Nano-Lipoidal Carriers of Isotretinoin with Anti-aging Potential: Formulation, Characterization and Biochemical Evaluation

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Running Title: Anti-photoaging Topical Formulation of Nano-colloidal Isotretinoin

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Abstract: Isotretinoin (ITR) is a drug of choice in the treatment of all types of acne, including recalcitrant, severe and nodulocystic. The most widely employed route of its administration, i.e., oral intake, is reported to be associated with severe side-effects including teratogenicity, skin dryness and psychological disorders. Topical delivery, though advised for ITR, is marked with several hiccups like irritation, erythema and peeling of skin. The current studies, therefore, were embarked upon to develop "optimized" SLNs of ITR employing Formulation by Design (FbD) approach. The developed system was characterized and evaluated for skin compliance, skin transport characteristics and anti-acne potential against testosterone-induced acne in male Laca mice. The SLNs were able to transport the drug to various skin layers effectively while formed drug micro-reservoirs. The nano-colloidal systems showed marked anti-acne potential and tolerability on the mouse skin vis-à-vis the marketed product. The optimized SLNs exhibited drug entrapment of 89.49±4.1 %, while the size was found to be in nano-range (i.e., 75.3±2.4 nm). The ITR formulation was found to be stable too as per ICH guidelines. The results vouch immense promise of the optimized SLNs of ITR in reducing dermal irritation and increasing the therapeutic performance, thus resulting in an efficacious and patient-complaint formulation.
Acne: An understanding of the disease and its impact on life

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Abstract
Acne is one of the most common skin diseases affecting majority of the teens and reaching its pinnacle during adulthood. In certain severe cases, it mounts to pronounced skin deformity. This appears to adversely dampen the self esteem of the affected which can eventually lead to depression and even suicides. The disease invariably diminishes in twenties but in some cases, it might even persist in thirties, forties and beyond and there is no such definite way to predict its spell. Majority of females suffer from mild to moderate acne at some stage of life. Although the pathogenesis still stands unknown, but some of the probable reasons could be: increased sebum production, ductal keratinization, bacterial colonization of the pilosebaceous ducts and inflammation. Effective approach towards the treatment of acne primarily rests on thorough understanding of its pathogenesis. An indispensable factor demanding consideration for its treatment is personal and family history. Apart from genetic makeup, food habits also affect the severity of the disease. This review will lay emphasis on a brief disease pathogenesis underlined in the very disease and its impact on life.

Key words:
Pimples, Quality of life, food habits, comedo, acne vulgaris, P.acne

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Introduction
Acne vulgaris is a human skin disease characterized by areas of seborrhea (red scaly skin), comedones (blackheads and whiteheads), papules, pustules, nodules and probably scarring[1]. Acne affects the skin regions with the densest sebaceous follicles populations which include face, upper part of chest and the back. Severe acne is marked by inflammation
but it can also manifest in non-inflammatory state in moderate cases[1,2]. The disease is supposed to be associated with anxiety and depression resulting in social disunion[3]. Hair follicles can be inflamed, especially on the face, chest and back. This inflammation is called acne. Acne affects many individuals, especially in their teens, but can persist well into adulthood. Acne is marked by excess oil production by hair follicles, when the follicles are irritated and when the pores get plugged which leads to (opening of the follicle) leads to increased bacteria in the follicles.

According to W.H.O: Acne is an inflammatory disease of the pilosebaceous units in the skin of the face, neck, chest and upper back. It initially appears during the onset of puberty at the time when androgenic stimulation triggers excessive production of sebum and abnormal follicular keratinization, colonization by a Gram-positive bacterium (Propionibacterium acnes) and local inflammation. P. acnes produces inflammation through the production of extracellular products such as lipases, proteases, hyaluronidases and chemotactic factors[4].

Types of Acne
- Mild acne—this includes whiteheads (closed clogged pores) and blackheads (clogged pores that are open at the skin surface and more easily noticeable).
- Moderate or severe inflammatory acne includes whiteheads and blackheads plus papules (reddened areas that are elevated above the skin surface) and areas of pustules (pimples—small bumps on the skin that contain visible fluid).
- Nodulocystic acne—nodules are deeply embedded solid, often painful lesions. These may develop additional infections and may eventually lead to scarring if not treated. Nodules can be greater than 5 mm in diameter.

The pathogenesis of Acne vulgaris is multifactorial, while the key factor is genetics[5]. If both parents had acne, 75% of children will have acne. If 1 parent had acne, then the probability becomes 25%. However, similar to other genetic conditions, not every family will have the same pattern and sometimes skipping of generations is observed.

Retention hyperkeratosis is the first identified event in the development of acne vulgaris[6]. The exact underlying cause of this hyperproliferation is not yet understood. Presently, 3 leading hypotheses have been proposed to explain why the follicular epithelium produces cells at a faster rate that are retained in individuals with acne.

First, androgenic hormones have been referred to as the initial trigger[7]. Comedones, result from follicular plugging. Additionally, it has been noticed that androgen hormone receptors are present in sebaceous glands. This gives the reason why individuals with malfunctioning androgen receptors do not develop acne[8].

Secondly, excess sebum production is another key factor in the development of acne vulgaris. Sebum production and excretion are regulated by a number of different hormones including androgens and mediators[9].

As a result, inflammation may be a primary phenomenon or a secondary phenomenon. Most of the evidence suggests a secondary inflammatory response to P acnes infestation. Interleukin 1-alpha expression has also been identified in microcomedones, and is supposed to have a role in the development of acne[10].

The main and the third underlying cause of acne is a genetic predisposition. The condition is inherited in an autosomal dominant pattern with incomplete penetrance.

Acne lesions: As already discussed, acne lesions mark the onset of the disease. Table 1 discusses various types of acne lesions which are associated in low or high frequency.
Table 1: Types of acne lesions

<table>
<thead>
<tr>
<th>Type</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comedo</td>
<td>A plug of keratin and sebum within the dilated orifice of hair follicle, invariably containing <em>P. acne</em>, <em>Staphylococcus aureus</em> and <em>Pityrosporum ovale</em></td>
</tr>
<tr>
<td>Microcomedo</td>
<td>The first acne lesion, often followed by inflammatory lesions</td>
</tr>
<tr>
<td>Open comedo</td>
<td>Comedo with a widely dilated orifice in which the pigmented impingement is invariably visible, seen as a blackhead.</td>
</tr>
<tr>
<td>Closed comedo</td>
<td>A comedo devoid of a widely dilated orifice which may rupture and cause inflammatory lesion in the dermis</td>
</tr>
<tr>
<td>Fistulated (polyporous) comedo</td>
<td>An assemblage of open comedos from interconnected hair follicles</td>
</tr>
<tr>
<td>Pustule</td>
<td>An inflammatory lesion (a small, elevated, circumscribed lesion) evolving from the microcomedo that displays a visible cap of pus.</td>
</tr>
<tr>
<td>Papule</td>
<td>A firm and more deep-engraved lesion than a pustule. Its emergence reflects a more severe form of acne than the pustule and often results in scarring.</td>
</tr>
<tr>
<td>Nodule</td>
<td>A larger and still deeper inflammatory lesion that invariably produces a deep scar. It can also be painful. Nodules may suppurnate and contain a core of purulent material.</td>
</tr>
<tr>
<td>Conglobate lesion</td>
<td>Severely inflamed lesions joined by sinus tracts seen in the most disfiguring forms of acne. <em>Draining sinuses</em>, chronic fistulae arising in conglobate lesions, also may develop</td>
</tr>
<tr>
<td>Hidradenitis suppurativa/ acne inversa</td>
<td>A chronic inflammatory scarring process of the axilla and groin that may accompany severe acne or stand alone</td>
</tr>
</tbody>
</table>

Affected Age Group

During adolescence, *acne vulgaris* is more common in males than in females. In adulthood, *acne vulgaris* is more common in women than in men.

*Acne vulgaris* may be present in the first few weeks and months of life, when a newborn is still under the influence of maternal hormones and when the androgen-producing portion of the adrenal gland is disproportionately large. This neonatal acne tends to resolve spontaneously. However, the neonate should be treated with a mild retinoid to clear out the impacted follicles. Adolescent acne usually begins with the onset of puberty, when the gonads begin to produce and release more androgens.

Acne is not limited to adolescence. Twelve percent of women and 5% of men at age 25 years have acne. By age 45 years, 5% of both men and women still have acne [11]. Table 2 represents age-wise prevalence of acne.

Table 2: Age-wise comparison of acne

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Body-location</th>
<th>Morphology</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below 12</td>
<td>Centro-facial</td>
<td>Comedonal</td>
<td>Both</td>
</tr>
<tr>
<td>12 to 20</td>
<td>Face, trunk</td>
<td>Mixed</td>
<td>Both</td>
</tr>
<tr>
<td>Above 20</td>
<td>Perioral, jawline, chin</td>
<td>Inflammatory</td>
<td>Women</td>
</tr>
</tbody>
</table>

Socioeconomic Factors and Acne

The spell of acne is not as widespread in the rural society as it is in the industrialized and modern society. Substantial attempts are being made to find concrete evidence of the relationship between diet and acne. However, alarming glycemic indices have seemed to play a pivotal role in the development of acne. The prevalence of acne in Inuit people (Eskimo) increased with their transition towards the western living (acclimation) has been reported by Schaefer and Bendiner. Surveys of disease in some rural African villages in Kenya, Zambia, and the Bantu in South Africa reported far less cases of acne than is found in the descendants of these communities now residing in UK or the United States. It has been reported by Necropsies and others that in a location where there was little use of modern facilities and vegetarian food habits, the rates of incidence of acne were found to be rare. In a report, two populations, i.e., Kitavan Islanders of Papua New Guinea and Ache hunter gatherers of Paraguay have been observed. These two communities have not shown even a single case of acne. It has been hypothesized that the populations in the urban area are more affected with acne than in non-urban stretches. Glycemic index has been proposed for the same, which is lower in non-western...
diets. Higher glycemic index are reported to cause hyperinsulinemia, which most probably, initiates sequence of endocrine events which affect the sebaceous glands and follicular keratinization. A cross-sectional study of 2214 healthy adolescents of Arequipa and Peru, reported a low prevalence of acne of moderate-to-severe type, i.e., significantly less in Indians (28%) than in Mestizos (43%) or whites (45%). It was hypothesized that ethnic differences are responsible for this difference rather than distinct socioeconomic situation or alimentary or hygienic habits. In another study on 9955 school children (age 6-16 years) in a rural region in Brazil, only 2.7% were found to suffer from acne vulgaris.

**Dietary Role in Acne**

A lot has been written on this subject over the past few years. The role of diet in acne is gradually being ascertained. Molecular mechanisms postulated as responsible for the stimulation of the pilosebaceous unit are logical and scientific. Reports are available to establish the relationship between dietary manipulation and the acne associated with biochemical and endocrine parameters. Also, the statistical association between acne and dairy is strong.

**Milk and dairy products**

Excessive milk and dairy products intake has been reported to induce acne either depending upon fats or due to hormones. Today 75% to 90% of marketed milk and milk products are being derived from pregnant cows. Hence, milk contains hormones like placenta-derived progesterone, dihydrotestosterone (DHT) precursors, including 5a-pregnanedione and 5a-androstanedione. These compounds can be easily converted to DHT (the accepted prime acnegen) in a few enzymatic steps. The enzymes required to mediate the change are present in the human pilosebaceous unit. It has been proposed that 5a-reductase inhibitors might block the final conversion to DHT. But, blockade of 5a-reductase cannot serve the purpose of acne management as the chemicals have already undergone 5a-reduction—in the bovine mammary gland. These precursors arrive at the pilosebaceous unit with no need for further 5a-reduction, a situation that exposes humans to potent agonists for which we are unprepared by any evolutionary defense mechanism.

Just the steroid hormones in milk are not of primary concern. It contains also prolactin, somatostatin, growth hormone releasing factor-like activity, gonadotropin-releasing hormone, luteinizing hormone, thyroid-stimulating and thyrotropin-releasing hormones, numerous steroid hormones, insulin, epidermal growth factor (EGF), nerve growth factor (NGF), IGF-1 and -2, transforming growth factors (TGFs), vitamin D, transferrin, lactoferrin, many prostaglandins including Pazzo, erythropoietin, bombesin, neurotensin, vasoactive intestinal peptide, various nucleotides, cyclic adenosine monophosphate and guanosine monophosphate, B-casomorphins, and even relaxin. The concentrations of above listed compounds vary among species and the lacta and pregnancy stage. Most of these are growth enhancers and increase sebum production. Milk is, after all, specifically designed to make things grow.

Intake of milk during adolescence is generally associated with history of teenage acne. There are evidences that these associations are not a result of fat content of milk. Instant breakfast drink, cream cheese and cottage cheese are also reported to be associated with acne. These associations may be because of the milk content of these foods.

**Fibrous diet**

Inclusion of fibres in the food is a good food habit and 30% dietary fibre per day is reported to decrease the acne instances. This effect would be a result of low glycemic load. High fibre and low fat diet is known to decrease the sebum production in adults and such diet has been reported by Smith et al.

**Fish and sea foods**

Fish and sea foods are rich in omega-3-fatty acids and intake of such foods is correlated with lower
rates of incidence of acne episodes. Omega-3-fatty acids is a proved leukotriene B4-inhibitor. Inhibition of latter reduces the sebum production and improves the inflammatory condition of acne. Apart from omega-3- fatty acids, fish and sea-foods are rich in polyunsaturated fats. Both omega-3- fatty acids and polyunsaturated fats are known to decrease the androgen levels. Hence, larger intake of sea-foods may decrease the incidences of acne [13-20].

Chocolate
Fulton et al. [21] proved that the intake of about ten times of chocolate that is found in chocolate bars did not affect the cause of acne vulgaris and sebum production. The age old idea of co-relation of chocolate intake with acne was proved wrong.

General Measures for Acne Skin
• Patience— Acne is reported to increase the stress. Therefore, it becomes essential to be patient and not to expect miracles [22]. Outburst of acne can be accompanied with an increase in anxiety levels and a decrease in confidence levels of those affected, mostly the teens, which might even end up into depression or suicidal tendencies. Stress management, therefore, needs to be kept into consideration for effective acne treatment [3].
• Face washing— It is believed that overwashing of acne afflicted area and scrubbing can exacerbate the condition. But studies recommend washing the face twice daily with a mild cleanser. This practice can increase convenience of the patient the anti-acne efficacy also [23]. Soaps should not be used to clean the acne-prone skin rather cleansers with a pH of 5.5 should be preferred [24].
• Picking on the lesions— Acne scars are reported to be one of the results of self-manipulations or picking of the comedos [25]. Hence, it is advised not to try picking on them which shall only render the condition worse.
• Cosmetics— Selection of over-the-counter cosmetics should be judicious as many ingredients can be allergic to the acne skin and can worsen the condition[26]. Skin care products containing nicotinamide, lactic acid, triethyl acetate/ethylélineolate, and certain plant extracts like Mahonia, tea tree oil and Saccharomyces may contribute to a decrease in acne lesions [24, 27].

• Acne inducing medication— No medication should be taken prior to consultation from a physician. Corticosteroids, neuropsychotherapeutic drugs, antituberculosis drugs and immunomodulating molecules are the classes of drugs known to induce acne [28].
• Regular follow-up visits— Regular follow-ups and appointment with the physician is recommended so as to keep the condition under control and to take care of any cases of allergy or adverse reactions without any delay. Online follow-ups have also been reported equivalent to the official visits [29]. It is worth noting that the primary goal of acne treatment is to minimize permanent skin damage and diminish the probability of recurrence of the same. Although, acne can’t be cured but it can kept under reasonable control adhering to proper guidelines [30]. Acne-care advice preferably should be individualized, and both clinician and patient aware of the limitations. Despite of sufficient evidence, the clinicians cannot issue strict recommendations in relation to diet, hygiene and face-washing and sunlight to patients with acne [31].

Conclusion:
Acne, a non-mortality disease, affects the overall performance of the affected person. Despite the established underlying pathogenesis reasons, food habits and the life-style of the person have also been recognized to affect the disease state. Foods rich in omega-3-fatty acids, polyunsaturated fats, and total fibre content and with low glycemic content are reported to significantly decrease the incidences of acne. Foods rich in milk and dairy products are known to increase the incidences of acne. Apart from
these, fast food and the stressful life style have also been reported to increase the acne instances in a few literature reports. Against the common perception of increase of acne with chocolate intake, the review points out a study against this belief. There are strong recommendations for the face-wash schedule and incorporation of agents in cosmetics which decrease the acne. However, there can not be a complete diet chart for the acne patients and set guidelines for the day-to-day life style, but the review has pointed the habits and precautions which have been established to be strongly correlated with acne.

References:

ORIGINAL ARTICLE

Design and evaluation of flexible membrane vesicles (FMVs) for enhanced topical delivery of capsaicin

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Abstract
Capsaicin, extracted from the fruits of Capsicum, is a powerful local stimulant with strong rubifacient action, devoid of vesication. Topical use of capsaicin is quite common in the treatment of various pain-associated musculo-skeletal disorders, itching and neuropathy. Despite its high pharmacodynamic potential, the patient compliance to the drug is reported to be poor owing to multiple skin problems like irritation, burning sensation, and erythema. The present study targets the encasement of drug in the interiors of flexible membrane vesicles (FMVs), as these are reported to have better penetration in the deeper layers of skin, thus leading to enhanced localization of drug and consequently, decreased skin irritation. Multilamellar drug-loaded FMVs, prepared by thin-film hydration were evaluated for their efficacy in vitro and in vivo. When compared with conventional liposomes, the formulated FMVs showed higher skin retention during ex vivo permeation studies employing LACA mice skin, higher analgesic potential using radiant tail-flick method in mice, and better flexibility in regaining their size. Being less of an irritant, these vesicular carriers were also found to be more comfortable on human skin. Thus, the capsaicin-loaded FMVs offer high potential as topical drug delivery technologies with improved patient acceptance and effectiveness.

Keywords: Transfersomes, pain, irritation, phospholipid, liposomes, edge activator

Introduction
Capsaicin, (8-methyl N-vanillyl-6-nonenamide), is a pungent capsaicinoid, extracted from the fruits of Capsicum (Lynn, 1990). It is known to execute a variety of pharmacological actions on cardiovascular, respiratory, and nervous systems (Monseerennosorn et al., 1982). The drug produces marked alterations in the function of unmyelinated sensory afferent fibers, which are believed to signal pain and initiate inflammatory responses (Lynn, 1990). It is a drug of choice for the topical treatment of some typical painful conditions such as rheumatoid arthritis, osteoarthritis, muscle strain, sprains, peripheral neuropathy, chronic post-herpetic neuralgia, and itching (Basha & Whitehouse, 1991; Rauns & Bryson, 1995). Also, it is a very useful adjunct topical therapy in many other soft tissue diseases because of its counter-irritant properties. However, despite its immense therapeutic profile, the capsaicin molecules are known to be patient-unfriendly owing to their potent tendency to cause skin irritation, burning, and erythema. This tends to limit the topical application of the drug, which needs to be significantly improved (Yosipovitch et al., 1999). On the other hand, as the topical application of capsaicin circumvents the significant hepatic first-pass metabolism that the drug is known to undergo when administered orally, and improves skin deposition of the drug, it is highly appropriate to develop its topical delivery system(s) (Wang et al., 2001).

The exquisitely structured mesophases made up of saturated phospholipids and the surfactants have shown great potential in ameliorating the performance of drug molecules by modifying and controlling their release. These also improve the overall action by improving the skin penetration, if modified for the flexibility in the vesicle membranes (Cevc & Blume, 2001). These flexible vesicles have been proposed and proved by several researchers (Cevc et al., 1998; El Maghraby et al., 2000; Hiruta et al., 2006; Loan Honeywell-Nguyen et al., 2006) for their efficient and enhanced drug delivery along with their compatibility with the biological barriers. These carries have been...
employed efficiently to entrap drugs with less aqueous solubility (Cevc & Blume, 2001) and can be a better option for the encapsulation of hydrophobic capsaicin within the lipidic layers. The current study endeavors to formulate the stable flexible capsaicin-loaded vesicles for its topical delivery and to assess the potential of these lipidic carriers to improve the patient compliance by decreasing its irritation potential, while maintaining or improving the drug efficacy.

Materials and methods
Materials
Samples of capsaicin, Phospholipon 90 H, and Carbopol® 934 were obtained ex-gratis from M/s Panacea Biotec Ltd., Lahu, Punjab, India; M/s Phospholipid GmbH, Nattermannallee, Germany, and M/s Lubrizol Co., OH, respectively. Sephadex G-50 (medium) was procured from M/s Sigma-Aldrich, St. Louis, MO. Polycarbonate membranes were procured from M/s Whatman, Kent, UK. All other chemicals used for the formulation development were of analytical grade. Double distilled water was employed throughout the study.

Preparation of flexible membrane vesicles and liposomes
Capsaicin-loaded flexible membrane vesicles (FMVs) were prepared by thin-film hydration (Cevc et al., 1997). Accurately weighed quantities of drug, phospholipid, and surfactant were dissolved in chloroform-methanol (2:1) in a round bottom flask. The solvent was evaporated at 50 ± 1°C and 100 rpm under reduced pressure using rotary film evaporator (Buchi RE 121, Switzerland). After complete evaporation of solvent, the flask was kept under vacuum for overnight to remove the residual solvent. The obtained thin lipid film was hydrated at 50 ± 1°C and 60 rpm under reduced pressure using rotary film evaporator to obtain a homogenous suspension of the FMVs (El Maghraby et al., 1999). The suspension was kept at room temperature for 2h for complete hydration (i.e. swelling of phospholipids) to take place before conducting any characterization and evaluation studies on it. Liposomes were also prepared using an identical method, except that surfactant was replaced with cholesterol.

Selection of edge activators
Attempts were made to study the interactions of different edge activators viz. Span 80 (SP), Tween 80 (TW), sodium cholate (SC), sodium deoxycholate (DC), and Brij 35 (BR) with capsaicin, and to obtain the stable drug-loaded FMVs. Selection criteria were based upon the microscopic studies, particle size distribution, turbidity, number of vesicles per cubic mm, and drug entrapment. The amounts of phospholipid and drug were kept as fixed, while varying only the amount of surfactant in each case. A total of five of such surfactants, viz. SP, TW, SC, DC, and BR employing five different ratios of each (Table 1) were selected and observed for the formation of FMVs.

Transmittance studies
Transmittance of different FMV formulations was observed spectrophotometrically at a wavelength of 560 nm by diluting 0.2 mL of the suspension up to 10 mL with water against water as the blank (set at 100% transmittance). The transmittance of the FMV formulations was compared with that of the liposomal formulation of capsaicin.

Morphology and structure of vesicles
The prepared capsaicin FMVs were characterized for morphology (i.e. shape uniformity and lamellarity) employing phase contrast optical microscope (Kyowoo, Medilux, Japan; Jain et al., 2003).

Micromeritic studies
The vesicle size and size distribution profile were determined using dynamic light scattering (DLS) method (Mastersizer® 2000, Malvern Instruments Ltd, Worcestershire, UK; El Maghraby et al., 2000).

Number of vesicles
The FMV dispersion was diluted five times with normal saline (0.9% w/v). The number of FMVs per cubic mm was counted microscopically employing an hemocytometer. The FMVs in 80 small squares were counted and their number density was calculated using the following formula (Jain et al., 2005):

\[
\text{Number of vesicles} = \frac{\text{Total no of squares counted} \times \text{dilution factor} \times 4000}{\text{Total no of FMVs per cubic mm}}
\]

Drug entrapment efficiency of prepared FMVs
Mini-column centrifugation technique was adopted for the removal of unentrapped drug (Fry et al., 1978). The prepared columns were pre-saturated with plain (i.e. without drug) FMVs (Reynolds et al., 1983; Grabielle-Madelmont et al., 2003). After pre-saturating the column, the drug-loaded FMV suspension (0.2 mL) was placed in the column. Centrifugation was carried out at 2000 rpm (1565g) for 3 min. The eluted vesicles were collected and observed under microscope for the absence of any unentrapped drug crystal(s). A reference slide of the drug suspension was made to compare the crystals using optical microscopy.
microscope (at 1000x). The column was washed again with 0.2 mL of water and the eluate obtained was pooled with the previously collected eluate. Thereafter, a volume of 0.2 mL of the eluted FMV suspension was digested with suitable volume of chloroform-methanol (2:1 v/v) mixture and solution was analyzed spectrophotometrically employing ultraviolet-visible spectrophotometer (Genesys, Thermospectronic, MA). The empty FMVs, treated in the analogous way, served as blank during the studies.

Degree of deformability
The deformability index of FMVs was determined using vesicle extruder (Eastern Sci. Inc., MD), and the results were compared with those of the standard liposomal formulations. The FMV suspension was passed through polycarbonate membrane filter of 1 μm pore size employing vesicle extruder. The particle size of vesicle suspension was determined before and after extrusion using DLS.

Development of secondary topical vehicle
In order to make the formulated FMVs rheologically acceptable for topical applications, these were further incorporated in Carbopol® 934 hydrogel (1% by weight).

Preparation of conventional without cream
The efficacy of FMV formulations of capsaicin was compared with that of the liposomes and the conventional cream-based formulation. It was composed of 0.025% capsaicin, 6% sorbitan mono-oleate, 3% white bees wax, 36% white soft paraffin, 15% liquid paraffin, and quantity sufficient to 100% with distilled water. The conventional cream was formulated by simple melting and mixing the ingredients under agitation.

In vitro drug permeation studies
In order to characterize the prepared FMV formulation, the in vitro drug permeation studies were carried out using Franz diffusion cells (PermeGear, Inc., PA). Permeation experiments were conducted employing excised abdominal skin of LACA mice (Agarwal et al., 2001). Various prepared formulations of capsaicin viz. CAP₁ (FMV suspension), CAP₂ (FMV Carbopol gel), CAP₃ (Liposomal suspension), CAP₄ (Liposomal Carbopol gel), and CAP₅ (conventional cream), each equivalent to 2 mg of capsaicin, were applied onto the mice skin in the donor compartment. As capsaicin is practically insoluble in water, 50% ethanol was required in receptor fluid to maintain sink conditions, hence, phosphate buffer pH 7.4: ethanol (1:1) was used as the diffusion medium (Magnusson & Koskinen, 2000).

Skin retention studies
Following permeation studies, the skin tissue mounted on the diffusion cell was removed and washed thrice with saline solution, followed by blotting between tissue paper to remove any adhering formulation from the surface. Subsequently, the skin tissue was cut into small pieces and homogenized with 10 mL of chloroform-methanol mixture (2:1 v/v) for extracting capsaicin. The homogenate suspension, thus, obtained was centrifuged at 5000 rpm (3913g) for 5 min. The supernatant was filtered using a 0.45 μm membrane filter (Millipore, MA) and quantified for drug content spectrophotometrically. Fresh skin tissue, treated in the similar manner, was taken as blank for the above study. Each experiment was conducted in triplicate (Singh et al., 2005).

In vivo pharmacodynamic evaluation
The analgesic effect of the formulation was evaluated in male LACA mice employing the popular radiant heat tail-flick latency test using an analgesiometer (Kulkarni, 1999). The studies were carried out after the obtaining requisite approval from Institutional Ethics Committee, Panjab University, Chandigarh, India. In brief, the animals were divided into four groups, i.e. A, B, C, and D, each group consisting of six animals each. The basal reaction time was measured for each group with a cut-off period of 10 sec. The drug-free FMV gel was applied to Group A animals, the conventional capsaicin-loaded cream was applied to Group B animals, the drug-loaded liposomal gel was applied to Group C animals, and the drug-loaded FMV gel to Group D animals. The reaction time was measured at the desired periodic time intervals for each group. The percent analgesic effect was determined using the following formula:

\[
\text{Percent analgesic effect} = \frac{\text{Observed reaction time} - \text{Basal reaction time}}{\text{Cut-off time period - Basal reaction time}} \times 100
\]

For discriminating the statistical significant difference among the studied groups, a one-way analysis of variance (ANOVA) was applied to the obtained data, followed by Studentized Tukey’s test (Daniel, 2008; Bhatia et al., 2007).

Determination of irritancy potential of FMV gel
Fifteen healthy human volunteers participated in the trials conducted at Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, after obtaining written informed consent from them. Requisite approval of the Institutional Ethics Committee was obtained prior to the clinical studies. During this study, capsaicin FMV gel was compared with that of the conventional cream containing capsaicin. Each subject was tested, with the FMV preparation on one arm and conventional cream on the other, in a randomized fashion. About 0.2 g of the preparation was applied to a 10 cm² area marked by ink on the volar surface of the forearm for an application time of 4 h. The subjects, at regular intervals of time, were asked to rate the intensity of burning sensation, itching and redness, all indicating the irrita­tion level. Each condition was ordinarily scored according to the strength of the observed reactions as per the following criteria (Table 2):
The resulting scoring values were summed to provide a global rank score for a particular condition. The effectiveness of FMV capsaicin gel was assessed vis-à-vis conventional capsaicin cream using the Student’s paired t-test.

**Stability studies: drug leakage studies**

The prepared FMV formulations were stored at different temperatures, i.e., 5 ± 3°C, 25 ± 2°C, and 40 ± 2°C and the amount of drug leaked was determined. After estimating the initial percentage entrapment of the drug in the formulations, three batches of the same formulation were stored in sealed glass ampoules (one each) at each of the studied temperatures at least for a period of 4 weeks. After every 7 days, percentage entrapment of the drug was determined as per the method described earlier under drug entrapment efficiency studies.

**Stability studies: integrity of vesicles**

The structural integrity of FMVs was monitored by microscopic and visual observations to check for the number of disrupted vesicles. Studies were carried out at three different temperatures, i.e., 5 ± 3°C, 25 ± 2°C, and 40 ± 2°C for a period of 4 weeks. The degrees of disruption and aggregation were calculated on the basis of an ordinal scale ranging between 1 and 4 (Table 3). For calculation of these degrees, the same method was adopted as discussed previously for the counting of vesicles employing hemocytometer. Numbers of disrupted and aggregated vesicles in the selected areas were counted and their percentage population out of total vesicles was calculated.

**Results and discussion**

**Selection of edge activators**

For selection of edge activators, the criteria were based upon the microscopic evaluation of various FMV formulations, particle size, particle size distribution, vesicle density (i.e., number of vesicles per cubic mm), vesicular morphology, lamellarity, and drug entrapment of various formulation batches (Table 4).

All the suspensions containing varying ratios of different surfactants, when kept at room temperature, yielded different results. It was observed that the suspensions containing SP, SC, and DC were stable and did not settle quickly. Suspensions containing BR and TW, on the other hand, settled completely. Moreover, very few vesicles were microscopically observed using the latter two surfactants. Best results were shown by SP containing vesicles. It may be attributed to the appropriate inter and intramolecular interactions between the surfactant and phospholipid molecules. The surfactant molecules activate the edges of the FMVs making them more flexible and hence, more permeable (Cevc, 1996). Hence on the basis of suspension stability, SP, SC, and DC were selected for further characterization. The results obtained were in close agreement with the previously published reports (Cevc, 1996; El Maghraby et al., 1999; El Maghraby et al., 2000).

**Formulation characterization**

**Transmittance studies**

After suitable dilution, various FMV formulations were compared with the liposomal formulation of capsaicin for the extent of turbidity (Table 5). With increase in concentration of surfactant in the FMV suspensions, there was an increase in transmittance, i.e., decrease in the turbidity of the suspension. Turbidity was found to decrease continuously with increased levels of surfactants till an optimal critical concentration was reached (i.e., 15% in all cases), after which turbidity started increasing again. On further increasing the concentration of surfactant, a reduction in percent transmittance was noticed, indicating increased extent of aggregation in the suspension resulting in larger particles. Concentration of edge activator(s) has pronounced effect on the various systemic formulation attributes like vesicle size, entrapment efficiency, and permeation flux. Hence, selection of proper concentration of the edge activator remains one of the crucial steps in the fabrication of deformable liposomes (El Maghraby et al., 2000). Transmittance of the vesicular suspension within a wavelength of 400-600 nm, when the substances contained in vesicles do not absorb, furnishes an idea about the degree of aggregation and particle assembly in the dispersions of lipid vesicles (Bezrukova & Rozenberg, 1981). Interaction between the surfactant molecules and phospholipids take place, which can even result in solubilization of the lipidic membranes. Such interactions can be assessed with the help of turbidimetric analysis (Simões et al., 2005). Henceforth, transmittance studies helped in the selection of appropriate concentration of surfactant(s) in the FMV formulations indicating that edge activator concentration above 15% leads to increased aggregation of vesicles.

**Number of vesicles per cubic mm**

This study revealed that on increasing the concentration of the edge activator, the numbers of FMVs initially

---

Table 2. Coding scores for skin irritancy potential during human trials.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Severity</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No irritation, burning, or redness</td>
<td>Nil</td>
<td>0</td>
</tr>
<tr>
<td>Barely perceptible stinging, burning, or itching</td>
<td>Slight</td>
<td>1</td>
</tr>
<tr>
<td>Definite stinging, burning, or itching</td>
<td>Mild</td>
<td>2</td>
</tr>
<tr>
<td>Distinctly uncomfortable stinging, burning, or itching</td>
<td>Moderate</td>
<td>3</td>
</tr>
<tr>
<td>Continuously stinging, burning or itching and intensely uncomfortable</td>
<td>Severe</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 3. Coding degree scores for vesicular aggregation and disruption.

<table>
<thead>
<tr>
<th>Degree of aggregation</th>
<th>Degree of disruption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5-10%</td>
</tr>
<tr>
<td>2</td>
<td>10-20%</td>
</tr>
<tr>
<td>3</td>
<td>20-30%</td>
</tr>
<tr>
<td>4</td>
<td>30-40%</td>
</tr>
<tr>
<td>5</td>
<td>5-10%</td>
</tr>
<tr>
<td>6</td>
<td>10-15%</td>
</tr>
<tr>
<td>7</td>
<td>15-20%</td>
</tr>
</tbody>
</table>
Table 4. Formulation attributes of various FMV batches for selection of edge activator.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Surfactant (mg)</th>
<th>Lipid: surfactant weight ratio</th>
<th>Abundance of various vesicular systems</th>
<th>Size Morphology</th>
<th>Number of lamella</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP 1</td>
<td>Span 80 (5.29 mg)</td>
<td>95:05</td>
<td>Few</td>
<td>Very small</td>
<td>Circular SUVs</td>
</tr>
<tr>
<td>SP 2</td>
<td>Span 80 (11.11 mg)</td>
<td>90:10</td>
<td>Numerous</td>
<td>Mostly small, few medium</td>
<td>Circular, few oval SUVs, few MLVs</td>
</tr>
<tr>
<td>SP 3</td>
<td>Span 80 (17.65 mg)</td>
<td>85:15</td>
<td>Numerous</td>
<td>Small to medium</td>
<td>Regular circular and oval SUVs and MLVs</td>
</tr>
<tr>
<td>SP 4</td>
<td>Span 80 (25.00 mg)</td>
<td>80:20</td>
<td>Several</td>
<td>Small to medium</td>
<td>Circular SUVs</td>
</tr>
<tr>
<td>SP 5</td>
<td>Span 80 (33.33 mg)</td>
<td>75:25</td>
<td>Few</td>
<td>Small</td>
<td>Circular SUVs</td>
</tr>
<tr>
<td>TW 1</td>
<td>Tween 80 (5.29 mg)</td>
<td>95:05</td>
<td>Few</td>
<td>Small</td>
<td>Irregular, some circular SUVs</td>
</tr>
<tr>
<td>TW 2</td>
<td>Tween 80 (11.11 mg)</td>
<td>90:10</td>
<td>Few</td>
<td>Small</td>
<td>Circular SUVs</td>
</tr>
<tr>
<td>TW 3</td>
<td>Tween 80 (17.65 mg)</td>
<td>85:15</td>
<td>Almost nil</td>
<td>Small</td>
<td>Circular SUVs</td>
</tr>
<tr>
<td>TW 4</td>
<td>Tween 80 (25.00 mg)</td>
<td>80:20</td>
<td>Almost nil</td>
<td>Small</td>
<td>Circular SUVs</td>
</tr>
<tr>
<td>TW 5</td>
<td>Tween 80 (33.33 mg)</td>
<td>75:25</td>
<td>Almost nil</td>
<td>Small</td>
<td>Circular SUVs</td>
</tr>
<tr>
<td>BR 1</td>
<td>Brij 35 (5.29 mg)</td>
<td>95:05</td>
<td>Few</td>
<td>Small</td>
<td>Irregular SUVs</td>
</tr>
<tr>
<td>BR 2</td>
<td>Brij 35 (11.11 mg)</td>
<td>90:10</td>
<td>Almost nil</td>
<td>Small</td>
<td>Irregular SUVs</td>
</tr>
<tr>
<td>BR 3</td>
<td>Brij 35 (17.65 mg)</td>
<td>85:15</td>
<td>Almost nil</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>BR 4</td>
<td>Brij 35 (25.00 mg)</td>
<td>80:20</td>
<td>Nil</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>BR 5</td>
<td>Brij 35 (33.33 mg)</td>
<td>75:25</td>
<td>Nil</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DC 1</td>
<td>Sodium deoxycholate (5.29 mg)</td>
<td>95:05</td>
<td>Numerous</td>
<td>Small to medium</td>
<td>Circular SUVs</td>
</tr>
<tr>
<td>DC 2</td>
<td>Sodium deoxycholate (11.11 mg)</td>
<td>90:10</td>
<td>Numerous</td>
<td>Small to medium</td>
<td>Circular and oval SUVs</td>
</tr>
<tr>
<td>DC 3</td>
<td>Sodium deoxycholate (17.65 mg)</td>
<td>85:15</td>
<td>Numerous</td>
<td>Small, large medium</td>
<td>Circular SUVs</td>
</tr>
<tr>
<td>DC 4</td>
<td>Sodium deoxycholate (25.00 mg)</td>
<td>80:20</td>
<td>Few</td>
<td>Small to medium</td>
<td>Circular SUVs</td>
</tr>
<tr>
<td>DC 5</td>
<td>Sodium deoxycholate (33.33 mg)</td>
<td>75:25</td>
<td>Few</td>
<td>Small</td>
<td>Circular SUVs</td>
</tr>
<tr>
<td>SC 1</td>
<td>Sodium cholate (5.29 mg)</td>
<td>95:05</td>
<td>Few</td>
<td>Small</td>
<td>Circular SUVs</td>
</tr>
<tr>
<td>SC 2</td>
<td>Sodium cholate (11.11 mg)</td>
<td>90:10</td>
<td>Few</td>
<td>Very Small</td>
<td>Circular SUVs</td>
</tr>
<tr>
<td>SC 3</td>
<td>Sodium cholate (17.65 mg)</td>
<td>85:15</td>
<td>Several</td>
<td>Very Small</td>
<td>Circular SUVs</td>
</tr>
<tr>
<td>SC 4</td>
<td>Sodium cholate (25.00 mg)</td>
<td>80:20</td>
<td>Few</td>
<td>Very Small</td>
<td>Circular SUVs</td>
</tr>
<tr>
<td>SC 5</td>
<td>Sodium cholate (33.33 mg)</td>
<td>75:25</td>
<td>Almost nil</td>
<td>Very Small</td>
<td>Circular SUVs</td>
</tr>
</tbody>
</table>

Translation of coded abundance in actual units: Nil, 0–500; Almost nil, 500–1000; Few, 1000–5000; Several, 5000–10,000; Numerous, > 10,000. BR, Brij 35; DC, sodium deoxycholate; FMV, flexible membrane vesicles; MLVs, multilamellar vesicles; SC, sodium cholate; SP, Span 80; SUVs, small unilamellar vesicles; TW, Tween 80.

Increased, but up to a particular concentration, the number of FMVs tends to exhibit a declining trend afterwards (Table 5).

The results are in consonance with the percent transmittance studies, where an analogous pattern was noticeable. Reduction in the number of vesicles can be attributed to increased tendency of aggregation and formation of larger vesicles after attaining a certain critical concentration of surfactant. Here, the formulations with 15% of surfactant tended to show maximum number of vesicles per cm³.

**Entrapment efficiency**

With increasing drug–lipid ratio, an initial increase in the entrapment efficiency as well as drug payload was observed (Table 6).

Maximum entrapment efficiency and drug loading were observed with the SP 3 formulation (0.085: 1; drug: lipid ratio), after which entrapment efficiency tended to decrease with increasing drug–lipid ratio. It may be attributed to the saturation of the lipids with drug, resulting eventually in the decreased drug loading and efficiency (Singh et al., 2005). After selection of formulation SP 3, further increase in drug amounts in SP 3 formulation, resulted in decrease in the entrapment efficiency, as depicted in Table 7.

The decreased entrapment efficiency beyond a certain optimum concentration of surfactant and drug–lipid ratio, can be ascribed to the saturation of lipid layers with drug coupled with the dissolution of phospholipids in the surfactant(s) (Simões et al., 2005). This study helped in optimizing the drug–lipid ratio so as to have a FMV system with maximum possible entrapment efficiency.

**Degree of deformability**

Degree of deformability is an important and specific parameter of FMVs, as it differentiates FMVs from other vesicular carriers like liposomes in terms of degree of
flexibility and hence, ability to cross the stratum corneum (Cevc, 1996; Cevc et al., 1998; Jain et al., 2003; Hiruta et al., 2006). The deformability index has been chosen here to assess the membrane flexibility of the vesicles. Alteration in particle size of the FMVs *vis-à-vis* the conventional liposomes by passing through the vesicle extruder (Eastern Sci. Inc., MD) was measured employing Malvern Mastersizer (Table 8).

In all the formulations where edge activators were employed, the vesicles were found to regain their respective original sizes. Here, the vesicular system containing SP as the edge activator showed the best deformability index, i.e. 1.09, in comparison to 0.491 of the conventional liposomal systems. The utility of SP in the attainment of an appropriate flexibility is in consonance with the earlier results (El Maghraby et al., 2000; van den Bergh et al., 2001). The observation clearly demonstrates the ability of SP as edge activator to increase the malleability of the vesicles.

**Morphology and structure of prepared capsaicin FMVs**

The FMV formulations were microscopically examined (optical microscope, magnification 1000X) for their structural attributes such as lamellarity, uniformity of shape, and physical stability characteristics like aggregation and/or fusion. The FMVs were found to be of multilamellar character with spherical shape and marked uniformity, as depicted in Figure 1.

**Micromeritic studies**

Micromeritic studies were carried out employing Malvern Mastersizer’ 2000 (Malvern Instruments Ltd.) to determine vesicular size distribution profile of the optimized FMV formulation of capsaicin (SP 3). The average vesicle size was found to be of 7.761 μm with apparently log normal distribution characteristics (Figure 2).

**Skin permeation studies**

The plots between amounts of drug permeated versus time (Figure 3) using various capsaicin containing formulations unambiguously depict that the amounts of drug permeated were far lower in case of FMV gel and FMV suspension *vis-à-vis* other capsaicin formulations. Both vesicular formulations (liposomes and FMVs) showed relatively reduced skin penetration than the conventional formulation. Further, the permeation

---

**Table 5. Percent transmittance and number of vesicles of various formulations.**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>No. of vesicles per cubic mm (x 1000)</th>
<th>Percent transmittance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP 1</td>
<td>35</td>
<td>70.8</td>
</tr>
<tr>
<td>SP 2</td>
<td>47</td>
<td>72.4</td>
</tr>
<tr>
<td>SP 3</td>
<td>60</td>
<td>75.6</td>
</tr>
<tr>
<td>SP 4</td>
<td>39</td>
<td>71.5</td>
</tr>
<tr>
<td>SP 5</td>
<td>31</td>
<td>69.8</td>
</tr>
<tr>
<td>SC 2</td>
<td>17</td>
<td>72.4</td>
</tr>
<tr>
<td>SC 3</td>
<td>30</td>
<td>75.6</td>
</tr>
<tr>
<td>SC 4</td>
<td>22</td>
<td>71.5</td>
</tr>
<tr>
<td>DC 2</td>
<td>29</td>
<td>79.3</td>
</tr>
<tr>
<td>DC 3</td>
<td>25</td>
<td>85.0</td>
</tr>
<tr>
<td>DC 4</td>
<td>21</td>
<td>81.5</td>
</tr>
<tr>
<td>Liposomes</td>
<td>10</td>
<td>57.6</td>
</tr>
</tbody>
</table>

DC, sodium deoxycholate; SC, sodium cholate; SP, Span 80.

**Table 6. Entrapment of capsaicin in FMVs in various formulation batches*.**

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Surfactant (mg)</th>
<th>Drug entrapped (mg)/100 mg lipid</th>
<th>Percent entrainment efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP 3 (a)</td>
<td>5.3</td>
<td>6.6</td>
<td>66.3</td>
</tr>
<tr>
<td>SP 3 (b)</td>
<td>11.1</td>
<td>7.0</td>
<td>70.1</td>
</tr>
<tr>
<td>SP 3 (c)</td>
<td>17.6</td>
<td>7.5</td>
<td>74.8</td>
</tr>
<tr>
<td>SP 3 (d)</td>
<td>25.0</td>
<td>6.5</td>
<td>64.8</td>
</tr>
<tr>
<td>SC 2</td>
<td>33.3</td>
<td>5.9</td>
<td>59.1</td>
</tr>
<tr>
<td>SC 3</td>
<td>5.3</td>
<td>3.5</td>
<td>35.0</td>
</tr>
<tr>
<td>SC 4</td>
<td>11.1</td>
<td>3.8</td>
<td>37.5</td>
</tr>
<tr>
<td>SC 5</td>
<td>17.6</td>
<td>4.2</td>
<td>42.5</td>
</tr>
<tr>
<td>DC 2</td>
<td>25.0</td>
<td>4.1</td>
<td>41.0</td>
</tr>
<tr>
<td>DC 3</td>
<td>33.3</td>
<td>4.1</td>
<td>35.3</td>
</tr>
</tbody>
</table>

DC, sodium deoxycholate; FMV, flexible membrane vesicles; SC, sodium cholate; SP, Span 80.

*A*Ratio of drug and phospholipid (1:10) were kept as constant for all batches.

**Table 7. Effect of varying drug concentration on the entrapment efficiency.**

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Drug (mg)</th>
<th>Drug entrapped (mg)/100 mg lipid</th>
<th>Percent entrapment efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP 3 (a)</td>
<td>5.0</td>
<td>4.6</td>
<td>91.7</td>
</tr>
<tr>
<td>SP 3 (b)</td>
<td>10.0</td>
<td>7.2</td>
<td>72.1</td>
</tr>
<tr>
<td>SP 3 (c)</td>
<td>15.0</td>
<td>8.7</td>
<td>57.9</td>
</tr>
<tr>
<td>SP 3 (d)</td>
<td>20.0</td>
<td>8.9</td>
<td>46.6</td>
</tr>
</tbody>
</table>

SP, Span 80.

Amounts of drug phospholipid (100 mg) and surfactant (17.6 mg) were kept constant for all batches.

**Table 8. Diameter of FMVs and liposomes before and after extrusion.**

<table>
<thead>
<tr>
<th>Batch</th>
<th>Diameter before extrusion (μm)</th>
<th>Diameter after extrusion (μm)</th>
<th>Degree of deformability</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP 2</td>
<td>8.26</td>
<td>8.66</td>
<td>1.05</td>
</tr>
<tr>
<td>SP 3</td>
<td>7.99</td>
<td>8.75</td>
<td>1.09</td>
</tr>
<tr>
<td>SP 4</td>
<td>16.61</td>
<td>9.49</td>
<td>0.57</td>
</tr>
<tr>
<td>SC 2</td>
<td>10.63</td>
<td>7.58</td>
<td>0.71</td>
</tr>
<tr>
<td>SC 3</td>
<td>5.19</td>
<td>5.08</td>
<td>0.98</td>
</tr>
<tr>
<td>DC 2</td>
<td>55.40</td>
<td>49.46</td>
<td>0.89</td>
</tr>
<tr>
<td>DC 3</td>
<td>43.92</td>
<td>39.92</td>
<td>0.91</td>
</tr>
<tr>
<td>Liposomes</td>
<td>16.91</td>
<td>8.31</td>
<td>0.49</td>
</tr>
</tbody>
</table>

DC, sodium deoxycholate; FMV, flexible membrane vesicles; SC, sodium cholate; SP, Span 80.
of drug from FMV formulation was found to be appreciably lower than that of the liposomal one. This may be assigned to the difference in the composition and size of the both vesicular carriers, affecting the drug release profile as well as the tendency to interact with skin. Large sized liposomes exhibited higher permeation flux which may due to the diffusional release of the drug from these carriers. However, in the case of conventional cream, the level of interaction with skin seems to be the least, thus reducing the resistance of drug dermal transport.

The values of permeation flux of capsaicin obtained from the optimized FMV systems, i.e. FMV suspension and FMV gel (CAPa and CAPb), were found to be 19.84 μg/cm²/h and 18.39 μg/cm²/h, respectively, significantly lower than those obtained with the liposomal formulations, i.e. CAPc (25.47 μg/cm²/h), CAPd (20.39 μg/cm²/h) and conventional cream-based formulation of capsaicin, i.e. CAPe (36.92 μg/cm²/h). Figure 4 gives a comparative profile of the same. Evidently, it indicates the controlled release nature of the FMV preparation, in accordance with the findings reported earlier (Knepp et al., 1988; Knepp et al., 1990; Honeywell-Nguyen et al., 2005).

**Skin retention studies**

In this study, notably higher values of drug retention were obtained within the skin layers with capsaicin FMVs formulation and liposomal formulation to the tune of 0.286 pg/cm² and that of the was 0.176 pg/cm², respectively (Figure 5). This may be ascribed to the depot-forming characteristic of the vesicular systems. The observation of low permeation flux and high drug retention with FMVs formulation may be accounted for the characteristic flexibility of the vesicular membranes which help in moving the drug across the tough horny layer of skin, while keeping it trapped in the dermal layers (Cevc, 1996; El Maghraby...
In vivo pharmacodynamic studies

Significant increase \((P < 0.05)\) in the basal reaction time of the FMV formulation group vis-à-vis the groups in which marketed and conventional formulations was applied, as revealed by one-way ANOVA followed by Studentized Tukey’s test. Diagrammatic representation in Figure 6 also illustrates the evident trend.

The study showed enhanced analgesic effect over other formulations. This finding at in vivo level in terms of pharmacodynamic behavior is a logical expression of the skin permeation and skin retention characteristics of the developed formulations. Enhanced analgesia offered by FMVs system is an account of its better skin permeation and enhanced skin deposition potential (Cevc, 1996).

Skin compatibility studies

The irritancy of the developed FMV system was compared with that of the conventional system containing capsaicin. The results at different time points reveal substantial difference in the irritancy potential of two kinds of systems. Relatively less discomfort was observed with the FMV gel vis-à-vis the conventional cream (Figure 7).

The scores of burning, redness, and itching were found to be significantly lower with the FMV formulation and the volunteers observed to be more comfortable with the FMV gel.

This may be ascribed to the better structural interaction of the FMVs with skin, owing to their flexible membranes and depot-forming potential so as not to allow drug molecules to come in direct contact with the cells, but in the entrapped vesicular form. The FMVs probably enter the skin and form drug reservoirs, from where the controlled delivery of the drug to the lower skin layers occurs, resulting in marked decrease of irritation. This has been hypothesized and proved by various other researchers too (Rao et al., 1993; Honeywell-Nguyen et al., 2003; Loan Honeywell-Nguyen et al., 2006).

Stability profile

From the stability data obtained, it is vivid that the formulations stored at the refrigerated condition showed minimal loss of entrapped drug (Figure 8). At this low temperature of 5±3°C, only 2.6% of drug was lost over a period of 28 days. In contrast to this, the FMV formulations stored at 40±2°C showed higher drug loss, i.e. 22.4% in 28 days. More drug loss (i.e. 22.4%) at accelerated temperature conditions may be attributed to the loss in overall rigidity of the phospholipid vesicles, and consequent inability to retain the drug in vesicles. Hydrolysis of phospholipids and degradation of drug at elevated temperatures, in turn, may be held responsible for the loss of structure. With increase in temperature, an augmentation in the fluidity of lipid bilayers is also

Figure 6. Percent analgesic effect of capsaicin formulations.

Figure 7. Scores of redness, burning and itching in human volunteers.

Figure 8. Bar diagram depicting the stability of capsaicin flexible membrane vesicles (SP 3) at different temperatures.
FMVs for enhanced topical delivery of capsaicin

Table 9. Aggregation and disruption behavior of capsaicin elastic vesicles (SP 3), during their storage at various temperature conditions.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Aggregation</th>
<th>Disruption</th>
<th>Aggregation</th>
<th>Disruption</th>
<th>Aggregation</th>
<th>Disruption</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 5 ± 3°C</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>At 25 ± 2°C</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>At 40 ± 2°C</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

SP: Span 80.

quite likely due to the phase transition, which may lead to unfavorable packing. The formulations stored at ambient temperature showed higher amount of drug retention within the vesicles than the formulations stored at elevated temperatures, but lower than that of the refrigerated formulations. Accordingly, to avoid the drug loss as well as maintain the integrity of the FMVs, the formulation should be stored at low temperature conditions.

Table 9 enlists the scores of aggregation behavior and degree of disruption of flexible vesicles, as observed during stability studies, where the vesicular formulation stored at different temperatures were evaluated by microscopic and visual means. Initially, the vesicles were observed to undergo negligible aggregation and disruption at 5 ± 3°C and 25 ± 2°C after 7 days of storage. Nevertheless, the aggregation was found to increase as the time progressed further. In the case of samples stored at higher temperature of 40 ± 2°C, however, the degree of aggregation and disruption were observed to be much higher, ascribable to the potential degradation of the phospholipid molecules.

In a nutshell, the above studies vouch that the aggregation behavior and vesicle rupturing characteristics depend primarily upon the temperature conditions. Hence, storage under the refrigerated conditions was found to be more appropriate for avoiding any problem of instability of such vesicular carriers.

Conclusions

The present work on the preparation of topical FMVs is an attempt to utilize the immense potential of flexible vesicular carriers to surmount the skin irritation problems due to capsaicin and consequently, improve its patient acceptance. Following these thorough investigations on the formulated FMVs, it could be inferred that these flexible carriers with optimal characteristics of entrainment, drug payload, size, and surface malleability, are able to penetrate, partition, and permeate the skin barrier quite effectively. Further, the physicochemical modification in the drug by means of closed supramolecular association of phospholipid molecules also promises to prolong the drug action as revealed by drug deposition studies. The results obtained with these carriers in the current studies can also be rationally extrapolated to other poorly soluble molecules with untoward effects like skin irritation. Conclusively, the experimental results and the supportive interpretation unambiguously vouch the enormous promise of the transfersomes in dermatological problems enhancing patient compliance of irritant molecules like capsaicin, without compromising erstwhile drug efficacy.

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Declaration of Interest

This work was funded by University Grants Commission (UGC), New Delhi, India. The authors report no declarations of interest.

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El Maghraby GM, Williams AC, Barry BW. (1999). Analysis Of Variance. Skin delivery of oestradiol from deformable and

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Novel dithranol phospholipid microemulsion for topical application: development, characterization and percutaneous absorption studies

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Abstract
The objective of this study was to develop and characterize a novel dithranol-containing phospholipid microemulsion systems for enhanced skin permeation and retention. Based on the solubility of dithranol, the selected oils were isopropyl myristate (IPM) and tocopherol acetate (TA), and the surfactants were Tween 80 (T80) and Tween 20 (T20). The ratios of cosurfactants comprising of phospholipids and ethanol (1:10) and surfactant to co-surfactant (1:1 and 2.75:1) were fixed for the phase diagram construction. Selected microemulsions were evaluated for globule size, zeta potential, viscosity, refractive index, per cent transmittance, stability (freeze thaw and centrifugation), ex vivo skin permeation and retention. The microemulsion systems composed of IPM and T80 with mean particle diameter of 72.8 nm showed maximum skin permeation (82.23%), skin permeation flux (0.281 mg/cm²/h) along with skin retention (8.31%) vis-a-vis systems containing TA and T20. The results suggest that the developed novel lecithinized microemulsion systems have a promising potential for the improved topical delivery of dithranol.

Keywords: dermal delivery, colloidal delivery, phase diagrams, topical, anthralin, surfactant

Introduction
Dithranol (1,8-dihydroxy-9-anthrone; anthralin) has been used to treat various psoriatic disorders since many years. Despite being effective and safe, its application is difficult and troublesome owing to its irritating, burning, staining and necrotizing effects on the normal as well as the diseased skin. Further, the drug is highly lipophilic (log p = 2.3), poorly water soluble (< 2 µg/mL) and quiet unstable as it gets readily photo-oxidized (Mustakalio, 1981; Wang et al., 1987; Hiller et al., 1995).

Vesicular systems of dithranol, i.e. liposomes and niosomes, have been employed to circumvent these undesirable effects (Agarwal et al., 2001). Microemulsion tends to possess several advantages over the vesicular carriers, namely, higher storage stability, lower preparation cost, good production feasibility, absence of organic solvents and obiviation of intensive sonication (Paolino et al., 2002). Basic components of these colloidal formulations are surfactant, co-surfactant, oil and water combined in an appropriate ratio (Changez and Varshney, 2000). These systems have several specific physicochemical properties such as transparency, optical isotropy, low viscosity and thermodynamic stability (Heuschkel et al., 2008).

Microemulsion systems can increase the dermal delivery of drug by different mechanisms. First, microemulsions can enhance the solubility of poorly water-soluble drugs via the finely dispersed oil droplet phase. Second, the increased thermodynamic activity of the drug favours its partitioning into the skin and third, the ingredients of microemulsion may increase the permeation rate of drug via skin by reducing the diffusional barrier of the skin (Delgado-Charro et al., 1997; Gasco, 1997; Mohammed and Manoj, 2000). Selection of various components for a microemulsion system should be conducted judiciously to prevent the skin irritation that might arise due to their specific physicochemical nature. It is well known that use of a blend of...
surfactants can proficiently reduce the surface tension between oil and water during preparation of microemulsions (Peltola et al., 2003). Polyoxymethylene sorbitan esters (Tweens) are the effective and cost-effective non-ionic surfactants frequently used in microemulsions (Subramaniam et al., 2004). Of late, much attention has been focused on the utilization of phospholipids in pharmaceutically acceptable microemulsions (Magdassi and Siman-Tov, 1990; Lundsberg, 1994). Phospholipids are zwitterionic surfactants known for their biocompatibility (Zhang et al., 1996; Lawrence and Rees, 2000). In order to facilitate phospholipid-based microemulsions' formation, the surfactants based on short-chain alcohols have been used as cosurfactants (Stilbs et al., 1983; Aboofazeli et al., 1994). Lyophilized lecithin-based o/w microemulsions have been reported as low-toxic delivery systems for Amphoterica B (Moreno et al., 2003).

As there are no reports regarding the novel dithranol formulation based on lecithinized microemulsion system, the prime objective of this study was to formulate different types of lecithin-stabilized o/w microemulsion vehicles and to evaluate physicochemical characteristics for cutaneous delivery of dithranol.

Materials and methods

Materials

Free gift samples of dithranol and Phospholipon 90G were obtained from M/s Sujalam Chemicals (Mumbai, India) and M/s Phospholipid GmbH (Nattermannhalle, Germany), respectively. Terpeneless oil, soybean oil and olive oil were obtained from M/s Protina (Kolkata, India) and isopropyl myristate (IPM). Tween 20 (T20) and Tween 80 (T80) were procured from M/s S.D. Fine Chemicals Ltd. (Mumbai, India). Tocopherol acetate (TA) was procured from M/s S-Merck Ltd. (Mumbai, India) while absolute ethanol from M/s Bengal Chemicals Ltd. (Kolkata, India).

All other chemicals used for formulation development were of analytical grade.

Methods

Screening of oils, surfactants and cosurfactant

To find out the appropriate oil phase and surfactant in microemulsions, solubility of dithranol in various oils, such as IPM, soybean oil, olive oil, terpeneless oil and TA, and surfactants including T80, T20, Cremorphor EL, Nikkol HCO 40, Nikkol HCO 50 and Nikkol HCO 60 were determined using shake flask method. In brief, an excess amount of dithranol was added to 5 mL of oil/surfactant and then the resulting mixture was shaken reciprocally at 37°C for 72 h followed by centrifugation at 12,000 rpm for 10 min. The supernatant was filtered through a membrane filter (0.45 μm) and the drug concentration in the filtrate was determined by UV-visible spectrophotometer after the appropriate dilution with chloroform-methanol mixture (2:1 v/v). Phospholipon 90G was selected as the cosurfactant along with ethanol for the formulation of microemulsions. The ratio of Phospholipon 90G and ethanol was selected on the basis of solubility of dithranol in the respective ratios starting from 1:2.5 to 1:30 (Phospholipon 90G: ethanol). The oil, surfactant and cosurfactant ratios that showed high solubility of dithranol were later used for the preparation of microemulsions containing 0.5% dithranol.

Phase diagram construction

On the basis of solubility studies of dithranol, IPM and TA were selected as the oil phase. T80 and T20 were used as surfactants and phospholipid and ethanol used as the corresponding cosurfactants. Acetate buffer (pH 3.3) was employed as the aqueous phase for the construction of phase diagrams. Oil, surfactants and cosurfactants were grouped in eight different combinations for phase studies. Two Smax ratios (ratio of surfactant to cosurfactant) 1:1 and 2.75:1 for each surfactant and consequently four different combinations for each oil selected (Table 1). The ratio of aqueous phase and mixture of surfactant and cosurfactant (Smax) was varied (1:9-9:1), which were then titrated with oil till the appearance of turbidity. Similarly, ratio of oil and mixture of surfactant and cosurfactant was varied (1:9-9:1), which were then titrated with aqueous phase comprising of acetate buffer (pH 3.3), till the appearance of turbidity. The physical state of the lecithinized microemulsion was marked on a pseudo-three-component phase diagram with one axis representing aqueous phase, the other representing oil and the third representing a fixed Smax ratio. The microemulsion region was identified as the area in the phase diagram where clear and transparent formulations were obtained based on visual inspection (Kawakami et al., 2002).

Selection of formulations on the basis of phase diagrams

From each of the phase diagrams constructed, different formulations were chosen with minimum amount of emulsifier to a higher fixed value (approximately 60%) of emulsifier. This was assumed to represent the whole phase diagram.

Microemulsion formulation

According to the microemulsion areas in selected eight phase diagrams, various lecithinized microemulsions were selected at different component ratios. All microemulsion systems were prepared using analogous procedure. The oil phase was mixed with the phospholipid with the aid of heating (50-60°C) and the system was gently stirred on a magnetic stirrer (700 rpm for 10 min). The temperature of the above system was allowed to decrease and the surfactant was added to it. Then, the drug was dissolved in the above system to obtain a clear yellow solution. Required amount of aqueous phase (acetate buffer at pH 3.3) was added slowly under continuous stirring. Finally, ethanol was added to obtain a clear and transparent yellow coloured microemulsion.
Characterization

Various studies were conducted to characterize and study the effect of different processes and formulation variables on the quality of microemulsions. The formulations were kept randomly for a stipulated period of time under the given experimental conditions to assess the sustenance of the characteristics of microemulsion.

Drug content

The amount of drug contained in the microemulsions was determined by extracting the drug in chloroform: methanol (2:1) mixture and analysed using UV spectrophotometer. The plain microemulsion (without drug) with same composition served as the blank. The absorbance of the drug in this solution was noted on the basis of which drug content was determined using the following equation:

\[
\text{Concentration (mg/mL)} = \frac{\text{absorbance}}{A_{1cm} \times \text{dilution factor \times 10}^1}
\]

Globule size and zeta potential

The mean globule size and polydispersity index were measured at 25°C by photon correlation spectroscopy (PCS) using Malvern Zetasizer, Nano ZS 90 (Malvern Instruments Co., Worcestershire, UK) (Kelmann et al., 2007). The same instrument was employed to determine the zeta potential of the formulations. The operating principle of this instrument is based on the Doppler shift caused by the movement of globules across interference fringes which are produced by the intersection of two laser beams (Skiba et al., 1996).

Globule morphology

Morphology and structure of lipid microspheres were determined using transmission electron microscopy (TEM) at Central Instrumentation Laboratory (CIL), Panjab University, Chandigarh, and photomicrographs were taken at suitable magnifications.

Viscosity and refractive index

Viscosity of the formulation was measured using Rheometer (Hlberol QC, Anton Paar, Germany) at 25°C. Apparent viscosity at shear rate (\(\gamma\)) 0.05-100 s\(^{-1}\), was obtained at 25 ± 1°C. Experiments were carried out in triplicate and the results were presented as mean ± SD (SD, standard deviation).

To quantify the clarity of prepared system, refractive index (RI) was determined using Abbe’s refractometer at 37 ± 0.1°C. RI of the prepared formulations was compared vis-a-vis the RI of water.

Per cent transmittance measurement

The per cent transmittance of all the formulations was measured at 650 nm on a UV spectrophotometer (UV 1601, M/s Shimadzu, Kyoto, Japan) keeping distilled water as a blank.

Preparation of skin for ex vivo studies

To obtain mice skin, animals were sacrificed after obtaining approval from the Institutional Animal Ethics Committee. Hair on the dorsal side of animals were removed with the help of 0.1 mm animal hair clipper, in the direction of tail to head, and the skin was separated from the animal body. Dermis part of the skin was wiped three to four times with a wet cotton swab soaked in isopropanol to remove any adhering fat material. A portion of the skin was further employed for permeation studies after washing with distilled water (Singh et al., 2005).

Ex vivo skin permeation studies

Ex vivo permeation studies were conducted using abdominal skin of Laca mice employing Franz diffusion cells (PermeGear, Inc., Hellertown, PA, USA) with an effective diffusion area of 3.14 cm\(^2\) and sink volume of 30.0 mL. The skin tissue was adhered to the upper surface of receptor compartment. The receptor compartment contained acetate buffer (pH 3.3) with ascorbic acid (1% w/v), sodium metabisulphite (0.5% w/v), ethylenediamine tetra acetate (EDTA; 0.5% w/v) and sodium chloride (0.9% w/v) as stabilizers along with Cremophor RH 40 (1% w/v) and methanol (20% v/v) as diffusion medium. Various formulations containing dithranol equivalent to 1 mg were applied onto the skin in the donor compartment. Plain dithranol drug suspension was also applied onto the skin of donor compartment for comparison. The formulations were uniformly spread and were maintained in intimate contact with the skin. At suitable time intervals, aliquots of 1 mL each were withdrawn through the sampling port and replaced with equal amounts of diffusion medium to maintain constant receptor volume. The samples were spectrophotometrically analysed after suitable dilution.

Calculation of the ex vivo data

The raw data obtained from permeation studies were analysed by applying factor for volume correction and drug losses during sampling using the following equation, which calculates the values of fraction of drug permeated
and mean per cent drug permeated along with SD at varied times.

\[ C_t = C_0 + \left( \frac{V_t}{V_d} \right) \sum_{i=1}^{n-1} C_i \]  

(2)

For the nth sample, \( V_t \) is the volume of sample withdrawn, \( V_d \) the total volume of receptor medium, \( C_0 \) the corrected concentration, \( C_t \) the uncorrected concentration of the nth sample and \( C_i \) the uncorrected concentration.

Further, the corrected concentrations were used to calculate the values of the amount of drug released, and per cent drug released at each time of sampling. The flux values were calculated from the graph between the amounts of drug released per unit surface area versus time. The slope of the linear portion of the curve was taken as flux value.

Skin retention studies

Following permeation studies, the skin tissue mounted on the diffusion cell was removed and washed three to four times with distilled water. The treated skin area was weighed and dried using lint free cotton swab. Subsequently, the skin tissue was mashed with tissue homogenizer. The homogenate suspension thus obtained was mixed with 20 mL chloroform-methanol mixture (2:1, v/v) and shaken for 2h at 37 ± 1°C for complete extraction of dithranol. Supernatant was filtered through a 0.45 μm membrane filter (M/s Millipore, Massachusetts, USA) and quantified for drug content spectrophotometrically. Fresh skin tissue, treated in a similar manner, served as blank for the above study. Each experiment was conducted in triplicate (Singh et al., 2005).

Statistical analysis

The permeation and skin retention data between different formulations were compared for statistical significance by the one-way analysis of variance (ANOVA) followed by Student’s t-test using Microsoft Excel.

Stability studies

All microemulsion formulations were subjected to drug loss studies at different temperatures. After measuring the initial drug content in all the formulations, these were stored in sealed glass ampoules (three each) at 5 ± 3°C, 30 ± 2°C and 45°C ± 2°C for a period of at least 45 days. After every 7 days, the drug content was determined as per the method described in drug content studies.

Results and discussion

Screening of components for microemulsions

For poorly soluble drugs like dithranol, screening of microemulsion components (oil, surfactant and cosurfactant) on the basis of solubility is desirable to maintain the drug in solubilized state. The solubility of dithranol in various media was analysed. In five oils studied, the solubility of dithranol was the highest in TA (3.47 mg/mL) followed by IPM (3.15 mg/mL). The solubility in other three oils was relatively lower (olive oil, 2.59 mg/mL; soybean oil, 2.53 mg/mL; and terpeneless oil, 2.44 mg/mL). Both oils of ester nature, i.e. IPM and TA offered better sink to the drug, and both these oils were selected for further studies.

Previous reports indicated that the superior dermal flux appeared mainly due to the large solubilizing capacity of the microemulsions, which led to larger concentration gradient towards the skin (Krellgaard, 2002; Skrlov and Shapiro, 2004). It has also been reported that IPM acts as penetration enhancer which helps in increasing the permeability of drug into the skin (Kogan and Garti, 2006), whereas tocopherol has antioxidant properties which will certainly enhance the photostability of the drug dithranol.

Proper selection of surfactants is necessary as larger amounts of surfactants may cause skin irritation on topical application. It is, therefore, important to determine the surfactant which, besides being used in minimum concentration, possesses the highest solubilization capacity for the drug.

Non-ionic surfactants are relatively less toxic than their ionic counterparts and typically have lower critical micelle concentrations (CMCs; Kawakami et al., 2002). Out of the six non-ionic surfactants, the maximum solubility of dithranol was obtained in T80 (6.20 mg/mL) followed by T20 (6.00 mg/mL). Other non-ionic surfactants (Cremophore EL, 4.22 mg/mL; Nikkol HCO 40, 4.12 mg/mL; Nikkol HCO 50, 3.10 mg/mL; and Nikkol HCO 60, 1.99 mg/mL) not only offered less solubility to dithranol, but also resulted in quick degradation of dithranol (visual blackening of erstwhile yellow drug) as compared to T80 and T20.

Phospholipid 90G along with ethanol was used as cosurfactant. Phospholipid 90G helps in increasing the permeation of drug into the skin as phospholipids are the essential components of all biological membranes. When they are applied to skin as vehicles, due to their physicochemical properties and structures, they can interact with the stratum corneum lipids, perturb their structures and facilitate drug delivery (Yokomizo and Sagitani, 1996; Williams and Barry, 2004). The absolute alcohol used as the cosurfactant, was preferred since it is safe and non-toxic (Lawrence and Rees, 2000). Also, the presence of alcohol in the interfacial region causes a reduction in the rigidity of the otherwise-condensed lecithin film, allowing the curvature necessary for droplet formation (Buch et al., 1995).

For the two cosurfactants, i.e. phospholipid and ethanol were selected on the basis of solubility of dithranol. The solubility values in all ratios were in the order 1:2.5 (2.48 mg/mL) > 1:0.5 (2.16 mg/mL) > 1:7.5 (2.08 mg/mL) > 1:10 (2.17 mg/mL) > 1:20 (1.32 mg/mL) > 1:25 (1.30 mg/mL) > 1:30 (1.28 mg/mL). Drug exhibited maximum solubility in the ratio 1:2.5, but a ratio of 1:10 was selected for further studies due to its comparable solubility to 1:2.5 ratio as well as being a cost-effective alternative.

Further, Suss ratio (ratio of surfactant to cosurfactant) was decided on the basis of solubility studies performed in five different ratios (1:1, 2:7.5:1, 5:1, 10:1 and 20:1). Suss ratios containing T80 offered good solubility with the

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maximum in ratio 2.75:1 (8.403 mg/mL) followed by 1:1 (8.148 mg/mL). The solubility values in other ratios were little less (5:1 = 7.65 mg/mL, 10:1 = 6.32 mg/mL and 20:1 = 5.86 mg/mL). Thus, two Smix ratios were fixed to carry out the phase diagram studies.

**Construction of phase diagram**

The relationship between the phase behaviour of a mixture and its composition can be established with the aid of a phase diagram (Craig et al., 1995). Various pseudo-ternary phase diagrams of o/w microemulsions comprising of IPM/TA, T80/T20, lecithin, ethanol and acetate buffer, pH 3.3 are shown in Figures 1 and 2. The surfactant and cosurfactant mass ratio had been found to be a key factor influencing the phase properties, i.e. size and position of microemulsion region (Kreilgaard et al., 2000; Hua et al., 2004). The kind and concentration of oil employed also play an important role (Malcolmson et al., 1998; Yuan et al., 2006). With same surfactant T80 and Smix ratio 1:1 (Figures 1(a) and 1(b)), it can be observed that for the given amount of emulsifier, a slightly bigger microemulsion region was obtained with IPM (Figure 1(b)) in comparison to that with TA (Figure 1(a)) as oil phase. The maximum amount of oil emulsified in case of TA was 10.10%, whereas in case of IPM it was 15.7%. This can be attributed to the fact that IPM is easily emulsified than TA, i.e. needs relatively lesser amount of the selected emulsifier for its emulsification. Effect of surfactant and cosurfactant mass ratio (Smix) on microemulsion formation was prominent. When the concentration of cosurfactant increased (Smix ratio 2.75:1), in case of TA as oily phase and T80 as surfactant (Figure 1(c)), the microemulsion area increased and the oil solubilized increased up to 20% w/w, with the total surfactant concentration of 52% w/w. Similar observation was also noted when IPM (Figure 1(d)) was used as oily phase and T80 as surfactant. The maximum amount of oil that could be accommodated in the higher ratio (2.75:1) was 24% that too at a lower concentration of surfactant mixture, i.e. 44% w/w. This may be due to the substantial reduction of the o/w interfacial tension and increase in the interface fluidity with increasing amounts of cosurfactants (Azeeem et al., 2009).

The microemulsion region was considerably smaller when T20 was used as surfactant instead of T80. In Figure 2(a) (TA as oily phase, Smix 1:1), maximum amount of oil emulsified was only 4.74% and in Figure 2(b) (IPM as oily phase, Smix 1:1), it was found to be 2.82%. On increasing the cosurfactant concentration (Smix 2.75:1), in case of TA as oily phase, the maximum amount of oil that could be emulsified was 5.16% with the use of 66.39% emulsifier (Figure 2(c)) and in case of IPM, maximum amount of oil emulsified was 20% with the use.
of 58% emulsifier (Figure 2(d)). Pseudo-ternary phase diagrams with varied oil, surfactant and cosurfactant ratios suggested their influence on the formulation attributes. It can be generalized that T80 was found to be a better emulsifier than T20 for given set of oil, and IPM was easily emulsified than TA.

Selection of formulations on the basis of phase diagrams

There are no reports of the proper basis of selecting different microemulsion formulations from a phase diagram, as hundreds of formulations can be prepared from microemulsion region of a single diagram. The variation in the number of formulations was because of the difference in the size of microemulsion region which could be due to the different surfactants employed; T80 for ternary diagrams (Figures 1(a)-1(d)) and T20 for ternary diagrams (Figures 2(a)-2(d)). From the first four pseudo-ternary phase diagrams (Figures 1(a)-1(d)), totally three formulations were selected from each phase diagram; one formulation comprised of minimum amount of emulsifier (irrespective of the amount of oil being emulsified), the other formulation consisted of 10% of oil, with a maximum amount of emulsifier being up to 65% and another formulation incorporated 70% emulsifier, irrespective of the amount of oil being emulsified. For the remaining ternary phase diagrams (Figures 2(a)-2(d)), two formulations were selected from each phase diagram – formulations employing about 10% of oil. The compositions of all 20 microemulsion formulations are given in Table 2.

Table 2. Composition of all the formulations.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Oil phase code</th>
<th>S_{oil} (surfactant:cosurfactant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F11</td>
<td>TA (4%)</td>
<td>T80: (Phospholipid 90 G: ethanol) 45%</td>
</tr>
<tr>
<td>F12</td>
<td>TA (10%)</td>
<td>T80: (Phospholipid 90 G: ethanol) 50%</td>
</tr>
<tr>
<td>F13</td>
<td>TA (13%)</td>
<td>T80: (Phospholipid 90 G: ethanol) 65%</td>
</tr>
<tr>
<td>F21</td>
<td>IPM (5%)</td>
<td>T80: (Phospholipid 90 G: ethanol) 50%</td>
</tr>
<tr>
<td>F22</td>
<td>IPM (11%)</td>
<td>T80: (Phospholipid 90 G: ethanol) 65%</td>
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<tr>
<td>F23</td>
<td>IPM (16%)</td>
<td>T80: (Phospholipid 90 G: ethanol) 60%</td>
</tr>
<tr>
<td>F31</td>
<td>TA (3%)</td>
<td>T80: (Phospholipid 90 G: ethanol) 45%</td>
</tr>
<tr>
<td>F32</td>
<td>TA (10%)</td>
<td>T80: (Phospholipid 90 G: ethanol) 50%</td>
</tr>
<tr>
<td>F33</td>
<td>TA (15%)</td>
<td>T80: (Phospholipid 90 G: ethanol) 60%</td>
</tr>
<tr>
<td>F41</td>
<td>IPM (3%)</td>
<td>T80: (Phospholipid 90 G: ethanol) 40%</td>
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<tr>
<td>F42</td>
<td>IPM (5%)</td>
<td>T80: (Phospholipid 90 G: ethanol) 50%</td>
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<tr>
<td>F43</td>
<td>IPM (10%)</td>
<td>T80: (Phospholipid 90 G: ethanol) 60%</td>
</tr>
<tr>
<td>F51</td>
<td>TA (5%)</td>
<td>T20: (Phospholipid 90 G: ethanol) 50%</td>
</tr>
<tr>
<td>F52</td>
<td>TA (15%)</td>
<td>T20: (Phospholipid 90 G: ethanol) 60%</td>
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<tr>
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<td>IPM (3%)</td>
<td>T20: (Phospholipid 90 G: ethanol) 45%</td>
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<td>IPM (5%)</td>
<td>T20: (Phospholipid 90 G: ethanol) 55%</td>
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<tr>
<td>F63</td>
<td>IPM (10%)</td>
<td>T20: (Phospholipid 90 G: ethanol) 65%</td>
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<td>TA (3%)</td>
<td>T20: (Phospholipid 90 G: ethanol) 40%</td>
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<td>F72</td>
<td>TA (7%)</td>
<td>T20: (Phospholipid 90 G: ethanol) 50%</td>
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<td>F81</td>
<td>IPM (3%)</td>
<td>T20: (Phospholipid 90 G: ethanol) 40%</td>
</tr>
<tr>
<td>F82</td>
<td>IPM (5%)</td>
<td>T20: (Phospholipid 90 G: ethanol) 50%</td>
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</tbody>
</table>

Note: Formulations F11-F23 and F51-F62 comprise of fixed S_{oil} ratio i.e. 1:1 and formulations F31-43 and F71-F82 contain S_{oil} ratio 2.75:1. Acetate buffer, pH 3.3 (q.s) was used as the aqueous phase.
Table 3. Physicochemical characterization of formulations.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug content (%)</th>
<th>Globule size (nm)</th>
<th>Zeta potential (mV)</th>
<th>PDI</th>
<th>Viscosity (cP)</th>
<th>RI</th>
<th>% Transmittance</th>
</tr>
</thead>
<tbody>
<tr>
<td>F11</td>
<td>99.48</td>
<td>313.06</td>
<td>-2.11</td>
<td>0.51</td>
<td>160.67 ± 0.15</td>
<td>1.38</td>
<td>96.1</td>
</tr>
<tr>
<td>F12</td>
<td>99.68</td>
<td>281</td>
<td>-0.122</td>
<td>0.49</td>
<td>136.67 ± 0.58</td>
<td>1.40</td>
<td>97.9</td>
</tr>
<tr>
<td>F13</td>
<td>99.57</td>
<td>197.9</td>
<td>-0.134</td>
<td>0.35</td>
<td>113.00 ± 0.00</td>
<td>1.41</td>
<td>98.1</td>
</tr>
<tr>
<td>F21</td>
<td>99.53</td>
<td>295.2</td>
<td>-0.10</td>
<td>0.32</td>
<td>83.00 ± 0.10</td>
<td>1.38</td>
<td>97.4</td>
</tr>
<tr>
<td>F22</td>
<td>99.47</td>
<td>259.6</td>
<td>-0.045</td>
<td>0.44</td>
<td>52.13 ± 0.25</td>
<td>1.38</td>
<td>97.5</td>
</tr>
<tr>
<td>F23</td>
<td>99.65</td>
<td>285</td>
<td>0.254</td>
<td>0.36</td>
<td>52.67 ± 0.21</td>
<td>1.38</td>
<td>97.8</td>
</tr>
<tr>
<td>F31</td>
<td>99.63</td>
<td>376</td>
<td>0.087</td>
<td>0.38</td>
<td>405.67 ± 0.38</td>
<td>1.40</td>
<td>95.12</td>
</tr>
<tr>
<td>F32</td>
<td>99.67</td>
<td>127</td>
<td>-0.0250</td>
<td>0.37</td>
<td>378.67 ± 0.08</td>
<td>1.42</td>
<td>96.75</td>
</tr>
<tr>
<td>F33</td>
<td>99.62</td>
<td>240.4</td>
<td>0.011</td>
<td>0.43</td>
<td>348.33 ± 0.08</td>
<td>1.44</td>
<td>97.1</td>
</tr>
<tr>
<td>F41</td>
<td>99.81</td>
<td>172.2</td>
<td>-0.0027</td>
<td>0.32</td>
<td>235.33 ± 0.08</td>
<td>1.38</td>
<td>97</td>
</tr>
<tr>
<td>F42</td>
<td>99.66</td>
<td>72.8</td>
<td>0.0731</td>
<td>0.32</td>
<td>220.67 ± 0.08</td>
<td>1.40</td>
<td>97</td>
</tr>
<tr>
<td>F43</td>
<td>99.47</td>
<td>92.7</td>
<td>-0.0180</td>
<td>0.40</td>
<td>220.33 ± 0.08</td>
<td>1.42</td>
<td>97</td>
</tr>
<tr>
<td>F51</td>
<td>99.43</td>
<td>339</td>
<td>0.0287</td>
<td>0.44</td>
<td>119.47 ± 0.15</td>
<td>1.38</td>
<td>97</td>
</tr>
<tr>
<td>F52</td>
<td>99.70</td>
<td>469</td>
<td>0.0796</td>
<td>0.42</td>
<td>113.57 ± 0.06</td>
<td>1.38</td>
<td>96.3</td>
</tr>
<tr>
<td>F61</td>
<td>99.67</td>
<td>474</td>
<td>0.055</td>
<td>0.45</td>
<td>182.17 ± 0.06</td>
<td>1.40</td>
<td>96.4</td>
</tr>
<tr>
<td>F62</td>
<td>99.58</td>
<td>512.1</td>
<td>0.167</td>
<td>0.35</td>
<td>190.03 ± 0.06</td>
<td>1.40</td>
<td>96.1</td>
</tr>
<tr>
<td>F71</td>
<td>99.54</td>
<td>289</td>
<td>0.0354</td>
<td>0.41</td>
<td>222.33 ± 0.08</td>
<td>1.42</td>
<td>97.6</td>
</tr>
<tr>
<td>F72</td>
<td>99.49</td>
<td>433</td>
<td>0.067</td>
<td>0.42</td>
<td>269.80 ± 0.39</td>
<td>1.40</td>
<td>97</td>
</tr>
<tr>
<td>F81</td>
<td>99.51</td>
<td>597</td>
<td>0.0355</td>
<td>0.32</td>
<td>290.83 ± 0.12</td>
<td>1.40</td>
<td>96.4</td>
</tr>
<tr>
<td>F82</td>
<td>99.82</td>
<td>771.5</td>
<td>-0.0523</td>
<td>0.38</td>
<td>393.27 ± 0.29</td>
<td>1.42</td>
<td>95</td>
</tr>
</tbody>
</table>

Note: PDI, polydispersity index and RI, refractive index.

### Drug content

The drug content of various formulations ranged from 99.43% to 99.81% with a mean value of 99.55% (Table 3). These higher values of drug content ensured minimal drug loss during various stages of formulation development, hence, authenticated the method of preparation.

### Micromeritics and zeta potential

The zeta potential of all the prepared microemulsions was found to be approximately zero (Table 3). This may be attributed to non-ionic nature of both surfactants and cosurfactants used (i.e. T80 or T20 and lecithin). They do not impart any charge to the droplet surface and provide better stability to the microemulsion against any ionic interactions. Of all these formulations, the smallest globule size was of formulation F42 (72.8 nm) followed by formulation F32 (127 nm) and the largest globule size was of formulation F82 (771.5). The differences in the globule size may be attributed to the use of different surfactants in these formulations which were T80 for F42 and F32 and T20 for formulation F82. Hence, these results point towards the better emulsifying capability of T80 in this study which resulted in smaller globule size of the oil droplets.

### Globule morphology

TEM revealed the spherical nature and size homogeneity of the microemulsion droplets (Figure 3).

### Viscosity and RI

The values for viscosity for the various formulations are given in Table 3. All microemulsions showed Newtonian flow behaviour, when shear stress was plotted against shear rate. These results were in accordance with the earlier reports (Cilek et al., 2006). The Newtonian flow behaviour indicated that the droplets were small and spherical in nature (Ktistis, 1990). The constancy in RI values (1.4 ± 2) of all the formulations is an indication of constant microemulsion structure (Cilek et al., 2006).

### Per cent transmittance measurement

The clarity of microemulsion was checked by transparency, measured in terms of per cent transmission (%T).
Per cent transmittance values of all the formulations were around 97%, indicating high clarity of the formulations.

Skin permeation study

The ex vivo permeation of dithranol through abdominal skin of Laca mice from all microemulsions was determined in terms of per cent mean cumulative amount diffused at each sampling time point during time period of 24 h. The key factors that may have aided in enhancement of skin permeation are the choice of oil components, surfactant/cosurfactant and cosolvent in the formulation, particle diameter, mobility of the bioactive ingredient in the designed formulation and the concentration gradient (Lin et al., 2009). Statistically, the data obtained for skin permeation studies are highly significant ($p < 0.001$). In our study, the results indicated that the smaller globule diameters, as in two cases F42 and F32, resulted in enhanced skin permeation, in comparison to all other formulations (Figure 4). As shown in Figure 4 and Table 4, the per cent permeation and flux value of F42 were 82.3%, 0.281 mg/cm²/h, respectively, as compared to F32 which were 80.61% and 0.125 mg/cm²/h, respectively. Statistically, the difference in % permeation and flux values of F42 was highly significant ($p < 0.005$) vis-a-vis that of F32 (Table 4). The cumulative amount released, average flux values of all 20 microemulsions are given in Table 4. Microemulsions containing minimum amount of emulsifier showed a lesser permeation flux but as the concentration of emulsifier was increased to a certain extent keeping rest of the components constant, the permeation flux increased considerably; however, with further increase in emulsifier content up to 60%, the permeation flux did not increase appreciably. This could be attributed to the fact that high surfactant concentration decreases the thermodynamic activity of the drug in the vehicle, and the affinity of the drug to the vehicle becomes greater, consequently decreasing the flux (Shinoda and Kaneko, 1988). However, at lower concentration, due to the incorporation of phospholipoidal structures in the skin layers, surfactants can loosen or fluidize the lipidic matrix of the skin and can act as permeation promoters (Yokomizo and Sagitani, 1996; Williams and Barry, 2004). Therefore, it is conceivable that the permeation of dithranol is accompanied by the environment favourable to the partitioning of drug into the skin. Further, the formulations containing IPM as oily phase showed high permeation flux values in comparison to TA as oily phase because of the better solubilizing power of IPM, its penetration enhancement effect and higher emulsification by the surfactant.

Skin retention studies

In this study, percentage skin retention was estimated after 24 h for all 20 microemulsions ($p < 0.001$) (Table 4). Skin retention decreased with increase of emulsifier after a certain value. The findings are in consonance with the skin permeation studies. This may be ascribed to the fact that high concentration of alcohol (cosurfactant) had dissolved more of phospholipid as well as drug, resulting in the formation of pseudosolutions, therefore, increased permeation and retention. High levels of emulsifier might have formed their own vesicular systems consisting of various lamellae in which drug solution (because of increased ethanol content) is channelized and face few lamellar barriers for its release.

Maximum skin retention was achieved with the use of T80, while other variables were kept constant, i.e. maximum for formula F42 followed by F32. This high value of skin retention in the case of F42 ($p < 0.005$) when compared to F32 can be attributed to the nature of oil phase. These globules may have interacted with skin phospholipids and formed a depot resulting in increased skin retention. However, with TA microemulsions, the
extent of emulsification with same concentration of emul-
gents was quite low. Therefore, in this case, there will be
formation of depot, but to a lower extent, and most of
the drug will be released by diffusion prior to permeation via

stability studies

Stability studies at 5 ± 3°C, 30 ± 2°C and 45 ± 2°C were
performed for all the 20 formulations prepared for a period
of at least 45 days. Formulation F42 showed minimal drug
loss after 45 days at all studied temperatures. The percent-
age drug loss were 0.49% at 5 ± 2°C, 0.84% at 30 ± 2°C and
1.34% at 45 ± 2°C.

The results obtained indicate that all the formulations
were more stable at refrigerated conditions, i.e. at 5 ± 3°C,
and all the formulations were fairly stable at 45 ± 2°C.
Based on these results (Table 5), it could be concluded
that the best storage temperature for the prepared microem-
ulsions was 5 ± 3°C.

Conclusion

In conclusion, this study resulted in successful produc-
tion of novel dithranol phospholipid-based o/w microemulsion
employing various oils and surfactants/cosurfactants.
Studies accomplished to establish the effect of various
components on the design and development of emulsified
microemulsion systems containing dithranol. Overall, our
studies illustrated the enhanced dermal delivery of dithra-

Declaration of interest

The authors report no conflicts of interest.

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the formation and characterization of phospholipid microemulsions.
III. Pseudo-ternary phase diagrams of systems containing water-le-
cithin IPM and either an alkanoic acid, amine, alkane-diol, polyethylene


Novel drug delivery systems in topical treatment of psoriasis: Rigors and vigors

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ABSTRACT
Psoriasis is a chronic inflammatory skin disorder that may drastically impair the quality of life of a patient. Among the various modes of treatments for psoriasis, topical therapy is most commonly used in majority of patients. The topical formulations based on conventional excipients could serve the purpose only to a limited extent. With the advent of newer biocompatible and biodegradable materials like phospholipids, and cutting-edge drug delivery technologies like liposomes, solid lipid nanoparticles (SLNs), microemulsions, and nanoemulsions, the possibility to improve the efficacy and safety of the topical products has increased manifold. Improved understanding of the dermal delivery aspects and that of designing and developing diverse carrier systems have brought in further novelty in this approach. Substantial efforts and the consequent publications, patents and product development studies on the subject are the matter of interest and review of this article. However, majority of the work is related to the preclinical studies and demands further clinical assessment in psoriasis patients.

Key words: Dermal drug delivery, Liposomes, Microemulsions, Nanoparticles, Phospholipids, Vesicles

INTRODUCTION
Topical therapy is the mainstay of treatment for mild to moderate psoriasis and serves as a useful adjunct support to systemic therapy in severe disease. However, efficacy and compliance to topical therapy in psoriasis have been a major concern. Approximately, 70% of the psoriasis patients in three large surveys found to be unsatisfied or moderately satisfied with their current treatment. Lack of effective delivery of drugs and undesirable skin interactions of the topical treatments are the main reasons for patient noncompliance. Nevertheless, newer developments in the formulation approaches have raised hopes in making topical therapy more useful and acceptable. The present paper endeavors to review the overall developments in the field of Novel Drug Delivery Systems (NDDS) pertaining to the topical treatment of psoriasis.

NOVEL DRUG DELIVERY SYSTEMS
In search of safe and effective therapy, the development of new drugs has been the common practice historically. However, it involved a long gestation period in terms of time, efforts, and huge cost. Later on, it was realized that the issues pertaining to efficacy and safety are largely influenced by the distribution of the drug within the biological system, as there is appreciable deviation from the desired site of action, i.e., the target site. In fact, Nobel laureate, Sir Paul Ehrlich in 1905 envisioned the drug molecules as “magic-bullets” to hit the specific target site to attain the absolute efficacy and safety. This objective, hitherto un-accomplished gave way to an alternate approach of drug delivery, wherein the carrier systems were used to deliver the molecules to specific receptor sites without afflicting the normal tissues and organs of the body. Interestingly, it turned out to be a transformation of the original idea of “magic-bullets” to that of the “magic-guns.” The fundamentals lie in hosting the drug in carefully designed carriers to bring favorable changess in its surrounding microenvironment, and consequently, its delivery. It is
the modification(s) in physicochemical characteristics of the molecules and in the barrier properties of the biological membranes at various locations, which lead to improved transportation of drugs toward the diseased locations. Further, it improves the chances of the availability of the drug at the specific receptor site and enhances drug-receptor interaction through mediation of specialized composition and design of the carrier systems. All these factors tend to potentiate the degree of pharmacodynamic response, the safety and patient compliance being the immediate benefits.

The novel carriers have been exploited through almost all the routes of administration. However, the topical route has been adjudged as one of the most relevant to treat dermatological disorders more effectively. In contrast to the conventional formulations based on creams and ointments, these novel dermatological systems are different in their composition and constructs including their exterior and interior design. Various pharmaceutical and dermatological variables influence the choice of the system as per the demand of the drug and disease. Phospholipids represent a special class of surfactants with two long fatty acid chains (lipid region) and a bulky polar head (hydrophilic region) linked with phosphogroup on glycerol as the backbone. The unique structural features allow phospholipids to interact with water to form well-organized supra-structures like liposomes. The variation in composition and methods influences the nature of such self-assembled supra-structures in terms of their shape, design, size, and surface properties. This leads to different classes of carriers, viz. liposomes, transfersomes, micro and nanoemulsions, niosomes, dendrimers, solid lipid nanoparticles (SLNs), and nano lipid carriers (NLCs) [Table 1]. These carrier systems provide the entrapment opportunities to the drug molecules within their interior locations as per their fitment of steric and physicochemical properties. Association of drugs with carriers is normally noncovalent, based on collective strength of weak binding forces. Many newer carriers are evolving with the advent of technology and the demand of targeted delivery like ethosomes, emulsomes, magnetic nanoparticles, rescaled erythroosomes and bilosomes.

Apart from projected advantages, the novel carriers have associated drawbacks of high cost of excipients, need of expertise in the production of such carriers, stability, and evaluation issues.

| Table 1: Various colloidal carriers employed during topical delivery of drugs |
|---------------------------------|------------------------------------------|
| Drug delivery carrier systems | Description                                                                 |
| Liposomes | Vesicular carriers composed of bilayers of phospholipid molecules and enclosed water in those bilayers |
| Niosomes | Vesicular carriers composed of non-ionic surfactants instead of phospholipids |
| Microemulsions | Thermodynamically stable, isotropically clear and transparent carriers composed of oil, aqueous phase and surfactant(s). They are supersolvents. |
| Lipid emulsions | Micro- and nano-emulsions containing phospholipids as one of their surfactants |
| Transfersomes, flexible membrane vesicles | Liposomes with edge activators, highly deformable, reported to penetrate stratum corneum as such |
| Ethosomes | Liposomal systems comprising of high alcohol content, flexible vesicles, high drug loading |
| Solid lipid nanoparticles | Nanocarriers composed of drug loaded in solid lipid particles |
| Emulsomes | Nanocarriers with solid lipid core along with bilayers of phospholipids |
| Nanolipid carriers | Nanocarriers composed of drug loaded in lipid core composed of both solid and liquid lipids |
| Invasomes | Liposomes containing penetration enhancers |
| Dendrimers | Repeatedly branched, roughly spherical large molecules also used for drug delivery |

Figure 1 illustrates the pictorial representation of such interactions of the carriers with skin. The novel carrier systems are versatile and flexible in handling the various issues associated with the drug and the diseases. Table 2 enumerates the meritorious roles of NDI in topical therapy. Various attempts have been made...
in the recent past in reporting many studies for the delivery of various drugs employing novel colloidal carriers. Table 3 enlists selected instances.\textsuperscript{125-641}

**CHALLENGES IN TOPICAL DELIVERY OF DRUGS IN PSORIATIC SKIN**

According to the studies reported recently, stratum corneum (SC) is not an inert layer, but an "active-wall," which opposes the penetration of xenobiotics.\textsuperscript{4} Though no molecule can readily and fully pass through this membrane, yet it allows penetration of nearly all the materials to some extent. It is also vivid that the major route of penetration across the SC is the intercellular lipids.\textsuperscript{5} The state of hydration of SC is one of the most important factors in determining the rate of percutaneous absorption of a given solute. The level of hydration is a function of the water concentration gradient between the dermis and the surface of the skin as well as the ability of the SC to "bind" water.\textsuperscript{10} Delivery of solutes through the skin is associated with a number of difficulties as shown in Table 4.

“Rigidization” of psoriatic skin has been attributed to a rise in the levels of cholesterol and fall in the levels of ceramides.\textsuperscript{6} Apart from this, normal moisturizing factors (NMFs) like water are almost absent in the psoriatic skin. As a result of various factors, targeting the psoriatic tissues using topical route poses a big challenge.

<table>
<thead>
<tr>
<th>Table 2: Role of Novel drug delivery systems</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Use of versatile carriers</td>
</tr>
<tr>
<td>• Imparting protection to the molecules</td>
</tr>
<tr>
<td>• Biocompatibility of the systems</td>
</tr>
<tr>
<td>• Passive targeting</td>
</tr>
<tr>
<td>• Loading a variety of drugs</td>
</tr>
<tr>
<td>• Modifications in the physicochemical properties</td>
</tr>
</tbody>
</table>

Table 3: List of drugs (topical) encapsulated in various carrier systems\textsuperscript{125-641}

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Indication</th>
<th>Type of Drug Delivery System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporin A</td>
<td>Allergic skin disorders (atopic dermatitis)</td>
<td>Solid lipid nanoparticles\textsuperscript{29}</td>
</tr>
<tr>
<td>Calcineurin inhibitors</td>
<td>Prevent DNA photodamage</td>
<td>Liposomes\textsuperscript{30}</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>Fungal infection</td>
<td>Liposomes\textsuperscript{31}</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>Fungal infection</td>
<td>Liposomal gel\textsuperscript{32}</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>Fungal infection</td>
<td>Ethosomes\textsuperscript{33}</td>
</tr>
<tr>
<td>NB-002</td>
<td>Fungal infection</td>
<td>Nanoemulsion\textsuperscript{34}</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>Fungal infection</td>
<td>Liposomes\textsuperscript{35}</td>
</tr>
<tr>
<td>Ciclopirox Ointment</td>
<td>Fungal infection</td>
<td>Liposomal gel\textsuperscript{36}</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Psoriasis</td>
<td>Liposomes\textsuperscript{37}</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Psoriasis</td>
<td>Ethosomes\textsuperscript{38}</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Psoriasis</td>
<td>Niosomes\textsuperscript{39}</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Psoriasis</td>
<td>Liposomes\textsuperscript{40}</td>
</tr>
<tr>
<td>Temoporfin</td>
<td>Photodynamic therapy- psoriasis</td>
<td>Liposomal gels\textsuperscript{41}</td>
</tr>
<tr>
<td>Dithranol</td>
<td>Psoriasis</td>
<td>Liposomes and niosomes\textsuperscript{42,43}</td>
</tr>
<tr>
<td>Coal tar</td>
<td>Psoriasis</td>
<td>Lecithinized coal tar formulation\textsuperscript{44}</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>Psoriasis</td>
<td>Liposomal gel\textsuperscript{45}</td>
</tr>
<tr>
<td>Cetirizine</td>
<td>Pruritus</td>
<td>Liposomes\textsuperscript{46}</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>Pruritus</td>
<td>Liposomes\textsuperscript{47}</td>
</tr>
<tr>
<td>Lauric acid</td>
<td>Inflammatory acne</td>
<td>Liposomes\textsuperscript{48}</td>
</tr>
<tr>
<td>Azelaic acid</td>
<td>Acne</td>
<td>Liposomes and Ethosomes\textsuperscript{49}</td>
</tr>
<tr>
<td>Tretinoin</td>
<td>Acne</td>
<td>Liposomal gel\textsuperscript{50}</td>
</tr>
<tr>
<td>Benzyl peroxide</td>
<td>Acne</td>
<td>Liposomal gel\textsuperscript{51}</td>
</tr>
<tr>
<td>Idoxuridine</td>
<td>Herpes simplex</td>
<td>Liposomal gel\textsuperscript{52}</td>
</tr>
<tr>
<td>Dipotassium Glycyrrhizinate</td>
<td>Acute and chronic dermatitis</td>
<td>Liposomes\textsuperscript{53}</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>Allergic dermatitis</td>
<td>Magnetic liposomes\textsuperscript{54}</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>Musculoskeletal pain</td>
<td>Flexible membrane vesicles\textsuperscript{55}</td>
</tr>
<tr>
<td>Nimexulide</td>
<td>Inflammation and pain</td>
<td>Liposomes\textsuperscript{56}</td>
</tr>
<tr>
<td>Finasteride</td>
<td>Acne, androgenetic alopecia</td>
<td>Liposomes\textsuperscript{57}</td>
</tr>
<tr>
<td>Corticosteroid</td>
<td>UV induced erythema</td>
<td>Skin-lipid liposomes\textsuperscript{58}</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>Certain skin disorders</td>
<td>Liposomes\textsuperscript{59}</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td>Skin Allergy</td>
<td>Liposomes\textsuperscript{60}</td>
</tr>
<tr>
<td>Vitamin D analogues</td>
<td>Antiperakeratosis function</td>
<td>Liposomes\textsuperscript{61}</td>
</tr>
<tr>
<td>Calcipotriol, tacalcitol, calcitriol</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Psoriasis and novel drug delivery systems

Table 4: Challenges for topical drug delivery
- Variability in percutaneous absorption due to site, disease, age, etc.
- Skin “first-pass” metabolic effect
- Reservoir capacity of the skin
- Irritation potential and other toxicities due to drug
- Heterogeneity and inducibility of the skin in turn-over and metabolism
- Inadequate definition of bioequivalence criteria
- Incomplete understanding of technologies to facilitate or reduce percutaneous absorption

Challenges. The intricacies of the topical delivery into the psoriatic skin have lately been proposed to be addressed by the lipoidal carrier systems, such as liposomes. The latter resolve the problem of lipid imbalance by imparting the unsaturated fatty acids like linoleic acid to restore the normal skin conditions. Hence, these liposomal and allied carriers can result in an effective delivery of drugs across the psoriatic skin.

Several topical therapeutic agents are available for the treatment of psoriasis. Nevertheless, none of them can be regarded as an ideal drug molecule. This may either be due to their inherent side effects or their improper incorporation in the conventional vehicles. It is a well-known fact that due to variation in the physicochemical characteristics of the carrier and of the active compounds used, the degree of drug absorption through skin may vary, and therefore, may be the drug efficacy. Hence, the carriers based on scientific approach can modify the physicochemical properties of the drugs and can help to decrease the intensity and frequency of side effects associated with these active moieties. Formulations like gels, creams, ointments, and lotions are frequently used for the topical delivery of the antipsoriatic agents. However, these formulations are often not able to mask the drug-related issues causing obvious problems with patient acceptance and compliance [Table 5]. The topical delivery vehicle must be suitably designed and developed to attain the desirable attributes for use in extremely dehydrated and thickened psoriatic skin having lipid imbalance and sensitive to irritants.

Table 5: Common skin barrier problems in psoriasis
- Thickened inflamed skin lesions covered with scales
- Dry and natural moisturizing factor deficient skin
- Sensitive skin
- Tethered hairy skin
- Imbalance of skin lipids
- Excessive growth and aberrant differentiation of corneocytes

Novel Drug Delivery Systems in Topical Therapy for Psoriasis

The NDDS with their unique advantageous features provide favorable skin interactions as desired in the diseased conditions like psoriasis. Considering the benefits, there have been several recent attempts to use the NDDS approach to improve the existing topical drug formulations in psoriasis. A brief account of the efforts presents here the current scenario.

Dithranol

Dithranol, with a long history of use spanning over more than 100 years, is one of the most effective topical therapies in psoriasis. But in the existing form of products, it has not been fully accepted, mostly because of its irritation and staining properties. This made a long-standing demand on the researchers world wide to search for the modified molecule or formulation. It included enormous efforts as reflected in more than 1500 publications, patents and exclusive meetings on the dithranol per se. Various efforts like chemical modifications of the molecule, formulation changes, new treatment modifications or strategies and other miscellaneous approaches like short-contact therapy did not provide any definite solution. Subsequent work on liposomal systems with dithranol led to the improvement in its skin penetration. Agarwal et al. developed dithranol entrapped in liposomal and niosomal vesicles (0.5%) and found both of them superior to conventional formulation, while liposomes showed better results than niosomes employing mice skin. They found both of them superior to conventional formulation, while liposomes showed better results than niosomes in their patent application revealed the usefulness of mixed vesicular systems of dithranol with and without salicylic acid. The formulations, when tested on more than 12 patients for 4 weeks, proved to be effective and devoid of irritation and staining.

The study on liposomal dithranol continued by Katare et al. resulted in the development of a product. This product when tested clinically in an open label as well as randomized double blind trials showed that dithranol in greatly reduced doses (0.5%) in liposomes could clear the psoriasis
psoriasis plaques to match that of 1.15% commercially available dithranol ointment. The advantages of liposomal dithranol in terms of efficacy and compliance (nonirritancy and nonstaining) have been attributed to the ability of strategic liposomal formulation design [Figure 2]. In the latter form, the reactivity of drug is moderated to the desired level, while favorable drug-skin interactions as a result of membranous layers of liposomes do not allow for irritancy and deep staining of clothes. In the latter form, the reactivity of drug is moderated to the desired level, while favorable drug-skin interactions as a result of membranous layers of liposomes do not allow for irritancy and deep staining of clothes.139 401

Methotrexate
Methotrexate (MTX) is the gold standard drug used systemically in psoriasis, though there are not many products available for its topical application. The key reason for this is its inability to penetrate adequately in the skin and get access to the target cells. But of late, several formulations and delivery techniques have been employed in order to improve its delivery through skin. Strategies include the use of different penetration enhancers, adhesive laminate tapes as occlusive covering, physical techniques like iontophoresis, and development of novel drug delivery vehicles. In a study of liposomal formulation of MTX conducted in six patients, it resulted in clearance of psoriasis lesions, while one patient recovered completely. Further modified version of liposomes, i.e., deformable liposome was found to be quite superior to that of aqueous solution and normal liposomes in vitro. In a double-blind placebo-controlled trial involving 40 psoriasis patients, niosomal systems in chitosan gel (0.25%) resulted in a better efficacy, tolerance, and patient compliance, when compared to a marketed formulation. Another version of liposomal system containing ethanol, i.e., ethosomes, showed favorable skin permeation characteristics. Trotta et al. developed oil in water (o/w) microemulsions of MTX having sixfold higher permeation flux than that of the corresponding solutions in mice skin. Recent MTX incorporated in a hydrogel formulation show zero-order kinetic release and antipsoriatic activity. This formulation was evaluated in 35 psoriasis patients and the application site was also irradiated with 80 J diode laser of wavelength 650 nm, thrice week. During 8 months' follow-up, up to 60% of patients treated with LMTX gel had no recurrence. Solid lipid nanoparticles (SLN) of MTX have show improved drug accumulation in human cadaver skin. This formulation was also investigated clinically on 24 psoriasis patients for 6 weeks period. T researchers reported that MTX SLN-gel significantly improved the therapeutic index in terms of avera percentile improvement in healing (APIH) of lesions a reduction in average score of degree of erythema a scaling.181

Retinoids
Tretinoin (TRE) is a widely used drug in the topical treatment of acne, photo-aged skin, psoriasis and other skin disorders but unpleasant side effects often appear in the form of scaling, erythena burning, and stinging. Several attempts have been made to incorporate the drug in various colloid carriers. For instance, the drug has been incorporated into liposomes, niosomes, SLNs, and nanocapsules. These studies have been carried out in various animal models and reported to perform quite well. Safe iontophoretic tretinoin delivery is also reported in human volunteers.187

Tamoxifen
Tamoxifen (TAM), an anti-estrogen compound given systemically, has recently been figured as a useful agent in the treatment of certain skin specific disorders like psoriasis. Enhanced epidermal transport TAM employing different penetration enhancers has been reported. Katare et al. (2004) developed TA liposomes of multilamellar nature, which exhibit appreciably enhanced skin permeation as well retention of drug molecules in the skin.28 Tamoxifen (TAM), an anti-estrogen compound given systemically, has recently been figured as a useful agent in the treatment of certain skin specific disorders like psoriasis. Enhanced epidermal transport TAM employing different penetration enhancers has been reported. Katare et al. (2004) developed TA liposomes of multilamellar nature, which exhibit appreciably enhanced skin permeation as well retention of drug molecules in the skin.

Vitamin D-analogues
Vitamin D analogues such as calcipotriol, maxacalcitocalcit, and calcitriol are the mainstay of treatment in mild-to-moderate plaque psoriasis. Local irritati- is the most frequently noted side effect, which managed by combining vitamin D analogues with topical corticosteroids. Lin et al. developed NL

![Figure 2: Inter- and Intralamellar distribution of dithranol in liposomal formulation](image-url)
loaded with both MTX and calcipotriol and reported enhanced drug permeation with limited skin irritation in animal models.\textsuperscript{[90]} Prüfer et al. incorporated 1,25-dihydroxyvitamin D\textsubscript{3} in liposomes and reported its superiority over un-encapsulated drug in efficacy as well as safety.\textsuperscript{[94]}

**Tacrolimus**
Tacrolimus (FK506), an effective and well-tolerated immunosuppressant, has also found its importance in the treatment of chronic plaque-psoriasis. Various clinical trials of tacrolimus in chronic plaque-psoriasis have been conducted with the conventional topical formulations.\textsuperscript{[96,98]} Only preclinical animal studies with liposomes and nanoparticles of tacrolimus have been reported with improved skin transport effect.\textsuperscript{[98,100]}

**Theophylline derivatives**
Dyphylline, a derivative of theophylline, inactivates cyclic AMP (cAMP), and is, therefore, used in the management of psoriasis.\textsuperscript{[96]} Touitou et al. (1992) reported significant increase in permeation of dyphylline across abdominal mouse skin using liposomal systems, thus corroborating its promise in topical delivery.\textsuperscript{[98]}

**Levulinic acid derivatives**
Topical photodynamic therapy (PDT) with 5-aminolevulinic acid (ALA), a second-generation photosensitizer is a treatment option for psoriasis covering large area.\textsuperscript{[103]} The major limitation of this strategy, however, is the poor penetration of ALA into the skin lesions. Recently, Fang et al. developed ethosomal system for topical delivery of ALA to overcome its penetration problem. The said work significantly contributed in understanding of the behavior and outcome of penetration of hyperproliferative murine skin.\textsuperscript{[100]}

**Temoporfin**
Temoporfin (mTHPC) is a very potent second-generation synthetic photosensitizer with high tumor selectivity on activation at 652 nm. However, due to low aqueous solubility and high lipophilicity, the drug is difficult to be delivered topically.\textsuperscript{[106]} Considering this, Dragicevic-Curic et al. formulated a different type of vesicular systems (invasomes) which improved topical delivery of mTHPC indicating promising advantage for the photodynamic therapy.\textsuperscript{[106,17]}

**Corticosteroids**
Corticosteroids, one of the most frequently used classes of drugs in dermatology, have been in practice to treat psoriasis too, either alone or in combination with other drugs.\textsuperscript{[103,106]} Korting et al. developed liposomes containing 0.039% betamethasone dipropionate (BDP) and compared it with a commercial propylene glycol gel containing 0.064% BDP in a double-blind, randomized, paired trial lasting 14 days in 10 patients with psoriasis vulgaris and eczema. This report documented improvement in case of eczema, but not in psoriasis.\textsuperscript{[105]}

**Psoralens**
Psoralens, mainly employed in PUVA are 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), and 4,5,8-trimethylpsoralen (TMP). Studies have shown that the application of an emulsion cream and a microemulsion of 8-MOP helps in localization of the drug. Baroli et al. developed microemulsions for topical delivery of 8-MOP at the target site and enhanced porcine skin accumulation of 8-MOP without systemic side effects.\textsuperscript{[106]} Fang et al. developed nanoparticulate lipid-based drug carriers viz. SLNs and NLCs, with increased skin permeation and controlled release properties for psoralens.\textsuperscript{[107]}

**Terpenoids**
Triptolide (TP), a diterpenoid triepoxide, is indicated in the clinical treatment of psoriasis via oral or intravenous route.\textsuperscript{[108]} However, the clinical use of triptolide is limited because of its severe systemic toxicity profile. Mei et al. developed SLNs and microemulsions in order to explore their potential for the topical delivery of TP. The results indicated that these SLN dispersions and microemulsions could serve as efficient promoters for the TP penetrating into skin.\textsuperscript{[109]} Chen et al. also developed microemulsions, and it showed an enhanced in vitro permeation through mouse skins compared to an aqueous solution with no obvious skin irritation. They also studied hydrogel microemulsion of TP and found improvement in its penetration.\textsuperscript{[110]}

**Cyclosporin A**
Cyclosporin A (CsA) is used in the treatment of psoriasis by oral as well as topical route. Its high molecular weight (more than 500 Da) and limited cutaneous permeation are the key challenges for topical delivery.\textsuperscript{[111]} Many attempts have been made to achieve localized site-specific immunosuppression using conventional topical formulations of CsA, e.g., at Novartis Research Centre (Vienna, Austria), but of without any avail.\textsuperscript{[112,113]} Duncan et al. in a small double-
blind, vehicle-controlled trial reported significant improvement in psoriasis lesions treated with topical CsA formulation with penetration enhancer(s).\textsuperscript{1114} Guo et al. developed lecithin vesicular carriers for the transdermal delivery of CsA. They observed in vitro permeation technique that the flexible vesicles are better carriers for dermal enhancement.\textsuperscript{1118} Ugazio et al. incorporated CsA in SLNs and proposed for the exploitation through various routes.\textsuperscript{1119} Boinpally et al. studied the effect of iontophoresis on topical delivery of CsA across human cadaver skin using lecithin-solubilized drug which resulted in appreciable drug transport across skin. Few reports demonstrated monooil as penetration enhancer for the topical and transdermal delivery of CsA in various liquid crystalline systems.\textsuperscript{113}\textsuperscript{115} Verma et al. reported increased transport of CsA across skin employing alcoholic liposomes.\textsuperscript{1110} Katare et al., demonstrated successful topical delivery of CsA through multicompartamental liposomes and microemulsified systems.\textsuperscript{1111}\textsuperscript{1112} Liu et al. reported that 40% ethanol and 10% menthol shortened the lag time of the penetration of CsA into deeper skin layers.\textsuperscript{1120}

Coal tar
Some studies have been conducted on very old but highly useful drug, coal tar, using novel phospholipid structured topical formulation. This approach has been reported to be beneficial in meeting the challenges of skin irritation and staining on cloth and skin.\textsuperscript{111} They also reported better anti-psoriatic activity of this novel formulation vis-à-vis the conventional formulation employing mouse tail model of psoriasis.\textsuperscript{1121}

CONCLUSIONS
The emergence of novel drug delivery systems and its further evolution has attracted the interest of researchers in psoriasis. A wide range of efforts has been made which are mostly centered on the development of carrier-based formulations like liposomes and other colloidal range supra structures. The fundamental interest of such carriers lies on making the existing drugs more effective, safe, and patient-compliant. The studies suggest the importance of these systems for the enhancement in the skin penetration and accumulation of drug along with improved patient compliance. The unique moisturizing ability of the vesicles and its interactions through liposomal lipids with the skin lipids are the possible reason for the improvement in cutaneous transport of drugs. The problem of bulkiness of the molecules could be overcome by way of carrier interactions with the skin cells as reported in the case of cyclosporin. Besides latter, the moderation in the reactivity of the drugs such as dithranol could be of great avail by way of strategic liposomal (carrier) design.

Further, despite so much of work done on development of technique, it could not be extended sufficiently to the clinical level, which reflects a gap between the two domains of research, i.e., clinical and pharmaceutical. However, out of few isolated attempts for clinical applications, the availability of liposome-based dithranol and coal tar gel in the market exemplifies the initiation of the progress. And it needs an all round effort from different stakeholders to work out the bottleneck problems especially the cost of raw materials, scale-up stability and quality issues to ensure the availability of the products, while the establishment of clinical efficacy in psoriasis is of paramount interest.

REFERENCES
Katare, et al. Psoriasis and novel drug delivery systems


Psoriasis and novel drug delivery systems

Katare, et al.


Novel drug delivery systems in topical treatment of psoriasis: Rigors and vigors

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ABSTRACT

Psoriasis is a chronic inflammatory skin disorder that may drastically impair the quality of life of a patient. Among the various modes of treatments for psoriasis, topical therapy is most commonly used in majority of patients. The topical formulations based on conventional excipients could serve the purpose only to a limited extent. With the advent of newer biocompatible and biodegradable materials like phospholipids, and cutting-edge drug delivery technologies like liposomes, solid lipid nanoparticles (SLNs), microemulsions, and nanoemulsions, the possibility to improve the efficacy and safety of the topical products has increased manifold. Improved understanding of the dermal delivery aspects and that of designing and developing diverse carrier systems have brought in further novelty in this approach. Substantial efforts and the consequent publications, patents and product development studies on the subject are the matter of interest and review of this article. However, majority of the work is related to the preclinical studies and demands further clinical assessment in psoriasis patients.

Key words: Dermal drug delivery, Liposomes, Microemulsions, Nanoparticles, Phospholipids, Vesicles

INTRODUCTION

Topical therapy is the mainstay of treatment for mild to moderate psoriasis and serves as a useful adjunct support to systemic therapy in severe disease. However, efficacy and compliance to topical therapy in psoriasis have been a major concern. Approximately, 71% of the psoriasis patients in three large surveys were found to be unsatisfied or moderately satisfied with their current treatment.[10] Lack of effective delivery of drugs and undesirable skin interactions of the topical treatments are the main reasons for patient noncompliance.[11] Nevertheless, newer developments in the formulation approaches have raised hopes in making topical therapy more useful and acceptable.[12] The present paper endeavors to review the overall developments in the field of Novel Drug Delivery Systems (NDDS) pertaining to the topical treatment of psoriasis.

NOVEL DRUG DELIVERY SYSTEMS

In search of safe and effective therapy, the development of new drugs has been the common practice historically. However, it involved a long gestation period in terms of time, efforts, and huge cost. Later on, it was realized that the issues pertaining to efficacy and safety are largely influenced by the distribution of the drug within the biological system, as there is appreciable deviation from the desired site of action, i.e., the target site. In fact, Nobel laureate, Sir Paul Ehrlich in 1905 envisioned the drug molecules as “magic-bullets” to hit the specific target site to attain the absolute efficacy and safety.[13] This objective, hitherto un-accomplished gave way to an alternate approach of drug delivery, wherein the carrier systems were used to deliver the molecules to specific receptor sites without afflicting the normal tissues and organs of the body. Interestingly, it turned out to be a transformation of the original idea of “magic-bullets” to that of the “magic-guns.” The fundamentals lie in hosting the drug in carefully designed carriers to bring favorable change(s) in its surrounding microenvironment, and consequently, its delivery. It is...
the modification(s) in physicochemical characteristics of the molecules and in the barrier properties of the biological membranes at various locations, which lead to improved transportation of drugs toward the diseased locations. Further, it improves the chances of the availability of the drug at the specific receptor site and enhances drug-receptor interaction through mediation of specialized composition and design of the carrier systems. All these factors tend to potentiate the degree of pharmacodynamic response, the safety and patient compliance being the immediate benefits.

The novel carriers have been exploited through almost all the routes of administration. However, the topical route has been adjudged as one of the most relevant to treat dermatological disorders more effectively. In contrast to the conventional formulations based on creams and ointments, these novel dermatological systems are different in their composition and constructs including their exterior and interior design.[15] Various pharmaceutical and dermatological variables influence the choice of the system as per the demand of the drug and disease. Phospholipids represent a special class of surfactants with two long fatty acid chains (lipid region) and a bulky polar head (hydrophilic region) linked with phosphor-group on glycerol as the backbone. The unique structural features allow phospholipids to interact with water to form well-organized supra-structures like liposomes. The variation in composition and methods influences the nature of such self-assembled supra-structures in terms of their shape, design, size, and surface properties. This leads to different classes of carriers, viz. liposomes,[15–16] transfersomes, micro and nanoemulsions,[16–18] piosomes,[13,14] dendrimers,[15] invasomes,[15–17] solid lipid nanoparticles [SLNs][18–20] and nano lipid carriers (NLCs)[21–23,26] [Table 1]. These carrier systems provide the entrapment opportunities to the drug molecules within their interior locations as per their fitment of steric and physicochemical properties. Association of drugs with carriers is normally noncovalent, based on collective strength of weak binding forces. Many newer carriers are evolving with the advent of technology and the demand of targeted delivery like ethosomes, emulsomes, magnetic nanoparticles, resealed erythrosomes and bilosomes.

Apart from projected advantages, the novel carriers have associated drawbacks of high cost of excipients, need of expertise in the production of such carriers, stability, and evaluation issues.

Table 1: Various colloidal carriers employed during topical delivery of drugs

<table>
<thead>
<tr>
<th>Drug delivery carrier systems</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomes</td>
<td>Vesicular carriers composed of bilayers of phospholipid molecules and enclosed within these bilayers</td>
</tr>
<tr>
<td>Niosomes</td>
<td>Vesicular carriers composed of non-ionic surfactants instead of phospholipids</td>
</tr>
<tr>
<td>Microemulsions</td>
<td>Thermodynamically stable, isotopically clear and transparent carriers composed of oil, water and surfactants. They are supersonicated</td>
</tr>
<tr>
<td>Lipid emulsions</td>
<td>Micro- and nano-emulsions containing phospholipids as one of their surfactants</td>
</tr>
<tr>
<td>Transfersomes, flexible membrane vesicles</td>
<td>Liposomes with edge activators, highly deformable, reported to penetrate stratum corneum as such</td>
</tr>
<tr>
<td>Ethosomes</td>
<td>Liposomal systems comprising of high alcohol content, flexible vesicles, high drug loading</td>
</tr>
<tr>
<td>Solid lipid nanoparticles</td>
<td>Nanocarriers composed of solid lipid particles</td>
</tr>
<tr>
<td>Emulsomes</td>
<td>Nanocarriers with solid lipid core along with bilayers of phospholipids</td>
</tr>
<tr>
<td>Nanolipid carriers</td>
<td>Nanocarriers composed of drug loaded in lipid core composed of both solid and liquid lipids</td>
</tr>
<tr>
<td>Invasomes</td>
<td>Liposomes containing penetration enhancers</td>
</tr>
<tr>
<td>Dendrimers</td>
<td>Repeatedly branched, roughly spherical large molecules also used for drug delivery</td>
</tr>
</tbody>
</table>

Figure 1 illustrates the pictorial representation of the interactions of the carriers with skin.[15] The nc carrier systems are versatile and flexible in handling the various issues associated with the drug and the carrier to possess high potential for better patient compliance.

Table 2 enumerates the meritorious roles of NE systems in topical therapy. Various attempts have been made...
in the recent past in reporting many studies for the delivery of various drugs employing novel colloidal carriers. Table 3 enlists selected instances.[125-641]

**CHALLENGES IN TOPICAL DELIVERY OF DRUGS IN PSORIATIC SKIN**

According to the studies reported recently, stratum corneum (SC) is not an inert layer, but an "active-wall," which opposes the penetration of xenobiotics.[4] Though no molecule can readily and fully pass through this membrane, yet it allows penetration of nearly all the materials to some extent. It is also vivid that the major route of penetration across the SC is the intercellular lipids.[6] The state of hydration of SC is one of the most important factors in determining the rate of percutaneous absorption of a given solute. The level of hydration is a function of the water concentration gradient between the dermis and the surface of the skin as well as the ability of the SC to "bind" water.[9] Delivery of solutes through the skin is associated with a number of difficulties as shown in Table 4.

"Rigidization" of psoriatic skin has been attributed to a rise in the levels of cholesterol and fall in the levels of ceramides.[6] Apart from this, normal moisturizing factors (NMFs) like water are almost absent in the psoriatic skin. As a result of various factors, targeting the psoriatic tissues using topical route poses a big

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Indication</th>
<th>Type of Drug Delivery System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporin A</td>
<td>Allergic skin disorders (atopic dermatitis)</td>
<td>Solid lipid nanoparticles[20]</td>
</tr>
<tr>
<td>Calcineurin inhibitors</td>
<td>Prevent DNA photodamage</td>
<td>Liposomes[17]</td>
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<td>Amphotericin B</td>
<td>Fungal infection</td>
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<tr>
<td>Fluconazole</td>
<td>Fungal infection</td>
<td>Ethosomes[20]</td>
</tr>
<tr>
<td>NB-002</td>
<td>Fungal infection</td>
<td>Nanosuspension[21]</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>Fungal infection</td>
<td>Liposomes[22]</td>
</tr>
<tr>
<td>Cliostraxin</td>
<td>Fungal infection</td>
<td>Liposomes[23]</td>
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<tr>
<td>Oleamine</td>
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<tr>
<td>Methotrexate</td>
<td>Psoriasis</td>
<td>Liposomes[24]</td>
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<tr>
<td>Methotrexate</td>
<td>Psoriasis</td>
<td>Ethosomes[25]</td>
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<td>Niosomes[26]</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Psoriasis</td>
<td>Liposomes[27]</td>
</tr>
<tr>
<td>Temoporfin</td>
<td>Photodynamic therapy- psoriasis</td>
<td>Liposomal gel[28]</td>
</tr>
<tr>
<td>Dilithinol</td>
<td>Psoriasis</td>
<td>Liposomes and niosomes[29,30]</td>
</tr>
<tr>
<td>Coal tar</td>
<td>Psoriasis</td>
<td>Lecithinized coal tar formulation[31]</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>Psoriasis</td>
<td>Liposomes[32]</td>
</tr>
<tr>
<td>Celocine</td>
<td>Pruritus</td>
<td>Nanoparticles and liposomes[33,34]</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>Pruritus</td>
<td>Liposomes[35]</td>
</tr>
<tr>
<td>Lauric acid</td>
<td>Inflammatory acne</td>
<td>Liposomes[36]</td>
</tr>
<tr>
<td>Azelaic acid</td>
<td>Acne</td>
<td>Liposomes and Ethosomes[37]</td>
</tr>
<tr>
<td>Tretinoin</td>
<td>Acne</td>
<td>Liposomal gel[38]</td>
</tr>
<tr>
<td>Benzyl peroxide</td>
<td>Acne</td>
<td>Liposomal gel[39]</td>
</tr>
<tr>
<td>Idoxudinol</td>
<td>Herpes simplex</td>
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</tr>
<tr>
<td>Dipotassium Glycycnizinate</td>
<td>Acute and chronic dermatitis</td>
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</tr>
<tr>
<td>Prednisolone</td>
<td>Allergic dermatitis</td>
<td>Magnetic liposomes[42]</td>
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<tr>
<td>Capsaicin</td>
<td>Musculoskeletal pain</td>
<td>Flexible membrane vesicles[43]</td>
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<tr>
<td>Nimueiside</td>
<td>Inflammation and pain</td>
<td>Liposomes[44]</td>
</tr>
<tr>
<td>Finasteride</td>
<td>Acne, androgenetic alopecia</td>
<td>Liposomes[45]</td>
</tr>
<tr>
<td>Corticosteroid</td>
<td>UV induced erythma</td>
<td>Skin-lipid liposomes[46]</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>Certain skin disorders</td>
<td>Emollient foam formulation of clobetasol propionate[47]</td>
</tr>
<tr>
<td>Hydroxyzine</td>
<td>Skin Allergy</td>
<td>Liposomes[48]</td>
</tr>
<tr>
<td>Vitamin D analogues:</td>
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</tr>
<tr>
<td>Calcipotriol, tacalcitol, calcitriol</td>
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<td></td>
</tr>
</tbody>
</table>

Table 2: Role of Novel drug delivery systems

- Use of versatile carriers
- Imparting protection to the molecules
- Biocompatibility of the systems
- Passive targeting
- Loading a variety of drugs
- Modifications in the physicochemical properties

Table 3: List of drugs (topical) encapsulated in various carrier systems[125-641]

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challenge. The intricacies of the topical delivery into the psoriatic skin have lately been proposed to be addressed by the lipoidal carrier systems, such as liposomes. The latter resolve the problem of lipid imbalance by imparting the unsaturated fatty acids like linoleic acid to restore the normal skin conditions.[41] Hence, these liposomal and allied carriers can result in an effective delivery of drugs across the psoriatic skin.[42]

Several topical therapeutic agents are available for the treatment of psoriasis. Nevertheless, none of them can be regarded as an ideal drug molecule. This may either be due to their inherent side effects or their improper incorporation in the conventional vehicles. It is a well-known fact that due to variation in the physicochemical characteristics of the carrier and of the active compounds used, the degree of drug absorption through skin may vary, and therefore, may be the drug efficacy. Hence, the carriers based on scientific approach can modify the physicochemical properties of the drugs and can help to decrease the intensity and frequency of side effects associated with these active moieties.[43] Formulations like gels, creams, ointments, and lotions are frequently used for the topical delivery of the antipsoriatic agents. However, these formulations are often not able to mask the drug-related issues causing obvious problems with patient acceptance and compliance [Table 5].[44,45] The topical delivery vehicle must be suitably designed and developed to attain the desirable attributes for use in extremely dehydrated and thickened psoriatic skin having lipid imbalance and sensitive to irritants.[46]

**NOVEL DRUG DELIVERY SYSTEMS IN TOPICAL THERAPY FOR PSORIASIS**

The NDDS with their unique advantageous features provide favorable skin interactions as desired in the diseased conditions like psoriasis. Considering the benefits, there have been several recent attempts to use the NDDS approach to improve the existing topical drug formulations in psoriasis. A brief account of the efforts presents here the current scenario.

**Dithranol**

Dithranol, with a long history of use spanning over more than 100 years, is one of the most effective topical therapies in psoriasis. But in the existing form of products, it has not been fully accepted, mostly because of its irritation and staining properties. This made a long-standing demand on the researchers world wide to search for the modified molecule or formulation. It included enormous efforts as reflected in more than 1500 publications, patents and exclusive meetings on the dithranol per se. Various efforts like chemical modifications of the molecule, formulation changes, new treatment modifications or strategies and other miscellaneous approaches like short-contact therapy did not provide any definite solution.[47-49] Subsequent work on liposomal systems with dithranol led to the improvement in its skin penetration.[50] Agarwal et al. developed dithranol entrapped in liposomal and niosomal vesicles (0.5%) and found both of them superior to conventional formulation, while liposomes showed better results than niosomes employing mice skin. They found both of them superior to conventional formulation, while liposomes showed better results than niosomes.[51] Gidwani et al. in their patent application revealed the usefulness of mixed vesicular systems of dithranol with and without salicylic acid. The formulations, when tested on more than 12 patients for 4 weeks, proved to be effective and devoid of irritation and staining.[52]

The study on liposomal dithranol continued by Katare et al. resulted in the development of a product.[53,54] This product when tested clinically in an open label[55] as well as randomized double blind trials[56] showed that dithranol in greatly reduced doses (0.3%) in liposomes could clear the psoriasis
plaques to match that of 1.15% commercially available dithranol ointment. The advantages of liposomal dithranol in terms of efficacy and compliance (nonirritancy and nonstaining) have been attributed to the ability of strategic liposomal formulation design [Figure 2]. In the latter form, the reactivity of drug is moderated to the desired level, while favorable drug–skin interactions as a result of membranous layers of liposomes do not allow for irritancy and deep staining of clothes.

Methotrexate
Methotrexate (MTX) is the gold standard drug used systemically in psoriasis, though there are not many products available for its topical application. The key reason for this is its inability to penetrate adequately in the skin and get access to the target cells. But of late, several formulations and delivery techniques have been employed in order to improve its delivery through skin. Strategies include the use of different penetration enhancers, adhesive laminate tapes as occlusive covering, physical techniques like iontophoresis, and development of novel drug delivery vehicles. In a study of liposomal formulation of MTX conducted in six patients, it resulted in clearance of psoriasis lesions, while one patient recovered completely. Further modified version of liposomes, i.e., deformable liposome was found to be quite superior to that of aqueous solution and normal liposomes in vitro. In a double-blind placebo-controlled trial involving 40 psoriasis patients, niosomal systems in chitosan gel (0.25%) resulted in a better efficacy, tolerance, and patient compliance, when compared to a marketed formulation. Another version of liposomal system containing ethanol, i.e., ethosomes, showed favorable skin permeation characteristics. Trotta et al. developed oil in water (o/w) microemulsions of MTX having sixfold higher permeation flux than that from the corresponding solutions in mice skin. Recently MTX incorporated in a hydrogel formulation showed zero-order kinetic release and antipsoriatic activity. This formulation was evaluated in 35 psoriasis patients and the application site was also irradiated with 80 J diode laser of wavelength 650 nm, thrice a week. During 8 months’ follow-up, up to 60% of the patients treated with LMTX gel had no recurrence. Solid lipid nanoparticles (SLN) of MTX have showed improved drug accumulation in human cadaver skin. This formulation was also investigated clinically on 24 psoriasis patients for 6 weeks period. The researchers reported that MTX SLN-gel significantly improved the therapeutic index in terms of average percent improvement in healing (APIH) of lesions and reduction in average score of degree of erythema and scaling.

Retinoids
Tretinoin (TRE) is a widely used drug in the topical treatment of acne, photo-aged skin, psoriasis and other skin disorders but unpleasant side effects often appear in the form of scaling, erythema, burning, and stinging. Several attempts have been made to incorporate the drug in various colloidal carriers. For instance, the drug has been incorporated into liposomes, niosomes, SLNs, and nanocapsules. These studies have been carried out in various animal models and reported to perform quite well. Safe iontophoretic tretinoin delivery is also reported in human volunteers.

Tamoxifen
Tamoxifen (TAM), an anti-estrogen compound given systemically, has recently been figured as a useful agent in the treatment of certain skin specific disorders like psoriasis. Enhanced epidermal transport of TAM employing different penetration enhancers has been reported. Katare et al. (2004) developed TAM liposomes of multilamellar nature, which exhibited appreciably enhanced skin permeation as well as retention of drug molecules in the skin.

Vitamin D-analogues
Vitamin D analogues such as calcipotriol, maxacalcitol, tacalcitol, and calcitriol are the mainstay of treatment in mild-to-moderate plaque psoriasis. Local irritant is the most frequently noted side effect, which is managed by combining vitamin D analogues with topical corticosteroids.
loaded with both MTX and calcipotriol and reported enhanced drug permeation with limited skin irritation in animal models.\textsuperscript{90} Prüfer et al. incorporated 1,25-dihydroxyvitamin D\textsubscript{3} in liposomes and reported its superiority over un-encapsulated drug in efficacy as well as safety.\textsuperscript{91}

**Tacrolimus**

Tacrolimus (FK506), an effective and well-tolerated immunosuppressant, has also found its importance in the treatment of chronic plaque-psoriasis. Various clinical trials of tacrolimus in chronic plaque-psoriasis have been conducted with the conventional topical formulations.\textsuperscript{94-96} Only preclinical animal studies with liposomes and nanoparticles of tacrolimus have been reported with improved skin transport effect.\textsuperscript{97-99}

**Theophylline derivatives**

Dyphylline, a derivative of theophylline, inactivates cyclic AMP (cAMP), and is, therefore, used in the management of psoriasis.\textsuperscript{96} Touitou et al. (1992) reported significant increase in permeation of dyphylline across abdominal mice skin using liposomal systems, thus corroborating its promise in topical delivery.\textsuperscript{100}

**5-Aminolevulinic acid derivatives**

Topical photodynamic therapy (PDT) with 5-aminolevulinic acid (ALA), a second-generation photosensitizer is a treatment option for psoriasis covering large area.\textsuperscript{101} The major limitation of this strategy, however, is the poor penetration of ALA into the skin lesions. Recently, Fang et al. developed ethosomal system for topical delivery of ALA to overcome its penetration problem. The said work significantly contributed in understanding of the behavior and outcome of penetration of hyperproliferative murine skin.\textsuperscript{102}

**Terpenoids**

Triptolide (TP), a diterpenoid triepoxide, is indicated in the clinical treatment of psoriasis via oral or intravenous route.\textsuperscript{103} However, the clinical use of triptolide is limited because of its severe systemic toxicity profile. Mei et al. developed SLNs and microemulsions in order to explore their potential for the topical delivery of TP. The results indicated that these SLN dispersions and microemulsions could serve as efficient promoters for the TP penetrating into skin.\textsuperscript{104} Chen et al. also developed microemulsions, and it showed an enhanced \textit{in vitro} permeation through mouse skins compared to an aqueous solution with no obvious skin irritation. They also studied hydrogel microemulsion of TP and found improvement in its penetration.\textsuperscript{105}

**Cyclosporin A**

Cyclosporin A (CsA) is used in the treatment of psoriasis by oral as well as topical route. Its high molecular weight (more than 500 Da) and limited cutaneous permeation are the key challenges for topical delivery.\textsuperscript{111} Many attempts have been made to achieve localized site-specific immunosuppression using conventional topical formulations of CsA, e.g., at Novartis Research Centre (Vienna, Austria), but of without any avail.\textsuperscript{112,113} Duncan et al. in a small double-
blind, vehicle-controlled trial reported significant improvement in psoriasis lesions treated with topical CsA formulation with penetration enhancer(s).\[11\] Guo et al. developed lecithin vesicular carriers for the transdermal delivery of CsA. They observed by in vitro permeation technique that the flexible vesicles are better carriers for dermal enhancement.\[11] Ugazio et al. incorporated CsA in SLNs and proposed for the exploitation through various routes.\[11] Boinpally et al. studied the effect of iontophoresis on topical delivery of CsA across human cadaver skin using lecithin-solubilized drug which resulted in appreciable drug transport across skin. Few reports demonstrated monoolein as penetration enhancer for the topical and transdermal delivery of CsA in various liquid crystalline systems.\[11,12] Verma et al. reported increased transport of CsA across skin employing alcoholic liposomes.\[12] Katare et al., demonstrated successful topical delivery of CsA through multicompartamental liposomes and microemulsified systems.\[13,14] Liu et al. reported that 40% ethanol and 10% menthol shortened the lag time of the penetration of CsA into deeper skin layers.\[12]  

Coal tar  
Some studies have been conducted on very old but highly useful drug, coal tar, using novel phospholipid structured topical formulation. This approach has been reported to be beneficial in meeting the challenges of skin irritation and staining on cloth and skin.\[15] They also reported better anti-psoriatic activity of this novel formulation vis-à-vis the conventional formulation employing mouse tail model of psoriasis.\[14]  

CONCLUSIONS  
The emergence of novel drug delivery systems and its further evolution has attracted the interest of researchers in psoriasis. A wide range of efforts has been made which are mostly centered on the development of carrier-based formulations like liposomes and other colloidal range supra structures. The fundamental interest of such carriers lies on making the existing drugs more effective, safe, and patient-compliant. The studies suggest the importance of these systems for the enhancement in the skin penetration and accumulation of drug along with improved patient compliance. The unique moisturizing ability of the vesicles and its interactions through liposomal lipids with the skin lipids are the possible reason for the improvement in cutaneous transport of drugs. The problem of bulkiness of the molecules could be overcome by way of carrier interactions with the skin cells as reported in the case of cyclosporin. Besides latter, the moderation in the reactivity of the drugs such as dithranol could be of great avail by way of strategic liposomal (carrier) design.  

Further, despite so much of work done on development of technique, it could not be extended sufficiently to the clinical level, which reflects a gap between the two domains of research, i.e., clinical and pharmaceutical. However, out of few isolated attempts for clinical applications, the availability of liposome-based dithranol and coal tar gel in the market exemplifies the initiation of the progress. And it needs an all round effort from different stakeholders to work out the bottleneck problems especially the cost of raw materials, scale-up stability and quality issues to ensure the availability of the products, while the establishment of clinical efficacy in psoriasis is of paramount interest.  

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