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Proniosomal Gel of Tretinoin for the Treatment of Acne Vulgaris

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ABSTRACT

The objective of the present study was to formulate and evaluate Tretinoin proniosomal gel and to carry out comparative skin irritation study with conventional Tretinoin solution and Tretinoin conventional gel. Topical Tretinoin (0.25%, 0.05%) has been a reliable treatment of acne vulgaris since 30 years but its major drawback is that it causes skin irritation on the applied area. The proniosomal dispersion was prepared using different grades of non-ionic surfactants and cholesterol in different ratios along with Tretinoin. The scanning electron microscopy revealed that the proniosome vesicles were of LUV type and spherical shape. The proniosome vesicles prepared with SPAN 60, 40 and cholesterol in formulation PN9 showed maximum entrapment efficiency (76.77±1.54) .The prepared proniosome vesicles were incorporated into Carbopol gel (1%) base to prepare Tretinoin proniosomal gel. The stability study was carried out at different accelerated and non-accelerated conditions. The In-vitro diffusion study carried out using sigma dialysis membrane showed sustained release pattern of Tretinoin from proniosomal gel formulation. The comparative skin irritation study carried out on 18 healthy Wistar Rats of either sex showed remarkable decrease in signs of skin irritation caused by Tretinoin.

INTRODUCTION

Human skin is the important target site for the application of drug especially in the treatment of local, topical diseases. The main obstacle for the delivery of drug through the skin is the stratum corneum a dead, impermeable barrier devoid of biological activity for the therapeutic activity (Dreher et al, 1996). Despite of several researches, the barrier function of the stratum corneum still remains a major problem, which makes the development of new transdermal drug delivery systems an interesting challenge. (Jain and El-Laithy, 1998) To pursue optimal drug action, functional molecules could be transported by a lipoid carrier to the site of action and released the drug in a controlled manner to perform their task. Penetration enhancement with special formulation approaches are mainly based on the usage of colloidal carriers. Colloidal carriers have attracted the main interest because they are promising systems having localized effect. These carrier, accumulate in stratum corneum or other upper skin layers are not expected to penetrate into viable skin (Hu, 1999; Touitou, 2000), Proniosomes offer a versatile vesicle drug delivery concept with potential for delivery of drugs via transdermal route. Proniosomes minimizes problems of niosomes physical stability such as aggregation, fusion and leaking and provide additional convenience in transportation, storage and dosing (Biju et al, 2006). Proniosomal system serves as a rate limiting barrier for absorption of drugs. These systems can overcome the permeation barrier of the skin and act as a penetration enhancers for the drugs. The vesicles may serve as non toxic penetration enhancer for drug because of the amphiphilic nature of the vesicles; they are more stable and compatible with the skin. (Sheth et al, 1984). Transdermal therapeutic system has generated an interest as this system provides the considerable advantage of a non-invasive parentral route for drug therapy, avoidance of first pass gut and hepatic metabolism, decreased side effects and relative ease of drug input termination in problematic cases .

Tretinoin is the acid form of vitamin A and is also known as all-*trans* retinoic acid is a first generation topical retinoid commonly used to treat acne vulgaris and keratosis pilaris (Griffiths et al, 1993). Tretinoin shows very low bioavailability only 5% in the site of the action. Topical Tretinoin works by both comedolysis and by normalizing the maturation of follicular epithelium so that comedo formation ceases. (Tripathi et al, 2003). The main drawback with Tretinoin produces irritation on applied skin so it is hypothesized that proniosomal form of Tretinoin can overcome this drawback. (Abubkar et al, 2000). The aim of the present study was to encapsulate Tretinoin in proniosome colloidal vesicles and further incorporate it into the Carbopol gel (Wu et al, 2000).

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Proniosome carriers, well known for their potential in topical drug delivery, have been used to transport Tretinoin molecule in the skin layer. The objective of this study was to determine the factors influencing the encapsulation of Tretinoin in proniosomal gel and to optimize encapsulation parameters in order to achieve a suitable delivery system.

EXPERIMENTAL

Materials

Tretinoin was a gift from Psycoremedies (Ludhiana, India). Soya lecithin, cholesterol, and dialysis tubing were purchased from Hi-Media Laboratories (Mumbai, India). Span 20, 40, 60, 80 were purchased from Central Drug House (Mumbai, India). Potassium Dihydrogen Phosphate and disodium hydrogen phosphate were purchased from BDH Laboratory (BDH Chemicals Ltd, Poole, UK). Carbopol 971 gel base was purchased from Lubrizol advanced materials, USA. All chemicals were of analytical grade.

DEVELOPMENT OF PRONIOSOME

Proniosomal gel was prepared by a coacervation-phase separation method (Dreher et al, 1996).

Precisely weighed amounts of surfactant, lecithin, cholesterol and drug were taken in a clean and dry wide mouthed glass vial of 5.0 ml capacity and alcohol (1ml) was added to it.
After warming, all the ingredients were mixed well with a glass rod; the open end of the glass bottle was covered with a lid to prevent the loss of solvent from it and warmed over water bath at 60-70°C for about 5-10 min until the surfactant mixture was dissolved completely. Then the aqueous phase (PBS pH 7.4) was added and warmed on a water bath till a clear solution was formed which was converted into proniosome on cooling. A total of 12 Tretinoin Proniosomal formulations were prepared differing from each other in surfactant cholesterol composition. Compositions of proniosomal gel formulations are given in Table 1.

FORMULATION OF TRETINOIN PRONIOSOMAL GEL

Carbopol (500 mg) was dispersed in 10 ml of water and proniosomal formulation dispersion to prepare the gel. The final weight of gel was adjusted to 100 g with addition of required amount of distilled water to get the final strength of the gel. The pH of the gel was adjusted between pH 5.5-6.5 (Eutech pH 5, India). The gel was transferred to collapsible tubes and stored at 2-8° C in refrigerator.

CHARACTERIZATION OF PRONIOSOMAL GEL

Vesicle Size Analysis:

Hydration of proniosomal gel (100mg) was done by adding saline solution (0.9% solution) in a small glass vial with occasional shaking for 10 min. Size evaluation was carried out using optical microscope at a magnification of 100 x magnification by means of a fitted camera (Nikon D-500, 8 Mega pixel). The sizes of 200-300 vesicles were measured using a calibrated ocular and stage micrometer (Erma, Tokyo) fitted in the optical microscope. (Figure 1) .

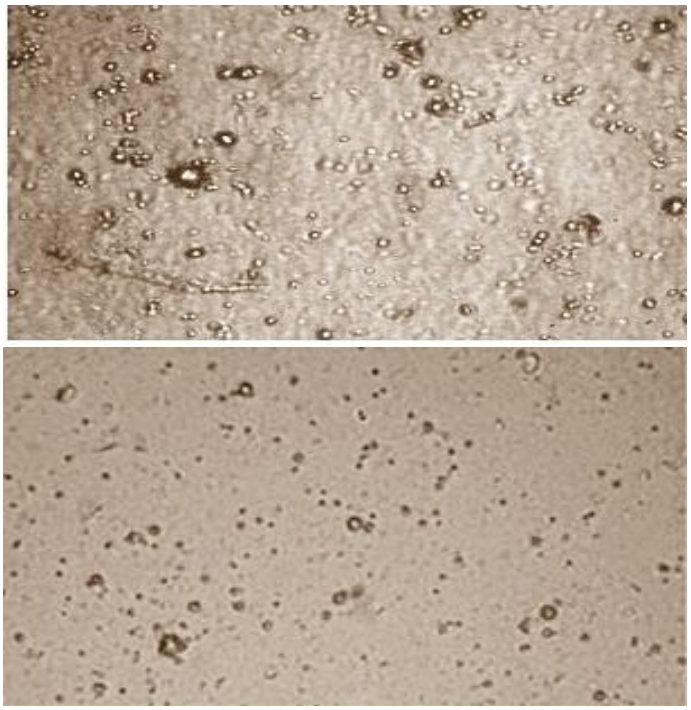

Fig. 1: Vesicle Size of proniosomal gel.

Rate of Spontaneity

Approximately 10 or 20 mg of proniosomal gel was transferred to the bottom of a clean stoppered glass bottle and spread uniformly around the wall of the glass bottle with the help of a glass rod. At room temperature, 2 ml of phosphate saline was added carefully along the walls of the glass bottle and left in a test-tube stand (Bhalerao et al, 2003). After 20 minutes, a drop of this saline solution was withdrawn and placed on Neubauers Chamber (Marienfeld, Germany) to count the number of vesicles. The data is reported in table no. 2

ENCAPSULATION EFFICIENCY

Formulated proniosomal formulations were centrifuged at 30,000 rpm at 25^0C for 30 min to separate unentrapped drug as supernatant. Supernatant was separated, filtered and sufficiently diluted with buffer pH 7.4 to determine the concentration of trapped drug spectro-photometrically (Varghese et al, 2004). The clear fraction was used for the determination of free drug at 350 nm spectrophotometrically. The percentage encapsulation efficiency was calculated from Equation 1.The data is reported in table no. 2

Percentage drug entrapment = [(Entrapped drug –Free drug)/Loaded drug] ×100 Eq. (1) (Azmin et al., 1985)

Results of encapsulation efficiency of Various Formulations are reported in Table no.2

Table. 1: Composition of the Proniosomal Formulation.

Formulation Code	Surfactant Type (mg)	Ratio*	Lecithin (mg)	Cholesterol (mg)	Alcohol (ml)	Water (ml)	Visual Observation
PN1	S20:S40	200:800	100	100	1	1	White Semi solid no proper vesicles
PN2	S20:S40	500:500	100	100	1	1	Light Brownish Semi-solid
PN3	S20:S60	200:800	100	100	1	1	White Semi-solid
PN4	S20:S60	500:500	100	100	1	1	White Semi-Solid
PN5	S20:S65	500:500	100	100	1	1	Pale Yellow Semisolid
PN6	S20:S65	200:800	100	100	1	1	Brown Liquid no vesicles found
PN7	S40:S80	500:500	100	100	1	1	Creamish Gel
PN8	S40:S60	200:800	100	100	1	1	Creamish Semisolid
PN9	S60:S40	500:500	100	100	1	1	Creamish Gel
PN10	S60:S80	200:800	100	100	1	1	Yellowish Semisolid needle shape particles
PN11	S65:S80	200:800	100	100	1	1	Yellowish Gel no proper vesicles
PN12	S65:S80	500:500	100	100	1	1	Yellowish gel

** Drug concentration used was 10 mg in each formulation.*

Table. 2: Encapsulation Efficiency of Various Preliminary Formulations.

Formulation Code	Vesicle Size ± SEM (μm)		Percent Drug Loading (± SEM)*	Rate of spontaneity (mm3 x 1000)	% Drug release after 18 hrs
	Without Agitation	With Agitation			
PN2	18.09±1.53	7.59±0.11	77.76(±1.51)	15.25±0.28	97.24±0.09
PN5	22.40±1.57	7.75±0.09	73.89±2.34	13.59±1.17	98.05±0.12
PN7	23.23±1.76	6.78±1.79	75.67±1.74	10.56±1.12	95.67±0.49
PN8	15.78±2.56	6.87±2.89	73.39±1.45	12.34±1.44	90.78±1.67
PN9	10.45±1.89	5.89±1.75	76.77(±1.54)	15.45±1.40	88.67±1.79
PN12	16.56±1.09	4.67±1.05	74.69±1.58	13.56±1.52	91.04±0.52

***Mean of three determinations*

Table. 3: Stability Tests for Optimized PN9 Formulation.

Condition	Time days	pH	Partcle size (μm)	%Entrapment
Refrigerated temperature	0	6.8	4.67±1.05	76.77(±1.54)
	15	6.76	4.71±.09	76.01(±1.93)
	30	6.72	4.76±0.87	74.23±2.98
	60	6.7	4.82±0.12	72.45±1.87
	90	6.69	4.90±1.09	71.23±0.23
Room temperature	0	6.8	4.67±1.05	76.77(±1.54)
	15	6.56	4.89±2.67	74.23±1.45
	30	6.23	5.12±0.67	72.34±0.78
	60	5.98	5.45±0.23	70.78±1.45
	90	5.7	5.76±1.42	69.12±0.71
Oven temperature	0	6.8	4.67±1.05	76.77(±1.54)
	15	FORMULATION CRACKED		
	30			
	60			
	90			

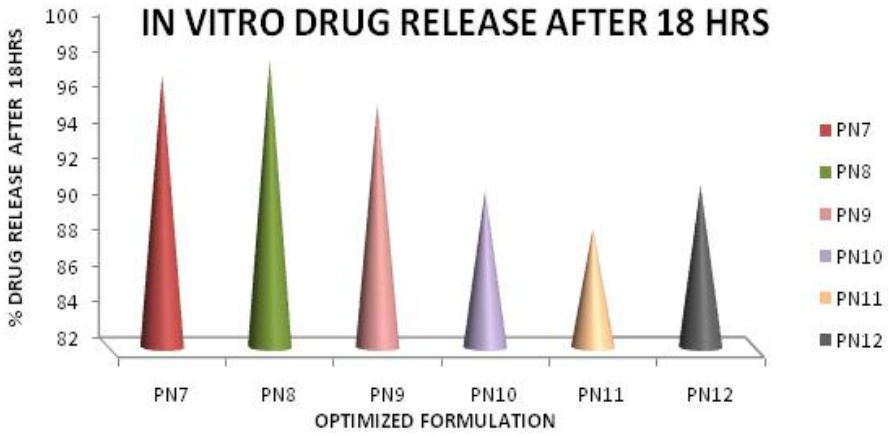

Fig. 2: Percentage Drug Release of the optimized formulation after 18 hrs .

***In Vitro* Release**

In vitro release studies of proniosomal gel were performed using locally manufactured Franz-diffusion cell. The capacity of receptor compartment was 10 ml. The dialysis cellophane membrane (Hi Media) was mounted between the donor and receptor compartment (Zhou et al, 1994).The receptor medium was phosphate saline buffer pH 7.4. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37.5 ± 2^0C. Heat was provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by a Teflon-coated magnetic bead fitted to a magnetic stirrer (Bio-Craft Scientific Systems Pvt. Ltd., Agra) (Franz, 1975).

Samples were withdrawn and were replaced by equal volumes of fresh receptor. Samples withdrawn were analyzed spectro-photometrically (Shimadzu-1700) at 350 nm for the study of the drug release over a period of 18 hrs.

The data reported in table No 2 and figure No. 2

Stability Studies

The ability of vesicles to retain the drug (Drug Retention Behaviour) was assessed by keeping the proniosomal gel at three different temperature conditions, i.e., Refrigeration Temperature ($4\text{-}8^0$C), Room Temperature (25 ± 2^0C) and oven temperature (45 ± 2^0C). Throughout the study, proniosomal formulations were stored in aluminium foil-sealed glass vials. (Domecq et al, 2001).

The samples were withdrawn at different time intervals over a period of 3 month and drug leakage from the formulations was analyzed for drug content spectro-photometrically.The data are reported in tablet No. 3

SKIN IRRITABILITY STUDY

The study was carried out with permission of animal ethical committee. Total 18 healthy Wister Rats of either sex having average weight of 3.5 kg were selected for study. The neck skin (4 cm^2 area) was shaved carefully. The animals were divided into two equal groups. The adequate amount of Tretinion proniosomal gel was applied to shaved skin area of one group. Same way, Tretinion conventional gel was applied to the shaved skin of other group which served as control. Both the formulations of same strength (0.05%) were applied on shaved rat skin for the determination of irritation characteristics. (Keshary et al, 1984). The applied area was covered by cotton and bandage. The visual observations were carried out at regular intervals of 12, 24, 48 hours for various symptoms such as scaling, lesions and erythema. The symptoms, lesions and erythema were graded as 3=severe, 2=moderate, 1=mild and 0=absent. The scaling was graded as 1=present, 0=absent

RESULTS AND DISCUSSION

Proniosomes have generated interest as a topical formulation as an approach to avoid the side effects associated with oral administration. To achieve the desirable therapeutic effect of proniosomes as drug carriers, they must be loaded with sufficient amount of active compound. Table 1 shows the effect of various sorbitan fatty acid esters and their ratio on the formation of Tretinoin in proniosomal gel. Tretinoin was best formulated by proniosomal prepared using Spans 40 and 60. This might be attributed to fact that Spans 40 and 60 are solid at room temperature and showed a higher phase transition temperatures [Tc] (Jain et al., 1998). It is reported in some research works that niosome vesicle can be formed if the HLB value of non-ionic surfactant is between 4.0 to 8.0. Thus, in current experiment, SPAN 60 (sorbitan monostearate) was selected as non ionic surfactant as its HLB value is 4.7. The phase transition temperature of SPAN 60 is very high as compare to other non-ionic surfactants (around 50°C) and thus the stability of prepared vesicle is high as compare to others (Yoshioka et al, 1994). The SPAN 85 has HLB value 1.8 thus the non-ionic surfactants were rejected. The surfactant: cholesterol ratio is very important to be optimized because the cholesterol acts as stabilizer and itself is lipophilic in nature, so increased concentration of cholesterol causes reduction in percentage drug entrapment of lipophilic drug. Thus, the optimum concentration of cholesterol is needed. (De et al, 1968) .

Determination of vesicle size is important for the topical application of vesicles. Size was reduced when the dispersion was agitated (Buhler et al, 1998). The reason for this is the energy applied in the agitation which results in the breakage of the larger vesicles to smaller vesicles. The size range was found to be 10.45 - 23.23 μm (without agitation) and 4.67 – 7.75 μm (with agitation). Vesicle size was found to be smallest in PN12 formulation (4.67±1.05μm) due to the presence of Span 80. Increasing hydrophobicity of the surfactant monomer led to a smaller vesicle, a result that is expected since surface energy decreases with increasing hydrophobicity.

For spontaneity studies, the formulations in Table 2 were treated with ethanol, propanol, butanol and isopropanol. It was found that proniosomes containing isopropanol and butanol were formed more spontaneously than proniosomes containing propanol and ethanol perhaps as a result of faster phase separation of isopropanol and butanol due to their lower solubility in water.

The morphology of proniosomal gel was studied using Scanning Electron Microcopy. SEM revealed that the niosomes formed were spherical and homogenous. Figure No 3

In vitro release studies are often performed to predict how a delivery system might work in an ideal situation as well as give some indications of its *in vivo* performance since drug release dictates the amount of drug available for absorption. The amount of drug released from different proniosomal gel formulation was found in the order of PN 9 >PN 8 > PN12 > PN 7 > PN 2 > PN 5 as shown in Fig. 2. It was found that PN9 showed a controlled release property over 18 hours. The release rate was constant from the 10th to the 18th hour. Thus, the formulation exhibited zero order release over this period. This may be attributed to the fact that the molecules of Spans 40 and 60 are in an ordered gel state at

the *in vitro* permeation condition of 25^{0}C, while the formulation PN 9 showed significantly higher release. The amount of drug retained within the vesicles under defined conditions ultimately governs the shelf life of the drug. The results showed that proniosomal gel formulation was quite stable at refrigeration and room temperatures as not much leakage of drug was found at these temperatures (Table 3). Percent drug retained at 45^{0}C might have decreased due to the melting of the surfactant (m.p: 48^{0}C) and lipid present in the formulation causes the cracking of the formulation and the phase separation taken place. Therefore, the proniosomal gel formulations can be stored at either refrigeration or room temperature.

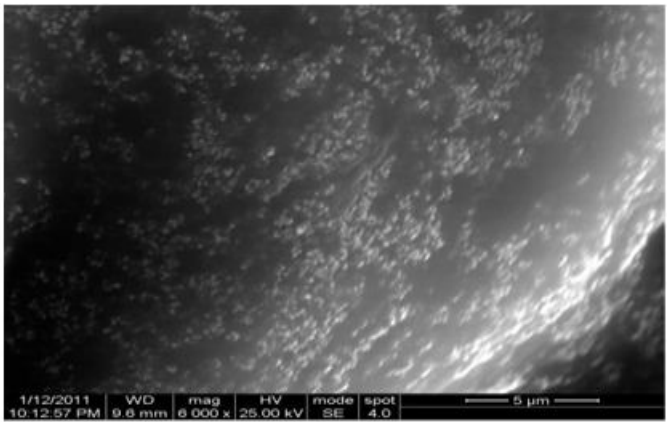

Fig. **3:** scanning electron microscopy of proniosomal gel.

Skin Irritation Study

The Figure 4 reveals that the rats treated with TRT solution shows more irritation characteristics. The Irritation signs are continuously increasing with time intervals. The data shows of the rats treated with TRT conventional gel which shows almost same irritation characteristics as the TRT solution. The graphical presentation of skin irritation studies of TRT proniosomal gel on rat skin. The irritation characteristics were found to be almost absent in treatment with proniosomal gel. This may be due to the sustained release pattern of TRT from the proniosomal gel. (Buhler et al, 1998). The irritation signs were totally absent throughout the treatment even after 18hours of continuous exposure.

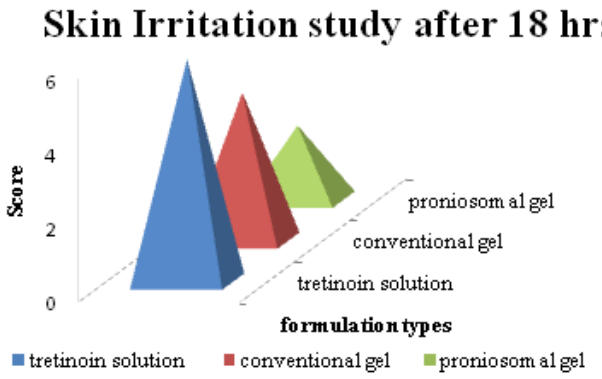

Fig. 4: Skin Irritation study.

CONCLUSION

In conclusion, we can state that besides imparting controlled systemic transdermal delivery to Tretinoin, proniosomal gel possesses high entrapment efficiency and utilizes alcohol, which itself can act as a penetration enhancer.

The *in-vitro* diffusion study shows the sustained release pattern of TRT from proniosomal gel and the skin irritation study reveals that the irritation symptoms due to TRT treatment are reduced to the remarkable level. The stability study proves that the proniosomes are more stable at 2-8°C hence proper attention should be paid in storage of proniosomal dosage forms.

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