Innovative Strategies for Enhancing Topical and Transdermal Drug Delivery

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Abstract: Historically, the skin was thought to be a simple homogenous barrier. However, it is now known to be a highly specialised organ, and plays a key role in homeostasis. The protective properties of the skin are provided by the outermost layer, the epidermis, which safeguards against chemical, microbial, and physical attack. The exceptional barrier properties of the skin result in it being a challenging route for the delivery of therapeutic agents. This article reviews strategies developed to enhance the skin penetration of drugs, ranging from conventional approaches, for example the use of chemical penetration enhancers to those in early-stage development, such as microscissioning.

Keywords: Transdermal delivery, stratum corneum, penetration pathways, skin penetration.

INTRODUCTION

The application of medicaments to the skin can be dated back many thousands of years. The ancient Greeks applied a mixture of water, olive oil and lead oxide as a balm to the skin. Lead oxide has astringent properties, and olive oil may act as an occlusive barrier, moisturising the skin [1]. Up until the end of the 19th century, the skin was widely regarded as being an impermeable barrier, even to the penetration of gases [2]. However, in 1893 Bourget and collaborators demonstrated that acute rheumatoid arthritis could be treated with topical salicylic acid [3]. In the early twentieth century it was recognised that more lipophilic agents had increased skin permeability [4]. The barrier properties of the skin were attributed to the outermost layers in 1919 [5]. Until the mid 20th century there was much debate as to which layer of the epidermis was responsible for the permeability barrier. In 1953, Blank [6] employed Wolf’s tape stripping technique [7] to assess water permeability of full thickness skin. Water loss remained almost normal until the lowest part of the stratum corneum had been removed, indicating that this layer was the principal permeability barrier. Scheuplein and coworkers thoroughly investigated skin permeability to a wide range of substances in vitro [2,8,9]. They modelled skin as a 3-layer laminate of stratum corneum, epidermis and dermis, with drug permeation driven by Fickian diffusion. By digesting the epidermal layer, stratum corneum was separated from the lower layers of the skin, and was determined to be the principal barrier for drug flux.

Until the mid 20th century, physicians prescribed topical preparations solely for the treatment of skin diseases. During World War II, angina attacks were observed to be less frequent amongst munitions employees working with nitroglycerin [10]. Subsequently, in 1954, nitroglycerin ointment was introduced for the management of angina [3]. This was the first commercial topical preparation specifically developed to treat a systemic disease. Thirty years later in the early 1980’s, the FDA approved the first transdermal patches; containing scopolamine for the prevention of motion sickness [11], and a patch system releasing nitroglycerin for the prevention of angina. Subsequently, patches containing clonidine, fentanyl, buprenorphine, levonorgestrel, lidocaine, norethisterone, estradiol, oxybutynin, testosterone and nicotine have received approval [11-13]. Transdermal products containing gransetron, human growth hormone, insulin, parathyroid hormone and rotigotine are currently undergoing clinical trials [11].

The majority of licensed preparations applied to the skin are aimed at delivering the drug for a local, rather than a systemic, action. These preparations tend to be relatively simple semi-solids such as creams, gels and ointments. Therapeutic categories include anaesthetics, anti-acne drugs, antimicrobials, corticosteroids, cytotoxics, retinoids, vitamin D analogues and phototherapy [13]. Over the past 25 years there has been increasing interest in transdermal drug delivery. A PubMed search for ‘transdermal’ in the year 1990 revealed 295 hits, compared to 947 hits in 2006. Between 1995 and 2002, the US transdermal market doubled from US$1.5 billion to over US$3 billion, and, it is predicted that in 2008, the market for transdermal systems will reach US$4.5 billion [14].

This review describes the barrier properties of the skin, how drugs penetrate the skin and the techniques that have been used to enhance drug penetration across skin.

THE stratum corneum BARRIER

The stratum corneum, or horny layer, is the outermost layer of the skin and has been identified as the principal barrier for penetration of most drugs [15]. The horny layer represents the final stage of epidermal cell differentiation. The thickness of this layer is typically 10 μm, but a number of factors, including the degree of hydration and skin location, influence this. For example, the stratum corneum on the palms and soles can be, on average, 400-600 μm thick [15], whilst hydration can result in a 4-fold increase in thickness [16]. The stratum corneum consists of 10-25 rows of dead keratinocytes (corneocytes) embedded in a lipid matrix [15]. The corneocytes are flattened, elongated, dead cells, lacking nuclei and other organelles [12]. The cells are joined together by desmosomes, maintaining the cohesiveness of this layer [17]. The heterogeneous structure of the stratum cor-
neum is composed of approximately 75-80% protein, 5-15% lipid and 5-10% unidentified on a dry weight basis [18].

The main lipids located in the stratum corneum are ceramides, fatty acids, cholesterol, cholesterol sulphate and sterol/wax esters [18,19]. These lipids are arranged in multiple bilayers called lamellae. Phospholipids are largely absent, a unique feature for a mammalian membrane. The ceramides are the largest group of lipids in the stratum corneum, accounting for approximately half of the total lipid mass [20], and are crucial to lipid organisation of the stratum corneum [17]. The brick and mortar model of the stratum corneum was first presented by Michaels et al. [16]. The bricks correspond to parallel plates of dead keratinised corneocytes, and the mortar represents the continuous interstitial lipid matrix (Fig. 1). It is important to note that the corneocytes are not brick shaped, but rather are polygonal, elongated and flat (0.2 - 1.5 μm thick and 34.0 - 46.0 μm in diameter) [12]. The mortar is not a homogenous matrix, but rather lipids are arranged in the lamellar phase (alternating layers of water and lipid bilayers), with some of the lipid bilayers in the gel or crystalline state [21]. The extracellular matrix is further complicated by the presence of intrinsic and extrinsic proteins such as enzymes. The barrier properties of the stratum corneum have been assigned to the multiple lipid bilayers residing in the intercellular space. These bilayers prevent desiccation of the underlying tissues by inhibiting water loss and limit the penetration of substances from the external environment [1,19].

TARGETING THE SKIN FOR DRUG DELIVERY

There are 3 principal targets for topical and transdermal drug delivery, the skin surface, the skin itself (epidermis or dermis) or the systemic circulation. The surface of the skin may be a target when considering disinfectants, insect repellents or cosmetics. Targeting the various layers of the skin is termed topical drug delivery and is relevant when the disease state presents within the organ itself. For example, treating neoplasias, inflammatory disorders and microbial infections of the skin [22]. Increasingly, transdermal or percutaneous delivery, whereby the systemic circulation is the principal target, is being considered as an alternative to conventional systemic and oral routes of administration. Advantages and limitations of this drug delivery route are summarised below [1,11,22-25].

Advantages

- Facilitates sustained delivery of drug, achieving a steady-state profile. This reduces the likelihood of peak-associated side effects, and ensures that drug levels are above the minimal therapeutic concentration
- Reduced dosing frequency – (e.g., fentanyl patch provides 72 hour pain relief)
- Avoids 1st pass metabolism
- Avoids variables that affect drug absorption in the gastrointestinal tract, such as pH, enzymatic activity and drug-food interactions
- Convenient, non-invasive means of drug delivery
- Ease of use negates the need for specialised healthcare staff to administer drugs, potentially reducing treatments costs
- Dosage form can be easily removed in the event of toxicity
- Provides an alternative route when the patient is unable to take drugs orally; e.g., nauseated and unconscious patients

Limitations

- Patches tend to be relatively complex systems, which are expensive to develop and manufacture

![Fig. (1). The ‘bricks and mortar’ representation of the stratum corneum with alternating lipid bilayers surrounding proteinaceous corneocytes (adapted from [18]).](image-url)
Transdermal expertise is limited to a relatively small number of companies

Suitable drugs are limited by stringent physicochemical factors, including molecular weight, partition coefficient and ionisation.

Owing to the excellent barrier properties of the skin, permeated amounts tend to be low. Therefore, only potent drugs are applicable to this administration route.

Some patients may not find patches aesthetically pleasing.

Skin irritation. Drugs and excipients may cause sensitisation, reactions such as erythema and oedema.

Misuse of transdermal patches has been associated with serious adverse effects and even death.

**DRUG PENETRATION PATHWAYS**

There are critically 3 ways in which a drug molecule can cross the intact stratum corneum: via skin appendages (shunt routes); through the intercellular lipid domains; or by a transcellular route (Fig. 2). A particular drug is likely to permeate by a combination of these routes, with the relative contributions of these pathways to the gross flux governed by the physicochemical properties of the molecule.

**The Appendageal Route**

Skin appendages provide a continuous channel directly across the stratum corneum barrier. However, their influence on drug penetration is hindered by a number of factors. The surface area occupied by hair follicles and sweat ducts is small (typically 0.1% of skin surface area), therefore limiting the area available for direct contact of the applied drug formulation. Sweat ducts are either empty or actively secreting an aqueous salt solution. Although an aqueous pathway across the skin is considered desirable for many drugs, permeation may be limited as sweat is travelling against the diffusion pathway of the permeant. Sebaceous glands are filled with a lipid rich sebum, which may present a barrier to hydrophilic drugs.

It is been suggested that the shunt route is frequently the dominant pathway shortly after application of the vehicle [2]. Once steady-state diffusion has been established, trans-epithelial routes become more important [8]. Although the contribution of the appendageal route is generally considered to have little influence on flux, it has been proposed that this route may be important for large polar molecules and ions [27].

**Transcellular Route**

Drugs entering the skin via the transcellular route pass through corneocytes. Corneocytes, containing highly hydrated keratin, provide an aqueous environment for which hydrophilic drugs can pass. The cells are surrounded by a lipid envelope which connects the cells to the interstitial lipids. Separating keratinised skin cells are multiple lipid bilayers; there are estimated to be up to 20 such lamellae between each corneocyte [28]. Therefore, the diffusion pathway for a drug via the transcellular route requires a number of partitioning and diffusion steps. After partitioning into and diffusing through the relatively aqueous corneocytes, the permeant must partition into the surrounding lipid envelope, and subsequently partition in and out of the multiple lipid bilayers separating corneocytes [19]. The physicochemical properties of the permeant will strongly influence whether the transcellular pathway is the predominate route taken. In particular, the relative ability to partition in and out of each skin phase is important. The transcellular pathway is, however, thought to be the predominant pathway for highly hydrophilic drugs during steady-state flux [18].

**Intercellular Route**

The intercellular pathway involves drug diffusing through the continuous lipid matrix. This route is a significant obstacle for 2 reasons. Firstly, recalling the ‘bricks and mortar’

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**Fig. (2).** Drug penetration pathways across skin (adapted from [26]).
model of the \textit{stratum corneum}, the interdigitating nature of the corneocytes yields a tortuous pathway for intercellular drug permeation, which is in contrast to the relatively direct path of the transcellular route. It has been estimated that water has 50 times further to travel by the intercellular pathway, than the direct thickness of the horny layer \cite{29}. Secondly, the intercellular domain is a region of alternating structured bilayers. Consequently, a drug must sequentially partition into, and diffuse through repeated aqueous and lipid domains. This route is generally accepted as the most common path for small uncharged molecules penetrating the skin \cite{18,30}.

**MODULATION OF TOPICAL AND TRANSDERMAL PENETRATION**

The \textit{stratum corneum} is the principal barrier to drug permeation across the skin. Consequently, there has been a concerted effort to investigate and develop novel strategies of maximising the amount of permeant crossing this barrier. Innovative approaches focus on altering the drug-vehicle interaction to enhance partitioning into the \textit{stratum corneum}, or modifying the structure of the \textit{stratum corneum} to make it less resistance to drug diffusion. Alternatively, energy-driven methods have been employed to propel drugs across the skin (Fig. 3).

**THERMODYNAMIC ACTIVITY AND SUPERSATURATION**

Steady-state flux of a drug through skin is described by Equation 1.

\[
\frac{dM}{dt} = \frac{DPC_v}{h} \tag{1}
\]

where \(dM/dt\) is the cumulative amount of permeant passing per unit area of membrane (flux), \(D\) is the diffusion coefficient, \(C_v\) is the concentration of the drug in the vehicle, \(P\) is the partition co-efficient between the membrane and the vehicle and \(h\) is membrane thickness. Higuchi \cite{31} rewrote this equation in terms of thermodynamic activities (Equation 2).

\[
\frac{dM}{dt} = \frac{aD}{\gamma h} \tag{2}
\]

where \(a\) is the thermodynamic activity of the permeant and \(\gamma\) is the effective activity coefficient in the membrane. The maximum flux of a drug across a membrane occurs when the drug is at its highest thermodynamic activity. At saturation, equilibrium exists between the solid and liquid phase and activity equals 1 \cite{32}. Therefore, all vehicles that contain a finely ground suspension exist as saturated solutions of the drug \cite{33}. Activity can exceed unity when supersaturated systems are formed, although such systems are inherently unstable. The degree of saturation of the solution is calculated by dividing the drug concentration in the prepared solution by the saturated solubility in the same solvent mix.

The most common method of creating a supersaturated system is the co-solvent method described by Poulson \textit{et al.} \cite{34}. Saturated solutions of co-solvent mixture are mixed with a poor solvent to create a supersaturated system \cite{35-37}. Megrab \textit{et al.} \cite{38} investigated estradiol penetration across human skin and a silicone membrane. Supersaturated systems (up to 18 degrees of saturation) were prepared by adding aqueous solutions to a saturated solution of estradiol in a propylene glycol/water (60:40) mix. Using the supersaturated system, drug flux was found to increase 13 and 9.3 times through excised human skin and silicone membranes, respectively.

Supersaturated systems can also be obtained, by evaporation of a volatile co-solvent. Moser \textit{et al.} \cite{39} prepared formulations containing the lipophilic drug, lavendustin, dis-

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**Fig. (3).** Summary of methods used to optimise topical and transdermal drug delivery.
solved in an ethanol-propylene glycol mix. In contrast to the co-solvent procedure described above, this formulation becomes supersaturated due to ethanol evaporation during the course of the experiment. The formulation was said to be stable for up to 8 hours at approximately 2 degrees of saturation, and the permeation rate of the supersaturated system across excised pigskin was approximately twice that of a saturated vehicle. Supersaturated systems can also be prepared by cooling a heated saturated solution down to skin temperature [40] or by moisture from the skin absorbing into a formulation, and acting as an antisolvent [41].

By tailoring the vehicle to maximise the thermodynamic activity of the drug, significant enhancement in drug flux can be achieved. This strategy is non-invasive and inexpensive to implement. However, supersaturated systems are, by their nature, thermodynamically unstable, and material in excess of the solubility limit will crystallise out. To inhibit drug crystallisation, and hence maintain the supersaturated state, various polymers have been used as antinucleants [35,36,38,42-44]. These agents are thought to adsorb onto initial crystals, preventing further crystal growth [38,45]. The use of polyvinylpyrrolidone (PVP) as an antinucleating agent was successfully demonstrated by Ma et al. [45]. Crystallisation of a steroid drug formulated in a matrix-type patch containing PVP, was delayed for a further 6 months compared to a patch void of an antinucleant.

**PRODRUGS**

Prodrugs are compounds which are usually, but not always, inactive in their parent form. Following administration, the parent drug is chemically transformed to active metabolites, which are largely responsible for exerting the pharmacological effect [46]. In terms of topical and transdermal delivery, the physicochemical characteristics of a drug can be optimised by altering its chemical structure. The aim of this is to develop an entity with improved partitioning and diffusion characteristics. Generally, prodrugs are designed to enhance the lipophilicity of the parent drug, thus increasing skin partitioning. The most common prodrug strategy is to covalently link an active drug with an inactive moiety via an ester bond [46]. Following administration, non-specific skin esterases cleave the active drug. The inactive moiety should be non-toxic and rapidly eliminated from the body.

The prodrug approach has been used to increase delivery of a range of drugs across the skin, including 6-mercaptopurine [47], 5-fluorouracil [48], naproxen [49,50]; ketoprofen [50]; diclofenac [50]; ketoralac [51], captopril [52], cyclosporine [53], buphenorphine [54], beta blockers [55], theophylline [56] and 5-aminolevulinic acid [57,58]. Permeation enhancement across skin can vary from negligible to over an order of magnitude. Van den Akker et al. [58] reported no significant increase in penetration of 5-aminolevulinic acid esters across mouse skin compared to the parent drug. In contrast, dialkylamnomethyl prodrugs of 6-mercaptopurine were found to have delivery rates up to 27-fold greater than the parent molecule [47]. One of the most successful commercial applications of the prodrug approach has been the use of the steroid ester betamethasone-17-valerate. However, as prodrugs are considered to be a new chemical entity, commercial development is generally limited [18].

**ION-PAIRS**

Ionised drugs do not readily permeate across human skin, indeed their permeation coefficient has been estimated to be approximately 10^4 times smaller than for the respective non-charged species [59]. The ion-pair strategy involves adding oppositely charged species to a charged drug, forming an ion-pair in which the charges are neutralised. The pair of oppositely charged ions is held together by Coulomb attraction without formation of a covalent bond [60]. The ion-pair should partition and diffuse through the stratum corneum, before dissociating in the viable epidermis, thereby releasing the active drug.

Megwa and coworkers [61] illustrated enhanced penetration of salicylic acid using the ion-pair strategy. A reduction in solution conductivity was observed in equimolar mixtures of salicylic acid and various alkyl amines, indicating ion-pair formation. Permeation experiments using excised human epidermis showed an almost 5-fold enhancement in salicylic acid penetration. Using a silicone membrane, Sarveiya et al. [60] reported a 16-fold increase in steady state flux of ibuprofen using triethylamine as a pairing agent. The authors confirmed ion-pair formation using NMR spectroscopy. The ion-pairing strategy has also been employed to enhance skin penetration of 5-aminolevulic acid [62], diclofenac [63], lidocaine [64], retinoic acid [65] and ondansetron [66].

**EUTECTIC MIXTURES**

The steady-state flux of a molecule across a membrane will be directly proportional to the solubility of the drug in the lipid phase of the membrane (assuming sink conditions at one side of the membrane and infinite dose of drug on the other). If a solution is ideal, log (solubility) should be directly related to the reciprocal of the melting point of the drug in a given solvent (Equation 3) [19,67].

\[
\log C_p = \frac{\text{CONSTANT} + \Delta H_d}{2.303RT_m}
\]

where, \( C_p \) is the mole fraction solubility of the drug, \( \Delta H_d \) is the drug lattice dissociation energy, \( R \) the gas constant; and \( T_m \) the drug melting temperature. Soluble drug is the only physical form that can diffuse. A linear correlation is seen when log (steady-state flux) is plotted against melting point, indicating that the lower the melting point (greater solubility) the better the penetration [33].

Eutectic mixtures can be used to reduce the melting point of a drug. A binary eutectic is a mixture of 2 components, which do not interact to form a new chemical entity. Instead, at certain ratios, they inhibit the crystallisation process of one another, resulting in a system with a lower melting point than either of the components. The simplest eutectic systems contain two constituents, and are described using a binary phase diagram, depicting compositional changes as a function of temperature. A generic binary phase diagram (A+B) is shown in Fig. (4). If a mixture (X) of B (65%) and A (35%) is heated to \( T_3 \), then one liquid phase would exist. When cooled, this would continue to be the situation until the temperature dropped to intersect the slope on the phase diagram, termed the liquidus line. Solid B would then pre-
cipitate out of solution as the temperature continued to decrease, resulting in a solid dispersion. At the eutectic composition (A 52%; B 48%) cooling from \(T_2\) progresses in a different manner to X. A liquid at this composition is the only one which, on cooling, would not deposit any pure A or B, but would go from a solution of A and B to a solid solution of A and B. Solid solutions are defined as two solids being present as a single crystalline phase, and may be termed substitutional or interstitial [68]. A substitutional solid solution is formed when 1 component takes the place of the other in the crystal lattice to form a mixed crystal. Interstitial solid solutions are formed when 1 component is small enough to fit into spaces between adjacent molecules in a crystal lattice of the other component.

The first commercial topical formulation based on a eutectic mixture was EMLA® cream. The oil-in water-cream formulation consists of a 1:1 eutectic mixture (melting point 18 °C) of lidocaine and prilocaine [69]. Having the active components in the liquid state allows direct emulsification of the ingredients without prior dissolution, thus maximising thermodynamic activity of the drugs in the external phase [18,68]. One disadvantage associated with EMLA is the delay in analgesia following application [70]. S-caine®, a eutectic mixture of lidocaine 2.5% and tetracaine 2.5% [71]. The cream formulation dries and forms a flexible membrane that is easily removed; meaning no occlusion is required. Furthermore, the delivery system has been shown to provide local anaesthesia within 30 minutes of application [72,73]. A 7.8-fold increase in testosterone penetration across full thickness excised mouse skin was reported when the drug was formulated as a eutectic mixture with menthol [74]. The authors suggested that the increase in flux was due to a combination of increased drug solubility and menthol interacting with skin lipids. Eutectic mixtures have also been employed to enhance topical delivery of ibuprofen [68] and propranolol [75].

**LIPOSOMES AND ANALOGUES**

Liposomes are thermodynamically stable vesicles composed of one or more concentric lipid bilayers [76]. Liposomes have 2 compartments, an aqueous central core, and a lipophilic region within the lipid bilayer. Hydrophilic drugs can be incorporated into the inner aqueous volume, whilst hydrophobic molecules can be entrapped in the lipid bilayers (Fig. 5). Conventional liposomes are composed of phospholipid, with or without cholesterol. The most common phospholipid is phosphatidylcholine from soybean or egg yolk, with cholesterol often used to stabilise the system [77]. As well as traditional liposomes, a range of structurally similar vesicles have been developed, including transfersomes, ethosomes and niosomes.

**Conventional Liposomes**

Conventional liposomes can be prepared in several ways [79]. Most frequently a film hydration method is employed [18], where a thin layer of lipid is deposited on the walls of a container by evaporation of a volatile solvent. An aqueous solution containing the drug to be entrapped is added at a temperature above the phase transition temperature of the lipids, resulting in formation of multilamellar vesicles (MLV). These systems contain several lipid bilayers surrounding an aqueous core. Further processing by sonication or filter extrusion produces large unilamellar vesicles (LUV, 1-5 μm diameter), or small unilamellar vesicles (SUV, 0.1-0.5 μm diameter).

The mechanism of liposomal action is not completely understood and a number of theories have been proposed. Early studies [80] suggested that intact vesicles penetrate the stratum corneum. Foldvari and coworkers [81] used large MLVs packed with colloidal iron (an electron dense marker) to demonstrate the presence of intact SUVs in the dermis.
with electron micrography. The authors proposed that large liposomes may lose external bilayers during penetration, facilitating permeation of smaller vesicles. However, when Du Plessis and collaborators [82], examined the influence of vesicular size on skin deposition of cyclosporin, they found that intermediate sized, rather than small liposomes induced better drug penetration. If liposomes penetrate the skin intact, clearly small vesicles would be expected to permeate better. An alternative explanation was sought when Kirjavainen et al. [83] reported a similar improvement in delivery when a fluorescent marker was incorporated into liposomes and when empty vesicles of similar composition were used to simply pre-treat the skin. The authors proposed that vesicles adsorbed onto, and fused with the skin surface. Thereafter, liposomal lipids act as skin penetration enhancers, disrupting the integrity of the outer layer, and facilitating enhanced drug permeation through it. However, the importance of liposome structure was demonstrated by [84]. The authors reported that liposome components in solution did have an additive influence on drug penetration, but was not the main factor. It was concluded that for optimum skin penetration, lipids and ethanol should be incorporated into vesicles [84]. The transappendegal route is limited to liposomal targeting into, but not necessarily through, the skin and is not thought to play a significant role in improving liposomal transdermal delivery [85,86].

Mezei and Gulasekharam [80] first demonstrated enhanced topical delivery using liposomes containing trimcinolone acetonide. The authors compared a liposomal lotion and an ointment containing the same drug concentration. The liposomal system was found to deliver 4-5 times more steroid to the epidermis and dermis, with lower levels of drug being delivered systemically. Subsequent work has confirmed that conventional liposomes are a useful means of targeting skin layers, but are generally little or no value as transdermal drug delivery carriers [87-90]. As well as targeting the skin layers, liposomal systems have been shown to target skin appendages. Lieb et al. [91], illustrated follicular targeting using liposomes containing a fluorescent dye (carboxyfluorescein). In contrast, dye formulated in simple aqueous solutions was restricted to the horny layer.

Niosomes

Niosomes or Non-ionic Surfactant Vesicles (NSV) are formed by the self-assembly of non-ionic surfactants in an aqueous dispersion. The mechanism of action of niosomes is thought to be similar to that described above for conventional liposomes [18]. Although niosomes have advantages in terms of cost, and chemical stability [92] they have been shown to be associated with reduced fluxes compared to conventional liposomes [93].

Transfersomes

Highly deformable, or elastic, liposomes, termed transfersomes, were first described by Cevc and Blume [94]. As with liposomes, transfersomes are composed of phospholipids, such as phosphatidylcholine, but, in addition, contain a surfactant (10-25%), such as sodium cholate, deoxycholate, Span 80, Tween 80 or dipotassium glycyrrhizinate [12,95]. Surfactant molecules act as an ‘edge activator’, which destabilises the lipid bilayers, conferring greater flexibility to the liposome [18]. Consequently, during deformation, surfactants tend to accumulate at the site of increased stress, due to their propensity for curved structures [85], thus reducing the energy required for changing shape. Transfersomes contain up to 10% ethanol, with the final aqueous lipid suspension having a total lipid concentration between 5 and 10% [18]. Preparation methods are similar to those employed with conventional liposomes. The film hydration method is used most commonly [95].

Typically, conventional rigid liposomes are 100-400 nm in diameter and are thought to be too large to fit between intercellular lipid domains of the stratum corneum [77]. Ultra deformable transfersomes are said to be able to squeeze through pores that are 10% of the vesicle diameter (approximately 20 nm pore diameter) [94]. Whereas the diffusion gradient is the driving force behind the topical delivery of drugs, the osmotic gradient across the skin is thought to be responsible for driving elastic vesicles [85]. The difference in water content varies from almost 100% at the basement membrane of the epidermis to approximately 20% at the skin surface. Upon application of transfersomes to the skin surface, the formulation will dry, and the vesicles start to partially dehydrate, resulting in the liposome becoming flattened or curved. To maintain stability, the vesicle will permeate deeper into the stratum corneum, where water content is higher [18]. The hydration theory mechanism is supported by the reduction in flux observed when skin is occluded [86]. Occlusion prevents skin desiccation, resulting in loss of the

**Fig. (5).** Distribution of hydrophilic and lipophilic drugs with a liposomal carrier (adapted from [78]).
hydration gradient – the proposed drive for transfersome transport.

Transfersomes have been successfully employed as topical and transdermal carriers for a number of drugs, including diclofenac [96], triamcinolone [97], dexamethasone [98], methotrexate [99], ketotifen [100,101], zidovudine [102], ethinylestradiol [103], retinol [104] and dipotassium glycyrrhizinate [105]. In vitro skin penetration of estradiol was found to be superior when transfersomes (17-fold increase) were compared to conventional rigid vesicles (9-fold increase) [86]. Applying empty vesicles in order to pre-treat the skin did not significantly influence drug flux. Furthermore, it was reported that the size of transfersomes had minimal effect on penetration enhancement. Subsequent work by the same group, investigated the shunt route as a possible pathway for these vesicles. The sandwich model was used, whereby a second stratum corneum membrane is placed on top of the epidermis. In theory, the probability of 2 shunt routes aligning between the 2 horny layers is extremely low. Hence, if the shunt pathway is the principal means of permeation, flux will drop dramatically. The authors reported that flux reduced in line with increased barrier thickness, thus discounted the shunt route as the main pathway for this vehicle [86]. Transdermal delivery of insulin (molecular weight approximately 6000 Da) using transfersomes has been shown to reduce blood glucose levels in mice [106,107] and humans [106]. Deformable liposomes applied in vivo have also been shown to be effective carriers for genetic material [108,109] and vaccines [110-112]. A recent phase III clinical trial compared topical application of ketoprofen in Tranferosome® gel to a placebo gel and oral ketoprofen for the treatment of pain associated with knee osteoarthritis [113]. Efficacy of the transfersome formulation was shown to be superior to placebo and comparable to 100 mg twice daily of the oral NSAID.

**Ethosomes**

Conventional liposomes can contain up to 10% ethanol, and it had been thought that higher levels of alcohol would have a detrimental effect on the lipid bilayers [95]. However, Touitou et al. [114] demonstrated that liposomes could be prepared containing much higher levels of ethanol (ethosomes). Similarly to liposomes, ethosomes are composed of phospholipids, but can contain 20-45% ethanol [95]. Ethosomes are frequently prepared by first dissolving the lipids and drug in ethanol, then adding the aqueous component as a fine stream with thorough mixing [115]. High ethanol content results in ethosomes being much smaller than liposomes, negating the need for size reduction. Furthermore, ethanol enhances solubility of more lipophilic drugs [95].

The exact mode of action of ethosomes remains unclear [116]. Ethanol is a well known permeation enhancer [117], and phospholipids can potentially cause disruption of the intercellular domains of the horny layer [118]. However, when compared; ethosomal preparations were found to be much more effective permeation enhancers than hydroethanolic solutions, ethanol or an ethanololic phospholipid solution [115]. An alternative theory that has been proposed is that ethanol initially acts to disrupt the lipid organisation of the stratum corneum [119]. Subsequently, ethosomes, which are thought to be more flexible than liposomes due to their increased alcohol component, squeeze through the compromised horny layer (Fig. 6).

Using formulations containing ethosomes, studies have reported increased in vitro skin permeation of trihexphenidyl hydrochloride [116], cannabidiol [120], minoxidil [115], ketotifen [101], testosterone [115] and acyclovir [119]. A clinical study comparing a proprietary cream containing acyclovir to 5% ethosomal acyclovir reported a significantly faster healing for the vesicular formulation [121]. Transdermal delivery of insulin using ethosomes has been demonstrated in vivo [119]. Lowering of blood glucose levels was seen in normal and diabetic rats, with a plateau effect observed for 8 hours, indicating percutaneous delivery of insulin and possible accumulation of insulin within the skin.

**NANOPARTICLES**

Solid lipid nanoparticles (SLN) and polymeric nanoparticles were originally developed for parenteral application. By incorporating drugs into nanoparticles, development obstacles, such as poor aqueous solubility or inadequate stability can be overcome. These systems can also be used to target specific anatomical sites, for example, the brain [122] or liver [123].

Fig. (6). Proposed mechanism of action of ethosomes (adapted from [115]).
In terms of topical drug delivery, most work to date has involved the use of SLNs. SLNs can be produced by high pressure homogenisation or by the microemulsion technique [124,125]. Drug distribution within the nanoparticle is said to a function of particle composition (lipid, drug, and surfactant) and of the method of production. Drug can be homogeneously dispersed throughout the matrix of the nanoparticle, or it can be localised within the core or the particle shell (Fig. 7). Drug distribution has the potential to influence drug release. SLNs with an enriched shell facilitate rapid or burst release, whereas particles with a drug loaded core lead to sustained release [125].

Maia et al. [126] compared prednicarbate penetration into freshly excised human skin from SLN dispersions and a cream. They reported a 30% increase in drug penetration into skin from the nanoparticulate system compared with the semi-solid preparation. Liu and colleagues [124] demonstrated selective skin targeting using SLN nanoparticles containing isotretinoin. In vitro penetration studies revealed that isotretinoin released from an ethanolic vehicle, could penetrate across excised rat skin. However, incorporation of the drug into SLNs, prevented transdermal delivery. Nanoparticulate formulations were shown to target isotretinoin to the upper skin layers, thus, avoiding systemic delivery. SLNs have also been successfully used to enhance topical penetration of co-enzyme Q10 [125] and retinol [127].

Several studies have investigated the use SLNs systems as sunscreens. SLNs, due to their particulate nature, can act as physical sunscreens, whereby particles reflect and scatter incoming UV radiation [128]. Alternatively, highly conjugated compounds that absorb UV radiation can be incorporated into SLNs [129]. Wissing and Muller [129] demonstrated sustained release of the molecular sunscreen, oxybenzone using a SLN system. Furthermore, SLNs were shown to reduce the rate of oxybenzone release compared to emulsion controls. Slower and more sustained release ensures that the active ingredient remains at the site of action, near the skin surface. The SLN also appears to be a promising system for skin care. An o/w cream, enriched with 4% SLN was applied twice daily to 25 volunteers for 4 weeks [128]. The increase in skin hydration (32%) was significantly greater compared to control (24%). Enhanced hydration is thought to be due to a thin film of nanoparticles acting as an occlusive barrier on the skin surface [125].

Nanoparticulate drug delivery systems may be a useful way of targeting skin layers, however, in terms of transdermal drug delivery they appear to be of limited benefit.

**CHEMICAL PENETRATION ENHANCERS**

Substances that reversibly reduce the barrier resistance of the stratum corneum are known as chemical penetration enhancers. Properties of an ideal penetration enhancer include [19,130]:

- It should be non-toxic, non-irritant and non-allergenic
- It should not elicit any pharmacological activity within the body
- It should have a rapid and reproducible effect
- It should be physicochemically compatible and stable with the other components of the formulation
- Its action should be unidirectional. i.e. it should facilitate enhanced drug absorption into the skin, but not promote the loss of endogenous substances from the body
- When the formulation is removed from the skin, barrier integrity should recover rapidly
- It should be an excellent solvent for drugs
- It should be inexpensive
- The substance should formulate easily into semi-solids, aerosols and skin adhesives
- It should be cosmetically acceptable in terms of odour, colour, taste and texture.

The most ideal penetration enhancer discovered to date is undoubtedly water. Hydration of the stratum corneum has been shown to increase the penetration of both hydrophilic and hydrophobic drugs [18, 33]. Hydration can be achieved by soaking the skin or using a formulation with high water content. More commonly, occlusion is used to prevent natural water loss from the skin, thus stratum corneum water content moves towards equilibrium with the underlying layers. Occlusion can be achieved by use of dressings, hydrophobic ointments or patch-based formulations [18]. The mechanism of action by which water increases transdermal drug penetration remains unclear. It has been suggested that small amounts of water are present in the head group regions. This water insertion loosens lipid packing, increasing mobility of the chains [131]. Larger amounts of water can exist as a separate phase in the intercellular space or can be taken up by the corneocytes [132].

A large number of other chemicals have been evaluated as potential permeation enhancers. This review aims to highlight several of the more frequently used agents.
**Alcohols**

Ethanol is one of the most commonly used permeation enhancers [133]. Estraderm®, containing estradiol was the first commercial transdermal system to use ethanol as a permeation enhancer [134]. A number of mechanisms have been proposed for permeation enhancing action of ethanol. As a solvent, ethanol can be included in the formulation to enhance the solubility of the drug. This is particularly important for poorly soluble permeants, as they are prone to depletion in the donor vehicle [18]. Ethanol is a relatively volatile solvent and will rapidly evaporate at skin temperature. Ethanol loss from a formulation may lead to the drug becoming supersaturated, which will influence drug flux across the membrane. In addition, ethanol is thought to alter the solubility properties of the stratum corneum, facilitating improved drug partitioning [38].

A recent study by Heard et al. [135] reported a close correlation between the permeation rates of ethanol and mafenamic acid across excised porcine ear skin. The authors proposed a ‘pull’ or ‘drag’ effect, whereby the permeation of the enhancer facilitates simultaneous drug penetration via a solvation or complexation interaction. Furthermore, it has been suggested that alcohols may extract lipids in the stratum corneum. Chloroform and methanol mixture is said to be the most effective extractant, removing all except the lipids covalently bonded to the corneocyte envelope. Ethanol is a milder solvent, extracting only some of the skin lipids [136].

Ethanol has been employed in vitro to enhance transdermal delivery of levonorgestrel, hydrocortisone and 5-fluorouracil across rodent skin [117], and estradiol across human skin in vivo [137]. Megrab and collaborators [138] noted that the enhancement effect of ethanol was concentration dependent. Estradiol flux across human epidermal membrane increased up to 60% ethanol, with higher concentrations causing reduced drug penetration. The authors investigated the effect of ethanol on skin water content and concluded that formulations containing high levels of alcohol were capable of dehydrating the skin, which may explain the concentration dependant action of ethanol.

**Azone**

Azone (1-dodecylazacycloheptan-2-one or laurocapram) has been specifically designed as a chemical permeation enhancer [18], and was first patented in 1976 [20]. At room temperature, Azone is a clear liquid, with a molecular weight of 281 Da. It is strongly lipophilic (log P_{ow} 6.2), and is compatible with most organic solvents. Its structure is comprised of a large polar head group and a C_{12} side chain (Fig. 8). An important advantage of Azone is its ability to enhance penetration at low concentrations [20]; with optimum enhancement frequently observed at loadings of 1-3% [18,133].

Barry [131] used thermal analysis of human stratum corneum to elucidate the mechanism of action for several permeation enhancers. No interaction was observed between Azone and proteins, indicating that the agent does not enter corneocytes in significant amounts. Differential scanning calorimetry (DSC) data suggested that lipophilic Azone partitions into lipid bilayers of the stratum corneum, resulting in distortion of lipid packing geometry.

Azone is an effective permeation enhancer for both hydrophilic and lipophilic permeants [139-141]. For example, Azone was shown to enhance 5-fluorouracil penetration by 100-fold across hairless rat skin [142]. Triamcinolone acetone, a more lipophilic drug, displayed a 2-5-fold increase in drug flux in vivo when Azone was employed [143].

**Dimethylsulphoxide**

Sulphoxides are compounds containing a sulfinyl group (S=O) attached to 2 carbon atoms. The most commonly used permeation enhancing sulphoxide is dimethylsulphoxide (DMSO, Fig. 9). It is miscible with both water and organic solvents, enabling it to be easily formulated into pharmaceutical preparations [19]. Owing to its aqueous solubility (log P_{ow} -1.35), DMSO is thought to act primarily at the polar head region of the lipid bilayers. At high concentrations (>60%), DMSO may also interact with stratum corneum lipids [144]. Furthermore, DMSO presence in the horny layer could aid partitioning of some drugs [131].

**Fatty Acids**

Fatty acids are carboxylic acids, often with long unbranched aliphatic tails. Examples of fatty acids employed as penetration enhancers include lauric acid, linoleic acid and oleic acid (Fig. 10). Penetration enhancement has been shown to be influenced by the number, position and type (cis/trans) of double bonds [19]. Generally, unsaturated fatty acids possessing the cis configuration are more effective enhancers of drug penetration [133]. The cis double bonds introduce a ‘kink’ into the alkyl tail. The bent or ‘kinked’ tail to thought to cause greater disruption to lipid bilayers than the straight trans configuration fatty acids, which differ little from saturated fatty acids [146]. Furthermore, alkyl chain length is also of significance, with C10 and C12 carbon chain lengths providing greatest permeation enhancement [155].
maintain for a group of substances found in the skin that are known to
Natural moisturising factor (NMF) is a composite term
pared to control.
By combining the use of the enhancer with iontophoresis, the
porcine epidermis using linolenic acid to pre-treat the skin.
reported a 4-fold increase in insulin delivery across excised
the vehicle to the
was due to oleic acid hindering meloxicam partitioning from
Jantharaprapap and Stagni [150] reported a 6-fold increase in meloxicam flux across human cadaver skin, from
gels containing 1% oleic acid, compared to control. When
oleic acid was increased above 1%, meloxicam penetration reduced. The authors suggested that
this was due to oleic acid hindering meloxicam partitioning from
the vehicle to the stratum corneum. Rastogi and Singh [167]
reported a 4-fold increase in insulin delivery across excised porcine epidermis using linolenic acid to pre-treat the skin. By combining the use of the enhancer with iontophoresis, the authors demonstrated a 15-fold increase in insulin flux compared to control.

Pyrrolidones

Natural moisturising factor (NMF) is a composite term for a group of substances found in the skin that are known to maintain stratum corneum hydration. One of these components is pyrrolidone carboxylic acid [168]. Although there remains some doubt as to the enhancing effect of pyrrolidone carboxylic acid, various analogues have been examined. N-methyl-2-pyrrolidone (NMP) and 2-pyrrolidone (2P) are the most widely used of these related compounds (Fig. 11).

NMP and 2P are polar solvents, and at low concentrations have been shown to primarily interact with keratinised regions of the horny layer. At higher levels they also interact with the polar head groups of the lipid bilayers. The high water solubility of the NMP and 2P probably inhibit their migration into lipid chains. Rather, the enhancers may displace water from the polar head groups creating a larger solvation shell (Fig. 12), which may in turn disrupt lipid packing [131].

Large increases in the transdermal penetration of several hydrophilic permeants (up to 450-fold) have been reported using NMP and 2P, whereas enhancement of lipophilic drugs is less remarkable [18,169]. Babu and Pandit [170] examined the effect of NMP and 2P on skin penetration (excised rat skin) of bupranolol from a reservoir-type patch system. NMP and 2P enhanced drug penetration rates 1.5- and 3.0-fold, respectively, compared to control. Liu et al. [148] reported enhanced topical deposition of cyclosporin A, into excised rat skin pre-treated with NMP, compared to control. However, the authors reported no significant increase in transdermal delivery of the drug. In a recent study, hydrogels containing NMP exhibited a significantly greater flux of griseofulvin across mouse skin in vitro than control [171]. Pyrrolidones are associated with local adverse reactions, including irritant dermatitis [172] and erythema [18], which precludes them from clinical use.

Surfactants

Surfactants are amphiphilic molecules, usually composed of a lipophilic alkyl chain (tail) connected to a hydrophilic head. They are classified by presence of charged groups on the head moiety. A non-ionic surfactant carries no charge, whereas negatively and positively charged head groups are associated with anionic and cationic surfactants respectively. If a surfactant contains a head with two oppositely charged groups, it is termed zwitterionic.

Sodium lauryl sulphate (SLS) is an anionic surfactant, and acts on the horny layer in a concentration-dependant

![Fig. (10). Chemical structure of oleic acid.](image)
manner. At 1%, SLS was shown to markedly disrupt both lipid and protein components of the stratum corneum in an at least partially reversible manner [131]. Barry proposed that the surfactant imbibes water, causing expansion of the intercellular spaces, with subsequent disruption of the lipid structures [13]. Baby et al. [173] examined the effect of surfactant charge on stratum corneum water content using DSC. The authors found that anionic surfactants were able to cause an increase in water content within the stratum corneum.

The majority of surfactants used in penetration enhancement studies have been anionic or non-ionic. Rat skin, pre-treated with SLS (0.5% w/w) was found to reduce the lag time for cyclosporin A penetration into deeper skin layers, and enhance the amount of cyclosporin A retained in the skin, compared to control [148]. Furthermore, the surfactant was shown to enhance the amount of drug permeated across rat skin 2-fold after 12 hours. Nokhodchi and coworkers [174] examined the influence of non-ionic (Tween 80), cationic (benzalkonium chloride and cetyltrimethylammonium bromide (CTAB)), and anionic (SLS) surfactants on the penetration of lorazepam across excised rat skin. The greatest enhancement effect was observed at 1% for Tween 80 (3.75-fold over control) and benzalkonium chloride (7.66-fold over control). Reduction in permeation rate above 1% was attributed to the formation of surfactant micelles. Incorporation of lorazepam into these micelles was suggested to reduce the thermodynamic activity of the drug and decrease drug flux. CTAB and SLS exhibited maximum permeation enhancing effect at 5% surfactant concentration. However, the authors proposed that this increase was due to the surfactants damaging the skin. Surfactants have also been used to augment physical penetration enhancement strategies, such as ultrasound [175] and iontophoresis [176].

Terpenes

Terpenes are non-aromatic compounds found in essential oils. They are lipophilic substances with relatively high log $P_{ow}$ values [133]. They are widely used as flavouring and fragrance agents. However, recently they have been examined as potential candidates for permeation enhancement. The FDA have classified a number of terpenes as GRAS (generally recognised as safe) and Kang et al. [177] demonstrated that the enhancing effects of terpenes are reversible. Examples of terpenes include carvone, cineole, menthone and eucarvone. The most common terpene employed as a penetration enhancer is menthol (Fig. 13).

Fig. (13). Chemical structure of Menthol.

The mode of action of terpenes has been attributed to their ability to disrupt intercellular packing of stratum corneum lipids [178-180]. However, Heard et al. [135] proposed a ‘pull’ mechanism was responsible for enhanced penetration of mefenamic acid, mediated by the terpene, 1.8-cineole. The authors examined the penetration of both mefenamic acid and 1.8-cineole across porcine ear skin. A linear near correlation was reported when the steady-state flux of mefenamic acid was plotted against the terpene. It was proposed that permeation of the terpene through the skin, ‘pulled’ or ‘dragged’ the solute by a solvation or complexation interaction.

Terpenes have been used in vitro in enhance skin penetration of caffeine [181], cyclosporin A [148], diclofenac [182], hydrocortisone [181], anipyrene [183], indomethacin [183], 5-fluorouracil [184], meloxicam [166], midazolam [185], and sumatriptan [186]. Amnuakit et al. [187] prepared polymeric films containing propranolol, and the terpene, cineole. Cineole (10% w/w) was shown to enhance propranolol flux across excised rat skin by almost 2-fold compared to control. Monti and colleagues [188] investigated the permeation enhancing effect of terpene-containing essential oils on the penetration of estradiol across excised hairless mouse skin. Niaouli oil, a complex mixture of 4 terpenes was found to enhance estradiol flux by almost 60-fold compared to control. Furthermore, the authors demonstrated a significantly improved enhancement effect for the complex oily mixture, compared to the components applied neat or in admixture. Recently Fang et al. [189] evaluated several terpenes as enhancers for transdermal delivery of tea catechines and theophylline in vitro and in vivo. Physicochemical characteristics of the terpenes were found to strongly influence drug penetration, with agents containing oxygen having increased enhancing activity, and bicyclic terpenes having reduced enhancing activity. Rastogi and Singh [167] reported that iontophoretic delivery of insulin was significantly enhanced through limonene pre-treated epidermis compared to control. The authors proposed that such a system would be able to delivery 50 IU of insulin within 3 h.

Other Chemical Permeation Enhancers

No compilation of permeation enhancers is likely to be exhaustive. However, other classes of compound used as chemical permeation enhancers include aminoacetates [190], phospholipids [191], oxazolidinones [20], esters [192], fatty alcohols [155], amides [155], amines [158], urea and derivatives [193], esters [194] and iminosulfuranes [195,196].

Penetration Retarders

Penetration retarders or reducers, have the opposite effect to permeation enhancers. In terms of drug delivery, they can be used to restrict drug localisation to the upper layers of the skin, reducing dermal and transdermal penetration [18]. Such agents may have application in formulations that contain sunscreens, pesticides or drugs with specific local skin targeting. The mechanism of action of permeation retardants is not fully understood. They may act as an antisolvent, preventing drug partitioning or add more lipid-like molecules to the lipid bilayers to enhance stratum corneum rigidity [18]. Hadgraft et al. [197] examined the influence of Azone and 5 of its analogues on the skin penetration of metronidazole and diethyl $m$-toluidine. They reported that 1 of the analogues acted as a retardant, producing an enhancement ratio of 0.2 at 40 hours compared to control. Furthermore, the compounds were tested for their ability to reduce the phase transition temperature of dipalmitoyl phosphatidylecholine multilamellar vesicles. The retarder, termed N-0915, was shown to actually increase the phase transition temperature, indicating that it was stabilising the lipid bilayers.
TAPE STRIPPING

Wolf’s skin-stripping technique [7], removes successive layers of the stratum corneum by repeated application of adhesive tape to the skin surface. Tsai et al. [198] examined the effect of tape stripping on the molecular weight cut-off of propylene glycol (PEG) penetration across mouse skin in vitro. Stripping of the stratum corneum facilitated permeation of PEGs up to 986 Da, whereas, control skin displayed a molecular cut-off of 414 Da. Tape stripping has also been shown to enhance topical penetration of both 5-aminolevulinic acid and hexyl aminolevulinate across mouse skin in vivo [199].

Although tape stripping in as inexpensive way of reducing the barrier function of the stratum corneum, it is inconvenient, shows poor reproducibility and is unlikely to be acceptable for routine use by patients.

LASER ABLATION

Laser ablation is a controlled technique which creates pores in the stratum corneum. Holes are produced by matching the energy wavelength to the main absorption peak of water (2790 nm) or tissue proteins (2940 nm). Vibrational heating causes skin components within the irradiated area to rapidly reach boiling point. The resultant vapour pressure induces a microexplosion, resulting in pores as the tissue vapourises. The surrounding skin is protected from heat-related damage due to the rapid reduction in energy from the ablated site.

Laser ablation has been successfully used to enhance skin penetration of 5-fluorouracil [200], 5-aminolevulinic acid [201,202], hydrocortisone [203], nalbuphine [204] and indomethacin [204]. The technique has also been successfully performed with peptides and DNA [205]. Irradiation of pig skin at 2790 nm using an erbium:yttrium-scandium-gallium-garnet (Er:YSGG) laser, resulted in a 2.1-fold increase in skin penetration of 5-fluorouracil [206]. Fang et al. examined the effect of molecular weight on the transdermal delivery of macromolecules using an erbium:yttrium-aluminium-garnet (Er:YAG) laser (2940 nm). The study reports transdermal delivery of model macromolecules up to 77 kDa across excised pig skin pre-treated using the laser.

Due to the relatively high cost of medical lasers, this type of technology may be restricted to the hospital setting for specialised procedures. In addition long-term safety concerns need to be addressed.

SUCTION ABLATION

Suction ablation utilises a vacuum to produce a small blister on the skin. Using an epidermatome, the upper surface of the blister is removed, providing a pathway of low resistance for drug penetration. This strategy has been employed to enhance transdermal delivery of the antidiuretic peptide 1-deamino-8-D-arginine [207], morphine [208], and antocin [209]. This technique is a multi-step process and is unlikely to be convenient for routine use by patients or clinicians. In addition, the time taken for blister formation can be up to 2.5 h [22]. There has been an absence of published work since 1996.

THERMAL ABLATION

Enhanced transdermal delivery using radiofrequency (RF) thermal ablation was first described by Sintov et al. [210]. Needle-like electrodes were inserted into the skin, while an alternating current at radiofrequency (100 kHz) was applied to each of the electrodes. Ions within the skin attempt to follow the change in direction of the alternating current, resulting in frictional heating and subsequent cell ablation. The authors reported an increase in transepithelial water loss (TEWL) following RF treatment. Upon microscopic examination, microchannels measured approximately 70 μm in depth and 30 μm in diameter. Significant improvements in transdermal delivery of diclofenac and grani- setron were demonstrated, both in vitro and in vivo using animal models.

Microscopic pores in the stratum corneum have been generated using an array of electrically resistant filaments [211]. The array was placed on rat skin, and briefly heated using an electrical current. The thermal energy delivered to the skin from each filament ablates the stratum corneum, culminating in an array of micropores. This strategy was used to delivery interferon-alpha-2B across hairless rat skin in vivo. Furthermore, the dose administered was doubled when microporation was combined with iontophoresis.

To date, thermal ablative methods rely on a 2-step process, whereby, the skin is first treated and then the formulation applied. For a system to be convenient for regular use, the processes of stratum corneum disruption and drug delivery need to be integrated within the same device. In addition, long-term safety data is required to determine if repeated use of such devices can cause irreversible skin damage.

MICROCSISSIONING

Herndon and coworkers [212] reported a novel strategy of disrupting the skin barrier involving the use of accelerated ‘sharp’ particles (microscissioning). Aluminium oxide particles (10-70 μm diameter) are accelerated under a pressurised stream of nitrogen, and directed towards the skin. Tissue is scized (cut) by high velocity particles, resulting in formation of microconduits (Fig. 14). Surrounding skin is protected by a Teflon or polyimide mask. The authors report that pores were fully open and up to 200 μm deep. Anaesthesia following topical application of lidocaine was significantly faster when the skin of human volunteers was pre-treated with microscissioning compared to control.

Microscissioning currently requires a skin preparation step, prior to application of the drug formulation, which will limit its appeal for routine use. Deposition of particles within the skin and potential to cause infection are safety concerns that need to be addressed before this experimental technology becomes more widely used.

MICRONEEDLE FACILITATED DRUG DELIVERY

The first report of microneedle (MN) assisted topical drug delivery was in the late 1990’s, whereby puncturing the skin using micron-sized needles was shown to increase permeability of human skin to a model drug, calcinein, by up to 4 orders of magnitude in vitro [213]. Subsequently, there has been intense interest in this technology with significant developments being made both in the fields of MN fabrication and drug delivery.
MN arrays (Fig. 15) are manufactured based on etching methods used by the microelectronics industry to create arrays of micron-sized needles [214,215]. The majority of studies to date have used silicon or metal MNs, although devices have also been made from dextrin [216,217], glass [218], maltose [219,220], and various polymers [221-224]. MNs can be made of varying length, as short as 25 μm and as long as 2000 μm. In addition, base diameter of the needle and needle density can also be altered. These devices have been shown to penetrate across the stratum corneum and into the viable epidermis, avoiding nerve fibres and blood vessels that reside primarily in the dermal layer. The overriding benefit of using MNs is the promise of pain-free injection of both small and large molecular weight active pharmaceutical ingredients [225].

In terms of structure, there are essentially two types of MN: solid and hollow needles. Solid MN can be employed by inserting the needles into the skin for a defined period of time. Following removal of the device, a drug-loaded vehicle is applied to the skin surface. Microchannels produced from the application of microneedles facilitate drug transport to the viable epidermis. Alternatively, solid needles can be coated with drug, and inserted into the skin. Upon removal of the device, drug remains deposited within the membrane [226,227]. Erodable solid MNs dissolve when the device is inserted into the skin. Drug can be incorporated directly into soluble needles, or a formulation applied to the MN-treated skin. Hollow MNs are somewhat more sophisticated, in that they have a hollow bore down through the centre of the needle [218,228,229]. When inserted into the skin, the hollow centre effectively bypasses the stratum corneum, creating a direct channel to the lower layers of the epidermis.

MN technology has been shown to enhance transdermal delivery of a wide range of molecules, including anthrax vaccine [230], β-galactosidase [231] calcein [213,221,222,224,232] bovine serum albumin [221,222,224,232] desmospressin [233]; diclofenac [228,234,235], erythropoietin [217], methyl nicotinate [236], ovalbumin [227,237] and plasmid DNA [231,238]. The most widely studied drug with regards to MN-mediated delivery is insulin, with enhanced penetration reported in vitro [218,224,239] and in vivo [216,218,224,228,229,234,235]. A recent study by Nordquist et al. [235] described a novel delivery system which combined an array of hollow MNs with an expandable insulin containing reservoir. Electrically heating the device causes silicone within the reservoir to expand, forcing the drug through the hollow needles. Using diabetic rats, the authors reported comparable insulin delivery between the MN- system and subcutaneous injection. Furthermore, by adjusting the silicone expansion rate, serum insulin levels could be controlled. Smart and Subramanian [240] described a novel MN-based system designed to painlessly monitor blood glucose. Under microprocessor control, a 2 mm MN was inserted in the skin of human volunteers. Blood was sampled via the MN (200 nanoliter), and glucose levels analysed. Glucose assay performance was shown to be comparable to an accepted commercial device, and pain perception in patients was reduced compared to a traditional lancet.

Although MN-based systems have shown promising results in animal models they have several limitations that need to be addressed. Firstly, devices are of restricted volume and surface area. Therefore, loading drug directly into needles or coating drug onto needles is only feasible for extremely potent drugs [241]. Hollow MNs have only one outlet and may become blocked by compressed dermal tissue [242]. The use of solid, non coated MNs generally requires a 2-step process, which is undesirable [243,244]. Silicon is not an FDA-approved biomaterial and safety concerns exist in
relation to the breaking of silicon and metal microneedles [241]. To date, no study has investigated the reversibility and consequences of long term applications of these devices or the potential to transmit infection. In addition, silicon MNs are expensive to produce, requiring dedicated clean room facilities.

**ULTRASOUND**

Acoustic waves with frequencies between 20 Hz and 20 KHz lie within the audible range. Ultrasound describes sound waves whose frequency is beyond 20 KHz [245]. The use of ultrasound to enhance topical or transdermal drug delivery is termed sonophoresis or phonophoresis and has been studied for over 50 years [246]. Permeation enhancement induced by ultrasound is particularly significant at low frequencies (<100 kHz) [247]. Ultrasound can be used to pre-treat the skin, prior to drug delivery, or the formulation can be applied at the same time.

The effects of ultrasound on the skin can be described as thermal or non-thermal. Skin absorption of ultrasound can result in significant local heating which, in turn, may accelerate drug diffusion, increase drug solubility, and enhance local blood flow [245,248]. However, enhanced drug penetration across skin can not solely be explained by the heat effect [18]. The most significant effect of ultrasound is thought to be the growth and oscillation of gaseous cavities, an effect termed cavitation [245,246]. In the low pressure portions of an ultrasound wave, dissolved gases can form air bubbles. Subsequent waves of higher pressure can cause these bubbles to grow in size and oscillate, which is thought to induce shear stresses on cells and tissues. Such shock waves are thought to disrupt the stratum corneum, thereby enhancing skin permeability [249].

Low-frequency ultrasound (frequencies below 100 KHz) has been used to enhance delivery of a range of low and high molecular weight drugs across the skin [245,250]. *In vitro* studies using human *stratum corneum* demonstrate enhanced transport (by several orders of magnitude) of the macromolecules insulin, interferon-γ, and erythropoietin using low frequency ultrasound [251]. Recently, Park and collaborators [252] used a compact, light weight low frequency transducer to enhance transdermal insulin delivery. Live adult pigs were anaesthetised, and xylazine administered to induce hyperglycaemia. The ultrasound-treated group showed a significant decrease in blood glucose, compared to control. The authors proposed that the device was capable of safely reducing blood glucose to a normal range.

*In vitro* and *in vivo* studies have demonstrated the efficacy of sonophoresis, with some studies reporting up to 1000-fold better penetration compared to simple topical application. However, challenges remain in terms of gaining a full understanding of how the technology operates and to fully evaluate its safety profile [246]. Singer et al. [253] demonstrated that low-intensity ultrasound induced only minor skin reactions in dogs, but high-intensity ultrasound was capable of causing second-degree burns.

**IONTOPHORESIS**

Iontophoresis is a century old technique whereby an electrical potential gradient is used to drive solute molecules across the skin. An electrophoretic device consists of a power source, terminating with a positive electrode (anode), and a negative electrode (cathode) (Fig. 16). Drug transport across the skin is facilitated by 2 primary mechanisms, electrorepulsion and electroosmosis. Using electrorepulsion, whereby, like charges repel each other, delivery of a positively charged drug (D⁺) can be achieved by dissolving the drug in a suitable vehicle in contact with an electrode of similar polarity (anode). Application of a small direct current (approximately 0.5 mA cm⁻²), causes the drug to be repelled from the anode, and it is attracted towards the oppositely charged electrode (cathode) [254]. This process is termed anodal iontophoresis. Conversely, cathodal iontophoresis occurs when anions (D⁻) are repelled from the cathode, towards the anode. Importantly, iontophoresis is not only reserved for charged drugs. Delivery of small neutral molecules may also be enhanced through electroosmosis. At pH values above 4, the skin is negatively charged, due to ionisation of carboxylic acid groups within the membrane. Positively charged ions, such as Na⁺, are more easily transported, as they attempt to neutralise the charge in the skin, hence there is a flow of Na⁺ to the cathode [254]. Owing to a net build up of NaCl at the cathodal compartment, osmotic flow of water is induced from the anode to the cathode. It is this net flow of water that facilitates transfer of neutral molecules across the skin.

The transappendageal route is thought to offer the path of least electrical resistance across the skin and is suggested to be the principal pathway taken by a permeant during electrophoresis [255]. Many factors influence electrophoresis, including pH of the donor solution, electrode type, buffer concentration, current strength and current type. These parameters have been reviewed extensively elsewhere [18,255-258].

Iontophoresis has been used to enhance transdermal delivery of a wide range of relatively small molecules, including apomorphine [259], rotigotine [260], 5-fluourouracil [261], 5-aminolevulinic acid [262], fentanyl [263], piroxicam [264], non steroidal anti-inflammatory drugs (NSAIDs) [265,266] and buspirone hydrochloride [267]. A recent study by Patel et al. [268] investigated delivery of sumatriptan from an iontophoretic patch system. *In vivo* pharmacokinetic studies, using the pig model, indicated that the iontophoretic patch achieved similar blood levels as those seen following oral, nasal and rectal delivery. However, the maximum concentration (Cmax) and time to Cmax (Tmax) were significantly lower and longer respectively, compared to subcutaneous injection. Vyteris, (Fair Lawn, NJ) has received approval for an iontophoretic patch containing lidocaine and adrenaline (LidoSite™) [76,255]. Adrenaline is a vasoconstrictor and reduces lidocaine clearance from the site. Phase III clinical studies demonstrated that both children and adults, experienced significantly less pain associated with venipuncture or IV cannulation when treated with the Vyteris system compared with the placebo system [256].

The most widely studied macromolecule, in terms of iontophoretic delivery is insulin. Monomeric human insulin has
a molecular weight of approximately 6000 Da [256]. In vivo studies have demonstrated increased insulin delivery in animals, using iontophoresis [269-271]. However, achieving even the basal insulin rate (0.5 – 1.0 IU h\(^{-1}\)) in humans is likely to be extremely challenging [256]. Other peptides that have been successfully delivered across the skin via iontophoresis include salmon calcitonin [272], human parathyroid hormone [273], luteinising hormone-releasing hormone (LHRH) [274], vasopressin [275] and somatostatin analogues [276,277]. Iontophoresis has traditionally been used to enhance transdermal drug delivery. However, by using ‘reverse iontophoresis’; substances can be extracted from the skin and analysed. This technique offers a non-invasive means of monitoring glucose and drug levels in the blood [278].

The main advantage of iontophoresis over other transdermal enhancement strategies is its ease of control. Electrical current is responsible for the increased delivery [134]. Therefore, by manipulating current density, and duration, the dose may be tailored to an individual patient’s needs. An example of this concept is the fentanyl HCl iontophoretic transdermal system (IONSYS\textsuperscript{TM}), which has been approved in the USA and Europe for the management of acute, moderate-to-severe postoperative pain. The system allows patients to self-administer fentanyl according to their personal requirements for pain relief (maximum 6 doses per hour) [263,279]. The patch has been shown to provide post-surgical pain control equivalent to a standard IV morphine regimen, delivered by a patient controlled analgesia (PCA) pump [280].

Safety concerns exist over the biophysical effects of iontophoresis, such as the possibility of irreversible skin damage [254]. Recent advances in the microelectronics industry have facilitated miniaturisation of iontophoretic systems. This has resulted in the development of devices which are much more convenient for the patient and have the potential to allow home use.

**ELECTROPORATION**

In contrast to iontophoresis, which uses small voltages (<10 V), electroporation employs relatively high voltage pulses (10 – 1000 V) for brief periods of time (< a few hundred milliseconds). When applied to stratum corneum, pulses are thought to induce formation of aqueous pores in the lipid bilayers. The aqueous pores may facilitate drug transport by passive diffusion, electroosmosis or iontophoresis during the pulse [18,76]. Furthermore, transdermal delivery of charged molecules may be further enhanced by iontophoretic transport through the transfollicular pathway during pulsation [281].

Electroporation has been used to deliver a number of small molecules, including tetracaine [282], lidocaine [283], nalbuphine [281], and cyclosporin [284]. Conjeevaram \textit{et al.} [285] reported no measurable permeation of fentanyl through human epidermis under passive conditions. However, iontophoresis gave a flux of approximately 80 µg cm\(^{-2}\) hr\(^{-1}\), with a 4-fold higher flux observed using electroporation. Larger molecules studied include heparin [286], parathyroid hormone [287] and DNA vaccines [141,288]. Medi and Singh [287] examined electrically facilitated transdermal delivery of human parathyroid hormone (PTH) using dermatomed porcine skin. Iontophoresis significantly enhanced the flux of PTH compared to passive delivery. Electroporation pulses of 100, 200 and 300 V significantly increased PTH flux in comparison to passive as well as iontophoretic flux. Furthermore, the authors demonstrated that by following electroporation pulses with iontophoresis, flux was further increased by several fold. Significant disruption of the stratum corneum was reported using light microscopy.
Electroporation has the potential to enhance transdermal delivery of macromolecules. However, more clinical studies are required to assess long-term safety of this technique and to gain a greater understanding of its mechanism of action [76]. Miniaturisation of such devices is essential to facilitate routine use by patients.

JET INJECTION

The conventional method of injectable drug delivery uses a needle and syringe. However, needle based methods have significant limitations, such as needle phobia and accidental needle stick injury [289,290]. An alternative method of injection is to deliver the drug as a high pressure jet (> 100 m/s), with sufficient intensity to pierce the skin [291]. This is referred to as needle-free or jet injection (Fig. 17). Devices can be powered by compressed air or by means of a compression spring [76]. There are essentially 2 types of jet injectors, liquid jet injector and powder jet injectors.

Liquid jet injectors have been shown to successfully deliver insulin [292], lidocaine [293,294] vaccines [290], human growth hormone [295], midazolam [296], bleomycin [297], interferon [298] and erythropoietin [299]. Needle-free liquid jet injectors have been associated with frequent bruising and pain, which offsets their advantages over the conventional needle and syringe approach [290]. Recent work by Arora et al. [300] demonstrated needle-free transdermal delivery of macromolecules using nanolitre-volume pulsed microjets. By using repeated injections of small injection volumes, 10-15 nanolitres, the authors proposed that pain and bruising would be minimised. In vitro studies using excised human epidermis demonstrated that on average, 48 pulses were required before the vehicle penetrated the membrane. After this lag-time (48 seconds), drug solution could be delivered at a rate of 1 µl min⁻¹. In the same study, microjet delivered insulin was shown to rapidly reduce blood glucose levels in rats. Furthermore, adverse effects, such as bleeding and erythema were significantly reduced with the microjet compared to a conventional liquid jet injector.

Transdermal powdered delivery is where the therapeutic compound is formulated as a fine powder (20-100 µm diameter) and is accelerated in a supersonic flow of helium gas to penetrate the skin [301]. The PMED® (Chiron Corporation) device, formerly known as PowderJect® [302] has been reported to successfully deliver vaccines [303,304], lidocaine [305] and testosterone [306]. Dry powder formulations are generally more stable than solutions and may negate the need for the ‘cold chain’ to be maintained when using vaccines, for example. This would be particularly advantageous for large-scale immunisation in developing countries with hot climates.

It is claimed that needle-free injection has several potential benefits. The fear of needles (belonephobia) and piercing (diatrypophobia) can be avoided [307]. In addition, several studies have shown that by adjusting injection parameters (e.g., injection volume) specific skin strata can be targeted. Furthermore, the use of jet injectors should avoid needle stick injuries. However, a number of limitations of needle-free injectors have been highlighted. Dosing accuracy and location of delivery may vary due to skin variability (thickness and hydration) between patients. Drugs are exposed to high shear stresses during injection, which may affect the structural integrity of proteins, vaccines and DNA. The long-term effect of bombarding the skin with high speed particles or liquids is not known; and in addition, jet injection has been associated with variable adverse reactions [307,308], and may be no less painful than conventional needle and syringe [290,309].

![Fig. (17). Representation of the jet injection process.](image-url)
OTHER PHYSICAL METHODS AND COMBINATION STRATEGIES

In addition to the techniques described above, the literature contains reports of several other innovative strategies for enhancing transdermal drug delivery. A pulse from a high powered laser can be used to create stress or compression waves. Termed photomechanical waves (PW), these pressure pulses can be directed onto the skin to increase permeability. Lee et al. [310] demonstrated transdermal delivery of insulin in a diabetic rat model using a single laser pulse, with blood glucose levels being reduced by approximately 80%. Controlled heat-assisted drug delivery (CHADD) is a disposable, self adhesive heating unit consisting of a heat-generating chemical component, sandwiched between a perforated cover film and a pressure-sensitive adhesive layer. Exposure of the powder to oxygen, results in an exothermic reaction; which is turns raises skin temperature in a controlled manner. Placing the CHADD patch over an S-Caine® patch (1:1 eutectic mixture of tetracaine and lidocaine) achieved significantly superior anaesthesia compared to placebo in a randomised double blind trial [311]. Further work by the same group, examined the influence of the CHADD system on transdermal delivery of testosterone in healthy male volunteers [312]. Placing the CHADD patch on top of a testosterone patch significantly increased the maximum serum drug concentration and reduced the time to peak testosterone concentration, compared to the androgen patch alone. Magnetoophoresis is where diamagnetic substances are repelled across the skin by a magnetic field. Murthy [313] reported enhanced flux of benzoic acid, across excised rat skin using this technique. Microdialysis devices can be used to measure drug uptake into skin. A thin (0.2 – 0.5 mm diameter) semi-permeable fibre is inserted into the dermis underlying the area where the formulation is applied. Physiological solution (perfusion) is passed through the fibre, facilitating passive diffusion of substances from the dermis into the perfusate, which is subsequently assayed. Based on this technology, a self-adhesive portable microinfuser device was used do deliver therapeutic levels of morphine and heparin in healthy male volunteers [314].

The enhancement approaches described above can be used alone or in conjunction with 1 or more other techniques. For example, combined strategies that have been used to improve iontophoretic drug delivery include chemical enhancement, electromperation, ultrasound and use of microneedles [167,257,315]. The combination of techniques selected will influence synergism. For example, 2 strategies that both enhance transdermal delivery by creating pores may not act synergistically [18]. A better approach may be to combine a microporation technique with a system designed to enhance the driving force for drug permeation.

CONCLUSION

To date the vast majority of topical and transdermal drug formulations are based on the passive diffusion of a low molecular weight drugs and several of which have been successfully employed in commercial systems. Transdermal delivery of macromolecules, such as insulin has generally been less successful. Recently strategies, such as microneedle arrays, elecetroporation and iontophoresis have been shown to enhance macromolecule delivery across the skin. However, more work is needed to establish in vivo efficacy, long-term safety data and cost-effectiveness of such methods.

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