cofactors were excluded. The three-dimensional (3D) structures were obtained from the protein data bank (PDB) and a ligand library was generated. All the structures were first optimized by energy minimization and the docking studies were then performed.

Ex vivo permeation study

Ex vivo permeation studies were performed on all CoQ10-loaded nanoemulsion creams using a Franz diffusion cell (Permear Gear Inc., Corona, CA). Sections of shaven Sprague Dawley rat skin were used as the model membrane. The donor and receptor compartments of the Franz diffusion cell were assembled using a clamp. The receptor chamber, with a contact area of 0.95 cm^2 and volume of 3.5 mL, was filled with cetrimide solution until it reached the sampling port. The cetrimide solution was continuously stirred at 300 rpm with a magnetic stir bar. The temperature of the system was maintained at 37 °C throughout the experiment. The donor compartment was equilibrated with the receiver compartment for 1 h to facilitate skin hydration. The formulations (infinite dosing, $n = 3$) were placed on the membrane surface of the donor compartment. Aliquots (0.5 mL) were withdrawn from the receptor medium at regular time intervals of 1, 2, 4, 6, 8, and 16 h. The samples were diluted with 2 mL of ethanol and analyzed using a UV-1800 spectrophotometer at a wavelength of 275 nm for detection and quantitation of CoQ10. The cumulative amount of CoQ10 that permeated through the set surface area (mg/cm^2) of skin was calculated and plotted against time (h). The rate of drug flux (mg/cm^2/h) was determined from the gradient of the plot. Each formulation was evaluated in triplicate.

Drug retention in skin

Following the drug permeation experiment, the skin section mounted on the Franz diffusion cell was carefully removed and the remaining portion of the formulation adhering to the skin was collected with a spatula. To determine the amount of CoQ10 retained in the skin, the sections were then soaked with 10 mL of ethanol and ultrasonicated for 2 h to extract the drug present in the skin sections. The resulting solutions were filtered using a

0.45 μm polytetrafluoroethylene (PTFE) membrane and the amount of CoQ10 was determined using a UV-1800 spectrophotometer.

Statistical analysis

Statistical analysis of the *in vitro* permeation study results was performed using one-way analysis of variance (ANOVA) and paired sample *t*-tests with SPSS version 20 software (IBM Cooperation, Armonk, NY). A multiple comparison test was used to compare different formulations and *p* values less than .05 were considered to be statistically significant.

Results and discussion

Fatty acid composition

Fatty acid composition of the edible oils was determined using GC. Linseed oil contained all the omega fatty acids with ALA (omega-3) being present at the highest concentration (52.57 ± 14.77%). Evening primrose oil was mainly composed of LA (70.60 ± 0.45%) and olive oil was mainly composed of OA (75.98 ± 0.15%). The detailed omega fatty acid composition in the linseed oil, evening primrose oil, and olive oil are shown in Table 2.

Droplet size

Droplet size distribution in nanoemulsion creams is one of the important physical characteristics which may affect the rate and extent of drug release as well as absorption and stability of system [22]. It was observed that CoQ10-loaded linseed oil nanoemulsion cream (LQ) showed the lowest mean droplet size (343.93 ± 10.35 nm)as compared to CoQ10-loaded evening primrose oil nanoemulsion cream (EQ) and CoQ10-loaded olive oil nanoemulsion cream (OQ) (361.75 ± 27.73 and 409.37 ± 50.82 nm, respectively) as shown in Figure 2(A). The droplet size distribution is influenced by varying amount of encapsulating agent or emulsifier, stages, and pressure of homogenization as well as composition of emulsion [23]. Emulsifiers with an unsaturated alkyl chain have an increased affinity for oils with unsaturated bonds. In this case, a blend of Tween 80 and Span 80 was used to emulsify the linseed, evening primrose and olive oils. Emulsifiers are surface-active molecules that adsorb to the surface of freshly formed

Table 2. Omega-3, -6, and -9 composition in oils.

Fatty acids	Linseed oil (%)	Evening Primrose oil (%)	Olive oil (%)
Oleic acid (C18:1)	20.45 ± 7.17	6.04 ± 2.45	75.98 ± 0.15
Linoleic acid (C18:2)	15.12 ± 8.38	70.60 ± 0.45	0.04 ± 0.03
Alpha-linolenic acid (C18:3)	52.57 ± 14.77	0.34 ± 0.08	0.59 ± 0.01

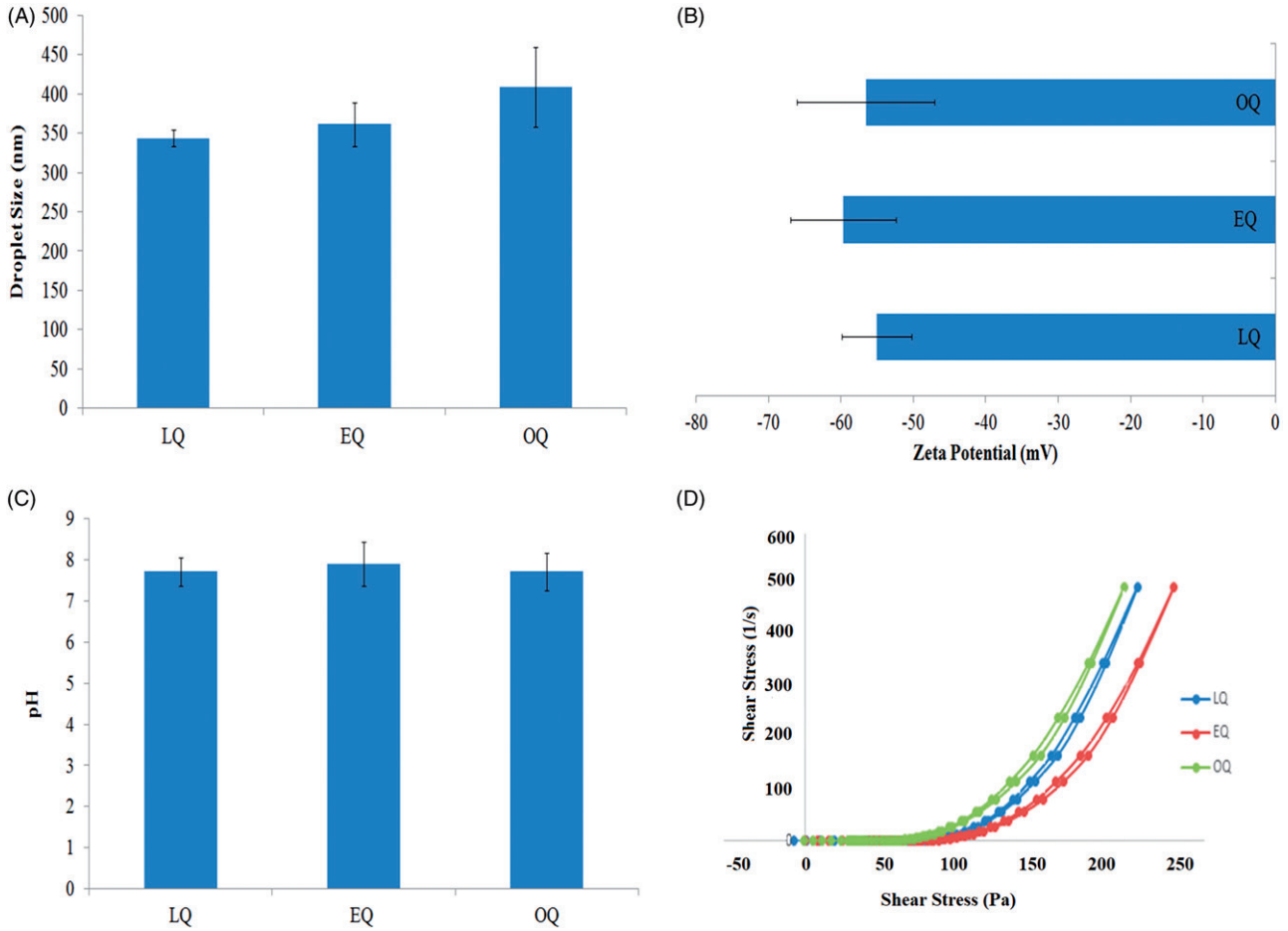

Figure 2. (A) Droplet size of CoQ10-loaded nanoemulsion creams (B) Zeta potential of CoQ10-loaded nanoemulsion creams (C) pH of CoQ10-loaded nanoemulsion creams (D) Rheological profile of CoQ10-loaded nanoemulsion creams (Data expressed as mean ± S.D).

droplets and act by two way: lowering interfacial tension and forming a protective layer that prevents the droplets from aggregating. The mixture of Tween 80 and Span 80 acted as hydrophilic and lipophilic surfactant that may have reduced the o/w interfacial tensions. There is direct relationship between the o/w interfacial tension and size of the internal droplets in which the lower the interfacial tension systems, the smaller the internal droplets [24]. Addition of rheological modifiers (cetyl alcohol and glyceryl monostearate) also influences the droplet size by increasing the surfactant-to-oil-ratio, facilitating the emulsification process and hence reducing the droplet size [25]. However, the droplet sizes of all creams in present study were still below 500 nm and were considered as nanoemulsion creams.

Zeta potential

The zeta potential indicates the charge related to ions moving with a droplet in the electric field. The zeta potentials of the CoQ10-loaded creams were found to be −55.09 ± 4.82 mV for LQ, −59.65 ± 7.28 mV for EQ, and −56.56 ± 9.5 mV for OQ (Figure 2(B)). Zeta potentials ranging between −40 and −60 mV are considered stable for a dispersion to resist aggregation and flocculation [23]. The nanoemulsion creams produced in the current study exhibited negative zeta potentials, which might have been due to the surfactants and free fatty acids or phospholipids present in the oil phase [22]. In addition, fatty acids contain both hydrophobic hydrocarbons and hydrophilic carboxylic acids in the same molecules. The combination of hydrophobic and hydrophilic groups allows the free fatty acids molecules to migrate and concentrate at the surface of the oil-water interfaces, thereby impacting the zeta potential and surface tension [26,27].

pH evaluation test

The pH for all the CoQ10-loaded nanoemulsion creams was evaluated (Figure 2(C)). The pH of all formulated creams was in the range of 7.71 ± 0.34 to 7.90 ± 0.54. The pH within this range might be attributed by the combination of Tween 80 and Span 80 [28]. The resulting pH range is deemed suitable for dermal use. The pH is also an important factor that influences the zeta potential of nanoparticles [29]. If the pH of the formulations is neutral or alkaline in nature, zeta potential of the formulations will be negative. The pH values were optimum in range to keep the zeta potential between −40 and −60 mV that is considered as most stable for dispersion system and there is minimal risk of flocculation and aggregation [23].

Table 3. Mechanical characterization of CoQ10-loaded creams (data expressed as mean ± S.D).

Formulation	Firmness (N)	Peak Stress (dyn/cm^2)	Adhesiveness (mJ)
LQ	0.82 ± 0.27	11,376.30 ± 3854.72	7.03 ± 2.35
EQ	0.50 ± 0.01	7181.63 ± 311.76	8.40 ± 0.40
OQ	0.52 ± 0.03	7510.85 ± 225.57	7.73 ± 0.61

Rheological studies

Rheological behavior for all the formulations was evaluated and the apparent viscosity (Pa.s) and shear stress (Pa) of nanoemulsion creams formulations were measured as a function of shear rate (1/s). The nanoemulsion creams exhibited pseudoplastic behavior in which shear stress vs shear rate curve convert toward the shear stress axis (Figure 2(D)). EQ showed the highest viscosity, followed by LQ and OQ. The pseudoplastic behavior of nanoemulsion creams suggests that the formulations are smooth in texture and easy to rub on the skin surface. Cetyl alcohol and glyceryl monostearate are thickening agents and might have modified the rheological properties of nanoemulsion creams. All the formulations displayed resistance to flow at low shear rates, and when the yield values are exceeded, the nanoemulsion creams begin to thin down (shear thinning) at high shear conditions, displaying non-Newtonian behavior [30].

Texture analysis

Texture analysis was performed to analyze the mechanical properties of the nanoemulsion creams formulations. The mechanical properties of formulations were evaluated based on the firmness (maximum positive force required to deform), peak stress (formulation on surface of the skin), and adhesiveness. The formulations were compared on the basis of values obtained for each parameter and recorded in Table 3. The texture of LQ was the firmest among the CoQ10-loaded creams with the value of 0.82 N. The same formulation also exhibited the highest value in peak stress evaluation 11376.30 dyn/cm^2 which indicates the intermolecular strength of emulsion and cetyl alcohol used as creaming agent. However, in adhesiveness test, EQ (8.40 ± 0.40 mJ) was more adhesive than OQ (7.73 ± 0.61 mJ) and LQ (7.03 ± 2.35 mJ).

Uniformity of content

Drug content uniformity studies were conducted for the CoQ10-loaded creams. The results demonstrated that CoQ10 was uniformly distributed throughout these creams, indicated by low S.D values as shown in Table 4. The results complied to pharmacopeial limit (90–110%) provided by British Pharmacopeia. It also demonstrated indirectly that the cream preparation method was reproducible, as CoQ10 was homogenously dispersed in the creams and did not degrade.

Molecular docking

The molecular docking studies of CoQ10 and the fatty acids are as shown in Figure 3. The average molecular binding affinity (docking energy) scores of the ALA and CoQ10 was −2.89 ± 0.05 kcal/mol, LA and CoQ10 was −3.21 ± 0.04, OA and CoQ10 was −2.82 ± 0.05 kcal/mol. The number of the binding modes was nine ($n = 9$). Based on the current molecular docking study, LA tended to bind CoQ10 the strongest; however, the differences among the binding energies of the fatty acids was not statically significant.

Table 4. Drug content (%) of CoQ10 loaded nanocreams, cumulative amount of CoQ10 permeated (mg/cm^2), and drug flux (mg/cm^2/h) of CoQ10-loaded nanoemulsion creams using rat skin membrane (data expressed as mean ± S.D).

Formulation	Drug content (%)	Cumulative amount of CoQ10 permeated (mg/cm^2)	Drug flux (mg/cm^2/h)
LQ	91.90 ± 0.10	3.97 ± 1.06	0.19 ± 0.05
EQ	92.47 ± 0.06	2.51 ± 1.11	0.12 ± 0.05
OQ	91.33 ± 0.15	2.77 ± 0.40	0.13 ± 0.02
Control (LP)	95.26 ± 0.72	1.91 ± 0.23	0.09 ± 0.01

The binding energy is released when a drug/molecule associates with a target, which leads to a lowering of the overall energy of the complex. The release of the binding energy also compensates for any transformation of the ligand resulting from its energy minimum relative to its conformation when bound with the macromolecule. Therefore, the greater the energy released upon binding of a ligand to the macromolecule, the greater the propensity of the ligand to associate with that macromolecule [11,31]. If the binding energy was a negative value that means the ligand was bound spontaneously without consuming energy. The molecules were also studied in Discovery Studio 2017 to visualize the binding site and bond length. It was found that all the fatty acids exhibit hydrophobic alkyl interaction with the CoQ10 molecule. The location and bond length for each fatty acid with CoQ10 were found to vary slightly. The C18 carbon atom of ALA interacts with C38 carbon atom of CoQ10 with bond length of around 3.92 A°. The alkyl interaction between LA and CoQ10 was found at C10 carbon atom of LA and C43 atom of CoQ10, having bond length of approximately 4.23 A°. The alkyl interaction between OA and CoQ10 was found between C1 atom of OA and C28 atom of CoQ10 having bond length of around 3.23 A°. The interactions between the fatty acids and the CoQ10 are shown in Figure 4. From this study, it could easily be analyzed that there is an interaction present between the fatty acids and CoQ10 which could be beneficial in terms of skin permeation of drugs in through pull mechanism [12].

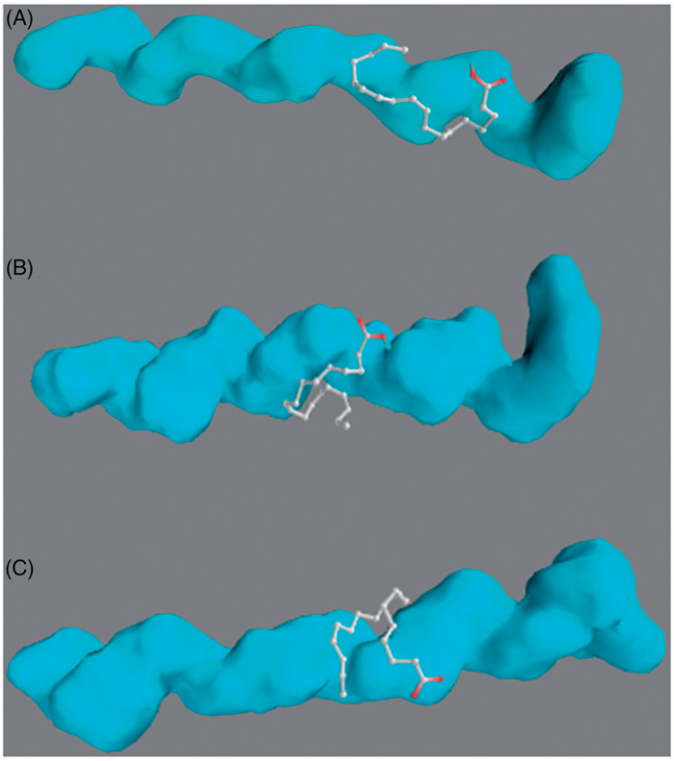

Figure 3. Docking studies using Autodock vina PyRx screening software of molecules (A) CoQ10 and α-linolenic acid (B) CoQ10 and linoleic acid (C) CoQ10 and oleic acid.

Ex vivo permeation study

Ex vivo permeation studies on rat skin were conducted to determine the permeation of nanoemulsion cream formulations. The extent of drug permeation highlighted the physicochemical properties of the drug itself and influence of vehicle system. LQ nanoemulsion cream exhibited significantly highest ($p<.05$, ANOVA) cumulative drug permeation and drug flux (3.97 ± 0.91 mg/cm^2 and 0.19 ± 0.05 mg/cm^2/h) as compared to all other formulations as shown in Figure 5. The control formulation cream (paraffin) displayed the lowest drug permeation and drug flux (1.91 ± 0.23 mg/cm^2 and 0.09 ± 0.01 mg/cm^2/h) as compared to edible oil nanoemulsion creams. The nanoemulsion cream along with edible oils that contain fatty acids might have influenced the drug permeation. It has been reported that unsaturated fatty acids enhance

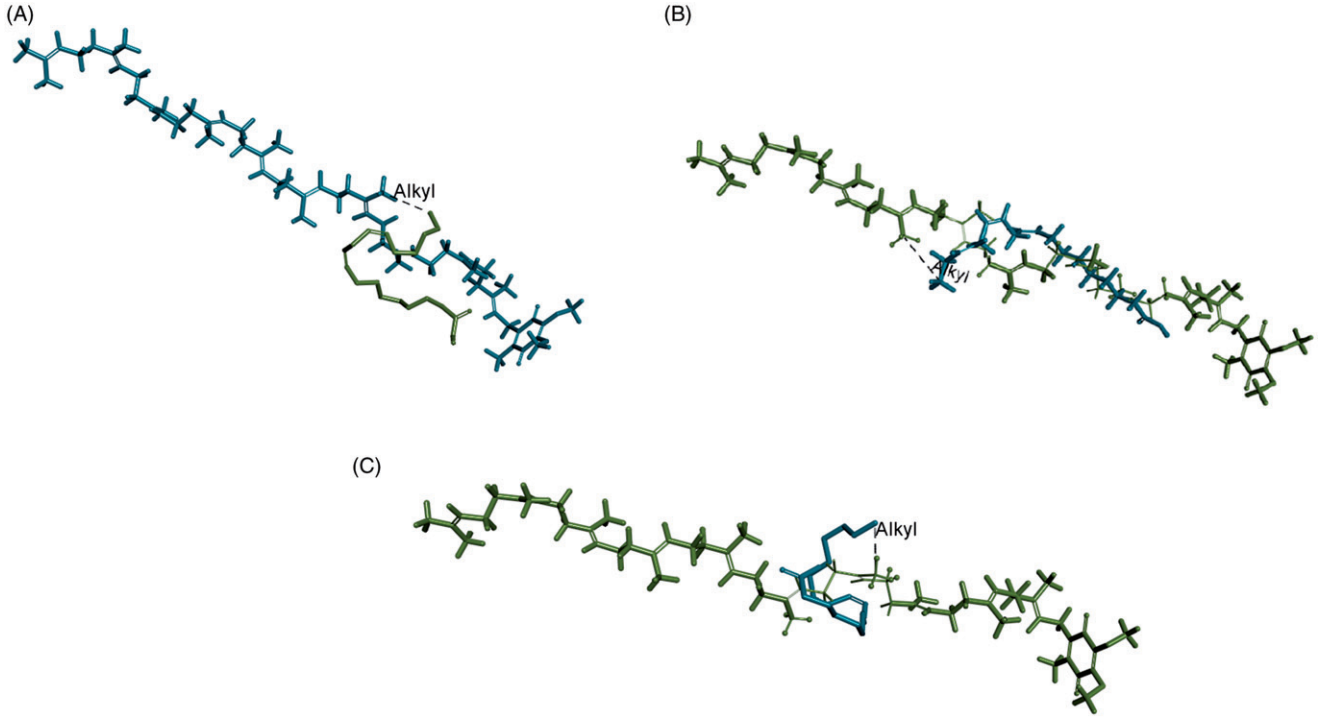

Figure 4. Weak intermolecular bonds between the molecules (A) CoQ10 and α-linolenic acid (B) CoQ10 and linoleic acid (C) CoQ10 and oleic acid.

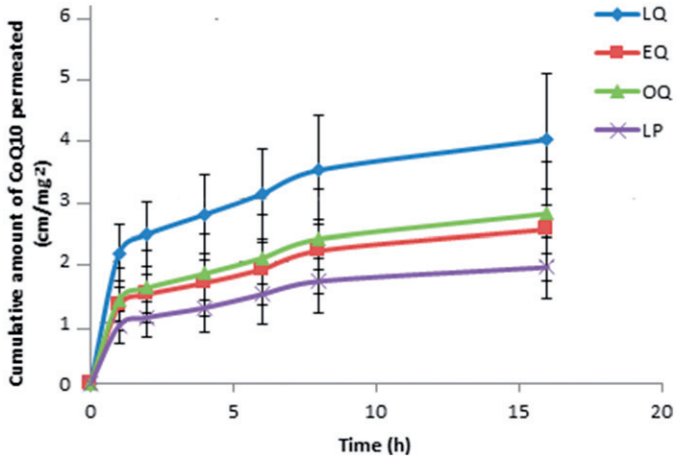

Figure 5. Cumulative amount of CoQ10 permeated (mg/cm^2) of CoQ10-loaded nanoemulsion creams through skin.

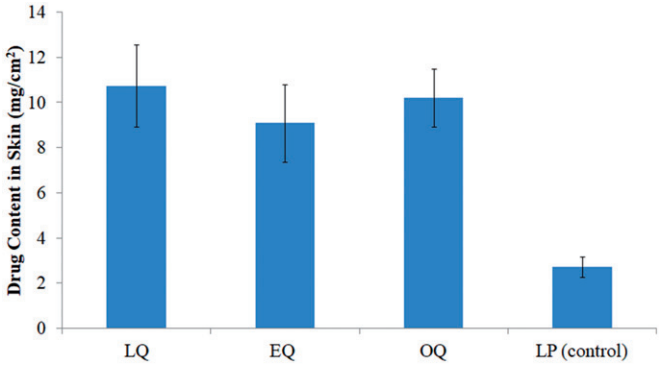

Figure 6. Drug deposition in skin (mg/cm^2) delivered by CoQ10-loaded nanoemulsion creams.

the skin permeation and this enhancement is related to degree of saturation and position of double bonds. Differences in the physicochemical properties of fatty acid which originate from differences in the double bond position most likely determine the efficacy of these compounds as skin penetration enhancers [20]. These permeation enhancers will form kinks (spaces) in the lipid structure of the skin that tends to increase with higher number of double bonds in the unsaturated fatty acids, which could further enhance the permeation of the drug. Kinks may be viewed as the mobile structural defect representing small, mobile, free volumes in the hydrocarbon phase of the membrane, resulting in increased fluidity permitting small molecules to enter and migrate across the membrane [32]. Furthermore, the influence of fatty acids in aiding permeation of CoQ10 through skin is achieved *via* what has been termed as the push and pull mechanism. The fatty acids showed some sort of weak interaction or a bond with CoQ10 and carry it or pull the drug into the SC by interacting with keratin and intercellular lipid domains [33], which were also suggested by the computerized docking studies reported earlier. The edible oils appeared to have also served as permeation enhancers in these formulations, thus improving the permeation of CoQ10 through the skin membrane. Linseed oil is composed of combination of omega-3, -6, and -9 fatty acids (ALA, LA, and OA, respectively), and resulted in the LQ cream demonstrating the highest level of permeation, followed by the OQ cream, which was only composed of a large proportion of OA fatty acids. As discussed above, the influence of omega fatty acids was also evaluated using computerized docking studies, which showed that all the omega fatty acids used in the current study demonstrated a weak interaction or bond with CoQ10 that was able to produce a push and pull mechanism to influence the permeation of CoQ10 through skin. The cumulative amount of permeated CoQ10 and drug flux for all the formulations is shown in Table 4.

Drug retention in skin

Determination of drug retention in skin was used to predict the drug content present in the skin (Figure 6). The results showed that LQ delivered the highest amount of CoQ10 into skin (10.73 ± 1.82 mg/cm^2) followed by OQ (10.21 ± 1.29 mg/cm^2) and then EQ (9.09 ± 1.72 mg/cm^2). The differences among the three creams were statistically different ($p < .05$, ANOVA). The lowest concentration of CoQ10 was delivered by the control formulation (2.72 ± 0.15 mg/cm^2). The results correlated with the cumulative permeation results and highlighted the role of fatty acids in drug distribution across the skin. Fatty acids are also known to interact with lipids in SC, disrupt their structures, increase their fluidity of lipid packing, decrease the diffusional resistance to drugs, and subsequently increasing the flux [34]. The degree of unsaturation of fatty acids also influences the efficacy of these compounds as skin penetration enhancers. Unsaturated fatty acids and the number of double bonds show higher enhancement of penetration compared to that of saturated fatty acids [20]. Therefore, the LQ nanoemulsion cream demonstrated better enhancement of drug penetration compared to that of the other formulations because of the combination of polyunsaturated fatty acids that comprises linseed oil. Fatty acids have also been postulated to decrease the diffusional path length due to the generation of pores on the surface of epidermal corneocytes of the SC and to thereby increase the absorption of drug into the skin [35,36].

Conclusion

Nanoemulsion creams containing CoQ10 and different sources of omega fatty acids were successfully developed using linseed oil, evening primrose oil, and olive oil. In this study, the LQ cream demonstrated the smallest droplet size, greatest firmness, and highest peak stress. All the nanoemulsion creams exhibited higher permeation of CoQ10 into the skin compared to that of the liquid paraffin control cream. The improved penetration could be attributed to the nano-sized droplets and the fatty acids present in the formulations. The LQ cream also showed the highest permeation and drug flux of CoQ10, which may have been due to the presence of omega-3, -6, and -9 fatty acids in the linseed oil, which served as permeation enhancers for the formulations. Thus, it can be concluded that nanoemulsion creams prepared from oils containing fatty acids may be beneficial for skin permeation of drugs. In addition, the combination of omega-3 and -9 fatty acids may also contribute to higher drug permeation.

Disclosure statement

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