Research Article

Nanostructured Lipid Carriers Improve Skin Permeation and Chemical Stability of Idebenone

Bei Li[1] and Zhi-Qiang Ge[1,2]

Abstract. Idebenone (IDB) is a synthetic antioxidant and analog of coenzyme Q10. The percutaneous permeation of IDB was investigated in guinea pig skin after application of different formulations. The enhancing effects of various formulations [nanostructured lipid carriers (NLCs), nanoemulsion (NE), or oil solution] on the permeation of IDB were evaluated using *ex vivo* guinea pig skins. Furthermore, stability of different formulations and in which chemical stability of IDB was determined during storage. Permeation experiments revealed that formulations varied in their ability to enhance the skin permeation of IDB. For NLC formulation, the cumulative amount of IDB in the epidermis, dermis, and acceptor medium of diffusion cells was approximately threefold more than NE or oil solution at the end of 24-h experiment. No significant difference between NE and oil solution was observed in the enhancement of penetration efficacy of IDB. Different formulations resulted in stability with different properties. NLC formulation revealed preferentially more stable than NE. The residual percentage of IDB loaded in NLCs, NE, and oil solution was 90.1%, 65.4%, and 51.3%, respectively, when stored at 40°C under 75% RH and 3,000 lx light conditions for 180 days. The results obtained here demonstrated that the abilities of NLCs to improve the chemical stability of IDB and enhance the skin permeation are much better than NE and oil solution. These suggest that NLCs containing IDB have significant potential use for skin care as an alternative topical formulation.

KEY WORDS: idebenone; nanostructured lipid carriers; skin permeation; stability.

INTRODUCTION

Idebenone (IDB) is a synthetic analog of coenzyme Q10 (CoQ10), which behaves as an antioxidant and free radical-scavenging molecule (1). The compound has been used to combat skin damage and aging. Currently, it is commercialized as an antiaging cosmetic (2). Compared with commonly known popular antioxidants in skin care products (vitamin C, vitamin E, alpha lipoic acid, kinetin, and coenzyme Q10), IDB is shown to be the most effective antioxidant in overall global assessment to prevent oxidative stress (3). As an antioxidant, the chemical stability of IDB is an essential guarantee for exerting desired antioxidant action. Unfortunately, IDB degraded by 60% and lost antioxidant activity by 30% exposed to a relative humidity of 75% and at 40°C during 45 days (4,5). For overcoming the disadvantages, a number of investigators designed formulations to protect the chemical stability of IDB. Palumbo *et al.* (5) encapsulated IDB with polyethyl-2-cyanoacrylate nanocapsules and obtained an improved antioxidant effect *versus* free drug. Amorim *et al.* (4) prepared the polymer nanoparticles based on chitosan loaded with IDB. The nanoparticles showed a tenfold increase of drug stability in comparison with the free drug and preserved antioxidant activity *in vitro*. Though the nanoparticles and nanocapsules provided effective protection for IDB stability, these formulations are still defective. For example, polyethyl-2-cyanoacrylate, up to date, has not been approved by the Food and Drug Administration of USA used for pharmaceutical excipients or drug carriers. The colloidal stability of the chitosan-based nanoparticles is still a major drawback for being a clinic therapeutic formulation because of the inherent physical and chemical properties of chitosan (derivatives) (6). Besides, the authors did not comment on whether chitosan (derivatives)-based nanoparticles aggregate or not. Moreover, the release percentages of IDB loaded in chitosan (derivatives) nanoparticles were higher than 50% after 24 h (4). This relatively fast release might be a manifestation of poor stability of the formulation. Therefore, it is useful to develop a novel formulation that can be applied to improve the chemical stability of IDB, minimize the drug leakage from the carrier systems, and delay the aggregation process of the carriers at storage period. Of course, it is also important that, as a cosmetic and pharmaceutical dermal formulation, the active ingredient of formulated products could efficiently penetrate into the skin and stay in the skin long enough to exert its desired effect.

Nanostructured lipid carriers (NLCs), lipid-based nanoparticles with a diameter usually less than 100 nm, are novel

[1] Department of Pharmaceutical Engineering, School of Chemical Engineering and Technology, Tianjin University, Education Ministry Key Laboratory of Systems Bioengineering, Tianjin 300072, People's Republic of China.

[2] To whom correspondence should be addressed. (e-mail: gezhiq@tju.edu.cn)

aaps®

drug delivery system for the delivery of lipophilic actives with high solubility, stability, powerful skin penetration, and low skin irritation (7). NLCs have been introduced for both pharmaceutical and cosmetic application, and more than 40 products are available on the cosmetic market (8). The advantages of NLCs over conventional delivery systems (liposomes, emulsions, polymeric nanoparticles, and solid lipid nanoparticles) include the protection of chemically labile compounds against chemical degradation, an increase of the loading capacity of the active compounds in the particles, a lower water content of the particle suspension and minimizing the expulsion of the active compound during storage (9). The smaller size of NLCs ensures a close contact to the stratum corneum and can increase the amount of the active compound penetrated into the skin. Being highly lipophilic, IDB is an excellent candidate for NLC encapsulation.

The aim of this study is to develop a carrier system that is able to improve the chemical stability of IDB and enhance the skin delivery of IDB. We would like to evaluate the stability improvement of the carrier systems by comparing the different formulations, *i.e.*, IDB-loaded NLCs, IDB-encapsulated nanoemulsion, and IDB-dissolved oil solution. The penetration-enhancing ability of these carrier systems was tested *ex vivo* using guinea pig skin as the model membrane. Investigation here will be useful for future application of IDB-loaded nanostructured lipid carriers.

MATERIALS AND METHODS

Materials

IDB was obtained from Shanghai Demo Chemical Co. Ltd. (China). Glyceryl palmito-stearate (Precirol ATO5) and medium chain fatty acid triglycerides (MCT), as solid lipid and liquid lipid, respectively, were gift samples from Gattefossé (Gennevilliers, France). Tween80 (Polysorbate 80) was purchased from Tianjin Jiangtian Chemical Technology Co. Ltd. (China). Soybean phosphatidylcholine (SPC) was purchased from Shanghai Sangon Co. Ltd. (China). Methanol (chromatographic pure) was purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained from a MilliQ Plus system, Millipore (Schwalbach, Germany).

Preparation of IDB-Loaded NLCs, NE, and Oil Solution

IDB-loaded NLC dispersions were produced by the modified high-shear homogenization and ultrasound method as previously reported (10). An optimized formulation was obtained by a great deal of single-factor prescription screening tests. The lipid and aqueous phases were prepared separately. The lipid phase consisted of glyceryl palmito-stearate (7% *w/v*) and MCT (3% *w/v*), emulsifier SPC (0.3% *w/v*), and IDB (0.5% *w/v*), while the aqueous phase consisted of Tween80 (2.4% *w/v*) and ultrapure water. Both phases were separately heated to 65°C for 15 min (temperature above melting point of the solid lipid). Under the same temperature, the hot water-surfactant solution was poured into the hot lipid phase and mixed using a high-shear homogenizer (FA25, Fluko, Germany), emulsifying at 10,000 rpm, 65°C for 5 min. Afterwards, the mixture was further treated using a probe-type ultrasonicator (Scientz JY88-IIN, China) for 5 min at 65°C. The resulting hot dispersions were filled in silanized glass vials and cooled to room temperature under ambient conditions to obtain the IDB-loaded NLCs. The total volume of the final product was 10 ml. In the case of NE, it was produced in the same manner with the same drug content (0.5% *w/v*), nonetheless, the solid lipid (glyceryl palmito-stearate) was replaced by MCT, and the amount of emulsifier increased greatly (5.8% *w/v*, SPC; 9.7% *w/v*, Tween80). The oil solution was obtained by directly dissolving IDB (0.5% *w/v*) in 10 ml MCT.

Characterization of IDB-Loaded NLCs and NE

Measurements of Size and Zeta Potential

The particle size and zeta potential was measured by a Malvern Zetasizer (Nano ZS ZEN3600, Malvern Instruments, UK) using a helium–neon laser with a wavelength of 633 nm. Malvern Zetasizer yields the mean particle size (z-ave) and the polydispersity index (PDI) which is a measure of the width of the size distribution. The z-ave and PDI values were obtained by averaging ten measurements at an angle of 173° in 1 cm diameter cells at 25°C with general purpose mode. A 1:50 dilution of all the formulations was made using ultrapure water to yield a suitable scattering intensity before the measurement. The zeta potential determination was based on the particle electrophoretic mobility in aqueous medium.

Morphology of IDB-loaded NLCs by Electron Microscopy

The morphology of IDB-NLCs was visualized by a field emission scanning electron microscope (S-4800, Hitachi, Japan), operating at an accelerating voltage of 5–30 kV. The samples were sputter coated with gold using a sputter coater (E-1045 ion Sputter, Hitachi, Japan). Prior to imaging, samples were diluted with ultrapure water, dropped on the copper specimen slice, and air-dried at room temperature.

Encapsulation Efficiency

Prior to filtration centrifugation, a volume of 0.5 ml of each samples was diluted with ultrapure water (1:20) to avoid deposition of free IDB (possibly crystallized in the aqueous phase) onto the NLC surface and thus measured as encapsulated. Diluted dispersion (0.5 ml) was then placed in the upper chamber of a centrifuge tube matched with an ultrafilter (Amicon Ultra, Millipore Co., USA, MWCO 50 kDa) and the unit was centrifuged at 10,000 rpm for 10 min. The aqueous dispersion medium containing the unloaded IDB was penetrated through the filter membrane into the sample recovery chamber. The amount of the unloaded IDB in the collected aqueous phase was detected by high-performance liquid chromatography (HPLC). As for the determination of the total drug loading in the dispersion medium, 0.5 ml IDB-loaded NLCs dispersion was dissolved in 9.5 ml methanol. After sonication for 10 min, the obtained suspension was centrifuged at 13,000 rpm for 10 min. The obtained supernatant was measured by HPLC. The resulting IDB content was regarded as the total amount of IDB first introduced.

The encapsulation efficiency (EE) could be calculated by the following equations:

$$EE(\%) = \frac{W_T - W_F}{W_T} \times 100\%$$

where W_T was the weight of total amount of IDB first introduced and W_F was the weight of unentrapped IDB in NLCs or NE dispersion, respectively.

Stability of Formulations During Storage

The long-term stability of NLCs and NE was investigated for 180 days. Prepared samples were respectively stored at three different conditions: 25°C in dark, 40°C in dark, and 25°C under daylight. Changes of the particle size, PDI, and zeta potential were detected during storage. To evaluate the possibility of IDB escaping from NLCs and NE, the encapsulation efficiency of NLCs and NE stored at 25°C in dark and under daylight was determined during storage.

IDB Determination

A reverse-phase-HPLC method was used to measure IDB concentration in the samples as previously reported (11). Detection wavelength was set at 278 nm. The mobile phase consisting of methanol and water (72:28 *v/v*) was pumped through the Waters μBondapak C18 column (10 μm, 3.9×300 mm) at a flow rate of 1.0 ml/min. All injections were performed at room temperature.

Chemical Stability of IDB

The evaluation of chemical stability of IDB loaded in NLCs, NE, and oil solution was carried out by comparing the amount of IDB incorporated in different formulations. Samples of the three formulations containing IDB were placed in a constant temperature and humidity box at temperature 40°C, a relative humidity of 75%, and intensity of illumination 3,000 lx for 180 days. The samples withdrawn at regular time intervals were extracted with methanol under sonication. The resulting solutions were detected for IDB by HPLC. The obtained results were expressed in residual percentage of the total amount of IDB first introduced.

Ex Vivo Skin Permeation Study

Skin Preparation

On the day of the experiment, guinea pigs (Experimental Animal Center of Tianjin Institute of Pharmaceutical Research, China) were selected as model animal. The animal experiment protocol was approved by the Animal Ethics Committee of Tianjin Institute of Pharmaceutical Research. Six guinea pigs used in the experiment are female, 8 weeks old, weighting 250±25 g. After anesthetized with ether, the guinea pigs were carefully shaved with razors to remove hairs and there was no visible change or red and swollen phenomenon of the skin. The dorsal skin composed of epidermis and underlying dermis was cut off from the animal surgically, and the adhering subcutaneous fat was carefully cleaned and washed out with phosphate-buffered saline (PBS) buffer (pH 7.0). To evidence the differences among the formulations, three parallel determinations were performed from the same donor for avoiding the variability of skin samples. Full-thickness skin was cut into approximately 2.4×2.4 cm pieces and there were totally 18 pieces of skins had been used in this study. The skins were then hydrated by dipping in 0.9% sodium chloride solution for 1 h to maintain sink condition before use.

Permeation Experiments

The permeation studies were performed in Franz-type diffusion cells (TT-6, Rightway Inc, Tianjin, China) with a diffusion area of 0.64 cm². The diffusion cells were mounted on a magnetic 6 stirrer and connected to a water bath at 32± 0.1°C. A dose of 0.3 ml of IDB-loaded NLC dispersion, IDB-encapsulated NE, and IDB-dissolved oil solution was applied on the skins, respectively. The excised skin was set in place with the epidermal side facing upward into the donor compartment and the dermis facing the acceptor compartment. The acceptor compartment of the cell was filled with 5.5 ml of 20% (*v/v*) ethanol in PBS (pH 7.4) to maintain sink conditions and to sustain permeant solubilization (12). Five hundred microliters aqueous of the acceptor medium was collected at 6 and 24 h and immediately replaced with equal volumes of fresh buffer. Then, the permeated amount of IDB was determined by extraction into a suitable solvent followed by HPLC analysis.

To further assess the effect of the different formulations on skin penetration, the amounts of IDB remaining in different layers of the skin were also determined at 6 h and at the end of the permeation study. The resulting skin was rinsed with PBS buffer (pH 7.0) and isopropanol and subsequently gently dried with a cotton swab to remove the residual donor sample. The epidermal layer of the skin dosing area was carefully separated from the dermis using the heat separation technique as described by Gillet *et al.* (13). The remaining skin was also collected separately and treated in a same manner as below: the skin was firstly cut into small pieces and extracted with the mixtures of methanol/chloroform (1:2 *v/v*) for 8 h due to the high solubility of both the drug and the intercellular lipids of epidermis (14). Samples were then homogenized with methanol using a tissue homogenizer and sonicated in an ultrasonic cell disruptor for 10 min. All the tissue samples were centrifuged at 13,000 rpm for 10 min. The supernatant was finally collected and analyzed for IDB content using HPLC.

Statistical Analysis

The data are reported as mean ± standard deviation (SD). The significance of the differences between formulations was tested using the Student's *t* test (Graph Pad Prism, version 4). $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISSCUSSION

Characterization of IDB-Loaded NLCs, NE, and Oil Solution

As shown in Table I, NLCs and NE are characterized by mean hydrodynamic diameters for 86.9±4.3 and 51.3±3.2 nm, respectively. PDIs are always lower than 0.3, indicating that

Table I. Parameters of NLC and NE Containing IDB

Formulations	z-ave (nm)	PDI	Zeta potential (mV)	EE (%)
NLC	86.9±4.3	0.21±0.04	−29.6±1.7	99.4±0.8
NE	51.3±3.2	0.27±0.02	−47.2±1.3	96.2±1.1

Each value represents the mean ± SD ($n=3$)
NLC nanostructured lipid carriers, *NE* nanoemulsion, *PDI* polydispersity index

the both NLCs and NE are homogeneous in size. Meanwhile, they show very good size reproducibility from batch to batch too. The scanning electron microscope imaging as shown in Fig. 1 is in good agreement with these results determined by photon correlation spectroscopy. Actually, the picture of NLCs shows very similar particles with a homogeneous size of ±80 nm.

Regarding the zeta potential values (Table I), both of the NLCs and NE possess a large negative charge and are therefore considered as an indication of good stability of the colloidal dispersions. Zeta potential values are about −30 mV for NLCs and −47 mV for NE. Exhibited smaller size and higher zeta potential value of NE than NLCs might be due to the application of larger quantities of SPC, whose anionic fractions are the origin of the negative charge of the emulsion, since greater ionization at the interface tends to increase the electrostatic repulsion (15).

Encapsulation efficiencies are reported in Table I. EE expresses the encapsulation efficiency as a function of the total drug concentration. Results are over 95% for each formulation. Such a high level of EE might be due to the high lipophilicity of IDB and the liquid state of MCT which is beneficial to encapsulate higher amount of drugs and reduce the particle crystallinity (16).

The stability of formulations was first evaluated by visual observations. We observed that NLCs and NE both revealed in homogeneous orange dispersions without sedimentation after 24 h, indicating that the dispersions were physically

Fig. 1. Scanning electron micrograph image of IDB-loaded NLCs. The IDB-loaded NLCs dispersion was diluted with ultrapure water in a ratio of 1:20 for image. The model of the scanning electron microscope (SEM) was s-4800, the accelerating voltage was 25 kV, and the images was captured under 40.0 k amplified times. Staff of 1.00 um at the left bottom of the figure indicated that the particle sizes were around 80 nm

stable. IDB-dissolved oil solution was also orange, clear, and transparent liquid. No drug crystal was found during storage.

Stability of Formulations During Storage

The stability of NLCs and NE was also evaluated by measuring their diameter and zeta potential after 30, 90, and 180 days of storage at 25°C, 40°C in dark, and 25°C under daylight. Meanwhile, changes of encapsulation efficiency were checked. The results are shown in Tables II and III.

As shown in Table II, the diameter and zeta potential of NLCs did not demonstrate large changes during 6 months from different storage conditions ($p>0.05$). Meanwhile, the PDI of the samples remained under 0.3, indicating a good physical stability of this colloidal system during 180 days of storage. When comparing the changes of parameters of NLCs and NE, a greater instability was observed for NE (Table III), especially for the samples stored at 40°C, a significant increase of the diameters and PDI was already detected after 30 days of storage ($p<0.01$). With increasing storage time, larger diameters appeared and the mean diameter increased tenfold over its initial and reached 550 nm at the end of 180 days of storage. This result could be due to the hydrogen bond break of surfactants induced by relatively high temperature, leading to a lower stability of NE (17). Concerning the samples stored at 25°C under daylight, the significant increase in diameter ($p<0.01$) delayed until 90 days of storage and the diameter grew about fivefold by the end of 180-day storage. No dramatic increase in diameter was observed for NE stored at 25°C in dark, but the change in diameter always existed during the storage period. Though the diameter of NE increased only onefold over its initial value, there is still statistical significance. Likewise, the PDI of NE stored at different conditions changed with time. PDI of NE only displayed a slight increase and remained under 0.3 after 180 days of storage at 25°C in dark ($p>0.05$), while a significant increase up to 0.35 was observed from the beginning 90 days under daylight condition (25°C) and maintained the level to the test end. By comparing with light, temperature has a more important influence on PDI of NE. The PDI of NE stored at 40°C had already increased to 0.35 only after 30 days of storage and never went down during the follow-up time. Changes regarding the zeta potential affected by temperature or light could be obtained from Tables II and III. No matter what conditions NE formulations were stored at, a sharp decrease of the absolute value of zeta potential was found by the end of 30 days of storage for all of them. Especially stored at 40°C, zeta potential of NE decreased about 37 mV after 180 days. At the same time, the zeta potential of NE stored at 25°C accompanied by light also fell by 32 mV. These results correlate well with the size analysis and also indicate an evident instability of the NE system. In contrast, smaller changes in zeta potential could be detected
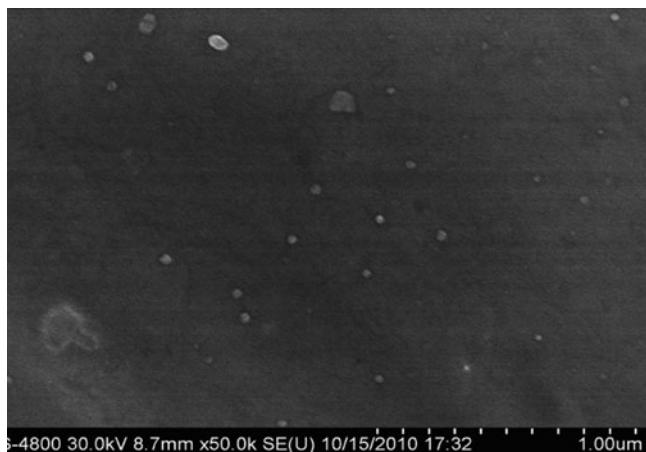
Table II. Change of Parameters of NLC During Storage

Storage time (days)	z-ave (nm)			PDI			Zeta potential (mV)			EE (%)	
	25°C	40°C	25°C, light	25°C	40°C	25°C, light	25°C	40°C	25°C, light	25°C, dark	25°C, light
30	90.3±4.4	81.5±3.2	91.4±2.9	0.19±0.04	0.22±0.05	0.19±0.05	−24.6±1.2	−23.3±1.5	−22.1±1.4	99.2±1.0	97.0±1.2
90	87.8±4.6	84.6±4.1	98.4±4.5	0.20±0.04	0.22±0.03	0.31±0.03	−25.4±0.9	−26.6±1.3	−30.1±1.7	98.4±0.8	93.3±1.4
180	86.7±3.8	84.4±4.7	106.8±6.6	0.20±0.02	0.20±0.03	0.25±0.04	−26.4±1.5	−28.5±1.1	−30.2±1.8	97.1±1.1	90.4±1.7

Each value represents the mean±SD (*n*=3)
PDI polydispersity index, *EE* encapsulation efficiency

Table III. Change of Parameters of NE During Storage

Storage time (days)	z-ave (nm)			PDI			Zeta potential (mV)			EE (%)	
	25°C	40°C	25°C, light	25°C	40°C	25°C, light	25°C	40°C	25°C, light	25°C, dark	25°C, light
30	52.9±3.2	96.4±2.9	52.9±2.6	0.24±0.06	0.32±0.04	0.24±0.04	−37.4±0.9	−27.3±1.5	−35.3±1.4	90.9±0.9	84.7±1.6
90	78.1±2.7	222.8±5.9	157.6±4.3	0.25±0.04	0.38±0.02	0.33±0.01	−25.3±1.7	−20.5±1.2	−23.2±1.2	81.3±1.0	71.2±1.9
180	102.3±4.3	552.4±5.7	321.3±5.1	0.30±0.05	0.42±0.03	0.38±0.05	−17.2±1.4	−10.3±1.3	−15.3±1.4	67.2±1.2	52.4±1.4

Each value represents the mean ± SD (*n*=3)
PDI polydispersity index, *EE* encapsulation efficiency

for NLCs during the test period regardless of the storage conditions (Table II). Statistical analysis did not show any significant inter-group difference in the zeta potential of NLCs among the three groups including 25°C, 40°C, and 25°C combined with daylight ($p > 0.05$).

By comparing Tables II and III, the encapsulation efficiency of freshly prepared NLCs and NE was comparable to each other (99.4% for NLCs, 96.2% for NE). But with the storage time went on, EE of NE stored at 25°C fell rapidly whether it was in dark or under daylight. The EE of NE had already declined to 67.2% (25°C, dark) and 52.4% (25°C, daylight) at the end of 180-day storage, respectively. Such pronounced decrease of EE may be related to the use of large quantities of surfactants in the NE formulation. NE is a dispersion of oil and water stabilized by an interfacial film of surfactant molecules whose hydrogen bond was likely to break during long-term storage, leading to the breaking of NE, thus a lower retention of the drug. In addition, the liquid character of oil droplets in o/w emulsions allows the active ingredients to partition between oil phase and water. IDB is likely to degrade in the water phase, re-partitions into the oil phase, and being replaced in the water phase by non-degraded IDB diffusing from the oil into the water phase. In contrast to this, NLCs consist of a mixture of solid lipid and the blend is also being solid at body temperature which leads to a very slow exchange between the solid particle phase and the external water phase (diffusion law by Einstein). As expected, there was no drastic decrease in EE of NLCs when tested under the same conditions. NLCs conditioned in dark showed only a slightly decrease ($p > 0.05$). Although it was relatively obvious ($p < 0.05$) that NLCs decreased in EE under the daylight compared to the initial value, EE above 90% after 180 days of storage was still acceptable. Formulations exposed to light showed some instability, which may attribute to the increased kinetic energy of the system caused by the light, thus resulting in the collision of particles to accelerate, and the probability of drug escaping from nanoparticles increased (18). The results of this study suggest that nanostructured lipid carrier is a way to enhance the stability of formulation. Different formulations result in different stability properties. NLC formulation reveals preferentially more stable than NE.

For the physical stability of oil solution containing IDB, the characteristics of oil solutions as a function of particle size, PDI, zeta potential, and encapsulation efficiency are unfeasible. The evaluation of the stability of oil solution has to be observed by visual inspection. Oil solutions containing IDB were always orange and bright. There was not any phenomenon of IDB recrystallization during 180-day storage, whatever if they were stored at 20°C or 40°C, under darkness or daylight.

Chemical Stability of IDB Entrapped in NLCs, NE, and Oil Solution

In order to investigate the improvement of the chemical stability of IDB after encapsulation, the residual percentage of IDB loaded in the different formulations was studied at 40°C under 75% RH and 3,000 lx light conditions for 180 days. Figure 2 is the residual percentage profiles of the IDB. It can be found that the stability of IDB was greatly improved by encapsulated in NLCs. IDB loaded in NLCs showed a
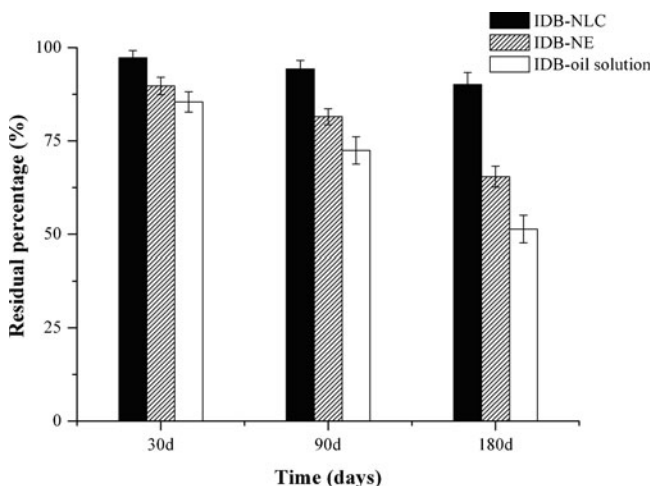

Fig. 2. Chemical stability of IDB entrapped in NLCs, NE, and oil solution stored at 40°C under 75% RH and 3,000 lx light conditions for 180 days. The residual percentage of IDB from NLCs (*black bar*), NE (*black and white bar*), and oil solution (*white bar*) was detected by HPLC after 1, 30, 90, and 180 days of production. Each value represents the mean ± SD ($n = 3$)

slight decomposition after 180 days of storage. The residual percentage was 90.1±3.2%. Comparatively, the residual percentage of IDB loaded in NE and oil solution declined gradually with time. After 180 days of storage, the residual percentage of IDB loaded in NE and in oil solution was 65.4±2.8% and 51.3±3.7%, respectively. Such a result could be interpreted in light of the occurrence of IDB decomposition in oil solution. IDB degradation induced by exposure to the light, heat, and humidity has been reported (4). Nanostructured lipid carrier represents a used strategy for protecting IDB against the harmful effects. NLCs have an imperfect matrix structure based on a mixture of solid and liquid lipid. MCT, as liquid lipid, possesses a higher solubility for IDB than solid lipids and is incorporated into the core of a solid lipid. The drug is probably in the liquid lipid which in turn is surrounded by the solid lipid. This provides some degree of mobility to the drug which contributes to stability even when the solid lipid undergoes polymorphic changes (19). A reduction in IDB expulsion from the disordered nanocompartments within the solid matrix avoids IDB degradation and maintains the residual percentage of IDB at relatively high level. In fact, it has been demonstrated that incorporation in lipid particles can effectively enhance the stability of drugs which are highly susceptible to light and humidity (20).

Ex Vivo Skin Penetration Studies

The *ex vivo* penetration of IDB into guinea pig skin treated with NLCs, NE, and oil solution was studied with Franz-type diffusion cells. Figure 3 depicts the cumulative amount of the compound in the epidermis, dermis, and acceptor medium of diffusion cells. During the first 6 h (left part in Fig. 3), the amount of IDB in epidermis treated with NLCs was approximately twice of that with NE or oil solution ($p < 0.05$), showing that incorporation of NLCs significantly enhanced the penetration of IDB compared with NE and oil solution. NE and oil solution revealed a similar drug content in the epidermis without statistical significance ($p > 0.05$). The
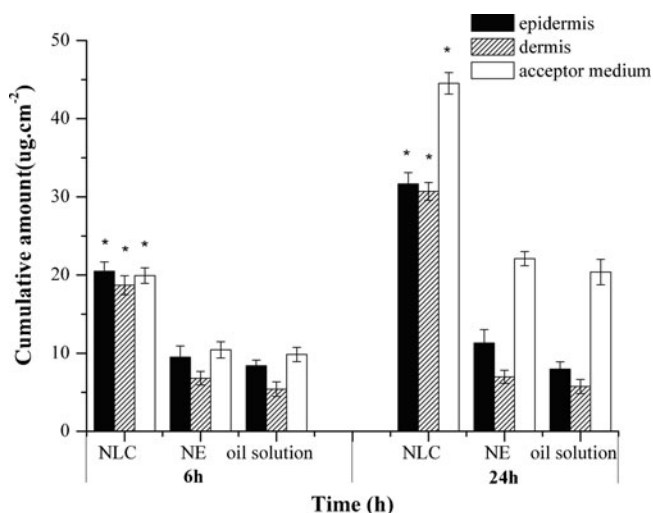
Fig. 3. *Ex vivo* skin permeation study of IDB-loaded NLCs, NE, and oil solution dispersions after applying for 6 h (*left*) and 24 h (*right*). Distribution of IDB in the skin (epidermis, *black bar*; dermis, *black and white bar*) and in the acceptor medium (*white bar*). Each value represents the mean ± SD (*n*=3). *$p<0.05$

accumulation amount of IDB in the dermis relied on the type of formulations and decreased in an order: NLCs, NE, and oil solution. This means that NLCs pass through epidermis and permeate dermis more easily than NE and oil solution. Moreover, cumulative amount of IDB in NLCs acceptor medium was also the highest ($p<0.05$) among the three formulations, with no significant difference between NE and oil solution ($p>0.05$).

After 24 h of experiment, the penetration of IDB from each formulation was shown in the right part of Fig. 3. When applied with NLCs, the accumulation amount of IDB in the epidermis, dermis, and acceptor medium all increased dramatically, but the growing tendency worked in compliance with the result of the first 6 h. The statistical difference among the three formulations at the end of 24 h was similar to results of the 6-h experiment, except the difference between NE and oil solution in epidermis. The amount of IDB in the epidermis was significantly higher for NE as compared to oil solution ($p<0.05$), but was still significantly lower than NLCs ($p<0.05$). When applied with NE and oil solution, respectively, the accumulation amount of IDB both in the epidermis and the dermis does not strikingly change compared to that of the end of 6-h test. Nevertheless, the amount of IDB in acceptor cells of both NE and oil solution increased by one time from 6 to 24 h.

IDB is a synthetic analog of CoQ10. The IDB *per se* lacks antioxidant activity *in vitro* as quinine form (21). It possesses an antioxidant activity only when it was converted to the hydroquinine form (IDBH$_2$) by intracellular CoQ10 and CoQ1 reductases. The purpose of the designed formulation is that more IDB molecules could penetrate through the epidermis and go into deeper layers to exert antioxidant activity for skin care after being applied to the topical skin.

At present, there are still two different opinions on the penetration ability of topically applied nanoparticles into and through the skin. In general, one opinion is that nanoparticles do not penetrate the stratum corneum into the epidermis (22). Another opinion is that nanoparticles can penetrate the stratum corneum through the epidermis into the dermis (23). The

existence of contradictory results could be due to the differences in experimental setting, type of skin, donor species, evaluation of skin integrity, nanoparticle type and dimensions, materials composed of nanoparticles, and investigation techniques. As drug vehicles, unlike industrial materials, topically applied nanoparticles should be paid more attention to the ability to protect drugs and delivery drugs to the target site rather than the final localization of nanoparticles itself, though the latter is also important for nanotoxicology and nanomaterial safeness. So far, the strict mechanism with the skin penetration of nanoparticles has not yet been clarified, but investigators have reached consensus on the routes of nanoparticle penetration through the skin. There are two routes. One is the appendageal route including the hair follicles, pilosebaceous, and sweat gland pores. Another is the intercellular route that is the lipidic matrix between the dead corneocytes in the stratum corneum. Owing to the hair follicle represents an invagination of the epidermis extending deep into the dermis, which results in a greater actual area for drug absorption (24). The hair follicle has become the most important penetration pathway for nanoparticles among the appendageal routes in recent years (25). Furthermore, the hair follicles represent an efficient reservoir for topically applied nanoparticles, which is usually extended deep into the tissue up to 2,000 μm (26). Lademann *et al.* (27) found that nanoparticles penetrate efficiently into the hair follicles, reaching deeper functional structures, where they can be stored for 10 days. However, in the case of non-particle form of the formulations as a control, such a long-term storage effect or such a deep penetration into the hair follicles behavior cannot be observed. In the present study, penetration of NLCs through guinea pig skin was twice or threefold higher than NE and oil solution, which was consistent with previous observation (25,28). This is partly associated with peculiar property of the nanoparticles, which can penetrate excellently into the hair follicles, reaching deeper functional structures and can be stored there for a long time (26). Another part of the reason could be that NLCs are composed of a mixture of lipids and surfactants, the latter can enhance disruption of lipid bilayers and keratin denaturation in stratum corneum, which results in drug-loaded NLCs passive permeation across the skin via the intercellular route (29). Alvarez-Roman *et al.* (22) once studied the distribution and skin permeation ability of non-biodegradable polystyrene nanoparticles labeled with fluorescein 5-isothiocyanate (FITC) using confocal laser scanning microscopy. They found that nanoparticles accumulated preferentially in the hair follicles and furrows that demarcate clusters of corneocytes. As controlled examination, subsequent to the application of a saturated FITC solution, localization of FITC in skin furrows and the hair follicles was not found from the surface images. Though the authors considered that polystyrene nanoparticles did not penetrate across the stratum corneum, they clearly showed that the nanoparticle systems preferentially accumulate in the hair follicles and skin furrows. In the present study, NE did not appear to promote IDB penetration through guinea pig skin with high efficiency. A part of the reason is that NE, which is dispersed oil droplet, not as solid nanoparticles or NLCs, does not have the advantage of sufficient accumulation in the hair follicles. Another part of the reason may be due to the higher concentration of the surfactants (Tween80 concentration of NE and NLCs are 9.7% and 2.4% *w/v* in formulations, respectively), which interact with the lipids to form oil droplets that enwrap IDB in its core.

Formation of the oil droplets not only occupies a large amount of the surfactants resulting in ability insufficiency to decompose lipid bilayers and keratin in stratum corneum, but also decreases the thermodynamic activity of the drug and the driving force of the drug absorption. Finally, these influence the efficacy of NE to facilitate drug penetration into the skin (29).

Oil solution applied in present study is only composed of MCT and IDB and does not have a geometrical shape. So it possesses neither the geometrical shape advantages of NLCs nor the disruption ability of the surfactant for lipid bilayers and keratin in the stratum corneum. But the results obtained here showed that IDB-dissolved oil solution formulation was also able to penetrate the skin through the stratum corneum, reaching the epidermis, the dermis, and acceptor cells. This can be explained by the physicochemical properties of IDB. IDB is a benzoquinone compound with a pKa of 15.19, a low molecular weight of 338.4, and calculated partition coefficient (octanol/water) of 3.49. These physicochemical properties can make it penetrate the skin passively (30).

CONCLUSION

The purpose of this study was to assess the feasibility of using NLCs to improve the stability and skin permeation of IDB. Through a comparative study on the three formulations, NLCs were found to achieve a significant improvement with respect to the chemical stability of IDB, skin permeation, and formulation stability compared to NE and oil solution. The results obtained in this study indicated that IDB-loaded NLCs could potentially be exploited as a novel IDB carrier for topical applications.

REFERENCES

1. Mcdaniel DH, Neudecker BA, Dinardo JC, Lewis JA, Maibach H. Clinical efficacy assessment in photodamage skin of 0.5% and 1.0% idebenone. J Cosmet Dermatol. 2005;4:167–73.
2. Farris P. Idebenone, green tea, and Coffeeberry® extract: new and innovative antioxidants. Dermatol Ther. 2007;20:322–9.
3. McDaniel DH, Neudecker BA, DiNardo JC, Lewis JA, Maibach HI. Idebenone: a new antioxidant—Part I. Relative assessment of oxidative stress protection capacity compared to commonly known antioxidants. J Cosmet Dermatol. 2005;4:10–7.
4. Amorim CM, Couto AG, Netz DJA, de Freitas RA, Bresolin TMB. Antioxidant idebenone-loaded nanoparticles based on chitosan and N-carboxymethylchitosan. Nanomedicine. 2010;6:745–52.
5. Palumbo M, Russo A, Cardile V, Renis M, Paolino D, Puglis G, Fresta M. Improved antioxidant effect of idebenone-loaded polyethyl-2-cyanoacrylate nanocapsules tested on human fibroblasts. Pharm Res. 2002;19:71–8.
6. Amidi M, Mastrobattista E, Jiskoot W, Hennink WH. Chitosan-based delivery systems for protein therapeutics and antigens. Adv Drug Deliv Rev. 2010;62:59–82.
7. Müller RH, Petersen RD, Hommoss A, Pardeike J. Nanostructured lipid carriers (NLC) in cosmetic dermal products. Adv Drug Deliv Rev. 2007;59:522–30.
8. Obeidat WM, Schwabe K, Müller RH, Keck CM. Preservation of nanostructured lipid carriers (NLC). Eur J Pharm Biopharm. 2010;76:56–67.
9. Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev. 2001;47:165–96.
10. Chen CC, Tsai TH, Huang ZR, Fang JY. Effects of lipophilic emulsifiers on the oral administration of lovastatin from nanostructured lipid carriers: physicochemical characterization and pharmacokinetics. Eur J Pharm Biopharm. 2010;74:474–82.
11. Artuch R, Colomé C, Vilaseca MA, Aracil A, Pineda M. Monitoring of idebenone treatment in patients with Friedreich's ataxia by high-pressure liquid chromatography with electrochemical detection. J Neurosci Meth. 2002;115:63–6.
12. Fang JY, Fang CL, Liu CH, Su YH. Lipid nanoparticles as vehicles for topical psoralen delivery: solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC). Eur J Pharm Biopharm. 2008;70:633–40.
13. Gillet A, Compre P, Lecomte F, Hubert P, Ducat E, Evrard B, et al. Liposome surface charge influence on skin penetration behaviour. Int J Pharm. 2011;411:223–31.
14. Junyaprasert VB, Teeranachaideekul V, Souto EB, Boonme P, Müller RH. Q10-loaded NLC versus nanoemulsions: stability, rheology and in vitro skin permeation. Int J Pharm. 2009;377:207–14.
15. Driscoll D. Lipid injectable emulsions: pharmacopeial and safety issues. Pharm Res. 2006;23:1959–69.
16. Müller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev. 2002;54:131–55.
17. Peltola S, Saarinen-Savolainen P. Microemulsions for topical delivery of estradiol. Int J Pharm. 2003;254:99–107.
18. Freitas C, Müller RH. Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLN™) dispersions. Int J Pharm. 1998;168:221–9.
19. Pathak P, Nagarsenker M. Formulation and evaluation of lidocaine lipid nanosystems for demal delivery. AAPS PharmSciTech. 2009;10:985–92.
20. Tursilli R, Casolari A, Iannuccelli V, Scalia S. Enhancement of melatonin photostability by encapsulation in lipospheres. J Pharmaceut Biomed. 2006;40:910–4.
21. Mordente A, Martorana GE, Minotti G, Giardina B. Antioxidant properties of 2,3-dimethoxy-5-methyl-6-(10-hydroxy-decyl)-1,4- benzoquinone (Idebenone). Chem Res Toxicol. 1998;11:54–63.
22. Alvarez-Roman R, Naik A, Kalia YN, Guy RH, Fessi H. Skin penetration and distribution of polymeric nanoparticles. J Control Release. 2004;99:53–62.
23. Rouse JG, Yang J, Ryman-Rasmussen JP, Barron AR, Monteiro-Riviere NA. Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin. Nano Lett. 2007;7:155–60.
24. Singh P, Sihorkar V, Jaitely V, Kanaujia P, Vyas SP. Pilosebaceous unit: anatomical considerations and drug delivery opportunities. Ind J Pharmacol. 2000;32:269–81.
25. Lademann J, Richter H, Schanzer S, Knorr F, Meinke M, Sterry W, et al. Penetration and storage of particles in human skin: perspectives and safety aspects. Eur J Pharm Biopharm. 2011;77:465–8.
26. Toll R, Jacobi U, Richter H, Lademann J, Schaefer H, Blume-Peytavi U. Penetration profile of microspheres in follicular targeting of terminal hair follicles. J Invest Dermatol. 2004;123:168–76.
27. Lademann J, Richter H, Teichmann A, Otberg N, Blume-Peytavi U, Luengo J, et al. Nanoparticles—an efficient carrier for drug delivery into the hair follicles. Eur J Pharm Biopharm. 2007;66:159–64.
28. Nam SH, Ji XY, Park JS. Investigation of tacrolimus loaded nanostructured lipid carriers for topical drug delivery. Bull Korean Chem Soc. 2011;32:956–60.
29. Nokhodchi A, Shokri J, Dashbolaghi A, Hassan-Zadeh D, Ghafourian T, Barzegar-Jalali M. The enhancement effect of surfactants on the penetration of lorazepam through rat skin. Int J Pharm. 2003;250:359–69.
30. Bos JD, Meinardi MMHM. The 500 Dalton rule for the skin penetration of chemical compounds and drugs. Exp Dermatol. 2000;9:165–9.