Original Article

Investigation of ethosomes as surrogate carriers for bioactives

Abstract

Background: Ethosomal vesicular system delivering a bioactive phytochemical, chrysin, was developed for transdermal delivery to increase its permeability and penetrability. **Materials and Methods:** Ethosomal system was optimized by keeping lecithin and ethanol concentration as independent variable while size and size distribution were taken as dependent variables. The optimized formulation was then subjected to various *in vitro* characterization parameters. **Results:** Ethosomal vesicle with an optimum size and polydispersity index of 134 ± 35 nm and 0.153, respectively, and entrapment efficiency of 80.05 ± 2.6% was considered as optimized and subjected to characterization. The scanning electron microscopy and transmission electron microscopy showed spherical entities with uniform surface whereas *in vitro* permeation and retention study showed the sustained mode of drug release and better skin retention as compared to hydroethanolic solution of the drug. The confocal laser scanning microscopy study reiterated high penetrability of vesicles into the skin. Histopathological and Fourier transform infrared spectroscopy analysis revealed its mechanism of penetration. **Conclusion**: The study thus demonstrated the ability of the ethosomal vesicles as surrogate carriers for delivery of bioactive agents through the skin for better amelioration of skin inflammation and other diseases.

Key words:
Bioactive, chrysin, ethosomal system, transdermal

Introduction

During the past two decades, transdermal drug delivery system has received much attention for circumventing ills, such as drug toxicity, bioavailability, first-pass metabolism, fluctuations in plasma levels, and achieving targeted localized effect, but the barrier nature of skin poses substantial challenge for dug penetration, permeation, and retention. Recent research has revealed an improved transdermal delivery through the development of phospholipid vesicular systems with increased elasticity called ethosomes.[1] Unlike liposomal vesicles and other classical transdermal systems, which become trapped within the top layer of stratum corneum (SC) cells, ethosomes penetrate the skin and allow enhanced delivery of various compounds to the deep strata of the skin or to the systemic circulation. In the present study, ethosomal vesicles were investigated for delivering a bioactive phytochemical, chrysin, natural flavone, an antioxidant which ameliorates ultraviolet (UV) induced oxidative stress in skin.

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Materials and Methods

Chemicals and reagents

Phospholipon 90 G was obtained as a gift sample from lipoid, Germany. Chrysin was purchased from Sigma-Aldrich Co.,

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USA. All other chemicals and reagents were from S. D. Fine Chemical, India.

Formulation of ethosomal vesicles

The ethosomal vesicular system was prepared by hot method.[2] Varying proportions of lecithin was dispersed in double distilled water maintained at 40°C. To this, different concentrations of ethanol with or without drug were added dropwise with continuous stirring in an in-house closed assembly to prevent the evaporation of ethanol. It was then allowed to cool at room temperature and centrifuged to obtain the ethosomal vesicles.

Characterization of ethosomal vesicles

Vesicle size and size distribution

The mean size and size distribution of ethosomal vesicles were analyzed by dynamic light scattering technique using Zetasizer 1000 h (Malvern instruments, UK). The sample was placed in a quartz cuvette diluted with distilled water, and size measurements were carried out at a scattering angle of 90°.

Vesicular shape and surface morphology

Scanning electron microscopy (SEM) was conducted to characterize the surface morphology of the ethosomal vesicles. One drop of ethosomal system was mounted on clear glass stub, air dried, and coated with Polaron E 5100 Sputter and visualized under SEM (JEOL JSM 840, USA).

Transmission electron microscope (Philips, CM-10 model) was used as a visualizing aid for ethosomal vesicles. Samples were dried on carbon-coated grid and negatively stained with an aqueous solution of phosphotungstic acid. After drying, the specimen was viewed under the microscope at 10–100 k-fold enlargements at an accelerating voltage of 100 KV.

Entrapment efficiency

Entrapment efficiency of chrysin loaded ethosomal vesicles was determined by ultracentrifugation method. The ethosomal suspension was centrifuged, and ethosomal vesicles were washed with water. The vesicles were then lysed by using Triton X-100 (0.5% v/v) and filtered through a 0.45 μm nylon disk filter. The entrapped drug content was analyzed by UV spectroscopy at 270 nm.

In vitro skin permeation and skin deposition

The rats were sacrificed, and the abdominal skin was removed. The hair and the adhering fat were carefully removed. The skin was mounted on the receptor compartment of the Franz diffusion cell. The receptor cell containing phosphate buffer saline pH 7.4 (30:70, v/v) and was constantly stirred. The donor compartment contained 1.0 mL of ethosomal formulations. Experiments were carried out for 24 h at 32°C ± 1°C. The study was also repeated with donor cell containing hydroethanolic solution of chrysin. Samples were withdrawn at regular intervals and analyzed for drug content by UV spectroscopy at 270 nm. On termination of experiment, the skin was washed, weighed, cut with scissors, and homogenized using Polytron homogenizer (Kinematica, Inc., Switzerland). The resulting solution was centrifuged, and the supernatant was analyzed for drug content by UV spectroscopy to determine the drug retained in the skin.

Depth of skin penetration

To determine the mechanism and extent of penetration of the ethosome through the skin, confocal scanning laser microscopy was performed**.** The rat skin was treated with ethosomes loaded with Rhodamine B dye for 24 h and then observed under confocal microscope with Fluorescence correlation spectroscope-Olympus Fluoview FV1000 with excitation at 488 nm and emission at 590 nm. The comparison was made with the skin treated with Rhodamine B hydroethanolic solution.

Vesicle skin interaction

Histopathological study of ethosomal treated and untreated skin

The ethosomal formulation was applied topically on the skin of rats for 24 h. After sacrificing the animals, skin was excised and fixed in buffered formalin (10% w/v). Sections of skin were then cut and stained with hematoxylin and eosin stain. Histological changes in SC, epidermis, and dermis were then examined under an optical microscope and compared with the control sample of skin.

Fourier transform infrared spectroscopy analysis of the ethosomal treated and untreated skin

Human skin (residual samples) was obtained from Sir Ganga Ram Hospital, New Delhi, India. SC was obtained by digesting the skin with trypsin.[3] The skin was treated with the formulation for 24 h. After treatment, the skin was removed and air dried for 3 h. Fourier transform infrared spectroscopy (FTIR) spectra of the treated and the untreated skin were recorded in the frequency range of 400–4000 cm^{-1} (Perkin Elmer, Germany).

Results

Lecithin and ethanol concentration influences vesicle size and entrapment efficiency

A total of 9 experimental runs were carried out [Table 1]. Soya lecithin was taken in a concentration varying from 2% to 3% w/w whereas the amount of ethanol was kept between 30 and 45% w/w. The dependent variable, i.e., particle size and polydispersity index demonstrated the conspicuous effect of ethanol concentration. The size of drug-loaded vesicles decreased with an increase in ethanol concentration, which may be attributed to the net negative charge induced by ethanol on the surface of the vesicles which gives it some degree of steric stabilization

Table 1: Optimization of placebo ethosomal vesicles

Formulation code	Soya lecithin (% w/v)	Ethanol (% w/v)	Water (% w/v)	Particle size (nm) ($n=3$)	PDI
CE 1	2.0	30	QS	170.13±11	0.124
CE 2	2.5	30	QS	175.71±20	0.115
CE 3	3.0	30	QS	179.73±20	0.140
CE 4	2.0	35	QS	159.79±17	0.124
CE 5	2.5	35	QS	161.51±20	0.146
CE 6	3.0	35	QS	169.54±23	0.115
CE 7	2.0	40	QS	116.60±15	0.151
CE 8	2.5	40	QS	124.30±12	0.190
CE 9	3.0	40	QS	134.40±19	0.153

QS – Quantity sufficient; PDI – Polydispersity index

and prevents it from aggregation.[4,5] However, increasing the ethanol concentration beyond 40% w/w yielded bigger particle size (324–428 nm), which could preclude its dermal penetration (data not shown). The size of the vesicles, however, did not vary with the lecithin concentration and was found to be comparable as the amount of lecithin was increased from 2% to 3%. Further on encapsulating chrysin, ethosomal vesicles (3% lecithin, 40% ethanol) with highest entrapment efficiency of 80.05 ± 2.60 and a corresponding size of 134 ± 35 nm was taken as final optimized formulation and was subjected for further characterization.

Characterization of ethosomal vesicles

The micrographs obtained by transmission electron microscopy [Figure 1a] and SEM [Figure 1b] analysis revealed the presence of spherical shaped entities with smooth and uniform morphology. *In vitro* release study showed sustained release of chrysin from the vesicles over 24 h in comparison to hydroethanolic solution of chrysin. Moreover, ethosomal systems also led to better skin deposition of 15.2% as compared to drug solution (4.23%). The confocal laser scanning microscopy (CLSM) showed the ability of the ethosomal vesicles to transverse the skin thickness to an extent of approximately 111 µm [Figure 2a] as compared to Rhodamine B solution, which was approximately 18 µm [Figure 2b]. This confirms the previous findings that ethosomes penetrate the skin by disrupting the lipid barrier of skin and reach the deeper layer of the skin.

Vesicle skin interaction

Histopathology study of the ethosomal treated and untreated skin was done to determine the safety of the formulation. Thinning of epidermal layer in the treated skin demonstrated the mechanism of penetration of the vesicles by disruption and extraction of lipid bilayer of the skin [Figure 3]. It also showed that ethanol did not cause any irritant effect as no signs of edema or erythema on the skin were observed. FTIR analysis of the SC depicts the fluidity of the proteins and lipids present in the SC. The bands at 2920 cm^{-1} and 2850 cm^{-1} were due to the asymmetric-CH_2 and symmetric-CH_2 vibrations of long chain hydrocarbons of lipids, respectively.[6] There was clear difference in the

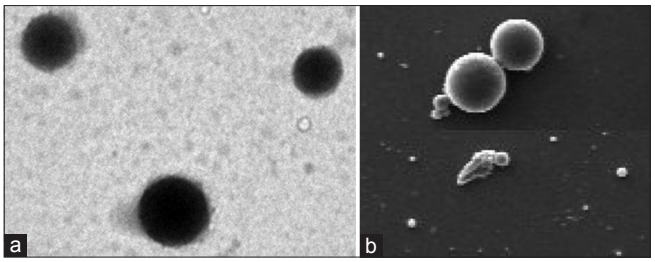

Figure 1: Transmission electron microscopy micrographs of optimized formulation (a); scanning electron microscopy micrographs of optimized formulation (b)

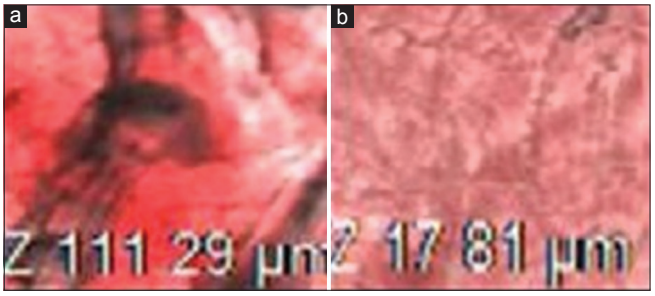

Figure 2: Confocal laser scanning microscopy images of cross sections of abdominal rat skin (a) after treating with Rhodamine B loaded ethosomal vesicles (b) after treating with hydroethanolic Rhodamine B solution

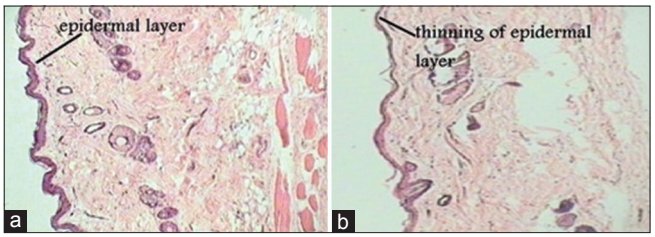

Figure 3: Histopathological sections of abdominal skin of rat (a) untreated (b) skin treated with ethosomal vesicles

FTIR spectra of untreated and treated SC with prominent decrease in asymmetric and symmetric CH-stretching of peak height and area indicating the permeation of the ethosomal vesicles via extraction of the SC lipids.[6]

Discussion

The size of drug-loaded vesicles decreased with an increase in ethanol concentration, which may be attributed to the net negative charge induced by ethanol on the surface of the vesicles which gives it some degree of steric stabilization and prevents it from aggregation.[4] However, increasing the ethanol concentration beyond 40% w/w yielded bigger particle size (324–428 nm), which could preclude its dermal penetration. *In vitro* release study showed sustained release of chrysin from the vesicles over 24 h in comparison to hydroethanolic solution of chrysin. Moreover, ethosomal systems also led to better skin deposition of 15.2% as

compared to drug solution (4.23%). The CLSM showed the ability of the ethosomal vesicles to transverse the skin thickness to an extent of approximately 111 μm. This confirms the previous findings that ethosomes penetrate the skin by disrupting the lipid barrier of the skin and reach the deeper layer of the skin. Histopathology study of the ethosomal treated and untreated skin was done to determine the safety of the formulation. Thinning of epidermal layer in the treated skin demonstrated the mechanism of penetration of the vesicles by disruption and extraction of the lipid bilayer of the skin.

There was clear difference in the FTIR spectra of untreated and treated SC with prominent decrease in asymmetric and symmetric CH-stretching of peak height and area indicating the permeation of the ethosomal vesicles via extraction of the SC lipids.[6]

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Nil.

Conflicts of interest
There are no conflicts of interest.

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