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Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol

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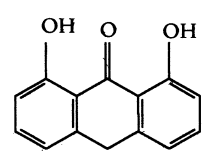
Abstract

Dithranol is one of the mainstays in the topical treatment of psoriasis. However, the use of dithranol in psoriatic condition is inconvenient and troublesome, as it has irritating, burning, staining and necrotizing effect on the normal as well as the diseased skin. The entrapment of drug in vesicles is viewed to help in the localized delivery of the drug and an improved availability of the drug at the site will reduce the dose and in turn, the dose-dependent side effects like irritation and staining. The investigations deal with critical parameters controlling the formulation and stabilization of dithranol loaded liposomes and niosomes. The entrapment efficiency of dithranol in liposomes was optimized by altering the proportion of phosphatidyl choline and cholesterol, and in case of niosomes it was between Span 60 and cholesterol. Hydration and permeation mediums were also established keeping in view the poor solubility and stability of dithranol. The mean liposome and niosomes sizes were 4 ± 1.25 and 5 ± 1.5 μm, respectively. The drug-leakage study carried out at different temperatures of 4–8, 25 ± 2 and 37 °C for a period of two months affirms that the drug leakage increased at a higher temperature. The in vitro permeation study using mouse abdominal skin shows significantly enhanced permeation with vesicles as indicated by flux of dithranol from liposomes (23.13 μg/cm^2/h) and niosomes (7.78 μg/cm^2/h) as compared with the cream base (4.10 μg/cm^2/h).

Keywords: Topical delivery; Liposomes; Niosomes; Dithranol; Entrapment efficiency; Stability; Skin-permeation

1. Introduction

Dithranol (1,8-dihydroxy-9-anthrone), first synthesized in 1916 have since been in clinical use in the treatment of psoriasis.



(1,8-dihydroxy-9-anthrone)

Psoriasis is characterized by four histopathological changes: hyper proliferation, hyper paraker-

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atosis, epidermal accumulation of polymorphonuclear leukocyte and dermal inflammation (Ashcroft et al., 2000). The target organelle for dithranol is mitochondria as therapeutic interaction occurs with the electron transport chain on the inner mitochondrial membrane resulting in a reduction of ATP synthesis (Mehrle et al., 1994). Simultaneously many enzymes associated with cell proliferation are reportedly inhibited by dithranol, viz. G-6-P dehydrogenase, ornithine decarboxylase, lipooxygenase, and protein kinase C (Reichert et al., 1985). Though the drug is highly effective in various psoriatic conditions yet its application is inconvenient and troublesome, as it has irritating, burning, staining and necrotizing effects on the normal as well as the diseased skin. Further, the drug is unstable due to its photosensitivity and gets readily photo-oxidized (Mustakalio, 1981; Hiller et al., 1995). The currently available dosage forms failed to moderate the undesired effects of dithranol and they also they could not improve the photochemical stability of drug. In addition, the conventional vehicles like soft paraffin bases possess limited solubilizing capacity for dithranol creating a need for the incorporation of an extra amount of drug in its crystalline form to maintain the desired drug concentration in bio-milieu and at the receptor site. The insoluble crystalline drug at the surface of psoriatic lesions and its uncontrolled distribution at perilesional site cause irritation and staining of normal skin. The continued research efforts, however, have yielded one commercially viable dithranol formulation (Micanol™) containing microcrystalline monoglyceride based microencapsulated drug. It is less irritating and easy to wash off from clothes (Vleuten et al., 1996; Marie-Jean et al., 1998). In the present study vesicular systems, i.e. liposomes and niosomes, were selected in order to circumvent the undesirable effects of the drug and to maximize its therapeutic indices. In one report Gehring et al. (1992) studied the effect of dithranol incorporated in liposomal gel on the severity of erythema on healthy skin. The current study entails the development of dithranol entrapped liposomal and niosomal systems and charaterization of these drug-loaded vesicular systems to optimize the various formulations and process-related variables. Assessment of the various characters, viz. degree of entrapment, size profile, drug-leakage profile and their ability to carry the drug across the skin barrier (skin permeation studies) has been carried out.

Liposomes are microscopic vesicles composed of one or more lipid bilayers arranged in a concentric fashion enclosing an equal number of aqueous compartments (Bangham et al., 1965). Analogous to liposomes, niosomes are formed from the self-assembly of non-ionic amphiphiles in combination with other lipidic surfactants in aqueous medium. Both liposomes and niosomes have attracted a great deal of attention in the delivery of dermal drugs because of many advantages, like they are biodegradable, non-toxic, amphiphilic in nature, penetration enhancers and effective in the modulation of drug release properties (Uchegbu and Vyas, 1998). These smectic mesophasic structures offer great opportunities in drug delivery as they can be modified in their structural characteristics like size, shape, lamellae nature and the type of composition being used.

2. Materials and methods

2.1. Chemicals

Dithranol (DTH) was a gift from M/s Glaxo (India) Ltd, Phospholipon 90H (PC) having 97.3% phosphatidyl choline content was a generous sample from Natterman phospholipids (Germany), Span 60 and Cremophor RH 40 was purchased from Koch-Light Laboratories (UK) and BASF (UK), respectively. Cholesterol (CHOL), Sephadex G-50 medium, Dicetyl phosphate (DCP), and Butylated Hydroxy Toluene (BHT) were procured from Sigma (USA). All other materials used in the study were of analytical grade.

2.2. Preparation of liposomes/niosomes

Multilamellar liposomes and niosomes were prepared by thin film hydration method. Accurately weighed quantities of the drug, lipid, sur-

Table 1
Effect of lipid composition on encapsulation efficiency of liposome

Batch	DTH:PC –CHOL (molar ratio) [a]	Entrapment levels (drug in mg/total lipid in mg)
DLS 1	1:2.5:2	0.97 ± 0.043/70
DLS 2	1:3.75:1.5	1.35 ± 0.065/90
DLS 3	1:2.5:2.5	1.49 ± 0.075/75
DLS 4	1:3.75:3	1.62 ± 0.034/105
DLS 5	1:5:2	2.00 ± 0.048/120
DLS 6	1:5:4	2.20 ± 0.059/140
DLS 7	1:10:4	3.00 ± 0.027/240
DLS 8	1:5:5	3.00 ± 0.054/150
DLS 9	1:6:6	3.51 ± 0.043/180

[a] A constant amount of DCP (0.4 molar ratio) was added to each formulation.

factant, CHOL, in the ratio shown in Tables 1 and 2 were dissolved in chloroform in a 250 ml round-bottom flask. A constant amount of DCP (0.4 molar ratio) was added to each formulation as a negative charger and BHT equivalent to 2% of the total lipids as an antioxidant in the organic phase in the flask. The chloroform was evaporated at 45 °C under reduced pressure at 150 rpm using Rotavapor Film Evaporator (Buchi 461, Switzerland). After complete evaporation of chloroform, the flask was kept under vacuum overnight under nitrogen atmosphere to remove the residual solvent. Hydration of the thin film was carried out using nitrogen purged acetate buffer pH 5.5 containing sodium chloride (0.9%) and sodium metabisulphite (0.5%) as stabilizers.

Table 2
Effect of surfactant and cholesterol composition on encapsulation efficiency of niosomes

Batch	DTH:SPAN 60:CHOL (molar ratio) [a]	Entrapment levels (drug in mg/total lipid in mg)
DNS 1	1:4:6.7	0.97 ± 0.027/110
DNS 2	1:16.47:3.2	1.86 ± 0.065/210
DNS 3	1:13.2:3.2	1.54 ± 0.059/174
DNS 4	1:13.2:6.7	1.97 ± 0.043/210
DNS 5	1:8:8	1.30 ± 0.059/166
DNS 6	1:7:7	1.08 ± 0.076/145

[a] A constant amount of DCP (0.4 molar ratio) was added to each formulation.

2.3. Determination of drug entrapment in vesicles

The separation of un-entrapped, free drug was achieved by mini column centrifugation method (New, 1990). Vesicular suspension (0.2 ml) was placed in column after presaturating it with empty vesicles. The centrifugation was carried out at 2000 rpm for 3 min and elutes containing drug-loaded vesicles were collected and observed under microscope for drug particles. The vesicular suspension thus obtained was digested in chloroform–methanol (2:1) solvent system, purged with nitrogen and analyzed using spectrophotometer (Lambda 15, Perkin–Elmer, USA) at 360 nm.

2.4. Reproducibility

One formulation each of liposomes and niosomes was prepared six times and studied to validate the reproducibility of formulation. The statistical analysis was avoided, as the results were highly reproducible each time.

2.5. Microscopy

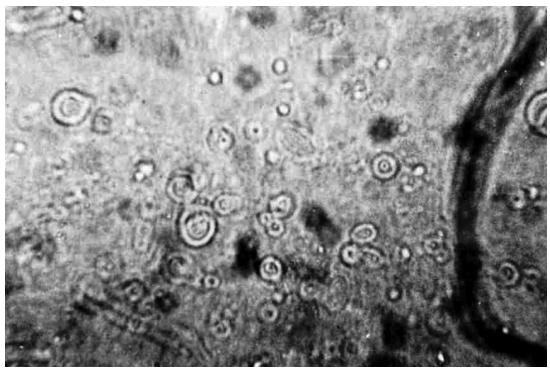
All the batches were viewed under optical microscope to observe the shape and lamellar nature of vesicles, as shown in the photomicrographs in Fig. 1a and b, respectively.

2.6. Determination of vesicle size

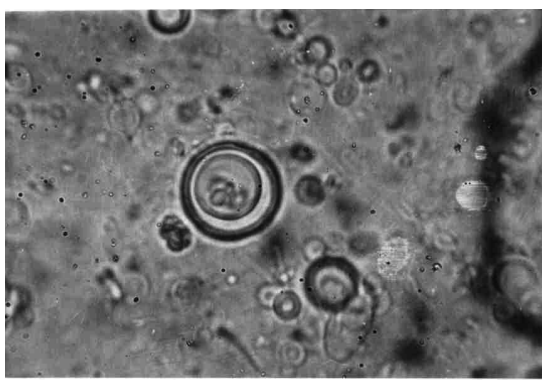
The vesicle sizes of liposomes and niosomes were determined by light scattering based on laser diffraction using the Malvern Mastersizer (Malvern, Model S, Ver. 2.15, UK). The apparatus consisted of a He–Ne laser (5 mW) and a small volume sample-holding cell. The sample was stirred using a magnetic stirrer bead to keep and maintain the sample in suspension.

2.7. Drug-leakage study from vesicles

The optimized liposomal (DLS 9) and niosomal batches (DNS 2, DNS 4) were sealed in 30 ml



(a)



(b)

Fig. 1. (a) Photomicrograph of dithranol loaded liposomes (DLS9). (b) Photomocrograph of dithranol loaded niosomes (DNS4).

vials after purging with nitrogen and stored at different temperatures 4–8, 25 ± 2 and 37 °C for a period of two months. The samples from each batch at each temperature were withdrawn at definite time intervals; the residual amount of the drug in vesicles (i.e. entrapment) was determined as described earlier (2.3). The results are shown in Fig. 2a and b.

2.8. Preparation of o/w cream

Cream was prepared by heating both oil phase and aqueous phase at 65 °C. The aqueous phase was added to the oil phase under mechanical stirring. The pH of the cream was adjusted to 5.5 with citric acid. The cream was allowed to cool down slowly under constant stirring and left overnight for air removal in a vacuum desiccator.

The Composition of cream base is as follows:

Composition	% (w/w)
Oil phase	
Dithranol	0.5
Light liquid paraffin	10
Isopropyl myristate	12
Cetyl alcohol	5
Primary Emulsifier	6
BHT	0.1
Glyceryl monostearate	5
Aqueous phase	
Secondary emulsifier	1.6
Propylene glycol	5
Citric acid	0.5
Water q.s.	100

2.9. Solubility study

The solubility study of dithranol was carried out in thermostatic water shaker bath at 37 °C for 24 h. Excess of drug was added in 10 ml modified buffer [acetate buffer pH 3.3 with stabilizers, viz. ascorbic acid (1%), sodium metabisulphite (0.5%), EDTA (0.5%), and sodium chloride (0.9%)] with an increasing concentration of surfactant Cremophor RH 40 in stoppered 25 ml volumetric flask after purging with nitrogen. After 24 h shaking, the samples were filtered using 0.45 μm membrane filter and analyzed after appropriate dilution using spectrophotometer at 360 nm. Solubility of dithranol in methanol was also determined.

2.10. In vitro permeation study

Permeation studies were carried out through hairless abdominal skin of laca mice using the modified Franz Diffusion Cell. The animals were sacrificed by an overdose of chloroform inhalation. The hairs on the dorsal side of animal were removed with the help of 0.1 mm animal hair clipper, in the direction of tail to head. The shaven part of the skin was separated from the animal and the hypodermis including blood vessels were surgically removed using a surgical blade (no. 23). The dermis part of the skin was wiped

off with a wet cotton swab soaked in isopropanol 3–4 times to remove any adhering fat material. The skin membrane surface area exposed to receptor phase was 4.15 cm^2 (2.3 cm diameter). In diffusion cell, degassed receptor phase acetate buffer with 1% w/v Cremophor RH 40 and 20% methanol purged with nitrogen (Section 2.9) was used for the evaluation of drug permeation. Various prepared formulations (liposomes, niosomes and cream base) each equivalent to 2.5 mg of drug was applied onto the prepared mice skin facing the donor chamber. An aliquot of 5 ml of samples was withdrawn at suitable time interval and replaced with same amount of medium to

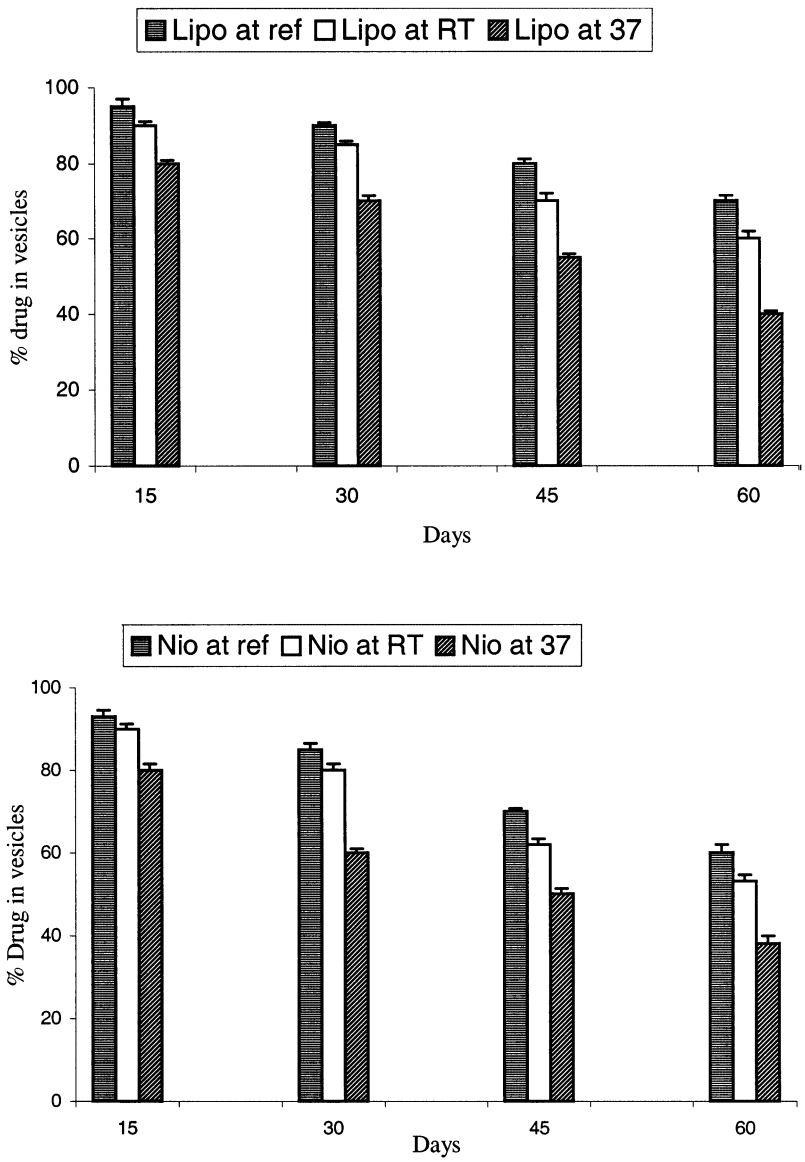


Fig. 2. (a) Bar diagram depicting the percent drug remained in liposomes after subjecting the liposomes (DLS9) to 4, 25 and 37 °C at 15, 30, 45 and 60 days. (b) Bar diagram depicting the percent drug remained in niosomes after subjecting the niosomes (DNS4) to 4, 25 and 37 °C at 15, 30, 45 and 60 days.

maintain the receptor phase volume as 150 ml. The samples were quantitiated by UV spectrophotometer at 360 nm.

3. Results and discussion

3.1. Entrapment efficiency

3.1.1. Influence of process parameters

Process variables, viz. vacuum, hydration medium, hydration time, speed of rotation of flask, and agitational method of size reduction were optimized to prepare lipid vesicles of dithranol. Acetate buffer of pH 5.5 with sodium metabisulphite (0.5%) and sodium chloride (0.9%) was found to be the best hydrating medium that ensured better drug stability. The rotational speed of the flask demonstrated discernible influence on the thickness and uniformity of the lipid film. The speed of 150 rpm yielded a uniform thin, lipid film yielding vesicular preparation of desired characteristics on hydration. While lower and higher rate of rotation resulted in preparation with noticeable aggregated non-vesicular lipid artifacts; multilamellar liposomes are first prepared by constant vortexing for 5 min on a vortex mixer and then sonicated for two 1 min periods using an ice bath in a probe sonicator (Ralsonics Model RP120, Mumbai, India). This gives the uniform size of liposomes in the size range of 4 ± 1.25 μm. The purpose of giving short period exposures of ultrasound is to obtain the liposomes of uniform and controlled size range avoiding any agglomerated mass of vesicles while maintaining the multilamellar nature of vesicles. In case of niosomes, 2 min vortexing is sufficient to give a uniform size of 5 ± 1.5 μm. The vortexing and sonication process did not affect the degree of dithranol entrapment in liposomes and niosomes.

3.1.2. Influence of formulation component variables

All the liposomal and niosomal systems were added with a constant quantity (0.4 molar ratio) of negatively charged DCP. The inclusion of charger into the lipidic layers could avoid the aggregation and fusion of vesicles to maintain their integrity and uniformity. In addition to the charger, an antioxidant BHT (2% of total lipids) was added to each formulation to minimize the oxidative degradation of phospholipids leading to stability problems.

The studies of various liposomes consisting of different ratios of drug, PC and CHOL describe the effect of these variables on the degree of entrapment (Table 1). At a lower level of PC, the entrapment efficiency was found to be less (DLS 1 to DLS 4) and on increasing the same while keeping the other constituent, CHOL, at a constant level the entrapment efficiency could be enhanced up to 3.0 mg/240 mg of total lipids (DLS 6 and DLS 7). This may be accounted to the saturation of lipid domains with reference to drug where low PC content provides limited entrapment capacity (Patel and Mishra, 1999). An increase in CHOL concentration with the same DTH and PC concentration (DLS5, DLS6, and DLS8) led to an increase in the entrapment levels of dithranol from 2.0 mg/120 mg to 2.2 mg/140 mg to 3.0 mg/150 mg of total lipids. The increase in the entrapment efficiency is attributed to the ability of CHOL to cement the leaking space in the bilayer membranes, which in turn allow enhanced drug level in liposomes (Plessis et al., 1996). In DLS 7 although the total lipid level is higher, entrapment is quite less as compared with DLS 9 indicating that the dithranol entrapment is enhanced when PC and CHOL are in equal molar ratio. The latter indicates the importance of appropriate proportions of the two (PC and CHOL) to maximize the entrapment.

Table 2 summarizes the various surfactant and lipid concentrations and drug-entrapment efficiencies of the niosomal systems prepared. An increase in the Span 60 concentration while maintaining constant DTH and CHOL concentrations (DNS3, DNS2), led to an increase in the entrapment of the drug from 1.54 mg/174 mg to 1.86 mg/210 mg of total lipids. This increased entrapment may be attributed to the increase in the availability of lipophilic ambience, which can now accommodate the drug molecules to a higher extent. Similarly, the difference in the entrapment of batches DNS1 and DNS4 can be explained. The rise in CHOL level equal to that of Span 60

Table 3
Effect of DCP on entrapment efficiency of liposomal batch (DLS 9) and niosomal batch (DNS 4)

DTH:PC:CHOL: DCP	Entrapment level (mg)/total lipid (mg)
1:6:6:0.4	$3.51 \pm 0.027/180$
1:6:6:0.8	$3.55 \pm 0.065/180$
1:6:6:1.2	$3.60 \pm 0.045/180$
1:6:6:1.6	$3.62 \pm 0.034/180$
DTH–SPAN 60–CHOL–DCP	Entrapment level (mg)/total lipid (mg)
1:13.2:6.7:0.4	$1.97 \pm 0.027/210$
1:13.2:6.7:0.8	$2.00 \pm 0.054/210$
1:13.2:6.7:1.2	$2.03 \pm 0.074/210$
1:13.2:6.7:1.6	$2.05 \pm 0.043/210$

(DNS5 and DNS6) resulted in loss of entrapment efficiency. Further, increasing the CHOL level increases the entrapment of drug from 1.54 mg/174 mg (DNS3) to 1.97 mg/210 mg (DNS4) but with a further increase in this level of CHOL the entrapment is reduced. This deciphers that the CHOL level beyond a certain level starts disrupting the bilayered structure leading to loss of drug-entrapment levels (Redziniak and Perrier, 1996). Further, in order to study the effect of DCP levels on the liposomes and niosomes varying amount of DCP is added and the degree of entrapment determined. It was found that an increased concentration of charger does not affect the entrapment efficiency and size characteristics (Table 3).

3.2. Drug-leakage study from vesicles

The bar diagram between percent drug remaining in liposomes (DLS 9) and niosomes (DNS 4) at 15, 30, 45 and 60 days at 4, 25 and 37 °C has been represented in Fig. 2a and b. The other batch of niosomes DNS 2 has been withdrawn from the study because the drug leakage was significantly greater in this batch, which may be accounted to the amount of CHOL, insufficient for the structural integrity of niosomes. Both liposomes and niosomes have shown a fairly high retention of the drug inside the vesicles at a refrigerated temperature up to a period of one month ($\approx 90\%$). While, storage at high temperature (37 °C) lead to a substantial loss ($\approx 40\%$) of drug from liposomes and niosomes at the end of one month period (Fig. 2a and b); the drug leakage at elevated temperatures may be related to the degradation of lipids in the bilayers resulting in defects in membrane packing making them leaky. The drug-retention capacity of liposome has been shown to be higher than niosomes at all conditions. The better stability of liposomes over niosomes may be explained on the basis of the greater affinity of phospholipid bilayers towards the drug molecule and also due to their better structural integrity. The same has been corroborated by comparatively enhanced accommodating capacity of phospholipid bilayer systems as indicated by their better entrapment efficiency in comparison with niosomes (Tables 1 and 2).

3.3. Permeation study

3.3.1. Diffusion medium

Since the drug is poorly soluble in water (≤ 2 μg/ml), it needed to develop an appropriate medium that can provide sufficient solubility for the drug to maintain the required sink condition during permeation studies. Initially, an acetate buffer saline at pH 3.3 was selected which has shown a better stability of dithranol (Wang et al., 1987). Further, addition of 0.5% EDTA, 0.5% sodium metabisulphite and 1% ascorbic acid was found to protect the drug from photolytic and oxidative degradation. The method of solubility enhancement for dithranol was also reported earlier (Cafaggi et al., 1998; Delneuville et al., 1998). In the present study to solubilize the drug, a surfactant Cremophor RH 40 was selected and taken in varying concentrations with and without different amount of methanol (Table 4). It has been found that on increasing the surfactant concentration and inclusion of methanol improved the solubility. A medium with 20% methanol and 1% Cremophor RH 40 gave a solubility of 0.157 mg/ml which was considered to be sufficient for the permeation study. An amount of 2.5 mg of dithranol was taken into the donor compartment and the volume of the receptor medium used was

150 ml. This permeation medium served the purpose as it had approximately ten times more capacity to solubilize the amount of drug added.

3.3.2. Diffusion study

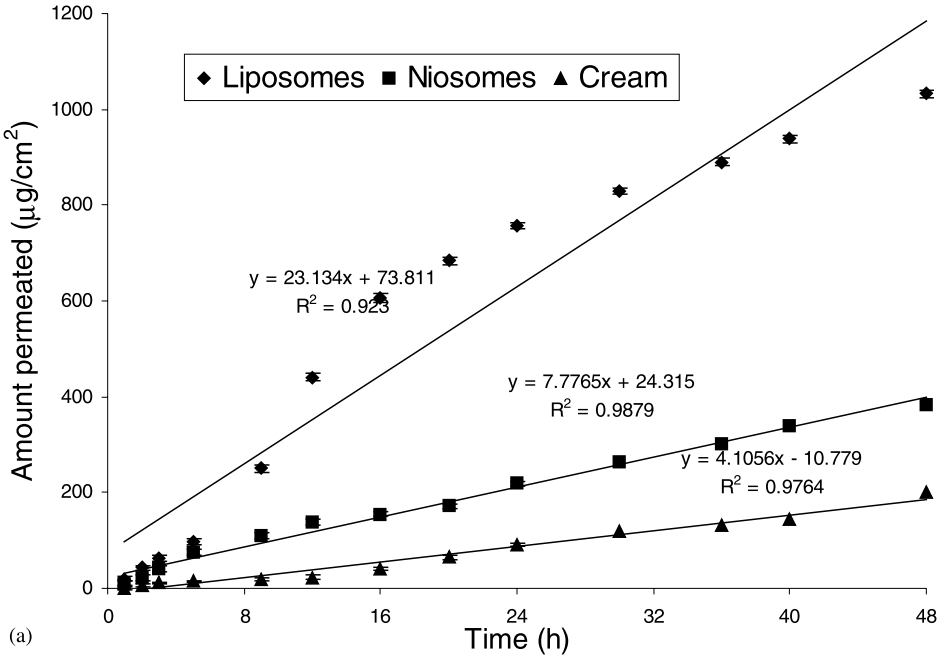
Fig. 3a shows the mean cumulative amount of drug released per unit surface area from cream and optimized batches of liposome and niosomes. The drug-flux was obtained by plotting the cumulative amount of dithranol in the receptor phase per square centimeter against time. Fig. 3b depicts the permeation flux of dithranol from liposomes (DLS 9), niosomes (DNS 4) and cream. The permeation rate constants, 23.13 μg/cm^2/h with liposomal and 7.78 μg/cm^2/h with niosomal systems in comparison with 4.10 μg/cm^2/h obtained for the cream base clearly indicate the enhancing effect of vesiculation on drug permeation and penetration on the skin. Both the liposomal and niosomal encapsulation of dithranol exhibited enhanced permeation through the mice skin in comparison with the conventionally prepared cream base formulation of dithranol. The improved skin penetration of drug and the consequently enhanced drug-transport abilities through liposomes and niosomes can be explained on the basis of the presence of drug molecules in a solubilized state. The later is achieved in an ambience of aqueous and non-aqueous phases of bilayered systems, most ideally suited for drug penetration (Whitefield, 1981). The prevailing hydrodynamic condition provides proper ground for better drug–skin partitioning. Further, the permeation of dithranol is observed to be more pronounced in case of liposomes vis-a-vis niosomes. The higher permeation of dithranol through mice skin in case of liposomes proves its merit over niosomes. This reflects the impact of composition of vesicles that are otherwise structured alike (i.e. MLVs). The liposomal phospholipids (natural constituent of skin lipids) proved to be better in generating and retaining the required physico-chemical state of the skin for enhanced permeation. This may be attributed to their ability to vesiculate independently as the phopholipids carry two bulky nonpolar lipid chains and a polar head group, which helps in its spontaneous structuring into closed bilayered systems (Cevc, 1996; Vrhovnik et al., 1998). Moreover, it results in their capacity to produce long lasting effect. Niosomes may serve well in the initial stages but not better than liposomes as their constituents, i.e. non-ionic surfactants are not inherently equipped to meet the demand of such spontaneity of vesiculation. They require other components like cholesterol for CPP (critical packing parameter) to acquire the shape, and once deformed during the penetration process may not regain the same required structures. This explains that niosomes are comparatively less efficient in sustaining the suitable skin status for drug transfer.

Table 4
Solubility study of dithranol using Cremophor RH 40 and methanol for selection of permeation medium

Solvent	Solubility (mg/ml)
Cremophor RH 40 0.5%	0.072
Cremophor RH 40 1.0%	0.121
Cremophor RH 40 1.5%	0.149
Cremophor RH 40 2.0%	0.181
Cremophor RH 40 1.0% and methanol 20%	0.157
Cremophor RH 40 2.0% and methanol 20%	0.251
Methanol	1.32

4. Conclusions

Entrapment of dithranol in liposomal and niosomal systems could be achieved after studying the effect of various process and formulation variables. The amount of drug loaded into these vesicles was in the range of 0.97 mg/70 mg to 3.51 mg/180 mg of total lipid. However, so much entrapment could suffice the amount of dithranol desired for the formulation purpose and comparable with the drug contents in commercial dosage forms. These systems have been found to be reasonably well in their size and stability characteristics and exhibited the improved permeation properties. The in vitro permeation study indicates that dithranol in exquisite amphiphillic atmosphere of closed lamellar system has an



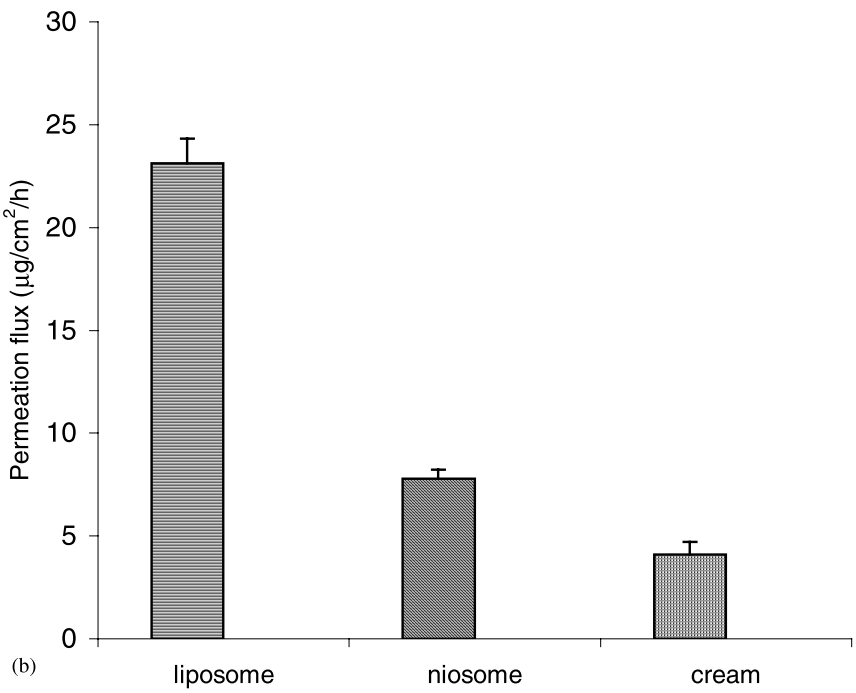


Fig. 3. (a) Diagram depicting the regressed lines between amount of dithranol permeated and the time for the optimized batches of liposomes (DLS9), niosomes (DNS4) and drug in cream base. (b) Bar diagram depicting the flux of dithranol permeated from the liposomal, niosomal and cream formulations.

enhanced access to deeper skin layers. The results obtained in this study reveal the merits of developed dithranol-loaded liposomes and niosomes and justify their potential in strengthening the efficacy and safety of the drug.

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