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Review

Liposomes and skin: From drug delivery to model membranes

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ARTICLE INFO

Article history:
Received 10 March 2008
Received in revised form
18 April 2008
Accepted 2 May 2008
Published on line 8 May 2008

Keywords:
Liposomes
Transdermal
Animal skin
Skin lipid liposomes
Ultradeformable vesicles
Vesicles

ABSTRACT

The early eighties saw the introduction of liposomes as skin drug delivery systems, initially promoted primarily for localised effects with minimal systemic delivery. Subsequently, a novel ultradeformable vesicular system (termed "Transfersomes" by the inventors) was reported for transdermal delivery with an efficiency similar to subcutaneous injection. Further research illustrated that the mechanisms of liposome action depended on the application regime and the vesicle composition and morphology.

Ethical, health and supply problems with human skin have encouraged researchers to use skin models. Traditional models involved polymer membranes and animal tissue, but whilst of value for release studies, such models are not always good mimics for the complex human skin barrier, particularly with respect to the stratum corneal intercellular lipid domains. These lipids have a multiply bilayered organization, a composition and organization somewhat similar to liposomes. Consequently researchers have used vesicles as skin model membranes. Early work first employed phospholipid liposomes and tested their interactions with skin penetration enhancers, typically using thermal analysis and spectroscopic analyses. Another approach probed how incorporation of compounds into liposomes led to the loss of entrapped markers, analogous to "fluidization" of stratum corneum lipids on treatment with a penetration enhancer. Subsequently scientists employed liposomes formulated with skin lipids in these types of studies.

Following a brief description of the nature of the skin barrier to transdermal drug delivery and the use of liposomes in drug delivery through skin, this article critically reviews the relevance of using different types of vesicles as a model for human skin in permeation enhancement studies, concentrating primarily on liposomes after briefly surveying older models. The validity of different types of liposome is considered and traditional skin models are compared to vesicular model membranes for their precision and accuracy as skin membrane mimics.

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doi:10.1016/j.ejps.2008.05.002

Contents

1. Introduction

Transdermal drug delivery offers many advantages over other traditional routes of administration. Unfortunately, the barrier nature of the skin presents a significant obstacle for most drugs to be delivered into and through it (Barry, 1983). Accordingly, researchers are investigating various strategies to overcome these barrier properties. Ideally, these investigations should employ human skin. However, samples of human skin of sufficient size and quality for penetration experiments are not readily accessible to most investigators and in any case are only available in limited amounts. Thus, many models have been explored to replace human tissue and this in itself is an active area of research. Liposomes have been claimed to serve both uses; they can improve transdermal drug delivery and can be used as a model for the skin membrane. This review deals with the potential of liposomes as a skin drug delivery system and on their use as a skin model.

2. Skin structure

The structure of the skin and its barrier functions have been extensively described in the literature (Scheuplein and Blank, 1971; Elias, 1981; Orland, 1983; Barry, 1983; Williams, 2003). The skin barrier in the healthy and diseased state has also been recently reviewed (Bouwstra and Ponec, 2006). Human skin comprises a series of layers penetrated by hair shafts and gland ducts (Fig. 1). The major skin layers, from inside to outside, comprise the fatty subcutaneous layer (hypodermis), the dermis of connective tissue and the stratified avascular, cellular epidermis.

The dermis, at 3–5 mm thick, is composed of fibrous proteins (collagen and elastin) and an interfibrillar gel of glycosaminoglycans, salts and water. Blood and lymphatic vessels, nerve endings, pilosebaceous units (hair follicles and sebaceous glands) and sweat glands are embedded within the dermis. The hair follicles and sweat ducts open directly into the environment at the skin surface and provide the so-called appendageal route of skin permeation (Barry, 1983; Bissett, 1987).

The epidermis contains no blood vessels so nutrients and waste products must diffuse across the dermal–epidermal junction to maintain its vitality. The epidermis consists of five layers, which from inside to outside are the stratum germinativum (basal layer), stratum spinosum (spinous layer), stratum granulosum (granular layer), stratum lucidum and stratum corneum (SC). Because the SC cells are dead, the epidermis without the SC is usually termed the viable epidermis (Barry, 1983; Bissett, 1987).

The SC is considered as the rate limiting barrier in transdermal permeation of most molecules (Barry, 1983). The SC comprises 15–20 layers of corneocytes and when dry it has a thickness of 10–15 μm (Christophers and Kligman, 1964; Christophers, 1971). Upon hydration, the SC swells and its thickness can reach 40 μm (Scheuplein, 1967). The structure of the SC is often depicted in the so-called bricks and mortar arrangement (Michaels et al., 1975), where the keratin-rich corneocytes (bricks) are embedded in the intercellular lipid-rich matrix (mortar). This arrangement is illustrated in Fig. 2.

For any molecules applied to the skin, two main routes of skin permeation have been defined; the transappendageal and transepidermal pathways (Scheuplein, 1965). The transappendageal routes are also known as the shunt routes and include permeation through the sweat glands and across the hair follicles with their associated sebaceous glands. Recent studies have re-examined the long held assumption that the follicles occupy approximately 0.1% of the surface area of human skin (Scheuplein, 1967). Otberg et al. (2004) showed that follicular number, opening diameter and follicular volume are important considerations in drug delivery through these appendages and indeed the forehead provides

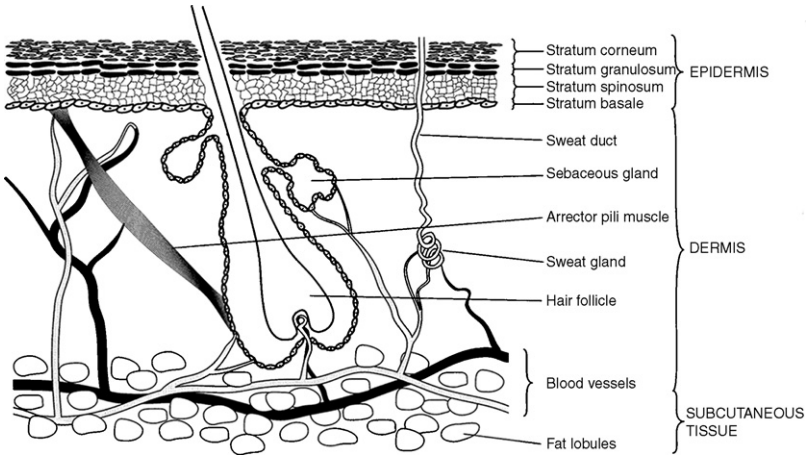

Fig. 1 – A diagrammatical representation of a cross-section through human skin showing the different cell layers and appendages (from Williams, 2003, with permission).

13.7 mm^2/cm^2 as the follicular infundibula, i.e. approximately 13.7% of the surface area of the forehead is available as follicles. Interestingly, the same study also showed that the historically held view of the follicles providing approximately 0.1% of the surface area of the stratum corneum appears to be valid for forearm skin.

The transepidermal pathway can be defined as the pathway where compounds permeate across the intact, unbroken stratum corneum. This pathway contains two micropathways. First, the intercellular route, which is a continuous but tortuous way through the intercellular lipid domains and secondly, the transcellular pathway through the keratinocytes, then across the intercellular lipids (Fig. 2) (Barry, 1991). As can be seen from Fig. 2, the transcellular pathway requires not only partitioning into and diffusion through the keratin bricks but also into and across the intercellular lipids. Thus, the intercellular lipids play a major role in the barrier nature of the SC.

The role of lipids in the barrier function has been investigated thorough permeation studies employing lipid extracted SC. Skin permeability to water was significantly increased after removing skin lipids (Scheuplein and Blank, 1971). Further, the permeability of the skin from different body sites can be related to total lipid content (Elias, 1981). Accordingly, the intercellular pathway is widely regarded as the main route of permeation of most compounds despite the relatively small surface area available for this route (Albery and Hadgraft, 1979; Guy and Hadgraft, 1989); naturally all molecules traverse by a combination of all three routes, the relative importance of which will vary depending on the molecules physico-chemical characteristics. As the lipid domains offer the primary barrier to permeation of most drugs, extensive research is being conducted to understand the composition and organization of these structures.

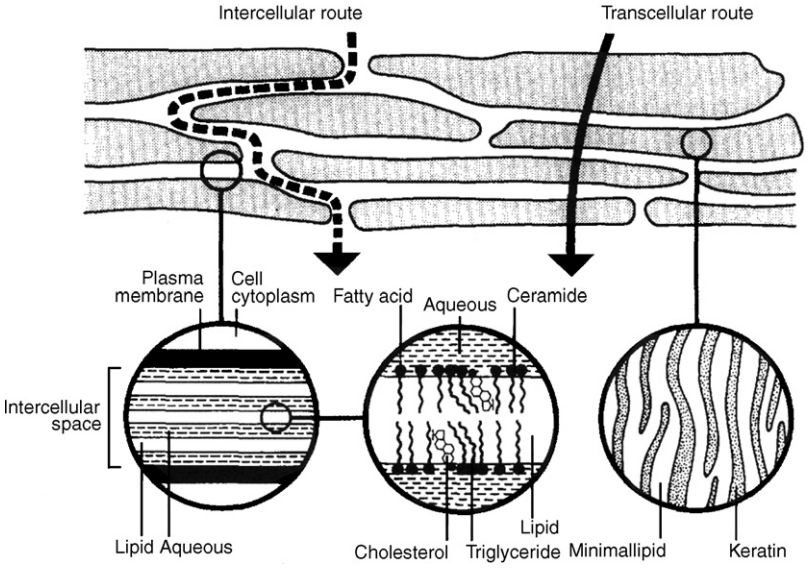

Fig. 2 – Diagram of the brick and mortar model of the stratum corneum with a simplified lamellar organization of intercellular domains showing the major stratum corneum lipids. Also shown are the possible drug permeation pathways through intact stratum corneum; the transcellular or the tortuous intercellular pathways (after Elias, 1981; Barry, 1991).

During epidermal differentiation, the lipid composition changes from a polar character to a neutral mixture (Lampe et al., 1983). For whole stratum corneum, the major fractions are neutral lipids (78%) and sphingolipids (18%) together with a small amount of polar lipid. There is a considerable quantity of non-polar material present (e.g. squalene) totalling about 11%. Both saturated and unsaturated fatty chains exist in all neutral lipid species, with unsaturated chains predominating except for the free fatty acids fraction. The ceramide (sphingolipid) fraction comprises primarily saturated fatty acid chains. Thus many lipid species exist in the horny layer, differing both in type and chain length; this complex lipid mixture forms bilayered structures (Fig. 2) (Barry, 1987).

3. Liposomes as skin drug delivery systems

Liposomes can exert different functions after topical application (El Maghraby et al., 2006). They can improve drug deposition within the skin at the site of action where the goal is to reduce systemic absorption and thus minimise side effects (i.e. provides a localising effect). They can provide targeted delivery to skin appendages in addition to their potential for transdermal delivery (i.e. increased systemic absorption). These effects are briefly reviewed with a description of differing mechanisms of action for liposomes as skin drug delivery systems. A more detailed description of liposome mechanisms of action can be found in the literature (El Maghraby et al., 2006).

3.1. *Localising effects*

Mezei and Gulasekharam (1980) were the first to employ liposomes as skin drug delivery systems. They reported that vesicles of dipalmitoylphosphatidylcholine (DPPC) and cholesterol (CH) (1.1:0.5, molar ratio) increased the concentration of triamcinolone acetonide in the epidermis and dermis by four- to fivefold and reduced percutaneous absorption compared with a standard ointment. Incorporating these liposomes in a gel dosage form, similar findings were observed compared with a gel containing free drug and the liposome components at the same concentrations (Mezei and Gulasekharam, 1982). This initial publication emphasised the importance of liposomal encapsulation and hence good entrapment efficiency of the drug in the vesicle for efficient skin drug delivery. Contrary to this, the topical input of 5α-dihydrotestosterone from similar vesicles was inferior to that from an acetone solution containing the same drug concentration when assessed by monitoring the size of the flank organs of the female hamster (Vermorken et al., 1984). The contradiction could be due to different experimental approaches; first, the use of different steroids; second, Mezei and Gulasekharam used skin deposition as a measure for activity whereas the Vermorken group measured a biological effect; further, the studies used different animal models, the schemes of application were different and finally it should be also noted that Mezei and Gulasekharam determined the skin deposition after washing the skin with ethanol. This washing step could have enhanced drug permeation into the skin. More recently, El Maghraby et al. (2006) explained that both groups used equal drug concentration rather than equal thermodynamic activity for their controls and that the biological effect on the hamster flank organs may require systemic drug delivery rather than a liposome-mediated localised effect.

Using a cutaneous herpes simplex virus guinea pig model, topical delivery of the peptide drug interferon from liposomes was superior to that from a water-in-oil emulsion or aqueous solution, with liposomes made from stratum corneum lipids (SCLs) showing the best effect. With respect to the method of nano-aggregate preparation, those produced by a dehydration/rehydration (DRV) method were the best (Weiner et al., 1989). The superiority of DRV liposomes could be due to raised entrapment efficiency. In a subsequent study employing a tape stripping technique on guinea pig skin in vitro, liposomes increased the deposition of interferon into stratum corneum and deeper skin strata with no drug detected in the receptor (Egbaria et al., 1990). This study indicated that the pharmacological effect obtained by Weiner et al. (1989) was due to improved skin accumulation (i.e. a localising effect). The build up of cyclosporin-A in the stratum corneum of hairless mice from various liposomal formulations was in the order of SCL liposomes > phospholipid liposomes > O/W emulsion > hydroalcoholic drug solution. This series of studies reflected the localising effects of liposomes and highlighted the dependence of deposition "efficiency" on the lipid composition and method of preparation. The anti-inflammatory effect from nano-structural delivery paralleled the skin accumulation results (Fresta and Puglisi, 1997).

The potential of liposome-encapsulated local anaesthetics to provide local anaesthesia to intact skin was investigated. Using the pin-prick assay, prolonged anaesthesia from a tetracaine vesicular formulation was shown, whereas a cream control formulation was ineffective (Gesztes and Mezei, 1988). Similarly, liposomes prolonged lidocaine anaesthesia compared with a conventional cream with a higher skin deposition being recorded after application of liposomes (Foldvari et al., 1990), indicating that the improved anaesthetic effect after vesicle delivery was probably due to improved skin accumulation. Both increased drug permeation through, and deposition into, human skin were recorded in vitro for liposomal tetracaine relative to an ointment containing the same drug concentration (Foldvari, 1994). Vesicles provided stronger and deeper anaesthesia relative to a commercial eutectic mixture of local anaesthetics (EMLA, 2.5% lidocaine and 2.5% prilocaine) in humans (Hung et al., 1997). Employing ethanol containing liposomes, the intensity and duration of the anaesthetic effect of benzocaine was increased (Mura et al., 2007).

Yu and Liao (1996) recorded increased retention and permeation of triamcinolone acetonide from liposomes compared with the ointment form. This finding is contrary to the early reports of Mezei and Gulasekharam (1980, 1982) which showed improved skin deposition and reduced permeation, and also differs from subsequent studies such as those of Egbaria et al. (1990) and Fresta and Puglisi (1997). This discrepancy could be due to the use of different membranes; permeability differences between species and especially the relatively poor correlation seen between rat skin as used by Yu and Liao (1996) and human skin which can make direct comparisons between laboratory protocols problematic (and highlights the importance of skin model selection).

The above studies all employed traditional liposomes made of phospholipids or skin lipids. Planas et al. (1992) reported an improved anaesthetic effect of lidocaine and tetracaine when delivered from ultradeformable vesicles. It is important to note that the authors applied the tested formulations under occlusion for 25 min, although this application protocol is contrary to the recommended "open" application for ultradeformable vesicles. Ultradeformable vesicles produced enhanced anaesthesia compared with drug solution or traditional liposomes. Surprisingly, topically applied ultradeformable vesicles generated an effect equivalent to that created after subcutaneous injection of the same formulation. Using heat-separated human abdominal epidermal membranes, El Maghraby et al. (2001a) recorded improved skin deposition of 5-fluorouracil (5-FU) from a similar vesicular formulation.

3.2. *Targeted delivery to skin appendages*

Several workers have studied the potential of such vesicular structures for targeting the appendages, especially to the pilosebaceous units (hair follicles with their associated sebaceous glands). This area was extensively reviewed by Lauer (1999) with an update by El Maghraby et al. (2006).

Carboxyfluorescein was selectively targeted into the pilosebaceous units of the hamster ears after application of liposomes. Liposomes showed better targeting than aqueous solutions even when these solutions contained 10% ethanol or 0.05% sodium lauryl sulphate, or when propylene glycol was the donor vehicle (Lieb et al., 1992). The deposition of γ-interferon into the skin of humans, hairless mice and hamster was greater from liposomes compared to an aqueous solution. The greatest accumulation was seen in hamster skin, which has the highest follicular density suggesting the follicular pathway as a route for drug deposition from liposomes (Du Plessis et al., 1992). Significant amounts of cimetidine were placed into the pilosebaceous glands and other skin strata of the Syrian male hamster ear, after topical application of the drug in 50% aqueous ethanol, nonionic surfactant vesicles, or in phospholipid liposomes (Lieb et al., 1994).

Contrary to the above reports, neither liposomes nor mixed micelles provided any advantage over an ethanolic gel with regard to follicular delivery of isotretinoin. This finding was attributed to the highly lipophilic nature of the drug which would intrinsically target the sebaceous gland (Tschan et al., 1997). The results were later explained by El Maghraby et al. (2006) on the basis that ethanol can enhance follicular delivery through partial solubilisation of the sebum or softening of the material in the duct. They also added that, whilst these findings could suggest a positive effect of liposomes and mixed micelles, they were only as effective as the ethanolic gel.

Vesicular preparations were superior in the treatment of acne vulgaris compared to conventional preparations including alcoholic lotions (Skalko et al., 1992). This was considered as strong evidence that vesicles can effectively target drug delivery to skin appendages (El Maghraby et al., 2006). Recently, the vitro permeation through hamster flank skin and in vivo deposition in hamster ear demonstrated the potentials of liquid-state liposomes and surfactant vesicles for successful delivery of finasteride to the pilosebaceous unit (Tabbakhian et al., 2006).

3.3. *Improved transdermal delivery*

Although the majority of reports dealing with standard liposomes concentrate on improved drug deposition into skin and its appendages, some early sources cited improved transdermal delivery from these nano-aggregates. After finite dose applications to hairless mouse skin, Ganesan et al. (1984) reported that, for lipophilic drugs, greater amounts were delivered from vesicles compared to aqueous solution. Liposome-encapsulated antibodies were distributed rapidly into the deep cutaneous regions of piglet skin with a clearly raised percutaneous absorption compared to aqueous solutions (Artman et al., 1990a,b). Fresta and Puglisi (1996) found that vesicles of unsaturated phospholipid (fluid liposomes), produced high percutaneous absorption and tissue distribution rather than skin accumulation. Employing a human skin graft model, a liposomal formulation of phosphatidylcholine (PC) and CH augmented the uptake of γ-interferon into the epidermis of viable human skin compared with aqueous solution (Short et al., 1996).

Whilst researchers were reporting mainly localised or rarely transdermal effects of liposomes, Cevc and Blume (1992) claimed that certain types of lipid vesicles (ultradeformable vesicles) can penetrate intact to the deep layers of the skin and may progress far enough to reach the systemic circulation, but they must be applied under non-occlusive conditions. The superiority of ultradeformable vesicles over "standard" liposomes for transdermal drug delivery was shown, and the importance of open (i.e. non-occluded) application was emphasised; however, a deviation from this protocol can be found (Planas et al., 1992) where an improved anaesthetic effect was reported after occluded treatment with anaesthetic ultradeformable vesicles. Transdermal immunization with large proteins by means of ultradeformable vesicles has also been reported (Paul et al., 1995). Further, ultradeformable vesicles improved the regio-specificity and the biological activity of the corticosteroids in vivo. The effect was dose-dependent and it was concluded that this carrier can target the drug into the viable skin and, when used in a higher dose, can distribute the medicament throughout the body (Cevc et al., 1997).

Ultradeformable vesicles provided arachidonic acid-induced oedema suppression equivalent to a lotion containing five times the drug concentration of that in ultradeformable vesicles, after 0.5 h. Subsequently, after 2 h, the *Transfersome* formulation was more efficacious than the lotion. When standard nano-carriers (PC, cholesterol) were evaluated, no oedema suppression was found after 0.5 h. After 2 h, however, liposomes produced a measurable suppression which was about one-third that of ultradeformable vesicles and about half that of the lotion (with five times more drug). The authors stated that the late effect of the vesicle formulation arose from free drug permeation following its release from liposomes (Cevc et al., 1997). El Maghraby et al. (2006) rejected this explanation and stated that vesicles should have provided one-fifth of the efficacy of the lotion (containing free drug) for this explanation to be valid, unless there is some penetration enhancing effect for such liposomes. Further, successful systemic delivery of insulin by ultradeformable vesicles has been reported from in vivo mice and human studies. The efficiency of the formulation was comparable to that obtained

after SC injection of the same preparation but with a longer lag time. This lag time may be required for vesicle skin penetration (Cevc et al., 1995, 1998). It is noteworthy that, in the 10 years since these reports, an ultradeformable insulin delivery system has not come to market which raises a question over the efficacy of these nano-aggregates as transdermal delivery systems.

In a series of studies involving an optimized experimental design, El Maghraby et al. (1999) investigated estradiol skin delivery from a variety of liposomes. The experiments probed optimized ultradeformable vesicles (El Maghraby et al., 2000a) relative to standard liposomes. The ultradeformable formulations included PC with sodium cholate, PC with Span 80 and PC with Tween 80. The standard nano-carriers encompassed pure PC vesicles (non-rigid), PC with CH (membrane stabilized liposomes), and two rigid vesicles of DPPC and DPPC/CH. The studies employed low dose open application of the formulations to human epidermal membranes hydrated by an "open hydration" protocol that maintained the transepidermal water gradient. The results indicated that all types of liposomes improved both estradiol deposition into and permeation through the epidermis compared with the saturated aqueous control. The ultradeformable vesicles were better than the standard liposomes with respect to transepidermal drug flux only.

The presence of a surfactant increases the elasticity of the lipid bilayers (El Maghraby et al., 2004). Accordingly, it was concluded that flexible liposomes are more efficient in transdermal drug delivery (El Maghraby et al., 2006). It was suggested that such surfactants (edge activators) can impart deformability to the nano-carriers, which allows for improved transdermal drug delivery (Cevc, 1996). The incorporation of ethanol in lipid vesicles is an alternative approach to fluidize the lipid membrane and thus enhance drug provision (Touitou et al., 2000). Ethanol containing vesicles (termed "ethosomes" by the inventors) improved the transdermal delivery of melatonin, an anti-jet lag agent with poor skin permeation and long lag time (Dubey et al., 2007). Also, flexible surfactant vesicles showed higher efficiency compared to rigid vesicles (Honeywell-Nguyen et al., 2003a).

Successful topical delivery of low molecular weight heparin was reported after incorporation into surface charged flexible vesicles made of lipids with Tween 80. These vesicles were termed flexosomes and the cationic structures were the most efficient (Song and Kim, 2006). The Span 80-based ultradeformable vesicles, initially optimized by El Maghraby et al. (2000a), have been recently employed for topical immunization. The results suggested that hepatitis B loaded ultradeformable vesicles are able to provide a positive immune response (D. Mishra et al., 2006; V. Mishra et al., 2006; Mishra et al., 2007).

A new type of fusogenic vesicles (vesosomes) have been introduced for topical immunization. In these, the tetanus toxoid was incorporated into cationic liposomes made of PC, dioleoyl phosphatidylethanolamine (DOPE) and dioleoyl trimethyl ammonium propane (DOTAP). These vesicles were subsequently entrapped in an interdigitated lipid bilayer to provide vesicles within a vesicle (vesosomes). These aggregates were claimed to offer a promising system for transcutaneous immunization (D. Mishra et al., 2006; V. Mishra et al., 2006).

4. Mechanisms of action of liposomes as skin drug delivery systems

Alternative mechanisms have been suggested (El Maghraby et al., 2006) for liposomes acting as skin drug delivery systems (Fig. 3). In the subsequent sections we will consider the proposed different mechanisms, illustrating both positive and negative findings. Examples of studies investigating these mechanisms are presented in Table 1 .

4.1. *Free drug mechanism (see Fig. 3 at A)*

According to this process, the drug permeates the skin independently after exiting from the vesicles (Ganesan et al., 1984).

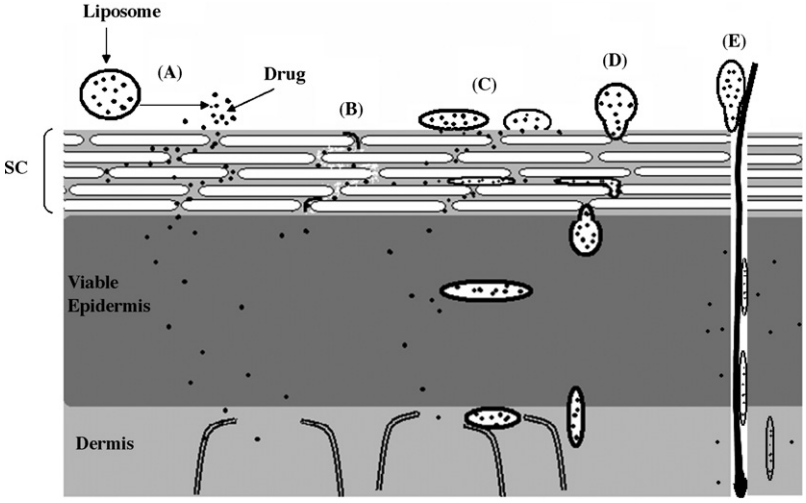

Fig. 3 – Possible mechanisms of action of liposomes as skin drug delivery systems. (A) is the free drug mechanism, (B) is the penetration enhancing process of liposome components, (C) indicates vesicle adsorption to and/or fusion with the stratum corneum (SC) and (D) illustrates intact vesicle penetration into or into and through the intact skin (not to scale) (modified from El Maghraby et al., 2006).

Table 1 – Examples of studies investigating the mechanisms of action of liposomes as skin drug delivery systems

Mechanism	Method	Results/conclusion
Free drug mechanism		
Positive findings	None	
Negative findings		
El Maghraby et al. (1999)	Comparing transdermal flux plots with in vitro release profiles	Peak flux appeared at a time during which drug release was negligible
Penetration enhancement		
Positive findings		
Kato et al. (1987)	Application of drug in lecithin solution in propylene glycol	Lecithin enhances the delivery by lowering the skin permeability barrier
Hofland et al. (1995)	Freeze fracture electron microscopy and X-ray scattering studies, performed after dipping human SC in a liposome	Ultrastructure changes in the intercellular lipids indicating penetration enhancement
Zellmer et al. (1995)	DSC studies of treating the SC with liposomes	Change in the enthalpy of SC lipid-related transitions
Yokomizo and Sagitani (1996)	Skin pre-treatment with PG, PC or PE	Promoted drug permeation and shortened lag time
El Maghraby et al. (1999)	PC liposome pre-treatment and permeation studies from medicated liposomes	Permeation increased by fourfold after pre-treatment and eightfold from medicated liposomes
Negative findings		
Weiner et al. (1989)	Application of drug with empty vesicles	No accelerant effect
Du Plessis et al. (1994a)	Skin pre-treatment with empty vesicles	No accelerant effect
El Maghraby et al. (2000b)	Delivery from liposomes or from lipid solution	Lipid solution was not efficient
Vesicle adsorption to or fusion with the SC		
Positive findings		
Kirjavainen et al. (1996)	SC pre-treatment with liposomes and interactions of vesicles with SCLL	Skin surface adhesion and fusion or mixing with SC lipid matrix
El Maghraby et al. (1999), Kirjavainen et al. (1999b)	Drug partitioning into the SC	Improved partitioning of various drugs
Negative findings	None	
Intact vesicular skin penetration		
Traditional liposomes		
Positive findings		
Foldvari et al. (1990)	Application of liposomes containing electron dense marker	Electron micrographs with intact liposomes in the dermis
Egbaria et al. (1990), Fresta and Puglisi (1996)	Skin deposition of the dual labelled PL or SCL liposome components	The ratio of radiolabelled components of liposomes was maintained constant throughout the skin strata
Negative findings		
Du Plessis et al. (1994b)	Monitoring effect of vesicle size on drug skin deposition	Higher deposition from intermediate size and not small size liposomes
Zellmer et al. (1995), Korting et al. (1995)	Confocal laser scanning or electron microscopy after application of liposomes	No evidence of intact carrier penetration
Transfersomes		
Positive findings		
Cevc and Blume (1992)	Monitoring the fate of the applied Transfersomes	Recovery of some lipid-associated radioactivity from the liver

Table 1 (*Continued*)

Mechanism	Method	Results/conclusion
Valenta and Janisch (2003), Verma et al. (2003)	Studying the effect of vesicle size on skin delivery	Small vesicles improved both deposition into and penetration through skin, with large structures improving deposition only
Cevc et al. (2002), Cevc and Gebauer (2003)	Measuring vesicle size before and after extrusion through microporous filter. Also skin permeation studies	Vesicles penetrated a pore with a diameter three times smaller than their own span. Fluorescent Transfersomes detected in the mice blood
Negative findings		
El Maghraby et al. (1999)	Comparing transdermal delivery from SUVs and LMLVs	Similar transdermal delivery was recorded
Transappendageal penetration		
Positive finding	None	Only deposition data available
Negative findings		
Cevc et al. (1998)	Comparing delivery through different animals or humans with diverse densities of hair follicles	No significant difference with respect to insulin delivery from transfersomes
El Maghraby et al. (2001b)	Comparing liposomal drug delivery through epidermis to that through SC–epidermal sandwich	No difference was recorded
Han et al. (2004)	Application of liposomes with or without iontophoresis	Transfollicular delivery was enhanced only after combination with iontophoresis

The role of this process in transdermal delivery of estradiol was shown to be negligible by El Maghraby et al. (1999) who compared the transepidermal flux plot with its in vitro drug release profile and reported that the peak flux of the drug through skin appeared at a time during which drug release from liposomes was negligible.

4.2. *Penetration enhancing mechanism (see Fig. 3 at B)*

Kato et al. (1987) first concluded that lecithin enhances transdermal delivery by lowering the permeability barrier of the skin. Changes in the ultrastructures of the intercellular lipids were seen after application of the vesicles suggesting a penetration enhancing effect. It was concluded that nano-aggregates containing relatively small hydrophilic head groups can interact with human SC in vitro (Hofland et al., 1995).

In another study, Zellmer et al. (1995) treated human SC (non-occlusively) with dimyristoylphosphatidylcholine (DMPC) liposomes, followed by differential scanning calorimetric investigations. DMPC vesicles did not penetrate into SC but the lipid can penetrate and change the enthalpy of the lipid-related transitions of the SC. In addition, Kirjavainen et al. (1996) revealed that, depending on composition, vesicles may produce an enhancing effect (shown by skin pre-treatment); their lipid components may penetrate deep into the SC or may fuse and mix with skin lipids to loosen their structure. Liposomes containing DOPE or lyso-PC produced the greatest effect. It was thought that the conical shape of DOPE was essential for this effect. Skin pre-treatment with propylene glycol (PG), PC or phosphatidylethanolamine (PE) promoted drug permeation and shortened the lag time. When repeating the same study on silastic membranes instead of skin, none of the phospholipids accelerated drug permeation. Furthermore, PG, PE or PC did not significantly affect the percutaneous absorption of indomethacin through skin lacking stratum corneum. This clearly indicated that phospholipids act directly on the permeability barrier of SC (Yokomizo and Sagitani, 1996).

Negative findings concerning penetration enhancement have also been reported. No accelerant effect was found for SCL liposomes when the empty vesicles were applied in combination with free interferon (Weiner et al., 1989). Du Plessis et al. (1994a) found that skin pre-treatment with empty vesicles did not give the advantages of encapsulated drug. They concluded that the hypothesis that liposomes interact with SC is invalid.

Employing a pre-treatment protocol with empty liposomes, El Maghraby et al. (1999) reported a possible accelerant effect only for non-rigid PC vesicles. However, comparing this promotion (fourfold) with the relative flux obtained after application of estradiol-loaded vesicles (eightfold), it was concluded that enhancement is not the only mechanism operating. For other traditional liposomes (containing CH or DPPC) and ultradeformable systems, skin pre-treatment was not effective, thus excluding an accelerant effect. Investigating the importance of liposome structure, El Maghraby et al. (2000b) compared estradiol skin delivery from standard and ultrade-

formable nano-aggregates with that obtained from propylene glycol solution containing the same components. The results indicated the importance of the colloidal structure, excluding a major role for a sorption promoting mechanism in improved skin delivery from such liposomes. It was also reported that drug molecules must be applied together with and entrapped within the nano-aggregates themselves, suggesting that elastic vesicles act as drug carrier systems and not as penetration enhancers (Honeywell-Nguyen et al., 2003a). Discrepancies in literature reports concerning the penetration enhancing effects of different formulations can be attributed to the use of different lipid components in the vesicles, with non-rigid lipids tending to produce the greatest enhancing effects.

4.3. *Vesicle adsorption to and/or fusion with the stratum corneum (see Fig. 3 at C)*

According to this mechanism 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. 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 SC; instead, they dissolve and form a unit membrane structure (Schaller and Korting, 1996). The processes of adhesion onto the skin surface and fusion or mixing with the lipid matrix of stratum corneum have been suggested for liposome lipids (Kirjavainen et al., 1996). Phospholipids increased the partitioning of estradiol, progesterone and propranolol into the stratum corneum lipid bilayers (Kirjavainen et al., 1999b). It was also suggested that the major component of liposomes, phospholipids, increased the continuity of the lipid matrix of the skin and thus facilitated the movement of lipophilic molecules (Keith and Snipes, 1982). Based on this suggestion we should expect improved drug uptake from saturated aqueous solution after skin pre-treatment with empty vesicles. To further clarify the previous concepts, an uptake study was conducted (El Maghraby et al., 1999) in which stratum corneum membranes were dipped into the test formulation or aqueous solution for a short time (10 min). Drug uptake was increased only from medicated carriers indicating the necessity of co-application of drug with the nano-structures. The uptake ratios (URs) between the vesicles and solution ranged from 23 to 29 with no significant differences between individual formulations. This significant uptake after such a short time may imply high affinity of the vesicles for the stratum corneum. Considering the superiority of deformable nano-aggregates over traditional liposomes in increasing transepidermal flux, and that no significant differences were found in the URs at short contact time, these finding suggest that ultradeformable vesicles could have promoted diffusion through the membrane rather than partitioning (uptake) into the tissue.

4.4. *Intact vesicular skin penetration mechanism (see Fig. 3 at D)*

4.4.1. *Traditional liposomes*

The possibility that intact vesicles penetrate human skin was suggested in the first report on liposomes as skin drug delivery systems (Mezei and Gulasekharam, 1980, 1982). Conceptually it was difficult to believe that large lipid vesicles could penetrate the densely packed stratum corneum in great numbers, and many workers have tested this hypothesis. Foldvari et al. (1990) applied DPPC, CH (2:1) liposomes loaded with an electron dense marker to guinea pigs. Electron micrography showed the presence of intact liposomes in the dermis. The authors proposed that liposomes carrying the drug can penetrate the epidermis. Nano-aggregates were better than a traditional gel in the treatment of eczema but not for psoriasis (Korting et al., 1990). It was thus concluded that vesicles can penetrate diseased skin with its ruptured SC (as in eczema) but cannot invade skin with hyperkeratosis, as in psoriasis. Subsequently, fluormicrographic studies showed that intact small unilamellar vesicles (SUVs) of PC and CH penetrated no deeper than the SC (Lasch et al., 1991).

Using dual labelled liposome components, the skin deposition of those derived from phospholipid and SCL carriers was studied. The ratio of radiolabelled components of liposomal preparations was constant throughout the skin strata. The authors explained this as possible molecular mixing of liposomal bilayers with the SC bilayers (Egbaria et al., 1990). When [^{14}C] inulin (hydrophilic marker) in liposomes whose lipid bilayer was radiolabelled with [^{3}H] cholesterol was applied, the ratio of inulin to cholesterol was also constant throughout skin strata. The explanation given by the authors (molecular mixing) would not justify equal ratios of the dual label in the deeper skin strata. These findings may suggest possible carrier skin penetration. Similar findings were reported again for both phospholipid and SCL liposomes (Fresta and Puglisi, 1996). The ratio of [^{3}H] DPPC to [^{14}C] tretinoin deposited into various skin strata of the hairless rat was monitored after application of liposomes (Masini et al., 1993). This ratio was constant throughout the SC but not in the nucleated epidermis and dermis. Liposomes and tretinoin co-transport into SC was thus accompanied by independent penetration of free drug, which could have escaped from liposomes on the skin surface. This report suggests that vesicles can penetrate only into the stratum corneum.

Contrary to the previous findings, Du Plessis et al. (1994b) found that intermediate-sized and not small-sized liposomes resulted in higher skin deposition. They considered this as an indication that intact liposomes did not penetrate the skin. Furthermore, no evidence of intact carrier penetration could be found after application of DMPC or soy-lecithin liposomes (Zellmer et al., 1995; Korting et al., 1995).

4.4.2. *Ultradeformable vesicles*

Ultradeformable liposomes have been reported to invade the skin intact and go deep enough to be absorbed by the systemic circulation. The transdermal hydration gradient is said to produce a force sufficient to drive ultradeformable vesicles through the intact stratum corneum and into the epidermis. Phospholipid hydrophilicity leads to xerophobia (tendency to avoid dry surrounding). Accordingly, for the vesicles to remain maximally swollen, those on the skin surface try to follow the local hydration gradient, moving into deeper skin strata (Cevc and Blume, 1992; Cevc et al., 1995).

The process of vesicle skin penetration was attributed to high deformability of these specialised vesicles, which results

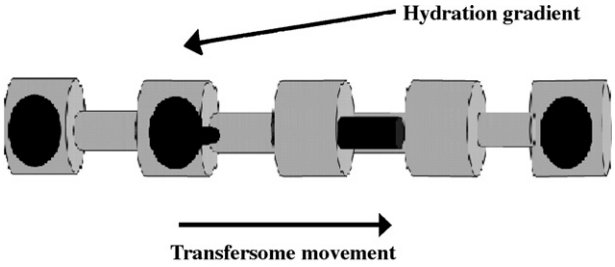

Fig. 4 – Hydration gradient and deformability-driven movement of *Transfersomes* through small pores (after Cevc et al., 1996).

from "edge activator" molecules (e.g. surfactants) accumulating at the site of high stress due to their raised propensity for greatly curved structures (Fig. 4). This rearrangement was claimed to reduce the energy required for deformation; the stress is reportedly produced upon drying of the vesicles which, being flexible, can follow the transdermal hydration gradient (Cevc et al., 1995).

It is difficult to believe that the presence of the so called edge activators in the vesicles can result in vesicle deformability so that they can penetrate into intact skin with its dense stratum corneum lipid packing and which contain very small "pores" relative to that of the vesicle diameter. To test this supposition, the epidermal permeation of estradiol from large multilamellar vesicles (LMLVs, at least 557 nm in diameter) was compared with that obtained from smaller entities of a mean size of 124–138 nm (SUVs). The concept was to investigate the possibility that intact nano-aggregates penetrate through skin, assuming that this infiltration is a function of the vesicle size (El Maghraby et al., 1999). The SUVs are less than the maximum dimension reported to enter skin and the minimum size of LMLVs is above the maximum volume which can invade skin (Cevc et al., 1995). SUVs were as effective as LMLVs, a finding which suggests that intact vesicles do not permeate through human epidermal membrane in vitro (El Maghraby et al., 1999). However, reduction of vesicle size improved drug deposition into deeper strata and penetration through skin, with large structures improving deposition only (Valenta and Janisch, 2003; Verma et al., 2003).

Trotta et al. (2002) incorporated dipotassium glycyrrhizinate (DG), an amphiphilic anti-inflammatory drug, into liposomes. They reported that the agent increased the elasticity of the entities. Measuring the vesicle size before and after extrusion through a microporous filter, elastic particles were capable of penetrating a pore with a diameter three times smaller than their own span. However, they were able to show only improved skin deposition of DG, not improved flux. The extrusion results may suggest vesicle elasticity but do not demonstrate skin penetration. Indeed the "pores" in the stratum corneum lipid matrix are at least 10 times smaller than the ultradeformable vesicle diameter (which generally exceeds 100 nm). Also, the largest pores on the skin surface are provided by the shunts (hair follicles, sweat ducts) which play no major role in liposomal transdermal drug penetration (see below). In similar studies, the size of ultradeformable vesicles was unchanged after extrusion through semi-permeable membrane barriers. The authors reported the presence of the carriers in mice blood after topical application of fluorescent labelled ultradeformable vesicles. Noteworthy, the size of these vesicles was similar to that of the starting liposome suspension. This was taken as a clear evidence for vesicle invasion into and through skin (Cevc et al., 2002; Cevc and Gebauer, 2003). However, it should be noted that a vesicular structure may form spontaneously after absorption of the components as monomers.

Fast delivery of intact elastic vesicles into the SC was recorded and was thought to be via channel-like regions in the SC. Again, elastic vesicles were superior to rigid nano-aggregates with non-occlusive application being best (Honeywell-Nguyen et al., 2003b). The transport of vesicle components and a model drug into human skin was monitored in vivo. Only elastic vesicle material can rapidly enter the SC reaching almost the SC-viable epidermal junction. However, the distribution profile of the drug in the lower SC layers was different to that of the vesicle material. This suggests that once the elastic vesicles partition into the SC, the drug releases from the carrier (Honeywell-Nguyen et al., 2004; Honeywell-Nguyen and Bouwstra, 2005).

In light of the above reports, it appears that some vesicles may penetrate intact to some extent into healthy skin. Questions remain as to how deep into the skin strata intact carriers move, and if indeed intact structures can carry their payload through the entire tissue.

4.5. *Transappendageal penetration (see Fig. 3 at E)*

Occlusive application and full skin hydration is supposedly detrimental for transdermal drug delivery from ultradeformable vesicles. This effect was attributed to inhibition of the transdermal hydration gradient, which is believed to be the driving force for vesicle-skin penetration (Cevc et al., 1995). Another possible explanation is that over-hydration of the skin can swell the corneocytes and thus close or at least minimise the size of shunts that may play a role in liposomal skin delivery.

Electron microscopy indicated that liposomes up to 600 nm diameter can penetrate through skin but those of 1000 nm or more remain interiorised in the SC. Deposition was higher in hairy guinea pigs but, with regard to penetration though skin, no difference could be found between hairless and hairy guinea pigs. Despite this finding, it was concluded that invasion is mainly along the hair sheath (Schramlova et al., 1997). However, these findings can reflect only delivery into, rather than through, the hair follicles. 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 *Transfersomal* input of insulin (Cevc et al., 1998).

A novel in vitro technique using human abdominal skin was developed to explore the role of appendageal transport on liposomal skin delivery of estradiol. The study monitored vesicular delivery through epidermis and compared this with penetration through a sandwich of SC and epidermis. As the orifices of these shunts occupy only a small fraction of the total skin surface area, there was a negligible chance that the shunts in the two membranes would superimpose. It was thus

assumed that the top layer of SC would block most of the shunts available in the bottom membrane (El Maghraby et al., 2001b). From this study, it was concluded that the shunt routes played a minor role in estradiol transdermal delivery from liposomes. Also, the transfollicular delivery from liposomes was enhanced only after combination with iontophoresis (Han et al., 2004).

In summary, it appears that the shunt routes play no major role in liposomal transdermal delivery. However, vesicle penetration into but not necessarily through hair follicles (i.e. targeting) is clearly demonstrated in numerous literature reports.

5. Liposomes as skin model membranes

Table 2 presents a summary of the specifications, advantages and limitations of alternative skin models commonly used in transdermal drug delivery research.

5.1. *Traditional models*

Skin penetration studies play an essential role in the selection of drugs for dermal or transdermal application. Therefore, the choice of predictive in vitro penetration models is highly important. Ideally, human skin should be used to evaluate penetration properties of candidate drugs. However, ethical problems, religious restrictions and limited availability made the use of human skin difficult for most investigators.

Whilst animal skin provides an alternative to human skin, there are clear differences in dermal absorption between different animals. These differences arise from physiological variations but can be compounded by researchers employing different percutaneous penetration measurement methodologies (e.g. Scheuplein, 1978; Panchagnula et al., 1997). Numerous animal models have been suggested as a substitute for human skin, including primate, porcine, mouse, rat, guinea pig and snake skins. Anatomical investigations show comparable characteristics between porcine ear skin and that of human skin with respect to the stratum coreneal and epidermal thickness as well as the follicular structure and the hair density (Wester and Maibach, 1989; Jacobi et al., 2007). Among the rodents, rat skin showed the closest anatomical similarity to that of human skin with mouse skin revealing markedly different features (Wester and Maibach, 1989).

The presence of fur may be considered as an important difference between experimental animals and humans and so several hairless species have also been used. These include hairless mice and guinea pigs, with athymic nude mice to which human skin has been grafted and scared tissue also employed as furless models (Reifenrath et al., 1984; Rougier et al., 1987; Bogen et al., 1992; Simon and Maibach, 1998). However, it is important to note that percutaneous absorption through animal skin may differ significantly from that obtained with human tissue. In general, drug absorption through rat skin can be one to three orders of magnitude greater than in humans, depending on permeant properties, experimental methods, and exposure site (Bartek et al., 1972; Poet et al., 2000). Comparing in vivo absorption of testosterone, a fivefold difference between percutaneous absorption in rabbits and humans was found, and the rank order of the amount absorbed was rabbit > rat > pig > human (Bartek et al., 1972). Percutaneous absorption in monkeys has been reported to compare well to that determined in man (Wester and Maibach, 1975; Wester et al., 1980). Other studies have suggested that absorption through pig skin may most closely predict human dermal absorption (Reifenrath et al., 1984; Lavker et al., 1991). Several different strains of pig have been employed; Reifenrath et al. (1984) compared the absorption of different chemicals through weanling Yorkshire pig and human skin. In general, the correlation between these two species was good. Of the tested chemicals, caffeine and parathion showed the largest difference between human and pig absorption. Caffeine absorption was over twofold greater in humans than pig tissue whereas parathion exhibited a twofold greater absorption through pig skin than human skin. These results were similar to the earlier finding reported by Bartek et al. (1972). Unfortunately, pigs and monkeys are less readily available and are more expensive research models than smaller laboratory animals.

The studies outlined above demonstrated two important issues. The first is that differences between animal models and human skin absorption are permeant specific, making general predictive conclusions a difficult task. The second issue is that the most readily available and easy to handle animals show less correlation to human percutaneous absorption than the less readily available and more difficult to house research animals such as pigs and monkeys.

Researchers have employed reconstructed human skin models as another option. These models can be very useful if the penetration barrier of the skin equivalents is similar to that of human tissue and such skin equivalents have been suggested for use in penetration studies (Kriwet and Parenteau, 1996). In a very elegant study, Schmook et al. (2001) compared the penetration properties of human, pig and rat skin with the Graftskin™ LSE™ (living skin equivalent) and the Skinethic™ HRE (human reconstructed epidermis) models using drugs with widely varying polarity. In agreement with published data, pig skin appeared as the most suitable model for human skin: the fluxes through the skin and concentrations in the skin were of the same order of magnitude for both tissues, with differences of at most two- or fourfold, respectively. Graftskin™ LSE™ provided an adequate barrier to salicylic acid, but was very permeable for the more hydrophobic compounds. It was thus concluded that the available reconstituted skin models could not be regarded as generally useful for in vitro penetration studies.

Reconstructed human epidermis has been also employed to investigate the mechanism of action of liposomes as skin drug delivery systems. After application of large unilamellar liposomes (mainly made from soybean PC) to human epidermis reconstituted in vitro, electron microscopic investigations revealed the presence of dose-dependent alterations to the morphology of both the stratum corneum and the viable part of the epidermis. Shrunken lipid droplets were found between the corneocytes. In addition, the corneocytes of various layers of the stratum corneum and the keratinocytes of the upper layer of the living epidermis showed lipid deposition. This finding indicates a possible penetration enhancing mechanism of liposome components (Korting et al., 1995), and is

Table 2 – Summary of the specifications, advantages and limitations of various models of human skin employed in transdermal drug delivery research

Model	Specification/advantages	Limitations
Animal models		
Monkeys		
Wester and Maibach (1975), Wester et al. (1980)	Percutaneous absorption compares well to human skin	Restricted use and high cost
Pigs		
Reifenrath et al. (1984), Lavker et al. (1991), Jacobi et al. (2007)	The ear showed anatomical similarities to human skin with respect to SC and epidermal thickness, follicular structure and the hair density. Permeability is close to human skin	Difficult to obtain and high cost
Rodents		
Bartek et al. (1972), Simon and Maibach (1998), Poet et al. (2000)	Relatively cheap and readily available. Rat may be the best rodent model. The problem of fur can be eliminated using hairless animals	More permeable than human skin
Reconstructed skin models		
Reconstructed epidermis		
Netzlaff et al. (2005)	Close to human skin with respect to general structure, and biochemical features. Useful in toxicity studies. More consistent in permeability	Relatively weak barrier nature especially for lipophilic drugs
Reconstructed full thickness skin		
Nakamura et al. (1990)	The presence of dermis may eliminate the weak barrier nature to lipophilic drugs	Absence of the vascular network may provide false barrier nature
Lamellar matrix		
Moghimi et al. (1996a,b,c)	DSC and X-ray revealed good structural correlation with the SC lipids. Permeation studies showed good barrier nature	The lipid composition may be dissimilar to SC lipids. Need further investigations with various drugs
Liposomal models		
Phospholipid liposomes		
Rolland et al. (1991), Bonina et al. (1994), El Maghraby et al. (2005)	Used mainly to study the mechanism of action of skin penetration enhancers. DPPC is the most widely used. Its T_m can be easily measured as it has a narrow main endothermic peak	Lipid arrangement is different from the of the SC lipid lamellae
		Provided misleading results with enhancers having two hydrogen-bonding sites in the anti position
SCL liposomes		
Kim et al. (1993), El Maghraby et al. (2005)	Successfully used to probe the mode of action of skin enhancers. Have the same lipid arrangement as SC lipids	Represent the intercellular route only
Yoneto et al. (1995, 1996), Kirjavainen et al. (1996, 1999a)	The effect of agents on the release of markers suggested the effects of these agents on skin	Cellular protein is absent
Proteoliposomes		
Lopez et al. (1996)	Comprise a mixture of lipids and proteins and was used to study effect of surfactant	Requires further investigation

similar to our data obtained after investigating the penetration enhancing effect of PC vesicles using human skin in vitro (El Maghraby et al., 1999). However, it should be noted that most reconstructed epidermal membranes are leakier than the normal human epidermis and thus offer greater potential for vesicle penetration, providing an exaggerated effect relative to normal human skin.

The use of reconstructed human epidermis has been recently reviewed and it was concluded that some models (SkinEthic®, EpiSkin®, and EpiDerm®) are close to human skin in some aspects; their general structure, composition and biochemical features are similar to human skin and so they are useful in toxicity studies. The models also have the advantage of being more consistent in permeability and responsiveness, compared to many human skin samples obtained from surgical procedures. Unfortunately, their major limitation is their relatively weak barrier nature which makes them far more permeable than excised human skin (Netzlaff et al., 2005).

A tighter, easily maintained reproducible organotypic epidermal culture model was developed, employing rat epidermal keratinocytes grown for 3 weeks on a collagen gel. This model exhibited normal stratum corneum structural and functional properties (Pasonen-Seppänen et al., 2001). The permeability of this model to drugs was comparable to that of human epidermal cadaver membranes with an average of only twofold higher permeability (Suhonen et al., 2003). This model showed only minor differences in the lipid composition and thermal phase behaviour compared to human skin explaining the minor differences in permeability (Pappinen et al., 2008).

It is useful to note that the above studies evaluated reconstructed human epidermal models, which were shown to be more permeable than human skin particularly for lipophilic drugs. This stimulated researchers to consider reconstructed full thickness models as more suitable models (Nakamura et al., 1990). The presence of dermal tissue may add some barrier to lipophilic drug permeation thus mitigating the higher permeability of epidermal model especially for lipophilic drugs. However, reconstructed or cultured full thickness skin in vitro lacks the vascular network present in vivo. This vascular network should minimise the barrier role of the dermis as it will clear any molecule crossing the dermoepidermal junction. Accordingly, the use of epidermal membranes in vitro tends to provide a more representative model mimicking the in vivo situation.

Accepting that the intercellular lipid domains are responsible for the barrier nature of the SC, a simple lamellar mesomorphic structure (matrix) was prepared and used to probe the barrier nature of the SC. This matrix comprised 20% cholesterol, 25% water and 55% a mixture of fatty acids and their salts. Thermal analysis and X-ray diffraction studies revealed that the matrix had a good structural correlation with the SC. In addition, release and permeation studies conducted using estradiol and 5-fluorouracil indicated that the matrix could provide a good barrier model for the SC intercellular pathway (Moghimi et al., 1996a,b,c). Subsequently, more refined models have been proposed containing more physiologically relevant lipid combinations, including ceramides, or using a combination of lipids extracted from skin.

5.2. *Liposomal models*

Since SC intercellular lipids form bilayers, liposomes have been proposed as models for skin membranes. Simple phospholipid liposomes comprising DPPC were initially used before Wertz et al. (1986) prepared liposomes from a lipid mixture approximating the lipid composition of the SC lipids (40% ceramides, 25% cholesterol, 25% palmitic acid and 10% cholesterol sulphate), termed SCL liposomes. These structures were used mainly to investigate the mechanisms of enhanced skin drug delivery. They were also used to investigate the possible oxidant or antioxidant effect of certain materials. This section will summarise these applications.

Thermal analysis (differential scanning calorimetry, DSC) has been successfully used to probe the mechanisms of action of skin penetration enhancers. The originators used human stratum corneum and tested a variety of penetration enhancers with different lipophilicities (Goodman and Barry, 1983, 1985, 1986, 1988; Barry, 1987). Based on the DSC results as well as permeation and partitioning data, Barry (1987) proposed four possible mechanisms of action of skin penetration enhancers:

(1) Disruption of the organization of the intercellular lipids of the SC increasing the fluidity and thus permitting easier drug permeation through the less rigid environment. Lipophilic enhancers may act primarily via this mechanism.
(2) Many accelerants also interact with intracellular protein. The exceptions were Azone and oleic acid; however these were most effective as enhancers when dissolved in a polar co-solvent such as propylene glycol (PG), which itself interacts with protein. Although drug flux can increase via lipid interaction alone, once the lipid barrier weakens, the protein-filled cells may still provide a significant diffusional resistance. Thus an enhancer which affects both lipid and protein domains could be more potent. Intracellular drug transport could be increased by a solvating action of enhancers on the protein helices. This mechanism encompasses the displacement of bound protein–water, the expansion of protein structure, and the competition with permeants for hydrogen-bonding sites.
(3) The diffusional resistance of the intracellular contents alters markedly with skin hydration—water itself is quite a potent penetration enhancer. The reason for this is that in the fully hydrated skin, the intracellular regions will be more fluid and water will compete for drug-binding sites, lowering the diffusional barrier.
(4) Small polar enhancers such as dimethylsulphoxide (DMSO) and its analogues, the pyrrolidones and PG may accumulate in both intercellular and protein regions of the tissue. The presence of these powerful solvents may then increase drug partitioning into the skin, yielding increased fluxes.

The action of a penetration enhancer has been related to its partition coefficient. Small polar enhancers may partition preferentially at low concentrations into the protein region of the stratum corneum. At high concentrations they could also

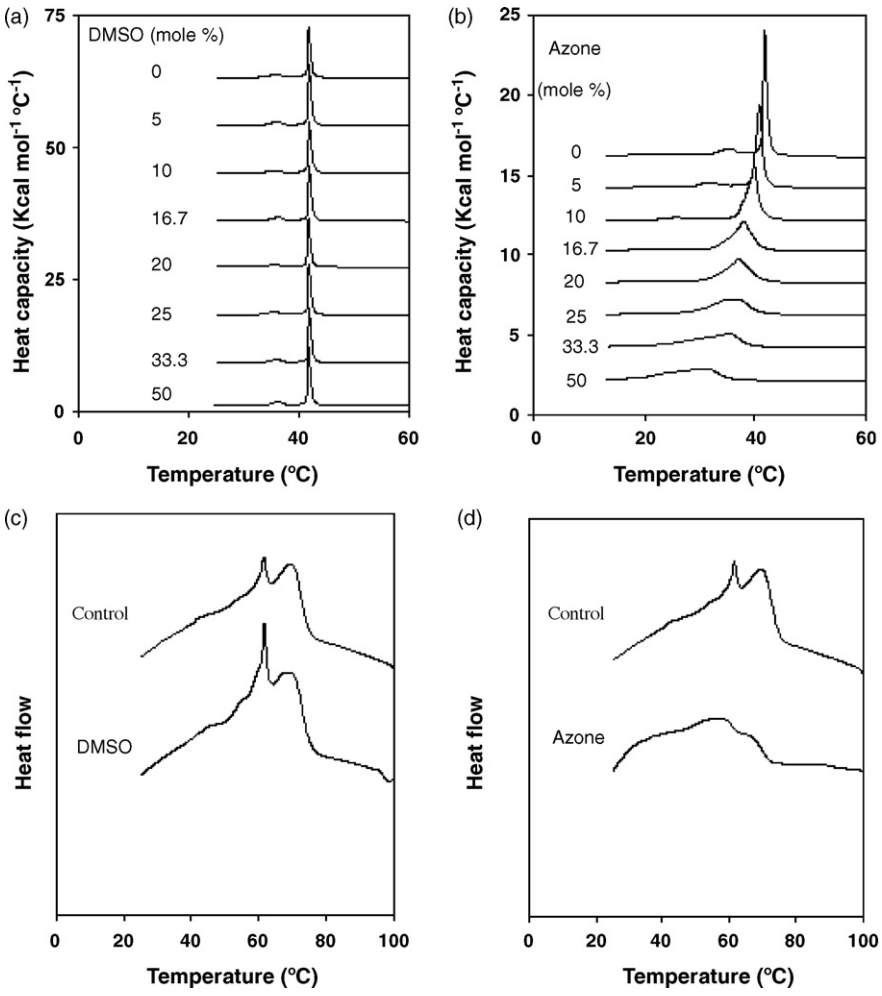

Fig. 5 – Examples of the HSDSC traces of DPPC liposomes, containing increasing concentrations of DMSO (a), or Azone (b) and traces of SCL liposomes containing 0 mmol (control) or 5 mmol of DMSO (c) or Azone (d) (after El Maghraby et al., 2005).

interact with stratum corneum lipids, increasing fluidity. Non-polar materials appear to enter the lipid regions only, where they disrupt the lipid bilayers whilst enhancers of intermediate polarity interact with both protein and lipid.

With thermal analysis providing good evidence for enhancer:skin interactions, and due to poor availability of human skin samples, liposomes have been employed as an alternative model membrane to investigate the mechanisms of action of skin penetration enhancers, typically using DSC. DPPC liposomes are typically selected for this purpose (e.g. Beastall et al., 1988; Rolland et al., 1991; Bonina et al., 1994; Inoue et al., 2001; El Maghraby et al., 2004; Auner et al., 2005) since DPPC is a widely used model for lipid bilayers and its transition temperature (T_m) can be easily measured as it has a narrow main endothermic peak (see Fig. 5a and b, top trace). These investigations revealed comparable results with those obtained with studies employing human SC, especially with lipophilic enhancers such as Azone and oleic acid which broadened the main endothermic peak and reduced its T_m suggesting lipid disruption. Further, Hadgraft et al. (1996) employed DPPC liposomes to demonstrate the mechanism of action of a skin penetration retarder; incorporating N-0915 (a compound having oxygen atoms in a *trans*-position) into DPPC liposomes increased the T_m of the main endothermic peak, a membrane stabilizing rather than disrupting effect.

Considering its lipid composition and organization (Fig. 2), it is clear that the organization of the SC lipids, in which there are alternating ceramides, cholesterol, cholesterol ester and fatty acids, is completely different from that of the phospholipid liposomes (PLLs) in which the phospholipids are arranged successively. This raises concerns over the validity of PLL as a skin model. This was recently investigated by El Maghraby et al. (2005) who studied the mechanisms of action of various novel and traditional skin penetration enhancers in both PLL and SCL liposomes. The enhancers included Azone (AZ), octyl salicylate (OS), padimate O (PADO) and 2-(1-nonyl)-1,3-dioxolane (ND, commercially known as SEPA^{TM}) as lipophilic enhancers with DMSO as a model hydrophilic enhancer. Incorporating AZ, OS or PADO in liposomes broadened the endothermic peaks and reduced the T_m, indicating a lipid disruption mechanism. This action was comparable in the DPPC liposomes and SCL liposomes (see e.g. Fig. 5b and d). Incorporating DMSO in vesicles sharpened the endothermic peak with no significant effect on the T_m. This effect is explained

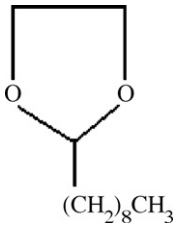

Fig. 6 – Chemical structure of the enhancer 2-(1-nonyl)-1,3-dioxolane (ND).

by increased hydration of the bilayers and the formation of a solvation shell around the head groups. Once again the effect was comparable in both PLL and SCL liposomes (Fig. 5a and c; El Maghraby et al., 2005). For ND, a dioxolane derivative (Fig. 6), the results were rather surprising. Incorporation into DPPC liposomes increased the T_m of the endothermic peak (Fig. 7a), an effect which is consistent with classifying the compound as a penetration retarder; as a dioxolane derivative, ND can form hydrogen bonds with each successive PL molecule. However, it is difficult to classify ND as a penetration retarder when other researchers have reported an enhancing effect, shown also by thermal and spectroscopic analysis of ND-treated human SC (Diani et al., 1995; Morganti et al., 1999). Incorporating ND in SCL liposomes broadened the endothermic peaks and reduced the T_m, indicating membrane disruption and a penetration enhancing effect (El Maghraby et al., 2005) in agreement with the work done on human skin (Morganti et al., 1999). This study thus shows a limitation in using PLL as a model of human skin and highlighted the importance of considering the lipid composition and the structural organization of the model membrane before making general conclusions of enhancer effects.

Elsewhere SCL liposomes were successfully used as a model to investigate the mechanism of action of enhancers (Kim et al., 1993). Employing a fluorescent probe and liposomal marker release studies, SCL liposomes were used to study the mechanism of action of 1-alkyl-2-pyrrolidones as skin penetration enhancers (Yoneto et al., 1995, 1996). The compounds increased the fluidity of the vesicular membrane and increased the rate of release of entrapped markers. These effects indicate penetration enhancement via a membrane disruption mechanism. Interestingly these effects were comparable with those obtained using hairless mouse skin (Yoneto et al., 1995).

In a series of investigations, liposomes prepared from linolenic acid, DPPC, cholesterol and ceramide III or ceramide IV have been used as skin model membranes to study the effect of UV radiation on the skin. In addition, the redox behaviour of materials such as ascorbic acid and hyaluronan on skin upon exposure to UV radiation was studied using these models. They concluded that ascorbic acid was degraded after a pro-oxidative effect and its incorporation in the topical formulations as an antioxidant may be deleterious. They added that considering human skin and its constant exposure to UV light and oxygen and an increased pool of iron in irradiated skin, incorporating hyaluronan or its fragments in cosmetic formulations or sunscreens could be helpful for tissue protection (Trommer et al., 2001, 2002, 2003). The same model

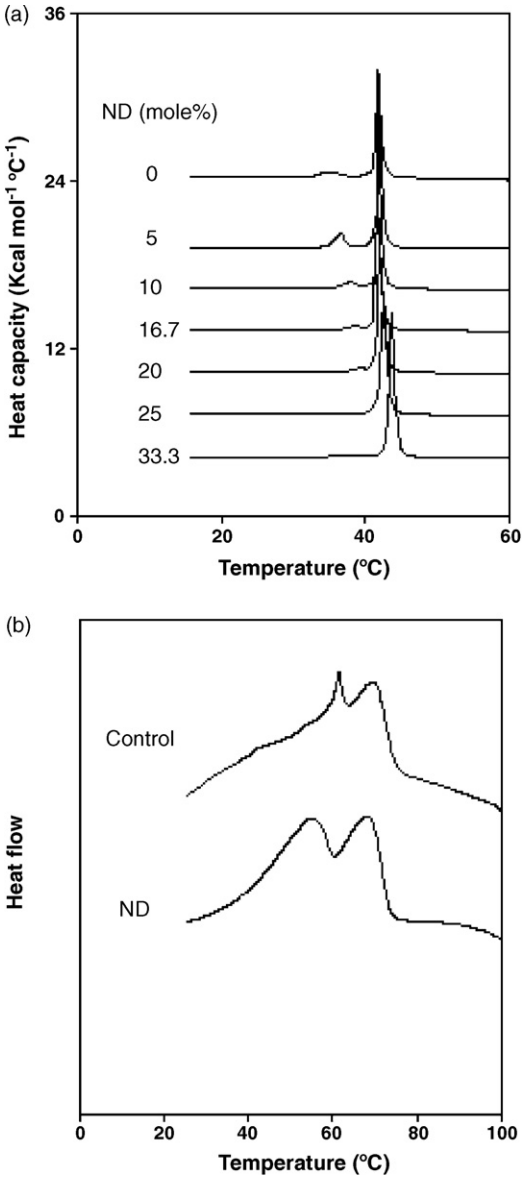

Fig. 7 – Examples of the HSDSC traces of DPPC liposomes, containing increasing concentrations of ND (a) and traces of SCL liposomes containing 0 mmol (control) or 5 mmol of ND (b) (after El Maghraby et al., 2005).

has been successfully used for screening a variety of compounds as antioxidants after topical application in an attempt to develop new compounds useful for skin protection against UV radiation (Trommer and Neubert, 2005).

SCL liposomes have also been used as a model membrane to investigate the mechanism of action of phospholipid vesicles as skin drug delivery systems (Kirjavainen et al., 1996). These researchers loaded calcein into SCL liposomes and incubated them with phospholipid vesicles before monitoring the effect of the later on calcein release from the SCL liposomes. Treatment with liposomes containing dioleylphosphatidylethanolamine (DOPE) or lyso-PC increased calcein release indicating destabilisation of the SCL liposomal membrane. These results supported those obtained after a

pre-treatment study conducted on human skin which showed enhanced permeation of the fluorescent marker following pre-treatment with liposomes containing DOPE or lyso-PC (Kirjavainen et al., 1996). This study indicates the potential value of SCL liposomes as a skin mimic and it was concluded that liposomes containing DOPE can fuse and mix with the skin lipids to loosen their structure providing a penetration enhancing effect.

In subsequent studies, liposome–skin interactions and their effects on skin permeation of drugs were probed in vitro (Kirjavainen et al., 1999a), employing both human skin and SCL liposomes. The SC penetration of a lipophilic fluorescent probe was deeper from PC liposomes containing 32% ethanol compared with ethanol-free liposomes. The penetration pattern from DOPE containing liposomes was not affected by ethanol. However, addition of ethanol increased the mixing of both PC and DOPE liposomes with the SCL liposomes. In addition, ethanol containing liposomes (both types) showed a destabilising effect on the skin lipid liposomes as evidenced by increased calcein release compared with control (containing the same concentration of ethanol). This indicated that liposomes could have a penetration enhancing effect. The improved effects seen with co-use of ethanol might result from the solvent in liposomes providing a looser structure which facilitates the transfer of liposome components into skin, producing the destabilising effect on skin lipids. This study again shows the potential value of SCL liposomes as a skin model.

The effects of phospholipids on fluidity of SC lipid bilayers and drug partitioning into them was evaluated, employing the SCL liposomes as a model for human stratum corneum (Kirjavainen et al., 1999b). The study revealed that different phospholipids have varied effects on drug partitioning into the SC lipid bilayers. Thus, incorporating egg-PC, soya-PC or DOPE into skin lipid liposomes increased the partitioning of drugs into these SC liposomes whereas distearyl-PC did not change this partitioning. It was suggested that fluid state phospholipids can disrupt the rigid structure of the skin lipids, thus increasing drug partitioning into the lipid phase. However, gel state phospholipids produce only minor or no effects. It was concluded that the phospholipid-improved skin permeation of drugs may be at least partially due to increased drug partitioning. These results are in good agreement with those obtained after a partitioning study employing human stratum corneum in vitro (El Maghraby et al., 1999) which further indicated the value of SCL liposomes as skin models.

A mixture of lipids and proteins, extracted from pig SC using a chloroform/methanol mixture, was successfully used to prepare liposomes (Lopez et al., 1996). These vesicles were termed “proteoliposomes” and were employed as skin models for studying the interactions of surfactants with skin. The authors monitored alterations in the release of an entrapped fluorescent marker from these liposomes after addition of surfactant, as a measure for the interaction of surfactants with the lipid membranes. The study compared proteoliposomes with PC vesicles. The results revealed similar changes in bilayer permeability in the presence of the same amounts of surfactants, but different release kinetics were evident (Lopez et al., 1996).

6. Conclusion

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 one such advance with claims of enhanced transdermal drug delivery to efficiencies comparable with subcutaneous administration. Claims such as non-invasive transcutaneous vaccination using these structures would be a significant clinical advance but the mechanisms of action of these vesicles as skin drug delivery systems require further clarification. Indeed, most literature reports have generated data from animal models and it is well known that animal skin permeability differs to that in humans (typically more permeable). Accordingly, confirmatory investigations on human skin are still required.

The use of liposomes as models for skin membranes provide another important application but it should be noted that the simple phospholipids-based liposomes may result in misleading conclusions. Those having a lipid composition similar to that of SC lipids can at least partially represent the SC membrane.

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