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FORMULATION AND EVALUATION OF GEL CONTAINING ETHOSOMES ENTRAPPED WITH TRETINOIN

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ABSTRACT

A skin disease, like acne, is very common and normally happens to everyone at least once in their lifetime. The structure of the stratum corneum is often compared with a brick wall, with corneocytes surrounded by the mortar of the intercellular lipid lamellae. One of the best options for successful drug delivery to the affected area of skin is the use of ethosomes which can be transported through the skin through channel-like structures. Tretinoin is a widely used retinoid for the topical treatment of acne, photo-aged skin, psoriasis and skin cancer which makes it a good candidate for topical formulation. Yet side effects, like redness, swelling, peeling, blistering and, erythema, in addition to its high lipophilicity make this challenging. Drug loaded ethosomes had been prepared using phospholipid and ethanol, were optimized and characterized for entrapment efficiency, vesicular size, shape, *In-vitro* skin permeation, skin retention, drug-membrane component interaction and stability. The ethosomal formulation having 0.5 %w/v of phospholipid and 20 %v/v of ethanol (F2) showing the greatest entrapment efficiency (80.25±0.23) with small particle size (205.40±2.31nm) was selected for further skin permeation studies. The skin permeation and skin retention studies were performed on ethosomal formulation, liposomal formulation (0.5 %w/v of phospholipid without alcohol), hydroethanolic drug solution and phosphate buffer saline (pH7.4) drug solution. Among them, ethosomal formulation showed higher cumulative percentage of drug permeation (93.36±0.45%) and 8 hours than the other formulations. Scanning electron microscopy confirmed the three dimensional nature of ethosomes. Dynamic light scattering technique proved that the ethosomes has smaller vesicular size than the liposomes prepared without alcohol. FT-IR studies revealed no interaction between the drug and membrane components. The ethosomal vesicles were incorporated in carbopol gel base and its anti-acne was compared with the marketed gel. Our results suggest that the ethosomes are an efficient carrier for dermal and transdermal delivery of tretinoin.

Keywords: Tretinoin, Ethosomes, Diffusion, Carbopol gels, Transdermal delivery.

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INTRODUCTION

The optimization of drug delivery through human skin is important in modern therapy. Clearly, the topical route of drug delivery for treating skin diseases offers an attractive alternative to the conventional drug delivery methods of oral administration/injection and it is becoming a most innovative research area in drug delivery. A skin disease like acne, is very common and normally happens to everyone once in their lifetime. Acne vulgaris is a chronic inflammatory dermatosis which is notable for open and/or closed comedones (blackheads and whiteheads) and inflammatory lesions including papules, pustules or nodules. It is a disorder of sebaceous follicles which are special pilosebaceous units located on the face, chest and back [1]. *Propionibacterium acnes* and *Staphylococcus epidermidis* have been recognized as pus-forming bacteria triggering inflammation in acne [2].

The organism produces extracellular lipases that hydrolyze sebum triglycerides to glycerol and free fatty acids that have proinflammatory properties [3]. The topical treatment of acne includes topical retinoids [4,5], benzoyl

peroxide (BPO) [4,6], azelaic acid [7], erythromycin [8], clindamycin [8] and combination therapies [9,10]. The adverse effects of topical antiacne agents include burning, erythema, scaling, flare-up, photosensitivity and bacterial resistance [4]. Tretinoin are used individually and in a cyclic manner for acne treatment. Various conventional topical medicines are available in the market for treatment but have a less therapeutic effect due to the efficient barrier properties of skin membranes. The structure of the stratum corneum is often compared with a brick wall made of corneocytes and surrounded by the mortar of the intercellular lipid lamellae [11]. The best alternative for successful drug delivery to an affected area of skin is elastic vesicles (ethosomes) which can be transported through the skin via channel-like structures. Moreover, they are too small in the nanometer size range to be detected by the immune system; furthermore, they can deliver the drug to the target site using lower drug doses in order to reduce side effects often experienced by topical routes by passing the complexity of the skin structure [12]. The main advantages of using nanocarriers arise from their peculiar features, such as their tiny size, high surface energy, high surface area, composition and architecture [12]. The use of lipid vesicles in delivery systems for skin treatment has attracted increasing attention in recent years [13, 14]. However, it is generally agreed that classic liposomes are of little or no value as carriers for transdermal drug delivery because they do not deeply penetrate the skin, but rather remain confined to the upper layer of the stratum corneum [13]. Only specially designed vesicles were shown to be able to allow transdermal delivery [15]. Ethanol is known as an efficient permeation enhancer [16]. However, due to the interdigitation effect of ethanol on lipid bilayers, it was commonly believed that vesicles cannot coexist with high concentrations of ethanol. Currently, ethanol can only be found in relatively low concentrations in liposomes formulations, if at all. We have discovered and have been investigating lipid vesicular systems embodying ethanol in relatively high concentrations, which we named ethosomes and that are very efficient at enhancing the skin permeation of a number of drugs [17–20]. These findings are supported by other recent reports [21,22]. The present paper focuses on the characterization of ethosomal systems and presents the enhancing delivery properties of these systems.

MATERIALS AND METHODS

Tretinoin was obtained as a gift sample from Bioplus Life science, Bangalore. Phosphatidylcholines from Himedia mumbai. Ethanol and Propylene glycol from SD Fine chemicals Mumbai. All other chemicals and reagent were of analytical grade.

Preparation of ethosomes

Ethosomal formulations were prepared by as reported by [23]. In brief the lecithin (1-4%w/v) was taken in small round bottom flask and solubilized with ethanol (10-50%) containing drug under mixing with a magnetic stirrer. The round bottom flask was covered to avoid ethanol evaporation. Distilled water was added slowly with continuous stirring to obtain the ethosomal colloidal suspensions. The final suspension of ethosomes was kept at room temperature for 30 min. under continuous stirring. Formulation was stored in the refrigerator. The composition of various ethosomal formulations and liposomal formulation were represented in table 1.

Table 1: Different Composition of ethosomes formulation

F. Code	Drug (mg)	Phospholipid (% w/v)	Ethanol (% w/v)	PEG (%w/v)	Water (%w/v)
F1	100	0.5	10	20	100
F2	100	0.5	20	20	100
F3	100	0.5	30	20	100
F4	100	1.0	10	20	100
F5	100	1.0	20	20	100
F6	100	1.0	30	20	100

Evaluation of ethosomes

Microscopic observation of prepared ethosomes

An optical microscope (cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared ethosomes formulation.

Vesicle size and zeta potential

Vesicle size and zeta potential of the Ethosomes were measured by photon correlation spectroscopy using a horiba scientific, nanoparticle analyzer instrument the results shown in table 2.

Entrapment efficiency

Entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium. About 1 ml of the drug loaded ethosomes dispersion was placed in the eppendorf tubes and centrifuged at 10,000 rpm for 30 min. The ethosomes along with encapsulated drug were separated at the bottom of the tubes. Plain ethosomes without Tretinoin was used as blank sample and centrifuged in the same manner. In order to measure the free drug concentration, the UV absorbance of the supernatant was determined at 322 nm. The results shown in table 2a.

Visualization by scanning electron microscopy (SEM)

The size and shape of the vesicles were observed in the scanning electron microscopy. One drop of ethosomal suspension (F2) was mounted on a clear glass stub. It was then air dried and gold coated using sodium auro

thiomalate to visualize under scanning electron microscope at 10,000 magnifications.

FTIR studies

The interaction between ethosomal membrane component and drug was observed from IR-Spectral studies by observing any shift in peaks of drug in the spectrum of physical mixture of drug and phosphatidylcholine.

Stability studies

Stability studies were carried out by storing the ethosomal formulations at two different temperatures 4°C and 25±2°C. The drug content was estimated for every 15 days to identify any change in the entrapment efficiency of ethosomal formulation.

Preparation of ethosomal gel

The incorporation of the drug loaded ethosomes (equivalent to 2%) into gels was achieved by slow mechanical mixing at 25 rpm (REMI type BS stirrer) for 10 minutes. The optimized formulation was incorporated into three different carbapol gel concentration 0.5, 1 and 2% w/w.

Evaluation of gel

Physical Characteristic

The Physical Characteristic was checked for gel formulations (homogeneity and texture) and observations were shown in Table 3.

Determination of pH

The pH of the gel was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times (table 4).

Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually and observations were shown in Table 3.

Extrudability study

The gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked in table 3.

Assay

Weight equivalent to 10 mg of ethosomal gel dissolved in 5 ml methanol in 10 ml volumetric flask, sonicate it for 10 min and volume make up to 10 ml and dilute suitably to 10µg/ml and take the absorbance at 322nm and calculate using calibration curve of linearity.

Spreadability

An important criterion for gels is that it must possess good spreadability. Spreadability is a term expressed to denote the extent of area to which the gel readily spreads on application area. The therapeutic efficacy of a formulation also depends on its spreading value. A special apparatus has been designed to study the spreadability of the formulations. Spreadability is expressed in terms of time in seconds taken by two slides to slip of from formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of two slides, better the spreadability. Two glass slides of standard dimensions (6×2) were selected. The gel formulation whose spreadability had to be determined was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6 cms along the slide. 100 grams of weight was placed up on the upper slide so that the gel formulation between the two slides was traced uniformly to form a thin layer. The weight was removed and the excess of the gel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20 gram load could be applied 50 with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6 cms and separate away from lower slide under the direction of the weight was noted. The experiment was repeated and the average of 6 such determinations was calculated for each gel formulation (Table3).

Viscosity

The measurement of viscosity of the prepared gel was done using Brookfield digital Viscometer. The viscosity was measured using spindle no. 6 at 10 rpm and 25^{0}C. The sufficient quantity of gel was filled in appropriate wide mouth container. The gel was filled in the wide mouth container in such way that it should sufficiently allow to dip the spindle of the Viscometer. Samples of the gels were allowed to settle over 30 min at the constant temperature (25 ±/1^{0}C) before the measurements (Table 4).

***In-vitro* drug release studies using the semipermeable membrane Preparation of semipermeable membrane for the diffusion studies:**

The semipermeable membrane approximately 25 cm x 2cm was taken and washed in the running water. It was then soaked in distilled water for 24 hours, before used for diffusion studies to remove glycerin present on it and was mounted on the diffusion cell for further studies.The prepared Ethosomes delivery system was evaluated for *in vitro* drug release. The drug release studies were carried out using modified franz diffusion cell. The dissolution study was carried out in 24 ml dissolution medium which was stirred at 50 rpm maintained at 37±0.2°C. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 10ml by PBS (pH 7.4). The samples withdrawn were assayed spectrophotometrically at 322.0 nm for Tretinoin and using UV visible spectrophotometer. The release of Tretinoin was calculated with the help of Standard curve of Tretinoin. The observations of drug release for the drug in uncoated formulation and coated formulation is tabulated in Table 5 and fig 1.

Release kinetics

In-vitro diffusion has been recognized as an important element in drug development. Under certain conditions it can be used as a surrogate for the assessment of bioequivalence. Several theories/kinetic models describe drug dissolution from immediate and modified release dosage forms. There are several models to represent the drug dissolution profiles where ft is the function of t (time) related to the amount of drug dissolved from the pharmaceutical dosage system. To compare dissolution profiles between two drug products model dependent (curve fitting), statistic analysis and model independent methods can be used. The following plots were made: cumulative % drug release vs. time (zero order kinetic models); log cumulative of % drug remaining vs. time (first order kinetic model); cumulative % drug release vs. square root of time (Higuchi model) results are shown in table 5 and fig 2-4.

Stability Studies

Stability studies were carried out with optimized formulation which was stored for a period of 45 days at 4±1°C, RT and 40±1°C. The particle size of formulation was determined by optical microscopy using a calibrated ocular micrometer. The vesicle size of the ethosomes was found to increase at RT, which may be attributed to the aggregation of ethosomes at higher temperature. At 45±2°C the aggregate i.e. these ethosomes were unstable at higher temperature like 45±2°C. Percent efficiency of ethosomes also decrease at higher temperature like 45±2°C.

RESULTS AND DISCUSSION

Preformulation studies are essential protocols for improvement of safety, efficacy and stability of dosage form as well. Thus it plays an important role in order to ensure optimum condition for clinically advantageous delivery system. Organoleptic property: Drug substances which irritates to skin should be handle with precautions. Flavors, dyes, excipients used will affect stability and bioavailability of dosage form. Color of the tretinoin was found to be white to almost white powder and no Odor. Solubility: One important goal of the pre-formulation studies is to device a method to check the solubility of drug. Solubility of the drug was determined by taking weighed quantity of drug (about 1-2 mg) in the test tube separately and added the 10 ml of the solvent. Tretinoin was soluble in methanol, Chloroform and 7.2 phosphate buffer, Slightly soluble in 0.1N NaOH, and 0.1N HCL and water. FTIR spectroscopy: Identification and authentication of drug sample was done by infrared spectroscopy. The IR spectra showed the presence of principal group like at 2876.86 C-H aromatic stretching; 1636.38 C=O stretch and 682.25.19 of C–H bending (aromatic) which confirm the drug is tretinoin. Loss on drying: Loss on drying is directly measured by IR moisture balance. Firstly instrument is calibrated by knob then 5.00 gm of drug (powder) was taken and temp was set at 100°C to 105°C for 15 minutes and constant reading was taken again set the knob and check % moisture. The percentage of loss on drying of tretinoin was found to be 0.25% w/w respectively. Melting point: Melting point was determined by Melting point apparatus using open capillary method and found to be in the range of 180-182°C for tretinoin. Moisture content: Moisture content determination was done by Karl Fischer colorimetric method. tretinoin Moisture content was found to be 0.13%. Determination of λ max of tretinoin: Identification of tretinoin was performed by UV/VIS Spectroscopy. The 10 µg/ml solutions of tretinoin was scanned in the range of 200-400nm to determine the wavelength of maximum absorption for drug. The λ_{max} of tretinoin was found to be 322.00nm. From the respective stock solution (1mg/ml) different concentration of 5,10,15,20 and 25 µg/ml tretinoin was prepared and scanned in UV region. Their absorbances were noted at ʎmax, 322 nm and calibration curve was plotted as absorbance Vs concentration and their linearity range was determined. Compatibility studies of drug and excipients: In the compatibility testing study blends of drug and excipients are prepared by triturating the drug with excipients. The lambda max does not deviate from 322nm shows that there is no interaction between drug and excipients. Ethosomal formulations were prepared by hand shaking method. Total six formulations were prepared using varying amount of phospholipids and ethanol. The prepared formulation was evaluated for Microscopic observation, Vesicle size, zeta potential and Entrapment efficiency. The maximum entrapment efficiency was observed in formulation F2, was further subjected for zeta potential and particle size. The particle size and zeta potential was found to be 205.4 and -0.9mv respectively. The optimized formulation of ethosomes was further incorporated into carbopol gel base (0.5, 1.0 and 1.5%) and evaluated for Physical Characteristic pH, Washability, Extrudability study, Spreadability, Viscosity and *In-vitro* Drug Release Studies Using the semipermeable membrane. In all formulation formulation F2 select as a optimized formulation because of its good Spreadability (15.56±0.35), Viscosity (3215±58) and pH (7.10±0.15). The maximum % assay was also found in formulation F2 (96.56±0.15). The *in vitro* drug release data of the formulation was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equation and Korsmeyer's models in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r' values of formulation was maximum i.e 0.978 hence indicating drug release from formulations was found to follow Pappas model of drug release kinetics.

Table 2: Result for Vesicle size and Entrapment efficiency of drug loaded Ethosomes

Formulation Code	Vesicle size	Entrapment Efficiency
F1	215.56±3.25	65.58±0.51
F2	232.56±4.56	72.65±0.84
F3	205.40±2.31	80.25±0.23
F4	265.50±4.58	75.65±0.21
F5	260.12±5.65	70.14±0.65
F6	272.56±4.10	76.65±0.45

Table 2a: Vesicle size and entrapment efficiency of optimized ethosomes

Formulation Code	Vesicle size (nm)	Entrapment Efficiency	Zeta potential
F2	205.4	80.25±0.23	-31mv

Table 3: Results of Homogeneity, Extrudability, Spreadability of gel formulation

Code	Homogeneity and Texture	Spreadability (gm.cm/sec.)	Extrudability	Washability
F1	+++	18.56±0.45	+++	**Good**
F2	+++	15.56±0.35	+++	**Good**
F3	++	13.56±0.25	+++	**Good**

+++ Good ++ Average

Table 4: Results of pH, Viscosity and % Assay

Code	pH	Viscosity (cps)	% Assay
F1	6.90±0.21	3325±45	90.25±0.21
F2	7.10±0.15	3215±58	95.56±0.12
F3	7.15±0.045	3014±60	91.25±0.25

Table 5: Cumulative % drug release of Tretinoin from optimized ethosomes formulation

Time (hrs)	% Cumulative Drug Release (Marketed formulation)	% Cumulative Drug Release Ethosomal gel
0.5	45.65±0.45	33.25±0.20
1	86.56±0.89	45.59±0.31
2	99.78±0.78	65.56±0.43
4	-	72.25±0.66
6	-	85.65±0.69
8	-	93.36±0.45
10	-	99.15±0.36

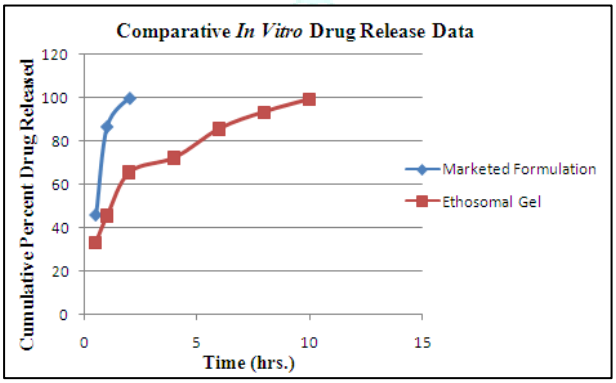

Figure 1: Comparative in vitro drug release, Cumulative Percent Drug Released Vs Time

Table 6: *In Vitro* Drug Release Data for optimized gel formulation F2

S. No.	Time (H)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release ± SD	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
1	0.5	0.707	-0.301	33.25	1.522	66.75	1.824
2	1	1.000	0.000	45.59	1.659	54.41	1.736
3	2	1.414	0.301	65.56	1.817	34.44	1.537
4	4	2.000	0.602	72.25	1.859	27.75	1.443
5	6	2.449	0.778	85.65	1.933	14.35	1.157
6	8	2.828	0.903	93.36	1.970	6.64	0.822
7	10	3.162	1.000	99.15	1.996	0.85	-0.071

* Average of three determinations

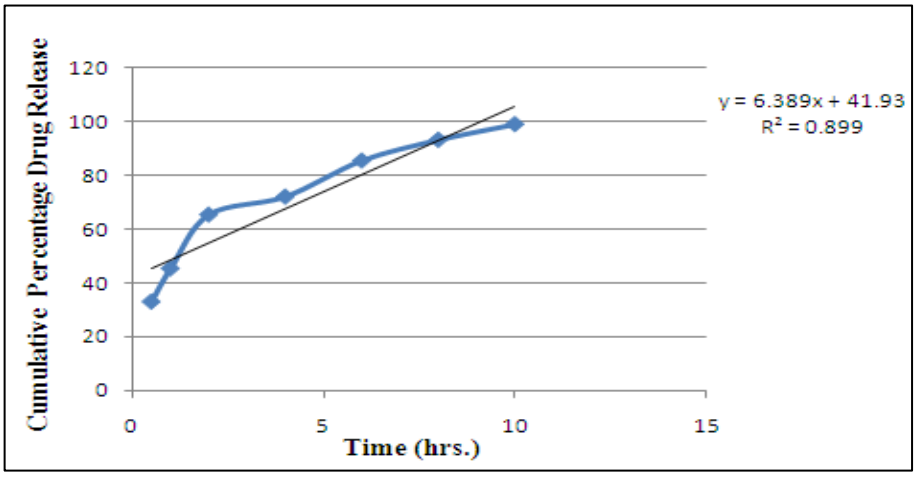

Figure 2: Cumulative Percent Drug Released Vs Time (Zero Order Plots)

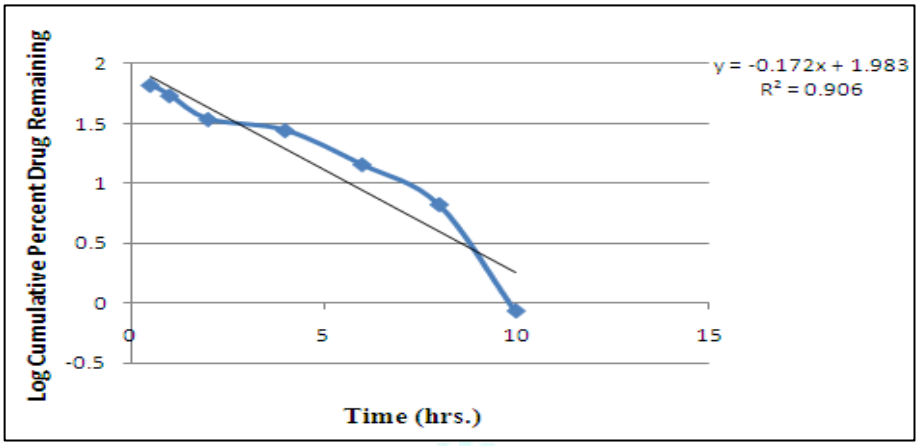

Figure 3: Log Cumulative Percent Drug Remaining Vs Time (First Order Plots)

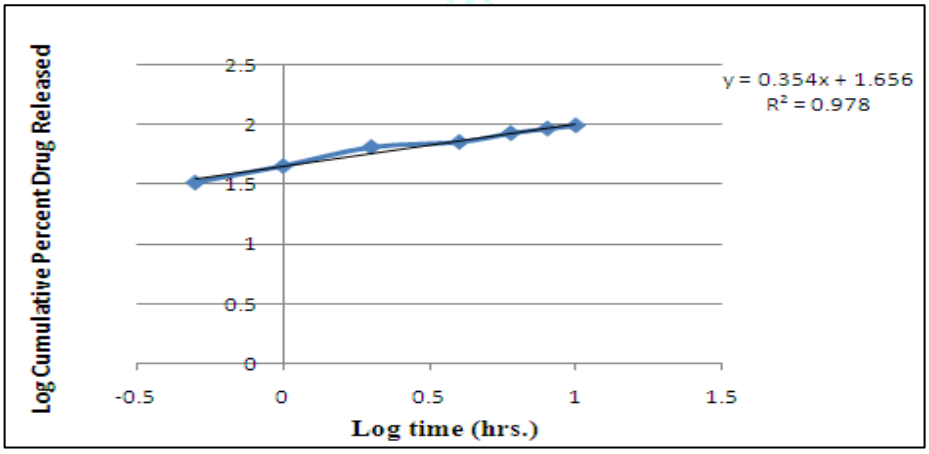

Figure 4: Log Cumulative Percent Drug Released Vs Log Time (Peppas Plots)

CONCLUSION

In this paper, ethosome formulations containing tretinoin as a lipophilic drug have been prepared successfully and characterized. It was observed that the presence of ethanol in the aqueous compartment of the vesicles favoured tretinoin encapsulation. Therefore, the inclusion of ethanol in ethosome might play a vital role in the enhancement of tretinoin permeation. The efficient drug delivery shown here together with the long-term stability of ethosomes made this system a promising candidate for topical delivery of tretinoin. Currently, further studies involving safety on skins, pharmacological effect on AD, and mechanism of inhibition action for the ethosomal delivery systems were being performed.

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