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FORMULATION AND EVALUATION OF DITHRANOL PRONIOSOMES FOR TREATMENT OF ACNE AND PSORIASIS

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ABSTRACT

The work's objective was to formulate and describe dithranol niosomal gel, put it into an appropriate dermatological basis, and evaluate its relative effectiveness in treating acne and psoriasis infections. DTL was discovered to have UV absorption maximums (λmax) of 254, 287, and 354 nm and possess a melting point of 178^0C. In compatibility studies, it was discovered through FTIR spectral analysis that the absorption maxima of functional groups in both the pure drug DTL and the drug polymer/stabilizer/gelling agent mixture did not change. Then, different ratios of surfactant, lecithin, cholesterol, drug, Carbopol 934 (gelling agent), and ethanol were employed to manufacture vesicular proniosomes of dithranol (DTL) using the coacervation phase separation method. The formulated proniosomes had a lamellar vesicular structure, and the carrier had vesicular properties, according to SEM and TEM visualisation. Vesicle size reduces as the surfactant's hydrophobicity increases (since surface energy falls with increased hydrophobicity). Because Span 40 and Span 60 were combined with soy lecithin and cholesterol, formulation R4 had the highest entrapment efficiency (95.92%). DTL was released in the following order in several formulations: R1, R2, R4, R3, R5, and R6. After 30 hours, the R1 formulation had the highest drug release (85.11%) and the R6 formulation had the lowest drug release (76.25%), according to this drug release order of several formulations. The *in-vitro* release of DTL from proniosomal formulations increased significantly as the alkyl chains of different Spans decreased. DTL was found to be released from R1, R2, R3,

R4, R5, and R6 formulations at rates of 83.27%, 81.65%, 76.79%, 82.28%, 76.51%, and 73.11%, respectively. The most effective proniosomal formulation was R1. The presence of phenolic hydroxyl groups is the primary cause of DTL's antioxidant activity. Dithranol proniosomal specialised formulations were found to be effective in treating acne and psoriasis.

KEYWORDS: Carbopol 934, dithranol, cholesterol, lecithin, drug, proniosomal, surfactant.

INTRODUCTION

According to Hamnerius (1997), the most prevalent skin condition that affects the face, back, and trunk areas with the most oil glands is *Acne vulgaris*. Although *Acne vulgaris* has long been treated with antibiotics, there is a rise in antibiotic resistance in dermatological settings. *Acne vulgaris* is a highly common skin condition that peaks in adolescence, but it also affects many men and women in their 20s and 40s.

According to Leyden (2001), the most prevalent type of acne, sometimes referred to as common acne, is *Acne vulgaris*. The skin's inflammatory reaction to the infection is what causes the redness. Skin bacteria can therefore proliferate rapidly. Skin becomes puffy and red due to infection. Due to elevated hormone levels during puberty, acne is fairly frequent. Oil and dead skin cells block the skin's pores, resulting in acne, a common skin ailment. Teenagers are most commonly affected by acne; about 85% of them have at least a mild case. The face, chest, back, shoulders, and neck are where it most frequently appears.

According to Cunliffe *et al*., 2004, the pathophysiology of acne is controlled by sebum hypersecretion in malformed follicles, which results in microcomedones. Inflammation is brought on by the follicular hyperproliferation of microcomedones. *Propionibacterium acnes*, the primary cause of acne, is more likely to proliferate anaerobically in this sebum-producing skin disease. Acne lesions can also contain Pitryosporum ovale and Staphylococcus epidermidis. Inflammatory lesions and severe acne are caused by the growth of these bacteria, primarily Propionibacterium acnes.

P. acnes is present in every typical human (Shehadeh & Kligman, 1963). It can cause acute infections and is frequently responsible for late chronic illnesses. *Propionibacterium acnes's* genome has a number of virulent components that demonstrate the bacterium's potential for pathogenicity. Though there are some distinctions, young men and women are affected by

acne nearly equally. Acne that is more severe and lasts longer is more common in men than in women. Women, on the other hand, are more likely to experience sporadic acne as a result of hormonal changes related to their menstrual cycle and acne brought on by cosmetics. Some people, particularly women, experience acne well into adulthood, but the majority overcome it by their 20s.

Swanson (2003), shown how the bacteria *Propionibacterium acnes* (P. acnes), Staphylococcus epidermidis, and Malassezia furfur cause acne, which is typically characterised by the development of seborrhoea, comedone, and inflammatory lesions. P. acnes is an anaerobic organism. It causes inflammatory acne by breaking down sebaceous triglycerides into fatty acids. Anti-acne medications target Staphylococcus epidermidis and *Propionibacterium acnes*. The long-term usage of antibiotics to treat acne is out of date due to increased antibiotic resistance.

According to Kanlayavattanakul *et al*., 2011, a number of anomalies in sebaceous gland function, such as water-soluble lipids, which are mostly face triglycerides in sebum, are variables that enhance inflammation and aid in the metabolism of normal flora, including *P. acnes*. *P. acnes* contributes to toll-like receptors (TLRs), stimulates CD4+ expression in keratinocytes, sebocytes, and neutrophil function, and has a mitogenic effect on T cells via heat-shock proteins (HSPs).

According to Clark (1993), comedolytics (benzoyl peroxide, tretinoin, azeleic acid, and isotretinoin) and antibiotics (tetracycline, erythromycin, etc.) are used in oral and topical therapy to treat *Acne vulgaris*. These medications have particular side effects in addition to creating bacterial resistance. These include isotretinoin, clindamycin, erythromycin, and tetracycline.

Herbal medications are safer than synthetic antibiotics (Jain, 2003). Because they have fewer or no adverse effects, doctors now favour herbal formulations containing *Azadirachta indica*, *Curcuma longa*, *Aloe vera*, *Rosa centifolia*, *Carica papaya*, etc. to allopathic medications. According to Tafin *et al*., 2012, intravenous antibiotics and occasionally surgery are used to treat severe infections brought on by *P. acnes*.

Numerous bacteria, including *P. acnes* and *S. epidermidis*, are being investigated in relation to the pathophysiology of acne. None of these bacteria have been proven to cause acne, but

they are present on normal skin along with others that may be connected to the pathogenesis of acne. According to Shoba (1998), *Azadirachta indica* has a number of essential oils with antipyretic and antihelmintic properties. Additionally, it cleanses the blood and aids in biliary secretion regulation. By eliminating *Propionibacterium acnes*, natural herbs including *Curcuma longa* and *Azadirachta indica* have been demonstrated to have anti-inflammatory properties.

According to Zhang *et al*., 2019, psoriasis is a chronic inflammatory skin condition that can significantly lower a person's quality of life. Psoriasis can be treated in a number of ways, with topical therapy being the most popular among most patients. Genetic and lifestyle factors can cause psoriasis; treatment recommendations call for sufferers to get lifelong care and constant observation. Psoriasis is a chronic, recurring condition that lasts a lifetime.

Psoriasis is characterised by red, scaly skin patches that can be unpleasant and itchy, according to Campanati *et al*., 2021. When healthy skin cells are mistakenly attacked by the immune system, they proliferate and divide more rapidly than usual, leading to psoriasis. The skin develops thick, scaly patches as a result. The various types of psoriasis include erythrodermic psoriasis, pustular psoriasis, inverse psoriasis, guttate psoriasis, and plaque psoriasis. Psoriasis can range in intensity from mild cases that only affect tiny portions of the skin to severe cases that impact huge areas of the body. Although there is no treatment for psoriasis, there are several strategies to control its symptoms.

According to Vicic *et al*., 2021, psoriasis is not communicable. However, it still has detrimental physical, psychological, and social effects that lower the quality of life for those who are affected. Psoriatic arthritis, metabolic syndrome, cardiovascular disease, depression, and other systemic disorders are also related conditions. Because psoriasis is a major global public health concern, it necessitates a multimodal approach to health care.

According to a National Psoriasis Foundation patient study conducted in the United States between 2001 and 2008, 60% of patients with moderate-to-severe psoriasis and 33% of people with mild psoriasis said their condition had a major impact on their daily lives. The histological basis of psoriasis, a chronic immune-mediated skin condition, involves keratinocyte proliferation and a failure in the process of differentiation.

Over 125 million people worldwide suffer from psoriasis, accounting for 2-3% of the global

population (Kimbal *et al*., 2005). However, the prevalence varies among ethnic groups and nations due to varying environmental, behavioural, and possibly genetic susceptibilities. According to studies, it is considerably lower in Asian and African American patients and higher in white people. For example, it ranges from 0.1 to 1.5 percent in China and Japan, but between 3.2 and 3.3 percent in North America and Europe. Corticosteroids, retinoids, and vitamin D analogues are topical therapies that can be administered directly to the afflicted skin to reduce inflammation and slow down the development of skin cells (Khan *et al*., 2023). Phototherapy, sometimes referred to as light therapy, uses UV radiation to reduce inflammation and slow down the growth of skin cells.

According to Zhang *et al*., 2016, nanogels a relatively new kind of delivery system seem to be highly popular right now, especially in dermatology. These three-dimensional cross-linked polymers have a number of special qualities, including high drug loading capacity, biocompatibility, and targeting of particular skin layers through controlled release mechanisms. First, nanogels have a number of significant qualities that could improve the effectiveness of topical treatments for psoriasis and other skin conditions. Methotrexate, cyclosporine, and biological agents are examples of systemic therapies that can be administered orally or by injection to lower inflammation and inhibit the immune system (Yiu *et al*., 2022). Adalimumab, a biological agent that targets a particular immune system molecule termed tumour necrosis factor-alpha (TNF-α), is one example of a medication used to treat psoriasis. Adalimumab can alleviate psoriasis symptoms by reducing inflammation and slowing the development of skin cells by inhibiting TNF-α.

According to Georgaki *et al*., 2022, corticosteroids, keratolytics, phototherapy, calcineurin inhibitors, biologics that target proinflammatory mediators, vitamin D analogues, and cyclosporine, an immunosuppressive medication that has long been used to treat a range of immune-mediated disorders and prevent organ transplant rejection. Inhibiting the activation of T-cells, which are immune cells involved in the development of autoimmune disorders and transplant rejection, is how it suppresses the immune system. Psoriasis symptoms such as redness, scaling, and skin thickness can be lessened by cyclosporine.

According to Biju and Sushama (2009), vesicles have emerged as the preferred drug delivery vehicle in recent years. It has been discovered that lipid vesicles are useful in membrane biology, immunology, diagnostic methods, and most recently, genetic engineering. According to Maryam *et al*., 2017, the vesicles can trap both water-soluble and water-insoluble

medications. The interior watery compartment can trap hydrophilic medications. On the other hand, lipophillic medications may partition between the vesicle bilayer and the aqueous phase or become trapped in the bilayer.

A niosome is a liposome based on non-ionic surfactants (Mujoriya *et al*., 2011). an excipient (other excipients can also be employed); greater penetration capacity compared to earlier emulsion preparations; structural similarity to liposomes; stability of niosomes with advantages over liposomes (Fig.1).

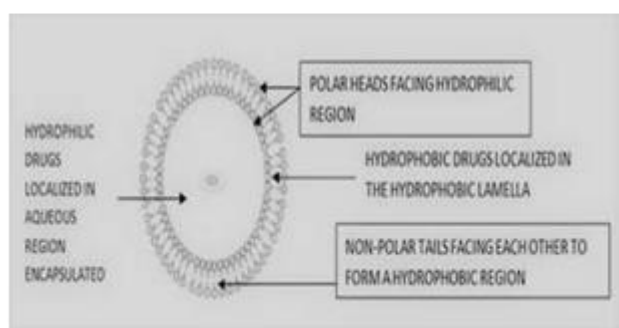

Fig.1: Structure of Niosomes.

A unique medicine delivery method called niosomes confines the medication within a vesicle. Niosomes get their name from the bilayer of non-ionic surface active substances that make up the vesicle. Because they are composed of a bilayer, niosomes and liposomes are comparable.

But in the case of niosomes, the bilayer is composed of phospholipids or non-ionic surface active substances. Depending on how they are prepared, niosomes can be either unilateral or multilateral. Niosomes are categorised either according to size or according to the number of bilayers (MLV, SUV, etc.).

"Dry niosomes" is another name for proniosomes. Drug encapsulation in the proniosomal vesicular formation increases penetration in the intended locations, maintains systemic circulation, offers controlled release, and lessens adverse effects. Proniosomes are free-flowing, dry formulations of the carrier coated with surfactant that can be rehydrated in a matter of minutes by revealing agitation in heated aqueous fluid. Proniosomes can trap both polar and non-polar, hydrophobic and hydrophilic medications. Proniosomes reduce the physical stability issues of niosomes, such as aggregation, fusion, and escape, and they also make transportation, distribution, storage, and dosage more convenient (Fig.2).

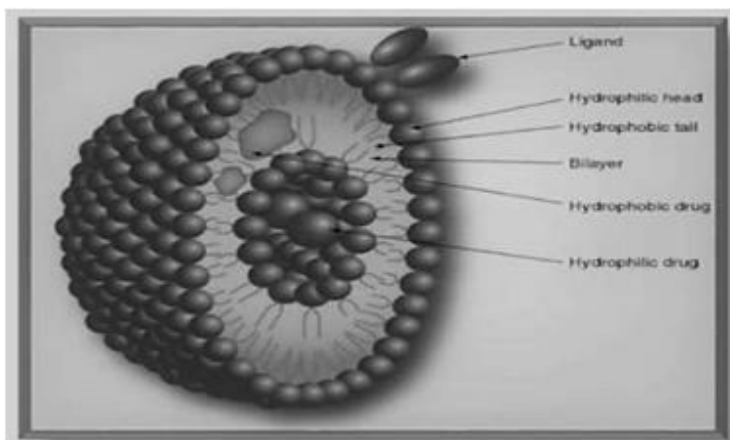

Fig.2: Structure of Proniosomes.

According to Mishra *et al*., 2011, upon hydration, proniosomes transform into niosomes and begin to function. When hydrated, proniosomes change into niosomes. The addition of aqueous solutions may cause the hydration. Proniosomes have the ability to trap both lipophilic and hydrophilic medicines (Fig.3-6).

Fig.3: Action of proniosomes

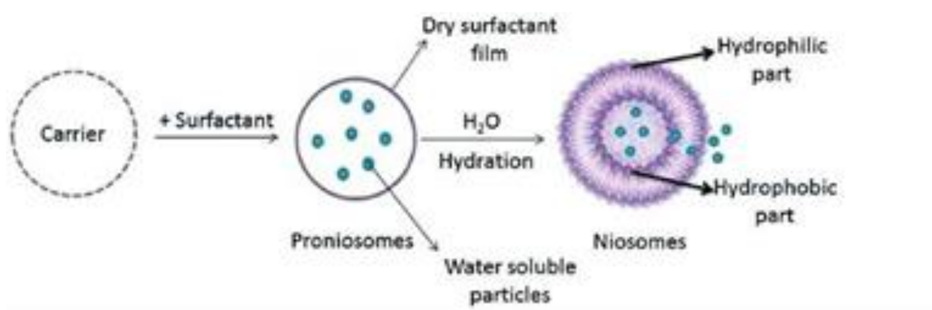

Fig.4: Conversion of Proniosomes to Niosomes.

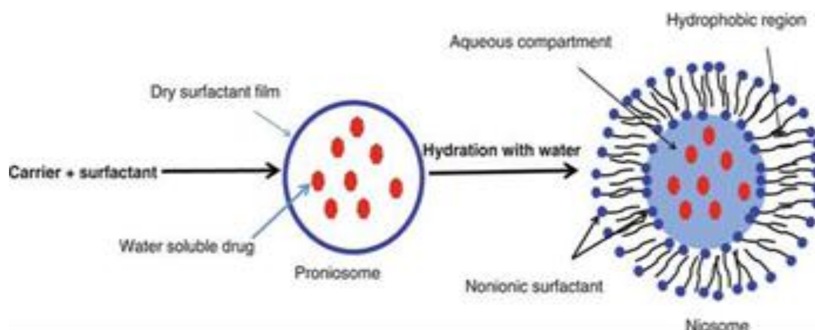

Fig.5: Conversion of Proniosomes.

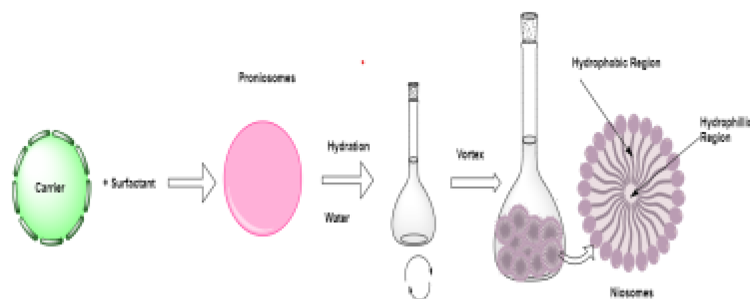

Fig.6: Proniosome hydration (hydrophilic & hydrophobic) areas of niosomes.

Niosomes produced from proniosomes for the administration of poorly soluble medications (Dua *et al*., 2012). These proniosomes are made of maltodextrin powder that has been coated with either a surfactant or a medication combination to produce a dry powder. The maltodextrin dissolves when hot water is added, and the surfactant creates a suspension of multilamellar vesicles (niosomes) that hold the poorly soluble medication. Drug is gradually released into solution by the niosomes (Fig.7).

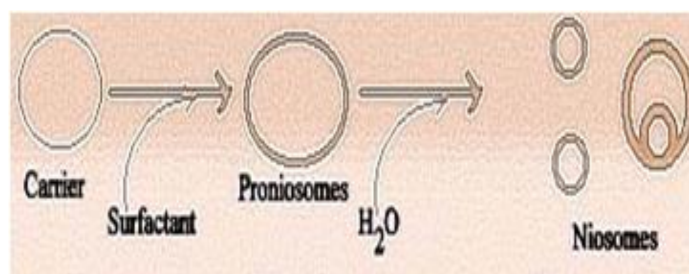

Fig.7: Proniosomes Methodology.

The following are a few techniques for proniosome preparation that have been documented: According to Walve *et al*., 2011, a round-bottom flask is filled with ether, methanol, or chloroform. In the rotary evaporator, the organic solvent evaporates at room temperature (20°C), leaving a thin coating of solid mixture on the flask's walls. The aqueous phase can be used to rehydrate the dried surfactant film at 0–60°C with minimal stirring. According to Walve *et al*., 2011, a chloroform: methanol (2:1) stock solution containing 250 µmol of surfactant dissolved in a 2:1 chloroform: methanol solution. If there is less surfactant loading, a slurry is formed by adding more organic solvent solution (Fig. 8-9).

According to Ali *et al*., 2022, nanocarriers like nanoparticles have a number of advantages over traditional treatments. First off, precision drug delivery is made possible by the ability of nanocarriers to target particular cells or tissues. Nanocarriers minimise adverse effects on healthy cells while maximising therapeutic advantages by delivering the medicine close to the

target cells. Additionally, the drug's bioavailability can be enhanced by nanoparticles. Their compact dimensions and high surface area to volume ratio allow for effective skin absorption and uptake. This improved bioavailability guarantees that a greater percentage of the medication reaches the intended spot, boosting its effectiveness.

Additionally, according to Guo and Jin (2023), nanocarriers protect the medication from deterioration and biodegradation. Dithranol's potency is preserved and its shelf life is increased because to this protection.

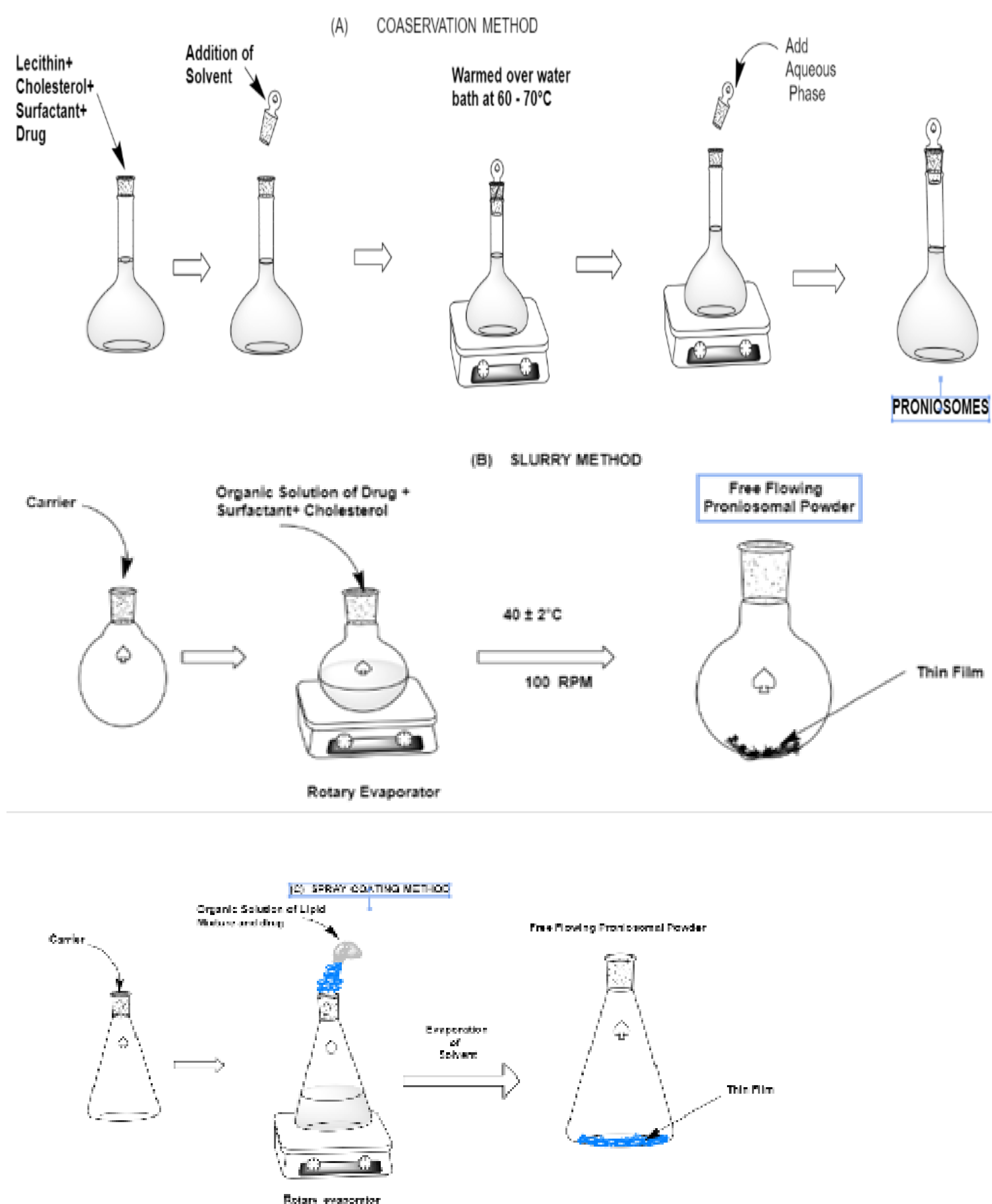

Fig.8: Various Methods of Preparation of proniosomal preparations.

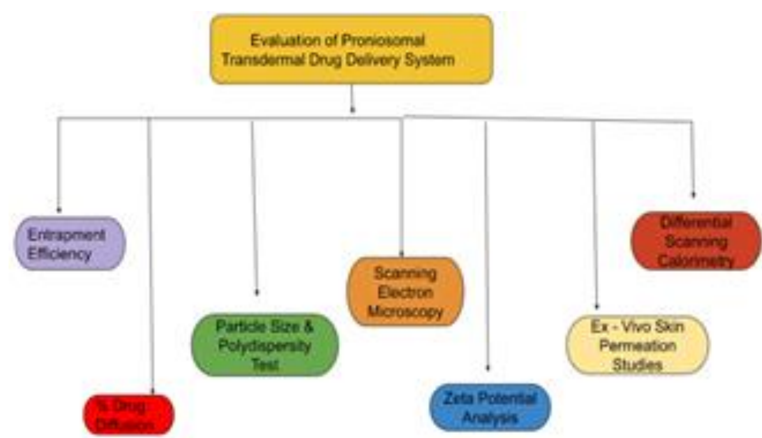

Fig.9: Proniosomal Transdermal Drug Delivery System Assessment.

Another benefit of using nanocarriers is regulated drug release (Mascarenhas *et al*., 2022). Because of their special qualities, the medication can be released under control for a long time at the appropriate concentrations. This regulated release lowers the frequency of administration while guaranteeing a long-lasting therapeutic impact. Overall, using anti-psoriatic medications in nanocarriers provides a cutting-edge and successful method of treating psoriasis.

Sehgal *et al*., 2014, state that dithranol or anthralin is a synthetic derivative of chrysarobin, a naturally occurring compound that has been applied topically to treat psoriasis (Fig. 10-11).

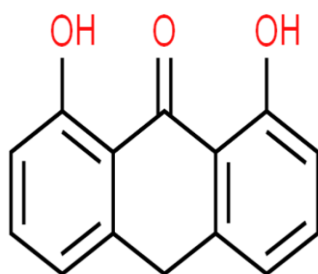

Fig. 10: Dithranol.

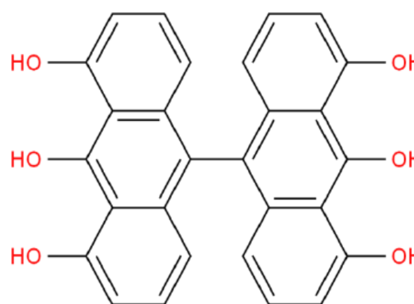

Fig.11: dithralon dimer.

To be precise, the objectives of the study are to optimize formulation parameters in order to obtain maximum efficiency of drug encapsulation, size of the particles and stability of the niosomal nanoparticles. In addition, the study aims to determine the *in-vitro* release kinetics of dithranol loaded the niosomal nanoparticles as well as to determine the *in-vitro* efficacy of the niosomal nanoparticles using psoriatic skin models. Through the realization of these goals, the study will help in the formulation of new topical formulations to effectively treat psoriasis, which overcome the drawbacks experienced with the

conventional formulations and helps improve patient outcomes. In relation to these challenges, a feasible solution could be to develop psoriasis therapy formulations derived from nanogels. Concerning the fact that psoriasis is a long-term disease, nanogels are introduced as a new perspective treatment method because of their ability to encapsulate the drug, deliver it in a controlled manner and target the site of action. This research program is proposed to design and analyze dithranol-loaded niosomal nanoparticles as a possible treatment of psoriasis.

Kammerau *et al.,* 1975, dithranol has both anti- inflammatory and anti-proliferative effects, which determined its high efficacy in the reduction of psoriatic lesions and the subsequent improvement in the appearance of skin. Although effective, the traditional dithranol preparations have certain drawbacks that include skin irritation, staining and poor patient compliance. Moreover, the pleasant physicochemical characteristics of dithranol, such as moderate organic solvent solubility and low molecular weight, provide the opportunity to encapsulate this compound in niosomal nanoparticles. Overall, dithranol represents a promising candidate for formulation in niosomal nanoparticles for the treatment of psoriasis.

In order to improve its solubility, stability, skin penetration, and therapeutic efficacy while reducing irritation and adverse effects, this study set out to create a unique niosomal gel loaded with dithranol. In order to conduct research on the creation and assessment of dithranol niosomal gel for the treatment of psoriasis and acne, this research study attempted to create scientific protocols. Preparing and characterising dithranol niosomal gel, including it into an appropriate dermatological basis, and evaluating its relative effectiveness in treating cutaneous infections were the objectives of the study. Using various drug and surfactant ratios, dithranol niosomal gel was created. The drug entrapment efficiency, size, form, and *in-vitro* diffusion research of vesicular carriers were all assessed. The optimised niosomes' zeta potential, vesicular size, and drug entrapment efficiency were also assessed. Thus, current research aim and objectives were as follows.

To conduct physiochemical characterization of the drug dithranol (DTL) (Identification of Drug: Appearance; Drug Authentication: FTIR & UV spectroscopy; Melting point and Solubility determination; Partition coefficient determination; Determination of λmax; Compatibility Studies).

To prepare calibration curve for quantitative estimation of drug dithranol.

To formulate / prepare niosomes (blank & dithranol loaded)

To evaluate dithranol loaded niosomes (vesicle size; entrapment efficiency)

To prepare niosomal gels with Cholesterol, Lecithin, Carbopol 934 and Span 20, Span 40, Span 60, Span 80.

To Evaluate dithranol loaded niosomes gel (determination of pH; viscosity; drug content: *in-vitro* diffusion study; stability studies)

According to Painsi *et al*., 2015, dithranol has a lipophilic character and can swiftly penetrate the skin, making it a valuable medicinal substance (Saville, 1981). Furthermore, its toxicity, low stability (it is readily oxidised and poor solubility prevent it from being used as a possible medicinal treatment (Behrangi *et al*., 2022). According to Kadian *et al*., 2021, dithranol inhibits keratinocyte growth and T cell activation, controls cell differentiation (accumulation in mitochondria and reactive oxygen species production), and triggers apoptosis.

Physical and Chemical Properties of Dithranol

Table 1: Dithranol Properties.

IUPAC name	:	1,8-Dihydroxy-9(10H)- anthracenone
Molecular Formula	:	$C_{14}H_{10}O_3$
Molecular Mass	:	226.231 $g.mol^{-1}$
Physical Nature	:	Solid (Crystalline powder)
Colour	:	Yellow
MP	:	178^oC (352^oF)
pH	:	Acidic pH (range between 2.5 and 5.5)
Boiling Point	:	464.1oC (± 45.0^oC) at 760 mmHg
pka	:	The pKa of free dithranol in solution is 7.9±0.4
Density	:	1.419 ±0.06 g/cm3
Absorbance maxima (λmax)	:	Three distinct maxima : 254–256 nm, 287–289 nm, 354–356 nm
Plasma Protein Binding	:	0% plasma protein binding
Half-life	:	1.2 hours at pH 7.4 (physiological pH) and roughly 2 to 3 hours in other environments
Refractive index	:	1.837
Partition coefficient (log P)	:	1.99
HPLC Retention Time	:	5.32 minutes (acetonitrile / acid / water) and 10.2 minutes (acetonitrile / water / TFA)
Storage	:	Tightly closed, light-protected containers at 2-8°C (36-46°F)
Solubility in Water	:	Practically insoluble (<0.1 g/100mL at 20 °C)
Solubility	:	Soluble in organic solvents; highest solubility in dimethylformamide (DMF) (~10 mg/mL) and [DMSO] (~5mg/mL); soluble in chloroform (20 mg/mL), acetone, benzene; ethanol and ether;

Pharmacological Properties of Dithranol

Psoriasis is treated with dithranol or anthralin as pharmaceuticals. Dithranol are also helpful for alopecia areata. In 30 minutes, damaged skin absorbs more dithranol than intact skin, which takes around 16 hours.

MATERIALS AND METHODS

Pre-formulations Studies

The overall objective of pre-formulation testing is to generate information useful to the formulation and development of stable dosage form with good bioavailability. Dithranol drug sample was purchased from HiMedia Laboratories Private Limited, India and it was authenticated / indentified by FTIR and UV Spectroscopy.

Identification of Drugs - Dithranol (DTL)

(a) Identification of Drug by FTIR
(b) Identification of Drug by Ultraviolet (UV) Spectroscopy
(c) Melting Point (mp) Determination
(d) Physical Appearance Analysis
(e) Partition Coefficient Determination

Purity Determination of Dithranol (DTL)

(a) Determination of Drug by Standard Curve
(b) Purity Determination of Drug by Solubility Analysis

Identification of Dithranol (DTL) by FTIR

- KBr dispersion pellets (free of moisture) were used for the scanning; 10 mg of spectroscopic grade KBr was mixed individually with 1 mg of sample and standard drug in a glass mortar.
- Figures display the FTIR spectra of the drug sample and the standard reference.
- To determine the identification and purity of Dithranol (DTL), characteristic FTIR peaks can be attributed to its functional groups.
- The drug sample's spectral peaks were determined to be comparable to the standard reference (Fig. 12-13).

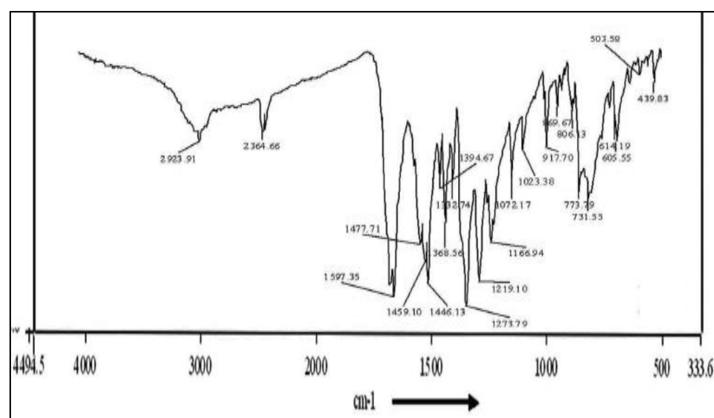

Fig.12: FTIR spectra of Dithranol (DTL; Pure reference).

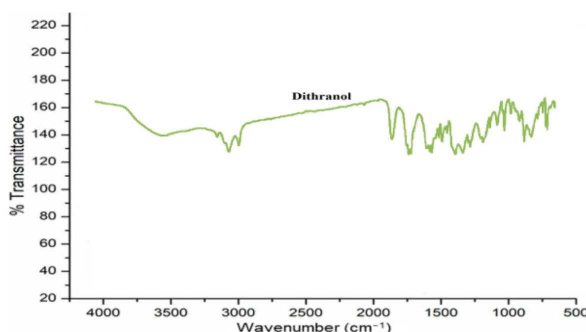

Fig.13: FTIR spectra of Dithranol (DTL) sample.

Identification of Dithranol (DTL) by Ultraviolet (UV) Spectroscopy

In order to determine λmax of dithranol stock solution in chloroform was prepared and scanned / analysed for absorbance at wavelength between 200-400 nm using Double Beam UV Spectrophotometer - Model 2202, Systronics, India.

Accurately weighed 100 mg of dithranol was dissolved in few ml of chloroform and volume was made upto 100 with chloroform (1 mg/ml and scanned between between 200-400 nm using to determine the absorption maxima (λmax) using Double Beam UV Spectrophotometer - Model Systronics -2202).

Melting Point Analysis of Dithranol (DTL)

Melting point (mp) is the temperature at which the pure liquid and solid exist equilibrium. MP of the dithranol was determined by using thieles tube method (Table 2).

Table 2: Melting point of Dithranol (DTL)

S. No.	Reported	Observed
1.	178-179°C	178^0C

Solubility Determination / Analysis of Dithranol (DTL)

DTL's solubility was investigated in a variety of solvents, including ethanol, distilled water, methanol, dichloromethane acetone, and chloroform. 10 mg of dithranol (DTL) was transferred in a series of different solvents (5 ml) in different conical flask. These flasks were shaken by mechanical shaker (Hicon India) for 24 hrs with constant vibration at constant temperature (25^0C).

Different solutions were then filtered and different filtrates were diluted with same/ appropriate solvent and analysed by double beam UV spectrophotometer (Systronics – 2202). Quantitative estimations of DTL were carried out for each sample to calculate the solubility of DTL in different solvents (Table 3).

Table 3: Solubility of dithranol (DTL) in different solvents.

Solvent	Solubility (µg/ml)
Distilled water	06.31 (insoluble)
Chloroform	980.21 (Freely Soluble)
Dichloromethane	968.48 (Freely Soluble)
Acetone	968.16 (Soluble Freely)
Methanol	954.16 (Soluble Freely)
Ethanol	935.16 (Less Freely Soluble)

Physical Appearance Analysis of Dithranol (DTL).

Colour	:	lemon yellow or orange powder
Nature	:	leaflets or plates (Solid)
Odour & Taste	:	No odour and No taste

Partition Coefficient Determination of Dithranol (DTL)

The ratio of unionised drug dispersed between the organic phase and aqueous phase at equilibrium is known as PC, and it is used to characterise and evaluate whether a drug is hydrophilic or lipophilic.The ability of a medicine to pass through the lipoidal cell membrane. In the solvent system, n-octanol/distilled water was determined.5 ml of octanol, 5 ml of DW, and 10 mg of medicationAfter eight hours of shaking in a vortex shaker, all phases were separated. The aqueous phase was then analysed for DTL using a Shimdzu-1700 E UV spectrophotometer against a reagent blank solution.It was established how much drug was in the octanol phase.P was determined as the partition coefficient value

$$P_{o/w} = (C_{organic}/C_{aqueous}) P_{w/0} = (C_{aqueous}/C_{organic})$$

Table 4: Dithranol (DTL) partition coefficient (log P) in various solvents.

S. No.	Solvent System	Reported	Observed
1.	n-octanol / water	1.99	1.96
2.	n-octanol / PBS (pH : 7.2)	1.82	1.77

The partition coefficient (log P value) of Dithranol (DTL) indicated that dithranol is a lipophilic drug with a high affinity for lipids rather than water, making it suitable for formulation within lipophilic carriers such as solid lipid nanoparticles (Table 4).

Compatibility Studies of Dithranol (DTL)

a. FTIR spectroscopy was used to assess the compatibility of the medication DTL with polymers and surfactants.
b. To verify compatibility, a physical mixture of DTL and polymer (1:1 ratio) was made, and the mixture was then subjected to FTIR analysis.
c. The combination FTIR spectra of physical mixtures had peaks that were comparable to those of pure drug FTIR, with no discernible changes.

Preparation of Calibration / Standard of Curve Dithranol (DTL)

i. A precisely weighed 100 mg of dithranol (DTL) was dissolved in a few ml of chloroform, and the volume was increased to 100 using distilled water (1 mg/ml Stock solution A).

ii. A 100 ml volumetric flask was filled with 1 ml of this solution, and the volume was increased to 100 ml by adding 10 µg/ml of chloroform (Stock solution B).

iii. To obtain concentrations of 0, 10, 20, 30, 40, 50, and 60 µg/ml, various aliquots of Stock solution B (10 µg/ml) were produced, absorbance was measured at 254 nm against the blank (Fig. 14).

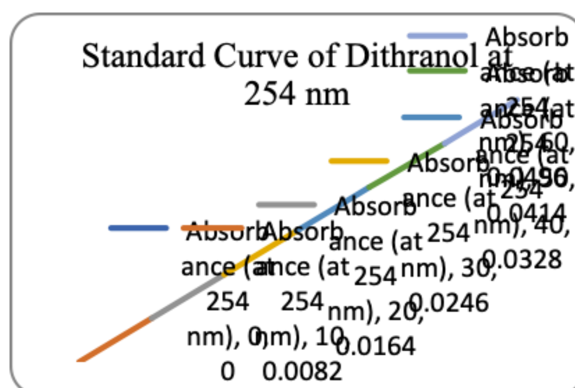

Fig. 14: Standard curve of dithranol at 254 nm.

Preparation of the Formulation

Method of Preparation: Coacervation Phase Separation Method.

A clean, dry glass vial (5 ml) was filled with precisely weighed amounts of surfactant, carrier (lecithin), cholesterol, carbopol 934 (gelling agent), and medication dithranol (DTL). Solvent was then added with straightforward mixing;

Vials were closed with cap and heated on water bath for 5 min;

The mixture were allowed to cool at room temperature till the dispersion got converted into proniosomes (Table 5). (Nagaswamy *et al*., 2014)

Table 5: Composition of Proniosomal Gel Formulations.

Formulation Code	Surfactant Type	Ratio (mg)	Soya Lecithin (mg)	Choles-terol (mg)	Carbopol 934 (% w/w)	Drug (DTL) (mg)	Ethanol (ml)
R1	S20:S40	250:250	500	100	1.5 %	10	0.5
R2	S20:S60	250:250	500	100	1.5 %	10	0.5
R3	S20:S80	250:250	500	100	1.5 %	10	0.5
R4	S40:S60	250:250	500	100	1.5 %	10	0.5
R5	S40:S80	250:250	500	100	1.5 %	10	0.5
R6	S60:S80	250:250	500	100	1.5 %	10	0.5

Characterisation of Proniosomes

Scanning Electron Microscope Analysis (SEM Analysis)

i. The Advanced Instrumentation Research Facility (AIRF), JNU, New Delhi, used scanning electron microscopy (Evo-40, Zeiss, Germany) to visualise the surface morphology and structure.

ii. The proniosomes were sparingly sprinkled on double-sided adhesive tape that had already been shucked to aluminium stubs in order to prepare the samples.

iii. After applying a gold coating to the stubs using a fine coat ion sputter, the samples were examined for surface morphology and particle size (Fig.15-16).

Fig.15: SEM Analysis of Proniosomes loaded with dithranol (R1).

Fig. 16: SEM Analysis of Proniosomes loaded with dithranol (R4).

Transmission Electron Microscope Analysis (TEM)

i. Using an accelerating voltage of 80 kV, proniosome vesicles were observed using TEM at AIRF (JNU);

ii. A drop of proniosome sample was applied to a carbon-coated grid to create a thin layer before the film dried;

iii. Phosphotungustic acid (PTA) was used to negatively stain the sample.

iv. After adding a drop of staining solution to the film, any extra solution was drained off using filter paper.

v. The material was examined in a TEM after the grid was fully air dried (Fig. 17-18).

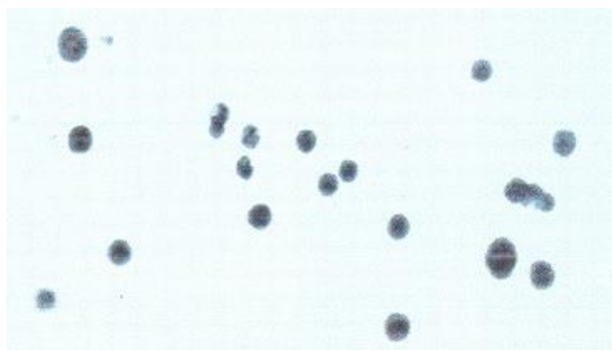

Fig.17: TEM Photograph of Proniosomes loaded with dithranol (R1).

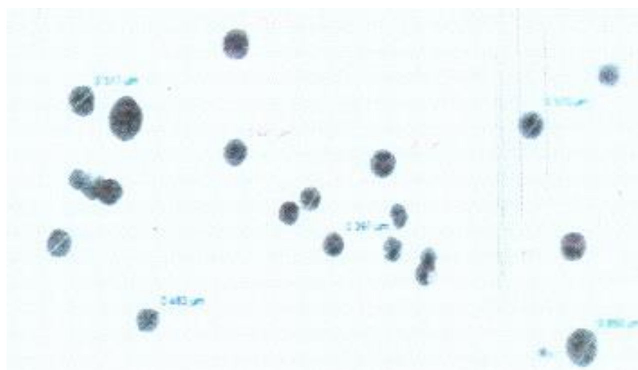

Fig.18: TEM Photograph of Proniosomes loaded with dithranol (R4).

Analysis of Particle Size

Table 6: Vesicle Size Analysis of Different Formulations.

S. No.	Formulation Code	Vesicle Size ± SD (µm)
1.	R1	25.23 ± 1.03
2.	R2	19.46 ± 1.86
3.	R3	17.64 ± 1.58
4.	R4	24.11 ± 1.72
5.	R5	18.34 ± 1.64
6.	R6	17.52 ± 1.54

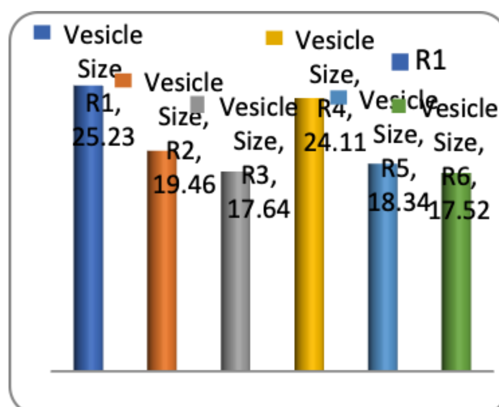

Fig. 19: Vesicle Size Analysis of Different Formulations.

Percentage Entrapment Efficiency

After the unentrapped DTL medication was removed, the amount of drug entrapment within the proniosomes was calculated;

Proniosome pellets and the DTL drug-free supernatant were separated from unentrapped DTL drug by centrifuging the dispersion in a cooling centrifuge (Remi Instruments, Mumbai) at 22000 rpm/min at 40C for 45 minutes.

Centrifugation was used to remove any unentrapped DTL drug, and the proniosome pellets were rinsed again with phosphate buffer;

After an appropriate dilution with phosphate buffer solution, the combined supernatant was measured for DTL drug content using a double beam UV spectrophotometer to measure absorbance at 275.6 nm.

Percentage of drug entrapment in the proniosomes was calculated from the difference between the initial drug added and the drug detected in the supernatant.

Amount of DTL drug exactly present within the proniosomes was also analysed by dissolving the proniosomes in methanol to countercheck the drug entrapment percentage.

Analysis of DTL drug in proniosomes was carried out using the empty proniosomes dissolved in methanol as blank in order to nullify interference of excipients (Table 7).

Table 7: Entrapment Efficiency of various Proniosomal Formulations.

S. No.	Formulation Code	Drug Loading (%)
1.	R1	94.86 ± 1.83
2.	R2	93.34 ± 1.02
3.	R3	90.79 ± 0.96
4.	R4	95.92 ± 1.36
5.	R5	93.45 ± 1.08
6.	R6	91.62 ± 1.72

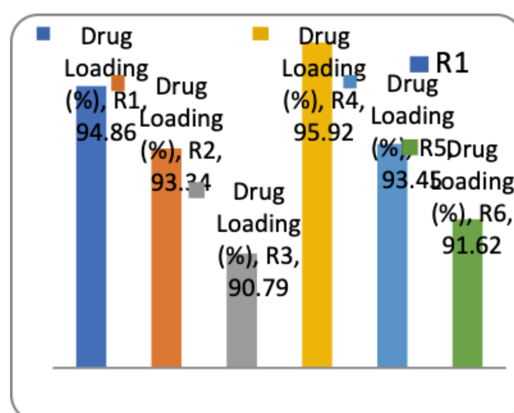

Fig. 20: Entrapment Efficiency of various Proniosomal Formulations.

In-vitro Drug Release Studies

i. A locally made diffusion cell was used to examine the in vitro release of DTL from proniosomal formulation;

ii. The diffusion cell's effective permeation area was 1 cm2, and the receptor cell's volume was 40 ml;

iii. A temperature of 37±20C was maintained;

iv. A magnetic stirrer operating at 100 rpm continuously agitated the receptor compartment, which held 40 ml of phosphate buffer solution (pH 7.2);

v. The donor and receptor compartments were separated by artificial semi-permeable barrier;

vi. The membrane was exposed to the proniosomal formulation;

vii. Samples were removed through the diffusion cell's sample port at prearranged intervals during a 30-hour period, and a UV spectrophotometer was used for analysis;

viii. An identical volume of pH 7.2 phosphate buffer solution was promptly added to the receptor phase (Table 8);

ix. The experiment was completed in triplicate for each research;

x. The sink condition was maintained throughout.

Table 8: Various parameters for *in-vitro* drug release study.

S. No.	Material	Quantity
1.	Membrane	Semi-permeable membrane
2.	Release Media	Phosphate buffer (pH 7.2)
3.	Temperature	37 ± 2^0C
4.	Volume of medium used	40 ml
5.	Absorbance wavelength of DTL	254 nm
6.	Assembly used	Diffusion cell
7.	Time	30 hrs
8.	Area of diffusion Cell	1 cm^2

Statistical methods were used to analyse and check whether there was any significant difference present in drug release pattern of different formulations or not (Table 9-12).

Table 9: Statistical Analysis.

Formulation	Drug Release	Drug Release	Drug Release
R1	83.27	86.75	79.79
R2	81.65	84.48	78.82
R3	76.79	78.8	74.8
R4	82.28	85.47	79.09
R5	76.51	80.3	72.72
R6	73.11	75.83	70.39

Release Kinetics Modeling

Table 10: Mathematical models used to describe drug release mechanism.

S. No.	Mathematical models	Equation
1.	Zero order	Qt = Qo + Kot
2.	First order	In Q = In Qo + K1t
3.	Higuchi	Qt = KH√t
4.	Korsmeyer-peppas	Qt / Q∞ = Kktn

Table 11: Different release mechanism of "n" value.

"n"	Mechanism
n >1	Super case-II Transport
1	Zero order release (Case-II Transport)
0.5< n<1	First order (Anomalous Transport)
0.5	Higuchi Matrix (Fickian Diffusion)

Table 12: Release modeling data for different DTL formulations.

S. No.	Formulation Code	Zero Order Release	First Order Release	Higuchi Release	Korsmeyer-Peppas Release	
		R^2	R^2	R^2	R^2	n
1	R1	0.977	0.988	0.975	0.993	0.740
2	R2	0.979	0.984	0.967	0.994	0.824
3	R3	0.988	0.988	0.957	0.995	0.865
4	R4	0.986	0.981	0.960	0.994	0.886
5	R5	0.990	0.985	0.954	0.994	0.907
6	R6	0.988	0.986	0.936	0.990	0.976

Stability Studies

Table 13: Storage stability of Formulation R1 and R4 after 4 weeks.

S. No.	Time (weeks)	Drug retained (%) at 2-8^{0}C		Drug retained (%) at Room Temp.		Drug retained (%) at 45^{0}C	
		R1	R4	R1	R4	R1	R4
1	0	100	100	100	100	100	100
2	1	98.6	98.9	95.8	96.9	91.3	92.6
3	2	98.2	98.3	93.6	94.3	85.7	87.3
4	3	97.8	97.2	90.2	93.6	79.2	81.6
5.	4	97.6	97.1	87.2	89.4	71.3	76.5

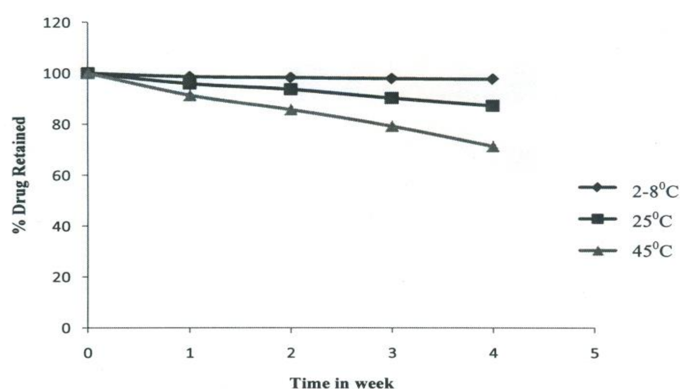

Fig. 21: Comparative stability of Formulation R1.

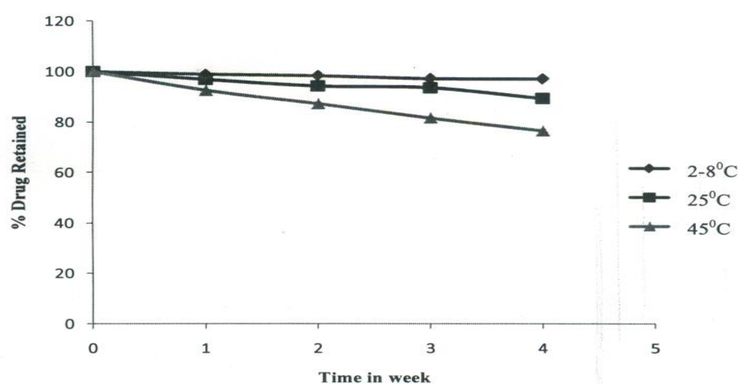

Fig. 22: Comparative stability of Formulation R4.

In-vitro antioxidant Activity by DPPH Radical Scavenging Test

Dithranol proniosomes (DTLPNS) and dithranol (DTL) were tested for their ability to scavenge the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical. DTL and DTLPNS concentrations ranging from 0 to 100 µg/ml were produced. 0.1 ml of DPPH (0.1 mM) methanolic solution combined with 2 ml of DTL or DTLPNS solution. Following a 30-minute incubation period, the solution was assessed using a UV-visible spectrophotometer at λmax 517 nm. Ascorbic acid (AA) was used as a positive standard to correlate the results, with methanol serving as the control (Fig. 23).

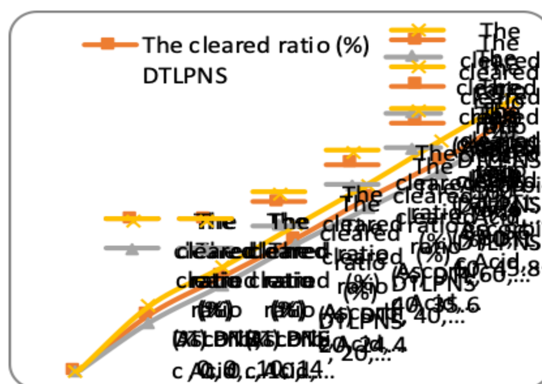

Fig. 23: DPPH regression curve values.

The DPPH assay was used to assess the antioxidant activity of 2,2-Diphenyl-1-picryl-hydrazyl-hydrate radical scavenging activity of ascorbic acid (AA), dithranol (DTL), and dithranol-loaded nanosponges (DTLPNS). This method enables the assessment of antioxidant responses of a particular sample based on concentration.

RESULTS AND DISCUSSION

In the current study, an attempt was made to use the vesicular drug delivery system (VDDS) to create the best proniosomal formulation to improve the penetration of the transdermal formulation of the poorly water-soluble drug dithranol (DTL). The proniosomal (dry-niosomes) formulation, which is known for its safety and high therapeutic efficacy, was converted to niosomes and added to the transdermal route to increase bioavailability (i.e., to lower the daily dosage schedule of dithranol, which would improve patient compliance and drug safety).

Pure dithranol (DTL) was procured from a commercial source (purchased from HiMedia Laboratories Private Limited, India) and sample drug were analysed for various parameters in different studies and results were obtained. FTIR analysis was performed for confirmation of

their identity and purity. Sample drug showed absorption peaks which were similar to reference standard drug. Absorption peaks (cm^{-1}) at 1605.67 (Aldehyde C-H stretching), 1453.93 (C=C stretching; aromatic), 1280.49 C=O stretching, 1220.23 (O-H bending; phenol) and 1165.13 (O-H bending; alcohol)) were found and both sample drug and reference drug were pure dithranol (DTL).

The UV absorption maximum (λmax) for Dithranol (DTL) was found to be 254 nm, 287, and 354 nm. Dithranol (DTL) mp was found to be 178^0C. Dithranol (DTL) was then found to be freely soluble in acetone (968.16 µg/ml), dichloromethane (968.48 µg/ml), methanol (954.16 µg/ml), and chloroform (980.21 µg/ml); less freely soluble in ethanol (935.16 µg/ml); and nearly insoluble in water (06.31 µg/ml). Additionally, DTL was discovered to be a yellow, crystalline powder that had no taste or odour. The partition coefficient of DTL was analysed and was found to be 1.96 (reported 1.99 in n-octanol/distilled water) and 1.77 (reported 1.82 in n-octanol/phosphate buffer pH -7.2).

FTIR spectrum of Cholesterol + dithranol (DTL) drug showed absorption peaks at 3450 (OH), 2980 (C-H stretching), 1470 (C=C aromatic), 1380 (CH bending), 1630 ($C{=}O$ stretching), 1453 ($C{-}C$ stretching (aromatic), 1280 (C-O stretching and phenolic OH-bending), and 3400 (O-H stretching). FTIR spectrum of Lecithin + dithranol (DTL) drug showed absorption peaks at 1630 ($C{=}O$ stretching), 1453 ($C{-}C$ stretching (aromatic), 1280 (C-O stretching and phenolic OH-bending), and 3400 (O-H stretching), 2950 (CH stretching), 1750 (C=O), and 1430 (CH bending).

FTIR spectrum of Carbopol 934 + dithranol (DTL) drug showed absorption peaks at 1630 ($C{=}O$ stretching), 1453 ($C{-}C$ stretching (aromatic), 1280 (C-O stretching and phenolic OH-bending), and 3400 (O-H stretching), 3500 ($O{-}H$ group), 1750 (carbonyl ($C{=}O$) stretching), 1450 (CH_2 bending vibration), 1250 (C-O stretching vibration), 1200 ($C{-}O{-}C$ stretching vibrations), and 850 (plane bending vibration of $=C{-}H$). FTIR spectrum of Span 20 + dithranol (DTL) drug showed absorption peaks at 1605.67 (Aldehyde C-H stretching), 1453.93 (C=C stretching; aromatic), 1280.49 (C=O stretching), 1220.23 (O-H bending), 1165.13 (O-H bending), 3600 (OH), 2835 (CH-CH stretching), 1755 (C=O), and 1470 (CH-CH bending).

FTIR spectrum of Span 40 + dithranol (DTL) drug showed absorption peaks at 1630 ($C{=}O$ stretching vibration of anthrone structure), 1460 ($C{-}C$ stretching), 1200 (C-O stretching and

OH-bending). FTIR spectrum of Span 60 + dithranol (DTL) showed absorption peaks at 1630 ($C=O$ stretching vibration of anthrone structure), 1453 ($C-C$ stretching), 1165 (C-O stretching and OH-bending). FTIR spectrum of Span 80 + dithranol (DTL) showed absorption peaks at 1630 ($C=O$ stretching vibration of anthrone structure), 1453 ($C-C$ stretching), 1280 (C-O stretching and OH-bending), 3400 (O-H phenolic stretching), 1740 (Sharp absorption peak for $C=O$ ester stretching), 2950 (Stretching vibrations of $-CH_2$- and $-CH_3$), 1173 (C-O-C stretching), 3400 (OH group). In FTIR spectral analysis, it was found that there was no change in absorption maxima of functional groups in pure drug dithranol (DTL) as well as drug polymer / stabilizer / gelling agent mixture hence drug and polymer / stabilizer / gelling agent were found to be compatible to each other.

Coacervation phase separation method was used for formulation of vesicular proniosomes of dithranol (DTL) and various ratio of surfactant, lecithin, cholesterol, drug, Carbopol 934 (gelling agent) and ethanol were used for the formulation of proniosomes. Different concentrations of Carbopol 934, cholesterol, lecithin, span 20, span 40, span 60, and span 80 were used to create distinct proniosomal formulations (R1, R2, R3, R4, R5, and R6). Vesicle size, SEM and TEM analysis, entrapment efficiency, *in-vitro* drug release study, and other factors were used to assess each formulation. Out of all these proniosomal formulations, the R4 formulation demonstrated the maximum entrapment effectiveness while the R6 formulation displayed the smallest vesicle size.

The visualization by SEM and TEM showed that formulated proniosomes has a lamellar vesicular structure and vesicular characteristics were shown by the carrier. When the dispersion was agitated, the size of the created vesicles decreased because the energy supplied caused the bigger vesicles to break into smaller ones, which ranged from 17.52±1.54 µm to 25.23±1.03 µm. Due to the presence of Span 60, formulation R1 had the highest vesicle size (25.23±1.03 µm), while formulation R6 had the smallest (17.52±1.54 µm). A reduced vesicle size results from the surfactant becoming more hydrophobic (since surface energy reduces with increased hydrophobicity). Due to the formulation's higher HLB value (7.65±1) and greater drug entrapment efficiency, Span 40 results in larger proniosome vesicles.

The dithranol (DTL) entrapment efficiency of proniosomes was found in the range of 90-95% and it was attributed to the lipophilic nature of the drug. Entrapment efficiency was found

maximum in formulation R4 (95.92%; because Span 40 and Span 60 were used alongwith soya lecithin and cholesterol). Soya lecithin contains saturated fatty acids and Span 40 produced larger vesicle size with higher dithranol (DTL) entrapment efficiency.

Permeation tests conducted *in-vitro* consistently provide important insights into the behaviour of the product. The amount of medicine accessible for absorption is determined by the drug permeated. *In-vitro* drug release studies were performed using semi-permeable dialysis membrane. The order of dithranol (DTL) drug release in different formulations was R1>R2>R4>R3>R5>R6. According to this drug release order of different formulations, R1 formulation showed the maximum drug release i.e. 85.11% and R6 showed the minimum drug release i.e. 76.25% after 30 hrs. Drug release from R1 was highest due to composition with S20 and S40 resulted into better in-flux properties than S60 and S80 (R6).

The release profile of bioactive substances in polymeric systems is being studied more and more using mathematical modelling since it might reveal crucial details regarding the release process. Zero order, first order, Higuchi, and Korsmeyer-Peppas models were used to analyse how the drug dithranol (DTL) is released from proniosomes. Table displays the regression coefficient and slopes for the various drug release kinetics models. The models deemed most suitable for the drug release were those with the highest regression coefficient. Formulations R1, R2, R3, R4, R5, and R6, with r2 values of 0.993, 0.994, 0.995, 0.994, 0.994, 0.0990, and n values of 0.740, 0.824, 0.865, 0.886, 0.907, and 0.976, respectively, followed the Korsmeyer-Peppas model. Release can be determined using the super case 2 transport mechanism (non-fickian transport) when n values are greater than 0.74. The stability study was carried out for formulation R1 and R4 at different temperature like refrigeration temperature (2-8^{0}C), room temperature (25±3^{0}C) and at 45^{0}C for 4 weeks and after evaluation of each work data, the data shows that at 2-8^{0}C formulation R1 has remained 97.6% and formulation R4 was remained 97.1%; at 25±3^{0}C, R1 has remained 87.2% and formulation R4 was remained 89.4%. At 45^{0}C, 71.3% and 76.5% were remained for formulation R1 and R4 respectively. So, there was nominal degradation at room temperature (stable) whereas degradation took place at high temperature (45^{0}C) after 4 weeks. After 4 weeks, best stability was found at refrigeration temperature (2-8^{0}C). So proniosomal formulation should be stored at 2-8^{0}C (refrigerator). Formulation R1 showed better storage stability than formulation R4 (either at room temperature or at refrigeration temperature).

The DTLPNS and DTL's antioxidant activity were directly correlated with their total phenolic content (TPC) concentrations. DTL exhibits an antioxidant action mostly because of the presence of phenolic hydroxyl groups. The DTL moiety's dose-dependent nature was demonstrated by the fact that the antioxidant activity of all the samples under investigation increased with concentration. Finally, it was found that dithranol proniosomal specialized formulations can be used for psoriasis and acne. It works by inhibiting cell turnover and reducing inflammation, though its use is limited by severe staining of skin and clothing and high irritation potential. Proniosomal specialized formulations can be used in short-contact therapy (10–30 minutes) and washed off to minimize irritation. Recent studies suggested that incorporating DTL into proniosomal specialized formulations improves its stability and antioxidant-like efficiency, allowing better scavenging of DPPH radicals compared to pure dithranol.

CONCLUSIONS

Pure dithranol (DTL) was obtained from a commercial source (HiMedia Laboratories Private Limited, India). DTL was discovered to have UV absorption maximums (λmax) of 254, 287, and 354 nm and possess a melting point of 178^{0}C. Further, DTL was then found to be freely soluble in chloroform, dichloromethane, acetone, and methanol; less freely soluble in ethanol; and nearly insoluble in water. After analysis, the partition coefficient of DTL was determined to be 1.96 (reported as 1.99 in n-octanol/distilled water) and 1.77 (reported as 1.82 in n-octanol/phosphate buffer pH -7.2). In compatibility studies, it was discovered through FTIR spectral analysis that the absorption maxima of functional groups in both the pure drug DTL and the drug polymer/stabilizer/gelling agent mixture did not change. The formulated proniosomes had a lamellar vesicular structure, and the carrier had vesicular properties, according to SEM and TEM visualisation. Formulation R1 had the largest vesicles while formulation R6 had the smallest because of Span 60. Vesicle size reduces as the surfactant's hydrophobicity increases (since surface energy falls with increased hydrophobicity). The *in-vitro* release of DTL from proniosomal formulations increased significantly as the alkyl chains of different Spans decreased. The total phenolic content (TPC) contents of the DTLPNS and DTL were directly correlated with their antioxidant properties. The presence of phenolic hydroxyl groups is the primary cause of DTL's antioxidant activity. Dithranol proniosomal specialised formulations were found to be effective in treating acne and psoriasis. It reduces inflammation and inhibits cell turnover, but its significant potential for irritation and severe skin discolouration restrict its use.

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