Original Article

The Depigmentation Effect of Hydroquinone-loaded Nanostructured Lipid Carriers (NLCs) on the Rat Skin

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Abstract

The goal of this research is the localization of hydroquinone (HQ) to the epidermis for the treatment of hyperpigmentation in rat skin. For this purpose, nanostructured lipid carrier (NLC) was selected for the dermal delivery of HQ. A 2^3 factorial design was used in this study, and eight NLCs were prepared with a cold homogenization technique. HQ entrapment efficiency (EE %), particle size, morphology, thermal behavior of NLCs, and permeability parameters through rat skin with NLC in comparison with HQ aqueous solution (HQ-S) with Franz diffusion cells were evaluated. Based on the optimization technique, the best NLC was selected and in the *in vivo* experiment, the depigmentation effect of optimized NLC in comparison with that of HQ-S was evaluated. The results showed that the main problem for HQ permeability was fast permeation and low concentration in the site of action. Partitioning from aqueous donor phase into skin rate was the limiting step for drug flux, and this can be solved using NLC. The decrease in maximum flux obtained by NLC was according to formulation 8. Regression analysis suggested a significant and direct effect of the S/L ratio and the percentage of liquid lipids on the drug loading. NLC decreased drug permeation through rat skin basically due to sustained release properties.

Keywords: *Depigmentation, hydroquinone, nanostructured lipid carrier, percutaneous absorption, rat*

Introduction

Hydroquinone (HQ) is a phenolic organic compound that demonstrated the anti-hyperpigmentation effect by blocking the enzyme responsible for melanin production. Topical HQ is frequently applied as a skin-lightening agent in concentrations up to 10% that may be caused irritation in sensitive individuals.

HQ preferentially has been reported to decrease the melanosomes formation, induce the degradation of melanosomes, changing in the structure of melanosomes, and destruction of the membranes of melanocytes by lipid peroxidation.[1-4]

Treatment of hyperpigmentation disorders, however, is often difficult and prolonged, requiring a great deal of patience and knowledge with a variety of therapeutic modalities to achieve success.[5]

HQ is a hydrophilic compound with low permeability through the skin. Therefore, a high dose is necessary for clinical uses resulting in a high incidence of skin irritation and causing diseases such as vitiligo.[6] Therefore, attention is now being focused on the potential toxicities and adverse effects of HQ itself. Nano-particulate drug delivery systems could improve the therapeutic effect of HQ by protecting it against degradation and improving drug partitioning into the skin.

Solid lipid nanoparticles are made by simply replacing the liquid lipid (oil) with a solid lipid in o/w emulsions, being solid at body temperature. The solid lipid nanoparticles (SLN) were introduced at the beginning of the 1990s. The second generation of the SLN is nanostructured lipid carriers (NLCs), which are produced by using a blend of a solid lipid with a liquid lipid.[7]

Lipid nanoparticles have been investigated for various pharmaceutical applications, e.g., parenteral,[8,9] dermal,[10,11] and ocular[12] administration.

The NLCs demonstrate great benefits as a drug carrier for the delivery of hydrophilic and lipophilic molecules such as perfect physical stability, low cost compared to liposomes, biocompatibility, and biodegradability.[13] NLCs can act as a drug carrier for epidermal targeting, follicular delivery, and controlled release of an active moiety that has been

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very well established.[14] NLCs are the new generation of SLNs that overcome some limitations of SLNs such as low drug loading, lipid polymorphism, and followed-up drug leakage during storage.[10] NLCs consist of a mixture of particularly different lipid molecules, i.e., solid and liquid lipids form a less perfect crystalline structure with many imperfections providing more space for drug loading.[15] NLCs' applicability for dermatological disorders such as skin dermatitis, psoriasis, alopecia, and burn was reported in the literature.[16-18] These colloidal carriers offered controlled release profiles for antibacterial drugs[19] and many other drugs.[20,21] Moreover, the low particle size of the NLCs cause close contact with stratum corneum (SC) and improve drug partitioning into the skin.[22,23] The epidermal drug targeting can be provided with NLCs as they are incorporated into the deeper layers of the skin.[24]

Lipid nanoparticles (SLNs, NLCs) have been introduced as suitable carriers to modulate the penetration/permeation of drugs throughout the different layers of skin.[25] Aqueous NLCs formulations were proposed for topical delivery of superoxide dismutase[26] and various glucocorticoids such as dexamethasone[27] and betamethasone valerate.[28] Most of these studies offered that SLNs and NLCs are promising carriers for topical drug delivery. Percutaneous absorption of HQ through human stratum corneum and full-thickness rat skin has been measured *in vitro* using 5% aqueous solutions of HQ as the donor solutions. The measured absorption rate (mean +/−S.D.) of HQ through human stratum corneum was 0.52 ± 0.13 µg/cm^2/h, while that for full-thickness rat skin was 1.1 +/−0.65 µg/cm^2/h.[29] Therefore, the aim of this study was to design and evaluate the NLC of HQ for increasing drug localization in the epidermis to decrease systemic absorption and increase drug concentration in the site of action.

Materials and Methods

Materials

Hydroquinone was obtained from Merck (Germany). Oleic acid, span 20, propylene glycol, and lecithin were provided by Merck company. Compritol 888 ATO (CA) was gifted from Gattefosse Company (France).

Experimental design

In the first part of our research, lipid screening (solubility test) was applied for finding the most suitable solid and liquid lipids and surfactants that are able to dissolve the required amount of hydroquinone in the nano lipid particles. After these experiments, compritol and oleic acid were selected as solid and liquid lipids, respectively. The full factorial design with three independent variables and two levels was used. The independent variables consist of liquid lipid percentage, surfactant/lipid ratio(S/L), and percentage of transcutol (%T). Therefore, eight formulations were prepared and the physicochemical and skin permeability properties were studied.

Manufacturing of NLC formulation

Cold homogenization technique has been used to prepare hydroquinone NLCs. In the first step, the hydroquinone powder was dispersed in a molten mixture of solid and liquid lipids. In the second step, the resulting mixture was cooled to solidify the drug–lipid mixture. then, ground into fine powder. Dispersing the lipid-drug powder in the cold surfactant and co-surfactant solution at 40°C. Finally, the resulting suspension was homogenized at high pressure (2,000 bar) at low (IKA®T25, Ultra-Torax, Germany) for three cycles.

Particle size determination

The average diameter of HQ-loaded NLCs was determined photon correlation spectroscopy using particle size instruments (QUIDIX, South Korea) at a fixed angle of 90° at 25°C. All hydroquinone NLC samples were appropriately diluted (20×) with distilled water to avoid multiple scattering phenomena.

Percentage of entrapment efficiency (EE%)

The quantity of EE% in NLCs was measured by ultracentrifugal technique in conditions of 25,000 rpm for 30 min and 4°C. The NLC sample with a defined amount of HQ was centrifuged and supernatant liquid was analyzed by UV spectrophotometry at 289 nm for determining free HQ. Additionally, the direct method, HQ loaded in each NLC was determined after NLC treatment with Triton X-100 (0.5% w/v) as a detergent. The amount of loaded HQ was calculated by the following equation.[26]

$$\%EE = \frac{\text{Amount of HQ loaded}}{\text{Amount of HQ initially added}} \times 100 \qquad (2)$$

Drug release behavior in NLC samples

The released amount of hydroquinone from NLCs was carried out by Franz diffusion cells. The NLC samples were dispersed in 5 ml of water as used in the donor phase and the receptor section was filled with 30 ml phosphate buffer solution (pH 7). Sampling was performed for 72 h in predetermined intervals.

Particle morphology

Prepared nanoparticles were characterized for shape by Atomic Force Microscopy (AFM) using a Nanowizard II.

The permeability of HQ through isolated rat skin

Male Wistar rats with a weight of 40–180 g and 9–12 weeks old were prepared from the animal center, Ahvaz Jundishapur University of Medical Sciences, and stored in standard conditions. This *ex vivo* study was performed by Franz diffusion cells (effective area, 0.693 cm2 for HQ-loaded NLC and HQ supersaturated solution in distilled water (HQ-S) at 37 ± 2°C. The hairs of isolated rat skin were removed, washed with water, and placed between donor and receptor chambers. The receptor medium consisted 30 ml phosphate buffer with pH 7 and donor phases filled with NLCs. During the experiment, 2 ml of receptor media was removed and displaced with a buffer

solution at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 28 h. HQ permeated amount was assayed by spectrophotometric method at 289 nm.

NLC stability evaluation

To investigate the stability, NLC samples were stored at temperature conditions 30°C and 75% ± 5% RH as per the ICH guidelines for three months and then evaluated by monitoring time- and temperature-dependent changes of particle size and drug loading. Increasing particle size and decreasing drug loading during storage are signs of nanoparticles fusion together and drug explosion because of lipid modification.

Thermal behavior of NLCs

The heating and cooling behaviors of NLC samples and HQ powder were evaluated by Differential Scanning Calorimetry (DSC) method. In thermal program, samples were heated and cooled at −30 to +120°C and +60 to −40°C, respectively, with a scan rate of 10°C/min.

Evaluation of depigmentation by HQ

Female rats (8–12 weeks old) were supplied from animal center of Ahvaz Jundishapur University of Medical Sciences and stayed for 5 days in feeding standard condition. A UVB lightbox was designed to perform this experiment. The rats were exposed to UVB radiation for 15 min every day with 20.8 ± 1.2 W/m^2 for a week and the number of melanocytes in the rat skin was counted and compared with the number of nonexposed skin. Hyperpigmentation was induced by UVB- irradiation. For this purpose, UVB-irradiation box was designed and for six days periods and 15 min in each day with 20.8 ± 1.2 W/m^2 amount of UVB irradiated, and the number of melanocytes was counted and compared with unirradiated skin area in the defined area of skin. Topical effect of HQ was done with daily application of NLC samples (containing 2% of HQ) on the rat skin for a six days period. After that, animals were sacrificed using ketamine/xylazine, and then skin samples were removed. Biopsy specimens for optical microscopy were obtained at intervals of 1, 2, and 3 weeks. The following biopsy specimens were obtained from epilated skin of each of the three animals: (a) untreated (control); (b) treated with an NLC containing all the ingredients except HQ; (c) treated with 2% HQ-loaded NLC; (d) treated with a Trademark o/w cream containing 2% HQ (manufactured by Tolid Daru pharmaceutical company, Tehran, Iran)

Results and Discussion

NLC formulations based on full-factorial design

This experiment was used a full-factorial design method with three variables and two high (+) and low (−) levels. The compositions of eight formulations are illustrated in Table 1.

HQ-loaded NLC characterization

Entrapment efficiency percent (EE %)

Table 2 summarizes the EE % for different formulations. The results indicate that the EE % has a significant relationship and direct effect with the S/L ratio (P = 0.029); so that, not significant with other independent variables. Therefore, the surfactant and its concentration demonstrate a critical role in drug loading and nanoparticle formation in this study. HQ-loaded in NLCs EE % was ranging from 32% to 48%. The highest and lowest EE% were provided by formulation 2 and 4, respectively, and may be suitable for loving water agents (e.g., hydroquinone).

Size of NLC samples

The size of NLCs has a critical role on the nanoparticle properties, such as permeability parameters. Table 3 shows the mean particle size and polydispersity index (PDI). NLCs sizes were between 28 and 70 nm.

The results indicate no significant relationship between size and independent variables. It seems that cold homogenization method can be suitable for preparing of NLC with proper particle size.[30]

Morphology evaluation by AFM

AFM picture of NLC formulation 2 was shown in Figure 1. The figure illustrates forming NLCs with uniform shape and size.

Drug release behavior

Release behavior of HQ through NLC formulations was showed 2 h and 72 h percentages of (%D2 and %D72) as burst and prolonged release [Figure 2 and Table 4].

Results demonstrated a significant effect of S/L ratio and L% on drug release percentages after 2 h of experiment, so that, any increase in the ratio of S/L and decrease in liquid lipid has been increased in the release amounts of HQ. In

Table 1: Formulation components for HQ-loaded NLC according to full-factorial design

Formulation number	State in full-factorial design	Drug(mg)	(O.A)%	Transcutol%	Surfactant%	Solid lipid%
1	+ + +	0.3	1	1	2	10
2	+ + −	0.3	1	0	2	10
3	+ − +	0.3	05	1	2	5
4	− − +	0.3	0.5	1	1	5
5	− + −	0.3	1	0	1	10
6	+ − −	0.3	0.5	0	2	5
7	− + +	0.3	1	1	1	10
8	− − −	0.3	0.5	0	1	5

Table 2: Loading efficiency for prepared formulations (mean±SD, *n* = 5)

Formulation number	State in full-factorial design	Loading efficiency (%)
1	+ + +	55.15 ± 4.83
2	+ + −	57.5 ± 3.95
3	+ − +	56.30 ± 5.15
4	− − +	32.9 ± 2.77
5	− + −	46.95 ± 3.05
6	+ − −	54.77 ± 6.25
7	− + +	43.17 ± 2.37
8	− − −	50.95 ± 7.24

Table 3: Mean particle size of nanoparticles and polydispersity index (mean±SD, *n* = 3)

Formulation number	Full-factorial state	Mean particle size(nm)	Polydispersity index
1	+ + +	69.7± 4.1	0.33±0.05
2	+ + −	28.5±2.49	0.31±0.06
3	+ − +	22.5±2.41	0.35±0.04
4	− + −	20.78±0.96	0.24±0.05
5	− + +	25.5±0.73	0.18±0.02
6	+ − −	33.1±1.64	0.16±0.01
7	− − +	24.4±1.85	0.26±0.02
8	− − −	30.2±2.33	0.44±0.03

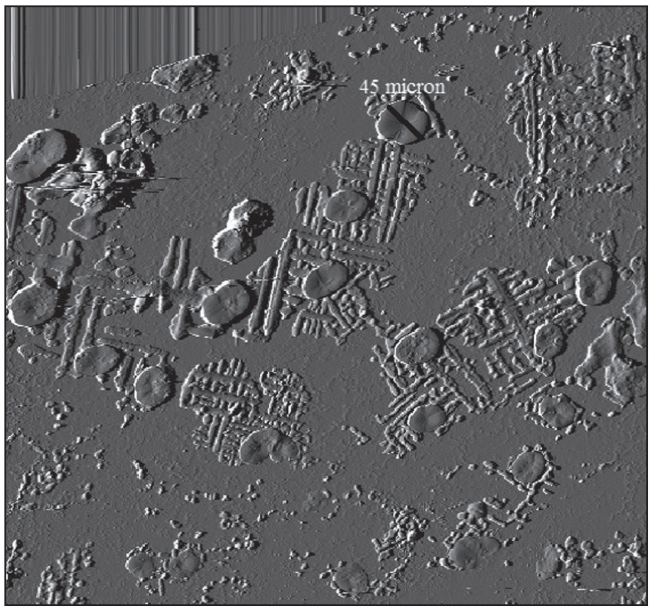

Figure 1: AFM picture of NLC formulation 1 at a magnification of 100

fact, the S/L ratio was the reason for increasing drug loading and drug release after 2 h. It seems that higher drug loading induced a higher concentration gradient between within and outside of nanoparticles that causes higher drug release. An increase in oleic acid reduced the HQ release rate but had no significant relationship with drug loading. It can be concluded that oleic acid with increased drug solubility in nanoparticles decreased drug release. Lower amounts in %D72 belonged to formulation 7 while formulation 3

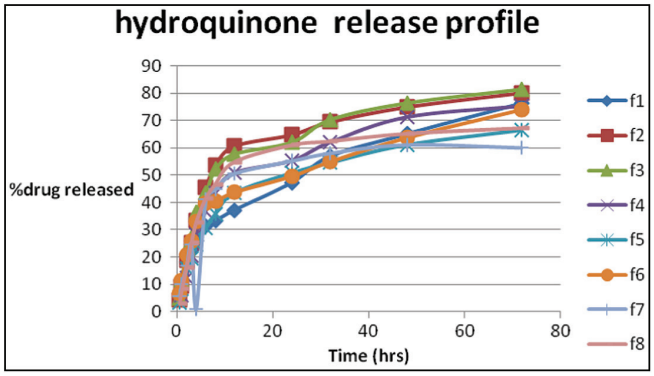

Figure 2: HQ release profile from different NLCs

Table 4: Different parameters regarding HQ release from NLCs (mean±SD, *n* = 5)

Formulation number	Full-factorial state	D2 (%)	D72 (%)
1	+ + +	18.2 ± 2.37	76.33 ± 6.95
2	+ + −	18.4 ± 0.66	80.11 ± 7.7
3	+ − +	22.8 ± 1.96	81.39 ± 6.65
4	− − +	16.7 ± 1.2	60.12 ± 5.1
5	− + −	12.4 ± 1.16	75.4 ± 6.1
6	+ − −	20.9 ± 2.2	74.1 ± 3.2
7	− + +	15.8 ± 1.3	66.5 ± 5.12
8	− − −	16.1 ± 1.2	67.12 ± 5.67

had the highest value of % D72. S/L ratio demonstrates the same significant and direct correlation with % D72 as with % D2. Higher in S/O ratio may be promoted drug loaded in the shell layer of NLCs and consequently can be increased rate of release. These findings suggest a core-shell model for HQ-loaded NLCs.

The HQ-loaded NLCs were shown two phases in release profiles so that the first hours contained immediate release and the second phase has a prolonged release. Therefore, the S/L ratio is a critical key in the selection of an optimized formulation.

Compritol ATO 888 is contained mono- and diglycerides (64–72%, melting point of 71.1°C and HLB 2) provided a prolonged release profile for HQ. Our findings are in agreement with the former study.[31]

Thermal behavior of NLCs

The thermal behavior of NLCs was performed by DSC technique that to measure heat flow amounts. DSC was used to investigate water state in nanoparticles including bulk (free) and bound (interfacial) water.[32] Cooling and heating thermograms according to NLC's were provided shown in Figure 3 and Table 5. In the cooling program, DSC profiles showed 1 exothermic peak at around −26 to −34°C which is attributed to the bounded water that is in accordance with the literature report.[32] There was a significant correlation between S/L ratios and enthalpy of transition so that any increase in the S/L ratios leads to an increase in enthalpy amounts. It

seems that all the water in the nanoparticles is interfacial water (bounded) and no free water was detected. Therefore, water behavior is affected by the concentration of surfactants. Heating thermograms demonstrated 2 endothermic peaks at −4 to −10°C that is attributed to the fusion of ice and 63–96°C that shows the fusion of the lipid phase. The effect of the S/L ratio on the enthalpy of transition at −4 to −10°C was direct and significant. Therefore, the concentration of the surfactant determined the energy for water freezing and the fusion of ice. Independent variables did not show any significant correlation with the enthalpy of transition at 63–96°C. HQ showed an endothermic peak at 172.5°C that indicates its melting temperature[33] which was disappeared in thermogram of HQ-NLC that demonstrates the compatibility between HQ and lipid. Additionally, these thermograms indicate that HQ may be dispersed as amorphous and homogeneous states in the lipid matrix.

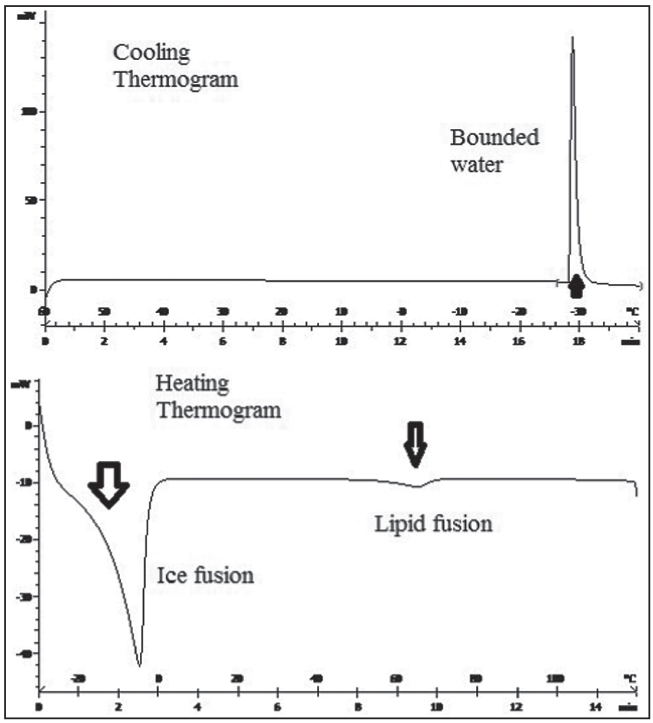

Figure 3: DSC cooling and heating thermograms of HQ-loaded NLC No 1 for evaluation of the thermal behavior of nanoparticles

The permeability of HQ-loaded NLCs through rat skin

The drug permeated quantities were determined for 28 h period. Various permeated factors such as 4 and 28 h (Q4 and Q28), flux (J), and permeability coefficient (P) were measured [Table 6].

The maximum and minimum amounts of Jss were 2.76 and 0.87 (mg/cm^2.h) belonging to formulations 1 and 8, respectively. Jss of control was 4.12 and significantly more than all NLC formulations. On the other hand, the maximum and minimum values of Q4 were 7.07 and 2.2 mg that are belonging to formulations 1 and 2, respectively. All formulations have a significantly lower value of Q4 in comparison to HQ-S (P = 0.001). All formulations decreased drug permeation through rat skin and formulation 8 demonstrated the highest effects.

All independent variables had a significant and direct effect on Q4. Surfactant mainly is affected on drug loading in NLCs and transcotul is a dose-dependent permeation enhancer. On the other hand, the maximum and minimum values of Q28 were 49.6 and 15.6 according to formulations 1 and 8 respectively, which were the same as those of Q4. The independent variables such as S/L ratio and transcotul percentage have a significant and direct correlation on the drug permeated after 28 h. All formulations have a significantly lower value of Q4 in comparison to control (P = 0.009). Salimi *et al.* performed an *in vitro* study based on HQ-loaded microemulsion permeability through rat skin.[34] They reported that 90.5% of HQ released after 24 h and microemulsions increased HQ permeability 4 times more than an aqueous suspension of HQ. In the other study, HQ-loaded SLN was used to overcome insufficient skin penetration and instability of HQ.[35] In previous studies, they established almost 3 folds higher HQ accumulation in the skin and 6.5 folds lower HQ entrance to receiving section of Franz diffusion cell from HQ-loaded SLN hydrogel compared to HQ carbopol hydrogel. They deposited around 45% of topical applied HQ-loaded SLN into the skin. In the present study, we deposited maximum HQ deposition into the skin was around 80% that belonged to formulation 8. Therefore, the amount of HQ permeability through rat skin is mainly is dependent on drug carriers. HQ with low molecular weight and intermediate affinity to aqueous and oily phases presents good

Table 5: Transition temperature and enthalpy of hydroquinone loaded NLC

Formulation No.	Factorial design condition	Heating				Cooling	
		First transition		Second transition			
		ΔH(J/g)	T_m(^{0}c)	ΔH(J/g)	T_m(^{0}c)	T_m(^{0}c)	ΔH(J/g)
1	+ + +	90.19±10.33	−5±0.8	2.79±0.4	66±7.52	−29±3.5	98.21±10.74
2	+ + −	96.34±8.21	−7±0.5	6.54±0.37	70±6.17	−33±2.80	110.53±13.54
3	+ − +	76.34±7.7	−5±0.6	9.99±1.10	62±6.38	−34±3.20	115.21±12.67
4	− + −	60.66±7.4	−4±0.5	1.5±0.87	88±5.44	−30±2.77	57.32±4.93
5	− + +	19.01±2.21	−5±0.4	1.7±0.12	94±7.63	−27±2.85	63.77±5.82
6	+ − −	71.90±5.11	−7±0.5	2.22±0.15	63±7.14	−26±2.60	70.32±6.54
7	− − +	7.44±6.72	−10±0.6	5.96±0.38	69±6.27	−26±2.30	13.87±10.90
8	− − −	6.39±7.25	−4±0.5	1.11±0.11	96±7.33	−29±2.20	20.32±1.63

permeability through the skin. HQ is readily absorbed through the skin and can be detected in urine as the intact substances after a 2% cream application.[36] This evidence indicates the high permeability of HQ through the skin. Therefore, a better therapeutic effect of HQ can be achieved by more drug localization into the skin. In the present study, HQ localization is issued by NLCs.

Table 6: HQ permeated amount (mg) through rat skin after 4 and 28 h (mean±SD)

Formulation number	State in full-factorial design	Q4 (mg)	Q28 (mg)
1	+ + +	44.37 ± 3.7	128.6 ± 13.6
2	+ + −	39.7 ± 3.12	118.45 ± 9.9
3	+ − +	41.2 ± 5.5	122.2 ± 13.3
4	− − +	33. 5 ± 2.9	103.5 ± 7.2
5	− + −	26.72 ± 2.4	93.5 ± 10.1
6	+ − −	35.27 ± 3.2	116.7 ± 12.6
7	− + +	37. 2 ± 2.8	113.9 ± 9. 7
8	− − −	24.1 ± 2.2	90.6 ± 10.6
Control	-	71.8 ± 8.5	263.4 ± 20.9

Anti-hyperpigmentation property of HQ-loaded NLCs

NLC formulation 2 with the highest EE % (57.5%), desirable particle size (28 nm) and sustained release profile was selected for these experiments. Melanosomes degeneration, clumped melanosomes, and unmelanized melanosomes were evaluated as signs of depigmentation effects.[1] As shown in Figure 4A–D, NLC increased the amount of degenerated melanocytes and unmelanized melanosomes in comparison with other groups. On the other hand, HQ-loaded NLC decreased the formation of melanosomes more than HQ cream and placebo. The percentage of reduction in the melanosomes after treatment with NLC 2 and HQ cream was 71% and 23%, respectively. Therefore, NLC decreased the number of melanosomes more than HQ cream significantly. These effects of HQ-loaded NLC

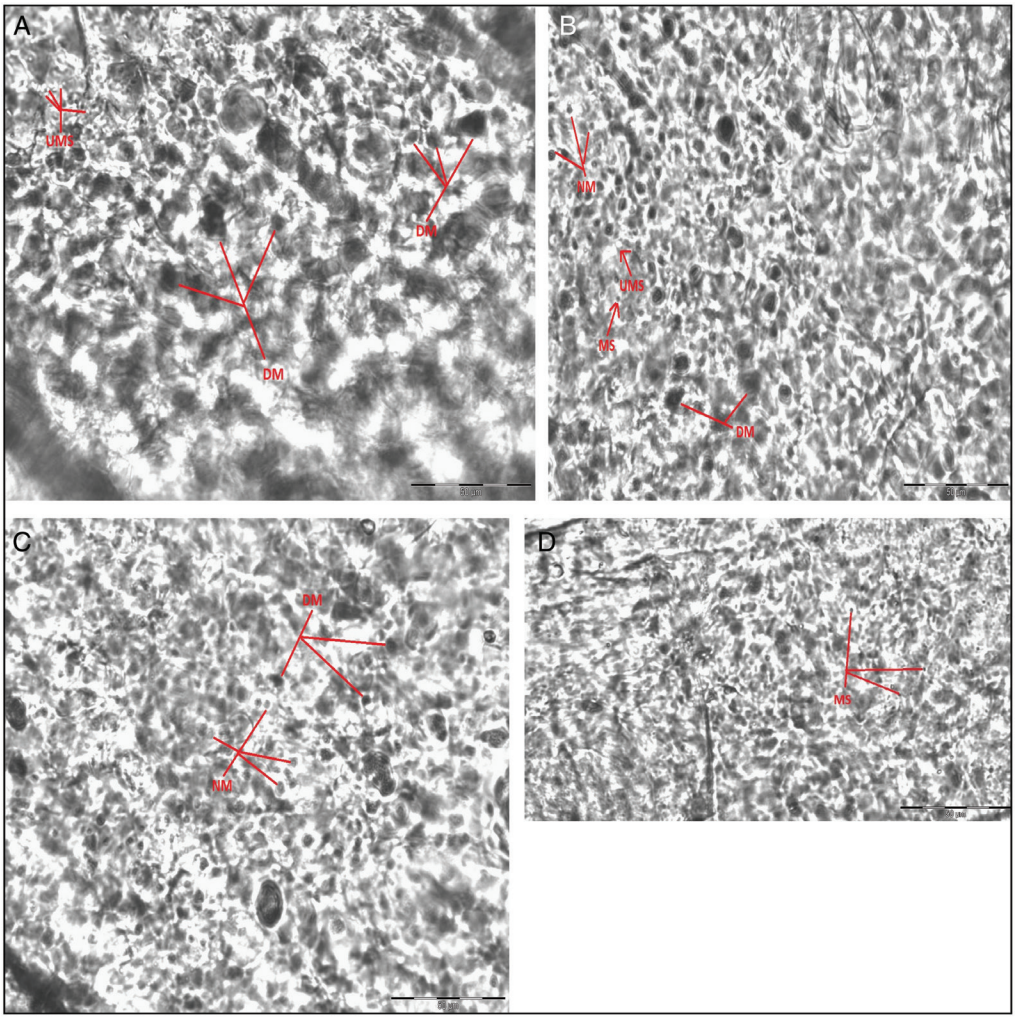

Figure 4: Optic microscopic pictures of skin samples 3 weeks after treatment with: (A) hydroquinone (HQ)-loaded nanostructured lipid carrier (NLC) formulation 2, (B) HQ trademark cream, (C) NLC placebo, and (D) H2O treated. DM, degenerating melanocytes; MS, clumped melanosomes; NM, normal-appearing melanocytes; UMS, melanosomes are unmelanized

have appeared after 3 weeks and no significant effect was seen after 1 and 2 weeks of treatment. Therefore, it seems that NLC increased HQ concentration into the epidermis significantly more than HQ trademark cream, placebo, and untreated skin that cases higher depigmentation effect. On the other hand, NLC with localization of HQ into the epidermis not only increased the depigmentation effect but also decreased HQ systemic absorption.

Stability of HQ-loaded NLCs

HQ-loaded NLCs did not show any significant changes in particle size and EE% after three months. These results demonstrate no nanoparticle fusion and lipid modification during storage conditions.

Discussion

HQ is an anti-hyperpigmentation compound with poor stability and its hydrophilic nature is a very important challenge in preparing effective topical formulations.[37] If HQ passes the skin it can demonstrate systemic absorption and lead to adverse effects such as nephrotoxicity and hepatotoxicity.[38] Human studies have indicated that topical bioavailability of HQ can assess to 45% after 24 h. The application of HQ topical formulations has been associated with ochronosis and other major side effects due to the carcinogenic potential of HQ.[39] Toxicity is related to high penetration of HQ because using of enhancers in topical formulations such as oleic acid.[40] Therefore the hypothesis followed by this work was the localization of HQ in the deep skin layers and increase its stability by loading in NLCs. The first step in drug permeation into the skin is partitioning from vehicle into the stratum corneum. This step is rate-limiting for HQ permeation through the skin as a hydrophilic compound. NLCs as lipid carriers resolved this limitation and increased HQ partitioning into the skin because they improve the dissolution of the hydrophilic drug in the lipid phase that was previously reported for some drugs such as Ketorolac.[41] The second step is diffusion through different skin layers. HQ showed sufficient diffusion through living epidermis that was associated with systemic toxicity.[38] NLCs with nano-size tightly adhered to the skin surface and released the HQ in a controlled manner. Thus lower HQ dose is needed for providing therapeutic response and reduction in local and systemic side effects can be achieved. This effect of NLCs was previously reported for Acitretin-loaded in NLCs.[42] Thus, NLCs with lipophilic nature demonstrate low partitioning into the living epidermis and release HQ in a slow manner into the living epidermis that in a result they can increase HQ localization into the deep layers of stratum corneum and near to the living epidermis. Ghanbarzadeh *et al.* used SLNs for HQ dermal delivery to overcome the insufficient skin penetration of HQ and its severe side effects as a result of systemic absorption.[35] They reported that the HQ-loaded SLNs with 86 nm particle size showed 3 times higher drug accumulation in the skin and 6.5 times lower drug entrance to receiving compartment of Franz diffusion cells compared with carbopol made hydrogel. In the present study NLCs demonstrated lower particle size (27–70 nm), higher loading capacity (20–30%) compared with SLNs, and showed 5 times higher HQ accumulation in the skin. Therefore it seems that NLCs can be introduced as novel and perfect HQ carriers for dermal delivery.

Conclusion

In this study, different NLC formulations of hydroquinone were prepared by a factorial design method for increasing drug localization in the epidermis. The results indicate that all of the selected NLC formulations have acceptable physicochemical properties, especially particle size, release behavior, percentage of entrapment efficiency, HQ permeated amount through rat skin after 4 and 28 h, and stability for topical use. The NLC samples had an average particle size range of 20.78–69.7 nm. The percentage of entrapment efficiency values of the NLCs were 32.9 and 57.5. It seems that cold homogenization method can be suitable for preparing NLC with proper particle size. The drug release profile showed that 81.39% of the drug was released from formulation No. 2 within the first 72 h of the experiment.

All formulations decreased drug permeation through rat skin and formulation No. 8 demonstrated the highest effects. The percentage of reduction in the melanosomes after treatment with formulation No. 2 and HQ cream was 71% and 23%, respectively. Therefore, NLC decreased the number of melanosomes more than HQ cream significantly.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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