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LECITHINISED MICROEMULSIONS FOR TOPICAL DELIVERY OF TRETINOIN

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ABSTRACT

Tretinoin is indicated for the management of acne, photoaged skin, psoriasis and other skin disorders and also for severe conditions like acute promyelocytic anaemia and squamous cell carcinoma of the skin. The potential of tretinoin in these conditions is limited due to want of a proper delivery vehicle. In order to develop alternate formulations for the topical administration of tretinoin, lecithinsed microemulsions were prepared and evaluated as delivery vehicles. These systems were prepared using phosphate buffer pH 5.5, isopropyl myristate, tween 80 and ethanol. The microemulsions were characterized using TEM. The ability of the system to deliver tretinoin into and through the skin was evaluated in vitro using the skin of laca mice. The in vitro permeation data showed that the novel microemulsions increased tretinoin penetration through the skin; higher flux (33.92 µg / cm² / hr) was obtained with microemulsion formulation and microemulsified gel (31.54 µg / cm² / hr) in comparison to the plain drug solution (22.33 µg / cm² / hr), plain drug in gel (28.67 µg / cm² / hr) and the marketed preparation (24.28 µg / cm² / hr). These results were supported by skin retention study and it was noted that the maximum amount retained was with microemulsified gel (96.28 µg / cm²) and non-gel (82.13 µg / cm²) respectively. The other systems; drug in solution, drug in gel (conventional) and marketed preparation were able to retain the drug at the level of 32.4 µg/ cm², 21.54 µg / cm² and 29.32 µg / cm² respectively. These results suggest that the studied microemulsions may be appropriate vehicles for topical delivery of tretinoin.

Keywords: *Tretinoin, microemulsions, acne, topical delivery, lecithin*

Introduction

Microemulsions have generated considerable interest over the years as potential drug delivery systems [1,2]. Formulations based on microemulsions have several characterstics viz., enhanced drug solubilization, good thermodynamic stability and ease of manufacturing [3,4]. Microemulsions are versatile systems and can be used to deliver drugs via several routes. These systems have been extensively studied for topical administration [5]. As topical vehicles, microemulsions can increase the local or systemic delivery of a drug by different mechanisms [4,6]. The existence of microdomains of different polarity within the same single phase solution enables both water soluble and oil soluble materials to be solubilized. The diffusional barrier of the skin may be modified depending on the composition of microemulsion. Also an increased thermodynamic activity of the drug may favor its partitioning in the skin.

In this work, the ability of microemulsion system to incorporate and deliver tretinoin to and into the skin has been investigated. Tretinoin, a retinoid, commonly referred to as vitamin A acid is a widely used drug in the topical treatment of acne, photoaged

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skin, psoriasis and other skin disorders. Topical tretinoin therapy has been reported to be superior in treatment of ichthyosis, erythroderma and pityriasis rubra pilaris in comparison to application of salicylic acid [7]. Chemically, tretinoin is all – trans retinoic acid.

Topical treatment with tretinoin has been used with varying success in wide range of cutaneous conditions [8]. Its use in acne is well established and is also reported in rosacea, keratinization disorders like darier's disease, and in pigmentation disorders like chloasma [28]. Tretinoin can reverse some of the skin changes associated with chronic exposure to sun light. However the effects seen with tretinoin are transient and the skin reverts to its pre treatment state once the application is stopped. Tretinoin exerts its action through two types of nuclear receptors – retinoid acid receptor (RAR) and retinoid – x- receptor (RXR) [9] belonging to the thyroid / steroid superfamily of ligand dependent nuclear transcription factors. These receptors exist as three distinct gene products α, β, γ. Tretinoin binds to RAR α that predominates in human epidermis. The expression of RAR β may also be induced by Tretinoin [10].

Unfortunately the potential of tretinoin in the said conditions is limited due to want of a proper delivery vehicle. Besides delivery; skin irritancy, poor aqueous solubility and photostability of tretinoin are some of the difficulties to deal with. The hitherto adopted formulation practices do not address these issues and fail to provide desired protection to drug molecule as well as transport. Thus in the light of these drug and dosage form restrictions, there has been a quest to develop drug delivery strategies in order to make topical delivery of drug more safe and meaningful. Many vescicular colloidal drug delivery systems like liposomes, niosomes, nanoparticles etc have been explored to obtain a formulation system with desired properties. Amongst these, microemulsions have been considered as promising careers to help place tretinoin molecule onto the target site thereby improving efficiency. The presence of phospholipids over the microstructures formed is an added advantage.

2. Materials and Methods

2.1 Materials

Tretinoin was obtained as a gift sample from Psycho Remedies, Ludhiana. Isopropyl myristate, Tween 80 and span 80 were purchased from S D Fine chemicals Ltd., Mumbai. Lecithin (Phospholipid) was purchased from Nattermann Phospholipids, Germany; Isopropyl alcohol and n-Octanol from E-Merck (India) Ltd and dehydrated ethanol from Bengal Chemicals Ltd., Kolkata respectively. All the products were used as received.

2.2 Determination of Solubility

Tretinoin was added in excess to a fixed volume of water, acidified IPA, methanol, ethanol, IPM and IPA: phosphate buffer pH 5.5. All the samples were shaken for 24 hours at room temperature and filtered through 0.22 microns filter. After suitable dilution with suitable solvents, analysed spectrophotometrically at their respective λmax. Amount of drug and solubility was determined using $E_{1cm}^{1\%}$ in the respective solvents.

2.3 Determination of Partition Coefficient

Partitioning of tretinoin was determined in

- Octanol and water system
- Isopropyl myristate and buffer system (pH 5.5) by calculating log P values in respective systems

The two phases were mutually saturated before use by taking them together in 1:1 ratio and shaking overnight on shaker at 37ºC for 24 hrs. The phases were than separated by centrifugation at 2000 rpm for 10 min. Further, final isolation of the two phases was carried out using separating funnel. 50 mg of tretinoin was added to a mixture of 5 ml of pre-saturated organic phase i.e. isopropyl myristate or octanol and 10 ml of presaturated phosphate buffer in 25 ml conical flasks. These conical flasks were rotated at 37ºC for 24 hrs in a water bath shaker. The two phases were then separated by centrifugation at 100 rpm for 10 min and the

concentration of tretinoin in the IPM layer was determined by UV spectrophotometric analysis after suitable dilution. The partition coefficient of the drug 'P' was calculated using the following formula:-

$$P = \frac{C_o V_w}{C_w V_o}$$

where P= partition coefficient

C_o=concentration of drug in organic phase

C_w=concentration of drug in aqueous phase

V_o=volume of organic phase

V_w= volume of aqueous phase

2.4 Composition of Microemulsions

All microemulsions were formulated with double-distilled water to avoid surface active impurities. The appropriate amount of aqueous phase, isopropyl myristate (IPM), lecithin, tween 80 and absolute alcohol were weighed into 10 ml glass tubes. Micremulsions were prepared by vortexing the mixtures vigorously till clear microemulsions were obtained. Drug loaded micremulsions were prepared by adding tretinoin to the oil phase before addition of aqueous phase and vortexing. Microemulsions and solutions containing tretinoin were protected from light by storing in dark – brown bottles wrapped with aluminium foil.

The choice of IPM as the internal phase of the microemulsion was based on the following consideration. With respect to its safety, IPM is regarded to be a very low toxic excipient suitable for its use as vehicle for many transdermal pharmaceutical dosage forms. It is included within the list of permitted additives of food products intended for human intake (FDA) and it has been demonstrated that it does not possess any significant risk in general terms, based on IPM's lack of carcinogenicity, mutagenicity and low acute toxicity from oral, dermal, inhalation or parentral toxicity studies. In addition, IPM is likely metabolized to isopropyl alcohol which is a class III solvent (low risk) according to ICH Guideline on Residual solvents, and myristic acid, which is an edible fatty acid, usually found in animals.

Lecithin and polysorbate 80 were chosen as surfactant combination in the formulation of microemulsion for topical use due to the safety consideration. Lecithin is a biocompatible zwitterionic naturally obtained surfactant and both lecithin and polysorbate 80 have been described as very low to non-toxic excipients appropriate for pharmaceutical formulation [11].

2.5 Characterization of microemulsion systems

In order to characterize the obtained systems more accurately; TEM, mechanical stress, drug carrying capacity and drug loss studies were carried out [29-31].

2.6 Penetration studies using human skin

In vitro permeation experiments were performed using skin removed from laca mice sacrificed by spinal disclocation[13-15]. The skin was dehaired and immediately frozen at -18^{o}C until further use. The day before the experiment, the skin was placed overnight in a refrigerator at 4 ^{o}C. The subcutaneous fat was carefully removed and the skin cut into 3x3 cm^2 pieces. The skin pieces were clamped between the donor and the receiver chambers of vertical Franz diffusion cells (7.14cm^2), the stratum corneum was kept towards the donor compartment. Donor solutions consisted of 1ml of either plain drug in solution, drug in plain gel, drug in microemulsion, drug-microemulsified gel and marketed drug preparation (gel). The acceptor compartment was filled with acidified IPA: phosphate buffer pH 5.5 (2:1) using a circulating water bath. At 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 24.0 hours, sampling was done through sampling port replacing the volume of the buffer in the acceptor compartment. The samples were filtered (0.45µm filter0 and analyzed by UV spectrophotometry.

Finally the amount of drug accumulated into the skin was recovered using the following procedure. At the end of the perfusion experiments, the skin was removed and rinsed with buffer. The cleaned skin piece

was meshed completely in a beaker and 10 ml of diffusion medium was added. The resulting solution was warmed slightly and mixed using a homogenizer. The skin homogenate was then centrifuged and the supernatant was separated which was filtered through Whatman filter. After suitable dilution, the drug content in the filtrate was determined spectrophotometricaly. All results were expressed as mean ±S.D.

2.7 Analytical Assay: The Tretinoin content of the skin cuts was determined by UV-spectrophotometry using spectrophotometer, Shimadzu, Japan at 352 nm.

3 Results and Discussion

3.1 Solubility

The medium to carry out the release and permeation profile was required so as to provide the requisite capacity to solubilize the drug and provide the desired sink conditions. Amongst the varied combinations tried, the desired solubility of 1.92 mg/ml was obtained with acidified IPA: phosphate buffer pH 5.5 (2:1) system which is approximately eight times higher than the aqueous solubility of the drug. Owing to skin irritancy and photolability of the drug, adequate care was exercised to avoid contact with body parts using proper protection gear for hands and face. Care was taken to avoid exposure of the drug to direct sunlight by working in a small dark hood within the laboratory. Table 1 summarises the solubility profile of tretinoin in different media.

Table 1: Solubility of Tretinoin

Solvent Medium	Solubility
Water	0.48 g/ml
$IPA_{(ac)}$	5.96 mg/ml
Methanol	3.15 mg/ml
Ethanol	1.33 mg/ml
IPA_{ac}:Buffer (5.5) (2:1)	1.92 mg/ml
IPM	1.12 mg/ml

3.2 Partitioning study

The partition coefficient of drug was determined in octanol and water and in IPM and phosphate buffer pH 5.5 system. The latter was used in microemulsion formulation. Value of log P exhibit the highly lipophilic nature of the molecules and also supports to favor drug skin partitioning.

Table 2: Partition coefficient study of tretinoin

System	log P
Octanol and Water	6.614
Isopropyl myristate and buffer (pH 5.5)	6.932

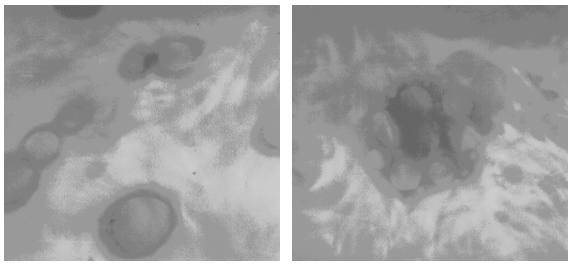
3.3 Formulation Variables Study

Formulation variables study was carried out by selecting the appropriate components and their ratio in order to achieve the composition with desired characteristics [17,18]. Typical components of microemulsion formulation include oil, surfactant, cosurfactant and aqueous phase [19,20]. Of the various oils screened, IPM was found to be most stable for the studied period of 45 days (sustainability >45 days) on account of emulsion stability / sustainability and also on solubility of tretinoin in the selected oil (1.12 mg/ml). the optimum level of IPM for tretinoin microemulsion was found to be 5% w/w.

In order to select the surfactants and other agents to bring the microemulsion characters in product, it was found that an appropriate combination of three such agents is essential to achieve a microemulsion product which can also act as a potential carrier. These include lecithin,Tween 80 and ethanol as cosolvent. Tween 80 contributed as a surfactant to a major extent at the optimized level of 30% w/w respectively while ethanol at 10% w/w was found necessary to bring the isotropic properties. Lecithin in the concentration of 1% w/w was included to bring the major skin related transport advantage [21-26]. Aqueous phase was chosen as phosphate buffer pH 5.5 to mimic the pH condition of the skin.

3.4 Characterization of the microemulsion system

The results of TEM pictures reveal that tretinoin microdroplets were almost spherical in shape and coating of lecithin was also evident. The average droplet size for tretinoin microemulsion was found to be of the order of 110 nm. The results of TEM further indicated the existence of an isotropic dispersion of spherical droplets, leading to the assumption of inverse micelles because of the proportion of the constituents.



Freshly prepared Tretinoin ME 54,000X Treinoin ME (5 weeks) 54,000X

3.5 Stability

Stability of the prepared systems was assessed using mechanical stress (centrifugation) and accelearated temperature study. Systems were centrifuged at 10,000 rpm and it was observed that a minimal degree (1%) of separation occurred after 240 mins of centrifugation indicating reasonable stability of tretinoin microemulsion.

Table 3: Effect of centrifugation on oil phase separation for Tretinoin Microemulsion .

Sr. No.	Centrifugation time (mins)	% oil phase remaining after centrifugation
1	0	100
2	15	100
3	30	100
4	45	100
5	60	100
6	120	100
7	180	100
8	240	99

3.6 Drug Loss Study

Phase separation and drug loss study was conducted [16] by storing the emulsified formulations at different temperature conditions mainly room temperature (25±5ºC), at higher temperature (40±5ºC) and at lower temperature (4ºC) as shown in Table 4.

Table 4: Effect of oil phase separation study and percent drug content of Tretinoin microemulsion at room temperature (25±5ºC), at higher temperature (40±5ºC) and at lower temperature (4ºC)

Formulation	% Drug content	% oil phase separation
Fresh Microemulsion at r. t.	98	-
Microemulsion stored at 40°C for 45 days	64	0.5
Microemulsion stored at 4°C for 45 days	80	-
Microemulsion stored at 25°C for 45 days	96	-

Micremulsions stored at 40 °C for 45 days showed a marked decrease in percentage drug content as well as oil phase separation. This can be explained on the basis of the fact that thermal energy affects the fluid state of lecithin interfacial film thereby causing its disruption and destabilization at the oil-water interface.

3.7 In vitro permeation study and skin retention studies

The permeation flux of different drug formulations were compared with the drug solution and also to the drug incorporated in non emulsified gel as well as marketed preparation. Maximum flux was obtained with microemulsion formulation (33.92 µg / cm^2 / hr) and microemulsion gel (31.54 µg / cm^2 / hr) comparable to the tretinoin solution (22.33 µg / cm^2 / hr). The extent of permeation with plain drug in gel and marketed preparation were noted to be at the flux level of 28.67 µg / cm^2 / hr and 24.28 µg / cm^2 / hr respectively. The formulation codes for different drug combinations used for permeation study are listed as under:

TrAp	:	Plain drug in solution
TrBp	:	Plain drug in gel
TrCp	:	Drug in microemulsion
TrDp	:	Drug in microemulsion gel
TrEp	:	Marketed preparation (Retino A from Shalaks Laboratories) hu

Table 5: Comparative results of permeation flux for different formulations containing tretinoin

Formulation Code	Permeation flux (g/cm^2/hr)
TrAp	22.33 ± 0.02
TrBp	28.67 ± 0.07
TrCp	33.92 ± 0.06
TrDp	31.54 ± 0.03
TrEp	24.28 ± 0.13

The following table 6 summarises the comparative drug release through the skin of laca mice for various combinations of Tretinoin, all results are expressed as mean ± S.D.

Table 6: Mean percentage drug release (±S.D.) with time for various drug combinations of Tretinoin using skin of laca mice.

Time (hrs)	Percent Drug Permeated (±S.D.)				
	TrAp	TrBp	TrCp	TrDp	TrEp
0.5	1.66±0.06	1.30±0.05	0.86±0.03	0.72±0.02	2.42±0.08
1.0	9.04±0.36	3.68±0.14	3.74±0.14	3.08±0.12	11.64±0.46
1.5	11.96±0.47	8.60±0.34	8.78±0.35	6.44±0.25	18.98±0.75
2.0	16.12±0.64	10.32±0.41	12.94±0.51	11.06±0.44	26.82±1.07
3.0	23.90±0.95	17.36±0.69	20.98±0.83	13.32±0.53	32.92±1.31
4.0	29.60±1.18	25.64±1.02	30.32±1.21	24.16±0.96	47.34±1.89
6.0	37.94±1.51	32.66±1.30	46.06±1.84	33.96±1.35	63.66±2.54
8.0	45.70±1.82	42.80±1.71	55.18±2.20	41.26±1.65	71.24±2.84
12.0	76.28±3.05	53.72±2.14	62.03±2.48	52.28±2.07	79.91±3.19
24.0	88.45±3.53	75.48±3.01	69.44±2.77	65.40±2.61	86.14±3.44

Diagram showing mean percent drug permeated (±S.D.) with time for various drug combinations of Tretinoin using skin of laca mice.

Skin Retention Study

The results obtained from skin retention studies indicate that maximum degree of depot formation is for lecithinised microemulsion (TrCp)that is 96.28 g/cm^2 . The gel form of tretinoin microemulsion showed similar results (82.13 g/cm^2) while the nonphospholipid gel and solution showed comparatively lower retention (21.54 g/cm^2 and 29.32 g/cm^2 respectively). This can be explained on the basis of the fact that lecithin can integrate with the skin lipids thereby causing depot effect by forming a micro-reservoir to hold the drug [27]. It is clearly seen that tretinoin in microemulsion system is able to cross the skin layer (higher permeation) to a greater extent also after epidermal transfer, is able to maintain its presence in a larger amount (depot effect).

Table 7: Comparative results of amount retained for different formulations containing Tretinoin

Formulation Code	Amount retained (g/cm^2)
TrAp	32.40 ± 0.04
TrBp	21.54 ± 0.03
TrCp	96.28 ± 0.09
TrDp	82.13 ± 0.11
TrEp	29.32 ± 0.13

The improved performance of microemulsion system in terms of higher permeation and retentability may be accounted to the carrier effect of the products. The latter includes enhanced skin carrier interaction, amphiphilic nature, hydration effect and also the size of microstructures. In the composition of microstructures, phospholipids are the key components that have the unique ability to produce all the above effects [32,33]. These are natural membrane molecules that can

integrate with skin tissues and thus form a favorable bio-ambience. In addition to phospholipids, presence of surfactant and cosolvent helps provide such nano range micro structures which hold the tretinoin molecule within their phase and serve as well composed carriers. These microstructures are able to penetrate the stratum corneum barrier forming micro reservoirs of drug in the vicinity of target tissues.

The enhanced permeation and retention of tretinoin into the skin caused by microemulsion are probably due to the compounds included in the formulation. Surfactants which can loosen or fluidize the lipidic matrix of the stratum corneum – principal diffusional barrier can act as permeation promoters. Therefore it is conceivable that the permeation of tretinoin is accompanied by components of microemulsions with the potentiality to create an environment favorable to the partition of drug into the skin.

Studied microemulsions showing a drug accumulation 1.5-4.5 fold higher than the corresponding percutaneous delivery are very appealing vehicles for the development of future topical treatment of skin diseases. These microemulsions have been formulated with surfactants considered as safe for topical applications. The high surfactant content of the formulation and the observed enhancer effect implies that the skin tolerance to a repeated application should be examined. It must be noticed that other microemulsion systems containing as much as 44 % of surfactants have been shown to be well tolerated [12].

Conclusion

In this study, the utility of microemulsions as vehicles for topical delivery of tretinoin in presence of phospholipids was studied. The results obtained suggest that these microemulsions can serve as efficient promoters of tretinoin localization into the skin. The enhanced skin retention of tretinoin could help significantly to optimize the targeting of drug without a concomitant increase of the systemic side effects. It can be concluded that tretinoin products with desired characteristics and performance were developed successfully[34].

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