

VESICLES – MECHANISM OF TRANSDERMAL PERMEATION: A REVIEW

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ABSTRACT

Transdermal drug delivery is an attractive alternative to conventional techniques for administration of systemic therapeutics. The major challenge in designing transdermal drug delivery systems is to overcome the natural transport barrier of the skin. One approach is the use of vesicle formulations. Liposomes were first shown to be of potential value for topical therapy by Mezei and Gulasekharan in 1980, since then investigation continued towards development of lipid vesicles as carriers for skin delivery of drugs. But still vesicles are considered as a controversial class of dermal and transdermal carriers. This review provides an overview of the effectiveness of conventional, deformable and ethosomal vesicles as drug delivery systems as well as their possible mode of action as permeation enhancers or transdermal drug carriers. Deformable liposomes and ethosomes penetrate stratum corneum, thus releasing their drugs or proteins into systemic circulation. Vesicles as carrier systems can give rise to development of novel transdermal drug delivery systems.

Keywords: Transdermal, Dermal, Liposomes, Transferosomes, Ethosomes, Invasomes.

INTRODUCTION

Transdermal drug delivery is a viable administration route for potent, low molecular weight therapeutic agents which cannot withstand the hostile environment of the gastro-intestinal tract and /or are subject to considerable first pass metabolism by the liver. It uses the skin as an alternative route for the delivery of systemically acting drugs. Dermal drug delivery is the topical application of drugs to the skin in the treatment of skin diseases, wherein high concentrations of drugs can be localised at the site of action, thereby reducing the systemic drug levels and side effects.¹⁻³

The skin covers a total surface area of approximately 1.8m² and provides the contact between the human body and its external environment. The stratum corneum, the outermost layer of the skin acts as the main barrier in the skin. The structure of the stratum corneum is often compared to a brick wall, with the keratin-rich corneocytes as the bricks surrounded by the mortar of the intercellular lipid lamellae. Two main routes of skin permeation are the transappendageal route and transepidermal route. The transappendageal route also known as the shunt route includes permeation through the sweat glands and across the hair follicles with their associated sebaceous glands. The transepidermal route contains two micropathways, the intercellular route and transcellular route as shown in fig 1⁴. The intercellular route is a continuous way through intercellular lipid domains and transcellular pathway through the keratinocytes, then across the intercellular lipids. Naturally all molecules traverse by a combination of all three routes, the relative importance of which route depends on the molecules physico-chemical characteristics.^{4,5}

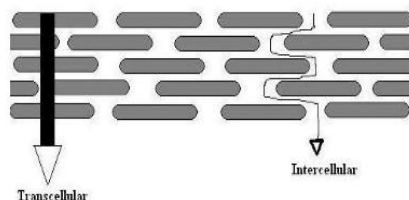


Fig. 1: A schematic representation of the skin showing the transcellular and intercellular routes of penetration.

It has been generally accepted that the highly organised crystalline lipid lamellae play an essential role in the barrier properties of the stratum corneum. Many techniques have been aimed to disrupt and weaken the highly organised intercellular lipids in an attempt to enhance drug transport across the intact skin or to increase the delivery force for the permeation of drugs across this skin barrier.^{5,6} One of the most convenient methods is the use of vesicle formulations as skin delivery systems.

The rationale for using vesicles in dermal and transdermal drug delivery is many folds.^{7,8} Vesicles might:

- Act as drug carriers to deliver entrapped drug molecules into or across the skin.
- Act as penetration enhancers for the penetration of the individual lipid components into the stratum corneum and subsequently altering the intercellular lipid lamellae within this skin layer.
- Serve as a depot for sustained release of dermally active compounds
- Serve as a rate limiting membrane barrier for the modulation of systemic absorption, hence providing a controlled transdermal delivery system.

Conventional liposomes as skin delivery systems

Liposomes are lipid vesicles that fully enclose an aqueous volume. Lipid molecules are usually phospholipids with or without some additives. Cholesterol may be included to improve bilayer characteristics of liposomes, increasing the microviscosity of the bilayers which in turn reduces the permeability of the membrane to water soluble molecules. This also results in the stabilization of the membrane and increase in the rigidity of the vesicles. Many methods for preparations of liposomes are described in literature. Most commonly used is the film hydration method.⁹

The potential value of liposomes for topical therapy was first introduced by Mezei and Gulasekharan (1980)¹⁰. In this study, greater four to five fold triamcinolone acetonide concentrations in the epidermis and dermis with lower systemic drug levels were observed when the drug was delivered from liposomal lotion in comparison with conventional formulations of the same drug concentrations.¹⁰

Several *in vivo* and *in vitro* transport studies reported that conventional liposomes only enhanced skin deposition, mostly by reducing (or no effect) the percutaneous permeation or systemic absorption of hydrocortisone,^{11,12,13} corticosteroids,¹⁴ lidocaine,¹⁵ tretinoin,¹⁶ and ciclosporin.¹⁷

These results suggested that conventional liposomes were useful for topical/dermal delivery of these drugs. Lipid composition, method of preparation and thermodynamic state of the bilayers of liposomes were all shown to greatly affect skin deposition behaviour of liposomes.¹⁸

Dermal delivery with skin-lipid liposomes was shown to be more effective than delivery with phospholipid vesicles.¹⁹ A decrease in cholesterol content in vesicular bilayers, which increases fluidity of the bilayers, resulted in increasing the drug transport across the stratum corneum. Recently it was evident that classic liposomes are of little or no value as carriers for transdermal delivery, as they do not deeply penetrate the skin. But rather remain confined to the upper layers of the stratum corneum. Confocal microscopy studies showed that intact liposomes were not able to penetrate into granular layers of the epidermis.²⁰

Due to the large size of the liposomes as stated by El Maghraby,²¹ they were not able to enter the capillary circulation and thus acted as reservoirs for the drug at the site of action. Though liposome lipids penetrate into the stratum corneum by adhering onto the surface of the skin and subsequently destabilising, fusing or mixing with the lipid matrix, they may act as penetration enhancers by loosening the lipid structure of the stratum corneum and promoting impaired barrier function of these layers to the drug with subsequent increase in the skin partitioning of drug.²² From the above observations we can infer that dermal delivery with skin-lipid liposomes is more effective than delivery with phospholipid vesicles.²³

Mechanism of action of liposomes

In the free drug mechanism (Fig. 2 at A) the drug permeates through skin after exiting from the vesicles²⁵. In the penetration enhancing mechanism (Fig. 2 at B), after application of vesicles, changes in the ultrastructures of the intercellular lipids were seen suggesting a penetration enhancing effect.²⁶ In vesicle adsorption to and/or fusion with the stratum corneum (Fig. 2 at C) the vesicles may adsorb to the stratum corneum surface with subsequent transfer of drug directly from vesicles to skin or vesicles may fuse and mix with the stratum corneum lipid matrix, increasing drug partitioning into the skin.

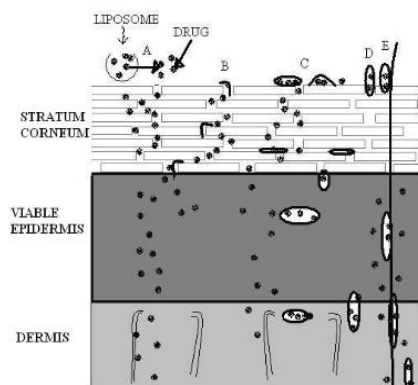


Fig.2: Possible mechanisms of action of liposomes as skin drug delivery systems. (A) is the free drug mechanism, (B) is the penetration enhancing process of liposome components, (C) indicates vesicle adsorption to and/or fusion with the stratum corneum (SC) and (D) illustrates intact vesicle penetration into and through the intact skin and (E) the transappendageal route.²⁴

The interaction of liposomes with human skin has been reviewed and it was concluded that they can be taken into the skin but cannot penetrate through intact healthy stratum corneum, instead they dissolve and form a unit membrane structure.²⁷ In intact vesicular skin penetration mechanism, (Fig.2 at D) traditional liposomes with intact vesicles cannot penetrate the human skin but ultradeformable

liposomes have been reported to invade the skin intact and go deep enough to be absorbed by the systemic circulation.¹⁶

The transappendageal penetration (Fig. 2 at E) route plays a minor role in transdermal delivery from liposomes. Electron microscopy indicated that liposomes upto 600nm of diameter can penetrate through skin but those of 1000nm or more remain interiorised in the stratum corneum. Deposition was higher in hairy guinea pigs but with regard to penetration through skin, no difference could be found between hairless and hairy guinea pigs. Also, vesicular delivery through shunts was excluded on the basis that there were no significant variations between different animals or humans with diverse densities of hair follicles, with regard to the transferosomal input of insulin.²⁸ The transfollicular delivery from liposomes was enhanced only after it was combined with iontophoresis technique.²⁹

Niosomes as skin delivery systems

Niosomes are composed of non-ionic amphiphiles (surfactants) and are similar in function to liposomes.

Niosomes constructed from a new non-ionic surfactant alpha, omega-hexadecyl-bis-(1-aza-18-crown-6) (bola-surfactant), span 80 and cholesterol show significantly improved percutaneous permeation of ammonium glycyrrhizinate³⁰ with respect to both the aqueous drug solution and a physical mixture between unloaded bola-niosomes and the aqueous drug solution. Niosomes constructed from cholesterol, span 60 and dicetyl phosphate were effective in increasing skin permeation of frusemide across mouse skin as compared to conventional formulations.³¹

The migration of cyclosporin A from cyclosporin glyceryl dilaurate/C₁₆EO₁₀ /cholesterol niosomes into the deeper skin strata has also been studied *in vitro* and it was found that factors such as dosing volume in non-occluded conditions affected the rate of uptake with smaller dose volumes giving rise to an increased uptake of the drug into deeper skin strata. This is due to the time required for dehydration of the formulation, a fundamental element of the penetration process. This same formulation was found to be beneficial for the deposition of cyclosporin A and alpha-interferon into pilosebaceous units of the hamster ear model. Based on the above studies, it does appear that transdermal drug delivery with niosomes appears to be a promising carrier for hydrophobic and amphiphilic drug molecules and would require that the dose be applied in high concentrations and within niosomes prepared from low phase transition surfactant mixtures.³² Niosomes mechanism is similar to conventional liposomes.⁹

Deformable Liposomes as skin delivery systems

Over the past 15 years, intensive research led to the introduction and development, of a new class of highly deformable (elastic or ultraflexible) liposomes that have been termed transferosomes.

Transferosomes are ultradeformable hydrophilic lipid vesicles that putatively cross the skin under the influence of a transepidermal water activity gradient. Transferosomes consist of phospholipids and an edge activator that increases the deformability of the bilayers and is often a single chain surfactant such as sodium cholate, sodium deoxycholate, Span 60, Span 65, Span 80, Tween 20, Tween 60, Tween 80 or dipotassium glycyrrhizinate.³³

The edge activators confer ultradeformability on the transferosomes and allow them to squeeze through channels in the stratum corneum that are less than one-tenth the diameter of the transferosome. The driving force for penetration into the skin is the "transdermal gradient" caused by the difference in water content between the relatively dehydrated skin surface (approx 20% water) and the aqueous viable epidermis (close to 100%).³⁴

Vesicles can be applied occlusively or non-occlusively. The difference in skin interaction between occlusive and non-occlusive application is of importance for deformable vesicles. As the transport of transferosomes is driven by the osmotic gradient across the skin occlusion would eliminate this osmotic gradient and is therefore detrimental for the actions of the deformable vesicles.^{35,36}

Preparation of deformable liposomes involves methods similar to those used in the preparation of traditional liposomes. Most commonly, the film hydration method is used. The pro-vesicular approach, proposed to enhance the stability of vesicles, has been extended to deformable liposomes and pro-ultraflexible lipid vesicles of levonorgestrel were developed and investigated.³⁷

Mechanism of action of deformable liposomes

Deformable liposomes can penetrate through stratum corneum by two mechanisms. First, vesicles can act as drug carrier systems, whereby intact vesicles enter the stratum corneum carrying vesicle-bound drug molecules into the skin. Secondly vesicles can act as penetration enhancers, whereby vesicle bilayers enter the stratum corneum and subsequently modify the intercellular lipid lamellae. This will facilitate penetration of free drug molecules into and across the stratum corneum.^{38,39}

In the first mechanism the driving force for the vesicles entering the skin is xerophobia (the tendency to avoid dry surroundings).^{35,40} The difference in penetration between deformable liposomes and traditional liposomes is the high and stress-dependent adaptability of such deformable vesicles which enables them alone to squeeze between the cells in the stratum corneum, despite the large average vesicle size (Fig.3). Thus, they can trespass the intact skin spontaneously, under the influence of the naturally occurring in-vivo transcutaneous hydration gradient intact without permanent disintegration.^{35,36,40}

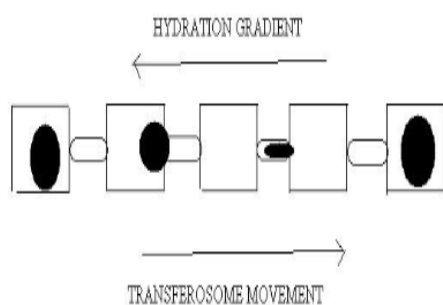


Fig.3: Ultradeformable transferosome squeezing through minute pores in the stratum corneum, driven by the water concentration gradient. The modified liposome thus penetrates from the horny layer surface (relatively dry) to the wet viable tissues.⁴

The intact vesicular permeation into the stratum corneum and the penetration enhancing effect play a role in the enhanced skin delivery of drugs by deformable liposomes under non-occlusive conditions and it suggests that one of the two mechanisms might predominate the other according to the physico-chemical properties of the drug. The transport of drug by deformable liposomes into the stratum corneum bypassing the main barrier for drug permeation will considerably improve skin delivery. Ultradeformable vesicles have been shown to be successful in delivering a range of different drugs across the skin including 5-fluorouracil,⁴¹ lidocaine,⁴² tetracaine,⁴² cyclosporin A,⁴³ insulin,⁴⁴ diclofenac,⁴⁰ triamcinolone acetamide,^{45,46} hydrocortisone,⁴⁷ dexamethasone,⁴⁷ levonorgestrel,³⁷ estradiol,⁴⁸ low molecular weight heparin,⁴⁹ methotrexate,⁵⁰ dipotassium glycyrrhizinate⁵¹ and zidovudine.⁵²

Ethosomes as skin delivery systems

Ethosomes are novel lipid carriers composed of phospholipid, ethanol and water and are recently developed by Touitou et al.^{53,54} Liposomal formulations containing upto 10% ethanol and upto 15% propylene glycol were previously described by Foldvari et al⁵⁵

but the use of high ethanol content was first described by Touitou et al⁵⁶ for ethosomes. Due to the interdigitation effect of ethanol on lipid bilayers, it was believed that high concentrations of ethanol are detrimental to liposomal formulations.

Ethosomes are novel permeation-enhancing lipid vesicles embodying high concentration (20-45%) of ethanol and are prepared by dissolving the lipids and drug in ethanol. The aqueous component is added slowly in a fine stream at a constant rate in a well sealed container with constant mixing. Mixing is then continued for additional five minutes.⁵³

A characteristic feature of ethosomes is their small size relative to the liposomes. This could be due to their incorporation of high ethanol concentration, which confers a surface negative net charge to the liposome which causes the size of vesicles to decrease. The size of ethosomal vesicles were reported to increase with decreasing ethanol concentration in the range of 20-45%. Presence of ethanol allows for better solubility of many drugs thus exhibiting high encapsulation efficiency for a wide range of molecules including lipophilic drugs.⁵³⁻⁵⁷

Ethosomes were reported to improve *in vivo* and *in vitro* skin delivery of various drugs both under occlusive and non-occlusive conditions.

Mechanism of action of ethosomes

The fig.4 illustrates a hypothetical model of how ethosomes may enhance penetration of drugs through stratum corneum lipids. The stratum corneum lipids multilayers are densely packed and highly conformationally ordered at physiological temperature.

Ethanol interacts with lipid molecules in the polar head group region, resulting in a reduction in the T_m of the stratum corneum lipids thus increasing their fluidity. The intercalation of ethanol into the polar head group can result in an increase in the membrane permeability. Ethanol may also provide the vesicles with soft flexible characteristics which allow them to more easily penetrate into deeper layers of the skin. The ethosome vesicles can penetrate through the disordered stratum corneum. The release of drug in the deeper layers of the skin and its transdermal absorption could be the result of fusion of ethosomes with skin lipids and drug release at various points along the penetration pathway.⁵⁸ The effect of ethanol on stratum corneum lipids and on vesicle fluidity as well as dynamic interaction between ethosomes and the stratum corneum may contribute to the superior delivery properties. Ethosomes have been used both *in vivo* and *in vitro* for the delivery of various drugs across skin such as minoxidil,⁵³ testosterone,⁵³ acyclovir,⁵⁹ cannabidiol,⁶⁰ erythromycin⁶¹, ammonium glycyrrhizinate,⁶² sotalol,⁶³ sodium salicylate,⁶³ propranolol,⁶³ trihexyphenidyl,⁶⁴ zidovudine⁶⁵ and azelaic acid.⁶⁶

Invasomes as carriers for skin delivery systems and their mechanism:

Several lipophilic and hydrophilic penetration enhancers (i.e. labrasol,transcutol and cineole) were tested and penetration enhancer - containing vesicles have been introduced where there is enhancement in penetration due to the penetration enhancers.⁶⁷ Such penetration enhancer containing vesicles with terpenes as penetration enhancers were termed as invasomes.

Invasomes composed of phosphatidylcholine, ethanol and a mixture of terpenes as penetration enhancers have been introduced by Verma and Fahr's group.⁶⁸ In addition to the mechanism of penetration enhancement of elastic vesicles terpenes which are considered as potent penetration enhancers increase drug permeation by disrupting lipid packaging of stratum corneum and/or disturbing the stacking of the bilayers.⁶⁹

Penetration enhancer-containing vesicles have been used as carriers for Minoxidil⁶⁸, Diclofenac⁶⁷ and Temoporfin⁶⁹.

Vesicles being studied as carriers for skin delivery and their proposed mechanism have been listed in table1 taking some examples into consideration.

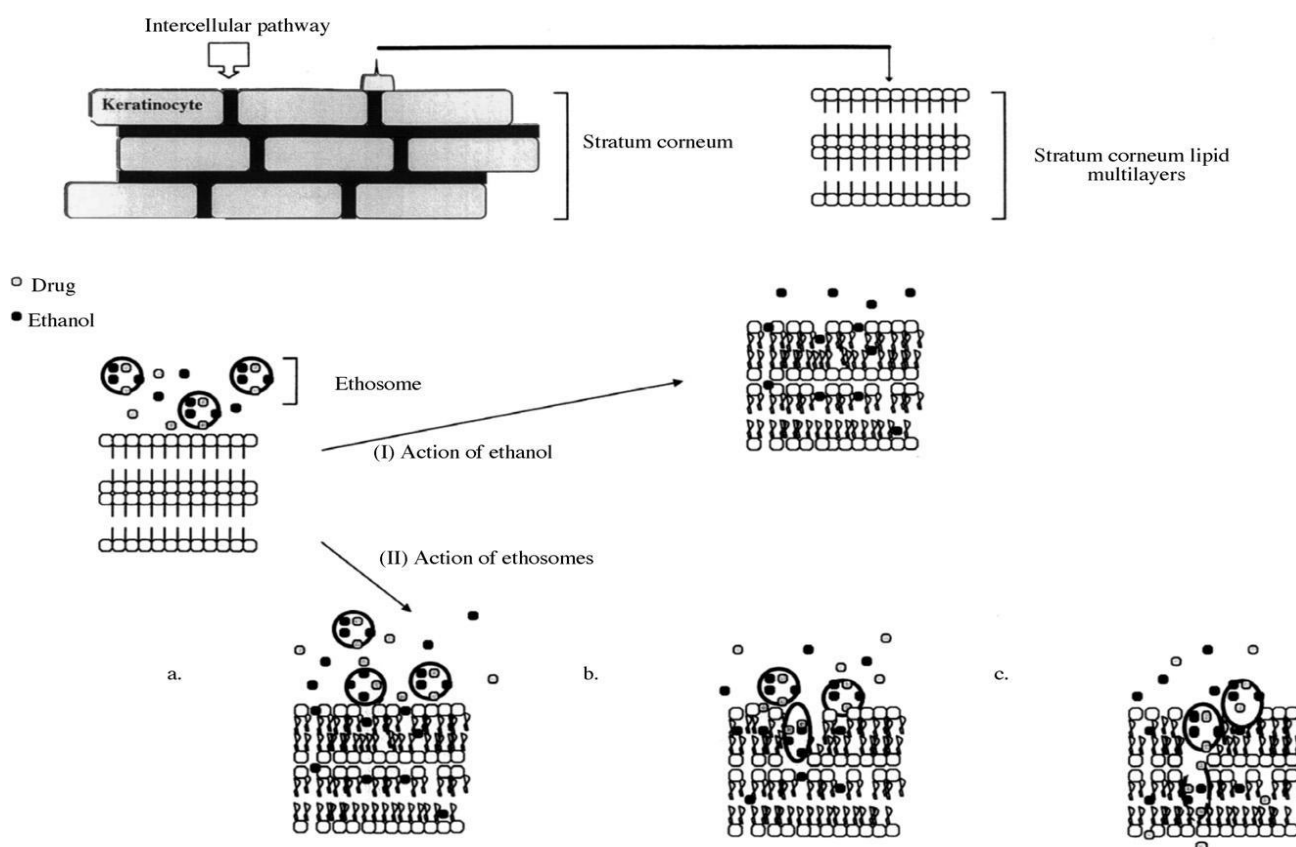
Fig.4: Mechanism of ethosomes as skin delivery systems.⁵³

Table 1:

S.no	Formulation	Drug	Vesicle Composition (%w/w)	Proposed mechanism	Conclusion	Ref
1	Liposomes & niosomes	Dithranol	PC:CH Span60: CH	The hydrodynamic condition present provides better drug-skin partitioning.	The in-vitro permeation study showed enhanced permeation with vesicles (liposomes & niosomes) when compared with cream base.	70
2	Liposomes & niosomes	enoxacin	SPC:CH (9:1) Span60: CH(1:1)	By permeation enhancer effect and direct vesicle fusion with stratum corneum.	Ability of Liposomes and niosomes to modulate drug delivery without significant toxicity makes the two vesicles useful to formulate topical enoxacin	71
3	Niosomes	Gallidermin	Tween61/CH/ DP(1:1:0.05 molar ratio)	No absorption due to large molecular structure of gallidermin as well as the large niosomal structure.	Gallidermin loaded in anionic niosomes and incorporated in gel is the superior topical anti-bacterial formulation because of high accumulation in the skin with no risk of systemic effect.	72
4	Bola-Niosomes	5-fluorouracil	Bola,span80,CH (2:5:2 molar ratio)	Bola surfactant contributes flexibility and deformability of the structure which enables them to pass through human skin similarly to ethosomes & transfersomes.	Bola-niosomes were able to promote the intracellular delivery thus improving the anti-cancer activity of the entrapped 5-fluorouracil.	73

5	Novel elastic niosomes	Diclofenac diethylammonium	DPPC/ Tween61/ span60: CH(1:1, 3:7,1:1 molar ratio)	In elastic niosomes ethanol may penetrate into the skin and influence the bilayer structure of stratum corneum leading to the enhancement of drug penetration	Enhancement of transdermal absorption through rat skin and in-vivo anti-inflammatory effect when entrapped in novel elastic tween61 niosomes	74
6	Elastic liposomes	Dipotassium glycyrrhizinate	PC:KG (2:1,4:1, 8:1) HPC:KG (2:1,4:1, 8:1)	By transcutaneous hydration force and further enhancement by fusion of vesicles with skin, facilitated by increase in fluidity of phospholipid bilayers containing KG.	Deformable liposomes when applied non-occlusively significantly improve the in-vitro skin delivery of KG when compared with aqueous solution	51
7	Deformable liposomes	Methotrexate	PC:KG (2:1) PC:KG (4:1) HPC:KG (2:1) HPC: KG(4:1)	By transcutaneous Hydration force	Deformable liposomes improve in-vitro skin delivery compared to either aqueous solution or normal liposomes.	50
8	Deformable liposomes	Ketotifen	PC:Tween 80 (84.5:15.5 w/w)	Under non-occlusive conditions, intact vesicle permeation into the stratum corneum.	The penetration enhancing effect appeared to be of greater importance in the enhanced skin delivery of ketotifen by deformable liposomes under non-occlusive conditions.	75
9	cationic transferosomes	Topical genetic vaccine against hepatitis B	DOTMA:SDC (95:5; 90:10; 85:15; 80:20; 75 :25 %w/w)	-----	Cationic transferosomes were capable of inducing strong humoral and cellular immune response after topical administration.	76
10	transferosomes	Non-invasive vaccine	SPC:SDC(85:15%w/w)	Increasing fluidity of intercellular lipid and weakening of stratum corneum, supports passage of transferosomes through very fine pores in the skin under suitable osmotic gradient.	Protein antigens can be safely delivered non-invasively through the skin using elastic carrier transferosomes.	77
11	ultradeformable vesicles	Diclofenac sodium	PC:EA (85:15%w/w)	Penetration enhancing effect and intact vesicle permeation under non-occlusive conditions.	Transferosomes can significantly improve the in-vitro skin delivery of diclofenac sodium compared to the marketed product (Olfen gel).	78
12	Ethanollic Liposomes	Melatonin	PC(2%) & Ethanol(30%)	Increase in thermodynamic activity due to evaporation of ethanol, increases penetration of drug molecule due to reduction in barrier property of stratum corneum by ethanol.	Ethosomes bearing melatonin offered a suitable approach for transdermal delivery when compared to liposomes & hydroethanolic solution.	79
13	Ethosomes	Lamivudine	PC& Ethanol	by lipid perturbation and increasing the intercellular lipid lamellae space of stratum corneum.	Lipid perturbation along with elasticity of ethosome vesicle seems to be the main contributor for improved skin permeation.	80
14	ethosomes in carbomer gel	bupirone	PC (2.5%)& Ethanol(38%) Carbomer gel(0.7%).	Bilayers fluidity of the soft phospholipid vesicle in conjunction with presence of high concentration of ethanol in the system.	Ethosomal bupirone transdermal system can be considered as a promising delivery system for the treatment of menopausal syndromes.	81
15	ethosomes	Ammonium glycyrrhizinate	PC (1-3%) & Ethanol (30-45%)	Combined effect of ethanol & phospholipids allowed sustained drug release that was determined by the formation of a reservoir of the drug in the skin.	In-vitro and in-vivo results showed that ammonium glycyrrhizinate ethosomes can ensure a sustained release of drug and prolongation of its therapeutic activity.	62
16	ethanollic liposomes	indinavir	PC (1-3%) & Ethanol (25-45%)	Dual function performed by ethanol i.e., fluidizing both the vesicular lipid bilayers	Ethosomes of indinavir showed better permeation when compared with	82

				and stratum corneum lipids, thus providing a greater malleability to the vesicles and enhancing permeability of the skin.	liposomes, ethanolic drug solution & plain drug solution.	
17	ethosomes	Ketotifen	PC (4.25%) & Ethanol (30%)	Ethanol increases the flexibility of the vesicles allowing them to more easily penetrate into deeper layers of the skin.	Ketotifen should be incorporated in ethosomal vesicles for optimum skin delivery as ethosomes were not able to improve skin delivery of non-entrapped ketotifen.	75
18	ethosomes	5-aminolevulinic acid	PE & Ethanol	Ethanol interacts with the skin and extracts lipids of the stratum corneum, and fluidized stratum corneum lipids create channels which allow the increased delivery of a drug.	The penetration ability of ethosomes was greater than that of liposomes.	83
19	Penetration enhancer-containing vesicles	minoxidil	Soy lecithin, Dicetylphosphate, Labrasol, Transcutol, Cineole	Intact vesicle penetration by entering the stratum corneum where they form a depot from which the drug is slowly released.	Penetration enhancer-containing vesicles can be a potential innovative carriers for improving topical delivery of minoxidil.	68
20	Invasomes	Temoporfin	PC: Ethanol (75:25) & Terpenes (0-1%)	Synergistic effect of liposomes, terpenes and ethanol.	Invasomes containing 1% of terpene mixture present an effective drug carrier system for delivering the highly hydrophobic drug Temoporfin into the stratum corneum and deeper layers of skin.	69

^a PC, phosphatidyl choline; CH, cholesterol; SPC, soya- phosphatidylcholine; DCP, dicetyl phosphate; DPPC, dipalmitoyl phosphatidylcholine; KG, dipotassium glycyrrhizinate; HPC, hydrogenated phosphatidylcholine; DOTMA, N-[1-(2,3-dioleoyloxy)-propyl]-N,N,N-trimethyl ammonium chloride; SDC, sodium deoxycholate; EA, edge activator; PE, phosphatidylethanolamine.

CONCLUSION

In summary, from the aforementioned studies, it is evident that liposomes offer potential value in dermal and transdermal drug delivery and recent advances and modifications appear to have generated increased therapeutic potential. Alteration in their composition and structure results in vesicles with tailored properties. Flexible and ultradeformable liposomes are such advances with claims of enhanced transdermal drug delivery to efficiencies comparable with sub-cutaneous administration.

However, a detailed knowledge of the mode of action is necessary in order to assess the full potential of elastic vesicles as skin delivery vehicles, such as the delivery of large molecules or targeting certain sites and cells within the skin. This is only possible when vesicles act as carrier systems and could give rise to the development of very interesting and novel transdermal drug delivery systems. Table 1 (vesicles being studied as carriers for skin delivery and their proposed mechanism) shows that deformable liposomes and ethosomes are better carriers for transdermal delivery when compared with liposomes and niosomes.

So, a vesicle formulation that rapidly enters the stratum corneum and remains in the deepest layers of stratum corneum releasing their drugs or proteins has useful advantages and is an important area of study to investigate such a promising approach.

REFERENCES

- Vyas SP, Khar RK. Transdermal drug delivery. In: Vyas SP & Khar RK, ed. Controlled drug delivery-concepts and advances. New Delhi, India: Vallabh Prakashan, 2002, 411-447.
- Yie W.Chein. Transdermal drug delivery & delivery systems. In: Yie W.Chein, ed. Novel drug delivery systems. New York: Marcel Dekker, 1992, 301-380.
- Yie W.Chein. Transdermal controlled systemic medications. New York: Marcel Dekker, INC. 1987.
- Brian Barry. Transdermal drug delivery. In: Michael E.Aulton, ed. Pharmaceutics- The science of dosage form design. New York: Churchill Livingstone, 2002, 499-533.
- J. Hadgraft. Skin, the final frontier. Int. J. Pharm. 2001; 224:1-18.
- Ashok K. Tiwary, Bharti Sapra and Subheet Jain. Innovations in Transdermal Drug Delivery: Formulations and Techniques. Recent Patents on Drug Delivery & Formulation. 2007; 1:23-36.
- Schreier H and Bouwstra J.A. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. J. Controlled Release. 1994; 30:1-15.
- Bouwstra J.A, Honeywell-Nguyen P.L. Vesicles as a tool for transdermal and dermal delivery. Drug Discovery Today: Technologies. 2005; 2(1): 67-74.
- Vyas SP, Khar RK. Targeted and controlled drug delivery. New Delhi, India: CBS publisher and distributor. 2001.
- Mezei M, Gulasekharan V. Liposomes- a selective drug delivery system for the topical route of administration. Lotion dosage form. Life Sci. 1980; 26: 1473-1477.
- Wohlrab W, Lasch J. Penetration kinetics of liposomal hydrocortisone in human skin. Dermatologica. 1987; 174: 18-22.
- Wohlrab W, Lasch J. The effect of liposomal incorporation of topically applied hydrocortisone on its serum concentration and urinary excretion. Dermatol. Monatsschr. 1989; 175: 348-352.

13. Kim M.K, Chung S.J, Lee M.H, Cho A.R, Shim C.K. Targeted and sustained delivery of hydrocortisone to normal and stratum corneum removed skin without enhanced skin absorption using a liposomal gel. *J. Control. Release.* 1997; 46: 243-251.
14. Fresta M, Puglisi G. Corticosteroid dermal delivery with skin-lipid liposomes. *J. Control. Release.* 1997; 44: 141-151.
15. Foldvari M, Gesztes A, Mezei M. Dermal drug delivery by liposome encapsulation: clinical and electron microscopic studies. *J. Microencapsul.* 1990; 7: 479-489.
16. Masini V, Bonte F, Meybeck A, Wepierre J. Cutaneous bioavailability in hairless rats of tretinoin in liposomes or gel. *J. Pharm. Sci.* 1993; 82: 17-21.
17. Egbaria K, Ramachandran C, Weiner N. Topical delivery of ciclosporin: evaluation of various formulations using in vitro diffusion studies in hairless mouse skin. *Skin Pharmacol.* 1990; 3: 21-28.
18. Bouwstra J.A, Honeywell-Nguyen P.L. Skin structure and mode of action of vesicles. *Adv. Drug Deliv. Rev.* 2002; 54: S41-S55.
19. Egbaria K, Ramachandran C, Weiner N. Topical application of liposomally entrapped cyclosporin evaluated by in vitro diffusion studies with human skin. *Skin Pharmacol.* 1991; 4: 21-28.
20. Kirjavainen M, Urtti A, Jaaskelainen I, Suhonen T.M, Paronen P, Valjakka-Koskela R et al. Interaction of liposomes with human skin in vitro – the influence of lipid composition and structure. *Biochim. Biophys. Acta.* 1996; 1304: 179-189.
21. El Maghraby G.M, Barry B.W, Williams A.C. Can drug-bearing liposomes penetrate intact skin? *J. Pharm. Pharmacol.* 2006; 58: 415-429.
22. Kirjavainen M, Monkkonen J, Saukkosaari M, Valjakka-Koskela R, Kiesvaara J, Urtti A. Phospholipids affect stratum corneum lipid bilayer fluidity and drug partitioning into the bilayers. *J. Control. Release.* 1999; 58: 207-214.
23. Zellmer S, Pfeil W, Lasch J. Interaction of phosphatidylcholine liposomes with the human stratum corneum. *Biochim. Biophys. Acta.* 1995; 1237: 176-182.
24. El Maghraby G.M, Barry B.W, Williams A.C. Liposomes and skin: From drug delivery to model membranes. *Eur J Pharm.* 2008; 34: 203-222.
25. Ganesan M.G, Weiner N.D, Flynn G.L, Ho N.F.H. Influence of liposomal drug entrapment on percutaneous absorption. *Int. J. Pharm.* 1984; 20: 139-154.
26. Kato A, Ishibashi Y, Miyake Y. Effect of egg yolk lecithin on transdermal delivery of bunazosin hydrochloride. *J. Pharm. Pharmacol.* 1987; 39: 399-400.
27. Schaller M, Korting H.C. Interaction of liposomes with human skin: the role of the stratum corneum. *Adv. Drug Deliv. Rev.* 1996; 18: 303-309.
28. Cevc G, Gebauer D, Stieber J, Schatzlein A, Blume G. Ultraflexible vesicles, Transferosomes, have an extremely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin. *Biochim. Biophys. Acta.* 1998; 1368: 201-215.
29. Han I, Kim M, Kim J. Enhanced transfollicular delivery of adriamycin with a liposome and iontophoresis. *Exp. Dermatol.* 2004; 13 (2): 86-92.
30. Paolino D. In vitro and in vivo evaluation of Bola-surfactant containing niosomes for transdermal delivery. *Biomed. Microdevices.* 2007; 9: 421-433.
31. Azeem A. Non ionic surfactant vesicles as a carrier for transdermal delivery of frusemide. *J. Dispers. Sci. Technol.* 2008; 29(5): 723-730.
32. Ijeoma F, Uchegbu, Suresh P. Vyas. Non-ionic surfactant based vesicles (niosomes) in drug delivery. *Int. J. Pharm.* 1998; 172: 33-70.
33. Pankaj Karande, Samir Mitragotri. Enhancement of transdermal drug delivery via synergistic action of chemicals. *Biochim. Biophys Acta.* 2009; 1788: 2362-2373.
34. Heather A.E. Benson. Transdermal drug delivery: penetration enhancement techniques. *Curr. Drug Delivery.* 2005; 2: 23-33.
35. Cevc G and Blume G. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. *Biochim. Biophys Acta.* 1992; 1104: 226-232.
36. Cevc G, Schatzlein A, Richardsen H. Ultraflexible lipid vesicles can penetrate the skin and other semi-permeable barriers unfragmented. Evidence from double label CLSM experiments and direct size measurements. *Biochim. Biophys Acta.* 2002; 1564: 21-30.
37. Jain S, Sapre R, Tiwary A.K, Jain N.K. Proultraflexible lipid vesicles for effective transdermal delivery of levonorgestrel: development, characterisation and performance evaluation. *AAPS PharmSciTech.* 2005; 6: E513-E522.
38. Honeywell-Nguyen P.L, Arenja S, Bouwstra J.A. Skin penetration and mechanisms of action in the delivery of the D2-agonist rotigotine from surfactant-based elastic vesicle formulations. *Pharm. Res.* 2003; 20: 1619-1625.
39. Honeywell-Nguyen P.L, Bouwstra J.A. The in vitro transport of pergolide from surfactant-based elastic vesicles through human skin: a suggested mechanism of action. *J. Control. Release.* 2003; 86: 145-156.
40. Cevc G and Blume G. New, highly efficient formulation of diclofenac for the topical, transdermal administration in ultraflexible drug carriers, Transferosomes. *Biochim. Biophys Acta.* 2001; 1514: 191-205.
41. El Maghraby G.M, Williams A.C, Barry B.W. Skin delivery of 5-fluorouracil from ultraflexible and standard liposomes in vitro. *J. Pharm. Pharmacol.* 2001; 53(8): 1069-1077.
42. Planas M.E. Noninvasive percutaneous induction of topical analgesia by a new type of drug carrier, and prolongation of local pain insensitivity by anesthetic liposomes. *Anesth. Analg.* 1992; 75(4): 615-621.
43. Guo J.X. Lecithin vesicular carriers for transdermal delivery of cyclosporin A. *Int. J. Pharm.* 2000; 194(2): 201-207.
44. Cevc G. Transdermal drug delivery of insulin with ultraflexible carriers. *Clin. Pharmacokinet.* 2003; 42(5): 461-474.
45. Cevc G and Blume G. Biological activity and characteristics of triamcinolone acetonide formulated with the self regulating drug carriers, Transferosomes(R). *Biochim. Biophys Acta, Biomembr.* 2003; 1614(2): 156-164.
46. Fesq H. Improved risk benefit ratio for topical triamcinolone acetonide in Transferosome (R) in comparison with equipotent cream and ointment: a randomized controlled trial. *Br. J. Dermatol.* 2003; 149(3): 611-619.
47. Cevc G and Blume G. Hydrocortisone and dexamethasone in very deformable drug carriers have increased biological potency, prolonged effect, and reduced therapeutic dosage. *Biochim. Biophys Acta, Biomembr.* 2004; 1663(1-2): 61-73.
48. Garg M. Ethinylestradiol loaded ultraflexible liposomes : pharmacokinetics and pharmacodynamics. *J. Pharm. Pharmacol.* 2006; 58(4): 459-468.
49. Song Y.K, Kim C.K. Topical delivery of low-molecular-weight heparin with surface charged flexible liposomes. *Biomaterials.* 2006; 27(2): 271-280.
50. Trotta M. Deformable liposomes for dermal administration of methotrexate. *Int. J. Pharm.* 2004; 270(1-2): 119-125.
51. Trotta M. Elastic liposomes for skin delivery of dipotassium glycyrrhizinate. *Int. J. Pharm.* 2002; 241(2): 319-327.
52. Jain.S, Tiwary A.K, Jain N.K. Sustained and targeted delivery of an anti-HIV agent using elastic liposomal formulation: mechanism of action. *Curr. Drug Deliv.* 2006; 3: 157-166.
53. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes- novel vesicular carriers for enhanced delivery: characterisation and skin penetration properties. *J. Control. Release.* 2000; 65: 403-418.
54. Touitou E, Godin B, Weiss C. Enhanced delivery of drugs into and across the skin by ethosomal carriers. *Drug Dev. Res.* 2000; 50: 406-415.
55. Foldvari M, Gesztes A, Mezei M, Cardinal L, Kowalczyk I, Behl M. Topical liposomal local anesthetics : design, optimization and evaluation of formulations. *Drug Dev. Ind. Pharm.* 1993; 19: 2499-2517.
56. Touitou E, Alkabes M, Dayan N. Ethosomes: novel lipid vesicular system for enhanced delivery. *Pharm. Res.* 1997; 14: 305-306.

57. Elsayed M.M, Abdallah O.Y, Naggar V.F, Khalafallah N.M. Deformable liposomes and ethosomes as carriers for skin delivery of ketotifen. *Pharmazie*. 2007; 62: 133-137.
58. Elsayed M.M, Abdallah O.Y, Naggar V.F, Khalafallah N.M. Lipid vesicles for skin delivery of drugs: Reviewing three decades of research. *Int J. Pharm.* 2007;332: 1-16.
59. Horwitz E, Pisanty S, Czerninski R, Helser M, Eliav E, Touitou E. A clinical evaluation of a novel liposomal carrier for acyclovir in the topical treatment of recurrent herpes labialis. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 1999; 87: 700-705.
60. 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: 377-387.
61. Godin B, Touitou E. Erythromycin ethosomal systems: physicochemical characterisation and enhanced antibacterial activity. *Curr. Drug Deliv.* 2005; 2: 269-275.
62. 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.
63. Kirjavainen M, Urtti A, Valjakka-Koskela R, Kiesvaara J, Monkkonen J. Liposome-skin interactions and their effects on the skin permeation of drugs. *Eur. J. Pharm. Sci.* 1999; 7: 279-286.
64. Dayan N, Touitou E. Carriers for skin delivery of trihexyphenidyl HCL: ethosomes vs. Liposomes. *Biomaterials.* 2000; 21: 1879-1885.
65. Jain S, Umamaheshwari R, Bhadra D, Jain N. Ethosomes: a novel vesicular carrier for enhanced transdermal delivery of an anti HIV agent. *Indian J. Pharm.Sci.*2004; 66: 72-81.
66. Esposito E, Menegatti E, Cortesi R. Ethosomes and liposomes as topical vehicles for azelaic acid: a preformulation study. *J.Cosmet. Sci.* 2004; 55: 253-264.
67. Maria Manconi, Carla Caddeo, Chiara Sinico, Donatella Valenti, Maria Cristina Mostallino, Giovanni Biggio, Anna Maria Fadda. Ex vivo skin delivery of diclofenac by transcutol containing liposomes and suggested mechanism of vesicle-skin interaction. *Eur. J. Pharm. Biopharm.* 2011 ;(article in press).
68. Simona Mura, Maria Manconi, Chiara Sinico, Donatella Valenti, Anna Maria Fadda. Penetration enhancer-containing vesicles (PEVs) as carriers for cutaneous delivery of Minoxidil. *Int. J. Pharm.* 2009;380: 72-79.
69. Nina Dragicevic-Curic, Dietrich Scheglmann, Volker Albrecht, Alfred Fahr. Temoporfin-loaded invasomes: Development, characterisation and in vitro skin penetration studies. *J. Control. Release.* 2008;127: 59-69.
70. Agarwal R, Katare O.P, Vyas S.P. Preparation and in-vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol. *Int. J. Pharm.* 2001; 228: 43-52.
71. Jia-You Fang, Chi-Tzong Hong, Wen-Ta Chiu, Ying-Yue Wang. Effect of liposomes and niosomes on skin permeation of enoxacin. *Int. J. Pharm.* 2001; 219: 61-72.
72. Aranya Manosroi, Penpan Khanrin, Warangkana Lohcharoenkal, Rolf G.Werner, Friedrich Gotz, Worapaka Manosroi, Jiradej Manosroi. Transdermal absorption enhancement through rat skin of gallidermin loaded in niosomes. *Int. J. Pharm.* 2010; 392: 304-310.
73. Donatella Paolino, Donato Cosco, Rita Muzzalupo, Elena Trapasso, Nevio Picci, Massimo Fresta. Innovative bola-surfactant niosomes as topical delivery systems of 5-fluorouracil for the treatment of skin cancer. *Int. J.Pharm.*2008; 353: 233-242.
74. Manosroi A, Jantrawut P, Manosroi J. Anti-inflammatory activity of gel containing novel elastic niosomes entrapped with diclofenac diethylammonium. *Int. J. Pharm.* 2008;360: 156-163.
75. Elsayed M.M.A, Abdallah O.Y, Naggar V.F, Khalafallah N.M. Deformable liposomes and ethosomes: Mechanism of enhanced skin delivery. *Int. J. Pharm.* 2006; 322: 60-66.
76. Sunil Manohar, Amit Rawat, Praveen K. Dubey, Prem N.Gupta, Kapil Khatri, Amit k. Goyal, Vyas S.P. Cationic transferosomes based topical genetic vaccine against hepatitis B. *Int.J.Pharm.* 2007; 340: 13-19.
77. Prem N.Gupta, Vivek Mishra, Amit Rawat, Praveen Dubey, Sunil Mahor, Suresh P. Vyas. Non-invasive vaccine delivery in transferosomes, niosomes and liposomes : a comparative study. *Int. J.Pharm.* 2005; 293: 73-82.
78. Ghada M. El Zaafarany, Gehanne A.S.Awad, Samar M.Holayel, Nahed D. Mortada. Role of edge activators and surface charge in developing ultradeformable vesicles with enhanced skin delivery. *Int.J.Pharm.* 2010; 397: 164-172.
79. Vaibhav Dubey, Dinesh Mishra, N.K.Jain. Melatonin loaded ethanolic liposomes: Physicochemical characterisation and enhanced transdermal delivery. *Eur.J.Pharm. Biopharm.*2007;67:398-405.
80. Subheet Jain, Ashok.k Tiwary, Bharti Sapra, Narendra K.Jain. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. *AAPS Pharm SciTech.*2007; 8: E1-E8.
81. Touitou E, Margarita Shumilov. Buspirone transdermal administration for menopausal syndromes, in vitro and in animal model studies. *Int. J. Pharm.* 2010;387:26-33.
82. Vaibhav Dubey, Dinesh Mishra, Manoj Nahar, Vikas Jain, Narendra Kumar Jain. Enhanced transdermal delivery of an anti-HIV agent via ethanolic liposomes. *Nanomedicine.* 2010;6: 590-596.
83. Yi-Ping Fang, Yi-Hung Tsai, Pao-Chu Wu, Yaw-Bin Huang. Comparison of 5-aminolevulinic acid – encapsulated liposome versus ethosome for skin delivery of photodynamic therapy. *Int. J.Pharm.* 2008; 356: 144-152.