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EFFECT OF INCORPORATING CERAMIDE IN NIOSOMES FOR TRANSDERMAL DELIVERY OF LIDOCAINE AND FLURBIPROFEN

Hadir F. Marei*, Mona F. Arafa, Ebtessam A. Essa and Gamal M. El Maghraby

Department of Pharmaceutical Technology, College of Pharmacy, University of Tanta, Tanta, Egypt.

***Corresponding Author: Hadir F. Marei**

Department of Pharmaceutical Technology, College of Pharmacy, University of Tanta, Tanta, Egypt.

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ABSTRACT

The permeation abilities and physical characters of niosomes can be altered by modulating the composition of vesicular membrane. Ceramides can be incorporated into the niosomal membrane altering the transdermal delivery of the entrapped drug. Accordingly, the objective of this study was to investigate the effect of incorporation of ceramides in niosomes on transdermal delivery of two model drugs (flurbiprofen and lidocaine). Niosomal formulation comprising Span 60 with cholesterol (4:1, w/w) were used with ceramide being incorporated in the vesicles (4:1:1, w/w) and saturated aqueous solution of drug serving as a control. The drug entrapment efficiency of all the tested formulation was monitored and skin permeation studies were conducted before and after incorporation of ceramides. The entrapment efficiency of flurbiprofen and lidocaine after 8 hours was 98.25% and 85.67% respectively. Niosomal formulation enhanced skin permeation of flurbiprofen but hindered permeation of lidocaine. Incorporation of ceramides into niosomes did not have any significant effect on entrapment efficiency nor skin permeation at the tested concentration.

KEYWORDS: Ceramide; Niosomes; Transdermal delivery; Lidocaine; Flurbiprofen; Entrapment efficiency.

1. INTRODUCTION

Despite of the advantages of transdermal drug delivery over other routes of administration, the penetration of most drugs is hindered by the barrier nature of the skin.[1] Many studies have investigated many penetration enhancing techniques trying to disrupt skin barrier and facilitate delivery of drugs into and through skin after topical administration. The most commonly used approaches include utilization of chemical penetration enhancers[2] and physical enhancement techniques which employs electric current, ultrasonic perturbation and also microneedling.[3,4,5] Supersaturated formulations were also reported to affect rate of drug input through the skin by influencing the drug chemical potential.[6] Microemulsion and self microemulsifying systems were also reported as promising alternatives for transdermal delivery of different model drugs.[7,8] Nanostructures have been drawing great attention for the last few years for enhanced transdermal drug delivery. These nanostructures can be in the form of lipid vesicles in which an aqueous component is entrapped in one or multiple lipid bilayers or may be in the form of solid lipid or polymeric nanoparticles. The vesicular systems encompass liposomes as the prototype which can be modified to transfersomes, ethosomes or niosomes. The pro-concentrates of such systems are being employed as well.[9,10,11,12,13,14,15] Mezei and Gulasekharam reported altered/increased skin deposition of model drug after being encapsulated with a liposome. This publication was the first to investigate efficacy of liposomes as a drug carrier appearing at 1980. Further investigations were made studying and modulating the composition of these systems trying to optimize transdermal drug delivery through these carriers. Surfactants (also called edge activators) were incorporated into vesicular membrane of liposomes resulting in a highly deformable vesicles or transfersomes that were claimed to invade the intact skin resulting in enhanced transdermal drug delivery.[16] Besides increasing deformity of vesicles by addition of edge activator another type of vesicles called ethosomes were developed by incorporation of high ethanol content. This type of vesicles was reported to enhance transdermal drug delivery.[12] The development of surfactant-based vesicles (niosomes) as alternative to liposomes for enhanced transdermal drug delivery have been studied with the goal of cost minimization and enhancing the stability of the carrier.[17]

The rigidity of vesicular membrane can be tuned by modulating the composition of vesicular membrane.[18,19,20] Fluidization of vesicle membrane was reported to influence the transdermal drug delivery from vesicles. This fact is observed with both for liposomes as well as niosomes.[18,21,22]

Ceramides are one of the major lipids of stratum corneum which have been employed recently in formulation of different transdermal drug delivery systems. Various types of ceramides including ceramide 2, ceramide 3, ceramide 6 and even a synthetic analogue of ceramides have been utilized as a major (or a part of) lipid component in many nanostructured vesicles and solid lipid nanoparticles. A series of pseudoceramide based solid lipid nanoparticles were reported to effectively carry the hydrophobic apigenin agents and showed enhanced transdermal delivery compared to drug propylene glycol solution and drug loaded nanoemulsion.[23] Guar and co-workers.[24] developed ceramide 2 nanovesicles which showed enhanced physical stability against temperature and humidity compared to phosphatidylcholine vesicles. This was reflected as reduction of drug leakage from vesicles, a better drug entrapment and controlled release of drug over time. Ceramide2: palmitic acid solid lipid nano particles were reported to enhance transdermal delivery of curcumin among other solid lipid nanoparticles developed from other types of lipids. The author also suggested that presence of ceramide 2 may influence the phase behaviour and transdermal absorption from its formulation.[25] Ceramide was doped into surfactant based vesicular phospholipid systems causing a significant membrane tubulation with a deviation from the conventional spherical morphology to create tubulated vesicles called cerosomes. These cerosomes showed increased entrapment of tazarotene (model anti-psoriatic drug), reduction in its release while enhancing its deposition within the skin.[26]

Thus, incorporation of ceramide into the niosomal membrane may result in alteration of physical properties of niosomes which will be reflected as altered drug entrapment efficiency and permeation behavior.

Accordingly, the main objective of this study was to investigate the effect of incorporation of ceramides in niosomes on their transdermal delivering abilities. To achieve this, a niosome formulation containing Span 60 and cholesterol at weight ratio of 4:1 was prepared and compared with that containing ceramides. Flurbiprofen and lidocaine were utilized as model drugs and their transdermal permeability from the tested niosomal formulations was monitored and compared with their permeability from saturated aqueous solution.

2. MATERIALS AND METHODS

2.1. Materials

Flurbiprofen was provided by Kahira Pharmaceuticals and Chemical Industries, Cairo, Egypt. Lidocaine was supplied as a gift sample from Eva pharma, Cairo, Egypt. Sorbitan monostearate (Span 60) was obtained from Central Drug House (P) Ltd (Daryaganj, New Delhi, India). Ceramide 2 was supplies as a simple gift from Givaudan Co. (Argenteuil, France). Pharmaceutical grade ethanol, potassium dihydrogen phosphate, glycerol and cholesterol were purchased from El Nasr Pharmaceuticals Chemicals Co., Cairo, Egypt. Acetonitrile (HPLC grade) was purchased from Fisher Scientific UK, Loughborough, Lecis, United Kingdom.

2.2. Preparation of control and tested formulations

The control formulation for each drug was in the form of saturated aqueous solutions of either flurbiprofen and lidocaine each prepared with addition of excess drug crystals to maintain saturation. The prepared saturated solutions were incubated in a temperature-controlled water bath maintained at 32 ^{o}C for 72 hours to attain saturation.

The composition of the tested formulations is presented in Table 1. The lipid components (Span 60, cholesterol and ceramide (if any) were mixed with tested drug and ethanol then heated in a water bath (at 65^{o}C for 5 minutes) until formation of clear liquid. The aqueous phase then was added and mixed gradually while heating until complete dispersion. Further dilution with aqueous phase produces the tested niosomes. The developed niosomal dispersion was left at ambient temperature for swelling overnight and sonicated (Bransonic 3510E – MTH, Branson UL trasonics corporation, Danbury, USA) for 45 minutes before permeation expirements.[27,28]

Table 1: Summary of composition of tested formulation.

Formulation	Span 60	cholesterol	ceramide	ethanol	Aqueous phase
Plain niosomes	2.16 g	0.54 g	----------	3.24 g	3.24 ml
Ceramide containg niosomes	2.16 g	0.54 g	0.54 g	3.24 g	3.24 ml

***The aqueous phase is 0.1% w/w glycerol in water. The amount of flurbiprofen added to flurbiprofen formulation was 0.216 g. The amount of lidocaine added to lidocaine formulation was 0.207 g.**

2.3. High pressure liquid chromatography (HPLC)

Table 2 presents the experimental conditions for drug analysis. HPLC was employed for analysis of samples and quantification of the tested drugs. The HPLC equipment was of 1260 infinity type which was attached to a VWD1260 UV detector in addition to TCC 1260 automatic sampling device (Agilent technologies, Deutschland, Germany). Isocratic elution was performed using stationary phase made of C18 (150 mm×4.6 mm) column with particle size of 5 µm (Intersil®, GL Sciences Inc., Tokyo, Japan). The analysis operation was conducted using Agilent Open LAB ChemStation software. Quantification of flurbiprofen employed the available method (Alshaikh et al., 2019). For lidocaine the experimental conditions were optimized in house.

Table 2: Summary of the conditions of the HPLC methods of analysis of the tested drugs.

Drug	Mobile phase composition	Detection wave length (nm)	Flow rate (ml/min)
Lidocaine	20% Acetonitrile: 80% 20mM potassium di hydrogen phosphate buffer (pH adjusted to 3.3 using ortho phosphoric acid)	210	1
flurbiprofen	60% Acetonitrile: 40% filtered water (pH adjusted to 3.3 using ortho phosphoric acid)	257	1.1

2.4. Entrapment efficiency

The values of entrapment efficiency were calculated after separation of the free (unentrapped) drug by dialysis (27). Dialysis sacs (cellulose tubing, cut off 14,000 Daltons, Sigma-Aldrich, St.Louis, MO, USA) were filled by 2 ml of each niosomal dispersion. Each sac was then incubated in 100 ml of the receptor media used in permeation studies (30% v/v ethanolic solution). Sample of ethanolic dialysis fluid was then taken after 8 hours and analyzed for quantification of free unentrapped drug. The following equation is used for calculation of entrapment efficiency (%) in which Ct refers to the total amount of drug loaded in the dialysis sac and Cf is the amount of free drug quantified in the dialysate (29,30) (Table 3 and 4):

Entrapment efficiency (%) = [(ct -cf) / ct] × 100.

Table 3: Summary of entrapment efficiency and the calculated permeation parameters for flurbiprofen.

Drug formulation	Entrapment efficiency (%)		Flux ($\mu g\ cm^{-2}\ h^{-1}$)	Lag time (Hours)
	After 8 hours	After 10 hours		
Saturated aqueous solution of flurbiprofen	———	———	19.93 (±4.53)	1.53 (±0.25)
Flurbiprofen plain niosomes	98.25 (±0.03)	96.50 (±0.052)	27.216 (±6.04)	1.69 (±0.27)
Flurbiprofen ceramide niosomes	97.32 (±0.47)	94.56 (±0.96)	23.66 (±4.31)	1.58 (±0.04)

Table 4: Summary of entrapment efficiency and the calculated permeation parameters for lidocaine.

Drug formulation	Entrapment efficiency (%)		Flux ($\mu g\ cm^{-2}\ h^{-1}$)	Lag time (Hours)
	After 8 hours	After 10 hours		
Saturated aqueous solution of lidocaine	———	———	163.03 (±26.59)	0.51 (±0.25)
Lidocaine plain niosomes	85.67 (±1.01)	71.47 (±1.94)	58.89 (±6.32)	0.08 (±0.03)
Lidocaine ceramide niosomes	88.54 (±1.9)	77.32 (±3.62)	44.95 (±10.27)	0.28 (±0.04)

2.5. Preparation of skin samples

Freshly excised albino male rabbit ear skin was employed as skin model for this study. Other investigators have successfully utilized this model to test the transdermal permeation of various drugs. (12,7). Animal treatment and skin preparation were conducted taking into consideration the guidelines of the Ethical Committee of Faculty of Pharmacy, Tanta University (approval number, 1932019). The full thickness membrane of freshly excised rabbit ears was isolated from the inner side by gentle easing after cutting along the tips of the ears.

2.6. Skin permeation studies

These permeation studies employed vertical glass Franz diffusion cells with 14.5 ml capacity of receptor compartment and diffusional surface area of 2.27 cm^2. The skin was cut into pieces of suitable size and was mounted immediately on the diffusion cells with the stratum corneal side facing up and the dermal side facing the receptor compartment. The receptor compartment was filled with 30% v/v ethanol in distilled water which can maintain sink conditions throughout the study. The cells were then maintained in water bath with the temperature being adjusted to preserve the skin surface temperature at 32 +1 ^oC to mimic vivo conditions. The cells were left to equilibrate under these conditions for overnight after which the donor compartment was loaded by 2 ml of the tested formulation and covered with aluminum foil to provide occlusive conditions. Samples of the receptor fluid were collected at 1hrs, 2hrs, 3hrs, 4hrs, 6hrs, 8hrs, 10hrs and 24 hrs and were refilled with fresh receptor immediately after sampling to maintain constant volume. These samples were then analyzed for permeant using HPLC. The distribution of tested formulations and the corresponding control on the cells ensured that the same rabbit skin being used in both cases (31). The transdermal permeation parameters were computed from the penetration profiles which were plotted from the amounts of drug diffused as a function of time. Extrapolation of this line to the X axis will provide the lag time.

2.7. Statistical analysis

Statistical analysis was performed using Kruskal Wallis test with Tukey`s multiple comparison being employed as post hoc test to compare between means.

3. RESULTS AND DISCUSSION

3.1. Entrapment efficiency

The entrapment of drugs in niosomes were estimated after separation of the free drug by dialysis. The samples were incubated in the dialysate for 8 and 10 hours respectively and the entrapment efficiency was determined accordingly. recorded entrapment efficiency values of different formulations containing flurbiprofen and lidocaine are presented in Table 3 and 4. The amount of free drug was subtracted from initial amount added and the percentage of the drug entrapped relative to the initial amount of drug used in niosomes preparation was calculated to determine the entrapment efficiency. The average of calculated entrapment efficiency values of flurbiprofen in plain niosomes at 8 and 10 hours were 98.25% and 96.50% respectively (Table3). Flurbiprofen is a lipophilic drug with a log p value of 4.16 so the recorded high entrapment efficiency is expected as the drug would be entrapped mainly in the vesicular membrane. Similar results were reported by other investigators for entrapment of estradiol (model lipophilic drug) into vesicular systems.[19] Incorporation of ceramide into niosomal formulation didn't result in significant change in drug entrapment efficiency which may indicate that the drug can coexist with the ceramide within the vesicular membrane.

For lidocaine the average value of calculated entrapment efficiency in plain niosomes at 8 and 10 hours were 85.67% and 71.47% respectively. This high entrapment efficiency value was expected for lidocaine base with a log p of 2.4. The recorded significant reduction in entrapment efficiency of lidocaine after incubation for 10 hours compared to 8 hours incubation in reflects the rapid release of lidocaine from vesicles. This can suggest rapid loss of the entrapped drug while being applied on the donor compartment during skin permeation studies at 32 ^{o}C. The recorded entrapment efficiency after incorporation of ceramide into niosomes at 8 and 10 hours were 88.54% and 77.32% respectively. This slight increase in entrapment efficiency after ceramide addition was found to be nonsignificant (P>0.05) with the same significant reduction in entrapment efficiency of lidocaine after incubation for 10 hours compared to 8 hours incubation.

The recorded entrapment efficiency values for tested drugs indicate saturation of the vesicles with tested drug and presence of excess drug in the continuous aqueous phase thus ensure that the composition of the niosomes would be the only factor that cause any difference in transdermal drug delivery from different formulations.[15]

3.2. Skin permeation studies

Fig. 1 and 2 show the permeation profiles of flurbiprofen and lidocaine from their saturated aqueous solutions and tested niosomes. The permeation profiles were linear after a lag time which is expected when excess drug/formulation is applied with the receptor maintaining sink conditions. These steady state plots were used to calculate the permeation parameters which were used for comparison between drug permeation from its saturated solutions and corresponding niosomes. The calculated permeation parameters are presented in Table 3 and 4.

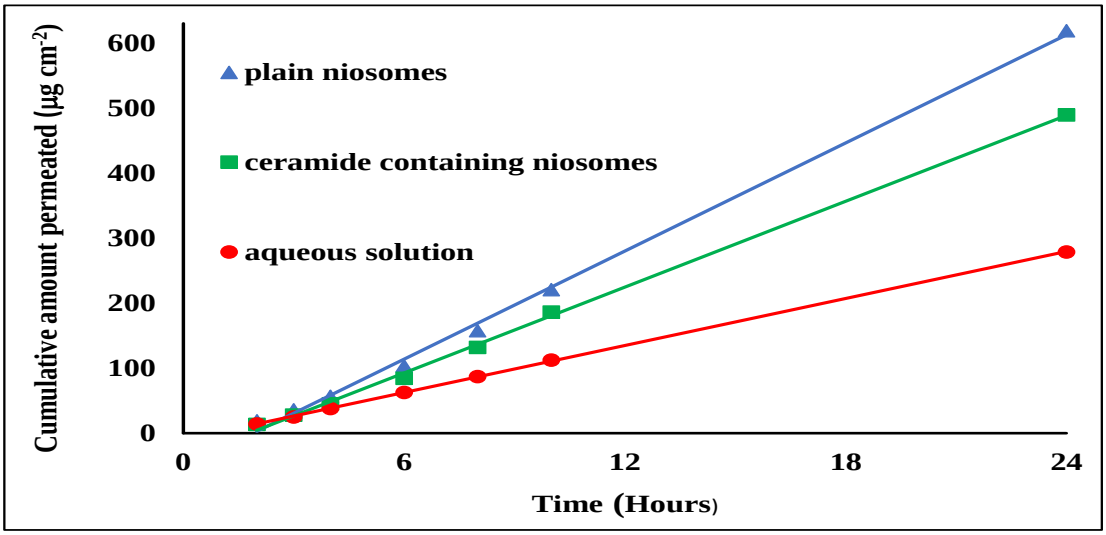

Fig. 1: Show the permeation profiles of flurbiprofen from its saturated aqueous solution, plain niosomes and ceramide containing niosomes.

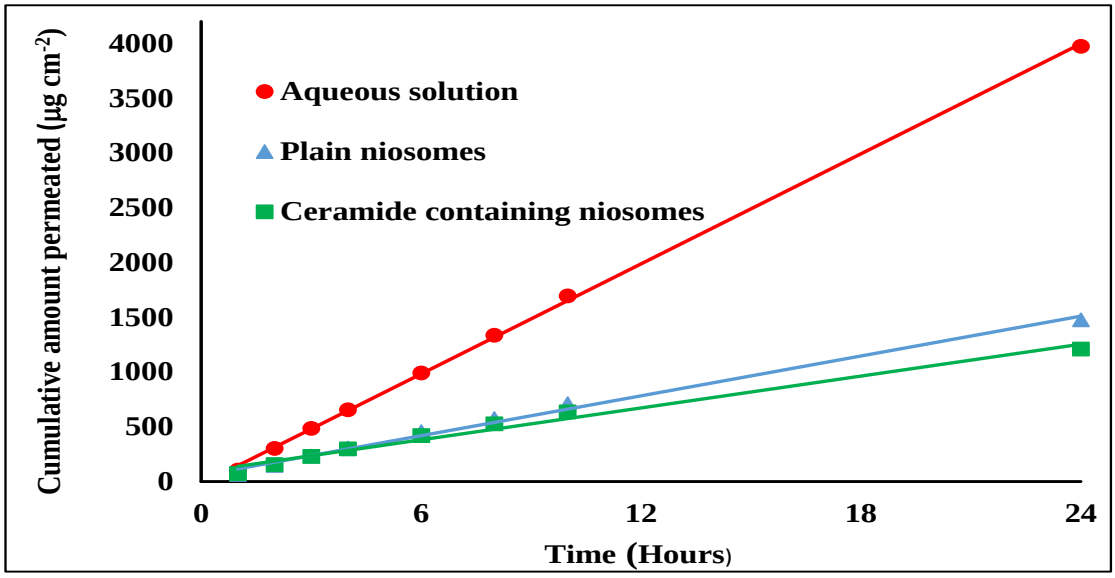

Fig. 2: Show the permeation profiles of lidocaine from its saturated aqueous solution, plain niosomes and ceramide containing niosomes.

Application of saturated aqueous solution of flurbiprofen on the full thickness rabbit skin produced transdermal flux of 19.93 µg cm^{-2} h^{-1}. Akhter and Barry reported similar flux value to that recorded in spite using human abdominal epidermal membrane in the permeation study (32). Regardless of composition all niosomal formulation enhanced transdermal delivery of flurbiprofen compared to saturated aqueous solution (Fig. 1and Table3).

Application of flurbiprofen in plain niosomes increased transdermal drug flux by 1.37-fold higher than saturated solution. This low enhancement value is expected due to rigid nature of formulation (Fig. 1 and Table 3).

The effect of incorporating ceramide into niosomal formulation on drug permeability was investigated. A slight non-significant reduction in flurbiprofen permeability was recorded after application of ceramide containing niosomes compared to plain niosomes (Fig. 1 and Table 3).

Application of saturated aqueous solution of lidocaine on the full thickness rabbit skin produced transdermal flux of 163.03 µg cm^{-2} h^{-1}. Similar steady state flux (140.6 ± 10.0 µg cm^{-2} h^{-1}) was reported for lidocaine from aqueous solution in spite using pig ear skin.[33] In contrast to flurbiprofen, all the tested niosomal formulation hindered transdermal delivery of lidocaine compared to saturated aqueous solution (Fig.2 and Table 4).

Application of lidocaine in plain niosomes produced an average transdermal flux of 58.89 µg cm^{-2} hr^{-1}. This flux is lower than that recorded after application of saturated aqueous solution of the drug (Fig.2 and Table 4). These results can be explained on the base of the expected fast liberation of entrapped lidocaine to the external aqueous phase. Unfortunately, the amount of drug liberated is far much lower than the saturated solubility of the drug in water. This can lead to sub-saturated solution with much lower thermodynamic activity compared to the corresponding saturated solution. This can explain the reduction in drug flux. The results of this study confirm the deleterious effect of rapid drug release from vesicular systems which is a documented problem in case of small molecular weight hydrophilic drugs.

The co-formulation of ceramide with niosomes revealed a trend of reducing lidocaine permeability (fig. 2 and table 4). However, this reduction in drug permeation was found to be not statistically significant ($P>0.05$).

In spite presence of many publications referring to positive effect of ceramide incorporation into vesicular structure either the ceramide-based vesicles or ceramide dopped vesicles.[25,26,34] the present study revealed that incorporation of ceramide into niosomal formulation did not have any significant effect on drug permeability and entrapment efficiency at tested ceramide concentration. The different lipid-based tested formulation among different publications may explain the controversial results recorded.

4. CONCLUSION

Incorporation of ceramide in niosomes did not alter the transdermal drug delivery of the entrapped drugs. The delivery of lipophilic drugs from niosomes was better than that of the hydrophilic molecule.

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