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RESEARCH

LIPOSOMAL GELS AS CARRIERS FOR SAFER TOPICAL DELIVERY OF TAZAROTENE

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ABSTRACT

The aim is to design a liposomal delivery system for topical administration of tazarotene capable of providing controlled and localized release of the encapsulated drug in order to minimize adverse effects associated with its topical use. Tazarotene loaded liposomes were prepared and characterized for entrapment efficiency, particle size and stability. Tazarotene liposomal gels were formulated and evaluated comparatively with commercial gel with respect to primary skin irritation and skin permeation . The effect of vesicular incorporation of tazarotene on its accumulation into hairless rat skin from liposomal suspension and gels were studied. The results of the study showed that the maximum entrapment efficiency recorded (93.49%±0.25) was achieved by formula having 1:1 lipoid S 100 to cholesterol molar ratio. The mean particle size of liposomal formulae ranged from (5.68µm-0.72µm). The stability profile of the selected system assessed for 90 days at refrigerated temperature showed no sign of sedimentation or color change with (86.27% ±0.44) of drug retained. All liposomal suspension and gels revealed higher drug retention compared to commercial gel, lesser skin irritancy and greater skin tolerance was also observed.

1 INTRODUCTION

Tazarotene is a third generation retinoid which is effective for topical treatment of psoriasis and acne vulgaris and has cosmetic benefits for photoaging [(1)]. However there are adverse effects accompanied with its use where local cutaneous irritation including burning, itching, erythema, peeling or dryness occurs in approximately one-quarter of patients using tazarotene [(2, 3)]. In comparative trials 0.1% tazarotene gel demonstrated the highest irritation score followed by tazarotene cream compared to different concentration of tretinoin cream [(4)]. In a recent study, tazarotene foam 0.1 % was found to be an alternative to tazarotene gel with less systemic exposure [(5)]. Therefore, the development of new effective topical drug delivery system intended to modulate tazarotene release rate, enhance its localization in the skin and reduce its percutanous absorption might minimize its adverse effects and could be of particular usefulness.

Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of phospholipids with or without some additives [(6)]. Among a variety of drug lipid carriers, liposomes seem to have the best potential as localizers of topically applied drugs [(7)].

It was reported that conventional liposomes only enhanced skin deposition, with mostly reduction effect on percutaneous permeation or systemic absorption of drugs with better drug localization at site of action [(8)]. The aim of this work is to minimize the adverse effects associated with tazarotene application on the skin. Incorporating tazarotene in suitable liposomal formulation may modify its diffusion parameters in the skin and hence reduce its systemic absorption and consequently its adverse effects. Also, its effectiveness could be improved by maximizing its accumulation into the skin.

2. MATERIALS AND METHODS

2.1. Materials

Soy phosphatidylcholine (Lipoid S 100) and cholesterol were obtained from Medical Union Pharmaceuticals, Egypt, tazarotene was provided by Marcyrl Pharmaceutical Industries, Egypt. Spectra/Pore dialysis membrane, 12,000 -14,000 molecular weight cut off was

purchased from Spectrum Laboratories Inc., USA. Isopropyl myristate minimum 90% GC was purchased from Sigma –Aldrich, USA. Carbopol 934, B. F., was from Goodrich Chemical Company, USA. Hydroxy propyl methylcellulose (HPMC) powder was purchased from Tama, Japan. Acnitaz is a commercial product produced by Marcyrl Pharmaceutical Industries, Egypt. All other reagents were of analytical grade.

2.2. Preparation of liposomes

Six different liposomal formulae of tazarotene shown in Table (1) were prepared by thin film Method [9]. Precisely, Lipoid S 100 and tazarotene (as well as cholesterol if added) were dissolved in chloroform: methanol mixture (2:1, v/v). The organic solvent system was slowly removed under reduced pressure, using a rotary evaporator, at (40°C) such that a very thin film of dry lipid was formed on the inner surface of the flask. The dry lipid film was slowly hydrated with 10 ml phosphate buffer saline (PBS) at pH 5.5.

Table 1
Composition of tazarotene liposomes

Formulae	Compostion		
	Tazarotene (mg)	**Lipoid S 100 (molar ratio)**	**Cholesterol (molar ratio)**
F1	10	10	0
F2	10	9	1
F3	10	7	2
F4	10	7	4
F5	10	7	6
F6	10	1	1

2.3. Characterization of liposomes Determination

Liposomes size distribution was determined by light scattering based on laser diffraction using LA 920 laser diffraction particle size analyzer (Horriba, Japan).

2.3.2. Encapsulation efficiency determination

The tazarotene containing liposomes were separated from the unentrapped drug by centrifugation at 13000 r.p.m. for one hour and at (2°C) using 3K 30 cooling centrifuge (Sigma,U S A). The cake, thus formed, was washed with 10 ml phosphate buffered saline and recentrifuged again for one hour under the same conditions.The liposomes were then lysed with methanol and sonication. The drug contents were then determined spectrophotometrically at 352 nm using UV- 1650 P.C spectrophotometer (Shimaduzu, Japan). The entrapment efficiency was calculated on the basis of drug incorporated to the total amount of drug applied [10].

2.3.3. Storage stability study

Samples of tazarotene liposomal suspension were stored at refrigerated temperature (4±2 °C) and room temperature (25±2 °C) for 90 days. The stored liposomal formulae were assessed for any signs of color change, sedimentation and drug leakage.

2.4. Preparation of gel

In order to adjust the rheological properties of liposomal suspension to facilitate its application by the patients [11], tazarotene liposomal suspension (F3) was incorporated into different gel bases as listed in Table (2).

Table 2
Composition of tazarotene liposomal gels

Gel name	Composition	
	Gelling agents	**% of gelling (W/W)**
Gel 1	Carbopol P 934	0.5
Gel 2	Carbopol P 934	1
Gel 3	HPMC	2
Gel 4	HPMC	4

Carbopol P 934 gel bases were prepared by dispersing gelling agent in buffer solution (pH 5.5), stirring for 24 hr at room temperature and then adding triethanlamine up to pH 7. The resulting gel was stored in capped glass containers, at 4 °C, in the dark. Tazarotene loaded liposomal formula (F3) was mixed into Carbopol base to obtain final drug concentration of 0.1% w/w in the gels [12].

Hydroxy propyl methyl cellulose gel bases (HPMC) were prepared by dissolving weighted amount of HPMC gradually in buffer solution (pH=5.5) by the aid of magnetic stirrer. The mixture is then stirred with magnetic stirrer at medium speed till the formation of gel base then left overnight for equilibration. Liposomal gel formulations were prepared by mixing the tazarotene liposomal formula (F3) with the gels in order to have a final tazarotene concentration of 0.1% w/w in the gels.

2.5. In vitro permeation studies through artificial membrane

Permeation studies of tazarotene from different liposomal formulae through artificial membrane were performed for 9 hr at 37±1°C, using 20 ml capacity plastic syringe, where its outer tube was cut smooth to whole diameter near the

nozzle, the effective permeation area was 3.14 cm^2. A cellulose nitrate membrane impregnated with isopropyl myristate as lipid phase was used as artificial lipophilic membrane simulating the epidermal barrier [13] . The cellulose membrane was fixed around the plastic syringe with a rubber band and its edges were sealed with an adhesive tape. The receiver compartment 50 ml consisted of a hydro-alcoholic solution [1:1 methanol to phosphate buffered saline pH=7.4] in the presence of 0.05% sodium lauryl sulphate to achieve sink conditions [14]. The temperature of the buffer system was maintained at 37 ± 0.5 °C by placing the beaker, containing 50 ml hydro-alcoholic solution of pH 7.4, with the hanged syringe in a water bath placed over a thermostatically controlled magnetic stirrer. The donor compartment was filled with 1 ml of liposomal suspension (0.1% tazarotene) or control preparation (plain liposomes). A commercial gel Acnitaz was used as control. Every hour a sample of 3ml was withdrawn from the receptor compartment and spectrophotometrically assayed for drug content at 352 nm using plain permeation media as a blank. The sample was replaced with an equal volume of fresh receptor medium. Experiments were performed in triplicate.

2.6. In vitro permeation studies through rat skin

The experiments were carried out using the whole skin of hairless rat skin. The hair was shaven using a mechanical hair clipper, without damaging skin then it was frozen at −18 °C. Before the experiments, the skin was hydrated for 2 hours in buffer solution of pH 5.5 simulating pH of the skin at room temperature [15]. In vitro skin permeation study was performed by using, 20 ml capacity plastic syringe. Its outer tube was cut smooth to whole diameter near the nozzle, with an effective diffusion area of 3.14 cm2. A circular piece of hairless rat, previously soaked in the buffer solution at pH=5.5, was then stretched around the cut end touching the liposomal suspension with the dermal side in direct contact with the receptor medium. The skin was fixed around the plastic syringe with rubber band and its edges were sealed with an adhesive tape. Accurately 1ml of liposomal suspension (0.1% tazarotene) or control preparation (plain liposomal suspension) was introduced into the tube from the top. A Commercial gel, Acnitaz was used as a control. The whole plastic syringe was hanged into 50 ml of [1:1 methanol to phosphate buffered saline] (pH=7.4) in the presence of 0.05% sodium lauryl sulphate to achieve sink conditions at 37 ± 0.5 °C, so that the cut end of syringe, covered with hairless rat skin, is in the center of the solution.

The temperature of the buffer system was maintained at 37 ± 0.5 °C by placing the beaker, containing 50 ml hydro-alcoholic solution of pH 7.4, with the hanged syringe in a water bath placed over a thermostatically controlled magnetic stirrer. The solution in the receptor was stirred magnetically at 150 rpm throughout the time of the diffusion studies. Before starting the experiment the donor cell was sealed with parafilm. Aliquots of 3 ml samples were withdrawn every hour for 9 hours then immediately analyzed by UV spectrophotometer at 352nm against a blank prepared without the drug. The samples were replaced with equal volumes of fresh receptor medium and the correction for the cumulative dilution was calculated. Experiments were performed in triplicate. The cumulative amount of drug transferred into the receptor side was calculated.

experiments 2.7

Data analysis and statistics of permeation

In vitro steady-state drug fluxes (μg/h cm^2) were calculated by least square linear regression analysis from the linear portion of the cumulative amount of drug diffused versus time plots. The permeability coefficients (K_p, cm/h) of the drug were determined by dividing the drug fluxes by the initial concentration of the drug in the donor phase. Results are expressed as means ± standard deviation (n = 3 independent samples). The significance of the differences between different formulations was tested using the one-way analysis of variance (ANOVA), followed by the Student–Newman–Keuls multiple comparison post test (Graph Pad Prism, Version 3). The differences were considered statistically significant when $P < 0.0001$.

2.8. Skin retention of tazarotene

At the end of the permeation study, the skin was removed and the surface was washed three times with PBS (pH=5.5). The skin samples were each soaked in a flask with 20 ml of methanol for 24 h. Then the methanolic samples were shaken in an ultrasound bath four times for 30 min each, in order to extract all the amount of drug accumulated in the skin pieces [16]. The methanolic solution was then filtered and analyzed by UV spectrophotometer at 352 nm against a blank.

2.9. Skin irritancy study

The study was carried out using parallel group design where rabbits weighing 2-2.5 kg were divided into four groups (each gp, n=3):

1-Group 1: Marketed formulation (Acnitaz).

2-Group 2: Liposomal gel containing tazarotene.

3-Group 3: Liposomal gel without tazarotene (Placebo gel).

4-Group 4: No application (control).

The animals were housed in an air-conditioned room (20 ° C) and the back of the rabbits were clipped free of hair 24 hour prior to application of the formulations, and the skin was observed for any visible change such as erythema for the next five days. Evaluation was carried out using the scale reported by Mandawgade and Patravale [17].

3. Results and discussion

3.1. Size distribution and entrapment efficiency

The particle size of tazarotene liposomes showed a corresponding decrease with the addition of cholesterol as shown in Table (3). This can be explained by the fact that the addition of cholesterol to the phospholipids film causes the membrane to be more compact [18].

Table 3
Diameter and entrapment efficiency (%) of the prepared tazarotene liposomes

Formulae	Entrapment efficiency (%)	Average size (μm± S.D.)
F1	72.60±0.46	5.68±1.71
F2	79.74±0.42	3.68±1.73
F3	82.75±0.12	1.85±0.65
F4	86.68±0.28	1.42±0.58
F5	90.78±0.18	1.02±0.44
F6	93.49±0.25	0.71±0.36

Values represent mean ± SD; n=3

Regarding encapsulation of tazarotene, it was found that entrapment efficiency increased linearly with the addition of cholesterol, where the maximum entrapment efficiency of tazarotene recorded (93.49% ±0.25) was achieved by F6 (1:1 Lipoid S 100 to cholesterol molar ratio). This was explained on the fact that cholesterol increased the microviscosity of the liposomal bilayers, lowered leakage of tazarotene from liposomes and hence led to higher drug retention [19].

3.2. Storage stability study

Liposomal formulation stored at room temperature showed gradual physical instability manifested by partial sedimentation and yellowish discoloration after 90 days. On the other hand liposomal formulation stored at refrigerated temperature showed no sign of sedimentation or color change. The stability profile of tazarotene loaded liposomal formulation evaluated for substantial loss of drug at various temperatures as shown in Fig. (2), suggested that liposomal formula should be stored at refrigerated temperature 4±2 ^{o}C to achieve optimum conditions for storage because at elevated temperature greater drug loss from the system was observed. This might be ascribed to the effect of temperature on the gel-to liquid transition of lipid bilayer together with possible chemical degradation of phospholipids leading to defects in the membrane packing [20].

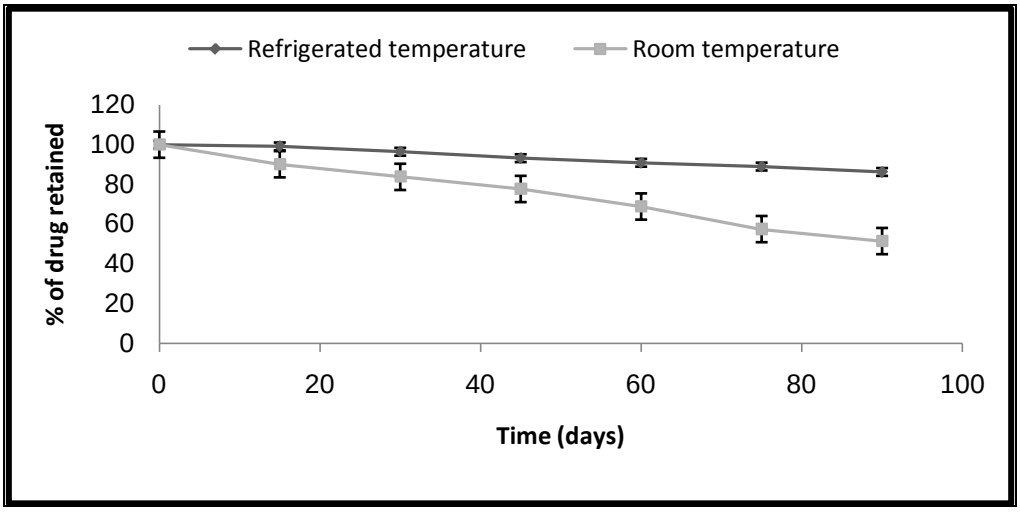

Fig.2. Extent of tazarotene leakage from its selected formula (F3) at different temperatures. Values represent mean ± SD; n=3

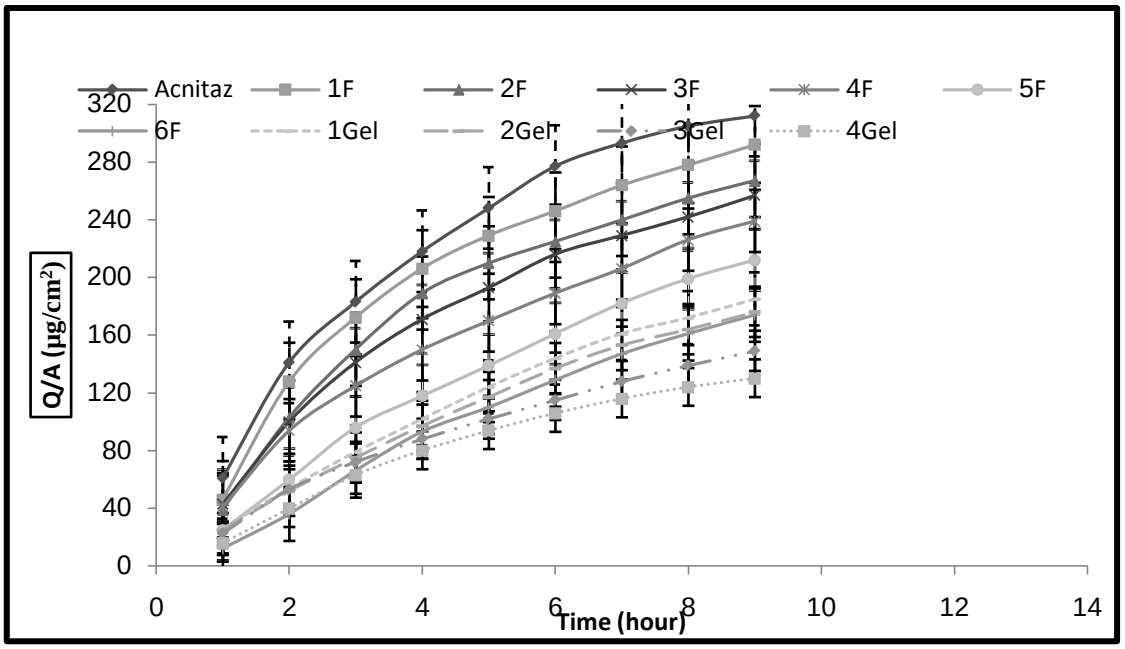

Fig.3. Permeation profiles through artificial membrane of tazarotene from different formulae. Values represent mean ± SD; n=3

3.3. In vitro permeation studies through artificial membrane

The results of permeation studies across artificial membrane of tazarotene from different liposomal dispersions and liposomal gels are compared to commercial gel (Acnitaz) and listed in Table (4) in terms of (K_p), while the corresponding drug permeation profiles are shown in Fig.(3). It can be observed that the release from all liposomal formulations (dispersion and gels) was slower ($p<0.001$) than commercial gel. This may be due to the formation of drug depot in the liposomes formulae which resulted in sustained and localized release of the drug [21]. The higher lag time obtained from liposomal formulations than commercial gel may be ascribed to the depot formation and selective drug lipid partitioning which presumably could retard permeation of the drug [22]. The release from liposomal dispersion was mainly dependant on cholesterol molar ratio as increasing cholesterol molar ratio led to subsequent decrease in release from liposomes, this can be explained by the fact that the presences of cholesterol in the bilayer modulate membrane fluidity by restricting movement of relatively mobile hydrocarbon chain, reducing bilayer permeability, decreasing efflux of encapsulated drug, resulting in prolonged drug retention [23]. The incorporation of drug in liposomes resulted in delayed release which was further delayed in case of liposomal gel due to formation of an additional diffusion barrier to drug release [24].

Table 4: Permeation parameters of tazarotene through artificial membrane from different formulae

Formulae	K_p (cm/hr)	Lag time (min.)
Acnitaz	0.041±0.0022	6 ± 1.1
F1	0.038±0.0036	12± 1.5
F2	0.036±0.0010	15± 1.4
F3	0.033±0.0026	15± 1.5
F4	0.028±0.0038	18± 2.0
F5	0.026±0.0027	21± 1.7
F6	0.023±0.0010	33± 1.7
Gel 1	0.019±0.0009	18± 1.2
Gel 2	0.018±0.0010	18± 2.0
Gel 3	0.015±0.0008	21± 2.0
Gel 4	0.013±0.0006	27± 1.2

Values represent mean ± SD; n=3

3.4. Permeation studies through rat skin

Permeation studies across rat skin were performed on the same formulations examined in the previous studies with artificial lipophilic membrane, under same conditions. The results are summarized in Table (5) in terms of permeability coefficient (K_p), and the corresponding permeation profiles are presented in Fig.(4). As a general observation, a reduced drug permeation rate was noted, due to the more complex permeation process through the rat skin than through the artificial membrane. Longer lag time was observed in skin permeation studies compared to artificial membrane. This can be explained that longer time is necessary to saturate the skin and reach pseudo-steady state flux condition between donor and receiver compartment. As stated before the commercial gel gave higher amount of permeated tazarotene than liposomal formulation. This was due to the formation of drug depot in liposomes formulae which resulted in sustained and localized release of drug. On comparing skin permeation data of liposomal gels and those obtained from liposomal suspension (F3), we can conclude that the amount of tazarotene permeated through the skin from all the liposomal gels formulations tested was significantly lower ($P < 0.0001$) than that released from the same liposomal suspension through skin. This is due to presence of an additional diffusion barrier to drug release in liposomal gel formulations [24]. These results are in agreement with Mura et al. [22] who observed that the presence of the polymeric network in gels led to a slower drug release and lower permeation rate from liposomal gels compared to liposome dispersions.

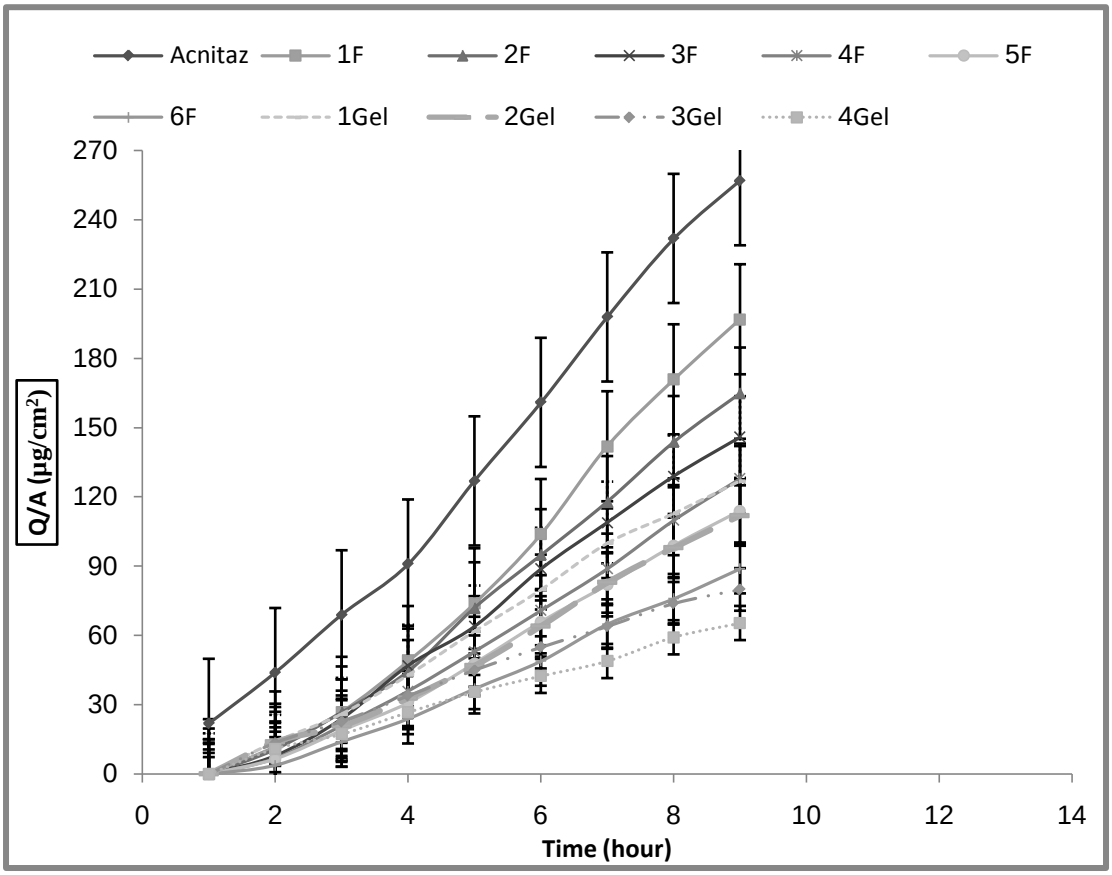

Fig.4. Permeation profiles through rat skin of tazarotene from different formulae.

Table:5

Permeation parameters of tazarotene through rat skin from different formulae

Formulae	K_p (cm/hr)	Lag time (min.)
Acnitaz	0.031±0.0015	24± 1.6
F1	0.027±0.0025	78± 2.1
F2	0.022±0.0011	84± 3.0
F3	0.020±0.0010	90± 1.2
F4	0.017±0.0016	96± 1.7
F5	0.015±0.0009	96± 2.5
F6	0.012±0.0010	108± 1.5
Gel 1	0.014±0.0010	93± 1.5
Gel 2	0.014±0.0013	93± 1.9
Gel 3	0.009±0.0003	99± 1.7
Gel 4	0.007±0.0004	105± 2.0

Values represent mean ± SD; n=3

Table 6

Tazarotene accumulated in skin after 9 hours from different formulae

Formulae	Tazarotene accumulated (μg/cm^2)
Acnitaz	24.75±0.29
F1	66.09±0.44
F2	61.84±0.60
F3	57.37±0.79
F4	52.77±0.65
F5	47.11±0.32
F6	45.09±0.31
Gel 1	45.55±1.39
Gel 2	43.78±1.82
Gel 3	40.62±0.62
Gel 4	33.08±0.86

Values represent mean ± SD; n=3

3.6. Skin retention of tazarotene

Higher amount of tazarotene was retained in the skin in case of all liposomes formulae compared to commercial gel as shown in Table (6). These results are in agreement with Masini et al [25] studying in vitro permeation through hairless mouse skin of radio labeled retinoic acid (RA) from liposomal suspension and gel formulation. They reported that RA absorption was higher from the gel but the percentages of drug found in the epidermis and dermis were higher from liposomal formulation which, therefore, affected RA skin distribution. Regarding liposomal gels, the amounts of tazarotene accumulated in the skin after 9 hours from liposomal gels were significantly lower (P <0.0001) than in case of liposomal suspension (F3).This is due to presence of an additional diffusion barrier to drug release in liposomal gel formulations. These results are in accordance with Krajiˇsnik et al [26] who found that liposome dispersions delivered higher temoporfin amounts to SC and deeper skin layers than liposomal gels. Also Padamwar and Pokharkar [27] found that there is a decrease in the skin deposition of drugs from gels as compared to the liposome dispersion, which might be due to increased viscosity of gels which retards the release from their structure.

3.7. Skin irritancy study

The skin-irritation studies indicated that liposomes-based tazarotene gel (Carbopol 1% liposomal gel) resulted in a considerably less irritation as compared to marketed tazarotene formulation (Acnitaz).Thus, liposomal-based gels demonstrated remarkable advantage over marketed formulation in improving the skin tolerability of tazarotene indicating their potential in improving patient acceptance and topical delivery of tazarotene. Cumulative applications of the liposomal gel as shown in Table (7) and illustrated in Fig. (5) revealed less skin irritancy (mean cumulative score = 0.66 ± 0.57) compared to marketed formulation (mean cumulative score = 3.66± 0.57) on intact rabbit skin; (p <0.0001). These results are in accordance with Contreras et al [(28)] who correlated the reduction in the retinoic acid permeation from liposomal gel with the reduction in the adverse effects of the drug. They accord also with Patil et al [(29)] who considered the liposomes as good strategy in improving the topical delivery of the drug by enhancing the dermal localization with a concomitant reduction in side effects.

Table 7 Mean cumulative irritancy score of different formula

Formulae	Mean cumulative irritancy score
Acnitaz	3.66 ± 0.57
Gel 1	0.66 ± 0.57
Control	0.00 ± 0.00
Placebo gel	0.33 ± 0.57

Values represent mean ± SD; n=3

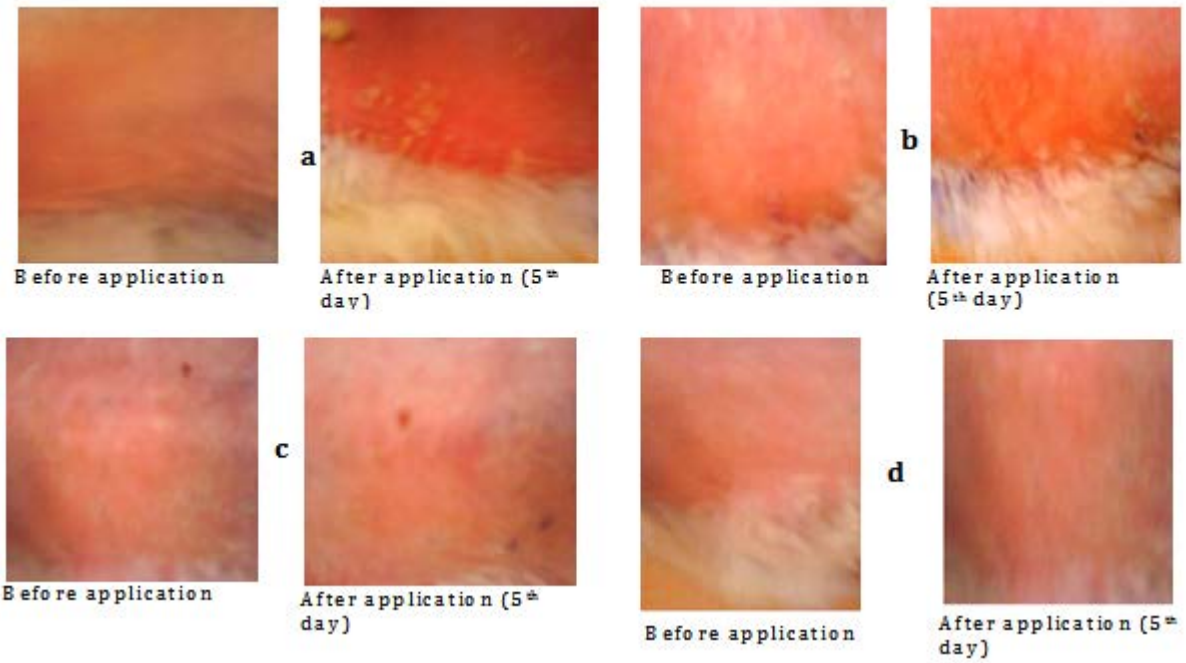

Fig.5. Monitoring irritation progress following various tazarotene formulations compared to placebo and control.
(a) commercial gel (Acnitaz). (b) liposomal gel.
(c) control. (d) placebo gel (plain liposomal gel)

Conclusion

Overall results obtained during this work have shown that liposomes may be an interesting carrier for tazarotene in skin disease treatment, where greater drug retention and reduced percutanous absorption were observed leading to less irritancy and better drug tolerance compared to commercial gel . Liposomes formulae should be stored at refrigerated temperature to minimize the drug leakage from the vesicles and avoid any physical change in the liposomes as sedimentation or color change. The release of tazarotene from liposomes dispersions was mainly dependant on lipid composition where the vesicle size showed no influence. Regarding liposomal gels, the amounts of tazarotene permeated through or accumulated in the skin after 9 hours were significantly lower ($P < 0.0001$) than in case of liposomal suspension.

Declaration of Interest

The authors report no declaration of interest.

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