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Development of Naftifine Hydrochloride Alcohol-Free Niosome Gel

Heba S. Barakat, Inas A. Darwish, Labiba K. El-Khordagui, and Nawal M. Khalafallah

Faculty of Pharmacy, Department of Pharmaceutics, University of Alexandria, Azarita, Alexandria, Egypt

Marketed topical gels of the antifungal drug naftifine hydrochloride contain 50% alcohol as cosolvent. Repeated exposure to alcohol could be detrimental to skin. The aim of this study is to develop an alcohol-free niosome gel containing 1% naftifine hydrochloride. Niosomes were prepared and formulation variables were optimized to achieve maximum entrapment coupled with stability. Maximum drug entrapment and niosome stability entailed imparting a negative charge to the vesicles where entrapment efficiency reached 50%. Niosomes were incorporated into a hydroxyethylcellulose gel. The final gel contained a total drug concentration of 1% (wt/wt) half of which was entrapped in the niosomes. The results suggest the potential usefulness of the niosome gel.

Keywords niosomes; gels; naftifine hydrochloride; alcohol-free

INTRODUCTION

Naftifine HCl (Naf.HCl) is a synthetic, broad-spectrum antifungal agent and is among the first choice drugs for the treatment of dermatophytosis. The problem in formulating Naf.HCl is that the required concentration in the topical preparations exceeds its aqueous solubility. Attempts have been made to formulate the drug as creams, where formulation pH liberates the drug base, which is emulsified as an o/w cream. Alternatively, Naf.HCl has been solubilized using alcohol and Tween 80 and formulated in the form of a hydroalcoholic gel containing 52% (vol/vol) alcohol (MerzPharmaceuticals, 2004)

A major concern with such hydroalcoholic products is the clinical implication and consequence of repeated skin exposure to high alcohol concentration. The skin irritating potential of alcohol has been well investigated and documented (Bacchi-Modena, Bolis, Campagnoli, & De Cicco, 1997; Baumanna, Rath, Fischer, & Iffland, 2000; Tornier, Rosdy, & Maibach, 2006). Ethanol is able to penetrate normal skin within a few minutes via the sweat and sebaceous gland ducts, and transepidermally via the stratum corneum. The permeability constant of the human abdominal skin for ethanol is about (1.2×10^{-3} cm/h), a value comparable to that of water (1.0×10^{-3} cm/h) (Scheuplein & Ross, 1970). Skin alteration as a result of repeated alcohol application has been clinically observed, and includes dryness, desquamation, brown maculae, inhibition of hair growth, erythema, urticaria, papules, vesicles, and erosions. Histologically, hyperkeratosis, acanthosis, epithelial atypism, and mast cell degranulation were confirmed. Such adverse effects of alcohol are attributed to the carbon atoms of alcohol (C–OH), which yield free radicals leading to fast and violent reactions especially on vulnerable skin (Baumanna et al., 2000; Hess, Molinari, Gleason, & Radecki, 1991).

Treatment of topical fungal infections is often lengthy. In addition, the integrity of the skin is disrupted especially in conditions, such as athletes foot, characterized by maceration and fissuring of the interdigital skin. The application of a strong alcoholic formulation will be painful. Therefore, the formulation of Naf.HCl in an alcohol-free delivery system with better pharmaceutical attributes is of practical importance.

Niosomes are closed bilayer structures formed from the self-assembly of nonionic amphiphiles, mainly surfactants, in aqueous media. They are different from micelles, which are another type of surfactant aggregates; micelles entrap lipophilic molecules in an aqueous medium, are much smaller than niosomes and contain no bilayers (Van Hal et al., 1996a; Narang, Delmarre, & Gaoc, 2007).

Niosomes are considered to be a useful drug carrier for a wide range of dermal products, because of their ability to modulate drug release, and serve as solubility and penetration enhancer (Manosroi et al., 2003). Transdermal drug delivery from niosomes appears promising particularly for hydrophobic and amphiphilic drugs. These drugs are more likely, when compared with more hydrophilic ones, to associate with these loosely bound surfactant vesicles (Uchegbu & Vyas, 1998).

Address correspondence to Heba S. Barakat, Faculty of Pharmacy, Department of Pharmaceutics, University of Alexandria, El-Khartom Square, Azarita, Alexandria 21521, Egypt. E-mail: h_barakat76@yahoo.com

In addition, the poor solubility of some of these drugs may limit their incorporation into topical vehicle in the required concentration. The biphasic character of niosomes offers a wide scope for enhancing their solubility.

A niosome gel formulation for Naf.HCl could be beneficial in two ways. First, niosomes may allow drug formulation in a topical gel in the required concentration without the need for a cosolvent. Secondly, niosomes may help localize the drug at the site of infection, in the vicinity of the infective fungus leading to increased efficacy. This is because the entrapment of a drug in vesicles improves solubility and helps in the localized delivery and deposition at the site of action (Ning, Guo, Pan, Chen, & Gu, 2005).

The aim of this study is to develop and characterize an alcohol-free niosome gel formulation for the antifungal drug Naf.HCl. Better patient compliance and product efficacy are anticipated benefits.

MATERIALS AND METHODS

Materials

Naf.HCl (98.0–102.0%) was a generous gift from Pharco Pharmaceutical Company, Egypt. Sorbitan monostearate (Span 60), polyoxyethylene sorbitan monopalmitate (Tween 40), dicetyl phosphate (DCP), and cholesterol were purchased from Sigma-Aldrich Chemie Gmbh, Germany. Hydroxyethylcellulose was a generous gift from Omya Ltd, UK. Visking dialysis tubing (size 3, 20/32 in.) was purchased from Medicell Int. Ltd., UK. All other reagents used were of analytical grade.

Methods

Preparation of Naf.HCl Niosomes

Niosomes were prepared by the film hydration method using a lipid mixture consisting of two surfactants (150 μM total): sorbitan monostearate (Span 60) and polyoxyethylene sorbitan monopalmitate (Tween 40), with cholesterol (molar ratio 1:1:2, respectively, 300 μM total lipid). Vesicles from single surfactant Span 60 and cholesterol in the molar ratio of (1:1) were also prepared for comparison. The surfactants and cholesterol were placed in a 100 mL round bottom flask and dissolved in 3 mL chloroform. Naf.HCl (and DCP in some formulations) was dissolved in the chloroform solution. The chloroform was evaporated under reduced pressure at 60°C using a rotary evaporator (Buchi Rotavapor, Switzerland) till the formation of a thin lipid film. The formed film was hydrated with 5 mL of distilled water in a mechanical shaking water bath (Gesellschaft Fur Labortechnik, Germany) for 1 h at 60ºC. Dispersions were allowed to cool to room temperature (~25ºC) and then left overnight at 4–5°C for complete annealing and swelling of the vesicles, yielding the niosome dispersion containing both free and entrapped drug. In some cases, the niosome-entrapped Naf.HCl was separated from unentrapped drug by ultracentrifugation at 28,621 × *g* for 60 min at 0°C (Sigma laborzentrifugen refrigerated centrifuge 3K-30, GMBH, Germany). The supernatant layer was removed and the drug-loaded niosome pellets were washed with distilled water, recentrifuged for another 60 min under the same conditions and separated from the supernatant by decantation yielding drug-loaded niosome pellets.

Characterization of Naf.HCl-Loaded Niosomes

Entrapment Efficiency. An aliquot of drug-loaded niosome pellets was dissolved in 10 mL of alcohol. The absorbance of the solution was measured at λ_{max} 255 nm after suitable dilution using UV–Visible spectrophotometer, Model UV-1601 PC (Shimadzu, Japan). Percentage of Naf.HCl entrapped in the niosomes was calculated relative to the corresponding initial drug amount added as follows:

$$\text{Naf.HCl EE}(\%) = \frac{\text{Amount of Naf.HCl entrapped} \times 100}{\text{Corresponding initial amount of Naf.HCl}}. \quad (1)$$

Optimization of Entrapment Efficiency. Formulation factors investigated to maximize entrapment efficiency (EE) included Tween 40 to Span 60 molar ratio, initial drug amount, and molar ratio of drug to the negative charge-imparting agent DCP.

Three niosome formulations were selected, from among the 11 formulations prepared, for further characterization.

Scanning Electron Microscope Photomicrographs. A drop of the niosome dispersion was mounted on an aluminum stub and dried in the vaccum evaporator chamber of the scanning electron microscope (JSM-5510). The vesicles were sputter-coated with gold/palladium (Au/Pd) and examined using a scanning electron microscope (JSM-5510, Joel Ltd., Japan) equipped with a digital camera at 20 kV accelerating voltage.

Vesicle Size Analysis. Niosome dispersion was diluted with distilled water and sonicated for 30 s. The number mean diameter was calculated using laser diffraction particle size analyzer, Model 1064 (Cilas, France) operated at a wavelength of 780 nm.

Drug Release from Niosome Dispersions. In vitro release of Naf.HCl from niosome dispersions was investigated by dialysis. Dialysis tubing (Visking 20/32; Medicell Int., UK) was washed and soaked in distilled water 1 day before the experiment. An aliquot of 0.5 mL of the niosome dispersion (equivalent to 5 mg Naf.HCl) was pipetted into the dialysis bag and the bag was sealed. Bags containing 0.5 mL of a hydroalcoholic solution of Naf.HCl (containing 5 mg Naf.HCl) were also prepared. The dialysis bags were then immersed in 25 mL distilled water in 50 mL stoppered conical flasks and shaken at 32°C. The volume of the release medium (25 mL of distilled water) was sufficient to maintain sink condition for drug release. Preliminary release runs using different buffers (pH 4.5–5.5), as release medium, indicated a tendency for drug precipitation in the presence of some buffer ingredients. All

release runs were carried out using distilled water. At time intervals (1, 2, 4, 6, and 12 h), 0.5 mL of the release medium was removed, diluted with alcohol and assayed spectrophotometrically at λ_{max} 255 nm. At each time interval, 0.5 mL of distilled water (kept at 32°C) was added to replace the removed sample.

Physical Stability of Niosomes. Niosome dispersions were placed in sealed tubes and stored at 4°C for 6 weeks. Leakage, vesicle size, and drug release rate were monitored.

Leakage of the entrapped Naf.HCl from niosomes was determined every 2 weeks. Unentrapped drug was separated from the niosome-retained drug by ultracentrifugation. Both niosome pellets and supernatant were assayed separately at λ_{max} 255 nm.

The change in the size of the niosomes was monitored every 2 weeks during storage. The mean vesicle size was determined by the same method detailed above.

The release experiment was repeated at the end of the storage period to check release stability.

Preparation of Niosome Gel

Aiming to prepare an alcohol-free niosome gel, the neutral polymer hydroxyethylcellulose (final concentration 1.5% [wt/wt]) was added to a selected niosome dispersion (F10), and gently stirred (Polamed magnetic stirrer, model MM5, Poland). Mixing was continued till thickening of the dispersion and formation of the niosome gel. The gel was left overnight at 4°C. The gel, so prepared, contained a total drug concentration of 1% (wt/wt) half of which was entrapped in the niosomes (based on EE of the niosome dispersion determined before gelling).

Drug Release from Gel. A dialysis method was used. Visking membrane was soaked in distilled water for 24 h then rinsed thoroughly with distilled water. The tested gel (0.25 g) was placed in round stainless steel cups (16 mm diameter and 3 mm depth). The gel sample completely filled the cup void. The Visking membrane was placed over the gel surface without entrapping air bubbles, and fixed to the cup with a stainless steel ring. The cup assembly was immersed in 25 mL distilled water in a 100 mL beaker as release medium. The volume of the release medium was sufficient to achieve the sink condition for the drug (2.5 mg) present in 0.25 g gel. The beaker was placed in a thermostatically controlled shaking water bath at 32°C. Samples of 0.5 mL were withdrawn at fixed time intervals (1, 2, 4, 6, and 12 h) and replaced with distilled water. Samples were assayed spectrophotometrically.

Physical Stability of Gel. Gel samples (15 g) were stored at 4°C in tightly sealed containers for 6 weeks. The stored gels were visually inspected every 2 weeks for any signs of drug crystallization. Drug release stability was assessed by repeating the release experiment at the end of the storage period. The release study was performed at 32°C in distilled water.

RESULTS AND DISCUSSION

Composition of the niosome formulations and percentage of drug entrapped are shown in Table 1.

TABLE 1
Composition and Entrapment Efficiency (EE) of Naf.HCl Niosome Formulations

Formulation		Composition (mg)					
N°	Code	Naf.HCl	Chol.	Span 60	Tween 40	DCP	EE (%)
Effect of Tween 40:Span 60 molar ratio							
F1	SP	50	58	64	–	–	9.5
F2	TWSP (1:5)[a]	50	58	53	32	–	33.0
F3	TWSP (1:2)	50	58	42	64	–	28.0
F4	TWSP (1:1)	50	58	32	96	–	23.0
F5	TWSP (2:1)	50	58	21	128	–	12.0
Effect of initial drug amount							
F6	TWSP-25	25	58	32	96	–	13.85
F4	TWSP-50	50	58	32	96	–	23.0
F7	TWSP-75	75	58	32	96	–	15.0
Effect of DCP:Naf.HCl molar ratio							
F4	TWSP	50	58	32	96	–	23.0
F8	$TWSP_{(-)}1:5$ [b]	50	58	32	96	17	29.0
F9	$TWSP_{(-)}2:5$	50	58	32	96	34	41.0
F10	$TWSP_{(-)}3:5$	50	58	32	96	51	49.0
F11	$TWSP_{(-)}4:5$	50	58	32	96	68	47.5

[a]Tween 40:Span 60 molar ratio.
[b]DCP:Naf.HCl molar ratio.

Optimization of Drug Entrapment in Niosomes

Effect of Tween 40:Span 60 Molar Ratio

Gradual increase in Tween 40:Span 60 molar ratio (F2–F5) led to a corresponding decrease in EE. However, complete lack of Tween 40 (F1) was not in favor of entrapment (Table 1). Formulae F2 and F3 showed good EE but were not stable; drug crystals separated on standing.

Micelle formation in Tween 40-containing niosome systems may take place even in the presence of cholesterol (Girigoswami, Das, & De, 2006). These micelles can solubilize a portion of the drug, under the conditions of vesicle formation (shaking at 60°C for 1 h), at the expense of drug entrapment within the niosomes. This could lead to sequestering a portion of the drug in the aqueous hydration medium and subsequently lower EE.

Drug crystallization in F2 and F3 indicated instability of these systems. By comparison, the 1:1 ratio (F4) did not show drug crystallization and was used thereafter.

Effect of Initial Drug Amount

Increasing the initial drug amount from 25 to 50 mg while keeping total lipid constant at 300 μM (F6 and F4) led to a two-fold increase in EE. Further increase in drug amount to 75 mg (F7) decreased EE (Table 1). Figure 1 shows the amount of drug entrapped (mg) with increasing initial drug amounts. At 75 mg initial drug amount, drug loading (mg) in the vesicles was the same as at 50 mg. The plateau observed indicates the capacity-limited loading caused probably by the limited number of niosomes available. EE (as %) calculated at 75 mg initial drug was consequently lower than at 50 mg. This trend has been documented for colchicine entrapment in Span 60 vesicles (Hao, Zhao, Li, Yang, & Li, 2002).

Effect of Dicetyl Phosphate

The influence of the negative charge-imparting agent (DCP) on EE was investigated at DCP:Naf.HCl molar ratios 1:5, 2:5, 3:5, and 4:5 (F8–F11). Uncharged niosomes (F4) EE (22%) increased upon incorporating DCP. Maximum EE (~50%) was attained with DCP:Naf.HCl molar ratio 3:5 (F10). Further increase in the DCP:Naf.HCl molar ratio to 4:5 (F11) did not increase EE (Table 1). The slight decrease in EE observed at DCP:Naf.HCl ratio of 4:5 may be attributed to increased repulsion within the double layers leading to decreased vesicle stability (Carafa et al., 1998).

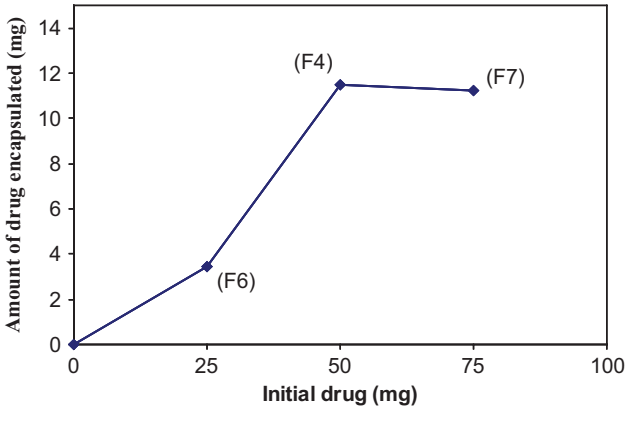

FIGURE 1. Effect of initial drug (mg) on the amount of Naf.HCl encapsulated in (TWSP 1:1) niosomes.

Inclusion of ionic surfactants, such as DCP, in niosomes generally serves to stabilize the vesicles against fusion or aggregation by means of increasing their ξ potential. It has also been reported that the presence of a charge in the membrane structure can increase water uptake within the double layer, increasing its thickness as well as the size of the aqueous compartment, resulting in larger vesicles with higher EE (Carafa et al., 1998). The increase in EE, brought about by DCP, could also be attributed to the electrostatic interaction between the opposite charges of the drug and the membrane components. This was the case with lidocaine HCl for which a direct proportionality between EE and DCP concentration was reported (Van Hal et al., 1996b). Similarly, positively charged stearylamine has been reported to increase EE of the anionic drug, all-*trans* retinoic acid, in Span niosomes (Desai & Finlay, 2002). The entrapment of the negatively charged DNA (Jain, Singh, Vyas, & Mishra, 2005) and the weakly acidic acetazolamide (Aggarwal, Garg, & Kaur, 2004) in Span 60 niosomes are additional examples.

Physical Characterization of Naf.HCl Niosomes

Three niosome formulations (F4, F8, and F10) were selected for characterization. Selection was based on the EE and the absence of drug crystals.

Niosome Size Analysis

Determination of the number mean diameter for the three selected niosome formulations yielded sizes ranging from 0.09 to 0.15 μm. The uncharged vesicles (F4) had the smallest mean diameter (0.09 μm). Increasing DCP was accompanied by a slight increase in the mean diameter (0.11 and 0.15 μm for F8 and F10, respectively).

Release Study

Percentage of Naf.HCl released during 12 h was determined for the three selected niosome formulations at 32°C (Figure 2). Niosomes showed biphasic drug release. An initial phase lasting 2 h was followed by a slow release phase extending from 2 to 12 h. In the initial phase, free unentrapped drug diffuses into the release medium, whereas in the slow release phase the entrapped drug leaks out gradually from the vesicles into the medium. Inclusion of DCP into niosomes reduced both rate and extent of drug release during the study period, the effect being concentration dependent. The slower release rate from the negatively charged niosomes could be attributed to retention of the positive drug by the negative bilayer (Carafa, Marianecci, Lucania, Marchei, & Santucci, 2004).

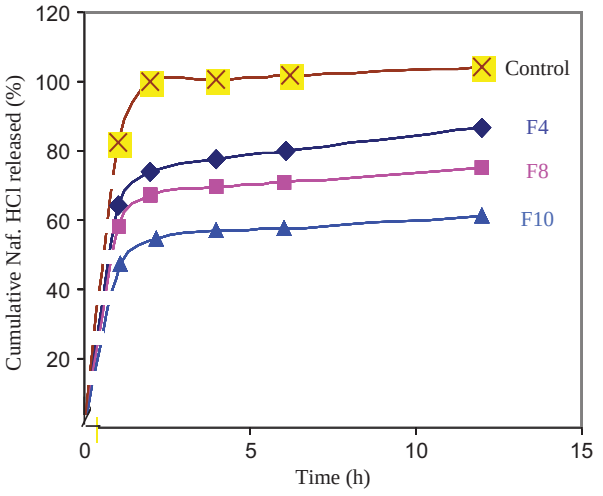

FIGURE 2. Release profiles of Naf.HCl niosome dispersions in distilled water at 32°C.

Kinetics of Drug Release from Niosomes

Linear regression analysis of the release data (2–12 h) indicated that the entrapped drug was released from niosomes, during that period, by diffusion-controlled mechanism (Higuchi release plots, Figure 3; and release parameters, Table 2). The values of the percentage of entrapped drug calculated from EE results are also included in the table for comparison with Higuchi plot intercept values. The observed rank-order correlation between Higuchi intercept values and percentage unentrapped drug supports the claim that drug appearing in the release medium (0–2 h) is mainly the unentrapped drug.

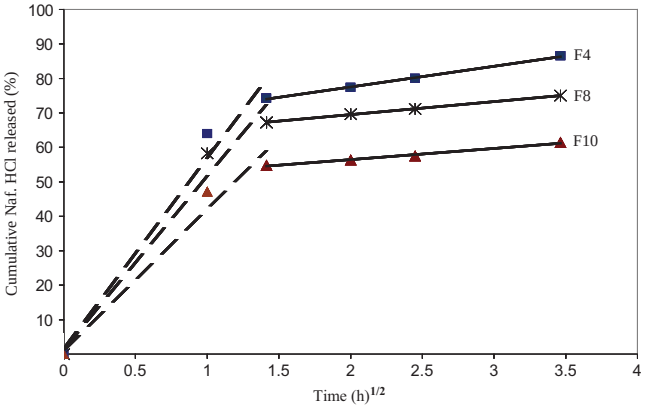

FIGURE 3. Higuchi release plots for freshly prepared Naf.HCl niosome dispersions.

Stability of Naf.HCl Niosome Dispersion

Drug Leakage from Niosomes

The three formulations stored at 4°C for 6 weeks were fairly resistant to drug leakage out of the niosomes. Percentage of drug retained in the vesicles at the end of the storage period, compared with initial values, ranged from 80 to 84%. The uncharged niosomes (F4) showed slightly higher drug leakage (20%), than the charged ones (F8 and F10, 18 and 16%, respectively). Incorporation of DCP increased the stability of the vesicles, the effect being concentration dependent. This trend is attributed to electrostatic interaction between the negative charge-imparting agent and the positively charged drug (Van Hal et al., 1996b).

Vesicle Size Stability

Monitoring vesicle size every 2 weeks for niosomes stored at 4°C revealed no appreciable change in the mean vesicle diameter for the neutral and charged niosome formulations during the 6-week study period.

Release Stability

The release study was repeated after 6 weeks storage at 4°C to check release reproducibility. Release data after storage (Table 3) indicated a slight rise in Higuchi release parameters. The intercept values, again showed a good match with the recorded rise in unentrapped drug, estimated from EE.

Niosome Gel Results

Drug Release from Niosome Gel

The release profiles of the niosome gel (containing F10) were biphasic (Figure 4) characterized by an initial phase (0–2 h) during which unentrapped drug diffuses across the dialysis membrane, followed by a second slower phase (2–12 h). The initial diffusion phase was faster for the niosome dispersion

TABLE 2
Higuchi Diffusion Model Release Parameters of Freshly Prepared Naf.HCl Niosome Dispersions

Formulation Code	Correlation Coefficient (*r*)	Slope	Intercept	Percentage of Unentrapped[a]
F4	.998	6.03	65.5	75
F8	.999	3.8	62	66
F10	.995	3.2	50.1	50.4

[a] Estimated from EE data.

TABLE 3
Release Parameters According to Higuchi Diffusion Model and Percentage of Unentrapped Before and After storage at 4°C for 6 Weeks

	Correlation Coefficient		Slope		Intercept		Percentage of Unentrapped	
Formulation Code	Before	After	Before	After	Before	After	Before	After[a]
F4	.998	.967	6.03	7.15	65.5	68.1	75	79
F8	.999	.967	3.77	3.9	62	63.7	66	71
F10	.995	.980	3.2	3.0	50.1	52.2	50.4	57.5

[a]Calculated from EE values determined after storage.

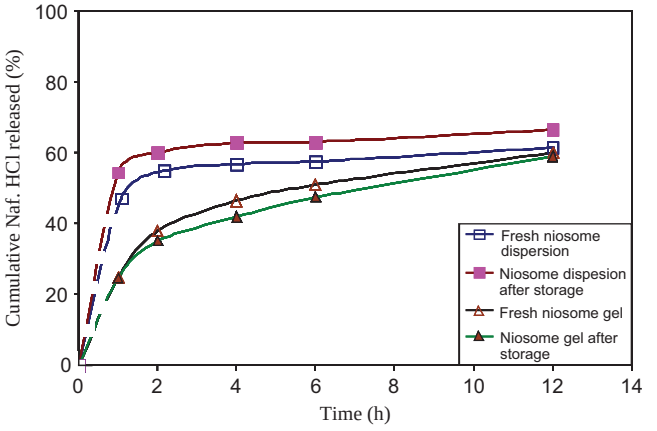

FIGURE 4. Release profiles of Naf.HCl niosome dispersion and gel before and after storage for 6 weeks at 4°C.

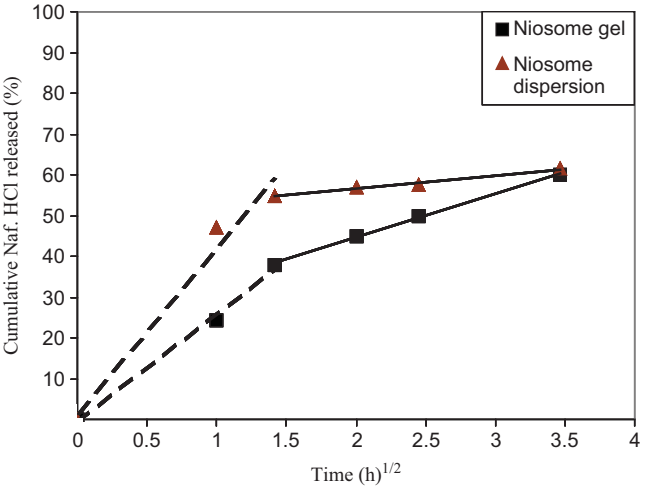

FIGURE 5. Higuchi release plots for freshly prepared Naf.HCl niosome dispersion (F10) and gel in distilled water at 32°C.

(also shown in Figure 4) compared with the niosome gel, but the extent of release at 12 h was comparable.

Incorporation of the niosomes into a structured gel vehicle resulted in a slower initial phase compared with niosome dispersion possibly because of the diffusion restriction imposed by the polymeric network of the gel (Glavas-Dodov, Fredro-Kumbaradzi, Goracinova, Calis, & Hincal, 2003; Ning et al., 2005; Turker, Erdogan, Ozer, Ergun, & Tuncel, 2005).

Kinetics of Naf.HCl Release from the Niosome Gel

The niosome gel showed Higuchi diffusion model release kinetics (release data 2–12 h) similar to the niosome dispersion (both shown in Figure 5), indicating matrix-controlled diffusion of the released drug (Glavas-Dodov et al., 2003; Shahiwala, Misra, & Gujarat, 2002; Zeljka & Natasa, 2004).

Stability Assessment of the Niosome Gel

Microscopic Examination. Microscopical examination of the gel samples revealed no sign of drug crystallization during storage (6 weeks at 4°C). The absence of drug crystallization could be attributed to the niosomes keeping the entrapped drug in a soluble state. Apart from vesicles, some of the hydrophilic surfactant molecules used in the preparation of niosomes probably formed micelles, which helped to maintain the free unentrapped drug in solution.

Release Stability. The niosome gel showed good release reproducibility as indicated by the close release profiles before and after storage (Figure 4).

CONCLUSION

An alcohol-free controlled delivery antifungal gel containing 1% (wt/wt) Naf.HCl was developed. The gel showed good stability and maintained drug delivery over a 12-h study period. The gel contains the drug in both the free form (50%) and niosome-entrapped form (50%) to generate an intended two-phase release profile.

Niosomes used in the gel formulation are negatively charged niosomes with 0.15 μm mean diameter, developed in the study. They contain Tween 40, Span 60, and cholesterol

in 1:1:2 molar ratio together with DCP and Naf.HCl in 3:5 molar ratio.

The Naf.HCl niosome gel developed in this study may offer great potentials in topical antifungal therapy.

REFERENCES

Aggarwal, D., Garg, A., & Kaur, I. P. (2004). Development of a topical niosomal preparation of acetazolamide: Preparation and evaluation. *J. Pharm. Pharmacol., 56*(12), 1509–1517.

Bacchi-Modena, A., Bolis, P., Campagnoli, C., & De Cicco, F. (1997). Efficacy and tolerability of estraderm MX®, a new estradiol matrix patch. *Maturitas, 27*(3), 285–292.

Baumanna, M. A., Rath, B., Fischer, J. H., & Iffland, R. (2000). The permeability of dental procedure and examination gloves by an alcohol based disinfectant. *Dent. Mater., 16*(2), 139–144.

Carafa, M., Marianecci, C., Lucania, G., Marchei, E., & Santucci, E. (2004). New vesicular ampicillin-loaded delivery systems for topical application: Characterization, in vitro permeation experiments and antimicrobial activity. *J. Control. Release, 95*(1), 67–74.

Carafa, M., Santucci, E., Alhaique, F., Coviello, T., Murtas, E., Riccieri, F. M., Lucania, G., & Torrisi, M. R. (1998). Preparation and properties of new unilamellar non-ionic surfactant vesicles. *Int. J. Pharm., 160*(1), 51–59.

Desai, T. R., & Finlay, W. H. (2002). Nebulization of niosomal all-trans-retinoic acid: An inexpensive alternative to conventional liposomes. *Int. J. Pharm., 241*(2), 311–317.

Girigoswami, A., Das, S., & De, S. (2006). Fluorescence and dynamic light scattering studies of niosomes-membrane mimetic systems. *Spectrochim. Acta A Mol. Biomol. Spectrosc., 64*(4), 860–866.

Glavas-Dodov, M., Fredro-Kumbaradzi, E., Goracinova, K., Calis, S., & Hincal, A. A. (2003). 5-Fluorouracil in topical liposome gels for anticancer treatment—formulation and evaluation. *Acta Pharm., 53*(4), 241–250.

Hao, Y., Zhao, F., Li, N., Yang, Y., & Li, K. (2002). Studies on a high encapsulation of colchicine by a niosome system. *Int. J. Pharm., 244*(1–2), 73–80.

Hess, J., Molinari, J. A., Gleason, M. J., & Radecki, C. (1991). Epidermal toxicity of disinfectants. *Am. J. Dent., 4*(1), 51–56.

Jain, S., Singh, P., Vyas, S. P., & Mishra, V. (2005). Mannosylated niosomes as adjuvant-carrier system for oral genetic immunization against hepatitis B. *Immunol. Lett., 101*(1), 41–49.

Manosroi, A., Wongtrakul, P., Manosroi, J., Sakai, H., Yuasa, M., Sugawara, F., & Abe, M. (2003). Characterization of vesicles prepared with various non-ionic surfactants mixed with cholesterol. *Colloids Surf. B Biointerf., 30*(1), 129–138.

MerzPharmaceuticals. (2004). Naftifine hydrochloride 1% gel, Retrieved June 17, 2008, from http://www.merzusa.com/pdfs/naftin%20gel%20PI%204-04b.pdf.

Narang, A. S., Delmarre, D., & Gaoc, D. (2007). Stable drug encapsulation in micelles and microemulsions. *Int. J. Pharm., 345*(1–2), 9–25.

Ning, M., Guo, Y., Pan, H., Chen, X., & Gu, Z. (2005). Preparation, in vitro and in vivo evaluation of liposomal/niosomal gel delivery systems for clotrimazole. *Drug Dev. Ind. Pharm., 31*(4-5), 375–383.

Scheuplein, R., & Ross, L. (1970). Effects of surfactants and solvents on the permeability of epidermis. *J. Soc. Cosmet. Chem., 21*, 853–873.

Shahiwala, A., Misra, A., & Gujarat, I. (2002). Studies in topical application of niosomally entrapped Nimesulide. *J. Pharm. Pharmaceut. Sci., 5*(3), 220–225.

Tornier, C., Rosdy, M., & Maibach, H. I. (2006). In vitro skin irritation testing on reconstituted human epidermis: Reproducibility for 50 chemicals tested with two protocols. *Toxicol. In Vitro, 20*(4), 401–416.

Turker, S., Erdogan, S., Ozer, A. Y., Ergun, E. L., & Tuncel, M. (2005). Scintigraphic imaging of radiolabelled drug delivery systems in rabbits with arthritis. *Int. J. Pharm., 296*(1–2), 34–43.

Uchegbu, I. F., & Vyas, S. P. (1998). Non-ionic surfactant based vesicles (niosomes) in drug delivery. *Int. J. Pharm., 172*(1), 33–70.

Van Hal, D. A., Bouwstra, J. A., & Junginger, H. E. (1996a). Nonionic surfactant vesicles containing estradiol for topical application. In S. Benita (Ed.), *Microencapsulation: Methods and industrial applications* (pp. 329–347). New York: Marcel Dekker, Inc.

Van Hal, D. A., Jeremiasse, J., Vringer, T., Junginger, H. E., & Bouwstra, J. A. (1996b). Encapsulation of lidocaine base and hydrochloride into non-ionic surfactant vesicles (NSVs) and diffusion through human stratum corneum in vitro. *Eur. J. Pharm. Sci., 4*(1), 147–157.

Zeljka, P., & Natasa, S. (2004). Liposomal gel with chloramphenicol: Characterisation and in vitro release. *Acta Pharm., 54*, 319–330.

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