ORIGINAL ARTICLE

Colloidal Dispersions (Liposomes and Ethosomes) for Skin Drug Delivery and their Role on Rheumatoid Arthritis

Nimmathota Madhavi, Beeravelli Sudhakar, J. Vijaya Ratna

Department of Pharmaceutical Technology, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India

Abstract

Aim: The aim of the present work was to focus on the applicability of liposomes and ethosomes for transdermal delivery. In the current review, we had focused on transdermal delivery transdermal delivery of vesicular system vesicular systems, i.e., liposome and ethosomes, factors affecting their permeation and penetration efficiency, limitations, applications, method of preparations, evaluation parameters, and selection of lipids. In this review, we considered the mechanism of controlled drug release of vesicles by transdermal delivery and the impact of their physicochemical properties. Rheumatoid arthritis (RA) is most frequently suffering disease in the geriatric population. By transdermal delivery, conventional anti-RA (ARA) therapy associated with several disadvantages. The modern ARA therapy; disease-modifying antirheumatic drugs (DMARDs) are more effective in reducing down disease progression. The basic mechanism of action of DMARDs in RA is not clear. The vesicles have a potential role in RA; especially, we focused on the impact of liposomes and ethosomes on RA by transdermal delivery, vesicular mechanism to justification in ARA therapy. **Conclusion:** The liposomes and ethosomes were beneficial tools for transdermal delivery. They have a potential role to control RA by transdermal delivery.

Key words: Ethosomes, flux and permeation, liposomes, phospholipid, rheumatoid arthritis

INTRODUCTION

The skin is the multilayered largest portion of an average adult body. It covers around 2 m^2 of surface area and receives approximately one-third of all blood circulating through the body. It controls influx of toxins, efflux of water and shows impermeable nature to the foreign molecules. The skin acts as a potential barrier due to stratum corneum, which represents the outer layer of the epidermis. Which imparts the barrier properties to the skin and inhibit the penetration of the active substances across the skin.[1]

The human skin consists of three main layers: epidermis, dermis and hypodermis. the stratum corneum is the first layer of the skin, plays the rate limiting barrier to various substances to permeate or penetrate through the skin. The human body contains 60% of water, whereas stratum corneum contains only 20% of water. It has 10-15 µm thickness and density 1.4 g/cm^3 depending on its state of hydration. The stratum corneum contains dried dead corneocytes, enriched keratin fibers with 0.2-0.4 µm thickness and 40 µm wide. The corneodesmosomes maintain structure stability by corneocytes is held together which confer structural stability to the stratum corneum. The lipids in the stratum corneum prevent excessive loss of water from the body. Every month, these corneocytes are usually regenerated. The epidermis is a multilayered membrane, and it contains 75-150 µm thickness. This layer does not contain blood vessels. The epidermis is hydrophilic in nature. The dermis contains thickness around 3-5 µm. The dermis layers full of with nerves, capillaries, sweat glands, sebaceous glands, and hair follicles. These are all frame into a network of connective tissue.[1,2]

Address for correspondence:
Nimmathota Madhavi, Research Scholar, Department of Pharmaceutical Technology, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh - 530003.
E-mail: nimmathota.madhavi@gmail.com.
Mobile: +919849153089.

Received: 12-05-2016
Revised: 23-06-2016
Accepted: 28-06-2016

The considerable advantages over the established and easier oral route are:[1-5]

Good patient compliance because of its little risk and simple way of administration. This mode thwarts the fluctuations which appear at gastrointestinal absorption.

- Self-medication is possible, removal of the dosage form at the will of the patient
- Controlled drug administration by controlling the drug plasma distribution and decrease the dosing frequency
- It outflanks hepatic metabolism and increases systemic absorption and bioavailbility
- Decreases dosing frequency, dose, and the cost
- Reduced toxic manifestations.

Factors affecting permeation across the skin[6-8]

Diffusion process controlled the transportation of active substances across the skin. The permeation of various active substances such hydrophilic and lipophilic is governed by Fick's first law. Several factors affect the permeation profiles of active substance through the skin such as solubility, charge, partition coefficient (Log P), molecular weight (molecular weight not exceeding 500 Daltons), and concentration gradient. The permeation efficiency is dependent on solubility and the partition coefficient of the drug. The partition coefficient (Log P) more than 2 is difficult to diffuse and does not partition into stratum corneum. At physiological temperature, stratum corneum (formidable barrier) maintains brick and mortar nature. Limited number of hydrophobic molecules able to cross the skin during this condition. Lipophilic molecules easily permeate across stratum corneum in presence of the delivery system by altering the order of stratum corneum, whereas hydrophilic molecules required some external energy to permeate across the stratum corneum. The aqueous solubility should be maintaining more than 1 mg/mL. pH-partition hypothesis states that molecules mainly cross biological membranes in unionized form.[9] Molecular weight is also one of the factors to choose the active substance. Molecules for transdermal drug delivery should have low molecular weight typically <500 Daltons. The lipophilicity should be in the range of 1-3. When we are selecting an active substance for transdermal delivery, the dose is also important parameter. Active substance should have a low dose and high potency.

Transport of drugs through the skin[10]

Transportation of active substances to the systemic ciculation through the skin is seen as a desirable alternative to oral medication. Transdermal drug delivery has advantage to bypass the gastrointestinal tract (GIT) which would obviate the GIT irritation that frequently occurs and avoid partial first pass metabolism by the liver. For many years, there was a speculation that active transport mechanisms occurring within skin were responsible for its unique barrier properties. The work of Scheuplein ultimately clarified the origin of skin permeability to molecular substances and established it as a passive diffusive process rather than a biologically active property. Through extensive studies, it was conclusively proven that the principle barrier to permeation is stratum corneum and that it is at least three to five times less permeable than the dermis and that permeability of the entire epidermis was indistinguishable from that of the stratum corneum alone. Skin permeation occurs by Fickian diffusion of the penetrating species, with the gradient impenetrate concentration across the entire skin localizes within the stratum corneum.

Routes of drug penetration through the skin[10-12]

Two pathways through the intact barrier may be identified, the intercellular and transcellular route. The intercellular lipid route is between the corneocytes, whereas transcellular route contemplates the crossing through the corneocytes and the intervening lipids. The permeation of drugs through skin includes diffusion through the intact epidermis through skin appendages. The skin appendages are hair follicles, sweat glands, and sebaceous glands form shunt pathways through the intact epidermis, occupying only 0.1% of the total human skin. It is known drug permeation through the skin is usually limited by the stratum corneum.

Recently, follicular penetration has become a major focus of interest due to the drug targeting to the hair follicle is of great interest in the treatment of skin diseases. However, follicular orifices occupy only 0.1% of the total skin surface area; it was assumed as a non-important route. However, a variety of studies shown the hair follicles could be a way to trough the skin. Skin being the largest organ of the human body and part of its physical and physiological functions; it is a vital and promising route of drug transport because of its abundance, large surface area, simple and highly preferred route because of the ideal advantages. This can be assuring via the research done on the transdermal route in the last few decades and increased market expected to reach $32 billion in 2015.[13]

Time has brought enough changes and drug delivering strategies to improve patient compliance and decrease toxic manifestations. Development in novel technologies associated with specific requirements majorly to accomplish patient suitability, targeted and appropriate usage of drug, illustrated and proven methodologies to overcome the drug-related drawbacks and formulation associated difficulties, physiological requirements had entertained the innovators to produce positive ways of new methodologies and logical determination of every tiny aspect opened the doors for supplementary portal to deliver drug in a safe and satisfactory way technically, ethically, and economically. The transdermal route of drug delivery has many advantages for administration of drugs in local and systemic therapy, but the skin is widely recognized for its effective barrier properties compared with other biological membranes.

The low permeability of the skin makes it a minor port of entry for drugs. During the past decades, there has been wide interest in exploring new techniques such as physical permeation enhancement technique (iontophoresis,[14,15] electroporation,[16] sonophoresis,[17] microneedles,[18,19] jet injectors, suction ablation, and thermophoresis) and passive penetration enhancement technique (saturated solutions[20] and penetration enhancers[21,22]) to increase drug absorption through skin. Transdermal drug delivery of drugs by lipid vesicles has evoked a considerable interest.[23,24] In the current review, we have been focused on liposomes and ethosomes for transdermal delivery.[25,26]

In general, dispersion systems are defined as systems where one immiscible phase is dispersed in another continuous phase. According to Banker Modern Pharmaceutics,[27] the dispersion systems are classified into three categories such as molecular level, colloidal level, and coarse level as mentioned in Table 1. The molecular dispersions are not visible by advanced electronic microscopes such as transmission electron microscopy (TEM). The coarse dispersion systems such as emulsions and suspensions could be visible by a compound microscope. The colloidal dispersions (liposomes, ethosomes, and nanoparticles) could be visible with scanning electron microscope (SEM) and TEM. The colloidal dispersions governed various carrier-based drug delivery systems such as niosomes, liposomes, ethosomes, nanoparticles, and mixed micelles [Table 1].[28]

Colloidal dispersions have a potential role on skin drug delivery. They have ability to overcome barrier properties of the skin without affecting skin structure and more potential than conventional dosage forms such as creams, ointments, and lotions.[28]

In the current review, we had focused on the applicability and limitations, preparation, evaluation parameters, selection of lipids for liposomal and ethosomal vesicular dispersions for transdermal delivery and their role in rheumatoid arthritis (RA).

Liposomes

Liposomes were first described by Bangham *et al.,* in 1965. Liposomes are microparticulate or colloidal carriers. Which are usually 0.05-5.0 μm in diameter and form spontaneously when certain lipids are hydrated in aqueous medium. Liposomes are microscopic spherical shaped vesicles composed of a bilayer of phospholipids with or without cholesterol. They can encapsulate and effectively deliver both hydrophilic and hydrophobic substances.[29-31]

Attractive biological properties of liposomes:[23-31]

- Liposomes are biocompatible, biodegradable, and non-toxic phospholipid-based systems
  - They enhance the solubility
  - They alter drug pharmacokinetics
  - They improve stability
  - They improve patient compliance
- Phospholipids are weak emulsifier, which contain well-tolerated surfactant molecules
- Liposomes can entrap water-soluble (hydrophilic) active pharmaceutical agents in their internal water compartment and water insoluble (hydrophobic) pharmaceuticals into the membrane
- Liposome-incorporated pharmaceuticals are protected from the inactivating effect of external conditions; yet do not cause undesirable side reactions
- Liposomes provide a unique opportunity to deliver pharmaceuticals into cells or even inside individual cellular compartments
- Size, charge, and surface properties of liposomes can be easily changed simply by adding new ingredients to the lipid mixture before liposome preparation and/or by variation of preparation methods
- On dermal delivery, liposomes increase the drug retention time at the desired site.

Liposomes as carrier for dermal drug delivery systems

Huge research was done on liposomes as drug delivery systems for dermal delivery. However, there was no clarity on its mechanism to transport active molecules across the skin. Different approaches were proposed for the mechanism of liposomes for dermal delivery.[30-33]

Few are as following: In general, stratum corneum is lipophilic in nature. It contains corneocytes, enriched keratin fibers (contain 50% phosphatidylcholine). These corneocytes are providing a fluidity (aqueous) environment for the diffusion of drug substances. When applying liposomes for dermal delivery, liposomes were interact with hydrophilic portion of the lipids in stratum corneum. At this condition, the phase transition temperature of the skin was decreased by increasing the fluidity in the skin helps the vesicles, could be easily alter

Table 1: Types of dispersion systems on the base of particle size of dispersed phase

Type of dispersion systems	Size ranges	Characteristics of systems	Examples
Molecular Level	<1 nm	Particles invisible by TEM	O_2, K, and Cl_2 ions dissolved in water
Colloidal level	1 nm-1 μm	Particles visible by SEM and TEM	Liposomes, ethosomes
Coarse level	>1 μm	Particles visible by microscope	Emulsions, suspensions

TEM: Transmission electron microscopy, SEM: Scanning electron microscope

the structure of the skin and easily squeezes into the skin and release drugs. It indicated that state of skin hydration played one of the key factors in the diffusion process through the skin. The permeation profile of drug substance strongly depends on the state of skin hydration. Human body contains water content around 10-20%. The occlusive conditions are more favorable to control the water content in the skin. In general, liposomes have occlusive properties to increase the skin humidity and consequently maintain the barrier functions of the skin. When treating liposomes for topical delivery, liposomes use to adsorb on the skin and maintain lipid concentration on the stratum corneum. The skin hydration is completely depends on phospholipid concentration on the skin. When we are developing liposomes for dermal delivery, natural lipids are more preferable compared to synthetic lipids. Natural lipids are maintained the barrier function and having ability to alter the permeation profile and decrease the side effects of the drug substance. The composition and properties of liposomes are the key factors in their permeation into the skin.

Basically, liposomes are colloidal suspensions. In this state, liposomal vesicles are in random in motion (thermal motion). At liquid state, liposomes are showing better results as skin drug delivery. However, the ultimate dosage form of skin delivery is semisolids such as ointments and gels. In general, semisolid dosage forms are usually maintained the higher skin contact time. As suspension for dermal delivery, it shows less skin contact time because they could easily evaporate. Now-a-day, gels more passionable dosage form to load liposomes compared to ointments and lotions. When loading liposomes into the polymeric gel barrier, vesicles movement completely restricted. The vesicles movement in the gels completely depends on viscosity profiles of gels. Hence, the pseudoplastic nature of viscosity is more favorable for gel preparation.

Recently, many research groups are using differential scanning calorimetry (DSC)[34] and spectroscopy (infrared [IR])[35] to predict the permeation profiles of liposomal vesicles through skin. DSC studies can be useful to predict the skin fluidity by exerting phase transition temperature of the skin and by Fourier transform IR studies, elucidation of lipid functional groups in the skin. When liposomes are applied on the skin, they could interact with a lipid portion of the stratum corneum; it leads to alter functional groups slightly from the normal skin stage to treated skin stage.

Liposomes can exert different functions by topical application. They could improve drug deposition (by adsorb on the skin) within the skin at the site of action (i.e. provides a localizing effect). The liposomal adsorption on the skin may promote a lipid exchange between phospholipid of bilayer and cellular skin lipids. The drug permeation and penetration profiles of liposomes were affected by several factors such as lipid compositions, vesicles size, and physicochemical properties of active molecules, vesicle membrane bilayer structure, and preparation methods.

Many researchers have given a statement about the role of liposomes for systemic delivery. They said that liposomes are basically rigid in nature, and they could easily adsorb on the skin and promote local action. Due to their rigid nature, liposomes were failed to enhance the systemic delivery (not enable to cross stratum corneum). The rigid liposomes decreased the bilayer membrane fluidity, and thus decrease the penetration rate of vesicles through the skin. The stratum corneum plays rate-limiting role in the modulation of systemic absorption of active substances through the skin. Vast research done on the conventional liposomal formulations for a transdermal application made them to think about the new developments but did not show fruitful results as they cannot pass the stratum corneum effectively.[29-32]

Here, we are giving some reference to support above statements. Fadda *et al*.[36] developed phycocyanin loaded liposomes by the film hydration techniques. They reported that phycocyanin (C-Pc) loaded liposomes were adsorbed on stratum corneum and accumulated C-Pc on stratum corneum. They reported that liposomes do not have ability to permeate the C-Pc into deep skin layers. In this study, liposomes were showed below 50% of encapsulation efficiency.

Recently Sudhakar *et al*.[37] developed terbinafine loaded liposomes by ethanol injection method using phospholipon 90H and DMPC. In this study, the encapsulation efficiency of liposomes showed 70%, and the *in vitro* permeation showed 72.62 ± 0.22% per 12 h. They reported that liposomal *in vitro* behavior completely affected the concentration of cholesterol. The above two references strongly indicated that liposomes were failure to increase the encapsulation efficiency above 90%. *In vitro* release studies indicated that liposomes failure to produce the systemic delivery. In general, formulation efficiency is dependent on physicochemical properties of the active substance, formulation properties, and biological barriers (skin). The low encapsulation, as well as low permeation profiles, leads to insufficient bioavailability of active substances from liposomal formulations through the skin.

To overcome this cusp (liposomal vesicular systems could not cross the stratum corneum), a new way which acted as viaduct to cross the stratum corneum and to deliver the drug considerably, a new liposomes-enriched (45% of ethanol) model has been developed and introduced a novel generation of vesicular elastic systems.

Ethosomes

Ethosomes was developed by Touitou. They are deformable liposomes embedded high alcohol content (up to 45%). Ethosomes are composed of phospholipid, ethanol, and water and with or without cholesterol. Liposomes and ethosomes are not alternative to each other. Ethosomes are quite different from liposomes on morphology, physicochemical properties, and mechanism of action. In general, ethosomes

use to maintain 45% of ethanol, due to this reason; ethosomes are differentiated from other vesicular systems. The particle sizes of ethosomes are available from 30 to 200 nm due to 45% of ethanol. The higher concentration of ethanol causes the vesicle bilayer self- degradation of interpenetrating character, leads to form lower particle size than conventional liposomes. It is proposed that alcohol fluidizes the lipid bilayer and stratum corneum bilayer lipids, thus acquiesces the soft, tractable ethosomes to penetrate through the stratum corneum. Naturally, ethosomes are soft, malleable sub-micron size spherical shaped vesicles. Ethosomes are permeated significantly through the skin layers more rapidly and enhanced steady state concentration (higher flux) in comparison to conventional liposomes. Although, the exact mechanism for better permeation into deeper skin layers from ethosomes is still not clear. The combined effect of phospholipid and high concentration of ethanol in vesicular formulations has been suggested to be responsible for deeper distribution and penetration in the skin lipid bilayers, but the highest ethanolic concentration is the devastating negative thing associated with ethosomes.[29,38]

Advantages of ethosomes[29,37-41]

- Ethosomes are versatile carrier-based drug delivery systems and could be encapsulate hydrophilic, lipophilic, and amphiphilic molecule
- By transdermal route, ethosomes can deliver drugs in a passive manner
- During development of ethosomes, phospholipids vesicles can exist higher ethanol concentration (up to 45%)
- Ethosomal vesicles are soft, malleable in nature due to the presence of ethanol in the vesicles; it has a fluidizing effect on lipid bilayer vesicles
- Ethosomes can alter the solubility properties of active ingredients in the vesicles leads to increase the permeation profile
- Ethosomal vesicles bilayer packed very less tightly, possess a high degree of fluidity and has lower transition temperature compared to liposomes
- Ethosomal vesicles maintain synergistic effect between ethanol, phospholipid vesicles, and skin, thus enhances drug transport compared to conventional liposomal rigid vesicles
- Ethosomes showing better stability compared to liposomes due to the negative charge of the net system avoiding aggregation of vesicles during their stability.

Touitou *et al.*[41] studied testosterone loaded ethosomes by cold preparation method. They were studied in *vitro* permeation studies on human cadaver skin. They reported the *in vitro* permeation was more for ethosomes (594.57 ± 39.9 µg) compared to marketed formulation (Androgel®) (92.27 ± 2.86 µg). They studied the effect of ethanol in receptor compartment by applying fluorescent solution on skin using confocal laser scanning microscopy (CLSM) studies. There were no changes were observed for tested and controlled skin. Hence, they reported the presence of ethanol in receptor compartment does not affect on penetration behavior of fluorescent solution.

Jain *et al.*[42] developed methotrexate loaded ethosomes by thin film hydration technique using soya (1-3w/v %) and 3:1 chloroform and methanol. In their study, different concentrations of phospholipid and ethanol were applied for the development of ethosomes. They reported that increasing concentration of lipid increased particles size, whereas increasing concentration of ethanol decrease the particle size. It indicated that higher ethanol concentration decreased the thickness of bilayer vesicle membrane. They study the effect of temperature on the storage of ethosomes. They reported that increasing temperature increased particle size from the initial stage. The percent encapsulation efficiency was decreased. They suggested that 2-8°C conditions were more favor for their better stability. *In vitro* drug permeation studies showed more permeation from ethosomes (57.2 ± 4.34 µg/h/cm^2) compared to liposomes (14.6 ± 1.65 µg/h/cm^2) and hydroethanolic solution (22.43 ± 0.24 µg/h/cm^2). They concluded that combined effect of ethanol and lipid, thus providing a mode for transdermal delivery.

Dubey *et al.*[35] developed melatonin-loaded ethosomes for transdermal delivery. Ethosomes were developed by cold method, and liposomes were developed by film hydration technique. The *in vitro* permeation of ethosomes showed 59.2 ± 1.22 µg/h/cm^{-1} flux, whereas hydroethanolic solution and conventional liposomes showed 22.43 ± 0.24 µg/h/cm^{-1} and 10.9 ± 1.65 µg/h/cm^{-1}, respectively. They said that the combined effect of ethanol and phospholipid providing a mode for sustained delivery of melatonin. In their study, DSC experiment was utilized to predict the fluidity condition of liposomes and ethosomes vesicles. They reported that liposomes showed higher phase transition temperature (T_m) 16.6°C, whereas ethosomes showed −8.8°C. This statement indicates that ethanol showed the fluidizing effect on ethosomal vesicles.

Preparation methods for liposomes and ethosomes

Various methods are available to prepare vesicular colloidal dispersions. The main goals of development of vesicles are to increase the encapsulation efficiency and to avoid leakage of drug from them. In general, passive loading and active loading methods are available to formulate the vesicular dispersions [Table 2].

Factors involved in active loading or remote loading[46,47]

The passive loading techniques are unable to produce 100% encapsulation efficiency of the active molecules into

Table 2: Preparation methods for liposomes and ethosomes[43-45]

Liposomes
1. Passive loading techniques
i. Mechanical dispersion technique
ii. Solvent dispersion technique
iii. Detergent removal method
i. Mechanical dispersion technique
Sonication
French pressure cell
Film hydration technique
Micro emulsion technique
Membrane extrusion technique
ii. Solvent evaporation technique
Ether injection method
Ethanol injection method
Reverse phase evaporation
iii. Detergent removal method
Dialysis process
Gel permeation
Detergent removal of mixed micelles
2. Active or remote loading technique
Tangential Flow Filtration
Ethosomes
Cold method
Hot method
Film hydration method

liposomes. The passive loading techniques have a lot of disadvantage such as low encapsulation, organic solvents retention in the lipid bilayers, and drug leakage during *in vivo* studies. The loading and encapsulation efficiency parameters are depended on various factors such as the source of lipids, drug/lipid ratios, thermal properties of lipid such as phase transition temperature, their saturation state (hydrogenated), carbon chain length in lipids, and cholesterol concentration.

Lipids that contain phosphorous know as phospholipid lipids. In general, phospholipid has (choline, phosphorus) hydrophilic head and lipophilic tails (fatty acid chains) both are linked with glycerol bridge. Selection of phospholipid affected development of liposomes. Natural phospholipids are extracted from soya bean, rape (canola) seed, wheat germ, sunflower and flax seed, and animal materials such as egg yolk, milk, or krill. Naturally, available phospholipids are more preferable. The large-scale production of natural lipids does not require more toxic organic solvents compared to synthetic. Lipid synthesis methods are lengthy and required toxic solvents. Unsaturated natural lipids are converted to saturate by hydrogenation using phospholipase-D enzyme. Saturated phospholipids have a high phase transition temperature, and they have highly flowing property.

Natural lipids are more preferable compared to synthetic, but saturated state is more important parameter rather than source of lipids. Saturated lipids have high phase transition temperature promotes high encapsulation efficiency and less leakage during storage. For example, DOXIL is the first liposomal product of doxorubicin. Even AMBISOMES is amphotericin B loaded liposomal product approved by USFDA. Both products were made up of hydrogenated soya phosphatidylcholine (HSPC). Carbon chain lengths also imp parameters to affect the drug loading efficiency. Long chain length lipids have higher Vandeeer Waals attractive forces promote cohesive nature to decrease the drug leakage. The lipid and cholesterol ratio influences the drug loading efficiency of liposomes. Cholesterol has an impact on lipid bilayer membrane structural organization and influences the stability of liposomes by reducing the rotational freedom of the phospholipid hydrocarbon chain length helps stabilize the lipid bilayers. During development of liposomes, cholesterol controls the penetration and permeation of water into liposomes. In general, lipid and cholesterol concentrations 60:40 and 70:30 are more preferable.

The active or remote loading is an alternative method to passive loading methods to overcome drawbacks associated with passive loading techniques. Many liposomal marketed products made by active loading technique such as doxorubicin, daunorubicin, citicoline loaded liposomal products. Active pharmaceutical ingredients should pose some desirable properties such as amphiphilic or hydrophilic in nature, log D at pH 7 in the range of −2.5-2.0 and pKa ≤11.

The pH imbalance is the driving force for active loading of active molecules into liposomes. Various ammonium salts such as citrates, phosphates, and sulfate are useful loading molecules, e.g., doxorubicin, daunorubicin, citicoline, and ciprofloxacin. Metal ion gradients are another remote loading technique. $MnSO_4$ and $MnCl_2$ are used for topotecan. Active molecules once encapsulated into liposomes will be available in precipitating state or protonated in liposomes because of this reason 100% encapsulation can be desirable and can be overcome the toxicity troubles. Tangential flow filters are working (TFF) more efficiently to purify the external concentrations of concentration gradients salts without wastage of samples.

Evaluation parameters during development of liposomes and ethosomes

During the development of liposomes and ethosomes, various physical and chemical parameters are involved followed as Table 3.

Table 3: Table summary of liposomes and ethosomes of various characterization parameters and their evaluation[43,44,48]

Characterization methods	Analytical methods/instrumentation
Physical characterization	
Vesicle shape and surface morphology	TEM, freeze-fracture electron microscopy, SEM
Vesicle size and size distribution	
Submicron range	Dynamic light scattering, TEM, Zetasizer
Micron range	TEM, freeze-fracture electron microscopy, photon correlation microscopy, laser light scattering, gel permeation and gel exclusion
Surface charge	Free-flow electrophoresis
Electrical surface potential and surface pH	Zeta potential measurements and pH probes
Lamellarity and lipid and ethanol interaction	Small angle X-ray scattering,[31] P-NMR, freeze-fracture electron microscopy
Phase behavior bilayer fluidity	Freeze-fracture electron microscopy, DSC
Purification	TFF
Percent capture	Mini column centrifugation, gel exclusion, ion-exchange chromatography, protamine aggregation, radio labeling
Drug release	Franz diffusion cell with artificial or biological membrane, dialysis bag diffusion
Vesicle fusion measurement	FRET assay, probe dilution assay
Turbidity	Nephalometer
Vesicle skin interaction	CLSM Fluorescence microscopy TEM Eosin-hematoxylin staining
Chemical characterization	
Phospholipid concentration	Barlett assay/Stewart assay
Cholesterol concentration	Cholesterol oxidase assay and HPLC
Drug concentration	Methods as per monograph for individual drug
Phospholipid peroxidation	UV absorbance, TBA (for endo peroxidase), Iodometric (for hydro peroxidase) and GLC
Phospholipid hydrolysis	HPLC and TLC and fatty acid concentration
Cholesterol auto-oxidation	HPLC and TLC
Antioxidant degradation	HPLC and TLC enzymatic diagnostic kit (Sigma)
Ethanol quantification	Oxidation of alcohol to acetaldehyde
Stability	Dynamic light scattering technique

TEM: Transmission electron microscopy, SEM: Scanning electron microscope, TFF: Tangential flow filters, HPLC: Hydrogenated soya phosphatidylcholine, TLC: Thin layer chromatography, TBA: Thiobarbituric acid, UV: Ultraviolet, CLSM: Confocal laser scanning microscopy, DSC: Differential scanning calorimetry

Excipients used in the formulation of liposomes and ethosomes[49]

Formulation of liposomes and ethosomes require similar components as listed in Table 4. Each of the components in the vesicular system has its own significance for their presence in the formulation, which is described in detail.

Phospholipids

Although liposomes and ethosomes can be made using a variety of surface-active molecules, most commonly, used reagents are phospholipids. All hydrated phospholipids do not form bilayers, e.g. phosphatidylethanolamine form inverted hexagonal phases and lysophospholipids form spherical micelles. The common thing that exists for bilayer forming compounds is their amphiphilicity. Phospholipids are the lipid amphiphiles with two acyl chains with same or different numbers of carbon atoms in each chain varying from 10 to 24-C in number linked to hydrophilic phosphate head via the glycerol bridge. There are different types of phospholipids based on their origin either natural phospholipids or synthetic phospholipids. Natural phospholipids are mostly suitable for transdermal applications and list of natural phospholipids available are given in Table 5. The synthetic phospholipids find their

Table 4: Constituents of liposomes and ethosomes

Excipients	Liposomes	Ethosomes
Base	Phospholipids Natural and synthetic lipids	Phospholipids Natural and synthetic lipids
Stabilizer	Cholesterol	Cholesterol
Permeation enhancer	-	Ethanol Propylene glycol
Solubilizer	Cyclodextrin	-
Vehicles	Different grades Carbopols Natural gums: Gum karaya	Different grades Carbopols Natural gums: Gum karaya
Charge inducers	Stearylamine, dicethylphosphate, and stearic acid	Stearylamine, dicethylphosphate, and stearic acid
Dispersion medium	Aqueous medium phosphate buffers	Aqueous medium phosphate buffers

Table 5: List of natural phospholipids

Name	Molecular weight in g/mol
HSPC	768.00
Phosphatidyl serine	744.03
Phosphatidyl inositol	886.56
Phosphatidyl ethanolamine	385.30

HSPC: Hydrogenated soya phosphatidylcholine

utility mainly when the parenteral route of administration is desired and different synthetic phospholipids available readily are mentioned in Table 6. Parameters that are to be considered in selection of phospholipids that would govern the geometry and packing (p) are:

- Molecular volume of the hydrophobic part (v)
- The critical length of the hydrophobic hydrocarbon region (l_c)
- The optimum surface per molecule at the hydrocarbon-water interface (α_o).

These are related to each other as per the below Equation:

$$P=v/(l_c\alpha_o) \quad (1)$$

Role of liposomes and ethosomes on RA[50-55]

Arthritis is two types: One is acute and another one is chronic. Acute arthritis is characterized by rapid joint destruction and the duration of this stage from hours too few days, e.g., infectious arthritis and gout. Chronic arthritis is mediated by adaptive immune response by T-cells and macrophages. Duration of this stage is from days to weeks. Ex: RA, RA associated with ankylosing spondylitis. RA is an autoimmune disease that causes our body to attacks its own immune system. RA can also cause firm lumps under the skin known as rheumatoid nodules. When our immune system attacks the synovium, RA can occur. Synovium is the lining of the membrane that surrounds the joint which eventually invade and destroy the cartilage and bone within the joint. Arthritis of the joint is called synovitis. In this case, synovium becomes inflamed and swollen the synovial membrane. In synovitis, cell proliferation will take place with respect to T-cells, B-cells, macrophages, and plasma cells. In case, RA lymphocytes and macrophages continuously enter into the joint cavity and multiply the release of inflammatory mediators such as cytokines, leukotrienes, and prostaglandins. Within weeks, the synovium becomes thickened, the mass of synovial tissue spreads over the top of cartilage in a rheumatic joint called as "pannus." This is made of white blood cells (WBC). WBC consists macrophages, B-cells, T-cells, neutrophils, plasma cells T-helper cells. These all will produce rheumatic factor, prostaglandins, cytokines, and other mediators. The disease prevalence is more in female compared to males in humans. In general, there is no proper medication for RA.

Non-steroidal anti-inflammatory drug (NSAIDS) has a lot of disadvantages such as low half-life, low bioavailability, frequent administration, and high doses to get desired concentration at the target site. On oral delivery, most of the NSAIDS such as etodolac, indomethacin, and ibuprofen are produce gastrointestinal bleeding, nausea, and vomiting. High doses lead to cause a toxic effect at the target site. They have first pass metabolism effect on the oral delivery. So, these drawbacks make increases the patient in-compliance.[50-53] Currently, in the market, NSAIDS loaded various dosage forms such as gels, ointments, lotions, and creams are available for RA. However, they are failed to control the disease. These conventional dosage forms do not have proper permeation and penetration ability across the skin. At present, disease-modifying antirheumatic drugs (DMARDs) are more effective in reducing down disease progression. The basic mechanism of action of DMARDs in RA is not clear. Vesicular systems have been extensively played predominantly role as drug delivery systems in the treatment of RA. Till now, huge research was done on liposomes and ethosomes for RA by dermal, transdermal, and intravenous (IV) administrations. Liposomes and ethosomes are lipid-based vesicular systems. Basically, phospholipids can act as permeability enhancers. They have ability to increase cutaneous and percutaneous absorption of the drugs by transdermal route.

Table 6: Summary of different synthetic phospholipids

Name of the phospholipid	Molecular weight (g/mol)	Transition temperature (T_c) (°C)
Dimyristoyl phosphatidyl choline (DMPC)	677.94	23
Distearoyl-sn-glycero phosphoethanolamine (DSPE)	748.06	74
Dipalmitoyl phosphatidyl choline (DPPC)	734.05	41
Disteroyl phosphatidyl choline (DSPC)	790.15	55
Dioleoyl phosphatidyl choline (DOPC)	786.12	-20
Dilauryl phosphatidyl ethanolamine (DLPE)	579.75	29
Disteroyl phosphatidyl ethanolamine (DSPE)	748.07	74
Dioleoyl phosphatidyl ethanolamine (DOPE)	744.04	-16
Dimyristoyl phosphatidic acid (DOPA)	721.94	-8
Disteroyl phosphatidyl serine (DSPS)	814.06	74
Dimyristoyl phosphatidyl glycerol (DMPG)	688.85	23
Dipalmitoyl phosphatidyl serine (DPPS)	757.96	63
Dilauryl phophatidic acid (DLPA)	557.65	31
Dipalmitoyl phosphatidic acid (DPPA)	669.87	67

Colloidal dispersions are played major role to control the RA, specially liposomes and ethosomes.

In RA, during the process of inflammation, inflammatory mediators act by enhancing vasopermeation in gap junctions present in the endothelial cells at the level of post capillary venues. Because of its low size (size <0.2 µm), colloidal dispersions easily involved extravasations in the gap junctions between adjacent endothelial cells. Depends on route of administration, liposomes were succeeded in antirheumatoid therapy. By parenteral (intra-arterial,[56] IV,[57] and intradermal[58]) administration, RA drugs loaded stealth liposomes were increasing the systemic circulation and deliver the drugs at targeted sight. When applying topically, liposomes act as reservoir and it can localize the drug substance at the skin level and decrease the systemic absorption. For treating pain located in the joints, topical, or transdermal route is suitable. For example, corticosteroids are a good choice for treatment of RA, but these drugs show rapid clearance at the site of inflammation. Many reports state that liposomes are a selective choice to encapsulate corticosteroids into liposomal vesicles, and they had ability to delay the release and increased retention in treatment of RA. The advantages of topical delivery of corticosteroids loaded liposomes improve and prolong drug exposure in the inflamed area. According to Srinath *et al*. statement, <200 nm size of colloidal dispersions is more preferable for preferable for effective localization at the inflammation sites.[58] Du Plessis *et al*.[59] reported that liposomal mechanism of drug transport across the skin. They suggested that naturally phospholipids have permeability nature to cross the biological barriers. Phospholipids may mix with intercellular lipids and leads to form swelling effect which provides to form intracutaneous depot. On the other hand, Mezei and Gulasekharam,[31] Mezei,[32] and Barry[60] suggested that liposomes are not unable to penetrate into deeper multilayer of skin.

Even ethosomes also had an impact on anti-RA.[61] Touitou *et al*.[62] developed betamethasone dipropionate loaded ethosomes for transdermal delivery. They reported that accumulation of drug concentration was three times higher than diprolene cream in epidermis and dermis. Moreover, the anti-inflammatory activity was also higher from ethosomes. Lodzki *et al*.[63] and Touitou *et al.,* developed cannabidiol loaded ethosomes for transdermal delivery. They reported that 49% of the loaded (845 g/cm^2) accumulated in within the viable skin tissue over 24-h period. In the *in vivo* studies also 110.7 ± 24.15 µg/cm^2 drug concentration was accumulated from ethosomes. ElSayed *et al*.[64] developed ketotifen loaded deformable liposomes and ethosomes for skin delivery. They reported that ethosomes showed higher drug concentration accumulated in the skin compared to deformable liposomes.

Chan *et al*.[65] developed nanosized ethosomes bearing ketoprofen for improved transdermal delivery. Ethosomal vesicles were prepared by the method reported elsewhere with some modifications. Size analysis of the optimized formulation showed that minimum vesicle size was observed to be 120.376 nm, whereas maximum was 410.2721 nm depending on the concentration of soya phosphatidylcholine and ethanol. Ethosomal formulation prepared with 3% SPC and 20% alcohol exhibited 175.8% entrapment efficiency, whereas alcohol concentration up to 40% entrapment efficiency was increased up to 78.77%. 1% soya phosphatidylcholine and 20% alcohol of formulations shows 81.475% of *in vitro* drug release. The flux was found to be 165.7719 µg/h/cm^{-1}, whereas lower flux 94.67103 µg/h/cm^{-1} was observed with hydroalcoholic drug solution. They reveal that ethosomal formulation showed better results as compared with a hydro alcoholic drug solution. CLSM confirmed that ethosomal system showing high fluorescent intensity of rhodamine 123 loaded formulations compared to hydroalcoholic drug solution of probe across skin.

Table 7: List of anti-inflammatory drugs loaded liposomes

Dosage form	Active ingredient	Excipients	Method of preparation	Conclusion	References
Liposomes	Indometacin	Phosphatidylcholine, cholesterol	High speed dispersion method	Liposomes are good choice to overcome the indometacin drawbacks by dermal delivery and indometacin loaded liposomes had strong anti-inflammatory effect	[58]
Ethosomes	Ketoprofen	Soya phosphatidylcholine	Modified ethanolic injection method	*ex vivo* permeation profile of ketoprofen loaded ethosomes showed better permeation than hydro ethanolic solution by human skin	[65]
Liposomes, ethosomes, and transferosomes	Dichlofenac sodium	Soya lecithin cholesterol, span 80, ethanol, carbopol 914	Liposomes by ethanol injection Ethosomes by cold method Transferosomes by rotary evaporation method	Transferosomes and ethosomes had better *ex vivo* profile than conventional liposomes	[67]
Liposomes, transferosomes, and ethosomes	Celecoxib	Phosphatidylcholine, dicethylphosphate, dipotassium lycyrrhizinate (DPG), sodium cholate	Liposomes and transferosomes by thin film evaporation and ethosomes were prepared by modified ethanol injection method	Ethosomes had higher permeation profile than transferosome, liposomes, and aqueous suspension.	[68]
Liposomal gel	Piroxicam	Soya phosphatidylcholine, cholesterol	Film hydration method	Encapsulated of piroxicam produced an increase of topical anti-inflammatory effect, suggested that inhibition of inflammation can be obtained with lower drug concentrations from liposomal gel	[69]
Ethosomal gel	Ibuprofen	Phosphatidylcholine cholesterol, ethanol	Film hydration method	Ibuprofen plasma concentration reached a C_{max} of 74.11±18.52 ng/ml 2 h post application on rat skin. Ibuprofen gel induced an analgesic effect 30 minutes following its application lasting for at least 6 h	[70]
Ethosomes	Dichlofenac sodium	Soya phosphatidylcholine, cholesterol, ethanol	Film hydration technique and followed sonication	Ethosomes showed 12.9±1.0 μg/h/cm^2 of flux across the skin over 24 h. The optimized ethosomal batch had 22.9% of ethanol, PC: CH ratio of 88.4:11.6	[71]

(*Contd*...)

Table 7: (*Continued*)

Dosage form	Active ingredient	Excipients	Method of preparation	Conclusion	References
Liposomes	Dichlofenac Sodium	Phosphatidylcholine cholesterol	Film hydration method	Diclofenac has shown promising effect as a safe and convenient treatment for lameness associated with osteoarthritis	[72]
Proliposomes	Aceclofenac	Phosphatidylcholine cholesterol	Film-deposition on carriers method	Proliposomes deliver aceclofenac in a sustained manner and exhibited superior stability	[73]
Elastic liposomes	Meloxicam	B-cyclodextrin, HSPC	Rotary evaporation sonication method	Cyclodextrin-based elastic liposomes showed 9.1 time higher than pure drug and 1.4 time higher than meloxicam elastic liposomes	[74]
Liposomes	Lornoxicam	Soybean L-α-PC and cholesterol, Tween 80	Homogenization followed evaporation and sonication	Elastic liposome had the ability to enhance lornoxicam permeation in 24 h with controlled drug penetration pattern	[75]
Ethosomes	Meloxicam	phospholipids 90G, ethanol	Film hydration method	Synergetic effect of ethanol and lipids promotes flux 10.42 (μg/cm^2/h) at 24 h	[76]

PC: Phosphatidylcholine, HSPC: Hydrogenated soya phosphatidylcholine

Paolino *et al*.[66] developed ethosomes for skin delivery of ammonium glycyrrhizinate i*n vitro* percutaneous permeation through human skin and *in vivo* anti-inflammatory activity in human volunteers. Ethosomes were prepared by the cold method. Ethosomes elicited an increase of the *in vitro* percutaneous permeation of both methyl nicotinate and ammonium glycyrrhizinate ethosomes. As compared with methyl nicotinate ethosomes, ammonium glycyrrhizinate ethosomes showed better vesicle size ranges were observed between 350 and 100 nm, and entrapment efficiency was found to be 78.45. Finally, they state that the differences between methyl nicotinate and ammonium glycyrrhizinate in the cumulative amount of drug permeated through human stratum corneum epidermis from the same type of formulations were probably due to the different physicochemical properties of the two molecules because methyl nicotinate erythema depends on two principal factors: Vehicle composition and duration of application. These findings were probably due to the presence of ethanol that can improve the skin accumulation of ammonium glycyrrhizinate. Some of the additional supporting data as mentioned in Table 7 for liposomes and ethosomes for transdermal delivery.

CONCLUSION

By transdermal delivery, stratum corneum restricts or obstacle to entry for active molecule. Three different generations of transdermal delivery systems were developed increase transdermal flux and localization viable skin multilayers. However, they have poor distribution profiles by the transdermal route. The above review indicates that vesicular systems are an alternative source to improve transdermal drug delivery system profile. In the vesicular dispersions from (liposomes) Mezei to Elka Touitou (ethosomes), huge research was done. However, they'll be no clarity on the vesicular drug release mechanisms for transdermal delivery. Phospholipid is a weak emulsifier and natural permeating agent. In association with cholesterol, the permeability was restricted. Liposomal vesicle permeation rate was inversely proportional to the concentration of cholesterol. The lower concentration of cholesterol makes liposomes more permeability, whereas higher concentration cause more rigid in nature causes to decrease permeation through the skin. When compared to liposomes, ethosomes better for increase flux concentration of drugs across the skin. Particularly in ethosomes, ethanol itself acts as co-solvency for water and

permeation enhancer. During drug release process, there might be the synergistic effect of lipid and alcohol promotes ethosomes are more advanced than liposomes. However, the exact drug release mechanism was not clear. Still, it required some extent of research work on liposomes and ethosomes for transdermal delivery. Compared to parenteral route, there was no liposomal or ethosomal transdermal products in the market. The above-mentioned literature stated that vesicle able to localize and permeate transdermal concentrations of active substances across/into the skin. Liposomes compared to transdermal delivery, parenteral (IV) route of stealth liposomes ahead in the market. Because Doxil, ambisomes were entering the market. RA is a chronic disease; traditional RA treatment has huge disadvantages which we had mentioned above. The applicability of liposomes and ethosomes in RA increase the confidence to control the disease status by skin delivery.

REFERENCES

1. Ita KB. Transdermal drug delivery: Progress and challenges. J Drug Deliv Sci Technol 2014;24:245-50.
2. Karadzovska D, Brooks JD, Monteiro-Riviere NA, Riviere JE. Predicting skin permeability from complex vehicles. Adv Drug Deliv Rev 2013;65:265-77.
3. Prausnitz MR, Mitragotri S, Langer R. Current status and future potential of transdermal drug delivery. Nat Rev Drug Discov 2004;3:115-24.
4. Godin B, Touitou E. Transdermal skin delivery: Predictions for humans from *in vivo, ex vivo* and animal models. Adv Drug Deliv Rev 2007;59:1152-61.
5. Arora A, Prausnitz MR, Mitragotri S. Micro-scale devices for transdermal drug delivery. Int J Pharm 2008;364:227-36.
6. Naik A, Kalia YN, Guy RH. Transdermal drug delivery: Overcoming the skin's barrier function. Pharm Sci Technol Today 2000;3:318-326.
7. Hadgraft J. Skin deep. Eur J Pharm Biopharm 2004;58:291-9.
8. Gerber M, Breytenbach JC, du Plessis J. Transdermal penetration of zalcitabine, lamivudine and synthesised N-acyl lamivudine esters. Int J Pharm 2008;351:186-93.
9. Hadgraft J, Valenta C. pH, pK(a) and dermal delivery. Int J Pharm 2000;200:243-7.
10. Knorr F, Lademann J, Patzelt A, Sterry W, Blume-Peytavi U, Vogt A. Follicular transport route--research progress and future perspectives. Eur J Pharm Biopharm 2009;71:173-80.
11. Andrews SN, Jeong E, Prausnitz MR. Transdermal delivery of molecules is limited by full epidermis, not just stratum corneum. Pharm Res 2013;30:1099-109.
12. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci 2001;14:101-14.
13. Jain KK. Transdermal Drug Delivery: Technologies, Markets, and Companies. Basel: Jain Pharma Biotech; 2005.
14. Ita KB, Banga AK. *In vitro* transdermal iontophoretic delivery of penbutolol sulfate. Drug Deliv 2009;16:11-4.
15. Djabri A, Guy RH, Delgado-Charro MB. Transdermal iontophoresis of ranitidine: An opportunity in paediatric drug therapy. Int J Pharm 2012;435:27-32.
16. Murthy SN, Sen A, Hui SW. Surfactant-enhanced transdermal delivery by electroporation. J Control Release 2004;98:307-15.
17. Sarheed O, Frum Y. Use of the skin sandwich technique to probe the role of the hair follicles in sonophoresis. Int J Pharm 2012;423:179-83.
18. Donnelly RF, Singh TR, Morrow DI, Woolfson AD. Transdermal delivery applications. In: Microneedle-Mediated Transdermal and Interdermal Drug Delivery. Hoboken: Wiley-Blackwell; 2012. p. 79-112.
19. Henry S, McAllister DV, Allen MG, Prausnitz MR. Microfabricated microneedles: A novel approach to transdemal drug delivery. J Pharm Sci 1998;87:922-5.
20. Leveque N, Raghavan SL, Lane ME, Hadgraft J. Use of a molecular form technique for the penetration of supersaturated solutions of salicylic acid across silicone membranes and human skin *in vitro*. Int J Pharm 2006;318:49-54.
21. Iervolino M, Cappello B, Raghavan SL, Hadgraft J. Penetration enhancement of ibuprofen from supersaturated solutions through human skin. Int J Pharm 2001;212:131-41.
22. Vinod PS, Gordon LF, Richard HG, Howard IM, Hans S, Jerome PS, *et al*. *In Vivo* percutaneous penetration/absorption, Washington, D.C., May 1989. Pharm Res 1991;8:1071-5.
23. Korting HC, Schafer KM. Carriers in the topical treatment of skin disease. Schäfer-Korting M, editor. Drug Delivery. Berlin, Heidelberg: Springer; 2010. p. 435-68.
24. Gillet A, Evrard B, Piel G. Liposomes and parameters affecting their skin penetration behaviour. J Drug Deliv Sci Technol 2011;21:35-42.
25. Juan EC, Marlen RC, Luisa DD, Roberto DT, Luisa RV, Norma CA. Research and reports in transdermal drug delivery. Nanocarrier Systems for Transdermal Drug Delivery. Vol. 1. Rijeka: InTech Publisher.2012. p. 202-240.
26. Gireesh T, Kuldeep C, Pramod K. Liposomal current status, evaluation and recent advances. Int J Curr Pharm Res 2013;5:4-14.
27. Banker GS. Revised and expanded. Modern Pharmaceutics. 4th ed. New York: Marcel Dekker; 2002.
28. Schubert MA, Muller-Goymann CC. Novel colloidal delivery systems for dermal application. J Drug Deliv Sci Technol 2004;14:423-34.
29. Touitou E. Drug delivery across the skin. Expert Opin Biol Ther 2002;2:723-33.
30. Mezei M, Gulasekharam V. Liposomes--a selective drug delivery system for the topical route of administration: Gel dosage form. J Pharm Pharmacol 1982;34:473-4.

31. Mezei M, Gulasekharam V. Liposomes--a selective drug delivery system for the topical route of administration. Lotion dosage form. Life Sci 1980;26:1473-7.
32. Mezei M. Multiphase liposomal drug delivery system. U.S. Patent 4761288, 1988.
33. Schmid MH, Korting HC. Therapeutic progress with topical liposome drugs for skin disease. Adv Drug Deliv Rev 1996;18:335-42.
34. Jurgen L, Sebastian Z, Wolfgang P, Rolf S. Interaction of liposomes with the human skin lipid barrier: HSclls as model system — DSC of intact stratum corneum and *in situ* CLSM of human skin. J of Liposomal Res 1995;5:99-108.
35. Dubey V, Mishra D, Jain NK. Melatonin loaded ethanolic liposomes: Physicochemical characterization and enhanced transdermal delivery. Eur J Pharm Biopharm 2007;67:398-405.
36. Manconia M, Pendás J, Ledón N, Moreira T, Sinico C, Saso L, *et al.* Phycocyanin liposomes for topical anti-inflammatory activity: *In-vitro in-vivo* studies. J Pharm Pharmacol 2009;61:423-30.
37. Sudhakar B, Varma JN, Murthy KV. Formulation, characterization and *ex vivo* studies of terbinafine HCl liposomes for cutaneous delivery. Curr Drug Deliv 2014;11:521-30.
38. Touitou E. Composition for applying active substances to or through the skin. U.S. Patent 1998; 5,716,638.
39. Godin B, Touitou E. Erythromycin ethosomal systems: Physicochemical characterization and enhanced antibacterial activity. Curr Drug Deliv 2005;2:269-75.
40. Godin B, Touitou E. Mechanism of bacitracin permeation enhancement through the skin and cellular membranes from an ethosomal carrier. J Control Release 2004;94:365-79.
41. Ainbinder D, Touitou E. Testosterone ethosomes for enhanced transdermal delivery. Drug Deliv 2005;12:297-303.
42. Dubey V, Mishra D, Dutta T, Nahar M, Saraf DK, Jain NK. Dermal and transdermal delivery of an anti-psoriatic agent via ethanolic liposomes. J Control Release 2007;123:148-54.
43. Vemuri S, Rhodes CT. Preparation and characterization of liposomes as therapeutic delivery systems: A review. Pharm Acta Helv 1995;70:95-111.
44. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, *et al.* Liposome: Classification, preparation, and applications. Nanoscale Res Lett 2013;8:102.
45. Abdulbaqi IM, Darwis Y, Khan NA, Assi RA and Khan AA. Ethosomal nanocarriers: The impact of constituents and formulation techniques on ethosomal properties, *in vivo* studies, and clinical trials. Int J Nanomedicine. 2016;25:2279-304.
46. Eloy JO, Claro de Souza M, Petrilli R, Barcellos JP, Lee RJ, Marchetti JM. Liposomes as carriers of hydrophilic small molecule drugs: Strategies to enhance encapsulation and delivery. Colloids Surf B Biointerfaces 2014;123:345-63.
47. Xu X, Khan MA, Burgess DJ. Predicting hydrophilic drug encapsulation inside unilamellar liposomes. Int J Pharm 2012;423:410-8.
48. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes - Novel vesicular carriers for enhanced delivery: Characterization and skin penetration properties. J Control Release 2000;65:403-18.
49. van Hoogevest P, Wendel A. The use of natural and synthetic phospholipids as pharmaceutical excipients. Eur J Lipid Sci Technol 2014;116:1088-107.
50. McDuffie FC. Morbidity impact of rheumatoid arthritis on society. Am J Med 1985;78:1-5.
51. Janssens HJ, Lagro HA, van Peet PG, Gorter KJ, van der Pas P, van der Paardt M, *et al.* NHG - Standaard artritis. Huisarts Wet 2009;52:439-53.
52. Olsen NJ, Stein CM. New drugs for rheumatoid arthritis. N Engl J Med 2004;350:2167-79.
53. Gaffo A, Saag KG, Curtis JR. Treatment of rheumatoid arthritis. Am J Health Syst Pharm 2006;63:2451-65.
54. de Silva M, Hazleman BL, Thomas DP, Wraight P. Liposomes in arthritis: A new approach. Lancet 1979;1:1320-2.
55. Metselaar JM, Storm G. Liposomes in the treatment of inflammatory disorders. Expert Opin Drug Deliv 2005;2:465-76.
56. López-García F, Vázquez-Autón JM, Gil F, Latoore R, Moreno F, Villalaín J, *et al.* Intra-articular therapy of experimental arthritis with a derivative of triamcinolone acetonide incorporated in liposomes. J Pharm Pharmacol 1993;45:576-8.
57. Metselaar JM, Mastrobattista E, Storm G. Liposomes for intravenous drug targeting: Design and applications. Mini Rev Med Chem 2002;2:319-29.
58. Srinath P, Vyas SP, Diwan PV. Preparation and pharmacodynamic evaluation of liposomes of indomethacin. Drug Dev Ind Pharm 2000;26:313-21.
59. Du plessis J, Ramachandran C, Weiner N, Müller DG. The influence of particle size of liposomes on the deposition of drug into skin. Int J Pharm 1994;103:277-82.
60. Barry BW. Drug delivery routes in skin: A novel approach. Adv Drug Deliv Rev 2002;54:S31-40.
61. Touitou E, Godin B. Ethosomes for skin delivery. Drug Deliv Sci Technol 2007;17:303-8.
62. Nudelman Z. Steroid Dermal System for Reservoir Formation in the Skin. - M.Sc. Thesis, The Hebrew University of Jerusalem; 2006.
63. Lodzki M, Godin B, Rakou L, Mechoulam R, Gallily R, Touitou E. Cannabidiol-transdermal delivery and anti-inflammatory effect in a murine model. J Control Release 2003;93:377-87.
64. Elsayed MM, Abdallah OY, Naggar VF, Khalafallah NM. Deformable liposomes and ethosomes: Mechanism of enhanced skin delivery. Int J Pharm 2006;322:60-6.
65. Chourasia MK, Kang L, Chan SY. Nanosized ethosomes bearing ketoprofen for improved transdermal delivery. Results Pharma Sci 2011;1:60-7.

66. Paolino D, Lucania G, Mardente D, Alhaique F, Fresta M. Ethosomes for skin delivery of ammonium glycyrrhizinate: *In vitro* percutaneous permeation through human skin and *in vivo* anti-inflammatory activity on human volunteers. J Control Release 2005;106:99-110.
67. Ghanbarzadeh S, Arami S. Enhanced transdermal delivery of diclofenac sodium via conventional liposomes, ethosomes, and transfersomes. Biomed Res Int 2013;2013:616810.
68. Bragagni M, Mennini N, Maestrelli F, Cirri M, Mura P. Comparative study of liposomes, transfersomes and ethosomes as carriers for improving topical delivery of celecoxib. Drug Deliv 2012;19:354-61.
69. Canto GS, Dalmora SL, Oliveira AG. Piroxicam encapsulated in liposomes: Characterization and *in vivo* evaluation of topical anti-inflammatory effect. Drug Dev Ind Pharm 1999;25:1235-9.
70. Shumilov M, Bercovich R, Duchi S, Ainbinder D, Touitou E. Ibuprofen transdermal ethosomal gel: Characterization and efficiency in animal models. J Biomed Nanotechnol 2010;6:569-76.
71. Jain S, Patel N, Madan P, Lin S. Quality by design approach for formulation, evaluation and statistical optimization of diclofenac-loaded ethosomes via transdermal route. Pharm Dev Technol 2015;20:473-89.
72. Schleining JA, McClure SR, Evans RB, Hyde WG, Wulf LW, Kind AJ. Liposome-based diclofenac for the treatment of inflammation in an acute synovitis model in horses. J Vet Pharmacol Ther 2008;31:554-61.
73. Gupta V, Barupal AK, Ramteke S. Formulation development and *in vitro* characterization of proliposomes for topical delivery of Aceclofenac. Indian J Pharm Sci. 2008;70:768-75.
74. Jain SK, Gupta Y, Jain A, Amin S. Elastic liposomes bearing meloxicam-beta-cyclodextrin for transdermal delivery. Curr Drug Deliv 2008;5:207-14.
75. Ehab IT. Lipid vesicular systems: Formulation optimization and *ex vivo* comparative study. J Mol Liq 2014;196:211-6.
76. Ahad A, Raish M, Al-Mohizea AM, Al-Jenoobi FI, Alam MA. Enhanced anti-inflammatory activity of carbopol loaded meloxicam nanoethosomes gel. Int J Biol Macromol 2014;67:99-104.

Source of Support: Nil. **Conflict of Interest:** None declared.