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# Dermal and transdermal delivery of an anti-psoriatic agent via ethanolic liposomes

Vaibhav Dubey [a,*], Dinesh Mishra [a], Tathagata Dutta [a], Manoj Nahar [a], D.K. Saraf [b], N.K. Jain [a]

[a] Pharmaceutics Research Laboratory, Department of Pharmaceutical Sciences, Dr. Hari Singh Gour University, Sagar (M.P.), 470003, India
[b] Department Of Zoology, Dr. Hari Singh Gour University, Sagar (M.P.), 470003, India

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**Abstract**

The aim of the current investigation is to evaluate the transdermal potential of novel vesicular carrier, ethosomes, bearing methotrexate (MTX), an anti-psoriatic, anti-neoplastic, highly hydrosoluble agent having limited transdermal permeation. MTX loaded ethosomes were prepared, optimized and characterized for vesicular shape and surface morphology, vesicular size, entrapment efficiency, stability, in vitro human skin permeation and vesicle-skin interaction. The formulation ($EE_9$) having 3% phospholipid content and 45% ethanol showing the greatest entrapment (68.71±1.4%) and optimal nanometric size range (143±16 nm) was selected for further transdermal permeation studies. Stability profile of prepared system assessed for 120 days revealed very low aggregation and growth in vesicular size (8.8±1.2%). MTX loaded ethosomal carriers also provided an enhanced transdermal flux of 57.2±4.34 μg/cm$^2$/h and decreased lag time of 0.9 h across human cadaver skin. Skin permeation profile of the developed formulation further assessed by confocal laser scanning microscopy (CLSM) revealed an enhanced permeation of Rhodamine Red (RR) loaded formulations to the deeper layers of the skin (170 μm). Also, the formulation retained its penetration power after storage. Vesicle skin interaction study also highlighted the penetration enhancing effect of ethosomes with some visual penetration pathways and corneocytes swelling, a measure of retentive nature of formulation. Our results suggests that ethosomes are an efficient carrier for dermal and transdermal delivery of MTX.


*Keywords:* Psoriasis; Methotrexate; Ethosomes; Confocal laser scanning microscopy

## 1. Introduction

Psoriasis, a T-lymphocyte mediated autoimmune disease of the dermis and epidermis is characterized by leukocyte infiltration into the skin and localized deregulated skin growth, which leads to the development of scaling erythematous plaques. Methotrexate (MTX), a dihydrofolate reductase enzyme inhibitor is used at high doses in the treatment of certain types of neoplasias. MTX is also useful as an immunosuppressant and anti-inflammatory agent, even at low doses and thus have potential in controlling recalcitrant psoriasis and adult rheumatoid arthritis, in a long-term therapy. MTX has been shown to selectively inhibit DNA synthesis in psoriatic epidermal cells, thus decreasing mitotic activity [1–4]. However, unacceptable low bioavailability, high inter-individual variability and severe GI side effects such as nausea and vomiting, limits its oral use at low and intermittent doses. Thus, oral MTX is often substituted by self-administered subcutaneous or intramuscular injections. Nevertheless, hepatotoxic and CNS effects precipitate on systemic use of this drug [5]. MTX enhances adenosine delivery in the CNS and its activation of A1 receptors in the brain can be responsible for induction of fatigue, lethargy, and headache [6,7].

To reduce such effects, it would clearly be preferable to administer MTX topically [8]. In spite of several advantages offered by transdermal route, only a few molecules are administered topically/transdermally because of formidable barrier nature of stratum corneum (SC). A major problem in this case is the hydrosolubility of the drug and its high molecular weight (454.56 g: mol). Also, the presence of drug mostly in dissociated form at physiological pH further limits its capacity for passive

* Corresponding author. Tel./fax: +91 7582264712.
*E-mail addresses:* jnarendr@yahoo.co.in, rxvaibhav@yahoo.com (V. Dubey).

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diffusion. However, techniques such as iontophoresis, electroporation or the use of appropriate vehicles such as hydrogels or microemulsions may enhance transdermal delivery of this drug [9–11].

Despite the ineffectiveness of systemically administered MTX in the treatment of psoriasis, clinical results with local therapy have been disappointing [12]. The enhanced delivery of various therapeutic agents via human cadaver skin mediated by ethanolic liposomes (ethosomes) has been encouraging [13–17]. The present work thus, focuses on developing novel ethosomal formulation with respect to dermal and transdermal delivery of MTX for the possible therapy of psoriasis and exploring possible mechanisms of better skin penetration of ethosomal carrier.

## 2. Materials and methods

Soya phosphatidylcholine (PC) (99%), phosphotungstic acid was purchased from Sigma, St. Louis MO USA. Rhodamine Red-X 1, 2 dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine trimethylammonium salt (RR) was purchased from Molecular Probes (Eugene, Oregon, USA). MTX was received as a gift sample from Dabur Ltd, India. All other solvents were of HPLC grade and triple distilled water was used wherever required.

### 2.1. Preparation of MTX loaded vesicles

Classic mechanical-dispersion method was followed to develop the MTX loaded ethosomes [18]. Briefly, Soya PC (1.0–3.0% w/v) was dissolved in chloroform:methanol (3:1) mixture in a clean, dry round bottom flask followed by removal of the organic solvents using rotary vacuum evaporator above the lipid transition temperature (Rotary Evaporator, Superfit, Ambala, India) to form a thin lipid film on the wall of the flask. Finally, the traces of solvent mixture were removed from the deposited lipid film under vacuum overnight followed by hydration with different concentration of hydroethanolic mixture containing MTX (1.0% w/v) by rotation (60 rpm, 1 h) at the corresponding temperature. The preparation was vortexed followed by sonication at 4 °C using probe sonicator (at 40 W, Imeco, Ultrasonics, Mumbai) in 3 cycles of 5 min with 5 min rest between the cycles. Similarly, RR loaded ethosomes were prepared by classic mechanical–dispersion method. In this case, Soya PC (3.0% w/v) and RR (0.03%) were dissolved in chloroform:methanol (3:1) mixture and a similar procedure were followed as described in the preparation of ethosomes.

MTX loaded conventional liposomes were prepared by Cast film method [19]. Briefly, Soya PC (3.0% w/v) was dissolved in chloroform:methanol (3:1) mixture in a clean, dry round bottom flask followed by removal of the organic solvents using rotary vacuum evaporator above the lipid transition temperature to form a thin film on the wall of the flask. After removal of solvent traces, thin lipid film was hydrated with 0.2 M phosphate buffer (pH 6.5) containing MTX (1.0% w/v) by rotation (60 rpm, 1 h) at the corresponding temperature, followed by sonication as described in the case of ethosomes. 45% hydroethanolic solution of drug acted as control.

### 2.2. Vesicular characterization

Transmission electron microscope (TEM) (Philips CM12 Electron Microscope, Eindhoven, Netherlands) was used as a visualizing aid for ethosomal vesicles. Scanning electron microscopy (SEM) (Leo-435 VP, Cambridge UK) was also conducted to characterize the surface morphology of the ethosomal vesicles. The size distribution of ethosomes was done in two sets of triplicates, in a multimodal mode, by Dynamic Light Scattering (DLS) technique using a computerized Malvern Autosizer 5002 inspection system (Malvern, UK). For vesicles size measurement, vesicular suspension was mixed with the appropriate medium (PBS, pH 6.5) and the measurements were taken in triplicate [20].

Prepared ethosomal vesicles were further characterized for entrapment efficiency by a Sephadex G-50 minicolumn centrifugation technique [21,22]. The method was repeated at least three times with a fresh syringe packed with gel each time till the fraction collected was free from unentrapped drug. The vesicles were lysed by Triton X-100 (0.5% w/w) and entrapped drug was estimated using HPLC.

### 2.3. Confocal laser scanning microscopy (CLSM)

Depth and mechanism of skin penetration of RR loaded ethosomes was investigated using CLSM, as reported previously [20]. Briefly, unentrapped probe was removed from probe-loaded vesicles by minicolumn ultracentrifugation thereafter formulation was applied non-occlusively for 8 h to the dorsal skin of 5–6 week old nude albino rat (Sprague Dawley strain). The rat was then sacrificed by heart puncture; dorsal skin was excised, washed, placed on aluminium foil and adhering fat and/or subcutaneous tissue was removed. The skin was then sectioned into the pieces of 1 $mm^2$ size and evaluated for depth of probe penetration for various formulations. The full skin thickness was optically scanned at different increments through the *z*-axis of a CLS microscope (LSM 510 with an attached universal Zeiss epifluorescence microscope). All investigations were performed as per the protocol approved by the Institutional Animals Ethical Committee of Dr. H. S. Gour University, Sagar, India.

### 2.4. In vitro drug release studies (skin permeation studies)

The in vitro skin permeation of MTX loaded ethosomal formulations was studied using locally fabricated Franz diffusion cell with an effective permeation area and receptor cell volume of 1.0 $cm^2$ and 10 ml, respectively. The temperature was maintained at 32±1 °C. The receptor compartment contained 10 ml PBS (pH 6.5) and was constantly stirred by magnetic stirrer (Expo India Ltd., Mumbai, India) at 100 rpm. Dermatomed (500 μm thickness) human cadaver skin from abdominal areas were obtained from District Hospital, Sagar, India and stored at −20 °C. The skin was then carefully checked through a magnifying glass to ensure that samples were free from any surface irregularity such as tiny holes or crevices in the portion that was used for transdermal permeation studies. After assurance, the skin was mounted on a receptor compartment with the stratum corneum side facing upward into the donor compartment. The ethosomal formulation (250 μl) was