Potential Therapeutic Applications of Advanced Novel Carrier Based Drug Delivery System: Ethosomes

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Abstract - There are numerous forms of administration methods including oral, transdermal, lung inhalation, mucosal, and intravenous injection, depending on the delivery route. The transdermal drug delivery system (TDDS) is one appealing strategy among them.

TDDS has emerged as one of the most extensively researched methods for noninvasive drug delivery into the body through the skin. TDDS has had a big impact on the way that several medicinal substances, particularly in the treatment of disorders of the central nervous system and cardiovascular system, hormone therapy, and pain management.

With almost 12% of the worldwide medication delivery market, transdermal dosage forms are really the most effective nonoral systemic drug delivery methods.

Nanocarriers is an advancement in the nanotechnology used in pharmaceutical sciences that are an effective therapeutic agents for controlled and specific delivery of drug molecules to tissues and cells . Ethosomes are one of the non invasive novel nanocarriers that are mainly made up of ethanol thus are biodegradable and biocompatible.

In this article we discuss the potentials of ethosomes as a therapeutic agent with examples of various drug loaded ethosomes currently used in the medical era.

Index terms – transdermal drug delivery system , nanocarriers, ethosomes , therapeutic application.

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1. Introduction

Recent research has led to the development of transdermal drug delivery systems (TDDS), which aim to administer systemic medication through topical administration on the entire skin surface. When given the topical application, self-contained and discontinuous dose forms that administer the medicine to the systemic circulation through the skin at a predetermined rate are referred to as transdermal therapeutic approaches. Several benefits of transdermal administration include increased safety and efficacy. As it is administered in semisolid form (gel or cream) and has numerous applications in the pharmaceutical, veterinary, and cosmetic fields, this method of drug delivery avoids the risks and pain connected with parenteral therapy and promotes patient compliance. In this regard, the transdermal route is an intriguing choice because it is economical and reliable (Kumar et al., 2020).

Only lipophilic compounds with molecular weights under 500 Da can flow through TDDS because of the stratum corneum's (s.c) barrier characteristics. Other therapeutic advantages of TDDS include bypassing the first pass metabolism effect for drugs with limited oral bioavailability, prolonged drug delivery to give a steady state plasma profile and subsequently fewer systemic adverse consequences, which may lead to greater patient compliance. Currently, vesicular and non-invasive drug delivery systems like liposomes, niosomes, transferosomes, and ethosomes are employed to improve the penetration of drugs via the s.c. Ethosomes have the potential to significantly penetrate the epidermal barrier, which is the primary limiting element in TDDS (Kumar et al., 2020).

2. Nanocarriers

The domain of delivery of drug has experienced enormous growth over the past 1.5 decades, which has been characterized by the emergence of new formulations and methods. Unsatisfactory physicochemical characteristics of medications and physiological hurdles found in the body, such as the skin and membrane coverings of different human organs, are the two main obstacles to effective drug distribution. It is well knowledge that the therapeutic efficacy of the delivery methods may be influenced by the type of the active pharmaceutical ingredient (API) and its bioavailability at the site of action. Drugs having limited physiological barrier permeability and poor biological fluid solubility are unable to fully demonstrate their efficacy. Several methods have been suggested in the past to address the issues with drug solubility and permeability. Particle size reduction, the synthesis of metastable polymorphs, prodrugs, solid dispersions, and drug complexation with hydrophilic carriers have all been found to be beneficial. The development of nanotechnology has helped to resolve various drug solubility-related problems. Researchers have discovered that the medicine can pass through a variety of biological hurdles, including those in the brain, nose, eyes, and bladder (Garg et al., 2017).

The insertion of both hydrophobic and hydrophilic active components, ideal target distribution and bioavailability, better stability and preservation of active ingredients from degrading are just a few advantages that nanocarriers provide. They also improve skin penetration (Santos et al., 2021).

3. Ethosomes

Ethosomes are soft, malleable phospholipid-based elastic nanovesicles composed mainly of phospholipids, ethanol (relatively high concentration), and water, developed by Touitou, 1997, as an additional novel lipid carriers that enable drugs to reach the deep skin layers and / or the systemic circulation. They are reported to improve the skin delivery of various drugs (Verma and Pathak, 2010). They are soft and malleable. The size of an ethosomal vesicle lies within the nanometer (nm) range (Mbah et al., 2014). Due to their size (approximately 150–200 nm) and high deformability, they are also referred to as elastic nanovesicles (Ascenso et al., 2015). Schematic diagram of ethosomes is shown in figure 1.

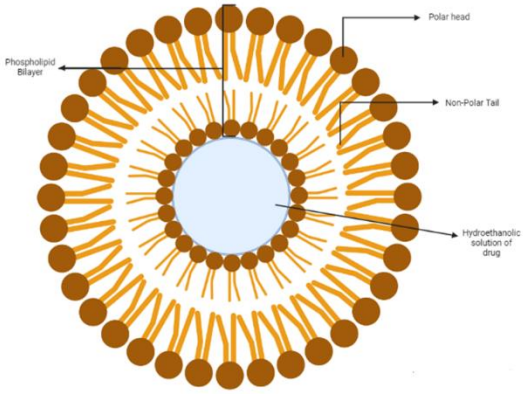

Figure 1: Schematic diagram of ethosomes

Studies have shown that coexistence of phospholipid vesicles with ethanol at high concentrations is feasible and produces an enhancing delivery carrier makes them unique, as ethanol is known for its disturbance of skin lipid bilayer organization (Godin and Touitou, 2003).

Ethosomes were reported to be effective at delivering molecules to and through the skin to the systemic circulation. The ethosomal carrier was previously tested for dermal delivery of the antiviral drug acylovir. The authors in their study reported a two-armed, double-blinded, randomized clinical trial, and demonstrated the efficiency of the ethosomal 5% acylovir system, compared to a 5% acylovir cream (Zovirax, ZC) for the topical treatment of herpetic infection. (20–45%) (Verma and Pathak, 2010).

These vesicles are capable of penetrating through pores of size smaller than their own size. They provide sustained drug delivery and can act as a carrier for both hydrophilic and hydrophobic drugs. These vesicles can be easily applied on skin in form of gel or ointment (Garg et al., 2017).

The lipid vesicles provide a controlled transdermal delivery system and also serve for the solubilization of poorly soluble drugs. Combination of phospholipids and high concentration of ethanol in vesicular formulations have been suggested to be responsible for deeper penetration and distribution in the skin lipid bilayers (Bodade et al., 2013).

3.1 Composition of ethosomes

Table 1: Different additives employed in formulation of ethosomes

Materials	Examples	Uses	References
Phospholipid	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmityl phosphatidyl choline	Vesicles forming component	Jain et al., 2011
Alcohol	Ethanol Isopropyl alcohol	For providing the softness for vesicle membrane As a penetration enhancer	Jain et al., 2011
Vehicle	Carbopol 934, HPMC	Gelling agent	Zahid et al., 2018
Edge activators	N-DMSO, Tween, Span	Enhances skin permeability	Zahid et al., 2018
Cholesterol	Cholesterol	For providing the stability to vesicle membrane	Aute et al., 2012
Dye	Rhodamine-123 Rhodamine red Fluorescene Isothiocynate (FITC) 6- Carboxy fluorescence	For characterization study	Patrekar et al., 2015
Polyglycerol	Propylene glycol, transcutol RTM	As a skin penetration enhancer	Gupta et al., 2012

3.2 Methods of preparation of ethosomes

3.2.1 Hot method: The drug is dissolved in a mixture of ethanol and propylene glycol and the mixture is added to the phospholipid dispersion in water at 40°C. The preparation is sonicated using the Probe Sonicator for three cycles of five minutes at 4°C, with a five-minute rest between each cycle. To obtain nano-sized ethosomes, the formulation is homogenised three times at 15,000 psi pressure in a high pressure homogenizer (Verma and Pathak, 2010).

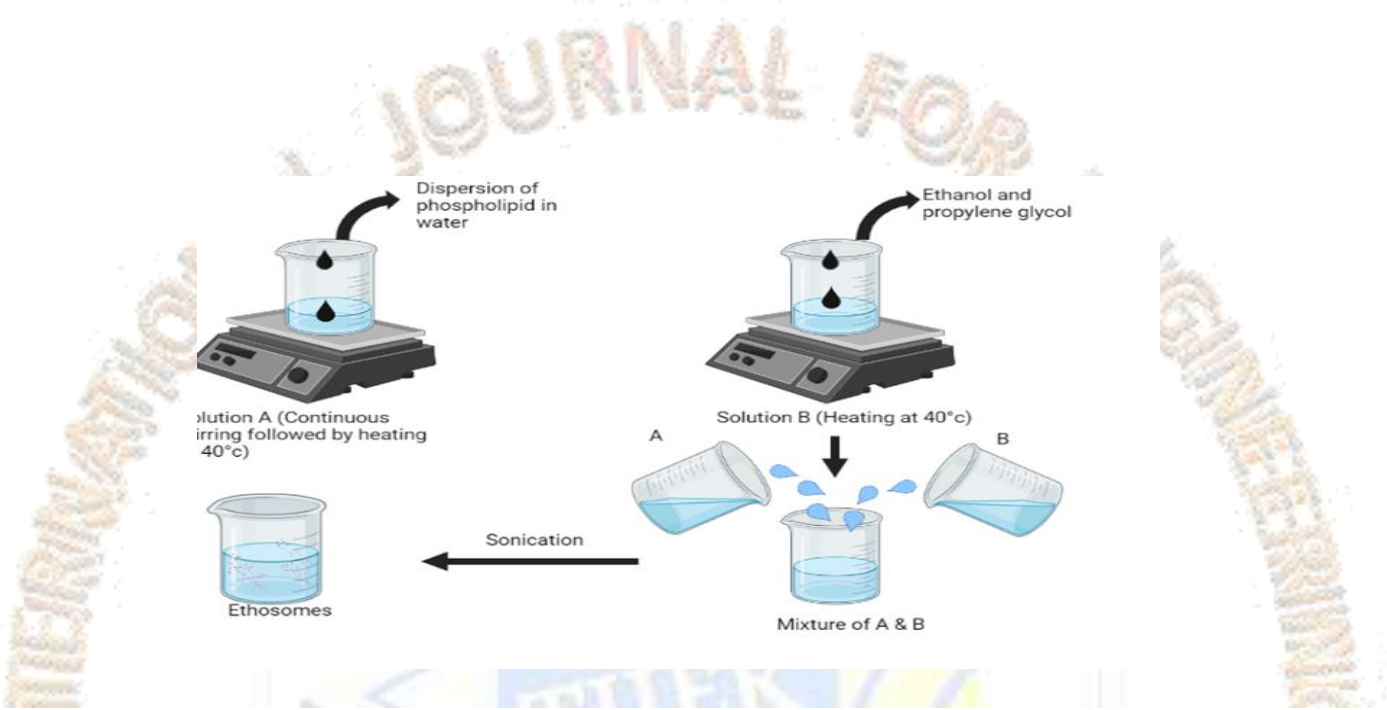

Figure 1: Diagram of hot method for the preparation of ethosomes (Pandey et al., 2014)

3.2.2 Cold injection method: In this method, ethanolic solution of lipids and drug is prepared by vigorous stirring. Polyols are added to the prepared solution and the mixture is heated to 30°C. To make homogeneous vesicles, preheated water is added drop by drop to the mixture and agitated constantly. Sonication, extrusion, and other methods can be used to attain the desired vesicle size. Closed vessel should be used in whole process

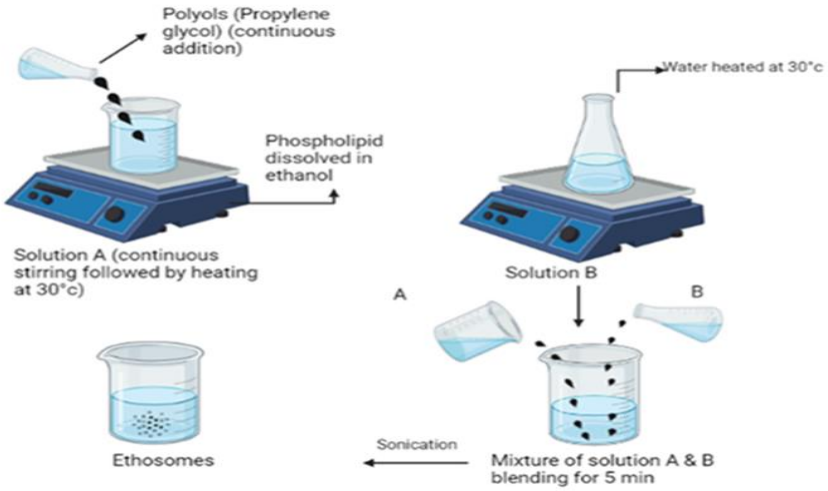

Figure 2: Diagram of hot method for the preparation of ethosomes (Pandey et al., 2014)

3.2.3 Classic Mechanical-Dispersion Method: Ethosome preparation by the classic mechanical-dispersion method can be accomplished by dissolving soya PC in a mixture of chloroform: methanol (3:1), in a round bottom flask. Above the lipid transition temperature of 60°C, the organic solvents are removed with the help of a rotary vacuum evaporator until a thin lipid film forms on the flask wall. By placing the contents under vacuum overnight, traces of solvent are removed from the deposited lipid film. This is followed by hydration with different concentrations of the hydroethanolic mixture, containing the active ingredient by rotating the flask at an accurate temperature (Santos et al., 2021).

3.3 Evaluation of ethosomes

Test	**Technique/instrument**
Particle size analysis and shape	Optical microscopy, Scanning Electron Microscopy, Transmission Electron Microscopy
Drug content	High performance liquid chromatography/UV
Zeta potential	Zeta meter
Transition temperature	Differential scanning calorimetry (DSC)
Penetration and Permeation Studies	Confocal laser scanning (CLS)
Drug Entrapment	Ultracentrifugation technique

3.3.1 Particle size: Ethosomes can be easily visualized by using Transmission electron microscopy (TEM) and by Scanning electron microscopy (SEM) (Satyam et al., 2010).

3.3.2 Drug content: Drug content of the ethosomes can be determined using UV spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic method (Zahid et al., 2018).

3.3.3 Zeta potential: Charge on the vesicles is another important parameter that indicates the stability of formulation. Charge is usually measured in terms of zeta potential (ZP). ZP is defined as an electric potential at the shear plane which is the boundary of the surrounding liquid layer attached to the moving particles in the medium. It is reported that value of ZP above 60 mV yields excellent stability, while 30, 20 and less than 5 mV generally results in good stability, acceptable short term stability and fast particle aggregation, respectively. The nature and ratio of surfactant used also influences the zeta potential (Garg et al., 2016).

Zeta potential of the formulation can be measured by Zeta meter (Satyam et al., 2010).

3.3.4 Stability studies: The stability of vesicles can be determined by assessing the size and structure of the vesicles over time (Satyam et al., 2010).

3.3.5 Transition temperature: The transition temperature of the vesicular lipid systems can be determined by using differential scanning calorimetry (DSC) (Wahid et al., 2011).

3.3.6 Penetration and Permeation Studies: Depth of penetration from ethosomes can be visualized by confocal laser scanning (CLS) (Parashar et al., 2013).

3.3.7 Drug Entrapment: The entrapment efficiency of ethosomes can be measured by the ultracentrifugation technique (Kumar et al., 2020).

3.4 Therapeutic applications of ethosomes

3.4.1 Ethosomes formulation for topical delivery of thymoquinone for improved therapy in skin acne

In their work, Kausar et al. (2018) created and refined the ethosomes formulation for topical administration of thymoquinone for enhanced acne treatment. The formulation was adjusted using a "3-factor 3-level Box-Behnken design" based on vesicle size, entrapment efficiency, and flux. The improved mixture was then tested for "anti-microbial, anti-inflammatory, anti-acne, and skin irritation activity." The improved formulation had vesicles with a diameter of 105.2 8.0 nm, an entrapment efficiency of 85.30 6.30%, and a flux of 65.40 7.6 g/cm2 /h. Confocal laser microscopy was used to determine the depth of penetration of rhodamine-loaded ethosomes across rat skin. Antimicrobial testing revealed that the marketed allopathic gel, manufactured ethosomes formulation, and marketed herbal gel had zones of inhibition of 29.0 1.00 mm, 20.33 0.57 mm, and 20.33 0.57 mmIn vivo anti-inflammatory efficacy showed that using produced ethosomes and commercialized diclofenac gel formulation reduced rat paw edema by 80.74% and 84.61%, respectively, in Wistar rats. An anti-acne study revealed that the created ethosomes formulation had a significant effect on sebaceous glands units by lowering the quantity and size of glands. Further skin irritation testing demonstrated that the produced formulation was safe, less irritating, and tolerated for topical administration. The new thymoquinone-loaded ethosomes formulation was shown to be a viable therapy option for acne vulgaris and other skin problems.

3.4.2 Ethosomes containing lamivudine

Using lamivudine as a model drug, ethosomal formulations were created and tested in vitro, ex vivo, and in vivo. The effect of ethosome on skin ultrastructure was studied using transmission electron microscopy, scanning electron microscopy, and fluorescence microscopy. T-lymphoid cell line (MT-2) was used to test cytotoxicity and cellular uptake of ethosomes. When compared to lamivudine solution (2.8 0.2 g/cm2 /h), the optimized ethosomal formulation demonstrated 25 times higher transdermal flux (68.4 3.5 g/cm2 /h). Microscopic examinations revealed that ethosomes had an effect on the ultrastructure of s.c. In the intercellular region of deeper skin layers, distinct regions with lamellar stacks derived from vesicles were observed. The results of the cellular uptake study revealed that ethosomes had significantly higher intracellular uptake (85.7% 4.5%). According to the findings of the characterization studies, lipid perturbation, along with the elasticity of ethosomes vesicles, appears to be the primary contributor to improved skin permeation (Jain et al., 2007).

3.4.3 Ethosomes entrapped with tretinoin

Tretinoin is a well-known retinoid used to treat acne, photo-aged skin, psoriasis, and skin cancer, making it an ideal candidate for topical formulation. However, its high lipophilicity and side effects such as redness, swelling, peeling, blistering, and erythema make this difficult. Drug-loaded ethosomes were prepared and characterized for entrapment efficiency, vesicular size, shape, in-vitro skin permeation, skin retention, drugmembrane component interaction, and permanence using phospholipid and ethanol. An ethosomal formulation containing 0.5%w/v phospholipid and 20%v/v ethanol (F2) with the highest entrapment efficiency (80.250.23) and small particle size (205.402.31nm) was chosen for further skin permeation studies. In terms of cumulative percentage of drug permeation (93.360.45%) and 8 hours, the ethosomal formulation outperformed the others. Scanning electron microscopy confirmed the three-dimensional nature of ethosomes. According to the dynamic light scattering technique, the ethosomes have smaller vesicular size than the liposomes prepared without alcohol. FTIR analysis revealed no interaction between the drug and the membrane components. The antiacne activity of ethosomal vesicles in carbopol gel base was compared to that of marketed gel. Our findings suggest that ethosomes are an effective carrier for dermal and transdermal tretinoin delivery (Mishra et al., 2018).

3.4.4 Diclofenac-loaded phospholipid vesicles (liposomes, ethosomes and penetration enhancer-containing vesicles)

Diclofenac-loaded phospholipid vesicles, including conventional liposomes, ethosomes, and PEVs (penetration enhancer-containing vesicles), were created and tested for efficacy in TPA (phorbol ester)-induced skin inflammation. Vesicles were created using a low-cost, unpurified mixture of phospholipids and diclofenac sodium; PEVs were created using Transcutol® P and propylene glycol, and ethosomes were created using ethanol. Transmission electron microscopy and small and wide angle X-ray scattering were used to investigate the structure and lamellar organization of the vesicle bilayer, as well as the main physicochemical features. The formulations, along with a diclofenac solution and commercial Voltaren Emulgel®, were tested for anti-inflammatory efficacy on TPA-treated mice dorsal skin in a comparative trial. Vesicles were around 100 nm in size, negatively charged, capable of encapsulating diclofenac in high yields, and revealed varying lamellarity depending on the formulation composition. Vesicular formulations increased drug accumulation while decreasing permeation. When vesicular diclofenac was applied to TPA-inflamed skin, it significantly reduced oedema and leucocyte infiltration, especially when PEVs were used. Histology confirmed the effectiveness of the vesicles, as they reduced the tissue damage caused by TPA. The proposed approach, which is based on vesicular nanocarriers, has the potential to be effective in treating a variety of inflammatory skin disorders (Caddeo et al., 2013).

3.4.5 Paclitaxel-loaded ethosomes

Topical anticancer drug application for the treatment of malignancies represents a new challenge in dermatology, potentially representing an alternative therapeutic approach for the effective treatment of nonmelanoma skin cancer, that is, actinic keratoses, and malignant lesions of the skin caused by ultraviolet radiation. Many taxanes, as well as anti-proliferative and antimitotic drugs, are being studied for the treatment of cutaneous malignant transformation of actinic keratoses, particularly squamous cell carcinoma. Because of their suitable physicochemical properties and enhanced skin penetration ability for deep dermal delivery, paclitaxel-loaded ethosomes are proposed as topical drug delivery systems for the treatment of this pathology. Our in vitro data show that the skin application of paclitaxel-loaded ethosomes improved the permeation of paclitaxel in s.c-epidermis membrane model and increased its anti-proliferative activity in a squamous cell carcinoma model as compared to the free drug. The results obtained encouraged the use of the paclitaxel-loaded ethosomes as the formulation for the potential treatment of squamous cell carcinoma, a malignant transformation of actinic keratosis (Paolino et al., 2012)

3.4.6 Methotrexate Incorporated Ethosomes and Salicylic Acid for the Treatment of Psoriasis

Ethosomal gel of methotrexate (MTX) and salicylic acid (SA) MTX-SA was prepared by the cold method and optimized by comparing it with MTX ethosomal gel and drug solution. The ex-vivo investigation, entrapment efficiency, particle size, and zeta potential were chosen as the essential quality-checking characteristics. Using the imiquimod-induced psoriasis model, the antipsoriatic potential of MTX-SA ethosomal gel was evaluated using the Psoriatic Area and Severity Index (PASI) score and histopathological examination (Chandra et al., 2019).

3.4.7 Novel elastic membrane vesicles (EMVs) and ethosomes-mediated effective topical delivery of aceclofenac

When compared to Elastic membrane vesicles (EMVs), ethosomes had higher vesicle density, drug loading, and deformability index. The drug permeation and retention provided by both vesicular systems were well tolerated on mice skin. Despite this, the in vivo performance of ethosomes was found to be superior to that of EMVs in both models studied. Phospholipid-based vesicular systems, particularly ethosomes, have the potential to improve the delivery and safety of aceclofenac (ACE) via the topical route (Sharma et al., 2016).

3.4.8 Indomethacin-pentapeptide modified ethosomes

The gel of indomethacin-pentapeptide modified ethosomes (IMC-KTTKS-Es) was used to transfer the lipophilic IMC through the skin to produce topical analgesia and anti-inflammatory effects. IMC-KTTKS-Es were successfully prepared using the thin-film evaporation and hydration method, fully characterized, and evaluated after being loaded. In vitro skin, transdermal study and confocal laser scanning microscopy (CLSM) evaluation showed that IMC-KTTKS-Es could enhance the skin retention effect of IMC when topical administration. The enhanced analgesic efficacy of IMC-KTTKS-Es gel was significant ($P < .05$) compared to IMC-Es gel of the same dose. Similarly, the anti-inflammatory activity of IMC-KTTKS-Es gel was stronger than that of IMC-Es

gel in dimethyl benzene-induced ear swelling of mice. Furthermore, irritation studies illustrated that the IMC-KTTKS-Es gel did not cause any skin irritation. Overall, these results showed that IMC-KTTKS-Es gel might be a promising transdermal delivery vector for topical analgesia and anti-inflammatory (Niu et al., 2022).

3.4.9 Ethosomes and organogels for cutaneous administration of crocin

Ethosome and Organogels (ETHO and ORG) have been demonstrated to be efficient vehicles for crocin (CRO) administration on skin. Particularly organogels was able to better control crocin labile stability. *In vivo* studies have demonstrated that thanks to the peculiar supramolecular organization of PC, both ETHO-CRO and ORG-CRO vehicles enhanced CRO absorption through skin, suggesting their suitability to treat inflammatory skin disorders. Moreover CRO can also have a protective effect on the surface of the skin, like sunscreens. This double effect could be useful for instance in melanoma prevention and therapy. Nonetheless, to verify this hypothesis animal studies should be performed (Esposito et al., 2016).

3.4.10 Co-loaded berberine chloride and evodiamine ethosomes for treatment of melanoma

In this study we successfully developed ethosomes co-loaded with Berberine chloride (BBR) and evodiamine (EVO). The desired ethosomes had mean size of 171 nm and 90% or above entrapment efficiency for both BBR and EVO. Following topical application to human skin, these ethosomes were shown to deliver BBR and EVO to the basal layers of human skin in vitro. Furthermore, co-loading BBR and EVO in ethosomes improved anti-melanoma effects on B16 cells in tissue culture. This increase in antitumor activity may be due to improved compound bioavailability and synergistic effects of BBR and EVO. This finding supports the ethosomes' suitability as potential topical carriers for the treatment of melanoma. Future research will include the use of an appropriate animal model to test BBR and EVO co-loaded in ethosome formulations (Lin et al., 2020).

3.4.11 Apigenin-loaded ethosomes

The goal of this study was to create and test a new topical delivery system for apigenin using ethosomes. Uniform design experiments were used to find the best apigenin-loaded ethosome formulation. In vitro and in vivo skin deposition and transdermal flux of apigenin loaded in ethosomes, liposomes, and deformable liposomes were compared. The amount of phospholipids in ethosome formulations increased the efficiency of apigenin encapsulation. Furthermore, skin deposition and transdermal flux of apigenin improved with higher levels of phospholipids (Lipoid S 75) and short-chain alcohols (propylene glycol and ethanol), but decreased with higher propylene glycol to ethanol ratios.The profiles of skin deposition versus time for ethosomes differed significantly between in vivo and in vitro studies when compared to liposomes or deformable liposomes. In vitro and in vivo, optimized ethosomes demonstrated superior skin targeting. Furthermore, they had the greatest effect on reducing cyclooxygenase-2 levels in mouse skin inflammation caused by UVB light. As a result, apigenin-loaded ethosomes are a promising therapeutic approach for treating UVB-induced skin inflammation (Shen et al., 2014).

3.4.12 Ethosomes entrapped with Terbinafine Hydrochloride

The current study looks into the entrapment of Terbinafine Hydrochloride (TH) in ethosomal vesicles using both the unsonicated and sonicated methods. Carbopol 934P was included in the best sonication-derived formulation, F6. The formulated ethosomal gel, i.e. F6, was used to achieve a zero order release profile of TH. The ingredients are phospholipid, ethanol, and propylene glycol. The prepared ethosomes were tested for drug entrapment efficiency (DEE), in-vitro and ex-vivo drug diffusion, FT-IR, and stability. The size and shape of F6 ethosomes vesicles was characterized by SEMIn-vitro drug release studies were carried out for 12 hours using a sigma dialysis membrane in phosphate buffer, pH 7.4, while drug content was determined using HPLC. DEE was ranked between 55.331.32% and 69.112.11%. The F6 ethosomal formulation produced the highest DEE with a vesicle size of 2481.02 nm. . FT-IR studies confirmed that there was no chemical interaction between the drug and the formulation's excipients. The drug diffusion observed after 12 hours from F6 and marketed cream (MR) formulations was 74.010.62% and 61.450.86%, respectively, according to ex-vivo results. The MR and F6 ethosomal gels had similarity factors (f2 values) of 85.14 and 42.63, respectively. It was discovered that F6 had dissimilar dissolution profiles. F6 and MR transdermal flux values were found to be 144.611.28 g/cm.Transdermal flux value for F6 and MR was found to be 144.61±1.28 µg/cm 2 /hr and 121.6±1.16 µg/cm^2 /hr respectively. This study disclosed that F6 resides at targeted site for a relatively longer period of time thereby signifying the improved patient compliance (Iizhar et al., 2016).

3.4.13 Ethosomes for Coenzyme Q10 Cutaneous Administration

The present investigation has demonstrated the suitability of ethosomes as a transcutaneous delivery system for Coenzyme Q10 (CoQ10). The significance of size distribution analysis in choosing the composition of ethosomal vesicles has been highlighted by the preformulatory study. The multilamellar structure of vesicles based on phosphatidylcholine and ethanol was demonstrated by SAXS and cryo-TEM.

In contrast to other nanosystems previously examined in other studies, ethosomes were notably able to increase the EC of CoQ10 and to better control its stability due to their unique supramolecular organization. This confirms the enormous potential of ethosomal vesicles for the solubilization and delivery of lipophilic drugs. The study's description of the composition and preparation methods of ETHO can be used to ensnare molecules that possess attributes beyond antioxidants, thereby enhancing their absorption and delaying their degradation.

. Ex-vivo studies have confirmed the hypothesis that ethosomes can be used as nanosystems for CoQ10 delivery through the skin. Indeed, TEM analysis confirmed ethosomal fibroblast uptake and passage through the more complex model reconstituted human epidermis (RHE). Furthermore, the decrease in fluorescence signal intensity down to the stratum basale supports the efficacy of ethosomes as transdermal delivery systems, making them a promising vehicle for CoQ10 encapsulationAdditional in vivo studies will be necessary to assess not only the comparable antioxidant delivery against other carrier systems, but also the prolonged antioxidant effect of ETHO-CoQ10, even though RHE can be regarded as reliable models for examining the effect of topical formulation on skin (Sguizzato et al., 2020).

3.4.14 Griseofulvin-loaded ethosomes

Griseofulvin (GRF) is a significant antifungal drug with low bioavailability; thus, a topical formulation with targeted action and minimal systemic effects appears to be a preferable solution. GRF's poor solubility has hampered the development and commercialization of topical formulations. The goal of this study was to create a new GRF formulation for topical application using lipid-based nanosystems, as well as to investigate its permeation and penetration, cell viability, and therapeutic action. GRF was incorporated into ethosomal systems made of soy bean phosphatidylcholine, ethanol, and water. Permeation through newborn pigs was performed using Franz diffusion cells after the vesicles were characterized in terms of size, charge, and penetrability. Cell viability at various concentrations of the chosen substance. Cell viability was determined at various concentrations of the chosen formulation. Finally, a skin-adapted agar diffusion test was performed to evaluate the formulation's therapeutic efficacy. The average size of GRF vesicles was 130 nm. Because drug retention in the s.c. was achieved, permeation and penetration assays revealed that GRF-loaded ethosomes have an adequate profile for use in a topical formulation. Cell viability tests revealed that this formulation had no cytotoxicity to HaCaT cells at concentrations less than 50 g/mL. The skin diffusion test demonstrated the developed formulation's ability to target skin dermatophytes. The findings of this study provide a new perspective on the topical treatment of fungal infections (Marto et al., 2016).

3.4.15 Ligustrazine phosphate ethosomes for treatment of Alzheimer’s disease

We investigated transdermal administration of ligustrazine phosphate (LP), an antioxidant, for the treatment of Alzheimer's disease (AD) in this study. The LP transdermal ethosomal system was created and tested. In vitro permeation studies were conducted using Franz-type diffusion cells and confocal laser scanning microscopy. Furthermore, the effect of the LP transdermal ethosomal system on Alzheimer's disease was assessed in scopolamine-induced amnesia rats using the Morris water maze test. The antioxidant enzyme activities and levels of the lipid peroxidation product malondialdehyde (MDA) in the brains of rats were also measured.The outcomes demonstrated that the LP ethosomal system's skin drug deposition and penetration ability were both noticeably greater than those of the aqueous one. Improvements in behavioral performance were also indirectly indicative of the antioxidant enzyme activities and MDA levels in the brains of the amnesic rats being restored to normalcy by the LP transdermal ethosomal system. In summary, ethosomes may be the preferred vesicles for LP transdermal delivery, and LP may provide a viable therapeutic alternative in the fight against AD (Shi et al., 2012).

3.4.16 Glimepiride-loaded ethosomes

Glimepiride is a sulfonylurea anti-diabetic medication of the third generation. It is practically insoluble in water; this inability to dissolve in water and slow dissolution may result in irreproducible clinical responses or therapeutic failure due to subtherapeutic plasma drug levels. A large portion of an oral dose is wasted due to low oral bioavailability. To overcome these limitations, glimepiride was entrapped in a novel vesicular carrier system (Ethosomes) to improve glimepiride therapeutic efficacy via the transdermal route. As a result, the current study sought to develop, characterize, and assess the transdermal potential of ethosomes encapsulating glimepiride. Optical microscopy, transmission electron microscopy, and minicolumn centrifugation were used to determine vesicular shape, surface morphology, and entrapment efficiency, respectively. Unlike liposomes, ethosomes had

a more condensed vesicular structure and were found to be negatively charged. In comparison to plain drug solution and liposomal formulation, ethosomal formulation was found to be more efficient delivery carriers with high entrapment, optimal nanometric size range, and low polydispersity index. The ethosomal formulations had entrapment efficiencies ranging from 42 to 78%. When ethosomes were used in in vitro percutaneous permeation experiments, the permeation of glimepiride through rat skin was significantly increased. In-vitro skin permeation kinetics revealed zero order drug release from formulations. The flux from ethosomes was three times that of liposomal solution. FT-IR studies revealed that when skin was treated with an ethosomal formulation, ceramides became loosened, resulting in the breakdown of hydrogen bond networks at the skin's surface.Results suggested ethosomes to be the most proficient carrier system for dermal and transdermal delivery of glimepiride (Bhulli and Sharma, 2012).

3.4.17 Tacrolimus-loaded ethosomes

Ethosomal formulations containing tacrolimus as a lipophilic drug have been prepared successfully and characterized for atopic dermatitis (AD). It was found that tacrolimus encapsulation was facilitated by the presence of ethanol in the aqueous compartment of the vesicles. Thus, ethanol incorporation into liposomes may be essential for improving tacrolimus penetration. This system showed great promise for the topical delivery of tacrolimus due to its effective drug delivery and long-term stability of ethosomes. Additional research was being done on the ethosomal delivery systems' mechanism of inhibition action, safety on skins, and pharmacological impact on AD (Li et al., 2012).

3.4.18 Psoralen-loaded ethosomes

Zhang, 2014 developed a novel psoralen transdermal delivery system employing ethosomes, flexible vesicles that can penetrate the s.c and target deep skin layers. According to an in vitro study on skin penetration, ethosomes loaded with psoralen had better permeability than liposomes. Psoralen transdermal flux and skin deposition with ethosomes were 38.89± 0.32 mg/cm2 /h and 3.87± 1.74 mg/cm2, respectively; these values were 3.50 and 2.15 times higher than those obtained with liposomes. After being applied to rat skin in vivo on a daily basis for seven days, the safety of the ethosomes and liposomes was determined. When compared to an equivalent ethanol solution, the ethosomes demonstrated superior biocompatibility with human embryonic skin fibroblasts, suggesting that the phosphatidylcholine contained in the ethosome vesicles enhanced their biocompatibility. These results suggested that ethosomes might enhance psoralen's transdermal delivery and perhaps that of other medications needing deep skin delivery.

3.4.19 Testosterone propionate via surfactant-modified ethosomes

Meng (2013) assessed the transdermal potential of newly developed liposomes and testosterone propionate (TP) ethosomes made by surfactant modification. The impact of cremophor EL-35 and hexadecyl trimethyl ammonium bromide on the prepared vesicles' zeta potential and particle size was examined. Using a variety of methods, including transmission electron microscopy, confocal laser scanning microscopy, differential scanning calorimetry, dynamic light scattering, and others, the entrapment efficiency and stability as well as in vitro and in vivo skin permeation were investigated. According to the findings, ethosomes are characterized as spherical, unilamellar structures that have a nanometric size of 156.5 ± 3.5 nm and a low polydispersity of 0.100 ± 0.015. In liposomal and ethosomal carriers, the efficiency of TP entrapment was 64.7% 2.1% and 92.7% 3.7%,

respectively. The prepared TP ethosomal system's stability profile was assessed for 120 days and revealed very low aggregation and vesicular size growth. TP ethosomes also improved transdermal flux to 37.85 2.8 g/cm2/hour and reduced lag time to 0.18 hours across mouse skin. The skin permeation efficiency of the TP ethosomes was further evaluated using confocal laser scanning microscopy, which revealed that rhodamine red-loaded formulations permeated deeper layers of the skin (260 m) than liposomal formation (120 m).

3.4.20 Amphoterecin-B ethosomal gel against *Candida albicans*

Amphotericin B (AmB) ethosomal gel (EF) formulation, evaluation of antifungal activity against human fungal isolates, and comparison of antifungal activity with marketed liposomal gel (MLG). The physical appearance, pH, spreadability, viscosity, drug content, zeta potential, in-vitro diffusion study, and in-vitro and in-vivo antifungal study of AmB ethosomal gel (EF) were all evaluated. The pH value was found to be within acceptable limits (6.2 0.021). EF demonstrated greater spreadability than MLG. To confirm the formulation's stability, EF had a higher drug content (97.3 0.43%) than MLG (76 0.32%), better spreadability, and a lower negative zeta potential. In comparison to MLG (24 0.13 mm), AmB EF had the largest zone of inhibition (28 0.20 mm) against Candida species. The in-vitro and in-vivo studies revealed that C. albicans induced dermal mycosis could be effectively treated. According to the findings of this study, ethosomal gel is the most effective carrier system for dermal and transdermal delivery of Amphotericin B for the treatment of dermatomycoses (Kaur and Maurya, 2020).

3.4.21 Ethosomes of Clobetasol propionate

Clobetasol propionate is a synthetic corticosteroid that is used topically in dermatology. It's a prednisolone derivative with low mineralocorticoid activity and high glucocorticoid activity. The current study aims to develop and test ethosomes of clobetasol propionate. Cold method ethosomes of clobetasol propionate were successfully prepared and characterized by pH, size, zeta potential, drug content, drug entrapment, in-vitro drug release, and formulation F9 was found to be the best of all formulations prepared (Richa et al., 2016).

3.4.22 Intranasal Deformable Ethosomes of Rasagiline Mesylate

Rasagiline mesylate intranasal deformable ethosomes were developed for the effective treatment of Parkinson's disease. The ethanol injection method was used to create ethosomes. For formulation optimization, Doptimal design was used. Ethanol, propylene glycol, and phospholipids were chosen as independent variables, with ethosome encapsulation efficiency (EE) as the dependent variable. The optimum formulation of RM ethosomes has a higher EE of 38% with a spherical bilayered structure revealed by TEM analysis, an average particle size range of 256 nm, and zeta potential values of -24.4mV. Subsequent in vitro drug diffusion experiments using sheep nasal mucosa yielded a cumulative amount diffused of 766µg/cm2. These results confirmed that ethosomes has potential for nose to brain delivery system of Rasagiline mesylate for treatment of Parkinson’s disease (Mishra et al., 2020).

3.4.23 Ethosomes and Transfersomes for Topical Delivery of Ginsenoside Rh1 from Red Ginseng

The goal of this study was to improve topical delivery of ginsenoside Rh1 isolated from red ginseng using a new vesicular system of ethosomes and transfersomes versus a conventional liposome. Particle size, zeta potential, entrapment efficiency (% EE), and transmission electron microscopy (TEM) studies were performed on ginsenoside Rh1-loaded vesicles. Furthermore, skin permeation profiles using frantz diffusion cells and rat dorsal

skin treated with ethosome and transfersome were obtained and compared to conventional liposomes. The size of vesicles range from 108.5 to 322.9 nm, and negatively charged from −20.95 to −31.37 mV. The % EE of ginsenoside Rh1 was obtained between 45.0 to 65.0%. Comparing transfersomes to ethosome and traditional liposomes, ginsenoside Rh1 skin penetration was much higher. Therefore, based on the current study, ginsenoside Rh1-loaded transfersomes can act as a topical therapeutic effects potential (Choi et al., 2015).

3.4.24 Ethosomes containing indomethacin for transdermal deliver

Ethosomes containing indomethacin (8mg/mL) were prepared using different concentrations of soybean phosphatidylcholine (SPC), ethanol, and dispersion media and additives. In the presence of 10%-30% ethanol in a pH 7.4 phosphate buffer, ethosomes with a good colloidal appearance were obtained. The physical appearances, size, and entrapment efficiency (EE) of ethosomes were studied and discussed in relation to their constituents. At 20% ethanol (2% and 4% SPC) and 30% ethanol (6% SPC), vesicular size was significantly reduced. The EE was adjusted to account for vesicular size and SPC concentration. The optimized ethosomes were made from a 4% w/v SPC:cholesterol:deoxycholic acid (6:2:1 molar ratio) dispersion in 20% v/v ethanol in a pH 7.4 phosphate buffer. The vesicular size was 55 8 nm, the EE was 52.51%, the zeta potential was -39.06 1.53 mV. Over 24 hours, these ethosomes resulted in significantly higher indomethacin permeation through pig skin than the commercial solution and the ethanolic solution of indomethacin. When stored at room temperature for three months, it retained its physical appearance, drug content, and EE. These findings suggested that ethosomes could be used as a transdermal drug carrier for indomethacin (Sakdiset et al., 2019).

3.4.25 Curcumin ethosomal nanocarriers for the skin cancer delivery

CUR loaded ethosomes were successfully developed and optimized in this study using a two factor three level (32) factorial design approach. The optimized formulation, which contained 3% (w/v) phospholipid and 40% (v/v) ethanol, demonstrated appropriate vesicle size in the nano range, good entrapment efficiency, increased drug permeation, and improved skin deposition capability. The ethosomes' amorphous nature was revealed by DSC and XRD studies, indicating successful encapsulation of CUR. A fluorescence microscopy study confirmed the potential of ethosomes in enhancing skin penetration and drug transport into deeper layers of the skin. In A375 human melanoma cell lines, In A375 human melanoma cell lines, the MTT assay revealed that the optimized CUR-ETH formulation had greater cytotoxic potential than CUR. The cellular uptake study demonstrated that A375 cells internalized CUR ethosomal formulations more effectively than pure CUR. The flow cytometric analysis revealed that CUR ethosomes cause apoptosis in A375 cells. These findings show that ethosomes can be used as a potential therapeutic strategy for melanoma treatment by delivering the CUR into the deepest layers of the skin, where cancer cells reside. Further research using appropriate animal models is being conducted to confirm the efficacy of these formulations in the treatment of melanoma. We propose ethosomes as an innovative drug delivery carrier for future clinical use (Peram et al., 2010).

3.4.26 Liposomes- and ethosomes-associated distamycins

This study compared the performance of liposomes and ethosomes as specialized delivery systems for distamycin A (DA) and two of its derivatives. Liposomes and ethosomes were made using traditional methods, extruded through polycarbonate filters, and measured for dimensions, morphology, and encapsulation efficiency. It was discovered that DA was associated with vesicles (either liposomes or ethosomes) by approximately 16.0%, while

both DA derivatives showed a percentage of association of approximately 80% in the case of liposomes and 50% in the case of ethosomes. In vitro antiproliferative activity experiments on cultured human and mouse leukemic cells revealed that vesicles could boost the activity of both DA derivatives. Furthermore, it was shown that the aging of both liposomes- and ethosomes-associated distamycin suspensions had little effect on vesicle size, while all samples showed significant drug leakage over time. Furthermore, based on the different physicochemical properties of DA and its derivatives (i.e., log P), vesicle-associated DA had the highest drug loss compared to both its derivatives. Finally, the enhancement of drug activity expressed by these specialized delivery systems-associated DD could be interesting in order to obtain an efficient therapeutic effect aimed at reducing or minimizing toxic effects associated with distamycin administration (Cortesi et al., 2010).

3.4.27 Azelaic Acid (AzA) Based Ethosomes for Topical Delivery for the Treatment of Acne

AzA ethosomal formulations were successfully prepared, optimized, and tested. In terms of % Entrapment efficiency and % Drug diffused, the thin film hydration method performed well. The size of the vesicle obtained was appropriate. Based on in-vitro, ex-vivo release profiles, and antimicrobial activity, ethosomal gel was superior and efficient to conventional formulations. In addition, the formulated gels provided sustained release of the medication. The prepared dosage form was non-irritant to the skin and thus can be used successfully as a topical formulation. AzA vesicular-based delivery systems are thus promising for acne treatment, and vesicular systems can be used topically to treat a variety of skin disorders (Mistry and Ravikumar, 2016).

3.4.28 pH-responsive ethosomes for vaginal delivery of metronidazole

pH-responsive metronidazole ethosomes (ME) were created using the solvent evaporation method and tested for vaginal delivery. Metronidazole and P90H did not interact according to differential scanning calorimetry. SEM analysis revealed the formation of spherically shaped vesicles. With a PDI of 0.338, the average vesicle size was 179.9 nm. Entrapment efficiency (EE) was 50.31 3.38% and loading capacity (LC) was 39.89 0.02%, respectively. Over a pH range of 6.5-5.0, permeation and flux were in the following order: ME 5.6 > ME 5.0 > ME 6.5, with ME 5.6 exhibiting the highest release profile (P 0.05).. The metronidazole molecules were entrapped by ethosome vesicles. When compared to the aqueous dispersion, the optimized ethosome gel (ME 5.6) exhibited pseudo-plastic flow behavior typical of non-Newtonian fluids and demonstrated potential for sustained delivery. This could be replicated in the vagina to increase metronidazole local and systemic bioavailability (Mbah et al., 2014).

3.4.29 Finasteride loaded ethosomes

The size, morphology, surface charge, and entrapment efficiency of finasteride-loaded ethosomes (FES) were measured using an ultra-probe sonicator. The ability of FES to permeate through rat skin and human cadaver frontal scalp skin was also tested. When compared to the unencapsulated FIN, the spherical shaped ethosomes of different batches ranged in size from 107.8 2.50 to 220.4 6.92 nm and showed good permeation across rat skin and frontal scalp skin of human cadaver. The findings demonstrated FES's ability to permeate the s.c. and reach the pilosebaceous unit (PSU) of the hair follicle. Although additional use of permeation enhancer increases the permeation of FIN across the skin, its addition may not be a favourable option for the deposition of ethosomes in the PSU (Wilson et al., 2017).

3.4.30 Ethosomes and ultradeformable liposomes containing clotrimazole

The aim of the study was to develop, assess, and contrast the transdermal potential of two new vesicular nanocarriers: ultradeformable liposomes and ethosomes that contain the antifungal bioactive clotrimazole (CLT). Ultradeformable liposomal (UL) formulation (TT3) and ethosomal formulation (ET4) demonstrated the smallest polydispersity index (0.027 ± 0.011 and 0.067 ± 0.009), optimal nanometric size range (132 ± 9.5 nm and 121 ± 9.7 nm), and highest entrapment (68.73 ± 1.4% and 55.51 ± 1.7%, respectively). The formulation ET_4 provided enhanced transdermal flux 56.25 ± 5.49 μg/cm^2/h and decreased the lag time of 0.9 h in comparison to TT_3 formulation (50.16 ± 3.84 μg/cm^2/h; 1.0 h. Studies on skin interaction and FT-IR showed that ET4's penetration-enhancing effect was greater than that of TT3 formulation. ET_4 formulation also had the highest zone of inhibition (34.6 ± 0.57 mm), in contrast to TT_3 formulation (29.6 ± 0.57 mm) and marketed cream formulation (19.0 ± 1.00 mm) against candidal species. Results suggested ethosomes to be the most proficient carrier system for dermal and transdermal delivery of clotrimazole (Maheshwari et al., 2012).

3.4.31 Psoralen in Ethosomes for Biofilm Treatment

Bagchi (2017) investigated the photoinduced dynamics of PSO, a photobiologically important drug, in ethosomes. PSO-ethosomes were characterized structurally and spectroscopically. Furthermore, we investigated solvent relaxation in confined environments using DCM as a model fluorophore. Picosecond-resolved FRET revealed drug molecule binding in the vesicles. It is assumed that when PSO is excited with UVA light, there is a nonradiative energy transfer from PSO to CV. Furthermore, it has been discovered that the increased cytotoxicity of PSO-loaded ethosomes is responsible for their improved antimicrobial activityPSO-loaded ethosomes have antibiofilm activity against both Gram-negative and Gram-positive bacteria. As a result of these findings, novel, high-potential therapeutic drugs with improved pharmacological efficacy to treat multi-drug-resistant bacteria-induced diseases will be developed.

3.4.32 Resveratrol loaded ethosomal hydrogel

The ethosomal hydrogel containing resveratrol was created and optimized using a systematic Quality by Design approach. First, the quality target product profile (QTPP) of an ethosomal formulation was defined, and critical quality attributes (CQAs) and critical material attributes (CMAs) were screened using fish bone diagram risk assessment studies. To optimize the selected CMAs, 32 full factorial designs were used with Design Expert software. As independent CMAs, phospholipid concentration (X1) and ethanol concentration (X2) were chosen. As dependent CQAs, vesicle size (Y1), entrapment efficiency (Y2), permeation flux (Y3), and drug deposition in the dermal layer (Y4) were assessed. The physicochemical and skin permeation properties of the optimized formulation were then evaluated. In comparison to a conventional cream, ethomal hydrogel significantly improved skin permeation parameters and resveratrol skin deposition. CLSM studies found ethosomal hydrogel to be widely distributed in the deeper skin layers, which corroborated the findings. Thus, there is evidence that systemically developed ethosomal gel can deliver increased amounts of bioactives into the skin, and a number of products for dermal/transdermal applications are expected to be developed based on it in the future (Arora and Nanda, 2019).

3.4.33 Hyaluronic acid-containing ethosomes

In this study, a hyaluronic acid-containing ethosomes (HA-ES) transdermal drug delivery system was developed, and rhodamine B (RB) was used as a model drug to be encapsulated. The surface morphology, entrapment efficiency, drug loading, and stability of the resulting HA-ES-RB were then evaluated. The prepared HA-ES-RB was found to be spherical, with good dispersion and stability and a particle size of less than 100 nm. Skin permeation experiments were performed in vitro using Franz diffusion cells and rat dorsal skins. The penetration effect of HA-ES-RB was found to be significantly greater than that of ES-RB. The fluorescence microscopy image revealed that HA-ES-RB penetrated deep into the dermis. The excellent transdermic drug delivery effect of HA-ES-RB may be attributed to its smaller size, hyaluronic acid hydration, and increased potential targeting to skin and skin appendages of liposomal carriers. Furthermore, the HA-ES delivery system did not cause cytotoxicity in normal cells, indicating good biocompatibility. This study developed hyaluronic acid-containing ethosomes that can provide a quick, high-efficiency, safe, and self-administered transdermal drug delivery system (Xie et al., 2018).

3.4.34 Ethosomes encapsulated with 5-fluorouracil (5-FU)

The goal of this study is to see how well ethosomes encapsulated with 5-fluorouracil (5-FU) work in treating laryngotracheal stenosis in rabbit models. The amorphous, size distribution, and encapsulation efficiency of the 5-FU ethosome were investigated using the thin film hydration method. The tracheal mucosa was scraped about 0.5 cm with a nylon brush to induce scar formation in the airway, and then models were divided into three groups: 5-FU ethosome group, 5-FU group, and saline group, with drug injected into scars of each group via paracentesis guided by endoscope, respectively. The stenosis states were observed using a laryngofiberscope immediately after administration, 7, 14, and 21 days later. The 5-FU ethosome group had no significant difference in airway stenosis when compared to the 5-FU group at 7 days after administration, but the 5-FU ethosome significantly reduced the airway stenosis after 21 days when compared to the 5-FU group again and had no restenosis during the observation period. Because ethosomes encapsulated with 5-FU were effective for laryngotracheal stenosis, it suggests that it has potential as a new method for treating airway stenosis caused by granulation tissue (Mao et al., 2016).

3.4.35 Thymoquinone-loaded ethosome

Thymoquinone (TQ), the main biologically active complex of Black Cumin seed, has been shown to have anticancer properties in a variety of tumors. The response surface method (RSM) was used to prepare ethosome for TQ encapsulation in this study. The central composite design (CCD) was used to optimize three effective parameters involved in ethosome structure: phospholipid, cholesterol, and ethanol concentration, as well as their combined effects. The experimentally validated optimum values for the variables were phospholipid 5% (W/W), ethanol 45% (V/V), and cholesterol 1.5% (W/W). The ethosomal formulation was more defined in terms of vesicle shape, size, zeta potential, and percentage entrapment efficiency. The drug entrapment efficiency was 99%, with average vesicle size and zeta potential of 201 nm and 632 mv, respectively. RSM and experimental assay produced a quadratic model with a high adequacy (R2) for size and zeta potential of 0.9319 and 0.9338, respectively. Optimized ethosome encapsulates thymoquinone (TQ). A cellular toxicity and release test was also performed. The toxicity and release curves were obtained, and the ethosomic TQ had greater cytotoxic activity

against MCF-7 cell lines than free TQ. The IC50 values for free TQ and ethosomic TQ were 1.10 g/ml and 0.95 g/ml, respectively. The developed model suggests a novel method for predicting and testing lipidic carriers (Nasri et al., 2019)

3.4.36 Silymarin-loaded liposomes and ethosomes

The goal of this study was to create Silymarin formulations (Silymarin entrapped in liposomes and ethosomes, referred to as LSM and ESM, respectively) to improve oral bioavailability of Silymarin and to evaluate its tissue distribution in free-moving rats using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Silibinin is the primary active constituent of Silymarin, which will be studied. In terms of precision, accuracy, and extraction recovery, a rapid, sensitive, and repeatable LC-MS/MS method was developed and validatedIn addition, the established method was used to investigate the pharmacokinetics and tissue distribution of Silymarin in rats. The formulations' size, zeta potential, and drug release were studied. These findings suggest that Silymarin encapsulated in LSM and ESM may provide more efficient tissue distribution and increased oral bioavailability, potentially improving its therapeutic bioactive properties in the body (Chang et al., 2014).

3.4.37 Curcumin-Loaded Hyaluronian-Modified Ethosomes

Zhang et al. (2019) successfully created HA-modified ethosomes with propylene glycol as a novel curcumin drug carrier. The HA gel network formed on the surface of phospholipid vesicles effectively reduced drug leakage, improved preparation stability, and enabled slow release of the loaded curcumin. The HA-ES system targeted the CD44 protein, which is overexpressed in inflamed psoriatic skin, to achieve targeted drug delivery, resulting in increased curcumin accumulation in inflamed skin. Topical application of HA-ES enhanced the therapeutic effect on mice's imiquimod-induced inflammatory skin that resembled psoriatic lesions. In conclusion, this promising topical delivery system may also be used to increase the accumulation of other anti-psoriatic medications by targeting the highly expressed CD44 protein in the inflammatory skin.

3.4.38 Ethosomes of Phenylethyl Resorcinol

Ethosome formulations containing phenylethyl resorcinol (PR) were developed. 0.5% w/v PR, 0.5% w/v cholesterol from lanolin, 3% w/v L-α-phosphatidylcholine from soybean, 30% v/v absolute ethanol, and water up to 100% v/v were used to create the formulation. With a vesicular size of 389 nm, a low polydispersity index of 0.266, a zeta potential of -34.19 ± 0.44 mV, a high PR entrapment efficiency of 71%, and good stability during 4 months of storage at 4 and 30 °C at 75% relative humidity, these characteristics were highlighted. The permeation coefficient of PR from ethosomes was found to be significantly higher than that from liposomes in in vitro studies using pig skin. The application of ethosome formulations resulted in 7.4-, 3.3-, and 1.8-fold higher PR accumulation in pig skin compared to liposomes, 20% propylene glycol solution, and 30% hydroethanolic solution, respectively. The inhibition value of PR's antityrosinase activity in pig skin was around 80%. In B16 melanoma cells, ethosomes consistently showed higher tyrosinase inhibition activity and melanin content reduction than other formulations. In albino rabbits, ethosomes did not cause acute dermal irritation. These findings show that ethosomes can efficiently deliver PR into the skin and hold promise for the topical application of skin lightening products (Limsuwan et al., 2017).

3.4.39 Ethosomes-based topical delivery system of antihistaminic drug

The goal of this study is to create a new topical formulation of cetirizine dihydrochloride based on ethosomes for efficient delivery. The optimal combination of medication, phospholipon 90 GTM, and ethanol was evaluated for its rheological behavior, drug content, vesicular size, spreadability, pH, and entrapment efficiency. The *ex vivo* permeation studies through mice skin showed highest permeation flux (16.300 ± 0.300 µg/h/cm^2) and skin retention (20.686 ± 0.517 µg/cm^2) for cetirizine-loaded ethosomal vesicles as compared to conventional formulations. The optimized formulation's in vivo pharmacodynamic evaluation was evaluated against oxazolone-induced atopic dermatitis (AD) in mice. Skin hyperplasia, erythema score, dermal eosinophil count, and reduction in scratching score were the parameters that were assessed. According to our findings, ethosomes are useful delivery systems for the antihistaminic medication cetirizine, which is used to treat AD (Goindi et al., 2014).

3.4.40 Curcumin loaded ethosomes

The current study aimed to create, develop, and optimize curcumin ethosomes formulations for transdermal application. Using the thin film hydration method, the preparation of ethosomes was optimized using the Box Behnken design (BBD) approach with three independent variables (concentration of lecithin, ethanol, and cholesterol) and three response variables (vesicle size (nm), percent entrapment efficiency, and flux). The percent drug entrapment eff Optimized F4 formulation containing 10mg lecithin, 4.5% ethanol and 10mg cholesterol demonstrated greater drug entrapment efficiency (81.2±3.12%) and smaller vesicle (228.8nm) with desired flux (10.5±2.6µg/cm2 /hr) through human cadaver skin (HCS). In-vivo study revealed significant increase in percent inhibition (58.8 % for7 h) of F4 formulation as compared ($P<0.05$) to oral administration. Stability study revealed no any significant change during three months study. Thus formulated curcumin loaded ethosomes could be the promising approach for transdermal delivery in pain management (Pathan et al., 2017).eficiency, vesicle size analysis, polydispersity index, zeta potential, in-vitro permeation study, in-vivo studies, and vesicular stability study of the ethosomes were all evaluated.

3.4.41 Tamoxifen citrate loaded ethosomes

Tamoxifen citrate-loaded ethosomes for transdermal applications were prepared and characterized. The formulations were evaluated for morphological characteristics, particle size distribution, calorimetric properties, zeta potential, and drug entrapment. The permeation profiles of prepared ethosomes, liposomes, and hydroethonalic solution were compared across cellophane membrane and human cadaver skin. According to the results of the permeation studies, ethosomes were able to deliver more than 90% of the drug within 24 hours of application, whereas liposomes and hydroethanolic solution delivered only 39.04% and 36.55%, respectively. Sarwa et al. (2013) also report on skin deposition and stability studies.

3.4.42 Ethosomes for transdermal delivery of caffeic acid

The current study describes a preliminary study aimed at developing ethosomes for caffeic acid transdermal administration. Because caffeic acid has both antioxidant potential and high instability, encapsulation appears to be an intriguing strategy. Ethosomes were created by adding water to a phosphatidylcholine ethanol solution while it was magnetically stirred. Photon correlation spectroscopy, small-angle X-ray spectroscopy, and cryogenic transmission electron microscopy were used to investigate the size distribution and morphology of

ethosome, while high-performance liquid chromatography was used to assess caffeic acid entrapment capacity. Caffeic acid stability in ethosome was compared to the molecule's stability in water as determined by mass spectrometry. Poloxamer 407 was used to thicken ethosomal dispersion, resulting in an ethosomal gel with rheological behavior and deformability. Caffeic acid diffusion kinetics were determined using Franz cells, and its skin penetration and antioxidant activity were assessed using a porcine skin membrane-covered biosensor based on an oxygen electrode. The mean diameter of ethosomes was 200 nm and nearly stable after three months. Caffeic acid entrapment in ethosome significantly prolonged drug stability in comparison to aqueous solution, reaching 77% w/w in ethosome after six months, whereas in water, almost complete degradation occurred within one month. The addition of poloxamer altered vesicle structure and size slightly while decreasing vesicle deformability. Caffeic acid diffusion coefficients from ethosome and ethosome gel were, respectively, 137- and 33-fold lower with respect to the aqueous solution. At last, the caffeic acid permeation and antioxidant power of ethosome were more intense with respect to the simple solution (Hallan et al., 2020).

3.4.43 Polyvinyl alcohol/hydroxyethylcellulose containing ethosomes

The purpose of this research is to create a scaffold for transdermal drug delivery method (TDDM) by electrospinning polyvinyl alcohol (PVA) and hydroxyethylcellulose (HEC). As a drug model, fluorescein isothiocyanate (FITC) loaded on ethosomes (FITC@Eth) was used. The prepared PVA/HEC/FITC@Eth scaffold was characterized using a scanning electron microscope (SEM), which revealed morphology changes caused by the addition of FITC@Eth. In addition, Fourier transform infrared spectroscopy (FTIR), mechanical properties, X-ray diffraction (XRD), and thermal gravimetric (TGA) analysis show that adding FITC@Eth to PVA/HEC has no effect on the scaffold properties. In vitro skin permeation experiments with rat dorsal skins were conducted using Franz diffusion cells. Because of the presence of ethosome, which enhances the potential skin targeting, FITC@Eth penetration was greater than free FITC. Finally, the prepared PVA/HEC/FITC@Eth scaffold appears to be a promising transdermal scaffold for long-term FITC release (Fawal et al., 2020).

3.4.44 Ethosomes for skin delivery of Ropivacaine

Ropivacaine, a novel long-acting local anesthetic, has proven to be superior. However, Naropin® Injection, the form used in clinic, can cause patient discomfort. The goal of this study was to formulate ropivacaine (RPV) in ethosomes and assess their potential for delivering RPV transdermally. The RPV-loaded ethosomes were prepared using a thin-film dispersion technique, and the formulation was evaluated using size, zeta potential, differential scanning calorimetry (DSC), and X-ray diffraction (XRD). The optimized RPV-ethosomes exhibited a typical lipid bilayer structure with a narrow size distribution of 73.86 2.40 nm and drug loading of 8.27 0.37%, EE of 68.92 0.29%. DSC and XRD analysis revealed that RPV was in an amorphous state when encapsulated into ethosomes . Furthermore, the results of an ex vivo permeation study demonstrated that RPV-ethosomes could promote permeability in a highly efficient and rapid manner (349.0 11.5 g cm2 at 12 h and 178.8 7.1 g cm2 at 0.5 h). According to the findings of a histopathology study, the interaction between ethosomes and skin could loosen the tight conjugation of corneocyte layers and weaken the permeation barrier. RPV-ethosomes were found to be a promising delivery system for encapsulating and delivering RPV for transdermal administration (Zhai et al., 2015).

3.4.45 Ethosomes containing diclofenac sodium for transdermal delivery

To investigate the effect of ethosomes on topical skin penetration, diclofenac sodium was used. The compatibility of Phospholipon 90H and diclofenac sodium was investigated using Fourier-transform infrared spectroscopy (FTIR) and no incompatibilities were discovered. In vitro permeation studies were carried out using a 282Franz diffusion cell to compare drug permeation and retention across pig skin of ethosomal systems against a hydroethanolic solution of drug and phospholipid and the commercially available Omnigel®. After 12 hours of testing, the ethosomal system had the highest drug permeation (18.76%), which was more than 6% higher than the marketed formulation. When compared to the hydroethanolic solution and the marketed product, the ehosomal system had the highest skin retention of diclofenac (3.62 g/cm2). This research supports the use of ethosomes as a more effective therapeutic tool for the topical delivery of poorly permeable drugs (Natarajan and Karri, 2018).

3.4.46 Topical Delivery of Tetrandrine by Ethosomes

The goal of this study was to see if ethosomes could be used to improve the antiarthritic efficacy of tetrandrine through topical application. Tetrandrine was discovered to be a weak base (pKa=7.06) with a pH-dependent partition coefficient. The pH gradient loading method was used to create the spherical-shaped ethosomes. Ex vivo permeation and deposition behavior of ethosomes revealed that drug flux across rat skin and drug deposition in rat skin were 2.1- and 1.7-fold higher than that of liposomes, respectively. Confocal laser scanning microscopy confirmed that ethosomes could improve drug delivery depth and quantity when compared to liposomesIn comparison to liposomes, ethosomes were shown to significantly improve the therapeutic efficacy of tetrandrine on Freund's complete adjuvant-induced arthritis. These findings suggested that ethosomes could be a promising carrier for topical tetrandrine delivery into and across the skin (Fan et al., 2013).

3.4.47 Transdermal mitoxantrone ethosmal gel

Mitoxantrone (MTO) ethosome gel was used to topically apply MTO for melanoma therapy. To begin, an ethosome was created by combining MTO, phospholipids, ethanol, and water, then adding hydroxypropyl methylcellulose to create an ethosome gel. The ethosome was identified. On an electrical cell-substrate impedance sensing system with a novel modified chip, the cytotoxicity on B16 melanoma cells was evaluated. The ethosome gel's anti-melanoma effect in vivo was investigated. Immunohistochemical and flow cytometric studies were carried out. The MTO ethosomes were 78 nm in size and had a zeta potential of 55 mV. The ethosomes were flexible vesicles with significantly higher in vitro permeability across rat skin than MTO aqueous solutions. The ethosomes were more cytotoxic and had a greater anti-melanoma effect in vivo than MTO solutions. The MTO ethosomes improved calreticulin membrane translocation in B16 cells and confirmed MTO cell uptake. The MTO ethosome gel is a promising transdermal delivery system for melanoma therapy that has the advantages of not invading the skin and having no severe side effects (Yu et al., 2015).

3.4.48 Ethosomes-based hydrogel formulations of methoxsalen

The creation and characterization of ethosomes-based hydrogel formulations of methoxsalen for improved topical delivery and effective vitiligo treatment. The ethosomes were created using a central composite design (CCD) and were tested for vesicle shape, size, zeta potential, lamellarity, drug entrapment, and drug leaching.

Following that, the optimized ethosomes were incorporated into Carbopol 934 gel and evaluated for drug content, rheological behavior, texture profile, in vitro release, ex vivo skin permeation and retention, skin photosensitization, and histopathological examination. Ethosomes were discovered to be spherical and multilamellar in nanometric size range structures with narrow size distribution and high encapsulation efficiency. Skin permeation and accumulation in the epidermal and dermal layers were observed with ethosomal formulations. The fluorescence microscopy study with 123 Rhodamine revealed that the drug-loaded ethosomes permeated deeper layers of skin more effectively. In addition, when compared to the conventional cream, the developed formulation demonstrated insignificant phototoxicity and erythema. Histopathological examination of skin segments was used to cross-validate the results. In summary, the ethosomes-based hydrogel formulation was discovered to be a promising drug delivery system, demonstrating enhanced percutaneous penetration of methoxsalen with reduced phototoxicity and erythema, resulting in improved patient compliance for vitiligo treatment (Garg et al., 2015).

3.4.49 Brucine Ethosomal Gel for Skin Cancer Delivery

The anticancer activity of brucine (BRU) loaded ethosomal gel was developed, optimized, and characterized. Essentially, the thin film hydration method was used to create BRU ethosomal preparations using the Central composite design (CCD), which was used to create a (32) factorial design. Two independent variables (phospholipid percentage and ethanol percentage) were assigned, each with three responses (vesicular size, encapsulation efficiency, and flux). One formula was chosen and incorporated into an HPMC gel base based on the desirability function to create BRU loaded ethosomal gel. Every physical attribute of the produced gel was evaluated. Ex vivo permeation, MTT calorimetric assay, and in vitro release studies were carried out. Acceptable values for the characterization parameters indicated that the BRU-loaded ethosomal gel was suitable for topical application. The investigation of in-vitro release was effectively extended for six hours. The optimal SSTF value was screened using the enhanced flux from the BRU loaded ethosome. Ultimately, an in-vitro cytotoxicity study demonstrated that the drug's anticancer activity against A375 human melanoma cell lines was markedly enhanced by the BRU-loaded ethosomal gel. Significantly, the research provided compelling evidence for additional research into the recently developed BRU loaded ethosomal gel as a potential therapeutic approach for the treatment of melanoma (Ismail et al., 2021).

3.4.50 Acyclovir-loaded ethosomes and solid lipid nanoparticles

A comparison of the efficacy of ethosomes and solid lipid nanoparticles as acyclovir delivery systems. Ethosomes were created by dissolving phosphatidylcholine and acyclovir in ethanol and then adding an aqueous buffer, whereas solid lipid nanoparticles were created by homogenization and ultrasonication. Cryo-transmission electron microscopy was used to characterize the morphology of both colloidal systems. Encapsulation efficiency for ethosomes was 94.22.8% and for solid lipid nanoparticles was 53.20.2%. Both formulations are close to neutral in terms of Z potential. The diffusion coefficients of the drug from ethosomes and solid lipid nanoparticles were 9.4 and 1.2-fold lower, respectively, when compared to free acyclovir in solution, demonstrating the ability of both colloidal systems to enhance drug diffusion. Plaque reduction assays in Vero cell monolayer cultures were used to assess both systems' antiviral activity against HSV-1. Acyclovir's antiviral activity was found to be

the same in both free and loaded forms, according to the data. Taken together, these findings suggest that colloidal systems could be useful in mediating acyclovir penetration within Vero cells (Cortesi et al., 2011).

Future Prospects

The discovery of the ethosome prompted a new approach in vesicular research for transdermal drug delivery. Furthermore, ethosomes have been shown to have exceptional encapsulation efficiency for a wide range of compounds, including lipophilic compounds. Ethosomes have the potential to significantly improve the efficacy of transdermal medication administration.

The current focus of ethosomal research is on target delivery. It has been successful in converting a variety of drugs into ethosomes with improved pharmacokinetic parameters. Because of the increased delivery provided by ethosomes, smaller amounts of chemicals are used in formulation to achieve the same or higher systemic bioavailability as traditional dose forms. The benefits of ethosomes in improved drug administration far outweigh the challenges of using them in formulation. Because it does not necessitate precise procedures or sophisticated equipment, the method is appealing for use in the pharmaceutical and cosmetic industries. Because the preparation methods are simple and inexpensive, commercialization of product development is straightforward. In TDD, the use of ethosomes may improve the delivery of drugs with both large and small molecular weights, such as vaccines and drugs with both lipophilic and hydrophilic properties. The goal of nanotechnology manufacturers is to create stable products with nanoscale particle sizes ranging from 1 to 100 nm that can effectively deliver the encapsulated active pharmaceutical ingredient (API). Ethosomes contribute to the achievement of this goal.

Ethosomes have been found to be far more effective at delivering drugs to the skin, and they have been used to encapsulate hydrophilic drugs, cationic drugs, proteins, and peptides. Ethosomal carriers present new challenges and opportunities for the development of novel and improved therapies. Ethosomes act as a penetrating enhancer, allowing for greater pervasion than other transdermal vesicular drug delivery systems. A wide range of active agents with various therapeutic functions were formulated into ethosomes for use in transdermal and dermal drug delivery systems. Based on the findings of these studies, we concluded that ethosomes are the present and future of the vesicle system in dermal and transdermal drug delivery.

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