Overcoming the Skin Permeation Barrier: Challenges and Opportunities

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Abstract: Stratum corneum (SC), the outermost layer of the skin, constitutes an excellent protective physiological barrier, and is the main challenge in transdermal drug delivery. Many approaches have been used to enhance the penetration of drugs through this layer, covering passive and active methods or the combination of both. This opens the opportunity to broaden the spectrum of drugs that can be administered through the skin, providing alternatives to existing products, and filling gaps that conventional routes failed to occupy. In this review, an overview of the different permeation enhancing methodologies is carried out, focusing on the combination of lipid nanoparticles with conventional chemical enhancers, as a proof of concept of a successful development strategy.

Keywords: Transdermal delivery, permeation enhancement, passive methods, active methods, chemical penetration enhancers, lipid nanoparticles, microneedles.

INTRODUCTION

Over time, the skin has become an important route for drug delivery encompassing topical, regional or systemic effects. The present work focuses on aspects that should be taken into consideration for transdermal drug delivery, i.e., when a drug is intended to be delivered through the skin in order to enter the bloodstream. The advantages of transdermal delivery include providing a non-invasive, painless and convenient means of drug administration, avoidance of possible infection and compliance issues related to injections, steadier and sustained drug levels over a prolonged period of time, reduced side effects associated with peaks and troughs in drug plasma concentration, avoidance of the liver first-pass metabolism and other variables associated with the GI tract (pH, gastric emptying), as such providing an alternative route when oral dosing is not possible (unconscious or nauseated patients), and the ease of dose termination when adverse effects occur [1].

Nevertheless, the skin constitutes an excellent natural barrier, which limits the number of drugs able to cross its external layer, the stratum corneum, in sufficient quantities to reach a therapeutic plasma concentration. This explains why ca. 30 years since the approval by FDA of the first transdermal patch, the Alza’s Transderm Scop® (scopolamine) to treat motion sickness, only approximately 20 drugs or drug combinations have been marketed in the United States and European Union [2].

Facing a declining output from innovative pharmaceutical research, before starting the development process of a new product, a careful analysis of the market is crucial in order to identify relevant needs. Thus, a problem must first be identified for which a solution with a discernible benefit can be found.

The above market trends indicate that TDDS are limited to some drugs/therapeutic areas, such as nitroglycerin for cardiovascular disorders, rivastigmine for Alzheimer’s disease, fentanyl for pain management, estradiol and testosterone for hormone replacement, ethinylestradiol/norelgestromin for female contraception and nicotine for smoking cessation.

In this context, the potential of the transdermal pathway is still far from exhausted and the development of different technologies opens the opportunity for new therapeutic areas being explored. This intends to combining improvement over an existing therapy, and providing a solution for problems with an existing drug.

Before proceeding into the strategies studied to deliver drugs through the transdermal route, an anatomophysiological perspective of the skin and a description of the factors influencing drug permeation are presented.

SKIN

Function

The skin is the largest organ of the human body, covering about 1.7 m² and accounting for more than 10% of the total body mass of an average person [1, 3].

Numerous functions have been attributed to the skin. As the human integument, it establishes the interface between body and external environment, ensuring a protective barrier against chemical, mechanical, microbial, physical, and ultraviolet (UV) radiation injuries, and preventing the loss of moisture and body nutrients [3-5]. Besides limiting the entrance of xenobiotics, it constitutes a physical barrier for the penetration of microorganisms. The production of the “acid mantle” (pH~5) by the sebaceous and sweat glands, the presence of a thin film on the skin surface comprised of sebum, corneocyte debris and residual material from sweat, the low surface water content, the resident microflora, and surface-deposited antimicrobial lipids contribute additionally to the antimicrobial protection [6, 7].

The skin also serves important metabolic, immunological and sensorial functions. It has an important role in the metabolism of melanin, vitamin D, lipids, collagen and keratin. Additionally, it presents immunological activity, as it contains Langerhans cells [8, 9]. The skin also provides sensory information, such as pressure, pain and temperature, since it encloses nerve endings and receptors [3].

Furthermore, its role in assuring the homeostasis of the body should be emphasized, not only in terms of the maintenance of the respective composition and excretory functions, but also in blood pressure control and thermoregulation [3, 9].
To properly assess and rationalize the ability of a drug to be delivered transdermally, an understanding of the fundamental aspects of skin anatomy and physiology is of crucial importance and will be firstly discussed.

Essentially, the skin consists of four layers: the stratum corneum (nonviable epidermis), the remaining layers of the epidermis (viable epidermis), dermis, and subcutaneous tissues (hypoderminis). A number of appendages are also associated with the skin, comprising hair follicles, apocrine and eccrine sweat glands, and nails (Fig. 1) [9].

Although the large surface area would indicate that the skin is a suitable pathway for drug delivery, from a permeation point of view, skin represents a challenge due to its outermost layer, the SC, which determines the formidable barrier described above. The qualification for this is the unique physicochemical composition and architecture of the SC. Therefore, the structure of this layer will be discussed in more detail. The other layers and appendages present important functions, constituting target sites for drug delivery.

Epidermis

The epidermis is a dynamic multilayered region, with a thickness ranging from ca. 0.06 mm on the eyelids to 0.8 mm on the soles of the feet and on the palms of the hands [3]. The epidermis is avascular, composed by keratinocytes (95% of cells) that undergo constant proliferation, differentiation, and keratinization, being responsible for the constant physiological renewal of the skin. Each layer is known to represent a different level of cellular or epidermal differentiation [1]. Thus, the structure and composition of the keratinocytes change during their migration from the stratum basale, through the stratum spinosum, stratum granulosum, and the stratum lucidum up to the outermost SC. During their maturation process, keratinocytes synthesize and express numerous different protein and lipids [11]. On reaching the SC, cells become enucleated (corneocytes) and flattened, being usually referred as nonviable epidermis, in contradistinction to the lower epidermal layers (viable epidermis) [3, 12]. Interspersed among the keratinocytes in the viable epidermis, another population exists (accounting for the remaining 5% [13] of cells), but do not participate in the process of keratinization. These include the melanocytes, Merkel cells (tactile epithelioid cells), and Langerhans cells (intraepidermal macrophages) [14]. In addition to the structured cellular components of skin, various skin appendages, such as hair, sweat and sebaceous glands exist as specializations of the epidermis. The epidermis also possesses many enzymes capable of metabolizing topicaly applied compounds, which are also involved in the physiological keratinocyte maturation and desquamation process, formation of natural moisturizing factor (NMF) and general homeostasis [8, 15, 16].

The stratum basale (SB), also referred to as the stratum germinativum or basal layer, is a single layer of columnar cells, presenting a high nucleo-cytoplasmatic ratio, attached to the basement membrane via hemidesmosomes (proteineaceous anchors). Desmosomes interconnect the keratin of adjacent and overlying cells, thereby ensuring the structural integrity of the skin. It is the only layer of the epidermis composed of keratinocytes able to undergo cell division (stem cells) [17]. It also contains the melanocytes, Langerhans cells, and Merkel cells.

Melanocytes are dendritic cells that synthesize melanin, a high molecular weight polymer that provide the pigmentation of the skin, hair, and eyes. Melanin is packaged in subcellular organelles, called melanosomes and transported to the neighboring basal keratinocytes [13]. The main function of melanin is to afford protection of the skin by absorbing potentially harmful UV radiation, thus minimizing the liberation of free-radicals in the basal layer [3].

Langerhans are bone marrow-derived dendritic cells and the major antigen presenting cells in the skin [13]. They are activated by the binding of antigen to the cell surface, then migrating from the epidermis to the dermis and on to the regional lymph nodes, where they sensitize T cells to generate an immunological response [3].

Merkel cells are associated with the nerve endings and are concentrated in the touch-sensitive sites of the body, such as the fingertips and lips, being associated to sensorial perception [3, 7, 18].

The stratum spinosum (SS) is composed of two to six rows of keratinocytes immediately above the basal layer. Their morphology changes from columnar to polygonal, and possess an enlarged cyto-
plasm containing a higher number of keratin filaments (tonofilaments) and organelles when compared with the SB. Beyond the typical cell organelles observed in SB, the presence of Odland bodies, lipid-enriched lamellar bodies (LB) is also evident in this layer. The increase in protein and lipid synthesis denotes the dual aspect of epidermal differentiation [3, 17].

The stratum granulosum (SG), also known as granular layer is composed by keratinocytes at a different level of differentiation. They contain intracellular keratohyalin granules, composed by keratin, as well as profilagrin, loricrin and a cysteine-rich protein. The fillagrin subunits of profilagrin are important as matrix molecules to promote the aggregation and alignment of the keratin filaments [17]. The rise in the protein synthesis is accompanied by an increased lipogenesis, as evidenced by the large number of LB, which are believed to be the precursors of the intercellular lipid lamellae of the SC [19]. The LB also contain hydrolytic enzymes, such as the SC chymotryptic enzyme, a serine protease which has been associated with the desquamation process [20]. As the cells ascend in the SG, the LB are extruded to the intercellular domains (Fig. 2) [3].

In the stratum lucidum (SL), a layer present in the palms and soles where the skin is particularly thick, the cell nucleus and other organelles disintegrate, keratinization increases, and cells become flattened and compact [3].

**Stratum Corneum: a Key Role**

The stratum corneum, also known as horny layer, is the outermost layer of the skin, consisting of 10-15 cells in depth, which corresponds to 10-20 μm cell layers of high density (1.4 g/cm³ in the dry state) with low hydration (10-20% in comparison to ca. 70% in viable epidermis). The SC has been described as a brick wall-like structure, in which the corneocytes represent the “bricks”, embedding in a “mortar” of the intercellular lipids, interlinked by desmosomes (Fig. 3a) [21]. The corneocytes are flat and elongated cells, usually up to 50 μm in length and 1.5 μm thick, devoid of nucleus, and composed of about 70-80% keratin and 20% lipid within a cornified cell envelope (~10 nm thick) [3]. The cornified cell envelope is a protein/lipid polymer structure formed just below the cytoplasmic membrane, surrounding the exterior of the corneocytes. It consists of two parts: a protein envelope and a lipid envelope (Fig. 3b). The protein envelope is thought to contribute to the biomechanical properties (e.g., impact resistance) of the cornified envelope, as a result of the cross-linking of specialized structural proteins, mainly involucrin and loricrin, by both disulfide bonds and N-(γ-glutamyl) lysine isopeptide bonds formed by transaminases [22].

The lipid envelope comprises α-hydroxyceramides covalently bound to the protein matrix of the cornified envelope [24, 25]. There are indications that it is essential in the assembly of the intercellular lipid lamellae, thus providing the structure and barrier function of the SC [26].

The unique composition of the SC intercellular lipids and their particular organization as multiple stacked membrane layers within a continuous lipid domain forming intercellular lipid lamellae are critical to the permeability barrier function of the SC (Fig. 3C). The major components of these lipid domains are ceramides, cholesterol, free fatty acids, cholesterol esters, and cholesterol sulfate, whose contents vary between individuals and with the anatomical site [3, 27-29]. Ceramides, the most abundant lipids in SC, consist of a sphingoid base (sphingosine, dihydrosphingosine, phytosphingosine, or 6-hydroxy-sphingosine), linked via an amide bond to a fatty acid (nonhydroxy, α-hydroxy, or ester-linked α-hydroxy) [30]. Both variations in the fatty acid carbon chain and the sphingoid base architecture result in a large number of ceramide subclasses, with a wide variation in chain length distribution, as depicted in Fig. 4.

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Fig. (2). Schematic representation of epidermal differentiation. Major events include extrusion of lamellar bodies, loss of nucleus, and increasing amount of keratin in the stratum corneum. Adapted from reference [9].
Another important lipid class in stratum corneum is cholesterol, the second most abundant, which is crucial for promoting the intermixing of different lipid species [17]. On the other hand, cholesterol sulfate, in spite of being present in small amounts (typically 2–5% w/w), plays an important role in the SC desquamation process [11, 31].

Free fatty acids have also been identified among the intercellular lipid, and consist of long chain saturated free fatty acids, especially lignoceric acid (C24) and hexacosanoic acid (C26), and trace amounts of very long-chain (C32-C36) saturated and monounsaturated free fatty acids [32].

The particular arrangement of the lipids in structured lipophilic (lipid packed hydrocarbon chains) and hydrophilic (lipid polar head groups surrounded by water) domains imposes a polar and lipid route of penetration that should be considered when an increase in the permeation by the disorder of SC is intended to be promoted (Fig. 3C, see Section 1.3.1).

Several models for the structural organization of lipids have been suggested so far. These include the “sandwich model” proposed by Bouwstra et al, in which a long periodicity phase, consisting of three regions (a central liquid crystalline layer surrounded by two crystalline gel phases in an orthorhombic arrangement on both sides) was identified [33] or the ‘single gel phase’ model propounded by Norlén [34], which suggests a lipid arrangement in a single coherent gel phase with no boundaries. However, there is no consensus about the existence of a single, general, model for lipid packing.

Thus, the impermeable character of the cornified envelope, together with the highly structured lipid lamellae that are oriented parallel to the corneocyte cells, defines a very densely packed structure in the SC, important to consider as main barrier for drug penetration.

**Dermis**

Beneath the epidermis, separated by a thin basement membrane, is the dermis, a layer of about 2-5 mm in thickness, composed of dense irregular connective tissue. It consists of collagen fibrils that provide support, and elastic connective tissue that ensures elasticity and flexibility, embedded in an amorphous ground substance of mucopolysaccharides. Predominant cell types of the dermis are fibroblasts, responsible for the synthesis and renewal of the components of the connective tissue, mast cells, and macrophages involved in immune and inflammatory response. As a result of its structure, dermis affords low resistance to drug permeation. Nevertheless, the transport of very lipophilic drugs to the deeper tissues may be compromised, due to the increased hydration degree [3, 8].

It provides a highly vascularized network that ensures the removal of the permeant molecules from the dermo-epidermal junction to the bloodstream, thus allowing a concentration gradient between the applied formulation on the skin surface and the dermis.

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Fig. (3). Schematic representation of (A) the “bricks and mortar” model for human stratum corneum, with the corneocyte “bricks”, the intercellular lipid “mortar”, and the corneodesmosomes connecting the corneocytes, (B) the corneocyte envelope. (from reference [23] with permission), and (C) intercellular lipid lamellae.
Lymph vessels within the dermis can also remove permeated molecules from the skin, which is particularly important in the case of large molecules. Moreover, it plays a crucial role in the physiological temperature maintenance and in tissue nutrition and metabolic changes. It also contains sensory nerves (free nerve endings and end corpuscles), including Pacinian corpuscles that sense vibration and Meissner corpuscles, responsible for tactile and pressure sensations [13].

Besides, a number of appendages are originated within the dermis, such as the hair follicles and associated sebaceous glands, eccrine and apocrine sweat glands.

**Hypodermis**

Beneath the dermis is a layer of loose connective tissue, commonly known as the hypodermis or subcutaneous tissue. It is composed of lipocytes, arranged into fat lobules with interconnecting collagen and elastin fibers [13]. Its primary functions are protection against physical shock, heat insulation, and energy storage. Blood vessels and nerves are supplied to the skin via the hypodermis. It also aids in binding the skin to the underlying fascia and skeletal muscle [9].

**Appendages**

The hair follicles are distributed across the entire skin surface with the exception of the soles of the feet, the palms of the hands and the lips, representing a fractional area of about 1/1000 of the total skin surface. The sebaceous gland associated with each hair follicle secretes sebum, which is composed of free fatty acids, triglycerides, and waxes. Sebum ensures protection and lubricates the skin, also maintaining the skin surface at a pH value of about 5. A smooth muscle, the erector pilorum, attaches the follicle to the dermal tissue, enabling hair to respond to fear or cold. Eccrine glands, present a fractional area of about 1 in 10,000 of the total body surface, and secrete sweat in response to exercise, high environmental temperature, as well as emotional stress. The apocrine glands are limited to specific body regions (axillae, nipples, and anogenital area), being about ten times the size of the eccrine glands [3, 9].

**PERCUTANEOUS ABSORPTION**

According to what was mentioned above, the process of drug absorption from a transdermal drug delivery system into the systemic circulation may be regarded as a passage through consecutive skin layers. Thus, it starts with the release of the permeant from the vehicle. On the other hand, all these steps are highly dependent on the solubility and diffusivity of the permeant within each environment. Release of the permeant from the vehicle and uptake into the lipophilic SC is dependent on the relative solubility in each environment, and the vehicle-SC partition coefficient. In turn, the diffusion coefficient of the speed at which the permeant moves within each environment is dependent on both the permeant properties and factors related to the environment, such as its viscosity and tortuosity, i.e. diffusional path length [3, 35].

Despite of the heterogeneity of the skin barrier, steady-state permeation or flux (J) of a drug through the SC can be simplistically described by Fick’s first law of diffusion

\[
J = \frac{dQ}{dt} = \frac{DKC_0}{h}
\]

where \(Q\) is the drug amount permeating a certain unit area of skin, \(D\) is the diffusion coefficient of the permeant in the skin, \(K\) is the partition coefficient between the stratum corneum and the vehicle, \(C_0\) is the applied concentration of permeant, and \(h\) the diffusional path length. The mechanism of drug penetration can be primarily considered driven by passive diffusion and, thus, dependent on the concentration gradient.

**Routes of Drug Penetration**

The transport of drugs through the skin may be carried out through three potential pathways: sweat ducts, hair follicles and associated sebaceous glands (transappendageal route), or across the continuous stratum corneum (transepidermal route) (Fig. 5). In what regards the latter, two particular pathways through intact SC may exist: the intercellular lipid route between the corneocytes (intercellular) and the transcellular route through the corneocytes and interleaving lipids (transcellular) [36, 37].

It should be noted that these pathways are not mutually exclusive, that is, a drug may use more than one penetration route, which will be dependent on its physicochemical properties.

The transcellular route has been regarded as a polar route through the SC, since the corneocytes contain an intracellular keratin matrix that is relatively hydrated and, thus, polar in nature. Therefore, permeation requires consecutive partitioning between this polar environment and the lipophilic domains involving the corneocytes [3, 35]. This is a preferential route for hydrophilic...
compounds, despite the need of the permeant to cross the intercellular lipids, in order to jump from one corneocyte to another [38]. Although this is the more direct route, the transport is predominantly carried out by the intercellular route, which provides the only continuous route through the SC [36]. In this case, the diffusional path length is much longer than the simple thickness of the SC (10-15 μm), since the intercellular domains are highly tortuous, and may be in excess of 150 μm. Within intercellular spaces, a diffusing molecule has to cross a variety of lipophilic (via lipid core) and hydrophilic (via polar head groups) domains of the structured lipids [35, 39].

Despite the appendages (glands and hair follicles) have been considered as low resistance shunts, their contribution was primarily estimated to be small, since they represent only 0.1-1% of the total skin surface area [40]. However, it is suggested that the appendageal route dominates during the lag phase of the diffusional process [3]. In recent years, there has been renewed interest in targeting follicular delivery through colloidal-based formulation approaches [14].

**FACTORS AFFECTING DRUG PERMEATION**

**Physiological Factors**

Skin permeability may be affected by a large number of physiological factors. This includes age, anatomical site, ethnicity, gender and some skin disorders.

Intrinsic aging leads the epidermis to become thinner and the corneocytes less adherent to one another, although the SC thickness has been shown not to significantly change [41]. It is also reported that the lipid composition suffers alterations with age, with decreased levels of all major lipid species, particularly for ceramides. Additionally, the dermis becomes atrophic and relatively acellular and avascular. The reduced hydration levels and lipid content of ageing skin may be responsible for a demonstrated reduction in skin permeability with hydrophilic compounds [35, 42]. Moreover, the skin barrier function in young children is significantly reduced, which may lead to an increased permeability [43].

Skin permeability may also vary with anatomical site. Several studies have revealed that distinct anatomic skin sites possess different morphologic and functional characteristics. These regional variations induce skin barrier function alterations in the following order: genitals (more permeable)>head and neck>trunk>arm and leg (less permeable).

Some differences have been reported across ethnic groups, although inconsistent, suggesting that ethnic differences are much less profound than interindividual differences within ethnic groups [28].

Concerning gender, only slight or no differences in the epidermal barrier have been reported as determined by basal transdermal water loss (TEWL) between male and female skin [3, 44, 45].

The state of the skin (normal, abraded, or diseased) may also influence permeation. A number of common skin disorders, such as eczema (dermatitis), psoriasis, ichthyosis, and acne vulgaris may compromise barrier function. Skin infections that manifest eruptions at the skin surface can also temporarily reduce the barrier [3, 10].

All these factors should be equated when developing a transdermal system, since the rate of drug delivery is determined by the system at the application site, so that physiological variables may raise safety issues [46]. These include the skin sites, which impacts e.g. on the membrane thickness and temperature, affecting the permeation rate, and blood flow, influencing drug clearance.

**Properties of the Drug**

The capacity of a drug to enter the skin depends on its ability to penetrate, the hydrophobic and hydrophilic domains of the skin. Ideal physicochemical properties of a molecule to penetrate and permeate through the SC can be extracted from Equation (1.1). These include [37, 47] a high, but balanced, partition coefficient (K). Thus, a log P<sub>o/w</sub> comprised between 1 and 3 is pointed as optimal, since drugs that are too hydrophilic are unable to partition from the vehicle into the SC. On the other hand, very lipophilic drugs will be retained in intercellular SC lipids, and will not partition to the more aqueous viable epidermis, thus limiting their skin permeation rate. Additionally, ionized species have also a lower permeability coefficient than the unionized counterpart, since the log P of the former is lower [39]. A low molecular weight is also desirable, since the size of the permeant will influence the diffusivity (D) within the SC. An inverse relationship between permeant size and skin permeation has been reported. As a general rule, permeants selected for transdermal delivery tend to be less than 500 Da, when D tends to be high. It should also possess an adequate lipid solubility (high diffusion coefficient, D), but also reasonable aqueous solubility (> 1mg/mL) (high donor concentration, C<sub>d</sub>, in order to ensure a high concentration gradient, the driving force for diffusion) to maximize flux. Finally, a low melting point (<200 ºC) is also a good characteristic, since it correlates with good solubility of the drug in the intercellular SC lipid domain.

In addition to these specific physicochemical properties, drug candidates should be characterized by a high therapeutic potency (deliverable dose ideally below 20 mg/day), poor oral bioavailability and short biological half-life, in order to take maximal advantage of a transdermal administration. The drug should also not be irritant to the skin or stimulate an immune reaction in the skin [48, 49].
Properties of the Vehicle

The successful development of a transdermal drug delivery system relies on the application of the skin permeation fundamentals to the design of an appropriate formulation. The latter assumes particular importance, since the vehicle may profoundly influence the drug release mechanism from a formulation (by modulating the vehicle/SC partition), alter the skin barrier properties (by SC modification/circumvention), or simply promoting an increase of the drug solubility in the SC. The effects upon the SC may range from the interaction with SC intrinsic elements, including the intercellular lipid lamellae and protein components, or promoting SC hydration by an occlusive effect [35, 50]. These different effects could be obtained e.g. through the incorporation of chemical enhancers in the formulation, by a hydration effect promoted by nanocarriers or simply by controlling the degree of occlusion of the backing layer and the porosity of the permeable membrane of a transdermal (reservoir type, in the latter case) system. Methods to enhance skin permeation are discussed in detail below.

PERMEATION ENHANCEMENT

Under normal circumstances, due to barrier properties of the skin, transport from simple vehicles will often be insufficient to achieve therapeutic drug concentrations at the site of action [51]. In order to overcome this limitation, a number of techniques have been developed aiming at increasing the range of penetrants and the rate of transdermal delivery. Strategies to achieve penetration enhancement can be categorized as passive and active, ranging from simple occlusion and formulation optimization, to the use of chemical and physical methods or combinations of both (Fig. 6). These rely on two main approaches: increasing skin permeability and/or providing a driving force acting on drug [10].

PASSIVE METHODS

Passive penetration enhancement can be achieved by manipulation of the formulation, increasing the thermodynamic activity of the drug in formulations (e.g., supersaturated and nanocarrier systems), drug modification, and/or by using chemical penetration enhancers (CPE) that interact with skin constituents to promote drug flux [47].

Supersaturated Systems

Supersaturation is a state reached when the amount of drug dissolved in a matrix exceeds its equilibrium solubility. Supersaturated formulations allow an increased driving force by increasing the concentration of the drug in the vehicle. However, since the thermodynamic activity of supersaturated systems is higher than that of saturated systems, they are inherently unstable, thus compromising long-term stability. The use of mixed cosolvent systems (e.g., mixtures of propylene glycol and water), with antinucleant polymers to inhibit or retard crystallization [52], or the induction of in situ changes in drug concentration with solvent evaporation [53] are some of the strategies employed to produce supersaturated systems and avoid stability problems [47, 48].

Drug Modification

Other approaches to increase drug delivery through the skin rely on chemical modification of a poorly penetrating drug into a pharmacologically inactive prodrug, which readily penetrates the skin, as a result of the increase in lipophilicity [54]. The ion-pair formation and the use of eutectic mixtures are other examples of drug modification. Ion pairs are defined as neutral species formed only by electrostatic attraction between oppositely charged ions, which exhibit sufficient lipophilicity to dissolve...
in the SC [47]. The ion pair diffuses subsequently to the aqueous viable epidermis, where dissociation into the charged species occurs, before diffusing onwards [37].

Eutectic mixtures take advantage of a reduction in the melting point of a permeant, which will have a direct effect on its solubility in skin and thus should increase skin permeability [55].

Chemical Penetration Enhancers

Chemical penetration enhancers (CPE) are pharmacologically inactive compounds that may temporarily diminish the barrier of the skin, by affecting drug partition and diffusion into the skin, thus interacting with the SC constituents [54]. Several substances have been identified as drug penetration enhancers. However, safety issues constitute the major concern that limits their clinical use. Therefore, there is an attempt to identify new CPE that are classified as GRAS (generally recognized as safe). Ideal properties of a chemical enhancer are depicted in Table 1.

Table 1. Properties of an ideal chemical penetration enhancer [56].

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<th>Ideal chemical penetration enhancer</th>
<th>Property</th>
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<tr>
<td></td>
<td>Pharmacologically inert.</td>
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<td></td>
<td>Nonallergenic, nonirritating, and nontoxic.</td>
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<td></td>
<td>Rapid onset of effect with a predictable duration of activity.</td>
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<td>Immediate and complete recovery of the normal barrier property of the SC, after removal of the enhancer.</td>
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<td>Decrease in one direction only of the barrier function of the skin, without loss of endogenous materials.</td>
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<td>Physically and chemically compatible with drugs and excipients in the dosage form; readily incorporated into the delivery system.</td>
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<td></td>
<td>Cosmetically acceptable when applied to the skin.</td>
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<td></td>
<td>Inexpensive, odorless, tasteless, colorless.</td>
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CPE are believed to affect permeation via one or more of three main mechanisms, relying on the lipid-protein-partitioning theory of skin penetration enhancement [57]. This concept comprises lipid bilayer interaction (intercellular route), protein modification (intracellular route), and partitioning promotion effects [37]. Therefore, enhancers can [58] modify the intercellular lipid domains, reducing the barrier resistance of the lipid bilayers, and thus, augmenting diffusivity in the SC. Multiple actions are described regarding interaction of the CPE within the intercellular lipid domain (Fig. 7A).

Thus, they may interact with the lipid polar headgroups by establishing H-bonding and/or ionic forces and causing alteration in the hydration spheres of the lipid bilayers, or insert between the hydrophobic lipid tails (e.g. terpenes, gemini surfactants [60, 61]). As a result, they are able to disturb the lipid packing, thus increasing lipid fluidity and promoting drug penetration. On the other hand, perturbation in the lipid order may arise when the enhancer forms pools in the skin, as a result of the respective structure (polar head and long saturated alkyl chain, e.g., oleic acid), allowing the permeant to diffuse faster either through them or through the defects between the pools and the structured lipids [47, 62]. Some CPE may also cause lipid extraction (e.g., ethanol).

They can also act on SC proteins, such as intracellular keratin, causing its denaturation or modification of its conformation, which leads to swelling and increased hydration (Fig. 7B); or, by interacting with desmosomes, responsible for the maintenance of the cohesion between corneocytes (Fig. 7C).

In some cases, they alter the partitioning of the drug or of a cosolvent into the skin, by promoting modifications in the solvent nature (aqueous domain) of the stratum corneum. Solvents such as propylene glycol, ethanol, and Transcutol® (diethylene glycol monoethyl ether) are believed to act in this way [63].

CPE may also indirectly increase the thermodynamic activity of the vehicle or promote the solubilization of the permeant in the formulation [58].

Nanocarrier Systems

Nanocarrier systems including liposomes and derivatives, microemulsions, nanoemulsions, polymer and lipid nanoparticles have deserved increasingly attention in the delivery of drugs to the skin.

Vesicles

Liposomes are lipid vesicles, mainly composed by phospholipids with or without some additives (e.g. cholesterol, stearylamine), enclosing an aqueous volume [64]. They may consist of a single (unilamellar), a few (oligolamellar) or many (multilamellar) concentric phospholipid bilayer(s) [65].

In what concerns liposomes (lipid vesicles, mainly composed by phospholipids with or without some additives, e.g. cholesterol or stearylamine, enclosing an aqueous volume [64]) and related vesicles, such as niosomes (non-ionic surfactants vesicles [66]), ethosomes (ethanol phospholipid vesicles [67]), or ultraformable liposomes (also termed Transfersomes®), composed by phospholipids and an edge activator, that simultaneously destabilizes lipid bilayers of the vesicles and increases deformability of the bilayers [68], several studies, sometimes controversial, have been published reporting their effectiveness as transdermal drug delivery vehicles [69, 70]. In general, the rationale for using vesicles for promoting the transport of compounds into or across the skin relies on their ability to entrap the drug molecules, acting as drug carriers and to behave as penetration enhancers due to alteration promoted in the intercellular lipid lamellar as a result of the interaction with the lipid components of the formulation and to act as a drug reservoir suitable for a controlled drug delivery system [69, 71]. How deep vesicles can penetrate into the skin remains a conflicting subject, with studies reporting that conventional liposomes are preferentially confined to the upper layers of the SC, while ultraformable liposomes cross the intact skin, under the influence of the natural hydration gradient [72, 73].

Microemulsions

Microemulsions are clear, thermodynamically stable, isotropic liquid mixtures composed by oil, water, and surfactant, usually combined with a cosurfactant, with a size typically below 100 nm [74, 75]. They can behave as a potential reservoir of lipophilic or hydrophilic drugs, depending on the nature of the dispersed phase, either lipophilic (O/W) or hydrophilic (W/O). These systems are known to increase drug absorption through the skin. This may be due to the penetration enhancement effect of the carrier promoted by several combined mechanisms, such as by exerting a direct action on the skin, by interaction with the proteins (e.g. denaturation of intracellular keratin or affection of desmosomes), intercellular lipid (modification of the lipid bilayers or lipid extraction) reducing the resistance to penetration, and induction of changes in the solvent properties of the SC, affecting drug partitioning, or indirectly on the formulation, by promoting a supersaturated state (resulting from the solvent evaporation) and incorporating enhancer molecules in the composition of the vehicle that not only favor partitioning but also induce skin disorder [74].
Lipid Nanoparticles

Solid lipid nanoparticles (SLN), considered the first generation of lipid nanoparticles, were introduced in 1991 as an alternative carrier system to emulsions, liposomes and polymeric nanoparticles [76]. SLN are colloidal carriers, with sizes typically ranging from 40 to 1000 nm, derived from o/w emulsions, in which the liquid lipid (oil) was replaced by biodegradable and biocompatible solid lipids (0.1-30% w/w), that is, lipids that are in the solid state at both room and body temperatures, and stabilized by aqueous emulsifiers (0.5-5%) solution. Solid lipids can vary from pure lipids or a mixture of lipid compounds, encompassing triglycerides, partial glycerides, fatty acids, and waxes. On the other hand, the emulsifiers, including a large variety of non-ionic and ionic surfactants, are chosen depending on the administration route, being often used in association, in order to prevent particle agglomeration more efficiently [77].

At the turn of the millennium, a second generation of lipid nanoparticles, consisting of a matrix composed of a blend of solid and liquid lipids (oils), led to the creation of the concept of nanostructured lipid carriers (NLC) [78]. NLC were introduced to overcome some potential limitations of the SLN, such as low drug loading and drug expulsion during storage [79]. These problems are a consequence of the SLN crystalline matrix (pure solid lipid or a mixture of solid lipids) and of the occurrence of polymorphic transitions, respectively [80-82]. Lipid molecules are known to exhibit polymorphism, that is, presenting different three-dimensional structures (polymorphic forms): unstable α, metastable β', and as the most stable the β modification [83]. After preparation, at least a part of the lipid particle crystallizes in a higher energy modification (α and β' polymorphic forms). During storage, rearrangement of the crystal lattice might occur evolving to a more thermodynamically stable configuration (β form), which corresponds to a lower free energy state and, consequently, to a highly ordered system, with less imperfections on the crystal lattice of the lipids [84]. This transformation leads to the expulsion of the drug and contributes also to the instability of the system. The drug expulsion as a consequence of the crystallisation process led to the development of NLC, in which the matrix remains solid, but not crystalline [85]. In the NLC, the incorporation of a liquid lipid in the solid matrix creates a less ordered structure. This characteristic allows accommodating higher amounts of drug, which can be located not only between the fatty acid chains and the lipid layers, but also in imperfections of the lipid matrix (Fig. 8). Additionally, the solubility of the drug in oils is usually higher than in solid lipids [79, 80, 86, 87].
Several mechanisms of drug incorporation have been considered for these nanocarriers. In the case of SLN, three models can be applied: SLN type I or the homogenous matrix of solid solution, in which the drug is molecularly dispersed in the particle matrix, SLN type II or the drug enriched shell and SLN type III or drug enriched core models, where the drug is concentrated in the particle shell and core, respectively. For NLC, also three types are described: NLC type I or the imperfect model, with many imperfections in the matrix which are able to accommodate the drug, being created when small amounts of the liquid lipids are employed, NLC type II or the structureless/amorphous model, which is created when mixing special lipids which avoid the occurrence of crystallization, and NLC type III or the multiple O/W model, which is characterized by small oil nanocompartments dispersed in the solid lipid matrix. This can be obtained when the liquid lipids are incorporated in the solid matrix, exceeding the respective solubility. These particles are able to accommodate higher amounts of drug, in particular drugs more soluble in the liquid than in the solid lipids [83, 85]. Thus, either the composition or the method of production contribute to obtain different types/structures of lipid nanocarriers, which therefore result in different performances in terms of drug loading and release.

The nature of lipid nanoparticles confers them distinct advantages over conventional carriers (emulsions, liposomes, and polymer nanoparticles), such as an excellent tolerability, resulting from the generally recognized as safe (GRAS) status of the excipients employed. Thus, there is a reduced danger of acute and chronic toxicity, improved physical stability, protection of incorporated labile drugs from degradation, low cost, possibility to modulate drug release due to the solid nature of the lipid matrix, drug targeting, feasibility of scaling-up, cost-effective production method, and relatively low cost of excipients [81, 87, 89-93]. Moreover, their unique properties such as reduced size, combined with a large surface area and high drug loading are attractive for a potential improvement in the performance of pharmaceuticals, in particular for transdermal delivery [94, 95].

Polymer Nanoparticles

Other nanosized drug carriers, beyond lipid nanoparticles, have attracted much attention in the past decade. These include polymeric nanoparticles, where chitosan as a natural bio-compatible cationic polysaccharide [96] and synthetic polymers, such as poly-glycolic acid (PGA) [97] or their co-polymers as poly-lactic-co-glycolic acid (PLGA) [98], have been investigated for drug permeation enhancement. Particular emphasis has been given, in this respect to hair follicular route. Other approaches rely on the use of dendrimers. These are hyperbranched, monodisperse, three-dimensional macromolecules with a defined molecular weight, able to host and facilitate transport of drugs through the skin [99].

ACTIVE STRATEGIES

Active methods involve the use of external energy to act as driving force and/or to reduce the barrier function of the SC, which allows expanding the range of permeants to deliver through the skin. These manipulations include the application of various forms of energy (e.g., heat, electrical, magnetic), or breaching, reducing, or weakening the SC barrier by mechanical means [1]. Focus will be given to electrically assisted and mechanical methods. However, other technologies, such as magnetophoresis, which involves the application of a magnetic field, to enhance the driving force on a penetrant [100], photomechanical waves [101, 102], or thermal ablation [103] to increase the “permeabilization” of the SC have also been investigated.

Iontophoresis and Electroporation

Iontophoresis is a noninvasive method that involves the application of a small electric current to drive ionic molecules across the skin. It is particularly effective for ionic and polar drugs such as peptides, which are very poorly absorbed by the skin under normal conditions [104]. The technology employs the use of two electrodes immersed in a solution containing the drug compound. The application of a potential difference (voltage) across the electrodes results in the motion of drug molecules, in the form of charged ions, which travel from the electrodes into the skin [105]. Transdermal drug transport enhancement by iontophoresis is promoted by three main mechanisms: electrorepulsion of charged solutes by the electrode, electroosmotic effects on unionized, polar species, and permeabilization of the skin by the electric current [48, 104, 106].

Electroporation involves the application of high voltage (>50V) pulses to the skin, lasting for a period in the microsecond-millisecond range, and leading to the creation of aqueous pores or pathways through the SC. The pores formed are small (<10nm), transient (from microseconds to seconds), sparse (0.1% of surface area), and congregated in discrete local transport regions. This technique also expands the range of drugs (small to macromolecules, lipophilic or hydrophilic, charged or neutral molecules) which can be delivered transdermally [104, 107].

Sonophoresis

Sonophoresis is used to describe enhanced transdermal delivery following application of ultrasound energy to the skin. Ultrasound at various frequencies (20 kHz – 16 MHz) has been employed to enhance skin permeability. However, low-frequency ultrasound (<100 kHz, usually 20 kHz) has been reported to be more effective in the transdermal transport enhancement in comparison to high frequency ultrasound [108]. It induces the disruption of the lipid bilayer of SC through several phenomena: cavitation, which leads to generation and oscillation of gas bubbles forming small hydrophilic channels through the SC, combined with thermal effects (temperature increase), induction of convective transport and mechanical effects (occurrence of stresses due to pressure variation induced by ultrasound) [109]. This technique has been employed in the enhancement of the delivery of drugs, macromolecules, oligonucleotides, DNA and vaccines [110].

Mechanical Methods

The microneedles technique uses small micron-sized needles as a mechanical approach to breach the SC, creating microchannels through the skin, so as to deliver a drug at a predetermined depth [111]. Microfabrication techniques have been developed for silicon, metal, biodegradable polymers, and sugar-based microneedle arrays, having solid and hollow bores with different geometries and sizes. There are four general approaches of transdermal delivery by microneedles: (a) hollow microneedles, whereby a drug in solution is active and passively delivered through the bore of the microneedle (“poke and flow” approach), (b) solid microneedles to pierce the skin, following by application of a patch to diffuse drug through the skin (“poke and patch” approach), (c) biodegradable polymeric microneedles, containing the drug encapsulated (dissolving or porous microneedles), allowing a controlled drug release by dissolution or diffusion from the pores into the skin (“poke and release” approach), and (d) drug coated microneedles, inserting them into the skin for subsequent release through hydration of the coating (“coat and poke” approach) [10, 112]. Microneedles have been used to deliver drugs, proteins, and particles across skin in a simple, painless and minimally invasive manner, since they do not penetrate up to the papillary dermis where nerve endings are located [111]. However, important issues concerning safety and difficulty in self-administration can be pointed as disadvantages, which may delaying the introduction of microneedles in the market [113].

Needleless jet injectors arise as a painless technique, alternative to conventional needle injection, that enable to overcome the SC barrier, in this case by propel powders or liquids at high velocity into skin. It has been particularly employed in the transdermal delivery of proteins and peptides [114].
Other mechanical techniques, such as microdermabrasion or tape stripping may enhance penetration by reducing the thickness of the SC barrier, whereas stretching or flexing can lead to a general weakening of the barrier [104, 115].

On the other hand, delivery through the pilosebaceous unit represents an interesting pathway to convey macromolecules or even ions, either through the sebaceous glands or the dense network of blood capillaries that embed the follicles, thereby avoiding the barrier of the SC [116].

**Magnetophoresis**

Magnetophoresis is a method that uses a magnetic field to promote drug penetration. The predominant effect responsible for enhanced transdermal drug permeation is the magnetokinesis, that is, a phenomenon that forces the propagation of drug molecules under a gradient magnetic field. Similarly to iontophoresis, two mechanisms are described: magnetorepulsion and magnetohydrokinesis. Magnetorepulsion can be described as a repulsive force that drives the drug molecules in the presence of an external magnetic field, while magnetohydrokinesis mediated drug transport results from the movement of water across the membrane under the influence of the same external magnetic field [117, 118].

**Photomechanical Waves**

Photomechanical waves (PW) are pressure waves generated by intense laser radiation, that can transiently permeabilize the SC. They can induce lipid disruption, by the creation of temporary pores allowing drug diffusion into deeper layers and/or affect the cell membrane, hence opening up transcellular routes [119, 120]. These pressure waves are compression waves, thus excluding biological effects induced by cavitation, such as those promoted by ultrasound. Their amplitude is of the order of hundreds of atmospheres (bar), with a duration ranging from nanoseconds to only a few microseconds [101]. The application of these waves to human volunteers was not associated with pain or discomfort and no damage or alteration in the skin appearance has been evidenced. PW have been reported to efficiently enhance skin delivery of macromolecules, such as insulin, plasmid DNA and proteins [121].

**Recent Combinational Techniques**

In order to make the transport through the skin more effective, combinations among several strategies have been investigated. These have involved the association of passive with active methods, such as CPE-iontophoresis [122], SLN-iontophoresis [123], CPE-electroporation [124], CPE-sonophoresis [125], and between active technologies, e.g., microneedles-iontophoresis [126], microneedles-sonophoresis [127], microneedles-electroporation [128], or electroporation-iontophoresis [129].

**DEVELOPMENT OF A TRANSDERMAL FORMULATION**

A transdermal pharmaceutical formulation may range from an aerosol spray to a semisolid or self-adhesive patch (reservoir, matrix, drug-in-adhesive or microreservoir). Whatever the dosage form, important technological concerns must be addressed through-

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**Fig. (9).** Transdermal administration of lipid nanoparticles. (A) When in contact with the skin, lipid nanoparticles create a thin film with very narrow inter-spaces between the particles. Adapted from [88] (with permission). (B) This film hinders water evaporation, leading to an occlusive effect, and subsequently, to increased skin hydration, resulting in drug penetration-increasing effects. Adapted from [83].
out the pharmaceutical development, including compatibility, stability, acceptability, as well as the bioavailability in order to ensure the therapeutic outcome. That implies an optimization of the applied formulation focused on the improvement of release and permeation through the skin [63]. Accordingly, the composition of the vehicle and the active substances deserves special emphasis, especially because a close contact with the skin is often accompanied by possible risks of adverse reactions. Furthermore, the lack of precision related to the variability of the amount of drug delivered through the skin as a result of the epidermis thickness, as well as to a possible mechanical removal of the applied formulation must be equated [83]. In order to overcome the above-mentioned issues, more efficient solutions have still to be found.

Lipid Nanoparticles
Lipid nanoparticles (SLN and NLC) claim several advantages that prompt an enhancement in the penetration of drugs through the skin. These include the skin similarity stemming from their hydrophobic character, the biocompatibility, and the existence of a solid matrix, which make these carriers appropriate for a long-term controlled-release transdermal administration. Furthermore, the small particle size, along with a high specific surface area made available a higher drug absorption rate, therefore providing a larger efficacy as a delivery system [130]. Additionally, from a mechanistic point of view, the adhesion of lipid nanoparticles to the skin leads to a formation of a thin film, that induces occlusion and a reduction in transepidermal water loss [131, 132]. The resulting hydration effect, which in turn corresponds to one of the passive enhancement strategies (Fig. 6), of the SC leads to a disorder in the corneocyte packing and a widening of the inter-corneocyte gaps, thus enabling drug penetration into deeper skin strata (Fig. 9) [132-134].

Ethanol and Terpenes
Chemical permeation enhancers has long been shown to increase the diffusivity and/or the solubility of the drugs by reversibly disordering or ‘fluidizing’ the lipid structure of the SC, hence compromising the barrier function of the skin [10, 58, 135]. Taking this into consideration, terpenes and ethanol as co-solvent incorporated in a lipid nanoparticles formulation arise as an appealing complementary approach, so as to maximize permeation, by combining the effect of the nanoparticles and chemical enhancers.

The aforementioned combination has recently been assessed, revealing a synergistic effect for the permeation rate of two distinct drugs, both from a physicochemical and pharmacotherapeutical point of view, olanzapine and simvastatin [50]. Note that a full characterization of this type of systems requires a combination of different techniques [136], and must be supported by demanding analytical methods [137], which make the system even more challenging. Figure 10 illustrates some of these findings.

A microneedle roller device (Dermaroller®) for the perforation of the SC was subsequently employed as an alternative methodological. Microneedles induce transient micropathways that allow lipid nanoparticles and drugs to bypass the SC, thus facilitating their permeation into the viable epidermis [10, 138]. Nevertheless, the findings have shown that, for this system, the skin pretreatment by the microneedle device only slightly increased the permeation rate. This emphasized that the formulation characteristics determined the main driving force for skin permeation [139].

CONCLUSION
Several approaches for the transdermal delivery of drugs were addressed in this review, and the pros and cons of different methods presented in an attempt to assess the respective features. It is well known that the skin barrier represents a formidable obstacle for drug delivery, but new multicomponent technologies, combining several strategies, have shown promising results within a field that remains largely unexplored.

![Fig. (10). Permeation rate of olanzapine and simvastatin using a combination of nanostructured lipid carriers and terpenes in ethanol as chemical enhancers. Results are expressed as mean±SEM. (n=6). Key: Sat sol = Saturated solution, 30%Et = 30% V/V Ethanol, 5%L = 5% w/V Limonene, NLC = Nanostructured lipid carriers, OL = Olanzapine, SV = Simvastatin, Jss = flux at steady-state.](image)
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