

Formulation and Performance Evaluation of *Calendula Officinalis* Linn Extract Loaded Ethosomal Cream

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ABSTRACT

Among the various species of the genus Calendula, C. officinalis is the only one, which is extensively used clinically throughout the world. Pharmacological studies reveal that C. officinalis exhibits antibacterial, antiviral, anti-inflammatory, anti-tumor and antioxidant properties, helps promote the healing of minor burns, scrapes and skin irritations and relieves sunburn and minor cuts. The objective of this study was to optimize conditions for encapsulating Calendula officinalis Linn extract. Thus a novel approach to effectively treat wrinkles, helps promote the healing of minor burns, scrapes and skin irritations and relieves sunburn and minor cuts by delivery of an antioxidant using a special lipid vesicular carrier, the Ethosomal cream was prepared.

Keywords: *Ethosomes, C. officinalis, Lipid vesicular carrier, Characterization, Evaluation.*

1. INTRODUCTION

C. officinalis has been included in number of herbal formulations, which are in clinical use for the treatment of various ailments like central nervous system disorders¹. Keeping in view the ethnopharmacology, phytochemical and pharmacological reports, low toxicity and frequency of use, *C. officinalis* seems to hold great potential for in depth investigation for various biological activities²⁻⁴. It difficult to formulate in an acceptable, stable composition for cosmetic use. Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation⁵⁻⁸. These are soft, malleable vesicles tailored for improved delivery of active agents. They are composed mainly of phospholipids, (phosphatidylcholine, phosphatidylserine, phosphatidic acid), high concentration of ethanol and water. Since *Calendula* contents are hydrophobic, it is considered to be a good candidate for ethosomal

incorporation, as it can be encapsulated in the lipid layer of the ethosomes⁹⁻¹². These observations facilitate the doorway of novel delivery systems in the development of antiwrinkle creams etc. There is no such Ethosomal cream formulation available in market which shows better efficacy. Considering the fact, that to treat wrinkles antioxidant has to reach to the level of dermis; *Calendula officinalis* Linn extract ethosomal application of on the skin can offer the advantage of delivering the antioxidant directly to the site and produce prompt effects. Ethosomal carriers are systems containing soft vesicles¹³⁻¹⁵. They are composed mainly of phospholipid (phosphatidylcholine) and ethanol at relatively high concentrations with water as non solvent. Ethanol interacts with lipid molecules in the polar head group region, resulting in a reduction in the phase transition temperature (T_m) of the stratum corneum lipids, increasing their fluidity¹⁶⁻²¹. The intercalation of ethanol into the polar head group environment results in an increase in the membrane permeability. In addition to the effects of ethanol on stratum corneum structure, the ethosome itself interacts with the stratum corneum barrier. Ethanol also provides the vesicles softness and flexibility²²⁻²⁵. Ethosomes are phospholipid-based elastic vesicles containing 20–45% ethanol and water. For the preparation of elastic vesicles; ethanol is a proven permeation enhancer that has been added in the vesicular systems. High flexibility imparted by ethanol of vesicular membranes permits the elastic vesicles to squeeze themselves through the pores²⁶⁻³⁴. The proposed mechanism of penetration enhancement with the ethosomal system suggests the intercalation of ethanol into the polar head group environment resulting in increased membrane permeability. With respect to stability, Ethosomes have been reported to be more stable than liposomes because of the presence of ethanol, which provides a net negative charge on the surface, which helps to avoid aggregation of vesicles due to electrostatic repulsion³⁵⁻⁴². Topically applied ethosomes can increase the residence time of active ingredients in the stratum corneum, epidermis and reduce the systemic absorption of drugs. These properties allow Ethosomes to permeate easily into the deeper layers of the skin⁴³⁻⁴⁸. Ethanol is an established efficient permeation enhancer and is present in quite high concentration (20-50%) in ethosomes. However, due to the interdigitation effect of ethanol on lipid bilayers, it was commonly believed that vesicles could not coexist with high concentration of ethanol⁴⁹⁻⁵⁴. Touitou discovered and investigated lipid vesicular systems embodying ethanol in relatively high concentration and named them ethosomes. The basic difference between liposomes and ethosomes lies in their composition. The synergistic effect of combination of relatively high concentration of ethanol

(20- 50%) in vesicular form in ethosomes was suggested to be the main reason for their better skin permeation ability⁵⁵⁻⁵⁸. The high concentration of ethanol (20-50%) in ethosomal formulation could disturb the skin lipid bilayer organization. Therefore, when integrated into a vesicle membrane, it could give an ability to the vesicles to penetrate the SC. Furthermore, due to high ethanol concentration the ethosomal lipid membrane was packed less tightly than conventional vesicles but possessed equivalent stability. This allowed a softer and malleable structure giving more freedom and stability to its membrane, which could squeeze through small openings created in the disturbed SC lipids. In addition, the vesicular nature of ethosomal formulations could be modified by varying the ratio of components and chemical structure of the phospholipids. The versatility of ethosomes for systemic delivery is evident from the reports of enhanced delivery of quite a few drugs like acyclovir, minoxidil, trihexyphenidyl, testosterone, cannabidiol and zidovudine⁵⁹⁻⁶².

2. MATERIALS AND METHODS

Acquisition of samples

Authentication of Calendula officinalis Linn

Authenticated samples were purchased from the local herb dealer and were again authenticated by the Department of Botany, Yashwantrao Chavan College of Science, Karad.

Preparation of extracts

Extraction of *Calendula officinalis* Linn was done by continuous hot extraction method by 95% v/v ethyl alcohol and 85% v/v ethyl alcohol at a temperature of 60 °C until complete exhaustion of the drug.

Preparation of Ethosomes

Ethosome colloidal suspensions were made up of 1–3% (w/v) soya phosphatidylcholine, 30–50% (v/v) ethanol, *Calendula officinalis* extract, ethosomes were prepared on trial and error batches. Hot method of preparation is used for ethosome formulation.

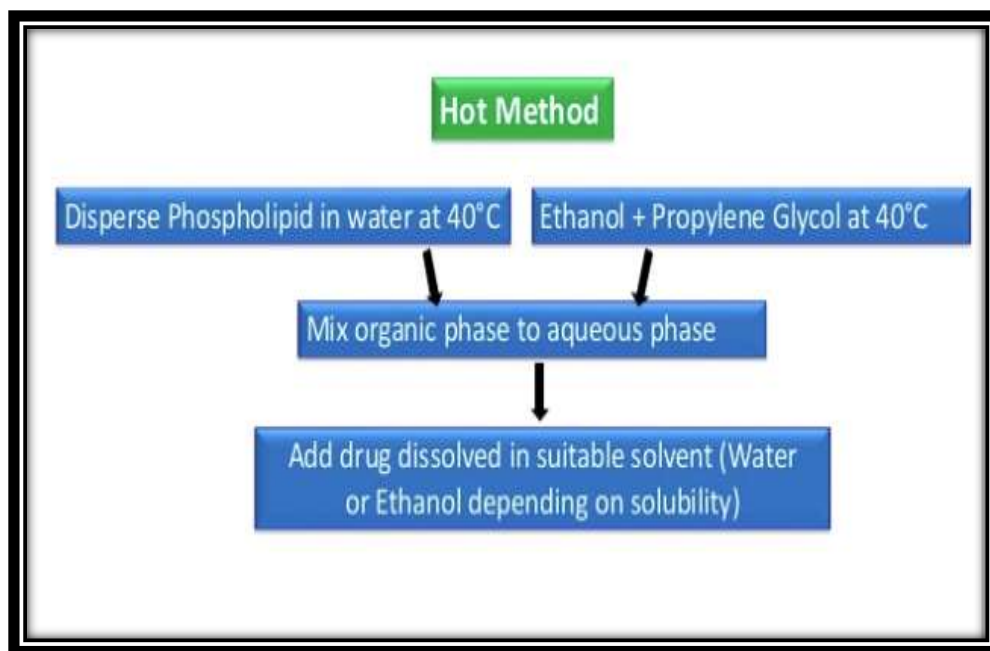


Figure 1. Preparation of Ethosomes

Characterization of Ethosomes

Morphological characterization of optimized vesicle was done by using a digital microscope with camera at 40x resolution.

Entrapment efficiency

Vesicle size, size distribution and zeta potential analysis-Zeta potential was a measure of colloidal property of ethosome which affects the permeation and stability of vesicles.

Preparation of cream

The phase inversion technique was used to prepare the cream base Briefly, lipid phase (olive oil, cetyl alcohol, stearic acid, sorbitanmonooleate, propylene glycol and glycerin) and aqueous phase (Tween 80 and water) was separately heated to 75 °C and then the hot water phase was added to the lipid phase under constant stirring at 600 rpm depending on trial and error batches. After complete melting and homogenous mixing, temperature was decreased to 30°C and ethosome suspension was added at an increased speed (275 ± 25 rpm). Few drops of rose oil was added during stirring to give good odor to the formulation. The base was prepared by the same method as used in the formulation.

Physicochemical evaluation of cream

Evaluations of the creams

Organoleptic properties, pH, homogeneity, viscosity, and rheology were evaluated.

In vitro penetration tests

Skin penetration tests were conducted using Franz diffusion cells with membrane areas of 2.01 cm² and a receptor compartment containing 15 mL of phosphate buffer at pH 5.5. The liquid compartment was maintained at 37°C±0.5°C and was stirred with a magnetic stirrer at 250 rpm. The skin specimens were placed between the donor and receptor compartments with the stratum corneum facing upward. Subsequently, 1.0 g samples of the creams were applied to the skin surfaces, and up to 2.0 mL samples were taken from the receptor compartment using a syringe after 10, 30, 60, 120, 240, 360, 480, 600, 720, 840, 960, 1080, 1200, 1320, and 1440 min. The EGCG concentrations were then determined in these samples using HPLC. In vitro penetration tests were performed 3 times.

3. RESULTS AND DISCUSSION

Extraction and Yields

Table 1. Solvents, extraction methods and respective yield

Sr. no.	Extract	Solvents	Colour	% yield w/w
1.	Soxhlet extraction	Ethanol	green	10.35±0.87

Characterization of cream

Microscopy

Optical microscopy was done with a digital microscope with camera in 40x resolution.

Qualitative chemical investigation

The results of qualitative chemical investigation of *Calendula officinalis* indicated the presence of mainly sterols, terpenoids, flavonoids, phenolic compounds and tannins etc.

Table 2. Qualitative chemical investigation of *Calendula officinalis*

Chemical Constituents	<i>Calendula officinalis</i>
Alkaloid (Dragendroff Test)	++
Glycosides (Borntragers Test)	+
Flavonoid (Shinoda Test)	+
Steroid (Salkovaski Test)	+
Saponins (Foam Test)	+
Carbohydrates (Molisch Test)	-
++ present - Absent	

Preparation of Ethosomes

Ethosome colloidal suspensions was made up of 1–3% (w/v) soya phosphatidylcholine, 30–50% (v/v) ethanol, *Calendula officinalis* extract, ethosomes was prepared. Hot method of preparation is used for ethosome formulation. The phase inversion technique was used to prepare the cream base Briefly, lipid phase (olive oil, cetyl alcohol, stearic acid, sorbitanmonooleate, propylene glycol and glycerin) and aqueous phase (Tween 80 and water) was separately heated to 75 °C and then the hot water phase was added to the lipid phase under constant stirring at 600 rpm depending on trial and error batches. After complete melting and homogenous mixing, temperature was decreased to 30 °C and ethosome suspension was added at an increased speed (275 ± 25 rpm). Few drops of rose oil was added during stirring to give good odor to the formulation. The base was prepared by the same method as used in the formulation.

Vesicular Size and Shape Analysis

Developed ethosomes were evaluated for size and shape by using optical microscopy method. Developed ethosomal vesicles were soft and spherical in shape.

Drug Encapsulation Efficiency

The percentage drug leakage from developed ethosomes were very less (< 6%) at refrigerated condition for the entire duration of the study.

Evaluation of cream

Organoleptic tests showed pale yellow color.. The creams were homogeneous when applied and had pH values of 5.40 and 5.45, respectively, indicating compatibility with the physiological pH of skin and the stability. The viscosity values of the creams were 11,800 and 11,200 cps, respectively, and both creams had thixotropic plastic rheology properties.

In vitro penetration tests

After applying the topical preparations, the cumulative amounts of EGCG that penetrated the Franz diffusion cells were $2442.57 \pm 93.47 \mu\text{g}/\text{cm}^2$ for ES, $3897.67 \pm 1380.29 \mu\text{g}/\text{cm}^2$ for ethosome suspensions (ET), $413.92 \pm 52.83 \mu\text{g}/\text{cm}^2$ for CE, and $905.75 \pm 49.47 \mu\text{g}/\text{cm}^2$ for CET. We also calculated the flux of the active substance (EGCG) through rat skin and expressed these data as circulating EGCG per unit area per unit time. The flux values for ES, ETS, CE, and CET were 109.032 ± 21.969 , 131.65 ± 66.03 , 12.66 ± 1.45 , and $40.96 \pm 5.56 \mu\text{g}.\text{cm}^{-2}/\text{h}$, respectively. Based on these results, we suggest that CET achieves better penetration than CE. In addition, the ethosomal suspension had better penetration than the ES, indicating that ethosomes in topical creams can improve EGCG penetration through rat skin. The present ethosomal suspensions have a high ethanol content, which enhances penetration through hydrogen bond interactions with the phospholipid layers of the stratum corneum. Ethanol also facilitates the passage of the active ingredient into deeper skin layers by enhancing flexibility of following fusion of the ethosome vesicles with lipids in skin membranes

4. CONCLUSION

Ethosomes elicit potential to deposit *Calendula officinalis* Linn into the deeper layers of skin in order to exert its antioxidant effect. *Calendula officinalis* linn entrapment was significantly improve with ethanol extract (% of ethanol on batches was determined on trial and error batches). It improved in overall elasticity, biological elasticity, recovery of deformed skin, firmness and reduction in fatigue which can be correlated with anti wrinkle properties of cream.

These beneficial effects might have been due to the synergistic antioxidant, anti-inflammatory and protective properties of the constituents of extract and hydrant, moisturizing and lipid components of ethosomes and cream. Therefore, it may be conclude that proposed cream is multipurpose use as anti-wrinkle cream, Sun screen cream, protective skin irritation cream, Wound healing cream which was more effective.

5. ACKNOWLEDGMENTS

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