RESEARCH ARTICLE

Enhanced Stability and Dermal Delivery of Hydroquinone Using Microemulsion-based System

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Abstract

Introduction: Hydroquinone (HQ), a famous anti-hyperpigmentation agent, suffers from several weaknesses such as instability due to rapid oxidation and insufficient skin penetration because of the hydrophilic structure. The aim of this research was to formulate, characterize and evaluation of *in vitro* skin permeability of HQ-loaded microemulsion (ME). **Methods:** HQ MEs were prepared by pseudoternary phase diagram method, the appropriate ratios of oil, s/c mixture, and water were chosen, and full factorial design was used with three variables at two levels for preparing eight formulations. The prepared MEs were evaluated regarding their droplet size, viscosity, pH, differential scanning calorimetry, stability, *in vitro* drug release, and *in vitro* skin permeability. **Results and Discussion:** The results showed that the mean droplet size range of ME samples was in the range of 7.05–79.56 nm and pH was 5.3–5.7, respectively. Viscosity range of MEs was 109–195 cps. Drug release profile showed that 90.51% of the drug released (ME-HQ-8) in the 24 h of the experiment. The kinetics of drug release from all selected MEs were approximately described by Higuchi model and showed prolonged release when compared to HQ solution. All ME formulations with different compositions and properties significantly increased flux and permeability coefficient from rat skin. J_{ss} and Papp parameters in ME-HQ-2 formulation were 0.404 mg/cm^2 h, 0.02 cm/h, and 4 times higher than those of control, respectively. The selective MEs have 99.9% HQ amount after 6 months storage. They have visually cleared and no any color changes, thus HQ MEs can could been protect drug for a long time without antioxidant. **Conclusion:** The present research established that the amount of components of water, oil, and S + C in ME formulation plays an important role in the physicochemical properties and permeability parameters. This study showed that any change in content and composition of MEs could be changed physicochemical properties and permeability parameters during drug permeation from ME samples. The studied MEs increased permeation rate and permeability coefficient through rat skin. Our results were showed that ME formulation could not be increase diffusivity of HQ in stratum corneum.

Key words: Dermal, hydroquinone, microemulsion, permeability, release

INTRODUCTION

Microemulsions (MEs) are thermodynamically stable and low viscose mixtures of oil and water that have been stabilized with a surfactant and usually in combination with a cosurfactant.[1,2]

Advantages of MEs in topical and transdermal drug delivery have been suggested by several studies. Conventional MEs can be categorized into oil-in-water, water-in-oil, and bicontinuous phase MEs.[3] MEs, as drug delivery systems, have several advantages such as high stability, enhanced drug solubility, ease of manufacturing drugs, and protection of the unstable drugs against environmental conditions. Moreover, they improve percutaneous penetration of drugs.[4] Oil and surfactant phases contribute to the potential enhancing effect of MEs rather than the specific MEs structure.[1] Oil phase like oleic acid can interact with the lipids in the stratum

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corneum leading to an increase in their fluidity, such that drug mobility is also increased.[5] While surfactants penetrate into the skin layers and enhance transdermal and dermal drug delivery either by disrupting the stratum corneum lipids or by increasing the partition coefficient of the drug between skin and formulation medium, thus improving the drug solubility in the skin.[1]

Hydroquinone (HQ) is the main choice for hyperpigmentation therapy. However, the long-term use of HQ is known to cause several unwanted side effects including contact dermatitis, post-inflammatory hyperpigmentation, and ochronosis. HQ is a pharmaceutical compound used not only as a skin-whitening agent but also to condense tyrosinase in the generation mechanism of melanin.[6] HQ, a famous anti-hyperpigmentation agent, suffers from several weaknesses such as instability due to rapid oxidation and insufficient skin penetration because of the hydrophilic structure. HQ is highly unstable into various topical vehicles, presenting low topical bioavailability and a relevant level of toxicity.[7]

In this study, 2% w/w HQ was incorporated into selected MEs, and their physical/chemical stabilities and physicochemical properties were investigated. *In vitro* drug permeation studies through excised rat skin were also evaluated to determine the permeation parameters.

MATERIALS AND METHODS

HQ, Oleic acid, Span 20, and Tween 80 were procured from Merck Chemical Company (Germany). Diethylene glycol monoethyl ether (Transcutol-P) was kindly purchased from GATTEFOSSE Company (France). The effect of variables on different responses was evaluated by experimental design using Minitab 17.

Animal studies

Male adult Wistar rats (weighing 150–250 g) aged 8–10 weeks in the current study were prepared from Animals Laboratory, Ahvaz Jundishapur University of Medical Sciences, which was conducted with the approval of the Animal Ethical Committee, Ahvaz Jundishapur University of Medical Sciences (permit B-9642). Their abdominal skin was shaved using an electric clipper, taking care not to damage the skin. Before sacrificing, they were killed with ketamine injection. Abdominal full-thickness skin was removed, and using cooled pure acetone solution with 4°C, any extraneous subcutaneous fats cleaned from the dorsal side. The thickness of whole skin was determined using a digital micrometer. The animals were treated according to the principles for the care, and use of laboratory animals and the procedures followed with the standard international guidelines.[8]

HQ solubility

The solubility of HQ in different oils (Transcutol-P, oleic acid), surfactants (Tween 80, Span 20), and cosurfactant (propylene glycol) was determined by dissolving an extra amount of HQ in 5 mL of each oil, surfactant, and cosurfactant. The samples were mechanically agitated by means of a shaking water bath functioning at 300 strokes per minute for 72 h at 25 ± 0.5°C to reach equilibrium. After equilibration, the samples were centrifuged at 10000 rpm for 30 min to exclude the undissolved drug. In the next step, the clear supernatants were filtered through a polytetrafluoroethylene membrane filter (φ = 0.45 μm), and the filtrates were analyzed using UV spectrophotometry at 220 nm.[9]

Pseudoternary phase diagram construction

In this research, various pseudoternary phase diagrams were prepared to obtain the concentration range of the components for the existing region of MEs. Two phase diagrams were prepared with the 1:1 and 3:1 weight ratios of (Tween 80/span 20) propylene glycol, respectively. For each phase diagram, the surfactant mixture was added into the oil blend (Oleic acid-Transcutol-P) (10:1), and the surfactant/cosurfactant mixture were then mixed at the weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. These mixtures were vigorously mixed using a magnetic stirrer and diluted dropwise with double-distilled water at 25 ± 1°C. The samples were classified as MEs when they appeared as clear liquids.[10]

Preparation of HQ MEs

Full factorial design was utilized regarding with three variables at two levels for preparing eight ME formulations. Main variables that considered in the determination of ME formulations include percentage of oil (%oil), water percentage (%W), and surfactant/co-surfactant ratio (S/C). Eight different formulations with low and high levels of oil (10% and 60%), water (5%, 10%), and S/C mixing ratio (1:1, 3:1) were selected for preparing ME formulations. Various MEs were selected from the pseudoternary phase diagram with 1:1 and 3:1 weight ratio of Tween 80-Span 20/propylene glycol [Table 1]. HQ (2%) was added to oil phase, and then, S/C mixture and an definite amount of double-distilled water was added to the mixture dropwise and continued by stirring the mixtures at ambient temperature until a uniform mixture was obtained.[10]

Droplet size measurements

The droplet size of MEs was measured at 25 ± 1°C by SCATTER SCOPE 1 QUIDIX (South Korea).

Viscosity measurements

The viscosity of MEs was determined at 25 ± 1°C using a Brookfield viscometer (DV-II + Pro Brookfield, USA)

Table 1: Composition of selected MEs of hydroquinone

Formulation	Factorial design	(S:C)	% Oil	%S+C	%Water
ME-HQ-1	+++	3:01	60	30	10
ME-HQ-2	++-	3:01	60	35	5
ME-HQ-3	+-+	3:01	10	80	10
ME-HQ-4	+--	3:01	10	85	5
ME-HQ-5	--+	1:01	10	80	10
ME-HQ-6	---	1:01	10	85	5
ME-HQ-7	-+-	1:01	60	35	5
ME-HQ-8	-++	1:01	60	30	10

ME: Microemulsions, HQ: Hydroquinone

through spindle no. 34, with shear 100 rpm. A 10 mL volume sample was used for viscosity measurements.[11]

Stability studies

MEs were studied for their physical and chemical stability by temperature, centrifuge stability tests, assay of HQ amount, pH, and mean droplet size in MES after 6 months storage. They were stored in different temperature conditions (4°C, 25°C, 37°C and 75% ± 5% RH for 6 months) according to the ICH guidelines and then visually inspected for phase separation, precipitation, pH, and droplet size changes. Furthermore, after centrifugation at 15000 rpm for 30 min at 25 ± 1°C in a high-speed apparatus ((MPV-350R, POLAND), the samples were visually inspected to detect any phase separation.[12] HQ amount in ME samples after 6 months storage was determined with HQ extraction using methanol then were analyzed using UV spectrophotometry at 220 nm.

Drug release study

Franz diffusion cells having contacted area of 3.46 cm^2 were used to determine the drug release from different formulations. Before each experiment, the cellulose membrane was first hydrated in distilled water at 25°C for 24 h. Then, it was clamped between donor and receptor compartments. HQ ME samples (5 g ME) were accurately weighed and placed on the membrane. 30 ml phosphate buffer solution (PBS) pH 7 was utilized as receptor medium. The receptor fluid was constantly stirred by externally driven magnetic bars at 200 rpm throughout the experiment. At definite time intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 24 h), 2 ml sample was removed from receptor compartments for spectrophotometric determination at 220 nm for the drug content and replaced immediately with an equal volume of fresh receptor medium. The cumulative percentage of released drug was plotted versus time, and their behavior was described by fitting on three different kinetic models such as zero, first, and Higuchi orders. The maximum r^2 was considered as the most probable mechanism.[13,14]

Differential scanning calorimetry (DSC)

DSC measurements were carried out by means of a Mettler Toldo DSC1 star ® system fitted with the refrigerated cooling system. Approximately 5–10 mg of each ME samples were weighted into hermetic aluminum pans and quickly sealed to stop water evaporation from samples. Concurrently, an empty hermetically closed pan was employed as a reference. ME samples were exposed in a temperature varying from +30°C to −50°C (scan rate: 10°C/min). Transitions of enthalpy quantities (ΔH) were computed from endothermic and exothermic peaks of thermograms.[14]

Permeability experiments

Specially designed vertical diffusion cells (with an effective diffusion area of approximately 3.4618 cm^2) were utilized to evaluate *in vitro* permeation. The receptor compartment was filled with 30 ml PBS (PBS, pH 7). Whole skin samples, hydrated before use, were mounted between the donor and receptor compartments of the cell with the stratum corneum facing the donor medium. The donor phase was filled with each HQ ME samples (5 g). The diffusion cells were placed on a heater-stirrer (37 ± 0.5°C), and the receptor phase was stirred continuously at 200 rpm using small magnetic bars. At each interval time (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 24, 28, 32, and 48 h), a 2 ml sample was withdrawn from the receptor medium and replaced by an equivalent volume of PBS to maintain sink condition. A UV spectrophotometer was utilized to detect the permeated amount of HQ in derived samples at 220 nm. The free drug MEs and 2% HQ water solution were used as negative and positive controls, respectively.[15]

Data analysis and statistics

Cumulative permeated HQ per unit area was calculated and plotted against time. Steady-state flux ($mg/cm^2/h$) was calculated from the linear portion of the slope of the permeation curve. Permeability coefficient (Kp,cm/h) through the skin for HQ was calculated as in Eq. 1:

$Kp = J_{ss}/Cv$ (1)

Where J_{ss} and Cv are steady-state flux and HQ concentration in donor medium, respectively.

The enhancement ratio (ER) was calculated to obtain the relative enhancement in the permeability parameters amount of ME samples in respect of the control (2% HQ water solution) permeability parameters. The ER was estimated as:

Enhancement ratio (ER) = Permeability parameter amount of ME formulation/permeability parameter amount of control.

All of the experiments were carried out in triplicate and the results were presented as means, using one-way analysis of variance (ANOVA), the data were statistically analyzed and $P < 0.05$ was considered as significant differences.

RESULTS AND DISCUSSION

Solubility study

The solubility of HQ shown in Table 2.

Phase studies

The pseudoternary phase diagrams of oleic acid-Transcutol-P (10:1)/Tween 80 Span 20/propylene glycol/water are presented in Figure 1. The phase diagrams clearly showed that ME existence boundary increased with increase in the weight ratio of surfactant/cosurfactant and lead to the presence of much more water amount in the MEs structure (Km = 1–3).

Characterization of HQ MEs

The viscosity, mean droplet size, polydispersity index (PI) and pH of HQ MEs are presented in Table 3.

The ME formulations in this research indicated the average viscosity range (109–195 cps), pH value (5.3–5.7), and droplet size (7.05–79.56 nm). ANOVA showed that correlation between ME droplet sizes with independent variables (%water, %oil) is significant ($P < 0.05$), so that the droplet sizes are increased with less percentage of the water phase and more percentage oil phase in ME sample. The decrease in droplet size is related to a great increase in surface area that would lead to improved bioavailability and skin permeation.[16] The polydispersity value described the uniformity of the droplet size. All polydispersity values were obtained smaller than 0.5. Hence, the results show the narrow distribution of droplet size in ME formulations. The ME formulations had appropriate observed pH value (5.3–5.7) that is the best one for topical application.

ANOVA is showed that correlation between viscosity with independent variables (s/c) is significant ($P < 0.05$), so that the viscosity is increased with less s/c ratio HQ MEs.

Figure 2 shows the release profile of HQ MEs. Drug release profile showed that 90.51% of the drug was released in 24 h of the experiment for ME-HQ-1. The percentage of the drug released and kinetics of release in the ME formulations are summarized in Table 4.

ANOVA represented that the correlation between drugs released in 2 hours (R2 h) and the independent variables (S/C, %oil) was significant ($P < 0.05$). It means that R2 h increased with less percentage of oil phase and more S/C ratio in HQ formulations. Furthermore, the correlation between drug released in 24 hours (R24 h) and the independent variable

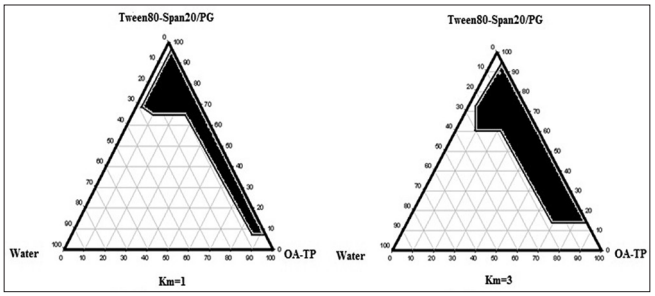

Figure 1: The pseudoternary phase diagrams of the oil-surfactant/cosurfactant mixture-water system at the 1:1 (km = 1) and 3:1 (km = 3) Weight ratio of Tween 80/Span 20/propyleneglycol [PG] at ambient temperature, dark area show microemulsions zone

Table 2: Solubility of hydroquinone in oil, surfactant, and cosurfactant (mean±SD, *n*=3)

Phase type	Excipient	Solubility (mg/ml)
Oil	Transcutol-P	6.2±0.1
	Oleic acid	9.1±0.1
	Oleic acid+Transcutol-P (10:1)	10.5±0.2
Surfactants	Tween 80	4.2±0.15
Cosurfactant	Span 20	3.4±0.17
	Propylene glycol	8±0.17

SD: Standard deviation

Table 3: Viscosity, mean droplet size, pH, polydispersity index of selected HQ MEs (mean±SD, *n*=3)

Formulation	pH	Viscosity (cps)	Mean droplet size (nm)	Polydispersity index	Mean droplet size (nm) (after 6 months)
ME-HQ-1	5.5±0.1	110±0.3	7.05±0.03	0.4±0.001	7.1±0.04
ME-HQ-2	5.5±0.2	109±0.1	79.56±0.58	0.35±0.002	80.1±0.1
ME-HQ-3	5.6±0.1	195±0.1	53.63±0.42	0.4±0.001	54±0.3
ME-HQ-4	5.7±0.3	185±0.1	10.26±0.15	0.4±0.001	11.1±0.1
ME-HQ-5	5.6±0.2	158±0.1	21.96±0.31	0.35±0.002	22.2±0.4
ME-HQ-6	5.4±0.1	121±0.1	34.4±0.41	0.35±0.002	35±0.1
ME-HQ-7	5.3±07	130±0.1	10.43±0.12	0.4±0.001	10.5±0.2
ME-HQ-8	5.3±0.2	134±0.1	11.3±0.3	0.35±0.002	11.5±0.1

ME: Microemulsions, HQ: Hydroquinone, SD: Standard deviation

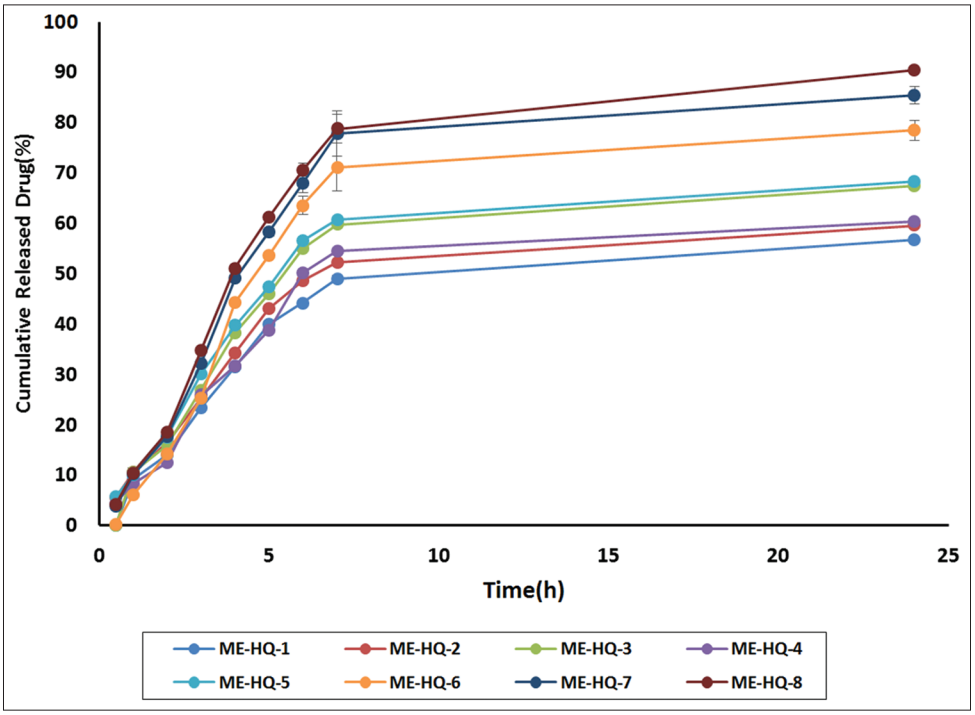

Figure 2: *In vitro* profile of microemulsion formulations of hydroquinone.

(%oil) was significant ($P < 0.05$), so that, any decrease in oil percentage phase increased R24h in ME samples. It is established that small droplet size contributes to the fast release.[17]

Figure 3 shows DSC cooling thermograms of HQ ME formulations. Cooling MEs transition temperature and enthalpy are represented in Table 5.

In cooling curves of the ME formulation, bulk water (free water) and bound water are obtained at 0°C and −15 to −18°C, respectively. According to the ANOVA results, a non-significant correlation ($P > 0.05$) was found between the bound melting transition temperature (Tm_2) and independent variables, also, the independent variables affected enthalpy of the exothermic peak of bound water ($P < 0.05$); i.e., the enthalpy increased due to the increase of water and decrease of oil phase percentage. The thermal behavior of water can be useful and rapid tools by which to understand the microstructure of MEs.[18] Our findings are in agreement with those of previous reports by Podlogar *et al*.[19] DSC has been utilized to calculate heat flow that is associated with transitions in materials as a function of temperature.

It was shown that all of the ME formulations have proper characteristics regarding their homogeneity and 6-month duration stability. Average droplet sizes and pH of MEs at the beginning and after 6 months of storage are shown no significant difference ($P > 0.05$) [Table 3]. The formulated drug-loaded ME systems were found physically and chemically stable for a period of 6 months with no phase separation, flocculation or coalescence during storage at different temperature conditions and also during centrifugation. Thus, the formulated system of HQ is said

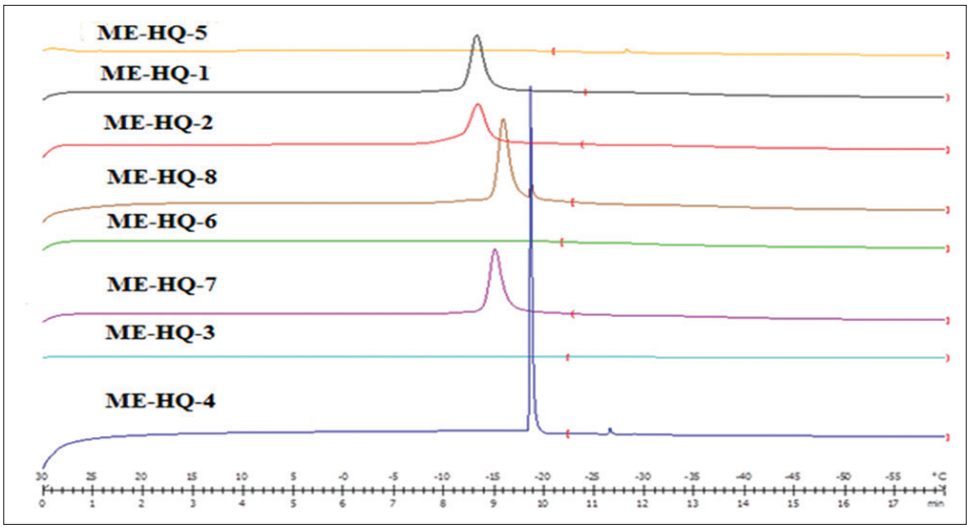

Figure 3: Differential scanning calorimetry cooling thermograms of hydroquinone microemulsions

Table 4: Percent release and kinetic release of selected microemulsions (mean±SD, *n*=3)

Formulation	% Release, 24 h	Kinetics of release	R^2	%Release, 2 h
ME-HQ-1	56.82±0.487	Higuchi	0.758	14.14±0.138
ME-HQ-2	59.55±1.352	Higuchi	0.758	16.44±0.20
ME-HQ-3	67.50±1.195	Higuchi	0.736	16.02±0.845
ME-HQ-4	60.36±1.153	Higuchi	0.777	12.53±0.248
ME-HQ-5	68.37±1.230	Higuchi	0.778	17.63±0.377
ME-HQ-6	78.44±2.042	Higuchi	0.762	14.31±0.679
ME-HQ-7	85.47±1.723	Higuchi	0.773	17.81±0.436
ME-HQ-8	90.51±0.520	First	0.825	10.57±0.359

ME: Microemulsions, HQ: Hydroquinone, SD: Standard deviation

Table 5: Transition temperature and enthalpy of HQ MEs (mean±SD, *n*=3)

Formulation	TM_2 (°C)	ΔH_2 (mJ/mg)
ME-HQ-1	−17±0.1	34.31±0.7
ME-HQ-2	−16.2±0.2	25.68±0.9
ME-HQ-3	−17±0.1	35.86±0.5
ME-HQ-4	−18±0.2	25.03±0.4
ME-HQ-5	−13±0.3	25.68±0.6
ME-HQ-6	−15±0.1	32.69±0.8
ME-HQ-7	−16±0.2	32.16±0.3
ME-HQ-8	−18±0.1	34.92±0.7

ME: Microemulsions, HQ: Hydroquinone, SD: Standard deviation

to exhibit good thermodynamic stability. The selective MEs have 99.9% HQ amount after 6 months storage. They have visually cleared and no any color changes, thus HQ MEs can could been protect drug for a long time without antioxidant.

The permeability parameters of different ME formulations are represented in Table 6. In permeability studies, ANOVA represented that the correlation between J_{ss} and the independent variables (%W, S/C ratio) were significant ($P < 0.05$). Therefore, any decrease in water phase percentage and an increase in s/c ratio caused increase J_{ss} parameter in ME formulation. Hence, J_{ss} of HQ for ME-HQ-2 was 0.404 mg/cm^2 h, which was 4 times higher than that of control (HQ solution, 2%). The correlation between apparent diffusivity coefficient (D_{app}) and the independent variables was not significant ($P > 0.05$). D_{app} parameter in ME formulations was less than those of control. Our results were showed that ME vehicle could not be increase diffusivity of HQ in stratum corneum.

The correlation between T_{lag} and the independent variable (%Oil) was significant so that any decrease in oil phase percentage significantly increased the T_{lag} parameter in ME formulations. Hence, the maximum T_{lag} of HQ for ME-HQ-6 was 4.1 h. All of ME formulations have higher T_{lag} amount than those of control. The correlation between permeability coefficient (P) and the independent variables was not significant (P > 0.05). Hence, permeability coefficient (P) of HQ for ME-HG-8 was 0.020cm/h, which was 4 times higher than that of control.

Table 6: *In vitro* permeability parameters of different ME formulations of HQ through excised rat skin (mean±SD, *n*=3)

Formulation No.	J_{ss}, mg/cm² h	T_{Lag}, h	$D_{app,}$ cm²/h	P_{app} (cm/h)	ER_{flux}	ER_D	ER_P
Control	0.1±0.0007	1.71±0.21	0.077±0.009	0.005±0.0003	-	-	-
ME-HQ-1	0.364±0.024	2.28±0.15	0.023±0.001	0.018±0.0012	3.61±0.27	0.311±0.6	3.61±0.27
ME-HQ-2	0.404±0.005	2.52±0.04	0.0229±0.04	0.020±0.0002	4±0.1	0.292±0.03	4±0.1
ME-HQ-3	0.314±0.004	3.19±0.12	0.017±0.0002	0.0157±0.0002	3.11±0.02	0.225±0.03	3.11±0.02
ME-HQ-4	0.308±0.012	2.95±0.32	0.197±0.002	0.015±0.0006	3.05±0.1	0.259±0.06	3.05±0.1
ME-HQ-5	0.316±0.025	3.3±0.7	0.017±0.004	0.015±0.001	3.13±0.23	0.226±0.09	3.13±0.23
ME-HQ-6	0.329±0.007	4.1±0.1	0.015±0.0003	0.0164±0.0003	3.26±0.05	0.197±0.3	3.26±0.05
ME-HQ-7	0.369±0.004	2.4±0.1	0.026±0.002	0.018±0.0002	3.66±0.01	0.341±0.078	3.66±0.01
ME-HQ-8	0.374±0.001	2.4±0.1	0.0246±0.001	0.0187±0.0003	3.7±0.018	0.321±0.05	3.7±0.02

ME: Microemulsions, HQ: Hydroquinone, SD: Standard deviation

The correlations between ERp and ER_{flux} with the independent variable (S/C ratio) were significant ($P < 0.05$). Hence, any increase in oil phase percentage significantly increased ERp and ER_{flux} parameters in ME formulations. All ME formulations with different compositions and characteristics significantly increased flux and permeability coefficient from rat skin. In addition, several previous studies showed the benefits of MEs vehicle as an enhancer in dermal delivery.[20-23] The content of S/C mixture in ME samples affected the skin permeation rate of HQ significantly. As the content of S + C mixture was decreased from 85% to 35% at S/C = 3 (Km = 3), the skin permeation rate of HQ increased by 4-fold (ME-HQ-2). This may be due to an increased thermodynamic activity of the drug in the ME at the lower content of S + C mixture. The higher permeability rate of HQ from ME formulations is most probably due to the S + C mixture, which act as penetration enhancers. The enhancer can increase the permeation through the skin by altering the diffusion or partitioning coefficient of the drug.[24] Several mechanisms have been proposed to explain the advantages of ME for the topical delivery of drugs. First, a large content of drugs can be incorporated in the ME formulation due to the high solubilizing capacity. Second, the steady-state flux of the drug from ME formulation may be increased, since the affinity of a drug to the internal phase in ME can be easily modified to favor partitioning into stratum corneum, using a different internal phase, changing its portion in ME or adjusting its property.

Drugs can permeate the SC through two pathways, the first is the intercellular route, and the other is the transcellular way. The intercellular route plays a major role in the percutaneous uptake of drugs. It is known that a complex mixture of essentially neutral lipids that are arranged as bilayers with their hydrophobic chains facing each other. Most of the lipophilic drugs permeate through this lipid pathway. Polar head groups of lipids face an aqueous area forming a polar route that hydrophilic drugs generally permeate through this pathway. Topically applied MEs are proposed to permeate the SC and to exist intact in the whole horny layer, modifying both the polar and the lipid pathways. The lipophilic domain of the ME can interact with the SC in many ways. The drug dissolved in the lipid domain of the ME can directly partition into the lipids of the SC, or the lipid vesicles themselves can occupied between the lipid chains of the SC, thereby disrupt its bilayer structure. In fact, these bilayer interactions will lead to increased permeability of the lipid pathway to HQ. On the other hand, the hydrophilic domain of the ME can hydrate the SC and acts an important role in the permeation of drugs. When the water phase of the ME enters the polar pathway, it will increase the inter-lamellar volume of the SC lipid bilayers, resulting in the disruption of its interfacial structure. Since some lipid chains are covalently attached to corneocytes, hydration of these proteins will also lead to the disorder of lipid bilayers.[25]

Aungst *et al.* studied different fatty acids, alcohols, sulfoxides, surfactants, and amide as enhancers for naloxone.[26] It was suggested that enhancers with an unsaturated C18 alkyl chain such as oleic acid appeared to be the optimal ones. Since the tail of the stratum corneum bilayer is hydrophobic, the fatty acids can enter the bilayer, disrupt it by creating separate domains, and in this way may induce highly permeable pathways in the stratum corneum. The most popular enhancer is oleic acid. It increased the flux of salicylic acid 28-fold and of 5-fluorouracil 56-fold through human skin membrane.[27] PG has been found to act as an enhanced by a mechanism similar to that of ethanol enhancing the permeability of hexyl nicotinate.[28] There is wide use of nonionic surfactants in topical formulations as solubilizing agents. Tween 80 was reported to accelerate hydrocortisone permeation.[29] Oleic acid, propylene glycol, and water in our ME formulations are considered as permeation enhancers that acting by several mechanisms including disruption of the organized intercellular lipid structure of the stratum corneum, fluidization of the stratum corneum lipids.[30] Therefore, it is reasonable to believe that the developed MEs in this study should be effective; however, additional *in vivo* efficacy studies are recommended to show the potential of these ME systems as a dermal delivery system of HQ.

CONCLUSION

The present research established that the amount of components of water, oil and S + C in ME formulation plays an important role in the physicochemical properties and permeability parameters. The kinetics of drug release from all selected MEs were approximately described by Higuchi model and showed prolonged release when compared to HQ solution. The stability of HQ enhanced using MEs system. The studied MEs increased permeation rate and permeability coefficient through rat skin.

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