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PREPARATION AND CHARACTERIZATION OF ACITRETIN-LOADED NIOSOMES FOR
PSORIASIS TREATMENT

A thesis

presented in partial fulfillment of requirements

for the degree of Master of Science

in the Department of Pharmaceutics and Drug Delivery

The University of Mississippi

by

MAREY A. ALMAGHRABI

May 2017

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ABSTRACT

Psoriasis is a chronic skin disease that manifests as impaired epidermal differentiation. The disease is typically treated with acitretin, an effective oral cytotoxic agent. However, due to its side effects, its use is highly limited. Topical delivery of acitretin may decrease the systemic toxicity and increase the drug's bioavailability at the pathological site. However, this approach has some limitations. The decrease in skin integrity due to psoriasis could lead to escape of drug into the systemic circulation system and consequently compromise the topical approach. Also, the high instability of acitretin in the presence of heat could limit its topical application. The aim of this work is to formulate and characterize niosomes for topical delivery of acitretin in order to decrease the drug's systemic side effects, provide a controlled method of delivery to the pathological site, and improve the thermal stability of acitretin. To achieve this goal, acitretin niosomes were prepared using the thin film hydration technique. The niosomes were then characterized and optimized for size, entrapment efficiency, and drug release. The characterized niosomes were evaluated and investigated as a topical drug delivery system. The lead formulation displayed an optimum particle size of 471 ± 1.15 nm with a PDI of 0.4 ± 0.04 , zeta potential of -21 ± 0.26 , entrapment efficiency of 92 ± 2.70 , and controlled drug release of 30.80 ± 0.21 %. *In vitro* permeation studies across a tab-stripped epidermis showed that niosomes can control the drug permeation of compromised skin. The cumulative amount of drug permeated from niosomes was 1.87 ± 0.09 $\mu\text{g}/\text{cm}^2$, compared to 3.6 ± 0.02 $\mu\text{g}/\text{cm}^2$ with drug control and 2.4 ± 0.08 $\mu\text{g}/\text{cm}^2$ with excipient control. Also, the *in vitro* deposition studies showed that the amount of the drug deposited from niosomes to the epidermis after stratum corneum removal (665 ± 0.2 ng/mg) was

significantly greater than the amount of the drug in the solution and excipient control (385 ± 0.1 and 205 ± 0.4 ng/mg, respectively). Moreover, *in vitro* thermal degradation studies confirmed that the acitretin niosome formulations have a longer half-life than the drug in solution (115.75 days for samples stored at 4 °C, 60.18 days for samples stored at 24 °C, and 45.59 days for samples stored at 40 °C). In summary, the results showed that the incorporation of acitretin into niosomes for topical delivery might be a promising approach for the treatment of psoriasis.

DEDICATION

I dedicate my thesis to my wonderful family. I am especially grateful to my loving parents, Abdulmootani and Barakah Almaghrabi, who provided encouragement and pushed me to do my best. They have been a source of strength throughout this program. I am also grateful to my wife, Wafa, for being there for me during the entire program; to all of my sisters and brothers, who supported me throughout the process; and to my son, Battal, who holds a special place in my heart. My love for all of you can never be quantified.

ACKNOWLEDGMENTS

I wish to thank my committee members, who were more than generous with their expertise and precious time. Special thanks to Dr. S. Narasimha Murthy, my committee chairman, for his countless hours of reflection, reading, encouragement, and, most of all, patience throughout the entire process. Also, thanks to Drs. Michael A. Repka and Seongbong Jo for agreeing to serve on my committee.

I would like to acknowledge and thank my department for allowing me to conduct my research. Special thanks to those in the staff development and human resources departments for their continued support.

I thank my colleagues, Dr. Murthy Group, Abhijeet Maurya, Purnendu Sharma, and Vijay Kumar Shankar, and all the members of my group for the stimulating discussions, the sleepless nights we worked together before deadlines, and all the fun we have had in the last four years.

Last but not least, I would like to thank my parents for nurturing and supporting me spiritually throughout my life.

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PSORIASIS

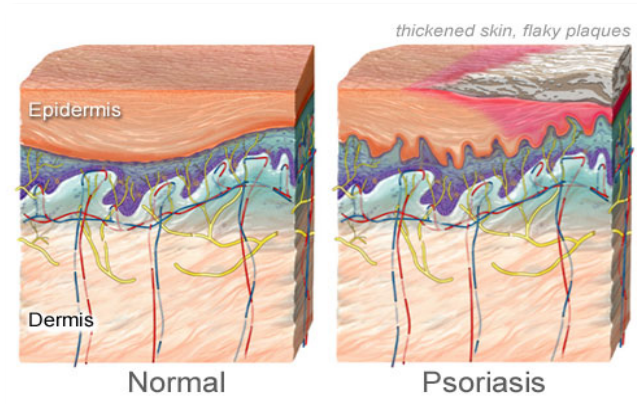


Figure 1. Psoriatic skin

Disease overview

Psoriasis is a chronic skin disease that can result in substantial morbidity and mortality (Rachakonda et al., 2014) (Armstrong et al., 2012) . The prevalence of psoriasis is about 3.2 % among US adults aged 20 years and older (Gelfand et al., 2007). The disease is characterized by the proliferation and keratinization of exaggerated and disordered epidermal cells as well as silvery scales on the skin epidermis. Many efforts have been made to understand the pathophysiology of disease. Unfortunately, the pathological sequence that causes keratinization has not yet been identified. However, the most widely accepted hypothesis is that psoriasis is an immune-mediated skin disease that affects genetically predisposed individuals who are exposed to triggering environmental factors. The scales appear on the skin due to hyperproliferation of keratinocytes and incomplete cornification and migration and accumulation of nuclei in the stratum corneum

(SC) (Fig. 1). The mitotic rate of keratinocytes is much higher than that of normal cells, resulting in thickening of the epidermis layer. This idea is supported by the efficacy of many immunomodulator agents for treatment of psoriasis. Psoriasis can manifest on any part of the body, including the elbows, knees, and sacral regions, and produces sharp, dry, and itchy feelings (Winterfield, Menter, Gordon, & Gottlieb, 2005) (Griffiths & Barker, 2007) (Langley, 2012). These symptoms can affect patients' quality of life both physically and psychologically (de Korte, Mommers, Bos, & Sprangers, 2004) (Fig. 2).



Figure 2. Histology of normal and psoriatic skin

Management of psoriasis

The goal of psoriasis treatment is to decrease and suppress cutaneous lesions so that the disease no longer affects patients' employment and/or social life. The majority of patients cannot achieve remission without treatment, so continuous therapy is required. The management of psoriasis can be divided into three types of therapy: topical, photo, and systemic.

The management of psoriasis usually starts with topical treatment (Drake et al., 1993), which is most useful in mild to moderate cases, when the disease covers less than 20 % of the body's surface area. Topical treatment is effective, convenient to use, and lacks the serious side effects produced by other types of treatment. The most widely used topical treatment in the United

States is corticosteroids (Wood, Greaves, & Weinstein, 1995), which have anti-inflammatory, anti-proliferative, and immunosuppressive properties. More than 80 % of US dermatologists prefer to prescribe topical corticosteroids for mild, limited psoriasis. The clinical efficacy of this drug is highly related to its potency. A highly potent corticosteroid is highly anti-inflammatory, so it is usually used for acute flare-ups of the disease, whereas medium-potent corticosteroids are used for maintenance therapy (Liem, McCullough, & Weinstein, 1995). Despite the clinical effectiveness of topical corticosteroids, their use may be accompanied by side effects, including local skin atrophy, tachyphylaxis, fast relapse times, irritation from the vehicle, and contact dermatitis. Using large amounts in cases of acute psoriasis can result in systemic absorption, leading to suppression of the hypothalamic–pituitary–adrenal axis (Takeda, Arase, & Takahashi, 1988). Other topical treatments include calcipotriene (a vitamin D analogue), tazarotene, coal tar, anthralins, and keratolytics.

When psoriasis is extensive and severe, affecting more than 15–20 % of the body’s surface area, topical treatment is impractical for management of disease. Frequent application of topical agents over a large surface area is extremely costly and difficult for patients to perform. Thus, severe cases of psoriasis should be treated using systemic medications. A systemic regimen is often complicated and may require specialized equipment and continuous monitoring.

Patients with severe psoriasis flare-ups may also require phototherapy during hospitalization. The Goeckerman regimen is a typical phototherapy regimen that involves twice-daily administration of ultraviolet B and coal tar. Once the condition is stabilized, systemic treatment can be provided on a less frequent basis. The only three systemic medications for psoriasis approved by the United States Food and Drug Administration (USFDA) are methotrexate, cyclosporine, and acitretin.

Many side effects are associated with systemic treatments. Long-term use of methotrexate can cause toxicity, leading to bone marrow suppression, drug-induced hepatic fibrosis, or cirrhosis (Roeningk et al., 1998). Administration of acitretin is also associated with a very high risk of teratogenic effects. Because of its potential teratogenicity, acitretin should not be used by women of child-bearing age (Gollnick et al., 1988). In addition, cyclosporine can cause severe nephrotoxicity if used long term (ZACHARIAE, KRAGBALLE, HANSEN, MARCUSSEN, & OLSEN, 1997). In a recent study, 34 of 122 (28 %) patients treated with cyclosporine for a period of 22 months discontinued the treatment due to renal failure (Grossman et al., 1996). Of the three systemic medications, acitretin is probably the safest for long-term treatment.

Acitretin

Acitretin is a free and active metabolite of etretinate, the first oral synthetic retinoid used to treat psoriasis. Because of the superior pharmacokinetic properties of acitretin compared to etretinate, the drug became the most widely used systemic retinoid (Orfanos, 1999). In fact, it is the only systemic retinoid approved by the USFDA, and its effectiveness has been reviewed by many researchers (Lee & Li, 2009).

Pharmacology of acitretin

Acitretin is available on the market as an oral capsule (10 and 25 mg). The bioavailability of acitretin ranges between 20 and 90 % after oral administration. It requires 1–4 h to reach the peak plasma level. It has much shorter half-life (2–4 days) than etretinate (120 days). Further, acitretin is 50 times less lipophilic than etretinate, and because of that, its elimination time is much shorter. Acitretin metabolizes to 13-cis-acitretin, and both are widely distributed throughout the body. One month after discontinuing acitretin, the amount of the drug in plasma is undetectable.

However, it can undergo re-esterification to produce etretinate, thereby increasing the risk of teratogenicity. Acitretin's mechanism of action is still unknown. However, it is believed that acitretin induces and modulates the expression of growth factors in psoriatic epidermis and its receptors. Overall, acitretin reduces the proliferation rate of psoriasis in the epidermis, promotes differentiation between keratinocytes, regulates the desquamation of corneocyte, and decreases the thickness of SC and inflammation in the epidermis and dermis.

Effectiveness of acitretin in psoriasis treatment

Acitretin has been found to be effective as a monotherapy for treating moderate to severe psoriasis. Several studies were conducted to determine the efficacy of different dosages of acitretin as a single agent for the treatment of different types of psoriasis. The efficacy of acitretin depends on the clinical type of psoriasis. The drug showed good response to erythrodermic and pustular psoriasis, However, acitretin exhibited moderate efficacy for treating chronic plaque-type psoriasis (Goldfarb et al., 1988) (Olsen, Weed, Meyer, & Cobo, 1989).

Acitretin also was found to be effective as a combination therapy. When acitretin is used in combination with calcipotriol, it can better clear psoriatic plaque (Rim, Park, Choe, & Youn, 2003). In a randomized paired comparison study, 60 % of patients achieved complete clearance of psoriasis faster when treated with a combination of acitretin and calcipotriol. Moreover, the use of acitretin combinations in photo(chemo)therapy improves the efficacy of such therapy and decreases the UV dose and duration of treatment required for chronic plaque psoriasis (Saurat et al., 1988) (Tanew, Guggenbichler, Hönigsmann, Geiger, & Fritsch, 1991).

Side effects of acitretin

Acitretin is considered a pregnancy category X drug. Fetal abnormalities associated with oral acitretin include meningomyelocele, meningoencephalocele, multiple bony malformations, facial dysmorphia, low-set ears, high palate, anophthalmia, decreased cranial volume alterations, abnormal appendages, hip malformations, multiple synostoses, and cardiovascular malformations. Due to the serious teratogenic toxicity of acitretin, psoriatic women of childbearing age are advised to take an extensive course of birth control for at least three years after discontinuing psoriasis therapy. Acitretin is also associated with mucocutaneous side effects like cheilitis, rhinitis, dry mouth, pruritus, alopecia, and hair pigmentation. Moreover, it was reported that acitretin can cause hyperlipidemia in 25–40 % of psoriatic patients (Katz, Waalen, & Leach, 1999). Generally, with respect to teratogenicity, the adverse effects produced by acitretin are mild and reversible in nature, and only a few cases might require discontinuation of the treatment.

Topical delivery of acitretin for the treatment of psoriasis

The current systemic therapies for psoriasis are highly limited by their side effects, as mentioned above. Many psoriatic patients around the world have clearly indicated that they are unsatisfied with their current treatment; in a survey conducted by the National Psoriatic Foundation, only 28 % of patients were satisfied with their psoriasis therapy (McKenna & Stern, 1997). This low satisfaction occurs because the treatment is time-consuming and often ineffective and systemic medications have adverse side effects.

During the search for safe and effective psoriasis treatment, interest in topical delivery systems has increased. It is believed that topical systemic medications could inhibit the undesirable side effects associated with conventional systemic treatment and increase the local availability of the drug molecules at the target site. In addition, topical delivery helps avoid the first pass

metabolism and offer continuous, controlled drug delivery. All of these advantages improve the thermodynamic response and safety profile of drugs for treating psoriasis. Thus, a topical approach to psoriasis treatment could be an ideal alternative to the conventional systemic approach.

Although topical drug delivery systems for treating psoriasis have many benefits, they are still associated with many challenges, including the following: the variability in skin absorption due to the application site, severity of disease, and patient's age; the first pass metabolic effect on skin; the reservoir capacity of the skin; and the potential for irritation and toxicity due to the drug. Furthermore, targeting psoriatic tissue using a topical approach poses a big challenge due to the fact that psoriatic skin is extremely dehydrated and thick, and thus usually has a lipid imbalance and high sensitivity to irritants. Also, the lack of SC integrity in psoriatic skin could allow the drug to escape into systemic circulation and consequently compromise topical delivery.

In addition to all the previous limitations of topical delivery for psoriasis treatment, topical delivery of acitretin poses additional disadvantages. Acitretin induces skin sensitivity and is highly unstable in the presence of air, heat, and light. Also, the drug has very low water solubility, which makes it challenging to formulate topical acitretin. For topical delivery of acitretin to psoriatic skin, the delivery vehicle must be suitably designed and developed to overcome all these challenges.

Niosomes: a topical delivery system for acitretin

Niosomes are non-ionic surfactants formed by the hydration of a mixture of single-alkyl chains of non-ionic surfactants and cholesterol, which results in a closed bilayer structure that was first reported by Handjani-Vila et al. in 1979 (Agrawal, Petkar, & Sawant, 2010). Niosomes can be produced using various types of non-ionic surfactants, including polyglycerol alkyl ethers, crown ethers, ester-linked surfactants, glucosyldialkyl ethers, polyoxyethylene alkyl ethers, Brij,

Tweens, and Spans. Niosomes have attracted a great deal of attention for their unique features, which are suitable for dermal and transdermal delivery. For example, they are biodegradable, biocompatible, and non-immunogenic. The vehicle is associated with better patient compliance and better therapeutic effects than conventional oily formulations. They are capable of controlling and sustaining the release of drugs due to depot formation. The shape, size, composition, and fluidity of niosome formulation can be controlled when required (Haran, Cohen, Bar, & Barenholz, 1993). Also, niosomes can entrap large amounts of materials in a small volume of vesicles (Nasr, Mansour, Mortada, & Elshamy, 2008). Interestingly, niosomes' bilayer structure includes hydrophobic tails, which are shielded from aqueous media, and hydrophilic head groups, which have the most contact with aqueous media. This structure is similar to the phospholipid vesicle structure of liposomes and is able to encapsulate both hydrophilic (in their inner spaces) and hydrophobic drugs (in the lipid bilayer area) (raja, Pillai, Udupa, & Chandrashekar, 2017) (Fig. 3). Niosomes can be considered an alternative to liposomes due to their ability to alleviate the drawbacks associated with liposomes, such as chemical instability, variable purity of phospholipids, and high cost. These features make niosomes an attractive option for topical delivery of acitretin.

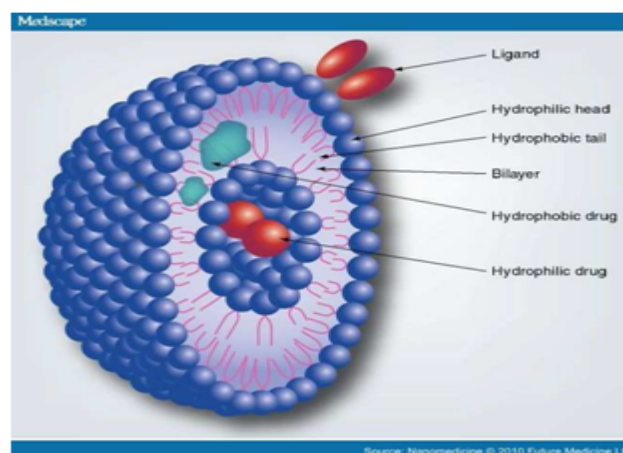


Figure 3. Arrangement of surfactants in niosomes

Niosomes have been evaluated in several previous works as a topical delivery system for psoriasis treatment. In a study done by M. Manconi et al., the efficacy of vesicular incorporation of tretinoin for dermal delivery through pig skin was investigated. The results demonstrated that niosomes have higher cutaneous drug retention than liposomes and commercial formulations and that the cutaneous delivery of tretinoin is strongly affected by the vesicles' composition and drug's thermodynamic activity (Mura, Pirot, Manconi, Falson, & Fadda, 2007).

In another study, the safety and efficacy of topical delivery of methotrexate via niosomes were examined. The study aimed to assess irritation and sensitization among healthy human volunteers caused by the prepared niosomal methotrexate compared to a placebo and marketed methotrexate gel. The results showed that the niosomal methotrexate formulation more significantly reduced ($P < 0.05$) the total PASI score (from 6.7378 ± 1.48576 to 2.0023 ± 1.13718) compared to a placebo and marketed methotrexate gel. The study concluded that the niosomal methotrexate formulation is more effective than the placebo or marketed methotrexate gel (Lakshmi, Devi, Bhaskaran, & Sacchidanand, n.d.).

HYPOTHESIS AND SPECIFIC AIMS

We hypothesize that incorporating acitretin in a niosomal formulation improves the thermal stability of the Active Pharmaceutical Ingredient (API) and result in controlled drug delivery at the pathological site.

Aim 1. To obtain niosomes that have high API entrapment efficiency and are 200–600 nm in size.

Different sonication and hydration times were initially applied to control the size and entrapment efficiency of the prepared niosomes. The niosomes were characterized according to size, PDI, zeta potential, and entrapment efficiency. Then, a second set of surfactants was incorporated into the optimized formulation to achieve higher entrapment efficiency. The resultant niosomes were characterized again according to size, PDI, zeta potential, and entrapment efficiency.

Aim 2: To determine the optimum molar ratio of the lead surfactant, span 60, and cholesterol for preparing acitretin-loaded niosomes.

Different molar ratios of the lead surfactant, span 60, and cholesterol were used to prepare six niosome formulations and were characterized according to size, PDI, zeta potential, entrapment efficiency, and drug release.

Aim 3: To evaluate the lead niosome formulation as a topical delivery system of acitretin for psoriasis treatment.

In vitro skin permeation and deposition studies were performed using intact and tape-stripped porcine epidermis

MATERIAL AND METHODS

Materials

Acitretin (98 %) was purchased from Pure Ontario Chemicals Inc., Canada. High Performance Liquid Chromatography (HPLC)-grade solvents like methanol, ethanol, and chloroform were purchased from Fisher Chemicals, USA. Span 60 and formic acid were purchased from Sigma Chemicals, St. Louis, MO. Brij[®] 93 was gifted by BASF. Brij[™] S100, Brij[™] S20, and Brij[™] C10 were gifted by CRODA Inc., USA. Cholesterol was purchased from Alfa Aesar, USA, and sephadex was purchased from MP Biochemicals, USA. Porcine skin was obtained from Pontotoc Slaughterhouse, Pontotoc, MS, USA. Dialysis membrane (10 kDa) was purchased from Spectrum Chemicals, New Brunswick, NJ, USA.

Methods

Development of niosomal formulations

Preparation of niosomes

Niosomes were prepared using the thin film hydration technique, for which sonication and hydration time were already optimized. Accurate quantities of surfactants, cholesterol, and the drug were obtained. The mixture was dissolved in an ethanol/chloroform solution (71.4:28.6 %) in a round-bottom flask. The solvents evaporated at 60 °C under reduced pressure using a rotary flash evaporator. After solvent evaporation, the flask was kept overnight in a vacuum evaporation chamber to remove residual chloroform and ethanol. The resultant thin film was hydrated with 10 ml of phosphate buffer saline (PBS; pH 7.4), and the flask was rotated at 60 °C and 150 rpm for

30 min. The niosome formulations underwent probe sonication for 20 s three times with 20 s intervals. The acitretin-entrapped vesicles were separated from the un-entrapped material using gel chromatography of a sephadex G-50 column. The filtration process was repeated three times before the filtered fraction was collected to ensure that there was no un-entrapped acitretin in the filtrate. The niosomes were then collected in amber-colored vials and stored in a refrigerator at 5-8 °C for future studies.

Effect of different sonication/hydration times on niosomes' characteristics

At a fixed molar ratio of nonionic surfactant, span 60, and cholesterol, nine niosome formulations were prepared using the thin film hydration technique as presented in Table 1. Different hydration (10, 20, and 30 min) and sonication (0.5, 1, and 2 min) times were applied in order to determine the best conditions for vesicle formation. Similar amounts of the drug (1 mg) were loaded into the formulations. The niosome formulations were characterized according to size, polydispersity index, zeta potential and entrapment efficiency.

Table 1. Design of the experiment investigating optimization of the thin film hydration method

Formulation (F)	Sonication Time (min)	Hydration Time (min)	Span 60/Cholesterol (molar ratio)	Drug Content (mg)
F1	0.5	10	1:1	1
F2	1	20	1:1	1
F3	2	30	1:1	1
F4	0.5	20	1:1	1
F5	1	30	1:1	1
F6	2	10	1:1	1
F7	0.5	30	1:1	1
F8	1	10	1:1	1
F9	2	20	1:1	1

Incorporation of secondary surfactant

Four niosome formulations were prepared using non-ionic surfactants (Brij c10, Brij s100, Brij s20, and Brij 93) in combination with the non-ionic surfactant span 60 and cholesterol at a molar ratio of 1:1:2. One mg of acitretin was loaded into all niosome formulations. The niosomes was characterized according to size, PDI, zeta potential, and entrapment efficiency.

Variability studies

To study the effect of different molar ratios of surfactants to cholesterol on niosomes' characteristics, six niosome formulations were prepared using our modified thin film hydration method. The secondary lead surfactant (Brij 93) was mixed with span 60 and cholesterol at different molar ratios, as presented in Table 2. Then, 1 mg of acitretin was loaded into all formulations and niosomes were characterized according to size, PDI, zeta potential, entrapment efficiency, and drug release.

Table 2. Design of variability studies

Formulation (F)	Span 60 (molar ratio)	Brij 93 (molar ratio)	Cholesterol (molar ratio)	Drug Content (mg)
F5-4A	1	1	2	1
F5-4B	1	2	2	1
F5-4C	2	1	2	1
F5-4D	1	2	1	1
F5-4E	2	1	1	1
F5-4F	1	1	1	1

Measurement of size, PDI, and zeta potential

Vesicle size and the distribution of prepared niosomes were measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS-90 instrument (Malvern Instruments, Worcestershire, UK). Measurement was performed at a scattering angle of 90° after diluting and equilibrating the suspension at 25 °C for 60 s. All measurements were performed in triplicate, and the results are presented as the average value ± the standard deviation.

Measurement and calculation of entrapment efficiency

The entrapment efficiency, which is expressed as a percentage of the total amount of acitretin encapsulated in niosomes, was determined using a centrifugation method. The prepared niosomes were first filtered using Sephadex column G-50 to separate the entrapped drug from the untrapped drug. After that, we collected the filtered portion of niosomes and ruptured the lipid structure using Triton-X100 (0.01 % v/v) to extract the entrapped drug. Then, the niosomal suspension was separated by centrifugation at 15,000 rpm for 15 min. Finally, the supernatant was taken and quantified using HPLC. The same process was performed with the non-filtered niosomes to obtain the untrapped drug. Entrapment efficiency was calculated by comparing the entrapped (filtered) and untrapped (non-filtered) amounts of the drug using the following equation:

$$E\% = \frac{D_{nf} - D_f}{D} \times 100$$

***In vitro* release studies**

An *in vitro* drug release study was carried out using a 250 ml glass beaker. A dialysis membrane (10 kDa) was filled with 0.5 ml of niosome formulations and immersed in 200 ml of ethanol/PBS (20:80) receiver medium. The temperature of the dispersion medium was maintained at 25 °C with magnetic stirring at 300 rpm throughout the experiment. A sample volume of 1 ml was taken from the receiver medium at different time points during the 12 h experiment and replaced with the same amount of fresh medium to maintain the conditions. The samples were analyzed and quantified using HPLC.

***In vitro* permeation studies**

A fresh porcine skin was brought from a local slaughterhouse. The abdominal skin regions were taken and shaved using an electric shaver. The hairless skin was cut into small rectangular pieces. Then, the pieces were covered with aluminum foil and immersed in a water bath maintained at 60 °C for 2 min. After 2 min, the aluminum foil was removed and the epidermis was carefully mounted by hand on clean glass slides. Then, the epidermis was kept for 12 h until it was completely dried and then was stored in a refrigerator at 5 °C for future use. The *in vitro* skin permeation study was performed using Franz diffusion cells with an effective diffusion area of 0.64 cm². A hairless porcine epidermis was sandwiched between the donor and receiver medium with the SC side facing the donor compartment. The receiver compartment was filled with 5 ml of an ethanol/PBS solution (20:80), maintained at 37 °C, and continuously stirred throughout the experiment. Then, 0.5 ml of niosome suspension was loaded into the donor compartment. Acitretin and excipient solutions were used as controls. One ml samples were withdrawn from the receiver medium at different time points and replaced by equal quantities of fresh medium. The amount of

acitretin in these samples was quantified and determined using HPLC. During the 24 h experiment, the donor and receiver media were covered with aluminum foil to prevent light exposure. The cumulative amount of drug permeated over time was plotted and compared with the drug and excipient controls.

***In vitro* skin deposition studies**

A skin deposition study was performed to estimate the amount of drug retained in the epidermis. At the end of the permeation study, the epidermis was carefully removed from the diffusion cells and washed with a water/methanol solution 3 times to remove excess formulations remaining on the top layer. The epidermis was kept for 6 hours until it was completely dry. The diffusion area was cut using a tube-puncturing cutter and accurately weighed. Next, 1 ml of NaOH (0.1 N) was added to the epidermis, which was then kept overnight in a rotary shaker until completely dissolved. Then, 1 ml of acetonitrile was added to the dissolved epidermis in order to extract the retained drug. The mixture was kept in a rotary shaker for 6 h until phase separation clearly appeared. The upper phase, which contains the drug, was removed and centrifuged for 15 min at 15,000 rpm. The supernatant was then removed and the drug content was determined using HPLC.

HPLC analysis

An isocratic HPLC method was developed for quantifying acitretin. The experiment was performed using a water HPLC system (Water 600 Controller, USA) equipped with a 600 pump unit, a 717 plus autosampler with an injection valve with a sample loop of 50 μ l, and a 2487 dual absorbance UV detector. The mobile phase consists of 0.01 % acetonitrile/formic acid solution (90:10 v/v) and was delivered at a flow rate of 1 ml/min⁻¹. Then, 20 μ l of the injection was eluted

in a LUNA 54, C18, 4.6×150 mm column (Phenomonex, USA) at room temperature. The column eluent was monitored at 360 nm, and the acitretin peaks were separated at a retention time of 5 min.

Stability studies

Physical stability studies were carried out to investigate the leaking of acitretin from niosomes and the thermal stability of niosomal acitretin. Niosome formulations were sealed in 10 ml amber-colored glass vials and stored at three different temperatures (5 °C, 25 °C, and 37 °C) for a period of 90 days. Samples from each patch were taken at time points of 0, 30, and 90 days. The niosomes' drug content, size, PDI, and zeta potential were measured and compared.

DEVELOPMENT AND OPTIMIZATION OF NIOSOME FORMULATIONS

Results

The effect of different sonication/hydration times on niosomes' characteristics

Table 3. Characterization of niosomal formulations

Formulation (F)	Entrapment Efficiency (EE %)	Particle Size (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)
F1	36±0.10	800±0.01	1.00±0.01	-16.00±0.15
F2	39±9.21	405±3.20	0.61±0.03	-9.33±0.58
F3	57±2.24	184±1.01	0.80±0.01	-5.00±0.65
F4	81±5.50	756±1.02	0.58±0.04	-4.33±0.57
F5	72±2.10	397±0.10	0.58±0.03	-13.33±0.57
F6	18±1.02	220±1.21	0.43±0.01	-5.67±1.52
F7	87±2.01	812±1.03	0.66±0.20	-12.17±0.29
F8	27±10.31	519±3.01	1.00±0.04	-3.67±0.58
F9	13±9.03	200±1.03	0.80±0.01	-4.33±0.58

The effect of different sonication times on niosomes' characteristics

Sonication time is an important variable during the preparation of niosomes. It can influence the size, PDI, and entrapment efficiency of niosomes. Proper sonication time can lead to less variation in vesicles' size and hence less particle aggregation. We used different sonication times (0.5, 1, and 2 min) to determine their effect on the entrapment efficiency of niosomes. The results of these trials demonstrated that sonication time had a significant effect ($P < 0.05$) on entrapment efficiency. As presented in Table 3, high average entrapment efficiency was obtained for niosome formulations sonicated for 0.5 min (68 ± 0.15 %), while medium average entrapment

efficiency was obtained for niosomes sonicated for 1 min ($46 \pm 0.30\%$) and low average entrapment efficiency was obtained for niosomes sonicated for 2 min ($29 \pm 1.01\%$). Also, the results showed that sonication time significantly affected the size of niosomes ($P < 0.05$). The average size of niosomes sonicated for 0.5 min was 789 ± 1.01 nm, compared to 440 ± 0.01 nm for those sonicated for 1 min and 272 ± 0.03 nm for those sonicated for 2 min. Sonication time was found to have no significant effect on PDI ($P > 0.05$).

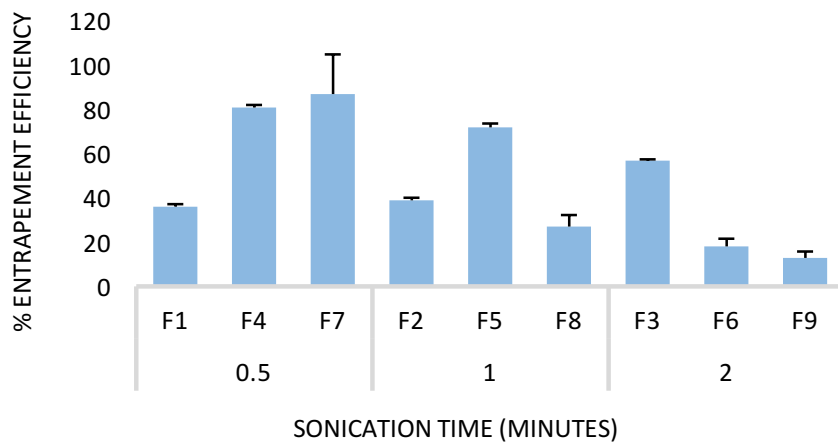


Figure 4. The entrapment efficiency of niosome formulations, obtained by applying different sonication times (0.5, 1 and 2 min)

The effect of different hydration times on niosomes' characteristics

Hydration time is another important factor in the formation of niosomes. Improper hydration time can result in the formation of fragile niosomes or leakage problems. To determine the optimal hydration time, several were applied. The results showed that high average entrapment efficiency was obtained for niosome formulations hydrated for 30 min ($72 \pm 2.24\%$), medium average entrapment efficiency was obtained for niosomes hydrated for 20 min ($44 \pm 1.03\%$), and very low average entrapment efficiency was obtained for niosomes hydrated for 10 min (27 ± 1.02

%) (Table 3). The results of this study did not show that hydration time had a significant effect on the niosomes' size, PDI, or zeta potential ($p>0.05$).

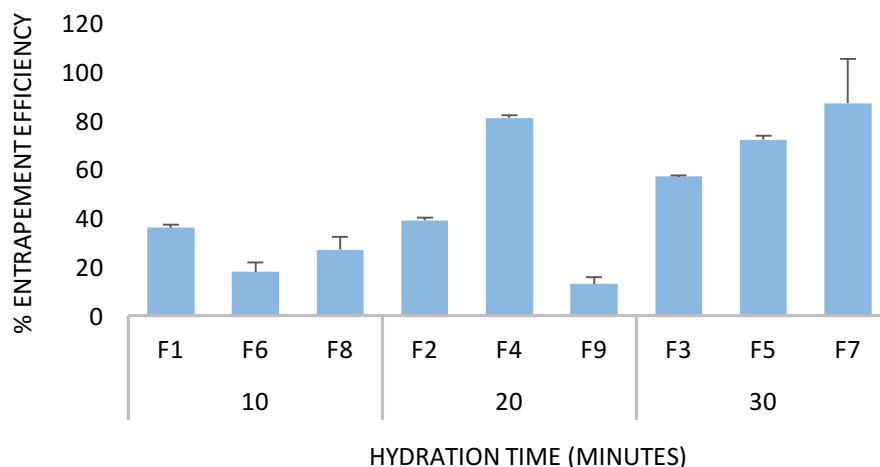


Figure 5. The entrapment efficiency of niosome formulations, obtained by applying different hydration times (10, 20, and 30 min)

Effect of incorporating a secondary surfactant on the characterized formulation

The main goal of incorporating a second surfactant into a niosome formulation (F5) is to further improve the entrapment efficiency of the system. Adding another surfactant may create a synergistic effect that could form niosomes with high entrapment efficiency. From a pharmaceutical viewpoint, entrapment efficiency is one of the most important factors affecting niosome formulations. Niosomes with high entrapment efficiency require less time and effort to remove unentrapped material during manufacturing. Additionally, during topical delivery, high niosome entrapment efficiency allows more API to be permeated and localized within the skin. To achieve this goal, four different nonionic surfactants were chosen based on their hydrophilic-lipophilic balance (HLB) to be incorporated into a previously characterized formulation (F5)

(Table 4). The results showed that, among the four surfactants, Brij 93 produced the highest entrapment efficiency. The entrapment efficiency of niosomes formed using Brij 93 as a secondary surfactant was 90 ± 0.03 %, compared to 30 ± 0.12 % for those formed with Brij c10, 13 ± 1.01 % for those formed with Brij s100, and 20 ± 0.10 % for those formed with Brij s20.

Table 4. Characterization of niosome formulations with secondary surfactants

Formulation (F)	Type of surfactant	(HLB) Value	Size (nm)	Polydispersity index (PDI)	Zeta Potential (mV)	Entrapment Efficiency (EE%)
F5-1	Brij C10	12	30 ± 0.12	353 ± 1.29	0.65 ± 0.54	-19 ± 0.65
F5-2	Brij S100	18	13 ± 1.01	279 ± 0.65	0.7 ± 0.05	-21 ± 1.51
F5-3	Brij S20	15	20 ± 0.10	312 ± 1.54	0.3 ± 0.04	-11 ± 0.54
F5-4	Brij 93	4	90 ± 0.03	420 ± 0.35	0.5 ± 0.05	-18 ± 0.75

Discussion

One of the most commonly used laboratory methods for niosome preparation and drug loading identified in the literature is the thin film hydration method. This method was previously described by Bangham et al. (1965) for the preparation of liposomes. However, this method requires further vesicle optimization for topical delivery of acitretin. Our aim was to obtain high API entrapment efficiency and a vesicle size of 200–600 nm. It was reported that a smaller particle size (≤ 200 nm) will easily permeate skin, especially psoriatic skin, where the SC lacks integrity, and enter into systemic circulation. This is not favorable for our purpose. In addition, it was reported that a larger particle size (≥ 600 nm) will not permeate the SC barrier (Verma, Verma, Blume, & Fahr, 2003). Thus, we believe that niosomes sized between 200 and 600 nm will help us achieve our aim.

To achieve our aim, different sonication (0.5, 1 and 2 min) and hydration (10, 20 and 30 min) times were applied to determine the effect of these parameters on the niosomes' characteristics. We found a negative relationship between sonication time and entrapment efficiency; as we increased the sonication time, entrapment efficiency decreased (Fig. 6). The highest entrapment efficiency was obtained with niosome formulations F4, F5, and F7. However, F4 and F7 were excluded because of their high average size (>600 nm). The optimum formulation in terms of sonication time was F5, which was sonicated for 1 min. Hydration time, on the other hand, was positively related to the entrapment efficiency of niosome formulations (Fig. 5). The highest entrapment efficiency was obtained with niosome formulations F5 and F7. However, F7 was excluded because of its size (>600 nm). The optimum formulation in terms of hydration time was F5, which was hydrated for 30 min, perhaps because new vesicles form during long hydration times. The hydration medium and time highly influence the self-assembly of non-ionic surfactants. Increasing the hydration time during self-assembly formation of niosomes will allow more vesicles that can accommodate more API to be formed.

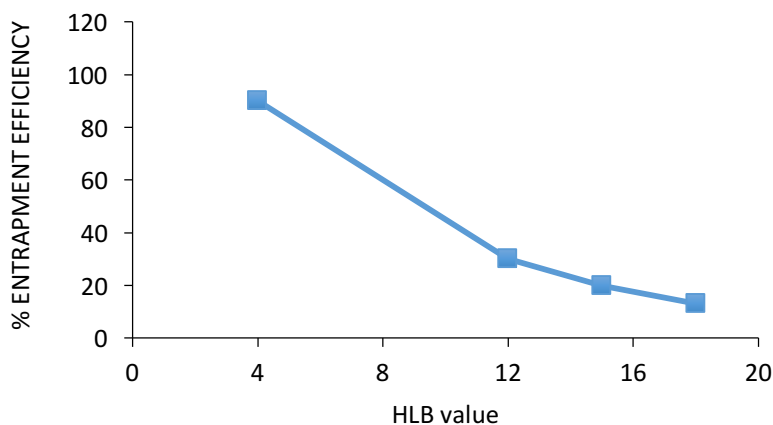


Figure 6. The entrapment efficiency of niosome formulations prepared using different HLB value surfactants

Also, a negative relationship was found between entrapment efficiency and the HLB values of the second surfactants (Fig. 4). The highest entrapment efficiency was obtained for niosomes prepared using surfactants with low HLB values, and vice versa. A low HLB value indicates a long hydrocarbon chain compared to hydrophilic surface area. A long hydrocarbon chain will increase the lipophilic character of surfactants. Surfactants with low HLB values, like Brij 93, have a more lipophilic environment to accommodate lipophilic drugs like acitretin ($\log p = 6.4$).

Conclusion

To sum up, the thin film hydration method with 1 min sonication time and 30 min hydration time was found to create the optimum niosome formulation (F5). The nonionic surfactant Brij 93 (HLB=4) is used as a lead secondary surfactant to form niosomes with the highest entrapment efficiency (F5-4).

VARIABILITY STUDIES OF THE CHARACTERIZED NIOSOMES

Results

The effect of various molar ratios of surfactants to cholesterol on niosomes' characteristics

After we determined the optimum surfactants for topical niosome formulations, we aimed to identify the optimal molar ratio of surfactants to cholesterol for obtaining the lead niosome formulation. We have conducted this set of experiments to determine the effect of various molar ratios of the lead secondary surfactant Brij 93, span 60, and cholesterol on niosomes' size, PDI, zeta potential, entrapment efficiency, and drug release.

Table 5. Characterization of niosomes and effect of various molar ratios of surfactants to cholesterol

Formulation (F)	Size (nm)	Polydispersity index (PDI)	Zeta Potential (mV)	Entrapment Efficiency (EE%)	Drug Release (%)
F5-4A	471±1.15	0.41±0.04	-21±0.26	92±2.70	30.80±0.21
F5-4B	549±1.06	0.80±0.10	-17±1.37	83±0.69	46.72±1.16
F5-4C	463±1.75	0.58±0.09	-20±0.61	81±1.30	40.17±1.10
F5-4D	361±1.03	0.58±0.02	-12±0.61	64±3.19	64.70±0.53
F5-4E	364±2.21	0.43±0.02	-13±1.31	68±2.27	70.00±0.64
F5-4F	404±1.71	0.66±0.09	-15±0.72	71±21.14	81.12±1.61

The effect of the lead secondary surfactant Brij 93 and span 60 on niosomes' characteristics

Using different molar ratios of span 60 to Brij 93 had no significant effect ($P>0.05$) on niosomes' characteristics. Maintaining the levels of cholesterol and Brij 93 and changing the level of span 60 showed no significant differences in the size, PDI, zeta potential, entrapment efficiency, or drug release of niosomes, nor did maintaining the levels of span 60 and cholesterol and changing the level of Brij 93 (Tables 2 and 5).

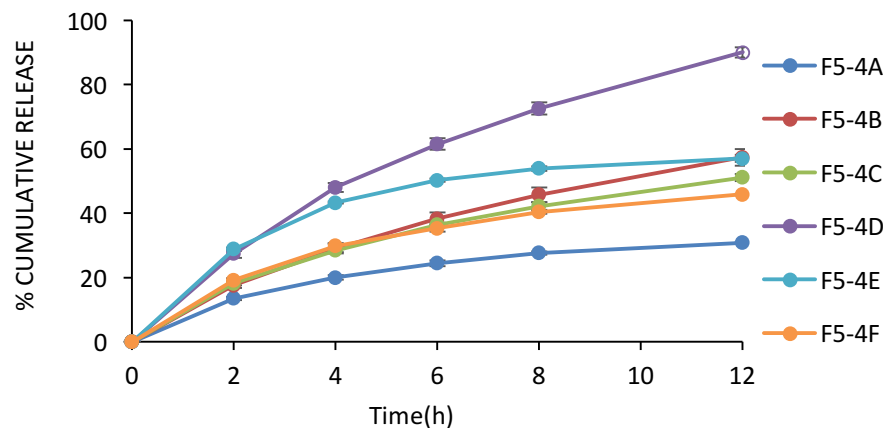


Figure 7. *In vitro* drug release of various formulations of acitretin niosomes

The effect of cholesterol on niosomes' characteristics

Using different molar ratios of cholesterol had no significant effect ($P>0.05$) on the size, PDI, or zeta potential of niosome formulations. Maintaining the levels of span 60 and Brij 93 and changing the level of cholesterol showed no significant difference in the size, PDI, or zeta potential of niosomes (Table 2 and 5). However, entrapment efficiency was significantly influenced by changing the cholesterol level in niosome formulations ($P<0.05$). The average entrapment efficiency of niosome formulations F5-4D, F5-4E, and F5-4F (cholesterol=molar ratio of 1) was low (64 ± 3.19 , 68 ± 2.27 , and 71 ± 1.14 %, respectively). However, high average entrapment

efficiency (92 ± 2.70 , 83 ± 0.69 and 81 ± 1.30 %, respectively) was obtained for niosome formulations F5-4A, F5-4B, and F5-4C (cholesterol=2 molar ratio) (Fig. 8). Moreover, the *in vitro* release studies of formulations revealed that using different molar ratios of cholesterol had a significant effect ($P < 0.05$) on niosomes' drug release. Formulations prepared using low concentrations of cholesterol (F5-4D, F5-4E, and F5-4F) resulted in faster drug release (64 ± 0.53 , 70 ± 0.64 , and 81 ± 1.61 %, respectively). However, the other formulations prepared using higher concentrations of cholesterol (F5-4A, F5-4B, and F5-4C) achieved slower and more controlled drug release (30 ± 0.21 , 46 ± 1.16 , and 40 ± 1.10 %, respectively) (Fig. 9).

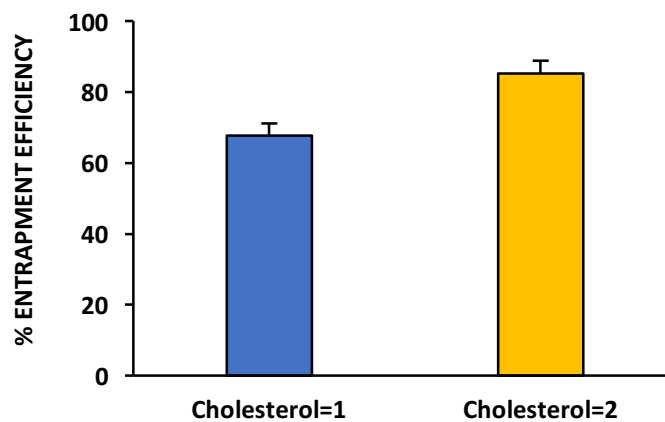


Figure 8. The average entrapment efficiency of niosome formulations F5-4A, F5-4B, and F5-4C (cholesterol=molar ratio of 1) compared to niosome formulations F5-4D, F5-4E, and F5-4F (cholesterol=molar ratio of 2)

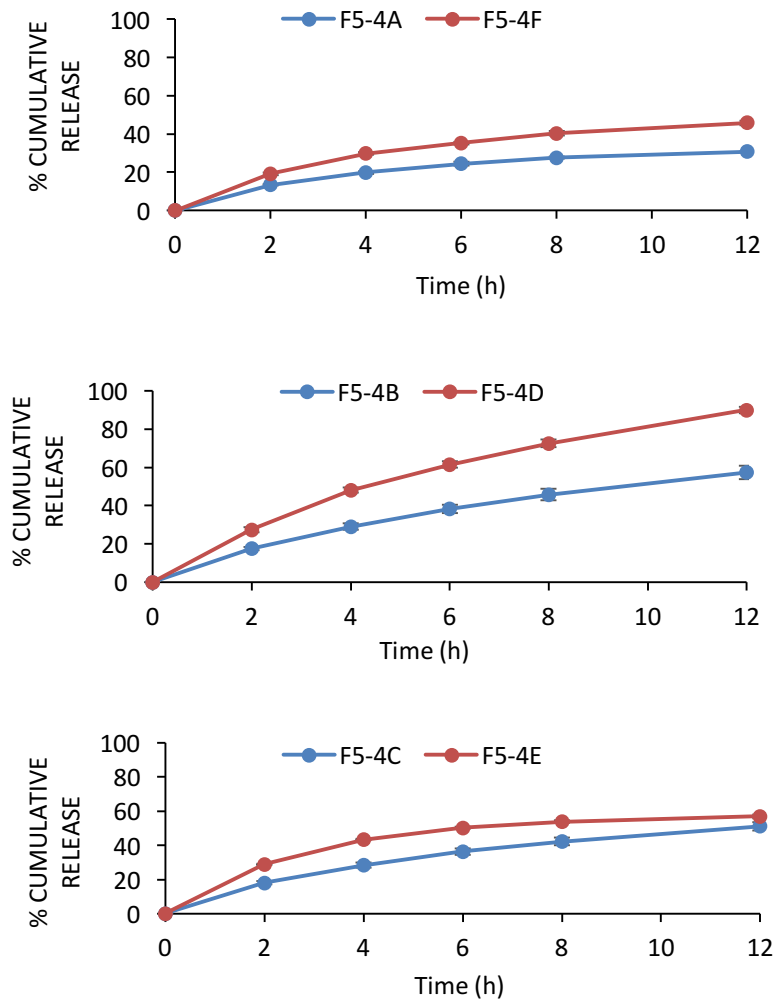


Figure 9. *In vitro* drug release of niosome formulations with molar ratio of 1 for cholesterol compared to formulations with a molar ratio of 2 for cholesterol at the same surfactant concentration

Discussion

Cholesterol has an important impact on niosomes' entrapment efficiency and drug release. Cholesterol influences the physical properties and structure of niosomes through its interaction with nonionic surfactants. It can affect formulations by modulating the cohesion, mechanical strength, and water permeability of the lipid bilayer. In fact, inclusion of cholesterol into niosome formulations could increase the viscosity and rigidity of the bilayer structure. Drug partitioning in

the lipid bilayer will be easier with a highly structured system of surfactants and cholesterol. By using more cholesterol, the lipid bilayer will become more rigid and, consequently, more drugs will be encapsulated within the bilayer structure and will be less likely to escape. This may explain the high entrapment efficiency and slow drug release of niosome formulations containing more cholesterol. The best entrapment efficiency and drug release were obtained for niosome formulation F5-4A, which was prepared with a 1:1:2 molar ratio of span 60, Brij 93, and cholesterol, respectively.

Conclusion

In conclusion, 1:1:2 is the optimum molar ratio of span 60, Brij 93, and cholesterol, respectively, for preparing niosomes for controlled topical delivery of acitretin. The lead niosome formulation was F5-4A.

LEAD NIOSOMAL FORMULATION STABILITY STUDIES

Results

Stability studies of vesicular size, PDI, and zeta potential

During a 90-day stability study, the results showed that at all temperatures (4 °C, 24 °C, and 40 °C), the niosome formulations showed no significant changes in size, PDI, or zeta potential ($P < 0.05$) (Fig. 10). After 90 days, the average niosome sizes were 458 ± 0.13 , 465 ± 0.43 , and 471 ± 0.34 nm at 4 °C, 24 °C, and 40 °C, respectively, compared to 425 ± 1.04 nm at day 0. Also, the niosomes' PDIs were 0.51 ± 0.01 , 0.54 ± 0.03 , and 0.56 ± 0.03 at 4 °C, 24 °C, and 40 °C, respectively, compared to 0.42 ± 0.03 at day 0. Finally, the niosomes' zeta potentials were -11 ± 0.32 , -10 ± 0.31 , and -9 ± 0.43 mV at 4 °C, 24 °C, and 40 °C, respectively, compared to -21 ± 0.51 mV at day 0.

Stability studies of niosomes' drug content

Ninety-day drug content stability studies were performed to study the drug release from the lead niosome formulation. The results showed non-significant drug release from niosomes over 90 days at all temperatures ($P > 0.05$) (Fig. 11). After 90 days, the percentages of drug retained in the niosomes were 88 ± 0.14 %, 86 ± 0.32 %, and 82 ± 0.21 % at 4 °C, 24 °C, and 40 °C, respectively, compared to 100 % at day 0.

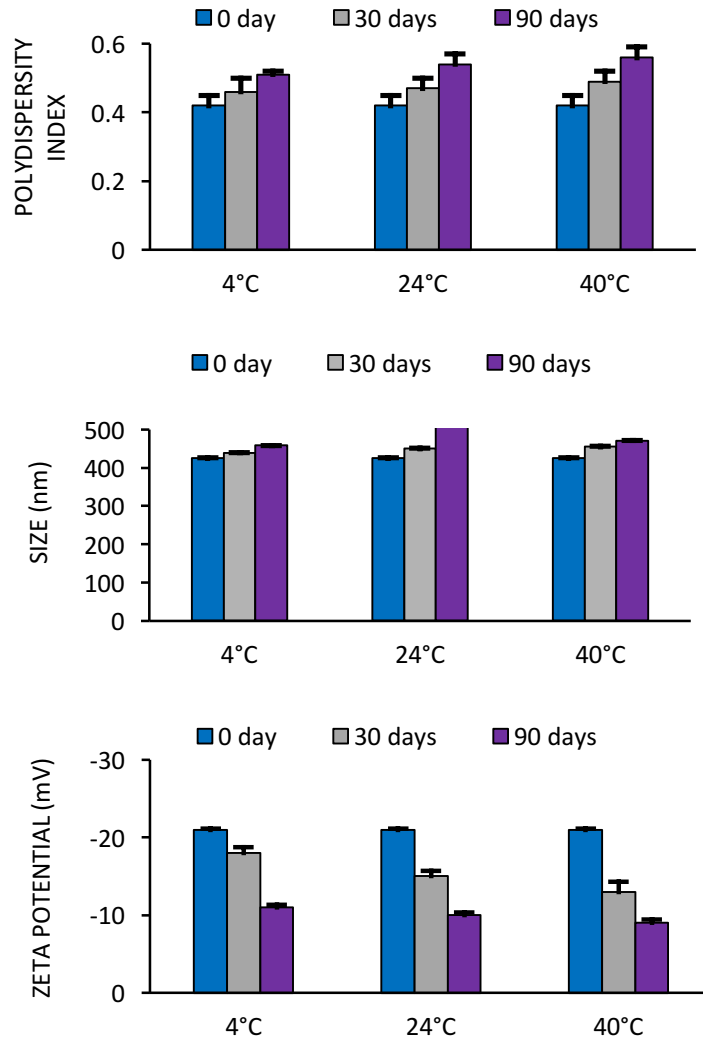


Figure 10. The size, PDI, and zeta potential of the best niosome formulation stored at different temperatures (4 °C, 24 °C, and 40 °C) for 0, 30, and 90 days

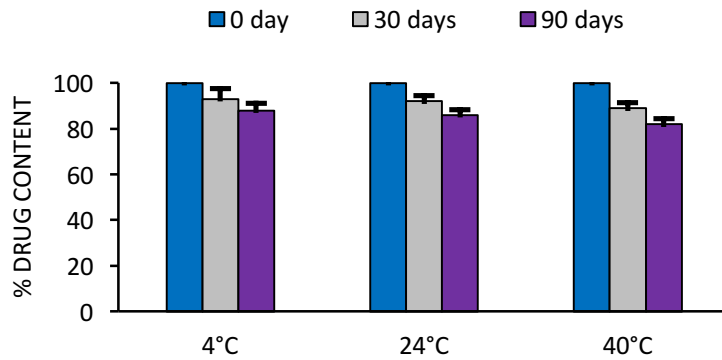


Figure 11. The drug content of the lead niosome formulation stored at different temperatures (4 °C, 24 °C, and 40 °C) for 0, 30, and 90 days

***In vitro* thermal degradation studies**

In vitro thermal degradation studies were performed to study and compare the thermal degradation of acitretin in solution with respect to the lead niosome formulation. The results showed less degradation of acitretin in the niosome formulation compared to acitretin in solution (Table 6). With the niosome formulation, the drug's half-life was 501.51, 429.87, and 334.34 days at 4 °C, 24 °C, and 40 °C, respectively, compared to 115.75, 60.81, and 45.59 days at 4 °C, 24 °C, and 40 °C, respectively, for acitretin in solution.

Table 6. *In vitro* thermal degradation studies of acitretin niosome formulation versus acitretin in solution

Acitretin Drug Formulation	Half-Life (Days)		
	4 °C	24 °C	40 °C
Acitretin Niosome Formulation	501.51	429.87	334.34
Acitretin in Solution	115.75	60.18	45.59

Discussion

The stability data demonstrates that the lead niosome formulation has good physical stability. Over a period of 90 days, there was no sign of vesicle aggregation in niosome samples stored at different temperatures. The high negative zeta potential indicates the stability of the colloidal system. If all the particles in the suspension have a high negative zeta potential, then they will tend to repel each other and there will be no chance for them to come together. Also, the data from these studies revealed that the lead niosome formulation has good chemical and thermal stability. The niosomes showed good ability to reduce the thermal degradation of acitretin. The

highly arranged bilayer structure of niosomes could play an important role in protecting the drug molecule from degradation as it might provide a cooler environment for the drug than the outside environment.

Conclusion

Highly physically, chemically, and thermally stable niosomes can be prepared using Brij 93, span 60, and cholesterol with a molar ratio of 1:1:2, respectively. After a period of 90 days, the prepared niosomes had low particle aggregation, drug release, and degradation.

EVALUATION OF LEAD NIOSOME FORMULATION USING ACITRETIN AS A TOPICAL DELIVERY SYSTEM

Results

In vitro skin permeation studies across intact porcine epidermis

In order to study the influence of niosome formulations on acitretin diffusion through the skin, we carried out an *in vitro* permeation study using a porcine epidermis and Franz diffusion cells. During this study, we compared the permeation data obtained from niosomal acitretin with those obtained from the drug and excipient controls. Fig. 12 shows the permeation profile (cumulative amount of acitretin permeated versus time) of acitretin through the epidermis obtained from niosomes in comparison with controls. The niosomes showed improvement in cumulative drug percutaneous permeation ($0.14 \pm 0.01 \mu\text{g}/\text{cm}^2$) compared to that of the drug control ($0.03 \pm 0.02 \mu\text{g}/\text{cm}^2$) and the excipient control ($0.04 \pm 0.01 \mu\text{g}/\text{cm}^2$). The flux rate was $0.004 \pm 0.01 \text{ ng}/\text{cm}^2/\text{h}$ for the niosome formulation, $0.0007 \pm 0.02 \text{ ng}/\text{cm}^2/\text{h}$ for the drug control, and $0.001 \pm 0.03 \text{ ng}/\text{cm}^2/\text{h}$ for the excipient control.

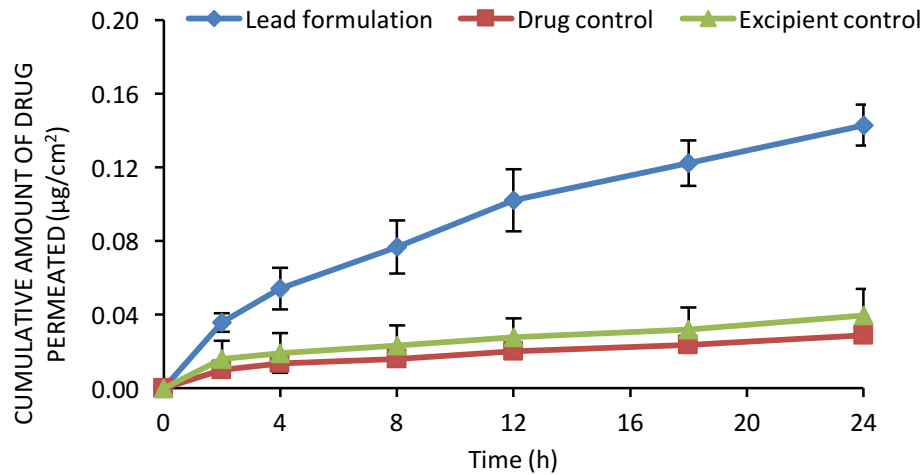


Figure 12. Cumulative amount of acitretin permeated from best niosome formulation (F5-4A) across a porcine epidermis

***In vitro* skin deposition studies using intact porcine epidermis**

At the end of the permeation studies, the epidermis was removed and the amount of drug retained was measured to assess the skin deposition capability of niosomes. The results showed that the drug deposition of niosomes was significantly higher than that of the controls (Fig. 13). The amount of drug deposited within the epidermis was 230 ± 01.02 ng/mg with niosomes, compared to 46 ± 0.02 ng/mg with the drug control and 84 ± 02.01 ng/mg with the excipient control.

***In vitro* skin permeation studies across tab-stripped porcine epidermis**

The previous permeation studies were performed using an intact epidermis. However, in cases of psoriasis, the SC might be compromised, so to better assess the permeability of our lead niosome formulation, we carried out a permeation study with an epidermis after SC was removed in order to simulate real psoriatic skin conditions. The results showed that the niosomes were able to control the permeation of the drug across damaged skin (Fig. 14). The skin permeability of

niosomes was $1.87 \pm 0.09 \mu\text{g}/\text{cm}^2$, compared to $3.6 \pm 0.02 \mu\text{g}/\text{cm}^2$ for the drug control and $2.4 \pm 0.08 \mu\text{g}/\text{cm}^2$ for the excipient control.

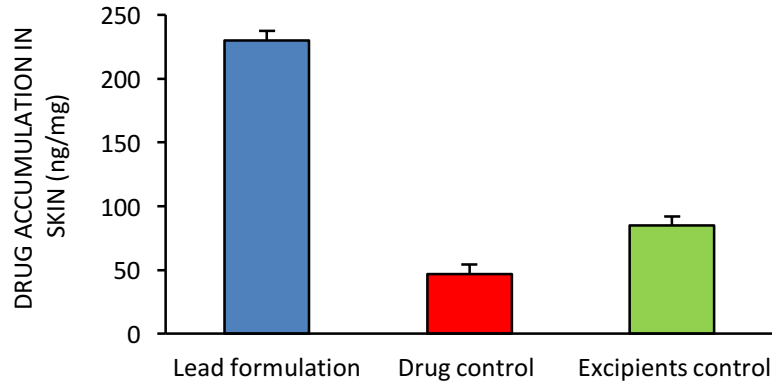


Figure 13. *In vitro* skin deposition study of acitretin-loaded best niosome formulation (F5-4A)

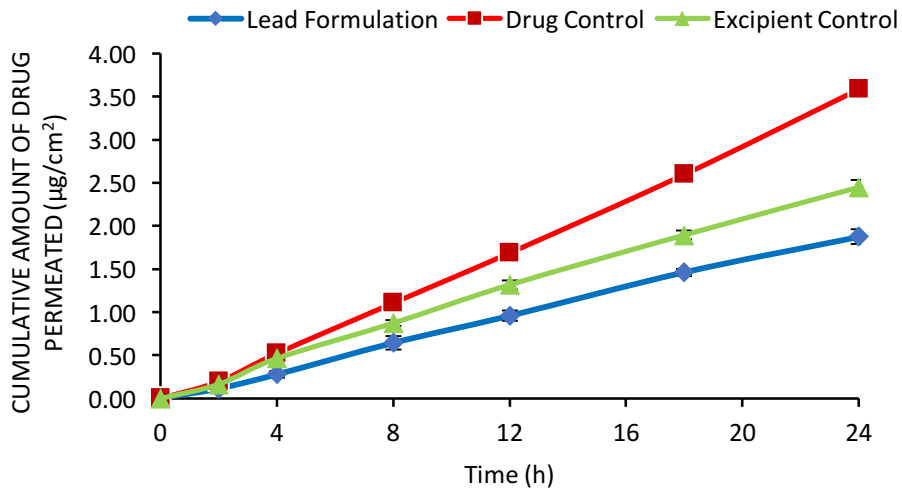


Figure 14. *In vitro* permeation study of best niosome formulation with acitretin (F5-4A) compared to drug and excipient controls across tab-stripped porcine epidermis

***In vitro* skin deposition studies using tab-stripped porcine epidermis**

We also performed a skin deposition study using an epidermis with tab-stripped SC in order to obtain accurate information about the ability of niosomes to deposit acitretin within a psoriatic epidermis. Again, the niosomes showed a significant improvement in the deposition of acitretin in skin compared to the controls ($P < 0.05$) (fig. 15). The amount of drug retained within epidermis was 665 ± 0.02 ng/mg for the niosomes, compared to 385 ± 0.01 ng/mg for the drug control and 205 ± 0.04 ng/mg for the excipient control.

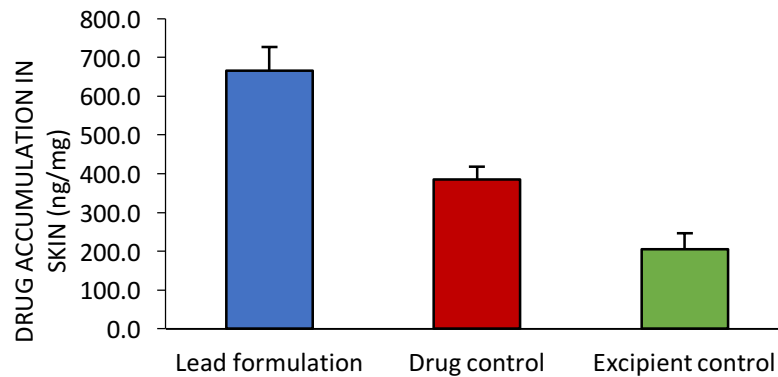


Figure 15. *In vitro* skin deposition study of best niosome formulation with acitretin (F5-4A) compared to drug and excipient controls within a tab-stripped porcine epidermis

Discussion

The higher permeability of niosomes across an intact epidermis compared to the controls might be due to the role of the nonionic surfactant span 60; it was reported that span 60 enhances skin permeation (J.-Y. Fang, Yu, Wu, Huang, & Tsai, 2001). Many mechanisms have been proposed to explain the ability of niosomes to modulate drug transfer across skin in order to understand niosomes' enhanced skin permeability (Uchegbu & Vyas, 1998) (J. Fang, Hong, Chiu, & Wang, 2001) (Vora, Khopade, & Jain, 1998). However, the most interesting mechanism is vesicles' ability to fuse on the surface of skin, which might lead to the accumulation of large

concentration gradients of intercalated drug within the skin layer and therefore increase permeation (Schreier & Bouwstra, 1994). However, when the epidermis is compromised, niosomes control topical acitretin permeation, which is considered to be an advantage of a topical approach to psoriasis treatment.

One of the main reasons to employ niosomes for topical delivery of acitretin was its dermal localization, which enhances the localized treatment of psoriasis and reduces its systemic toxicity. For this purpose, *in vitro* skin deposition of niosomes was investigated using intact and compromised porcine epidermises, and the results from these experiments revealed significant drug deposition in the epidermis with niosomes compared to the controls ($P < 0.05$) (Figs. 13 and 15). The results from these studies supported the hypothesis that incorporating acitretin into niosomes enhances drug deposition into skin epidermis. The results are also in agreement with several previous studies reporting that niosomes improve the dermal localization of several topical therapeutic agents (Abdelbary & Aboughaly, 2015) (Manconi, Sinico, Valenti, Lai, & Fadda, 2006) (Goyal et al., 2015).

Conclusion

In the current work, topical application of acitretin-loaded niosomes for management of psoriasis was investigated to avoid systemic toxicity. The lead niosomal formulation displayed an optimum particle size and high entrapment efficiency. Also, the *in vitro* thermal degradation studies confirmed that niosomes can induce the thermal stability of acitretin. Moreover, the *in vitro* skin permeation and deposition studies suggested that niosomes can control the topical delivery of acitretin and improve the drug localization at the skin pathological site. In conclusion, the results confirmed that acitretin niosomes may have profound therapeutic application in a topical approach

to the treatment of psoriasis. However, further studies and investigations using a suitable animal model are required in order to establish the superiority and safety of the developed niosomes compared to the current systemic acitretin therapy.

BIBLIOGRAPHY

- Abdelbary, A. A., & Aboughaly, M. H. H. (2015). Design and optimization of topical methotrexate loaded niosomes for enhanced management of psoriasis: Application of Box-Behnken design, in-vitro evaluation and in-vivo skin deposition study. *International Journal of Pharmaceutics*, 485(1–2), 235–243. <http://doi.org/10.1016/j.ijpharm.2015.03.020>
- Agrawal, Y., Petkar, K. C., & Sawant, K. K. (2010). Development, evaluation and clinical studies of Acitretin loaded nanostructured lipid carriers for topical treatment of psoriasis. *International Journal of Pharmaceutics*, 401(1), 93–102. <http://doi.org/10.1016/j.ijpharm.2010.09.007>
- Armstrong, A. W., Schupp, C., Wu, J., Bebo, B., Stern, R., Nijsten, T., ... Gourraud, P. (2012). Quality of Life and Work Productivity Impairment among Psoriasis Patients: Findings from the National Psoriasis Foundation Survey Data 2003–2011. *PLoS ONE*, 7(12), e52935. <http://doi.org/10.1371/journal.pone.0052935>
- de Korte, J., Mommers, F. M. C., Bos, J. D., & Sprangers, M. A. G. (2004). Quality of Life in Patients with Psoriasis: A Systematic Literature Review. *Journal of Investigative Dermatology Symposium Proceedings*, 9(2), 140–147. <http://doi.org/10.1046/j.1087-0024.2003.09110.x>
- Drake, L. A., Ceilley, R. I., Cornelison, R. L., Dobes, W. A., Dorner, W., Goltz, R. W., ... Dobes, W. L. (1993). Guidelines of care for psoriasis. *Journal of the American Academy of Dermatology*. [http://doi.org/10.1016/S0190-9622\(08\)81783-5](http://doi.org/10.1016/S0190-9622(08)81783-5)
- Fang, J.-Y., Yu, S.-Y., Wu, P.-C., Huang, Y.-B., & Tsai, Y.-H. (2001). In vitro skin permeation of estradiol from various proniosome formulations. *International Journal of Pharmaceutics*, 215(1), 91–99. [http://doi.org/10.1016/S0378-5173\(00\)00669-4](http://doi.org/10.1016/S0378-5173(00)00669-4)

- Fang, J., Hong, C., Chiu, W., & Wang, Y. (2001). Effect of liposomes and niosomes on skin permeation of enoxacin. *International Journal of*. Retrieved from <http://www.sciencedirect.com/science/article/pii/S0378517301006275>
- Gelfand, J. M., Troxel, A. B., Lewis, J. D., Kurd, S. K., Shin, D. B., Wang, X., ... HK, C. (2007). The Risk of Mortality in Patients With Psoriasis. *Archives of Dermatology*, 143(12), 136–139. <http://doi.org/10.1001/archderm.143.12.1493>
- Goldfarb, M. T., Ellis, C. N., Gupta, A. K., Tincoff, T., Hamilton, T. A., & Voorhees, J. J. (1988). Acitretin improves psoriasis in a dose-dependent fashion. *Journal of the American Academy of Dermatology*, 18(4), 655–662. [http://doi.org/10.1016/S0190-9622\(88\)70086-9](http://doi.org/10.1016/S0190-9622(88)70086-9)
- Gollnick, H., Bauer, R., Brindley, C., Orfanos, C. E., Plewig, G., Wokalek, H., & Hoting, E. (1988). Acitretin versus etretinate in psoriasis. *Journal of the American Academy of Dermatology*, 19(3), 458–468. [http://doi.org/10.1016/S0190-9622\(88\)70198-X](http://doi.org/10.1016/S0190-9622(88)70198-X)
- Goyal, G., Garg, T., Malik, B., Chauhan, G., Rath, G., & Goyal, A. K. (2015). Development and characterization of niosomal gel for topical delivery of benzoyl peroxide. *Drug Delivery*, 22(8), 1027–1042. <http://doi.org/10.3109/10717544.2013.855277>
- Griffiths, C. E., & Barker, J. N. (2007). Pathogenesis and clinical features of psoriasis. *The Lancet*, 370(9583), 263–271. [http://doi.org/10.1016/S0140-6736\(07\)61128-3](http://doi.org/10.1016/S0140-6736(07)61128-3)
- Grossman, R. M., Chevret, S., Abi-Rached, J., Blanchet, F., Dubertret, L., Mueller W, H. B., ... Pim D, C. M. B. L. (1996). Long-term Safety of Cyclosporine in the Treatment of Psoriasis. *Archives of Dermatology*, 132(6), 623. <http://doi.org/10.1001/archderm.1996.03890300039008>
- Haran, G., Cohen, R., Bar, L. K., & Barenholz, Y. (1993). Transmembrane ammonium sulfate gradients in liposomes produce efficient and stable entrapment of amphipathic weak bases.

Biochimica et Biophysica Acta (BBA) - Biomembranes, 1151(2), 201–215.

[http://doi.org/10.1016/0005-2736\(93\)90105-9](http://doi.org/10.1016/0005-2736(93)90105-9)

Katz, H. I., Waalen, J., & Leach, E. E. (1999). Acitretin in psoriasis: An overview of adverse effects. *Journal of the American Academy of Dermatology*, 41(3), S7–S12.

[http://doi.org/10.1016/S0190-9622\(99\)70359-2](http://doi.org/10.1016/S0190-9622(99)70359-2)

Lakshmi, P. K., Devi, G. S., Bhaskaran, S., & Sacchidanand, S. (n.d.). Niosomal methotrexate gel in the treatment of localized psoriasis: phase I and phase II studies. *Indian Journal of Dermatology, Venereology and Leprology*, 73(3), 157–61. Retrieved from

<http://www.ncbi.nlm.nih.gov/pubmed/17558046>

Langley, R. G. (2012). Effective and sustainable biologic treatment of psoriasis: what can we learn from new clinical data? *Journal of the European Academy of Dermatology and Venereology*, 26(s2), 21–29. <http://doi.org/10.1111/j.1468-3083.2011.04412.x>

Lee, C. S., & Li, K. (2009). A review of acitretin for the treatment of psoriasis. *Expert Opinion on Drug Safety*, 8(6), 769–779. <http://doi.org/10.1517/14740330903393732>

Liem, W. H., McCullough, J. L., & Weinstein, G. D. (1995). Effectiveness of topical therapy for psoriasis: results of a national survey. *Cutis*, 55(5), 306–10. Retrieved from

<http://www.ncbi.nlm.nih.gov/pubmed/7614843>

Manconi, M., Sinico, C., Valenti, D., Lai, F., & Fadda, A. M. (2006). Niosomes as carriers for tretinoin: III. A study into the in vitro cutaneous delivery of vesicle-incorporated tretinoin.

International Journal of Pharmaceutics, 311(1–2), 11–19.

<http://doi.org/10.1016/j.ijpharm.2005.11.045>

McKenna, K. E., & Stern, R. S. (1997). The impact of psoriasis on the quality of life of patients from the 16-center PUVA follow-up cohort. *Journal of the American Academy of*

- Dermatology*, 36(3), 388–394. [http://doi.org/10.1016/S0190-9622\(97\)80214-9](http://doi.org/10.1016/S0190-9622(97)80214-9)
- Mura, S., Pirot, F., Manconi, M., Falson, F., & Fadda, A. M. (2007). Liposomes and niosomes as potential carriers for dermal delivery of minoxidil. *Journal of Drug Targeting*, 15(2), 101–108. <http://doi.org/10.1080/10611860600991993>
- Nasr, M., Mansour, S., Mortada, N. D., & Elshamy, A. A. (2008). Vesicular aceclofenac systems: A comparative study between liposomes and niosomes. *Journal of Microencapsulation*, 25(7), 499–512. <http://doi.org/10.1080/02652040802055411>
- Olsen, E. A., Weed, W. W., Meyer, C. J., & Cobo, L. M. (1989). A double-blind, placebo-controlled trial of acitretin for the treatment of psoriasis. *Journal of the American Academy of Dermatology*, 21(4), 681–686. [http://doi.org/10.1016/S0190-9622\(89\)70236-X](http://doi.org/10.1016/S0190-9622(89)70236-X)
- Orfanos, C. E. (1999). Treatment of psoriasis with retinoids: present status. *Cutis*, 64(5), 347–53. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10582161>
- Rachakonda, T. D., Schupp, C. W., Armstrong, A. W., Armstrong, A. W., Schupp, C., Wu, J., ... Schupp, C. W. (2014). Psoriasis prevalence among adults in the United States. *Journal of the American Academy of Dermatology*, 70(3), 512–6. <http://doi.org/10.1016/j.jaad.2013.11.013>
- raja, Pillai, G., Udupa, N., & Chandrashekar, G. (2017). Anti-inflammatory activity of niosome encapsulated diclofenac sodium in arthritic rats. *Indian Journal of Pharmacology*, 26(1), 46.
- Rim, J. H., Park, J. Y., Choe, Y. B., & Youn, J. Il. (2003). The Efficacy of Calcipotriol + Acitretin Combination Therapy for Psoriasis. *American Journal of Clinical Dermatology*, 4(7), 507–510. <http://doi.org/10.2165/00128071-200304070-00006>
- Roenigk, H. H., Auerbach, R., Maibach, H., Weinstein, G., Lebwohl, M., Gubner, R., ... al., et. (1998). Methotrexate in psoriasis: Consensus conference. *Journal of the American Academy*

- of Dermatology*, 38(3), 478–485. [http://doi.org/10.1016/S0190-9622\(98\)70508-0](http://doi.org/10.1016/S0190-9622(98)70508-0)
- Saurat, J.-H., Geiger, J.-M., Amblard, P., Beani, J.-C., Boulanger, A., Claudy, A., ... Tapernoux, B. (1988). Randomized Double-Blind Multicenter Study Comparing Acitretin-PUVA, Etretinate-PUVA and Placebo-PUVA in the Treatment of Severe Psoriasis. *Dermatology*, 177(4), 218–224. <http://doi.org/10.1159/000248567>
- Schreier, H., & Bouwstra, J. (1994). Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. *Journal of Controlled Release*, 30(1), 1–15. [http://doi.org/10.1016/0168-3659\(94\)90039-6](http://doi.org/10.1016/0168-3659(94)90039-6)
- Takeda, K., Arase, S., & Takahashi, S. (1988). Side Effects of Topical Corticosteroids and their Prevention. *Drugs*, 36(Supplement 5), 15–23. <http://doi.org/10.2165/00003495-198800365-00005>
- Tanew, A., Guggenbichler, A., Hönigsmann, H., Geiger, J. M., & Fritsch, P. (1991). Photochemotherapy for severe psoriasis without or in combination with acitretin: A randomized, double-blind comparison study. *Journal of the American Academy of Dermatology*, 25(4), 682–684. [http://doi.org/10.1016/0190-9622\(91\)70253-X](http://doi.org/10.1016/0190-9622(91)70253-X)
- Uchegbu, I. F., & Vyas, S. P. (1998). Non-ionic surfactant based vesicles (niosomes) in drug delivery. *International Journal of Pharmaceutics*, 172(1), 33–70. [http://doi.org/10.1016/S0378-5173\(98\)00169-0](http://doi.org/10.1016/S0378-5173(98)00169-0)
- Verma, D. D., Verma, S., Blume, G., & Fahr, A. (2003). Particle size of liposomes influences dermal delivery of substances into skin. *International Journal of Pharmaceutics*, 258(1–2), 141–51. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12753761>
- Vora, B., Khopade, A., & Jain, N. (1998). Proniosome based transdermal delivery of levonorgestrel for effective contraception. *Journal of Controlled Release*. Retrieved from

<http://www.sciencedirect.com/science/article/pii/S0168365997001004>

Winterfield, L. S., Menter, A., Gordon, K., & Gottlieb, A. (2005). Psoriasis treatment: current and emerging directed therapies. *Annals of the Rheumatic Diseases*, 64 Suppl 2(suppl 2), ii87-90; discussion ii91-2. <http://doi.org/10.1136/ard.2004.032276>

Wood, A. J. J., Greaves, M. W., & Weinstein, G. D. (1995). Treatment of Psoriasis. *New England Journal of Medicine*, 332(9), 581–589.
<http://doi.org/10.1056/NEJM199503023320907>

ZACHARIAE, H., KRAGBALLE, K., HANSEN, H. E., MARCUSSEN, N., & OLSEN, S. (1997). Renal biopsy findings in long-term cyclosporin treatment of psoriasis. *British Journal of Dermatology*, 136(4), 531–535. <http://doi.org/10.1046/j.1365-2133.1997.6101586.x>

VITA

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